Unexpected Fragmentation of β -Substituted *meso*-Tetraphenylporphyrins Induced by High-Energy Collisional Activation

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The protonated molecules and radical cations of *meso*-tetraphenylporphyrins with β -pyrrolic substituents, when formed by fast atom bombardment (FAB) and subjected to high-energy collisions, give rise to unexpected fragment ions. The reaction involves hydrogen migration from the ortho position of the phenyl ring to the α atom of the substituent, with formation of an intramolecular, six-membered ring. The process is analogous to condensed-phase cyclizations described for the same type of compounds. The fragmentation requires the presence of a double bond in the substituent group attached to the pyrrolic ring. A rearrangement process involving anchimeric assistance by the phenyl group (analogous to an ortho effect) is proposed for the formation of these ions. (J Am Soc Mass Spectrom 2002, 13, 1427-1431) © 2002 American Society for Mass Spectrometry

The porphyrin macrocycle is present in a variety of systems found in nature and participates in numerous important biochemical functions. New porphyrins are being synthesized to mimic the natural systems. Research on porphyrins has accelerated recently because they are applicable in a variety of fields of chemistry, physics, medicine, and biology [1]. Most promising are applications as agents in photodynamic therapy in cancer treatment [2], receptor models in molecular recognition [3], and use in materials chemistry [4] owing to their optical properties and their ability to form microporous solids, conductive polymers, and sensors. The functionalization of the β -pyrrolic positions of *meso*-tetraphenylporphyrins is particularly of interest because the properties of the porphyrin macrocycle can be modified by small changes in the β -pyrrolic substituents [5]. The ability of these substituents to interact with the adjacent phenyl groups may lead to deviations of planarity of the porphyrin rings, affecting the chemical, spectroscopic, and other properties of the substituted porphyrins [6, 7]. An example is the role of non-planar conformations of chlorophyll, vitamin B12, and cytochrome P450 in biological processes [8].

Despite the increasing interest in this class of compounds, the mass spectrometric analysis of porphyrins is still relatively unexplored [9], particularly that of substituted *meso*-tetraphenylporphyrins [10–16]. The few studies that describe the fragmentation of tetraphenylporphyrins in the MS/MS mode [12–16] show that this approach gives valuable structural information about the peripheral substituents. MS/MS is expected to be useful for the chemical characterization of porphyrins because it allows a rapid identification of porphyrins in mixtures, especially those resulting from synthetic procedures. Further, the assembly of spectral databases will make widely available the valuable structural data from MS [17, 18]. Nevertheless, mass spectral data of tetraphenylporphyrins substituted in β -pyrrolic positions comprise only two recent reports: One on β -bromotetraphenylporphyrins [19, 20] and another on the fragmentation of β -nitrotetraphenylporphyrin [16], which is the precedent for this research.

In this report, we discuss the unexpected fragmentation of the molecular ions formed under FAB of *meso*-tetraphenylporphyrins substituted in the β -pyrrolic position, when subjected to high-energy collisional activation. The porphyrins studied are represented in Figure 1 and include porphyrins as the free bases and as Ni (II) and Cu (II) complexes. This work extends what we reported recently in a communication in this journal [16].

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	M	RX=Z
1 TPP β NO ₂	H, H	NO ₂
2 Cu-TPP β NO ₂	Cu(II)	NO ₂
3 Cu-TTPvinyl	Cu(II)	CH=CH ₂
4 Ni-TTPvinyl	Ni(II)	CH=CH ₂
5 TPPacrylate	H, H	CH=CHCO ₂ Me
6 Cu-TPPacrylate	Cu(II)	CH=CHCO ₂ Me
7 Ni-TPPacrylate	Ni(II)	CH=CHCO ₂ Me
8 Ni-TTPpropenal	Ni(II)	CH=CHCHO
9 Ni-TPPmethylhydroxypropionate	Ni(II)	CHOHCH ₂ CO ₂ Me

Figure 1. β -Substituted *meso*-Tetraphenylporphyrins studied here.

Experimental

The instrument was a VG ZAB-T (Manchester, UK), a four-sector instrument, consisting of two high-mass, double focusing mass spectrometers of an overall BEBE design [21]. The instrument was equipped with a Cs⁺ gun, operated at 25 kV.

The samples to be subjected to FAB were dissolved in chloroform, and a 1 μ L aliquot was loaded on the probe tip with 1 μ L of 3-nitrobenzyl alcohol. To obtain product-ion mass spectra, the precursor ions were selected with MS-1 and activated with collisions with helium gas at pressure sufficient to give 50% main-beam suppression, and detected with the single-point detector. The accelerating potential and the collision cell were at 8 and 4 kV, respectively. Data acquisition was carried out with a VG OPUS 3.1 data system and interfaced to the mass spectrometer by a VG SIOS I unit.

The porphyrins and their metal complexes were synthesized by using published methods [22, 23]. Purity of compounds was checked by thin-layer chromatography and proton nuclear magnetic resonance, confirming that the purity was at least 95%. The metal complex 6 was formed in the FAB source by addition of the free base porphyrin 5 and copper acetate to the matrix, according to a previously published procedure [24].

Scheme 1

Results and Discussion

The FAB mass spectra of the various porphyrins show formation of the protonated molecules or the molecular radical cations, depending on the porphyrin in question. Thus, we found peaks corresponding to the protonated molecules, $[M + H]^+$, for the free-base porphyrins 1 and 5 and for metalloporphyrin 8, whereas for the other metalloporphyrins, we saw peaks corresponding to the radical cations $M^{\cdot+}$.

Previously we reported that fragmentation of two isomeric nitro-tetraphenylporphyrins is sufficient to distinguish the isomers [16]. For example, the loss of water from the $[M + H]^+$ of β -nitro-*meso*-tetraphenylporphyrin (Scheme 1) occurs only for this isomer and not for one in which the nitro group is on the phenyl ring. This result encouraged us to design a test of whether the reactions are applicable to other compounds that have the structural features shown for the substructure in Scheme 1. In this article, we demonstrate that various other test compounds (Figure 1) undergo the rearrangement/elimination reaction in Scheme 1, a process that involves an interaction between the phenyl group and the substituent on the pyrrolic ring. The mechanism for fragmentation of the radical cations appears to be similar to that of the protonated molecule, suggesting that the structural utility of the fragmentation is independent of the nature of the charge. The results, organized for discussion purposes around the substituents, strongly suggest that the process is general.

Nitro Group ($-NO_2$)

High-energy (HE) collisional activation of the protonated molecule of the TPP β NO₂ (1, m/z 660), and of the radical cation of its copper complex, Cu-TPP β NO₂ (2, m/z 720), leads to similar fragmentation (the spectra were previously published [16]). According to the general mechanism, Compound 1 should lose H₂O, and this occurs to give a product ion of $\sim 10\%$ relative abundance (RA) (Scheme 2). Accompanying the water elimination is the loss of two OH groups, which gives a product ion of $\sim 15\%$ RA (Scheme 3). These reactions distinguish 1 from an isomer in which the nitro group is shifted to the para position of the phenyl ring [16] and

Scheme 2

are in accord with the mechanistic proposal that the elimination involve anchimeric assistance from the phenyl group (Scheme 1A). The radical cation of the copper complex (2) loses, via similar mechanisms, H_2O and ($\text{H}_2\text{O} + \text{OH}$) to give products of ~ 10 and 35% RA, respectively). The reactions of the copper complex also distinguish it from an isomer in which the nitro group is located in the phenyl ring.

An analogous elimination of OH takes place for the radical cations of *o*-nitrodiphenylmethane, which shares the structural motif of two aromatic rings separated by a bridge containing one heavy atom. These reactions, however, are true "ortho effects" in which a hydrogen atom must be present on the atom bound *ortho* to the nitro group, as determined by isotopic labeling [25, 26]. The reactions seen in the porphyrin systems reported here are special because this reaction does not occur for the *o*-nitrodiphenylether radical cation, for which the requisite hydrogen atom is missing.

Ortho-Vinyl Group ($-\text{CH}=\text{CH}_2$)

The HE CAD spectra of the molecular ions $\text{M}^{+\cdot}$ of the Cu-TPP β vinyl (3) and Ni-TPP β vinyl (4) complexes (m/z 701 and 696 respectively) are very similar. Although the main fragmentation pathways involve loss of C_6H_6 and two phenyl groups ($2 \text{ C}_6\text{H}_5$), an important fragmentation is the loss of CH_3 from the radical cation of 3 (see Figure 2) and from $[\text{Ni-TPP}\beta\text{vinyl}]^{+\cdot}$ (Scheme 4), as predicted from Scheme 1B.

Acrylate Group ($-\text{CH}=\text{CHCO}_2\text{CH}_3$)

For the $[\text{M} + \text{H}]^+$ of Compound 5, we expect the loss of $\text{CH}_3\text{COOCH}_3$ according to Scheme 1A, and this loss occurs to give a product ion of $\sim 25\%$ RA. Furthermore, interaction at the α -olefin carbon atom and at the carbonyl will give larger ring intermediates that may offer an explanation for losses of HCOOCH_3 and CH_3OH , respectively. The former product ion is most

Scheme 3

Figure 2. Portion of the high-energy CAD spectrum of the $\text{M}^{+\cdot}$ ion of Cu-TPP β vinyl (magnified $13\times$ with respect to the relative intensity of the $\text{M}^{+\cdot}$).

abundant (100%), and the latter has a RA of $\sim 40\%$. It is possible, of course, that they are expelled directly and without any interaction with (or anchimeric assistance by) the phenyl ring. The radical cations of the copper and nickel complexes behave as expected. An example is the HE CAD spectrum (Figure 3) of the former complex where we see evidence for the facile loss of the $\text{CH}_2\text{COOCH}_3$, COOCH_3 , and OCH_3 radicals.

Propenal Group ($-\text{CH}=\text{CHCHO}$)

If Scheme 1 is correct, the HE CAD spectrum of the protonated Ni-TPP β propenal (8) should show loss of CH_3CHO (Scheme 5), and the resulting product ion is $\sim 65\%$ RA. Interaction at the carbonyl would lead to an eight-membered ring intermediate that could lose water. Indeed, that resulting product has an abundance of

Scheme 4

Figure 3. High-energy CAD spectrum of the $M^{+\bullet}$ ion of Cu-TPP β acrylate (magnified 10 $\times$ with respect to the relative intensity of the $M^{+\bullet}$).

~50% RA. These unexpected eliminations are in accord with Scheme 1 and are difficult to rationalize by other means.

Methylhydroxypropionate Group ($-\text{CHOHCH}_2\text{CO}_2\text{CH}_3$)

The fragmentation of the radical cation of the Ni-TPP β methylhydroxypropionate (**9**) seems to violate the rule that the side chain contains a double bond so that anchimeric assistance can occur. The fragmentation of Compound **9**, however, does fit the general scheme if a loss of water occurs first to introduce a double bond in conjugation with the porphyrin ring. This loss of water may occur as a 1,2-elimination, in accord with the expectations of a charge-remote (thermal-like) fragmentation. We were able to see a product ion resulting from the combined loss of H_2O and $\text{CH}_2\text{COOCH}_3$, with an abundance of ~25% RA. We propose that the loss of water gives an unsaturation along the side chain, and the presence of this new double bond promotes the loss of $\text{CH}_2\text{COOCH}_3$. Furthermore, this compound undergoes facile losses of COOCH_3 (~5% RA), $\text{CH}_2\text{COOCH}_3$ (~47% RA) and, unexpectedly, $\text{COCH}_2\text{COOCH}_3$ (~100% RA). The loss of $\text{CH}_2\text{COOCH}_3$ indicates that some radical losses occur probably without anchimeric assistance.

Conclusion

Collisional activation of protonated molecules or radical cations of various *meso*-tetraphenylporphyrins leads to a distinct fragmentation involving anchimeric assistance

Scheme 5

tance by a phenyl group and the double bond of the substituent. This behaviour is markedly different from that of *meso*-tetraphenylporphyrins substituted in the *para* positions of the phenyl rings, where simple elimination of the substituent occurs [12, 14]. The presence of the metal center does not change significantly the type of fragmentation of *meso*-tetraphenylporphyrins with the same β -pyrrolic group, although the neutrals expelled from the radical cations are radicals whereas they are mainly closed-shell molecules for the $[M + H]^+$ ions. A mechanism of intramolecular cyclisation with formation of a six-membered ring linking the α atom of the substituent and the *ortho* carbon of the neighbouring phenyl group serves as a unifying rationalization for these results. The broader implication of this reaction is confirmed by a similar reaction mechanism that occurs for these compounds in acidic solution [27–29].

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