

Table II. ^{13}C Chemical Shift Changes for Selected Carbon Resonances of Glutathione upon Complexation^a

Carbon	Δ , ppm			
	Zn ²⁺	Cd ²⁺	Pb ²⁺	Hg ²⁺
Cys-C _{β}	3.1	3.3	3.5	4.4
Cys-C _{α}	1.0	1.5	1.6	1.0
Cys-CO ₂ H	0.5	0.5	0.8	0.2
Glu-COOH	6.0	3.9	<i>b</i>	<i>b</i>
Glu-C _{β}	2.6	1.8	<i>b</i>	<i>b</i>
Gly-C _{α}	0.1	0.2	0.3	<i>c</i>

^a The chemical shifts of the carbons in noncomplexed glutathione at pD 7.0 were used in the above calculations. The differences listed for the Gly-C _{α} carbon are the differences between the solid and dashed curves for this carbon in Figures 3, 6, and 8. ^b No coordination of Hg²⁺ or Pb²⁺ to the glutamyl end was detected. ^c No coordination of Hg²⁺ to the glycyl carboxylic acid group was detected.

The differences between the chemical shifts of the Glu-COOH and Glu-C _{β} carbon resonances of the form in which the glutamyl amino and carboxyl groups are simultaneously coordinated by Cd²⁺ and by Zn²⁺, and the form in which the glutamyl carboxyl group is ionized and the amino group is protonated, are also listed in Table II. The difference is larger for Zn²⁺ than for Cd²⁺, the same as the relative stabilities of the glutamic acid complexes of these metal ions.²⁶ Complexation affects the chemical shift of the Glu-COOH carbon more than the chemical shift of the Glu-C _{β}

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carbon, which in turn experiences a larger effect than the Glu-C _{α} carbon. The larger effect at the Glu-C _{β} carbon compared with the effect at the Glu-C _{α} carbon is similar to what is observed on protonation of the amino group of amino acids and peptides.²⁷

Also listed in Table II are the differences between the solid and dashed curves for the Gly-C _{α} carbon at pD 7.0 in Figures 3, 6, and 8. The order of decreasing difference is Pb²⁺ > Cd²⁺ > Zn²⁺, the same order as the relative affinities of these metal ions for acetylglycine and the C-terminal end of polyglycine peptides.^{2,17}

Conclusions

Carbon-13 chemical shift data indicate that the metal ions Zn²⁺, Cd²⁺, Pb²⁺, and Hg²⁺ bind to the potential coordination sites of glutathione with a high degree of specificity. All four metal ions bind to the sulfhydryl group while only Zn²⁺ and Cd²⁺ bind to the glutamyl amino group under the conditions used in this work. These results demonstrate the potential of carbon-13 magnetic resonance as a technique for elucidating the mode of binding of metal ions by biological molecules.

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Proton Nuclear Magnetic Resonance Line Widths and Spin Relaxation in Paramagnetic Metalloporphyrins of Chromium(III), Manganese(III), and Iron(III)

Gerd N. La Mar*^{1a} and F. Ann Walker^{1b}

Contribution from the Departments of Chemistry, University of California, Davis, California 95616, and California State University, San Francisco, California 94132. Received June 4, 1973

Abstract: The proton nmr line widths of a series of metalloporphyrins of general structure PMX (P = tetra-*p*-tolylporphyrin, M = trivalent transition ion, X = halide or azide) have been investigated as a function of metal ion and apical ligand, X. The line widths depend strongly on M, decreasing in the order Cr > Mn > Fe. For fixed M, the sensitivity of the line width to X depends on the metal ion. In the case of Cr(III), the insensitivity of the line width to X is interpreted in terms of a dipolar relaxation mechanism where the correlation time is the tumbling time of the complex. The iron system exhibits dramatic variations in line width as a function of X which parallel the reverse trend in the zero-field splitting, ZFS, parameter *D*. This is consistent with a dipolar relaxation mechanism where the correlation time is the electron spin relaxation time, *T*_{1e}, which is in turn determined by the modulation of the ZFS by the motions of the complex. A semiquantitative relationship holds between the relative line width as a function of X and the relative value for *D*⁻² for the related deuterohemins. The proton relaxation mechanism for the Mn(III) complexes is intermediate between that of the Cr(III) and Fe(III) systems, with the tumbling time in solution and *T*_{1e} making comparable contributions to the correlation time for dipolar relaxation. The relationship between narrow nmr lines and large ZFS is also demonstrated for the iron(III) complexes in the unusual intermediate *S* = 3/2 spin state, the bis(diethylidithiocarbamate)iron halides. This investigation reveals that in cases where *T*_{1e} makes significant contributions to the correlation time for paramagnetic relaxation of nuclei, the nmr spectral resolution can be controlled, permitting high resolution studies in high-spin iron systems. In favorable cases, the nmr line width data may be used to determine the sign of the ZFS parameter, *D*.

The utility of nuclear magnetic resonance as a tool for investigating electronic structure and dynamic

(1) (a) University of California, Fellow of the Alfred P. Sloan Foundation, 1972-1974. (b) California State University.

properties of paramagnetic molecules² has developed

(2) G. N. La Mar, W. D. Horrocks, Jr., and R. H. Holm, Ed., "Chemical Applications of NMR in Paramagnetic Molecules," Academic Press, New York, N. Y., 1973.

remarkably in the past decade. A particularly important sub-area has been the application of nmr toward elucidating the active site in metalloproteins.³⁻⁵ The advantage of investigating the structure and structure-function relationships in such proteins is that the hyperfine fields^{6,7} generated in the vicinity of the metal ion induce large chemical shifts outside the normal diamagnetic range characteristic of the bulk of the protein. Since the electron-nuclear interactions are relatively short ranged, the hyperfine shifted resonances can serve as sensitive probes for the environment of the active site.

In addition to hyperfine shifts,^{6,7} the paramagnetic center usually causes line broadening^{8,9} for signals originating from nuclei in the vicinity of the metal ion. These relaxation effects⁸⁻¹¹ are sometimes sufficiently severe so as to preclude resolution of the signals near the active site. Although the quantitative interpretation may also yield useful structural data on the active site environment, such information can be realized only if line broadening is small relative to chemical shift differences between nonequivalent nuclei. The factors governing the paramagnetic shifts^{6,7} in complexes of most common transition metal ions are now well understood. However, considerably less attention has been directed toward elucidating the factors influencing nuclear and electron spin relaxation mechanisms¹⁰⁻¹² and the relationships between line width and electronic structure in a given complex.

In the case of the iron hemoproteins^{3,5} (myoglobin, hemoglobin, and cytochrome *c*), which have been subjected most extensively and successfully to such nmr studies, the bulk of the work has focused on the low-spin, LS ($S = 1/2$), ferric proteins and model heme complexes. The high-spin, HS, ferric and ferrous forms of both the proteins¹³ and inorganic models¹⁴ have received much less attention. The initial preference for investigating the LS over the HS ferric species⁵ can be traced to the much more favorable proton relaxation¹⁵ in the former complexes. However, recent work on HS ferrous¹⁶ and ferric¹⁷ hemoproteins as well as heme complexes¹⁸⁻²⁰ has demonstrated the value for extending the nmr studies to HS iron species.

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As an aid toward understanding proton relaxation in HS heme proteins and model heme complexes, we have investigated the proton line widths of a series of paramagnetic porphyrin complexes as a function of metal ion and axial ligand, using the synthetic ligand,²¹ *meso*-tetra-*p*-tolylporphyrin, *p*-CH₃TPP. The choice of the synthetic porphyrin was dictated by the requirement of fourfold symmetry in order that spectral resolution not interfere with the determination of the true line width for the signal from a set of equivalent protons. The metal ions selected are iron(III),²² manganese(III),^{23,24} and chromium(III),²¹ whose complexes magnetic studies have revealed to exist in HS states, with $S = 5/2$, 2, and $3/2$, respectively.^{25,26} Line-width studies for the LS Co(II) porphyrins have been reported elsewhere.²⁷ The complexes of interest can be formulated as *p*-CH₃TPPMX, where X is a halide or azide. X-Ray structural studies of the related complexes of tetraphenylporphyrin and TPPMCl, with M = Fe(III)²⁸ and Mn(III),²⁹ have revealed them to be isostructural, with the metal ion displaced above the porphyrin plane by 0.38 and 0.27 Å, respectively. The structure of the Cr(III) complex may be assumed to be very similar, although a reduced out-of-plane displacement is anticipated.

The present study demonstrates that the factors determining the proton line widths may be ascertained such that qualitative information on the electronic structure of the metal chromophore may be deduced from the relaxation data. Moreover, it is shown that a judicious choice of axial ligand may result in a proton nmr spectrum for a HS ferric porphyrin which is as well resolved as that for the more frequently studied LS forms.

Experimental Section

The Cr, Mn, and Fe porphyrins were prepared by the method of Adler, *et al.*²¹ Approximately 0.5 g of H₂-*p*-CH₃TPP was dissolved in 100 ml of DMF and heated to boiling. The divalent metal chloride (anhydrous CrCl₂ or FeCl₂, hexahydrate of MnCl₂) was added slowly until the spectrum of the free porphyrin disappeared. The solution was cooled for several hours. An equal volume of distilled water was added and the resulting crystals filtered, washed with water, and dried. The crystals were then dissolved in methylene chloride and chromatographed on silica gel (Baker chromatographic grade). Any free porphyrin passed through fairly rapidly, whereas the trivalent metal porphyrin was held on the column. It was eluted with 50% methanol-methylene chloride. The sample was evaporated to dryness and dissolved in methylene chloride; gaseous HCl was bubbled into the solution for about 5 min to convert the trivalent metal porphyrin to the chloride form.

Metathesis of the anion was accomplished by preparing a saturated aqueous solution of the sodium salt of the desired anion and adjusting the pH to 3.0. The desired portion of the metal porphyrin

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chloride in methylene chloride was added and stirred vigorously with the aqueous salt solution for several hours. The methylene chloride layer was then separated and dried over the sodium salt of the desired anion. After filtration, the methylene chloride was evaporated and the sample recrystallized from benzene.

The extinction coefficients and absorption maxima of *p*-CH₃-TPPCrCl were in close agreement with those of Adler, *et al.*,²¹ for the similar TPPCrCl. These values are listed in Table I and are

Table I. Absorption Maxima and Extinction Coefficients of Representative *p*-CH₃TPPCrX Complexes

Absorption max, nm/log extinction coeff	TPPCrCl ^a	<i>p</i> -CH ₃ -TPPCrCl	<i>p</i> -CH ₃ -TPPCrN ₃	<i>p</i> -CH ₃ -TPPCrI
λ ₁		658 (sh)	648	648
ε ₁		2.95	3.11	3.16
λ ₂	600	606	605	602
ε ₂	3.90	3.98	3.97	3.81
λ ₃	560	566	565	564
ε ₃	4.00	4.00	3.98	3.83
λ ₄	520	525	522	519
ε ₄	3.70	3.74	3.80	3.74
λ ₅	448	450	448	451
ε ₅	4.19	5.31	5.24	4.92
λ ₆			422	423
ε ₆			4.72	4.66
λ ₇	394	398	401	399
ε ₇	4.53	4.59	4.61	4.52

^a Reference 21.

compared with those for the azide and iodide metathesis products. The peaks at ~650 and 422 nm may possibly reflect slight contamination of the samples with the free porphyrin, H₂-*p*-CH₃TPP, although this was not detected by nmr.

For the Mn(III) complexes, previous reports on complexes with natural²⁴ as well as synthetic²³ porphyrins have suggested the possible presence of coordinated water in addition to the halide, formulating the complex *p*-CH₃TPPMnCl(H₂O) instead of *p*-CH₃TPPMnCl. However, a recent single crystal X-ray structure²¹ has revealed that the related TPPMnCl is a five-coordinated complex without the additional water. Furthermore, addition of a large excess of *N*-methylimidazole to CDCl₃ solutions of *p*-CH₃TPPMnCl failed to yield evidence for the diamagnetic water resonance, although it has been reported²⁶ that such nitrogen bases completely displace the axial ligands to form the bis adduct. The extinction coefficients and absorption maxima of *p*-CH₃TPPMnCl were in good agreement with those reported by Boucher²⁴ for the complex formulated as TPPMnCl(H₂O), and the visible bands for the other halide complexes were shifted in accordance with data reported by Boucher²³ for (protoporphyrin dimethyl ester, MnX(H₂O)). We therefore formulate these complexes as *p*-CH₃TPPMnX. The purity of the various Mn(III) complexes was also established by noting characteristic shifts of other porphyrin resonances as X is varied.

The Fe(III) complexes of deuterohemin dimethyl ester with a series of anions have been prepared and characterized by Caughey, *et al.*,²² The analogous complexes of *p*-CH₃TPP were prepared in the same manner. The peak positions (particularly for *m*-H and *p*-CH₃) differed significantly as a function of the anion (see Table III), so that the proton nmr spectra served as proof of the purity of the complexes. Solutions containing two complexes with different anions yielded superimposed spectra of the two components, indicating that anion exchange is slow on the nmr time scale. The complexes, *p*-CH₃TPPFeX, with X = Cl, Br, I, and N₃, were obtained pure: repeated metathesis with fluoride anions failed to yield a complex with a spectrum which was not dominated by the original chloride salt, although evidence for a new minor component was present. Our inability to determine the characteristic line widths for any resonance for the X = F complex prevented us from including this complex in our analysis.

The nmr samples were prepared by dissolving 10–15 mg of complex in 0.4 ml of chloroform-*d*. For the complexes with X = Cl and I, changes in solution concentrations of a factor of four did not affect the line widths noticeably, which indicates that the association must be unimportant.

The proton nmr spectra were recorded on a Varian HA-100 spectrometer operating at 100.0 MHz modified to operate with variable frequency modulation using a PAR HR-8 lock-in amplifier. Audio side bands were used to calibrate peak positions, with TMS serving as internal calibrant. All shifts are reported in parts per million, referenced either against TMS or the diamagnetic nickel(II) porphyrin. Line widths are defined as the full width at half-height under nonsaturating conditions and are given in hertz at 100 MHz.

Theory

Nuclear relaxation in a paramagnetic complex can occur through both dipolar¹¹ and scalar¹⁰ coupling to the electron magnetic moment of the metal ion. The combined equations for *T*_{1H} and *T*_{2H} relaxation are

$$T_{1H}^{-1} = 2B[3\tau_c + 7\tau_c/(1 + \omega_S^2\tau_c^2)] + 2C[\tau_c/(1 + \omega_S^2\tau_c^2)] \quad (1)$$

and

$$T_{2H}^{-1} = B[7\tau_c + 13\tau_c/(1 + \omega_S^2\tau_c^2)] + C[\tau_c + \tau_c/(1 + \omega_S^2\tau_c^2)] \quad (2)$$

where $B = S(S + 1)\gamma^2g^2\beta^2/15r^6$ (r = metal-proton distance), and $C = 1/3S(S + 1)(A/\hbar)^2$ (A/\hbar = contact coupling constant in radians sec⁻¹). τ_c is the correlation time for the dipolar interaction and is given³⁰ by

$$\tau_c^{-1} = \tau_r^{-1} + T_{1e}^{-1} \quad (3)$$

and τ_c is the correlation time for the hyperfine exchange mechanism, which can be written³⁰

$$\tau_c^{-1} = \tau_{ex}^{-1} + T_{1e}^{-1} \quad (4)$$

where τ_r , τ_{ex} , and T_{1e} are the tumbling time of the complex in solution, the chemical exchange rate, and the electron spin-lattice relaxation time, respectively. Since the proton line width, δ , and *T*_{2H} are related by

$$\pi\delta = T_{2H}^{-1} \quad (5)$$

experimental proton line widths directly yield estimates of the correlation times if r and A/\hbar are known. In the cases to be considered here, the proton of interest generally experiences zero or negligible contact shifts, so that $A/\hbar \sim 0$. Hence, we can focus only on the first term in eq 2 arising from dipolar relaxation.

The ability to observe well-resolved nmr spectra in paramagnetic complexes is generally attributed⁹ to the condition $T_{1e} < \tau_r$, such that the quality of the spectral resolution is determined by the operation of an efficient electron spin relaxation mechanism.¹² For complexes with inefficient electron spin relaxation (long T_{1e}), $\tau_c = \tau_r$. Comparison of line widths for a given type of proton in a series of isostructural complexes, *p*-CH₃-TPPMX, where M is fixed and X is a variable halogen, will yield τ_c 's which reflect only the difference in the tumbling times, τ_r . The fixed porphyrin ligand is so large in the present complexes that a variation in X is not likely to significantly alter τ_r . Hence, the proton line widths in a series of complexes with fixed M and with $\tau_c = \tau_r$ should be relatively insensitive to the nature of X, perhaps increasing slightly as X increases in size. Furthermore, as long as $\tau_c = \tau_r$, the relative line widths in a series of complexes *p*-CH₃TPPMX are predicted to vary as $S(S + 1)$ if the metal ion is varied and X is kept fixed.

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For complexes of metal ions which exhibit very efficient electron spin relaxation (*i.e.*, $T_{1e} \ll \tau_r$), so that $\tau_c = T_{1e}$, eq 3 and 5 predict that

$$\delta \propto T_{1e} \quad (6)$$

Hence, relative line widths in a series of isostructural complexes must reflect the relative T_{1e} 's. Analysis of the proton relaxation therefore involves analysis of the mechanisms of electron spin relaxation.

The dominant electron spin relaxation mechanism in complexes with $S \geq 1$ has been shown¹² to result from modulation of the zero-field splittings, ZFS, by the tumbling of the complex. Although the exact equation governing T_{1e} differs for the various metal ions being considered, the electron spin relaxation rate for a given metal ion can always be expressed^{12,31} as

$$T_{1e}^{-1} \propto D^2 f(\tau) \quad (7)$$

where D is the ZFS parameter³² in an axially distorted complex, and $f(\tau)$ is the appropriate spectral density function for the motion which modulates the ZFS. In a series of complexes of a given metal ion it may be reasonable to assume that this motion will not depend significantly on X, due to the large bulk of the $p\text{-CH}_3\text{-TPPM}$ moiety. Based on this assumption, eq 6 and 7 can be combined to yield

$$\delta \propto D^{-2} \quad (8)$$

If it can be shown that $\tau_c = T_{1e}$, the relative line widths will yield relative values of the ZFS parameter, D . Trends in the ZFS parameter with variable X reflect trends in the axial field strength³² and can be interpreted by existing models. Changes in proton line widths in a series of distorted nickel(II) complexes have been shown³³ to be consistent with the expected change in the ZFS parameter as a function of the distortion angle.

Results

The proton nmr spectra of TPPCrX exhibit a single broad peak at -7.5 to -8.0 ppm from TMS which is a composite of several phenyl resonances. For $p\text{-CH}_3\text{-TPPCrX}$, the peak at -7.5 ppm is decreased in intensity, and a new peak is present at ~ 2.4 ppm from TMS, near where the diamagnetic methyl group resonates; this latter resonance is assigned to $p\text{-CH}_3$ of the complex. The 3:2 area ratios of the upfield and downfield peaks suggest that the broad peak at -8.0 ppm is due to $m\text{-H}$. The $o\text{-H}$ and pyrrole-H peaks are apparently much too broad to detect. The observed $p\text{-CH}_3$ shifts, which are all in the diamagnetic region, and the methyl line widths for $X = \text{Cl, I, and N}_3$ are given in Table II. Within experimental error, the methyl line widths for the three complexes are identical. The absence of isotropic shifts for both $p\text{-CH}_3$ and $m\text{-H}$ dictates that $A/\hbar \sim 0$ for both; hence, dipolar relaxation alone must be considered.

The spectra for the ferric complex, $p\text{-CH}_3\text{TPPFcCl}$, have already been assigned unambiguously.^{18,19} The traces as a function of X are very similar, except for a

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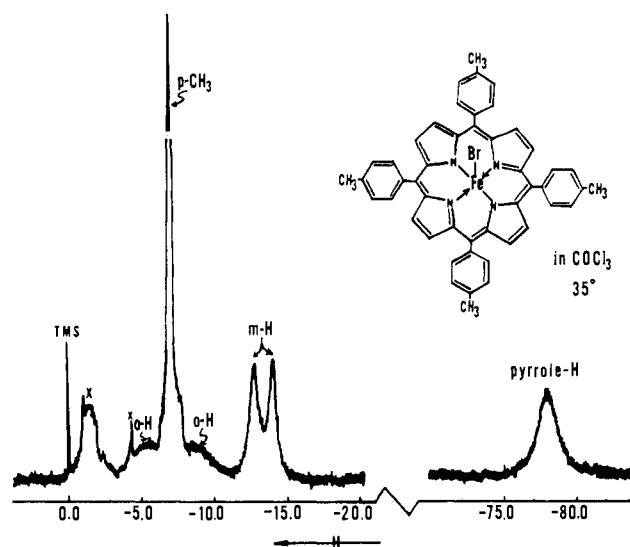


Figure 1. Proton nmr spectrum (100 MHz) of $p\text{-CH}_3\text{TPPFcBr}$ in CDCl_3 solution at 35° . Note that the nonequivalent $o\text{-H}$ resonances are resolved; \times indicates impurity peaks.

Table II. Methyl Proton Line Widths in $p\text{-CH}_3\text{TPPCrX}$ as a Function of X^a

	Cl	I	N ₃
Shift ^b	-2.34	-2.37	-2.35
Line width ^c	65 ± 5	70 ± 5	70 ± 5

^a In CDCl_3 solution, at 35° . ^b Shift in ppm, referenced against TMS. ^c Line width in Hz at 100 MHz.

general downfield bias for all of the phenyl resonances. Most noticeable is a dramatic dependence of line width on X, with the pyrrole-H line width differing by a factor of ~ 3 between $X = \text{I}$ and N_3 . For $X = \text{Br}$, whose proton nmr trace is displayed in Figure 1, the peaks are sufficiently narrow to permit the resolution of the two nonequivalent $o\text{-H}$ peaks. The isotropic shifts for three ligand positions and line width data for the clearly resolved pyrrole-H and $p\text{-CH}_3$ peaks for the complexes with $X = \text{Cl, Br, I, and N}_3$ are listed in Table III.

The $p\text{-CH}_3$ peak in $p\text{-CH}_3\text{TPPMnX}$ complexes is assigned on the same basis as for the Cr(III) complexes; substitution of $p\text{-CH}_3$ for the $p\text{-H}$ in TPPMnX introduces a new, well-resolved peak in the vicinity of the diamagnetic methyl region. An additional downfield peak near -8 ppm from TMS probably arises from $m\text{-H}$. An upfield resonance, which is severely broadened, is due to either $o\text{-H}$ or pyrrole-H. Assignment of peaks other than that due to $p\text{-CH}_3$ is unimportant here. A more detailed analysis of the isotropic shifts in a variety of manganese(III) porphyrin complexes will be reported elsewhere.³⁴ The proton nmr spectra of some Mn(III) complexes with natural porphyrins have been also reported.³⁵ The insignificant phenyl proton shifts again argue for $A/\hbar \sim 0$, indicating dominant dipolar relaxation. The $p\text{-CH}_3$ peaks are also dependent on X, although less dramatically than for the ferric species. The shift and line width data for the $p\text{-CH}_3$ peaks in the complexes with $X = \text{Cl, Br, I, F, and N}_3$ are found in Table IV.

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Table III. Proton Isotropic Shifts and Line Widths in $p\text{-CH}_3\text{TPPFeX}$ as a Function of X^a

Position	I	Br	Cl	N ₃
Pyrrole-H				
Shift ^b	-67.6	-69.1	-68.7	-67.0
Line width	95 ± 10	145 ± 10	250 ± 15	290 ± 20
<i>m</i> -H				
Shifts ^b	-7.58	-6.51	-5.52	-5.28
	-6.28	-5.18	-4.60	-3.96
<i>p</i> -CH ₃				
Shift ^b	-4.98	-4.27	-3.70	-3.08
Line width ^c	9 ± 1	14 ± 1	16 ± 1	18 ± 1
<i>D</i> from line width ^d	~18.5 (1.64) ^f	~14.5 (1.28)	11.3 (1.00)	~10.4 (0.92)
<i>D</i> from DPDMFeX ^e	16.4 (1.83) ^f	11.8 (1.32)	8.95 (1.00)	7.32 (0.82)

^a In CDCl₃ solution at 35°. ^b Shift in ppm, referenced against diamagnetic $p\text{-CH}_3\text{TPPNI}$. ^c Line width in Hz at 100 MHz. ^d Calculated using eq 6 and 7 in text; *D* (cm⁻¹) for X = Cl from ref 18. ^e DPDM = deuteroporphyrin dimethyl ester; far-ir esr data taken from ref 37. ^f *D* in cm⁻¹. ^g Relative values of *D* are given in parentheses, arbitrarily normalized to *D* = 1.0 for X = Cl.

Table IV. Methyl Proton Line Widths in $p\text{-CH}_3\text{TPPMnX}$ as a Function of X^a

	I	Br	Cl	N ₃	F
Shift ^b	-3.08	-3.04	-2.94	-2.93	-2.93
Line width ^c	32 ± 2	52 ± 3	42 ± 2	34 ± 2	29 ± 2
Rel <i>D</i> from line width ^d	-(1.13)	(0.90)	(1.00)	(1.12)	(1.20)
<i>D</i> for PDPMEFeX ^f		-1.10 (0.66) ^g	-2.53 (1.00)	-3.08 (1.12)	

^a In CDCl₃ solution at 35°. ^b Shift in ppm, referenced against TMS. ^c Line width in Hz at 100 MHz. ^d Obtained using eq 8 in text. ^e Relative values of *D* in parentheses; *D* for X = Cl normalized to unity. ^f DPDM = deuteroporphyrin dimethyl ester; far-ir esr data taken from ref 37.

The shifts and line widths for two additional, non-porphyrin complexes of the novel intermediate ferric spin state,³⁶ $S = 3/2$, are included in Table V. The line

Table V. Shift and Line Width Data for (Et₂-dtc)₂FeX^a

	Br	Cl
$\alpha\text{-CH}_2$		
Shift ^b	-26.5	-23.8
Line width ^c	215 ± 10	350 ± 20
$\beta\text{-CH}_3$		
Shift ^b	-3.68	-3.55
Line width ^c	65 ± 5	105 ± 5
<i>D</i> , ^d cm ⁻¹	+7.5	-1.93

^a In CDCl₃ solution at 35°. ^b Shift in ppm, referenced against TMS. ^c Line width in Hz at 100 MHz. ^d Far-ir esr data taken from ref 37.

width data for these diethyldithiocarbamatoiron(III) halide chelates, (Et₂-dtc)₂FeX, with X = Cl and Br, are included for comparative purposes, since ZFS data for these complexes have also been reported.³⁷

Discussion

The data in Tables II–IV show that the sensitivity of methyl line width to X in the isostructural complexes, $p\text{-CH}_3\text{TPPMX}$, differs for the three metal ions considered. If the same correlation time were operative for all three metal ions (*i.e.*, $\tau_c = \tau_r$), then the relative line widths depend only on $S(S + 1)$. This would predict relative methyl line widths of 1:1.6:2.3 for Cr:Mn:Fe; the experimental ratios for X = Cl are 1.0:0.68:0.25, respectively. Therefore T_{1e} must contribute significantly to τ_c for at least the Mn(III) and

(36) B. F. Hoskin, R. L. Martin, and A. H. White, *Nature (London)*, **211**, 627 (1966).

(37) G. C. Brackett, P. L. Richards, and W. S. Caughey, *J. Chem. Phys.*, **54**, 4383 (1971).

Fe(III) species. The actual value for τ_c for each complex may be calculated using the experimental methyl line width (or pyrrole-H for Fe(III)) in Tables II–IV and eq 3; $r(\text{M-}p\text{-CH}_3)$ is estimated at 9.6 Å from X-ray data on the closely related TPP complexes.^{28, 29}

Chromium(III) Porphyrins. The observed line width is essentially independent of X, indicating identical τ_c 's for all complexes. Equation 2 yields $\tau_c \sim 4 \times 10^{-10}$ sec. The rotational correlation time for hemin in water has been obtained from dielectric studies,³⁸ with $\tau_r \sim 5 \times 10^{-10}$ sec. The present $p\text{-CH}_3\text{TPP}$ ligand is more bulky than protoporphyrin IX, but the viscosity of CDCl₃ is only half that of water, so that a τ_r of comparable or slightly smaller magnitude can be expected for $p\text{-CH}_3\text{TPPCrX}$. We therefore conclude that for the Cr(III) species $\tau_c = \tau_r$ and $T_{1e} \gg \tau_r$. This conclusion accounts for the insensitivity of the line width to X since $T_{1e} \propto D^2$, which depends on X (*vide supra*). The size of the complex for X = I or N₃ is slightly larger than for X = Cl, and this may account for the small increase in τ_r for the iodide and azide relative to the chloride complex.

Proton relaxation for the chromium species therefore proceeds *via* dipolar coupling to the electrons, which is modulated directly by the tumbling of the complex in solution. The observation that $\tau_c = \tau_r$ cannot be considered surprising, since Cr(III) complexes generally exhibit well-resolved esr spectra and very poorly resolved nmr spectra, indicative of a long T_{1e} . The long T_{1e} can be traced to the small ZFS parameters ($D < 0.5 \text{ cm}^{-1}$) characteristic of distorted Cr(III) complexes.³⁹

Iron(III) Porphyrins. Contact contributions to the isotropic shifts are not negligible for HS iron(III) porphyrins, as discussed elsewhere,^{13, 19} which necessitates retaining both relaxation terms in eq 2. Since

(38) D. A. Pitt and C. P. Smyth, *J. Phys. Chem.*, **63**, 582 (1959).

(39) Reference 31, Chapter 10.

the complexes are dynamically stable on the nmr time scale, $\tau_{\text{ex}}^{-1} = 0$, and hence $\tau_e = T_{1e}$ in eq 4. Neglecting the hyperfine exchange term at first, eq 2 yields $\tau_e \lesssim 4 \times 10^{-11}$ sec. Since τ_r is much longer than this, τ_e must be T_{1e} . Retaining the hyperfine exchange term in eq 2, with both τ_e and τ_e equal to T_{1e} , we obtain $T_{1e} \sim 3 \times 10^{-11}$ sec (the hyperfine exchange term contributes $\gtrsim 10\%$ to the overall relaxation rate). Relaxation in the Fe(III) system therefore proceeds primarily *via* the dipolar interaction modulated by the electron spin relaxation time, T_{1e} .⁴⁰

From the discussion of nuclear and electron spin relaxation in the second section, we may therefore expect the relative proton line widths for the ferric species to be given by eq 8. The ZFS parameter, D , for the chloride complex has been estimated independently by quantitatively analyzing¹⁸ the quadratic temperature dependent component of the pyrrole-H shift using the theory of Kurland and McGarvey,⁶ with $D_{\text{Cl}} \sim 11.3$ cm⁻¹. Using this value for D_{Cl} , the experimental relative pyrrole-H line widths for other X, and eq 8, values for D for the remaining complexes of Fe(III) are estimated. These estimates are listed in the next to last line in Table III; the relative values of D , obtained directly from eq 8, with D_{Cl} arbitrarily normalized to unity, are also included in parentheses. Also included in the last line of Table III are the values of D for the structurally related complexes of deuteroporphyrin IX dimethyl ester, DPDMEFeX, obtained directly by Brackett, *et al.*,³⁷ using far-ir esr techniques (the relative D values, with D_{Cl} normalized to 1.0, are also found in parentheses).

Comparison of our line width data and the far-ir D values as a function of X reveals that not only do the line widths decrease in the order $\text{N}_3 < \text{Cl} < \text{Br} < \text{I}$, the reverse order of the magnitude of D , but the relative D values calculated from the line width data *via* eq 8 are in semiquantitative agreement with the relative D values determined from the far-ir esr work^{37,41} on DPDME-FeX complexes (the data in parentheses in the last two lines of Table III). Since our evaluation of D for the various $p\text{-CH}_3\text{TPPFeX}$ complexes is based on the assumption of identical spectral density functions for electron spin relaxation, the quality of the agreement in trends strongly supports our conclusion that $\tau_e = T_{1e}$. We therefore conclude that the proton relaxation mechanisms are modulated by T_{1e} , which in turn is determined by modulation of the zero-field levels of the complex.

This analysis suggests that useful data on the ZFS parameters may be obtained by monitoring line width changes of ferric hemes in solution. The reduction in line width by a factor of ~ 2.6 upon substituting iodide for chloride suggests that a *judicious selection of axial ligand may improve considerably the resolution of the proton nmr spectra of HS iron(III) species*, thereby permitting a detailed probing of the environment of HS iron hemoproteins.

It is noteworthy that the trend in isotropic shifts for the *meso*-phenyl peaks, given in Table III, also indicates

(40) M. Rubinstein, A. Baram, and Z. Luz, *Mol. Phys.*, **20**, 67 (1971).

(41) A similar trend in D as a function of X, namely $\text{I} > \text{Br} > \text{Cl}$, has been determined by analyzing the Mössbauer data of the complexes TPPFeX: C. Maricondi, D. K. Straub, and L. M. Epstein, *J. Amer. Chem. Soc.*, **94**, 4157 (1972).

an increase in D in the order $\text{N}_3 < \text{Cl} < \text{Br} < \text{I}$. We have shown elsewhere^{18,19} that the non-Curie temperature dependence permitted determination of the dipolar shift with a T^{-2} dependence which results from the ZFS. For TPPFeCl, the phenyl proton shifts were demonstrated to exhibit large downfield dipolar contributions. The increasing downfield bias for the phenyl shifts which parallels the decreasing pyrrole-H line widths in this study reconfirms the large dipolar contribution to the shifts. At the pyrrole position, the dipolar term represents only a very small fraction of the shift, so that the pyrrole-H isotropic shift is quite insensitive to X. If dipolar shifts are calculated using the D values estimated from the line width data for various X (Table III), the resulting contact shifts for the phenyl group are very similar for all X.

Manganese(III) Porphyrins. The shift data for the Mn(III) complexes also indicate the need to consider only dipolar relaxation. The line width data for $p\text{-CH}_3\text{TPPMnCl}$ yield $\tau_e \sim 1.5 \times 10^{-10}$. If we take τ_e for the chromium(III) porphyrins as τ_r , then the calculated τ_e for the Mn(III) complex suggests that τ_r and T_{1e} have comparable magnitudes, with both contributing to τ_e according to eq 3. Since τ_r should be fairly insensitive to X (as found for Cr(III)), while T_{1e} will vary as D^2 (as found for Fe(III)), it may be expected that the experimental line width will exhibit a dependence on X which is intermediate between the Cr(III) and Fe(III) cases.

The observed line width trend in Table IV ($\text{I} < \text{Br} > \text{Cl} > \text{N}_3 > \text{F}$) is qualitatively consistent with the trend based on the far-ir esr data available for DPDMEMnX complexes,³⁷ reproduced in the last line of Table IV (the relative D 's are given in parentheses). The relative values of D for our complexes, calculated from the line width data and assuming $\tau_e = T_{1e}$, are found in the third line of Table IV. A comparison of the relative D values from our line width data and the esr data reveals that the calculated fractional change in D is much smaller than that observed for the deuterohemins, which is consistent with comparable contributions of τ_r and T_{1e} to τ_e .

It should be noted that the trend in line widths as a function of X is reversed from that found for iron(III) porphyrins, since the sign of D is reversed.³⁷ The relaxation mechanism depends^{12,31} only on the absolute magnitude of D . Although DPDMEMnI was also studied by far-ir techniques, Brackett, *et al.*,³⁷ were unable to determine the ZFS parameter because of poor resolution. The extrapolated trend in D as a function of X, however, led them to suggest³⁷ that the sign of D changes on going from the Br to the I complex. A smooth variation in D as a function of X could therefore yield a D for the X = I complex which is numerically larger (though positive) than for the X = Br complex. The observation that the line width increases $\text{N}_3 < \text{Cl} < \text{Br}$ and then decreases for I lends strong support for a positive D for the iodide complex.

Proton relaxation in manganese(III) porphyrins is concluded to arise from the dipolar interaction modulated by both the direct tumbling of the complex and the electronic spin relaxation time, the latter of which is in turn determined by the modulation of the ZFS.

Miscellaneous Fe(III) Complexes. The line width data for the $(\text{Et}_2\text{-dtc})_2\text{FeX}$ complexes³⁶ with X = Cl

and Br, illustrated in Table V, also confirm a relaxation mechanism similar to that discussed for the HS iron(III) and manganese(III) porphyrins. Estimates³⁶ of $r(\text{Fe}-\text{CH}_2)$ yield $\tau_c \leq 5 \times 10^{-11}$ sec, which is again indicative of $\tau_c \sim T_{1e}$. For these complexes, however, changing X causes major changes in the size and tumbling characteristics of the complex, such that neither τ_r nor the spectral density function for electron spin relaxation is likely to be independent of X. This invalidates use of eq 8. The qualitative trend of decreasing line widths with increasing ZFS parameters, which are available for these complexes from similar far-ir esr work and are reproduced in Table V, clearly reveal that the correlation time is predominantly T_{1e} , which is in turn determined by the ZFS mechanism.

Implications. Our present investigation suggests that for HS porphyrins for which T_{1e} contributes significantly to the correlation time for nuclear relaxation, the nmr spectral resolution can, in principle, be controlled to some extent by the choice of axial ligand. Since D in the axially distorted complexes of interest always increases^{32,37} with decreasing ligand field strength of X, the trend in line width with X will be determined

by the sign of D . Weaker axial ligands will narrow nmr lines if D is positive but will broaden the lines if D is negative. In systems for which no independent esr data on D are available, the trend in line widths with axial ligand could serve to determine the sign of the ZFS parameters.

It may be noted that the present demonstration of a simple relationship between short electron relaxation time (narrow nmr lines) and the magnitude of the ZFS parameter suggests that similarly useful^{3,5,16,17} nmr studies should be applicable to ferrous porphyrins and related complexes. The large ZFS for ferrous phthalocyanine⁴² ($D \sim 64 \text{ cm}^{-1}$), which exhibits the interesting intermediate spin state $S = 1$, indicates that well-resolved proton nmr spectra should be readily obtainable.

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(42) C. G. Barraclough, R. L. Martin, and S. Mitra, *J. Chem. Phys.*, **53**, 1643 (1970).

Spin Density Distribution in Unsymmetrical Sandwich Complexes. II.¹ Electron Spin Resonance Studies of π -Cyclopentadienyl- π -cycloheptatrienechromium(I) and π -Cyclopentadienyl- π -cycloheptatrienylchromium(-I) Radical Anion

Ch. Elschenbroich,* F. Gerson, and F. Stohler

Contribution from Physikalisch-Chemisches Institut der Universität Basel, 4056 Basel, Switzerland. Received May 25, 1973

Abstract: Reaction of π -cyclopentadienyl- π -cycloheptatrienylchromium (CpCrTr) with potassium in 1,2-dimethoxyethane yields the relatively stable radical anion CpCrTr \cdot^- which is characterized by its esr spectrum. In the presence of protic impurities, π -cyclopentadienyl- π -cycloheptatrienechromium (CpCrCHT) is formed instead of CpCrTr \cdot^- . However, CpCrCHT \cdot can be converted into CpCrTr \cdot^- on contact with potassium under strictly aprotic conditions. ESR evidence strongly suggests a dominant π -ligand character for the lowest unoccupied MO of CpCrTr which thus correlates with the orbital e_{2u} of bisbenzenechromium. Reduction of π -cyclopentadienyl- π -methylcycloheptatrienylchromium (CpCrMeTr) leads to the unstable radical anion CpCrMeTr \cdot^- which, in contrast to the parent CpCrTr \cdot^- , cleaves off the seven-membered ligand. The latter is identified by esr spectroscopy in the form of the ion triple MeTr $\cdot^-/2\text{K}^+$.

Whereas the radical cations of bisarene-transition metal- π complexes have provided sufficient data to characterize the highest occupied orbital (HOMO) in this class of compounds,² conclusive information on the nature of the lowest unoccupied orbital (LUMO) has not yet been gathered. Reports on the radical anions of bisarene-transition metal- π complexes are scarce,³ in contrast to numerous studies of noncoordi-

nated aromatic radical anions. In a few cases,⁴⁻⁶ negatively charged paramagnetic ions were obtained from ferrocene derivatives bearing strongly electron-attracting substituents, such as benzoyl or nitro groups. However, such radical anions cannot be regarded as suitable model compounds for a study of spin density distribution between the ligands and the central atom, since in these cases the spin population is essentially localized on the substituent groups. It was therefore

(1) Part I: Ch. Elschenbroich and F. Gerson, *J. Organometal. Chem.*, **49**, 445 (1973).

(2) See part I¹ and the references therein.

(3) To our knowledge, there is only one paper in which the formation of such a radical anion is proposed: G. Henrici-Olivé and S. Olivé, *J. Amer. Chem. Soc.*, **92**, 4831 (1970).

(4) Ch. Elschenbroich and M. Cais, *J. Organometal. Chem.*, **18**, 135 (1969).

(5) J. J. McDonnell, G. Capen, and R. Michealson, *Tetrahedron Lett.*, 4251 (1969).

(6) W. C. Danen and C. T. West, *Tetrahedron Lett.*, 219 (1970).