Biosynthesis of Porphyrins and Related Macrocycles. Part III.[1] Rational Syntheses of [β-^{13}C]-, [γ-^{13}C]-, and [δ-^{13}C]-Protoporphyrin-IX: Assignment of the ^{13}C Nuclear Magnetic Resonance Signals from the *meso*-Carbon Atoms of Protoporphyrin-IX Dimethyl Ester

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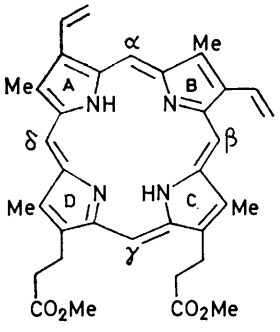
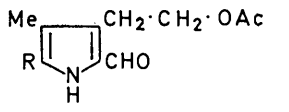
The ^{13}C signals arising from the *meso*-carbon atoms of protoporphyrin-IX are of key importance for biosynthetic studies. Rigorous assignment is made of the signals from the β- and δ-carbon atoms by synthesis of [β-^{13}C]- and [δ-^{13}C]-protoporphyrin-IX dimethyl ester from unsymmetrical pyrromethenes in 42% yield. The formation of porphyrins from *a,c*-biladienes and formaldehyde is studied and leads to synthesis of [γ-^{13}C]protoporphyrin-IX dimethyl ester. The spectrum of this product allows assignment of the two remaining signals, from the γ- and α-carbon atoms. The three ^{13}C-labelled samples of protoporphyrin-IX dimethyl ester are converted into 2,4-diacetyldeuteroporphyrin-IX dimethyl ester; assignment is thereby made of the well-spread ^{13}C signals from the *meso*-carbon atoms of this porphyrin. ^{13}C Chemical shifts are reported for a variety of pyrrole derivatives and porphyrins.

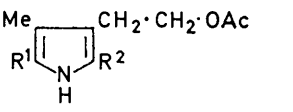
PROTOPORPHYRIN-IX dimethyl ester (1) shows four signals in its ^{13}C n.m.r. spectrum near δ 97 (from tetramethylsilane) which have been proved [1,2] to arise from the *meso*-carbon atoms [α, β, γ, and δ on structure (1)]. These signals potentially provide a key to understanding the mechanism by which the macrocycle of the natural type-III porphyrins is biosynthesised from porphobilinogen apparently with reversal of ring D. Our approach is based on the use of doubly ^{13}C-labelled porphobilinogen to detect intramolecular rearrangement(s) and to determine which site(s) are affected by such migrations. Clearly the four signals on which attention has been focused must be unambiguously assigned to the individual *meso*-carbon atoms; this was achieved by synthesis of three samples of protoporphyrin-IX dimethyl ester singly labelled with carbon-13 at the β-, γ-, and δ-positions. The present paper describes the synthetic sequences and the spectroscopic assignments.



There have been important developments over the past fifteen years in the field of porphyrin synthesis [3] and a number of reaction sequences are available. The choice here was based upon the possibility of preparing the β- and δ-labelled materials by the same route provided that the macrocycle could be constructed in good yield from *unsymmetrical* building blocks; here, Johnson's valuable variation [4] of Fischer's porphyrin synthesis from pyrromethenes [5] was selected. Conversely, the aim for the γ-labelled material was to take advantage of the symmetry about the CD-ring system.

Synthesis of [β-^{13}C]protoporphyrin-IX dimethyl ester (1) involved building the macrocycle from the pyrromethenes (2) and (3); the ^{13}C label was to be introduced at the site marked ■. Synthesis of the pyrromethene (2) was achieved by the following sequence.

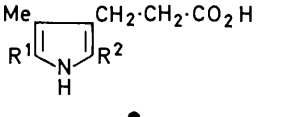


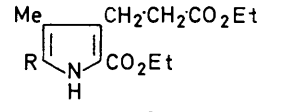


(9) R = $PhCH_2 \cdot O_2C$
(10) R = HO_2C
(11) R = I
(12) R = H

(13) $R^1 = PhCH_2 \cdot O_2C$, R^2 = Me■
(14) $R^1 = HO_2C$, R^2 = Me■
(15) R^1 = H, R^2 = Me■
(16) $R^1 = PhCH_2 \cdot O_2C$, $R^2 = CO_2H$
(17) $R^1 = PhCH_2 \cdot O_2C$, R^2 = H

Series *(a)* ■ = ^{12}C Series *(b)* ■ = ^{13}C





(18) R^1 = Me●, $R^2 = CO_2H$
(19) R^1 = Me●, R^2 = H
(20) $R^1 = HO_2C$, R^2 = CHO

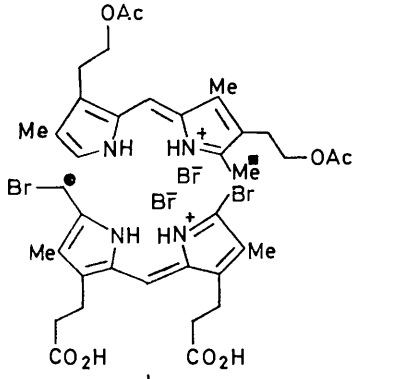
(21) R = Me●
(22) R = H
(23) R = HO_2C
(24) R = OHC

Series *(a)* ● = ^{12}C Series *(b)* ● = ^{13}C

The unlabelled pyrrole (13a), available from the work of Kenner and Jackson,[6d] was converted by bishalogenation

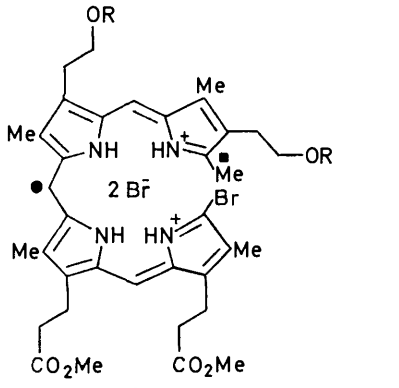
[1] Part II, A. R. Battersby, E. Hunt, E. McDonald, and J. Moron, preceding paper.
[2] A. R. Battersby, J. Moron, E. McDonald, and J. Feeney, *J.C.S. Chem. Comm.*, 1972, 920.
[3] (*a*) R. L. N. Harris, A. W. Johnson, and I. T. Kay, *Quart. Rev.*, 1966, **20**, 211; (*b*) H.-H. Inhoffen, J. W. Buchler, and P. Jäger, *Fortschr. Chem. org. Naturstoffe*, 1968, **26**, 284; (*c*) K. M. Smith, *Quart. Rev.*, 1971, **25**, 31.
[4] R. L. N. Harris, A. W. Johnson, and I. T. Kay, *J. Chem. Soc.* (*C*), 1966, 22; P. Bamfield, R. L. N. Harris, A. W. Johnson, I. T. Kay, and K. W. Shelton, *J. Chem. Soc.*, (*C*) 1966, 1436.
[5] *E.g.* H. Fischer and A. Kirrmann, *Compt. rend.*, 1929, **189**, 467.
[6] For earlier syntheses of protoporphyrin-IX see (*a*) H. Fischer and K. Zeile, *Annalen*, 1929, **468**, 114 and references therein; (*b*) R. P. Evstigneeva, V. N. Guryshev, A. F. Mironov, and G. Ya. Volodarskaya, *Zhur. obshchei Khim.*, 1969, **39**, 2558; (*c*) R. Grigg, A. W. Johnson, and M. Roche, *J. Chem. Soc.* (*C*), 1970, 1928; (*d*) R. P. Carr, A. H. Jackson, G. W. Kenner, and G. S. Sach, *J. Chem. Soc.* (*C*), 1971, 487; (*e*) A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *Chem. Comm.*, 1971, 1304.

and hydrolysis into the aldehyde (9). In some experiments, the pyrromethane (25) was formed as a significant by-product and it clearly arises from the monohalogenated intermediate. Hydrogenation of the aldehyde in the presence of triethylamine afforded the acid (10), which was iodinated, and the product (11) was reduced catalytically to yield the aldehyde (12). If triethylamine was omitted from the first step, reduction

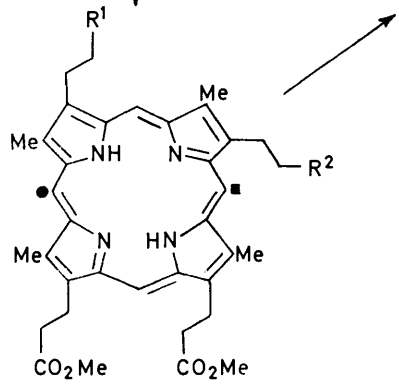


(2)

(3)



(4)

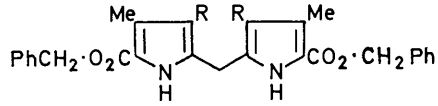


(1)

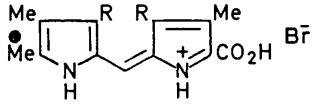
(5) $R^1 = R^2 = OAc$
(6) R^1 or R^2 = H, R^2 or R^1 = OAc
(7) $R^1 = R^2 = OH$
(8) $R^1 = R^2 = Cl$

Series *(a)* ● = ■ = ^{12}C Series *(b)* ■ = ^{13}C ● = ^{12}C
Series *(c)* ■ = ^{12}C ● = ^{13}C

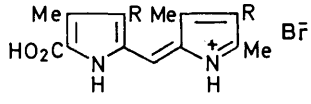
of the formyl residue occurred. The acid (14a), derived from ester (13a), underwent decarboxylation in a melt at 180° to generate the air-sensitive α-free pyrrole (15a), which was treated directly with the aldehyde (12); the pyrromethene (2a) was isolated in 69% yield.



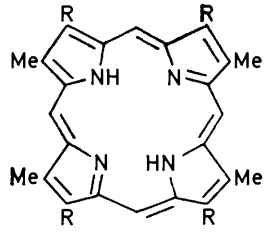
(25) $R = CH_2 \cdot CH_2 \cdot OAc$



(26) $R = CH_2 \cdot CH_2 \cdot CO_2H$



(27) $R = CH_2 \cdot CH_2 \cdot OAc$



(28) $R = CH_2 \cdot CH_2 \cdot CO_2Me$
(29) $R = CH_2 \cdot CH_2 \cdot CO_2Et$
(30) $R = CH_2 \cdot CH_2 \cdot CO_2H$

We then required the dibromopyrromethene (3a) which had been prepared earlier [7] in unlabelled form from the known [8] monopyrroles (19a) and (20). Essentially the published route was used and the chemical and spectroscopic properties of all the products closely matched the reported ones. The pyrromethene (3a) was obtained in 55% yield from the monopyrroles (19a) and (20).

The pyrromethenes (2a) and (3a) reacted smoothly in the presence of tin(IV) chloride [4] to form the *a,c*-biladiene [*cf.* (4a)] as its tin(IV) complex. Treatment with methanolic hydrogen bromide yielded the *a,c*-biladiene dihydrobromide (4a) which was cyclised in pyridine–dimethyl sulphoxide [4] to give three separable porphyrins. The n.m.r. spectrum of the most polar product proved the absence of *O*-acetyl residues, that of the porphyrin(s) of intermediate polarity showed one such group, and the least polar product contained two *O*-acetyl groups. This last porphyrin was produced by *O*-acetylation of either of the others and the most polar porphyrin was formed by treatment of the other materials with methanolic sulphuric acid. It was clear that these products were the bis-hydroxyethylporphyrin (7a), its mono-*O*-acetate(s) (6a), and the *OO*-diacetate (5a). Accordingly, the total porphyrin from the cyclisation step was subjected to methanolysis to afford the bis-hydroxyethylporphyrin (7a) in the high yield of 69% from pyrromethenes. Cyclisation of the *a,c*-biladiene (4a) in hot *o*-dichlorobenzene [4] was less satisfactory (45% yield). Also, an attempt to use the carboxylic acid (27), derived from

[7] P. Bamfield, R. Grigg, A. W. Johnson, and R. W. Kenyon, *J. Chem. Soc.* (*C*), 1968, 1259.

[8] F. Morsingh and S. F. MacDonald, *J. Amer. Chem. Soc.*, 1960, **82**, 4377; G. M. Badger, R. L. N. Harris, and R. A. Jones, *Austral. J. Chem.*, 1964, **17**, 987.

pyrroles (15a) and (10), in place of the α-free pyrromethene (2a) gave a low yield of the required material together with a small amount of coproporphyrin-II tetramethyl ester (28) formed from two units of the pyrromethene (3a).

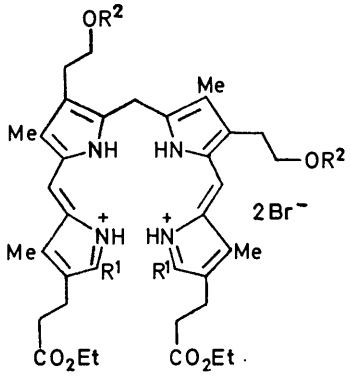
Jackson, Kenner, and their co-workers [6d] had previously prepared the dihydroxyprophyrin (7a) by different syntheses and had carried it forward *via* the dichloride (8a) to protoporphyrin-IX dimethyl ester. With some modifications (see Experimental section), these two final steps were applied here and afforded protoporphyrin-IX dimethyl ester in 42% overall yield from the pyrromethenes; the synthetic product was identical with the ester derived from natural protoporphyrin-IX.

The way was now open to prepare [β-^{13}C]- and [δ-^{13}C]-protoporphyrin-IX. The pyrrole (13a) was trihalogenated (sulphuryl chloride) and the product hydrolysed to yield the acid (16) which was thermally decarboxylated. Reductive methylation of the resultant α-free pyrrole (17) by MacDonald's method [9] at −7° with [^{13}C]formaldehyde (90% enrichment) gave the labelled pyrrole (13b). When the reaction was carried out at higher temperatures, the yield was low and the pyrromethane (25) was formed together with other by-products. The [2-*methyl*-^{13}C]pyrrole (13b) was then carried through the complete sequence *via* (15b), (2b), (4b), (7b), and (8b) to yield [β-^{13}C]protoporphyrin-IX dimethyl ester (1).

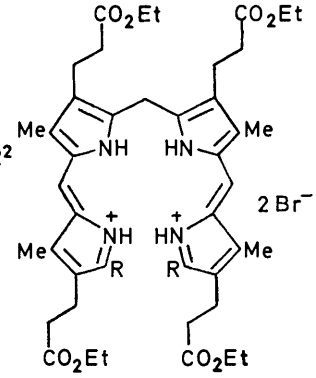
The starting material for introduction of the label at the δ-position was the α-free pyrrole (22); this is prepared [8] from the acid (23) by iodination and hydrogenation. During conversion of the pyrrole (21a) into the acid (23) by the usual sequence of halogenation and hydrolysis, the aldehyde (24) was isolated in *ca.* 20% yield; this was readily converted into the required product (23) by permanganate oxidation. Reductive methylation at the vacant α-position of pyrrole (22) with [^{13}C]formaldehyde then afforded the labelled pyrrole (21b) from which [δ-^{13}C]protoporphyrin-IX dimethyl ester (1) was synthesised by repetition of the synthesis already outlined.

The ^{13}C spectra of the two labelled samples of protoporphyrin-IX dimethyl ester allowed rigorous assignment of the signals at δ 96·8 and 96·4 (down-field from Me_4Si) to the β- and δ-carbon atoms, respectively. Table 1 collects the assignments for the ^{13}C spectra of the porphyrins and Table 2 those for the synthetic intermediates.

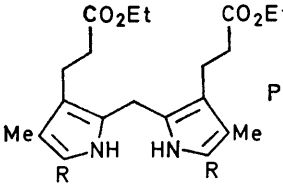
The synthetic plan for [γ-^{13}C]protoporphyrin-IX involved condensation of [^{13}C]formaldehyde with the *a,c*-biladiene (31). This scheme is attractive for three reasons: (*a*) the labelled atom is introduced late in the sequence, (*b*) the symmetry about rings C and D in the required porphyrin is used to advantage, and (*c*) [^{13}C]-formaldehyde is commercially available. Since few cases of porphyrin formation in this way have been reported,[10] a synthesis of coproporphyrin-II tetraethyl ester (29) was carried out first to assess the isomeric purity of the product. It is known that coproporphyrin-II (30) is separable chromatographically from its type-I and type-III plus type-IV isomers.[11]



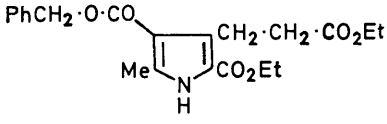
(31) $R^1 = CO_2H$, R^2 = H or Ac
(32) R^1 = H, R^2 = H or Ac



(33) R = CO_2H
(34) R = CO_2Et



(35) R = $CO_2 \cdot CH_2Ph$
(36) R = CO_2H
(37) R = H



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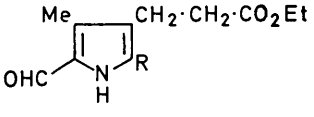
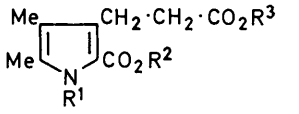


(43) R = $CO_2 \cdot CH_2Ph$
(44) R = CO_2H
(45) R = I
(46) R = H



(39) $R^1 = CH_2I$, R^2 = Et, R^3 = H
(40) $R^1 = CH_2OMe$, R^2 = Et, R^3 = Me
(41) R^1 = H, $R^2 = CH_2Ph$, R^3 = H
(42) R^1 = H, $R^2 = CH_2Ph$, R^3 = Et

The required *a,c*-biladiene (33) was prepared by condensation of two molecules of the aldehyde (44) with one of the symmetrical pyrromethane (37). The latter was obtained from the known dibenzyl ester [12] (35) by catalytic hydrogenation in the presence of triethylamine followed by decarboxylation of the resultant acid (36). The aldehyde (44) was synthesised from the available pyrrole [13] (38) by reductive methylation [9] with formaldehyde, hydrogen iodide, and hypophosphorous acid which afforded the iodomethyl derivative (39). This was hydrolysed and esterified to provide the pyrrole (21a) in overall yield of 44%. The nature of the immediate

[9] M. W. Roomi and S. F. MacDonald, *Canad. J. Chem.*, 1970, **48**, 139.
[10] A. W. Johnson and I. T. Kay, *J. Chem. Soc.*, 1965, 1620.
[11] J. E. Falk, 'Porphyrins and Metalloporphyrins,' Elsevier, Amsterdam, 1964, p. 193.
[12] A. W. Johnson, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1959, 3416.
[13] A. R. Battersby, D. A. Evans, K. H. Gibson, E. McDonald, and L. Nixon, *J.C.S. Perkin I*, 1973, 1546.

product (39) from the reductive methylation step was indicated by the lack of N–H stretching (i.r.) and by the presence (n.m.r.) of a singlet at τ 4·47 (2H). Structure (39) followed from the mass spectrum and elemental analysis and from conversion of this product by methanolic diazomethane into the *N*-methoxymethylpyrrole (40).

Treatment of the dimethylpyrrole (21a) with sodium hydride and benzyl alcohol gave a mixture of monobenzyl biladiene dihydrobromide (34) so obtained was sufficiently stable for isolation and spectroscopic characterisation. However, the corresponding dicarboxylic acid (33), prepared from (37) and (44), was less stable and it was therefore converted directly into coproporphyrin-II tetraethyl ester (29) (15% yield) by treatment with a five-fold excess of paraformaldehyde in acidic ethanol. Hydrolysis of this product gave coproporphyrin-II (30),

TABLE 1

^{13}C Chemical shifts ($CDCl_3$) for porphyrins at 25·2 MHz; δ values (downfield from Me_4Si)

Compound	Molarity	Ar–CH_2–CH_2–Cl	Ar CH=CH_2		Ar CH_2–CH_2–CO–OMe				Ar–Me	*meso*-H α	β	γ	δ
(8) [a]	0·025	30·2 45·5			21·8	36·8	*k*	51·6	12·1	96·2	95·9 [j]	96·2	96·8
(8) [b]	0·020	30·2 45·5			21·8	36·8	*k*	51·6	11·9	96·2	96·0	96·2	96·9 [j]
(1) [c]	0·044		129·8	120·1	21·7	36·8	172·8	51·6	11·4, 12·7	97·3	96·8 [j]	95·4	96·4
(1) [d]	0·013		129·8	120·4	21·9	36·8	*k*	*k*	11·6, 12·7	97·7	97·1 [j]	95·8	96·7
(1) [e]	0·044		129·8	120·1	21·7	36·6	172·8	51·6	11·5, 12·7	97·3	96·8	95·4	96·4 [j]
(1) [f]	0·013		129·9	120·3	21·9	36·8	*k*	*k*	11·6, 12·7	97·7	97·1	95·8 [j]	96·7
		Ar–CH(OH)–Me											
(62)	0·12	65·3 26·0			21·6	36·7	173·0	51·7	11·3, 11·5	(97·8	97·5	95·9	95·2 [l])
		Ar–CO–Me											
(63) [g]	0·016	*k* 33·2			21·7	36·6	*k*	51·7	11·8, 14·2 14·4	102·4	99·9 [j]	95·6	97·4
(63) [h]	0·02	*k* 33·1			21·6	36·6	172·8	51·7	11·4, 11·7 14·2	102·3	99·9	95·4	97·3 [j]
(63) [i]	0·004	*k* 33·3			21·8	36·7	*k*	*k*	*k*	102·7	100·2	96·0 [j]	97·9

[a] Using [β-^{13}C]-(8) at *ca.* 8% enrichment. [b] Using [δ-^{13}C]-(8) at *ca.* 7% enrichment. [c] Using [β-^{13}C](-1) at *ca.* 5% enrichment. [d] Using [β-^{13}C]-(1) at *ca.* 6% enrichment. [e] Using [δ-^{13}C]-(1) at *ca.* 5% enrichment. [f] Using [γ-^{13}C]-(1) at *ca.* 6% enrichment. [g] Using [β-^{13}C]-(63) at *ca.* 5% enrichment. [h] Using [δ-^{13}C]-(63) at *ca.* 5% enrichment. [i] Using [γ-^{13}C]-(63) at *ca.* 6% enrichment. [j] Signal strongly enhanced. [k] Very weak or not observed. [l] Not assigned to individual *meso*-carbon atoms. The parameters used here and for Table 2 were: spectral width 5000 Hz; acquisition time 0·4 s; pulse width 75 μs; sensitivity enhancement 0·4 s.

TABLE 2

^{13}C Chemical shifts for pyrrole derivatives at 25·2 MHz; δ values (downfield from Me_4Si)

Compound	Molarity	Ar CH_2–CH_2O–CO–CH_3				Ar COO–CH_2–Ph			α-Me	β-Me	CHO	$ArCH_2Ar$	Ring carbon atoms [d]
(13)	0·095 [a]	23·6	64·1	170·3	20·9	160·0	65·3	127·6	11·5 [e]	10·6			117·1, 128·0, 130·3, 136·1
	0·159 [b]	24·0	64·1	169·6	20·6	161·1	65·4	127·6	11·1	11·1			117·2, 128·0, 130·5, 136·9
(9)	0·304 [a]	23·8	63·6	170·1	20·8	159·9	66·5	127·9		9·7	178·7		126·8, 128·9, 130·3, 134·9
(11)	0·171 [a]	23·8	63·8	170·8	20·9					11·7	175·9		81·8, 129·6, 139·0
(12)	0·410 [a]	23·5	64·0	170·6	20·8					9·7	176·9		115·8, 120·6, 124·8, [e] 129·6
(25)	0·081 [a]	23·8	64·0	170·8	20·8	160·4	65·5	127·0		10·8		22·9	117·4 or 117·7, 128·1, 130·1, 135·8
(2)	0·061 [a]	23·3 and 24·6	62·8 and 63·6	169·7 and 170·3	20·6				13·3 [e]	9·9 and 10·6			121·3, [e] 124·6, 125·9, 127·8, 138·3 [e] 139·1, 139·5, 145·3, 156·0
		$ArCH_2$–CH_2–X				Ar–COO–CH_2–CH_3			α-Me	β-Me			
(21)	0·094 [b]	21·6	35·7			171·1 and 172·2	59·5 and 59·9	14·3 and 14·5	10·9 [e]	8·8			

[a] Solvent $CDCl_3$. [b] Solvent C_6D_6. [c] Assigned from spectrum of ^{13}C-enriched material. [d] Includes signal from bridge –CH= for compound (2). [e] Strong sharp signal assigned to unsubstituted position (–CH=). The ^{13}C spectra of a large number of similar pyrrole derivatives have been measured and the chemical shifts agree to within 1—2 p.p.m. with those quoted for the representative examples above.

(41) and the corresponding dibenzyl esters;[14] the latter by controlled hydrolysis gave the former and all the ester (41) was converted into pyrrole (42). Oxidation of this with lead tetra-acetate[15] afforded the 2-formylpyrrole (43), from which the required aldehyde (44) was derived by hydrogenolysis in the presence of triethylamine.

The conditions for formation of the *a,c*-biladiene (33) were first studied by using the pyrromethane (37) and the formyl ester (24), the latter being prepared by oxidation of the pyrrole (21a) with lead tetra-acetate. The *a,c*-which was compared by t.l.c. on cellulose with the four authentic coproporphyrin isomers * [*i.e.* (I)—(IV)]. Our synthetic coproporphyrin-II was free from the isomeric porphyrins and thus the reaction sequence could be modified with confidence for the synthesis of [γ-^{13}C]protoporphyrin-IX dimethyl ester (1) as follows.

Rings C and D of protoporphyrin-IX were to be provided as before by the formylpyrrole (44), and rings A and B were to be built in as the pyrromethane (60).

* Generously provided by Professor G. W. Kenner and Dr. K. M. Smith (Liverpool).

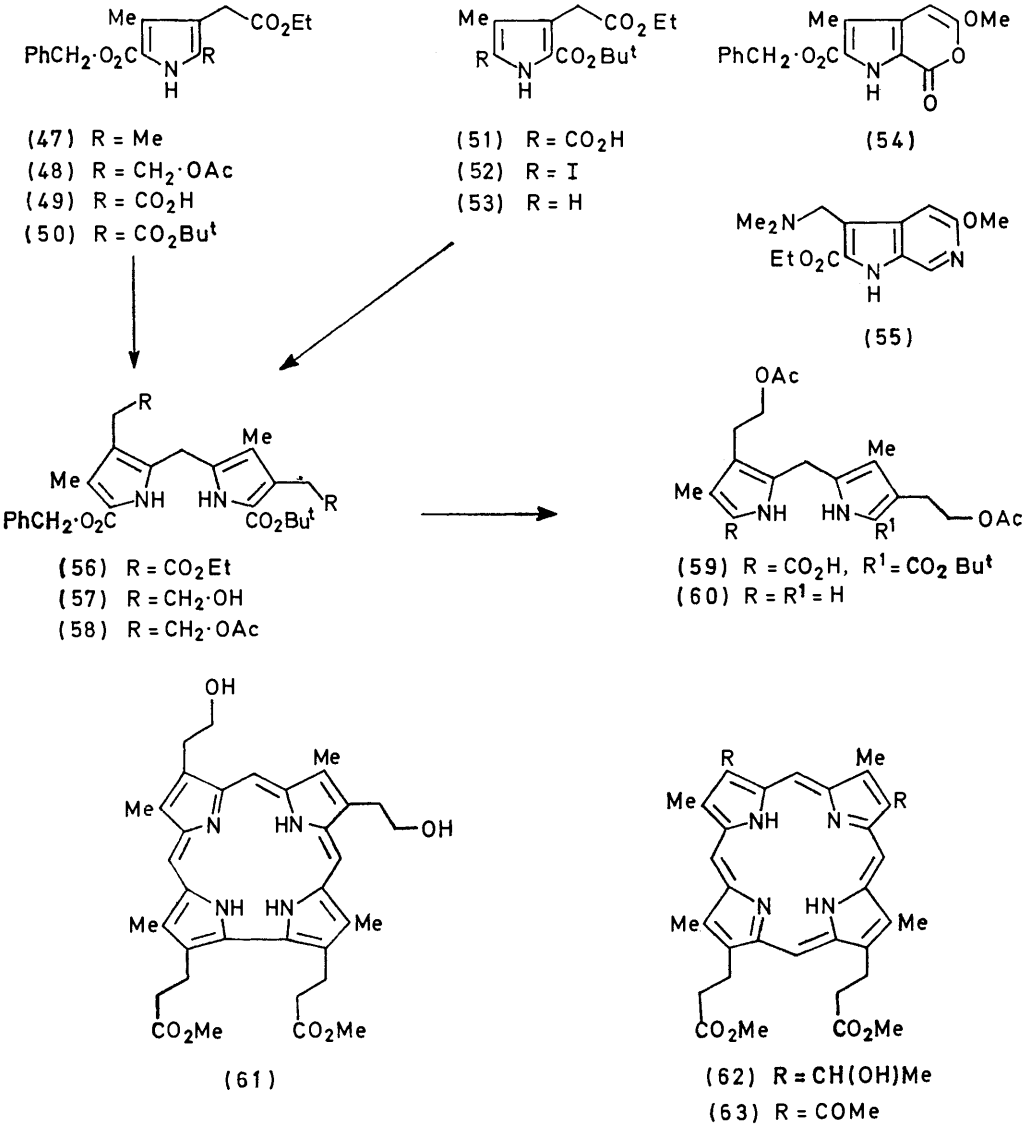
[14] A. Hayes, G. W. Kenner, and N. R. Williams, *J. Chem. Soc.*, 1958, 3779.

[15] G. M. Badger, R. L. N. Harris, and R. A. Jones, *Austral. J. Chem.*, 1964, **17**, 996.

Both pyrrole residues of the latter substance were planned to originate in the same pyrrole (47) which simply by reaction with lead tetra-acetate was converted into the ring A precursor (48). Construction of the precursor of ring B involved conversion of the pyrrole (47) by the standard sequence of halogenation and hydrolysis into the acid (49). Treatment of the corresponding acid chloride with t-butyl alcohol and dimethylaniline[6d] afforded the t-butyl ester (50). An interesting by-product with molecular weight corresponding to a product of dehydration of the acid (49) was isolated in the methyl ester series and it was converted into the ester (50) by prolonged heating with t-butyl alcohol. The assigned structure (54) is in accord with an n.m.r. singlet (1H) at τ 4·50 and with its u.v. spectrum being very similar to that[13] of the azaindole (55). Hydrogenolysis of the ester (50) gave the monocarboxylic acid (51), which was decarboxylated by way of the iodopyrrole (52) and reduction to yield the precursor of ring B (53).

Alkylation of this α-free pyrrole (53) with the acetoxymethylpyrrole (48) afforded in 72% yield the pyrromethane (56), which was reduced with diborane[16] to the diol (57). After acetylation to yield the ester (58) and hydrogenation to cleave the *O*-benzyl system, the acid (59) was treated with trifluoroacetic acid which effected both removal of the t-butyl group and bis-decarboxylation. The resultant bis-α-free pyrromethane (60) was immediately condensed with two equivalents of the aldehyde (44) to provide the key intermediate (31); we did not examine the extent to which deacetylation occurred in this step. This product reacted with formaldehyde in the presence of air to close the macrocyclic ring and, after methanolysis, the diol (7a) was isolated in 7% overall yield from the pyrromethane (59).

Repetition of the foregoing sequence [(31) ⟶ (7)] with [^{13}C]formaldehyde (90% enrichment) gave the



[16] K. M. Biswas and A. H. Jackson, *Tetrahedron*, 1968, **24**, 1145.

[γ-^{13}C]diol (7), which was converted as in earlier cases *via* the dichloride (8) into [γ-^{13}C]protoporphyrin-IX dimethyl ester (1), showing 50% ^{13}C enrichment. The ^{13}C n.m.r. spectrum of this product established that the γ-carbon atom gives rise to the signal at δ 95·8 (downfield from Me_4Si). The cause of the surprising decrease in ^{13}C content from the paraformaldehyde added to the isolated protoporphyrin-IX ester became clear when it was found that porphyrin is produced when the *a,c*-biladiene (31) is heated in acidic ethanol *without* paraformaldehyde, a side-reaction which leads to dilution of the labelled material. It is probable here that the γ-carbon atom is derived from one of the terminal carboxy-groups of the biladiene (31); analogous reactions have been reported.[17]

We also explored the reaction of the 1,19-unsubstituted *a,c*-biladiene (32) with formaldehyde for construction of the macrocycle but the yield of diol (7) was similar to that from the 1,19-dicarboxylic acid (31). In addition, a blue by-product was formed which is formulated as the corrole (61) on the basis of its mode of formation and its u.v. and mass spectra. The u.v. spectrum of our product was virtually identical with that of 8,12-diethyl-2,3,7,13,17,18-hexamethylcorrole.[10]

The biladiene (32) was prepared by condensation of the pyrromethane (60) with the aldehyde (46), which was available from the acid (44) by decarboxylation [by the iodination–reduction sequence *via* the iodopyrrole (45)].

The foregoing syntheses allow rigorous assignment to be made of the four ^{13}C signals from the *meso*-carbon atoms of protoporphyrin-IX dimethyl ester (1). When one ' reads ' from low to high field, the order is α, β, δ, γ and the difference in chemical shift between the signals from the α- and γ-carbons atoms is 1·9 p.p.m.* (see Table 1).

The ^{13}C signals from the *meso*-carbon atoms of diacetyldeuteroporphyrin-IX dimethyl ester (63) are more widely spread [18] than those just assigned. It was clear that the diketone (63) could offer advantages for spectroscopic study of material from biosynthetic experiments if it could be prepared reproducibly from protoporphyrin-IX on a small scale. Treatment of protoporphyrin-IX dimethyl ester (1) with hydrobromic acid, hydrolysis of the product,[19] and esterification afforded haematoporphyrin-IX dimethyl ester (62) in 74% yield. Brief oxidation with chromic acid then gave the diacetylporphyrin (63) in 43% overall yield. This sequence was applied to each of our synthetic ^{13}C-labelled samples of protoporphyrin-IX dimethyl ester and the ^{13}C spectra were recorded for the resultant [β-^{13}C]-, [γ-^{13}C]-, and [δ-^{13}C]-diacetyldeuteroporphyrin-IX dimethyl esters [as (63)]. The results (Table 1) allow unambiguous assignment of the ^{13}C signals from the *meso*-carbon atoms of porphyrin (63); reading from low to high field, the order is α, β, δ, γ. Importantly, there is a chemical shift difference of 6·9 p.p.m. between the signals from the α- and γ-carbon atoms (Table 1).

All the foregoing spectroscopic assignments [20] provide the firm foundation for work which has established [21] the nature of the rearrangement process involved in the biosynthesis of the natural porphyrins.

* The chemical shifts are dependent to a small extent on the concentration of the porphyrin and a systematic study of this aspect is in hand.

[17] H. Fischer, *Ber.*, 1927, **60**, 2638; H. Fischer and P. Halbig, *Annalen*, 1926, **448**, 200; 1926, **450**, 158.

EXPERIMENTAL

The general directions in Part I [13] were followed save that magnesium sulphate was used in addition as a drying agent.

Benzyl 4-(2-*Acetoxyethyl*)-5-*formyl*-3-*methylpyrrole*-2-*carboxylate* (9).—A stirred solution of the pyrrole [6d] (13a) (1 g) in anhydrous ether (14 ml) was treated dropwise at 20° with a solution of sulphuryl chloride (0·94 g) in ether (6 ml). After 45 min, the solvents were removed and a further addition of ether (10 ml) was also evaporated off. The residue was stirred for 15 min at 100° with sodium acetate (2 g) in water (12 ml), then cooled and extracted with ether. The ethereal solution was washed with N-sodium carbonate solution and saturated aqueous sodium chloride, dried, and evaporated to give the 5-*formylpyrrole* (0·99 g, 95%), m.p. 69—70° (from benzene–n-hexane) (Found: C, 65·7; H, 5·7; N, 4·1. $C_{18}H_{19}NO_5$ requires C, 65·6; H, 5·8; N, 4·2%); $\lambda_{max.}$ 232 and 306 nm; $\nu_{max.}$ 3420, 1730, 1700, and 1655 cm^{-1}; *m/e* 329 (M^+, 25%), 284 (42), 283 (30), 270 (30), 269 (100), 256 (29), 255 (28), and 240 (20); τ 0·19 (1H, s, CHO), 2·52—2·68 (5H, m, Ph), 4·66 (2H, s, CH_2Ph), 5·82 and 6·96 (each 2H, t, J 7 Hz, CH_2·CH_2·O), 7·68 (3H, s, Me), and 8·01 (3H, s, Ac).

3,3'-*Bis*-(2-*acetoxyethyl*)-5,5'-*bisbenzyloxycarbonyl*-4,4'-*dimethyl*-2,2'-*pyrromethane* (25).—This was occasionally formed as a by-product in the foregoing preparation and was separable by chromatography in ether on alumina. Crystallised from benzene–n-hexane, the *pyrromethane* had m.p. 118—119° (Found: C, 68·6; H, 6·1; N, 4·75. $C_{35}H_{38}N_2O_8$ requires C, 68·4; H, 6·2; N, 4·6%), $\lambda_{max.}$ 292 nm; $\nu_{max.}$ 1720 and 1660 cm^{-1}; τ 0·26 (2H, s, 2NH), 2·74 (10H, s, 2Ph), 4·82 (4H, s, 2CH_2Ph), 5·98 and 7·48 (each 4H, t, J 7 Hz, 2 CH_2·CH_2·O), 6·14 (2H, s, bridge CH_2), 7·74 (6H, s, 2Me), and 8·07 (6H, s, 2Ac).

4-(2-*Acetoxyethyl*)-5-*formyl*-3-*methylpyrrole*-2-*carboxylic Acid* (10).—A solution of the pyrrole (9a) (2 g) in tetrahydrofuran (40 ml) containing triethylamine (2 drops) was stirred at 20° with 10% palladised charcoal (0·3 g) and hydrogen for 2·3 h (uptake had then ceased). The filtered solution was evaporated and the residue crystallised from chloroform–light petroleum (b.p. 60—80°) to afford the *pyrrolecarboxylic acid* (1·4 g, 97%), m.p. 160—165° (decomp.) (Found: C, 55·3; H, 5·6; N, 6·3. $C_{11}H_{13}NO_5$ requires C, 55·2; H, 5·5; N, 5·9%), $\lambda_{max.}$ 227 and 307 nm (shifted to 225 and 310 nm on addition of N-sodium hydroxide); $\nu_{max.}$ 3410, 1735, 1690, and 1655 cm^{-1}; *m/e* 239 (M^+, 12%), 180 (25), 179 (100), 166 (15), 134 (18), 133 (65), and 121 (30); τ [$(CD_3)_2SO$] 0·16 (1H, s, CHO), 5·9 and 6·98 (each 2H, t, J 7 Hz, CH_2·CH_2·O), 7·75 (3H, s, Me), and 8·02 (3H, s, Ac).

[18] See D. Doddrell and W. S. Caughey, *J. Amer. Chem. Soc.*, 1972, **94**, 2510 for ^{13}C spectrum of (63) at natural abundance.

[19] Ref. 13, p. 176.

[20] Preliminary report, A. R. Battersby, G. L. Hodgson, M. Ihara, E. McDonald, and J. Saunders, *J.C.S. Chem. Comm.*, 1973, 441.

[21] A. R. Battersby, E. Hunt, and E. McDonald, *J.C.S. Chem. Comm.*, 1973, 442.

4-(2-*Acetoxyethyl*)-5-*formyl*-2-*iodo*-3-*methylpyrrole* (11).—Iodine (0·52 g) in ethanol (10 ml) was added over 5 min to a hot, stirred solution of the foregoing pyrrole (0·5 g) in water (8 ml) containing potassium hydrogen carbonate (0·42 g). After the mixture had been boiled until the colour was discharged, it was poured into water (50 ml) and extracted with benzene; the extracts were washed with N-sodium carbonate and water, dried, and evaporated, and the residue was chromatographed on alumina in ether. The *iodopyrrole* crystallised from chloroform–n-hexane (0·4 g, 60%); m.p. 81—82° (Found: C, 37·5; H, 3·7; N, 4·4. $C_{10}H_{12}INO_3$ requires C, 37·4; H, 3·8; N, 4·4%), $\lambda_{max.}$ 315 nm; $\nu_{max.}$ 3410, 1728, and 1640 cm^{-1}; *m/e* 321 (M^+, 48%), 278 (5), 261 (100), 249 (20), 248 (28), 233 (55), 220 (5), and 193 (5); τ 0·60 (1H, s, CHO), 5·87 and 7·0 (each 2H, t, *J* 7 Hz, $CH_2{\cdot}CH_2{\cdot}O$), and 8·03 (6H, s, Me and Ac).

3-(2-*Acetoxyethyl*)-2-*formyl*-4-*methylpyrrole* (12).—The foregoing aldehyde (0·3 g), 10% palladised charcoal (0·6 g), and magnesium oxide (0·3 g) in tetrahydrofuran (20 ml) were shaken for 48 h with hydrogen (1 atm) at 20°. The filtered solution was evaporated, the residue was shaken with chloroform and water, and the organic layer was dried and evaporated. Chromatography of the products on alumina (10 g) in ether gave the *formylpyrrole* (160 mg, 88%), m.p. 64—65° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 61·8; H, 6·4; N, 6·9. $C_{10}H_{13}NO_3$ requires C, 61·5; H, 6·7; N, 7·2%), $\lambda_{max.}$ 301 nm; $\nu_{max.}$ 3430, 1725, and 1638 cm^{-1}; *m/e* 195 (M^+, 35%), 152 (5), 135 (100), 134 (20), 123 (35), 122 (75), 107 (75), and 106 (35); τ 0·39 (1H, s, CHO), 3·13 (1H, d, *J* 3 Hz, 5-H), 5·82 and 6·98 (each 2H, t, *J* 7 Hz, $CH_2{\cdot}CH_2{\cdot}O$), 7·95 (3H, s, Me), and 8·00 (3H, s, Ac).

3-(2-*Acetoxyethyl*)-2,4-*dimethylpyrrole* (15a).—The acid [6d] (14a) derived from the ester (13a) showed $\lambda_{max.}$ 278 nm (shifted to 269 nm on addition of N-sodium hydroxide); $\nu_{max.}$ (KBr) 3300, 1720, and 1660 cm^{-1}; τ [$(CD_3)_2SO$] 6·05 and 7·42 (each 2H, t, *J* 7 Hz, $CH_2{\cdot}CH_2{\cdot}O$), 7·85 and 7·88 (each 3H, s, 2Me), and 8·03 (3H, s, Ac).

This acid (0·5 g) was heated under nitrogen at 180° until gas evolution ceased (a few min); chromatography of the product on alumina (5 g) in ether gave the *dimethylpyrrole* as a gum (376 mg, 94%) (Found: M^+, 181·1101. $C_{10}H_{15}NO_2$ requires *M*, 181·1103), $\nu_{max.}$ 3470 and 1730 cm^{-1}; τ 3·62 (1H, d, *J* 3 Hz, 5-H), 5·92 and 7·31 (each 2H, t, *J* 8 Hz, $CH_2{\cdot}CH_2{\cdot}O$), 7·82 and 7·97 (each 3H, s, 2Me), and 7·97 (3H, s, Ac).

Alternatively, the acid (14a) (0·5 g) was heated for ½ h under reflux in xylene (30 ml) in the dark under nitrogen. The residue left on evaporation was purified as before to give the same product (370 mg, 92%). This α-free pyrrole was unstable and was used directly for the next step.

3,4′-*Bis*-(2-*acetoxyethyl*)-3′,4,5′-*trimethyl*-2,2′-*pyrromethene Hydrobromide* (2a).—Hydrobromic acid (0·2 ml; 48%) was added at 0° to a solution in methanol (0·5 ml) of the aldehyde (12) (197 mg) and the foregoing pyrrole (183 mg). After 1 h at 0°, the solid was collected and recrystallised from chloroform–light petroleum (b.p. 60—80°) to give the *pyrromethene hydrobromide* (303 mg, 69%), m.p. 159—160° (Found: C, 54·7; H, 6·1; N, 6·4. $C_{20}H_{27}BrN_2O_4$ requires C, 54·7; H, 6·2; N, 6·4%), $\lambda_{max.}$ ($CHCl_3$) 366 and 479 nm (log ε 3·85 and 4·82) (shifted to 426 nm on addition of piperidine); $\nu_{max.}$ 1730 and 1620 cm^{-1}; τ 2·46 (1H, d, *J* 4 Hz, 5-H), 2·71 (1H, s, =CH–), 5·83—5·99 (4H, m, 2 $CH_2{\cdot}OAc$), 7·03 and 7·28 (each 2H, t, *J* 7 Hz, $2CH_2$), 7·31, 7·68, and 7·94 (each 3H, s, 3Me), and 8·04 (6H, s, 2Ac).

2,4-*Bis*-(2-*hydroxyethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (7a).—The foregoing pyrromethene hydrobromide (237 mg) and the dibromopyrromethene hydrobromide [7] (308 mg) were dissolved at 40° in glacial acetic acid (13 ml). After cooling to 20°, the solution was diluted with chloroform (130 ml), treated with tin(IV) chloride (6·5 ml) and then stirred for 1 h in the dark. The residue from evaporation of the solution showed $\lambda_{max.}$ ($CHCl_3$) 456 and 540 nm. It was treated with stirring at 20° for 2 h with dry methanol (65 ml) and hydrobromic acid (13 ml; 48%) and then the mixture was added to chloroform (325 ml). This solution was washed with aqueous hydrobromic acid (3 × 70 ml; 10%), dried, and evaporated; $\lambda_{max.}$ values for the *a,c*-biladiene hydrobromide were 458 and 531 nm. A solution of this product in dimethyl sulphoxide (64 ml) and pyridine (1·3 ml) was kept in the dark at 20° for 2 days and then evaporated. T.l.c. of the residue (silica; 1 : 9 methanol–chloroform) showed three components, R_F 0·25, 0·46, and 0·54. The total material was dissolved in methanol (195 ml) containing 5% (w/v) of concentrated sulphuric acid; the solution was kept at 0° for 16 h and then poured onto ice and water (*100 g*). After basification with ammonia, the mixture was extracted with chloroform and the extracted product crystallised on treatment with chloroform–ether. It was recrystallised from pyridine–ether to yield the bis-hydroxyethylporphin (7a) (238 mg), m.p. 226—227° (lit.,[6d] 225—226·5°); chromatography on alumina in chloroform of the material in the mother liquors gave the same diol, m.p. 226—227° (6 mg; total yield 69%) (Found: C, 68·9; H, 6·45; N, 9·0. Calc. for $C_{36}H_{42}N_4O_6$: C, 69·0; H, 6·75; N, 8·9%), *m/e* 626 (M^+, 100%), 595 (30), 567 (6), 566 (4), 565 (5), 564 (3), 553 (15) 522 (5), and 521 (4); τ (0·03M in $CF_3{\cdot}CO_2D$) 5·25—5·58 (12H, m, $2CH_2{\cdot}CH_2{\cdot}OH$ and $2CH_2{\cdot}CH_2{\cdot}CO$) 6·24, 6·30, and 6·32 (12H, 3H, and 3H, 6Me), and 6·70—6·90 (4H, m, $2CH_2{\cdot}CH_2{\cdot}CO$).

Protoporphyrin-IX Dimethyl Ester (1).—A modification of the published conditions [6d] for the conversion of (7a) into (8a) proved advantageous. A stirred solution of the foregoing diol (70 mg) in dry dimethylformamide (18 ml) was treated with methanesulphonyl chloride (4·9 ml) and anhydrous lithium chloride (4·9 g) at 75° for 40 min in the dark. After basification with aqueous ammonium hydroxide, the solution was extracted with methylene chloride and the product was purified as earlier [6d] to give the bischloroethylporphyrin (8a) (63 mg, 85%), m.p. 217—218° (lit.,[6d] 216—217°) (Found: C, 65·35; H, 5·7; N, 8·4. Calc. for $C_{36}H_{40}Cl_2N_4O_4$: C, 65·3; H, 6·1; N, 8·4%), $\lambda_{max.}$ ($CHCl_3$) 400 (log ε 5·30), 498 (4·22), 532 (3·98), 568 (3·86), and 619 nm (3·68); *m/e* 622 (M^+, 100%), 628 (40), 627 (30), 626 (35), 613 (10), and 589 (15); τ (0·038M in $CDCl_3$) 0·04 and 0·30 (each 1H, α- and γ-H, not necessarily respectively), 0·17 (1H, s, δ-H), 0·22 (1H, s, β-H), 5·60—5·86 (12H, m, $2CH_2{\cdot}CH_2Cl$ and $2CH_2{\cdot}CH_2{\cdot}CO$), 6·37 (6H, s, 2OMe), 6·48 6·51, 6·55, and 6·59 (3H each s, 4Me), and 6·72—6·87 (4H, m, $2CH_2CO$). The assignment of the β- and δ-signals was made by observing which signals were split (^{13}C–^1H coupling) in the ^1H spectrum of the ^{13}C-labelled porphins prepared later.

Various methods were studied for the conversion of the bischloroethylporphin into protoporphyrin-IX dimethyl ester. Jackson and Kenner's procedure [6d] (a 4-day sequence) consistently gave the best yield (72%); the following quicker method affords 66%. 1,5-Diazabicyclo[4.3.0]-non-5-ene (1·5 ml) and the bischloroethylporphin (25 mg) in dry tetrahydrofuran (6 ml) were heated under reflux in nitrogen for 4 h in the dark. After evaporation of the

solvents, the residue in methylene chloride was neutralised with glacial acetic acid and washed with water. The products from evaporation of the methylene chloride were chromatographed on alumina (4 g) in 1 : 1 benzene–methylene chloride and the protoporphyrin-IX dimethyl ester was crystallised (plates) from chloroform–methanol; yield 17·4 mg (66%), m.p. 217—219° unchanged in admixture with authentic diester (m.p. 217—219°) prepared from natural protoporphyrin-IX (Found: C, 73·1; H, 6·3; N, 9·2. Calc. for $C_{36}H_{38}N_4O_4$: C, 73·2; H, 6·5; N, 9·5%). Crystallisation of this product from methylene chloride–methanol afforded needles of m.p. 227—228°, *m/e* 590 (M^+, 100%) (remainder of spectrum identical with that from authentic material); u.v., i.r., n.m.r., and t.l.c. properties of synthetic and naturally derived samples were also identical.

Attempted Porphin Formation from the Pyrromethene (27).—The acid (10) (0·1 g) and the α-free pyrrole (15a) (0·1 g) in acetic acid (0·4 ml) and 45% hydrobromic acid in acetic acid (0·1 ml) were kept at 20° in the dark for 16 h. The solid was collected and washed with acetic acid and ether to give the pyrromethene hydrobromide (0·1 g), which was characterised spectroscopically because of its instability: $\lambda_{max.}$ 475 nm (shifted to 428, 470, and 500 nm by addition of N-sodium hydroxide); τ 2·49 (1H, s, =CH–), 5·72—5·92 and 6·86—7·25 (each 4H, m, $CH_2{\cdot}CH_2{\cdot}O$), 7·20, 7·54, and 7·70 (each 3H, s, 3Me), and 7·96 (6H, s, 2Ac).

This product (17 mg) and the bromopyrromethene (3) (20 mg) were dissolved at 40° in glacial acetic acid (1 ml) and to the cooled solution were added chloroform (8 ml) and tin(IV) chloride (0·5 ml). The mixture was stirred at 20° for 2 h and then worked up as the *a,c*-biladiene hydrobromide as in the synthesis of the porphin (7a). A solution of this product in chloroform (10 ml) was added dropwise over 10 min to *o*-dichlorobenzene (30 ml) at 180°; the chloroform was allowed to distil out. After the mixture had been heated under reflux for a further 15 min, it was evaporated and the residue, by p.l.c. on silica in 1 : 1 chloroform–ether, gave the bishydroxyethylporphin (7a) (0·4 mg) and the corresponding monoacetate (6a) (0·6 mg), both identified by full comparison with the samples prepared earlier. A fast-running porphin (1·1 mg) was identified by u.v.–visible, accumulated n.m.r., and mass spectra as a coproporphyrin tetramethyl ester containing a trace of the diacetate (5a); it was proved to be the type-II isomer (28) by spectroscopic and chromatographic comparison with an authentic sample.*

4-(2-Acetoxyethyl)-2-benzyloxycarbonyl-3-methylpyrrole-5-carboxylic Acid (16).—A solution of sulphuryl chloride (3·07 g) in dry ether (20 ml) was added dropwise over 5 min at 20° to a stirred solution of the pyrrole (13a) (2 g) in ether (50 ml). The solution was heated under reflux for 1 h, then evaporated (and evaporated twice again after addition of ether) and the residue was stirred for 15 min with a hot solution of sodium acetate (4 g) in water (24 ml). Extraction with ether gave a solution which was shaken with an excess of aqueous N-sodium carbonate; the aqueous solution was acidified (SO_2) and extracted with ether to give the *pyrrole-5-carboxylic acid* (1·32 g, 60%), m.p. 138—139° (decomp.) [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 62·7; H, 5·5; N, 4·0. $C_{18}H_{19}NO_6$ requires C, 62·6; H, 5·55; N, 4·05%), $\lambda_{max.}$ 281 and 290sh nm; $\nu_{max.}$ 1730—1680 cm^{-1}; *m/e* 345 (M^+, 10%), 301 (15), 285 (90), 243 (20), and 242 (100); τ 2·50—2·66 (5H, m, Ph), 4·64 (2H, s, CH_2Ph), 5·78 and 6·89 (2H each, t, *J* 7 Hz, $CH_2{\cdot}CH_2{\cdot}O$), 7·66 (3H, s, Me), and 7·97 (3H, s, Ac).

Benzyl 4-(2-Acetoxyethyl)-3-methylpyrrole-2-carboxylate (17).—The foregoing acid (0·5 g) was heated under nitrogen at 230° until gas evolution ceased (a few min) and the resultant oil was fractionated on alumina in ether to give the *α-free pyrrole* (418 mg) which was used immediately for the following preparation (Found: M^+, 301·1298. $C_{17}H_{19}NO_4$ requires *M*, 301·1313), $\lambda_{max.}$ 273 nm; $^4\nu_{max.}$ 1730—1680 cm^{-1}; *m/e* 301 (M^+, 20%), 257 (1), 241 (100), 228 (12), 194 (10), and 150 (12).

Benzyl 4-(2-Acetoxyethyl)-3-methyl-5-[^{13}C]methylpyrrole-2-carboxylate (13b).—A stirred mixture of hydriodic acid (6 ml) and hypophosphorus acid (1·2 ml) at −5° was treated dropwise with acetic anhydride (6 ml) and stirring was continued until a pale straw colour resulted. [^{13}C]Paraformaldehyde (11·7 mg; 90% enrichment) was then added followed by the foregoing pyrrole (105 mg) in acetic acid (1·5 ml) and the mixture was stirred at −6 to −8° for 3 h. After basification with aqueous ammonium hydroxide (containing 50 mg of sodium thiosulphate), the solution was extracted with chloroform and the product was fractionated by p.l.c. on silica in 7 : 1 benzene–ether. The main fraction crystallised from benzene–light petroleum (b.p. 60—80°) to give the [5-methyl-^{13}C]*pyrrole* (13b) (73 mg, 66%), m.p. 73—74° (Found 89% enrichment in ^{13}C by mass spectrum); *m/e* 316 (M^+ for ^{13}C material, 32%), 315 (M^+ for ^{12}C material, 4), 256 (100), 255 (13), 243 (30), 241 (40), 228 (35), and 165 (30), n.m.r. spectrum virtually identical with spectrum of ^{12}C material save for τ 7·79 (0·89 × 3H, d, *J* 127 Hz, 5-$^{13}CH_3$ and 0·11 × 3H, s, 5-$^{12}CH_3$).

[β-^{13}C]*Protoporphyrin-IX Dimethyl Ester* (1).—The foregoing ^{13}C-pyrrole was converted as earlier into the [5′-*methyl*-^{13}C]pyrromethene (2b), m.p. 159—160° showing n.m.r. data almost identical with those of unlabelled material except for the signal at τ 7·31 (0·89 × 3H, d, *J* 128 Hz, 5′-$^{13}CH_3$ and 0·11 × 3H, s, 5′-$^{12}CH_3$). The derived bishydroxyethyl[β-^{13}C]porphin (7b), m.p. 226—227°, gave *m/e* 627 (M^+ for ^{13}C material, 100%), 626 (M^+ for ^{12}C material, 18), 596 (18), 568 (5), 567 (4) 566 (4), 565 (3), 554 (7), 523 (4), and 522 (3). This was converted into the bischloroethyl[β-^{13}C]porphin (8b), m.p. 217—218°, *m/e* 663 (M^+ for ^{13}C material, 100%), 662 (M^+ for ^{12}C material, 18), 629 (30), 628 (20), 627 (25), 614 (10), and 590 (14); τ (0·038M in $CDCl_3$) 0·22 (0·89H, d, *J* 154 Hz, β-^{13}C and 0·11H, s, β-^{12}C) with the remainder of the signals as for unlabelled material. Dehydrochlorination gave [β-^{13}C]-protoporphyrin-IX dimethyl ester (116 mg), m.p. 217—219°, *m/e* 591 (M^+ for ^{13}C material, 100%), 590 (M^+ for ^{12}C material, 14), and 518 (12); τ (0·023M in $CDCl_3$) 0·0 (*ca.* 0·94, s, *J* 148 Hz, β-^{13}C and *ca.* 1·1H, s, β-^{12}C overlapped with 1H).

Ethyl 3-(2-Ethoxycarbonylethyl)-5-formyl-4-methylpyrrole-2-carboxylate (24).—Sulphuryl chloride (891 mg) in dry ether (2 ml) was added dropwise at 20° over 10 min to a stirred suspension of the pyrrole [14] (21a) (534 mg) in ether (7 ml) and the mixture was heated under reflux for 1 h. The solvent, and then two further additions of ether, were evaporated off, the residue was stirred for 15 min with sodium acetate (1 g) in hot water (6 ml), and then the mixture was extracted with ether. Extraction of the ethereal solution with N-sodium carbonate followed by the usual work-up gave the acid (23) (290 mg, 49%) m.p. 202—204° (lit.,[8] 202—203°). The ether layer containing the

* See footnote p. 2926.

neutral products yielded a gum (0·2 g) which on alumina in ether gave the *pyrrole aldehyde* (120 mg, 21%), m.p. 85—85·5° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 59·7; H, 6·8; N, 5·2. $C_{14}H_{19}NO_5$ requires C, 59·8; H, 6·8; N, 5·0%). $\lambda_{max.}$ 232 and 303 nm; $\nu_{max.}$ 3420, 1720, 1710, and 1655 cm^{-1}; *m/e* 281 (M^+, 33%), 236 (18), 207 (100), and 194 (30); τ 0·20 (1H, s, CHO), 5·66 and 5·91 (each 2H, q, *J* 7 Hz, $2O{\cdot}CH_2$), 6·98 and 7·50 (each 2H, t, *J* 8 Hz, $CH_2{\cdot}CH_2{\cdot}CO$), 7·70 (3H, s, Me), and 8·66 and 8·80 (each 3H, t, *J* 7 Hz, $2O{\cdot}CH_2{\cdot}CH_3$).

This aldehyde (1 g) in acetone (30 ml) and acetic acid (1·5 ml) was stirred at 20° for 5 h as potassium permanganate (1·2 g) was added in portions. After the solution had been decolourised (SO_2), it was acidified and extracted with chloroform to yield the acid (23) (0·75 g, 71%), m.p. 202—204°, identical with that already described.

Ethyl 3-(2-*Ethoxycarbonylethyl*)-4-*methyl*-5-[^{13}C]*methylpyrrole*-2-*carboxylate* (21b).—The straw coloured reagent (total vol. 6·6 ml) was prepared as for the synthesis of (13b). [^{13}C]Paraformaldehyde (30 mg; 90% enrichment) was added, followed by the α-free pyrrole [8] (22) (250 mg) and the mixture was stirred at 0° for 1·25 h. Unlabelled paraformaldehyde (15 mg) was added and after a further 0·5 h, the mixture was basified with aqueous ammonium hydroxide (containing thiosulphate) and extracted with chloroform. The product was fractionated on alumina in ether to give the [5-methyl-^{13}C]*pyrrole* (202 mg, 75%), m.p. 90—91° (from n-hexane and aqueous ethanol); 85% enrichment in ^{13}C by mass spectrum, *m/e* 268 (M^+ for ^{13}C material, 100%), 267 (M^+ for ^{12}C material, 17), 223 (33), 195 (69), 194 (67), 193 (18), and 181 (45); τ 7·85 (0·85 × 3H, d, *J* 128 Hz, 5-$^{13}CH_3$ and 0·15 × 3H, s, 5-$^{12}CH_3$).

[δ-^{13}C]*Protoporphyrin-IX Dimethyl Ester* (1).—The foregoing [^{13}C]pyrrole was hydrolysed and decarboxylated to yield the acid (19b), m.p. 127—129°, *m/e* 168 (M^+), which as earlier was converted first into the [5′-*methyl*-^{13}C]pyrromethene hydrobromide (26) and then into the dibromo[5′-*methylene*-^{13}C]pyrromethene hydrobromide (3c). The route then led to the bishydroxyethyl[δ-^{13}C]porphin (7c), m.p. 226—227°, *m/e* 627 (M^+ for ^{13}C material, 100%), 626 (M^+ for ^{12}C material, 20), 596 (25), 568 (3), 567 (2), 566 (3), 565 (2), 554 (7), 523 (3), and 522 (2). The derived bischloroethyl[δ-^{13}C]porphin (8c), m.p. 217—218°, showed *m/e* 663 (M^+ for ^{13}C material, 100%), 662 (M^+ for ^{12}C material, 20), 629 (35), 628 (20), 627 (25), 614 (5), and 590 (7); τ (0·04M in $CDCl_3$) 0·17 (0·85H, d, *J* 154 Hz, δ-^{13}C and 0·15H, s, δ-^{12}C). Dehydrochlorination finally afforded [δ-^{13}C]protoporphyrin-IX dimethyl ester (117 mg), m.p. 217—219°, *m/e* 591 (M^+ for ^{13}C material, 100%), 590 (M^+ for ^{12}C material, 16), and 518 (12); τ (0·023M in $CDCl_3$) 0·06 (*ca.* 0·9H, d, *J* 148 Hz, δ-^{13}C and *ca.* 1·1H, s, δ-^{12}C overlapped with 1H).

Ethyl 3-(2-*Carboxyethyl*)-1-*iodomethyl*-4,5-*dimethylpyrrole* 2-*carboxylate* (39).—Acetic anhydride (80 ml) was added dropwise with stirring at 0—5° to hydriodic acid (50 ml; freshly distilled under nitrogen from red phosphorus) followed by hypophosphorous acid (14 ml). After this mixture had warmed to 20°, ethyl 4-benzyloxycarbonyl-3-(2-ethoxycarbonylethyl)-5-methylpyrrole-2-carboxylate [22] (38) (7·76 g) was added and the mixture was warmed to 55° over 40 min. After a further 20 min at 55°, a precipitate appeared; acetic anhydride (40 ml) and acetic acid (20 ml) were then added over 10 min and the temperature was raised to 65—75°. The precipitate had dissolved after 1 h and the heating was continued for 20 min. The mixture at 40—45° was treated in portions with paraformaldehyde (1·8 g) over 20 min and then stirred for a further 20 min at 40—45°. The solvents were evaporated off, water (20 ml) was added, and the suspension was kept for 16 h at 0°. The solid was collected, washed with water, and dried; this material was used directly in the following paragraph. For analysis the N-*iodomethylpyrrole* was recrystallised from ethyl acetate; m.p. 182—186° (Found: C, 41·7; H, 4·7; I, 34·2; N, 3·7. $C_{13}H_{18}INO_4$ requires C, 41·3; H, 4·7; I, 33·6; N, 3·7%); $\nu_{max.}$ (Nujol) (no NH stretch) 1705 and 1680 cm^{-1}; *m/e* 379 (M^+, 1%), 252 (90), 206 (17), 178 (17), 164 (100), and 134 (52), *m** 168 (379 ⟶ 252); τ [$(CD_3)_2SO$] 4·47 (2H, s, CH_2I), 5·80 (2H, q, *J* 7 Hz, ester CH_2), 7·0—7·7 (4H, m, $CH_2{\cdot}CH_2$), 7·83 (3H, s, CH_3), 8·12 (3H, s, CH_3), and 8·73 (3H, t, *J* 7 Hz, ester CH_3).

Treatment of this product (0·1 g) in methanol (4 ml) with an excess of distilled ethereal diazomethane gave the *N*-methoxymethyl ester (40), $\lambda_{max.}$ (MeOH) 285 nm; $\nu_{max.}$ (no NH stretch) 1735 and 1680 cm^{-1}; *m/e* 297 (M^+) and 266; τ 4·36 (2H, s, $N{\cdot}CH_2$), 5·72 (2H, q, *J* 7 Hz, ester CH_2), 6·34 and 6·73 (each 3H, s, 2OMe), 6·9—7·6 (4H, m, $CH_2{\cdot}CH_2$), 7·78 and 8·05 (each 3H, s, $2CH_3$), and 8·65 (3H, t, *J* 7 Hz, ester CH_3).

Ethyl 3-(2-*Ethoxycarbonylethyl*)-4,5-*dimethylpyrrole*-2-*carboxylate* (21a).—A solution of all the foregoing crude iodomethyl derivative in ethanol (30 ml), water (90 ml), and concentrated hydrochloric acid (4 ml) was heated under reflux for 0·5 h and then cooled to 0°. The precipitated solid was collected, dried, and esterified by heating under reflux with anhydrous ethanolic 7% hydrogen chloride. The residue from evaporation was recrystallised from aqueous ethanol to give the pyrrole (21a) (2·3 g), m.p. 88—90°, identified by comparison with material already prepared.

Benzyl 3-(2-*Ethoxycarbonylethyl*)-4,5-*dimethylpyrrole*-2-*carboxylate* (42).—The foregoing pyrrole ester (6·4 g) was stirred at 110° and 10 mmHg for 12 h with a solution of sodium hydride (1·28 g of 50% dispersion) in benzyl alcohol (32 ml). The residue from evaporation was partitoned between ethyl acetate (100 ml) and water (40 ml) and the organic layer was washed with 2N-sodium hydroxide and water. The residue from the organic layer was recrystallised from benzene–light petroleum (b.p. 60—80°) to give the dibenzyl ester (3·86 g), m.p. 130—131° (lit.,[14] 130°), $\lambda_{max.}$ ($CHCl_3$) 288 nm; $\nu_{max.}$ (Nujol) 3280, 1715, and 1660 cm^{-1}; *m/e* 391 (M^+, 30%), 300 (4), 256 (6), 198 (5), 194 (6), 192 (9), 152 (10), 148 (5), 120 (3), and 91 (100), *m** 230 (391 ⟶ 300).

Acidification of the combined alkaline solutions with sulphur dioxide gave the monobenzyl ester (41) (4·0 g), m.p. 164—165° (lit.,[14] 165—167°), $\lambda_{max.}$ 288 nm; $\nu_{max.}$ (Nujol) 3305, 3200—2400, 1695, and 1660 cm^{-1}; *m/e* 301 (M^+, 35%), 256 (2), 242 (5), 239 (4), 210 (8), 192 (16), 167 (8), 166 (7), 152 (6), 134 (5), and 91 (100), *m** 175·5 (210 ⟶ 192) and 146·5 (302 ⟶ 210).

The foregoing dibenzyl ester (3·9 g) was stirred at 20° in ethanol (100 ml) and 4N-sodium hydroxide (15 ml) until all dissolved (2·5 h). After addition of water (100 ml), the solution was evaporated to 100 ml and acidified with sulphur dioxide to yield the monobenzyl ester (2·72 g), m.p. 165—166°.

A solution of the monobenzyl ester (4 g) in anhydrous ethanolic 7% hydrogen chloride (80 ml) was heated under reflux for 10 min and then evaporated. The residue in chloroform was washed with water, saturated sodium

[22] S. F. MacDonald, *J. Chem. Soc.*, 1952, 4176, 4184.

hydrogen carbonate solution, and water, and then dried and evaporated. The residue crystallised on trituration with light petroleum (b.p. 40—60°) to give the *benzyl ethyl ester* (42) (3·7 g), m.p. 81—82° (from aqueous ethanol) (Found: C, 69·1; H, 7·0; N, 4·5. $C_{19}H_{23}NO_4$ requires C, 69·3; H, 7·0; N, 4·3%), $\lambda_{max.}$ ($CHCl_3$) 288 nm; $\nu_{max.}$ (Nujol) 3280, 1715, and 1655 cm^{-1}; *m/e* 329 (M^+, 48%), 284 (7), 238 (11), 198 (7), 194 (11), 192 (50), 149 (15), and 91 (100), *m** 172 (329 ⟶ 238) and 158 (238 ⟶ 194); τ 1·1br (1H, s, NH) 2·64 (5H, m, ArH), 4·72 (2H, s, $ArCH_2$), 5·92 (2H, q, *J* 7 Hz, $CO_2{\cdot}CH_2Me$), 6·96 and 7·52 (each 2H, each m, $CH_2{\cdot}CH_2$), 7·84 and 8·06 (each 3H, each s, ring Me), and 8·78 (3H, t, *J* 7 Hz, $CO_2{\cdot}CH_2Me$).

Benzyl 3-(2-*Ethoxycarbonylethyl*)-4-*methyl*-5-*formylpyrrole*-2-*carboxylate* (43).—The foregoing benzyl ethyl ester (4·4 g) was heated at 95—100° for 4 h in glacial acetic acid (80 ml) containing lead tetra-acetate (14·3 g). The solvent was removed, and the residue partitioned between ethyl acetate (100 ml) and saturated sodium hydrogen carbonate solution (200 ml). After separation, the aqueous layer was extracted with ethyl acetate (2 × 50 ml); the combined organic extracts were washed with water (50 ml), dried, and evaporated. The residue was recrystallised from benzene–light petroleum (b.p. 40—60°) to give the *formylpyrrole* (3·6 g), m.p. 92·5—93° (Found: C, 66·8; H, 6·3; N, 4·1. $C_{19}H_{21}NO_5$ requires C, 66·5; H, 6·2; N, 4·1%); $\lambda_{max.}$ ($CHCl_3$) 304 nm; $\nu_{max.}$ (Nujol) 3130, 1730, 1705, and 1650 cm^{-1}; *m/e* 343 (M^+, 11%), 298 (2), 252 (7), 210 (5), 207 (6), 206 (33), and 91 (100), *m** 185 (343 ⟶ 252), 171·5 (252 ⟶ 207), and 168 (252 ⟶ 206); τ 0·23 (1H, s, CHO), 0·4br (1H, s, NH), 2·62 (5H, m, ArH), 4·68 (2H, s, $PhCH_2$), 5·91 (2H, q, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$), 6·94 and 7·49 (each 2H, each m, $CH_2{\cdot}CH_2$), 7·66 (3H, s, ring Me), and 8·77 (3H, t, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$).

3-(2-*Ethoxycarbonylethyl*)-5-*formyl*-4-*methylpyrrole*-2-*carboxylic Acid* (44).—The benzyl ester (43) (0·4 g) in dry tetrahydrofuran (20 ml) containing triethylamine (1 drop) was hydrogenated over 10% palladium–charcoal (100 mg) at 20° and 760 mmHg for 1 h. The solvent was removed and the residue was recrystallised from chloroform–light petroleum (b.p. 60—80°) to give the *pyrrole acid* (267 mg), m.p. 156—157° (Found: C, 56·7; H, 6·0; N, 5·7. $C_{12}H_{15}NO_5$ requires C, 56·9; H, 6·0; N, 5·5%), $\lambda_{max.}$ 303 nm; $\nu_{max.}$ (Nujol) 3300—2400, 1730, 1705, and 1635 cm^{-1}; *m/e* 253 (M^+, 34%), 209 (23), 208 (25), 207 (23), 179 (100), 166 (44), 161 (23), and 148 (40), *m** 173 (253 ⟶ 209), 169 (253 ⟶ 207), 155 (207 ⟶ 179), and 145 (179 ⟶ 161); τ [$(CD_3)_2SO$] 0·21 (1H, s, CHO), 6·97 (2H, q, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$), 7·06 (2H, m), 7·75 (3H, s, ring Me), and 8·82 (3H, t, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$).

Ethyl 3-(2-*Ethoxycarbonylethyl*)-4-*methyl*-5-*formylpyrrole*-2-*carboxylate* (24).—A solution of the dimethylpyrrole (21a) (1·07 g) and lead tetra-acetate (4·25 g) in acetic acid (24 ml) was stirred at 80—90° for 6 h, then treated with water (0·2 ml) and, after 10 min, was evaporated. The residue was partitioned between ethyl acetate and half-saturated aqueous sodium hydrogen carbonate. The material from the organic solution was crystallised from aqueous ethanol to yield the formylpyrrole (0·89 g), m.p. 85°, identified by comparison with the material already prepared; *m/e* 281 (M^+, 75%), 236 (41), 207 (100), and 194 (68), *m** 197 (281 ⟶ 236) and 153 (281 ⟶ 207).

3,3′-*Bis*-(2-*ethoxycarbonylethyl*)-4,4′-*dimethylpyrromethane*-5,5′-*dicarboxylic Acid* (36).—The corresponding *dibenzyl ester* (35), m.p. 102—103°, was prepared by the reported method [12] and further characterised (Found: C, 69·3; H, 6·6; N, 4·3. $C_{37}H_{42}N_2O_8$ requires C, 69·2; H, 6·5; N, 4·4%), $\lambda_{max.}$ 292 nm; $\nu_{max.}$ 3415, 3330, and 1720—1675br cm^{-1}; *m/e* 642 (M^+, 6%), 551 (23), 533 (4), 460 (3), 417 (5), 252 (38), and 91 (100), *m** 514·5 (551 ⟶ 533) and 472 (642 ⟶ 551); τ 0·7—1·0br (2H, 2NH), 2·65 (10H, s, ArH), 4·73 (4H, s, $PhCH_2$), 5·95 (4H, q, *J* 7 Hz, ester CH_2), 6·03 (2H, s, bridge CH_2), 7·1—7·6 (8H, m, $CH_2{\cdot}CH_2$), 7·71 (6H, s, Me), and 8·85 (6H, t, *J* 7 Hz, ester Me).

This ester (0·5 g) in warm ethanol (100 ml) containing triethylamine (1 ml) was shaken with 10% palladised charcoal (50 mg) and hydrogen for 4 h. More triethylamine (2 ml) was added and the solution was filtered and evaporated under nitrogen. A suspension of the residue in water (4 ml) was adjusted to pH 4 with sulphur dioxide and the *pyrromethanedicarboxylic acid* was collected (290 mg), m.p. 210—211° (Found: C, 59·5; H, 6·6; N, 6·0. $C_{23}H_{30}N_2O_8$ requires C, 59·7; H, 6·5; N, 6·1%), $\lambda_{max.}$ (MeOH–Et_3N) 272 nm; $\nu_{max.}$ (Nujol) 3340 and 1720 cm^{-1}; *m/e* 462 (M^+, <1%), 418 (11), 374 (89), 273 (100), and 194 (87); τ [$(CD_3)_2SO$] 5·99 (4H, q, *J* 7 Hz, ester CH_2), 6·12 (2H, s, bridge CH_2), 7·88 (6H, s, Me), and 8·84 (6H, t, *J* 7 Hz, ester Me).

1′,8′-*Bis*(*ethoxycarbonyl*)-1,4,5,8-*tetrakis*-(2-*ethoxycarbonylethyl*)-2,3,6,7-*tetramethylbiladiene*-a,c *Dihydrobromide* (34).—The pyrromethane-5,5′-dicarboxylic acid (36) (46 mg) was treated with trifluoroacetic acid (8 ml) at 20° under nitrogen in the dark for 20 min. After the solvent had been evaporated off the formylpyrrole ester (24) (56 mg) was added, followed with vigorous stirring by hydrogen bromide in acetic acid (50% solution; 1 ml). The mixture was kept at 20° for 1 h, then diluted with ether (15 ml) and kept at 0° to give the biladiene dihydrobromide (84 mg), which was exhaustively washed with ether; *m/e* 900 (M^+ for free base, 15%), 696 (100), and 446 (>900); τ 2·55br (2H, s, $-CH=$), 4·45br (2H, s, bridge CH_2), 5·56 (4H, q, *J* 7 Hz, ring $CO_2{\cdot}CH_2Me$), 6·00 (8H, overlapping q, *J* 7 Hz, aliphatic ester CH_2), 6·9—7·8 (16H, $CH_2{\cdot}CH_2$), 7·64 (12H, s, Me), 8·55 (6H, t, *J* 7 Hz, ring $CO_2{\cdot}CH_2Me$), and 8·79 and 8·89 (each 6H, each t, each *J* 7 Hz, aliphatic ester Me).

Coproporphyrin-II *Tetraethyl Ester* (29).—The pyrromethane-5,5′-dicarboxylic acid (36) (23·1 mg) was converted as before into the bis-α-free pyrromethane and was treated immediately with the formylpyrrole acid (44) (25·3 mg) as in the preceding experiment. The hydrogen bromide and acetic acid were evaporated off and the biladiene dihydrobromide (33) became solid on trituration with ether (yield 44 mg); $\lambda_{max.}$ ($CHCl_3$) 248 (log ε 3·90), 278 (3·85), 380 (3·97), 464 (4·40), 485 (4·43), and 520 nm (4·38).

This product (20 mg) was immediately heated under reflux in ethanol (5 ml) with paraformaldehyde (3 mg) and hydrogen bromide in acetic acid (1 drop of 50% solution) for 16 h. The solvent was evaporated off and the residue was fractionated by p.l.c. on silica in ether–chloroform (1 : 1) and benzene–chloroform (1 : 1) to give coproporphyrin-II tetraethyl ester (2·3 mg), m.p. 256—259° (from ethanol–chloroform) (lit.,[23] 258°), $\lambda_{max.}$ ($CHCl_3$) 402 (log ε 5·27), 498 (4·18), 534 (4·04), 568 (3·95), and 624 nm (3·60); *m/e* 766 (M^+ 100%), 721 (11), 694 (10), 693 (10), and 679 (11).

The coproporphyrin-II ester (1 mg) was kept for 16 h at 20° in 7N-hydrochloric acid (0·5 ml) and the solution was evaporated. T.l.c. of the residue on cellulose in lutidine–ammonia [11] against standard samples * of coproporphyrin-

* See footnote p. 2926.

[23] H. Fischer and H. Orth, ' Die Chemie des Pyrrols,' vol. II, Akademische Verlag, Leipzig, 1937, p. 567.

I, -II, -III, and -IV showed our sample to be pure coproporphyrin-II.

Benzyl 4-Ethoxycarbonylmethyl-3,5-dimethylpyrrole-2-carboxylate (47).—To a stirred solution of benzyl acetoacetate (33·5 g) in acetic acid (55 ml) at 5—10° was added a solution of sodium nitrite (12·6 g) in water (45 ml). After the solution had been kept at 4° for 2 h, it was added dropwise to a stirred solution of ethyl 3-acetyl-4-oxopentanoate (33 g) in acetic acid (40 ml) while portions of a mixture of zinc powder (32·2 g) and sodium acetate (32·2 g) were added simultaneously so that the temperature was maintained at 60—80°. Finally the mixture was heated under reflux for 1 h, then poured into ice–water (1 kg); the solid was collected and dissolved in chloroform (200 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate. The material recovered from the chloroform, on crystallisation from ethanol, gave the *pyrrole ester* (17·8 g), m.p. 83—83·5° (Found: C, 68·2; H, 6·7; N, 4·3. $C_{18}H_{21}NO_4$ requires C, 68·5; H, 6·7; N, 4·4%), $\lambda_{max.}$ ($CHCl_3$) 281 nm; $\nu_{max.}$ (Nujol) 3320, 1735, and 1670 cm^{-1}; *m/e* 315 (M^+, 33%), 242 (39), 224 (1), 208 (7), 181 (7), 180 (8), 152 (7), 150 (4), and 91 (100), *m** 186 (315 ⟶ 242) and 159 (315 ⟶ 224).

Benzyl 5-Acetoxymethyl-4-ethoxycarbonylmethyl-3-methylpyrrole-2-carboxylate (48).—The foregoing pyrrole (6·3 g) was stirred at 20° in acetic acid (600 ml) with lead tetra-acetate (10·2 g) for 5 h. The solution was evaporated and the residue in warm chloroform (300 ml) was washed with an excess of saturated aqueous sodium hydrogen carbonate and then with water. Crystallisation of the product from the chloroform [from benzene–light petroleum (b.p. 60—80°)] gave the *acetoxymethylpyrrole* (6·3 g), m.p. 125·5—127°. (Found: C, 64·4; H, 6·3; N, 3·5. $C_{20}H_{23}NO_6$ requires C, 64·3; H, 6·2; N, 3·7%), $\lambda_{max.}$ ($CHCl_3$) 273 nm; $\nu_{max.}$ (Nujol) 3280, 1720, and 1660 cm^{-1}; *m/e* 373 (M^+, 100%), 314 (29), 300 (25), 240 (31), 222 (83), 127 (37), and 91 (50), *m** 263 (373 ⟶ 314) and 192 (300 ⟶ 240); τ 0·65br (1H, s, NH), 2·62 (5H, s, ArH), 4·69 (2H, s, $PhCH_2$), 4·93 (2H, s, CH_2·OAc), 5·88 (2H, q, *J* 7 Hz, ester CH_2), 6·52 (2H, s, ring CH_2), 7·69 and 7·95 (each 3H, s, ring Me, $COCH_3$), and 8·74 (3H, t, *J* 7 Hz, ester Me).

5-Benzyloxycarbonyl-3-ethoxycarbonylmethyl-4-methylpyrrole-2-carboxylic Acid (49).—Sulphuryl chloride (10·8 ml) in ether (200 ml) was added over 0·5 h to a stirred solution of the dimethylpyrrole (47) (12·6 g) in ether (400 ml) at 0° and then the mixture was kept for a further 0·5 h at 20°. The solvent was evaporated off, ether (2 × 200 ml) was added and removed each time, and the residue was heated under reflux for 4 min in water (400 ml) containing hydrated sodium acetate (40 g). After rapid cooling, the solution was extracted with ether (400 ml) and the ethereal solution was washed with saturated aqueous sodium hydrogen carbonate (3 × 100 ml). The combined aqueous washings were acidified with sulphur dioxide and extracted with ethyl acetate (3 × 100 ml) to give the *pyrrole acid* (11 g), m.p. 121·5—122° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 62·5; H, 5·6; N, 4·1. $C_{18}H_{19}NO_6$ requires C, 62·6; H, 5·6; N, 4·1%), $\lambda_{max.}$ 280 and 290sh nm; $\nu_{max.}$ (Nujol) 3240, 1725, 1705, and 1660 cm^{-1}; *m/e* 345 (M^+, 12%), 300 (2), 299 (2), 272 (6), 271 (7), 254 (3), 253 (1), 238 (2), 228 (1), 164 (4), and 91 (100), *m** 259 (345 ⟶ 299), 246 (299 ⟶ 271), 203 (253 ⟶ 228), and 187 (345 ⟶ 254); τ [$(CD_3)_2SO$] 2·6 (5H, m, ArH), 4·72 (2H, s, $PhCH_2$), 5·95 (2H, q, *J* 7 Hz, ester CH_2); 6·24 (2H, s, ring CH_2), 7·82 (3H, s, ring CH_3), and 8·81 (3H, t, *J* 7 Hz, ester Me).

Benzyl 5-Methoxy-3-methyl-7-oxopyrano[3,4-b]*pyrrole-2-carboxylate* (54).—The 3-methoxycarbonyl analogue of the foregoing acid (0·2 g), prepared in a strictly analogous way, was heated at 40° for 40 min with thionyl chloride (0·4 ml). After 1 h further at 20°, the solvent was evaporated off, benzene was then added and also evaporated off. t-Butyl alcohol (0·4 ml) and *NN*-dimethylaniline (0·33 ml) were added and after the mixture had been heated at 70° for 2 h, it was kept at 20° for 16 h. A solution of the mixture in chloroform (20 ml) was washed in turn with water, 6N-sulphuric acid, water, 1N-sodium carbonate, and water. The product from the organic solution crystallised from benzene–light petroleum (b.p. 60—80°) to yield the *pyranopyrrole* (110 mg), m.p. 173—174° (Found: C, 65·5; H, 4·9 N, 4·3. $C_{17}H_{15}NO_5$ requires C, 65·2; H, 4·9; N, 4·5%), $\lambda_{max.}$ ($CHCl_3$) 258, 284, 294, and 346 nm; $\nu_{max.}$ 3410, 1775—1705, and 1620 cm^{-1}; *m/e* 313 (M^+, 100%), 285 (1), 252 (5), 222 (4), 205 (55), 190 (8), 162 (8), 121 (11), and 91 (92), *m** 260 (313 ⟶ 285); τ 0·5br (1H, s, NH), 2·63 (5H, m, ArH), 4·50 (1H, s, ring CH), 4·66 (2H, s, $PhCH_2$), 6·16 (3H, s, OMe), and 7·64 (3H, s, ring Me).

t-Butyl 5-Benzyloxycarbonyl-3-ethoxycarbonylmethyl-4-methylpyrrole-2-carboxylate (50).—The pyrrolecarboxylic acid (49) (8 g) was heated at 40° with thionyl chloride (16 ml) for 40 min, and after 1 h at 20° the solution was evaporated. Dry benzene was added and evaporated off and after addition of t-butyl alcohol (16 ml) and *NN*-dimethylaniline (13 ml) the mixture was heated at 80° for 16 h. Chloroform (200 ml) was added and the solution was washed successively with water, 6N-sulphuric acid, water, 1N-sodium carbonate, and water. The product from the chloroform was chromatographed on alumina in 1 : 1 benzene–chloroform to yield the *pyrrole t-butyl ester* (6·1 g), m.p. 67—68° [from light petroleum (b.p. 60—80°)] (Found: C, 65·8; H, 6·7; N, 3·4. $C_{22}H_{27}NO_6$ requires C, 65·8; H, 6·8; N, 3·5%), $\lambda_{max.}$ ($CHCl_3$) 284 and 294sh nm; $\nu_{max.}$ (film) 3440, 3280, and 1730—1680 cm^{-1}; *m/e* 401 (M^+, 7%), 345 (13), 300 (6), 271 (14), 254 (6), 164 (10), and 91 (100), *m** 297 (401 ⟶ 345), 260 (345 ⟶ 300), and 246 (300 ⟶ 271).

4-Ethoxycarbonylmethyl-3-methyl-5-t-butoxycarbonylpyrrole-2-carboxylic Acid (51).—The foregoing pyrrole ester (5 g) in tetrahydrofuran (80 ml) containing triethylamine (4 drops) was shaken with 10% palladised charcoal and hydrogen at 20° and 760 mmHg. After uptake was complete (1 h), the catalyst was removed and the *product* (3·9 g) from the solution was recrystallised from benzene–light petroleum (b.p. 60—80°); m.p. 132·5—133° (Found: C, 58·0; H, 6·8; N, 4·4. $C_{15}H_{21}NO_6$ requires C, 57·9; H, 6·8; N, 4·5%), $\lambda_{max.}$ (EtOH) 282 and 290sh nm; $\nu_{max.}$ (Nujol) 3510, 3460, 3320, 3400—2220, 1740, 1720, and 1690—1665 cm^{-1}; *m/e* 311 (M^+, 17%), 255 (63), 238 (6), 210 (27), 209 (29), 182 (52), 181 (100), and 164 (55), *m** 209 (311 ⟶ 255), 182 (311 ⟶ 238), 171 (255 ⟶ 209), 157 (209 ⟶ 181), and 148 (181 ⟶ 164).

t-Butyl 3-Ethoxycarbonylmethyl-5-iodo-4-methylpyrrole-2-carboxylate (52).—Iodine (2·97 g) in ethanol (48 ml) was added at 60° under nitrogen over 1 h to the foregoing pyrrole-2-carboxylic acid (3·6 g) in water (60 ml) and ethanol (12 ml) containing potassium hydrogen carbonate (2·37 g). The mixture was stirred for a further 1·75 h at 60° before water (60 ml) was added dropwise with cooling. After 2 h at 0°, the *pyrrole* was collected; yield 3·85 g, m.p. 90—91° (from aqueous ethanol) (Found: C, 42·9; H, 5·1; N, 3·5 $C_{14}H_{20}INO_4$ requires C, 42·8; H, 5·1; N, 3·6%), $\lambda_{max.}$ ($CHCl_3$) 282 nm; $\nu_{max.}$ (Nujol) 3280, 1730, and 1660 cm^{-1};

m/e 391 (M^+, 17%), 336 (100), 335 (52), 291 (23), 290 (35), 264 (48), 263 (51), 246 (37), and 202 (23), *m** 288 (391 → 336 or 335); τ 0·9br (1H, s, NH), 5·87 (2H, q, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$), 6·20 (2H, s, ring CH_2), 8·02 (3H, s, ring Me), 8·44 (9H, s, Bu^t), and 8·75 (3H, t, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$).

5-Benzyl 5'-t-Butyl 3,4'-Bisethoxycarbonylmethyl-3',4-dimethylpyrromethane-5,5'-dicarboxylate (56).—The iodopyrrole (52) (0·6 g) was hydrogenated in tetrahydrofuran (30 ml) over 10% palladised charcoal (0·6 g) and magnesium oxide (0·6 g) for 24 h at 20° and 760 mmHg. The solution was filtered and evaporated, and the residue in ether (60 ml) was washed with water (3 × 25 ml). The product (420 mg) from the ether was used directly but was characterised spectroscopically; $\lambda_{max.}$ ($CHCl_3$) 273 nm; $\nu_{max.}$ (film) 3290, 1765, 1725, and 1680 cm^{-1}; *m/e* 267 (M^+, 18%), 211 (75), 194 (7), 166 (38), 165 (49), 138 (100), 137 (95), and 120 (59), *m** 177 (211 → 194), 167 (267 → 211), 129 (211 → 165), 114 (165 → 137), 105 (137 → 120), and 104 (138 → 120); τ 1·0br (1H, s, NH), 3·36 (1H, d, *J* 2 Hz, ring H), 5·86 (2H, q, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$), 6·21 (2H, s, ring CH_2), 7·97 (3H, s, ring Me), 8·44 (9H, s, Bu^t), and 8·74 (3H, t, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$).

The α-free pyrrole was heated at reflux for 1·5 h under nitrogen in acetic acid (15 ml) containing anhydrous sodium acetate (450 mg) and the acetoxymethylpyrrole (48) (373 mg). Chloroform (60 ml) was added and the solution was washed with water, an excess of saturated aqueous sodium hydrogen carbonate, and water. The oil from the chloroform was triturated first with light petroleum and then with 5% ether in light petroleum (b.p. 60—80°) and the *solid* (420 mg) was recrystallised from benzene–light petroleum (60—80°); m.p. 129° (Found: C, 66·2; H, 6·9; N, 4·6. $C_{32}H_{40}N_2O_8$ requires C, 66·2; H, 6·9; N, 4·8%), $\lambda_{max.}$ ($CHCl_3$) 275 and 285 nm; $\nu_{max.}$ (Nujol) 3295, 3340, 1745, 1720, 1685, and 1660 cm^{-1}; *m/e* 580 (M^+, 1%), 524 (1), 489 (1), 480 (1), 433 (2), 373 (28), 314 (9), 300 (7), 240 (11), 222 (24), 164 (9), and 91 (100), *m** 474 (580 → 524), 440 (524 → 480), 413 (580 → 489), 398 (500 → 480), and 263 (373 → 314); τ 0·7br and 1·2br (each 1H, s, NH), 2·65 (5H, m, ArH), 4·73 (2H, s, $PhCH_2$), 5·84 and 5·86 (each 2H, q, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$), 6·15 and 6·21 (each 2H, s, ring CH_2), 6·60 (2H, s, bridge CH_2), 7·70 and 8·00 (each 3H, s, ring Me), 8·47 (9H, s, Bu^t), and 8·74 (6H, t, *J* 7 Hz, 2 × $CO_2{\cdot}CH_2{\cdot}CH_3$).

5-Benzyl 5'-t-Butyl 3,4'-Bis-(2-hydroxyethyl)-3',4-dimethylpyrromethane-5,5'-dicarboxylate (57).—The foregoing pyrromethane (0·7 g) in tetrahydrofuran (10 ml) was reduced with diborane [generated externally over 3 h by adding boron trifluoride–ether complex (5·08 ml) dropwise to sodium borohydride (1 g) in bis-(2-methoxyethyl) ether (4 ml), and carried over by a slow stream of nitrogen]. The mixture was stirred for a further 1 h, then treated under nitrogen with methanol and evaporated to yield the *pyrromethane* as an amorphous solid (Found: M^+, 496·2580. $C_{28}H_{36}N_2O_6$ requires *M*, 496·2572), $\lambda_{max.}$ ($CHCl_3$) 278 and 289 nm; $\nu_{max.}$ 3600—3150, 3440, 1770, and 1675 cm^{-1}; *m/e* 496 (M^+, 10%); 462 (2), 440 (1), 349 (11), 283 (21), 164 (18), 146 (90), 137 (26), and 91 (100); τ ($CDCl_3$, $CDCl_3$–D_2O) 0·3 and 1·2br (each 1H, s, NH), 2·68 (5H, m, ArH), 4·76 (2H, s, $PhCH_2$), 6·21 (2H, s, bridge CH_2), 6·28 and 7·07 (each 4H, m, $CH_2{\cdot}CH_2$), 7·73 and 8·00 (each 3H, s, ring Me), 8·50 (9H, s, Bu^t), and 7·85br (2H, s, OH).

5-Benzyl 5'-t-Butyl 3,4'-Bis-(2-acetoxyethyl)-3',4-dimethylpyrromethane-5,5'-dicarboxylate (58).—The foregoing diol was treated at 20° with acetic anhydride (2 ml) in pyridine (10 ml) under dry nitrogen for 2 h, then poured into iced water (100 ml) and shaken with chloroform (3 × 30 ml). The combined solution was washed with N-hydrochloric acid (50 ml), water, saturated aqueous sodium hydrogen carbonate, and water. Chromatography of the product from the chloroform on silica in 1 : 1 ether–chloroform gave the *pyrromethane* (0·6 g) as an amorphous solid (Found: M^+, 580·2756. $C_{32}H_{40}N_2O_8$ requires *M*, 580·2782), $\lambda_{max.}$ ($CHCl_3$) 275 and 287 nm; $\nu_{max.}$ (film) 3340 and 1745—1660 cm^{-1}; *m/e* 580 (M^+, 11%), 546 (3), 524 (7), 434 (20), 390 (13), 374 (13), 255 (16), 179 (13), 164 (17), and 91 (100), *m** 473 (580 → 524) and 321 (434 → 374); τ 0·9br and 1·1br (each 1H, s, NH), 2·69 (5H, m, ArH), 4·76 (2H, s, $PhCH_2$), 5·86, 5·98, 7·00, and 7·28 (each 2H, t, *J* 7 Hz, $CH_2{\cdot}CH_2$), 6·16 (2H, s, bridge CH_2), 7·72 (3H, s, ring Me), 8·00 (9H, s, ring Me, OAc), and 8·49 (9H, s, Bu^t).

3,4'-Bis-(2-acetoxyethyl)-3',4-dimethyl-5'-t-butoxycarbonylpyrromethane-5-carboxylic Acid (59).—A solution of the foregoing pyrromethane (570 mg) in tetrahydrofuran (15 ml) containing triethylamine (1 drop) was hydrogenated over 10% palladium–charcoal (0·3 g) at 20° and 760 mmHg. After uptake ceased (2 h) the solution was filtered and evaporated and the residue was triturated with light petroleum to give the *pyrromethane-5-carboxylic acid* (420 mg), m.p. 146—149° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 61·2; H, 7·1; N, 5·5. $C_{25}H_{34}N_2O_8$ requires C, 61·2; H, 7·0; N, 5·7%), $\lambda_{max.}$ (MeOH) 275 and 289 nm; $\nu_{max.}$ (Nujol) 3320, 3225, 3350—2500, 1745, and 1670 cm^{-1}; *m/e* 490 (M^+, 1%), 446 (75), 390 (56), 389 (50), 345 (25), 330 (44), 315 (25), 303 (19), 283 (38), and 146 (100), *m** 341 (446 → 390), 339 (446 → 389), and 279 (390 → 330); τ [$(CD_3)_2SO$] 5·98, 6·14, 7·12, and 7·32 (each 2H, m, $CH_2{\cdot}CH_2$), 6·23 (2H, s, bridge CH_2), 7·81 (3H, s, ring Me), 8·04 (9H, s, ring Me, OAc), and 8·47 (9H, s, Bu^t).

2,4-Bis-(2-hydroxyethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphin (7a).—The pyrromethane (59) (107 mg) was kept at 20° in trifluoroacetic acid (16 ml) for 15 min under nitrogen and protected against light. The solvent was evaporated off and the 2-formylpyrrole (44) (101 mg) was added, followed by hydrogen bromide in acetic acid (4 ml; 50% solution). The mixture was swirled vigorously and then kept under nitrogen in darkness for 30 min, before evaporation. The residue was triturated with ether (3 × 2 ml) to give the biladiene (31) (190 mg). This was treated directly with paraformaldehyde (27 mg) in ethanol (40 ml) containing hydrogen bromide [1 drop of 50% solution in acetic acid, in ethanol (100 ml)]. After the solution had been heated under reflux for 8 h, it was evaporated, and p.l.c. of the residue on silica with 5% methanol in chloroform gave three porphyrin bands. These were combined and treated with 5% (w/v) sulphuric acid in methanol (40 ml) overnight at 20°. Isolation of the bishydroxyethylporphin (10·8 mg) was performed as earlier; m.p. 224—225·5°, $\lambda_{max.}$ ($CHCl_3$) 403 (log ε 5·12), 499 (4·11), 535 (3·95), 570 (3·60), and 624 nm (3·38); *m/e* 626 (M^+, base peak).

[γ-^{13}C]*Protoporphyrin-IX Dimethyl Ester* (1).—The foregoing bishydroxyethylporphin (10 mg) was converted as before into the bischloroethylporphin (8a) (8 mg), $\lambda_{max.}$ ($CHCl_3$) 403, 499, 534, 570, and 625 nm; *m/e* 664 (70), 663 (54), 662 (M^+, 100%), 628 (32), 627 (19), 626 (22), 613 (12), and 589 (15), identical with previous samples. It was dehydrochlorinated as earlier to yield protoporphyrin-IX dimethyl ester (5·4 mg), m.p. 215—217° (Found: C, 72·9; H, 6·5; N, 9·6. Calc. for $C_{36}H_{38}N_4O_4$: C, 73·2; H, 6·5;

N, 9·5%), $\lambda_{max.}$ ($CHCl_3$) 408 (log ε 5·22), 507 (4·24), 540 (4·12), 577 (3·93), and 631 nm (3·82); *m/e* 590 (M^+, 100%) and 517 (15).

Repetition of the foregoing sequence with [^{13}C]paraformaldehyde gave [γ-^{13}C]protoporphyrin-IX dimethyl ester (50% enrichment by mass spectrometry). Part (3·5 mg) was diluted with protoporphyrin-IX dimethyl ester (23 mg) from natural sources and, after chromatography on alumina and recrystallisation twice from chloroform–methanol, was used for measurement of the ^{13}C spectrum.

8,13-*Bis*-(2-*hydroxyethyl*)-2,18-*bis*-(2-*methoxycarbonylethyl*)-3,7,12,17-*tetramethylcorrole* (61).—A solution of the pyrrole-2-carboxylic acid (44) (0·1 g) in water (1·5 ml) containing potassium hydrogen carbonate (82 mg) was stirred under nitrogen at 60° as iodine (107 mg) in ethanol (1·5 ml) was added over 20 min. After a further 20 min, the cooled mixture was poured on ice (20 g) and the organic material was extracted into benzene. The benzene solution was washed with M-sodium thiosulphate, then water, and the product recovered from it was chromatographed on alumina in 1 : 1 ether–chloroform to give the iodopyrrole (45) (62 mg), $\lambda_{max.}$ ($CHCl_3$) 317 nm; *m/e* 335 (M^+, <1%), 208 (49), and 122 (100); τ 0·59 (1H, s, CHO), 5·86 (2H, q, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$), 7·1—7·7 (4H, m, $CH_2{\cdot}CH_2$), 7·68 (3H, s, Me), and 8·76 (3H, t, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$).

All the foregoing product in tetrahydrofuran (4 ml) was shaken with hydrogen, palladised charcoal (62 mg), and magnesium oxide (62 mg) at 20° and 760 mmHg for 24 h. The solution was filtered and evaporated. Chromatography of the residue on alumina in 1 : 1 ether–chloroform gave the α-free pyrrole (46) (31 mg). This was immediately treated with the pyrromethane (60) exactly as for the preparation of the biladiene (31), and this in turn was treated with paraformaldehyde as for the synthesis of the bishydroxyethylporphin (7a). Chromatography of the final product, after methanolysis, gave the porphin (7a) in yield similar to that obtained before, together with the *corrole* as a slower-running blue product (Found: M^+, 614·3114. $C_{35}H_{42}N_4O_6$ requires *M*, 614·3102), $\lambda_{max.}$ ($CHCl_3$) 399 (log ε 5·04), 409 (4·97), 541 (4·15), 556 (4·15), and 595 nm (4·17).

Diacetyldeuteroporphyrin-IX Dimethyl Ester (63.)—The following preparation of [δ-^{13}C] material is typical. A solution of [δ-^{13}C]-protoporphyrin-IX dimethyl ester (99·7 mg) in glacial acetic acid–hydrogen bromide (4 ml of 50% w/v solution) was stirred at 20° for 29 h, then poured into water (75 ml) and kept under nitrogen at 0° for 12 h. The solution was treated with an excess of saturated aqueous sodium acetate and extracted with ether and the ethereal solution was washed with water. The residue from evaporation, in methanol (5 ml), was treated with ether (20 ml) and then with an excess of ethereal diazomethane for 1 h at 0°. Chromatography of the product on alumina (6 g) with 4 : 1, and then 3 : 1 benzene–chloroform removed less polar products; chloroform eluted [δ-^{13}C]haematoporphyrin-IX dimethyl ester (74% by u.v.).

A solution of this porphyrin in acetone (80 ml) was stirred by rapid passage of nitrogen and treated with Jones reagent (25 drops, *ca.* 1 ml). The reaction was quenched after exactly 1 min by addition with shaking of ether (160 ml) and saturated aqueous sodium acetate (20 ml). Water was added and the organic layer was washed twice with water. After back-extraction of the aqueous solutions with chloroform, the combined ethereal and chloroformic solutions were evaporated. Chromatography of the residue on alumina in 3 : 1 benzene–chloroform gave 2,4-diacetyl-[δ-^{13}C]deuteroporphyrin-IX dimethyl ester, which crystallised from chloroform–methanol (yield 41 mg). More of this product (4·4 mg) was obtained by repeating the oxidation on material from the mother liquors and other chromatographic fractions; $\lambda_{max.}$ ($CHCl_3$) 424 (log ε 5·15), 517 (4·11), 550 (3·85), 588 (3·77), and 640 nm (3·33); $\nu_{max.}$ 1732 and 1658 cm^{-1}; *m/e* 622 (M^+, 7%), 549 (2), 488 (4), 484 (2) 252 (19), 224 (25), and 218 (100); τ (0·03M) −0·17, −0·01, 0·72, and 1·01 (each 1H, s, *meso*-H), 6·0 and 6·9 (4H each, m, $CH_2{\cdot}CH_2$), 6·39, 6·41, 6·58, 6·62, 6·77, 6·86, 6·90, and 7·08 (each 3H, s, 4 × ring Me, 2 × COMe, 2 × CO_2Me); the spectrum was very dependent on the concentration and at 0·008M showed −0·54, −0·46, 0·36, and 0·41 (each 1H), 5·8 and 6·8 (each 4H), 6·37 (9H), and 6·26, 6·50, 6·60, 6·75, and 6·82 (each 3H).

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