The Identification of Deuteroetiohaem IX in Colorado Coal

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Using paramagnetically-shifted ^{1}H NMR spectroscopy of the dicyano-iron(III) complexes at 600 MHz as the principal tool, the identification of etiohaem III in Colorado coal is confirmed (1.63 μg g^{-1}), and deuteroetiohaem IX (0.52 μg g^{-1}) is identified for the first time, by comparison with samples made by total synthesis.

Bituminous coal from the Cretaceous of Colorado, USA, contains about 10 μg g^{-1} iron porphyrins.[1] We have been attempting the daunting task of identifying individual haem components in order to throw light on the coalification process.[2] Recently it has been shown that the identification can in principle be considerably aided by using the paramagnetically-shifted ^{1}H NMR spectra of the dicyano-iron(III) complexes prepared following fractionation of the coal haems by TLC.[3] In these spectra, β-methyl groups are shifted downfield (to *ca.* δ 15) while β-H appear upfield (at *ca.* δ

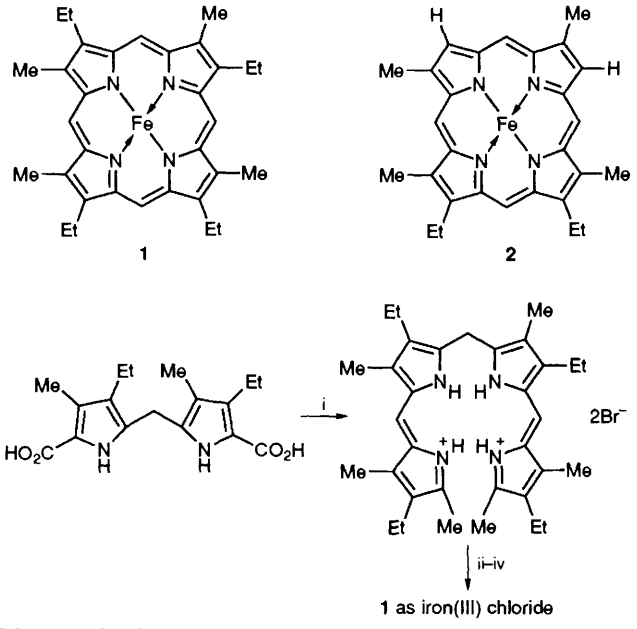
−15), allowing clear separation from the (inevitable) impurities in the 'normal' organic region (and especially in the δ 1–3 range).

In this way we have identified the C_{32} compound etiohaem III **1** in Colorado coal on the basis of analytical evidence.[4]

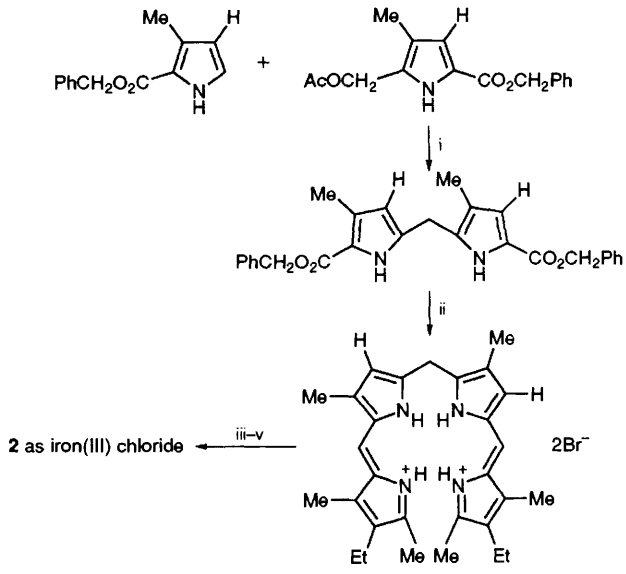
Fractionation of haems from a dried powdered Colorado coal sample (PSOC 850, US classification HVC, 79.6% C) was carried out by multiple and sequential TLC following extraction with 7% sulfuric acid in methanol (v/v) at room temperature for 48 h.[2,3] This gave nineteen fractions ranging from C_{32} (most mobile) to C_{26} (least mobile). The major and most mobile haem which appeared in fractions 1 and 2 was isolated in the amount of 1.63 μg per g of coal. Its identity was confirmed as etiohaem III by comparison of the paramagnetically-shifted ^{1}H NMR spectrum of the dicyano-iron(III) complex with that of an authentic sample prepared by total synthesis using the Johnson biladiene-*a*,*c* approach[5] (Scheme 1).

In fractions 12–17 C_{28} haems were identified by accurate measurement of the molecular ion: if cleavage of side chains ($C_{32} \rightarrow C_{28}$) occurs *via* the bituminous cracking progression[2] these fractions may still be expected to be mixtures. However, fraction 15 was purified until it appeared to be a single substance present to the extent of 0.52 μg g^{-1}. The molecular ion appeared at *m*/*z* 476.166 ($C_{28}H_{28}N_4$ ^{56}Fe requires 476.166): the VIS spectrum in chloroform–methanol is shown in Fig. 1(*a*). Attempts to observe the paramagnetically-shifted spectrum of the dicyanoferrihaem (CD_3OD saturated with KCN, 300 K) at 250 MHz were unsuccessful, but at 600 MHz the spectrum shown in Fig. 2(*a*) was obtained. Impurities are still present, but quite clearly this haem has four β-methyl groups and two β-hydrogens.

Deuteroetiohaemin IX [**2**, as iron(III) chloride, chloro-iron(III) 2,18-diethyl-3,7,12,17-tetramethylporphyrin][6] was synthesised (again using the Johnson biladiene-*a*,*c* method) as shown in Scheme 2.



Scheme 1 *Synthesis of etiohaem III*: i, krytopyrrole aldehyde/H$^+$, 69%; ii, $CuCl_2$–DMF (dimethylformamide); iii, 5% H_2SO_4–TFA (trifluoroacetic acid), ii + iii = 36%; iv, $FeCl_3$



Scheme 2 *Synthesis of deuteroetiohaem IX*: i, K10 montmorillonite clay, CH_2Cl_2, 94%; ii, H_2/Pd; kryptopyrrole aldehyde/H$^+$, 86%; iii, $CuCl_2$–DMF; iv, 15% H_2SO_4–TFA, iii + iv = 22%; v, $FeCl_3$

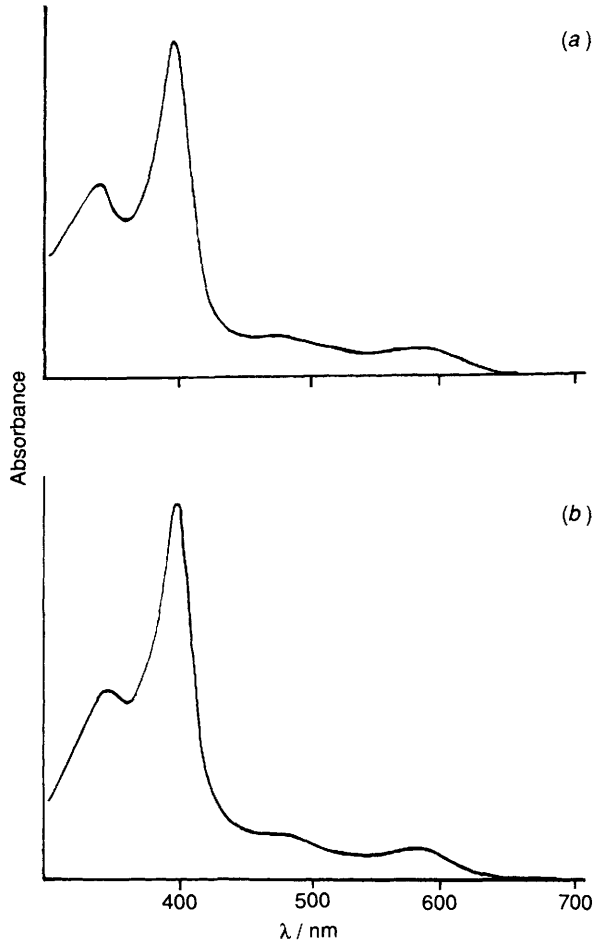


Fig. 1 VIS spectra in chloroform–methanol; (*a*) deuteroetiohaem IX from Colorado coal (fraction 15) and (*b*) synthetic deuteroetiohaem IX

Table 1

Deuteroetiohaem IX	β-Methyl/δ	β-Hydrogen/δ
Natural, coal-derived	+18.59, +16.02, +15.56, +13.46	−15.31, −15.52
Synthetic	+18.60, +16.03, +15.565, +13.46	−15.29, −15.51

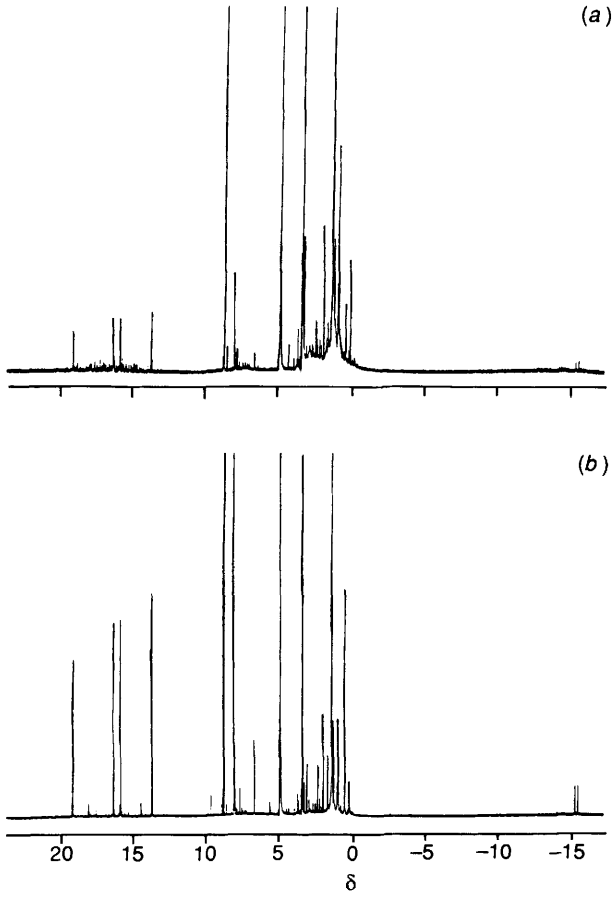


Fig. 2 ^{1}H NMR spectra of dicyano-iron(III) complexes of deuteroetioporphyrin IX *ca.* 10^{-3} mol dm^{-3} in CD_3OD saturated with KCN at 300 K; (*a*) sample from Colorado coal (fraction 15) and (*b*) synthetic compound (Scheme 2). (Chemical shifts relative to Me_4Si).

The synthetic material had its molecular ion at *m/z* 476.164 and proved to be indistinguishable from fraction 15 on TLC, on comparison of VIS spectra [Figs. 1(*a*) and (*b*)] and, most significantly, on comparison of the paramagnetically-shifted ^{1}H NMR spectra of the $(CN)_2Fe$(III) complexes [Figs. 2(*a*) and (*b*)]. The numerical comparison between the key signals is shown in Table 1.

We conclude that deuteroetiohaem IX is one of the principal C_{28} components of the mixture which is formed by side-chain cracking during the coalification process. Cytochrome *c*, rather than chlorophyll *a*/*b*, seems to us to be the more plausible precursor of structure **2**: but a firm conclusion about the origin of this material must await the identification of the other major components, and the expected recognition of structures indicative of chlorophyll precursors, as are found in the porphyrins from crude oil.[7]

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