Effects of Axial Ligand upon Paramagnetic Shifts in ^{1}H Nuclear Magnetic Resonance Spectra of High-spin Deuterohemins

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Summary Certain protons in high-spin deuterohemin esters in $CDCl_3$ exhibit large paramagnetic shifts to high or low field with the extent of shift for most, but not all, protons significantly influenced by the nature of the axial ligand.

RECENT ^{1}H n.m.r. studies on paramagnetic hemeproteins suggest the paramagnetic shifts will prove extremely useful in the interpretation of structure–function relationships in these proteins.[1] The extent of these shifts vary with protein and with axial ligand. Knowledge of ^{1}H chemical shifts for protein-free iron(III) porphyrins is important for interpretations of protein spectra and for computation of delocalized spin densities.[2] Data for low-spin cyanohemins were recently reported.[2] Here we report spectra for $CDCl_3$ solutions of high-spin iron(III) deuteroporphyrin dimethyl esters with different axial ligands. Certain protons of the porphyrin ligand exhibited large paramagnetic shifts to high or low field with the extent of shift for most, but not all, protons dependent upon the nature of axial ligand. Protons of an axial ligand (phenoxo) exhibited even larger shifts to high and low field. These results suggest significant contributions of π-type orbitals for the transmission of isotropic hyperfine interactions from iron and stand in contrast to data reported for chlorohemins in $(CD_3)_2SO$ where no high-field shifts were found and it was concluded that transmission was principally through the σ-bond system of the porphyrin ring.[1b]

The spectra were obtained at 100 MHz with a Varian HA-100 spectrometer using field-sweep mode. Signals were detected as upper sidebands using a modulation frequency of 15—25 KHz. Solutions examined contained *ca.* 10 mg. of hemin derivative[3] in 0·4 ml. $CDCl_3$.

Spectra for two derivatives are in the Figure. Assignments were made on the basis of proton counting and of comparisons with other porphyrins (Table). All protons, except for the relatively isolated ester OCH_3 and β-CH_2 of the 6,7-propionic acid ester groups, were shifted well outside the region for diamagnetic metal porphyrins.[5] Paramagnetic shifts and band widths were much greater

TABLE

^{1}H *chemical shifts*[a] *for deuterohemin esters in* $CDCl_3$ *at* 35°

Axial ligand	$H_{2,4}$	$CH_{3(1,3,5,8)}$[b]	α-CH_2[c]	Meso-H[d]
Phenoxo[e]	−75	−39	— — −31 (2)	38
Fluoro	−76	−43	— — −33 (2)	35
Azido	−72	−46	−41 (1) −39 (1) −35 (2)	46
Chloro	−75	−49	−44 (1) −42 (1) −39 (2)	57
Bromo	−72	−51	−45 (1) −42 (1) −38 (2)	57

[a] P.p.m. from $SiMe_4$: negative values downfield, positive values upfield. [b] Chemical shift at maximum peak height. [c] Numbers in parentheses represent proton counts. [d] Chemical shifts measured less accurately than for other protons. [e] Assignments of the −83 and −75 resonances to phenoxo-ligand and 2,4 protons respectively, were made because the 2,4 protons in other derivatives were found near −75.

than was found for low-spin hemins.[2] As noted earlier for paramagnetic nickel(II) porphyrins,[6] only meso-protons were shifted to high field where they appeared as a broad absorption. The 2,4 protons experienced the greatest low-field shift, *ca.* 60 p.p.m. Methyl protons at positions 1, 3, 5, and 8, though not equivalent, were not clearly resolved. Multiple resonances (two one-proton resonances well resolved from a two-proton resonance at higher field) on the high-field side of the ring methyls were assigned to α-CH_2 groups and indicate that these four protons are nonequivalent, presumably due to steric interactions between bulky propionic acid ester groups at the 6,7-positions. With the phenoxo-derivative, resonances at 97 and -83 p.p.m. were assigned to 2,6 phenyl protons and 3,5 phenyl protons, respectively, since the protons nearer the iron could be expected to experience the greater paramagnetic shift; the 4 phenyl proton was not definitely located.

The shifts for ring methyl, α-CH_2, and meso-protons varied with a change in axial ligand (Table) and generally followed the order ($\phi O < F < N_3 < Cl < Br$) also found for zero-field splittings in the solid,[4] Mössbauer quadrupole splittings,[7,8] and shifts in electronic spectra.[7] The effectiveness of transmission of isotropic hyperfine interactions from the paramagnetic iron to protons at the periphery of the porphyrin ring is expected to depend upon the extent of bonding interactions between porphyrin nitrogens and iron which in turn is influenced by differences in axial ligand to iron bonding. The shifts thus provide further support that the above order is the order of increasing "interaction" between porphyrin nitrogens and iron.[7] It should be noted, however, that paramagnetic shifts for 2,4 protons were relatively insensitive to a change in axial ligand. The 2,4 protons of chlorodeuterohemin in $(CD_3)_2SO$ and the eight pyrrole protons of chloro-$\alpha\beta\gamma\delta$-tetraphenylporphiniron(III) in $CDCl_3$ were found at comparable frequencies, 75 and 79 p.p.m. respectively.[2,6,9]

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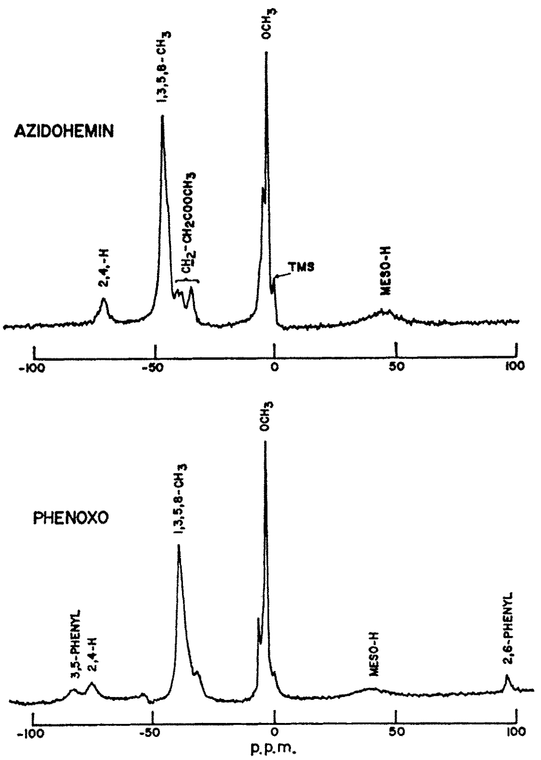

FIGURE. ^1H *n.m.r. spectra of azido* (*upper*) *and phenoxo* (*lower*) *derivatives of deuteroporphyrin IX dimethyl ester iron*(III) *in* $CDCl_3$.

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