The *meso*-Reactivity of Porphyrins and Related Compounds. Part V.[1] The *meso*-Oxidation of Metalloporphyrins [2]

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The action of hydrogen peroxide in pyridine on a variety of metal complexes (Mn, Fe, Co, Ni, Cu, and Zn) of octaethylporphyrin under specified conditions has been shown to give metal octaethyloxophlorin derivatives in those cases where the complexed metal ion has a readily available higher oxidation level. Manganese(III) octaethyloxophlorin and dipyridinecobalt(III) octaethyloxophlorin prepared in this way have been characterised; they are identical with the products of appropriate metallation of octaethyloxophlorin prepared by ring synthesis. Although iron(III) octaethyloxophlorin has not been isolated from the reaction, direct demetallation gives octaethyloxophlorin, and benzoylation followed by demetallation gives 5-benzoyloxyoctaethylporphyrin, again in each case identical with an authentic sample prepared by ring synthesis. Iron(III) octaethyloxophlorin readily undergoes autoxidation in pyridine to give, after hydrolysis, octaethylbilatriene-*abc*. The implications of these observations for the chemistry of haem catabolism are discussed.

In Part IV [1] we reported the introduction of oxygen functions at the β- and *meso*-positions of octaethylporphyrin. In relation to the chemistry of haem catabolism [3] it is *meso*-substitution in an iron complex that is expected to be relevant. Earlier work in this area is extensive, although much of it was carried out in the late thirties when facilities for the confirmation of structure by spectroscopic means were not generally available. Lemberg and his colleagues undertook pioneering studies on the coupled oxidation of protohaemochrome with hydrogen peroxide–ascorbic acid. An intermediate ($\lambda_{max.}$ 639 nm) was initially thought to be a haem–hydrogen peroxide complex,[4] but later work led to the suggestion that it was a *meso*-hydroxyporphyrin iron complex and the free *meso*-hydroxyporphyrin † was isolated.[5] Elemental analysis was not reported; and it now appears certain that the hydroxylation process is not positionally specific under these conditions.[6] Further evidence for the *meso*-hydroxyporphyrin was obtained by Fischer and his colleagues, who showed that *meso*-hydroxyhaemins were obtained by treating haemochromes with hydrogen peroxide in pyridine: [7,8] *meso*-hydroxycoproporphyrin I tetramethyl ester was obtained crystalline and analysed. Both groups demonstrated the ready autoxidation of the initial intermediate to the verdohaemochrome stage, and the transformation of this, after demetallation, to the bilatriene system. We have studied the *meso*-oxidation of a symmetrical model, octaethylhaem, in order to confirm and clarify these results by spectroscopic observations and, for the first time, by a direct comparison of the products with authentic compounds prepared by ring synthesis.[1] We have also observed the effect of varying the co-ordinated metal ion in the hope that a knowledge of the way the metal ion affects reactivity in the heterocyclic ligand might throw some light on the role of iron in haem catabolism.

Each metalloporphyrin was treated, under nitrogen, with hydrogen peroxide (*ca.* 1·5 mol. equiv.) in pyridine at 50—60° for 15 min. Under these conditions octaethylporphyrin was largely recovered.

Iron Complexes.—Although octaethylhaemin [chloroiron(III) octaethylporphyrin] did not react (75% recovery of octaethylporphyrin), the dipyridinehaemochrome (I) [dipyridineiron(II) octaethylporphyrin], which crystallised as dark purple prisms from oxygen-free pyridine, and which gave the normal n.m.r. spectrum expected for a low-spin iron(II) complex, reacted rapidly to give an olive-green solution ($\lambda_{max.}$ 649 nm). Reduction with dithionite gave a wine-red solution showing a typical sharp haemochrome spectrum ($\lambda_{max.}$ 519 and 547 nm; $\varepsilon_\beta > \varepsilon_\alpha$) similar to that of the original haemochrome (I) ($\lambda_{max.}$ 517 and 545·5 nm; $\varepsilon_\alpha > \varepsilon_\beta$) except that the relative intensities of the two bands were reversed. Lemberg observed [5] a similar reversal of intensities with 'oxyprotohaemochrome,' and Clezy [9] has reported such behaviour for iron complexes of *meso*-hydroxyporphyrins made by ring synthesis. This suggested that the intermediate ($\lambda_{max.}$ 649 nm) was the iron(III) complex of a hydroxyporphyrin.

Direct removal of iron from the olive-green intermediate gave octaethyloxophlorin (II) in low yield (12%) together with other pigments. It proved more satisfactory to treat the intermediate, still under nitrogen, with benzoyl chloride, whereupon the solution became red once more. Demetallation gave 5-benzoyloxyoctaethylporphyrin (III) (75%), identical with a sample prepared by the ring-synthetic route,[1] thus establishing directly that substitution had occurred at a *meso*-position.[10]

† Tautomeric with the oxophlorin system. The hydroxy name is used in this paragraph for simplicity.

[1] Part IV, R. Bonnett, M. J. Dimsdale, and G. F. Stephenson, *J. Chem. Soc.* (*C*), 1969, 564.

[2] Presented in summary at the 5th International Symposium on the Chemistry of Natural Products, IUPAC, London, 1968.

[3] For a review see R. Lemberg, *Rev. Pure Appl. Chem.*, 1956, **6**, 1; for initial observation see O. Warburg and E. Negelein, *Ber.*, 1930, **63**, 1816.

[4] R. Lemberg, B. Cortis-Jones, and M. Norrie, *Nature*, 1937, **139**, 1016.

[5] R. Lemberg, B. Cortis-Jones, and M. Norrie, *Biochem. J.*, 1938, **32**, 171.

[6] R. Bonnett and A. F. McDonagh, *Chem. Comm.*, 1970, 237.

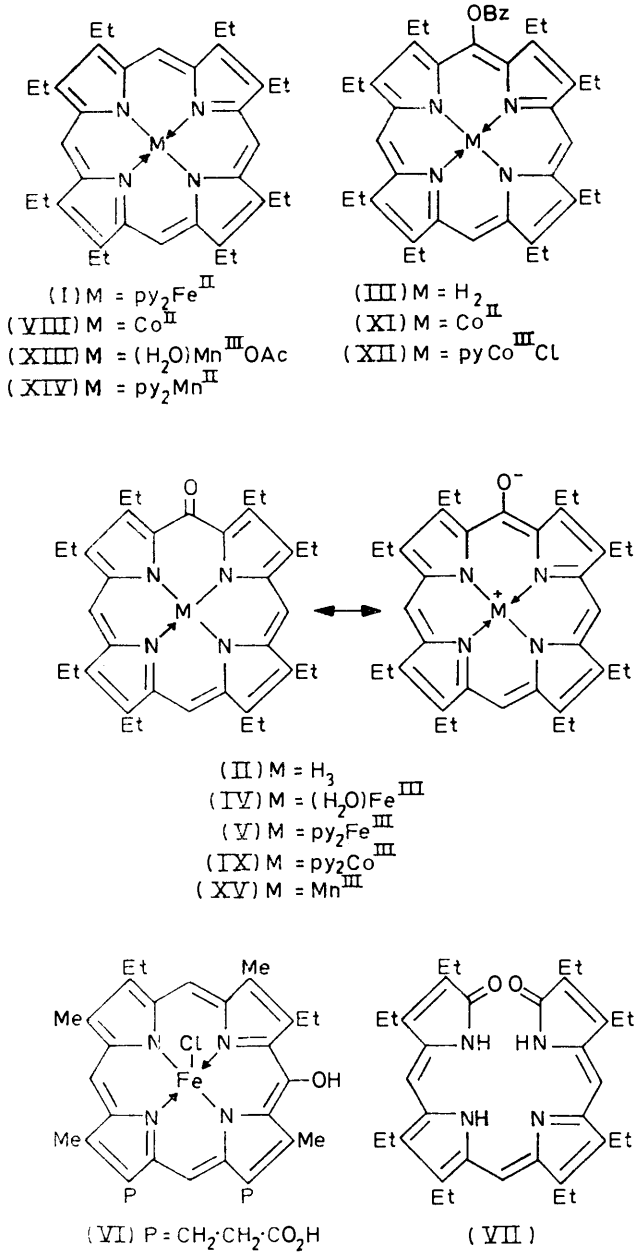
[7] H. Libowitsky and H. Fischer, *Z. physiol. Chem.*, 1938, **255**, 209.

[8] H. Libowitsky, *Z. physiol. Chem.*, 1941, **265**, 191; E. Stier, *ibid.*, 1942, **272**, 239; **273**, 47.

[9] P. S. Clezy and A. W. Nichol, *Austral. J. Chem.*, 1965, **11**, 1835.

[10] Preliminary communication, R. Bonnett and M. J. Dimsdale, *Tetrahedron Letters*, 1968, 731.

The intermediate ($\lambda_{max.}$ 649 nm) could not be isolated from the peroxide oxidation, largely because it was



readily autoxidised (Figure 1). However, aquairon(III) octaethyloxophlorin (IV) was obtained as black needles by metallation of octaethyloxophlorin, and its spectroscopic properties in oxygen-free pyridine, both before ($\lambda_{max.}$ 648 nm) and after ($\lambda_{max.}$ 520, 548 nm; $\varepsilon_\beta > \varepsilon_\alpha$) reduction confirmed that the olive-green intermediate was the iron(III) octaethyloxophlorin system, presumably as its dipyridinehaemichrome (V). Compound (IV) gave analytical figures expected for an aqua-complex. The i.r. spectrum showed a strong band at 1560 cm^{-1} ascribed to the strongly polarised carbonyl group of an oxophlorin, and we therefore prefer the structure shown. This stands in contrast to the chloroiron(III) 10-hydroxymesoporphyrin structure (VI) assigned to the haemin obtained from 10-oxomesophlorin by Kenner and his colleagues: [11] however, compound (VI) was crystallised from methanolic hydrogen chloride and under these conditions addition of hydrogen chloride to the iron(III) oxophlorin system would be expected.

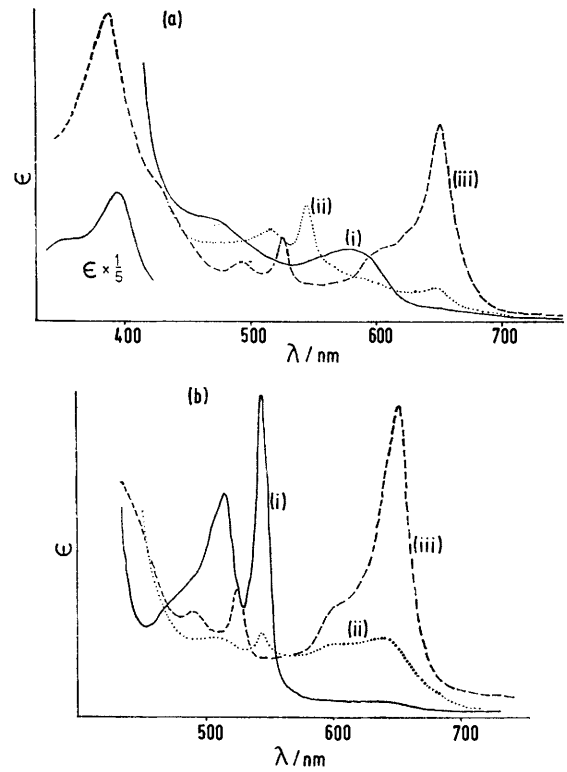


FIGURE 1 Iron porphyrins. (a) The course of the *meso*-oxidation with ascorbic acid–air (Lemberg); solvent 5% pyridine in chloroform: (i) octaethylhaemin, (ii) within 1 min of addition of ascorbic acid. The maxima at *ca.* 520 and 550 nm are ascribed to the iron(II) porphyrin (pyridine haemochrome): this in turn gives the iron(III) oxophlorin, which is rapidly autoxidised to octaethylverdohaemochrome, (iii) (after 16 min with occasional shaking in air). (b) The course of the *meso*-oxidation using hydrogen peroxide on the haemochrome (Libowitsky) followed by aeration; solvent degassed aqueous pyridine, Thunberg cell: (i) dipyridineoctaethylhaemochrome, (ii) 9 min after addition of 1 mol. equiv. of H_2O_2. This spectrum is that of the iron(III) oxophlorin: benzoyloxylation at this stage gives a *meso*-benzoyloxy-derivative (see Experimental section). (iii) On exposure to air, and shaking, with formation of octaethylverdohaemochrome (*cf.* Figure 1a). On treating this solution with benzoyl chloride the spectrum is virtually unchanged *

Aeration of the pyridine solution of the olive-green intermediate (V) [from (I) or (IV)] caused rapid conversion into the verdohaemochrome [3,12] ($\lambda_{max.}$ 387, 525, and 650 nm) (Figure 1). Hydrolysis of this gave

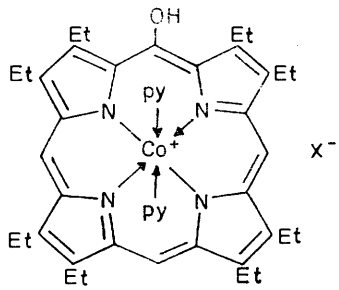
* We thank Dr. A. F. McDonagh for this series of spectra.

[11] A. H. Jackson, G. W. Kenner, and K. M. Smith, *J. Chem. Soc.* (*C*), 1968, 302.

[12] *Cf.* E. Y. Levin, *Biochemistry*, 1966, **5**, 2845, and references therein.

octaethylbilatriene-*abc* (VII) [31% from (I); 50% from (IV)]. In each experiment the bilatriene was accompanied by red pigments which are thought to belong to the bilipurpurin group.[13]

Cobalt Complexes.—When subjected to the reaction with hydrogen peroxide, bromo(pyridine)cobalt(III) octaethylporphyrin was recovered (87%). Cobalt(II) octaethylporphyrin (VIII) readily reacted, however, the solution becoming lime-green in colour. The new species was much more stable towards autoxidation than the iron(III) intermediate (V), and was isolated as deep blue prisms, analysis of which gave results in agreement with the dipyridinecobalt(III) octaethyloxophlorin structure (IX). The same compound was obtained when octaethyloxophlorin (II) was metallated ($CoCl_2$–HOAc) and the crude product was crystallised from pyridine in the presence of air, again demonstrating that *meso*-oxidation had occurred. Spectroscopic data were in accord with the oxophlorin structure (IX) rather than the *meso*-hydroxyporphyrin structure (X); indeed the latter, requiring a counter ion, was excluded by the microanalytical data. The visible spectrum (Figure 2) was unlike those of known cobalt(III) porphyrins,[14] but became like these when the solution was acidified, presumably because *O*-protonation [to give (X), axial ligands no longer specified; *cf.* (VI)] had occurred. The i.r. spectrum of a solution in chloroform displayed no absorption attributable to a hydroxy-function, but a strong band was present at 1545 cm^{-1} indicative of a strongly polarised carbonyl group [*cf.* octaethyloxophlorin (1565 cm^{-1}) and the iron complex (IV)]. As



(X)

expected for an octahedral cobalt(III) complex it was possible to observe n.m.r. spectra, although that of a solution in deuteriochloroform showed considerable broadening of the methylene signals and the *meso*-signals were not detected: it is possible that some degree of paramagnetic character, already noted for the free base,[15] remains associated with the macrocycle even when it is complexed. Indeed, Clezy has recently reported [16] a paramagnetic zinc complex of this series. In deuteriopyridine the methyl and methylene groups of (IX) gave rise to fairly well resolved triplets (τ 8·08 and 8·38) and quartets (τ 5·81 and 6·45). A solution in deuteriotrifluoroacetic acid gave two signals at −0·49 (2H) and −0·09 (1H), presumably attributable to the *meso*-protons: however, in view of the low percentage recovery of the compound from this solution, we regard this assignment with some reservation.

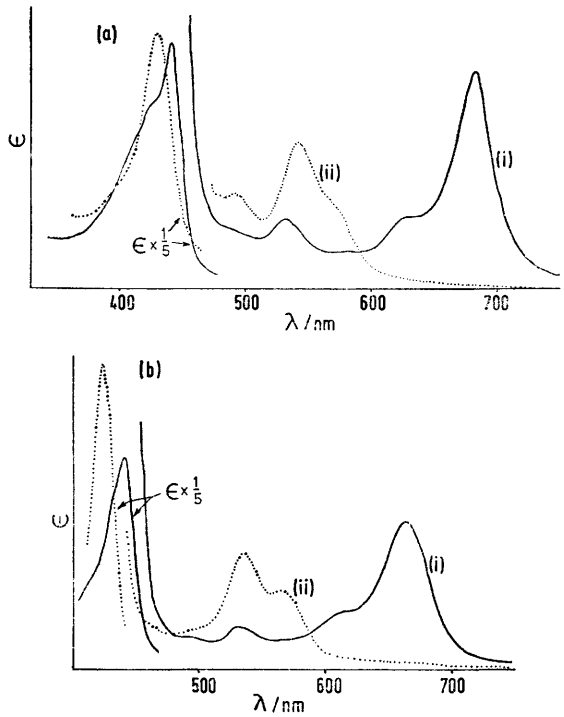


FIGURE 2 Cobalt porphyrins. Electronic spectra of dipyridinecobalt(III) octaethyloxophlorin (a) (i) in ether, (ii) in this solution treated with a trace of 10N-HCl; (b) (i) in 5% pyridine in chloroform, (ii) in this solution treated with a small amount of benzoyl chloride

The complex (IX) was benzoylated (*cf.* Figure 2b) to give red-purple prisms of a new cobalt complex which was identical with the product formed by aerating a solution in pyridine of cobalt(II) 5-benzoyloxyoctaethylporphyrin (XI) [prepared by metallation of (III) in the normal way] in the presence of hydrochloric acid. This product displayed the electronic spectrum expected [14] for a cobalt(III) porphyrin, and a sharp i.r. band at 1740 cm^{-1} confirmed the presence of a benzoyloxy-group. The n.m.r. spectrum of this complex (see Experimental section) confirmed the identification as chloro(pyridine)-cobalt(III) 5-benzoyloxyoctaethylporphyrin (XII). Hydrolysis with sodium hydroxide in aqueous pyridine regenerated the oxophlorin complex (IX).

Manganese Complexes.—The electronic spectrum of acetatoaquamanganese(III) octaethylporphyrin (XIII) resembled those reported by Calvin [17] and Boucher [18] for

[13] W. Siedel and W. Fröwis, *Z. physiol. Chem.*, 1940, **267**, 37; W. Siedel and E. Grams, *ibid.*, p. 49.
[14] A. W. Johnson and I. T. Kay, *J. Chem. Soc.*, 1960, 2979.
[15] R. Bonnett, M. J. Dimsdale, and K. D. Sales, *Chem. Comm.*, 1970, 962.
[16] P. S. Clezy, A. J. Liepa, and G. A. Smythe, *Austral. J. Chem.*, 1970, **23**, 603.
[17] P. A. Loach and M. Calvin, *Biochemistry*, 1963, **2**, 361; G. Engelsma, A. Yamamoto, E. Markham, and M. Calvin, *J. Phys. Chem.*, 1962, **66**, 2517.
[18] L. J. Boucher, *J. Amer. Chem. Soc.*, 1968, **90**, 6640; 1970, **92**, 2725.

other manganese(III) porphyrins. Reduction of compound (XIII) in pyridine (brown solution) with aqueous dithionite gave a magenta solution from which water precipitated a magenta solid. When dissolved in pyridine in air, this was rapidly reoxidised to the manganese(III) level, but it was isolable as magenta prisms, relatively stable in air, by crystallisation from oxygen-free aqueous pyridine containing dithionite. Microanalytical data accorded with the formulation of this substance as dipyridinemanganese(II) octaethylporphyrin (XIV), and this structure was supported by the close parallel between its electronic spectrum and that reported [17] for manganese(II) haematoporphyrin. The manganese porphyrins have received comparatively little attention until recently: [17,18] indeed, the complex (XIV) appears to be the first manganese(II) porphyrin isolated in a crystalline analytically pure condition.

Treatment of the manganese(III) porphyrin (XIII) with hydrogen peroxide in pyridine gave a brown solution, not markedly sensitive to autoxidation, which afforded a black crystalline solid (31%). The same product was obtained (74%) from the manganese(II) porphyrin (XIV). Analytical data accorded with a manganese oxyoctaethylporphyrin formula, and since the complex could also be obtained by metallation of octaethyloxophlorin, it was evident that once again *meso*-oxidation had occurred. Analytical and spectroscopic evidence indicated that the product was to be formulated as manganese(III) octaethyloxophlorin (XV). Thus analysis indicated the absence of counter ions, and, indeed, of further axial ligands.* The electronic spectrum of a solution in chloroform was unlike that of the manganese(III) porphyrin (XIII), but possessed four broad bands and an inflection, all of relatively low molar extinction, suggesting reduced conjugation in the macrocycle. On acidification ($CF_3 \cdot CO_2H$) the spectrum reverted to that of a normal manganese(III) porphyrin owing to *O*-protonation [Figure 3; *cf.* the analogous reaction with (IX)]. The i.r. spectrum had strong bands at 1550 and 1570 cm^{-1} attributed to the polarised carbonyl group of (XV): no hydroxy-stretching mode was apparent. It appears, therefore, that whereas divalent metal complexes of the oxophlorins exist predominantly in the *meso*-hydroxyporphyrin form,[11,16] complexes of trivalent metal ions prefer the oxophlorin system which may be seen [*e.g.* (IV), (IX), and (XV)] to accommodate a trivalent ion satisfactorily.

Nickel, Copper, and Zinc Complexes.—The hydrogen peroxide–pyridine reagent was without appreciable action on the nickel, copper, and zinc complexes of octaethylporphyrin (see Experimental section).

Discussion.—Within the first transition series metal complexes studied here it is apparent that *meso*-substitution has occurred with those metal porphyrins (Mn^{II}, Fe^{II}, and Co^{II}) in which an easily accessible higher oxidation state is available to the metal ion. Conversely, ligand oxidation is not observed, or is only detected to a minor degree, with the metal complexes containing iron(III), cobalt(III), nickel(II), copper(II), and zinc(II) ions. (The fate of the hydrogen peroxide in these cases has not been determined: it is possible that some catalytic decomposition occurs.) Ligand reactivity here does not appear to depend in a simple way on ionic charge (Fe^{II} reacts; Cu^{II} does not), on axial ligandation (neither Zn^{II}, which would be expected to co-ordinate readily with pyridine ligands, nor Cu^{II}, which would not,[20] reacts) or on *d*-electron configuration, although the reactive compounds are all complexes of metal ions with the *d* orbital just over half complete (d^5, d^6, d^7) and in the low spin state. The only example so far encountered where a higher oxidation state might be regarded as not readily available has been the reaction with the manganese(III) compound [which gave a 31% yield of manganese(III) oxophlorin]. However, the Mn^{IV} oxidation state appears to be stabilised by the porphyrin ligand. Thus Loach and Calvin [17] report that treatment of manganese(III) haematoporphyrin with sodium hypochlorite gave a manganese(IV) porphyrin, which was observed to revert spontaneously to the manganese(III) compound, presumably by oxidation of the solvent. It is therefore conceivable that a manganese(IV) oxophlorin may be an

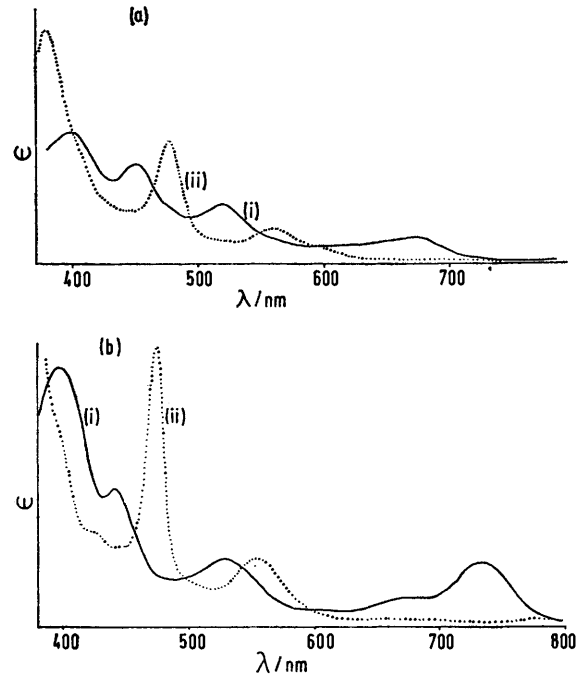


FIGURE 3 Manganese porphyrins. Electronic spectrum of manganese(III) octaethyloxophlorin (a) (i) in chloroform, (ii) in this solution treated with a trace of trifluoroacetic acid; (b) (i) in 5% pyridine in chloroform, (ii) in this solution treated with a small amount of benzoyl chloride

* Pyridine complexes of manganese porphyrins have been observed to lose the axial ligands readily on heating *in vacuo*; presumably this loss occurs here during drying for analysis (*cf.* ref. 19).

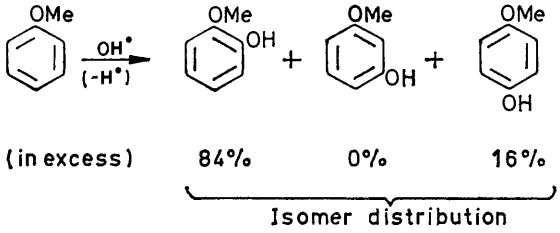
[19] A. Yamamoto, L. K. Phillips, and M. Calvin, *Inorg. Chem.*, 1968, **7**, 847.
[20] A. H. Corwin, D. G. Whitten, E. W. Baker, and G. G. Kleinspehn, *J. Amer. Chem. Soc.*, 1963, **85**, 3621.

intermediate in the *meso*-hydroxylation of the manganese(III) octaethylporphyrin complex, but this is evidently a less favourable process than the corresponding reaction of the manganese(II) system (74% yield).

It is emphasised that our conclusions apply to the pyridine–hydrogen peroxide reagent, first used in this series by Libowitzky and Fischer,[7] and cannot be extended, without further study, to other systems. Thus hydrogen peroxide in dilute solution is reported [21] not to react with the copper, cobalt, and magnesium derivatives of protoporphyrin but, under other conditions, does react with chloroiron(III) protoporphyrin, although the products have not been identified, and an initial autoxidative attack at the vinyl groups may be involved.[22] In various chemical and electrochemical systems stable cation radicals may be formed by one-electron oxidation of the macrocycle:[23] however, these processes, which give products with spectra reminiscent of those of the oxophlorins, are unlikely to be involved in the present reaction, since, for example, the order of the first ligand oxidation potentials assigned to the metalloporphyrins (zinc < cobalt) [23] suggests a reactivity order in the opposite sense to that observed with the hydrogen peroxide–pyridine reagent.

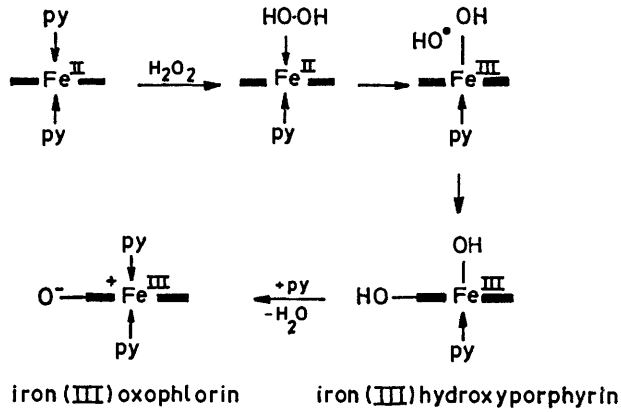
Since both *meso*-position and metal ion are oxidised, it is possible that a mechanism analogous to that proposed for aromatic hydroxylation with Fenton's reagent (H_2O_2–$FeSO_4$) may apply here. This reagent is thought to generate the hydroxyl radical, which may attack

$$Fe^{II} + H_2O_2 \longrightarrow Fe^{III} + OH^- + OH^{\bullet}$$



SCHEME 1

benzenoid systems, and in doing so demonstrates electrophilic character (for example [24] Scheme 1). It has been shown in earlier parts of this series, and elsewhere, that the *meso*-positions of porphyrins and metalloporphyrins are susceptible to electrophilic attack.[25] It is therefore suggested that hydrogen peroxide [26] displaces one of the pyridine ligands [27] from the pyridine haemochrome and that there follows a reaction of the Fenton type in which, as a consequence of the Fe^{II} ion being embedded in the heteroaromatic system, the hydroxyl radical is generated in the vicinity of the *meso*-position. Since the *meso*-position is readily attacked by electrophilic species, and since the hydroxyl radical is highly reactive, the



SCHEME 2

meso-hydroxylation is expected to occur efficiently (which, overall, is reflected in the yields observed here). Viewing the porphyrin nucleus edge-on, the proposed mechanism may be drawn as in Scheme 2. Analogous *meso*-hydroxylation processes can be envisaged for the manganese(II) and cobalt(II) porphyrins.

Haem Catabolism.—The chemistry of haem catabolism is complex and uncertain. As others, particularly Lemberg,[5] have recognised, reactions of the sort studied here are attractive as model processes because they proceed from iron porphyrins to bilatrienes rapidly and under mild (if not physiological) conditions. Evidently for the *in vitro* process the vinyl and propionic acid side chains are not essential for the reactions to occur, nor do they direct the reaction to the α-bridge (C-5) to more than a minor extent.[6] It follows that, in spite of the refutation [28] of earlier reports on the isolation of an enzyme fraction from mammalian liver,[29] the reaction *in vivo* must, if it follows the same general sequence of chemical steps, be directed to C-5 in some way, and presumably enzymically.[30] However, Ó Carra and Colleran [31] have observed that the globin system may

[21] F. Haurowitz, *Enzymologia*, 1937, **2**, 9.
[22] M. Rothschild, *Arch. Biochem. Biophys.*, 1960, **90**, 229; S. B. Brown and P. Jones, *Trans. Faraday Soc.*, 1968, **64**, 994.
[23] A. Stanienda and G. Biebl, *Z. phys. Chem.* (*Frankfurt*), 1967, **52**, 254; A. Wolberg and J. Manassen, *J. Amer. Chem. Soc.*, 1970, **92**, 2982; J. H. Fuhrhop and D. Mauzerall, *ibid.*, 1969, **91**, 4174; R. H. Felton, D. Dolphin, D. C. Borg, and J. Fajer, *ibid.*, 1969, **91**, 197.
[24] R. O. C. Norman and G. K. Radda, *Proc. Chem. Soc.*, 1962, 138; C. R. E. Jefcoate, J. R. L. Smith, and R. O. C. Norman, *J. Chem. Soc.* (*B*), 1969, 1013.
[25] For a review see H. H. Inhoffen, J. W. Buchler, and P. Jäger, *Fortschr. Chem. org. Naturstoffe*, 1968, **26**, 284.
[26] For a discussion of hydrogen peroxide adducts of haem systems in this connection, see R. Lemberg, *Rev. Pure Appl. Chem.*, 1956, **6**, p. 16.
[27] *Cf.* J. N. Phillips, *Rev. Pure Appl. Chem.*, 1960, **10**, 35.
[28] E. Y. Levin, *Biochem. Biophys. Acta*, 1967, **136**, 155.
[29] H. Nakajima, T. Takemura, O. Nakajima, and K. Yamaoka, *J. Biol. Chem.*, 1963, **238**, 3784.
[30] Recent reports on the isolation of enzyme preparations include: O. Nakajima and C. H. Gray, *Biochem. J.*, 1969, **111**, 23P; R. Tenhunen, H. S. Marver, and R. Schmid, *J. Biol. Chem.*, 1969, **244**, 6388.
[31] P. Ó Carra and E. Colleran, *FEBS Letters*, 1969, **5**, 295.

play some part in directing the reaction, and McDonagh, in experiments with freshly isolated crystalline human haemoglobin, has made broadly similar observations.[32] The chemical detail of the first stage *in vivo* is obscure: it may take a course analogous to that suggested for the model system, although an alternative pathway involving ligand oxidation followed by *meso*-attack of water must also be considered. Either route would generate the iron(III) hydroxyporphyrin, and the present work indicates that this would exist as the iron(III) oxophlorin system. This (presumably as its protein haemochrome) would be expected to be so readily autoxidised[33] that enzymic activation might not be required. This ease of autoxidation stands in contrast to the behaviour of the cobalt(III) and manganese(III) derivatives and points up a previously unsuspected role of the metal ion in the reactivity of these systems.

EXPERIMENTAL

General experimental conditions were as indicated previously.[34] Wavelengths (in nm for electronic spectra) and wave numbers (in cm^{-1} for i.r. spectra) refer to maxima unless otherwise stated: molar extinctions appear in parentheses. N.m.r. data are given in τ values with reference to internal tetramethylsilane, with integration and multiplicity in parentheses. Mass spectra were measured on an A.E.I. MS902 instrument, by use of the direct insertion technique. Light petroleum refers to the fraction of b.p. 60—80°. Solvent compositions are given as volume ratios. Yields refer to isolated materials, except in the case of the direct preparation of octaethyloxophlorin. 1% Hydrogen peroxide in pyridine was prepared by appropriate dilution of aqueous hydrogen peroxide (100 vol.). In naming the porphyrins the positions of the eight ethyl groups (2, 3, 7, 8, 12, 13, 17, 18) are omitted throughout.

Iron Porphyrins

Octaethylhaemin [*Chloroiron*(III) *Octaethylporphyrin*].*—Octaethylporphyrin (250 mg) was extracted (Soxhlet) into a refluxing solution of iron(III) chloride (125 mg) and anhydrous potassium acetate (150 mg) in glacial acetic acid (90 ml). Dark purple prisms of octaethylhaemin were removed and were washed (water, aqueous sodium hydrogen carbonate, and water). The product was recrystallised (Soxhlet) from chloroform–benzene (1 : 3) to give dark purple prisms (239 mg, 82%) of *octaethylhaemin*, m.p. >300° (Found: C, 69·3; H, 6·95; Cl, 5·7; N, 8·8. $C_{36}H_{44}ClFeN_4$ requires C, 69·3; H, 7·1; Cl, 5·7; N, 9·0%), λ($CHCl_3$) 380 (96,400), 462infl (7300), 507 (9400), 535 (9700), 582infl (2600), and 634 (4500).

Pyridine Octaethylhaemochrome [*Dipyridineiron*(II) *Octaethylporphyrin*).—Hydrazine hydrate (0·50 ml) was added to octaethylhaemin (150 mg) stirred under nitrogen in pyridine (22·5 ml) at *ca.* 50° (colour change red-brown ⟶ wine-red). After 5 min the solution was cooled (ice bath, 5 min) and acetic acid (0·75 ml) was added, followed, after 2 min, by water (22·5 ml). After a further hour at 0° the precipitate was washed with water and dried to give crude *pyridine octaethylhaemochrome* as a red-brown powder (152 mg, 91%). For analysis this was recrystallised from pyridine (nitrogen flush) containing a little water, to give dark reddish-purple prisms (98 mg), m.p. >300° (Found: C, 73·65; H, 7·25; N, 11·2. $C_{46}H_{54}FeN_6$ requires C, 74·0; H, 7·3; N, 11·25%), λ(pyridine) 407·5 (125,000), 508infl (15,700), 517 (17,300), and 545·5 (30,500), τ(C_5D_5N) −0·03 (4H, s), 5·09—5·59 (3·75H, m, co-ordinated pyridine?), 5·94 (16H, q), and 8·06 (24H, t).

meso-*Oxidation of Pyridine Octaethylhaemochrome*.—A stirred solution of pyridine octaethylhaemochrome (112·5 mg) in pyridine (75 ml; nitrogen-flushed) was treated with 1% hydrogen peroxide in pyridine (0·68 ml, 1·3 mol. equiv.). The solution was warmed to *ca.* 60° (colour change red ⟶ olive-green) and stirred at that temperature for 15 min. An excess of benzoyl chloride (2·7 ml) was added (colour change to red-brown), and stirring was continued at room temperature for 30 min. The residue obtained after removing the solvent under reduced pressure was dissolved in acetic acid (175 ml) and stirred (nitrogen, room temp., 5 min) with a saturated solution (20 ml) of iron(II) chloride in concentrated hydrochloric acid. Dilution with water, extraction with chloroform, washing of the extract (aqueous sodium hydrogen carbonate and water), drying, and column chromatography (grade II alkaline alumina) gave octaethylporphyrin (15 mg; eluted with 1 : 1 benzene–light petroleum) and a second red band, eluted with benzene. The second fraction was rechromatographed and gave, after recrystallisation from chloroform–methanol, purple prisms (74 mg, 75%) of 5-benzoyloxyoctaethylporphyrin, m.p.† 276—278° (lit.,[1] m.p. 280—283°) identical (i.r., t.l.c., and electronic spectroscopy) with an authentic sample prepared by the ring-synthetic route.[1] Hydrolysis of the benzoyloxy-derivative gave octaethyloxophlorin as already described.[1]

Octaethyloxophlorin directly from the meso-*Oxidation of Pyridine Octaethylhaemochrome*.—The preceding experiment was repeated with 33 mg of the haemochrome. Benzoyl chloride was not added to the olive-green solution, which was concentrated under reduced pressure. The residue was dissolved, under nitrogen, in acetic acid (75 ml; nitrogen-flushed) and treated with saturated iron(II) chloride in concentrated hydrochloric acid (10 ml). The solution was stirred for 5 min at room temperature, diluted with water, and extracted with chloroform. The extract was washed (water, sodium hydrogen carbonate, and water), dried, concentrated, and chromatographed on alkaline alumina (grade V). Benzene eluted octaethyloxophlorin (3 mg, 12%; estimated spectroscopically) as a blue-green substance, identical (t.l.c. and electronic spectrum) with authentic material, save that t.l.c. revealed a trace of octaethylbilatriene-*abc*.

Attempted meso-*Oxidation of Octaethylhaemin*.—Octaethylhaemin (31 mg) in pyridine (25 ml) was treated as before with hydrogen peroxide in pyridine (1·3 mol. equiv.). Excess of benzoyl chloride was added, and demetallation and purification were carried out as for the reaction of

* Octaethylhaemin has been characterised before but the analysis was unsatisfactory.[35] Since in our work this was a key compound, we thought it necessary to obtain an analytically satisfactory sample.

† Given incorrectly as 267—268° in ref. 10.

[32] R. Bonnett and A. F. McDonagh, unpublished work.

[33] A. H. Jackson and G. W. Kenner, in 'Porphyrins and Related Compounds,' Biochemical Symposium No. 28, ed. T. W. Goodwin, Academic Press, London, 1968, p. 3; see also T. Kondo, D. C. Nicholson, A. H. Jackson, and G. W. Kenner, *Biochem. J.*, 1971, **121**, 601.

[34] R. Bonnett and G. F. Stephenson, *J. Org. Chem.*, 1965, **30**, 2791.

[35] H. Fischer and R. Baumler, *Annalen*, 1929, **468**, 58.

pyridine haemochrome. Chromatography on grade II alkaline alumina gave octaethylporphyrin (20 mg, 75%; benzene elution) and a trace of a blue pigment (chloroform elution) having the same visible spectrum as octaethylbilatriene-*abc*. 5-Benzoyloxyoctaethylporphyrin was not detected.

Iron(III) *Octaethyloxophlorin*.—Octaethyloxophlorin (30 mg) was extracted (Soxhlet) under nitrogen in the dark into a solution of anhydrous iron(III) chloride (45 mg) and anhydrous sodium acetate (75 mg) in acetic acid (12 ml). The precipitate of needles was washed (water, aqueous sodium hydrogen carbonate, and water) and dried *in vacuo* to give black needles (28 mg, 83%) of *aquairon*(III) *octaethyloxophlorin*, m.p. >300°, which was recrystallised from benzene–light petroleum (Found: C, 69·65; H, 6·85; N, 8·9. $C_{36}H_{45}FeN_4O_2$ requires C, 69·55; H, 7·3; N, 9·0%), λ($CHCl_3$) 392 (58,000), 493infl (15,200), and 528infl (13,000), λ(pyridine) 425·5 (49,000), 648 (5300), λ(pyridine–$Na_2S_2O_4$) Soret band, 540infl (7000), 520 (11,600), and 548 (8000), ν(KBr) 1590, 1560, 1060, 1055, 1015, 955, and 885.

Octaethylbilatriene-abc.—(a) *From the haemochrome*. Pyridine octaethylhaemochrome (38 mg) in pyridine (25 ml; nitrogen-flushed) was treated with hydrogen peroxide in pyridine (1%; 0·25 ml). The solution was warmed to *ca.* 60° and stirred for 15 min. Air was then bubbled through the solution for 10 min, and the solvent was removed. The green residue was dissolved in chloroform (10 ml; nitrogen flushed) and treated with methanolic 2N-potassium hydroxide (20 ml), followed by methanolic 50% hydrogen chloride (25 ml). The dark blue solution was kept for 5 min, diluted with water (100 ml), and exhaustively extracted with chloroform. The extract was washed (water, aqueous sodium hydrogen carbonate, and water) and dried. Chromatography on alkaline alumina (grade V) with benzene followed by crystallisation from methanol gave dark blue prisms (9 mg, 31%) of *octaethylbilatriene*-abc, m.p. 251—257° (decomp.) (Found: C, 75·85; H, 8·4; N, 10·9%; M^+, 554·362. $C_{35}H_{46}N_4O_2$ requires C, 75·75; H, 8·35; N, 10·1%; *M*, 554·362), λ(EtOH) 300infl (23,500), 367 (55,500), and 648·5 (17,300), λ(EtOH–$CF_3{\cdot}CO_2H$) 296 (20,000), 361 (58,600), 634infl (24,000), and 689 (35,500), ν(KBr) 3413, 3340, 1687, 1616, 1587, 1210, 1150, 1053, 1010, and 943, τ($CDCl_3$) 1·81br (3H, s), 3·36 (1H, s), 4·10 (2H, s), *ca.* 7·50 (12H, overlapping q), 7·74 (4H, q), and *ca.* 8·90 (24H, m). Addition of D_2O at room temperature caused the signal at τ 1·81 to disappear.

(b) *From iron*(III) *octaethyloxophlorin*. Iron(III) octaethyloxophlorin (10 mg) was dissolved in pyridine (7 ml) under nitrogen ($\lambda_{max.}$ 424, 525, 605, and 650 nm). The solution was aerated for 10 min ($\lambda_{max.}$ 387, 525, and 650 nm). After removal of solvent under reduced pressure, the residue was dissolved, under nitrogen, in methanolic 2N-potassium hydroxide (5 ml; brown solution), and treated with methanolic 20% hydrogen chloride (6 ml). The deep blue solution was stirred at room temperature for 5 min, then diluted with water (100 ml) and extracted exhaustively with ether. The ethereal extract was washed (water, aqueous sodium hydrogen carbonate, and water), dried and concentrated. Chromatography (alkaline alumina, grade V) with benzene eluted a bluish-green fraction, followed by the main, deep blue product. The first band (thought to be the iron verdin complex) was re-treated with methanolic hydrogen chloride and worked up as before to give, after chromatography, a further quantity of the blue product, which was obtained from methanol as dark blue prisms (4·6 mg, 51%), identical (t.l.c. and electronic spectrum) with the sample of octaethylbilatriene-*abc* obtained before.

Cobalt Porphyrins

Cobalt(II) *Octaethylporphyrin*.—Octaethylporphyrin (75 mg) was refluxed for 5 min in glacial acetic acid (80 ml) with an excess of cobalt(II) chloride hexahydrate and sodium acetate. The crystalline product was washed (water, aqueous sodium hydrogen carbonate, water, and methanol) and then dried to give red hair-like needles (59 mg, 71%) of cobalt(II) octaethylporphyrin, m.p. >300°, which was recrystallised from chloroform–light petroleum (Found: C, 73·4; H, 7·25; N, 9·45. $C_{36}H_{44}CoN_4$ requires C, 73·1; H, 7·5; N, 9·45%), λ($CHCl_3$) 391 (215,000), 518 (10,300), and 551 (21,700).

Bromo(*pyridine*)*cobalt*(III) *Octaethylporphyrin*.—Cobalt(II) octaethylporphyrin [from octaethylporphyrin (50 mg)] in pyridine (10 ml) was treated with hydrobromic acid (48%; 0·5 ml) and heated on a steam-bath for 30 min. The solvent was removed under reduced pressure. The residue in chloroform was washed with water and dried. The filtered concentrated solution was treated with hot light petroleum to give purple needles (33·5 mg, 48%) of *bromo*(*pyridine*)-*cobalt*(III) *octaethylporphyrin*, m.p. >300° (Found: C, 65·9; H, 6·3; N, 9·45. $C_{41}H_{49}BrCoN_5$ requires C, 65·6; H, 6·6; N, 9·35%), λ($CHCl_3$) 346 (25,500), 416 (134,000), 529 (10,900), and 561 (11,500), τ($CHCl_3$) 0·0 (4H, s), 3·7—4·3 (2H, m), 5·1—5·4 (3H, m), 5·91 (16H, q), and 8·13 (24H, t).

meso-*Oxidation of Cobalt*(II) *Octaethylporphyrin*.—Cobalt(II) octaethylporphyrin (60 mg) in pyridine (75 ml) was treated, under nitrogen, at *ca.* 60° with 1% hydrogen peroxide in pyridine (0·6 ml, 1·7 mol. equiv.) and the solution, which slowly became lime-green in colour, was stirred for 15 min. The solvent was removed under reduced pressure, and the residue was chromatographed (alkaline alumina, grade V). After benzene had eluted a small amount of a pink fraction [λ($CHCl_3$) 521 and 553 nm], chloroform–benzene (1 : 4) eluted the main product (lime-green solution) which was obtained, from pyridine–water, as dark-blue prisms (58 mg, 75%) of *dipyridinecobalt*(III) *octaethyloxophlorin*, m.p. >300° (Found: C, 72·45; H, 7·23; Co, 7·75 (from residue, as Co_3O_4); N, 11·1. $C_{46}H_{53}CoN_6O$ requires C, 72·25; H, 7·0; Co, 7·7; N, 11·0%), λ(pyridine) 430infl (68,000), 442 (102,000), 489 (2300), 531 (3500), 582infl (2600), 629infl (6600), and 675 (20,100), λ(pyridine–HBr) 430 (170,000), 540 (11,000), and 551infl (6700), ν(KBr) 1600w, 1580, 1545, 1210, 1060, 1015, 955, 890, 800, 760 and 690, ν($CHCl_3$) 1608w, 1583, 1065, 1055, 1020, 960, and 890, τ($CDCl_3$) 3·81br (2·44H, t), 4·74br (4·25H, t), 5·9—7·1 (17H) and 8·45br (24H, t) (the resolution was not improved at −28°), τ(C_5D_5N) 5·31br (1H, s), 5·81 and 6·45 (both br q, total 17H), and 8·08 and 8·38 (both t, total 24H).

Dipyridinecobalt(III) *Octaethyloxophlorin by Metallation*.—Octaethyloxophlorin (5 mg) was dissolved in refluxing acetic acid (7·5 ml) and treated with an excess of a solution of cobalt(II) chloride hexahydrate and sodium acetate in acetic acid. The solution was refluxed for 5 min and the red-brown needles which had formed were removed, washed, and dried [λ($CHCl_3$) 400, 525infl, 648infl, 676, and 766]. Crystallisation from pyridine–water gave dark blue prisms (2·2 mg, 32%) of dipyridinecobalt(III) octaethyloxophlorin, identical (i.r., t.l.c., and electronic spectrum) with the product from the *meso*-oxidation of cobalt(II) octaethylporphyrin.

Chloro(pyridine)cobalt(III) *5-Benzoyloxyoctaethylporphyrin.*—Dipyridinecobalt(III) octaethyloxophlorin (20 mg) in pyridine (15 ml) was treated with benzoyl chloride (10 μl) (colour change green ⟶ red). After 30 min, the solvent was removed and the residue was crystallised from benzene–light petroleum to give purple microprisms (20·5 mg, 95%) of *chloro(pyridine)cobalt*(III) *5-benzoyloxyoctaethylporphyrin*, m.p. >300° (Found: C, 69·75; H, 6·7; Co (as Co_3O_4 from residue) 7·1; N, 8·25. $C_{48}H_{53}ClCoN_5O_2$ requires C, 69·75; H, 6·45; Co, 7·15; N, 8·5%), λ($CHCl_3$–pyridine) 352 (27,000), 424 (145,000), 536 (10,900), and 565 (6900), ν(KBr) 1740, 1603, 1020, and 710, τ($CDCl_3$) −0·12 (2H, s), 0·28 (1H, s), 1·43—1·83 (2H, m), 2·19—2·58 (3H, m), 3·9—4·9 (1H, m), 5·18—5·5 (2H, m), 5·66—6·5 (16H, m), and *ca.* 8·15 (24H, overlapping t).

The same compound was obtained by the metallation ($CoCl_2,6H_2O$–NaOAc–HOAc) of 5-benzoyloxyoctaethylporphyrin, followed by treatment of the product with pyridine and hydrogen chloride [*cf.* the preparation of bromo(pyridine)cobalt(III) octaethylporphyrin] and crystallisation from benzene–light petroleum.

Hydrolysis of Chloro(pyridine)cobalt(III) *5-Benzoyloxyoctaethylporphyrin.*—Chloro(pyridine)cobalt(III) 5-benzoyloxyoctaethylporphyrin (5 mg) in pyridine (3 ml) and 2N-sodium hydroxide (0·5 ml) was refluxed for 15 min (colour change red ⟶ green). The solution was carefully diluted with water until crystallisation occurred; dark blue prisms (2·5 mg) of dipyridinecobalt(III) octaethyloxophlorin, identical (i.r., t.l.c., and electronic spectrum) with authentic material, were obtained.

Attempted meso-*Oxidation of Bromo(pyridine)cobalt*(III) *Octaethylporphyrin.*—Bromo(pyridine)cobalt(III) octaethylporphyrin (24 mg) in pyridine (20 ml) was treated in the usual way with hydrogen peroxide (1·5 mol. equiv.) in pyridine. The solution was stirred for 15 min at 60°, but showed no change in visible absorption. The solvent was removed and the residue was crystallised from chloroform–light petroleum to recover (87%) the starting material (i.r., t.l.c., and electronic spectrum).

Manganese Porphyrins

Acetatoaquamanganese(III) *Octaethylporphyrin.*—Octaethylporphyrin (100 mg) was extracted from a thimble into a solution of an excess of manganese(II) acetate in acetic acid (120 ml). The solution was diluted with water (250 ml) and then extracted with chloroform until no further pigment was removed. The extract was washed (water, aqueous sodium hydrogen carbonate, and water) and evaporated; the residue crystallised from chloroform–light petroleum to give *acetatoaquamanganese*(III) *octaethylporphyrin*, m.p. >300°, as brownish-black needles (100 mg, 81%) (Found: C, 68·65; H, 7·85; N, 8·8. $C_{38}H_{49}MnN_4O_3$ requires C, 68·65; N, 7·45; N, 8·45%), λ($CHCl_3$) 358 (75,000), 420 (17,200), 464 (57,000), 556 (10,800), 687 (6300), and 775 (1400), ν($CHCl_3$) 3175, 1693, and 1608.

Dipyridinemanganese(II) *Octaethylporphyrin.*—Acetatoaquamanganese(III) octaethylporphyrin (40 mg) in pyridine (20 ml, nitrogen-flushed and stirred) was treated with saturated aqueous sodium dithionite (4 ml). After 4 min the magenta mixture was diluted with water (20 ml; nitrogen-flushed). The precipitate was removed, washed with water, and recrystallised under nitrogen from pyridine–water containing sodium dithionite to give magenta prisms (30 mg, 67%) of *dipyridinemanganese*(II) *octaethylporphyrin*, m.p. >300° (Found: C, 73·85; H, 7·45; N, 11·45. $C_{46}H_{54}N_6Mn$ requires C, 74·05; H, 7·3; N, 11·25%), λ(pyridine–water 1 : 1, containing $Na_2S_2O_4$, under nitrogen) 424·5 (156,000), 510infl (3500), 550 (18,000), and 584 (9700).

meso-*Oxidation of Acetatoaquamanganese*(III) *Octaethylporphyrin.*—Acetatoaquamanganese(III) octaethylporphyrin (25 mg) in pyridine (25 ml) was stirred for 15 min at *ca.* 60° with 1% hydrogen peroxide in pyridine (0·2 ml, 1·6 mol. equiv.). The solvent was removed (reduced pressure); chromatography of the residue on alumina (grade V, alkaline) with chloroform gave a greenish-brown fraction, and methanol–chloroform (1 : 4) eluted starting material (6 mg). The material in the chloroform fraction was obtained from chloroform–light petroleum as a black amorphous solid (7·0 mg, 31%). Crystallisation from pyridine–water gave black needles of *manganese*(III) *octaethyloxophlorin*, m.p. >300°, dried under high vacuum (60°, 2 h) for analysis (Found: C, 71·5; H, 7·1; N, 9·45. $C_{36}H_{43}MnN_4O$ requires C, 71·75; H, 7·2; N, 9·3%), λ($CHCl_3$) 398 (35,000), 451·5 (26,800), 520 (16,200), and 679 (7200), λ($CHCl_3$ + trace $CF_3{\cdot}CO_2H$) 380, 476, and 560), λ(pyridine) 394 (27,700), 438 (22,300), 494infl (6400), 528 (9320), 678 (4000), and 743 (12,000), λ(pyridine–10N-HCl, 1 : 1) 380, 474, and 557, λ(pyridine–aq. $Na_2S_2O_4$) 431, 525infl, 557, 584infl, and 604, ν(KBr) 1608, 1568, 1546, 1148, 1054, and 890. A Nujol mull showed no absorption bands attributable to an OH group.

meso-*Oxidation of Dipyridinemanganese*(II) *octaethylporphyrin.*—Dipyridinemanganese(II) octaethylporphyrin (15 mg) in pyridine (15 ml; nitrogen-flushed) was treated at *ca.* 60° with 1% hydrogen peroxide in pyridine (0·1 ml, 1·56 mol. equiv.). The solution, which rapidly changed from magenta to brown, was stirred for 15 min, then worked up as in the preceding experiment to give manganese(III) octaethyloxophlorin (9·0 mg, 74%) as a black powder, identical (i.r., t.l.c., and electronic spectrum) with samples of this compound obtained by other methods.

Manganese(III) *Octaethyloxophlorin.*—Octaethyloxophlorin (11 mg) in boiling acetic acid (15 ml) was treated with excess of manganese(II) acetate in acetic acid (colour change green ⟶ brown). After 5 min under reflux, the solution was cooled, diluted with water, and extracted with chloroform. Chromatography of the washed extract on alumina as before gave (after removal of solvent) a greenish-brown residue. This was washed with a little ether (to remove unchanged oxophlorin) and was obtained from chloroform–light petroleum as a black powder (2·7 mg), identical (i.r., t.l.c., and electronic spectrum), with the manganese(III) octaethyloxophlorin from the *meso*-oxidation reactions.

Other Porphyrins

Copper(II),[36] nickel(II),[37] and zinc(II)[38] complexes of octaethylporphyrin were prepared as described elsewhere.

Attempted meso-*Oxidation.*—(a) *Copper complex.* Copper(II) octaethylporphyrin (10 mg) in pyridine (10 ml) under nitrogen was treated at *ca.* 60° with 1% hydrogen peroxide in pyridine (0·1 ml, 1·75 mol. equiv.). The solution was stirred for 15 min, during which time no change in visible absorption occurred. The solvent was removed, and the residue was crystallised from chloroform–methanol to give

[36] U. Eisner, *J. Chem. Soc.*, 1957, 3461.
[37] G. F. Stephenson, Ph.D. Thesis, London, 1964.
[38] O. Somaya, Dissertation, Brunswick, 1967.

the starting material (i.r., t.l.c., and electronic spectrum) in virtually quantitative recovery.

(b) *Zinc complex.* The zinc porphyrin behaved in an analogous way, and was recovered (80%).

(c) *Nickel complex.* The nickel porphyrin behaved in a similar way. Column chromatography on alumina (grade V, alkaline) with benzene recovered starting material (90%): a trace of a green-brown compound [$\lambda(CHCl_3)$ 520, 554, and 640] was eluted with chloroform.

(d) *Free base.* Octaethylporphyrin (53 mg) was treated with hydrogen peroxide in pyridine as before. The solution remained red. Excess of benzoyl chloride was added and the mixture was worked up in the usual way and chromatographed on alumina (grade II, alkaline). Benzene eluted octaethylporphyrin (30 mg, 56%); chloroform–benzene (1 : 1) eluted a trace of a second porphyrin which was not 5-benzoxyloxyoctaethylporphyrin (t.l.c.).

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