Biosynthetic Intermediates between Coproporphyrinogen-III and Protoporphyrin-IX

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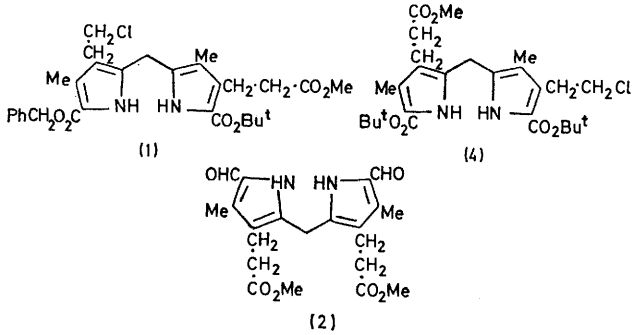
Summary New syntheses of the isomeric tripropionic porphyrins (**3a**) and (**3b**) have been carried out, and the feeding of the porphyrinogens (**5b**) and (**5c**) (labelled with tritium) to an enzyme system from *Euglena gracilis* has confirmed the hypothesis that the 2-propionic acid side-chain is modified before the 4-substituent in the biosynthesis of protoporphyrinogen-IX (**5d**) from coproporphyrinogen-III (**5a**).

ISOLATION of harderoporphyrin from the Harderian gland of the rat has been described,[1] and the hypothesis that the biosynthesis proceeds in the sequence, coproporphyrinogen-III (**5a**) ⟶ harderoporphyrinogen (**5b**) ⟶ protoporphyrinogen-IX (**5d**) (*i.e.* that the propionic side-chain at position 2 is transformed into vinyl before that at position 4) has been advanced. We now substantiate this hypothesis with experimental results.

The former syntheses[1,2] of harderoporphyrin and isoharderoporphyrin trimethyl esters (**3a**) and (**3b**) respectively, have been superseded by more efficient routes† [from the pyrromethanes (**1**) or (**4**) and (**2**)][3] patterned after our syntheses[4,5] of deuteriated derivatives of protoporphyrin-IX dimethyl ester.

The two isomeric porphyrins (**3a**) and (**3b**) were labelled with tritium for the biosynthetic experiments, using our new method[5,6] of *meso*-proton exchange with hexapyridylmagnesium iodide and tritiated water in pyridine.‡ The



(1) (4) (2) (3) (5)

a ; R^1 = CH:CH_2; R^2 = CH_2·CH_2·CO_2Me
b ; R^1 = CH_2·CH_2·CO_2Me ; R^2 = CH : CH_2

a ; R^1=R^2=CH_2·CH_2·CO_2H
b ; R^1= CH : CH_2 ; R^2= CH_2·CH_2·CO_2H
c ; R^1=CH_2·CH_2·CO_2H ; R^2= CH : CH_2
d ; R^1 = R^2 = CH : CH_2

† The m.p.s. of (**3a**), 203—204°, and (**3b**), 216—218°, are considerably higher than those reported previously.[1] Repetition of the original syntheses has yielded samples of these porphyrins with m.p.s. agreeing with the new values.

‡ Acceptable counting efficiencies were obtained by bleaching in sunlight, in the presence of a small quantity of benzoyl peroxide.

labelled porphyrinogen tricarboxylic acids required for feeding were obtained by hydrolysis of the porphyrin esters with potassium hydroxide in water–tetrahydrofuran, and then reduction to the hexahydro-derivatives with sodium amalgam.§ The labelled isomeric porphyrinogens (**5b**) and (**5c**) were fed separately to a divided batch of an enzyme extract from *Euglena gracilis*,[7] along with similarly tritiated coproporphyrinogen-III (**5a**) as a standard reference.[8] Protoporphyrin-IX dimethyl ester was isolated by chromatography after esterification, and the incorporations were respectively 27% of (**5a**), 33% of (**5b**), and 4·5% of (**5c**). After the overnight incubations of (**5a**), (**5b**), and (**5c**) with the enzyme extract, it was noticeable that the solution from (**5c**) was the only one of the three which had any porphyrinic colouration prior to oxidation in daylight. This indicates that (**5c**) was not being accepted by the enzyme system.

We conclude that a biosynthetic sequence in which the propionate residue at position 2 in (**5a**) is modified before that at position 4 is preferred.

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§ Reduction of coproporphyrin-III and aerial re-oxidation gave 96% retention of the tritium label, in accordance with the expected dominance of a primary isotope effect. Incorporations reported herein have been adjusted to take account of this small tritium loss.

[1] G. Y. Kennedy, A. H. Jackson, G. W. Kenner, and C. J. Suckling, *FEBS Letters*, 1970, **6**, 9; 1970, **7**, 205.

[2] C. J. Suckling, Ph.D. Thesis, Liverpool, 1970.

[3] Pyrromethane (**1**) was prepared by condensation of benzyl 2-acetoxymethyl-3-(2-chloroethyl)-4-methylpyrrole-5-carboxylate with t-butyl 4-(2-methoxycarbonylethyl)-3-methylpyrrole-5-carboxylate in acetic acid containing a catalytic quantity of toluene-*p*-sulphonic acid hydrate: *cf.* A. M. d'A. R. Gonsalves, G. W. Kenner, and K. M. Smith, *Tetrahedron Letters*, 1972, 2203. Pyrromethane (**4**) was likewise prepared from t-butyl 2-acetoxymethyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-5-carboxylate and t-butyl 4-(2-chloroethyl)-3-methylpyrrole-5-carboxylate. The (2-chloroethyl)pyrroles and the pyrromethane (**1**) are new compounds which gave satisfactory elemental analyses and mass and n.m.r. spectra compatible with the assigned structures.

[4] A. M. d'A. R Gonsalves, G. W. Kenner, and K. M. Smith, *Chem. Comm.*, 1971, 1304.

[5] G. W. Kenner and K. M. Smith, *Ann. N.Y. Acad. Sci.*, in the press.

[6] G. W. Kenner, K. M. Smith, and M. J. Sutton, *Tetrahedron Letters*, submitted for publication.

[7] E. F. Carell and J. S. Kahn, *Arch. Biochem. Biophys.*, 1964, **108**, 1.

[8] The incorporation of side-chain ^{3}H and ^{14}C labelled coproporphyrinogen-III into protoporphyrin-IX using this system has recently been reported: A. R. Battersby, J. Staunton, and R. H. Wightman, *J.C.S. Chem. Comm.*, 1972, 1118.