795. *The Nitration and Hydroxylation of Ætioporphyrin I*

By A. W. Johnson and Diane Oldfield

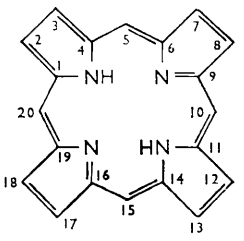
Nitration of ætioporphyrin I has been shown to occur at the *meso*-positions and mono-, di-, and trinitro-derivatives of ætioporphyrin I are described. The physical and chemical properties of the nitro-compounds are described, including reduction of mononitroætioporphyrin I to the mono-amino-derivative. Reaction of *meso*-aminoætioporphyrin I with nitrous acid gives a mixture of products including a *meso*-nitroaminoætiophyrin I.

Hydroxylation of ætioporphyrin I in the presence of sulphuric acid gives a β-oxochlorin formed by a pinacol rearrangement of the intermediate dihydroxychlorin.

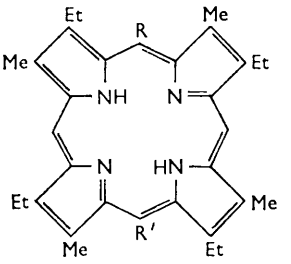
Although several physical properties of the porphyrin ring system (I) indicate that it possesses aromatic character,[1] there have been relatively few studies made of porphyrin substitution reactions. Both calculations [1b] and experimentation suggest that free β-positions (2, 3, 7, 8, 12, 13, 17, and 18 in I; numbering of porphyrins [2]) in the ring are more susceptible to electrophilic attack than are the *meso*-positions (5, 10, 15, and 20 in I)

[1] For example: (*a*) W. T. Simpson, *J. Chem. Phys.*, 1949, **17**, 1218; (*b*) H. C. Longuet-Higgins, C. W. Rector, and J. R. Platt, *ibid.*, 1950, **18**, 1175; (*c*) R. J. Abraham, *Mol. Phys.*, 1961, **4**, 145; (*d*) E. B. Fleischer, *J. Amer. Chem. Soc.*, 1963, **85**, 146; (*e*) A. Stern and G. Klebs, *Annalen*, 1933, **505**, 295.
[2] I.U.P.A.C. rules for porphyrin nomenclature, *J. Amer. Chem. Soc.*, 1960, **82**, 5582.

and bromination has been recommended [3] as a method which will permit the recognition of a free β-position in a porphyrin. However, porphyrins which are substituted in all eight β-positions are also subject to electrophilic substitution and Hans Fischer and his co-workers have described the preparation of a number of halogeno,[4] nitroso,[5] nitro,[5] and sulphonic acid [6] substitution products from such compounds. It is possible that some nitrosoporphyrins may have physiological significance.[7] The orientation of the sub-



(I)



(II)

stituents in these compounds was not, however, always established with certainty. Although Fischer regarded certain of the halogeno and sulphonic acid derivatives as *meso*-substitution products, the structure of the nitroporphyrins has been variously formulated as containing side-chain nitro-substituents [8] and *meso*-nitro-substituents.[9] We have re-examined this problem using ætioporphyrin I (II; R = R′ = H) following the earlier work of Fischer,[10,11] and largely on the basis of n.m.r. spectra, we have shown that nitration involves *meso*-substitution. A preliminary account of our work has been given [12] and a similar conclusion has been reached by Bonnett and Stephenson [13] who studied the nitration of octaethylporphyrin. Fischer and his school have also described nitration products from ætioporphyrin II,[10] tetramethyltetrapropylporphyrin,[14] coproporphyrin I and its tetramethyl ester,[15] and " isouroporphyrin II " octamethyl ester.[16] With phylloporphyrin and pyrroporphyrin, both of which contain an unsubstituted 13-position, it is possible [17] that nitration occurs at this position, but this has not yet been proved.[9]

We have found that nitration of a solution of ætioporphyrin I in 63% sulphuric acid with 25% nitric acid at 12—13° for 30 min. gave 55% of a mononitro-derivative contaminated with a little dinitroætioporphyrin I, which was removed by chromatography on alumina of a solution in carbon tetrachloride. Thin-layer chromatography of benzene–light petroleum solutions is particularly effective for the separation of the mixed nitro-derivatives. In agreement with the observations of Fischer and Neumann,[11] mononitroætioporphyrin I formed a bright red copper complex, which was also obtained by nitration of the porphyrin with cupric nitrate in acetic anhydride at room temperature for 2 hr. Using a longer nitration period, ætioporphyrin I gave a dinitro-derivative and the corresponding copper complex has also been obtained directly by reaction with cupric nitrate and acetic anhydride. Two isomeric *meso*-dinitroætioporphyrins I can exist, the 5,10- and 5,15- (II; R = R′ = NO_2) dinitroporphyrins, and it was demonstrated by thin-layer

[3] H. Fischer and A. Treibs, *Annalen*, 1928, **466**, 188; A. Treibs and E. Wiedemann, *ibid.*, p. 264.
[4] H. Fischer and H. Orth, " Die Chemie des Pyrrols," Vol. II, i, Leipzig, 1937, p. 230.
[5] Ref. 4, p. 263.
[6] Ref. 4, p. 554.
[7] J. B. Fox and J. S. Thomson, *Biochemistry*, 1964, **3**, 1323.
[8] A. Stern and H. Molvig, *Z. phys. Chem.*, 1936, *A*, **177**, 365.
[9] H. Fischer and W. Klendauer, *Annalen*, 1941, **547**, 123.
[10] H. Fischer and A. Treibs, *Annalen*, 1928, **466**, 188.
[11] H. Fischer and W. Neumann, *Annalen*, 1932, **494**, 225.
[12] A. W. Johnson and D. Oldfield, *Tetrahedron Letters*, 1964, 1549.
[13] R. Bonnett and G. F. Stephenson, *Proc. Chem. Soc.*, 1964, 79.
[14] H. Fischer, M. Goldschmidt, and W. Nüssler, *Annalen*, 1931, **486**, 21.
[15] H. Fischer and J. Hilger, *Z. physiol. Chem.*, 1925, **149**, 68; H. Fischer and W. Fröwis, *ibid.*, 1931, **195**, 74.
[16] H. Fischer and E. Thurnher, *Z. physiol. Chem.*, 1932, **204**, 76.
[17] H. Fischer, M. Speitmann, and H. Meth, *Annalen*, 1934, **508**, 156.

chromatography on alumina as well as by steady-state distribution between benzene and 30% sulphuric acid that both isomers were present. We have so far been unable to obtain sufficient of the individual compounds to permit complete characterisation. Under still more vigorous conditions of nitration, trinitroætioporphyrin I could be obtained and this also was converted into its copper derivative.

Reduction of mononitroætioporphyrin I with stannous chloride and hydrochloric acid has given the corresponding amino-derivative (II; R = NH_2, R′ = H, see below), the first *meso*-aminoporphyrin to be characterised. The copper derivative and *N*-acetyl derivative of monoaminoætioporphyrin I have also been prepared, and the visible spectra of the various porphyrin *meso*-substitution products are summarised in Table 1.

TABLE 1

Visible spectra of *meso*-substituted porphyrins (in chloroform) $\lambda_{max.}$ ($\varepsilon_{max.} \times 10^{-3}$)

	Visible max.						
Porphyrin	Soret	IV	III	II	Ia	I	Remarks
Ætio I	397	496	531	564	595	615	Ætio type
	(174)	(14·1)	(10·2)	(6·76)	(1·36)	(4·47)	
Mononitroætio I	398·5	504	538	573		627	Ætio type
	(117·5)	(11·9)	(7·95)	(5·9)		(4·91)	
Dinitroætio I (mixed isomers)	380 397	504	537	575		629	Ætio type
	(99·4) (98·2)	(11·7)	(7·27)	(5·4)		(4·01)	
Trinitroætio I	403	509	537	585		637	
	(91·2)	(11·5)	(6·05)	(4·9)		(2·9)	
Monoaminoætio I	416	519	553	586·5		646	
	(158)	(11·75)	(5·42)	(3·63)		(6·38)	
Monoacetamidoætio I	406	505	537	575		633	Ætio type
	(158)	(13·9)	(8·2)	(6·3)		(4·4)	
Ketonic oxidation product	408	510	549	589	618 *	646	
	(150)	(7·5)	(10·6)	(5·05)	(1·7)	(29·7)	
Oxime of ketonic oxidation product	403	502	534	572	630	656	
	(187)	(10·9)	(13·6)	(4·35)	(2·8)	(37·1)	

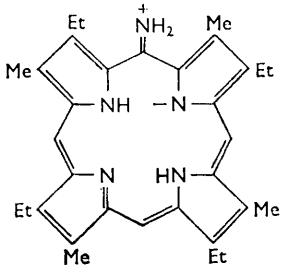
* Inflection.

Largely through the work of Stern and his collaborators,[18] porphyrin spectra are divided into types according to the order of intensity of the four main maxima (I—IV, see Table 1) other than the Soret band (around 400 mμ). It was on this basis that Stern and Molvig,[8] incorrectly, stated that the mononitroporphyrins could not be *meso*-nitro-derivatives. It will be seen from Table 1 that the spectra of the mononitro-, monoacetamido, and dinitro-ætioporphyrins I are still of the ætio- (intensities IV > III > II > I) rather than the phyllo-type (IV > II > III > I), and it seems that the empirical rules formulated for the porphyrins known at that time will not apply to porphyrins containing *meso*-substituents showing strong mesomeric effects. The markedly decreased intensity of the Soret bands of the nitro-compounds compared with unsubstituted ætioporphyrin I are an indication of the loss of overall aromatic character of these derivatives presumably because of the electron-attracting character of the nitro-groups as well as their size and location. However, the same effect is not observed in the spectrum of the *meso*-acetamido-derivative where the Soret band has an intensity comparable with that of the unsubstituted ætioporphyrin. The spectrum of the *meso*-amino derivative is of a type (IV > I > III > II) not described previously and possibly indicates a contribution from mesomeric structures such as (III).

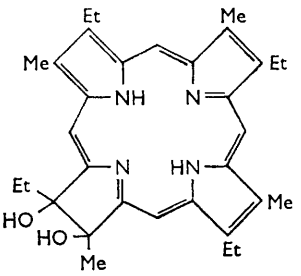
N.m.r. spectra of porphyrins [19] and related macrocyclic systems such as chlorins [20,21] and corroles [22] have been studied extensively during recent years, and provide an ideal method

[18] A. Stern and F. Pruckner, *Z. phys. Chem.*, 1937, *A*, **180**, 321, and earlier Papers.
[19] Review: A. Kowalsky and M. Cohn, *Ann. Rev. Biochem.*, 1964, **33**, 499.
[20] R. B. Woodward and V. Škarič, *J. Amer. Chem. Soc.*, 1961, **83**, 4676.
[21] J. J. Katz, M. R. Thomas, and H. H. Strain, *J. Amer. Chem. Soc.*, 1962, **84**, 3587; J. W. Mathewson, W. R. Richards, and H. Rapoport, *ibid.*, 1963, **85**, 364.
[22] A. W. Johnson and I. T. Kay, *Proc. Chem. Soc.*, 1964, 89; *J.*, 1965, 1620.

for the determination of the orientation of substituents. The spectra of the substitution products of ætioporphyrin I were measured in trifluoroacetic acid (TFA) and deuterotrifluoroacetic acid (DTFA), the porphyrins being present as the dications. The n.m.r. spectrum of



(III)



(IV)

mononitroætioporphyrin I, compared with that of ætioporphyrin I itself,[23] clearly shows the loss of symmetry caused by the introduction of the substituent (Table 2). The single peaks associated with the imino-protons, the *meso*-protons and the ring-substituent methyl proton in the ætioporphyrin I spectrum are all replaced by multiple peaks, in the spectrum

TABLE 2

N.m.r. signals of ætioporphyrins I (TFA solution except where otherwise stated)

Compound	*Meso*-groups (s)	Imino-groups (s)	β-Methyl-groups (s)	β-Ethyl groups CH_2 (q)	β-Ethyl groups CH_3 (t)
Ætioporphyrin I[3]	−1·0	14·8	6·2	5·7	8·16
Mononitroætioporphyrin I	−0·77(2; βδ)	13·72	6·36	5·89	8·29
	−0·68(1; γ)	14·17	6·46		
			6·76		
Trinitroætioporphyrin I	−0·54	11·7	6·63	6·55	8·53
			6·79		
			6·93		
Monoaminoætioporphyrin I	+0·56(2; βδ)	9·01	6·73	6·37	8·33
	+0·99(1; γ)	10·49	6·78		
			6·92		
Monoacetamidoætioporphyrin I	−0·68(2; βδ)	13·3	6·3	5·9	8·26
	−0·59(1; γ)	13·48	6·55		
		14·3	6·69		
		14·4			
Ketonic oxidation product *	−0·52(1)	—	6·42(6)	5·8(6)	8·26(9)
	−0·34(1)		6·50(3)	7·11(2)	9·24(3)
(in DTFA)	−0·23(1)		7·75(3)		
	+0·69(1)				

s = singlet; q = quartet; t = triplet. * Determined on 100 Mc Varian instrument.

of the nitro-compound. In particular, the *meso*-proton signals are present as two singlets (C-10 + C-20 protons; C-15 proton) and correspond in all to only three protons, which suggests that one of the original *meso*-protons has been replaced by the nitro-substituent. A similar observation was made in the spectrum of *meso*-methylætioporphyrin I.[24] The imino-protons differ appreciably in their environment in the mononitro-compound, and the corresponding signals are widely spaced. The position of the signals corresponding to the protons of the meso-, β-methyl, and β-methylene (of the ethyl substituents) groups in the spectrum of nitroætioporphyrin I are at higher field than the corresponding proton signals in the ætioporphyrin I spectrum and those associated with the imino-groups are at

[23] R. J. Abraham, A. H. Jackson, and G. W. Kenner, *J.*, 1961, 3468.
[24] R. J. Abraham, A. H. Jackson, G. W. Kenner, and D. Warburton, *J.*, 1963, 853.

lower field. This represents a substantial decrease in the strength of the induced ring current and hence (cf. refs. 23 and 25) of the degree of aromatic character of the nitroporphyrin. The magnitude of these effects, together with the number and nature of the signals leave no doubt that the nitro-group must occupy a *meso*-position on the macrocyclic ring.

Displacements of the signals associated with the various types of proton in the trinitroætioporphyrin I compared with ætioporphyrin I itself are in the same direction as those observed with the mononitro-compound but are even greater as expected.

The n.m.r. spectrum, like the visible spectrum, of the aminoporphyrin (in TFA, when the amine was present as its salt) shows a remarkable divergence from that of ætioporphyrin I; in fact, the effect of the protonated amino-group on the positions of absorption of the *meso*- and imino-protons exceeds that of three nitro-groups. The spread of absorption (τ 0·19) associated with the side-chain methyl groups is appreciably less in the case of the amino compound than that observed (τ 0·40) in the spectrum of mononitroætioporphyrin I. This effect may be due to the fact that the amino-group, being smaller than nitro, causes less distortion of the planar porphyrin molecule. The effect of the *meso*-acetamido-group on the spectrum of ætioporphyrin I, approximated to that of one *meso*-nitro-group. N.m.r. spectra of solutions of the *meso*-substituted ætioporphyrins in deuterotrifluoroacetic acid were also determined. No exchange of *meso*-protons with deuterium was observed at room temperature, in common with the behaviour of other porphyrins (*e.g.*, ref. 20) although the exchange is known to occur at elevated temperatures in certain cases.[26] However, in the case of monoaminoætioporphyrin I, the *meso*-proton at C-15 exchanged with deuterium at room temperature after about 20 min. to give the monodeuterio-derivative (II; R = NH_2, R′ = D). When the amino-compound was heated in DTFA at 100° for 1 hr., the remaining *meso*-protons were also exchanged for deuterium. This is the first example of deuterium exchange in the porphyrin series at room temperature (cf. ref. 26), the electrophilic substitution no doubt being facilitated by the electron-donating properties of the amino-group.

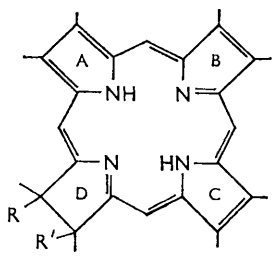
Reaction of aminoætioporphyrin I with nitrous acid gave a mixture of products, among which was a *meso*-nitroaminoporphyrin, presumably 5-amino-15-nitroætioporphyrin I (II; R = NH_2, R′ = NO_2) by analogy with the structure of the deuteration product of aminoætioporphyrin. Nitration of amines by nitrous acid presumably involves the intermediate formation of the nitroso-amine which is oxidised to the nitro-amine; several examples of the reaction are known.[27]

The direct introduction of hydroxyl groups into the porphyrin nucleus can be accomplished by various oxidising reagents and is known to involve either the *meso*-positions by substitution, or the β-positions of the pyrrole rings by addition, *i.e.*, with the formation in this case of 2,3-dihydroxychlorins, *e.g.*, (IV). The oxidation of hæmoproteins to bile pigments in Nature involves oxidation of the porphyrin ring at a *meso*-position and eventual loss of a *meso*-carbon atom as carbon monoxide (cf., *e.g.*, refs. 28 and 29). This type of oxidation of porphyrins has been studied extensively with model compounds, and the oxidation by hydrogen peroxide or peroxy-radicals has been shown to give rise in the first instance to *meso*-hydroxyporphyrins [30] which have been purified, and in certain cases [30a] characterised as *O*-acyl derivatives. When porphyrins are oxidised with lead dioxide or

[25] J. A. Elvidge and L. M. Jackman, *J.*, 1961, 859; W. S. Caughey and W. S. Koski, *Biochemistry*, 1962, **1**, 923.
[26] R. Bonnett and G. F. Stephenson, *Proc. Chem. Soc.*, 1964, 291.
[27] H. H. Hodgson and A. Kershaw, *J.*, 1930, 277; H. H. Hodgson and J. H. Crook, *J.*, 1932, 1812, 2976.
[28] C. H. Gray, " The Bile Pigments," Methuen, London, 1953.
[29] R. Lemberg and J. W. Legge, " Hæmatin Compounds and Bile Pigments," Interscience, New York, 1949; R. Lemberg, *Reviews Pure Appl. Chem.*, 1956, **6**, 1.
[30] (*a*) H. Libowitsky and H. Fischer, *Z. physiol. Chem.*, 1938, **255**, 209; (*b*) H. Fischer and K. Herrle, *ibid.*, **251**, 85; (*c*) R. Lemberg, B. Cortis-Jones, and M. Norrie, *Biochem. J.*, 1938, **32**, 149; K. Anan and H. S. Mason, *J. Biochem.* (*Tokyo*), 1961, **49**, 765.

lead tetra-acetate, four atoms of oxygen are introduced into the molecule, with the formation of the yellow xanthoporphyrinogens,[31] which can be reduced back to the porphyrins. These compounds, which do not form stable metal derivatives, also contain oxygen substituents in the *meso*-positions, although the precise structure of these compounds has not yet been defined.

The so-called " dioxychlorins " which were obtained by oxidation of chlorins (V; R = R′ = H), *e.g.*, by silver oxide and oxygen, and which were believed by Fischer [32] to be 17,18-dihydroxychlorins, have been shown [20] to be 20-chloro-chlorins formed by the action of the nascent chlorine liberated during the " purification." However in the porphyrin series, hydroxylation reactions were investigated extensively by Fischer, and shown to produce two types of product. Reaction of porphyrins with osmium tetroxide, usually in pyridine solution, led to the formation of 17,18-dihydroxychlorins by direct hydroxylation.[33] When the hydroxylation of porphyrins was effected with hydrogen peroxide in presence of sulphuric acid [34] or when the 17,18-dihydroxychlorins were treated with sulphuric acid or oleum,[35] the so-called " anhydrides " of the 17,18-dihydroxychlorins were formed. It is possible that the product obtained from mesoporphyrin by irradiation of a pyridine solution in presence of air [36] also belonged to this type. The dihydroxychlorins and the corresponding " anhydrides " had visible spectra of the chlorin type. Although the " anhydrides " were formulated as epoxides they did not exhibit typical epoxide reactions, *e.g.*, the " epoxide " ring was not split with hydrogen halides, acetic anhydride, or methylamine.[35]



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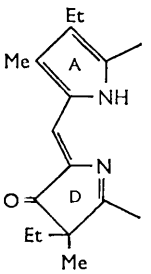
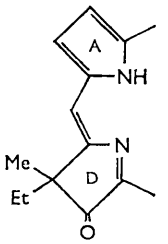


(VI)



(VII)

A reinvestigation [37] has shown that the main product formed by oxidation of ætioporphyrin I with hydrogen peroxide in presence of sulphuric acid [38] is a ketone formed in *ca.* 20% yield and produced by a pinacol rearrangement of the intermediate diol (V; R = R′ = OH).[39] The ketone was characterised by the formation of the corresponding copper derivative and the oxime.

Two structures (VI) and (VII) are possible for the ketonic rearrangement product and these have not yet been distinguished although (VI) is favoured on the basis of the superior " migratory aptitude " of the ethyl as compared with the methyl group.[40] The product contained a strong ketonic band at 1715 cm.$^{-1}$ (KBr disc) in its infrared spectrum

[31] (*a*) H. Fischer and H. Orth, " Die Chemie des Pyrrols," vol. II, ii, Leipzig, 1940, p. 423; (*b*) J. M. Osgerby and S. F. MacDonald, *Canad. J. Chem.*, 1962, **40**, 1585.
[32] Ref. 31*a*, p. 106.
[33] H. Fischer and C. G. Schröder, *Annalen*, 1939, **537**, 250; H. Fischer and H. Eckoldt, *ibid.*, 1940, **544**, 138; H. Fischer and H. Pfeiffer, *ibid.*, 1944, **556**, 131; H. Wenderoth, *ibid.*, 1947, **558**, 53.
[34] H. Fischer, H. Gebhardt, and A. Rothhaas, *Annalen*, 1930, **482**, 1.
[35] H. Fischer and H. Pfeiffer, *Annalen*, 1944, **556**, 131.
[36] H. Fischer and H. Bock, *Z. physiol. Chem.*, 1938, **255**, 1.
[37] Preliminary Communication, R. Bonnett, D. Dolphin, A. W. Johnson, D. Oldfield, and G. F. Stephenson, *Proc. Chem. Soc.*, 1964, 371.
[38] H. Fischer, H. Gebhardt, and A. Rothhaas, *Annalen*, 1930, **482**, 1.
[39] Professor H. H. Inhoffen has recently informed us that he (Thesis, G. Klotmann, Braunschweig, 1964) has recognised that the rearrangement product of the dihydroxychlorin obtained from octaethylporphyrin (cf. ref. 37) is a ketone formed by pinacol rearrangement.
[40] C. J. Collins, *Quart. Rev.*, 1960, **14**, 357.

and details of the visible spectrum are given in Table 1. The n.m.r. spectrum (Table 2) (kindly determined for us by Dr. J. Beynon and his associates at Imperial Chemical Industries Limited, Dyestuffs Division) of a solution in DTFA on a 100 Mc. Varian instrument showed four separate peaks associated with the four *meso*-protons, one of which differed appreciably from the other three and was attributed to the *meso*-proton adjacent to the bulky gem-dialkyl grouping. Likewise one methyl and one ethyl group differed from the other three as expected, although the low values of the signals associated with the methyl group (τ 7·75) and methylene (τ 7·11) of the ethyl group showed that they were still appreciably influenced by the aromatic ring system. The *meso*-proton signal at τ 0·69 was lost by deuterium exchange when the solution in deuterotrifluoroacetic acid was kept for 1 hr. at room temperature. The diol (V; R = R′ = OH) has been obtained from ætioporphyrin I by oxidation with osmium tetroxide in pyridine and it has been confirmed that it undergoes the pinacol rearrangement on treatment with sulphuric acid. These rearrangements are the first recognised examples of transformation from the porphyrin ββ′-alkyl substitution pattern to the ββ-arrangement of substituents, found in the naturally occurring corrins, including vitamin B_{12}.[41]

Experimental

Ultraviolet and visible spectra were determined on chloroform solutions on a Unicam S.P. 700 spectrometer. Infrared spectra were determined on a Perkin-Elmer Infracord, model 137, and refer to potassium bromide discs. N.m.r. spectra were determined on trifluoroacetic acid (TFA) or deuteriotrifluoroacetic acid (DTFA) solutions on an AEI RS2 instrument operating at 60 Mc./sec., tetramethylsilane being used as internal reference. Light petroleum refers to the fraction, b. p. 40—60°.

Mononitroætioporphyrin I.—Ætioporphyrin I (100 mg.) was dissolved in concentrated sulphuric acid (35 ml.), water (20 ml.) was added cautiously and the deep red solution cooled to 12°. Dilute nitric acid (0·2 ml. of 25%) was then added and the solution stirred for 30 min., the temperature being kept at 12—13°, during which the solution changed from deep red to deep green. The product was poured into water, and the brown precipitate separated and dissolved in chloroform (25 ml.). The extract was washed, dried, and then chromatographed on alumina using more chloroform for elution. The product was obtained as a red-brown solution and was crystallised from chloroform–methanol to give deep blue needles (64 mg.). Examination of this product by thin-layer chromatography [alumina with benzene–light petroleum (b. p. 40—60°) (13 : 47) as solvent] showed that it contained small amounts of the dinitro-compound. The crude product was rechromatographed on alumina, carbon tetrachloride being used as solvent and eluent, when the dinitro-compound was obtained as an initial brown band, closely followed by the main reddish-brown band of the mononitro-compound. The solvent was removed and the product [11] crystallised from chloroform–methanol when it formed deep blue needles (55 mg.) (Found: C, 73·2; H, 7·25; N, 13·6. Calc. for $C_{32}H_{37}N_5O_2$: C, 73·4; H, 7·15; N, 13·4%); $\nu_{max.}$ 1370, 1383, 1444, 1460, 1535, 2872, 2931, 2966, and 3004 cm.$^{-1}$.

The copper complex [11] formed (i) from the mononitro-derivative with cupric acetate in chloroform–methanol containing 1 drop of ammonium hydroxide was obtained as bright red needles (chloroform–methanol) (Found: C, 66·1; H, 5·7; N, 12·4. Calc. for $C_{32}H_{35}CuN_5O_2$: C, 65·8; H, 6·05; N, 12·0%); $\lambda_{max.}$ 401, 530, and 566 mμ ($\varepsilon_{max.}$ 164,400, 12,710, and 20,320, respectively).

(ii) A solution of cupric nitrate trihydrate (25 mg.) in acetic anhydride (10 ml.) was added to ætioporphyrin I (100 mg.) in glacial acetic acid (100 ml.) and the solution stirred at room temperature for 2 hr. The product was poured into water, and the brick-red precipitate collected, dissolved in chloroform, and chromatographed on alumina. The main bright red band was collected, and the product (35 mg.) crystallised from chloroform–methanol, forming bright red needles (Found: C, 66·1; H, 5·95; N, 11·95%).

Dinitroætiophyrin I.—Ætioporphyrin I (100 mg.) in sulphuric acid was nitrated with nitric acid exactly as described above but the time of reaction was extended to 2 hr. The reaction mixture was treated as before, dissolved in carbon tetrachloride (200 ml.), and chromatographed

[41] R. Bonnett, *Chem. Reviews*, 1963, **63**, 573.

on alumina in order to separate the mononitro-derivative. The initial brown band was collected and the product [11] crystallised from chloroform–methanol; it formed reddish-blue needles (54 mg.; 45%) (Found: C, 67·5; H, 6·15; N, 14·55. Calc. for $C_{32}H_{36}N_6O_4$: C, 67·6; H, 6·4; N, 14·8%). Thin-layer chromatography revealed the presence of two isomers, that with the lower R_F value being formed in the greater amount.

The copper complex was prepared from ætioporphyrin I (100 mg.) in chloroform (50 ml.) by the action of hydrated cupric nitrate (100 mg.) in acetic anhydride (40 ml.) as described above for the mononitro-derivative, and was obtained as bright red needles (76 mg.; 57%) (Found: C, 61·1; H, 5·55; N, 13·0. Calc. for $C_{32}H_{35}CuN_6O_4$: C, 61·0; H, 5·45; N, 13·35%); $\lambda_{max.}$ 402, 536, and 570 mμ ($\varepsilon_{max.}$ 163,600, 12,630, and 19,380, respectively).

Trinitroætioporphyrin I.—(i) Ætioporphyrin I (100 mg.) in sulphuric acid was nitrated with nitric acid exactly as described for the mononitro-derivative above, but the reaction time was extended to 8 hr. No traces of the mono-, di-, or tetranitro-compounds could be detected in the product which was isolated by the procedure described above. The product [10,11] was crystallised from chloroform–methanol and formed deep purple needles (Found: C, 62·3; H, 5·75; N, 15·7. Calc. for $C_{32}H_{35}N_7O_6$: C, 62·6; H, 5·75; N, 16·0%); $\nu_{max.}$ 1374, 1459, 1547, 2882, 2939, 2969, 2991, and 3317 cm.$^{-1}$.

(ii) Ætioporphyrin I (1·0 g.) was dissolved in concentrated nitric acid (80 ml.), a dark green solution being obtained. This was kept for 36 hr., then poured into water, and the green precipitate separated and dissolved in ether. The ethereal solution was washed, dried, and the solvent removed. The residue was dissolved in chloroform and chromatographed on alumina, more chloroform being used for elution. The yellow-brown band was collected and the product crystallised from chloroform–methanol, forming purple crystals (120 mg.; 13%), identical with the foregoing trinitro-derivative.

The copper complex formed red needles (chloroform–methanol) (Found: C, 57·2; H, 4·85; N, 14·4. Calc. for $C_{32}H_{33}CuN_7O_6$: C, 56·9; H, 4·95; N, 14·5%); $\lambda_{max.}$ 401, 538, and 575 mμ ($\varepsilon_{max.}$ 161,000, 12,300, and 17,200, respectively).

Monoaminoætioporphyrin I.—Mononitroætioporphyrin I (100 mg.) was suspended in concentrated hydrochloric acid (70 ml.), and stannous chloride (300 mg.) was added. The mixture was stirred for 4 hr., a green precipitate being formed, and it was then poured into water. The precipitate was separated, washed, and extracted with chloroform. The chloroform extract was washed, dried, and chromatographed on alumina using more chloroform for elution and protecting the column from light. The first fraction consisted of unchanged nitro-compound and was followed by a green band of the amino-derivative. This was obtained as a mauve-brown solution and the product was crystallised from chloroform–methanol, forming deep purple needles (40 mg.; 42%) (Found: C, 77·7; H, 7·9; N, 14·35. $C_{32}H_{39}N_5$ requires C, 77·85; H, 7·95; N, 14·2%); $\nu_{max.}$ 1060, 1117, 1275, 1315, 1462, 1470, 1619, 2960, 3297, 3415, and 3499 cm.$^{-1}$.

The copper derivative formed purple needles (chloroform–methanol) (Found: C, 69·1; H, 6·7; N, 12·7. $C_{32}H_{37}CuN_5$ requires C, 69·25; H, 6·65; N, 12·65%); $\lambda_{max.}$ 416·5, 540·5, 571·5, and 591·5 mμ ($\varepsilon_{max.}$ 227,000, 10,420, 4630, and 5180, respectively); $\nu_{max.}$ 1059, 1147, 1274, 1466, 1657, 2867, 2928, 2961, and 3396 cm.$^{-1}$.

The N-*acetyl derivative* was formed by suspending the amino-compound (30 mg.) in acetic anhydride (5 ml.) and adding 1 drop of perchloric acid. The blue solution turned deep red after 30 min. Water (20 ml.) containing ammonia solution (3 drops of *d* 0·88) was added and the brown precipitate separated and extracted into chloroform. The solution was chromatographed on alumina, more chloroform being used for elution, the *N*-acetyl derivative appearing as a narrow red band. This was collected, the solvent was removed, and the product crystallised from chloroform–light petroleum as deep red needles (18 mg.) (Found: C, 75·8; H, 7·7; N, 13·05. $C_{34}H_{41}N_5O$ requires C, 76·2; H, 7·65; N, 13·1%); $\nu_{max.}$ 731, 1454, 1470, 1668, 1687, 2961, 3280, and 3352 cm.$^{-1}$.

Action of Nitrous Acid on Monoaminoætioporphyrin I.—Monoaminoætioporphyrin I (200 mg.) was dissolved in sulphuric acid (80 ml. of 5N), the solution diluted with water (160 ml.), and the deep blue solution cooled to 5°. An aqueous solution of sodium nitrite (10 mg./ml.) was added dropwise to the stirred porphyrin solution until a slight excess of nitrous acid was present (starch–iodide). The deep green solution was kept at 5° for 4 hr., urea (50 mg.) was then added, and after a further 30 min., the solution was allowed to warm to room temperature. It was then neutralised ($NaHCO_3$), extracted with chloroform (2 × 50 ml.), and the chloroform

extract washed, dried, and chromatographed on alumina (Spence type H, deactivated with 6% water), chloroform being used for elution. The initial pink and orange bands were followed by a mauve band of unchanged monoaminoætioporphyrin I (17 mg.). This was followed by a brown and a narrow pink band which were difficult to separate on this chromatogram but were subsequently separated by chromatography on alumina (Spence type H, deactivated with 9% water). Isolation of the product from the brown band, followed by crystallisation from chloroform–methanol gave blue crystals (30 mg.) (Found: C, 71·1; H, 7·3; $C_{32}H_{38}N_6O_2$ requires C, 71·35; H, 7·1); $\lambda_{max.}$ 416, 529, 567, 591, and 651 mμ ($\varepsilon_{max.}$ 135,000, 8900, 6500, 5700, and 4900, respectively); $\nu_{max.}$ 1360 (NO_2), 1460, 1525 (NO_2), and 1620 cm.$^{-1}$.

Hydrogen Peroxide Oxidation of Ætioporphyrin I.—Ætioporphyrin I (500 mg.) was dissolved in concentrated sulphuric acid (50 ml.), and the stirred solution cooled to 0°. Ice-cold hydrogen peroxide (7 ml. of 3%) was added dropwise over 30 min. keeping the temperature below 10°, during which time the solution turned from red to green. It was stirred for a further 15 min. and then poured into ice–water. The product was extracted with ether and the extract washed ($NaHCO_3$) and dried. After removal of the solvent the residue was dissolved in chloroform and chromatographed on alumina (15 × 2 in.), chloroform being used for elution. The first band (mauve) was collected and the second band (red) proved to be unchanged ætioporphyrin I, (40 mg.). The *product* from the first band was crystallised from chloroform–methanol, forming dark blue needles (32 mg.). A low intensity band at 688 mμ in the visible spectrum disappeared after two further crystallisations (Found: C, 77·7; H, 7·75; N, 10·8. $C_{32}H_{58}N_4O$ requires C, 77·7; H, 7·75; N, 11·3%); $\nu_{max.}$ (i) 1189, 1457, 1588, 1715, 2869, 2925, 2961, and 3338 cm.$^{-1}$, (ii) ($CHCl_3$ solution) 1705 (C=O) cm.$^{-1}$.

The corresponding *copper derivative* was obtained from the foregoing ketone (30 mg.) in chloroform (10 ml.) by the addition of a solution of cupric acetate (15 mg.) in methanol (5 ml.), the initial red solution immediately turning blue. The product was heated under reflux for 5 min. and the solvent removed until crystallisation commenced. After cooling, the product was collected, dissolved in chloroform, and chromatographed on alumina. The single broad blue band was collected and the product crystallised from chloroform–methanol, forming deep red needles (25 mg.) (Found: C, 68·7; H, 6·45; N, 10·35. $C_{32}H_{36}CuN_4O$ requires C, 69·05; H, 6·5; N, 10·1%); $\lambda_{max.}$ 378, 417, 518, 575, and 624 mμ ($\varepsilon_{max.}$ 42,500 159,400, 3550, 8430, and 41,000, respectively) with λ_{infl} 397 mμ (ε_{infl} 55,000); $\nu_{max.}$ 1400, 1456, 1585, 1715, 2865, 2925, 2960. and 3050 cm.$^{-1}$.

The *ketoxime* was prepared by reaction of hydroxylamine hydrochloride (50 mg.) and sodium acetate (50 mg.) in aqueous methanol with a solution of the ketone (50 mg.) in pyridine (25 ml.). The mixture was heated under reflux for 24 hr., the solvent then removed under reduced pressure, and the residue dissolved in chloroform and chromatographed on alumina. After elution of unchanged material (20 mg.) the oxime was obtained as a greenish-mauve dichroic solution. The solid was crystallised from chloroform–methanol, forming purple prisms (12 mg.) (Found: C, 75·3; H, 7·3. $C_{32}H_{39}N_5O$ requires C, 75·4; H, 7·7; N, 13·75%); $\lambda_{max.}$ 403, 502, 534, 572, 630, and 656 mμ ($\varepsilon_{max.}$ 187,000, 10,900, 13,600, 4350, 2800, and 37,900, respectively).

2,3-Dihydroxyætiochlorin I (cf. ref. 42).—A solution of osmium tetroxide (500 mg.) in dry ether (10 ml.) was added to ætioporphyrin I (500 mg.) in dry chloroform (250 ml.). Pyridine (1 ml.) was added and the solution kept at room temperature for 2 days. During this time the colour of the solution changed from red to yellow to green. The chloroform and ether were removed, and pyridine (17 ml.) was added to the residue together with a solution of sodium metabisulphite (0·9 g.) in water (15 ml.). The mixture was stirred for 24 hr. and then poured into water. The precipitate was separated, dissolved in chloroform (100 ml.), and chromatographed on alumina with elution by chloroform. The initial diffuse pale green band was followed by a pink band consisting of unchanged ætioporphyrin I (50 mg.). The column was washed with methanol and the remaining green band eluted with glacial acetic acid. On contact with acetic acid, the green band changed to deep pink. Removal of the solvent gave the product as a deep green solid which was crystallised from chloroform–methanol when it formed deep green prisms [42] (120 mg.); $\lambda_{max.}$ 389, 495, and 649 mμ ($\varepsilon_{max.}$ 52,500, 4650, and 11,430, respectively).

Acid Rearrangement of 2,3-Dihydroxyætiochlorin I.—The dihydroxychlorin (75 mg.) was dissolved in concentrated sulphuric acid (70 ml.), and the green solution was stirred at room

[42] H. Fischer and H. Eckoldt, *Annalen*, 1940, **544**, 138.

temperature for 30 min. The solution was poured on to ice, then extracted with ether, and the ethereal extract washed and dried. Most of the solvent was removed and the concentrated solution then chromatographed on alumina, chloroform being used for elution. The first mauve band was collected, the solvent removed, and the residue crystallised from chloroform–methanol to yield dark blue needles (12 mg.) identical with the ketone obtained directly by the oxidation of ætioporphyrin I with hydrogen peroxide and sulphuric acid (above); $\lambda_{max.}$ 406·5, 508·5, 550, 589, and 646·5 mμ ($\varepsilon_{max.}$ 140,000, 6980, 8050, 4020, and 23,500); λ_{infl} 622 mμ (ε_{infl} 2050).

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