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58. Hyperfine Shifts of the ^{13}C-NMR. in Low Spin Iron(III) Porphyrin Complexes

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Summary. The ^{13}C-NMR. in Zn(II)(Porphin), Fe(III)(Porphin)(CN$_2$), Zn(II)(Tetraphenyl-porphin), and Fe(III)(Tetraphenylporphin)(CN$_2$) have been identified, and the ^{13}C hyperfine shifts in the iron complexes evaluated. It was found that dipole-dipole coupling with the electron spin localized in the π-orbitals of the aromatic carbon atoms makes an important contribution to the ^{13}C hyperfine shifts. In a preliminary analysis the experimental spin density distribution obtained from the combined ^{1}H- and ^{13}C-NMR.-data is compared with theoretical models of the iron porphyrin complexes.

Introduction. – Investigations of the electronic states in the heme groups of hemoproteins and their significance for the biological roles of these proteins have for many years attracted the interest of a considerable number of researchers. Some years ago high resolution ^{1}H-NMR.-spectroscopy was added to the techniques suited for studies in this field [1] [2]. The application of NMR. is particularly attractive for low spin ferric (Fe(III), $S = \frac{1}{2}$) heme compounds where the longitudinal electronic relaxation time is very short and hence the line-width of the nuclear resonances is very little affected by the interactions with the unpaired electron [2]. These studies are now being complemented by ^{13}C-NMR.-investigations [3]. The present paper describes the ^{13}C-resonance assignments in Zn(II)(POR)[1], Zn(II)(TPP)[1], Fe(III)(POR)(CN)$_2$[1], and Fe(III)(TPP)(CN)$_2$[1], and presents a preliminary interpretation of the combined data on ^{1}H- and ^{13}C-NMR. hyperfine shifts in the low spin iron(III) porphin complex. As may be seen from the following considerations, studies of the ^{13}C-NMR. hyperfine shifts can be expected to provide some of the data needed to remove the ambiguities in the spin density distributions for iron porphyrin compounds derived on the basis of the ^{1}H-NMR.-data [2] [4] [5] [6].

The nuclear resonance positions Δv observed in paramagnetic species can be decomposed into two terms,

$$\Delta v = \Delta v_{\text{diam}} + \Delta v_{\text{hf}} \tag{1}$$

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[1]) Abbreviations used: POR: porphin; TPP: tetraphenyl porphin; EPR.: electron paramagnetic resonance; FT: *Fourier* transform.

where $\Delta\nu_{\text{diam}}$ would be the resonance positions in the absence of the electronic paramagnetism, and $\Delta\nu_{\text{hf}}$ are the hyperfine shifts which arise from the interactions with the unpaired electrons. In the present and in previous work [2] $\Delta\nu_{\text{hf}}$ was obtained as the difference between the positions of the corresponding resonances in the paramagnetic iron porphyrins and the diamagnetic zinc porphyrin complexes. In the interpretation of $\Delta\nu_{\text{hf}}$ the following contributions have to be considered

$$\Delta\nu_{\text{hf}} = \Delta\nu_{\text{pc}}^{\text{M}} + \Delta\nu_{\text{pc}}^{\text{L}\sigma} + \Delta\nu_{\text{pc}}^{\text{L}\pi} + \Delta\nu_{\text{c}}^{\sigma} + \Delta\nu_{\text{c}}^{\pi} \qquad (2)$$

$\Delta\nu_{\text{pc}}^{\text{M}}$ is the pseudocontact shift arising from dipole-dipole coupling with the electron spin localized on the central metal ion [7] [8], $\Delta\nu_{\text{pc}}^{\text{L}\sigma}$ and $\Delta\nu_{\text{pc}}^{\text{L}\pi}$ are the pseudocontact shifts from interactions with the electron spin delocalized to the ligands through the σ- and π-bonds, respectively [8], $\Delta\nu_{\text{c}}^{\sigma}$ the contact shift arising from *Fermi* contact coupling [9] with the electron spin delocalized to the ligand atoms through the σ-bond system, and $\Delta\nu_{\text{c}}^{\pi}$ the contact shifts from spin delocalization in the π-bond system. When dealing with low spin iron(III) porphyrin compounds it appears to be a reasonable assumption that $\Delta\nu_{\text{pc}}^{\text{L}\sigma}$ and $\Delta\nu_{\text{c}}^{\sigma}$ do not contribute appreciably to $\Delta\nu_{\text{hf}}$ [2].

For the hyperfine shifts of ^{1}H-NMR. pseudocontact coupling with the electron spin on the ligand atoms appears not to be important [8]. Of the two terms which thus remain in equation [2] when the proton resonances in low spin ferric hemes are considered, $\Delta\nu_{\text{pc}}^{\text{M}}$ could in principle be computed from the electronic g-tensor and the atomic coordinates of the complex [8], and the electron spin density localized in the π-orbitals of the aromatic ring carbon atoms to which the protons are bonded could then be obtained from $\Delta\nu_{\text{c}}^{\pi}$ [10]. However, EPR.[1]) spectra of low spin ferric porphyrin compounds have so far only been observed at low temperature, and the present evidence is that the g-values effective in producing the $\Delta\nu_{\text{pc}}^{\text{M}}$ observed in solutions of the iron porphyrin complexes at ambient temperature are not identical with those measured by EPR. in the frozen solutions [2][2]) Hence there is some ambiguity in the evaluation of $\Delta\nu_{\text{c}}^{\pi}$ from the proton hyperfine shifts [2] [4].

With the addition of ^{13}C-NMR. the hyperfine shifts can be measured for almost all the nuclei of the porphyrin molecules. From studies of the symmetric model compounds described in this paper it should be possible to assess the importance of $\Delta\nu_{\text{pc}}^{\text{L}\pi}$ for the ^{13}C-NMR. hyperfine shifts (equation (2)), and to narrow down the limits of uncertainty in the determination of $\Delta\nu_{\text{pc}}^{\text{M}}$. The more accurate data on the spin density distribution thus obtained should warrant more detailed investigations of the electronic wave functions on these complexes, which one may expect to provide a basis for a more detailed investigation of the phenomena observed in the hemoproteins.

Experimental. – Zn(II)(POR), Zn(II)(TPP), Fe(III)(POR) chloride, and Fe(III)(TPP) chloride were obtained from the *Mad River* Chemical Company, Yellow Springs, Ohio, and the deuterated solvents from *Ciba AG* in Basel. For the ^{13}C-NMR.-studies ca. 0.02 M solutions were prepared. To obtain the low spin ferric compounds a large excess of KCN was added. On the basis of earlier, as yet unpublished studies on the complex formation between hemin and cyanide ion

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[2]) In this respect the situation seems to be more favorable in some low spin ferric hemoproteins where the g-tensor was determined by single crystal EPR.-studies at low temperature [11] [12] or by NMR. at ambient temperature [13].

in various solvents it appears that the NMR. spectra presented in this paper correspond to the dicyano-complexes.

The solvents used for the individual experiments are described in the figure captions. Important criteria for the choice of the solvents were that the same solvent should be suitable to prepare sufficiently concentrated solutions of the zinc and the iron complexes, and there should be minimal interference between the resonances of the solvent and those of the porphyrin complexes. In the NMR. spectra of the systems which were finally chosen (Fig. 1–5) there appears to be no indication that the porphyrin complexes form dimers or higher aggregates, a difficulty which is often encountered in work with porphyrin compounds [14].

^{1}H-NMR.-spectra were recorded at 220 or 100 MHz on the *Varian* spectrometers HR-220 and XL-100. Resonance positions are in ppm from internal TMS, where shifts to lower field at constant frequency are indicated by negative numbers.

^{13}C-NMR.-spectra at 25.14 MHz were obtained with the FT[1]) technique. For this our XL-100 spectrometer is equipped with a *Varian* FT attachment including a 16 K *Varian* 620-i computer. Sample tubes with 12 mm outer diameter were employed. The sample temperature, which was maintained constant during each experiment, varied from ca. 29° to 34° depending on the conditions of the particular experiment. The following are typical parameters as they were used for studies of the diamagnetic Zn(II) complexes, and the paramagnetic Fe(III) complexes, respectively. (i) Zn(II)(TPP), 0.02 M in CDCl$_3$ (Fig. 5D): spectral width 5000 Hz, pulse width 100 μsec, proton decoupler noise band width 5.5 KHz, acquisition time 0.8 sec, 75,000 transients accumulated, sensitivity enhancement 0.2 sec. (ii) Fe(III)(TPP)(CN)$_2$, 0.02 M in d$_5$-pyridine-D$_2$O 4:1 (Fig. 2A): Spectral width 10,000 Hz, pulse width 120 μsec, proton decoupler noise band width 5.5 KHz, acquisition time 0.4 sec, 20,000 transients accumulated, sensitivity enhancement 0.2 sec.

Results. – The identification of the ^{13}C-resonances in the diamagnetic and paramagnetic porphyrins is based on heteronuclear proton-carbon-13 double resonance experiments and thus depends critically on the proton resonance assignments. Therefore, with the exception of those which have been published previously, the ^{1}H-NMR.-spectra will also be discussed in this section. The experimental data are summarized in Tables 1 and 2.
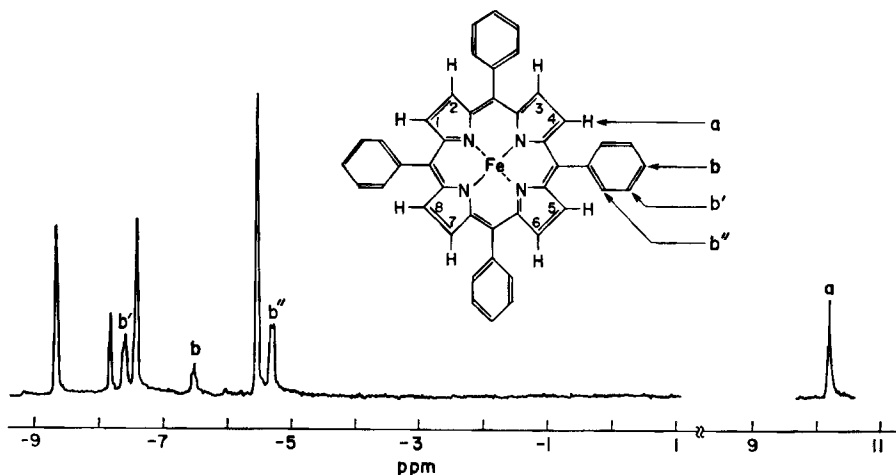

Fig. 1. ^{1}H-NMR.-spectrum at 220 MHz of Fe(III)(TPP)(CN)$_2$ in d$_5$-pyridine/D$_2$O 4:1 at 26°. The solvent gives rise to the four singlet resonances between -5 and -9 ppm. The structure of the complex, in which the two axial cyanide ligands have been omitted, and the resonance assignments are also given.

Fig. 1 shows the ^{1}H-NMR.-spectrum of Fe(III)(TPP)(CN)$_2$. The resonance assignments given in the figure follow from the relative intensities of the resonances and their multiplet structures which arise from proton-proton spin-spin coupling.
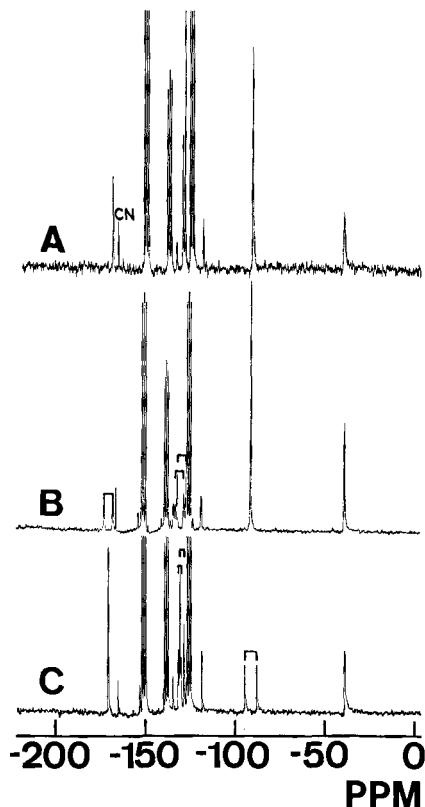

Fig. 2. ^{13}C-NMR.-spectra at 25.14 MHz of Fe(III)(TPP)(CN)$_2$ in d$_5$-pyridine/D$_2$O 4:1. The seven resonances of TPP (see Fig. 1) are at – 40, – 91, – 118, – 128, – 129, – 133, and – 168 ppm. The three triplets at – 124, – 137, and – 150 ppm are the resonances of d$_5$-pyridine, and the line at – 165 ppm comes from the cyanide.

A. ^{1}H-noise decoupled, at 34°. B. Selective double resonance irradiation at proton resonance a (Fig. 1), at 29°. C. Selective double resonance irradiation at proton resonance b″ (Fig. 1), at 29°.

Three ^{13}C-NMR.-spectra of Fe(III)(TPP)(CN)$_2$ observed in different double resonance experiments are shown in Fig. 2. In the proton noise decoupled spectrum (Fig. 2A) seven resonances are observed which correspond to the seven different types of carbon atoms in TPP (Fig. 1). Selective irradiation of the proton resonance a (Fig. 1) yields spectrum 2B, and irradiation of the proton line b″ gives spectrum 2C. The resonance at – 91 ppm corresponds thus to the β-carbon atoms (1–8 in Fig. 1), the resonances at – 128, – 129, and – 168 to the carbon atoms b′, b, and b″ of the phenyl rings (Fig. 1), respectively, and the lines at – 40, – 118, and – 133 ppm to the three quarternary carbon atoms in the molecule. From comparison with Fe(III) (POR)(CN)$_2$ (Fig. 3) the resonance at – 40 ppm is assigned to the quarternary carbon

atoms of the pyrrole rings (Fig. 1). The identification of the two remaining quarternary carbon resonances will be further dealt with in the discussion section.

In the ^{1}H-NMR.-spectrum of Fe(III)(POR)(CN)$_2$ at 34° the resonances of the β- and meso-protons are at 14.8 and -1.3 ppm, respectively [2]. When both these resonances were irradiated in proton noise-decoupling experiments, the ^{13}C-NMR.-spectra A and C in Fig. 3 were obtained. Off-resonance proton irradiation at 6.5 ppm yielded the spectra B and D in Fig. 3 [3]). From the multiplet structures and the relative resonance intensities in these spectra the resonance assignments in Table 1 were derived.
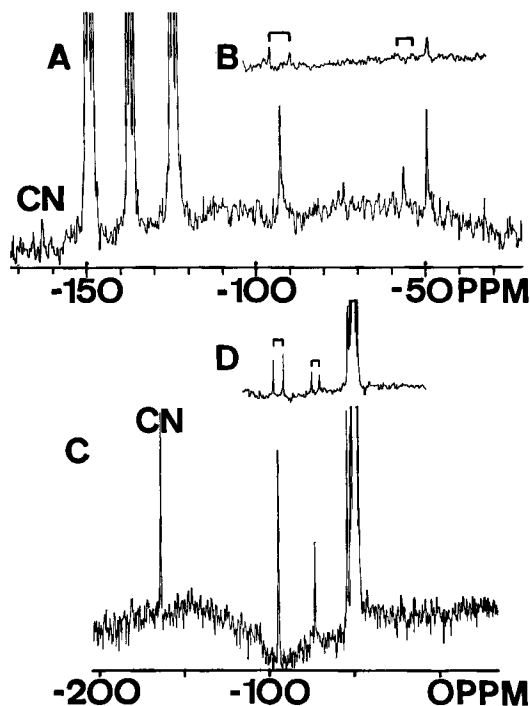

Fig. 3. ^{13}C-NMR.-spectra at 25.14 MHz of Fe(III)(POR)(CN)$_2$. A and B: in d$_5$-pyridine/D$_2$O 4:1, solvent resonances at -124, -137, and -150 ppm. A: proton noise decoupled at 33°, B: proton off-resonance irradiation, at 30°. C and D: in d$_4$-methanol, solvent resonance at -49 ppm. C: proton noise decoupled, at 33°. D: proton off-resonance irradiation, at 30°.

In the spectra of Fig. 2 and 3 only one ^{13}C-resonance can be assigned to the cyanide carbon atom. The position of this line is essentially identical to that of the resonance observed in a solution of KCN in the respective solvent. This is what one would expect if there were no rapid exchange of cyanide ions between the axial positions of the iron porphyrin complexes and the bulk of the solution. If this were actually so it should in principle be possible to detect the resonances of the bound cyanide ions. However, from the stoichiometry of the compounds these resonances,

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[3]) The rather poor quality of the spectra in Fig. 3 is due to less than optimal performance of the spectrometer during the period of time when these particular experiments were carried out.

which may also be somewhat broadened by the interactions with the electron spin [2], have to be comparatively weak. We hope to settle this question in the near future with experiments with ^{13}C-enriched KCN. (See note added in the proofs, p. 595).

In the ^{1}H-NMR.-spectrum of Zn(II)(POR) the β- and meso-protons are observed at -9.8 and -10.6 ppm, respectively [2]. The spectrum of Zn(II)(TPP) is shown in
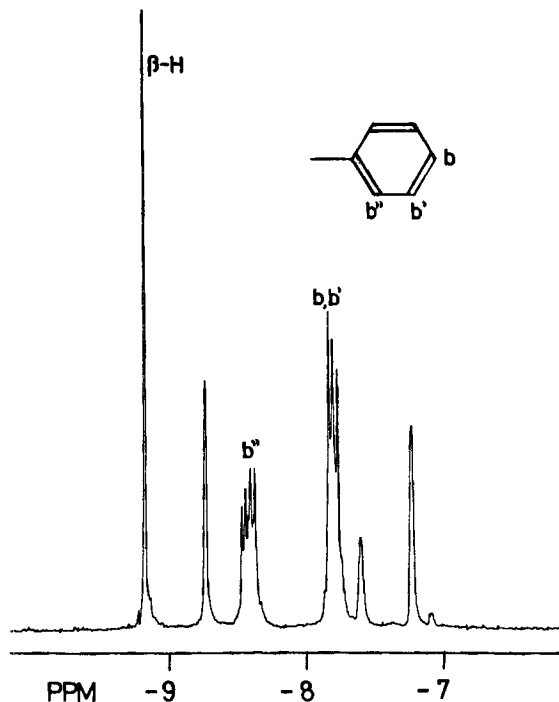

Fig. 4. ^{1}H-NMR.-spectrum at 100 MHz of Zn(II)(TPP) in d$_5$-pyridine/D$_2$O 10:1, at 29°. The identification of the resonances in Zn(II)(TPP) is also given. The three additional singlet resonances between -7 and -9 ppm come from d$_5$-pyridine.

Fig. 4. The identification of the resonances β and b is based on the relative resonance intensities, and those of the resonances b' and b" on comparison with the spectra simulated with characteristic coupling constants for substituted benzene [15] for the two possible assignments of these lines.

In the context of the investigation of the hyperfine shifts in the iron complexes we were mainly interested in the zinc porphyrin complexes in a mixed solvent of pyridine and D$_2$O. Resonance assignments in Zn(II)(POR) and Zn(II)(TTP) in this solvent were obtained from the combination of the data in Fig. 5. In the spectrum 5A there are two resonances of Zn(II)(POR) at -106 and -133 ppm which can be assigned to the meso- and β-positions, respectively, from the off-resonance ^{1}H–^{13}C double resonance experiment in Fig. 5B. In the spectrum of Zn(II)(TPP) in Fig. 5C six resonances of the complex can be observed at -122, -127, -128, -132, -135, and -143 ppm. An off-resonance double irradiation experiment showed that the resonance of one of the quarternary carbon atoms had not been detected, just as in

Zn(II)(POR) (Fig. 5, A und B). In a solution in CDCl$_3$ (Fig. 5D) all the seven resonances of Zn(II)(TPP) can be observed, six of them being in essentially identical positions as those seen in Fig. 5C, and the seventh at − 151 ppm. From the multiplet structures in Fig. 5F the β- and b″-carbon lines can be identified, and the resonances b and b′ were assigned on the basis of their relative intensities. It is then seen that the positions of the β-carbon resonances are essentially identical in the three spectra 5A,
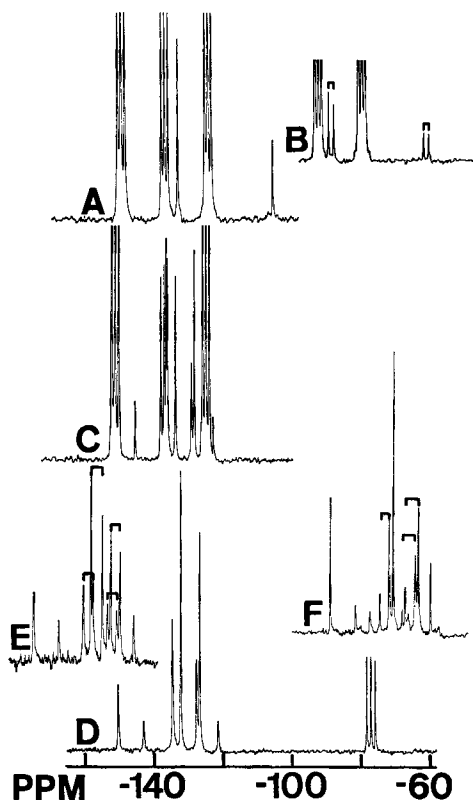

Fig. 5. ^{13}C-NMR.-spectra at 25.14 MHz of Zn(II)(POR) and Zn(II)(TPP). A. Zn(II)(POR) in d$_5$-pyridine/D$_2$O 4:1, proton noise decoupled, at 34°. B. Same sample as in A, proton off-resonance irradiation, at 32°. C. Zn(II)(TPP) in d$_5$-pyridine/D$_2$O 10:1, proton noise decoupled, at 34°. D. Zn(II)(TPP) in CDCl$_3$, proton noise decoupled, at 34°. E. Same sample as in D, proton off-resonance irradiation, at 31°. F. Same sample as in D, selective double resonance irradiation of the resonance of the β-protons, at 29°. The solvent resonances are at − 124, − 137 and − 150 ppm for d$_5$-pyridine, and at − 77 ppm for CDCl$_3$. Note that the horizontal scale applies to the spectra A, C, and D, whereas B, E, and F have been inserted in arbitrary positions.

5C, and 5D, and the same is true for the other corresponding resonances in Fig. 5,C and D. It appears then likely that a quarternary resonance which would correspond to the line at − 151 ppm in spectrum 5D is buried by the solvent resonances in the spectra 5A and 5C. On this basis the resonance of the quarternary carbon atom of pyrrole (Fig. 1) was assigned a chemical shift of − 150.8 ppm in all the three spectra

Fig. 5A, C, and D (Table 2). The identification of the two remaining quarternary carbon atoms in Zn(II)(TPP) will be discussed in the following section.

Discussion. – In the first part of this section, a preliminary analysis of the hyperfine shifts in Fe(III)(POR)(CN)$_2$ (Table 1) is outlined. This is followed by some comments on the extension of the method to the TPP complex and to biological heme compounds.

As was already pointed out in the introduction, it appears that equation (2) can to a reasonable approximation be reduced to (3) for the proton resonances and to (4) for the ^{13}C-resonances in Fe(III)(POR)(CN)$_2$

$$\Delta\nu_{hf}(^1H) = \Delta\nu_{pc}^M + \Delta\nu_c^\pi \tag{3}$$

$$\Delta\nu_{hf}(^{13}C) = \Delta\nu_{pc}^M + \Delta\nu_{pc}^{L\pi} + \Delta\nu_c^\pi \tag{4}$$

In what follows a series of arbitrary values for $\Delta\nu_{pc}^M$ will be chosen, and the corresponding spin density distributions evaluated using the relations proposed by *McConnell* [10], and *Karplus & Fraenkel* [16] [17]. The resulting spin density distributions will be compared with those expected from molecular orbital calculations [5] [18].

The NMR.-data of Fig. 3 show that the g-tensor effective in producing the $\Delta\nu_{pc}^M$'s in Fe(III)(POR)(CN)$_2$ has axial or higher symmetry, where the principal symmetry axis would be perpendicular to the plane of the porphin ligand [2]. The pseudocontact shifts for the different nuclei in porphin are then related by

$$\frac{\Delta\nu_{pc}^M(i)}{\Delta\nu_{pc}^M(j)} = \frac{r_j^3}{r_i^3} \tag{5}$$

where $\Delta\nu_{pc}^M(i)$ and $\Delta\nu_{pc}^M(j)$ are the pseudocontact shifts in ppm for the nuclei i and j, and r_i and r_j the distances of these nuclei from the iron [7]. Hence if a value for $\Delta\nu_{pc}^M$ is chosen for one nucleus the corresponding $\Delta\nu_{pc}^M$'s for all the other nuclei can readily be obtained from the known atomic coordinates of the porphin skeleton [19].

If it is assumed for simplicity that only the electronic ground state is populated and that the influence of the g-tensor anisotropy on the NMR. contact shifts can be omitted from consideration [2], $\Delta\nu_c^\pi$ in ppm is given by [8] [20].

$$\Delta\nu_c^\pi = -A^I \frac{|\gamma_e|}{|\gamma_I|} \frac{S(S+1)}{3kT} \tag{6}$$

A^I is the contact interaction constant, γ_e and γ_I are the gyromagnetic ratios of the electron and the nucleus, S is the electronic spin, k the *Boltzmann* constant, and T the absolute temperature. For an aromatic proton the contact interaction constant A^H can be related to the integrated spin density centered on the π-orbital of the carbon atom to which it is bound, ϱ_C^π, by [10]

$$A^H = Q_{CH}^H \cdot \varrho_C^\pi \tag{7}$$

where $Q_{CH}^H \sim -63$ MHz was found for a large number of compounds.

For an aromatic carbon atom the contact interaction constant A^C can be related to the spin densities ϱ_C^π and $\varrho_{X_i}^\pi$ centered on its π-orbital and on the π-orbitals of the three atoms X_i to which it is bonded [16] [17].

$$A^C = (S^C + \sum_{i=1}^{3} Q^C_{CX_i})\, \varrho_C^\pi + \sum_{i=1}^{3} Q^C_{X_iC}\, \varrho_{X_i}^\pi \tag{8}$$

In equation (8) S^C accounts for the polarization of the 1s-electrons, the $Q^C_{CX_i}$'s for the polarization of the 2s-electrons by the π-electron spin density on the carbon atom, and the $Q^C_{X_iC}$'s for the polarization of the 2s-electrons by the π-electron spin densities on the neighboring atoms. The factors Q depend on the atom X. From theoretical calculations for an aromatic CHC_2 fragment, $S^C = -35$ MHz, $Q^C_{CH} = 54$ MHz, $Q^C_{CC'} = 40$ MHz, and $Q^C_{C'C} = -39$ MHz were obtained [16]. Contact coupling constants computed with equation (8) and using these constants were found to be in good agreement with the experimental data in a variety of radicals [17].

For $\varDelta\nu_{pc}^L$ in equation (4) it is assumed that only the interaction with the π-electron spin density centered on the observed carbon atom has to be considered. In view of the rapid decrease of dipole-dipole coupling with increasing distance, this appears to be a reasonable approximation. We then write

$$\varDelta\nu_{pc}^L = D\varrho_C^\pi \tag{9}$$

where the empirical constant D describes the dipole-dipole coupling of the carbon-13 nuclear spin with the electronic spin localized in the π-orbital.

The hyperfine shifts for the β- and meso-carbon resonances in $Fe(III)(POR)(CN)_2$ can thus be expressed by

$$\varDelta\nu_{hf}(\beta) - \varDelta\nu_{pc}^M(\beta) = D\varrho_C^\pi(\beta) + (S^C + 2Q^C_{CC'} + Q^C_{CH})\varrho_C^\pi(\beta) + Q^C_{C'C}$$
$$[\varrho_C^\pi(\beta) + \varrho_C^\pi(\text{quart.})] \tag{10}$$

$$\varDelta\nu_{hf}(\text{meso}) - \varDelta\nu_{pc}^M(\text{meso}) = D\varrho_C^\pi(\text{meso}) + (S^C + 2Q^C_{CC'} + Q^C_{CH})\varrho_C^\pi(\text{meso})$$
$$+ 2Q^C_{C'C}\, \varrho_C^\pi(\text{quart.}) \tag{11}$$

For any arbitrary value of $\varDelta\nu_{pc}^M$, $\varrho_C^\pi(\beta)$ and $\varrho_C^\pi(\text{meso})$ can be obtained with equations (3), (5), (6), and (7) from the ^{1}H-NMR.-data. The corresponding values for D and $\varrho_C^\pi(\text{quart})$ were then computed with equations (10) and (11). The results for two values of $\varDelta\nu_{pc}^M$ are given in Table 3. Estimates of the spin density on the nitrogen atoms were obtained from $\varDelta\nu_{hf}$ of the quarternary carbon atom (Table 1) using equation (8) and assuming that $Q^C_{NC} \sim Q^C_{C'C} = -39$ MHz, and $Q^C_{CN} \sim Q^C_{CC'} = 40$ MHz.

It seems appropriate at this point to emphasize that we consider table 3 to present preliminary results which may be subject to modification at a later stage of the investigation. The approximations involved in the above analysis will have to be examined more carefully, in particular also the validity for the iron porphyrin complexes of the values used for the coefficients S and Q in equation (8). Yet, it appears that two qualitative conclusions can be drawn. First, it seems that $\varDelta\nu_{pc}^L$ makes an important contribution to the observed carbon-13 hyperfine shifts. Second, it would

Table 1. ^1H- and ^{13}C-resonances in $Zn(II)(POR)$[a]) and $Fe(III)(POR)(CN)_2$[a])

Resonance assignments	Zn(II)(POR) [b])	Fe(III)(POR)(CN)$_2$ [b])	$\Delta\nu_{\text{hf}}$
H$_\beta$	− 9.8	14.8	24.6
meso-H	− 10.6	− 1.3	9.3
C$_\beta$	−133.2	−92.6	40.6
meso-C	−105.7	−56.0	49.7
quart. C	−150.8 [c])	−49.4	101.4

[a])   In d$_5$-pyridine/D$_2$O 4:1 at 34°.
[b])   Chemical shifts in ppm from internal TMS. Negative numbers indicate shifts to lower field.
[c])   From comparison with Zn(II)(TPP) in CDCl$_3$ (Fig. 5).

Table 2. ^1H- and ^{13}C-resonances in $Zn(II)(TPP)$[a]) and $Fe(III)(TPP)(CN)_2$[b])

Resonance Assignments[c])	Zn(TPP) [d])	Fe^{3+}(TPP)(CN)$_2$ [d])	$\Delta\nu_{\text{hf}}$
H$_\beta$	− 9.2	9.9	19.1
H$_b$	− 7.8	− 6.5	1.3
H$_{b'}$	− 7.8	− 7.6	0.2
H$_{b''}$	− 8.4	− 5.4	3.0
C$_\beta$	−132.5	− 90.6	41.9
C$_b$	−128.0	−129.0	− 1.0
C$_{b'}$	−127.5	−128.0	− 0.5
C$_{b''}$	−134.9	−168.2	−33.3
quart. C pyrrole	−150.8 [e])	− 39.8 [f])	111.0
quart. C meso	} { −143.3	} { −132.8	} { −11.2 <
quart. C phenyl	} { −121.6	} { −118.8	} { $\Delta\nu_{\text{hf}}$ < 24.5

[a])   In d$_5$-pyridine/D$_2$O 10:1, at 34°.
[b])   In d$_5$-pyridine/D$_2$O 4:1, at 34°.
[c])   See Fig. 1 and 4 for nomenclature. H$_\beta$ = a in Fig. 1.
[d])   Chemical shifts in ppm from internal TMS. Negative numbers indicate shifts to lower field.
[e])   From comparison with Zn^{2+} (TPP) in CDCl$_3$ (see Fig. 5).
[f])   From comparison with Fe(III)(POR)(CN)$_2$ (see Fig. 3).

Table 3. Experimental spin densities in $Fe(III)(POR)(CN)_2$ corresponding to different arbitrary values of $\Delta\nu_{\text{pc}}^M$ (see text)

Position	A	B
$\Delta\nu_{\text{pc}}^M$ meso-H	10 ppm	3 ppm
ϱ_C^π [a]) C$_\beta$	1.0	1.4
ϱ_C^π [a]) meso-C	0.0	0.4
ϱ_C^π [a]) quart C	0.3	0.7
ϱ_N^π [a])	0.8	0.9
D [b])	74	67

[a])   In per cent of one unpaired electron.
[b])   D describes $\Delta\nu_{\text{pc}}^L$ for the ^{13}C-resonances (equation 9), and is given in ppm per 1% of one unpaired electron.

appear that $\Delta\nu^{M}_{pc}$ is considerably smaller than what one might have expected from the g-values measured in a frozen solution of $Fe(III)(POR)(CN)_2$ [2] [21]. In Table 3, $\Delta\nu^{M}_{pc}$ of example A corresponds approximately to what one would compute from the low temperature g-values[4]), and in B a much smaller value for $\Delta\nu^{M}_{pc}$ had been chosen. If the corresponding spin density distributions are compared with those estimated from *Hückel* calculations on porphyrin complexes [5] [18], it appears that B is a better assumption than A (Table 3). This would be in agreement with earlier observations in the ^{1}H-NMR.-spectra of low spin ferric hemes [4][5]). With the experimental data now available it seems worthwhile to extend the theoretical investigations on the spin density distribution in $Fe(III)(POR)(CN)_2$ to more elaborate models. This should hopefully provide for a better position to make an optimal guess of $\Delta\nu^{M}_{pc}$ and hence to improve the reliability of the experimental spin densities.

In $Fe(III)(TPP)(CN)_2$ a greater number of experimental data are available than in the porphin complex (Table 2), and it will be interesting to compare the resulting values for $\Delta\nu^{M}_{pc}$ and D with those in Table 3. With some good luck, it should be possible to derive the assignments of the two quarternary carbon resonances in the meso-position and in the phenyl ring in $Zn(II)(TPP)$ and $Fe(III)(TPP)(CN)_2$ (Table 2) from considerations similar to those outlined above for $Fe(III)(POR)(CN)_2$. This work is currently in progress.

The ^{13}C-resonances have also been identified in $Fe(III)(protoporphyrin$ $IX)$ $(CN)_2$ [3] [22], $Fe(III)(deuteroporphyrin$ $IX)(CN)_2$ [22], several metal-free porphyrins [23], and work on the zinc complexes with protoporphyrin IX and deuteroporphyrin IX is currently in progress in our laboratory. It is also to be expected that at least some of the ^{13}C-resonances of the heme groups in several low spin ferric hemoproteins will be identified before long [24]. The data on the symmetric model complexes thus get added interest in view of the interpretation of the ^{13}C-NMR. hyperfine shifts in the biological heme compounds.

In addition to the importance for future investigations of biological heme compounds, the present results could possibly be quite generally of some relevance for ^{13}C-NMR.-studies of paramagnetic metal complexes. Previous investigations in this field dealt with systems where both σ- and π-delocalization of the electron spin appear to be important. All the terms in equation (2) had thus to be considered even in a qualitative analysis of the data [25] [26], and the contributions from σ- and π-delocalization could not be evaluated separately. It may now for example be interesting to analyse the empirical parameter D (equation (9) and Table 3) in terms of theoretical considerations on the ligand contributions to the pseudocontact shift [8].

Note added in the proofs: Studies with ^{13}C-enriched KCN showed that there is rapid exchange of cyanide ions between the axial positions of the iron porphyrin complexes and the bulk of the solution. In Fig. 2 and 3 of the present paper the cyanide resonance is only little

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[4]) $g_1 = 3.64$, $g_2 \sim 2.29$, and $g_3 \sim 1.0$ were observed in a frozen solution of $Fe(III)(POR)(CN)_2$ at 1.4 °K [2] [21]. With $g_{||} \sim 3.6$ and $g_{\perp} \sim 1.6$, $\Delta\nu^{M}_{pc}$ (meso-H) ~ 10 ppm is found with the formalism of ref. [8].

[5])   M. *Karplus* (private communication) found in a recent, as yet unpuslished, detailed analysis that the ^{1}H-NMR.-data on low spin ferric hemes imply positive spin density on the meso carbon atoms. This would then also be in qualitative agreement with the results of the present paper.

affected by the presence of the iron porphyrin complexes becouse the solutions contained a large excess of KCN.

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