Nitrosation and Nitrosylation of Haemoproteins and Related Compounds. Part 1. Porphyrins and Metalloporphyrins

By **Raymond Bonnett,* Antonios A. Charalambides,** and **Ronald A. Martin,** Department of Chemistry, Queen Mary College, Mile End Road, London E1 4NS

Acidified nitrite mixtures react with octaethylporphyrin, mesoporphyrin dimethyl ester, and (less effectively) octaethylhaemin to give *meso*-nitro-derivatives. Such products are also furnished by the reaction of sodium nitrite or nitric oxide with a preparation of the radical cation of zinc(II) octaethylporphyrin. The radical cation also gives *meso*-substituted derivatives with chloride, thiocyanate, and benzoate ions.

Nitrosylhaems are characterised from the reactions of nitric oxide with octaethylhaemin and protohaemin dimethyl ester. The pigment of cured meat, nitrosylprotohaem, has been fully characterised (as its dimethyl ester) for the first time. The reaction of excess of acidified nitrite with octaethylhaemin for a short period gives a product regarded as a mixture of mono- and di-nitrosyl derivatives, but in the presence of ascorbic acid nitrosyloctaethylhaem is formed cleanly. Under suitable conditions nitrosylhaems react with secondary amines to generate nitrosamines. The results are discussed in the context of the chemistry of meat curing processes.

SODIUM NITRITE is employed in strictly regulated amounts as an antibacterial and cosmetic agent [1] in the processing of certain meat products. The use of nitrite in the curing of meat probably has its origins in antiquity, the nitrite arising from the microbiological reduction of nitrate present as an impurity in the salt used in curing. The characteristic effect on colour seems first to have been recorded in the late Roman period.[2] The pink pigment of cured meat was first recognised as a nitrosylhaem † by Haldane [3] in 1901, and is currently most reasonably viewed as a denatured nitrosylmyoglobin (Scheme 1).

However, the reaction is more complex than this. First, considering only reactions at the metal, the stoicheiometric formation of the nitrosyl using acidified nitrite requires a reductant (Scheme 1):‡[6] this may be one of a number of natural reducing agents occurring in the tissue, but sometimes ascorbic acid is added to the cure. In the absence of other reductants the iron(II) porphyrin may fulfil this role, with the consequent formation of the nitrosyl complex and the iron(III) complex (metmyoglobin) in a 1 : 1 ratio.[6,7] In the presence of

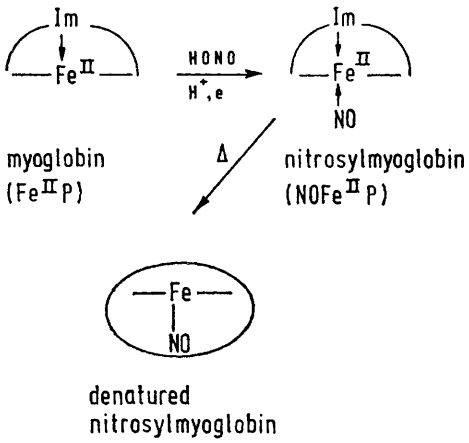
$$2Fe^{II}P + HONO \longrightarrow NO{\cdot}Fe^{II}P + Fe^{III}P^{+} + OH^{-}$$

oxygen the product is metmyoglobin.[8a,§] The latter reaction is significant in another context: high nitrite levels in drinking water are especially dangerous to

$$2Fe^{II}P + HONO + O_2 + H_2O \longrightarrow 2Fe^{III}P^{+} + HNO_3 + 2OH^{-}$$

infants because of the analogous formation of methaemoglobin, which cannot transport oxygen, and which the infants cannot efficiently convert back into haemoglobin because the enzymic capability to do this is still developing.[9]

Secondly, reactions may occur at sites other than the metal. There are several indications of this.[3,10] Thus methaemoglobin made by oxidising haemoglobin with



SCHEME 1 Generation of denatured nitrosylmyoglobin from myoglobin. The horizontal line represents protoporphyrin viewed edge on. The globin is represented by the thickened curved line, one amino-acid (histidine F8) [4] furnishing an axial iminazole (Im) ligand. In denatured nitrosylmyoglobin the exact structure is not known: there is evidence for pentacoordinate iron.[5] The ellipse is an attempt to convey the protective effect that the denatured protein exerts on the pigment of cured meat.

P stands for porphyrin dianion throughout this paper.

† We follow the convention of employing nitrosyl to refer to NO liganded to a metal ion, and nitroso to refer to C–NO, S–NO *etc.*

‡ This problem does not arise when nitric oxide is used as the reagent ($Fe^{II}P + NO \rightarrow NO{\cdot}Fe^{II}P$) but this does not appear to be employed in current curing practice.

§ There is also evidence that this reaction can lead to hydrogen peroxide.[8b]

[1] K. Mohler, Proceedings of the International Symposium on Nitrite in Meat Products, Zeist, 1973, Centre for Agricultural Publishing and Documentation, Wageningen, 1974, p. 13; J. Meester, *ibid.*, p. 265.

[2] Review: E. F. Binkered and O. E. Kolari, *Food. Cosmet. Toxicol.*, 1975, **13**, 655.

[3] J. Haldane, *J. Hygiene*, 1901, **1**, 115.

[4] H. C. Watson, *Progr. Stereochem.*, 1969, **4**, 299.

[5] R. Bonnett, K. D. Sales, and P. A. Scourides, unpublished work.

[6] J. Brooks, *Proc. Roy. Soc.*, 1937, **B123**, 368; N. W. Wakid and K. Y. Helou, *Internat. J. Biochem.*, 1973, **4**, 257.

[7] F. Jung and H. Remmer, *Arch. exptl. Path. Pharmakol.*, 1949, **206**, 459.

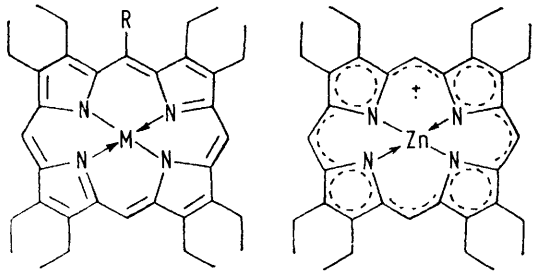
[8] (*a*) J. H. Austin and D. L. Drabkin, *J. Biol. Chem.*, 1935, **112**, 67; L. A. Greenberg, D. Lester, and H. W. Haggard, *J. Biol. Chem.*, 1943, **151**, 665; M. Bartik and J. Kupka, *Folia. Vet.*, 1967, **11**, 43 (*Chem. Abs.*, 1968, **68**, 92,904c); (*b*) G. Cohen, M. Martinez, and P. Hochstein, *Biochemistrt*, 1964, **3**, 901.

[9] J. D. Ross and J. F. Desforges, *Pediatrics*, 1959, **23**, 718.

[10] *E.g.* H. Uchida and M. H. Klapper, *Biochim. Biophys. Acta*, 1970, **221**, 640.

nitrite has been reported [11] to be different from the oxidation product obtained when ferricyanide is used. Occasionally the curing process goes wrong, and green pigments are produced (' nitrite greening ').[12] Clearly side reactions could occur in the porphyrin ligand, or in the protein chain, and we are examining both these possibilities. With reference to the protein part of the molecule, we have already discussed the reaction of tryptophan derivatives (1-nitrosation),[13,14] and we report here some reactions of porphyrins and metalloporphyrins. These side reactions may have a special importance if they lead to *N*-nitrosamines, or to substances which have the potential to nitrosate ingested secondary amines, since a number of *N*-nitrosamines are known to be powerful carcinogens.[15]

Reaction at Carbon.—In order to examine the reactivity of the porphyrin system with acidified nitrite mixtures, the model systems octaethylporphyrin (1) and octaethylhaemin (2) were employed. Both compounds gave products of *meso*-nitration. Thus octaethylporphyrin reacted with sodium nitrite in aqueous acetic acid–sulphuric acid at room temperature during 90 min to give 5-nitro-octaethylporphyrin (3) (69%). Similarly, mesoporphyrin dimethyl ester (in chloroform–acetic acid–water) gave the *meso*-nitro-derivative (presumably a

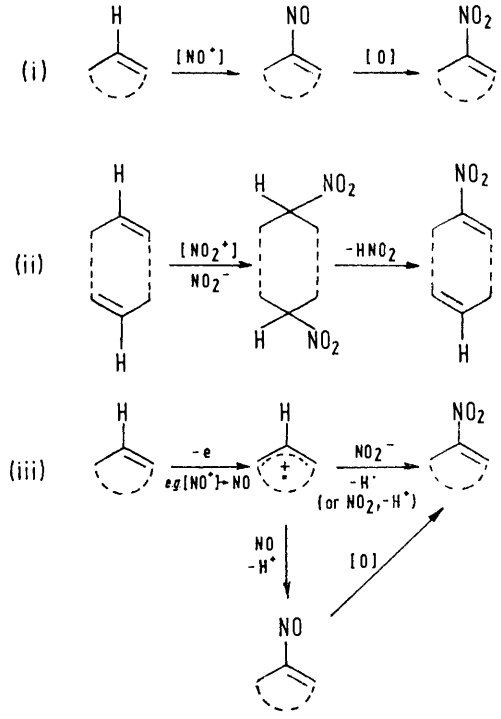


(1) M = 2H, R = H
(2) M = Fe^{III}Cl, R = H
(3) M = 2H, R = NO_2
(4) M = Fe^{III}Cl, R = NO_2
(10) M = Fe^{II}NO, R = H

(5)

mixture of isomers, 65%). Small amounts of a number of by-products, including dinitro-derivatives,[16] have been observed in small-scale reactions, but we have not recognised nitroso-derivatives as such. Fischer and Neumann [17] reported a dinitro-nitroso-derivative as the major product from etioporphyrin I and nitrous acid, but this product does not appear to have been subjected to chromatographic purification.

Under similar conditions the iron(III) complex (2) gave the corresponding 5-nitro-derivative (4), although the yield was low (23%) and much octaethylhaemin was recovered. (During a short term reaction, nitrosylation was observed, see below).



SCHEME 2 Aromatic nitration with acidified nitrite solutions: three possible routes. The abbreviated structures refer to *meso*-positions of the porphyrin skeleton

Clearly, *meso*-substitution of the porphyrin system occurs readily under mild nitrosating conditions. The reaction presumably involves electrophilic *C*-nitrosation, followed by oxidation of the nitroso-group to nitro (i, Scheme 2), but other pathways are conceivable. One such is the addition of N_2O_3 (or N_2O_4) followed by elimination (see ii, Scheme 2): addition–elimination processes across the *meso*-bridges of the porphyrin system have been postulated before,[18] and this pathway finds analogy in the reaction of mixed nitrogen oxides with anthracene.[19] Another possible route involves the oxidation of the heteroaromatic system to an intermediate radical cation which is then attacked by the nitrite ion or by nitric oxide (iii, Scheme 2).

Experiments to test this last possibility with octaethylporphyrin and octaethylhaemin did not give promising results, although the reaction of octaethylporphyrin with silver nitrite and iodine (conditions used

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[13] K. L. Agarwal, G. W. Kenner, and R. C. Sheppard, *J. Chem. Soc.* (*C*), 1969, 954.
[14] R. Bonnett and R. Holleyhead, *J.C.S. Perkin I*, 1974, 962.
[15] P. N. Magee and J. M. Barnes, *Brit. J. Cancer*, 1956, **10**, 14; *Adv. Cancer Res.*, 1967, **10**, 163.
[16] R. Bonnett and G. F. Stephenson, *J. Org. Chem.*, 1965, **30**, 2791.
[17] H. Fischer and W. Neumann, *Annalen*, 1932, **494**, 225.
[18] *E.g.* R. Bonnett, I. A. D. Gale, and G. F. Stephenson, *J. Chem. Soc.* (*C*), 1967, 1168.
[19] J. Meisenheimer and E. Connerade, *Annalen*, 1904, **330**, 133; E. D. B. Barnett, *J. Chem. Soc.*, 1925, 2040.

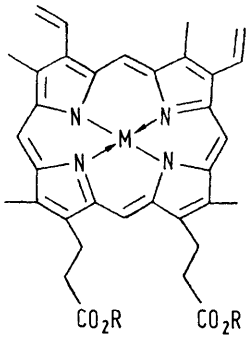
successfully in the nitration of perylene presumably *via* a radical cation pathway [20]) did give a low yield of (3). More success was achieved with zinc(II) octaethylporphyrin, which is more readily oxidised than either the parent base or the iron(III) complex (half wave oxidation potentials, $E_{\frac{1}{2}}$, for (1; M = Fe^{III} 1.00 V, 2 H 0.81 V, and Zn^{II} 0.63 V).[21] Successful substitution reactions along this route have also been reported recently for magnesium(II) octaethylporphyrin [22] ($E_{\frac{1}{2}} \sim 0.54$ V) [21] and for zinc(II) complexes of octaethylchlorin [22] and *meso*-tetraphenylporphyrin.[23]

The radical cation (5) of zinc(II) octaethylporphyrin was generated either chemically (with bromine) or electrochemically (at a controlled potential), its formation ($\lambda_{max.}$ 650 nm) being followed spectroscopically. Treatment with an excess of sodium nitrite solubilised in an organic solvent with a crown ether gave appreciable yields of the 5-nitro-derivative (25—33%). Treatment of the radical cation (chemical oxidation) with nitric oxide gave the 5-nitro-derivative (35%) and a mixture of the 5,10- and 5,15-dinitro-derivatives (13%). These results suggest that both pathways indicated in Scheme 2(iii) are feasible.

Dolphin has argued [24,25] that nucleophiles do not react with porphyrin radical cations (but only with the dications): we are not converted to this view. The radical cation appears to be set up to react both with radical reagents and with nucleophiles, and the simplest interpretation of the reactions with nitric oxide and nitrite, respectively, would be in such terms. Moreover, examples of nucleophilic attack on radical cations appear to be securely demonstrated in other areas.[26] Further examples were provided in small-scale experiments. Following the generation of the radical cation (5) by either oxidative method, treatment with sodium chloride gave a mixture of the 5-chloro- and 5,15-dichloro-derivatives,[27] while treatment with potassium thiocyanate gave the 5-thiocyanato-derivative.[28] (Chemical oxidation gave the better yields in these reactions.) Reaction of (5) with sodium benzoate gave a low yield of the 5-benzoyloxy-derivative: this exemplifies a likely pathway in the reaction of benzoyl peroxide with metalloporphyrins in the cold.[29]

The reaction of nitrous acid with protoporphyrin and protohaemin [as their dimethyl esters, (6) and (7)] gave mixtures of products which are under investigation. Presumably reaction occurs both at *meso*-positions and at vinyl groups. Some of the products are greenish in colour and hence may be responsible for the 'nitrite greening' referred to earlier.

Reaction at Iron.—Under certain conditions the reaction of octaethylhaemin with acidified nitrite solutions gave not the 5-nitro-derivative but a mixture containing iron nitrosyls (see below). It was important to have the authentic nitrosylhaem for comparison purposes, and this



(6) R = Me, M = 2H Protoporphyrin dimethyl ester
(7) R = Me, M = FeCl Protohaemin dimethyl ester
(8) R = Me, M = FeNO Nitrosylprotohaem dimethyl ester
(9) R = H, M = FeNO Nitrosylprotohaem

was prepared from octaethylhaemin or from methoxo-iron(III) octaethylporphyrin with an excess of nitric oxide in tetrahydrofuran–water mixtures. Nitrosylprotohaem dimethyl ester (8) was prepared in an analogous manner. This appears to be the first time that the pigment responsible for the colour of ham and other cured meats has been fully characterised (albeit as its dimethyl ester). The corresponding free acid (9) has also been prepared, but was not obtained analytically pure. These preparations are regarded as reductive nitrosylations:

$$MeOFe^{III}P + NO \longrightarrow MeONO + Fe^{II}P$$
$$Fe^{II}P + NO \longrightarrow NOFe^{II}P$$

Similar reactions have been reported for tetraphenylhaemin, but we have not isolated the species Cl·NO·FeP detected in that system,[30,31] although such a species could have been formed as an intermediate.

The formation of the model nitrosyloctaethylhaem (10) was signalled by the appearance of a bright pink-red colour ($\lambda_{max.}$ in $CHCl_3$ 389, 478, 531, and 557 nm). The nitrosyl complex gradually decomposed in solution in the presence of air, but the solid complex, brought out of tetrahydrofuran solution by the addition of oxygen-free water, was a bright red solid which could be handled satisfactorily. It could even be subjected to t.l.c. examination, although it decomposed on the plate. The i.r. spectrum showed a strong band at 1 670 cm^{-1} ascribed to the stretching mode of the bent Fe–NO group.*

* Scheidt and Frisse give $\nu_{max.}$ 1 670 cm^{-1} and ∠Fe–N–O = 149.2° for nitrosotetraphenylhaem.[32]

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[21] J. H. Fuhrhop, *Structure and Bonding*, 1974, **18**, 1.
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[25] E. C. Johnson and D. Dolphin, *Tetrahedron Letters*, 1976, 2197.
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[27] R. Bonnett, I. A. D. Gale, and G. F. Stephenson, *J. Chem. Soc.* (*C*), 1966, 1600.
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[31] B. B. Wayland and L. W. Olsen, *J.C.S. Chem. Comm.*, 1973, 897; *J. Amer. Chem. Soc.*, 1974, **96**, 6037.
[32] W. Scheidt and M. E. Frisse, *J. Amer. Chem. Soc.*, 1975, **97**, 17.

Magnetic subsceptibility measurements indicated one unpaired electron ($\mu = 2.4$ B.M.). Similarly, nitrosylprotohaem dimethyl ester had $\lambda_{max.}$ 398, 487, 546, and 570 nm and $\nu_{max.}$ 1 660 cm^{-1}. The e.s.r. spectrum of a polycrystalline sample of this compound was typical of a radical in a rhombic environment ($g = 2.097$, 2.052, and 2.010),[33] the g values resembling those recorded for 'relaxed' subunits of nitrosylhaemoglobin ($g = 2.10$, 2.06, and 2.010).[34]

Nitrosylhaem formation was also observed using acidified nitrite solution, although here the reaction was more complex. (We term this a Type II reaction to distinguish it from the Type I reaction with nitric oxide.) Short term treatment of octaethylhaemin with an excess of nitrite gave a red solid, the i.r. spectrum of which showed bands at 1 664 and 1 875 cm^{-1}. Over several days the bands at 1 875, 1 290, and 800 cm^{-1} decreased relatively. These bands are ascribed to a second nitrosyl complex, probably $(NO)_2FeP$, since in the tetraphenylhaem series [31] the corresponding compound has $\nu =$ 1 870 cm^{-1}. The haemin, NOFeClP, $\nu = 1\ 880\ cm^{-1}$ in the tetraphenylhaem series,[31] is less likely since the Fe–Cl stretch at *ca.* 350 cm^{-1} is missing for the Type II preparation, which is thus regarded as a mixture of (6) and dinitrosyliron octaethylporphyrin. Occasionally the i.r. spectrum of the product also contained a band at 1 535 cm^{-1}, evidence for the presence of *C*-nitration products. A much cleaner reaction occurred with a small excess of sodium nitrite in the presence of ascorbic acid (a source of nitric oxide [35]): the product here was indistinguishable (i.r.) from nitrosyloctaethylhaem.

Transnitrosation. It was considered important to determine whether or not the iron nitrosyl system could act as a nitrosating agent. Such a reaction could be direct, since the odd electron is delocalised,[33,36] and, in valence-bond terms, one of the canonical forms has cationoid character at the ligand. Alternatively, the

$$Fe^{II}\text{–}NO \longleftrightarrow Fe^{III}\text{–}NO^{-} \longleftrightarrow Fe^{I}\text{–}\overset{+}{N}O$$

reaction could be an indirect one, a nitrosating species being liberated in the presence of traces of moisture and oxygen, formally

$$Fe^{II}NO \rightleftharpoons Fe^{II} + NO \xrightarrow{O_2,H_2O} Fe^{III} + HNO_3 + HNO_2$$

It was of interest to discover if, under suitable conditions, a nitrosamine could be produced in this way from a nitrosylhaem. It was found that nitrosyloctaethylhaem in aqueous acetic acid–tetrahydrofuran did cause a small amount (4%) of nitrosation [14] of *N*-acetyltryptophan methyl ester. Treatment of diphenylamine with nitrosylprotohaem dimethyl ester in air led to *N*-nitrosodiphenylamine (37%) and the μ-oxo-haem as the only products. The reaction is regarded as an indirect one, since under nitrogen a much lower yield (10%) of nitrosamine was obtained, together with some unchanged nitrosylhaem. Evidently under suitable

$$NOFe\ P + Ph_2NH \xrightarrow[60°C]{air} Ph_2NNO + PFe\text{–}O\text{–}FeP$$

conditions a nitrosylhaem can furnish a nitrosating species. However, we have so far been unsuccessful in attempts to observe the nitrosation of proline using nitrosylhaemoglobin as the source of the nitrosating species. This may be ascribed, in part at least, to the high stability constant of the iron–nitrosyl system in nitrosylhaemoglobin which is about fifty times greater than the corresponding value for simple nitrosylhaems.[37]

EXPERIMENTAL

General experimental procedures and conventions employed in presenting spectroscopic data have been given previously.[14,28]

Methoxoiron(III) *Octaethylporphyrin.*—Octaethylhaemin (227 mg) was dissolved in chloroform (50 ml) under reflux and triethylamine (1 ml) was added to it. The solution was concentrated to *ca.* 10 ml, methanol (*ca.* 5 ml) was added, and the solution was set aside at 4 °C to yield crystals (163 mg, 72%) of methoxoiron(III) octaethylporphyrin, m.p. >300 °C, darkening at 200 °C (Found: C, 71.3; H, 7.5; N, 9.15. $C_{37}H_{47}FeN_4O$ requires C, 71.7; H, 7.65; N, 9.05%), $\lambda_{max.}$ 394 (ε 110 000), 460i (16 700), and 582 nm (11 300); *m/e* (172 °C) 620 (10), 619 (M^+, 27), 590 (15), 589 (61), 588 (M — OMe, 100), 587 (9), 586 (11), 561 (5), and 560 (10).

μ-*Oxo-bis*(*iron*(III) *Octaethylporphyrin*).[38]—(i) Octaethylhaemin (76 mg) was dissolved in diethylamine (100 ml) under reflux. The solution was filtered, water (10 ml) was added to it, and the solution was concentrated to *ca.* 25 ml. Crystals of the μ-oxo-compound (49 mg, 67%) appeared when the solution was set aside. (ii) Methoxoiron(III) octaethylporphyrin (25 mg) was dissolved in tetrahydrofuran (10 ml) under reflux and addition of water (40 ml) resulted in the immediate formation of crystals of the μ-oxo-compound (21 mg, 87%), $\lambda_{max.}$ 395 (ε 117 000), 472i (25 400), and 584 nm (17 500); *m/e* (227 °C) 1 194 (5), 1 193 (10), 1 192 (M^+, 13), 674 (4), 590 (12), 589 (56), 588 (100), 587 (6), and 586 (14).

Zinc(II) 5-*Nitro-octaethylporphyrin.*—5-Nitro-octaethylporphyrin (26.5 mg) [16] in chloroform (10 ml) and acetic acid (30 ml) was treated with zinc(II) acetate (50.4 mg) dissolved in acetic acid. After 5 min a two-banded spectrum of the zinc complex had developed. The solution was diluted with chloroform (40 ml) and washed (aq. $NaHCO_3$, water). The dried product was crystallised from chloroform methanol to give zinc(II) 5-nitro-octaethylporphyrin (27.2 mg, 92%) (Found: M^+, 641.261. Calc. for $C_{36}H_{43}N_5O_2{}^{64}Zn$: M, 641.271), $\lambda_{max.}$ 406 (ε 197 000), 540 (14 300), and 578 nm (15 100); $\nu_{max.}$ 1 530, 1 375, and 1 370 cm^{-1}.

Reaction of Sodium Nitrite with Porphyrins under Acidic

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[38] J. W. Buchler and H. H. Schneehage, *Z. Naturforsch.*, 1973. **28B**, 433.

Conditions.—(a) *Octaethylporphyrin.* Octaethylporphyrin (105 mg) in conc. sulphuric acid–acetic acid–water (25 : 15 : 25; 65 ml) was cooled in an ice-bath and purged with nitrogen (20 min). Sodium nitrite (200 mg) in water (1 ml) was added to it, and the solution was kept under nitrogen in the ice-bath for 1.5 h. Chloroform (200 ml) was added to the mixture which was then washed with aqueous $NaHCO_3$ and with water, and then dried, concentrated, and subjected to preparative t.l.c. (40 × 40 cm silica gel; light petroleum–acetone 95 : 5). The major component was extracted (spectroscopic yield 82%) and crystallised from chloroform–methanol to give dark purple needles (7.9 mg, 69%) of 5-nitro-octaethylporphyrin identical [electronic spectrum (e.s.), t.l.c.] with an authentic sample.[16]

(b) *Mesoporphyrin dimethyl ester.* A solution of mesoporphyrin dimethyl ester (97 mg) in chloroform (5 ml) and acetic acid (94 ml) was purged with nitrogen (15 min) and treated with sodium nitrite (500 mg) in water (1 ml). The solution was stirred under nitrogen for 90 min, during which time it changed from colour from pink-red to brown-red. The solution was extracted as before and chromatographed on alumina (activity III) with chloroform–light petroleum (1 : 1). Crystallisation from chloroform–methanol gave nitromesoporphyrin dimethyl esters (isomers 68 mg, 65%) identical (e.s., t.l.c.) with the authentic sample [16] (Found: M^+, 639.304; $C_{36}H_{41}N_5O_6$ requires M, 639.306).

(c) *Small-scale experiments.* These were carried out with octaethylporphyrin on a 1—2 mg scale at *ca.* 25 °C for 90 min and yields were determined spectroscopically. The results are summarised in Table 1 (NOEP = *meso*-nitro-octaethylporphyrin).

Reaction of Sodium Nitrite with Octaethylhaemin under Acidic Conditions.—(a) *C-Substitution.* Octaethylhaemin [chloroiron(III) octaethylporphyrin, 26 mg] in chloroform (55 ml) and acetic acid (94 ml) was treated under nitrogen with sodium nitrite (500 mg) in water (1 ml). After 90 min at room temperature the product was worked up in the usual way and chromatographed on alumina (grade III). Elution with chloroform–light petroleum (3 : 2) gave, first, *chloroiron(III) 5-nitro-octaethylporphyrin* (5-nitro-octaethylhaemin) (6.3 mg, 23%; 42% conversion) identical (e.s., t.l.c.) with the sample made by metallation; and, secondly, octaethylhaemin (12 mg, 46%). The t.l.c. separation was difficult, but could be accomplished using glass-backed silica gel H irrigated with light petroleum–ether–methanol 65 : 30 : 10.

The authentic sample of 5-nitro-octaethylhaemin was prepared by extracting 5-nitro-octaethylporphyrin (13.4 mg) from a washed thimble into a solution of anhydrous ferric chloride (11 mg) and anhydrous potassium acetate (11 mg) in acetic acid (15 ml). The crystalline product was washed with acetic acid, and dried *in vacuo* to give bluish rods (13.7 mg, 89%) (Found: C, 64.8; H, 6.6. $C_{36}H_{43}ClFeN_5O_2$ requires C, 64.6; H, 6.5%), $\lambda_{max.}$ 377 (ε 81 900), 509 (7 800), 537 (7 500), and 637 nm (3 500); $\nu_{max.}$ 1 530, 1 375, and 350 cm^{-1}.

(b) *Reaction at metal. Type II preparation.* (i) Octaethylhaemin (32 mg) in tetrahydrofuran (30 ml) was vigorously shaken with sodium nitrite (4 g) in 0.5M-HCl (20 ml). (Colour change orange-red to bright wine red). The tetrahydrofuran solution was immediately concentrated to a few ml and water (100 ml) was added. The resulting precipitate was washed with distilled water and dried under reduced pressure to give a red solid (26 mg) regarded as a mixture of mono- and di-nitrosyliron octaethylporphyrins; $\nu_{max.}$ 1 875, 1 664, 1 444, 1 370, 1 290, 1 270, 1 222, 1 144, 1 110, 1 055, 1 018, 993, 962, 837, 800, 749, and 700 cm^{-1}. (ii) Octaethylhaemin (14.5 mg) in tetrahydrofuran (6 ml) was treated with an aqueous solution (2 ml) of sodium nitrite (23 mg) and ascorbic acid (40 mg) under nitrogen. After 60 s water (45 ml) was added and the red precipitate (6.5 mg) was dried in air. The i.r. spectrum resembled that of nitrosyloctaethylhaem (see below) but had an additional strong band at 1 655 cm^{-1}, which did not disappear after 3 days *in vacuo*. However, when the sample was redissolved and reprecipitated the i.r. absorption was identical to that of nitrosyloctaethylhaem.

TABLE 1

Small-scale reactions of octaethylporphyrin (OEP)

Solvent (volumes in ml)	$NaNO_2$ (mg)	OEP	NOEP	diNOEP	No. of other bands on t.l.c.
H_2SO_4–HOAc–H_2O (25 : 15 : 25)	200	0.4	86	2	6
H_2SO_4–HOAc–H_2O (25 : 15 : 25)	10	61	13		4
$CHCl_3$–py–HOAc–H_2O (10 : 5 : 89 : 1)	200	54	11		3
CHCl–HOAc–H_2O (5 : 94 : 1)	200	5	31		6
$CHCl_3$–HOAc–H_2O (5 : 94 : 1)	*	86			0
THF–MeCN (20 : 5)	†	50	12		2

* Sodium nitrate (200 mg). † Silver nitrite (2.7 mg) and iodine (2.3 mg) in MeCN (5 ml).

Reaction of Nitrite and Other Anions with Oxidised Zinc Octaethylporphyrin.—(a) *Oxidation with bromine.* A typical procedure is as follows. Zinc(II) octaethylporphyrin (10 mg) in dry benzene (50—60 ml) was treated in the dark, after nitrogen purging, with a solution of bromine (*ca.* 5 mg) in benzene (*ca.* 0.5 ml). The formation of the radical cation ($\lambda_{max.}$ 650 nm; colour change pink to purple green) was confirmed by e.s. The solution was then treated with a large excess (100—200 mg) of a salt dissolved in sufficient benzene (*ca.* 5 ml) containing dicyclohexyl-18-crown-6 (100 mg) and stirred (N_2, dark) for some hours. The solution was washed with aqueous $NaHCO_3$ (2 × 100 ml) and water (100 ml) and then dried (Na_2SO_4); the products were separated by t.l.c. and estimated either spectroscopically or by isolation. In some cases a demetallation step (brief treatment with 20% HCl) was introduced before the chromatographic separation. The results are summaried in Table 2.

(b) *Electrochemical oxidation.* A typical procedure is as follows. Zinc(II) octaethylporphyrin (10 mg) in dry redistilled dichloromethane (20 ml) containing tetrabutylammonium perchlorate (0.1M) was electrolysed at 0.78 V using a carbon rod as the working electrode. The formation of the radical cation was followed by e.s. When the formation of the radical cation ($\lambda_{max.}$ 650 nm) appeared to be maximised the mixture was stirred for several hours (N_2, dark) with an excess of the reagent (100—200 mg) in dichloromethane (*ca.* 5 ml) containing the crown ether (100 mg).

The products were examined as before, usually after demetallation with 20% aq. HCl. The results are summarised in Table 2.

Nitrosyl Complexes.—(a) *Nitrosyliron*(II) *octaethylporphyrin* (*nitrosyloctaethylhaem*). *Type I preparation.* (i) Octaethylhaemin (76 mg) was dissolved in tetrahydrofuran (50 ml). After purging of the solution with dry oxygen-free nitrogen, nitric oxide was bubbled through it. At the same time oxygen-free water (100 ml) was slowly added. The bright red microcrystals of *nitrosyloctaethylhaem* (71 mg, 94%) were washed with water and ethanol, and dried (Found: C, 70.1; H, 7.35; N, 10.85. $C_{36}H_{44}FeN_5O$ requires C, 69.9; H, 7.15; N, 11.3%), $\lambda_{max.}$ 389 (83 500), 478 (10 200), 531 (9 300), and 557 nm (9 400); $\nu_{max.}$ 2 960, 2 930, 2 865, 1 670vs, 1 550w, 1 505w, 1 465, 1 445, 1 385w, 1 370m, 1 365m, 1 315m, 1 270, 1 222, 1 145, 1 128w, 1 110m, 1 055, 1 015, 990, 958, 920w, 840, 750, 718w, and 700m cm^{-1}.

(ii) Methoxoiron(III) octaethylporphyrin (29 mg) in tetrahydrofuran (20 ml) was purged with oxygen-free nitrogen and then treated with nitric oxide as above. The solution became bright red, and was purged with nitrogen before being treated with oxygen-free water (glove box) to give the microcrystalline red nitrosyloctaethylhaem (20 mg, 69%) which was washed with water and dried *in vacuo*.

(b) *Nitrosyliron*(II) *protoporphyrin dimethyl ester* (*nitrosylprotohaem dimethyl ester*). The reaction was carried out under argon using a Schlenck tube. Protohaemin dimethyl ester (45 mg) was dissolved in tetrahydrofuran (20 ml) and the solution was carefully degassed. Nitric oxide was bubbled through the solution. After 2 min water (5 ml, oxygen-free) was added. The stream of nitric oxide was continued for a total of 20 min, when more deoxygenated water (50 ml) was added to cause precipitation. The red precipitate was filtered under argon and washed several times with light petroleum and dried *in vacuo* to give red microcrystals (42 mg, 94%) of *nitrosylprotohaem dimethyl ester* (Found: C, 64.15; H, 5.3; N, 10.05. $C_{36}H_{36}FeN_5O_5$ requires C, 64.1; H, 5.4; N, 10.4%), $\lambda_{max.}$ 398 (81 600), 487 (11 300), 546 (10 500), and 570 nm (11 000); $\nu_{max.}$ 1 737, 1 660, 1 625, 1 550, 1 434, 1 358, 1 237, 1 168, 1 125, 1 090, 1 058, 988, 952, 906, 840, 752, 718, and 709 cm^{-1}. This compound was also prepared (62%) from ethoxoiron(III) protoporphyrin and dried *in vacuo* to give red microcrystals (42 mg, 94%) of *nitrosylprotohaem dimethyl ester* (Found: C, 64.15; H, 5.3; N, 10.05. $C_{36}H_{36}FeN_5O_5$ requires C, 64.1; H, 5.4; N, 10.4%), $\lambda_{max.}$ 398 (81 600), 487 (11 300), 546 (10 500), and 570 nm (11 000); $\nu_{max.}$ 1 737, 1 660, 1 625, 1 550, 1 434, 1 358, 1 237, 1 168, 1 125, 1 090, 1 058, 988, 952, 906, 840, 752, 718, and 709 cm^{-1}. This compound was also prepared (62%) from ethoxoiron(III) protoporphyrin dimethyl ester in a manner analogous to that employed with the corresponding methoxoiron(III) octaethyl compound (above).

TABLE 2

Reactions of oxidised zinc octaethylporphyrin (ZnOEP)

Reaction mixture	Reagent	Products	Yields (%)
(a) Oxidation with bromine			
(1) ZnOEP (10 mg)	$NaNO_2$ (170 mg)	ZnOEP	
Br_2 (2.3 mg) [a]	C.E. (110 mg); 2 h	zinc 5-nitroOEP	33 [b,c]
(2) ZnOEP (11.6 mg)	NaCl (103 mg)	OEP	15
Br_2 (5 mg)	C.E. (113 mg), 20 h (demetallated)	5-chloroOEP	20 [d]
		5,15-dichloroOEP	11 [d]
(3) ZnOEP (6.6 mg)	NaOAc (106 mg)	Complex mixture	
Br_2 (3 mg)	C.E. (100 mg), 3 h	(8 components)	
(4) ZnOEP (8.3 mg)	KSCN (116 mg)		
Br_2 (5 mg)	C.E. (102 mg), 2 h (demetallated)	OEP	16, 23
		5-thiocyanatoOEP	48,[b] 53 [b,e]
(5) ZnOEP (10 mg)	NO (bubbled for 5 min)	zinc 5-nitroOEP	35 [b,c]
Br_2 (14 mg)		zinc dinitroOEP	13 [b,f]
(b) Electrochemical oxidation			
(6) ZnOEP (9 mg)	$NaNO_2$ (105 mg)	OEP	0.5
	C.E. (120 mg), 3 h	ZnOEP	29
		zinc 5-nitroOEP	25 [c]
		zinc dinitroOEP	Trace
(7) ZnOEP (8.3 mg)	KSCN (103 mg)	OEP	29
	C.E. (110 mg), 2 h (demetallated)	5-thiocyanatoOEP	12 [e]
(8) ZnOEP (7.2 mg)	NaCl (103 mg)	OEP	29
	C.E. (114 mg), 15 h (demetallated)	5-chloroOEP	5 [d]
		5,15-dichloroOEP	0.8 [d]
(9) ZnOEP (7.3 mg)	$PhCO_2Na$ (150 mg)	OEP	20
	C.E.(115 mg), 3 h (demetallated)	5-benzoyloxyOEP	6

C.E. = crown ether (dicyclohexyl-18-crown-6)

[a] In the absence of oxidant there was no reaction between sodium nitrite and ZnOEP. [b] Isolated: other yields are spectroscopic. [c] Identified with sample described before. [d] Identified with authentic.[26] [e] Comparison sample kindly furnished by Dr. Peter Clezy, University of New South Wales.[27] [f] Demetallation gave 5,10- and 5,15-dinitroporphyrin isomers. Isolated yield refers to this mixture.

Transnitrosation Reactions.—(a) *With nitrosyloctaethylhaem.* Nitrosyloctaethylhaem (1 mg) and *N*-acetyltryptophan ester (2 mg) were dissolved in tetrahydrofuran (4.9 ml) and treated with aqueous acetic acid (0.1 ml, 1 : 1, colour change pink to brown). The solution was kept at room temperature in air for 1 h. The mixture was diluted with chloroform, washed (aq. $NaHCO_3$, H_2O), dried, and submitted to t.l.c. (light petroleum–ether–methanol 65 : 30 : 10). The products, estimated spectroscopically, were recovered *N*-acetyltryptophan methyl ester (69%) and *N*-acetyl-N^1-nitrosotryptophan methyl ester (4%).

(b) *With nitrosylprotohaem dimethyl ester.* (i) Diphenylamine (29.8 mg) and nitrosylprotohaem dimethyl ester (3.4 mg) were heated in dry tetrahydrofuran (2 ml) under nitrogen (60 °C, 3 h). The product was then purified by t.l.c. (benzene) and *N*-nitrosodiphenylamine was obtained in 10% yield. Unchanged nitrosyl haem and the μ-oxo-haem were also detected. In an analogous experiment in air, 37%

of *N*-nitrosodiphenylamine were formed, and the μ-oxo-haem was the only haem derivative detected.

(ii) Diphenylamine (47.6 mg) and nitrosylprotohaem dimethyl ester (4.4 mg) were kept molten (60 °C) for $2\frac{1}{4}$ h in air. The reaction mixture was submitted to t.l.c. (benzene) to give *N*-nitrosodiphenylamine (38%).

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