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PETROPORPHYRINS—III[1]

CHARACTERISATION OF A C_{32} AETIOPORPHYRIN FROM GILSONITE AS THE *BIS*[PORPHYRINATO-MERCURY(II) ACETATO]MERCURY(II) COMPLEX. ORIGIN AND SIGNIFICANCE

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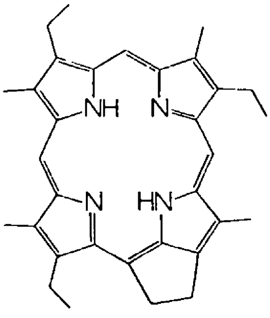
Abstract—A C_{32} aetio porphyrin isolated from Gilsonite bitumen (*ca.* 60 × 10^6 yr) was assigned unambiguously as aetioporphyrin—III by ^{1}H NMR analysis of its *bis*[porphyrinato-mercury(II) acetato]mercury(II) complex and by comparison with an authentic sample. The occurrence of this compound provides the first direct evidence that the petroporphyrins of Gilsonite are the product of reductive degradation of naturally-occurring chlorophylls, and conversion of the chlorin to the porphyrin system.

The reported occurrence of deoxophylloerythroetioporphyrin **1** and aetioporphyrin—III **2** as metal complexes in a Triassic sediment[2] and the proposal that geological porphyrins are derived from chlorophyll *a*[3,4] laid the foundation of molecular organic geochemistry. Mass spectrometric analysis of the alkyl porphyrins in this sediment, and in a variety of shales, bitumens and crude oils[5-7] showed, however, that the porphyrins occur as mixtures of Ni and/or V = O complexes of two major series: the deoxophylloerythroetio, DPEP, and the aetio types, with carbon numbers ranging from C_{26} to C_{39}. It was proposed that the members with additional carbon atoms ($>C_{32}$) arise either from transalkylation or from chlorobium chlorophylls.[7] These hypotheses were based on visible absorption and mass spectrometric data for total petroporphyrin mixtures. Oxidation to maleimides of the demetallated porphyrins from Boscan crude oil (Cretaceous, W. Venezuela) indicated that neither of these processes is a major one.[1] It was essential to isolate individual components to study further the origin(s) of these compounds. The structural elucidation of the C_{32} aetio and C_{32}DPEP porphyrins is of particular importance because the occurrence of these compounds in sedimentary organic matter is the basis of the hypotheses of Treibs[3] and Corwin.[4]

The bitumen Gilsonite (Eocene, Uinta Basin, Utah, U.S.A.) has a simple distribution of nickel petroporphyrins, in relatively high concentration (100 ppm),[7-11] allowing the isolation of the C_{32} aetio porphyrin present. We report the unambiguous structural determination of this compound, together with an improved isolation procedure and discussion of the origins of the petroporphyrins of Gilsonite.

RESULTS AND DISCUSSION

Powdered Gilsonite forms a viscous tar on contact with most common organic solvents, although it is soluble in dichloromethane and chloroform. Therefore, to concentrate selectively the Ni porphyrins, it was necessary to modify existing schemes[7-11] by dispersing the bitumen on alumina from solution in dichloromethane, and by selectively extracting the porphyrins with a toluene-methanol mixture. This method provides an extract *ca.* twice as rich in nickel porphyrins (1200 ppm) as obtained previously. The C_{32} aetio porphyrin was isolated, demetallated and purified using a variation of a previous method,[8] and was shown to give one peak by high performance liquid chromatography. Analysis of the porphyrin by ^{1}H NMR (Fig. 1) and by mass spectrometry indicated that it contained four methyl and four ethyl β substituents but no *meso* (bridge) alkyl substituents. Degradation using chromic acid[13] produced only 3-ethyl-4-methyl-1H-pyrrole-2,5-dione (3-ethyl-4-methyl maleimide). Therefore, the C_{32} aetio porphyrin was either one, or a mixture of, the four type (I, II, III, IV) isomers (**3**, **4**, **2**, **5**, respectively). Although the four isomers have similar chemical and physical properties, it is possible to distinguish them by comparing the ^{1}H NMR spectra of their *bis*[porphyrinato-mercury(II) acetato]mercury(II) complexes (proposed as **6** by Hudson and Smith[14]). The ^{1}H NMR data of the mercury "double sandwiches" of **2**, **3**, **4** and **5**, together with that of the isolated compound (see Fig. 2) are summarised in Table 1. Clearly the C_{32} aetio porphyrin is



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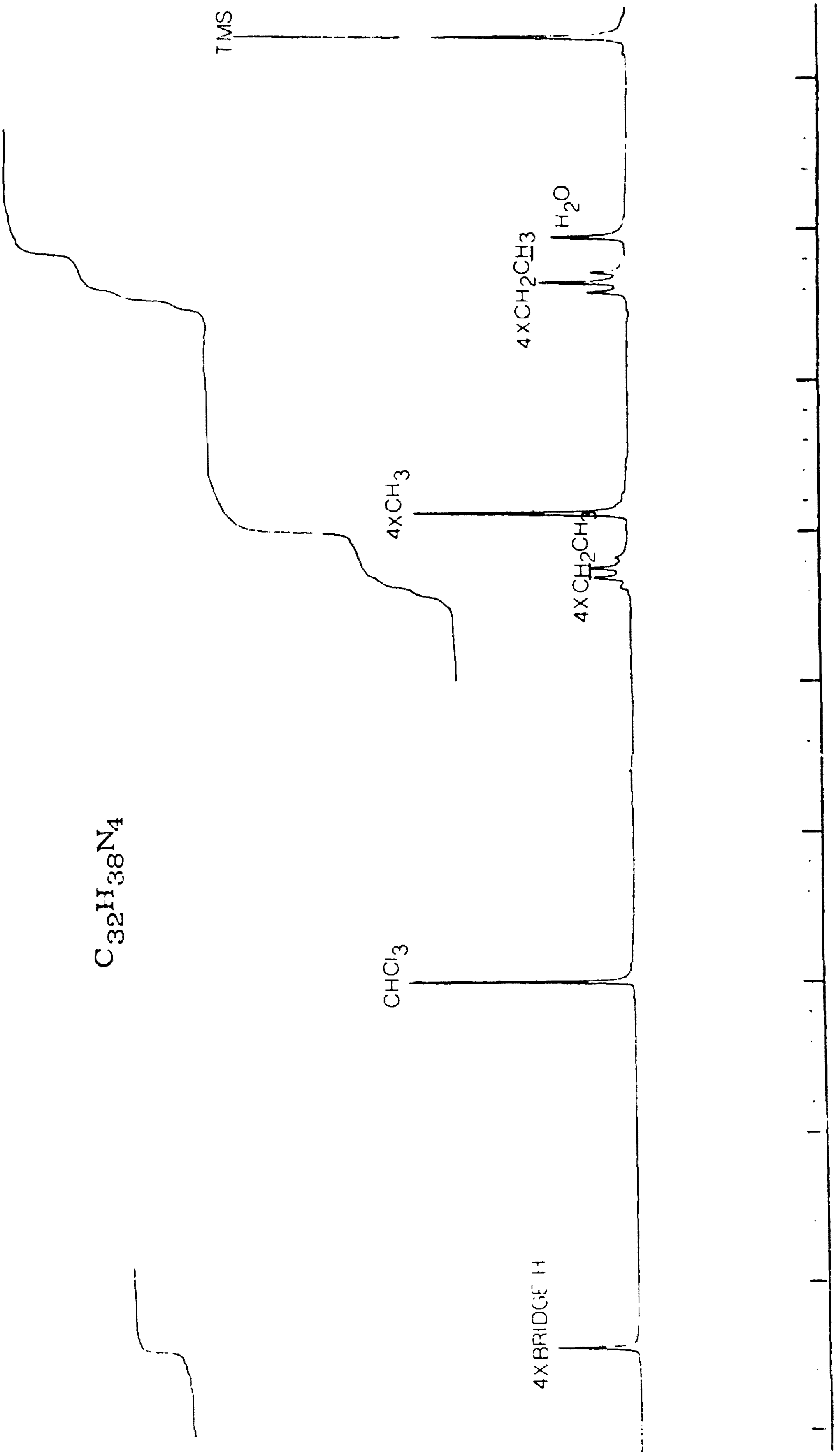


Fig. 1. ^{1}H NMR spectrum ($CDCl_3$, JEOL-PFT 100 instrument) of the C_{32} aetio porphyrin isolated from Gilsonite as one of the major aetio porphyrins.

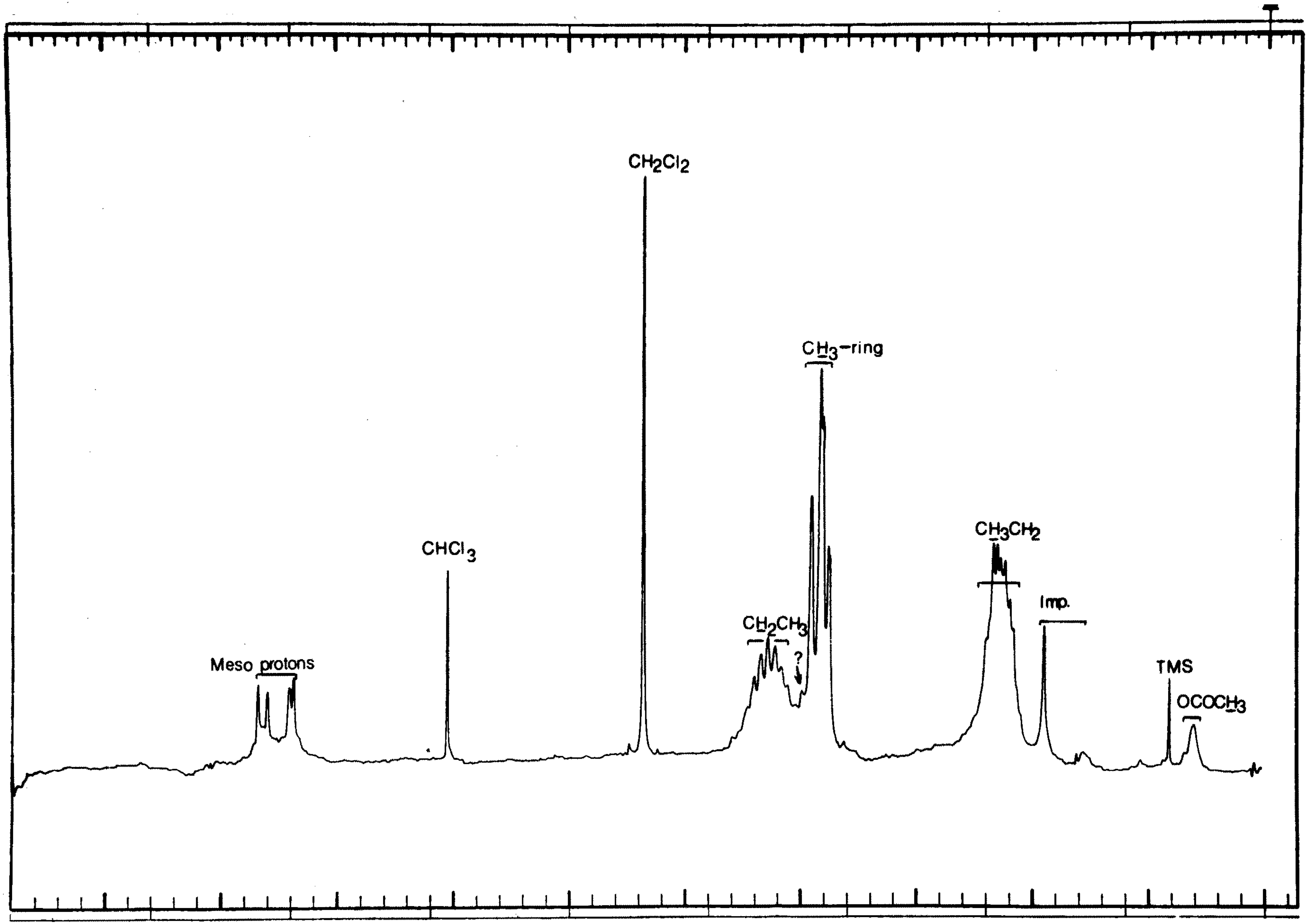
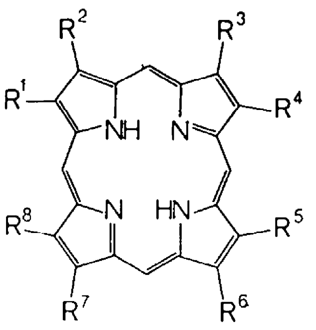


Fig. 2. ^{1}H NMR spectrum ($CDCl_3$, JEOL:PFT 100 instrument) of the *bis*[porphyrinato-mercury(II) acetato]mercury(II) complex of the C_{32} aetio porphyrin isolated from Gilsonite (Imp. = impurity; ? probably arises from decomposition of the sandwich complex as there is an intensity increase in this signal after standing overnight).

Table 1. Chemical shifts (δ) of mercury "double sandwich" complexes **6** of aetio porphyrins and the C_{32} aetio porphyrin from Gilsonite in $CDCl_3$, and HPLC data for corresponding metal-free compounds.

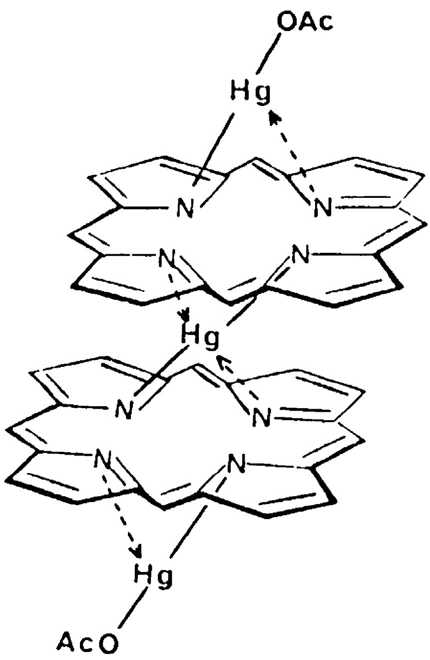
Compound	Chemical Shift				HPLC Retention Time (min) on NARP
	meso-H[a]	CH_3	$C\underline{H}_2$-CH_3	CH_2-$C\underline{H}_3$	
Aetio porphyrin I (**3**)	9.00 (s)	3.50 (s) 3.54 (s)	4.0 (m)	1.60 (t) 1.72 (t)	38.8
Aetio porphyrin II (**4**)	9.04 (s)[b] 9.00 (s)	3.5 (s)[b]	4.0 (m)[b]	1.70 (t)[b]	37.2
Aetio porphyrin—III (**2**)	8.80 (s) 8.83 (s) 9.07 (s) 9.16 (s)	3.40 (d)[c] 3.48 (s) 3.50 (s) 3.59 (d)	4.1 (m)	1.7 (m)	37.5
Aetio porphyrin—IV (**5**)	8.68 (s) 8.98 (s) 9.26 (s)	3.42 (s) 3.59 (s)	4.0 (m)	1.62 (t) 1.81 (t)	37.2
C_{32} Aetio porphyrin[d]	8.79 (s) 8.83 (s) 9.05 (s) 9.14 (s)	3.41 (d) 3.47 (s) 3.49 (s) 3.59 (bs)	4.2 (m)	1.7 (m)	37.5[e]

[a] Satellite peaks observed due to ^1H-^{193}Hg Coupling. [b] Reference 14. [c] Additional fine structure.
[d] Traces of decomposed sandwich observed (possibly HgII porphyrin) t = triplet, m = multiplet.
[e] Did not co-chromatograph with aetio porphyrin—I, but co-chromatographed with aetio porphyrin—III.



2: $R^1=R^3=R^5=R^8=CH_3$; $R^2=R^4=R^6=R^7=C_2H_5$
3: $R^1=R^3=R^5=R^7=CH_3$; $R^2=R^4=R^6=R^8=C_2H_5$
4: $R^1=R^4=R^5=R^8=CH_3$; $R^2=R^3=R^6=R^7=C_2H_5$
5: $R^1=R^3=R^6=R^8=CH_3$; $R^2=R^4=R^5=R^7=C_2H_5$

aetioporphyrin—III **2**. It is interesting that mixtures of **2**, **3**, **4**, **5**, when subjected to HPLC using a non-aqueous reverse phase (NARP) column, gave only separation of **3** from **2**, **4** and **5** which were not resolved (Table 1). The isolated C_{32} aetio porphyrin behaved similarly and did not co-chromatograph with **3**. The combined spectrometric and chromatographic data for this compound provide the best evidence to date that the porphyrins of Gilsonite have been produced from the defunctionalisation of chlorophyll *a*, the most abundant of the naturally-occurring chlorophylls. It seems likely that the compound is formed via the opening of the isocyclic ring present originally in chlorophyll. Although the point on the degradative pathway at which this process occurs is unknown, one possibility is that it takes place after formation of the DPEP porphyrin skeleton. It has been observed by *in vitro* experiments that DPEP porphyrins are converted into aetio prophyrins on heating with clays.[15] Similarly it is known that the concentration of aetio alkyl porphyrins increases with respect to DPEP alkyl porphyrins, with increasing burial depth, in samples from a uniform stratigraphic sequence.[16] Alternatively, the ring opening may occur at an earlier (chlorin) stage.[17]



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Clearly it is important to determine the structure of the C_{32}DPEP porphyrins in sedimentary samples before it is possible to investigate fully the hypotheses of Treibs[3] and Corwin.[4]

EXPERIMENTAL

^{1}H NMR spectra were run on JEOL PS-100 and JEOL PFT-100 spectrometers; $CDCl_3$ (previously passed through alumina) was used as solvent with TMS as internal reference. Chemical shifts are expressed on the δ scale. The following abbreviations are used for ^{1}H NMR data: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. U.V./visible absorption data (in CH_2Cl_2) were obtained on a Pye-Unicam SP-1800 spectrophotometer. Quantitation of nickel porphyrins was carried out using the molar extinction coefficient of 34820 at 550 nm.[18] GC analyses were performed on a Carlo-Erba 2150 filled with a glass column (25 m × 0.3 mm i.d. capillary) coated with OV-1 using N_2 as carrier gas with a flow rate of *ca.* 1 ml min^{-1}, programming from 60°C to 260°C at 6°C min^{-1}. Direct insertion probe mass spectra were obtained using a AEI MS902 mass spectrometer. High resolution mass spectra were obtained on the same instrument using the peak matching technique with perfluorokerosene as internal standard. Elemental compositions from accurate mass measurements were obtained on a Digital Equipment Corporation LINC-8 computer. The HPLC equipment comprised two Waters M6000 D pumps, a Waters M660 solvent progammer, and a Varian Variscan 2635 M detector fitted with 8 μl flow cells. Analyses on 5 μ Partisil (25 cm × 4.6 mm stainless steel) were carried out using toluene and hexane (1:9 vol:vol) as solvent A and toluene and chloroform (1:1 vol:vol) as solvent B, programmed (10 min) from 25% B to 75% B with a linear program and flow rate of 1.5 ml min^{-1}: HPLC grade solvents (Rathburn Chemicals Ltd.) were used. Thin layer chromatography (TLC) was carried out using Keiselgel H Type 60 (0.5 mm) pre-eluted with $CH_3CO_2C_2H_5$ and reactivated at 100° (2 h). Continuous elution TLC[19] was carried out on Kieselgel H Type 60 (40 cm × 20 cm × 0.5 mm), treated as above. The sample was applied to the shorter edge. All solvents were distilled before use, except those used in the isolation of the total organic extract. The *bis* (porphyrinato-mercury (II) acetato)mercury(II) complexes of aetio porphyrin—I **3**, aetio porphyrin—III **2** and aetio porphyrin—IV **5** were synthesised by the method of Hudson and Smith.[14]

Isolation of the nickel porphyrins. Powdered gilsonite (700 g) was dissolved in CH_2Cl_2 (3 l) and alumina (B.D.H., Grade 11, 10 Kg) was added. The solvent was removed under reduced pressure, and the dry, impregnated alumina was extracted with toluene-CH_3OH (120 l, 1:2 vol:vol, recycled) until the visible spectra showed that the eluates contained no porphyrins. The eluates were evaporated to dryness, and the crude extract, a black tar (70 g), was chromatographed on alumina (B.D.H., Grade 11, 500 g) eluting with C_6H_{14}, C_6H_{14} toluene, toluene, toluene-CH_2Cl_2, $CHCl_2$, $CHCl_2$-CH_3OH and CH_3OH. The eluates were monitored by visible absorption spectrometry, and the petroporphyrin-containing fractions were combined and evaporated to dryness leaving a dark red oil (4 g, containing *ca.* 80 mg porphyrins). This fraction was purified further by column chromatography on silica gel (200 g, Hopkin & Williams Ltd., MFC. 100–200 mesh) using gradient elution of CH_2Cl_2 in C_6H_{14} and monitoring as above. The initial elution with hexane produced a black tar (1 g, containing 10 mg porphyrin) which was set aside. The remainder of the crude metalloporphyrins (300 mg total; 66 mg porphyrin, quantitated from U.V./visible spectrometry) was separated into 5 fractions labelled "A" (15 mg; 6 mg porphyrin), "B" 30 mg, 13 mg porphyrin), "C" (50 mg; 23 mg porphyrin), "D" (30 mg; 16 mg porphyrin) and "E" (10 mg; 3 mg porphyrin) by continuous elution TLC[19] (9 h) with toluene C_6H_{14} (3:4 vol:vol) as eluant. Mass spectrometry showed the C_{32} aetio porphyrin was concentrated in fraction E.

Isolation of the C_{32} aetio porphyrin from fraction E. Fraction E (10 mg; 3 mg porphyrin) was demetallated with methane sulphonic acid (0.5 ml)[20] at 110°C (0.5 h). The solid solution was poured into saturated aqueous CH_3CO_2Na (10 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The organic layers were combined, washed with water, and evaporated to dryness. Separation of the demetallated porphyrins (3 mg; 2.5 mg porphyrin) was carried out by continuous elution TLC (9 h) using toluene–$CHCl_3$ (3:4 vol:vol) as eluant. The C_{32} aetio porphyrin (*ca.* 0.7 mg), contaminated with *ca.* 15% C_{30} aetio porphyrin and C_{31} aetio porphyrin (by MS), was the least polar of the 3 fractions observed.

Purification of the C_{32} aetio porphyrin. To the crude C_{32} aetio porphyrin (0.7 mg) in CH_2Cl_2 (2 ml) and glacial CH_3CO_2H (1 drop) was added a saturated solution of zinc acetate in methanol (0.5 ml). The mixture was heated under reflux (10 min) and metal insertion was shown to be complete by visible absorption spectrometry. The zinc porphyrin mixture was poured into water (5 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic phase was washed with water (3 ml) and evaporated to dryness. Separations of the zinc complex into two fractions was carried out by TLC, eluting (×3) with toluene hexane (1:1 vol:vol). The major component was demetallated with trifluoroacetic acid (6 drops), and the acid mixture was diluted with CH_2Cl_2 (5 ml), washed with water (2 × 1 ml) and evaporated to dryness. The C_{32} aetio porphyrin (0.5 mg) was purified further by heating (10 h) at 140°C *in vacuo* (*ca.* 1 × 10^{-6} mm) to remove volatile impurities. HPLC analysis on 5 μ Partisil showed the sample to be >95% pure (t_R 8.8 min); the HPLC retention data for analysis on the NARP column are summarised in Table 1. ^{1}H NMR δ 1.87 (t, 4 × $C\underline{H}_3$–CH_2); 3.64 (s, 4 × $C\underline{H}_3$-ring); 4.10 (q, 4 × $CH_3C\underline{H}_2$); 10.09 (s, 4 × *meso* H). MS (70 eV) Significant ions 478 (100%; $M^{+\cdot}$), 463 (30; M-15^+), 239 (16; M^{2+}); λ_{max} (CH_2Cl_2) 398, 498, 532, 568 and 620 nm; (Found $M^{+\cdot}$ 478.310; $C_{32}H_{38}N_4$ requires: 478.312).

C_{32} Aetio bis *[porphyrinato-mercury (II) acetato]mercury(II) complex.* Mercury(II) acetate (5 mg, 8 equiv.) was added to a stirred solution of the porphyrin (0.5 mg) in CH_2Cl_2 (2 ml) and THF (1 ml). The mixture was stirred and warmed (10 min). Visible absorption spectrometry showed that metal insertion was complete, and the mixture was passed through a bed of Kieselgel H (0.2 g) eluting with CH_2Cl_2 THF (1:1). The combined filtrates were evaporated. The sample was left *in vacuo* (10^{-2} mm) for 10 h prior to ^{1}H NMR analysis (Table 1); λ_{max} 404, 544 (inflection) 574 and 610 (shoulder) nm.

Oxidation of the C_{32} aetio porphyrin. To the C_{32} aetio porphyrin (100 μg) in trifluoroacetic acid (2 drops) was added a solution of Cr_2O_3 (0.33 g) in dilute H_2SO_4 (2.5 ml, 25% w/v, pre-extracted with CH_2Cl_2 3 × 10 ml). The yellow solution was cooled to 0°C (2 h) and left to stand at ambient (2 h). The solution was extracted with CH_2Cl_2 (4 × 2 ml), and the organic phase was washed with water (2 ml). The organic phase was evaporated to dryness and analysed by GLC. The product, 3-ethyl-4-methyl-1 $\underline{H}$-pyrrole-2,5-dione was confirmed by coinjection with a standard.

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