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MAGNETIC AND MODEL STUDIES OF CYTOCHROME C OXIDASE

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MAGNETIC AND MODEL STUDIES OF CYTOCHROME C OXIDASE

by

Vinai Chunplang

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IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE:

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Houston, Texas

May, 1985
ABSTRACT

Magnetic and Model Studies of Cytochrome c Oxidase

PART I: Magnetochemical Studies of Bovine Cytochrome c Oxidase.

PART II: A Synthetic Model Compound Approach to the Active Site Structure of Cytochrome c Oxidase.

By Vinai Chunplang

PART I: The variable-temperature (20-200 K) magnetic susceptibility properties of bovine cytochrome c oxidase in its fully-oxidized or resting state (Hartzell-Beinert and Yonetani preparations), its fully-reduced state (Hartzell-Beinert) and its resting-CN⁻ state (Hartzell-Beinert and Yonetani) have been examined. The Hartzell-Beinert resting (g₁₂ form), fully-reduced (from g₁₂), and resting-CN⁻ (from g₁₂) studies are repeat experiments of Tweedle, et al., [J. Biol. Chem. (1978), 253, 8065], performed in our laboratory about nine years ago. The Hartzell-Beinert resting enzyme without a g₁₂ epr signal (g₁₂-less) and Yonetani resting enzyme (g₁₂) studies are new to this work. In general, the magnetochemical results for the Hartzell-Beinert (g₁₂ and g₁₂-less) and Yonetani resting enzyme forms are nearly Curie in nature and identical to those of Tweedle. All three sets of data are consistent with a strong antiferromagnetic exchange-coupled \([\text{cyt.}\text{A}^{3+}(S = 5/2)-\text{Cu}^{2+}(S = 1/2)]\) pair at the active site with \(-J \geq 200\text{cm}^{-1}\) to give an epr inactive \(S = 2\) gound state. The
Hartzell-Beinert reduced enzyme displays magnetochemical behavior like that previously reported by Tweedle, where only cyt.b$_3$$^{2+}$ (S = 2) is a paramagnetic center. Finally, the magnetochemistry of the Hartzell-Beinert and Yonetani resting-CN$^-$ derivatives were also found to be nearly identical to that of Tweedle, in that the data are non-Curie in nature and can be interpreted in terms of a weak antiferromagnetic exchange-coupled [cyt.b$_3$$^{3+}$ (S = 1/2) - Cu$_{II}$$^{2+}$ (S = 1/2)] pair at the active site. The coupling constants are $-J \sim 20$ cm$^{-1}$ and $\sim 45$ cm$^{-1}$ for H.B$_{12}$-less and Yonetani resting-CN, respectively, (Tweedle: $-J \sim 30$ cm$^{-1}$). The past and present magnetochemical results for bovine cytochrome c oxidase are discussed as they relate to some current inconsistencies in the literature.

**PART II:** Twelve new $\mu$-imidazolato heterobinuclear metal complexes have been synthesized to model the proposed imidazolate-bridged [cyt.b$_3$$^{3+}$(imid)Cu$_{II}$$^{2+}$] active site structure of resting cytochrome c oxidase where $-J_{(Fe_{III}-Cu_{II})} \geq 200$ cm$^{-1}$ may be the case. The model compounds have been derived from [LFe$_{III}$(TPP)] (TPP$^{2-}$ = tetraphenylporphyrinato and L = OSO$_2$CF$_3^-$) and [M$^{Ii}$(2-mimidH)$_2$DAP]$^{2+}$ or [M$^{II}$(imidH)$_2$DAP]$^{2+}$ (M$^{II} = $ Zn and Cu) to yield species containing [LFe$_{III}$(2-mimid)Zn$^{II}]^+$ [compound 1], [LFe$_{III}$(2-mimid)Cu$^{II}]^+$ [compound 2], [LFe$_{III}$(imid)Zn$^{II}]^+$ [compound 3], and [LFe$_{III}$(imid)Cu$^{II}]^+$ [compound 4] cores. Furthermore, reaction of [M$^{II}$(TPP)] (M$^{II} = $ Co or Mn) with [M$^{II}$(imid)DAP]$^{2+}$ (M$^{II} = $ Zn and Cu) has produced new model compounds
containing [Co^{II}(imid)Zn^{II}]^+ [compounds 5 (BF$_4^-$) and 7 (OSO$_2$CF$_3^-$)],
[Co$^{II}$(imid)Cu$^{II}$]$^+$ [compounds 6 (BF$_4^-$) and 8 (OSO$_2$CF$_3^-$)], [Mn$^{II}$(imid)Zn$^{II}$]$^+$ [compounds 9 (BF$_4^-$) and 11 (OSO$_2$CF$_3^-$)] and [Mn$^{II}$(imid)Cu$^{II}$]$^+$ [compounds 10 (BF$_4^-$) and 12 (OSO$_2$CF$_3^-$)] centers. The
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considered to be good spin mimics of the [cyt. $\Delta_3^{3+}(S = 5/2)$–Cu$^{2+}(S$
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DEDICATION

This thesis is dedicated to my parents
who have always loved and believed in me and given
me the support and encouragement necessary to achieve my goals.

In a more special way, it is also dedicated to Nathanaht,
who has always been loving and understanding.
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INTRODUCTION

Cytochrome c oxidase (cyt. c ox.) is the terminal enzyme in the respiratory metabolism of all aerobic organisms, including plants, animals, yeasts, algae and some bacteria. The enzyme is responsible for catalyzing the four electron reduction of one mole of molecular oxygen or dioxygen ($O_2$) to produce two moles of water with the release of 23.8 kcal mole$^{-1}$ of energy [1],

$\text{(reduced cyt. c ox.)} + O_2 + 4H^+ \rightarrow \text{(oxidized cyt. c ox.)} + 2H_2O + E.$

This provides most of the free energy needed for the life processes of aerobic organisms by coupling electron transport to the synthesis of adenosine triphosphate, ATP [2]. It is not surprising, therefore, that the enzyme is found in high concentration in tissue with high energy requirements [3]. Another gauge of its importance in aerobic biological systems comes from the suggestion of Malmström [4] that 90% of the biological dioxygen consumption is directed through cytochrome c oxidase. The enzyme is tightly associated with either the inner membrane of mitochondria, the respiratory organelle of higher organisms, or the cell membrane in bacteria. The fundamental importance of the dioxygen reaction with cytochrome c oxidase has stimulated many investigations aimed at ascertaining the structure/function relationship of the active site.

The study of the cytochrome first began in the late 1800's by MacMunn [5], a British physician, who discovered it to be a respiratory pigment. He found the pigment to be distributed in most the plant and
animal tissue and called it myohematin [6]. MacMunn's important
discovery received very little attention from scientists and became
effectively lost in the literature [7] until the 1920's when Keilin and
Warburg initiated their studies of the pigment. In 1925, Warburg [8]
postulated that the cell contains an iron compound in its bivalent state
and that this state reacts with dioxygen and passes into a higher
valency state; he called it "Atmungsferment". At the same time, by
using microspectroscopy, Keilin [9] confirmed the validity of MacMunn's
discovery [10] and demonstrated that the pigment in its reduced form
contains a spectrum with four bands at 604, 564, 550, and 521 nm.
Later, Keilin realized that this spectrum arose from three different
components, none of which was autooxidizable, and he named them
cytochromes a, b, and c. In 1926, Warburg [11], by ingenious use of the
light-induced reversal of CO inhibition of the respiration in yeast,
definitely proved the heme protein nature of Atmungsferment by its
photochemical action spectrum [11,12]. Because the indophenol reaction
of nonrespiring cells or cell-free preparation of heart muscle was also
inhibited by CO [13] and this inhibition, like the respiratory activity
of intact yeast cells [11], was reversed by light, Keilin [14]
postulated the existence of a respiratory "chain" responsible for
cellular respiration. In his proposed scheme, substrate dehydrogenases
reduced the cytochromes and the dehydrogenases were further oxidized by
CO-sensitive oxidase, which also probably contain copper [15,16].
However, since Keilin was unsuccessful in demonstrating the dissociation
of CO of cytochrome a, Warburg [17] rejected the cytochrome as a pigment
arising from the degradation of the Atmungsferment. It was not until 1938 that Keilin and Hartee [16,18,19] were able to demonstrate that, in fact, the cytochrome actually contained two distinct heme groups which he later named cytochrome $a$ and $a_3$. In addition, Keilin characterized Warburg's previously reported green heme, Atmungsferment, as cytochrome $a_3$. Furthermore, in work associated with Hartree, Keilin also found that cytochrome $a_3$ reacts with CO, CN$^-$, and possibly dioxygen, while cytochrome $a$ remained inert towards these substances. More than ten years later, Chance and co-workers [20,21] studied the photo-dissociation spectrum of the cytochrome $a_3$-CO compound from yeast and heart muscle and showed it to be identical to the photochemical action spectrum of Warburg. This provided final proof of the identity of cytochrome $a_3$ and cytochrome c oxidase. The term cytochrome c oxidase was coined for the protein containing cytochromes $a$ and $a_3$ by Keilin because it could directly oxidize cytochrome c. Keilin [15,16] also postulated that the enzyme contained copper, however, he could not demonstrate the necessity of copper in the oxidation of cytochrome c by cytochrome c oxidase. It was not until 1963 that the definite evidence, that the loss of the copper corresponded to a loss in activity, was obtained, which implied that copper is an important part of the active site of cytochrome c oxidase [22]. Extensive studies by Japanese scientists in the 1930's (see, for example: Tamiya and Ogura [23]) resulted in the isolation of purified cytochrome c oxidase free of all other cytochromes [24].
The mechanism of oxidation of cytochrome c by cytochrome c oxidase was previously described by two widely accepted theories: (1) the theory of unified cytochrome c oxidase activity, postulated by Okunuki [25], which stated that cytochrome a did not exist as two parts (cytochrome a and a$_3$) but as one individual unit, and (2) the concerted action theory of George and later Malmstrom [26] which asserted that the electron transfer between the cytochromes and dioxygen proceed along multi-electron transfer paths, minimizing the individual role of each metal center in the electron transfer process. These two theories were well-known and widely accepted among scientists until the mid 1970's when Chance and his coworkers [27], using low-temperature flash photolysis techniques in rapid kinetic experiments, demonstrated an alternative mechanism for the electron transfer from cytochrome c through cytochrome c oxidase to dioxygen. They demonstrated that the electron transferred reaction was a stepwise reaction, by which cytochrome c first transferred its electron to cytochrome a and then to the binuclear complex of copper and cytochrome a$_3$. Furthermore, they showed evidence of a peroxo intermediate associated with the binuclear complex, which was a crucial piece of evidence supporting the stepwise mechanism [28]. Reduction potential studies of each of the metal centers lead to the postulate that the electron transfer was first to cytochrome a, then to copper, then to cytochrome a$_3$, and finally to dioxygen [29]. Based upon a collective body of work, the theory of Chance and co-workers is now widely accepted.
Cytochrome c oxidase is not only important in the respiratory chain, but it is also one of the most complex metalloproteins. Hence, it is of great interest to study in order to elucidate its structural properties as well as to understand its reaction mechanism. As mentioned previously, the enzyme is the terminal electron acceptor in the mitochondrial respiratory chain and it plays an important role as the catalytic enzyme in the reduction of dioxygen to produce water. To date, the exact mechanism whereby the enzyme affects the oxidation of four moles of ferrocytochrome c with the concomitant reduction of dioxygen is still one of the most vigorously pursued areas in all of biochemistry.

In pursuing an understanding of the mechanism whereby cytochrome c oxidase functions, it is crucial to know the nature of the active site. To this end, there have been several investigations of the structural properties of the enzyme active site [31] in order to arrive at a picture of its overall topology. It is now generally agreed that the enzyme contains two iron and two copper atoms. An essential requirement for the thorough characterization of the enzyme mechanism is to firmly establish the electronic structures of the four metal centers. This realization has lead to a great deal of effort aimed at determining the exact electronic and structural properties of the metal centers in the fully-oxidized, fully-reduced and intermediate-reduced forms of the enzyme. The existence of two different cytochromes, a and a₃, was first proposed by Kielin and Hartree [16,18,19] to explain a series of
observations about the effect of inhibitors on the absorbance spectra of the cytochromes in respiring yeast and from heart muscle. This fact, combined with spectroscopic data, has now firmly established that the two iron heme centers cyt.A and cyt.A3, in cytochrome c oxidase exist in different environments. Through various spectroscopic and other studies, it is also now generally agreed that cytochrome A3 contains a pentacoordinate high-spin iron(II) center (cyt.A32+) in the fully-reduced form of the enzyme. The sixth coordination site of cyt.A32+ is thus vacant and available for an interaction with simple ligands such as CO, CN⁻ or with dioxygen to form an oxidized, hexacoordinate iron heme (cyt.A33+) in the fully-oxidized or resting enzyme. On the other hand, cytochrome A is a low-spin, six-coordinated iron heme in both the reduced (cyt.A2+) and oxidized (cyt.A3+) forms. The fifth and sixth axial positions of the heme in cytochrome A are occupied by imidazole group from histidine residues, and they are unreactive toward dioxygen or other ligands.

- Keilin was the first to suggest that copper was essential for the enzyme to have normal activity [15,17,19]. Mackler and Penn [32] found that copper and heme were present in a 1:1 ratio in oxidase from beef heart. Similar to the iron heme groups, it was also found that there were two types of epr-measurable copper which were also isolated from one another. The iron and copper in the native enzyme are assumed to exist as Fe(III) and Cu(II) in the fully-oxidized or resting form, and Fe(II) and Cu(I) in the fully-reduced form [33]. This assumption is
based on the fact that a mole of native enzyme contains two moles of iron and two moles of copper and requires four moles of electrons to complete the reduction of one mole of dioxygen. The four metal sites are referred to as Cu_D, Cu_U, cyt_a, and cyt_a[3], where D and U indicate epr-detectable and undetectable, respectively. In addition, it is known that in the oxidized form of the enzyme, cytochrome_a is a low-spin Fe(III) heme site (S = 1/2) and Cu_D is an epr-detectable Cu(II) site (S = 1/2). Cytochrome_a, reported to be the primary electron acceptor from cytochrome_c, and Cu_D are isolated from one another and there is no direct metal-metal interaction between the two centers [34].

One of the most important and perplexing features of many studies of cytochrome oxidase has been the reoccurring indication that the high-spin (S = 5/2) Fe(III) heme center of cytochrome_a[3]^{3+} exists as an intergal part of native cytochrome oxidase, and should, therefore, be epr detectable [35]. However, no S = 5/2 iron epr signal (g ~ 6, 2) of the fully-oxidized enzyme has ever been detected, nor has more than half of the Fe(III) or Cu(II) ever been accounted for by epr. An explanation for the systematic absence of both Cu(II) and Fe(III) epr signals resides in a suggestion by Van Gelder and Beinert [36] that both the epr-silent Fe(III) and Cu(II) in oxidized cytochrome oxidase are present as an antiferromagnetically coupled binuclear center of even-spin: cytochrome_a[3]^{3+} (S = 5/2) coupled to copper(II) (S = 1/2) to yield [cyt_a[3]^{3+} - Cu^{2+}] (S = 2 as the ground state with an S = 3 excited state, 3J above the ground state). This possibility has been explored
theoretically by Griffith [37]. With Fe(III) \((S = 5/2)\) and Cu(II) \((S = 1/2)\) thus coupled to produce an even-spin system, no epr signal should be observed. The comprehensive model of Palmer, Babcock and Vickery [PBV] [38] thus proposed that the functioning unit of resting cytochrome oxidase is composed of three metal-centered units: an isolated low-spin \((S = 1/2)\) ferrihemoprotein center (cytochrome \(a^3\))\(^{+}\), an isolated Cu(II) \((S = 1/2)\) center (Cu\(D^2\))\(^{+}\), and an antiferromagnetically spin-coupled \((S = 2)\) binuclear center comprised of Cu(II), \(\text{Cu}_U^2\)\(^{+}\), and a high-spin ferrihemoprotein center (cytochrome \(a_3^3\))\(^{+}\) bridged via an imidazolate anion from a histidine residue. Recently, in a variable-temperature (6–300 K) magnetochemical study, Tweedle, et al., [39] in conjunction with other studies, including optical [40], MCD [41,42] and resonance Raman [43] spectroscopy, have suggested for the fully-oxidized or resting form of the enzyme, that the iron center of cytochrome \(a_3^3\)\(^{+}\), Fe(III) \((S = 5/2)\) and Cu\(U^2\) (II) \((S = 1/2)\) are strongly coupled antiferromagnetically \((-J \geq 200 \text{ cm}^{-1})\) to give a resultant \(S = 2\) ground state for the binuclear active site. These studies support the PBV model [38] of a \([\text{cyt.}a_3^3+(B)\text{-Cu}_U^2]\) structure for the active site, however, the nature of the bridging ligand is still very much in question. Biochemically feasible bridges such as \(B = \text{imidazolato from}\) histidine [38,39], \(\text{oxo from water, dioxygen or tyrosine}\) [44,45] and \(\text{mercapto from cysteine}\) [46,47] have all been suggested as possible mediators of the "strong" magnetic exchange interaction. However, recent Mössbauer studies of cytochrome c oxidase, from \textit{Thermus Thermophilus} and beef heart, by Münck and Fee, et al., [80] have
questioned both the $S = 5/2$ assignment for $\text{c}yt_a^3{^3}^+$ and the antiferromagnetic coupling scheme.

An alternative explanation for the absence of the epr signal for $\text{c}yt_a^3{^3}^+$ and $\text{Cu}_U^{2+}$ has also been suggested by Seiter and Angelos [48]. In this model, the lack of epr signals was postulated to be due to an $\text{c}yt_a^4{^4}^+$ ($S = 2$) center and an $\text{Cu}_U^{+}$ center. The Seiter and Angelos model has never gained wide-spread support [50] because it assumes a redox inactive role for $\text{Cu}_U^{+}$, while necessitating a relatively rare situation of an Fe(IV) hemoprotein center. In fact, the recent Mössbauer studies of cytochrome c oxidase from *Thermus Thermophilus* by Mühle and Fee [49] tend to rule out the possibility of an Fe(IV) heme in the resting state, while also raising the possibility of heterogeneity [81-85] at the active site in both the bacterial and bovine enzymes [51].

As mentioned previously, the nature of the bridging ligand in the $[\text{c}yt_a^3{^3}^+-\text{(B)}-\text{Cu}_U^{2+}]$ model for the active site of cytochrome c oxidase is still in question. This realization has inspired an interesting line of research aimed at elucidating the identity structure of the bridging ligand, and therefore, of the active site in the native enzyme. Among the three leading possible candidates, imidazolato from histidine [38,39] receives the most attention in this thesis. In addition, an oxo bridge possibility, as first proposed by Blumberg [44,45], and a mercapto bridge possibility, as recently suggested by Powers and Chance [46,47] on the basis of their EXAFS studies, round out the possible
candidates. Figure 1 illustrates proposed structures for each of the three possibilities for the \([\text{cyst.} \mathbf{Fe}_3^{3+}] \rightarrow \text{B} \rightarrow \text{Cu}_U^{2+}\) binuclear active site.

Figure 1b shows the \(\mu\)-imidazolato model, first proposed by Palmer, et al., [38]. The model incorporates the \([\text{cyst.} \mathbf{Fe}_3^{3+} \text{imid} \text{Cu}_U^{2+}\) unit as the active site structure in the resting enzyme. In this proposal, an imidazolate group from a proximal histidine residue of the protein occupies the fifth coordination site of \(\text{cyst.} \mathbf{Fe}_3^{3+}\) as a bridge between the Fe(III) and Cu(II) centers. Thus, the \(\text{cyst.} \mathbf{Fe}_3^{3+}\) center will have a vacant sixth, axial site. During the catalytic cycle of Figure 2, which shows the 4e reduction of dioxygen to two moles of water, the dioxygen molecule can be depicted as binding to the distal site of \(\text{cyst.} \mathbf{Fe}_3^{2+}\) in the binuclear unit. The \(\mu\)-imidazolato proposal is based upon some circumstantial results which include: (1) studies of the \(\text{Fe}^{15} \text{NO}\) reduced enzyme [52] which illustrate that the Fe(III) center of \(\text{cyst.} \mathbf{Fe}_3^{3+}\) has a nitrogen atom as an apical donor atom, (2) epr studies of \(\text{Fe}^{15} \text{N}\) labelled histidine by Chan, et al., [53] which have indicated that, in the fully-reduced enzyme, the axial position of \(\text{cyst.} \mathbf{Fe}_3^{2+}\) is occupied by endogeneous imidazole, and (3) X-ray structural data of superoxide dismutase (SOD) which demonstrate the presence of an imidazolate bridge in a mixed-metal binuclear \([\text{Cu}^{II}(\text{imid})\text{Zn}^{II}]\) center [54,55].

Furthermore, preliminary magnetic studies of the cobalt derivative of SOD, \([\text{Co}^{II}(\text{imid})\text{Cu}^{II}]\) [58-58], indicate strong (-J > 300 cm\(^{-1}\)) antiferromagnetic coupling between the metal centers. To date, several model compounds containing \([\text{M(imid)M}']\) porphyrin centers have been
Figure 1

Three Proposed Ligand-Bridged Models for the
Active Site Structures of Resting Cytochrome c Oxidase

a) The μ-Oxo Model
b) The μ-Imidazolato Model
c) The μ-Mercapto Model
Figure 2

Proposed Catalytic Mechanism Incorporating
The μ-Imidazolato Model as the Active Site Structure
in Resting Cytochrome c Oxidase
4 Cyt. c\(^{3+}\)  
Cyt. \(\alpha^{+2}\) Cu\(_u^+\)  
4 Cyt. c\(^{2+}\)  
Cyt. \(\alpha^{+3}\) Cu\(_u^{+2}\)  

4H\(^+\), Cyt. \(\alpha^{+2}\), Cu\(_u^+\)  
2H\(_2\)O, Cyt. \(\alpha^{+3}\), Cu\(_u^{+2}\)

REDUCED OXIDASE

RESTING OXIDASE (\(-\nu_2 \geq 200 \text{ cm}^{-1}\))
reported [59-63]. Most available information obtained from studies of these model compounds have indicated that imidazolate cannot foster antiferromagnetic exchange interactions as large as that presumably present in resting cytochrome oxidase.

Blumberg [64], among others [38,39], first described a $\mu$-oxo model as an alternative for the active site structure of cytochrome oxidase. The $\mu$-oxo model, with its $[\text{cvt}_{2}\text{Fe}_{3}^{3+}-\text{O}-\text{Cu}_{2}^{2+}]$ active site structure as shown in Figure 3, employs only a single oxygen atom ($O^{2-}$) from dioxygen as the bridging ligand between the $\text{cvt}_{2}\text{Fe}_{3}^{3+}$ and $\text{Cu}_{2}^{2+}$ centers in resting oxidase with the imidazolate moiety of a histidine residue occupying the sixth, backside coordination site [65]. In comparison to the $\mu$-imidazolato proposal, the $\mu$-oxo possibly perhaps seems more acceptable as a structure for the active site of the resting oxidase, primarily because several $\mu$-oxo binuclear complexes are known which exhibit a strong antiferromagnetic exchange interaction of the magnitude proposed to be operative in resting oxidase [66]. Among these is a mixed-metal, binuclear porphyrin complex containing a proposed $[\text{Fe}_{\text{III}}-\text{O}-\text{Cu}_{\text{II}}]$ core [67] which may exhibit an antiferromagnetic exchange interaction as large as $-J \gtrsim 200 \text{ cm}^{-1}$. In addition, Chang et al., [57] have reported another mixed-metal, $\mu$-oxo species containing an $[\text{Fe}_{\text{III}}-\text{O}-\text{Cu}_{\text{II}}]$ unit which shows antiferromagnetic coupling with $-3J = 132 \text{ cm}^{-1}$, and West and co-workers [69] have reported a similar $[\text{Cr}_{\text{III}}-\text{O}-\text{Fe}_{\text{III}}]$ species with $-J = 153 \text{ cm}^{-1}$. Furthermore, available magnetic and spectroscopic data [70], including some very recent EXAFS results [71a,71b], suggest an
Figure 3

Proposed Catalytic Mechanism Incorporating
The μ-Oxo Model as the Active Site Structure
in Resting Cytochrome c Oxidase
[Fe–Cu] separation of only ca. 3.0 Å in resting oxidase which, of course, favors a single atom such as oxygen or perhaps sulfur as the bridging ligand; in contrast, ca. 5 Å would be required for the [Fe–Cu] separation, if imidazolate were the bridge. Furthermore $^{18}O_2/^{16}O_2$ labelling studies, by Beinert, et al., [72] to determine whether the formation of an oxo bridge from dioxygen is a reasonable possibility, indicated that neither atom of dioxygen (as $O^{2-}$) is incorporated in the enzyme as a bridging ligand. In the experiment, the enzyme was cycled through one oxidation turn over and then all H$_2$O$^{18}$ produced was monitored by mass spectrometry to determine whether any $^{18}O_2$ was retained by the fully-oxidized enzyme. Within experimental error, all of the labelled $^{18}O_2$ was found in H$_2$O$^{18}$ rather than only half, as would be expected if an $^{18}O^{2-}$ bridge were retained by the enzyme. Other evidence opposing the μ-oxo possibility include resonance Raman studies by Palmer, et al., [73] in which $^{18}O_2$ instead of $^{16}O_2$ was utilized in preparing the resting enzyme. In the experiment, no evidence of any isotope effect on the resonance Raman spectrum was observed which might have have arisen from the formation of different [Fe$^{II}_3$-$^{16,18}O$–Cu$^{II}$] bridging site structures. Although the results of these two $^{18}O/^{16}O$ labelling studies do not eliminate the possibility of the μ-oxo model, they do question its validity.

Primarily on the basis of EXAFS data of cytochrome c oxidase, Powers, et al., [47] recently suggested an alternative μ-mercapto model for the active site structure of the enzyme. In this model, as
illustrated in Figure 1c, a sulfur moiety from a methionine or cysteine residue of the protein backbone is proposed as the bridging ligand between cyt.a$_3^{3+}$ and Cu$_U^{2+}$. In addition to the EXAFS data, a resonance Raman experiment [74] has suggested that the Cu$_U^{2+}$ site in the oxidized or resting enzyme may be a Type I or blue copper site, although a recent electronic spectroscopy study by Brudvig, et al., [75] has indicated that Cu$_U^{2+}$ is not a Type I copper center. Figure 4 depicts a proposed mechanism for dioxygen reduction involving the μ-mercapto model.

Following the breaking of the Fe-S bond, this scheme proceeds to the dioxygen binding step where a μ-peroxo iron-copper intermediate is postulated [62]. Essentially, this mechanism is a "special case" of the catalytic cycle of the μ-oxo model.

Heterotrinuclear and binuclear complexes containing sulfur bridging ligand have recently been synthesized. Elliott and Akabori [77] have reported an [Fe$^{III}$-Cu$^{II}$-Fe$^{III}$] trinuclear complex containing sulfur bridges between the (S = 1/2) copper center and two Fe(III) centers of intermediate spin (S = 3/2) to give an epr silent center [78]. The complex was magnetochemically characterized as having three uncoupled metal centers, and the epr silent behavior was interpreted as arising from a combination of dipolar coupling and a small exchange interaction between the copper center and the rapidly relaxing S = 3/2 iron centers. Furthermore, Holm and co-workers [79] have recently reported a [Fe$^{III}$-S-Fe$^{III}$] bridged compound which exhibits a strong antiferromagnetic exchange interaction (−J = 176 cm$^{-1}$) between the two
Figure 4

Proposed Catalytic Mechanism Incorporating
The μ-Mercapto Model as the Active Site Structure
in Resting Cytochrome c Oxidase
iron centers through a mercapto bridge. This is one of the first well-characterized model compounds to demonstrate that a μ-mercapto bridge can foster an antiferromagnetic exchange interaction as strong as that which may exist for the active site of resting oxidase.

Since the catalytic mechanism by which cytochrome c oxidase reduces dioxygen to water is dependent on the nature of the bridging ligand between cyt$_{A3}^3+$ and Cu$_{U}^{2+}$, studies aimed at revealing the active site structure are of fundamental importance before the catalytic activity can be fully understood and appreciated. Thus, further elucidation of the nature of bridging ligand of the oxidase active site was one of the ultimate goals of the thesis. In order to help achieve the goal, this work has been divided into the following two parts:

**Part I** involves full-temperature (ca. 20-200 K) magnetic susceptibility studies on resting oxidase and its cyano derivative. Studies using Hartzell-Beinert preparations of these enzyme forms are, for the most part, repeated experiments of work performed in our laboratories about nine years ago [39] and have been reexamined here because of some inconsistencies between our earlier data and recent literature results [81]. Furthermore, we have also performed new experiments on the resting and cyano-derivatized resting enzyme obtained by the Yonetani isolation procedure, for the purpose of comparison with the Hartzell-Beinert data.
Part II involves the synthesis, isolation, and study of twelve new μ-imidazolato heterobimetallic compounds as model compounds for Palmer’s proposed \([\text{cyt.}a_3^{3+}(\text{imid})Cu_2^{2+}]\) structure [38] for the active site of resting cytochrome c oxidase. This work concerns model compounds of metalloporphyrins with \([\text{Co}^{II}(\text{imid})\text{Cu}^{II}]^+\), \([\text{Mn}^{II}(\text{imid})\text{Cu}^{II}]^+\), and \([\text{Fe}^{III}(2\text{-meimid})\text{Cu}^{II}]^+\) cores and follows-up two of our earlier reports which involved other \([\text{Fe}^{III}(\text{imid})\text{Cu}^{II}]^+\) model compound systems [59, 61]. Through full-temperature (ca. 20-300 K) magnetic susceptibility studies and various spectroscopic investigations (UV-vis, epr) these new μ-imidazolato model compounds have yielded additional insight about the suitability of Palmer’s proposal for the active site structure of cytochrome c oxidase.
PART I

Magnetochemical Studies of Bovine Cytochrome c Oxidase

Knowledge of the oxidation and spin states of the four metal sites in oxidized, reduced, partially oxidized and partially reduced cytochrome c oxidase is vital for an understanding of the catalytic mechanism of the enzyme. Essential information about the heme spin states in a cytochrome c oxidase can be obtained from Mössbauer, MCD, epr spectroscopies and magnetic susceptibility studies of the enzyme. The Mössbauer technique is very useful for an $^{57}$Fe enriched sample such as cytochrome $c_{1a3}$ from Thermus Thermophilus, but it is very limited when applied to the bovine enzyme [80] due to the lack of $^{57}$Fe enrichment [86]. MCD spectroscopy is not quantitatively diagnostic of all iron spin-states [87] and the epr technique is not applicable to even spin systems which may occur at the active site (eg. $S = 2$). The magnetic susceptibility measurement, however, is inherently quantitative, giving a direct measure of the heme spin state. Hence, magnetic susceptibility data are crucial experimental information. In addition, magnetochemical studies are especially important to the cytochrome c oxidase question since they supply a general method, not available through other physical/spectroscopic techniques, for directly probing the nature of a bridging ligand at the active site, in addition to the metal centers being bridged.

The paramagnetic susceptibility of high molecular weight proteins such as cytochrome c oxidase is only a small part of the measured bulk magnetic susceptibility. The small amount of paramagnetic
susceptibility must be extracted from the overwhelming diamagnetic background due to the apoprotein. Hence, it is very difficult and troublesome to obtain accurate and useful magnetic susceptibility data for cytochrome c oxidase (MW ~ 2x10^5 Daltons). In spite of the difficulties, a few successful magnetic susceptibility measurements have been made. Ehrenburg and Yonetomi reported the first set of magnetic susceptibility data in 1961 [88]. Their experimental results suggested that cytochrome a_3 was in a mixture of high- and low-spin states, which were in thermal equilibrium. This interpretation was disproved by Tsuzuki and Okunuki who reported another set of magnetic susceptibility data in 1971 [89]. These two sets of data, however, are both considered questionable due to the presence of contaminating extrinsic copper or other metals [90]. In 1968, Ehrenburge and Vanneste [91] repeated the measurements on "better" preparations, and extended the former measurements to include valuable determinations at intermediate states of reduction. Later in 1977, Falk, et al., [92] reported the ambient temperature magnetic susceptibility of fully-oxidized and fully-reduced cytochrome c oxidase as determined by an NMR technique. These experimental data gave strong support to a picture of the cytochrome c oxidase with one heme in a high-spin state (S = 5/2) antiferromagnetically coupled to a copper(II) center (S = 1/2) and with one low-spin heme (S = 1/2) and one copper(II) (S = 1/2) being magnetically isolated centers. Their interpretation was in good agreement with the prediction of the PBV model, i.e., one S = 2 binuclear [Fe/Cu] center and two S = 1/2 centers [38]. To date, the most thoroughly conclusive magnetic
susceptibility study of the protein was reported in 1978 by Tweedle, et al., [39]. In the study, the temperature dependence of the paramagnetic susceptibility of cytochrome c oxidase in its fully-oxidized, fully-reduced and CN⁻-derivatized form of the resting enzyme was measured from 6 to 200 K. The magnetic susceptibility data for the fully-reduced enzyme indicated that the only paramagnetic center present was a simple high-spin (S = 2) ferrous species, previously identified as cytochrome a₃²⁺ on the basis of MCD [93] and solution magnetic susceptibility measurements [92]. Furthermore, the study indicated two possible cases of an antiferromagnetic exchange interaction between cyt.a₃³⁺ and Cu₄²⁺ at the active site of bovine cytochrome c oxidase. The results obtained for the fully-oxidized (resting) enzyme corresponded perfectly to the requirements of the PBV model [38], in which the enzyme possesses two magnetically isolated spin S = 1/2 centers and a spin-coupled S = 2 center. The S = 2 center paramagnetism was interpreted to arise from a cyt.a₃³⁺(S = 5/2)—Cu₄²⁺(S = 1/2) antiferromagnetically coupled [Fe/Cu] binuclear site of total spin S = 2 with −J ≥ 200 cm⁻¹. In addition, the wide temperature range of the data permitted an analysis of other available lower temperature (T < 4K) data [94]. A second case of an antiferromagnetic interaction was observed for the CN⁻ derivative of the resting enzyme. In this case, the magnetic susceptibility data was interpreted in terms of an antiferromagnetic exchange interaction at the [cyt.a₃³⁺·CN (S = 1/2)—Cu₄²⁺ (S = 1/2)] binuclear active site with −J ~ 40 cm⁻¹. From recent ⁵⁷Fe-enriched Mössbauer spectroscopy studies of the resting enzyme from T. Thermophilus [85] it was concluded that (1)
two different cyt.\textsubscript{a}\textsuperscript{3+} sites are spectroscopically observed, indicating active site heterogeneity which may be dependent on the isolation procedure and (2) both of the spectroscopically observed cyt.\textsubscript{a}\textsuperscript{3+} sites have indications of spectral behavior incompatible with an $S = 2$ spin-couple ground state, contradicting Tweedle's [39], Gray's, and Scott's [85] susceptibility results on the mammalian resting oxidase. Rather, as these authors point out, "with the limited information available (the initial Mössbauer data), the results may be consistent with a model involving weak antiferromagnetic coupling with $-J \ll D$ ($D$ is the zero field splitting, typically $5-10$ cm$^{-1}$)." In addition, the recent EXAFS and kinetics studies by Powers [51] indicate that there exist significant molecular heterogeneity and inconsistencies among between preparations of cytochrome c oxidase by different methods and even among by the same method preparations. Furthermore, such heterogeneity has been observed in the reaction of oxidase with hydrogen peroxide [81-83], cyanide [51] and with other ligands [84]. These results raise several questions of central importance to the magnetic and modeling studies of the oxidase active site: (1) does the active site structure differ in a fundamental way between bacterial and mammalian oxidase? (2) is active site heterogeneity a universal problem in preparations of resting oxidase and its derivatized forms? and (3) is there a viable alternative model for the active site of resting oxidase whereby $-J$ is "small" between cyt.\textsubscript{a}\textsuperscript{3+} and Cu\textsuperscript{2+}?
It is unlikely that the active site structure of bacterial and mammalian oxidase differ in any significant way, especially since they are spectroscopically indistinguishable by UV-vis, epr, and MCD spectroscopy [80]. The second of the above concerns is more difficult to address, but it is of central importance. Without the availability of Mössbauer data for mammalian oxidase, the situation for the bovine preparations will be less definitive but no less important because the literature abounds with quotations of our "-J > 200 cm\(^{-1}\)" report as a benchmark number for judging the success or failure of various model compounds. If heterogeneity is also a problem with bovine preparations, the susceptibility measurement previously reported by Tweedle, resulting in the often quoted "-J > 200 cm\(^{-1}\)" number, may simply reflect a mole-fraction-weighted average of two or more magnetically active sites, since magnetic susceptibility is a bulk property measurement. Therefore, this situation definitely needs clarification due to its key role in model compound studies.

Since Tweedle's, et al., [39] initial report on the resting and CN\(^-\)-derivative of resting oxidase, two reports differing with some of Tweedle's interpretations have appeared in the literature [80,86,95]. In variable-temperature (1.5-200 K) MCD studies of the Yoneton resting, resting-CN\(^-\), and partially reduced-CN\(^-\) forms of Yoneton oxidase by Thomson, et al., [99] the magnetization characteristics of cyt.\(\mathbb{A}^3\)\(^{2+}\)-CN clearly showed the ground state to be an electronic doublet with another state, probably a spin singlet, lying > 10 cm\(^{-1}\) above the ground state.
These features are well accounted for by an electronic state of spin $S = 1$ with a predominantly axial distortion which leaves the doublet, $M_S = \pm 1$, as the ground state and the $M_S = 0$ component as the excited state. This ground state would not give an epr signal. Such an electronic state could arise either from ferromagnetic coupling between cyt.$\alpha_3^{3+}$-CN and the Cu$_{IV}^{2+}$, or from a home in the Fe(IV) state. The latter possibility, however, is diminished by the Mössbauer studies of bacterial cytochrome c oxidase [80,86] which indicate that the cyt.$\alpha_3^{3+}$ site of CN$^{-}$-derivatized oxidase is typical of low-spin ferric heme-CN complexes.

The low-temperature Mössbauer spectrum taken in an applied magnetic field shows that the electronic ground state of the cyt.$\alpha_3^{3+}$-CN complex has integer electronic spin, suggesting ferromagnetic coupling of the low-spin ferric heme cyt.$\alpha_3^{3+}$ ($S = 1/2$) to Cu$_{IV}^{2+}$($S = 1/2$) to yield a resultant $S = 1$ ground state. In addition, the Mössbauer spectra [86] of oxidized bovine cyt.$\alpha_3^{3+}$-CN shows that the heme iron is also low-spin ferric and that the ground state has integer spin $S \geq 1$, which plausibly results from ferromagnetic coupling of an $S = 1/2$ heme to an $S = 1/2$ Cu(II) ion. The similarity in the spectra obtained for the bovine [86] and bacterial [80] preparations leaves little doubt that the electronic structures of the cyanide complexes in both enzymes is substantially the same. Thus, the Mössbauer studies support, in considerable detail, the basic conclusions derived from the low-temperature MCD studies [95]. These conclusions are inconsistent with earlier deductions drawn from magnetic susceptibility data [39]. Furthermore, the Mössbauer and MCD results require that the cyanide derivative exhibit a magnetic moment.
twice that actually observed [39]. This discrepancy cannot be explained on the basis of paramagnetic impurities in the sample. Such impurities would have led to an erroneously high value for the magnetic moment. A more plausible explanation appears to be that, at the high concentrations used in the susceptibility measurements (1-2 mmol in heme a), the enzyme undergoes substantial autoreduction which was reversed during the dilution used for optical quantitation [39]. In the present work, this potential problem has been avoided by obtaining the electronic spectra on exactly the same (undiluted) samples as used in the magnetic susceptibility experiments. Finally, the recent \(\mu\)-cyanogen model compound work of Murray and co-workers [96]) also suggests that a cyanide bridge between a low-spin iron(III) porphyrin and a copper(II) center can foster a weak ferromagnetic \(+J = 0.25 \text{ cm}^{-1}\) interaction.

In order to help further clarify the source(s) of the above discrepancies, the variable-temperature (ca. 20-200 K) magnetic susceptibility of bovine resting cytochrome c oxidase and its CN\(^-\) derivative (modified Hartzell-Beinert) have been carefully reexamined. In addition, the resting enzyme and its cyanide derivative have been obtained by the Yonetani procedure and examined by variable-temperature magnetochemistry for the first time. These studies have revealed a possible source where the inconsistencies between Thomson's MCD measurements (Yonetani resting oxidase·CN\(^-\)) and our earlier magnetochemical measurements (modified Hartzell-Beinert resting oxidase·CN\(^-\)) may have arisen.
**Experimental Section**

**Enzyme Preparation and Characterization:** In this work, solubilized bovine cytochrome c oxidase was prepared by two different methods: modified Hartzell-Beinert [97] kindly supplied by Dr. G. Palmer and Yonetani [98] kindly supplied by Dr. G. Babcock. Spectroscopic ratios for the final solubilized product OD\(_{\text{reduced}}\) at 605 nm/OD\(_{\text{oxidized}}\) at 600 nm and OD\(_{\text{reduced}}\) at 443 nm/OD\(_{\text{oxidized}}\) at 424 nm were in agreement with (or better than) those reported by Lamberg [99] of 2.0-2.3 and 1.25-1.40, respectively. Enzyme concentration on a per heme basis was determined spectroscopically using $\varepsilon_{\text{reduced}}$ at 605 nm = 21.2 mM\(^{-1}\) cm\(^{-1}\) and $\varepsilon_{\text{oxidized}}$ at 600 nm = 9.6 mM\(^{-1}\) cm\(^{-1}\). Optical spectra were recorded on a Cary 17 or an IBM model 9430 UV-VIS spectrophotometer.

**Resting Cytochrome c Oxidase:** In the present work, three samples of fully-oxidized cytochrome c oxidase were studied by variable-temperature (ca. 20-300 K) magnetochemistry. The first two samples were isolated by a modified Hartzell-Beinert procedure. 

H.B. w/g\(_{12}\) and H.B. g\(_{12}\)-less represent Hartzell-Beinert preparation containing a g\(_{12}\) and containing no g\(_{12}\) signal in the epr spectrum, respectively. The electronic spectra of these samples are shown in Figure 5a and 5b for H.B. w/g\(_{12}\) and H.B. g\(_{12}\)-less respectively. The third sample was prepared according to the procedure described by Yonetani; its optical spectrum is shown in Figure 5c. The spectra of Figure 5 are the same (undiluted) samples used in the magnetic susceptibility experiments. All three samples have been characterized by epr and optical spectroscopy as well as by their activities. All of
Figure 5

Electronic Spectrum of:

a) Resting Cytochrome c Oxidase (H.B. w/g\textsubscript{12})
b) Resting Cytochrome c Oxidase (H.B. g\textsubscript{12}-less)
c) Resting Cytochrome c Oxidase (Yonetani)
d) Resting Cytochrome c Oxidase-CN (from H.B. g\textsubscript{12}-less), Sample 1
e) Resting Cytochrome c Oxidase-CN (from H.B. g\textsubscript{12}-less), Sample 2
f) Resting Cytochrome c Oxidase-CN (from Yonetani)
b. Resting Oxidase (H.B. 912-less)

- Oxidized Oxidase
- Reduced Oxidase

Absorbance vs. Wavelength (nm)

Absorbance

Wavelength (nm)

400
500
600
700
800

0.0
1.0
2.0

x5
d. Oxidized Cyt. Oxidase-CN (H.E. g_{12}-less)

Sample # 1
Oxidized Cyt. Oxidase-CN (H.B. g12-less)
Sample #2
f.

Resting Oxidase
(Yonemoto)
the enzyme preparations were "well-behaved," showing spectral properties and activities as reported earlier in the literature [97-99].

**Fully-Reduced Cytochrome c Oxidase:** The fully-reduced enzyme was made directly from the H.B.w/g_{12} sample without removing it from the Faraday quartz vessel. Reduction was accomplished by adding 10 μl of freshly prepared 0.2 M dithionite (95%, Virginia Smelting) in a buffer solution to the sample and allowing it to stand (after purging with dry He gas for ~5 min) in ice for 1.5 hours, by which time the formerly red-brown solution had turned distinctly green (especially to reflected light).

**Resting Cytochrome c Oxidase-CN:** Three samples of resting oxidase-CN were studied in this work. The first two samples of the enzyme were made from H.B.g_{12}-less and the third sample was made from Yonetani resting oxidase by incubation with KCN. The electronic spectra of the samples are shown in Figures 5d, 5e and 5f. The concentration of the samples was determined by optical spectroscopy of the final concentrated samples without dilution. Optical spectra were taken before and after the magnetochemical measurements to be sure that the samples had not undergone autoreduction during manipulation. There was no evidence of autoreduction of any sample.

**Procedure For Handling The Enzyme:** Samples for the magnetic susceptibility measurements were taken as frozen pellets (77 K) and made
ca. 1.0-1.5 mM in heme a by suspension in an appropriated buffer solution. These enzyme solutions were always kept below 16 °C and in ice whenever possible. Contact with metal utensils or containers of any kind was avoided, and the glass, quartz, and plastic utensils used for handling the enzyme solutions were cooled before use. Great care was taken to avoid contaminants or conditions which might be harmful to the enzyme samples.

In the experiment, 200 μl of concentrated sample solutions were micro-pipetted into the quartz magnetic susceptibility sample vessel. The quartz vessel and the pipet were weighed before and after transfer of the sample to determine the exact amount of enzyme in the quartz sample vessel. After transferring the sample into the quartz cell, 10 μl of 1500 units/ml of glucose oxidase, 5 μl of 1.0 M of β-o-glucose, and 1 μl of 16 units catalase were added to the enzyme. The glucose oxidase, glucose, and catalase were used as effective scavengers of paramagnetic dioxygen. Subsequently, the enzyme solution was purged with He gas for ca. 3 min and the quartz vessel capped. The enzyme sample was then allowed to stand in ice for ca. 5 min before being frozen in liquid N₂ prior to the magnetic susceptibility measurements. The sample vessel was then transferred to the sample chamber of the Faraday susceptometer while the temperature of the chamber was below 250 K and decreasing. The sample chamber was then evacuated and refilled with dry He gas several times. Finally, the sample chamber was filled with ca. 1 mm Hg of He gas at the lowest temperature (ca. 20 K). The magnetic
susceptibility measurements were then obtained in the temperature range of 20–200 K.

**Instrumentation:** The magnetic susceptibility measurements were measured on a Faraday magnetic susceptometer. A detailed description of the instrument has been previously published [100]. The basic instrument consists of a Cahn MS 6000-1 Faraday balance modified to improve the sensitivity to that required for accurate measurements on magnetically dilute protein samples. The value of the applied field was 9.1 kilogauss and \( \frac{dH}{dx} \) was \( 1.1 \times 10^7 \) gauss/cm. The sensitivity of the Cahn RG null-type electrobalance can be used to 1µg sensitivity on weight changes of 10 mg, even with ca. 1.2 g load. The cryogenics consists of an Air Products Interface Model DMX-19 vacuum shroud and an LT-3-110 B Heli-tran system. The APD-TL digital temperature read out monitoring an iron-doped gold versus chromel thermocouple was replaced with a more accurate Scientific Instruments Model 3800 temperature indicator/controller equipped with an LFE model 4427 voltmeter monitoring a Scientific Instruments Model Si-400 silicon diode sensor. The sample container was made from a cut-off quartz epr tube, with the end blown into a small bulb and capped with an mmr pressure cap. The balance was calibrated using the difference in gram susceptibility between a 0.1 M solution of analyzed NiCl\(_2\)·6H\(_2\)O and triply distilled water [101].
**Computation:** The calculation of the electrobalance "\( \beta \)" constant is given in Appendix I. The computer program used for the non-linear least squares fitting of the magnetic susceptibility data is shown in Appendix II. A PDP 11/70 computer was used for the calculations.
RESULT AND DISCUSSION

Magnetic Susceptibility Measurements. The variable-temperature (ca. 20-200 K) magnetic susceptibility studies of resting cytochrome c oxidase and its cyano derivative are the main result of Part I of this thesis. The observed susceptibility, \( X_{\text{obs}} \), of the sample at a given temperature is simply the product of the weight difference (\( \Delta \)) of the sample in and out of the magnetic field and the electrobalance calibration constant, \( \beta \). The electrobalance calibration constant, \( \beta \), was obtained by calibration of the balance using the difference in susceptibility between a 0.1 M solution of analyzed NiCl\(_2\cdot6\)H\(_2\)O and triply distilled water. After the observed susceptibility of the sample was obtained, the magnetic susceptibility was analyzed by the procedure of Kotani [102] to obtain \( X_{\text{para}} \) and \( X_{\text{molar}} \). Basically, \( X_{\text{obs}} \) is the sum of \( X_{\text{dia}} \) and \( X_{\text{para}} \). \( X_{\text{dia}} \) is the correction for the temperature independent diamagnetism associated with the buffer solution and apoprotein. \( X_{\text{para}} \) is the paramagnetic susceptibility of the paramagnetic centers, copper and heme iron in the case of cytochrome c oxidase. \( X_{\text{para}} \) is the temperature dependent susceptibility which vanishes when \( T^{-1} \) becomes zero. Hence, \( X_{\text{dia}} \) can be determined by extrapolating the \( X_{\text{obs}} \) vs. \( T^{-1} \) curve to the point where \( T = \infty \) (where the paramagnetism disappears), leaving \( X_{\text{obs}} \) as the intercept. \( X_{\text{para}} \) at various temperatures is the difference between \( X_{\text{obs}} \) and \( X_{\text{dia}} \) at each temperature. Besides the diamagnetic correction, the temperature dependent susceptibility due to the presence of some of paramagnetic impurities must be considered and subtracted from the total magnetic susceptibility. The paramagnetic impurities come mainly from the quartz
vessel and plastic cap, as well as from the buffer solution and other foreign salts (e.g., KCN). The quartz vessel possesses some small temperature dependent component [103], which is amplified in these experiments where the vessel itself has many times the mass of the enzyme sample. The contribution of these paramagnetic impurities have been determined by measuring the $X_{\text{obs}}$ of the buffer solutions. The final results were then calculated from the difference in the value of $X_{\text{para}} = X_{\text{obs}} - X(T = \infty)$ for the enzyme solution and $X_{\text{para}}$ for an identically treated buffer solution without enzyme. Finally, the paramagnetic susceptibility per mole of enzyme, $\chi_{\text{molar}}'$ or $\chi_M'$ is then simply obtained by dividing $X_{\text{para}}$ by the number of moles of paramagnetic centers in the sample. The $X_M'$ is then plotted vs. $T^{-1}$ to obtain a slope for use in the calculations below.

The results of the experiments in this study will be discussed in terms of $\mu_{\text{eff}}$ or $n_{\text{eff}}$ (the effective Bohr magneton number), which is defined for Curie behavior as

$$\left( \mu_{\text{eff}} \right)^2 = \frac{3k}{N\beta^2} \cdot \text{slope of } X_M' \text{ vs. } T^{-1} \quad ------(1)$$

or for non-Curie behavior as

$$\left( \mu_{\text{eff}} \right)^2 = \frac{3k}{N\beta^2} \cdot (X_M' \cdot T) \quad ------(2)$$

where $X_M'$ is the molar paramagnetic susceptibility, $\mu_{\text{eff}}$ the effective Bohr magneton number, $k$ the Boltzmann constant, $\beta$ the Bohr magneton, and $N$ Avogadro's number.
In order to test the overall precision and accuracy of the electrobalance measurements, variable-temperature magnetic susceptibilities of two samples of metmyoglobin fluoride [\textit{Met-Mb}(F^-)] and a sample solution of CuSO\textsubscript{4}·5H\textsubscript{2}O solutions have been determined. The [\textit{Met-Mb}(F^-)] solutions were prepared as previously described, and the concentration determined spectrophotometrically by the known extinction coefficient in the visible region [104]. The samples were carefully transferred into the sample container using a micro-pipet, and the sample vessel capped under an Ar atmosphere. In order to eliminate the paramagnetic dioxygen, 5 μl of 0.1 M of glucose, 10 μl of 1500 units/ml glucose oxidase, and 1 μl of 0.1 M catalase were added into the protein solution samples. The temperature dependent susceptibility associated with any adventitious paramagnetism was compensated for by subtracting the susceptibility of the buffer solution containing 0.2 M KF from that of the protein sample. The final data were manipulated as previously described [38,100].

The two data sets for 200 μl of 0.959 mM and 1.108 mM of [\textit{Met-Mb}(F^-)] are tabulated in Tables 1a and 1b and the plots of \(X'_M\) vs. \(T^{-1}\) are shown in Figures 6a and 6b, respectively. The slopes of these \(X'_M\) vs. \(T^{-1}\) plots were found to be identical when least-squares fitted and in good agreement with the results previously reported by Tweedle [104] and Kotonii [102]. The \(\mu_{\text{eff}}\) value for [\textit{Met-Mb}(F^-)] is in the range of 5.9 ± 0.1 \(\mu_B\), corresponding to a high-spin \(S = 5/2\)
### Magnetic Susceptibility Data

For \([\text{Met-Mb}(F^-)]\) Sample 1

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<tr>
<th>T(K)</th>
<th>1/T(x10^{-3})</th>
<th>$X'_M(x10^{-2})^a$</th>
<th>$\mu_{\text{eff}}(\mu_B)$</th>
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<td>5.83</td>
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*a. in cgs-emu (mole enzyme)^{-1}
### TABLE Ib

**Magnetic Susceptibility Data**

*For [Met-Mb(F^-)] Sample 2*

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<tr>
<th>T(K)</th>
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<th>X_M'(x10^{-2})^a</th>
<th>(\mu_{\text{eff}}(\mu_\text{B}))</th>
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<td>6.07</td>
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</tr>
<tr>
<td>206.5</td>
<td>4.84</td>
<td>2.09</td>
<td>5.87</td>
</tr>
</tbody>
</table>

^a. in cgs-emu (mole enzyme)^{-1}
Figure 6

$X'_M$ vs. $T^{-1}$ Plot for:

a) [Met-Mb($F^-$)], Sample 1
b) [Met-Mb($F^-$)], Sample 2
a. [Met-Mb (F^-)]

Sample 1

Slope = 4.4
Intercept = -7.58E-5
n^2 = 35.3

b. [Met-Mb (F^-)]

Sample 2

Slope = 4.4
Intercept = 6.73E-5
n^2 = 35.2
ferrihemoprotein center. The $\mu_{\text{eff}}$ vs. $T$ plots for these two samples are shown in Figures 7a and 7b.

Furthermore, to compare the $\mu_{\text{eff}}$ value of an $S = 1/2$ system of CuSO$_4 \cdot 5H_2O$ to that of the $S = 5/2$ system of [Met-Mb(F$^-$)], the temperature dependent susceptibility of a 20.14 mM aqueous solution of CuSO$_4 \cdot 5H_2O$ was also determined. The copper sulfate solution was micro-pipetted into the sample container and capped under an Ar atmosphere. A blank was run using glass, multidistilled water and used to corrected for any temperature dependent susceptibility associated with factors other than the copper. The data were treated identically to that of the protein.

The data for 216 $\mu$l of a 20.14 mM aqueous solution of CuSO$_4 \cdot 5H_2O$ are tabulated in Table II and plotted as $X'_M$ vs. $T^{-1}$ plot in Figure 8a. The slope of the $X'_M$ vs. $T^{-1}$ plots indicates the $\mu_{\text{eff}}$ value for the copper center is $2.0 \pm 0.1 \mu_B$. The $\mu_{\text{eff}}$ vs. $T$ plot is shown in Figure 8b. This value is in good agreement with the value reported previously for a sample of CuSO$_4 \cdot 5H_2O$ [105].

**Fully-Oxidized (Resting) Enzyme.** In this study, the magnetic susceptibility of three samples of resting oxidase are determined. The first two samples are prepared by the modified Hartzell-Bienert method [97]. A characteristic of the first sample is a $g_{12}$ signal in the epr spectrum and its multiphasic reaction with KCN. It is referred to as
Figure 7

$\mu_{\text{eff}}$ vs. $T$ Plot for:

a) [Met-Mb(F$^-\text{)}$], Sample 1
b) [Met-Mb(F$^-\text{)}$], Sample 2
a. [Met-Mb (F^-)]

Sample 1

\[ \mu_{\text{eff}}, \mu_B \]

\[ T, \text{ K} \]

b. [Met-Mb (F^-)]

Sample 2

\[ \mu_{\text{eff}}, \mu_B \]

\[ T, \text{ K} \]
Figure 8

a) $\bar{x}_M \times T^{-1}$ Plot for the CuSO$_4$·5H$_2$O Solution (20.14 mM Cu)
b) $\mu_{\text{eff}} \times T$ Plot for the CuSO$_4$·5H$_2$O Solution (20.14 mM Cu)
a. [CuSO$_4$·5H$_2$O Solution]

\[ X', \text{ cgs-emu mol}^{-1}/10^{-1} \]

\[ 0.04 \quad 0.06 \quad 0.08 \quad 0.10 \quad 0.12 \]

\[ 0.02 \quad 0.04 \quad 0.06 \]

Slope = 0.45
Intercept = -1.43E-6
\( n^2 = 3.6 \)

b. [CuSO$_4$·5H$_2$O Solution]

\[ \mu_{\text{eff}}/\mu_B \]

\[ 0 \quad 2.0 \quad 4.0 \]

\[ 40.00 \quad 80.00 \quad 120.00 \quad 160.00 \quad 200.00 \quad 240.00 \]

\( T, \text{ K} \)
<table>
<thead>
<tr>
<th>T(K)</th>
<th>1/T(x10^{-3})</th>
<th>$X'_M$(x10^{-3})*a</th>
<th>$\mu_{\text{eff}}$(\mu_B)</th>
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</thead>
<tbody>
<tr>
<td>85.0</td>
<td>11.77</td>
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<td>5.94</td>
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<td>1.89</td>
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</tbody>
</table>

*a. in cgs-emu mole^{-1}
H.B.w/g₁₂ throughout this thesis. The second sample lacks a g₁₂ epr signal, and it is referred to as the H.B.g₁₂-less enzyme throughout this thesis; its reaction with KCN is monophasic and about 100 fold faster than the H.B.g₁₂-less sample. The third sample has been prepared by the modified Yonetani procedure [98]. The variable-temperature magnetic susceptibilities of the three samples are tabulated in Tables III, IV and V and the $X'_M$ vs. 1/T plots for the H.B.w/g₁₂, H.B.g₁₂-less and Yonetani resting enzymes are shown in Figures 9a, 9b and 9c, respectively. The $X'_M$ vs. 1/T plots of all three samples appear linear over the entire 20-200 K range, and therefore conform to the Curie Law, $X'_M = C/T$ where

$$C = \frac{(\bar{g})^2 N\theta^2 S(S+1)}{3k} \quad (3)$$

The quantity $(\bar{g})^2[S(S+1)]$ is $(\mu_{\text{eff}})^2$, the effective magnetic moment squared and has the implicit dimensions of $\beta^2$. Each metal center in the enzyme is presumed to affect this quantity with the contribution of low-spin cyt.A³⁺ and Cu_D²⁺ being established directly from the above definition with $(\bar{g})^2$, the mean square g-value, equal to 1/3 ($g_x^2 + g_y^2 + g_z^2$). For these two centers with $S = 1/2$ the respective g-values are: cyt.A³⁺, $g_z = 3.03$, $g_y = 2.21$, $g_x = 1.45$ and Cu_D²⁺, $g_z = 2.18$, $g_y = 2.02$, $g_x = 1.99$ [106]. Thus, $\mu_{\text{eff}}^2$ values for cyt.A³⁺ and Cu_D²⁺ are 4.0 and 3.2, respectively.

In the present work, all three samples of the resting enzyme exhibit nearly identical values of $(\mu_{\text{eff}})^2$, with the actual values being
**TABLE III**

**Magnetic Susceptibility Data**

For Resting Cytochrome Oxidase (H.B.w/g.)

<table>
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<th>x'_M(x10^{-2})^a</th>
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^a. in cgs-emu (mole enzyme)^{−1}
TABLE IV
Magnetic Susceptibility Data
For Resting Cytochrome Oxidase (H.B. g_yy-less)

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<td>206.0</td>
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^a. in ogs-emu (mole enzyme)^{-1}
TABLE V
Magnetic Susceptibility Data
For Resting Cytochrome Oxidase (Yonetani)

<table>
<thead>
<tr>
<th>T(K)</th>
<th>1/T(x10^{-3})</th>
<th>(X'_M(x10^{-2})) a</th>
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<td>22.05</td>
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<td>26.61</td>
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<td>51.80</td>
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<td>191.3</td>
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<td>1.98</td>
</tr>
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</table>

a. in cgs-emu (mole enzyme)^{-1}
Figure 9

$X'_M \text{ vs. } T^{-1}$ Plot for:

a) Resting Cytochrome c Oxidase (H.B. w/g$_{12}$)

b) Resting Cytochrome c Oxidase (H.B. g$_{12}$-less)

c) Resting Cytochrome c Oxidase (Yonetani)
a. Resting Oxidase

\( \chi \), M, \( \text{mg-emu mol}^{-1} \text{enzyme}^{-1} \)

- Slope: 3.9
- Intercept: -1.47E-3
- \( n_2 = 31.4 \)

1/T (K^-1) / 10^-1
b. Resting Oxidase

(H.B. 912-960)

Slope = 4.1
Intercept = -2.9196E-4

\[ n^2 = 32.8 \]

\[ \frac{1}{T} \text{ (K)} \frac{1}{10^{-1}} \]

\[ 0.50 \]

\[ 0.40 \]

\[ 0.30 \]

\[ 0.20 \]

\[ 0.10 \]

\[ 0.0 \]

\[ 0 \]

\[ 0.0 \]

\[ 1.0 \]

\[ 2.0 \]

\[ X_m, \text{ g} \text{ - eqm mol}^{-1} \text{ enzyme}^{-1} \]
Resting Oxidase
(Yonemori)

\[ \frac{X_M, \text{ cgs-emu mol-1 enzyme}^{-1}}{0.20} \]

\[ \frac{1}{T} \text{ (K}^{-1}) / 10^{-1} \]

Slope = 4.1
Intercept = -1.73E-3
\[ n^2 = 32.8 \]
31.5, 32.8 and 32.8 ± 1.5, for H.B.w/g₁₂, H.B.g₁₂-less and Yonetani oxidase, respectively. These values are also identical to the one previously reported (31.5 ± 1.0) by Tweedle, et al., [39] for a sample of H.B.w/g₁₂. In addition, these values of $\mu_{\text{eff}}^2$ are also the same as that obtained by H.B. Gray and R. Scott for a Hartzell-Beinert preparation [107]. Thus, the contributions of cyt.$\alpha_3^{3+}$ plus Cu$^{2+}_U$ to the total squared magnetic moment is ca. $(31.5 - 7.2) = 24.3$. The interpretation of this value requires consideration of the possible alternative magnetic states for isolated and magnetically-coupled cyt.$\alpha_3^{3+}$ and Cu$^{2+}_U$ as presented in Table VI. Inspection of the table immediately establishes that the only three alternatives which fit the experimental results are: (1) a high-spin Fe(IV) (cyt.$\alpha_3^{4+}$) center uncoupled to Cu(I) (Cu$^+_U$), (2) a strongly coupled ferromagnetic pair of Cu$^{2+}_U$ and intermediate-spin (S = 3/2) cyt.$\alpha_3^{3+}$, or (3) a strongly-coupled antiferromagnetic pair comprised of Cu$^{2+}_U$ and high-spin (S = 5/2) cyt.$\alpha_3^{3+}$. The Fe(IV) proposal, first advanced by Seiter and Angelos [49] has been essentially invalidated by the recent Mössbauer spectroscopy study by Münck and Fee of bovine oxidase and $^{57}$Fe-enriched oxidase from Thermus Thermophilis [80] which showed $\delta$ and $\Delta E_Q$ parameters typical of only Fe(III). In addition, an unpublished time-resolved RR observation of $O_2$-binding in reduced bovine oxidase has shown porphyrin indicator bands typical of only Fe(II) and Fe(III) [108]. The second alternative mentioned above has gained some credibility in the last few years due to possible detection by both Mössbauer and MCD spectroscopy [80,109] of an ferromagnetic interaction (albeit weak at < 5 cm$^{-1}$)
<table>
<thead>
<tr>
<th>Cytochrome a₃₊</th>
<th>Cu²⁺</th>
<th>Coupling Scheme</th>
<th>Total Spin</th>
<th>$\mu_{\text{eff}}^2$ (Theory)ᵃ,ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>S=1/2 (low-spin)</td>
<td>S=1/2</td>
<td>Antiferromagnetic</td>
<td>S=0</td>
<td>0(a), 6(w)</td>
</tr>
<tr>
<td>S=1/2 (low-spin)</td>
<td>S=1/2</td>
<td>None (isolated)</td>
<td>(S=1/2; S=1/2)</td>
<td>6</td>
</tr>
<tr>
<td>S=1/2 (low-spin)</td>
<td>S=1/2</td>
<td>Ferromagnetic</td>
<td>S=1</td>
<td>8(a), 6(w)</td>
</tr>
<tr>
<td>S=3/2 (intermediate-spin)</td>
<td>S=1/2</td>
<td>Antiferromagnetic</td>
<td>S=1</td>
<td>8(a), 18(w)</td>
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<tr>
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<td>S=1/2</td>
<td>None (isolated)</td>
<td>(S=3/2; S=1/2)</td>
<td>18</td>
</tr>
<tr>
<td>S=3/2 (intermediate-spin)</td>
<td>S=1/2</td>
<td>Ferromagnetic</td>
<td>S=2</td>
<td>24(a), 18(w)</td>
</tr>
<tr>
<td>S=5/2 (high-spin)</td>
<td>S=1/2</td>
<td>Antiferromagnetic</td>
<td>S=2</td>
<td>24(a), 38(w)</td>
</tr>
<tr>
<td>S=5/2 (high-spin)</td>
<td>S=1/2</td>
<td>None (isolated)</td>
<td>(S=5/2; S=1/2)</td>
<td>38</td>
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<tr>
<td>S=5/2 (high-spin)</td>
<td>S=1/2</td>
<td>Ferromagnetic</td>
<td>S=3</td>
<td>48(a), 38(w)</td>
</tr>
<tr>
<td>S=2(Fe²⁺₃₉₉)</td>
<td>S=0 (Cu⁺)</td>
<td>None</td>
<td>S=2</td>
<td>24</td>
</tr>
</tbody>
</table>

ᵃ) Theoretical values of $\mu_{\text{eff}}^2$ are calculated as spin-only with $\mu_{\text{eff}}^2 = g^2S(S+1)$ and $g = 2.0$.

ᵇ) Strong (s) and weak (w) refer to the cases where $|J| >> kT$ (strong) and $g\beta H < |J| < kT$ (weak).
between cyt.₃³⁺ (S = 1/2) and Cu₄²⁺ (S = 1/2) in both mammalian and bacterial resting-CN oxidase. Still, on the negative side of this interpretation is the fact that evidence for intermediate-spin hemes in proteins is of recent origin [110] and is confined to cytochrome c of *Chromatium* and *Rhodospirillum*; however, the interpretation of these data is controversial and has been challenged by Rawling, et al., [111] on the basis of near-infrared MCD spectra which suggest that these proteins are very heterogeneous with contributions from high-spin and low-spin species. Thus, the most reasonable interpretation of the susceptibility data lies with the third and final alternative where cyt.₃³⁺ (S = 5/2) and Cu₄²⁺ (S = 1/2) are strongly coupled antiferromagnetically (−J ≥ 200 cm⁻¹) to yield an S = 2 resultant ground state. In fact, resonance Raman results [115] support the presence of an S = 5/2 spin state for cyt.₃³⁺ in Bovine resting oxidase, although the results do not address the possible presence of a magnetic interaction involving cyt.₃³⁺. A strong antiferromagnetic exchange interaction in resting oxidase is also consistent with the epr spectrum where there appears only one g = 2 signal which is assigned to magnetically isolated Cu₄²⁺ and quantitates to only approximately 40% of the total copper present [112]. In addition, an antiferromagnetic scheme seems quite plausible since an S = 5/2 epr signal, attributable to cyt.₃³⁺, is apparently observed with the cytochrome c oxidase CO complex photolyzed at low-temperature [113] and with partially reoxidized enzyme [114]. Moreover, moderate to strong antiferromagnetic interactions are much in evidence in other biochemical systems as such ferredoxins [115], hemerythrins [116], type III copper pairs in laccase
[117] and the copper-and cobalt-substituted derivatives of superoxide dismutase [118,119].

For the above reasons, it is reasonable to conclude, as did Tweedle, et al., [39] that the residual magnetic moment of fully-oxidized cytochrome c oxidase is best interpreted as arising from an antiferromagnetically coupled binuclear complex of \([\text{cyt.}A^3_3 - \text{Cu}_U^2^+]\) and have, therefore, computed the paramagnetic susceptibility of the enzyme as the sum of three terms:

\[
X_{\text{tot}} = X_a + X_D + X_{(\text{pair})}
\]  

(4)

due to \(\text{cyt.}A^3_3\), \(\text{Cu}_D^2^+\), and the \([\text{cyt.}A^3_3 - \text{Cu}_U^2^+]\) pair, respectively. The first two terms are due to \(S = 1/2\) species and give a simple, linear (Curie) contributions to the total susceptibility. The \(X_{(\text{pair})}\) term potentially introduces a variety of complications. Insofar as the total spin of \(S = 2\) arises from an exchange coupled state, the Hamiltonian is

\[
H = -2JS_1 \cdot S_2
\]  

(5)

where \(S_1\) and \(S_2\) are quantized spin moments of \(\text{cyt.}A^3_3\) and \(\text{Cu}_U^2^+\); these have been taken to be \(S_1 = 5/2\) and \(S_2 = 1/2\), respectively. For negative values of \(J\), the ground state is spin \(S = 2\) with an \(S = 3\) excited state at \(3J\) above the ground state. A plot of \(X_M^T\) vs. \(T^{-1}\) for this system should be linear when \(T\) is either much larger or much smaller than \(3J\), with the slope \(\mu_{\text{eff}}^2\) of either 24 (\(T \ll |3J|\text{ case}\)) or 38 (\(T \gg |3J|\text{ case}\)). Only the former case is consistent with the observed results, and from
the linearity of the data at temperatures even as high as 200 K, it can be concluded that \(-J > 200 \text{ cm}^{-1}\) for the \([\text{cyt.} h_3^+ \text{Cu}^{2+}_U]\) pair.

**The Reduced Enzyme.** Reduced cytochrome c oxidase is obtained readily by the reduction of resting oxidase with dithionite. The magnetic susceptibility of the reduced enzyme is tabulated in Table VII and the \(X'_M \text{ vs. } T^{-1}\) plot is shown in the Figure 10. Without the presence of any ligands, the reduced enzyme is highly paramagnetic. The \(X'_M \text{ vs. } T^{-1}\) plot over the range of 20–200 K is linear, with the slope of the data yielding a value of \(n^2 = 26\). This value is identical to the value which was reported previously by Tweedle, et al., [39]. Thus, the observed value of \(n^2\) or \(\mu_{\text{eff}}^2\) in this study confirms the previous interpretation. The four metal centers in the reduced enzyme are assumed to be in their reduced valence states. Thus, the observed paramagnetic susceptibility must arise exclusively from reduced cytochrome \(h_3^{2+}\) which, if in the high-spin Fe(II) configuration, is expected to have a value of 24–29 for \(n^2\), in excellent agreement with observation. The two Cu(I) ions will be diamagnetic as will the reduced cytochrome \(h_3\), which is a low-spin ferrohemoprotein \((S = 0)\), with no contribution to the paramagnetic susceptibility. The present magnetic susceptibility results are also in agreement with: (1) the room temperature magnetic susceptibility measurement of Vangard, where \(n^2 = 21\) [2,92], (2) the unpublished magnetic susceptibility titration data of Ehrenburg and Van Neste (where \(n^2 = 24–29\) [3]), (3) reported MCD measurements that concluded that reduced cytochrome c oxidase is
Figure 10

$X'_M$ vs. $T^{-1}$ Plot for Reduced Cytochrome c Oxidase from the H.B. w/s$_{12}$ Resting Enzyme
<table>
<thead>
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<th>T(K)</th>
<th>1/T(x10^{-3})</th>
<th>\chi''_M(x10^{-2})^a</th>
</tr>
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<td>22.18</td>
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<td>26.44</td>
<td>37.82</td>
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<tr>
<td>205.0</td>
<td>4.89</td>
<td>1.62</td>
</tr>
</tbody>
</table>

a. in cgs-emu (mole enzyme)^{-1}
paramagnetic and similar to deoxyhemoglobin, which is well-known to be in the high-spin $S = 2$ Fe(II) state [120,121]. Therefore, there seems to be little reason to question the interpretation that cytochrome $a_3^{3+}$ is the only paramagnetic component in the fully-reduced oxidase. From the tendency for non-linearity at $T < 1.0$ K, together with the observation of Moss, et al., [119] that the susceptibility of reduced oxidase below 4.2 K is essentially independent of temperature, it can be estimated that the zero field splitting (D) of this $(S = 2)$ Fe(II) state is on the order of 5-10 cm$^{-1}$, as compared to a value of 5 cm$^{-1}$ obtained for deoxyhemoglobin [123]. A more precise estimate will require additional magnetochemical data between 5-10 K or, alternatively, the use of variable-temperature far-infrared spectroscopy [124].

**Resting-CN Enzyme.** The cyanide derivative of resting cytochrome $c$ oxidase can be easily obtained by incubating of the protein with KCN. According to MCD measurements [120,125], cyt.$a_3^{3+}$ is low-spin in this derivative, presumably with CN$^-$ ligated as in Figure 11D or 11H (Note that this interpretation assumes preservation of the Fe-N (imidazolate) (11D) and probably Fe-N (imidazole) (11H) bonds, an assumption which seems reasonable since the position of the imidazole moiety in protein should be relatively "fixed" due to attachment to the polypeptide back-bone; for the μ-mercapto possibility, an 11D structure, with mercapto replacing imidazolato, would be most likely). In terms of μ-imidazolato, μ-oxo, or μ-mercapto models, it would therefore be expected that the [cyt.$a_3^{3+}$-Cu$_{II}^{2+}$] binuclear center would have modified
Figure 11

Comparison of The Alternative Models for
The Active Site of Cytochrome c Oxidase
* For a third μ-mercapto alternative resting oxidase is postulated to be like structure (C) with a mercapto bridge replacing imidazolato, and likewise for [resting-CN⁻] in (D).
properties since the \textbf{antiferromagnetic} interaction is now between two S = 1/2 centers with a total spin of S = 0 in the ground state and S = 1 for the excited state at some higher energy. If J \gg kT, the total susceptibility will be simply \[X_a + X_d + X_{\text{pair}}\] with \(X_{\text{pair}}\) being zero and thus, the predicted value for \((\mu_{\text{eff}})^2 = 7.2 (\mu_B)^2\). Conversely, if J \ll kT, the total susceptibility will be \[X_a + X_d + X_{\text{pair}}\], with the contribution of \(X_{\text{pair}}\) being appropriate to a thermally randomized S = 1 manifold with the predicted value for \((\mu_{\text{eff}})^2 = 15.2 (\mu_B)^2\). Finally, if J \sim kT, the expectation for the \(X_M' = \delta T^{-1}\) plot is a curved line with limiting slopes of \((\mu_{\text{eff}})^2 = 7.2 (\mu_B)^2\) and 15.2 \((\mu_B)^2\) in the low and high temperature limits, respectively. In this experiment, \(X_{(\text{tot})}\) is defined as,

\[X_{(\text{tot})} = X_a + X_d + X_{\text{pair}}\]  \hspace{1cm} (5)

with \(X_{\text{pair}}\) now appropriate to a pair of \textbf{antiferromagnetically} coupled S=1/2 ions. The classic model for this two spin interaction yields the well-known Bleaney-Bowers Equation \(6,\)

\[X_M' = \frac{2(\delta)^2 N B^2}{3kT} \left[1 + \frac{1}{3}\exp\left(-\frac{2J}{kT}\right)\right]^{-1} + \frac{P + C}{T}\]  \hspace{1cm} (6)

for the molar susceptibility \(X_M'\) where 2J is the difference in energy between the singlet and triplet levels and \(P\) is the temperature independence paramagnetism. In the present analysis, \(P\) is simply a scaling factor which permits a direct comparison of calculated and observed \(X_M'\) values. The \((\delta)^2\) values for cyt.\(\mathbf{a}^{3+}\) and Cu\(\mathbf{b}^{2+}\) of 4.0 and 3.2, respectively, were obtained directly from their epr spectra for use
in the C/T term to give C = 0.88. The \((g^2)\) value of the magnetically coupled \([\text{cyt.} A_{3}^{3+} - \text{Cu}_{u}^{2+}]\) center was assumed to be given by \((g^2) = \frac{1}{2}(g^2)_{\text{cyt.} A_{3}^{3+}} + (g^2)_{\text{Cu}_{u}^{2+}}\) with the range being 4.7 - 5.1 in these experiments; a value of 4.75 gave the best fit to the experimental data. The Curie (C/T) term has been included in Equation (6) in order to account for the susceptibility contribution from the magnetically isolated paramagnetic centers, \([\text{cyt.}A_{3}^{3+} + \text{Cu}_{D}^{2+}]\).

In the magnetochemical studies by Tweedie, et al., [39] the calculated value of 7.2 for the contribution of \([\text{cyt.} A_{3}^{3+} + \text{Cu}_{D}^{2+}]\) to \(\mu_{\text{eff}}^2\) and an initial estimate for C of 0.90 was used in fitting Equation 6. To obtain an initial value for the dependence of \(X'_{M}(\text{cyt.} A_{3}^{3+} - \text{Cu}_{U}^{2+})\) pair on T, \(X'_{M}(\text{cyt.} A_{3}^{3+} + \text{Cu}_{D}^{2+})\) was substracted from \(X'_{M}(\text{obs})\). In this format, Tweedle, et al., demonstrated that the nature of the proposed antiferromagnetic interaction is more clearly visualized and a Neel temperature of \(~45\) K was obtained as an initial estimate for \(-J\). Thus with input parameters of \(C = 0.90\) and \(-J = 45\) cm\(^{-1}\), Equation 6 was fit by the usual Gauss-Newton nonlinear least squares procedure, minimizing the summation of \([X'_{M}(\text{obs}) - X'_{M}(\text{cal.})]^2\). The final parameters obtained by this procedure were \(-J = 38.5 \pm 1.3\) cm\(^{-1}\), \(C = 0.95 \pm 0.004\), and \(P = 1.0 \times 10^{-3}\).

In the present work, three samples of resting cytochrome c oxidase-CN have been studied. The first two samples were prepared from H.B. \(_{12}\)-less resting oxidase. Hence, the samples are referred to as
H.B.812-less samples 1 and 2, respectively. The third sample was prepared according to the procedure of Yonetani. The magnetic susceptibility data are tabulated in Table VIII. In general, the samples exhibit nearly identical magnetochemical data, showing a temperature dependent behavior which is typical of antiferromagnetism, with a Neel temperature of ca. 40 K. The Neel temperature is more easily visualized if the molar susceptibility of only the [cyt.A33+-CuU2+.CN−] pair is plotted against temperature as shown in Figure 13. The $X'_M (\text{cyt.A3}^{3+}-\text{CuU}^{2+}.\text{CN}^-)$ pair data in the Table IX was obtained by way of Equation (7),

$$X'_M (\text{cyt.A3}^{3+}-\text{CuU}^{2+}.\text{CN}^-) = X'_M (\text{enzyme}) - \left[ \frac{C}{T} + P \right] \quad (7)$$

The data in Figure 13 compare very favorably to the classic example of an antiferromagnetically coupled $S = 1/2$ two spin system in the anhydrous Cu(II) acetate dimer case [127]. From the position of the Neel points in Figure 13, it is clear that the results from these experiment are very similar to the previous report of Tweedle, [39]. In order to determine more precise values for the antiferromagnetic coupling constants, the Bleaney-Bowers equation (Equation 6), has been applied to the data. The sum of the $g^2$ values for cyt.A33+.CN− and CuU2+ were limited to the range of 4.7 to 5.1, and the value that gave the best fit in this work is 4.75. A constant value of $C = 0.88$ (vide supra) was used in the data fitting and P was allowed to vary, with the initial estimate being $2.3 \times 10^{-3}$. The observed magnetic susceptibility data are then fitted to Equation 6 by the usual Gauss-Newton non-linear
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a. in cgs-emu (mole enzyme)^{-1} observed
b. in cgs-emu (mole enzyme)^{-1} calculated
### TABLE VIIIb

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*a.* in cgs-emu (mole enzyme)^-1 observed  
*b.* in cgs-emu (mole enzyme)^-1 calculated
Figure 12

$X'_M$ vs. $T^{-1}$ Plot for:

a) Resting Cytochrome c Oxidase-CN (from H.B. $g_{12}$-less), Sample 1
b) Resting Cytochrome c Oxidase-CN (from H.B. $g_{12}$-less), Sample 2
c) Resting Cytochrome c Oxidase-CN (from Yonetani resting)
b. Resting Oxidase-CN (H.B. g₁₂-less)
(Sample #2)

\[ \chi_M, \text{cgs-emu mól-enzyme}^{-1} \]

\[ 1/T \ (K^{-1}) / \times 10^{-1} \]

\[ -J = 22.7 \pm 1.8 \]
\[ C = 0.88 \]
\[ P = 6.8E-3 \pm 1.0E-4 \]
(c) Resting Oxidase-CN (Yonetani)

\[ J = 45.5 + 1.8 \]

\[ C = 0.88 \]

\[ P = 4.2 \times 10^{-3} + 3.4 \times 10^{-5} \]
Figure 13

$\bar{X}'_M$ vs. T Plot for:

a) cyt. $\text{B}_3^{3+}$ - $\text{Cu}_U^{2+}$ - CN (from H.B. $g_{12}$ - less Resting Oxidase), Sample 1
b) cyt. $\text{B}_3^{3+}$ - $\text{Cu}_U^{2+}$ - CN (from H.B. $g_{12}$ less Resting Oxidase), Sample 2
c) cyt. $\text{B}_3^{3+}$ - $\text{Cu}_U^{2+}$ - CN (from Yonetani Resting Oxidase)
b. Resting Oxidase-CN (H.B. g_{12}-less)
(Sample # 2)

\[ -J = 22.7 \pm 1.8 \]

\[ C = 0.88 \]

\[ P = 6.8E-3 \pm 1.0E-4 \]
a. Resting Oxidase-CN (H.B. g_{12}-lase)

(Sample # 1)

\[-J = 20.3 \pm 2.5\]
\[C = 0.88\]
\[P = 3.6E-3 \pm 1.7E-4\]
TABLE IXa

Magnetic Susceptibility Data
For the \([\text{Cyt.}_{2}\text{Fe}^{3+}\text{-Cu}_{2}^{2+}\text{-CN}]\) Pair (from H.B. \(g_{12}\)-less Resting Oxidase-CN)

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<td>130.2</td>
<td>7.68</td>
<td>5.72</td>
<td>5.68</td>
</tr>
<tr>
<td>145.2</td>
<td>6.89</td>
<td>5.21</td>
<td>5.19</td>
</tr>
<tr>
<td>162.3</td>
<td>6.16</td>
<td>4.65</td>
<td>4.72</td>
</tr>
<tr>
<td>180.0</td>
<td>5.55</td>
<td>4.23</td>
<td>4.30</td>
</tr>
<tr>
<td>201.0</td>
<td>4.97</td>
<td>3.66</td>
<td>3.90</td>
</tr>
</tbody>
</table>

a. in cgs-emu (Cyt₃³⁺-Cu₂⁺CN pair)^⁻¹ observed
b. in cgs-emu (Cyt₃³⁺-Cu₂⁺CN pair)^⁻¹ calculated
TABLE IX

Magnetic Susceptibility Data
For the \([\text{Cyt}_{2}^{3+}\text{Cu}_{2}^{2+}\cdot\text{CN}]\) Pair (from Yonetani Resting Oxidase-CN)

<table>
<thead>
<tr>
<th>T(K)</th>
<th>(1/T(\times 10^{-3}))</th>
<th>(X_M'(\times 10^{-3})^a)</th>
<th>(X_M'(\times 10^{-3})^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.52</td>
<td>60.53</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>20.56</td>
<td>48.64</td>
<td>0.24</td>
<td>0.21</td>
</tr>
<tr>
<td>24.96</td>
<td>40.06</td>
<td>0.57</td>
<td>0.63</td>
</tr>
<tr>
<td>29.90</td>
<td>33.44</td>
<td>1.26</td>
<td>1.31</td>
</tr>
<tr>
<td>34.73</td>
<td>28.79</td>
<td>2.20</td>
<td>2.05</td>
</tr>
<tr>
<td>40.08</td>
<td>24.95</td>
<td>2.85</td>
<td>2.85</td>
</tr>
<tr>
<td>45.19</td>
<td>22.13</td>
<td>3.34</td>
<td>3.53</td>
</tr>
<tr>
<td>50.00</td>
<td>20.00</td>
<td>4.02</td>
<td>4.04</td>
</tr>
<tr>
<td>54.80</td>
<td>18.25</td>
<td>4.47</td>
<td>4.44</td>
</tr>
<tr>
<td>60.10</td>
<td>16.64</td>
<td>4.68</td>
<td>4.77</td>
</tr>
<tr>
<td>65.50</td>
<td>15.17</td>
<td>5.06</td>
<td>4.98</td>
</tr>
<tr>
<td>70.00</td>
<td>14.29</td>
<td>5.12</td>
<td>5.11</td>
</tr>
<tr>
<td>79.10</td>
<td>12.64</td>
<td>5.28</td>
<td>5.21</td>
</tr>
<tr>
<td>90.40</td>
<td>11.06</td>
<td>5.30</td>
<td>5.19</td>
</tr>
<tr>
<td>99.10</td>
<td>10.09</td>
<td>5.12</td>
<td>5.04</td>
</tr>
<tr>
<td>108.00</td>
<td>9.26</td>
<td>4.95</td>
<td>4.95</td>
</tr>
<tr>
<td>121.50</td>
<td>8.23</td>
<td>4.77</td>
<td>4.72</td>
</tr>
<tr>
<td>134.50</td>
<td>7.44</td>
<td>4.33</td>
<td>4.47</td>
</tr>
<tr>
<td>151.20</td>
<td>6.61</td>
<td>4.19</td>
<td>4.17</td>
</tr>
<tr>
<td>168.30</td>
<td>5.94</td>
<td>3.87</td>
<td>3.89</td>
</tr>
<tr>
<td>183.10</td>
<td>5.46</td>
<td>3.70</td>
<td>3.66</td>
</tr>
<tr>
<td>204.40</td>
<td>4.89</td>
<td>3.40</td>
<td>3.38</td>
</tr>
</tbody>
</table>

\(^a\) in cgs-emu (Cyt\(_2\)^{3+}\text{Cu}_{2}^{2+}\cdot\text{CN} \text{pair})^{-1} \text{ observed}
\(^b\) in cgs-emu (Cyt\(_2\)^{3+}\text{Cu}_{2}^{2+}\cdot\text{CN} \text{pair})^{-1} \text{ calculated}
least squares procedure to minimized the summation of \( [X_M^\prime (\text{obs.}) - X_M^\prime (\text{cal.})]^2 \). The final parameters by this procedure are \(-J = 20.3 \pm 2.5\), \(C = 0.88\), \(P = 3.6 \times 10^{-3} \pm 1.7 \times 10^{-4}\), and \(-J = 22.7 \pm 1.8\), \(C = 0.88\), \(P = 6.5 \times 10^{-3} \pm 1.0 \times 10^{-4}\) for H.B. g\(_{12}\)-less 1 and 2, respectively. The Yonetani resting oxidase\cdot-CN gave \(-J = 45.5 \pm 1.8\) cm\(^{-1}\), \(C = 0.88\), and \(P = 4.1 \times 10^{-3} \pm 3.4 \times 10^{-5}\). The \(-J\) value for Yonetani resting oxidase\cdot-CN is slightly different from the first two samples. However, this difference is not significant to change the general interpretation of the data.

The agreement factors defined by the summation of \( [X_M^\prime (\text{obs.}) - X_M^\prime (\text{cal.})]^2 \) are \(2.7 \times 10^{-7}\) and \(9.5 \times 10^{-8}\) for H.B. g\(_{12}\)-less sample 1 and 2, respectively.

In these experiments, optical spectra were obtained before and after manipulation of the enzyme sample (without any dilution) in order to insure that the samples did not undergo autoreduction; no autoreduction was observed. The results of these magnetochemistry experiments (and that of Tweedle, et al.,) are still in disagreement with the MCD results of Thomson [120,125], who concluded that the enzyme active site exhibited a weak ferromagnetic interaction between cyt.e\(_3\)\(^{3+}\)\cdot-CN \((S = 1/2)\) and Cu\(_{11}\)\(^{2+}\) \((S = 1/2)\) to give an \(S = 1\) ground state. It is still not clear why this discrepancy exists, but it may be useful to note here that the MCD experiment required that ethandiol be added to the enzyme sample (50% in diol) to obtain a high quality optical glass. In studies designed to explore any possible effects of additives, such as ethandiol, we have recently performed preliminary magnetochemical
studies of ethandiol treated H.B.$g_{12}$-less cyanide derivative with 50% ethandiol present [126]. In these experiments, initial indications are that the $X'_M/Y_M$ $T^{-1}$ plot appears Curie in nature from ca. 20 to 200 K with no indication of a Neel temperature being found. This observation indicates that the antiferromagnetic interaction may be lost in enzyme samples treated ethandiol in high concentration. Obviously, more extensive magnetochemical data of this nature will be needed before any definite conclusions can be drawn. Such data is in the process of being collected in our laboratories, although it may require $<5$ K studies to see any ferromagnetism of the nature indicated by Thomson's MCD studies.

Finally, comparing the results of this work with those of the earlier findings of Tweedle, et al., [39], it is obvious that nearly identical magnetochemical data have been obtained. In all three sets of experiments, the cyanide treated H.B.$g_{12}$-less and Yonetani samples of resting cytochrome c oxidase display variable-temperature magnetic susceptibility data consistent with an antiferromagnetic exchange interaction between cyt.$g_3^3(S = 1/2)$ and Cu$^2_4(S = 1/2)$ with $-J \approx 21$ cm$^{-1}$ and $\sim 45$ cm$^{-1}$, respectively. In fact, if Tweedle's data [39] are analyzed by the same procedure used in this work, $-J = 31.3 \pm 1.3$ cm$^{-1}$ is obtained. The inconsistency between this result (for bovine H.B.$g_{12}$-less-CN$^-$ and Yonetani-CN$^-$) and that of Thomson (from bovine Yonetani-CN$^-$) and Fee and Münck (for Thermus Thermophilus-CN$^-$) still remains to be resolved; however, we now feel very confident of the available magnetic susceptibility data on the CN$^-$ forms of the enzyme
derivatives in this work. It is anticipated that presently on-going collaborative magnetic susceptibility experiments between ourselves and the laboratories of Professor Babcock and Dye at Michigan State University using SQUID methodology will lend even greater confidence to the conclusions drawn above.
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11. Warburge, O. Biochim. Z. (1926), 177, 471.

12. Warburg, O.; Negelein, E. Biochim. Z. (1929), 214, 64.


107. Private Communication, Professor Gray, H.B. and Professor Scott, R. California Institute of Technology.

108. Private Communication, Professor Woodruff, W. Department of Chemistry, University of Texas at Austin. Austin, Texas.


APPENDIX I

General Theory and Practice of Magnetic Susceptibility Measurements

The Theory. Magnetochemical measurements have been used in inorganic chemistry mainly to investigate valency and stereochemical problems in transition metal complexes. More extensive studies are able to throw some light on the nature of the coordination bond in these complexes. Reviews of magnetic properties of transition metal complexes from the point of view of both theory and experiment at an introductory [1-5] and advanced level [6,7] have been compiled. Magnetic susceptibility is a measure of how susceptible a substance is to magnetic polarization. All substances are affected in some ways by the application of a magnetic field.

If a substance is placed in a magnetic field of $H$ oersteds then $B$, the magnetic induction, or the density of lines of force within the substance, is given by $H$ plus a contribution, $4\pi I$, due to the substance itself.

$$B = H + 4\pi I \quad (1)$$

where $I$ is the intensity of magnetization or magnetic moment per unit volume. Dividing equation (1) by $H$:

$$P = 1 + 4\pi \frac{I}{H} = 1 + 4\pi X_v \quad (2)$$

Where $P$ and $X_v$ are the permeability and susceptibility per unit volume respectively and which may be considered dimensionless.
In practice, susceptibility is usually more conveniently expressed per unit mass (gram susceptibility $X_g$) than per unit volume. The gram susceptibility can be obtained by dividing the $X_v$ by the density ($d$) of the substance.

$$X_g = \frac{X_v}{d} \quad \text{(cm}^3/\text{gram})$$

---

(3)

Multiplying $X_g$ by the molecular weight produces a molar susceptibility, $X'_M$.

$$X'_M = X_g \cdot MW \quad \text{(cm}^3/\text{mole})$$

---

(4)

The value of $X$ is negative for diamagnetic substance and positive for a paramagnetic one.

There are four types of magnetic behavior: diamagnetism, paramagnetism, ferromagnetism, and antiferromagnetism. The behaviors corresponding to these various classifications are described in Table I.

The behavior of the susceptibility as a function of temperature is also quite characteristic for these different substances. This is illustrated in Figure 1. The temperature at which the maximum occurs in the plot of antiferromagnetic behavior is referred to as the Néel temperature. The temperature at which the break occurs in the ferromagnetic plot is called the Curie temperature.
### TABLE 1

**VARIOUS TYPES OF MAGNETIC BEHAVIOR**

<table>
<thead>
<tr>
<th>Type</th>
<th>Sign</th>
<th>Magnitude</th>
<th>Field Dependence of $x$</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamagnetism</td>
<td>$-$</td>
<td>$10^{-9}$ emu units</td>
<td>Independent</td>
<td>Field induced, paired electron circulations</td>
</tr>
<tr>
<td>Paramagnetism</td>
<td>$+$</td>
<td>0 to $10^{-5}$ emu units</td>
<td>Independent</td>
<td>Angular momentum of the electron</td>
</tr>
<tr>
<td>Ferromagnetism</td>
<td>$+$</td>
<td>$10^{-5}$ to $10^{-3}$ emu units</td>
<td>Dependent</td>
<td>Spin alignment from dipole-dipole interaction of moments on adjacent atoms [1]</td>
</tr>
<tr>
<td>Antiferromagnetism</td>
<td>$+$</td>
<td>0 to $10^{-4}$ emu units</td>
<td>Dependent</td>
<td>Spin pairing, $\parallel$, from dipole-dipole interactions</td>
</tr>
</tbody>
</table>

#### Figure 1

Temperature dependence of ferromagnetic, paramagnetic, and antiferromagnetic behavior.
Paramagnetism is generated by the tendency of magnetic angular momentum to orient itself in a magnetic field. Pierre Curie derived an empirical equation from magnetic susceptibility experiments that described the magnetic susceptibility of a paramagnetic sample as being inversely proportional to the temperature,

\[ \chi = \frac{C}{T} \]  

\[ \text{(5)} \]

where \( \chi \) is the susceptibility per volume, mass, or mole, \( T \) is the absolute temperature and \( C \) is the Curie constant. The Curie constant, can be derived classically or quantum mechanically [4], and is dependent on the number of unpaired electron and the \( g \) value of the compound. In order to express the magnitude of susceptibility in temperature independent terms, a quantity known as the effective Bohr magneton number can be derived [4];

\[ \mu_{\text{eff}} = 2.828 (X'_M \cdot T)^{1/2} \]  

\[ \text{(6)} \]

where \( \mu_{\text{eff}} \) (also called \( n_{\text{eff}} \)) is the effective Bohr magneton, \( X'_M \) is the paramagnetic susceptibility per mole and \( T \) the absolute temperature.

The theoretical relationship of \( \mu_{\text{eff}} \) and the number of unpaired electrons (\( n \)) in a paramagnetic substance can be described as:

\[ (\mu_{\text{eff}})^2 = n \cdot (n + 2) \]  

\[ \text{(7)} \]

Equation (5) and (7) represent ideal behavior which is often not the case, and in fact, most of the important information to be gained from magnetic studies comes from deviations from ideality which may then
imply electronic and structural aspects of the paramagnetic system under study.

The Practice (Faraday Method): In the Faraday method, a small amount of substance is suspended in an inhomogeneous magnetic field and the small force developed is measured by one of several means. When a substance is placed in a magnetic field which is uniform in an x direction and non-uniform in an z direction, the force exerted on the sample in the z direction, F_z, is related to its magnetic susceptibility can be described by,

\[ F_z = \frac{1}{2} x \frac{\Delta H}{x \Delta z} \]

where \( x \) is the magnetic susceptibility, \( H_x \) is the strength of the x field, and \( \frac{\Delta H}{\Delta z} \) is the field gradient. While it is easy to measure \( F_z \) and \( H_x \), it is very difficult to accurately measure the gradient. It is much easier, however, to reproduce a field strength and gradient. Therefore, in practice, the force exerted on a standard sample of known susceptibility is measured and equated to the force exerted on an unknown. A balance is generally used for this purpose so the z direction is oriented perpendicular to gravity. A reproducible field gradient is maintained by reproducing a current through an electromagnet fitted with pole pieces designed to maintain a constant field and gradient with in a region large enough to encompass the sample. With this arrangement it is desirable (1) to use small samples to fit well within the "constant force" region of the field, (2) to measure the
force on the samples in the presence and absence of the magnetic field in order to obtain $X_g$ (susceptibility per gram), and (3) to use a null-type force measuring device to insure that the gradient felt by the sample is not altered by physical movement into the field. These purposes are well served by a servo-nulled electronic microbalance.

In the work, the balance was calibrated using the difference in susceptibility between a triply distilled water and 0.1 M solution of analyzed NiCl$_2$·6H$_2$O [8]. The reason for using this procedure, as opposed to measuring the force exerted on the sample container alone and then sample container plus contents, is that large loads on the balance deflect the beam supporting ribbon downward varying in amount with load. Adding several hundred microliters of liquid to the sample container thus changes its position and its measured force in the magnetic field. Therefore, the sample position is established, and all calibrations and measurements are made with approximately the same load on the balance. In the general, 200 µl of H$_2$O and 0.1 M solution of NiCl$_2$·6H$_2$O were weighed in and out the magnetic field at room temperature. Then, the electrobalance constant ($\beta$) was obtained from the following relationship,

$$\beta = \frac{X_1 m_1 - X_2 m_2}{\Delta_1 - \Delta_2}$$

where $\Delta_1$, $\Delta_2$ are [weight (field off) - weight (field on)] of NiCl$_2$·6H$_2$O solution and H$_2$O and $m_1$ and $m_2$ are the masses of the NiCl$_2$·6H$_2$O solution and H$_2$O in the sample container, $X_1$ and $X_2$ are susceptibilities of
NiCl\textsubscript{2}·6H\textsubscript{2}O solution and H\textsubscript{2}O, respectively. The susceptibility of water is taken to be -0.720 \times 10^{-6} cgs unit/g. The susceptibility per gram of NiCl\textsubscript{2}·6H\textsubscript{2}O at 20 C is given by the following equation [9],

$$\chi_g = [23.21p - 0.720(1-p)] \times 10^{-6}$$  \hspace{1cm} (10)

where p is the weight fraction of nickel chloride in the solution. The susceptibility of this solution was found to be independent of concentration near 30% NiCl\textsubscript{2} by weight. A typical value of $\beta$ (at I = 27 amp) was about $1.10 \times 10^{-7}$ cgs-emu mg$^{-1}$. 
References

1. Nyholm, R.S. *Quart. Rev.*, 7, 377 (1953)


APPENDIX II

The Non-Linear Least Squares Fitting Program. This general program package was originally produced in Fortan II by Moor and Zeigler at Los Alamos for use with the IBM 704 computer. In this work, the program has been updated in Fortan IV for use with the PDP 11/70 computer.
300 FORMAT(24I3)
READ(2,400)(PG(K),K=1,IK)
400 FORMAT(6E12.7)
C READ IN DATA CARDS
IF(IEQ.01)GOTO3
C BLOCK LOADING
READ(2,400)(Y(I),I=1,N)
DO 10 J=1,M
10 READ(2,400)(X(J,I),I=1,N)
IF(IWME.1G003)READ(2,400)(W(I),I=1,N)
GOTO8
C POINTWISE LOADING
3 IF(IWNE.1G004)
DGDO1=1,N
DGJ03=1,N
30 READ(2,400)(Y(I),(X(J,I),J=1,M),W(I))
GOTO8
4 DGDO1=1,N
40 READ(2,400)(Y(I),X(J,I),J=1,M)
GOTO8
C SET UP WEIGHTS IF NOT READ IN
5 IF(IWNE.0)GOTO6
DO401=1,N
40 W(I)=1.0
GOTO8
6 IF(IWNE.2)GOTO7
DO501=1,N
50 W(I)=1.0/Y(I)
GOTO8
7 DO601=1,N
60 W(I)=1.0/Y(I)++2
C READ IN DUM AND TEST CARDS IF CALLED FOR
8 IF(IDUM.0T.0READ(2,400)(DUM(L),L=1,IDUM)
IF(ITEST.EQ.0)TEST=0.000001
IF(ITEST.NE.0READ(2,400)TEST
CLOSE(UNIT=2,DISPOSE=SAVE)
RETURN
END
SUBROUTINE PSLAX
C CALCULATION ROUTINE
C REVISED MAY. 1969
DOUBLE PRECISION X,Y,W,PC,DUM,P,SP,YC,DY,BM,Z,PART,XT,TET,TEST,VAR,
*SSQ,DET,AM,DP,PC,AN,H,SPCC
DIMENSION Y(300),X(3,150),W(300),Z(3),YC(300),DY(300)
DIMENSION IX(40),PC(40),P(40),SP(40),BM(20,21),PART(40)
DIMENSION ALAB(20),DUM(100)
DIMENSION AM(20,20),DP(40),PC(40),AN(40)
COMMON Y,X,W,PC,DUM,P,SP,YC,DY,BM,Z,PART,XT,TET,TEST,VAR,SSQ,DET,ALAB
*,N,IK,IV,MI,IB,ITEST,IDUM,NDUM,IPR,IFDG,IM,IDF,ISN,Ip,DLT,ISC,IX,INTT
C DOUBLE PRECISION ADDED FOR IBM360/40 ETSU MAY. 1969
C INITIAL OPTIONAL PRINTOUT
IF(IREQ.0)GOTO1
WRITE(6,100)(ALAB(L),L=1,20)
100 FORMAT(20A4)
WRITE(6,200)IDUM
200 FORMAT(//10H THERE ARE,14,36H ENTRIES FOR THE VARIABLE CALLED DUM)
IF(IDUM.0T.0)WRITE(6,300)(L,DUM(L),L=1,IDUM)
300 FORMAT(/40H DUM(L).13.3H =.1PE15.7))
WRITE(6,400)TEST
400 FORMAT(/8H TEST = .1PE15.7))
C INITIALIZATION FOR MAIN ITERATION LOOP
1 KFREE=IK-IM
IF=KFREE+1
IDF=N-KFREE
DF=IDF
IT=0
DO 10 K=1,IK
DP(K)=0.0
SP(K)=0.0
PC(K)=PG(K)
10 P(K)=PG(K)
LASTIT=0
H2SC=0
IF(KFREE.EQ.0)GOTO03
C MAIN ITERATION LOOP
2 IT=IT+1
   K=1,0
   DO 30 K=1,KFREE
      DO 20 KK=1,KFREE
         AM(K,KK)=0.0
      20 BM(K,KK)=0.0
      BM(K,KP)=0.0
      K1=K+1
      DO 30 BM(K,K1)=1.0
      3 VAR=VAR+1
      SSG=SSG+DY(I)**2
50 CONTINUE
C LOOP TO SET UP NORMAL EQUATIONS
   DO 90 I=1,N
   C CALL YP ROUTINE, CALCULATE SUM OF SQUARES
   DO 40 J=1,M
50 Z(J)=X(J,I)
   C CALL YPS(I)
      YC(I)=YT
      DY(I)=Y(I)-YC(I)
      VAR=VAR+DY(I)**2
      SSG=SSG+DY(I)**2
   IF(KFREE.EQ.0)GOTO03
C SET UP AN AS VECTOR OF PARTIAL DERIVATIVES
   KI=0
   DO 60 K=1,IK
      IF(KM.EQ.0)GOTO04
      DO 50 KK=1,KM
         IF(K.EQ.IX(KK))GOTO05
      50 CONTINUE
   4 K2=K-K1
      AN(K2)=PART(K)
   GOTO06
50 KI=KI+1
60 CONTINUE
C FORM A AND B MATRICES
   DO 70 K=1,KFREE
      DO 70 KK=1,KFREE
         AM(K,KK)=AM(K,KK)+AN(K)*AN(KK)*W(I)
      70 BM(K,1)=BM(K,1)+AN(K)*DY(I)*W(I)
90 CONTINUE
IF(KFREE.EQ.0)GOTO03
C OPTIONAL PRINTOUT OF A AND B MATRICES ON LAST ITERATION
   IF(LASTIT.EQ.0)GOTO06
      WRITE(6,100)(ALAB(L),L=1,20)
60 FORMAT(6,500)
   WRITE(6,5000)
      DO 40 J=1,IK
         WRITE(6,500)(BM(K,1),K=1,IK)
   50 FORMAT(6,6000)
C SOLVE THE NORMAL EQUATIONS
   IF(KFREE.GT.0)GOTO07
   DET=AM(1,1)
   BM(1,1)=BM(1,1)/AM(1,1)
   BM(1,2)=1.0/AM(1,1)
   GOTO08
   DO 7 CALL LSS(KFREE,KP,20,AM,BM,DET)
C WRITE THE VALUE OF THE DETERMINANT, A INVERSE, AND NO. OF ITERATIONS
   IF(LASTIT.EQ.0)GOTO11
      WRITE(6,8000)DET
800 FORMAT(24 HOVALUE OF DETERMINANT = ,1PIE14 7/)
IF(IPR.EQ.0)GOTO9
WRITE(6,900)
900 FORMAT(3X,1HK,35X,17HSUBVERSE OF A(K,L)//)
DO 120 K=1,KFREE
WRITE(6,700)K,(BM(K,KK),KK=2,KP)
120 WRITE(6,1000)
1000 FORMAT(1HO)
9 WRITE(6,1100)IT
1100 FORMAT(///16,11H ITERATIONS)
C CALCULATE NEW PARAMETER VALUES AND CHECK FOR SIGN CHANGES IF NECESSARY
11 K1=0
DO 140 K=1,IK
IF(IM.EQ.0)GOTO12
DO 130 KK=1,IM
IF(K,EQ.IX(KK))GOTO16
130 CONTINUE
12 K2=K1
DP(K)=BM(K2,1)
13 PC(K)=P(K)+H*DP(K)
IF(LASTIT.NE.0)GOTO140
IF(IFG.EQ.1)GOTO140
14 IF(IT.GT.5)GOTO140
15 IF(P(K).LT.PC(K).GE.0)GOTO140
H=H/2
16 IF(GE.1.OE.-10)GOTO13
WRITE(6,1200)
1200 FORMAT(75H THE PROGRAM QUIT ITERATING SINCE THE PARAMETER(S) INSIST ON CHANGING SIGNS)
RETURN
16 K1=K1+1
140 CONTINUE
C OPTIONAL PRINTOUT FOR EACH ITERATION
1 IF(LASTIT.NE.0)GOTO19
IF(IPR.EQ.0)GOTO17
WRITE(6,1300)IT,H,VAR
1300 FORMAT(1HO,13,1P2E17.7)
DO 150 K=1,IK
150 WRITE(6,700)K,PG(K),P(K),PC(K),DP(K)
C TEST FOR CONVERGENCE
17 KK=0
DO 160 K=1,IK
IF(IPR.EQ.0)GOTO18
SPCC=PC(K)-P(K)/P(K)
IF(DABS(SPCC)-TEST)160,160,19
18 KK=KK+1
160 CONTINUE
IF(KK.EQ.IK)GOTO19
M2SC=1
C SET PARAMETER VALUES FOR THE NEXT ITERATION
19 DO 170 K=1,IK
170 P(K)=PC(K)
C AFTER LAST ITERATION GO BACK FOR FINAL CALCULATION OF YC, DY, ETC.
IF(LASTIT.EQ.0)GOTO21
KFREE=0
GOTO3
C TEST WHETHER 25 ITERATIONS HAVE BEEN TAKEN
21 IF(M2SC.EQ.1)GOTO22
IF(IT.LT.200)GOTO2
C GO BACK FOR LAST ITERATION
22 LASTIT=1
GOTO2
C CALCULATE WEIGHTED VARIANCE, STANDARD DEVIATION OF THE PARAMETERS
23 WW=VAR/DF
K1=0
DO 200 K=1,IK
IF(IM.EQ.0)GOTO24
DO 180 KK=1,1 IM
IF(K.EQ.IX(KK))GOT025
180 CONTINUE
24 K2=K-1
K3=K2+1
SP(K)=DSRT(BM(K2,K3)*WVAR)
GOT0190
25 K1=K1+1
190 CONTINUE
RETURN
END
SUBROUTINE RSUNK
C
C OUTPUT ROUTINE
C
C REVISED MAY. 1968
C
C DOUBLE PRECISION Y, X, W, PQ, DUM, P, SP, YC, DY, BM, Z, PART, YT, TEST, WVAR,
*C SSG, DET, A, B, TS
C
C DIMENSION Y(300), X(5,150), W(300), Z(5), YC(300), DY(300)
C DIMENSION IX(40), P(40), P1(40), SP(40), BM(20, 21), PART(40)
C DIMENSION ALAB(20), DUM(100)
C COMMON Y, X, W, PQ, DUM, P, SP, YC, DY, BM, Z, PART, YT, TEST, WVAR, SSG, DET, ALAB
*, M, IX, IM, IB, TEST, IDUM, NDUM, IPR, IFG, IIM, IDF, IIS, IPI, IJL, ISC, IX, INTT
C
C DOUBLE PRECISION ADDED FOR IBM360/40 ETSU MAY. 1969
C
C PAGE ONE OF THE STANDARD OUTPUT
C
C WRITE THE PROBLEM ID, FUNCTION ID, AND A SUMMARY PARAGRAPH
C
WRITE(6,100)(ALAB(I),I=1,20)
100 FORMAT(20A4)
    I=1
    CALL YPS(I)
    WRITE(6,200)N, M, IK, IM, WVAR, SSG
200 FORMAT(///'20H THIS PROBLEM CONTAINS 13, 14H DATA POINTS. 12, 30H IN
//'103 I DEPENDENT VARIABLE(S), AND 12, 15H PARAMETER(S) (12, 24H OF THEM HEL
//'2D CONSTA NTS) //26H THE WEIGHTED VARIANCE IS 1PE14, 7, 5EH AND THE UNW
//'20 SUM OF SQUARES OF THE DEVIATIONS IS 1PE14, 7, 1EH // ///)
C
C WRITE PARAMETER GUESSES, FINAL VALUES, AND STANDARD DEVIATIONS AND
C CALCULATE AND WRITE THE EXACT LEAST SQUARES EQUATIONS
C
WRITE(6,300)
300 FORMAT(' Q U ESTIMATE OF FINAL VALUE OF S. D. OF
1 Exact Least squares Equation s'/'
2 K K-TH PARAMETER K-TH PARAMETER K-TH PARAMETER
3 Fitted function Input data'///)
    KFREE=IK-IM
    DO 40 K=1, IK
    IF(KFREE.EQ.0)GOT02
    IF(IM.EQ.0)GOT02
    DO 10 KK=1, IM
    IF(K.EQ.IX(KK))GOT01
10 CONTINUE
GOT02
    1 WRITE(6,400)K,PG(K),P(K),SP(K)
400 FORMAT(1HO, I3, '1PE17.7, 29H THIS PARAMETER WAS HELD FIXED')
    QOT040
2 A=0. 0
B=0. 0
DO 30 I=1, N
    DO 20 J=1, M
20 Z(J)=X(J, I)
    CALL YPS(I)
    A=A+W(I)*Y(J)*PART(K)
30 B=B+W(I)*Y(J)*PART(K)
    IF(KFREE.EQ.0)QOT03
    WRITE(6,500)K,PQ(K),P(K),SP(K), A, B
500 FORMAT(1HO, I3, '1PE17.7, 31X, '1PE17.7')
    QOT040
3 WRITE(6,600)K,PQ(K),P(K),SP(K), A, B
600 FORMAT(1HO, I3, '1PE17.7, 29H THIS PARAMETER WAS HELD FIXED, DY, IPSE17
IF (M1.EQ.0) GO TO 21
M3 = M1
DO 18 J = 1, M3
DO 17 L = 1, M3
M1 = M1 - L
S1 = 0.
M2 = M1 + 1
DO 16 K = M2, M3
16 S1 = S1 + A(M1, K) * B(K, J)
17 B(M1, J) = B(M1, J) - S1
18 CONTINUE
GO TO 21
19 CALL LABRT (1, COM2, 2)
GO TO 21
20 CALL LABRT (1, COM3, 3)
21 RETURN
END

SUBROUTINE LABRT(ISW, LHOL, INX)
INTEGER*2 LHOL(24)
LOGICAL PS, TS
DATA NP/10/, PS/. TRUE.,/ TS/. FALSE./
IF((ISW.EQ.0) OR (ISW .GT. 5)) RETURN
GOTO(1, 2, 3, 4, 5) ISW
1 IF(PS .AND. (NP .GT. 0)) PRINT 27, LHOL, INX
27 FORMAT(1X, 9X, 24A2, 3X, 13)
NP = NP - 1
IF(TS) CALL EXIT
RETURN
2 PS = . FALSE.
RETURN
3 PS = TRUE.
NP = INX
RETURN
4 TS = TRUE.
RETURN
5 TS = FALSE.
RETURN
END

SUBROUTINE YPS(I)
C PARTIALS AND FUNCTION DEFINITION ROUTINE
C REVISION MAY, 1968
C DOUBLE PRECISION Y, X, W, PG, DUM, P, SP, VC, DV, BM, Z, PART, YT, TEST, WVAR,
C *SSG, DET
C DOUBLE PRECISION A, B, C, D, E, F, G, H, PI, G, R
C DIMENSION Y(30), X(150), W(150), Z(5), VC(300), DV(300)
C DIMENSION ALAB(20), DUM(100)
C COMMON Y, X, W, PG, DUM, P, SP, VC, DV, BM, Z, PART, YT, TEST, WVAR, SSG, DET, ALAB
C * N, IX, IW, MI, IB, TEST, IDUM, NDUM, IPA, IFO, IM, IDF, ISW, IPLT, ISC, IX, INTT
C CALL ERROR MESSAGE ROUTINE WITH OPTIONAL ARGUMENT
C
C CALL LABRT(ISW, LHOL, INTT)
C
IF(1) 3, 3
1 WRITE(6, 2)
2 FORMAT (' BLEANEY - BUCHS EQUATION ')
GOTO 4
3 YT = 0.0
B = 0.69458
A = (1.207*4. 75)/4.99
YT = A/(1. + 3.333*EXP(2. *P(1)/(B*Z(1))))*Z(1) + P(2)/Z(1) + P(3)
D = 0.33333*EXP(2. 9986*P(1)/Z(1))
PART(1) = -A*(1. + 3 88*D/(Z(1)**(1. + D))**2
PART(2) = 1./Z(1)
PART(3) = 1
4 RETURN
END
17)
40 CONTINUE
C CALCULATE AND WRITE THE CORRELATION MATRIX
   IF(KFREE.EQ.0)GOT04
   WRITE(6,700)
700 FORMAT('///47H MATRIX OF CORRELATIONS BETWEEN FREE PARAMETERS/')
   DO 60 K1=1,KFREE
   DO 60 K2=1,KFREE
   50 TS(K2)=BM(K1,K2+1)/DSQRT(BM(K1,K1+1)*BM(K2,K2+1))
   60 WRITE(6,800)(TS(K),K=1,KFREE)
800 FORMAT(1HO,14,14F8.3/(F12.3,13F8.3))
C PAGE TWO OF THE STANDARD OUTPUT
C WRITE THE PROBLEM ID. FOR EACH POINT WRITE THE WEIGHT, INDEPENDENT
C VARIABLE, DEPENDENT VARIABLE, CALCULATED FUNCTION, DEVIATION, AND
C CALCULATE AND WRITE THE STANDARD DEVIATION OF THE PREDICTED MEAN.
4 WRITE(6,1000)(ALAB(L),L=1,20)
WRITE(6,900)
900 FORMAT(1HO,28X,11HINDEPENDENT,7X,9HDEPENDENT,7X,10HCALCULATED,34X,
11HSTD. DEV. OF/5X,1H1,7X,6HWEIGHT,11X,6HVARIABLE,9X,6HVARAIBLE,9X,
2HFUNCTION,9X,9HDEVIATION,16X,14HPREDICTED MEAN)
   DO 130 I=1,N
   DO 70 J=1,M
   70 Z(J,J)=X(J,I)
   CALL YPSI(I)
   A=0.0
   IF(KFREE.EQ.0)GOTO7
   K=0
   DO 90 K=1,IK
   IF(M.EQ.0)GOTO5
   DO 80 K2=1,IM
   IF(K.EQ.IX(K2))GOTO6
   80 CONTINUE
   5 KJ=K-K1
   TS(KJ)=PART(K)
   GOT090
   6 K1=K1+1
90 CONTINUE
   DO 110 K=1,KFREE
   DD 110 KK=1,KFREE
   110 A=A+TS(K)*TS(KK)*BM(K,KK+1)
   A=DSQRT(A+MVVAR)
   7 J=1
   IF(M.GT.1)GOT08
   WRITE(6,1100)I,W(I),X(I,I),Y(I),Y(C(I),D(Y)),A
1100 FORMAT(1HO,15,1PE17.7,1PE18.7,1PE17.7,1PE27.7)
   QOT09
   8 WRITE(6,1200)I,W(I),J,X(J,I),Y(I),Y(C(I),D(Y)),A
1200 FORMAT(1HO,15,1PE17.7,13,1PE15.7,1PE17.7,1PE27.7)
   DO 120 J=2,M
120 WRITE(6,1300)J,X(J,I)
130 CONTINUE
   RETURN
END

C MATRIX SOLVER ROUTINE
DOUBLE PRECISION A,B,DET,T,X
DIMENSION A(I,N),B(I,M)
DOUBLE PRECISION S1,S2
INTEGER*4 COM1(24),COM2(24),COM3(24)
C DOUBLE PRECISION ADDED FOR IBM360/40 ETSU MAY.1969 (ARGUMENTS ONLY)
C ERROR MESSAGES
DATA COM1,'LS','S','NE','AR','S','IN','QU','LA','R','SY','
I','ST','EM','CA','LC','UL','AT'.
2 '10', 'N', 'CO', 'NT', 'IN', 'VE', 'D', '
2 'ST', 'RO', 'YE', 'D', '
2 'ST', 'RO', 'YE', 'D', '
NN = N
IF (NN.EQ.0) GO TO 20
MM = M
X = 0.
DO 1 J = 1, NN
DO 1 K = 1, NN
T = DABS(A(K,J))
IF (T.GT.X) X = T
1 CONTINUE
IF (X.EQ.0.) GO TO 19
IF (X.GT.1.E-15) GO TO 2
CALL LABRT (1, COM1, 1)
2 SN = 1.
DO 14 J = 1, NN
L = J - 1
IF (L.EQ.NN) GO TO 11
T = DABS(A(J,J))
M1 = J
M2 = J + 1
DO 13 K = M2, NN
X = DABS(A(K,J))
IF (X.LE.T) GO TO 3
T = X
M1 = K
3 CONTINUE
IF (M1.EQ.J) GO TO 6
DO 4 K = 1, NN
T = A(J,K)
A(J,K) = A(M1,K)
4 A(M1,K) = T
DO 5 K = 1, MM
T = B(J,K)
B(J,K) = B(M1,K)
5 B(M1,K) = T
SN = -SN
6 IF (A(J,J).EQ.0.) GO TO 19
DO 10 K = M2, NN
S1 = 0.
S2 = 0.
IF (L.EQ.0) GO TO 8
DO 7 MQ = 1, L
S1 = S1 + A(J,MQ)*A(MQ,K)
8 A(J,K) = (A(J,K) - S1)/A(J,J)
DO 9 MQ = 1, J
S2 = S2 + A(K,MQ)*A(MQ,M2)
9 A(K,M2) = A(K,M2) - S2
10 A(K,M2) = A(K,M2) - S2
11 DO 13 K = 1, MM
S1 = 0.
IF (L.EQ.0) GO TO 13
DO 12 MQ = 1, L
S1 = S1 + A(J,MQ)*B(MQ,K)
13 B(J,K) = (B(J,K) - S1)/A(J,J)
14 CONTINUE
DET = A(1,1)*SN
IF (DET.EQ.0.) GO TO 19
IF (N.EQ.1) GO TO 21
DO 15 J = 2, NN
15 DET = DET*A(J,J)
IF (DET.EQ.0.) GO TO 19
A Typical Data File for Non-Linear Least Squares Fitting

of the Magnetic Susceptibility Data of an Enzyme Sample

---

`/ MAGNETIC DATA, OXIDASE-CN 3/14/85
27 3 1 0 1 1 0 0 1 1 0 0

2
3.52  +018.80  -012.23  -03
4.973 -022.583  +01
4.619 -022.855  +01
4.418 -023.038  +01
4.265 -023.193  +01
4.226 -023.239  +01
4.130 -023.346  +01
4.025 -023.478  +01
3.881 -023.656  +01
3.690 -023.960  +01
3.440 -024.400  +01
3.287 -024.730  +01
3.143 -025.090  +01
2.980 -025.585  +01
2.846 -026.055  +01
2.741 -026.510  +01
2.645 -026.920  +01
2.559 -027.415  +01
2.482 -027.850  +01
2.415 -028.350  +01
2.310 -028.610  +01
2.203 -029.100  +01
2.021 -021.002  +02
1.879 -021.091  +02
1.743 -021.188  +02
1.590 -021.3225 +02
1.360 -021.595  +02
1.178 -021.9065 +02
STOP`
PART II

A SYNTHETIC MODEL COMPOUND APPROACH TO THE ACTIVE SITE

STRUCTURE OF CYTOCHROME C OXIDASE
Part II A Synthetic Model Compound Approach to the Active Site Structure of Cytochrome c Oxidase

In 1976, Palmer, et al., [1] proposed a comprehensive model for the metal-containing sites of cytochrome c oxidase. In this proposal, fully-oxidized or resting oxidase is composed of three units: an isolated low-spin \((S = 1/2)\) ferriheme protein center \((\text{cyt.} a^3)\), an isolated \((S = 1/2)\) Cu(II) center, \((\text{Cu} D^2)\), and an antiferromagnetically spin-coupled binuclear center \((S = 2\) with \(-J \gtrsim 200\) cm\(^{-1}\)) comprised of an \((S = 1/2)\) Cu(II) site, \((\text{Cu} U^2)\) and a high-spin ferriheme protein site \((S = 5/2)\) \((\text{cyt.} a^3)\) bridged via an imidazolate anion from a histidine residue.

In view of the fact that several model complexes containing imidazolate bridges between two metal centers have been synthesized [2-7], there is no question of the bridging capability of the imidazolate anion. However, the ability of an imidazolate bridge to mediate a "strong" antiferromagnetic exchange interaction \((-J \gtrsim 200\) cm\(^{-1}\)) between the bridged metal centers has been questioned repeatedly [8]. The \(\mu\)-imidazolato complexes with \(M = M' = \text{Cu}\) have been reported by several workers to exhibit moderately strong antiferromagnetic coupling in the range of \(-J \lesssim 90\) cm\(^{-1}\) [9]. Others also have reported imidazolate-bridged complexes of \(M = M' = \text{Fe}\) [3], Mn [10] and Co [13]. A synthetic model for the active site of cytochrome c oxidase, reported by Landrum, et al., [11] contains an imidazolate bridge between Mn(II) \((S = 5/2)\) and Co(II) \((S = 1/2)\) and exhibits very weak antiferromagnetic coupling \((-J \lesssim 5\) cm\(^{-1}\)) between the two metal centers. Furthermore, a
solution state study of a 2-methylimidazolate-bridged
[Fe$^{III}$ (2-imid) Cu$^{II}$] complex has been reported by Kovacs and Shepherd
[12]. However, the complex was incompletely characterized due to their
inability to obtain an analytically pure solid sample. In fact, most
past model complexes studies by ourselves [13-15] and other
workers[2-14], have suggested that, in synthetic systems, imidazolate
and other multi-atom heterocyclic bridges are incapable of fostering
antiferromagnetic exchange interactions much greater than 100 cm$^{-1}$ with
$-J << 100$ cm$^{-1}$ in most cases. Dessens, et al., [13] recently reported a
complex containing an imidazolate bridge between Fe(III) and Cu(II),
[LiFe$^{III}$ (imid) Cu$^{II}$]$^+$, where L = OSO$_2$CF$_3^-$ . In this report, the
heterobinuclear complex was found to display magnetic and epr properties
consistent with "strong" coupling ($-J \gtrsim 200$ cm$^{-1}$) between the two metal
centers across the imidazolate bridge, similar to that proposed for the
cytochrome c oxidase active site. However, another $\mu$-imidazolato
iron-copper binuclear complex, with L = Cl$^-$, also reported by Dessens,
[13] displayed weak ($J \lesssim 15$ cm$^{-1}$) antiferromagnetic coupling. The fact
that the OSO$_2$CF$_3^-$ ligand induced a mixture of high-spin ($S = 5/2$) and
low-spin ($S = 1/2$) states for the iron center, each being strongly
antiferromagnetically coupled to a Cu(II) ($S = 1/2$) center, indicates
that the spin state of the iron center may be one of the important
factors to be considered when modeling the active site of the resting
enzyme. With this in mind and in order to further investigate
imidazolato model compounds for the active site of resting cytochrome c
oxidase, twelve new heterobinuclear $\mu$-imidazolato model complexes have
been synthesized, characterized and investigated in this work. The new
heterobinuclear complexes are:
[L(TPP)Fe\textsuperscript{III}(2-meimid)Zn\textsuperscript{II}(2-meimidH)DAP]\text{(OSO}_2\text{CF}_3\text{)} \text{ compound 1},

[L(TPP)Fe\textsuperscript{III}(2-meimid)Cu\textsuperscript{II}(2-meimidH)DAP]\text{(OSO}_2\text{CF}_3\text{)} \text{ compound 2},

[L(TPP)Fe\textsuperscript{III}(imid)Zn\textsuperscript{II}(imidH)DAP]\text{ (OSO}_2\text{CF}_3\text{)} \text{ compound 3},

[L(TPP)Fe\textsuperscript{III}(imid)Cu\textsuperscript{II}(imidH)DAP]\text{ (OSO}_2\text{CF}_3\text{)} \text{ compound 4},

[(TPP)Co\textsuperscript{II}(imid)Zn\textsuperscript{II}(imidH)DAP]\text{ (BF}_4\text{)} \text{ compound 5},

[(TPP)Co\textsuperscript{II}(imid)Cu\textsuperscript{II}(imidH)DAP]\text{ (BF}_4\text{)} \text{ compound 6},

[(TPP)Co\textsuperscript{II}(imid)Zn\textsuperscript{II}(imidH)DAP]\text{ (OSO}_2\text{CF}_3\text{)} \text{ compound 7},

[(TPP)Co\textsuperscript{II}(imid)Cu\textsuperscript{II}(imidH)DAP]\text{ (OSO}_2\text{CF}_3\text{)} \text{ compound 8},

[(TPP)Mn\textsuperscript{II}(imid)Zn\textsuperscript{II}(imidH)DAP]\text{ (BF}_4\text{)} \text{ compound 9},

[(TPP)Mn\textsuperscript{II}(imid)Cu\textsuperscript{II}(imidH)DAP]\text{ (BF}_4\text{)} \text{ compound 10},

[(TPP)Mn\textsuperscript{II}(imid)Zn\textsuperscript{II}(imidH)DAP]\text{ (OSO}_2\text{CF}_3\text{)} \text{ compound 11}, \text{ and}

[(TPP)Mn\textsuperscript{II}(imid)Cu\textsuperscript{II}(imidH)DAP]\text{ (OSO}_2\text{CF}_3\text{)} \text{ compound 12}

where TPP\textsuperscript{2-} = tetraphenylporphyrinato and L = OSO\textsubscript{2}CF\textsubscript{3}\textsuperscript{-}. Proposed structures for the comounds are shown below in Figures 1 and 10.

The reasons for choosing [Co\textsuperscript{II}(TPP)] and [Mn\textsuperscript{II}(TPP)] as metalloporphyrin fragments are related to the spin state of their derivatives. [LCo\textsuperscript{II}(TPP)] compounds with L = nitrogeous bases are usually five-coordinate and low-spin \(d^7\) (\(S = 1/2\)) systems \([14]\), with unpaired electron density in the \(e_g\) orbital with correct symmetry to interact with the \(\sigma\)-bonding orbital framework of an imidzolato bridge. In contrast, [LMn\textsuperscript{II}(TPP)] compounds with L = nitrogeous bases are usually five-coordinate and high-spin \(d^5\) (\(S = 5/2\)) systems \([15]\), which are isoelectronic with high-spin \(S = 5/2\) cyt.\textsubscript{a} \(3^+\) in resting oxidase, where both \(\sigma\)- and \(\pi\)-bonding \(d\)-orbitals are occupied with unpaired electrons. The pentacoordinated ligand of the M' fragment in the [L(TPP)Fe\textsuperscript{III}(2-meimid)M\textsuperscript{II}(2-meimidH)DAP]\textsuperscript{+} complexes (Figure 1) is
Figure 1

Synthetic Scheme for The μ-Imidazolato Binuclear Fe(III) Compounds
\[ \text{[Fe}^{\text{III}}(\text{TPP})L]\]
\[L = \text{OSO}_2\text{CF}_3^-\]

\[M = \text{Cu}^{\text{II}} \text{ or Zn}^{\text{II}}\]

CH\(_2\)Cl\(_2\)/CH\(_3\)CN (10%);
proton sponge;
Reflux

\[ \text{(1) } M' = \text{Zn}, R = \text{Me} \]
\[ \text{(2) } M' = \text{Cu}, R = \text{Me} \]
\[ \text{(3) } M' = \text{Zn}, R = \text{H} \]
\[ \text{(4) } M' = \text{Cu}, R = \text{H} \]
\[ L \]

\[ (\text{OSO}_2\text{CF}_3) \]
\[ R = \text{H or Me} \]
derived from the Schiff base reaction of 2-methylhistamine, rather than histamine as used earlier by Dessens, \textit{et al.}, [13]. It was hoped that the methyl group on the 2-position of the imidazolato ring (Figure 2) would sterically extend the Fe–N(imidazolate) bond upon formation of the mixed-metal binuclear complexes, thereby producing a pure high-spin (S = 5/2) six-coordinate Fe(III) center. Collman, \textit{et al.}, [16] employed a similar strategy to produce five-coordinate high-spin Fe(II) picket-fence compounds using 2-methylimidazole. Furthermore, it was felt that the steric and spin state involvement of the methyl group might be sufficient to move the Fe(III) center out of the porphyrin plane enough to discourage the formation of the tri- or poly-nuclear complexes that seemed to plague Dessens, \textit{et al.}, [13] when they tried to extend their studies into the solution state. In addition to the new \([\text{L(TPP)Fe}^{\text{III}}(2\text{-meimid})\text{M}^{\text{II}}(2\text{-meimidH})\text{DAP}]^{+}\) compounds, the analogous \([\text{L(TPP)Fe}^{\text{III}}(\text{imid})\text{M}^{\text{II}}(\text{imidH})\text{DAP}]^{+}\) complexes of Dessens, \textit{et al.}, has also been reinvestigated as \(\text{OSO}_{2}\text{CF}_{3}^{-}\) salts for the purpose of comparison with the earlier work.
EXPERIMENTAL SECTION

Materials: Histamine, free base, was purchased from Sigma Chemical Company and 2,6-diacetylpyridine (DAP), 2-(2-aminoethyl)pyridine, and 1,3-dihydroxyacetone from Aldrich Chemical Company; Cu(OSO₂₂ CF₃)₂·6H₂O, Cu(BF₄)₂·6H₂O, Ag(OSO₂₂ CF₃), Zn(BF₄)₂·6H₂O, Mn(OAc)₂·2H₂O, Co(OAc)₂·2H₂O were purchased from Alfa Products. Raney nickel was obtained from Aldrich Chemical Company. Solvents (reagent grade THF, CH₂Cl₂, CH₃CN, CH₃OH, EtOH, isopropanol, isopropylacetate, hexane, DMSO and toluene) were obtained from Fisher. Electrochemical grade tetrabutyl ammonium perchlorate (TBAP) was purchased from GFS Chemicals, recrystallized twice from ethyl acetate and cyclohexane and dried for several hours at 90 °C. Industrial grade Ar, N₂, and He gases were obtained from Big Three Industrials and streamed through H₂SO₄, KOH(s), a heated copper catalyst, and a P₂O₅ scrub train to insure dry, dioxygen-free gases.

Dry and purified reagents and solvents were obtained as follows:
CH₃CN was refluxed and distilled over 10g Na₂CO₃ and 15g KMnO₄ for every 800 ml; EtOH was refluxed under N₂ over CaO for several hours then distilled under N₂; DMF was first dried with KOH overnight and then distilled from CaO or BaO; CH₂Cl₂ was predried with Na₂CO₃ and then distilled from P₂O₅; MeOH was distilled from P₂O₅ onto molecular seives (Linde 4a); SOCl₂ was first refluxed with flowers of sulfur overnight and then distilled under N₂; toluene was refluxed and distilled from Na/benzophenone ketyl under N₂; and pyrrole (Aldrich) was distilled prior to use. All other reagents were used as received without special precautions. Degassing was achieved by the standard freeze–thaw method using argon gas. Air sensitive or hygroscopic compounds were
manipulated in an N₂ dry box (a Vacuum Atmospheres Drilab with a HE-35 Dri-Train) or on a Schlenkline using standard techniques [17].

**Instrumentation:** Electronic spectra were recorded on a Cary Model 17 recording spectrophotometer. Infrared spectral studies were obtained using a Beckman IR-4230 spectrophotometer, using nujol mulls or KBr disks. Elemental analyses were performed in-house on a Perkin Elmer Model 240c Elemental Analyzer (C,H,N) or by Galbraith Laboratories Inc.; air-sensitive materials were loaded within an inert atmosphere box into preweighed, airtight glass vials. Metal analyses (Co, Fe, Mn, Cu, Zn) were obtained in-house on an Instrumentation Laboratories Inc. 253 Atomic Absorption Spectrophotometer or by Galbraith Laboratories Inc. Proton nmr spectra were recorded on JEOL FX-90Q spectrometer operating in the fourier transform mode. Solution conductivities were obtained using a Model 31 YSI conductivity bridge.

Electrochemical studies were performed under dry N₂. A PAR Model 174A Analyzer was employed for the cyclic voltammetry measurements. 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. A Metrohm K901 saturated calomel electrode (SCE) was used as a reference electrode, a 16 guage platinum wire as the counter electrode, and a platinum button electrode served as the working electrode. The reference electrode was partially separated from the bulk solution by means of a fritted-glass bridge containing supporting electrolyte solution. The electrolyte
solution was 0.5 M TBAP in CH₂Cl₂ and deaerated with purified N₂ saturated with CH₂Cl₂ for at least 30 minutes.

Magnetic susceptibilities were obtained by the Faraday method, using a Cahn Model 6600-1 research magnetic susceptibility system equipped with a continuous liquid He flow cryostat. The cryogenic apparatus consisted of an Air Products Interface Model DMX-19 vacuum shroud, an LT-3-110 B Heli-tran system, and a Scientific Instruments Model 3800 temperature indicator/controller equipped with LFE Model 4427 voltmeter monitoring a Scientific Instruments Model Si-400 silicon diode sensor. The diamagnetic corrections in cgsu (mol⁻¹ 10⁻⁶) were calculated from Pascal's constants:

\[[\text{Co}^{II}\text{(TPP)}] = -222,\]
\[[\text{ClFe}^{III}\text{(TPP)}] = -238,\]
\[[\text{(OSO}_2\text{CF}_3)\text{Fe}^{III}\text{(TPP)}] = -260,\]
\[[\text{Mn}^{II}\text{(TPP)}] = -222,\]
\[[\text{Cu}^{II}(\text{imidH})_2\text{DAP}] (\text{BF}_4)_2 = -230,\]
\[[\text{Zn}^{II}(\text{imidH})_2\text{DAP}](\text{BF}_4)_2 = -245,\]
\[[\text{Cu}^{II}(2-\text{meimidH})_2\text{DAP}](\text{OSO}_2\text{CF}_3)_2 = -289,\]
\[[\text{Zn}^{II}(2-\text{meimidH})_2\text{DAP}](\text{OSO}_2\text{CF}_3)_2 = -296,\]
\[[\text{(TPP)Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}] (\text{BF}_4) = -409,\]
\[[\text{(TPP)Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}] (\text{BF}_4) = -424,\]
\[[\text{(TPP)Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3) = -422,\]
\[[\text{(TPP)Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3) = -437,\]
\[
\begin{align*}
\{(\text{OSO}_2\text{CF}_3)\text{(TPP)}\text{Fe}^{III}(2\text{-meimid})\text{Cu}^{II}(2\text{-meimidH})\text{DAP}\}(\text{OSO}_2\text{CF}_3) &= -553, \\
\{(\text{OSO}_2\text{CF}_3)\text{(TPP)}\text{Fe}^{III}(2\text{-meimid})\text{Zn}^{II}(2\text{-meimidH})\text{DAP}\}(\text{OSO}_2\text{CF}_3) &= -568, \\
\{(\text{OSO}_2\text{CF}_3)\text{(TPP)}\text{Fe}^{III}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}\}(\text{OSO}_2\text{CF}_3) &= -542, \\
\{(\text{OSO}_2\text{CF}_3)\text{(TPP)}\text{Fe}^{III}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}\}(\text{OSO}_2\text{CF}_3) &= -557, \\
\{(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}\}(\text{BF}_4) &= -409, \\
\{(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}\}(\text{BF}_4) &= -424, \\
\{(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}\}(\text{OSO}_2\text{CF}_3) &= -426, \text{ and} \\
\{(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}\}(\text{OSO}_2\text{CF}_3) &= -441.
\end{align*}
\]

Epr spectra of polycrystalline solids or $10^{-4}$ M CH$_2$Cl$_2$ solution were obtained at 10 K on a Varian E-line spectrometer with an Air Products interface Model LTD-3-110 Heli-Tran cryogenic system. Diphenylpicrylhydrazyl (dpph) was used as the reference for the magnetic field position. The polycrystalline solids were prepared with analytically pure ammonium sulfate, ~1.0% in metal compound.

**Syntheses**

**Ethyl acetimidate hydrochloride**: The compound was prepared by a Pinner synthetic method [18]. A mixture of 0.5 mole (23.04 g, 29.18 ml) of absolute ethanol and 0.5 mole (20.53 g, 26.12 ml) of dry acetonitrile CH$_3$CN in 62 ml of anhydrous diethyl ether was cooled in an ice-salt bath for about 10 min. After cooling, anhydrous HCl gas was bubbled through the solvent mixture until the reaction mixture increased by 18 g (0.5 mole) in weight; this procedure took approximately 15 min. The flask containing the solvent mixture was tightly capped and allowed to stand.
overnight, during which time a crystalline solid was obtained. The white crystalline solid was collected by suction filtration, and after being washed with ether, it was dried under vacuum at room temperature for 15 hours. Yield was 26 g (42%). mp 110-113 °C (lit. 112-114) [19].

**Anal. Calcd.** For $\text{C}_4\text{H}_{10}\text{NOCl}$: C, 38.33%; H, 8.16%; N, 11.33%;. **Found:** C, 39.05%; H, 8.42%; N, 11.0%.

2-methylhistamine was synthesized according to general procedure of Dziuron and Schumack [20] or Durant, et al., [21]. During this procedure, three intermediates are isolated including 2-methyl-4-hydroxymethylimidazole hydrochloride, 2-methyl-4-chloromethylimidazole hydrochloride and 2-methyl-4-cyanomethylimidazole hydrochloride. The synthesis of these intermediates are given directly below.

**2-methyl-4-hydroxymethylimidazole hydrochloride:** After transferring 200 ml of liquid $\text{NH}_3$ into a 300 ml precooled autoclave (from Par instrument Co.), ethyl acetimidate hydrochloride (22.6 g, 0.18 mol) was added gradually to the liquid. Then, 1,3-dihydroxyacetone (16.5 g, 0.18 mol) was also added slowly to the reaction mixture. The autoclave was closed and the contents were stirred and heated at 60-70 °C (350-450 psi) in an oil bath for 6 hours. After the autoclave was cooled to room temperature, the contents were filtered and the filtrate was concentrated with stirring at 45 °C. The resulting white residue was then extracted with three, 100 ml portions of dry acetone.
Subsequently, the acetone solution was concentrated under vacuum to obtain an oily liquid as a crude product. The product was further purified by recrystallization from an iso-propanol/ether solvent mixture to give the 2-methyl-4-hydroxymethylimidazole free base. Yield was 11 g (54%), mp 119-120 °C (lit. 120-122 °C) [21]. The free base carbinol was then converted to a hydrochloride salt by dissolving the free base (10 g, 89 mmol) in 50 ml iso-propanol followed by addition of ethanol saturated with HCl until pH = 1. The crude product was then obtained as a solid by addition of diethyl ether to the solution mixture. Upon cooling, a white solid precipitated and was collected by suction filtration. Yield 7 g (52%), mp 145-147 °C (lit. 149-151 °C) [21].

Anal. Calc. For C<sub>5</sub>H<sub>8</sub>N<sub>2</sub>O: C, 53.56%; H, 7.19%; N, 24.98%. Found. C, 53.63%; H, 7.41%; N, 24.91%. <sup>1</sup>H NMR (D<sub>2</sub>O, external TMS): δ 2.8 (s, CH<sub>3</sub>), 4.38 (s, CH<sub>2</sub>), 6.8 (s, imidazole H<sup>5</sup>).

2-methyl-4-chloromethylimidazole hydrochloride: 6.5 g, (43.74 mmol) of 2-methyl-4-hydroxymethylimidazole hydrochloride was added, in portions, with stirring to SOCl<sub>2</sub> (15 ml) at room temperature. The mixture was stirred and heated at 100 °C for 45 min and the excess SOCl<sub>2</sub> was allowed to evaporate to obtain a dark brown residue. The solid residue was dissolved in 35 ml ethanol and the solution was decolorized by boiling with 5 g of activated charcoal. After filtration to remove the charcoal, diethyl ether was added to the filtrate until the solution turn cloudy, and a white solid product started forming upon cooling. The solid was collected by suction filtration. Yield 5 g (68%), mp
148-150 °C (lit. 149-150 °C) [22]. Anal. Calcd. For C₅H₈N₂Cl₂: C, 35.95%; H, 4.83%; N, 16.77%. Found: C, 36.47%; H, 4.72%; N, 16.90%. ¹H nmr (CD₃CN, external TMS): δ 2.8 (s, CH₃), 4.42 (s, CH₂), 6.8 (s, imidazole H⁵).

2-methyl-4-cyanomethylimidazole hydrochloride: 4.5 g (27 mmol) of 2-methyl-4-chloromethylimidazole hydrochloride was added in portions to a stirring mixture of NaCN (5.75 g, 82 mmol) in dry DMSO (50 ml) at room temperature. The reaction mixture was stirred and heated at 40 °C for 1 hour and the solvent was removed by vacuum distillation to obtain a dark brown oily liquid residue. The residue was then dissolved in 100 ml distilled water and the desired product was obtained by continuously extracting the water solution with iso-propylacetate. The iso-propylacetate layer was evaporated to dryness and the residue was recrystallized from an iso-propylacetate/diethyl ether solvent mixture to obtain 2-methyl-4-cyanomethylimidazole hydrochloride. Yield was 2.8 g (66%), mp. 167 °C (lit. 168-170 °C) [21]. Anal. Calcd. For C₆H₈N₂Cl: C, 59.49%; H, 5.82%; N, 34.69%. Found: C, 59.20%; H, 5.87%; N, 33.97%. ¹H nmr (D₂O, external TMS): δ 2.38 (s, CH₃), 3.68 (s, CH₂), 6.8 (s, imidazole H⁵).

2-methylhistamine: The 2-methylhistamine was obtained by the chemical reduction of 2-methyl-4-cyanomethylimidazole hydrochloride. 2.5 g (16 mmol) of 2-methyl-4-cyanomethyl-imidazole hydrochloride in 75 ml ethanol was saturated with NH₃ at 10 °C in an autoclave. After
addition of 1 g of Raney nickel to the solution mixture, the autoclave was pressurized to 2000 psi H₂. The contents were then stirred and heated at 145-150 °C (2000-2300 psi) for 3 hours. After allowing the reaction mixture to cool to room temperature, it was filtered through a layer of activate charcoal to remove the Raney nickel and the filtrate was evaporated to dryness. The residue was free base 2-methylhistamine, as an oily liquid. Yield 1.3 g (65%). Anal. Calc. For C₆H₁₁N₃: C, 57.57%; H, 8.86%; N, 33.57%. Found: C, 57.39%; H, 8.80%; N, 33.40%. ¹H nmr (CDCl₃, internal TMS): δ 2.38 (s, CH₃), 2.65 (t) (CH₂), 3.0 (t) (CH₂), 4.64 (b) (NH), 6.62 (s, imidazole H⁵).

[Zn⁺⁺(OSO₂CF₃)₂]·6H₂O: A solution of 1.36g ZnCl₂ (10 mmol) and 5.14 g AgOSO₂CF₃ (20 mmol) in 100 ml of water was stirred with boiling for 15 min. The AgCl was removed by filtration and the filtrate reduced to dryness under vacuum. The resulting solid was then dried under vacuum at room temperature for 4 hrs. Yield 3.0 g (72% assuming 6H₂O).

[Cu⁺⁺(imidH)₂DAP](OSO₂CF₃)₂: The ligand solution was prepared according to the general procedure of Simmons, et al., [23] by dissolving 0.16 g of 2,6-diacetylpyridine (1 mmol) and 0.22 g of histamine, free base, (2 mmol) in 25 ml MeOH. The reaction mixture was refluxed for 1 hour to yield a yellow solution. This solution was then combined with 0.47 g of [Cu⁺⁺(OSO₂CF₃)₂]·6H₂O (1 mmol) and stirred for another 10 min. The green solution was filtered and reduced to dryness under vacuum at room temperature to yield a green solid residue. This
residue was then recrystallized from MeOH/ether and dried under vacuo at 60 °C for 6 hours; yield 0.44 g (62%) of green crystals. Anal.
Calcd. For CuC_{21}H_{23}N_7S_2O_6F_6: C, 35.47%; H, 3.26%; N, 13.79%; Cu, 8.94%. Found: C, 36.19%; H, 3.40%; N, 13.87%; Cu, 8.68%.

\( \mu_{\text{eff}}(\text{solid, RT}) = 2.0 \ \mu_B \).

\( [\text{Zn}^{\text{II}}(\text{imidH})_2\text{DAP}](\text{OSO}_{2}\text{CF}_3)_2 \): The compound was prepared using \( [\text{Zn}^{\text{II}}(\text{OSO}_{2}\text{CF}_3)_2] \cdot 6\text{H}_2\text{O} \) instead of \( [\text{Cu}^{\text{II}}(\text{OSO}_{2}\text{CF}_3)_2] \cdot 6\text{H}_2\text{O} \) and treated identically to the analogous copper complex. The product is a light yellow crystalline solid. Yield 0.45 g (63%) Anal. Calcd. for ZnC_{21}H_{23}N_7O_6S_2F_6: C, 35.38%; H, 3.25%; N, 13.75%; Zn, 9.17%. Found C, 36.14%; H, 3.46%; N, 13.79%; Zn, 9.00%.

\( [\text{M}^{\text{II}}(\text{imidH})_2\text{DAP}](\text{BF}_4)_2 \cdot \text{H}_2\text{O} \): where \( \text{M} = \text{Zn} \) and \( \text{Cu} \): The zinc(II) and copper(II) complexes were prepared and purified by the method of Simmons, et al., [23].

\( [\text{Cu}^{\text{II}}(2\text{-meimidH})_2\text{DAP}](\text{OSO}_{2}\text{CF}_3)_2 \): The ligand solution was prepared by refluxing 25 ml of a methanolic solution of 2,6-diacetylpyridine (0.16 g, 1 mmol) and 2-methylhistamine, free base, (0.25 g, 2 mmol) for 1 hour. The yellow solution was allowed to cool to room temperature before it was filtered, and the filtrate was combined with a \( [\text{Cu}^{\text{II}}(\text{OSO}_{2}\text{CF}_3)_2] \cdot 6\text{H}_2\text{O} \) (0.47 g, 1 mmol). The solution turned green upon addition of the copper salt and the reaction mixture was stirred at room temperature for another 15 min. The green solution was then filtered
and the filtrate was evaporated under vacuum to dryness to obtain a green solid. The solid was purified by recrystallization from a methanol/diethyl ether solvent mixture and dried under vacuum at room temperature for 6 hours. Yield 0.5 g (68%) *Anal. Cald.* For CuC₂₅H₂₇N₇O₆S₂F₆: C, 37.37%; H, 3.68%; N, 13.26%; Cu, 8.60%. *Found:* C, 36.94%; H, 3.92%; N, 13.00%; Cu, 8.4%. μ<sub>eff</sub> (Solid, RT) = 2.02 μ<sub>B</sub>.

\[\text{[Zn}^{II}\text{(2-imidH)}_2\text{DAP)}\text{(OSO}_2\text{CF}_3\text{)}_2\text{]}\] The compound was prepared using [Zn<sup>II</sup>(OSO<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>].6H₂O instead of [Cu<sup>II</sup>(OSO<sub>2</sub>CF<sub>3</sub>)<sub>2</sub>].6H₂O and treated identically to the analogous copper complex. The recrystallized product was a yellow solid. Yield 0.54 g (73%). *Anal. Cald.* For ZnC₂₃H₂₇N₇O₆S₂F₆: C, 37.28%; H, 3.67%; N, 13.23%; Zn, 8.57%. *Found:* C, 37.00%; H, 3.98%; N, 13.57%; Zn, 8.67%.

\[[\text{ClFe}^{III}\text{(TPP)}]\]. The metallation of meso-tetrathienylporphyrine (TPPH₂) was accomplished according to the general procedure of Alder, et al., [24]. A mixture of two g (16.5 mmol) of anhydrous FeCl₂ and two g (3.3 mmol) of meso-tetrathienylporphyrine was refluxed in 350 ml of reagent grade DMF for 6 hours. The solvent was evaporated under vacuum to obtain a dark purple solid residue which was dry under vacuum at room temperature for 14 hours. The crude product was then dissolved in 150 ml dried CH₂Cl₂ and suction filtered to remove impurities. The filtrate was reduced to dryness by flash evaporation. Subsequently, the purple \[[\text{ClFe}^{III}\text{(TPP)}]\] product was dissolved in a minimum amount (200 ml) of hot
THF and recrystallized by the addition of 100 ml 6 N HCl and enough distilled water for dilution to a total volume of 800 ml. The recrystallized product precipitated upon cooling, after which it was collected by filtration, washed with water, and dried under vacuum at 60 °C for 14 hours. Anal. Cald. For \( \text{FeC}_{44}\text{H}_{28}\text{N}_{4}\text{Cl} \): C, 75.06%; H, 4.00%; N, 7.96%; Fe, 7.93%. Found: C, 75.43%; H, 3.79%; N, 8.03%; Fe, 7.80%. \( \mu_{\text{eff}} \) (Solid, RT) = 6.1 \( \mu_B \).

\[ ((\text{OSO}_2\text{CF}_3)\text{Fe}^{III}(\text{TPP})) \] The Metathesis of [ClFe\( ^{III} \)(TPP)] with Ag(\( \text{OSO}_2\text{CF}_3 \)) by the method of Reed, et al., [25] was utilized to prepare \[ ((\text{OSO}_2\text{CF}_3)\text{Fe}^{III}(\text{TPP})) \]. One g (1.42 mmol) of [ClFe(TPP)] and a mole equivalent of Ag(\( \text{OSO}_2\text{CF}_3 \)) (0.36 g) were refluxed in 30 ml THF for 30 min. After filtration, 90 ml of reagent grade hexane was added to the filtrate and purple solid crystals started to form upon cooling. The solution was then stored in the freezer overnight. The solid product was collected by filtration and recrystallized from 40 ml of hot toluene by the slow addition of 75 ml heptane. The shiny purple crystals which formed with cooling were collected by filtration. After washing the crystalline product with heptane, the solid was dried under vacuum at 60 °C for 14 hours. IR (KBr pellet): \( \nu \) (\( \text{OSO}_2\text{CF}_3 \)) = 1340(s), 1240(s), 1205(s), and 630(s) cm\(^{-1} \). Anal. Cald. For \( \text{FeC}_{45}\text{H}_{26}\text{N}_{4}\text{O}_3\text{S}_3 \): C, 66.10%; H, 3.45%; N, 6.85%; Fe, 6.83%. Found: C, 65.86%; H, 3.68%; N, 6.60%; Fe, 6.74%. \( \mu_{\text{eff}} \) (Solid, RT) = 5.25 \( \mu_B \).
[Co\textsuperscript{II}(TPP)]: [Co\textsuperscript{II}(TPP)] was prepared according to the procedure described by Rothemund and Menotti [26]. TPPH\textsubscript{2} (0.5 g; 0.82 mmol) in 50 ml of chloroform and [Co(oAc)\textsubscript{2}].4H\textsubscript{2}O (0.25 g; 1 mmol) dissolved in 50 ml of glacial acetic acid were combined and refluxed for 0.5 hours. Upon cooling, maroon crystals formed which were recrystallized by soxhlet extraction using diethylether. The product was then chromatographed on silica gel (0.5 g of [Co\textsuperscript{II}(TPP)] on a 12x1/2 in. column), using CH\textsubscript{2}Cl\textsubscript{2} as the eluent. Yield 0.15 g (27%) of dark maroon crystals. \textit{Anal. Calcd.} For CoC\textsubscript{44}H\textsubscript{28}N\textsubscript{4}: C, 78.68%; H, 4.20%; N, 8.34%; Co, 8.78%. \textit{Found:} C, 78.33%; H, 4.39%; N, 7.90%; Co, 8.93%. μ\textsubscript{eff}(solid, RT) = 2.7 μ\textsubscript{B}. λ\textsubscript{max} (CH\textsubscript{2}Cl\textsubscript{2}): 410 nm (ε=3.1x10\textsuperscript{4}), 527 nm (ε=1.6x10\textsuperscript{4}).

[C1Mn\textsuperscript{III}(TPP)]: [C1Mn\textsuperscript{III}(TPP)] was synthesized according to the procedure of Rothemund and Menotti [26]. TPPH\textsubscript{2} (0.5 g; 0.82 mmol) in 50 ml of chloroform and Mn(OAc)\textsubscript{2}.4H\textsubscript{2}O (0.5 g; 1 mmol) and 0.1 g of NaCl in 70 ml of glacial acetic acid were combined and refluxed for six hours. The solvent was removed under vacuum, 50 ml of glacial acetic acid was added to the dry residue, and the resulting solution concentrated to approximately 15 ml. The crystals which formed upon cooling were collected by filtration, washed with glacial acetic acid, air dried, and recrystallized by soxhlet extraction using diethylether. The crystalline product was then further purified by silica gel column chromatography, as above, using CH\textsubscript{2}Cl\textsubscript{2} as the eluent. Yield 0.20 g (35%) of dark green lustrous crystals. \textit{Anal. Calcd.} For
MnC\textsubscript{44}H\textsubscript{28}N\textsubscript{4}Cl: C, 75.16%; H, 4.01%; N, 7.97%; Mn, 7.82%. Found: C, 74.82%; H, 4.04%; N, 7.76%; Mn, 7.70%. $\mu_{\text{eff}}$ (solid; RT) = 4.94 $\mu_B$.

$\lambda_{\text{max}}$ (CH\textsubscript{2}Cl\textsubscript{2}): 340 nm ($\epsilon$=13.2x10$^4$), 389 nm ($\epsilon$=15.2x10$^4$), 475 nm ($\epsilon$=16.0x10$^4$), 568 nm ($\epsilon$=40.5x10$^3$), 605 nm (35.4x10$^4$), 694 nm ($\epsilon$=18x10$^3$).

Bis (2,4-pentanedionato)chromium(II). [Cr\textsuperscript{II}(acac)$_2$] was prepared by the general method of Ocone, et al., [27].

$\text{Mn}^{\text{II}}$(TPP): $\text{Mn}^{\text{II}}$(TPP) was synthesized by the method of Reed, et al., [28] in an inert atmosphere. [ClMn\textsuperscript{III}(TPP)] (1.0 g; 1.42 mmol) in 20 ml of toluene was heated almost to boiling and [Cr\textsuperscript{II}(acac)$_2$] (0.60 g; 2.4 mmol) was then added until the color of the solution changed from green to claret. The hot solution was then quickly filtered and 20 ml of hexane was added to the filtrate. After 4 hours, the crude product was collected by filtration and the visible spectrum was checked for the absence of a [ClMn\textsuperscript{III}(TPP)] band at 475 nm. The product was recrystallized from hot toluene/hexane to give sparkling purple crystals. Yield 0.50 g (53%). Anal. Calc. For MnC\textsubscript{44}H\textsubscript{28}N\textsubscript{4}: C, 79.15%; H, 4.23%; N, 8.39%; Mn, 8.23%. Found: C, 78.84%; H, 4.32%; N, 8.10%; Mn, 8.25%. $\mu_{\text{eff}}$ (Solid, RT) = 6.1 $\mu_B$.

$\lambda_{\text{max}}$ (CH\textsubscript{2}Cl\textsubscript{2}): 359 nm ($\epsilon$=5.3x10$^4$), 434 nm ($\epsilon$=2.5x10$^5$), 520 nm ($\epsilon$=6x10$^3$), 562 nm ($\epsilon$=1.1x10$^4$), 602 nm (1.0x10$^4$).

[(OSO\textsubscript{2}CF\textsubscript{3})(TPP)Fe\textsuperscript{III}(2-meimid)Zn\textsuperscript{II}(2-meimidH)DAPJ(OSO\textsubscript{2}CF\textsubscript{3})\cdot CH\textsubscript{2}Cl\textsubscript{2}]

(Compound 1): The heterobinuclear complex was prepared by the general
method of Dessens, et al., [13]. A 1:(1.2) molar ratio mixture of
\[(\text{OSO}_2\text{CF}_3)_2\text{Fe}^{\text{III}}(\text{TPP})\] (0.19 g, 0.23 mmol) and \([\text{Zn}^{\text{II}}(2\text{-meimidH})_2\text{DAP}]\)(\text{OSO}_2\text{CF}_3)_2\] (0.21 g, 0.28 mmol) was refluxed for 4 hours in the
presence of one mole equivalent of proton sponge (0.046 g, 0.23 mmol) in
a 50 ml of a dry \(\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}\) (10%) solvent mixture. After refluxing,
the solvent was filtered and the filtrate was evaporated to dryness,
yielding a dark purple crystalline solid. The excess
\([\text{Zn}^{\text{II}}(2\text{-meimidH})_2\text{DAP}]\)(\text{OSO}_2\text{CF}_3)_2\] was removed by washing the crude product
with water until the wash was clear. The solid was then washed with
heptane and dried under vacuum at 45 °C for 10 hours. Anal. Cald. For:
\[\text{FeZnC}_{67}\text{H}_{54}\text{N}_{11}\text{O}_{16}\text{S}_{2}\text{F}_6\text{CH}_2\text{Cl}_2\]: C, 54.69%; H, 3.78%; N, 10.32%; Fe,
3.74%; Zn, 4.38%. Found: C, 54.90%; H, 4.09%; N, 10.07%; Fe,
3.23%; Zn, 3.86%. \(\mu_{\text{eff}}\) (Solid, RT) = 2.31 \(\mu_B\)

\[\text{[(OSO}_2\text{CF}_3)_2(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{Cu}^{\text{II}}(2\text{-meimidH})\text{DAP}]\)(\text{OSO}_2\text{CF}_3)_2\cdot\text{CH}_2\text{Cl}_2\]
(Compound 2). The compound was prepared using \(\text{Cu}^{\text{II}}(2\text{-meimidH})_2\)
\(\text{DAP}]\)(\text{OSO}_2\text{CF}_3)_2\) instead of \([\text{Zn}^{\text{II}}(2\text{-meimidH})_2\text{DAP}]\)(\text{OSO}_2\text{CF}_3)_2\) and treated
identically to the analogous iron-copper binuclear complex. The desired
product was a dark purple solid. Anal. Cald. For:
\[\text{FeCuC}_{67}\text{H}_{54}\text{N}_{11}\text{S}_{2}\text{O}_{6}\text{F}_6\text{CH}_2\text{Cl}_2\]: C, 54.75%; H, 3.79%; N, 10.33%; Fe,
3.74%; Cu, 4.26%. Found: C, 54.92%; H, 3.99%; N, 9.96%; Fe, 3.55%;
Cu, 3.97%. \(\mu_{\text{eff}}\) (Solid, RT) = 3.15 \(\mu_B\).

\[\text{[(OSO}_2\text{CF}_3)_2(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{M}^{\text{II}}(\text{imidH})\text{DAP}]\)(\text{OSO}_2\text{CF}_3)_2\cdot\text{CH}_2\text{Cl}_2\] with \(\text{M}^+ =
\text{Zn}\) (Compound 3) and \(\text{Cu}\) (Compound 4): The iron-zinc and iron-copper
binuclear complexes were prepared by first combining 0.12 g (0.15 mmol)
of \( [(\text{OSO}_2\text{CF}_3)\text{Fe}^{\text{III}}(\text{TPP})] \) and 0.13 g (0.18 mmol) of \([\text{M}^{\text{II}}(\text{imidH})_2\text{DAP}]\) \( (\text{OSO}_2\text{CF}_3)_2 \) \( (M' = \text{Cu}, \text{Zn}) \) in a 1:(1.2) molar ratio in 150 ml of a dry
\( \text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN} \) (10%) solvent mixture. The solution was then refluxed for
6 hours in the presence of 0.032 g (0.15 mmol) of proton sponge.
Subsequently, the solution was reduced to dryness and the resulting dark
purple solid was washed with water to remove any excess
\([\text{M}^{\text{II}}(\text{imidH})_2\text{DAP}]\) \( (\text{OSO}_2\text{CF}_3)_2 \) and then with hexane. The product was dried
under vacuum at room temperature for 14 hours.

**Compound 3:** Anal. Calc. For \( \text{FeZnC}_{65}\text{H}_{50}\text{N}_{11}\text{O}_{6}\text{S}_2\text{F}_6\cdot\text{CH}_2\text{Cl}_2 \): C, 54.09%; H, 3.58%; N, 10.51%; Fe, 3.81%; Zn, 4.46%. Found: C, 54.43%;
H, 3.69%; N, 10.21%; Fe, 3.57%; Zn, 4.00%. \( \mu_{\text{eff}} \) (Solid, RT) = 4.51
\( \mu_{\text{B}} \).

**Compound 4:** Anal. Calc. For \( \text{FeCuC}_{65}\text{H}_{50}\text{N}_{11}\text{O}_{6}\text{S}_2\text{F}_6\cdot\text{CH}_2\text{Cl}_2 \): C, 54.16%; H, 3.58%; N, 10.53%; Fe, 3.81%; Cu, 4.34%. Found: C, 53.90%;
H, 3.70%; N, 10.00%; Fe, 3.75%; Cu, 4.46%. \( \mu_{\text{eff}} \) (Solid, RT) = 3.78
\( \mu_{\text{B}} \).

\( [(\text{TPP})\text{Co}^{\text{II}}(\text{imid})(\text{imidH})\text{DAP}]\cdot(\text{BF}_4)_2\cdot\text{CH}_2\text{Cl}_2 \) with \( M' = \text{Zn} \) (Compound
5) and Cu (Compound 6): The heterobinuclear complexes were prepared
according to the general procedure of Dessens, et al., [13]. A 1:(1.2)
molar ratio mixture of \( [\text{Co}^{\text{II}}(\text{TPP})] \) (0.21 g) and \([\text{M}^{\text{II}}(\text{imidH})_2\text{DAP}]\) \( (\text{BF}_4)_2 \)
(0.26 g for \( M' = \text{Zn} \) and 0.25 g for \( M' = \text{Cu} \)) was refluxed for 6 hours in
the presence of one mole equivalent of proton sponge (0.096 g) or
$\text{-BuO}^+\text{K}^+$ (0.050 g) in a CH$_2$Cl$_2$/CH$_3$CN (90/10) dry solvent mixture. The
solvent was then removed in vacuo and the resulting crystalline product
washed with 50 ml of water to remove any excess $[\text{M'}^\text{II}(\text{imidH})_2\text{DAP}](\text{BF}_4)_2$
and then with 50 ml of hexane. The solid was further purified by
recrystallization from CH$_2$Cl$_2$/hexane. The dark purple product was dried
over P$_2$O$_5$ in vacuo at 60 °C for 15 hours.

**Compound 5:**  
Anal.  Calcd.  For CoZnC$_{63}$H$_{50}$N$_{11}$BF$_4$.CH$_2$Cl$_2$  
C, 61.14%; H, 4.17%; N, 12.26%; Co, 4.46%; Zn, 5.20%.  Found: C,
61.35%; H, 4.50%; N, 11.97%; Co, 4.49%; Zn, 5.30%.  $\mu_{\text{eff}}$ (solid, RT)
= 2.21 $\mu_B$.  $\lambda_{\text{max}}$ (CH$_2$Cl$_2$): 582 nm ($\varepsilon=4.0\times10^3$), 555 nm ($\varepsilon=8.1\times10^3$), 434 nm
($\varepsilon=14.4\times10^4$).  $\Delta_\text{c} = 40 \mu$hos cm$^{-1}$ mole$^{-1}$ at 25 °C in CH$_2$Cl$_2$.

**Compound 6:**  
Anal.  Calcd.  For CoCuC$_{63}$H$_{50}$N$_{11}$BF$_4$.CH$_2$Cl$_2$  
C, 61.23%; H, 4.18%; N, 12.27%; Co, 4.69%; Cu, 5.65.  Found: C,
61.35%; H, 4.46%; N, 12.30%; Co, 4.54%; Cu, 5.63%.  $\mu_{\text{eff}}$ (solid, RT)
= 3.10 $\mu_B$.  $\lambda_{\text{max}}$ (CH$_2$Cl$_2$): 583 nm ($\varepsilon=4.0\times10^3$), 556 nm ($\varepsilon=4.75\times10^3$),
436 nm ($\varepsilon=11.6\times10^4$).  $\Delta_\text{c} = 40 \mu$hos cm$^{-1}$ mole$^{-1}$ at 25 °C in CH$_2$Cl$_2$.

$\text{[(TPP)Co}^\text{II}(\text{imid})\text{M'}^\text{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)_2$.CH$_2$Cl$_2$ with M' = Zn
(compound 7), and Cu (compound 8): The compounds were prepared using
$[\text{Zn}^\text{II}(\text{OSO}_2\text{CF}_3)_2]_2\cdot\text{6H}_2\text{O}$ or $[\text{Cu}^\text{II}(\text{OSO}_2\text{CF}_3)_2]_2\cdot\text{6H}_2\text{O}$ instead of the BF$_4^-$ salts
and treated identically to the analogous
$\text{[(TPP)Co}^\text{II}(\text{imid})\text{M'}^\text{II}(\text{imidH})\text{DAP}](\text{BF}_4)_{\text{ compounds above.}}$
Compound 7: Anal. Calcd. For CoZnC$_{64}$H$_{50}$N$_{11}$SO$_3$F$_3$. CH$_2$Cl$_2$: C, 59.17%; H, 3.79%; N, 11.68%; Co, 4.47%; Zn, 4.95%. Found: C, 59.52%; H, 4.00%; N, 11.45%; Co, 4.25%; Zn, 4.80%. $\mu_{\text{eff}}$ (solid, RT) = 2.23 $\mu_B$. $\lambda_{\text{max}}$ (CH$_2$Cl$_2$): 594 nm ($\varepsilon$=3.72x10$^3$), 552 nm ($\varepsilon$=7.14x10$^3$), 433 nm ($\varepsilon$=14.67x10$^3$), 301 nm ($\varepsilon$=21.5x10$^3$). $\Lambda_c$ = 42 $\mu$mhos cm$^{-1}$ mole$^{-1}$ at 25 °C in CH$_2$Cl$_2$.

Compound 8: Anal. Calcd. For CoCuC$_{64}$H$_{50}$N$_{11}$SO$_3$F$_3$. CH$_2$Cl$_2$: C, 59.25%; H, 3.98%; N, 11.69%; Co, 4.47%; Cu, 4.82%. Found: C, 59.03%; H, 4.15%; N, 11.45%; Co, 4.31%; Cu, 4.67%. $\mu_{\text{eff}}$ (Solid, RT) = 3.14 $\mu_B$. $\lambda_{\text{max}}$ (CH$_2$Cl$_2$): 590 nm ($\varepsilon$=3.25x10$^3$), 554 nm ($\varepsilon$=6.75x10$^3$), 432 nm ($\varepsilon$=12.73x10$^3$), 282 nm ($\varepsilon$=18.31x10$^3$). $\Lambda_c$ = 41 $\mu$mhos cm$^{-1}$ mole$^{-1}$ at 25 °C in CH$_2$Cl$_2$.

{(TPP)Mn$^{II}$ (imid)M$^{II}$ (imidH)DAP}$(BF_4)_2$.CH$_2$Cl$_2$ with M$'=\text{Zn}$ (Compound 9) and Cu (Compound 10): Since these derivatives of [Mn$^{II}$(TPP)] complexes are very air sensitive, all manipulations were performed in an inert atmosphere. Compounds 9 and 10 were prepared by first combining [Mn$^{II}$(TPP)] (0.12 g; 0.18 mmol) and [M$'$$^{II}$(imidH)$_2$DAP]$(BF_4)_2$ (0.13 g; 0.21 mmol for M$'=\text{Zn}$ and 0.132 g; 0.21 mmol for M$'=\text{Cu}$) in a 1:(1.2) molar ratio in 150 ml of a dry CH$_2$Cl$_2$/CH$_3$CN (90/10) solvent mixture. The solution was refluxed for 6 hours in the presence of proton sponge (0.04 g; 0.21 mmol) or t-BuO$^-$K$^+$ (0.02 g; 0.21 mmol) and then reduced to dryness under vacuum. The resulting solid was washed with water, and then with hexane, and recrystallized from CH$_2$Cl$_2$/hexane to give a dark
green solid which was then dried at room temperature for 6 hours in vacuo.

**Compound 9: Anal. Calcd.** For MnZnC\(_{63}H_{50}N_{11}\)BF\(_4\)·CH\(_2\)Cl\(_2\): C, 61.34%; H, 4.18%; N, 12.9%; Mn, 4.38%; Zn, 5.22%. **Found:** C, 60.98%; H, 4.05%; N, 12.43%; Mn, 4.43%; Zn, 5.15%. \(\mu_{\text{eff}}\) (solid, RT) = 5.78 \(\mu_B\). \(\lambda_{\text{max}}\) (CH\(_2\)Cl\(_2\)): 616 nm (\(\varepsilon=10.3\times10^3\)), 583 nm (\(\varepsilon=9.6\times10^3\)), 532 nm (\(\varepsilon=7.6\times10^3\)), 458 nm (\(\varepsilon=6.2\times10^4\)), 403 nm (\(\varepsilon=3.2\times10^4\)), 381 nm (\(\varepsilon=4.34\times10^4\)), 347 nm (\(\varepsilon=1.0\times10^4\)). \(\Delta_c\) = 44 \(\mu\)mhos cm.\(^{-1}\) mole\(^{-1}\) at 25 °C in CH\(_2\)Cl\(_2\).

**Compound 10: Anal. Calcd.** For MnCuC\(_{63}H_{50}N_{11}\)BF\(_4\)·CH\(_2\)Cl\(_2\): C, 61.43%; H, 4.19%; N, 12.31%; Mn, 4.39%; Cu, 5.08%. **Found:** C, 60.84%; H, 4.06%; N, 12.19%; Mn, 4.20%; Cu, 4.98%. \(\mu_{\text{eff}}\) (solid, RT) = 5.11 \(\mu_B\). \(\lambda_{\text{max}}\) (CH\(_2\)Cl\(_2\)): 618 nm (\(\varepsilon=1.0\times10^4\)), 579 nm (\(\varepsilon=8.8\times10^3\)), 530 nm (\(\varepsilon=5.7\times10^3\)), 460 nm (\(\varepsilon=8.5\times10^4\)), 397 nm (6.0\times10^4), 380 nm (\(\varepsilon=6.64\times10^4\)), 348 nm (\(\varepsilon=6.2\times10^4\)). \(\Delta_c\) = 40 \(\mu\)mhos cm.\(^{-1}\) mole\(^{-1}\) at 25 °C in CH\(_2\)Cl\(_2\).

\[([\text{TPP}]\text{Mn}^{\text{II}}(\text{imid})\text{M}^{\text{II}}(\text{imidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\cdot\text{CH}_2\text{Cl}_2\text{ with }\text{M}^{\text{I}}=\text{Zn}\]

(Compound 11) and Cu (Compound 12): The compounds were prepared using [Zn\(^{\text{II}}\)(OSO\(_2\)CF\(_3\))\(_2\)] or [Cu\(^{\text{II}}\)(OSO\(_2\)CF\(_3\))\(_2\)] instead of the BF\(_4^-\) salts and treated identically to the analogous (TPP)Mn\(^{\text{II}}\)(imid)Mn\(^{\text{II}}\) (imidH)DAP][BF\(_4^-\)] compounds above.

**Compound 11: Anal. Calcd.** For MnZnC\(_{64}H_{50}N_{11}\)SO\(_3\)F\(_3\)·CH\(_2\)Cl\(_2\): C, 59.35%; H, 3.98%; N, 11.71%; Mn, 4.18%; Zn, 4.97%. **Found:** C,
60.15%; H, 3.91%; N, 12.11%; Mn, 4.08%; Zn, 4.79%. $\mu_{\text{eff}}$ (solid, RT) = 5.94 $\mu_B$. $\lambda_{\text{max}}$ (CH$_2$Cl$_2$): 614 nm ($\varepsilon=12.25\times10^3$), 576 nm ($\varepsilon=11.0\times10^3$), 526 nm ($\varepsilon=6.02\times10^3$), 458 nm ($\varepsilon=4.93\times10^4$), 402 nm ($\varepsilon=5.27\times10^4$), 369 nm ($\varepsilon=6.62\times10^4$), 345 nm ($\varepsilon=5.74\times10^4$), 265 nm ($\varepsilon=3.50\times10^4$). $\Delta_c = 43$ $\mu$hos cm.$^{-1}$ mole.$^{-1}$ at 25 $^\circ$C in CH$_2$Cl$_2$.

Compound 12: Anal. Calcd. For MnCuC$_{64}$H$_{50}$N$_{11}$S$_3$F$_3$·CH$_2$Cl$_2$: C, 59.43%; H, 3.99%; N, 11.73%; Mn, 4.18%; Cu, 4.84%. Found: C, 59.70%; H, 3.69%; N, 12.16%; Mn, 4.20%; Cu, 4.68%. $\mu_{\text{eff}}$ (solid, RT) = 5.14 $\mu_B$. $\lambda_{\text{max}}$ (CH$_2$Cl$_2$): 616 nm ($\varepsilon=9.91\times10^3$), 580 nm ($\varepsilon=8.9\times10^4$), 5.28 nm ($6.91\times10^3$), 459 nm ($8.75\times10^4$), 402 nm ($8.50\times10^4$), 378 nm ($9.65\times10^4$), 349 nm ($9.0\times10^4$), 287 nm ($5.91\times10^3$). $\Delta_c = 45$ $\mu$hos cm.$^{-1}$ mole.$^{-1}$ at 25 $^\circ$C in CH$_2$Cl$_2$. 
RESULTS AND DISCUSSION

The Binuclear Iron(III) System

The synthetic scheme for the binuclear \( \mu \)-imidazolato Fe(III) compounds synthesized and studied in this work is shown in Figure 1. The new species, \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^\text{III}(2\text{-meimid})\text{M}^\text{II}(2\text{-meimidH})\text{DAP}]^+ \text{OSO}_2\text{CF}_3^-\) with \( \text{M}^' = \text{Zn} \) (compound 1) and \( \text{Cu} \) (compound 2) and \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^\text{III}(\text{imid})\text{M}^\text{II}(\text{imidH})\text{DAP}] \text{OSO}_2\text{CF}_3^-\) with \( \text{M}^' = \text{Zn} \) (compound 3) and \( \text{Cu} \) (compound 4) have been prepared by the general method of Dessens, et al., [13]. In general, the synthesis consisted of the reaction of 1 mole equivalent of \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^\text{III}]\) and 1,2 mole equivalents of \([\text{M}^\text{II}(2\text{-meimidH})\text{DAP}] \text{OSO}_2\text{CF}_3^-\) or \([\text{M}^\text{II}(\text{imidH})\text{DAP}] \text{OSO}_2\text{CF}_3^-\) ( \( \text{M}^' = \text{Cu}, \text{Zn} \) ) in the presence of 1 mole equivalent of a base such as \([1,2\text{-bis-(dimethylamino)}\text{napthalene}] \text{(proton sponge)}\) or \(\text{t-BuO}^-\text{K}^+\) in a dry \(\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN} \) (90/10) solvent mixture.

Similar to the \( \mu \)-imidazolato heterobinuclear complexes, \([(\text{X})(\text{TPP})\text{Fe}^\text{III}(\text{imid})\text{M}^\text{II}(\text{imidH})\text{DAP}] \text{BF}_4^-\) with \( \text{X} = \text{Cl}^- \) and \( \text{OSO}_2\text{CF}_3^- \), previously reported by Dessens, et al., [13] the new \( \mu \)-imidazolato heterobinuclear complexes in this study also exhibited rather complicated properties in solution due mainly to an equilibrium process involving (binuclear) \(\rightleftharpoons\) (porphyrin fragment + copper fragment). For this reason, the Fe(III) compounds of this work could not be purified by recrystallization or studied in the solution state.

The metalloporphyrin fragment of compounds 1-4 was obtained by metathesis of \([\text{ClFe}^\text{III}(\text{TPP})]\) with \([\text{Ag(OSO}_2\text{CF}_3)]\) in a dry, non-coordinating solvent according to the method of Reed, et al., [28].
\([\text{ClFe}^{\text{III}}(\text{TPP})]\) was prepared by the metallation of tetraphenylporphyrin (TPPH$_2$) with anhydrous FeCl$_2$ according to Alder, et al., [24].

The pentacoordinated M' (II) fragments (M' = Cu, Zn) of compounds 3 and 4 were prepared as previously described by Dessens, et al., [13]. Unlike compounds 3 and 4, the pentacoordinated M' (II) fragments, \([M'^{\text{II}}(2\text{-meimidH})_2\text{DAP}](\text{OSO}_2\text{CF}_3)_2\), of compounds 1 and 2 were derived from the Schiff base condensation of free base 2-methylhistamine (instead of free base histamine) with 2,6-diacetylpyridine, as shown in Figure 2.

2-methylhistamine was prepared by a modified procedure of Dziuron and Schunack [20]. As shown in the first step of Figure 3, the reaction of ethyl acetimidate hydrochloride with 1,3-dihydroxyacetone in liquid NH$_3$ at ~ 60 °C and ~ 400 psi gave the 2-methyl-4-hydroxymethylimidazol as a free base. This carbinol was then converted to a hydrochloride salt by reaction with HCl in EtOH. Next the hydrochloride salt of the carbinol was reacted with SOCl$_2$ to form 2-methyl-4-chloromethylimidazol. Upon the reaction of this chloro compound with NaCN in dry DMSO, 2-methyl-4-cyanomethylimidazol was obtained. Subsequently, 2-methylhistamine was obtained in free base form by the hydrogenation of the nitrile compound with Raney nickel and hydrogen at elevated pressure and temperature. The carbinol, chloro, nitrile, and 2-methylhistamine compounds were characterized by IR spectroscopy (Figure 4), $^1$H nmr spectroscopy (Figure 5) and by melting point determination for the solids (see Experimental Section).
Figure 2

Synthetic Scheme for

\[
[M'\text{II}(2\text{-meimidH})_2\text{DAP}]\text{(OSO}_2\text{CF}_3)_2
\]

with $M' = \text{Cu, Zn}$
DAP + 2-methylhistamine

\[ \text{CH}_3\text{OH} \]

\[ [(2\text{-meimidH})_2\text{DAP}] \]

\[ \text{CH}_3\text{OH} \quad \text{M}^{III}(\text{OSO}_2\text{CF}_3)_2 \]

\[ [\text{M}^{III}(2\text{-meimidH})_2\text{DAP}](\text{OSO}_2\text{CF}_3)_2 \]

\[ [\text{M'} = \text{Cu or Zn}] \]
Figure 3

Synthetic Scheme for the Preparation of 2-methylhistamine
HOCH₂COCH₂OH + NH₃ → liq. NH₃

CH₃CO Et.HCl

60°C/400 p.s.i. / 6h

CH₂-OH

HCl/EtOH

HCl.HN=N

CH₂-CI

SOCl₂ → 100°C/45 min.

NaCN

DMSO

40°C/1h

HCl.HN=N

CH₂-OH

HCl.HN=N

CH₂-CN

H₂-Ni → 150°C/2000 p.s.i. / 3h

EtOH/NH₃

CH₂-CH₂-NH₂
**Figure 4**

Infrared Spectrum as a Nujol Mull of:

a) 2-methyl-4-hydroxymethylimidazol·HCl
b) 2-methyl-4-chloromethylimidazol·HCl
c) 2-methyl-4-cyanomethylimidazol·HCl
d) 2-methylhistamine
Figure 5

\[^1H\ mM\]n spectrum of:

a) 2-methyl-4-hydroxymethylimidazol·HCl (D\(_2\)O (\(*\))
   with external TMS)

b) 2-methyl-4-chloromethylimidazol·HCl (CD\(_3\)CN (\(*\))
   with external TMS)

c) 2-methyl-4-cyanomethylimidazol·HCl (D\(_2\)O (\(*\))
   with external TMS)

d) 2-methylhistamine (CDCl\(_3\) (\(*\)) with TMS)
Infrared Spectroscopy. Infrared spectra of the mononuclear and binuclear \( \mu \)-imidazolato Fe(III) compounds are shown in Figure 6. For \([(\text{OSO}_2\text{CF}_3)\text{Fe}^{\text{III}}(\text{TPP})]\), bands at 1340(s), 1240(s), 1205(s), and 630(s) cm\(^{-1}\) are observed in the IR spectrum as previously reported by Reed, et al., [25]. The spectra of the binuclear compounds \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{M}^{\text{II}}(\text{imidH})\text{DAP}(\text{OSO}_2\text{CF}_3)]\) appear similar to those of the analogous \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{M}^{\text{II}}(\text{imidH})\text{DAP}(\text{BF}_4^-)]\) compounds which indicates that the \([\text{M}^{\text{II}}(2\text{-meimidH})\text{DAP}]^{2+}\) and \([\text{M}^{\text{II}}(\text{imidH})_2\text{DAP}]^{2+}\) fragments react in a similar nature to form the binuclear compounds. Furthermore, it also shows that the 2-methyl substituent of the imidazole ring, while introducing a more sterically encumbering \(\text{M}^{\text{II}}\) fragment, does not prevent the formation of \(\mu\)-imidazolato binuclear complexes. Similar to the infrared spectra for the \(\mu\)-imidazolato binuclear complexes of Dessens, et al., [13] the infrared spectra of compounds 1-4 in the 2000-600 cm\(^{-1}\) region, contain absorption peaks corresponding to the appropriate functional groups which are present in the mononuclear compounds and peaks characteristic of the porphyrin fragments, as is shown in Figure 6. In particular, a C=N imine stretching frequency is observed for all the compounds around 1580-1600 cm\(^{-1}\), and two broad peaks centered around 1260 and 1160 cm\(^{-1}\) are due to the presence of the \(\text{OSO}_2\text{CF}_3^-\) anion [25]. The characteristic peaks of \(\text{OSO}_2\text{CF}_3^-\) as an axial ligand are also observed for all the binuclear complexes at ca. 1340(s), 1240(s), 1205(s) and 630 cm\(^{-1}\) [25].
Figure 6

Infrared Spectrum as a KBr Disk (a,b) or Nujol Mull (c-h) of:

a) \([\text{ClFe}^{\text{III}}(\text{TPP})]\)

b) \([\text{(OSO}_2\text{CF}_3\text{)}\text{Fe}^{\text{III}}(\text{TPP})]\)

c) \([\text{Zn}^{\text{II}}(2\text{-meimidH})_2\text{DAP}(\text{OSO}_2\text{CF}_3)_2]\)

d) \([\text{Cu}^{\text{II}}(2\text{-meimidH})_2\text{DAP}(\text{OSO}_2\text{CF}_3)_2]\)

e) \([\text{(OSO}_2\text{CF}_3\text{)}(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{Zn}^{\text{II}}(2\text{-meimidH})\text{DAP}(\text{OSO}_2\text{CF}_3)_2]\) (1)

f) \([\text{(OSO}_2\text{CF}_3\text{)}(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{Cu}^{\text{II}}(2\text{-meimidH})\text{DAP}(\text{OSO}_2\text{CF}_3)_2]\) (2)

g) \([\text{(OSO}_2\text{CF}_3\text{)}(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP}(\text{OSO}_2\text{CF}_3)_2]\) (3)

h) \([\text{(OSO}_2\text{CF}_3\text{)}(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{Cu}^{\text{II}}(\text{imidH})\text{DAP}(\text{OSO}_2\text{CF}_3)_2]\) (4)
C. \([Zn^{II}(2\text{-meimidH})_2DAP](OSO_2CF_3)_2\)

D. \([Cu^{II}(2\text{-meimidH})_2DAP](OSO_2CF_3)_2\)
e. \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{Zn}^{\text{II}}(2\text{-meimidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\]\)

f. \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{Cu}^{\text{II}}(2\text{-meimidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\]\)
g. \([(\text{OSO}_2\text{CF}_3)\text{(TPP) Fe}^{\text{III}}\text{(imid)}\text{Zn}^{\text{II}}\text{(imidH)}\text{DAP})\text{(OSO}_2\text{CF}_3]\)

h. \([(\text{OSO}_2\text{CF}_3)\text{(TPP) Fe}^{\text{III}}\text{(imid)}\text{Cu}^{\text{II}}\text{(imidH)}\text{DAP})\text{(OSO}_2\text{CF}_3]\)
Magnetochemistry. Variable-temperature (ca. 20–300 K) magnetic susceptibility data for the μ-imidazolato binuclear Fe(III) compounds 1–4 are displayed as plots of \( \mu_{\text{eff}} \) (\( \mu_B \)) vs. \( T \) in Figure 7 and \( \chi_M \) vs. \( T^{-1} \) in Figure 8. The actual data are found in Table I. Magnetic data for the mononuclear parent complexes have also obtained at room temperature. The [ClFe\(^{III}\)(TPP)] compound exhibits a room temperature magnetic moment of 5.7 \( \mu_B \) which is typical of high-spin (\( S = 5/2 \)) Fe(III). The [(OSO\(_2\)CF\(_3\))Fe\(^{III}\)(TPP)] complex exhibits a more unusual magnetic moment of 5.15 \( \mu_B \) which has been attributed to an intermediate admixed spin state by several investigators [25,29,30]. [Cu\(^{II}\)(2-imimidH)\(_2\)DAP](OSO\(_2\)CF\(_3\))\(_2\) and [Cu\(^{II}\)(imidH)\(_2\)DAP](OSO\(_2\)CF\(_3\))\(_2\) have magnetic moments of 2.0 \( \mu_B \) and 2.1 \( \mu_B \) respectively; these values are typical values for (\( S = 1/2 \)) Cu(II) complexes and in good agreement with the results reported previously by Simmon, et al., [23] for [Cu\(^{II}\)(imid)\(_2\)DAP](X)\(_2\) with X = ClO\(_4\)\(^-\) and BF\(_4\)\(^-\).

The room temperature magnetic moment for compound 1, containing the [(OSO\(_2\)CF\(_3\))\(_2\)(TPP)Fe\(^{III}\)(2-imimid)Zn\(^{II}\)(2-imimidH)DAP]\(^+\) core, is 2.31 \( \mu_B \). This value is in good agreement with the 2.2–2.4 \( \mu_B \) range normally found for low-spin Fe(III) having an orbital contribution to the 1.73 \( \mu_B \) spin-only moment. The value is clearly indicative of a low-spin (\( S = 1/2 \)) ground state for 1, and this interpretation is also supported by the epr results reported below. A low-spin ground state for 1 is not necessarily unexpected since several other hexacoordinate, low-spin Fe(III) porphyrin compounds have been reported by others [25,31]. In addition, Dessens, et al., [13] recently found that the Fe(III)
Figure 7

µ_{eff} (µ_B) vs. Temperature (K) Plots of:

a) [(OSO₂CF₃)(TPP)Fe^{III}(2-meimid)Zn^{II}(2-meimidH)DAP](OSO₂CF₃) (1)
b) [(OSO₂CF₃)(TPP)Fe^{III}(2-meimidCu^{II}(2-meimidH)DAP](OSO₂CF₃) (2)
c) [(OSO₂CF₃)(TPP)Fe^{III}(imid)Zn^{II}(imidH)DAP](OSO₂CF₃) (3)
d) [(OSO₂CF₃)(TPP)Fe^{III}(imidCu^{II}(imidH)DAP](OSO₂CF₃) (4)
a. [Fe$^{III}$-\(2\text{-meimid}\)-Zn$^{II}$](OSO$_2$CF$_3$)
\[ L = \text{OSO}_2\text{CF}_3^- \]

\[
\mu_{\text{eff}} \text{, } \mu_B
\]

\[
\begin{array}{c}
\text{TEMPERATURE (K)}
\end{array}
\]

b. [Fe$^{III}$-\(2\text{-meimid}\)-Cu$^{II}$](OSO$_2$CF$_3$)
\[ L = \text{OSO}_2\text{CF}_3^- \]

\[
\mu_{\text{eff}} \text{, } \mu_B
\]

\[
\begin{array}{c}
\text{TEMPERATURE (K)}
\end{array}
\]
C. \([\text{Fe}^{III}-(\text{imid})-\text{Zn}^{II}]\left(\text{OSO}_2\text{CF}_3\right)\)

\[L = \text{OSO}_2\text{CF}_3^-\]

\[
\mu_{\text{eff}}, \mu_B
\]

\[
\text{TEMPERATURE (K)}
\]

d. \([\text{Fe}^{III}-(\text{imid})-\text{Cu}^{II}]\left(\text{OSO}_2\text{CF}_3\right)\)

\[L = \text{OSO}_2\text{CF}_3^-\]

\[
\mu_{\text{eff}}, \mu_B
\]

\[
\text{TEMPERATURE (K)}
\]
Figure 8

$X'_M \ vs. \ T^{-1} \ (K^{-1})$ Plots of:

a) $[(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{III}(2\text{-meimid})\text{Zn}^{II}(2\text{-meimidH})\text{DAP}]\text{(OSO}_2\text{CF}_3)$ (1)
b) $[(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{III}(2\text{-meimid})\text{Cu}^{II}(2\text{-meimidH})\text{DAP}]\text{(OSO}_2\text{CF}_3)$ (2)
c) $[(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{III}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}]\text{(OSO}_2\text{CF}_3)$ (3)
d) $[(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{III}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}]\text{(OSO}_2\text{CF}_3)$ (4)
\( [\text{Fe}^{III}-(2\text{-imid})-\text{Zn}^{II}] (\text{OSO}_2\text{CF}_3) \)

\[ L = \text{OSO}_2\text{CF}_3^- \]

Slope = 0.40
Intercept = 1.34E-3

\( [\text{Fe}^{III}-(2\text{-imid})-\text{Cu}^{II}] (\text{OSO}_2\text{CF}_3) \)

\[ L = \text{OSO}_2\text{CF}_3^- \]

Slope = 0.63
Intercept = 2.67E-3
C. $[\text{LFe}^{III}-(\text{imid})-\text{Zn}^{II}] (\text{OSO}_2\text{CF}_3)$

$L = \text{OSO}_2\text{CF}_3^-$

Slope = 1.81
Intercept = 3.01E-3

\[ \chi_M, \text{emu-cgs mole}^{-1} \]

\[ 1/T \ (K^{-1}) \]

---

d. $[\text{LFe}^{III}-(\text{imid})-\text{Cu}^{II}] (\text{OSO}_2\text{CF}_3)$

$L = \text{OSO}_2\text{CF}_3^-$

Slope = 0.87
Intercept = 2.76E-3

\[ \chi_M, \text{emu-cgs mole}^{-1} \]

\[ 1/T \ (K^{-1}) \]
TABLE Ia

Magnetic Susceptibility Data
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*a. in cgs-emu mole^{-1}
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a. in cgs-emu mole\(^{-1}\)
porphyrin center in the related binuclear compound, \([\text{Cl(TPP)}\text{Fe}^{\text{III}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP}]^+\) is also low-spin \((S = 1/2)\) \text{Fe(III)} with \(\mu_{\text{eff}}(\text{RT}) = 2.67\ \mu_\text{B}\). Thus, contrary to the hope and expectation that a 2-methyl substituent on the \(\mu\)-imidazolato ring would be sterically demanding enough to promote a high-spin \((S = 5/2)\) \text{Fe(III)} porphyrin center, such is clearly not the case, even though a previous study had shown the ground state of pentacoordinate \([\text{Fe}^{\text{III}}(2\text{-meimidH})(\text{TPP})]\) is high-spin [32]. The room temperature magnetic moment of compound 2 is 3.15 \(\mu_\text{B}\), a value which gradually decreases to 2.35 \(\mu_\text{B}\) by 20 K. The \(\mu_{\text{eff}}\) vs. T curve for compound 2 parallels that of compound 1, but with the additional paramagnetism expected for an \((S = 1/2)\) \text{Cu(II)} center having \(\mu_{\text{eff}} \approx 2.0\ \mu_\text{B}\). From a magnetochemical point of view, by comparing the full-temperature magnetochemical data for 1 and 2, it is reasonable to conclude that the iron center of compound 2 is also low-spin \((S = 1/2)\) \text{Fe(III)}. The epr studies reported below also support this assignment.

For compound 3, \([\text{OSO}_2\text{CF}_3(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)\), the room temperature magnetic moment is 4.51 \(\mu_\text{B}\) (gradually decreasing to 3.87 \(\mu_\text{B}\) by \(~20\ K\)), which is a somewhat unusual value for a simple \text{Fe(III)} porphyrin compound. However, this value is only slightly higher than the 3.36 \(\mu_\text{B}\) value reported by Dessens, \textit{et al.}, [13] for the analogues binuclear complex, 
\([\text{OSO}_2\text{CF}_3(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP}](\text{BF}_4^-)\), as a BF\(_4^-\) rather than as an OSO\(_2\)CF\(_3^-\) salt. Regardless of this unusual \(\mu_{\text{eff}}\) value for the BF\(_4^-\) salt, Dessens, \textit{et al.}, could rationalize the result by assuming the
presence of a mixture of two different Fe(III) spin states \( S = 1/2; S = 5/2 \) in the polycrystalline sample, an interpretation which was also supported by epr measurements on a polycrystalline sample. Compound 4 is analogous to 3 with \( M' = \text{Cu}^{II} \) rather than \( \text{Zn}^{II} \), and as such, there is an additional paramagnetic \( (S = 1/2) \text{Cu}^{II} \) center present. However, 4 possesses a room temperature magnetic moment of only 3.78 \( \mu_B \), which gradually decreases to 2.73 \( \mu_B \) by \( \sim 20 \, \text{K} \). This room temperature \( \mu_{\text{eff}} \) value is only slightly greater than that of the \( \text{BF}_4^- \) salt reported by Dessens, but more importantly, it is substantially lower than that of 1 where \( M' = \text{Zn}^{II} \). This result is also true throughout the entire temperature range \( (\text{ca.} 20-300 \, \text{K}) \) of these studies. Thus, the variable-temperature magnetic properties of compounds 3 and 4 closely parallel the \( \mu \)-imidazolato cation species reported earlier by Dessens, et al., with the only difference between the compounds in this study \( (X = \text{OSO}_2\text{CF}_3^-) \) and those of Dessens \( (X = \text{BF}_4^-) \) being the counter ions. The slight differences in the magnetic data can, therefore, be attributed to a small anion effect.

**Epr Spectroscopy.** The epr spectra for compounds 1-4 in the polycrystalline state at 10 K are shown in Figure 9. The compounds could only be studied in the solid state because of their instability in solution (vide supra). The epr parameters for each compound are documented in Table II. Compound 1 exhibits a well-defined, broad multiple resonance in the \( g \sim 2 \) region of the spectrum which is characteristic of low-spin \( (S = 1/2) \) Fe(III) porphyrin centers in
Figure 9

Epr Spectrum at 10 K as a Polycrystalline Solid

(~ 1.0% Fe(III) in Ammonium Sulfate) for:

a) \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{Zn}^{\text{II}}(2\text{-meimidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\] (1)

b) \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(2\text{-meimid})\text{Cu}^{\text{II}}(2\text{-meimidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\] (2)

c) \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\] (3)

d) \([(\text{OSO}_2\text{CF}_3)(\text{TPP})\text{Fe}^{\text{III}}(\text{imid})\text{Cu}^{\text{II}}(\text{imidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\] (4) (x10 of a-c)
a. $[\text{Fe}^{III}(2\text{-meimid})\text{Zn}^{II}]^+$

b. $[\text{Fe}^{III}(2\text{-meimid})\text{Cu}^{II}]^+$

$T = 10K$

\[
\begin{align*}
\text{INTENSITY} & \\
\text{MAGNETIC FIELD, G} & \\
0 & 1000 & 2000 & 3000 & 4000 \\
\downarrow & \downarrow & \downarrow & \downarrow & \\
g = 6 & g = 2 & \\
\end{align*}
\]
TABLE II
Epr Parameters at 10 K for the Fe(III) Compounds
as Polycrystalline Solids (~ 1% Fe(III) in Ammonium Sulfate)

<table>
<thead>
<tr>
<th>Compound</th>
<th>g factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
</tr>
<tr>
<td>[Fe$^{III}$ (2-meimid)Zn$^{II}$] (OSO$_2$CF$_3$) (1)</td>
<td>$g_1 = 5.89$</td>
</tr>
<tr>
<td></td>
<td>$g_2 = 1.99$ ($g_{zz} = 2.70$)</td>
</tr>
<tr>
<td></td>
<td>($g_{yy} = 2.27$)</td>
</tr>
<tr>
<td></td>
<td>($g_{xx} = 1.69$)</td>
</tr>
<tr>
<td>[Fe$^{III}$ (2-meimid)Cu$^{II}$] (OSO$_2$CF$_3$) (2)</td>
<td>$g_1 = 5.89$</td>
</tr>
<tr>
<td></td>
<td>$g_2 ~ 2$</td>
</tr>
<tr>
<td>[Fe$^{III}$ (imid)Zn$^{II}$] (OSO$_2$CF$_3$) (3)</td>
<td>$g_1 = 5.89$</td>
</tr>
<tr>
<td></td>
<td>$g_2 ~ 2$</td>
</tr>
<tr>
<td>[Fe$^{III}$ (imid)Cu$^{II}$] (OSO$_2$CF$_3$) (4)</td>
<td>$g_1 ~ 6$</td>
</tr>
</tbody>
</table>
tetragonally distorted fields [24]; the small \( g \sim 6 \) resonance can be attributed to a slight \( (S = 5/2) \) impurity. Compound 2 displays a similar but significantly broadened, spectral pattern in the \( g \sim 2 \) region, with an additional semi-superimposed component centered around \( g = 2 \) which can be assigned to the \((S = 1/2) \) Cu(II) center; this spectrum also displays a small \( g \sim 6 \) signal which is likely due to an \((S = 5/2)\) impurity. The \( g \) factors and spectral patterns attributed to low-spin Fe(III) in 1 and 2 are similar to those observed for the low-spin Fe(III) \( \mu \)-imidazolato binuclear complexes, 

\[[\text{Cl(TPP)}\text{Fe}^{III}_{\text{imid}}\text{Ni}^{II}_{\text{imidH}}\text{DAP}(\text{BF}_4)]\], reported by Dessens et al. [13]. The \( g_{zz}, g_{yy}, \) and \( g_{xx} \) values for 1 (Table II) are also in excellent agreement with those reported for a low-spin [(protoporphyrinato IX)iron(III)] complex with one axially coordinated imidazole anion [33]. The \( g \) factors for this species (\( g_{zz} = 2.78, g_{yy} = 2.26, g_{xx} = 1.72 \)) are quite different from those of [(protoporphyrinato IX)iron(III)] with one axially coordinated, neutral imidazole ligand (\( g_{zz} = 3.02, g_{yy} = 2.24, \) and \( g_{xx} = 1.51 \)), thereby indicating that the imidazolato anion in 1 has significantly altered the epr signal in a characteristic manner. In addition to epr signals attributable to \((S = 1/2) \) Fe(III), compounds 1 and 2 also exhibit a weak resonances around \( g = 6 \) which is likely due to a small impurity with an \((S = 5/2) \) spin state. From relative integration of the \( g = 2 \) and \( g = 6 \) signals, the \((S = 1/2) \) state in 1 and 2 is present in \( \geq 90 \% \) abundance. Regardless of whether the \( g = 6 \) signals in question arise from small \((S = 5/2) \) impurity or from an electronic structure for Fe(III) that is
somewhat more complex than a "pure" (S = 1/2) ground state, the basic conclusion is unchanged: antiferromagnetic exchange through imidazolate in 2 is vanishingly small (−J ~ 0 cm⁻¹) where the Fe(III) center is essentially low-spin. For −J > 0, no epr spectrum would be observed since, in this case, the ground state would be S = 0 and the excited state would be S = 1. This is the same as concluded by Dessens, et al., for [Cl(TPP)Fe^{III}(imid)Cu^{II}(imidH)DAP](BF₄) where Fe(III) was also essentially low-spin.

In contrast to the vanishingly small exchange interaction found in compound 2, magnetic and epr data on 4 indicate quite different behavior concerning the ability of imidazolate to foster magnetic coupling between Fe(III) and Cu(II). From the epr spectra of [(OSO₂CF₃)(TPP)Fe^{III}(imid)M^{II} (imidH)DAP](BF₄) (M' = Cu, Zn), Dessens, et al., [13] concluded that the sample in the solid state contained a spin-mixture of (S = 5/2; ca. 20 %) and (S = 1/2; ca. 80 %) states. This interpretation can also be extended to interpret the epr spectrum of the analogue, compound 3, in this work. For compound 3, changing the counter-anion to OSO₂CF₃⁻ (from BF₄⁻) could, of course, somewhat alter the mixture of spin states, which might explain the slightly greater magnetic moment data for 3. Assuming 5.9 μₘ and 2.0 μₘ as limiting value for the (S = 5/2) and (S = 1/2) iron(III) spin states in 1, a 65 % (S = 5/2) and 35% (S = 1/2) distribution would explain the observed 4.51 μₘ value at room temperature.
To explain the epr "quiet" behavior of \((\text{OSO}_2 \text{CF}_3)(\text{TPP})\text{Fe}^\text{III}(\text{imid})\text{Cu}^\text{II}(\text{imidH})\text{DAP}(\text{BF}_4)\), Dessens, et al., [13] proposed that a strong antiferromagnetic coupling interaction across imidazolate between \(S = 1/2\) Cu(II) and a spin-mixture of Fe(III) [80% \((S = 5/2); 20\% \((S = 1/2)]\) gives rise to a resultant [80% \((S = 0); 20\% \((S = 2)]\) spin-mixture which would be epr silent. In fact, the compound did display very weak epr signals in both the \(g = 6\) and \(g = 2\) spectral regions, but these signal accounted for < 1% of Fe(III) and Cu(II) present when integrated against signal intensities from samples of \((\text{OSO}_2 \text{CF}_3)\text{Fe}^\text{III}(\text{TPP})\) and \([\text{Cu}^\text{II}(\text{imidH})]_2\text{DAP}(\text{BF}_4)_2\) under the same conditions. In this study, \(\{(\text{OSO}_2 \text{CF}_3)(\text{TPP})\text{Fe}^\text{III}(\text{imid})\text{Cu}^\text{II}(\text{imidH})\text{DAP}\} (\text{OSO}_2 \text{CF}_3)\) exhibits nearly identical epr and magnetochemical properties to its analogous BF\(_4^–\) salt, including the very weak epr spectrum as shown in Figure 9d (10x). From this point of view, it is reasonable to conclude that the physical properties of \((\text{OSO}_2 \text{CF}_3)(\text{TPP})\text{Fe}^\text{III}(\text{imid})\text{Cu}^\text{II}(\text{imidH})\text{DAP}\) are similar as BF\(_4^–\) or OSO\(_2\)CF\(_3^–\) salts. Furthermore, by comparing the magnetochemical results of this study to the previous experimental results reported by Dessens, et al., it is relevant to conclude that \(\{(\text{OSO}_2 \text{CF}_3)(\text{TPP})\text{Fe}^\text{III}(\text{imid})\text{Cu}^\text{II}(\text{imidH})\text{DAP}\} (\text{OSO}_2 \text{CF}_3)\) also exhibits a strong antiferromagnetic coupling interaction between \(S = 1/2\) Cu(II) and a spin-mixture \((S = 5/2); S = 1/2\) of Fe(III) centers to produce a resultant \([(S = 0); S = 2)]\) spin-coupled ground state with \(-J (\text{Cu}^\text{II}-\text{Fe}^\text{III}) \gtrsim 200 \text{ cm}^{-1}\).
The Binuclear Cobalt(II) System

A synthetic scheme for the four cobalt compounds, compounds 5-8, is shown in Figure 10. In general, they were prepared by refluxing a mixture of 1 mole equivalent of [Co$^{II}$ (TPP)] and 1.2 mole equivalents of $[M''^{II} (imidH)_{2} DAP](X)_{2}$ ($M''$ = Cu, Zn and $X$ = BF$_4^{-}$, OSO$_2$CF$_3^{-}$) for 6 hours in the presence of 1 mole equivalent of a base such as proton sponge or t-BuO$^-K^+$ in a dry CH$_2$Cl$_2$/CH$_3$CN (90/10) solvent mixture. The reaction does not occur in the absence of base, and the zinc(II) and copper(II) complexes, $[M''^{II} (imidH)(Py)DAP]^{2+}$ (with one terminal pyridine group replacing an imidazole moiety) [13], also forms an analogous $[(TPP)M''^{II}(imid)M''^{II}(Py)DAP]^+$ compound in the presence (but not in the absence) of base. The use of $[Cu^{II}(Py)_{2}DAP]^{2+}$ in the reaction procedure (two terminal pyridine groups) [13], produces no binuclear products.

These synthetic results, as well as the evidence indicated below by the spectroscopic and other studies, support the formation of the new heterobinuclear μ-imidazolato complexes shown in Figure 10. Compounds 5-8 possess satisfactory elemental analysis and the solution state conductivity of the compounds in CH$_2$Cl$_2$ are consistent with uni-univalent electrolyte behavior as required by the molecular formulation (see Experimental Section).

The metalloporphyrin fragment of the heterobinuclear complexes has been derived from well-known procedures. Four coordinate [Co$^{II}$ (TPP)] was prepared by the general method of Rothemund and Menotti [26]. The fine maroon crystals which formed were recrystallized by using an extraction thimble with diethyl ether. The product was further purified
Figure 10

Synthetic Scheme for the Preparation of [(TPP)M\textsuperscript{II} (imid)M\textsuperscript{II} (imidH)DAP](X)

with \( M = \text{Co, Mn} \); \( M' = \text{Cu, Zn} \); and \( X^- = \text{BF}_4^-; \text{OSO}_2\text{CF}_3^- \)
\[ \text{[M}^{II} \text{(TPP)]} \]
\[ M = \text{Co}^{II} \text{ or Mn}^{II} \]

\[ \text{CH}_2\text{Cl}_2 / \text{CH}_3\text{CN (10%)}; \]
\[ \text{PROTON SPONGE or } t\text{-buO}^-\text{K}^+; \]
\[ \text{REFLUX} \]

\[ \text{(X}^-) \]

- (5) \( M = \text{Co}, \quad M' = \text{Zn}, \quad X = \text{BF}_4^- \)
- (6) \( M = \text{Co}, \quad M' = \text{Cu}, \quad X = \text{BF}_4^- \)
- (7) \( M = \text{Co}, \quad M' = \text{Zn}, \quad X = \text{OSO}_2\text{CF}_3^- \)
- (8) \( M = \text{Co}, \quad M' = \text{Cu}, \quad X = \text{OSO}_2\text{CF}_3^- \)
- (9) \( M = \text{Mn}, \quad M' = \text{Zn}, \quad X = \text{BF}_4^- \)
- (10) \( M = \text{Mn}, \quad M' = \text{Cu}, \quad X = \text{BF}_4^- \)
- (11) \( M = \text{Mn}, \quad M' = \text{Zn}, \quad X = \text{OSO}_2\text{CF}_3^- \)
- (12) \( M = \text{Mn}, \quad M' = \text{Cu}, \quad X = \text{OSO}_2\text{CF}_3^- \)
on a silica gel column using CH₂Cl₂ as the eluent. At room temperature and above, the dioxygen adduct of [Co²⁺(TPP)L] (L = nitroeneous bases) is unstable [34], and therefore, the present binuclear Co(II) compounds could be conveniently manipulated on the open lab bench. A general procedure for preparing the pentacoordinate \( [M^{\text{II}} \text{(imidH)}_2 \text{DAP}]^{2+} \) complexes \((M' = \text{Zn, Cu})\) was previously described by Simmons, et al., [23]. The pentacoordinate ligand was prepared by a Schiff base condensation of 2,6-diacetylpyridine and free base histamine in refluxing methanolic solution. Subsequently, BF₄⁻ or OSO₃CF₃⁻ salts of Cu(II) or Zn(II) were added to the ligand solution. The solvent was then removed to obtain the solid products, and the products were further purified by recrystallization from a hot methanol/ether solvent system.

**Infrared Spectroscopy.** The infrared spectra of the Co(II) compounds obtained using nujol mulls, are shown in Figure 11. The spectrum of [Co²⁺(TPP)] is characterized by metal-sensitive bands at 1600, 1491, 1351, 1005, and 997 cm⁻¹, which are observed in the Figure 11a. This spectrum is typical of [Co²⁺(TPP)], as reported previously by Kincaid, et al., [35]. The spectra of compounds 5-8 as nujol mulls are also shown in Figure 11. The data indicate the successful formation of the binuclear complexes. For example, a C=N stretching frequency around 1580-1600 cm⁻¹ for the \([M^{\text{II}} \text{(imidH)}_2 \text{DAP}]^{2+}\) fragment is always observed. A broad B-F stretch centered around 1050 cm⁻¹ is observed for compound 5 and 6 which contain BF₄⁻ as the counter anion. These spectral results are similar to those obtained by Dessens, et al., [13] for
Figure 11

Infrared Spectrum as a Nujol Mull of:

a) $[\text{Co}^{II}(\text{TPP})]$

b) $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$ (5)

c) $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$ (6)

d) $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$ (7)

e) $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$ (8)
a. $[\text{Co}^{II}(\text{TPP})]$
b. $[(\text{TPP})\text{Co}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP}](\text{BF}_4^-)$

c. $[(\text{TPP})\text{Co}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}(\text{imidH})\text{DAP}](\text{BF}_4^-)$
d. \[(TPP)Co^{II}(imid)Zn^{II}(imidH)DAP](OSO_2CF_3)\]

e. \[((TPP)Co^{II}(imid)Cu^{II}(imidH)DAP](OSO_2CF_3)\]
[(OSO₂CF₃)(TPP)Fe³⁺(imid)M⁺⁺(imidH)DAP](BF₄⁻) complexes. In contrast to compounds 5 and 6, compounds 7 and 8, which contain only OSO₂CF₃⁻ as a counter anion, have infrared absorptions characteristic of OSO₂CF₃⁻ at 635 and 1225 cm⁻¹ [28].

Electronic Spectroscopy. The electronic spectra of the complexes have been obtained to further characterize the new heterobinuclear species. The solution state electronic spectra in CH₂Cl₂ for mononuclear [Co²⁺(TPP)], as well as for the binuclear compounds 5-8, are shown in Figure 12. Spectral parameters are listed in Table III.

Electronic spectra of metalloporphyrins are characterized by absorptions in the UV (soret bands) and visible regions of the spectra [36]. The soret transitions are electronically allowed and are usually an order of magnitude more intense than the visible bands, which acquired intensity from a vibronic coupling mechanism [37-40].

As seen in the Figure 12, the binuclear complexes 5-8 all exhibit similar electronic spectra, indicating a similar electronic environment for the cobalt. However, the five-coordinate binuclear complexes all exhibit a considerably different spectrum from that of the four-coordinate parent porphyrin compound [Co¹⁺(TPP)]. For [Co¹⁺(TPP)], the observed spectrum is shown in Figure 12a or b, with the visible region α band at 527 nm and the β or soret band at 410 nm [41]. For the new binuclear compounds, 5-8, these bands are red shifted by as much as
Figure 12

UV-Visible Electronic Absorption Spectrum for:

a) [Co$^{II}$ (TPP)] in CH$_2$Cl$_2$, [Cu$^{II}$ (imidH)$_2$DAP](BF$_4$) in CH$_3$CN, and [(TPP)Co$^{II}$ (imid)Cu$^{II}$ (imidH)DAP](BF$_4$)$_2$, (6)

b) [Co$^{II}$ (TPP)] in CH$_2$Cl$_2$

c) [(TPP)Co$^{II}$ (imid)Zn$^{II}$ (imidH)DAP](BF$_4$)$_2$, (5) in CH$_2$Cl$_2$

d) [(TPP)Co$^{II}$ (imid)Cu$^{II}$ (imidH)DAP](BF$_4$)$_2$, (6) in CH$_2$Cl$_2$

e) [(TPP)Co$^{II}$ (imid)Zn$^{II}$ (imidH)DAP](OSO$_2$CF$_3$)$_2$, (7) in CH$_2$Cl$_2$

f) [(TPP)Co$^{II}$ (imid)Cu$^{II}$ (imidH)DAP](OSO$_2$CF$_3$)$_2$, (8) in CH$_2$Cl$_2$
a. [Co$^9$TPP]
b. [Cu$^2$imidH$_2$DAP]$^{2+}$
c. δ

\[ \varepsilon \times 10^4, \text{M}^{-1}\text{cm}^{-1} \]

\[ \varepsilon \times 10^3, \text{M}^{-1}\text{cm}^{-1} \]

\( \lambda, \text{nm} \)

\[ \varepsilon \times 10^4, \text{M}^{-1}\text{cm}^{-1} \]

[b. [Co$^{II}$TPP]]

\( \lambda, \text{nm} \)
c. \([(\text{TPP})\text{Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}]\text{(BF}_4^-)\)

\[\varepsilon \times 10^4, \text{M}^{-1} \text{cm}^{-1}\]

\[\lambda, \text{nm}\]

\[\varepsilon \times 10^3, \text{M}^{-1} \text{cm}^{-1}\]

d. \([(\text{TPP})\text{Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}]\text{(BF}_4^-)\)

\[\varepsilon \times 10^4, \text{M}^{-1} \text{cm}^{-1}\]

\[\lambda, \text{nm}\]

\[\varepsilon \times 10^3, \text{M}^{-1} \text{cm}^{-1}\]
e. [(TPP)Co^{II}(imid)Zn^{II}(imidH)DAP](OSO_2CF_3)

\[ \varepsilon \times 10^4, \text{M}^{-1} \text{cm}^{-1} \]

\[ \lambda, \text{nm} \]

f. [(TPP)Co^{II}(imid)Cu^{II}(imidH)DAP](OSO_2CF_3)

\[ \varepsilon \times 10^3, \text{M}^{-1} \text{cm}^{-1} \]

\[ \lambda, \text{nm} \]
### TABLE III

**Electronic Spectral Data in CH$_2$Cl$_2$ for the Co(II) Compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{max}$ (nm)</th>
<th>$\varepsilon \times 10^3$ (M$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Co$^{II}$(TPP)]</td>
<td>527</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>410</td>
<td>230.0</td>
</tr>
<tr>
<td><a href="BF$_4$">Co$^{II}$(imid)Zn$^{II}$</a> (5)</td>
<td>582</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>555</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>434</td>
<td>144.0</td>
</tr>
<tr>
<td></td>
<td>296</td>
<td>28.0</td>
</tr>
<tr>
<td><a href="BF$_4$">Co$^{II}$(imid)Cu$^{II}$</a> (6)</td>
<td>582</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>556</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>435</td>
<td>116.4</td>
</tr>
<tr>
<td></td>
<td>328</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>284</td>
<td>15.0</td>
</tr>
<tr>
<td><a href="OSO$_2$CF$_3$">Co$^{II}$(imid)Zn$^{II}$</a> (7)</td>
<td>590</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>554</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>432</td>
<td>127.3</td>
</tr>
<tr>
<td></td>
<td>282</td>
<td>18.3</td>
</tr>
<tr>
<td><a href="OSO$_2$CF$_3$">Co$^{II}$(imid)Cu$^{II}$</a> (8)</td>
<td>594</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>552</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>433</td>
<td>146.7</td>
</tr>
<tr>
<td></td>
<td>301</td>
<td>21.5</td>
</tr>
</tbody>
</table>
compounds 5-8 display a new shoulder at ca. 580-590 nm. If [Co\textsuperscript{II}(TPP)] is gradually added to a CH\textsubscript{2}Cl\textsubscript{2} solution containing the μ-imidazolato cobalt complexes and the spectrum recorded after each addition, the 527 nm and 410 nm bands of [Co\textsuperscript{II}(TPP)] gradually increase in intensity, but the corresponding red-shifted bands assigned to the binuclear compounds (and the shoulders at 580-590 nm) remain constant in their intensities. This observation indicates that the equilibrium constant for the dissociation reaction,

\[
[(\text{TPP})\text{Co}^{\text{II}}(\text{imid})\text{M'}^{\text{II}}(\text{imidH})\text{DAP}]^+ + \text{H}^+ \rightarrow [\text{Co}^{\text{II}}(\text{TPP})] + [\text{M'}^{\text{II}}(\text{imidH})_2\text{DAP}]^{2+}
\]

is small in CH\textsubscript{2}Cl\textsubscript{2} and that the five-coordinate μ-imidazolato complexes predominate in solution. This conclusion is also supported by the electrochemistry results below. It is not possible to directly compare spectra of the binuclear compounds of Figure 12 with 1:1 mixtures of [Co\textsuperscript{II}(TPP)] and [M\textsuperscript{II}(imidH)_2DAP](X)_2 in CH\textsubscript{2}Cl\textsubscript{2} due to the insolubility of the M' = Cu and Zn compounds for either X = BF\textsubscript{4}^{-} or OSO\textsubscript{2}CF\textsubscript{3}^{-}.

**Electrochemistry.** Electrochemical data, as cyclic voltammograms, were obtained for compounds 5-8, at a platinum electrode in CH\textsubscript{2}Cl\textsubscript{2} at 5x10\textsuperscript{-4} M in Co(II) and 0.1 M TBAP as supporting electrolyte. The cyclic voltammograms (C.V.'s) for the compounds vs. S.C.E. are seen in Figure 13, and the half-wave potentials are compiled in Table IV. For each C.V., the potential was initially set at ca. -1.5 V and scanned in the
Figure 13

Cyclic Voltammograms at a Platinum Electrode

for the Co(II) Compounds \(5 \times 10^{-4} \text{ M}\) in \(\text{CH}_2\text{Cl}_2\) with 0.1 M TBAP,

Relative to S.C.E. at a Scan Rate of 200 mV/sec for:

a) [Co\(^{II}\)(TPP)]

b) [(TPP)Co\(^{II}\)(imid)Cu\(^{II}\)(imidH)DAP](X); \(X^- = BF_4^-\) or \(OSO_2CF_3^-\)

c) [(TPP)Co\(^{II}\)(imid)Zn\(^{II}\)(imidH)DAP](X); \(X^- = BF_4^-\) or \(OSO_2CF_3^-\)
### TABLE IV

Cyclic Voltammetric Data for the Co(II) Compounds \(5 \times 10^{-4} \text{ M}\)

in \(\text{CH}_2\text{Cl}_2\) with 0.1M in TBAP.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(E_{1/2}(\text{V}), (\text{relative to S.C.E.})) (pps*)</th>
<th>(M(\text{II})/M(\text{III}))</th>
<th>(M(\text{II})/M(\text{I}))</th>
<th>(M'(\text{II})/M'(\text{I}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(<a href="%5Ctext%7BBF%7D_4">\text{Cu}^{\text{II}}(\text{imid})_2\text{DAP}</a>)_2</td>
<td>-0.32</td>
<td>(0.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Cu}^{\text{II}}(\text{imid})_2\text{DAP}</a>)_2</td>
<td>-0.33</td>
<td>(0.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Co}^{\text{II}}(\text{TPP})])</td>
<td>+0.56</td>
<td>-0.87</td>
<td>(1.30)</td>
<td>(0.75)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BBF%7D_4">\text{Co}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}</a>) (5)</td>
<td>+0.48</td>
<td>-0.85</td>
<td>(1.20)</td>
<td>(0.73)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BBF%7D_4">\text{Co}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}</a>) (6)</td>
<td>+0.48</td>
<td>-0.84</td>
<td>-0.42</td>
<td>(1.20)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Co}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}</a>) (7)</td>
<td>+0.47</td>
<td>-0.86</td>
<td>(1.25)</td>
<td>(0.80)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Co}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}</a>) (8)</td>
<td>+0.46</td>
<td>-0.85</td>
<td>-0.46</td>
<td>(1.23)</td>
</tr>
</tbody>
</table>

*pps = Peak to Peak Separation at a Scan Rate of 200 mV/sec.*
anodic direction to ca. +1.5 V with a scan rate of 200 mV/sec. The $E_{1/2}$ values are the potentials lying midway between the oxidation and reduction waves for a given couple, as confirmed by differential pulse polarography. A typical C.V. of [Co$^{II}$(TPP)] is shown in Figure 13a. In general, the electrochemical processes of [Co$^{II}$(TPP)] have been identified with the following electrode reactions [42]:

\[
\begin{align*}
[\text{Co}^{III}(\text{TPP})]^{3+} + e^- & \rightarrow [\text{Co}^{III}(\text{TPP})]^{2+} \\
[\text{Co}^{III}(\text{TPP})]^{2+} + e^- & \rightarrow [\text{Co}^{III}(\text{TPP})]^+ \\
[\text{Co}^{III}(\text{TPP})]^+ + e^- & \rightarrow [\text{Co}^{II}(\text{TPP})]^0 \\
[\text{Co}^{II}(\text{TPP})]^0 + e^- & \rightarrow [\text{Co}^{I}(\text{TPP})]^-
\end{align*}
\]

Reactions 5 is outside the solvent range of CH$_2$Cl$_2$, and is only observed in DMF and DMSO [42,43]. The potential for the reduction of [Co$^{II}$(TPP)]$^0$ to [Co$^{I}$(TPP)]$^-$ (reaction 4) is only slightly dependent on the solvent and has been reported in the literature to occur at −0.82 V in DMSO [44,45] and at −0.76 V in DMF [46], and in this study, it is observed at −0.87 V vs. S.C.E.

In contrast to the invariance with solvent of the Co$^{II}$/Co$^I$ couple for reaction 4, the Co$^{III}$/Co$^{II}$ couple of reaction 3 is markedly dependent on the coordinating ability of solvent [46,47]. In the present work, $E_{1/2}$ values for this Co$^{III}$/Co$^{II}$ couple are observed at +0.56 V in CH$_2$Cl$_2$. In strongly coordinating solvents such as DMSO and pyridine, a facile oxidation of Co(II) is observed and in nonbonding
solvent such as CH₂Cl₂, oxidation of Co(II) was irreversible [43]. In nonaqueous media, porphyrin and metalloporphyrins may be oxidized in two, single-electron transfer steps to yield π-cation radicals or dications [46] or reduced in two, single-electron transfer steps to yield π-anion radicals or dianions [47]. In this study [Co^{II}(TPP)] was not observed to undergo a reversible reduction of the porphyrin ring to yield an anion diradical radical (reaction 5), however, two oxidation steps of porphyrin ring (reactions 1 and 2) were apparently observed (Figure 13) at E_{1/2} = +1.1 V and +1.27 V vs S.C.E. This result is similar to that reported by Walker, [35].

Costa and coworkers [48] have found that the Co(II)/Co(I) couple is considerably more sensitive to equatorial ligand substituents than redox potentials for the Co(II)/Co(III) couple, which is more sensitive to axial ligand substituents. They interpret these results as an indication that the Co(II)/Co(III) couple is dominated by a strong axial ligand interaction. However, in the Co(II)/Co(I) couple case, axial ligand interaction is weak and equatorial ligand electronic properties are thus more important. Similar effects were observed in our study.

The Co(II)/Co(III) redox potential was shifted anodically, from +0.56 V for [Co^{II}(TPP)] to +0.48 V for compounds 5 and 6 and +0.47 and +0.46 V for compounds 7 and 8, respectively, upon formation of the heterobinuclear complexes. The anodic shift of the Co(II)/Co(III) couple indicates preferential stabilization of the binuclear complexes relative to [Co^{II}(TPP)], and can be accounted for by the formation of
more stable five-coordinate binuclear complexes. However, the 
Co(II)/Co(I) redox potential was unchanged upon formation of the 
binuclear complexes, with the $E_{1/2}$ values being $-0.85$ V for compounds 5 and 8 and $-0.84$ and $-0.86$ V for compounds 6 and 7, respectively. In 
addition to the redox potentials of the cobalt centers, half-wave 
potentials at $-0.42$ V and $-0.46$ V were also observed for compounds 6 and 
8 due, presumably, to the Cu(II)/Cu(I) couples. Furthermore, the 
reduction of the porphyrin ring to form a π-anion radical and dianion 
radical were also observed at $E_{1/2} \approx -1.2$ and $-1.3$, respectively, for 
the compounds 5-8. In the case of compounds 5 and 7, the $E_{1/2}$ values at 
ca. $-0.9$ V may due to the reduction of the penta-coordinated ligand of 
the Zn(II) fragments [49]. However, the Co(II)/Co(III) and Co(II)/Co(I) 
redox potentials are nearly identical for compounds 5-8, indicating 
similar electronic environments about the Co(II) centers. Hence, the 
counter anions (BF$_4^-$ or OSO$_2$CF$_3^-$) seem to have little affect on the 
electronic properties of the Co(II) center.

Magnetochemistry. The magnetochemical properties of the 
mononuclear parent compound, [Co$^{II}$(TPP)], as well as the binuclear 
Co(II) compounds were studied from ca. 20-300 K. The $\mu_{\text{eff}}$ (solid, RT) 
value for [Co$^{II}$(TPP)] was found to be 2.69 $\mu_B$, a value in good agreement 
with the value previously reported by Sato, et al., [50]. This value is 
somewhat higher than the theoretical spin-only value for an $S = 1/2$ 
($\mu_{\text{eff}} = 1.73 \mu_B$), nevertheless it may be accounted for by assuming an 
orbital contribution to the spin-only magnetic moment. This observed
\( \mu_{\text{eff}} \) value is in reasonable agreement with the value of 2.51 \( \mu_B \) predicted from the epr \( g \)-values [50].

The variable-temperature (ca. 20-300 K) magnetic susceptibilities for compounds 5-8 have also been determined. The magnetochemical data are documented in Table V and displayed in Figure 14 as \( \mu_{\text{eff}}'(\mu_B) \) vs. \( T \) (K) plots and Figure 15 as \( x'_M \) vs. \( T^{-1} \) plots. Compounds 5 and 7, containing \([\text{Co}^{II}(\text{imid})\text{Zn}^{II}]^+\) cores, exhibit room temperature magnetic moments of 2.21 \( \mu_B \) and 2.23 \( \mu_B \) respectively. These values are somewhat lower than those of \([\text{Co}^{II}(\text{TPP})]\) but in reasonable agreeable with a low-spin (\( S = 1/2 \)) ground state for a five-coordinate cobalt(II) porphyrin center, assuming an orbital contribution to the 1.73 \( \mu_B \) spin-only magnetic moment. As the temperature is lowered, these values gradually decrease to 1.74 \( \mu_B \) and 1.78 \( \mu_B \) by ca. 20 K. Compounds 6 and 8 contain one additional unpaired electron from a Cu(II) center in addition to the one unpaired electron for low-spin Co(II). These compounds, with \([\text{Co}^{II}(\text{imid})\text{Cu}^{II}]^+\) cores, possess \( \mu_{\text{eff}} \) (solid, RT) values of 3.10 \( \mu_B \) and 3.14 \( \mu_B \), respectively. These values are in a good agreement with theory if it is assumed that \([\mu_{\text{eff}} \text{binuclear}] = [(\mu_{\text{eff}}\text{Co})^2 + (\mu_{\text{eff}}\text{Cu})^2]^{1/2} \), as would be the case if the two \( S = 1/2 \) centers were magnetically isolated from one another. These \( \mu_{\text{eff}} \) values also gradually decreased to 2.47 \( \mu_B \) and 2.51 \( \mu_B \) by ca. 20 K.

Furthermore, the magnetic susceptibility data over the entire temperature range for the corresponding \([\text{Co}^{II}(\text{imid})\text{Zn}^{II}]^+\) and \([\text{Co}^{II}(\text{imid})\text{Cu}^{II}]^+\) compounds are parallel to one another, with their \( x'_M \)
TABLE V

Magnetic Susceptibility Data
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\(^a\) in cgs-emu mole\(^{-1}\)
### Magnetic Susceptibility Data

For \([\text{Co}^{II}(\text{imid})\text{Cu}^{II}]\text{(BF}_4\text{)}\)

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a. in cgs-emu mole^{-1}
**Figure 14**

$\mu_{\text{eff}}(\mu_B)$ vs. Temperature (K) Plot of:

1. $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$
2. $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$
3. $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$
4. $[(\text{TPP})\text{Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$
a. [Co$^{II}$-(imid)-Zn$^{II}$] (BF$_4$)

b. [Co$^{II}$-(imid)-Cu$^{II}$] (BF$_4$)
c. [Co\textsuperscript{II}-(imid)-Zn\textsuperscript{II}] (OSO\textsubscript{2}CF\textsubscript{3})

\[ \mu_{\text{eff}}, \mu_B \]

\[ \begin{array}{c}
\text{TEMPERATURE (K)} \\
0 & 100.00 & 200.00 & 300.00 \\
0 & 4.00 \\
\hline
0 & 2.00 & 4.00 \\
6.00 & 2.00 & 4.00 \\
6.00 & 2.00 & 4.00 \\
6.00 & 2.00 & 4.00 \\
\end{array} \]

\[ \begin{array}{c}
\text{TEMPERATURE (K)} \\
0 & 100.00 & 200.00 & 300.00 \\
0 & 4.00 \\
\hline
0 & 2.00 & 4.00 \\
6.00 & 2.00 & 4.00 \\
6.00 & 2.00 & 4.00 \\
6.00 & 2.00 & 4.00 \\
\end{array} \]

d. [Co\textsuperscript{II}-(imid)-Cu\textsuperscript{II}] (OSO\textsubscript{2}CF\textsubscript{3})
Figure 15

$X'_N \times S \times T^{-1} (K^{-1})$ Plot of:

a) $[(TPP)\text{Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$ (5)
b) $[(TPP)\text{Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$ (6)
c) $[(TPP)\text{Co}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$ (7)
d) $[(TPP)\text{Co}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$ (8)
a. \([\text{Co}^{II}-(\text{imid})-\text{Zn}^{II}] (\text{BF}_4)\)

\[
\chi_M, \text{emu-cgs mole}^{-1}/10^{-1}
\]

\[
\begin{array}{c}
\text{Slope} = 0.36 \\
\text{Intercept} = 1.06E-3
\end{array}
\]

\[
1/T \ (K^{-1})
\]

b. \([\text{Co}^{II}-(\text{imid})-\text{Cu}^{II}] (\text{BF}_4)\)

\[
\chi_M, \text{emu-cgs mole}^{-1}
\]

\[
\begin{array}{c}
\text{Slope} = 0.72 \\
\text{Intercept} = 2.42E-3
\end{array}
\]

\[
1/T \ (K^{-1})
\]
C. $\text{[Co}^{II-\text{(imid)}}-\text{Zn}^{II}]\text{(OSO}_2\text{CF}_3\text{)}$

$\chi_M, \text{emu-cgs mole}^{-1}/10^{-1}$

Slope = 0.37
Intercept = 1.03E-3

1/T (K$^{-1}$)

---

d. $\text{[Co}^{II-\text{(imid)}}-\text{Cu}^{II}]\text{(OSO}_2\text{CF}_3\text{)}$

$\chi_M, \text{emu-cgs mole}^{-1}$

Slope = 0.74
Intercept = 2.13E-3

1/T (K$^{-1}$)
vs. $T^{-1}$ plots being nearly linear from ca. 20 - 300 K. This magnetochemical pattern for compounds 5-8 demonstrates nearly Curie behavior for all four compounds, with 6 and 8 possessing magnetically non-interacting $S = 1/2$ Co(II) and Cu(II) centers (i.e., $-J \sim 0$). This conclusion is further supported below from epr spectroscopy studies. For comparison purposes, it should be mentioned that Sinn and coworkers [51] have recently reported that a closely related compound (immediately below) of 6 and 8 possess a magnetic interaction of $-J_{(\text{Co II}-\text{Cu II})} \sim 35 \text{ cm}^{-1}$ across an imidazolate bridge between the two $S = 1/2$ Co(II) and Cu(II) centers.

EPR Spectroscopy. The binuclear cobalt(II) compounds have also been examined by epr spectroscopy as crystalline powders and in frozen solution as CH$_2$Cl$_2$ glasses at 10 K. The epr spectra of compounds 5-8 in frozen solution as CH$_2$Cl$_2$ glasses are shown in Figure 16 and the spectral parameters are set out in Table VIa. The $g$-values given in the table are only approximate descriptions of the field-frequency of the spectral lines and are not meant to be taken as the true $g$ tensors of the Zeeman term in the spin Hamiltonian. The cobalt(II) epr spectra for
Figure 16

Epr Spectra at 10 K of the Co(II) Compounds
in Frozen Solution as CH₂Cl₂ Glasses

a) [{Co}^{II}(TPP)]

b) [(TPP){Co}^{II}(imid){Zn}^{II}(imidH)DAP]_(X); \(X^- = BF_4^-; OSO_2CF_3^-\)

c) [(TPP){Co}^{II}(imid){Cu}^{II}(imidH)DAP]_(X); \(X^- = BF_4^-; OSO_2CF_3^-\)
**TABLE VIa**

Epr Parameters at 10 K for the Co(II) Compounds in Frozen Solution as CH₂Cl₂ Glasses

<table>
<thead>
<tr>
<th>Compound</th>
<th>g factor</th>
<th>Co</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Co\textsuperscript{II}(TPP)]</td>
<td></td>
<td>$g_1 = 3.32$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$g_2 = 1.80$</td>
<td></td>
</tr>
<tr>
<td><a href="BF%E2%82%84">Co\textsuperscript{II}(imid)Zn\textsuperscript{II}</a> (\text{5})</td>
<td>$g_1 = 2.01$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="BF%E2%82%84">Co\textsuperscript{II}(imid)Cu\textsuperscript{II}</a> (\text{6})</td>
<td>$g_1 = 2.01$</td>
<td>$g_1 = 2.08$</td>
<td></td>
</tr>
<tr>
<td><a href="OSO%E2%82%82CF%E2%82%83">Co\textsuperscript{II}(imid)Zn\textsuperscript{II}</a> (\text{7})</td>
<td>$g_1 = 2.01$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="OSO%E2%82%82CF%E2%82%83">Co\textsuperscript{II}(imid)Cu\textsuperscript{II}</a> (\text{8})</td>
<td>$g_1 = 2.01$</td>
<td>$g_1 = 2.08$</td>
<td></td>
</tr>
</tbody>
</table>
the [Co\textsuperscript{II}(imid)Zn\textsuperscript{II}]\textsuperscript{+} and [Co\textsuperscript{II}(imid)Cu\textsuperscript{II}]\textsuperscript{+} cores as either BF\textsubscript{4}\textsuperscript{−} or OSO\textsubscript{2}CF\textsubscript{3}\textsuperscript{−} salts are essentially identical which again confirms that the electronic structures of the Co(II) centers in all four compounds can be considered to be the same.

A typical spectrum for a low-spin, four-coordinate d\textsuperscript{7} Co(II) center was observed for [Co\textsuperscript{II}(TPP)] as show in Figure 16a. The low-field hyperfine splitting is composed of eight line arising from the interaction of the unpaired electron on cobalt with the cobalt I = 7/2 nuclear spin [53]. A number of theoretical treatments have shown the g-values in low-spin d\textsuperscript{7} (S = 1/2) systems depend on the magnitudes of the separation between the four sets of d-orbitals. The g values deviate appreciably from 2.0 (2.3-2.4), with the deviation inversely proportional to the \textsuperscript{2}A\textsubscript{1}→\textsuperscript{2}E separation [54]; g tends to be relatively close to 2.0 (1.8-2.1). For Co(II) complexes with a fixed planar ligand fields, the energy of the \textsuperscript{2}A\textsubscript{1} state is merely affected by axial interaction, increasing the \textsuperscript{2}A\textsubscript{1}→\textsuperscript{2}E splitting and thereby decreasing g toward 2.0 [55]

\begin{align*}
\begin{array}{c}
\text{Energy (milli eV scale)} \\
\text{a) Orbital Energies b) State Energies}
\end{array}
\end{align*}

\begin{align*}
\text{\textsuperscript{2}A\textsubscript{1} (e\textsuperscript{b},a,\textsuperscript{b})} \\
\text{\textsuperscript{2}E (e\textsuperscript{b'},a')} \\
\text{\textsuperscript{2}E (e\textsuperscript{b},a')} \\
\text{\textsuperscript{2}E (e\textsuperscript{b'},a')} \\
\end{align*}

Assumed order of d orbital and state energies for planar, low-spin cobalt(II). The relative positions of e and b\textsuperscript{a} are unimportant.
A similar epr phenomena was also observed in this study in that, the \( g - g \) decreased upon formation of the binuclear compounds 5-8. This result further confirms the presence of a five-coordinate binuclear compound in \((\text{CH}_2\text{Cl}_2)\) solution. For compounds 5-8, it appears that the cobalt(II) centers all exist in a low-spin state, with each spectrum displaying a prominent \( g = 2 \) signal, which is characteristic of an isotopic \( (S = 1/2) \) spin-state for five coordinate Co(II) complexes. For compounds 6 and 8, the \( g = 2 \) Co(II) signal is seen to be significantly broadened relative to that of compounds 5 and 7 which can be attributed to the presence of another \( S = 1/2 \) Cu(II) center. This result is, therefore, consistent with the above magnetochemical data, indicating an absence of any significant antiferromagnetic interaction across the imidazolate bridge in the \([\text{Co}^{\text{III}}(\text{imid})\text{Cu}^{\text{II}}]^{+}\) species. In fact, for \(-J \neq 0\), no Co(II) or Cu(II) epr spectrum would be detected since the ground state would be \( S = 0 \) and the excited state \( S = 1 \) in this case. The epr spectra for compounds 5-8 as polycrystalline solids at 10 K were also determined, as shown in Figure 17 and Table VIb contains the epr spectral parameters. The similarities of the spectra in frozen \( \text{CH}_2\text{Cl}_2 \) glasses to those as polycrystalline powders indicates that compounds 5-8 possess the same oxidation states and electronic structures in both the solid and solution states.
Figure 17

EPR Spectra at 10 K of the μ-Imidazolato Co(II) Compounds
as Polycrystalline Solids (~1.0% Co(II) in Ammonium Sulfate) for:

a) [Co\textsuperscript{II}(TPP)]

b) [(TPP)Co\textsuperscript{II}(imid)Zn\textsuperscript{II}(imidH)DAP](X); X\textsuperscript{−} = BF\textsubscript{4}\textsuperscript{−} or OSO\textsubscript{2}CF\textsubscript{3}\textsuperscript{−}

c) [(TPP)Co\textsuperscript{II}(imid)Cu\textsuperscript{II}(imidH)DAP](X); X\textsuperscript{−} = BF\textsubscript{4}\textsuperscript{−} or OSO\textsubscript{2}CF\textsubscript{3}\textsuperscript{−}
T = 10K

a. [Co\textsuperscript{II} (imid) Zn\textsuperscript{II}]\textsuperscript{+}

b. [Co\textsuperscript{II} (imid) Cu\textsuperscript{II}]\textsuperscript{+}

\[ g = 6 \quad \text{and} \quad g = 2 \]

MAGNETIC FIELD, G

INTENSITY
**TABLE VIIa**

EPR Parameters at 10 K for the Co(II) Compounds as Polyammonia Solids (1.0% Co(II) in Ammonium Sulfate)

<table>
<thead>
<tr>
<th>Compound</th>
<th>g factor</th>
<th>Co</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>$<a href="%5Ctext%7BBF%7D_4">\text{Co}^{II}(\text{imid})\text{Zn}^{II}</a>$ (5)</td>
<td>$g_1 = 2.01$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$<a href="%5Ctext%7BBF%7D_4">\text{Co}^{II}(\text{imid})\text{Cu}^{II}</a>$ (6)</td>
<td>$g_1 = 2.01$</td>
<td></td>
<td>$g_1 = 2.08$</td>
</tr>
<tr>
<td>$<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Co}^{II}(\text{imid})\text{Zn}^{II}</a>$ (7)</td>
<td>$g_1 = 2.01$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Co}^{II}(\text{imid})\text{Cu}^{II}</a>$ (8)</td>
<td>$g_1 = 2.01$</td>
<td></td>
<td>$g_1 = 2.08$</td>
</tr>
</tbody>
</table>
The Binuclear Iwawase (II) System

The four μ-imidazolato binuclear complexes containing the Mn(II) tetraporphyrin fragment, compounds 9-12, were synthesized according to the general procedure described above for the Co(II) system, except that all synthesis and handling procedures were performed anaerobically. The synthetic scheme is shown in Figure 10. The binuclear compounds are obtained by the reaction of 1 mole equivalent of [Mn^{II}(TPP)] and 1.2 mole equivalents of [M'+^{II}(imidH)_2DAP]^+ (M' = Cu, Zn) as a 0SO_2CF_3^- or BF_4^- salt in the presence of a base such as [1,2-bis-(dimethylamino) napthalene] (Proton Sponge) or t-BuOK in a dry CH_2Cl_2/CH_3CN (90/10) solvent mixture. Compounds 9-12 all possess satisfactory elemental analyses and solution state conductivities that are consistent with uni-univalent electrolyte behavior as required by the molecular formulation in Figure 10 (see Experimental Section).

[Mn^{II}(TPP)] was obtained by the reduction of [Mn^{III}(TPP)Cl] with [Cr^{II}(acac)_2] [12], according to the reaction,

[Mn^{III}(TPP)Cl] + [Cr^{II}(acac)_2] \rightarrow [Mn^{II}(TPP)] + [Cr^{III}(acac)_2Cl]

The advantages of [Cr^{II}(acac)_2] over other reductants are the chloride ligand removal and transfer in the redox reaction, as well as its solubility in organic solvent such as toluene. This allows one to avoid more polar solvents which might otherwise serve as interfering ligands [56].
Unlike the previous Co(II) and Fe(III), complexes of this work, [Mn^{II}(TPP)] and its binuclear derivatives are readily and irreversibly oxidized upon exposure to air. Hence, it was necessary to synthesize and manipulate all the Mn(II) compounds in an anaerobic environment. Another important property of [Mn^{II}(TPP)] and its axially-ligated derivatives are their preference to form only five-coordinate compounds even in the presence of excess base [12].

**Infrared Spectroscopy.** The infrared spectra of the binuclear Mn(II) compounds are shown in Figure 18. Like in the case of the preceding Co(II) and Fe(III) compounds, the infrared spectroscopic data for the Mn(II) compounds are consistent with binuclear compound formation. Spectral peaks corresponding to the appropriate functional groups expected for the binuclear compounds are observed mainly in the 600-1700 cm\(^{-1}\) spectral region. Compound 9 and 10, which are BF\(_4\)\(^-\) salts, show a broad B-F stretch center around 1050 cm\(^{-1}\). The characteristic peaks for the OSO\(_2\)CF\(_3\)\(^-\) moiety at 635 cm\(^{-1}\) and 1225 cm\(^{-1}\) [25] are observed for the compounds 11 and 12 which contain OSO\(_2\)CF\(_3\)\(^-\) as the counter anion. A C=N imine stretching frequency around 1580-1600 cm\(^{-1}\) is observed for all the compounds.

**Electronic Spectroscopy.** The electronic spectra in CH\(_2\)Cl\(_2\) for [Mn^{II}(TPP)] as well as the new binuclear compounds are shown in Figure 19. The spectral parameters are listed in Table VII. Mononuclear [Mn^{II}(TPP)] displays a typical spectrum for a metalloporphyrin compound.
Figure 18

Infrared Spectrum as a Nujol Mull of:

a) $[\text{Mn}^{II}(\text{TPP})]$

b) $[(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$ (9)

c) $[(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{BF}_4)$ (10)

d) $[(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$ (11)

e) $[(\text{TPP})\text{Mn}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3)$ (12)
a. $[\text{Mn}^{II}(\text{TPP})]$
b. \([\text{TPP}]\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP}]\text{(BF}_4\text{)}\)

c. \([\text{TPP}]\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}(\text{imidH})\text{DAP}]\text{(BF}_4\text{)}\)
d. \[ ([\text{TPP}]\text{Mn}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP})(\text{OSO}_2\text{CF}_3) \]

e. \[ ([\text{TPP}]\text{Mn}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP})(\text{OSO}_2\text{CF}_3) \]
Figure 19

UV-Visible Electronic Absorption Spectrum in CH₂Cl₂ for:

a) [Mn
\text{II}(\text{TPP})]\text{ in CH₂Cl₂}, [Cu
\text{II}(\text{imidH})₂ \text{DAP}](\text{BF}_4)\text{ in CH₃CN},
and [(TPP)Mn
\text{II}(\text{imid})Cu
\text{II}(\text{imidH})\text{DAP}](\text{BF}_4), (10)
b) [Mn
\text{II}(\text{TPP})]
c) [(TPP)Mn
\text{II}(\text{imid})Zn
\text{II}(\text{imidH})\text{DAP}](\text{BF}_4) (9)
d) [(TPP)Mn
\text{II}(\text{imid})Cu
\text{II}(\text{imidH})\text{DAP}](\text{BF}_4) (10)
e) [(TPP)Mn
\text{II}(\text{imid})Zn
\text{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3) (11)
f) [(TPP)Mn
\text{II}(\text{imid})Cu
\text{II}(\text{imidH})\text{DAP}](\text{OSO}_2\text{CF}_3) (12)
c. \([\text{TPP}]\text{Mn}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP}](\text{BF}_4^-(\lambda, \text{nm})

\[\varepsilon \times 10^4, \text{M}^{-1} \text{cm}^{-1}\]

\[\varepsilon \times 10^3, \text{M}^{-1} \text{cm}^{-1}\]

---

d. \([\text{TPP}]\text{Mn}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP}](\text{BF}_4^-(\lambda, \text{nm})

\[\varepsilon \times 10^4, \text{M}^{-1} \text{cm}^{-1}\]

\[\varepsilon \times 10^3, \text{M}^{-1} \text{cm}^{-1}\]
e. \((\text{TPP})\text{Mn}^{II}(\text{imid})\text{Zn}^{II}(\text{imidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\)

\[ \varepsilon \times 10^4, \text{M}^{-1} \text{cm}^{-1} \]
\[ \lambda, \text{nm} \]

f. \((\text{TPP})\text{Mn}^{II}(\text{imid})\text{Cu}^{II}(\text{imidH})\text{DAP})(\text{OSO}_2\text{CF}_3)\)

\[ \varepsilon \times 10^4, \text{M}^{-1} \text{cm}^{-1} \]
\[ \lambda, \text{nm} \]
<table>
<thead>
<tr>
<th>Compound</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>( \varepsilon M \times 10^3 ) (M(^{-1}) cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Mn}^{\text{II}}(\text{TPP})])</td>
<td>602</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>562</td>
<td>11.2</td>
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<tr>
<td></td>
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<tr>
<td>(<a href="%5Ctext%7BBF%7D_4">\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}</a>) (9)</td>
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<td>10.3</td>
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<td>(<a href="%5Ctext%7BBF%7D_4">\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}</a>) (10)</td>
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<td>62.1</td>
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(continued)
<table>
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<tr>
<th>Compound</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>( c \times 10^3 ) (M(^{-1}) cm(^{-1}))</th>
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</thead>
<tbody>
<tr>
<td>(<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}</a>) (11)</td>
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<td></td>
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<tr>
<td></td>
<td>526</td>
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<td>49.3</td>
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<td>265</td>
<td>35.0</td>
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<tr>
<td>(<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}</a>) (12)</td>
<td>616</td>
<td>9.9</td>
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The visible region has three bands with relatively comparable intensities at 602 nm, 562 nm, and 520 nm. In addition, two soret bands with greater intensities are found at 434 nm and 359 nm. The positions and intensities of these bands are in good agreement with the values previously reported by Reed, et al., [15]. Similar to the Co(II) system, bathometric shifts (red shifts) in the visible and soret bands, as large as 20 nm, are observed upon formation of the μ-imidazolato binuclear complexes. This same spectral behavior was also observed by Reed, et al., [15] upon treatment of a toluene solution of [Mn^{II}(TPP)] with various ligands (L = 1-methylimidazole, 2-methylimidazole or pyridine) to form intense green solutions of the five-coordinate [Mn^{II}(TPP)L] derivatives. The soret bands position of the present binuclear compounds are also shifted from that of the parent compound, [Mn^{II}(TPP)]. However, the presence of a large molecule like [M^{II}(imidH)_{2}DAP]^+ may account for this difference. Unfortunately, the molar absorptivities of the [M^{II}(imidH)_{2}DAP]^{+} bands are relatively small compared to those of the porphyrin fragment. Hence, it is difficult to observe bands corresponding to only the [M^{II}(imidH)DAP]^+ fragment (especially Cu(II) d-d bands) in the electronic spectrum of the binuclear complexes. All four compounds 5-8 exhibit electronic spectra quite different from that of [LMn^{III}(TPP)], and this indicates the absence of [LMn^{III}(TPP)] in the binuclear complexes. It is not possible to directly compare spectra of the binuclear compounds of Figure 19 with that of a 1:1 mixture of [Mn^{II}(TPP)] and [M^{II}(imidH)_{2}DAP](X)_{2} in CH_{2}Cl_{2}, due to the insolubility of the M' = Cu and Zn compounds for
either $X^-$ = BF$_4$- or OSO$_2$CF$_3$-. In general, the electronic spectra of compounds 9, 11, 10, and 12 are comparable, indicating a similar electronic environment about Mn(II) in both the Zn(II) and Cu(II) derivatives. In particular, this helps establish the oxidation state of the manganese centers in compounds 10 and 12 as 2+ rather than 3+, a fact that has considerable importance as discussed below in the magnetochemistry and epr sections.

**Electrochemistry.** Electrochemistry studies were performed on the binuclear Mn(II) compounds 9–12 in CH$_2$Cl$_2$ at 5x10$^{-4}$ M in Mn(II) compound and 0.1 M TBAP as supporting electrolyte. Figure 20 shows the cyclic voltammograms (C.V.'s) for the Mn(II) compounds and the half-wave potentials ($E_{1/2}$ values) are listed in Table VIII. For each C.V., the potential was initially set at ca. -1.5 V and scanned in the anodic direction to ca. +1.5 V with a scan rate of 200 mV/sec. The $E_{1/2}$ values were measured as that potential lying midway between the oxidation and reduction peaks for a given couple, as determined by differential pulse polarography. The C.V. of [Mn$^{II}$(TPP)] is shown in Figure 20a. Analogously to the above Co(II) compounds, the electrochemical processes for [Mn$^{II}$(TPP)] can be rationalized by the following reactions:

\[
\begin{align*}
[Mn^{III}(TPP)]^{2+} + e^- &\rightarrow [Mn^{III}(TPP)]^+ \\
[Mn^{III}(TPP)]^+ + e^- &\rightarrow [Mn^{II}(TPP)]^0 \\
[Mn^{II}(TPP)]^0 + e^- &\rightarrow [Mn^{II}(TPP)]^- \\
[Mn^{II}(TPP)]^- + e^- &\rightarrow [Mn^{II}(TPP)]^{2-}
\end{align*}
\]
Figure 20

Cyclic Voltammograms at a Platinum Electrode

for the Co(II) Compounds (5x10^{-4} M) in CH_{2}Cl_{2} with 0.1 M TBAP.

Relative to S.C.E. at a Scan Rate of 200 mV/sec for:

a) [Mn^{II}(TPP)]

b) [(TPP)Mn^{II}(imid)Cu^{II}(imidH)DAP](X); X^{-} = BF_{4}^{-}; OSO_{2}CF_{3}^{-}

c) [(TPP)Mn^{II}(imid)Zn^{II}(imidH)DAP](X); X^{-} = BF_{4}^{-}; OSO_{2}CF_{3}^{-}
# TABLE VIII

**Cyclic Voltammetric Data for the Mn(II) Compounds \((5\times10^{-4})\)**

in CH\(_2\)Cl\(_2\) with 0.1M in TRAP.

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<td>([\text{Mn}^{II}(\text{imid})\text{Cu}^{II}]\text{(BF}_4\text{)}) ((10))</td>
<td>(-0.24) (-0.37) ((0.75)) ((0.86))</td>
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<td>([\text{Mn}^{II}(\text{imid})\text{Zn}^{II}]\text{(OSO}_2\text{CF}_3\text{)}) ((11))</td>
<td>(-0.26) ((0.80))</td>
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<tr>
<td>([\text{Mn}^{II}(\text{imid})\text{Cu}^{II}]\text{(OSO}_2\text{CF}_3\text{)}) ((12))</td>
<td>(-0.26) (-0.36) ((0.78)) ((0.89))</td>
</tr>
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* pps = Peak to Peak Separation at Scan Rate of 200 mV/sec.
The half-wave potential for the Mn$^{II}$/Mn$^{III}$ couple (reaction 2) has been normally observed at ca. −0.33 V in CH$_2$Cl$_2$ [56], but under the conditions of this study, the $E_{1/2}$ value for the oxidation of [Mn$^{II}$(TPP)] to form [Mn$^{III}$(TPP)]$^+$ was found to be −0.27 V. The porphyrin ring oxidation (reaction 1) which forms a π-cation radical was not observed in this study due to the narrowness of the solvent (CH$_2$Cl$_2$) window. However, two, single electron reduction processes of the porphyrin ring, similar to those of the above Co(II) compounds, are observed at −1.14 V and −1.24 V to form π-anion and dianion radicals (reactions 3 and 4), respectively. These values are in good agreement with the values previously reported [56–58].

In contrast to the Co(II) compounds, the redox potentials of the Mn(II) compounds, particularly, the Mn(II)/Mn(III) couple are not substantially affected upon binuclear compounds formation. The $E_{1/2}$ values for the Mn(II)/Mn(III) couple for compounds 9 and 10 are −0.24 V and for compounds 11 and 12 they are −0.26 V. The similarity of the half-wave potentials for the manganese centers in compounds 9–12 indicates a similar electronic environment for manganese in all four compounds. This information can be used to help interpret the magnetochemistry and epr spectroscopic data below.

In addition to the redox chemistry associated with the manganese centers, half-wave potentials at −0.37 V were also observed for compounds 10 and 12, and are presumably due to the Cu$^{II}$/Cu$^{I}$ couple.
Furthermore, similar to \([\text{Mn}^{\text{II}}(\text{TPP})]\), the reduction of porphyrin rings in the binuclear compounds to form \(\pi\)-anion and dianion radicals were observed at ca. -1.2 and ca. -1.3 V for all four compounds. Finally, in the case of compounds 9 an 11, the half-wave potentials at ca. -0.9 V is possibly due to a reduction of the penta-coordinated ligand in the Zn(II) fragments; no such reduction process was observed for the Cu(II) containing binuclear compounds.

**Magnetochemistry.** Variable-temperature (ca. 20-300 K) magnetic susceptibility data for the \(\mu\)-imidazolato binuclear Mn(II) compounds are listed in Table IX and displayed as \(\mu_{\text{eff}} (\mu_B) \text{ vs.} T\). Temperature (K) plots in Figure 21 and \(X_M' \text{ vs.} T^{-1}\) plots in Figure 22. The room temperature magnetic moment of \([\text{Mn}^{\text{II}}(\text{TPP})]\) is 6.12 \(\mu_B\). This value is typical of a high-spin \(d^5\) \((S = 5/2)\) center and is in good agreement with that previously reported [15]. Compound 9, containing a \([\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}]\) core, exhibits a \(\mu_{\text{eff}}\) value of 5.84 \(\mu_B\) at room temperature, which gradually decreases to 5.08 \(\mu_B\) by 23.9 K. A value of 5.84 \(\mu_B\) is slightly lower than that theoretically expected (5.9 \(\mu_B\)) for a high-spin \(d^5\) \((S = 5/2)\) system, but it is within the range reported for other \((S = 5/2)\) Mn(II) compounds of from 5.7 to 6.5 \(\mu_B\) [50]. For compound 11, also with a \([\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}]^+\) core, the \(\mu_{\text{eff}}\) value varies from 5.94 \(\mu_B\) at 293 K to 5.07 \(\mu_B\) by 23.2 K. Furthermore, the \(X_M' \text{ vs.} T^{-1}\) plots for 9 and 11 (Figure 22a and 22c), are almost linear from ca. 20-300 K, indicating nearly Curie behavior. Compound 10, with a \([\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}]^+\) core, contains an additional unpaired electron from the Cu(II) center when
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a. in cgs-emu mole$^{-1}$
## TABLE IXc

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a. in cgs-emu mole^{-1}
TABLE IXd
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<td>5.73</td>
<td>1.715</td>
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<tr>
<td>189.0</td>
<td>5.29</td>
<td>1.598</td>
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<tr>
<td>201.5</td>
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<td>1.513</td>
<td>4.94</td>
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<tr>
<td>218.0</td>
<td>4.59</td>
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<td>1.372</td>
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<td>5.07</td>
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<td>3.77</td>
<td>1.236</td>
<td>5.10</td>
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<tr>
<td>293.8</td>
<td>3.40</td>
<td>1.124</td>
<td>5.14</td>
</tr>
</tbody>
</table>

\(^a\) in cgs-emu mole\textsuperscript{-1}
Figure 21

$\mu_{\text{eff}}$ ($\mu_B$) vs. Temperature (K) Plot of:

a) \([(\text{TPP})\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP)}(\text{BF}_4)\] (9)

b) \([(\text{TPP})\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}(\text{imidH})\text{DAP)}(\text{BF}_4)\] (10)

c) \([(\text{TPP})\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP)}(\text{OSO}_2\text{CF}_3)\] (11)

d) \([(\text{TPP})\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}(\text{imidH})\text{DAP)}(\text{OSO}_2\text{CF}_3)\] (12)
a. $[\text{Mn}^{\text{II}}-\text{imid}-\text{Zn}^{\text{II}}]\langle\text{BF}_4\rangle$

b. $[\text{Mn}^{\text{II}}-\text{imid}-\text{Cu}^{\text{II}}]\langle\text{BF}_4\rangle$
c. \([\text{Mn}^{\text{II}}-(\text{imid})-\text{Zn}^{\text{II}}]\text{(OSO}_2\text{CF}_3)\)

\[\mu_{\text{eff}}, \mu_B\]

\[\text{TEMPERATURE (K)}\]

---

d. \([\text{Mn}^{\text{II}}-(\text{imid})-\text{Cu}^{\text{II}}]\text{(OSO}_2\text{CF}_3)\)

\[\mu_{\text{eff}}, \mu_B\]

\[\text{TEMPERATURE (K)}\]
Figure 22

$X'_M$ vs. $T^{-1}$ $(K^{-1})$ Plot of:

a) [(TPP)Mn$^{II}$ (imid)Zn$^{II}$ (imidH)DAP](BF$_4$) (9)

b) [(TPP)Mn$^{II}$ (imid)Cu$^{II}$ (imidH)DAP](BF$_4$) (10)

c) [(TPP)Mn$^{II}$ (imid)Zn$^{II}$ (imidH)DAP](OSO$_2$CF$_3$) (11)

d) [(TPP)Mn$^{II}$ (imid)Cu$^{II}$ (imidH)DAP](OSO$_2$CF$_3$) (12)
\[ a. [\text{Mn}^{II}-(\text{imid})-\text{Zn}^{II}][\text{BF}_4] \]

\[ X'_M, \text{emu-cgs mole}^{-1} \]

\[ \frac{1}{T} (K^{-1}) \]

Slope = 3.13
Intercept = 5.36E-3

\[ b. [\text{Mn}^{II}-(\text{imid})-\text{Cu}^{II}][\text{BF}_4] \]

\[ X'_M, \text{emu-cgs mole}^{-1} \]

\[ \frac{1}{T} (K^{-1}) \]

Slope = 2.03
Intercept = 5.91E-3
C. $[\text{Mn}^{II}-(\text{imid})-\text{Zn}^{II}]\langle \text{OSO}_2\text{CF}_3 \rangle$

\[ X'_M, \text{emu-cgs mole}^{-1} \]

\[ 1/T \ (K^{-1}) \]

Slope = 3.09

Intercept = 6.01E-3

d. $[\text{Mn}^{II}-(\text{imid})-\text{Cu}^{II}]\langle \text{OSO}_2\text{CF}_3 \rangle$

\[ X'_M, \text{emu-cgs mole}^{-1} \]

\[ 1/T \ (K^{-1}) \]

Slope = 2.04

Intercept = 5.21E-3
compared to its Zn(II) analogues. The expected \( \mu_{\text{eff}} \) value for

\([\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}]^+\), in the absence of any interaction between the \((S = 5/2)\) Mn(II) and \((S = 1/2)\) Cu(II) metal centers, is in the range of

6.3–6.5 \( \mu_B \). This value is simply derived from \( [\mu_{\text{eff}}(\text{binuclear})] = (\mu_{\text{eff}}(\text{Cu}(\text{II})) + \mu_{\text{eff}}(\text{Mn}(\text{II})))^{1/2} \) and assumes that the \( \mu_{\text{eff}}(\text{Mn}(\text{II})) = 6.0 \mu_B \) and \( \mu_{\text{eff}}(\text{Cu}(\text{II})) = 2.0 \mu_B \). Somewhat unexpectedly, the experimental magnetic moment for compound 10 at room temperature is 5.11

\( \mu_B \), a value which gradually decreases to 4.15 \( \mu_B \) by ca. 20 K. Similar to compound 10, compound 12 exhibits a \( \mu_{\text{eff}} \) value of 5.14 \( \mu_B \) at room temperature which gradually decreases to 4.16 at ~ 20 K. The depression in the magnetic moment for the two \([\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}]^+\) compounds relative to their analogous \([\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}]^+\) species, throughout the full ca. 20–300 K temperature range, could arise from at least four different possibilities. The first possibility assumes an autoreduction of Cu(II) to Cu(I) by the Mn(II) center to form \([\text{Mn}^{\text{III}}(\text{imid})\text{Cu}^{\text{I}}]^+\) binuclear centers in compounds 10 and 12. The half-wave potential for the Mn(II)/Mn(III) couple in \([\text{Mn}^{\text{II}}(\text{TPP})]\) is \(-0.27\) V (SCE/CH\(_2\)Cl\(_2\)) which is very close to that of Cu(I)/Cu(II) couple of \([\text{Cu}^{\text{II}}(\text{imidH})_2\text{DAP}]^{2+}\) of \(-0.32\) V (SCE/CH\(_3\)CN). Thus, autoreduction might possibly occur upon formation of the \( \mu \)-imidazolato compounds. In fact, if the nature of the binuclear centers in compounds 10 and 12 consisted of \([\text{Mn}^{\text{III}}(\text{imid})\text{Cu}^{\text{I}}]^+\) cores, the expected \( \mu_{\text{eff}} \) values should be near 5.0 \( \mu_B \) or that for a high-spin \( S = 2 \) Mn(III) species, and this is near the value experimentally observed for both compounds. While the above UV–vis electronic spectral and electrochemical data for compounds 10 and 12
show no evidence for the presence of Mn(III), additional studies (ESCA, EXAFS, resonance Raman, and/or X-ray structural) will be needed to establish with certainty the oxidation state of manganese in these [Mn(imid)Cu]⁺ binuclear compounds. In contrast, the epr studies discussed below have definitively established the presence of $S = 5/2$ Mn(II) in compounds 9 and 11 which are the $M' = Zn$ analogues of 10 and 12. A second option that deserves consideration is the possible presence of an $S = 3/2$ Mn(II) center in 10 and 12, but not in 9 and 11. This situation would lead to a value of $\mu_{\text{eff}} \sim 5.0 \ \mu_B$ for 10 and 12 if one assumes no magnetic interaction between Mn$^{\text{II}}$ ($S = 3/2$) and Cu$^{\text{II}}$ ($S = 1/2$) and values of 4.5 $\mu_B$ for the intermediate-spin Mn(II) and 2.0 $\mu_B$ for Cu(II). This possibility can not be discounted by magnetochemistry alone, but in conjunction with the epr data below, it appears unlikely. A third option also invokes an intermediate spin state ($S = 3/2$) for the Mn$^{\text{II}}$ centers in 10 and 12, but assumes a strong ferromagnetic interaction ($+J \geq 200 \ \text{cm}^{-1}$) between Mn$^{\text{II}}$ ($S = 3/2$) and Cu$^{\text{II}}$ ($S = 1/2$) to give a resultant $S = 2$ spin state for the binuclear $[\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}]^+$ centers; this situation also leads to a value of $\mu_{\text{eff}} \sim 5.0 \ \mu_B$, and as discussed below, it is not inconsistent with the available epr data. However, no $\mu$-imidazolato model compounds to date, have shown a ferromagnetic interaction of any magnitude between metal centers, so this possibility is also considered to be unlikely in view of available information. The fourth and final option involves postulating a "strong" antiferromagnetic exchange interaction ($-J \geq 200 \ \text{cm}^{-1}$) between Mn(II) ($S = 5/2$) and Cu(II) ($S = 1/2$) to give a resultant $S = 2$
spin-coupled ground state for the binuclear center with $\mu_{\text{eff}} \sim 5.0$ $\mu_B$.
Like the ferromagnetic option above, the antiferromagnetic option invokes strong magnetic coupling across imidazolate. In this case, there is ample evidence, from model compound studies, for antiferromagnetism in $\mu$-imidazolato systems, albeit with $-J$ usually $\lesssim 100$ cm$^{-1}$. As discussed below, this interpretation involving antiferromagnetism, is also consistent with epr data on the present system, especially since compounds 9 and 11 (M' = Zn(II)) have been shown by the epr studies to contain $S = 5/2$ Mn(II). Furthermore, in this interpretation, compound 10 and 12 would serve as good electronic, but not necessarily structural, model compounds for the [Cyt.$\text{A}_3^{3+}$($S = 5/2$)-B-Cu$^{2+}(S = 1/2)$] active site of resting oxidase.

**Epr Spectroscopy.** Compounds 10-12 have also been examined by epr spectroscopy in both the polycrystalline and solid solution (CH$_2$Cl$_2$ glasses) states at 10 K. Epr spectral for the Mn(II) compounds are shown in Figure 23 and 24. The epr of [Mn$^{II}$(TPP)] as shown in Figure 23a, is anisotropic ($g_1 = 6.0$ and $g_2 = 2.04$), with a six-line hyperfine splitting pattern due to the $^{55}$Mn ($I = 5/2$) nucleus [61]. Compounds 9 and 11, both containing [Mn$^{II}$(imid)Zn$^{II}$]$^+$ cores, exhibit similar epr spectra to that of [Mn$^{II}$(TPP)] with $g_1 = 5.9$ and $g_2 = 2.04$. These observed $g$ values are typical of high-spin ($S = 5/2$) $d^5$ systems with zero field splittings greater than the microwave quantum [62,63]. This result confirms that the manganese porphyrin centers in 9 and 11 contain high-spin $d^5$ ($S = 5/2$) Mn(II). In addition, it indicates that the
Figure 23

Epr Spectra at 10 K of the $\mu$-Imidazolato Mn(II) Compounds in Frozen Solution as $\text{Cl}_2\text{Cl}_2$ Glasses

a) $[\text{Mn}^{\text{II}}(\text{TPP})]$  

b) $[(\text{TPP})\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}(\text{imidH})\text{DAP}](\text{X}); \text{X}^- = \text{BF}_4^-, \text{OSO}_2\text{CF}_3^-$  

c) $[(\text{TPP})\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}(\text{imidH})\text{DAP}](\text{X}); \text{X}^- = \text{BF}_4^-, \text{OSO}_2\text{CF}_3^-$
T = 10 K

a. \([\text{Mn}^{II}(\text{TPP})]\)

b. \([\text{Mn}^{II}(\text{imid})\text{Zn}^{II}]^+\)

c. \([\text{Mn}^{II}(\text{imid})\text{Cu}^{II}]^+\)

\(g = 6\)
\(g = 2\)
Figure 24

Epr Spectra at 10 K of the μ-Imidazolato Mn(II) Compounds as Polycrystalline Solids (~1.0% Mn(II) in Ammonium Sulfate)

a) [(TPP)Mn$^{II}$(imid)Zn$^{II}$(imidH)DAP](X); $X^- = BF_4^-$; $OSO_2CF_3^-$

b) [(TPP)Mn$^{II}$(imid)Zn$^{II}$(imidH)DAP](X); $X^- = BF_4^-$; $OSO_2CF_3^-$
\[ T = 10 \text{ K} \]

\[ \text{[Mn}^{II}(\text{imid})\text{Zn}^{II}]\text{BF}_4 \]

\[ \text{[Mn}^{II}(\text{imid})\text{Zn}^{II}]\text{OSO}_2\text{CF}_3 \]

\[ \uparrow \quad g = 6 \quad \downarrow \quad g = 2 \]

**Magnetic Field, G**

**Intensity**
<table>
<thead>
<tr>
<th>Compound</th>
<th>g factor</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Mn}^{\text{II}}(\text{TPP})])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BBF%7D_4">\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}</a>) (9)</td>
<td></td>
<td>(g_1 = 6.00)</td>
<td>(g_1 = 5.90) (weak)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BBF%7D_4">\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}</a>) (10)</td>
<td></td>
<td>(g_2 = 2.04)</td>
<td>(g_1 = 2.04) (weak)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Mn}^{\text{II}}(\text{imid})\text{Zn}^{\text{II}}</a>) (11)</td>
<td></td>
<td>(g_1 = 5.90)</td>
<td></td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BOSO%7D_2%5Ctext%7BCF%7D_3">\text{Mn}^{\text{II}}(\text{imid})\text{Cu}^{\text{II}}</a>) (12)</td>
<td></td>
<td>(g_1 = 4) (weak)</td>
<td>(g_1 = 2) (weak)</td>
</tr>
</tbody>
</table>

Table: Epr Parameters at 10 K for the Mn(II) Compounds in Frozen Solution as \(\text{CH}_2\text{Cl}_2\) Glasses
TABLE Xb

**Epr Parameters at 10 K for the Mn(II) Compounds as Polycrystalline Solids (~ 1% Mn(II) in Ammonium Sulfate)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>g factor</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Mn}^{II} (\text{imid})\text{Zn}^{II}] (\text{BF}_4)) (9)</td>
<td></td>
<td>(g_1 = 5.90)</td>
<td>(g_1 \sim 2) (weak)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(g_2 = 2.04)</td>
<td>(g_1 \sim 2) (weak)</td>
</tr>
<tr>
<td>([\text{Mn}^{II} (\text{imid})\text{Cu}^{II}] (\text{BF}_4)) (10)</td>
<td></td>
<td>(g_1 \sim 4) (weak)</td>
<td>(g_1 \sim 2) (weak)</td>
</tr>
<tr>
<td>([\text{Mn}^{II} (\text{imid})\text{Zn}^{II}] (\text{O}_2\text{S}_2\text{CF}_3)) (11)</td>
<td></td>
<td>(g_1 = 5.90)</td>
<td>(g_2 = 2.04)</td>
</tr>
<tr>
<td>([\text{Mn}^{II} (\text{imid})\text{Cu}^{II}] (\text{O}_2\text{S}_2\text{CF}_3)) (12)</td>
<td></td>
<td>(g_1 \sim 4) (weak)</td>
<td>(g_1 \sim 2) (weak)</td>
</tr>
</tbody>
</table>
electronic environment about the Mn(II) centers in 9 and 11 are similar to that of [Mn\textsuperscript{II}(TPP)]. This conclusion is also reiterated by comparing the spectrum of [Mn\textsuperscript{II}(TPP)] with the solid state spectra of 9 and 11 as shown in Figure 24. Furthermore, this observation is in good agreement with the previously described magnetochemical data.

As discussed above, compounds 10 and 12, with their [Mn(imid)Cu]\textsuperscript{+} cores, appear to possess unusual magnetochemical properties when compared to their Zn(II) counterparts. Specifically, their $\mu_{\text{eff}}$ values at all temperature are considerably below those expected for a binuclear compound containing isolated $S = 5/2$ Mn(II) and $S = 1/2$ Cu(II) centers. The epr spectra of 10 and 12 in Figure 23, consist of very weak and broad multiple resonances at $g \sim 4$ (Mn) and $g \sim 2$ (Mn and/or Cu). The $g \sim 4$ value for Mn is considerably different from those of a typical axial high-spin ($S = 5/2$) Mn(II) center. Integration of the $g = 4$ and $g = 2$ signals against epr signals for known concentrations of [Mn\textsuperscript{II}(TPP)] and [Cu\textsuperscript{II}(imid\textsuperscript{H})\textsubscript{2}DAP](OSO\textsubscript{3}CF\textsubscript{3})\textsubscript{2} in frozen CH\textsubscript{2}Cl\textsubscript{2} or CH\textsubscript{3}CN glasses at 10 K, indicate that the observed signals in Figure 23c account for < 1% of Mn and Cu present. Thus, it can be concluded that the [Mn(imid)Cu]\textsuperscript{+} cores of 10 and 12 are effectively epr silent, with the observed very weak signals probably being due to small amounts of manganese and copper impurities. Attempts to obtain solid state epr spectra of 10 and 12, under the experimental condition used in Figure 24, were also unsuccessful. Furthermore, these same epr results for 10 and 12 were
obtained for three different sample preparations on three separate occasions.

In the above magnetochemistry section, it was noted that at least four alternatives could adequately account for the reduced magnetism of compounds 10 and 12 relative to their Zn(II) analogues (9 and 11, respectively): (1) 9 and 11 contain [Mn^{II}(imid)Zn^{II}]^+ cores while 10 and 12 contain [Mn^{III}(imid)Cu^{I}]^+ cores with high-spin S = 2 Mn(II), (2) 9 and 11 contain [Mn^{II}(imid)Zn^{II}]^+ cores with S = 5/2 Mn(II) while 10 and 12 contain [Mn^{II}(imid)Cu^{II}]^+ cores with S = 3/2 Mn(II) and no magnetic interaction between Mn(II) and S = 1/2 Cu(II), (3) 10 and 12 contain [Mn^{II}(imid)Cu^{II}]^+ cores with S = 3/2 Mn(II) and S = 1/2 Cu(II) spin-coupled through a strong ferromagnetic interaction to give a resultant S = 2 ground state, and (4) 10 and 12 contain [Mn^{II}(imid)Cu^{II}]^+ cores with S = 5/2 Mn(II) and S = 1/2 Cu(II) strongly coupled antiferromagnetically to give a resultant S = 2 ground state.

The present epr (and above UV-vis spectroscopy) results comment very constructively concerning these four alternatives. For possibility (1), a high-spin Mn(III) center would be an even spin S = 2 system, and therefore the silent epr spectra for 10 and 12 support this possibility. Then again, the above UV-vis electronic spectral data do not argue convincingly in favor of a Mn(III) porphyrin compound. For possibility (2), an isolated intermediate-spin (S = 3/2) Mn(II) center should possess a strong epr spectrum at g ~ 4 as does the S = 3/2 Mn(II)
compounds similar to those of intermediate-spin \((S = 3/2)\) Fe(III) \([25]\). Unless some rapid relaxation process, similar to that proposed by Elliott and Akaburi \([64]\) for their \([\text{Fe}-\text{S}-\text{Cu}]\) system, is operating to broaden the Mn(II) and Cu(II) signals, possibility (2) therefore also seems to be unlikely. Additionally, it is difficult to rationalize why the Mn(II) spin state would differ so in compound 9 and 11 \((S = 5/2)\) and 10 and 12 \((S = 3/2)\). This leads to consideration of possibilities (3) and (4), which both assume strong magnetic coupling across imidazolate to give spin-coupled, epr silent \(S = 2\) ground states for the binuclear centers. For possibility (3), the proposed coupling would be ferromagnetic in nature \((+J \gtrsim 200 \text{ cm}^{-1})\), whereas for (4) it would be antiferromagnetic \((-J \gtrsim 200 \text{ cm}^{-1})\). Since there is presently no evidence for an \(S = 3/2\) spin state for Mn(II) in compounds 10 and 12 and because ferromagnetism is undocumented for all known \(\mu\)-imidazolato species, possibility (3) seems quite unlikely, leaving (4) as the leading candidate at this time. This interpretation, echoes the conclusions of Dessens, et al., \([13]\) and those of the above discussed binuclear Fe(III) chemistry, in ascerting that imidazolate bridges can, in certain circumstances, foster strong antiferromagnetic exchange interactions \((-J \gtrsim 200 \text{ cm}^{-1})\). This conclusion is contrary to most other \(\mu\)-imidazolato model compounds results (including some of our own \([8c]\)) which have indicated an upper limit of only ca. \(-J \lesssim 100 \text{ cm}^{-1}\) for imidazolate bridges. It thus appears that \(\mu\)-imidazolato chemistry will remain a futile area of investigation for some time to come, and in this connection, the PBV model of \([\text{cyt.} A_{3}^{3+}(\text{imid})\text{Cu}_{4}^{2+}]\) with \(-J \gtrsim 200 \text{ cm}^{-1}\) for the active site
structure of resting oxidase continues survival, albeit tenuous, as a viable alternative.
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27. Ocone, L.R.; Block, B.P. Inorganic Synthesis. (1939), 2, 125.


