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SYNTHESIS OF BILIVERDIN IXγ (PTEROBILIN)[1]

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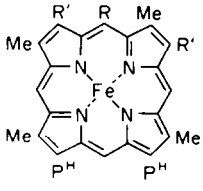
Abstract—Condensation of a bis(acetoxyethyl)pyrromethane dicarboxylic acid (**11d**) with a diformylpyrroketone (**12b**) afforded a bis(acetoxyethyl)-γ-*meso*-hydroxyporphyrin (**13d**) which was converted into the related bis(chloroethyl)-γ-benzoyloxyporphyrin (**14f**). The Zn complex of the latter was transformed by brief treatment with base, followed by chloromethylethyl ether into the zinc bis(chloroethyl)-γ-ethoxymethoxyporphyrin (**20b**). Dehydrochlorination with potassium t-butoxide in t-butanol, and acid catalysed demetallation and deprotection then afforded the somewhat unstable blue γ-oxyprotoporphyrin dimethyl ester (**21**). The Fe-complex of the latter readily underwent oxidative ring opening by aerial oxidation in pyridine, and after demetallation gave biliverdin IXγ (pterobilin) dimethyl ester (**22**).

THE vast majority of naturally occurring bile pigments are derived by oxidative ring opening of haem (**1**) at the α-position.[2,3] In mammals they appear to be largely waste products arising from the oxidative breakdown of blood haemoglobin, and haem enzymes, but in plants they may play an important physiological role, e.g. as photosynthetic receptors (in red and blue-green algae) or as growth regulators (e.g. phytochrome).[4] Both the mammalian and plant bile pigments are thought to be formed by *meso*-oxidation of haem (by haem oxygenase) followed by oxidative ring-opening of the intermediate α-oxyhaem (**2**), and demetallation, to form biliverdin IXα (**L4**, the green pigment of bile, and of birds' eggs).[2] The latter is subsequently reduced to bilirubin IXα (**L6**) by biliverdin reductase,[3] and a further series of bacterial reductions in the gut then gives rise to the urobilinoid pigments found in faeces.[5] In plants alternative reductive transformations occur with the formation of the plant bile pigments such as phycocyanobilin, phycoerythrobilin and phytochrome.



1 R = H, R' = CH=CH$_2$
2 R = OH, R' = CH=CH$_2$
3 R = OH, R' = Et

P^H = $CH_2CH_2CO_2H$

Unequivocal evidence for the origin of the mammalian, avian and plant bile pigments has been obtained from metabolic studies with labelled haem precursors, as well as with haem itself.[2,3,6] However, the precise mechanism of the ring-opening process is still not entirely clear. Evidence for the intermediacy of an α-oxyhaem (**2**) was originally based on analogies with processes occurring in the *in vitro* chemical oxidations of haem (with peroxide, or oxygen in pyridine) to *meso*-oxyhaems, followed by oxidative ring-opening and demetallation to bile pigments.[2,3] Subsequently we provided indirect evidence for this pathway by showing that the enzymic conversion of α-oxy-mesohaem (**3**) into mesobilirubin IXα (**L7**) occurred *in vivo*.[7] The oxidative ring-opening of haem into biliverdin appears to proceed by the same mechanism, whether in plants or in mammals, or even if carried out by purely chemical means; experiments with mixtures of oxygen-16 and oxygen-18 show that in both the enzymic and chemical processes two separate molecules of oxygen are needed for the ring opening (in addition to that required for the initial *meso*-oxidation).[8]

The reasons for the high degree of specificity of the position of ring-opening in the biological oxidations are as yet unknown,[cf 2,3] and the chemical oxidation of haem affords a mixture of all four isomeric biliverdins. For this reason the discovery of a new family of blue bile pigments based on biliverdin IXγ (**22**) in the Lepidoptera has excited considerable interest.[9] The main butterfly pigment is biliverdin IXγ and the other pigments such as neopterobilin, phorcabilin and sarpedobilin are presumably derived from it by ring closures of the side-chain vinyl groups onto the neighbouring pyrrole rings. Biosynthetic studies in the caterpillar of *Actias selene* (*Attaccidae*) have clearly demonstrated that aminolaevulinic acid and protoporphyrin IX (**L1**) are precursors of phorcabilin, but the intermediacy of haem itself has not yet been proven.[10] It is of course tempting to suggest that the mechanism of the ring-opening to biliverdin IXγ parallels that to the mammalian and plant bile pigments, the only difference being the specificity of the initial *meso*-oxidation process. In order to study this possibility in more detail we have now synthesised γ-oxyprotoporphyrin and transformed it into biliverdin IXγ as essential preliminaries to biosynthetic studies.[11]

The plan was to prepare a symmetrical diformylpyrroketone corresponding to rings C and D, and to condense this with a suitably substituted dipyrromethane corresponding to rings A and B of the oxyporphyrin.[12] This approach is similar to the MacDonald porphyrin synthesis[13,14] which utilises a diformylpyrromethane and an α-free pyrromethane (or the corresponding dicarboxylic acid). As in our earlier syntheses of vinyl substituted porphyrins[15] it was decided to generate the vinyl groups from chloroethyl side-chains at a late stage in the synthesis. It was also necessary to protect the *meso*-oxygen substituent, and, as will be seen in the sequel, it became necessary

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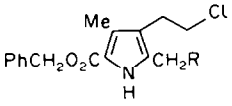
to change from a base-labile, to an acid-labile, protecting group at a late stage.

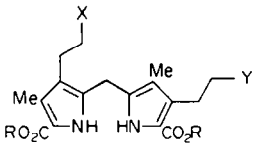


4 a R = H
b R = OAc

The pyrrole (**4a**) required for the A-ring of the oxyporphyrin was prepared by well-established methods, but the pyrrole (**9c**) corresponding to the B-ring required the development of a new synthetic route as indicated in Scheme 1. The individual steps in this pathway generally gave good yields, and the introduction of the β-Me group by amino-alkylation followed by catalytic hydrogenation (**5** → **6** → **7**) paralleled a method previously used in earlier studies of a different pyrrole.[16]

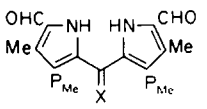
The pyrrole (**4a**) was converted into the acetoxymethyl analogue (**4b**) by treatment with lead tetraacetate, and coupled with the α-free pyrrole (**9c**) by heating in methanol containing a catalytic amount of *p*-toluenesulphonic acid.[17] The product formed in 75% yield, however, was the hydroxyethylpyrromethane (**10a**) rather than the expected acetoxyethylpyrromethane (**10b**), and evidently acid-catalysed methanolysis of the acetoxyethyl group had occurred. Although the hydroxyethyl side-chain could easily be converted into the acetoxyethyl side-chain by treatment with acetic anhydride in pyridine, it was found that the methanolysis could be avoided by the use of acetic acid/sodium acetate in the coupling reaction, although the yield was lower.

The diformylpyrroketone (**12b**) required for the C and D rings of the oxyporphyrin was synthesised from the well-known symmetrical diformylpyrromethane[18] (**12a**) in moderate yield by oxidation with sulphuryl chloride in acetic acid.[19] Attempts to improve the yield by the use of other oxidising agents, e.g. selenium dioxide, manganese dioxide, lead tetraacetate and chromyl chloride, were all unsatisfactory; only with selenium dioxide were appreciable amounts of the



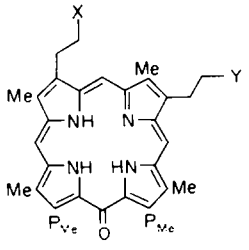
(a) X = Cl, Y = OH
(b) X = Cl, Y = OAc
(c) X = Y = OH
(d) X = Y = OAc

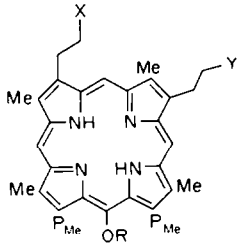
10 R = CH_2Ph **11** R = H



P^{Me} = $CH_2CH_2CO_2Me$

12 (a) X = H_2
(b) X = O



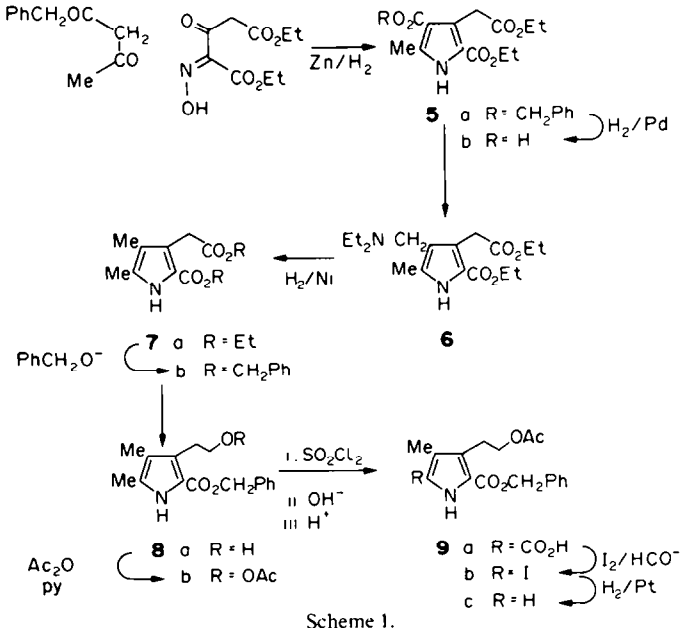


13 (a) X = Cl, Y = OAc
(b) X = Y = Cl
(c) X = Y = OH
(d) X = Y = OAc

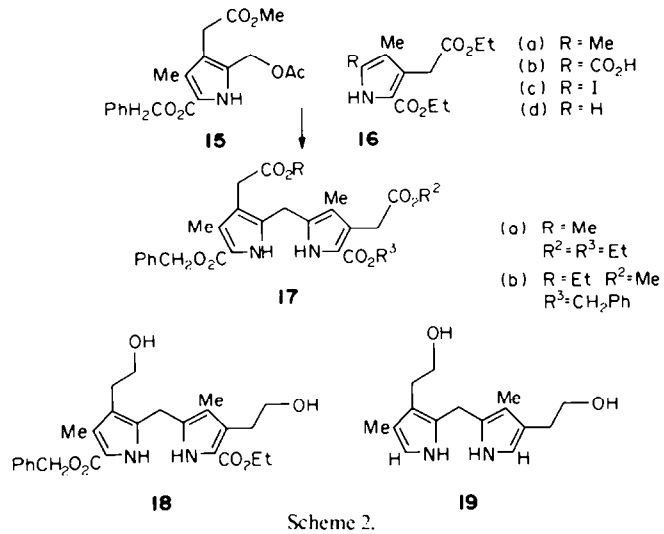
14 (a) X = Cl, Y = OAc, R = Ac
(b) X = Cl, Y = OAc, R = Mes
(c) X = Y = Cl, R = Mes
(d) X = Y = OAc, R = COPh
(e) X = Y = OH, R = COPh
(f) X = Y = Cl, R = COPh

pyrroketone formed, but it was difficult to remove selenium by-products, and moreover, some over-oxidation to a monoformyl-monocarboxylic acid also occurred.

The pyrromethane dicarboxylic acid (**11b**), prepared by catalytic hydrogenolysis of the dibenzyl ester (**10b**) was then coupled with the diformylpyrroketone (**12b**) in methylene chloride containing a catalytic amount of methanesulphonic acid. Visible spectro-



Scheme 1.



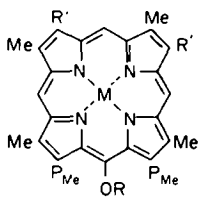
Scheme 2.

scopic studies showed that the formation of oxyporphyrin (**13a**) was essentially complete in under 30 min and it was isolated as the *meso*-acetoxy-derivative (**14a**) after treatment with acetic anhydride and pyridine. In subsequent experiments derivatisation was carried out with methanesulphonyl chloride in pyridine but two products were obtained, the monochloroethylmonoacetoxyethylmesyloxyporphyrin (**14b**) and the bis(chloroethyl)mesyloxyporphyrin (**14c**). The latter was presumably formed by partial hydrolysis of the acetoxyethyl group during the cyclisation process, and the resulting hydroxyethyl group had been transformed into chloroethyl by the mesyl chloride. The monoacetoxyethylporphyrin (**14b**) was also converted into the bis(chloroethyl) mesyloxyporphyrin (**14c**) by acid-catalysed methanolysis to the hydroxyethyl analogue, followed by treatment with methanesulphonyl chloride in pyridine, but the overall conversion (**11b** + **12b** → **14c**) was inefficient, and two alternative syntheses were devised.

Firstly the pyrromethane (**19**) was synthesised by the route shown in Scheme 2 from the α-free pyrrole (**16d**) and the readily available acetoxymethylpyrrole (**15**). The initial pyrromethane (**17a**) obtained in this way was then reduced with diborane[20] to afford the bis(hydroxyethyl)pyrromethane (**18**), and this was subsequently hydrolysed and decarboxylated by heating with dilute sodium hydroxide (containing a trace of hydrazine as antioxidant) in a sealed tube.[cf 13] The resulting di-α-free pyrromethane (**19**) was then coupled with the diformylpyrroketone (**12b**) in methylene chloride containing a catalytic amount of methanesulphonic acid. The resulting bis(hydroxyethyl)oxyporphyrin (**13c**) was converted directly into the bis(chloroethyl)mesyloxyporphyrin (**14c**) by treatment with methanesulphonyl chloride in pyridine. Subsequently the bis(acetoxyethyl)pyrromethane (**10d**) became available as an intermediate in other studies,[21] and this was converted into the di-acid (**11d**) by hydrogenolysis and coupled with the diformylpyrroketone (**12b**) in presence of trifluoroacetic acid to form the bis(acetoxyethyl)oxyporphyrin (**13d**). The latter was then converted into the *meso*-benzoyloxy derivative (**14d**) by treatment with benzoyl chloride in pyridine, in preference to the *meso*-mesyloxyderivative, for reasons which will become apparent below. Acid-catalysed methanolysis of the acetoxyethyl groups then gave the bis(hydroxyethyl)-*meso*-benzoyloxyporphyrin (**14e**) which was transformed directly into the bis(chloroethyl) analogue (**14f**) by treatment with thionyl chloride in dimethyl formamide or methanesulphonyl chloride in pyridine.

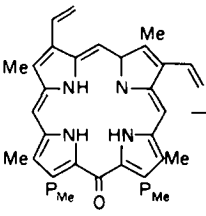
The next stage in the synthesis was to generate the vinyl groups of the desired oxyporphyrin by elimination of HCl from the chloroethyl groups of the protected oxyporphyrins. However, all attempts to carry out this process with the mesyloxyporphyrin (**14c**) using a variety of bases (e.g. potassium t-butoxide, lithium 2,2,6,6-tetramethylpiperidine, and 1,8-bis(dimethylamino)-naphthalene) in dry, or aprotic, solvents, and using either the porphyrin free base, or better, the zinc complex, were all unsuccessful owing to the extreme ability of the mesyloxy group; it was evident from the blue colour which rapidly developed in most experiments that oxophlorin was being formed, and that this was then undergoing decomposition as the starting material was rarely recovered. Unfortunately the corresponding bis(chloroethyl)-*meso*-benzoyloxyporphyrin (**14f**) also proved to be unstable when a similar series of attempts were made to convert it into the divinyl analogue. We, therefore, concluded that it would be necessary to replace the base-labile mesyl, or benzoyl, groups by an alternative protecting group which could be removed under mild acidic conditions.

With our earlier experiences of oxophlorins (oxyporphyrins) in mind we carried out preliminary studies of the use of tetrahydropyranyl, and benzyl groups but neither proved satisfactory. In the event we treated the zinc complex (**20a**) of the oxyporphyrin (**13b**) (obtained by brief alkaline hydrolysis of the *meso*-benzoyloxyporphyrin (**14f**) with 1,8-bis(dimethylamino)-naphthalene ("proton sponge") and the blue-green zinc oxophlorin anion formed was then alkylated with chloromethylethyl ether to form the zinc *meso*-ethoxymethoxyporphyrin (**20b**). (The use of pyridine as a base in this reaction was unsatisfactory, partly owing to reaction with the chloromethyl ether.) Dehydrochlorination of the zinc complex (**20b**) was then carried out by use of potassium t-butoxide in t-butanol at 20° for three days, and the desired zinc divinyl-*meso*-ethoxymethoxyporphyrin (**20c**) was



(a) M = Zn, R' = CH_2CH_2Cl, R = H
(b) M = Zn, R' = CH_2CH_2Cl, R = CH_2OEt
(c) M = Zn, R' = $CH{=}CH_2$, R = CH_2OEt
(d) M = $Fe^{III}Cl$, R' = $CH{=}CH_2$, R = H

20



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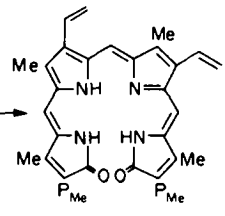

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obtained in moderate yield. The protecting group was cleaved, and the zinc was removed, by brief treatment with boron trifluoride in methanol, and the blue γ-oxyprotoporphyrin dimethyl ester (**21**) was obtained after chromatographic purification. Owing to its relative instability the latter was converted directly into the iron complex (**20d**) and oxidised by air in pyridine solution.[cf 2,21] The green verdohaemochrome formed was then treated successively with potassium hydroxide in methanol, and boron trifluoride in methanol, to remove the iron, and after work-up and chromatography on alumina, the blue fraction finally obtained was purified by thick layer chromatography on silica gel. Several blue bands were obtained but the major fraction on elution from the plate, and crystallisation, afforded biliverdin IXγ dimethyl ester (**22**) in 20% overall yield from the Zn complex (**20c**). The other minor products which had the same general visible spectral characteristics as the biliverdin IXγ were thought to be intramolecular cyclisation products related (or identical) to phorcabilin, sarpedobilin etc.[cf 9] The biliverdin IXγ dimethyl ester proved to be identical in all respects (m.p., spectra etc) with material obtained by the direct oxidation of haem, and the separation of the four isomeric biliverdins.[22,23]

In a series of related experiments we have now also prepared the γ-oxyprotoporphyrin dimethyl ester (**21**) by direct oxidation of protoporphyrin IX dimethyl ester and HPLC separation of the four isomeric oxyprotoporphyrins formed.[24] Biosynthetic experiments are now in progress.

EXPERIMENTAL

M.p.s were determined on a hot stage apparatus. NMR spectra were determined with a Perkin–Elmer R32 (90 MHz) instrument, and mass spectra with a Varian CH5 double focusing instrument (both for e.i. and f.d. spectra). Reactions were monitored wherever possible by TLC on silica gel, and by UV-visible spectroscopy. Column chromatography was carried out on alumina (Brockmann Grade III). Drying agent: Na_2SO_4.

Pyrroles

Ethyl 4-diethylaminomethyl-3-(ethoxycarbonylmethyl)-5-methylpyrrole-2-carboxylate (**6**). **5b**[24] (7.98 g, 30 mmol) was taken up in abs alcohol (21.6 ml) aqueous formaldehyde soln (3.6 ml; 37–41% w/v), and Et_2NH (5.9 ml) and the mixture was boiled under reflux for 5 hr. The resulting yellow soln was slowly poured with continuous stirring into water (150 ml). Initially a yellow oil separated which solidified on cooling. This was filtered off and the filtrate was acidified with dil HCl to pH 2, to recover a small amount of unchanged acid. The Mannich base was recrystallised from aqueous EtOH to give plate-like crystals (6.63 g, 79%), m.p. 54–55° (Found: C, 62.85; H, 8.9; N, 8.5. $C_{17}H_{28}N_2O_4$ requires: C, 62.9; H, 8.7; N, 8.6%). λ_{max} ($CHCl_3$) 282 nm (ε 16,000). τ ($CDCl_3$) 9.01 (6H, t, J = 7 Hz, N ($CH_2C\underline{H}_3)_2$), 8.69 (6H, q, J = 7 Hz, $OCH_2C\underline{H}_3$), 7.75 (3H, s, 5-CH_3), 7.59 (4H, q, J = 7 Hz, N ($C\underline{H}_2CH_3)_2$), 6.68 (2H, s, $C\underline{H}_2N$), 6.04 (2H, s, $C\underline{H}_2CO_2CH_2CH_3$), 5.77 (4H, m, J = 7 Hz, $OC\underline{H}_2CH_3$), and 0.88 (1H, s, N$\underline{H}$). *m/e* 324 (M^+, 22%), 252 ($M^+ - NEt_2$, 55%) and 74 (100%).

Ethyl 3-(ethoxycarbonylmethyl)-4,5-dimethylpyrrole-2-carboxylate (**7a**). **6** (4.0 g, 10 mmol) was dissolved in abs EtOH (50 ml) and treated with Raney Ni (W_6; 2 ml). The mixture was hydrogenated (100 atm) at 140° overnight. The resulting mixture was filtered through Celite. The Celite was washed with $CHCl_3$ and the combined filtrates were evaporated to dryness. The residue was recrystallised from aqueous EtOH to give needle-like crystals of the desired pyrrole (2.29 g, 73%), m.p. 106–108° (Found: C, 61.4; H, 7.8; N, 5.8. $C_{13}H_{19}O_4N$ requires: C, 61.6; H, 7.6; N, 5.5%). λ_{max} ($CHCl_3$) 283 nm (ε 16,000). τ ($CDCl_3$) 8.75 (3H, t, J = 7 Hz, $CH_2CO_2CH_2C\underline{H}_3$), 8.69 (3H, t, J = 7 Hz, $CO_2CH_2C\underline{H}_3$), 8.09 (3H, s, 4-CH_3), 7.81 (3H, s, 5-CH_3), 6.20 (2H, s, $C\underline{H}_2CO_2CH_2CH_3$), 5.78 (4H, m, J = 7 Hz, OCH_2CH_3), and 0.83 (1H, s, NH). *m/e* 253 (M^+, 86%), and 180 ($M^+ - CO_2CH_2CH_3$, 100%).

Benzyl 3-(benzyloxycarbonylmethyl)-4,5-dimethylpyrrole-2-carboxylate (**7b**). Na pellets (0.23 g, 10 mmol) were dissolved in benzyl alcohol (20 ml) and the soln added to a soln of **7a** (4.73 g, 20 mmol) in benzyl alcohol (20 ml). The resulting mixture was heated under reduced pressure (20 mm Hg) at 100° for 2.5 hr. The benzyl alcohol was removed by distillation under reduced pressure (b.p. 82° at 0.55 mm Hg). The residual yellow oil was dissolved in abs EtOH (40 ml) and a few small pieces of solid CO_2 were added. The soln was treated with water and the solid which crystallised was recrystallised from aqueous EtOH to give dibenzyl ester (5.18 g, 74%) m.p. 83–84° (Found: C, 72.85; H, 6.2; N, 3.8. $C_{23}H_{23}NO_4$ requires: C, 73.2; H, 6.1; N, 3.7%). λ_{max} ($CHCl_3$) 285 nm (ε 17,000). τ ($CDCl_3$) 8.12 (3H, s, 4-CH_3), 7.86 (3H, s, 5-CH_3), 6.14 (2H, s, $C\underline{H}_2CO_2CH_2Ph$), 4.94 (2H, s, $CH_2CO_2C\underline{H}_2Ph$), 4.78 (2H, s, $CO_2C\underline{H}_2Ph$), 2.71 (10H, s, 2 × Ph), and 1.05 (1H, s, N$\underline{H}$). *m/e* 377 (M^+, 18%), 286 ($M^+ - CH_2C_6H_5$, 9%) and 91 ($C_7H_7^+$, 100%).

Benzyl 3-(2-hydroxyethyl)-4,5-dimethylpyrrole-2-carboxylate (**8a**). Diborane gas was generated externally by addition of

BF_3 diethyl etherate (16.0 ml, 0.13 mol) to a vigorously stirred suspension of $NaBH_4$ (3.15 g, 0.1 mol) in diglyme (6.5 ml). The diborane was passed during 3 hr in a slow stream of dry N_2, into a soln of **7b** (2.56 g, 10 mmol) in dry THF (12.5 ml). Abs MeOH (10 ml) was then carefully added dropwise with stirring until the vigorous effervescence had ceased. The soln was evaporated to dryness under reduced pressure, and the resulting yellow oil crystallised from benzene-light petroleum (b.p. 60–80°) to give the hydroxyethylpyrrole (1.39 g, 75%) as needles, m.p. 64–66° (Found: C, 70.2; H, 7.3; N, 5.0. $C_{16}H_{19}NO_3$ requires: C, 70.3; H, 7.0; N, 5.1%). λ_{max} ($CHCl_3$) 284 nm (ε 17,300). τ ($CDCl_3$) 8.09 (3H, s, 4-CH_3), 7.87 (3H, s, 5-CH_3), 7.01 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OH$), 6.28 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OH$), 4.74 (2H, s, $CO_2C\underline{H}_2Ph$), 2.64 (5H, s, Ph), and 0.98 (1H, s, NH). *m/e* 273 (M^+, 24%), 242 ($M^+ - CH_2OH$, 6%) and 91 ($C_7H_7^+$, 100%).

Benzyl 3-(2-*acetoxyethyl*)-4,5-*dimethylpyrrole*-2-*carboxylate* (**8b**). Ac_2O (1.4 g, 10 mmol) was added dropwise to a stirred soln of **8a** (1.38 g, 5 mmol) in dry pyridine (12.0 ml). The resulting soln was stirred at room temp for 4.5 hr before being added dropwise, with vigorous stirring, to ice-water (50 ml). The buff-coloured ppt was filtered off, washed with water and recrystallised from light petroleum (b.p. 60–80°) to give the acetoxyethylpyrrole (1.35 g, 85%) as needles, m.p. 84–85° (Found: C, 68.5; H, 6.6; N, 4.5. $C_{18}H_{21}NO_4$ requires: C, 68.6; H, 6.7; N, 4.4%). λ_{max} ($CHCl_3$) 284 nm (ε 18,400). τ ($CDCl_3$) 8.06 (3H, s, 4-CH_3), 8.02 (3H, s, $OCOCH_3$), 7.84 (3H, s, 5-CH_3), 6.95 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OCOCH_3$), 5.84 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OCOCH_3$), 4.71 (2H, s, $CO_2C\underline{H}_2Ph$), 2.63 (5H, s, CH_2Ph), and 0.91 (1H, s, NH). *m/e* 315 (M^-, 14%), 164 ($M^- - OCOCH_3 - CH_2C_6H_5 - H$, 53%) and 91 ($C_7H_7^+$, 100%).

5-*Benzyloxycarbonyl*-4-(2-*acetoxyethyl*)-3-*methylpyrrole*-2-*carboxylic acid* (**9a**). **8b** (0.2 g, 1 mmol) was dissolved in Na-dried ether (50 ml) and freshly distilled sulphuryl chloride (0.26 g, 2 mmol) was added dropwise with stirring. The soln was stirred at 20° for 3 days, and then evaporated to dryness under reduced pressure. The resulting orange coloured oil was dissolved in dioxan (2 ml) and the soln treated with a soln of $NaOAc \cdot 3H_2O$ (0.27 g, 2 mmol) in water (1.0 ml). The mixture was heated to 70° and maintained at this temp for 1.5 hr, and then stirred at room temp overnight. Ether (8.0 ml) was added to the yellow soln and the lower aqueous layer was separated. The organic extract was washed with Na_2CO_3aq (1 M; 3 × 5 ml). The aqueous extracts were combined and saturated with SO_2 gas. The white ppt which formed was filtered off, washed well with water and recrystallised from $CHCl_3$-light petroleum (b.p. 40–60°) to give the acid (0.11 g; 49%), m.p. 137–140° (Found: C, 62.4; H, 5.3; N, 4.3. $C_{18}H_{19}NO_6$ requires: C, 62.6; H, 5.5; N, 4.05%). λ_{max} ($CHCl_3$) 280 nm (ε 13,700) and 289 nm (infl.). τ (DMSO-d_6) 8.12 (3H, s, 3-CH_3), 7.82 (3H, s, $CH_2CH_2OCOC\underline{H}_3$), 7.06 (2H, t, J = 7 Hz, $CH_2CH_2OCOCH_3$), 5.99 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OCOCH_3$), 4.74 (2H, s, $C\underline{H}_2Ph$), and 2.63 (5H, m, $CH_2\underline{P}h$). *m/e* 345 (M^+, 3%) 194 ($M^+ - OCOCH_3 - H - CH_2C_6H_5$, 58%), and 91 ($C_7H_7^+$, 100%).

Benzyl 5-*iodo*-3-(2-*acetoxyethyl*)-4-*methylpyrrole*-2-*carboxylate* (**9b**). **9a** (0.2 g, 1 mmol) was dissolved in MeOH (2.6 ml), the soln was heated to 60–65° and then treated with a soln of $NaHCO_3$ (0.15 g, 2 mmol) in water (1.5 ml). Whilst maintaining the temp at 60–65°, a soln of I_2 (0.15 g, 1 mmol) and KI (0.24 g, 1 mmol) in water (2 ml), and MeOH (4 ml) was added dropwise with stirring during 1–1.5 hr. (Addition of one drop of I_2 soln caused the mixture to become yellow in colour. The next drop was not added until the soln had become colourless again.) When addition was complete the soln was maintained at 60–65° for a further hr, and then kept at 0° overnight. The crystalline produce was filtered off, washed well with hot water, dried under vacuum and then recrystallised from CH_2Cl_2-light petroleum (b.p. 40–60°) to give the iodopyrrole (0.2 g, 80%), m.p. 107.5–108.5° (Found: C, 48.1; H, 4.05; N, 3.7. $C_{17}H_{18}NO_4I$ requires: C, 47.8; H, 4.25; N, 3.3%). λ_{max} ($CHCl_3$) 280 nm (ε 17,000). τ ($CDCl_3$) 8.06 (3H, s, 3-CH_3), 8.04 (3H, s, $OCOCH_3$), 6.95 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OCOCH_3$), 5.88 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OCOCH_3$), 5.88 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OCOCH_3$). 4.71 (2H, s, $C\underline{H}_2Ph$), 2.65 (5H, s, $CH_2\underline{P}h$), and 0.60 (1H, s, NH). *m/e* 427 (M^+, 15%), 276 ($M^+ - OCOCH_3 - CH_2C_6H_5 - H$, 37%), and 91 ($C_7H_7^+$, 100%).

Benzyl 3-(2-*acetoxyethyl*)-4-*methylpyrrole*-2-*carboxylate* (**9c**). **9b** (0.40 g, 1 mmol) and Adams PtO_2 catalyst (0.018 g) were added to a soln of anhyd NaOAc (0.18 g, 2 mmol) in MeOH (20 ml). The mixture was hydrogenated at 20° and 760 mm until the uptake of H_2 had ceased (2 hr; *ca* 50 ml; 0.002 mol). The soln was filtered through Celite, and the combined filtrates, after washing the Celite with MeOH, were evaporated to dryness. The resulting yellow oil was dissolved in EtOAc (15 ml) and washed with water (15 ml). The organic extract was washed with Na_2CO_3aq (1M; 3 × 5 ml), water (3 × 5 ml) and then dried, filtered and evaporated to dryness. The yellow oil so obtained was induced to crystallise from benzene-light petroleum (b.p. 40–60°) to give the α-free-pyrrole (0.28 g, 99%) as plates, m.p. 96–97° (Found: C, 67.6; H, 6.2; N, 4.7. $C_{17}H_{19}NO_4$ requires: C, 67.8; H, 6.4; N, 4.65%). λ_{max} ($CHCl_3$) 274 nm (ε 15,000). τ ($CDCl_3$) 8.05 (3H, s, 4-CH_3), 7.99 (3H, s, $CH_2CH_2OCOC\underline{H}_3$), 6.95 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OCOCH_3$), 5.84 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OCOCH_3$), 4.70 (2H, s, $C\underline{H}_2Ph$), 3.34 (1H, d, 5-H), 2.62 (5H, m, $CH_2\underline{P}h$), and 1.08 (1H, s, NH). *m/e* 301 (M^-, 6%), 150 ($M^- - OCOCH_3 - H - CH_2C_6H_5$, 64%), and 91 ($C_7H_7^+$, 100%).

5-*Ethoxycarbonyl*-4-(*ethoxycarbonylmethyl*)-3-*methylpyrrole*-2-*carboxylic acid* (**16b**). Sulphuryl chloride (1.6 g, 12 mmol) was added dropwise to a stirred soln of **16a** = **7a** (1.0 g, 4 mmol) in dry ether (20 ml). The resulting pale yellow soln was stirred at 20° for 3 days and then evaporated to dryness. The residual brown viscous oil was taken up in dioxan (10 ml) and then treated with a soln of NaOAc (1.0 g, 12 mmol) in water (10 ml). The mixture was stirred at 70° for 1.5 hr and then at 20° overnight. Ether (10 ml) was added and the colourless aqueous layer separated. The organic phase was extracted with a soln of Na_2CO_3aq (2M; 3 × 10 ml) and the combined aqueous extracts were saturated with SO_2 gas. The resulting white ppt was filtered off, washed with water, dried *in vacuo*, and recrystallised from $CHCl_3$-light petroleum (b.p. 40–60°) to afford the acid (0.83 g, 74%), m.p. 155–157° (dec.) (Found: C, 55.2; H, 6.1; N, 5.0. $C_{13}H_{17}NO_6$ requires: C, 55.1; H, 6.1; N, 4.9%). λ_{max} ($CHCl_3$) 280 (ε 19,000), and 290 nm (infl.) (16,300). τ ($CDCl_3$/DMSO-d_6) 8.76 (3H, t, J = 7 Hz, $CH_2CO_2CH_2C\underline{H}_3$), 8.66 (3H, t, J = 7 Hz, $CO_2CH_2C\underline{H}_3$), 7.74 (3H, s, 3-CH_3), 6.21 (2H, s, $C\underline{H}_2CO_2CH_2CH_3$), 5.86 (2H, q, J = 7 Hz, $CH_2CO_2C\underline{H}_2CH_3$), 5.67 (2H, q, J = 7 Hz, $CO_2C\underline{H}_2CH_3$), and 0.23 (1H, s, NH). *m/e* 283 (M^+, 38%), 209 ($M^+ - CO_2CH_2CH_3 - H$, 100%), and 164 ($M^+ - CO_2CH_2CH_3 - OCH_2CH_3 - H$, 77%).

Ethyl 5-*iodo*-3-(*ethoxycarbonylmethyl*)-4-*methylpyrrole*-2-*carboxylate* (**16c**). A soln of $NaHCO_3$ (1.05 g, 10 mmol) in water (2 ml) was added at 65° to a stirred soln of **16b** (1.17 g, 4 mmol) in MeOH (20 ml). A soln of I_2 (1.05 g, 10 mmol) and KI (1.37 g, 10 mmol) in MeOH (10 ml) and water (5 ml) was added dropwise during 1 to 1.5 hr while the temp was maintained at 65°. After stirring at 65° for a further hr the mixture was allowed to stand at 0° overnight. The ppt was filtered off, washed with hot water, dried under vacuum, and recrystallised from CH_2Cl_2-light petroleum (b.p. 40–60°) to give the iodopyrrole (1.26 g, 84%), m.p. 115–118° (Found: C, 39.4; H, 4.2; N, 4.1. $C_{12}H_{16}NO_4I$ requires: C, 39.5; H, 4.4; N, 3.8%). λ_{max} ($CHCl_3$) 281 nm (ε 13,700). τ ($CDCl_3$) 8.72 (6H, q, J = 7 Hz, $OCH_2C\underline{H}_3$), 8.04 (3H, s, 4-CH_3), 6.18 (2H, s, $C\underline{H}_2CO_2CH_2CH_3$), 5.77 (4H, m, J = 7 Hz, $OC\underline{H}_2CH_3$), and 0.70 (1H, s, NH). *m/e* 365 (M^+, 100%), 291 ($M^+ - CO_2CH_2CH_3 - H$, 63%), and 246 ($M^+ - OCH_2CH_3 - CO_2CH_2CH_3 - H$, 43%).

Ethyl 3-(ethoxycarbonylmethyl)-4-methylpyrrole-2-carboxylate (**16d**). **16c** (470 mg, 1 mmol) was dissolved in MeOH (30 ml) containing anhyd NaOEt (220 mg, 3 mmol) and Adams PtO_2 (22 mg) and the mixture hydrogenated at 20° and 760 mm until the uptake of H_2 had ceased (30 min). The resulting soln was filtered through Celite, which was then washed with CH_2Cl_2, and the combined filtrates evaporated to dryness to give a yellow residue. EtOAc (13 ml) and water (13 ml) was added and the organic phase washed with Na_2CO_3aq (1M; 3 × 5 ml), water (3 × 5 ml), dried, filtered and evaporated to dryness to give a colourless oil which solidified after drying *in vacuo*. The α-free pyrrole (0.30 g, 99%) crystallised from aqueous EtOH, m.p. 44–45° (Found: C, 60.1; H, 7.5; N, 5.8. $C_{12}H_{17}NO_4$ requires: C, 60.2; H, 7.2; N, 5.85%). λ_{max} ($CHCl_3$) 272 nm (ε 11,700). τ ($CDCl_3$) 8.71 (6H, q, J = 7 Hz, $OCH_2C\underline{H}_3$), 7.99 (3H, s, 4-CH_3), 6.19 (2H, s, $C\underline{H}_2CO_2CH_2CH_3$), 5.77 (4H, m, J = 7 Hz, $OC\underline{H}_2CH_3$), 3.35 (1H, s, 5-H), and 0.76 (1H, s, NH). *m/e* 239 (M^+, 61%), 166 ($M^+ - COOCH_2CH_3$, 99%), and 120 ($M^- - COOCH_2CH_3 - OCH_2CH_3 - H$, 100%).

Benzyl 5-acetoxymethyl-4-methoxycarbonylmethyl-3-methylpyrrole-2-carboxylate (**15**). A soln of benzyl 4-methoxycarbonylmethyl-3,5-dimethylpyrrole-2-carboxylate[25] (4.0 g, 10 mmol) in glacial AcOH (48 ml) was treated with lead tetraacetate (5.88 g, 10 mmol) and the resulting mixture was stirred at room temp for 4 hr. After stirring for approximately 1 hr a creamy white ppt was deposited. When the reaction was complete the mixture was added to stirred water (200 ml), and the white crystalline solid separated by filtration, washed well with water, and recrystallised from aqueous MeOH to give the acetoxymethylpyrrole (3.3 g, 69%), m.p. 125–126° (Found: C, 63.6; H, 5.7; N, 4.1. $C_{19}H_{21}NO_6$ requires: C, 63·5; H, 5·9; N, 3.9%). λ_{max} ($CHCl_3$) 272 nm (ε 19,000). τ ($CDCl_3$) 7.99 (3H, s, $CH_2OCOC\underline{H}_3$), 7.74 (3H, s, 3-CH_3), 6.54 (2H, s, $C\underline{H}_2CO_2CH_3$), 6.37 (3H, s, $CH_2CO_2C\underline{H}_3$), 4.99 (2H, s, $C\underline{H}_2OCOCH_3$), 4.74 (2H, s, $C\underline{H}_2Ph$), 2.68 (5H, s, $CH_2\underline{Ph}$), and 2.72 (1H, s, NH). *m/e* 359 (M^+, 9%), 300 ($M^+ - CO_2CH_3$, 7%), and 91 ($C_7H_7^+$, 100%).

Benzyl 4-(2-chloroethyl)-3,5-dimethylpyrrole-2-carboxylate (**4a**). $SOCl_2$ (0.7 ml, 10 mmol) was added rapidly, but dropwise, to a stirred soln of benzyl 4-(2-hydroxyethyl)-3,5-dimethylpyrrole-2-carboxylate (2.73 g, 10 mmol) in CH_2Cl_2 (10 ml) containing DMF (0.8 ml) and heated to 50°. When addition was complete dry N_2 was passed through the soln for 1 hr. After cooling to room temp the mixture was diluted with CH_2Cl_2 (20 ml) and washed with 2M HCl (3 × 15 ml), $NaHCO_3$aq (3 × 20 ml), and water (3 × 20 ml). The organic extract was dried and evaporated to dryness. The residue was dissolved in benzene and filtered through a short column of alumina (50 g; elution with benzene). The filtrate was evaporated to dryness and the residue was crystallised from CH_2Cl_2–n-hexane to give the chloroethylpyrrole (1.98 g, 68%), m.p. 123–124° (lit.[26] m.p. 121–122°). λ_{max} ($CHCl_3$) 283 nm. τ ($CDCl_3$) 7.81 (3H, s, ring CH_3), 7.72 (3H, s, ring CH_3), 7.18 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2Cl$), 6.51 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2Cl$), 4.71 (2H, s, $C\underline{H}_2Ph$), 2.61 (5H, s, Ph), and 0.05 (1H, s, NH).

Pyrromethanes

Dibenzyl 3′-(2-chloroethyl)-4-(2-acetoxyethyl)-3,4′-dimethylpyrromethane-5,5′-dicarboxylate (**10b**). **4b** (259 mg) and **9** (220 mg) were suspended in distilled MeOH (7 ml) and stirred during 5 min at 65°, under N_2. *p*-Toluenesulphonic acid monohydrate (9.6 mg) was then added and the mixture stirred for a further 5 min before the temp was reduced to 55–57°, and stirring was continued at this temp for 6 hr. CH_2Cl_2 (10 ml) was added and the red soln was washed with water (10 ml), $NaHCO_3$aq (2M; 3 × 5 ml), water (3 × 5 ml), dried, filtered and evaporated to dryness. The oil was crystallised from CH_2Cl_2–n-hexane to give **10a** (300 mg, 75%) as pink plate-like crystals, m.p. 71–73°. λ_{max} ($CHCl_3$) 273 (ε 243,000), and 285 nm (24,000). τ ($CDCl_3$) 8.04 (3H, s, CH_3), 7.74 (3H, s, CH_3), 7.18 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2Cl$), 7.02 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OH$), 6.55 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2Cl$), 6.28 (2H, t, J = 7 Hz, CH_2CH_2OH), 6.15 (2H, s, CH_2), 4.76 (4H, d, CH_2Ph), 2.68 (10H, s, Ph), and 0.98 (2H, s, NH). *m/e* 549 (M^+, 2%), 551 (0.7%), and 91 (100%).

A soln of Ac_2O (0.08 g) in dry pyridine (1.5 ml) was added dropwise with stirring to a soln of the foregoing pyrromethane (0.314 g) in pyridine (6 ml). The resulting soln was stirred at room temp for 9 hr and then allowed to stand at 0° overnight before dropwise addition to ice-water (15 ml). $CHCl_3$ (3 × 10 ml) was added and the organic phase was washed with water (2 × 10 ml), HCl (2M; 3 × 5 ml), water (1 × 10 ml), $NaHCO_3$aq (2M; 3 × 10 ml), water (3 × 10 ml), and finally dried, filtered and evaporated to dryness. Traces of pyridine were removed by azeotroping with toluene (3 × 5 ml). The resulting oil solidified when triturated with cyclohexane, and was recrystallised from CH_3Cl MeOH to give pale pink needles of the acetoxyethylpyrromethane (0.32 g, 93%), m.p. 152–154° (Found: C, 67.4; H, 5.9; N, 4.3. $C_{33}H_{35}N_2O_6Cl$ requires: C, 67.1; H, 6.0; N, 4.7%). λ_{max} ($CHCl_3$) 272 (ε 140,700), and 285 nm (140,700). τ ($CDCl_3$) 8.06 (3H, s, $OCOCH_3$), 8.04 (3H, s, CH_3), 7.75 (3H, s, CH_3), 7.19 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2Cl$), 6.98 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OCOCH_3$), 6.56 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2Cl$), 6.15 (2H, s, CH_2), 5.86 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OCOCH_3$), 4.75 (4H, s, $C\underline{H}_2Ph$), 2.68 (10H, s, Ph), and 1.02 (2H, s, NH). *m/e* 591 (M^+, 3%), 593 (0.9%), 439 ($M^+ - OCOCH_3 - H - CH_2C_6H_5$, 21%) and 91 ($C_7H_7^+$, 100%).

5,5′-Dicarboxy-3′-(2-chloroethyl)-4-(2-acetoxyethyl)-3,4′-dimethylpyrromethane (**11b**). A soln of **10b** (50 mg) in dry THF (10 ml) containing Et_3N (5 drops) was hydrogenated at room temp and atmospheric pressure over 10% Pd-C (13 mg) until the uptake of H_2 had ceased (2 hr). The catalyst was filtered off through Celite, which was washed with THF, and the combined filtrates were evaporated to dryness to give the diacid as a brown oil (34 mg, 97%). λ_{max} ($CHCl_3$) 274 and 287 nm (infl.). τ ($CDCl_3$) 8.04 (3H, s, CH_3), 7.92 (3H, s, $OCOCH_3$), 7.74 (3H, s, CH_3), 7.15 (2H, t, $C\underline{H}_2CH_2Cl$), 6.94 (2H, t, $C\underline{H}_2CH_2OCOCH_3$), 6.65 (2H, t, CH_2CH_2Cl), 6.14 (2H, s, CH_2), 5.81 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OCOCH_3$), and −0.3 (2H, s, NH). *m/e* 323 ($M^+ - 2CO_2$, 0.23%), and 44 (100%).

Benzyl 3-(methoxycarbonylmethyl)-4′-(ethoxycarbonylmethyl-3′,4-dimethyl-5′-ethoxycarbonylpyrromethane-5-carboxylate (**17a**). (i) Benzyl 5-acetoxymethyl-4-methoxycarbonylmethyl-3-methylpyrrole-2-carboxylate (150 mg) and ethyl 3-(ethoxycarbonylmethyl)-4-methylpyrrole-2-carboxylate (99 mg) were suspended in glacial AcOH (10 ml), treated with anhyd NaOAc (240 mg) and heated at reflux during 4 hr under N_2. $CHCl_3$ (10 ml) was added to the cooled soln, and the mixture was washed with water (5 ml), satd $NaHCO_3$aq (5 × 5 ml), and finally water (3 × 5 ml). The organic extract was dried, filtered and evaporated to dryness. The red oil was crystallised from CH_2Cl_2–n-hexane, affording a pale pink residue (210 mg, 93%).

TLC analysis indicated that the crude residue contained traces of the symmetrical pyrromethane. The unsymmetrical pyrromethane was isolated by thick-layer chromatography and with the exclusion of light. The product (120 mg, 53%) was recrystallised from CH_2Cl_2–n-hexane, m.p. 148–150° (Found: C, 64.61; H, 6.61; N, 5.90. $C_{29}H_{34}N_2O_8$ requires: C, 64.66; H, 6.38; N, 5.2%). λ_{max} ($CHCl_3$) 274 (ε 30,700), and 285 nm (32,000). τ ($CDCl_3$) 8.75 (6H, m, $OCH_2C\underline{H}_3$), 8.05 (3H, s, CH_3), 7.76 (3H, s, CH_3), 6.62 (3H, s, $CH_2CO_2C\underline{H}_3$), 6.32 (2H, s, $CH_2CO_2CH_3$), 6.22 (2H, s, CH_2), 6.16 (2H, s, $C\underline{H}_2CO_2CH_2CH_3$), 5.84 (4H, m, $OC\underline{H}_2CH_3$), 4.75 (2H, s, CH_2Ph), 2.68 (5H, m, Ph), 1.04 (1H, s, NH), and 0.54 (1H, s, NH). *m/e* 538 (M^+, 24%), 447 ($M^+ - CH_2C_6H_5$, 99%), 373 ($M^+ - CH_2C_6H_5 - CO_2Et$, 77%), and 91 (100%).

(ii) A suspension of benzyl 5-acetoxymethyl-4-methoxycarbonylmethyl-3-methylpyrrole-2-carboxylate (82 mg) and ethyl 3-(ethoxycarbonylmethyl)-4-methylpyrrole-2-carboxylate (59 mg) in distilled MeOH (6 ml) was treated with *p*-toluenesulphonic acid hydrate (8.5 mg; 0.04 mmol) and stirred under N_2 at 50–55° overnight. The red soln was

diluted with water (5 ml) and extracted with CH_2Cl_2. The organic phase was washed with water (5 ml), $NaHCO_3$aq (2M, 3 × 5 ml), and then water (3 × 5 ml), dried, filtered and evaporated to dryness. The residue was chromatographed as above to afford an oil which crystallised from CH_2Cl_2 n-hexane to give the required pyrromethane (68 mg, 51%), m.p. 148–150° (identical in all respects with the previous product).

Benzyl 3,4′-*bis*-(2-*hydroxyethyl*)-3′,4-*dimethyl*-5′-*ethoxycarbonylpyrromethane*-5-*carboxylate* (**18**). Diborane gas was generated externally by the dropwise addition of BF_3 diethyl etherate (6.4 ml) on to a suspension of $NaBH_4$ (1.26 g) in diglyme (2.6 ml), and passed in a stream of dry N_2 into a soln of **17a** (259 mg) in THF (5 ml) during 2 hr at room temp. Abs MeOH (approx 10 ml) was then carefully added dropwise with stirring to the THF soln until the vigorous effervescence had ceased. The mixture was evaporated to dryness and the resulting pink oily residue was chromatographed, on silica thick layer plates with $CHCl_3$ MeOH (19 : 1) in the dark. The bis-(hydroxyethyl)-pyrromethane (214 mg, 95%) was crystallised from CH_2Cl_2-cyclohexane, m.p. 68–70° (Found: C, 67.0; H, 6.65; N, 6.0. $C_{26}H_{32}N_2O_6$ requires: C, 66.6; H, 6.9; N, 6.0%). λ_{max} ($CHCl_3$) 277 (ε 22,500), and 289 nm (24,600). τ ($CDCl_3$) 8.71 (3H, t, J = 7 Hz, $CO_2CH_2C\underline{H}_3$), 9.97 (3H, s, CH_3), 7.75 (3H, s, CH_3), 7.34 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OH$), 7.04 (2H, t, J = 7 Hz, $C\underline{H}_2CH_2OH$), 6.40 (2H, t, J = 7 Hz, CH_2CH_2OH), 6.28 (2H, t, J = 7 Hz, $CH_2C\underline{H}_2OH$), 6.17 (2H, s, CH_2), 5.74 (2H, q, J = 7 Hz, $CO_2CH_2CH_3$), 4.72 (2H, s, CH_2Ph), 2.64 (5H, m, Ph), −0.05 (1H, s, NH), and −0.46 (1H, s, NH). *m/e* 468 (M^+, 30%) 377 ($M^+ - CH_2C_6H_5$, 47%), and 91 (100%).

3,4′-*Bis*-(2-*hydroxyethyl*)-3′,4-*dimethylpyrromethane* (**19**). A suspension of the foregoing benzyl 3,4′-bis-(2-hydroxyethyl)-3′,4-dimethyl-5′-ethoxycarbonylpyrromethane-5-carboxylate (100 mg) in NaOHaq (6 ml, 4%) containing hydrazine hydrate (2 drops) was heated in a sealed glass tube at 170° for 2 hr. After cooling to room temp overnight the yellow soln was diluted with water (5 ml) and extracted with CH_2Cl_2 (4 × 5 ml). The organic phase was washed with water (3 × 5 ml), dried, filtered and evaporated to dryness, affording the crude pyrromethane as a brown oil (52.4 mg, 95%) which was dried *in vacuo*. τ ($CDCl_3$) 8.05 (3H, s, CH_3), 8.02 (3H, s, CH_3), 7.40 (2H, t, J = 7 Hz, CH_2CH_2OH), 7.38 (2H, t, J = 7 Hz, CH_2CH_2OH), 6.35 (4H, t, J = 7 Hz, $CH_2C\underline{H}_2OH$), 6.25 (2H, s, CH_2), 5.38 (2H, s, OH), 3.64 (2H, s, 5,5′-H), 2.28 (1H, s, NH), and 1.70 (1H, s, NH).

5,5′-*Diformyl*-3,3′-*bis*(2-*methoxycarbonylethyl*)-4,4′-*dimethylpyrromethane* (**12a**). Finely ground 5,5′-dicarboxy-3,3′-bis(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane (0.5 g) was added in portions to trifluoroacetic acid (3 ml) and the mixture stirred at room temp for 5 min. The red soln was cooled to 0° and triethylorthoformate (0.7 ml) was added dropwise and the stirring continued for a further 5 min at 0°. The soln was added dropwise with stirring to an ice-water mixture (40 ml). A red oil separated which solidified on scratching. The ppt was filtered off, washed with water, and added to a mixture of abs EtOH (5 ml) and NH_4OH (1M; 10 ml). The yellow residue was isolated by filtration after 10 min, washed with water and dried *in vacuo*. The diformylpyrromethane (0.30 g, 64%) crystallised from abs EtOH to give pale yellow needles m.p. 175–177° (lit.[28] m.p. 179–181°). λ_{max} ($CHCl_3$) 300 and 315 nm. τ ($CDCl_3$) 7.74 (6H, s, CH_3), 7.51 (4H, t, $CH_2CH_2CO_2CH_3$), 7.22 (4H, t, $CH_2C\underline{H}_2CO_2CH_3$), 6.31 (6H, s, $CH_2CH_2CO_2CH_3$), 5.96 (2H, s, CH_2), 0.51 (2H, s, CHO), and −0.26 (2H, s, NH). *m/e* 402 (M^+, 100%), and 373 ($M^+ - CHO$, 63%).

Dibenzyl 3-*ethoxycarbonylmethyl*-4′-*methoxycarbonylmethyl*-4,3′-*dimethylpyrromethane*-5,5′-*dicarboxylate* (**17b**). Benzyl 5-acetoxymethyl-4-methyl-3-methoxycarbonylmethylpyrrole-2-carboxylate (730 mg) in CH_2Cl_2 (50 ml) was added to benzyl 4-ethoxycarbonylmethyl-3-methylpyrrole-2-carboxylate (615 mg) in CH_2Cl_2 (150 ml) containing $SnCl_4$ (20 drops) cooled in salt-ice to −10° with vigorous stirring. The soln was stirred for 1½ hr allowing the temp to rise slowly to 0°. The soln was washed well with water, dried and the solvent was removed. The residue was crystallised from aqueous MeOH giving the pyrromethane (1.0 g, 81%) as needles m.p. 150–152°, with softening at 145° (Found: C, 67.7; H, 5.9; N, 4.5. $C_{34}H_{36}N_2O_8$ requires: C, 68.0; H, 6.0; N, 4.7%). τ ($CDCl_3$) 0.4 (1H, s, NH), 1.1 (1H, s, NH), 2.65 (10H, s, Ph), 4.75 (4H, br, $PhCH_2$), 5.95 (2H, q, $CO_2C\underline{H}_2CH_3$), 6.17 (4H, s, CH_2CO_2R), 6.4 (3H, s, CO_2CH_3), 6.62 (2H, s, CH_2 bridge), 7.75 (3H, s, CH_3), 8.05 (3H, s, CH_3), 8.82 (3H, t, $CO_2CH_2CH_3$). *m/e* (F.D.) 600 (M^+, 100%).

Dibenzyl 3,4′-*bis*(2-*hydroxyethyl*)-4,3′-*dimethylpyrromethane*-5,5′-*di-carboxylate* (**10c**). The foregoing pyrromethane (210 mg) was dissolved in dry THF (10 ml) and diborane gas (generated from $NaBH_4$ (1 g) in diglyme (10 ml) and BF_3 etherate (5 ml)) was passed through the soln in a slow stream of N_2 during 4½ hr. MeOH was added until effervescence ceased and the solvents were removed. The residue was crystallised from CH_2Cl_2/petrol 40–60° giving the diol, (165 mg, 90%), m.p. 101–103° (Found: C, 70.1; H, 6.4; N, 5.2. $C_{31}H_{34}N_2O_6$ requires: C, 70.2; H, 6.5; N, 5.3%). τ ($CDCl_3$) 1.1 (1H, br, NH), 2.67 (10H, s, Ph), 4.77 (2H, s), 4.85 (2H, s, $PhC\underline{H}_2$), 6.25 (2H, s, CH_2 bridge), 6.4 (4H, m, $CH_2C\underline{H}_2OH$), 7.05 (2H, t), 7.4 (2H, t, $C\underline{H}_2CH_2OH$), 7.8 (3H, s, CH_3), 8.05 (3H, s, CH_3).

When the reaction was scaled up to 1.0 g the reduction was incomplete after 5 hr, and so the reaction vessel was stoppered and left overnight. The dissolved diborane completed the reduction and afforded 770 mg (87%) of the required product but contaminated with a trace of a fluorescent material which was inseparable on TLC and not removed by recrystallisation or decolourising charcoal.

Dibenzyl 3,4′-*bis*(2-*acetoxyethyl*)-4,3′-*dimethylpyrromethane*-5,5′-*dicarboxylate* (**10d**). The foregoing diol (500 mg) in pyridine (10 ml) was treated with Ac_2O (2 ml); the soln was stirred under N_2 for 2 hr at room temp then poured into ice-water (100 ml). The pyrromethane was extracted with CH_2Cl_2 (3 × 25 ml) which was washed with 1N HCl (50 ml), satd $NaHCO_3$aq (50 ml) and water (50 ml). The soln was dried and the solvent was removed to give an oil which was chromatographed on silica (Merck, Kieselgel 60) with CH_2Cl_2 : ether (1 : 1). The required fractions were combined and evaporated to give the title pyrromethane as a pale yellow oil which could not be induced to crystallise (520 mg, 90%). It was used without further purification. τ ($CDCl_3$) −0.27 (2H, br, NH), 2.83 (10H, s, Ph), 4.83 (4H, s, $PhC\underline{H}_2$), 5.95 (4H, m, $CH_2C\underline{H}_2OAc$), 6.2 (2H, s, CH_2 bridge), 6.98 (2H, t), 7.3 (2H, t, CH_2CH_2OAc), 7.75 (3H, s, CH_3), 8.0 (3H, s, CH_3), 8.1 (6H, br, $OCOC\underline{H}_3$). *m/e* (F.D.) 614 (M^+, 100%).

3,4′-*Bis*(2-*acetoxyethyl*)-4,3′-*dimethylpyrromethane*-5,5′-*dicarboxylic acid* (**11d**) The foregoing dibenzyl ester (100 mg) in MeOH (30 ml) containing Et_3N (5 drops) was treated (under N_2) with 10% Pd-C (20 mg). The suspension was hydrogenated overnight at 20°/760 mm. The catalyst was filtered off and the solvent was removed to give the diacid as a pale pink gum which rapidly turned dark red on standing. After drying under vacuum the gum was used without further purification in subsequent porphyrin preparations.

5,5′-*Diformyl*-3,3′-*bis*(2-*methoxycarbonylethyl*)-4,4′-*dimethylpyrroketone* (**12b**). (i) Sulphuryl chloride (0.15 ml) was added dropwise during 5 min to a stirred soln of **12a** (267 mg) in glacial AcOH (7 ml). The red soln was stirred at room temp for 1 hr. Abs EtOH (1 ml) and water (8 ml) were then added dropwise and the stirring was continued for a further 30 min. A yellow ppt was isolated by filtration, washed with water, and dried *in vacuo* to give the pyrroketone (0.1 g, 36%), m.p. 202–204° (lit.[19] m.p. 207–208°). λ_{max} ($CHCl_3$) 276, 317, and 355 nm. τ ($CDCl_3$) 7.35 (4H, t, J = 7 Hz, $C\underline{H}_2CH_2CO_2CH_3$), 6.95 (4H, t, J = 7 Hz, $CH_2CH_2CO_2CH_3$), 6.38 (6H, s, $CH_2CH_2CO_2C\underline{H}_3$), 0.82 (2H, s, CHO), and −1.34 (2H, s, NH). *m/e* 416 (M^+, 68%), and 342 ($M^+ - CH_2CO_2CH_3 - H$, 100%).

(ii) **12a** (99.8 mg) was dissolved in dioxan (10 ml) and selenium dioxide (301 mg) was added. The mixture was

vigorously stirred and heated to reflux for 3 hr, and then allowed to stand at room temp overnight. The red mixture was filtered through Celite, to remove the finely divided black Se, and the Celite was washed with CH_2Cl_2. The combined filtrates were evaporated to dryness affording a red oil which was taken up in $CHCl_3$ (10 ml), washed with water (3 × 5 ml), dried, filtered and evaporated to dryness. The dark red oil remaining was chromatographed on 20 × 20 cm silica preparative thick layer plates with MeOH-$CHCl_3$ (1 : 19). Two bands were removed and extracted from the silica by washing with $CHCl_3$.

The upper, less polar band gave a yellow residue which was a single spot on TLC, and identical spectroscopically with the required pyrroketone prepared as above (12.3 mg, 12%), m.p. 201–203°.

The more polar band also gave a yellow residue which was recrystallised from aqueous MeOH to give yellow, fine, needle-like crystals regarded as 5′-formyl-3,3′-bis(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane 5-carboxylic acid (11.1 mg, 10%) m.p. 192 195°. λ_{max} ($CHCl_3$) 260, 268, 300, 399, and 421 nm. τ ($CDCl_3$) 8.02 (3H, s, CH_3), 7.68 (3H, s, CH_3), 7.42 (4H, t, J = 7 Hz, $C\underline{H}_2CH_2CO_2CH_3$), 7.10 (4H, t, J = 7 Hz, $CH_2C\underline{H}_2CO_2CH_3$), 6.36 (3H, s, $CH_2CH_2CO_2C\underline{H}_3$), 6.32 (3H, s, $CH_2CH_2CO_2C\underline{H}_3$), 3.85 (1H, s), 0.24 (1H, s, CHO), −0.79 (1H, s, NH), and −0.96 (1H, s, NH). *m/e* 432 (M^+, 0.2%), 388 ($M^+ - CO_2$, 62%), and 314 ($M^+ - CO_2 - CH_2CO_2CH_3 - H$, 58%).

Porphyrins

2,4-*Bis*-(2-*chloroethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-γ-*mesyloxyporphyrin* (**14b**). (i) A soln of 3,4′-bis(2-hydroxyethyl)-3′,4-dimethylpyrromethane (45.2 mg) in CH_2Cl_2 (3 ml) was added to 5,5′-diformyl-3,3′-bis(2-methoxycarbonylethyl)-4,4′-dimethylpyrroketone (65.7 mg) in CH_2Cl_2 (3 ml), and the mixture was stirred at 20° under N_2, and protected from light. Methanesulphonic acid (2 drops) was added and the resulting red soln was stirred at room temp for a further 2 hr, and then evaporated to dryness under a stream of N_2. The oily residue was treated with excess methanesulphonyl chloride (1 ml) in pyridine (5 ml) and heated at 75° for 35 min When the mixture had cooled to room temp, water (5 ml) was added and the porphyrin product was carefully extracted with CH_2Cl_2. The dark red organic extract was washed with water (3 × 5 ml), dried, filtered and evaporated to dryness. The residue was chromatographed on an alumina grade III column (40 g, eluting with 1 : 1 (v/v) benzene : CH_2Cl_2 (1 : 1). The single porphyrin band was eluted, evaporated to dryness and crystallised from $CHCl_3$-MeOH affording dark red needles of the mesyloxyporphyrin (13.2 mg, 11%), m.p. 181–183° (Found: C, 58.7; H, 5.5; N, 7.5. $C_{37}H_{42}N_4O_7SCl_2$ requires: C, 58.6; H, 5.6; N, 7.4%). λ_{max} ($CHCl_3$) 406, 506, 540, 581, and 635 nm. τ ($CDCl_3$) 13.6 (2H, s, NH), 6.61 (14H, m, CH_3, CH_2CH_2Cl, and $C\underline{H}_2CH_2CO_2CH_3$), 6.42 (6H, s, CH_3), 6.34 (6H, s, $CH_2CH_2CO_2CH_3$), 5.80 (11H, m, $CH_2C\underline{H}_2Cl$, $CH_2CH_2CO_2CH_3$ and OSO_2CH_3), 0.28, 0.09, 0.01 (all 1H, s, *meso*-H) *m/e* (F.D.) 757 (M^+, 100%), 677 ($M^+ - SO_2CH_3 - H$, 93%).

(ii) A soln of 5,5′-dicarboxy-3′-(2-chloroethyl)-4-(2-acetoxyethyl)-3,4′-dimethylpyrromethane (20 mg) and 5,5′-diformyl-3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrroketone (21.5 mg) in CH_2Cl_2 (4 ml) was treated with methanesulphonic acid (2 drops) and stirred at 20° under N_2 and in the dark for 2 hr. The red mixture (λ_{max} ($CHCl_3$) 417, 495, 559, and 621 nm) was then evaporated to dryness under a stream of dry N_2. The residue was dissolved in dry pyridine (3 ml), affording a green soln, and methanesulphonyl chloride (1 ml) was added. The mixture was stirred at room temp for 10 min and then heated at 75° for 35 min. After cooling to room temp, water (5 ml) was added and the mixture was extracted with CH_2Cl_2 (20 ml). The combined red organic extracts were washed with water (3 × 5 ml), dried, filtered, evaporated to dryness and finally dried *in vacuo*. The brown residue was chromatographed on grade III alumina (25 g; eluting with benzene : CH_2Cl_2 (1 : 1). Two porphyrin fractions were collected.

The first fraction was identified as the bis-chloroethyl-γ-mesyloxyporphyrin (7.0 mg, 18%) by mass and NMR spectrometry, and proved to be identical with the product obtained in the foregoing preparation.

The second fraction gave violet red needles of **14b** (8.8 mg, 22%) from $CHCl_3$-MeOH. λ_{max} ($CHCl_3$) 406, 505, 539, 580 and 634 nm. *m/e* (F.D.) 702 ($M^+ - SO_2CH_3$, 100%). This was stirred at room temp overnight in the dark in 5% (v/v) sulphuric acid in MeOH (1 ml). The resulting soln was added to ice-cold water (5 ml), the last traces being washed into the flask with $CHCl_3$ (5 ml). The mixture (pH ~ 2) was neutralised with dil NH_4OH (3.5% to pH ~ 7–8). The organic phase was separated and the aqueous layer was extracted with $CHCl_3$ (10 ml). The combined organic extracts were washed with water (3 × 5 ml), dried, filtered and evaporated to dryness. The red residue was chromatographed on grade IV alumina (18 g; elution with CH_2Cl_2 initially and then gradually increasing the polarity until eluting with $CHCl_3$ and finally with 2% MeOH in $CHCl_3$. A single red porphyrin band was isolated, together with a blue oxophlorin band.

The latter was shown by visible spectroscopy (λ_{max} ($CHCl_3$) 404, 590 and 643 nm, inflections at 510 and 545 nm) and mass spectrometry (*m/e* (F.D.) 660 (M^+, 94%) and 659 ($M^+ - 1$, 100%)) to be **13c** (1.1 mg, 23%) and was identical with the product prepared in a different manner directly from **18b** and **12b** (see below).

The red band crystallised from $CHCl_3$-MeOH and gave the bis(hydroxyethyl)-γ-mesyloxyporphyrin (2.8 mg, 53%). λ_{max} ($CHCl_3$) 405, 505, 539, 580, and 633 nm. τ ($CDCl_3$) 13.58 (2H, s, NH), 6.68, 6.58, 6.51, 6.46, 6.41 and 6.38 (complex m), 5.72 (broad m), 1.19, 1.02, −0.06 (all 1H, s, *meso*-H). *m/e* (F.D.) 739 (M^+, 33%) and 738 ($M^+ - H$, 100%).

Treatment of this material with methanesulphonyl chloride (0.5 ml) in pyridine (2 ml) as in (i) above, followed by work-up in the usual way and chromatography on alumina (grade III) gave the bis(chloroethyl)-γ-mesyloxyporphyrin (2 mg) identical in all respects (m.p., spectroscopy and TLC) with material prepared as described above.

(iii) To a stirred CH_2Cl_2 soln (6 ml) of **19** (47.1 mg) and **12b** (74.6 mg) under N_2, was added methanesulphonic acid (2 drops). After stirring for 2 hr in the dark at 20° the red soln was treated with water (5 ml). The organic extract was separated, washed with NH_4OH (2M; 3 × 5 ml), water (3 × 5 ml), dried, filtered and evaporated to dryness. The dark greenish-blue residue was chromatographed on grade IV alumina column (20 g) eluting initially with CH_2Cl_2, and gradually increasing the polarity to 5% MeOH in $CHCl_3$. The bluish-green fractions were collected and rechromatographed using $CHCl_3$ as eluant, and after evaporation to dryness afforded the dark blue **13c** (26.4 mg, 23%). λ_{max} ($CHCl_3$) 405, 500i, 510i, 591 and 643 nm. *m/e* (F.D.) 644 (100%), and 642 (M^+, 26%). This was immediately converted into the desired bis(chloroethyl)-γ-mesyloxyporphyrin by treatment with excess methanesulphonyl chloride in pyridine as in the previous preparation described above. The product was identical with those described in (i) and (ii).

2-(2-*Chloroethyl*)-4-(2-*acetoxyethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-γ-*acetoxyporphyrin* (**14a**). A soln of **11a** (10.0 mg) and **12b** (10.7 mg) in dry CH_2Cl_2 (2 ml) was treated with methanesulphonic acid (2 drops) and stirred at room temp under N_2 and protected from light for 2 hr. Spectroscopic sampling indicated that the Soret band (417 nm) had then attained its maximum intensity. The red soln was diluted with CH_2Cl_2 (5 ml) and water (5 ml) was added. The mixture was neutralised with dil NH_4OH (2M; 2 × 3 ml) and the blue-green organic extract was separated, washed with water (3 × 3 ml), dried, filtered and evaporated to dryness. The residue was chromatographed on grade III alumina (16 g, eluting with $CHCl_3$). The blue eluates were

evaporated to dryness giving a dark blue residue of the oxophlorin (16 mg, 89%). λ_{max} ($CHCl_3$) 403, 500, 506, 590, and 641 nm. *m/e* (F.D.) 703 (M^+, 100%). This was then treated with Ac_2O (0.5 ml) in pyridine (3 ml) and stirred at 20° in the dark overnight. The resulting red soln was added to water (5 ml) and the porphyrin was extracted with CH_2Cl_2 (3 × 3 ml). The organic phase was separated and washed with water (3 × 5 ml), dried, filtered and evaporated to dryness. The red residue was chromatographed on alumina (35 g, grade III) eluting with benzene : CH_2Cl_2 (1 : 1) and gradually increasing the polarity of the eluant until using 100% CH_2Cl_2. The red porphyrin fractions were collected, evaporated to dryness, and dried *in vacuo* to yield the acetoxyporphyrin (10 mg, 61%). λ_{max} ($CHCl_3$) 403, 500, 533, 576, and 627 nm. *m/e* (F.D.) 745 (M^+, 57%), and 744 ($M^+ - H$, 100%).

2,4-*Bis*(2-*acetoxyethyl*)-γ-*benzoyloxy*-6,7-*bis*(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin* (**14d**). A mixture of **11d** (100 mg) and **12b** (96 mg) in trifluoroacetic acid (10 ml) was stirred for 2 hr. The soln was evaporated to dryness under reduced pressure and the residue was taken up in pyridine (10 ml). Benzoyl chloride (2 ml) was added to the green soln which immediately turned red. The soln was kept for 30 min before addition of CH_2Cl_2 (200 ml). The porphyrin soln was washed with 1N HCl (2 × 100 ml), 1N NH_3 (2 × 100 ml) and water (2 × 100 ml). The solvent was removed and the residue was chromatographed on alumina with 2% acetone in CH_2Cl_2. The required fractions were combined and crystallised from CH_2Cl_2-MeOH to give the title porphyrin as shiny red plates (60 mg, 31%), m.p. 165–167° (Found: C, 68.2; H, 5.9; N, 7.1%. $C_{47}H_{50}N_4O_{10}$ requires: C, 67.95; H, 6.0; N, 6.75%). τ ($CDCl_3$) (0.05M) −0.05 (1H, s), 0.04 (1H, s), 0.23 (1H, s, 3 × *meso*-H) 1.25 (2H, m), 2.15 (3H, m, Ph), 5.20 (6H, m), 5.85 (10H, m, 2 × CH_2CH_2OAc, 2 × $CH_2CH_2CO_2Me$), 6.42, 6.45, 6.5, 6.55, 6.6 (18H, 4 × ring CH_3 and 2 × CO_2CH_3), 8.0 (6H, s, 2 × $OCOCH_3$), 13.65 (2H, br, NH). *m/e* (F.D.) 830 (100%). λ_{max} ($CHCl_3$) (log ε) 404 (5.23), 503 (4.16), 535 (3.68), 578 (3.71) and 631 nm (3.21).

γ-*Benzoyloxy*-2,4-*di*(2-*chloroethyl*)-6,7-*di*(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin* (**14f**). The foregoing diacetoxyethyl porphyrin (60 mg) was taken up in methanolic H_2SO_4 (5% v/v) and heated under reflux for 1 hr. On cooling, the di(2′-hydroxyethyl)porphyrin was extracted with $CHCl_3$, and was washed with aqueous ammonia and then water. The solvent was removed and the residue was crystallised from CH_2Cl_2-hexane giving **14e** as needles, m.p. 253–255°, 52 mg (96%).

This diol (40 mg) was not further characterised but was dissolved in a mixture of $CHCl_3$ (50 ml) and N,N-DMF (25 ml) and anhyd K_2CO_3 (5 g) was added. $SOCl_2$ (1.5 ml) was added and the mixture was stirred, whilst being protected from moisture for 2 hr. The mixture was poured into water, and the organic layer was separated and washed with aqueous ammonia and water. The solvent was removed and the residue was chromatographed on alumina with CH_2Cl_2. The required fractions were combined and evaporated to dryness, and the residue was crystallised from CH_2Cl_2-MeOH to give the required porphyrin (30 mg, 72%) as jagged, purple needles, m.p. 256–258°. (Found: C, 66.1; H, 5.7; N, 6.8%. $C_{43}H_{44}N_4O_6Cl_2$ requires: C, 65.9; H, 5.6; N, 7.1%). τ ($CDCl_3$) (0.1M) 0.38 (1H, s), 0.47 (1H, s), 0.92 (1H, s, 3 × *meso*-H), 1.30 (2H, m), 2.20 (3H, m, Ph), 5.54–6.35 (16H, m, 2 × CH_2CH_2Cl, 2 × $CH_2CH_2CO_2Me$), 6.53, 6.57 (18H, 4 × CH_3 and 2 × CO_2CH_3), 14.05 (2H, br, NH). *m/e* (F.D.) 783 (42%), 784 (37%), 785 (57%), 786 (100%), 787 (21%). λ_{max} ($CHCl_3$) (log ε) 404 (5.29), 503 (4.18), 525 (3.72), 578 (3.74) and 630 nm (3.21).

In a similar reaction the diol (20 mg) in dry N,N-DMF (20 ml) containing LiCl (5 g) was treated with methanesulphonyl chloride (5 ml) at 75°. The soln was kept at this temp for 40 min before being worked up in the same way as described above. The required porphyrin, identical to the above, was obtained in 65% yield.

2,4-*Bis*(2′-*chloroethyl*)-γ-*ethoxymethoxy*-6,7-*di*(2′-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin, zinc complex* (**20b**). The foregoing γ-benzoyloxyporphyrin (15 mg) in pyridine (10 ml) was treated with 10% methanolic KOH (10 ml) and the soln was kept until the peak at 503 nm had disappeared (approx 5 min). $CHCl_3$ (50 ml) was added and the soln was washed with 10% aqueous citric acid (2 × 50 ml) and water (2 × 50 ml). The soln was evaporated, dried under vacuum and the residue was taken up in 14% BF_3 in MeOH (5 ml). The blue soln was boiled (under N_2) for 30 min before extraction with $CHCl_3$ (50 ml), washing with aqueous ammonia, water and drying. Thus a soln of **13b** was obtained λ_{max} 408, 545 (sh), 510 (sh), 592, 645 nm.

A saturated soln of zinc acetate in MeOH (2 ml) was added to the oxophlorin in $CHCl_3$ (50 ml); after 5 min the soln had changed to a reddish-pink colour and the visible spectrum (λ_{max} 408, 430, 500 (sh), 537, 574 nm) showed the formation of zinc porphyrin to be complete. The soln was washed with water (2 × 50 ml), dried and the solvent removed.

The residue of zinc oxophlorin was taken up in pure, dry acetone (25 ml) and stirred with anhyd K_2CO_3 (1 g) under N_2. The suspension was heated under reflux until the green colour of the oxophlorin anion was pronounced (2 min) and 1,8-bis(dimethylamino)-naphthalene (0.75 g) was added and the mixture was again refluxed for 2 min before addition of chloromethyl ethyl ether (2 drops). The soln, which immediately turned pink, was heated for 5 min before adding a further 2 drops of the chloromethyl ethyl ether and heating for a further 5 min. The pink soln was kept at room temp for 30 min before pouring into $CHCl_3$ (100 ml). The K_2CO_3 was filtered off and washed with small portions of $CHCl_3$. The combined solns were washed with aqueous ammonia (2 × 50 ml), 10% citric acid (3 × 50 ml), water (2 × 50 ml) and dried. The solvent was removed and the residue was chromatographed on alumina (grade V) with CH_2Cl_2. The required fractions were combined and evaporated, and the residue on crystallisation from CH_2Cl_2-hexane gave the zinc porphyrin as shiny purple plates (8.0 mg, 56%), m.p. 84–86° (with softening at 72°). Elemental analysis was not attempted. τ (0.02M) 0.23 (2H, s), 1.03 (1H, s, 3 × *meso*-H), 4.3 (2H, s, OCH_2O), 5.2–6.5 (12H, m, 2 × CH_2CH_2Cl and 2 × $CH_2CH_2CO_2Me$), 6.28, 6.38 (18H, 4 × CH_3 and 2 × CO_2CH_3), 6.5–7.0 (6H, m, OCH_2CH_3 and 2 × $CH_2CH_2CO_2Me$), 9.07 (3H, t, OCH_2CH_3). *m/e* (F.D.) 798 (58%), 799 (44%), 800 (100%), 801 (38%), 802 (50%), 803 (80%), 804 (18%). λ_{max} (CH_2Cl_2) (log ε) 409 (5.55), 500 (3.40), 537 (4.21) and 572 nm (3.95).

γ-*Ethoxymethoxy*-6,7-*di*(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinylporphyrin, zinc complex.* (γ-*Ethoxymethoxyprotoporphyrin IX dimethyl ester zinc complex*) (**20c**). The foregoing Zn complex (**20b**) (6.7 mg) in dry THF (5 ml) was treated with 1M t-BuOK in t-BuOH (30 ml) under N_2. The resulting soln was kept in a dessicator in the dark for 72 hr before adding EtOAc (100 ml), washing with 10% citric acid (2 × 50 ml), water (3 × 50 ml) and evaporating to dryness (finally by azeotroping with abs EtOH).

Re-esterification of the propionic acid functions was achieved by addition of a soln of diazomethane (from 2 g of nitrosomethylurea) in ether (75 ml) to the porphyrin in MeOH (10 ml). After 5 min the mixture was diluted with CH_2Cl_2 (25 ml) and washed with 10% citric acid (2 × 50 ml), aqueous ammonia (2 × 50 ml), water (3 × 50 ml) and dried. The solvents were removed and the residue was chromatographed on alumina (grade V) with CH_2Cl_2. The required fractions were combined, evaporated and crystallised from CH_2Cl_2-hexane giving the title porphyrin (5.2 mg, 85%) as a red amorphous powder, m.p. 220–222°. Elemental analysis was not attempted. τ ($CDCl_3$) (0.014M) 0.05 (1H, s), 0.15 (1H, s), 0.82 (1H, s, 3 × *meso*-H), 1.8–2.2 (2H, m, $CH{=}CH_2$), 3.7–4.15 (4H, m, $CH{-}CH_2$), 4.32 (2H, s, OCH_2O), 5.1–5.8 (4H, br, $CH_2CH_2CO_2Me$), 6.25 (18H, br, 4 × CH_3 and 2 × CO_2CH_3), 6.5–6.85 (6H, m, 2 × $CH_2CH_2CO_2Me$ and OCH_2CH_3), 9.05 (3H, t, OCH_2CH_3). *m/e* (F.D.) 727 (89%), 728 (35%), 729 (72%), 730 (71%), 731 (100%), 732 (59%),

733 (12%). λ_{max} (CH_2Cl_2) (log ε) 416 (5.48), 508 (3.40), 543 (4.24) and 583 nm (4.05).

Bile pigment

Biliverdin IXγ dimethyl ester (**22**). The foregoing γ-ethoxymethoxyporphyrin (5 mg) was dissolved in hot AcOH (10 ml) under N_2 in the dark. After 10 min K_2CO_3 (0.5 g) was added to the green soln. The buffered soln of **21** thus formed was heated to 100° (oil bath) and a saturated, aqueous soln containing both NaCl and $FeSO_4$ (0.5 ml) was added. This mixture was kept at this temp for a further 20 min before being cooled and poured into 20% CH_2Cl_2 in ether (150 ml). The AcOH was washed out with water (6 × 100 ml), and the organic layer was dried and the solvent was removed. The γ-oxyhemin (λ_{max} (ether) 408 and 470–560 nm) was taken up in pyridine (10 ml) giving a green soln (λ_{max} 438 and 660 (br) nm). The pyridine soln was stirred in a darkened flask which was flushed with O_2 for 3 hr. After this time the visible spectrum (λ_{max} (ε ratios) 399 (4.3), 495 (1.56), 531 (1.5), 605 (1.0) and 657 nm (2.3) showed no more formation of the verdohaemochrome. The pyridine was removed on a rotary evaporator and the residue was taken up in N_2-flushed MeOH (12.5 ml); a soln of methanolic KOH (2N, 1 ml) was added (under N_2) and after 1 min 14% BF_3 in MeOH (12.5 ml) was added to the soln which turned a blue-green colour. The soln was heated under reflux for 15 min under N_2 and then kept at 20° overnight before being poured into water (75 ml): the pigments were then extracted with $CHCl_3$ (2 × 50 ml). The extract was washed with water (2 × 50 ml), and the solvent was removed. The residue was dissolved in a little $CHCl_3$ and again washed with water (2 × 50 ml), and the solvent was removed. The residue was chromatographed on alumina (grade V) with CH_2Cl_2 (containing 0.5% MeOH). The blue eluates were evaporated and applied to a thick-layer plate coated with silica and developed with $CHCl_3$ containing 5% acetone. The major blue band was scraped off and the biliverdin IXγ was extracted into $CHCl_3$. Crystallisation from a very small volume of CH_2Cl_2-light petroleum gave the bile pigment (17 mg, 20%) as green needles, m.p. 205–207° (lit. m.p. 207–209°). *m/e* (F.D.) 610 (M^+, 100%). λ_{max} ($CHCl_3$) 377 and 642 nm. This product was identical in all respects with material obtained by direct oxidation of haem in pyridine, and separation of the mixture of isomeric biliverdins formed.

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