MASS SPECTROMETRY DIFFERENTIATION OF ISOMERIC ALKYL PORPHYRINS. FRAGMENTATION ANALYSIS OF *MESO*-TETRAOXOPORPHYRINOGENS

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Abstract—The mass spectra of lead dioxide oxidation products of three etiotype porphyrins and one *meso*-substituted etiotype porphyrin have been analyzed. The *meso*-tetraoxo products formed, fragment upon electron-impact to give mono-, di-, and tri-pyrrole fragments with the pyrrole substituents still intact. From this fragment information it is possible to differentiate many isomeric alkyl porphyrins. Due to the inability to assign exact positions to the substituents on each pyrrole unit, however, definite isomeric identification is impossible without further information. High resolution gas chromatography retension data of volatile (trimethylsiloxy) Si^{IV} derivatives may eventually provide the necessary additional information to make absolute structural assignment possible.

The described method has been designed to prove detailed structural information about geoporphyrin materials. Geochemical conclusions from such data should help provide information about the origin of organic materials and thus help establish possible connections with early life processes.

INTRODUCTION

RECENT advances in the synthesis of volatile porphyrin derivatives have led to compounds that can be gas chromatographed at moderate conditions.¹ This technique has been extended to the micro-analysis of alkylporphyrin materials found in ancient geological organic residues.² In order to study the structure of isolated geoporphyrins, new micromethods have been developed.

Analysis of alkylporphyrins by mass spectrometry provides only general structural information such as molecular weight, equivalent unsaturation and element composition. Since the predominant fragmentations occur in the β positions of pyrrole alkyl substituents (Fig. 1), information regarding the types and number of substituents on the macrocyclic ring can be obtained. For instance, the mass spectrum of etioporphyrin (I) Fig. 1a with four ethyl substituents shows a moderate $[M - 15]^+$ ion, m/e 463 whereas the hydrogenated deuteroporphyrin spectrum Fig. 1b contains propyl substituents that lead to a moderate β cleavage ion at $[M - 29]^+$, m/e 421. The intensity of the β fragmentation ions is proportional to the number of alkyl substituents leading to that fragment. Table 1 contains relative intensity data for the mass spectrometric analysis of several alkyl porphyrins containing methyl and ethyl substituents. Compound I with two ethyl groups gives an $[M - 15]^+/[M]^+$ intensity of 22.5%, whereas compound IV with four ethyl substituents gives a β fragment/ molecular ion ratio of 27.0%. The relative intensities are very sensitive to probe and source temperature, ionization potential and acceleration potential. Thus, in order to make intensity comparisons, standards and unknowns must be run under identical conditions. Any change in the basic structure, such as meso-substitution (V), renders comparison data with other structures, i.e. etiotype (I to IV), impossible.



Further information regarding actual substituent position on the porphyrin ring periphery can only be determined through selective cleavage of the aromatic macrocyclic portion. Several available techniques that can effect ring cleavage involve pyrolysis gas chromatography³ and oxidative or reductive cleavage.⁴ Each of these techniques has the disadvantage of the formation of complex reaction products and

Table 1. Ratio of $[M - 15]^+$ to $[M]^{+*}$ in the spectra of alkylporphyrins (I to VI)



Compound	Mol. wt.	1	2	3	4	5	6	7	8	δ	$\frac{[M-15]^+(\%)}{[M]^+}$
(I)	(450)	Et	Me	Me	Me	Me	Me	Et	Me	_	22.5
(II)	(464)	Me	Et	Me	Me	Me	Et	Me	Et		25.0
(III)	(478)	Me	Et	Me	Et	Me	Et	Me	Et	— -	26.0
(IV)	(478)	Me	Me	Me	Et	Et	Et	Me	Et	_	27.0
(V)	(464)	Et	Me	Me	Me	Me	Me	Et	Me	Me	16.5
(VI)	(450)	Me	Et	Me	Et	Me	H	Me	Et	-	no data

* $[M]^+$ = Molecular ion; all spectra were run on an MS-9 instrument (70 eV, 8 kV accelerating voltage, 100 μ a emission, ion chamber 250°C using direct insertion probe).

low yields making interpretation of the results difficult, especially when working in the micro-region. Further, the monopyrrole fragments formed are not useful for the determination of substituent positions around the macrocycle periphery.

Meso-tetraoxoporphyrinogens:

Inhoffen and co-workers have recently investigated the mass spectrum of a mesotetraoxo-octaethylporphyrinogen.⁵ Instead of the expected β fragmentations of ethyl substituents, tri-, di- and mono-pyrrole fragments were observed with alkyl pyrrole substituents still intact. Following this lead, we have examined by mass spectrometry the meso-tetraoxoderivatives of several alkyl porphyrins in order to determine the use of fragmentation data in alkyl group position assignment.

Using the procedure described by Inhoffen *et al.*,⁶ the *meso*-tetraoxoderivatives of compounds III, IV and VI (Table 1) were synthesized. The mass spectrum of the symmetrical *meso*-tetraoxoetiporphyrinogen (I, Mol. wt. 540) is shown in Fig. 2a. A small amount of a further oxidized product containing one more oxygen (Mol. wt. 556) was also observed. More volatile than the related tetraoxoderivative, this



compound could be separated via temperature programmed insertion probe. The suggested structure (VII) and mass spectrum are shown in Fig. 2b.

Comparison of the two spectra show that the *meso*-tetraoxoderivatives lead to intense dipyrrole and monopyrrole fragments whereas the proposed pentaoxoderivative (VII) leads predominantly to tri- and monopyrrole fragments. High resolution data obtained is in agreement with the following proposed fragmentation scheme.



All meso-tetraoxoporphyrinogens and related pentaoxoderivatives should follow the same fragmentation scheme. Relative intensities of individual ions within the mono-, di- and tri-pyrrole groupings should be a function of the number of fragments produced of a particular molecular weight. In order to demonstrate this, compound VI, an unsymmetrical substituted porphyrin was converted to its meso-tetraoxoporphyrinogen derivative and analyzed by mass spectrometry (Fig. 3). The small amount of pentaoxoderivative formed (m/e 520) accounts for the lack of intense tri-pyrrole fragments. Background interference and the presence of slight impurities lead to inaccurate intensity ratios of pyrrole fragments. Despite the interference, however, much information can be gained. High resolution isolation of individual peaks and comparison of peak intensities would lead to more accurate comparisons.



Proposed fragments are given below:---



The fragments described are all the fragments that would be expected from random fragmentation of the *meso*-tetraoxoporphyrinogen. Intensity ratios of fragments in the mono-, di- and tri-pyrrole regions are shown below in Table 2.

Other less common fragments can be accounted for by proposing di- and tri-pyrrole units of the type:



where X = 0 or 1. All assignments are based on high resolution analysis of individual fragments.

Mol. wt.	Pred. Intens. Ratio	Observed Ratio
Tripyrrole units		
404)	1/3	1/2·2
376)	·	-
Dipyrrole units		
269)	1/1	1/1.08
241	·	
Monopyrrole units		
162)	3/1	2/1
134	•	

TABLE 2. INTENSITY RATIOS OF M.S. (FIG. 4)

Compound IV was converted to the *meso*-tetraoxoporphyrinogen derivative and analyzed by mass spectrometry. The spectra are shown in Figs. 4a and b. Fig. 4a is predominantly the tetraoxoderivative (m/e 540) whereas Fig. 4b is a combination of the m/e 540 component and m/e 556 (proposed pentaoxoderivative). Mono-, di and tri-pyrrole units and corresponding intensity ratios are given in Table 3.

The observed fragment ion intensity ratios within the mono-, di- and tri-pyrrole groupings are very close to the predicted values. Such results can be extremely useful in structural assignment. For instance, if Figs. 4a and b were spectra of an unknown sample, and low resolution information of the molecular alkyl porphyrin were recorded, the following could be concluded: Low resolution mass spectrometric analysis of compound before oxidation indicates no substituent greater than ethyl. The $[M - 15]^+/[M]^+$ intensity ratio (Table 1, compound IV) is 27%, suggesting the presence of 4 ethyl and thus 4 methyl substituents. Analysis of *meso*tetraoxo and



Fragment	Structural Assignment	Predicted Intens. Ratio	Actual Intens. Ratio
556 540	[M]+ [M]+		
446	O = O = O	1	0-94
432	$ \begin{array}{c} & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & $	2	2.0
418	$O \rightarrow O \rightarrow$	1	1.06
283	$\left(\begin{array}{c} & & & \\ & & & \\ & & & \\ & &$	1	1.0
255	$\left(\begin{array}{c} & & & \\ & & & & \\ & & & \\ & & & & \\$	1	1.03

TABLE	3.	FRAGMENTS	OF	THE	MASS	SPECTRA	OF	Meso-TETRAOXOPORPHYRINOGEN AND PENTAOX	0-
			DEF	RIVAT	ive (T	YPE VII)) OF	COMPOUND IV (TABLE 1)	

Fragment	Structural Assignments	Predicted Intens. Ratio	Actual Intens. Ratio
176		1	0.67
162		2	2.0
148		1	1.3

TABLE 3. (cont'd)

pentaoxoderivatives would limit the structures of this compound to three possibilities (a, b, c).



Further narrowing of structural possibilities would not be possible without the development of high resolution gas chromatographic separations that are able to effect the isolation of individual geometrical isomers. Toward this result g.c. studies using volatile bis (trimethylsiloxy) Si^{IV} derivatives are being improved.²

The lead dioxide oxidation products of compound V (Table 1) were investigated. The reaction products of this *meso*-substituted methyl porphyrin are composed of three major components, of which two can be accounted for by loss of the *meso*-methyl, followed by oxidation to the *meso*-tetraoxo and pentaoxoderivatives. These compounds fragment in the predicted manner to yield mono-, di- and tri-pyrrole fragments. A possible structure for the third oxidation product $[M]^+ m/e 526$ (VIII) is suggested. The proposed conversion of the *meso*-methyl into a *meso*-formaldehyde group is supported by a large $[M - 29]^+$, m/e 497 fragment.



The mass spectrum of this compound is very difficult to analyze due to the effect of unsymmetrical *meso*-substitution on the fragmentation pattern. There appears to be a preference for cleavage at the *meso*-oxopositions, however, thus leading to only two tri-pyrrole fragments whose structures are suggested.



This information is of a preliminary nature and must yet be supported by high resolution data. If the assigned structures are correct, the *meso*-substituent would have to be either at position a or b. There is no doubt, however, that all tri-, di- and mono-pyrroles and other fragments produced are specific and will eventually allow one to assign an exact position to the *meso* substituent.

CONCLUSION

Mass spectral analysis of lead dioxide oxidation products of porphyrins allows differentiation between geometrical isomers. Substituents on the pyrrole ring and in the *meso* position can be identified and located on the basis of fragmentation data. Definite isomer identification is limited, however, by the inability to assign exact positions to the substituents on each pyrrole unit. It is hoped that high resolution gas chromatography of volatile bis (trimethylsiloxy) Si^{IV} derivatives will eventually complement this technique by allowing partial resolution of geometrical isomers.

Application of this technique to the analysis of geoporphyrin materials should provide the necessary information that is needed to draw geochemical conclusions about the origin of organics found and thus establish a possible relation to early life processes.

EXPERIMENTAL

Oxidation of alkyl porphyrins was performed using conditions described by Inhoffen *et al.*⁵ Porphyrin (≈ 1 mg) was added to two ml of 1/5 (V/V) solution of glacial acetic acid in chloroform. Lead dioxide (10 mg) was added and the mixture allowed to stand for four hours.⁸ The solution was then filtered, the solvent removed (in vacuum) and the residue dissolved in chloroform forming a yellow-brown solution. Silicic acid (Merck-dried at 120°/1 hr.) thin layer chromatography was performed using a 10% acetone/benzene solvent mixture. The light yellow (RF \approx 0.7) band was eluted with acetone and analyzed by mass spectrometry.

The methyl esters of deuteroporphyrin were reduced to the corresponding diols using excess lithium aluminum hydride in tetrahydrofuran. This dihydroxy porphyrin was then sulphonated with methylsulfonyl chloride and further reduced with lithium aluminum hydride to yield the corresponding alkylporphyrin derivative. This method is described in detail by Inhoffen *et al.*⁶

All mass spectra were run on an MS-9 (AEI) instrument (at 70 eV, 8 kV accelerating voltage, 100 μ a emission and 245°C ion chamber temperature) and a CEC 21-104 instrument (at 70 eV, 800 V accelerating voltage, 40 μ a emission and source temperature at 245°C). Both instruments were equipped with direct insertion probes; however, the CEC instrument permitted temperature programmed runs.

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