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Nuclear magnetic resonance of paramagnetic metalloporphyrins

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Abstract. The present article reviews and discusses proton magnetic resonance studies on metalloporphyrins which provide good models for heme proteins in their physical and chemical properties. Emphasis is given on the discussion of the ^{1}H NMR work done in our laboratory.

Keywords. Nuclear magnetic resonance; proton magnetic resonance; metalloporphyrins; paramagnetic shift; iron porphyrins; cobalt porphyrins.

1. Introduction

Metalloporphyrins are an important class of compounds which have been the subject of most extensive research in recent years (Adler 1973; Smith 1975; Dolphin 1978; Lever and Gray 1983). The presence of these compounds as prosthetic groups in hemoproteins has added considerable significance to their studies as models for the active site of the proteins (figure 1). The large size and complexity of hemoproteins present formidable problems in the elucidation of the structure, function and structure–function relationships in biological macromolecules. As an aid toward atleast partial understanding of the function of such complex systems on a molecular basis, enormous amount of research is being done on model metalloporphyrins which exhibit some of the salient physical or spectroscopic properties of the active site in the proteins.

The present article reviews and discusses proton magnetic resonance (PMR) studies in solution on selected paramagnetic metalloporphyrins. The choice of examples is based on two considerations. Only those paramagnetic metalloporphyrins have been discussed here, which are good models of heme proteins and have been investigated by PMR method in detail. The examples selected for discussion are mostly those studied in our laboratory and reflect the current interest of our group. Obviously this review is not intended to be an exhaustive one, and interested readers are referred to other articles for an exhaustive and up-to-date survey (Wüthrich 1970; Phillips 1973; Scheer and Katz 1975; Morrow and Gurd 1975; Morishima *et al* 1978; La Mar and Walker 1979; La Mar 1979; Goff 1983). Further the present article will cover only the proton magnetic resonance studies since ^{1}H is still the most widely studied nucleus by NMR.

The PMR of paramagnetic metalloporphyrins has been utilised for a variety of studies. It has been used to determine distribution of unpaired spin density across the porphyrin skeleton, to study the dynamics of ligand binding, to study relaxation processes and so on (La Mar and Walker 1979; Goff 1983). In the present article the emphasis will be on the application of PMR to determine the electronic structure of the metal ion and metal-ligand bonding in the iron and cobalt porphyrins.

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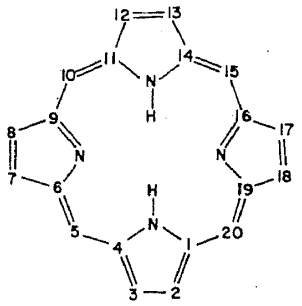

Compound	5,10,15,20	2	3	7	8	12	13	17	18
TPP	Ph	H	H	H	H	H	H	H	H
OEP	H	Et	Et	Et	Et	Et	Et	Et	Et
EP	H	Me	Et	Me	Et	Me	Et	Me	Et
MP	H	Me	Et	Me	Et	Me	P	P	Me
PP	H	Me	V	Me	V	Me	A	A	Me

Ph = − Phenyl Me = $-CH_3$
Et = $-C_2H_5$ V = $-CH=CH_2$
A = $-CH_2-CH_2-COOH$
P = $-CH_2-CH_2-CO_2CH_3$

Figure 1. Metal-free porphyrin with various substitutions and nomenclature.

2. Theory of paramagnetic shift and analysis of results

The nature and shift pattern of the PMR spectra in paramagnetic systems is different from that in the diamagnetic compounds, principally because of the presence of unpaired electron in the former. The shift arising purely from the presence of unpaired electron is called paramagnetic shift or isotropic shift. The isotropic shift for a particular proton in a complex can be obtained from the difference in the shift of proton in a paramagnetic compound and in an analogous diamagnetic metal complex or ligand;

$$(\Delta H/H)_{\text{iso}} = (\Delta H/H)_{\text{para}} - (\Delta H/H)_{\text{dia}}. \tag{1}$$

The isotropic proton shift (IPS) consists of Fermi contact shift and dipolar shift:

$$(\Delta H/H)_{\text{IPS}} = (\Delta H/H)_{\text{CS}} + (\Delta H/H)_{\text{DS}}. \tag{2}$$

Fermi contact term involves interaction of the nuclear magnetic moment with the unpaired electron spin density at the nucleus and is given by

$$H_F = \mathbf{A}s\,\mathbf{S}\cdot\mathbf{I}, \tag{3}$$

where $\mathbf{A}s$ is the hyperfine coupling constant and is given as

$$\mathbf{A}s = (8\pi/3)\gamma_N g\beta\,\delta(\mathbf{r}), \tag{4}$$

$\mathbf{r}$ is a vector specifying the position of the electron with respect to the nucleus. $\delta(\mathbf{r})$ is the Dirac delta function which is 1 for $r = 0$ and zero otherwise. γ_N is magnetogyric ratio of the nucleus; g is rotationally averaged g-value for the complex, β the Böhr magneton. The magnitude of $\mathbf{A}s$ reflects the amount of unpaired spin density at the nucleus and positive or negative spin density is given by sign of $\mathbf{A}s$. The dipolar shift arises because of

a dipolar interaction between the electronic magnetic moment and nuclear spin, and is given by,

$$H_D = \hbar\gamma_N g\beta \left\{ \frac{3(\mathbf{r}\cdot\mathbf{S})(\mathbf{r}\cdot\mathbf{I})}{\mathbf{r}^5} - \frac{\mathbf{S}\cdot\mathbf{I}}{\mathbf{r}^3} \right\}, \tag{5}$$

where $\mathbf{r}$ is a vector locating the electron relative to the nucleus. This term becomes zero for spherical electron distribution.

For a paramagnetic compound with electron spin S and nuclear spin $I = \frac{1}{2}$ (*i.e.* proton), McConnell and Robertson (1958) deduced following expressions for the contact and dipolar shifts:

$$(\Delta H/H)_{\mathrm{CS}} = -\frac{g\beta S(S+1)}{(\gamma_N/2\pi)3kT}\mathbf{A}s, \tag{6}$$

$$(\Delta H/H)_{\mathrm{DS}} = \frac{\beta^2 S(S+1)}{3kT}\cdot\frac{(3\cos^2\theta - 1)}{r^3}\cdot F(g), \tag{7}$$

where $F(g)$ is an algebraic function of the g-tensor values, r the distance between the metal ion and the nucleus, and θ is the angle between the symmetry axis and the vector connecting the metal ion and the nucleus. Equations (6) and (7) predict an ideal Curie-law type temperature dependence for the IPS, which is however not often observed for the metalloporphyrins and other transition metal complexes (Mitra 1977).

In the derivation of eqs. (6) and (7) several assumptions have been made, which drastically restrict their application. First, it was assumed that the ground state of the paramagnetic ion is an orbital singlet with no thermally accessible excited state. Second, it was implicitly assumed that for $S \geqslant 1$ the zero-field splitting (ZFS) of the ground state could be ignored. Third, it was assumed that the orbital contribution to the ground state could be taken into account through the use of g-tensor components. All these assumptions are however not valid for a large number of metal complexes, especially metalloporphyrins. For example, it is known that there may be low-lying states in several metalloporphyrins, which would be appreciably populated at room or higher temperatures. In cases for $S \geqslant 1$ the ZFS could be quite large; in fact the ZFS in many iron porphyrins lies in the range 10–60 cm^{-1}, and hence its effect becomes very significant. It has also now become evident that orbital contribution to the IPS may not always be taken through g-values (Horrocks and Greenberg 1973; Dhingra *et al* 1975). The effect of these factors manifests in the strong deviation from $1/T$ temperature dependence of IPS, which indeed is observed for many metalloporphyrins.

In view of these limitations, Kurland and McGarvey (1970) and Bleaney (1972) have deduced general expressions for the Fermi contact and dipolar shifts of paramagnetic complexes. Here all the above limitations of the theory have been taken into account, and the expressions have wider applicability as both the contact and dipolar shifts have been expressed in terms of the principal molecular susceptibilities. The general expressions in rhombic symmetry are given as follows:

$$(\Delta H/H)_{\mathrm{CS}} = -\frac{\mathbf{A}s}{3(\gamma_N/2\pi)}\left(\frac{K_x}{g_x} + \frac{K_y}{g_y} + \frac{K_z}{g_z}\right), \tag{8}$$

$$(\Delta H/H)_{\mathrm{DS}} = \frac{1}{3N}\{\tfrac{1}{2}(K_x + K_y) - K_z\}\left\langle\frac{3\cos^2\theta - 1}{r^3}\right\rangle + \frac{1}{2N}(K_x - K_y)\left\langle\frac{\sin^2\theta\cos 2\phi}{r^3}\right\rangle. \tag{9}$$

Here K_x, K_y and K_z are the principal molecular susceptibilities along three orthogonal axes. r, θ and ϕ are the polar coordinates of the resonating proton. In axial symmetry which obtains in most metalloporphyrins, $K_x = K_y = K_\perp$ and $K_z = K_\parallel$, and equations (8) and (9) can be written as

$$(\Delta H/H)_{CS} = -\frac{As}{3(\gamma_N/2\pi)}\left(\frac{K_\parallel}{g_\parallel}+\frac{2K_\perp}{g_\perp}\right), \tag{10}$$

$$(\Delta H/H)_{DS} = \frac{1}{3N}(K_\perp - K_\parallel)\left\langle\frac{3\cos^2\theta - 1}{r^3}\right\rangle. \tag{11}$$

Clearly, a knowledge of principal susceptibilities is required not only for calculating the dipolar shift but in determining the hyperfine coupling constant. It would be clear that for highly anisotropic systems, use of eq. (6) for obtaining As is erroneous, and eq. (10) must be used.

Several features of eqs. (8)–(11) are noteworthy. First, it is evident that the temperature dependence of the contact and dipolar shifts follows that of the susceptibilities, and hence it need not follow the $1/T$ dependence as demanded in eqs. (6) and (7). For example, the principal susceptibilities of complexes having an orbital triplet ground term, and those having an orbital singlet ground term with large ZFS, do not obey a $1/T$ variation. Evidently the contact and the dipolar shifts in such cases are expected to show deviation from $1/T$ behaviour. Second, a customary method to calculate the dipolar shift is to use the g-tensor values (vide eq. (7)). In situations where such ESR data were not available (as in the case of orbital triplet ground term complexes), the contribution of this term was earlier generally ignored, which is evidently not correct. Further, in many cases the ESR experiment is done at very low temperatures (*ca* 4 K) and resonance is observed from the lowest spin level, while IPS experiments are done in the temperature range (340–230 K) where all the levels within 200–300 cm^{-1} are populated. This factor can easily be taken through the use of magnetic susceptibility rather than g-values.

When the experimental data of principal magnetic susceptibilities are available, analysis of the IPS data becomes straightforward. When such data are not however available, computed susceptibilities based on ligand (or crystal) field theory are useful. Such calculation uses parametrisation of the ligand field in terms of the electronic structure of the metal ion, which suggests a way to deduce the electronic structure of the metal ion through the analysis of the IPS data.

Finally some comments are necessary about the evaluation of geometric factor in eq. (9) or (11). The values of r, θ and ϕ can be obtained if the x-ray structural data are available. For complexes with rigid geometry the geometric factor can be calculated by simple trigonometric relations using known or estimated structural parameters. For situations involving internal rotations appropriate averaging must be considered. The use of solid state x-ray structural data in calculating geometric factor assumes that there is no structural change in solution. This is largely true for metalloporphyrins.

We now discuss some experimental results on iron and cobalt porphyrins. In our discussion of the proton shift we have taken the down-field shift to be negative. All the shifts are corrected with reference to the free ligand and are with respect to TMS.

3. Survey of experimental results

3.1 *Iron (III) porphyrins*

The ferric ion in iron (III) porphyrins may exist in three discrete spin-states: high-spin ($S = 5/2$), low-spin ($S = \frac{1}{2}$) and intermediate-spin ($S = 3/2$). Majority of the ferric

porphyrins exist either in high- or low-spin state; the intermediate-spin state for the ferric porphyrins is a rarity and has only recently been stabilised in a few complexes. There is also the possibility of thermal spin-equilibrium between any two of these spin-states (George *et al* 1964; Martin and White 1968) and/or quantum mechanical mixing of spin states (Harris 1966; Maltempo 1974; Mitra *et al* 1980, 1983). We discuss here PMR of some high-spin and 'intermediate-spin' (showing spin-mixed ground state) iron (III) porphyrins.

3.1*a* *High-spin iron(III) porphyrins*: PMR of high-spin iron(III) porphyrins has been extensively studied for various reasons. They form a good model system for the ferric heme proteins. The PMR spectra of these compounds consist of well-resolved relatively narrow lines for most protons and show large paramagnetic shifts. In many cases the resonance lines can be easily assigned. Further the analysis of the IPS data becomes relatively easy since the ground state of the metal ion is an orbital singlet ($^6A_1$).

Majority of the iron(III) porphyrins are five-coordinated. Among them tetraphenylporphyrin iron(III) halides, Fe(TPP)X, X = Cl, Br, I, are the best examples in which the iron atom is coordinated to four basal pyrrole nitrogen atoms and a fifth apical halogen. The iron is about 0.5 Å above the mean porphyrin plane and is drawn towards the halogen. Proton magnetic resonance studies on this system over a range of temperature (Behere *et al* 1982) have revealed several interesting features (figure 2a). The large IPS for the pyrrole protons, the observation of two resonances for the meta and ortho phenyl protons, and the non-Curie temperature dependence (figure 3) of the IPS are some of the noteworthy features. The appearance of separate resonances for the two meta and two ortho protons arises because (i) the iron atom is above the mean plane of the porphyrin ring making the two phenyl meta and ortho protons magnetically inequivalent and (ii) the phenyl ring rotation in the solution is slow on

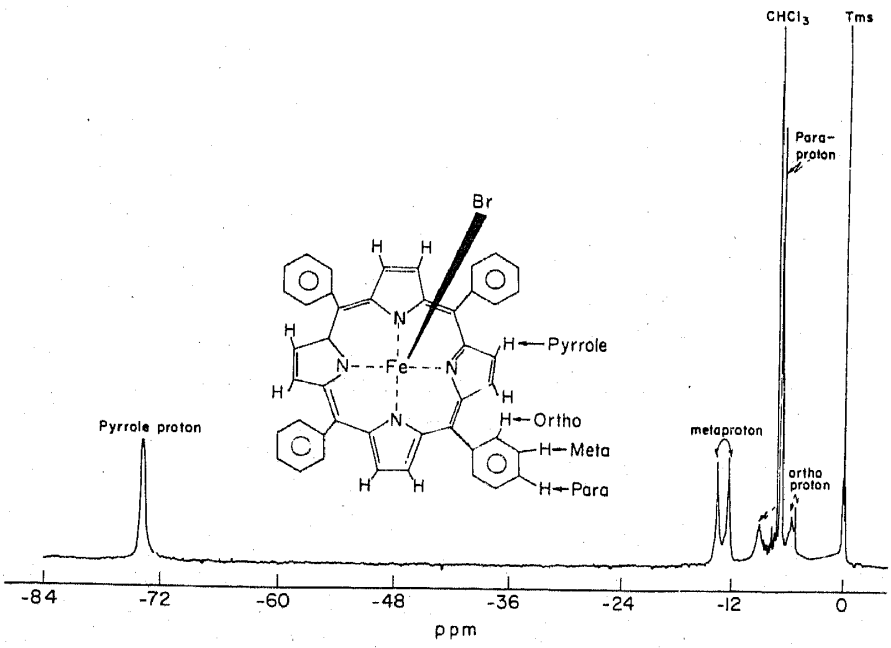

Figure 2. (a) Proton NMR spectrum of Fe(TPP)Br. Two ortho- and meta-proton peaks are indicated. The chemical shift is in ppm. (Behere *et al* 1982).

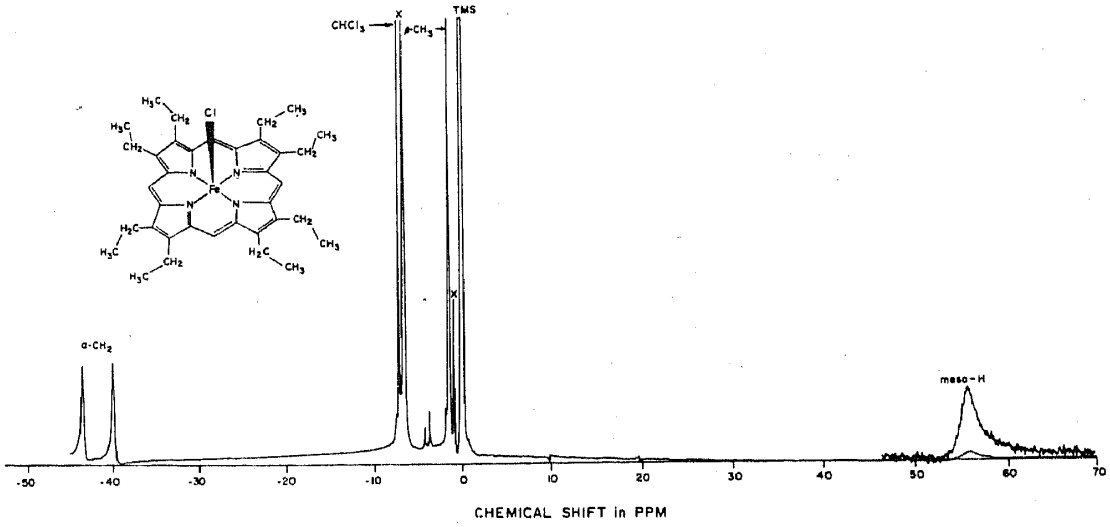

Figure 2. (b) Proton NMR spectrum of Fe(OEP)Cl. The two methylene protons are 'magnetically' inequivalent with respect to the iron atom which is pulled above the porphyrin core toward the chlorine atom.

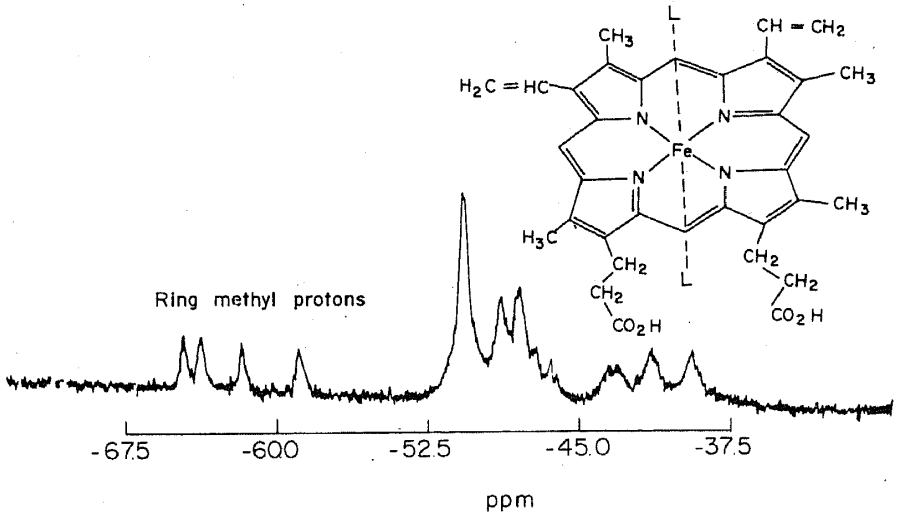

Figure 2. (c) A part of the proton NMR trace of $[Fe(PP)L_2]^+ Cl^-$, L = DMSO. The four methyl protons show well-resolved down-field shift (Behere *et al* 1984).

NMR time-scale. At higher temperatures ($t > 60\,°C$) the rotation of the phenyl ring becomes fast and the doublets coalesce into one peak. The PMR spectrum of closely related octaethylporphyrin iron(III) chloride, Fe(OEP)Cl, shows two resonances for the pyrrole α-CH_2 protons and a broad up-field resonance for the meso proton (figure 2b). As in the Fe(TPP)X, the iron here is displaced out-of-plane, which renders the methylene protons diastereotopic. A similar large IPS is shown by the four methyl groups in the six-coordinated $[Fe(PP)(DMSO)_2]$ Cl, where PP is protoporphyrin (figure 2c). The well-resolved four methyl resonances are clearly separate from other proton resonances (Behere *et al* 1984) and provide a sensitive probe to characterise and

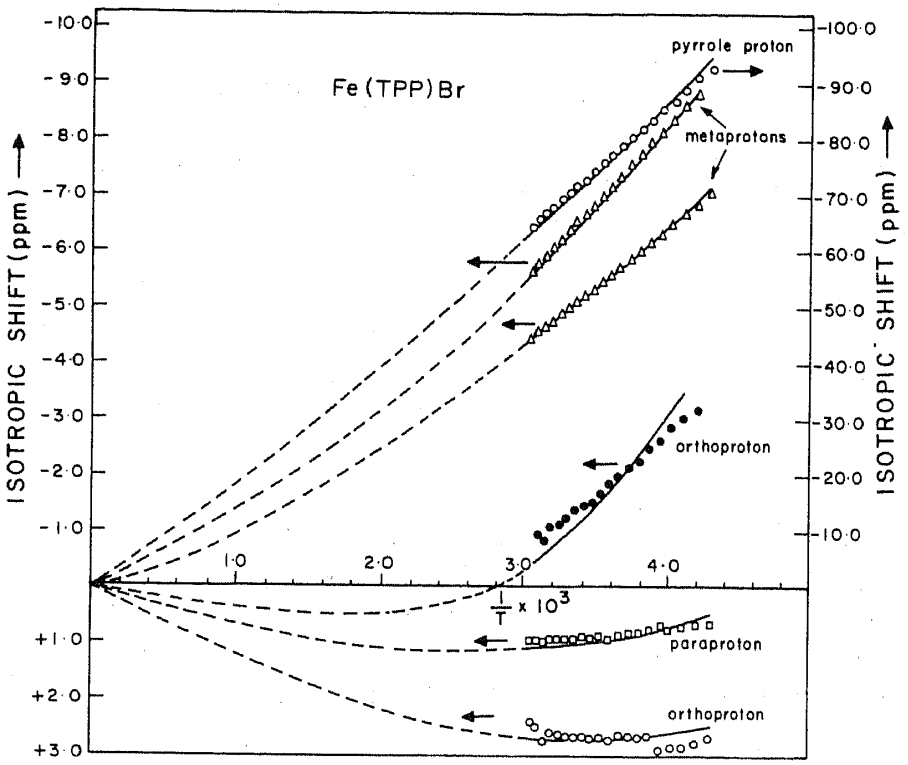

Figure 3. Temperature dependence of the isotropic proton shifts for Fe(TPP)Br in *d*-chloroform (Behere *et al* 1982).

Table 1. Isotropic proton shifts (ppm) for the metal-free and Fe(TPP)X complexes at 293 K. The shifts of the paramagnetic complexes are corrected for the diamagnetic ligand (Behere *et al.* 1982).

Compound	pyrrole H	Ortho H	Ortho H′	meta H	meta H′	para H	NH
TPP[a]	−8·85	−8·22		−7·75		−7·75	2·78
TTP[b]	−8·85	−8·09		−7·75		−2·75[c]	2·78
Fe(TPP) (NCS)	−67·30	−0·21	2·79	−5·62	−4·42	1·20	—
Fe(TPP)Cl	−72·40	*d*	*d*	−5·76	−4·55	1·38	—
Fe(TPP)Br	−73·32	−1·48	2·66	−6·72	−5·27	0·93	—
Fe(TPP)I	−72·30	−4·33	1·51	−7·82	−5·98	0·14	—

[a] TPP, tetraphenylporphyrin; [b] tetra-*p*-tolylporphyrin; [c] shift for the (p-CH_3) protons; [d] not resolved.

understand the heme environmental changes and protein–heme interaction (Budd *et al* 1979).

Returning to the discussion on Fe(TPP)X we examine the IPS data at room temperature, which are listed in table 1. While there is similarity in the shift pattern among the members of the series, some anomalies are immediately obvious. It is seen, for example, that the sign of the shifts for the two ortho protons is different, and their magnitude is smaller than that of the corresponding meta protons (Behere *et al* 1982).

Table 2. Contact and dipolar shifts (ppm) for Fe(TPP)Br at two extreme temperatures (Behere *et al* 1982).

	328 K		233 K	
Protons	CS	DS	CS	DS
pyrrole-H	−55·10	−8·73	−77·56	−17·29
ortho-H	6·05	−6·40	8·51	−12·69
ortho-H′	5·14	−2·39	7·24	−4·74
meta-H	−3·10	−2·50	−4·36	−4·96
meta-H′	−2·74	−1·69	−3·86	−3·35
para-H	3·01	−1·91	4·24	−3·79

There is no a priori reason for the two ortho protons to have IPS of opposite sign. Likewise, the ortho protons being closer to the metal ion are expected to show larger shift than the meta protons. It has been shown (Behere *et al* 1982) that these discrepancies arise due to a substantially large dipolar contribution, especially to the shift of the phenyl protons. The non-Curie temperature dependence of the IPS especially of the phenyl protons is also interpreted to arise from the substantial dipolar contribution.

An accurate evaluation of dipolar shift is possible in the Fe(TPP)X series since both the principal susceptibility tensor values and geometric factors are accurately known (Behere *et al* 1977, 1979, 1981; Behere and Mitra 1979; Scheidt 1978). Using (11) the dipolar shift at various temperatures was therefore accurately estimated; a typical result for Fe(TPP)Br is listed in table 2 at two extreme temperatures of measurements. Several features of the results are interesting. For example, the above anomalies now disappear when the contact shifts of the Fe(TPP)X series are compared. The contact shifts of the two ortho and meta protons have the same sign, and the magnitude of the contact shift of the ortho protons is larger than that of the meta protons. The most significant observation is however that both the dipolar and contact shifts are of similar magnitude for all the phenyl protons. The finding of relatively large contact shift for the phenyl protons is significant since the phenyl proton shifts are generally assumed (Goff *et al* 1977) to be dipolar in view of the C–C single bond joining the phenyl groups to the porphyrin skeleton. The dipolar contribution to the pyrrole proton shift is also significant especially at lower temperatures. It is important to realise that the dipolar shift in the high-spin iron(III) porphyrin arises almost wholly from the zero-field splitting of the ground state ($^6A_1$) since the g-values are here nearly isotropic. Thus an analysis of the IPS data based on eqs. (6) and (7) would lead to erroneous conclusion.

Equations (10) and (11) can be rewritten in another form by substituting expressions for K_i derived on the basis of spin Hamiltonian formalism for the $^6A_1$, and the new expressions are:

$$(\Delta H/H)_{\mathrm{CS}} = -\frac{35g\beta}{12(\gamma_N/2\pi)kT}\mathbf{A}s, \tag{12}$$

$$(\Delta H/H)_{\mathrm{DS}} = 28\, g^2\beta^2 \frac{\langle 3\cos^2\theta - 1\rangle}{(3kT)^2 r^3} D, \tag{13}$$

where D is the ZFS parameter of the $^6A_1$ state, and $g_{\parallel} = g_{\perp} = g$. Hence

$$(\Delta H/H)_{\text{IPS}} = \alpha/T + \varepsilon/T^2, \tag{14}$$

where

$$\alpha = 35g\beta \mathbf{A}s/12(\gamma_N/2\pi)k,$$

$$\varepsilon = (28g^2\beta^2 D/9k^2)(3\cos^2\theta - 1)/r^3.$$

Equation (14) suggests that the temperature dependence of the IPS can afford an estimate of ZFS and of course $\mathbf{A}s$ values. The result of such an attempt is generally successful (table 3) though it is advisable to estimate only D from the fit of eq. (14), $\mathbf{A}s$ being determined separately from ambient temperature data.

The analysis of the IPS data on six-coordinated [Fe(PP) $(DMSO)_2$] Cl on the basis of eq. (14) is rather instructive (Behere *et al* 1984). The four methyl resonances are very prominent in the PMR spectrum (figure 2c), which can be conveniently studied at various temperatures. Such results are shown in figure 4. Here the IPS data are plotted as IPS $\times T$ vs. $1/T$ since eq. (14) can be written as

$$(\Delta H/H)_{\text{IPS}} \times T = \alpha + \varepsilon/T. \tag{15}$$

The slope and intercept of such a plot give D and $\mathbf{A}s$ uniquely. Such an analysis of the data in figure 4 gives $D = 8{\cdot}4\ \text{cm}^{-1}$ and $\mathbf{A}s = 0{\cdot}1$ MHz (Behere *et al* 1984). This method of analysing the data has obviously distinct advantage.

Thus an accurate and proper analysis of the IPS data on high-spin iron(III) porphyrins has highlighted several points:

(i) The dipolar shift is not negligible even for an S-state ion having isotropic g-values. This is because of the fact that the ZFS makes important second order contribution to the IPS. Single crystal magnetic susceptibility data are therefore useful to evaluate the dipolar shift.

(ii) The contact shift for the phenyl protons in the Fe(TPP)X complexes is significant. This was considered earlier to be negligible.

(iii) A reasonably accurate estimate of ZFS of the $^6A_1$ ground state is possible from the analysis of the temperature dependent IPS.

The mechanisms involved in the delocalisation of the unpaired spin across the porphyrin skeleton can be discussed on the basis of the hyperfine coupling constants (table 3). The $\mathbf{A}s$-values of the phenyl protons are similar in magnitude but alternate in sign, indicating that π-type ligand molecular orbitals are involved in the spin-

Table 3. Fermi contact coupling constants, $\mathbf{A}s$ (MH_z), and D (cm^{-1}) values for the Fe(TPP)X series (Behere *et al* 1982).

Proton	FE(TPP)(NCS)	Fe(TPP)Cl	Fe(TPP)Br	Fe(TPP)I
pyrrole-H	0·200	0·210	0·196	0·188
ortho-H	−0·009	—	−0·022	−0·014
ortho-H′	−0·012	—	−0·018	—
meta-H	0·014	0·014	0·011	0·014
meta-H′	0·011	0·011	0·010	0·012
para-H	−0·007	−0·008	−0·011	−0·009
D	5·1	6·0	12·5	13·5

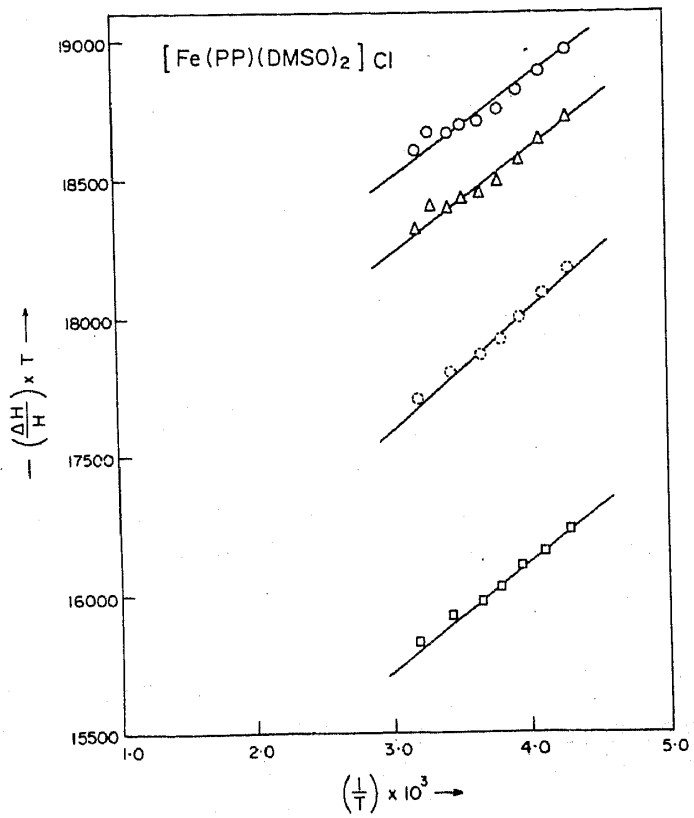

Figure 4. Temperature dependence of the isotropic proton shifts for the four methyl protons of Fe(PP)(DMSO)$_2$Cl. See the text for the reason of the plot as $(\Delta H/H) \times T$ vs $1/T$ (Behere *et al* 1984).

delocalisation mechanism. This π-spin transfer occurring mainly from the meso carbon onto the phenyl ring is due to the fact that the phenyl ring in solution is not perpendicular to the porphyrin ring. The pyrrole protons in the TPP series show contact shifts that are negative and lie in the range of -60 to -70 ppm. In Fe(OEP) Cl, where the pyrrole protons are replaced by the ethyl groups, the α-methylene protons show a negative contact shift of about -30 ppm. This attenuation in the contact shift indicates that the unpaired electron spin density of the ferric ion has delocalised through σ-type molecular orbitals to the pyrrole proton positions (Chakravorty 1970).

3.1b *Spin-mixed iron(III) porphyrins*: As discussed earlier, a ferric porphyrin may have the rare intermediate-spin ground state as well as the possibility of a spin-mixed ground state. In recent years the above possibility has been achieved in a number of examples, the most celebrated among them are the perchlorato iron(III) porphyrins (Mitra *et al* 1983; Dolphin *et al* 1977; Masuda *et al* 1980). These porphyrins have a 'square' pyramidal geometry around the iron atom, similar to that of the Fe(TPP)X series, with oxygen of the perchlorate being coordinated to the iron at the fifth apical position. These porphyrins have intermediate magnetic moments (4·8–5 BM) at room temperature. A detailed solid state magnetic study in the 1·2–300 K temperature range has conclusively established that the ground state of the iron in these molecules is spin-mixed with predominant $S = 3/2$ spin character admixed with a low-lying $S = 5/2$ spin-state (Mitra *et al* 1983).

The PMR characteristics of these molecules are quite revealing. In $CDCl_3$ solution the temperature dependence of the IPS for the $Fe(TPP)ClO_4$ and $Fe(OEP)ClO_4$ complexes are shown in figures 5 and 6. The magnitude and pattern of the shift for the CH_2 and CH_3 protons in $Fe(OEP)ClO_4$ complex are qualitatively similar to that of the high-spin Fe(OEP)Cl though the meso-H shift is much smaller. The temperature dependence of the various proton shifts shows deviations from a simple $1/T$ law. However the most remarkable temperature dependence is observed for the pyrrole protons in $Fe(TPP)ClO_4$ (Goff and Shimomura 1980; Dugad *et al* 1984a). A down-field shift at higher temperatures changes into an up-field shift at lower temperatures (figure 6). These features suggest an unusual electronic structure of the metal ion in these complexes, perhaps similar to that deduced from the solid state magnetic data.

A theoretical analysis of the above data is evidently not possible on the basis of the model of Kurland and McGarvey (1970), which assumes the ground state to be purely of one discrete spin-state and with no close-lying excited state. A complete crystal-field analysis of the data for d^5 electron configuration is therefore needed. Such an analysis of the IPS data has recently been done (Dugad *et al* 1984a), which not only explains quantitatively the 'anomalous' temperature dependence of the pyrrole-H shift but also highlights the application of PMR studies in the deduction of the electronic structure of the metal ion.

The model considers a d^5 ion in tetragonal symmetry with five d-orbitals split into three orbital singlets and one orbital doublet denoted as $b_1(x^2-y^2)$, $a_1(3z^2-r^2)$,

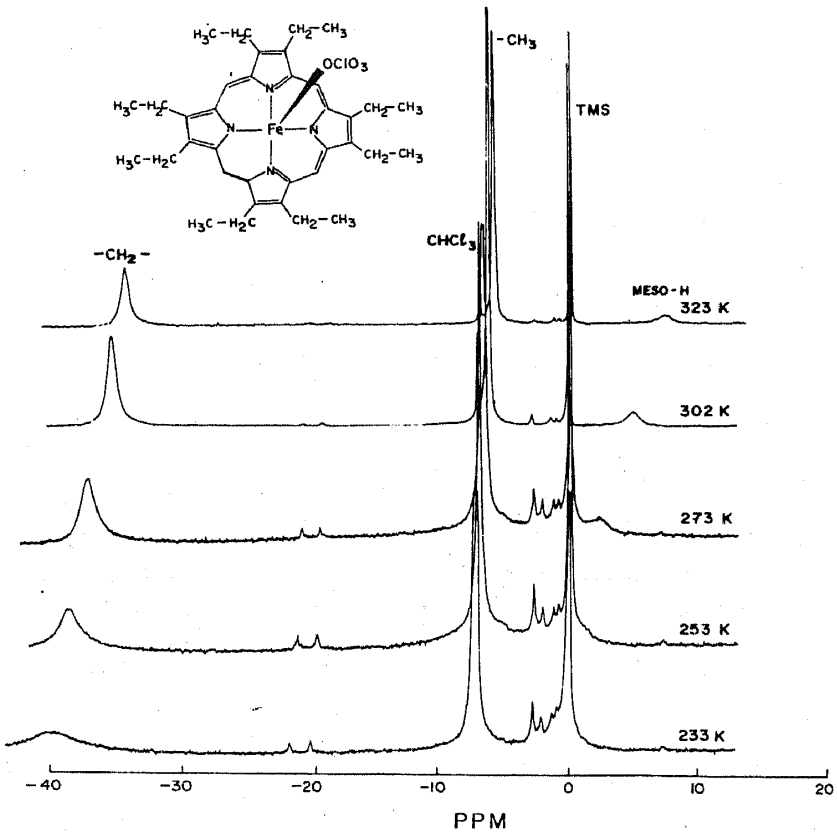

Figure 5. Proton NMR spectra and temperature dependence of various proton shifts in $Fe(OEP)ClO_4$ (Dugad *et al* 1984a).

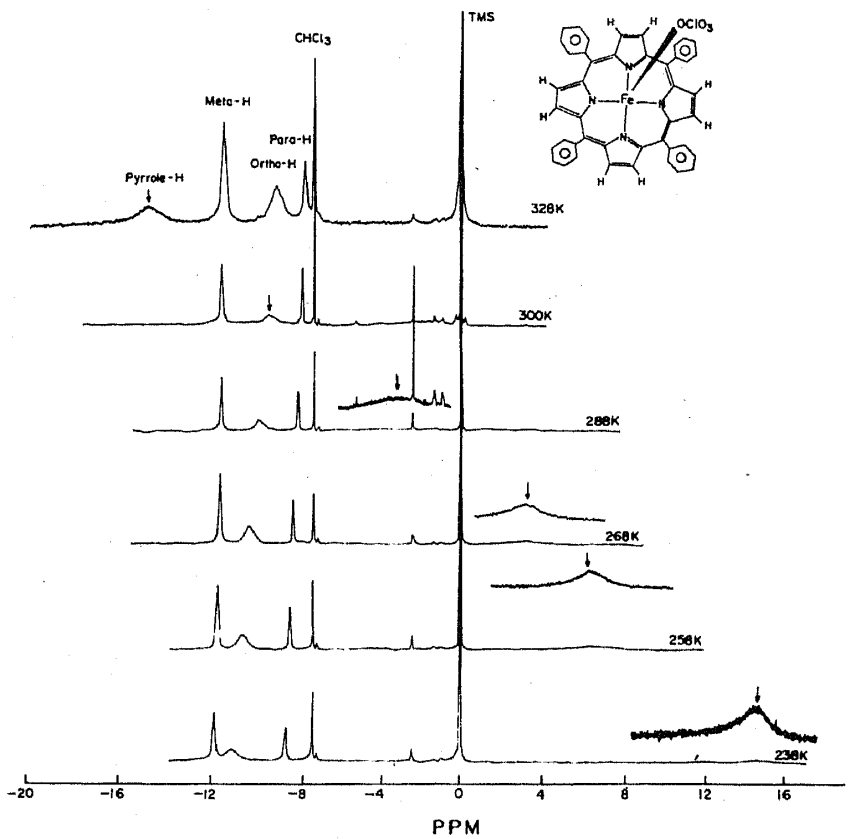

Figure 6. Proton NMR spectra and temperature dependence of various proton shifts in $Fe(TPP)ClO_4$. Note the anomalous temperature dependence of the pyrrole proton (marked by arrow) which turns from a down-field to an up-field shift as the temperature decreases (Dugad *et al* 1984a).

$b_2(xy)$ and $e(xz, yz)$. The relative energies of these orbitals are given by three parameters defined as (Marathe and Mitra 1976):

$$E(b_1) - E(b_2) = \Delta,$$
$$E(a_1) - E(b_2) = \Delta - \delta_2,$$
$$E(e) - E(b_2) = \delta_1.$$

A d^5 ion possesses a total of 43 high, low and intermediate spin multiplet states. Of these only the low-lying sextet ($^6A_1$), the quartets ($^4A_2$ and ^4E) and doublets (^2E and $^2B_2$) mix significantly *via* spin-orbit coupling and hence merit consideration. The energies of these terms are expressed in terms of above parameters (Δ, δ_1 and δ_2) and the Racah parameters B and C. Matrix elements of the spin-orbit coupling is calculated using the operator,

$$\mathscr{H}_{SO} = \zeta \sum_{i=1}^{5} \mathbf{l}_i \cdot \mathbf{S}_i,$$

where ζ is the spin-orbit coupling parameter. A set of 24 wavefunctions corresponding to the above five terms are obtained. Using these wavefunctions as a basis set, 24×24 matrix elements of spin-orbit coupling are calculated. The magnetic susceptibility can then be evaluated in a straightforward manner. Incorporation of (10) and (11) enables the calculation of contact and dipolar shifts and hence the IPS. In doing such a

Table 4. Crystal field parameters for the perchlorato iron (III) porphyrins (Dugad *et al* 1984a).

Parameter		$Fe(TPP)ClO_4$	$Fe(OEP)ClO_4$
Δ		34020 cm^{-1}	34200 cm^{-1}
δ_1		1228 cm^{-1}	1270 cm^{-1}
δ_2		12280 cm^{-1}	12700 cm^{-1}
η		0·3970	0·4081
Ground state	$^4A_2$	54%	62%
Eigen function	$^6A_1$	23%	21%
	^2E	22%	16%

calculation there are in fact a number of disposable parameters: three crystal field (Δ, δ_1 and δ_2), two Racah (B and C) and the ζ. In addition to these are the hyperfine coupling parameters. To reduce the number of parameters, B, C and ζ are taken as free-ion values. Some estimate of Δ, δ_1 and δ_2 are available from the fit to the magnetic data. Attempt is then made to fit the temperature variation data of IPS for various protons in the same molecule with fixed set of Δ, δ_1 and δ_2 and different As. Such an analysis gave excellent fit to the data in figures 5 and 6. The values of the crystal field parameters and the energies and wave functions of various states are summarised in table 4. The results clearly show the spin-mixed behaviour of the ground state, with $^4A_2$ lying lowest and mixed very extensively with a very low-lying $^6A_1$. All other states are observed to be lying very high in energy except the doublet. The main success of the calculation is to reproduce the 'anomalous' temperature dependence of the pyrrole-H in $Fe(TPP)ClO_4$. The analysis of the data, despite its over-parametric nature, has the merit to give information relating to the electronic structure of the metal ion, and hence it may be useful in studying paramagnetic metalloproteins.

3.2 *Iron(II) porphyrins*

Though iron(II) porphyrins provide the closest analogy to the oxy- and deoxy-hemoproteins the NMR studies on these systems are still rather limited. This is largely because the paramagnetic iron(II) porphyrins are highly sensitive to aerial oxidation, and extreme care is needed in handling the sample. Also, the analysis of the data is not as simple as in the high-spin iron(III) porphyrins.

The iron(II) porphyrins exist in three spin states, namely high-spin ($S = 2$), low-spin ($S = 0$) and intermediate-spin ($S = 1$). While most of the iron(II) porphyrins are either high-spin or low-spin, there are now definite examples of the intermediate-spin species.

The best synthetic models for high-spin deoxyhemoglobin are the five-coordinated 2-methylimidazole iron(II) porphyrins. The PMR data on several of them are summarised in table 5 (La Mar *et al* 1977; Goff and La Mar 1977). The down-field pyrrole-H and α-CH_2 shifts indicate a large σ-delocalisation. The relatively small up-field meso-H shift suggests a moderate π-delocalisation to the meso-position. This pattern of IPS is similar to that found for high-spin iron(III) porphyrins which contain an unpaired electron in the $d_{x^2-y^2}$ orbital. The ground state of the high-spin iron(II) porphyrin is therefore likely to have a configuration either $(d_{xy})^2$ $(d_{xz}, d_{yz})^2$ $(d_{z^2})^1$ $(d_{x^2-y^2})^1$ or $(d_{xz}, d_{yz})^3$ $(d_{xy})^1$ $(d_{z^2})^1$ $(d_{x^2-y^2})^1$. In case of the former the ground

Table 5. Isotropic proton shift (in ppm) for high-spin iron(II) porphyrins at 298 K. The data are taken from Goff and La Mar (1977).

Position	TPP	p-CH_3 TPP	OEP
Pyrrole-H	−43·4	−43·7	
Pyrrole-CH_2	—	—	−8·5
-CH_3	—	—	0·37
meso-H	—	—	6·7
Phenyl ortho-H	1·00	1·05	—
Phenyl meta-H	0·70	0·67	—
Phenyl para-H	1·02	CH_3 : 0·37	—

state will be an orbital singlet and in the latter, an orbital doublet. In either of the cases several low-lying excited states are possible. A detailed analysis of the variable temperature data based on crystal field model is therefore needed to determine the actual ground state of the ferrous ion.

Planar Fe(TPP) and Fe(OEP) have been proved to be the unique examples of $S = 1$ spin situation. Since these molecules are highly anisotropic the dipolar contribution is expected to be very large (Boyd *et al* 1979). The shift for the pyrrole-H is small and up-field (Goff *et al* 1977) but this arises from the contact and dipolar shift being of comparable magnitude and opposite sign. The phenyl protons show relatively large down-field shift but an approximate analysis of the data suggests the phenyl proton shifts to be almost wholly dipolar in origin. Magnetic studies on Fe(TPP) have established $^3A_2$ as ground state arising from $(d_{xy})^2$ $(d_{z^2})^2$ $(d_{xz}d_{yz})^2$ electron configuration, and the existence of several low-lying excited states (Boyd *et al* 1979). An analysis of the IPS data based on detailed crystal field model is therefore needed to account for the observed shift.

3.3 *Low-spin Co(II) porphyrins*

Cobalt(II) porphyrins do not occur naturally but the related vitamin B_{12} corrin system is believed to catalyse molecular rearrangements via the Co(II) oxidation state in atleast some of its enzymatic reactions. In addition, Co(II)-reconstituted hemoglobin and myoglobin have been the subject of extensive studies to obtain informations about the heme–heme interaction. These have evoked interest in the electronic properties of cobalt(II) porphyrins.

PMR of several low-spin ($S = \frac{1}{2}$) planar cobalt(II) porphyrins has been studied over a range of temperature. A typical spectrum is shown in figure 7. The shifts are all down-field and some typical results are listed in table 6. The pyrrole and phenyl proton shifts are comparable. The most interesting is however, the temperature dependence of the IPS. A typical result is shown in figure 8. The IPS data show distinct deviation from a $1/T$ dependence, which is not expected of an $S = \frac{1}{2}$ system.

The low-spin cobalt(II) ion in the porphyrins has a $(dxy)^2$ $(d_{xz,\,yz})^4$ $(d_{z^2})^1$ electron configuration with the unpaired electron residing in the (d_{z^2}) orbital. The ground state is then $^2A_1$. The ESR results show large g-anisotropy. In such a case the IPS should obey a $1/T$ dependence. There are two possible explanations for the observed departure from the $1/T$ dependence. First is the possibility of axial solvation at lower temperatures.

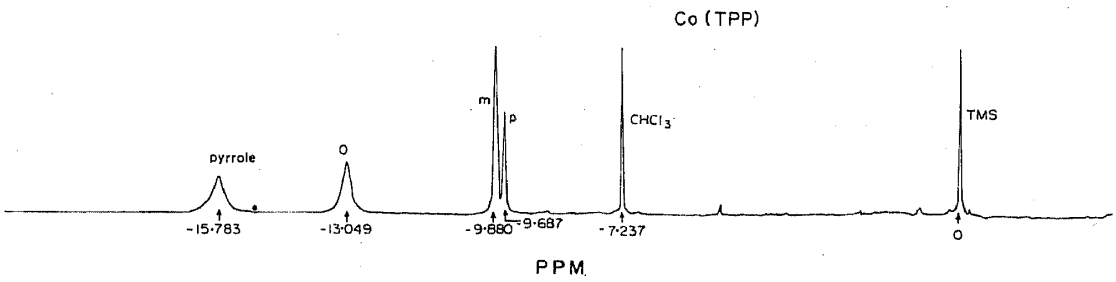

Figure 7. Proton NMR spectrum of Co(TPP).

Table 6. Isotropic shift data for various protons of low spin cobalt(II) porphyrin complexes at room temperature (~ 300 K).

Complex	Pyrrole	Ortho	Meta	Para	Ref.
TPPCo	−6·94	−4·80	−2·12	−1·92	1
(p-CH_3) TPPCo	−6·96	−4·87	−1·29	+0·03 (p-CH_3)	1
(p-OCH_3) TPPCo	−6·96	−4·82	−1·66	−1·46 (p-OCH_3)	1
OEPCo	pyr·CH_2 −3·55	CH_3 −4·05		meso −19·0	2
EPCo	pyr·CH_3 −4·73	CH_2 −4·66	CH_3 −3·92	meso −17·57	3
MPDMECo	pyr·CH_3 −4·35	CH_2 −4·4	CH_3 −3·67	meso −16·62	3

1. Dugad *et al* (1984b); 2. La Mar and Walker (1973); 3. Hill *et al* (1973).

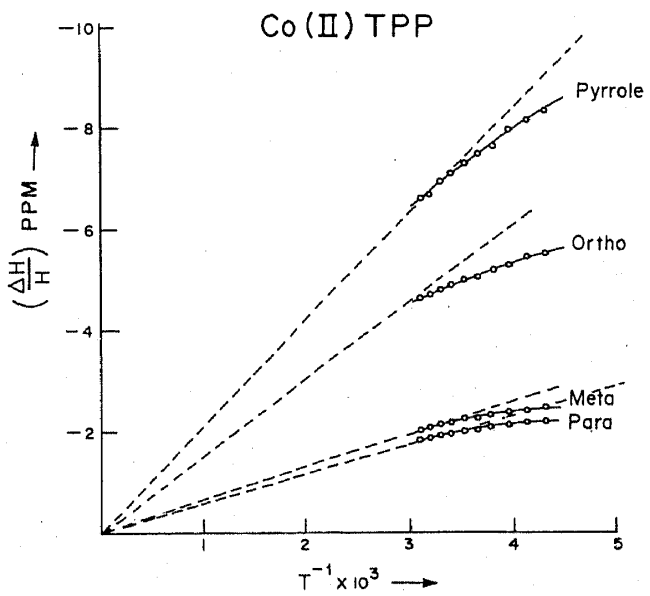

Figure 8. Temperature dependence of the isotropic proton shifts in Co(TPP) (Dugad *et al* 1984b).

Second is the existence of low-lying excited states of higher spin multiplicity. Since the measurements were done in $CDCl_3$ which does not show solvation effect in other metalloporphyrins, the first possibility does not appear to be very likely. However the existence of low-lying excited states has been suspected in many planar low-spin

Table 7. Crystal field parameters for Co(II)TPP (Dugad *et al* 1984b).

Δ_a		10000 cm^{-1}
Δ_b		425 cm^{-1}
ζ		300 cm^{-1}
$g_{\parallel}$		2·00 (1·97)
$g_{\perp}$		2·54 (2·38)
Ground state	$^2A_1$	76%
Eigen function	$^4A_2$	23%

Δ_a and Δ_b are the energies of ^2E and $^4A_2$ with reference to $^2A_1$ respectively; ζ is the one electron spin orbit coupling parameter; values in parentheses are experimental g values taken from LaMar and Walker (1973).

cobalt(II) systems including analogous cobalt(II) phthalocyanine (Gregson *et al* 1976; Murray and Sheahan 1973). On this basis an attempt has recently been made to account for the temperature dependence of the IPS of figure 8, which has given excellent fit to the data and an insight into the electronic structure of the cobalt(II) porphyrins (Dugad *et al* 1984b). Results of such attempt are included in table 7. The deduced parameters also reproduce correctly the observed magnetic moment at room temperature. The presence of the low-lying quartet is perhaps a general phenomenon in low-spin planar cobalt(II) complexes and is responsible for their high magnetic moment (2·2–2·6 BM) at room temperature.

4. Concluding remarks

From the above discussion we find that the proton magnetic resonance spectroscopy of biologically important paramagnetic molecules is very useful in understanding electronic structure of the metal ion. The method is obviously useful for paramagnetic heme proteins in solution where the electronic structure of the iron plays important role in modulating function of the protein. This is in addition to several other applications, which the PMR spectroscopy has in studying the heme systems. Studies in some of these areas on heme proteins are currently being undertaken in our laboratory to understand the electronic structure, interaction of small molecules with the proteins and concommitant conformational changes.

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