Biosynthesis of Porphyrins and Related Macrocycles. Part 25.[1] Synthesis of Analogues of Coproporphyrinogen-III and Studies of their Interaction with Coproporphyrinogen-III Oxidase from *Euglena gracilis*

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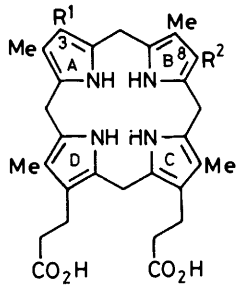
Analogues of coproporphyrinogen-III have been synthesized in which the propionate groups respectively on ring-A and on ring-B are modified either by homologation or esterification. Coproporphyrinogen-III oxidase from *Euglena gracilis* acts on the analogues which possess normal substituents on ring-A to generate a vinyl group on that ring. The enzyme does not affect the analogues in which the ring-A propionate group has been changed.

Conditions have been defined for the MacDonald synthesis of porphyrins which yield products of high isomeric purity.

Coproporphyrinogen-III oxidase catalyses decarboxylation of coproporphyrinogen-III (copro'gen-III) (**1**) to afford protoporphyrinogen-IX (proto'gen-IX) (**2**), the penultimate precursor of protohaem which is the prosthetic group of many haem proteins. The propionate group at position-3 of the substrate (**1**) is transformed into a vinyl group before that at position-8, and 8-carboxyethyl-3-vinyldeuteroporphyrinogen (harderoporphyrinogen) (**3**) can be detected as an enzyme-free intermediate.[2-4] Each propionate side chain is processed enzymically in a stereospecific fashion, through an antiperiplanar elimination of carbon dioxide and the *Si*-hydrogen atom at the methylene group adjacent to the macrocycle.[5-8]

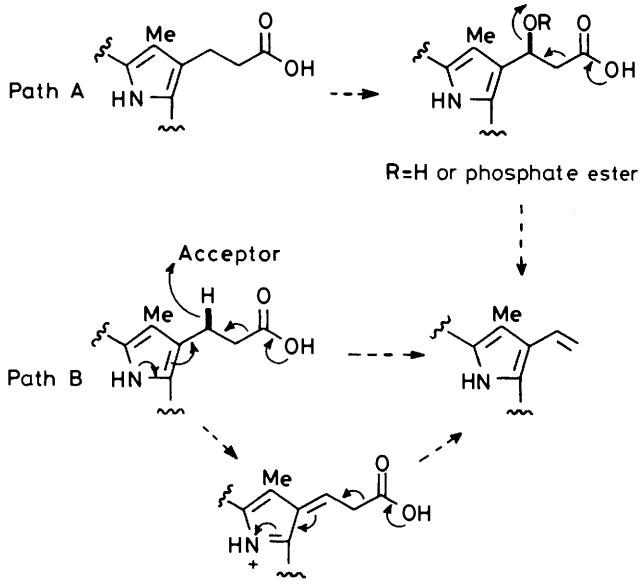


(**1**) $R^1 = P^H$, $R^2 = P^H$
(**2**) $R^1 = CH{=}CH_2$, $R^2 = CH{=}CH_2$
(**3**) $R^1 = CH{=}CH_2$, $R^2 = P^H$
(**4**) $R^1 = CHCH_2CO_2H$ (OH), $R^2 = P^H$
(**5**) $R^1 = B^H$, $R^2 = P^H$
(**6**) $R^1 = P^H$, $R^2 = B^H$
(**7**) $R^1 = P^{Me}$, $R^2 = P^H$
(**8**) $R^1 = P^H$, $R^2 = P^{Me}$

$P^H = CH_2CH_2CO_2H$
$P^{Me} = CH_2CH_2CO_2Me$
$B^H = CH_2CH_2CH_2CO_2H$

The recent purification of coproporphyrinogen-III oxidase from bovine liver[9] has shown that the enzyme contains no metal ions, does not require haem, flavin, nor pyridine nucleotide coenzymes and uses only molecular oxygen as a final electron acceptor. Moreover, a tyrosine residue at the active site has been implicated in the oxidation process.[10] The exact mechanism for formation of the vinyl group is still uncertain but the intermediacy of a β-hydroxypropionate side chain has found support.[11] In particular, the 3-β-hydroxypropionate derivative (**4**) of synthetic origin was transformed very efficiently by the purified bovine enzyme into proto'gen-IX.[10] Plausible steps for this possible sequence are shown as Path A in Scheme 1.

Coproporphyrinogen-III oxidase activity is also present in strictly anaerobic bacteria[12] which suggests that more than one mechanistic path has evolved to generate protoporphyrinogen-IX (**2**) necessary for the formation of cytochromes, chlorophylls, and bacteriochlorophylls. For example, a single protein fraction isolated from yeast has been reported[13] to catalyse both the aerobic and anaerobic conversion of copro'gen-III into proto'gen-IX, although the latter process requires the presence of several co-factors. A mechanism involving removal of hydride from the β-carbon atom[14] may be operative in the anaerobic process, Path B, Scheme 1.

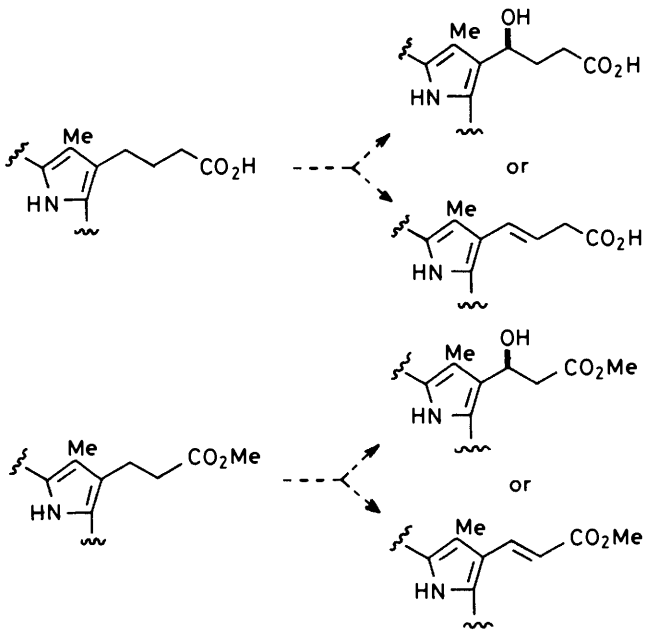


Scheme 1. Two possible paths for the oxidative decarboxylation of propionate groups

Although copro'gen-III is the natural substrate for coproporphyrinogen-III oxidase, the enzyme is not absolutely specific and it will also catalyse the conversion of copro'gen-IV into proto'gen-XIII,[15,16] of 8-propionyl deuteroporphyrinogen-IX

into 8-vinyldeuteroporphyrinogen-IX,[17] and of mesoporphyrinogen-IV into protoaetioporphyrinogen.[18]

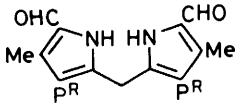
The plan in the present work was to test the action of coproporphyrinogen-III oxidase on substrates which had been structurally modified so as to intercept sequences of the type illustrated in Scheme 1. Thus it was conceivable that the homologues (**5**) and (**6**), carrying a butyrate side chain respectively on rings A and B rather than a propionate residue, might be oxidized (or dehydrogenated) to allow the alcohol or olefin in Scheme 2 to be isolated. The monomethyl esters (**7**) and (**8**) of copro'gen-III were of similar interest in that the



Scheme 2. Possible enzymic products from modified propionate groups.

decarboxylation step would clearly be impossible thus offering the possibility of gaining information about oxidized intermediates (Scheme 2). The synthesis of the required porphyrins will be described first.

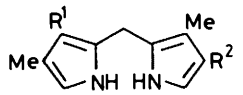
Synthesis of Analogues of Coproporphyrinogen-III (**1**).—MacDonald's approach[19] was used for synthesis of (**5**)—(**8**) involving condensation of a symmetrical methylenedipyrrole dialdehyde with an unsymmetrical bis-α-free methylenedipyrrole. The known dialdehyde[20] (**9**) was used as the



(**9**) $P^R = CH_2CH_2CO_2Me$
(**10**) $P^R = CH_2CH_2CO_2CH_2Ph$

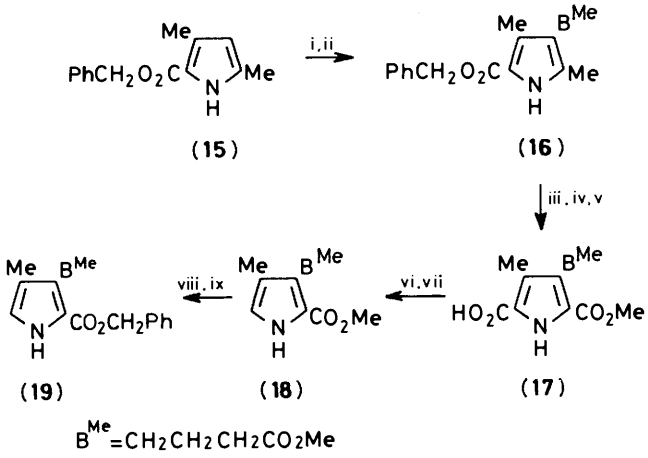
southern building block for systems (**5**) and (**6**), whilst transesterification of (**9**) using sodium benzyl oxide furnished (**10**), which was used in a similar way for synthesis of the porphyrinogens (**7**) and (**8**). The four bis-α-free methylenedipyrroles (**11**)—(**14**) were then required as the respective northern building blocks for macrocycles (**5**)—(**8**).

A key intermediate for synthesis of methylenedipyrroles (**11**) and (**12**) was the pyrrole (**16**), which was prepared from the

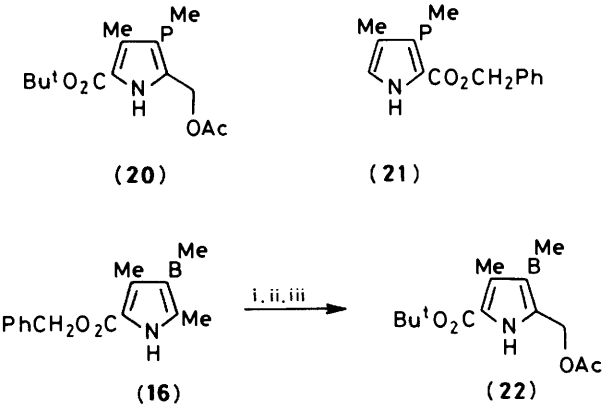


	R^1	R^2
(**11**)	P^{Me}	B^{Me}
(**12**)	B^{Me}	P^{Me}
(**13**)	P^H	P^{Me}
(**14**)	P^{Me}	P^H

$P^{Me} = CH_2CH_2CO_2Me$
$P^H = CH_2CH_2CO_2H$
$B^{Me} = CH_2CH_2CH_2CO_2Me$

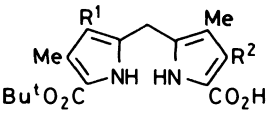


Scheme 3. *Reagents:* i, $MeO_2CCH_2CH_2COCl$, $SnCl_4$, CH_2Cl_2; ii, B_2H_6, THF–MeOAc; iii, SO_2Cl_2, then H_2O; iv, CH_2N_2; v, Pd–C, H_2; vi, KI–I_2; vii, Pt, H_2; viii, $PhCH_2ONa$, $PhCH_2OH$; ix, MeONa, MeOH

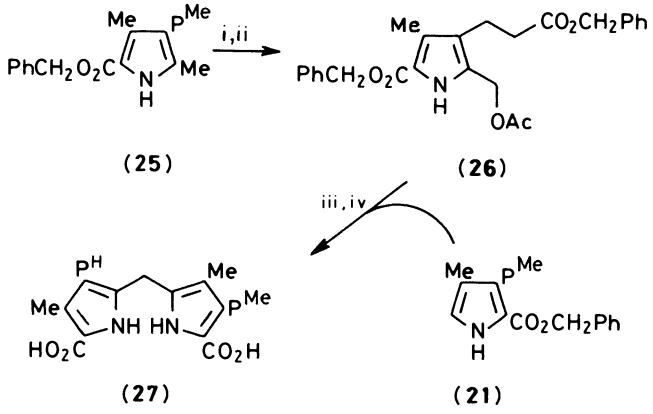


Scheme 4. *Reagents:* i, Pd–C, H_2; ii, Bu^{t}OH, DCC, THF; iii, $Pb(OAc)_4$, HOAc

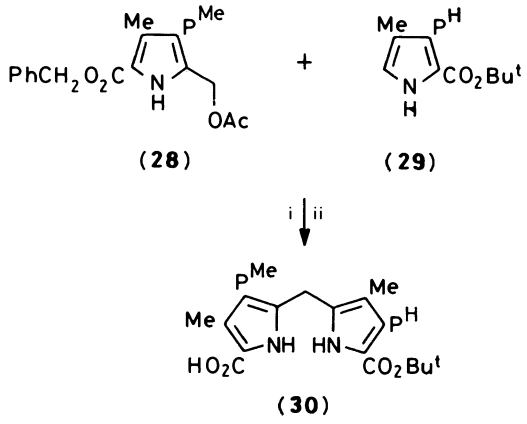
known[21] pyrrole (**15**), and converted (*a*) into the α-free pyrrole (**19**), as in Scheme 3, and (*b*) into the α-acetoxymethylpyrrole (**22**) as in Scheme 4. Treatment of the latter (**22**) with the known pyrrole[22] (**21**) in methylene chloride containing a catalytic quantity of toluene-*p*-sulphonic acid afforded, after subsequent hydrogenolysis over palladized charcoal, the methylenedipyrrole (**23**). This was separable from minor symmetrical by-products by thin-layer chromatography. Similarly, the α-free pyrrole (**19**) and the known[23] material (**20**) were combined and transformed into the pure methylenedipyrrole (**24**).



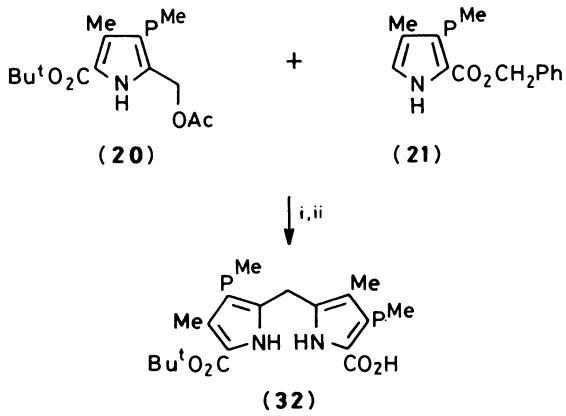
	R^1	R^2
(23)	B^{Me}	P^{Me}
(24)	P^{Me}	B^{Me}



Scheme 5. *Reagents:* i, $PhCH_2ONa$, $PhCH_2OH$; ii, $Pb(OAc)_4$, HOAc; iii, *p*-$TolSO_3H$, CH_2Cl_2; iv, Pd–C, H_2



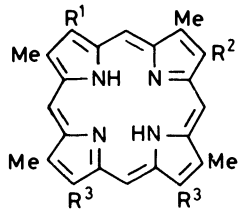
Scheme 6. *Reagents:* i, *p*-$TolSO_3H$, CH_2Cl_2; ii, Pd–C, H_2



Scheme 7. i, *p*-$TolSO_3H$, CH_2Cl_2; ii, Pd–C, H_2

The northern methylenedipyrroles (**13**) and (**14**), used for synthesis of the mono-methyl esters (**7**) and (**8**), were derived from intermediates (**27**) and (**30**), which were synthesized as in Schemes 5 and 6. Finally, the pyrroles (**20**) and (**21**) were combined to afford a methylenedipyrrole, which after hydrogenolysis and purification gave the crystalline acid (**32**) (Scheme 7). This was used for synthesis of coproporphyrin-III tetramethyl ester (copro-III Me_4 ester) (**33**).

Our initial studies of the MacDonald condensation process were carried out with the methylenedipyrrole (**32**) which with trifluoroacetic acid afforded the corresponding bis-α-free methylenedipyrrole. This was coupled directly with the dialdehyde (**9**) under the standard MacDonald conditions[19] to yield copro-III Me_4 ester (**33**) in 61% yield. However, analysis of this product by h.p.l.c.[24] showed that small amounts (4—6%) of other coproporphyrin isomers accompanied the main product (**33**). Similar problems had been noted previously by other workers.[2] A systematic investigation of this reaction showed that if each of the methylenedipyrroles (**23**), (**24**), and (**32**), after being stirred in trifluoroacetic acid, was added directly to the dialdehyde (**9**) in methylene chloride–methanol, then work-up gave the corresponding porphyrins (**34**), (**35**), and (**33**) in *ca.* 45—55% yield and >99% isomeric purity.



	R^1	R^2	R^3
(33)	P^{Me}	P^{Me}	P^{Me}
(34)	B^{Me}	P^{Me}	P^{Me}
(35)	P^{Me}	B^{Me}	P^{Me}
(36)	P^{H}	P^{Me}	P^{CH_2Ph}
(37)	P^{Me}	P^{H}	P^{CH_2Ph}
(38)	$CH{=}CH_2$	$CH{=}CH_2$	P^{Me}
(39)	$CH{=}CH_2$	P^{Me}	P^{Me}
(40)	$CH{=}CH_2$	B^{Me}	P^{Me}

$P^{Me} = CH_2CH_2CO_2Me$ $P^{H} = CH_2CH_2CO_2H$
$P^{CH_2Ph} = CH_2CH_2CO_2CH_2Ph$ $B^{Me} = CH_2CH_2CH_2CO_2Me$

In a similar manner, the methylenedipyrroles (**27**) and (**30**) were used with the dialdehyde (**10**) to afford the pure porphyrins (**36**) and (**37**) in *ca.* 40% yield.

The porphyrinogens (**1**), (**5**), and (**6**) were then readily prepared from the porphyrins (**33**), (**34**), and (**35**) by alkaline hydrolysis followed by reduction using sodium-amalgam. Formation of the mono-esters (**7**) and (**8**) from the porphyrins (**37**) and (**36**) involved hydrogenolysis over palladized charcoal and reduction in buffered solution with sodium amalgam.

Incubation Experiments with Coproporphyrinogen-III Oxidase.—When copro'gen-III (**1**) was incubated with the cell-free system from *Euglena gracilis*,[25] which contains coproporphyrinogen-III oxidase, it was largely transformed. Work-up involved oxidation of porphyrinogens to porphyrins, extraction of the latter, esterification, and chromatography. Protoporphyrin-IX dimethyl ester (**38**), harderoporphyrin trimethyl ester (**39**), and small amounts of unchanged copro-III Me_4 ester (**33**) were obtained. These incubation conditions were then used on each of the porphyrinogens (**5**), (**6**), (**7**), and (**8**); only substances (**6**) and (**8**) served as significant substrates for the enzyme.

The porphyrins isolated after work-up as above from incubation of the homologue (**6**) included (**35**), arising from unchanged starting material, and small amounts of the homologue of harderoporphyrin as its ester (**40**). This material had been formed by oxidative decarboxylation of the propionate side chain on ring-A of the substrate (**6**). The assigned structure (**40**) is based upon u.v.-vis., mass, and ^{1}H n.m.r. spectra. In particular, addition of [Eu(fod)$_3$] to this material in chloroform caused a pronounced downfield shift of just one ^{1}H n.m.r. signal arising from a *meso*-proton.[26] This demonstrated that the propionate side chains of rings C and D had remained intact, and so the vinyl groups obvious in the n.m.r. spectrum must have been formed from the ring-A propionate residue.

The porphyrinogen esterified at ring-B (**8**) also acted as a substrate for the enzyme, but a very poor one; after incubation and work-up, trace amounts of harderoporphyrin trimethyl ester (**39**) were isolated together with copro-III Me$_4$ ester (**33**).

Discussion

The results from the foregoing enzymic experiments show that coproporphyrinogen-III oxidase will generate a vinyl group on ring-A of an analogue of copro'gen-III in which the substituents on ring-B have been modified. However, both of the studied changes on ring-B caused a large reduction in the turnover rate by the enzyme. Further, changes to the propionate on ring-A of copro'gen-III (homologation or esterification) prevented any significant enzymic action. These results interlock with the previous findings that for the normal substrate (**1**), the ring-A propionate group is converted into a vinyl group before that on ring-B. Our results are also consistent with the view[4] that the formation of the two vinyl groups of proto'gen-IX (**2**) is a strictly ordered process with the ring-B propionate group only being enzymically modified after generation of a vinyl group on ring-A.

A valuable development in synthesis arising from the present work is the definition of conditions for the MacDonald synthesis of porphyrins which generate products of high isomeric purity though the need for homogeneous methylene-dipyrroles as starting materials is emphasized.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Unless otherwise stated, u.v. visible spectra were determined on Unicam SP1800 or SP800 spectrophotometers in methanol solutions. I.r. spectra were recorded on a Perkin-Elmer 257 instrument for Nujol mulls, and n.m.r. spectra on Varian HA-100 (100 MHz, C.W.) or CFT-20 (80 MHz, F.T.) instruments, as solutions in $CDCl_3$. Mass spectra were obtained on MS9 or MS12 instruments, with high resolution spectra being obtained on the MS902. The h.p.l.c. system comprised a Waters Associates ALC202-41 instrument with a Cecil CE212 variable wavelength monitor set to 400 nm with (*a*) Waters μ-Bondapak-C_{18} using aqueous acetonitrile as eluant or (*b*) Waters μ-Bondapak-CN using combinations of acetonitrile–toluene–hexane as eluant.

All solvents were redistilled before use and were evaporated under reduced pressure on a rotary evaporator unless otherwise stated. Ether refers to diethyl ether. Solutions were dried over magnesium sulphate. Analytical t.l.c. was carried out on commercial Merck plates layered with silica GF_{254}. For preparative work larger plates (20 × 20 cm) were used. Column chromatography was carried out on Spence Grade-H or Woelm Grade-III alumina or on Merck Kieselgel-60 (70—230 mesh).

Pyrroles

Benzyl 3-(2-*Methoxycarbonylethyl*)-2,4-*dimethylpyrrole*-5-*carboxylate* (**25**).—This compound was prepared essentially as described in ref. 27 (18 g, 30%), m.p. 98—100 °C (lit.,[27] 99—101 °C) (Found: C, 68.8; H, 6.8; N, 4.4. $C_{18}H_{21}NO_4$ requires: C, 68.6; H, 6.7; N, 4.4%); $\lambda_{max.}$ 283 nm; $\nu_{max.}$ 3 310, 1 740, and 1 665 cm^{-1}; δ 9.35 (1 H, s, NH), 7.29 (5 H, s, ArH), 5.27 (2 H, s, ArCH$_2$), 3.58 (3 H, s, OMe), 2.5 (4 H, m, CH$_2$CH$_2$CO$_2$), 2.22 (3 H), 2.18 (3 H) (s, pyrrMe); *m/z* 315 (*M*$^+$, 82%), 284 (5), 242 (98), and 91 (100).

Benzyl 5-*Acetoxymethyl*-4-(2-*methoxycarbonylethyl*)-3-*methylpyrrole*-2-*carboxylate* (**28**).—The foregoing pyrrole (**25**) (3.2 g) in glacial acetic acid (55 ml) containing acetic anhydride (1.2 ml) was treated during 1 h with lead tetra-acetate (5 g) and then stirred overnight. The mixture was poured into water (250 ml), the product collected, washed with water, and dissolved in methylene chloride (50 ml). The solution was washed with water, dried, evaporated, and the residue was recrystallized from methylene chloride–n-hexane to give the acetoxymethylpyrrole (2.74 g, 80%), m.p. 110—111 °C (lit.,[28] 111—112 °C) (Found: C, 64.4; H, 6.2; N, 3.7. $C_{20}H_{23}NO_6$ requires C, 64.3; H, 6.2; N, 3.8%), $\lambda_{max.}$ 274 nm.; $\nu_{max.}$ 3 310, 1 740, and 1 680 cm^{-1}; δ 9.38 (1 H, s, NH), 7.35 (5 H, s, ArH), 5.28, (2 H, s, ArCH$_2$), 5.05 (2 H, s, OCH$_2$), 3.63 (3 H, s, OMe), 2.6 (4 H, m, CH$_2$CH$_2$CO$_2$), 2.28 (3 H, s, pyrrMe), and 2.02 (3 H, s, Ac); *m/z* 373 (M$^+$, 100%), 342 (20), and 314 (48).

5-*Benzyloxycarbonyl*-3-(2-*methoxycarbonylethyl*)-4-*methylpyrrole*-2-*carboxylic Acid*.—To a solution of the pyrrole (**25**) (31.6 g) in methylene chloride (100 ml) was added ether (400 ml) followed by a solution of sulphuryl chloride (55 g) in methylene chloride (130 ml). The mixture was stirred for 1.5 h and then evaporated to an oil, which was poured into water (100 ml) and acetone (500 ml) and heated on a hotplate. After a further 20 min, sodium acetate (85 g) in water (125 ml) was added, the acetone was evaporated during 1 h, and the mixture was cooled to 18 °C. The aqueous layer was decanted off and the oil in boiling methanol (150 ml) was treated with a slurry of sodium hydrogen carbonate (13 g) in water (100 ml) followed by water (250 ml). The filtered solution was extracted with ether (200 ml), acidified with concentrated hydrochloric acid (25 ml) and extracted with methylene chloride (250 ml), the extracts being dried and evaporated. Trituration of the residue with methanol gave the carboxylic acid (22.8 g, 66%), m.p. 143—145 °C (lit.,[29] 149—150 °C) (Found: C, 62.2; H, 5.6; N, 4.2. $C_{18}H_{19}NO_6$ requires C, 62.6; H, 5.6; N, 4.1%), $\lambda_{max.}$ 283 and 289 nm; $\nu_{max.}$ 3 200, 3 000, 1 735, 1 690, and 1 670 cm^{-1}; δ 9.60 (1 H, s, NH), 7.37 (5 H, s, ArH), 5.31 (2 H, s, ArCH$_2$), 3.62 (3 H, s, OMe), 3.17—2.98 (2 H, m, C*H*$_2$CH$_2$CO$_2$), 2.61—2.41 (2 H, m, CH$_2$CH$_2$CO$_2$), and 2.28 (3 H, s, pyrrMe); *m/z* 345 (*M*$^+$, 11%), 327 (0.2), 314 (2.1), 301 (3.3), 285 (10.5), and 91 (100).

2-*Methoxycarbonyl*-3-(*methoxycarbonylethyl*)-4-*methylpyrrole*-5-*carboxylic Acid*.—This compound was prepared from the foregoing acid (9.8 g) essentially as in ref. 22 (7.3 g, 99%), m.p. 180—182 °C (lit.,[22] 179—180 °C) (Found: C, 53.5; H, 5.7; N, 5.3. $C_{12}H_{15}NO_6$ requires C, 53.5; H, 5.6; N, 5.2%), $\lambda_{max.}$ 282 nm; $\nu_{max.}$ ($CHCl_3$ soln.) 3 310, 1 745, 1 705, and 1 670 cm^{-1}; δ 9.57 (1 H, s, NH), 8.07 (1 H, s, OH), 3.89 (3 H, s, pyrrCO$_2$Me), 3.67 (3 H, s, CH$_2$CO$_2$*Me*), 3.1 (2 H, m C*H*$_2$CH$_2$CO$_2$), 2.5 (2 H, m, CH$_2$C*H*$_2$CO$_2$), 2.32 (3 H, s, pyrrMe); *m/z* 269 (M$^+$, 55%), 252 (1.1), 238 (21.5), and 209 (100).

Methyl 5-*Iodo*-3-(2-*methoxycarbonylethyl*)-4-*methylpyrrole*-2-*carboxylate*.—The foregoing pyrrolecarboxylic acid (7.3 g) and sodium hydrogen carbonate (7.5 g) were warmed until

dissolved in water (50 ml) and 1,2-dichloroethane (50 ml) and a solution of iodine (9.6 g) and sodium iodide (10.6 g) in water (50 ml) were added. The mixture was heated under reflux for 40 min, the excess of iodine was removed with sodium metabisulphite, the organic phase was separated, and the aqueous layer was extracted with methylene chloride. The combined organic layers were dried, evaporated, and the residue crystallized from methylene chloride–n-hexane to give the iodopyrrole (9 g, 95%), m.p. 129—130 °C (lit.,[22] 130—131 °C) (Found: C, 37.1; H, 3.9; N, 4.0. $C_{11}H_{14}INO_4$ requires C, 37.6; H, 4.0; N, 4.0%), $\lambda_{max.}$ 281 nm; $\nu_{max.}$ 3 270, 1 735, and 1 680 cm^{-1}; δ 9.05 (1 H, s, NH), 3.87 (3 H, s, pyrrCO_2Me), 3.67 (3 H, s, CH_2CO_2*Me*), 3.1 (2 H, m, $CH_2CH_2CO_2$), 2.5 (2 H, m, $CH_2CH_2CO_2$), and 2.0 (3 H, s, pyrrMe); *m/z* 225 (M^+, 31.3%), 194 (11), 165 (45.5), and 43 (100).

Methyl 3-(2-*Methoxycarbonylethyl*)-4-*methylpyrrole*-2-*carboxylate*.—This compound was prepared from the foregoing iodopyrrole as in ref. 22.

Benzyl 3-(2-*Benzyloxycarbonylethyl*)-4-*methylpyrrole*-2-*carboxylate*.—The foregoing dimethyl ester (10.5 g) in dry benzyl alcohol (35 ml) was treated with sodium benzyl oxide (1 ml; 4 g sodium in 100 ml benzyl alcohol) at 209 °C under nitrogen. Vigorous evolution of methanol ensued and the temperature dropped to *ca.* 195 °C. When the temperature had again reached 209 °C, a further aliquot of sodium benzyl oxide was added. This procedure was repeated until addition of sodium benzyl oxide caused no further evolution of methanol and no subsequent drop in temperature. The cooled mixture was then poured into a mixture of water (100 ml), methanol (100 ml), and glacial acetic acid (2.2 ml) and the product was extracted into methylene chloride (2 × 150 ml), which was washed with water, dried, and evaporated. The residual oil was pumped at 0.1 mmHg and then was filtered in ether down a short column of grade-H alumina. The eluate was partially evaporated and trituration with n-hexane gave the crystalline product (15.2 g, 86%), m.p. 62—63 °C from n-hexane–ether (lit.,[22] 63—65 °C) (Found: C, 73.3; H, 6.1; N, 3.8. $C_{23}H_{23}NO_4$ requires C, 73.2; H, 6.1; N, 3.7%); $\lambda_{max.}$ 248 and 275 nm; $\nu_{max.}$ 3 320, 1 730, and 1 680 cm^{-1}; δ 8.95 (1 H, s, NH), 7.30 (10 H, s, ArH), 6.58 (1 H, d, pyrrH), 5.25 (2 H), 5.05 (2 H) (s, $ArCH_2$), 3.1 (2 H, m, $CH_2CH_2CO_2$), 2.5 (2 H, m, $CH_2CH_2CO_2$), and 2.00 (3 H, s, pyrrMe); *m/z* 377 (M^+, 11%), 286 (11), and 91 (100).

Benzyl 3-(2-*Methoxycarbonylethyl*)-4-*methylpyrrole*-2-*carboxylate* (**21**).—This compound was prepared from the foregoing dibenzyl ester essentially as in ref. 22; yield 70%, m.p. 57.5—58.5 °C from ether–light petroleum (b.p. 60—80 °C) (lit.,[22] 57—58 °C) (Found: C, 67.7; H, 6.4; N, 4.8. $C_{17}H_{19}NO_4$ requires C, 67.8; H, 6.4; N, 4.7%), $\lambda_{max.}$ 250 and 274 nm; $\nu_{max.}$ 3 270, 1 740, and 1 665 cm^{-1}; δ 8.80 (1 H, s, NH), 7.29 (5 H, s, ArH), 6.55 (1 H, d, pyrrH), 5.20 (2 H, s, $ArCH_2$), 3.52 (3 H, s, CO_2Me), 2.9 (2 H, m, $CH_2CH_2CO_2$), 2.4 (2 H, m, $CH_2CH_2CO_2$), and 1.94 (3 H, s, pyrrMe); *m/z* 301 (M^+, 26%), 270 (2.4), and 256 (1.3).

Ethyl 2,4-*Dimethylpyrrole*-5-*carboxylate*.—To diethyl malonate (50 g) in glacial acetic acid (57 ml) and water (81 ml), cooled in an ice-bath, was added sodium nitrite (65 g) in portions over 90 min such that the temperature remained below 5 °C. The solution was then stirred for a further 4 h at room temperature, washed with ether (2 × 50 ml), and the combined ether extracts were evaporated. The resultant oil was added dropwise, along with zinc dust (40.5 g), to a solution of acetylacetone (37 g) in glacial acetic acid (60 ml) at 75 °C. Further acetic acid (60 ml) was added during the addition to moderate the exothermic reaction and to keep the products in solution. When the addition was complete (*ca.* 1 h), the mixture was heated at 85 °C for a further 1 h and then poured into ice cooled water (300 ml). The product was collected, washed with water, dried, and recrystallized from methanol to give the pyrrole (32.5 g, 63.5%), m.p. 122—123 °C (lit.,[30] 124—125 °C) (Found: C, 64.4; H, 8.0; N, 8.3. $C_9H_{13}NO_2$ requires C, 64.7; H, 7.8; N, 8.4%), $\lambda_{max.}$ 277 nm; $\nu_{max.}$ 3 300, 1 660, and 1 500 cm^{-1}; δ 9.80 (1 H, s, NH), 5.67 (1 H, d, pyrrH), 4.25 (2 H, q, CH_2Me), 2.21 (6 H, 2, pyrr*Me*), and 1.37 (3 H, t, CH_2*Me*); *m/z* 167 (M^+, 100%), 138 (22.5), and 122 (24).

Benzyl 2,4-*Dimethylpyrrole*-5-*carboxylate* (**15**).—The foregoing ethyl ester (23.4 g) in dry benzyl alcohol (42 ml) was treated with a stock solution of sodium benzyl oxide (1.5 ml; 4 g sodium in 100 ml benzyl alcohol) at 209 °C under nitrogen. Ethanol was evolved vigorously and the temperature dropped to *ca.* 200 °C. This procedure was repeated until no further ethanol was evolved and the resultant cooled solution was poured into a solution of methanol (280 ml), water (280 ml), and glacial acetic acid (7.3 ml). After the mixture had been stirred for *ca.* 30 min, the product was collected, washed with 50% aqueous methanol, redissolved in methylene chloride and the solution, dried and evaporated. The resultant benzyl ester crystallized from methylene chloride–n-hexane (30.4 g, 95%), m.p. 102—103 °C (lit.,[21] 102—104 °C) (Found: C, 73.1; H, 6.6; N, 5.8. $C_{14}H_{15}NO_2$ requires C, 73.3; H, 6.6; N, 6.1%), $\lambda_{max.}$ 250 and 278 nm; $\nu_{max.}$ 3 295, 1 670, 1 580, and 1 500 cm^{-1}; δ 9.55 (1 H, s, NH), 7.38 (5 H, m, ArH), 5.68 (1 H, d, pyrrH), 5.23 (2 H, s, $ArCH_2$), 2.22 (3 H), 2.18 (3 H, s, pyrrMe); *m/z* 229 (M^+, 25%), 182 (2), 138 (6), 122 (8), and 91 (100).

Benzyl 3-(3-*Methoxycarbonylpropionyl*)-2,4-*dimethylpyrrole*-5-*carboxylate*.—The foregoing pyrrole (34.4 g) and 3-methoxycarbonylpropionyl chloride[31] (32.4 g) were dissolved in methylene chloride (200 ml), and anhydrous $SnCl_4$ (25 ml, 56.5 g) was added over 30 s with stirring at 0 °C in an ice-bath. After 15 min, the mixture was poured into water–concentrated hydrochloric acid (1:1) (300 ml) and stirred vigorously for 5 min. The organic layer was then separated, washed with 2M-aqueous hydrochloric acid (300 ml) and then with saturated sodium hydrogen carbonate, and partially evaporated; the *acylated pyrrole* was displaced from solution by n-hexane (39.5 g, 77%), m.p. 115—116 °C from methanol (Found: C, 66.4; H, 6.2; N, 4.0. $C_{19}H_{21}NO_5$ requires C, 66.5; H, 6.2; N, 4.1%), $\lambda_{max.}$ 234, 257, and 284 nm; $\nu_{max.}$ 3 300, 1 725, and 1 660 cm^{-1}; δ 9.50 (1 H, s, NH), 7.37 (5 H, s, ArH), 5.30 (2 H, s, $ArCH_2$), 3.64 (3 H, s, OMe), 3.02 (2 H, t, pyrr$COCH_2$), 2.71 (2 H, t, CH_2CO_2), 2.57 (3 H), and 2.45 (3 H) (s, pyrrMe); *m/z* 343 (M^+, 9%), 312 (2), 256 (11), and 91 (100).

Benzyl 3-(3-*Methoxycarbonylpropyl*)-2,4-*dimethylpyrrole*-5-*carboxylate* (**16**).—The foregoing pyrrole (30.7 g) in tetrahydrofuran (200 ml) and methyl acetate (300 ml) was stirred at 0 °C under nitrogen, treated with sodium borohydride (5.4 g), and then dropwise with $BF_3{\cdot}Et_2O$ (28.7 g). The mixture was stirred for 15 min, quenched with methanol (100 ml) and when effervescence ceased, the insoluble material was removed and the solvent was evaporated. The residue in methanol (400 ml) was refluxed for 15 min, evaporated, and the residue was extracted again with carbon tetrachloride (500 ml). The filtered solution was evaporated and triturated with ethanol to give the *pyrrole* (23.9 g, 81%) which was chromatographed on silica with ether–methylene chloride (1:9) as eluant (86% recovery), m.p. 66.5–67.5 °C (Found: C, 69.4; H, 7.1; N, 4.2. $C_{19}H_{23}NO_4$ requires C, 69.3; H, 7.0; N, 4.3%) $\lambda_{max.}$ 255 and 285 nm; $\nu_{max.}$ 3 305, 1 735, and 1 670 cm^{-1}; δ 8.98 (1 H, s, NH), 7.39 (5 H, m, ArH), 5.30 (2 H, s, $ArCH_2$), 3.65 (3 H, s, OMe), 2.35 (4 H, m, $CH_2CH_2CH_2$), 1.8 (2 H, m, $CH_2CH_2CH_2$), 2.28 (3 H), and 2.18 (3 H), (s, pyrrMe); *m/z* 329 (M^+, 20%), 298 (4), 255 (1.5), 242 (31), and 91 (100).

2-Benzyloxycarbonyl-4-(3-methoxycarbonylpropyl)-3-methylpyrrole-5-carboxylic Acid.—The pyrrole (**16**) (9.9 g) in dry tetrahydrofuran (300 ml) was treated with sulphuryl chloride (16.2 g) in dry tetrahydrofuran (50 ml) and the solution was heated under reflux for 30 min and then evaporated. The resulting oil was poured into water–acetone (1:4; 2 000 ml) and boiled for 20 min after which sodium acetate (15 g) was added and the solution was stirred and heated for a further 20 min. The acetone was evaporated and the material extractable from the residue with methylene chloride was dissolved in hot methanol (150 ml) and to it was added sodium hydrogen carbonate (12 g) as a slurry in water (150 ml). When effervescence ceased, the solution was diluted with water (250 ml) and extracted with ether (3 × 250 ml). The ether extracts were back-extracted with aqueous sodium hydrogen carbonate and the combined aqueous solutions were acidified with concentrated hydrochloric acid. The *pyrrolecarboxylic acid* was extracted with methylene chloride and subsequently crystallized from ether–n-hexane and from methylene chloride–ether (8.6 g, 79%), m.p. 101—103 °C (Found: C, 63.2; H, 6.1; N, 3.6. $C_{19}H_{21}NO_6$ requires C, 63.5; H, 5.9; N, 3.9%), $\lambda_{max.}$ 283 nm; $\nu_{max.}$ 3 220, 2 300—2 400br, 1 705, and 1 665 cm^{-1}; δ 9.71 (1 H, s, NH), 9.60 (1 H, s, CO_2H), 7.39 (5 H, s, ArH), 5.34 (2 H, s, $ArCH_2$), 3.63 (3 H, s, CO_2Me), 2.81 (2 H, m, pyrr$CH_2CH_2CH_2$), 2.35 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.27 (3 H, s, pyrrMe), and 1.90 (2 H, m, pyrr$CH_2CH_2CH_2$); *m/z* 359 (M^+, 2.6%), 341 (0.6), 328 (0.6), and 91 (100).

Benzyl 2-Methoxycarbonyl-3-(3-methoxycarbonylpropyl)-4-methylpyrrole-5-carboxylate.—The foregoing acid (26.2 g) was esterified in ether solution using distilled diazomethane. The pyrrole *ester* was an oil (27.3 g, 100%) $\lambda_{max.}(CHCl_3)$ 284 and 293 nm; $\nu_{max.}(CHCl_3)$ 3 435 and 1 705 cm^{-1}; δ 9.34 (1 H, s, NH), 7.35 (5 H, s, ArH), 5.31 (2 H, s, $ArCH_2$), 3.84 (3 H, s, pyrrCO_2Me), 2.78 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.30 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.27 (3 H, s, pyrrCH_3), and 1.81 (2 H, m, $CH_2CH_2CH_2$); *m/z* 373 (M^+, 13%), 342 (5), 314 (3), 299 (11), and 91 (100).

2-Methoxycarbonyl-3-(3-methoxycarbonylpropyl)-4-methylpyrrole-5-carboxylic acid (**17**).—The foregoing benzyl ester (22.6 g) was hydrogenolysed over palladized charcoal as earlier. The *carboxylic acid* (**17**) crystallized from methanol (16.6 g, 96%), m.p. 164—165.5 °C (Found: C, 54.9; H, 6.1; N, 5.2. $C_{13}H_{17}NO_6$ requires C, 55.1; H, 6.1; N, 5.0%), $\lambda_{max.}$ 282 nm; $\nu_{max.}$ 3 300, 3 240, 1 740, 1 705, and 1 670 cm^{-1}; δ 9.51 (1 H, s, CO_2H), 9.48 (1 H, s, NH), 3.87 (3 H, s, pyrrCO_2Me), 3.63 (3 H, s, CH_2CO_2Me), 2.79 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.33 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.29 (3 H, s, pyrrMe), and 1.85 (2 H, m, $CH_2CH_2CH_2$); *m/z* 283 (M^+, 83%), 252 (43), 239 (25), 224 (34), and 209 (100).

Methyl 5-Iodo-3-(3-methoxycarbonylpropyl)-4-methylpyrrole-2-carboxylate.—The foregoing acid (**17**) (7.08 g) was converted into the title pyrrole by iodination as described earlier. This *iodopyrrole* crystallized from ether–n-hexane (7.56 g, 83%), m.p. 115—116.5 °C (Found: M^+, 366.0131. $C_{12}H_{16}INO_4$ requires 366.0103), $\lambda_{max.}$ 255 and 280 nm; $\nu_{max.}$ 3 280, 1 720, and 1 675 cm^{-1}; δ 9.03 (1 H, s, NH), 3.82 (3 H, s, pyrrCO_2Me), 3.63 (3 H, s, CH_2CO_2Me), 2.81 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.33 (2 H, m, $CH_2CH_2CH_2CO_2$), 1.97 (3 H, s, pyrrMe), and 1.82 (2 H, m, $CH_2CH_2CH_2$); *m/z* 365 (M^+, 100%), 334 (24), 306 (13), and 291 (75).

Methyl 3-(3-Methoxycarbonylpropyl)-4-methylpyrrole-2-carboxylate.—The foregoing iodopyrrole (9.13 g) was hydrogenated over Adams platinum and worked up as described earlier to give the *α-free pyrrole* as an oil (5.61 g, 94%) (Found: M^+, 239.1148. $C_{12}H_{17}NO_4$ requires 239.1157), $\lambda_{max.}$ 271 nm; $\nu_{max.}(CHCl_3)$ 3 460, 1 720, and 1 685 cm^{-1}; δ 8.87 (1 H, s, NH), 6.65 (1 H, d, pyrrH), 3.80 (3 H, s, pyrrCO_2Me), 3.63 (3 H, s, CH_2CO_2Me), 2.8 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.34 (2 H, m, $CH_2CH_2CH_2CO_2$), 2.01 (3 H, s, pyrrMe), and 1.89 (2 H, m, $CH_2CH_2CH_2$); *m/z* 239 (M^+, 73%), 208 (31), 180 (38), 165 (69), and 120 (100).

Benzyl 3-(3-Benzyloxycarbonylpropyl)-4-methylpyrrole-2-carboxylate.—The foregoing pyrrole dimethyl ester (5.53 g) was transesterified using benzyl alcohol and sodium benzyl oxide as above. The *benzyl ester* crystallized from ether–n-hexane (6.19 g, 68%), m.p. 82—83 °C (Found: C, 73.3; H, 6.5; N, 3.4. $C_{24}H_{25}NO_4$ requires C, 73.6; H, 6.4; N, 3.6%), $\lambda_{max.}$ 250 and 275 nm; $\nu_{max.}$ 3 290, 1 725, and 1 675 cm^{-1}; δ 8.73 (1 H, s, NH), 7.28 (10 H, s, ArH), 6.59 (1 H, d, pyrrH), 5.21 (2 H, s, pyrrCO_2CH_2), 5.03 (2 H, s, $CH_2CH_2CH_2CO_2CH_2$), 2.72 (2 H, m, pyrr$CH_2CH_2CH_2CO_2$), 2.28 (2 H, m, $CH_2CH_2CH_2CO_2$), 1.94 (3 H, s, pyrrMe), and 1.79 (2 H, m, $CH_2CH_2CH_2$); *m/z* 391 (M^+, 4%), 300 (4), 256 (4), and 91 (100).

Benzyl 3-(3-Methoxycarbonylpropyl)-4-methylpyrrole-2-carboxylate (**19**).—The foregoing dibenzyl ester (0.36 g) was transesterified using sodium methoxide and methanol as for the pyrrole (**21**). The *pyrrole* crystallized from ether–light petroleum (b.p. 60—80 °C) (0.23 g, 78%), m.p. 50—51 °C (Found: C, 68.4; H, 6.8; N, 4.3. $C_{18}H_{21}NO_4$ requires C, 68.6; H, 6.7; N, 4.4%), $\lambda_{max.}$ 252 and 275 nm; $\nu_{max.}$ 3 475 and 1 710 cm^{-1}; δ 9.16 (1 H, s, NH), 7.23 (5 H, m, ArH), 6.49 (1 H, d, pyrrH), 5.16 (2 H, s, $ArCH_2$), 3.50 (3 H, s, CO_2Me), 2.64 (2 H, t, $CH_2CH_2CH_2CO_2$), 2.11 (2 H, t, $CH_2CH_2CH_2CO_2$), 1.91 (3 H, s, pyrrMe), and 1.72 (2 H, m, $CH_2CH_2CH_2$); *m/z* 315 (M^+, 82%), 284 (10), 270 (7), 240 (10), and 91 (100).

Benzyl 3-(2-Benzyloxycarbonylethyl)-2,4-dimethylpyrrole-5-carboxylate.—The pyrrole ester (**25**) (3.15 g) in dry benzyl alcohol was transesterified using the procedure with sodium benzyl oxide outlined above. The *dibenzyl ester* crystallized from ether–n-hexane (3.1 g, 80%), m.p. 88—89 °C (Found: C, 73.7; H, 6.6; N, 3.5. $C_{24}H_{23}NO_4$ requires C, 73.6; H, 6.4; N, 3.6%), $\lambda_{max.}$ 283 nm; $\nu_{max.}$ 3 440, 1 720, and 1 670 cm^{-1}; δ 8.43 (1 H, s, NH), 7.36 (5 H), 7.29 (5 H) (s, ArH), 5.29 (2 H, s, $ArCH_2$), 5.10 (2 H, s, $ArCH_2$), 2.62 (4 H, m, CH_2CH_2), and 2.30 (3 H) and 2.18 (3 H) (both s, pyrrMe); *m/z* 391 (M^+, 31%), 300 (14), 284 (4), and 91 (100).

Benzyl 5-Acetoxymethyl-4-(2-benzyloxycarbonylethyl)-3-methylpyrrole-2-carboxylate (**26**).—The foregoing pyrrole (2.5 g) was oxidized with lead tetra-acetate (4 g) as for the pyrrole (**28**). The *acetoxymethylpyrrole* crystallized from methylene chloride–ether–n-hexane (1.95 g, 68%), m.p. 113.5—115.5 °C (Found: C, 69.5; H, 6.0; N, 3.3. $C_{26}H_{27}NO_6$ requires C, 69.5; H, 6.1; N, 3.1%), $\lambda_{max.}$ 274 nm; $\nu_{max.}$ 3 420 and 1 720— 1 680 cm^{-1}; δ 8.87 (1 H, s, NH), 7.37 (5 H), 7.29 (5 H) (s, ArH), 5.31 (2 H), 5.10 (2 H) (s, $ArCH_2$), 5.06 (2 H, s, CH_2OAc), 2.67 (4 H, q, $CH_2CH_2CO_2$), and 2.30 (3 H) and 2.05 (3 H) (both s, pyrrMe); *m/z* 449 (M^+, 14%), 390 (3), 316 (9), 298 (7), and 91 (100).

t-Butyl 4-(3-Methoxycarbonylpropyl)-3,5-dimethylpyrrole-2-carboxylate.—The pyrrole (**16**) (0.66 g) in tetrahydrofuran (25 ml) was hydrogenated over palladized charcoal (10% Pd; 60 mg) at room temperature and atmospheric pressure. When uptake of hydrogen ceased, the catalyst was removed and the filtrate was evaporated, leaving the *pyrrole carboxylic acid* as a pink solid. This was used below without further purification. For analysis, a sample was recrystallized from methanol, m.p. 136—138 °C (decomp.) (Found: C, 60.2; H, 7.2; N, 5.7. $C_{12}H_{17}NO_4$ requires C, 60.2; H, 7.2; N, 5.9%), $\lambda_{max.}$ 250 and 273 nm;

$\nu_{max.}$ 3 310, 2 600, 1 725, and 1 645 cm^{-1}; δ(C_5D_5N) 3.63 (3 H, s, CO_2Me), 2.64 (3 H, s, pyrrMe), 2.46 (4 H, m, $CH_2CH_2CH_2$), 2.30 (3 H, s, pyrrMe), and 1.90 (2 H, m, $CH_2CH_2CH_2$); *m/z* 239 (M^+, 42%), 208 (35), 195 (30), and 152 (10).

All of the carboxylic acid above in dry t-butyl alcohol (5 ml) and dry tetrahydrofuran (10 ml) was treated with dicyclohexylcarbodi-imide (1.1 g) as a solution in tetrahydrofuran (10 ml). The mixture was stirred for 16 h at 20 °C and then filtered, evaporated, and the residue purified by repeated chromatography on grade-H alumina eluting with ether. The *t-butyl ester* was crystallized from ether–n-hexane (392 mg, 67%), m.p. 82.5—83.5 °C (Found: C, 65.1; H, 8.2; N, 4.4. $C_{16}H_{25}NO_4$ requires C, 65.1; H, 8.5; N, 4.7%), $\lambda_{max.}$250 and 282 nm; $\nu_{max.}$ 3 300, 1 730, and 1 650 cm^{-1}; δ 8.60 (1 H, s, NH), 3.64 (3 H, s, CO_2Me), 2.37 (4 H, m, pyrr$CH_2CH_2CH_2$), 2.21 (3 H) and 2.18 (3 H) (both s, pyrrMe), 1.77 (2 H, m, $CH_2CH_2CH_2$), and 1.56 (9H, s, Bu^t); *m/z* 295 (M^+, 26%), 264 (5), 239 (58), 222 (14), and 152 (100).

t-Butyl 5-Acetoxymethyl-4-(3-methoxycarbonylpropyl)-3-methylpyrrole-2-carboxylate (**22**).—The foregoing t-butyl ester (470 mg) was oxidized by the procedure outlined above for the pyrrole (**28**) to yield the *acetoxymethylpyrrole* (403 mg, 72%), m.p. 75—76 °C from ether–n-hexane (Found: C, 61.3; H, 7.7; N, 4.0%), $\lambda_{max.}$ 274 nm; $\nu_{max.}$ 3 320, 1 740, and 1 670 cm^{-1}; δ 8.99 (1 H, s, NH), 5.01 (2 H, s, CH_2OAc), 3.68 (3 H, s, CO_2Me), 2.45 (4 H, m, pyrr$CH_2CH_2CH_2$), 2.25 (3 H, s, pyrrMe), 2.06 (3 H, s, pyrrMe), 1.80 (2 H, m, $CH_2CH_2CH_2$), and 1.59 (9 H, s, Bu^t); *m/z* 353 (M^+, 12%), 322 (3), 309 (3), 294 (7), and 83 (100).

t-Butyl 5-Acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (**20**).—The acetoxymethylpyrrole (**20**) was prepared from the corresponding α-methylpyrrole by the procedure described above for the synthesis of (**28**). The product was chromatographed on alumina with ether and crystallized from ether–n-hexane (5.28 g, 78%), m.p. 81.5—82.5 °C (lit.,[23] 82—83 °C) (Found: C, 60.1; H, 7.4; N, 4.0. $C_{17}H_{25}NO_6$ requires C, 60.2; N, 7.4; N, 4.1%), $\lambda_{max.}$ 272 nm; $\nu_{max.}$ 3 300, 1 740, and 1 660 cm^{-1}; δ 9.39 (1 H, s, NH), 5.00 (2 H, s, CH_2OAc), 3.60 (3 H, s, CO_2Me), 2.71 (2 H, m, pyrrCH_2), 2.38 (2 H, m, CH_2CO_2), 2.20 (3 H, s, pyrrMe), 2.00 (3 H, s, OAc), and 1.52 (9 H, s, Bu^t); *m/z* 339 (M^+, 35%), 308 (8), 283 (15), and 223 (100).

t-Butyl 5-Iodo-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate.—t-Butyl 5-carboxy-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate[32] (7 g) was iodinated with decarboxylation by the procedure outlined earlier. The product was chromatographed on alumina with ether and crystallized from ether–n-hexane (8.2 g, 93%), m.p. 127—129 °C (lit.,[32] 133—134 °C) (Found: C, 43.1; H, 5.2; N, 3.5. $C_{14}H_{20}INO_4$ requires C, 42.8; H, 5.1; N, 3.6%), $\lambda_{max.}$($CHCl_3$) 280 nm; $\nu_{max.}$ 3 420, 1 725, and 1 675 cm^{-1}; δ 8.59 (1 H, s, NH), 3.69 (3 H, s, CO_2Me), 3.08 (2 H, m, $CH_2CH_2CO_2$), 2.52 (2 H, s, $CH_2CH_2CO_2$), 2.02 (3 H, s, pyrrMe), and 1.59 (9 H, s, Bu^t); *m/z* 393 (M^+, 52%) and 337 (100).

t-Butyl 3-(2-Carboxyethyl)-4-methylpyrrole-2-carboxylate (**29**).—The foregoing iodopyrrole (15.8 g) was converted as outlined above into the corresponding α-free pyrrole (10.6 g, 99%), m.p. 58—64 °C (lit.,[33] 63—64 °C); it was used without further purification; δ 9.45 (1 H, s, NH), 6.60 (1 H, d, pyrrH), 3.66 (3 H, s, CO_2Me), 3.0 (2 H, m, $CH_2CH_2CO_2$), 2.5 (2 H, m, $CH_2CH_2CO_2$) 2.02 (3 H, s, pyrrMe), and 1.58 (9 H, s, Bu^t); *m/z* 267 (M^+).

This product (2.11 g) in methanol (25 ml) and saturated aqueous sodium carbonate (10 ml) were heated on a steam-bath until hydrolysis of the side-chain ester was complete [reaction assayed by t.l.c. on silica with ether–methylene chloride (20:80) as eluant]. The cooled solution was washed with ether, adjusted to pH 4—5 with 15% aqueous hydrochloric acid, and the product extracted into methylene chloride; the extract was washed with water, dried, and evaporated to leave the *acid* (1.73 g, 87%), m.p. 174—177 °C from aqueous methanol (Found: C, 61.4; H, 7.5; N, 5.4. $C_{13}H_{19}NO_4$ requires C, 61.4; H, 7.6; N, 5.5%), $\lambda_{max.}$($CHCl_3$) 272 nm; $\nu_{max.}$ 3 340, 3 240, 1 710, and 1 670 cm^{-1}; δ 8.94 (1 H, s, NH), 6.66 (1 H, d, pyrrH), 3.08 (2 H, m, $CH_2CH_2CO_2$), 2.60 (2 H, m, $CH_2CH_2CO_2$), 2.08 (3 H, s, pyrrMe), and 1.60 (9 H, s, Bu^t); *m/z* 253 (M^+, 11%), 231 (9), 197, (12), and 151 (100).

2,2′-*Methylenedipyrroles*

Dibenzyl 3,3′-Bis-(2-methoxycarbonylethyl)-4,4′-dimethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylate.—The acetoxymethylpyrrole (**28**) (18.6 g) in water–acetic acid (1:4) (180 ml) was heated on a steambath for 1 h. The cooled solution was mixed with water (200 ml) and the product was extracted with ether (400 ml); the extract was washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated to give the product (12.5 g, 81.5%), m.p. 99—100 °C from ethanol (lit.,[23] 99—100 °C) (Found: C, 68.4; H, 6.3; N, 4.6. $C_{35}H_{38}N_2O_8$ requires C, 68.4; H, 6.2; N, 4.6%), $\lambda_{max.}$($CHCl_3$) 275 and 287 nm; $\nu_{max.}$ 3 360, 3 340, 1 735, 1 705, 1 580, and 1 500 cm^{-1}; δ 9.60 (2 H, s, NH), 7.27 (10 H, s, ArH), 5.19 (4 H, s, $ArCH_2$), 3.93 (2 H, s, pyrrCH_2), 3.53 (6 H, s, OMe), 2.73 (4 H, m, $CH_2CH_2CO_2$), 2.43 (4 H, m, $CH_2CH_2CO_2$), and 2.22 (6 H, s, pyrrMe); *m/z* 614 (M^+, 14%), 523 (30), 505 (5.5), and 91 (100).

5,5′-Diformyl-3,3′-bis(2-methoxycarbonylethyl)-4,4′-dimethyl-2,2′-methylenedipyrrole (**9**).—The preceding methylenedipyrrole (20 g) in tetrahydrofuran (500 ml) was hydrogenated over 10% palladized charcoal (2 g). When uptake of hydrogen was complete, 2M-aqueous ammonia (60 ml) was added and the filtered solution was adjusted to pH 7 with acetic acid. The tetrahydrofuran was evaporated and the methylenedipyrroledicarboxylic acid collected, washed with water, and dried. It was used without further purification (14 g, 99%), m.p. 194—198 °C (decomp.).

This acid (14 g) was then added in portions over 10 min to trifluoroacetic acid (85 ml) at 0 °C and the resulting solution was stirred for a further 5 min. Trimethyl orthoformate (40 ml) was then added and the mixture was stirred at 0 °C for 7 min and then poured into water (1 200 ml). The precipitate was collected, added to a mixture of ethanol (140 ml) and N-aqueous ammonia (280 ml), stirred for 10 min, and then filtered off. The solid was washed with water and recrystallized from methanol to give the diformylmethylenedipyrrole (7.8 g, 60%). The product was chromatographed on activity-III alumina first in ether–methylene chloride (10:90) and then with methanol–methylene chloride (10:90). The recovered material crystallized from methanol to give the diformylmethylenedipyrrole (90% recovery), m.p. 180—181 °C (lit.,[34] 180—181 °C) (Found: C, 62.6; H, 6.7; N, 6.9. $C_{21}H_{26}N_2O_6$ requires C, 62.7; H, 6.5; N, 6.9%) $\lambda_{max.}$ 300 and 322 nm; $\nu_{max.}$ 3 270, 3 260, 3 200, 1 740, and 1 625 cm^{-1}; δ 9.40 (2 H, s, CHO), 4.30 (2 H, s, pyrrCH_2pyrr), 3.65 (6 H, s, CO_2Me), 2.76 (4 H, m, $CH_2CH_2CO_2$), 2.46 (4 H, m, $CH_2CH_2CO_2$), and 7.75 (6 H, s, pyrrMe), *m/z* 402 (M^+, 100%), 373 (42), and 329 (15).

5,5′-Diformyl-3,3′-bis(2-benzyloxycarbonylethyl)-4,4′-dimethyl-2,2′-methylenedipyrrole (**10**).—The foregoing diformylmethylenedipyrrole (**9**) (0.4 g) was added at 18 °C to a stirred solution of sodium (0.12 g) in dry benzyl alcohol (15 ml). After 18 min, the solution was poured into a mixture of methanol (25 ml), water (50 ml), and acetic acid (1 ml) and the

product was extracted with methylene chloride; the extract was washed with water and evaporated. The residue was pumped at 0.1 mmHg and then filtered through alumina, in ether–methylene chloride (1:4) and then with methanol–chloroform (1:19). The *methylenedipyrrole* crystallized from ether–n-hexane (375 mg, 68%), m.p. 118—118.5 °C (Found: C, 71.6; H, 6.4; N, 5.1. $C_{33}H_{34}N_2O_6$ requires C, 71.5; H, 6.2; N, 5.1%), $\lambda_{max.}$ 275, 305, and 321 nm; $\nu_{max.}$ 3 300, 1 725, 1 635, and 1 150 cm^{-1}; δ 9.93 (2 H, s, NH), 9.44 (2 H, s, CHO), 7.28 (10 H, s, ArH), 5.15 (4 H, s, $ArCH_2$), 3.95 (2 H, s, pyrrCH_2pyrr), 2.68 (8 H, m, $CH_2CH_2CO_2$), and 2.27 (6 H, s, pyrrMe); *m/z* 554 (M^+, 20%), 528 (8), 478 (9), and 463 (100).

t-Butyl 5-Carboxy-3,4′-bis(2-methoxycarbonylethyl)-3′,4-dimethyl-2,2′-methylenedipyrrole-5-carboxylate (**32**).—The acetoxymethylpyrrole (**20**) (678 mg) in methylene chloride (50 ml) was added dropwise over 20 min to a solution of the α-free pyrrole (**21**) (602 mg) in methylene chloride (50 ml) containing toluene-*p*-sulphonic acid (10 mg). After being stirred at room temperature under nitrogen for 90 min, the solution was washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated. The resulting methylenedipyrrole was an oil which was used directly in the next step; $\lambda_{max.}$ 277 and 292 nm; δ 9.93 (1 H, s, NH), 9.00 (1 H, s, NH), 7.22 (5 H, m, ArH), 5.18 (2 H, s, $ArCH_2$), 3.83 (2 H, s, pyrrCH_2pyrr), 3.55 (6 H, s, CO_2Me), 2.82 (2 H), 2.64 (2 H) (m, $CH_2CH_2CO_2$), 2.38 (4 H, m, $CH_2CH_2CO_2$), 2.15 (3 H) and 2.05 (3 H) (both s, pyrrMe), and 1.48 (9 H, s, Bu^t); *m/z* 580 (M^+, 25), 523 (11), and 433 (100).

The foregoing methylenedipyrrole was dissolved in THF (100 ml) and hydrogenated over 10% palladized charcoal (100 mg) at atmospheric pressure and room temperature. When uptake was complete, the solution was filtered and evaporated and the product purified by t.l.c. on silica with 7% methanol in chloroform as eluant. The major band was the required methylenedipyrrole monoester (**32**), but a minor band with a higher R_F was identified as the di-t-butyl ester (M^+, 546). The former crystallized from ether–n-hexane and from methylene chloride–ether–n-hexane (618 mg, 63%), m.p. 99—101 °C. In subsequent preparations, samples with m.p. 149—153 °C were also obtained (Found: C, 61.2; H, 7.2; N, 5.6. $C_{25}H_{34}N_2O_8$ requires C, 61.2; H, 7.0; N, 5.7%), $\lambda_{max.}$ 275 and 289 nm; $\nu_{max.}$ 3 320, 3 250, 1 735, and 1 660 cm^{-1}; δ 11.32 (1 H) and 10.83 (1 H) (both s, NH), 3.89 (2 H, s, pyrrCH_2pyrr), 3.70 (3 H) and 6.33 (3 H) (both s, CO_2Me), 2.95 (4 H, m, $CH_2CH_2CO_2$), 2.5 (4 H, m, $CH_2CH_2CO_2$), 2.21 (3 H) and 2.04 (3 H) (both s, pyrrMe), and 1.58 (9 H, s, Bu^t); *m/z* 446 ($M^+ - CO_2$, 11%), 390 (11), 373 (3.5), 372 (3.5), and 135 (100).

t-Butyl 5-Carboxy-3-(2-methoxycarbonylethyl)-4′-(3-methoxycarbonylpropyl)-3′,4-dimethyl-2,2′-methylenedipyrrole-5-carboxylate (**24**).—The acetoxymethylpyrrole (**20**) (678 mg) and the α-free pyrrole (**18**) (630 mg) were combined by the method outlined above for the methylenedipyrrole (**32**). The resultant methylenedipyrrole was used without further purification; $\lambda_{max.}$ 250, 278, and 293 nm; δ 9.10 (1 H) and 8.41 (1 H) (both s, NH), 7.37 (5 H, s, ArH), 5.24 (2 H, s, $ArCH_2$), 3.90 (2 H, s, pyrrCH_2pyrr), 3.62 (3 H) and 3.58 (3 H) (both s, CO_2Me), 2.9—1.6 (10 H, complex multiplet, 5 × CH_2), 2.22 (3 H) and 2.02 (3 H) (both s, pyrrCH_3), and 1.53 (9 H, s, Bu^t); *m/z* 594 (M^+, 7%), 563 (1.4), 537 (2.8), 521 (2.0), and 91 (100). This methylenedipyrrole was hydrogenated in tetrahydrofuran and the product was purified by t.l.c. and crystallized as outlined above for the methylenedipyrrole (**32**). The *methylenedipyrrole* was recrystallized from ether–methylene chloride–n-hexane (589 mg, 59%), m.p. 130—133 °C (Found: C, 61.9; H, 7.3; N, 5.4. $C_{26}H_{36}N_2O_8$ requires C, 61.9; H, 7.2; N, 5.6%), $\lambda_{max.}$ 250, 275, and 284 nm; $\nu_{max.}$ 3 315, 3 220, 1 735, and 1 660 cm^{-1}; δ 3.89 (2 H, s, pyrrCH_2pyrr), 3.71 (3 H) and 3.65 (3 H) (both s, CO_2Me), 2.84 (4 H, m, $CH_2CH_2CO_2$), 2.40 (4 H, m, $CH_2CH_2CO_2$ and $CH_2CH_2CH_2CO_2$), 2.22 (3 H) and 7.97 (3 H) (both s, pyrrMe), 1.75 (2 H, m, $CH_2CH_2CH_2$), and 1.58 (9 H, s, Bu^t); *m/z* 504 (M^+, 0.6%), 460 (100), 447 (2), and 405 (81).

t-Butyl 5′-Carboxy-3-(3-methoxycarbonylpropyl)-4′-(2-methoxycarbonylethyl)-3′,4-dimethyl-2,2′-methylenedipyrrole-5-carboxylate (**23**).—The acetoxymethylpyrrole (**22**) (254 mg) and α-free pyrrole (**21**) (216 mg) were combined as in the foregoing experiment to give a methylenedipyrrole as an oil, $\lambda_{max.}$ 250, 277, and 293 nm; δ(CCl_4) 9.90 (1 H, s, NH), 9.05 (1 H, s, NH), 7.23 (5 H, s, ArH), 5.19 (2 H, s, $ArCH_2$), 3.79 (2 H, s, pyrrCH_2pyrr), 3.58 (3 H) and 3.54 (3 H) (both s, CO_2Me), 2.93 (2 H, m, $CH_2CH_2CO_2$), 2.50—1.98 (6 H, m, $CH_2CH_2CO_2$ and $CH_2CH_2CH_2CO_2$), 2.15 (3 H) and 1.97 (3 H) (both s, pyrrMe), 1.65 (2 H, s, $CH_2CH_2CH_2$), and 1.48 (9 H, s, Bu^t); *m/z* 594 (M^+, 6.3%) 574 (9), 537 (5), 517 (11), and 277 (100).

This was then taken through the same steps as in the foregoing experiment to yield the required *methylenedipyrrole* (**23**) (256 mg, 71%), m.p. 129.5—130.5 °C from methylene chloride–ether–n-hexane (Found C, 61.8; H, 7.3; N, 5.3. $C_{26}H_{36}N_2O_8$ requires C, 61.9; H, 7.2; N, 5.6%), $\lambda_{max.}$ 248, 274, and 290 nm; $\nu_{max.}$ 3 360, 3 260, 1 740, 1 730, 1 665, 1 640, and 3 000—2 400 cm^{-1}; δ 3.87 (2 H, s, pyrrCH_2pyrr), 3.70 (3 H) and 3.68 (3 H) (both s, CO_2Me), 3.07 (2 H, m, $CH_2CH_2CO_2$), 3.71—2.25 (6 H, m, $CH_2CH_2CH_2CO_2$ and $CH_2CH_2CO_2$), 2.22 (3 H), 2.05 (3 H), and 2.05 (3 H) (all s, pyrrMe), 1.85 (2 H, m, $CH_2CH_2CH_2$ and CH_2CH_2), and 1.60 (9 H, s, Bu^t); *m/z* 504 (M^+, 5%), 460 (100), and 447 (3).

Dibenzyl 3-(2-Benzyloxycarbonylethyl)-4′-(2-methoxycarbonylethyl)-3′,4-dimethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylate.—The acetoxymethylpyrrole (**26**) (449 mg) in methylene chloride (40 ml) was added dropwise over 30 min to a stirred solution of the α-free pyrrole (**21**) (301 mg) in methylene chloride (40 ml) containing toluene-*p*-sulphonic acid (6 mg). The mixture was stirred under nitrogen at room temperature for 90 min and then washed with aqueous sodium hydrogen carbonate and evaporated. The residue was purified by t.l.c. on silica with ether–methylene chloride (10:90) as eluant. The pure *methylenedipyrrole* crystallized from ether–n-hexane (452 mg, 66%), m.p. 76—79 °C (Found: C, 71.3; H, 6.0; N, 3.8. $C_{41}H_{42}N_2O_8$ requires C, 71.3; H, 6.1; N, 4.1%), $\lambda_{max.}$ 277 and 292 nm; $\nu_{max.}$ 3 440, 1 720, and 1 680 cm^{-1}; δ 8.95 (1 H) and 8.35 (1 H) (both s, NH), 7.33 (5 H), 7.31 (5 H), and 7.25 (5 H) (all s, ArH), 5.26 (2 H), 5.25 (2 H), and 5.05 (2 H) (all s, $ArCH_2$), 3.84 (2 H, s, pyrrCH_2pyrr), 3.63 (3 H, s, CO_2Me), 3.05—2.40 (8 H, complex multiplets, side chain CH_2's), and 2.29 (3 H) and 2.00 (3 H) (both s, pyrrMe); *m/z* 690 (M^+, 7%), 659 (3), 599 (100), and 582 (4).

t-Butyl 5-Carboxy-3-(2-methoxycarbonylethyl)-4′-(2-carboxyethyl)-3′,4-dimethyl-2,2′-methylenedipyrrole-5′-carboxylate (**30**).—The acetoxymethylpyrrole (**28**) (746 mg) was treated with the α-free pyrrole (**29**) (506 mg) as for the foregoing preparations of methylenedipyrroles. The amorphous product was used directly in the next stage (Found: C, 65.8; H, 6.9; N, 4.8. $C_{31}H_{38}N_2O_8$ requires C, 65.7; H, 6.8; N, 4.9%), $\lambda_{max.}$($CHCl_3$) 277 and 286 nm; $\nu_{max.}$ 3 420, 3 400—3 000, 1 720, and 1 680 cm^{-1}; δ 8.83 (1 H) and 8.46 (1 H) (both s, NH), 7.34 (5 H, s, ArH), 5.26 (2 H, s, $ArCH_2$), 3.89 (2 H, s, pyrrCH_2pyrr), 3.67 (3 H, s, CO_2Me), 3.06—2.30 (8 H, m, $CH_2CH_2CO_2$), 2.27 (3 H) and 2.00 (3 H) (both s, pyrrMe), and 1.56 (9 H, s, Bu^t); *m/z* 566 (M^+, 7%), 522 (1.3), 509 (11), and 466 (100).

The methylenedipyrrole in tetrahydrofuran (100 ml) was hydrogenated over 10% palladized charcoal (100 mg), and when uptake ceased, the solution was filtered and evaporated. A solution of the residue in a small volume of 2M-aqueous

ammonia was carefully neutralized with acetic acid. The precipitated methylenedipyrrole was collected, washed with water, and dried (601 mg, 63%), m.p. 158—160 °C from methylene chloride–n-hexane (Found: C, 60.7; H, 6.8; N, 5.7. $C_{24}H_{32}N_2O_8$ requires C, 60.5; H, 6.8; N, 5.9%), $\lambda_{max.}$($CHCl_3$) 278 and 286 nm; $\nu_{max.}$ 3 300, 3 220, 1 720, and 1 655 cm^{-1}; δ 11.26 (1 H) and 10.96 (1 H) (both s, NH), 3.91 (2 H, s, pyrrCH_2pyrr), 3.72 (3 H, s, CO_2Me) 3.04—2.40 (8 H, m, $CH_2CH_2CO_2$), 2.32 (3 H) and 2.13 (3 H) (both s, pyrrMe), 1.59 (9 H, s, Bu^t); *m/z* 432 ($M^+ - CO_2$, 70%) 376 (78), 375 (67), and 331 (100).

Porphyrins

Coproporphyrin-III Tetramethyl Ester (**33**).—(*a*) *Using hydriodic acid.* The methylenedipyrrole (**32**) (113 mg) in trifluoroacetic acid (12 ml) was stirred at 2—3 °C under nitrogen in the dark and the decarboxylation was followed by u.v. spectroscopy. After 85 min, the solvent was removed by freeze drying and the residue was dissolved in acetic acid (50 ml) and added to the diformylmethylenedipyrrole (**9**) (88 mg), also in acetic acid (50 ml); subsequently hydriodic acid (0.4 ml) was added. The mixture was stirred at 18 °C for 45 min and then sodium acetate (1 g) was added and the solution aerated in the dark overnight. The solvents were evaporated and the residue was taken up in methylene chloride (100 ml) and washed successively with water, saturated aqueous sodium hydrogen carbonate, and water, and then dried and evaporated. The product was purified by t.l.c. with ether–methylene chloride (1:9) as eluant and crystallized from methylene chloride–methanol (94 mg, 61%), m.p. 144—148 °C (lit.,[35] 150—153 °C). Analysis by h.p.l.c. on a C18 μ-Bondapak reverse-phase column showed that the product contained *ca.* 4% coproporphyrin-I and *ca.* 2% of coproporphyrin-II esters as isomeric impurities (analysis for the type-IV isomer was not attempted).

(*b*) *Using trifluoroacetic acid in methanol–methylene chloride.* The methylenedipyrrole (**32**) (200 mg) in trifluoroacetic acid (25 ml) was stirred at 2—3 °C, under nitrogen in the dark for 95 min. The solution was then added to the diformylmethylenedipyrrole (**9**) (158 mg) in methylene chloride (100 ml) and methanol (15 ml) and stirred at 18 °C in the dark overnight; it was then washed with aqueous sodium hydrogen carbonate and water, evaporated, and dried at 0.1 mmHg. The product was purified by t.l.c. on silica with ether–methylene chloride (1:9) as eluant and crystallized from methanol–methylene chloride (140 mg, 48%), m.p. 149—152 °C. Analysis by h.p.l.c. showed the product to be *ca.* 99% pure.

(*c*) *Using trifluoroacetic acid and sodium acetate.* The methylenedipyrrole (**32**) (150 mg) was stirred in trifluoroacetic acid (20 ml) in the dark under nitrogen at 2—3 °C, for 90 min. The solution was then added to a solution of the diformylmethylenedipyrrole (**9**) (123 mg) in methylene chloride (120 ml) and methanol (24 ml). The solution was stirred at 18 °C for 5 min after which sodium acetate (6 g) was added and the mixture stirred for a further 50 min; it was then washed with aqueous sodium acetate and water, evaporated, and dried at 0.1 mmHg. The product was purified by t.l.c. and obtained crystalline as described in (*b*) above (127 mg, 58%), m.p. 147—150 °C. Analysis by h.p.l.c. showed the product to be essentially free of other isomers, from methylene chloride–methanol, m.p. 152.5—153.5 °C (Found: C, 67.3; H, 6.7; N, 7.6. $C_{40}H_{46}N_4O_8$ requires C, 67.6; H, 6.5; N, 7.9%), $\lambda_{max.}$($CHCl_3$) 400, 500, 532, 566, 594, and 621 nm; δ 10.03 (4 H, s, *meso*-H), 4.44 (8 H, t, $CH_2CH_2CO_2$), 3.68 (24 H, s, Me), 3.31 (8 H, t, $CH_2CH_2CO_2$), −3.67 (2 H, s, NH); *m/z* 710 (M^+, 100%), 679 (6.3), 652 (5.6), 651 (6.3), and 637 (35).

The Homologue at Position-8 of Coproporphyrin-III Tetramethyl Ester (**35**).—The methylenedipyrrole (**24**) (150 mg) was decarboxylated, condensed with the diformyl methylenedipyrrole (**9**) (120 mg), and worked up to give the required *porphyrin* (**35**) (122 mg, 56%), using procedure (*b*) outlined above, m.p. 141—142 °C. Analysis by h.p.l.c. using the conditions outlined for (**33**) above showed the product to be *ca.* 98% pure (Found: C, 67.7; H, 7.0; N, 7.4. $C_{41}H_{48}N_4O_8$ requires C, 67.9; H, 6.7; N, 7.7), $\lambda_{max.}$($CHCl_3$) 400, 500, 534, 568, 593, and 621 nm; δ 10.08 (4 H, s, *meso*-H), 4.45 (6 H, t, $CH_2CH_2CO_2$) 4.05 (2 H, m, $CH_2CH_2CH_2CO_2$), 3.68 (24 H, s, Me), 3.31 (8 H, t, CH_2CO_2Me), 2.69 (2 H, br s, $CH_2CH_2CH_2CO_2$), and −3.67 (2 H, s, NH); *m/z* 724 (M^+, 100%), 693 (9), 666 (8), 665 (10), and 651 (32).

The Homologue at Position-3 of Coproporphyrin-III Tetramethyl Ester (**34**).—The methylenedipyrrole (**23**) (150 mg) and the diformylmethylenedipyrrole (**9**) (120 mg) by method (*b*) similarly gave the required *porphyrin* (97 mg, 45%), m.p. softens 152—153 °C, melts 181.5—182.5 °C. Analysis by h.p.l.c. as outlined above for (**33**), showed the product to be *ca.* 98% pure (Found: C, 67.8; H, 6.7; N, 7.7. $C_{41}H_{48}N_4O_8$ requires C, 67.9; H, 6.7; N, 7.7%), $\lambda_{max.}$($CHCl_3$) 400, 500, 534, 568, 593, and 621 nm; δ 10.08 (4 H, s, *meso*-H), 4.45 (6 H, t, $CH_2CH_2CO_2$), 4.05 (2 H, m, $CH_2CH_2CH_2CO_2$), 3.68 (24 H, s, Me), 3.31 (8 H, t, $CH_2CH_2CO_2$ and $CH_2CH_2CH_2CO_2$), 2.69 (2 H, br s, $CH_2CH_2CH_2CO_2$), and −3.67 (2 H, s, NH); *m/z* 724 (M^+, 100%), 693 (10), 665 (7), and 651 (14).

13,17-*Bis*(2-*benzyloxycarbonylethyl*)-3-(2-*carboxyethyl*)-8-(2-*methoxycarbonylethyl*)-2,7,12,18-*tetramethylporphin* (**36**).—Dibenzyl 3-(2-benzyloxycarbonylethyl)-4′-(2-methoxycarbonylethyl)-3′,4-dimethyl-2,2′-methylenedipyrrole-5,5′-dicarboxylate (230 mg) in tetrahydrofuran (50 ml), was hydrogenated over 10% palladized charcoal (25 mg). When uptake was complete, the filtered solution was evaporated and the residue redissolved in 2M-aqueous ammonia (10%) and then diluted with water to 30 ml. This was then neutralized with acetic acid and the precipitated methylenedipyrroletricarboxylic acid (**27**) was collected by centrifugation, washed with water, and dried (96 mg, 69%), m.p. 167—169 °C (decomp.). This acid (75 mg), without further purification, was dissolved in trifluoroacetic acid (12 ml) and stirred under nitrogen at 2—3 °C for 70 min. The solution was added to the diformylmethylenedipyrrole (**10**) (99 mg) in methylene chloride (60 ml) and methanol (12 ml) and stirred at 18 °C for 2 min. Sodium acetate (3 gm) was then added and the solution was stirred for a further 20 min, washed with aqueous sodium hydrogen carbonate and water and finally evaporated. The *porphyrin* was purified by t.l.c. on silica with methanol–chloroform (1:20) as eluant (63 mg, 41%), m.p. 196—198 °C from methylene chloride–methanol (Found: C, 72.3; H, 6.1; N, 6.4. $C_{51}H_{52}N_4O_8$ requires C, 72.2; H, 6.1; N, 6.7%), $\lambda_{max.}$($CHCl_3$), 403, 500, 534, 570, 593, and 620 nm; δ 9.84 (4 H, br s, *meso*-H), 7.00 (10 H, s, ArH), 5.03 (4 H, s, $ArCH_2$), 4.30 (8 H, br m, $CH_2CH_2CO_2$), 3.49 (15 H, singlets, Me), 3.25 (8 H, br m, $CH_2CH_2CO_2$), and −4.32 (2 H, br s, NH); *m/z* 848 (M^+, 83%), 804 (50), 790 (38), 786 (33), 772 (83), 758 (33), and (862 corresponding to M^+ of methyl ester of porphyrin; 100%).

The foregoing porphyrin (**36**) (3 mg) in methylene chloride was treated with an excess of ethereal diazomethane, stirred for 3 min, and then evaporated. The residue was chromatographed on alumina using ether–methylene chloride (1:5) and the product crystallized from methylene chloride–methanol, m.p. 160—163 °C; $\lambda_{max.}$($CHCl_3$) 400, 500, 534, 570, 593, and 620 nm; δ 10.01, 10.05 (4 H, 2 × s, *meso*-H), 7.02 (10 H, s, ArH), 5.04 (4 H, s, $ArCH_2$), 4.44 (8 H, t, $CH_2CH_2CO_2$), 3.79—3.63 (18 H, singlets, Me), 3.34 (8 H, m, $CH_2CH_2CO_2$), and −3.66 (2 H, s, NH); *m/z* 862 (M^+, 100%) and 785 (31).

13,17-*Bis*(2-*benzyloxycarbonylethyl*)-8-(2-*carboxyethyl*)-3-(2-*methoxycarbonylethyl*)-2,7,12,18-*tetramethylporphin* (**37**).—

The methylenedipyrrole (**30**) (95 mg) and the diformylmethylenedipyrrole (**10**), gave as in the foregoing synthesis the *porphyrin* (68 mg, 40%), m.p. 190—192 °C from methylene chloride–methanol (Found: M^+, 848.3780; $C_{51}H_{52}N_4O_8$ requires M, 848.3784); $\lambda_{max.}$($CHCl_3$) 402, 500, 534, 570, 593, and 620 nm; δ 9.83 (4 H, br s, *meso*-H), 7.00 and 6.95 (10 H, s, ArH), 5.02 and 4.96 (4 H, s, $ArCH_2$), 4.25 (8 H, br m, $CH_2CH_2CO_2$), 3.49 to 3.04 (23 H, overlapping s and m, $CH_2CH_2CO_2$ and Me), and −4.7 (2 H, br s, NH).

This porphyrincarboxylic acid (**37**) (3 mg) was esterified with diazomethane as for (**36**). The product had m.p. 162—164 °C from methylene chloride–methanol, $\lambda_{max.}$($CHCl_3$) 400, 500, 534, 569, 593, and 620 nm; δ 10.04 (4 H, s, *meso*-H), 7.03 (10 H, s, ArH), 5.06 (4 H, s, $ArCH_2$), 4.45 (8 H, t, $CH_2CH_2CO_2$), 3.71 and 3.64 (18 H, 2 × s, Me), 3.35 (8 H, m, $CH_2CH_2CO_2$), and −3.65 (2 H, s, NH); *m/z* 862 (M^+, 100%), 848 (81), 804 (30), 793 (36), and 792 (38). This porphyrin and the material obtained by esterification of (**36**) above were inseparable by h.p.l.c.

Coproporphyrin-III Monomethyl Ester (*at the* C-8 *Propionate Residue*).—The dibenzyl ester (**36**) (50 mg) in glacial acetic acid (25 ml) was hydrogenated over 10% palladized charcoal (10 mg) in the dark under ambient conditions for 4 h. The resulting solution was stirred in daylight and air for 10 min and then filtered and evaporated. A solution of the residue in 2M-ammonia was neutralized with acetic acid, the precipitated *porphyrin* (39.5 mg, 100%) being collected, washed with water and dried. Paper chromatography of the product on Whatman No. 1 paper, with 2,6-dimethylpyridine–water (5:3.5) as eluant gave one spot, R_F 0.45. Coproporphyrin-III showed R_F 0.36 and haemin chloride had R_F 0.59 (Found: C, 64.4; H, 6.0; N, 7.8. $C_{37}H_{40}O_8N_4 \cdot H_2O$ requires C, 64.7; H, 6.2; N, 8.2%), δ(CF_3CO_2D) 11.50 (CF_3CO_2H; reference), 13.3 (1 H), 11.24 (1 H), 11.18 (1 H), and 11.13 (1 H) (all s, *meso*-H), 4.81 (8 H, t, $CH_2CH_2CO_2$), 3.96 (15 H, s, Me), and 3.55 (8 H, t, $CH_2CH_2CO_2$); δ(C_5D_5N) 10.61 (1 H), 10.35 (1 H), 10.22 (1 H), and 10.10 (1 H) (all s, *meso*-H), 4.51 (8 H, m, $CH_2CH_2CO_2$), 3.49 and 3.43 (15 H, s, Me), 3.35 (8 H, m, $CH_2CH_2CO_2$), and −3.22 (2 H, s, NH).

Coproporphyrin-III Monomethyl Ester (*at the* C-3 *Propionate Residue*).—The dibenzyl ester (**37**) (50 mg) was hydrogenated and worked up as in the foregoing preparation (37.3 mg, 95%), single spot by paper chromatography as above, R_F 0.56; on the same chromatogram coproporphyrin-III showed R_F 0.35 and haemin chloride R_F 0.56 (Found: C, 65.15; H, 6.0; N, 7.8. $C_{35}H_{40}N_4O_8 \cdot H_2O$ requires C, 64.7; H, 6.2; N, 8.2%), δ(CF_3CO_2D) 11.50 (CF_3CO_2H; reference), 11.34 (1 H), 11.24 (1 H), 11.19 (1 H), and 11.12 (1 H) (all s, *meso*-H), 4.82 (8 H, t, $CH_2CH_2CO_2$), 3.96 (15 H, s, Me), and 3.46 (8 H, t, $CH_2CH_2CO_2$); δ(C_5D_5N) 10.62 (1 H), 10.36 (1 H), 10.22 (1 H), and 10.09 (1 H) (all s, *meso*-H), 5.50 (8 H, m, $CH_2CH_2CO_2$), 3.50, 3.47, 3.45, and 3.43 (15 H, 4 × s, Me), 3.34 (8 H, m, $CH_2CH_2CO_2$), and −3.22 (2 H, s, NH).

Enzymic Experiments

Effect of Enzymes from Euglena gracilis *on Coproporphyrinogen-III*.—Coproporphyrin-III tetramethyl ester (**33**) (11 mg) in redistilled tetrahydrofuran (10 ml) and aqueous 2M-potassium hydroxide (12 ml) was stirred overnight at 20 °C under nitrogen in the dark. The organic layer was then separated and the aqueous layer was freed of tetrahydrofuran by evaporation and was then neutralized with dilute hydrochloric acid. The precipitated porphyrin was redissolved with the minimum amount of aqueous 2M-potassium hydroxide.

Freshly prepared 3% sodium amalgam (10 g) was stored over sodium hydroxide pellets *in vacuo* for *ca.* 2 h and then divided into two portions, each being added to half of the porphyrin solution prepared above. The mixture was shaken under nitrogen in the dark for *ca.* 3—4 min, during which time the solution became colourless. The solution of coproporphyrinogen-III was filtered and used immediately.

The cell-free extract (200 ml) from broken *Euglena gracilis* cells[36] was adjusted to pH 7.0 with Tris buffer and after addition of EDTA (1.5 g per 200 ml of enzyme solution) with stirring was again adjusted to pH 7.0. The solution of porphyrinogen was then added, the pH was adjusted to 7.5—8.0, and the resulting solution was incubated with shaking in air and in the dark at 30 °C. The incubation mixture was then added to ethyl acetate–acetic acid (3:1) (250 ml) and stirred in the light for 20 min. The mixture was centrifuged at 1 500 g for 10 min after which the organic layer was syphoned off, and the aqueous layer was decanted from the protein. The protein was washed with more ethyl acetate–acetic acid (3:1) (100 ml), and the solids were again removed by centrifugation.

The aqueous layer was also extracted with ethyl acetate–acetic acid (3:1) (2 × 100 ml). The combined organic fractions were washed once with saturated aqueous sodium acetate (250 ml), and once with aqueous 3% sodium acetate (250 ml). The organic layer was finally extracted with 1.5M-hydrochloric acid (3 × 20 ml and 4 × 10 ml), the combined acidic extracts were adjusted to pH 4 by addition of sodium hydrogen carbonate, and the porphyrins were extracted into ether. The residue from the ether was treated with 5% H_2SO_4 in methanol (20 ml) overnight at 4 °C in the dark until it was mixed with chloroform (50 ml) and washed with saturated aqueous sodium hydrogen carbonate. Chromatography of the residue from the chloroform on alumina was with (*a*) benzene, (*b*) methylene chloride (elutes protoporphyrin-IX dimethyl ester and then harderoporphyrin trimethyl ester), (*c*) ether–methylene chloride (1:10) (elutes coproporphyrin-III tetramethyl ester). The separated porphyrins were crystallized from chloroform–methanol.

The optimum incubation time was *ca.* 30 min which yielded protoporphyrin-IX dimethyl ester (*ca.* 2.0 mg), m.p. 232—234 °C (lit.,[37] 231 °C); harderoporphyrin trimethyl ester (*ca.* 0.5 mg), m.p. 202—205 °C (lit.,[1] 203—204 °C); and coproporphyrin-III tetramethyl ester (*ca.* 0.2 mg), m.p. 148—150 °C (see above 152.5—153.5 °C). Satisfactory u.v.–vis., n.m.r., and mass spectral data were obtained for all three porphyrins.

Effect of Enzymes from Euglena gracilis *on Homologues* (**5**) *and* (**6**) *of Coproporphyrinogen-III*.—The homologue (**35**) (11 mg) was hydrolysed as above and reduced to the porphyrinogen (**6**), which was incubated with the cell-free system from *Euglena gracilis* (200 ml) for 18 h at 30 °C. Extraction as before gave the harderoporphyrin homologue (**40**) (0.5 mg), m.p. 173—175 °C from methanol–chloroform, homogeneous by h.p.l.c. (Found: M^+, 664.3221. $C_{39}H_{44}N_4O_6$ requires M, 664.3259), $\lambda_{max.}$($CHCl_3$) 404, 503, 539, 572, and 623 nm; δ 10.20 (1 H), 10.13 (1 H), and 10.08 (2 H) (all s, *meso*-H), 8.2 (1 H, m, $CH{=}CH_2$), 6.2 (2 H, m, $CH{=}CH_2$), 4.44 (6 H, m, $CH_2CH_2CO_2$), 4.16 (2 H, m, $CH_2CH_2CH_2CO_2$), 3.73 and 3.66 (24 H, s, Me), 3.31 (8 H, m, $CH_2CH_2CO_2$ and $CH_2CH_2CH_2CO_2$), 2.68 (2 H, m, $CH_2CH_2CH_2CO_2$), and −3.59 (2 H, s, NH); *m/z* 664 (M^+ 100%), 633 (6), 605 (5), 591 (16.5), and 577 (8). Recovered starting material (**35**) was also isolated.

In parallel experiments, it was found that the isomeric homologue (**5**) was unaffected by the enzyme system.

Effect of Enzymes from Euglena gracilis *on Coproporphyrinogen-III Mono Methyl Esters* (**7**) *and* (**8**).—Coproporphyrin-III monomethyl ester (at the C-8 propionate residue) (10 mg) was shaken with 3% sodium amalgam (10 g) in pH 7.5M-phosphate buffer (15 ml). The resultant colourless solution was filtered and the pH was adjusted to *ca.* 8.0 with dilute aqueous acetic acid.

This solution was added to the enzyme preparation (250 ml) as above in the presence of EDTA (0.75 g) at pH. 7.6. After incubation at 37 °C for 18 h the tetrapyrroles were extracted by the method described above and analysed by h.p.l.c. The main component was copro-III Me_4 ester together with a very minor product (*ca.* 10 g) which was identical with harderoporphyrin trimethyl ester (**39**) by t.l.c. on silica in ether–methylene chloride (1:9) and by h.p.l.c. (co-injection with authentic material) on μ-Bondapak-C_{18} in aqueous acetronitrile.

Repetition of this experiment with the isomeric ester modified on ring A (**7**) afforded only copro-III Me_4 ester (**33**).

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References

1 Part 24, A. R. Battersby, I. A. Chaudhry, P. S. Clezy, C. J. R. Fookes, T. T. Hai, E. McDonald, and M. Thompson, *J. Chem. Soc., Perkin Trans. 1*, 1985, 135.
2 J. A. S. Cavaleiro, G. W. Kenner, and K. M. Smith, *J. Chem. Soc., Chem. Commun.*, 1973, 183; *J. Chem. Soc., Perkin Trans 1*, 1974, 1188.
3 D. E. Games, A. H. Jackson, J. R. Jackson, R. V. Belcher, and S. G. Smith, *J. Chem. Soc., Chem. Commun.*, 1976, 187.
4 G. H. Elder, J. O. Evans, J. R. Jackson, and A. H. Jackson, *Biochem. J.*, 1978, **169**, 215.
5 Z. Zaman, M. M. Aboud, and M. Akhtar, *J. Chem. Soc., Chem. Commun.*, 1972, 1263.
6 A. R. Battersby, J. Baldas, J. Collins, D. H. Grayson, K. J. James, and E. McDonald, *J. Chem. Soc., Chem. Commun.*, 1972, 1265.
7 A. R. Battersby, E. McDonald, H. K. W. Wurziger, and K. J. James, *J. Chem. Soc., Chem. Commun.*, 1975, 493; A. R. Battersby, *Experientia*, 1978, **34**, 1.
8 M. Akhtar, M. M. Aboud, G. Bernard, P. Jordan, and Z. Zaman, *Philos. Trans. R. Soc. London, Ser. B*, 1976, **237**, 117.
9 T. Yoshinaga and S. Sano, *J. Biol. Chem.*, 1980, **255**, 4722.
10 T. Yoshinaga and S. Sano, *J. Biol. Chem.*, 1980, **255**, 4727.
11 A. H. Jackson, D. M. Jones, G. Philip, T. D. Lash, A. M. Alcira, and S. G. Smith, *Int. J. Biochem.*, 1980, **12**, 681.
12 M. Mori and S. Sano, *Biochim. Biophys. Acta.*, 1972, **264**, 252.
13 R. Poulson and W. J. Poulgase, *J. Biol. Chem.*, 1974, **249**, 6367.
14 S. Sano and S. Granick, *J. Biol. Chem.*, 1961, **236**, 1173.
15 L. Mombelli, E. McDonald, and A. R. Battersby, *Tet. Lett.*, 1976, 1037; A. R. Battersby, A. D. Hamilton, E. McDonald, L. Mombelli, and O.-H. Wong, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1283.
16 H. M. Al-Hazimi, A. H. Jackson, D. J. Ryder, G. H. Elder, and S. G. Smith, *J. Chem. Soc., Chem. Commun.*, 1976, 188.
17 R. J. Porra and J. E. Falk, *Biochem. J.*, 1964, **90**, 69.
18 A. H. Jackson, D. M. Jones, S. G. Smith, and G. H. Elder, *J. Chem. Soc., Chem. Commun.*, 1976, 322.
19 G. P. Arsenault, E. Bullock, and S. F. MacDonald, *J. Am. Chem. Soc.*, 1960, **82**, 4384.
20 P. S. Clezy and A. J. Liepa, *Aust. J. Chem.*, 1970, **23**, 2443.
21 A. Hayes, G. W. Kenner, and N. R. Williams, *J. Chem. Soc.*, 1958, 3779.
22 J. A. S. Cavaleiro, G. W. Kenner, and K.M. Smith, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2478.
23 P. S. Clezy and A. J. Liepa, *Aust. J. Chem.*, 1970, **23**, 2443.
24 A. R. Battersby, D. G. Buckley, G. L. Hodgson, R. E. Markwell, and E. McDonald, in 'High Pressure Liquid Chromatography in Clinical Chemistry,' eds. P. F. Nixon, C. H. Gray, C. K. Lim, and M. S. Stoll, Academic Press, London, 1976.
25 A. R. Battersby, J. Staunton, and R. H. Wightman, *J. Chem. Soc., Chem. Commun.*, 1972, 188.
26 M. S. Stoll, G. H. Elder, D. E. Games, P. O'Hanlon, D. S. Millington, and A. H. Jackson, *Biochem. J.*, 1973, **26**, 2697.
27 A. W. Johnson, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1958, 4254.
28 A. W. Johnson, I. T. Kay, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc., C*, 1959, 3416.
29 A. H. Jackson, G. W. Kenner, and D. Warburton, *J. Chem. Soc.*, 1965, 1328.
30 G. G. Kleinspehn, *J. Am. Chem. Soc.*, 1955, **77**, 1546.
31 J. Cason, *Org. Synth.*, Coll. Vol. III, 1955, 169.
32 A. H. Jackson, G. W. Kenner, and K. M. Smith, *J. Chem. Soc., C*, 1971, 502.
33 R. J. Abraham, G. H. Barnett, E. S. Bretschneider, and K. M. Smith, *Tetrahedron*, 1973, **29**, 553.
34 P. S. Clezy, C. J. R. Fookes, and A. J. Liepa, *Aust. J. Chem.*, 1972, **25**, 1979.
35 T. H. Fuhrhop and K. M. Smith in 'Falk's Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam, 2nd edn. 1975.
36 A. R. Battersby, J. F. Beck, K. H. Gibson, G. L. Hodgson, K. E. Markwell, E. McDonald, J. Moron, and L. N. Nixon, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2771.
37 J. E. Falk, 'Porphyrins and Metalloporphyrins,' Elsevier, Amsterdam, 1964.

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