Cyclization of Biliverdins to Verdohaemochromes

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Iron(II) octaethylverdohaemochrome was obtained both by cyclization of octaethylbiliverdin in the presence of $FeSO_4$ and pyridine, and by coupled oxidation of octaethylhaemin and ascorbic acid with O_2 in the presence of pyridine. The compound obtained by cyclization of biliverdin IXα dimethyl ester was, however, different from that obtained by the methylation of iron(II) protoverdohaemochrome IXα, the product of coupled oxidation of the protohaem in myoglobin. The electronic absorption spectra of the pyridine and tosylmethyl isocyanide ($TsCH_2NC$) complexes, (**14**) and (**15**), of the product of cyclization were different from the spectra of the same complexes, (**11**) and (**12**), of the product of coupled oxidation. The product of cyclization was reduced to the product of coupled oxidation by sodium dithionite. It is postulated that compounds (**14**) and (**15**) are bis(pyridine) and bis(isocyanide) coordination complexes of iron(III) protoverdohaemochrome dimethyl ester. The positions of the maxima in the absorption spectra of compounds (**16**) and (**17**), the pyridine and $TsCH_2NC$ complexes of both the product of coupled oxidation of mesohaemin dimethyl ester and the product of cyclization of mesobiliverdin dimethyl ester, indicated that these compounds are analogues, not of (**11**) and (**12**), but of (**14**) and (**15**). Iron(II) azaporphyrins (**7**) and (**13**) were prepared by treatment of the corresponding iron(II) oxaporphyrins (**5**) and (**11**) with ammonia.

Haem is oxidized by O_2 in the presence of pyridine and a reducing agent such as hydrazine or ascorbic acid to verdohaemochrome, the pyridine complex of iron(II) verdohaem.[1] One of the *meso* carbon atoms of the porphyrin ring of haem is replaced by an oxygen atom in the verdohaem ring.[2] Because neither alone is oxidized under the conditions of the experiment, the concurrent oxidation of haem and reducing agent was termed coupled oxidation.[3] Removal of pyridine by HCl from verdohaemochrome in the presence of air produces verdohaemin, the dichloride of iron(III) verdohaemin.[2] The oxahaem structure suggested for verdohaemin[2] was confirmed with the synthesis of mesoverdohaemin.[4] A structure that would result from ring closure of biliverdin by a hemiacetal-like oxygen bridge was suggested for verdohaemochrome;[1b,2] recent work has shown, however, that verdohaemochrome has the same oxaporphyrin ring structure as verdohaemin.[5–7]

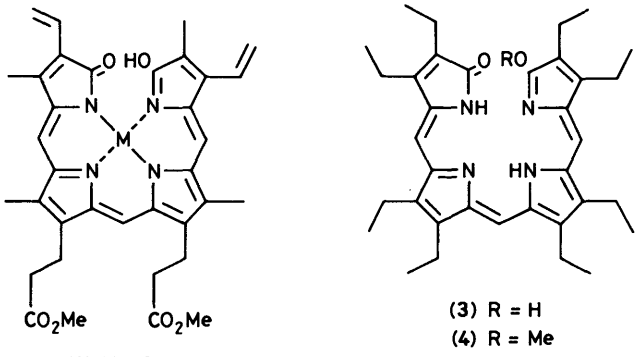
The observation that verdohaemochrome was converted into biliverdin by hydrolysis[2] led to the assumption that verdohaem is the precursor of biliverdin in coupled oxidation. When [$^{18,18}O_2$]-labelling experiments showed that neither oxygen atom of biliverdin was derived from water in both physiological and chemical conversion of haem into biliverdin,[8] verdohaem seemed to be excluded as the precursor of biliverdin. Recent demonstrations of a non-hydrolytic route in the conversion of verdohaemochrome to biliverdin have reinstated verdohaem as a feasible intermediate in the oxidative degradation of haem.[5,7,9]

Enzyme-mediated cleavage of the protoporphyrin ring is specific for the α-*meso* bridge and produces biliverdin IXα;[10] therefore a rigorous examination of the role of protoverdohaem in physiological haem degradation requires the use of its IXα isomer. Knowledge that the coupled oxidation *in situ* of the haem in myoglobin yields only the IXα isomer of biliverdin[11] was applied to the preparation of protoverdohaemochrome IXα.[5] A cleaner approach, ring closure of the iron complex of biliverdin IXα, was suggested by reports of the synthesis of zinc complexes of oxaporphyrins from zinc biliverdins.[12] This report describes the preparation of verdohaems from biliverdins and of azahaemochromes[13] from verdohaemochromes.

Results and Discussion

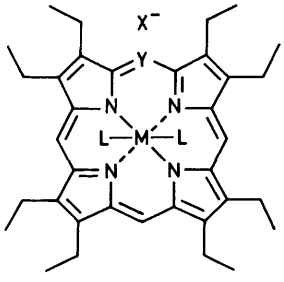
Note on Nomenclature.—The pyridine complexes of iron(II) protoverdohaem and iron(II) mesoverdohaem were named verdohaemochromogen and mesoverdohaemochromogen, respectively,[1a] and the hydroxide and chloride of iron(III) verdohaem were named verdohaematin[14] and verdohaemin,[2] respectively. The name haemochromagen has been shortened to haemochrome, and the definition of haemochrome now includes complexes of both iron(II) and iron(III) haems, axial ligands other than pyridine, and porphyrins other than protoporphyrin.[15] Names of verdohaemochromes to be described below will designate explicitly their oxidation states, ligands, and side chain structures.

Previously zinc octaethyl-5-oxaporphyrin[12a] and zinc 5-oxamesoporphyrin IXα dimethyl ester[12b] were obtained by the cyclization of the corresponding zinc biliverdins. Biliverdin IXα dimethyl ester (**1**) was treated with zinc acetate to furnish the zinc complex (**2**), which was cyclized with acetic anhydride and treated with $NaBF_4$ to give the tetrafluoroborate of zinc oxaprotoporphyrin IXα dimethyl ester (**9**). Electronic absorption spectra of compounds (**1**), (**2**), and (**9**) are shown in Figure 1. Recovery of the dimethyl ester (**1**) after hydrolysis of compound (**9**) and methylation of the product of hydrolysis showed that the vinyl groups were intact in (**9**). The oxaprotoporphyrin structure of (**9**) was supported by the base peak at *m/z* 655, which corresponds to the cation $C_{35}H_{35}N_4O_5{}^{64}Zn^+$ in the FAB mass spectrum, and the diamagnetic ring-current shifts 9.28, 9.05, and 8.97 in its ^1H n.m.r. spectrum. The elemental analysis of (**9**) agreed with the presence of a tetrafluoroborate counter ion to the positive charged oxonium bridge. For comparison with compound (**9**), ^1H n.m.r. spectra of the diester (**1**) and zinc protoporphyrin IX dimethyl ester (**10**) were taken. The vinyl protons of (**9**) were assigned (Table 1) by the decoupling method (Table 2). These data were used to analyse the spectra of iron oxaprotoporphyrin derivatives. The zinc complex (**9**) was stable in the absence of a ligand. Iron(II) verdohaems, on the other hand, are unstable in the absence of a ligand such as pyridine or tosylmethyl



(1) M = 2H
(2) M = Zn

(3) R = H
(4) R = Me



	M	Y	L	X
(5)	Fe^{II}	O^+	C_6H_5N	Cl
(6)	Fe^{II}	O^+	$TsCH_2NC$	BF_4
(7)	Fe^{II}	N	$TsCH_2NC$	
(8)	2H	N		

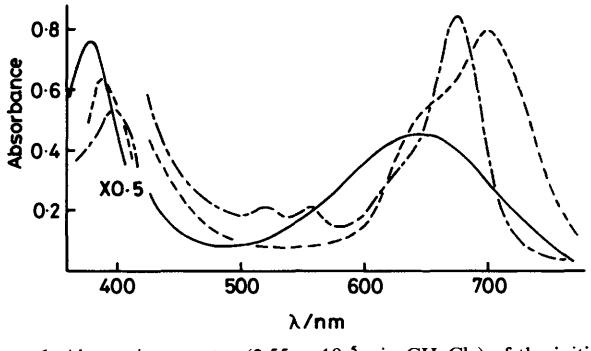
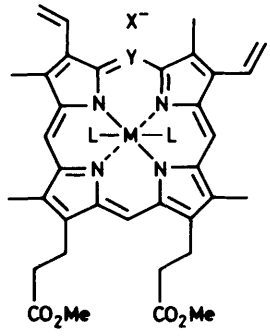
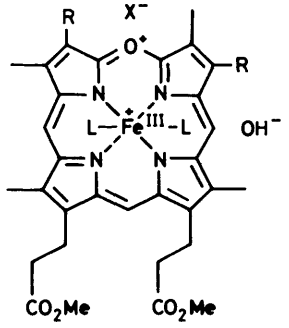


Figure 1. Absorption spectra (2.55×10^{-5}M in CH_2Cl_2) of the initial, intermediate, and final compounds in the cyclization of a biliverdin dimethyl ester in the presence of a metal ion (——) biliverdin IXα dimethyl ester (**1**); (— — —) the zinc complex (**2**) of (**1**); (— · —) zinc oxaprotoporphyrin IXα dimethyl ester (**9**).

isocyanide ($TsCH_2NC$).[5-7] The method of Fuhrhop *et al.*[12] was therefore modified: pyridine was added to the ring closure mixture in order to stabilize the iron(II) oxaporphyrin as it formed. The product was then purified as the $TsCH_2NC$ complex, which is more stable than the pyridine complex.

Coupled oxidation of octaethylhaemin and sodium ascorbate with O_2 in the presence of pyridine yielded iron(II) bis(pyridine)octaethylverdohaemochrome (**5**),[6,7] from which compound (**6**) was obtained by treatment with $TsCH_2NC$ and $NaBF_4$. The elemental analysis of (**6**) indicated the presence of a single counter ion, consistent with the iron(II) oxidation state. The base peak at *m/z* 591 in the FAB mass spectrum of compound (**6**) corresponds to the cation $C_{35}H_{43}FeN_4O^+$. The ^1H n.m.r. data of (**6**) are given in Table 3. Cyclization of octaethylbiliverdin (**3**) with acetic anhydride in the presence of $FeSO_4$ and a small amount of pyridine, followed by the addition of an excess of $TsCH_2NC$ and treatment with aqueous $NaBF_4$, yielded a blue pigment identical with compound (**6**). Hydrolysis of compound (**5**) with KOH–MeOH followed by 2M-HCl gave two blue pigments. The major product was (**3**), and the minor product was *O*-methyl octaethylbiliverdin (**4**) according to its ^1H n.m.r. (Table 3) and mass spectra. Treatment of compound (**5**) with concentrated ammonia[13] and then with $TsCH_2NC$ gave a red-purple pigment. The elemental analysis of this product showed no counter ion, and the base peak in its FAB mass spectrum was at *m/z* 589, which corresponds to $C_{35}H_{43}FeN_5$, or ligand-free iron azaporphyrin. These data and the ^1H n.m.r. spectrum (Table 3) identified the product as iron(II) bis(tosylmethyl isocyanide)octaethyl-5-azaporphyrin (**7**). Demetallation of compound (**7**) yielded the azaporphyrin (**8**).





	M	Y	L	X
(9)	Zn^{II}	O^+		BF_4
(10)	Zn^{II}	CH		
(11)	Fe^{II}	O^+	C_6H_5N	Cl
(12)	Fe^{II}	O^+	$TsCH_2NC$	BF_4
(13)	Fe^{II}	N	$TsCH_2NC$	

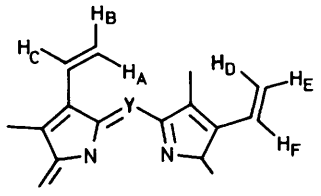
	R	L	X
(14)	$CH=CH_2$	C_6H_5N	Cl
(15)	$CH=CH_2$	$TsCH_2NC$	BF_4
(16)	Et	C_6H_5N	Cl
(17)	Et	$TsCH_2NC$	BF_4

(16) and (17) are mixtures of their IXα, IXβ, IXγ, and IXδ isomers.

The bis(pyridine) complex of iron(II) protoverdohaemochrome IXα dimethyl ester chloride (**11**) was treated with $TsCH_2NC$ and $NaBF_4$. The FAB mass spectrum of the product showed a base peak at *m/z* 647, which corresponds to the unliganded cation $C_{35}H_{35}FeN_4O_5^+$ of (**11**) or (**12**), and a fragment ion at *m/z* 1 037, which corresponds to the cation of the $(TsCH_2NC)_2$ complex (**12**). The ^1H n.m.r. data of compound (**12**) are given in Table 1. The product of the reaction between compound (**11**) and concentrated ammonia[13] was esterified and treated with $TsCH_2NC$ and $NaBF_4$. The resulting compound was identified as the $(TsCH_2NC)_2$ complex of iron(II) azaprotoporphyrin IXα dimethyl ester (**13**) from ^1H n.m.r. data (Table 1), FAB mass spectral data, and elemental analysis. The product contained no counter ion.

Biliverdin IXα dimethyl ester (**1**) was heated together with $FeSO_4$ in acetic anhydride containing a small amount of pyridine to furnish compound (**14**), which was treated with $TsCH_2NC$ and $NaBF_4$ to give the diester (**15**). Hydrolysis of (**14**) followed by esterification gave compound (**1**); therefore the vinyl groups remained intact during the cyclization and subsequent hydrolysis, and the vinyl protons in the n.m.r. spectrum of (**15**) were all assigned (Table 1). According to their mass spectra, the protoverdohaem dimethyl ester nuclei of compounds (**12**) and (**15**) have the same mass. Though the

Table 1. ^{1}H N.m.r. data (δ in $CDCl_3$, multiplicity and *J*/Hz in parentheses)



Y = O$^+$, N, CH

	(9)	(12)	(15)	(13)	(1)	(10)
meso-H	9.28, 9.05, 8.97	9.27, 9.20, 8.91	9.19, 9.12, 9.08	9.66, 9.54, 9.54	6.67, 6.00, 5.96	8.89, 8.63, 8.52, 8.38
H_A	6.69 (d, 17)	6.72 (d, 16)	6.80 (d, 16)	7.42 (d, 17)		
H_B	6.00 (d, 9)	6.09 (d, 10)	5.99 (d, 10)	5.99 (d, 11)	6.62 and 6.50	
H_C	7.35 (dd, 17, 9)	7.76 (m)	7.78 (m)	8.20 (dd, 17, 11)	(dd, 18, 12) (H_C and H_F)	7.76 and 7.38 (H_C and H_F)
H_D	6.17 (d, 17)	6.21 (d, 16)	6.20 (d, 16)	6.28 (d, 17)	6.12, 5.98, 5.61,	
H_E	6.10 (d, 9)	5.95 (d, 10)	6.08 (d, 10)	6.00 (d, 11)	5.38 (H_A, H_B,	6.06—5.82 (H_A,
H_F	7.58 (dd, 17, 9)	7.76 (m)	7.96 (dd, 16, 10)	8.22 (dd, 17, 11)	H_D, and H_E)	H_B, H_D, H_F)
Me on pyrrole	3.64, 3.09, 3.06, 3.03	3.29, 3.18, 3.07, 3.05	3.28, 3.23, 3.18, 3.07	3.61, 3.52, 3.45, 3.45	2.16, 2.06, 2.01, 1.85	3.25, 3.12, 3.11, 3.00
$\rangle$—CH_2	3.85 (t, 6.5)	3.73 (m)	3.79 (m)	4.21 (t, 7.5)	2.93	3.92 and 3.87
CH_2CO	3.02 (t, 6.5)	3.00 (m)	3.00 (m)	3.18 (t, 7.5)	2.55	2.89
OMe	3.65	3.65	3.65	3.68	3.68	3.68 and 3.65
ortho-H on $TsCH_2NC$		7.10 (d, 9)	7.06 (d, 9)	7.05 (broad s)		
meta-H on $TsCH_2NC$		6.59 (d, 9)	6.60 (d, 9)	5.83 (broad s)		
CH_2 on $TsCH_2NC$		3.10	3.07	2.42		
Me on $TsCH_2NC$		2.41	2.41	2.34		
NH					8.61	

Table 2. Decoupled ^{1}H n.m.r. data of compound (**9**) (same vinyl protons as in Table 1)

irradiated protons	H_F (δ 7.58) (dd, 17, 9)	H_C (δ 7.35) (dd, 17, 9)	H_A (δ 6.69) (d, 17)	H_D (δ 6.17) (d, 17)	H_E (δ 6.10) (d, 9)	H_B (δ 6.00) (d, 9)
H_F				s*	s*	
H_C			s*			s*
H_A		d, 9				sharpened
H_D	d, 9					
H_E	d, 17					
H_B		d, 17				

* Each singlet showed a small coupling constant (*J ca.* 1.8 Hz).

Table 3. ^{1}H N.m.r. data (δ in $CDCl_3$)

	(6)	(7)	(8)
meso-H	9.15, 9.15, 8.80	9.59, 9.52, 9.52	10.02, 9.88, 9.88
CH_2on pyrrole	3.61—3.44	4.03—3.87	4.10—3.91
Me on pyrrole	1.73—1.55	1.96—1.83	1.88—1.80
ortho-H on $TsCH_2NC$	7.06 (d, 8)	7.00 (br s)	
meta-H on $TsCH_2NC$	6.65 (d, 8)	5.94 (br s)	
CH_2on $TsCH_2NC$	3.00	2.40	
Me on $TsCH_2NC$	2.35	2.30	
NH			−2.68

proton assignments in the ^{1}H n.m.r. spectra of (**12**) and (**15**) were the same, the protons showed slightly different chemical shifts. The electronic absorption spectra of (**14**) and (**15**) had the same shapes as those of (**11**) and (**12**), but the absorption maxima of (**14**) and (**15**) are all shifted toward the red from those of (**11**) and (**12**). Reduction of (**14**) with sodium dithionite produced (**11**) (Figure 3), from which compound (**12**) was obtained by treatment with $TsCH_2NC$ and $NaBF_4$. Thus, (**14**) is oxidized to (**11**), and (**15**) is oxidized to (**12**). Protoverdohaemochrome IXα, of which compounds (**11**) and (**12**) are esterified derivatives, is an iron(II) oxaprotoporphyrin with a chloride counter ion to the positively charged oxonium bridge.[5] The elemental analysis of compound (**15**), which is consistent with the presence of two univalent anions, suggests that the difference in the oxidation state from that of (**11**) or (**12**) is one electron.

Either the central metal atom or its porphyrin ligand may lose an electron in a one-electron oxidation of a metalla-porphyrin. Oxidation of the porphyrin produces a metallo-porphyrin π cation radical without change in the oxidation state of the metal atom.[16,17] Binding of $TsCH_2NC$ suggests that compound (**15**) is an iron(II) oxaporphyrin because isocyanides are known to bind to iron(II), but not to iron(III), porphyrins;[18] however, a radical formulation of (**15**) is unsupported by

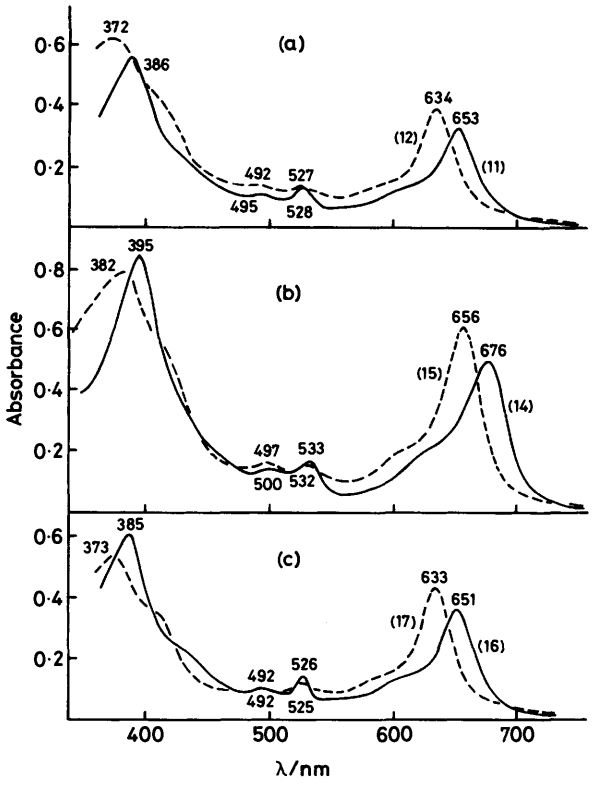


Figure 2. Absorption spectra (5% pyridine–CH_2Cl_2, v/v) of the (——) bis(pyridine) and (— — —) bis(tosylmethyl isocyanide co-ordination complexes of verdohaemochrome dimethyl esters: (a) 1.2×10^{-5}M (**11**) and (**12**); (b) 1.6×10^{-5}M (**14**) and (**15**); (c) 1.4×10^{-5}M (**16**) and (**17**)

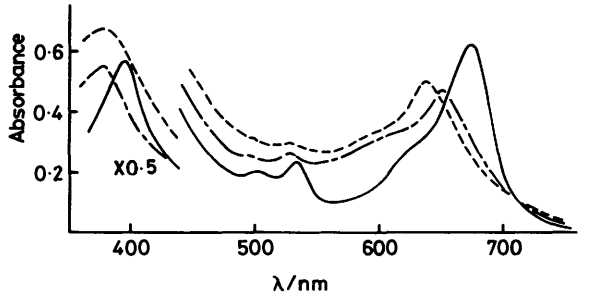


Figure 3. Absorption spectra (5% pyridine–CH_2Cl_2, v/v) in the reaction of (**14**) with $Na_2S_2O_4$ in pyridine: (——) (**13**); (— · —) 1 min after addition of an excess of $Na_2S_2O_4$; (— — —) after addition of an excess of $TsCH_2NC$ to the reaction mixture

spectral data. Whereas the electronic (Figure 2) and n.m.r. (Table 1) spectra of compound (**15**) are similar to those of compound (**12**), the electronic[16] and n.m.r.[17] spectra of a metalloporphyrin π cation radical are grossly different from those of a non-radical metalloporphyrin. The small change in the electronic spectrum that resulted from the one-electron oxidation of the pyridine–CO complex of osmium(II) octaethylporphyrin was taken to indicate that the product was the osmium(III) compound and not the osmium(II) porphyrin radical.[19] Accordingly, although there is no precedent for the binding of an isocyanide by an iron(III) porphyrin, spectral evidence favours the iron(III) protoverdohaemochrome structure over the iron(II) cation radical structure for (**15**). The novel affinity may be the result of the replacement of a methene bridge in the porphyrin ring by an oxonium bridge in the oxaporphyrin ring. An analogous structure for compound (**14**) poses no problem as pyridine is a ligand of both iron(II) and iron(III) porphyrins.[20]

Both coupled oxidation of mesohaemin IX dimethyl ester and cyclization of mesobiliverdin IX dimethyl ester resulted in the same green pigment (**16**), from which the $TsCH_2NC$ complex (**17**) was prepared. The base peak in its mass spectrum indicated that (**17**) is a complex of mesoverdohaem dimethyl ester, and its elemental analysis agreed more closely with the presence of one anion rather than two; however, the absorption spectrum of (**16**) has the same maxima as those reported for the spectrum in pyridine of mesohaemin IXβ dimethyl ester, an iron(III) compound, which was obtained as analytically pure dichloride.[4] Absorption maxima in the spectra of mesoporphyrin derivatives are invariably shifted 5—6 nm or more toward the violet of the corresponding maxima of analogous protoporphyrin derivatives.[21] Large differences in the same direction, 18 nm in pyridine–water and 13 nm in pyridine–chloroform, were observed between the absorption maxima in the red region of the spectra of protoverdohaemochrome and mesoverdohaemochrome.[1a] The difference in the position of this peak in pyridine–CH_2Cl_2 was only 2 nm between (**16**) and (**11**) and 1 nm between (**17**) and (**12**) (Figure 2). The small differences between the spectra of (**16**) and (**11**) and between the spectra of (**17**) and (**12**) argue against the analogy of the mesoporphyrin derivatives (**16**) and (**17**) with the protoporphyrin derivatives (**11**) and (**12**). On the other hand, the direction and magnitude of the differences between the spectra of (**16**) and (**14**) and between the spectra of (**17**) and (**15**) are typical of differences between mesoporphyrin and protoporphyrin derivatives;[1a,21] we accordingly infer that compounds (**16**) and (**17**) are analogues of compounds (**14**) and (**15**), and thus are the pyridine and $TsCH_2NC$ complexes, respectively, of iron(III) mesoverdohaemochrome IX dimethyl ester. Inasmuch as coupled oxidation of mesohaemin produces a mixture of the isomers of mesobiliverdin,[22] (**16**) and (**17**) are mixtures of their IXα, IXβ, IXγ, and IXδ isomers.

In previous studies, mesoverdohaemochrome from the reaction of iron oxamesoporphyrin (a mixture of isomers) with O_2,[23] protoverdohaemochrome IXα from the coupled oxidation of myoglobin,[5] and octaethylverdohaemochrome (**5**) from the coupled oxidation of octaethylhaemin,[6,7] were all shown to be iron(II) compounds. In the present work, the product of the cyclization of octaethylbiliverdin was found to be identical with the iron(II) product of the coupled oxidation of octaethylhaemin. The products (**14**) and (**15**) of the cyclization of compound (**1**) were, however, found to be at a higher oxidation state than the corresponding complexes (**11**) and (**12**), respectively, of esterified iron(II) protoverdohaemochrome IXα. The absorption spectra of compounds (**16**) and (**17**) suggested that these oxamesohaem products of both coupled oxidation and cyclization are at the iron(III) state postulated for compounds (**14**) and (**15**). The oxidation of a verdohaemochrome to its iron(III) state thus appears to be associated with the nature of its substituents and not to the method of its synthesis.

Iron(II) protoverdohaemochrome IXα was shown to be an intermediate in the degradation of haem to biliverdin IXα by coupled oxidation of myoglobin in the presence of ascorbic acid and pyridine.[5] The shape of the absorption spectrum in pyridine of the precursor of biliverdin IXα in the enzymic degradation of haem[24] is similar to that of iron(II) protoverdohaemochrome IXα;[5] however, the maxima in the spectrum of the enzymic product are toward the red from the corresponding maxima of iron(II) protoverdohaemochrome IXα. In particular, the major absorption in the red region, which is at 660 nm in the spectrum of iron(II) protoverdohaemochrome IXα in pyridine,[5] is at 679 nm in the spectrum of the enzyme-produced intermediate. In the

spectra of the esterified verdohaemochromes prepared in this study (Figure 2), the spectrum of iron(II) bis(pyridine)-protoverdohaemochrome (**11**) has its red peak at 653 nm, and the spectrum of iron(III) bis(pyridine)protoverdohaemochrome (**14**) spectrum has its red peak at 676 nm. These comparisons suggest that in pyridine solution the intermediate isolated from the enzymic system[24] may have been the pyridine complex of iron(III) protoverdohaemochrome IXα.

The coupled oxidation of an asymmetrically substituted iron porphyrin results in a mixture of four isomers of biliverdin.[11,22,25] A specific isomer of a verdohaem can, therefore, be prepared by coupled oxidation of a haemin, chromatographic separation of the products,[22,25] and cyclization of the required isomer of biliverdin. The present work has shown the feasibility of this approach.

Experimental

Myoglobin, type III from horse heart, was purchased from Sigma. Biliverdin IXα was prepared by oxidation of bilirubin IXα, purchased from Aldrich, with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone. Octaethylporphyrin was prepared according to a published procedure.[26] Mesohaemin IX dimethyl ester was prepared by the insertion of iron[27] into mesoporphyrin IX dimethyl ester purchased from Sigma. Tosymethyl isocyanide ($TsCH_2NC$), purchased from Aldrich, was recrystallized from ethanol after treatment with active carbon in ethanol. All other solvents and chemicals employed in this study were of reagent grade. Analytical t.l.c. was performed on Merck Kieselgel 60 F_{254}. Preparative t.l.c. was performed on Merck Kieselgel 60 HF_{254} (20 cm × 20 cm × 2 mm). Electronic absorption spectra were recorded on the Hitachi Model 100-50 spectrophotometer and millimolar extinction coefficients, ε_{mM} in l $mmol^{-1}$ cm^{-1} were calculated. Melting points were determined with the Yanoco Micro Melting Point Apparatus. ^1H N.m.r. spectra at 270 MHz of samples in $CDCl_3$ solution containing internal Me_4Si were recorded with the JEOL JNM-GX 270 FT n.m.r. spectrometer. Mass spectra were obtained on an LKB type 9000 spectrometer at ionizing energies of 70 and 20 eV by the direct inlet method. FAB Mass spectra were obtained on a Kratos MS 50 mass spectrometer equipped with an Ion Tech saddle-field atom gun at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, Nebraska.

(5-*Oxaprotoporphyrinato* IXα *dimethyl ester*)*zinc*(II), *Tetrafluoroborate* (*Zinc* 5-*oxaprotoporphyrin* IXα *Dimethyl Ester, Tetrafluoroborate*) (**9**).—To a solution of biliverdin IXα dimethyl ester (**1**) (100 mg, 0.16 mmol), a solution of zinc acetate dihydrate (180 mg, 0.82 mmol) in methanol (10 ml) was added. The solvent was evaporated, and the residue was dissolved in dichloromethane (10 ml) and acetic anhydride (30 ml). After the mixture had been heated at 120 °C for 10 min under argon, the solvent was removed with a vigorous stream of argon at 140 °C. The resulting residue, which showed a major green spot (R_F 0.7) on t.l.c. [CH_2Cl_2–MeOH (9:1)], was chromatographed on silica gel with CH_2Cl_2–MeOH (gradient up to 5%) to give the major green pigment. The pigment was dissolved in dichloromethane, washed with saturated aqueous $NaBF_4$ and water, dried, and evaporated under reduced pressure to give a residue, which after recrystallization from chloroform–light petroleum (1:1), gave the *tetrafluoroborate* (**9**) (89 mg, 73.1%), m.p. >300 °C (Found: C, 56.5; H, 4.7; N, 7.5. $C_{35}H_{35}BF_4N_4O_5Zn$ requires C, 56.4; H, 4.8; N, 7.5%); FAB mass *m/z* 659 (39%), 657 (66), and 655 (100) (*m/z* 659, 657, and 655 correspond to $M^+ - BF_4$ in which *M* contains ^{68}Zn, ^{66}Zn, and ^{64}Zn, respectively).

Hydrolysis of Compound (**9**).—To a solution of the salt (**9**) (50 mg, 0.067 mmol) in methanol (10 ml), a saturated solution of KOH–MeOH (10 ml) was added. This reaction mixture was stirred for 3 min and then poured into 2M-HCl (100 ml) at 0 °C with stirring. The mixture was extracted with CH_2Cl_2 (2 × 50 ml), and the combined organic extracts were washed with water, dried, and evaporated to give a blue residue which, after methylation, showed a major blue spot (R_F 0.49) and minor blue and pink spots (R_F 0.92 and 0.89, respectively) on t.l.c. [benzene–acetone (8:2)]. The major blue pigment (**1**) (27.9 mg, 67.9%) was isolated by preparative t.l.c. [benzene–acetone (8:2)] and recrystallized from $CHCl_3$–light petroleum (1:5); *m/z* 610 (M^+, 100%). This compound was identical with authentic (**1**)[5] according to its ^1H n.m.r. and mass spectra.

(2,3,7,8,12,13,17,18-*Octaethyl*-5-*oxaporphyrinato*)*bis*(*tosylmethyl isocyanide*)*iron*(II) *Tetrafluoroborate* [*Iron*(II) *Bis*(*tosylmethyl isocyanide*)-2,3,7,8,12,13,17,18-*octaethylverdohaemochrome Tetrafluoroborate*] (**6**).—To a solution of octaethylhaemin (150 mg, 0.27 mmol) in pyridine (50 ml) and water (10 ml), a solution of sodium ascorbate (1 g, 5 mmol) in water (10 ml) was added. Oxygen was bubbled through the mixture for 45 min at room temperature after which it was diluted with water (150 ml) and extracted with CH_2Cl_2 (3 × 150 ml). The combined organic extracts were washed successively with aqueous NaCl and water, dried, and evaporated to give a residue, which by preparative t.l.c. [CH_2Cl_2–pyridine–MeOH (9:0.6:0.4)] yielded compound (**5**),[7] $\lambda_{max.}$ (5% pyridine in CH_2Cl_2) 651, 605 (sh), 526, 493, 430 (sh), and 384 nm (ε_{mM} 41.1, 10.6, 13.1, 7.7, 17.2, and 53.4). Compound (**5**) was dissolved together with $TsCH_2NC$ (300 mg, 1.6 mmol) in dichloromethane (5 ml) and the solution was washed with saturated aqueous $NaBF_4$, dried, and filtered. The filtrate was applied to a preparative t.l.c. plate [CH_2Cl_2–MeOH (9:1)] to obtain the *title compound* (**6**), as a blue oil (83 mg, 28.8%) (Found: C, 59.4; H, 5.8; N, 7.6. $C_{53}H_{61}BF_4FeN_6O_5S_2$ requires C, 59.6; H, 5.8; N, 7.8%); $\lambda_{max.}$ (5% pyridine–CH_2Cl_2 containing an excess of $TsCH_2NC$) 631, 590 (sh), 525, 491, 405 (sh), and 371 nm (ε_{mM} 44.8, 12.9, 11.2, 9.9, 35.9, and 53.4); FAB *m/z* 591 ($M^+ - 2 \times TsCH_2NC - BF_4$, 100%).

Cyclization of 2,3,7,8,12,13,17,18-*Octaethylbiliverdin* (**3**).—To a solution of compound (**3**) (70 mg, 0.13 mmol) in acetic anhydride (30 ml) and pyridine (5 ml), a solution of $FeSO_4 \cdot 7H_2O$ (350 mg, 1.26 mmol) in methanol (5 ml) was added under argon. The reaction mixture was heated for 15 min at 110 °C under argon, and the solvent was removed by a vigorous flow of argon at 140 °C to give a green residue (**5**). The residue was extracted with CH_2Cl_2 (3 × 30 ml) and the combined organic extracts were washed with water, dried, and evaporated at <20 °C to give a residue. The residue was dissolved together with $TsCH_2NC$ (300 mg, 1.54 mmol) in CH_2Cl_2 (30 ml), and the solution washed with saturated aqueous $NaBF_4$ (twice), dried, and evaporated at <20 °C to give a blue residue. The pure blue pigment (**6**) (53 mg) was isolated by preparative t.l.c. [CH_2Cl–MeOH (19:1)]. This pigment was identical with (**6**) obtained from the coupled oxidation of octaethylhaemin.

Hydrolysis of Compound (**5**).—Hydrolysis of compound (**5**) (20 mg, 0.025 mmol) with KOH–MeOH followed by 2M-HCl gave two blue pigments (**3**) (6.9 mg, 48.9%) and (**4**) (1.8 mg, 12.5%). Pigment (**3**) was identical with authentic octaethylbiliverdin according to its mass and ^1H n.m.r. spectrum.[28] O-*Methyl octaethylbiliverdin* (**4**) had δ($CDCl_3$) 13.01 and 10.29 (each s, NH), 6.60, 6.27, and 5.73 (each s, *meso*-H), 4.01 (s, OMe), 2.59—2.42 (m, 6 × CH_2), 2.28 (q, 7.3, CH_2), 2.18 (q, 7.3, CH_2), and 1.25—1.00 (m, 8 × Me); $\lambda_{max.}$(CH_2Cl_2) 665, 620 (sh), and 369 nm (ε_{mM} 12.3, 10.0, and 38.2); *m/z* (20 eV) 568 (M^+, 100%), 553 ($M^+ - 15$, 24.1), and 539 (9.6); *m/z* (70 eV) 568 (M^+, 100%), 553 ($M - 15$, 66.7), 539 (22.1), 524 (5.0), 523 (6.0), 510

(5.3), 509 (9.3), 286 (12.7), 284 (29.2), 283 (12.6), 269 (19.1), 262 (6.8), 255 (7.1), 254 (5.1), 247 (6.2), and 240 (5.7).

(2,3,7,8,12,13,17,18-*Octaethyl-5-aza-porphyrinato*)*bis*(*tosyl methylisocyanide*)*iron*(II) [*Iron*(II) *Bis*(*tosyl methylisocyanide*)-2,3,7,8,12,13,17,18-*octaethyl-5-azahemochrome*] (**7**).—To a solution of compound (**5**) (163 mg, 0.21 mmol) in dichloromethane (2 ml) and methanol (10 ml) concentrated ammonia solution (10 ml) was added under argon.[13] The mixture was set aside overnight at room temperature after which it was diluted with water (50 ml) and extracted with CH_2Cl_2 (3 × 30 ml). The combined organic extracts were washed with water, dried, and evaporated at < 20 °C to give a residue. The residue was dissolved together with $TsCH_2NC$ (500 mg, 2.6 mmol) in CH_2Cl_2 (5 ml) and then applied on a preparative t.l.c. plate [CH_2Cl_2–MeOH (99:1)] to provide the title compound (**7**) as a red-purple *pigment* (68 mg, 36%), which was recrystallized from CH_2Cl_2–light petroleum, m.p. > 300 °C (Found: C, 64.7; H, 6.3; N, 9.6. $C_{53}H_{61}FeN_7O_4S_2$ requires C, 64.9; H, 6.3; N, 10.0%); $\lambda_{max.}$(CH_2Cl_2) 555, 490, and 364 nm (ε_{mM} 16.2, 13.2, and 114.7); *m*/*z* (FAB) 589 (M^+ − 2 × $TsCH_2NC$, 100%) and 588 (98).

2,3,7,8,12,13,17,18-*Octaethyl-5-azaporphyrin* (**8**).—To a solution of compound (**7**) (50 mg, 0.05 mmol) in acetic acid (20 ml), a solution of saturated $FeSO_4$ in concentrated HCl (2 ml) was added under argon. After 5 min, water (100 ml) was added, and the reaction mixture was extracted with CH_2Cl_2 (2 × 100 ml). The combined extracts were washed with water, dried, and evaporated to give a residue from which the *octaethylazaporphyrin* (**8**) (17.2 mg, 63.0%) was isolated by preparative t.l.c. (CH_2Cl_2) (Found: C, 78.4; H, 8.5; N, 12.8. $C_{35}H_{45}N_5$ requires C, 78.5; H, 8.5; N, 13.1%); $\lambda_{max.}$(CH_2Cl_2) 610, 558, 536, 505, and 376 nm (ε_{mM} 26.7, 9.6, 26.6, 8.2, and 131.5); *m*/*z* 535 (M^+, 100%), 520 (M^+ − 15, 31.4), 490 (10).

(5-*Oxaprotoporphyrinato* IXα *Dimethyl Ester*)*bis*(*tosylmethyl isocyanide*)*iron*(II) *Tetrafluoroborate* [*Iron*(II) *Bis*(*tosylmethyl isocyanide*)*protoverdohaemochrome* IXα *Dimethyl Ester Tetrafluoroborate*] (**12**).—Iron(II) protoverdohaemochrome IXα was prepared by the coupled oxidation of myoglobin and sodium ascorbate. Details of this procedure and of the methylation of the verdohaemochrome have been reported.[5] The product was treated with pyridine to give iron(II) bis(pyridine)protoverdohaemochrome IXα dimethyl ester chloride (**11**) (35 mg, 0.06 mmol) which was dissolved together with $TsCH_2NC$ (110 mg, 0.6 mmol) in CH_2Cl_2 (5 ml) under argon. The solution was washed with saturated aqueous $NaBF_4$, dried, and evaporated at < 20 °C to give a residue, from which bluish green pigment (**12**) (31 mg, 48%) was isolated by preparative t.l.c. [CH_2Cl_2–MeOH (19:1)]. This oil showed only one spot on t.l.c., whereas its n.m.r. spectrum indicated the presence of a small amount of an impurity. This compound was too unstable to allow further purification: *m*/*z* (FAB) 647 (M^+ − 2 × $TsCH_2NC$ − BF_4, 100%), 648 (84.2), and 1 037 (M^+ − BF_4, 3.6).

(5-*Azaprotoporphyrinato* IXα *Dimethyl Ester*)*bis*(*tosylmethyl isocyanide*)*iron*(II) [*Iron*(II) *Bis*(*tosylmethyl isocyanide*)-5-*azaprotohemochrome* IXα *Dimethyl Ester*] (**13**).—A solution of compound (**11**) (50 mg, 0.068 mmol) in CH_2Cl_2 (2 ml) and methanol (10 ml) was treated with ammonia under argon as described for the preparation of (**7**); the product of this reaction was then methylated. A green pigment (48 mg), obtained by preparative t.l.c. [CH_2Cl_2–MeOH (9:1)] of the methylated product, was dissolved in a solution of $TsCH_2NC$ (130 mg, 0.67 mmol) in CH_2Cl_2 (3 ml). The solution was washed with saturated aqueous $NaBF_4$, dried, and evaporated to give a red-purple residue. Preparative t.l.c. [CH_2Cl_2–MeOH (49:1)] of the residue and recrystallization from CH_2Cl_2–light petroleum gave the title ester (**13**) as a red-purple *compound* (24.7 mg, 35%), m.p. > 300 °C (Found: C, 61.4: H, 5.2; N, 9.5. $C_{35}H_{53}FeN_7O_8S_2$ requires C, 61.0; H, 5.4; N, 9.1%); *m*/*z* (FAB) 645 (M^+ − 2 × $TsCH_2NC$, 100%).

Cyclization of Biliverdin IXα *Dimethyl Ester* (**1**).—The procedure used to cyclize compound (**3**) was applied to compound (**1**) (150 mg, 0.25 mmol). Preparative t.l.c. [CH_2Cl_2–MeOH–pyridine (45:4:1)] of the crude product gave a green pigment (**14**). A solution of compound (**14**) in CH_2Cl_2 (5 ml), to which $TsCH_2NC$ (480 mg, 2.46 mmol) was added, was washed with saturated aqueous $NaBF_4$, dried, and evaporated at < 20 °C to give a residue, from which a bluish green pigment (5-*oxaprotoporphyrinato* IXα *dimethyl ester*)*bis*(*tosylmethyl isocyanide*)*iron*(III), *tetrafluoroborate*, [*iron*(III) *bis*(*tosylmethyl isocyanide*)*protoverdohaemochrome*[*iron*(III) *bis*(*tosylmethyl isocyanide*)*protoverdohaemochrome* IXα *dimethyl ester tetrafluoroborate hydroxide*] (**15**) (97.6 mg, 35.3%), was obtained by preparative t.l.c. [CH_2Cl_2–MeOH (19:1)] (Found: C, 55.9; H, 4.6; N, 7.1. $C_{53}H_{54}N_6O_{10}S_2BF_4FeO$ requires C, 55.75; H, 4.8; N, 7.4%); *m*/*z* (FAB) 647 (M^+ − 2 × $TsCH_2NC$ − OH − BF_4, 100%), and 1 037 (M^+ − OH − BF_4, 1.4%).

Hydrolysis of Compound (**14**).—To a solution of compound (**14**) (35 mg, 0.04 mmol) in CH_2Cl_2 (1 ml), saturated KOH–MeOH (2 ml) and 2M-HCl (5 ml) were added in succession at 0 °C under argon. The residue from the washed and dried CH_2Cl_2 extract of the reaction mixture was methylated, and the product was purified by preparative t.l.c. [benzene–acetone (8:2)] to give compound (**1**) (13 mg, 63%). The spectra of compound (**1**) were identical with those previously reported.[5]

Treatment of Compound (**14**) *with Sodium Dithionite*.—A solution of compound (**14**) (24.3 mg, 0.029 mmol) in pyridine (5 ml), to which saturated aqueous $Na_2S_2O_4$ (1 ml) had been added, was stirred for 1 min at room temperature. The reaction mixture was diluted with water (10 ml) and extracted with CH_2Cl_2 (2 × 10 ml). The combined organic extracts were washed with water, dried, and evaporated at < 20 °C. The residue was dissolved in CH_2Cl_2 (10 ml), washed with saturated aqueous $NaBF_4$ (10 ml), dried, and filtered. The filtrate, to which an excess of $TsCH_2NC$ had been added, was applied to a preparative t.l.c. plate [CH_2Cl_2–MeOH (9:1)] to give a bluish green pigment (2.7 mg, 11.4%). This pigment was identical with compound (**12**) according to its absorption and ^1H n.m.r. spectrum.

Coupled Oxidation of Mesohaemin Dimethyl Ester.—To a solution of mesohaemin dimethyl ester (200 mg) in CH_2Cl_2 (5 ml) and pyridine (50 ml), a solution of sodium ascorbate (500 mg) in water (5 ml) was added over 30 min under O_2 at room temperature. Water (70 ml) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 × 70 mm). The combined extracts were washed with saturated aqueous NaCl, dried (Na_2SO_4), and evaporated to give a residue from which a green pigment (**16**), was purified as an oil (86 mg, 32.8%) by preparative t.l.c. [CH_2Cl_2–MeOH–pyridine (9:0.5:0.5)]. The green pigment was dissolved together with $TsCH_2NC$ (50 mg) in CH_2Cl_2 (30 ml), washed (twice) with saturated aqueous $NaBF_4$, dried, and evaporated to give a bluish green pigment [a mixture of the IXα, IXβ, IXγ, and IXδ isomers of (5-*oxamesoporphyrinato* IX *dimethyl ester*)*bis*(*tosylmethyl isocyanide*)-*iron*(III) *tetrafluoroborate, hydroxide* [*iron*(III) *bis*(*tosylmethyl isocyanide*)*mesoverdohaemochrome* IX *dimethyl ester tetrafluoroborate hydroxide*] (**17**), which was purified by preparative t.l.c. [CH_2Cl_2–MeOH (19:1)]; yield 15 mg (53.8%) (Found: C, 56.2; H, 5.4; N, 7.0. $C_{53}H_{57}BF_4FeN_6O_9S_2$ requires C, 56.4; H, 5.1; N, 7.4. $C_{53}H_{58}BF_4FeN_6O_{10}S_2$ requires C, 55.6; H, 5.1; N,

7.3%); FAB *m/z* 651 ($M^+ - 2 \times TsCH_2NC - OH - BF_4$, 100%).

Hydrolysis of Compound (**16**).—The green pigment (**16**) (53 mg) obtained above was hydrolysed with saturated KOH–MeOH and 2M-HCl. The bluish residue from the washed and dried CH_2Cl_2 extract of the reaction mixture was methylated to produce mesobiliverdin dimethyl ester, which was isolated by preparative t.l.c. [benzene–acetone (8:2)] (30 mg).

Cyclization of Mesobiliverdin Dimethyl Ester.—Mesobiliverdin dimethyl ester (30 mg) was cyclized in the same manner as for compounds (**1**) and (**3**). The reaction mixture, to which pyridine (10 ml) and water (50 ml) had been added, was extracted with CH_2Cl_2 (2 × 50 ml). The combined extracts were washed with saturated aqueous $NaBF_4$, dried (Na_2SO_4) and evaporated to give a greenish residue (**16**). The residue was dissolved together with $TsCH_2NC$ (50 mg) in CH_2Cl_2 (3 ml) and purified by preparative t.l.c. [CH_2Cl_2–MeOH (19:1)] to give compound (**17**) [18 mg, 24.4% from (**16**)] *m/z* (FAB) 651 ($M^+ - 2 \times TsCH_2NC - OH - BF_4$, 100%).

Acknowledgements

This work was supported in part by a grant from the National Institutes of Health. We thank Kenneth B. Tomer, Assistant Director of the Midwest Center for Mass Spectrometry for FAB mass spectra.

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Received 13*th July* 1984; *Paper* 4/1213