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ENZYMIC FORMATION OF A TRICARBOXYLIC PORPHYRIN AND PROTOPORPHYRIN-XIII FROM COPROGEN-IV†

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The late stages of porphyrin biosynthesis[1] involve enzymes which are not highly specific in that they catalyse not only the normal pathway urogen-III (1) → coprogen-III (2) → protoporphyrin-IX (3) but also operate on isomers of (1) and (2), though often at a slower rate. One such isomer is coprogen-IV (5) which was reported[2] enzymically to yield a product isomeric with protoporphyrin-IX (3); it was of unknown structure. We now report that coproporphyrinogenase from beef liver mitochondria converts coprogen-IV (5) into protoporphyrin-XIII (6) (Fischer's numbering[3]) together with a tricarboxylic acid (as 17).

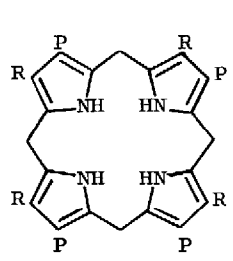
Coproporphyrin-IV tetramethyl ester (10) was synthesised in 66% yield from the pyrromethanes (8) and (9) by S. F. MacDonald's method and the product was shown to be entirely free from coproporphyrin-I and, -II esters by h.p.l.c.[4] The derived coproporphyrin-IV (11) was reduced with Na/Hg to coprogen-IV (5) which was incubated at ca. pH 7.8 in the dark with beef liver mitochondria. Esterification of the resultant porphyrins (recovery 34-46%) and chromatography gave fractions corresponding to di-, tri-, and tetra-carboxylic esters and the proportions of these depended on the length of incubation (Table). These results are consistent with the view that with the mitochondrial enzyme, oxidative decarboxylation of the second propionate side-chain is significantly slower for coprogen-IV (5) than for the natural isomer, coprogen-III (2).

The dicarboxylic porphyrin ester fraction from Expt. 3 was shown by h.p.l.c. on 10μ Porasil to contain protoporphyrin-IX ester≠ (4) by compari-

† Shortened names will be used throughout; urogen ≡ uroporphyrinogen; coprogen ≡ coproporphyrinogen; protogen ≡ protoporphyrinogen.

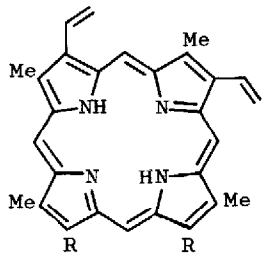
≠ This may arise from the liver mitochondrial preparation or by rapid conversion of traces of coprogen-III conceivably present in the synthetic IV-isomer; this is being examined.

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



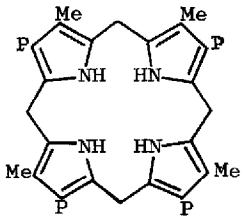
(1) R = CH_2CO_2H

(2) R = Me

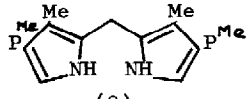


(3) R = P

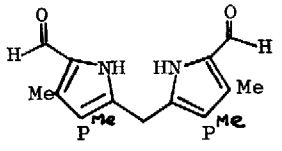
(4) R = P^{Me}



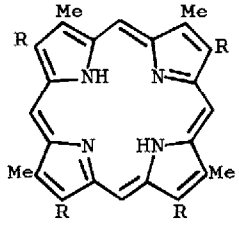
(5)



(8)

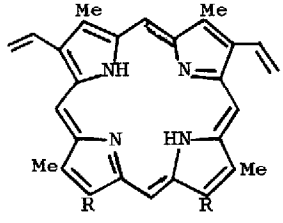


(9)



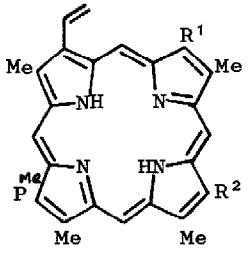
(10) R = P^{Me}

(11) R = P



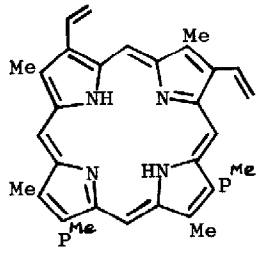
(6) R = P

(7) R = P^{Me}

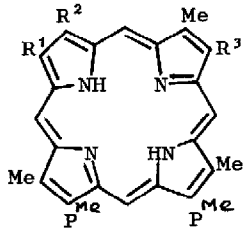


(12) R^1 = vinyl, R^2 = P^{Me}

(13) R^1 = P^{Me}, R^2 = vinyl



(14)



(15) R^1 = Me, R^2 = vinyl, R^3 = P^{Me}

(16) R^1 = Me, R^2 = P^{Me}, R^3 = vinyl

(17) R^1 = vinyl, R^2 = Me, R^3 = P^{Me}

TABLE Action of coproporphyrinogenase on coprogen isomers

Expt. No.	Incubation time	Coprogen isomer	Total porphyrin recovered	Composition of porphyrin mixture		
				4 CO_2Me	3 CO_2Me	2 CO_2Me
1	1 hr.	Type-III (2)	40%	69%	1%	30%
2	1 hr.	Type-IV (5)	46%	52%	47%	1%
3	22 hr.	Type-IV (5)	34-39%	15%	37%	48%

son with authentic material and a separable isomer (M^+ at m/e 590) shown to be protoporphyrin-XIII dimethyl ester (7) as follows. The n.m.r. spectrum of the dicarboxylic ester fraction run with increasing quantities of $Eu(fod)_3$ showed the rapid movement downfield[5] of one meso-proton signal (integrating for 1H). Thus the isomer, like protoporphyrin-IX ester (4), has a meso-proton flanked by two propionate side-chains. Of the four possible dicarboxylic porphyrin esters (7), (12), (13) and (14) derivable from coprogen-IV (5) only isomer (7) matches this requirement and so this structure, that of protoporphyrin-XIII diester, can be assigned to the ester of the product from coprogen-IV (5). The lanthanide shifts (or virtual lack of shifts) for the signals from the methylene, O-methyl, and two C-methyl groups, (vinyl and two C-methyl groups) of the porphyrin ester (7) were entirely consistent with this structural assignment. Synthesis of structure (7) is in hand.

H.p.l.c. analysis of the tricarboxylic porphyrin trimethyl ester enzymically produced from coprogen-III (2) revealed a single peak running identically with harderoporphyrin ester (15) but differing from the ester of isoharderoporphyrin (16); this result is in agreement with the earlier findings of Kenner and Smith for a different enzyme system using ^{14}C-incorporations and dilution analysis.[6] The foregoing tricarboxylic ester (M^+ m/e 650) similarly derived from coprogen-IV (5) also gave a single peak on h.p.l.c. and its n.m.r. spectrum supported a monovinyl porphyrin structure. In the presence of $Eu(fod)_3$, one meso-proton signal from the new tricarboxylic ester shifted massively downfield in support of structure (17) for this product.

Thus, coprogen-IV (5) is converted rapidly by coproporphyrinogenase from mitochondria into the tricarboxylic porphyrinogen and more slowly into protoporphyrin-XIII (6). These results cast light on the structural requirements of the enzyme. Further, because of the symmetry of protoporphyrin-XIII (6) and its distinction by h.p.l.c. from protoporphyrin-IX (3), they have valuable implications for current studies of the role of pyrromethanes in porphyrin biosynthesis.

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