Assignments of the Paramagnetically Shifted Methyl Resonances in the Nuclear Magnetic Resonance Spectrum of Iron(III) Protoporphyrin-IX Cyanide by Selective Deuteriation

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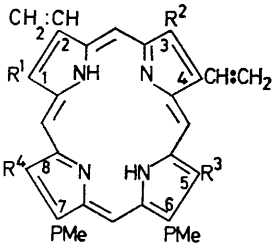
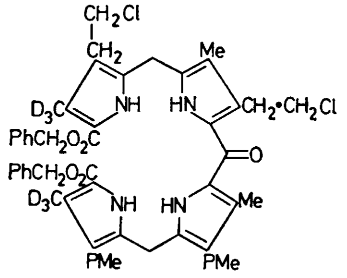
Summary Syntheses of 1,8- and 1,3-bis(trideuteriomethyl) protoporphyrin-IX dimethyl esters [(**1c**) and (**1d**) respectively] are outlined, and these compounds, together with the 5,8-bis(trideuteriomethyl) isomer (**1b**) are used to specifically assign the paramagnetically shifted methyl resonances in the n.m.r. spectrum of iron(III) protoporphyrin-IX cyanide.

PARAMAGNETICALLY shifted n.m.r. lines can be observed in the n.m.r. spectra of low-spin iron(III) porphyrins.[1-4] The complexes with cyanide have been particularly useful,[3] firstly because their short electron-relaxation times give narrow well-resolved resonances, and secondly, because of the conceptual simplicity of the low spin d^5 configuration with $S = \frac{1}{2}$, where the unpaired spin is in an iron orbital suitable for π-bonding to the porphyrin ring. This simplicity has been used[5] in a molecular-orbital analysis of the shifts in terms of the spin densities, with simple Hückel molecular orbitals as a basic set.



(1)

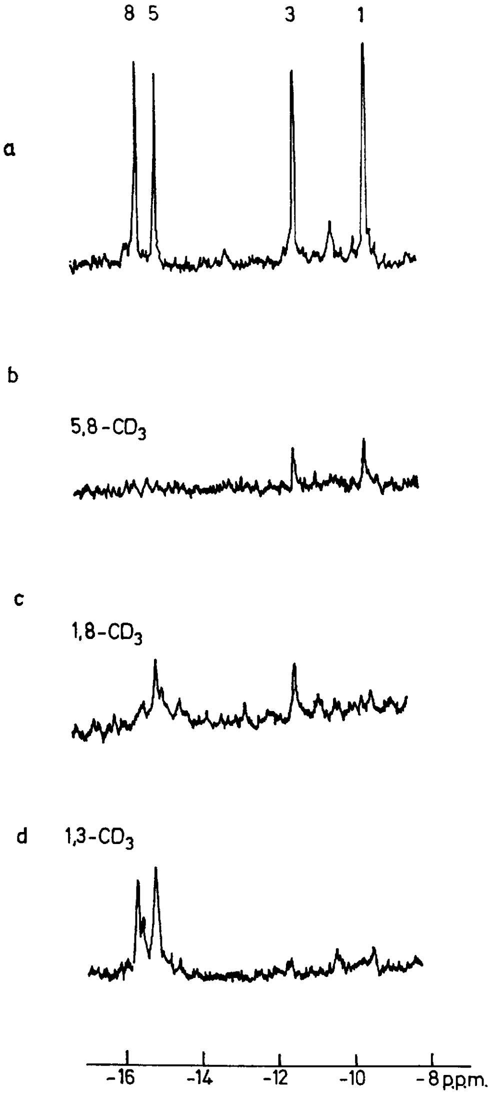


(2)

a; $R^1 = R^2 = R^3 = R^4 = Me$ **c**; $R^1 = R^4 = CD_3$, $R^2 = R^3 = Me$
b; $R^1 = R^2 = Me$, $R^3 = R^4 = CD_3$ **d**; $R^1 = R^2 = CD_3$, $R^3 = R^4 = Me$
$PMe = CH_2{\cdot}CH_2{\cdot}CO_2Me$

Although it is easy to identify the resonances from the four methyl groups in the iron(III) cyanide complex of protoporphyrin-IX and its di-ester (**1a**) on the basis of their integrated intensities, it is important to assign individual resonances to particular methyl groups in order to test the analysis.[5] We now outline syntheses of 1,8- and 1,3-bis(trideuteriomethyl)protoporphyrin-IX dimethyl esters [(**1c**) and (**1d**) respectively] and the n.m.r. spectra of the iron(III) cyanide dicarboxylic acids of (**1b**—**d**) which allow unambiguous assignment of the four methyl resonances.

The route to the 5,8-bis(trideuteriomethyl) derivative (**1b**) has already been outlined;[6] the 1,3-bis(trideuteriomethyl) compound (**1d**) was prepared† using the same concept. The 1,8-bis(trideuteriomethyl)protoporphyrin-IX (**1c**) posed more formidable problems because this molecule no longer possessed the synthetic symmetry elements of its isomers (**1b**) and (**1d**). It was therefore synthesised from the *b*-oxobilane (**2**).[7]



FIGURE

The Figure compares the low-field region in the n.m.r. spectrum‡ of iron(III) protoporphyrin-IX cyanide (a), with

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† By condensation of 3,4′-bis(2-chloroethyl)-3′,4-bis(trideuteriomethyl)pyrromethane with 5,5′-diformyl-3,3′-bis(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane, followed by vinylation of the resulting 2,4-bis(2-chloroethyl)porphyrin in the usual way.[6]

‡ Measurements were made with a Varian HR-220 spectrometer, operating at 220 MHz in the field-swept mode. Shifts are given in p.p.m. from an internal standard of 2,2-dimethyl-2-silapentane-5-sulphonate with negative signs indicating lower fields. Solutions were made by adding 3 to 5 mg of the iron(III) chloride complexes to a solution of 25 mg potassium cyanide in a mixture of 0·4 ml C_5D_5N and 0·1 ml deuterium oxide.

those of the samples prepared[8] from the selectively deuteriated compounds. The only other resonances in this spectral region are from the two vinyl CH groups which, at 25°, occur at $-10{\cdot}5$ and $-9{\cdot}5$ p.p.m. These can be seen most clearly in Figure (a), where the higher-field CH proton resonance is superimposed upon the highest-field methyl resonance. The specific assignments are indicated in the Figure and since only two of the three deuteriated compounds suffice for a positive identification of all four methyls, the third compound affords an independent verification.

It has been shown that the downfield shifts of the methyl resonances from their positions in diamagnetic metalloporphyrins (near zero p.p.m.) arise mainly from contact interactions with the delocalised unpaired spins,[5] and the large separations between the resonances are particularly significant. Considering the symmetry allowed by the porphyrin (assuming the eight peripheral positions to be equivalent), it has been tempting[5] to try to understand the resonance shifts in terms of the symmetry elements. The unpaired-electron spins are in orbitals which are even under inversion. Hence, in this approximation, one would expect the methyl groups at positions 1 and 5 to have identical shifts. However, from the present work it is clear that they are widely split by almost 6 p.p.m. The second symmetry element for the four methyls which is allowed by considering all peripheral positions to be equivalent is a reflection plane interchanging the methyls at positions 3 and 8. Once again, the data in the Figure shows a relatively large splitting between these two lines. We therefore conclude that the approximate symmetry of the porphyrins does not help in assignment of these resonances.

If the peripheral positions are not assumed to be equivalent and the molecular orbitals for low-spin iron(III) protoporphyrin-IX cyanide are calculated, we can find reasonable values of the Hückel parameters such that the spin densities at positions 1 and 5 are comparable with the observed splittings. However, before adjusting these parameters it is necessary to evaluate other contributions to the shifts. For example, we have observed reproducible differences of a few p.p.m. between the spectra in the Figure and those of the corresponding iron(III) cyanide dimethyl esters which cannot be reflecting a direct electronic effect. A detailed analysis of these results will be attempted in conjunction with the n.m.r. results of these compounds in haemoproteins.

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[1] W. S. Caughey and L. F. Johnson, *Chem. Comm.*, 1969, 1362.

[2] H. A. O. Hill and K. G. Morallee, *Chem. Comm.*, 1970, 266.

[3] K. Wüthrich, R. G. Shulman, B. J. Wyluda, and W. S. Caughey, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **62**, 636.

[4] R. J. Kurland, R. G. Little, D. G. Davis, and C. Ho, *Biochemistry*, 1971, **10**, 2237.

[5] R. G. Shulman, S. H. Glarum, and M. Karplus, *J. Mol. Biol.*, 1971, **57**, 93.

[6] A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *Chem. Comm.*, 1971, 1304.

[7] *Cf.* G. W. Kenner and K. M. Smith, *Annals New York Acad. Sci.*, 1973, **206**, 138.

[8] J. E. Falk, 'Porphyrins and Metalloporphyrins,' Elsevier, Amsterdam, 1964, p. 133.