# Biosynthesis of Porphyrins and Related Macrocycles. Part 13.[1] Structure of the Protoporphyrin Isomer derived from Coproporphyrinogen IV by the Action of Beef-Liver Coproporphyrinogenase: Synthesis of Protoporphyrin XIII

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Coproporphyrinogen IV (8) is synthesised and is oxidatively decarboxylated by coproporphyrinogenase from beef-liver to produce, after aromatisation, a porphyrin proved to be protoporphyrin XIII (11) by spectroscopy and by unambiguous synthesis; the synthetic route is described. A monovinylporphyrin, derived from an intermediate for the conversion (8) ⟶ (11), is also isolated. The importance of these results for earlier biosynthetic studies with pyrromethanes is discussed.

AN important step in the biosynthetic pathway leading to the haems and chlorophylls[2] is the double oxidative decarboxylation of coproporphyrinogen III (1) to protoporphyrinogen IX (2) catalysed by the enzyme coproporphyrinogenase. Early work on the substrate specificity of the enzyme from ox-liver revealed[3] that copro-

porphyrinogen isomers I and II were unaffected by the enzyme but coproporphyrinogen IV (8) was transformed into an unidentified protoporphyrin isomer. More recently, further studies of the enzyme's substrate specificity have been made,[4] the stereochemistry of the overall change in the propionate side chains has been
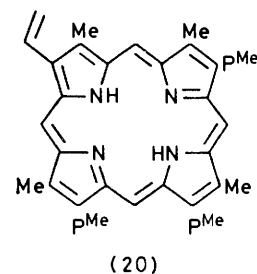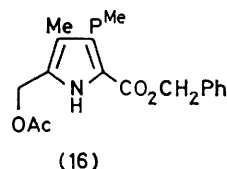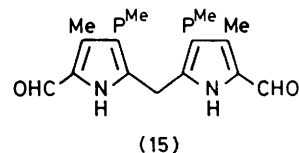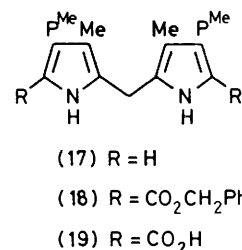

(4)

(1)

(3)

(2)

(5)

(6) R = H

(7) R = Me
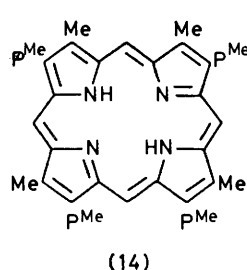
A = CH_2CO_2H

P = $CH_2CH_2CO_2H$
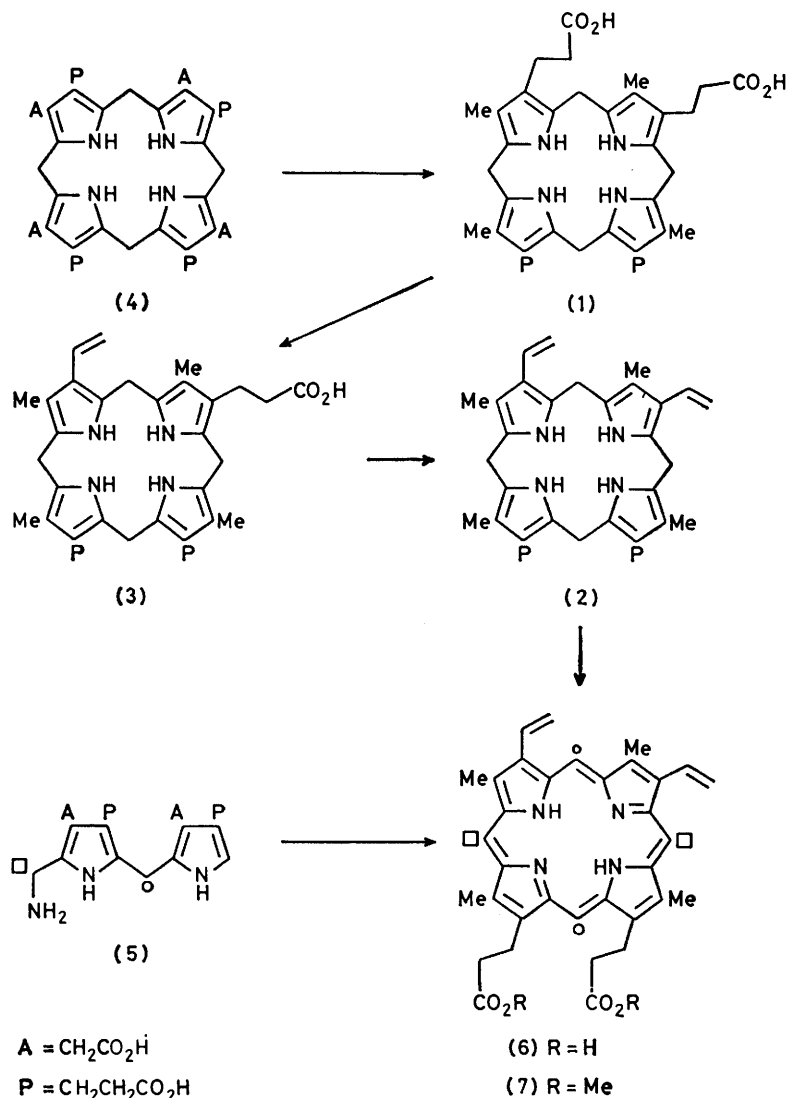
elucidated,[5] and harderoporphyrinogen (3) has been shown to be an intermediate.[6]

In connection with related studies [7] on the biosynthesis of uroporphyrinogen III (4), the specific incorporation of ^{14}C-, and ^{13}C-labelled pyrromethane (5) into protoporphyrin-IX (6) had been reported from this laboratory.[8] These incorporations were questioned [9] and it was suggested that the observed results could be due to incorporation of (5) into a protoporphyrin isomer via uroporphyrinogen IV and coproporphyrinogen IV (8).
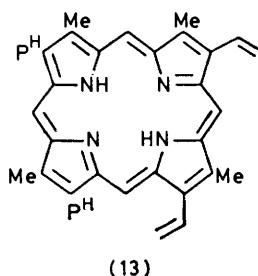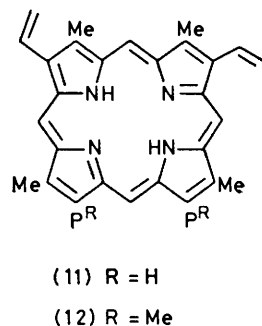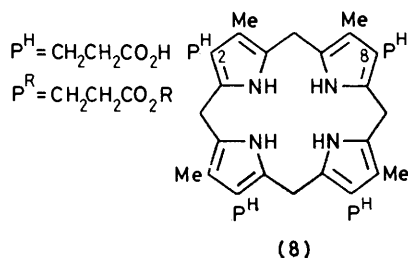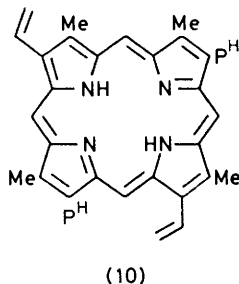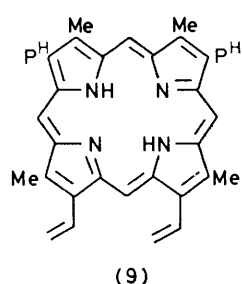
Coproporphyrin IV tetramethyl ester (14) was synthesised via a MacDonald condensation of the pyrromethanes (15) [13] and (17). The latter compound was prepared from the acetoxymethylpyrrole (16) [14] via (18) and (19) (see Experimental section). The isomeric purity of the coproporphyrin IV ester (14) was checked by h.p.l.c. and n.m.r., and when it was prepared from carefully purified (17) no isomeric porphyrins could be detected.

Coproporphyrin IV, prepared from (14) by hydrolysis, was reduced by sodium amalgam to coproporphyrinogen IV (8). This was then incubated in air at pH 7.8, for 22 h at 38 °C with beef-liver mitochondria. The air, required for coproporphyrinogenase, also slowly oxidises the various porphyrinogens to porphyrins, a process which was essentially complete at the end of the incubation. The total free porphyrins were extracted and esterified, the recovery ranging from 34—39% in

(9)

(10)

P^H = $CH_2CH_2CO_2H$

P^R = $CH_2CH_2CO_2R$

(8)

(14)

(17) R = H

(18) R = CO_2CH_2Ph

(19) R = CO_2H

(15)

(16)

(11) R = H

(12) R = Me

(13)

(20)

The extensive precautions taken to ensure that protoporphyrin IX (6) was indeed being handled,[10] rather than an isomer, made the suggestion a remote possibility. Nevertheless, it was important to identify the protoporphyrin isomer obtained enzymically from coproporphyrinogen IV (8) and to show that it differed and was separable from protoporphyrin IX.[11]

Enzymic and Synthetic Studies. Provided that the porphyrinogen macrocycle remains intact throughout the enzyme-catalysed reaction [and this has been proved [12] for coproporphyrinogen III (1)], then only four isomers of protoporphyrin may be obtained from coproporphyrinogen IV (8), after aromatisation, namely the protoporphyrins (9), (10), (11), and (13). These isomers may in principle be distinguished on the basis of their differing symmetry properties, and we shall return to this point later.

several experiments. Chromatography on silica afforded three fractions corresponding to coproporphyrin esters (15%), harderoporphyrin esters [11],* (37%), and protoporphyrin esters (48%).

The ^{1}H n.m.r. spectrum of the fraction containing protoporphyrin esters exhibited 5 lines (ratio

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* The fraction containing harderoporphyrin ester was homogenous by h.p.l.c. Its n.m.r. spectrum and a molecular ion at m/e 650 supported a porphyrin structure with one vinyl group, and in the presence of Eu(fod)$_3$ *one* of the ' bridge ' methine-H n.m.r. signals shifted markedly downfield. These results are consistent with structure (20), the intermediate expected to lie between coproporphyrinogen IV and protoporphyrin XIII (11) proved in the sequal to be the final product. The tricarboxylic porphyrin ester (20) has recently been synthesised [15] and found to be indistinguishable from the product isolated from incubations of coproporphyrinogen IV (8) with duck-blood hemolysate.

1 : 3 : 1 : 1 : 2) in the region characteristic of the ' bridge ' protons: clearly a mixture was present, and this was confirmed when analytical h.p.l.c. revealed two peaks in the ratio *ca.* 1 : 1 (Figure). One of the peaks had a retention time identical to that of protoporphyrin IX dimethyl ester (7) and was inseparable from authentic (7) on co-injection. Moreover, when n.m.r. spectra of the mixture from the incubation were run in the presence of different
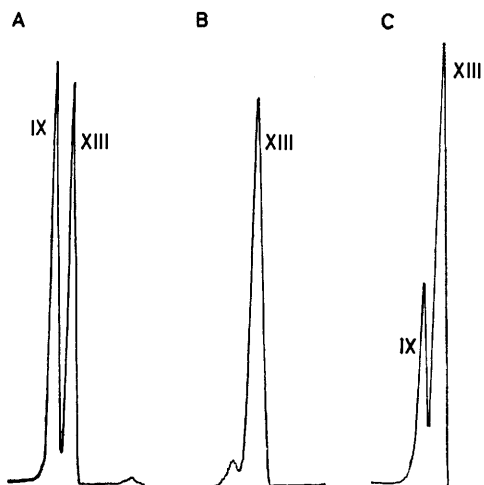
Separation of dimethyl esters of protoporphyrin-IX (7) and protoporphyrin-XIII (12) by h.p.l.c. (see Experimental section for conditions). Trace A, mixture of (7) and (12) from enzymic run on coproporphyrinogen IV (8); trace B, protoporphyrin XIII dimethyl ester (12) isolated from product giving trace A; trace C, co-injection of synthetic protoporphyrin XIII dimethyl ester (12) with mixture shown in trace A

concentrations of $Eu(fod)_3$,[16] one complete set of resonances shifted in exactly the manner found for authentic protoporphyrin IX dimethyl ester (7). This component of the mixture almost certainly came from endogenous porphyrin in the mitochondrial preparation.* The behaviour of the remaining set of resonances in the [1]H n.m.r. spectrum of the mixture led to the conclusion that they arise from protoporphyrin XIII dimethyl ester (12); so the enzymic product produced from coproporphyrinogen IV (8) is the corresponding acid (11). This follows from the observation that only three signals in the n.m.r. spectrum of the ester (12) were strongly shifted in the presence of $Eu(fod)_3$: (*a*) the resonances from the ester methyls; (*b*) the resonances of the propionate side chains; and (*c*) one of the ' bridge '-H atoms: † importantly, the vinyl resonances were unaffected.

Parallel work [17] using haemolysed blood from chickens also showed protoporphyrin XIII is the enzymic product from coproporphyrinogen IV and subsequently the same was found with a similar preparation from ducks' blood.[18] With *Euglena gracilis* [12b] we observed no significant conversion of coproporphyrinogen IV (8) into protoporphyrin XIII (11) with an enzyme preparation

* The alternative explanation that protoporphyrin IX (6) arises from contaminating coproporphyrin III in the synthetic coproporphyrin IV can be rejected since n.m.r. analysis proved <5% of other isomers were present, yet a contamination of >20% would be needed to account for the results.

which was shown readily to convert coproporphyrinogen III (1) into protoporphyrin IX (6).

This assignment of structure (12) to the ester of the enzymic product from coproporphyrinogen IV was confirmed by comparing the biosynthetically prepared sample with a specimen of protoporphyrin XIII dimethyl ester (12) prepared by unambiguous synthesis as follows. The symmetry in the target porphyrin again permitted the use of a MacDonald condensation, between the unsubstituted pyrromethane (21) and the dialdehyde [13] (15): acetoxyethyl side chains are convenient precursors of vinyl groups.[19]

Reductive methylation [20] of the β-free pyrrole (22) using formaldehyde–hydriodic acid–phosphinic acid gave the *N*-iodomethyl derivative (*cf.* reference 19) (25) of the required product (23) but the conversion (25) ⟶ (23) was easily achieved in high yield (85%) by acid-catalysed hydrolysis. The reductive methylation worked equally well on (24), the precursor of (22), shortening the overall route. The ester side chains of (23) were differentiated by the standard transformation (23) ⟶ (26) ⟶ (27) before lead tetra-acetate oxidation to produce the acetoxymethylpyrrole (28). Pyrromethane
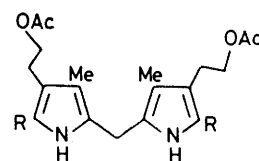
(21) R = H
(32) R = CO_2H
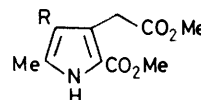
(22) R = H
(23) R = Me
(24) R = I
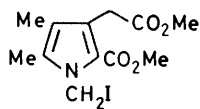
(25)

(26) R = CH_2Ph
(27) R = Me

(28)
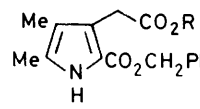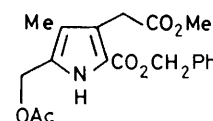
(29) was then obtained in 87% yield by heating (28) in aqueous acetic acid, a method found to be superior in this instance to the use of stannic chloride, or toluene-*p*-sulphinic, trifluoroacetic, or hydrochloric acids under various conditions. Specific reduction of the side-chain esters of (29) by diborane gave the diol (30) which was acetylated to yield the ester (31). Hydrogenolysis of (31) afforded the dicarboxylic acid (32).

For the synthesis of porphyrin (33), the acid (32) was decarboxylated by treatment with cold trifluoroacetic acid. The reaction was followed by u.v. spectroscopy and the product (21) was condensed *in situ* with the diformylpyrromethane (15) to give (33). The acetoxy-

† Addition of $Eu(fod)_3$ strongly shifts the signal from a ' bridge '-H when the ' bridge ' is flanked by *two* ester groups;[16] structure (12) is the only one of the four possibilities which has this feature.

ethyl side chains of (33) were transformed into chloro-ethyl groups by acid-catalysed methanolysis to yield (34) followed by reaction with mesyl chloride–lithium chloride in dimethylformamide.[19b] Elimination of hydrogen chloride from (35) to give protoporphyrin XIII ester (12) was accomplished by basic treatment of the zinc derivative [19a] of (33) followed by demetallation in acidic methanol.

The synthetic protoporphyrin XIII ester (12) was indistinguishable from the ester of the product produced enzymically from coproporphyrinogen IV (8). The comparison methods used were t.l.c., h.p.l.c. (see Figure), and u.v.–visible, i.r., and [1]H n.m.r. spectroscopy including the powerful test of mixing the natural and
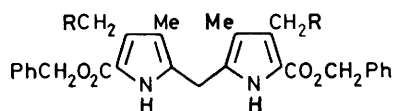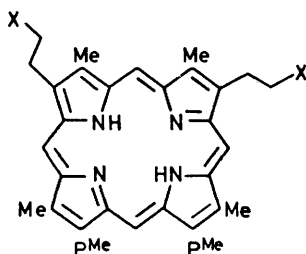

(29) R = CO$_2$Me

(30) R = CH$_2$OH

(31) R = CH$_2$OAc

(33) X = OAc

(34) X = OH

(35) X = Cl

synthetic samples followed by direct [1]H n.m.r. and also running spectra in the presence of Eu(fod)$_3$.

During the foregoing synthetic work,[21] related syntheses of protoporphyrin XIII ester (12) were reported from other laboratories.[17,18,22]

Conclusions.—The above enzymic and synthetic work shows that coproporphyrinogenase from beef liver accepts coproporphyrinogen IV (8) as a substrate and catalyses specific oxidative decarboxylation of the propionate side chains at C-2 and C-8 to produce, after oxidative work-up, protoporphyrin-XIII (11). That this is *not*, as suggested,[9] the product being produced from incorporation experiments with deaminase–cosynthetase involving aminomethylpyrromethanes [8] is shown by (*a*) the proof that protoporphyrin IX and protoporphyrin XIII esters (7) and (12) are distinguishable and separable; (*b*) the protoporphyrin IX ester (7) produced biosynthetically from substrate (5), ^{13}C-labelled at ○ and □ gives *four separate* [13]C-*resonances* (1 : 1 : 1 : 1) from the bridges; only three signals (1 : 2 : 1) are possible from protoporphyrin XIII ester (12) because of its

symmetry properties. All the earlier studies from this laboratory of the type III problem in porphyrin bio-synthesis involving pyrromethanes [7a] are thus confirmed.

EXPERIMENTAL

Except as noted below, general directions are given in references 16*b* and 23. Exposure of any of the substances to light was avoided as far as possible.

Dibenzyl 4,4'-Bis-(2-methoxycarbonylethyl)-3,3'-dimethyl-pyrromethane-5,5'-dicarboxylate (18).—2-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methyl-5-(acetoxymethyl)-pyrrole [14] (16) (3.93 g) in glacial acetic acid containing 20% water (35 ml) was heated at 90 °C during 60 min. Water (10 ml) was added, and the mixture was cooled to precipitate the pyrromethane (18). Two recrystallisations from methanol afforded the *pyrromethane* (2.50 g, 77.5%), m.p. 107.5—109 °C (Found: C, 68.3; H, 6.3; N, 4.4. C$_{35}$H$_{36}$N$_2$O$_8$ requires C, 68.4; H, 6.2; N, 4.55%), *m/e* 614 (M^+), $\nu_{max.}$ 3 440, 1 730, and 1 685 cm^{-1}, δ 1.97 (6 H, s, 2 × Me), 2.2—3.2 (8 H, m, 2 × pyrrCH$_2$CH$_2$CO), 3.55 (6 H, s, 2 × OMe), 3.71 (2 H, s, pyrr$_2$CH$_2$), 5.13 (4 H, s, 2 × OCH$_2$Ph), 7.21 (10 H, s, 2 × C$_6$H$_5$), and 9.72 (2 H, br s, NH).

4,4'-Bis-(2-methoxycarbonylethyl)-3,3'-dimethylpyrro-methane-5,5'-dicarboxylic Acid (19).—The foregoing dibenzyl ester (18) in ethanol (25 ml) containing palladium–charcoal (10%; 40 mg) was hydrogenated at room temperature and atmospheric pressure until uptake ceased (90 min). The filtered solution was partially evaporated and water was added to precipitate the *acid* (18) (210 mg, 75%), m.p. 175—180 °C (decomp.) (Found: C, 57.6; H, 6.0; N, 6.4. C$_{21}$H$_{26}$N$_2$O$_8$ requires C, 58.05; H, 6.0; N, 6.45%), $\nu_{max.}$ (Nujol) 3 350, 1 720, and 1 680 cm^{-1}, δ 1.9 (6 H, s, 2 × Me), 2.1—3.0 (8 H, m, 2 × pyrrCH$_2$CH$_2$CO), 3.56 (6 H, s, 2 × OMe), and 3.71 (2 H, s, pyrr$_2$CH$_2$), *m/e* 346 (M^+ − 2 × CO$_2$, no parent ion).

Coproporphyrin IV Tetramethyl Ester (14).—The foregoing dicarboxylic acid (18) (80 mg) was heated to reflux under N$_2$ in dry dimethylformamide (6 ml) during 30 min and the solution was then evaporated. After the solid residue had been kept at 0.1 mmHg during 30 min, it was dissolved in glacial acetic acid (70 ml) and added to 4,4'-dimethyl-3,3'-bis-(2-methoxycarbonylethyl)pyrromethane-5,5'-biscarbaldehyde [13] (15) (55 mg) in glacial acetic acid (70 ml). Hydriodic acid (0.3 ml) in glacial acetic acid (70 ml) was added to the stirred mixture followed after 30 min by sodium acetate (1.3 g) in glacial acetic acid (20 ml). Air was then blown into the solution during 20 h. The solvent was largely evaporated off and the residue mixed with water (50 ml) and extracted with chloroform. The organic layer was washed with aqueous 2N-potassium perborate (50 ml) then with water and evaporated. The residue was chromatographed on silica gel and the fraction containing the coproporphyrin ester crystallised from methanol–chloroform (76 mg, 66%), m.p. 182—184 °C (lit.,[24] 185 °C) (Found: C, 67.6; H, 6.6; N, 7.9. C$_{40}$H$_{46}$N$_4$O$_8$ requires C, 67.8; H, 6.3; N, 7.9%), *m/e* 710 (M^+), $\lambda_{max.}$ 403, 500, 534, 569, and 620 nm, δ 3.2 and 4.3 (each 8 H, m, 4 × porphCH$_2$CH$_2$CO), 3.47 (6 H, s, 2 × Me), 3.63 (6 H, s, 2 × OMe), 9.81 (1 H, s, H-5), 9.85 (2 H, s, H-10 and H-20), and 9.95 (1 H, s, H-15). Analysis [25] by h.p.l.c. excluded the presence of isomeric coproporphyrin esters.

Action of Coproporphyrinogenase on Coproporphyrinogen IV (8).—Typically coproporphyrin IV tetramethyl ester

(5 mg) in tetrahydrofuran (5 ml) and aqueous 2N-potassium hydroxide (6 ml) was stirred under nitrogen for 24 h at 20 °C. The coloured aqueous layer was separated, dissolved tetrahydrofuran was removed under reduced pressure, and the solution was neutralised with 10% hydrochloric acid (ca. 2.5 ml). After the solid had been redissolved with a few drops of 2N-aqueous potassium hydroxide, freshly ground sodium amalgam (3%; ca. 10 g) was added, and the mixture was shaken in the dark under nitrogen until it was colourless (ca. 5 min). The solution was decanted from the amalgam, and added to beef-liver mitochondria [16b] (100 ml; from ca. 170 g liver) and 0.2M-Tris *-HCl buffer (70 ml; pH 7.8). The incubate, in

Water was then added and the suspension was kept for 16 h at 0 °C to give a further crop (0.1 g) of the pyrrole (25) (total yield (77%), m.p. 165—166 °C (decomp.). $\nu_{max.}$ (Nujol) 1 690 cm^{-1}, no N–H stretch, $\lambda_{max.}$ 287 nm, δ ([$^2H_6$]DMSO) 1.89 and 2.21 (each 3 H, s, 2 × Me), 3.61 (2 H, s, pyrr-CH$_2$CO), 3.71 (3 H, s, OMe), and 5.52 (2 H, s, NCH$_2$I).

The pyrrole (25) was also prepared in 74% yield from the β-free pyrrole [26] (22) by a similar procedure.

A solution of the foregoing crude N-iodomethylpyrrole (25) (13 g) in ethanol (100 ml), water (300 ml), and concentrated hydrochloric acid (10.6 ml) was heated under reflux for 0.5 h. The solution was cooled at 0 °C for 2 h, and the solid was collected, dried, and esterified by heating

TABLE

Enzymic runs using coproporphyrinogenase

Isomer of coproporphyrinogen used (weight/mg)	Incubation time/h	% Recovery of porphyrin	Yield of different fractions in total porphyrin recovered (%)		
			Coproporphyrin fraction	Harderoporphyrin fraction	Protoporphyrin fraction
III (5)	1	40	69	1	30
IV (5)	1	46	51	46	3
IV (5)	23	38	15	37	48
IV (10)	24	26	55	6	39
IV (20)	22	39	54	9	37

2 × 250 ml-Erlenmeyer flasks, each fitted with a loose cotton plug, was swirled gently at 38 °C for 22 h (see Table). Ethyl acetate–acetic acid (3 : 1 v/v) was added and the mixture was kept in dim light for 15 min. The mitochondria were removed by centrifugation and the porphyrins were transferred from EtOAc–HOAc into 10% HCl and then into ether as described earlier.[16b] The crude porphyrins were esterified at 25 °C in methanol (20 ml) containing H$_2$SO$_4$ (1 ml) and the esters were separated by preparative t.l.c. on silica gel, eluting with 5% Et$_2$O in CH$_2$Cl$_2$, to give protoporphyrin dimethyl ester (R_F 0.59), harderoporphyrin trimethyl ester (R_F 0.44), and coproporphyrin tetramethyl ester (R_F 0.31). The proportions obtained in various incubations are summarised in the Table.

The protoporphyrin ester fraction was separated into two components corresponding to isomers IX and XIII by h.p.l.c. on a pair of 10μ Porasil columns (Waters Associates) eluting with wet ether–hexane (2 : 3 v/v) (the wet ether being prepared from dry ether and water-saturated ether in a 3 : 2 v/v ratio). Numerous injections were made, each of ca. 12 μg porphyrin ester in 5 μl CH$_2$Cl$_2$, and the peaks corresponding to the two isomers were collected separately and pooled until sufficient was obtained for ^{1}H n.m.r. analysis. Protoporphyrin XIII dimethyl ester (Found: m/e 590.288 6. C$_{36}$H$_{38}$N$_4$O$_4$ requires M^+, 590.289 3) had $\lambda_{max.}$ (CHCl$_3$) 634, 579, 544, 508, and 404 nm, δ 3.20 and 4.35 (each 4 H, t, J 6 Hz, 2 × porphCH$_2$CH$_2$CO), 3.56 and 3.58 (12 H, 4 × porphMe), 3.65 (6 H, s, 2 × OMe), 6.20 (4 H) and 8.21 (2 H) (2 × CH$_2$=CH), and 10.01, 10.15, and 10.17 (1 H, 1 H, 2 H, 4 × meso-H).

Methyl 4,5-Dimethyl-3-(methoxycarbonylmethyl)pyrrole-2-carboxylate (23).—Acetic anhydride (11.6 ml) was added dropwise with stirring at 0—5 °C to hydriodic acid (7.25 ml) followed by phosphinic acid (2 ml). After the solution had warmed to 20 °C, the iodopyrrole [26] (24) (1 g) was added, and when the iodine colour had faded to orange, paraformaldehyde (0.26 g) was added in small portions. The mixture was heated at 60—65 °C for 1 h and after being kept at 20 °C for 2 h, the precipitate (0.69 g) was filtered off and the filtrate was evaporated to dryness (finally 0.6 mmHg).

* Tris(hydroxymethyl)aminomethane.

under reflux with anhydrous methanolic 7% hydrogen chloride (60 ml) for 0.5 h. After cooling, the crystalline product was collected and the filtrate was evaporated to dryness to give a second crop of the dimethylpyrrole (23) (total 7.05 g; 85%), m.p. 121.5—122 °C (Found: C, 58.4; H, 6.8; N, 6.2. C$_{11}$H$_{15}$NO$_4$ requires C, 58.7; H, 6.7; N, 6.2%), $\nu_{max.}$ 3 440, 1 725, and 1 695 cm^{-1}, $\lambda_{max.}$ 286 nm, δ 1.9 and 2.18 (each 3 H, s, Me-pyrr), 3.67 (3 H, s, OMe), and 3.78 (5 H, s, OMe and pyrrCH$_2$CO).

Benzyl 3-(Benzyloxycarbonylmethyl)-4,5-dimethylpyrrole-2-carboxylate (26).—The foregoing dimethyl ester (23) (5.1 g) in benzyl alcohol (23 ml) in a 50-ml conical flask under a stream of nitrogen was heated to boiling (ca. 200 °C) on a hot plate. A 1M-solution of sodium benzyloxide in benzyl alcohol (0.4 ml) was added and when the vigorous evolution of methanol had slowed down the solution was heated again to boiling and more sodium benzyloxide solution (0.4 ml) was added. Additions were repeated until no further methanol was evolved and the cooled solution was poured into methanol–water (1 : 1; 255 ml) and kept at 0 °C for 3 h to precipitate the dibenzyl ester (26) (7.4 g, 85%), m.p. 89—90 °C (Found: C, 73.3; H, 6.2; N, 3.7. C$_{23}$H$_{23}$NO$_4$ requires C, 73.3; H, 6.2; N, 3.7%), $\nu_{max.}$ 3 440, 1 725, and 1 675 cm^{-1}, $\lambda_{max.}$ 287 nm, δ 1.86 and 2.12 (each 3 H, s, Me-pyrr), 3.79 (2 H, s, pyrrCH$_2$CO), 4.98 and 5.13 (each 2 H, s, OCH$_2$Ph), and 7.1 (10 H, s, 2 × Ph).

Benzyl 3-(Methoxycarbonylmethyl)-4,5-dimethylpyrrole-2-carboxylate (27).—The foregoing dibenzyl ester (26) 6.9 g) in dry tetrahydrofuran (8 ml) and dry methanol (32 ml) was treated with a 1M-solution of sodium methoxide in methanol (16 ml). After 3.5 h at 20 °C water was added and extraction with ether gave the product which was used for the next stage without purification. A small sample was chromatographed on alumina using ether as eluant to afford the pure ester (27), m.p. 66—67 °C (Found: C, 66.9; H, 6.3; N, 4.5. C$_{17}$H$_{19}$NO$_4$ requires C, 67.8; H, 6.3; N, 4.65%), $\nu_{max.}$ 3 440, 1 725m, and 1 675 cm^{-1}, $\lambda_{max.}$ 287 nm, δ 1.9 and 2.13 (each 3 H, s, Me-pyrr), 3.56 (3 H, s, OMe), 3.77 (2 H, s, pyrrCH$_2$CO), 5.23 (2 H, s, OCH$_2$Ph), and 7.3 (5 H, s, Ph).

Benzyl 5-Acetoxymethyl-3-(methoxycarbonylmethyl)-4-

methylpyrrole-2-carboxylate (28).—The foregoing crude methyl benzyl ester (27) (15 g) was stirred overnight at 20 °C with lead tetra-acetate (25 g) in acetic acid (1.5 l). The solvent was evaporated off and the residue partitioned between chloroform (1.84 l) and an excess of 10% w/v aqueous sodium hydrogencarbonate. The product obtained by evaporation of the organic layer was recrystallised from benzene–light petroleum to give the *pyrrole* (28) (7 g, 40%), m.p. 122—124 °C (Found: C, 63.3; H, 5.8; N, 3.8. $C_{19}H_{21}NO_6$ requires C, 63.5; H, 5.85; N, 3.9%), $v_{max.}$ 3 420, 1 725, and 1 695 cm⁻¹, $\lambda_{max.}$ 277.5 nm, δ 2.02 (3 H, s, OAc), 2.04 (3 H, s, Me-pyrr), 3.59 (3 H, s, OMe), 3.81 (2 H, s, pyrrCH₂CO), 5.01 (2 H, s, pyrrCH₂OAc), 5.28 (2 H, s, OCH₂Ph), and 7.3 (5 H, s, Ph).

Dibenzyl 3,3′-Dimethyl-4,4′-bis(methoxycarbonylmethyl)-pyrromethane-5,5′-dicarboxylate (29).—The foregoing acet-oxymethylpyrrole (3 g) in aqueous acetic acid (30 ml, 20% H_2O) was heated on a steam-bath for 1 h. After cooling to 20 °C the solution was poured into water (33 ml) and extracted with ether to give the *pyrromethane* (29) which was recrystallised from ethanol (2.1 g, 87.5%), m.p. 108—111 °C (Found: C, 67.3; H, 5.9; N, 4.6. $C_{33}H_{34}N_2O_8$ requires C, 67.6; H, 5.8; N, 4.8%), M^+ 586, $v_{max.}$ 3 430, 3 330, 1 740, and 1 690 cm⁻¹, $\lambda_{max.}$ 294 (shoulder, 278) nm, δ 1.93 (6 H, s, 2 × Me-pyrr), 3.56 (6 H, s, 2 × OMe), 3.74 (2 H, s, pyrr₂CH₂), 3.78 (4 H, s, 2 × pyrrCH₂CO), 5.22 (4 H, s, 2 × OCH₂Ph), and 7.3 (10 H, s, 2 × Ph).

Dibenzyl 3,3′-Bismethyl-4,4′-bis-(2-hydroxyethyl)pyrro-methane-5,5′-dicarboxylate (30).—The pyrromethane (29) (500 mg) in dry tetrahydrofuran (10 ml) was reduced by stirring for 16 h with diborane in tetrahydrofuran (80 ml) under nitrogen at 20 °C. [The diborane was generated by adding sodium borohydride (7.82 g) in diglyme (200 ml) dropwise to a stirred solution of boron trifluoride–ether (50 ml) (redistilled) and was carried over by a slow stream of nitrogen into dry tetrahydrofuran (150 ml).]

The mixture was then refluxed for 3.5 h and on cooling to 20 °C was treated under nitrogen with dry methanol before evaporation. Trituration of the residue with ether gave the *diol* (30) (0.32 g, 60%), m.p. 121—123 °C (Found: m/e 530. $C_{31}H_{34}N_2O_6$ requires M^+, 530), $v_{max.}$ 3 433, 3 330, and 1 680 cm⁻¹; $\lambda_{max.}$ 294 (shoulder 277) nm; δ 1.98 (6 H, s, 2 × Me-pyrr), 2.99 and 3.72 (each 4 H, t, *J* 6 Hz, 2 × pyrrCH₂CH₂-OH), 3.81 (2 H, s, pyrr₂CH₂), 5.24 (4 H, s, 2 × OCH₂Ph), and 7.3 (10 H, s, 2 × Ph).

3,3′-Dimethyl-4,4′-bis-(2-acetoxyethyl)pyrromethane-5,5′-dicarboxylic Acid (32).—The foregoing diol (30) (0.6 g) was treated with acetic anhydride (10 ml) and pyridine (8.5 ml) under nitrogen for 1.5 h and then poured into iced water (85 ml). The mixture was extracted several times with chloroform and the combined organic extracts were washed with 1M-hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water. Evaporation of the solvent gave the diacetate (31) as a gum, $v_{max.}$ 3 430 and 1 725—1 685 cm⁻¹, $\lambda_{max.}$ 293 (shoulder 278) nm, δ 1.97 and 2.00 (each 6 H, s, 2 × Me-pyrr and 2 × AcO), 3.02 and 4.13 (each 4 H, t, *J* 6 Hz, 2 × pyrrCH₂CH₂OAc), 3.78 (2 H, s, pyrr₂CH₂), 5.23 (4 H, s, 2 × OCH₂Ph), and 7.3 (10 H, s, 2 × Ph). The pyrromethane ester (31) (0.9 g) in warm ethanol (180 ml) was hydrogenated over 10% palladium–charcoal (0.09 g) at 20 °C and 760 mmHg. After 4 h, the filtered solution was evaporated to dryness and trituration of the residue with light petroleum gave the *dicarboxylic acid* (32) (0.54 g, 85%), m.p. 168—170 °C from ethanol (Found: C, 57.9; H, 6.1; N, 6.3. $C_{21}H_{28}N_2O_8$ requires C, 58.05;

H, 6.0; N, 6.45%), $v_{max.}$ (Nujol) 3 330, 2 800 (broad), and 1 740 cm⁻¹, $\lambda_{max.}$ 288 nm (shoulder 275), δ ([²H₆]DMSO) 1.97 and 1.98 (each 6 H, s, 2 × Me-pyrr and 2 × AcO), 2.94 and 4.05 (each 4 H, t, *J* 6 Hz, 2 × pyrrCH₂CH₂OAc), and 3.77 (2 H, s, pyrr₂CH₂).

2,8-Bis-(2-acetoxyethyl)-13,17-bis-(2-methoxycarbonylethyl)-3,7,12,18-tetramethylporphin (33).—The pyrromethanedi-carboxylic acid (31) (0.05 g) was treated with trifluoroacetic acid (5 ml) for 1.5 h at 0 °C and the diformylpyrromethane (15) (0.05 g) in 3 : 1 v/v chloroform–methanol (25 ml) was then added. After the solution had been stirred at 20 °C for 4 h, it was washed with sodium hydrogencarbonate solution and water, and dried. Chromatography of the product from evaporation on silica gel gave the *porphin* (33) (28.2 mg, 24%), m.p. 209—211 °C (from chloroform–methanol) (Found: C, 67.6; H, 6.4; N, 7.95%; m/e, 710.332 9. $C_{40}H_{46}N_4O_8$ requires C, 67.6; H, 6.5; N, 7.9%; M^+, 710.331 4), $v_{max.}$ 3 320 and 1 740 cm⁻¹, $\lambda_{max.}$ 624, 571, 534, and 499 nm, δ 2.03 (6 H, s, 2 × Ac), 3.27 and 4.82 (each 4 H, t, *J* 6 Hz, porphCH₂CH₂OAc), 3.58 (6 H, s, 2 × OMe), 3.62 and 3.63 (12 H, s, 4 × Me-porph), 4.38 (8 H, m, porphCH₂CH₂CO), and 10.04 (4 H, s, 4 × meso-H).

2,8-Bis-(2-hydroxyethyl)-13,17-bis-(2-methoxycarbonyl-ethyl)-3,7,12,18-tetramethylporphin (34).—The foregoing bis-acetoxyethyl porphin (33) (0.22 g) in sulphuric acid in methanol (5% w/v; 20 ml) was kept for 16 h at 20 °C, then poured into iced water (10 ml), basified with aqueous 2N-ammonia, and extracted with chloroform. The organic phase was evaporated and the residue chromatographed on silica gel using chloroform–methanol (20 : 1 v/v). The *hydroxyethylporphin* (34) crystallised from chloroform–methanol (19 mg, 98%), m.p. 214—216 °C (Found: M^+, 626.307 0. $C_{36}H_{42}N_4O_6$ requires M^+, 626.310 3), $v_{max.}$ 3 310 and 1 728 cm⁻¹, $\lambda_{max.}$ (CHCl₃) 622, 570, 535, 500, and 375 nm, δ ([²H₅]pyridine) 3.14 (4 H, t, *J* 6 Hz, 2 × CH₂CO), 3.23 (6 H, s, 2 × OMe), 3.32 and 3.33 (12 H, s, 4 × Me-porph), 4.21 (12 H, m, 2 × porphCH₂CH₂OH and 2 × porphCH₂), and 9.93 (1 H, s), 10.11 (2 H, s), and 10.18 (1 H, s) 4 × meso-H).

2,8-Bis-(2-chloroethyl)-6,7-bis-(2-methoxycarbonylethyl)-3,7,12,18-tetramethylporphin (35).—The foregoing bis-hydroxyethylporphin (34) (16 mg) in dimethylformamide (5 ml) was treated with anhydrous lithium chloride (1.0 g) and methanesulphonyl chloride (1.2 ml) at 70—75 °C for 40 min. The cooled solution was basified with aqueous 2N-ammonia and the product, extracted with dichloromethane, was chromatographed on silica gel (dichloromethane–benzene) to give the *chloroethylporphyrin* (35) which was recrystallised from ether–hexane (7.3 mg, 44%) (Found: m/e, 662.245 0. $C_{36}H_{40}Cl_2N_4O_4$ requires M^+, 662.242 6), $\lambda_{max.}$ (Et₂O) 619, 566, 529, 497, and 389 nm, δ 3.25 (4 H, t, *J* 6 Hz, 2 × CH₂CO), 3.48 (6 H, s, 2 × OMe), 3.64 and 3.55 (12 H, 4 × Me-porph), 4.29 (12 H, m, 2 × porphCH₂CH₂Cl and 2 × porphCH₂), and 9.79, 9.86, and 9.96 (1 H s, 1 H s, 2 H s, 4 × meso-H).

2,8-Divinyl-13,17-bis-(2-methoxycarbonylethyl)-3,7,12,18-tetramethyl-1,4-divinylporphin (12).—The dichloroethyl-porphin (35) (7.3 mg) in dichloromethane (3 ml) was treated with a saturated aqueous solution of zinc acetate (0.5 ml) and warmed for 5 min. Evaporation gave the metalloporphyrin which in tetrahydrofuran (0.5 ml) was treated with 2M-potassium t-butoxide in t-butyl alcohol (5.0 ml). After stirring the solution under nitrogen for 4 days at 20 °C it was poured into ethyl acetate (20 ml) and acidified with glacial acetic acid. The organic layer was

washed with water and the solid remaining after evaporation was dissolved in tetrahydrofuran and treated with sulphuric acid–methanol (5% w/v, 15 ml) for 16 h at 0 °C. The solution was then basified with aqueous 2N-ammonia and extracted with chloroform. After washing with water, the product from the organic extracts was chromatographed on silica gel (chloroform–methanol, 20 : 1 v/v) to give the protoporphyrin-ester (12) which crystallised from chloroform–methanol (5.9 mg, 90%), m.p. 210—212 °C [lit. 198—200,[17] 208—200,[18] and 228—229, 180 °C (decomp.)[21]] (Found: C, 71.8; H, 6.45; N, 9.05%; m/e 590.288 8. $C_{36}H_{38}N_4O_4\cdot CH_4O$ requires C, 71.35; H, 6.8; N, 8.9%; $C_{36}H_{38}N_4O_4$ requires M^+ 590.289 2), $\nu_{max.}$ 3 320, 2 930, 1 740, 1 630, and 1 110 cm^{-1}, $\lambda_{max.}$ (CHCl$_3$) 634, 579, 542, 508, and 405 nm, δ 3.22 and 4.35 (each 4 H, t, J 6 Hz, 2 × COCH$_2$CH$_2$-porph), 3.59 and 3.61 (12 H, 4 × Me-porph), 6.21 (4 H) and 8.25 (2 H) (2 × CH$_2$=CH), and 10.01 (1 H), 10.14 (1 H), and 10.16 (2 H) (4 × meso-H).

This ester was identical to the sample prepared biosynthetically (see Figures). Exact comparisons were made by visible spectroscopy, h.p.l.c. on 10μ Porasil with 3 : 2 hexane–ether as eluant (the ether being a 3 : 2 mixture of dry ether and water-saturated ether), and n.m.r. of pure and mixed samples with and without added shift reagent [Eu(fod)$_3$]. Addition of 0.35 mg Eu(fod)$_3$ to a solution of 0.10 mg of porphyrin ester (12) in 0.3 ml of CDCl$_3$ caused one ' bridge '-H signal to move downfield by 0.75 p.p.m.

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