Anal. Chem. 1981, 53, 2014–2020

Structural Analysis of Tetrapyrroles by Hydrogen Chemical Ionization Mass Spectrometry

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The H_2 chemical ionization mass spectra of several standard porphyrins have been determined. Fragmentation proceeds via hydrogenation yielding porphyrinogens, followed by cleavage at the meso (bridge) positions to produce mono-, di-, and tripyrrolic ions. Secondary fragmentation by cleavage of substituent groups attached to the pyrrole rings may then occur. Identification of these fragments and the corresponding molecular ions produces considerable structural information on the parent molecule. In certain cases these data are sufficient to distinguish porphyrin type (positional) isomers. In addition information of substituents at meso positions may be retained.

Initial investigations of the chemical ionization mass spectrometry (CIMS) of porphyrins (1), using methane as the reagent gas, have shown evidence for the cleavage of the parent macrocycle into fragments containing one, two, or three pyrrole rings (*1*) (mono-, di-, and tripyrrolic fragments) in addition to the expected quasi-molecular ion cluster (*2*). This effect is particularly noticeable at lower source temperatures, and mass fragmentography has suggested the existence of two competing ionization processes. The spectra were complicated by reactions of the porphyrin macrocycle and its fragments with larger alkyl ions, which are quite abundant in the methane gas plasma. The fragmentation pathways observed showed similarities to the electron impact mass spectra (EIMS) of porphyrinogens (2), prepared in vitro (*3*) by hydrogenation of porphyrins, which are known to fragment by cleavage at the meso positions in the macrocycle. Further preliminary studies, now employing H_2 as reagent gas, yielded mass spectra of a less complex nature, again showing ions due to mono-, di-, and tripyrrolic fragments (*1*). This paper describes the extension of this work and attempts to rationalize the fragmentation modes observed and their relevance to the identification of porphyrin structures and stereochemistries, particularly with reference to positional isomers and meso substituents. We also outline potential applications of the method and compare it with alternative methods of porphyrin structural analysis.

EXPERIMENTAL SECTION

Apparatus. Mass spectral data were obtained on a Finnigan 4000 quadrupole mass spectrometer operating in the CI mode. Reagent gas was hydrogen (99.99%) at a source pressure of 0.15 torr. Source temperature was 200 °C and the emission current was 350 μA. Electron voltage was 40 eV. Samples were introduced by direct insertion probe, the probe temperature being programmed ballistically from ambient to 350 °C in 10 min. During this period, the spectrometer was scanned cyclically from m/e 50 to 750 every 8 s. Data were acquired and processed by a Finnigan INCOS data system. All spectra were averaged over the period of distillation of the porphyrins (ca. 225–275 °C) and subtracted for background ions. The wide dynamic range of the acquisition system enables extremely low intensity ions to be detected. In order to differentiate these peaks from random noise, mass fragmentography was employed to determine whether ions detected were significant.

Samples. 7,12-Diethyl-3,8,13,17-tetramethyl-21*H*,23*H*-porphine-2,18-dipropanoic acid (mesoporphyrin-IX dimethyl-d_3 ester, 1a) was prepared by dissolving the diacid (2 mg) in CD_3OD (1 mL, 99%, BDH) and adding concentrated H_2SO_4 (10% w/v). The reaction mixture was then left for 18 h at 0 °C. An excess (ca. 10 mL) of saturated aqueous sodium acetate was then added. The product was then extracted into CH_2Cl_2 (2 × 10 mL) and washed with water (2 × 10 mL) and the solvent removed by rotary evaporation. EIMS showed no significant undeuterated product (<0.5%) to be present.

All other samples were characterized by the donors and were >99% pure by EIMS.

RESULTS AND DISCUSSION

The H_2 CI mass spectra of the porphyrins analyzed contain two pronounced characteristic ions in the region of the molecular ion. They are the true molecular ion ($M^{+\cdot}$) and an ion at $(M + 6)^{+\cdot}$ whose mass is identical with that of the corresponding porphyrinogen (2), formed by addition of four meso and two imido hydrogen atoms to the parent porphyrin. It would seem, therefore, that extensive in situ addition of hydrogen is taking place. Intermolecular hydrogenation processes are well-known in mass spectrometry, a particular example being the reduction of vinyl groups attached to porphyrin macrocycles (*4*). In previous work using H_2 CIMS, this effect has also been noted in various aromatic systems (*5*). The porphyrinogen ions formed are expected to be unstable, especially with regard to cleavage at the meso positions. The formation of mono-, di-, and tripyrrolic fragments has previously been observed in the electron impact mass spectra of porphyrinogens by this fragmentation mechanism (*3*). Additionally, porphyrinogens are known to rearrange via meso cleavage under acid conditions (*6*).

A_4-Type Porphyrins. In this paper the term A_4-porphyrins is used to describe porphyrins where the four pyrrole rings all contain the same pairs of β-substituents. In the case of 2,3,7,8,12,13,17,18-octaethyl-21*H*,23*H*-porphine (octaethylporphyrin, OEP, 3) there are two ethyl groups on each ring. A_2B_2 then describes a porphyrin containing two pairs of different pyrrole rings, each pair having identical pairs of β-substituents, etc. The H_2 CI mass spectrum of octaethylporphyrin (Figure 1) may be divided into four distinct regions, corresponding to mono-, di-, tri-, and tetrapyrrolic ions. Nonspecific attack by the reagent gas at the meso positions of porphyrinogen ions would yield three monopyrrolic fragments, containing 0, 1, or 2 meso carbon atoms, respectively, in an approximate 1:2:1 ratio. Thus, in the H_2 CI mass spectrum of OEP, abundant ions are observed at m/e 123, 137, and 151 (4) in such a ratio and are presumably formed by this process. These primary monopyrrolic ions are liable to cleavage β to the pyrrole ring (benzylic-type cleavage) losing a methyl radical to give the more stable secondary species (5) at m/e 108, 122, and 136, in a similar ratio. A further fragment at m/e 150 and the increased abundance of the ion at m/e 136 are indicative that this process is supplemented by the formation of less hydrogenated species (6).

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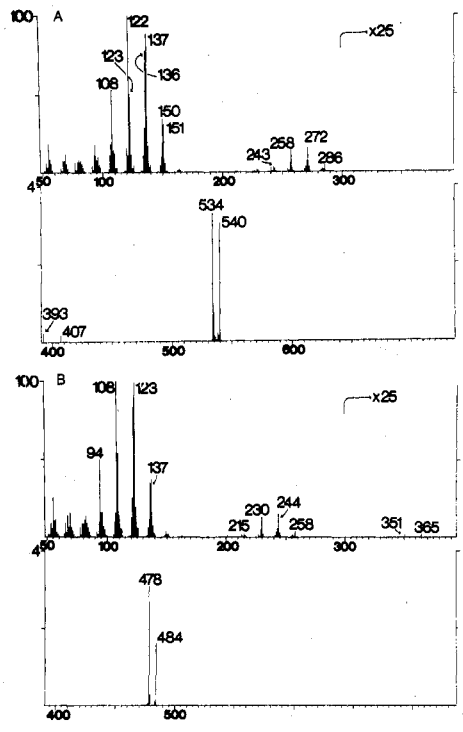


Figure 1. H_2 CI mass spectra of symmetric porphyrins: (A) octaethylporphyrin, (B) aetioporphyrin-I.

The three primary dipyrrolic ions (7), at m/e 258, 272, and 286, are formed analogously and may also fragment by loss of methyl radical to give ions at m/e 243, 257, and 271 (8). It is noticeable that a distortion of the expected 1:2:1 ratio occurs with the more highly substituted fragment being of lower intensity, the ratios approximating to 3:3:1. This effect is further observed in the tripyrrolic region of the mass spectrum where the fragment containing all four meso carbon atoms is not observed, leaving the trace ions at m/e 393 and 407 (9). It is also noticeable that the di- and tripyrrolic ions formed contain saturated meso carbon atoms (pyrrane structure) rather than the pyrrene structure reminiscent of open chain tetrapyrroles, further evidence for the formation of a porphyrinogen species as an intermediate. These pyrrane structures are themselves unstable (7) and are liable to further attack to yield smaller fragments. Given a sufficiently long residence time in the source, it is likely that monopyrrolic fragments are formed by this stepwise cleavage as well as by direct formation from the porphyrinogen. Thus a competition could be established between side chain cleavage of primary di- and tripyrrolic fragments, to yield the more stable benzylic type ions, and further attack of the reagent gas, to yield smaller fragments. This provides a possible explanation for the low abundance of secondary di- and tripyrrolic fragments, compared with their monopyrrolic analogues. The mechanism of the cleavage processes involved in the fragmentation of the porphyrin macrocycle is, as yet, unknown. However, the extensive formation of odd electron species could be interpreted in terms of the continual addition of neutral H_2 molecules, rather than attack by ionized species. The postulated fragmentation scheme for OEP is shown in Figure 2. The complex reaction mechanisms that do take place are necessarily dependent on source conditions (pressure and temperature of reagent gas, effect of source and lens voltages on source residence times) and thus, while the same fragmentation pathways are always observed, the relative abundance of the ions may alter between different sets of analyses. The data previously presented (1) therefore show slight differences from those shown here.

The H_2 CI mass spectrum of 2,7,12,17-tetraethyl-3,8,13,18-tetramethyl-21*H*,23*H*-porphine (aetioporphyrin-I,

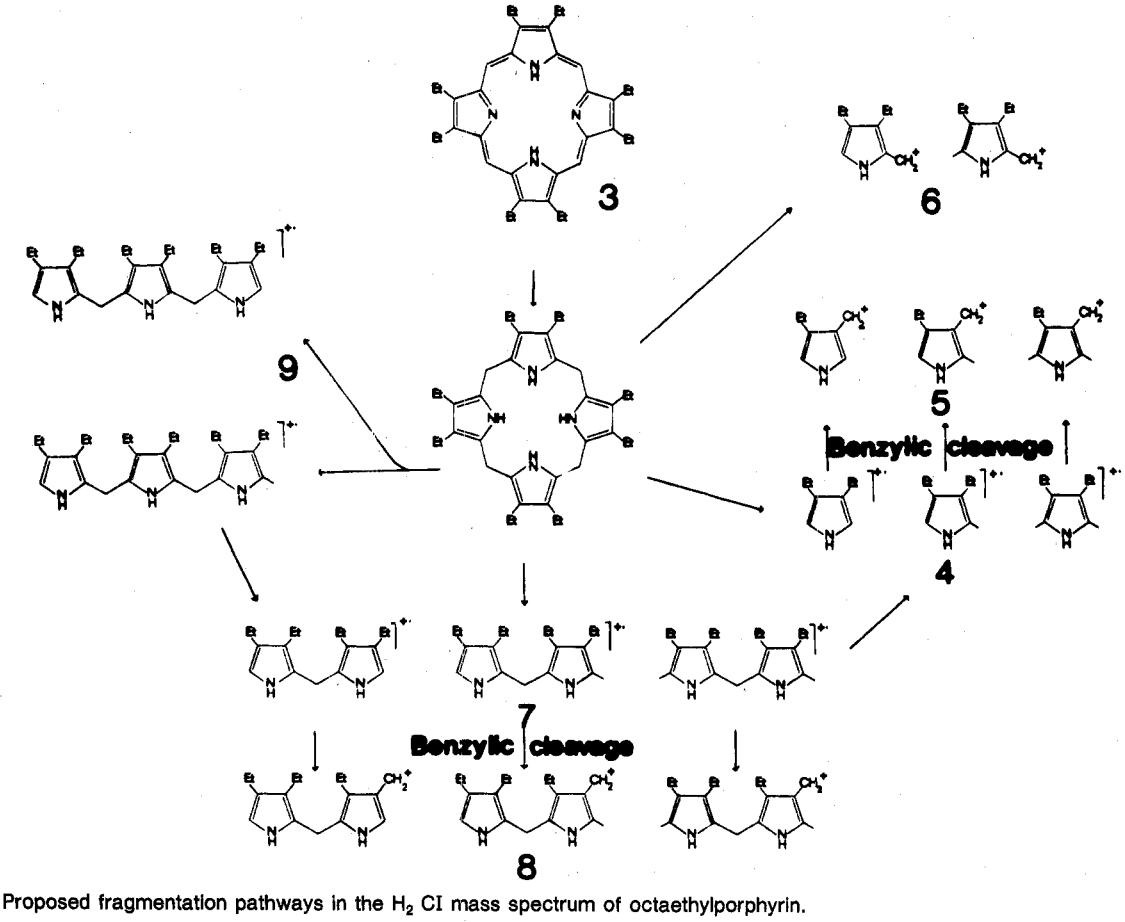


Figure 2. Proposed fragmentation pathways in the H_2 CI mass spectrum of octaethylporphyrin.

Table I. Abbreviated H_2 CI Mass Spectra of Porphyrins[a]

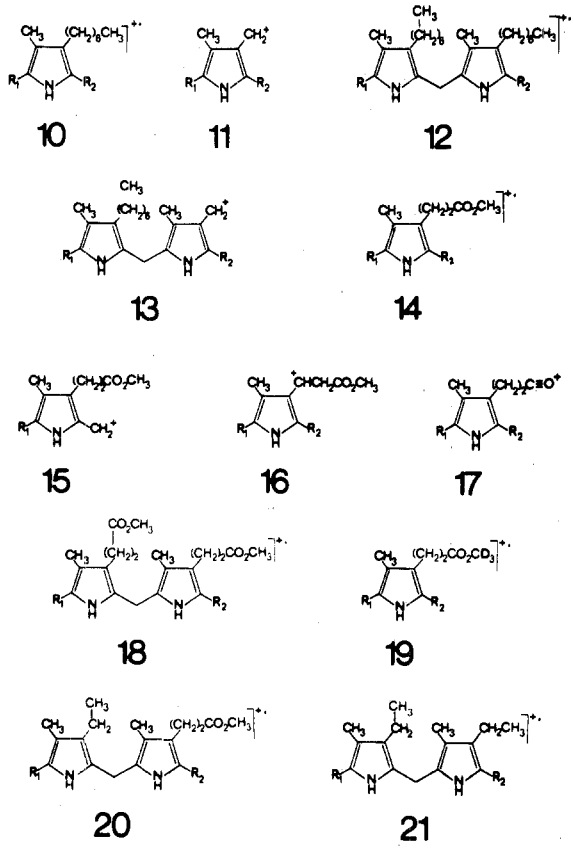
compd	fragment type	ions (intens, % rel to base peak)
tetraheptylporphyrin (1c)	tetrapyrrolic	764 (0.2), 758 (1.5), 730 (0.4)
	tripyrrolic	575 (0.05), 561 (0.05)
	dipyrrolic	398 (0.6), 397 (0.6), 384 (4.3), 383 (4.3), 370 (3.9), 369 (3.1), 355 (0.9), 341 (0.4), 313 (0.2), 299 (1.1), 285 (1.5)
	monopyrrolic	207 (17.8), 206 (24.5), 194 (37.0), 193 (55.5), 192 (68.6), 180 (22.1), 179 (24.9), 178 (34.8), 164 (11.2), 150 (4.7), 136 (4.8), 122 (32.8), 108 (100.0), 94 (69.0)
	other	83 (11.3), 82 (11.3), 69 (20.2), 57 (48.0), 56 (17.1), 55 (42.6)
coproporphyrin-IV TME (1d)	tetrapyrrolic	710 (0.1)
	tripyrrolic	
	dipyrrolic	374 (0.2), 373 (0.2), 360 (1.2), 359 (0.7), 346 (1.0), 329 (0.1), 315 (0.1), 287 (0.3), 273 (0.6), 259 (0.5)
	monopyrrolic	195 (11.7), 194 (9.4), 181 (27.8), 180 (22.6), 167 (11.2), 166 (7.9), 150 (7.9), 136 (7.5), 122 (31.8), 108 (100.0), 94 (65.8)
	other	57 (15.1), 55 (20.1)
mesoporphyrin-II DME (1e)	tetrapyrrolic	600 (0.1), 595 (0.4), 594 (0.4)
	tripyrrolic	481 (0.05), 423 (0.1), 422 (0.1), 409 (0.1)
	dipyrrolic	316 (0.4), 315 (0.2), 302 (2.4), 301 (1.2), 288 (2.2), 287 (1.2), 273 (0.5), 271 (0.4), 257 (0.5), 244 (0.7), 230 (0.9), 229 (0.9), 215 (1.1), 201 (1.0)
	monopyrrolic	195 (9.8), 194 (7.7), 181 (21.5), 180 (17.8), 167 (7.5), 166 (5.7), 164 (2.3), 150 (7.4), 137 (15.9), 136 (20.8), 123 (44.1), 122 (69.2), 109 (27.3), 108 (100.0), 96 (16.7), 94 (47.7)
	other	82 (12.0), 55 (14.5)
mesoporphyrin-IX DME (1a)	tetrapyrrolic	600 (0.4), 595 (0.4), 594 (0.5)
	tripyrrolic	481 (0.05), 480 (0.05), 467 (0.05), 423 (0.05), 422 (0.1), 409 (0.1), 408 (0.05)
	dipyrrolic	374 (0.1), 360 (0.6), 346 (0.3), 316 (0.4), 315 (0.3), 302 (1.7), 301 (1.0), 288 (1.3), 287 (1.0), 273 (0.8), 271 (0.4), 258 (0.8), 257 (0.8), 244 (2.7), 243 (1.9), 230 (2.6), 229 (2.0), 215 (0.9)
	monopyrrolic	195 (9.4), 194 (7.7), 181 (20.0), 180 (16.5), 167 (7.4), 166 (5.6), 164 (2.2), 150 (6.4), 137 (16.4), 136 (19.8), 123 (45.6), 122 (67.2), 109 (27.7), 108 (100.0), 96 (17.5), 94 (49.3)
	other	82 (12.2), 55 (10.9)
mesoporphyrin-IX DME (1a) -d_6	tetrapyrrolic	606 (0.1), 601 (0.1), 600 (0.3)
	tripyrrolic	487 (0.05)
	dipyrrolic	366 (0.2), 365 (0.05), 352 (0.1), 319 (0.1), 318 (0.1), 305 (0.6), 304 (0.4), 291 (0.5), 290 (0.3), 276 (0.3), 271 (0.2), 258 (0.5), 257 (0.4), 244 (1.7), 243 (1.0), 230 (1.7), 229 (1.2), 215 (0.6)
	monopyrrolic	198 (9.1), 197 (6.1), 184 (20.0), 183 (14.6), 170 (6.9), 169 (4.4), 164 (1.8), 150 (6.4), 137 (15.2), 136 (16.9), 123 (44.5), 122 (62.0), 109 (28.3), 108 (100.0), 96 (16.9), 94 (49.9)
	other	82 (12.0), 55 (10.0)
γ-phylloporphyrin XV (1f)	tetrapyrrolic	528 (1.4), 522 (1.0)
	tripyrrolic	409 (0.05), 395 (0.05), 351 (0.05)
	dipyrrolic	316 (0.4), 302 (2.5), 301 (0.6), 288 (7.6), 287 (2.8), 274 (6.3), 273 (3.5), 259 (2.9), 258 (2.5), 244 (10.3), 243 (4.9), 230 (12.5), 229 (6.6), 216 (6.4), 215 (5.7), 202 (7.9), 201 (6.6), 187 (5.7)
	monopyrrolic	209 (3.7), 208 (4.7), 195 (7.4), 194 (6.2), 181 (12.0), 180 (8.1), 167 (13.1), 150 (6.1), 137 (17.9), 136 (31.8), 123 (60.3), 122 (79.8), 109 (52.2), 108 (100.0), 94 (84.3)
	other	82 (15.6), 81 (20.6), 80 (19.9), 69 (11.3), 67 (15.3), 57 (11.9), 57 (11.9), 55 (25.4), 53 (10.6)

[a] All major peaks over 10% of base peak, except those due entirely to ^{13}C-containing ions, have been included. Additionally, all principal ions in the upper mass region which are significant in terms of fragmentation assignments are tabulated. Full names for the compounds are given in the text.

1b) (Figure 1) is essentially identical with that of octaethylporphyrin, allowing for a shift of 14, 28, 42, and 56 amu in the mono-, di-, tri-, and tetrapyrrolic regions, respectively. It seems reasonable, therefore, to deduce that the fragmentation pathways of all alkylporphyrins are similar.

The H_2 CI mass spectrum of 2,7,12,17-tetra-*n*-heptyl-3,8,13,18-tetramethyl-21*H*,23*H*-porphine (tetraheptylporphyrin, **1c**) (Table I) similarly confirms the fragmentation pathways described above. Production of primary monopyrrolic ions (**10**) at *m/e* 179, 193, and 207 is followed by benzylic cleavage to give the secondary fragments (**11**). Ions at intermediate masses are observed, due to cleavage at various positions along the alkyl side chain, but these are of low intensity with respect to the preferred mechanism. Similar processes are observed in the dipyrrolic region, leading to primary fragments (**12**) at *m/e* 370 and 384 and secondary ions (**13**) at *m/e* 285 and 299 due to benzylic cleavage.

The H_2 CI mass spectrum of 3,8,12,17-tetramethyl-21*H*,23*H*-porphine-2,7,13,18-tetrapropanoic acid tetramethyl ester (coproporphyrin-IV TME, **1d**) (Table I) presents a further relatively simple case for structural assignment. The major ions at *m/e* 167, 181, and 195 correspond to the formation of the three monopyrrolic fragments (**14**), again by attack at the meso positions yielding products containing 0, 1, or 2 meso carbon atoms, respectively, in an approximate 1:2:1 ratio. Secondary fragmentation of these ions by benzylic-type cleavage, losing 73 mass units, occurs to give the fragments (**11**), at *m/e* 94, 108, and 122, in a similar ratio. Additional ions are also observed at *m/e* 166, 180, and 194, which may have structure **15**, due to ions produced directly



from meso cleavage, or conceivably **16** by benzylic loss of hydrogen from the primary monopyrrolic fragments. Further side chain fragmentations are also possible, such as loss of the methoxy group which is a fragmentation generally characteristic of methyl esters, to give minor ions (**17**) at m/e 136, 150, and 164. In the dipyrrolic region of the mass spectrum of coproporphyrin-IV TME analogous ions may be observed, but of considerably lower intensity. The general structure of the primary dipyrrolic ions (**18**) would be consistent with the proposed mechanisms yielding ions at m/e 346, 360, and 374. Ions at 1 and 31 mass units lower are again found. The major secondary fragments, however, appeared at 87 mass units less than the primary dipyrrolic ions. It is conceivable that this might be due to the loss of the complete propanoic acid ester group, but this is unlikely to be a more favourable process than benzylic cleavage to lose 73 mass units. In this case, further studies, using metastable peaks, isotopic labeling, or accurate mass measurement, are necessary to determine the fragmentation processes involved. There are no peaks corresponding to tripyrrolic fragments in the mass spectrum of coproporphyrin-IV TME and analogously the intensity of the dipyrrolic fragments is significantly smaller than in the spectra of the alkylporphyrins. This appears to be a reflection of the instability of pyrrane structures containing functionalized side chains. Indeed, the monopyrrolic fragments show a similar trend, the ratio of primary to secondary ions being much lower than for the alkylporphyrins.

A_2B_2-Type Porphyrins and the Identification of Isomeric Differences. 7,17-Diethyl-3,8,12,18-tetramethyl-21*H*,23*H*-porphine-2,12-dipropanoic acid dimethyl ester (mesoporphyrin-II DME, **1e**) and mesoporphyrin-IX DME (**1a**) are isomeric compounds both containing two pyrrole rings having methyl and ethyl substituents and two rings having methyl and propanoic acid methyl ester substituents in the porphyrin macrocycle. For convenience, these will be called a-type and b-type rings, respectively. The isomers differ in the arrangement of these pyrrole rings around the macrocycle. Mesoporphyrin-II DME has a structure with the two a-type rings diagonally opposed (abab structure) whereas mesoporphyrin-IX DME has the two rings adjacent (aabb structure). This, however, has no effect on the relative abundances of the primary and secondary monopyrrolic ions, the H_2 CI mass spectrum of the two compounds (Table I) being identical below m/e 200. The ions formed are a mixture of those described for aetioporphyrin-I and coproporphyrin-IX TME whose molecular structures contain only four a-type and four b-type rings, respectively.

The formation of b-type monopyrrolic fragments and their secondary fragmentations, outlined for coproporphyrin-IX TME above, are clarified by labeling each methyl ester group of mesoporphyrin-IX DME with three deuterium atoms. A shift of 3 mass units is only observed for the primary fragments to give ions at m/e 170, 184, and 198 (**19**), indicating that secondary fragmentation always involves loss of label, by acyl cleavage losing $\cdot OCD_3$ or benzylic cleavage losing $\cdot CH_2CO_2CD_3$.

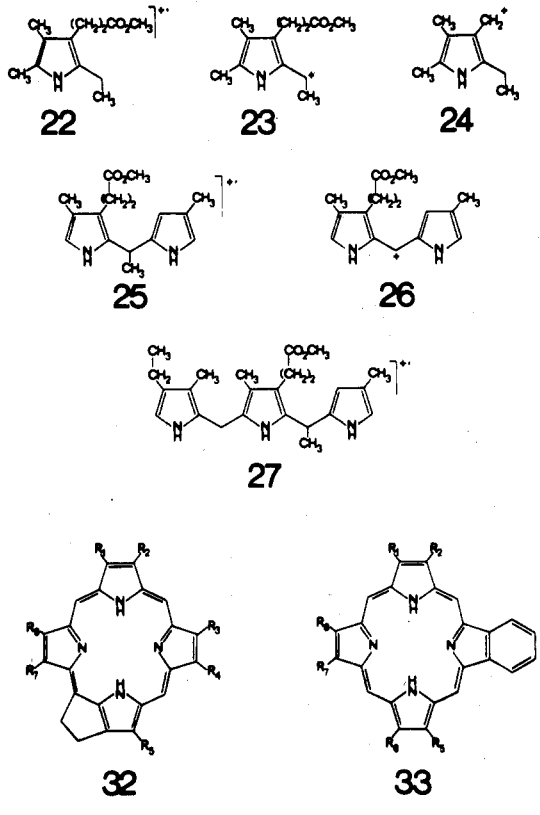
The isomeric difference is reflected, though, in the dipyrrolic fragments observed for the respective compounds. Thus while mesoporphyrin-II DME only has significant peaks at m/e 288 and 302 (**20**), as each dipyrrolic fragment can only be composed of a combination of a- and b-type pyrrole rings, mesoporphyrin-IX DME has additional peaks at m/e 230 and 244 (**21**) due to an a_2 combination and m/e 346, 360, and 374 (**18**) from a b_2 combination. The ions at m/e 230 and 244 which appear in the former spectrum and could correspond to an a_2 combination are of considerably reduced intensity and can be attributed to fragmentations from the propanoic side chains. These assignments are confirmed by labeling the methyl ester group. The mass spectrum then shows a shift of 3 mass units in the primary ab-type dipyrrolic fragments and 6 mass units in the primary b_2-type fragments. Thus, while it is known that the pyrrole rings of porphyrinogens rearrange under acid conditions, yielding all-isomer mixtures (*6*), this process cannot occur in the H_2 CIMS of porphyrins. Recombination of the monopyrrolic fragments to give random dipyrrolic ions can similarly be discounted. The absence of these characteristics is essential for the method to be useful in the structural differentiation of certain isomeric pairs.

The relative abundances of the dipyrrolic fragments in the spectrum of mesoporphyrin-IX DME can be seen to reflect the same trends as observed in the mass spectrum of coproporphyrin-IV TME, that is, the tendency for fragments containing extended side chains to be less stable than those with simple alkylation patterns. Thus it is observed that a_2-type dipyrrolic fragments are more abundant than the ab combination which in turn are more intense than the b_2 combination, even allowing for the effects of secondary fragmentation.

In the tripyrrolic region of the mass spectra, we again find no differences between the isomers as both structures are capable of degrading to give a_2b- and ab_2-type tripyrrole configurations.

Retention of Meso Substituent Information. The H_2 CI mass spectral fragmentations of 8,13-diethyl-3,7,12,17,20-pentamethyl-21*H*,23*H*-porphine-2-propanoic acid methyl ester (γ-phylloporphyrin-XV, **1f**) (Table I) are of great importance as the structure of the latter includes a methyl meso substituent. As such, it is possible to investigate the influence of this group on the fragmentation pathways and, if so, to see whether this can be used to infer the presence or absence of such substituents in structurally undetermined porphyrins. In addition, the compound presents a more difficult problem for mass spectral fragmentation analysis, having a more complex structure than the porphyrins described so far. Due to this complexity, though a description of every ion observed in the mass spectrum is possible, only the more important fragmentations, especially those concerning the meso substituent, will be discussed. Thus, in the monopyrrolic region

of the mass spectrum, ions are observed at masses expected for the fragmentation processes outlined above. This includes two ions, at m/e 208 and 209, which can only correspond to the structures **22** and **23** which retain the meso methyl substituent. In addition, the ion due to benzylic cleavage of **22** at m/e 136 (**24**) is of much greater abundance than might be expected in the absence of the meso methyl group. The dipyrrolic region of the spectrum, being that region where most structural information is available, is extremely complex. However, it is possible to separate the ions observed into groups corresponding to the various combinations of two pyrrolic units. From this it is possible to determine that the ion at m/e 274 can only be rationalized on the basis of structure **25**, which contains the meso methyl substituent. A further noticeable ion is that at m/e 259, which may be due to loss of this methyl substituent, by benzylic cleavage (**26**). In the tripyrrolic region of the spectrum only three trace ions are observed, at m/e 351, 395, and 409. Again, the ion at m/e 395 could only be rationalized on the basis of structure **27**, which once more includes the meso methyl group.



Applications in the Analysis of Different Porphyrin Structural Types. Porphyrins may be divided into five separate structural types: A_4, A_3B, A_2B_2, A_2BC, and ABCD, depending on the nature of their β substituents. In the H_2 CIMS method for any structural type, the mono- and tripyrrolic fragments should be independent of any specific arrangement of pyrrole rings about the macrocycle, as they inevitably represent ions due to the presence or absence of individual rings. The dipyrrolic fragments, however, will give direct evidence of the presence of adjacent pairs of pyrrole rings. The data summarized in Table II show that for all structural types any specific arrangement of pyrrole rings about the macrocycle should be identifiable. It is important to note here that the structural information available does not extend to the substitution pattern on each individual pyrrole ring as the fragmentation pathways involved lead only to intact pyrrole units. It is highly unlikely, then, that this method will be capable of positively identifying type isomers, i.e., those which differ in the same manner as aetioporphyrin-I (**1b**) and

Table II. Determination of Structural Information from the Dipyrrolic Fragments of Porphyrins in H_2 CIMS

structural type		possible dipyrrolic fragments
general	specific[a]	
A_4	AAAA	A_2
A_3B	AAAB	A_2, AB
A_2B_2	AABB	A_2, AB, B_2
	ABAB	AB
A_2BC	AABC	A_2, AB, AC, BC
	ABAC	AB, AC
ABCD	ABCD	AB, BC, CD, AD
	ACBD	AC, BC, BD, AD
	ABDC	AB, BD, CD, AC

[a] This refers to the specific sequence of pyrrole rings about the porphyrin macrocycle.

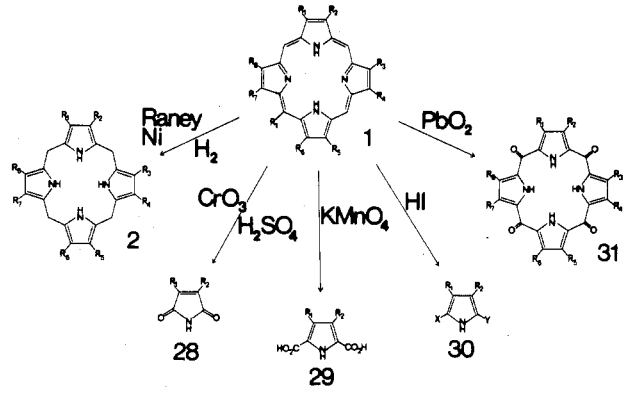


Figure 3. Methods for the partial structural analysis of porphyrins by degradative and chemical alteration techniques. For structure **1**. (a) $R_1 = R_3 = R_5 = R_8 = CH_3$, $R_2 = R_4 = C_2H_5$, $R_6 = R_7 = (CH_2)_2CO_2CH_3$, $R_\gamma = H$; (b) $R_1 = R_3 = R_5 = R_7 = CH_3$, $R_2 = R_4 = R_6 = R_8 = C_2H_5$, $R_\gamma = H$; (c) $R_1 = R_3 = R_5 = R_7 = CH_3$, $R_2 = R_4 = R_6 = R_8 = n\text{-}C_7H_{15}$, $R_\gamma = H$; (d) $R_1 = R_4 = R_6 = R_7 = CH_3$, $R_2 = R_3 = R_5 = R_8 = (CH_2)_2CO_2CH_3$, $R_\gamma = H$; (e) $R_1 = R_3 = R_5 = R_7 = CH_3$, $R_4 = R_8 = C_2H_5$, $R_2 = R_6 = (CH_2)_2CO_2CH_3$, $R_\gamma = H$; (f) $R_1 = R_3 = R_5 = R_8 = R_\gamma = CH_3$, $R_2 = R_4 = C_2H_5$, $R_7 = (CH_2)_2CO_2CH_3$, $R_6 = H$; (g) $R_1 = R_3 = R_5 = R_8 = CH_3$, $R_2 = R_4 = R_6 = R_7 = C_2H_5$, $R_\gamma = H$. For structure 30, X, Y = H, CH_3.

aetioporphyrin-III (2,7,12,18-tetraethyl-3,8,13,17-tetramethyl-21*H*,23*H*-porphine, **1g**), whose structures are different only by the interchange of the methyl and ethyl substituents at the 17 and 18 positions in the fourth pyrrole ring. The presence of meso substituents will, of course, complicate further the problems of porphyrin structural analysis by the H_2 CIMS method by increasing the probability of the formation of isomeric dipyrrolic fragments.

The limitations of the H_2 CIMS method outlined above fortunately do not accurately reflect the situation in practice. As biosynthetic pathways are enzymatically controlled, the number of isomeric possibilities for any given tetrapyrrole can be significantly reduced. This, in turn, renders any information regarding meso substituents more accessible. In addition, these limitations do not detract from the ability of the H_2 CIMS method to give detailed structural information on the individual pyrrole rings present.

Comparison of Methods. Of the physical methods for the structural analysis of porphyrins, most, such as X-ray crystallography (*8*), the method of choice at present for the exact structures it provides, require much larger, purer samples which are, in general, unnecessary for mass spectrometric techniques. Of these techniques EIMS of porphyrins only provides very limited structural information (*9*), so attempts have been made, either by degradation or chemical alteration, to obtain compounds which might yield more useful data (Figure 3). These methods are summarized in Table III.

Table III. Comparison of Degradative and Chemical Alteration Techniques for the Structural Analysis of Porphyrins

method	quality[a]	product	analytical method	product		information			comment
				stable[b]	unstable[b]	meso	β[c]	di- and tripyrroles	
CrO_3 oxi.	100 μg	pyrrole-2,5-diones	GC-MS	√		-	√	-	
HI/CH_3CO_2H red.	1 mg	pyrrole	GC-MS		√	√	√	-	the pyrroles can be stabilized by alkylation with HCHO/HI
$KMnO_4$ oxi.	1 mg	pyrrole-2,5-dicarboxylic acid	PC[d]		√	-	√	-	
PbO_2 oxi.	1 mg	5,10,15,20-tetraoxoporphyrinogens	EIMS	√		-	√	√	Tri- and tetrapyrrolic species are produced
Ni/H_2 red.	1 mg	porphyrinogens	EIMS		√	√	√	√	similar data to H_2/CIMS
H_2/CIMS	1 μg		CIMS	-	-	√	√	√	

[a] Quantity required for one compound. [b] Stability of the products toward oxygen and acid. [c] β = β substituent. [d] PC = Paper chromatography.

Degradative techniques, which convert the macrocycle into monopyrrolic units, by oxidation to maleimides (pyrrole-2,5-diones) (**28**) (*10–12*) or pyrrole-2,5-dicarboxylic acids (**29**) (*13*) or by reduction to pyrroles (**30**) (*14*), inevitably lose all information concerning the relative position of each pyrrole ring identified in the porphyrin macrocycle which is obtainable from dipyrrolic units.

The first two methods additionally lose information on meso substituents. All these techniques are also affected by product instability and purity, which are major factors in microscale reactions. Chemical alteration, by reduction to porphyrinogens (**2**) (*3*) or oxidation to *meso*-tetraoxoporphyrinogens (**31**) (*15*), has proved more successful analytically, giving further information under EI mass spectrometry via fragments containing one, two, or three pyrrole rings. However, the former compounds are notoriously unstable (*6*) and the latter reaction gives side products and, once more, removes information on the meso substituents. In comparison with all these methods, H_2 CIMS yields detailed structural data, including information regarding meso substituents. The method is also clean, using only the parent porphyrins themselves, thereby requiring no prior chemical treatment and avoiding any problems with side reactions or product instability.

From the comparisons presented above it would seem, therefore, that the H_2 CIMS method is the most useful yet obtainable though at present it is restricted to single compounds. For mixtures of porphyrins simple EI mass spectrometry combined with degradation to maleimides (*10*) would probably remain the methods of choice for obtaining the maximum structural information. However, if developments in the gas chromatography of porphyrins and their derivatives continue (*16*), the prospect of GC-CIMS of porphyrin mixtures may also be possible, potentially giving a full structural analysis of mixtures of petroporphyrins, for example.

Meanwhile, for single components the potential for the structural analysis of tetrapyrroles by H_2 CIMS lies mainly in the biosynthetic and geochemical fields. At a very basic level it may be used in the former to trace isotopic labels. More importantly, given the accessibility to meso substituent data which enzymatic specificity allows, the method may be employed in studies such as those into the biosynthesis of vitamin B_{12} (*17*) or chlorobium chlorophylls (*18*). There also would seem to be no reason why the method could not be applied to slightly different compound classes, like the open-chain tetrapyrroles, to study bilanes or bile pigments (*19*).

In organic geochemistry the structural analysis of petroporphyrins isolated by preparative HPLC should be of immediate relevance. The retention of the isocyclic ring of DPEP porphyrins (**32**) on fragmentation would be of particular importance for certain of these preparative fractions (*20*). Similarly, useful data may be obtained on rhodoporphyrins (**33**) (*21*) whose structure is, as yet, uncertain. Studies are in progress along these lines.

ACKNOWLEDGMENT

Mesoporphyrin-II DME and mesoporphyrin-IX DME were the gift of E. Macdonald, University of Cambridge, England. Coproporphyrin-IV TME, γ-phylloporphyrin-XV, and mesoporphyrin-IX diacid were the gift of T. W. Griffiths, Department of Biochemistry, University of Bristol, England. Aetioporphyrin-I, tetraheptylporphyrin, and octaethylporphyrin were the gifts of K. M. Smith, University of California, Davis, and the late G. W. Kenner, University of Liverpool, England.

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RECEIVED for review January 21, 1981. Accepted July 21, 1981. This work was supported by the Natural Environment Research Council (Grant No. GR3/2951 and GR3/3758) for instrumentation. We thank the Science Research Council for a studentship (G.J.S.) and the National Aeronautics and Space Administration for support through a subcontract from the University of California at Berkeley (Contract No. NGL 05-003-003).

Speciation at Trace Levels by Helium Microwave-Induced Plasma Emission Spectrometry

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An evolved gas analysis/emission spectrometer system capable of identifying inorganic compounds at trace levels in solid samples, especially environmental particulates, is described. As a sample is heated from 25 to 1000 °C at 140 °C/min, its components vaporize at characteristic temperatures into an atmospheric pressure, helium, microwave-induced plasma, which acts as an atomic emission source by which the chemical composition of the evolved vapors is determined. Unique excitation conditions in He allow trace amounts of both metals and nonmetals to be determined. Identification of a given compound is based on the coincident observation of its metal and nonmetal components at its temperature of vaporization. This coincidence exists for the pure halide, sulfide, and sulfate salts of Cd, Hg, Pb, and Zn. A number of obstacles may hamper identification, most importantly, chemical reactions in the sample which alter the identity of compounds before vaporization.

"Chemical form" is becoming recognized as an important concept in evaluating the environmental impact of inorganic pollutants. At present, analytical chemistry falls short of the challenge of compound identification in solids because the elements typically of interest to environmental chemists, namely, toxic metals such as Cd, Hg, and Pb, exist at concentrations too small for established methods of speciation, such as X-ray diffraction and infrared spectrometry, to be applicable. Consequently, the development of new methods capable of distinguishing the forms of trace-level metals in the solid state is highly desirable.

One approach that shows significant promise is evolved gas analysis (EGA)—a thermal analytical method which monitors gases released from a sample as a function of temperature. Of the several types of analyzers used for EGA, the mass spectrometer has become the most important. Recent applications of EGA/MS have included minerals (*1*, *2*), oil shales (*3*), polymers (*4*), and lunar soils (*3*). Environmental studies using evolved gas analysis are few (*5*). High-resolution mass spectrometry, used primarily to study organic compounds in urban airborne particulates (*6*), identified several inorganic species as well, namely, H_2SO_4, NH_4HSO_4, $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, $NaHSO_4$, $NaNO_3$, S_8, As_4O_6, Cd, a Zn compound, and I_2. In settled urban dust, evolved gas analysis using conductivity detectors for evolved CO_2 and SO_2 showed the presence of organic carbon, inorganic carbonates, and inorganic sulfates (*7*, *8*).

Despite the obvious cost advantages of atomic spectrometry over mass spectrometry as a selective detector, the former has barely been investigated. Geochemists were first to recognize that speciation of metal compounds by EGA/atomic spectrometry may be possible and profitable. The natural association of Hg with lead and zinc sulfides allows low-level mercury to be used as a tracer for locating potential areas of lead and zinc sulfide mineralization. By means of EGA, $HgCl_2$ was distinguished clearly from HgS by the difference in the Hg vaporization temperature (*9*, *10*). Since only HgS was indicative of sulfide ore deposits, EGA was superior to total Hg determinations as a prospecting tool. Less detailed studies of Cd and Pb minerals by EGA have also been reported (*11*). Hanamura (*12*) vaporized pure arsenic oxides, mercury chlorides, and Si and SiO_2 into an Ar microwave-induced plasma to demonstrate that the different species did indeed volatilize at different temperatures and that speciation based on this phenomenon was feasible. Robinson and Rhodes (*13*) gave a similar prognosis by observing Pb and Cd compound

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0003-2700/81/0353-2020$01.25/0