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ENHANCED STRUCTURE DETERMINATION OF BETA-PYRROLE AND N-SUBSTITUTED PORPHYRINS BY DESORPTION CHEMICAL IONIZATION MASS SPECTROMETRY.[1]

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Summary: The extensive macrocycle—but minimal substituent—fragmentation occurring in the chemical ionization mass spectra of porphyrins when ammonia, rather than hydrogen, is the reagent gas leads to more detailed structure determinations.

Conventional electron-impact mass spectrometry (EIMS) has proved to be of relatively little value for the structural elucidation of porphyrins for two reasons.[2] First, the macrocycle does not cleave to any appreciable extent; thereby, necessarily, precluding gaining any information about the substituents attached to particular pyrrole rings. Second, while some diagnostic substituent cleavages do occur (most notably, loss of $\cdot CH_2CO_2R$ from propionic ester groups) other important groups (e.g., vinyl and formyl) are silent under EIMS conditions.

Although the advent of GC/MS techniques encouraged the return to classical oxidative and reductive degradation methods as a means of identifying some of the particular individual pyrrole ring substituents,[3] a more insightful approach was taken by Budzikiewicz, et al.[4] in their observation that the hexahydro derivatives (porphyrinogens) underwent extensive cleavage under EI conditions to produce mono-, di-, and tripyrrolic fragments. Subsequently, Shaw, et al.[5] in their study of alkyl substituted porphyrins obtained similar results via the porphyrinogens produced in situ in the mass spectrometer under H_2 CI conditions, thereby avoiding the need to produce, handle, or isolate the fragile porphyrinogens themselves.

We attempted to extend the H_2 CI method via the desorption chemical ionization technique (D/CI) to porphyrins with vinyl, formyl, acyl, ethoxycarbonyl, cyano, and propionic ester substituents which have arisen during the course of other studies but found that their H_2 D/CIMS were sufficiently cluttered with abundant secondary cleavage (benzylic) fragments as to make structural elucidation of the porphyrins difficult.

Preliminary work indicates that much better results can be obtained through the use of the milder reagent gas ammonia since as shown (Figure 1) in the NH_3 D/CIMS of 4-acetyldeuteroporphyrin IX DME (1) extensive cleavage of the macrocycle occurs but without the excessive formation of secondary cleavage ions.

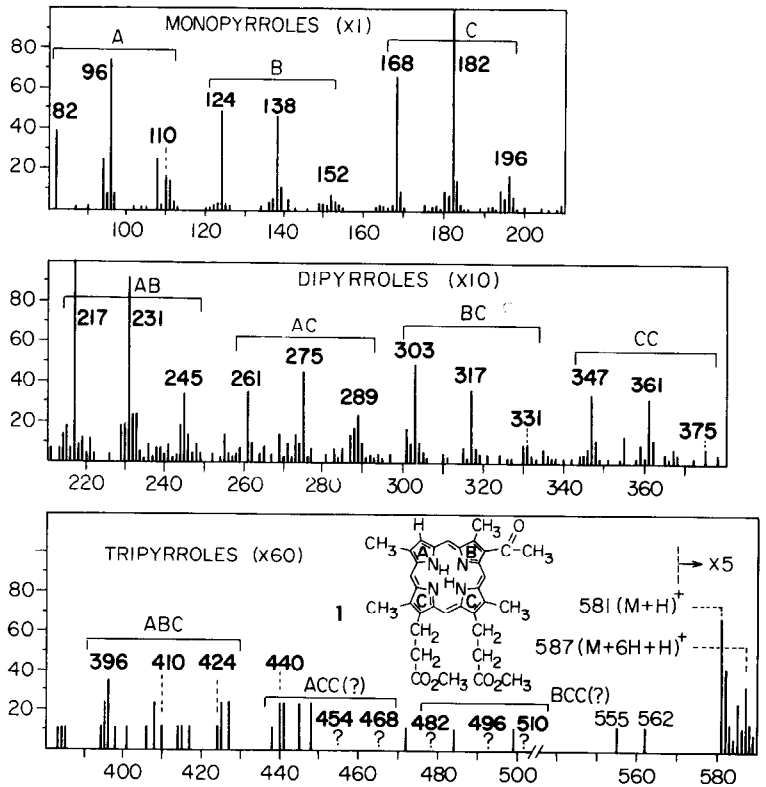


Figure 1. (above) NH_3 D/CIMS of 4-acetyldeuteroporphyrin IX DME. See ref. 7 for conditions.

Figure 3. (right) NH_3 D/CIMS of N-ethoxycarbonylmethyloctaethylporphyrin in the monopyrrolic mass region.

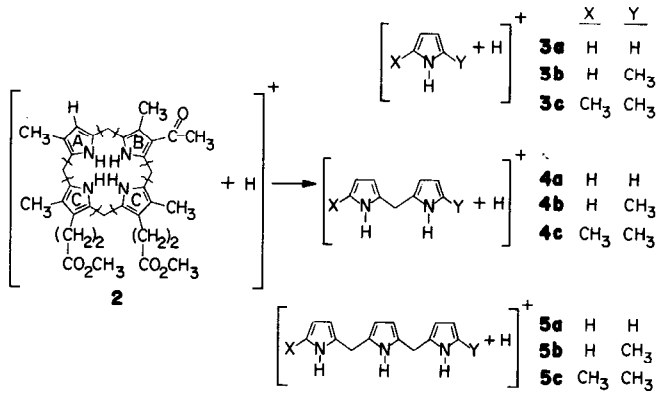
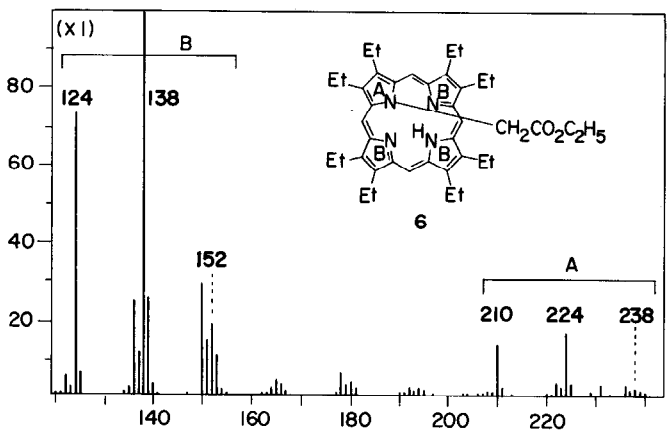


Figure 2. Proposed fragmentation pathway in the NH_3 D/CIMS of porphyrins.



A mechanism (Figure 2) which accounts for the more abundant fragment ions in the NH_3 D/CIMS of 1 involves initial attack on the porphyrin by the reagent gas to form the protonated porphyrin molecular ion (m/z 581) and the protonated porphyrinogen molecular ion (2, m/z 587). Further nonspecific attack by the reagent gas at the meso positions of the unstable porphyrinogen ion (2) results in cleavage of the macrocycle and the formation of protonated mono- (3), di- (4), and tripyrrolic (5) fragments, each of which may contain 0, 1, or 2 meso carbon units.

Ring I of the acetylporphyrin (1) contains the methyl, hydrogen substituent pair and yields the triad of protonated monopyrrolic fragments (3a,b,c) labeled A in Figure 1 at m/z 82, 96 and 110. The triad labeled B (m/z 124, 138 and 152) arises from the methyl, acetyl substituent combination on ring II and the triad labeled C derives from both of the identically substituted rings III and IV of 1. The other ions present in the monopyrrolic mass region are generally in much lower abundance indicating that other pathways not involving protonated species (e.g., odd-electron species) and secondary cleavages from them are of minor importance. This retention of the structural integrity of the pyrrole substituents is an important feature of NH_3 D/CIMS since it greatly facilitates ready determination of the substituents on each pyrrole ring of the porphyrin via tabulation of the masses of pyrrole fragments 3a,b,c with the common or expected beta-pyrrole substituents.

The dipyrrolic mass region of the NH_3 D/CIMS provides definitive information about the sequence of the differently substituted rings about the porphyrin macrocycle. Without regard to isomeric structures due to interchange of the pairs of substituents on a particular pyrrole ring, porphyrins can be classified according to the number of differently substituted pyrrole rings (A_4, A_3B, A_2B_2, ABC_2, and ABCD) which they may contain. The acetylporphyrin (1) is of the ABC_2 type and has its rings in the specific sequence ABCC. Thus, it can (and does, Figure 1) give rise to the four sets of protonated dipyrrolic fragment triads (4a,b,c) labeled AB, AC, BC, and CC. Notice that had the three different pyrrole rings been in the specific order ACBC, then only two sets of triads (AC and BC) would have been observed. In the tripyrrolic mass region the protonated fragment ions appear in very low abundance and contain no unique structural information not otherwise contained in the mono- and dipyrrolic mass regions.

The NH_3 D/CIMS of N-ethoxycarbonylmethyloctaethylporphyrin (6) (Figure 3, monopyrrolic mass region only) presages an important application of this MS technique for drug metabolism studies since a number of substances containing terminal olefinic or acetylenic groups interact with cytochrome P-450 by a suicidal process to produce N-substituted derivatives of heme. The position alkylated depends both on the particular agent and on the form of P-450;[6] however, at present such information, indicative of the topologies of the active sites, can be obtained only via NMR which necessitates the use of substantial quantities of material. In Figure 3, the triad (B) arising from the rings without the N-substituent is found at m/z 124, 138 and 152; whereas the triad from the ring (A) with the $N\text{-}CH_2CO_2C_2H_5$ group is at m/z 210, 224, and 238. Thus, the structural integrity of this relatively fragile N-substituent is also retained under NH_3 D/CI conditions. In the biological experiment, HPLC of only a small amount

of liver microsomal P-450 extract would be required in order to determine by the presence of a new triad of fragments whether alkylation (e.g.,[6] R = $-CH_2CH(OH)CH_3$ or R = CH_2COCH_3) had occurred on rings I and/or II or III and/or IV of protoporphyrin IX.

Although further work will be required on porphyrins containing vinyl, formyl, nuclear ethoxycarbonyl, cyano, and other acyl substituents in order to properly judge the limits of the method, the preliminary spectra presented here indicate that NH_3 D/CIMS holds high potential as a means of elucidating all aspects of porphyrin structures except for the specific positional isomerism of the groups on each individual pyrrole ring.

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7. Conditions: Ribermag R10-10B quadrupole mass spectrometer; source temperature 200°; emission current, 0.2 mA; electron energy, 70 eV; NH_3 (99.998%) pressure, 0.4 T; current programming through the D/CI coil, 7 mA s^{-1} from 40 to 500 mA.

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