Biosynthesis of Porphyrins and Related Macrocycles. Part VIII.[1,2] Enzymic Decarboxylation of Uroporphyrinogen-III: Structure of an Intermediate, Phyriaporphyrinogen-III, and Synthesis of the Corresponding Porphyrin and of Two Isomeric Porphyrins

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A porphyrin formed when porphobilinogen (1) is incubated with an enzyme system from avian erythrocytes is proved to be heptacarboxylic and of the type III series; its properties correspond with those of phyriaporphyrin-III. Two of the four possible structures for this porphyrin are eliminated by preparing it from [2,11-$^{13}C_2$]porphobilinogen and carrying out a ^{13}C n.m.r. analysis of the product, with use of a praseodymium shift reagent.

Synthesis of all four heptacarboxylic porphyrins shows that the natural product has undergone specific decarboxylation of the acetic acid side chain on ring D to form (after aromatisation) the ring D methylporphyrin [as (12)]. Our results are consistent with the view that the initial enzymic decarboxylation on the pathway from uroporphyrinogen-III (2) to coproporphyrinogen-III (4) occurs at ring D.

Studies are outlined leading to effective large-scale syntheses of key pyrrolic building blocks for this and other work and several methods of general interest in this area are reported.

EARLIER work had demonstrated that the biosynthetic pathway [3] from porphobilinogen (1) to haem (7) involves stepwise decarboxylation of the four acetic acid side-chains of uroporphyrinogen-III (2). Porphyrins had been detected having the properties of hepta-, hexa-, and penta-carboxylic acids [4-8] (Scheme 1); these are derived by chemical oxidation of the corresponding porphyrinogens (hexahydroporphyrins) which are the true biosynthetic intermediates. One well characterised substance of unknown structure which had been isolated was a heptacarboxylic acid named [8a] phyriaporphyrin-III; this name has superseded earlier ones, *viz.* porphyrin-208 [8b] and pseudouroporphyrin.[8c] Phyriaporphyrin-III yielded coproporphyrin-III (5) on decarboxylation and there was good evidence [8a] for biosynthetic conversion of the corresponding phyriaporphyrinogen-III into protoporphyrin-IX (6).

Our interest in this area arose (*a*) from the need for heptacarboxylic porphyrins in concurrent studies [9] on the biosynthesis of vitamin B_{12} and (*b*) from our incubations of porphobilinogen (1) with the enzyme system from avian erythrocytes [10] which had been freed from ferrochelatase,[3] the enzyme responsible for iron insertion into protoporphyrin-IX (6) to form haem (7). These runs [11] consistently yielded † a separable mixture of uroporphyrin-III (3), coproporphyrin-III (5), protoporphyrin-IX (6), and a porphyrin isolated as its methyl ester, $C_{46}H_{52}N_4O_{14}$. This composition corresponds to the isolated porphyrin being a heptacarboxylic acid; confirmation was gained from the n.m.r. spectrum of the methyl ester and, more rigorously, by forming the ester by use of a 1 : 1 mixture of methanol and trideuteriomethanol [12] (CD_3OH). An octet of molecular ions was observed in the mass spectrum of the product with relative intensities corresponding to the calculated binomial series (1 : 7 : 21 : 35 : 35 : 21 : 7 : 1). Acid-catalysed decarboxylation of the heptacarboxylic porphyrin afforded coproporphyrin-III (4), and therefore it must be one of the four monomethyl porphyrins (11)—(14). Furthermore, the isolated amount of this heptacarboxylic porphyrin was less when long incubation times were used, in agreement with the corresponding porphyrinogen being converted enzymically into porphyrinogens appearing later on the biosynthetic pathway.

All the reported properties of phyriaporphyrin-III correspond closely with the more extensive data outlined above and there is little doubt that we are handling the same substance.

Two of the structures (11)—(14) for phyriaporphyrin-III were eliminated by the following studies based on ^{13}C labelling. The porphyrin was prepared enzymically from [2,11-$^{13}C_2$]porphobilinogen [11] [(8)—(10)] carrying 90 atom % ^{13}C at each labelled site; this corresponds to 81% of doubly-labelled molecules (8) and *ca.* 9% of each of the singly-labelled species (9) and (10). No matter

† In all such experiments, the initially formed porphyrinogens were oxidised by air and light to give the stable porphyrins for isolation.

[1] Part VII, A. R. Battersby, M. Ihara, E. McDonald, J. Saunders, and R. J. Wells, *J.C.S. Perkin I*, 1976, 283.

[2] Preliminary account, A. R. Battersby, E. Hunt, M. Ihara, E. McDonald, J. B. Paine III, F. Satoh, and J. Saunders, *J.C.S. Chem. Comm.*, 1974, 994.

[3] Reviewed by A. R. Battersby and E. McDonald in 'Falk's Porphyrins and Metalloporphyrins,' 2nd edn., ed. K. M. Smith, Elsevier, Amsterdam, 1975.

[4] L. Bogorad and S. Granick, *Proc. Nat. Acad. Sci. U.S.A.*, 1953, **39**, 1176.

[5] E. I. B. Dresel and J. E. Falk, *Biochem. J.*, 1956, **63**, 80 and 388.

[6] D. Mauzerall and S. Granick, *J. Biol. Chem.*, 1958, **232**, 1141.

[7] R. B. Frydman, M. L. Tomaro, A. Wanschelbaum, and B. Frydman, *F.E.B.S. Letters*, 1972, **26**, 203.

[8] (*a*) A. M. del C. Batlle and M. Grinstein, *Biochim. Biophys. Acta*, 1964, **82**, 1, 13 and references therein; (*b*) M. Grinstein, S. Schwartz, and C. J. Watson, *J. Biol. Chem.*, 1945, **157**, 323; (*c*) J. E. Falk, E. I. B. Dresel, A. Benson, and B. C. Knight, *Biochem. J.*, 1956, **63**, 87.

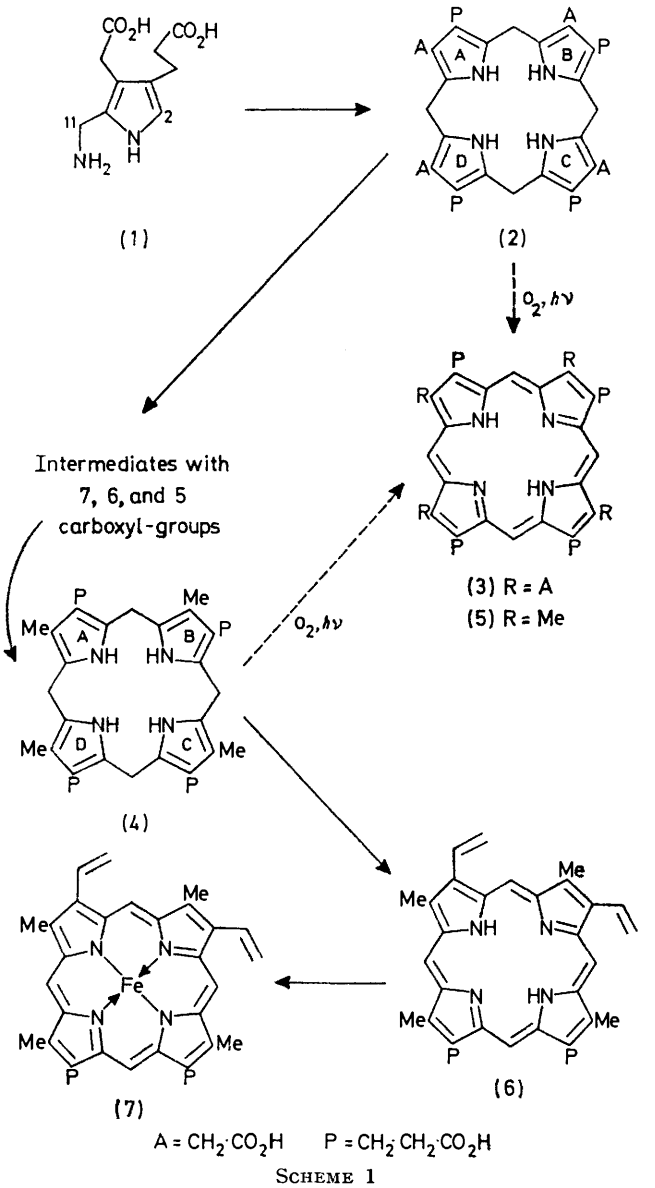
[9] A. R. Battersby, M. Ihara, E. McDonald, F. Satoh, and D. C. Williams, *J.C.S. Chem. Comm.*, 1975, 436 and references therein; see also A. I. Scott, N. Georgopapadakou, K. S. Ho, S. Kloize, E. Lee, S. L. Lee, G. H. Temme III, C. A. Townsend, and I. M. Armitage, *J. Amer. Chem. Soc.*, 1975, **97**, 2548.

[10] *E.g.* D. Shemin, T. Abramsky, and C. S. Russell, *J. Amer. Chem. Soc.*, 1954, **76**, 1204 and ref. 5 above.

[11] Part VI, A. R. Battersby, G. L. Hodgson, E. Hunt, E. McDonald, and J. Saunders, *J.C.S. Perkin I*, 1976, 273; A. R. Battersby, E. Hunt, and E. McDonald, *J.C.S. Chem. Comm.*, 1973, 442.

[12] E. Hunt and H. R. Morris, *Biochem. J.*, 1973, **135**, 833.

by what mechanism the type-III macrocycle [*e.g.* (2)] is built,* the labelling pattern for phyriaporphyrin-III illustrated in structures (11)—(14) must inevitably be produced. Simple calculation shows that *ca.* 43% of the phyriaporphyrin-III molecules carry all eight labels ($^{13}C_8$ species) and *ca.* 38% carry seven ^{13}C atoms, *i.e.* they lack either one of the ^{13}C atoms at a *meso*-bridge or one of those in the pyrrolic rings.

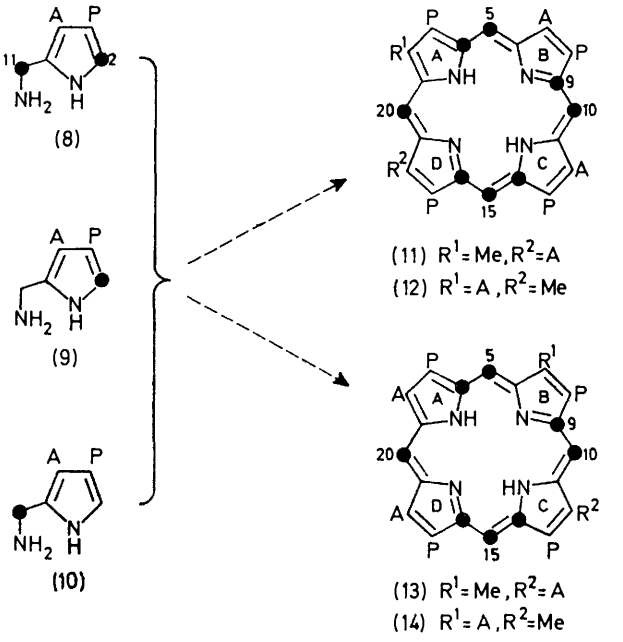


SCHEME 1

Work in this laboratory has shown that two directly bonded ^{13}C atoms in a porphyrin macrocycle show a 72 Hz splitting [11] whereas two ^{13}C atoms set in the same molecule, as at sites C-5 and C-9 [or in an equivalent relationship; see (11)], give rise to a 5.5 Hz splitting.[1] It follows that the splitting pattern for the *major* ^{13}C n.m.r. signals from the four *meso*-bridges is predicatable.

* For work which precisely defines the characteristics of the rearrangement process, see ref. 11.

As a result, unambiguous assignments of signals to bridge carbon atoms can be made; the 5.5 Hz triplet



SCHEME 2

observed in the ^{13}C n.m.r. spectrum of labelled phyriaporphyrin-III is thus from C-20, the 72 Hz triplet arises from C-15 and the double doublets (J 72 and 5.5 Hz) correspond to C-5 and C-10 which must be considered together.

When the praseodymium shift reagent Pr([$^2H_9$]fod)$_3$ was added in portions to a solution of labelled phyriaporphyrin-III methyl ester in chloroform, the signals from the four *meso*-bridge carbon atoms were affected as shown in Figure 1. The shift reagent had been shown [11] to affect most strongly the ^{13}C signals from *meso*-bridges

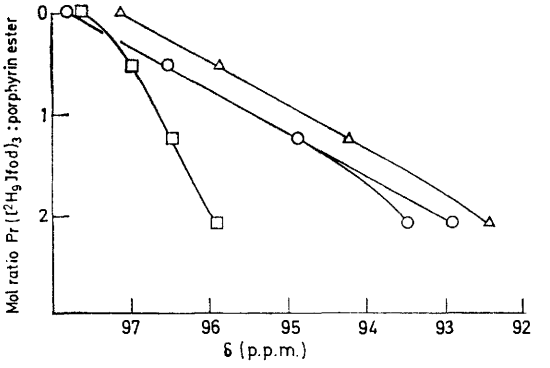


FIGURE 1 Effect of praseodymium shift reagent on ^{13}C signals from *meso*-bridges of ^{13}C-enriched phyriaporphyrin-III heptamethyl ester (74); (○) from C-5 and from C-10; (△) from C-15; (□) from C-20

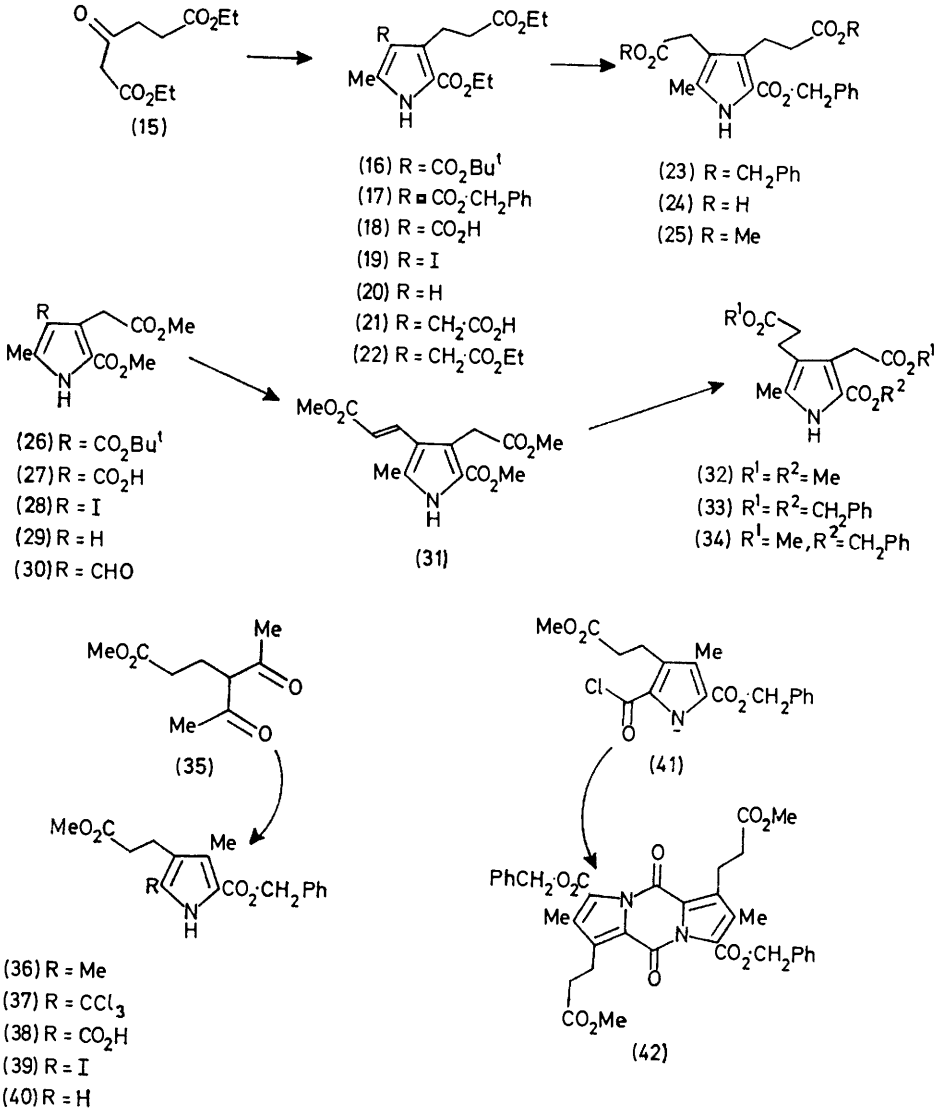
flanked by two ester groups, and this had earlier been shown to be so for ^1H n.m.r. spectroscopy.[13] Figure 1

[13] M. S. Stoll, G. H. Elder, D. E. Games, P. O'Hanlon, D. S. Millington, and A. H. Jackson, *Biochem. J.*, 1973, **131**, 429.

shows that the signal already assigned to C-20 was shifted far less, for a given quantity of praesodymium reagent, than were the signals from C-5, -10, and -15, and therefore structures (11) and (12) were strongly favoured for phyriaporphyrin-III over structures (13) and (14). The decision between the former pair was made by synthesis of both porphyrins.

The two key building blocks (25) and (34) for these syntheses were also needed for several other projects, and much improved methods have therefore been developed which are now routinely used for their preparation on 50—200 g scales. Close attention has been given to achieving high yields and to simplicity of operation for all the compounds described in this paper and the methods should be generally useful. If a material has been prepared previously in other ways, reference is given with the appropriate experimental directions.

Diethyl β-oxoadipate (15), more efficiently prepared *via* the magnesium ethoxide enolate of ethyl acetoacetate, gave the pyrrole (16) by a known Knorr synthesis.[14] This product has the advantage over the benzyl ester (17) used earlier that deprotection can be carried out essentially quantitatively on a large scale to give the crystalline acid (18) by using trifluoroacetic acid in boiling 1,2-dichloroethane. A high-yielding process for two-phase iodination of the acid (18) then gave the iodopyrrole (19) and we believe this procedure will be widely applicable; yields >90% have been obtained consistently for both



α- and β-iodopyrroles. The product (19) was used directly in MacDonald's reductive alkylation process[15] with glyoxylic acid since the iodopyrrole is rapidly reduced in the reaction medium to afford the β-free system (20). For this purpose, it is best to prepare the reducing agent from phosphorous acid and acetic acid rather than the original acetic anhydride. The acid (21)

[14] A. Treibs and K. Hintermeier, *Chem. Ber.*, 1954, **87**, 1167.
[15] M. W. Roomi and S. F. MacDonald, *Canad. J. Chem.*, 1970, **48**, 139.

was obtained thereby from the iodopyrrole (19) in >90% yield and esterification to the triester [16] (22) was essentially quantitative with ethanol–sulphuric acid containing triethyl orthoformate.

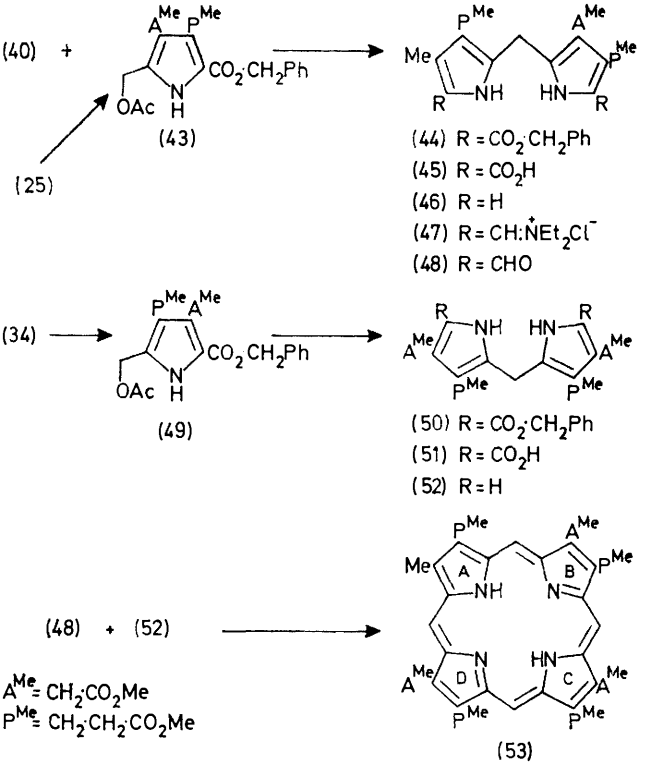
The transbenzylation method used in our earlier work [17] was unreliable and the proportions formed of the tribenzyl ester (23) and diacid (24) varied considerably. A rapid high-temperature ester exchange process (base-catalysed), initially developed for another purpose,[18] is consistently effective in giving 80—95% yields of the tribenzyl ester (23), from which the required dimethyl monobenzyl ester (25) was obtained by selective base-catalysed exchange (methoxide–methanol) at room temperature. The rate of this exchange depends on the structure of the pyrrolic ester involved but high yields of the required dimethyl monobenzyl esters have been obtained in all cases by this procedure.

The improved route to the isomeric pyrrole (34) again started with a t-butyl ester (26) which was converted as in the foregoing sequence *via* (27) into the iodopyrrole (28). This was reduced with sodium iodide and hydrochloric acid followed by sodium disulphite to remove the iodine formed and the β-free pyrrole (29) (>95% yield) was formylated as earlier.[17] Large-scale work was simplified by condensing the resultant aldehyde (30) with methyl hydrogen malonate in toluene–pyridine–piperidine at reflux under a Dean–Stark trap into which the ternary azeotrope of toluene–pyridine–water was condensed. This afforded the ester (31) in >80% yield. The required building block for porphyrin synthesis was then reached by hydrogenation to yield the ester (32) followed by the transesterification sequence (32) ⟶ (33) ⟶ (34) as described above.

Synthesis of the third pyrrole (40) brings out some points of general interest. The required β-diketone (35) was prepared from acetylacetone and methyl acrylate by using (in contrast to earlier methods) potassium carbonate in butan-2-one. A mild base and a non-nucleophilic solvent avoided retro-Claisen deacetylation of the product. It was now necessary to convert the pyrrole (36) prepared from this diketone into the acid (38), and use of sulphuryl chloride in dichloromethane and ether (see Experimental section) afforded a high yield of the trichloromethyl derivative (37). The subsequent hydrolysis of such trichloromethylpyrroles in aqueous sodium acetate is often bedevilled by loss of material to pyrrocoll [(42) in this case]. Reasoning that formation of pyrrocoll (42) is probably stimulated by base [*via* the anion(41)], we carried out the hydrolysis in boiling aqueous acetone, and the hydrochloric acid formed was neutralised with sodium acetate only as a final step. In this way, the pyrrole (36) was converted into the acid (38) in >90% yield on a 0.25 mol scale. The remaining steps *via* (39) to (40) were analogous to those already described.

Of the two possible structures (11) and (12) for phyriaporphyrin-III, the former is symmetrical about the CD ring system and so is accessible by MacDonald's route. For this, rings A and B of (11) are provided by one pyrromethane and rings C and D by another.

The pyrromethane (44) was prepared by use of mild trifluoroacetic acid catalysis from pyrroles (40) and (43) and the product was converted into the dicarboxylic acid (45) by hydrogenation. This was decarboxylated only slowly in boiling dimethylformamide but at a convenient rate in boiling diethylformamide. The resultant bis-α-free pyrromethane (46) was chosen for conversion into the dialdehyde (48) because (*a*) it was more readily prepared than the other pyrromethane (52) and (*b*) the unsymmetrical system allowed us to check structural integrity in both the decarboxylation and



formylation steps. Thus, addition of benzoyl chloride [19] to the solution of pyrromethane (46) in diethylformamide gave the isolable bis-imine salt (47) from which the dialdehyde (48) was obtained by hydrolysis.

The ring CD component (52) was prepared analogously *via* (50) and (51) from two units of the acetoxymethylpyrrole (49). The pyrromethane (52) reacted with the foregoing dialdehyde (48) and after re-esterification by using methanol–sulphuric acid with trimethyl orthoformate, the ring A methyl porphyrin (53) was isolated in 45% yield. Its n.m.r. spectrum (Figure 2) was

[16] *Cf.* A. Treibs and W. Ott, *Annalen*, 1958, **615**, 137.
[17] A. R. Battersby, D. A. Evans, K. H. Gibson, E. McDonald, and L. Nixon, *J.C.S. Perkin I*, 1973, 1546.
[18] (*a*) D. Dolphin, J. B. Paine III, and R. B. Woodward, unpublished work; (*b*) D. Dolphin and L. Vegh, unpublished work.
[19] R. Chong, P. S. Clezy, A. J. Liepa, and A. W. Nichol, *Austral. J. Chem.*, 1969, **22**, 229.

clearly different from that of the methyl ester of phyriaporphyrin-III. By exclusion therefore, phyriaporphyrin-III has structure (12), *i.e.* it is the ring D methyl isomer.[2]

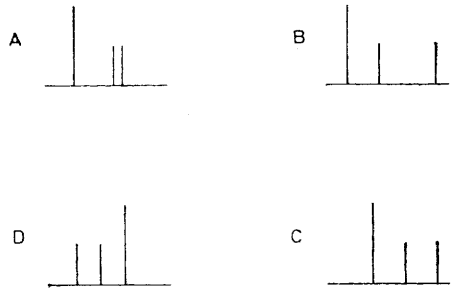


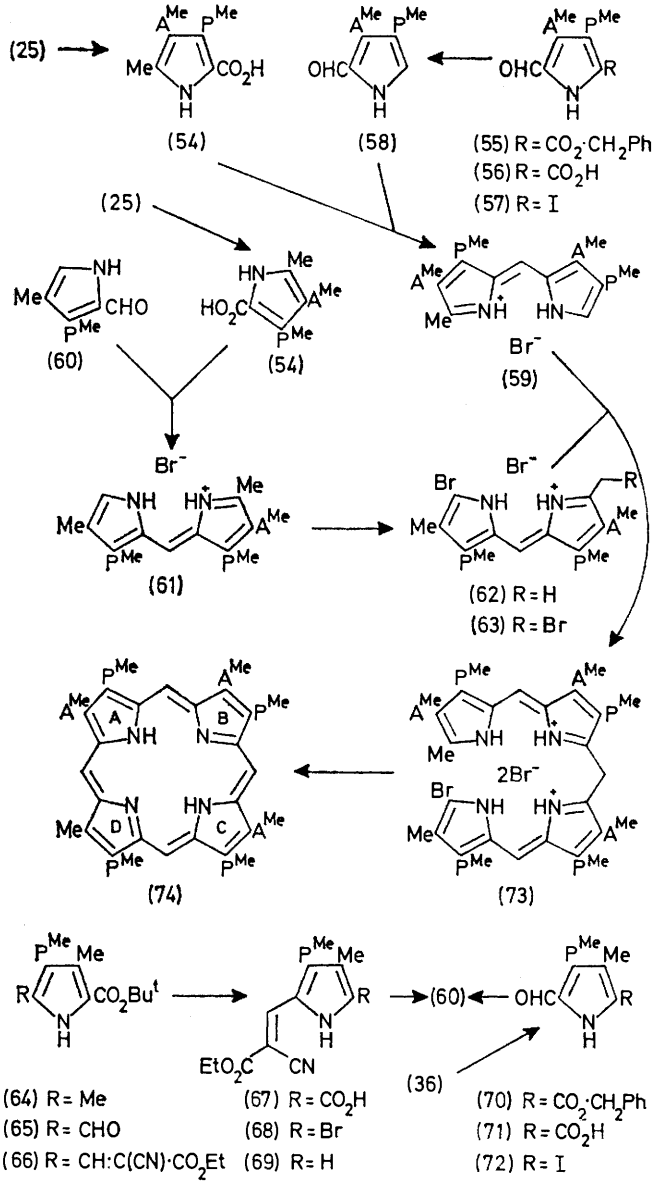
FIGURE 2 Patterns of ^1H n.m.r. signals from the *meso*-bridges (near δ 10.0) for the four isomeric heptacarboxylic porphyrin methyl esters in $CDCl_3$ at low concentration (4—12 mmol l^{-1}); (A) 2-methyl, (B) 7-methyl, (C) 12-methyl, and (D) 18-methyl isomer

The synthetic plan for this unsymmetrical isomer (12) required the preparation of the pyrromethanes (59) and (63), which were to be converted into the porphyrin by Johnson's *a,c*-biladiene method.[20] This scheme involved some risk, for MacDonald [21] was unable to effect the necessary brominations of certain pyrromethenes bearing acetic and propionic side chains. For the present and future work, it was important to overcome this limitation.

Accordingly, the pyrromethene (59) was prepared from the pyrroles (54) and (58), the latter being derived *via* (56) and (57) from the aldehyde (55). This in turn was obtained in high yield by halogenation of pyrrole (25) with sulphuryl chloride and subsequent hydrolysis. The second pyromethene (61) resulted from condensation of the aldehyde (60) with the acid (54). In both pyrromethene preparations, a re-esterification step (methanol–hydrobromic acid–trimethyl orthoformate) was included after the condensation reaction which considerably improved the yield and ease of crystallisation of the product. We confirmed that the α-free positions of the products (59) and (61) were unaffected by this procedure.

Initially, we had difficulty in devising a satisfactory route to the aldehyde (60). Lead tetra-acetate smoothly converted the pyrrole (64) into the aldehyde (65), isolated as the acrylate [18] (66). Deprotection to form the acid (67), bromination to give (68), and hydrogenation were effective in yielding the α-free pyrrole (69). Alkaline hydrolysis then removed the protecting cyanoacrylate group [18] and re-esterification led to the required product (60). This time-consuming route was fortunately superseded when the aldehyde (70), from oxidation by lead tetra-acetate of the benzyl ester (36), was crystallised. The standard steps *via* (71) and (72) then readily afforded the aldehyde (60) in quantity.

Bromination of the pyrromethene (61) was studied with a large excess of bromine in boiling 1,2-dichloroethane (in contrast to the commonly used solvents for such brominations, *e.g.* acetic or formic acid). These conditions had been devised earlier [18b] for a simpler unreactive pyrromethene. Analysis by n.m.r. showed rapid substitution at the α-free position to give (62) but much slower attack on the α-methyl group, and the reaction was run until the signal for this methyl group had been completely eliminated. It was essential at this stage to evaporate to dryness and to treat with an excess of cyclohexene (removal of perbromides) before direct reaction of the product (63) with the ring A–ring B



component (59). Crystalline *a,c*-biladiene dihydrobromide (73) was isolated in 25% yield. Ring-closure in pyridine–dimethyl sulphoxide [20] then gave the

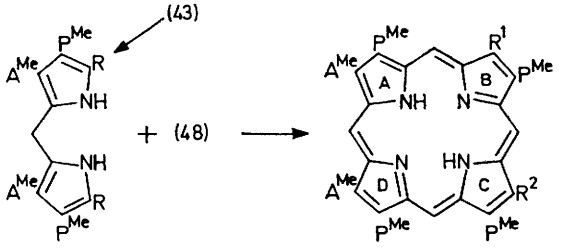
[20] R. L. N. Harris, A. W. Johnson, and I. T. Kay, *J. Chem. Soc.* (*C*), 1966, 22; P. Bamfield, R. L. N. Harris, A. W. Johnson, I. T. Kay, and K. W. Shelton, *ibid.*, *p.* 1436.

[21] A. Markovac, B. D. Kulkarni, K. B. Shaw, and S. F. MacDonald, *Canad. J. Chem.*, 1966, **44**, 2329.

product (74) in 67% yield from the biladiene (73). This ring D methyl porphyrin was identical with the ester of natural phyriaporphyrin-III.

Following our preliminary structural account [2] and shortly before completion of the foregoing synthesis of the ring D methyl porphyrin, Professor P. Clezy (University of New South Wales) kindly sent us a sample of this porphyrin which he and his colleagues had synthesised and we established its identity with the ester of phyriaporphyrin-III by ^{1}H n.m.r. spectroscopy. More recently, Professor A. H. Jackson and his co-workers have prepared this porphyrin synthetically [together with its three isomers (53), (78), and (79)] and have shown it to be identical with the heptacarboxylic porphyrin isolated from poisoned rats.[22]

The ring C methyl porphyrin (79) was synthesised for concurrent research on the biosynthesis of vitamin B_{12} and will be described in a paper on that topic.[2,23]



To complete the set, the ring B methyl porphyrin ester (78) was built by methods analogous to those used for the ring A methyl isomer (53). The pyrromethanes (48) and (77) gave a 48% yield of the porphyrin ester (78), and its difference from the ester of the natural porphyrin added further strength to the earlier conclusions.

Professor Clezy's group also built these three porphyrins [(53), (78), and (79)] in parallel work, and his samples have been shown by direct comparison in Cambridge to be identical with ours; Professor Clezy's kindness in sending us these samples is gratefully acknowledged.

In conclusion, the foregoing spectroscopic and synthetic work establishes that phyriaporphyrin-III carries the methyl group on ring D (74). Our findings are consistent with the corresponding heptacarboxylic porphyrinogen being the first enzymic decarboxylation product of uroporphyrinogen-III (2).

EXPERIMENTAL

For general directions, see refs. 17 and 24. ^{13}C N.m.r. spectra were measured for solutions in $CDCl_3$ unless otherwise stated under the conditions described in ref. 11.

Isolation of Phyriaporphyrin-III Heptamethyl Ester and ^{13}C-Labelled Porphyrin.—Earlier work had shown [11] that higher yields of phyriaporphyrin-III were obtained in the presence of considerable concentrations of sodium chloride. Accordingly, the following conditions were used.

Porphobilinogen (1) (40 mg) in 1M-Tris–hydrochloric acid buffer (15 ml; pH 7.8) was mixed with enzyme preparation A[11] from chicken blood erythrocytes (100 ml; activity 0.12 μmol h^{-1} ml^{-1}) containing sodium chloride (2 g). The mixture was gently shaken in the dark in air at 37 °C for 24 h and then worked up as for the enzymic formation of protoporphyrin-IX by use of the coupled enzyme system.[11] Phyriaporphyrin-III was in the aqueous sodium acetate solution, which was adjusted to pH 3 with concentrated hydrochloric acid and extracted with ethyl acetate (100 ml portions) until the extracts were colourless. The combined solution in ethyl acetate was shaken with 2% hydrochloric acid (50 ml portions) until no further porphyrin was extracted. After the combined acidic extracts had been overlayered with an equal volume of ethyl acetate, small amounts of solid sodium carbonate were added with shaking until all the porphyrin had been extracted into the ethyl acetate. The organic extract was dried over sodium chloride and the recovered porphyrin was esterified with methanol–sulphuric acid as usual.[11]

When a blank experiment was run exactly as above but without the porphobilinogen, no porphyrins were detected by u.v.–visible spectroscopy.

A solution of the porphyrin esters in dichloromethane was run onto a column of silica gel (20 g) and elution was performed with 1 : 2 dichloromethane–chloroform (containing 2% ethanol) Phyriaporphyrin-III was eluted after a small amount of coproporphyrin-III tetramethyl ester had been removed The *heptacarboxylic ester* was recrystallised (yield 2.5 mg) from chloroform–light petroleum (b.p. 60—80°); m.p. 213—214° (Found: C, 62.3; H, 6.0; N, 6.0. $C_{46}H_{52}N_4O_{14}$ requires C, 62.4; H, 5.9; N, 6.3%); $\lambda_{max.}$ ($CHCl_3$) 404, 501, 535, 570, 595sh, and 622 nm; *m/e* 886 ($M^+ + 2$, 18%), 885 ($M^+ + 1$, 55), 884 (M^+, 100), 854 (2.5), 853 (4), 826 (11), 825 (16), 812 (9), 811 (22), and 442 (M^{2+}, 5.5), *m** 744 (calc. for 884 ⟶ 811, 744.03); τ −0.23, −0.205, and −0.17 (1 H, 1 H, and 2 H, s, *meso*-H), 4.81 and 4.88 (14 H, br, overlapping $ArCH_2$·CO), 5.55 (total 8 H, overlapping t, *J* 7.5 Hz, CH_2·CH_2·CO), 6.21, 6.30, 6.31, and 6.33 (total 24 H, s, 7 CO_2Me, 1 ArMe), and 6.47—6.78 (total 8 H, overlapping t, *J* 7.5 Hz, CH_2·CH_2·CO).

A solution of the foregoing porphyrin ester (1 mg) in methanol (2.5 ml), CD_3OH (2.5 ml), and concentrated sulphuric acid (0.25 ml) was kept at 20 °C for 24 h and then worked up as above. The product showed molecular ions with indicated relative intensity at *m/e* 905 ($^2H_{21}$ *ca.* 2), 902 ($^2H_{18}$ 14), 899 ($^2H_{15}$ 35), 896 ($^2H_{12}$ 54), 893 ($^2H_9$ 54), 890 ($^2H_6$ 32), 887 ($^2H_3$ 11), and 884 ($^2H_0$ *ca.* 2).

[2,11-$^{13}C_2$]Porphobilinogen [11] (undiluted; 31 mg) was converted enzymically as above into ^{13}C-labelled phyriaporphyrin-III heptamethyl ester (2.4 mg). The ^{13}C n.m.r. results are summarised in Figure 1.

Diethyl β-Oxoadipate [25] (15).—Grignard-quality magnesium turnings (22 g) and anhydrous ethanol (130 ml) were stirred in protected equipment and treated with carbon tetrachloride (2 ml). When the reaction slowed, anhydrous ether (125 ml) was added and further additions were made

[22] A. H. Jackson, G. H. Elder, and co-workers, *Phil. Trans. Roy. Soc., Series B*, 1976, **273**, 191.
[23] Part X, A. R. Battersby, M. Ihara, E. McDonald, F. Satoh, and D. C. Williams, in preparation.
[24] A. R. Battersby, J. F. Beck, and E. McDonald, *J.C.S. Perkin I*, 1974, 160.
[25] M. Viscontini and N. Merckling, *Helv. Chim. Acta*, 1952, **35**, 2280; M. Viscontini and H. Köhler, *ibid.*, 1954, **37**, 41.

occasionally to allow the thick mixture to be stirred. Finally, the flask was heated at 40 °C until all the magnesium had reacted and the mixture was then cooled to 0 °C. Ethyl acetoacetate (115 g) in anhydrous ether (100 ml) was added over 45 min at 0 °C and the mixture was then stirred rapidly for 30 min. β-Ethoxycarbonylpropionyl chloride (145 g) in anhydrous ether (100 ml) was added over 45 min to the stirred mixture at −5 °C, and the mixture was then further stirred at 20 °C for 1.5 h and kept at 20° for 16 h. After addition of ice (25 g) and ether (100 ml) with stirring, the mixture was adjusted at 0° to pH 2 with 2N-sulphuric acid, the ether layer was separated, and the aqueous layer was extracted twice with ether. The combined ethereal solution was washed with water, saturated sodium hydrogen carbonate, and water, and dried. The product from the ether (usually 206—210 g) is sufficiently pure for the deacetylation step on the whole product with ammonia carried out essentially as previously.[25] Diethyl β-oxoadipate (110 g) was obtained, b.p. 110—119° at 0.7 mmHg.

Ethyl 3-(2-*Ethoxycarbonylethyl*)-4-*iodo*-5-*methylpyrrole*-2-*carboxylate* (19).—The foregoing oxo-ester was converted [14] into the pyrrole (16), m.p. 112.5—114.5° (lit.,[14] 112.5°). This (88.2 g) in 1,2-dichloroethane (400 ml) and trifluoroacetic acid (20 ml) was stirred and heated under reflux for 2 h. Water was then added and the solid acid (18) was collected and washed with water and dichloromethane (yield 72.9 g, 98%).

All this product was added to a solution of sodium hydrogen carbonate (75.5 g) in water (500 ml) at 50 °C, and after the addition of 1,2-dichloroethane (500 ml) the stirred mixture was heated quickly to reflux. A solution of iodine (70.1 g) in water (300 ml) containing sodium iodide (75 g) was added over several min, the mixture was heated under reflux for 40 min, then sodium disulphite was added in portions to remove the excess of iodine. The product from the organic phase (89 g) was sufficiently pure for the next stage; the *iodopyrrole* was recrystallised from methanol m.p. 109—110.5° (Found: C, 41.2; H, 4.8; I, 33.2; N, 3.7. $C_{13}H_{18}INO_4$ requires C, 41.2; H, 4.8; I, 33.5; N, 3.7%); τ −0.02br (1 H, NH), 5.67 and 5.84 (each 2 H, q, *J* 7.5 Hz OEt), 6.7—7.65 (4 H, m, $CH_2{\cdot}CH_2$), 7.71 (3 H, s, pyrr-Me), and 8.64 and 8.74 (each 3 H, q, *J* 7.5 Hz, OEt).

Ethyl 3-(2-*Ethoxycarbonylethyl*)-4-*ethoxycarbonylmethyl*-5-*methylpyrrole*-2-*carboxylate* [17] (22).—The iodopyrrole (19) (89 g) in glacial acetic acid (500 ml) was treated at 20 °C with hydriodic acid (135 ml; s.g. 1.7) followed by phosphorous acid (50%; 145 ml). To the mixture, which had warmed to 40 °C, was added glyoxylic acid hydrate (50 g) in several portions, and the temperature rise was reversed by cooling to 40 °C. After 50 min further at 40 °C, water (1.5 l) was added and after 16 h at 20 °C the solid acid (21) was collected (65—66 g). This (38.3 g) was treated in order with absolute ethanol (250 ml), triethyl orthoformate (100 ml), and concentrated sulphuric acid (10 ml). After the mixture had been stirred until homogeneous, it was kept anhydrous for 18 h at 20 °C then was diluted with dichloromethane (250 ml) followed by water (500 ml). The organic phase was shaken with aqueous sodium hydrogen carbonate, filtered, and evaporated. The residue was dissolved in the minimum of boiling n-hexane and the solution was mixed with a suspension of the crystalline ester (*ca.* 100 mg) in n-hexane to give the pyrrole (22) (37.6 g), m.p. 63—65° (lit.,[17] 62—65°; lit.,[15] 66—66.5°).

Benzyl 3-(2-*Benzyloxycarbonylethyl*)-4-*benzyloxycarbonylmethyl*-5-*methylpyrrole*-2-*carboxylate* (23).—A solution of the foregoing triethyl ester (50.8 g) in benzyl alcohol (350 ml; distilled from potassium carbonate) in a 1 l conical flask was stirred under a nitrogen stream and heated to reflux so that the benzyl alcohol condensed at the top of the flask. After a few min, a solution (1 ml) of sodium (1 g) in benzyl alcohol (25 ml) was added dropwise, and when the vigorous evolution of ethanol slowed, more of this solution (0.5—1 ml) was added every few min until no further ethanol was evolved; heating was continued throughout and when the original b.p. (*ca.* 209° in the solution) was reached, a final portion (1 ml) of sodium benzyloxide was added. A few min later (15—20 min from start of process) the hot solution was poured into a stirred mixture of water (540 ml), acetic acid (15 ml), and methanol (960 ml). The solid product was collected, washed with 1 : 1 water–methanol (1 l) and water (1 l) and then, still moist, was dissolved in dichloromethane (*ca.* 500 ml). After removal of the water layer and filtration, the solution was evaporated (to 300 ml) and mixed with ether (300 ml) then n-hexane (500 ml) to give the tribenzyl ester, which was washed with 1 : 4 ether–n-hexane; yield 67.3 g; m.p. 106.5—107.5° (lit.,[26] 108°; lit.,[27] 106—108°).

Benzyl 3-(2-*Methoxycarbonylethyl*)-4-*methoxycarbonylmethyl*-5-*methylpyrrole*-2-*carboxylate* (25).—The ester (23) (69 g) in anhydrous tetrahydrofuran (250 ml) and magnesium-dried methanol (350 ml) was treated at 20 °C with a stock solution of sodium methoxide (30 ml) [from sodium (4.83 g) in methanol (220 ml)]. After 3.5 h, t.l.c. showed no further change; glacial acetic acid (10 ml) was added and the solution was evaporated. The residue crystallised from aqueous methanol and after thorough drying was re-treated as above in tetrahydrofuran (150 ml), methanol (250 ml), and sodium methoxide (50 ml of above solution) for 1.5 h. Acetic acid (10 ml) was added, then water (3 l), and the crystalline ester was collected and dissolved in dichloromethane; the organic layer was then separated from water and evaporated. The product in ether (250 ml) was diluted with n-hexane (550 ml) to give the ester (25), which was washed with 1 : 4 ether–n-hexane; yield 45.1 g, m.p. 80—81.5° (lit.,[17] 78.5—79.5; lit.,[27] 78°).

This was converted essentially as earlier [1] into the acetoxymethylpyrrole (43), which yielded [1] the pyrromethane (75); 70% yield from (25) to (75).

Methyl 3-*Methoxycarbonylmethyl*-5-*methyl*-4-*t*-*butyloxycarbonylpyrrole*-2-*carboxylate* (26).—Dimethyl 3-oxopentanedioate (174 g) and concentrated hydrochloric acid (1 ml) were stirred and treated dropwise at 20 °C with freshly distilled pentyl nitrite (135 ml) over 2 h. After 16 h more at 20 °C, glacial acetic acid (200 ml) was added. This solution was added slowly over 2 h to a well stirred mixture of t-butyl acetoacetate (158 g), acetic acid (400 ml), and ammonium acetate (105 g) at 50—70 °C while zinc dust (270 g) was simultaneously added in portions so as to maintain an excess of zinc. After a further 0.5 h at 60 °C, the solution was decanted from the inorganic sludge, which was washed several times with acetic acid. The combined organic solution was diluted to 3 l with water, and the solid was collected, washed with water, and dissolved in dichloromethane. The water was separated, and the filtered organic solution was evaporated. Crystallisation of the

[26] J. M. Osgerby, J. Pluscec, Y. C. Kim, F. Boyer, N. Stojanac, H. D. Mah, and S. F. MacDonald, *Canad. J. Chem.*, 1972, **50**, 2652.

[27] A. Valasinas, E. S. Levy, and B. Frydman, *J. Org. Chem.*, 1974, **39**, 2874.

residue from aqueous methanol then from dichloromethane–n-hexane gave the *pyrrole ester* (26), m.p. 131—131.5° (143.9 g) (Found: C, 57.8; H, 6.8; N, 4.5. $C_{15}H_{21}NO_6$ requires C, 57.85; H, 6.8; N, 4.5%); τ −0.06br (1 H, NH), 5.77 (2 H, s, CH_2CO), 6.22 and 6.27 (each 3 H, s, OMe), 7.60 (3 H, s, pyrr-Me), and 8.47 (9 H, s, Bu^t).

Methyl 4-Iodo-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (28).—A stirred solution of the ester (26) (93.3 g) in 1,2-dichloroethane (400 ml) and trifluoroacetic acid (30 ml) was heated under reflux for 75 min. Water (250 ml) was then added and when crystallisation was complete the acid (27) was collected and washed with dichloromethane and water (73.7 g). This was added to a stirred solution at 50 °C of sodium hydrogen carbonate (75 g) in water (500 ml). When foaming started, 1,2-dichloroethane (500 ml) was added and the mixture was heated to reflux to dissolve the pyrrole. A solution of iodine (85 g) in water (500 ml) containing sodium iodide (90 g) was added during 3 min and heating under reflux was continued for 0.5 h. Sufficient sodium disulphite was then added to remove the excess of iodine, and the organic phase was separated, filtered, and evaporated to give the iodopyrrole, which was recrystallised from dichloromethane–n-hexane; yield 93.9 g, m.p. 124—124.5° (lit.,[17] 121.5—122°).

Methyl 3-Methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (29).—The iodopyrrole (28) (97.7 g) in boiling methanol (500 ml) was treated with sodium iodide (77.6 g) in water (75 ml) followed by concentrated hydrochloric acid (40 ml). Iodine was rapidly formed. After several min sodium disulphite (45 g) in water (75 ml) was added, and the mixture was diluted with water and extracted with dichloromethane to give the β-free pyrrole (60.2 g), which was pure enough (m.p. after recrystallisation 64.5—65.5°; lit.,[17] 59—61.5°) for the next stage. This involved formylation essentially as earlier [17] to give the 4-formylpyrrole (30).

Methyl 3-Methoxycarbonylmethyl-4-(2-methoxycarbonylvinyl)-5-methylpyrrole-2-carboxylate (31).—A stirred solution of the 4-formylpyrrole (30) (38.2 g) in pyridine (154 ml) and toluene (160 ml) was heated under reflux in equipment fitted with a Dean–Stark trap. When traces of initial water had been thus removed, piperidine (4.2 ml) in acetic acid (10 ml) was added, followed by malonic acid monomethyl ester in portions (20, 5, 5, 5, and 5 ml) added each time formation of water slowed; total time of reaction *ca.* 2 h. The solvents were then evaporated off, the residue in dichloromethane was washed with 2N-hydrochloric acid, and the filtered organic phase was evaporated. Crystallisation from methanol gave the acrylic ester (38.6 g), m.p. 142—144° (lit.,[17] 143—145°).

This was hydrogenated as previously [17] but work-up involved addition of sulphur (*ca.* 200 mg) to deactivate traces of palladium which were found otherwise to cause difficulties in the next step.

Benzyl 3-Methoxycarbonylmethyl-4-(2-methoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (34).—The foregoing product (29.7 g) was converted as for the related case (22) into the tribenzyl ester (42.9 g), m.p. 100—102° (lit.,[26] 92—93°; lit.,[27] 94—96°).

This (66.2 g) was converted into the monobenzyl ester as for the isomer (25) by using tetrahydrofuran (200 ml), anhydrous methanol (430 ml), and sodium methoxide (80 ml) [from sodium (7.09 g) in methanol (250 ml)]; reaction was complete in 4 h and work-up as before gave the monobenzyl ester (34) (35.8 g), m.p. 114—117° (from ether–n-hexane) (lit.,[17] 113—116°; lit.,[27] 111°).

Benzyl 3-Methyl-4-(2-methoxycarbonylethyl)-5-carboxypyrrole-2-carboxylate (38).—Pentane-2,4-dione (1 552 ml), dry butan-2-one (1 l), and anhydrous potassium carbonate (50 g) were heated under reflux and methyl acrylate (1 269 ml) was steadily added. Reaction was complete after *ca.* 2 h, and after cooling the inorganic solids were filtered off. Distillation of the organic material afforded the diketone (35) (1 859 g, 71.4%).[28] This was converted [29] into the pyrrole (36) and part (63.1 g) was dissolved in stirred dry dichloromethane (200 ml) in a 3 l conical flask. Ether (800 ml), redistilled from phosphoric oxide and kept over sodium wire, was then added, followed *immediately* by a solution of sulphuryl chloride (110.3 g) in dichloromethane (130 ml) poured in as rapidly as the very vigorous reaction allows (usually *ca.* 30 s). After the mixture had been stirred for 1.5 h more, it was evaporated and the residue was added to water (200 ml) and acetone (500 ml); more acetone (500 ml) was used to wash in. The solution was heated and after *ca.* 30 min was treated with a solution of anhydrous sodium acetate (100 g) in water (250 ml). During boiling for 1 h, the acetone was allowed to evaporate off and on cooling the resultant aqueous suspension, the acid solidified. It was collected, washed with water, dissolved in boiling methanol (300 ml), and treated cautiously with a slurry of sodium hydrogen carbonate (26 g) in water (200 ml). The solution was then diluted with water (1 l), filtered, and extracted with ether. Acidification of the aqueous phase with concentrated hydrochloric acid (50 ml) gave the acid (38), m.p. 146—151° (63.6 g, 92%) (lit.,[30] 149—150°); τ −1.35 (1 H, s, CO_2H), 0.28br (1 H, NH), 2.65 (5 H, s, Ar), 4.66 (2 H, s, CH_2Ar), 6.33 (3 H, s, OMe), 6.89 and 7.42 (total 4 H, m, $CH_2{\cdot}CH_2$), and 7.70 (3 H, s, pyrr-Me).

Benzyl 5-Iodo-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (39).—The acid (38) (17.2 g) was iodinated as above by using sodium hydrogen carbonate (17.9 g), water (100 ml), and 1,2-dichloroethane (100 ml), with iodine (17.9 g) and sodium iodide (20.4 g) in water (100 ml). The product (39) (19.6 g) had m.p. 95—96° (polymorph, 87—88°) (from ether–n-hexane) (lit.,[31] 95°).

This (19.5 g) was reduced with hydrogen as earlier [31] to the pyrrole (40), which was worked up as usual; m.p. 42.5—44.5° (12.2 g) (after crystallisation at −78 °C from ether–n-hexane) (lit.,[31] m.p. 41—42°).

Dibenzyl 3,4′-Bis(methoxycarbonylethyl)-3′-methoxycarbonylmethyl-4-methylpyrromethane-5,5′-dicarboxylate (44).—A solution of the α-free pyrrole (40) (6.02 g) and the acetoxymethylpyrrole (43) (8.62 g) in dry dichloromethane (115 ml) and trifluoroacetic acid (1 ml) was kept in the dark without air at 20 °C. After 2 days, more trifluoroacetic acid (1 ml) was added, and after a total of 6 days t.l.c. showed complete reaction and the solvents were evaporated off. The residue in ether (200 ml) was diluted with n-hexane (50 ml), and when crystallisation was well established 1 : 4 ether–n-hexane (250 ml) was added with stirring. After 16 h the

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[29] A. W. Johnson, I. T. Kay, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1959, 3416.

[30] A. H. Jackson, G. W. Kenner, and D. Warburton, *J. Chem. Soc.*, 1965, 1328; J. A. S. Cavaleiro, G. W. Kenner, and K. M. Smith, *J.C.S. Perkin I*, 1973, 2478.

[31] P. J. Crook, A. H. Jackson, and G. W. Kenner, *J. Chem. Soc.* (*C*), 1971, 474.

solid (11.9 g) was collected and washed with ether–n-hexane. Recrystallisation from methanol gave the *pyrromethane* (9.96 g), m.p. 98.5—102.5° (Found: C, 66.4; H, 6.1; N, 4.1%; M^+, 672. $C_{37}H_{40}N_2O_{10}$ requires C, 66.1; H, 6.0; N, 4.15%; M^+, 672); τ 0.16br (2 H, NH), 2.70br (10 H, 2Ar), 4.78 (4 H, s, $ArCH_2$), 6.09 (2 H, s, $pyrr_2CH_2$), 6.39 and 6.42 (each 3 H, s, OMe), 6.49 (5 H, s, OMe and CH_2CO), 6.88—7.60 (8 H, m, $CH_2{\cdot}CH_2$), and 7.73 (3 H, s, pyrr-Me).

3,4′-*Bis*(*methoxycarbonylethyl*)-3′-*methoxycarbonylmethyl*-4-*methylpyrromethane*-5,5′-*dicarbaldehyde* (48).—A solution of the pyrromethane (44) (3.36 g) in tetrahydrofuran (170 ml) was stirred with 10% palladised charcoal (1.15 g) and hydrogen until uptake ceased. The filtered solution and washings were evaporated and the residue (2.53 g) was heated under reflux and nitrogen with *NN*-diethylformamide (20 ml; freed from formic acid and distilled from potassium carbonate); samples were checked for loss of the 285 nm absorption (pyrrole-α-carboxylic acid). This took 3 h and the solution of (46), cooled to *ca.* 3 °C, was then treated rapidly with benzoyl chloride (3 ml) and kept at 20 °C for 18 h. Ether (85 ml) was added with stirring and the iminium salt (47) which crystallised was collected, washed with ether and dissolved in water (250 ml). To the filtered solution was added sodium hydrogen carbonate (*ca.* 5 g) and the mixture was then heated to 80 °C. When separation of the solid was complete, it was collected, washed with water, and dissolved in dichloromethane. The material from the organic phase crystallised from methanol to give the *diformylpyrromethane* (1.42 g), m.p. 157—163° (Found: C, 59.5; H, 6.2; N, 6.35. $C_{23}H_{28}N_2O_8$ requires C, 60.0; H, 6.1; N, 6.1%); *m/e* 460 (M^+, 100) and 431 as large peaks with no peaks corresponding to symmetrical pyrromethanes; τ −0.62 and −0.46 (each 1 H, br, NH), 0.48 and 0.54 (each 1 H, s, CHO), 5.97 (2 H, s, $pyrr_2CH_2$), 6.26, 6.30, and 6.37 (each 3 H, s, OMe), 6.46 (2 H, s, CH_2CO), 6.86—7.57 (8 H, m, $CH_2{\cdot}CH_2$), and 7.73 (3 H, s, pyrr-Me).

Dibenzyl 3,3′-*Bis*(*methoxycarbonylethyl*)-4,4′-*bis*(*methoxycarbonylmethyl*)*pyrromethane*-5,5′-*dicarboxylate* (51).—(a) *Stepwise.* A stirred solution of the pyrrole (34) (3.73 g) in glacial acetic acid (30 ml) and acetic anhydride (5 ml) was treated with lead tetra-acetate (5.26 g of commercial quality) in one portion. The mixture was heated to 80 °C until t.l.c. showed no starting material; occasionally addition of more lead tetra-acetate was necessary. Ethylene glycol (3 ml) was added, then water (200 ml), and the precipitated acetoxymethylpyrrole (49) was collected, washed with water, dried, and recrystallised from ether–n-hexane (yield 3.22 g).

Part (1.01 g) was heated under reflux with water (10 ml) and acetic acid (20 ml) for 3 h and the cooled mixture was extracted with dichloromethane. The extracted material was treated, in order, with methanol (10 ml), trimethyl orthoformate (10 ml), and concentrated sulphuric acid (20 drops). After 18 h at 20 °C, the mixture was partitioned between dichloromethane and water and the organic material was crystallised from methanol to give the pyrromethane (51) (441 mg), identical with material synthesised in a different way.[1]

(b) *Direct.* The pyrrole (34) (7.47 g) in glacial acetic acid (50 ml) was treated as above with lead tetra-acetate (11.4 g) and heated to 88 °C over 30 min; all starting material had then reacted. Water (20 ml) was added at 65—70° and heating was continued up to 98 °C with addition of more water (20 ml) after 1.5 h. After a total of 6 h, the mixture was extracted with dichloromethane; further work-up as above gave the pyrromethane (51) (2.75 g), identical with that obtained by method (*a*).

3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-7,12,18-*tris*-(*methoxycarbonylmethyl*)-2-*methylporphin* (*Ring* A *Methylporphyrin*) (53).—A solution of the pyrromethane (51) (1.46 g) in tetrahydrofuran (100 ml) was stirred with hydrogen and 10% palladised charcoal (0.3 g) until uptake ceased. The filtered solution was diluted to 250 ml with tetrahydrofuran to give a stock solution of the acid (51) which was stable for months in the dark at 4 °C. Part of this solution (22.4 g) was evaporated to dryness and the residue was decarboxylated by boiling for 2.5 h with *NN*-diethylformamide (5 ml) as for (45) above. A solution of the residue from evaporation in glacial acetic acid (30 ml) was added to a solution of the dialdehyde (48) (95 mg) in glacial acetic acid (50 ml), followed immediately by hydriodic acid in acetic acid [60 ml of solution made by adding 1 ml of concentrated hydriodic acid in acetic acid (redistilled from red phosphorus) to 100 ml of acetic acid]. The mixture was kept in the dark for 20 min then was treated with sodium acetate (3.23 g) and a stream of air overnight in the dark. The residue from evaporation was partitioned between water and dichloromethane and the material from the latter was dissolved in dichloromethane (20 ml) and methanol (100 ml) and treated with concentrated sulphuric acid (2 ml) and trimethyl orthoformate (30 ml). After 5 h, the product was partitioned between water and dichloromethane and the solid from the latter was crystallised from dichloromethane–methanol to give the *methylporphyrin* (53) (99 mg), which was further purified by p.l.c. on silica with 2% methanol–chloroform (final elution with chloroform); recrystallisation from chloroform–methanol gave material of 213—216°, undepressed by admixture with natural phyriaporphyrin-III heptamethyl ester; however, n.m.r. (Figure 2) showed the synthetic and natural samples to be different (Found: C, 62.5; H, 6.0; N, 6.35. $C_{46}H_{52}N_4O_{14}$ requires C, 62.4; H, 5.9; N, 6.3%).

^1H *N.m.r. Spectra of Heptacarboxylic Porphyrin Esters.*—The ^1H n.m.r. spectra of porphyrins vary with concentration [32] and this was taken into account when comparing the spectra of natural and synthetic samples. Each of the four isomers gave rise to a unique pattern from the *meso*-bridges (Figure 2) which was independent of concentration over the range 4—12 mmol l^{-1}; different patterns were found with 48mM-solutions. The low concentration is the appropriate one for such comparisons and the spectra were recorded (1—5 mg of sample) by the pulsed Fourier transform technique. The four isomers are best distinguished by the *patterns* of signals, and the separations (p.p.m.) of low-field signal from middle signal (*p*) and of middle signal from high field signal (*q*) were consistently as follows: ring A methyl isomer, (*p*) 0.05—0.06, (*q*) 0.00—0.02; ring B methyl isomer, (*p*) 0.04—0.05, (*q*) 0.07; ring C methyl isomer, (*p*) 0.04—0.05, (*q*) 0.04; ring D methyl isomer (*p*) 0.02—0.03, (*q*) 0.03—0.04

3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,12,18-*tris*-(*methoxycarbonylmethyl*)-7-*methylporphin* (*Ring* B *Methylporphyrin*) (78).—The pyrromethane (75) (0.36 g) was hydrogenated to give the carboxylic acid (76), which was decarboxylated as in the foregoing experiment The product (77) was treated with the dialdehyde (48) (232 mg) in glacial acetic acid (125 ml) and immediately mixed with

[32] R. J. Abraham, P. A. Burbidge, A. H. Jackson, and G. W. Kenner, *Proc. Chem. Soc.*, 1963, 134.

diluted hydriodic acid in acetic acid (150 ml) prepared as above. After 25 min, the mixture was worked up as before (by using 8.45 g of sodium acetate) to give the *methylporphyrin* (78) (266 mg) (from dichloromethane–methanol). It was further purified as for the ring A methylporphyrin; m.p. 227—228° (Found: C, 61.55, 61.7; H, 5.95, 6.0; N, 6.05, 6.1. $C_{46}H_{52}N_4O_{14}$,MeOH requires C, 61.55; H, 6.15; N, 6.1%).

Benzyl 5-Formyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate (55) (with G. S. MORGAN).—A solution of the pyrrole (25) (11.2 g) in dry dichloromethane (300 ml) was stirred at 0 °C in protected equipment while redistilled sulphuryl chloride (8.92 g) in dry dichloromethane (150 ml) was added during 30 min. After a further 1 h, water (500 ml) was added, and the organic phase was separated and evaporated. The residue in tetrahydrofuran (100 ml) and water (50 ml) was warmed on a steam-bath while sodium hydrogen carbonate was added in portions until the pH of the solution remained at 9. Extraction with dichloromethane gave the *aldehyde*, m.p. 77—81° (from ether–n-hexane) (Found: C, 61.8; H, 5.45; N, 3.5. $C_{20}H_{21}NO_7$ requires C, 62.0; H, 5.45; N, 3.6%); τ −0.02br (1 H, NH), 0.24 (1 H, s, CHO), 2.62br (5 H, Ar), 4.67 (2 H, s, $ArCH_2$), 6.18 (2 H, s, CH_2CO), 6.31 and 6.40 (each 3 H, s, OMe), and 6.79—7.13 and 7.31—7.68 (each 2 H, m, CH_2CH_2).

4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carbaldehyde (58) (with G. S. MORGAN).—The aldehyde (55) (7.75 g) in tetrahydrofuran (90 ml) and triethylamine (1.0 ml) was stirred with hydrogen and 10% palladised charcoal at 760 mmHg and 20 °C until uptake was complete (4 h). The filtered solution was evaporated, the residue in methanol (60 ml) was treated with acetic acid (3 ml) and water (60 ml), and the methanol was evaporated off. The precipitated acid (56) was collected (4.62 g).

This (2.97 g) with water (20 ml), sodium hydrogen carbonate (3.06 g), and chloroform (30 ml) was heated under reflux while a solution of iodine (2.6 g) and sodium iodide (5.14 g) in water (30 ml) was added over 7 min. After heating for 45 min more, the organic layer was separated and evaporated to give the iodide (57). This was used as such for dissolution in tetrahydrofuran (100 ml) and was stirred with magnesium oxide (3.03 g), 10% palladised charcoal, and hydrogen at 760 mmHg and 20 °C for 4 days; uptake was then complete. The filtered solution was mixed with dichloromethane and washed with water and the organic layer was evaporated. Extraction of the residue with boiling water gave a solution which yielded the *aldehyde* (1.27 g), m.p. 97—98° (Found: C, 57.2; H, 6.0; N, 5.45. $C_{12}H_{15}NO_5$ requires C, 56.9; H, 6.0; N, 5.5%); τ −0.17br (1 H, NH), 0.37 (1 H, s, CHO), 3.06 (1 H, d, J 3.5 Hz, pyrr-H), 6.23 (2 H, s, CH_2·CO), 6.31 and 6.35 (each 3 H, s, OMe), and 7.02—7.60 (4 H, m, CH_2·CH_2).

3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bis(methoxycarbonylmethyl)-5-methylpyrromethene Hydrobromide (59).—The benzyl ester (25) (1.26 g) was hydrogenolysed as above and the resultant acid in methanol (20 ml) was mixed with a solution of the aldehyde (58) (0.87 g) in methanol (30 ml). The solution was boiled and immediately treated with concentrated hydrobromic acid (48%; 1 ml), and boiling was continued for 20 min. Trimethyl orthoformate (10 ml) was then added cautiously and the solution was evaporated over 6 min to *ca.* 15 ml. Addition of diethyl ether (55 ml) caused crystallisation of the *pyrromethene* (1.55 g), which was washed with 15 : 85 methanol–ether; m.p. 153—156° (Found: C, 52.0; H, 5.7; Br, 14.1; N, 4.9. $C_{24}H_{31}BrN_2O_8$ requires C, 51.9; H, 5.6; Br, 14.4; N, 5.0%); τ −3.52 and −3.40 (each 1 H, br, NH), 2.37 (1 H, d, J 4 Hz, pyrr-H), 2.42 (1 H, s, *meso*-H), 6.15 (2 H, s, 3′-CH_2·CO), 6.30, 6.33, and 6.38 (6 H, 3 H, 3 H, s, 4 OMe), 6.46 (2 H, s, 4-CH_2·CO), 6.82—7.50 (8 H, m, CH_2·CH_2), and 7.27 (3 H, s, pyrr-Me).

t-Butyl 5-Formyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (65) *and its Cyanoacrylate Derivative* (66).—A solution of the pyrrole [29] (64) (140.5 g) in glacial acetic acid (1 l) and acetic anhydride (10 ml) was added with stirring to lead tetra-acetate (607.6 g). The mixture was kept at 80—90 °C until t.l.c. showed complete reaction. Water (4 l) was added, the mixture was extracted with dichloromethane, and the organic solution was washed with aqueous sodium hydrogen carbonate before evaporation. A small part of the residual gum was crystallised, in poor recovery, from ethanol and methanol to give the *aldehyde*, m.p. 91—94° (dimorphic prisms and long needles) (Found: C, 60.9, 60.9; H, 7.1, 7.2; N, 4.7, 4.6. $C_{15}H_{21}NO_5$ requires C, 61.0; H, 7.2; N, 4.75%); τ 0.16 (1 H, s, CHO), 0.33br (1 H, NH), 6.35 (3 H, s, OMe), 6.76—7.17 and 7.27—7.59 (4 H, m, CH_2·CH_2), 7.71 (3 H, s, pyrr-Me), and 8.41 (9H, s, Bu^t).

The remaining crude aldehyde in ethanol (500 ml) was heated close to reflux for 30 min with ethyl cyanoacetate (60 g) and piperidine (2 ml), then diluted with 6 : 4 ethanol–water (500 ml). The product was collected and washed with 7 : 3 ethanol–water (2 l), and the filtrate was extracted with dichloromethane to yield more product which was crystallised from 4 : 1 ethanol–water (total yield 136.2 g); m.p. 82.5—84.3° (Found: C, 61.5; H, 6.9; N, 7.1%; M^+, 390. $C_{20}H_{26}N_2O_6$ requires C, 61.5; H, 6.7; N, 7.2%; M, 390); τ −0.25br (1 H, NH), 1.93 (<1 H, s, C=CH of major isomer), 2.53 (small s, C=CH of minor isomer), 5.64 (2 H, q, OEt), 6.36 (3 H, s, OMe), 6.8—7.75 (4 H, m, CH_2·CH_2), 7.70 (3 H, s, pyrr-Me), 8.40 (9 H, s, Bu^t), and 8.61 (3 H, t, OEt).

5-(2-Cyano-2-ethoxycarbonylvinyl)-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylic Acid (67).—A solution of the foregoing product (31.2 g) in 1,2-dichloroethane (100 ml) and trifluoroacetic acid (10 ml) was heated under reflux for 2 h. A small sample (see below for the remainder) was evaporated and the *acid* was recrystallised from methanol; m.p. 161—169° for the major isomer (the minor isomer persists with m.p. *ca.* 205°) (Found: C, 57.7; H, 5.4; N, 8.4. $C_{16}H_{18}N_2O_6$ requires C, 57.5; H, 5.4; N, 8.4%); τ −1.04br (1 H, CO_2H), −0.27br (1 H, NH), 1.88 (<1 H, s, C=CH of major isomer), 2.50 (small s, C=CH of minor isomer), 5.64 (2 H, q, OEt), 6.36 (3 H, s, OMe), 7.05 and 7.48 (each 2 H, t, CH_2·CH_2), 7.68 (3 H, s, pyrr-Me), and 8.62 (3 H, t, OEt).

2-Bromo-5-(2-cyano-2-ethoxycarbonylvinyl)-4-(2-methoxycarbonylethyl)-3-methylpyrrole (68).—The remaining solution (above) was heated under reflux while a solution (18 ml), made from bromine (10 ml) and 1,2-dichloroethane (15 ml) was added over 1 h. When an excess of bromine persisted, the solvent was evaporated off and the residue crystallised from methanol to give the *bromopyrrole*, m.p. 94.5—101° (for the major isomer; the minor one had m.p. *ca.* 118°) (21.8 g), and chromatography of the mother liquors (silica gel; dichloromethane) gave more product (1.47 g) (Found: C, 48.8; H, 4.75; Br, 21.6; N, 7.6. $C_{15}H_{17}BrN_2O_4$ requires C, 48.8; H, 4.65; Br, 21.6; N, 7.6%); τ 0.28br (1 H, NH), 2.06 (<1 H, s, C=CH of major isomer), 2.71 (small s, C=CH of minor isomer), 5.67 (2 H, q, OEt) 6.35 (3 H, s, OMe),

7.05—7.49 (4 H, m, CH_2·CH_2), 7.99 (3 H, s, pyrr-Me), and 8.62 (3 H, t, OEt).

2-(2-*Cyano*-2-*ethoxycarbonylvinyl*)-3-(2-*methoxycarbonylethyl*)-4-*methylpyrrole* (69).—A solution of the bromopyrrole (68) (22.1 g) in tetrahydrofuran (220 ml) containing anhydrous sodium acetate (8 g) and glacial acetic acid (10 ml) was stirred with hydrogen and 10% palladised charcoal (2.2 g) until uptake ceased (*ca.* 3 h). The filtered solution was evaporated and the residue crystallised from methanol to give the α-*free pyrrole* (15.8 g), m.p. 108—110° (Found: C, 62.1; H, 6.35; N, 9.4. $C_{15}H_{18}N_2O_4$ requires C, 62.05; H, 6.25; N, 9.65%); τ 0.17br (1 H, NH), 1.98 (1 H, s, C=CH), 2.97 (1 H, d, *J* 3 Hz, pyrr-H), 5.68 (2 H, q, OEt), 6.36 (3 H, s, OMe), 7.06 and 7.69 (each 2 H, t, CH_2·CH_2), 7.95 (3 H, s, pyrr-Me), and 8.65 (3 H, t, OEt).

3-(2-*Methoxycarbonylethyl*)-4-*methylpyrrole*-2-*carbaldehyde* (60).—(a) via *The cyanoacrylate*. A solution of the foregoing product (11.6 g) in methanol (40 ml) was heated under reflux in a nitrogen stream for 2.5 h with sodium hydroxide (20.2 g) in water (60 ml). The solution was cooled to 0 °C and mixed with tetrahydrofuran (200 ml) and (slowly) with sulphuric acid at 0 °C (25.2 g of concentrated acid with 40 ml of water). Dichloromethane (200 ml) was quickly added and sufficient water to slurry the inorganic salts. Triethylamine (4 ml) was added to the separated organic phase, which was then evaporated, and the residue in water (25 ml) was acidified with acetic acid (3 ml). The precipitated acid (5.62 g) had m.p. 122—124° (decomp.). A solution of it in methanol (60 ml) was treated with an excess of distilled ethereal diazomethane; after a few min acetic acid (12 ml) was added, and the solution was evaporated; it was then evaporated twice again after successive additions of toluene (2 × 50 ml). The residue was dissolved in methanol (50 ml) and mixed with ether (200 ml) and n-hexane before filtration. The oil left by evaporation of the filtrate was crystallised from methanol (30 ml) and water (30 ml) to give in three crops the *formylpyrrole* (5.69 g), m.p. 91—92.5° (Found: C, 61.6; H, 6 9; N, 7.0. $C_{10}H_{13}NO_3$ requires C, 61.5; H, 6.7; N, 7.2%).

(b) via *The benzyl ester*. The aldehyde (70) (32.9 g), prepared as earlier,[33] was debenzylated in tetrahydrofuran and triethylamine with hydrogen and palladium as for cases above. The resultant acid [34] (21.7 g) was dissolved in water (750 ml) containing sodium hydrogen carbonate (30 g). Chloroform (500 ml) was added and the stirred mixture was heated under reflux while iodine (25.6 g) and sodium iodide (30.3 g) in water (250 ml) were added during 15 min. After heating for a further 10 min, the cooled organic phase was separated and evaporated with addition of a little magnesium oxide, then again evaporated twice after two successive additions of methanol. The final methanolic solution was filtered and evaporated to give the crystalline iodopyrrole, which was hydrogenated immediately. A solution of it in tetrahydrofuran (500 ml) and methanol (150 ml) was stirred with magnesium oxide (30 g), 10% palladised charcoal (3.27 g), and hydrogen at normal temperature and pressure until uptake ceased (6 days). The filtered solution was evaporated to dryness and the residue was partitioned between water and dichloromethane. The product from the latter was extracted with hot water and, by careful cooling and seeding, the aldehyde was crystallised (total 11.3 g); m.p. 91—92°, identical with product from method (a); τ 0.15br (1 H, NH), 0.42 (1 H, s, CHO), 3.13 (1 H, d, *J* 3 Hz, pyrr-H), 6.36 (3 H, s, OMe), 6.94 and 7.43 (each 2 H, t, CH_2·CH_2), and 7.96 (3 H, s, pyrr-Me).

3,3′-*Bis*-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethyl*-4′,5-*dimethylpyrromethene Hydrobromide* (61).—The benzyl ester of the pyrrole (25) (7.46 g) was hydrogenolysed as above and the resultant acid in methanol (100 ml) was mixed with the foregoing aldehyde (3.92 g). The filtered solution was heated under reflux for 10 min with concentrated hydrobromic acid (48%; 6 ml), then trimethyl orthoformate (50 ml) was added slowly and the solution was concentrated to *ca.* 100 ml. The cold solution was mixed with ether (400 ml) to crystallise the *pyrromethene* (2.75 g), and two further crops were obtained by reworking the mother liquors (total yield 6.74 g); m.p. 124—133° (Found: C, 53.1; H, 5.9; Br, 15.9; N, 5.6. $C_{22}H_{29}BrN_2O_6$ requires C, 53.1; H, 5.9; Br, 16.05; N, 5.6%); τ −3.33 and −3.21 (each 1 H, br, NH), 2.32 (1 H, s, *meso*-H), 2.42 (1 H, d, *J* 2 Hz, pyrr-H), 6.30, 6.37, and 6.39 (each 3 H, s, OMe), 6.44 (2 H, s, CH_2CO), 6.76—7.0 (4 H, m, CH_2·CH_2), 7.28 (3 H, s, 5-pyrr-Me), 7.20—7.48 (4 H, m, CH_2·CH_2), and 7.88 (3 H, s, 4′-pyrr-Me).

3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,12-*tris*-(*methoxycarbonylmethyl*)-18-*methylporphin* (*Ring* D *Methylporphyrin*) (74).—The pyrromethane (61) (502 mg) was heated under reflux with 1,2-dichloroethane (20 ml) so that some solvent boiled away, removing any water. Then at full reflux, bromine (3.72 g) in 1,2-dichloroethane (11 ml) was added and the mixture was heated under reflux for 1 h and then was evaporated. The residue was dissolved in dichloromethane (10 ml) and cyclohexene (5 ml) and, after 2 h at 20 °C, the solution was evaporated. To the residue was added a solution of the pyrromethene (59) (532 mg) in dichloromethane (40 ml) followed by tin(IV) chloride (3.3 ml) in dichloromethane (10 ml). After 18 h at 20 °C, the solution was decanted from the precipitated material; the solution was shown to yield negligible porphyrin on treatment as for the precipitate.

Methanol (30 ml) and 48% concentrated hydrobromic acid (5 ml) were added to the precipitate to give red crystals, which were collected after addition of ether (100 ml) and washed with a little methanol and then ether (282 mg). This biladiene hydrobromide (73) in dimethyl sulphoxide (30 ml) and pyridine (2 ml) was kept at 20 °C in the dark for 5 days. Methanol (100 ml) and water (300 ml) were added and the precipitate was collected and dissolved in dichloromethane; the solution was filtered and evaporated. The residue was re-esterified as for the isomeric porphyrins above and finally was crystallised from chloroform–methanol to give the *methylporphyrin* (74) (148 mg), m.p. 223—225° (Found: C, 62.55; H, 5.95; N, 6.2%; M^+, 884. $C_{46}H_{52}N_4O_{14}$ requires C, 62.4; H, 5.9; N, 6.3%; *M*, 884).

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