Pyrroles and Related Compounds. Part XXIII.[1] Protoporphyrin-I

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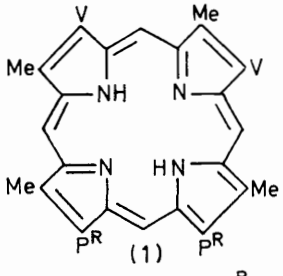
The synthesis of protoporphyrin-I dimethyl ester (2) *via* the *b*-oxobilane route is reported. Fractional crystallisation was unsuccessful in the separation of the dimethyl esters of protoporphyrin-I and protoporphyrin-IX and also the tetramethyl esters of coproporphyrins -I and -III; the significance of these observations, in relation to biosynthetic investigations, is discussed.

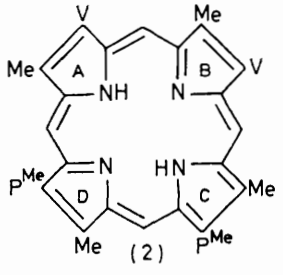
We have confirmed [2] that protoporphyrin-IX is a biosynthetic precursor of the plant chlorophylls, using an isolated chloroplast system.[3] In order to guard against spurious results due to radioactive contamination, we decided to test the specificity of the chloroplast system in a blank experiment, by feeding a porphyrin structurally similar to protoporphyrin-IX (1a), but which could not be incorporated into the chlorophylls. This paper reports the synthesis of protoporphyrin-I dimethyl ester (2), and also a limited investigation into the efficiency of fractional crystallisation for the separation of biologically significant porphyrin type-isomers.

Protoporphyrin-I dimethyl ester (2) was chosen as the synthetic objective on account of its close structural relationship to protoporphyrin-IX dimethyl ester (1b); the only difference between the two compounds is the order of the substituents in ring D. We considered that this was a particularly significant difference because it is ring D which is stereospecifically reduced at the end of chlorophyll biosynthesis.

Though derived from the regular ' type-I ' substituent pattern, protoporphyrin-I dimethyl ester (2) presented substantial difficulties in its synthesis. Presumably on account of these difficulties, compound (2) had not been synthesised before; it could only be obtained isomerically pure from a general synthetic route having no symmetry restrictions. Neither has protoporphyrin-I ever been isolated from any natural source, even though it is of possible biological significance since it is the potential end-product of vinylation of coproporphyrinogen-I, a



(1)



(2)

a ; R = H
b ; R = Me

$P^R = CH_2 \cdot CH_2 \cdot CO_2R$
V = CH:CH$_2$

natural product produced under certain rare pathological conditions (porphyrias).

In view of our past experience,[4] we selected the *b*-oxobilane route for the synthesis of (2). Division of the molecule into AB and CD units presented us with the pyrromethanes (3) and (4) as the initial synthetic objectives. We chose to generate the vinyl groups from 2-acetoxyethyl functions at the porphyrin stage (as we had done in our earlier syntheses [5] of protoporphyrin-IX

[1] Part XXII, J. A. S. Cavaleiro, A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, preceding paper.

[2] See footnote 1 in M. T. Cox, T. T. Howarth, A. H. Jackson, and G. W. Kenner, *J. Amer. Chem. Soc.*, 1969, **91**, 1232.

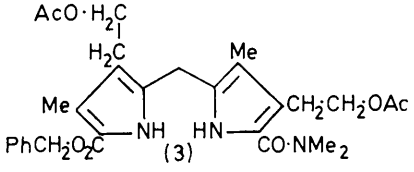
[3] J. M. Charlton, K. J. Treharne, and T. W. Goodwin, *Biochem. J.*, 1967, **105**, 205.

[4] K. M. Smith, *Quart. Rev.*, 1971, **25**, 31.

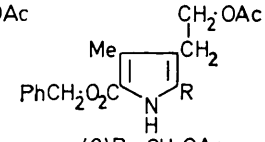
[5] R. P. Carr, A. H. Jackson, G. W. Kenner, and G. S. Sach, *J. Chem. Soc.* (*C*), 1971, 487.

dimethyl ester). Thus, catalytic hydrogenation of the dimethylcarbamoylpyrrole (5) afforded the corresponding carboxylic acid (6), which was decarboxylated by iodination [to give (7)] followed by hydrogenation over Adams catalyst. The resultant pyrrole (8) was condensed with the 2-acetoxymethylpyrrole (9) [obtained from the corresponding 2-methylpyrrole (10) with lead tetra-acetate] in acetic acid * in the presence of a catalytic amount of toluene-*p*-sulphonic acid hydrate during 3 h at 40°, to give a 78% yield of the known pyrromethane (3); our earlier synthesis of this compound had given an inferior yield.[1,5]

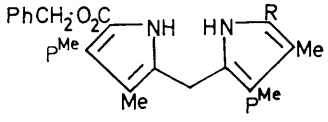
The CD pyrromethane (11) was prepared from the 2-acetoxymethylpyrrole (12) and the 2-unsubstituted pyrrole (13) in methanol containing a catalytic quantity of toluene-*p*-sulphonic acid (35°; 6 h). Though a 75% yield was obtained in this way, only a limited quantity



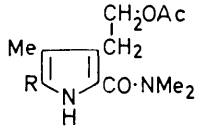
(3)



(9) R = CH_2OAc
(10) R = Me

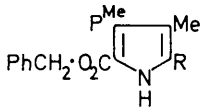


(4) R = H
(11) R = CO_2Bu^t

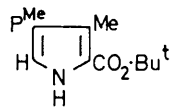


(5) R = $PhCH_2O_2C$
(6) R = HO_2C
(7) R = I
(8) R = H

of the 2-methylpyrrole (14) [from which the 2-acetoxymethylpyrrole (12) was obtained with lead tetra-acetate] was available from earlier work,[7] and so larger quantities of (11) were prepared by an entirely different route which avoided the difficultly accessible pyrrole (14). Thus, trichlorination and hydrolysis of the readily available pyrrole (15) gave the carboxylic acid (16), which afforded a quantitative yield of the oily mixed ester (17) when treated with diazomethane. Hydrogenation over palladised charcoal furnished a 98% yield of the carboxylic acid (18) which was iodinated [to give an 84% yield of (19)] and then hydrogenated over Adams catalyst, to give a high yield of the oily 2-unsubstituted pyrrole (20). With sodium and benzyl alcohol, (20) gave a high yield of the dibenzyl ester (21), which afforded a 91% yield of the required 2-unsubstituted pyrrole (22) when treated with methoxide in methanol during 8 h at



(12) R = CH_2OAc
(14) R = Me
(22) R = H



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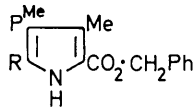
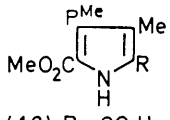
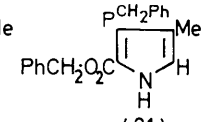


(15) R = Me
(16) R = HO_2C
(17) R = MeO_2C



(18) R = CO_2H
(19) R = I
(20) R = H



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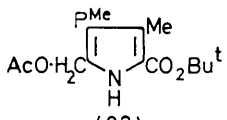
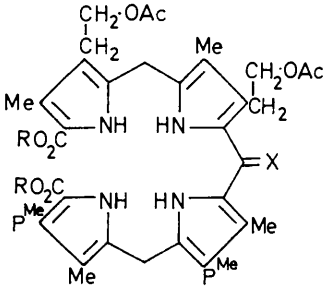


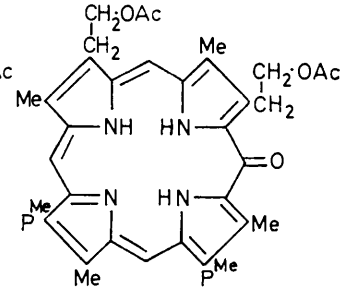
(23)

room temperature. Finally, the pyrromethane (11) was obtained in 75% yield by condensation of (22) with the known [8] 2-acetoxymethylpyrrole (23) in acetic acid containing toluene-*p*-sulphonic acid hydrate. In cold trifluoroacetic acid, the pyrromethane (11) gave the 5-unsubstituted compound (4) required for the *b*-oxobilane preparation.

The phosphoryl chloride complex of the pyrromethane amide (3) was treated with the 5-unsubstituted pyrromethane (4) under the usual conditions [9] to give the tetrapyrrolic imine salt (24); chromatographic purification [9] followed by hydrolysis, gave a 46% overall yield † of the *b*-oxobilane dibenzyl ester (25) which was hydrogenated over palladised charcoal to give the *b*-oxobilane-1′,8′-dicarboxylic acid (26). Cyclisation to



(24) R = $PhCH_2$, X = $\overset{+}{N}Me_2$
(25) R = $PhCH_2$, X = O
(26) R = H, X = O



(27)

the oxophlorin (27) was accomplished in the usual fashion,[9] with trimethyl orthoformate and trichloroacetic acid in methylene chloride; the oxophlorin was not isolated for characterisation, but was treated with acetic anhydride in pyridine to give the corresponding *meso*-acetoxyporphyrin (28) in a yield of 47% from the

* When methanol was used as the solvent (*cf.* ref. 6) the product was contaminated with the corresponding 3,4′-bis-(2-hydroxyethyl)pyrromethane, owing to methanolysis.[1]

† The fore-running eluates from the imine salt chromatography yielded a significant amount of the pyrromethane amide (3); if we take this into account, the corrected yield of the *b* oxobilane (25) is 59%.

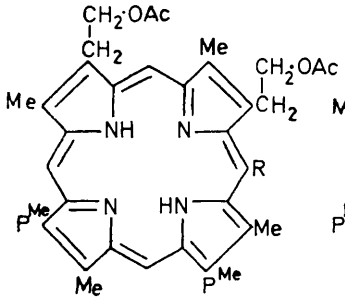
[6] A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *Tetrahedron Letters*, 1972, 2203.

[7] P. J. Crook, A. H. Jackson, and G. W. Kenner, *Annalen*, 1971, **748**, 26.

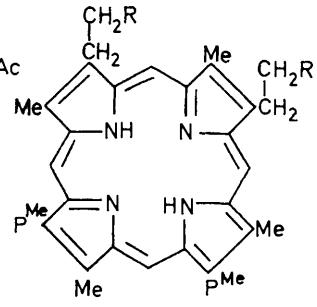
[8] C. J. Suckling, Ph.D. Thesis, Liverpool, 1970.

[9] A. H. Jackson, G. W. Kenner, G. McGillivray, and K. M. Smith, *J. Chem. Soc.* (*C*), 1968, 294.

dibenzyl *b*-oxobilane-1′,8′-dicarboxylate (25). Transformation into the *meso*-unsubstituted porphyrin (29) was accomplished in 86% yield by hydrogenation (until



(28) R = OAc
(29) R = H



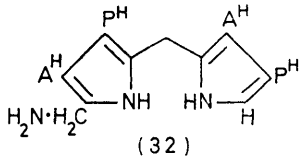
(30) R = OH
(31) R = Cl

colourless) over palladised charcoal, followed by re-oxidation of the porphyrinogen with dichlorodicyanobenzoquinone.[10] In 5% sulphuric acid–methanol compound (29) afforded a 95% yield of the 2,4-bis-(2-hydroxyethyl)porphyrin (30) from which the corresponding 2,4-bis-(2-chloroethyl)porphyrin (31) was obtained in 80% yield after treatment with thionyl chloride and dimethylformamide. Finally, vinylation was accomplished on the zinc chelate of (31) with t-butoxide in t-butyl alcohol, giving an 84% yield of protoporphyrin-I dimethyl ester (2) (m.p. 206—208°) after esterification in methanol–sulphuric acid. Admixture with protoporphyrin-IX dimethyl ester (1b) (m.p. 229°) caused pronounced depression of the m.p. to 185—215°.

As expected,[11] the mass spectra of the dimethyl esters of protoporphyrins -I and -IX showed no major differences. However, the methyl regions in the ^1H n.m.r. spectra allowed differentiation between the isomers, the resonances of the type-I isomer being observed as a 1 : 2 : 1 triplet (0·05M in deuteriochloroform); all four *meso*-protons were resolved in each spectrum. The n.m.r. spectra in trifluoroacetic acid allowed the unambiguous differentiation between protoporphyrin-I dimethyl ester and its type-IX isomer; the *meso*-protons of the former appeared as the expected[12] equal intensity doublet whereas those of the latter are observed[12] as a 1 : 3 doublet.

The isomers (1b) and (2) were inseparable by t.l.c.; * this encouraged us to examine other, more classical methods for their separation. It is well known[13] that mixtures of porphyrin type-isomers tend to co-crystallise, presumably on account of the planar nature of these macrocycles. Consequently, there exists a serious danger of error in the interpretation of data obtained from biosynthetic feedings of radiochemically labelled precursors. Redistribution of the pyrrole rings after uroporphyrinogen-III formation can be discounted because of recent work,[14] and therefore, feedings involving intact porphyrins or porphyrinogens as substrates should be beyond suspicion with regard to type-isomer contamination. However, it is known[15] that pyrromethanes [*e.g.* (32)] tend to give porphyrins [*e.g.* uroporphyrin-I (33)] under relatively mild conditions, and there exists a possibility that type-I porphyrins, produced non-enzymically from labelled porphobilinogen (34) or pyrromethanes [*e.g.* (32)], might contaminate normal metabolic products (having the type-III skeleton), produced by genuine incorporations of the labelled substrates. Therefore we decided to examine the separation of the dimethyl esters of labelled protoporphyrins -I (2) and -IX (1b) by fractional crystallisation under conditions similar to those of a normal radiochemical work-up.†

Tritiation of the *meso*-positions of the two protoporphyrins was accomplished by application of our new



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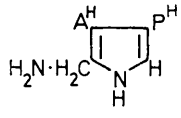

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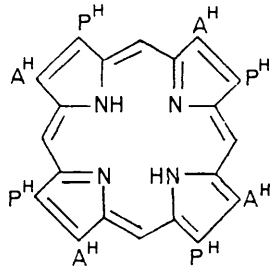


(33)

A^R = $CH_2 \cdot CO_2R$

method;[16] we used hexapyridylmagnesium di-iodide in pyridine with a tritium source. In this way, protoporphyrin-I dimethyl ester (2) with a specific activity of $1{\cdot}25 \times 10^7$ disint. mg^{-1} s^{-1} and protoporphyrin-IX dimethyl ester (1b) with activity $6{\cdot}93 \times 10^6$ were obtained. After admixture of labelled protoporphyrin-I dimethyl ester (0·4 mg) with unlabelled protoporphyrin-IX dimethyl ester (52·0 mg), crystallisations were

* However, we have more recently separated this and other mixtures of porphyrin type-isomers using high pressure liquid chromatography with chloroform–n-heptane or tetrahydrofuran–n-heptane solvent mixtures.

† The usual procedure for work-up of biosynthetic feeding experiments is to dilute the crude incubation products with inactive starting material and products. After a suitable separation and crystallisation to constant activity (if possible), the recoveries and incorporations are calculated. Though protoporphyrin-I is not a known natural product, the use of disrupted enzymic systems makes it a possible product from a pyrromethane feeding, through a combination of non-enzymic and enzymic transformations.

[10] S. W. McCombie, Ph.D. Thesis, Liverpool, 1972.
[11] A. H. Jackson, G. W. Kenner, K. M. Smith, R. T. Aplin, H. Budzikiewicz, and C. Djerassi, *Tetrahedron*, 1965, **21**, 2913.
[12] R. J. Abraham, A. H. Jackson, and G. W. Kenner, *J. Chem. Soc.*, 1961, 3468.
[13] *E.g.* F. Morsingh and S. F. MacDonald, *J. Amer. Chem. Soc.*, 1960, **82**, 4377; E. J. Tarlton, S. F. MacDonald, and E. Baltazzi, *ibid.*, p. 4389.
[14] B. Franck, D. Gantz, F. P. Montforts, and F. Schmidtchen, *Angew. Chem. Internat. Edn.*, 1972, **11**, 421; A. R. Battersby, J. Staunton, and R. H. Wightman, *J.C.S. Chem. Comm.*, 1972, 1118; J. A. S. Cavaleiro, G. W. Kenner, and K. M. Smith, *ibid.*, 1973, 183.
[15] *E.g.* R. B. Frydman, A. Valasinas, and B. Frydman, *Biochemistry*, 1973, **12**, 80.
[16] G. W. Kenner, K. M. Smith, and M. J. Sutton, *Tetrahedron Letters*, 1973, 1303.

carried out, from methylene chloride–n-hexane and occasionally methylene chloride–methanol. Care was taken with each crystallisation to avoid precipitation, and in each case the solution was left between 24 and 48 h before collection of the crystals (Experimental section). Table 1 shows the specific activities and recoveries of material from each crystallisation, as well as the results from the inverse experiment in which 1·02 mg of labelled protoporphyrin-IX dimethyl ester and 50·0 mg of unlabelled protoporphyrin-I dimethyl ester were used.

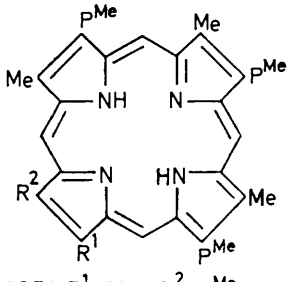
TABLE 1

Attempted separation by fractional crystallisation of the dimethyl esters of protoporphyrin-I and protoporphyrin-IX

	Labelled Proto-I (2) unlabelled Proto-IX (1b)		Labelled Proto-IX (1b) unlabelled Proto-I (2)	
Crystallisation no.	Specific activity *	Recovery (mg)	Specific activity *	Recovery (mg)
0 (Admixture)	$9{\cdot}54 \times 10^4$	(52·4)	$1{\cdot}39 \times 10^5$	(52·04)
1	$5{\cdot}13 \times 10^4$	47·5	$9{\cdot}16 \times 10^4$	40·0
2	$3{\cdot}44 \times 10^4$	44·7	$8{\cdot}63 \times 10^4$	38·0
3	$2{\cdot}08 \times 10^4$	41·1	$8{\cdot}34 \times 10^4$	33·8
4	$1{\cdot}72 \times 10^4$	39·0	$7{\cdot}31 \times 10^4$	31·8
5	$9{\cdot}15 \times 10^3$	35·8	$6{\cdot}74 \times 10^4$	26·8
6	$6{\cdot}26 \times 10^3$	30·8	$5{\cdot}34 \times 10^4$	20·4
7	$4{\cdot}75 \times 10^3$	25·8	$4{\cdot}90 \times 10^4$	17·1
8	$2{\cdot}96 \times 10^3$	24·0		

* Disint. mg^{-1} s^{-1}.

In a possibly more significant separation, crystallisations of 0·60 mg of coproporphyrin-I tetramethyl ester (35) (specific activity $4{\cdot}13 \times 10^6$ disint. mg^{-1} s^{-1}) and 45·3 mg of unlabelled coproporphyrin-III tetramethyl



(35) R^1 = Me, R^2 = P^{Me}
(36) R^1 = P^{Me}, R^2 = Me

ester (36) were carried out, as well as the inverse with 1·14 mg of coproporphyrin-III tetramethyl ester (specific activity $2{\cdot}77 \times 10^6$ disint. mg^{-1} s^{-1}) and 50·5 mg of unlabelled coproporphyrin-I tetramethyl ester. The results from these crystallisations are presented in Table 2.

The data in Tables 1 and 2 lead one to conclude that only a slow, gradual separation between the type-isomers is occurring. Table 1 shows that it is possible to remove the protoporphyrin-I impurity from protoporphyrin-IX at a greater rate than the type-IX isomer could be removed from type-I. This can be accounted for by the noticeably increased solubility of protoporphyrin-I dimethyl ester over protoporphyrin-IX dimethyl ester. Table 2 shows a gradual fall in activity in each of the coproporphyrin mixtures; in no case was anything approaching a complete separation obtained.

TABLE 2

Attempted separation by fractional crystallisation of the tetramethyl esters of coproporphyrin-I and coproporphyrin-III

	Labelled Copro-I (35) unlabelled Copro-III (36)		Labelled Copro-III (36) unlabelled Copro-I (35)	
Crystallisation no.	Specific activity *	Recovery (mg)	Specific activity *	Recovery (mg)
0 (Admixture)	$5{\cdot}40 \times 10^4$	(46·5)	$6{\cdot}12 \times 10^4$	(52·78)
1	$5{\cdot}02 \times 10^4$	39·3	$5{\cdot}43 \times 10^4$	46·7
2	$3{\cdot}91 \times 10^4$	34·6	$5{\cdot}13 \times 10^4$	44·0
3	$3{\cdot}14 \times 10^4$	33·2	$3{\cdot}81 \times 10^4$	42·1
4	$3{\cdot}00 \times 10^4$	31·6	$2{\cdot}16 \times 10^4$	40·9
5	$2{\cdot}75 \times 10^4$	30·0	$2{\cdot}08 \times 10^4$	38·0
6			$8{\cdot}24 \times 10^3$	35·0

* Disint. mg^{-1} s^{-1}.

It is thus dangerous to assume that a separation can be achieved by exhaustive crystallisation to small bulk of a large quantity of a porphyrin used in a dilution analysis.

We conclude that it is essential to ensure that porphyrinic products from feeding radiochemically labelled non-macrocyclic precursors to enzymic systems are rigorously crystallised *to constant activity*; otherwise, there is no guarantee that some or all of an apparent incorporation may not be due to contamination (from non-enzymic processes) which has been picked up by the compound used for the dilution analysis, and from which it is inseparable.

The results from feeding protoporphyrin-I to the chloroplast system will be reported elsewhere, together with those of the feedings of genuine precursors of the plant chlorophylls.

EXPERIMENTAL

M.p.s were measured on a Kofler hot-stage apparatus. Neutral alumina (Merck; Brockmann Grade III) was used for all chromatographic separations, and reactions were followed by t.l.c. and by spectrophotometry, as described in earlier parts of this series. Electronic absorption spectra were determined (solutions in methylene chloride) with a Unicam SP 800 spectrophotometer, ^1H n.m.r. spectra (solutions in deuteriochloroform with tetramethylsilane as internal standard) with a Varian HA-100 instrument, and mass spectra with either an A.E.I. MS902 or MS12 spectrometer (at 50 μA and 70 eV; direct inlet with source temperature 200—220°).

Pyrroles

5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylic Acid (16).—5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-2,4-dimethylpyrrole (15) (42·15 g) was suspended in carbon tetrachloride (400 ml), and, with stirring at room temperature, sulphuryl chloride (34·0 ml) was added dropwise during 4 h. The solution was set aside overnight before evaporation to a viscous oil, which was taken up in dioxan (1·5 l) and treated with sodium acetate

trihydrate (216 g) in water (1 l).* The mixture was refluxed with stirring on a boiling water-bath during 2 h before being left to cool and then diluted with ether (1 l). The organic phase was extracted several times with aqueous sodium carbonate; the extracts were combined and then flushed with a stream of compressed air during 20 min. Sulphur dioxide gas was then passed into the solution (to pH 6; care to avoid excessive frothing). The carboxylic acid (33 g, 72%) was filtered off and dried; it was normally used without further purification and had m.p. 145° (lit.,[17] 149—150°).

2-Methoxycarbonyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-5-carboxylic Acid (18).—The pyrrole carboxylic acid (16) (11·6 g) in ether (500 ml) was treated with an excess of ethereal diazomethane. After 5 min the solution was evaporated, to leave a viscous oil, which was chromatographed, with benzene as eluant. The oil (17) obtained by evaporation of the eluates could not be induced to crystallise, even after many weeks; yield 12 g (100%), τ 0·48br (1H, NH), 2·64 (5H, m, Ph), 4·70 (2H, s, CH_2Ph), 6·18 and 6·38 (each 3H, s, OMe), 6·9 and 7·4 (each 2H, m; CH_2·CH_2·CO), and 7·70 (3H, s, Me).

The mixed ester (17) (12 g) was dissolved in tetrahydrofuran (200 ml) containing triethylamine (4 drops) and hydrogenated at room temperature and atmospheric pressure over palladised charcoal (10%; 1·4 g). After uptake of hydrogen had ceased, the catalyst was filtered off through Celite, which was washed with tetrahydrofuran, and the combined filtrates were evaporated to dryness, giving the *pyrrolecarboxylic acid* (8·95 g, 99%), m.p. 179—180° (from methylene chloride–n-hexane) (Found: C, 53·6; H, 5·7; N, 5·1. $C_{12}H_{15}NO_6$ requires C, 53·5; H, 5·6; N, 5·2%), τ [$(CD_3)_2SO$] −1·42 (1H, s, CO_2H), 6·25 and 6·45 (each 3H, s, OMe), 7·1 and 7·6 (each 2H, t; CH_2·CH_2·CO), and 7·81 (3H, s, Me).

Methyl 5-Iodo-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (19).—The foregoing pyrrolecarboxylic acid (8·90 g) was dissolved in methanol (170 ml) at 70°. After reduction of the temperature to 60° sodium hydrogen carbonate (7·0 g) in water (50 ml) was added dropwise. At the same temperature, with stirring, a mixture of iodine (9·3 g) and potassium iodide (12·1 g) in water (30 ml) and methanol (60 ml) was added dropwise during 1 h. After a further 30 min, water (200 ml) was added and the suspension was set aside in the refrigerator during 1 h. The *iodopyrrole* was filtered off, washed well with water, dried, and crystallised from methylene chloride–n-hexane; yield 9·75 g (84%), m.p. 130—131° (Found: C, 37·9; H, 4·1; N, 4·05. $C_{11}H_{14}INO_4$ requires C, 37·6; H, 4·0; N, 4·0%), τ 0·68br (1H, NH), 6·20 and 6·39 (each 3H, s, OMe), 7·0 and 7·5 (each 2H, m; CH_2·CH_2·CO), and 8·06 (3H, s, Me).

Methyl 3-(2-Methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (20).—The foregoing iodopyrrole (9·5 g) in methanol (100 ml) containing sodium acetate trihydrate (9·5 g) and Adams platinum oxide was hydrogenated at atmospheric pressure and room temperature until uptake of hydrogen had ceased (2 h). After filtration through Celite, the solution was evaporated to dryness and then methylene chloride (100 ml) was added. The solution was washed with water, dried (Na_2SO_4), and evaporated to dryness, to give an oil (6·0 g, 98%) which over a period of many weeks could not be induced to crystallise; τ 0·97br (1H, NH), 3·32 (1H, d, *J* 3 Hz, α-H), 6·20 and 6·36 (each 3H, s, OMe), 7·0 and 7·5 (each 2H, m; CH_2·CH_2·CO), and 7·96 (3H, s, Me).

Benzyl 3-(2-Benzyloxycarbonylethyl)-4-methylpyrrole-2-carboxylate (21).—The foregoing dimethyl ester (6·0 g) was dissolved in distilled benzyl alcohol (100 ml). After addition of sodium (100 mg), the mixture was heated on a boiling water-bath during 6 h at *ca.* 15 mmHg (water pump). The mixture was set aside to cool, and pieces of solid carbon dioxide were added. The benzyl alcohol was removed, under nitrogen, at 0·4 mmHg and 65—70°, and the resultant syrup was taken into ether; the solution was washed with water, dried ($MgSO_4$), and then evaporated *in vacuo*. Chromatography of the residue in mixtures of petroleum (b.p. 60—80°) and benzene afforded an oil after evaporation of the eluates. The oil was kept briefly under high vacuum, and then crystallised from methylene chloride–n-hexane to give the *pyrrole dibenzyl ester* (8·6 g, 85%), m.p. 63—65° (Found: C, 73·0; H, 6·2; N, 3·65. $C_{23}H_{23}NO_4$ requires C, 73·2; H, 6·1; N, 3·7%), τ 1·07br (1H, NH), 2·71 (10H, s, 2Ph), 3·42 (1H, d, *J* 3 Hz, α-H), 4·74 and 4·93 (each 2H, s, CH_2Ph), 6·9 and 7·4 (each 2H, m; CH_2·CH_2·CO), and 7·98 (3H, s, Me).

Benzyl 3-(2-Methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (22).—The foregoing dibenzyl ester (4·0 g) was dissolved in distilled methanol (100 ml) and treated with sodium (150 mg). After stirring at room temperature during 8 h, solid carbon dioxide was added and the solution was evaporated almost to dryness. Methylene chloride (100 ml) was added and the solution was washed with water, dried (Na_2SO_4), and evaporated to dryness. The product was filtered through a short column of alumina (Merck; Grade III) with methylene chloride as eluant. Evaporation of the eluates and crystallisation from ether–petroleum (b.p. 60—80°) gave the 2-unsubstituted pyrrole as white needles (2·91 g, 91%), m.p. 56—58° (lit.,[18] 57—58°), τ 0·97br (1H, NH), 2·7 (5H, m, Ph), 3·46 (1H, d, *J* 3 Hz, α-H), 4·81 (2H, s, CH_2Ph), 6·49 (3H, s, OMe), 7·0 and 7·5 (each 2H, m; CH_2·CH_2·CO), and 8·05 (3H, s, Me).

Benzyl 5-Acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (12).—Benzyl 3-(2-methoxycarbonylethyl)-4,5-dimethylpyrrole-2-carboxylate (14) (3·2 g) in acetic acid (55 ml) containing acetic anhydride (1·2 ml) was treated during 2 h with lead tetra-acetate (5·0 g) in portions. The solution was stirred overnight before addition dropwise to water (250 ml). The product was filtered off, washed well with water, dried, and recrystallised from methylene chloride–n-hexane to give the *acetoxymethylpyrrole* (3·6 g, 95%), m.p. 83—84° (Found: C, 64·6; H, 6·2; N, 3·85. $C_{20}H_{23}NO_6$ requires C, 64·3; H, 6·2; N, 3·75%), τ 0·69br (1H, NH), 2·58 (5H, s, Ph), 4·67 (2H, s, CH_2·Ph), 4·96 (2H, s, CH_2·O), 6·35 (3H, s, OMe), 7·0 and 7·5 (each 2H, m; CH_2·CH_2·CO), and 7·92 (6H, s, β-Me and COMe).

4-(2-Acetoxyethyl)-5-(dimethylcarbamoyl)-3-methylpyrrole (8).— 4-(2-Acetoxyethyl)-5-(dimethylcarbamoyl)-2-iodo-3-methylpyrrole [19] (7) (3·55 g) was dissolved in methanol (55 ml) containing sodium acetate trihydrate (3·6 g) and Adams platinum oxide (40 mg) and the mixture was hydrogenated at room temperature and atmospheric pressure until hydrogen uptake had ceased (3 h). The

* This hydrolysis was performed at high dilution in order to avoid formation of the corresponding pyrrocoll, which is a major product from hydrolyses at high concentration.

[17] A. H. Jackson, G. W. Kenner, and D. Warburton, *J. Chem. Soc.*, 1965, 1328.

[18] P. J. Crook, Ph.D. Thesis, Liverpool, 1968.

[19] A. H. Jackson, G. W. Kenner, and K. M. Smith, in preparation.

solution was filtered through Celite and the filtrate was evaporated almost to dryness. Methylene chloride (100 ml) was added and the resultant solution was washed with water, dried (Na_2SO_4), and then evaporated to dryness. The residue was recrystallised from methylene chloride–n-hexane to give the 2-*unsubstituted pyrrole* (2·135 g, 92%), m.p. 128—129° (Found: C, 60·3; H, 7·7; N, 11·7. $C_{12}H_{18}N_2O_3$ requires C, 60·5; H, 7·6; N, 11·8%), τ 0·76br (1H, NH), 3·38 (1H, d, *J* 3 Hz, α-H), 5·81 (2H, t, CH_2·O), 6·90 (6H, s, NMe_2), 7·10 (2H, t, CH_2·CH_2·O), 7·90 and 7·93 (each 3H, s; β-Me and COMe).

Pyrromethanes

Benzyl 3′,4-Bis-(2-methoxycarbonylethyl)-3,4′-dimethyl-5-t-butoxycarbonylpyrromethane-5-carboxylate (11).—(*a*) Benzyl 5-acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (0·525 g) and t-butyl 3-(2-methoxycarbonylethyl)-4-methylpyrrole-5-carboxylate [20] (0·384 g) were suspended in distilled methanol (10 ml) and stirred during 5 min at 45°. The temperature was then reduced to 35—38° and toluene-*p*-sulphonic acid hydrate (14·5 mg) was added; stirring was continued at this temperature during 6 h and the reaction was followed by t.l.c. Water (1 ml) containing a little sodium acetate trihydrate was added dropwise before addition of methylene chloride (50 ml). The organic phase was washed with aqueous sodium hydrogen carbonate and then water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed in petroleum (b.p. 60—80°)–benzene mixtures to afford an oil (0·611 g, 75%) which could not be induced to crystallise; τ 0·74br and 1·50br (each 1H, NH), 2·70 (5H, s, Ph), 4·80 (2H, s, CH_2Ph), 6·16 (2H, s, CH_2), 6·42 and 6·48 (each 3H, s, OMe), 6·9—7·7 (8H, m, $2CH_2$·CH_2), 7·82 and 8·01 (each 3H, s, β-Me), and 8·51 (9H, s, Bu^t).

(*b*) Benzyl 3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (2·953 g) and t-butyl 2-acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-5-carboxylate [8] (3·270 g) were suspended in acetic acid (25 ml). To the stirred solution at 37—39° was added toluene-*p*-sulphonic acid hydrate (92 mg) and the reaction was continued for a further 3 h. Methylene chloride (100 ml) was added and the solution was washed with water, aqueous sodium hydrogen carbonate, and water again, dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed as in (*a*) to afford an oil (4·20 g, 75%), identical (t.l.c. and n.m.r.) with the material described in (*a*).

Benzyl 3,4′-Bis-(2-acetoxyethyl)-5′-(dimethylcarbamoyl)-3,4′-dimethylpyrromethane-5-carboxylate (3).—Benzyl 3-(2-acetoxyethyl)-2-acetoxymethyl-5-methylpyrrole-5-carboxylate [21] (2·430 g) and 4-(2-acetoxyethyl)-5-(dimethylcarbamoyl)-3-methylpyrrole (1·628 g) were dissolved in acetic acid (30 ml) and treated with toluene-*p*-sulphonic acid hydrate (66 mg). The mixture was stirred at 38—40° during 3·5 h before dilution with methylene chloride (100 ml). The solution was washed with water, aqueous sodium hydrogen carbonate, and water once more, dried ($MgSO_4$), and evaporated to dryness. The residue was crystallised from methylene chloride–n-hexane to give the pyrromethane (2·796 g, 78%), m.p. 147—150° (lit.,[5] 142—143°), τ −0·06br and 0·08br (each 1H, NH), 2·74 (5H, s, Ph), 4·80 (2H, s, CH_2Ph), 5·97 and 6·07 (each 2H, t, CH_2·O), 6·29 (2H, s, CH_2), 7·06 (6H, s, NMe_2), 7·25 and 7·31 (each 2H, t, CH_2·CH_2·O). 7·78 (3H, s, β-Me), and 8·04 and 8·07 (6H, s and 3H, s; 2COMe and β-Me).

b-*Oxobilane*

Dibenzyl 2,4-Bis-(2-acetoxyethyl)-6,8-bis-(2-methoxycarbonylethyl)-1,3,5,7-tetramethyl-b-oxobilane-1′,8′-dicarboxylate (25).—Benzyl 3,4′-bis-(2-acetoxyethyl)-5′-(dimethylcarbamoyl)-3,4′-dimethylpyrromethane-5-carboxylate (3·375 g) was dissolved in phosphoryl chloride (20 ml) and kept at 40° during 1 h before evaporation *in vacuo*; last traces of phosphoryl chloride were scavenged by addition and evaporation of 1,2-dibromoethane (20 ml). The residue was kept under high vacuum during a further 2 h. Meanwhile, benzyl 3′,4-bis-(2-methoxycarbonylethyl)-3,4′-dimethyl-5-t-butoxycarbonylpyrromethane-5-carboxylate (oil; 4·269 g, 1·2 equiv.) was dissolved in trifluoroacetic acid and kept at room temperature during 45 min with a stream of dry nitrogen gas passing through the solution. The solution was evaporated to dryness and methylene chloride (100 ml) was added. After successive washings with water, aqueous sodium hydrogen carbonate, and water, the methylene chloride phase was dried (Na_2SO_4) and evaporated to dryness. To this was added the phosphoryl chloride complex described above, in methylene chloride (15 ml), and the mixture was heated at 38—40° during 26 h under a slow stream of nitrogen (saturated with methylene chloride from an external wash-bottle). Methylene chloride (100 ml) was added and the solution was washed briefly with water before being dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed, with benzene and then benzene–ethyl acetate mixtures as eluant. [Evaporation of these eluates, and trituration with a small quantity of ether afforded 0·818 g of the starting 5′-dimethylcarbamoylpyrromethane (3).] Further elution of the column with ethyl acetate and ethyl acetate–methanol mixtures afforded the imine salt (24). This was dissolved in methylene chloride (150 ml) and saturated aqueous sodium hydrogen carbonate (150 ml) was added. The mixture was stirred vigorously during 40 h at room temperature; the organic layer was then washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed in solvent mixtures containing initially benzene alone, and then benzene–ethyl acetate. Evaporation of the eluates gave the *b*-oxobilane (2·70 g; 46 or 59% based on starting material consumed) as a pale yellow foam which could not be induced to crystallise; τ −0·66br, −0·50br, −0·20br, and −0·05br (each 1H, NH), 2·76 and 2·78 (each 5H, s, Ph), 4·82 and 4·86 (each 2H, s, CH_2·Ph), 6·1 (4H, m, $2CH_2$·O), 6·25 (4H, s, $2CH_2$), 6·43 (6H, s, 2OMe), 6·9—7·7 (12H, m, CH_2·CH_2·O and CH_2·CH_2·CO), and 7·75, 7·98, 8·02, 8·04, 8·09, and 8·20 (each 3H, s; 4 β-Me and 2COMe).

Porphyrins

β-Acetoxy-2,4-bis-(2-acetoxyethyl)-6,8-bis-(2-methoxycarbonylethyl)-1,3,5,7-tetramethylporphin (28).—The foregoing dibenzyl *b*-oxobilane-1′,8′-dicarboxylate (2·7 g) in tetrahydrofuran (150 ml) containing triethylamine (4 drops) and 10% palladised charcoal (0·3 g) was hydrogenated at room temperature and atmospheric pressure until uptake of hydrogen ceased (3 h). (Total hydrogenolysis was confirmed in a spectrophotometric experiment; the maximum centred on 285 nm disappeared completely within 20 min when a sample of the hydrogenated material in methylene chloride was treated with a little trichloroacetic acid in a u.v. cell.) The catalyst was filtered off through Celite,

[20] A. M. d'A. Rocha Gonsalves, Ph.D. Thesis, Liverpool, 1972.
[21] M. T. Cox, Ph.D. Thesis, Liverpool, 1969.

which was thoroughly washed with methylene chloride and tetrahydrofuran. The combined filtrates were evaporated to dryness and the foamy residue was put under high vacuum during 1 h, before it was taken up in purified methylene chloride (920 ml) to which was subsequently added M-trichloroacetic acid in methylene chloride (180 ml). The resulting solution was treated with distilled trimethyl orthoformate (7·0 ml), and the deep red solution ($\lambda_{max.}$ 338 and 510 nm) was stirred in the dark during 5 h. Pyridine (15·8 ml) was then added and the solution was then stirred for 19 h. The deep red solution was then exposed to indirect sunlight (daylight is sufficient) during 3 h and a change in colour to green became apparent. The solution was evaporated to about 500 ml, washed with water and then dried (Na_2SO_4). The solvent was completely removed to give the monocation of the oxophlorin (27), which was taken up in pyridine (80 ml) and acetic anhydride (20 ml). The solution was kept at room temperature with stirring, during 1 h, then evaporated. The residue was placed under high vacuum and then chromatographed in methylene chloride. Crystallisation of the porphyrin from the eluates afforded the meso-*acetoxyporphyrin* (986 mg, 47%), m.p. 206—208° (Found: C, 65·8; H, 6·3; N, 7·35. $C_{42}H_{48}N_4O_{10}$ requires C, 65·6; H, 6·3; N, 7·3%), τ −0·4, 0·04, and 0·26 (each 1H, s, *meso*-H), 5·22 (4H, t, 2CH_2·O), 5·5—6·0 (8H, m, CH_2·CH_2·CO and CH_2·CH_2·O), 6·34, 6·36, and 6·37 (each 3H, s; 2OMe and 5-Me), 6·52, 6·54, and 6·55 (each 3H, s; 1-, 3-, and 7-Me), 6·83 (4H, t, 2CH_2·CO), 7·04 (3H, s, β-OAc), 7·89 and 7·95 (each 3H, s, COMe), and 13·70 (2H, s, 2NH), $\lambda_{max.}$ 401 (ε 180,000), 500 (15,000), 532 (5400), 572 (5900), and 625 nm (1350); $\lambda_{max.}$ (CH_2Cl_2–CF_3·CO_2H) 413 (ε 333,000), 558 (12,100), and 595 nm (3200); *m/e* 769 (14%), 726 (27), and 666 (100).

2,4-*Bis*-(2-*acetoxyethyl*)-6,8-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,7-*tetramethylporphin* (29).—The foregoing *meso*-acetoxyporphyrin (386 mg) in tetrahydrofuran (85 ml) containing triethylamine (3 drops) and 10% palladised charcoal (170 mg) was hydrogenated at room temperature and atmospheric pressure until uptake of hydrogen had ceased (6 h). The colourless solution was filtered through Celite, which was washed with more tetrahydrofuran, and the combined filtrates were treated immediately with dichlorodicyanobenzoquinone (0·353 g) in dry benzene (5 ml). The solution was evaporated to dryness and the residue was chromatographed (elution with methylene chloride). The porphyrinic eluates were evaporated to dryness and the residue was crystallised from methylene chloride–n-hexane to give the *porphyrin* (305 mg, 86%), m.p. 244—246° (Found: C, 67·4; H, 6·7; N, 7·8. $C_{40}H_{46}N_4O_8$ requires C, 67·6; H, 6·5; N, 7·9%), τ −0·10 and −0·04 (each 2H, s; 4 *meso*-H), 5·20 (4H, t, 2CH_2·O), 5·6—5·9 (8H, m, CH_2·CH_2·O and CH_2·CH_2·CO), 6·34 (6H, s, 2OMe), 6·50br (12H, s, 4 β-Me), 6·81 (4H, t, CH_2·CH_2·CO), 7·94 (6H, s, 2COMe), and 14·1br (2H, 2NH); $\lambda_{max.}$ 398 (ε 160,000), 497 (13,500), 531 (8800), 567 (5800), and 621 nm (3500); $\lambda_{max.}$ (CH_2Cl_2–CF_3·CO_2H) 405 nm (ε 365,000), 550 (15,300), and 592 (6000); *m/e* 710 (100%).

2,4-*Bis*-(2-*hydroxyethyl*)-6,8-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,7-*tetramethylporphin* (30).—The foregoing bisacetoxyethylporphyrin (232 mg) was set aside overnight in the dark in 5% v/v sulphuric acid in methanol. Chloroform and aqueous sodium acetate were added and the organic phase was washed with water, dilute aqueous sodium hydrogen carbonate, and water once more, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on Grade V alumina (elution with chloroform). The porphyrinic eluates were evaporated to dryness, and the residue was crystallised from methylene chloride–n-hexane, to give the required *porphyrin* (195 mg, 95%), m.p. 211—213° (Found: C, 69·1; H, 6·8; N, 9·0. $C_{36}H_{42}N_4O_6$ requires C, 69·0; H, 6·8; N, 8·9%), τ 0·00 and 0·02 (each 2H, s; 4 *meso*-H), 5·5—5·9 (12H, m, CH_2·CH_2·O and CH_2·CH_2·CO), 6·38 (6H, s, 2OMe), 6·44, 6·46, 6·48, and 6·50 (each 3H, s, β-Me), and 6·80 (4H, t, CH_2·CH_2·CO); $\lambda_{max.}$ 399 (ε 200,000), 498 (14,000), 531 (9000), 568 (6000), and 621 nm (4000); $\lambda_{max.}$ (CH_2Cl_2–CF_3·CO_2H) 407 (ε 317,000), 551 (16,000), and 593 nm (6000); *m/e* 627 (100%).

2,4-*Bis*-(2-*chloroethyl*)-6,8-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,7-*tetramethylporphin* (31).—The foregoing bis-(2-hydroxyethyl)porphyrin (176 mg) was treated with chloroform (80 ml), dimethylformamide (15 ml), and potassium carbonate (5·5 g) before addition of thionyl chloride (5 ml). The mixture was stirred during 6 h and then poured carefully into water. The organic phase was washed with water, dilute aqueous sodium hydrogen carbonate, and then water again, dried (Na_2SO_4), and evaporated. Last traces of dimethylformamide were removed under high vacuum. The residue was chromatographed in methylene chloride, and the porphyrinic eluates were evaporated to dryness, giving a red residue which was crystallised from methylene chloride–n-hexane to give the *bis*-(2-*chloroethyl*)*porphyrin* (149 mg, 80%), m.p. 233—235° (Found: C, 65·0; H, 6·0; N, 8·4. $C_{36}H_{40}Cl_2N_4O_4$ requires C, 65·15; H, 6·1; N, 8·4%), τ 0·02 and 0·16 (each 2H, s; 4 *meso*-H), 5·6—5·9 (12H, m, CH_2·CH_2Cl and CH_2·CH_2·CO), 6·36 (6H, s, 2OMe), 6·48 and 6·51 (each 6H, s, 4 β-Me), 6·80 (4H, t, CH_2·CH_2·CO), and 14·03 (2H, s, 2NH); $\lambda_{max.}$ 399 (ε 170,000), 499 (14,000), 532 (8000), 569 (5500), and 622 nm (3400); $\lambda_{max.}$ (CH_2Cl_2–CF_3·CO_2H) 404 (ε 326,000), 551 (15,000), and 593 nm (6000); *m/e* 666 (10%), 664 (75), 662 (100), 628 (12), 627 (15), 626 (12), and 589 (22).

6,8-*Bis*-(2-*methoxycarbonylethyl*)-1,3,5,7-*tetramethyl*-2,4-*divinylporphin* (2) (*Protoporphyrin*-I *Dimethyl Ester*).—The foregoing bis-(2-chloroethyl)porphyrin (140 mg) in methylene chloride (100 ml) was treated with a saturated solution of zinc acetate in methanol (5 ml). After slight warming, the solution was poured into water and the zinc chelate was extracted into more methylene chloride (100 ml). The organic phase was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was taken up in dry tetrahydrofuran (25 ml) and M-potassium t-butoxide in t-butyl alcohol (50 ml) was added. The mixture was set aside in the dark during 72 h before addition of acetic acid (2 ml), chloroform (100 ml), and pyridine (3 ml). The organic phase was washed with water, dried (Na_2SO_4), and then evaporated to dryness. The residue was treated with 5% v/v sulphuric acid in methanol (100 ml) overnight; the solution was then diluted with methylene chloride, and washed with water containing sodium acetate and then with water alone. The solvent was evaporated off, leaving a red residue, which was chromatographed in methylene chloride. The *product* obtained by evaporation of the eluates was crystallised from methylene chloride–n-hexane (105 mg, 84%); m.p. 206—208° (Found: C, 73·0; H. 6·55; N, 9·3. $C_{36}H_{38}N_4O_4$ requires C, 73·2; H, 6·5; N, 9·5%), τ 0·33, 0·35, 0·43, and 0·47 (each 1H, s, *meso*-H), 1·8—2·2 (2H, m, 2CH:CH_2), 3·7—4·1 (4H, m, 2CH:CH_2), 5·7—6·1 (4H, m, CH_2·CH_2·CO), 6·40 (6H, s, 2OMe), 6·55, 6·65, and 6·72 (3H, s, 6H, s, and 3H, s, 4 β-Me), 6·94 (4H, t, CH_2·CH_2·CO), and 14·64 (2H, s, 2NH); τ (CF_3·CO_2H) (*cf.*

ref. 12) −1·02 and −0·99 (each 2H, s; 4 *meso*-H), 1·6—2·0 (2H, m, 2C*H*:CH_2), 3·4—3·8 (4H, m, 2CH:CH_2), 5·40 (4H, t, CH_2·CH_2·CO), 6·2—6·35 (18H, m, 4 β-*Me* and 2OMe), 6·74 (4H, t, CH_2·CH_2·CO), and 14·14br (4H, 4NH); $\lambda_{max.}$ 406 (ε 153,000), 506 (13,300), 539 (10,500), 576 (5800), and 629 nm (4000); $\lambda_{max.}$ (CH_2Cl_2–CF_3·CO_2H) 412 nm (ε 237,000), 556 (15,300), and 599 nm (6400), *m/e* 590 (100%) and 517 (40).

Typical Porphyrin Tritiation Procedure.—A mixture of magnesium turnings (0·5 g) and iodine (1·0 g) in ether (40 ml) was heated under reflux in an atmosphere of dry nitrogen, until colourless (1·5 h). The ether was then evaporated off, leaving a white residue which was treated with pyridine (25 ml) and heated with stirring at 130° during 30 min under nitrogen. Protoporphyrin-IX dimethyl ester (20 mg) in pyridine (5 ml) was then added; spectrophotometry showed complete metallation after 25 min at 125°. Tritiated water (*ca.* 0·1 ml; *ca.* 0·5 Ci) was added under nitrogen, and the mixture was stirred and heated at 125° during 3 h. Acetic acid (30 ml) and chloroform (50 ml) were added to the cooled solution and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was taken into 5% v/v sulphuric acid in methanol (50 ml) and set aside overnight. Chloroform was then added and the solution was washed with aqueous sodium acetate, aqueous sodium hydrogen carbonate, and then water. The organic phase was dried (Na_2SO_4), evaporated to dryness, and then chromatographed (elution with methylene chloride). The porphyrinic eluates were evaporated to dryness and the residue was crystallised from methylene chloride–n-hexane to give 14 mg (70% recovery) of protoporphyrin-IX dimethyl ester, having unchanged m.p. and of specific activity $6·93 \times 10^6$ disint. mg^{-1} s^{-1}.

In a similar way, protoporphyrin-I dimethyl ester (specific activity $1·25 \times 10^7$ disint. mg^{-1} s^{-1}), coproporphyrin-I tetramethyl ester (specific activity $4·13 \times 10^6$), and coproporphyrin-III tetramethyl ester (specific activity $2·77 \times 10^6$) were labelled.

Typical Crystallisation Procedure.—The radioactive porphyrin mixture (*ca.* 50 mg) was dissolved in methylene chloride (*ca.* 5 ml) and then treated dropwise with n-hexane (*ca.* 5 ml) *or* methanol (*ca.* 5 ml). A check was then made that the n-hexane or methanol addition had not induced precipitation, and the solution was set aside, at room temperature in the dark, covered with a small piece of aluminium foil, for between 24 and 48 h. (The solutions were not placed in the refrigerator because this usually induced rapid crystallisation.) At no time were the solutions allowed to go dry. The crystals were then filtered off, washed with a little n-hexane or methanol, and dried *in vacuo* at 100°. Other combinations of solvents, including tetrahydrofuran, ethyl acetate, ether, and benzene, were unsatisfactory, since they either produced an oily product or else were unacceptably sacrificial from the recovery point of view.

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