Mechanism of Biosynthesis of the Vinyl Groups of Protoporphyrin-IX

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Summary Coproporphyrinogen-III (**1**) is shown to be converted biochemically into protoporphyrin-IX (**4**) with loss of one hydrogen atom from each propionate residue on rings A and B and the ^3H-tracer results are consistent with a stereospecific process.

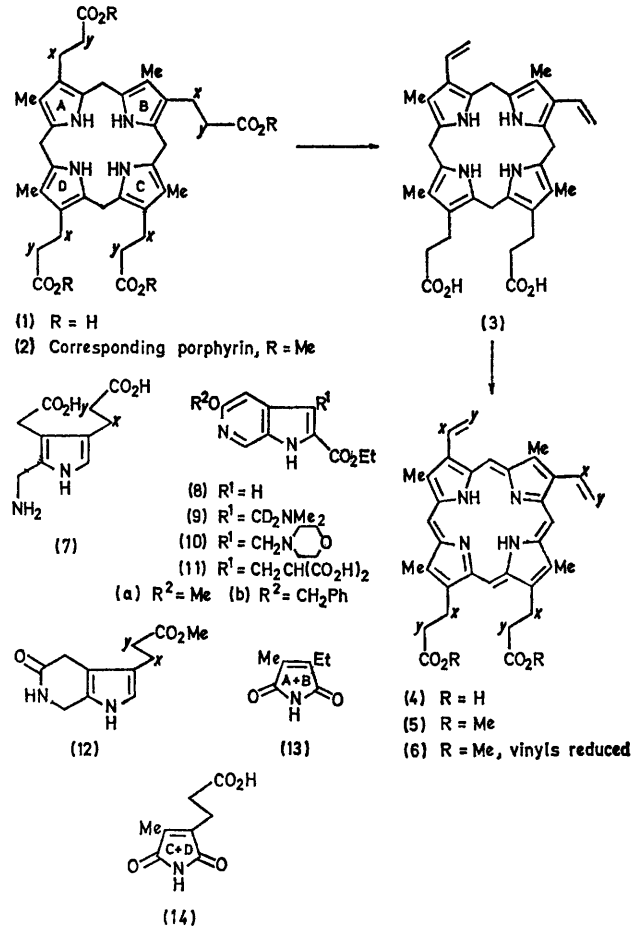
COPROPORPHYRINOGEN-III (**1**) is converted into protoporphyrinogen-IX (**3**) by coproporphyrinogenase, an oxidative enzyme system;[1] (**3**) is the precursor of protoporphyrin-IX (**4**). Several mechanisms have been suggested[2] which differ in the oxidation levels that the two methylenes of the propionate groups on rings A and B of (**1**) would experience over the reaction sequences. Incorporation experiments with deuteriated porphobilinogen, (**7**) were therefore undertaken.

[$^2H_2$]Formaldehyde reacted with the azaindole[3] (**8a**) and dimethylamine to give the [$^2H_2$-*methylene*] base (**9a**) which *via*[3] (**11a**) yielded porphobilinogen lactam ester (**12**) labelled at *x*, > 98% $^2H_2$ species. Unlabelled (**11b**) was exchanged with D_2O and decarboxylated in pyridine–D_2O; the derived (**12**) labelled at *y* contained 89% $^2H_2$ and 11% $^2H_1$ species. Alkaline hydrolysis of the two samples of (**12**) gave two porphobilinogens (**7**) labelled at *x* and at *y* which were reconverted with diazomethane into (**12**) for mass spectrometry; no detectable loss of deuterium had occurred in either case.

Porphobilinogen (**7**) labelled at *x* was incubated with an enzyme system from *Euglena gracilis*[4] and the pools of (**1**) and (**3**) so formed were converted photochemically[5] into the corresponding porphyrins which were isolated and analysed as the esters (**2**) and (**5**). Eight deuterium atoms were present in (**2**) whereas (**5**) contained six (Table).

Similarly, (**7**) labelled at *y* yielded (**2**) containing $^2H_8$ as the major species. A slight fall in ^2H content occurred over the incubation and isolation processes (Table) but the analyses show that the four hydrogens at sites *y* of (**1**, rings A and B) are preserved as the vinyl groups of (**4**) are formed. The locations of the ^2H labels were confirmed by n.m.r.

These results eliminate mechanisms for (**1**) → (**3**) in *Euglena gracilis* based on intermediate ketones or acrylic acids; they are consistent with (i) hydroxylation–fragmentation[6] or (ii) oxidative fragmentation.[7]



Deuterium content of labelled porphyrins

	From (**7**) labelled at *x*		From (**7**) labelled at *y*		
	Found	Calc.	Found	Calc.[a]	Calc.[b]
Coproporphyrin-III tetramethyl ester (**2**)	93±3% $^2H_8$	92% $^2H_8$	50±2% $^2H_8$	63% $^2H_8$	50% $^2H_8$
	6±2% $^2H_7$	7% $^2H_7$	35±2% $^2H_7$	31% $^2H_7$	38% $^2H_7$
			15±2% $^2H_6$	6% $^2H_6$	11% $^2H_6$
					Calc.[c]
Protoporphyrin-IX dimethyl ester (**5**)	90±3% $^2H_6$	92% $^2H_6$	44±2% $^2H_8$	63% $^2H_8$	45% $^2H_8$
	10±2% $^2H_5$	7% $^2H_5$	37±2% $^2H_7$	31% $^2H_7$	40% $^2H_7$
			19±2% $^2H_6$	6% $^2H_6$	13% $^2H_6$

[a] Based upon 89% $^2H_2$, 11% $^2H_1$ in (**7**). [b] Based upon 84% $^2H_2$, 16% $^2H_1$ in (**7**). [c] Based upon 82% $^2H_2$, 18% $^2H_1$ in (**7**).

The base (**10b**) prepared from (**8b**), [^{14}C]formaldehyde, and morpholine gave (**7**) as above ^{14}C-labelled at *x* which was mixed with (**7**) labelled at *x* with ^3H synthesised as in the ^2H series. Incorporation experiments with this ^3H;^{14}C-labelled (**7**) were carried out in enzyme systems from algal[4] and avian[8] sources; the former gave free protoporphyrin-IX (**4**, 8·6% incorpn.), the latter mainly haem [66% incorpn.

with 17% into free (**4**)]. The three samples of (**5**) so obtained were converted into mesoporphyrin-IX dimethyl ester (**6**). Oxidation[9] of the derived acids gave (**13**) from rings A and B and haematinic acid (**14**) from rings C and D. The $^{3}H;^{14}C$ ratios consistently found for haematinic acid were essentially that of the administered porphobilinogen (100, 99, 97% ^{3}H-retention, respectively) whereas the (**13**) contained, within experimental error, 50% of the tritium present in (**7**) (49, 51, 49% ^{3}H-retention, respectively). These results are consistent[10] with stereospecific attack at the two methylene groups marked *x* on rings A and B of (**1**).

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