Biosynthesis of Porphyrins and Related Macrocycles. Part VII.[1] Synthesis of Specifically Labelled [$^{14}C_1$]Uroporphyrin-III and of [10,14-$^{13}C_2$]-Uroporphyrin-III. Conversion of the Latter into [10,14-$^{13}C_2$]Protoporphyrin-IX; Biosynthetic Significance of its ^{13}C Nuclear Magnetic Resonance Spectrum

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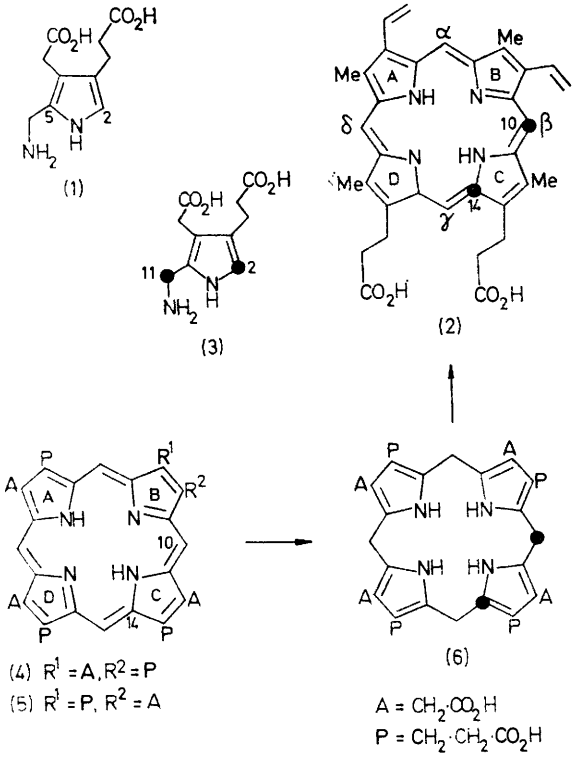
Uroporphyrin-III (4) has been synthesised by a route which allows [2,11-$^{13}C_2$]porphobilinogen (3) to be elaborated to give [10,14-$^{13}C_2$]uroporphyrin-III (4). This, after reduction to the corresponding porphyrinogen, has been transformed enzymically into [10,14-$^{13}C_2$]protoporphyrin-IX (2). The ^{13}C n.m.r. signal from position 10 (β-*meso*-carbon) of the methyl esters of both porphyrins (2) and (4) appears as a 5.5 Hz doublet. This demonstration of long-range coupling is of decisive importance for related research on the nature of the rearrangement process by which the natural type III porphyrins are biosynthesised.

A new procedure has been developed for pyrromethane formation, based on catalysis by tin(IV) chloride, which appears to be of general value.

RESEARCH described in the preceding paper established the nature of the enzymic rearrangement which occurs as the type III porphyrins are biosynthesised from porphobilinogen (1). The characteristics of this process were



shown to be that (*a*) the porphobilinogen unit which provides ring D of protoporphyrin-IX (2) undergoes rearrangement which is intramolecular with respect to that porphobilinogen unit; (*b*) what was originally the aminomethylene carbon atom of this rearranging unit migrates from its initial pyrrolic α-position (position 5) to the other α-position (position 2); (*c*) the porphobilinogen units which provide rings A, B, and C together with the connecting *meso*-bridge carbon atoms are incorporated *intact*. This last statement depends on producing a rigorous proof that the 5.5 Hz splitting observed [1] for the ^{13}C n.m.r. signal (for example from the β-*meso*-carbon of the biosynthetic protoporphyrin-IX) is correctly interpreted as arising from long-range coupling through three bonds, *i.e.* from two ^{13}C labels set *in the same molecule* as in structure (2). Synthetic work reported here provides the necessary proof by affording a sample of protoporphyrin-IX in which the majority of molecules unambigously carry two ^{13}C atoms so disposed [see (2)].

The synthetic strategy was dictated by the ready availability of [2,11-$^{13}C_2$]porphobilinogen [1] (3), which contrasts with the impracticability of synthetic routes to other doubly ^{13}C-labelled pyrrolic starting materials. Starting from the labelled porphobilinogen (3), the initial target must be labelled uroporphyrin-III (4) from which protoporphyrin-IX (2) can be derived by reduction to uroporphyrinogen-III (6) followed by enzymic transformation. It is established [2,3] that no biochemical scrambling of rings and bridges of the macrocycle (6) occurs during these enzymic steps.

Uroporphyrin-III (4) was first synthesised by MacDonald's group [4] by their important method of condensing a bis-α-free pyrromethane with a diformylpyrromethane. The porphyrin-forming step in the present work is essentially the same as MacDonald's and involves the pyrromethanes (7) and (8), though our syntheses of these units differ from the earlier work, especially for the pyrromethane (8). This synthetic plan was selected to

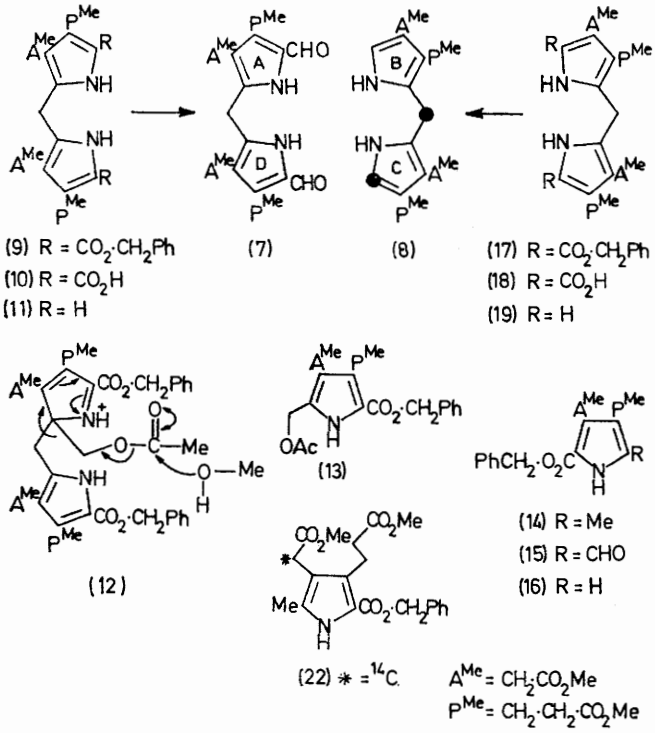
[1] Part VI, A. R. Battersby, G. L. Hodgson, E. Hunt, E. McDonald, and J. Saunders, preceding paper.

[2] B. Franck, D. Gantz, and F. Hüper, *Angew. Chem. Internat. Edn.*, 1972, **11**, 420.

[3] A. R. Battersby, J. Staunton, and R. H. Wightman, *J.C.S. Chem. Comm.*, 1972, 1118; A. R. Battersby, E. McDonald, J. Staunton, J. R. Redfern, and R. H. Wightman, *J.C.S. Perkin I*, 1976, 266.

[4] E. J. Tarlton, S. F. MacDonald, and E. Baltazzi, *J. Amer. Chem. Soc.*, 1960, **82**, 4389.

allow specific labelling around ring c by building the labels into the unsymmetrical unit (8). This then settled the formylation step, which should be of the less precious unlabelled unit to give the dialdehyde (7). Franck and



his co-workers [2] have used a similar approach to uroporphyrin-III, the labels in their case being in the formyl groups of the symmetrical pyrromethane.

Symmetrical pyrromethane dicarboxylic esters [*e.g.* (9)] are often prepared from an acetoxymethylpyrrole [*e.g.* (13)] by treatment with acid [5] but the action of methanolic hydrogen chloride on the pyrrole (13) gave the pyrromethane (9) in only 31% yield. However, a new procedure in which the pyrrole (13) was treated with tin(IV) chloride at 0 °C in methylene chloride containing a trace of methanol afforded the pyrromethane (9) in 80% yield. A rationalisation of the beneficial effect of methanol is illustrated (12). We have used this method here and in other studies to synthesise a range of symmetrical and unsymmetrical pyrromethanes and it appears to be generally useful. In addition, treatment of the bromomethylpyrrole (13; Br in place of OAc) with silver perchlorate in undried ethyl acetate gave a high yield of the symmetrical pyrromethane (9) and we have prepared similar systems in this way. An example of the use of silver ion for the synthesis of unsymmetrical pyrromethanes is given in the Experimental section. For all cases studied so far, the yields have been in excess of 80%. However, the ease of preparation in high yield and relative stability of the acetoxymethylpyrroles make the tin(IV) chloride procedure the current method of choice.

The ester (9) was catalytically debenzylated and the resultant dicarboxylic acid (10) was thermally decarboxylated in dimethylformamide. Formylation of the product (11) *in situ* by using benzoyl chloride [6] gave the diformylpyrromethane (7) in 43% overall yield from the ester (9).

The α-free pyrrole (16) required for the BC component (8) was prepared by standard conversion of the 5-methylpyrrole (14) into the 5-formylpyrrole (15), which was deformylated by tristriphenylphosphinerhodium chloride [7,8] in boiling toluene to give pyrrole (16) in 82% yield. Improved methods for the synthesis of the pyrroles (14) and (22; unlabelled) and some miscellaneous pyrrole derivatives are described in the Experimental section.

The reaction between the pyrroles (13) and (16) in refluxing acetic acid was slow but catalysis by tin(IV) chloride in methylene chloride afforded the pyrromethane (17) in 81% yield; the conditions were designed to avoid self-condensation of the acetoxymethylpyrrole (13). The ester (17) was hydrogenolysed, and decarboxylation of the resultant acid (18) to the BC precursor (19) was achieved at reflux in acetic acid for 12 min (75% isolated yield); other methods for decarboxylation, *e.g.* trifluoroacetic acid at 20 °C, proved unsatisfactory in this case. In preparative runs, the pyrromethane (19) [or (8) in ^{13}C series] was not isolated, but the total product was condensed with the diformylpyrromethane (7) to give, after oxidation in air and esterification, uroporphyrin-III (4) as its octamethyl ester in good yield.

The isomeric purity of the product was checked by acid-catalysed decarboxylation [9] of the derived uroporphyrin to yield the corresponding coproporphyrin isomer(s), isolated as the tetramethyl ester(s) [(20) and (21), respectively, show coproporphyrin-III and coproporphyrin-II]. Chromatographic methods were available [10] to separate coproporphyrins-I and -II from each other and from the mixture of coprophyrins-III and -IV; no separation of the latter pair had been carried out previously. Recently, the separation of all four coproporphyrin isomers (as their tetramethyl esters) has been achieved in Cambridge by high-pressure liquid chromatography (h.p.l.c.).[11] Analysis of the coproporphyrins derived from the above synthetic uroporphyrin showed that the original synthetic product was mainly the required type III isomer (4) together with *ca.* 15% of the type II isomer (5). Current studies aim to pinpoint the

[5] For discussion and leading references on pyrromethane formation see J. A. S. Cavaleiro, A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *J.C.S. Perkin I*, 1973, 2471.

[6] R. Chong, P. S. Clezy, A. J. Liepa, and A. W. Nichol, *Austral. J. Chem.*, 1969, **22**, 229.

[7] J. Tsuji and K. Olmo, *Tetrahedron Letters*, 1965, 3969.

[8] A related decarbonylation of azaindole aldehydes occurs at 300 °C in decalin with the rhodium catalyst: B. Frydman, S. J. Reil, J. Boned, and H. Rapoport, *J. Org. Chem.*, 1968, **33**, 3762.

[9] J. E. Falk, 'Porphyrins and Metalloporphyrins,' Elsevier, Amsterdam, 1964, p. 147.

[10] Ref. 9, p. 189.

[11] G. L. Hodgson and R. E. Markwell, unpublished work, Cambridge.

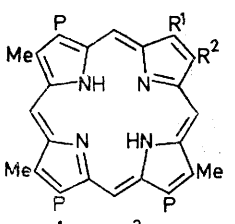
source of the uroporphyrin-II from which this coproporphyrin-II is derived. However, by modifying the conditions of the decarboxylation and condensation steps, pure uroporphyrin-III (4) octamethyl ester was obtained in 60—70% yield.

With an efficient route to uroporphyrin-III octamethyl ester available, which was suitable for specific labelling, we first synthesised a sample labelled at the methylene group of the acetic acid residue on ring C. This was required for biosynthetic studies on vitamin B_{12}. The ^{14}C-labelled pyrrole (22) was available from earlier work [12] and it was converted by lead tetra-acetate into the labelled form of the acetoxymethyl derivative (13) which was built specifically into ring C of uroporphyrin-III by application of the foregoing synthesis.

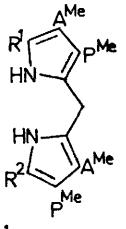
Attention turned next to the preparation of the doubly ^{13}C-labelled pyrromethane (8) from [2,11-$^{13}C_2$]porphobilinogen (3). It was necessary first to block the reactive 2-position of (3) with an electron-withdrawing substituent, and to allow this the amine and carboxylic acid functionalities were protected as the t-butoxycarbonyl [13] and methyl ester derivatives, respectively, to provide the urethane (23). This was converted (*cf.* ref. 4) into the 2-iodopyrrole (24), but removal of the *N*-protecting group with cold trifluoroacetic acid resulted in extensive decomposition and formation of porphyrins. Evidently the iodo-substituent is not sufficiently electron-withdrawing and the 2-bromo-derivative (25) suffered from the same drawback. However, the 2-formylpyrrole * (26) prepared from the urethane (23) in the standard way [6] proved to be ideal, and treatment of it with trifluoroacetic acid gave the salt (27). Diazotisation in acetic acid with sodium nitrite [13] provided a suitable ring C precursor (28) in admixture with a little hydroxymethyl derivative (29), which together reacted smoothly with the α-free pyrrole [catalysed by tin(IV) chloride]. The desired reaction to yield the pyrromethane (30) occurred without significant competition from pyrromethene formation.

The pyrromethane (30) was decarbonylated with the rhodium catalyst as above and the resultant monobenzyl ester (31) was hydrogenolysed to yield the monocarboxylic acid (32). Brief treatment with hot acetic acid effected decarboxylation and, as before, the total product (19) [or (8) in the labelled series] was immediately condensed with the dialdehyde (7) to provide, after esterification of a little (*ca.* 10%) partially hydrolysed porphyrin, uroporphyrin-III (4) octamethyl ester in 65% yield uncontaminated by isomers. In the labelled series, the majority of molecules in this product carried two ^{13}C labels, at C-10 (β-*meso*) and C-14 [see (4)]. This labelled material was hydrolysed to [10,14-$^{13}C_2$]uroporphyrin-III (4), which was reduced with sodium amalgam to the corresponding uroporphyrinogen-III (6). The latter was converted by the large scale enzyme preparation from *Euglena gracilis* [15] into [10,14-$^{13}C_2$]-protoporphyrin-IX (2), isolated as its dimethyl ester.

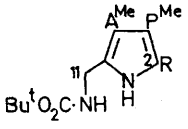
The proton decoupled ^{13}C n.m.r. spectra of the [10,14-$^{13}C_2$]uroporphyrin-III (4) octamethyl ester and of the foregoing labelled protoporphyrin-IX (2) dimethyl ester each showed a strong doublet signal, J 5.5 Hz, centred respectively at δ 98.0 and 97.4 p.p.m. downfield from tetramethylsilane. These signals correspond in



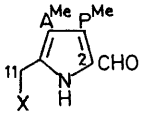
(20) R^1 = Me, R^2 = P
(21) R^1 = P, R^2 = Me



(30) R^1 = CO_2·CH_2Ph, R^2 = CHO
(31) R^1 = CO_2·CH_2Ph, R^2 = H
(32 R^1 = CO_2H, R^2 = H
(33) R^1 = R^2 = CHO

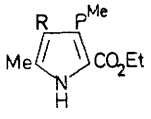


(23) R = H
(24) R = I
(25) R = Br

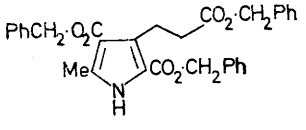


(26) X = NH·CO_2Bu^t
(27) X = $\overset{+}{N}H_3$ CF_3·CO_2^-
(28) X = OAc
(29) X = OH

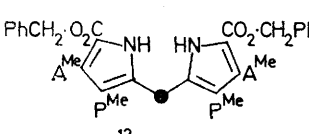
P = CH_2·CH_2·CO_2H A^{Me} = CH_2·CO_2Me P^{Me} = CH_2·CH_2·CO_2Me



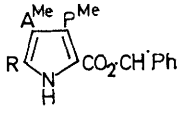
(34) R = CO_2H
(35) R = CHO
(36) R = CO_2Et



(37)



(41) ● = ^{12}C
(42) ● = ^{13}C



(38) R = Me
(39) R = CHO
(40) R = H

*Attempts to oxidise the aldehyde to the corresponding acid uniformly failed; permanganate, silver oxide, silver oxide–sodium cyanide,[14] chromium trioxide–pyridine, and dichromate in acetic acid were tested.

[12] A. R. Battersby, D. A. Evans, K. H. Gibson, E. McDonald, and L. Nixon, *J.C.S. Perkin I*, 1973, 1546.

[13] (*a*) E. Schnabel, *Annalen*, 1967, **702**, 188; (*b*) B. Frydman, S. Reil, A. Valasinas, R. Frydman, and H. Rapoport, *J. Amer. Chem. Soc.*, 1971, **93**, 2738.

[14] E. J. Corey, N. W. Gilman, and B. E. Ganem, *J. Amer. Chem. Soc.*, 1968, **90**, 6516.

[15] For small scale see E. F. Carell and J. S. Kahn, *Arch. Biochem. Biophys.*, 1964, **108**, 1.

each case to the β-*meso*-carbon atom * (position 10). Our original interpretation [1,18] of the 5.5 Hz coupling as being a long-range one between carbon-13 atoms set as in structure (2) is thus confirmed. The deduction that the porphobilinogen units which form rings A, B, and C of protoporphyrin-IX (2) are built in intact in the living system is thereby secure.

EXPERIMENTAL

For general directions, see refs. 12 and 19. ^{13}C N.m.r. spectra were measured for solutions in $CDCl_3$, unless otherwise stated, under conditions described in ref. 1.

Benzyl 5-Acetoxymethyl-2,3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate (13).—Benzyl 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (1 g) was stirred at 20 °C for 6 h with lead tetra-acetate (1.38 g) in acetic acid (80 ml) with exclusion of moisture. The residue from evaporation was partitioned between chloroform (100 ml) and an excess of aqueous 10% sodium hydrogen carbonate and the aqueous layer was extracted with fresh chloroform (4 × 20 ml). The *product* from the combined chloroform extracts was recrystallised from benzene–light petroleum (b.p. 60—80°); yield 1.06 g (92%); m.p. 107—108° (Found: C, 61.6; H, 5.9; N, 3.3. $C_{22}H_{25}NO_8$ requires C, 61.3; H, 5.8; N, 3.3%); $\lambda_{max.}$ ($CHCl_3$) 276 nm; $\nu_{max.}$ (Nujol) 3 295, 1 740, and 1 670 cm^{-1}; *m/e* 431 (M^+, 10%), 400 (3), 372 (12), 371 (9), 340 (3), 298 (9), 280 (27), 266 (14), 108 (18), 107 (13), and 91 (100), *m** 320 (431 ⟶ 372) and 268 (431 ⟶ 340); τ 0.65br (1 H, s, NH), 2.68 (5 H, m, ArH), 4.74 (2 H, s, PhCH_2), 4.98 (2 H, s, ring CH_2O), 6.37 and 6.43 (each 3 H, s, OMe), 6.49 (2 H, s, CH_2·CO_2Me) 6.99 and 7.49 (each 2 H, m, CH_2·CH_2), and 7.99 (3 H, s, COMe).

The starting material for the foregoing synthesis was prepared (with G. L. Hodgson and J. B. Paine, tert.) by the following improved route. Ethyl 3-(2-ethoxycarbonylethyl)-4-ethoxycarbonylmethyl-5-methylpyrrole-2-carboxylate [12] (710 mg) was heated with anhydrous benzyl alcohol (8 ml) in which sodium (8 mg) had previously been dissolved. The temperature was raised to *ca.* 200 °C over 1 h with stirring; when 195—200 °C was reached the solution began to darken. It was immediately cooled to *ca.* 100 °C and glacial acetic acid (0.1 ml), methanol (15 ml). and water (10 ml) were added in that sequence. After 2 h at 20 °C, the corresponding tribenzyl ester was collected, recrystallised from aqueous methanol (1.02 g, 93%), m.p. 106—107°, and identified with authentic material.[12] Part (250 mg) was stirred with methanol (15 ml), ether (20 ml), and acetyl chloride (1 ml) until fully dissolved (1 h); the solution was kept at 20 °C for 20 h and then evaporated. The residue in ether (25 ml) was stirred with sodium hydrogen carbonate (1 g) until neutral; the suspension was filtered and the product from the ether was crystallised from 3 : 7 water–methanol to give the dimethyl monobenzyl ester [12] (159 mg, 90%), m.p. 76—78°.

* The natural abundance ^{13}C n.m.r. signals from the four *meso*-carbon atoms of uroporphyrin-III ester have the same chemical shift whereas those of protoporphyrin-IX ester appear as four separate signals.[16] The enhanced 5.5 Hz doublet from the labelled uroporphyrin-III ester thus stands on a smaller singlet, but for the labelled protoporphyrin-IX ester only the rigorously assigned signal from the β-carbon [17] appears as an enhanced 5.5 Hz doublet, the other three signals from the α-, γ-, and δ-*meso*-carbon atoms being small singlets.

Dibenzyl 4,4′-Bis-(2-methoxycarbonylethyl)-3,3′-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylate (9). —(a) *By using tin*(IV) *chloride*. The foregoing acetoxymethylpyrrole (0.4 g) in dry methylene chloride (20 ml) and methanol (4 drops) was treated under nitrogen with tin(IV) chloride (0.4 ml) at 0 °C. After 5 h, methanol (4 ml) was added, followed by chloroform (25 ml), and the mixture was washed with 2N-hydrochloric acid, saturated sodium hydrogen carbonate solution, and then water. The residue from the chloroform was recrystallised from methanol to give the *pyrromethane* (270 mg, 80%), m.p. 145—146° (Found: C, 64.0; H, 5.9; N, 3.7. $C_{39}H_{42}N_2O_{12}$ requires C, 64.1; H, 5.8; N, 3.8%); $\lambda_{max.}$ ($CHCl_3$) 276sh and 286 nm; $\nu_{max.}$ (Nujol) 3 325, 3 280, 1 745, 1 735, 1 710, and 1 695 cm^{-1}; *m/e* 730 (M^+, 16%), 671 (4), 639 (100), 563 (24), 505 (12), and 341 (20), *m** 559 (730 ⟶ 639); τ 2.70 (10 H, m, ArH), 4.76 (4 H, s, PhCH_2), 6.18 (2 H, s, bridge CH_2), 6.42br (16 H, s, OMe and CH_2CO), and 6.97 and 7.49 (each 4 H, m, CH_2·CH_2).

(b) *By using silver perchlorate*. A solution of the bromomethylpyrrole (13; Br in place of OAc) (51 mg) in ethyl acetate (1 ml) was added at 20 °C to a stirred solution of silver perchlorate (30 mg) in ethyl acetate (5 ml). After 1 h, saturated aqueous sodium chloride was added followed, after stirring for 5 min, by powdered sodium hydrogen carbonate (0.5 g) and anhydrous sodium sulphate (2 g). The mixture was stirred for 30 min, filtered, and evaporated. Recrystallisation of the residue from methanol gave the pyrromethane (9), m.p. 146—147° (36 mg, 90%) (Found: C, 64.3; H, 6.0; N, 3.9%), n.m.r. as under (a).

4,4′-Bis-(2-methoxycarbonylethyl)-3,3′-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylic Acid (10).—The above dibenzyl ester (100 mg) in dry tetrahydrofuran (15 ml) containing triethylamine (2 drops) was hydrogenated over 10% palladium–charcoal (50 mg) at 20 °C and 760 mmHg. After 2 h, the mixture was filtered through Celite, which was washed with hot triethylamine–tetrahydrofuran (1 : 100). The combined filtrates were evaporated to *ca.* 1 ml and water (3 ml) was added, followed by acetic acid (to pH 6). The *pyrromethanedicarboxylic acid* crystallised at 0 °C (68 mg, 90%); m.p. 257—260° (decomp.). It was recrystallised by dissolving in methanol containing triethylamine and, after filtration, neutralisation with acetic acid and dilution with water (Found: C, 54.6; H, 5.6; N, 5.15. $C_{25}H_{30}N_2O_{12}$ requires C, 54.5; H, 5.5; N, 5.1%); $\lambda_{max.}$ 277 nm shifting to 266 nm on addition of 1N-sodium hydroxide; $\nu_{max.}$ (Nujol) 3 460—2 150, 3 325, 1 725, and 1 670 cm^{-1}; *m/e* 506 (M^+ − 44, 1%), 462 (M^+ − 88, 77), 431 (14), 403 (15), 389 (100), 375 (6), 329 (12), 255 (14), 238 (27), 237 (22), 206 (13), 134 (11), 120 (10), 118 (15), 106 (27), and 98 (15); τ [$(CD_3)_2SO$] 6.18 (2 H, s, bridge CH_2), 6.42 (12 H, overlapping, OMe), 6.57 (4 H, s, CH_2·CO), and 7.10—7.25 and 7.45—7.62 (each 4 H, m, CH_2·CH_2).

4,4′-Bis-(2-methoxycarbonylethyl)-3,3′-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarbaldehyde (7) (with E. Hunt).—The foregoing pyrromethane-5,5′-dicarboxylic acid (50 mg) in dry, degassed dimethylformamide (0.3 ml)

[16] A. R. Battersby, E. Hunt, E. McDonald, and J. Moron, *J.C.S. Perkin I*, 1973, 2917.
[17] A. R. Battersby, G. L. Hodgson, M. Ihara, E. McDonald, and J. Saunders, *J.C.S. Perkin I*, 1973, 2923.
[18] A. R. Battersby, E. Hunt, and E. McDonald, *J.C.S. Chem. Comm.*, 1973, 442.
[19] A. R. Battersby, J. F. Beck, and E. McDonald, *J.C.S. Perkin I*, 1974, 160.

was heated in an evacuated (at −78 °C), sealed tube at 190—200 °C for 4.5 h. After cooling to −78 °C, the tube was opened and redistilled benzoyl chloride (0.15 ml) was added with use of a nitrogen bubbler to stir the mixture. After 1.5 h at 20 °C under nitrogen, the mixture was heated at 60 °C for 0.5 h and cooled to 20 °C. Dry benzene (1 ml) was added, and the precipitated imine salt was collected and washed with benzene (2 × 1 ml). The dried solid was treated with water (1.5 ml) and aqueous 10% sodium carbonate (0.38 ml) and heated at 70 °C for 3 min. After a further 1 h at 20 °C, the mixture was extracted with chloroform to give the dialdehyde [20] (7), m.p. 198—199° [from chloroform–light petroleum (b.p. 60—80°)] (24 mg, 48%); $\lambda_{max.}$ ($CHCl_3$) 297sh and 311 nm; $\nu_{max.}$ (Nujol) 3 215, 1 730, and 1 620 cm^{-1}; *m/e* 518 (M^+, 37%), 489 (33), 460 (46), 431 (53), 429 (17), 85 (66), and 83 (100), *m** 461 (518 → 489), 434 (489 → 460), 404 (460 → 431), and 377.5 (431 → 403); τ 0.46 (2 H, s, CHO), 6.08 (2 H, s, bridge CH_2), 6.22 and 6.39 (each 6 H, s, OMe), 6.44 (4 H, s, CH_2·CO), and 6.96 and 7.41 (each 4 H, m, CH_2·CH_2).

Benzyl 4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate [12] (14).—Methyl 4-benzyloxycarbonyl-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate [21] (17.2 g) in dimethylacetamide (38 ml) was shaken with 10% palladised charcoal (1 g) and hydrogen until uptake was complete (3 h). The filtered solution was diluted with water (300 ml) to give the pyrrole-4-carboxylic acid (12 g, 93%). Part (1 g) was heated under reflux in nitrogen with trifluoroacetic acid (5 ml) for 2 h, then treated at 0 °C with ethyl orthoformate [22] (1.5 ml) over 5 min. After 4 h at 0 °C the mixture was adjusted to pH 8 with aqueous ammonia and extracted with methylene chloride to give the 3-formylpyrrole (650 mg), m.p. 173—175° (from ethyl acetate). This was converted into the title compound largely as previously,[12] with the following modification of the ester exchange step.

Methyl 4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate [12] (250 mg) in dry benzyl alcohol (3 ml) in which sodium (4 mg) had been dissolved was treated as for the preparation of the isomeric tribenzyl ester (above). The product, *benzyl 4-(2-benzyloxycarbonylethyl)-3-benzyloxycarbonylmethyl-5-methylpyrrole-2-carboxylate* (403 mg), crystallised from aqueous methanol and chloroform–hexane; m.p. 100—102° (Found: C, 71.5; H, 6.0; N, 2.3. $C_{32}H_{31}NO_6$ requires C, 71.5; H, 5.9; N, 2.65%); τ 1.2br (1 H, s, NH), 2.68 (5 H, ArH), 2.72 (10 H, ArH), 4.80, 4.94, and 4.96 (each 2 H, s, ArCH_2), 6.15 (2 H, s, CH_2·CO), 7.42 (4 H, m, CH_2·CH_2), and 7.85 (3 H, s, Me). This was converted as for the isomer (above) into the corresponding dimethyl monobenzyl ester [12] (14), m.p. 113—115° (91%).

Benzyl 5-Formyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (15).—Sulphuryl chloride (448 mg) in ether (5 ml) was added dropwise at 20 °C during 5 min to a stirred suspension of the pyrrole (14) (500 mg) in anhydrous ether (50 ml). Two further additions of sulphuryl chloride (90 mg each in 3 ml of ether) were made after 1.5 and 2.5 h and the solution was stirred for a further 2.5 h. The residue from evaporation was dissolved in ether (20 ml) and the solution evaporated. Sodium acetate (1.5 g) in water (20 ml) was then added and the mixture was heated at 100 °C for 10 min. Extraction with ether (washing with N-sodium carbonate and saturated brine) gave the *aldehyde* (446 mg, 86%), m.p. 113—115° (from n-hexane–benzene); a lower m.p. form (96—98°) was obtained on one occasion (Found: C, 62.1; H, 5.6; N, 3.8. $C_{20}H_{21}NO_7$ requires C, 62.0; H, 5.4; N, 3.6%) $\lambda_{max.}$ 302 nm; $\nu_{max.}$ (Nujol) 3 270, 1 726, 1 702, and 1 662 cm^{-1}; τ 0.22 (1 H, s, CHO), 0.3br (1 H, NH), 2.63 (5 H, ArH), 4.70 (2 H, s, ArCH_2), 6.17 (2 H, s, CH_2·CO), 6.37 and 6.40 (each 3 H, s, OMe), and 6.93 and 7.42 (each 2 H, t, *J* 7.5 Hz, CH_2·CH_2·CO).

Benzyl 4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (16).—The foregoing aldehyde (0.5 g) in dry toluene (80 ml) was heated and stirred in nitrogen under reflux with tris(triphenylphosphine)rhodium chloride (1.19 g) for 1.5 h. The residue left after evaporation was treated with ether (100 ml), the solution was filtered, and the filtrate and washings were evaporated. P.l.c. on silica in 1 : 1 benzene–ether gave the α-free pyrrole (380 mg), m.p. 62—64°, identified by direct comparison.[12]

Dibenzyl 3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylate (17).—(a) *By using tin*(IV) *chloride*. Tin(IV) chloride (0.1 ml) was added to a solution of the foregoing pyrrole (16) (90 mg) in anhydrous methylene chloride (5 ml) at −20 °C under nitrogen. A solution of the acetoxymethylpyrrole (13) (107 mg) in methylene chloride (3 ml) was then added with stirring, and after 10 min at −20 °C more methylene chloride (20 ml) was added and the solution was washed with N-hydrochloric acid (10 ml), saturated sodium hydrogen carbonate (10 ml), and saturated brine (10 ml). The product from the organic layer was crystallised from ethyl acetate–n-hexane to give the *pyrromethane* (147 mg), m.p. 103—104° (raised to 118—119° by one recrystallisation) (Found: C, 63.9, 64.2; H, 5.7, 5.9; N, 4.0, 3.65. $C_{39}H_{42}N_2O_{12}$ requires C, 64.1; H, 5.8; N, 3.8%); $\lambda_{max.}$ 288 nm; $\nu_{max.}$ 3 300, 1 725, and 1 690 cm^{-1}; *m/e* 730 (M^+, 3%), 699 (2), 671 (2), 640 (10), 639 ($M^+ - C_7H_7$, 100), 607 (2), 596 (3), 595 (5), 578 (4), 577 (14), 563 (3), 549 (3), 535 (3), 531 (3), 505 (9), and 503 (12); τ −0.12br and 0.10br (each 1 H, s, NH), 2.60—2.79 (10 H, m, ArH), 4.75 and 4.78 (each 2 H, s, ArCH_2), 6.05, 6.20, and 6.45 (each 2 H, pyrr$_2CH_2$, 2 × pyrr-CH_2·CO), 6.39, 6.41, and 6.48 (3 H, 6 H, and 3 H, each s, 4 × OMe), and 6.90—7.59 (8 H, m, 2 × CH_2·CH_2).

(b) *By using silver perchlorate*. A solution of the bromomethylpyrrole (13; Br in place of OAc) (452 mg) in dry ethyl acetate (5 ml) was added dropwise over 5 min to a stirred mixture of benzyl 4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate [12] (359 mg) and silver perchlorate (218 mg) in dry ethyl acetate (40 ml). After stirring for 1 h (in the dark under nitrogen), the product was isolated as for the isomer (9) above and was crystallised from methanol (−10 °C) to yield the pyrromethane, m.p. 116—119° (677 mg, 93%), raised to 118—119° by recrystallisation from ethyl acetate–n-hexane, identical with the product from (a).

The reaction was equally successful (92%; m.p. 117—119°) with the alternative combination of α-free and bromomethyl pyrroles.

3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylic Acid (18).—This was prepared from the foregoing dibenzyl ester as for the isomer (10) above, in 95% yield; m.p. 198—202°

[20] G. P. Arsenault, E. Bullock, and S. F. MacDonald, *J. Amer. Chem. Soc.*, 1960, **82**, 4384.

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

[22] P. S. Clezy, C. J. R. Fookes, and A. J. Liepa, *Austral. J. Chem.*, 1972, **25**, 1979.

(decomp.) (Found: C, 54.7; H, 5.5; N, 5.1. $C_{25}H_{30}N_2O_{12}$ requires C, 54.5; H, 5.5; N, 5.1%); $\lambda_{max.}$ 282 nm; $\nu_{max.}$ 3 300, 1 720, and 1 652 cm^{-1}; *m/e* 462 ($M^+ - 2CO_2$), 431, 403, 389, 375, 329, 266, 255, 238, 237, 236, 220, 206, and 205; τ [$(CD_3)_2SO$] 6.18, 6.30, and 6.57 (each 2 H, s, pyrr$_2$CH$_2$, 2 × pyrr-CH$_2$·CO), 6.45 (12 H, s, 4 × OMe), and 7.10—7.90 (m, overlapped with Me_2SO, 2 × CH$_2$·CH$_2$).

3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole (19).—A stirred solution of the foregoing acid (10 mg) in degassed glacial acetic acid (1.5 ml) was heated in the dark under nitrogen for 12 min in an oil-bath at 125—135 °C. The solvent was rapidly evaporated (0.2 mmHg) and the residue was purified by p.l.c. in 1 : 1 chloroform–ether under nitrogen in the dark. The product was eluted with 1 : 9 methanol–chloroform to give the pyrromethane (6 mg, 72%), m.p. 51—53° (lit.,[4] 53.5—54.5°); $\lambda_{max.}$ 222 nm; τ 3.45 and 3.58 (each 1 H, d, *J* 3 Hz, pyrrole α-H), 6.18 (2 H, s, pyrr$_2$CH$_2$), 6.27, 6.32, and 6.38 (3 H, 6 H, and 3 H, each s, 4 × OMe), 6.49 and 6.55 (each 2 H, s, 2 × pyrr-CH$_2$·CO), and 7.15—7.51 (8 H, m, 2 × CH$_2$·CH$_2$).

Uroporphyrin-III Octamethyl Ester [*Ester of* (4)].—The dicarboxylic acid (18) (30 mg) was decarboxylated in glacial acetic acid (4.1 ml) as above and the solution was quickly cooled to 0 °C. A solution of the diformylpyrromethane (7) (21.4 mg) in glacial acetic acid (16.4 ml) was added, followed by a mixture of 1 part of 56% hydriodic acid (stored over phosphine hydriodide [23]) and 100 parts of acetic acid (14.8 ml). The solution was stirred under nitrogen in the dark for 20 min, sodium acetate (516 mg) in acetic acid (7.4 ml) was then added, and air was slowly bubbled through the solution for 16 h. The residue from evaporation was dissolved in chloroform and washed with aqueous ammonium hydroxide (containing a little sodium thiosulphate) and then water. The product from the chloroform was esterified with 5% (w/v) sulphuric acid in methanol (41 ml) at 4° for 16 h and the ester was purified by p.l.c. (two developments) with 1.5% methanol in chloroform. The main fraction (eluted with 1 : 9 methanol–chloroform) was recrystallised from chloroform–methanol yielding uroporphyrin-III octamethyl ester (27.4 mg, 70% from the dialdehyde), m.p. 259—261° (lit.,[4] 255—258, 258—260°); $\lambda_{max.}$ ($CHCl_3$) 406, 501, 535, 571, and 624 nm; *m/e* (M^+) 942.

This product (2.73 mg) was heated in an evacuated sealed tube with glacial acetic acid (0.25 ml) and aqueous 1% hydrochloric acid (1.25 ml) for 4.5 h at 185—187 °C. The resultant solution was neutralised with sodium hydroxide and treated with sodium dihydrogen phosphate to precipitate the coproporphyrin. This was converted into the tetramethyl ester as above, and the latter was chromatographed on alumina in methylene chloride. The total fraction containing coproporphyrin tetramethyl ester(s) was checked by h.p.l.c.; [11] it proved to be pure coproporphyrin-III tetramethyl ester, m.p. 162—165° (from methylene chloride–methanol; yield 1.44 mg); $\lambda_{max.}$ ($CHCl_3$) 399, 496, 531, 567, and 621 nm.

[12-methylene-^{14}C]*Uroporphyrin-III Octamethyl Ester.*—The [^{14}C]pyrrole [12] (22) (47.1 mg; total 205 μCi) was converted into the labelled acetoxymethylpyrrole (13) (56.4 mg, 97%) and this was used as above for the synthesis of the [^{14}C]-pyrromethane (17) (80 mg, 84%). The derived dicarboxylic acid (18) (55 mg, 90%) was decarboxylated and converted into the labelled uroporphyrin-III octamethyl ester, which was recrystallised twice from chloroform–methanol [yield 46 mg, 39% from the starting pyrrole (22)]; m.p. 260—261°; total activity 81.7 μCi.

3-(2-Carboxyethyl)-4-carboxymethyl-5-t-butoxycarbonylaminomethylpyrrole.—t-Butoxycarbonyl azide (537 mg) was added to a stirred suspension of porphobilinogen (732 mg) in 1 : 19 dioxan–water (30 ml) at 20 °C in the dark. The pH of the mixture was adjusted to 9.4—10 at the outset and maintained there by addition of 2N-potassium hydroxide. After 6 h, water was added, the mixture was extracted with ether (2 × 20 ml), and the aqueous phase was acidified to pH 3.5 (citric acid). Extraction with ethyl acetate (80 ml total) gave the *t-butoxycarbonylaminomethylpyrrole* (890 mg, 92%), m.p. 140.5—141° (Found: C, 55.1; H, 6.8; N, 8.7. $C_{15}H_{22}N_2O_6$ requires C, 55.2; H, 7.1; N, 8.6%); $\nu_{max.}$ (Nujol) 3 375, 3 305, 1 710, and 1 665 cm^{-1}; τ[$(CD_3)_2$-SO] −0.5br (1 H, s, NH), 3.65 (1 H, d, *J* 2 Hz, α-H), 5.96 (2 H, dd, *J* 3 Hz, CH$_2$·N), 6.69 (2 H, s, CH$_2$·CO), 7.34—7.76 (4 H, m, CH$_2$·CH$_2$), and 8.61 (9 H, s, Bu^t).

3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-t-butoxycarbonylaminomethylpyrrole-2-carbaldehyde (26) *and* [$^{13}C_2$]-*Material.*—The foregoing pyrrole (1.09 g) in methanol was esterified with distilled diazomethane in ether [prepared from *N*-nitroso-*N*-methylurea (3 g)] to give the corresponding dimethyl ester (23); $\nu_{max.}$ (film) 3 480—3 140 and 1 740—1 685 cm^{-1}; *m/e* 354 (M^+, 13%), 323 (3), 298 (10), 297 (15), 253 (6), 239 (16), 238 (16), 237 (7), 225 (14), 223 (9), 181 (11), 163 (10), 106 (15), 87 (39), 59 (44), and 57 (100), *m** 251 (354 → 298), 249 (354 → 297), 216 (297 → 253), and 215.5 (298 → 253); τ 1.34br (1 H, s, NH), 3.56 (1 H, d, *J* 2 Hz, α-H), 4.80br (1 H, t, *J* 6 Hz, amide NH), 5.84 (2 H, d, *J* 6 Hz, CH$_2$N), 6.36 (6 H, s, OMe), 6.59 (2 H, s, CH$_2$·CO), 7.14—7.58 (4 H, m, CH$_2$·CH$_2$), and 8.59 (9 H, s, Bu^t). All the ester in dry dimethylformamide (10 ml) at 0 °C was treated with redistilled benzoyl chloride (2 ml) in portions under nitrogen during 1 h. After a further 3 h at 20 °C, the solvent was evaporated off and the residue was stirred vigorously for 16 h with chloroform and aqueous 10% potassium hydrogen carbonate. The aqueous layer was then extracted with chloroform and the extract was chromatographed on alumina in 1 : 1 chloroform–benzene to give the *formylpyrrole* (1.12 g, 87%) homogeneous by t.l.c. in 1 : 1 ether–chloroform and in 5% methanol–chloroform (Found: M^+, 382.1748. $C_{18}H_{26}N_2O_7$ requires *M*, 382.1738); $\lambda_{max.}$ 303 nm; $\nu_{max.}$ (film) 3 360—3 250, 1 745—1 685, and 1 635 cm^{-1}; *m/e* 382 (M^+, 67%), 326 (100), 325 (45), 297 (50), 281 (22), 267 (22), 266 (55), 265 (55), 253 (34), and 206 (33), *m** 278.5 (382 → 326), 270.5 (326 → 297), and 243 (326 → 281); τ −0.40br (1 H, s, NH), 0.40 (1 H, s, CHO), 4.50br (1 H, s, amide NH), 5.67 (2H, d, *J* 6 Hz, CH$_2$N), 6.26 and 6.30 (each 3 H, s, OMe), 6.45 (2 H, s, CH$_2$·CO), 6.91 and 7.38 (each 2 H, m, CH$_2$·CH$_2$), and 8.50 (9 H, s, Bu^t); δ_C 177.4 (CHO), 172.7 and 171.9 (*C*O$_2$·CH$_3$), 156.3 (*C*O$_2$Bu^t), 136.9, 133.5, 128.3, and 114.7 (ring c), 80.0 [*C*(CH$_3$)$_3$], 52.0 and 51.5 (ester CH$_3$), 35.8 (*C*H$_2$·CO and *C*H$_2$·NH), 29.1 (pyrr-CH$_2$·*C*H$_2$), 28.2 [C(*C*H$_3$)$_3$], and 18.8 (pyrr-*C*H$_2$·CH$_2$).

The [$^{13}C_2$]-series started with [2,11-$^{13}C_2$]porphobilinogen [1] (144 mg; 40 atom % ^{13}C at C-2 and C-11), which was converted as above into its t-butoxycarbonyl derivative, diluted with unlabelled material (85 mg), esterified, and formylated to yield the [2,11-$^{13}C_2$]formylpyrrole [as (26)] having 20 atom % ^{13}C at C-2 and C-11 (180 mg); δ_C as above except for 20-fold enhancement of the signals at 128.3 and 35.7.

[23] R. V. Gregorovich, K. S. Y. Liang, D. M. Clugston, and S. F. MacDonald, *Canad. J. Chem.*, 1968, **46**, 3291.

5-Acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carbaldehyde (28) *and* [$^{13}C_2$]-*Material*.—Dry trifluoroacetic acid (8 ml) was added at 20 °C in the dark to the foregoing t-butoxycarbonylaminomethylpyrrole (440 mg) under nitrogen, and after 0.5 h the mixture was evaporated. The residue in acetic acid (12 ml) under nitrogen was stirred as sodium nitrite (1 g) was added over 6 h and after a further 1 h the solvent was evaporated off. The residue was partitioned between chloroform (50 ml) and an excess of aqueous 10% potassium hydrogen carbonate, the aqueous layer was extracted with chloroform (2 × 20 ml), and the product from the chloroform was chromatographed on alumina in 2% methanol–chloroform to give the *acetoxymethylpyrrole* (220 mg, 59%), m.p. 70—71° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 55.6; H, 6.0; N, 4.2. $C_{15}H_{19}NO_7$ requires C, 55.4; H, 5.9; N, 4.3%); $\lambda_{max.}$ 301 nm; $\nu_{max.}$ (Nujol) 3 225, 1 745—1 730, 1 655, and 1 640 cm^{-1}; *m/e* 325 (M^+, 100%), 294 (24), 283 (24), 266 (60), 265 (25), 222 (32), 206 (56), 146 (35), 85 (22), and 83 (33), *m** 266 (325 ⟶ 294) and 246.5 (325 ⟶ 283); τ 0.20br (1 H, s, NH), 0.39 (1 H, s, CHO), 4.91 (2 H, s, CH_2O), 6.33 and 6.38 (each 3 H, s, OMe), 6.48 (2 H, s, CH_2·CO), 6.95 and 7.41 (each 2 H, m, CH_2·CH_2), and 7.95 (3 H, s, $COCH_3$); δ_C 177.9 (CHO), 172.8 and 171.5 (CO_2·CH_3), 133.0, 132.5, 129.0, and 116.7 (ring c), 56.8 (CH_2·O), 52.0 and 51.5 (ester CH_3), 35.6 (CH_2·CO), 29.2 (pyrr-CH_2·CH_2·CO), 20.5 (CH_3CO), and 18.7 (pyrr-CH_2·CH_2).

The later fractions from the above chromatographic purification contained a mixture of the acetoxymethylpyrrole (28) and the corresponding hydroxy-compound (29). The latter was isolated by p.l.c. on silica in 1 : 1 ether–chloroform; $\lambda_{max.}$ 300 nm; *m/e* 283 (M^+); τ 0.5 (1 H, s, CHO), 5.32 (2 H, s, CH_2O), 6.32 and 6.38 (each 3 H, s, OMe), 6.51 (2 H, s, CH_2CO), and 6.95 and 7.42 (each 2 H, m, CH_2·CH_2). The mixture was acetylated with acetic anhydride (0.5 ml) in pyridine (2.5 ml) for 16 h at 20 °C and worked up as usual to give pure acetoxymethylpyrrole (28); this raised the total yield to 71%.

The preparation was repeated starting with the [2,11-$^{13}C_2$]-pyrrole [as (26)] (180 mg) to yield the [2,11-$^{13}C_2$]acetoxymethylpyrrole [as (28)] (90 mg) and the hydroxymethyl analogue [as (29)] (15 mg); δ_C for the former as above but with 20-fold enhancement of the signals at 129.0 and 56.8, the latter being shown in an expanded spectrum to be a doublet, *J* 2.4 Hz, owing to weak long-range coupling.

Benzyl 3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bismethoxycarbonylmethyl-5′-formyl-2,2′-methylenedipyrrole-5-carboxylate (30) *and* [$^{13}C_2$]-*Material*.—The foregoing unlabelled acetoxymethylpyrrole (80 mg) in dry methylene chloride (3 ml) added over 5 min to benzyl 4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-5-carboxylate [12] (89 mg) in dry methylene chloride (3 ml) and tetrahydrofuran (8 drops) containing redistilled tin(IV) chloride (6 drops) under nitrogen at 20 °C. After 25 h, methanol (0.2 ml) was added and the mixture in chloroform (10 ml) was washed with saturated aqueous sodium hydrogen carbonate and then water. The residue from the chloroform by p.l.c. on silica in 4% methanol in chloroform gave the *pyrromethane* (30) (110 mg, 72%), m.p. 155—156° [from benzene–light petroleum (b.p. 60—80°)] (Found: C, 61.8; H, 5.8; N, 4.5. $C_{32}H_{36}N_2O_{11}$ requires C, 61.6; H, 5.8; N, 4.5%); $\lambda_{max.}$ ($CHCl_3$) 305 and 281 nm; $\nu_{max.}$ (Nujol) 3 335, 3 225, 1 735, 1 725, 1 685, and 1 645 cm^{-1}; *m/e* 624 (M^+, 12%), 533 (48), 328 (6), 298 (9), 297 (9), 278 (19), 277 (35), 268 (21), 266 (21), and 91 (100), *m** 456 (624 ⟶ 533); τ −0.32br and −0.21br (each 1 H, s, NH), 0.41 (1 H, s, CHO), 2.70 (5 H, m, ArH), 4.76 (2 H, s, $PhCH_2$), 6.01 (2 H, s, $pyrr_2CH_2$), 6.18 and 6.44 (each 2 H, s, CH_2·CO), 6.23, 6.35, and 6.44 (12 H, s, OMe), and 6.86—7.54 (8 H, m, CH_2·CH_2); δ_C 176.2 (CHO), 174.8, 173.4, 172.7, and 171.9 (ester CO), 135.9 132.8, 130.5, 128.7 122.6, 120.0, and 114.7 (ring c), 128.3 and 128.0 (aromatic), 65.6 ($PhCH_2$), 52.3 and 51.6 (OMe), 35.9 and 30.9 (CH_2·CO), 34.1 and 29.2 (pyrr-CH_2·CH_2), 22.3 ($pyrr_2CH_2$), and 19.0 and 18.6 (pyrr-CH_2·CH_2).

In the ^{13}C series [2,11-$^{13}C_2$]-5-acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carbaldehyde (90 mg, *ca.* 20% of molecules being $^{13}C_2$) reacted with the α-free pyrrole (100 mg) in the presence of tin(IV) chloride as above to yield the [$^{13}C_2$]pyrromethane (138 mg); $\lambda_{max.}$ ($CHCl_3$) 305 and 281 nm; *m/e* 626 (M^+ for $^{13}C_2$ material, 6%), 625 (M^+ for ^{13}C material, 7), 624 (M^+ for ^{12}C material, 16), 535 (32), 534 (37), and 533 (100); τ 0.45 (1 H, m, CHO), 2.71 (5 H, m, ArH), 4.80 (2 H, s, $PhCH_2$), 6.04 (2 H, 80% of signal as s, 20% as br d, *J* 128 Hz, $pyrr_2$-CH_2), 6.22 and 6.48 (each 2 H, s, CH_2·CO), 6.27, 6.39, and 6.48 (12 H, s, OMe), and 6.85—7.57 (8 H, m, CH_2·CH_2); δ_C as above except that the signals at 128.7 and 22.3 were enhanced *ca.* 20 times.

Benzyl 3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole-5-carboxylate (31) *and* [$^{13}C_2$]-*Material*.—The unlabelled formylpyrromethane (30) (70 mg) was heated under reflux (nitrogen) in dry toluene (14 ml) with tris(triphenylphosphine)rhodium chloride (113 mg) for 7 h. The residue from evaporation was triturated with dry ether (5 × 5 ml) and the ether-soluble material was purified by p.l.c. on silica in 1 : 1 ether–chloroform, and recrystallised from benzene–light petroleum (b.p. 60—80°) to afford the decarbonylated *pyrromethane* (31) (56 mg, 84%), m.p. 82° (Found: C, 62.3; H, 6.3; N, 4.9. $C_{31}H_{36}N_2O_{10}$ requires C, 62.4; H, 6.1; N, 4.7%); $\lambda_{max.}$ ($CHCl_3$) 283 nm; $\lambda_{max.}$ (Nujol) 3 380, 3 285, 1 738, 1 720, and 1 690 cm^{-1}; *m/e* 596 (M^+, 19%), 565 (6), 537 (4), 505 (78), 461 (11), 459 (7), 445 (7), 262 (22), and 91 (100), *m** 428 (596 ⟶ 505), 421 (505 ⟶ 461), and 418 (505 ⟶ 459); τ 0.05br and 1.25br (each 1 H, s, NH), 2.70 (5 H, m, ArH), 3.58 (1 H, d, *J* 2 Hz, α-H), 4.78 (2 H, s, $PhCH_2$), 6.14 (2 H, s, $pyrr_2CH_2$), 6.19 and 6.52 (each 2 H, s, CH_2·CO), 6.36, 6.39, and 6.42 (12 H, s, OMe), and 7.11—7.61 (8 H, m, CH_2·CH_2); δ_C (carbonyl C not observed) 132.4, 126.5, 122.6, 121.1, 114.4, and 111.1 (ring c), 128.3 and 127.9 (aromatic), 65.5 ($PhCH_2$), 52.2, 51.6, and 51.4 (OMe), 34.8 and 34.4 (pyrr-CH_2·CH_2·CO), 30.8 and 29.8 (CH_2·CO), 22.3 ($pyrr_2CH_2$), and 20.6 and 18.9 (pyrr-CH_2·CH_2).

In the $^{13}C_2$-series, decarbonylation of the labelled benzyl 5′-formylpyrromethane-5-carboxylate (93 mg) by use of the rhodium complex (152 mg) gave the α-free [$^{13}C_2$]pyrromethane (62 mg).

[10,14-$^{13}C_2$]*Uroporphyrin-III* (4) *Octamethyl Ester*.—The foregoing α-free [$^{13}C_2$]pyrromethane (62 mg) was hydrogenated over 10% palladium–charcoal (56 mg) at 20 °C and 760 mmHg in ethanol (7 ml). After 1.5 h the solution was filtered under nitrogen and the residue from evaporation was triturated with dry ether to afford the solid pyrromethane-5-carboxylic acid (40 mg), which was used directly in the next stage. For unlabelled material: $\lambda_{max.}$ (EtOH) 277 nm shifting to 267 nm on addition of N-sodium hydroxide; *m/e* 462 (M^+ − 44, 28%), 284 (100), and 202 (84).

The labelled acid was heated under reflux for 12 min in degassed acetic acid (6 ml) under nitrogen in the dark. After rapid cooling, the 5,5′-diformylpyrromethane (7) (30.6

mg) in acetic acid (21 ml) was added, followed immediately by hydriodic acid in acetic acid (1 : 100; 20 ml), and the mixture was stirred at 20 °C for 25 min. Sodium acetate (660 mg) in acetic acid (10 ml) was added, air was bubbled through the mixture in the dark for 16 h, and the solvents were then evaporated off. The residue in chloroform was washed with an excess of aqueous 2N-ammonia containing a little sodium thiosulphate and then water. Work-up as for [^{14}C]uroporphyrin-III octamethyl ester (above) gave [10,14-$^{13}C_2$]uroporphyrin-III octamethyl ester (40.4 mg, 73% from the diformylpyrromethane), m.p. 259—262° (lit.,[4] m.p. 255—258°), identified by comparison with authentic material [Found (for unlabelled material): C, 61.2; H, 6.0, N, 6.2. Calc. for $C_{48}H_{54}N_4O_{16}$: C, 61.1, H, 5.8; N, 5.9%]; $\lambda_{max.}$ ($CHCl_3$) 405, 500, 533, 571, and 626 nm; *m/e* 942 (M^+ for unlabelled material).

[10,14-$^{13}C_2$]*Protoporphyrin-IX* (2) *Dimethyl Ester*.—A solution of the foregoing [10,14-$^{13}C_2$]uroporphyrin-III octamethyl ester (5 mg) in tetrahydrofuran (15 ml) was stirred with aqueous 2N-potassium hydroxide (15 ml) for 16 h. The solution was washed with ether, acidified with concentrated hydrochloric acid (2.3 ml), and treated with 2N-potassium hydroxide until the porphyrin just redissolved. This vigorously stirred solution was treated with freshly prepared 2% sodium amalgam (2.5 g) under nitrogen for 0.5 h, the colourless decanted solution was adjusted to pH 8 with 1 : 1 acetic acid–water and then incubated for 0.5 h with the enzyme preparation from two standard batches of *Euglena gracilis*;[1] the protoporphyrin-IX was isolated, esterified, and purified as usual.[1] This gave [10,14-$^{13}C_2$]-protoporphyrin-IX dimethyl ester (1.12 mg), which was combined with the product from an identical run (total 2.19 mg) and then diluted with protoporphyrin-IX dimethyl ester from natural sources (6.57 mg) to give material in which 5% of the molecules were $^{13}C_2$-labelled. After recrystallisation, the final product was used for ^{13}C n.m.r. spectroscopy.

2-*Iodo*-3-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethyl*-5-*t*-*butoxycarbonylaminomethylpyrrole* (24).—The α-free pyrrole (23) (84 mg) and potassium hydrogen carbonate (28.5 mg) in stirred ethanol (2 ml) and water (2 ml) were treated with iodine (61.4 mg) in ethanol (1 ml) over 1.5 h at 0 °C under nitrogen. After removal of the slight excess of iodine (aqueous sodium thiosulphate) the solution was evaporated and the residue was partitioned between chloroform and water. Chromatography of the product from the chloroform on alumina in 1 : 1 chloroform–benzene gave the 2-*iodopyrrole* (100 mg, 88%) (Found: M^+, 480.0752. $C_{17}H_{25}IN_2O_6$ requires *M*, 480.0758); τ (60 MHz) 1.30br (1 H, s, NH), 4.86br (1 H, s, amide NH), 5.85 (2 H, d, *J* 6 Hz, CH_2·N), 6.35 (6 H, s, OMe), 6.55 (2 H, s, CH_2·CO), 7.21—7.58 (4 H, m, CH_2·CH_2), and 8.56 (9 H, s, Bu^t).

2-*Bromo*-3-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethyl*-5-*t*-*butoxycarbonylaminomethylpyrrole* (25).—*N*-Bromosuccinimide (28 mg) in methylene chloride (1.5 ml) was added over 1.5 h at 0 °C under nitrogen to a stirred solution of the α-free pyrrole (23) (48 mg) in methylene chloride (1 ml) and water (1 ml). After a further 1 h, the organic layer was washed with 2M-sodium sulphite solution and water and evaporated. The residue (57 mg, 97%) was homogeneous on silica in 1 : 1 ether–chloroform, but deteriorated rapidly in air and was not further purified (Found: M^+, 432.0902. Calc. for $C_{17}H_{25}{}^{78}BrN_2O_6$: *M*, 432.0894); τ (60 MHz) 1.28br (1 H, s, NH), 4.89br (1 H, s, amide NH), 5.90 (2 H, d, *J* 6 Hz, CH_2·N), 6.36 (6 H, s, OMe), 6.60 (2 H, s, CH_2·CO), 7.25—7.62 (4 H, m, CH_2·CH_2), and 8.61 (9 H, s, Bu^t).

Ethyl 3-(2-*Ethoxycarbonylethyl*)-4-*formyl*-5-*methylpyrrole*-2-*carboxylate* (35).—The acid (34) (5 g) in trifluoroacetic acid (15 ml) was heated at 70 °C under nitrogen for 2.5 h then cooled at −3 °C and treated with ethyl orthoformate (4 ml) in one portion. The temperature of the stirred mixture rose to 10 °C and after being left at 20 °C for 4 h more the solution was poured into ice–water (150 ml) and adjusted to pH 8 with ammonia. Extraction with dichloromethane gave the *aldehyde* (3 g), further purified by p.l.c. on silica in ethyl acetate; m.p. 121—123° (from ethyl acetate) (Found: C, 57.2; H, 6.5; N, 9.3. $C_{14}H_{19}NO_5$ requires C, 57.0; H, 6.4; N, 9.5%); τ −0.02 (1 H, s, CHO), 0.4br (1 H, s, NH), 6.62 and 6.90 (each 2 H, q, CH_2·O), 6.62 and 7.39 (each 2 H, t, CH_2·CH_2), 7.42 (3 H, s, Me), and 8.60 and 8.75 (each 3 H, t, CH_3·CH_2).

Dibenzyl 3-(2-*Benzyloxycarbonylethyl*)-5-*methylpyrrole*-2,4-*dicarboxylate* (37).—A solution of the ester (36) (2.3 g) in benzyl alcohol (18 ml) in which sodium (10 mg) had been dissolved was heated with stirring to 200 °C over 1.5 h. The solution was cooled to 100 °C, and glacial acetic acid (0.2 ml) was added followed by methanol (25 ml) and water (15 ml). The precipitated product (from 9 : 1 methanol–water) had m.p. 118—119° (yield 3.4 g, 86%) (Found: C, 71.4; H, 5.6; N, 2.5. $C_{31}H_{29}NO_6$ requires C, 72.1; H, 5.7; N, 2.7%); τ 0.17 (1 H, s, NH), 2.7 (15 H, ArH), 4.74, 4.76, and 4.98 (each 2 H, s, $ArCH_2$), 6.56 and 7.44 (each 2 H, t, CH_2·CH_2), and 7.54 (3 H, s, Me).

Benzyl 3-(2-*Methoxycarbonylethyl*)-4-*methoxycarbonylmethylpyrrole*-2-*carboxylate* (40).—The following modified method (*cf.* ref 12) was used. A stirred solution of the pyrrole (38) (1 g) in dry ether (100 ml) was treated at 0 °C with sulphuryl chloride (0.27 ml) in ether (10 ml) during 15 min. After 30 min, the mixture was warmed to 20 °C and after 2 h was evaporated and re-evaporated after addition of ether (50 ml). The residue was stirred for 5 min with a boiling solution of sodium acetate (3 g) in water (50 ml) and then cooled and extracted with ether to give the aldehyde (39) (819 mg), m.p. 80—82° (from chloroform–ether).

This aldehyde (250 mg) was decarbonylated as earlier to give, after p.l.c., the α-free pyrrole (40) (213 mg), m.p. 60—63° (from ether–pentane), identical with an authentic sample.[12]

Dibenzyl 3,3′-*Bis*-(2-*methoxycarbonylethyl*)-4,4′-*bismethoxycarbonylmethyl*-2,2′-*methylenedipyrrole*-5,5′-*dicarboxylate* (41) *and the* [^{13}C]-*Form* (42).—(a) *Unlabelled*. A solution of benzyl 5-bromomethyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (48 mg) in ethyl acetate (1 ml) was added to a stirred solution of silver perchlorate (30 mg) in ethyl acetate (5 ml). After 5 min, the product was worked up as earlier to give the *pyrromethane* (33 mg), m.p. 135—136.5° (from chloroform–pentane) (Found: C, 63.9; H, 6.0; N, 3.7. $C_{39}H_{42}N_2O_{12}$ requires C, 64.1; H, 5.8; N, 3.8%); τ 0.55br (2 H, s, NH), 2.7 (10 H, s, ArH), 4.79 (4 H, s, $ArCH_2$), 6.02 (2 H, s, $pyrr_2CH_2$), 6.21 (4 H, s, CH_2·CO), 6.44 (12 H, s, OMe), and 7.24 and 7.5 (each 4 H, diffuse t, CH_2·CH_2).

(b) ^{13}*C-Labelled*. [^{13}C]Paraformaldehyde (29.5 mg; 90 atom % ^{13}C) was added at 0 °C to a stirred solution of the α-free pyrrole (16) (718 mg) in trifluoroacetic acid (10 ml). After 15 min at 0 °C and 1 h at 20 °C the solution was evaporated and the residue in ethyl acetate (50 ml) was stirred with sodium hydrogen carbonate (2 g), filtered, and evaporated. From methanol, the [^{13}C]*pyrromethane* had m.p.

134—136° (690 mg); ^{1}H n.m.r. spectrum was as in (*a*) except that the signal at 6.02 appeared as a doublet, *J* 127 Hz, centred on a singlet; ratio of lines *ca.* 4 : 1 : 4; *m/e* 731 (M^+, 100%), 730 (28), and 729 (5).

3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bismethoxycarbonylmethyl-2,2′-methylenedipyrrole-5,5′-dicarbaldehyde (33).—A solution of the dicarboxylic acid (18) (100 mg) in freshly distilled dimethylformamide (0.6 ml) was heated in an evacuated sealed tube at 190 °C for 4 h. The tube was cooled to −50° and opened, and benzoyl chloride (0.3 ml) was added. After the solution had been stirred with a nitrogen stream for 1.5 h at 20 °C, it was heated under nitrogen at 50 °C for 0.5 h. Benzene (3 ml) was added to the cooled mixture, and the precipitated iminium salt was washed with benzene (2 ml) and then hydrolysed with water (2 ml) and aqueous 10% sodium carbonate (0.5 ml) by heating rapidly to 80 °C. The *dialdehyde* which separated from the cooled solution had m.p. 142—145° (from methanol); yield 30—55% (Found: C, 57.9; 58.2; H, 6.0, 5.9; N, 5.3, 5.4. $C_{25}H_{30}N_2O_{10}$ requires C, 57.9; H, 5.8; N, 5.4%); τ −0.43br (2 H, 2 NH), 0.42 (2 H, s, CHO), 5.98 (2 H, s, $pyrr_2CH_2$), 6.25 and 6.43 (each 2 H, s, CH_2·CO), 6.35, 6.31, 6.24, and 6.19 (each 3 H, s, OMe), and 6.95, 7.22, and 7.36 (2 H, 2 H, and 4 H, all t, CH_2·CH_2·CO).

We thank Drs. G. L. Hodgson, E. Hunt, and J. B. Paine, tert., for their contributions to the indicated experiments, and Professor G. W. Kenner and Dr. K. M. Smith, Liverpool, for samples of the four coproporphyrins. R. J. W. and J. S., respectively, thank the Roche Research Institute of Marine Pharmacology (Sydney) for leave and finances, and the S.R.C. for a research studentship. We are indebted to the Nuffield Foundation and the S.R.C. for financial support.

[5/1410 *Received*, 17*th July*, 1975]