Synthetic and Biosynthetic Studies of Porphyrins. Part 1. Synthesis of the ' S-411 ' Porphyrin obtained from Meconium [1]

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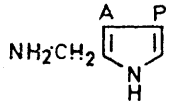
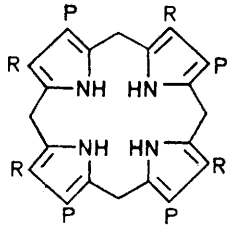
S-411 porphyrin is shown to be 2-(2-carboxyvinyl)-1,3,5,8-tetramethylporphin-4,6,7-tripropionic acid † by synthesis *via* the *b*-oxobilane and MacDonald routes. The isomeric 4-(2-carboxyvinyl)porphyrin was also prepared by the MacDonald route. In each case the acrylic acid side chain was introduced at the porphyrin stage. The two isomeric porphyrins were clearly distinguished from one another by m.p., n.m.r. spectra, and steady-state countercurrent distribution. The role of the S-411 porphyrin in relation to porphyrin biosynthesis is discussed.

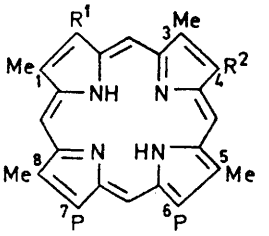
ALTHOUGH the main landmarks in porphyrin biosynthesis, porphobilinogen (PBG) (1), uroporphyrinogen-III (2a), coproporphyrinogen-III (2b), and protoporphyrin-IX (3a), have now been recognised for 15—20 years,[2] little was known until recently about the intermediates and sequence of steps between these compounds. However the way in which the unsymmetrical uroporphyrinogen-III is formed by reversal of one of the constituent PBG units has now been defined by the Yale group.[3] The nature of the hepta-, hexa-, and penta-carboxylic intermediates between uroporphyrinogen-III and coproporphyrinogen-III has also been deduced recently,[4] and details of this work will form the basis of later papers in this series. The sequence of steps between coproporphyrinogen-III and protoporphyrin-IX has now also been defined,[5-8] and in this paper we describe the



(1)



(2) a; R = A
b; R = Me



(3) a; $R^1 = R^2 = CH:CH_2$
b; $R^1 = CH:CH_2, R^2 = P$
c; $R^1 = CH:CH\cdot CO_2H, R^2 = P$
d; $R^1 = P, R^2 = CH:CH\cdot CO_2H$

A = $CH_2\cdot CO_2H$
P = $CH_2\cdot CH_2\cdot CO_2H$

† In this and the following paper, porphyrin skeletons are numbered according to the Fischer system.

[1] Preliminary account, A. H. Jackson and D. E. Games, *Ann. New York Acad. Sci.*, 1975, **244**, 591.

[2] G. S. Marks, *Ann. Reports*, 1962, **59**, 385.

[3] A. I. Scott, K. S. Ho, M. Kajiwara, and T. Takahashi, *J. Amer. Chem. Soc.*, 1976, **98**, 1589.

[4] A. H. Jackson, H. A. Sancovich, A. M. Ferramola, N. Evans, D. E. Games, S. A. Matlin, G. M. Elder, and S. G. Smith, *Phil. Trans. Roy. Soc.*, 1976, *B*, **273**, 191.

[5] G. Y. Kennedy, A. H. Jackson, G. W. Kenner, and C. J. Suckling, *F.E.B.S. Letters*, 1970, **6**, 9; **7**, 205.

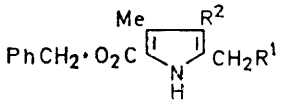
[6] J. A. S. Cavaleiro, G. W. Kenner, and K. M. Smith, *J.C.S. Perkin I*, 1974, 188.

[7] A. H. Jackson, D. E. Games, J. R. Jackson, R. V. Belcher, and S. G. Smith, *J.C.S. Chem. Comm.*, 1976, 187; A. H. Jackson D. E. Games, P. Couch, J. R. Jackson, R. V. Belcher, and S. G. Smith, *Enzymes*, 1974, **17**, 81; Z. Zaman, M. M. Aboud, and M. Akhtar, *J.C.S. Chem. Comm.*, 1972, 1263.

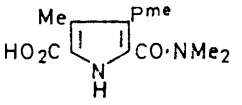
[8] A. R. Battersby, J. Baldas, J. Collins, D. H. Grayson, K. J. James, and E. McDonald, *J.C.S. Chem. Comm.*, 1972, 1265.

synthesis and proof of structure of S-411 porphyrin, a didehydrocoproporphyrin found in meconium.

Meconium is an accumulated end-product of the metabolism of the foetus in animals and is excreted during the first few hours of life of the new-born. It is a rich source of free porphyrins,[9] and is free from the action of micro-organisms, unlike normal mammalian faeces. Nicholas and Rimington [10] in a comparative study of the meconium of different animal species found that coproporphyrin-III was the major porphyrin present. Later



(4)



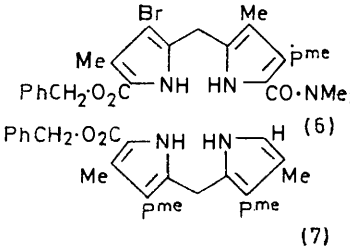
(5)

a; R^1 = H, R^2 = COMe
b; R^1 = R^2 = Br
c; R^1 = NC_5H_5 Br^-, R^2 = Br
d; R^1 = $\overset{+}{N}H{\cdot}CHO$, R^2 = Br
e; R^2 = H, R^1 = OAc

French and Thonger,[11] using micro-scale counter-current distribution techniques, detected small amounts of protoporphyrin and two other porphyrins: one of these is probably harderoporphyrin (3b); the other, the so-called ' S-411 ' porphyrin is the subject of this paper.

The designation ' S-411 ' refers to the position of the confirmed by catalytic hydrogenation, in which 1 mol. equiv. of hydrogen was taken up, to give a product which had an ' aetio-type ' spectrum, and which was indistinguishable from coproporphyrin-III on paper chromatography. Two alternative structures for S-411 (3c and d) were therefore proposed, on the basis of this evidence and biogenetic speculations.[13] We now show by synthesis of both isomers and comparisons with natural material that the structure of S-411 porphyrin is (3c).

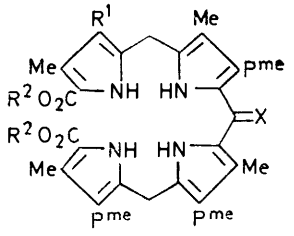
The direct synthesis of porphyrins with an acrylic acid side-chain is not feasible by any of the existing methods and it was, therefore, decided to prepare the related β-free porphyrins and to introduce the acrylic acid side-chains at the end of the syntheses. The *b*-oxobilane method [14] was chosen for the synthesis of the first porphyrin, the free β-position being protected with a bromine atom during the initial stages. The β-acetylpyrrole (4a), prepared from benzyl 2-hydroxyimino-3-oxobutyrate and acetylacetone in a Knorr-type synthesis, was treated with 4 equiv. of bromine to give the bromo(bromomethyl)pyrrole (4b). The latter was converted into the corresponding pyridiniomethylpyrrole (4c) and coupled in hot formamide with the lithium salt of the pyrrolecarboxylic acid (5) to give the pyrromethane amide (6); a small amount of the formamidomethylpyrrole (4d) was also formed. The pyrromethane amide (6) was then converted into its phosphoryl chloride complex and treated with the α-free-pyrromethane (7) in hot



(6)

(7)

P^{me} = $CH_2{\cdot}CH_2{\cdot}CO_2Me$



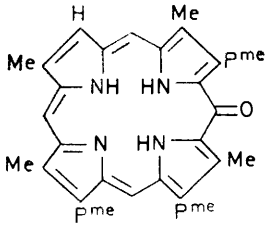
(8) a; X = $\overset{+}{N}Me_2$ R^1 = Br, R^2 = CH_2Ph
b; X = O, R^1 = Br, R^2 = CH_2Ph

(9) X = O, R^1 = R^2 = H

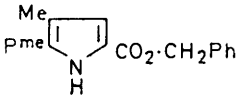
Soret band in the near u.v. at 410—411 nm (in 5% w/v hydrochloric acid). The free base exhibited a ' rhodo-type ' visible spectrum and ran as a tetracarboxyporphyrin in the lutidine–ammonia paper chromatographic system. The mass spectrum of the tetramethyl ester showed a parent ion at *m/e* 708, corresponding to a didehydrocoproporphyrin ester,[12] and this evidence was consistent with an analogue of coproporphyrin in which one of the propionic acid side-chains was replaced by an acrylic acid group.[13] The presence of the latter was methylene chloride. The resulting imine salt (8a) was purified by chromatography, and the *b*-oxobilane (8b) obtained after hydrolysis with aqueous sodium carbonate (50% yield from the pyrromethanes). Prolonged hydrogenation of the *b*-oxobilane over palladium–charcoal effected hydrogenolysis both of the benzyl ester groups and of the β-bromo-substituent, as shown by mass spectrometry. The crude dicarboxylic acid (9), was then cyclised directly to the oxophlorin (10) by treatment with trimethyl orthoformate and trichloroacetic acid in

[9] J. Waldenstrom, *Z. physiol. Chem.*, 1936, **239**, III.
[10] R. H. Nicholas, Ph.D. Thesis, London, 1951.
[11] J. M. French and E. Thonger, *Clinical Sci.*, 1966, **31**, 337.
[12] A. H. Jackson, G. W. Kenner, and K. M. Smith, unpublished work; K. M. Smith, Ph.D. Thesis, Liverpool, 1967.
[13] J. M. French, D. C. Nicholson, and C. Rimington, *Biochem. J.*, 1966, **120**, 393.
[14] *Cf.* A. H. Jackson and K. M. Smith in ' The Total Synthesis of Natural Products,' ed. J. Apsimon, Wiley, London and New York, 1974, p. 144.

methylene chloride followed by aerial oxidation; the reaction was followed spectroscopically and when oxophlorin production had reached a maximum it was converted without isolation into the related acetoxyporphyrin (11a) (38% yield from the pyrromethanes). The acetoxy-group was next removed by catalytic hydrogenation to porphyrinogen followed by re-oxidation to porphyrin. If the re-oxidation was carried out by aeration the yield was only 30% and a small amount of a chlorin ($\lambda_{max.}$ 650 nm) was obtained as a by-product; the low yield in comparison with previous syntheses was attributed to the presence of a free β-position. Iodine could not be used to oxidise the intermediate porphyrinogen owing to iodination of the free β-position, but



(10)

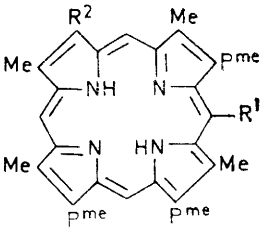


(12)

dichlorodicyanobenzoquinone was a more efficient oxidising agent and the porphyrin (11b) was obtained in 53% yield.

The use of the MacDonald route [14] (coupling of a diformylpyrromethane with an αα′-unsubstituted pyrromethane, or the related dicarboxylic acid) was also explored for the synthesis of this porphyrin. Initially the bromomethylpyrrole (4b) was condensed with the α-free pyrrole (12) in boiling glacial acetic acid containing sodium acetate, and the bromopyrromethane (13a) was obtained in 35% yield after careful chromatography. However, even after hydrogenation for several days over palladium or platinum not all the bromine was removed (as shown by mass spectrometry) and a mixture of the pyrromethane acids (13b and c) was obtained; preliminary small-scale experiments showed that this mixture could be converted into a mixture of porphyrins by acid-catalysed condensation with the diformyl pyrromethane (14) in air, but the two porphyrins (11b and c) could not be separated chromatographically. In view of these results we turned to the use of an unprotected β-position, and the acetoxymethylpyrrole (4c) was condensed with the α-free pyrrole (12) in methanol containing toluene-*p*-sulphonic acid to give the desired pyrromethane dibenzyl ester (13d) in 20% yield. [Low yields were also obtained in other syntheses with this acetoxymethylpyrrole (see Experimental section), and this was attributed to self-condensation and polymerisation resulting from the presence of the free β-position.] Hydrogenolysis gave the dicarboxylic acid (13c) which was condensed with the diformylpyrromethane (14) in methylene chloride–methanol containing toluene-*p*-sulphonic acid; subsequent addition of zinc acetate and aeration afforded the required porphyrin (11b) in 18% yield after re-esterification and removal of the zinc. Coproporphyrin-II tetramethyl ester (4%) was also obtained.

Initial attempts to introduce a formyl group into the



(11) a; R^1 = OAc, R^2 = H
b; R^1 = R^2 = H
c; R^1 = H, R^2 = Br
d; R^1 = H, R^2 = CHO
e; R^1 = H, R^2 = CH:CH·CO_2Me
f; R^1 = H, R^2 = CH:CH_2

free β-position involved Vilsmeier type formylation of the copper complex with the phosphoryl chloride complex of dimethylformamide. However after hydrolysis t.l.c. showed that four products were formed, and mixtures of porphyrins were also obtained in similar attempts to formylate deuteroporphyrins (*cf.* ref. 15). The brown iron(III) complex was, therefore, formylated with an excess of dichloromethyl methyl ether in the presence of tin(IV) chloride,[16] and a green intermediate with a chlorin-type spectrum was obtained; this intermediate may have structure (15) in view of the probable mechanism of these formylation reactions. Removal of iron by treatment with aqueous iron(II) sulphate then afforded the deep purple formylporphyrin (11d) which had a characteristic rhodo-type visible spectrum. The desired acrylic ester (11e) was obtained by condensation with the methyl hydrogen malonate in hot pyridine containing a trace of piperidine.

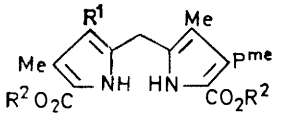
An alternative route to this porphyrin was also envisaged, by use of the pyrromethane amide (17); it was hoped that the β-ester group could be selectively converted into the free acid at the porphyrin stage and transformed into a formyl group *via* the corresponding phenyl thioester.[17] The pyrrole amide (16a) was prepared

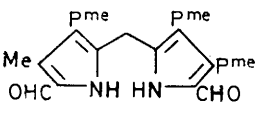
[15] (*a*) A. H. Jackson, G. W. Kenner, and J. Wass, *J.C.S. Perkin I*, 1974, 480; (*b*) H. Brockmann, K. M. Bliesener, and H. K. Inhoffen, *Annalen*, 1968, **718**, 148.

[16] H. Fisher and A. Schwarz, *Annalen*, 1934, **512**, 239.

[17] *Cf.* P. S. Clezy and A. J. Liepa, *Austral. J. Chem.*, 1970, **23**, 246.

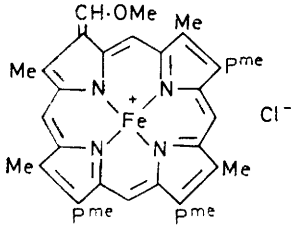
by Knorr synthesis from dimethylacetoacetamide oxime and ethyl acetoacetate and converted into the related acetoxymethylpyrrole (16b) with lead tetra-acetate. The latter was coupled with the α-free pyrrole (12) in hot acetic acid containing acetic acid, but the desired pyrromethane (17) was only obtained in poor yield after extensive chromatography; this approach was therefore





(13) a; R^1 = Br, R^2 = CH_2Ph
b; R^1 = Br, R^2 = H
c; R^1 = R^2 = H
d; R^1 = H, R^2 = CH_2Ph

(14)



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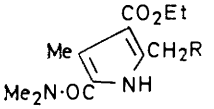
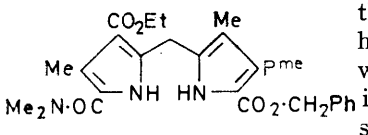
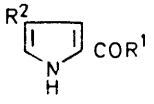


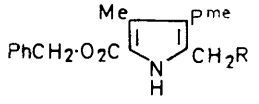
(16) a; R = H
b; R = OAc



(17)



(18) a; R^1 = NMe_2, R^2 = H
b; R^1 = NMe_2, R^2 = Me
c; R^1 = OMe, R^2 = CHO
d; R^1 = OMe, R^2 = Me
e; R^1 = O·CH_2Ph R^2 = Me



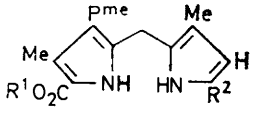
(19) a; R = H
b; R = OAc

abandoned, as the route to the formylporphyrin was proving more satisfactory.

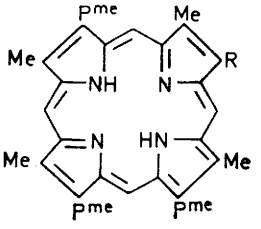
For the synthesis of the 4-acrylic acid porphyrin, *b*-oxobilane synthesis through the pyrromethane amide (21a) was also considered. This required preparation of the 4-methylpyrrole (18b), but although the amide (18a) was obtained in moderate yield by successive treatment of pyrrole with phosgene and dimethylamine, it could not be methylated even by strong methylating agents such as methyl fluorosulphate. Formylation, followed by reduction (as in the synthesis of 4-methylpyrrole-2-carboxylates) [18] was not possible because of the formation of amide complexes with Lewis acids. In view of these results we turned to the application of the MacDonald method to the pyrromethanes (14) and (20c).

Methyl pyrrole-2-carboxylate (prepared from trifluoroacetylpyrrole by hydrolysis and methylation) [12] was formylated with butyl dichloromethyl ether in presence of aluminium trichloride. The product (18c) was hydrogenated over platinum to give the 4-methylpyrrole (18d) and transesterified to yield the benzyl ester (18e). Condensation with the acetoxymethylpyrrole (19b) in methanol in presence of toluene-*p*-sulphonic acid [19] at 30 °C then afforded the pyrromethane (20b) in excellent yield. The latter was hydrogenolysed to the corresponding diacid (20c) and coupled with the diformylpyrromethane (14) in methylene chloride in the presence of toluene-*p*-sulphonic acid at 35 °C; saturated aqueous zinc acetate was then added, the solution was aerated, and the porphyrin ester (21a) was obtained in 15% yield after re-esterification with methanolic sulphuric acid; coproporphyrin-II tetramethyl ester (3%) was also obtained. The yield of the β-free porphyrin (21a) was disappointing, but cyclisations at lower or higher temperatures afforded even lower yields. Formylation and conversion into the related acrylic ester (21c) were then accomplished in a fashion similar to that for the isomer (11e).

Mixed m.p. comparisons of the ester of the natural material with the two synthetic compounds suggested that it was identical with the 2-acrylic isomer (11e); however, relatively little natural material (<0.5 mg) was available and it was thought desirable to confirm the identity by other means. The n.m.r. spectra of the synthetic materials were slightly different from each other, but in view of the small amount of natural



(20) a; R^1 = CH_2Ph, R^2 = CO·NMe_2
b; R^1 = CH_2Ph, R^2 = CO_2·CH_2Ph
c; R^1 = H, R^2 = CO_2H

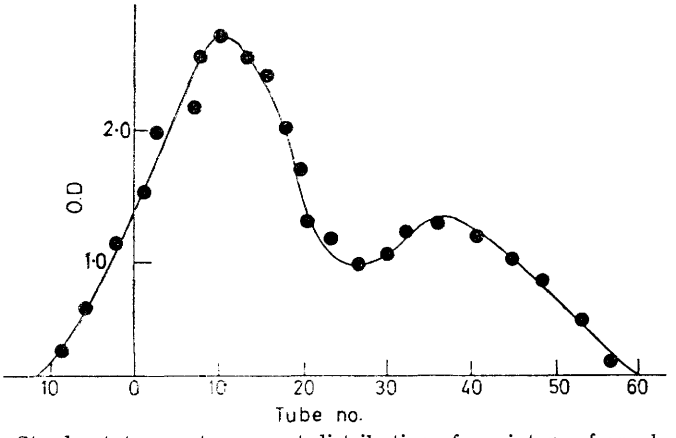


(21) a; R = H
b; R = CHO
c; R = CH:CH·CO_2Me
d; R = $CH:CH_2$

material and the concentration effects known to occur in porphyrin n.m.r. spectra,[20] comparisons of n.m.r. spectra were not possible. The mass spectra of the two isomers were virtually identical with each other, as were their visible spectra. We, therefore, turned to counter-current distribution, which had originally been used to isolate natural material.[11,13] However a different solvent system, isobutyl methyl ketone–benzene–sulphuric acid [5,21] was used (rather than ether–hydrochloric acid)

[18] P. E. Sonnet, *J. Medicin. Chem.*, 1971, **15**, 97.
[19] *Cf.* G. W. Kenner and K. M. Smith, *Ann. New York Acad. Sci.*, 1973, **206**, 138.
[20] R. J. Abraham, P. A. Burbridge, A. H. Jackson, and G. W. Kenner, *J. Chem. Soc.* (*B*), 1966, 620.
[21] A. H. Jackson, G. W. Kenner, and J. Wass, *J.C.S. Perkin I*, 1972, 1475.

and separations were carried out in a steady-state instrument. A mixture of equal amounts of each of the two synthetic porphyrins and the natural material was separated into two well-defined bands after *ca.* 800 transfers (see Figure); the larger slower moving band corresponded to the 2-acrylic acid isomer and thus confirmed conclusively the structure of the natural product. More recently, we have also attempted to separate the two isomeric esters (11e) and (21c) by high-pressure liquid chromatography, but without success,*



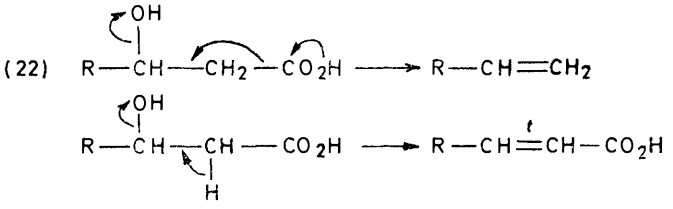
Steady state counter-current distribution of a mixture of equal amounts of natural S–411 porphyrin and the synthetic porphyrins (3c and d) between benzene–isobutyl methyl ketone (1 : 1 v/v) and 0.87M-sulphuric acid in a 120-tube Quickfit instrument with 10 ml upper and lower phases (640 upper phase and 160 lower phase transfers). The position of the larger of the two peaks corresponds to the porphyrin (3c) and the smaller to the porphyrin (3d), as determined in separate experiments with the individual isomers

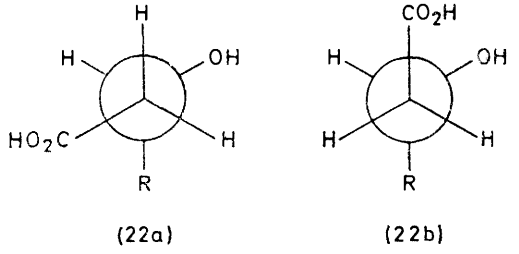
even though hardero- and isohardero-porphyrin isomers (11f) and (21d) are readily separated from each other.[6,22]

In the original paper concerning the structure of the S-411 porphyrin it was suggested that the acrylic acid residue might be in the *cis*-form.[13] However, both our synthetic porphyrins were clearly *trans* because of their method of preparation and as confirmed by their n.m.r. spectra. Moreover, molecular models show that a *cis*-acrylic acid could not be formed unless the side-chain is twisted out of conjugation with the nucleus, whereas there is little steric hindrance to coplanarity for a *trans*-acrylic acid side chain. The natural product also shows a characteristic 'rhodo-type' spectrum due to conjugation of the acrylic acid residue with the macrocycle, and as it is identical with the synthetic 2-isomer we conclude that the natural product must also have the *trans*-configuration.

The role of the S-411 porphyrin (3c) in relation to porphyrin biosynthesis is of considerable interest, and the possibility that the related porphyrinogen might be the first intermediate between coproporphyrinogen-III (2b) and protoporphyrin-IX (3a) was considered. However, this was excluded by the finding that oxidative decarboxylation of the 2- and 4-propionate side chains of coproporphyrinogen-III occurred with retention of both hydrogen atoms of each of the methylene groups neighbouring the carboxy-groups, as shown by deuterium labelling studies.[8] Recent work in Liverpool[6] and in Cardiff[1,7] has shown that the 2-propionate group of coproporphyrinogen-III is degraded before that at position 4, and a 2-vinylporphyrin [harderoporphyrin (3b) has been isolated, originally from the rat harderian gland,[5] but more recently[7] from red-cell hemolysates and liver homogenates after incubation with coproporphyrinogen-III]. The 2-acrylic acid structure of the S-411 porphyrin is in accord with these results.

It has been suggested[23] that hydroxylation of the methylene group neighbouring the macrocyclic ring is the first stage in the metabolism of coproporphyrinogen-III, although hydride ion removal may also be a possibility especially in anaerobic systems.[24] Thus fragmentation of the hydroxypropionate side-chains could occur either with simultaneous loss of the hydroxy- and carboxy-groups, or by loss of water alone. However Newman projections indicate that the *trans*-conformation (22a)





(22a) (22b)

required for the former process is less stable than that (22b) required to give the acrylic acid. It thus seems possible that the normal metabolic process requires enzymic assistance to hold the side-chain in the correct conformation for fragmentation to the vinyl group, whereas formation of the S-411 porphyrin in meconium may be due to non-enzymic loss of water from the more stable conformer of the hydroxypropionate; alternatively the S-411 porphyrin may be an artefact, produced on work-up, from the corresponding hydroxypropionate porphyrin (22).

EXPERIMENTAL

M.p.s were determined on a hot-stage apparatus. N.m.r. spectra were determined with a Perkin-Elmer R14 100 MHz

* *Note added in proof:* This has now been achieved by Mr. R. Towill and Dr. K. R. N. Ras using a specially prepared reversed phase column.

[22] N. Evans, D. E. Games, A. H. Jackson, and S. A. Matlin, *J. Chromatog.*, 1975, **115**, 325.
[23] S. Sano, *J. Biol. Chem.*, 1966, **241**, 5276.
[24] G. H. Tait, *Biochem. J.*, 1972, **128**, 1159; S. Granick and S. Sano, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1961, **20**, 376.

spectrometer, and mass spectra with a Varian CH5D instrument. Reactions were monitored whenever possible by t.l.c. on silica gel, and by u.v.–visible spectroscopy. Column chromatography was carried out on alumina (Brockmann grade III).

Pyrroles

5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylic Acid.—Freshly distilled sulphuryl chloride (24.3 ml; 3.3 mol. equiv.) was added over 1 h at 20 °C to a stirred solution of benzyl 4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (31.5 g) in dry ether (1 100 ml). The solution was stirred for a further 3 h, and left overnight; a starch–iodide test then indicated that the reaction was complete. The solvent was removed under reduced pressure, and the oily residue was immediately dissolved in dioxan (150 ml) and stirred vigorously with sodium acetate trihydrate (14 g) in water (200 ml) for 3 h at 70 °C. Ether (200 ml) was added and the aqueous layer was separated and re-extracted with ether.

The organic extracts were back-extracted with aqueous 10% sodium carbonate (4 × 50 ml) and the aqueous layers were subsequently saturated with sulphur dioxide. The product was filtered off, washed well with hot water, and dried *in vacuo*. Recrystallisation from chloroform–light petroleum (b.p. 40—60°) gave the required pyrrolecarboxylic acid (24.1 g, 70%) as pale yellow prisms, m.p. 149—150° (lit.,[15a] 149—150°), τ ($CDCl_3$) 7.70 (3 H, s, Me), 7.45 (2 H, t) and 6.93 (2 H, t) (CH_2·CH_2), 6.34 (3 H, s, OMe), 4.66 (2 H, s) and 2.60 (5 H, s) (CH_2Ph), 0.29 (1 H, s, NH), and −0.15 (1 H, s, CO_2H).

An insoluble impurity filtered from the original ether layer, was the corresponding *pyrrocol* (2.3 g, 8%), m.p. 160—161° (Found: C, 65.8; H, 5.0; N, 4.1. $C_{36}H_{34}N_2O_{10}$ requires C, 66.0; H, 5.2; N, 4.3%), τ ($CDCl_3$) 7.91 (6 H, s, Me), 7.40 (4 H, t) and 6.90 (4 H, t) (CH_2·CH_2), 6.33 (6 H, s, OMe), and 4.63 (4 H, s) and 2.61 (10 H, s) (CH_2Ph), *m/e* 654 (3%) and 91 (100).

5-Dimethylcarbamoyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylic Acid (5).—Benzyl 5-dimethylcarbamoyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (3.72 g) in dry tetrahydrofuran (100 ml) containing triethylamine (3 drops) was hydrogenated over 10% palladium–charcoal (300 mg) at room temperature and pressure. After 30 min, uptake of hydrogen ceased; the catalyst was filtered off and the solvent removed under reduced pressure giving the *pyrrolecarboxylic acid* (2.8 g, 100%) as a crystalline solid, m.p. 72—75°, τ ($CDCl_3$) 7.70 (3 H, s, Me), 7.50 (2 H, t) and 7.15 (2 H, t) (CH_2·CH_2), 6.35 (3 H, s, CO_2Me), 6.96 (6 H, s, $CONMe_2$), −0.17 (1 H, s, NH), and −0.37 (1 H, s, CO_2H).

Benzyl 4-Bromo-3-methyl-5-pyridiniomethylpyrrole-2-carboxylate Bromide (4c).—Benzyl 4-bromo-5-bromomethyl-3-methylpyrrole-2-carboxylate [21] (3.87 g) was dissolved in the minimum amount of hot pyridine and the solution diluted with a large volume of dry ether. The *pyridinium salt* (4.2 g, 90%) crystallised as needles, m.p. 153—154°, τ ($CDCl_3$) 7.78 (3 H, s, Me), 4.77 (2 H, s, $PhCH_2$), 3.77 (2 H, s, NCH_2), 2.70 (5 H, s, Ph), 2.35 (1 H, t), 1.95 (2 H, d), and 0.60 (2 H, d) (pyridine ring H), and −2.45 (1 H, s, NH).

Ethyl 5-Dimethylcarbamoyl-2,4-dimethylpyrrole-3-carboxylate (16a).—(*a*) Sodium nitrite (90 g) in water (200 ml) was added slowly to *NN*-dimethylacetoacetamide (160 g) in glacial acetic acid (200 ml) at 0 °C at such a rate that the temperature did not rise above 10 °C. This solution was added slowly with vigorous stirring to ethyl acetoacetate (162 g) in glacial acetic acid (300 ml) which had been heated to 60 °C. A mixture of zinc dust (200 g) and anhydrous sodium acetate (175 g) was also added simultaneously in small portions and at such a rate that the temperature of the mixture did not exceed 90 °C. The mixture was then heated at 100 °C for 1 h and poured into ice–water (10 l). The precipitated pyrrole was filtered off, washed well with water, and dried *in vacuo*. Crystallisation from aqueous methanol, and then methylene chloride–light petroleum (b.p. 60—80 °C) yielded the *pyrrole* (164 g, 60%) as needles, m.p. 117—118° (Found: C, 60.1; H, 7.6; N, 11.5. $C_{12}H_{18}N_2O_3$ requires C, 60.05; H, 7.6; N, 11.8%), τ ($CDCl_3$) 8.67 (3 H, t) and 5.75 (2 H, q) (Et), 7.74 (3 H, s, Me), 7.60 (3 H, s, Me), 6.94 (6 H, s, $CONMe_2$), and −1.20 (1 H, s, NH), *m/e* 238 (46%) and 193 (100).

(*b*) 4-Ethoxycarbonyl-3,5-dimethylpyrrole-2-carboxylic acid (4.5 g) was added to thionyl chloride (15 ml) and the mixture was heated at 50 °C for 1 h. The solution was evaporated under reduced pressure and the resulting red oil taken up in dry benzene (35 ml). An excess of anhydrous dimethylamine was bubbled through and the benzene solution was washed with dilute acid and water until the washings were neutral, dried ($MgSO_4$), and evaporated under reduced pressure. Recrystallisation of the residue from ether–light petroleum (b.p. 60—80°) yielded the pyrrole (0.35 g, 7%) as pale brown needles, m.p. 116—117°.

Benzyl 4-Carbamoyl-3,5-dimethylpyrrole-2-carboxylate.—This was prepared from benzyl acetoacetate (10 g) and acetoacetamide (4.15 g) in the same manner as in the foregoing preparation. Recrystallisation from aqueous methanol gave the *pyrrole* (2 g, 22%) as off-white clusters of needles, m.p. 231—233° (Found: C, 65.8; H, 5.7; N, 10.1. $C_{15}H_{16}N_2O_3$ requires C, 66.2; H, 5.5; N, 9.7%).

Ethyl 2-Acetoxymethyl-5-dimethylcarbamoyl-4-methylpyrrole-3-carboxylate (16b).—Lead tetra-acetate (9.74 g, 1.1 mol. equiv.) was added in portions during 15 min to a solution of ethyl 5-dimethylcarbamoyl-2,4-dimethylpyrrole-3-carboxylate (4.76 g) in glacial acetic acid (200 ml) and the mixture was stirred overnight. The solution was poured onto water (500 ml) and immediately extracted with chloroform (2 × 100 ml). The extracts were washed with 10% sodium carbonate solution (50 ml) and water (2 × 50 ml), dried ($MgSO_4$), and evaporated under reduced pressure. Recrystallisation of the solid residue from methylene chloride–light petroleum (b.p. 60—80°) gave the *pyrrole* (2.5 g, 44%) as needles, m.p. 123—125° (Found: C, 56.5; H, 6.5; N, 9.7. $C_{14}H_{20}N_2O_5$ requires C, 56.75; H, 6.75; N, 9.5%), τ ($CDCl_3$) 8.67 (3 H, t) and 5.71 (2 H, q) (Et), 7.99 (3 H, s, OAc), 7.72 (3 H, s, Me), 6.93 (6 H, s, $CONMe_2$), 4.71 (2 H, s, CH_2), and −0.70 (1 H, s, NH), *m/e* 296 (7%), 238 (30), and 193 (100).

NN-*Dimethylpyrrole-2-carboxamide.*—Freshly distilled pyrrole (6.7 g) was dissolved in dry ether (30 ml) and a stream of phosgene gas was bubbled quickly through the solution until it was saturated. The excess of phosgene was removed under dry nitrogen. Fresh ether (30 ml) was added to the yellow residue and anhydrous dimethylamine was bubbled through the solution. The ether solution was then washed with dilute hydrochloric acid, 10% sodium carbonate solution, and finally water until the washings were neutral, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was chromatographed on alumina (Spence grade III), with first benzene–light petroleum

(b.p. 60—80 °C) (1 : 1 v/v) and then benzene as eluant. The solvent was removed under reduced pressure; recrystallisation of the residue from benzene–light petroleum (b.p. 60—80 °C) yielded the *pyrrole* (9.9 g, 70%), m.p. 95° (Found: C, 60.65; H, 7.0; N, 20.6. $C_7H_{10}N_2O$ requires C, 60.9; H, 7.25; N, 20.3%), τ ($CDCl_3$) 6.83 (6 H, s, $CONMe_2$), 3.16 (1 H, s, 3-H), 3.48 (1 H, d, 4-H), 3.83 (1 H, d, 5-H), and −1.00 (1 H, s, NH), *m/e* 138 (100%).

Pyrromethanes

Benzyl 3-Bromo-5′-dimethylcarbamoyl-4′-(2-methoxycarbonylethyl)-3′,4-dimethylpyrromethane-5-carboxylate (6).—5-Dimethylcarbamoyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylic acid (2.82 g) and lithium methoxide (0.38 g) were suspended in dry formamide (35 ml) and the mixture was shaken until all the solid had dissolved. Benzyl 4-bromo-3-methyl-5-pyridiniomethylpyrrole-2-carboxylate bromide (4.66 g) was then added and the solution heated under nitrogen for 20 h at 100 °C. During this time a viscous oil separated and when the mixture was cooled a solid crystallised from the formamide solution. The crystals were filtered off, washed well with water, and dried. The oil was taken up in methylene chloride (50 ml) and washed successively with dilute hydrochloric acid and water, until the washings were neutral, and dried ($MgSO_4$). Evaporation gave a brown oil which crystallised on triturating with ether. The two crops were combined; recrystallisation from methylene chloride–light petroleum (b.p. 60—80 °C) gave the *pyrromethane* (2.23 g, 41%) as needles, m.p. 205—207° (Found: C, 57.1; H, 5.6; Br, 14.45; N, 7.7. $C_{26}H_{30}BrN_3O_5$ requires C, 57.4; H, 5.5; Br, 14.7; N, 7.3%), τ ($CDCl_3$) 7.74 (3 H, s, Me), 7.97 (3 H, s, Me), 7.58 (2 H, t) and 7.30 (2 H, t) (CH_2·CH_2), 6.98 (6 H, s, $CONMe_2$), 6.39 (3 H, s, CO_2Me), 6.22 (2 H, s, CH_2), 4.67 (2 H, PhCH_2), 2.61 (5 H, s, Ph), and −0.31 (1 H, s), −0.45 (1 H, s) (NH), *m/e* 543 (26%), 545 (28), and 91 (100). An insoluble impurity (0.3 g, 5%; m.p. 221—223°) filtered off in the recrystallisation was benzyl 4-bromo-5-formamidomethyl-3-methylpyrrole-2-carboxylate (lit.,[21] m.p. 222°), τ [$(CD_3)_2SO$] 7.79 (3 H, s, 3-Me), 5.74 (2 H, d, CH_2), 4.76 (2 H, s, PhCH_2), 2.63 (5 H, s, Ph), 2.00 (1 H, s, CHO), 1.87 (1 H, m), and −1.8 (1 H, s, NH), *m/e* 350 (2%), 352 (2), and 91 (100).

Dibenzyl 3-Bromo-4′-(2-methoxycarbonylethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylate (13a).—Benzyl 4-bromo-5-bromomethyl-3-methylpyrrole-2-carboxylate (1.16 g) and benzyl 3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (0.903 g) were dissolved in benzene (60 ml) and heated under reflux under nitrogen for 2 h. The dark red solution was poured into water and extracted with methylene chloride, and the extracts were washed with water until the washings were neutral. The methylene chloride was removed under reduced pressure and the residue chromatographed on alumina (Merck, activity III). Elution first with benzene–light petroleum (b.p. 60—80 °c) (7 : 3) and then with benzene yielded an orange oil, which crystallised at 0 °C. Recrystallisation from methylene chloride–light petroleum (b.p. 60—80 °C) gave the *pyrromethane* (0.63 g, 35%) as needles, m.p. 129—131° (Found: C, 61.1; H, 4.7; N, 4.8. $C_{31}H_{31}BrN_2O_6$ requires C, 61.3; H, 5.1; N, 4.6%), τ ($CDCl_3$) 7.98 (3 H, s) and 7.85 (3 H, s) (Me), 7.60 (2 H, t) and 7.03 (2 H, t) (CH_2·CH_2), 6.44 (3 H, s, CO_2Me), 6.19 (2 H, s, CH_2), 4.85 (2 H, s, $PhCH_2$), 2.81 (5 H, s, Ph), and −0.16 (1 H, s) and −0.34 (1 H, s) (NH), *m/e* 606 (10%), 608 (10), and 91 (100).

Dibenzyl 4′-(2′-Methoxycarbonylethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylate (13d).—A suspension of benzyl 5-acetoxymethyl-3-methylpyrrole-2-carboxylate (587 mg) and benzyl 3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (600 mg) in methanol (10 ml) was treated with toluene-*p*-sulphonic acid hydrate (10 mg) and heated under nitrogen at 35 °C for 4 h. Chloroform was added and the solution washed with 10% sodium carbonate solution and water, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was chromatographed on neutral alumina (Spence grade III), with benzene–light petroleum (b.p. 60—80 °C) (7 : 3) as eluant. The pyrromethane was obtained as an oil (216 mg, 20%) which could not be induced to crystallise, τ ($CDCl_3$) 8.05 (3 H, s) and 7.75 (3 H, s) (β-Me), 7.60 (2 H, t) and 7.05 (2 H, t) (CH_2·CH_2), 6.45 (3 H, s, CO_2Me), 4.83 (4 H, s, PhCH_2), 4.23 (1 H, s, β-H), 2.75 (10 H, s, Ph), and 0.10 (2 H, s, NH).

Dibenzyl 3-(2-Methoxycarbonylethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylate (20b).—A suspension of benzyl 4-methylpyrrole-2-carboxylate [25] (215 mg) and benzyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (373 mg) in methanol (7 ml) was treated with toluene-*p*-sulphonic acid (12 mg) and heated under nitrogen at 35 °C overnight. The mixture was cooled to 0 °C, and the product filtered off and recrystallised from methylene chloride–light petroleum (b.p. 60—80 °C) to give the *pyrromethane* (475 mg, 90%) as needles, m.p. 144—146° (Found: C, 70.4; H, 6.1; N, 5.3. $C_{31}H_{32}N_2O_6$ requires C, 70.5; H, 6.2; N, 5.2%), τ ($CDCl_3$) 7.94 (3 H, s) and 7.75 (3 H, s, Me), 7.60 (2 H, t) and 7.31 (2 H, t) (CH_2·CH_2), 6.39 (3 H, s, CO_2Me), 6.16 (2 H, s, CH_2), 4.79 (4 H, s, PhCH_2), 3.26 (1 H, s, 4′-H), 2.72 (10 H, s, Ph), and 0.58 (1 H, s) and 0.12 (1 H, s) (NH), *m/e* 528 (10%) and 91 (100).

3-(2-Methoxycarbonylethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylic Acid (20c).—The corresponding dibenzyl ester (412 mg) in dry tetrahydrofuran (30 ml) containing triethylamine (2 drops) was hydrogenated over 10% palladium–charcoal (100 mg) at 20 °C and 760 mmHg until uptake had ceased. The catalyst was filtered off and the solvent removed under reduced pressure. The residual foam was recrystallised by addition of a little ether, yielding the *pyrromethane* (240 mg, 100%) as needles, m.p. 200° (decomp.) (Found: C, 58.8; H, 6.0; N, 8.1. $C_{17}H_{20}N_2O_6$ requires C, 58.6; H, 5.7; N, 8.0%), *m/e* 260 (3%) and 41 (100) (no M^+).

Oxobilanes

Dibenzyl 2-Bromo-4,6,7-tris-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-b-oxobilane-1′,8′-dicarboxylate (8b).—Benzyl 3-bromo-5′-dimethylcarbamoyl-4′-(2-methoxycarbonylethyl)-3,4-dimethylpyrromethane-5-carboxylate (1.36 g) was dissolved in phosphoryl chloride (15 ml) at 50 °C. The excess of phosphoryl chloride was removed under reduced pressure and dry 1,2-dibromoethane (2 × 10 ml) was then added and distilled off under reduced pressure to ensure that the last traces of phosphoryl chloride had been removed. The residual oil ($\lambda_{max.}$ 385 and 286 nm) was taken up in methylene chloride (10 ml) and mixed with a solution of benzyl 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5-carboxylate [obtained by decarboxylation of the corresponding pyrromethane-5′-carboxylic acid (1.4 g)] in methylene chloride (10 ml). The mixture was

[25] Part 2, D. E. Games, A. H. Jackson, and P. J. O'Hanlon, following paper.

heated under reflux in the dark for 64 h under nitrogen until spectroscopic analysis showed that the new band which developed at 410 nm had reached its maximum intensity. After addition of methylene chloride (30 ml), the mixture was washed with water (200 ml), dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was chromatographed on alumina (Merck, activity III), with first benzene, then benzene containing increasing proportions of ethyl acetate, and finally ethyl acetate as eluants. The column was finally washed with methanol; evaporation of the methanolic fraction gave the intermediate *imine salt* (8a) (1.5 g) as a yellow foam (Found: C, 60.8; H, 6.2; Br, 7.4; N, 6.5. $C_{53}H_{61}BrN_5O_{10}$ requires C, 61.0; H, 5.85; Br, 7.5; N, 6.7%), τ ($CDCl_3$) 7.76 (12 H, s, Me), 7.30 (12 H, m, CH_2·CH_2), 6.80 (6 H, s, NMe_2), 6.36, 6.42, and 6.42 (each 3 H, s, CO_2Me), 5.97 (2 H, s) and 6.04 (2 H, s) (CH_2), 4.80 (4 H, s, $PhCH_2$), and 2.70 (10 H, s, Ph).

The imine salt was taken up in methylene chloride (50 ml) and hydrolysed by stirring under reflux with 10% sodium carbonate solution (90 ml) for 90 min. The organic layer was separated, washed with water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The residue was chromatographed on alumina (Merck activity III) and eluted with benzene–ethyl acetate (7 : 3). The b-*oxobilane* (1.34 g, 50%) was obtained as a brown foam (Found: C, 62.0; H, 5.8; N, 5.6. $C_{51}H_{55}BrN_4O_{11}$ requires C, 62.4; H, 5.6; N, 5.6%), $\lambda_{max.}$ ($CHCl_3$) 294 and 352 nm, $\lambda_{max.}$ ($CHCl_3$–HCl) 292, 323, and 450 nm, τ ($CDCl_3$) 7.76 (12 H, s, Me), 7.80 (6 H, t) and 7.30 (6 H, t) (CH_2·CH_2), 6.58, 6.51, and 6.43 (each 3 H, s, CO_2Me), 6.20 (2 H, s) and 6.22 (2 H, s) (CH_2), 4.84 (4 H, s, $PhCH_2$), 2.80 (10 H, s, Ph), and 0.34, −0.07, and −0.33 (each 1 H, s, NH), *m/e* 978 (0.01%), 980 (0.01), and 91 (100).

4,6,7-*Tris*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-b-*oxobilane*-1′,8′-*dicarboxylic Acid* (9).—The corresponding dibenzyl ester (1.1 g) in dry tetrahydrofuran (125 ml) containing triethylamine (4 drops) was hydrogenated over 10% palladium–charcoal (0.4 g) at 20 °C and 1 atm for 5 days. The catalyst was filtered off through Celite and the solvent removed under reduced pressure, yielding the b-*oxobilane* as a brown foam (0.75 g, 92%) which was used without further purification (Found: C, 61.3; H, 6.0; N, 7.4. $C_{37}H_{44}N_4O_{11}$ requires C, 61.6; H, 6.1; N, 7.8%), $\lambda_{max.}$ (EtOH) 277 and 350 nm, *m/e* 632 (100%) (no M^+). The n.m.r. spectrum ($CDCl_3$) was very similar to that of the corresponding dibenzyl ester except for the lack of peaks at τ 4.80 and 2.70, and the appearance of a peak at 4.15 (1 H, s).

Porphyrins

β-*Acetoxy*-4,6,7-*tris*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (11a).—4,6,7-Tris-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-*b*-oxobilane-1′,8′-dicarboxylic acid (3.13 g) was treated successively with 1M-trichloroacetic acid (27.82 g) in methylene chloride (150 ml) and trimethyl orthoformate (9.1 g) in methylene chloride (650 ml). The deep red solution ($\lambda_{max.}$ 502 nm) was stirred for 3 h in the dark before addition of pyridine (7.5 ml), and the mixture was then stirred in air overnight in the dark. The resulting green oxophlorin solution ($\lambda_{max.}$ 404, 492, 575, 640, and 698 nm) was evaporated to dryness under reduced pressure and the residue taken up in pyridine (60 ml) and acetic anhydride (20 ml). After stirring in the dark at room temperature for 1 h, the deep red solution was evaporated to low volume under reduced pressure. The residue was dissolved in methylene chloride (150 ml) and washed with 10% sodium carbonate (2 × 50 ml) and then with water until the washings were neutral, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The residue was dissolved in the minimum volume of methylene chloride and chromatographed twice on alumina (Merck, activity III) first in methylene chloride, and then in methylene chloride–benzene (1 : 1). The porphyrinic fraction was evaporated to dryness under reduced pressure and the residue recrystallised from methylene chloride–methanol to give the *acetoxyporphyrin* (0.91 g, 30%) as red-brown needles, m.p. 165—167° (Found: C, 66.6; H, 6.15; N, 8.1. $C_{38}H_{42}N_4O_8$ requires C, 66.9; H, 6.1; N, 8.2%), $\lambda_{max.}$ ($CHCl_3$) 404 (log ε 5.24), 502 (4.08), 511 (3.68), 536 (3.68), and 623 nm (2.93), $\lambda_{max.}$ ($CHCl_3$–CF_3·CO_2H) (dication) 424 (5.33), 561 (4.06), and 602 nm (3.49), τ ($CDCl_3$) 7·00 (3 H, s, OAc), 6.76 and 5.87 (each 6 H, t, CH_2·CH_2), 6.48 (6 H, s) and 6.42 (6 H, s) (Me), 6.36, 6.31, and 6.18 (each 3 H, s, CO_2Me), 1.06 (1 H, s), 0.17, 0.06, and −0.06 (each 1 H, s, *meso*-H), and 13.71 (2 H, s, NH), *m/e* 682 (13%) and 640 (100).

4,6,7-*Tris*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (11b).—(*a*) β-Acetoxy-4,6,7-tris-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphin (110 mg) in dry tetrahydrofuran (125 ml) containing triethylamine (5 drops) was hydrogenated over 10% palladium–charcoal (150 mg) at 20 °C and 1 atm for 16 h. The catalyst was filtered off and more tetrahydrofuran (400 ml) containing pyridine (2 ml) was added. Air was bubbled through the stirred solution, irradiated by an electric lamp bulb overnight, and the solution was concentrated to a small volume under reduced pressure. Benzene (30 ml) was added and the solution reconcentrated to a small volume (*ca.* 2 ml) under reduced pressure. This solution was chromatographed twice on alumina (Merck, activity III), with methylene chloride–benzene (1 : 1) as eluant, and the porphyrinic fractions were evaporated to dryness under reduced pressure. Recrystallisation of the residue from methylene chloride–methanol afforded the *porphyrin* (32 mg, 35%) as purple needles, m.p. 182—184° (Found: C, 69.2; H, 6.6; N, 9.1. $C_{36}H_{40}N_4O_6$ requires C, 69.2; H, 6.4; N, 9.0%), $\lambda_{max.}$ ($CHCl_3$) 398 (log ε 5.23), 496 (4.08), 531 (3.86), 568 (3.72), and 622 nm (3.50), $\lambda_{max.}$ ($CHCl_3$–CF_3·CO_2H) (dication) 416 (5.45), 553 (4.18), and 597 nm (3.80), τ ($CDCl_3$) 14.35 (2 H, s, NH), 6.48 (6 H, s, Me), 6.37 (9 H, s, CO_2Me), 6.78 (6 H, t) and 5.87 (6 H, t) (CH_2·CH_2), 1.18 (1 H, s, 2-H), 0.30 (1 H, s), 0.23 (2 H, s), and 0.15 (1 H, s) (*meso*-H), *m/e* 624 (100%), 625 (41), and 551 (40).

When the oxidation to the porphyrin was carried out with 1,2-dichloro-4,5-dicyanoquinone (DDQ) (200 mg) in benzene (200 ml) instead of air, the yield of porphyrin was increased to 53%.

(*b*) 4′-(2-Methoxycarbonylethyl)-3,4′-dimethylpyrromethane-5,5′-dicarboxylic acid (108 mg) and 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarbaldehyde (132 mg) were dissolved in methanol (2 ml) and methylene chloride (50 ml), treated with toluene-*p*-sulphonic acid (0.25 g) in methanol (2 ml), and stirred for 24 h at 35 °C with exclusion of light. A saturated solution of zinc acetate in methanol (7 ml) was added to the resulting solution ($\lambda_{max.}$ 410 and 505 nm), which was stirred in air until the Soret band ($\lambda_{max.}$ 400 nm) had reached a maximum. The solvent was removed under reduced pressure and the residue re-esterified with methanol–5% concentrated sulphuric acid (20 ml) overnight. The acid was neutralised

with concentrated ammonia, and the porphyrin taken up in chloroform; the solution was washed with water, dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed twice on alumina (Merck, activity III) with methylene chloride–benzene (1 : 1) as eluant. Recrystallisation from methylene chloride–methanol gave the porphyrin (22 mg, 15%) as purple needles, m.p. 181—183°, which did not depress the m.p. of an authentic sample obtained by the *b*-oxobilane route.

A second porphyrin fraction obtained by elution with chloroform was coproporphyrin-II tetramethyl ester (4 mg, 2.5%), m.p. 285—288°.

2,6,7-*Tris*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (21a).—This was obtained from 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarbaldehyde (1.3 g) and 3-(2-methoxycarbonylethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylic acid (1.08 g) by the same procedure as in the foregoing preparation. The *porphyrin* (220 mg, 15%) was obtained as red needles, m.p. 190—191° (Found: C, 68.7; H, 6.45; N, 9.4. $C_{36}H_{40}N_4O_6$ requires C, 69.2; H, 6.4; N, 9.0%), $\lambda_{max.}$ ($CHCl_3$) 398 (log ε 5.22), 498 (4.14), 531 (3.77), 568 (3.66) and 621 nm (3.49), $\lambda_{max.}$ ($CHCl_3$–CF_3CO_2H) (dication) 417 (5.41), 554 (4.20), and 597 nm (3.81), τ ($CDCl_3$) 14.43 (2 H, s, NH), 6.62, and 6.46 (each 3 H, s, Me), 6.40 (9 H, s, CO_2Me), 1.18 (1 H, s, 2-H), 0.44 (1 H, s), 0.36 (1 H, s), and 0.31 (2 H, s) (*meso*-H), *m/e* 624 (100%), 625 (41), and 551 (43).

2-*Formyl*-4,6,7-*tris*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (11d).—4,6,7-Tris-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphin (62 mg) in refluxing acetic acid (32 ml) was treated with a freshly prepared solution of iron(II) acetate in acetic acid. Conversion into the haem was almost instantaneous but refluxing was continued for 5 min and the acetic acid then removed under reduced pressure. The residue was taken up in methylene chloride (120 ml) and water (110 ml) and the organic phase washed with 10% sodium hydrogen carbonate solution (3 × 100 ml) and with water until the washings were neutral. The dried ($MgSO_4$) solution of the haem ($\lambda_{max.}$ 383, 407, 507, 548, and 650 nm) in dry methylene chloride was treated with butyl dichloromethyl ether (3.5 ml) and then tin(IV) chloride (0.7 ml) and the mixture kept at room temperature for 30 min. During this time the colour of the solution changed from brown to green ($\lambda_{max.}$ 408, 556, 587, and 675 nm). The mixture was poured into water, and the organic phase was washed with water only until the washings were neutral, dried ($MgSO_4$), and evaporated to dryness. The haem was dissolved in the minimum quantity of pyridine and the solution diluted with glacial acetic acid (420 ml). To this stirred solution (under nitrogen) was added a freshly prepared solution of iron(II) sulphate (1.8 g) in concentrated hydrochloric acid (17 ml); passage of nitrogen was continued until the reaction was complete (*ca.* 5 min). The mixture was poured into methylene chloride and saturated aqueous sodium acetate, and the porphyrinic fraction extracted into the organic phase. The combined extracts were washed with 10% sodium carbonate solution, and then water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The residue was dissolved in the minimum volume of methylene chloride and chromatographed twice on alumina (Merck, activity III) with first methylene chloride and then methylene chloride–benzene (1 : 1) as eluants. The porphyrinic fractions were evaporated to dryness and the residue recrystallised from chloroform–methanol to give the *porphyrin* (26 mg, 10%) as violet needles, m.p. 250—252° (Found: C, 67.8; H, 6.4; N, 8.5. $C_{37}H_{40}N_4O_7$ requires C, 68.0; H, 6.1; N, 8.6%), $\lambda_{max.}$ ($CHCl_3$) 412 (log ε 5.23), 516 (3.99), 558 (4.23), 582 (4.03), and 645 nm (3.36), $\lambda_{max.}$ ($CHCl_3$–$CF_3{\cdot}CO_2H$) (dication) 431 (5.23), 571 (4.08), and 618 nm (4.02), τ ($CDCl_3$) 15.70 (2 H, s, NH), 7.09 (6 H, s), 7.26 (3 H, s), and 6.66 (3 H, s) (Me), 6.9 (6 H, m) and 5.9 (6 H, m) ($CH_2{\cdot}CH_2$), 6.38 (6 H, s) and 6.09 (3 H, s) (CO_2Me), 0.83 (1 H, s), 0.68 (2 H, s), and 0.58 (1 H, s) (*meso*-H), and −1.68 (1 H, s, CHO), *m/e* 652 (100%), 653 (41), 621 (52), 579 (29), and 551 (18).

4-*Formyl*-2,6,7-*tris*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (21b).—This was obtained from 2,6,7-tris-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphin (186 mg) by the foregoing procedure as red *needles* (84 mg, 42%), m.p. 235—236° (Found: C, 67.8; H, 6.3; N, 8.9. $C_{37}H_{40}N_4O_7$ requires C, 68.0; H, 6.1; N, 8.6%), $\lambda_{max.}$ ($CHCl_3$) 412 (log ε 5.23), 516 (4.00), 556 (4.23), 581 (4.03), and 645 nm (3.31), $\lambda_{max.}$ ($CHCl_3$–$CF_3{\cdot}CO_2H$) (dication) 431 (5.23), 569 (4.08), and 617 nm (4.03), τ ($CDCl_3$) 15.31 (2 H, s, NH), 7.1 (6 H, m) and 5.9 (6 H, m) ($CH_2{\cdot}CH_2$), 6.93, 6.79, 6.69, and 6.65 (each 3 H, s, Me), 6.47, 6.36, and 6.32 (each 3 H, s, CO_2Me), 1.33, 0.66, 0.60, and −0.1 (*meso*-H), and −0.97 (1 H, s, CHO), *m/e* 652 (100%), 653 (41), 624 (44), 579 (43), and 551 (24).

4,6,7-*Tris*-(2-*methoxycarbonylethyl*)-2-(2-*methoxycarbonylvinyl*)-1,3,5,8-*tetramethylporphin* (11e).—A solution of methyl hydrogen malonate (3.2 g) in pyridine (40 ml) and piperidine (0.60 ml) was added to 2-formyl-4,6,7-tris-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (65 mg) in pyridine (55 ml) and piperidine (0.02 ml) at 95 °C, during 4 h. Further methyl hydrogen malonate (1 g) in pyridine (12 ml) and piperidine (0.02 ml) was added and the mixture heated under reflux during the addition. The solvent was removed under reduced pressure and the residue chromatographed twice on alumina (Merck, activity III), with methylene chloride–benzene (1 : 1) as eluant. The porphyrin fractions were evaporated to dryness under reduced pressure; recrystallisation of the residue from chloroform–methanol gave the *porphyrin* (57 mg, 80%), m.p. 236—238° (lit.,[13] m.p. 232°) (Found: C, 67.5; H, 6.2; N, 7.7. $C_{40}H_{44}N_4O_8$ requires C, 67.8; H, 6.3; N, 7.9%), $\lambda_{max.}$ ($CHCl_3$) 413 (log ε 5.08), 510 (3.97), 548 (4.12), 577 (3.92), and 635 nm (3.44), $\lambda_{max.}$ ($CHCl_3$–$CF_3{\cdot}CO_2H$) (dication) 425 (5.17), 563 (4.09), and 610 nm (3.92), τ ($CDCl_3$) 15.12 (2 H, s), 6.8 (6 H, m), and 5.9 (6 H, m) ($CH_2{\cdot}CH_2$), 7.06, 6.90, 6.86, and 6.54 (each 3 H, s, Me), 6.33 (6 H, s) and 6.28 (3 H, s) (CO_2Me), 3.40 and 1.28 (each 1 H, d, *J* 17 Hz, vinyl H), and 1.00, 0.88, 0.48, and 0.36 (each 1 H, s, *meso*-H), *m/e* 708 (100%), 709 (46), and 635 (39).

2,6,7-*Tris*-(2-*methoxycarbonylethyl*)-4-(2-*methoxycarbonylvinyl*)-1,3,5,8-*tetramethylporphin* (21c).—This was obtained from 4-formyl-2,6,7-tris-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (46 mg) by the same procedure as in the foregoing preparation, as red *needles* (33 mg, 75%), m.p. 219—220° (Found: C, 67.85; H, 6.2; N, 7.5. $C_{40}H_{44}N_4O_8$ requires C, 67.8; H, 6.3; N, 7.9%), $\lambda_{max.}$ ($CHCl_3$) 413 (log ε 5.08), 510 (3.97), 548 (4.14), 577 (3.92), and 635 nm (3.44), $\lambda_{max.}$ ($CHCl_3$–$CF_3{\cdot}CO_2H$) (dication) 425 (5.17), 563 (4.09), and 616 nm (3.92), τ ($CDCl_3$) 15.04 (2 H, s, NH), 7.0 (6 H, m) and 5.9 (6 H, m) ($CH_2{\cdot}CH_2$), 6.92, 6.88, 6.74, and 6.61 (each 3 H, s, Me), 6.42, 6.35, and 6.32 (each 3 H, s, CO_2Me), 3.21 and 1.06 (each 1 H, d, *J* 17 Hz, vinyl H), and 0.96, 0.58, 0.49, and 0.43 (each 1 H, s, *meso*-H), *m/e* 708 (100%), 709 (45), and 635 (30).

Comparisons of Natural S-411 Porphyrins and the two Synthetic Acrylic Acid Porphyrins (3c *and* d).—A small sample (<0.5 mg) of the tetramethyl ester of natural S-411 porphyrin kindly made available to us by Dr. D. C. Nicholson had m.p. 215—220° (lit.,[13] 232°). The mixed m.p. with our synthetic 2-acrylic isomer (11e) was 210—213°, whereas a mixture of the synthetic 4-acrylic isomer (21c) and the naturally derived ester melted over the range 165—210°. This clearly indicated that the natural product was the isomer with the acrylic acid side chain in the 2-position, and this was confirmed by the counter-current distribution experiments described below.

Following preliminary studies with both the natural and the synthetic porphyrins a mixture of S-411 porphyrin (0.2 mg), synthetic porphyrins (3c) (0.2 mg), and synthetic porphyrin (3d) (0.2 mg) (obtained by hydrolysis with hydrochloric acid of the corresponding tetramethyl esters) was subjected to steady-state counter-current distribution between benzene–isobutyl methyl ketone (1 : 1 v/v) and 0.87M-sulphuric acid. The upper and lower phases were each 10 ml, and after a total of 800 transfers (640 upper and 160 lower) the distribution curve obtained (see Figure) clearly showed two bands in the ratio 2 : 1. The larger band corresponded to the 2-acrylic isomer (3c) ,as shown by a related experiment in which a similar distribution curve was obtained from a mixture of 2 parts of synthetic (3c) and 1 part of synthetic (3d). Thus the natural product was clearly identical with the synthetic (3c).

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