Biosynthesis of Porphyrins and Related Macrocycles. Part 12.[1] Vitamin B_{12}: Studies with 1-Methylbilanes

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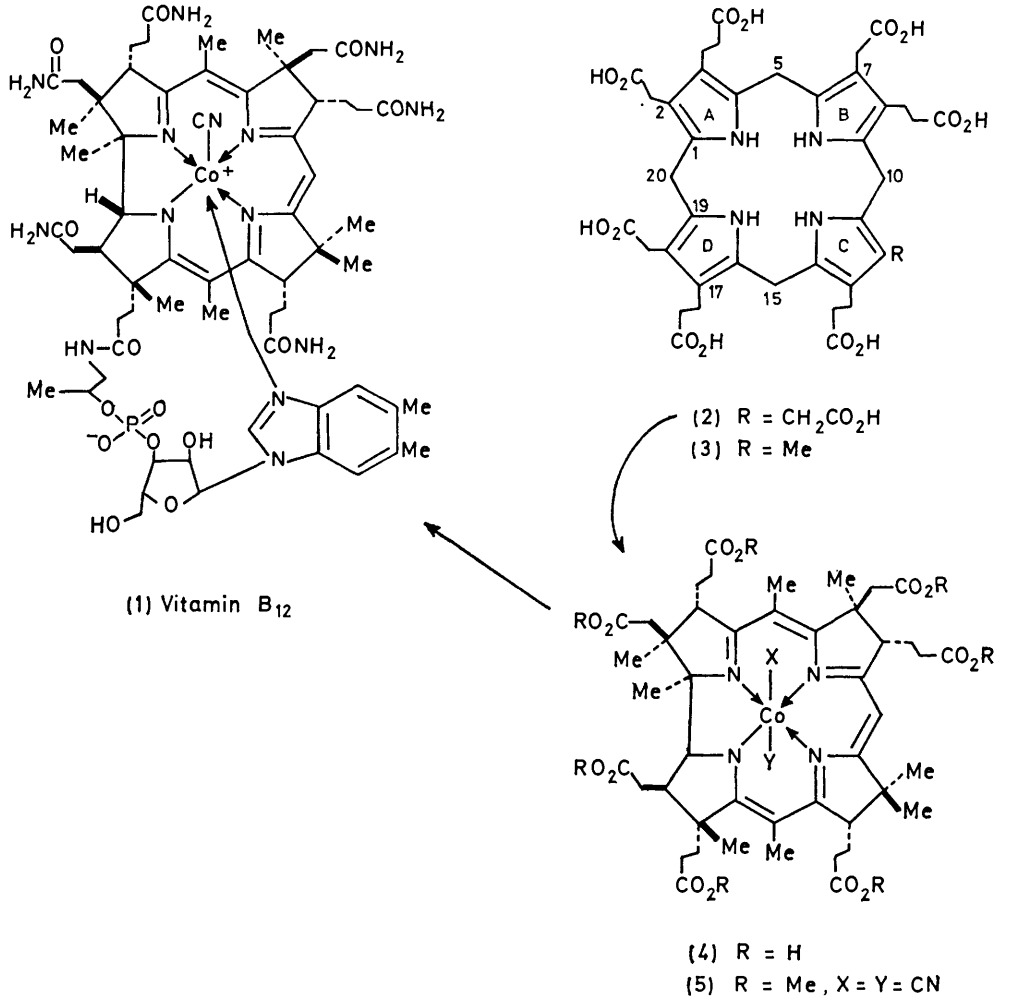
The search for intermediates which closely follow uro'gen-III (2) on the biosynthetic pathway to vitamin B_{12} (1) has involved studies with 1-methylbilanes. Four such bilanes, ^{14}C-labelled at the C-1 methyl group, have been synthesised by rational methods and tested as precursors of the corrin macrocycle [*e.g.* (4)] using an enzyme system from *Propionibacterium shermanii*. None of the bilanes was incorporated into (4) and this outcome is in full agreement with more recent studies.

EARLIER parts of this series [2,3] have described our work on the biosynthesis of vitamin B_{12} (1) including (*i*) incorporation of ^{13}C-labelled δ-aminolaevulinic acid and methionine together with stereochemical studies, (*ii*) the development of an effective broken-cell enzyme system which biosynthesises the corrin macrocycle [*e.g.* (4)], and (*iii*) rigorous proof that uro'gen-III (2) is a biosynthetic precursor of cobyrinic acid (4) and so of vitamin B_{12} itself (1) [4] (see Scheme 1). Similar independent and often complementary studies have appeared from several other laboratories.[5,6]

It is evident by inspection of the structures of uro'gen-III (2) and cobyrinic acid (4) that conversion of the former into the latter requires the following steps: (*a*) *C*-methylation at C-1, -2, -5, -7, -12, -15, and -17; (*b*) decarboxylation of the acetic acid residue at C-12; (*c*) extrusion of C-20; (*d*) bond formation between C-1 and C-19; (*e*) insertion of a cobalt atom; and (*f*) possible

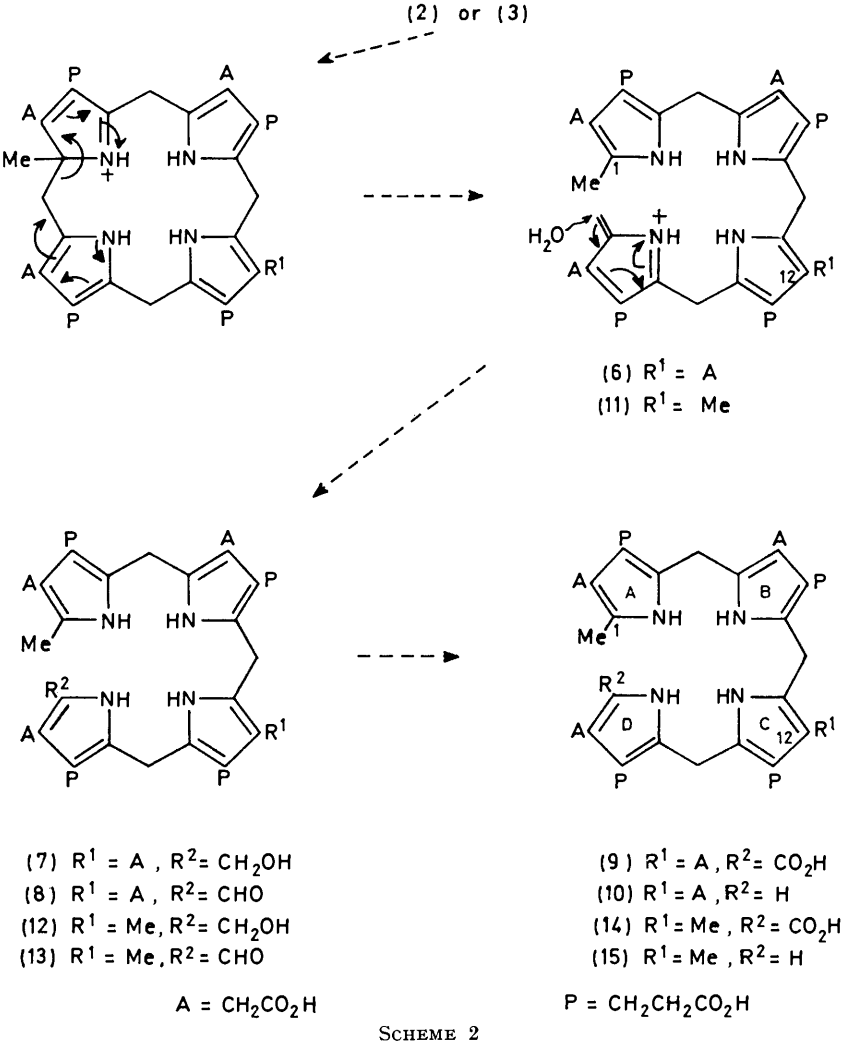


SCHEME 1

redox changes. These steps could in principle be carried out in a very large number of sequences * and it was therefore essential at this stage (1975) to focus attention on discovering the next reasonably stable intermediate on the pathway beyond uro'gen-III (2). Analysis of the operations (*a*)→(*f*) above, taking account of reactivity and mechanistic probability, led to the view [4] that the first step to take place on uro'gen-III (2) was probably one of the following: (*i*) *C*-methylation at C-1; (*ii*) decarboxylation of the acetic acid residue at C-12; or (*iii*) *C*-methylation at C-2, C-7, or C-17.

Possibility (*i*) could bring about cleavage of the macrocycle, as illustrated in Scheme 2, to yield the methylenepyrrolenine (6) which by reaction with water could form the hydroxymethylbilane (7). The aldehyde (8) and acid (9) are then available, in principle, by oxidation and the α-free bilane (10) could arise by obvious simple steps from (7), (8), or (9). Similar speculative schemes have been considered by others.[7] If one of these four bilanes (7), (8), (9), or (10) is a true biosynthetic intermediate between uro'gen-III (2) and the corrin ring system, then it should be enzymically incorporated into cobyrinic acid (4). It was possible, *a priori*, that several of the bilanes might be so incorporated since they represent a sequentially formed set.

Possibility (*ii*) would produce the heptacarboxylic acid (3) and methylation at C-1 of this macrocycle could similarly produce, *via* (11), the four 1,12-dimethylbilanes (12), (13), (14), and (15) (see Scheme 2). Because of the timing of our experiments with the various 1-methylbilanes, the 1,12-dimethyl series was included in the work and will be described here; however, it was subsequently proved [3] that decarboxylation at the C-12 acetic acid group is *not* the next step on the pathway beyond uro'gen-III (2) towards corrins, so this approach was a *cul-de-sac*. Finally, possibility (*iii*) will be referred to again at the end of this paper.

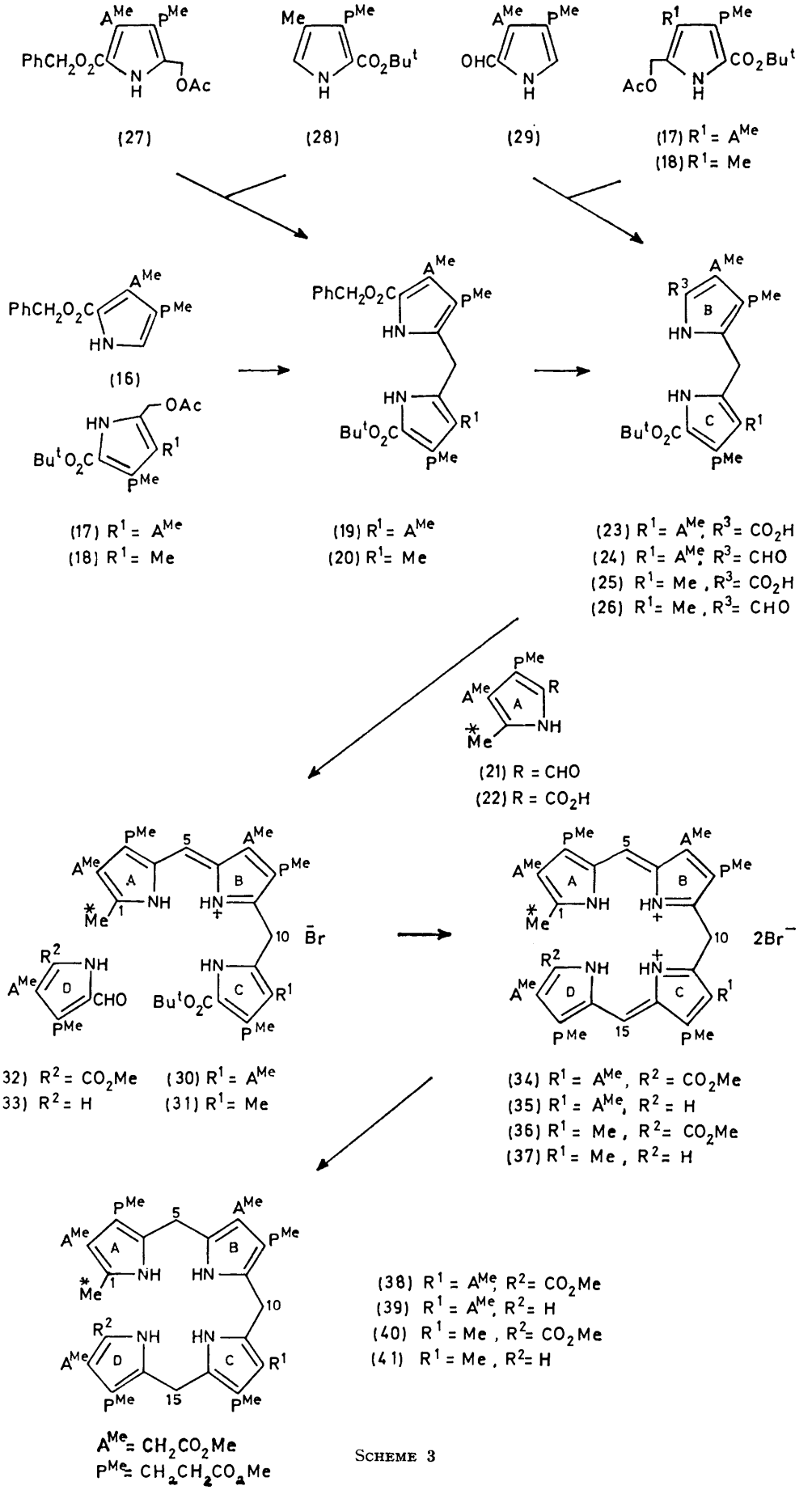


SCHEME 2

* Because the possibilities are so enormous, we have refrained from publishing our biogenetic speculations at each stage of the investigation, preferring first to test them rather than add confusion by publishing yet more unsupported schemes.

RESULTS AND DISCUSSION

The foregoing reasoning led to the initial plan to


(27)
(28)
(29)
(17) R1 = AMe
(18) R1 = Me
(16)
(17) R1 = AMe
(18) R1 = Me
(19) R1 = AMe
(20) R1 = Me
(23) R1 = AMe, R3 = CO2H
(24) R1 = AMe, R3 = CHO
(25) R1 = Me, R3 = CO2H
(26) R1 = Me, R3 = CHO
(21) R = CHO
(22) R = CO2H
(32) R2 = CO2Me
(33) R2 = H
(30) R1 = AMe
(31) R1 = Me
(34) R1 = AMe, R2 = CO2Me
(35) R1 = AMe, R2 = H
(36) R1 = Me, R2 = CO2Me
(37) R1 = Me, R2 = H
(38) R1 = AMe, R2 = CO2Me
(39) R1 = AMe, R2 = H
(40) R1 = Me, R2 = CO2Me
(41) R1 = Me, R2 = H
AMe = CH2CO2Me
PMe = CH2CH2CO2Me

SCHEME 3

carry out rational syntheses of the [1-^{14}C-*methyl*]bilanes (9), (10), (14), and (15) for incorporation experiments with the broken-cell enzyme system [3] from *Propionibacterium shermanii*. The bilanes were expected to be unstable substances,[8] so the approach was to be *via* the corresponding *a,c*-biladienes (34), (35), (36), and (37); substances of this type are relatively stable and are often crystalline.[9]

The various synthetic routes are summarised in Scheme 3. For one route, the pyrrole providing the final ring A was the aldehyde (21) and the unit for rings B and C was the pyrromethane acid (23) or (25), both being available from the differentially protected esters (19) and (20). The ester (19) was prepared by standard methods from the α-free pyrrole (16) and the ester (17) while ester (20) came from the α-free pyrrole (28) combined with the ester (27).

The second sequence involved conversion of the foregoing pyrromethane acids (23) and (25) into the aldehydes (24) and (26); these now allowed the ring A component to be the readily available acid (22). An alternative convenient preparation of the pyrromethane aldehydes (24) and (26) involved alkylation of the α-free aldehyde [10] (29) with one or other of the acetoxymethylpyrroles (17) or (18).

These different approaches all eventually led to the same pair of tripyrrene salts (30) and (31) and these, after removal of the t-butyl ester group with trifluoroacetic acid, were condensed with the aldehyde (32), or alternatively with (33), to yield the set of four crystalline *a,c*-biladiene hydrobromides (34), (35), (36), and (37).

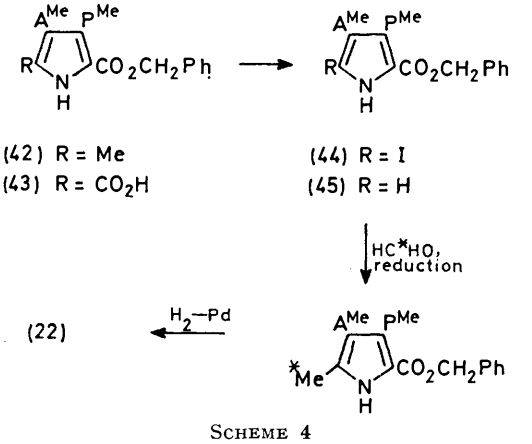
Several methods were studied for reduction of the *a,c*-biladienes to the required bilanes; *e.g.* (*a*) catalytic hydrogenation of the free biladienes liberated from their salts by a variety of inorganic and organic bases; and (*b*) chemical reduction with sodium amalgam or with borohydride. In this series, the best procedure was to treat a methanolic solution of the red dibromide salt with sodium acetate and immediately to hydrogenate the resultant green solution of the *a,c*-biladiene free base over pre-reduced Adams platinum catalyst. This afforded a virtually colourless solution of the bilane, *e.g.* (38), which was sufficiently stable for purification by careful chromatography. Each of the bilane esters (38), (39), (40), and (41) was fully characterised by n.m.r. and mass spectrometry, including accurate mass determination.

The foregoing synthetic routes were worked out with unlabelled materials. For the ^{14}C-labelled set of bilanes, we chose the second sequence above *via* the pyrromethane aldehydes (24) and (26), because the radiochemical yield for the synthesis of the acid (22) was much higher than for the aldehyde (21). The label was introduced from ^{14}C-formaldehyde by reductive methylation,[11] as shown in Scheme 4.

Repetition of the four syntheses using the ^{14}C-labelled acid (22) afforded the [1-^{14}C-*methyl*]bilanes (38)—(41). The required bilanepolycarboxylic acids (9), (10), (14), and (15), ^{14}C-labelled at the C-1 methyl group, were then prepared by alkaline hydrolysis immediately prior to the enzymic experiments.

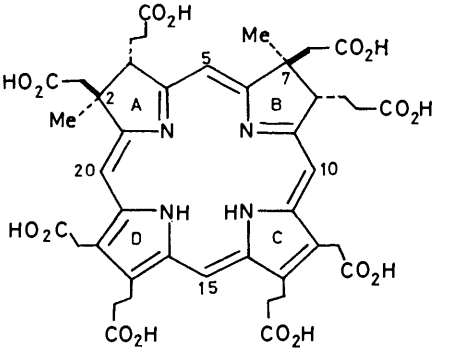
The bilane esters (39) and (41) have been independently synthesised in unlabelled form by Gossauer and Engel.[12]

Each ^{14}C-labelled bilanepolycarboxylic acid was incubated in the usual way [3] with the broken-cell enzyme system from *P. shermanii*; [3] unlabelled cobyrinic acid (4) was then added as carrier and the corrin was isolated as crystalline cobester (5). The efficacy of the enzyme preparation was demonstrated by a parallel run in which [12-*methylene*-^{14}C]uro'gen-III (as 2) was used as a precursor; the activity of the isolated cobester (5) corresponded to an incorporation of 2.9%. The clear outcome of these runs was that none of the four bilanes (9), (19), (14), and (15) was incorporated into the corrin macrocycle.



SCHEME 4

These findings are in complete agreement with recent work on the possibility [(*iii*)] considered at the outset, which was that the next step beyond uro'gen-III (2) might be *C*-methylation at C-2, C-7, or C-17. This work,[13] which post-dates the foregoing experiments on bilanes, has shown that possibility (*iii*) is in fact the correct one and that sirohydrochlorin (46), or its dihydroderivative, is an intermediate beyond uro'gen-III (2) on



(46)

the biosynthetic pathway to cobyrinic acid (4) and vitamin B_{12} (1).

EXPERIMENTAL

General directions are given in ref. 3.

Pyrroles

2-Formyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole (21).—A solution of the pyrrole [10] (42) (25 g) in methanol (200 ml) was shaken with hydrogen and 10% palladium–charcoal (0.4 g). When uptake of hydrogen ceased, the filtered solution was evaporated to give *3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylic acid* (22) (18.7 g), pure enough for use in the next stage. A sample was recrystallised from chloroform–light petroleum, m.p. 127—129 °C (Found: C, 55.0; H, 6.1; N, 4.8%; M^+, 283. $C_{13}H_{17}NO_6$ requires C, 55.1; H, 6.1; N, 5.0%; M, 283); $\nu_{max.}$ 3 430, 2 920, 1 725, and 1 655 cm^{-1}; $\lambda_{max.}$ 278 nm (ε 12 026); δ 2.24 (3 H, s, Me), 2.61 and 3.06 (each 2 H, t, J 7 Hz, 2 CH_2), 3.46 (2 H, s, CH_2CO_2), 3.65 and 3.67 (each 3 H, s, 2 CO_2Me), 8.3 and 9.01 (each 1 H, broad, CO_2H and NH).

This acid (3.8 g) in trifluoroacetic acid (20 ml) was stirred at 50 °C for 15 min and then cooled to 0 °C. Trimethyl orthoformate [14] (50 ml) was added and after the mixture had been stirred at 0 °C for 20 min, the temperature was raised to 20 °C for 55 min. Water (100 ml) was added and after stirring for 2 h at 0 °C, 2M-aqueous ammonia solution (400 ml) was added and the mixture was extracted with dichloromethane. The product was chromatographed on silica (250 g), eluant ether, and crystallised from benzene–hexane or chloroform–light petroleum to give the aldehyde (21) (69—78%), m.p. 112—114 °C (lit.,[15] m.p. 114 °C) (Found: C, 58.2; H, 6.4; N, 5.1%; M^+, 267. $C_{13}H_{17}NO_5$ requires C, 58.4; H, 6.4; N, 5.2%; M, 267); $\nu_{max.}$ 3 430, 3 240, 2 940, 2 830, 1 730, and 1 630 cm^{-1}; $\lambda_{max.}$ 268 and 309 nm (ε 4 581 and 13 793), δ 2.27 (3 H, s, Me), 2.59 and 3.05 (each 2 H, t, J 7 Hz, 2 CH_2), 3.42 (2 H, s, CH_2CO_2), 3.64 and 3.66 (each 3 H, s, CO_2Me), and 9.49 (1 H, s, CHO).

3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-[^{14}C]methylpyrrole-2-carboxylic Acid (22).—The required α-free pyrrole (45) had been prepared earlier [16] but an improved route was used here as follows. The benzyl ester (42) (1.12 g) in dry ether (30 ml) was mixed with sulphuryl chloride (1.35 g) and the solution was refluxed for 1 h. More sulphuryl chloride (0.27 g) was added and, after refluxing for a further 1 h, the solvent was evaporated, water–acetone (1 : 4) (60 ml) was added, and the solution was refluxed for 5 min. Sodium acetate (2.4 g) in water was then added and after heating the mixture under reflux for 5 min, the acetone was evaporated off and the suspension was extracted with chloroform. The extracted material was partitioned between water [(100 ml) containing sodium hydrogencarbonate (6 g)], and ether and the aqueous layer was acidified with 2N-hydrochloric acid. Chloroform extraction afforded the acid (43), m.p. 124—125 °C (0.67 g), after crystallisation from ether–hexane.

To a stirred mixture of this acid (6.05 g) in water (130 ml), sodium hydrogencarbonate (3.78 g), and dichloroethane (130 ml) was added a solution of iodine (4.32 g) in water (95 ml) and sodium iodide (4.5 g). After the mixture had been refluxed for 1 h, chloroform (300 ml), with sufficient sodium metabisulphite to remove excess of iodine, was added. The phases were well shaken and the organic phase was washed with dilute aqueous sodium metabisulphite and then with water. Evaporation of the chloroform and crystallisation of the residue from ether (50 ml)–hexane (100 ml) gave the iodopyrrole (44) (6.54 g), m.p. 100—101 °C.

A solution of this iodopyrrole (6.45 g) in methanol (500 ml) [containing sodium acetate (6.4 g), sodium hydroxide (532 mg), and water (5 ml)] was shaken with Adams platinum oxide (55 mg) and hydrogen for 2.5 h. The filtered solution was evaporated and the residue partitioned between chloroform and water. After the organic layer had been washed with saturated aqueous sodium hydrogencarbonate, it was dried and evaporated. Chromatography of the residue on silica (150 g) in benzyl–ethyl acetate (9 : 1) gave the α-free pyrrole (45), m.p. 61—62°, from ether–hexane (1 : 1) (yield 2.75 g); it was identified by direct comparison with the earlier sample,[16] m.p. 60—63 °C.

Unlabelled paraformaldehyde (10 mg) was added to the ampoule of [^{14}C]paraformaldehyde (0.5 mCi, 0.59 mg) and the mixture was dissolved in the whole of the following solution. Hypophosphorous acid (0.85 ml) was added dropwise at 0 °C to hydriodic acid (specific gravity 1.94, 2.2 ml) followed by acetic anhydride (2.2 ml). The reducing mixture containing the labelled aldehyde was then added to a solution at 0 °C of the α-free pyrrole (45) (125 mg) in acetic acid (3 ml) and stirred for 5 h. The mixture was then poured into water (90 ml) containing ammonia solution (specific gravity 0.880) (10 ml) and the product was extracted into chloroform. After the chloroform solution had been washed with saturated aqueous sodium chloride, it was evaporated and the residue was chromatographed on silica (20 g) in benzene–ethyl acetate (9 : 1) to yield the ^{14}C-labelled pyrrole (Scheme 4), m.p. 78—80 °C, from ether–hexane (33.7 mg), specific activity 660 μCi $mmol^{-1}$ (lit.,[16] m.p. 78.5—79.5 °C).

To the mother liquors was added unlabelled pyrrole (42) (10 mg) and on concentration a second crop (24.8 mg), m.p. 77—79 °C, was obtained, specific activity 470 μCi $mmol^{-1}$. This dilution of the mother liquors was repeated once more.

These three crops were mixed and the whole (99 mg; radiochemical yield 23%) was stirred in methanol (10 ml) with 5% palladium–charcoal (25 mg) and hydrogen for 1 h. Evaporation of the filtered solution and crystallisation of the residue from chloroform–hexane gave the 5-[^{14}C]methylpyrrolecarboxylic acid (22) (69.4 mg), specific activity 450 μCi $mmol^{-1}$, m.p. 124—127 °C, identified by comparison with an earlier unlabelled sample.[10]

Benzyl 5-Iodo-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate.—This has been prepared previously [3] as an intermediate for the α-free pyrrole (16) but has now been more fully characterised: m.p. 125—127 °C, from ether–light petroleum (Found: C, 46.7; H, 4.1; N, 2.6%; M^+, 485. $C_{19}H_{20}NO_6I$ requires C, 47.0; H, 4.2; N, 2.9%; M, 485); $\nu_{max.}$ 3 420, 2 940, 1 730, and 1 690 cm^{-1}; $\lambda_{max.}$ 253 and 282 nm (ε 6 626 and 17 942); δ 2.47 and 2.72 (each 2 H, t, J 7 Hz, CH_2), 3.58 and 3.66 (each 3 H, s, CO_2Me), 3.86 (2 H, s, CH_2CO_2), 5.28 (2 H, s, PhCH_2), 7.35 (5 H, s, $Ph$$CH_2$), and 9.20 (1 H, broad, NH).

t-Butyl 3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate.—The unlabelled pyrrolic acid [10] (22) (24 g) was stirred with thionyl chloride (40 ml) at 45 °C for 35 min and the residue from evaporation was treated with t-butyl alcohol (40 ml) and *NN*-dimethylaniline (35 ml). The mixture was stirred at 75 °C for 2 h, then cooled and, in dichloromethane (500 ml), was washed

with 2N-hydrochloric acid and then water. The product from the organic phase in ether was filtered through a short column of alumina and the residue from the percolate crystallised from ether–light petroleum to give the *t-butyl ester* (23.7 g), m.p. 126—127 °C (Found: C, 60.1; H, 7.4; N, 3.9%; M^+, 339. $C_{17}H_{25}NO_6$ requires C, 60.2; H, 7.4; N, 4.1%; *M*, 339); $\nu_{max.}$ 3 425, 2 900, and 1 730 cm^{-1}; $\lambda_{max.}$ 282 nm (ε 5 360); δ 1.54 (9 H, s, Bu^t), 2.23 (3 H, s, Me), 2.56 and 3.01 (each 2 H, t, *J* 7 Hz, CH_2), 3.42 (2 H, s, CH_2CO_2), 3.66 (6 H, s, 2 CO_2Me), and 9.96 (1 H, broad, NH).

t-Butyl 5-Acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate (17).—The foregoing ester (20.3 g) was stirred in acetic acid (300 ml) with lead tetra-acetate (32 g) for 9 h at 18 °C. The residue from evaporation was partitioned between water (500 ml) and dichloromethane and the material from evaporation of the organic phase was dissolved in ether and filtered through a short column of alumina. The product from the percolate was crystallised from ether–light petroleum to give the *acetoxymethylpyrrole* (18.5 g), m.p. 52—53 °C (Found: C, 57.6; H, 6.8; N, 3.5%; M^+, 397. $C_{19}H_{27}NO_8$ requires C, 57.4; H, 6.9; N, 3.5%; *M*, 397); $\nu_{max.}$ 3 420, 2 900, and 1 730 cm^{-1}; $\lambda_{max.}$ 272 nm (ε 16 907); δ 1.54 (9 H, s, Bu^t), 2.05 (3 H, s, MeCO), 2.56 and 3.01 (each 2 H, t, *J* 7 Hz, CH_2), 3.55 (2 H, s, CH_2CO_2), 3.65 and 3.67 (each 3 H, s, CO_2Me), 5.05 (2 H, s, OCH_2), and 9.22 (1 H, broad, NH).

Methyl 5-Formyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (32).—A stirred solution of methyl 4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate [16] (3.7 g) in dichloromethane (50 ml) was treated dropwise at 0 °C during 45 min with a solution of sulphuryl chloride (3 g) in dichloromethane (70 ml). After 15 min further stirring water–acetone (1 : 4) (100 ml) was added and the organic solvents were evaporated. The suspension was extracted with dichloromethane and the extracts were washed with saturated aqueous sodium hydrogencarbonate before evaporation. The residue was chromatographed on silica (200 g) in benzene–ethyl acetate (2 : 1) to give the *formylpyrrole* (2.4 g), m.p. 97—101 °C, from benzene–hexane * (Found: C, 53.8; H, 5.6; N, 4.3%; M^+, 311. $C_{14}H_{17}NO_7$ requires C, 54.0; H, 5.5; N, 4.5%; *M*, 311); $\nu_{max.}$ 3 410, 2 940, and 1 725 cm^{-1}; $\lambda_{max.}$ 235 and 301 nm (ε 11 049 and 16 134); δ 2.59 and 3.67 (each 2 H, t, *J* 7 Hz, CH_2), 3.63 and 3.68 (each 3 H, s, CO_2Me), 3.84 (2 H, s, CH_2CO_2), 3.85 (3 H, s, CO_2Me), and 9.81 (1 H, s, CHO).

2-Formyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole (33) (*with* Dr. R. Hollenstein *and* C. J. R. Fookes).—A solution of benzyl 5-formyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate [17] (3.5 g) in tetrahydrofuran (100 ml) containing triethylamine (4 drops) was shaken with hydrogen on 10% palladium–charcoal (220 mg) until uptake of hydrogen ceased. The filtered solution was evaporated and the residue was dissolved in water (100 ml) containing sodium hydrogencarbonate (5 g). 1,2-Dichloroethane (100 ml) was added and the mixture was heated under reflux while iodine (3 g) and sodium iodide (3.2 g) in water (30 ml) were added during 15 min. After refluxing had been continued for a further 45 min, the cooled organic layer was separated and washed with aqueous sodium metabisulphite and then with water. Evaporation of the organic phase left the crude 5-iodopyrrole which was used without further purification.

This product in tetrahydrofuran (60 ml) and methanol (60 ml) containing sodium acetate (2.2 g) was shaken with hydrogen and 10% palladium–charcoal (300 mg) until uptake ceased (22 h). The filtered solution was evaporated, the residue was extracted with chloroform and the inorganic matter was filtered off. Chromatography of the residue from the chloroform solution on alumina (eluant chloroform) gave the *aldehyde* (33) which was recrystallised from chloroform–ether–hexane (1.13 g), m.p. 82.0—82.5 °C (Found: *m/e* 253.095. $C_{12}H_{15}NO_5$ requires *m/e* 253.095); δ 2.62 and 3.09 (each 2 H, t, *J* 7 Hz; pyrrole-$CH_2CH_2CO_2Me$), 3.52 (2 H, s, pyrrole-CH_2CO_2Me), 3.66 and 3.72 (each 3 H, s, 2 OMe), 7.04 (1 H, d, *J* 3 Hz, pyrrole-H), 9.62 (1 H, s, CHO), and 9.9 (1 H, broad, NH).

t-Butyl 3-(2-Methoxycarbonylethyl)-4,5-dimethylpyrrole-2-carboxylate.—A solution of benzyl 3-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (3.15 g) in tetrahydrofuran (50 ml) was shaken with 5% palladium–charcoal (0.4 g) and hydrogen. When uptake of hydrogen ceased, the solution was filtered and then stirred at 20 °C for 12 h with t-butyl alcohol (15 ml) and dicyclohexylcarbodiimide [18] (3.3 g). The filtered solution was evaporated, the residue was freed from urea, and the product was crystallised from ether–light petroleum to give the title t-butyl ester (2.5 g), m.p. 91—92 °C (lit.,[19] m.p. 99—100 °C) (Found: C, 64.2; H, 8.3; N, 4.9%; M^+, 281. $C_{15}H_{23}NO_4$ requires C, 64.0; H, 8.2; N, 5.0%; *M*, 281); $\nu_{max.}$ 3 440, 2 910, 1 727, and 1 670 cm^{-1}; $\lambda_{max.}$ 249 and 285 nm (ε 5 123 and 15 620); δ 1.58 (9 H, s, Bu^t), 1.95 and 2.20 (each 3 H, s, Me), 2.52 and 3.02 (each 2 H, t, *J* 8 Hz, CH_2), 3.69 (3 H, s, CO_2Me), and 8.98 (1 H, broad, NH).

t-Butyl 5-Acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (18).—The foregoing product (2.5 g) in acetic acid (20 ml) was stirred at 18 °C for 16 h with lead tetra-acetate (4 g) and the solvent was then evaporated. The residue was partitioned between ether and water and the separated organic phase was washed with saturated aqueous sodium hydrogencarbonate. The product from the ether was crystallised from ether–light petroleum to yield the *acetoxymethylpyrrole* (2.4 g), m.p. 88—89 °C (Found: C, 60.3; H, 7.6; N, 4.1%; *M*, 339. $C_{17}H_{25}NO_6$ requires C, 60.2; H, 7.4; N, 4.1%; *M*, 339); $\nu_{max.}$ 3 430, 2 910, 1 725, and 1 680 cm^{-1}; $\lambda_{max.}$ 275 nm (ε 14 917); δ 1.58 (9 H, s, Bu^t), 2.06 (6 H, s, Me and OAc), 2.51 and 3.02 (each 2 H, t, *J* 8 Hz, CH_2), 3.68 (3 H, s, CO_2Me), 5.03 (2 H, s, CH_2O), and 9.32 (1 H, broad, NH).

Pyrromethanes

5-Benzyl 5′-t-Butyl 3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bis(methoxycarbonylmethyl)pyrromethane-5,5′-dicarboxylate (19).—A solution of the pyrrole [3] (16) (0.91 g) and the foregoing acetoxymethylpyrrole (17) (1.01 g) in dichloromethane (20 ml) was stirred under nitrogen at 20 °C with toluene-*p*-sulphonic acid monohydrate [20] (35 mg). After being shaken with excess of saturated aqueous sodium hydrogencarbonate, the organic layer was evaporated and the residue was chromatographed on alumina (5 g) in dichloromethane. The product was crystallised from ether–hexane to give the *pyrromethane* (19), m.p. 98—100 °C (1.06 g) (Found: C, 61.9; H, 6.4; N, 4.0%; M^+, 696. $C_{36}H_{44}N_2O_{12}$ requires C, 62.1; H, 6.3; N, 4.0%; *M*, 696); $\nu_{max.}$ 3 420, 2 960, 1 725, and 1 685 cm^{-1}; $\lambda_{max.}$ 241, 273, and 289 nm (ε 10 098, 24 857, and 32 003); δ 1.50

* The m.p. of this substance had by transcription error been incorrectly reported [16] as 147—148 °C.

(9 H, s, Bu^t), 2.4—3.1 (8 H, m, 4 × CH_2), 3.54 (2 H, s, CH_2CO_2Me), 3.60, 3.61, 3.65, and 3.73 (each 3 H, s, CO_2Me), 3.82 (2 H, s, CH_2CO_2Me), 3.93 (2 H, s, CH_2), 5.24 (2 H, s, $PhCH_2$), 7.3 (5 H, m, aromatic-*H*), and 9.6 and 10.05 (each 1 H, broad, NH). The above preparation was also carried out equally successfully using acetic acid as initial solvent in place of dichloromethane.

3,4′-Bis-(2-methoxycarbonylethyl)-3′,4-bis(methoxycarbonylmethyl)-5′-t-butyloxycarbonylpyrromethane-5-carboxylic Acid (23).—The foregoing product (6.84 g) in methanol (200 ml) was shaken with 5% palladium–charcoal (200 mg) and hydrogen until uptake of hydrogen ceased. The filtered solution was evaporated and the residue was crystallised from ether or ether–hexane to yield the *pyrromethane acid* (23), m.p. 135—137 °C (5.5 g) (Found: C, 57.1; H, 6.2; N, 4.4%; *m/e* 561, $[M - CO_2H]^+$. $C_{29}H_{38}N_2O_{12}$ requires C, 57.4; H, 6.3; N, 4.6; *M*, 606); $\nu_{max.}$ 3 300, 3 220, 2 940, 1 730, and 1 665 cm^{-1}; $\lambda_{max.}$ 250 and 282 nm; δ 1.55 (9 H, s, Bu^t), 2.3—3.1 (8 H, m, CH_2), 3.63 (2 H, s, CH_2CO_2), 3.68 (3 H, s, CO_2Me), 3.71 (6 H, s, 2 CO_2Me), 3.71 (3 H, s, CO_2Me), 3.85 (2 H, s, CH_2CO_2), 3.98 (2 H, s, CH_2), and 8.76 and 9.14 (each 1 H, broad, NH).

5′-t-Butyl 5-Formyl-3,4′-bis-(2-methoxycarbonylethyl)-3′,4-bis(methoxycarbonylmethyl)pyrromethane-5′-carboxylate (24).—A solution of the foregoing pyrromethane acid (2.05 g) in dichloromethane (50 ml) and methanol (10 ml) containing toluene-*p*-sulphonic acid monohydrate (1 g) was stirred at 18 °C for 8 h. The solution was then washed with saturated aqueous sodium hydrogencarbonate, dried, and evaporated. The residue, in the minimum volume of *NN*-dimethylformamide, was added to a mixture of benzoyl chloride (15 ml) in *NN*-dimethylformamide (50 ml) and calcium carbonate (5 g). After the mixture had been stirred at 20 °C for 2 h, a solution of sodium acetate (15 g) in water (50 ml) was added and after 25 min of stirring, water (50 ml) and chloroform (100 ml) were added, shaken, and the organic phase was separated. It was washed with saturated aqueous sodium hydrogencarbonate, the residue from evaporation of the chloroform was dissolved in ether and the solution was washed twice with water, dried, and evaporated. Chromatography of the product on silica (300 g) in benzene–ethyl acetate (3 : 1) gave the *formylpyrromethane* (24) as a gum (1.68 g) (Found: *M*, 590.245 0. $C_{29}H_{38}N_2O_{11}$ requires *M*, 590.247 3); $\nu_{max.}$ 3 420, 2 920, 1 730, and 1 680 cm^{-1}; $\lambda_{max.}$ 276 and 309 nm; δ 1.54 (9 H, s, Bu^t), 2.2—3.1 (8 H, m, CH_2), 3.56 and 3.75 (each 2 H, s, CH_2CO_2), 3.66, 3.70, 3.73, and 3.80 (each 3 H, s, CO_2Me), 3.97 (2 H, s, CH_2), 9.58 (1 H, s, CHO), and 9.70 (1 H, broad, NH).

The same material was also prepared by stirring for 1.5 h at 20 °C a solution of 4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carbaldehyde [10] (29) (0.5 g) with the acetoxymethylpyrrole (17) (0.8 g) in dichloromethane (75 ml) containing toluene-*p*-sulphonic acid monohydrate (0.45 g). The solution was then washed with saturated aqueous sodium hydrogencarbonate and worked up as above; yield 0.74 g, identical with foregoing product.

5′-t-Butyloxycarbonyl-3,4′-bis-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-3′-methylpyrromethane-5-carboxylic Acid (25).—A solution of the α-free pyrrole (28) (5.9 g) and the acetoxymethylpyrrole [10,21] (27) (9.5 g) in dichloromethane (200 ml) was stirred at 20 °C for 2 h with toluene-*p*-sulphonic acid monohydrate (0.85 g). The product (20) was isolated and hydrogenated and worked up as above to give the *pyrromethane acid* (25) (9.3 g), m.p. 165—166 °C (Found: C, 58.9; H, 6.4; N, 4.9%; M^+, 548. $C_{27}H_{36}N_2O_{10}$ requires C, 59.1; H, 6.6; N, 5.1%; *M*, 548); $\nu_{max.}$ 3 300, 3 240, 2 940, 1 730, and 1 665 cm^{-1}; $\lambda_{max.}$ 242m, 273, and 290 nm (ε 10 299, 22 166, and 27 430); δ 1.54 (9 H, s, Bu^t), 2.10 (3 H, s, Me), 2.2—3.1 (8 H, m, CH_2), 3.67 (9 H, s, 3 CO_2Me), 3.85 (2 H, s, CH_2CO_2), and 3.91 (2 H, s, CH_2).

5′-t-Butyl 5-Formyl-3,4′-bis-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-3′-methylpyrromethane-5′-carboxylate (26).—This was prepared from the foregoing pyrromethane acid by the same procedures used for synthesis of the aldehyde (24) above. The formylpyrromethane was obtained as a gum (81% yield) (Found: M^+, 532.238 6. $C_{27}H_{36}N_2O_6$ requires *M*, 532.241 9); $\nu_{max.}$ 3 420, 2 920, 2 840, 1 730, 1 675, and 1 640 cm^{-1}; $\lambda_{max.}$ 281 and 311 nm; δ 1.53 (9 H, s, Bu^t), 2.01 (3 H, s, Me), 2.3—3.1 (8 H, m, CH_2), 3.67 (6 H, s, 2 CO_2Me), 3.71 (3 H, s, CO_2Me), 3.76 (2 H, s, CH_2CO), 4.00 (2 H, s, CH_2), 9.53 (1 H, s, CHO), and 10.16 and 11.10 (each 1 H, broad, NH). The same material was also obtained by reacting the formylpyrrole [10] (29) (0.25 g) with the acetoxymethylpyrrole (18) (0.35 g), essentially as for the alternative preparation of pyrromethane (24) described above; the yield of pyrromethane (26) was 69%.

Tripyrrenes

t-Butyl 3,8,13-Tris-(2-methoxycarbonylethyl)-2,7,12-tris-(methoxycarbonylmethyl)-1-methyltripyrrene-a-14-carboxylate Hydrobromide (30).—*Method* (*a*). A solution of the pyrromethane acid (23) (606 mg) and the aldehyde (21) (267 mg) in dry dichloromethane (120 ml) were mixed with a solution of toluene-*p*-sulphonic acid monohydrate (761 mg) in dry methanol (30 ml) and stirred under nitrogen in the dark at 18 °C for 17 h. Dichloromethane (500 ml) was then added, the solution was washed with saturated aqueous sodium hydrogencarbonate, dried, and mixed with 48% hydrogen bromide in acetic acid (510 mg). The residue from evaporation was dissolved in benzene and the filtered solution was diluted with ether to produce red-orange crystals of the *tripyrrene* (580 mg), m.p. 165—167 °C (decomp.) (Found: C, 54.8; H, 6.1; N, 4.8. $C_{41}H_{54}BrN_3O_{14}$ requires C, 55.05; H, 6.1; N, 4.7%); δ 1.54 (9 H, s, Bu^t), 2.28—3.14 (12 H, m, 3 $CH_2CH_2CO_2$), 2.72 (3 H, s, 1-Me), 3.52, 3.59, and 3.69 (each 2 H, s, 3 CH_2CO_2), 3.61—3.71 (18 H, overlapping, 6 CO_2Me), 4.47 (2 H, s, CH_2), 7.53 (1 H, s, methene), and 10.34, 13.51, and 13.58 (each 1 H, br, NH).

Method (*b*). To a stirred solution of the [^{14}C]methylpyrrole (22) (69.4 mg) and the formylpyrromethane (24) (150 mg) in dry dichloromethane was added a solution of toluene-*p*-sulphonic acid monohydrate (186 mg) in methanol (6 ml). After the mixture had been stirred for 17 h, it was worked up as above to yield the [1-^{14}C-*methyl*]tripyrrene (30) (184 mg), identical apart from radioactivity with the previous sample.

t-Butyl 3,8,13-Tris-(2-methoxycarbonylethyl)-2,7-bis(methoxycarbonylmethyl)-1,12-dimethyltripyrrene-a-14-carboxylate Hydrobromide (31).—*Method* (*a*). A solution of the pyrromethane acid (25) (1.15 g) and the aldehyde (21) (0.57 g) in methanol (10 ml) at 0 °C was treated with 48% hydrogen bromide in acetic acid (1.4 ml) and after the mixture had been stirred at 0 °C for 10 min, the red-orange *tripyrrene hydrobromide* (1.3 g) was collected, m.p. 142—143 °C, from ethyl acetate–light petroleum [Found: C, 56.1; H, 6.4; N, 4.8; Br, 9.4%; M^+, 754 (—HBr). $C_{39}H_{52}BrN_3O_{12}$

requires C, 56.2; H, 6.2; N, 5.0; Br, 9.6%; *M*, 754]; $\nu_{max.}$ 3 140, 2 940, 1 730, and 1 675 cm^{-1}; $\lambda_{max.}$ 280, 362, and 498 nm (ε 16 051, 5 388, and 68 244); δ 1.54 (9 H, s, Bu^t), 2.04 (3 H, s, 12-Me), 2.2—3.1 (6 H, m, CH_2), 2.71 (3 H, s, 1-Me), 3.50 (2 H, s, CH_2CO_2), 3.63, 3.66, 3.68, and 3.70 (15 H, s, 5 CO_2Me), 3.78 (2 H, s, CH_2CO_2), 4.38 (2 H, s, CH_2), 7.50 (1 H, s, methene), 10.2, 13.5, and 13.6 (each 1 H, broad, NH). Conditions essentially the same as those used above [method (*a*)] for the tripyrrene (30) were also used equally successfully.

Method (*b*). The pyrromethane aldehyde (26) (14 mg) and the acid (22) (7 mg) were condensed together as in method (*a*) above to yield the same tripyrrene hydrobromide (16 mg). This method was used for synthesis of the ^{14}C-labelled materials.

Biladienes and Bilanes

Methyl 3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,12,18-*tetrakis*(*methoxycarbonylmethyl*)-1-*methylbiladiene*-a,c-19-*carboxylate Dihydrobromide* (34).—To a stirred solution of the tripyrrene dihydrobromide (30) (178 mg) and the aldehyde (32) (62 mg) in trifluoroacetic acid (5 ml) was added 45% hydrogen bromide in acetic acid (20 drops). The mixture was stirred in the dark under nitrogen for 2.5 h, then evaporated and the residue was dissolved in dichloromethane (2 ml). Hydrogen bromide in acetic acid (6 drops, 45%) was added followed gradually by ether (20 ml) to give the *biladiene dihydrobromide* (34) (203 mg), which crystallised from dichloromethane–ether as brown-red needles, m.p. 139—142 °C (decomp.) (Found: C, 51.7; H, 5.4; N, 5.0. $C_{50}H_{62}Br_2N_4O_{18}$ requires C, 51.5; H, 5.2; N, 4.8%); δ 2.75 (3 H, s, 1-CH_3), 3.32, 3.43 (each 3 H, s, 2 CO_2Me), 3.53, 3.70, 3.90, and 3.94 (each 2 H, s, 4 CH_2CO_2), 3.63—3.73 (18 H, overlapping, 6 CO_2Me), 4.00 (3 H, s, 19-CO_2Me), 5.46 (2 H, br s, bridge CH_2), and 7.57 and 8.00 (each 1 H, s, methenes).

3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,12,18-*tetrakis*(*methoxycarbonylmethyl*)-1-*methylbiladiene*-a,c *Dihydrobromide* (35).—This was prepared from the salt (30) (367 mg) and the aldehyde (33) (105 mg) in trifluoroacetic acid (7.5 ml) and 45% hydrogen bromide in acetic acid (*ca.* 480 mg), essentially as for the previous preparation. The *biladiene dihydrobromide* (35) crystallised as above (404 mg) was orange-red, m.p. 108—110 °C (decomp.) (Found: C, 51.9; H, 5.45; N, 5.3. $C_{48}H_{60}Br_2N_4O_{16}$ requires C, 52.0; H, 5.45; N, 5.05%); δ 2.73 (3 H, s, 1-Me), 3.27, 3.38 (each 3 H, s, CO_2Me), 3.52, 3.57, 3.77, and 3.87 (each 2 H, s, 4 CH_2CO_2), 3.62 (9 H, s, 3 CO_2Me), 3.67 (3 H, s, CO_2Me), 3.71 (6 H, s, 2 CO_2Me), 5.27 (2 H, s, bridge-CH_2), 7.54 (1 H, s, methene), and 7.87 (2 H, br s, methene + 19-H).

Methyl 3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,18-*tris*(*methoxycarbonylmethyl*)-1,12-*dimethylbiladiene*-a,c-19-*carboxylate Dihydrobromide* (36).—The tripyrrene salt (31) (124 mg) and the aldehyde (32) (56 mg) were stirred in trifluoroacetic acid (1.5 ml) with 45% hydrogen bromide in acetic acid (0.1 ml) for 45 min at 18 °C in the dark under nitrogen. Addition of ether caused crystallisation of the *biladiene dihydrobromide* (151 mg) which was recrystallised from methyl acetate, m.p. 126—130 °C (decomp.) [Found: C, 51.7; H, 5.5; N, 5.2%; M^+, 947 (−2HBr). $C_{48}H_{60}Br_2N_4O_{16}$ requires C, 52.0; H, 5.5; N, 5.1%; *M*, 947]; $\nu_{max.}$ 2 950, 1 730, and 1 610 cm^{-1}; $\lambda_{max.}$ (for chloroform solution containing HBr) 373, 456, and 526 nm (ε 12 808, 36 696, and 118 067); δ 2.0—3.2 (16 H, m, CH_2), 2.01 (3 H, s, 12-Me), 2.74 (3 H, s, 1-Me), 3.45 (3 H, s, CO_2Me), 3.55 (2 H, s, CH_2CO_2), 3.62, 3.64, 3.70, and 3.71 (18 H, s, 6 CO_2Me), 3.82 and 3.88 (each 2 H, s, CH_2CO_2), 3.99 (3 H, s, CO_2Me), 5.47 (2 H, s, CH_2), 7.53 and 7.92 (each 1 H, s, methenes), and 13.6 and 13.7 (each 2 H, broad, NH).

3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,18-*tris*-(*methoxycarbonylmethyl*)-1,12-*dimethylbiladiene*-a,c *Dihydrobromide* (37).—This was prepared as in the preceding experiment from the tripyrrene salt (31) (110 mg) and the aldehyde (33) (35 mg); the *biladiene dihydrobromide* (122 mg) crystallised from methyl acetate, m.p. 145—148 °C (Found: C, 52.7; H, 5.6; N, 5.4. $C_{46}H_{58}Br_2N_4O_{14}$ requires C, 52.6; H, 5.6; N, 5.3%); $\nu_{max.}$ 2 940, 1 730, and 1 610 cm^{-1}; $\lambda_{max.}$ (for chloroform solution containing HBr) 372, 457, and 523 nm (ε 14 674, 28 632, and 140 298); δ 1.9—3.2 (12 H, m, CH_2), 1.99 (3 H, s, 12-Me), 2.74 (3 H, s, 1-Me), 3.41 (3 H, s, CO_2Me), 3.51 and 3.55 (each 2 H, s, CH_2CO_2), 3.61, 3.63, 3.68, and 3.71 (18 H, s, 6 CO_2Me), 3.79 (2 H, s, CH_2CO_2), 5.33 (2 H, s, CH_2), 7.51 and 7.68 (each 1 H, s, methene), 7.70 (1 H, d, 19-H), and 13.7 (4 H, broad, NH).

Methyl 3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,12,18-*tetrakis*(*methoxycarbonylmethyl*)-1-*methylbilane*-19-*carboxylate* (38).—To a solution of the biladiene dihydrobromide (34) (105 mg) in methanol (20 ml) was added a saturated solution of hydrated sodium acetate in methanol (1.8 ml). The green solution was *immediately* added to a suspension of pre-reduced platinum (prepared from 90 mg PtO_2) in methanol (10 ml) and shaken under hydrogen at room temperature and pressure. When uptake of hydrogen ceased, the solution was virtually colourless. It was filtered, mixed with sodium hydrogencarbonate (15 mg) and water (1 ml), and then evaporated. The residue was partitioned between dichloromethane and water and the product from the organic layer was quickly chromatographed on silica (25 g) in ethyl acetate–benzene (1 : 2). The product was precipitated (micro-crystals) from benzene by hexane to give the *bilane ester* (38) (35 mg), m.p. 99—101 °C (Found: C, 59.6; H, 6.5; N, 5.3; M^+, 1 008.422 0. $C_{50}H_{64}N_4O_{18}$ requires C, 59.5; H, 6.4; N, 5.55%; *M*, 1 008.421 0); δ 2.10 (3 H, s, 1-Me), *ca.* 2.14 and *ca.* 2.7 (16 H, m, 4 $CH_2CH_2CO_2Me$), 3.36—3.85 (45 H, m, 9 OMe + 4 CH_2CO, 3 bridging CH_2), and 8.6—9.3 (4 H, broad m, 4 NH). The ^{14}C-labelled material (38) was prepared exactly as above; specific activity 460 μCi $mmol^{-1}$.

3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,12,18-*tetrakis*(*methoxycarbonylmethyl*)-1-*methylbilane* (39).—This was prepared as above from the biladiene dihydrobromide (35) (99 mg) to give the *bilane* (39) (23 mg), m.p. 121—124 °C (Found: C, 60.7; H, 6.7; N, 5.65; M^+, 950.415 9. $C_{48}H_{62}N_4O_{16}$ requires C, 60.6; H, 6.6; N, 5.9%; *M*, 950.415 9); δ 2.10 (3 H, s, 1-Me), *ca.* 2.4—2.7 (16 H, m, CH_2CH_2CO), 3.3—3.8 (38 H, m, 8 OMe + 4 CH_2CO_2Me + 3 bridging CH_2), 6.47 (1 H, d, *J* 2 Hz, 19-H), and 8.27, 8.40, 8.75, and 8.87 (each 1 H, s, 4 NH). The ^{14}C-labelled series gave bilane (39) of specific activity 496 μCi $mmol^{-1}$.

Methyl 3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,18-*tris*(*methoxycarbonylmethyl*)-1,12-*dimethylbilane*-19-*carboxylate* (40).—The biladiene dihydrobromide (36) (66 mg) was converted into the corresponding base and hydrogenated as above to yield the *bilane* (40) as a gum (48 mg) (Found: M^+, 950.414 0. $C_{48}H_{62}N_4O_{16}$ requires *M*, 950.415 9); δ 1.83 and 2.10 (each 3 H, s, Me), 2.4—2.8 (16 H, m, CH_2), 3.35 and 3.43 (each 2 H, s, CH_2), 3.51 (4 H, s, CH_2 and CH_2CO_2), 3.59, 3.61, 3.66, 3.68, 3.71, and 3.74 (24 H, s, 8 CO_2Me), 3.79 and 3.83 (each 2 H, s, CH_2CO_2), 7.22, 7.42, 7.82, and

8.09 (each 1 H, broad, NH). The radioactive sample prepared similarly had specific activity 653 μCi mmol^{-1}.

3,8,13,17-*Tetrakis*-(2-*methoxycarbonylethyl*)-2,7,18-*tris*-(*methoxycarbonylmethyl*)-1,12-*dimethylbilane* (41).—The biladiene salt (37) (45 mg) was converted as above into the corresponding *bilane* (41) as a gum (48 mg) (Found: M^+, 892.408 2. $C_{46}H_{60}N_4O_{14}$ requires M, 892.410 5); δ 1.91 and 2.11 (each 3 H, s, Me), 2.2—2.5 (16 H, m, CH_2), 3.43 (4 H, s, 2 CH_2), 3.48 and 3.55 (each 2 H, s, CH_2CO_2), 3.60, 3.64, 3.67, and 3.71 (21 H, s, 7 CO_2Me), 3.78 (2 H, s, CH_2CO_2), 6.48 (1 H, d, 19-H), 7.98, 8.42, and 8.90 (4 H, broad, NH). The labelled sample prepared identically had specific activity 667 μCi mmol^{-1}.

Incorporation Experiments using Enzymes from P. shermanii.—Each labelled bilane (9), (10), (14), and (15) was tested as a precursor of cobyrinic acid in exactly the same way, which will be described for the bilane (9).

The [^{14}C]bilane ester (38) (total activity 5.34 × 10^6 disintegrations min^{-1}) was stirred with tetrahydrofuran (3 ml) and aqueous 2N potassium hydroxide (3 ml) under nitrogen in the dark for 24 h. The separated aqueous layer was washed twice with tetrahydrofuran (3 ml) and then neutralised with 10% aqueous hydrochloric acid. After adjustment to pH 8 with 2N potassium hydroxide, the solution was incubated with one batch of the broken-cell enzyme preparation from *P. shermanii* [3] at 37 °C for 24 h. Cobyrinic acid (30 mg) was added and the mixture was worked up in the usual way [3] with isolation of the cobester (5).

For all four bilanes tested, the isolated cobester was essentially radioinactive, in contrast to a good incorporation (2.9%) from [12-*methylene*-^{14}C]uro'gen-III (as 2) in a parallel run.

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