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# 37. Hyperfine Shifts of the $^{13}$C-NMR. in Protoporphyrin IX Iron (III) Dicyanide and Deuteroporphyrin IX Iron (III) Dicyanide

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(31. VIII. 73)

*Summary.* The $^{13}$C-NMR. in Zn(II) (Protoporphyrin IX), Fe(III) (Protoporphyrin IX) $(CN)_2$, Zn(II) (Deuteroporphyrin IX dimethylester), and Fe(III) (Deuteroporphyrin IX) $(CN)_2$ have been identified, and the $^{13}$C hyperfine shifts in the iron complexes evaluated. In a partial analysis of these data the parameters $Q^{H}_{CCH_3}$, $Q^{H}_{CCH}$, and $Q^{C}_{C'CH}$, which characterize the isotropic coupling through hyperconjugation between the $^1$H and $^{13}$C nuclei of the porphyrin side chains and the unpaired electron spin density on the aromatic ring carbon atoms, have been re-examined. This paper is part of an investigation of the electronic states in low spin ferric hemes and hemoproteins, and the relations between the electronic structures and the biological roles of these molecules.

**Introduction.** – Several years ago high resolution $^1$H-NMR. spectroscopy was found to be a suitable method for the investigation of the unpaired electron spin density distribution in low spin ferric hemes and hemoproteins [1] [2]. It became soon apparent, however, that additional data were needed to remove certain ambiguities in the spin density distributions derived from proton NMR. hyperfine shifts alone [2] [3]. Recently we have started to complement the $^1$H-NMR. results by studies of the $^{13}$C-NMR. hyperfine shifts in the fourfold symmetrical low spin iron (III) complexes with porphin and tetraphenylporphin [4]. In the present paper the $^{13}$C-NMR. hyperfine shifts for Fe(III) (PROTO $(CN)_2$1)) and Fe(III) (DEUT) $(CN)_2$1)) are discussed.

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1) Abbreviations used: NMR.: nuclear magnetic resonance; ppm: parts per million; TMS.: tetramethylsilane; PROTO: protoporphyrin IX; DEUT: deuteroporphyrin IX; DEUTME: deuteroporphyrin IX dimethyl ester; EPR.: electron paramagnetic resonance; FT.: *Fourier*-transform.

**Analysis of the Experimental $^1$H- and $^{13}$C-NMR. Hyperfine Shifts.** – The nuclear resonance positions $\Delta\nu$ observed in paramagnetic species can be decomposed into two terms,

$$\Delta\nu = \Delta\nu_{\text{diam}} + \Delta\nu_{\text{hf}} \tag{1}$$

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 the previous work [2] [4] $\Delta\nu_{\text{hf}}$ was obtained as the difference between the positions of the corresponding resonances in the paramagnetic iron porphyrins, and in the diamagnetic zinc porphyrin complexes. It appears that only very small contributions to the hyperfine shifts in low spin ferric hemes should come from electron spin delocalization through $\sigma$-bonds [2] [4–6], and hence $\Delta\nu_{\text{hf}}$ would to a good approximation be given by

$$\Delta\nu_{\text{hf}} = \Delta\nu^{\text{M}}_{\text{pc}} + \Delta\nu^{\text{L}\pi}_{\text{pc}} + \Delta\nu^{\pi}_{\text{c}} \tag{2}$$

$\Delta\nu^{\text{M}}_{\text{pc}}$ is the pseudocontact shift arising from dipole-dipole coupling with the electron spin localized on the central metal ion [7] [8], $\Delta\nu^{\text{L}\pi}_{\text{pc}}$ is the pseudocontact shift from interactions with the electron spin delocalized to the ligands through the $\pi$-bonds [8], and $\Delta\nu^{\pi}_{\text{c}}$ the contact shift from spin delocalization in the $\pi$-bond system [9] [10].

The first step in the evaluation of the spin density distribution consists in determining the term $\Delta\nu^{\pi}_{\text{c}}$ from equation (2). For protons, $\Delta\nu^{\text{L}\pi}_{\text{pc}}$ appears not to be important [8], so that $\Delta\nu^{\text{c}}_{\pi} = \Delta\nu_{\text{hf}} - \Delta\nu^{\text{M}}_{\text{pc}} \cdot \Delta\nu^{\text{M}}_{\text{pc}}$ could in principle be computed from the electronic *g*-tensor [7] [8]. However, because of the extremely short longitudinal electronic relaxation times [2], no EPR. spectra of low spin ferric hemes at ambient temperature have as yet been reported. Therefore the separation of the terms $\Delta\nu^{\pi}_{\text{c}}$ and $\Delta\nu^{\text{M}}_{\text{pc}}$ in the proton hyperfine shifts has so far remained somewhat ambiguous [2] [3] [5] [6] [11]. For $^{13}$C, $\Delta\nu^{\text{L}\pi}_{\text{pc}}$ can also be an important contribution to the hyperfine shifts of the aromatic carbon atoms [4]. Again, as for protons, the evaluation of $\Delta\nu^{\pi}_{\text{c}}$ from equation (2) is not straightforward. However, combining the data on $^1$H- and $^{13}$C-NMR. hyperfine shifts appears to allow a considerable reduction of the uncertainties in the estimates of the pseudocontact shift contributions [4]. For the partial analysis of the hyperfine shifts in this paper, we will assume that the electronic *g*-tensor in Fe(III) (PROTO) $(CN)_2$ and Fe(III) (DEUT) $(CN)_2$ has axial symmetry, where the principal symmetry axis would be perpendicular to the heme plane. The relative pseudocontact shifts for the different nuclei are then given by

$$\frac{\Delta\nu^{\text{M}}_{\text{pc}}(\text{i})}{\Delta\nu^{\text{M}}_{\text{pc}}(\text{j})} = \frac{\text{r}_\text{j}^3}{\text{r}_\text{i}^3} \tag{3}$$

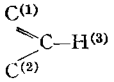
where $\Delta\nu^{\text{M}}_{\text{pc}}(\text{i})$ and $\Delta\nu^{\text{M}}_{\text{pc}}(\text{j})$ are the pseudocontact shifts in ppm for the nuclei i and j, and $\text{r}_\text{i}$ and $\text{r}_\text{j}$ the distances of these nuclei from the iron [7].

With $\Delta\nu^{\pi}_{\text{c}}$ determined through equation (2), one can proceed to the computation of the contact interaction constants $\text{A}^\text{I}$. For the purpose of the discussion in this paper, it is assumed 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]. $\Delta\nu^{\pi}_{\text{c}}$ in ppm is then given by [8] [12]

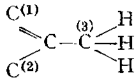
$$\Delta\nu^{\pi}_{\text{c}} = -\text{A}^\text{I}\left|\frac{\gamma_\text{e}}{\gamma_\text{I}}\right| \frac{\text{S}(\text{S}+1)}{3\,\text{kT}} \tag{4}$$

where $\gamma_e$ and $\gamma_I$ are the gyromagnetic ratios of the electron and the nucleus, S is the electronic spin, k the *Boltzmann* constant, and T the absolute temperature.

The contact interaction constants are related to the unpaired electron spin densities $\varrho^\pi_c$ localized on the aromatic carbon atoms by empirical parameters $Q^I$. For the present analysis of the heme data, we will primarily deal with the fragments I and II, in which finite spin density can be localized in the $\pi$-orbitals of the three aromatic carbon atoms.



I



II

For the aromatic proton $H^{(3)}$ in I, we have [10]

$$A^H = Q^H_{CH} \cdot \varrho^\pi_c \tag{5}$$

A corresponding relation holds for the methyl protons in II [13] [14]

$$A^H = Q^H_{CCH_3} \cdot \varrho^\pi_c \tag{6}$$

For an aromatic carbon atom the contact interaction constant $A^C$ can be related to the spin densities $\varrho^\pi_c$ and $\varrho^\pi_{x_i}$ centered on its $\pi$-orbital and on the $\pi$-orbitals of the three atoms $X_i$ to which it is bonded [15] [16]. For the central carbon atoms in the fragments I and II one has

$$A^C = \left(S^C + \sum_{i=1}^{3} Q^C_{CX_i}\right) \varrho^\pi_C + \sum_{i=1}^{3} Q^C_{X_iC}\, \varrho^\pi_{X_i} \tag{7}$$

where $S^C$ accounts for the polarization of the 1s-electrons, the $Q^C_{CX_i}$'s for the polarization of the 2s-electrons by the $\pi$-electron spin density on the observed carbon atom, and the $Q^C_{X_iC}$'s for the polarization of the 2s-electrons by the $\pi$-electron spin densities on the neighboring atoms. For the methyl carbon atom in (II), one then obtains from equation (7)

$$A^C = Q^C_{C'C} \cdot \varrho^\pi_{C'} \tag{8}$$

where C′ denotes the aromatic carbon to which the methyl group is bound. The parameters $Q^H_{CH}$, $S^C$, $Q^C_{CX_i}$, and $Q^C_{X_iC}$ describe electron-nucleus interactions through spin polarization [10] [13] [15]–[18]. The following numerical values have been derived from theoretical considerations, and were found to be in good agreement with the experimental data in a variety of organic radicals: $Q^H_{CH} \approx -63$ MHz, $S^C \approx -35$ MHz, $Q^C_{CH} \approx 54$ MHz, $Q^C_{CC'} \approx 40$ MHz, and $Q^C_{C'C} \approx -39$ MHz.

In equation (6) the parameter $Q^H_{CCH_3}$ accounts for electron-nuclear spin-spin coupling which is mainly due to hyperconjugation [13] [14]. For uncharged aromatic radicals, $Q^H_{CCH_3} \approx 75$ MHz was proposed [19] [20]. However from studies of a large number of aromatic radicals, including paramagnetic metal ion complexes, $Q^H_{CCH_3}$ was also found to be quite different in different molecules [14] [20] [21].

In the $^1$H-NMR. studies of hemes and hemoproteins, $Q^H_{CCH_3}$ is of particular interest because the methyl resonances **a** are most easily observable, and their positions can be measured very accurately also in proteins with high molecular weight [2]. In the past, different values for $Q^H_{CCH_3}$ in low spin ferric porphyrin complexes had been proposed [2] [3] [5] [6] [11]. It is the main purpose of the discussion in this paper to reexamine the parameter $Q^{H}_{CCH_3}$ in equation (6), and to derive the corresponding parameters $Q^{H}_{CCH}$ and $Q^{C}_{C'CC}$, which characterize the isotropic coupling of the unpaired electron with the $\beta$-methylene protons and the $\beta$-carbon atoms of the propionic acid side chains (Fig. 1, d), from the combined $^1$H- and $^{13}$C-NMR. data.

**Experimental.** – Iron(III) protoporphyrin IX chloride was purchased from *Eastman Chemicals*, Rochester N.Y. Iron(III) deuteroporphyrin IX chloride was prepared by Mr. *J. Hochmann* according to the procedure described by *Winterhalter et al.* [22].

Zn(II) (PROTO) was prepared from protoporphyrin IX ‚B grade', which had been purchased from *Calbiochem*, and Zn(II)acetate according to the method of *Fischer et al.* [24]. The substance was characterized by comparison of its $^1$H- and $^{13}$C-NMR. spectra with those of Zn(II) protoporphyrin IX dimethylester obtained from Dr. *W. S. Caughey*, and Zn(II) (PROTO) obtained from Dr. *T. Yonetani*. *ca.* 10 mg of Zn(II) (DEUTME) were obtained from Dr. *W. S. Caughey*. For the $^{13}$C-NMR. studies *ca.* 0.006 to 0.05 M solutions were prepared, depending on the solubility of the complexes, and the available quantities of substance. 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 in various solvents, it appears that the NMR. spectra of the iron compounds presented in this paper correspond to the dicyanide complexes.

The solvents used for the individual experiments are described in the figure captions. Important criteria for the choice of the solvents were that similar solvents should be used 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. 1–6) 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 [23].

$^{13}$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) (PROTO), 0.01 M in $d_5$-pyridine (Fig. 5 A): spectral width 5,000 Hz, pulse width 60 $\mu$s, proton decoupler noise band width 5.5 KHz, acquisition time 0.8 s, pulse delay 0.8 s, 150,000 transients accumulated, sensitivity enhancement 0.2 s (ii) Fe(III) (PROTO) $(CN)_2$, 0.05 M in $d_5$-pyridine/$D_2O$ 4:1 (Fig. 2 A): Spectral width 10,000 Hz, pulse width 100 $\mu$s, proton decoupler noise band width 6.0 KHz, acquisition time 0.4 s, 120,000 transients accumulated, sensitivity enhancement 0.2 s. The chemical shifts are given in ppm[1]) from internal TMS[1]), where shifts to lower field at fixed frequency are indicated by negative numbers.

**Results.** – The resonance assignments in the $^{13}$C-NMR. spectra of Fe(III) (PROTO) $(CN)_2$, Fe(III) (DEUT) $(CN)_2$, Zn(II) (PROTO), and Zn(II) (DEUTME) described in this section are based on heteronuclear $^1$H-$^{13}$C double resonance experiments, and on comparison with the spectra of the iron and zinc complexes with porphin and tetraphenylporphin [4]. The $^{13}$C chemical shift data are summarized in Table 1.

To illustrate the procedures used, the $^1$H-NMR. spectrum in a solution in methanol of Fe(III) (PROTO) $(CN)_2$ is shown in Figure 1, where the resonance assignments correspond to those found previously in different solvents [2]. The proton NMR. spectra of the solutions of Fe(III) (DEUT) $(CN)_2$, Zn(II) (PROTO), and Zn(II) (DEUTME) used in the present study were also found to be essentially identical to

Tabelle 1. *$^{13}$C-NMR. positions in Fe(III) (PROTO) (CN)$_2$, Fe(III) (DEUT) (CN)$_2$, Zn(II) (PROTO), and Zn(II) (DEUTME) in ppm from internal TMS at 29°*

| Resonance Assigments (see Fig. 1) | Fe(III) (PROTO) (CN)$_2$ |  | Fe(III) (DEUT)(CN)$_2$ | Zn(II) (PROTO) | Zn(II) (DEUTME) |
|---|---|---|---|---|---|
|  | in CD$_3$OD | in d$_5$-pyridine/D$_2$O 4:1 | in CD$_3$OD | in d$_5$-pyridine | in CDCl$_3$ |
| solvent | – 49.0 | centerline – 137.0 | – 49.0 | centerline – 137.0 | – 77.3 |
| methyls (a) | 37.0a) | 38.8a) | 39.1 | – 13.2 | – 11.6 |
|  | 36.2a) | 38.4a) | 34.6 | – 13.2 | – 11.6 |
|  | 29.2b) | 29.2b) | 28.6 | – 14.5 | – 13.7 |
|  | 27.0b) | 25.5b) | 24.9 | – 14.5 | – 13.7 |
| propionates (c) | 17.6 | 18.1 | 19.7 | – 24.3 | – 21.9 |
|  | 16.1 | 16.6 | 14.0 | – 24.3 | – 21.9 |
| (d) | – 93.6 | – 90.8 | – 90.0 | – 39.8 | – 37.2 |
|  | – 95.9 | – 93.6 | – 99.2 | – 39.8 | – 37.2 |
| —COO— | – 184.4 | – 185.5 | – 184.3 | – 177.2 | – 173.9 |
|  | – 184.4 | – 185.8 | – 185.0 | – 177.2 | – 173.9 |
| —OCH$_3$ |  |  |  |  | – 51.9 |
|  |  |  |  |  | – 51.9 |
| vinyls (f) | – 67.8 | – 66.2 |  | – 133.0 |  |
|  | – 69.6 | – 70.3 |  | – 133.0 |  |
| (g) | – 164.6 | – 161.4 |  | – 121.1 |  |
|  | – 166.3 | – 165.0 |  | – 121.1 |  |
| β-carbons (1–8) | – 112.9 | – 104.8 | – 96.4c) | ......d) | – 129.2c) |
|  | – 122.6 | – 115.4 | – 106.4c) | ......d) | – 129.2c) |
|  | – 124.3 | – 120.3 | – 115.2 | – 138.6 | ≈ – 137 to – 141 |
|  | – 124.8 | – 133.4 | – 116.3 | – 139.0 |  |
|  | – 130.2 | – 135.6 | – 123.6 | – 139.3 |  |
|  | – 133.8 | – 140.8 | – 126.6 | – 139.3 |  |
|  | – 134.2 | – 164.4 | – 138.6 | – 142.4 |  |
|  | – 139.6 | ......d) | – 148.6 | – 142.4 |  |
| quaternary carbons next to the pyrrole nitrogens (q in Fig. 3) | – 31.4 | – 29.6 | – 21.6 | – 148.9 | ≈ – 147 to – 150 |
|  | – 36.4 | – 29.8 | – 32.6 | – 149.4 |  |
|  | – 38.2 | – 35.9 | – 40.9 | ......d) |  |
|  | – 42.4 | – 36.6 | – 52.6 | ......d- |  |
|  | ......d) | – 48.0 | – 55.9 | ......d) |  |
|  | ......d) | – 48.0 | – 56.3 | ......d) |  |
|  | ......d) | – 54.4 | – 58.4 | ......d) |  |
|  | ......d) | – 56.0 | ......d) | ......d) |  |

Table 1. (*continued*)

| | | | | | |
|---|---|---|---|---|---|
| *meso*-carbons | – 50 | – 34.4 | – 73.4 | – 99.8 | – 95.9 |
| (b) | – 62.6 | – 48.0 | – 75.5 | – 99.8 | – 97.2 |
| | – 69.6 | – 56.0 | ...... d) | – 99.8 | – 99.9 |
| | – 80.0 | – 71.2 | ...... d) | – 100.3 | – 100.7 |

a) These two lines correspond to the methyl groups which have their proton resonances near – 17 ppm (Fig. 1) [25] [26].

b) Methyl substituents with proton resonances between – 11 and – 14 ppm (Fig. 1) [25] [26].

c) These two resonances in Fe(III)(DEUT)$(CN)_2$ correspond to the $\beta$-carbon atoms 2 and 4 (**f** in Fig. 4; see also Fig. 1).

d) The resonances corresponding to these atom positions (Fig. 1) have not been observed. They are probably hidden by the solvent resonances.

those published earlier [2]. The structures of the different compounds are described in the caption of Figure 1.

Three $^{13}$C-NMR. spectra of Fe(III) (PROTO) $(CN)_2$, which were essential for the resonance assignments, are reproduced in the Figures 2 and 3. In the proton noise decoupled spectra in two different solvents (Fig. 2A and 3), all the 34 resonances expected from the molecular structure (Fig. 1) can be detected. Because the proton resonances in this paramagnetic complex are rather widely separated (Fig. 1), the resonances of all the carbon atoms which are directly bound to protons could be identified from the residual $^{13}$C–$^1$H-spin-spin couplings in the off-resonance proton

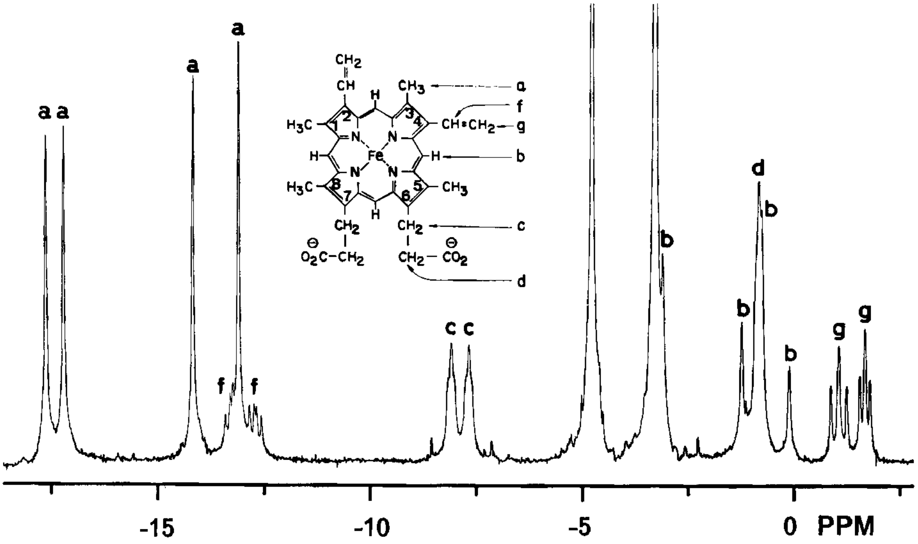


Fig. 1. Fourier *transform proton NMR. spectrum at 100 MHz of Fe(III) (PROTO)* $(CN)_2$ *in* $CD_3OD$. T = 20°C. The structure of the complex, where the axial cyanide ligands have been omitted, and the resonance assignments [2] are also indicated. Besides the replacement of Fe by Zn, the different compounds discussed in this paper contain the following substitutions: In DEUT the vinyl groups in positions 2 and 4 are replaced by protons, and an additional modification in DEUTME concerns the two propionic acid side chains 6 and 7, which are in the form of the methyl esters.

double irradiation experiment shown in Figure 2B. Those resonances of Fe(III) (PROTO) $(CN)_2$ which are hidden by the solvent in Figure 2 could be observed in Figure 3, and *v.v.* The quaternary carbon resonances were assigned from comparison with Fe(III) (Porphin) $(CN)_2$ and Fe(III) (tetraphenylporphin) $(CN)_2$ [4], and on the basis of the temperature-insensitive low field position of the carboxylic acid lines In additional $^1H$-$^{13}C$ double resonance experiments, the corresponding methyl resonances **a** in the $^1H$- and $^{13}C$-NMR. spectra (Tables 1 and 3) could be identified, as was described in more detail elsewhere [25] [26].

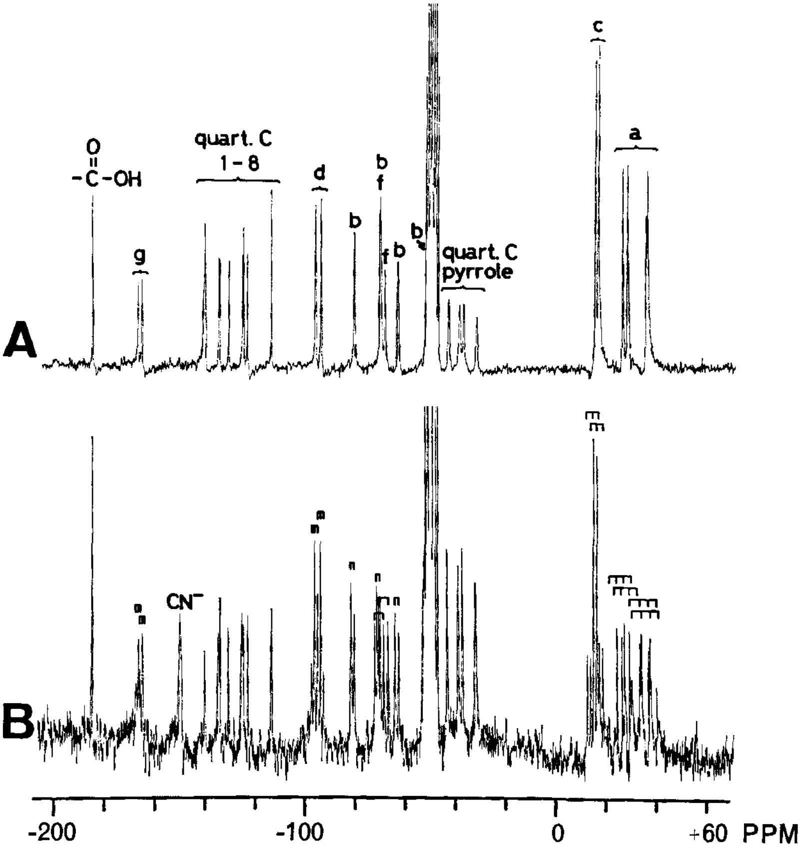


Fig. 2. Fourier *transform* $^{13}$*C-NMR. spectra at 25.14 MHz of Fe(III) (PROTO)* $(CN)_2$ *in* $CD_3OD$. T = 29°C. The strong resonance at – 49 ppm comes from the solvent. A: proton noise decoupled. B: off-resonance proton irradiation at + 5 ppm (see Fig. 1). The resonance assignments are in the nomenclature of Fig. 1.

In Fe(III) (DEUT) $(CN)_2$ (Fig. 4) we were mainly interested in the postions of the 2,4-ring carbon resonances **f** (Fig. 1). Since at 29° the 2,4-proton resonances are at *ca.* +17 ppm [3], and hence widely separated from the other proton resonances, these carbon lines could readily be assigned from a double resonance experiment in which the proton lines **f** were selectively irradiated (Fig. 4C). The other resonance assign-

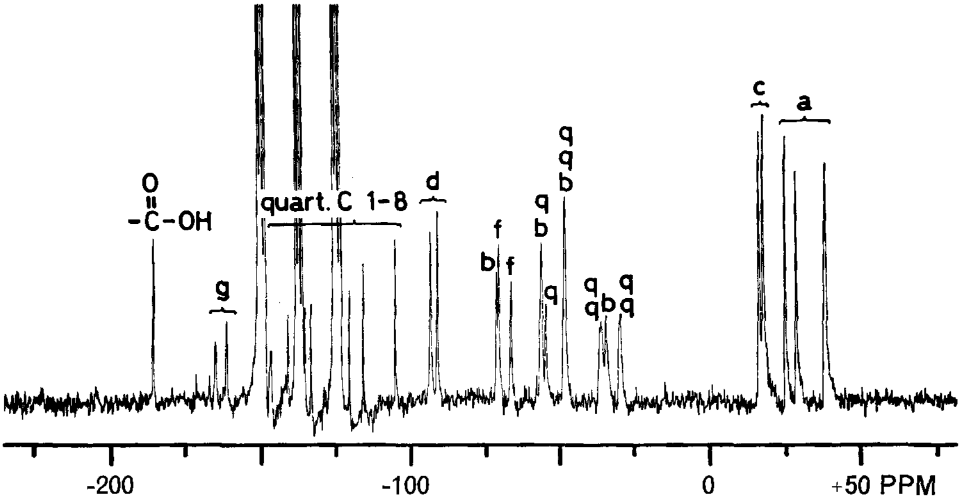


Fig. 3. *Proton noise decoupled $^{13}C$-NMR. spectrum of Fe(III) (PROTO) $(CN)_2$ in $d_5$-pyridine/$D_2O$ 4:1.* T = 29 °C. The solvent resonances are at −124, −137, and −150 ppm. The resonance assignments are in the nomenclature of Fig. 1, and q designates the quaternary carbon atoms next to the pyrrole nitrogen atoms.

ments in Figure 4A are based on the off-resonance proton irradiation experiment in Figure 4B. Two of the *meso*-carbon resonances (**b** in Fig. 1) are probably hidden by the solvent resonance. The comparison of Fe(III) (PROTO) $(CN)_2$ and Fe(III) (DEUT) $(CN)_2$ confirms also the assignment of the resonances **g** (Fig. 2 and 3), since these are missing in Figure 4.

In addition to the resonances of the porphyrin molecules and the solvent, the presence of $CN^-$ should also be manifested in the spectra of Figures 2,3, and 4. Only one cyanide line could be detected, and the position and appearance of this resonance differs somewhat in the different experiments [4]. Also the line has not in all cases been positively identified (Fig. 2–4). We have therefore started additional experiments with $^{13}C$-enriched KCN. These have so far shown that the variations between the cyanide resonances in the different spectra arise from the solvent dependence of the equilibrium between the rapidly interconverting species KCN and $CN^-$.

While many of the $^{13}C$-NMR. assignments in Figures 2–4 depend on the assignments of the $^1H$-NMR., an unambiguous identification of the methylene proton resonances **c** and **d** of the propionic acid side chains results on the other hand from the combined $^{13}C$- and $^1H$-NMR. data. Spectroscopic arguments based solely on the $^1H$-NMR. could not differentiate between these lines [2] [3] [5] [6]. The close similarity of the positions of the $^{13}C$ resonances **a** and **c** (Fig. 2–4) now shows that the assignment of the proton resonances **c** and **d** indicated in Fig. 1, which has also been proposed by different authors [5][6], is correct.

The $^{13}C$-NMR. spectra of Zn (II) (PROTO) and Zn (II) (DEUTME) are shown in Figures 5 and 6. All the resonances of these diamagnetic complexes are in the usual spectral region for organic compounds between – 10 and – 180 ppm. From the off-resonance proton double resonance irradiation experiments (Fig. 5 B and 6 B), the

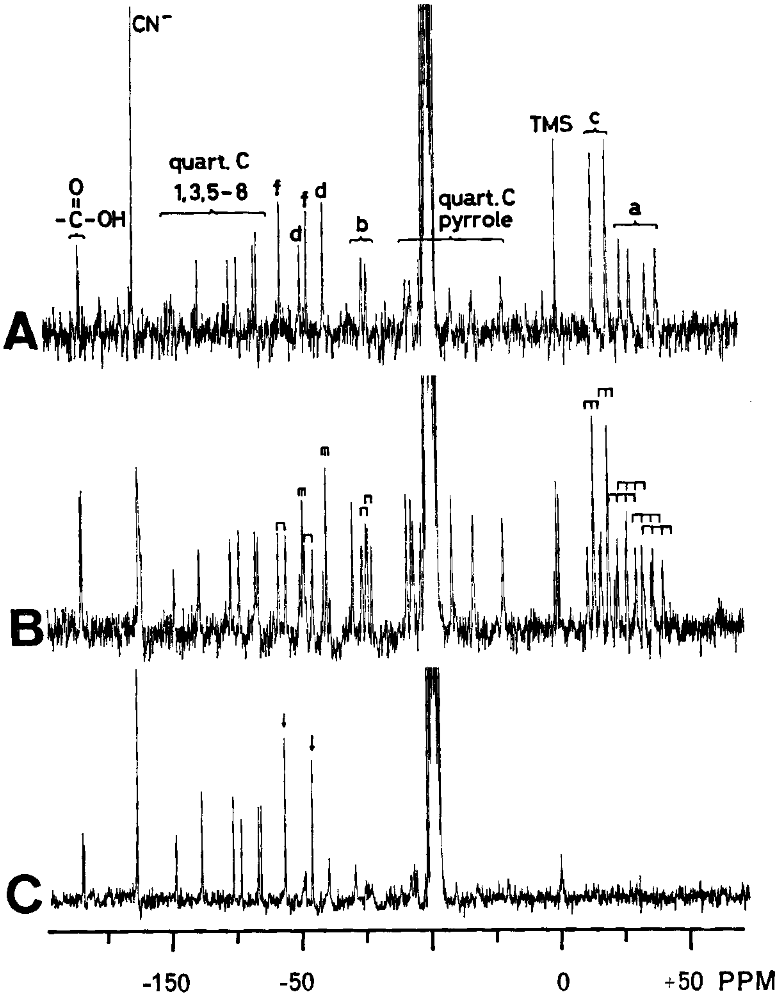


Fig. 4. $^{13}$*C-NMR. spectra of Fe(III) (DEUT) (CN)$_2$ in CD$_3$OD*. T = 29 °C. The solvent resonance is at – 49 ppm. A: proton noise decoupled; B: off-resonance proton irradiation at + 5 ppm. C: selective irradiation of the resonances of the 2- and 4-ring protons, which are at *ca.* + 17 ppm at 29° [2–3]. The resulting resonance assignments are also given (the nomenclature is that of Fig. 1, except that **f** now designates the $\beta$-carbon atoms 2 and 4). The arrows in spectrum C point to the the resonances **f**.

assignments of the methyl resonances **a**, the methylester line in Zn (II) (DEUTME), and the carboxylic acid resonances are straightforward. The identification of the resonances **f** and **g** followed from the comparison of Figures 5 and 6, and hence the four methine carbon resonances at ca. – 100 ppm had to come from the *meso*-carbon atoms (Fig. 1b). The propionic acid methylene carbons were assigned on the basis of the common rules for substituent effects on $^{13}$C-NMR. positions [27]. Finally, the two groups of quaternary ring carbon lines centered at – 138 and – 148 ppm, respectively,

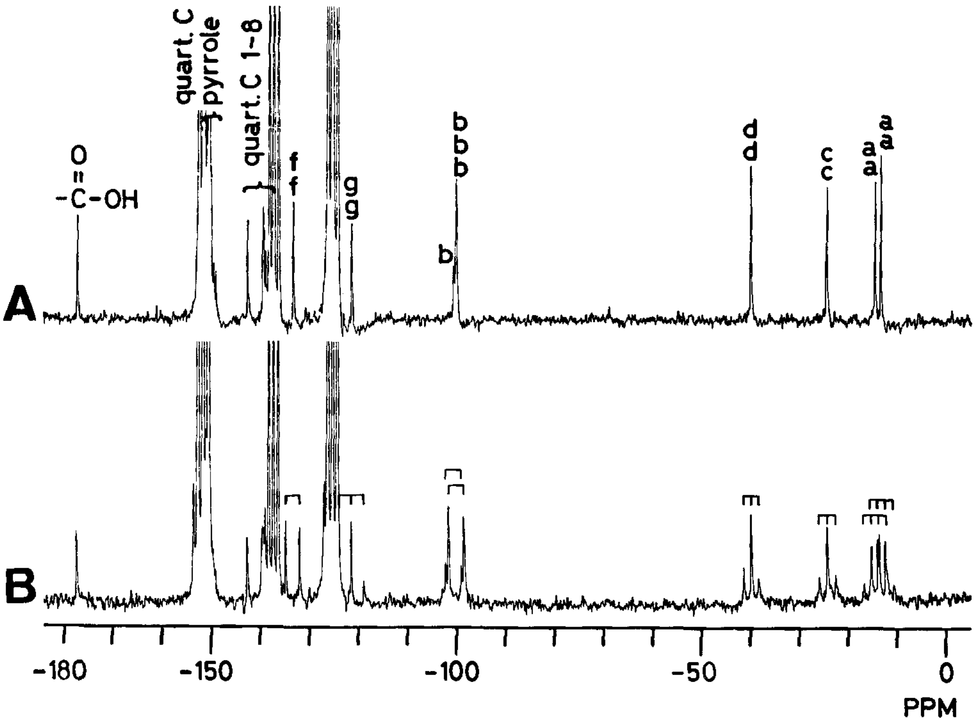


Fig. 5. *$^{13}$C-NMR. spectra of a ca. 0.01* M *solution of Zn(II) (PROTO) in $d_5$-pyridine.* A: proton noise decoupled. B: proton off-resonance irradiation at + 5 ppm. The resonance assignments are in the nomenclature of Fig. 1.

were identified from comparison with the spectra of the zinc complexes with porphin and tetraphenylporphin [4].

It is quite gratifying that single carbon lines could be detected in a solution which contained less than 0.01 M of Zn (II) (DEUTME) (Fig. 6). A somewhat lesser sensitivity was obtained with the paramagnetic iron complexes, probably mainly because small sample temperature variations during the experiment can lead to less than optimal resolution. This is best seen when the quaternary carbon resonances between –20 and –60 ppm are considered. These resonances have particularly large hyperfine shifts (Table 2), and hence are strongly dependent on temperature (eq. 4). In the spectrum of Figure 2 B, where the temperature regulation during the measurement was more reliable than in the experiment of Figure 2 A with proton broad band irradiation, these lines are noticeably narrower.

**Discussion.** – In the experiments described in the foregoing section, the $^{13}$C-NMR. of the different types of carbon atoms, *e.g.* the methyl carbon atoms (**a**) and the *meso*-carbon atoms (**b**) (Fig. 1), have been identified. Corresponding $^{13}$C resonance assignments had been described for different coproporphyrins and the Zn (II) complex of coproporphyrin I [28], and for various metalfree porphyrins with more diverse substituents [29] [30]. The data on the zinc (II) compounds in Table 1 are in good agree-

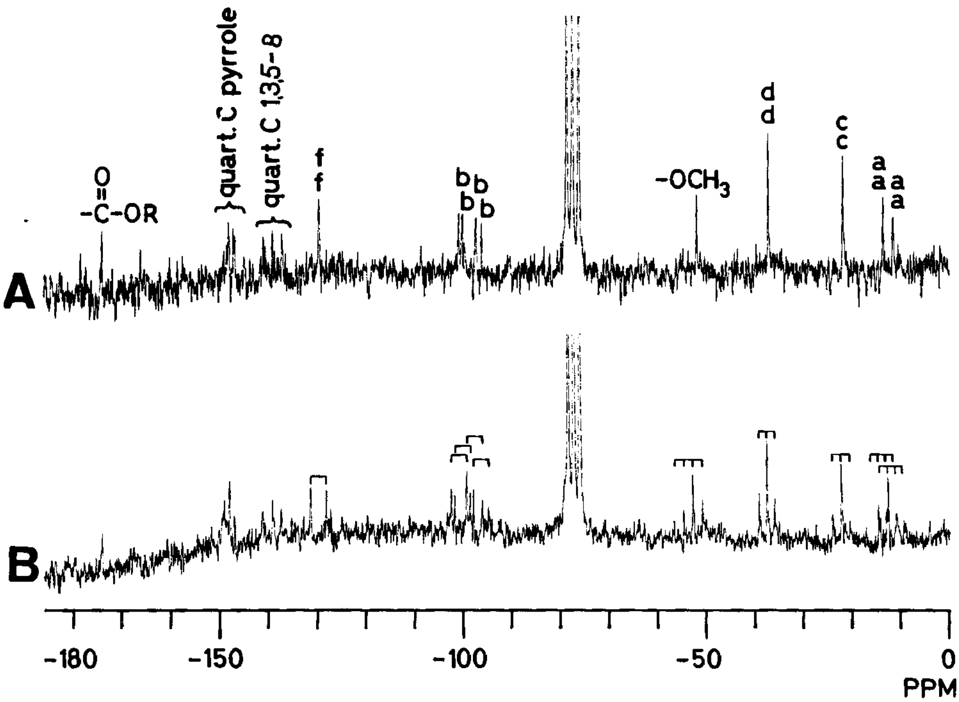


Fig. 6. *$^{13}$C-NMR. spectra of a ca. 0.006* M *solution of Zn(II) (DEUTME) in d-chloroform.* A: proton noise decoupled. B: proton off-resonance irradiation at + 5 ppm. The resonance assignments are in the nomenclature of Fig. 1, where **f** designates the 2.4-ring carbon atoms.

ment with these earlier studies. The different groups of proton resonances had also previously been assigned [2–3]. It appears that NMR. spectroscopic arguments which would lead to the identification of the individual resonances would have to be based on experiments with isotopically labelled porphyrins, as was described for the $^{13}$C-NMR. of the *meso* carbon atoms in the protoporphyrin IX dimethyl ester [31].

With the individual resonance assignments not available, the following approximate procedure was used in the computation of the hyperfine shifts. Since the relative chemical shifts between the $^{13}$C-NMR. of any given type of atoms in the diamagnetic Zn (II) compounds are small compared to the hyperfine shifts of the corresponding resonances in the paramagnetic iron complexes, an average value of $\Delta\nu_d$ was used for each type of atoms (Table 2). For practical reasons different solvents had to be used for the corresponding Zn (II) and Fe (III) complexes (Table 1). However, since the $^{13}$C-NMR. in diamagnetic porphyrins are quite generally little influenced by the solvent (Table 1, [28]–[30]), this should again produce only small errors in $\Delta\nu_{hf}$, so that for essentially all the carbon atoms the uncertainty in the hyperfine shifts of Table 2 is estimated to be much less than 10%.

On the basis of the combined $^1$H- and $^{13}$C-NMR. hyperfine shifts a quite detailed picture of the electronic states in the iron porphyrins should finally emerge. Several aspects of this analysis have previously been discussed in a paper on the more highly

Table 2. *$^{13}$C-Hyperfine shifts in Fe(III) (PROTO) $(CN)_2$ and Fe(III) (DEUT) $(CN)_2$ at 29°.*

| Resonances (see Fig. 1) | Fe(III) (PROTO) $(CN)_2$ | | Fe(III) (DEUT) $(CN)_2$ | |
|---|---|---|---|---|
| | average $\Delta\nu_d$ $d_5$-pyridine | $\Delta\nu_{hf}$ $d_5$-pyridine/$D_2O$ 4:1 | average $\Delta\nu_d$ $CDCl_3$ | $\Delta\nu_{hf}$ $CD_3OD$ |
| methyls | − 13.9 | + 52.7 | − 12.7 | + 51.8 |
| (**a**) | | + 52.3 | | + 47,3 |
| | | + 43.1 | | + 41.3 |
| | | + 39.4 | | + 37.6 |
| propionates | − 24.3 | + 42.4 | − 21.9 | + 41.6 |
| (**c**) | | + 40.9 | | + 35.9 |
| (**d**) | − 39.8 | − 51.0 | − 37.2 | − 52.8 |
| | | − 53.8 | | − 62.0 |
| vinyls | − 133.0 | + 66.8 | | |
| (**f**) | | + 62.7 | | |
| (**g**) | − 121.1 | − 40.3 | | |
| | | − 43.9 | | |
| *β*-carbon atoms | − 140 | + 35 | − 129.2 | + 32.8 [a] |
| (1–8) | | + 25 | | + 22.8 [a] |
| | | + 20 | − 139 | + 24 |
| | | + 7 | | + 23 |
| | | + 4 | | + 15 |
| | | − 1 | | + 12 |
| | | − 6 | | 0 |
| | | ..... [b] | | − 10 |
| quaternary carbon atoms next to the pyrrole nitrogen atom (**q** in Fig. 3) | − 150 | + 120 | − 148 | + 126 |
| | | + 120 | | + 115 |
| | | + 114 | | + 107 |
| | | + 113 | | + 95 |
| | | + 102 | | + 92 |
| | | + 102 | | + 92 |
| | | + 96 | | + 90 |
| | | + 94 | | ..... [b] |
| *meso*-carbon atoms | − 99.9 | + 65.5 | − 98.4 | + 25.0 |
| (**b**) | | + 51.9 | | + 22.9 |
| | | + 43.9 | | ..... [b] |
| | | + 28.7 | | ..... [b] |

[a] These two resonances in Fe(III) (DEUT) $(CN)_2$ correspond to the *β*-carbon atoms 2 and 4 (**f** in Fig. 4, see also Fig. 1).

[b] The resonances corresponding to these atom positions have not been positively identified.

symmetrical iron complexes with porphin and tetraphenylporphin [4], and further work is currently in progress. Therefore the present discussion will be limited to some observations on the side chains of the porphyrin ring carbon atoms 1–8 (Fig. 1), which bear on the parameters $Q^I$ which characterize the electron-nucleus scalar coupling through hyperconjugation.

Approximate numbers for the contact shifts of the side chain resonances were derived from Table 2 on the following basis: For the $^1$H-NMR., and the $^{13}$C-NMR. of the nonaromatic side chain carbon atoms, $\Delta\nu_{pc}^{L\pi}$ in equation (2) is supposed to be negligibly small [8] [4]. As in earlier work [4] we chose two limiting values for $\Delta\nu_{pc}^{M}$ of the *meso*-protons, *i.e.* (A) $\Delta\nu_{pc}^{M}$ *meso*-H = 10 ppm, which is approximately the pseudocontact shift that one would expect from the electronic *g*-values in the frozen solutions [2] [4] [5] and is taken to represent an upper limit for $\Delta\nu_{pc}^{M}$ in solution at ambient temperature, and (B) $\Delta\nu_{pc}^{M}$ *meso*-H = 3 ppm, which would represent the case of very small $\Delta\nu_{pc}^{M}$ as indicated by previous $^1$H-NMR. observations [2] [3].

Approximate values for the corresponding $\Delta\nu_{pc}^{M}$ of the other carbon and proton positions were then obtained with equation (3), using r = 4.5 Å, 5.3 Å, 5.7 Å, and 6.4 Å for the *meso*-protons (**b**), the 2.4-ring protons in DEUT (**f**), the methyl and methylene carbon atoms (**a**), and (**c**), and the methyl protons (**a**), methylene protons (**c**), and methylene carbon atoms (**d**), respectively. The resulting contact shifts $\Delta\nu_{c}^{\pi}$ are given in Table 3 in as far as they are used in the present discussion. As was pointed out earlier [4], more definite values for $\Delta\nu_{pc}^{M}$ which should result at a later stage of the analysis, will most probably lie between the values (A) and (B) of Table 3. Since $\Delta\nu_{pc}^{M}$

Table 3. *Approximate contact shifts of selected side chain $^1$H- and $^{13}$C-NMR. in Fe(III) (PROTO) $(CN)_2$* in a mixed solvent of $d_5$-pyridine/$D_2O$ 4:1 at 29°.

| | A<br>($\Delta\nu_{pc}^{M}$ *meso*-H = 10 ppm) | | | B<br>($\Delta\nu_{pc}^{M}$ *meso*-H = 3 ppm) | | |
|---|---|---|---|---|---|---|
| Resonances (see Fig. 1) | $\Delta\nu_{c}^{\pi}$ of $^1$H [a] | $\Delta\nu_{c}^{\pi}$ of $^{13}$C | $\varrho_{c}^{\pi}$ [b] | $\Delta\nu_{c}^{\pi}$ of $^1$H [a] | $\Delta\nu_{c}^{\pi}$ of $^{13}$C | $\varrho_{c}^{\pi}$ [b] |
| methyls | – 10.8 | 34.4 | 0.84 | – 8.3 | 37.9 | 0.92 |
| (**a**) | – 12.6 | 38.1 | 0.92 | – 9.1 | 41.6 | 1.02 |
| | – 16.2 | 47.3 | 1.15 | – 13.7 | 50.8 | 1.25 |
| | – 16.7 | 47.7 | 1.18 | – 14.2 | 51.2 | 1.25 |
| propionates | | 35.9 | 0.87 | | 39.4 | 0.97 |
| (**c**) | | 37.4 | 0.92 | | 40.9 | 1.00 |
| (**d**) | | – 54.5 | | | – 52.0 | |
| | | – 57.3 | | | – 54.8 | |

[a] Computed using data from ref. [2] and [3].

[b] In % of one unpaired electron. Computed from $\Delta\nu_{c}^{\pi}$ of $^{13}$C with equation (8), using $Q_{C'C}^{C}$ = – 39 MHz.

makes rather small contributions to the hyperfine shifts of the porphyrin side chain resonances (Tables 2 and 3), the future modifications of this quantity will therefore probably not greatly affect the conclusions of the present discussion.

The contact shifts of the $^1$H- and $^{13}$C-NMR. of the porphyrin side chains are related to the unpaired spin density on the ring carbon atom by equations (5), (6), and (8). If it is assumed that the generally used values for those parameters $Q^I$ which account for coupling through spin polarization are also valid for the low spin ferric hemes, which seems to be a reasonable assumption if the observations in other classes of compounds are considered [14–16] [18], equation (8) with $Q^C_{C'C} = -39$ MHz [15] yields the spin densities given in Table 3. If we now consider the methyl lines, equation (6) yields the following values for $Q^H_{CCH_3}$ if the spin densities and the proton contact shifts of Table 3 are used: With the assumptions (A) 47 MHz $\lesssim Q^H_{CCH_3} \lesssim$ 53 MHz, and for (B) 34 MHz $\lesssim Q^H_{CCH_3} \lesssim$ 42 MHz. In a corresponding approach for the calibration of $Q^H_{CCH_3}$, where the average of the proton contact shifts of the 2.4-ring protons and the 1.3.5.8-methyl protons in Fe(III) (DEUT) $(CN)_2$ are used with equations (5) and (6), and $Q^H_{CH} = -63$ MHz [2] [3] [5], one obtains for (A) that $Q^H_{CCH_3} \approx 48$ MHz, and for (B) that $Q^H_{CCH_3} \approx 33$ MHz. The close agreement between the results obtained from the two different approaches is quite encouraging. The envisaged more complete analysis of these data, in which the hyperfine shifts of the porphyrin ring carbons will also be considered [4], should make a more definite estimate of $\Delta\nu^M_{pc}$, and hence of $Q^H_{CCH_3}$, possible.

This preliminary analysis shall be concluded with some observations on the contact shifts of the $^{13}$C-resonances **c** and **d** of the propionic acid side chains (Fig. 1). As one would have expected, equation (8) and $\Delta\nu^\pi_c$ of the resonances **c** yield spin densities on the ring carbon atoms 6 and 7 (Fig. 1) which are very similar to those on the ring carbon atoms 1,3,5, and 8 (Table 3). Using these spin densities in equation (9), one

$$A^C = Q^C_{C'CC} \cdot \varrho^\pi_c \qquad (9)$$

finds for the parameter operative in producing the downfield contact shift of the resonances **d** the values (A) $Q^C_{C'CC} \approx 60$ MHz, and with the assumptions (B) $Q^C_{C'CC} \approx$ 51 MHz. These large positive values for $Q^C_{C'CC}$ imply that the contact shifts of the $^{13}$C resonances **d** of the propionic acid side chains (Fig. 1) are mainly caused by hyperconjugation [13-14]. It is then interesting to note that the effective $Q^C_{C'CC}$ for the carbon atoms **d** appears to be slightly larger than $Q^H_{CCH_3}$ for the methyl protons **a**, but considerably larger than $Q^H_{CCH}$ for the methylene protons **c**, which is estimated in the case (A) to be $Q^H_{CCH} \approx 20$ MHz, and in (B) $Q^H_{CCH} \approx 9$ MHz. These differences come probably mostly from the different steric orientations of the methyl and propionic acid side chains with respect to the heme plane [32], which are thus in principle also accessible for investigation by NMR.

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