Pyrroles and Related Compounds. Part XX.[1] Syntheses of Coproporphyrins

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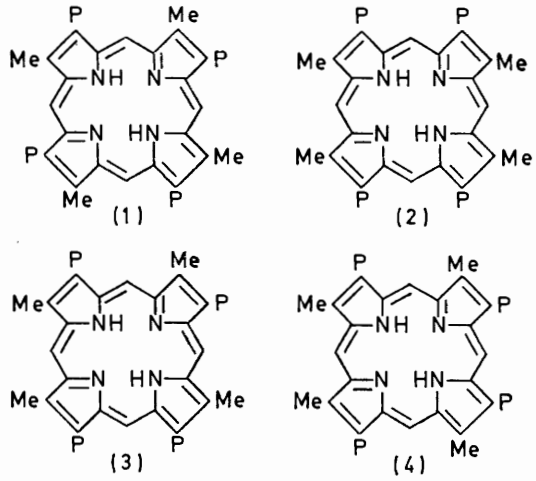
Coproporphyrin-I has been synthesised by modifications of Fischer's original methods; Coproporphyrin-II has been synthesised by three methods including a variant of the MacDonald method, involving self-condensation of a 5-formylpyrromethane-5'-carboxylic acid. Coproporphyrins-III and -IV and a related porphyrin tricarboxylic acid have been synthesised by application of the *a*-oxobilane route. The products in each case were single isomerically pure species as shown by spectroscopic, chromatographic, *X*-ray, and m.p. comparisons with each other and with authentic samples prepared by other workers.

THE coproporphyrins (1)—(4) are important reference compounds in biochemical work, but it is difficult to distinguish the four isomers on the basis of m.p. determinations alone (particularly if the compounds are not pure and in view of the tendency of porphyrins to exhibit polymorphism [2,3]). For this reason we undertook an intensive study of their ^{1}H n.m.r. spectra and showed that the four isomers could be easily distinguished from one another by comparing the fine structure of the spectra of the free bases in deuteriochloroform, and of the dications in trifluoroacetic acid.[4] We now describe the related synthetic studies, in the course of which we extended the use of our then newly developed *a*-oxobilane route to porphyrins.[5]

The problems inherent in designing unambiguous syntheses of pure porphyrin ' type ' isomers were adumbrated several years ago by MacDonald with particular reference to the copro- and uro-porphyrins.[2,6] All four coproporphyrin isomers were originally synthesised by variants of the classical Fischer method, by fusion of pyrromethenes, or by thermal decarboxylation of the corresponding uroporphyrins;[7] coproporphyrin-II had also been prepared by condensation of a pyrromethane-5,5'-dicarboxylic acid with formic acid [7] (see later).

In the present work, we employed the Fischer method in the case of coproporphyrin-I. Bromination of the pyrrolecarboxylic acid (5b), obtained by hydrogenolysis



of the corresponding benzyl ester (5a), afforded the pyrromethene hydrobromide (6a), and the latter, on

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[1] Part XIX, M. T. Cox, A. H. Jackson, and G. W. Kenner, *J. Chem. Soc.* (*C*), 1971, 1974.

[2] F. Morsingh and S. F. MacDonald, *J. Amer. Chem. Soc.*, 1960, **82**, 4377.

[3] *Cf.* J. C. Archibald, D. M. Walker, K. B. Shaw, A. Markovac, and S. F. MacDonald, *Canad. J. Chem.*, 1966, **44**, 345.

[4] R. J. Abraham, P. A. Burbridge, A. H. Jackson, and G. W. Kenner, *Proc. Chem. Soc.*, 1963, 134; R. J. Abraham, P. A. Burbidge, A. H. Jackson, and D. MacDonald, *J. Chem. Soc.* (*C*), 1966, 620.

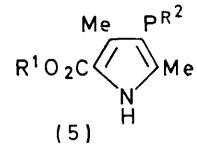
[5] A. H. Jackson, G. W. Kenner, and G. S. Sach, *J. Chem. Soc.* (*C*), 1967, 2045.

[6] G. P. Arsenault, E. Bullock, and S. F. MacDonald, *J. Amer. Chem. Soc.*, 1960, **82**, 4384; E. J. Tarlton, S. F. MacDonald, and E. Baltazzi, *ibid.*, p. 4389.

[7] H. Fischer and H. Orth, ' Die Chemie des Pyrroles,' vol. II (i), Akademische Verlag, Leipzig, 1937.

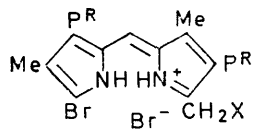
fusion in succinic acid, gave coproporphyrin-I, isolated as its tetramethyl ester in 35% yield. Heating of the same pyrromethene (6a) in formic acid gives only a 19% yield of coproporphyrin-I tetramethyl ester after methanolysis. This yield can be improved [8] to 50% by addition of 1 equiv. of free bromine to the reaction mixture; in essence the system then contains the perbromide of the pyrromethene (6a).[9]

An alternative preparation [10] involved bromination of the pyrrole t-butyl ester (5c) to give the bromomethylpyrromethene (6b), fusion of which in succinic acid also afforded coproporphyrin-I, but in lower yield.[11]



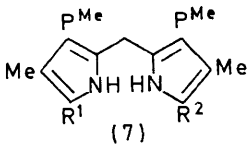
(5)

a; $R^1 = CH_2Ph, R^2 = Et$
b; $R^1 = H, R^2 = Et$
c; $R^1 = Bu^t, R^2 = Et$



(6)

a; X = R = H
b; X = Br, R = Et



(7)

a; $R^1 = R^2 = CO_2H$
b; $R^1 = R^2 = CHO$
c; $R^1 = CHO, R^2 = CO_2H$
d; $R^1 = R^2 = CO_2 \cdot CH_2Ph$
e; $R^1 = CO_2H, R^2 = CO_2 \cdot CH_2Ph$

Here and elsewhere $P^R = CH_2 \cdot CH_2 \cdot CO_2R$

Coproporphyrin-II tetramethyl ester (2) was synthesised by three methods. Burbidge originally investigated [11] a simple variation of one of Fischer's syntheses *viz.* heating the pyrromethanedicarboxylic acid (7a) with formic acid in presence of 48% hydrobromic acid in acetic acid for 1 h. After work-up and esterification, coproporphyrin-II tetramethyl ester was obtained in 8% yield, and was shown to be a single pure isomer by m.p., n.m.r., and chromatographic comparisons with other samples, and other coproporphyrins. This method sometimes gives mixtures of isomers, depending on the nature of the β-substituents in the pyrromethane, because cleavage at the methane bridge can occur under the acidic conditions of the reaction.[12] In later work, therefore, we modified the conditions of condensation in the light of our extensive studies of the use of one-carbon units for cyclisation of open-chain tetrapyrrolic intermediates in the *a*-oxobilane route to porphyrins.[5] Use of trimethyl orthoformate as the one-carbon unit and trichloroacetic acid as the catalyst in the cyclisation of the dicarboxylic acid afforded pure coproporphyrin-II tetramethyl ester in 25% yield. No attempt was made to optimise the conditions of this reaction, but the new procedure seems likely to prove of value in syntheses of other isomerically pure porphyrins with 'type-II' symmetry.

The second method was a simple extension of the MacDonald synthesis, which he first applied to the syntheses of the uroporphyrins-II, -III, and -IV.[6] The required diformylpyrromethane (7b) was prepared directly from the dicarboxylic acid (7a) by the Gattermann reaction, although the yield (28%) was low. Attempts to decarboxylate the diacid (7a), either thermally or by acid catalysis, led to mixtures of products (as shown by n.m.r., t.l.c., *etc.*), presumably because of cleavage reactions at the methane bridge. Condensation of the dialdehyde (7b) with the diacid (7a) in glacial acetic acid containing a little hydriodic acid (*cf.* ref. 6) followed by aeration then gave pure coproporphyrin-II tetramethyl ester (2) in 42% yield. Cyclisations of this type can now be accomplished [13] with toluene-*p*-sulphonic acid and zinc acetate in methanol, giving yields of porphyrin in the region of 35—40%, and these are more consistently obtained than by the conventional hydriodic–acetic acid method.

Shortly after this phase of the work was completed, Russian workers described a much improved synthesis of the diformylpyrromethane (7b), involving decarboxylation of the diacid (7a) in hot alkali (in the presence of hydrazine as stabiliser) followed by direct formylation of the resulting 5,5′-diunsubstituted pyrromethane; [14] more recently Clezy has reported [15] that decarboxylation may be effected by heating in dimethylformamide, and formylation is then readily carried out *in situ* by activation of the dimethylformamide with phosphoryl chloride or benzoyl chloride.

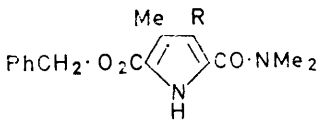
The third route to coproporphyrin-II was a variant [16] of the MacDonald porphyrin synthesis [6] in which the 5′-formylpyrromethane-5-carboxylic acid (7c) was self-condensed in glacial acetic acid containing hydriodic acid; the yield of pure type-II porphyrin obtained was 21%.

We originally hoped to extend the MacDonald method to syntheses of coproporphyrins-III and IV. However, owing to the difficulties experienced at the time in the preparation of the required diformylpyrromethane (7b), we turned instead to the *a*-oxobilane route, which had just been developed.[5] The route adopted is shown in the Scheme; in addition we also synthesised the analogous porphyrintripropionic acid (18a).

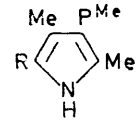
The key intermediates required for the syntheses of the pyrroketones (11) were the pyrrole amides (8) and (9) and the 5-unsubstituted pyrrole (10c). The amides

[8] K. M. Smith, preceding paper.
[9] K. M. Smith, *Tetrahedron Letters*, 1971, 2325.
[10] *Cf.* J. Ellis, A. H. Jackson, A. C. Jain, and G. W. Kenner, *J. Chem. Soc.*, 1964, 1935.
[11] P. A. Burbidge, M.Sc. Thesis, Liverpool, 1963.
[12] *Cf.* D. Mauzerall, *J. Amer. Chem. Soc.*, 1960, **82**, 2601; A. Treibs and H. G. Kohn, *Annalen*, 1958, **614**, 199.
[13] A. M. d'A.R. Gonsalves, G. W. Kenner, and K. M. Smith, *Chem. Comm.*, 1971, 1304.
[14] R. P. Evstigneeva and N. A. Preobrezhenskii, *Zhur. obshchei Khim.*, 1966, 806.
[15] R. Chong, P. S. Clezy, A. J. Liepa, and A. W. Nichol, *Austral. J. Chem.*, 1969, **22**, 229.
[16] *Cf.* also H. H. Inhoffen and J. Ullrich, unpublished work referred to in H. H. Inhoffen, J. W. Buchler, and P. Jäger, *Fortschr. Chem. org. Naturstoffe*, 1968, **26**, 284.

(8a) and (8b) had been synthesised previously [17] by trichlorination of the corresponding 2-methylpyrrole followed by treatment with dimethylamine, but in the

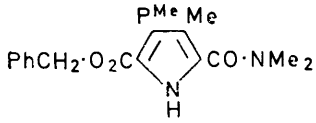


(8) a; R = Et
b; R = P^{Me}



(10)

a; R = $CO_2 \cdot CH_2Ph$
b; R = CO_2H
c; R = H
d; R = $CO \cdot NMe_2$



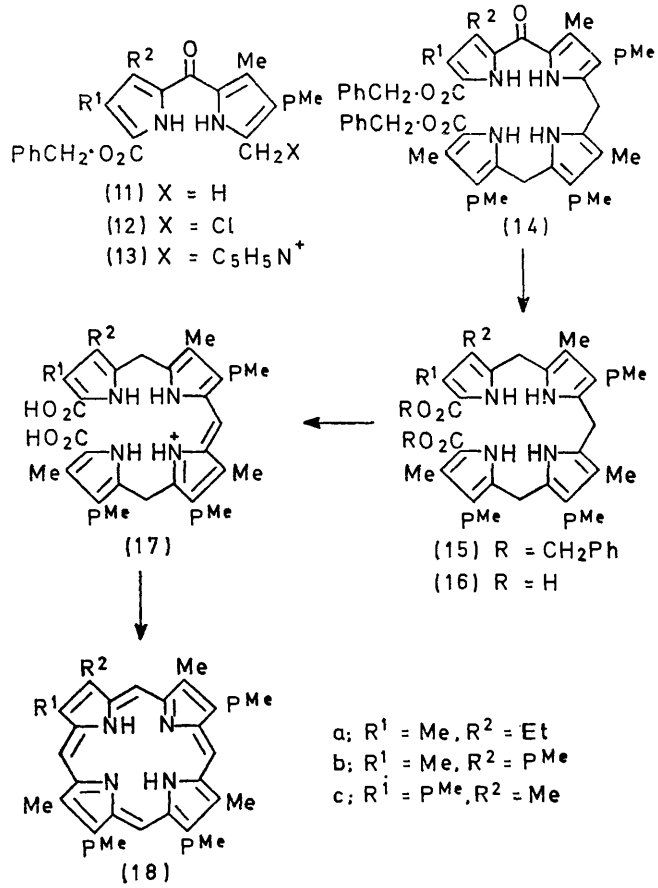
(9)

case of (8b), better results were obtained by the more conventional route from (10), *i.e.* $CH_3 \longrightarrow CCl_3 \longrightarrow CO_2H \longrightarrow COCl \longrightarrow CO \cdot NMe_2$. A similar method could have been employed for the preparation of the amide (9), but the reported synthesis requiring the 2-methylpyrrole precursor [18] was lengthy, and we turned to an alternative approach from the pyrrole (10a). Hydrogenolysis and decarboxylation, followed by successive treatment with phosgene and dimethylamine afforded the amide (10d); the α-methyl group of the latter was then chlorinated with t-butyl hypochlorite and the resulting trichloromethyl derivative on treatment with benzyl alcohol gave the amide (9).

Coupling of the amides (8a), (8b), and (9) with the α-unsubstituted pyrrole (10c) was effected with phosphoryl chloride, and afforded the required pyrroketones (11a—c) in yields of 77, 66, and 75%, respectively. The corresponding chloromethylpyrroketones (12) were readily prepared by chlorination with t-butyl hypochlorite in 80—85% yields, and the desired pyridinium salts (13) were each coupled with the lithium salt of the pyrromethanecarboxylic acid (7e) in formamide at 50°. The initial oily products were crystallised from ether and afforded the desired *a*-oxobilanes (14) in yields of 26, 35, and 38%, respectively; the structures of these compounds were confirmed by elemental analyses, and by ^1H n.m.r. spectrometry.

The oxobilanes (14) were then converted into the porphyrins (18) by the same sequence of reactions as had been developed in the earlier work,[5] *i.e.* reduction by diborane of the oxo-function [to (15)], hydrogenolysis of the terminal benzyl ester groups [to (16)], oxidation to the bilenes (17) with t-butyl hypochlorite, and cyclisation to the porphyrins (18) by treatment with trimethyl orthoformate and trichloroacetic acid in methylene chloride followed by aeration. Each of the various stages was monitored spectroscopically, and the porphyrins (18a), (18b) [*i.e.* coproporphyrin-III tetramethyl ester (3)], and (18c) [*i.e.* coproporphyrin-IV tetramethyl ester (4)] were obtained in overall yields of 23, 23, and 24%, respectively, from the corresponding *a*-oxobilanes (14). Only one porphyrin was obtained in each case, and isomeric purity was established by thin-layer and paper chromatography, and by ^1H n.m.r. spectrometry; satisfactory elemental analyses were obtained.

Dr. S. F. MacDonald compared the m.p.s of our tetramethyl esters of coproporphyrins-III and -IV, with his own samples [2,6] in Ottawa, and we made similar comparisons in Liverpool. He also prepared the copper complexes from our samples, and compared them with his own; *X*-ray powder photographs were also taken in Ottawa by Dr. M. Pryzbylska. Good overall agreement was obtained, although the m.p.s of the Liverpool samples were a little lower than those of the Ottawa samples. However, there was no doubt of the identity of our specimens, and of their isomeric purity, particularly in the light of the ^1H n.m.r., *X*-ray, and t.l.c. data.



(11) X = H
(12) X = Cl
(13) X = $C_5H_5N^+$

(14)

(15) R = CH_2Ph
(16) R = H

(17)

(18)

a; R^1 = Me, R^2 = Et
b; R^1 = Me, R^2 = P^{Me}
c; R^1 = P^{Me}, R^2 = Me

SCHEME

This was gratifying in view of the polymorphism shown by porphyrins, especially unsymmetrical derivatives such as coproporphyrin-III tetramethyl ester. The four coproporphyrin esters all exhibited virtually identical

[17] J. A. Ballantine, A. H. Jackson, G. W. Kenner, and G. McGillivray, *Tetrahedron*, 1966, **22**, Suppl. I, 241.

[18] *Cf.* A. Hayes, G. W. Kenner, and N. R. Williams, *J. Chem. Soc.*, 1958, 3779.

visible and mass spectroscopic properties;[19] data for naturally derived materials were very similar.

By comparing n.m.r. spectra of solutions in deuteriochloroform and in trifluoroacetic acid, the four coproporphyrins can be easily distinguished from one another.[4] However, separation of a mixture of coproporphyrins is difficult, and the best chromatographic procedure so far discovered, *i.e.* paper chromatography in lutidine–ammonia[20] only achieves a partial separation into three fractions: coproporphyrins-III and -IV are not resolved and run at an intermediate R_F value between the isomers I and II. We hoped that application of electrophoresis or counter-current distribution might be a more useful way of distinguishing between the four isomers, and might even be of use preparatively, as mixtures of coproporphyrins are readily available by polymerisation of suitable monopyrroles. However, electrophoretic experiments in a variety of buffer solutions were discouraging; no evidence of separation was obtained. Following earlier work,[11] we developed a new system for the counter-current distribution of porphyrin carboxylic acids, namely isobutyl methyl ketone–t-butyl alcohol (1 : 1 v/v) and dilute sulphuric acid (*ca.* 0·5—2M, depending on the porphyrins concerned). This system has two main advantages over the more common ether–hydrochloric acid used by earlier workers:[20] (i) the higher solubility of porphyrins and (ii) its greater chemical stability (ether tends to form peroxides and thus decomposition of porphyrins results unless special precautions are taken to exclude light and oxygen). Unfortunately, this new system failed to give any separation of mixtures of coproporphyrins, even after one thousand transfers. However, it is useful for separating other porphyrin carboxylic acids, *e.g.* harderoporphyrin[21] and the ' S-411 ' porphyrin from meconium.[22]

EXPERIMENTAL

M.p.s are uncorrected and were determined for samples in capillaries, except for those of the porphyrins, which were determined with a Kofler hot-stage apparatus and are corrected. Neutral alumina (Woelm grade III) was used for all chromatographic separations, and reactions were followed by t.l.c. and by spectroscopy as described in earlier parts of this series. Electronic spectra were determined with a Unicam SP 800 spectrometer, ^{1}H n.m.r. spectra with Varian A-60 and HA 100 spectrometers, and mass spectra with an A.E.I. MS9 spectrometer (at 50 μA and 70 eV; direct inlet heated to 200—220°).

Pyrroles

Benzyl 5-Dimethylcarbamoyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (8b).—(*a*) 5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylic acid (12·0 g) was dissolved in thionyl chloride (96 ml) with gentle warming at 40° for 30 min. Removal of the solvent under reduced pressure at 20° yielded a pale yellow oil which crystallised on triturating with light petroleum (b.p. 60—80°) to give the *pyrrole acid chloride* (11·3 g, 89%) as needles, m.p. 96° [from light petroleum (b.p. 60—80°)], τ ($CDCl_3$) 7·70 (4-Me), 6·95(m), 7·39(t), and 6·34 ($[CH_2]_2$·CO_2Me), 2·60 and 4·61 ($PhCH_2$), and *ca.* 0·45 (NH).

A solution of the foregoing chloride (8·5 g) in dry benzene (100 ml) was saturated with dimethylamine gas. After 30 min at 20° the benzene layer was washed thoroughly with water, 2N-hydrochloric acid (2 × 100 ml), and water again until the washings were neutral, dried ($MgSO_4$), and evaporated under reduced pressure. The resulting oil crystallised on triturating with ether to give the *pyrrole dimethylamide* (8·0 g, 92%) as pale yellow needles, m.p. 76—78° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 64·4; H, 6·5; N, 7·6. $C_{20}H_{24}N_2O_5$ requires C, 64·5; H, 6·5; N, 7·5%), τ ($CDCl_3$) 7·70 (3-Me), 7·0—7·6 (m) and 6·34 ($[CH_2]_2$·CO_2Me), 6·96 (NMe_2), 2·59 and 4·68 ($PhCH_2$), and *ca.* 0·5 (NH).

(*b*) Benzyl 4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (10·0 g) was dissolved in dry ether (400 ml) and a catalytic quantity of benzoyl peroxide was added. Sulphuryl chloride (8·4 ml, 3·25 mol. equiv.) was then added during 1 h to the stirred solution, which was then stored overnight at 20° under anhydrous conditions. The solution was boiled under reflux for 2 h and then concentrated to dryness under reduced pressure at 20°. The residual green oil was dissolved in dry benzene (100 ml) and dimethylamine gas was passed into the solution until saturation was reached. Water (100 ml) was introduced and the heterogeneous mixture vigorously stirred under reflux for 1 h. After work-up as in (*a*) the *pyrrole dimethylamide* (6·3 g, 53%) was obtained as needles, m.p. 76—78° identical with the material prepared as in (*a*) (mixed m.p. and i.r. spectrum).

Benzyl 5-Dimethylcarbamoyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (9).—2-Dimethylcarbamoyl-4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole (17·0 g) dissolved in carbon tetrachloride (680 ml) was stirred at 0° under nitrogen while a solution of t-butyl hypochlorite (24·1 ml, 3·0 mol. equiv.) in carbon tetrachloride (120 ml) was added during 30 min. After a further 15 min stirring at 0° followed by 40 min at 20° a starch–iodide reaction gave a negative result. Removal of the solvent under reduced pressure yielded orange crystals, which were not isolated but dissolved in a mixture of methylene chloride (170 ml) and carbon tetrachloride (800 ml). Benzyl alcohol (21·1 ml) dissolved in methylene chloride (170 ml) was added at room temperature, during 15 min, to the stirred solution of trichloromethylpyrrole, which was subsequently heated under reflux for 90 min. Removal of the solvent under reduced pressure yielded a brown oil which crystallised on triturating with ether to give *pyrrole dimethylamide* (13·7 g, 55%) as pale yellow needles, m.p. 123·5—125° [from benzene–ether–light petroleum (b.p. 60—80°)] (Found: C, 64·6; H, 6·3; N, 7·5. $C_{20}H_{24}N_2O_5$ requires C, 64·5; H, 6·5; N, 7·5%), τ ($CDCl_3$), 7·91 (3-Me), *ca.* 7·0 (m), *ca.* 7·55 (m), and 6·32 ($[CH_2]_2$·CO_2Me), 5·13 (NMe_2), 2·66 and 4·74 ($PhCH_2$), and *ca.* 0·4 (NH).

In another experiment the trichloromethylpyrrole was isolated as colourless needles (69%), the structure being confirmed by n.m.r. spectroscopy: τ ($CDCl_3$) 7·76 (3-Me), *ca.* 6·85 (m), *ca.* 7·35 (m), and 6·20 ($[CH_2]_2$·CO_2Me), and 6·72 (NMe_2) (NH not observed).

[19] A. H. Jackson, G. W. Kenner, K. M. Smith, R. T. Aplin, H. Budzikiewicz, and C. Djerassi, *Tetrahedron*, 1965, **21**, 2913.
[20] *Cf.* J. E. Falk, ' Porphyrins and Metalloporphyrins,' Elsevier, Amsterdam, 1964.
[21] G. Y. Kennedy, A. H. Jackson, G. W. Kenner, and C. J. Suckling, *FEBS Letters*, 1970, **6**, 9; 1970, **7**, 205.
[22] P. Couch and A. H. Jackson, unpublished work.

2-Dimethylcarbamoyl-4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole (10d).—4-(2-Methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylic acid (35·3 g) was decarboxylated by heating at 120—140° under nitrogen until no further effervescence took place. Phosgene gas was passed into a stirred, ice-cold solution of the resulting α-unsubstituted pyrrole in dry ether (300 ml) for 10 min and through the solution at room temperature for 10 min. Nitrogen was bubbled through the solution to remove excess of phosgene; evaporation of the solvent at 20° under reduced pressure then gave a brown solid, which was dissolved in dry benzene (720 ml). The solution was saturated with dimethylamine gas and set aside at 5° overnight. It was then washed with water (until the washings were neutral), dried ($MgSO_4$), and evaporated under reduced pressure to yield a brown oil which crystallised on triturating with ether to give *pyrrole dimethylamide* as pale yellow needles (22·8 g, 58%), m.p. 105·5—106·5° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 62·0; H, 7·9; N, 11·2. $C_{13}H_{20}N_2O_3$ requires C, 61·9; H, 8·0; N, 11·1%), τ ($CDCl_3$) 7·93 (5-Me), 7·80 (3-Me), 7·1—7·7 (m) and 6·28 ($[CH_2]_2{\cdot}CO_2Me$), and *ca.* 0·5 (NH).

Attempts to prepare the intermediate pyrrole acid chloride from the carboxylic acid and thionyl chloride were not successful.

4-(2-Methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylic Acid (10b).—Benzyl 4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (10·0 g) dissolved in dry tetrahydrofuran (125 ml) was hydrogenated at atmospheric pressure and room temperature over 10% palladium–charcoal (1·0 g) with triethylamine (2 drops) added as catalyst. Within 30 min the theoretical uptake of hydrogen had occurred (730 ml at N.T.P. including 20 ml for catalyst). Filtration and evaporation gave the pyrrolecarboxylic acid as a solid (7·0 g, 98%), m.p. 131° (decomp.), which was used without further purification.

5-Dimethylcarbamoyl-4-ethyl-3-methylpyrrole-2-carboxylic Acid.—Benzyl 5-dimethylcarbamoyl-4-ethyl-3-methylpyrrole-2-carboxylate (8a) (14·0 g) was dissolved in dry tetrahydrofuran (250 ml) containing triethylamine (6 drops) and the solution was hydrogenated over 10% palladium–charcoal (1·4 g) at room temperature and atmospheric pressure. After 20 min 1180 ml of hydrogen had been taken up. Filtration and evaporation gave the pyrrolecarboxylic acid (9·9 g, 100%) as a crystalline solid, m.p. 205° (decomp.), which was used without further purification.

Pyrromethanes

5,5′-Diformyl 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane (7b).—A slow stream of dry hydrogen chloride was passed through a suspension of 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarboxylic acid (800 mg) in methylene chloride (100 ml) at 0°. After 1 h hydrogen cyanide (6 ml) was added and hydrogen chloride was slowly bubbled through the suspension at 0° for a further 6 h. The mixture was stirred in a stoppered flask at 20° for 13 days, during which time complete dissolution was never obtained. The solvent was removed under reduced pressure at 20° and a solution of dipotassium hydrogen phosphate (6 g) in water (75 ml) was added to the brown viscous residue. The heterogeneous mixture was heated under reflux for 5 min with stirring. Methylene chloride (75 ml) was added and the mixture was vigorously stirred under reflux for a further 5 min. The organic layer was separated, washed with water until the washings were neutral, dried ($MgSO_4$), and evaporated under reduced pressure. Crystallisation of the residue from ether afforded the *pyrromethane* (207 mg, 28%) as needles, m.p. 184—185° (Found: C, 62·4; H, 6·4; N, 7·0. $C_{21}H_{26}N_2O_6$ requires C, 62·7; H, 6·5; N, 7·0%), τ ($CDCl_3$) 7·76 (4,4′-Me_2), 7·26 (t), 7·59 (t), and 6·39 ($[CH_2]_2{\cdot}CO_2Me$), 6·01 (CH_2), 0·61 (CHO), and −0·72 (NH).

Pyrromethenes

5-Bromo-3,4′-bis-(2-carboxyethyl)-3′,4,5′-trimethylpyrromethene Hydrobromide (6a) (with P. Burbidge).—Benzyl 4-(2-benzyloxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (3·0 g) in dry tetrahydrofuran (50 ml) containing triethylamine (10 drops) was hydrogenated at room temperature and atmospheric pressure over 10% palladium–charcoal (0·33 g) until uptake was complete. Filtration and evaporation afforded the corresponding diacid, which was just wetted with glacial acetic acid and then treated with a solution of bromine (1·08 ml, 2 mol. equiv.) in glacial acetic acid (8 ml). The pyrromethene hydrobromide (1·1 g, 58%) slowly crystallised and was obtained as a deep red granular product after washing with light petroleum (b.p. 40—60°); m.p. 205—210° (decomp.) [lit.,[23] 220° (decomp.)].

5-Bromo-5′-bromomethyl-3,4′-bis-(2-ethoxycarbonylethyl)-3,4-dimethylpyrromethene Hydrobromide (6b) (with P. Burbidge).—t-Butyl 4-(2-ethoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (5 g) was dissolved in dry ether (200 ml) and stirred with anhydrous potassium carbonate during the rapid addition of bromine (0·98 ml, 1 equiv.) in dry ether (200 ml). The mixture was stirred for a further 1 h, then filtered from potassium carbonate, and added dropwise during 1 h to a stirred solution of bromine (1·96 ml, 2 equiv.) in dry ether (400 ml). The solution was stirred for a further 0·5 h and then kept overnight at 0°. Evaporation under reduced pressure afforded a viscous red oil, which crystallised on triturating with light petroleum (b.p. 60—80°) to give the *pyrromethene hydrobromide* (1·9 g, 36%) as deep red granular crystals, m.p. 155° (Found: C, 42·2; H, 4·7; N, 4·3. $C_{22}H_{29}Br_3N_2O_4$ requires C, 42·2; H, 4·7; N, 4·5%), τ ($CDCl_3$) 8·79, 8·75, 5·94, 5·83, and 6·8—7·8 (m) ($EtO_2C{\cdot}[CH_2]_2$), 7·92 and 7·61 (2 × Me), 5·03 (CH_2Br), 2·52 (CH=), and −4·0br (NH). This material was used directly without further purification.

Pyrroketones

Benzyl 3-Ethyl-4′-(2-methoxycarbonylethyl)-3′,4,5′-trimethylpyrroketone-5-carboxylate (11a).—Benzyl 5-dimethylcarbamoyl-4-ethyl-3-methylpyrrole-2-carboxylate (15·5 g) was dissolved in dry ethylene dichloride (75 ml) with warming, and phosphoryl chloride (5·1 ml, 1·1 mol. equiv.) was added rapidly. The solution was stirred under nitrogen for 60 min at 20° and subsequently heated on a water-bath (at 88°) for 5 h. Examination of the u.v. spectrum of a sample at a concentration of 40 μmol l^{-1} then indicated that the peak at 283 nm had reached a minimum and that at 370 nm a maximum.

3-(2-Methoxycarbonylethyl)-2,4-dimethylpyrrole (9·9 g, 1·1 mol. equiv.) in dry ethylene dichloride (10 ml) was added during 20 min to the stirred solution at room temperature, while a slow stream of nitrogen was bubbled through. The solution was boiled under reflux until the u.v. spectrum of a sample at a concentration of 80 μmol l^{-1}

[23] Ref. 7, p. 86.

indicated that the peak at 400 nm had reached a maximum (90 min). A solution of sodium carbonate (10%; 100 ml) was added and the heterogeneous mixture was vigorously stirred under reflux for 2 h. The organic layer was separated, washed with water until the washings were neutral, dried ($MgSO_4$), and evaporated. The resulting viscous red oil crystallised on triturating with methanol to give the *pyrroketone* (14·2 g) as pale yellow prisms, m.p. 153—154° [from chloroform–ether (charcoal)]. Concentration of the mother liquors yielded a residue, which crystallised from ether to give a second crop of the pyrroketone (2·8 g), m.p. 152—154° (Found: C, 69·0; H, 6·7; N, 6·2. $C_{26}H_{30}N_2O_5$ requires C, 69·3; H, 6·7; N, 6·2%), τ ($CDCl_3$) 8·95 and 7·35 (3-Et), 8·03, 7·76, and 7·67 (3 × Me), *ca.* 7·1—7·7 (m) and 6·33 ($[CH_2]_2$·CO_2Me), 2·61 and 4·68 ($PhCH_2$), and 0·55br and 0·75br (2 × NH).

Benzyl 3,4′-Bis(2-methoxycarbonylethyl)-3′,4,5′-trimethylpyrroketone-5-carboxylate (11b).—Benzyl 5-dimethylcarbamoyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (8·9 g) was dissolved in dry ethylene dichloride (25 ml) with warming, and phosphoryl chloride (2·5 ml, 1·1 mol. equiv.) was added rapidly. The solution was stirred under nitrogen for 60 min at 20° and subsequently heated under reflux on a water-bath at 88° for 4 h. The u.v. spectrum of a sample at a concentration of 40 μmol l^{-1} then indicated that the peak at 285 nm had reached a minimum and the peak at 368 nm a maximum.

3-(2-Methoxycarbonylethyl)-2,4-dimethylpyrrole (4·7 g, 1·1 mol. equiv.) in dry ethylene dichloride (5 ml) was added during 10 min to the stirred solution at 20°, while a slow stream of nitrogen was bubbled through. The solution was stirred at 20° for 45 min and then boiled under reflux until the u.v. spectrum of a sample at a concentration of 80 μmol l^{-1} indicated that the peak at 400 nm had reached a maximum (*ca.* 2 h). A solution of sodium carbonate (10%; 60 ml) was added and the heterogeneous mixture was vigorously stirred under reflux for 2 h. The organic layer was washed with water until the washings were neutral, dried ($MgSO_4$), and evaporated. The resulting viscous purple oil crystallised on triturating with methanol to give the *pyrroketone* (8·0 g, 66%) as pale yellow prisms, m.p. 122° [from chloroform–ether (charcoal)] (Found: C, 66·3; H, 6·5; N, 5·6. $C_{28}H_{32}N_2O_7$ requires C, 66·1; H, 6·3; N, 5·5%), $\nu_{max.}$ (Nujol) 1560, 1680, and 1725 cm^{-1}, τ ($CDCl_3$) 8·04, 7·77, and 7·68 (3 × Me), 7·0—7·7 (m), 6·43, and 6·35 ($[CH_2]_2$·CO_2Me), 2·61 and 4·69 ($PhCH_2$), and 0·45br and 0·65br (2 × NH).

Benzyl 4,4′-Bis-(2-methoxycarbonylethyl)-3,3′,5′-trimethylpyrroketone-5-carboxylate (11c).—Benzyl 5-dimethylcarbamoyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (8·9 g) was coupled (as its phosphoryl chloride complex) with 3-(2-methoxycarbonylethyl)-2,4-dimethylpyrrole (4·7 g, 1·1 mol. equiv.) as in the foregoing experiment. Recrystallisation from chloroform–ether afforded the *pyrroketone* (9·1 g, 75%) as pale yellow prisms, m.p. 123—124° (Found: C, 66·1; H, 6·3; N, 5·8. $C_{28}H_{32}N_2O_7$ requires C, 66·1; H, 6·3; N, 5·5%), $\nu_{max.}$ (Nujol) 1575, 1680, and 1720 cm^{-1} (C=O), τ ($CDCl_3$) 8·00, 7·83, and 7·74 (3 × Me), 7·2—7·7 (6H, m), *ca.* 7·0 (2H, m), 6·35, and 6·33 ($[CH_2]_2$·CO_2Me), 2·62 and 4·67 ($PhCH_2$), and −0·1br (NH).

Benzyl 5′-Chloromethyl-3-ethyl-4′-(2-methoxycarbonylethyl)-3′,4-dimethylpyrroketone-5-carboxylate (12a).—Benzyl 3-ethyl-4′-(2-methoxycarbonylethyl)-3′,4,5′-trimethylpyrroketone-5-carboxylate (2·4 g) was dissolved in the minimum volume of dry tetrahydrofuran; dry ether was added (60 ml) and the solution cooled to 3°. The stirred solution was treated with t-butyl hypochlorite (0·7 ml, 1·1 mol. equiv.) dissolved in dry ether (30 ml) during 4 min, with the temperature kept below 3°. Within 5 min of the addition a negative starch–iodide reaction was obtained, indicating that all the t-butyl hypochlorite had reacted. The solvent was removed under reduced pressure, dry ether (25 ml) was added, and the solution was again evaporated to dryness under reduced pressure. Trituration with dry ether afforded pale orange crystals, which were filtered off and washed with a little ice-cold dry ether. The mother liquors were concentrated under reduced pressure and a second crop was obtained after trituration with light petroleum (b.p. 40—60°). Recrystallisation from benzene–light petroleum (b.p. 60—80°) gave the *chloromethylpyrroketone* (2·2 g, 85%) as fawn needles, m.p. 114—116° (Found: C, 64·5; H, 6·1; N, 5·7. $C_{26}H_{29}ClN_2O_5$ requires C, 64·4, H, 6·0; N, 5·8%), τ ($CDCl_3$), 8·90 (t) and *ca.* 7·5 (m) (Et), 8·02 and 7·69 (2 × Me), 7·1—7·7 (m) and 6·33 ($[CH_2]_2$·CO_2Me), 2·61 and 4·68 ($PhCH_2$), and *ca.* 0·5br and *ca.* 1·1br (2 × NH).

Benzyl 5′-Chloromethyl-3,4′-bis-(2-methoxycarbonylethyl)-3′,4-dimethylpyrroketone-5-carboxylate (12b).—This was prepared like the foregoing compound in 86% yield. It formed pale cream *needles*, m.p. 108—109° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 61·9; H, 6·0; N, 5·0. $C_{28}H_{31}ClN_2O_7$ requires C, 61·9; N, 5·8; N, 5·2%), τ ($CDCl_3$) 8·02 and 7·65 (2 × Me), 6·9—7·3 (2H, m), 7·3—7·6 (6H), 6·36, and 6·31 ($[CH_2]_2$·CO_2Me), 5·35 (CH_2Cl), 2·61 and 4·69 ($PhCH_2$), and *ca.* 0·1 and *ca.* 0·7 (2 × NH).

Benzyl 5′-Chloromethyl-4,4′-bis-(methoxycarbonylethyl)-3,3′-dimethylpyrroketone-5-carboxylate (12c).—This was prepared in 80% yield like its analogues. Crystallisation from benzene–light petroleum (b.p. 60—80°) gave pale cream *needles*, m.p. 92—94° (Found: C, 61·9; H, 5·8; N, 4·9. $C_{28}H_{31}ClN_2O_7$ requires C, 61·9; H, 5·8; N, 5·2%), τ ($CDCl_3$) 7·96 and 7·83 (2 × Me), 6·8—7·1 (2H, m), 7·1—7·7 (6H), 6·30, and 6·25 ($[CH_2]_2$·CO_2Me), 5·27 (CH_2Cl), 2·52 and 4·61 ($PhCH_2$), and 0·2 and −0·1 (2 × NH).

Oxobilanes

1′,8′-Bisbenzyloxycarbonyl-2-ethyl-1,3,5,8-tetramethyl-4,6,7-tris-(2-methoxycarbonylethyl)-a-oxobilane (14a).—Benzyl 5′-chloromethyl-3-ethyl-4′-(2-methoxycarbonylethyl)-3′,4-dimethylpyrroketone-5-carboxylate (6·4 g) was dissolved in pyridine (12 ml) by warming gently on a water-bath. 5′-Benzyloxycarbonyl-3,3′-bis(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5-carboxylic acid (6·92 g) was suspended in formamide (200 ml), lithium methoxide (0·502 g) was added, and the suspension was shaken until the solid had dissolved. The reactants were combined and the resulting clear amber solution was heated at 50° under nitrogen for 18 h. An oil slowly precipitated during the heating period and formed a viscous lower layer. After a further 17 h at room temperature under nitrogen the oil had partially solidified and the supernatant was decanted. The gummy deposit was washed thoroughly with water and then dissolved in methylene chloride (100 ml). The solution was washed thoroughly with water, dried ($MgSO_4$), and evaporated under reduced pressure. Re-concentration of the residue with dry peroxide-free ether (50 ml) under reduced pressure yielded a brown solid foam, which was redissolved in dry peroxide-free ether (100 ml) and set aside overnight under nitrogen. The crystallised material was filtered off and washed with a little ice-cold ether to give the yellow product (3·22 g, 26%), m.p. 119—122°.

Recrystallisation from methylene chloride–light petroleum (b.p. 40—60°) afforded the a-*oxobilane* as yellow prisms, m.p. 120—122° (Found: C, 68·3; H, 6·5; N, 6·0. $C_{33}H_{60}N_4O_{11}$ requires C, 68·5; H, 6·5; N, 6·0%), $\nu_{max.}$ (Nujol) 1550, 1655, 1695, and 1730 cm^{-1} (C=O), τ ($CDCl_3$) 9·03 (t) and *ca.* 7·5 (m) (Et) 8·12, 8·04, 7·78, and 7·71 (4 × Me), 7·2—7·7 (m), 6·42, and 6·38 ($[CH_2]_2{\cdot}CO_2Me$), 6·23 and 6·19 (2 × CH_2), 2·70, 2·64, 4·95, and 4·70 (2 × $PhCH_2$), and 0·4, 0·65, 0·85, and 1·35 (4 × NH).

The formamide layer from the reaction was poured into water (*ca.* 1·0 l) and set aside for 24 h; a black viscous oil separated. After decantation of the aqueous phase the oil was taken up in methylene chloride (100 ml), washed thoroughly with water, and dried ($MgSO_4$). Removal of the solvent under reduced pressure followed by reconcentration of the residue with dry peroxide-free ether (50 ml) afforded a dark brown foam, which was redissolved in dry ether (50 ml) and set aside for 48 h under nitrogen. A crystalline product (0·8 g) was filtered off and washed with a small volume of ice-cold ether to give prisms, m.p. 119—121°, identical (i.r. spectrum and mixed m.p.) with the main crop of *a*-oxobilane (total yield 32%).

1′,8′-*Bisbenzyloxycarbonyl*-2,4,6,7-*tetrakis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-a-*oxobilane* (14b).—This was prepared in the same manner from the appropriate chloromethylpyrroketone. The product (3·2 g, 35%) crystallised from methylene chloride–ether–light petroleum (b.p. 40—60°) as yellow *prisms*, m.p. 123—124° (Found: C, 66·8; H, 6·4; N, 5·7. $C_{55}H_{62}N_4O_{13}$ requires C, 66·9; H, 6·3; N, 5·7%), $\nu_{max.}$ (Nujol) 1550, 1645, 1690, and 1730 cm^{-1} (C=O) τ ($CDCl_3$) 8·05, 8·03, 7·75, and 7·69 (4 × Me), 7·0—7·7 (m), 6·41, 6·37, 6·36, and 6·35 ($[CH_2]_2{\cdot}CO_2Me$), 2·59, 2·65, 4·66, and 4·84 ($PhCH_2$), and *ca.* 1·0br (NH).

1′,8′-*Bisbenzyloxycarbonyl*-1,4,6,7-*tetrakis*-(2-*methoxycarbonylethyl*)-2,3,5,8-*tetramethyl*-a-*oxobilane* (14c).—This was prepared similarly. The product (38%) crystallised from methylene chloride–ether–light petroleum (b.p. 40—60°) as yellow prisms, m.p. 121—122·5° (Found: C, 66·9; H, 6·6; N, 5·7. $C_{55}H_{62}N_4O_{13}$ requires C, 66·9; H, 6·3; N, 5·7%), $\nu_{max.}$ (Nujol) 1560, 1660, 1710, and 1735 cm^{-1} (C=O), τ ($CDCl_3$) 8·08, 8·04, 8·04, and 7·80 (4 × Me), 7·0—7·3 (m), 6·44, and 6·42 ($[CH_2]_2{\cdot}CO_2Me$), 6·26 and 6·20 (2 × CH_2), 2·67, 2·75, 4·75, and 5·0 (2 × $PhCH_2$), and 0·3—0·5 (NH).

Porphyrins

Coproporphyrin-I *Tetramethyl Ester* (1).—(*a*) 5-Bromo-3,4′-bis-(2-carboxyethyl)-3′,4,5′-trimethylpyrromethene hydrobromide (1·0 g) was ground intimately with succinic acid (5·0 g) and dried overnight (NaOH pellets) *in vacuo*. The mixture was fused by heating in an oil-bath at 200°, with frequent stirring to ensure that the subliming succinic acid re-entered the melt. After 1 h the melt was extracted with hot aqueous alkali, and filtered from a small amount of insoluble material. (The ' spectroscopic ' yield of porphyrin at this stage was about 35%.)

The alkaline extract was cooled in ice and acetic acid was added until pH 5·5 had been attained. A dark brown solid precipitated which was separated from the supernatant by centrifuging and dried overnight at 100° *in vacuo*. After esterification with methanolic hydrogen chloride (120 ml) for 12 h the methanolic solution was evaporated to dryness under reduced pressure. The residue was taken up in a little methylene chloride and filtered through alumina (Woelm activity III), and the porphyrin fraction was evaporated to dryness (yield 224 mg, 33%). Rechromatography on alumina (Woelm activity III) in benzene–chloroform (70 : 30 v/v) gave a red solid (126 mg) which was recrystallised from chloroform–methanol to give the porphyrin (110 mg, 16%) as purple needles, m.p. 252—255° (lit.,[24] 248—252°). After hydrolysis (6M-HCl) paper chromatography in lutidine–aqueous ammonia showed only a single spot with an R_F value consistent with that for coproporphyrin-I.

During the chromatographic purification in benzene–chloroform it was observed that a quantity of porphyrinic material was retained at the top of the alumina column. The porphryrin was removed from the column by elution with methanol and the eluate evaporated to dryness under reduced pressure to give a red residue (38 mg). Paper chromatography indicated that this material was coproporphyrin-I free acid.

(*b*) 5-Bromo-5′-bromomethyl-3,4′-bis-(2-ethoxycarbonylethyl)-3′,4-dimethylpyrromethene hydrobromide (1·0 g) was ground intimately with succinic acid (5·0 g) and dried (NaOH pellets) *in vacuo* overnight. The fusion mixture was heated in an oil-bath at 200° with frequent stirring to ensure that subliming succinic acid re-entered the melt, and after 1 h the melt was extracted with hot aqueous alkali, any soluble residue being removed by filtration (' spectrophotometric ' yield 7%).

The porphyrinic product was isolated as in (*a*) and coproporphyrin-I tetramethyl ester (40 mg, 5%) was obtained as purple needles, m.p. 252—254°, identical with the previous sample (mixed m.p., i.r. spectrum, and paper chromatography of the free acid).

Coproporphyrin-II Tetramethyl Ester (2).—(*a*) (with P. Burbidge) Dibenzyl 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarboxylate (3 g) was converted into the corresponding 5,5′-dicarboxylic acid by catalytic hydrogenation and the residue, after filtration and evaporation, was heated on a steam bath with formic acid (98%; 10 ml) and hydrobromic acid–glacial acetic acid (48%; 5 ml) for 1 h. The solution was evaporated to dryness and the residue esterified with methanolic hydrogen chloride overnight. The methanolic solution was poured into water, neutralised with ammonia, and extracted with chloroform. The extracts were washed with water, dried ($MgSO_4$), evaporated to low bulk, and chromatographed on alumina in chloroform. The porphyrin fraction was re-chromatographed on alumina in chloroform–benzene (30 : 70 v/v) and the coproporphyrin-II tetramethyl ester (135 mg, 8%) crystallised as purple needles, m.p. 282—284° (lit.,[25] 287°).

(*b*) 3,3′-Bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarboxylic acid (550 mg) was treated with M-trichloroacetic acid in methylene chloride (72 ml) and trimethylorthoformate (2·5 g) in methylene chloride (408 ml). The solution was stirred overnight at 20° in the dark, under oxygen, during which period the original absorption at 285 nm decayed, and a broad absorption at 505 nm due to methene formation first increased and then also decayed as porphyrin salt ($\lambda_{max.}$ 410, 550, and 595 nm) was formed. The solution was then washed with sodium carbonate solution (10%; 200 ml) and water (2 × 200 ml), dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on alumina (grade III) in methylene chloride, and the porphyrinic eluates were evaporated to give

[24] Ref. 7, p. 483.
[25] Ref. 7, p. 487.

coproporphyrin-II tetramethyl ester (104 mg, 24%) as a violet solid. Recrystallisation from methylene chloride–methanol gave purple plates (63 mg), m.p. 285—288°.

(*c*) 5,5′-Diformyl-3,3′-bis(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane (110 mg) and 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarboxylic acid (120 mg) in glacial acetic acid (100 ml) were treated with 56% hydriodic acid (1 ml) in glacial acetic acid (40 ml), and the mixture was kept at 20° in the dark for 3 h. Anhydrous sodium acetate (2·8 g) in glacial acetic acid (40 ml) was then added and the mixture was aerated overnight. It was then diluted with water (200 ml) and cooled in ice, and dilute sodium carbonate solution was added until pH 5 was attained. The porphyrin was extracted with methylene chloride (4 × 40 ml); the combined extracts were washed with water, dried ($MgSO_4$), and evaporated. The residue was chromatographed twice on alumina (activity III) in methylene chloride, and the violet eluates were concentrated to give the porphyrin as a purple solid. Recrystallisation from methylene chloride–methanol gave the coproporphyrin-II tetramethyl ester (88 mg, 42%) as violet needles, m.p. 286—289°.

(d) 5′-Formyl-3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5-carboxylic acid (1·0 g) in glacial acetic acid (500 ml) was combined with 56% hydriodic acid (4 ml) in glacial acetic acid (200 ml), and the mixture was kept at 20° in the dark for 3 h. The product was then worked up as in (*c*) and after chromatography on alumina (activity III) in methylene chloride (twice) the porphyrinic eluate was concentrated to give a purple solid (189 mg, 22%). Crystallisation from methylene chloride–methanol afforded the porphyrin (120 mg) as purple plates, m.p. 286—288°, identical with that prepared in (*a*).

(e) 3,3′-Bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarboxylic acid (2·12 g) was decarboxylated under nitrogen by heating at 195—200° in an oil-bath until evolution of carbon dioxide had ceased (*ca.* 1 h). The resulting red-brown oil was heated on a steam-bath with formic acid (5 ml) and hydrobromic acid–glacial acetic acid solution (5 ml) (0·3 ml of 45% hydrobromic acid solution diluted to 10 ml with glacial acetic acid) for 1 h under nitrogen and then for 1 h in oxygen. The solution was evaporated to dryness; the residue was dried at 100° overnight under reduced pressure and then esterified with methanolic hydrogen chloride (120 ml) for 12 h. The product was worked up as in the other coproporphyrin preparations and gave a mixture of coproporphyrin tetramethyl esters (420 mg), as violet needles, m.p. 140—200°. The n.m.r. spectrum showed that this product was a mixture, and this was confirmed by paper chromatography of the free acids in lutidine–aqueous ammonia (*cf.* ref. 20).

Coproporphyrin-III *Tetramethyl Ester* (3).—1′,8′-Bis-benzyloxycarbonyl-2,4,6,7-tetrakis-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-*a*-oxobilane (1·18 g, 1·2 mmol) in dry tetrahydrofuran (35·2 ml) and dry ethyl acetate (35·2 ml) was reduced with diborane [generated from sodium borohydride (0·58 g) in bis-(2-methoxyethyl) ether (21 ml) and boron trifluoride ether complex (5·8 ml) in bis-(2-methoxyethyl) ether (16 ml)] during 5 min in an apparatus similar to that described previously. The diborane was swept into the reaction vessel by a slow stream of nitrogen, and initially the solution darkened due to the formation of a small amount of bilene borane complex (which is presumably an intermediate in the reduction of the ketone group). After *ca.* 15 min the colour of the solution began to lighten and after 85 min the absorption (at $\lambda_{max.}$ 360 nm) due to the pyrroketone system had disappeared.

The resulting colourless solution was evaporated to dryness under reduced pressure (nitrogen), treated with methanol (26·0 ml), and set aside under nitrogen until the oil had dissolved. Tetrahydrofuran (26·0 ml) was then added together with 10% palladium–charcoal (520 mg) and a solution of triethylamine in tetrahydrofuran (3%; 8 drops). The solution was then hydrogenated at atmospheric pressure and room temperature for 17 h; theoretical uptake was achieved. As a check on the completeness of hydrogenation a sample was removed from the flask (by means of a hypodermic syringe inserted through a serum cap in a side-neck) and diluted to a concentration of 50 μmol l^{-1} with ethanol; the rapid disappearance of the absorption (at 285 nm) due to the terminal pyrrolecarboxylic acid groups, on addition of a few drops of concentrated hydrochloric acid to the sample cell, then confirmed that the benzyl groups had been removed. The main solution from the hydrogenation was filtered through Hiflosupercel under nitrogen and then concentrated under reduced pressure. It was then reconcentrated with dry peroxide-free ether to remove traces of tetrahydrofuran and methanol, yielding the intermediate bilane diacid as a buff coloured gummy solid, which was used immediately in the next reaction.

This bilane diacid was taken up in tetrahydrofuran (140 ml) and the solution diluted to 280 ml with dry peroxide-free ether. The reaction vessel was flushed with nitrogen and a slow stream of nitrogen was passed while the solution was cooled to −15°. t-Butyl hypochlorite (0·144 ml) in dry peroxide-free ether (50 ml) cooled to 0° was then added during 60 min in the dark. A starch–iodide test gave a negative result 15 min after the end of the addition, and the bilene suspension formed was allowed to warm to room temperature. The suspension was evaporated to dryness, and the residue triturated with dry ether to give the purple bilene, [$\lambda_{max.}$ (CH_2Cl_2) 505 nm].

The bilene (assumed 1200 μmol), dissolved in methylene chloride (240 ml) containing trimethyl orthoformate (2·56 ml), was added to dry trichloroacetic acid (12·3 g) in methylene chloride (240 ml); the resulting mixture was stirred overnight under oxygen in the dark. The amounts of reactants (per ml of reaction mixture) were 2·5 μmol bilene, 50 μmol trimethyl orthoformate, and 150 μmol trichloroacetic acid. The methylene chloride solution was washed with N-sodium carbonate solution (4 × 100 ml) and then with water (5 × 100 ml), dried ($MgSO_4$), and evaporated. The residue was taken up in a little methylene chloride, filtered through alumina (Woelm activity III) to remove methene-like by-products, and finally chromatographed on alumina (Woelm activity III) in benzene–methylene chloride (1 : 1). The porphyrinic eluate gave a purple solid (262 mg) which was triturated with ether; the mixture was filtered to give coproporphyrin-III tetramethyl ester (198 mg, 23%). Crystallisation from methylene chloride–methanol (twice) gave the porphyrin (169 mg) as purple needles, softening at 150—155° and melting at 179—182° (lit.,[2] 153—155° and 178—182°; natural material, 153—156° and 178—182°) (Found: C, 67·3; H, 6·5; N, 7·8. Calc. for $C_{40}H_{46}N_4O_8$: C, 67·6; H, 6·5; N, 7·9%).

Coproporphyrin-IV *Tetramethyl Ester* (4).—The corresponding *a*-oxobilane dibenzyl ester (0·90 g) was carried through the same sequence of reactions as in the preparation of the isomeric coproporphyrin-III tetramethyl ester. A solution of the intermediate bilene (695 mg) in methylene

chloride (168 ml) containing trimethyl orthoformate (0·88 ml) was added to dry trichloroacetic acid (8·2 g) in methylene chloride (168 ml), and the resulting mixture was stirred overnight under oxygen in the dark. (Amounts of reactants per ml of reaction mixture were 2·5 μmol bilene, 25 μmol trimethyl orthoformate, and 150 μmol trichloroacetic acid.) After chromatography the porphyrinic eluate was concentrated to give a purple solid (220 mg), which on trituration with ether followed by filtration gave coproporphyrin-IV tetramethyl ester (158 mg, 24%). Crystallisation from methylene chloride–methanol–ether gave the porphyrin (131 mg) as purple needles, m.p. 168—170 and 183—185° (lit.,[2] 183—184° and 182—184°) (Found: C, 67·5; H, 6·3; N, 8·2. Calc. for $C_{40}H_{46}N_4O_8$: C, 67·6; H, 6·5; N, 7·9%).

2-*Ethyl*-4,6,7-*tris*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin*.—The corresponding *a*-oxobilane dibenzyl ester (1·12 g, 1·2 mmol) was carried through the same sequence of reactions as in the preparation of the foregoing coproporphyrin-III tetramethyl ester. In the preparation of the intermediate bilene the bilane diacid was taken up in tetrahydrofuran (60 ml) and diluted with dry peroxide-free ether (288 ml). This stirred solution was cooled to −25° and t-butyl hypochlorite (0·144 ml) in dry peroxide-free ether (80 ml) cooled to 0° was added during 2 h in the dark. Cyclisation under the standard conditions afforded the *porphyrin* (181 mg, 23%), which crystallised from methylene chloride–methanol as purple needles, m.p. 231—233° (Found: C, 70·3; H, 6·8; N, 8·6. $C_{38}H_{44}N_4O_6$ requires C, 69·9; H, 6·8; N, 8·6%), τ ($CDCl_3$; 0·15M), 0·85, 0·87, and 0·09 (2H) (4 × *meso*-H), 6·04 (m), 7·02 (m), and 6·61 ($[CH_2]_2{\cdot}CO_2Me$), 6·73 and 6·80 (9H) (4 × Me), *ca.* 6·0 (m) and 8·37 (t) (Et), and 13·85 (NH).

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