Mesoporphyrinogen-VI: a Substrate for Coproporphyrinogen Oxidase

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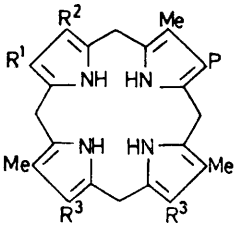
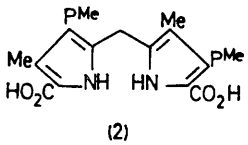
Summary Mesoporphyrinogen-IV is metabolised by coproporphyrinogen oxidase to 'protoaetioporphyrin,' thus showing that the two vicinal propionic acid groups which are present in all other known substrates are not essential for substrates of this enzyme.

COPROPORPHYRINOGENS-III (**1a**) and -IV (**1b**) are both substrates for coproporphyrinogen oxidase;[1] this oxidatively degrades the propionic acid side chains in rings A and B of both compounds to vinyl groups.[2] On the basis of this and other evidence we concluded[2] that the minimum structural requirement for substrates of coproporphyrinogen oxidase was the sequence of substituents (methyl† methyl-propionate methyl).

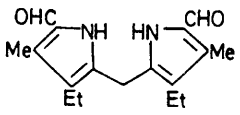


(1)

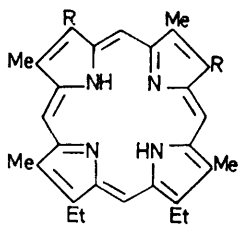
a; R^1 = Me, R^2 = R^3 = P
b; R^2 = Me, R^1 = R^3 = P
c; R^1 = Me, R^2 = P, R^3 = Et
d; R^1 = Me, R^2 = V, R^3 = Et



(2)



(3)



(4)

a; R = P^{Me}
b; R = V

P = $CH_2CH_2CO_2H$
P^{Me} = $CH_2CH_2CO_2Me$
V = $CH{=}CH_2$

The neighbouring propionic acid groups in rings C and D of both coproporphyrinogen-III (**1a**) and -IV (**1b**) are not metabolised and hitherto it has been tacitly assumed[3] that they are essential for binding to coproporphyrinogen oxidase. We therefore decided to study the metabolism of mesoporphyrinogen-VI which has the same arragement of side-chains in rings A and B as coproporphyrinogen-III, but only methyl and ethyl groups in rings C and D.

Acid catalysed condensation of the two pyrromethanes (**2**) and (**3**) followed by aerial oxidation afforded mesoporphyrin-VI dimethyl ester (**4a**), m.p. 195—197 °C (lit.[4] 199 °C), in good yield. Hydrolysis and reduction with sodium amalgam then gave the corresponding dicarboxylic porphyrinogen (**2c**) (2 mg) which was incubated with chicken red-cell haemolysates at 37 °C for 2 h. After extraction, the product was treated with methanolic sulphuric acid to convert any porphyrin free acids into their methyl esters. High pressure liquid chromatography, however, showed that the only porphyrin present (*ca.* 1 mg) had a retention time very similar to aetioporphyrin; the visible absorption spectrum (λ_{max} 400, 504, 538, 555, and 629 nm in $CHCl_3$) was also very similar to that of aetioporphyrin but, as expected, all the bands were shifted slightly to longer wavelength. Field desorption mass spectrometry gave a molecular ion at *m/e* 474 and thus confirmed that the product was protoaetioporphyrin[5] (**4b**); the 90 MHz n.m.r. spectrum (19 accumulations in $CDCl_3$) was also in accord with this structure.

Time-course studies showed that mesoporphyrinogen-VI (**1c**) was metabolised at a rate very similar to that for coproporphyrinogens-III (**1a**) and -IV (**1b**) (at equivalent substrate concentrations); *ca.* 40% of a monopropionate porphyrinogen, presumably[6] (**1d**), was formed during the course of the reaction. In similar experiments with rat liver homogenates the monopropionate porphyrinogen was the major product. We conclude therefore that the neighbouring propionate groups in the C and D rings of coproporphyrinogen-III are not an essential structural requirement for a substrate of coproporphyrinogen oxidase.

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† Can be replaced by H or vinyl.

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[2] H. M. G. Al-Hazimi, A. H. Jackson, D. J. Ryder, G. H. Elder, and S. G. Smith, *J.C.S. Chem. Comm.*, 1976, 188.
[3] *Cf.* G. Y. Kennedy, A. H. Jackson, G. W. Kenner, and C. J. Suckling, *FEBS Letters*, 1970, **6**, 9 and 205.
[4] H. Fischer and A. Rothhaas, *Annalen*, 1930, **484**, 90 and 107.
[5] H. Fischer, A. Kirstahler, and B. V. Zychlinski, *Annalen*, 1932, **500**, 3 and 9.
[6] By analogy with the metabolism of coproporphyrinogen-III, *cf.* J. A. S. Cavaleiro, G. W. Kenner, and K. M. Smith, *J.C.S. Perkin I*, 1974, 1188; D. E. Games, A. H. Jackson, J. R. Jackson, R. V. Belcher, and S. G. Smith, *J.C.S. Chem. Comm.*, 1976, 187, and ref. 3.