Synthesis, X-ray Crystal Structure, and Solution Behavior of $Fe(NO)_2(1\text{-MeIm})_2$: Implications for Nitrosyl Non-Heme-Iron Complexes with $g = 2.03$

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The biological importance of nitric oxide (NO) has attracted much attention in the past decade.[1,2] Extensive EPR studies have identified nitrosyl non-heme-iron complexes as products after biosynthetic evolution of NO in vitro,[3−6] and from the addition of NO to iron-centered proteins.[7,8] Muller *et al.*[9] and other groups[10,11] have also reported evidence for nitric oxide storage as nitrosyl non-heme-iron complexes. Recently, a light-sensitive nitrile hydratase from *Rhodococuss* sp. N771 was found to have an NO molecule bound to the non-heme-iron center.[12] This enzyme is activated upon irradiation, followed by the release of NO from the iron center. These nitrosyl non-heme-iron complexes are paramagnetic complexes of the type "$Fe(NO)_2$", more commonly referred to as "$g = 2.03$" complexes because of their characteristic isotropic *g*-factor. However, the isolation and structural determination of these compounds by means other than IR and EPR are both extremely tedious and difficult, and to our knowledge none are known. To gain understanding of the structures of these nitrosyl non-heme-iron complexes, we have recently isolated the first non-heme-iron complex that incorporates an imidazole moiety as well as nitrosyl ligands and is of the $g = 2.03$ family. Here we report the synthesis, X-ray crystal structure, and NMR and EPR studies of $Fe(NO)_2$(1-methylimidazole)$_2$ (**1**).

Syntheses were carried out under dry nitrogen atmosphere using glovebag and drybox techniques. Reactions of $Fe(NO)_2(CO)_2$ (1.82×10^{-3} mol) and excess of 1-methylimidazole (3.77×10^{-3} mol) were carried out in diethyl ether in test tubes with ground glass joints which were stoppered with Teflon valve stopcocks. The reactions were very rapid, changing color from red to green immediately and with gas evolution. After 1 h, green crystals formed that were of X-ray crystallographic quality.[13] These crystals were placed under paraffin oil which had been previously degassed and dried over sodium. One was selected and sealed in a capillary tube under nitrogen.

The X-ray crystal structure[14] of **1**, shown in Figure 1, provides the first direct structural insight concerning the "2.03" family of non-heme-iron nitrosyls. Compound **1** is monoclinic and crystallizes in a *C*2/*c* space group, with eight molecules per unit cell. The complex is pseudo-tetrahedral with a d^{10} iron center. The nitrosyl groups are linear (167.5(3)°, 170.1(3)°). The Fe−N(1) and Fe−N(2) bond distances of the nitrosyls are 1.648(3) and 1.650(3) Å, respectively. The Fe−N−O groups are bent symmetrically, with a O−Fe−O angle of 107.3°, as compared with the N(NO)−Fe−N(NO) angle of 116.6°. This is considered an "attracto" conformation because the N−M−N bond angle is less than 130° and the two oxygen atoms bend toward each other.[15] "Attracto" conformations are generally favored for first-row transition-metal dinitrosyls containing ligands that are good π-acceptors.[16] The dihedral angel between the planes of the two 1-methylimidazole ligands is 106.7°. The Fe−N(3) and Fe−N(5) bond distances of the 1-methylimidazole are 2.048(3) and 2.044(3) Å, respectively. Crystal packing reveals a layering of nitrosyl ligands and a layering between imidazole ligands, both down the *bc* plane. This crystal structure can be compared with the tetrahedral compound [(DAD)$Fe(NO)_2$] (DAD = diazadiene), in which the Fe−NO distances and the Fe−N(DAD) distances are 1.642, 1.641 and 2.037, 2.043 Å, respectively.[17] It is also of interest to compare this structure with the 3,5-dimethylpyrazolyl iron dinitrosyl dimer, $[(N_2C_5H_7)Fe(NO)_2]_2$, in which the mean Fe−NO distance is 1.696(2) Å and the mean Fe−N(pyrazolyl) distance is 2.009(5) Å.[18] Both Fe−N−O groups in the dimer are bent symmetrically, with an average O−Fe−O angle of 97.9(1)° with respect to the average N−Fe−N angle of 110.6(1)°, which also possess an "attracto" conformation. Rettig *et al.* recently published a polymeric iron(II) imidazole complex $[Fe(Im)_4]_n$, involving octahedral and tetrahedral iron centers.[19] The tetrahedral iron centers have an average Fe−N(Im) distance of 2.046 Å, which is not significantly different from the average Fe−N distance for the $Fe(NO)_2(1\text{-MeIm})_2$ compound. In the effort of modeling non-heme-iron enzymes, Hagadorn *et al.* recently reported the X-ray crystal structure of iron with methylimidazole and carboxylate 2,6-dimethylbenzoate ligands, $(Mes_2\text{-}ArCO_2)_2$-$Fe(MeIm)_2$, in which the average distance of the Fe−N(MeIm) was 2.062(2) Å, which is longer than the average Fe−N distance for the $Fe(NO)_2(1\text{-MeIm})_2$ compound.[20]

^{1}H and ^{13}C NMR spectra were measured by reacting 2 equiv of ligand with 1 equiv of $Fe(NO)_2(CO)_2$ in deuterated methanol. After gas evolution subsided, an aliquot was removed and placed in an NMR tube, where the sample was degassed by the use of freeze−pump−thaw procedures and then flamed-sealed under vacuum. The proton spectrum for **1** shows a mixture of 1-MeIm and $Fe(NO)_2(1\text{-MeIm})_2$ in solution. The broad signal at 8.8 ppm is attributed to H4 and H5 of 1-MeIm. The signal at 5.0 ppm is assigned to H2, and the broad peak at 4.0 ppm is attributed to the N-CH_3 group. The ^{13}C spectrum for **1** also shows a mixture of 1-MeIm and $Fe(NO)_2(1\text{-MeIm})_2$ in solution. The signals at 226.2 and 225.6 ppm are attributed to the C1 and C3 carbons,

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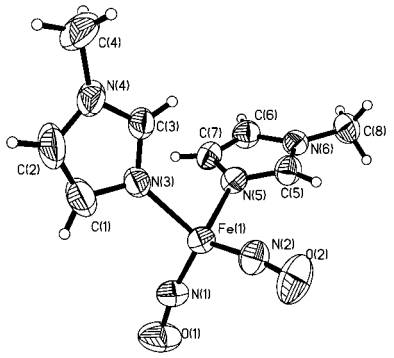

Figure 1. X-ray structure of $Fe(NO)_2(1\text{-MeIm})_2$, showing the atomic numbering scheme. Aniostropic thermal displacement ellipsoids are shown at the 50% probability level. Selected bond distances and angles: Fe−N1/Fe−N2 = 1.648(3)/1.650(3) Å; Fe−N3/Fe−N5 = 2.044(3)/2.048(3) Å; N1−O1/N2−O2 = 1.189(3)/1.188(4) Å; N1−Fe−N2 = 116.57(14)°; N3−Fe−N5 = 91.20(11)°; N1−Fe−N3/N1−Fe−N5 = 111.28(13)/112.76(12)°; N2−Fe−N3/N2−Fe−N5 = 114.43(13)/107.78(13)°.

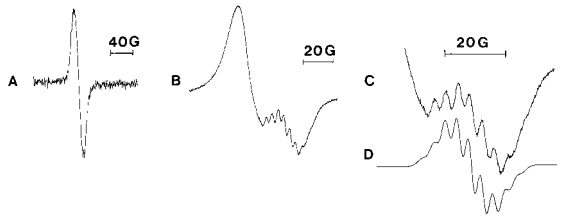

Figure 2. First derivative EPR spectra of the reaction of $Fe(NO)_2(CO)_2$ with 1-MeIm in diethyl ether. (a) EPR spectrum of $Fe(NO)_2(CO)_2$ (g = 2.0275) at room temperature. (b) EPR spectrum reveals new radical (g = 2.0151) formation (nine-line) after the addition of 1-MeIm at room temperature. (c) Experimental expansion of the nine-line EPR spectrum at 240 K. (d) Computer simulation with a_{N1} = 3.6 G and a_{N2} = 3.9 G for the signal of $Fe(NO)_2(1\text{-MeIm})_2^+$.

respectively. The peak at 143.4 ppm is assigned to the C2 carbon, and the broad peak at 38.3 ppm is attributed to the C4 (methyl) carbon. The tetrahedral d^{10} should be diamagnetic; however, it appears that some paramagnetic effects of iron on line broadening are occurring. The reaction proceeded rapidly, which leads us to believe that a 17-electron iron radical intermediate facilitates the rapid substitution of carbonyls by 1-methylimidazole ligands. This phenomenon was observed in iron carbonyl-based substitution reactions using pyridine[21] and iron nitrosyl carbonyl substitutions with tetracyanoethylene[22] or in the presence of reducing ligands.[23]

The reaction of $Fe(NO)_2(CO)_2$ and 1-methylimidazole with an approximate initial concentration of 0.7 M was monitored with EPR spectroscopy, as shown in Figure 2. At room temperature, the starting material, $Fe(NO)_2(CO)_2$, shows a broad peak with g = 2.0275 and ΔH_{pp} = 18.5 G that is attributed to the presence of $[Fe(NO)_2(CO)_2]^+$.[24] Upon addition of 1-MeIm ligand, a new signal (g = 2.0151) was observed which can be resolved to a well-split, nine-line spectrum at 240 K. Simulation of the second radical[25] gave rise to two sets of equivalent nitrogens (^{14}N, I = 1), with hyperfine coupling constants of 3.6 and 3.9 G, respectively. The hyperfine structure is attributable to the coupling of the two equivalent ^{14}N nuclei from the nitrosyls and two equivalent nuclei from the 1-MeIm, yielding a structure of $Fe(NO)_2(1\text{-MeIm})_2^+$, the 17-electron intermediate. These EPR signals arising from the intermediates disappeared upon the formation of the crystalline compound, $Fe(NO)_2(1\text{-MeIm})_2$.

To mimic biological systems, the reaction of $Fe(NO)_2(CO)_2$ with 4-methyl imidazole, imidazole, benzimidazole, 5,6-dimethylbenzimidazole, and L-histidine were also investigated by EPR spectroscopy. The g-values for these radicals fall in the range of 2.0151−2.0352 and a_N in the range of 1.88−3.90 G. These g values are typical for iron nitrosyl radicals with an unpaired electron localized on the Fe center.[25] These EPR spectra are obviously the same as those previously observed, obtained by directly reacting an Fe^{2+} salt and gaseous NO.[26]

Addition of 1-MeIm shifted the IR stretching frequencies of nitrosyls from 1810 and 1767 cm^{-1} [ν_{NO} for $Fe(NO)_2(CO)_2$] to 1673 and 1616 cm^{-1}, suggesting that 1-MeIm acts as a strong σ-donor. To explain the trend in the IR stretching frequencies, EHMO calculations were performed using X-ray data of **1** directly and with idealized structures possessing C_2 and C_s symmetry.[27] Interaction diagrams from these three sets of calculations show that the occupied molecular orbitals from the 1-MeIm fragment and the $Fe(NO)_2$ fragment have similar energy levels. However, in each case, the energy gap between the 1-MeIm fragment (in the range of 3.7−3.9 eV) is larger than that of the $Fe(NO)_2$ fragment (∼1.3 eV). In other words, the FMO LUMO of the imidazole unit is of higher energy than the FMO of $Fe(NO)_2$ unit. Therefore, the electrons from the imidazole fragment fill in the molecular orbitals first, followed by the occupation from the d electrons of the $Fe(NO)_2$ fragment to the HOMO. These d electrons are easier to back-donate into the lower FMO LUMO of the $Fe(NO)_2$ unit than to the LUMO of the imidazole fragment. Therefore, the imidazole ligands act as electron donors rather than π-acceptors. In addition, the HOMO has characteristics of the metal d-orbitals, which is also consistent with the EPR results that the unpaired electrons reside on the iron center. The net positive charge on Fe decreases when 1-MeIm ligands replace the CO ligands, while the negative charges on the nitrogens of the imidazole ligands also decrease, again inferring that the 1-MeIm ligands are donating electron density onto iron. The donation of electron density onto the iron center also results in a decrease of the positive charges on the nitrogens of the nitrosyls, which indicates the increase of the back-bonding into the π^* orbital of the NO ligand. This explains the weakening of the NO bond and shifting of the NO stretches.

In conclusion, we have demonstrated that iron nitrosyl complexes interact with imidazole based ligands to yield "g = 2.03" radicals, and **1** was fully characterized by NMR, IR, MS, and X-ray crystallography. These findings challenge biological chemists to determine whether the radicals they observe at g = 2.03 could be not only iron nitrosyl with thiols or cysteine residue, but also iron nitrosyl attached to other amino acids of proteins. Further studies are underway to isolate other g = 2.03 species and to establish the structures in biological systems containing nitrosyl non-heme-iron and amino acids of proteins.

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Supporting Information Available: X-ray structural data for **1**, including a summary of crystallographic parameters, atomic coordinates, bond distances and angles, and anisotropic thermal parameters (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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