Order of Assembly of the Four Pyrrole Rings during Biosynthesis of the Natural Porphyrins

By ALAN R. BATTERSBY,* CHRISTOPHER J. R. FOOKES, GEORGE W. J. MATCHAM, and EDWARD MCDONALD
(*University Chemical Laboratory, Lensfield Road, Cambridge* CB2 1EW)

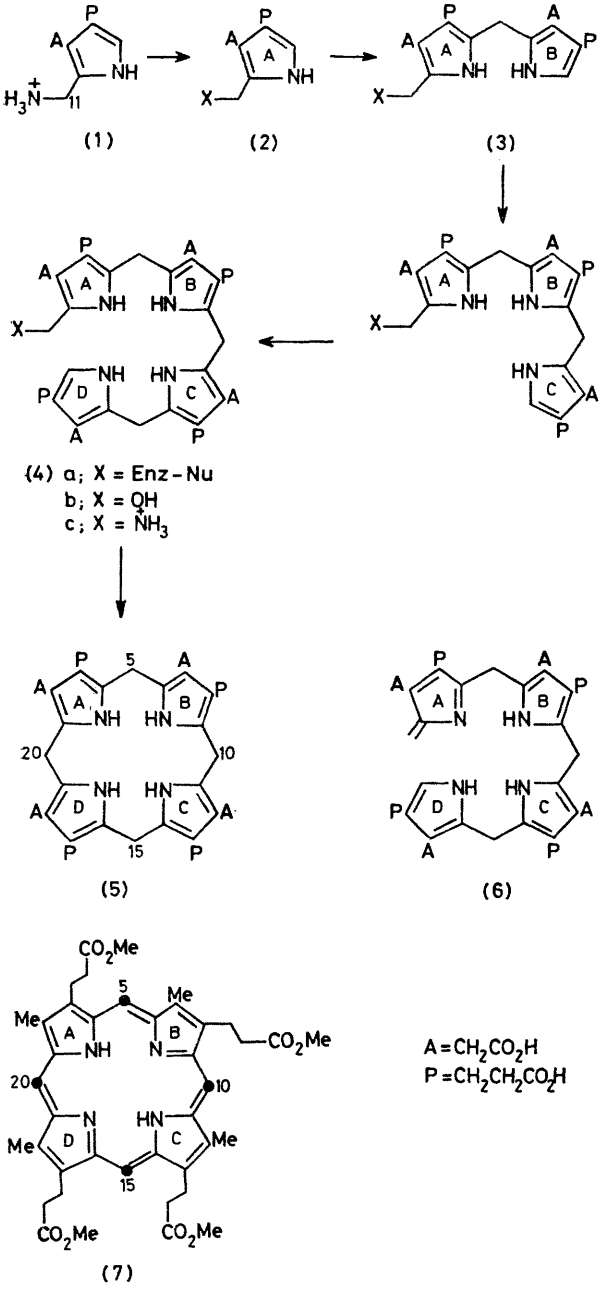
Summary ^{13}C-Labelling experiments involving relatively large quantities of deaminase-cosynthetase show that the four pyrrole rings of uro'gen-III (**5**), and thus also of the natural porphyrins, are assembled starting from ring A with sequential addition of rings B, C, and finally ring D; ring D is that which undergoes rearrangement.

THE way in which the enzyme system deaminase-cosynthetase converts four molecules of porphobilinogen (**1**), PBG, into uro'gen-III (**5**) has been largely defined over the last ten years.[1] Four PBG molecules are joined head-to-tail to generate the unrearranged bilane (**4**), in which the amino group of that molecule forming ring A has additionally been displaced, presumably by a nucleophilic group on the enzyme (**4a**). In the absence of cosynthetase this unrearranged bilane is released into the medium from deaminase as the hydroxymethylbilane[2] (**4b**) which cyclises chemically to uro'gen-I. However, when cosynthetase is also present, the hydroxymethylbilane (**4b**) is not detected and uro'gen-III (**5**) is rapidly produced.[2] The rearrangement which lies at the heart of the type-III problem thus takes place intramolecularly at this late stage in the overall conversion of PBG (**1**) into uro'gen-III (**5**). The order of assembly of the four pyrrole rings of the bilane (**4**) (and therefore of uro'gen-III) was, however, unknown; the following experiments provide this knowledge.

The deaminase-cosynthetase system, *ca.* 0·25 μmol, was isolated from *E. gracilis* and a *deficiency of unlabelled PBG* (**1**), 0·5 μmol, was added at 4 °C. After a short time, it would be expected that the 'loading' of the site on to which the first PBG unit binds should be highest whilst the corresponding 'loadings' at the other sites should decrease such that the last site to be filled carries least. The 'loading' referred to covers pyrrolic material on that site whether it be monopyrrole (*e.g.* **2**) or part of a di- (*e.g.* **3**), tri-, or tetrapyrrole.

At this point, an excess of 90 atom % [11-^{13}C]PBG (as **1**) was added to the partially loaded enzyme to sweep the bound pyrrolic species through to form uro'gen-III (**5**). This was dehydrogenated to uroporphyrin-III and, after

chemical decarboxylation and esterification, the resultant enriched coproporphyrin-III tetramethyl ester (**7**) was studied by ^{1}H n.m.r. spectroscopy using Eu(fod)$_3$ shift reagent.



The unambiguous signal assignments on the Figure are based on synthetic specifically ^{13}C-labelled samples of (**7**). The Figure reveals that the ^{12}C-content is greatest at C-20 and that the ^{12}C-levels in the other bridge carbons fall in the order C-5 > C-10 > C-15. Quantitatively, 30% of all the ring A of the uro'gen-III (**5**) formed is derived from the original unlabelled PBG and similarly, 18, 8, and 3 ± 1%, respectively, of rings B, C, and D. These values take into account that the enrichment of the [11-^{13}C]PBG is 90 atom %. Thus the first PBG unit to bind becomes ring-A (with C-20), the second ring B (with C-5), the third ring-C (and C-10), and the fourth ring-D (and C-15). Attention is drawn to the use here of ^{12}C-'labelling' against a background of ^{13}C-material which in this case affords a greater sensitivity of detection than would the reverse approach.

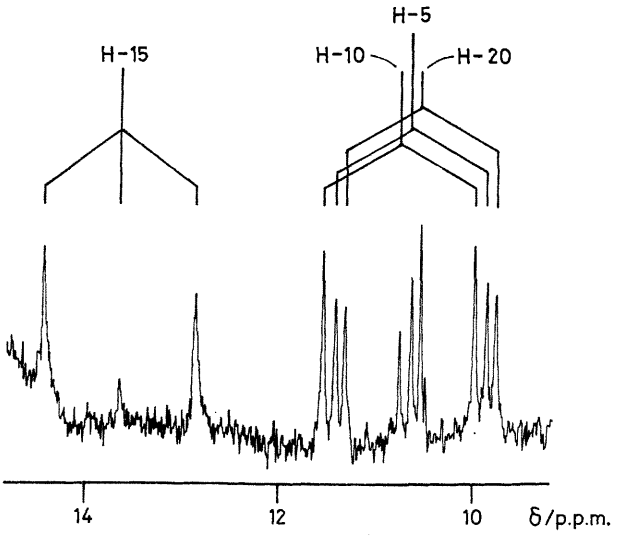


FIGURE. ^{1}H-N.m.r. signals from the bridges (positions 5, 10, 15, and 20) of coproporphyrin-III tetramethyl ester in the presence of Eu(fod)$_3$ from experiment to discover the order of assembly of the pyrrolic rings.

These results not only have their intrinsic value but they mesh with earlier findings. Thus, experiments with diluted doubly labelled PBG had proved[1] that uro'gen-III is formed *via* a single intramolecular rearrangement of ring D. Since it is now clear that ring D is the last pyrrole ring to be built into the tetrapyrrole, it follows that rearrangement *must* be at the tetrapyrrole level rather than earlier, which is precisely the conclusion drawn from studies with synthetic aminomethylbilanes[3] (*e.g.* **4c**). This latter conclusion would be even stronger [bearing in mind the formation of the hydroxymethylbilane (**4b**) from PBG by deaminase] if the aminomethylbilane (**4c**) were shown to be converted into the same hydroxymethylbilane (**4b**) by deaminase.

Accordingly, [*aminomethyl*-^{13}C]bilane (as **4c**) was treated with highly purified deaminase from *Euglena gracilis* and the enzymic reaction was stopped before completion by adjustment of the mixture to pH 12. The ^{13}C n.m.r. spectrum of the total reaction mixture showed significant accumulation (15—20%) of [*hydroxymethyl*-^{13}C]-bilane (as **4b**), δ 57·20 p.p.m. (relative to $NaO_2CCD_2CD_2$-$SiMe_3$) for $HO^{13}CH_2$-pyrrole group (*cf.* ref. 2). The amount of the hydroxymethylbilane (**4b**) accumulated from (**4c**) is inevitably much less than from PBG (**1**) since the rate of its enzymic formation is lower[2] whereas the chemical rate of ring-closure remains the same.

The combined results on order of assembly and on formation of hydroxymethylbilane show that synthetic aminomethylbilane (**4c**) enters the normal biosynthetic

pathway at an advanced stage by enzymic replacement of the amino group to form a reactive species such as (**4a**) or (**6**), a possibility which has been emphasised earlier.[1,2,4]

Grateful acknowledgement is made to the Nuffield Foundation, S.R.C., and Roche Products Ltd. for financial support.

(*Received, 8th March* 1979; *Com.* 232.)

[1] A. R. Battersby and E. McDonald, *Accounts Chem. Res.*, 1979, **12**, 14.
[2] A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, E. McDonald, and (in part) K. E. Gustafson-Potter, *J.C.S. Chem. Comm.*, 1979, 316.
[3] A. R. Battersby, C. J. R. Fookes, E. McDonald, and M. J. Meegan, *J.C.S. Chem. Comm.*, 1978, 185 and references therein.
[4] A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham and E. McDonald, *J.C.S. Chem. Comm.*, 1978, 1064.