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Enhanced Structural Determination of Substituted Porphyrins by Ammonia Desorption Chemical Ionization Mass Spectrometry[1]

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Abstract: The desorption chemical ionization mass spectra of porphyrins containing a variety of substituents at the external β-pyrrole positions and at the internal nitrogen positions have been investigated by using ammonia as the reagent gas. Extensive fragmentation of the porphyrin macrocycle occurs with the predominant formation of protonated mono- and dipyrrolic fragment ions. The substituents largely retain their structural integrity since secondary cleavage processes occur to a far lesser extent under NH_3 D/CI than under H_2 D/CI conditions. The particular pyrrole rings containing combinations of alkyl, propionic ester, formyl, acyl, and alkoxycarbonyl are readily identified. Cyano substituents yield somewhat more ambiguous but interpretable spectra. Vinyl groups undergo hydrogen addition to some extent—a phenomenon which could be readily identified by using ND_3 as the reagent gas. Sequence information about the pyrrole rings comes from the fragment ions in the dipyrrolic mass region. The extension of the method to the identification of isomeric N-alkylated derivatives of protoporphyin IX which arise from the suicidal inactivation of cytochromes P-450 by a number of drugs is assayed via model N-substituted porphyrins.

The first extensive and systematic investigation of the electron-impact mass spectra (EIMS) of porphyrins[2] predicted that mass spectral studies should be particularly useful in structural work on porphyrins by providing molecular weights, empirical formulas, and information about the substituents present. In the ensuing 2 decades, the reality of the first two visions has been well-documented.[3] The third prediction has, however, been less well-fulfilled. This lack arises from two sources. First, under EI conditions, very little cleavage of the macrocyclic ring occurs; instead, fragmentation is limited to the peripheral substituents. Second, while some substituents such as propionic ester groups are clearly evident by well-defined cleavage (e.g., benzylic) processes, other substituents (notably the biologically important vinyl and formyl groups) do not fragment to any extent. Thus, information about the presence of important substituents, the location of discernible substituents on a particular pyrrole ring, and the sequence of the pyrrole rings in the macrocycle are not generally accessible from the EIMS of porphyrins.

Two approaches for circumventing some of these limitations have been assayed. One avenue involves either oxidative (CrO_3, H_2SO_4, or $KMnO_4$)[4] or reductive degradation of the prophyrin to produce maleic imides and pyrrole fragments, which can then be subjected to GC/MS analysis. However, the identities of some pyrrole substituents may be lost by these procedures, and the pyrrole sequence information is necessarily lost. The other avenue involves the conversion of the porphyrin ring to an intact derivative which can then fragment more readily. Although some success has been obtained by using the *meso*-tetraoxo derivatives of porphyrins,[6] the porphyrinogens produced by hydrogenation (Raney Ni) are the preferred derivatives. Both derivatives fragment under EI conditions to produce ions containing one, two, and three pyrrole rings.

More recently, two groups[8,9] have demonstrated that porphy-

† Deceased Dec 8, 1984.

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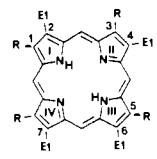

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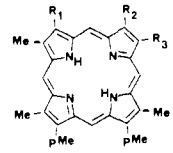
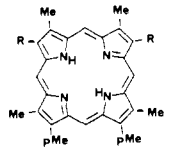
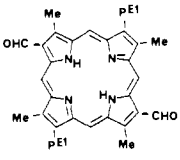
2 R = Et



	R_1	R_2	R_3
3	CH_3	CH_3	CH_3
4	H	CH_3	$COCH_3$
5	H	CH_3	$COCH_2CH_3$
6	$COCH_3$	CH_3	$COCH_3$
7	$COCH_2CH_3$	CH_3	$COCH_2CH_3$
8	$CO_2C_2H_5$	CH_3	$CO_2C_2H_5$
9	$CH_2CH_2CO_2CH_3$	H	$CO_2C_2H_5$
10	CH_3	CH_3	$CH{=}CH_2$
11	$CH{=}CH_2$	CH_3	$CH{=}CH_2$
12	$CH_2CH_2CO_2CH_3$	CH_3	CN
13	CN	CH_3	CN



14 R = CHO
15 R = CN



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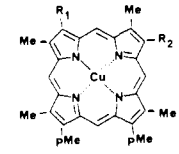
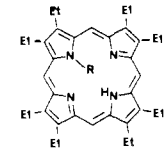
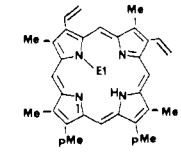


17 R_1 = CH_3 R_2 = $\triangle$-$CO_2C_2H_5$
18 R_1 = R_2 = $\triangle$-$CO_2C_2H_5$



19 R = CD_3
20 R = $CH_2CO_2C_2H_5$



+ Isomers

21

Figure 1. Structures of the porphyrins studied by NH_3 D/CIMS. P^{Me} = $CH_2CH_2CO_2CH_3$. P^{Et} = $CH_2CH_2CO_2C_2H_5$.

rinogens appear to be produced in the mass spectrometer in situ during the course of H_2 CIMS measurements since fragment ions with one, two, and three pyrrole rings are observed directly. A single spectrum also demonstrated that a similar process can be made to occur (at lower source temperatures) when methane is used as the reagent gas.[8a]

Prompted by these successes and by the possibility of enhancing the structural information content in the CIMS through the use of a milder reagent gas, the NH_3 desorption chemical ionization mass spectra (D/CIMS) were measured for a number of porphyrins (Figure 1) containing alkyl, vinyl, formyl, acyl, ethoxycarbonyl, and cyano substituents at the β-pyrrole positions. In addition, porphyrins with alkyl substituents at the internal nitrogen positions have also been measured. The results indicated that extensive fragmentation of the porphyrin macrocycle occurs with the predominant formation of protonated mono-, di-, and tripyrrolic species. The occurrence of side chain cleavage species was reduced compared to the results obtained from H_2 CIMS. Moreover, the structural integrity of each of the substituents was maintained since fragments containing each of the intact substituents could be easily identified in either the mono- or dipyrrolic mass regions. Although the ordering of the types of pyrrole rings can be readily determined, the specific substitutional isomers on the individual pyrrole rings remain indeterminate.

Experimental Section

Mass Spectrometry. Mass spectra were measured with a Ribermag R10-10C quadrupole mass spectrometer interfaced with a PDP-8-based data system using the SADR software. The source temperature, the emission current, and the electron energy were maintained at 200 °C (except for **18**, ~80 °C), 0.2 mA, and 70 eV, respectively. Special care was taken to adjust the reagent gas pressures to optimize the D/CI fragmentation. For NH_3 (99.998%) and H_2 (99.99%), the pressures were ca. 0.4 and ca. 0.2 torr, respectively. Etioporphyrin III (**1**) was used as a routine standard sample to ensure that the experimental parameters were adjusted and maintained to yield optimal spectra during each separate measurement period.

The samples were dissolved in methylene chloride and then transferred to a gold/tungsten coil. After solvent evaporation, the samples were introduced by the D/CI technique[10] and the heating current through the filament was programmed from 40 to 500 mA at a rate of 7 mA s^{-1}. The data and spectra presented here are selected from a single scan chosen on the basis of the total ion current which gives a monopyrrolic fragment as the base peak and the dipyrrolic fragments as intense as possible while still retaining the parent $(M + H)^+$ peak. All spectra were corrected for background ions. Careful control of the experimental conditions and the use of a standard calibration sample allows reasonably good spectral reproducibility. The peak intensity ratios will, of course, be somewhat different from measurement to measurement (and from instrument to instrument); the basic patterns, however, are consistently reproduced.

Materials. Several porphyrin samples were obtained from the collections of other investigators. A number of other porphyrins were prepared at Stanford during the course of separate magnetic circular dichroism spectroscopy studies. In all cases, their spectroscopic and physical properties indicated that the porphyrins were of high purity.

1,2,3,4,5,6,7,8-Octaethylporphyrin (OEP, **2**) was obtained from H. H. Inhoffen. K. M. Smith donated 1,3,5,8-tetramethyl-2,4,6,7-tetraethylporphyrin (etioporphyrin III, **1**) as well as the 2,4-dimethyl and 2-methyl-4-vinyl derivatives of deuteroporphyrin IX dimethyl ester (**3** and **10**), P. S. Clezy provided 2,4-(diethoxycarbonyl)deuteroporphyrin IX DME (**8**) and 1,5-diformyldeuteroporphyrin II diethyl ester (**16**).

The 4-acetyl, 4-propionyl, 2,4-diacetyl and 2,4-dipropionyl derivatives of deuteroporphyrin IX DME (**4**-**7**, respectively) were prepared[11] by a recently described acylation procedure.[12] The synthesis of 2-[2-(methoxycarbonyl)ethyl]-4-cyano- and 2-[2-(methoxycarbonyl)ethyl]-3-demethyl-4-(ethoxycarbonyl) derivatives of deuteroporphyrin IX DME (**12** and **9**) have been described recently.[13,14] Protoporphyrin IX DME (**11**) was prepared from hematoporphyrin and converted[15] to 2,4-dicyanodeuteroporphyrin IX DME (**13**) by standard procedures. Similarly, the 1,4-diformyl and 1,4-dicyano derivatives of deuteroporphyrin XIII DME (**14** and **15**) were prepared[16] from the corresponding divinyl porphyrin synthesized at Stanford. The copper complexes **17** and **18** were prepared[17] by reaction of the copper complexes of **10** and **11**, respectively, with ethyl diazoacetate according to a known procedure.[20a] The *N*-trideuteriomethyl and *N*-(ethoxycarbonyl)methyl derivatives of octaethylporphyrin (**19** and **20**) were prepared[17] from the free base and from the cobalt complex by reaction with trideuteriomethyl trifluoromethanesulfonate and ethyl diazoacetic ester, respectively, according to published methods. The isomeric mixture of *N*-ethylprotoporphyrin IX DME (**21**) was synthesized by reaction of protoporphyrin IX DME (**11**)

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(15) Lu, Y. C., unpublished work.
(16) Knierzinger, A., unpublished work.
(17) Tolf, B.-R., unpublished work.
(18) The full NH_3 D/CIMS of 4-acetyldeuteroporphyrin IX DME (**4**) is presented in the preliminary communication: Jiang, X.-Y.; Wegmann-Szente, A.; Tolf, B.-R.; Kehres, L. A.; Bunnenberg, E.; Djerassi, C. *Tetrahedron Lett.* **1984**, *25*, 4083.
(19) We have confirmed this by converting **8** to the tetramethyl ester derivative and find the new fragment at m/z 140 (38.7), 154 (66), and 168 (100).
(20) (a) Budzikiewicz, H.; Taraz, K. *Liebigs Ann Chem.* **1970**, *737*, 128. (b) Callot, H. J. *Bull. Soc. Chim. Fr.* **1972**, 4387.

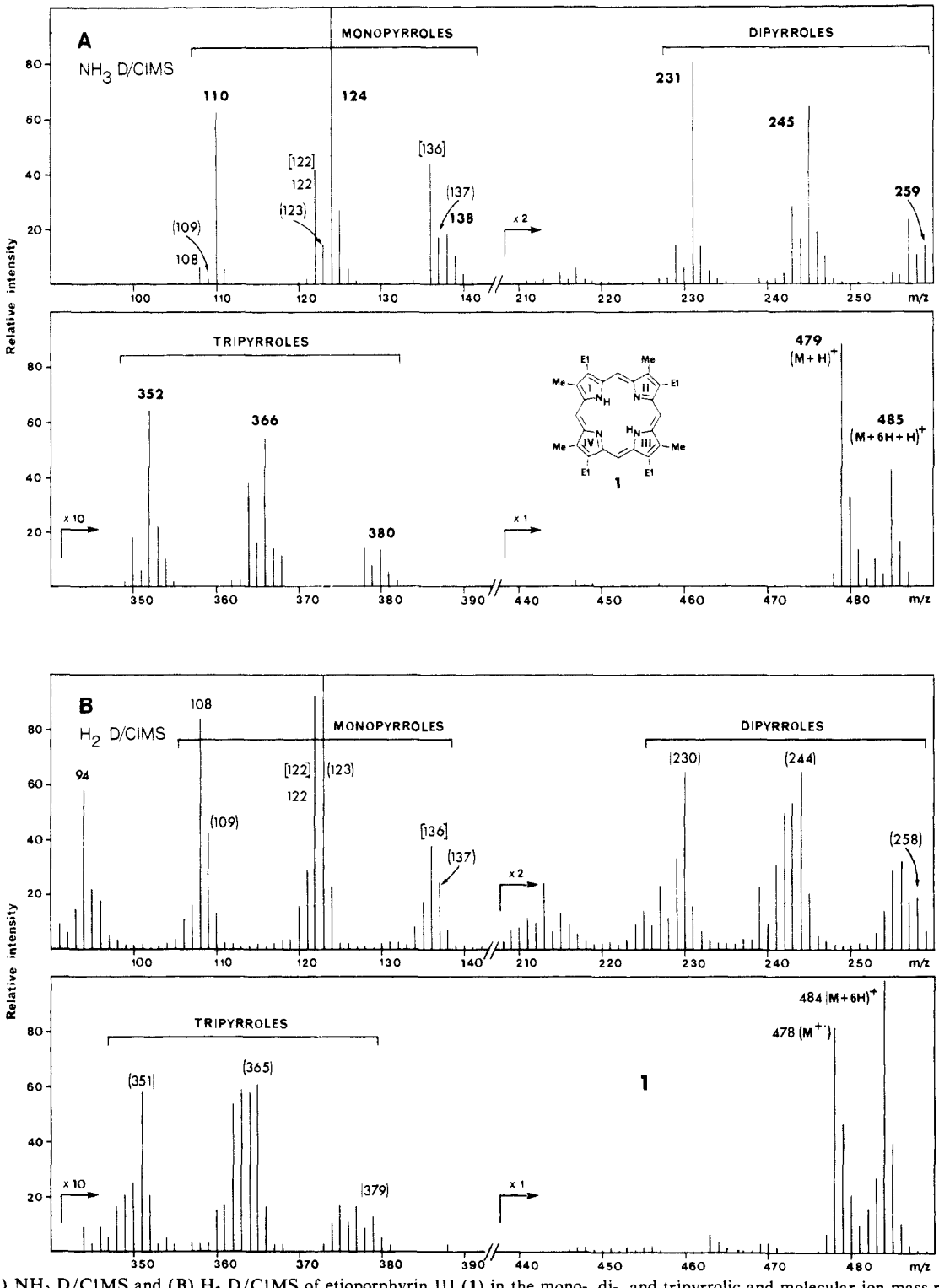


Figure 2. (a) NH_3 D/CIMS and (B) H_2 D/CIMS of etioporphyrin III (**1**) in the mono-, di-, and tripyrrolic and molecular ion mass regions. Ions of low relative abundance between the regions have been omitted. In the monopyrrolic region, bold numbers in A designate protonated ions. Parentheses enclose masses assumed to be odd-electron species (**28**). Brackets indicate fragments formed by β-hydrogen cleavage (**32**). Unenclosed mass numbers are for ions formed by β-alkyl cleavage (**30**). Only the protonated species **24** and **25** are annotated in the di- and tripyrrolic regions, respectively.

with ethyl trifluoromethanesulfonate.[17]

Results and Discussion

The D/CIMS spectra of etioporphyrin III (etio III, **1**) obtained with ammonia and with hydrogen as the reagent gases are shown in Figure 2A and B, respectively. In parallel with the EIMS of porphyrinogens reported by Budzikiewicz et al.,[7] extensive fragmentation occurs with both reagent gases. These spectra exhibit similar peaks in the molecular ion region and three triads of peaks in each of the mono-, di-, and tripyrrolic regions in addition to some other groupings of ions. It is particularly noteworthy that the spectrum obtained in the NH_3 D/CI mode is considerably less complex than that obtained with hydrogen. This feature will subsequently be seen to markedly facilitate the identification of pyrrolic and dipyrrolic fragments bearing particular substituents.

Fragmentation Pathways. The principal fragmentation pathway which appears to occur for porphyrins in the NH_3 D/CI mode is illustrated for etio III (**1**) in Figure 3. Initial attack by the reagent gas results in the formation of a protonated *porphyrin* molecular ion, m/z 479. Further attack by the reagent gas then occurs with the formation of the protonated *porphyrinogen* **22**, m/z 485 $(M + 6H + H)^+$, which has four additional hydrogens

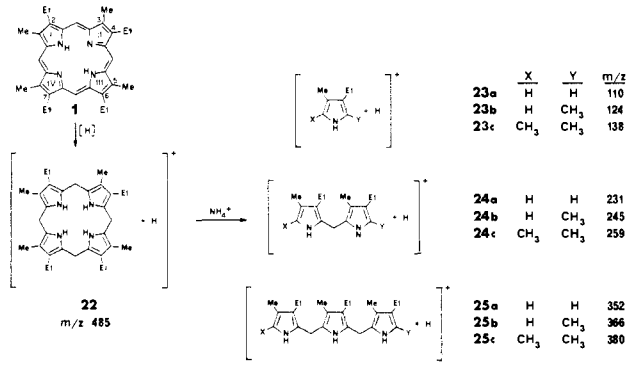


Figure 3. Suggested principal fragmentation pathway for etioporphyrin III (**1**) under NH_3 D/CI conditions. Specific tripyrrolic fragments incorporating pyrrole rings III and IV cannot be distinguished and are not explicitly shown.

at the meso positions and two additional imido hydrogens.

The porphyrinogen ions are unstable and are subject to continued nonspecific attack by the reagent gas at the meso carbon positions. This can result in formation of mono-, di-, and tripyrrolic fragments (**23**, **24**, and **25**), each of which may contain 0, 1, or 2 meso carbon atoms. These fragments are commonly found in the NH_3 D/CIMS as protonated ions since ions at one mass unit higher than the mass number of the expected fragments are more often the most abundant ions. In Figure 2A and subsequent figures, these protonated ions are indicated by bold numerals.

A second fragmentation pathway which may contribute ions to the NH_3 D/CIMS of porphyrins is fortunately of minor importance when compared to what is oberved in H_2 D/CIMS. In this pathway, which is analogous to that proposed by Shaw et al.[8b] to occur under H_2 CI conditions, odd-electron monopyrrolic (**28**, Figure 4), dipyrrolic (**29**), and tripyrrolic (no structure given) ions are formed. Here, we annotate their occurrence (mass numbers enclosed in parentheses in Figure 2A) and further fragmentation only for the monopyrrolic region of etio III. These ions readily undergo further fragmentation by β cleavage to produce more stable secondary ions. The secondary ions formed directly from the porphyrinogen or by benzylic hydrogen cleavage (hereafter we will assume for convenience the latter occurrence and will collectively represent it by structure **32**) are enclosed in square brackets in Figure 2A, whereas the mass numbers of those formed by benzylic cleavage of a methyl radical (**30**) are unenclosed.

In contrast to the ammonia spectrum (Figure 2A), the H_2 D/CIMS of etio III (Figure 2B) is much more complex and cluttered with ions arising from secondary fragmentations. The large cluster of ions in the monopyrrolic region, for example, represents an overlapped region of extra ion peaks with those from the main triad sets of ions. This would make the elucidation of a nonsymmetrically substituted porphyrin very difficult. Thus, the abundance of the protonated species formed in the NH_3 D/CIMS makes NH_3 the clearly preferred reagent gas.

NH_3 D/CIMS of Substituted Porphyrins. The following data for the structural elucidation of various substituted porphyrins have been arranged in a highly organized and systematic manner since it has not been heretofore presented. In addition to pertinent select spectra, we have collected relative intensity data for the more significant fragment ions in Tables I (monopyrrolic), II (dipyrrolic), and III (molecular ion clusters).

Table I provides an extensive listing of the fragments which can be reasonably assumed to contribute in some measure to the NH_3 D/CIMS of the substituted porphyrins via the two pathways elaborated above for etio III. For a particular porphyrin, entry into the table is made by noting which of the substituent pairs are found on the individual pyrrole rings of the porphyrin. The remaining columns give the mass numbers of the fragments for that pyrrole ring which *could* arise as the protonated species (**26a–c**); the odd-electron species (**28a–c**); or the fragments derived from **28** by benzylic hydrogen cleavage (**32a** and **b**), benzylic alkyl side-chain cleavage (**30a–c**), or from the loss of a fragment ($\cdot CH_3$, $\cdot CH_2CH_3$, or $\cdot OC_2H_5$) from a nuclear carbonyl substituent

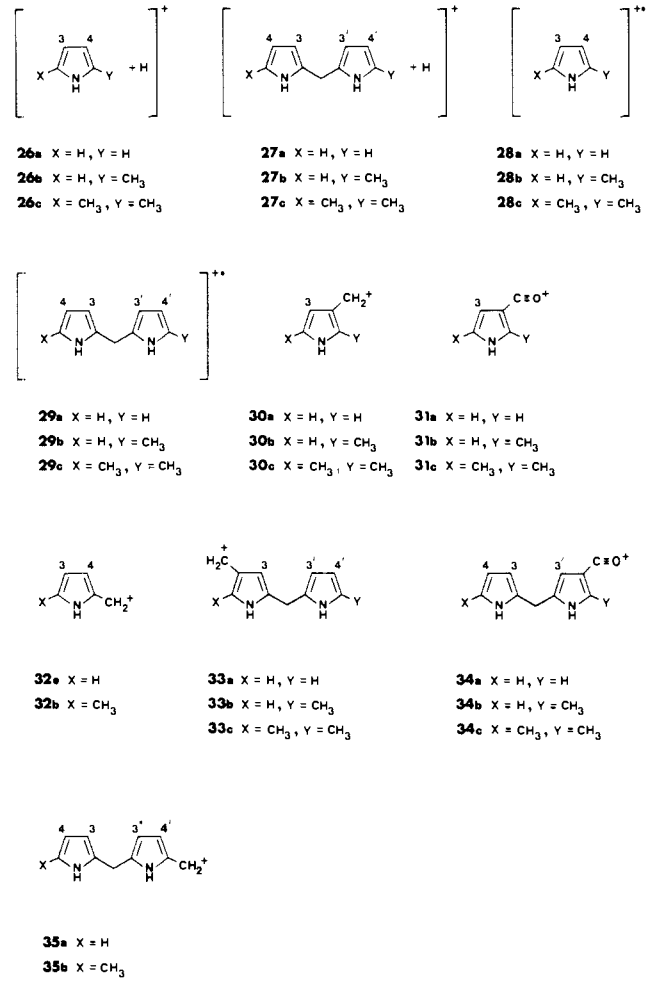


Figure 4. Schematic representation of the ions which may contribute to the NH_3 D/CIMS of the substituted porphyrins. Substituents in the 3- and 4-positions of the monopyrrolic fragments are given in Table I. Those in the 4,3,3′- and 4′-positions of the dipyrrolic fragments are entered in Table II. In relating the substituents on the porphyrin ring to those entered for the dipyrrolic frgments, note should be made of footnote c of Table II.

(**31a–c**). It is important to note that while fragment ions (**28**, **30–32**) arising via the secondary pathways may be coincident for different pyrrole rings in the same porphyrin (and therefore greatly confuse structure elucidation using H_2 D/CIMS), *the mass numbers of the protonated species* (**26a–c**) *found only in the NH_3 D/CIMS are not (for the porphyrins studied) coincident with secondary ion fragments.* A single (and unimportant, vide infra) exception is found for the methyl/vinyl combination in porphyrins **10** and **11** (see entry number 12 in Table I). *More important is the fact that there are no coincidences among the more abundant protonated ions* (**26a–c**) *of the differently substituted pyrrole rings* other than the partial coincidences observed for the (ethoxycarbonyl)porphyrins **8** and **9** (see entry numbers 10 and 11 in Table I). This, however, is a problem which can be readily surmounted (vide infra).

Monopyrrolic Fragment Ions. This region of the NH_3 D/CI mass spectrum is particularly useful for identifying the number of differently substituted pyrrole rings which a porphyrin may have and for determining the particular substituents which are attached to each of the pyrrole rings.

(i) Carbonyl-Substituted Porphyrins. The NH_3 D/CIMS of 2,4-dipropionyldeuteroporphyrin IX DME (**7**) is shown in Figure 5A. The ions at m/z 138, 152, and 166 are protonated species derived from the pyrrole rings which contain the methyl and propionyl groups (rings I and II), whereas those at m/z 168, 182, and 196 are the protonated species arising from the methyl and methyl propionic ester substituted rings III and IV. The ions in each triad contain 0, 1, and 2 meso carbon units, respectively, and

Table I. Relative Intensities of the Principal Monopyrrolic Fragments Found in the NH_3 D/CI Mass Spectra of the Substituted Porphyrins[a]

entry no.[b]	β-substituents[c] C3	β-substituents[c] C4	porphyrin no. (ring no.)[d]	structure **26a**; X = H, Y = H	**26b**; X = H, Y = CH_3	**26c**; X = CH_3, Y = CH_3	**28a**; X = H, Y = H	**28b**; X = H, Y = CH_3	**28c**; X = CH_3, Y = CH_3	**30a**[e] and **31a**; X = H, Y = H	**30b**[e] and **31b**; X = H, Y = CH_3	**30c**[e] and **31c**; X = CH_3, Y = CH_3	**32a**; X = H	**32b**; X = CH_3
1	CH_3	CH_3		96	110	124	95	109	123	94	108	122	108	122
			31 (I, II)	(84.2)	(100)	(15.9)	(2.6)	(13.7)	(11.4)	(7.6)	(26.1)	(20.2)	(26.1)	(20.2)
			10 (I)	(42.0)	(74.3)	(26.2)	(–)	(6.9)	(9.4)	(4.7)	(28.0)	(34.9)	(28.0)	(34.9)
2	CH_3	CH_2CH_3		110	124	138	109	123	137	94	108	122	122	136
			1 (I–IV)	(62.5)	(100)	(17.7)	(1.6)	(14.3)	(17.1)	(4.2)	(6.2)	(41.5)	(41.5)	(43.9)
3	CH_2CH_3	CH_2CH_3		124	138	152	123	137	151	108	122	136	136	150
			2 (I–IV)	(60.1)	(100)	(12.0)	(0.2)	(7.8)	(7.6)	(–)	(4.3)	(26.2)	(26.2)	(26.6)
4	CH_3	P^{ME}		168	182	196	167	181	195	94	108	122	180	194
			3*–15 (III, IV)	(39.7)	(47.8)	(11.9)	(1.8)	(1.3)	(3.0)	(7.6)	(26.1)	(20.2)	(4.4)	(8.6)
			12 (I)	(51.1)	(85.5)	(12.2)	(1.3)	(4.0)	(4.4)	(–)	(–)	(1.3)	(5.1)	(7.8)
5	CH_3	P^{Et}		182	196	210	181	195	209	94	108	122	194	208
			16 (II, IV)	(62.3)	(100)	(13.2)	(1.9)	(6.2)	(25.0)	(10.1)	(36.2)	(19.6)	(10.8)	(8.2)
6	CH_3	H		82	96	110	81	95	109	NCS[e]	NCS	NCS	94	108
			4 (I)	(39.1)	(75.3)	(17.1)	(0.3)	(8.7)	(2.4)				(25.0)	(24.8)
			5 (I)	(32.8)	(55.7)	(6.3)	(0.4)	(7.1)	(7.3)				(11.3)	(20.8)
7	CH_3	CHO		110	124	138	109	123	137	108	122	136	122	136
			14 (I, II)	(42.4)	(56.3)	(22.7)	(5.8)	(7.1)	(0.4)	(24.1)	(14.0)	(6.3)	(14.0)	(6.3)
			16 (I, III)	(49.5)	(66.6)	(20.1)	(10.3)	(8.8)	(3.9)	(36.2)	(19.6)	(11.3)	(19.6)	(11.3)
8	CH_3	$COCH_3$		124	138	152	123	137	151	108	122	136	136	150
			4 (II)	(48.5)	(46.4)	(8.3)	(3.3)	(6.2)	(2.7)	(24.8)	(2.6)	(3.5)	(3.5)	(3.3)
			6 (I, II)	(70.2)	(98.4)	(33.7)	(5.9)	(8.3)	(8.3)	(15.1)	(10.4)	(10.4)	(10.4)	(12.7)
9	CH_3	$COCH_2CH_3$		138	152	166	137	151	165	108	122	136	150	164
			5 (II)	(31.7)	(43.1)	(10.2)	(2.2)	(2.9)	(1.9)	(20.8)	(4.5)	(2.1)	(3.4)	(2.3)
			7 (I, II)	(61.6)	(100)	(31.0)	(4.3)	(7.9)	(7.4)	(7.0)	(7.9)	(8.9)	(10.6)	(9.2)
10	CH_3	$CO_2C_2H_5$		154	168[f]	182[g]	153	167	181	108	122	136	166	180
			8 (I, II)	(23.4)	(100)	(79.6)	(2.3)	(1.1)	(7.4)	(–)	(2.0)	(–)	(3.2)	(9.7)
11	H	$CO_2C_2H_5$		140	154	168[f]	139	153	167	94	108	122	152	166
			9 (II)	(4.4)	(13.2)	(94.9)	(–)	(1.1)	(6.2)	(2.3)	(3.1)	(0.3)	(–)	(2.9)
12	CH_3	$CH{=}CH_2$		108	122	136	107	121	135	NCS	NCS	NCS	120	134
			10 (II)	(28.0)[h]	(34.9)[i]	(11.2)	(1.1)	(0.3)	(0.4)				(1.1)	(1.8)
			11 (I, II)[l]	(9.7)[j]	(19.2)[k]	(14.3)	(0.7)	(1.0)	(1.2)				(1.6)	(1.9)
13	CH_3	CN		107	121	135	106	120	134	NCS	NCS	NCS	119	133
			12 (II)	(–)	(2.1)	(1.7)	(0.7)	(1.6)	(4.2)				(2.2)	(5.0)
			13 (I, II)	(3.0)	(21.4)	(15.3)	(0.3)	(5.4)	(8.5)				(3.0)	(8.1)
			15 (I, II)	(3.2)	(26.8)	(24.1)	(1.6)	(5.6)	(8.6)				(4.6)	(11.0)

[a] Relative intensities are given as the percentage of the base peak. [b] Notice that the entry number in combination with the structure number can be used to indicate a specific fragment ion. [c] $p^{Me} = CH_2CH_2CO_2CH_3$; $p^{Et} = CH_2CH_2CO_2C_2H_5$. [d] An asterisk indicates that the relative intensities cited were observed for this porphyrin. Ring numbers are indicated in structures **1** and **2** in Figure 1. [e] Note that structures **30a–c** are applicable for pyrrolic fragments containing only alkyl and propionic ester substituents. Structures **31a–c** apply for nuclear carbonyl substituents. NCS means no corresponding structure. [f] Coincident with fragment (4) **26a**. [g] Coincident with fragment (4) **26b**. [h] Coincident with fragments (1) **32a** and (4) **30b**. [i] Coincident with fragments (1) **32b** and (4) **30c**. [j] Coincident with fragment (4) **30b**. [k] Coincident with fragment (4) **30c**. [l] Fragments at *m/z* 110 (21.2), 124 (34.7), and 138 (6.4) probably arise by the addition of hydrogen to the vinyl groups to form ethyl substituents. Further indications of hydrogen addition are found in the dipyrrolic (Table II) and molecular ion cluster mass regions (Table III).

Table II. Relative Intensities of the Principal Protonated Dipyrrolic Fragments Found in the NH_3 DCI Mass Spectrum of the Substituted Porphyrins[a]

entry no.[b]	β-substituents[c] (27) pyrrole 1		pyrrole 2		from porphyrins[d]	structures 27a; X = H, Y = H	27b; X = H, Y = CH_3	27c; X = CH_3, Y = CH_3
1	CH_3	CH_3	CH_3	CH_3		203	217	231
					3 (I + II)	(11.2)	(8.8)	(1.2)
2	CH_3	CH_2CH_3	CH_3	CH_2CH_3		231	245	259
					1 (all pairs)	(39.9)	(32.2)	(7.4)
3	CH_2CH_3	CH_2CH_3	CH_2CH_3	CH_2CH_3		259	273	287
					2 (all pairs)	(12.2)	(9.4)	(1.7)
4	CH_3	p^{Me}	p^{Me}	CH_3		347	361	375
					3*-15 (III + IV)	(9.3)	(8.2)	(1.9)
					9 (I + IV)	(12.6)	(6.4)	(0.7)
5	p^{Me}	CH_3	CH_3	CH_3		275	289	303
					3 (I + IV, II + III)	(10.4)	(12.7)	(2.6)
					10 (I + IV)	(10.9)	(11.2)	(4.9)
6	p^{Me}	CH_3	CH_3	H		261	275	289
					4 (I + IV)	(3.5)	(4.5)	(2.4)
					5 (I + IV)	(8.4)	(6.0)	(2.7)
7	p^{Me}	CH_3	CHO	CH_3		289	303	317
					14 (I + IV, II + III)	(3.9)	(4.7)	(0.4)
8	p^{Et}	CH_3	CHO	CH_3		303	317	331
					16 (all pairs)	(8.0)	(6.5)	(2.2)
9	CHO	CH_3	CH_3	CHO		231	245	259
					14 (I + II)	(2.5)	(3.0)	(3.0)
10	CH_3	H	CH_3	$COCH_3$		217	231	245
					4 (I + II)	(10.7)	(9.2)	(3.4)
11	CH_3	$COCH_3$	CH_3	p^{Me}		303	317	331
					4 (II + III)	(4.9)	(3.7)	(0.9)
					6 (I + IV, II + III)	(8.3)	(10.1)	(3.9)
12	CH_3	$COCH_3$	CH_3	$COCH_3$		259	273	287
					6 (I + II)	(8.1)	(10.0)	(4.9)
13	CH_3	H	CH_3	$COCH_2CH_3$		231	245	259
					5 (I + II)	(13.1)	(15.6)	(4.2)
14	CH_3	$COCH_2CH_3$	CH_3	p^{Me}		317	331	345
					5 (II + III)	(6.8)	(6.3)	(2.1)
					7 (I + IV, II + III)	(5.3)	(5.6)	(2.2)
15	CH_3	$COCH_2CH_3$	CH_3	$COCH_2CH_3$		287	301	315
					7 (I + II)	(4.8)	(6.0)	(3.2)
16	p^{Me}	CH_3	CH_3	$CO_2C_2H_5$		333	347[e]	361[f]
					8 (I + IV, II + III)	(6.0)	(7.0)	(3.3)
17	CH_3	p^{Me}	H	$CO_2C_2H_5$		319	333	347[e]
					9 (I + II)	(5.2)	(6.4)	(12.6)
18	CH_3	$CO_2C_2H_5$	CH_3	$CO_2C_2H_5$		319	333	347[e]
					8 (I + II)	(1.5)	(6.0)	(7.0)
19	CH_3	CH_3	CH_3	$CH{=}CH_2$		215	229	243
					10 (I + II)[g]	(2.8)	(3.9)	(2.6)
20	CH_3	$CH{=}CH_2$	CH_3	p^{Me}		287	301	315
					10 (II + III)[h]	(5.2)	(4.2)	(1.4)
					11 (I + IV, II + III)[i]	(1.6)	(1.9)	(1.2)
21	CH_3	$CH{=}CH_2$	CH_3	$CH{=}CH_2$		227	241	255
					11 (I + II)[j]	(0.3)	(1.0)	(0.7)
22	CH_3	p^{Me}	CH_3	CN		286	300	314
					12 (I + II, II + III)	(3.8)	(4.7)	(1.1)
					13 (I + IV, II + III)	(7.8)	(9.8)	(3.7)
					15 (I + IV, II + III)	(6.0)	(8.2)	(3.4)
23	CH_3	CN	CH_3	CN		225	239	253
					13 (I + II)	(1.0)	(2.3)	(2.3)
					15 (I + II)	(0.4)	(1.4)	(3.0)

[a] Relative intensities are given as the percentage of the base peak. [b] As in Table I, entry numbers in combination with structure numbers can be used to designate particular fragment ions. [c] A number of the porphyrins contain dipyrrolic units having permutations of the same substituents. NH_3 CIMS is not able to distinguish between isomerically substituted dipyrrolic fragments. Consequently, the ordering of the substituents as depicted here may not conform to the ordering indicated in the structural formulas in Figure 1. [d] An asterisk indicates that the relative intensities were recorded for this porphyrin. [e] Coincident with fragment (4) **27a**. [f] Coincident with fragment (4) **27b**. [g] Peaks at m/z 217 (5.5), 231 (4.5), and 245 (1.7) are probably due to hydrogen addition. [h] For **10**, peaks at m/z 289 (11.2), 303 (4.9), and 317 (1.2) are probably due to hydrogen addition. [i] For **11**, peaks at m/z 289 (4.4), 303 (3.9), and 317 (0.8) are probably due to hydrogen addition. [j] Fragments due to hydrogen addition for rings I and II of **11** are at 4 mass units higher: m/z 231 (3.4), 245 (4.3), and 259 (2.1).

are schematically represented by structures **26a**, **26b**, and **26c** in Figure 4. It is noteworthy that the protonated ions having only one meso carbon atom (**26b**) are the more abundant of the three species (see also Table I). Since only a few other ions of much lower abundance are found, it appears that the formation of odd-electron species (**28**) and their secondary cleavage fragments (**30**, **31**, and **32**) as well as corresponding cleavage fragments derived from the protonated ions (**26**) is not a particularly favored process in the NH_3 D/CI mode. Thus, it is an easy matter to determine by NH_3 D/CIMS that porphyrin **7** contains two differently substituted pyrrole rings and what the particular substituents are. Pyrrole ring sequence information for this porphyrin will be commented on in a subsequent section.

The H_2 D/CIMS of **7** (Figure 5B) is in stark contrast to the structurally informative NH_3 D/CI spectrum. Odd-electron species (**28**) (masses enclosed in parentheses in Figure 5B) and

Table III. Relative Intensities of the Ions in the Molecular Ion Clusters of the Substituted Porphyrins[a]

porphyrin no.	$M^{+\cdot}$	$(M + H)^+$	$(M + 6H + H)^+$	other
1	478 (5.4)	479 (88.7)	485 (42.7)	
2	534 (0.1)	535 (6.6)	541 (6.3)	
3	566 (2.6)	567 (43.6)	573 (9.0)	
4	580 (0.2)	581 (13.8)	587 (6.7)	
5	594 (4.3)	595 (66.6)	601 (11.8)	
6	622 (1.3)	623 (39.8)	629 (5.1)	
7	650 (2.4)	651 (56.1)	657 (8.5)	
8	682 (1.5)	683 (56.5)	689 (12.0)	
9	682 (5.6)	683 (78.5)	689 (17.9)	
10	578 (2.5)	579 (70.2)	585 (4.2)	587 (6.0) $(M + 8H + H)^+$
11	590 (3.5)	591 (68.7)	597 (-)	601 (-) $(M + 10H + H)^+$
12	649 (2.3)	650 (100.0)	656 (14.8)	
13	588 (2.0)	589 (58.1)	595 (12.2)	
14	594 (0.8)	595 (46.7)	601 (11.1)	
15	588 (1.8)	589 (56.8)	595 (9.8)	
16	622 (0.6)	623 (35.2)	629 (6.5)	
19	551 (10.9)	552 (80.7)	558 (66.2)	535 (0.7) (loss of $\cdot CD_3$)
20	620 (0.2)	621 (9.5)	627 (0.2)	535 (5.0) (loss of $\cdot CH_2CO_2C_2H_5$)

[a] Relative intensities are given as the percentage of the base peak. [b] The source temperature was about 80 °C in contrast to the 200 °C used for all other porphyrins.

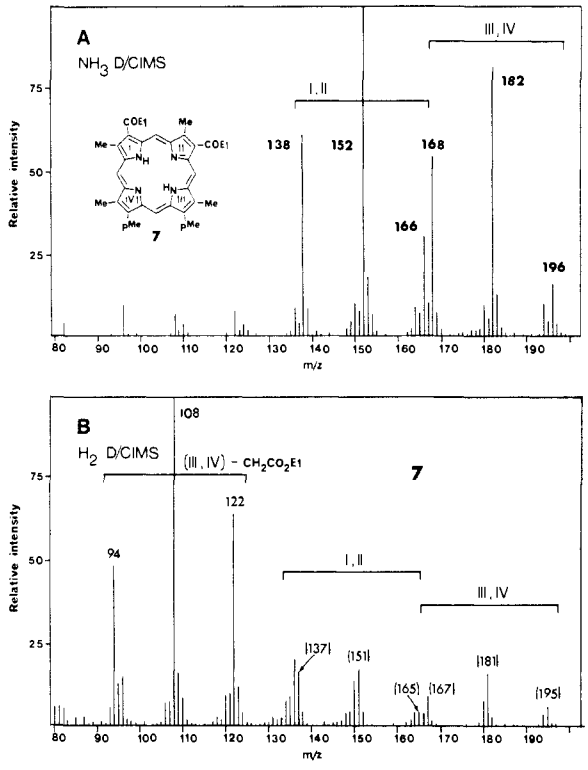


Figure 5. (A) NH_3 D/CIMS and (B) H_2 D/CIMS of 2,4-dipropionyldeuteroporphyrin IX DME in the monopyrrolic fragment mass region. Only protonated species (**27**) are designated in A (bold numerals). In B, odd-electron fragments (**28**) are enclosed in parentheses. The mass numbers of benzylic propionic ester cleavage fragments are unenclosed.

their β-hydrogen cleavage fragments (not designated) are found in only relatively low abundance. Instead, fragment ions (**30**) at m/z 94, 108, and 122 which are due to benzylic cleavage of the propionic ester side chains and ions at m/z 108, 122, and 136 which additionally may be formed by loss of $\cdot CH_2CH_3$ from the propionyl substituents (**31**) dominate the spectrum. Such side-chain-cleavage fragments would present a major interpretation problem for a porphyrin such as a monopropionyldeuteroporphyrin (e.g.,**5**) since fragments from its methyl/hydrogen-substituted ring I also fall in the β-cleavage mass region (see Table I).

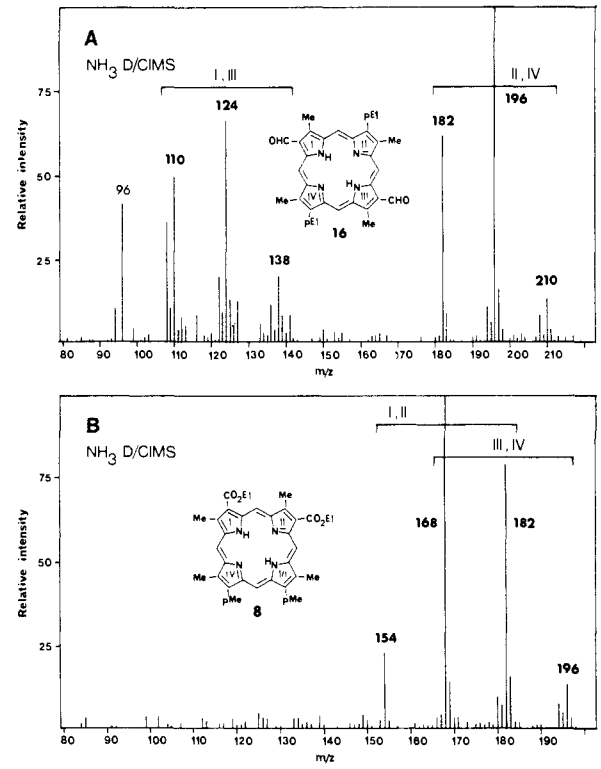


Figure 6. (A) NH_3 D/CIMS of 1,5-diformyldeuteroporphyrin II DEE (**16**) and (B) the NH_3 D/CIMS of 2,4-(diethoxycarbonyl) deuteroporphyrin IX DME (**8**) in their monopyrrolic fragment mass regions. In both spectra, bold mass numbers designate protonated species.

NH_3 D/CIMS were also obtained for a mono- and diacetyl derivative of deuteroporphyrin IX DME (**4** and **6**). The masses of the protonated species arising from the acetyl-substituted rings were shifted by the expected 14 mass units (Table I), but no problems were encountered in the interpretation of their spectra. Both of the monoacyl-substituted porphyrins (**4** and **5**) unambiguously showed the presence of the three differently substituted rings in the monopyrrolic mass region.[18]

Formyl groups are "silent" in the conventional EIMS of porphyrins in that they have no characteristic fragments. Figure 6A gives the monopyrrolic mass region of a formyl-substituted porphyrin (**16**). The ions at m/z 110, 124, and 138 show that the formyl groups (like the acetyl and propionyl groups) retain their structural integrity. A very similar spectrum was recorded for another formylporphyrin (**14**) except that the fragments arising from the pyrrole rings containing the methyl and propionic ester groups appeared 14 mass units lower due to the change from the ethyl to the methyl esters. The origin of the relatively abundant ion at m/z 96 (Figure 6A) is unknown. The a priori most plausible explanation—loss of $\cdot CH_2CO_2C_2H_5$) from a fragment ion of ring II or IV—is unlikely, since the corresponding methyl ester does not show this fragmentation.

Porphyrins having nuclear carboxylic acid substituents are often produced in synthetic studies as the ethyl esters. They commonly have a methyl group adjacent to the carboxylic acid substituent and one or more of the other pyrrole rings usually have the methyl and methylpropionic ester substituent pair. The NH_3 D/CIMS of a porphyrin having this combination of substituents, 2,4-(diethoxycarbonyl)deuteroporphyrin IX DME (**8**), is given in Figure 6B. Typical of such porphyrins is the *single tetrad* of ions at m/z 154, 168, 182, and 196, arising as a result of coincidences among the triads of protonated species at m/z 154, 168, and 182 and at m/z 168, 182, and 196 owing to rings I and II and III and IV, respectively. While this pattern, two satellite peaks of relatively low abundance accompanied by two abundant central fragment peaks, is quite diagnostic, further confirmation of the presence of the methyl/carboxylic ester substituent pair of substituents on one of the pyrrole rings can be easily gained in two ways. First, the nuclear ethoxycarbonyl group can be converted to the

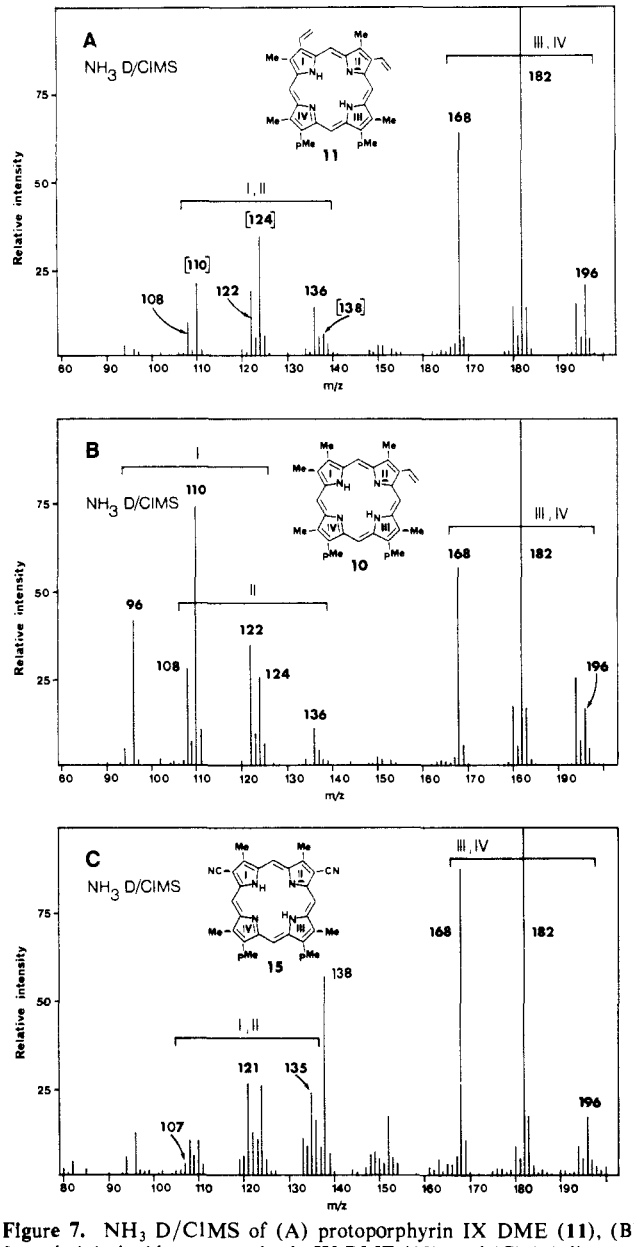


Figure 7. NH_3 D/CIMS of (A) protoporphyrin IX DME (**11**), (B) 2-methyl-4-vinyldeuteroporphyrin IX DME (**10**), and (C) 1,4-dicyanodeuteroporphyrin XIII DME (**15**) in their monopyrrolic fragment regions. The bracketed mass numbers indicate fragments which may be largely due to saturation of the vinyl group.

methoxycarbonyl function. The protonated fragment ions which arise from the methoxycarbonyl-containing rings are then shifted by 14 mass units and appear[19] at m/z 140, 154, and 168. Alternatively, the methyl propionic ester side chains can be easily converted to the ethyl esters. The methyl/ethyl propionic ester substituted pyrrole-protonated fragments are then shifted 14 mass units higher and would then appear (entry 5, Table I) at m/z 182, 196, and 210, with coincidence for only the satellite ions at m/z 182. In the (monoethoxycarbonyl)porphyrin **9**, the virtually ubiquitous adjacent methyl group has been replaced by a hydrogen atom; the protonated fragments associated with ring II appear at m/z 140, 154, and 168 as expected (Table I).

(ii) Other Substituted Porphyrins. Vinyl groups are also "silent" in the usual EIMS of porphyrins. They exhibit no characteristic fragmentations and often undergo hydrogen addition to yield ethyl groups. They would almost certainly also undergo hydrogen addition in the H_2 CI mode. Since one might expect hydrogen addition to nuclear vinyl groups to also occur when ammonia is used as the reagent gas, we have investigated the NH_3 D/CIMS of porphyrins containing one, 2-methyl-4-vinyldeuteroporphyrin IX DME (**10**), and two, 2,4-divinyldeuteroporphyrn IX DME (i.e.,

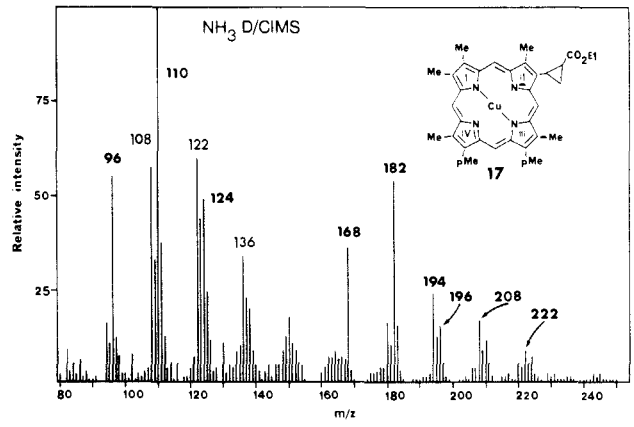


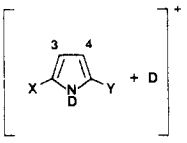
Figure 8. Monopyrrolic mass region of the NH_3 D/CIMS of compound **17**.

protoporphyrin IX DME, **11**), vinyl groups.

The molecular ion cluster (Table III) of protoporphyrin IX DME (**11**) did not show the peak at m/z 601 $(M + 10H + H)^+$ which would be expected if saturation of the vinyl groups was a major process. However, fragment ions in the dipyrrolic mass region (Table II) corresponding to the addition of two (for rings I + IV and II + III, entry20 in Table II) and four mass units (for rings I + II, entry 21) were found to be more abundant than those which would correspond to the protonated fragments containing unaltered vinyl groups. Further evidence for significant addition of hydrogen to the vinyl groups is also found in the monopyrrolic fragment mass region shown for **11** in Figure 7A. The fragments at m/z 108, 122, and 136 correspond to those expected for the methyl/vinyl substituent combination of rings I and II, whereas the generally more abundant triad of fragments at m/z 110, 124, and 138 are those anticipated for the methyl/ethyl combination which would arise from rings I and II after saturation of the vinyl groups. It might be noted that some of the intensity of these reputed methyl/vinyl pyrrolic fragments may also be due to the pathway involving benzylic hydrogen cleavage of odd-electron species derived from methyl/ethyl-substituted pyrrole rings.

The monopyrrolic mass region of the NH_3 D/CIMS of the monovinylporphyrin **10** shown in Figure 7B illustrates a somewhat more complicated situation which might well arise for a porphyrin of unknown structure. Here, assuming fragments from secondary pathways to be of minor importance, the protonated fragment ions at m/z 108, 122, and 136 appear to be due to the methyl/vinyl substituent pair of ring II. The protonated fragment ions at m/z 96, 110, and 124 are those expected for the methyl/methyl substituent combination of ring I. Of these latter fragments, only the one at m/z 96 is unique to ring I; the fragments at m/z 110 and 124 could also be due in part to the methyl/ethyl combination of ring II obtained after saturation of the vinyl group. Hence, the overlap of fragment ions from rings I and II as well as the possibility of coincidences would render the structure determination of porphyrin **10** tenuous.

In an attempt to overcome this problem, we converted the vinyl group of **10** into the cyclopropyl derivative **17** by treatment with ethyl diazoacetic ester. This reaction can be carried out on a microscale using the copper complex.[20] Since preliminary studies by us show that metals are extruded under NH_3 D/CI conditions, the metal complex could be used directly. The cyclopropyl function was expected to survive intact and (assuming an adjacent methyl substituent) the protonated fragments containing 0, 1, and 2 meso carbon atom (**26a–c**) should be found at m/z 194, 208, and 222. This region is well out of significant overlap with the ubiquitous methyl/methyl propionic ester triad at m/z 168, 182, and 196 but short of the dipyrrolic fragment mass region. To our disappointment, the mass spectrum (Figure 8) of the cyclopropyl derivative **17** proved to be virtually identical with that (Figure 7) of its precursor **10**. For instance, the expected fragment triad associated with ring II at m/z 194, 208, and 222 is weak and cannot be unambiguously identified. The reason for this unex-



36a X = D, Y = D
36b X = D, Y = CD_2H
36c X = D, Y = CD_3
36d X = CD_2H, Y = CD_2H
36e X = CD_2H, Y = CD_3
36f X = CD_3, Y = CD_3

Figure 9. Schematic representation of the deuterated ions which may contribute to the ND_3 D/CIMS in the monopyrrolic fragment mass region.

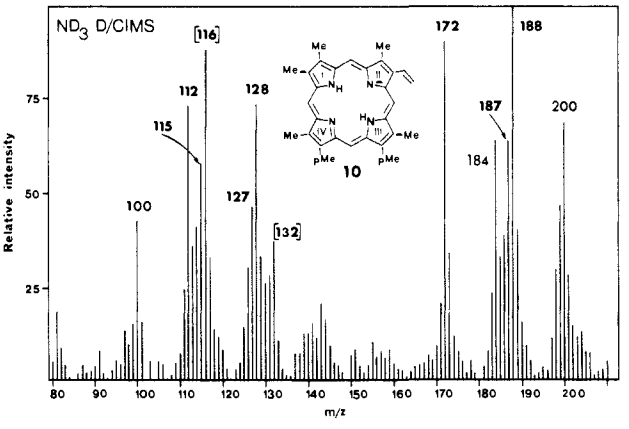


Figure 10. Monopyrrolic mass region of the ND_3 D/CIMS of 2-methyl-4-vinyldeuteroporphyrin IX DME (**10**). The mass numbers in brackets indicate fragments that may be partly due to the addition of deuterium to the vinyl group.

pected behavior, i.e., an apparent loss of (ethoxycarbonyl)carbene from 17 under NH_3 D/CI conditions, is currently not understood.

Facing the problems of using carbene addition products as diagnostic derivatives for identification of vinyl groups, we reasoned that the use of ND_3 as the reagent gas instead of NH_3 and an ensuing comparison of the spectra obtained under both conditions should rectify these problems. It was anticipated that the fragment triad at m/z 110, 124, and 138 in the NH_3 D/CIMS, which corresponded to a reduced methyl/vinyl-subsituted pyrrole, would be shifted in the ND_3 D/CIMS and a triad of deuterated fragments (**36a** and **d**, Figure 9) at m/z 116, 131 and 146 observed.

The monopyrrolic mass region of the ND_3 D/CIMS of **10** is shown in Figure 10. The spectrum is more complex and cluttered with fragment ions than the corresponding NH_3 D/CIMS (Figure 7B), which makes it more difficult to interpret. This is largely due to isotope-exchange reactions, which are apparent in the molecular ion region where a cluster of quasi-molecular ions are found separated by 1 mass unit.

The deuterated fragment ions at m/z 100, 115, and 130 are the triad (**36a**, **b**, and **d**) derived from the dimethyl-substituted ring I, whereas the fragment ions at m/z 112, 127, and 142 and m/z 116, 131, and 146 are the corresponding triads for the methyl/vinyl and the methyl/1,2-deuterioethyl substituent combinations of ring II, respectively. The fragment ions at m/z 172, 187, and 202 are the analogous species originating from rings III and IV. The fragment ions at m/z 128 and 188 may be largely due to species such as **36c**. The origin of the relatively abundant fragment ions at m/z 184 and 200 is not clear but appears to be due to species such as **32a** and **b** (Figure 4; deuterium instead of hydrogen), respectively.

Cyano-substituted porphyrins have yielded the least satisfying NH_3 D/CIMS in the monopyrrolic mass region. The protonated fragments (**26a–c**) corresponding to ring II of the monocyanoporphyrin **12** were found in only very low abundance (entry 13, Table I). 1,4-Dicyanodeuteroporphyrin XIII DME (**15**) did provide a measurable series of fragment ions in the methyl/cyano-substituted pyrrole region (Figur 7C). However, even here the triad does not show the normal characteristic appearance wherein the protonated fragment (**26b**, here at m/z 121) is clearly the most abundant peak of the triad. Additionally, this region is cluttered with other fragment ions (e.g., at m/z 138). The spectrum obtained from 2,4-dicyanodeuteroporphyrin IX DME (13) was only marginally different (Table I). We do not have a satisfactory explanation for this poor showing of the cyanoporphyrins under NH_3 D/CI conditions other than to assume that processes that favor the formation of uncharged species and adducts (e.g., amidines) may be more dominant in some cases than with the other substituted porphyrins. The fragment triad at m/z 124, 138, and 152, which is observed in the NH_3 D/CIMS of all cyanoporphyrins (**12**, **13**, and 15) investigated, may indeed originate from the addition of ammonia to the cyano groups. Substantiation of cyano substitution is more clearly revealed in the dipyrrolic mass region (see Figure 9A).

Dipyrrolic Fragment Ions. While the presence of particular fragment ions in the dipyrrolic mass region of the NH_3 D/CIMS helps to substantiate the data from the monopyrrolic mass region fragments. the principal utility of the dipyrrolic region lies in the establishment of the sequence of the pyrrole rings about the porphyrin macrocycle.

Table II gives the mass numbers and the relative intensities of the protonated dipyrrolic species which contains 0, 1, and 2 extra *meso* carbon units (structures **27a**, **b**, and **c**, respectively). Fragment ions which may arise via the secondary pathway are omitted from the table since they are generally of lower abundance (e.g., structures **29**, **33**, and **34**) and therefore do not obscure the elucidation of porphyrin structures. The number of entries is further truncated by omitting specific dipyrrolic fragments which involve permutation of the substituents on individual pyrrole rings because positional isomerism of this kind cannot be ascertained by NH_3 D/CIMS. Thus, the substitution pattern indicated for the dipyrrole rings in Table II may not always correspond to that in the structures of the individual porphyrins (Figure 1). Again, as indicated by footnotes e and f, coincidences among the protonated fragment ions occur only for the porphyrins bearing nuclear ethoxycarbonyl substituents.

Porphyrins are generally 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. Here the designations A, B, C, and D distinguish only the presence of different pairs of substituents on the pyrrole rings but do not further distinguish positional isomers arising from the interchange of the same two substituents on a particular pyrrole ring. These porphyrins comprise examples from each of these categories except for ones having four differently substituted rings.

(i) **A_4-Type Porphyrins.** Etio III (**1**), which contains only the methyl/ethyl substituent pair, is a prime example of this porphyrin class. Its NH_3 D/CIMS in the dipyrrolic region was shown in Figure 2A. The fragments arising from the protonated AA combinations are found at m/z 231, 245, and 259.

(ii) **A_2B_2-Type Porphyrins.** The spectrum in Figure 11A of 1,5-diformyldeuteroporphyrin II DME (**16**) illustrates the case of a porphyrin having two differently substituted rings arranged in the sequence ABAB for which only AB dipyrrolic fragments (m/z 303, 317, and 331) are possible. The mass spectrum obtained for **16** also demonstrates that rearrangements of dipyrrolic fragments and/or recombination of monopyrrolic fragments does not occur under NH_3 D/CI conditions since triads at m/z 231, 245, and 259 (due to the recombination of rings I and III) and at m/z 375, 389, and 403 (due to the recombination of rings II and IV) were of extremely low abundance.

When the two pairs of rings are arranged in the sequence AABB as for 2,4-dipropionyldeuteroporphyrin IX DME (**7**), the three dipyrrolic fragments AA (rings I + II), AB (rings I + IV and rings II + III), and BB (rings III + IV) which were formed (Figure 11B) provide a facile means of distinguishing between the two isomeric types of A_2B_2-type porphyrins. Notice that the ordering of the abundance of the fragment ions is variable within

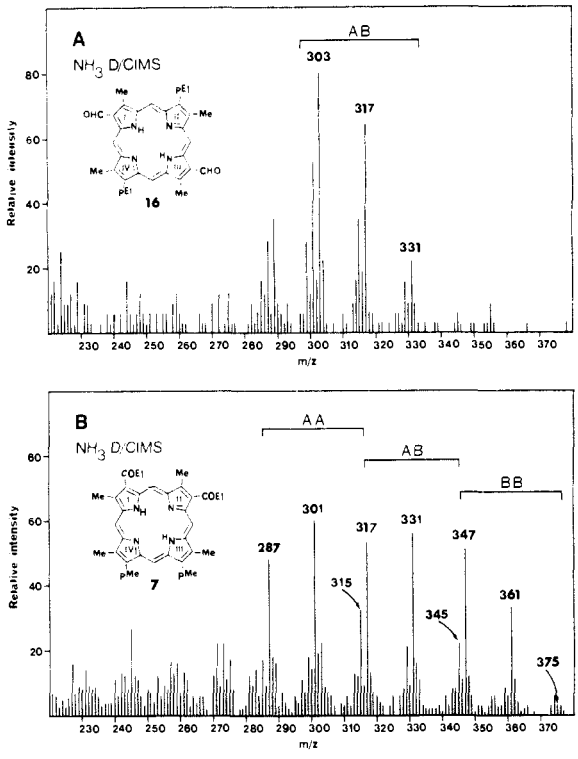


Figure 11. (A) Dipyrrolic mass region of the NH_3 D/CIMS of a porphyrin, 1,5-diformyldeuteroporphyrin II DEE (**16**), which contains two pairs of identically substituted rings arranged in the sequence ABAB. (B) The corresponding mass region of the NH_3 D/CIMS of 2,4-dipropionyldeuteroporphyrin IX DME (**7**) in which the two pairs of identically substituted rings occur in the sequence AABB.

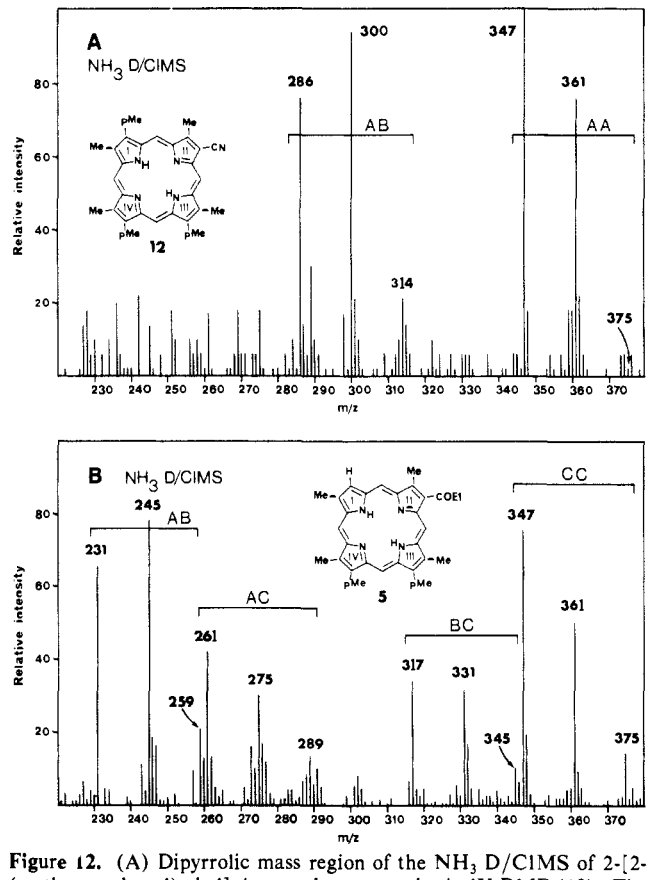


Figure 12. (A) Dipyrrolic mass region of the NH_3 D/CIMS of 2-[2-(methoxycarbonyl)ethyl]-4-cyanodeuteroporphyrin IX DME (**12**). The two differently substituted rings are in the sequence ABAA. (B) The corresponding region of the NH_3 D/CIMS of 4-propionyldeuteroporphyrin IX DME (**5**). The three differently substituted pyrrole rings are in the sequence ABCC.

the two triads. Frequently the dipyrrolic fragment without extra meso carbon units (**27a**), e.g., at m/z 347 in Figure 11B, occurs most often. In the monopyrrolic region, the fragment with one meso carbon unit was the most abundant ion of the triad. Thus, some care must be taken in examining spectra in the dipyrrolic mass region.

(iii) **A_3B-Type Porphyrins.** The monocyanoporphyrin, 2-[2-(methoxycarbonyl)ethyl]-4-cyanodeuteroporphyrin IX DME (**12**), contains three identically substituted pyrrole rings (rings I, III, and IV). Therefore, it can give rise (Figure 12A) to only the two dipyrrolic fragment triads AA (m/z 347, 361, and 375) and AB (m/z 286, 300, and 314). These fragment ions provide ample demonstration of the presence of the cyano substituent in ring II—a determination which could not be unambiguously made in the monopyrrolic mass region (vide supra). This conclusion is supported in the results obtained for the dipyrrolic fragments of the other two cyanoporphyrins (**13** and **15**) which contain only a single cyano group (entry 22, Table II). When the dipyrrolic fragment contains two cyano groups, (entry 23), these conclusions are less certain since the characteristic intensity pattern found for triads is not found for them.

(iv) **ABC_2-Type Porphyrins.** Since rings III and IV of 4-propionyldeuteroporphyrin IX DME (**5**) bear the same substituents, its specific sequence of pyrrole rings is ABCC. Four triads of dipyrrolic fragments are expected (AB, AC, BC, and CC) and are indeed observed (Figure 12B). Notice that if the sequence had been ACBC, only two triads of dipyrrolic fragments (AC and BC) would have been observed.

(v) **ABCD-Type Porphyrins.** No porphyrins of this type were investigated. The sequence variations possible are ABCD, ACBD, and ABDC, with each giving rise to four sets of dipyrrolic fragment triads. Since two of the dipyrrolic fragment triads are unique in each of the three sequence types, the ordering of the pyrrolic units about the porphyrin ring can be determined. The four dipyrrolic fragments will crowd the mass region (see Figure 12B) and possibly overlap, and in some cases there will be coincidences. Consequently, the most efficient procedure involves determining the pyrrolic substituents from the monopyrrolic mass region and then determining which of the possible dipyrrolic mass peaks are actually present.

Other Ions and the Molecular Ion Cluster. No discussion has been given of the tripyrrolic fragments; they are invariably of very low abundance and in any case do not provide unique structural information.

In the above discussion, we have briefly considered secondary cleavage ions which we presumed to arise by a different fragmentation pathway involving initial formation of odd-electron species. Ions derived from the protonated ions via substitution or elimination, for example, may also occur.[21,22] They do not, however, obscure the structural information provided by the generally more abundant simple protonated fragment ions.

The true molecular ion ($M^{+\cdot}$) is always formed in low abundance (Table III), whereas the protonated molecular ion $(M + H)^+$ is much more intense. The protonated porphyrinogen ions $(M + 6H + H)^+$ are usually much less abundant than the $(M + H)^+$ ions. An additional protonated quasi-molecular ion at 2 mass units higher, $(M + 8H + H)^+$, corresponding to the saturated vinyl group, was observed for the monovinylporphyrin **10**, although no such ion at 4 mass units higher, $(M + 10H + H)^+$, was found for protoporphyrin IX DME (**11**).

N-Substituted Porphyrins. N-Substituted porphyrins constitute an important class of porphyrins because of the discovery that a number of compounds which contain terminal olefinic or acetylenic groups, among them several drugs such as quinine, novonal (2,2-diethyl-4-pentenamide), norethisterone, ethynylestradiol, and ethchlorvynol, in addition to the well-known porphyrogenic compound, 4-alkyl-2,4-dihydropyridine, inactive cytochromes P-450

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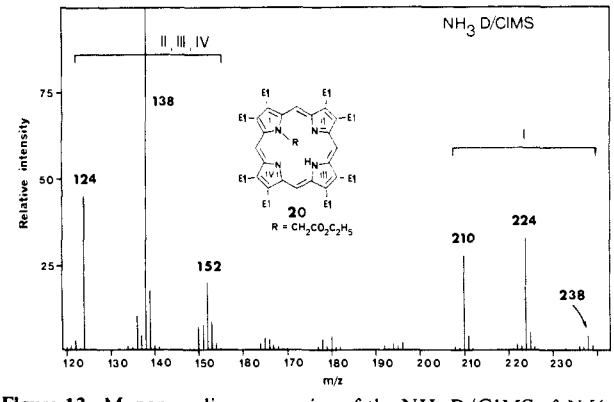

Figure 13. Monopyrrolic mass region of the NH_3 D/CIMS of *N*-[(ethoxycarbonyl)methyl]octaethylporphyrin (**20**). In contrast to the other porphyrins, the source temperature was 80 °C.

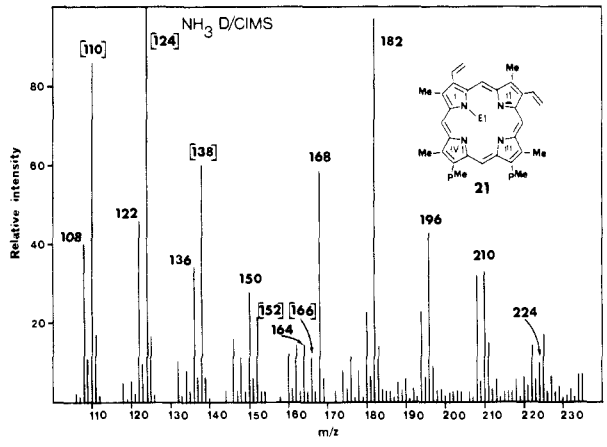

Figure 14. Monopyrrolic mass region of the NH_3 D/CIMS of the isomeric mixture of *N*-ethylprotoporphyrin IX DME (**21**). Mass numbers in brackets indicate fragments which may be largely due to saturation of the vinyl groups.

are reduced by a suicidal process with the ultimate formation of isomeric N-alkylated derivatives of iron protoporphyrin IX.[23] The suicide substrates appear to react preferentially with different forms of P-450, and the isomeric distribution of *N*-alkylprotoporphyrin IX depends on the particular substrate.[23] With propene, for example, the predominant isolated metal-free N-adduct of protoporphyrin IX DME contains a 2-hydroxyethyl substituent on the nitrogen of ring IV, whereas with propyne the nitrogen of ring I bears a 2-oxopropyl group.[23d,j] Structural information about these compounds has largely been derived from their NMR and field desorption mass spectra.[24]

Encouraged by our results in the β-substituted pyrrole series which show that the peripheral substituents of the protonated mono- and dipyrrolic fragments undergo further cleavages only to a minor extent, we have investigated the NH_3 D/CIMS of two N-substituted derivatives of octaethylporphyrin, **19** and **20**. These derivatives were chosen as models of the kinds of porphyrins produced during suicidal inactivation of cytochrome P-450. Preliminary work on *N*-(trideuteriomethyl)octaethylporphyrin (**19**) indicated N-dealkylation from one of the molecular ions was not a primary process under NH_3 D/CI conditions at a common source temperature of 200 °C. The monopyrrolic mass region clearly exhibited fragment ions associated with retention of the trideuteriomethyl group. Because inactivation of cytochrome P-450 with a majority of suicide substrates results in the formation of porphyrins containing more functionalized and hence more labile N-alkyl substituents, a more appropriate model N-substituted porphyrin, *N*-[(ethoxycarbonyl)methyl]octaethylporphyrin (**20**), was assayed. The NH_3 D/CI mass spectrum obtained for **20** indicated that porphyrins having sterically more demanding and labile N-substituents were more advantageously measured at lower source temperature (80–100 °C). The monopyrrolic mass region of the NH_3 D/CIMS of **20** is presented in Figure 13. The protonated fragment ions at *m/z* 124, 138, and 152 comprise the triad (**26a–c**) arising from the three pyrrole rings without the N-substituent, whereas the triad at *m/z* 210, 224, and 238 comes from the ring which bears the *N*-[(ethoxycarbonyl)methyl] group. Further fragmentation via the loss of the alkyloxy function is of relatively minor importance. Thus, it appears reasonable to suppose that, in general, N-substituents will largely retain their structural integrity under NH_3 D/CI conditions like their β-pyrrole counterparts.

On the basis of these results and the very small quantities of the porphyrins which are required, we believe that NH_3 D/CIMS should find a very useful application for investigating the topologies of the active sites of the cytochromes P-450 which are involved in the metabolism of particular drugs. When, for example 4-alkyl-1,4-dihydropyridines, which have found widespread use as probes in biochemical and mechanistical studies of cytochromes P-450, are administered as inactivators of cytochromes P-450, the 4-alkyl substituent is transferred to the nitrogens of iron protoporphyrin IX.[23e,f] These agents exhibit a relatively low regioselectivity, and all four isomers are formed. Depending, however, on the pretreatment with different inducers of cytochromes P-450, particular N-alkylated isomers are prevalent in the mixture of abnormal porphyrins isolated.[23e,f,g] An exception is the isomers corresponding to alkylation of ring II which are generally found in minor amounts. As an example of the usefulness of NH_3 D/CIMS in these types of studies, the mass spectrum of the isomeric mixture of *N*-ethylprotoporphyrin IX DME (**21**) was assayed which is isolated when 3,5-(diethoxycarbonyl)-4-ethyl-2,6-dimethyl-1,4-dihydropyridine has been administered as suicide substrate. The individual isomers were not separated (which can easily be accomplished), in order to demonstrate the usefulness of NH_3 D/CIMS in determining the regiochemistry of heme alkylation. Therefore, the NH_3 D/CI mass spectrum of **21** can simply be regarded as the sum of the mass spectra of individual isomers.

The monopyrrolic mass region of the NH_3 D/CIMS of **21** is shown in Figure 14. Careful comparison of this spectrum with that obtained for protoporphyrin IX DME (**11**) itself, notwithstanding the coincidences that do occur in the spectrum of **21**, leads to the ready distinction of the fragment ions which have originated from *N*-alkylation. Alkylation of rings I or II results in the observation of an additional triad of protonated fragment ions at *m/z* 136, 150, and 164 in addition to ions at *m/z* 138, 152, and 166 due to saturation of the vinyl group. Alkylation of rings III or IV is detected by the occurrence of protonated fragment ions at *m/z* 196, 210, and 224. The actual viability of this particular application of NH_3 D/CIMS in biological studies will have to be further experimentally assessed. NH_3 D/CIMS does not provide information regarding which of the pairs of rings (I or II and III or IV) are alkylated since unique fragments in the dipyrrolic mass region required for such diagnosis are not formed. Its main advantage is that partial isomer information can be derived from very small quantities of samples—a feature not currently shared by NMR.

Conclusions. The results presented here demonstrate that NH_3 D/CIMS is a very useful method for the elucidation of porphyrin

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structures. It provides specific structural information about the number of differently substituted pyrrole rings, the particular pairs of substituents (alkyl, side-chain ester, nuclear ester, vinyl, formyl, acyl, and cyano) at the β-pyrrole positions as well as substituents on the internal nitrogens, and the sequence of adjacent pyrrole rings about the porphyrin macrocycle. A particularly illustrative example was given in the full spectrum of 4-acetyldeuteroporphyrin IX DME (**4**) presented in the preliminary communication.[18] It does not, however, distinguish isomeric porphyrins which arise from interchange of the substituents on an individual pyrrole ring. Although not examined here, NH_3 D/CIMS may also be of value for elucidation of other kinds of porphyrin structures such as chlorins and corroles.

The NH_3 D/CIMS of porphyrins themselves is much more informative than EIMS of the separately formed and isolated porphyrinogens.[7] The use of ammonia rather than hydrogen as the reagent gas is to be preferred since the extensive β-cleavage fragments formed by using the latter gas clutter the MS and may obscure the identification of particular intact pyrrolic and dipyrrolic fragment ions.

Finally, NH_3 D/CIMS does not, of course, supplant porphyrin structural analysis via other physical methods—notably, by X-ray crystallography, NMR, or absorption and IR spectroscopy. Its advantage is that much smaller samples are required in order to obtain a nearly complete description of the porphyrin structure.

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Registry No. **1**, 26608-34-4; **2**, 2683-82-1; **3**, 32464-39-4; **4**, 15295-25-7; **5**, 100230-02-2; **6**, 10591-31-8; **7**, 90993-65-0; **8**, 56674-10-3; **9**, 91238-18-5; **10**, 100230-03-3; **11**, 5522-66-7; **12**, 90508-48-8; **13**, 7253-86-3; **14**, 100230-04-4; **15**, 100230-05-5; **16**, 100230-06-6; **17**, 100230-47-5; **18**, 100230-48-6; **19**, 100230-07-7; **20**, 55580-23-9; **21**, 80418-43-5; **22**, 100230-08-8; **23a**, 100230-09-9; **23b**, 93565-19-6; **23c**, 100230-10-2; **24a**, 100230-11-3; **24b**, 100230-12-4; **24c**, 100230-13-5; **25a**, 100230-14-6; **25b**, 100230-15-7; **25c**, 100230-16-8; **26a**, 35745-44-9; **26b**, 100230-17-9; **26c**, 100230-18-0; **27a**, 100230-19-1; **27b**, 100230-20-4; **27c**, 100230-21-5; **28a**, 34468-30-9; **28b**, 100230-22-6; **28c**, 85805-84-1; **29a**, 100230-23-7; **29b**, 100230-24-8; **29c**, 100230-25-9; **30a**, 100230-26-0; **30b**, 100230-27-1; **30c**, 100230-28-2; **31a**, 100230-29-3; **31b**, 100230-30-6; **31c**, 100230-31-7; **32a**, 40079-18-3; **32b**, 100230-32-8; **33a**, 100230-33-9; **33b**, 100230-34-0; **33c**, 100230-35-1; **34a**, 100230-36-2; **34b**, 100230-37-3; **34c**, 100230-38-4; **35a**, 100230-39-5; **35b**, 100230-40-8; **36a**, 100230-41-9; **36b**, 100230-42-0; **36c**, 100230-43-1; **36d**, 100230-44-2; **36e**, 100230-45-3; **36f**, 100230-46-4; NH_3, 7664-41-7; H_2, 1333-74-0; cytochrome P-450, 9035-51-2.

On the Mechanism of Alkyne-Forming Elimination Reactions: A Theoretical Study

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Abstract: A theoretical study employing ab initio molecular orbital calculations on the elimination of HX from halosubstituted alkenes to form alkynes is described. Upon substitution of fluorine in the β-position of ethylene anion, the inversion barrier drops ~20 kcal/mol while an α-fluoro or α-chloro substituent raises the inversion barrier of a vinyl anion by ~50 kcal/mol. The activation barrier for the elimination of fluoride ion from syn 2-fluorovinyl anion was calculated to be 4.1 kcal/mol higher in energy than that of the corresponding anti anion. We conclude that essentially all of the activation barrier in these alkyne-forming elimination reactions may be ascribed to electronic effects associated with inversion at the carbanionic center.

The stereochemistry of alkene- and alkyne-forming E2 eliminations has been intensively studied, and many factors that contribute to the overall syn or anti stereochemical course of these reactions have been identified.[1] Mechanistic rationale for this important class of reactions developed simultaneously with stereochemical studies on the S_N2 nucleophilic substitution reaction. Hughes[2] recognized that 1,2-elimination reactions may be thought of as an internal nucleophilic substitution at the carbon bearing the leaving group by the developing electron pair at the β carbon. One of the features essential for the description of a concerted 1,2-elimination process is the dihedral angle about the C_α–C_β bond in the activated complex. It was first suggested by DePuy,[3] and supported by experiments on various systems, that arrangements in which the C_α–X and the C_β–H bonds are antiperiplanar (dihedral angle 180°) or synperiplanar (dihedral angle 0°) will be preferred in the transition state of an HX elimination reaction. Ingold[4] and Sicher[5] have suggested that if C_β–H bond cleavage in a concerted syn elimination were considerably advanced in the transition state (such as in the E1cb-like process), sufficient ionicity could develop on the β carbon for the charge to spread on both sides of this carbon and thus proceed with inversion of configuration at the β carbon prior to elimination. In today's mechanistic parlance such alkene-forming reactions could be said to proceed by an antarafacial mode of elimination but still exhibit syn-stereospecificity with respect to the leaving group and the β-hydrogen undergoing elimination.[6]

In a recent theoretical study, we corroborated the above suggestion.[6] Employing ab initio calculations on a series of model carbanions, we found that halogenated aliphatic compounds do

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