

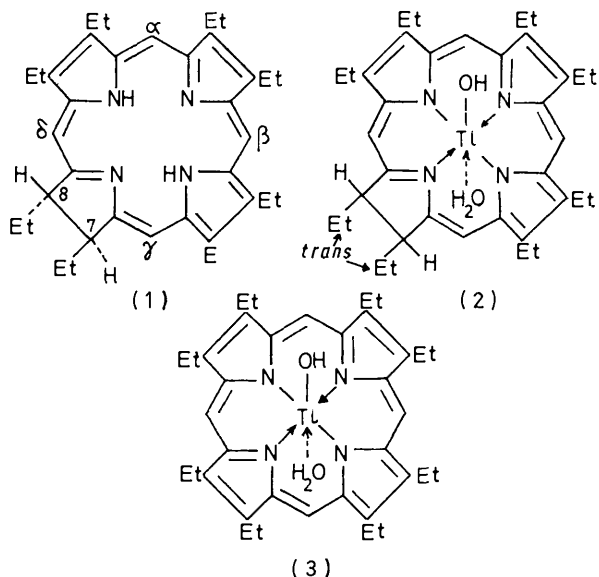
Transformation of Chlorins into Bile Pigment Analogues¹

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Treatment of *trans*-octaethylchlorin (1) with one equivalent of thallium(III) trifluoroacetate affords the corresponding aquo-hydroxide chelate (2) after chromatography on deactivated alumina; with four equivalents of the reagent, the ring opened dihydrobiliverdin (4) is obtained. When zinc *trans*-octaethylchlorin is treated with one equivalent of thallium(III) trifluoroacetate, the zinc *meso*-trifluoroacetoxy-*trans*-octaethylchlorin (12) is produced; hydrolysis of the trifluoroacetoxy-function in (12) in the presence of oxygen furnishes the dihydrobiliverdin (4) in high yield.

FOLLOWING our earlier experiments² on the synthesis and properties of thallium(III) porphyrins, we turned our attention to the reactions of chlorins (7,8-dihydroporphyrins) with thallium(III) trifluoroacetate (TTFA). We felt that further insight into metal-ligand interactions might be gained from the ¹H n.m.r. spectra of thallium(III) chlorins.

Treatment of *trans*-octaethylchlorin (1) with one equivalent of TTFA (in methylene chloride and tetrahydrofuran) during 1.5 min, followed by chromatography on deactivated alumina (which we have shown² accomplishes ligand exchange) gave the required aquochlorinatothallium(III) hydroxide (2) in 22% yield. As might be expected, the other products were the thallium(III) porphyrin (3) (26%) and the starting material (1) (40%), along with a small quantity of an unidentified



blue substance. The recovery of starting material and thallium(III) porphyrin was overcome by using thallium(III) acetate, a much less potent oxidising agent than TTFA, for the metal insertion, and in this way a 90% yield of the chelate (2) was obtained after chromatography.

† The chelate (2) is depicted as an octahedral species for simplicity, though there is no direct evidence that the water molecule is co-ordinated to the thallium atom. The thallium(III) atom (crystal ionic radius⁴ 0.95 Å) is presumably too large for the metal ion to be situated in the plane of the macrocyclic ring,² and hence, irrespective of the precise situation of the axial ligands, an asymmetric environment is created merely by the presence of the thallium atom.

graph. Elemental analysis and mass spectrometry³ indicated the presence of the axial ligands in (2), and this view was reinforced by the i.r. spectrum (KBr) which showed OH stretching bands at 3430 and 3330 cm⁻¹; such bands were difficult to observe² in the i.r. spectra of the corresponding porphyrin chelates, probably on account of intermolecular hydrogen bonding. The n.m.r. spectrum showed couplings of the methylene protons, peripheral protons (7, 8), and *meso*-protons with the thallium atom. In particular, the *meso*-protons (which are observed² as a simple doublet with $J_{\text{TI-H}}$ 44.4 Hz in the octaethylporphyrin chelate) all had different chemical shifts and different coupling constants, reflecting the asymmetry of the *trans*-octaethylchlorin chelate (2), which is an enantiomeric mixture (chiral environment about the thallium atom).†

The yield of the minor blue by-product was enhanced by performing the reaction as described earlier, but with four equivalents of TTFA. Chromatography afforded the thallium(III) porphyrin chelate (3) as the major product, along with only 3% of the chlorin chelate (2). The third band on the column was green in colour, but the colour became purple-blue when the solution was exposed to air during collection of the eluates. Rechromatography of this purple-blue solution afforded the unknown blue substance in 25% yield from *trans*-octaethylchlorin (1).

This blue compound had a visible absorption spectrum (λ_{max} 347 and 591 nm) which indicated interruption of the macrocyclic conjugation, or even rupture of the ring, since the 'Soret' band was absent. The dihydrobiliverdin structure (4) was assigned on the basis of the following evidence. No deshielded *meso*-protons were observed in the ¹H n.m.r. spectrum; these were replaced by three one-proton resonances (τ 3.44, 4.03, and 4.56) [Figure, (a)] at shifts characteristic of those found for the methine protons of conjugated bile pigments.⁵ The mass spectrum indicated a molecular weight of 556 and

¹ Preliminary communication, J. A. S. Cavaleiro and K. M. Smith, *Chem. Comm.*, 1971, 1384.

² (a) K. M. Smith, *Chem. Comm.*, 1971, 540; (b) R. J. Abraham, G. H. Barnett, E. S. Bretschneider, and K. M. Smith, *Tetrahedron*, 1973, 29, 553; R. J. Abraham, G. H. Barnett, and K. M. Smith, preceding paper.

³ Cf. K. M. Smith, *Org. Mass Spectrometry*, 1972, 6, 1401.

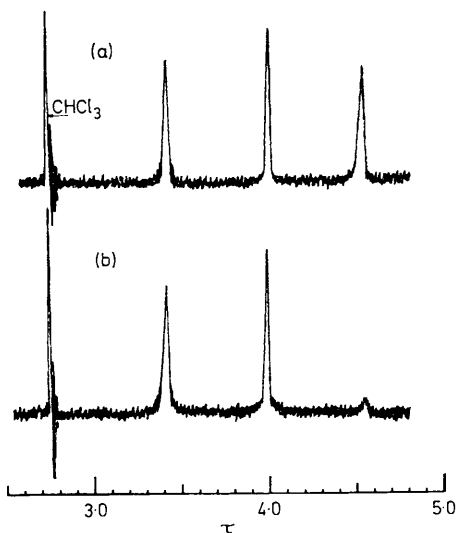
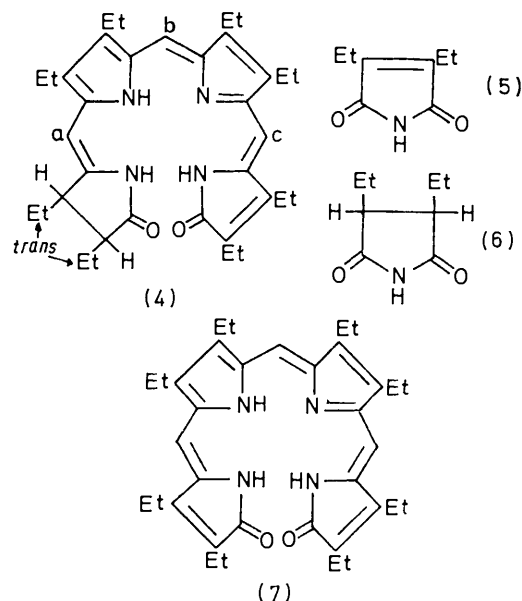
⁴ 'Handbook of Chemistry and Physics,' Chemical Rubber Co., 47th edn., 1966, p. F-124.

⁵ E.g. A. H. Jackson, G. W. Kenner, and K. M. Smith, *J. Chem. Soc. (C)*, 1968, 302; K. M. Smith, *J.C.S. Perkin I*, 1972, 1471.

the molecular formula $C_{35}H_{48}N_4O_2$ was confirmed by elemental analysis. In addition, the fragmentation pattern in the mass spectrum was similar to those of other bile pigments prepared in these laboratories^{5,6} and was characteristic of an open-chain tetrapyrrole. Evidence for the terminal lactam rings was obtained from i.r. spectroscopy, strong bands (KBr) at 1720 and 1685 cm^{-1} being observed.

Micro-scale chromic acid degradation⁷ of (4) gave diethylmaleimide (5) and diethylsuccinimide (6) (t.l.c. comparison); the yield of the former was greater than that of the latter, but it was not possible to confirm a 3:1 ratio of (5) to (6) on account of the scale of the oxidation. Chemical evidence of the tetrapyrrolic nature of (4) was obtained by treatment with iron(III)

rupture of the chlorin, or metallochlorin, macrocycle at the γ - or δ -position. Since the reaction was brought



Methine region in the n.m.r. spectra (HA-100 instrument; $CDCl_3$) of (a) the dihydro-octaethylbiliverdin (4) and (b) the dihydrobiliverdin prepared from $\gamma\delta$ -dideuterio-*trans*-octaethylchlorin (10)

chloride, which afforded a moderate yield of a compound which had the spectral characteristics of the biliverdin (7). The visible absorption spectrum compared well with published data⁵ and the n.m.r. spectrum showed a 1:2 doublet for the methine protons. Mass spectroscopy indicated a molecular weight of 554 and the fragmentation pattern was compatible with structure (7). The i.r. spectrum had strong bands at 1695 (shoulder) and 1674 cm^{-1} , and chromic acid degradation gave only diethylmaleimide (5). The identity of the iron(III) chloride oxidation product was positively confirmed as (7) by comparison with authentic octaethylbiliverdin (7), synthesised in low yield⁸ by coupled oxidation⁹ of octaethylhaemin (8).

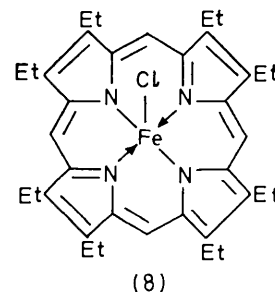
Nominally, the dihydrobiliverdin (4) is produced by

⁶ A. H. Jackson and G. W. Kenner, 'Porphyrins and Related Compounds,' Biochemical Symposium No. 28. ed. T. W. Goodwin, Academic Press, London, 1968, p. 3.

⁷ W. Rüdiger, *Z. physiol. Chem.*, 1969, **350**, 1291; ref. 6, p. 121.

⁸ A more efficient version of this general procedure has recently been published: R. Bonnett and A. F. McDonagh, *J.C.S. Perkin I*, 1973, 881.

about by a strongly electrophilic reagent,¹⁰ this appeared to be more likely than rupture at the α - or β -position, since chlorins are known to undergo electrophilic substitution most readily at the *meso*-positions adjacent to the reduced ring.¹¹ However, the possibility still remained that the dihydrobiliverdin might have the structure (9) [with a reduced internal ring rather than terminal ring], formed by rupture of the macrocycle at the α - or β -position. The n.m.r. spectrum was not wholly compatible with structure (9), and neither was the i.r. spectrum, but most of the other evidence [*e.g.* chromic acid oxidation, iron(III) chloride oxidation] could be accounted for by structure (9). This possibility was eliminated by synthesis of $\gamma\delta$ -dideuterio-*trans*-octaethylchlorin (10) (n.m.r. showed 95% deuteration) by



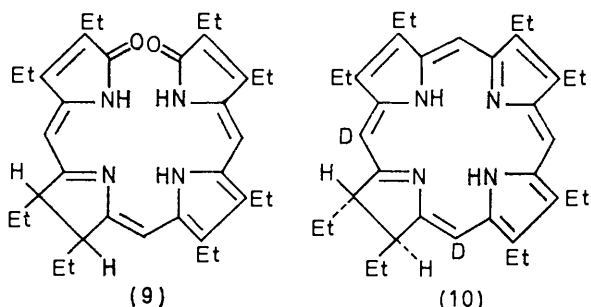
reaction of *trans*-octaethylchlorin (1) with deuterioacetic acid at 95° during 6 h (*cf.* ref. 11). When com-

⁹ *E.g.* A. W. Nichol and D. B. Morell, *Biochim. Biophys. Acta*, 1969, **184**, 173.

¹⁰ E. C. Taylor and A. McKillop, *Accounts Chem. Res.*, 1970, **3**, 338.

¹¹ R. B. Woodward and V. Skaric, *J. Amer. Chem. Soc.*, 1961, **83**, 4676; see also, R. Bonnett, I. A. D. Gale, and G. F. Stephenson, *J. Chem. Soc. (C)*, 1967, 1169.

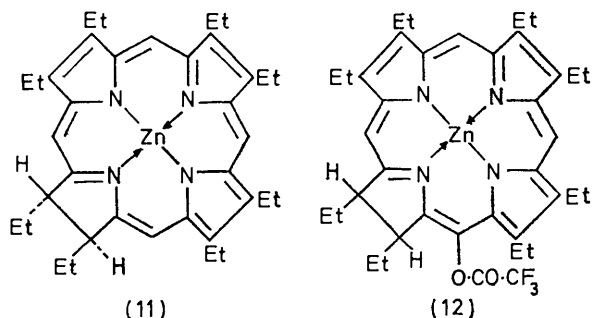
pound (10) was subjected to the TTFA reaction, the n.m.r. spectrum of the resulting dihydrobiliverdin [Figure, (b)] showed only two methine protons; the small residual peak at τ 4.56 was presumably observed because of incomplete deuteration of the starting material (10). This result is compatible only with



rupture of the macrocyclic ring at the γ - or δ -position; ring opening at α or β would yield a dihydrobiliverdin with two methine resonances absent from its n.m.r. spectrum.

The oxidative rupture of metalloporphyrins to their open-chain bile pigment counterparts is of great biological significance, since this is the pathway by which animals dispose of their waste haemoproteins. Only two procedures have been reported which accomplish the *in vitro* analogue of this process. The first is the much used and well established coupled oxidation process¹² in which iron porphyrins are treated with reaction mixtures containing hydrogen peroxide to give biliverdins. The second procedure is the photo-oxidation of magnesium porphyrins, the products of which were claimed¹³ to be biliviolins. This latter method has recently been re-examined¹⁴ and the formulation of the products has been criticised.

Treatment of zinc *trans*-octaethylchlorin (11) with one equivalent of TTFA gave a mixture consisting only of

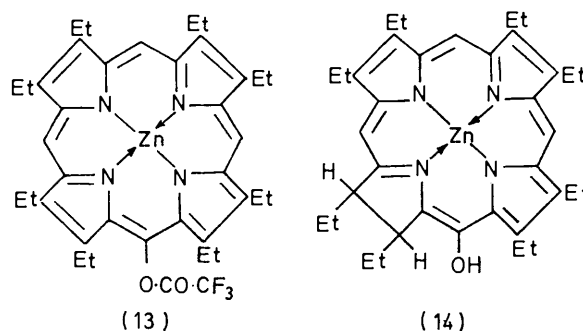


zinc octaethylporphyrin and a green, air-stable compound possessing a visible absorption spectrum similar

* This was readily accomplished, owing to the high insolubility of zinc octaethylporphyrin in methylene chloride-*n*-hexane.

¹² E.g. R. Lemberg, B. Cortis-Jones, and M. Norrie, *Biochem. J.*, 1938, **32**, 171; R. Lemberg, *Rev. Pure Appl. Chem.*, 1956, **6**, 1; H. Fischer and H. Libowitzsky, *Z. physiol. Chem.*, 1938, **251**, 198; H. Libowitzsky, *ibid.*, 1941, **265**, 191; E. Stier, *ibid.*, 1942, **272**, 239.

to, but not identical with that of the starting zinc chlorin (11). Chromatography of this mixture on alumina, with methylene chloride as eluant, gave a readily separable mixture of zinc octaethylporphyrin and the dihydrobiliverdin (4), after exposure of the eluates to air and rechromatography. It was thus apparent that the chromatographic step was accomplishing a transformation of the green intermediate which rendered it susceptible to attack by oxygen. Hence, the crude reaction mixture was purified of thallium salts by passage through a bed of Kieselgel G, and the two components of the mixture were separated by crystallisation.* The green compound was shown to be the zinc *meso*-trifluoroacetoxychlorin (12) on the basis of the following evidence. N.m.r. spectroscopy showed three *meso*-proton resonances (τ 0.42, 0.56, and 1.35), indicating *meso*-substitution adjacent to the reduced ring; the ¹⁹F n.m.r. spectrum featured a singlet 6.83 p.p.m. downfield from CF₃·CCl₃, and the i.r. spectrum (KBr) possessed a strong sharp band at 1800 cm⁻¹ [cf. the



CO·CF₃ stretching frequency (1795 cm⁻¹) for compound (13)¹⁵. A satisfactory elemental analysis was obtained, and the mass spectrum confirmed the molecular weight (⁶⁴Zn) as 710, with peaks attesting the expected¹⁵ loss of 97 mass units (CO·CF₃). Treatment with dichlorodicyanobenzoquinone afforded a high yield of the known¹⁵ zinc *meso*-trifluoroacetoxyporphyrin (13), and when treated with aqueous alkali, or (better) when chromatographed on alumina, in the presence of oxygen, (12) gave a high (spectroscopic) yield of the dihydrobiliverdin (4).

We have already suggested¹⁵ a mechanism for the trifluoroacetylation of magnesium and zinc porphyrins, involving the metalloporphyrin π -cation radical, and we see no reason why similar arguments should not be applied to the chlorin transformation. It is significant that the π -cation radical of thallium(III) porphyrins cannot be obtained with TTFA, whereas thallium(III) chlorins are readily transformed into the dihydrobiliverdin (4) [presumably *via* the metallochlorin π -cation radical and the thallium(III) *meso*-trifluoroacetoxychlorin]. These observations can be rationalised by the reasonable assumption that the oxidation potential of

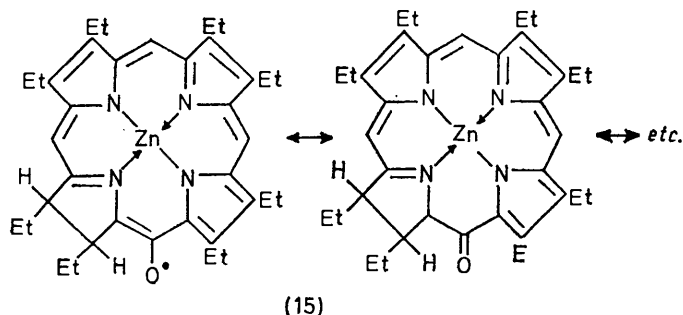
¹³ J. Barrett, *Nature*, 1967, **215**, 733.

¹⁴ J.-H. Fuhrhop and D. Mauzerall, *Photochem. Photobiol.*, 1971, **13**, 453.

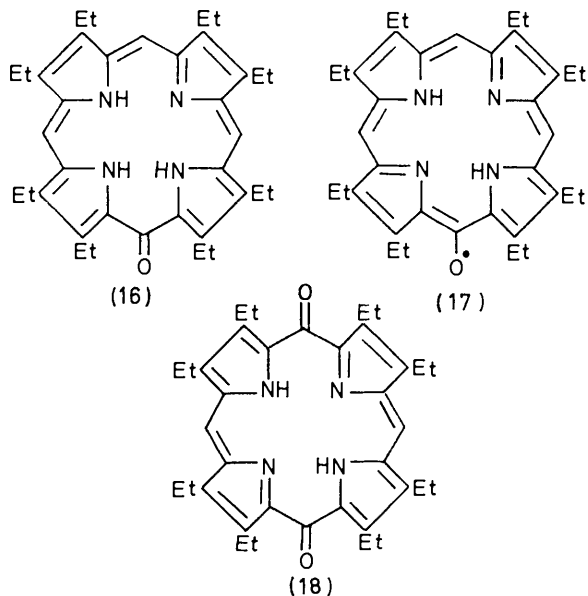
¹⁵ G. H. Barnett, M. F. Hudson, S. W. McCombie, and K. M. Smith, *J.C.S. Perkin I*, 1973, 691.

the thallium(III) chlorin is lower than that of the corresponding thallium(III) porphyrin.*

Chromatography of zinc *meso*-trifluoroacetoxy-porphyrins [e.g. (13)] on alumina affords the corresponding zinc oxophlorins, owing to hydrolysis of the trifluoroacetoxy-function on the support. It therefore seems reasonable that the zinc *meso*-trifluoroacetoxy-*trans*-octaethylchlorin (12) is hydrolysed under the same conditions to the zinc γ -hydroxychlorin (14); this compound (14) might be expected to add oxygen across the *meso*-function, but it seems more likely that the species



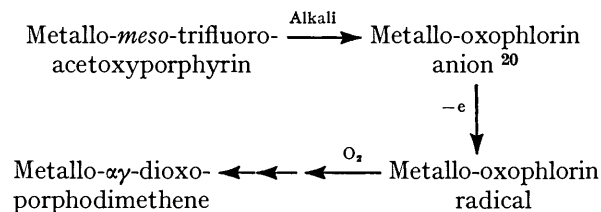
which adds oxygen is the radical (15) produced from (14) by loss of a proton and an electron. This view is supported by the recent work of Fuhrhop and his co-workers,¹⁷ who showed that chromatography of octaethyloxophlorin (16) on alumina gave the free radical (17); the zinc chelate of the oxophlorin radical was also



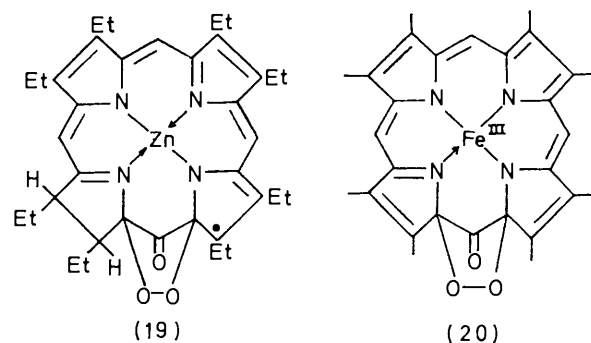
prepared. The oxidation potential of the oxophlorin was given¹⁷ as -50 mV (*vs.* s.c.e.) and crude calculations suggest that the oxidation potential of the zinc γ -hydroxychlorin (14) should be lower still than this value,

* Note added in proof: E_1^0 of thallium(III) octaethylporphyrin = 1.0 V; E_1^0 of thallium(III) octaethylchlorin = 0.79 V [in butyronitrile-tetramethylammonium perchlorate (0.1M), *vs.* s.c.e.]. We thank Dr. J.-H. Fuhrhop (Braunschweig, Germany) for these measurements.

indicating high susceptibility towards loss of an electron. The oxophlorin radical (17) reacted with oxygen to give the $\alpha\gamma$ -dioxoporphodimethene (18), and such compounds have been obtained^{2a,18} from other sources. Indeed, we have shown¹⁹ that metal derivatives (Mg^{II} , Zn^{II} , Tl^{III}) of *meso*-trifluoroacetoxyoctaethylporphyrin yield the corresponding metallo- $\alpha\gamma$ -dioxoporphodimethenes when hydrolysed under alkaline conditions in the presence of oxygen, with an ease dependent upon the central metal ion. The reaction sequence is presumably:



Addition of oxygen across the carbonyl group of the zinc-*meso*-oxychlorin radical (15) would afford the species (19) [or one of its resonance forms bearing the



radical on nitrogen], which could lose carbon monoxide in a cheletropic type of fragmentation, to give the dihydrobiliverdin (4) after replacement of the zinc ion with two protons and capture of a hydrogen atom. The suggested intermediate (19) is similar to the species (20) recently postulated²¹ as an intermediate in the *in vivo* catabolism of haemoproteins to bile pigments. Whilst some intermediates in the irreversible bleaching of the plant chlorophylls have been isolated,²² little is known about the breakdown of chlorophylls-*a* and -*b* in the senescent leaf. *meso*-Oxychlorins or their magnesium chelates might be intermediates in the process, and rupture of the chlorin macrocycle of the chlorophylls could be brought about by transformations which are, in essence, similar to those which we report here.

¹⁶ G. H. Barnett and K. M. Smith, unpublished results.

¹⁷ J.-H. Fuhrhop, S. Besecke, and J. Subramanian, *J.C.S. Chem. Comm.*, 1973, 1.

¹⁸ J.-H. Fuhrhop, *Chem. Comm.*, 1970, 781.

¹⁹ G. H. Barnett and K. M. Smith, manuscript in preparation.

²⁰ A. H. Jackson, G. W. Kenner, and K. M. Smith, *J. Amer. Chem. Soc.*, 1966, **88**, 4539; see also ref. 5.

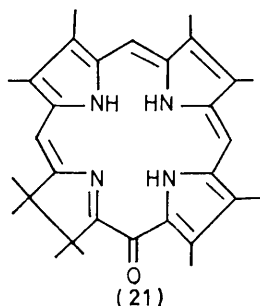
²¹ T. Kondo, D. C. Nicholson, A. H. Jackson, and G. W. Kenner, *Biochem. J.*, 1971, **121**, 601; see also ref. 6, p. 16.

²² J. J. Jen and G. Mackinney, *Photochem. Photobiol.*, 1970, **11**, 297, 303.

π -Cation radicals figure prominently in the chemistry of the chlorophylls in the leaf,²³ and if formed, a magnesium *meso*-hydroxychlorin would be expected to have an oxidation potential lower than that of the zinc *meso*-hydroxychlorin.

Further investigations of metallo-*meso*-hydroxychlorins and *meso*-oxychlorins (21)* will be reported elsewhere; in this context, it is fortunate that the *meso*-trifluoroacetoxychlorins are an accessible and stable source of these compounds.

More prolonged treatment (15 min) of *trans*-octaethylchlorin with TTFA, followed by addition of methanol to the crude products (in a vain attempt to isolate pure compounds prior to column separation) and chromatography, gave a large quantity of thallium(III) octaethylporphyrin and a 32% yield of the 'bilipurpurin' ²⁴-type compound (22). The n.m.r. spectrum of this substance showed methine proton signals at τ 3.30 and 4.10 and a methoxy-singlet at τ 6.63. Mass spectrometry indicated a molecular weight of 602 with loss of methanol (metastable ion) to give the base peak at m/e 570; elemental analysis confirmed the molecular formula as $C_{36}H_{50}N_4O_4$.



the figures being unaltered by extended drying of the sample at 100° and 0.1 mmHg. We had initially suspected that the resonance at τ 6.63 might be due to the presence of one molecule of methanol of crystallisation; however, the mass spectrum eliminated this possibility. The i.r. spectrum featured strong bands at 1725, 1705, and 1640 cm^{-1} and the continued presence of the reduced ring in the molecule was established by micro-scale chromic acid degradation, which yielded diethylmaleimide (5) and diethylsuccinimide (6). The final piece of evidence for the structural assignment was the visible absorption spectrum (λ_{max} 326, 505, and 536 nm), which was very similar to that reported for tripyrrenic compounds [e.g. (23)] synthesised by Plieninger and Stumpf,²⁵ which contain the same chromophore as (22).

The 'bilipurpurin' (22) is similar to compounds isolated from the well-known, but little understood, Gmelin reaction.²⁶ We have found²⁷ that the Gmelin

* On the basis of published studies on oxophlorins⁵ these compounds are depicted as the keto-tautomers, though this has not yet been established.

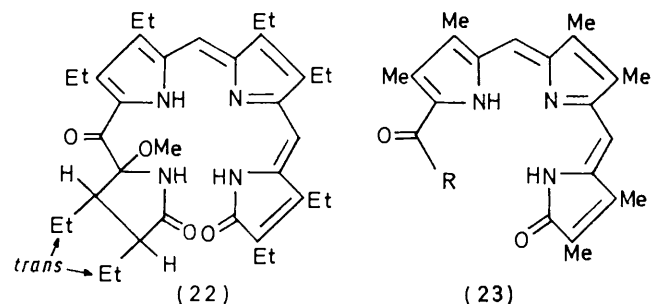
²³ E.g. P. Loach and J. J. Katz, *Photochem. Photobiol.*, 1973, **17**, 195.

²⁴ R. Lemberg and J. W. Legge, 'Haematin Compounds and Bile Pigments,' Interscience, New York, 1949, p. 130.

²⁵ H. Plieninger and K. Stumpf, *Chem. Ber.*, 1970, **103**, 2562.

²⁶ Ref. 24, p. 109.

reaction can be carried out in a stepwise fashion using lead tetra-acetate as the oxidant, in place of the more usual nitric acid; the results will be reported elsewhere.



EXPERIMENTAL

M.p. and spectroscopic measurements and chromatographic separations were carried out as described in the preceding paper.

Thallium(III) acetate²⁸ and trifluoroacetate²⁹ were prepared from thallic oxide (B.D.H.) following literature procedures. *trans*-Octaethylchlorin (1) was prepared from octaethylporphyrin by the procedure of Whitlock *et al.*³⁰ These workers reported that their material (m.p. 231.8—232°) gave persistently low carbon figures on combustion analysis; our product (m.p. 231°) was satisfactorily analysed (Found: C, 80.4; H, 9.0; N, 10.2. Calc. for $C_{36}H_{48}N_4$: C, 80.55; H, 9.0; N, 10.4%).

Aquo-trans-octaethylchlorinatothallium(III) Hydroxide (2). —(a) *From trans-octaethylchlorin and thallium(III) trifluoroacetate.* *trans*-Octaethylchlorin (123 mg) in methylene chloride (20 ml) was stirred with TTFA (120 mg) in tetrahydrofuran (2 ml) during 1.5 min. Methylene chloride (100 ml) and dilute aqueous sodium hydrogen carbonate (100 ml) were added. After shaking, the organic phase was removed, washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed, with benzene as eluant. The first fraction contained unchanged *trans*-octaethylchlorin (49 mg), which was crystallised from methylene chloride–*n*-hexane. The required *thallium(III) chlorin* (23 mg, 22%), eluted next, had m.p. 208—209° (from methylene chloride–*n*-hexane) (Found: C, 56.0; H, 6.5; N, 7.0. $C_{36}H_{48}N_4O_2Tl$ requires C, 55.58; H, 6.4; N, 7.2%). λ_{max} 408 (ϵ 161,000), 512 (5300), 577 (5800), and 625 nm (45,000), τ 0.37 (d, J 44 Hz), 0.38 (d, J 42 Hz), 1.33 (d, J 51 Hz), and 1.35 (d, J 46 Hz) (*meso*-H), 5.3—5.8 (m, 7-, 8-H), 6.0—6.5 (12H, m, $ArCH_2\cdot CH_3$), 7.4—8.0 (4H, m, 7-, 8- $CH_2\cdot CH_3$), 8.24 (18H, t, $ArCH_2\cdot CH_3$), and 8.85 and 9.05 (each 3H, t, 7-, 8- $CH_2\cdot CH_3$), m/e 944 (1%), 942 (1), 940 (0.5), 774 (4), 772 (2), 739 (7), 737 (3), 536 (100), 507 (25), 298.5 (5), 268 (19), 205 (13), and 203 (5), ν_{max} (KBr), 3430w and 3330m cm^{-1} (OH).

Continued elution of the column afforded aquo-octaethylporphinatothallium(III) hydroxide² (36 mg) and then a small quantity of a blue compound (1 mg), m.p. 219—

²⁷ M. F. Hudson and K. M. Smith, unpublished results.

²⁸ L. F. Fieser and M. Fieser, 'Reagents for Organic Synthesis,' vol. 1, Wiley, New York, 1967, p. 1150.

²⁹ A. McKillop, J. D. Hunt, M. J. Zelesko, J. S. Fowler, E. C. Taylor, G. McGillivray, and F. Kienzle, *J. Amer. Chem. Soc.*, 1971, **93**, 4841.

³⁰ H. W. Whitlock, R. Hanauer, M. Y. Oester, and B. K. Bower, *J. Amer. Chem. Soc.*, 1969, **91**, 7485.

221°, identical with the dihydro-octaethylbiliverdin (4) described later.

(b) From *trans*-octaethylchlorin and thallium(III) acetate. *trans*-Octaethylchlorin (27 mg) in methylene chloride (20 ml) was treated with a solution of thallium(III) acetate (75 mg, *ca.* 3 equiv.) in methanol (15 ml) and heated on a water-bath; after 30 min, spectrophotometry showed metal insertion to be complete. Methylene chloride (50 ml) and dilute aqueous sodium hydrogen carbonate (50 ml) were added; after shaking, the organic phase was separated, washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed, with methylene chloride as eluant, and evaporation followed by crystallisation of the green residue gave the thallium(III) chlorin (35.4 mg, 90%), identical with the product from (a).

trans-Octaethylchlorinatozinc(II) (11).—*trans*-Octaethylchlorin (107 mg) in methylene chloride (30 ml) was treated with a saturated solution of zinc acetate in methanol (10 ml) and heated on a water-bath during 10 min. Methylene chloride (100 ml) and water (100 ml) were added; after shaking, the methylene chloride layer was dried (Na_2SO_4) and evaporated to dryness. The residue was crystallised from methylene chloride (passed through alumina to remove traces of acid)—*n*-hexane to give the zinc chlorin (115 mg, 96%), m.p. *ca.* 280° (decomp.) (Found: C, 72.0; H, 7.7; N, 9.3. $\text{C}_{36}\text{H}_{46}\text{N}_4\text{Zn}$ requires C, 72.0; H, 7.7; N, 9.3%), λ_{max} 397 (ϵ 142,000), 500 (5700), 536 (4300), 569 (6500), and 616 nm (38,000); the n.m.r. spectrum showed only broad, undefined resonances; *m/e* 598 (100%) and 299 (32).

$\gamma\delta$ -Dideuterio-*trans*-octaethylchlorin (10).—*trans*-Octaethylchlorin (120 mg) in dioxan (5 ml) and deuterioacetic acid (15 ml) was heated under dry nitrogen during 6 h at 95°. Methylene chloride (50 ml) was added and the solution was washed with water (100 ml) containing sodium acetate (15 g) before being dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed in methylene chloride–benzene and afforded the $\gamma\delta$ -dideuteriochlorin (113 mg, 94% recovery). N.m.r. spectroscopy indicated >95% deuteration at the *meso*-positions adjacent to the reduced ring.

1,2-*trans*-Dihydro-octaethylbiliverdin (4).—(a) From *trans*-octaethylchlorin (1). *trans*-Octaethylchlorin (120 mg) in methylene chloride (20 ml) was stirred with TTFA (480 mg) in tetrahydrofuran (8 ml) during 1.5 min. Methylene chloride (100 ml) was added and the solution was washed with dilute aqueous sodium hydrogen carbonate (100 ml) and then water. After drying (Na_2SO_4), the methylene chloride layer was evaporated to dryness and the residue was chromatographed, with benzene as eluant. The least polar band was green, but on exposure to air during collection of the eluates, a colour change to purple-blue was apparent; this purple-blue benzene solution (A) was evaporated to dryness and set aside. Continued elution gave thallium(III) octaethylporphyrin (3) (70 mg) and then the dihydrobiliverdin (8 mg), m.p. 219–221° (from methylene chloride–*n*-hexane). Rechromatography of the residue from (A) gave a further 18 mg of (4) (elution with methylene chloride and crystallisation from methylene chloride–*n*-hexane; total yield 26 mg, 25%) (Found: C, 75.65; H, 8.9; N, 10.2. $\text{C}_{35}\text{H}_{48}\text{N}_4\text{O}_2$ requires C, 75.5; H, 8.7; N, 10.1%), λ_{max} 347 (ϵ 45,000) and 591 nm (18,000), τ 1.35br ($3 \times \text{NH}$), 3.44, 4.03, and 4.56 (3 methine H), 7.1–7.9 (14H, *m*, $\text{ArCH}_2\text{-CH}_3$ and 1-, 2-H), 8.0–8.6 (4H, *m*, $\text{CH-CH}_2\text{-CH}_3$), and 8.6–9.3 (24H, *m*, $\text{CH}_2\text{-CH}_3$), *m/e*

556 (100%, *M*⁺), 527 (15), 418 (12), 285 (53), 278 (11), 272 (28), and 255 (11), *m*^{*} 500 (556 → 527) and 314 (556 → 418), ν_{max} (KBr) 1720s and 1685s cm^{-1} .

Repetition of the reaction with $\gamma\delta$ -dideuterio-*trans*-octaethylchlorin (10) afforded a 25% yield of dihydrobiliverdin, the n.m.r. spectrum of which lacked only the high-field (τ 4.56) methine resonance.

(b) From *trans*-octaethylchlorinatozinc(II) (11). *trans*-Octaethylchlorinatozinc(II) (60 mg) in methylene chloride (10 ml) was treated with TTFA (58 mg, *ca.* 1 equiv.) in tetrahydrofuran (2 ml). After 45 s, methylene chloride (50 ml) was added and the solution was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed in methylene chloride, but the column was stripped with chloroform containing 2% ethanol because of conversion of the initial green material into dihydroverdin on the column, which resulted in streaking. The eluates were evaporated to dryness and the residue was treated with benzene (5 ml); filtration afforded zinc octaethylporphyrin (40 mg). Rechromatography of the benzene fraction, with methylene chloride as eluant, afforded the dihydrobiliverdin (14 mg, 24%) after evaporation of the blue eluates and crystallisation from methylene chloride–*n*-hexane. The product from this reaction was identical with the material prepared in (a).

Octaethylbiliverdin (7).—(a) From octaethylhaemin (8) (*cf. ref.* 9). Octaethylhaemin (500 mg)³⁰ dissolved in a mixture of pyridine (150 ml) and water (400 ml) was treated with ascorbic acid (18 g), and the pH was adjusted to *ca.* 7.5 with aqueous sodium hydroxide. Oxygen was bubbled into the stirred solution at 40–45° during 3 h; the mixture was then extracted with methylene chloride. The organic phase was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was set aside overnight in 5% v/v sulphuric acid in methanol and then diluted with water and extracted with methylene chloride. The methylene chloride layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography of the residue with methylene chloride gave a minor green band, material from which crystallised from methylene chloride–*n*-hexane to give the octaethylbiliverdin (24 mg, 5%), m.p. 249–251° (Found: C, 75.6; H, 8.6; N, 10.2. $\text{C}_{35}\text{H}_{46}\text{N}_4\text{O}_2$ requires C, 75.8; H, 8.4; N, 10.1%), λ_{max} 366 (ϵ 58,000) and 645 nm (15,000), τ 1.70 ($3 \times \text{NH}$), 3.39 (1H, *s*) and 4.13 (2H, *s*) (methine H), 7.52 (12H, *q*, $\text{CH}_2\text{-CH}_3$), 7.75 (4H, *q*, 1-, 8- $\text{CH}_2\text{-CH}_3$), and 8.6–9.1 (24H, *m*, $\text{CH}_2\text{-CH}_3$), ν_{max} (KBr) 1695sh and 1674 cm^{-1} , *m/e* 554 (100%), 539 (9), 525 (15), 496 (4), 481 (3), 416 (4), 283 (8), 277 (11), 272 (3), 255 (7), and 135 (10), *m*^{*} 498 (554 → 525).

(b) From the dihydrobiliverdin (4). The dihydrobiliverdin (4) (5 mg) dissolved in chloroform (5 ml) and methanol (5 ml) was treated with 20% iron(III) chloride in concentrated hydrochloric acid (3 ml). The mixture was heated on a boiling water-bath during 10 min; a further 3 ml of the iron chloride solution was added and the heating was continued for another 10 min. The mixture was poured into aqueous sodium acetate and then extracted with methylene chloride. The organic phase was washed with dilute aqueous sodium hydrogen carbonate and water, and then dried (Na_2SO_4), and evaporated to dryness. The residue was purified by chromatography on preparative silica plates (Kieselgel G), with benzene–hexane–methanol (50 : 10 : 6) for development. The green band was removed from the plate and extracted from the silica with 5% methanol in methylene chloride. Evaporation and crystal-

lisation of the residue (2 mg) from methylene chloride-n-hexane gave the biliverdin, m.p. 249–251°, identical (t.l.c., mixed m.p., and i.r. spectrum) with the material described in (a).

meso-Trifluoroacetoxy-trans-octaethylchlorinatozinc(II) (12).—trans-Octaethylchlorin (101 mg) in methylene chloride (30 ml) was treated with a saturated solution of zinc acetate in methanol (15 ml) and heated gently during 5 min. Methylene chloride (100 ml) was added and the solution was washed with water, dried (Na_2SO_4), and then evaporated to dryness. The residue was dissolved in methylene chloride (20 ml) and treated with TTFA (102.6 mg) in tetrahydrofuran (10 ml) during 50 s. The mixture was then passed through a bed of Kieselgel G, which was washed with a small quantity of chloroform. The combined eluates were evaporated to dryness and the residue was taken up in methylene chloride-n-hexane (1:1). After 1 h, zinc octaethylporphyrin (40 mg) was filtered off and the filtrate was left in air during evaporation of the methylene chloride. The meso-trifluoroacetoxychlorin was filtered off (80 mg, 55%); m.p. >300° (Found: C, 64.2; H, 6.3; N, 8.0; Zn, 9.0. $\text{C}_{38}\text{H}_{45}\text{F}_3\text{N}_4\text{O}_2\text{Zn}$ requires C, 64.1; H, 6.4; N, 7.9; Zn, 9.2%), λ_{max} 399 (ϵ 156,000), 501 (5000), 536 (3000), 572 (7500), and 617 nm (49,300), τ 0.42, 0.56, and 1.35 (meso-H), 5.65br (2H, t, $\text{CH}\cdot\text{CH}_2\cdot\text{CH}_3$), 5.9–6.8 (12H, m, $\text{CH}_2\cdot\text{CH}_3$), 7.6–8.1 (4H, m, $\text{CH}\cdot\text{CH}_2\cdot\text{CH}_3$), 8.1–8.5 (18H, m, $\text{CH}_2\cdot\text{CH}_3$), and 8.7–9.2 (6H, m, $\text{CH}\cdot\text{CH}_2\cdot\text{CH}_3$). δ_{F} 6.83 (s) p.p.m. (downfield from $\text{CF}_3\cdot\text{CCl}_3$), ν_{max} (KBr) 1800 cm^{-1} (CO), m/e (^{64}Zn) 710 (10%, M^+), 613 (I), 598 (90), and 596 (100).

1,2,3,4,5,6,7,8-Octaethyl-1,2-trans-dihydro-2'-methoxy-a-oxobilidiene-bc (22).—trans-Octaethylchlorin (100 mg) in

methylene chloride (20 ml) was treated with TTFA (480 mg) in dry tetrahydrofuran (2 ml) and the mixture was stirred during 15 min. Methylene chloride (100 ml) was added and the mixture was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was taken into a small volume of methylene chloride and methanol was added; the solution was warmed on a water-bath to remove methylene chloride. The solid which was precipitated was filtered off and chromatographed, with chloroform as eluant. The pink eluates were evaporated to dryness and the residue was crystallised from methylene chloride-n-hexane to give the bilipupurin (36 mg, 32%), m.p. 198–200° (Found: C, 71.55; H, 8.3; N, 9.2. $\text{C}_{36}\text{H}_{50}\text{N}_4\text{O}_4$ requires C, 71.7; H, 8.4; N, 9.3%), λ_{max} 326 (ϵ 52,000), 505 (25,000), and 536 (26,000), τ 1.80, 0.78, and 3.16 ($3 \times \text{NH}$), 3.30 and 4.10 (2 methine H), 6.63 (3H, s, OCH_3), and 7.1–8.0 (14H, m, $\text{CH}\cdot\text{CH}_2\cdot\text{CH}_3$ and $\text{CH}_2\cdot\text{CH}_3$), 8.0–8.6 (ca. 4H, m, $\text{CH}\cdot\text{CH}_2\cdot\text{CH}_3$), and 8.5–9.2 (24H, m, $\text{CH}_2\cdot\text{CH}_3$), ν_{max} (KBr) 1725s, 1705s, and 1640s cm^{-1} , m/e 602 (5%), 570 (100), 555 (7), 541 (7), 433 (100), 432 (70), 418 (3), 405 (8), 404 (7), 390 (8), 376 (24), 360 (3), 347 (5), 285 (6), and 170 (4), m^* 540 (602 \rightarrow 570), 514 (570 \rightarrow 541), 379 (433 \rightarrow 405), 349 (405 \rightarrow 376), and 329 (570 \rightarrow 433).

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