Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support (G.R.E. and S.S.E.) of this work. Work at the University of Colorado at Denver was partially supported by a University of Colorado C.R.C.W. grant. Elemental analyses were performed by Spang Microanalytical Laboratories.

References and Notes

(1) S. S. Eaton, G. R. Eaton, and R. H. Holm, *J. Organomet. Chem.*, **39**, 179 (1972).
(2) J. J. Bonnet, S. S. Eaton, G. R. Eaton, R. H. Holm, and J. A. Ibers, *J. Am. Chem. Soc.*, **95**, 2141 (1973).
(3) S. S. Eaton and G. R. Eaton, *J. Chem. Soc., Chem. Commun.*, 576 (1974).
(4) S. S. Eaton and G. R. Eaton, *J. Am. Chem. Soc.*, **97**, 3660 (1975).
(5) J. E. Maskasky, Ph.D. Thesis, Case Western Reserve University, 1972, pp 20–25.
(6) P. Fournari, R. Guilard, M. Fontesse, J.-M. Latour, and J.-C. Marchon, *J. Organomet. Chem.*, **110**, 205 (1976).
(7) W. Bhatti, M. Bhatti, S. S. Eaton, and G. R. Eaton, *J. Pharm. Sci.*, **62**, 1574 (1973).
(8) F. A. Walker and G. N. LaMar, *Ann. N.Y. Acad. Sci.*, **206**, 328 (1973).
(9) M. Moet-Ner and A. D. Adler, *J. Am. Chem. Soc.*, **94**, 4763 (1972).
(10) R. V. Snyder and G. N. LaMar, *J. Am. Chem. Soc.*, **98**, 4419 (1976).
(11) S. S. Eaton and G. R. Eaton, *Inorg. Chem.*, **16**, 72 (1977).
(12) M. Moet-Ner and A. D. Adler, *J. Am. Chem. Soc.*, **97**, 5107 (1975).
(13) F. A. Walker, E. Hui, and J. M. Walker, *J. Am. Chem. Soc.*, **97**, 2390 (1975).
(14) F. A. Walker, M.-W. Lo, and M. T. Ree, *J. Am. Chem. Soc.*, **98**, 5552 (1976).
(15) J. D. Satterlee, G. N. LaMar, and J. S. Frye, *J. Am. Chem. Soc.*, **98**, 7275 (1976); J. D. Satterlee, G. N. LaMar, and T. J. Bold, *ibid.*, **99**, 1088 (1977).
(16) F. A. Walker, D. Beroiz, and K. M. Kadish, *J. Am. Chem. Soc.*, **98**, 3484 (1976).
(17) G. C. Vogel and B. A. Beckmann, *Inorg. Chem.*, **15**, 483 (1976).
(18) K. M. Kadish and M. M. Morrison, *J. Am. Chem. Soc.*, **98**, 3326 (1976).
(19) M. Tezuka, Y. Ohkatsu, and T. Osa, *Bull. Chem. Soc. Jpn.*, **49**, 1435 (1976).
(20) K. M. Kadish, M. M. Morrison, L. A. Constant, L. Dickens, and D. G. Davis, *J. Am. Chem. Soc.*, **98**, 8387 (1976).
(21) K. M. Kadish and M. M. Morrison, *Inorg. Chem.*, **15**, 980 (1976).
(22) Abbreviations used throughout: *p*-R-TPP, tetrakis(*p*-R-phenyl)porphyrin dianion; *p*-CF_3-TPP, tetrakis(*p*-trifluoromethylphenyl)porphyrin dianion; *p*-Cl-TPP, tetrakis(*p*-chlorophenyl)porphyrin dianion; *p*-*i*-Pr-TPP, tetrakis(*p*-isopropylphenyl)porphyrin dianion; *p*-OMe-TPP, tetrakis(*p*-methoxyphenyl)porphyrin dianion; *p*-OH-TPP, tetrakis(*p*-hydroxyphenyl)porphyrin dianion; *p*-Et_2N-TPP, tetrakis(*p*-diethylaminophenyl)porphyrin dianion; *t*-Bupy, 4-*tert*-butylpyridine.
(23) M. Bhatti, W. Bhatti, and E. Mast, *Inorg. Nucl. Chem. Lett.*, **8**, 133 (1972).
(24) A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour, and L. Korsakoff, *J. Org. Chem.*, **32**, 476 (1967).
(25) D. W. Thomas and A. E. Martell, *J. Am. Chem. Soc.*, **78**, 1338 (1956).
(26) N. Datta-Gupta and T. J. Bardos, *J. Heterocycl. Chem.*, **3**, 495 (1966).
(27) J.-H. Fuhrhop, K. M. Kadish, and D. G. Davis, *J. Am. Chem. Soc.*, **95**, 5140 (1973).
(28) A. L. Van Geet, *Anal. Chem.*, **40**, 2227 (1968).
(29) D. A. Kleier and G. Binsch, *J. Magn. Reson.*, **3**, 146 (1970).
(30) T. Drakenberg and R. E. Carter, *Org. Magn. Reson.*, **7**, 307 (1975).
(31) D. Kost, E. H. Carlson, and M. Raban, *Chem. Commun.*, 656 (1971).
(32) Values of σ_p are taken from J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions", Wiley, New York, N.Y., 1963, p 172. Since σ_p is approximately the same for Me and Et and also σ_p is approximately the same for $NH(CH_3)$ and $N(CH_3)_2$ the assumption is made that σ_p for $N(CH_3)_2$ is a good estimate for $N(Et)_2$.
(33) S. S. Eaton and G. R. Eaton, to be published.
(34) C. H. K. Ling and M. M. Harris, *J. Chem. Soc.*, 1825 (1964).
(35) M. Oki and G. Yamamoto, *Bull. Chem. Soc. Jpn.*, **44**, 266 (1971).
(36) R. G. Little and J. A. Ibers, *J. Am. Chem. Soc.*, **95**, 8583 (1973).
(37) G. P. Fulton and G. N. LaMar, *J. Am. Chem. Soc.*, **98**, 2119, 2124 (1976), and references cited therein.

High-Spin Ferrous Porphyrin Complexes as Models for Deoxymyoglobin and -hemoglobin. A Proton Nuclear Magnetic Resonance Study

Harold Goff and Gerd N. La Mar*[1]

Contribution from the Department of Chemistry, University of California, Davis, California 95616. *Received March 18, 1977*

Abstract: The ^1H NMR spectra for a variety of high-spin ($S = 2$) ferrous porphyrin complexes have been recorded and analyzed. The five-coordinate high-spin species were generated from the corresponding unligated, intermediate-spin, $S = 1$, complexes by addition of a methyl-α-substituted imidazole or pyridine axial ligand. The isotropic shifts for synthetic porphyrin complexes are consistent with a predominant contact origin, with the high-spin iron(II) exhibiting only very small magnetic anisotropy. The patterns of porphyrin contact shifts in both synthetic and natural porphyrin complexes are very similar and reflect primarily σ spin transfer. Evidence against significant iron $\rightarrow$ porphyrin π back-bonding is presented. The insensitivity of the porphyrin shifts to the nature of the axial base suggests that heme resonances will not be useful probes for detecting histidine–iron tension in deoxyhemoglobins. The shifts for the axial imidazole, on the other hand, are consistent with primarily σ spin transfer and should serve as ideal indicators of such histidine–iron tension. The natural porphyrin resonance positions in the model complexes support earlier heme methyl assignments in deoxymyoglobin and deoxyhemoglobin and facilitate the assignment in the proteins of other single proton heme peaks as well as those of the elusive proximal histidyl imidazole.

Analysis of the ^1H NMR spectra of complexes of paramagnetic iron porphyrins in different oxidation and spin states has proved to be very useful both because the method provides some direct information on the π bonding, thought to be critical in the biological role, and because these complexes serve as models for assigning and interpreting the ^1H NMR spectra of the various paramagnetic forms of hemoproteins.[2,3] Sufficient parallel has been shown to exist between the pattern of proton resonance positions in model complexes[2-5] and hemoproteins[3,6,7] in both the high-spin[4,6] and low-spin[3,5,7] ferric forms, such that the data on the model systems can be used to make probable assignments not only for the isotropically shifted heme resonances[2,3] but also for the coordinated histidine.[5] In the case of the low-spin ferric systems where most of the work has centered, studies on model compounds[3] have provided a basis for interpreting changes in rotational positions and rotational mobility of pyrrole substituents with pH-induced structural changes in the protein.[8]

Although considerable effort has also been expended on the study of the ^1H NMR spectra of the physiologically more important deoxyhemoproteins[3,9-11] (ferrous, high spin, $S = 2$), the isotropic shifts have yielded much more qualitative

empirical rather than specific structural information for the proteins. The spectra do appear to be less well resolved than the ferric forms in that fewer resonances are clearly seen over a narrower frequency range.[9] However, the paramagnetically shifted peaks have been found to be very sensitive to mutations in and quaternary structure of hemoglobins.[9-11] At least part of this difficulty in making full use of the deoxy protein NMR spectra is the lack of any work on model compounds to provide confirmation of the probable methyl assignments and to suggest where other heme resonances may be expected to be located. In model complexes containing an axial imidazole, observation of the axial ligand proton signals may also allow the peak assignments of the proximal histidine in proteins. Most importantly, it has been shown by work on other metalloporphyrin complexes[2,5,12,13] that analysis of the isotropic shifts in well-defined models will yield information on the physical origin of the isotropic shifts. These results will also provide information as to whether the resonances in the protein arise from coordinated ligands or other amino acid residues in the vicinity or within the heme cavity. The sensitivity of the porphyrin shifts in model compounds to the nature of the axial ligand would also indicate whether heme resonances can be expected to be sensitive to tension in the proximal histidine–iron bond.[14] This latter mechanism has been invoked as the source of oxygen-affinity changes associated with both cooperativity and the effect of allosteric effectors in tetrameric hemoglobins.[14,15]

It has been demonstrated by optical spectroscopy and solution susceptibility measurements that ferrous, high-spin porphyrin complexes can be readily generated in solution by using a sterically hindered imidazole, such as 2-methylimidazole, which yields exclusively the five coordinate, monoligated, $S = 2$ species.[16-20] In the case of the synthetic porphyrin complex of tetraphenylporphyrin, TPPFe, the five-coordinate species has been isolated and characterized by x-ray crystallography.[21] The out-of-plane position of the high-spin ferrous iron and the vacant sixth site[21] thus establish this complex as an ideal model for deoxymyoglobin and hemoglobins.

We have shown[12,13] elsewhere that the phenyl resonances in TPP complexes are excellent indicators of the degree of axial magnetic anisotropy, $\chi_{\parallel} - \chi_{\perp}$, permitting a direct measure of the dipolar contribution to the isotropic shift in both low-spin ferric[12] and the intermediate spin,[13] $S = 1$, ferrous complexes. This dipolar shift for axial symmetry is given[22] by

$$(\Delta H/H)_{dip} = -(1/3N)(\chi_{\parallel} - \chi_{\perp})(3\cos^2\theta - 1)r^{-3} \quad (1)$$

where θ is the angle between the iron–proton vector and the unique axis, and r is the length of this vector. Since the observed isotropic shift is the vector sum of the dipolar and contact contributions, we can write

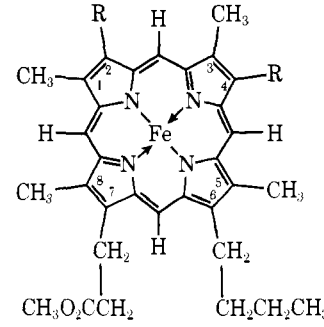
$$(\Delta H/H)_{iso} = (\Delta H/H)_{dip} + (\Delta H/H)_{con} \quad (2)$$

Hence, determining $(\Delta H/H)_{dip}$ will yield $(\Delta H/H)_{con}$. This latter term is given by the well-known equation[22]

$$(\Delta H/H)_{con} = -A\frac{g\beta S(S+1)}{(\gamma/2\pi)3kT} \quad (3)$$

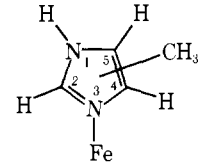
where A is the Fermi contact coupling constant which will indicate the dominant type of spin transfer[23] from the iron to the porphyrin and/or axial imidazole.

The analysis of the porphyrin contact shifts is again most effective using simple fourfold symmetrical complexes of synthetic porphyrins,[2,12,13] TPP, and octaethylporphyrin, OEP, which provide the needed probes for the pyrrole spin density. Natural porphyrins, which will be investigated to provide the information on the resonance positions in the proteins, are the derivatives of I with the 2,4 substituents indicated below.



Ia, R = ethyl, MPDMEFe
b, R = vinyl, PPDMEFe
c, R = proton, DPDMEFe
d, R = bromine, Br_2DPDMEFe
e, R = acetyl, Ac_2DPDMEFe

The axial ligands used to form the deoxy model compounds will include the methyl sterically hindered imidazoles, whose numbering system for the coordinated ligand, depicted in II, will be utilized.



II

Experimental Section

The preparation and characterization of iron(III) porphyrins have been described previously.[12,13] Specifically deuterated protoporphyrin was supplied by Professor K. Smith.[24] Crystalline TPPFe, prepared by chromous reduction of TPPFeCl,[25] was a gift from Professor C. A. Reed. Other iron(II) porphyrins were prepared in situ in benzene-d_6 or toluene-d_8 using the aqueous dithionite reduction technique.[12,17] Reductions were carried out in septum-capped NMR sample tubes. Solid iron(III) porphyrin and solid sodium dithionite were placed in the tube which was then flushed with nitrogen. Deaerated D_2O (~0.02 mL) and deaerated solvent were added by syringe and the tube was shaken vigorously for several minutes. The aqueous suspension was separated by centrifugation before recording the NMR spectrum. The solid methyl-substituted imidazole was either added initially with the solid iron(III) porphyrin and sodium dithionite or added in solution by syringe after reduction. When added as a solid before reduction a large excess of the methyl-substituted imidazole was necessary since most of the ligand was extracted into the aqueous layer. A small excess of sodium dithionite was employed to reduce TPPSFe(III) in deaerated D_2O solutions in septum-capped NMR tubes. Solutions of TPPFe in tetrahydrofuran-d_8 were prepared by adding deaerated solvent to solid TPPFe.

2-Methylimidazole, 2-CH_3Im (Sigma), was recrystallized three times from benzene. 2,5-Dimethylimidazole, 2,5-CH_3Im, was purified by sublimation at 80 °C under dynamic vacuum (~10 Torr). 1,2-Dimethylimidazole, 1,2-CH_3Im, was obtained as a gift from F. A. Walker. 4,5-Dimethylimidazole, 4,5-CH_3Im, was prepared by standard methods.[26] 2-Methylpyridine, 2-CH_3Py, was dried over solid potassium hydroxide and distilled.

2-Methylimidazole was deuterated at the 1 position by dissolution in D_2O followed by vacuum evaporation. The 1-H of this and other methyl-substituted imidazoles was also exchanged by deuterium in the presence of D_2O during the in situ reduction by sodium dithionite. The 1-H resonance was observed, however, in benzene solutions (dried over 3A molecular sieves) prepared from solid TPPFe.

A JEOL PS 100 FT NMR instrument operating at 99.5 MHz was used to obtain the ^1H NMR spectra as described previously.[13] Shifts are reported in parts per million. The uncertainties in shift position, relative to Me_4Si, are ±0.05 ppm for porphyrin resonances and ±0.1 ppm for axial imidazole resonances.

Results and Discussion

Resolution and Assignment of High-Spin Resonances. The

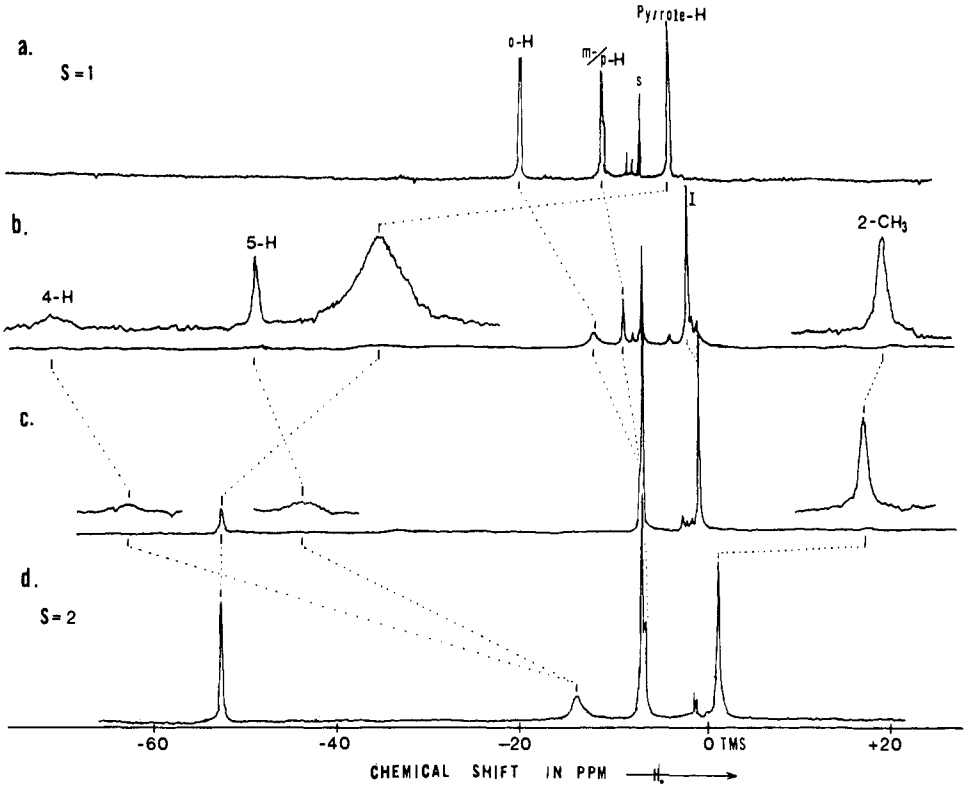


Figure 1. Titration of iron(II) porphyrins with 2-methylimidazole-*1-d*. Solvent, C_6D_6, 25 °C: (a) [TPPFe] = 3 mM; (b) [*p-i*-PrTPPFe]$_0$, 8 mM, [2-CH_3Im-*1-d*]$_0$ = 5 mM, the signal at "I" corresponds to the isopropyl resonance. (c) [*p-i*-PrTPPFe]$_0$ = 8 mM, [2-CH_3Im-*1-d*]$_0$ = 8 mM. (d) [TPPFe]$_0$ = 3 mM, [2-CH_3Im-*1-d*]$_0$ = 20 mM. The more soluble *p-i*-PrTPPFe is used in b and c to illustrate the coordinated imidazole resonances which are obtainable only at high iron(II) porphyrin concentrations. S = solvent peak.

desired high-spin, five-coordinated species were generated by adding an excess of 2-CH_3Im to a solution of TPPFe or *p-i*-PrTPPFe[21] to drive the equilibrium

$$\text{PFe} + \text{2-CH}_3\text{Im} \overset{K}{\rightleftharpoons} \text{PFe(2-CH}_3\text{Im)} \qquad (4)$$

completely to the right. However, in order to understand the resulting spectra we will first consider the sequential addition of 2-CH_3Im. In a of Figure 1 we reproduce the well-characterized proton trace[12] of the unligated TPPFe. Addition of 2-CH_3Im-*1-d* has two effects on the spectrum: the porphyrin resonances shift (the pyrrole H downfield and the phenyl resonances upfield), and the set of three new peaks at −77, −48, and +21 ppm of areas 1, 1, and 3 appear as illustrated in b of Figure 1. As we have demonstrated in detail elsewhere,[27] use of 2-CH_3Im results in an additional peak of area 1 at −85 ppm (NH) and clearly identifies the new peaks as arising from the axially coordinated ligand. In b of Figure 1, 35% of the iron(II) is still *p-i*-PrTPPFe, and 65% is *p-i*-PrTPPFe(2-CH_3Im-*1-d*); the known value[18] of $K = 2.4 \times 10^4$ M^{-1} is consistent with these ratios and indicates that $[\text{2-CH}_3\text{Im}]_{\text{free}} \sim 7 \times 10^{-5}$ M. Since the coordinated 2-CH_3Im peaks grow in intensity at fixed positions, ligand exchange must be slow. This conclusion is also strongly supported by the observation of separate 4-H and 5-H peaks; fast ligand exchange would average these shifts owing to the very rapid tautomerism in the free ligand. The observation of collapsed porphyrin peaks suggests some mechanism which averages the two environments without involving exchange with free ligand. However, since we can experimentally demonstrate slow axial ligand exchange, and can therefore determine the coordinated ligand shifts, we choose to postpone detailed investigation[28] of the kinetic implications, since we are interested here solely in the coordinated imidazole shifts.

As more 2-CH_3Im is added, the coordinated ligand peaks broaden and shift toward their diamagnetic positions as shown in c of Figure 1. This reflects the onset of rapid ligand exchange. The porphyrin peaks have almost reached their limiting positions for the $S = 2$ species. This again is consistent with a prediction[18] of ~95% conversion to the $S = 2$ form and $[\text{2-CH}_3\text{Im}]_{\text{free}} \sim 0.5$ mM. The decrease of the lifetime of coordinated ligand with added ligand indicates that exchange is by an associative mechanism.[28]

A large excess (7:1) of 2-CH_3Im drives equilibrium in eq 4 completely to the right and yields the pure $S = 2$ porphyrin spectrum as illustrated in d of Figure 1. Axial ligand exchange is now rapid, with the positions completely averaged not only between free and coordinated ligands but also for the 4-H and 5-H resonances via tautomerization in the free ligand.

Thus, the conditions required for obtaining the resonance positions for the axial ligand and for in-plane porphyrin are mutually exclusive for these high-spin systems.[27] When enough axial ligand is added to ensure complete high-spin complexes, the ligand exchange by an associative mechanism is rapid on the NMR time scale even at the lowest temperatures. The axial ligand exchange rate can be slowed to yield slow exchange NMR spectra by adding subequivalent amounts of base to minimize the amount of free base, but this results in incomplete conversion to the high-spin iron porphyrin. The combination of experiments illustrated in b and d of Figure 1, however, permit the resolution of all desired resonances for the high-spin species.

Isotropic shifts for the synthetic porphyrins and a variety of sterically hindered imidazoles, referenced to appropriate diamagnetic complexes,[29-31] are listed in Tables I and II, respectively. The porphyrin resonances were assigned by their titrations from the characterized $S = 1$ complexes. The 2-CH_3Im resonances have been assigned based on deuteration[27] and line width arguments as discussed elsewhere,[27] and are confirmed by the permutation of methyl and dimethyl sub-

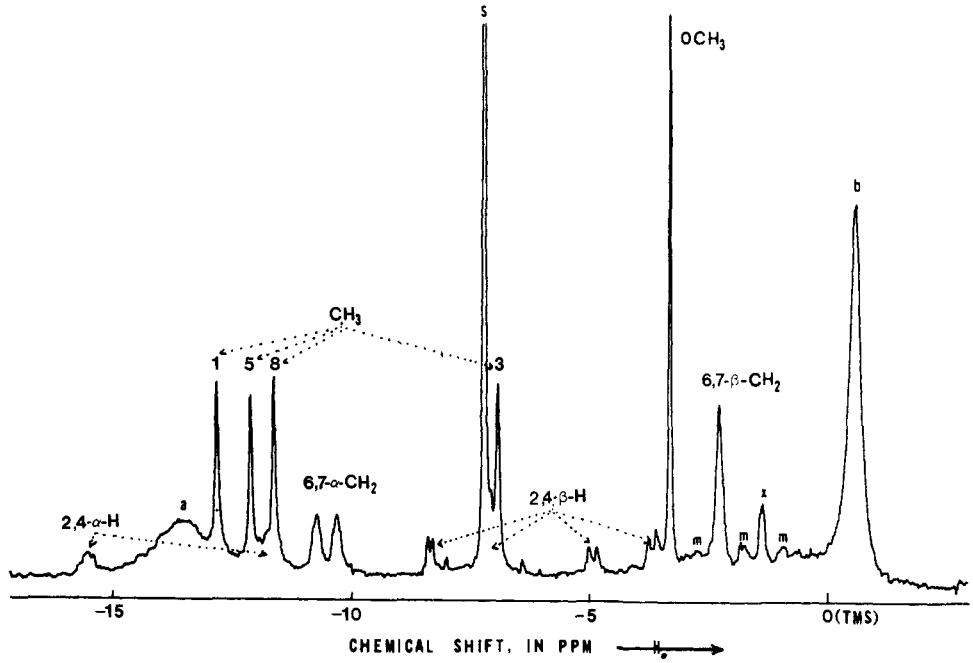


Figure 2. PPDMEFe(2-CH_3Im) in C_6D_6 solvent, 25 °C; $[PPDMEFe]_0$ = 3 mM, $[2\text{-}CH_3Im]_0$ = 10 mM. S = solvent peak, a, b are averaged axial ligand 4,5-H and CH_3 peaks, respectively.

Table I. Isotropic Shifts for Synthetic Porphyrins in High-Spin PFe(2-CH_3Im)[a,b]

		Meso-aryl			
P	pyrr-H	*o*-H	*m*-H	*p*-H	CH_3
TPP	−43.4	+1.45	+1.11	+1.02	
m-CH_3-TPP	−43.4	+1.52	+1.15	+1.08	+0.69
p-CH_3-TPP	−43.7	+1.58	+1.25		+0.37
	Pyrrole			Meso-H	
	α-CH_2	β-CH_3			
OEP	−8.40	+0.28		+6.96	

[a] Shifts in parts per million (±0.10 ppm) at 25 °C in toluene-d_8; *o*-H, *m*-H referenced to diamagnetic R-TPPInCl (ref 30), *p*-H, *m*-CH_3, *p*-CH_3 referenced to diamagnetic R-TPPNi (ref 12). [b] All data for systems with $[PFe]_0 \simeq 3$ mM, $[2\text{-}CH_3Im]_0 \simeq 15$ mM.

Table II. Isotropic Shifts for Methyl-Substituted Imidazoles in High-Spin *p-i*-Pr-TPPFe(RIm)[a]

	RIm			
Position	2-CH_3Im	2,5-$(CH_3)_2$Im	1,2-$(CH_3)_2$Im	4,5-$(CH_3)_2$Im
1-H	−73 (100)[b]	*c*		*c*
1-CH_3			−8.3 (25)	
2-H				−53 (400)
2-CH_3	+16 (105)	+15 (110)	+18 (115)	
4-H	−71 (300)	−68 (300)	−74 (300)	
4-CH_3				+24 (300)
5-H	−44 (30)		−48 (50)	
5-CH_3		−11.7 (40)		*d*

[a] Shifts in parts per million (±2%) at 25 °C in benzene-d_6 or toluene-d_8 solvent, referenced to diamagnetic Ru(II) porphyrin complexes (ref 5, 31). [b] Approximate line width, in hertz, given in parentheses. [c] The 1 position was deuterated to simplify assignments. [d] 5-CH_3 not located and probably lies under solvent or porphyrin resonances.

stitution reported here. The porphyrin shifts in TPPFeL were found to be independent of the choice of the five imidazoles used.

The proton trace of the deoxymyoglobin model, PPDMEFe(2-CH_3Im), is displayed in Figure 2. The averaged 4,5-H and 2-CH_3 peaks of the excess ligand are designated by a and b in the figure. The trace of the related derivative,[18] DPDMEFe(2-CH_3Im), is found in Figure 3. All resonances in the natural porphyrin derivatives are assigned on the basis of areas, multiplet structure, comparison with synthetic porphyrins, and by variation in the 2,4 substituents. The porphyrin isotropic shifts, referenced to diamagnetic complexes,[29] are listed in Table III. The individual methyl resonances of three porphyrins were assigned by specific deuteration of individual methyl groups.[4] Comparison of the shifts for identical substituents in Tables I and III reveals very similar shifts for identical groups in the synthetic and natural porphyrins.

The meso-H peaks in the natural porphyrin derivatives appeared in the region 0 to −6 ppm from Me_4Si, and it was not possible to resolve all four peaks in any of the derivatives owing to their small size, larger width, and overlap with other porphyrin peaks as well as some persistent impurities. Those located, however, resonated in the same region as the meso-H in OEPFe(2-CH_3Im). Hence, the meso-H's are not included in Table III.

The spectra in Figures 1-3 clearly exhibit excellent resolution indicative of very rapid electron spin relaxation. The pyrrole-H line width of 18 Hz is to be compared with that of 7 Hz for the low-spin ferric biscyano species. The assumption of dominant dipolar relaxation[32] yields $T_{1e} = 6 \times 10^{-13}$ s. This extremely short T_{1e} suggests that these high-spin ferrous complexes have very large zero-field splittings.

Analysis of Isotropic Shifts. The meso-phenyl resonances of paramagnetic TPP complexes have been shown[12,13] to yield characteristic isotropic shift patterns depending on whether the contact or dipolar shifts dominate. Shift direction alternation around the phenyl ring is characteristic if contact shifts dominate; the ring proton and methyl groups also exhibit shifts of opposite sign for any position.[23] On the other hand, dominant dipolar shifts[22] are characterized by shifts in the same direction for all ring positions with relative magnitudes given by the relative geometric factors in eq 1, $(3\cos^2\theta - 1)r^{-3}$, which have been previously computed for TPP.[12,13]

The test for dominant dipolar contributions to the phenyl

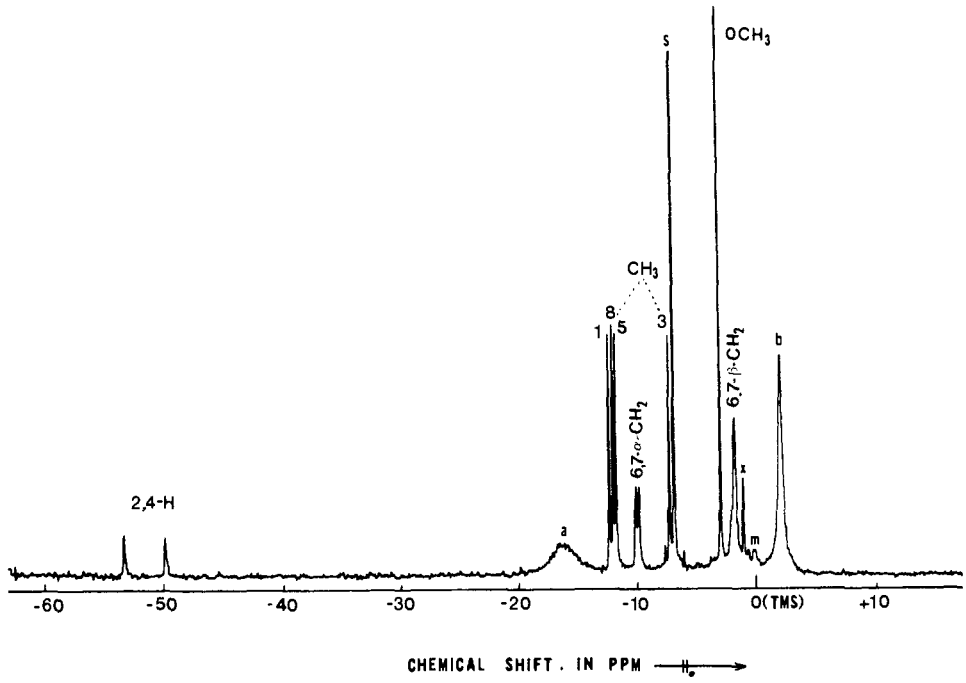


Figure 3. DPDMEFe(2-CH_3Im) in C_6D_6 solvent, 25 °C; $[DPDMEFe]_0$ = 3 mM, $[2\text{-}CH_3Im]_0$ = 10 mM. S = solvent peak. a, b are averaged axial ligand 4,5-H and CH_3 peaks, respectively.

resonances is a linear relationship between the observed isotropic shift and this geometric factor.[12] Such a plot for R-TPPFe(2-CH_3Im) is shown in Figure 4. Shifts are indeed small and all upfield. The In(III) complexes[30] used as diamagnetic references to obtain the isotropic shifts have *o*-H at lower field than either Ni(II) or Fe(II) complexes, thereby yielding an upper limit to the isotropic shift consistent with the spectra. Although a perfect fit to a straight line is not found, possibly owing to very small contact contributions or, more probably, to ±0.05 ppm uncertainties in the diamagnetic references used to calculate these small isotropic shifts, it is clear that there is only a small contribution to the dipolar shift. The best fit straight line to the points in Figure 4 yields an estimate of $(\Delta H/H)_{dip}^{o\text{-}H} < 1.6$ ppm.

The computed relative geometric factors based on available x-ray data for all other porphyrin positions as well as for the axial imidazoles are reproduced[5] in Table IV. This permits the direct determination of $(\Delta H/H)_{dip}$ for all other positions, which are also included in Table IV. Subtraction of the dipolar shift from the isotropic shift (eq 2) yields the value of $(\Delta H/H)_{con}$ for each position, as listed in the last column of Table IV. This last column indicates that all shifts are overwhelmingly contact in origin, except at the meso position, where there appears to be negligible spin density. Since the shifts for identical substituents are very close in synthetic and natural porphyrins we must conclude that the electronic structures are very similar and that both the contact and dipolar contributions to the shifts are the same as in the synthetic porphyrins.

The effect of temperature on the isotropic shifts in *p-i*-PrTPPFe(2-CH_3Im) is illustrated in Figure 5 in the form of a Curie plot. Porphyrin shifts were determined under conditions of excess axial ligand, while the axial ligand shifts were obtained as discussed above. The fact that the pyrrole-H shift is perfectly linear with T^{-1} indicates that only the high-spin, mono adduct is present in solution. It had previously been shown[19] that a bis adduct, which would be diamagnetic, can be induced to form at very low temperatures and in the presence of a very large excess of 2-CH_3Im. At the porphyrin concentrations required for our NMR experiments (~8 mM), only a small excess of 2-CH_3Im is needed to drive the equilibrium completely to the $S = 2$ form. The pyrrole-H and 5-H

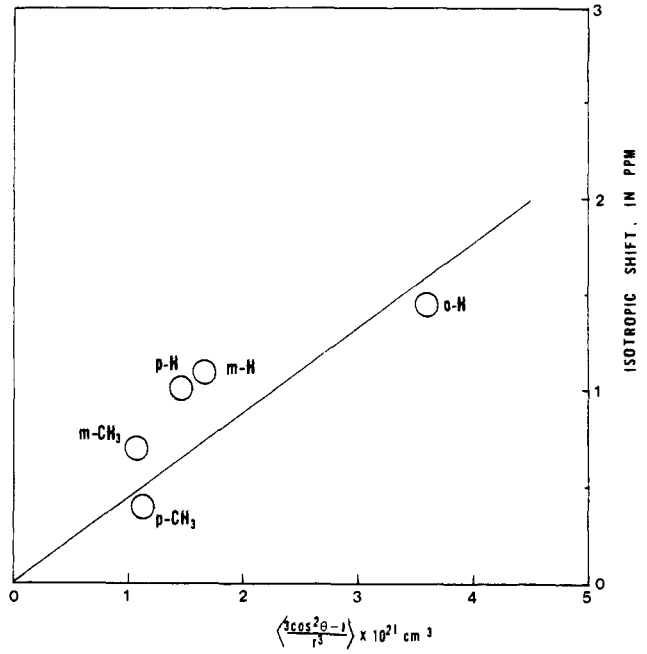


Figure 4. Plot of the isotropic shifts for meso-aryl substituents in R-TPPPe vs. the calculated geometric factor, $(3\cos^2\sigma - 1)r^{-3}$.

shifts adhere reasonably well to the Curie law, exhibiting relatively small nonzero intercepts at $T^{-1} = 0$. The phenyl shifts were too small to be measured with sufficient accuracy. The axial ligand resonances near the iron, 2-CH_3 and 4-H, both showed different behavior. The data for 2-CH_3 exhibit curvature at high temperature, possibly owing to rotational effects of the methyl group. The 4-H shift data fall on a straight line which exhibits a very substantial, (~ +35 ppm), nonzero intercept at $T^{-1} = 0$. The unusually large nonzero intercept of the 4-H signal could occur from a complex temperature dependence for the dipolar shift[22] since this position has the largest fractional contribution from this term. The very large zero-field splittings[32] suggested by the extremely short T_{1e} could give rise to a dipolar shift contribution with a T^{-2} de-

Table III. Isotropic Shifts for Natural Porphyrins in High-Spin PFe(2-CH_3Im)[a]

P	MPDME	DPDME	PPDME	Br_2DPDME	Ac_2DPDME
1,3,5,8-CH_3[b]	−9.8 −9.6 −9.5 −5.6	− 9.3 (1)[c] − 9.0 (8) − 8.8 (5) − 4.3 (3)	−9.5 (1)[c] −8.8 (5) −8.3 (8) −3.6 (3)	−9.7 −9.7 −9.5 −3.5	−6.7 (1)[c] −6.7 (5) −5.8 (8) +1.9 (3)
Δ[d]	4.2	5.0	5.9	6.2	8.6
6,7-α-CH_2	−7.3 −7.1	− 7.3 − 7.0	−7.6 −7.2	−7.6 −7.4	−6.2 −5.2
6,7-β-CH_2	+1.0	+ 1.1	+0.9	+0.9	+0.6
6,7-γ-OCH_3	+0.4	+ 0.4	+0.4	+0.4	+0.3
2,4-R	−7.3, −7.1[e] 0, +0.6[f]	−44.8[g] −41.3[g]	−7.4, −3.6[h] −2.2, −0.9[i] +1.4, +2.7[j]		+1.2[k] +2.1[k]

[a] Iron (II) porphyrin 3 mM, 2-methylimidazole 10 mM, in benzene-d_6, 25 °C. Shifts in parts per million (± 0.10 ppm) referenced to diamagnetic metalloporphyrins. Diamagnetic reference resonances with respect to Me_4Si are 1,3,5,8-CH_3, −3.3; α-CH_2, −3.1; β-CH_2, −3.1; −OCH_3, −3.3; vinyl α-CH, −8.1; vinyl β-CH (cis), -6.1; vinyl β-CH (trans), −6.3; β-CH_3, −1.7; acetyl, −3.5; pyrr-H, −8.7 (ref 5, 29, 31). [b] Average 1,3,5,8-methyl line width, 8 Hz. [c] Methyl assignments are given in parentheses. [d] Spread of the four methyl signals. [e] α-CH_2. [f] β-CH_3. [g] Pyrrole-H. [h] Vinyl α-CH. [i] Vinyl β-CH (cis). [j] Vinyl β-CH (trans). [k] Acetyl methyl.

Table IV. Separation of Isotropic Shifts in High-Spin PFe(RIm)[a]

		Relative $(3\cos^2\theta - 1)/r^{-3}$	Upper limit dipolar shift	Isotropic shift	Contact shift
P	*o*-H	+1.00[b]	+1.60	+1.5	−0.1
	m-H	+0.46	+0.80	+1.1	+0.3
	p-H	+0.41	+0.70	+1.0	+0.3
	pyrr-H	+1.95	+3.1	−43.4	−46.5
	pyrr-α-$CH_{2,3}$	+1.18	+1.9	−8.4	−10.3
	meso-H	+3.05	+4.9	+7.0	+2.1
RIm	1-H	−3.75	−6.0	−73	−67.
	1-CH_3	−2.22	−3.6	−8.3	−4.7
	2-H	−6.0	−9.6	−53	−43.
	2-CH_3	∼0	∼0	+16	+16.
	4-H	−5.8	−9.3	−71	−62.
	4-CH_3[c]	∼0	∼0	+24	+24.
	5-H	−3.74	−6.0	−44	−38.
	5-CH_3	−2.14	−3.4	−11.7	−8.3

[a] Shifts in parts per million at 25 °C. [b] The *o*-H geometric factor is normalized to +1.00; data taken from ref 5, 33. [c] The 2-CH_3 and 4-CH_3 are very close to the magic angle so that geometric factors cannot be determined more accurately in absence of crystallographic data on the subject complex.

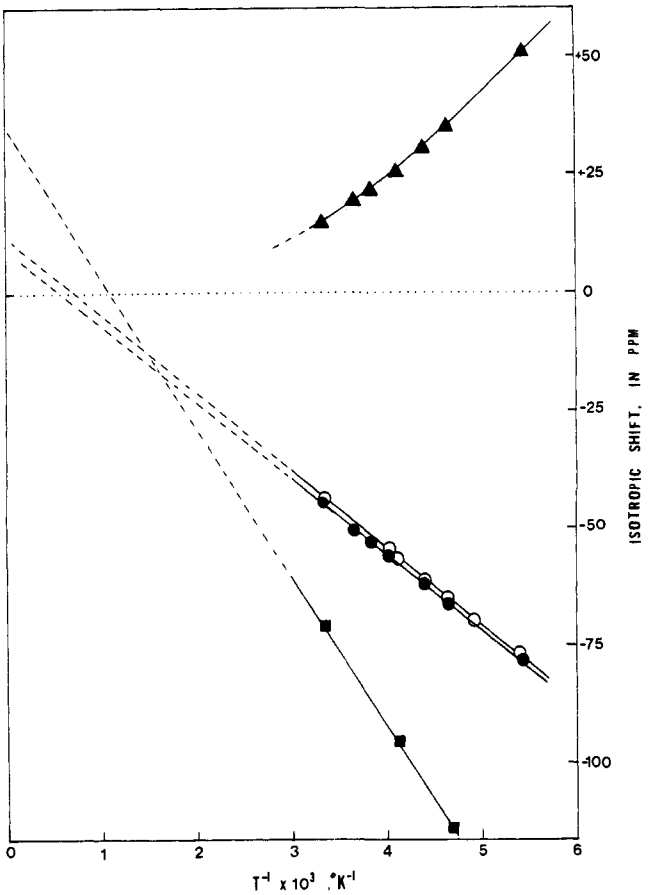


Figure 5. Curie plot for *p-i*-PrTPPFe(2-CH_3Im) in toluene-d_8. [*p-i*-PrTPPFe]$_0$ = 8 mM; [2-CH_3Im]$_0$ = 5 mM for ligand resonances and 15 mM for *p-i*-PrTPPFe resonances. -○-, pyrrole-H -●-, 5-H of 2-CH_3Im; ■, 4-H of 2-CH_3Im; ▲, 2-CH_3 of 2-CH_3Im.

pendence as previously observed[33] for high-spin ferric systems. More detailed discussion of the temperature dependence must await the development of a more rigorous theory for the shifts in the subject complexes.

Bonding in High-Spin Ferrous Complexes. The contact contributions to the isotropic shifts for the porphyrin substituents give no direct evidence for sizable π bonding. The meso-H contact shift is very small so that contributions from Fe→P π back-bonding, traditionally thought to be important in ferrous porphyrins, appear to be negligible (the π spin density is expected to be maximum at the meso position for the lowest vacant porphyrin π acceptor orbital). The pyrrole-H and -α-$CH_{2,3}$ contact shifts are both downfield, with the former larger, indicative of primarily σ spin transfer. Since high-spin iron is probably in a $(d_{xy})^2(d_{xz}, d_{yz})^2(d_{z^2})(d_{x^2-y^2})$ configuration, it possesses the necessary π bonding unpaired d electrons. The apparent dominant σ spin transfer also argues against sizable contributions from P→Fe π bonding. Any small amount of π spin transfer which exists must be masked by the more extensive σ spin transfer mechanism. The dominance of the σ spin transfer is probably due to the strongly σ antibonding $d_{x^2-y^2}$ orbital possessing an unpaired spin. This orbital is vacant in both $S = 1$ ferrous and $S = \frac{1}{2}$ ferric porphyrins which have been shown to exhibit predominant π contact shifts.

In all probability, the pyrrole-H and CH_3 shifts reflect both σ and π contributions, which will make it much more difficult to interpret changes in the shifts with perturbations in complexes or in the protein than with the $S = \frac{1}{2}$ ferric system.[34] The data for the natural porphyrin derivatives in Table III reveal that 2,4 substituents modulate the spread of the four methyl shifts, with the order of increasing spread, ethyl < vinyl ∼ proton < bromine < acetyl, the same as found in these

porphyrins with $S = 1$, ferrous,[13] and both the $S = 1/2$ and $5/2$ ferric[35,36] complexes. Assignment of the individual methyls reveals that primarily 3-CH_3 moves upfield as the 2,4 substituent is made more electron withdrawing. In fact, 3-CH_3 actually moves sufficiently upfield that in Ac_2DPDMEFe(2-CH_3Im) it has a *positive* isotropic shift. Since the shifts for other functional groups are almost independent of 2,4-R, the upfield 3-CH_3 shift must be contact in origin. We are unable to offer an explanation for this observation at this time.

The unique behavior of the 3-methyl group suggests the possibility that this effect comes from increasing asymmetry in the π electron distribution, which increasingly orients the axial 2-CH_3Im to interact with this unique pyrrole. This asymmetry has been characterized recently by both methyl group proton acidities[37] and donor/acceptor interactions among individual pyrroles.[38] This hypothesis could be confirmed using porphyrins where the axial ligand is covalently linked to the porphyrin side chain. However, if confirmed the methyl shift pattern could provide information on the orientation of the axial imidazole.

The axial imidazoles exhibit only downfield proton contact shifts which are indicative of σ spin transfer. The relative contact shifts, taken from Table IV, for 5-H/4-H/2-H/1-H are 1.00/1.41/0.98/1.52, and parallel reasonably well the relative proton spin densities from a CNDO calculation[39] for the σ radial of imidazole, i.e., 1.00/1.62/0.83/1.17, respectively. The small upfield 2,4-CH_3 contact shifts probably arise from some polarization mechanism as found earlier for analogous pyridine complexes,[40] or possibly from some direct interaction with the porphyrin π cloud. The dominance of σ spin transfer and the large shifts are due to the σ bonding with the spin containing d_{z^2}. In the case of low-spin ferric porphyrins, the small π shifts of the axial imidazoles were resolved[5] because d_{z^2} was vacant.

Other Axial Ligands. In order to assess the sensitivity of porphyrin shifts to the nature of the axial ligand, the proton spectra in a number of solvents and in the presence of 2-methylpyridine,[19] 2-CH_3Py, were recorded. Clearly resolved spectra for TPPFe derivatives in THF-d_8 and D_2O were obtained, with the pyrrole-H and the phenyl shifts listed in Table V. The THF complex has been previously suggested[16,7] to be a $S = 2$ species. Although these complexes were not characterized in any other manner, the shifts are very close to those of the 2-CH_3Im complexes and are strongly suggestive of the $S = 2$ state.

In the case of 2-CH_3Py, the interaction of the porphyrin with TPPFe was much weaker[19] than with 2-CH_3Im, so that the shifts for the pure 5-coordinate, $S = 2$ species had to be obtained by graphical procedure.[41] The extrapolated shifts are listed in Table V; K for the reaction was estimated at 70 M^{-1}.

As can be seen from the comparison of the data in Tables I and V, the pyrrole-H shifts ~−50 to −52 ppm from Me_4Si in all complexes are indistinguishible for L = 2-CH_3Py and 2-CH_3Im. Since the steric effect of the α-CH_3 in a six-membered heterocyclic base is expected to be much larger than for the five-membered base, the former is likely to have the weaker and/or longer Fe–N bond. This weaker interaction with 2-CH_3Py is also suggested by the ~350-fold decrease in K. The nearly identical pyrrole-H shifts with the different bases indicate that porphyrin shifts are rather insensitive to the nature of axial ligands. Since both pyrrole-H and phenyl shifts are similar for the different axial ligands, it suggests that both the contact and dipolar shifts (magnetic anisotropy) are insensitive to the nature of L.

Prospects for Deoxy Protein Spectra. The demonstration that the isotropic shifts in the model compounds are primarily contact in origin and that the magnetic anisotropy of the high-spin iron(II) is therefore very small is perhaps our most important conclusion pertinent to the protein NMR spectra. The very similar shifts of identical functional groups in model compounds and proteins (vide infra) indicate that electronic structures of the iron in the two environments are very similar. Moreover, since contact shifts arise only for the directly coordinated ligands, protoporphyrin IX and the proximal histidine, these groups should give rise to all isotropically shifted peaks in the proteins. This is to be contrasted to the more extensively studied low-spin ferric proteins with a large magnetic anisotropy which yields large isotropic (dipolar) shifts for noncoordinated amino acid side chains in the heme pocket.[42] It should be mentioned, however, that if amino acid side chains make close contacts with the iron(II), even though no formal bond exists, polarization mechanisms[23] could induce contact shifts in such residues.

Table V. Observed Shifts in High-Spin Ferrous Complexes[a]

Porphyrin	Axial ligand	Solvent	Shift from Me_4Si	
			pyrr-H	Phenyl H
TPPFe	2-CH_3Im	Toluene-d_8	−52.5	−7.0, −6.7
TPPFe	2-CH_3Py	Toluene-d_8	−52	−7
TPPFe	THF (?)	THF-d_8	−50.2	−7.7
TPPSFe	D_2O (?)	D_2O	−50.8[b]	−8.0[b]

[a] All shifts in parts per million (±0.2 ppm) at 25 °C. [b] Shifts relative to DSS.

The origin of two signals of intensity three in the region −10 to −15 ppm from DSS has been attributed to heme methyls in sperm whale myoglobin.[43,44] Similarly, three presumed methyl signals, one for the α and two for the β chains, in the tetrameric hemoglobins have been identified[10] in the region −12 to −24 ppm from DSS. The observation of heme methyls in the model compounds in the region −2 to −14 ppm from Me_4Si indicates that these earlier protein assignments are totally reasonable. The further downfield bias for one of the observed heme methyls in the proteins is probably due to the increased in-plane asymmetry in the protein relative to that of the model compounds. This has been shown to be the case for both the high-spin and low-spin ferric proteins.[6,7] The upfield methyl shift in Ac_2DPDMEFe(2-CH_3Im) suggests that an upfield, isotropically shifted peak of area 3 found in sperm whale myoglobin[43,44] and monomeric insect hemoglobins[44] may also be a heme methyl. In the case of the tetrameric hemoglobins, the remaining three heme methyls for each chain may all resonate in the diamagnetic envelope, and therefore will have to be resolved by the use of ^2H NMR on methyl-deuterated prosthetic groups. Other heme resonances likely to be found outside the 0 to −10 ppm from DSS region are the single proton peaks of the H_α of the vinyls, and α-CH_2 of the propionic acid side chains. A number of single proton resonances in this region have been located[44] but not assigned in myoglobin and hemoglobins.

The insensitivity of the pyrrole-H shift to the nature of the axial ligand (Table V) suggests that heme resonances will not serve as useful probes for any change in the tension in the proximal histidine–iron linkage.[14] However, this information can probably be determined readily from the unambiguously assigned proximal histidyl imidazole resonances.[27]

We have shown elsewhere that the location and identity of the 1-, 2-, and 4-H imidazole resonances in the model compounds has permitted the location of the same peaks of the proximal histidyl imidazole in deoxymyoglobin, and at least the 1-H peaks for the nonequivalent α and β chains in tetrameric hemoglobins. In addition to these imidazole ring protons, the data in Table II suggest that the 5-α-CH_2 group is also likely to give rise to a pair of single proton resonances in the region −10 to −15 ppm from DSS. The type of information about the orientation of the methylene group relative to the

imidazole plane available from these isotropic shifts has been discussed in detail for the related low-spin ferric hemoproteins.[5]

Although this work has provided confirmation of likely assignments of heme methyls as well as direct information which led to the location and assignment of the elusive proximal histidine resonances in deoxyhemoproteins,[27] unambiguous assignments of the heme resonances must await detailed studies for specifically deuterated prosthetic groups. Such studies are currently in progress in this laboratory.

The large downfield shifts for porphyrin 2,4-H suggest that the deuteroporphyrin-reconstituted heme proteins may provide a particularly well-resolved probe for monitoring protein structural changes at the heme periphery. Preliminary work with sperm whale deoxydeuteroporphyrin-myoglobin has yielded[45] the expected pair of single proton resonances in the region −48 to −54 ppm from DSS, precisely flanking the shifts in the model compounds.

Acknowledgments. The authors are indebted to C. A. Reed for a gift of TPPFe and to T. J. Bold for the preparation of 4,5-$(CH_3)_2$Im. This work was supported by a research grant from the National Institutes of Health, HL-16087.

References and Notes

(1) Alfred P. Sloan Fellow, 1972–1976; Fellow of the John Simon Guggenheim Memorial Foundation, 1975–1976.
(2) G. N. La Mar and F. A. Walker in "The Porphyrins", D. Dolphin, Ed., Academic Press, New York, N.Y., in press.
(3) W. D. Phillips in "NMR of Paramagnetic Molecules", G. N. La Mar, W. D. Horrocks, Jr., and R. H. Holm, Ed., Academic Press, New York, N.Y., 1973, Chapter 11; K. Wuthrich, *Struct. Bonding*, **8**, 53 (1970).
(4) D. L. Budd, G. N. La Mar, and K. M. Smith, to be published.
(5) G. N. La Mar, J. S. Frye, and J. D. Satterlee, *Biochim. Biophys. Acta*, **428**, 78 (1976).
(6) I. Morishima and T. Iizuka, *J. Am. Chem. Soc.*, **96**, 7365 (1974); T. Iizuka, S. Ogawa, T. Inubushi, T. Yonegawa, and I. Morishima, *FEBS Lett.*, **64**, 156 (1976).
(7) A. Mayer, S. Ogawa, R. G. Shulman, T. Yamane, JAS Cavaleiro, A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *J. Mol. Biol.*, **86**, 799 (1974).
(8) G. N. La Mar, D. B. Viscio, K. Gersonde, and H. Sick, submitted for publication in *Biochemistry*.
(9) J. S. Morrow and F. R. N. Gurd, *CRC Crit. Rev. Biochem.*, **3**, 221 (1975).
(10) D. G. Davis, T. R. Lindstrom, N. M. Mook, J. J. Baldassare, S. Carache, R. T. Jones, and C. Ho, *J. Mol. Biol.*, **60**, 10 (1971).
(11) T. R. Lindstrom, J. J. Baldassare, H. F. Bunn, and C. Ho, *Biochemistry*, **12**, 4212 (1973).
(12) G. N. La Mar and F. A. Walker, *J. Am. Chem. Soc.*, **95**, 1782 (1973).
(13) H. Goff, G. N. La Mar, and C. A. Reed, *J. Am. Chem. Soc.*, **99**, 3641 (1977).
(14) M. F. Perutz and L. F. TenEyck, *Nature (London)*, **237**, 495 (1972).
(15) A. Szabo and M. F. Perutz, *Biochemistry*, **15**, 4427 (1976).
(16) J. P. Collman and C. A. Reed, *J. Am. Chem. Soc.*, **95**, 2048 (1973).
(17) D. Brault and M. Rougee, *Biochemistry*, **13**, 4591, 4598 (1974).
(18) D. Brault and M. Rougee, *Biochem. Biophys. Res. Commun.*, **57**, 654 (1974).
(19) G. C. Wagner and R. J. Kassner, *J. Am. Chem. Soc.*, **96**, 5593 (1974); *Biochim. Biophys. Acta*, **392**, 319 (1975).
(20) T. G. Spiro and J. M. Burke, *J. Am. Chem. Soc.*, **98**, 5482 (1976).
(21) J. P. Collman, N. Kim, J. L. Hoard, G. Lang, L. J. Radonovich, and C. A. Reed, Abstracts, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1974, No. INOR-29. Abbreviations used: TPPFe, iron(II) tetraphenylporphyrin; *m*-CH_3-TPPFe, iron(II) *meso*-tetra-*m*-tolylporphyrin; *p*-CH_3-TPPFe, iron(II) *meso*-tetra-*p*-tolylporphyrins; *p*-*i*-PrTPPFe, iron(II) *meso*-tetra-*p*-isopropylphenylporphyrin; OEPFe, iron(II) octaethylporphyrin; TPPSFe, iron(II) *meso*-tetra-*p*-sulfonatophenylporphyrin; MPDMEFe, iron(II) mesoporphyrin IX dimethyl ester; DPDMEFe, iron(II) deuteroporphyrin IX dimethyl ester; PPDMEFe, iron(II) protoporphyrin IX dimethyl ester; Br_2DPDMEFe, iron(II) 2,4-dibromodeuteroporphyrin IX dimethyl ester; Ac_2DPDMEFe, iron(II) 2,4-diacetyldeuteroporphyrin IX dimethyl ester.
(22) J. P. Jesson in "NMR of Paramagnetic Molecules", G. N. La Mar, W. D. Horrocks, Jr., and R. H. Holm, Ed., Academic Press, New York, N.Y., 1973, Chapter 1.
(23) G. N. La Mar, in ref 22, Chapter 3; J. P. Collman, J. L. Hoard, N. Kim, G. Lang, and C. A. Reed, *J. Am. Chem. Soc.*, **97**, 2676 (1975).
(24) J. A. S. Cavaleiro, A. M. d'A Rocha Gonsalves, G. W. Kenner, K. M. Smith, R. G. Shulman, A. Mayer, and T. Yamane, *J. Chem. Soc., Chem. Commun.*, 392 (1974).
(25) J. P. Collman, J. L. Hoard, N. Kim, G. Lang, and C. A. Reed, *J. Am. Chem. Soc.*, **97**, 2676 (1975).
(26) H. Bredereck and G. Theilig, *Chem. Ber.*, **86**, 88 (1953).
(27) G. N. La Mar, D. L. Budd, and H. Goff, *Biochem. Biophys. Res. Commun.*, **77**, 104 (1977).
(28) The detailed kinetics of both ligand exchange in the high-spin species and ligand hopping from one porphyrin to another are under current investigation.
(29) H. Scheer and J. J. Katz in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975, Chapter 10.
(30) The diamagnetic shifts in a variety of five-coordinated porphyrin complexes, *p*-R-TPPMX, have been reported (S. S. Eaton and G. R. Eaton, *J. Am. Chem. Soc.*, **97**, 3660 (1975); in press). The *o*-H shifts are independent of MX = InOH, InCl, and TiO, with *o*-H = −8.20 ± 0.05 ppm and *m*-H, −7.60 ± 0.05 ppm from Me_4Si. These *o*-H shifts are slightly further downfield than that for the analogous planar nickel(II) complex (−7.95 ± 0.05 ppm). This insensitivity of the phenyl shifts to metal and axial ligand suggests that the In(III) complexes are the most reasonable diamagnetic references which introduce ≤0.05 ppm uncertainties into the isotropic shifts.
(31) C.-C. Chen, Ph.D. Thesis, Yale University, 1972.
(32) G. N. La Mar and F. A. Walker, *J. Am. Chem. Soc.*, **95**, 6950 (1973).
(33) G. N. La Mar, G. R. Eaton, R. H. Holm, and F. A. Walker, *J. Am. Chem. Soc.*, **95**, 63 (1973).
(34) R. G. Shulman, S. H. Glarum, and M. Karplus, *J. Mol. Biol.*, **57**, 93 (1971).
(35) D. B. Viscio, Ph.D. Thesis, University of California, Davis, 1977.
(36) D. L. Budd, M. Zobrist, G. N. La Mar, and K. M. Smith, manuscript in preparation.
(37) K. M. Smith and G. N. La Mar, to be published.
(38) G. N. La Mar and D. B. Viscio, *J. Am. Chem. Soc.*, **96**, 7354 (1974).
(39) M. Wicholas, R. Mustacich, B. Johnson, T. Smedley, and J. May, *J. Am. Chem. Soc.*, **97**, 2113 (1975).
(40) W. D. Horrocks, Jr., and D. L. Johnson, *Inorg. Chem.*, **10**, 1835 (1971).
(41) For large excess of axial ligand, L, the expression $\delta_{obsd} = \delta_2 - K^{-1}(\delta_{obsd} - \delta_1)[L]^{-n}$ holds, where δ_{obsd}, δ_2, and δ_1 are the chemical shifts which are observed, and those for the $S = 2$ and $S = 1$ species, respectively, K is the equilibrium constant, and n is the number of bound L in the $S = 2$ complex. A plot of δ_{obsd} vs. $[\delta_{obsd} - \delta_1][L]^{-n}$ will yield a straight line of slope K^{-1} and intercept δ_2. Excellent linear plots were obtained only for $n = 1$, indicating the expected mono adduct, with $K = 70\ M^{-1}$ and the shifts for the $S = 2$ species listed in Table V.
(42) B. Sheard, T. Yamane, and R. G. Shulman, *J. Mol. Biol.*, **53**, 35 (1970).
(43) M. E. Johnson, L. W. M. Fung, and C. Ho, *J. Am. Chem. Soc.*, **99**, 1245 (1977).
(44) G. N. La Mar, D. L. Budd, K. Gersonde, and H. Sick, manuscript in preparation.
(45) D. L. Budd and G. N. La Mar, unpublished observations.