Contribution from the Departments of Chemistry, University of Iowa, Iowa City, Iowa 52242, and University of Minnesota, Minneapolis, Minnesota 55455

Hydrogen and Deuterium NMR Studies of Carboxylate Coordination to Iron(III) Complexes: Diverse Chemical Shift Values for Coordinated Carboxyl Residues

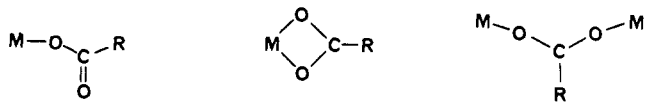
Isam M. Arafa,[1a] Harold M. Goff,*[1a] Sheila S. David,[1b] Bruce P. Murch,[1b] and Lawrence Que, Jr.*[1b]

Received September 24, 1986

Proton and deuterium NMR signals for coordinated carboxyl residues of high-spin iron(III) and manganese(III) porphyrin complexes have been unambiguously assigned through examination of the deuteriated carboxylate derivatives. Signals are shifted downfield in the region between 15 and 32 ppm for these paramagnetic compounds contained in chlorinated solvents. However, this chemical shift value is attenuated with respect to that observed at 140 ppm for the acetate proton signal of the (*N,N'*-ethylenebis(salicylideneaminato))(acetato)iron(III) Schiff base complex. Other non-heme iron(III) carboxylate complexes also give coordinated acetate proton signals in the 140 ppm region. The metalloporphyrin acetate signal is highly solvent dependent, and the signal does not follow Curie law behavior in variable-temperature experiments. Specific solvation effects for the carbonyl residue are suggested. These effects and the differing magnitudes for carboxylate chemical shifts in iron(III) porphyrin complexes and non-heme iron(III) complexes suggest monodentate carboxylate coordination to the iron(III) porphyrin and bidentate coordination in the other iron(III) complexes investigated. Given the wide range of carboxyl chemical shift values, identification of amino acid carboxylate binding in metalloproteins by NMR chemical shift values alone is thus highly problematic.

Introduction

Carboxyl groups may exhibit a number of metal coordination geometries, including monodentate (**1**), bidentate (**2**), and bridging bidentate (**3**). The carboxyl moieties of aspartic and glutamic



1 2 3

acids provide important metal binding sites in metalloproteins, and carboxyl bridging as in **3** has recently been delineated for the hemerythrins.[2,3] Appropriate model compounds have been prepared in which both μ-oxo and μ-carboxylato residues are incorporated.[4]

The potential for carboxyl binding to monomeric iron centers in proteins clearly must be considered. Evidence for aspartate–heme binding has been offered for mutant hemoglobins in which the proximal histidine amino acid has been replaced by aspartic acid.[5] Possibilities for assignment of axial amino acid or bound substrate carboxylate proton NMR signals in heme and non-heme iron proteins merit detailed examination of appropriate model compounds. Previous NMR studies with amino acids and polydentate amine–carboxylate ligand systems have focused on solution conformation and structure determinations of paramagnetic nickel(II) complexes.[6] These adducts unfortunately do not serve as appropriate model compounds for metalloprotein NMR signal assignments, due to differences in the central metal ion and the structural dissimilarities between the polydentate framework and protein side chains. The model compound studies described here will serve to demonstrate an exceedingly wide range of carboxylate chemical shift values for heme and non-heme iron(III) and manganese(III) complexes.

Experimental Section

Tetraarylporphyrins[7] and etioporphyrin I[8] were prepared by literature methods. Standard metal incorporation and purification methods were employed.[9] The acetate and propionate complexes of iron(III) tetraphenylporphyrin, (tpp)Fe^{III}, were prepared as recently described.[10,11] Preparation consisted of a 20-min reflux reaction step in which the μ-oxo dimeric $[(tpp)Fe]_2O$ compound was dissolved in the neat carboxylic acid. The warm reaction mixture was concentrated under reduced pressure and allowed to cool to room temperature. Crystalline product was recovered by filtration and was recrystallized from the appropriate carboxylic acid. The final crystalline product was dried under vacuum at 120 °C. The (acetato)iron(III) etioporphyrin I complex was prepared by the same procedure. The 2,2-dichloropropionate and bromoacetate complexes of (tpp)Fe^{III} were prepared by stirring $[(tpp)Fe]_2O$ with a 10-fold excess of the corresponding acid in CH_2Cl_2 solution. Crystalline products were recovered as described above.

Analogous manganese(III) tetraphenylporphyrin complexes were prepared by a 20-min reflux of "(tpp)Mn(OH)" in the neat acid.[12] Excess acid was evaporated under a nitrogen stream. The resulting solid was recrystallized from toluene and the crystalline product was dried under vacuum at 120 °C.

The (tpp)Fe(O-*t*-Bu) complex was generated in benzene solution through a metathesis reaction with potassium butoxide and (tpp)Fe(ClO_4). The new complex exhibited proton NMR signals at 80 ppm for the pyrrole protons and at 32 ppm for the *tert*-butyl protons (C_6D_6 solvent).

The dimeric (*N,N'*-ethylenebis(salicylideneaminato))(μ-oxo)iron(III) Schiff base complex, $[(salen)Fe]_2O$, was prepared as described in the literature.[13] A suspension of the solid in ethanol or methanol was allowed to react with a 100-fold excess of acetic acid. The carboxylate complex was formed immediately. The homogeneous solution was evaporated to dryness with a nitrogen stream. The solid was recrystallized three times by dissolution in chloroform with subsequent reduction of the volume and addition of heptane. The solid product was removed by filtration and dried under high vacuum at 60 °C. Extensive heating must be avoided, as the product is susceptible to decomposition in the solid state. Complete removal of free acetic acid was checked periodically by NMR spectroscopy, and a drying time of 4 h was found to be optimal. The analogous propionate complex was prepared by dissolution of (salen)Fe(OAc) in acetone in the presence of excess propionic acid. After being heated for a few minutes, and subsequent hot filtration, the solution yielded crystals on cooling. The (salen)Fe(O_2CCH_2Cl), (salen)Fe(OCr), and (saloph)Fe(O_2CCH_2Cl) complexes were prepared in a manner similar to that previously reported for (salen)Fe(O_2CCCl_3).[14] The binuclear

(1) (a) University of Iowa. (b) University of Minnesota.
(2) Hendrickson, W. A. In *Invertebrate Oxygen-Binding Proteins: Structure, Active Site, and Function*; Lamy, J., Lamy, J., Eds.; Marcel Dekker: New York, 1981; pp 503–515.
(3) Stenkamp, R. E.; Sieker, L. C.; Jensen, L. H. *J. Am. Chem. Soc.* **1984**, *106*, 618–622.
(4) Armstrong, W. H.; Spool, A.; Papaefthymiou, G. C.; Frankel, R. B.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 3653–3667.
(5) Brooks, R. C.; Brinigar, W. S. *J. Am. Chem. Soc.* **1984**, *106*, 4273–4274.
(6) Holm, R. H.; Hawkins, C. J. In *NMR of Paramagnetic Molecules*; La Mar, G. N., Horrocks, W. D., Jr., Holm, R. H., Eds.; Academic: New York, 1973; pp 243–332.
(7) Adler, A. D.; Longo, F. R.; Finarelli, J. D.; Goldmacher, J.; Assour, J.; Korsakoff, L. *J. Org. Chem.* **1967**, *32*, 476.
(8) Fuhrhop, J.-H.; Smith, K. M. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; pp 765–769.
(9) Adler, A. D.; Longo, F. R.; Vardi, V. *Inorg. Synth.* **1976**, *16*, 213–220.
(10) Oumous, H.; Lecomte, C.; Protas, J.; Cocolios, P.; Guilard, R. *Polyhedron* **1984**, *3*, 651–659.
(11) Abbreviations: OAc, acetate ion; OPr, propionate ion; OCr, crotonate ion; O-*t*-Bu, *tert*-butoxide ion; etio, the dianion of etioporphyrin I; tpp, the dianion of 5,10,15,20-meso(tetraphenylporphine); salen, the dianion of *N,N'*-ethylenebis(salicylideneamine); saloph, the dianion of *N,N'*-*o*-phenylenebis(salicylideneamine); hxta, the pentaanion of *N,N'*-(2-hydroxy-5-methyl-1,3-xylylene)bis(*N*-(carboxymethyl)glycine); HB($pz)_3$, the monoanion of tri-1-pyrazolylboron.
(12) Meunier, B.; Guilmet, E.; De Carvalho, M.; Poilblanc, R. *J. Am. Chem. Soc.* **1984**, *106*, 6668–6676.
(13) (a) Lewis, J.; Mabbs, F. E.; Richards, A. *J. Chem. Soc. A* **1967**, 1014–1018. (b) Lewis, J.; Mabbs, F. E.; Richards, A.; Thornley, A. S. *J. Chem. Soc. A* **1969**, 1993–1997.
(14) Wollmann, R. G.; Hendrickson, D. N. *Inorg. Chem.* **1978**, *17*, 926–930.

0020-1669/87/1326-2779$01.50/0

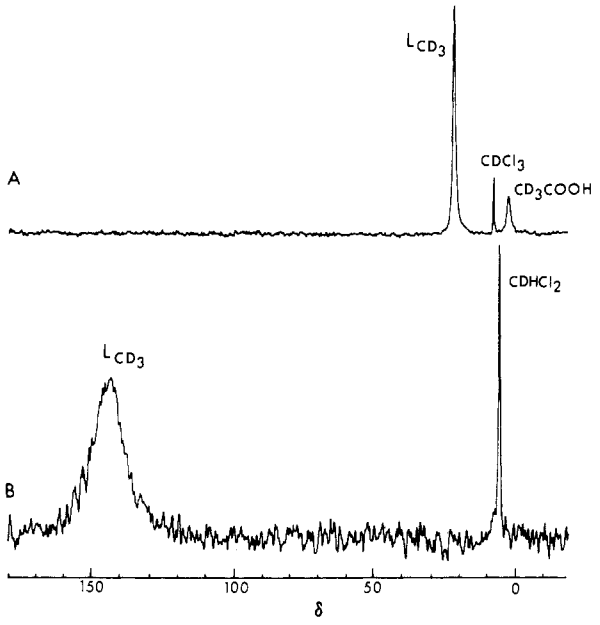


Figure 1. Deuterium NMR spectra of (A) (tpp)Fe(OAc-d_3) in $CHCl_3$ and (B) (salen)Fe(OAc-d_3) in CH_2Cl_2 referenced to $Si(CD_3)_4$ in ppm, at 25 °C. The coordinated acetate signal is labeled L_{CD_3}.

$(Me_4N)[(hxta)Fe_2(carboxylato)_2]$ complexes were synthesized by the route previously described for $(Me_4N)[(hxta)Fe_2(OAc)_2]$.[15]

Chlorinated solvents were washed with concentrated sulfuric acid, washed with water, washed with aqueous sodium carbonate, dried over solid calcium chloride, and distilled from solid P_4O_{10}. Solvents were stored over 3A molecular sieves under a nitrogen atmosphere at −15 °C.

Proton and deuterium NMR spectra were recorded on JEOL FX-90Q, Nicolet NT-300, and Bruker WM-360 spectrometers at respective frequencies of 90, 300, and 360 MHz for protons, and 13.7, 46, and 55 MHz for deuterium. Solution concentrations ranged from 1.0 to 5.0 mM in metal complex. Tetramethylsilane was utilized as a reference, and downfield chemical shifts are given a positive sign. Temperature calibration was carried out by the method of van Geet.[16] Infrared measurements were performed with IBM Model 98 and Matson Cygnus 25 Fourier transform systems.

Results

Iron(III) and Manganese(III) Porphyrin Carboxylate Complexes. The iron(III) carboxylate compounds examined here were characterized by comparison of proton NMR spectra with those reported in the literature.[10] Solutions of the (tpp)Fe–carboxylate compounds were consistently contaminated with the μ-oxo dimeric derivative, as was evidenced by presence of a pyrrole proton signal at 13.5 ppm. Other workers have noted that the carboxylate complex is readily hydrolyzed in solution, presumably by persistent traces of water.[10,17]

Preparations were carried out with acetic acid-d_4, and deuterium NMR spectra were recorded for the resulting complexes. Figure 1 summarizes the distinctive chemical shift values for the acetate CD_3 group when bound to an iron(III) porphyrin as compared to the iron(III) salen complex. A signal is observed at 21.4 ppm in spectrum A for the (tpp)Fe(OAc-d_3) complex in chloroform solution. A signal for free acetic acid is also apparent in this spectrum at 2.07 ppm. The free acetic acid is derived from solvent of crystallization or from hydrolysis of a portion of the complex to the μ-oxo dimeric product. Coexistence of the free and bound acetate signals indicates that ligand exchange is slow on the NMR time scale for the iron(III) porphyrin complex. No change was observed in the position of the 21.4 ppm coordinated acetate signal

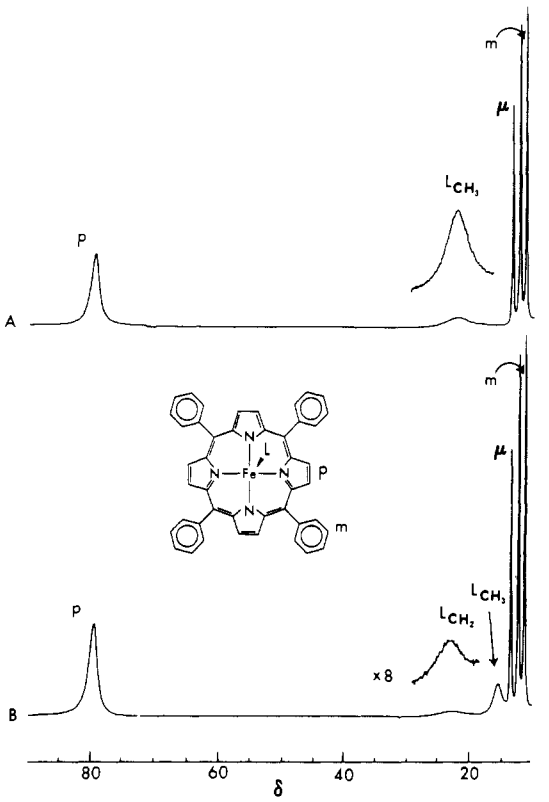


Figure 2. Downfield region of proton NMR spectra of (A) (tpp)Fe(OAc) and (B) (tpp)Fe(OPr) referenced to $Si(CH_3)_4$ in ppm, at 25 °C. Carboxylate ligand signals are labeled L_{CH_3} and L_{CH_2}. The signal labeled "μ" is for the pyrrole proton of the dimeric $[(tpp)Fe]_2O$ species.

Table I. Proton/Deuteron Resonances for Coordinated Carboxylate Ligands[a]

compd	solvent		
	$CHCl_3$	CH_2Cl_2	C_6H_6
(tpp)Fe(OAc)	21.4	32.1	53.6
(tpp)Fe(O_2CH_2Br)	25.0		
(tpp)Fe(OPr)			
CH_2	22.8	23.8	33.1
CH_3	15.5	15.9	16.1
(tpp)Fe($O_2CCCl_2CH_3$)	8.2		
(etio)Fe(OAc)	12.0	14.7	48.6
(salen)Fe(OAc)	138.2	143.0	*b*
(salen)Fe(O_2CCH_2Cl)	140		
(salen)Fe(OCr)			
2-H	129		
3-H	−10		
CH_3	23		
(saloph)Fe(O_2CCH_2Cl)	135		
(tpp)Mn(OAc)	14.2	18.9	37.2

[a] Values in ppm, referenced to tetramethylsilane, 25 °C. [b] Low solubility precluded NMR spectroscopy in benzene.

or in the 2.07 ppm free acetic acid signal with titration of up to 2.0 equiv of acetic acid into a $CDCl_3$ solution of (tpp)Fe(OAc).

Proton NMR spectra of the downfield region for both the (tpp)Fe–acetate and –propionate complexes dissolved in $CDCl_3$ are shown in Figure 2. The spectra are typical for a high-spin iron(III) tetraarylporphyrin complex,[18,19] with the pyrrole signal at 79.3 ppm and split phenyl meta proton signals at 12.2 and 11.3 ppm. The pyrrole signal for the μ-oxo dimer is seen at 13.5 ppm (labeled "μ"). Other phenyl signals in the aromatic region are now shown. Although proton NMR spectra have been reported for the carboxylate complexes,[10] no signals for axial ligands were

(15) Murch, B. P.; Bradley, F. C.; Que, L., Jr. *J. Am. Chem. Soc.* **1986**, *108*, 5027–5028.
(16) Van Geet, A. C. *Anal. Chem.* **1968**, *40*, 2227–2229.
(17) Samples prepared under rigorous dry box conditions likewise contained some μ-oxo dimer signals, as well as a sharp resonance at 2.4 ppm that matches the chemical shift value for acetic anhydride. An equilibrium that involves loss of acetate ligands with formation of μ-oxo dimer and acetic anhydride remains a possibility.
(18) La Mar, G. N.; Walker (Jensen), F. A. In *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1979, Vol. 4; pp 61–157.
(19) Goff, H. M. In *Iron Porphyrins—Part I*; Lever, A. B. P., Gray, H. B., Eds.; Addison-Wesley: Reading, MA, 1983, pp 237–281.

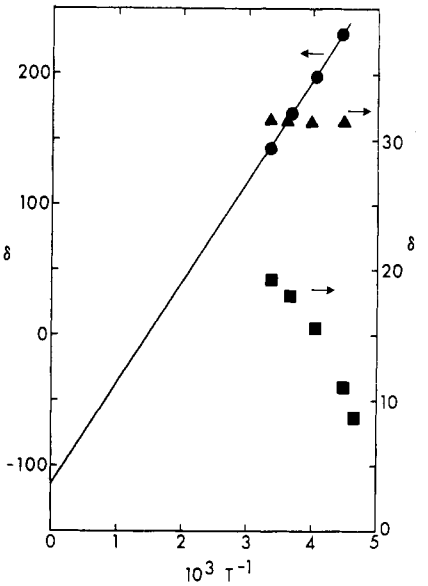


Figure 3. Curie plots for the coordinated CD_3 acetate deuterium NMR signal, in CH_2Cl_2 solution, with chemical shift referenced to $Si(CD_3)_4$: (●) (salen)Fe(OAc-d_3); (▲) (tpp)Fe(OAc-d_3); (■) (tpp)Mn(OAc-d_3).

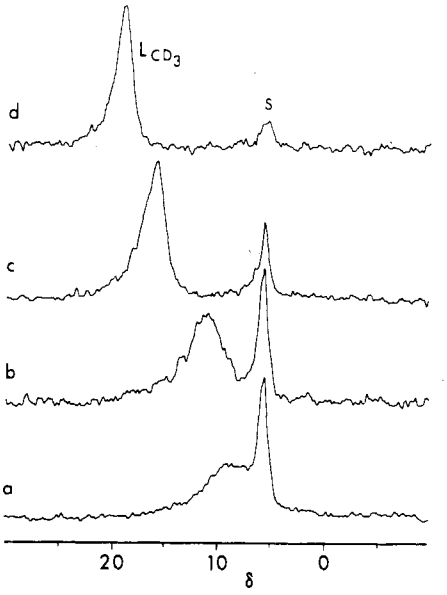


Figure 4. Variable-temperature deuterium NMR spectra of (tpp)Mn(OAc-d_3) in CH_2Cl_2 solution (S), referenced to $Si(CD_3)_4$: (a) 215 K; (b) 223 K; (c) 247 K; (d) 298 K. The coordinated acetate signal is labeled L_{CD_3}.

detected, perhaps due to the large line widths. A signal for the coordinated acetate methyl group is clearly apparent in Figure 2A at 21.9 ppm (matching the deuterium signal at 21.4 ppm). A corresponding signal is assigned to the coordinated propionate methylene group at 22.8 ppm in Figure 2B. A distinctive signal located at 15.5 ppm is not associated with any porphyrin resonance and hence is assignable to the propionate methyl group. The 2,2-dichloropropionate complex was prepared to facilitate assignment of the propionate methyl signal. In this instance it was necessary to utilize the phenyl deuteriated porphyrin in order to clearly resolve the broad CH_3 signal of bound $^-O_2CCl_2CH_3$ at 8.2 ppm.

Coordinated carboxyl signals are quite solvent dependent, as is evident from values listed in Table I. The acetate deuteron signal for (tpp)Fe(OAc-d_3) ranges from 21.4 to 53.6 ppm for the respective noncoordinating chloroform and benzene solvents. The signal is detected at 32.1 ppm for a dichloromethane solution and at 27.8 ppm for a 1:1 chloroform/dichloromethane solution. The pyrrole proton signal for this complex in the 79 ppm region differs by no more than 1.0 ppm for the solvents investigated. Solvent dependence is less dramatic for the propionate complex, with CH_2 signals covering a range of 22.8–33.1 ppm for the respective solvents. In attempts to evaluate the role of a highly polar, hydrogen-bonding solvent, methanol and *tert*-butyl alcohol were titrated into dichloromethane solutions of (tpp)Fe(OAc-d_3). Unfortunately, acetate displacement was evident for the (tpp)Fe^{III} complex, as the 32.1 ppm signal disappeared with addition of excess alcohol.

Variable-temperature measurements served to demonstrate a highly anomalous chemical shift dependence for the coordinated acetate resonance in (tpp)Fe(OAc). Idealized Curie law behavior would have the NMR signal in a paramagnetic complex shifted from the position for an analogous diamagnetic complex by a T^{-1} dependence. However, the coordinated acetate signal exhibits decidedly non-Curie behavior (dichloromethane solution) with chemical shift values relatively unchanged between −50 °C and ambient temperature. The results are summarized in Figure 3.

The chemical shift value for coordinated O-*t*-Bu methyl protons in the (tpp)Fe(O-*t*-Bu) complex (toluene solvent) is relevant for comparison with the carboxylate values. The signal is observed at 32 ppm—a value not significantly different from that detected for the acetate group of (tpp)Fe(OAc). This observation is significant in that the *tert*-butyl methyl and acetate methyl groups are the same number of bonds away from the metal center. Unlike the carboxyl complex, however, the coordinated butyl signal of the (tpp)Fe(O-*t*-Bu) complex exhibits Curie law behavior over the temperature range from 220 to 310 K (diamagnetic intercept of 5.0 ppm).

Deuterium NMR spectra were recorded for the (tpp)Mn(OAc-d_3) complex. A dramatic solvent dependence is apparent from the listings in Table I, wherein the CD_3 signal is at 14.2 ppm in chloroform solution and at 37.2 ppm in benzene solution. In the presence of free acetic acid at 25 °C this signal moved upfield, and no separate signal was seen for free acetic acid. Rapid ligand exchange is thus apparent for the manganese(III) derivative. Proton NMR spectra confirmed the presence of a coordinated acetate signal in the same spectral region, and also indicated the typical high-spin character of the manganese(III) carboxylate complex (pyrrole proton signal at −21.9 ppm).

Variable-temperature deuterium NMR spectra (dichloromethane solution) exhibit highly anomalous chemical shift values for the axial ligand signal of (tpp)Mn(OAc-d_3). Deuterium NMR spectra are shown at various temperatures in Figure 4, and a plot of chemical shift vs. $1/T$ is reported in Figure 3. A negative slope is noted that indicates essentially inverse Curie law behavior. Perturbations responsible for this anomalous behavior are not transmitted to the manganese porphyrin unit, however, as the pyrrole proton signal of (tpp)Mn(OAc) follows a Curie law dependence with deviations no greater than those observed for the chloride complex, (tpp)MnCl.[20] Hence the nearly inverse Curie law behavior for coordinated acetate ion is *not* due to gross electronic structural perturbations at the metal center but rather must reflect perturbations of the Mn–OAc linkage.

Non-Heme Iron(III) Complexes. The NMR spectra of several mononuclear and binuclear non-heme iron(III) complexes were examined for comparison with the iron(III) porphyrin carboxylate complexes. The (salen)FeX derivatives have been the subject of numerous physical studies.[21,22] The deuterium NMR spectrum of (salen)Fe(OAc-d_3) in dichloromethane at 25 °C is shown in Figure 1B. The very broad (800-Hz line width) bound CD_3 signal is observed at 143.0 ppm, in agreement with the previously reported proton chemical shift value of 138 ppm at 29 °C.[21] The chemical shift value in chloroform at 138 ppm is only marginally different considering the magnitude of the isotropic shift.

Variable-temperature deuterium NMR spectra for (salen)Fe(OAc-d_3) are shown in Figure 5. Although the variation in signal

(20) La Mar, G. N.; Walker, F. A. *J. Am. Chem. Soc.* **1975**, *97*, 5103–5107.
(21) La Mar, G. N.; Eaton, G. R.; Holm, R. H.; Walker, F. A. *J. Am. Chem. Soc.* **1973**, *95*, 63–75.
(22) Fanning, J. C.; Resce, J. L.; Lickfield, G. C.; Kotun, M. E. *Inorg. Chem.* **1985**, *24*, 2884–2889.

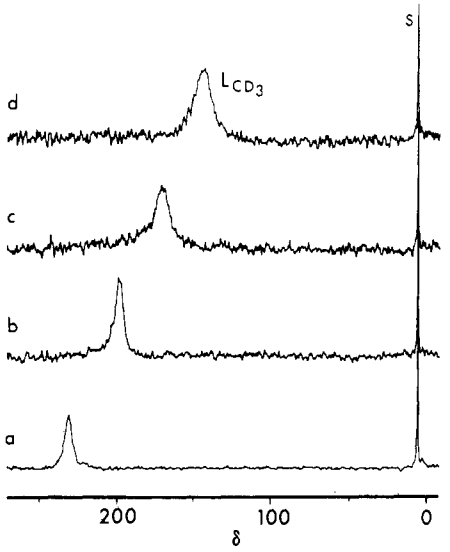


Figure 5. Variable-temperature deuterium NMR spectra of (salen)Fe(OAc-d_3) in CH_2Cl_2 solution (S), referenced to $Si(CD_3)_4$: (a) 215 K; (b) 223 K; (c) 247 K; (d) 298 K. The coordinated acetate signal is labeled L_{CD_3}.

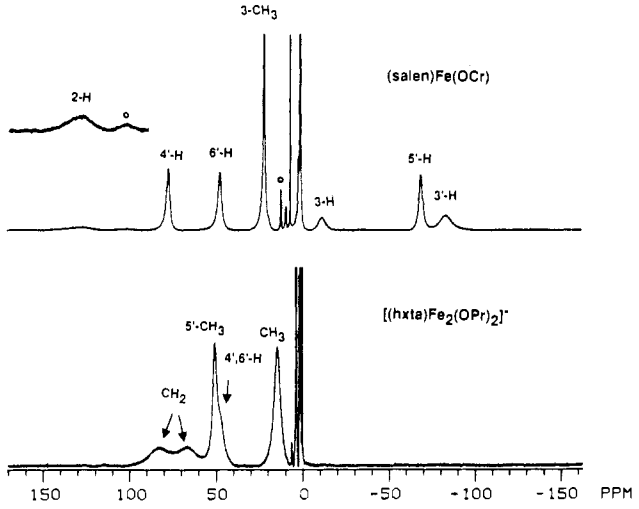


Figure 6. Proton NMR spectra of (salen)Fe(OCr) in $CDCl_3$ and $[(hxta)Fe_2(OPr)_2]^-$ in CD_3OD, at 25 °C. Primed numbers refer to proton signals from salen or hxta, whereas unprimed numbers refer to coordinated carboxylate signals. Resonances denoted by O arise from $[(salen)Fe]_2O$.

line widths is not understood, chemical shift values do increase as the temperature is lowered. A chemical shift vs. $1/T$ plot in Figure 3 reveals an intercept value of −115 ppm rather than an idealized value for acetate ion in a diamagnetic complex.

The CH_2 resonances of chloroacetate coordinated to (salen)Fe^+ and (saloph)Fe^+ are found at 140 and 135 ppm, respectively. Use of the more acidic chloroacetic acid improves the hydrolytic stability of the resulting carboxylate complex, thus minimizing the problems posed by appearance and exchange of free ligand. The resonances of (salen)Fe(OCr) (Figure 6) exhibit isotropic shifts with alternating signs. The coordinated crotonate 2-H, 3-H, and 3-CH_3 peaks are found at 129, −10, and 23 ppm, respectively. Taken together, the mononuclear non-heme iron carboxylate complexes exhibit significantly larger shifts for protons geminal to the carboxylate function than do the iron porphyrin complexes.

The binuclear complexes studied here also follow this general trend, when the observed shift is corrected for loss of paramagnetism due to antiferromagnetic coupling. The $[(hxta)Fe_2(OAc)_2]^-$ complex exhibits an acetate resonance at 89 ppm and an antiferromagnetic coupling constant, J, of −10 cm^{-1} (derived from a fit of the temperature dependence of the chemical shift values[15]). This J value would result in a 27% loss of paramagnetism per iron

Table II. Proton NMR Resonances for Coordinated Carboxylate Ligands in Binuclear Iron(III) Complexes

complex	carboxyl group	δ	J, cm^{-1}	"corrected" shift
$[(HB(pz)_3Fe)_2O(OAc)_2]$	CH_3	10.5	−122[a]	112
$[(HB(pz)_3Fe)_2OH(OAc)_2]^+$	CH_3	68.7	−17[b]	119
$[(hxta)Fe_2(OAc)_2]^-$	CH_3	89	−10	122
$[(hxta)Fe_2(OPr)_2]^-$	CH_2	83, 67	−10	113, 91
	CH_3	15		20
$[(hxta)Fe_2(OCr)_2]^-$	2-H	94	−10	126
	3-H	−3		−6
	3-CH_3	28		38

[a] Reference 4. [b] Reference 23.

center at 25 °C, relative to the uncoupled case. Application of this proportionality converts the 89 ppm value to 122 ppm for the uncoupled "monomeric" case. The corresponding propionate complex exhibits a diastereotopic pair of methylene resonances centered at 75 ppm and a methyl resonance at 15 ppm (Figure 6). The diastereotopism is a result of the C_2 symmetry of the molecule and serves to demonstrate that carboxylate exchange is slow on the NMR time scale. Indeed, free ligand resonances are readily observed for these complexes when excess carboxylic acid is added. When corrected for antiferromagnetic coupling, the CH_2 and CH_3 shifts for $[(hxta)Fe_2(OPr)_2]^-$ are 102 and 20 ppm, respectively. The crotonate complex exhibits isotropic shifts of alternating sign with the 2-H, 3-H, and 3-CH_3 resonances found at 95, −3, and 28 ppm, respectively.

The proton NMR spectra of two complexes with bridging carboxylates have been reported, namely $[(HB(pz)_3Fe)_2O(OAc)_2]$ and $[(HB(pz)_3Fe)_2OH(OAc)_2]^+$. These well-characterized bis(acetato)-bridged iron(III) pyrazolylborate complexes serve as models for the binuclear cluster in methemerythrins.[4,23] The acetate resonances of the oxo- and hydroxo-bridged complexes are found at 10.5 and 68.7 ppm, respectively. The values become 112 and 119 ppm when corrected with J values of −122 and −17 cm^{-1}, respectively. The limiting shifts for the acetate methyl group of the binuclear complexes thus consistently approach those of the mononuclear non-heme iron complexes as summarized in Table II and differ from those observed for the porphyrin complexes.

Infrared Spectroscopy. Solution infrared spectral measurements were carried out in an attempt to delineate monodentate as opposed to bidentate coordination geometries. Previous IR measurements made on (tpp)Fe(OAc) in the solid state reportedly show bands at 1665 and 1276 cm^{-1}, indicative of the monodentate coordination mode.[10] The analogous (acetato)iron(III) octaethylporphyrin ((oep)Fe(OAc)) and (acetato)iron(III) protoporphyrin dimethyl ester derivatives have also been reported,[24,25] and the solid-state infrared spectrum of (oep)Fe(OAc) reveals signals at 1640, 1340, and 1270 cm^{-1} consistent with monodentate coordination.[24]

Chloroform and dichloromethane solutions of the (tpp)Fe(OAc) species subjected to difference spectral comparisons with solutions of (tpp)FeCl or $[(tpp)Fe]_2O$ did not unambiguously show signals diagnostic of either monodentate or bidentate coordination. Infrared spectra of the (salen)Fe(OAc) complex likewise were complicated by extensive overlap of salen signals in the critical 1600-cm^{-1} carbonyl region. With respect to the signal overlap problem, other workers have commented as to the uncertainty of IR measurements for delineation of the carboxyl binding mode in the (salen)Fe(OAc) complex.[13b]

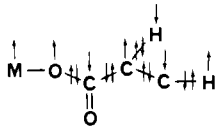
Discussion

Striking differences are seen in the NMR chemical shifts of carboxylate proton/deuteron residues coordinated to iron(III) and manganese(III) porphyrins as compared to those bound at non-heme iron(III) centers. This observation is at first surprising in

(23) Armstrong, W. H.; Lippard, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 4632–4633.
(24) Buchler, J. W.; Schneehage, H. H. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1973**, *28*, 433–439.
(25) Tang, S. C.; Koch, S.; Papaefthymiou, G. C.; Foner, S.; Frankel, R. B.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 2414–2434.

view of the similarity of phenolate and imidazole axial ligand resonance positions in heme and non-heme complexes.[18,19,26-29] One possible explanation for the differences may lie in variable coordination modes. Monodentate coordination is clearly the case for iron(III) porphyrins in the solid state as characterized by vibrational spectroscopic and X-ray crystallographic studies;[10] however, direct solution structural information is lacking. Bridging bidentate coordination has been established by X-ray crystallography for the carboxylates in binuclear complexes.[4,15,23] That this structure is maintained in solution is indicated by the persistence of antiferromagnetic coupling in all the binuclear complexes in solution and the observation of diastereotopic methylene resonances in $[(hxta)Fe_2(OPr)_2]^-$. The mode of coordination in (salen)Fe(OAc) is not known. In the solid, a dimeric structure similar to that of $[(salen)FeCl]_2$ is suspected on the basis of magnetic susceptibility measurements. This suggests that the acetate ion would be monodentate in such a structure. In solution this dimer would be broken as evidenced by the observation of a susceptibility value expected for a high-spin ferric center and titration studies that indicate a 1:1 complex.[28] The availability of an open coordination site on the iron may result in a bidentate coordination mode.

The diverse chemical shift values observed for the various carboxylate complexes may be a result of different spin delocalization mechanisms that are modulated by the coordination mode. For the monodentate mode, delocalization of unpaired spin density can occur from a σ-symmetry metal orbital through the σ-bond framework, which would contribute to downfield NMR shifts for all atoms in the carboxyl ligand. A second mechanism involving spin polarization may be operative, as well, in which spin polarization yields alternate positive ($\uparrow$) and negative ($\downarrow$) unpaired spin density along the ligand backbone.[30]



Negative spin density at a proton 1s orbital would provide an upfield contribution to the isotropic shift. Hence, the net chemical shift may reflect the relative importance of competing or additive terms. Spin polarization effects generally are important over short distances from the metal center, and direct σ-spin delocalization contributions are also known to diminish nearly an order of magnitude with each intervening bond. Precedent for a dominant spin polarization mechanism is found in the upfield CH_2 signals observed for the nickel(II) malonate and the nickel(II) β-alanine complexes, which have monodentate carboxylates as a result of the multidentate nature of the ligands used.[6,31]

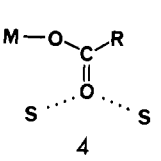
The bidentate mode of carboxylate coordination affords yet a third mechanism for spin delocalization via π-spin delocalization. This binding mode forces the carboxylate moiety to be coplanar with the metal ion(s). Unpaired spin density from a π-symmetry orbital can then be delocalized into the carboxylate function and onto the alkyl group by a mechanism analogous to that for alkyl groups of coordinated phenolates. Downfield shifts would be expected for acetate methyl protons. This pathway is in principle also available for the monodentate mode, but the likelihood of free rotation of the C–O single bond in a monodentate carboxylate ligand would greatly diminish the contribution of this mechanism.

The observed acetate proton NMR shifts are large and downfield for the binuclear complexes. Of the three possible mechanisms, it is clear that the σ-polarization mechanism cannot be dominant, in that upfield shifts would be expected. Between the σ- and the π-delocalization pathways, the latter would be expected to have the larger effect for the distant proton residues.

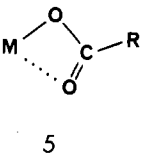
Further support for a dominant π-delocalization mechanism in bridging bidentate carboxylate complexes can be obtained from a comparison of the shifts in the $[(hxta)Fe_2(OOCR)_2]^-$ complexes. The acetate CH_3 resonance is found at 89 ppm, whereas the propionate CH_3 resonance is at 15 ppm, owing to the attenuation of unpaired spin density via the σ bond. In the crotonate complex, the 2-proton signal is found at 95 ppm, similar to that found for the acetate methyl proton, and the 3-H and 3-CH_3 resonances alternate in sign. If the delocalization of unpaired spin density was predominantly via σ bonds, the 3-H shift would have been similar to that of the propionate CH_3 and the 3-CH_3 resonance would have been in the diamagnetic region. However, the upfield shift of the 3-proton and the downfield shift of the corresponding CH_3 protons are both diagnostic of delocalization of unpaired spin density via π orbitals.

The seemingly anomalous NMR behavior (i.e., shift, solvent dependence, and temperature dependence) for the iron porphyrin complexes is at first puzzling, but explanations can be offered. The comparable shift values for the methyl and methylene protons of (tpp)Fe(OPr) may be rationalized if both σ-polarization and σ-delocalization mechanisms are operative. Competing terms would serve to diminish the methylene shift value, whereas downfield contributions from both mechanisms would augment the methyl shift value. In support of the monodentate coordination mode for (acetato)iron(III) porphyrins and predominant σ-spin polarization/delocalization are the comparable chemical shift values for the methyl protons of (tpp)Fe(O-*t*-Bu) and (tpp)Fe(OAc).

The solvent dependence for coordinated carboxylate signals in the metalloporphyrin complexes is notable and merits speculation as to the molecular basis. Variable solvation of the "carbonyl" residue provides a likely mechanism. The significant dipole moment for chloroform would dictate the most efficient interaction by this solvent and the least effective solvation (of the three solvents investigated) by benzene. Efficient solvation (**4**) would infer that

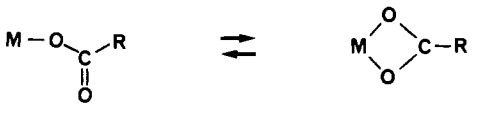


4



5

the carbonyl moiety is unavailable for interaction with the metal center. When left unsolvated, the carbonyl residue could interact weakly with the metal center (**5**), or an equilibrium involving the monodentate (**1**) and bidentate (**2**) complexes could be established.



1 2

The position of this rapid equilibrium, and hence the chemical shift value, would be dictated by the solvent. The equilibrium would lie to the left for chloroform and further to the right for benzene.

The variable-temperature NMR results for coordinated acetate signals are highly anomalous in terms of the pattern expected for a paramagnetic compound. The metalloporphyrin results *cannot* be explained by spin state or antiferromagnetic coupling effects at the metal center, as the β-pyrrole proton NMR signal of the metallotetraphenylporphyrins follows reasonable Curie law behavior over the temperature ranges investigated. The gross deviations from Curie law behavior for acetate signals may be explained by solvation effects, in that lower temperatures would favor caboxyl oxygen solvation of the form shown for structure **4**. Enhanced solvation would then presumably dictate an upfield

(26) Goff, H. M.; Shimomura, E. T.; Lee, Y. J.; Scheidt, W. R. *Inorg. Chem.* **1984**, *23*, 315–321.
(27) Pyrz, J. W.; Roe, A. L.; Stern, L. J.; Que, L., Jr. *J. Am. Chem. Soc.* **1985**, *107*, 614–620.
(28) Heistand, R. H., II; Lauffer, R. B.; Fikrig, E.; Que, L., Jr. *J. Am. Chem. Soc.* **1982**, *104*, 2789–2796.
(29) Lauffer, R. B.; Antanaitis, B. C.; Aisen, P.; Que, L., Jr. *J. Biol. Chem.* **1983**, *258*, 14212–14218.
(30) La Mar, G. N. In ref 6, pp 85–126.
(31) Milner, R. S.; Pratt, L. *Discuss. Faraday Soc.* **1962**, *34*, 88–95.

chemical shift contribution that would oppose the normal Curie law term. Moreover, the realization of Curie law behavior for the coordinated O-*t*-Bu proton signals of (tpp)Fe(O-*t*-Bu) points to a role for the acetate carbonyl group in dictating anomalous temperature and solvent effects for the acetate NMR signal.

From the foregoing discussion, one might infer that the carboxylate group in (salen)Fe(OAc) is chelated, given the large value of the acetate methyl shift and the alternating signs of the crotonate proton shifts. Examples of chelated acetate complexes are limited in number (examples include $UO_2(OAc)_4$, $Zn(OAc)_2 \cdot 4H_2O$,[32] and (acetato)ruthenium(II) phosphine complexes[33]), because the small bite angle of the carboxyl group generally disfavors a chelated structure. However, the nitrate ligand in (tpp)Fe(NO_3)[34] and in (salen)Fe(NO_3)[22] has been shown crystallographically and by IR spectroscopy to be unsymmetrically chelated in the solid state, lending credence to the potential for such anions to chelate to iron(III) under appropriate circumstances. Several (salen)FeX complexes exhibit dimeric structures in the solid state, indicating a tendency for these iron centers to be six-coordinate. Upon dissolution, $[(salen)Fe(OAc)]_2$ presumably dissociates into monomeric units, and the tendency toward six-coordination may be satisfied by chelation of the carboxylate ligand.

Conclusions

Regardless of the mechanisms possible for Curie law deviations and solvent dependence, at the empirical level it is clear that protons at the α-positions with respect to an iron(III) bound carboxylate residue can exhibit chemical shift values ranging from at least the normal aromatic region to some 140 ppm downfield. Even the β-proton signals can be shifted as far as 16 ppm downfield. The hypothesis relating coordination mode with chemical shift value would place signals for α-position protons in the 100–140 ppm region for a bidentate carboxylate ion, whereas monodentate coordination would give rise to significantly smaller shifts. Thus, identification of amino acid carboxylate binding in metalloproteins by NMR chemical shift values alone is highly problematic,[35] and other approaches should be used to corroborate suspected carboxylate coordination. On the other hand, once carboxylate coordination and signal assignment are established, NMR spectroscopy would be of value for detection of subtle structural, hydrogen-bonding, and solvation effects at the metal–carboxylate site.

Acknowledgment. This work was supported by NIH Grant GM-28831 (H.M.G.) and by NSF Grant DMB-8314935 (L.Q.). Technical assistance by Gregory Godziela is also acknowledged.

Registry No. (tpp)Fe(OAc), 33393-26-9; (tpp)Fe(O_2CCH_2Br), 109365-20-0; (tpp)Fe(OPr), 109365-21-1; (tpp)Fe($O_2CCCl_2CH_3$), 109365-22-2; (etio)Fe(OAc), 109365-23-3; (salen)Fe(OAc), 41742-84-1; (salen)Fe(O_2CCH_2Cl), 109365-24-4; (salen)Fe(OCr), 109365-25-5; (saloph)Fe(O_2CCH_2Cl), 24844-47-1; (tpp)Mn(OAc), 58356-65-3; $[(HB(pz)_3Fe)_2O(OAc)_2]$, 86177-70-0; $[(HB(pz)_3Fe)_2OH(OAc)_2]^+$, 90886-30-9; $[(hxta)Fe_2(OAc)_2]^-$, 103322-76-5; $[(hxta)Fe_2(OPr)_2]^-$, 109365-18-6; $[(hxta)Fe_2(OCr)_2]^-$, 109365-19-7; $CHCl_3$, 67-66-3; CH_2Cl_2, 75-09-2; C_6H_6, 71-43-2.

(32) Mehrotra, R. C.; Bohra, R. *Metal Carboxylates*; Academic: London, 1983; p 53.
(33) Robinson, S. D.; Uttley, M. F. *J. Chem. Soc., Dalton Trans.* **1973**, 1912–1920.
(34) Phillippi, M. A.; Baenziger, N.; Goff, H. M. *Inorg. Chem.* **1981**, *20*, 3904–3911.
(35) Glutamate binding to the heme iron center of cytochrome *c'* in the alkaline state has been suggested on the basis of proton NMR signals detected in the far downfield region at 111 and 135 ppm.[36] However, no support for previous assignment of these signals to the β-protons of a glutamate side chain is offered by our model compound study, in that the corresponding propionate methyl signals are found only 16 ppm downfield. In addition, the X-ray crystal structure of the neutral pH form of the protein reveals no proximal carboxylate side chains available for iron binding without large conformational perturbations.[37]
(36) La Mar, G. N.; Jackson, J. T.; Bartsch, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4405–4410.
(37) Weber, P. C. *Biochemistry* **1982**, *21*, 5116–5119.

Contribution from the Department of Chemistry, University of California, Riverside, California 92521

Nuclear Magnetic Resonance Studies of the Solution Chemistry of Metal Complexes. 24. Arylmercury(II) Complexes of Sulfhydryl-Containing Ligands

Dallas L. Rabenstein* and Jorge Bravo†

Received August 28, 1986

Equilibrium constants were determined by ^1H NMR for the complexes of *p*-mercuribenzenesulfonate (PMBS) with hydroxide and chloride ions and with the thiols glutathione, cysteine, penicillamine, mercaptosuccinic acid, and mercaptoethanol in aqueous solution. The formation constants of the PMBS–thiol complexes are very similar to those of the analogous methylmercury(II) complexes, suggesting that the stability of organomercury(II)–thiol complexes in aqueous solution is not strongly dependent on the nature of the organic moiety. The formation constants do, however, depend strongly on the Brønsted basicity of the sulfhydryl donor group, with the formation constant increasing as the sulfhydryl pK_a increases.

Introduction

The mercury of alkyl- and arylmercury(II) cations shows a strong preference for coordination to only one additional donor atom, and both bind most strongly to deprotonated sulfhydryl groups.[1,2] The kinetics and equilibria for the binding of methylmercury(II) by a variety of sulfhydryl-containing biological molecules has been characterized in detail;[1,3-7] however, similar information is lacking for sulfhydryl binding of arylmercury(II) compounds. The finding by X-ray analysis that the C–Hg–S system of methylmercury(II) and phenylmercury(II) complexes of cysteine is linear and that the Hg–S bond lengths in the two complexes are not significantly different[2] might suggest that the stabilities of alkyl- and arylmercury(II)–thiol complexes are similar; however, the formation constants that would allow a quantitative comparison have not been reported for any aryl-

†Permanent address: Departmento de Quimica Inorganica, Universidad de Santigo, Santiago de Compostela, Spain.

(1) Rabenstein, D. L. *Acc. Chem. Res.* **1978**, *11*, 100.
(2) Carty, A. J.; Malone, S. F. In *The Biochemistry of Mercury in the Environment*; Nriagu, J. O., Ed.; Elsevier/North-Holland Biomedical: New York, 1979; pp 433–479.
(3) Reid, R. S.; Rabenstein, D. L. *Can. J. Chem.* **1981**, *59*, 1505.
(4) Arnold, A. P.; Canty, A. J. *Can. J. Chem.* **1983**, *61*, 1428.
(5) Rabenstein, D. L.; Isab, A. A.; Reid, R. S. *Biochim. Biophys. Acta*, **1982**, *696*, 53.
(6) Reid, R. S.; Rabenstein, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 6733.
(7) Arnold, A. P.; Canty, A. J.; Reid, R. S.; Rabenstein, D. L. *Can. J. Chem.* **1985**, *63*, 2430.

0020-1669/87/1326-2784$01.50/0