Biosynthesis of Porphyrins and Related Macrocycles. Part I. Synthesis of ^{14}C-Labelled Pyrromethanes

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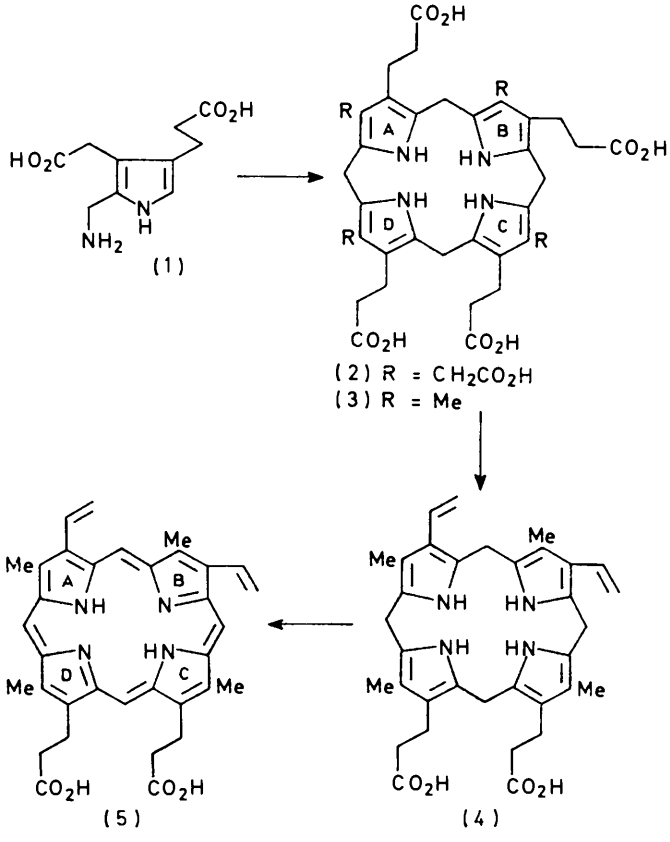
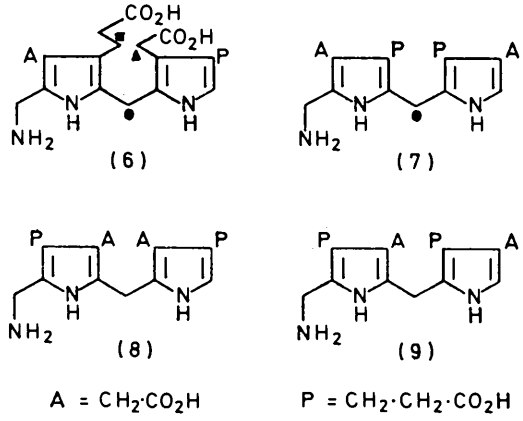
The biosynthetic problem posed by the macrocycle of the natural porphyrins, chlorins, and corrins is outlined together with the approaches being used for its solution. Routes are developed as follows for rational synthesis of the labelled (aminomethyl)pyrromethanes (6) and (7), which are of importance for incorporation studies on haem, protoporphyrin-IX, and vitamin B_{12}. An improved sequence affords porphobilinogen lactam methyl ester (16) labelled in the propionic ester residue. The 5-(chloromethyl)pyrrole (24) is synthesised carrying ^{14}C-labels at the C-4 methylene and C-5 methylene groups. A similar synthesis yields the labelled isomeric (chloromethyl)pyrrole (52). These three building blocks are used for synthesis of the pyrromethane lactams (27) and (55) of proven homogeneity labelled specifically with carbon-14 in the side-chains and at the bridge methylene group. Hydrolysis of the lactams affords the labelled (aminomethyl)pyrromethanes (6) and (7).

PROTOPORPHYRIN-IX (5) is known [1] to be biosynthesised from succinate and glycine *via* δ-aminolaevulinic acid and the monopyrrole porphobilinogen (PBG) (1). The



co-operative action of two enzymes, porphobilinogen deaminase and uroporphyrinogen-III cosynthetase, converts PBG into uroporphyrinogen-III (2), which undergoes enzymic decarboxylation to afford coproporphyrinogen-III (3). The propionate side chains carried by rings A and B of structure (3) are then oxidatively converted into vinyl groups, and the resultant protoporphyrinogen-IX (4) is dehydrogenated to give the aromatic porphyrin [1] (5). Evidence for the later stages of this sequence came mainly from enzymic and mutant studies,[1] but recently the conversion of uroporphyrinogen-III [2] (2) and coproporphyrinogen-III [3] (3) into protoporphyrin-IX (5) without rearrangement has been confirmed by radioactive tracer experiments.

The natural porphyrins and chlorins found in blood and plant pigments are all based on the type-III skeleton [as (2)—(5)] and there has been intense speculation about the mechanism by which PBG (1) is converted into (2) *apparently* with the reversal of ring D. One of the main objects in our current work on porphyrins is the solution of this biosynthetic problem. Two interlocking approaches were selected. The first, which makes use of ^{13}C-labelling, will be described in subsequent papers. The second involves synthesis of the four isomeric pyrromethanes (6)—(9) in ^{14}C-labelled form for studies



of incorporation into protoporphyrin-IX; the reaction sequence used has been outlined.[4] Pluscec and Bogorad [5] have demonstrated incorporation of the unlabelled isomer (6) in the presence of PBG into uroporphyrinogen-I [as (2), R and $CH_2 \cdot CH_2 \cdot CO_2H$

[1] Reviewed by L. Bogorad in ' The Chlorophylls,' eds. L. P. Vernon and G. R. Seeley, Academic Press, New York, 1966, p. 481 and by B. F. Burnham in ' Metabolic Pathways,' ed. D. M. Greenberg, Academic Press, New York, 1969, vol. III, 3rd edn., p. 403.

[2] B. Franck, D. Gantz, F. P. Montforts, and F. Schmidtchen, *Angew. Chem. Internat. Edn.*, 1972, **11**, 421.

[3] A. R. Battersby, J. Staunton, and R. H. Wightman, *J.C.S. Chem. Comm.*, 1972, 1118.

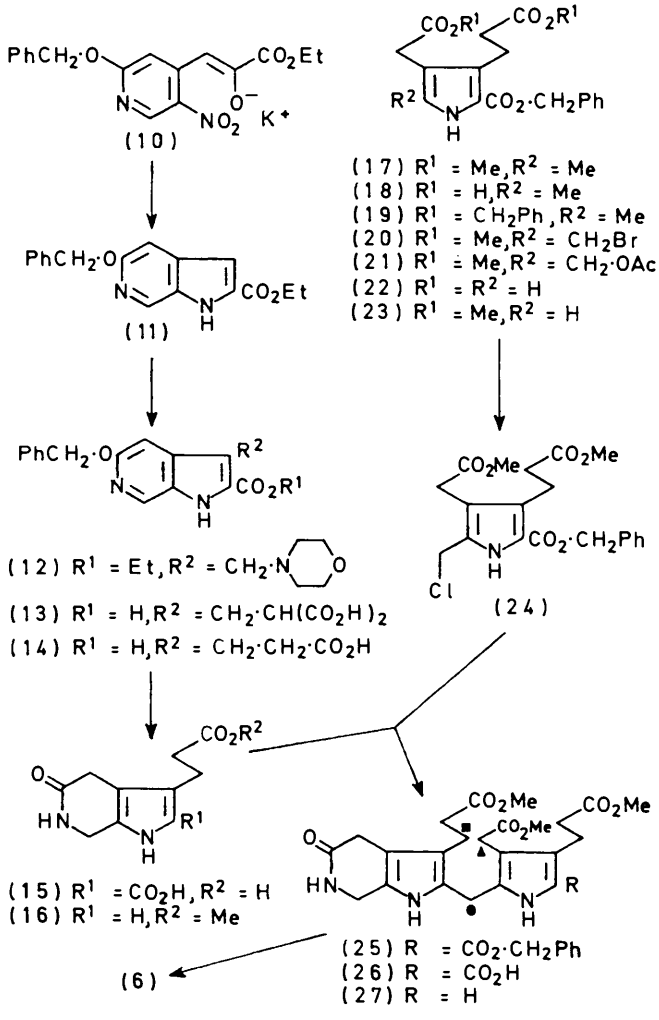
[4] A. R. Battersby, 23rd International Congress of Pure and Applied Chemistry, Special Lectures, 1971, vol. 5, p. 1.

[5] J. Pluscec and L. Bogorad, *Biochemistry*, 1970, **9**, 4736.

interchanged on ring D] by porphobilinogen deaminase. Recently, Frydman and Rapoport *et al.*[6] have reported syntheses of (6) and (7) also in unlabelled form, and the route used by S. F. MacDonald *et al.* to prepare the pyrromethane (6) for Bogorad has just been published.[7] This paper describes the development of efficient routes for synthesis of the pyrromethanes (6) and (7) labelled specifically with carbon-14 at the positions marked ●, ■, and ▲. The synthesis of (6) will be considered first.

It is essential for the envisaged biosynthetic work to be confident that the labelled substances used are pure and, in particular, are free from isomers. Yet the pyrromethane (6) was expected to be an unstable, acid-labile substance, unreceptive to handling and this proved to be so. Our synthetic target was therefore the lactam triester (27), from which (6) would be derived by one mild step of alkaline hydrolysis. One building block for (27) is the known lactam (16), but of the two published synthetic routes,[8] the desirable one for our purpose *via* (11) gave an unsatisfactory yield. Accordingly, the pyruvate salt [8] (10) was reduced with zinc and acetic acid to the diazaindene (11) in 77% yield; reduction of (10) with (*a*) ammoniacal ferrous sulphate and (*b*) alkaline dithionite gave lower yields of (11). The diazaindene (11) reacted with formaldehyde and morpholine hydrochloride and the resultant Mannich base (12) was converted by diethyl sodiomalonate into the triethyl ester of (13). Alkaline hydrolysis afforded the acid (13). Hot pyridine effected decarboxylation of the malonic acid and the product (14) was transformed into (15) by mild hydrogenation.[8] This sequence was run in the ^{14}C-series without isolation of intermediates to give (15) in 32% radiochemical yield from ^{14}C-formaldehyde. Decarboxylation of (15) in boiling water [9] and esterification by diazomethane then provided one of the required units (16), labelled or unlabelled, for the pyrromethane (6). The foregoing procedures are such that (16) can be prepared on a small scale (10—30 mg) in 35% overall yield from (11).

The pyrrolic component to be coupled with the lactam (16) must carry functions which allow the α-free position of (27) to be generated late in the synthesis. Our preliminary experiments were aimed at the system (34) and it was hoped to hydrolyse the esters selectively before decarboxylation of the pyrrolic α-carboxy-group. The required starting material (30) had previously been prepared [10] from readily available (28) but a different reaction sequence was used here to allow labels to be introduced conveniently. Debenzylation of (28) over palladium followed by carefully controlled thermal decarboxylation gave the β-free pyrrole (29). Reductive alkylation [11] of (29) using glyoxylic acid and esterification of the product with diazoethane gave (30) in 44% overall yield from (28). Since the reductive alkylation worked

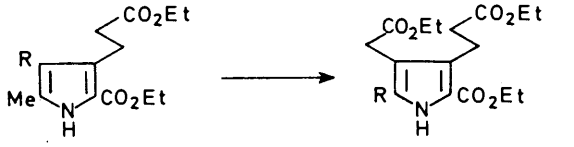


as well with 1 mol. equiv. of [2-^{14}C]glyoxylic acid as it did when higher molar ratios were used in the radioinactive series, an effective route was available to (30) ^{14}C-labelled at the methylene group of the acetate side-chain.

Unlabelled (30) was converted by dioxan dibromide [12] in dioxan into the bromide (31), which reacted with the lactam (16) in hot buffered acetic acid to provide the pyrromethane (34) in good yield. An alternative synthesis of pyrromethanes, which had worked well in other examples,[13] here requires reaction of the pyridinium salt (32), readily derived from the bromide (31), with the lithium salt of (15); (34) was again obtained after methylation but a modification of the former approach was used for subsequent work.

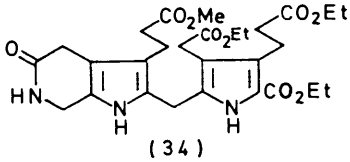
[6] B. Frydman, S. Reil, A. Valasinas, R. B. Frydman, and H. Rapoport, *J. Amer. Chem. Soc.*, 1971, **93**, 2738.
[7] J. M. Osgerby, J. Pluscec, Y. C. Kim, F. Boyer, N. Stojanac, H. D. Mah, and S. F. MacDonald, *Canad. J. Chem.*, 1972, **50**, 2652.
[8] B. Frydman, S. Reil, M. E. Despuy, and H. Rapoport, *J. Amer. Chem. Soc.*, 1969, **91**, 2338.
[9] A. H. Jackson and S. F. MacDonald, *Canad. J. Chem.*, 1957, **35**, 715.
[10] S. F. MacDonald, *J. Chem. Soc.*, 1952, 4176, 4184.
[11] M. W. Roomi and S. F. MacDonald, *Canad. J. Chem.*, 1970, **48**, 139.
[12] J. D. Billinoria and N. F. MacLagan, *J. Chem. Soc.*, 1954, 3257.
[13] A. H. Jackson, G. W. Kenner, and D. Warburton, *J. Chem. Soc.*, 1965, 1328.

Alkaline hydrolysis of the lactam ester (34) over a range of conditions soon showed that ring opening of the lactam was occurring readily. Mild conditions gave a



(28) R = $CO_2 \cdot CH_2Ph$
(29) R = H

(30) R = Me
(31) R = CH_2Br
(32) R = $CH_2 \cdot N^+$
(33) R = H



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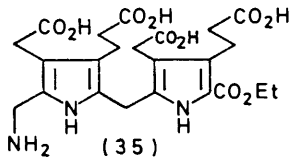

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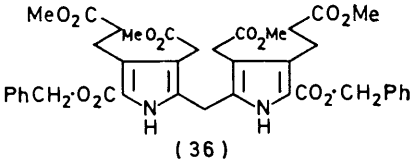

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mixture which, on mass spectrometric evidence, contained the amino-acid (35). Support for this interpretation was gained by comparing the ease of alkaline hydrolysis of the monopyrroles (30) and (16). The rates of attack at the various functions decreased in the order (*a*) aliphatic ethoxycarbonyl groups, (*b*) the lactam residue, (*c*) the ethoxycarbonyl group at position 2 of (30). Thus, selective hydrolysis of all the ester functions of (34) while retaining the lactam intact is not possible. The necessary differentiation of the α-ester was achieved by using the benzyl ester (17) prepared as follows.

When the triester (30) was heated with sodium benzyloxide in benzyl alcohol,[14] the monoester (18) was consistently obtained in 65% yield though on rare occasions 95% was isolated. Methylation of the monoester (18) with diazomethane then readily afforded the monobenzyl dimethyl ester (17). The tribenzyl ester (19) was isolated from the neutral fraction obtained in the ester exchange reaction and selective hydrolysis of the triester as a result of moisture entering the reaction could explain the formation of (18). Since strict precautions had been taken to exclude water, an alternative possibility was considered, that of nucleophilic attack by benzyloxide anion at the aliphatic ester groups. However, no trace of dibenzyl ether was found in the reaction mixture by g.l.c. under conditions which were shown readily to detect the stoicheiometric quantity.

The monobenzyl dimethyl ester (17) underwent bromination as earlier to give (20), which reacted with the lactam (16) in sodium acetate–acetic acid to yield the pyrromethane (25). However, more reproducible results were obtained in the benzyl series with the chloride (24), prepared from (17) and sulphuryl chloride, and with the reaction in sodium acetate–acetic acid being run at 100°. In agreement with Kenner *et al.*,[15] it was found that at least part of the coupling reaction between (16) and (24) occurs through the intermediacy of the acetate (21); the latter formed readily when (20) was treated with sodium acetate in acetic acid. Attempted recrystallisation of (21) from ethanol afforded the symmetrical product [16] (36).

The pyrromethane (25) is a stable crystalline product suitable for storage of labelled materials. It was converted by hydrogenation into the acid (26), which was decarboxylated in boiling aqueous methanol or (better) with trifluoroacetic acid [17] at 20°. The resultant pyrromethane (27) was sufficiently stable for normal handling and chromatography.

Application of the foregoing steps to the building blocks (16) and (24) which had been labelled as described earlier at sites ■ and ▲ provided two samples of the labelled lactam (27). The third label at ● required the pyrrole (17) carrying carbon-14 at the C-2 methyl group. Initially this substance was prepared by reductive methylation [11] of the pyrrole [18] (33) with [^{14}C]-formaldehyde followed by the exchange process with benzyl alcohol already described. However, a much higher radiochemical yield was achieved by carrying out the exchange reaction on (33) and methylation of the product (22) to give (23) followed by a mild step of reductive methylation. The [^{14}C]pyrrole (17) was then converted as before into the lactam (27) labelled at position ●.

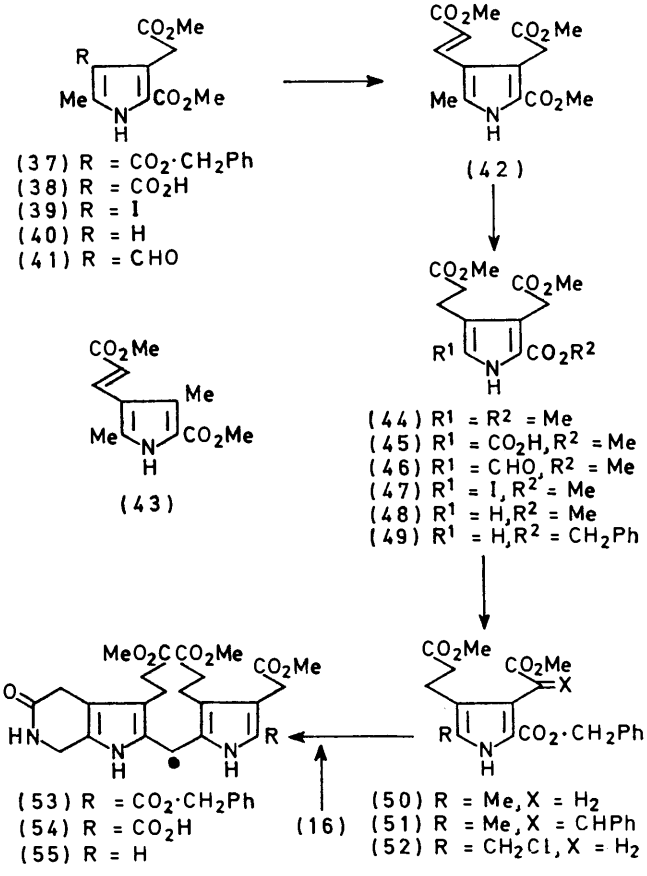
Our synthesis of the lactam (55), from which (7) was to be derived, was completed by a scheme similar to that used for the isomer (27). In the present case, the pyrrole (52) was required for coupling with lactam (16). The standard sequence (*cf.* ref. 10) (37) ⟶ (38) ⟶ (39) ⟶ (40) gave β-free material which was formylated with phosphoryl chloride–dimethylformamide. Condensation of the resultant aldehyde (41) with malonic acid and esterification gave the acrylic ester (42), together with a small quantity of the diester (43) presumably arising by selective hydrolysis and decarboxylation under acidic conditions. Catalytic hydrogenation converted (42) into (44) and exchange of the methyl ester for benzyl, as developed earlier, led to the required pyrrole (50); modification of the original conditions was necessary to avoid serious amounts of the by-product (51) being formed. Labelling with carbon-14 was carried out by the sequence (44) ⟶ (45) ⟶ (47) ⟶ (48) ⟶ (49), with a final step of reductive methylation of (49) with [^{14}C]formaldehyde. In some cases, the con-

[14] A. Hayes, G. W. Kenner, and N. R. Williams, *J. Chem. Soc.*, 1958, 3779.
[15] A. M. d'A. R. Gonsalves, G. W. Kenner, and K. M. Smith, *Tetrahedron Letters*, 1972, 2203.
[16] *E.g.* A. W. Johnson, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1959, 3416.
[17] A. H. Jackson, G. W. Kenner, and K. M. Smith, *J. Chem. Soc.* (*C*), 1971, 502.
[18] S. F. MacDonald, *Canad. J. Chem.*, 1955, **33**, 573.

version (44) ⟶ (45) by sulphuryl chloride yielded mainly the aldehyde (46), which was further oxidised to the acid (45) by permanganate.

Coupling of the lactam (16) with the chloromethylpyrrole (52) occurred smoothly and the last steps of the synthesis, (53) ⟶ (54) ⟶ (55), were carried out by the methods already described for the isomeric lactam (27). The pyrromethanes (53)—(55) are all less stable than their isomers (25)—(27), but again the benzyl ester (53) is the compound of choice for storage. The α-free pyrromethane (55) is stable if carefully manipulated.

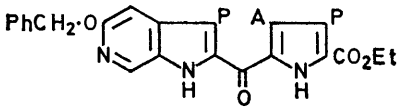
Before the successful syntheses of the lactams (27) and (55), preliminary studies were made of an alternative route[19] involving the dipyrrolyl ketone (56). The anhydrous sodium salt of the acid (57), derived from the diazaindene ester (11), with oxalyl chloride–dimethylformamide gave the acid chloride (58) from which the amide (59) was obtained in good yield. This with formaldehyde–dimethylamine gave the base (60), contaminated with by-product ether [*e.g.* (61)] derived from the alcoholic solvent; the side reaction was minimised by using propan-2-ol. Treatment of (60) with diethyl



sodiomalonate gave the ester (62), which could be hydrolysed to the tricarboxylic acid (13) or, under milder conditions, to the amide (63). Decarboxylation of (63) in hot pyridine and esterification of the product provided the diazaindene (64).

[19] J. A. Ballantine, A. H. Jackson, G. W. Kenner, and G. McGillivray, *Tetrahedron*, 1966, Suppl. 7, p. 241.

In a trial experiment, the amide (59) and 2,4-dimethyl-3-ethoxycarbonylpyrrole[20] were treated with phosphoryl chloride in hot dichloroethane. The reaction was monitored by observing the absorption spectrum of the salt (65), and hydrolysis of this intermediate gave



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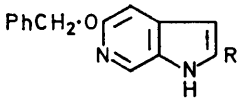
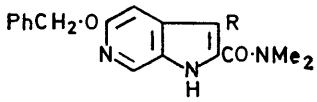
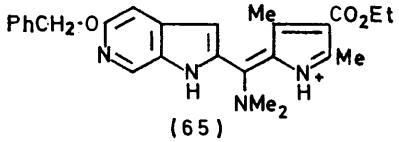


(57) R = CO_2H
(58) R = COCl
(59) R = $CO{\cdot}NMe_2$
(67) R = CHO



(60) R = $CH_2{\cdot}NMe_2$
(61) R = $CH_2{\cdot}O{\cdot}CHMe_2$
(62) R = $CH_2{\cdot}CH(CO_2Et)_2$
(63) R = $CH_2{\cdot}CH(CO_2H)_2$
(64) R = $CH_2{\cdot}CH_2{\cdot}CO_2Me$



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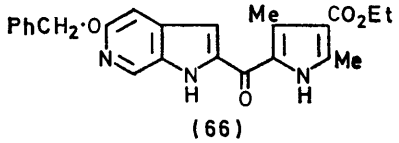

(66)

the ketone (66) in excellent yield. However, the pyrrole (33) did not react under these conditions nor under a variety of more forcing ones. Similarly no reaction could be induced between the diazaindene (64) and the pyrrole (33). Evidently deactivation by the 2-ester group of the pyrrole (33) is overwhelming[21] here [but *cf.* conversion of (33) into (30) and of (23) into (17)]. Preparation of the α-formyl diazaindene (67) is described in the Experimental section.

With both lactams (27) and (55) available, it was established by t.l.c. that they were separable and that there was no detectable quantity of one in the other. Controls with mixtures of known composition showed that 0·4% of (27) in (55) (and *vice versa*) was readily observed.

The lactam and ester residues of (27) and (55) were hydrolysed with sodium hydroxide and the reactions were followed to completion by n.m.r. spectroscopy.[22] The products (6) and (7), as their sodium salts, were handled almost entirely at 20° above pH 7 in subdued light and, as far as possible, under nitrogen because of their instability, especially in acid. The availability of singly

[20] L. Knorr, *Annalen*, 1886, **236**, 325.
[21] A. Treibs and G. Fritz, *Annalen*, 1958, **611**, 162.
[22] Y. C. Kim, *Canad. J. Chem.*, 1969, **47**, 3259.

and multiply labelled pyrromethanes (6) and (7) opens the way for a full study of their chemistry and of their effectiveness as substrates for the porphyrin-forming enzymes. Preliminary experiments [4] have shown that when (6) is incubated with the enzyme system from ducks' blood,[23] specifically labelled protoporphyrin-IX (5), as haem, is formed with little or no scrambling. Current work will determine the extent to which enzymic and specific chemical formation of labelled uroporphyrinogen-III (2) from (6) contribute to the observed result. The chemical and enzymic research on compounds (6)—(9) will be reported in subsequent papers.

EXPERIMENTAL

General Directions.—Solutions were dried over anhydrous sodium sulphate and evaporated at $<50°$. T.l.c. was carried out on Merck plates precoated with silica GF_{254}, and neutral grade III alumina was used for column chromatography. Samples for analysis were dried at 0·03 mmHg (over P_2O_5–NaOH) for several hours at 110°. M.p.s were determined on a Kofler hot-stage apparatus. The following spectrometers were used: Unicam SP 200 and SP 1000 and Perkin-Elmer 257 (i.r. in $CHCl_3$); Unicam SP 800 (u.v. in MeOH); Varian HA-100 and XL-100 (n.m.r. in $CDCl_3$); and A.E.I. MS9 and MS12 (mass spectra). Exceptions are noted in context.

Ethyl 5-Benzyloxy-1H-pyrrolo[2,3-c]pyridine-2-carboxylate (11).—The potassium enolate of ethyl (2-benzyloxy-5-nitro-4-pyridyl)pyruvate [8] (10) (7·64 g) was dissolved in cold methanol (160 ml) and added to a vigorously agitated suspension of zinc dust (78·4 g) in acetic acid (160 ml), resulting in an exothermic reaction. After 15 min, the mixture was filtered (glass sinter) and the filtrate reduced to a small volume before pouring into warm (50°) water (500 ml). The crystalline diazaindene (11) was collected after cooling; yield 4·05 g (69%), m.p. 149·5—150° (from chloroform) (lit.,[8] 150—152°); M^+ 296; $\nu_{max.}$ 1627, 1711, and 3470 cm^{-1}; $\lambda_{max.}$ 279, 288, and 338 nm; τ 0·5br (1H, s, NH), 1·48br (1H, s, H-7), 2·65 (5H, m, Ph), 2·97 and 3·01 (both 1H, s, H-3 and H-4), 4·64 (2H, s, O·CH_2Ph), and 5·61 and 8·62 (2H, q, and 3H, t, J 7 Hz, OEt).

Ethyl 5-Benzyloxy-3-morpholino[^{14}C]methyl-1H-pyrrolo-[2,3-c]pyridine-2-carboxylate (12).—The diazaindene (11) (29·6 mg), morpholine hydrochloride (18·3 mg), and [^{14}C]-paraformaldehyde (0·5 mCi; 2·78 mg) were heated together under reflux in isopropyl alcohol (1 ml) and water (0·1 ml). After 17 h, an excess of radioinactive paraformaldehyde (5 mg) was added, and heating was continued for 9 h. The residue from evaporation was partitioned between ether and N-hydrochloric acid; the aqueous layer was basified (solid sodium carbonate) and extracted with chloroform to give the radioactive *Mannich base* (12) (33 mg; 0·31 mCi) in 62% radiochemical yield (84% chemical yield); it was homogeneous by alumina t.l.c./radioscan. Radioinactive material had m.p. 110° (from ether–pentane); M^+ 395; $\nu_{max.}$ 1626, 1709, and 3460 cm^{-1}; $\lambda_{max.}$ 285, 293, and 346 nm; τ 0·52br (1H, s, NH), 1·53 (1H, s, H-7), 2·6 (5H, m, Ph), 2·70 (1H, s, H-4), 4·59 (2H, s, CH_2Ph), 5·58 and 8·60 (2H, q and 3H, t, J 7 Hz, OEt), 6·02 (2H, s, 3-CH_2·N), 6·34 and 7·50 (4H each, m, O·CH_2·CH_2·N). From ethanolic hydrogen chloride, the *trihydrochloride* was formed, m.p. 152—153° (decomp.) [Found (after drying at 20° over P_2O_5): C, 52·5; H, 5·5; N, 8·3%; M^+, 395. $C_{22}H_{25}N_3O_4$,3HCl requires C, 52·3; H, 5·6; N, 8·3%; M, 395 (for free base)]. On heating in vacuo, the *monohydrochloride* was obtained, m.p. 192—194° (Found: C, 61·4; H, 6·1; N, 10·0. $C_{22}H_{25}N_3O_4$,HCl requires C, 61·2; H, 6·1; N, 9·8%).

5-Benzyloxy-2-carboxy-1H-pyrrolo[2,3-c]pyridin-3-yl-[^{14}C]methylmalonic Acid Hydrochloride [*as* (13)].—The crude morpholinomethyldiazaindene (33 mg) was heated at 110° with a solution of sodium (12 mg) in diethyl malonate (0·4 ml). After 1·5 h, the mixture was hydrolysed by heating under reflux for 7 h with 3N-sodium hydroxide (8·5 ml). Sufficient 11N-hydrochloric acid was added dropwise to the cold solution to precipitate the *hydrochloride* (49 mg), m.p. 156—158° (from acetic acid) (Found: C, 51·8; H, 4·3; N, 6·2. $C_{19}H_{16}N_2O_7$,HCl,H_2O requires C, 52·0; H, 4·4; N, 6·4%); $\nu_{max.}$ (KBr) 1725, 1685, 1650, and 1615 cm^{-1}; $\lambda_{max.}$ 288, 296, and 344 nm; τ [$(CD_3)_2SO$] 1·66 (1H, s, H-7), 2·5 (5H, m, Ph), 3·02 (1H, s, H-4), 4·68 (2H, s, $ArCH_2$·O), 6·58 [2H, s, 3-CH_2·$CD(CO_2D)_2$].

3-(2-Carboxy-4,5,6,7-tetrahydro-5-oxo-1H-pyrrolo[2,3-c]-pyridin-3-yl)[3-^{14}C]propionic Acid (15).—The foregoing hydrochloride (49 mg) was decarboxylated by heating in pyridine (1 ml) at 110° for 1·5 h. After evaporation of solvent, the crude product (14) was suspended in water (1 ml) and solid potassium carbonate was added to give a slightly alkaline solution which was hydrogenated over 10% palladised charcoal (30 mg). The filtered solution was acidified (acetic acid) to give the lactam (14) (11 mg), m.p. 283° (lit.,[8] 295°); $\nu_{max.}$ (Nujol) 1705 and 1650 cm^{-1}; $\lambda_{max.}$ (alkaline solution) 268 nm; τ [$(CD_3)_2SO$] 2·30 (1H, s, NH), 5·76br (2H, s, CH_2·N), 6·80br (2H, s, CH_2·CO·N), and 7·20 and 7·60 (each 2H, t, J 7 Hz, CH_2·CH_2·CO). Treatment of the acid with diazoethane gave the *diethyl ester*, m.p. 234—236° (lit.,[24] 242°) (Found: M^+, 308. $C_{15}H_{20}N_2O_5$ requires M, 308), $\nu_{max.}$ 1725 and 1650 cm^{-1}; $\lambda_{max.}$ 275 nm.

[^{14}C]*Porphobilinogen Lactam Methyl Ester* (16).—The 2-carboxy-derivative (15) (11 mg) was boiled in water (4 ml) for 4 h. On cooling, PBG lactam (7·1 mg; 0·13 mCi) crystallised, m.p. 282—283° (lit.,[9] 280—283°). This was treated in methanol–chloroform with an excess of ethereal diazomethane to give the methyl ester, m.p. 248—251° (lit.,[6] 240—242°) $\nu_{max.}$ (Nujol) 3200, 1745, 1650, and 1615 cm^{-1}; $\lambda_{max.}$ none >220 nm; τ [$(CD_3)_2SO$] −0·3 and 2·3 (each 1H, broad s, 2 × NH), 3·50 (1H, d, J 3 Hz, 2-H), 5·72 (2H, m, CH_2·N), 6·40 (3H, s, OMe), 6·82 (2H, m, CH_2·CO·N), and 7·47br (4H, s, CH_2·CH_2).

Ethyl 3-(2-Carbethoxyethyl)-5-methylpyrrole-2-carboxylate (29).—A solution of the benzyl ester [10] (28) (0·5 g) in ethanol (30 ml) and ethyl acetate (20 ml) was hydrogenated at normal temperature and pressure over 10% palladised charcoal (2·5 g). The solution was then filtered and evaporated to give the acid, m.p. 234—236° (from ethanol) (lit.,[10] 240°). This was heated alone under nitrogen at 260—270° for 9 min; evolution of carbon dioxide had then ceased. The hot product was added to stirred hexane (50 ml) and unchanged starting material was filtered off. Evaporation of the filtrate and crystallisation of the residue from hexane (charcoal) gave the β-free pyrrole (195 mg, 59%), m.p. 58—60° (lit.,[10] 65—65·5°). An isomorph, m.p. 63—64·5° (from ethanol–hexane) has identical n.m.r., mass, and u.v. spectra and t.l.c. behaviour, but shows marked differences in the fingerprint region of the i.r. spectrum (Nujol) (Found: C, 61·3; H, 7·6; N, 5·3%; M^+, 253. Calc. for $C_{13}H_{19}NO_4$:

[23] D. Shemin, T. Abramsky, and C. S. Russell, *J. Amer. Chem. Soc.*, 1954, **76**, 1204.

[24] A. Treibs and W. Ott, *Annalen*, 1958, **615**, 137.

C, 61·6; H, 7·6; N, 5·5%; *M*, 253); $\nu_{max.}$ (Nujol) 3300, 1730, and 1660 cm^{-1}; $\lambda_{max.}$ 278 nm; τ 0·6br (1H, s, NH), 4·1 (1H, d, *J* 3 Hz, 4-H), 5·6 and 5·9 (each 2H, q, *J* 7 Hz, O·C*H*$_2$Me), 6·9 and 7·2 (each 2H, m, CH_2CH_2CO), 7·75 (3H, s, 5-Me), and 8·62 and 8·72 (each 3H, t, *J* 7 Hz, O·CH_2·C*H*$_3$).

5·[^{14}C]*Methyl-, and 4-Ethoxycarbonyl*[^{14}C]*methyl Samples of Ethyl 3-(2-Ethoxycarbonylethyl)-4-ethoxycarbonylmethyl-5-methylpyrrole-2-carboxylate* (30).—(*a*) A solution of the diester (29) (63·3 mg) in hydriodic acid (s.g. 1·94; 0·63 ml), and hypophosphorous acid (50%; 0·13 ml) was treated at 40° with sodium glyoxylate (28·5 mg), added in three portions over 15 min. Stirring was continued at 35—40° for a further 15 min, and the solution was evaporated at 25—30° (0·2 mmHg) to give an orange solid which was suspended in ethanol (1 ml) and treated with an excess of ethereal diazoethane. Evaporation gave the triester (63 mg, 72%), m.p. 62—65° (from ether–hexane) (lit.,[11] 66—66·5°) (an isomorph has been observed, m.p. 58°, and the two forms differ in the fingerprint region of solid phase i.r. spectra); $\nu_{max.}$ 3450, 3300, 1730, and 1680 cm^{-1}; $\lambda_{max.}$ 282 nm; τ 0·7br (1H, s, NH), 5·64 (2H) and 5·82 (4H) (each q, *J* 7 Hz, 3 × O·C*H*$_2$Me), 6·55 (2H, s, 4-CH_2CO), 6·9 and 7·4 (each 2H, m, 3-$[CH_2]_2$·CO), 7·75 (3H, s, 5-Me), and 8·63 (3H) and 8·75 (6H) (each t, *J* 7 Hz, 3 × O·CH_2·C*H*$_3$). ^{14}C-Labelled material was made similarly by use of sodium [2-^{14}C]glyoxylate.

(*b*) Acetic anhydride (1 ml) was added dropwise with cooling to hydriodic acid (1 ml; s.g. 1·94) then hypophosphorous acid (0·2 ml) was added, followed after 15 min by the pyrrole[25] (33) (54 mg) and paraformaldehyde (5·0 mg). The mixture was stirred at 20° for 45 min, then evaporated, and the residue was stirred overnight with ethanolic 12% hydrogen chloride (3 ml). This was then partitioned between ether and water, and the organic phase was washed with aqueous ammonia before evaporation to give crude product. P.l.c. on silica [10% chloroform–ethyl acetate (two developments)] and crystallisation from ether–pentane gave the hexaester (5 mg) corresponding to (36) (by mass spec., M^+ 662). Evaporation of the mother liquor and crystallisation of the residue from pentane gave the ester (30) (40 mg, 71%), homogeneous by t.l.c., and identical with the foregoing product. ^{14}C-Labelled material was obtained by use of [^{14}C]paraformaldehyde.

Ethyl 5-Bromomethyl-3-(2-ethoxycarbonylethyl)-4-ethoxycarbonylmethylpyrrole-2-carboxylate (31).—The triester (30) (1·7 g) in dry dioxan (15 ml) was treated with dioxan dibromide[12] (1·07 g) in small portions over 2 min, and the solution was stirred at room temperature for 2·5 h. Evaporation and recrystallisation of the residue from toluene–hexane gave the bromomethylpyrrole (1·4 g, 67%), m.p. 89—93° (lit.,[24] 92°); $\nu_{max.}$ (Nujol) 3300, 1730, and 1670 cm^{-1}; τ −0·06br (1H, s, NH), 5·46 (2H, s, CH_2Br), 5·63, 5·88, and 5·91 (each 2H, q, *J* 7 Hz, 3 × O·C*H*$_2$Me), 6·50 (2H, s, 4-CH_2·CO), 7·0 and 7·5 (each 2H, m, 3-CH_2·CH_2·CO), and 8·66, 8·89, and 8·92 (each 3H, t, *J* 7 Hz, 3 × O·CH_2·C*H*$_3$).

Benzyl 3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylate (23).—Ethyl 3-(2-ethoxycarbonylethyl)-4-ethoxycarbonylmethylpyrrole-2-carboxylate[20] (33) (340 mg) was added to a solution of sodium hydride (0·55 g) in dry benzyl alcohol (25 ml). The mixture was stirred at 80—90° under nitrogen for 3 h, then added to 3N-sulphuric acid (15 ml). The solution was adjusted to pH 8—9 (solid potassium carbonate) and any solid was dissolved by adding water. Ethyl acetate (15 ml) was added and the equilibrated organic phase was extracted with aqueous sodium hydrogen carbonate. Acidification of the combined aqueous solutions (3N-sulphuric acid) afforded the *monobenzyl ester* (22) (208) mg, 60%), m.p. 192—196° (from methanol) (Found: C, 61·4; H, 5·4; N, 4·1. $C_{17}H_{17}NO_6$ requires C, 61·6; H, 5·2; N, 4·2%). Ethereal diazomethane was added at 0° to a suspension of the acid (22) in methanol (2 ml) to give the *monobenzyl dimethyl ester* (23) (201 mg, 89%), m.p. 60—63° or m.p. 49—52° (from ether–pentane) (Found: C, 63·2; H, 6·0; N, 3·7. $C_{19}H_{21}NO_6$ requires C, 63·5; H, 5·9; N, 3·9%); $\nu_{max.}$ (Nujol) 3310, 1738, 723, and 1685 cm^{-1}; $\lambda_{max.}$ 271 nm; τ 1·0br (1H, s, NH), 2·63 (5H, s, Ph), 3·18 (1H, d, *J* 2·5 Hz, 5-H), 4·71 (2H, s, PhC*H*$_2$·O), 6·32 and 6·38 (each 3H, s, OMe), 6·50 (2H, s, CH_2·CO_2Me), and 7·22 (4H, m, CH_2·CH_2·CO).

Methyl 2-[3-Ethoxycarbonylmethyl-4-(2-ethoxycarbonylethyl)-5-ethoxycarbonylpyrrol-2-ylmethyl]-4,5,6,7-tetrahydro-5-oxo-1H-pyrrolo[2,3-c]*pyridine-3-propionate* (34).—Porphobilinogen lactam methyl ester (16) (30·8 mg) and the bromomethylpyrrole (31) (58 mg) were kept at 100° for 100 min in acetic acid (1·2 ml) containing sodium acetate (12 mg). Hot water (3 ml) was added slowly, followed by a further 20 ml, and the precipitate from the cold solution was recrystallised from ethanol to give the *pyrromethane* (57 mg, 70%), m.p. 206—208° (Found: C, 59·7; H, 6·3; N, 7·2%; M^+, 559. $C_{28}H_{37}N_3O_9$ requires C, 60·1; H, 6·65; N, 7·5%; *M*, 559); $\nu_{max.}$ (Nujol) 3350, 3220. 1730, 1710, and 1675 cm^{-1}; $\lambda_{max.}$ 285 nm; τ ($CDCl_3$–CD_3OD) 1·92 (1H, s, NH), 5·3—6·0 (8H, m), 6·13 (2H, s, bridge CH_2) 6·36 (3H, s, OMe), 6·50 (2H, s, 3-CH_2·CO), 7·0—7·6 (8H, m, 2 × CH_2·CH_2), and 8·6—8·9 (9H, t, 3 × O·CH_2*Me*).

Selective Hydrolysis Experiments.—(a) *Hydrolysis of aliphatic esters.* The pyrrole triethyl ester (30) (339 mg) in ethanol (4·5 ml) was treated with aqueous 4N-potassium hydroxide (4·5 ml) at 20° for 24 h. Dilution with water (20 ml) and acidification (acetic acid) led to precipitation of ethyl 3-(2-carboxyethyl)-4-carboxymethyl-5-methylpyrrole-2-carboxylate, m.p. 240—241° (from aqueous ethanol); *m/e* 283 (M^+), 266, 238, 237, and 211; τ (C_5D_5N) 5·82 (2H, q, O·C*H*$_2$Me), 6·31 (2H, s, CH_2CO), 6·48 and 7·02 (each 2H, t, CH_2·CH_2·CO), 7·74 (3H, s, Me), and 8·91 (3H, t, O·CH_2*Me*).

By t.l.c. monitoring of the reaction, it was shown that hydrolysis to the dicarboxylic acid monoester level was complete after 5½ h.

(b) *Hydrolysis of aliphatic ester in presence of lactam.* Porphobilinogen lactam methyl ester (16) (22·2 mg) in ethanol (0·15 ml) was hydrolysed as in (a) for 3½ h. After acidification, porphobilinogen lactam (15 mg, 73%) was isolated. Serious attack at the lactam function occurred when the hydrolysis period was extended (see also later work on hydrolysis of pyrromethane lactams).

Benzyl 3-(2-Methoxycarbonylethyl)-4-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (17).—(a) *By benzyl exchange.* The triester (30) (67 mg) in dry benzyl alcohol (5 ml) was treated with sodium hydride (102 mg; 50% dispersion in oil) at 80° for 3 h, with protection against moisture. After cooling the solution to 0°, water (10 ml) was added, then ether (10 ml); the pH was then adjusted to 11 by dropwise addition of 6N-sulphuric acid. The equilibrated organic phase was extracted with aqueous sodium carbonate, and the combined aqueous phases were acidified to pH 3 (3N-sulphuric acid). The precipitated

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

(65 mg) monoester (18) had m.p. 238—241° (from ethanol); M^+ 345; $\nu_{max.}$ (Nujol) 3300, 2700, 1700, and 1680 cm^{-1}; $\lambda_{max.}$ 283 (shifting to 293 nm with hydroxide). The neutral fraction in the foregoing organic phase afforded the tri-(benzyl ester) (19), m.p. 103—104° (from toluene–hexane) (Found: M^+, 525. Calc. for $C_{32}H_{31}NO_6$: M, 525); $\lambda_{max.}$ 280 nm; τ 1·1br (1H, s, NH), 2·76 (15H, s, 3 × Ph), 4·81, 4·99, and 5·03 (each 2H, s, 3 × $ArCH_2$·O), 6·63 (2H, s, CH_2·CO), 7·04 and 7·52 (each 2H, t, J 8 Hz, $[CH_2]_2$·CO), and 7·92 (3H, s, Me).

A suspension of the ester (18) in methanol (2 ml) with ethereal diazomethane gave a product which was fractionated on silica gel (60—120; H) in 3 : 7 ethyl acetate–hexane to give the *dimethyl benzyl triester* (17), m.p. 78·5—79·5 (from toluene–cyclohexane) (Found: C, 64·5; H, 6·2; N, 3·65%; M^+, 373. $C_{20}H_{23}NO_6$ requires C, 64·3; H, 6·2; N, 3·75%; M, 373); $\nu_{max.}$ (Nujol) 3310, 1738, and 1670 cm^{-1}; $\lambda_{max.}$ 283 nm; τ 0·90 (1H, s, NH), 2·62 (5H, s, Ph), 4·72 (2H, s, CH_2Ph), 6·36 and 6·41 (3H, both s, OMe), 6·58 (2H, s, CH_2·CO), 6·98 and 7·48 (2H, both t, CH_2·CH_2·CO), and 7·80 (3H, s, Me). Application of this procedure to the two different ^{14}C-labelled samples of the pyrrole (30) gave samples of the benzyl ester (17) labelled at the *C*-methyl group and in the acetate side-chain.

(b) *By reductive methylation.* Acetic anhydride (4·25 ml) was added dropwise to cold stirred hydriodic acid (4·25 ml; s.g. 1·49), followed by hypophosphorous acid (1·7 ml). The mixture was allowed to reach 20° and to achieve a pale-straw colour (*ca.* 10 min). Formaldehyde (20 mg) in acetic acid (6·4 ml) was added, followed by the pyrrole (23) (250 mg). After 50 min at 20° the mixture was added to saturated aqueous sodium carbonate (20 ml) and ether (15 ml). The organic phase was washed with aqueous carbonate, aqueous sodium thiosulphate, and water before evaporation. P.l.c. on silica [4 : 6 ethyl acetate–hexane (two developments)] gave the pyrrole (17) (116 mg, 45%), m.p. 75—79°. ^{14}C-Labelled material was prepared analogously on a smaller scale, by use of [^{14}C]paraformaldehyde.

Ethyl 3-(2-*Ethoxycarbonylethyl*)-4-*ethoxycarbonylmethyl*-5-(1-*pyridiniomethyl*)*pyrrole*-2-*carboxylate* (32).—The bromomethylpyrrole (31) (2·58 g) was dissolved in hot pyridine (10 ml). Addition of anhydrous ether (100 ml) gave an oil which crystallised from 1 : 2 ethanol–hexane to give the *pyridinium salt* (2·6 g, 84%), m.p. 82—86°; this salt showed various degrees of hydration in different preparations (Found: C, 50·2; H, 5·7; N, 5·6. $C_{22}H_{29}BrN_2O_6$,1·5H_2O requires C, 50·2; H, 6·1; N, 5·3%); $\nu_{max.}$ (Nujol) 3400, 3350, 1722, 1712, and 1633 cm^{-1}; $\lambda_{max.}$ 264 nm; τ (CD_3·OD), 1·02 (2H, d, pyridine α-H), 1·36 (1H, t, pyridine γ-H), 1·87 (2H, t, pyridine β-H), 4·07 (2H, s, CH_2·$\overset{+}{N}$), 5·71, 5·93, and 5·97 (2H, each q, O·CH_2Me), 6·30 (2H, s, CH_2·CO), 7·0 and 7·5 (2H, both t, CH_2·CH_2·CO), and 8·8 (9H, t, 3 × O·CH_2-*Me*).

Coupling of the Pyridinium Salt.—The salt (32) (125 mg) in formamide (2·0 ml) was added to the dilithium salt of 5-carboxyporphobilinogen lactam (15) (68·0 mg) and the mixture was heated in an evacuated sealed tube at 100° for 70 h. Water (25 ml) was added to the mixture, which was then extracted with ether, and the aqueous phase was neutralised (CO_2) and extracted with ethyl acetate (5 × 15 ml) to afford a gum (113 mg) having the spectroscopic properties expected for the pyrromethane. This, in dry ethanol (1 ml), was treated with an excess of ethereal diazoethane to give the ethyl ester (34), identical with the material described earlier.

^{14}C-*Labelled Samples of Methyl* 2-[5-*Benzyloxycarbonyl*-4-(2-*methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-4,5,6,7-*tetrahydro*-5-*oxo*-1H-*pyrrolo*[2,3-c]*pyridine*-3-*propionate* (25).—A solution of the 2-methylpyrrole (17) (278 mg) in ether (9 ml) was treated at 0° with ethereal 0·1M-sulphuryl chloride (9 ml). After 30 min, evaporation gave the chloride (24), which occasionally crystallised after a pentane wash. This material was checked by n.m.r. [τ 5·44 (CH_2Cl)] and then immediately treated with a solution of the lactam methyl ester (16) (149 mg) in 0·2M-acetate in acetic acid (3·7 ml); the mixture was heated at 100° for 20 min. Evaporation of the solvent and trituration of the residue with water and then with ethanol gave the *pyrromethane benzyl ester* (346 mg, 81%), m.p. 174—177° from ethanol; [*cf.* ref. 6, m.p. 166—170° (from aqueous methanol)] (Found: C, 62·5; H, 6·1; N, 7·5%; M^+, 593. $C_{31}H_{35}N_3O_9$ requires C, 62·7; H, 5·95; N, 7·1%; M, 593) $\nu_{max.}$ 1660, 1725, and 3300 cm^{-1}; τ −0·1br, 0·5br, and 3·4br (each 1H, s, 3 × NH), 2·7 (5H, m, Ph), 4·80 (2H, s, PhCH_2·O), 5·66br and 6·70br (each 2H, s, lactam CH_2·N and CH_2·CO), 6·16 (2H, s, bridge CH_2), 6·30, 6·44, and 6·50 (each 3H, s, 3 × OMe), 6·44 (2H, s, CH_2·CO), and 7·0 (2H, t) and 7·5 (6H, m) (2 × CH_2·CH_2·CO).

This compound was also formed from the bromide (20) under similar conditions, but the reaction mixture frequently became dark and the yield was variable.

The ^{14}C-labelled pyrromethanes were prepared similarly from the appropriately labelled 2-methylpyrrole (17) synthesised earlier.

Benzyl 5-*Acetoxymethyl*-3-(2-*methoxycarbonylethyl*)-4-*methoxycarbonylmethylpyrrole*-2-*carboxylate* (21).—The chloromethylpyrrole (24) (80 mg) was treated at room temperature for 40 min with a solution of anhydrous sodium acetate (15 mg) in acetic acid (1·5 ml). The residue obtained by evaporation was chromatographed on silica in 9 : 1 (v/v) chloroform–methanol to give the unstable acetoxymethylpyrrole (66 mg); τ 0·70 (1H, s, NH), 2·65 (5H, s, Ph), 4·74 (2H, s, PhCH_2·O), 4·99 (2H, s, CH_2·OAc), 6·38 and 6·43 (each 3H, s, 2 × OMe), 6·49 (2H, s, CH_2·CO), and 7·01 and 7·51 (each 2H, t, CH_2·CH_2·CO). Attempted recrystallisation of this ester from ethyl acetate resulted in conversion into the known pyrromethane (36), m.p. 141—145°, identical with an authentic sample.

The acetoxymethylpyrrole (21) (21·5 mg) in acetic acid (0·5 ml) containing anhydrous sodium acetate (5 mg) and porphobilinogen lactam methyl ester (11 mg) was heated at 65° for 12 h. Virtually complete conversion of (21) into the pyrromethane (25) occurred. A similar experiment at 20° (6 h) showed starting materials to be unchanged.

^{14}C-*Labelled Samples of Methyl* 2-[4-(2-*Methoxycarbonylethyl*)-3-*methoxycarbonylmethylpyrrol*-2-*ylmethyl*]-4,5,6,7-*tetrahydro*-5-*oxo*-1H-*pyrrolo*[2,3-c]*pyridine*-3-*propionate* (27).—A solution of the benzyl ester (25) (200 mg) in acetic acid (40 ml) was hydrogenated over palladium black (200 mg) until uptake had ceased (usually 3 h). The catalyst was filtered off under nitrogen in subdued light and washed with acetic acid. Evaporation of the filtrate gave the sensitive pyrromethanecarboxylic acid (26), which was immediately dissolved at 0° in trifluoroacetic acid (7 ml) under nitrogen. The solution was allowed to warm to 20° and after a further 20 min was evaporated. Benzene was added and evaporation gave the pyrromethane (133 mg, 62%). P.l.c. on silica with 10% methanol–chloroform followed by recrystallisation from methanol gave the product in 52% yield, m.p. 195—197° (*cf.* ref. 6, m.p. 192—

194°) (Found: C, 60·4; H, 6·4; N, 9·25%; M^+, 459·1988. $C_{23}H_{29}N_3O_7$ requires C, 60·1; H, 6·4; N, 9·15%; M, 459·2005); $\nu_{max.}$ (Nujol) 3340, 3295, 3190, 1732, 1706, 1702, and 1655 cm^{-1}; $\lambda_{max.}$ 232 nm (ε 11,000); τ 0·64, 0·91, 3·43 (1H, each s, NH), 3·56 (1H, d, J 2·5 Hz, 2-H), 5·64br (2H, s, CH_2·NH), 6·22 (2H, s, bridge CH_2), 6·30, 6·37, and 6·40 (each 3H, s, OMe), 6·50 (2H, s, CH_2·CO), 6·70 (2H, t, J 3 Hz, CH_2·CO·NH), and 7·3—7·5 (8H, m, 2 × CH_2·CH_2·CO).

Methyl 4-Benzyloxycarbonyl-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (37).—Redistilled pentyl nitrite (29·2 g) was added over 45 min to a stirred mixture at <40° of dimethyl acetonedicarboxylate (49·4 g) and concentrated hydrochloric acid (0·4 ml). The solution was kept overnight and then added over 45 min to a stirred mixture of ammonium acetate (37·5 g), benzyl acetoacetate (48 g), and zinc dust (12·5 g) in acetic acid (250 ml) held at 60—65° by cooling; more zinc dust (25 g total) was added in portions during this period. The mixture was heated at 95° for 2 h then decanted from the zinc into stirred ice–water (3 l). The crude product was washed with 1 : 1 water–ethanol and recrystallised from ethanol (250 ml) to give the *pyrrole* (48·5 g, 56%), m.p. 149—151° (Found: C, 62·6; H, 5·5; N, 3·8%; M^+, 345. $C_{18}H_{19}O_6N$ requires 62·6; H, 5·5; N, 4·1%; M, 345), $\nu_{max.}$ (Nujol) 3300, 1758, 1706, and 1670 cm^{-1}; $\lambda_{max.}$ 275 nm (ε 15,700); τ 0·40 (1H, NH), 2·66 (5H, s, Ph), 4·79 (2H, s, $PhCH_2$·O), 5·83 (2H, s, CH_2CO), 6·26 and 6·44 (each 3H, s, 2 × OMe), and 7·62 (3H, s, Me).

5-Methoxycarbonyl-4-methoxycarbonylmethyl-2-methylpyrrole-3-carboxylic Acid (38).—The benzyl ester (37) (20 g) in ethanol (230 ml) was hydrogenated over Raney nickel (7 ml) for 6 h at 125° and 150 atm. After filtration and evaporation the crude product was dissolved in aqueous ammonia (4M; 75 ml) and the solution was filtered before acidification (acetic acid). The precipitate was recrystallised from ethanol to give the *pyrrole acid* (13·4 g, 90·5%), m.p. 251—253° (Found: C, 51·9; H, 5·3; N, 5·2%; M^+, 255. $C_{11}H_{13}NO_6$ requires C, 51·8; H, 5·1; N, 5·5%; M, 255); $\nu_{max.}$ 3260, 3350vbr, 1746, and 1670br cm^{-1}; $\lambda_{max.}$ 276 nm (ε 16,400); τ (C_5D_5N) 5·52 (2H, s, CH_2·CO), 6·62 and 6·64 (each 3H, s, 2 × OMe), and 7·54 (3H, s, Me).

Methyl 4-Iodo-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (39).—Iodine (180 mg) in ethanol (2 ml) was added dropwise over 15 min to a stirred solution of the acid (38) (208 mg) and potassium hydrogen carbonate (250 mg) in 1 : 1 water–ethanol (4 ml). After a further 15 min the solution was boiled (to discharge the excess of iodine), then poured into water (40 ml). Filtration afforded the *iodopyrrole* (253 mg, 85%), m.p. 121·5—122° [from chloroform–light petroleum (b.p. 60—80°)] (Found: C, 35·5; H, 3·7; I, 37·8; N, 4·2%; M^+, 337. $C_{10}H_{12}INO_4$ requires C, 35·6; H, 3·6; I, 37·6; N, 4·2%; M, 337). $\nu_{max.}$ 3280, 1740, and 1675 cm^{-1}; $\lambda_{max.}$ 282 nm (ε 16,100); τ 0·33 (1H, NH), 6·21 (5H, s, CH_2·CO and CO_2Me), 6·30 (3H, s, OMe), and 7·75 (3H, s, Me).

Methyl 3-Methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (40).—The iodopyrrole (39) (11·7 g) in ethanol (325 ml) was hydrogenated over 5% palladised charcoal (5·7 g) and magnesia (5·7 g). Hydrogen uptake ceased after 4 h; the filtered solution was evaporated and the residue was partitioned between ether and water. Evaporation of the ether layer gave the *pyrrole* (6·77 g, 92%), m.p. 59—61·5° (from ether-pentane) (Found: C, 57·0; H, 6·3; N, 6·8%; M^+, 211. $C_{10}H_{13}NO_4$ requires C, 56·9; H, 6·2; N, 6·6%; M, 211); $\nu_{max.}$ (Nujol) 3290, 1762, 1750, and 1680 cm^{-1}; $\lambda_{max.}$ 278 nm (ε 17,200); τ 0·57 (1H, NH), 4·07 (1H, d, J 3 Hz, 4-H), 6·20 (5H, s, CH_2·CO and OMe), 6·31 (3H, s, OMe) and 7·75 (3H, s, Me).

Methyl 4-Formyl-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (41).—Phosphoryl chloride (0·10 ml) was added dropwise at 15° to dimethylformamide (0·09 ml). After 15 min the mixture was cooled at 5°, a solution of the pyrrole (40) (211 mg) in dichloroethane (0·25 ml) was added over 5 min, and the solution was heated under reflux for 20 min. After a solution of sodium acetate hydrate (750 mg) in water (1 ml) had been added, the stirred mixture was heated under reflux for 15 min, then cooled and extracted with dichloromethane. The extracted product was recrystallised from chloroform–light petroleum (b.p. 60—80°) to give the *aldehyde* (217 mg, 91%), m.p. 173—174·5° (Found: C, 55·5; H, 5·8; N, 5·7%; M^+, 239. $C_{11}H_{13}NO_5$ requires C, 55·2; H, 5·5; N, 5·9%; M, 239), $\nu_{max.}$ (Nujol) 3200, 1740, 1718, and 1660 cm^{-1}; $\lambda_{max.}$ 238 and 289 nm (ε 19,000 and 10,900); τ 0·10 (1H, s, CHO), 5·82 (2H, s, CH_2·CO), 6·21 and 6·32 (each 3H, s, 2 × OMe), and 7·53 (3H, s, Me).

Methyl 3-Methoxycarbonylmethyl-4-(2-methoxycarbonylvinyl)-5-methylpyrrole-2-carboxylate (42).—A solution of the formylpyrrole (41) (116 mg) and malonic acid (100 mg) in pyridine (6 ml) and piperidine (0·1 ml) was stirred at 100° under nitrogen with a molecular sieve (6 pellets, Linde 4A). After 5 h, the cooled mixture was diluted with dichloromethane (10 ml) and extracted with saturated aqueous sodium carbonate. The aqueous extract (after backwashing) was acidified (2N-sulphuric acid) to give the *acrylic acid* (118 mg, 87%), m.p. 246—248° (from ethanol–water) (the mother liquor *A* was examined as below) (Found: C, 55·4; H, 5·2; N, 5·2%; M^+, 281. $C_{13}H_{15}NO_6$ requires C, 55·5; H, 5·4; N, 5·0%; M, 281); $\nu_{max.}$ 3300, 2800vbr, 1715, 1695, and 1665 cm^{-1}; $\lambda_{max.}$ 250 and 314 nm (ε, 16,800 and 19,100); τ (C_5D_5N) 1·64 and 3·34 (each 1H, d, J 16 Hz, *trans*-CH=CH–CO), 5·53 (2H, s, CH_2·CO), 6·21 and 6·25 (each 3H, s, 2 × CO_2Me), and 7·53 (3H, s, Me).

This acid in methanol was treated with an excess of ethereal diazomethane to give the *methyl ester* (42), m.p. 143—145° [from dichloromethane–light petroleum (b.p. 40—60°)] (Found: C, 56·9; H, 5·9; N, 4·8%; M^+, 295. $C_{14}H_{17}NO_6$ requires C, 56·9; H, 5·8; N, 4·7%; M, 295).

Similar methylation of the material in mother liquor *A* and chromatography of the product gave *methyl 4-(2-methoxycarbonylvinyl)-3,5-dimethylpyrrole-2-carboxylate* (43), m.p. 183—184° (from chloroform–hexane) (Found: C, 61·1; H, 6·2; N, 5·7%; M^+, 237. $C_{12}H_{15}NO_4$ requires C, 60·8; H, 6·4; N, 5·9%; M, 237); $\nu_{max.}$ 3290, 1730, and 1680 cm^{-1}; $\lambda_{max.}$ 261 and 320 nm (ε 17,900 and 19,900); τ 0·58 (1H, s, NH), 2·32 and 3·96 (each 1H, d, J 16 Hz, *trans*-CH=CH·CO), 6·20 and 6·27 (each 3H, s, 2 × OMe), and 7·61 and 7·65 (each 3H, s, 2 × Me). Catalytic hydrogenation of this compound as below gave methyl 4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate, m.p. 105—106° (lit.,[6] 108°).

Methyl 4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (44).—The unsaturated ester (42) (87 mg) in ethanol (10 ml) was hydrogenated over 10% palladised charcoal (50 mg). When hydrogen uptake had ceased, the solution was filtered and evaporated to afford the *triester* (85 mg, 98%), m.p. 82—83° (from ether–hexane) (Found: C, 56·7; H, 6·4; N, 4·5%; M^+, 297. $C_{14}H_{19}NO_6$ requires C, 56·6; H, 6·4; N, 4·7%; M, 297);

$\nu_{max.}$ 3290, 1730, 1690, and 1680 cm^{-1}; $\lambda_{max.}$ 283 nm (ε 9000); τ 1·05 (1H, s, NH), 6·18 (2H, s, CH_2·CO), 6·19, 6·30, and 6·34 (each 3H, s, 3 × OMe), 7·42 (4H, m, $[CH_2]_2$·CO), and 7·76 (3H, s, Me).

Benzyl 4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethyl-5-methylpyrrole-2-carboxylate (50) (with J. F. Beck).—The triester (44) (669 mg) in benzyl alcohol (40 ml) containing sodium hydride (1·0 g of oily dispersion) was stirred at 70° ± 4° for 7 h, kept overnight at 20°, and added to N-sulphuric acid (12 ml). The solution was shaken with ether (100 ml), water (15 ml), and enough solid potassium carbonate to basify the aqueous layer. Acidification of the latter gave the *benzyl ester* (597 mg, 72%), m.p. 218—221° (from methanol) (Found: C, 62·4; H, 5·55; N, 4·1%; M^+, 345. $C_{18}H_{19}NO_6$ requires C, 62·6; H, 5·55; N, 4·1%; *M*, 345), $\nu_{max.}$ (Nujol) 3290, 2850br, and 1675br cm^{-1}; $\lambda_{max.}$ 286 nm (ε 18,800); τ (C_5D_5N) −2·37 (2H, 2 × CO_2H), 2·6 (5H, m, Ph), 4·60 (2H, s, $PhCH_2$·O), 5·53 (2H, s, CH_2·CO), 6·93 (4H, m, $[CH_2]_2$·CO), and 7·67 (Me).

Ethereal diazomethane was added to a suspension of the acid in methanol to give the *benzyl dimethyl ester*, m.p. 113—116° (from chloroform–pentane) (Found: C, 64·2; H, 6·2; N, 3·5%; M^+, 373. $C_{20}H_{23}NO_6$ requires C, 64·3; H, 6·2; N, 3·7%; *M*, 373); $\nu_{max.}$ (Nujol) 3300, 1743, 1723, and 1677 cm^{-1}; $\lambda_{max.}$ 285 nm (ε 19,300); τ 1·10 (1H, s, NH), 2·6 (5H, s, Ph), 4·74 (2H, s, $PhCH_2$·O), 6·19 (2H, s, CH_2·CO), 6·36 and 6·42 (each 3H, s, 2 × OMe), 7·44 (4H, m, $[CH_2]_2$·CO), and 7·79 (3H, s, Me).

5-Methoxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrole-2-carboxylic Acid (45).—The 5-methylpyrrole (44) (374 mg) in anhydrous ether (40 ml) was stirred at 0° for 5 min with sulphuryl chloride (0·27 ml). After 30 min more at 20° the solvent was evaporated off and the residue was treated with boiling aqueous sodium acetate (20 ml); the acidic product from aqueous methanol gave the *acid* (20—89% yield), m.p. 193—195° (from methanol–water) (Found: C, 51·15; H, 5·2; N, 4·2%; M^+, 327. $C_{14}H_{17}NO_8$ requires C, 51·4; H, 5·2; N, 4·3%; *M*, 327); $\nu_{max.}$ (Nujol) 3300, 1728, 1718, and 1673 cm^{-1}.

The neutral fractions from the poor reactions afforded the *2-carbaldehyde* (46), m.p. 147—148° (from chloroform–hexane) (Found: C, 54·2; H, 5·65; N, 4·4%; M^+, 311. $C_{14}H_{17}NO_7$ requires C, 54·0; H, 5·5; N, 4·5%; *M*, 311); $\nu_{max.}$ (Nujol) 3280, 1765, 1738, 1700, and 1680 cm^{-1}; $\lambda_{max.}$ 233 and 301 nm (ε 13,900 and 19,900); τ 0·14 (1H, s, CHO), 6·18 (2H, s, CH_2·CO), 6·16, 6·33, and 6·39 (each 3H, s, 3 × OMe), and 6·97 and 7·44 (each 2H, t, *J* 7 Hz, CH_2·CH_2·CO).

This aldehyde (46) was oxidised by potassium permanganate in acetone–acetic acid at 20° to afford the acid (45) in 45% yield.

Methyl 5-Iodo-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (47).—The foregoing acid (45) was iodinated as for the pyrrole (39), giving the *5-iodopyrrole* (78%), m.p. 147—148° (from chloroform–cyclohexane) (Found: C, 37·95; H, 4·0; N, 3·4; I, 31·25%; M^+, 409. $C_{13}H_{16}INO_6$ requires C, 38·15; H, 3·9; N, 3·4; I, 31·0%; *M*, 409); $\nu_{max.}$ (Nujol) 3250, 1738, and 1693 cm^{-1}; $\lambda_{max.}$ 279 nm (ε 16,300); τ 0·70 (1H, s, NH), 6·17 (2H, s, CH_2·CO), 6·21, 6·35, and 6·37 (each 3H, s, 3 × OMe), and 7·5 (4H, m, $[CH_2]_2$·CO).

Methyl 4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (48).—The 5-iodopyrrole (47) was hydrogenated as for compound (40) to give the *α-free pyrrole* (65%), m.p. 78—80° (from ether–pentane) (Found: C, 55·1; H, 6·1; N, 4·7%; M^+, 283. $C_{13}H_{17}NO_6$ requires C, 55·1; H, 6·05; N, 4·95%; *M*, 283); $\nu_{max.}$ (Nujol) 3230, 1700, and 1655 cm^{-1}; $\lambda_{max.}$ 273 nm (ε 14,600); τ 0·90br (1H, NH), 3·28 (1H, d, *J* 3 Hz coupled to NH, 5-H), 6·19 (2H, s, CH_2·CO), 6·22, 6·34, and 6·37 (each 3H, s, 3 × OMe), and 7·4 (4H, m, $[CH_2]_2$·CO).

Benzyl 4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (49).—The triester (48) was treated with sodium benzyloxide and the product was esterified as for compound (50) to give the *benzyl dimethyl ester* (49) (33%), m.p. 63·5—66° (from ether–pentane) (Found: C, 63·7; H, 5·9; N, 3·65%; M^+, 359. $C_{19}H_{21}NO_6$ requires C, 63·5; H, 5·9; N, 3·9%; *M*, 359); $\nu_{max.}$ (Nujol) 3230, 1700, and 1655 cm^{-1}; $\lambda_{max.}$ 275 nm (ε 16,700); τ 0·92 (1H, s, NH), 2·64 (5H, m, Ph), 3·29 (1H, d, *J* 3 Hz, 5-H), 4·75 (2H, s, $PhCH_2$·O), 6·18 (2H, s, CH_2·CO), 6·37 and 6·43 (each 3H, s, 2 × OMe), and 7·37 (4H, m, $[CH_2]_2$CO).

Benzyl 4-(2-Methoxycarbonylethyl)-3-methoxycarbonylmethyl-5-[^{14}C]methylpyrrole-2-carboxylate (50).—The pyrrole (49) (44 mg) was added at 0° to a solution of [^{14}C]paraformaldehyde (3·9 mg; 0·56 mCi) in acetic anhydride (1·0 ml), hydriodic acid (1·0 ml), and hypophosphorous acid (0·2 ml) prepared as previously. After the mixture had been kept at 0° for 1·5 h, radioinactive paraformaldehyde (1·5 mg) was added and stirring was continued for 40 min. The mixture then partitioned between aqueous ammonia containing sodium thiosulphate and chloroform, and the residue from the chloroform was purified by p.l.c. in 1 : 1 ethyl acetate–hexane to give the [^{14}C]*methylpyrrole* (32·2 mg; 0·38 mCi; 68%), identical with authentic material prepared earlier.

The first experiments on the preparation of the pyrrole (50) by reductive methylation were carried out under the conditions used for the conversion of (30) into (17). The total product (by n.m.r.) contained (50) and (51) in *ca.* 1 : 1 ratio; they could not be separated by t.l.c. Hydrolysis of the mixture (56 mg) with ethanolic 5% potassium hydroxide (10 ml) at 20° for 15 min gave a mixture of acids which was separated by p.l.c. on silica in 1 : 9 (v/v) methanol–chloroform to give the monocarboxylic propionic acid corresponding to the pyrrole (51), M^+ 447 (required 447). Treatment of this acid in methanol with an excess of diazomethane gave the triester (51), indistinguishable on t.l.c. from the original product (Found: M^+, 461·1825. Calc. for $C_{27}H_{27}NO_6$: *M*, 461·1838); $\nu_{max.}$ 3430, 3230, 2800br, 1710, and 1630 cm^{-1}; τ 0·40 (1H, s, NH), 2·20 (1H, s, olefinic), 2·74 and 2·88 (each 2H, s, Ph), 4·82 (2H, ABq, *J* 12, Hz, O·CH_2Ph), 6·54 (3H, s, OMe), 7·8 (4H, m, $[CH_2]_2$-CO), and 7·83 (3H, s, Me).

Methyl 2-[5-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methoxycarbonylmethylpyrrol-2-yl[^{14}C]methyl]-4,5,6,7-tetrahydro-5-oxo-1H-pyrrolo[2,3-c]pyridine-3-propionate (53).—The foregoing [^{14}C]methylpyrrole was chlorinated by sulphuryl chloride in ether and the product was coupled with the lactam methyl ester (16) in hot acetic acid–sodium acetate as for the isomer (25) to give the [^{14}C]*pyrromethane* (53) (49%), m.p. 152—155° (from ethanol) (*cf.* ref. 6, m.p. 150—153°) (Found: C, 62·7; H, 6·25; N, 7·0%; M^+, 593. $C_{31}H_{35}N_3O_9$ requires C, 62·7; H, 5·95; H, 7·1%; *M*, 593); $\nu_{max.}$ 3310, 1725, and 1655 cm^{-1}; $\lambda_{max.}$ 287 nm (ε 20,400); τ 0·22, 1·23, and 3·24 (each 1H, s, 3 × NH), 2·7 (5H, m, Ph), 4·84 (2H, s, $PhCH_2$·O), 5·69br (2H, s, CH_2·N), 6·13 (2H, s, bridge CH_2), 6·25 (2H, s, CH_2·CO), 6·43, 6·48, and 6·48 (each 3H, s, 3 × OMe), 6·69br (2H, s, CH_2·CO·N), and 7·4 (8H, m, 2 × $[CH_2]_2$·CO).

Methyl 2-[3-(2-*Methoxycarbonylethyl*)-4-*methoxycarbonylmethylpyrrol*-2-*yl*[^{14}C]*methyl*]-4,5,6,7-*tetrahydro*-5-*oxo*-1H-*pyrrolo*[2,3-c]*pyridine*-3-*propionate* (55).—Hydrogenation of the [^{14}C]benzyl ester (53) gave the acid (54), which was directly decarboxylated in cold trifluoroacetic acid, as for the isomer (27). The [^{14}C]*pyrromethane* (40% overall) had m.p. 159·5—161·5° (from methanol) (*cf.* ref. 6) (Found: M^+, 459·1988. $C_{23}H_{29}N_3O_7$ requires M, 459·2005); $\lambda_{max.}$ 230 nm (ε 10,400); τ 1·30, 1·40, and 3·44 (each 1H, s, 3 × NH), 3·44 (1H, s, 2-H), 5·66br (2H, s, CH_2·N), 6·18 (2H, s, bridge CH_2), 6·37, 6·40, and 6·40 (each 3H, s, 3 × OMe), 6·58 (2H, s, CH_2·CO), 6·65br (2H, s, CH_2·CO·N), and 7·4 (8H, m, 2 × $[CH_2]_2$·CO).

Hydrolysis of the Pyrromethane Lactam Esters (27) *and* (55).—These lactam esters can be clearly distinguished by t.l.c. on silica in 1 : 9 (v/v) methanol–chloroform: lactam (27) has the higher R_F value. Hydrolysis was monitored by n.m.r. (*cf.* ref. 19) in the following way. The lactams (20—25 mg) were treated separately in n.m.r. tubes with 2N-sodium deuteroxide in D_2O (0·4 ml) and the mixtures were stirred with a slow stream of nitrogen for 15 min. Six spectra were recorded over the period up to 74 h and a peak gradually appeared at τ 6·02 ± 0·02 corresponding to CH_2·NH_2; this peak had fully formed after 25 h.

Larger quantities of the two lactams were similarly hydrolysed with aqueous 2N-sodium hydroxide at 20° for 48 h. Small amounts of IRC-50 resin (H^+) were added to the solution until the pH fell to 6·8—7·0. The filtered solution and washings were evaporated below 20°, the residue was redissolved in D_2O, and the solution was evaporated. The final residue in D_2O was used for measurement of the n.m.r. spectra; the spectra of the products (6) and (7) were significantly different. The pyrromethane (6) showed δ (p.p.m. from HOD) −1·88 (1H, s, pyrrole α-H), 0·62 (2H, s, CH_2), 0·82 (2H, s, CH_2), 1·26 (4H, s, 2 × CH_2), 2·0 and 2·3 (total 8H, m, 2 × CH_2·CH_2); and (7) showed rapid exchange of pyrrole α-H and δ 0·61 (2H, s, CH_2), 0·80 (2H, s, CH_2), 1·29 and 1·34 (2H each, s, 2 × CH_2), and 2·0 and 2·4 [total 8H, m, 2 × CH_2·CH_2, pattern different from (6)].

5-*Benzyloxypyrrolo*[2,3-c]*pyridine*-2-*carboxylic Acid* (57).—A solution of the ethyl ester (11) (2·96 g) in ethanol (50 ml) was heated under reflux for 2 h with sodium hydroxide (0·6 g) in water (5 ml). The hot solution was diluted with water (50 ml) and partially evaporated (occasionally resulting in the crystallisation of the sodium salt). Acidification (glacial acetic acid) gave the crystalline *acid* (57) (2·68 g, 100%), m.p. 205° (from aqueous acetic acid) (Found: C, 65·2; H, 4·7; N, 10·4%; M^+, 268. $C_{15}H_{12}N_2O_3$,0·5H_2O requires C, 65·0; H, 4·7; N, 10·1%; M, 268); $\nu_{max.}$ (Nujol) 1650, 1700, and 3250 cm^{-1}, $\lambda_{max.}$ 278, 287, and 340 (changing in alkali to 279, 288, and 329 nm)); τ [$(CD_3)_2SO$] 1·48br (1H, s, H-7), 1·94 (1H, s, NH), 3·00 (2H, s, H-3 and H-4), 3·0 (5H, m, Ph), and 4·67 (2H, s, PhCH_2·O).

5-*Benzyloxypyrrolo*[2,3-c]*pyridine*-2-NN-*dimethylcarboxamide* (59).—The sodium salt of the acid (57) (2·67 g) was carefully dried *in vacuo* at 125°, suspended in dry tetrahydrofuran (250 ml), and treated at room temperature with oxalyl chloride (1·0 ml) and dimethylformamide (0·1 ml). After 10 min a second addition of these reagents was made, and after a further 20 min excess of dimethylamine (5·0 ml) was added. The solvents were evaporated off and the residue was washed successively with aqueous sodium hydrogen carbonate, water, and methanol, leaving the crystalline product (1·71 g, 62%) [unchanged acid was recovered from the aqueous washings (0·36 g, 15%)]. The *amide* had m.p. 207·5—208·5° (from aqueous acetic acid) (Found: C, 69·3; H, 5·7; N, 14·7%; M^+, 295. $C_{17}H_{17}N_3O_2$ requires C, 69·1; H, 5·8; N, 14·2; M, 295); $\nu_{max.}$ 1625, 3300, and 3500 cm^{-1}; $\lambda_{max.}$ 281 and 325 nm; τ 0·06br (1H, s, NH), 1·45br (1H, s, H-7), 2·6 (5H, m, Ph), 3·02 and 3·32 (each 1H, s, H-3 and H-4), 4·62 (2H, s, PhCH_2·O−), and 6·7br (6H, s, NMe_2).

5-*Benzyloxy*-3-*dimethylaminomethylpyrrolo*[2,3-c]*pyridine*-2-NN-*dimethylcarboxamide* (60).—Paraformaldehyde (0·14 g) was added to a hot solution of the amide (59) (0·89 g) and dimethylamine hydrochloride (0·97 g) in isopropyl alcohol (90 ml). The mixture was heated under reflux for 4 h, the solvent was evaporated off, and the residue was partitioned between ether and aqueous buffer (pH 4). The aqueous layer was made alkaline (solid sodium carbonate) and extracted with 3 : 1 ether–chloroform to give the Mannich base (0·69 g, 82%). Treatment with ethanolic hydrogen chloride gave the crystalline *dihydrochloride*, m.p. 120—122° (from acetonitrile) (Found: C, 51·6; H, 6·4; N, 12·1%; M^+, 352. $C_{20}H_{24}N_4O_2$,2HCl,2H_2O requires C, 52·05; H, 6·55; N, 12·1%; M, 352); $\nu_{max.}$ (Nujol) 1650 and 3300 cm^{-1}, $\lambda_{max.}$ 282 and 324 nm; τ [$(CD_3)_2SO$] 1·18 (1H, s, H-7), 1·56 (1H, s, H-4), 2·5 (5H, m, Ph), 4·46 (2H, s, PhCH_2·O), 5·43 (2H, s, CH_2·N), 6·97 (6H, s, CO·NMe_2), and 7·26 (6H, s, CH_2·NMe_2).

Evaporation of the ethereal extract gave the oily isopropyl ether (61) (120 mg, 14%) (Found: M^+, 367. Calc. for $C_{21}H_{25}N_3O_3$: M, 367); $\nu_{max.}$ 1630 and 3400 cm^{-1}; $\lambda_{max.}$ 279 and 322 nm; τ 1·31 (1H, s, NH), 1·8br (1H, s, H-7), 2·7 (5H, m, Ph), 3·01 (1H, s, H-4), 4·82 (2H, s, PhCH_2·O), 5·50 (2H, s, CH_2·O), 7·10 (6H, s, CO·NMe_2), and 6·45 (1H, septet) and 8·97 (6H, d) (each J 6 Hz, O·$CHMe_2$).

Diethyl (5-*Benzyloxy*-2-*dimethylcarbamoylpyrrolo*[2,3-c]*pyridin*-3-*yl*)*methylmalonate* (62).—The crystalline hydrochloride of the foregoing Mannich base (0·64 g) was added to a solution of sodium (0·16 g) in diethyl malonate (6 ml). After 1 h at 110° the mixture was cooled and shaken with ether (10 ml) and 2N-hydrochloric acid (10 ml) to give the crystalline *hydrochloride* (0·62 g, 82%), m.p. 107—111° [Found (after drying at room temperature): C, 52·0; H, 5·7; N, 7·3%; M^+, 467. $C_{25}H_{29}N_3O_6$,2HCl,2H_2O requires C, 52·1; H, 6·1; N, 7·3%; M, 467); $\nu_{max.}$ (Nujol) 1635, 1725, and 3450 cm^{-1}; $\lambda_{max.}$ 283 and 327 nm; τ (CD_3·OD) 1·30 (1H, s, H-7), 2·30 (1H, s, H-4), 2·5 (5H, m, Ph), 4·52 (2H, s, PhCH_2·O), 5·93 (4H, q) and 8·88 (6H, t) (each J 7 Hz, 2 × OEt), 6·17 (1H, t) and 6·64 (2H, d) (each J 8 Hz, CH·CH_2), and 6·86 and 7·01 (each 3H, s, NMe_2).

(5-*Benzyloxy*-2-*dimethylcarbamoylpyrrolo*[2,3-c]*pyridin*-3-*yl*)*methylmalonic Acid* (63).—The corresponding diethyl ester (0·15 g) dissolved in ethanol (1·5 ml) was heated under reflux for 1 h with potassium hydroxide (95 mg) in water (0·5 ml). The residue obtained after evaporation was redissolved in water (2 ml) and acidification by acetic acid gave the crystalline *malonic acid* (88 mg, 71%), m.p. 124—125·5° (from aqueous acetic acid) (Found: C, 58·9; H, 5·1; N, 9·95%; M^+, 411. $C_{21}H_{21}N_3O_6$,H_2O requires C, 58·7; H, 5·4; N, 9·8%; M, 411); $\nu_{max.}$ 1650, 1708, and 3300 cm^{-1}; $\lambda_{max.}$ 281 and 325 nm; τ (CD_3·OD) 1·6br (1H, s, H-7), 2·6 (5H, m, Ph), 2·82 (1H, s, H-4), 4·74 (2H, s, PhCH_2·O), 6·66 (2H, s, CH_2), and 6·98 (6H, s, CO·NMe_2).

Ethyl (5-*Benzyloxy*-2-*dimethylcarbamoylpyrrolo*[2,3-c]*pyridin*-3-*yl*)*propionate* (64).—A solution of the malonic acid (63) (0·41 g) in pyridine (2 ml) was heated at 125° for 2 h. Evaporation gave the *propionic acid* (271 mg, 74%), m.p. 205—210° (from methanol); $\nu_{max.}$ (Nujol) 1615, 1705,

and 3250 cm^{-1}; $\lambda_{max.}$ 281 and 325 nm; τ (C_5D_5N) 1·32 (1H, s, H-7), 2·4 (5H, m, Ph), 2·82 (1H, s, H-4), 4·38 (2H, s, PhCH_2·O), 6·63 and 7·07 (each 2H, t, J 8 Hz, [CH_2]$_2$·CO), and 7·14 (6H, s, CO·NMe_2).

Treatment of this acid with diazoethane in ether–methanol gave the *ethyl ester*, m.p. 113·5—114·5° (from ethanol) (Found: C, 67·1; H, 6·4; N, 10·2%; M^+, 395. $C_{22}H_{25}N_3O_4$ requires C, 66·8; H, 6·4; N, 10·6%; M, 395); $\nu_{max.}$ 1630, 1720, and 3400 cm^{-1}; $\lambda_{max.}$ 281 and 326 nm; τ 1·62 (1H, s, H-7), 2·6 (5H, m, Ph), 3·06 (1H, s, H-4), 4·62 (2H, s, PhCH_2·O), 5·94 (2H, q) and 8·82 (3H, t) (each J 7 Hz, OEt), 6·84 and 7·40 (each 2H, t, J 7 Hz, CH_2·CH_2·CO), and 7·00 (6H, s, CO·NMe_2).

5-Benzyloxypyrrolo[2,3-c]*pyridin-2-yl 4-Ethoxycarbonyl-3,5-dimethylpyrrol-2-yl Ketone* (66).—A solution of the amide (59) (29 mg) and ethyl 2,4-dimethylpyrrole-3-carboxylate (16 mg) in 1,2-dichloroethane (0·15 ml) was heated under reflux for 4 h with phosphoryl chloride (0·2 ml). The presence of intermediate (65) was indicated by $\lambda_{max.}$ 292, 314, and 400 nm. The solvent was evaporated off and the residue heated for 90 min with potassium carbonate (0·1 g) in water (5 ml). Extraction of the cold mixture with chloroform, and chromatography of the extract on neutral alumina (Woelm grade III; 5 g) gave the *ketone* (42 mg, 100%), eluted by 10% methanol–chloroform, m.p. 134·5—135·5° (from methanol) (Found: C, 68·6; H, 5·6; N, 9·9%; M^+, 417. $C_{24}H_{23}N_3O_4$ requires C, 69·05; H, 5·55; N, 10·1%; M, 417); $\nu_{max.}$ 1570, 1695, 3300, and 3450 cm^{-1}; $\lambda_{max.}$ 297 and 341 nm; τ 1·50 (1H, s, H-7), 2·6 (5H, m, Ph), 3·08 and 3·14 (each 1H, s, H-3 and H-4), 4·67 (2H, s, PhCH_2·O), 5·70 (2H, q) and 8·65 (3H, t) (each J 7 Hz, OEt), and 7·43 (6H, s, 2 × Me).

5-Benzyloxypyrrolo[2,3-c]*pyridin-2-ylmethanol*.—A solution of the ethyl ester (11) (0·94 g) in dry tetrahydrofuran (12 ml) was added to a stirred suspension of lithium aluminium hydride (0·24 g) in the same solvent (3 ml) at such a rate that the solvent boiled gently under reflux. After 1 h the excess of hydride was destroyed by careful addition of 2N-sodium hydroxide and the mixture was diluted with chloroform and filtered through Celite. The filtrate was washed with water and evaporated to give the *alcohol* (0·52 g, 65%), m.p. 137—138·5° (from ethyl acetate) (Found: C, 71·1; H, 5·7; N, 11·2%; M^+, 254. $C_{15}H_{14}N_2O_2$ requires C, 70·85; H, 5·55; N, 11·0%; M, 254); $\nu_{max.}$ 1625 and 3400 cm^{-1}; $\lambda_{max.}$ 276 and 313 nm; τ (CD_3·OD) 1·76 (1H, s, H-7), 2·6 (5H, m, Ph), 3·12 (1H, s, H-4), 3·72 (1H, s, H-3), 4·80 (2H, s, PhCH_2·O), and 5·30 (2H, s, CH_2·O).

5-Benzyloxypyrrolo[2,3-c]*pyridine-2-carbaldehyde* (67).—A solution of the foregoing alcohol (127 mg) in methanol (1 ml) and dichloromethane (14 ml) was heated under reflux for 5 h with a large excess of manganese dioxide. Filtration and evaporation gave the amorphous *aldehyde* (122 mg, 96%), which was crystallised from aqueous methanol to give needles, m.p. 155° (Found: C, 71·4; H, 4·9; N, 10·9%; M^+, 252. $C_{15}H_{12}N_2O_2$ requires C, 71·4; H, 4·8; N, 11·1%; M, 252); $\nu_{max.}$ 1625, 1675, and 3400 cm^{-1}; $\lambda_{max.}$ 290, 300, and 324 nm; τ 0·03 (1H, s, CHO), 1·4 (1H, s, NH), 1·6 (1H, s, H-7), 2·6 (5H, m, Ph), 2·85 (2H, s, H-3 and H-4), and 4·64 (2H, s, PhCH_2·O).

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