Biosynthesis of Protoporphyrin-IX from Coproporphyrinogen-III

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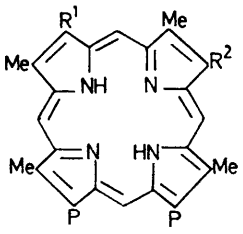
Summary By use of tritium labelled substrates, and h.p.l.c., it is shown that protoporphyrin-IX (**1d**) is formed from coproporphyrinogen-III (**2a**) *via* a specific pathway involving harderoporphyrinogen (**2b**) rather than its isomer (**2c**).

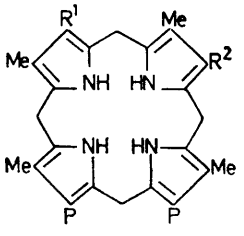
TRICARBOXYLIC porphyrins have been detected in a number of natural sources,[1] and one of the most prolific sources appears to be the harderian gland of the rat. The rat tricarboxylic porphyrin, harderoporphyrin (**1b**)[2] appears to be identical[1] with the naturally occurring tricarboxylic porphyrin isolated from human erythrocytes.[3] It seemed likely, therefore, that harderoporphyrinogen (**2b**) is an intermediate in porphyrin biosynthesis between coproporphyrinogen-III (**2a**) and protoporphyrinogen-IX (**2d**),[2] and it was suggested[2] that the 2-propionic acid group of coproporphyrinogen-III (**2a**) is preferentially transformed into vinyl before that at position 4. Evidence for this pathway in plants has been provided by experiments[4] with an enzyme preparation from *Euglena gracilis;* harderoporphyrinogen (**2b**) was incorporated some 10 times more effectively than its isomer (**2c**), although the absolute incorporations were low.

The two isomeric porphyrin esters (**3b**) and (**3c**) (labelled with tritium in the *meso*-positions by exchange with hexapyridylmagnesium iodide and tritiated water in pyridine[5]) were hydrolysed to the corresponding acids with alkali and reduced to the hexahydro derivatives (**2b**) and (**2c**) by treatment with sodium amalgam. The latter were shaken separately with haemolysates[6] of mature chicken erythrocytes for 2 h at 37 °C. After addition of cold protoporphyrin-IX followed by extraction, esterification, and purification by high pressure liquid chromatography (h.p.l.c.) the specific incorporations of (**2b**) and (**2c**) were found to be 34 and 0·8% respectively.

Studies of the incorporation of coproporphyrinogen-III (**2a**) (doubly labelled with tritium at the *meso*-positions and with ^{14}C in the β-methylene group of the 2-propionate side-chain) led us to the conclusion that the final dehydrogenation to protoporphyrin-IX was a stereospecific process in nature.[7] Taking this result into account the absolute incorporations of harderoporphyrinogen (**2b**) and isoharderoporphyrinogen (**2c**) determined with the singly labelled substrates should be corrected to *ca.* 70 and 1·5% respectively.



(1)



(2)

a; $R^1 = R^2 = P$
b; $R^1 = CH{=}CH_2$, $R^2 = P$
c; $R^1 = P$, $R^2 = CH{=}CH_2$
d; $R^1 = R^2 = CH{=}CH_2$
$P = CH_2CH_2CO_2H$
(3) Methyl ester of (1)

Kinetic studies of the conversion of coproporphyrinogen-III (**2a**) into protoporphyrin-IX (**3c**) in this avian system (using h.p.l.c. analysis[8] of the porphyrin methyl esters formed on work-up) show that only harderoporphyrinogen is formed as an intermediate.† Similar results have been obtained in experiments with rat liver homogenates[9] which also showed that only harderoporphyrinogen (**2b**) is formed as an intermediate *en route* to protoporphyrin-IX. We thus conclude that this route is highly specific in both animal and plant systems, and full details of these and related experiments will be published elsewhere. The high levels of incorporation obtained show that the haemolysate system is an excellent medium for biosynthetic experiments.

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† Harderoporphyrin (**1b**) can be clearly distinguished from its isomer (**1c**) by h.p.l.c. (*cf.* refs. 4 and 8).

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