Pyrroles and Related Compounds. Part 38.[1] Porphobilinogen Synthesis [2]

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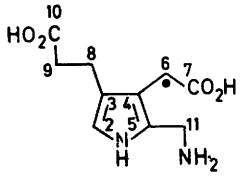
A new synthesis of porphobilinogen (1) and its [6-^{13}C]- and [6-^{14}C]-derivatives, *via* porphobilinogen lactam, is reported. For the most part the synthesis follows established methods in pyrrole chemistry, but there are two significant advances which make the route attractive: (1) transformation of readily available acetylpyrroles into methoxycarbonylmethylpyrroles by using thallium(III) nitrate in methanol, and (2) introduction of the aminomethyl function into porphobilinogen by cleavage of a phthalimidomethyl substituent.

PORPHOBILINOGEN (PBG) (1) is the key building block in the biosynthesis of the haemoproteins, the plant pigments, and vitamin B_{12},[3a] and their catabolic products



(1)

• = ^{13}C or ^{14}C label

P^R = $CH_2 \cdot CH_2 \cdot CO_2R$

A^R = $CH_2 \cdot CO_2R$

(*e.g.* bile pigments).[3b] The molecule is formed naturally by condensation of two molecules of δ-aminolaevulinic acid (ALA); this process has recently been used to accomplish large-scale preparations of PBG from δ-aminolaevulinic acid by using ALA dehydratase from *P. shermanii*[4] or from *R. spheroides* covalently bound to deactivated sepharose.[5] However, these new developments were preceded by a great deal of purely chemical ground work,[6] and this type of approach is still fundamentally important if PBG molecules with only one label are required. In this paper we report an efficient synthetic approach to PBG and derivatives carbon-labelled in the acetic acid side-chain.

Previous syntheses can be broadly classified into two groups, namely those following the somewhat classical

[1] Part 37, A. H. Jackson, G. W. Kenner, K. M. Smith, and C. J. Suckling, *Tetrahedron*, in the press.

[2] Preliminary publications, (*a*) G. W. Kenner, K. M. Smith, and J. F. Unsworth, *J.C.S. Chem. Comm.*, 1973, 43; (*b*) G. W. Kenner, J. Rimmer, K. M. Smith, and J. F. Unsworth, *Phil. Trans. Roy. Soc., Ser. B*, 1976, **273**, 255.

[3] For reviews see chapters in 'Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam, 1975, (*a*) A. R. Battersby and E. McDonald, p. 61; (*b*) P. O'Carra, p. 123.

[4] G. Müller, *Z. Naturforsch.*, 1972, **27b**, 473.

[5] D. Gurne and D. Shemin, *Science*, 1973, **180**, 1188.

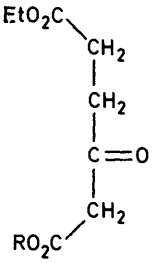
[6] For a review see, A. H. Jackson, and K. M. Smith, in 'Total Synthesis of Natural Products,' ed. J. W. ApSimon, Wiley, New York, vol. 1, 1973, p. 155.

route through 2-methylpyrrole-5-carboxylates [7] and those proceeding through the 'azaindole' route.[8] Although the azaindole route is extremely versatile in that it offers several opportunities for introduction of labelled atoms, it may only be used for preparation of PBG-like molecules which, by and large, are unsuitable for use as intermediates in chemical syntheses of porphyrins. On the other hand, the classical route (so named on account of the now classical series of papers by MacDonald [7c-j] which describe its early development) had several pyrrolic intermediates which can be utilised in a variety of syntheses of biologically significant porphyrins, as well as for PBG itself. Since the problems to which we were addressing ourselves at that time [2b] required not only labelled PBG but also labelled uroporphyrinogen-III and other 'sub-uroporphyrinogens, we chose to investigate the classical approach.

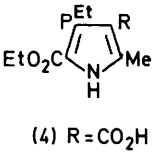
DISCUSSION

2-Methylpyrrole-5-carboxylates are readily available.[6] One major difficulty encountered by MacDonald was in the construction of the acetic acid side-chain, which can only be incorporated directly with difficulty.[9-11] Eventually, a method involving reductive *C*-alkylation using the 3-unsubstituted pyrrole and glyoxylic acid was devised,[7j] but such 3-unsubstituted pyrroles are not directly accessible from Knorr-type pyrrole syntheses. Our approach hinged upon the transformation of acetylpyrroles (for which acetylacetone is the readily available precursor) into the corresponding methoxycarbonylmethylpyrroles by using thallium(III) nitrate in methanol, wherein the methyl group in the acetyl is transformed into the methylene of the acetic side-chain by means of an aryl migration.[12]

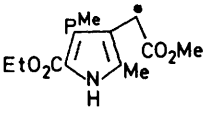
Thus, treatment of the acetylpyrroles (6) and (8) [derived from diethyl β-oxoadipate (2) and α-benzyl ω-ethyl β-oxoadipate (3), respectively] with 1.1 equiv. of thallium(III) nitrate in methanol containing nitric acid led to rearrangement and concomitant transesterification at the propionate groups, to give compounds (7) (79%) and (12) (83%). Labelled analogues were obtained by Friedel–Crafts acetylation of the 3-unsubstituted pyrroles (5) and (10) with [2-^{14}C]- and [2-^{13}C]-acetyl chloride, prepared, with oxalyl chloride, from the correspondingly labelled sodium acetate. An 82% yield of the 3-unsubstituted pyrrole (5) was meanwhile obtained by decarboxylation (copper–quinoline) [13] of the pyrrolecarboxylic acid (4); acetylation with the labelled acetyl chlorides and aluminium trichloride gave the [7-^{14}C]-acetylpyrrole (6) in 47% yield, and the [7-^{13}C]acetylpyrrole (6) (enhanced peak at 32.1 p.p.m.) in 58% yield



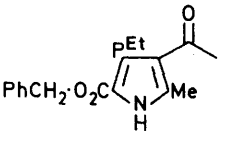
(2) R = Et
(3) R = CH_2Ph



(4) R = CO_2H
(5) R = H
(6) R = $COMe$



(7)



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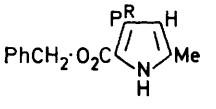
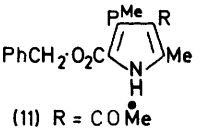


(9) R = CH_2Ph
(10) R = Me



(11) R = $COMe$
(12) R = CH_2CO_2Me

from the appropriate sodium acetate samples [for numbering see (1)].

The pyrrole (10) was obtained by a double transesterification with the pyrrole (5): treatment with sodium in benzyl alcohol gave an 86% yield of the dibenzyl ester (9), which was transformed into the pyrrole (10) in 84% yield by methanol containing nitric acid. Acetylation of (10) gave the [7-^{14}C]acetylpyrrole (11) or the [7-^{13}C]acetylpyrrole (11) (enhanced peak at 30.99 p.p.m.) in 31 and 69% yield, respectively, from the appropriate sodium acetate.

The Friedel–Crafts reaction gave substantially lower yields with the benzyl ester pyrroles than with the corresponding ethyl esters. Trial acetylations with the pyrrole (13) showed anhydrous tin(IV) chloride to be

[7] (*a*) K. S. N. Prasad and R. Raper, *Nature*, 1955, **175**, 629; (*b*) C. Rimington and S. Krol, *ibid.*, p. 630; (*c*) A. H. Jackson, D. M. MacDonald, and S. F. MacDonald, *J. Amer. Chem. Soc.*, 1956, **78**, 505; (*d*) A. H. Jackson and S. F. MacDonald, *Canad. J. Chem.*, 1957, **35**, 715; (*e*) D. M. MacDonald and S. F. MacDonald, *ibid.*, 1955, **33**, 573; (*f*) G. P. Arsenault and S. F. MacDonald, *ibid.*, 1961, **39**, 2043; (*g*) S. F. MacDonald and R. J. Stedman, *ibid.*, 1955, **33**, 458; (*h*) *J. Amer. Chem. Soc.*, 1953, **75**, 5448; (*i*) S. F. MacDonald, *J. Chem. Soc.*, 1952, 4176, (*j*) M. W. Roomi and S. F. MacDonald, *Canad. J. Chem.*, 1970, **48**, 139; (*k*) A. Treibs and K. Hintermeier, *Chem. Ber.*, 1954, **87**, 1167; (*l*) A. Treibs and W. Ott, *Annalen*, 1958, **615**, 137; (*m*) A. R. Battersby, J. Moron, E. McDonald, and J. Feeney, *J.C.S. Chem. Comm.*, 1972 920; (*n*) A. R. Battersby E. Hunt, E. McDonald, and J. Moron, *J.C.S. Perkin I*, 1973, 2917. A novel approach to PBG employing the latter stages of MacDonald's route has very recently been reported; (*o*) M. I. Jones, C. Froussios, and D. A. Evans, *J.C.S. Chem. Comm.*, 1976, 472.

[8] (*a*) B. Frydman, S. Reil, M. E. Despuy, and H. Rapoport, *J. Amer. Chem. Soc.*, 1969, **91**, 2338; (*b*) B. Frydman, G. Buldain, and J. C. Repetto, *J. Org. Chem.*, 1973, **38**, 1824; (*c*) A. Valasinas, E. S. Levy, and B. Frydman, *ibid.*, 1974, **39**, 2872; (*d*) A. R. Battersby, K. J. James, E. McDonald, and H. K. W. Wurtziger, *J.C.S. Chem. Comm.*, 1975, 493.

[9] H. Plieninger, P. Hess, and J. Ruppert, *Chem. Ber.*, 1968, **101**, 240.

[10] See footnote 3 in ref. 2*a*.

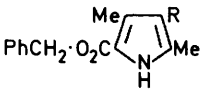
[11] P. S. Clezy and V. Diakiw, *Austral. J. Chem.*, 1973, **26**, 2697.

[12] A. McKillop, B. P. Swann, and E. C. Taylor, *J. Amer. Chem. Soc.*, 1971, **93**, 4919; 1973, **95**, 3340.

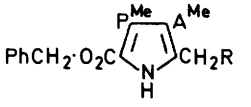
[13] T. Cohen and R. A. Schambach, *J. Amer. Chem. Soc.*, 1970, **92**, 3189.

superior to aluminium trichloride for catalysis of the reaction, which required only *ca.* 30 min for completion (t.l.c.) and gave yields of acetylpyrrole (14) consistently *ca.* 70% as compared with 35% or less for the same reaction with aluminium trichloride.

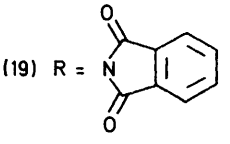
Thallium(III) nitrate-promoted rearrangement of the labelled acetylpyrroles (6) and (11) gave high yields, in all cases, of the corresponding [6-^{14}C]- and [6-^{13}C]-methoxycarbonylmethylpyrroles (7) and (12). There was no loss of radioactivity in the ^{14}C series, and the fact that an aryl migration had occurred was confirmed in the ^{13}C series, in which only the methylene in the acetic side-chain possessed ^{13}C content above natural abundance. The dimethylacetylpyrrole (14) was also treated with thallium(III) nitrate and gave a high yield of the pyrrole (15); this pyrrole, which is also accessible [14] by conventional ring fabrication, is now the precursor most often chosen for syntheses [15] of vinyl-substituted porphyrins.



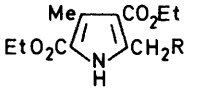
(13) R=H
(14) R=COMe
(15) R=$CH_2 \cdot CO_2Me$
(16) R=$CH_2 \cdot CO_2 \cdot CH_2 \cdot CH_2 \cdot OH$
(17) R=$CO \cdot CH_2 \cdot O \cdot NO_2$



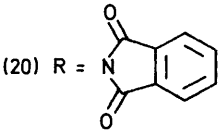
(18) R=OAc



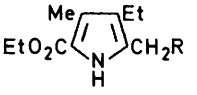
(19) R=



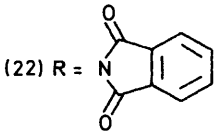
(20) R=



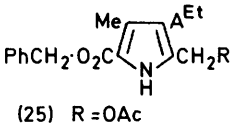
(21) R=Br



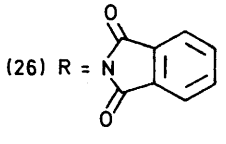
(22) R=



(23) R=Br
(24) R=NH_2



(25) R=OAc



(26) R=

Attempts to vary the esterifying alcohol in the acetic side-chain were only partially successful. Use of thallium(III) nitrate and ethylene glycol with the pyrrole (14) gave starting material and a 41% yield of the glycol ester (16). However, when benzyl alcohol was used a 74% yield was obtained of the (2-nitro-oxyacetyl) derivative (17), presumably by attack of nitrate anion upon the expected *C*-thalliated intermediate. t-Butyl alcohol reacted rapidly with thallium(III) nitrate to give a brown sludge of (presumably) thallium(III) oxide, and this reaction was not further investigated.

The methoxycarbonylmethylpyrrole (12) was to be the key pyrrole in our PBG synthesis, which is described below for the unlabelled series. All developments equally apply for the synthesis of the [6-^{14}C]- and [6-^{13}C]-derivatives; details of the labelled compounds are in the Experimental section.

Treatment of the pyrrole (12) with lead tetra-acetate in acetic acid gave the acetoxymethyl derivative (18) in 85% yield. This was converted in 89% yield into the phthalimidomethylpyrrole (19), a highly crystalline compound, by stirring with 1 equiv. of the potassium salt of phthalimide in dimethyl sulphoxide. Lower yields of (19) were obtained when other solvents (pyridine or dimethylformamide) were used. This type of Gabriel synthesis has been used before in the pyrrole series. In 1958 Treibs and Ott [7] prepared the phthalimidomethylpyrrole (20) in 54% yield by fusing the 2-bromomethylpyrrole (21) with potassiophthalimide; in the same year, Hayes [16] obtained the pyrrole (22) in 38% yield from the 2-bromoethylpyrrole (23) and potassiophthalimide in dimethylformamide. The phthalimidomethylpyrrole (22) was cleaved with hydrazine hydrate in methanol at 25 °C to give the 2-aminomethylpyrrole (24) in 91% yield. These promising results invited us to attempt to introduce the aminomethyl group into PBG in such a manner.

The next stage required removal of the benzyloxycarbonyl group in compound (19). The phthalimidomethylpyrrole (22) was reported [16] to be stable towards hydrogenolysis, and it was likewise discovered that the pyrroles (19) and (26) [obtained from the pyrrole (25)] could not be deprotected in the normal way. However, we had earlier shown [17] that benzyl esters in the porphyrin series can be cleaved acidolytically, and, by using the pyrrole (26) in trial reactions, conditions were developed to give the 2-unsubstituted pyrrole (27) in 63% yield from (19) by using a 1 : 1 mixture of 10% sulphuric acid in trifluoroacetic acid (TFA) and anisole. Treatment of (26) with 5% H_2SO_4 in TFA and methylene chloride gave the carboxylic acid (29) (identified by n.m.r.). Repetition of the reaction in the absence of methylene chloride gave a mixture of the required 2-unsubstituted pyrrole (28) and another product, identified by mass and n.m.r. spectroscopy as the 2-benzylpyrrole (30). By varying the percentage of H_2SO_4 in the TFA and the concentration of pyrrole substrate it was seen that formation of 2-benzylpyrrole was favoured by low acid strength and high pyrrole concentration. To avoid formation of the 2-benzylpyrrole in the synthesis of the

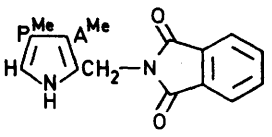
[14] R. P. Carr, A. H. Jackson, G. W. Kenner, and G. S. Sach, *J. Chem. Soc.* (*C*), 1971, 487.

[15] For general reviews on contemporary porphyrin synthesis see K. M. Smith, *Quart. Rev.*, 1971, **25**, 31; ref. 3, p. 29; ref. 6, p. 166.

[16] A. Hayes, Ph.D. Thesis, Cambridge, 1958.

[17] M. J. Sutton, Ph.D. Thesis, Liverpool, 1974.

pyrrole (27), anisole was added as a benzylium ion scavenger. In this way, a 69% yield of the model 2-unsubstituted pyrrole (28) was obtained from (26).



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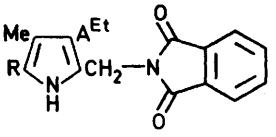
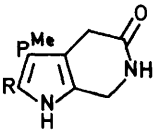
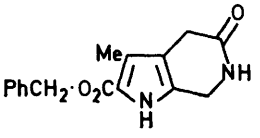


(28) R = H
(29) R = CO_2H
(30) R = $PhCH_2$



(31) R = H
(32) R = $CO_2 \cdot CH_2Ph$



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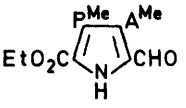

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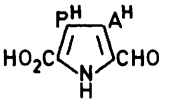

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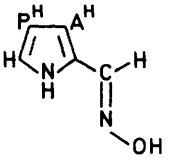

(36)

The phthalimide protecting group was next cleaved by treatment of the 2-unsubstituted pyrrole (27) with hydroxylamine hydrochloride, to give PBG lactam methyl ester (31) in 62% yield. The lactam was also prepared by using methylhydrazine, but though yields were comparable the former procedure was manipulatively more easy, particularly in small-scale reactions of the type used in the preparation of labelled products. The phthalimidomethylpyrroles (19) and (26) were also converted into the corresponding lactams (32) and (33) by using an excess of hydrazine hydrate in methanol (76 and 74% yield, respectively). However, this last method suffers from irreproducibility and attempts to cleave the benzyl ester by catalytic hydrogenation of the lactams over either palladised charcoal or Raney nickel were unsuccessful. When treated with 10% H_2SO_4 in TFA with anisole, the lactams (32) and (33) gave only a low yield of their respective 2-benzyl and 2-unsubstituted pyrroles.

Finally, alkaline hydrolysis [8a] of PBG lactam (31) gave a 67% yield of PBG (1), obtained as a white crystalline solid. The identity of the material was established by n.m.r. spectroscopy, and its purity was confirmed in the usual way by ascending paper chromatography.[7f]

The methoxycarbonylmethylpyrrole (7) and its [6-^{14}C]-derivative were also transformed [2a] into PBG by the efficient route developed by MacDonald.[7f] Dichlorination and hydrolysis gave the formylpyrrole (34) in 86% yield, and this was hydrolysed in 10% NaOH to give the tricarboxylic acid (35). Treatment of the pyrrole (35) with hydroxylamine hydrochloride gave the corresponding oxime (36), which was hydrogenated over palladised charcoal to give (in our hands) erratic yields ($\leqslant$30%) of PBG (1). This final reduction step is also reported by MacDonald to be troublesome, a by-product of undefined structure being produced during the hydrogenation.

EXPERIMENTAL

M.p.s were measured with a hot-stage apparatus. Unless otherwise stated, neutral alumina (Fluka; Brockmann grade III) was used for all chromatographic separations. Reactions were followed by t.l.c. as described in earlier parts of this Series. Carbon-13 and proton n.m.r. spectra were determined (solutions in $CDCl_3$) with a Varian XL-100 (^{13}C and ^{1}H) or HA-100 (^{1}H) instrument, with Me_4Si as internal standard and conditions as previously described.[18] Mass spectra were measured with an A.E.I. MS12 or MS902 spectrometer (at 50 μA and 70 eV; direct insertion probe with source temperature $\leqslant$200 °C).

Diethyl β-Oxoadipate (2).—Ethyl t-butyl malonate (45 g) in dry ether (200 ml) was stirred and refluxed with magnesium ethoxide (27.3 g) during 15 min. β-Ethoxycarbonylpropionyl chloride (43.3 g, 1.1 equiv.) in ether (200 ml) was added to the cooled cloudy solution, which was then refluxed for a further 3 h. The solution was acidified with 4N-sulphuric acid and the ether layer was separated. The aqueous phase was extracted with ether (3 × 50 ml) and the combined organic extracts were dried (Na_2SO_4) and evaporated to give an oil which was stirred with trifluoroacetic acid (150 ml) during 2 h at 25 °C. Evaporation *in vacuo* gave an oil which was distilled to give the product (31 g, 60%), b.p. 126—130° at 1 mmHg, τ 5.78 and 5.84 (4 H, q, 2 × OCH_2), 6.48 (2 H, s, CH_2CO), 7.0—7.5 (4 H, m, $CH_2 \cdot CH_2$), and 8.71 and 8.74 (6 H, t, 2 × $CH_2 \cdot CH_3$).

α-Benzyl ω-Ethyl β-Oxoadipate (3).—This compound was prepared by adaptation of a procedure developed by Neuberger *et al.*[19] A solution of the foregoing adipate (44.4 g) in ether was washed with saturated aqueous sodium hydrogen carbonate before being dried (Na_2SO_4) and evaporated. Benzyl alcohol (100 ml) was distilled from calcium oxide and dissolved in ether; the solution was washed with brine, dried (Na_2SO_4), and evaporated. The adipate and benzyl alcohol were mixed and heated at 140 °C under a stream of nitrogen (to remove ethanol) for 3 h. The reaction was monitored by n.m.r. spectroscopy; completion of the reaction was indicated by appearance of a signal at τ 4.89 ($PhCH_2$) equal in intensity to the two-proton singlet at τ 6.60 ($PhCH_2 \cdot O_2C \cdot CH_2$). Benzyl alcohol

[18] R. J. Abraham, R. D. Lapper, K. M. Smith, and J. F. Unsworth, *J.C.S. Perkin II*, 1974, 1004.

[19] W. G. Laver, A. Neuberger, and J. J. Scott, *J. Chem. Soc.*, 1959, 1474.

and unchanged diethyl β-oxoadipate were removed by distillation (0.5 mmHg; temperatures up to 160 °C). The product, which decomposed when distillation was attempted, was used without further purification; yield 45 g (80%), τ 2.68 (5 H, s, Ph), 4.89 (2 H, s, PhCH_2), 5.92 (2 H, q, CH_2·CH$_3$), 6.50 (2 H, s, CH_2CO), 7.2—7.6 (4 H, m, CH_2·CH_2), and 8.77 (3 H, t, CH_2·CH_3).

Ethyl 3-(2-Ethoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (5).—2-Ethoxycarbonyl-3-(2-ethoxycarbonylethyl)-5-methylpyrrole-4-carboxylic acid [7i] (4) (15.2 g) in quinoline (75 ml) containing copper(II) acetate (250 mg) was heated under nitrogen at 180—230 °C during 3 h. The solution was allowed to cool (1 h) before addition of ether (300 ml) and extraction with 2N-hydrochloric acid (2 × 250 ml). The organic layer was washed with water (2 × 250 ml) and saturated brine (250 ml), then dried (Na_2SO_4) and evaporated to give an oil. Crystallisation from methylene chloride–n-hexane gave pale yellow plates (10.6 g, 82%), m.p. 63—64° (lit.,[7i] 65—67°), τ 0.76br (NH), 4.20 (1 H, d, *J* 3 Hz, 4-H), 5.73 (2 H, q, CH_2·CH$_3$), 5.91 (2 H, q, CH_2·CH$_3$), 6.9—7.4 (4 H, m, CH_2·CH_2), 7.78 (3 H, s, 5-Me), 8.67 (3 H, t, CH_2·CH_3), and 8.78 (3 H, t, CH_2·CH_3).

Benzyl 3-(2-Benzyloxycarbonylethyl)-5-methylpyrrole-2-carboxylate (9).—The foregoing pyrrole (2 g) in benzyl alcohol (30 ml) containing sodium (30 mg) was heated at 100 °C and 15 mmHg during 5 h. After cooling, solid carbon dioxide was added and the excess of benzyl alcohol was distilled off (1 mmHg). The resulting brown oil was chromatographed (elution with toluene–methylene chloride). Evaporation gave a pale yellow solid which was recrystallised from methylene chloride–n-hexane to give the *pyrrole* (2.6 g, 86%) as white needles, m.p. 75—76° (Found: C, 73.05; H, 6.0; N, 3.8. $C_{23}H_{23}NO_4$ requires C, 73.2; H, 6.1; N, 3.7%), τ 0.7—0.9br (NH), 2.74 (10 H, s, 2 × Ph), 4.27 (1 H, d, *J* 3 Hz, 4-H), 4.78 (2 H, s) and 4.97 (2 H, s) (2 × PhCH_2), 6.8—7.5 (4 H, m, CH_2·CH_2), and 7.86 (3 H, s, 5-Me).

Benzyl 3-(2-Methoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (10).—The pyrrole (9) (4 g) was stirred overnight at 25 °C in methanol (50 ml) containing concentrated nitric acid (3 drops). Methylene chloride (50 ml) and water (50 ml) were added, and the organic layer was washed with aqueous sodium hydrogen carbonate and water, and then dried (Na_2SO_4) and evaporated. The resultant oil was chromatographed (elution with toluene–methylene chloride) to give an oil which soon solidified. Recrystallisation from methylene chloride–n-hexane gave the *pyrrole* (2.7 g, 84%) as white needles, m.p. 69—70° (Found: C, 67.6; H, 6.4; N, 4.8. $C_{17}H_{19}NO_4$ requires C, 67.8; H, 6.4; N, 4.65%), τ 1.08br (NH), 2.68 (5 H, s, Ph), 4.23 (1 H, d, *J* 3 Hz, 4-H), 4.78 (2 H, s, PhCH_2), 6.42 (3 H, s, OMe), 6.9—7.5 (4 H, m, CH_2CH_2), and 7.82 (3 H, s, 5-Me).

Ethyl 4-Acetyl-3-(2-ethoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (6).—(a) *By ring synthesis.* Diethyl β-oxoadipate (9.2 g) containing concentrated hydrochloric acid (0.1 ml) was cooled in an ice–salt bath, and freshly distilled pentyl nitrite (6 ml) was added with stirring during 1 h. The mixture was set aside overnight at 0 °C, then added during 30 min to a vigorously stirred solution of acetylacetone (4.4 ml) in acetic acid (30 ml) containing zinc powder (6 g). The temperature was maintained at 65 °C throughout the addition and a mixture of zinc powder (12 g) and ammonium acetate (10 g) was added in portions so that zinc was always present in excess over adipate. When the addition was complete the mixture was heated at 95—100 °C for 2 h; while still hot, the mixture was decanted from the excess of zinc into well stirred ice–water (500 ml). The precipitated pyrrole was filtered off, washed with water, and dried. It was recrystallised from methylene chloride–n-hexane–ether to give white *needles* (6.2 g, 50%), m.p. 107—108° (Found: C, 61.0; H, 7.2; N, 4.75. $C_{15}H_{21}NO_5$ requires C, 61.0; H, 7.2; N, 4.7%), τ 0.2—0.3br (NH), 5.70 (2 H, q, CH_2·CH$_3$), 5.90 (2 H, q, CH_2·CH$_3$), 6.6—7.6 (4 H, m, CH_2·CH_2), 7.46 and 7.55 (each 3 H, s, 5-Me and COMe), 8.64 (3 H, t, CH_2CH_3), and 8.76 (3 H, t, CH_2·CH_3), $\nu_{max.}$ ($CHCl_3$) 3 450 (NH), 1 730 (ArCO), and 1 680 cm^{-1} (CH_2CO).

(b) *Labelled samples prepared from the pyrrole* (5). Sodium [2-^{13}C]acetate (64.5% enriched; 100 mg) was diluted with unlabelled sodium acetate (200 mg) and stirred for 3 h at 0 °C in dry methylene chloride (12 ml) containing oxalyl chloride (0.3 ml). Ethyl 3-(2-ethoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (0.93 g) and aluminium trichloride (0.9 g) were added and the mixture was stirred at 50 °C during 1 h and then at 25 °C overnight. Methylene chloride was added and the mixture was washed with water, dried (Na_2SO_4), and evaporated to dryness. Preparative t.l.c. (18 20 cm × 20 cm × 1 mm plates coated with Kieselgel G; 20% ethyl acetate in cyclohexane) gave 626 mg (58% from sodium acetate) of product identical with the material described in (a) except for the ^{13}C n.m.r. spectrum [18] which showed an enhanced peak at 32.1 p.p.m.

When sodium [2-^{14}C]acetate (1 mCi) was used in place of the ^{13}C-labelled material, and mixed with inactive sodium acetate (250 mg), the above acetylpyrrole was obtained (437 mg, 47% from NaOAc); activity 303 mCi mol^{-1}.

Benzyl 4-Acetyl-3,5-dimethylpyrrole-2-carboxylate (14).—Anhydrous sodium acetate (125 mg, 1 equiv.) and oxalyl chloride (0.26 ml, 2 equiv.) were stirred in dry methylene chloride (10 ml) at 0 °C during 2.5 h. Benzyl 3,5-dimethylpyrrole-2-carboxylate (420 mg, 1.2 equiv.) and anhydrous tin(IV) chloride (0.26 ml, 1.5 equiv.) were added and the mixture was refluxed for 35 min before dilution with methylene chloride and washing with water and brine. The organic phase was dried (Na_2SO_4) and evaporated to give an oil which was chromatographed (preparative t.l.c.; 12 20 cm × 20 cm × 1 mm plates coated with Kieselgel G; 25% ethyl acetate in cyclohexane). The product was removed from the silica by stirring for 1 h with 5% methanol in methylene chloride (100 ml). Filtration and evaporation gave a white solid which was recrystallised from methylene chloride–n-hexane to give the acetylpyrrole (284 mg, 69% from NaOAc) as white needles, m.p. 134.5—135.5° (lit.,[20] 135°) (Found: C, 71.0; H, 6.4; N, 5.4. Calc. for $C_{16}H_{17}NO_3$: C, 70.8; H, 6.3; N, 5.2%), τ 0.6—0.9br (NH), 2.65 (5 H, s, Ph), 4.71 (2 H, s, PhCH_2), and 7.42, 7.52, and 7.59 (each 3 H, s, 3 × Me).

Benzyl 4-Acetyl-3-(2-ethoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (8).—A solution of concentrated hydrochloric acid (0.5 ml) and α-benzyl ω-ethyl β-oxoadipate (44.6 g) was first treated with pentyl nitrite (22.3 ml) and then added to a stirred suspension of acetylacetone (16.5 ml), ammonium acetate (50 g), and zinc powder (80 g) in acetic acid (150 ml) as described in the synthesis of the pyrrole (6) [method (a)]. Recrystallisation from di-isopropyl ether gave the *acetylpyrrole* (23.8 g, 42%) as white needles, m.p. 90—91° (Found: C, 67.35; H, 6.4; N, 4.0. $C_{20}H_{23}NO_5$ requires C, 67.2; H, 6.5; N, 3.9%), τ 0.2—0.4br (NH),

[20] A. W. Johnson, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1958, 4254.

2.67 (5 H, s, Ph), 4.73 (2 H, s, PhCH_2), 5.95 (2 H, q, CH_2·CH_3), 6.5—7.7 (4 H, m, CH_2CH_2), 7.50 and 7.56 (each 3 H, s, 2 × Me), and 8.78 (3 H, t, CH_2·CH_3), $\nu_{max.}$ ($CHCl_3$) 3 440 (NH), 1 725 (ArCO), and 1 685 cm^{-1} (CH_2CO). When large-scale preparations of this pyrrole were carried out, small quantities of benzyl 3,5-dimethylpyrrole-2-carboxylate were obtained from the recrystallisation mother liquors; m.p. 102—103° (lit.,[21] 102—104°).

Benzyl 4-Acetyl-3-(2-methoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (11).—Anhydrous sodium acetate (125 mg, 1 equiv.) and oxalyl chloride (0.26 ml, 2 equiv.) were stirred in dry methylene chloride (10 ml) at 0 °C during 2.5 h. Benzyl 3-(2-methoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (525 mg, 1.1 equiv.) and anhydrous tin(IV) chloride (0.26 ml, 1.5 equiv.) were added, and the mixture was refluxed for 35 min. The solution was diluted with methylene chloride (20 ml), washed with water and then brine, and dried (Na_2SO_4) before evaporation. The resultant red oil was chromatographed (preparative t.l.c.; 12 20 cm × 20 cm × 1 mm plates coated with Kieselgel G; 25% ethyl acetate in cyclohexane). The product was removed from the silica by stirring during 1 h with 5% methanol in methylene chloride (100 ml); filtration and evaporation gave a white solid which was recrystallised from methylene chloride–n-hexane to give the *pyrrole* (360 mg, 69% from NaOAc) as white needles, m.p. 105.5—106.5° (Found: C, 66.6; H, 5.9; N, 4.1. $C_{19}H_{21}NO_5$ requires C, 66.5; H, 6.2; N, 4.1%), τ 0.8br (NH), 2.70 (5 H, s, Ph), 4.76 (2 H, s, PhCH_2), 6.46 (3 H, s, OMe), 6.6—7.5 (4 H, m, CH_2·CH_2), 7.54 (3 H, s, COMe), and 7.62 (3 H, s, 5-Me).

The corresponding ^{13}C-labelled pyrrole was prepared in an analogous manner by using sodium [2-^{13}C]acetate (91 atom % ^{13}C). The only resonance in its ^{13}C n.m.r. spectrum which was enhanced was that at 30.996 p.p.m. A similar preparation in the ^{14}C series, with 4-unsubstituted pyrrole (700 mg), sodium [2-^{14}C]acetate (1 mCi) plus inactive acetate (250 mg), oxalyl chloride (0.26 ml), and aluminium trichloride (700 mg) gave a 31% yield (from NaOAc) of the ^{14}C-labelled pyrrole, activity 167 mCi mol^{-1}.

Ethyl 3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (7).—Ethyl 4-acetyl-3-(2-ethoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (6 g) in dry methanol (50 ml) was stirred with a solution of thallium(III) nitrate trihydrate (9.2 g) in methanol (50 ml) containing concentrated nitric acid (0.3 ml) during 48 h at 25 °C. The white precipitate of thallium(I) nitrate was filtered off and the filtrate was diluted with water and methylene chloride. The organic phase was dried (Na_2SO_4) and evaporated to give an oil which crystallised upon addition of n-hexane. Recrystallisation from methylene chloride–n-hexane–ether gave the *pyrrole* (5 g, 79%) as white needles, m.p. 90—91° (Found: C, 57.7; H, 6.7; N, 4.6. $C_{15}H_{21}NO_6$ requires C, 57.9; H, 6.8; N, 4.5%), τ 0.9—1.0br (NH), 5.73 (2 H, q, CH_2·CH_3), 6.37 (6 H, s, 2 × OMe), 6.59 (2 H, s, CH_2CO), 6.8—7.6 (4 H, m, CH_2·CH_2), 7.79 (3 H, s, 5-Me), and 8.67 (3 H, t, CH_2·CH_3), $\nu_{max.}$ ($CHCl_3$) 3 460 (NH), 1 735 (ArCO), and 1 680 cm^{-1} (CH_2CO). In the ^{13}C-labelled series the product showed an enhanced resonance in its ^{13}C n.m.r. spectrum at 30.8 p.p.m., all other resonances being at natural abundance. A similar preparation with the ^{14}C-labelled acetylpyrrole (435 mg), thallium(III) nitrate, and nitric acid (0.1 ml) in methanol (10 ml) gave an 80% yield of methoxycarbonylmethylpyrrole.

Benzyl 4-Methoxycarbonylmethyl-3,5-dimethylpyrrole-2-carboxylate (15).—Benzyl 4-acetyl-3,5-dimethylpyrrole-2-carboxylate (2 g, 1 equiv.) in methylene chloride (10 ml) and methanol (10 ml) was stirred overnight at 25 °C with a solution of thallium(III) nitrate trihydrate (3.5 g, 1.1 equiv.) and concentrated nitric acid (0.1 ml) in methanol (20 ml). After work-up as described for the analogue above the product was obtained as white needles (1.6 g, 72%), m.p. 91—92° (lit.,[14] 93—94°) (Found: C, 67.45; H, 6.3; N, 4.75. Calc. for $C_{17}H_{19}NO_4$: C, 67.8; H, 6.4; N, 4.65%), τ 0.2—0.4br (NH), 2.68 (5 H, s, Ph), 4.75 (2 H, s, PhCH_2), 6.37 (3 H, s, OMe), 6.66 (2 H, s, CH_2), and 7.72 and 7.83 (each 3 H, s, 2 × Me), $\nu_{max.}$ ($CHCl_3$) 3 450 (NH), 1 730 (ArCO), and 1 675 cm^{-1} (CH_2CO).

Benzyl 3,5-Dimethyl-4-(2-nitro-oxyacetyl)pyrrole-2-carboxylate (17).—Benzyl 4-acetyl-3,5-dimethylpyrrole-2-carboxylate (1 g) in dry methylene chloride (10 ml) was stirred overnight at 25 °C with a solution of thallium(III) nitrate trihydrate (1.7 g), concentrated nitric acid (0.5 ml), and benzyl alcohol (0.8 g, 2 equiv.) in dry bis-(2-methoxyethyl) ether (15 ml). After removal of the precipitated thallium(I) nitrate, methylene chloride (20 ml) and water (20 ml) were added and the organic layer was washed with water, dried (Na_2SO_4), and evaporated (1 mmHg) to give a brown solid. Recrystallisation from methylene chloride–n-hexane–ether gave a white crystalline *solid* (0.9 g, 74%), m.p. 133—135° (Found: C, 58.0; H, 4.8; N, 8.15. $C_{16}H_{16}N_2O_6$ requires C, 57.8; H, 4.85; N, 8.4%), τ 0.2—0.4br (NH), 2.55 (5 H, s, Ph), 4.66 (2 H, s, PhCH_2), 4.71 (2 H, s, CH_2·ONO_2), and 7.38 and 7.46 (each 3 H, s, 2 × Me), $\nu_{max.}$ ($CHCl_3$) 3 440 (NH), 1 715 (ArCO), 1 655 (CH_2CO), and 1 655 and 1 280 cm^{-1} (ONO_2), *m/e* 332.098 8 (M^+, $C_{16}H_{16}N_2O_6$, 0.5%), 271.120 3 ($C_{16}H_{17}NO_3$, 9.8), 257.101 8 ($C_{15}H_{15}NO$, 20), and 256.098 1 ($C_{15}H_{14}NO_3$, 100).

Benzyl 4-(2-Hydroxyethoxycarbonylmethyl)-3,5-dimethylpyrrole-2-carboxylate (16).—Benzyl 4-acetyl-3,5-dimethylpyrrole-2-carboxylate (1 g) in methylene chloride (20 ml) and ethylene glycol (20 ml) was stirred during 48 h at 25 °C with a solution of thallium(III) nitrate trihydrate (1.7 g) and concentrated nitric acid (0.1 ml) in ethylene glycol (20 ml). After removal of the precipitated thallium(I) nitrate, methylene chloride (20 ml) and water (20 ml) were added and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. The resultant oil was shown by t.l.c. to be a mixture of starting material and product, so it was chromatographed on alumina (elution with ether). The eluates containing the more polar compound gave an oil which was crystallised from carbon tetrachloride–ether to give white *crystals* (500 mg, 41%), m.p. 75—79° (Found: C, 65.1; H, 6.5; N, 4.35. $C_{18}H_{21}NO_5$ requires C, 65.2; H, 6.4; N, 4.2%), τ 0.7—1.0br (NH), 2.68 (5 H, m, Ph), 4.76 (2 H, s, PhCH_2), 5.8—5.9 and 6.2—6.3 (each 2 H, m, OCH_2·CH_2·OH), 6.63 (2 H, s, CH_2CO), and 7.73 and 7.84 (each 3 H, s, 2 × Me), $\nu_{max.}$ ($CHCl_3$) 3 600 (OH), 3 450 (NH), 1 730 (ArCO), and 1 670 cm^{-1} (CH_2CO), *m/e* 331 (M^+, 50%) and 242 (100).

Benzyl 3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (12).—Benzyl 4-acetyl-3-(2-methoxycarbonylethyl)-5-methylpyrrole-2-carboxylate (20.0 g) in dry methanol (150 ml) was stirred for 48 h at 25 °C with a solution of thallium(III) nitrate trihydrate (24.9 g) in dry methanol (150 ml) and concentrated nitric acid (2 ml). After removal of the precipitated thallium(I)

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

nitrate, methylene chloride (30 ml) was added and the solution was washed with water and brine, dried (Na_2SO_4), and evaporated to give a brown oil which crystallised. Recrystallisation from methylene chloride–n-hexane gave the pyrrole (17.3 g, 83%) as white needles, m.p. 78—79° (lit.,[22] 78.5—79.5°), τ 0.9—1.1br (NH), 2.67 (5 H, s, Ph), 4.76 (2 H, s, PhCH_2), 6.36 (3 H, s, OMe), 6.41 (3 H, s, OMe), 6.59 (2 H, s, CH_2), 6.9—7.6 (4 H, m, CH_2·CH_2), and 7.80 (3 H, s, 5-Me), $\nu_{max.}$ 3 450 (NH), 1 730 (ArCO), and 1 670 cm^{-1} (CH_2CO). When the reaction was carried out with only 300 mg of starting acetylpyrrole, a 91% yield of the product was obtained. In the ^{13}C-labelled series the product showed an enhanced resonance at 29.732 p.p.m. in its ^{13}C n.m.r. spectrum. A similar small-scale preparation with the ^{14}C labelled acetylpyrrole (300 mg), thallium(III) nitrate (412 mg), and nitric acid (0.1 ml) in dry methanol (10 ml) gave a 98% yield of the labelled methoxycarbonylmethylpyrrole.

Benzyl 5-Acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate (18).—Lead tetra-acetate (6 g, 1.1 equiv.) was added with warming to a solution of benzyl 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (5 g, 1 equiv.) in acetic acid (100 ml) containing acetic anhydride (2 ml). The solution was stirred overnight at 25 °C and then added dropwise to vigorously stirred water during 30 min. The precipitate was filtered off, washed with water, and dissolved in methylene chloride; the solution was then dried (Na_2SO_4) and evaporated to yield a white solid which was recrystallised from methylene chloride–n-hexane–ether to give the *pyrrole* (4.9 g, 85%) as fine white needles, m.p. 106—107° (Found: C, 61.5; H, 5.8; N, 3.45. $C_{22}H_{25}NO_3$ requires C, 61.25; H, 5.8; N, 3.25%), τ 0.4—0.6br (NH), 2.63 (5 H, s, Ph), 4.75 (2 H, s, PhCH_2), 4.99 (2 H, s, CH_2OAc), 6.37 (3 H, s, OMe), 6.42 (3 H, s, OMe), 6.49 (2 H, s, CH_2), 6.9—7.6 (4 H, m, CH_2·CH_2), and 7.95 (3 H, s, MeCO). In the ^{14}C-labelled series, the active methylpyrrole (317 mg) [diluted with inactive material (317 mg)] with lead tetra-acetate (864 mg) in acetic acid (20 ml) and acetic anhydride (1 ml) gave a 92% yield of the acetoxymethylpyrrole.

Benzyl 4-Ethoxycarbonylmethyl-3-methyl-5-phthalimidomethylpyrrole-2-carboxylate (26).—Benzyl 5-acetoxymethyl-4-ethoxycarbonylmethyl-3-methylpyrrole-2-carboxylate [23] (25) (1 g) in dry dimethyl sulphoxide (10 ml) was stirred during 2 h at 25 °C with a solution of potassiophthalimide (0.55 g, 1.1 equiv.) in dry dimethyl sulphoxide (20 ml). After filtration to remove potassium acetate the solvent was evaporated off (1 mmHg) to give a brown solid. Methylene chloride was added and the solution was washed with water (2 × 250 ml), dried (Na_2SO_4), and evaporated. The residue was crystallised from methylene chloride–n-hexane to give the *phthalimidomethylpyrrole* (1.1 g, 90%) as white needles, m.p. 138° (Found: C, 67.6; H, 5.2; N, 6.25. $C_{26}H_{24}N_2O_6$ requires C, 67.8; H, 5.25; N, 6.1%), τ 0.6—0.8br (NH), 2.1—2.4 (4 H, m, C_6H_4), 2.6—2.7 (5 H, m, Ph), 4.76 (2 H, s, PhCH_2), 5.23 (2 H, s, CH_2N), 5.94 (2 H, q, CH_2·CH_3), 6.42 (2 H, s, CH_2·CO), 7.77 (3 H, s, 3-Me), and 8.78 (3 H, t, CH_2·CH_3), $\nu_{max.}$ ($CHCl_3$) 3 440 (NH), 1 770 (N·CO), and 1 720 cm^{-1} (CH_2CO and ArCO), *m/e* 460 (M^+, 100%).

Benzyl 3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-phthalimidomethylpyrrole-2-carboxylate (19).—Potassiophthalimide (4.4 g, 1.1 equiv.) in dry dimethyl sulphoxide (150 ml) was added to benzyl 5-acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate (18) (8 g, 1 equiv.) in dry dimethyl sulphoxide (50 ml). After stirring during 2 h at 25 °C a white precipitate of potassium acetate was evident. The solvent was evaporated off (1 mmHg) and the residual brown solid was dissolved in methylene chloride (50 ml); the solution was washed with water (2 × 250 ml), dried (Na_2SO_4), and evaporated to give a light brown solid. Recrystallisation from methylene chloride–n-hexane–ether gave the *phthalimidomethylpyrrole* (8.5 g, 89%) as off-white needles, m.p. 132—133° (Found: C, 64.8; H, 5.1; N, 5.3. $C_{28}H_{26}N_2O_8$ requires C, 64.9; H, 5.05; N, 5.4%), τ 0.4—0.6br (NH), 2.1—2.4 (4 H, m, C_6H_4), 2.6—2.7 (5 H, m, Ph), 4.69 (2 H, s, PhCH_2), 5.17 (2 H, s, CH_2N), 6.31 (3 H, s, OMe), 6.33 (3 H, s, OMe), 6.38 (2 H, s, CH_2), and 6.9—7.6 (4 H, m, CH_2·CH_2), $\nu_{max.}$ ($CHCl_3$) 3 430 (NH), 1 770 (N·CO), 1 735 (ArCO), and 1 710 cm^{-1} (CH_2CO), *m/e* 518 (M^+, 100%) and 426 (100). In the ^{14}C series, labelled acetoxymethylpyrrole (80 mg) was diluted with inactive material (400 mg) and treated with potassiophthalimide (270 mg) in dimethyl sulphoxide (15 ml) to give an 85% yield of labelled phthalimidomethylpyrrole.

4-Ethoxycarbonylmethyl-3-methyl-5-phthalimidomethylpyrrole (28).—Benzyl 4-ethoxycarbonylmethyl-3-methyl-5-phthalimidomethylpyrrole-2-carboxylate (26) (600 mg) in anisole (60 ml) was stirred vigorously with 10% v/v concentrated sulphuric acid in trifluoroacetic acid (60 ml) during 1 h at 25 °C. The solution was extracted thoroughly with methylene chloride and the combined extracts were washed with water, saturated sodium hydrogen carbonate solution, and brine, dried (Na_2SO_4), and evaporated to dryness to give an oil which crystallised upon trituration with ether to give the *pyrrole* (28) (292 mg, 69%), m.p. 130—133° (Found: C, 66.1; H, 5.6; N, 8.3. $C_{18}H_{18}N_2O_4$ requires C, 66.2; H, 5.6; N, 8.6%), τ 1.4—1.6br (NH), 2.1—2.4 (4 H, m, C_6H_4), 3.58 (1 H, d, *J* 3 Hz, 2-H), 5.23 (2 H, s, CH_2N), 5.90 (2 H, q, CH_2·CH_3), 6.41 (2 H, s, CH_2·CO), 8.02 (3 H, s, 3-Me), and 8.76 (3 H, t, CH_2·CH_3), *m/e* 326 (M^+, 55%) and 252 (100). In one of several trial experiments, the phthalimidomethylpyrrole (100 mg) in 1% v/v concentrated sulphuric acid in trifluoroacetic acid (10 ml) was stirred during 1 h at 25 °C. A fine precipitate developed after 10 min and isolation of product as described above gave an oil which was subjected to preparative t.l.c. (elution of Kieselgel G plates with 1 : 1 ethyl acetate–n-hexane) and gave two fractions. N.m.r. spectroscopy showed the more polar substance to be the 2-unsubstituted pyrrole described above. The less polar product was identified as 2-benzyl-4-ethoxycarbonylmethyl-3-methyl-5-phthalimidomethylpyrrole (30), τ 2.1—2.4 (4 H, m, C_6H_4), 2.6—2.7 (5 H, m, Ph), 5.28 (2 H, s, CH_2N), 5.90 (2 H, q, CH_2·CH_3), 6.15 (2 H, s, PhCH_2), 6.41 (2 H, s, CH_2), 8.08 (3 H, s, 3-Me), and 8.76 (3 H, t, CH_2·CH_3), *m/e* 402 (100%). In another trial experiment, the phthalimidomethylpyrrole (250 mg) in methylene chloride (40 ml) containing 5% v/v concentrated sulphuric acid in trifluoroacetic acid (10 ml) was stirred during 30 min at 25 °C. Isolation of product as above gave an oil which crystallised upon trituration with methylene chloride; the compound was identified, from its n.m.r. spectrum, as 4-ethoxycarbonylmethyl-3-methyl-5-phthalimidomethylpyrrole-2-carboxylic acid (29),

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[23] M. T. Cox, A. H. Jackson, G. W. Kenner, S. W. McCombie, and K. M. Smith, *J.C.S. Perkin I*, 1974, 516.

τ ($CDCl_3$ + trace of TFA) 0.5—0.7br (NH), 2.1—2.4 (4 H, m, C_6H_4), 5.30 (2 H, s, CH_2·N), 5.86 (2 H, q, CH_2·CH_3), 6.35 (2 H, s, CH_2), 7.80 (3 H, s, 3-Me), and 8.74 (3 H, t, CH_2·CH_3).

3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-phthalimidomethylpyrrole (27).—Benzyl 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-phthalimidomethylpyrrole-2-carboxylate (19) (2 g) in anisole (200 ml) was stirred vigorously with 10% v/v concentrated sulphuric acid in trifluoroacetic acid (200 ml) at 25 °C during 2 h. The solution was thoroughly extracted with methylene chloride (3 × 200 ml) and the combined extracts were washed with water, aqueous sodium hydrogen carbonate solution, and water, then dried (Na_2SO_4) and evaporated to give a brown oil which crystallised upon trituration with ether. Recrystallisation from ethanol gave the *2-unsubstituted pyrrole* (925 mg, 63%) as off-white needles, m.p. 127—128° (Found: C, 62.4; H, 5.4; N, 7.5. $C_{20}H_{20}N_2O_6$ requires C, 62.5; H, 5.2; N, 7.3%), τ 1.3—1.5br (NH), 2.2—2.4 (4 H, m, C_6H_4), 3.53 (1 H, d, *J* 3 Hz, 2-H), 5.23 (2 H, s, CH_2N), 6.36 and 6.39 (8 H, s, 2 × OMe and CH_2CO), and 7.2—7.6 (4 H, m, CH_2·CH_2), $\nu_{max.}$ ($CHCl_3$) 3 460 (NH), 1 770 (N·CO), 1 730, and 1710 cm^{-1} (CH_2CO). In the ^{14}C-labelled series, the active phthalimidomethylpyrrole benzyl ester (450 mg) in 10% v/v sulphuric acid in trifluoroacetic acid (45 ml) and anisole (45 ml) gave a 60% yield of the 2-unsubstituted pyrrole.

Benzyl 4,5,6,7-Tetrahydro-3-methyl-5-oxo-1H-pyrrolo[2,3-c]pyridine-2-carboxylate (33).—To a solution of benzyl 4-ethoxycarbonylmethyl-3-methyl-5-phthalimidomethylpyrrole-2-carboxylate (26) (1.28 g) in methanol (150 ml) was added an excess of hydrazine hydrate (5 ml). The solution was stirred overnight at 25 °C before removal of the white precipitate by filtration and concentration of the filtrate to give a second crop of precipitate. The combined precipitates were recrystallised from aqueous ethanol to give the *lactam* (600 mg, 76%) as white crystals, m.p. 275—278° (decomp.) (Found: C, 67.4; H, 5.7; N, 9.9. $C_{16}H_{16}N_2O_3$ requires C, 67.6; H, 5.7; N, 9.85%), τ (TFA) 0.1—0.3br and 0.5—0.6br (2 × NH), 2.62 (5 H, s, Ph), 4.63 (2 H, s, $PhCH_2$), 5.26br (2 H, CH_2·NH), 6.26br (2 H, CH_2CO), and 7.71 (3 H, s, ArMe), *m/e* 284 (M^+, 100%).

Benzyl 4,5,6,7-Tetrahydro-3-(2-methoxycarbonylethyl)-5-oxo-1H-pyrrolo[2,3-c]pyridine-2-carboxylate (Benzyloxycarbonyl-PBG Lactam Methyl Ester) (32).—*Method A*. Benzyl 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-phthalimidomethylpyrrole-2-carboxylate (2 g) in methanol (80 ml) was stirred during 1 h at 25 °C with an excess of hydrazine hydrate (10 ml). The solution was poured into water (400 ml) and stirred for 30 min, then the precipitate was filtered off and recrystallised from aqueous methanol to give the *lactam* (1 g, 74%) as white crystals, m.p. 229—230° (Found: C, 64.1; H, 5.8; N, 8.1. $C_{19}H_{20}N_2O_5$ requires C, 64.0; H, 5.7; N, 7.9%), τ (TFA) −0.1 to 0.0br and 0.7—0.9br (2 × NH), 2.60 (5 H, s, Ph), 4.63 (2 H, s, $PhCH_2$), 5.27br (2 H, CH_2NH), 6.21br (2 H, CH_2CO), 6.28 (3 H, s, OMe), and 6.8—7.5 (4 H, m, CH_2·CH_2), $\nu_{max.}$ (KBr) 3 330 (NH), 1 740 (ArCO), and 1 655 cm^{-1} (CH_2CO), *m/e* 356 (M^+, 81%) and 265 (100). When this method was used the product was occasionally contaminated with starting material or a more polar by-product.

Method B (better). Benzyl 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-phthalimidomethylpyrrole-2-carboxylate (100 mg) in methanol (10 ml) was refluxed with tri-n-butylamine (2 ml) and methylhydrazine (1 ml) during 6 h. Water (50 ml) was added and after 10 min the precipitated solid was filtered off and dried (yield 58 mg, 86%). The product was identical with the lactam obtained by method A.

Methyl 4,5,6,7-Tetrahydro-5-oxo-1H-pyrrolo[2,3-c]pyridine-3-propionate (Porphobilinogen Lactam Methyl Ester) (31).—*Method A*. To a solution of hydroxylamine hydrochloride (640 mg, 3 equiv.) in methanol (30 ml) was added 4N-sodium methoxide in methanol (4.5 ml, 7 equiv.). The resulting suspension was stirred with a solution of compound (27) (1 g, 1 equiv.) in tetrahydrofuran (30 ml) during 20 min at 25 °C. The orange emulsion was added to water (200 ml) and extracted with chloroform (6 × 200 ml), and the combined extracts were dried (Na_2SO_4) and evaporated to give a white solid. Recrystallisation from methanol gave the lactam as white plates (356 mg, 62%), m.p. 245—246° (lit.,[24] 240—242°; lit.,[25] 248—250°; lit.,[22] 248—251°) (Found: C, 59.2; H, 6.2; N, 12.7. Calc. for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.6%), τ [$(CD_3)_2SO$] 1.9—2.1br (NH), 3.22 (1 H, d, *J* 3 Hz, pyrrole H), 5.3—5.5br (2 H, CH_2N), 6.10 (2 H, s, CH_2), 6.34 (3 H, s, OMe), and 7.1—7.2 (4 H, m, CH_2CH_2), $\nu_{max.}$ (KBr) 3 360—3 150 (NH), 1 730, and 1 660 cm^{-1} (CO), *m/e* 222 (M^+, 100%). The ^{14}C-labelled analogue was similarly prepared from phthalimidomethylpyrrole [200 mg (active) and 150 mg (inactive)], hydroxylamine hydrochloride (224 mg), 4N-methoxide (1.6 ml), and tetrahydrofuran (20 ml).

Method B. Tri-n-butylamine (20 ml) and methylhydrazine (10 ml) were refluxed with a solution of the 2-unsubstituted phthalimidomethylpyrrole (27) (1 g) in methanol (50 ml) during 1 h. Methylhydrazine and methanol were evaporated off *in vacuo* and the product was crystallised from the tri-n-butylamine. Filtration and recrystallisation from methanol gave the lactam as white plates (373 mg, 65%), identical with the material obtained by method A. Evaporation of the tri-n-butylamine liquors gave an oily residue which was chromatographed on silica gel (MFC) (elution with ethyl acetate). Evaporation gave a white solid shown to be *N*-methylphthalohydrazide, m.p. 239—240° (from methanol) (lit.,[26] 239.5—240.5°), τ [$(CD_3)_2SO$] 1.4—1.9 (4 H, m, C_6H_4) and 6.13 (3 H, s, NMe), *m/e* 176 (M^+, 100%).

Ethyl 5-Formyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate (34).—To a stirred solution of ethyl 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (7) (5 g) in dry carbon tetrachloride (50 ml) was added during 17 h at 25 °C a solution of sulphuryl chloride (2.7 ml, 2 equiv.) in dry carbon tetrachloride (50 ml). The solvent was evaporated off *in vacuo* at 25 °C and the resulting brown oil was boiled during 10 min with sodium acetate hydrate (10 g) in water (250 ml). Methylene chloride was added and the organic extract was dried (Na_2SO_4) and evaporated to give an oil which was crystallised from methylene chloride–n-hexane–ether to give the *formylpyrrole* (4.5 g, 86%) as white crystals, m.p. 83—85° (Found: C, 55.5; H, 6.1; N, 4.2. $C_{15}H_{19}NO_7$ requires C, 55.4; H, 5.9; N, 4.3%), τ 0.0—0.1br (NH), 0.28 (1 H, s, CHO), 5.65 (2 H, q, CH_2·CH_3), 6.17 (2 H, s, CH_2), 6.31 and 6.36 (2 × 3 H, s, 2 OMe), 6.9—7.6 (4 H, m, CH_2·CH_2), and 8.63 (3 H, t,

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$CH_2 \cdot CH_3$), $\nu_{max.}$ ($CHCl_3$) 3 420 (NH), 1 730 (ArCO), and 1 660 cm^{-1} (CH_2CO and CHO).

3-(2-Carboxyethyl)-4-carboxymethyl-5-formylpyrrole-2-carboxylic Acid (35).[7e]—The foregoing pyrrole (4.5 g) in aqueous 10% sodium hydroxide (56 ml) was heated under nitrogen on a steam-bath during 2 h. The cooled solution was passed through an Amberlite IR-120 ion-exchange column [H^+ form; from Na^+ form (100 g wet wt.)] with distilled water as eluant. The eluates (*ca.* 1 l) were evaporated (50 °C; 1 mmHg) under nitrogen and the resulting pink solid was immediately dissolved in refluxing acetone (500 ml). The solution was filtered, the filtrate was evaporated to *ca.* 50 ml, and ether (100 ml) was added. The precipitated coloured material was filtered off and the filtrate concentrated to 30 ml before addition of ether (60 ml). Upon cooling a pink-white solid was precipitated. A second crop was obtained by further concentration of the mother liquors; yield 2.7 g (73%), τ [$(CD_3)_2SO$] −2.3 to −2.5br (NH), 0.30 (1 H, s, CHO), 6.18 (2 H, s, CH_2), and 6.8—7.6 (4 H, m, $CH_2 \cdot CH_2$), $\nu_{max.}$ (KBr) 3 500—2 200 (CO_2H) and 1 650—1 730 cm^{-1} (CO).

Oxime (36) *of 3-(2-Carboxyethyl)-4-carboxymethyl-5-formylpyrrole.*[7f]—Hydroxylamine hydrochloride (6 g) in water (35 ml) was brought to pH 6—7 with aqueous 10% sodium hydroxide (24 ml). During purging with nitrogen the solution was added to 3-(2-carboxyethyl)-4-carboxymethyl-5-formylpyrrole-2-carboxylic acid (2.7 g) and the mixture was refluxed during 7 h at 150 °C in the dark. The cooled solution was acidified with 2N-hydrochloric acid (Congo Red); crystallisation then occurred. After 2 h at 0 °C the pale brown solid was collected and recrystallised from 0.1N-sodium hydroxide solution by adjusting the pH to 3 with 2N-hydrochloric acid; yield 1.35 g (69%), τ [$(CD_3)_2SO$] −1.0 to −0.8br (NH), −0.5 to 0.5br (3 H, 3 × OH), 2.66 (1 H, s, CH:N), 3.28 (1 H, d, *J* 3 Hz, 2-H), 6.50 (2 H, s, CH_2), and 7.3—7.5 (4 H, m, $CH_2 \cdot CH_2$).

5-Aminomethyl-3-(2-carboxyethyl)-4-carboxymethylpyrrole (*Porphobilinogen*) (1).—*Method A*. Porphobilinogen lactam methyl ester (31) (475 mg) was stirred during 3 days at 25 °C in 2N-potassium hydroxide (2 ml). The pale yellow solution was filtered, the sinter being washed with distilled water (1 ml). Adjustment of the pH to 7 with 7N-acetic acid induced crystallisation, and after 2 h at 0 °C the product was filtered off, washed with water, then methanol and ether, dried, and stored at −70 °C; yield of white crystals 350 mg (67%) [paper chromatography, R_F 0.50 (lit.,[25] 0.50)], τ (TFA) 0.7—0.9br (NH), 2.7—3.3br (3 H, $\overset{+}{N}H_3$), 3.25br (1 H, 2-H), 5.5—5.6 (2 H, d, collapsed to singlet upon addition of D_2O, CH_2N), 6.25 (2 H, s, CH_2), and 7.0—7.2 (4 H, m, $CH_2 \cdot CH_2$), τ (M-NaOD–D_2O) 3.56 (1 H, s, 2-H), 6.51 (2 H, s, $CH_2 \cdot N$), 6.82 (2 H, s, CH_2), and 7.4—7.9 (4 H, m, $CH_2 \cdot CH_2$). In the ^{14}C series, the labelled lactam (50 mg) in 2N-potassium hydroxide (0.5 ml) gave a 66% yield of [6-^{14}C]-PBG, activity 7.2 mCi mol^{-1}.

Method B.[7g,h] The oxime (36) (400 mg) and 10% palladised charcoal (160 mg) in distilled water (15 ml) were hydrogenated at 25 °C and 1 atm during 4 h (until uptake of hydrogen ceased). Addition of ammonia (s.g. 0.880; 0.5 ml) and filtration through Celite gave a yellow solution which was acidified to pH 7 with acetic acid. Paper chromatography showed the presence of PBG (R_F 0.50) and starting oxime (R_F 0.7). Fresh catalyst (160 mg) was added and the solution was rehydrogenated. After the same work-up the solution (at pH 7) was passed through an alumina column (5 mm × 50 mm; neutral alumina pre-washed with water; compound eluted with distilled water until the eluates were Ehrlich-negative). The eluates (*ca.* 100 ml) were freeze-dried to a white solid; recrystallisation from ammonium hydroxide–acetic acid (to pH 4.5) gave PBG as white plates, which were washed with water, ethanol, then ether, and dried. The product (130 mg, 30%) was stored at −70 °C, and was identical with the material obtained by method A.

Ethyl 3-(2-Carboxyethyl)-4-carboxymethyl-5-methylpyrrole-2-carboxylate.—Ethyl 3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (7) (311 mg) in tetrahydrofuran (50 ml) was stirred overnight at 25 °C with 0.1N-sodium hydroxide (50 ml). Acidification with N-hydrochloric acid (9 ml) and extraction with methylene chloride (3 × 100 ml) gave, after drying (Na_2SO_4) and evaporation, a solid. Recrystallisation from aqueous methanol gave white plates (271 mg, 96%), m.p. 225—235° (lit.,[7g,h] 237—238°) (Found: C, 54.9; H, 6.3; N, 5.1. Calc. for $C_{13}H_{17}NO_6$: C, 55.1; H, 6.05; N, 4.95%), τ (TFA) 5.50 (2 H, q, $CH_2 \cdot CH_3$), 6.25 (2 H, s, $CH_2 \cdot CO$), 6.7—7.3 (4 H, m, $CH_2 \cdot CH_2$), 7.68 (3 H, s, 5-Me), and 8.52 (3 H, t, $CH_2 \cdot CH_3$), $\nu_{max.}$ (KBr) 3 250 (NH), 3 500—2 000 (OH), 1 700 (ArCO), and 1 635 cm^{-1} (CH_2CO).

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