Biosynthesis of Porphyrins and Related Macrocycles. Part IV.[1] Syntheses of Isomeric Aminomethylpyrromethanes for Biosynthetic Study

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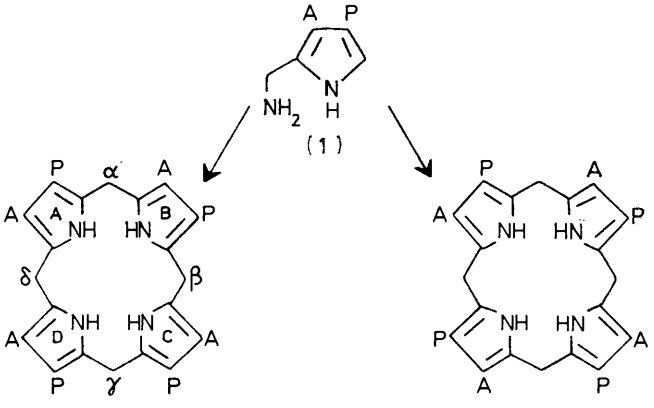
The aminomethylpyrromethanes (6) and (7) were required for biosynthetic studies on haem, protoporphyrin-IX and vitamin B_{12}; rational syntheses of both from methyl (1,4,5,6,7,8-hexahydro-6-oxopyrrolo[2,3-*c*]azepin-3-yl)-acetate (10) are described. A key stage in the synthesis of the lactam (10) is the ring closure of the amino-ester (26) catalysed by 2-pyridone. The pyrromethane lactams (8) and (9), derived by several stages from the lactam (10) were hydrolysed to yield the required aminomethylpyrromethanes, which were characterised by n.m.r. spectroscopy.

THE natural porphyrins and chlorins, *e.g.* haem, chlorophylls a and b, and the cytochromes, are all derived from the type-III macrocycle [see (2)] in which the substituents on ring D are, surprisingly, of reversed sequence. The macrocycle (2) is constructed in nature from four units

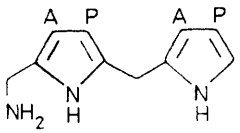


of porphobilinogen (1) without loss of carbon;[2] straightforward head-to-tail assembly would lead to the type-I isomer (3). Research in Cambridge[3,4] has recently established that the rearrangement process which generates uroporphyrinogen-III (2) is characterised by the following features: (*a*) only the porphobilinogen unit which forms ring D [see (2)] undergoes rearrangement; (*b*) this rearrangement is *intramolecular* with respect to the porphobilinogen unit forming ring D and the migrating carbon atom provides the *γ-meso* bridge of the porphyrinogen; and (*c*) the porphobilinogen units forming rings A, B, and C of (2) and their attached carbon atoms are built *intact* into the macrocycle.

These facts put strict limitations on the reaction sequences which can be considered for the rearrangement process, and few of the hypothetical schemes[5] now survive. Further progress, particularly towards pinpointing the stage at which rearrangement occurs, depends upon studies of intermediates between porphobilinogen (1) and uroporphyrinogen-III (2). To this end, unambiguous syntheses of the aminomethylpyrromethanes (4) and (5) were devised in our laboratory[6] and elsewhere.[7,8] Chemical and enzymic experiments in various systems with the pyrromethanes (4) and (5) have

[1] Part III, A. R. Battersby, G. L. Hodgson, M. Ihara, E. McDonald, and J. Saunders, *J.C.S. Perkin I*, 1973, 2923.
[2] B. F. Burnham, in 'Metabolic Pathways,' ed. D. M. Greenberg, Academic Press, New York, 1969, vol. III, 3rd edn., p. 403.
[3] A. R. Battersby, G. L. Hodgson, M. Ihara, E. McDonald, and J. Saunders, *J.C.S. Chem. Comm.*, 1973, 441.
[4] A. R. Battersby, E. Hunt, and E. McDonald, *J.C.S. Chem. Comm.*, 1973, 442.
[5] Leading references to over 20 schemes in J. H. Mathewson and A. H. Corwin, *J. Amer. Chem. Soc.*, 1961, **83**, 135; E. B. C. Llambias and A. M. del C. Battle, *Biochem. J.*, 1971, **121**, 327; R. Radmer and L. Bogorad, *Biochemistry*, 1972, **11**, 904.
[6] A. R. Battersby, D. A. Evans, K. H. Gibson, E. McDonald, and L. Nixon, *J.C.S. Perkin I*, 1973, 1546.
[7] B. Frydman, S. Reil, A. Valasinas, R. B. Frydman, and H. Rapoport, *J. Amer. Chem. Soc.*, 1971, **93**, 2738.
[8] 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.

been described.[7,9] We now report rational syntheses of the aminomethylpyrromethanes (6) and (7).



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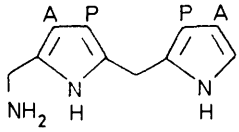

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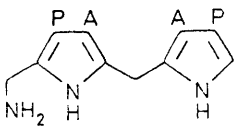

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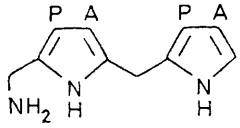
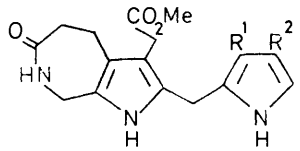


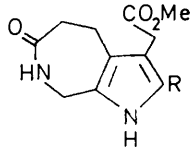
(7)

A=$CH_2{\cdot}CO_2H$; P=$CH_2{\cdot}CH_2{\cdot}CO_2H$



(8) R^1=$CH_2{\cdot}CO_2Me$, R^2=$CH_2{\cdot}CH_2{\cdot}CO_2Me$

(9) R^1=$CH_2{\cdot}CH_2{\cdot}CO_2Me$, R^2=$CH_2{\cdot}CO_2Me$



(10) R=H
(11) R=CO_2Me
(12) R=$CO_2{\cdot}CH_2Ph$

As in our earlier work [6] on the pyrromethanes (4) and (5), the aim was to synthesise the lactams (8) and (9) from which it was expected that the required compounds (6) and (7) could be derived in one mild step (alkaline hydrolysis). Accordingly, the lactam (10) became the first synthetic target.

Sulphuryl chloride converted the pyrrole [6] (17) into the corresponding chloromethyl derivative, which smoothly yielded the azide (18) on treatment with sodium azide. Hydrogenation of the azide in acidic medium afforded the amine hydrochloride (19) and this was hydrolysed to give the known [7] amino-acid (22). This did not yield the desired lactam on treatment with acetic anhydride in aqueous acetic acid [10] but gave the *N*-acetyl derivative, which was identified spectroscopically as its trimethyl ester (21).

Attention was therefore turned to alternative methods for protection of the amino-residue and the *N*-formyl group seemed to meet our requirements. Thus the *N*-formyl ester (20) was hydrolysed to the amino-acid under mild alkaline conditions but was unaffected by sodium acetate in hot acetic acid, the conditions used for pyrromethane formation. Formic acetic anhydride [11] converted the amino-acid (22) into its *N*-formyl derivative (23), which was decarboxylated in boiling water, and the product was esterified to yield the *N*-formyl ester (24).

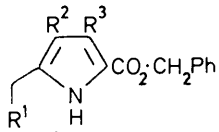
[9] (*a*) A. R. Battersby, *23rd International Congress of Pure and Applied Chemistry, Special Lectures*, 1971, vol. 5, p. 1; (*b*) J. Pluscec and L. Bogorad, *Biochemistry*, 1970, **9**, 4736; (*c*) R. B. Frydman, A. Valasinas, and B. Frydman, *Biochemistry*, 1973, **12**, 80; (*d*) A. R. Battersby, K. H. Gibson, E. McDonald, L. N. Mander, J. Moron, and L. N. Nixon, *J.C.S. Chem. Comm.*, 1973, 768.

[10] A. H. Jackson and S. F. MacDonald, *Canad. J. Chem.*, 1957, **35**, 715.

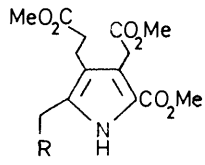
This was sufficiently stable for spectroscopic characterisation but the rapidity with which it decomposed rendered it valueless, and the lactam (10) even more attractive, for the main synthesis.

Attempts to cyclise the amino-acid (22) with *NN*-dicyclohexylcarbodi-imide or thermally gave only traces of lactam. However, when the amino-ester derived from the salt (19) was heated in dioxan with 2-pyridone as catalyst,[12] the lactam (11) was formed in 82% yield. The amino-acid (22) was therefore converted first into its dimethyl ester [7] (25), and this was treated with phenyldiazomethane [13] to afford the benzyl ester (26). The lactam (12) was then obtained in 71% yield by ring closure catalysed by 2-pyridone and was further transformed by hydrogenolysis and decarboxylation of the resultant acid. In this way the key building block (10) was obtained for construction of the pyrromethanes (8) and (9).

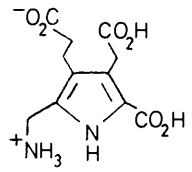
Alkylation of the lactam (10) with the chloromethylpyrrole (15) prepared from pyrrole (13) as earlier [6] gave



(13) R^1=H, R^2=$CH_2{\cdot}CO_2Me$, R^3=$CH_2{\cdot}CH_2{\cdot}CO_2Me$
(14) R^1=H, R^2=$CH_2{\cdot}CH_2{\cdot}CO_2Me$, R^3=$CH_2{\cdot}CO_2Me$
(15) R^1=Cl, R^2=$CH_2{\cdot}CO_2Me$, R^3=$CH_2{\cdot}CH_2{\cdot}CO_2Me$
(16) R^1=Cl, R^2=$CH_2{\cdot}CH_2{\cdot}CO_2Me$, R^3=$CH_2{\cdot}CO_2Me$



(17) R=H
(18) R=N_3
(19) R=$\overset{+}{N}H_3$ Cl^-
(20) R=NH·CHO
(21) R=NH·COMe



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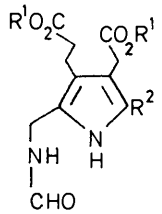
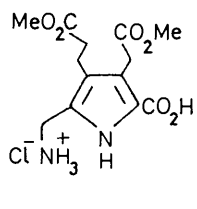


(23) R^1=H, R^2=CO_2H
(24) R^1=Me, R^2=H



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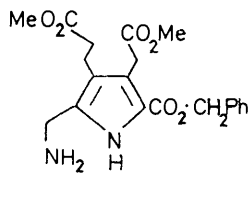


(26)

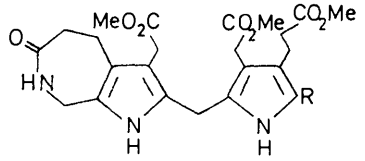
the crystalline pyrromethane (27), from which the *O*-benzyl group was removed by hydrogenolysis. Decarboxylation of the acid (28) in cold trifluoroacetic acid then

[11] T. Muramatsu, M. Murakami, T. Yoneda, and A. Hagitani, *Bull. Chem. Soc. Japan*, 1965, **38**, 244.

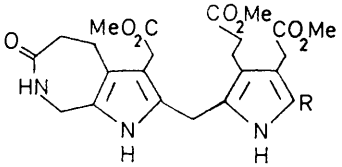
[12] H. C. Beyerman and W. Maassen van den Brink, *Proc. Chem. Soc.*, 1963, 266; H. T. Openshaw and N. Whittaker, *J. Chem. Soc.* (*C*), 1969, 89.

[13] C. G. Overberger and J. P. Anselme, *J. Org. Chem.*, 1963, **28**, 592.

afforded the α-free pyrromethane (8) as a moderately stable crystalline product.



(27) R=$CO_2 \cdot CH_2Ph$
(28) R=CO_2H



(29) R=$CO_2 \cdot CH_2Ph$
(30) R=CO_2H

The benzyl ester (29), synthesised in a similar way from the lactam (10) and the chloromethylpyrrole (16), was stable to careful handling. However, the α-free pyrromethane (9), prepared from it by hydrogenation and decarboxylation of the resultant acid (30), proved to be very labile. Special precautions against decomposition by air, light, and heat are necessary for its preservation; it was further shown during these studies that the lactams (8) and (9) are separable by t.l.c.

Alkaline hydrolysis of the simple lactam (10) occurred smoothly and the n.m.r. spectrum of the resultant potassium salt showed the hydrolysis to be complete in 24 h (or less) and that no decomposition had occurred after 70 h. Accordingly, hydrolysis under these conditions for 48 h was used for the two lactams (8) and (9) to generate the aminomethylpyrromethanes (6) and (7). These unstable substances were characterised as their potassium salts by ^{1}H n.m.r. (Fourier transform) spectroscopy [see the Table, which includes measurements

^{1}H Fourier transform n.m.r. spectra of aminomethylpyrromethanes (τ values) [a]

	Pyrromethanes			
Group	(4)	(5)	(6)	(7)
pyrr*H*	3·43	3·42 [b]	3·44	3·42
$H_2N \cdot CH_2$	5·94	5·90	5·89	5·88
$(pyrr)_2CH_2$	6·14	6·09	6·18	6·14
pyrrCH_2·CO	6·58	6·58	6·57	6·57
pyrrCH_2·CO	6·58	6·63	6·57	6·63
pyrr$CH_2 \cdot CH_2$·CO	7·28	7·29	7·33	7·31
pyrr$CH_2 \cdot CH_2$·CO	7·56	7·71	7·56	7·64

[a] Solutions of potassium salts in D_2O (see Experimental section for details); 0·02M-solution for (4) and (5), 0·05M-solution for (6) and (7). Spectral width 1000 Hz, acquisition time 2 s, pulse width 30 μs, 1000—2000 transients. [b] This signal was not observed in earlier spectra [6] collected by CAT on the HA-100 spectrometer.

under equivalent conditions on the isomeric aminomethylpyrromethanes (4) and (5)]. The spectrum of the pyrromethane (4) differs markedly from that of (5); similarly, the spectrum of the pyrromethane (6) is obviously different from that of (7). However, the spectra of the pair (4) and (6) are clearly distinguishable only when they are sharp and of good quality. The same holds true for the pair (5) and (7). This is of importance for current and future work on the isolation of biosynthetic intermediates.

The foregoing syntheses allow the preparation of specifically labelled forms of the pyrromethanes (6) and (7) since the pyrroles (13) and (14) are available [6] carrying ^{14}C at the chloromethyl group and in the side chains.

Enzymic work with the pyrromethane (6) has been reported in preliminary form [14] but the synthesis used has not been published. Our own research, both chemical and enzymic, with the pyrromethanes (6) and (7) will be published when the essential experiments are completed to prove specificity and site(s) of labelling in the derived porphyrins.

EXPERIMENTAL

The general directions in Part I [6] were followed with the following modifications and additions. T.l.c. was also carried out on plates coated with Reeve Angel Scientific silica gel/CT and on Merck plates precoated with aluminium oxide F_{254} (type T); i.r. spectra were determined for Nujol mulls; reported τ values for n.m.r. multiplets refer to the apparent centres of these signals.

Methyl 5-Azidomethyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (18).—The 5-methylpyrrole (17) (1 g) in anhydrous ether (50 ml) was stirred at 0° under nitrogen for 5 min with distilled sulphuryl chloride (490 mg). After a further 15 min at 20°, the solvent was evaporated off, and anhydrous ether (3 × 50 ml) was added and evaporated off to remove sulphuryl chloride; the dry residue showed a new n.m.r. signal τ 5·39 (2H, s, CH_2Cl) and absence of the original *C*-methyl signal (τ 7·70).

This product was immediately treated in acetone (15 ml) with aqueous sodium azide (453 mg in 7·5 ml) and the solution was stirred at 20° under nitrogen for 30 min. The acetone was evaporated off and the mixture was diluted with water (40 ml) and extracted with methylene chloride (3 × 50 ml) to give the crystalline *azide* (1·03 g, 90%) sufficiently pure for the next stage. Recrystallised from ether–pentane, the product had m.p. 53·5—55·5° (Found: C, 49·9; H, 5·3; N, 16·6%; M^+, 338. $C_{14}H_{18}N_4O_6$ requires C, 49·7; H, 5·4; N, 16·6%; *M*, 338); $\nu_{max.}$ 3270, 2090, 1728, and 1668 cm^{-1}; $\lambda_{max.}$ 276 nm (ε 15,600); τ 0·71 (1H, s, NH), 5·67 (2H, s, CH_2N_3), 6·19 (5H, s, OMe and pyrrCH_2·CO), 6·32 and 6·36 (each 3H, s, 2 × OMe), and 7·39 (4H, m, pyrr$[CH_2]_2$·CO).

Methyl 5-Aminomethyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate Hydrochloride (19).—The azide (18) (553 mg) in methanol (40 ml) containing conc. hydrochloric acid (0·34 ml) was shaken with 10% palladised charcoal (50 mg) and hydrogen at *ca.* 20° and 760 mmHg. The reduction was followed to completion by t.l.c.; the solution was then filtered (Celite) and evaporated. The dried residue was washed with ether (2 × 5 ml) and crystallised from methanol–ether to yield the *hydrochloride* (503 mg, 88%), m.p. 170—172° (Found: C, 48·3; H, 6·05; N, 8·3%; M^+, 348. $C_{14}H_{21}ClN_2O_6$ requires C, 48·3; H, 6·05; N, 8·05%; *M*, 348); $\nu_{max.}$ 3150, 2780, 2710, 2675, 2570, 1725, and 1683 cm^{-1}; $\lambda_{max.}$ 277 nm (ε 15,000); τ (D_2O) 5·80 (2H, s, pyrrCH_2·N), 6·25 and 6·35 (each 3H, s, 2 × OMe), 6·44 (5H, s, OMe and pyrrCH_2·CO), and 7·43 (4H, m, pyrr$[CH_2]_2$·CO).

[14] R. B. Frydman, A. Valasinas, and B. Frydman, *FEBS Letters*, 1972, **25**, 309.

5-Aminomethyl-4-(2-carboxyethyl)-3-carboxymethylpyrrole-2-carboxylic Acid (22).—A stirred solution of the amine hydrochloride (19) (0·5 g) in aqueous 2N-potassium hydroxide (7·5 ml) was heated at 90° for 30 min with a stream of nitrogen passing, which caused evaporation to 5 ml. This solution at 0° was adjusted to pH 5 with sulphur dioxide and after 1 h at 0° the *solid* was collected, washed with water (1 ml) and ether (10 ml), and dried (360 mg, 93%); m.p. 170—180° (decomp.) (*cf.* ref. 7) (Found: C, 47·5; H, 5·15; N, 10·1. $C_{11}H_{14}N_2O_6,0{\cdot}5H_2O$ requires C, 47·3; H, 5·4; N, 10·0%); $\nu_{max.}$ 3270, 1715br, and 1555br cm^{-1}; $\lambda_{max.}$ 265 and 224 nm; τ (2N-KOH in D_2O) 6·40 (2H, s, pyrr-CH_2·N), 6·43 (2H, s, pyrrCH_2·CO), and 7·52 and 7·86 (each 2H, m, pyrr$[CH_2]_2$·CO).

Attempted Lactam Formation from the Amino-acid (22).—A stirred suspension of the amino-acid (22) (69 mg) in pyridine (0·7 ml) and water (2·0 ml) under nitrogen at 0° was treated dropwise with ammonium hydroxide. When a clear solution had been obtained, acetic acid (0·35 ml) and acetic anhydride (0·35 ml) were added dropwise during 20 min and the mixture was kept at 0° for 23 h. The residue obtained by evaporation was treated in methanol (5 ml) with an excess of ethereal diazomethane and the major product (R_F 0·5) was isolated by p.l.c. on silica in 9 : 1 chloroform–methanol; it showed τ 5·67 (2H, m, pyrrCH_2·N), 6·22 (5H, s, pyrrCH_2·CO and OMe), 6·32 and 6·39 (each 3H, s, 2 × OMe), 7·32 (4H, m, pyrr$[CH_2]_2$·CO), and 8·02 (3H, s, NAc). This product was identical (t.l.c. and n.m.r.) with that obtained by acetylation of the amino-ester derived from the salt (19).

Methyl 5-Formylaminomethyl-4-(2-methoxycarbonylethyl)-3-methoxycarbonylmethylpyrrole-2-carboxylate (20).—A solution of the hydrochloride (19) (104 mg) in saturated aqueous sodium carbonate (3 ml) was extracted with methylene chloride (4 × 4 ml). The organic phase was washed with brine (8 ml), dried (NaCl), and evaporated. The residue (93 mg) in anhydrous tetrahydrofuran (3 ml) was treated under nitrogen at 0° dropwise during 15 min with a mixture (0·72 ml) of acetic anhydride (0·93 ml, 9·8 mmol) and formic acid (0·37 ml, 9·8 mmol). After a further 1 h, the solution was added at 0° to stirred saturated sodium carbonate solution (5 ml). The organic solvents were evaporated off and the aqueous residue was extracted with methylene chloride (3 × 5 ml) to yield an oil which by p.l.c. on silica in ethyl acetate gave the *N-formyl derivative* (87 mg, 85%), m.p. 97—98·5° (from ethyl acetate–ether) (Found: C, 52·85; H, 5·95; N, 8·05%; M^+, 340. $C_{15}H_{20}N_2O_7$ requires C, 52·9; H, 5·9; N, 8·2%; *M*, 340); $\nu_{max.}$ 3350, 3280, 1735, 1680, and 1650 cm^{-1}; $\lambda_{max.}$ 277 and 248sh nm; τ 0·36br (1H, s, pyrrNH), 1·81 (1H, s, N·CHO), 3·27br (1H, s, HN·CO), 5·57 (2H, m, pyrrCH_2·N), 6·22 (5H, s, OMe and pyrrCH_2CO), 6·32 and 6·39 (each 3H, s, 2 × OMe), and 7·37 (4H, m, pyrr$[CH_2]_2$·CO).

Study of Formylation of the Amino-acid (22) *and Decarboxylation of the Product.*—A suspension of the amino-acid (22) (0·5 g) in triethylamine (6 ml) was vigorously stirred at 20° as methanol (25 ml) and chloroform (25 ml) were added. The virtually clear solution was evaporated and the residue in chloroform (50 ml) was treated at 0° under nitrogen with formic acetic anhydride [11] in portions (0·3 ml) at 5 min intervals. After 6·6 ml of anhydride had been added, the mixture was stirred for 10 min then treated with methanol (10 ml) and stirred for 10 min. The residue from evaporation was dried under high vacuum for 48 h and triturated with acetone and ether to give the hygroscopic *N*-formyl derivative (23) (444 mg, 80%), which was stable *in vacuo*. It was identified by methylation (diazomethane) to yield a product identical with the foregoing trimethyl ester (20).

A solution of the *N*-formyl acid (23) (50 mg) in water (5 ml) was heated vigorously under reflux (bath 120—125°) in nitrogen for 2·25 h; the decarboxylation was followed by the decrease in absorption at 273 nm. The residue from evaporation was treated in methanol (10 ml) with an excess of distilled ethereal diazomethane for 1 h. The product was fractionated by p.l.c. on silica in ethyl acetate to yield an unstable solid (8 mg), τ 1·30br (1H, s, pyrrNH), 1·86 (1H, s, N·CHO), 3·45br (2H, m, pyrrH and HNCO), 5·65 (2H, m, pyrrCH_2·N), 6·35 and 6·42 (each 3H, s, 2 × OMe), 6·60 (2H, s, pyrrCH_2·CO), and 7·37 (4H, m, pyrr$[CH_2]_2$·CO).

Methyl 1,4,5,6,7,8-Hexahydro-3-methoxycarbonylmethyl-6-oxopyrrolo[2,3-c]azepine-2-carboxylate (11).—The amino-triester (48 mg), recovered as above from the salt (19) (56 mg), was heated under reflux in anhydrous dioxan (N_2) (25 ml) containing 2-pyridone [12] (50 mg) for 26 h. The residue from evaporation was purified by p.l.c. on silica in 3 : 7 methanol–ethyl acetate to give the *lactam* (35·2 mg, 81%), m.p. 211—212° (from methanol–ether) (Found: C, 55·6; H, 5·7; N, 10·05%; M^+, 280. $C_{13}H_{16}N_2O_5$ requires C, 55·7; H, 5·75; N, 10·0%; *M*, 280); $\nu_{max.}$ 3290, 1735, 1685, and 1662 cm^{-1}; $\lambda_{max.}$ 277 nm; τ (CD_3OD) 5·67 (2H, s, pyrr-CH_2·N), 6·23 (5H, s, OMe and pyrrCH_2·CO), 6·36 (3H, s, OMe), and 7·24 (4H, s, pyrr$[CH_2]_2$·CO).

Benzyl 1,4,5,6,7,8-Hexahydro-3-methoxycarbonylmethyl-6-oxopyrrolo[2,3-c]azepine-2-carboxylate (12).—The amino-acid (22) was converted essentially as in ref. 7 into the dimethyl ester hydrochloride (25) (64%), τ (CD_3OD) 5·78 (2H, s, pyrrCH_2·N), 6·18 (2H, s, pyrrCH_2·CO), 6·36 and 6·37 (each 3H, s, 2 × OMe), and 7·32 (4H, m, pyrr$[CH_2]_2$·CO). This product (431 mg) was treated at 20° in methanol (40 ml) with a slight excess of ethereal phenyldiazomethane [13] and when the reaction was complete (t.l.c.) the excess of reagent was destroyed with acetic acid. The residue from evaporation was partitioned between 1 : 3 chloroform–ether (15 ml) and 0·1N-hydrochloric acid (12 ml) and the organic phase was successively extracted with 0·02N-hydrochloric acid (1 × 10 ml) and water (1 × 10 ml). The combined aqueous phase was extracted with 1 : 3 chloroform–ether (10 ml), then basified with saturated aqueous sodium carbonate and extracted with methylene chloride (3 × 15 ml). The organic phase was washed with saturated brine (1 × 10 ml) and dried (NaCl). The product from evaporation was dried *in vacuo* and was immediately dissolved with 2-pyridone (0·5 g) in anhydrous dioxan (250 ml). After the solution had been heated under reflux (N_2) for 25 h, it was evaporated, and the residue was partitioned between methylene chloride (100 ml) and water (50 ml). The organic phase was further extracted with water (2 × 50 ml), 0·02N-hydrochloric acid (1 × 50 ml), water (2 × 50 ml), and brine (1 × 50 ml), then dried and evaporated to yield the *lactam* (326 mg, 71%), m.p. 225—225·5° (from 95% ethanol) (Found: C, 64·2; H, 5·6; N, 7·7%; M^+, 356. $C_{19}H_{20}N_2O_5$ requires C, 64·0; H, 5·7; N, 7·9%; *M*, 356); $\nu_{max.}$ 3290, 1740, 1690, and 1669 cm^{-1}; $\lambda_{max.}$ 279 and 247sh nm (ε 18,700 and 6390); τ 0·80 (1H, s, NH), 2·64 (5H, s, Ph), 3·60 (1H, m, NH), 4·73 (2H, s, CO·CH_2Ph), 5·78 (2H, m, pyrrCH_2·N), 6·25 (2H, s, pyrrCH_2·CO), 6·41 (3H, s, OMe), and 7·24 (4H, s, pyrr$[CH_2]_2$·CO).

Methyl (1,4,5,6,7,8-Hexahydro-6-oxopyrrolo[2,3-c]azepin-3-yl)acetate (10).—The lactam (12) (205 mg) in methanol (30 ml) was shaken with hydrogen and 10% palladised charcoal

at *ca.* 20° and 760 mmHg until uptake ceased. The solution was filtered (Celite) and evaporated and the residue was immediately dissolved in water (20 ml). Nitrogen was passed through the solution for 20 min and the solution was then heated under reflux (bath 125—130°) for 1·5 h, after which it showed no absorption above 204 nm. Triethylamine (4 drops) was added to the cooled solution and extraction with methylene chloride (7 × 5 ml) afforded the *α-free lactam* (72 mg, 56%), m.p. 156—157·5° (from methanol) (Found: C, 59·7; H, 6·4; N, 12·7%; M^+, 222. $C_{11}H_{14}N_2O_3$ requires C, 59·45; H, 6·35; N, 12·6%; *M*, 222); $\nu_{max.}$ 3160, 1740, and 1655 cm^{-1}; τ 1·96br (1H, s, pyrrNH), 3·50br (2H, m, pyrrH and HNCO), 5·83 (2H, m, pyrrCH$_2$·N), 6·35 (3H, s, OMe), 6·63 (2H, s, pyrrCH$_2$·CO), and 7·24 (4H, s, pyrr[CH$_2$]$_2$CO).

Methyl [2-(2-*Benzyloxycarbonyl*-3-β-*methoxycarbonylethyl*-4-*methoxycarbonylmethylpyrrol*-5-*ylmethyl*)-1,4,5,6,7,8-*hexahydro*-6-*oxopyrrolo*[2,3-c]*azepin*-3-*yl*]*acetate* (27).—A solution of the pyrrole (13) (149 mg) in anhydrous ether (30 ml) was treated at 0° with ethereal 0·124M-sulphuryl chloride (3·3 ml), also at 0°. After 15 min at 20°, the solution was evaporated at 20° and anhydrous ether (2 × 20 ml) was added to the residue and evaporated off. To the final residue, which was dried *in vacuo* for 15 min, was added a solution of the α-free lactam (10) (87·8 mg) in 0·2M-sodium acetate in acetic acid (2 ml). This solution was heated under nitrogen at 100° for 30 min, then evaporated and the residue was chromatographed on Mallinckrodt CC-7 silica gel in ethyl acetate. This yielded, after p.l.c. of side fractions on silica in 1 : 9 methanol–ethyl acetate, the *pyrromethane* (108 mg, 45%), m.p. 187·5° (decomp.) (from aqueous ethanol) (Found: C, 61·75; H, 5·85; N, 6·75%; M^+, 593. $C_{31}H_{35}N_3O_9$,0·5H_2O requires C, 61·8; H, 6·0; N, 7·0%; *M*, 593); $\nu_{max.}$ 3330, 3190, 1740, 1730, 1705, and 1668 cm^{-1}; $\lambda_{max.}$ 284, 227sh, and 212 nm (ε 19,700, 12,200, and 18,700); τ −0·18 and 0·58 (each 1H, br, s, 2 × pyrrNH), 2·66 (5H, m, Ph), 3·30 (1H, m, CONH), 4·77 (2H, s, CH$_2$Ph), 5·85 (2H, m, pyrrCH$_2$·N), 6·26 (4H, s, pyrr$_2$CH$_2$ and pyrrCH$_2$·CO), 6·40 (3H, s, OMe), 6·43 (6H, s, 2 × OMe), 6·60 (2H, s, pyrrCH$_2$·CO), 7·00 and 7·50 (each 2H, m, pyrr[CH$_2$]$_2$·CO), and 7·26 (4H, s, pyrr[CH$_2$]$_2$·CON).

Methyl [1,4,5,6,7,8-*Hexahydro*-2-(3-β-*methoxycarbonylethyl*-4-*methoxycarbonylmethylpyrrol*-5-*ylmethyl*)-6-*oxopyrrolo*[2,3-c]*azepin*-3-*yl*]*acetate* (8).—The pyrromethane (27) (20·4 mg) in methanol (10 ml) was stirred with hydrogen and palladium black (20 mg) at *ca.* 20° and 760 mmHg until uptake ceased. The solution was filtered (Celite) under nitrogen and the filtrate and washings were evaporated at 20°. The resultant solid at 0° was treated with trifluoroacetic acid (1 ml) also at 0° under nitrogen and the mixture was kept at 20° for 20 min before being evaporated at 20°. After the residue had been dried *in vacuo*, it was purified by p.l.c. on silica in 1 : 9 methanol–ethyl acetate to give the *α-free pyrromethane* (10 mg, 63%), m.p. 124—125° (from methanol) (Found: C, 59·4; H, 6·7; N, 9·3%; M^+, 459. $C_{23}H_{29}N_3O_7$,0·25H_2O requires C, 59·5; H, 6·4; N, 9·1%; *M*, 459); $\nu_{max.}$ 3333, 3280, 1735, 1710, and 1675 cm^{-1}; $\lambda_{max.}$ 224sh and 216 nm (ε 11,200 and 11,500); τ 0·61 and 0·80 (each 1H, br, s, pyrrNH), 3·59 (2H, m, pyrrH and CONH), 5·89 (2H, m, pyrrCH$_2$·N), 6·28 (8H, s, pyrr$_2$CH$_2$ and 2 × OMe), 6·36 (3H, s, OMe), 6·50 and 6·59 (each 2H, s, 2 × pyrrCH$_2$CO), 7·26 (4H, s, pyrr[CH$_2$]$_2$·CON), and 7·25 and 7·51 (total 4H, m, pyrr[CH$_2$]$_2$·CO).

Methyl [2-(2-*Benzyloxycarbonyl*-4-β-*methoxycarbonylethyl*-3-*methoxycarbonylethylpyrrol*-5-*ylmethyl*)-1,4,5,6,7,8-*hexahydro*-6-*oxopyrrolo*[2,3-c]*azepin*-3-*yl*]*acetate* (29).—This was prepared as for the isomer (27). The pyrrole (14) (146·4 mg) was chlorinated with ethereal 0·124M-sulphuryl chloride (3·55 ml) and the product was dissolved in 0·2N-sodium acetate–acetic acid solution (1·75 ml) containing the lactam (10) (86·5 mg). Further steps as earlier gave the *pyrromethane*, purified by p.l.c. on silica in 3 : 17 methanol–ethyl acetate; yield 88·6 mg (38%), m.p. 148—149° (from methyl acetate–ether) (Found: C, 59·9; H, 5·9; N, 6·8%; M^+, 222. $C_{31}H_{35}N_2O_9$,1·5H_2O requires C, 59·95; H, 6·2; N, 6·8%; *M*, 222); $\nu_{max.}$ 3370, 3320, 1735, 1705, and 1662 cm^{-1}; $\lambda_{max.}$ 286, 230sh, and 211 nm (ε 13,400, 6730, and 12,900); τ −0·08 and 1·03 (each 1H, br, s, 2 × pyrrNH), 2·70 (5H, m, Ph), 3·54 (1H, m, CONH), 4·81 (2H, s, CH$_2$Ph), 5·89 (2H, m, pyrrCH$_2$·N), 6·22 (4H, s, pyrr$_2$CH$_2$ and pyrrCH$_2$·CO), 6·41, 6·43, and 6·45 (each 3H, s, 3 × OMe), 6·64 (2H, s, pyrrCH$_2$·CO), 7·28 (4H, s, pyrr[CH$_2$]$_2$·CON), and 7·33 (4H, m, pyrr[CH$_2$]$_2$·CO).

Methyl [1,4,5,6,7,8-*Hexahydro*-2-(4-β-*methoxycarbonylethyl*-3-*methoxycarbonylmethylpyrrol*-5-*ylmethyl*)-6-*oxopyrrolo*[2,3-c]*azepin*-3-*yl*]*acetate* (9).—The foregoing benzyl ester (21 mg) was debenzylated as earlier over palladium black (19 mg) in methanol (10 ml). The slightly pink crystalline product from evaporation of the filtered solution was treated at 0° with trifluoroacetic acid (1 ml) also at 0° then kept at 20° for 20 min. The dried residue from evaporation was fractionated by p.l.c. on alumina with 1 : 9 methanol–ethyl acetate containing hydroquinone (1%). The product was eluted from the alumina with methanol, the solution was evaporated, and a solution of the residue in methyl acetate was filtered and evaporated to give the very unstable lactam, characterised by n.m.r.: τ 3·50 (1H, s, pyrrH), 5·76 (2H, s, pyrrCH$_2$·N), 6·17 (2H, s, pyrr$_2$CH$_2$), 6·29, 6·31, and 6·33 (each 3H, s, 3 × OMe), 6·54 and 6·60 (each 2H, s, 2 × pyrrCH$_2$·CO), 7·23 (4H, s, pyrr[CH$_2$]$_2$·CON), and 7·29 and 7·68 (total 4H, m, pyrr[CH$_2$]$_2$·CO).

Hydrolysis of the Pyrromethane Lactam Esters (8) *and* (9).—A suspension of the lactam (10—12 mg) in aqueous 2N-potassium hydroxide (0·5 ml) in a tube was agitated with a fine stream of nitrogen until the solid dissolved (3·5 h). The tube was then sealed under nitrogen, kept at 24° in the dark for a further 41—44 h, then diluted with water (2 ml). Portions of IRC-50 resin (H-phase) were added to the solution until the pH was 6·5; the resin was then removed and washed with water. The total filtrate (10 ml) was evaporated at 20° and the residue was dried *in vacuo* and dissolved in D_2O (0·5 ml) for n.m.r. analysis. The results are collected in the Table.

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