Tetrahedron Vol. 40, No. 19, pp. 3777 to 3786, 1984
Printed in the U.S.A.

0040-4020/84 $3.00+.00
Pergamon Press Ltd.

STRUCTURE ANALYSIS OF NATURALLY OCCURRING ALKYL PORPHYRINS BY HYDROGEN CHEMICAL IONISATION MASS SPECTROMETRY

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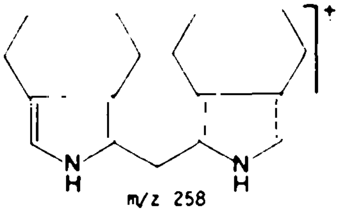
(*Received in UK* 14 *June* 1984)

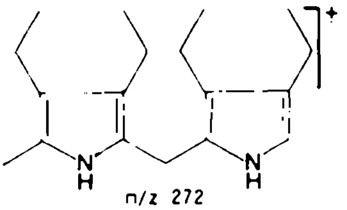
Abstract - Chemical ionisation (CI) mass spectrometry with H_2 as reagent gas has been used to obtain spectra of a number of demetallated alkyl porphyrins from an oil shale and a bitumen. The spectra give more structural information than the electron impact spectra, by way of odd electron dipyrrolic fragment ions. Thus, the structural possibilities for a novel C_{30} aetio porphyrin have been narrowed to two, although ^1H NMR only reduced the possibilities to six. The spectra of four types of components containing a five, six, seven or methyl-substituted six membered exocyclic ring show, for example, that three out of four C_{32} isomers can be distinguished. The technique offers promise as a tool in structural elucidation of other novel naturally occurring porphyrins.

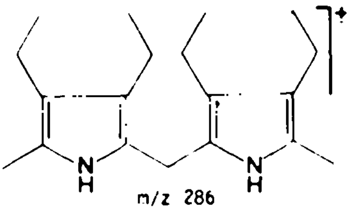
The porphyrins of ancient sediments and petroleums occur mainly as alkyl derivatives complexed to nickel (II) or vanadyl. Analytical techniques such as electron impact mass spectrometry (EIMS)[1], high pressure liquid chromatography (HPLC)[2-4], and combined gas chromatography-mass spectrometry (GC-MS)[5,6] have shown that they occur as complex mixtures of components containing several carbon number series and structural isomers of a given carbon number. These findings have been confirmed by isolation and characterisation of several components from Gilsonite bitumen (Eocene, U.S.A.)[7-9], Julia Creek oil shale (Cretaceous, Australia)[10-12], Marl slate (Permian, U.K.)[13], Serpiano oil shale (Triassic, Switzerland)[14] and Messel oil shale (Eocene, Germany)[15]. Among these are the metal complexes of 1 (R=H or CH_3), presumably derived from chlorophyll a[9,10,16], 2 which appears to originate from chlorophyll c [15] and components (3, R=H,CH_3,C_2H_5) with a seven membered exocyclic alkano ring which may also arise from chlorophyll[12,14].

Positive ion chemical ionisation mass spectrometry (CIMS)[17] using H_2 as reagent gas, is useful in the structural analysis of porphyrins of the aetio type (e.g. 4a) with no exocyclic alkano ring. Fragmentation proceeds via hydrogenation to yield the porphyrinogen, followed by cleavage at the meso (bridge) positions, to give mono- di-, and tripyrrolic ions. The spectra have similarities to those observed in the EIMS of porphyrinogens[18]. Identification of fragment ions, especially the dipyrrolic ions, and of molecular ions, can give rise to information on the sequence of the pyrrole rings and, in certain cases, can distinguish positional isomers. Information on meso substituents can also be obtained[17]. To date only synthesised standards have been analysed.

In the spectra of porphyrins of the aetio type, the major odd electron dipyrrolic ions occur as triplets containing 0, 1 and 2 meso carbons. Thus, the dipyrrolic region in the spectrum of 4a shows one such triplet at m/z 258, 272 and 286, in the approximate intensity ratio of 3:3:1 respectively, arising from the following ions[17]:


m/z 258


n/z 272


n/z 286

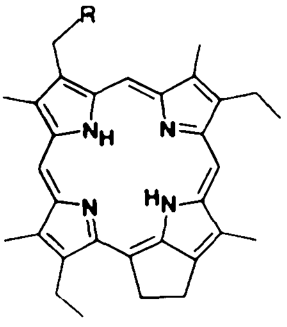
For ease of discussion of structural information from these ions, porphyrin types are described using A, B, etc. for the pyrrole rings[17]. Hence, 4a is described as an A_4 or AAAA type, since all four pyrrole rings contain the same pairs of β-substituents (Table 1).

Table 1. Possible dipyrrolic fragments from H_2 CIMS of porphyrins (after Ref.17)

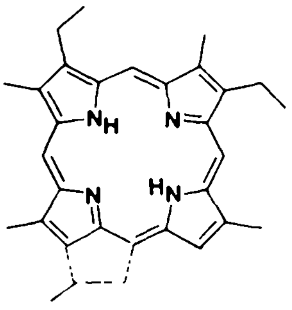
Porphyrin Structural Type		Dipyrrolic Fragments*
General	Pyrrole sequence	
A_4	AAAA	AA
A_3B	AAAB	AA, AB
A_2B_2	AABB	AA, 2AB, BB
	ABAB	AB
A_2BC	AABC	AA, AB, AC, BC
	ABAC	AB, AC
ABCD	ABCD	AB, BC, CD, AD
	ACBD	AC, BC, BD, AD
	ABDC	AB, BD, CD, AC

* Each fragment comprises a triplet of ions containing 0, 1 and 2 meso carbons (see text).

In this paper we report the first application of H_2 CIMS to the structural analysis of naturally occurring alkyl porphyrins of sedimentary origin. Furthermore, we extend the compound types to those having an exocyclic alkano ring (DPEP-type porphyrins)*. For brevity, the DPEP components are described by a shorthand method incorporating the carbon number and size of the exocyclic ring. Thus, 1 (R=CH_3) is a C_{32} DPEP-5 component and 5 (R=H) is a C_{31} DPEP-6 Me^1 component, to indicate a methyl substituent at C-15^1 within the six membered exocyclic ring.



1



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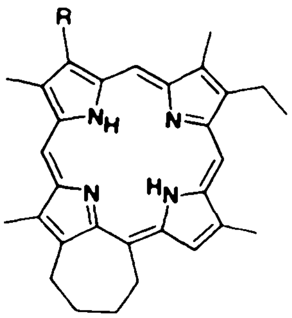

3

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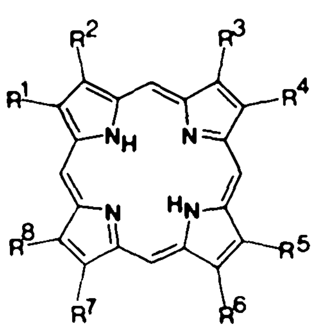
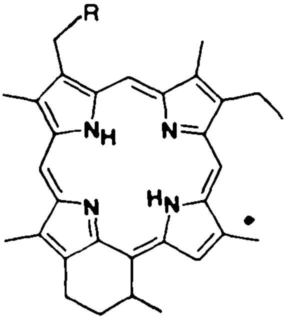
* Although the term deoxophylloerythroetioporphyrin applies only to components of type 1, DPEP is used herein for all compounds with an exocyclic ring.



4a R^{1-8}=CH_2CH_3; 4b $R^{1,4}$=CH_2CH_3 $R^{2,3,5-8}CH_3$
4c $R^{4,8}$=CH_2CH_3 $R^{1-3,5-7}$ CH_3; 4d $R^{4,7}$=CH_2CH_3
$R^{1-3,5,6,8}CH_3$; 4e $R^{4,5}$=CH_2CH_3 $R^{1-3,6-8}CH_3$
4f $R^{2,4}$=CH_2CH_3 $R^{1,3,5-8}CH_3$; 4g $R^{3,4}$=CH_2CH_3 $R^{1,2,5-8}CH_3$



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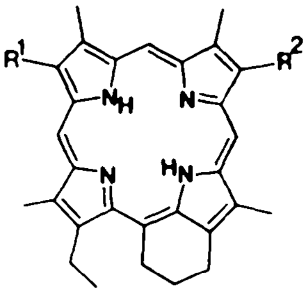


6a R^1=CH_3; R^2=CH_2CH_3
6b R^1=CH_2CH_3; R^2=CH_3

RESULTS AND DISCUSSION

C_{30} Aetioporphyrin from Gilsonite

^1H NMR analysis of a C_{30} aetioporphyrin isolated by HPLC from Gilsonite (Peak I, Fig.1), clearly indicated six methyl and two ethyl β-substituents[19]. Nuclear Overhauser enhancement (n.O.e.) experiments[20] were not possible since the four meso protons were not resolved at 200 MHz. Hence, NMR evidence indicated the component to be one of 4b-4g.

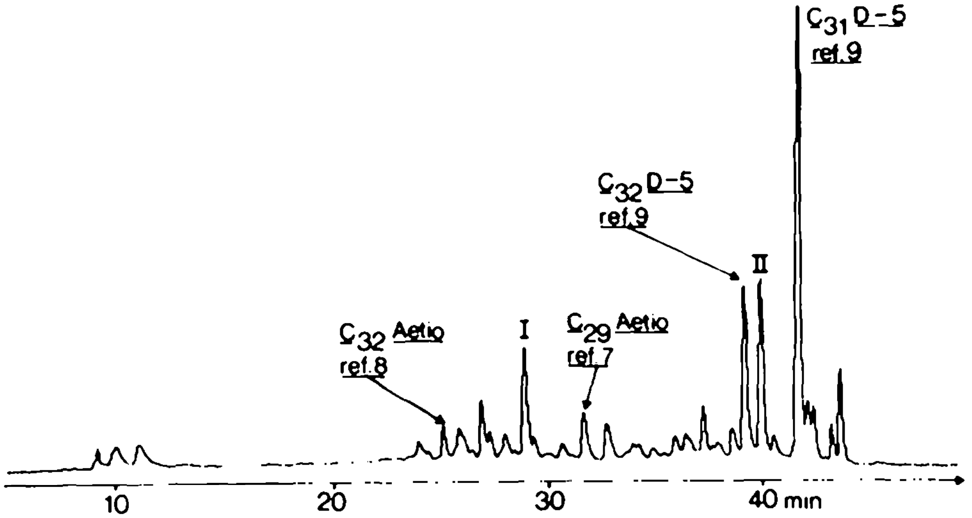
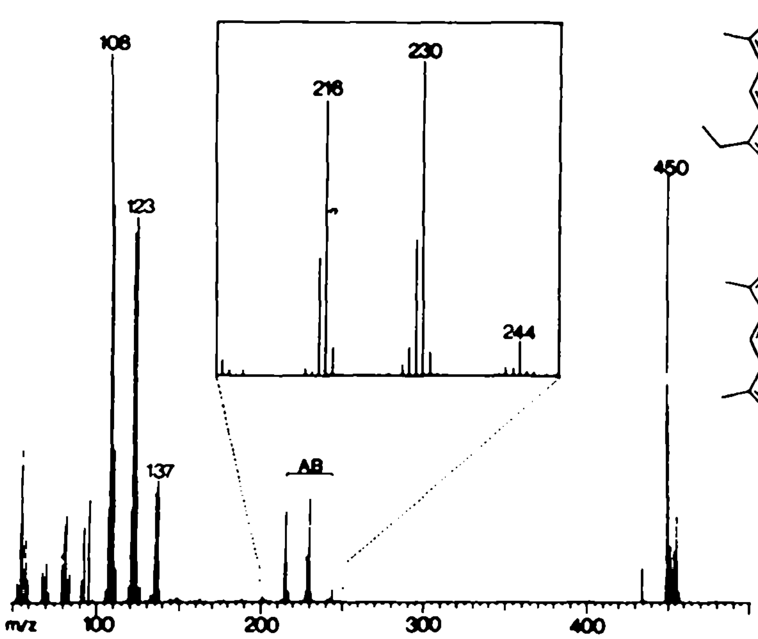
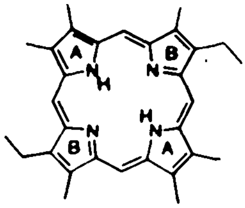


Figure 1. HPLC distribution of demetallated Ni(II) porphyrins from Gilsonite bitumen.

The H_2 CI spectrum yielded mono-, di- and tetrapyrrolic ions, tripyrrolic ions being too weak in intensity for consideration (Fig.2). Ions at m/z 450 and 456 correspond to the porphyrin $M^{+\cdot}$, and the porphyrinogen $(M+6)^{+\cdot}$ respectively. The dipyrrolic region showed one triplet of odd electron ions at m/z 216, 230 and 244, although minor even electron ions were also observed, e.g. m/z 201, arising by secondary cleavage from m/z 216 (cf. Ref.17). Such a simple pattern implies either an AAAA or an ABAB type structure, giving only one triplet of dipyrrolic fragments due to AA or AB respectively (Table 1), the former type being ruled out by ^1H NMR.

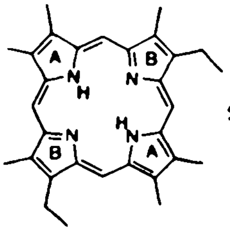
This was substantiated by the mass spectra of two of the possible structural isomers, 4b of type AABB, and 4c of type ABAB. Both gave similar distributions of ions to the Gilsonite C_{30} aetioporphyrin in the mono- and tetrapyrrolic regions of the spectra, as expected. Figure 3A shows the observed pattern of odd electron dipyrrolic ions from fragmentation at the meso positions in 4b and 4c and the Gilsonite component. Whilst 4c of type ABAB gave a similar distribution of dipyrrolic ions to the Gilsonite component, as expected, compound 4b of type AABB gave a more complex pattern





4c

OR



4d

Figure 2. H_2 CI spectrum of a C_{30} aetioporphyrin from Gilsonite bitumen.

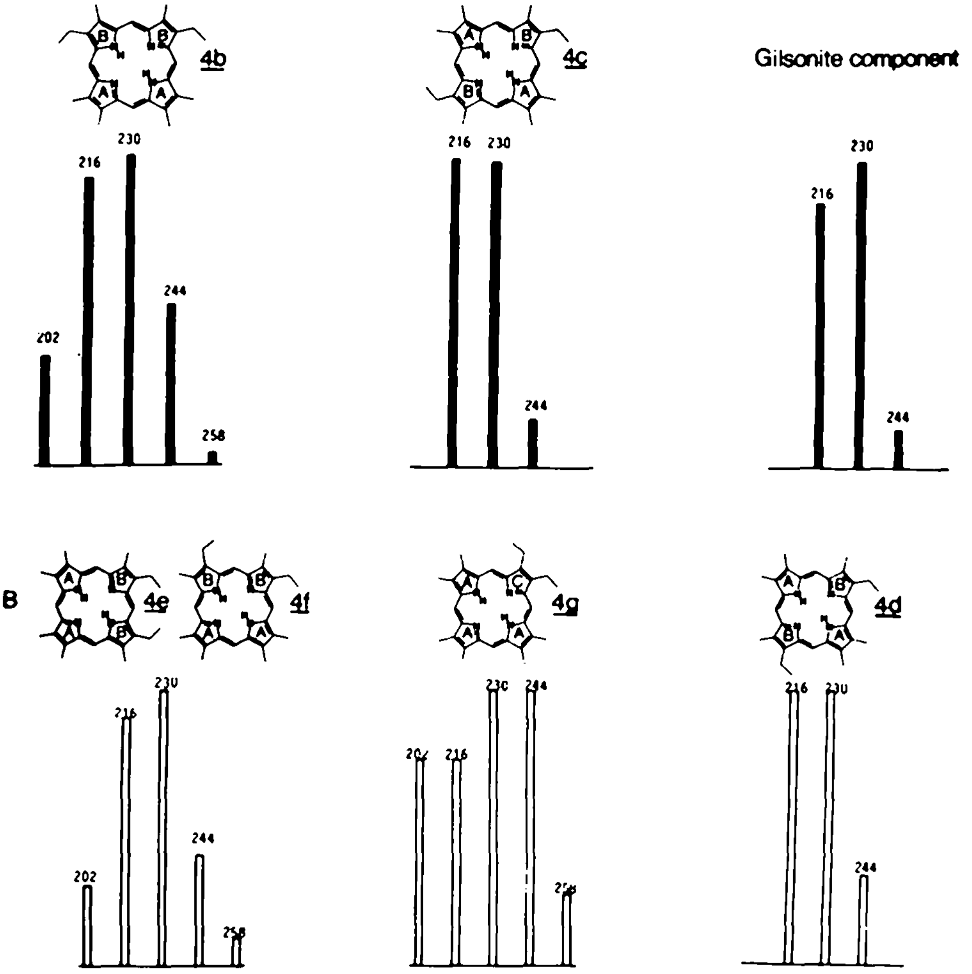


Figure 3. Patterns of odd electron dipyrrolic ions for C_{30} aetioporphyrins. A: Observed for 4b, 4c and Gilsonite component. B: Predicted for 4a-g.

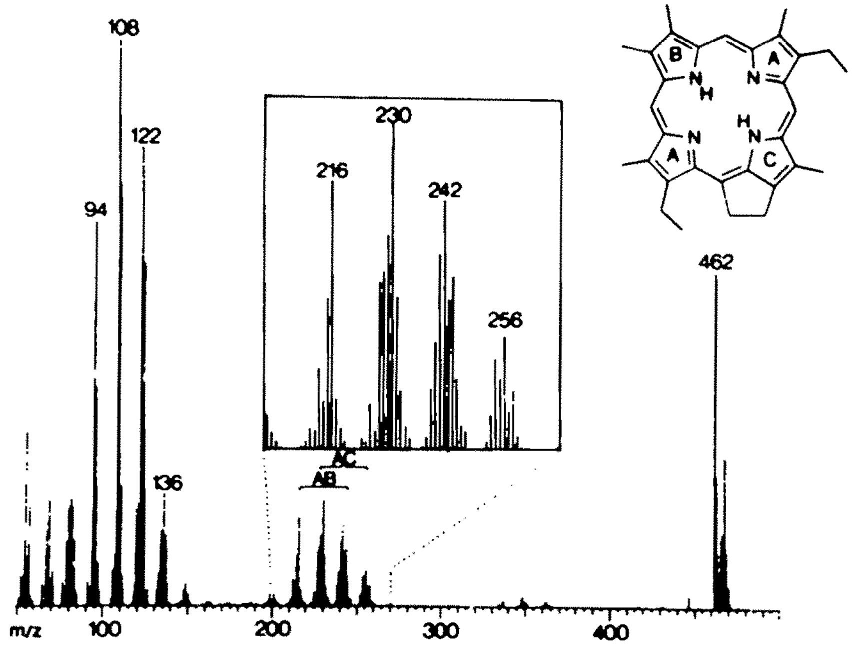


Figure 4. H_2 CI spectrum of <u>1</u> (R= H) from Serpiano shale.

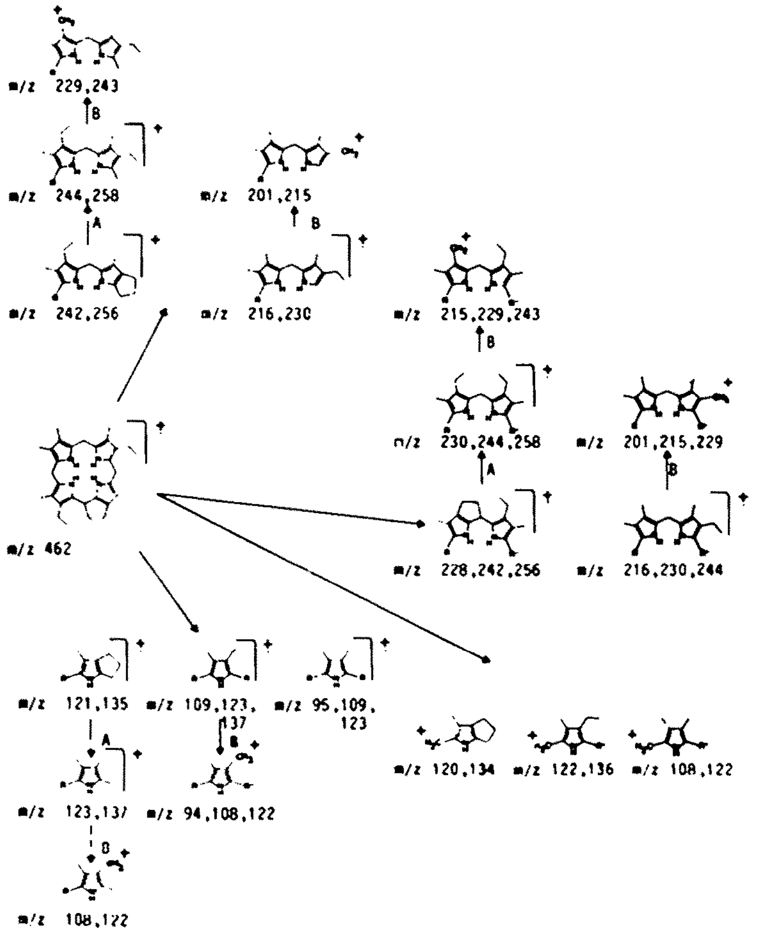


Figure 5. Proposed pathway to key dipyrrolic and monopyrrolic ions of 1 (R=H). Key: A = Exocyclic ring opening and hydrogenation. B = Benzylic cleavage.

arising from three overlapping triplets due to the AA, 2AB and BB fragments (Table 1). On the crude assumption that each dipyrrolic fragment gives rise to a 3:3:1 triplet, Fig.3B predicts the patterns of odd electron dipyrrolic ions expected in the spectra of 4d (ABAB type), 4e and 4f (AABB type) and 4g (AAAC type). Although the observed fragments containing both *meso* carbons are somewhat lower in intensity than might be expected in 4c (m/z 244) and 4b (m/z 258), it appears that the Gilsonite component corresponds to either 4c or 4d and the structural possibilities are limited to one or a mixture of these two isomers.

DPEP-5 Porphyrins

Two components which occur in Gilsonite (Fig.1) as Ni complexes[7,9] and in Serpiano oil shale as vanadyl complexes, have been fully characterised recently in Julia Creek oil shale[10,11] as 3,4-didehydro-3,14-diethyl-4,8,9,13,18-pentamethylphorbine (1, R=H) and 3,4-didehydro-3,9,14-triethyl-4,8,13,18-tetramethylphorbine (1, R=CH_3). The H_2 CI spectrum of 1 (R=H) from Serpiano (Fig.4) shows $M^{+\cdot}$ at m/z 462 and the porphyrinogen ion at m/z 468, and an $(M+4)^{+\cdot}$ ion at m/z 466. Such an $(M+4)^{+\cdot}$ ion is absent from the spectra of aetioporphyrins and presumably arises from incomplete hydrogenation of the macrocyclic ring. This is likely to result from a kinetic effect, in that the substituted *meso* carbon does not become hydrogenated until ring strain is reduced by hydrogenation at the unsubstituted *meso* positions. Mono- and dipyrrolic ions are also present at the expected masses (Figs. 4 and 5). The dipyrrolic region of this ABAC porphyrin shows only two overlapping triplets of odd electron ions, AB and AC, at m/z 216, 230 and 244 and m/z 228, 242 and 256 respectively, the latter triplet containing the exocyclic ring. Cleavage at the substituted *meso* carbon, followed by further hydrogenation, also occurs (Fig.5), as indicated by the ion at m/z 258. Such behaviour has been observed previously in the spectrum of 3,4-didehydro-9,13-di(2-methoxycarbonylethyl)-3,4,8,14,18-pentamethylphorbine[21], and presumably also contributes to m/z 230 and 244. The presence of the exocyclic ring adds a complication to interpretation of the relative intensities of the dipyrrolic ions, since cleavage at a substituted *meso* carbon occurs to a lesser extent[17,21], to contribute to m/z 216 (no *meso* carbons) and 230 (1 *meso* carbon), but not to m/z 244 (2 *meso* carbons) in the AB triplet and m/z 242 (1 *meso* carbon) and 256 (2 *meso* carbons) but not to m/z 228 (no *meso* carbons) in the AC triplet (Fig.5). Despite these complications, it is possible to rationalise in a crude manner the relative intensities of the odd electron dipyrrolic ions if the following assumptions are made: (i) there is a 3:3:1 ratio for the intensities of the "aetio-type" AB triplet, (ii) the AC triplet occurs in a 1.5:3:1.5 ratio, (iii) cleavage at the *meso* substituted carbon contributes an equal intensity to the appropriate ions within the AB and AC triplets, this intensity being 20% of the major ions in (i), and (iv) each odd electron ion containing the exocyclic ring contributes an amount, equivalent to 50% of its observed intensity, to the corresponding ring-opened fragment. The patterns of odd electron dipyrrolic ions arising from the porphyrinogen molecular ion predicted on this basis and the same observed ions for 1(R=H), and for 1(R=CH_3) also isolated from Serpiano, are shown in Fig.6. There is reasonably good agreement in the patterns.

It should be emphasised that the complex fragmentations are necessarily dependent on source conditions and although the same pathways are always observed, the relative abundances of the ions can alter. In practice, a commercially-available alkyl aetioporphyrin (e.g. 4a) is run first and the conditions (H_2 pressure, source temperature, source and lens voltage) are optimised to obtain the expected spectrum.

DPEP-6 Porphyrin

The spectrum of a C_{32} DPEP-6 from Gilsonite (peak II in Fig.1) is shown in Fig.7. ^1H NMR experiments reduce the structural possibilities to one of two isomers[19], differing only in the ordering of the β-substituents in the top half of each molecule represented by 6a (ABAC type) and 6b (AABC type). The fragmentations giving the odd electron dipyrrolic ions are similar to those in the spectra of the DPEP-5 components, and two obvious triplets occur at m/z 216, 230 and 244 and at m/z 242, 256 and 270, the latter containing the exocyclic ring. Ions at m/z 258 and 272 arise solely from opening of the exocyclic ring followed by hydrogenation, and this process presumably also contributes to m/z 244.

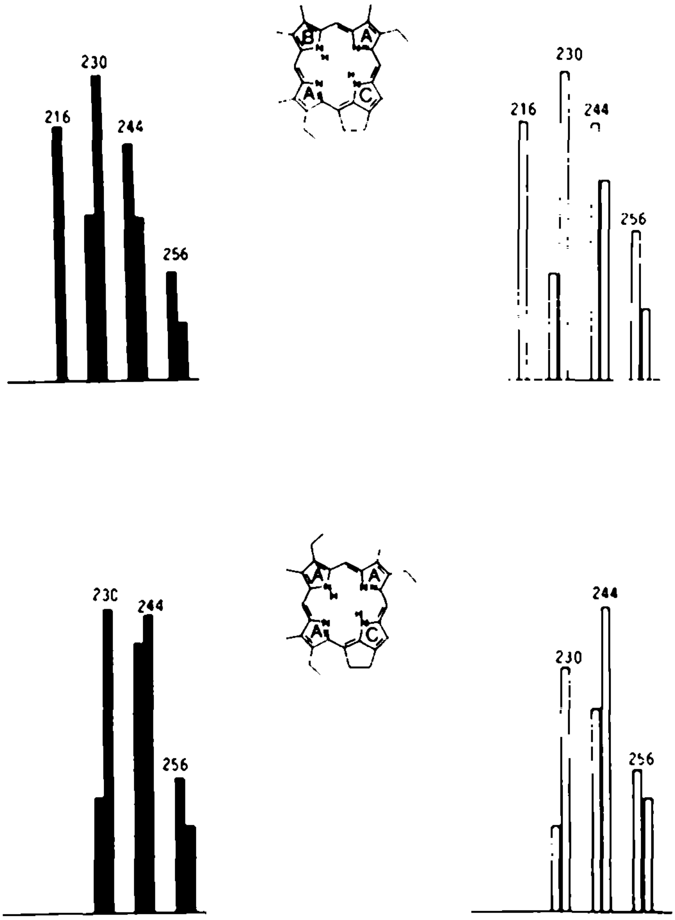
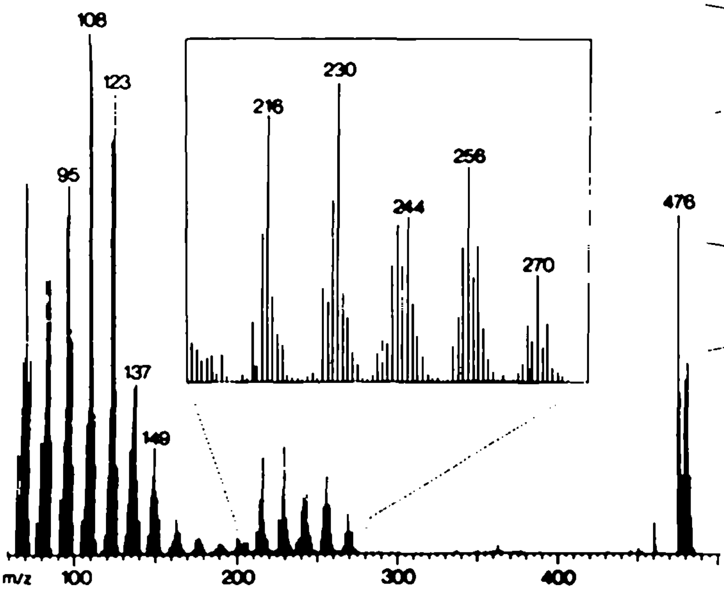
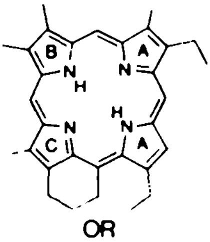


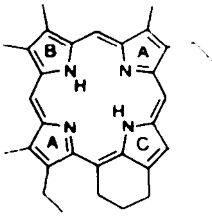
Figure 6. Patterns of odd electron dipyrrolic ions for C_{31} and C_{32} DPEP-5. Shaded = observed; Unshaded = predicted.





6b

OR



6a

Figure 7. H_2 CI spectrum of a C_{32} DPEP-6 from Gilsonite.

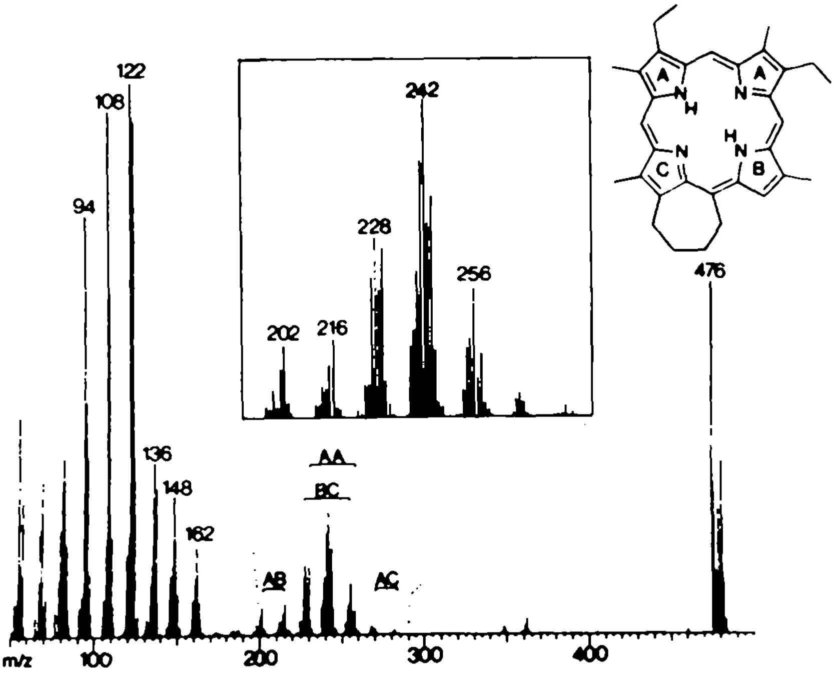


Figure 8. H_2 CI spectrum of 3(R=CH_2CH_3) from Serpiano shale.

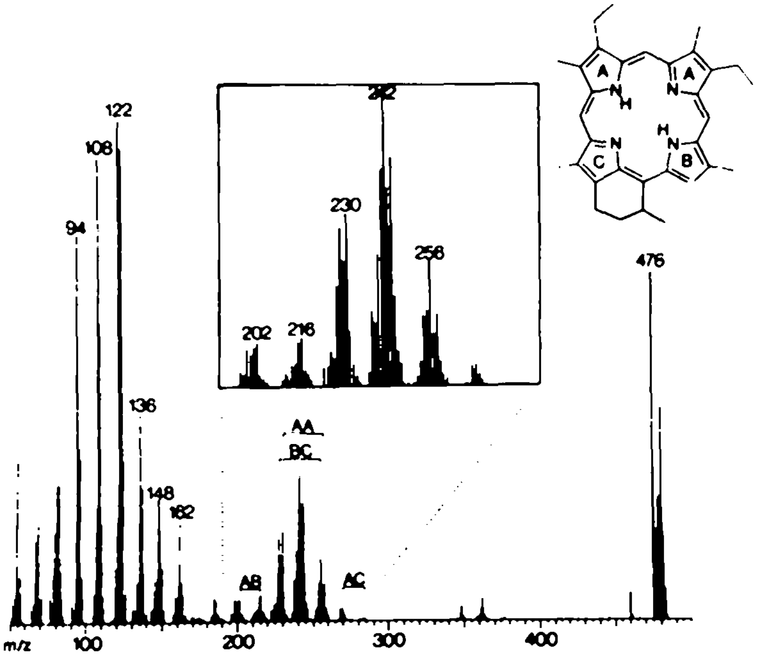


Figure 9. H_2 CI spectrum of 5 (R=CH_3) from Serpiano shale.

Making the same assumptions as for the DPEP-5 components, the predicted patterns for the odd electron dipyrrolic ions for 6a and 6b can be obtained, which are similar to each other and to the observed pattern for the Gilsonite component. The similarity in the predicted patterns arises because fragmentation at the substituted *meso* carbon in 6b to give AA and BC fragments is expected to be a minor process (*cf*. DPEP-5 components) and would only affect slightly the predicted pattern. Hence, the spectrum does not distinguish the two isomers.

DPEP-7 Porphyrins

A C_{32} DPEP-7 component from Serpiano oil shale has been assigned as 15,17-butano-3,8-diethyl-2,7,12,18-tetramethylporphyrin (3, R=CH_2CH_3)[14]. The spectrum (Fig.8) of this AABC porphyrin shows three obvious sets of dipyrrolic fragments: AA triplet (m/z 230, 244 and 258), BC triplet (m/z 228, 242 and 256), and AB doublet (m/z 202 and 216), as would be expected. The expected AC doublet (m/z 270 and 284) is of negligible intensity. Furthermore, the BC triplet containing the exocyclic ring is more intense than the AA triplet, unlike the DPEP-5 and DPEP-6 compounds. This C_{32} isomer is readily distinguished from its DPEP-5 and DPEP-6 counterparts.

Demetallation of the Serpiano vanadyl porphyrins also allowed isolation of a C_{31} DPEP-7 component as the free base. This component also occurs in Julia Creek oil shale as the Ni complex[12]. The H_2 CI spectrum (not shown) of this ABCD component, is strictly analogous to that of its C_{32} counterpart (Fig.8).

DPEP-6 Me^1 Porphyrins

Two novel DPEP components (5, R=CH_3 and H) with a methyl-substituted exocyclic ring, have also been isolated from Serpiano oil shale [22]. The spectrum of each (Fig.9 for the C_{32} component) is essentially identical to its DPEP-7 counterpart, each isomeric pair being of the AABC and ABCD types respectively.

CONCLUSIONS

1. H_2 CIMS of naturally occurring aetio and DPEP porphyrins gives rise to spectra containing considerably more structural information than the corresponding EI spectra.
2. A C_{30} aetio porphyrin from Gilsonite has been shown to be one or a mixture of two compounds, although ^{1}H NMR (200 MHz) only reduced the structural possibilities to six.
3. Of the four basic types of DPEP examined, three were readily distinguished by H_2 CIMS.
4. The techniques should be useful in providing structural information about unknown porphyrins, either where insufficient material is available for NMR spectroscopy, or where NMR data are limited. For example, the spectrum of a C_{31} DPEP isolated from the Messel shale, after demetallation, in insufficient quantity for ^{1}H NMR analysis, gave a spectrum consistent with 2 (AABC) type, whose nickel complex has been reported as a major porphyrin component[15]. The pattern of odd electron dipyrrolic ions was analagous to that observed for the C_{32} DPEP-6 Me^1. Two major triplets were observed at m/z 214, 228 and 242 (BC) and m/z 230, 244 and 258 (AA). In addition, at lower intensity, a doublet at m/z 202 and 216 (AC) was also observed. Ions at m/z 256 and m/z 270 (AB) were, however, much less intense than predicted by the method described above, as was the case for the C_{32} DPEP-6 Me^1. Similarly, a C_{32} DPEP-5 component isolated from Messel oil shale has been assigned as 1 (R=CH_3) from HPLC coinjection and H_2 CIMS alone[23].
5. Prior to analysis, care must be taken to obtain reproducible spectra by optimising source conditions using one or more known compounds as standards.

EXPERIMENTAL

Mass Spectrometry

Mass spectra were obtained on a Finnigan 4000 quadrupole spectrometer, in the CI mode, samples being introduced by direct probe. The reagent gas was H_2 (99.99%) and the source pressure was 0.14-0.16 torr. Typical operating conditions were: source 200°C; emission current 350 µA; electron voltage 40 eV. The probe was programmed ballistically from 100°C to 350°C (*ca*. 5 min.), after an initial period of 5 min. During this time, the spectrometer was scanned cyclically (8s) from m/z 50 to 700. Data were acquired and processed using a Finnigan INCOS data system. Spectra were averaged over the period of volatilisation (*ca*. 225-275°C) and were subtracted for background ions.

High Performance Liquid Chromatography

HPLC analyses were performed on three columns (Spherisorb 3W; 3 x 150, 4.6mm i.d.) connected in series, using a Spectra Physics SP 8700 tertiary solvent delivery system, and Rheodyne 7125 injector valve. Detection (400 nm) was carried out using an LDC 1202 Spectromonitor II variable wavelength detector. Solvent programme conditions were similar to those described previously[2].

Samples

The isolation of the demetallated porphyrin fractions from Gilsonite and Serpiano oil shale has been described previously[7,22]. Single components were isolated by preparative HPLC (Spherisorb 5W; 250 x 10mm i.d.), and were purified by TLC (Silica gel G, 5% acetone/toluene) and by distillation of volatile impurities (100°C, 10^{-6} torr, 6h) prior to MS analysis. Where only small amounts were available (< 10μg) single components were analysed by MS without TLC purification and distillation of volatile impurities.

ACKNOWLEDGEMENTS

We are grateful to Professor A.H. Jackson for providing samples of 4b and 4c. We thank the Natural Environment Research Council for mass spectrometric facilities (GR3/2951 and GR3/3758) and the British Petroleum plc for HPLC facilities. We also thank the Brazilian National Research Council (CNPq) (M.I.C.) and the Science and Engineering Research Council (G.W. and G.J.S.), respectively, for Studentships. R.P.E. thanks the British Petroleum plc for funding.

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