Cytochrome C Oxidase: Isolation, Crystallisation, and Synthesis of Porphyrin A Dimethyl Ester

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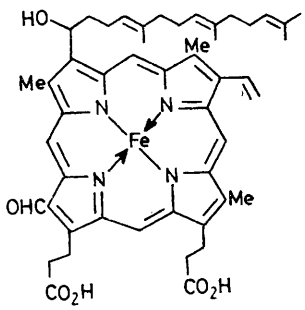
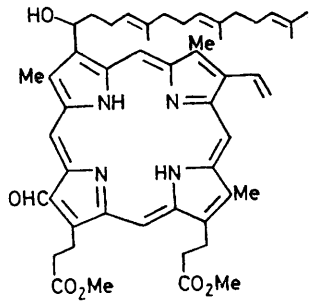
Summary Haem A preparations from beef heart are found to be heterogeneous and demetallation yields a polar porphyrin together with porphyrin A, isolated as its crystalline dimethyl ester (**2**) which is studied spectroscopically and its structure confirmed by unambiguous synthesis.

CYTOCHROME C OXIDASE† is a key respiratory enzyme and its prosthetic group(s) have been studied since Warburg's isolation[1] of the first crude metallo-porphyrin preparation called haem A. Excellent structural work on haem(s) A (and derivatives) by the groups of Lemberg, Lynen, MacDonald and Caughey, has been reviewed;[2] their evidence was accommodated by structure (**1**), the distinction between the illustrated nature of the long side-chain and possible reduced or cyclic forms being made by n.m.r. spectroscopy.[3]

The block to further progress was the difficulty of demonstrating homogeneity for haem A preparations or, more importantly, of isolating the corresponding pure metal-free porphyrin(s) A for comparison with synthetic samples. Advances in isolation and synthesis are described below.



(1)



(2)

'Porphyrin A' dimethyl ester, isolated from beef heart by acidic extraction[4] or by the basic method *via* the haem A dipyridyl complex,[3] was 'homogeneous' by t.l.c. but the product from both extractions was found by field desorption (F.D.) mass spectrometry and high pressure liquid chromatography[5] (h.p.l.c.) to be a similar mixture. The same two components were isolated in roughly equal amounts from each mixture by preparative h.p.l.c.‡

† Systematically cytochrome C: O_2 oxidoreductase, E.C.1.9.3.1.

‡ A sample of haem A pyridine complex generously provided by Professor W. S. Caughey, was converted here into the metal-free ester and the same two main components were present; *cf.* earlier indications (ref. 3) of more than one component.

The structure of the more polar of these two porphyrin esters will be considered in a future paper; the less polar ester crystallised, m.p. 128—130 °C, and will be referred to as porphyrin A dimethyl ester§ (λ_{max} 647, 584, 560, 517, and 412 nm in Et_2O; bands III:IV ratio 2·28:1). Re-insertion of iron into the latter ester (using dimethylformamide–$FeCl_2$[6]) gave pure haem A (76%) which was again demetallated; the product was >96% homogeneous (h.p.l.c.). Thus the more polar porphyrin is not an artefact of the metal removal process.

Structure (**2**), $C_{51}H_{62}N_4O_6$, *M* 826, was established for porphyrin A dimethyl ester by spectroscopy and synthesis as follows. F.D. mass spectrometry, using emitters activated at high temperature, showed a molecular ion at *m/e* 826 (anode wire current 16 mA) establishing the molecular size for the first time; M^+ 868 for *O*-acetylporphyrin A dimethyl ester gave confirmation.

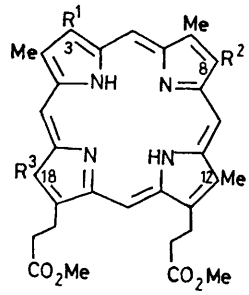
Degradation of (**2**) with hot resorcinol and re-esterification gave cytodeuteroporphyrin ester§ (**3**, 21%), M^+ 524, which was inseparable after 5 h.p.l.c. recycles from the original synthetic sample of this material[2b] kindly provided by Dr. S. F. MacDonald. Further it was readily separated from the isomer§ of (**3**) having hydrogen at positions 3, 8, and 12 prepared by resorcinol melt from the synthetic ester (**7**); see later.

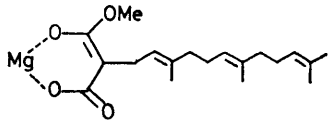
The n.m.r. spectra of porphyrin A dimethyl ester at 270 MHz without and with Eu([$^2H_9$]fod)$_3$ were in full agreement with structure (**2**) and the *O*-acetate showed the expected shifts, but, because of the anisotropy of the macrocycle, n.m.r. spectroscopy alone did not settle the stereochemistry of the side-chain. Unambiguous proof came from synthesis.

The formyl ester (**4**) was available by an unambiguous synthesis.[7] *trans,trans*-Farnesyl bromide and dimethyl malonate provided the enolate (**6**) which was acylated by the acid chloride corresponding to ester (**4**). Demethylation of the resultant β-keto ester with lithium iodide gave porphyrin A ketone (**5**) in which the aldehyde was selectively protected by acetal formation. Borohydride reduction of the ketone and hydrolysis of the acetal followed by esterification gave crystalline (*R,S*)-porphyrin A dimethyl ester§ (**2**), m.p. 124—125 °C.

This product was inseparable from the natural ester after 5 h.p.l.c. recycles and the u.v.–visible and n.m.r. spectra of synthetic and natural samples were superimposible; the n.m.r. spectrum of a mixture of synthetic and natural materials run with Eu([$^2H_9$]fod)$_3$ confirmed identity. The correspondence of the synthetic and natural *O*-acetates was established by a similar full h.p.l.c. and spectroscopic comparison.

Strength is added to the foregoing work by showing non-identity of (**2**) from natural material with closely related synthetic samples. Thus, the isomeric ester§ (**7**) was synthesised by a route analogous to that above; the product was readily separated from naturally derived (**2**).

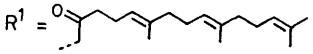




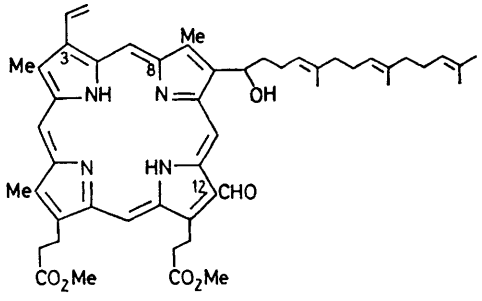
(6)

(3) $R^1 = R^2 = R^3 = H$

(4) $R^1 = CO_2Me$, $R^2 = CH{=}CH_2$, $R^3 = CHO$

(5) $R^1 =$ 

$R^2 = CH{=}CH_2$ $R^3 = CHO$



(7)

Structure (**2**) is thus established for porphyrin A dimethyl ester. Its synthesis provides material for studies of the relation of structure to function for the corresponding pure haem A and similarly for the haems from the *cis–cis*, *cis–trans*, and *trans–cis* isomers of (**2**) which are currently being synthesised.

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§ Satisfactory elemental analysis or accurate mass data obtained.

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