The Direct Characterisation of Iron Porphyrins from Coal using Paramagnetic Shift Effects on Proton NMR Spectra

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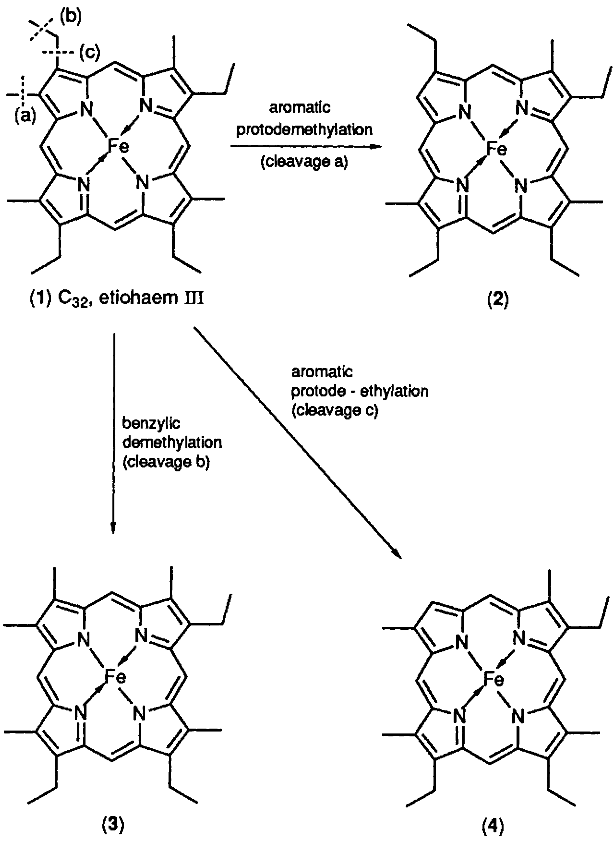
Iron porphyrins isolated from a Colorado bituminous coal have been investigated, and in favourable cases identified [etiohaem III, and the three suites (**2**), (**3**), (**4**) of its monodealkylation products], by the study of the paramagnetically shifted ^{1}H NMR spectra of the low spin iron(III) dicyanide complexes.

Although the presence of metalloporphyrins in fossil fuels has been recognised for more than fifty years,[1] it is only recently that iron porphyrins have been securely identified in coals and lignites.[2—4] The study of the chemical structures of these complexes, which is important in the context of understanding chemical changes during the catagenesis of organic deposits, has been hampered by their low abundance (< a few ppm) and by the need heretofore to remove the co-ordinated iron atom. This was done partly to facilitate chromatographic manipulation but mainly to produce diamagnetic complexes for structural ^{1}H NMR spectroscopic work. Thus mesohaem IX was identified in Victoria lignite; the iron was replaced by zinc, and the non-aggregating bis-pyrrolidine complex was employed for the structural assignment.[4]

In recent years there has been extensive study of the paramagnetically shifted ^{1}H NMR spectra of iron porphyrins especially in relation to haemoprotein structure.[5] Such spectra are sensitive to ligation, oxidation state, and spin state. We have now found that if the spin state and oxidation state are set as low-spin iron(III) by the use of high-field ligands (cyanide),[6] then the iron porphyrins from coal can be *directly* examined by ^{1}H NMR spectroscopy. The resulting paramagnetically shifted spectra provide important structural information, so avoiding the wasteful demetallation/metallation steps on these precious samples.

The coal chosen for examination was a bituminous coal from Colorado (Cretaceous, US classification HVC, PSOC 850) which contains appreciable amounts of iron porphyrins



Scheme 1. The primary dealkylation steps in the catagenesis of etiohaem III in coal. (**2**), C_{31}, product of nuclear demethylation; (**3**), C_{31}, product of benzylic demethylation; (**4**), C_{30}, product of nuclear de-ethylation. For (**2**)—(**4**), only one of the four isomers is shown.

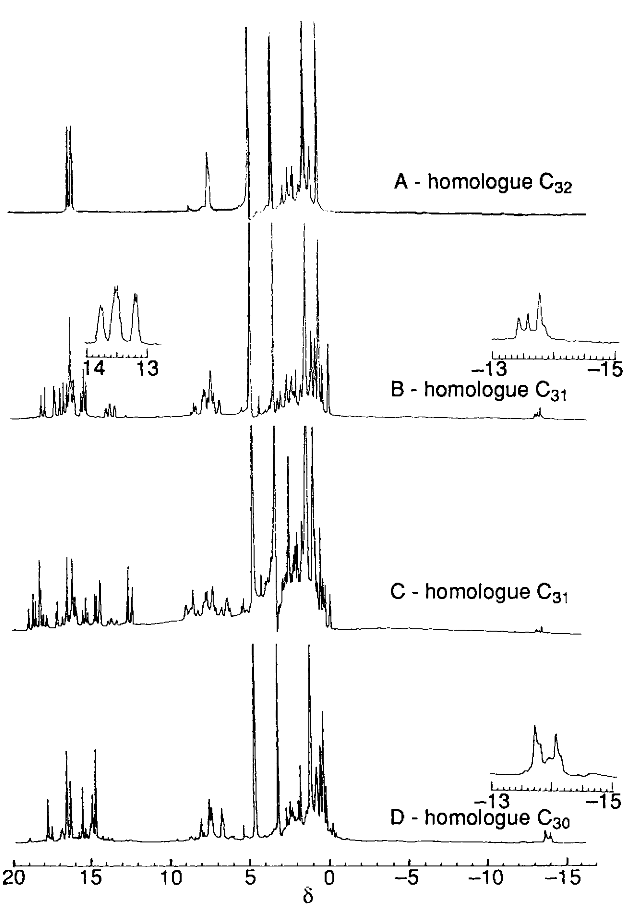


Figure 1. ^{1}H NMR spectra of iron porphyrins separated from Colorado coal (*ca.* 3×10^{-3} M, CD_3OD saturated with KCN, 301 K). A, C_{32} fraction [dicyanoiron(III), etioporphyrin III, dicyano-(**1**)]; B, C_{31} fraction, cleavage a [dicyano-(**2**) isomers]; C, C_{31} fraction, cleavage b [dicyano-(**3**) isomers] (the pyrrole β-H resonances at δ – 14 in this spectrum indicate incomplete removal of (**2**) isomers); D, C_{30} fraction, cleavage c [dicyano-(**4**) isomers]. The *meso* and $-CH_2C\mathit{H}_3$ resonances are located in the δ 0—6 region. Additional strong peaks in this region at δ *ca.* 4.6, 3.2, and 1.2 are due to -OH, $-OCD_2H$, and hydrocarbon chain impurities respectively.

Table 1. Paramagnetic shift effects on peripheral protons in dicyano-iron(III) porphyrin complexes (δ, ppm).[a]

	meso-H	β-H	β-Me	β-$C\mathit{H}_2CH_3$	β-$CH_2C\mathit{H}_3$
Octamethylhaem	1.1	—	16.0	—	—
Haem	1.3	−14.0	—	—	—
Etiohaem I	1.2	—	15.8	7.3	0.3

[a] Measurements on Bruker AM250 with ~ 3×10^{-3} M solutions in CD_3OD saturated with KCN; 301 K; shifts measured with respect to methanol (CH) and presented with respect to tetramethylsilane (δ 0).

(*ca.* 10—15 µg/g). We had earlier detected C_{32}–C_{28} polyalkylhaems in this material by high resolution mass spectrometry.[7,8] The powdered coal was extracted with 7% H_2SO_4–MeOH as previously described,[2,9] and the iron porphyrin fraction was separated and fractionated by careful and repeated preparative TLC on silica gel to give fractions some of which were shown by mass spectrometry to be essentially single homologues (but presumably still isomeric mixtures). The fractions described here are designated A to D: this is roughly the order of increasing polarity (decreasing R_f).

Standardised conditions (3×10^{-3} M solutions in [$^2H_4$]-methanol saturated with KCN at 301 K) were used for the NMR measurements. The protons at the periphery of the low-spin dicyanoferrihaems formed under such conditions are reported to give well separated narrow signals over the δ range 20 to −20.[5a] Typical shifts are: *meso*-H, δ 1.4; β-H, δ −14; β-methyl, δ 16; and β-methylene, δ 7.3.[10] These values were confirmed for appropriate model ferrihaems (based on the parent system, octamethylporphyrin, and etioporphyrin I) under our operating conditions as shown in Table 1.

The most mobile major component, A, of the iron porphyrins from the Colorado coal extract had a molecular ion corresponding to $C_{32}H_{36}FeN_4$, consistent with an etioferrihaem which had lost its axial ligand. For geobiogenetic reasons[1,4] we might suppose that the principal component here would be etiohaem III (**1**). The ^{1}H NMR spectrum provided direct experimental evidence for this in that the β-methyl region (δ *ca.* 16, Figure 1A) showed a clean set of four singlets of equal intensity at δ 16.10, 16.05, 15.84, and 15.80. The three other type isomers (I, II, IV) of etiohaem would each be expected to show different behaviour (I, one signal, II, one signal, IV, two signals); and a mixture of type isomers would be expected to show a more complex spectrum in this region. Hence we conclude that fraction A contains essentially only etiohaem III.

Fraction B was shown by mass spectrometry to have a molecular formula (axial ligands lost) of $C_{31}H_{34}FeN_4$. The appearance of signals at δ *ca.* −14 (Figure 1B) shows that this material has a free β-position. B must therefore be a mixture of C_{31}-haems formed from (**1**) by the loss of one ring methyl group [illustrated at (**2**) by one example]. Ring demethylation causes a redistribution of spin density[11] which particularly affects the chemical shift of the protons of the methylene group at the neighbouring position; these methylene groups appear as quartets (spin-spin coupling to CH_3 of ethyl group) shifted downfield to the δ 14 region (Figure 2B, inset). Four isomeric compounds could conceivably be formed by the mono ring-demethylation of etiohaem III; the NMR integration evidence is consistent with the presence of all four in about equal abundances.

Fraction C was particularly difficult to separate from fraction B. Mass spectrometry showed it to be an isomer (C_{31}) and the NMR spectrum (which showed the presence of a small amount of B, Figure 1C) was consistent with its formulation (**3**) as the product of benzylic mono-demethylation.

Fraction D was shown to consist mainly of the next lower homologue (C_{30}). The predominant signals in the ^1H NMR spectrum (Figure 1D) were consistent with structure of the type (**4**) in which the loss of a ring ethyl group had occurred, and isomers of this type can be recognised in the spectrum.

Subsequent fractions in which further dealkylations have occurred to give C_{29} and C_{28} polyalkylhaems remain to be studied in detail; the separations are more time-consuming and the ^1H NMR spectra are naturally more complex. However, our approach to the difficult question of the structures of coal haems using this novel TLC–NMR method has allowed the initial stages of the two distinct dealkylation processes (aromatic protiodealkylation and benzylic cleavage, Scheme 1), which are frequently postulated, to be separately identified, and in so doing has provided significant experimental support for current theories of porphyrin catagenesis.

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