Structure of a Heptacarboxylic Porphyrin Enzymically Derived from Porphobilinogen

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Summary A porphyrin heptacarboxylic acid, probably identical with phyriaporphyrin-III, is isolated and evidence from ^{13}C-n.m.r. and synthetic studies shows that it has structure (**10**); general synthetic methods are outlined.

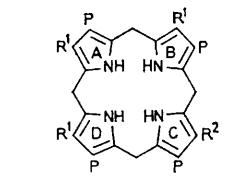
THE conversion of uroporphyrinogen-III (**1**) into coproporphyrinogen-III (**2**) on the porphyrin biosynthetic pathway[1] involves enzymic decarboxylation of four different acetic acid residues. The structures of the intermediates are unknown despite (*a*) their importance for porphyrin biosynthesis and (*b*) the attractive possibility that a heptacarboxylic acid (**3**) is an intermediate[2] for the biosynthesis of vitamin B_{12}.

Incubation of porphobilinogen (**4**) with a modified form[3] of the enzyme system from avian erythrocytes[4] afforded (after aeration to generate porphyrins from porphyrinogens) uroporphyrin-III (**5**), coproporphyrin-III (**6**) and protoporphyrin-IX. In addition, a porphyrin was isolated as its crystalline methyl ester, $C_{46}H_{52}N_4O_{14}$, m.p. 213—214°, which corresponds to the isolated substance being a heptacarboxylic acid. Its properties and analysis correspond with those of the methyl ester[5] of phyriaporphyrin-III (identical[5] with porphyrin-208[6] and pseudo-uroporphyrin[7]) and it is highly probable that this is the same substance. Phyriaporphyrin-III yielded coproporphyrin-III on decarboxylation[5] and evidence was given[5] for the corresponding porphyrinogen being a biosynthetic intermediate between uroporphyrinogen-III (**1**) and coproporphyrinogen-III (**2**).

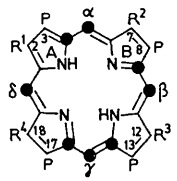
Our porphyrin was proved to be a heptacarboxylic acid by forming the ester with a 1:1 mixture of CH_3OH and CD_3OD.[8] The product's mass spectrum showed an octet of molecular ions with relative intensities of the required binomial pattern (1:7:21:35:35:21:7:1). Decarboxylation of the porphyrin gave only coproporphyrin-III and therefore its structure is limited to one of the four monomethyl porphyrins (**7**), (**8**), (**9**), or (**10**). The F.T. ^1H n.m.r. spectrum of its methyl ester was in agreement.

The possibilities were further reduced by enzymic preparation of the heptacarboxylic porphyrin from [2,11-$^{13}C_2$] porphobilinogen[9] (**4**) (90 atom % at each labelled site) which

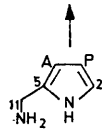
generates the porphyrin ring with 90 atom % of ^{13}C at the illustrated sites; see (**7**)—(**10**). The ^{13}C-signals from the γ- and δ-*meso* carbons of the corresponding methyl ester



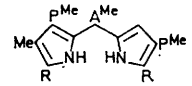
(1) ; $R^1 = R^2 = A$
(2) ; $R^1 = R^2 = Me$
(3) ; $R^1 = A$, $R^2 = Me$



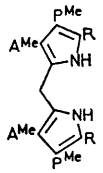
(5) ; $R^1 = R^2 = R^3 = R^4 = A$
(6) ; $R^1 = R^2 = R^3 = R^4 = Me$
(7) ; $R^1 = Me$, $R^2 = R^3 = R^4 = A$
(8) ; $R^2 = Me$, $R^1 = R^3 = R^4 = A$
(9) ; $R^3 = Me$, $R^1 = R^2 = R^4 = A$
(10) ; $R^4 = Me$, $R^1 = R^2 = R^3 = A$



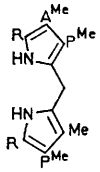
(4)



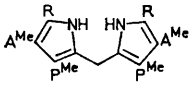
(11) ; R = CO_2CH_2Ph
(12) ; R = CO_2H
(13) ; R = H
(14) ; R = $CH=\overset{+}{N}Et_2Cl^-$
(15) ; R = CHO



(18) ; R = CO_2CH_2Ph
(19) ; R = H
(20) ; R = CHO



(21) ; R = CO_2CH_2Ph
(22) ; R = H



(16) ; R = CO_2CH_2Ph
(17) ; R = H

A = CH_2CO_2H $A^{Me} = CH_2CO_2Me$

P = $CH_2CH_2CO_2H$ $P^{Me} = CH_2CH_2CO_2Me$

● = ^{13}C

could be unambiguously assigned by their unique patterns[9] (72 Hz triplet and 5 Hz triplet, respectively) whereas those from the α- and β-*meso* carbons were both double doublets (J 5 and 72 Hz) and so must be considered together. Stepwise addition of Pr ([$^2H_9$] fod)$_3$ to a $CDCl_3$ solution of the labelled porphyrin ester was approximately twice as effective in causing upfield shift of the ^{13}C-signals from the α-, β-, and γ-*meso* carbons as it was for the δ-*meso* signal. It is known for ^1H spectroscopy, that lanthanide shift reagents affect most strongly the signals from protons at *meso*-carbons flanked by two ester groups[10] (*e.g.* at C-3 and C-7 for the α-*meso* position). Accordingly, the ^{13}C-results point to structure (**7**) or (**10**) for the heptacarboxylic porphyrin.

Synthesis of (**7**) was based on the pyrromethanes† (**11**) and (**16**). The diacid (**12**) from hydrogenolysis of (**11**) was decarboxylated in boiling diethylformamide and the product (**13**) without isolation was treated with benzoyl chloride;[11] hydrolysis of the resultant salt (**14**) gave the dialdehyde (**15**) in 62% overall yield from (**11**). This reacted with the pyrromethane (**17**) obtained as above from (**16**) to yield the ring-A methyl heptamethyl ester (**7**), 45% yield, m.p. 213—216°. This porphyrin was found to differ from the above natural product by normal and lanthanide-shifted ^1H-n.m.r. spectra.

The ring-B methyl porphyrin ester (**8**) (48% yield, m.p. 227—228°) and the ring-C methyl isomer (**9**) (32% yield, m.p. 238—240°) were synthesised by combination, respectively, of (**19**) with (**15**) and of (**20**)[12] with (**22**). These building units were prepared as above from the esters (**18**) and (**21**).

Current research[13] on the biosynthesis of vitamin B_{12} involves the ring-C methyl porphyrin (**9**). In addition, the syntheses of (**8**) and (**9**) confirmed the foregoing deduction that the ester of the natural porphyrin does not have either structure (**8**) or (**9**). Both synthetic samples were readily distinguishable from the natural material.

Two methods used in the above synthetic work are of general value in the porphyrin field: (*a*) smooth reaction of a 2-acetoxymethylpyrrole with an α-free pyrrole occurs in 10 min at −20° catalysed by a 2% solution of $SnCl_4$ in dichloromethane [preparation of (**21**), 86%]; (*b*) decarboxylation of pyrromethane dicarboxylic acids (*e.g.* **12**) in refluxing diethylformamide is complete in 2 h and *C*-formylation can be carried out *in situ*.

These studies show that the natural porphyrin heptacarboxylic acid has structure (**10**); its synthesis is in hand.

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† Many of the sequences used for the monopyrrolic building blocks are new and will be described in our full paper.

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