Autoxidation and Solvolysis Products of Octaethylverdohaemochrome

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Aerobic degradations of (i) octaethylverdohaemochrome (**1**) bis(pyridine)iron(II) 2,3,7,8,12,13,17,18-octaethyl-5-oxaporphyrinate in 5% pyridine–methanol, (ii) octaethylbilindione (**2**) in the presence of thallium(III) acetate in methanol, and (iii) (**2**) upon t.l.c. have been investigated. These degradations were caused by solvolysis and autoxidation. Eight degradation products obtained from reaction (i) were isolated, and their structures were elucidated by electronic absorption spectrophotometry and mass and n.m.r. spectroscopy as follows: octaethylbilindione (**2**), 2,3,7,8,12,13,17,18-octaethyl-19-methoxy-21*H*-bilin-1-one (**3**), 2,3,7,8,12,13-hexaethyl-14-methoxy-15*H*,16*H*-tripyrrin-1-one (**4**), methyl 2,3,7,8,12,13-hexaethyl-1-oxo-15*H*,16*H*-tripyrrin-14-carboxylate (**5**), 2,3,7,8,12,13-hexaethyl-1-oxo-15*H*,16*H*-tripyrrin-14-carbaldehyde (**6**), (4*RS*,5*RS*)- (**7**) and (4*RS*,5*SR*)- 2,3,7,8,12,13,17,18-octaethyl-4,5-dimethoxy-4,5-dihydro-21*H*,24*H*-bilin-1,19-dione (**8**), and 2,3,7,8,12,13-hexaethyl-14-pyridinio-15*H*,16*H*-tripyrrin-4-yl tetrafluoroborate (**9**). The products (**6**), (**7**), and (**8**) obtained from reaction (ii), and (**6**) obtained from reaction (iii) were identical with those obtained from reaction (i). Both (4*RS*,5*RS*)- (**7**) and (4*RS*,5*SR*)-dimethoxy adduct (**8**) were obtained from reactions (i) and (ii). Only the (4*RS*,5*RS*)-ethylenedioxy adduct (**10**) was obtained when ethylene glycol instead of methanol was used as solvent.

Haem compounds are particularly susceptible to oxidative attack at the *meso*-carbons which link the four pyrrole rings of the porphyrin macrocycle. Oxidation leads to the rupture or elimination of the carbon bridge and results in cleavage of the porphyrin ring and formation of an open-chain tetrapyrrolic structure.[1] In human beings and many animals, endogeneous haem (protohaem IX) is degraded to give the blue pigment, biliverdin, which ultimately is reduced enzymatically to bilirubin.[1c,2] Foulkes *et al.*,[1b] postulated the presence of an intermediate, verdohaemochrome, in the analogous chemical process that was termed a coupled oxidation by Lemberg *et al.*[1d] Biliverdin is the final product in the coupled oxidation of haem in the pyridine solution.[3] Jackson *et al.*,[4a] and Bonnett and Dimsdale[4b] observed the formation of the verdohaemochromes by oxygenation of the corresponding oxyporphyrins; their results thus supported Lemberg's proposal for the formation of verdohaemochrome.[3b]

We previously prepared verdohaemochrome IXα by the coupled oxidation of haemoglobin and myoglobin and identified it as (pyridinyl)iron(II) oxaprotoporphyrinate IXα[5]. Lagarias[6] and Hirota and Itano[7] elucidated the structure of octaethylverdohaemochrome obtained by the coupled oxidation of octaethylhaemin as bis(pyridinyl)iron(II) octaethyloxaporphyrinate. Recently, we synthesized verdohaemochrome by ring closures of the corresponding bilindiones.[8]

Verdohaemochromes upon treatment with an excess of mineral acid undergo hydrolysis and demetallation to give bilindiones. This reaction is accompanied by autoxidation of the chromophore to give bilipurpurins.[9] Bonnett *et al.*,[10] reported the *meso*-reactivity of octaethylbilindione, from which the 4,5-dialkoxy compounds were produced by oxidation with bromine in ethanol or methanol.

In this paper, we report the isolation and structural investigation of the degradation products of octaethylverdohaemochrome (1) in 5% pyridine–methanol and octaethylbilindione (2) with thallium(III) acetate in methanol; a comparison is also made between the products obtained from (1) and (2) on t.l.c. The autoxidation of (1) in ethylene glycol–pyridine (1:1), and the reaction of (2) with thallium(III) acetate in ethylene glycol–CH_2Cl_2 (1:1) are also discussed.

Results and Discussion

Solvolysis and autoxidation of octaethylverdohaemochrome (1) in pyridine–methanol, gave eight degradation products (**A**—**H**), isolated by column chromatography and preparative t.l.c. Compounds **E**, **F**, and **G** were also obtained by oxidation of octaethylbilindione (2) with thallium(III) acetate in methanol, and **F** was obtained by autoxidation of (2) on silica gel t.l.c. Yields of the degradation products (**A**—**H**) are given Table 1. Electronic absorption maxima, ^{1}H n.m.r. and mass spectral data are given in Tables 2, 3, and 4, respectively. Product **D** was identified as authentic octaethylbilindione (2)[6,7] by electronic absorption, ^{1}H n.m.r. and mass spectroscopies, and mixed t.l.c. analysis.

The ^{1}H n.m.r. spectrum of **A** shows signals for 3 *meso* protons and for eight ethyl and methoxy protons (Table 3). These results indicate that **A** is 2,3,7,8,12,13,17,18-octaethyl-19-methoxy-21*H*-bilin-1-one (3). Support of this deduction is provided by the e.i. mass spectrum which reveals a molecular ion peak at *m/z* 568 (base peak) and fragment in peaks at *m/z* 553 (M^+ − Me), 539 (M^+ − CH_2Me), and 537 (M^+ − OMe).

The ^{1}H n.m.r. spectrum of product **B** reveals signals for two methine, six ethyl, and one methoxy protons. Mass spectroscopy of product **B** provide a molecular ion peak at *m/z* 435 (base peak) and fragment ion peaks at *m/z* 420 (M^+ − Me), 406 (M^+ − CH_2Me), and 404 (M^+ − OMe). These results suggest that product **B** is 2,3,7,8,12,13-hexaethyl-14-methoxy-15*H*,16*H*-tripyrrin-1-one (4).

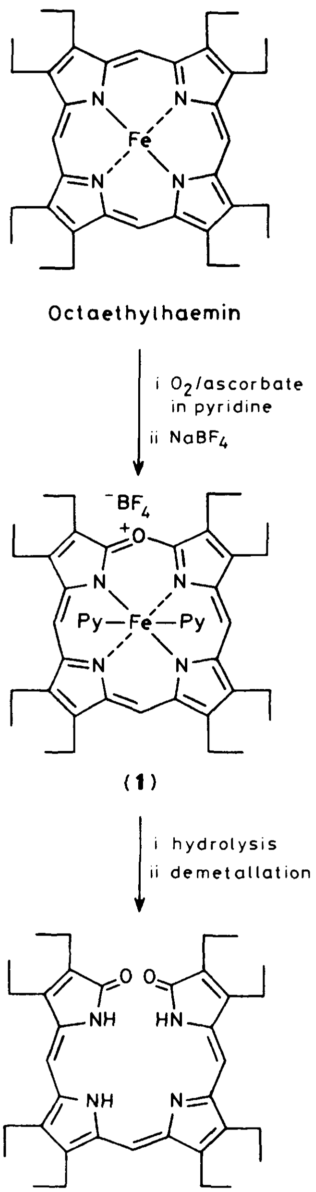
It is well known that compound (1) is susceptible to solvolysis.[11] Hydrolysis and methanolysis of (1) followed by demetallation gave compounds (2) and (3), respectively. Formation of compound (4) may result from further oxidative degradation of (3). However, the low yield of (4) suggests that the contribution from this type of degradation is small.

The ^{1}H n.m.r. spectrum of product **C** shows proton signals of

Table 1. R_F Values and yields of degradation products

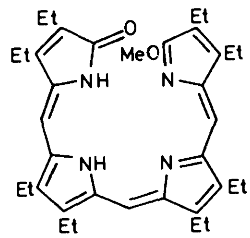
Product	Compd.	Colour	R_F value[a]	Yields (%): Products obtained by the reaction of (1) in 5% pyridine–MeOH	Yields (%): Products obtained by the reaction of (2) with $Tl(OAc)_3$ in MeOH[4a]	Yields (%): Products obtained from (2) on t.l.c.
A	(3)	Blue	0.84	8.1	0	0
B	(4)	Red	0.73	1.1	0	0
C	(5)	Red	0.68	2.3	0	0
D	(2)	Blue	0.41	10.8	8.4	13.3
E	(7)	Red	0.39	1.5	4.1	0
F	(6)	Red	0.30	21.0	11.7	66.9
G	(8)	Red	0.11	32.4	64.8	0
H	(9)	Blue	0.00 (0.35)[b]	8.4	0	0

[a] Solvent system was benzene–acetone (9:1). [b] This value (0.35) was obtained with the solvent system CH_2Cl_2–MeOH (9:1).

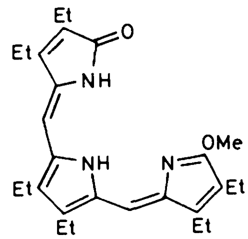


(2) (D)

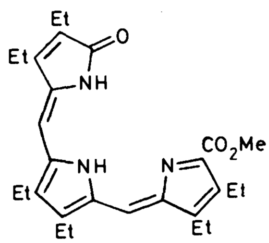
Scheme. Coupled oxidation of octaethylhaemin with ascorbate in pyridine exposed to air



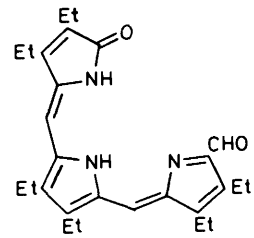
(3) (A)



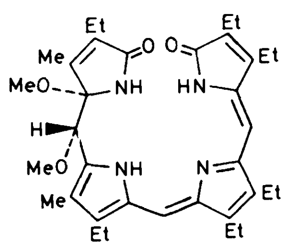
(4) (B)



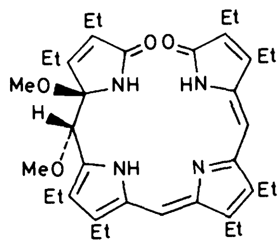
(5) (C)



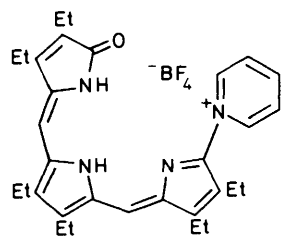
(6) (F)



(7) (E)



(8) (G)



(9) (H)

Table 2. Electronic absorption spectra of degradation products in CH_2Cl_2

Compd.	Free bases $\lambda_{max.}$/nm (ε mM)				Zinc complexes[a] $\lambda_{max.}$/nm (ε mM)			
(2)	650	369			710	371		
	(12.2)	(40.6)			(14.7)	(31.7)		
(3)	665	620sh[b]	369		793	730sh	403	375sh
	(12.3)	(10.0)	(38.2)		(12.9)	(8.9)	(26.6)	(21.8)
(4)	518	503	323		617	572	530	339
	(16.3)	(16.4)	(21.9)		(21.4)	(11.1)	(9.0)	(21.7)
(5)	532	500	465sh	315	616	570	339	
	(11.6)	(9.7)	(5.1)	(17.1)	(16.9)	(10.2)	(16.2)	
(6)	540	505	475sh	320	625	580	332	
	(5.8)	(5.8)	(3.7)	(21.2)	(5.8)	(4.0)	(18.8)	
(7)	555	520sh	325		627	577	543sh	338
	(18.5)	(15.2)	(25.0)		(40.0)	(15.2)	(5.5)	(31.5)
(8)	554	520sh	329		629	579	545sh	338
	(18.9)	(14.6)	(26.2)		(47.6)	(14.8)	(4.9)	(35.1)
(9)	643	385sh	337		604	520	400sh	335
	(11.3)	(8.3)	(18.5)		(8.3)	(5.2)	(7.7)	(15.4)
(10)	556	520sh	325		627	576	545sh	338
	(18.0)	(14.2)	(25.1)		(38.1)	(15.1)	(6.0)	(31.4)

[a] 100 μl Of zinc acetate (0.91M) in MeOH was added to each sample cuvette. [b] sh = Shoulder.

Table 3. ^1H N.m.r. (270 MHz) data of degradation products in $CDCl_3$

Compd.	*meso*-Protons	$-CH_2-$	–Me	D_2O exchangeable protons	Others
(3)	6.00 (1 H)	2.62—2.40 (12 H)	1.23—0.98 (24 H)	13.01 (1 H)	4.01 (3 H, s, OMe)
	6.27 (1 H)	2.28 (2 H)		10.29 (1 H)	
	5.74 (1 H)	2.19 (2 H)			
(4)	6.38 (1 H)	2.59—2.28 (12 H)	1.25—1.07 (18 H)	11.78 (1 H)	4.20 (3 H, s, OMe)
	5.98 (1 H)			7.49 (1 H)	
(5)	6.69 (1 H)	2.79 (2 H)	1.29—1.13 (18 H)	13.00 (1 H)	3.98 (3 H, s, OMe)
	5.91 (1 H)	2.65—2.41 (10 H)		9.40 (1 H)	
(6)	6.67 (1 H)	2.77—2.40 (12 H)	1.29—1.13 (18 H)	12.65 (1 H)	9.82 (1 H, s, CHO)
	5.88 (1 H)			9.36 (1 H)	
(7)	6.63 (1 H)	2.60—2.42 (10 H)	1.22—0.68 (24 H)	9.90 (2 H)	4.63 (1 H, s, C*H*OMe)
	5.93 (1 H)	2.19—2.02 (6 H)		8.66 (1 H)	3.47 (3 H, s, OMe)
					3.19 (3 H, s, OMe)
(8)	6.76 (1 H)	2.67—2.00 (16 H)	1.22—0.99 (24 H)	9.30—8.90 (2 H)	4.41 (1 H, s, C*H*OMe)
	5.91 (1 H)			6.31 (1 H)	3.33 (3 H, s, OMe)
					2.98 (3 H, s, OMe)
(9)	7.14 (1 H)	2.74—2.38 (12 H)	1.29—1.04 (18 H)	7.60 (broad signal)	9.16 (2 H, d, *J* 7 Hz, α-protons on pyridinio group)
	6.01 (1 H)				8.60 (1 H, t, *J* 7 Hz, γ-proton on pyridinio group)
					8.34 (2 H, t, *J* 7 Hz, β-protons on pyridinio group)
(10)	6.62 (1 H)	2.56—2.24 (12 H)	1.26—1.09 (24 H)	10.10—9.90 (2 H)	4.51 (1 H, td, *J* 12.1, 3.3 Hz)
	5.94 (1 H)	2.12—2.00 (4 H)		9.17 (1 H)	4.13 (1 H, dd, *J* 11.7, 2.9 Hz)
					4.01 (1 H, td, *J* 11.7, 3.3 Hz)
					3.85 (1 H, dd, *J* 12.1, 2.9 Hz) (four protons on ethylenedioxy group)

Chemical shifts were obtained in p.p.m. from internal tetramethylsilane.

two methine groups, six ethyl groups, and one methoxy group, whereas the i.r. spectrum of **C** reveals a band at 1 730 cm^{-1} (CO). The e.i. mass spectrum of **C** shows a molecular ion peak at *m/z* 463 (base peak) and fragment ion peaks at *m/z* 448 (M^+ – Me), 434 (M^+ – CH_2Me), and 432 (M^+ – OMe). Such results indicate that product **C** is methyl 2,3,7,8,12,13-hexaethyl-1-oxo-15*H*,16*H*-tripyrrin-14-carboxylate (**5**).

The ^1H n.m.r. spectrum of product **F** reveals proton signals for two *meso*-methine groups, six ethyl groups, and the presence of an aldehyde group. The e.i. mass spectrum showed a molecular ion peak at *m/z* 433 (base peak) and fragment ion peaks at *m/z* 418 (M^+ – Me) and 404 (M^+ – CH_2Me). These results suggest that product **F** is 2,3,7,8,12,13-hexaethyl-1-oxo-15*H*,16*H*-tripyrrin-14-carbaldehyde (**6**).

Product **H** in CH_2Cl_2 solution is blue, as are compounds (**2**) and (**3**). The absorption spectrum of **H** in CH_2Cl_2 provides a $\lambda_{max.}$ at 643 nm (Table 2). Addition of zinc acetate produces a hypsochromic shift (643 ⟶ 604 nm). Whereas, compounds (**2**) and (**3**) undergo bathocromic shifts, 650 ⟶ 710 nm and 665 ⟶ 793 nm, respectively. The ^1H n.m.r. spectrum of product **H** shows proton signals for two methine and six ethyl groups as well as those of a pyridinio group. The f.a.b.-m.s. of

Table 4. Mass spectral data of degradation products

Compd.[a]	Mass number (relative intensity)[b]
(3)	568 (100), 553 (74.2), 539 (30.1), 537 (5.1), 523 (5.6), 524 (5.6), 509 (11.7), 225 (5.7)
(4)	435 (100), 422 (3.0), 420 (29.7), 406 (6.8), 405 (4.4), 404 (5.7), 319 (5.5), 390 (4.4), 376 (5.5)
(5)	463 (100), 448 (26.1), 449 (17.9), 475 (14.9), 434 (43.2), 433 (14.9), 432 (17.0), 431 (33.8), 421 (10.1), 416 (21.0), 403 (21.0), 402 (42.5), 388 (34.6), 377 (23.0)
(6)	433 (100), 418 (4.2), 404 (37.5), 390 (11.9), 389 (7.5), 376 (14.2), 285 (11.8), 272 (11.0), 269 (12.4), 255 (36.9), 253 (20.4), 241 (11.1), 239 (14.0), 237 (12.1), 227 (11.6), 225 (19.8)
(7)	585 (16.0), 570 (3.0), 555 (8.0), 449 (81.7), 448 (100), 435 (8.8), 434 (13.7), 418 (7.3), 404 (6.7)
(8)	585 (6.4), 555 (9.4), 449 (64.1), 448 (100), 435 (8.1), 434 (15.2), 419 (5.6), 418 (7.3), 404 (6.7)
(9)	483 (80.1), 252 (82.0), 180 (93.1), 123 (93.3), 122 (91.5), 106 (56.8), 94 (72.7), 79 (44.9), 78 (49.8), 71 (100)
(10)	614 (63.2), 600 (17.9), 554 (5.3), 448 (5.4), 434 (70.6), 433 (100), 432 (7.4), 419 (31.8), 418 (22.4), 404 (41.5), 402 (5.0), 390 (11.2), 376 (11.9), 375 (7.9)

[a] Data of (3)—(8) and (10) were obtained with e.i.–m.s. The data of (9) was obtained with f.a.b.–m.s. [b] Relative intensity is % of base peak.

product **H** shows the fragment ion peak at *m/z* 438 (80% of base peak). These results, together with those from elemental analysis, suggest that product **H** is 2,3,7,8,12,13-hexaethyl-14-pyridinio-15*H*,16*H*-tripyrrin-14-yl tetrafluoroborate (**9**).

A possible mechanism to explain formation of compound (**9**) involves (i) attack at the carbon atom attached to the oxonium cation in (**1**) by a nucleophile (in this case pyridine). This causes the tetrapyrrolic ring to open. (ii) Oxidative degradation and demetallation follow to give (**9**). The exact mechanism of the formation of (**9**) from (**1**) remains obscure, but it is unlikely that compounds (**2**) and (**4**) are involved since when (**2**) or (**4**) was dissolved in a solution of 5% pyridine–MeOH, each compound was recovered unchanged.

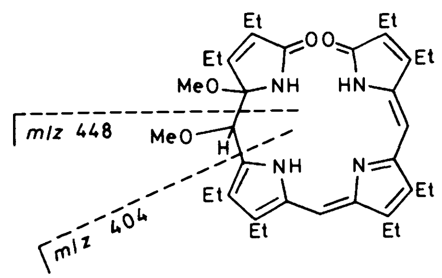


Figure 1. Mass fragmentation of compounds (**7**) and (**8**)

Products **E** and **G** showed very similar absorption spectra (Table 2). Thus each product showed n.m.r. signals for two *meso*-protons (6.63 and 5.93 p.p.m. in product **E**, and 6.67 and 5.91 p.p.m. in product **G**), suggesting that they were tripyrrins. However, each compound showed proton signals for eight ethyl groups and one methine group (4.63 p.p.m. in product **E**, and 4.31 p.p.m. in **G**) as well as two methoxy groups (3.37 and 3.19 p.p.m. in **E**, 3.33 and 2.98 p.p.m. in **G**). The e.i. mass spectra of products **E** and **G** showed fragment ion peaks at *m/z* 585 (M^+ $-OCH_3$), 448 (base peak), and 404, respectively (Figure 1). Products **E** and **G** were therefore characterized as dimethoxy derivatives of the bilindione (**2**).

Dialkoxy adducts of bilindiones have previously been reported by Fischer *et al.*[12] and Siedel *et al.*[13] Fischer found that treatment of 2,7,13,18-tetramethyl-3,8,12,17-tetraethyl-bilindione with quinone in methanol gave the 5,15-dimethoxy compound, whilst Siedel[13] obtained the 4,5-dimethoxy and 4,5,15,16-tetramethoxy compounds by reaction of meso-biliverdin with bromine in the presence of methanol; the 4,5-ethylenedioxy adduct was also obtained by the same reaction using ethylene glycol instead of methanol. Autoxidation has been postulated as a mechanism for the formation of dialkoxy adducts by Bonnett *et al.*[10] and Smith *et al.*[14] Furthermore Bonnett *et al.*[10] established that the general structure of the 4,5-dialkoxy adduct was (4*RS*,5*SR*) by *X*-ray analysis.

Treatment of compounds **E** and **G** with mineral acid in the presence of air, which resulted in both hydrolysis and autoxidation, gave only one product (**6**). These results showed that both compounds **E** and **G** are diastereoisomers of 4,5-dimethoxy adducts. Although Bonnett *et al.*[10] and Smith *et al.*[14] obtained only one isomer, the (4*RS*,5*SR*)-dimethoxy compound, both of the diastereoisomers **E** and **G** were obtained in the ratio 1:22 from reaction (i) and in the ratio 1:16 from reaction (ii), respectively. Although the melting point of **G** was different from that of the 4,5-dimethoxy compound that Bonnett *et al.*[10] obtained (crystallization from different solvents may have produced different crystalline forms), the ^{1}H n.m.r. spectrum of compound **G** was similar to that of the (4*RS*,5*SR*)-dimethoxy adduct but that of compound **E** was different. These results indicate that compound **G** is (4*RS*,5*SR*)-2,3,7,8,12,13,17,18-octaethyl-4,5-dimethoxy-4,5-dihydro-21*H*,24*H*-bilin-1,19-dione (**8**) and that compound **E** is (4*RS*,5*RS*)-2,3,7,8,12,13,17,18-octaethyl-4,5-dimethoxy-4,5-dihydro-21*H*,24*H*-bilin-1,19-dione (**7**).

The bilindione isomer (**8**) is by far the major product in the degradation of (**1**) in 5% pyridine–MeOH and in the reaction of (**2**) with thallium(III) acetate in MeOH. Compound (**7**), after treatment with 5% H_2SO_4–MeOH, is found to give the starting material (**7**) and its isomer (**8**) in the ratio 1:3. Similarly, the starting material (**8**) and its isomer (**7**) were obtained from compound (**8**) in the ratio 3:1.

The preparation of compound (**10**) from reactions (i) and (ii) with ethylene glycol instead of methanol as solvent was attempted. As both compounds (**1**) and (**2**) are virtually insoluble in ethylene glycol, ethylene glycol–pyridine (1:1) was used in reaction (i) and ethylene glycol–CH_2Cl_2 (1:1) was used in reaction (ii).

Autoxidation of verdohaemochrome (**1**) in ethylene glycol–pyridine (1:1) gave the products (**1**), (**6**), (**9**) and an unknown compound. The ^{1}H n.m.r. spectrum of the latter product shows signals for two *meso* protons, eight ethyl groups, and four protons of an ethylenedioxy group. These results indicate that the product is the 4,5-ethylenedioxy adduct (**10**). This is also

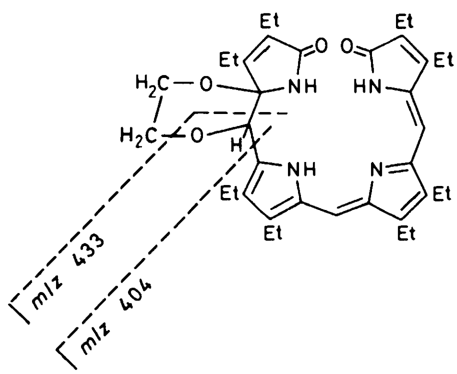


Figure 2. Structure and mass fragmentation of compound (**10**)

suggested by the mass spectrum, which shows molecular ion peaks at m/z 433 (100%) and 404 (41.5%) (Table 4 and Figure 2).

Compound (**10**) was also obtained together with (**6**) and unchanged starting material (**2**) by the reaction of (**2**) with thallium acetate in ethylene glycol–CH_2Cl_2 (1 : 1).

Experimental

Reagents and Materials.—Bis(pyridine)iron(II) octaethyloxaporphyrinate (**1**) was obtained by the method of Lagarias.[6] All chemicals and solvents were of reagent grade and obtained from commercial sources.

Analyses.—Analytical t.l.c. and preparative t.l.c. were performed on DC-Alufolien Kieselgel 60 HF_{254} (Merck) and Kieselgel 60 HF_{254} (Merck), respectively. Wacogel C-200 was used for column chromatography. M.p.s were determined with a Yanaco micro-melting point apparatus. ^1H N.m.r. (270 MHz) spectra of samples in $CDCl_3$ solution containing internal tetramethylsilane were recorded with a JEOL JNM-GX 270 FT NMR Spectrometer. Mass spectra were obtained with an LKB type 9000 spectrometer at an ionizing energy of 70 eV by the direct inlet method. F.a.b.–m.s. spectra were obtained with a JEOL JMS-DX 300 Mass Spectrometer. High resolution mass spectra were obtained with a JEOL OISG-2 Mass Spectrometer. I.r. spectra were recorded with a Hitachi 215 Grating Infrared Spectrophotometer. Electronic absorption spectra were recorded with a Hitachi Model 100–50 Spectrophotometer.

Isolation of Degradation Products.—Bis(pyridine)iron(II) octaethyloxaporphyrinate (**1**) (500 mg, 606.3 μmol) dissolved in 5% pyridine–MeOH (100 ml), was allowed to stand under air for 2 days at room temperature. The reaction mixture was poured into ice–water (100 ml) and extracted with CH_2Cl_2 (100 ml × 3). The combined organic extracts were washed (H_2O), dried (Na_2SO_4), and filtered. The filtrate was evaporated and the residue dissolved in CH_2Cl_2 (2 ml), and subjected to column chromatography (CH_2Cl_2–MeOH, gradient up to 10%) to give four fractions (I, II, III, and IV).

Products **A**, **B**, and **C**. Fraction I was evaporated and the residue dissolved in a small volume of CH_2Cl_2 was subjected to preparative t.l.c.; it showed one blue and two red bands. Each band was removed and extracted with CH_2Cl_2–MeOH (9 : 1) to give crude products **A**, **B**, and **C**, respectively. Each of the crude products **A**, **B**, and **C** was applied on column chromatography for further purification (benzene–acetone, gradient up to 10%). Product **A** (27.2 mg), m.p. 175—176 °C (after recrystallization from CH_2Cl_2), f.a.b.–m.s. m/z 568 (M^+, 100%) (Found: M^+, 568.3776. $C_{36}H_{48}N_4O_2$ requires M, 568.3776). Product **B** (2.8 mg), m.p. 168—169 °C, f.a.b.–m.s. m/z 435 (M^+, 100%) (Found: M^+, 435.2923. $C_{27}H_{37}N_3O_2$ requires M, 435.2885). Product **C**, (6.3 mg), m.p. 147—149 °C, $\nu_{max.}$($CHCl_3$) 3 600—3 200 (OH, NH) and 1 730 cm^{-1} (CO) (Found: M^+, 463.2826. $C_{28}H_{37}N_4O_3$ requires M, 463.2835).

Products **D**, **E**, *and* **F**. Fraction II was evaporated and the residue was subjected to preparative t.l.c. to give crude products **D**, **E**, and **F**. Each product was further purified by column chromatography (benzene–acetone, gradient up to 10%). Product **D**, (35.9 mg) was identical with authentic octaethylbilindione by t.l.c., ^1H n.m.r., and e.i.–m.s. Product **E** (5.6 mg) (Found: C, 71.95; H, 8.5; N, 9.0. $C_{37}H_{52}N_4O_4$ requires C, 72.04; H, 8.50; N, 9.08%). Product **F** (50.3 mg) m.p. 198—200 °C (decomp.), f.a.b.–m.s. m/z 433 (M^+, 100%) (Found: M^+, 433.2756. $C_{27}H_{35}N_3O_2$ requires M, 433.2729).

Product **G**. Fraction III was evaporated and the residue was subjected to column chromatography (CH_2Cl_2–acetone, gradient up to 20%) to give product **G** (119.4 mg), m.p. 175—176 °C (after recrystallization from CH_2Cl_2) (Found: C, 72.0; H, 8.5; N, 8.85. $C_{37}H_{52}N_4O_4$ requires C, 72.04; H, 8.50; N, 9.08%).

Product **H**. Fraction IV was evaporated and the crude bluish residue dissolved in CH_2Cl_2 (30 ml) was washed with saturated aqueous $NaBF_4$ (50 ml × 3), dried (Na_2SO_4), and filtered. The filtrate was evaporated to give a residue, which was subjected to column chromatography (CH_2Cl_2–MeOH, gradient up to 10%) to give product **H** (28.8 mg) m.p. 167—168 °C (after recrystallization from CH_2Cl_2). (Found: C, 65.05; H, 6.90; N, 9.70. $C_{31}H_{39}N_4O\cdot BF_4$ requires C, 65.27; H, 6.89; N, 9.82%).

Hydrolysis of Compound (**1**) *to Compound* (**2**).—1M-HCl (2 ml) was added to a solution of compound (**1**) (20 mg) in acetone (10 ml), and the resulting solution was allowed to stand overnight at room temperature. The mixture was poured into ice–water (30 ml) and extracted with CH_2Cl_2 (20 ml × 3). The combined organic extracts were washed (H_2O), dried (Na_2SO_4), filtered and the filtrate evaporated to give a residue, which was subjected to column chromatography (CH_2Cl_2–acetone, gradient up to 10%) to give compound (**2**) (16.8 mg, 86%). This was identical with authentic octaethylbilindione by t.l.c. and ^1H n.m.r.

Reaction of Compound (**7**) *with* 5% H_2SO_4–MeOH.—Compound (**7**) (18 mg) was dissolved in 5% H_2SO_4–MeOH (5 ml) and allowed to stand overnight at room temperature. The reaction mixture was poured into ice–water (30 ml) and extracted with CH_2Cl_2 (20 ml × 3). The combined organic extracts were washed (H_2O), dried (Na_2SO_4), and filtered and the filtrate evaporated to give a residue, which was subjected to column chromatography (benzene–acetone, gradient up to 10%) to give the starting material (4 mg, 22.2%), compound (**8**) (10 mg, 55.6%); and a trace of compound (**6**).

Reaction of Compound (**8**) *with* 5% H_2SO_4–MeOH.—Compound (**8**) (28 mg) was dissolved in 5% H_2SO_4–MeOH and allowed to stand overnight at room temperature. The reaction mixture was worked-up according to the procedure described above for (**7**). The resulting residue gave compounds (**6**) (1.3 mg, 6.6%), (**7**) (5.6 mg, 20%), and (**8**) (17.2 mg, 61.4%).

Degradation of (**7**) *to* (**6**).—1M-HCl (1 ml) was added to a solution of compound (**7**) (5 mg) in acetone (5 ml) and the mixture was allowed to stand overnight at room temperature. It was then poured into ice–water (30 ml), and extracted with CH_2Cl_2 (10 ml × 3). The combined extracts were washed (H_2O), dried (Na_2SO_4), filtered and the filtrate evaporated to give a residue, which was subjected to column chromatography (benzene–acetone, gradient up to 10%) to give compound (**6**) (2.8 mg, 80%).

Degradation of Compound (**8**) *to Compound* (**6**).—1M-HCl (1 ml) was added to a solution of compound (**8**) (15 mg) in acetone (10 ml) and the reaction mixture was allowed to stand overnight under air at room temperature. Further treatment of the mixture followed the procedure described for (**7**). After column chromatography, pure compound (**6**) (9 mg, 85%) was obtained.

Autoxidation of Compound (**2**) *on T.l.c.*—Bilindione (**2**) (30 mg) dissolved in CH_2Cl_2 (1 ml) was subjected to preparative t.l.c. (benzene–acetone, 8 : 2). After development, the t.l.c. plate was exposed to air for 2 days at room temperature; when the coloured band was then scratched out and extracted with 10% MeOH–CH_2Cl_2. The extract was evaporated and the residue subjected to column chromatography (benzene–acetone, gradient up to 10%) to give starting material (**2**) (3.4 mg) and compound (**6**) (22.2 mg).

Reaction of **(2)** *with Thallium*(III) *Acetate in* MeOH.—Thallium(III) acetate sesquihydrate (35.5 mg) was added to a solution of compound **(2)** (48 mg) in MeOH (10 ml) in the absence of light. After 30 min exposure to air, the mixture was poured into water (50 ml), and extracted with CH_2Cl_2 (20 ml × 3). The combined extracts were washed (H_2O), dried (Na_2SO_4), filtered and the filtrate evaporated to give a residue. This was subjected to column chromatography (benzene–acetone, gradient up to 10%) to give compounds **(2)** (4 mg), **(6)** (4.4 mg), **(7)** (2.2 mg), and **(8)** (34.5 mg).

Autoxidation of **(1)** *in Ethylene Glycol–Pyridine* (1:1).—Octaethylverdohaemochrome **(1)** (100 mg) dissolved in ethylene glycol–pyridine (1:1) (50 ml), was exposed to air for one month at room temperature. Evaporation of the solvent under reduced pressure and preparative t.l.c. (CH_2Cl_2–acetone, 9:1) of the residue gave unchanged green starting material **(1)** (R_F 0.64, 51.7 mg, 51.7%); blue compound **(9)** (R_F 0.14, 2.7 mg, 4.7%) and two red compounds **(6)** (R_F 0.71, 10.2 mg, 19.8%) and **(10)** (R_F 0.81, 5.5 mg, 7.6%). The absorption spectrum and ^1H n.m.r. spectrum of **(10)** are given in Tables 2 and 3, respectively. Fragments from the mass spectrum are shown in Table 4 and Figure 2. Analysis of **(10)** (Found: M^+, 614.3839. $C_{37}H_{50}N_4O_4$ requires *M*, 614.3832).

Reaction of Compound **(2)** *with Thallium*(III) *Acetate in Ethylene Glycol*–CH_2Cl_2 (1:1).—Thallium(III) acetate sesquihydrate (75 mg) was added to a solution of **(2)** (100 mg) in ethylene glycol–CH_2Cl_2 (1:1) (50 ml). After 30 min exposure to air, the mixture was poured into ice–water (100 ml), and extracted with CH_2Cl_2 (50 ml × 3). The combined extracts were washed (H_2O), dried (Na_2SO_4), filtered and the filtrate evaporated to give a residue. This was subjected to column chromatography (benzene–acetone, gradient up to 10%) to give compounds **(2)** (21.2 mg, 21.3%), **(6)** (19.4 mg, 25.%), and **(10)** (34.6 mg, 31.3%).

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