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Notes

^{13}C NMR Spectra of Carboxylate-Bridged Paramagnetic Dinuclear and Tetranuclear Iron Oxo Complexes

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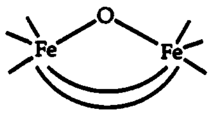
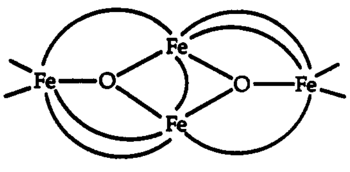
Nuclear magnetic resonance (NMR) spectroscopy has become a valuable technique for the identification of ligands coordinated to iron in polyiron oxo complexes and proteins.[1] Current understanding of the solution structure and magnetic properties of several diiron centers relies substantially upon the interpretation of ^1H and ^2H isotropic shifts, as well as ^1H T_1, 2D, nuclear Overhauser effect (NOE), and saturation transfer experiments on proteins[2] and model complexes.[3] ^1H NMR resonances of carboxylate ligands bound to polynuclear iron(III) oxo centers with appreciable magnetic exchange interactions are characterized by small isotropic contact shifts and broad linewidths. In diiron proteins, these weak resonances appear near or within the diamagnetic region of the protein ^1H NMR spectrum (~0–15 ppm)[4] and have been difficult to detect;[2] the first definitive NMR assignment of a coordinated carboxylate in a paramagnetic iron protein was demonstrated only recently.[2b] Despite the poor receptivity of ^{13}C versus ^1H nuclei, we were interested to learn whether ^{13}C NMR spectroscopy could be used as a structural tool, for example to distinguish the carboxylate-bridged μ-oxo dinuclear and tetranuclear iron(III) cores shown as follows:



$\{Fe_2O(O_2CR)_2\}^{2+}$



$\{Fe_4O_2(O_2CR)_7\}^{+}$

In this initial study, we report the first ^{13}C NMR spectra of isotopically enriched and natural abundance carboxylates of the (μ-oxo)bis(μ-carboxylato)diiron(III) unit. The results demonstrate the potential of this approach to detect and study carboxylate coordination to paramagnetic polyiron oxo centers.

Experimental Section

The compounds $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$, $[Fe_2O(O_2{}^{13}CH)_2(HBpz_3)_2]$ and $(Et_4N)[Fe_4O_2(O_2C^{13}CH_3)_7(H_2Bpz_2)_2]$ were synthesized from 99% $NaO_2C^{13}CH_3$ (ICON Services Inc.) and 99% $NaO_2{}^{13}CH$ (CIL) along with their natural abundance analogues by published procedures.[3a,5] All solvents, reagents, and other chemicals were obtained from commercial sources and used without additional purification. $\{^1H\}^{13}C$ NMR spectra were obtained on Bruker WM270 or Varian VXR500 spectrometers. A large number of transients, often greater than 50 000, were required to observe clearly all resonances. Delay times could be reduced to 0.5 seconds or less because of the fast relaxation properties of the paramagnetic complexes, shortening the length of time required to collect data. All NMR spectra were recorded at room temperature (22 °C) with saturated or nearly saturated solutions of iron complexes. Diamagnetic chemical shift values for the acetate anion in $CDCl_3$, methyl and carbonyl shifts of 20.8 and 177.6 ppm, respectively, and the formate anion in H_2O, 171.3 ppm, were used to calculate isotropic shifts.[6]

Results and Discussion

Proton decoupled ^{13}C NMR spectra are shown in Figure 1 for $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$, $[Fe_2O(O_2{}^{13}CH)_2(HBpz_3)_2]$ and $(Et_4N)[Fe_4O_2(O_2C^{13}CH_3)_7(H_2Bpz_2)_2]$, where $HBpz_3^-$ and $H_2Bpz_2^-$ represent hydrotris- and dihydrobis(1-pyrazolyl)borate. Labeling with $^{13}CH_3CO_2^-$ was necessary in order to observe the methyl resonance of the coordinated acetate ligand shown Figures 1a and 1c. The acetate methyl carbon of $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$ is apparent at 250 ppm, as are weaker broad resonances at 228 and 163 ppm expected to result from the pyrazole ring carbons. The narrower resonance at 191 ppm is assigned to the isotopically unenriched carbonyl carbon of the acetate ligand, since it is absent in the spectrum of the formate analogue. As in the ^1H NMR spectrum of $[Fe_2O(O_2CCH_3)_2(HBpz_3)_2]$, the ^{13}C spectrum is also consistent with the integrity of the structure being maintained in solution.[3a] The large isotropic shift (230 ppm)[6] and significant broadening (4800 Hz) of the acetate methyl ^{13}C resonance may be compared with the corresponding properties (8.4 ppm and 400 Hz respectively) of the methyl ^1H NMR resonance for this compound.[3a] To our knowledge, there are no reports of ^{13}C NMR resonances of acetate coordinated to comparable paramagnetic high spin iron(III) complexes, e.g. $[Fe(TPP)(O_2CCH_3)]$ and $[Fe(salen)(O_2CCH_3)]$.[2b,7]

The carbon atom of the formate group is observed as a prominent resonance at 142 ppm in the spectrum of $[Fe_2O(O_2{}^{13}CH)_2(HBpz_3)_2]$ (Figure 1b). The two pyrazole ring carbons appear as broad, much weaker resonances at the same positions as in the acetate complex. As for the acetate carbonyl carbon, the formate ^{13}C resonance can be observed in the natural abundance spectrum. The ^1H NMR resonances of the formate group in the $\{Fe_2O(O_2CH)_2\}^{2+}$ center are not observed owing to

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0020-1669/93/1332-1030$04.00/0

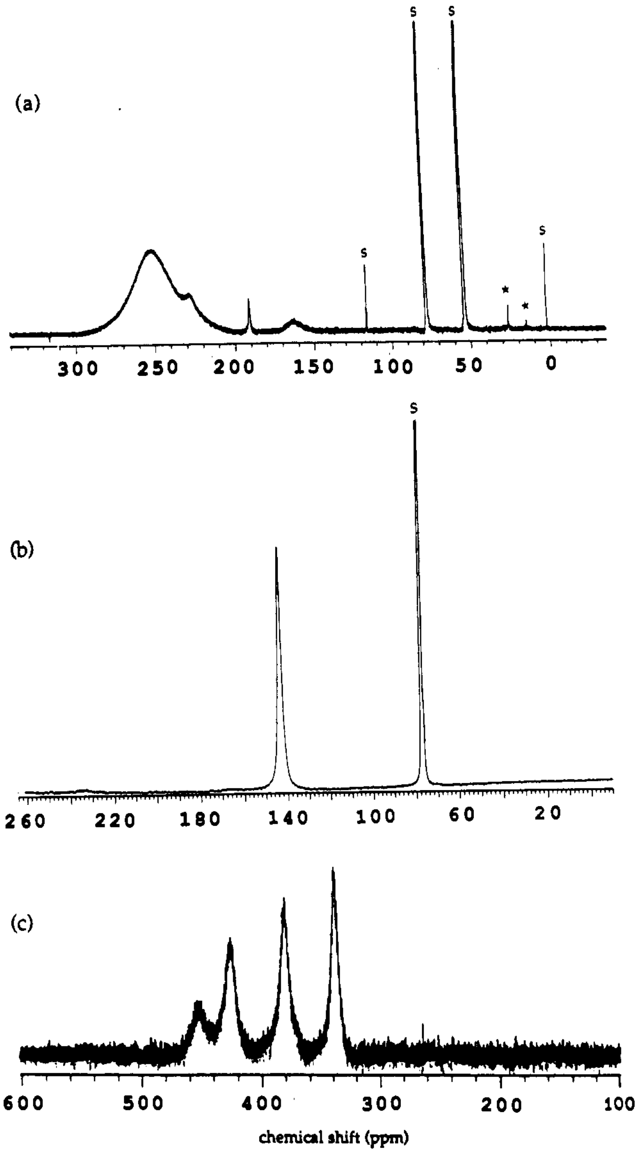

Figure 1. $\{^1H\}^{13}C$ NMR spectra of ^{13}C labeled carboxylate bridged polyiron oxo complexes at 125.7 MHz: (a) $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$ in $CD_2Cl_2/CDCl_3$; (b) $[Fe_2O(O_2{}^{13}CH)_2(HBpz_3)_2]$ in $CDCl_3$; (c) $[Fe_4O_2(O_2C^{13}CH_3)_7(H_2Bpz_2)_2](Et_4N)$ in CD_2Cl_2. The peaks assigned to solvent and residual acetonitrile resonances are marked with an S. Spectra were obtained at 295 K.

large linewidths.[3a,h] The relatively narrow linewidth and considerable intensity of the carbonyl resonance in $[Fe_2O(O_2{}^{13}CH)_2(HBpz_3)_2]$ illustrate the effectiveness of ^{13}C NMR spectroscopy to observe formate, as well as acetate, bridging bidentate coordination to (μ-oxo)diiron(III) complexes. The intensity of the formate carbonyl resonance may arise in part from the NOE observed in proton decoupled ^{13}C NMR. Typically, this effect is reduced or eliminated by paramagnetic nuclei;[8] however, ^1H NMR NOE and 2D NOESY measurements have been reported for ligands coordinated to the dimetallic center in reduced and $Fe^{III}Co^{II}$ uteroferrin.[2b,2c]

Both the acetate and formate carbonyl carbon resonances exhibit surprisingly smaller linewidths (150 and 300 Hz) and isotropic shifts (13 and –29 ppm) compared to the methyl carbon resonance. Small line broadening of ^{13}C enriched and natural abundance carbonyl resonances is also observed in the ^{13}C NMR spectra of ethylenediaminetetraacetate and amino acid Ni(II) complexes.[9] Moreover, the ^{13}C resonances of carbon atoms more remote from the ligand donors in these nickel complexes are downfield shifted and broadened compared to the carbonyl resonance, properties shared by the acetate methyl resonance of $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$. Interestingly, the downfield shift of the acetate carbonyl carbon of $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$ is in the opposite direction from the shift of the formate analogue. A similar shift reversal occurs for some pyridine ring carbon atoms in Ni(II) complexes upon substitution of a methyl group for a ring proton.[10]

The isotropic shifts,[11] a consequence of transferring unpaired electron spin density from a metal center out onto a ligand, of the Ni(II) ^{13}C NMR examples discussed above are dominated by a π-spin delocalization mechanism.[9] These shifts are contact in origin since dipolar interactions are negligible for octahedral Ni(II) complexes with orbitally non-degenerate ground states.[11] Since high-spin Fe(III) complexes are also orbital singlets, this delocalization pathway is likely to be the prevailing mechanism for our complexes. This conclusion is consistent with the π-spin delocalization mechanism deduced from ^1H NMR studies of bridging bidentate carboxylates coordinated to high spin diiron(III) complexes.[3b,12] We suggest that there is little or no unpaired electron spin density at the carbonyl carbon atom, which accounts for the lack of significant line broadening and shifts for these atoms. Other possible explanations are not ruled out, however.

The spectrum of $(Et_4N)[Fe_4O_2(O_2C^{13}CH_3)_7(H_2Bpz_2)_2]$ (Figure 1c) exhibits resonances at 454, 425, 380, and 338 ppm assigned to the methyl carbon atoms. The poor solubility of this complex in CD_2Cl_2 precluded identification of the remaining resonances. The occurrence of four distinct bands is in good agreement with the presence of four chemically inequivalent bridging carboxylate ligands in this complex.[5] The ^{13}C resonances are shifted much farther downfield than the corresponding acetate methyl peak in $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$. The large chemical shift difference between acetate bound to $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$ versus $(Et_4N)[Fe_4O_2(O_2C^{13}CH_3)_7(H_2Bpz_2)_2]$ emphasizes the difference between the magnetic moment of the tetranuclear $\{Fe_4O_2\}^{7+}$ species ($\mu_{eff/Fe} = 2.34\,\mu_B$)[5] compared to the more strongly coupled $\{Fe_2O\}^{4+}$ center ($\mu_{eff/Fe} = 1.71\,\mu_B$).[3a] Variable temperature studies of high-spin μ-oxodiiron(III) porphyrins have been reported that take advantage of the superior dispersion of ^{13}C NMR spectroscopy to study small changes in magnetic coupling.[13]

Distinguishing between different types of polyiron oxo centers, e.g. $\{Fe_2O\}^{4+}$ versus $\{Fe_4O_2\}^{7+}$, and structurally inequivalent bridging carboxylate ligands is sometimes difficult to accomplish by ^1H NMR spectroscopy because of its smaller chemical shift range.[5,14] For example, in the ^1H NMR spectrum of $[Fe_2O(O_2CCH_3)_2(HBpz_3)_2]$, the acetate methyl resonance is observed at 10.5 ppm overlapping a pyrazole ring peak at 9.1 ppm.[3a] In the same chemical shift region, $[Fe_4O_2(O_2CCH_3)_7(HBpz_3)_2]^-$ exhibits several ^1H NMR peaks, three of which were tentatively

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assigned to four structurally unique carboxylates;[5] again they overlap several pyrazole ring resonances. In the ^{13}C NMR spectrum of $(Et_4N)[Fe_4O_2(O_2C^{13}CH_3)_7(H_2Bpz_2)_2]$, the magnetic inequivalence of the four bridging carboxylates is clearly delineated. Moreover, the chemical shifts of these resonances differ substantially from the isotropically shifted methyl group in $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$.

From these results we conclude that ^{13}C NMR spectroscopy is useful for determining the number of inequivalent carboxylates bound to oxygen-bridged diiron centers and provides a means of detecting differences in the magnetic coupling in these complexes, complementing the established correlation between magnetic coupling and ^1H isotropic shifts.[3b,3d] As demonstrated in ^1H NMR studies,[3b] different carboxylate binding modes in paramagnetic complexes are likely to result in disparate ^{13}C NMR shift patterns. ^{13}C NMR spectroscopy should therefore be used in conjunction with other available characterization methods. Although a more comprehensive study is required to reveal the generality of this technique, we found ^{13}C NMR spectroscopy in combination with other solution data to be valuable in assigning the structure of the diiron oxo complex $[Fe_2O(MPDP)(HBpz_3)_2]$, ($MPDP^{2-}$ = *m*-phenylenedipropionate), prior to its confirmation by X-ray crystallography.[3g,15]

The chemical shift of the methyl resonance in $[Fe_2O(O_2C^{13}CH_3)_2(HBpz_3)_2]$ falls well outside the diamagnetic range of ^{13}C NMR shifts observed in proteins (~0–200 ppm).[4] Considering the breadth and intensity of the lines, and the labeling requirement for the methyl carbon, ^{13}C NMR studies of paramagnetic polyiron oxo centers in proteins may not be practical. Protein ^{13}C NMR resonances of carboxylate bound to diiron oxo proteins may be enhanced through biosynthetic incorporation of a labeled carboxylate amino acid by using an auxotroph. ^{13}C enriched histidine coordination to the active site of the metalloprotein alkaline phosphatase was studied in this manner.[16] Alternatively, the presence of many equivalent carboxylate iron binding sites, as may occur in ferritin (see ref 1a, p. 124), could amplify the intensity of the ^{13}C carboxylate resonance. Addition of ^{13}C-labeled carboxylates as a method for probing the binding and exchange of exogenous ligands to the diiron core may be more feasible.

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