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Biosynthesis of Type-III Porphyrins: Proof of Intact Enzymic Conversion of the Head-to-Tail Bilane into Uro'gen-III by Intramolecular Rearrangement

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Summary Synthesis of doubly ^{13}C-labelled forms of the unrearranged bilane (**2**) allows proof beyond doubt that the biosynthesis of natural porphyrins (and relatives) occurs by head-to-tail assembly of 4 PBG units *followed* by intramolecular rearrangement.

The long-standing problem of biosynthesis of the natural type-III porphyrins from four molecules of porphobilinogen (PBG) (**1**) was opened up by first proving[1] that *a single rearrangement* occurs which is intramolecular with respect to the PBG unit forming ring-D of uro'gen-III (as **3**). Then it was shown[2] that the [15-^{13}C]bilane (as **2**), which corresponds to combination of 4 PBG units without rearrangement, was converted efficiently (30% yield) into [15-^{13}C]-uro'gen-III (as **3**) by a purified preparation of the enzymes deaminase–cosynthetase from *Euglena gracilis*. This finding strongly supported the view[2] that the rearrangement step occurs *after straightforward assembly of* 4 *PBG units*. However, others have thought[3,4] that the enzymic process involves initial rearrangement by head-to-head reaction of the first 2 PBG units and enzymic transfer of a C_1-unit. It was thus essential to prove that enzymic conversion of the bilane (**2**) into uro'gen-III (**3**) takes place without breakdown and by an intramolecular process. The necessary experiments now outlined used two synthesised samples of doubly ^{13}C-labelled bilane, one labelled at ● (**2a**) and the other at ▲ (**2b**).

The pyrrole (**5**), prepared by Clezy's method[5] from dibenzyl [2-^{13}C]malonate (90 atom%) and the ketone (**4**), was converted into the corresponding 5-aldehyde and decarbonylated.[6] Formylation with dimethyl [formyl-^{13}C]-formamide (90 atom%) gave the [$^{13}C_2$]-aldehyde (**6**) from which was prepared[7] the aldehyde (**7**). This, after dilution with 3 parts of unlabelled material, was converted as earlier[2] into the bilane (**2a**) containing 20·3% of doubly labelled molecules. When the bilane (**2a**) was incubated with deaminase–cosynthetase (pH 7·1, 37 °C, 16 h), it gave, after the usual work up,[2] a mixture containing 75% of uroporphyrin-III ester (**9a**) (34% yield) and 25% of uroporphyrin-I ester (11% yield). Analysis was by high pressure liquid chromatography[2] after decarboxylation and re-esterification to the corresponding coproporphyrin-III ester (**10a**) and the type-I isomer, respectively.

The ^{13}C-n.m.r. spectrum† of the isolated coproporphyrin-III ester (**10a**) showed at *ca.* 97 p.p.m. a strong 72 Hz doublet[1] centred on a smaller singlet and this pattern moved 5·7 p.p.m. upfield on addition of 1 equiv. of $Pr(fod)_3$. It follows‡ that ^{13}C at C-15 is directly bonded to ^{13}C at C-16 as illustrated (**10a**), and this holds also for uro'gen-III (**3a**).

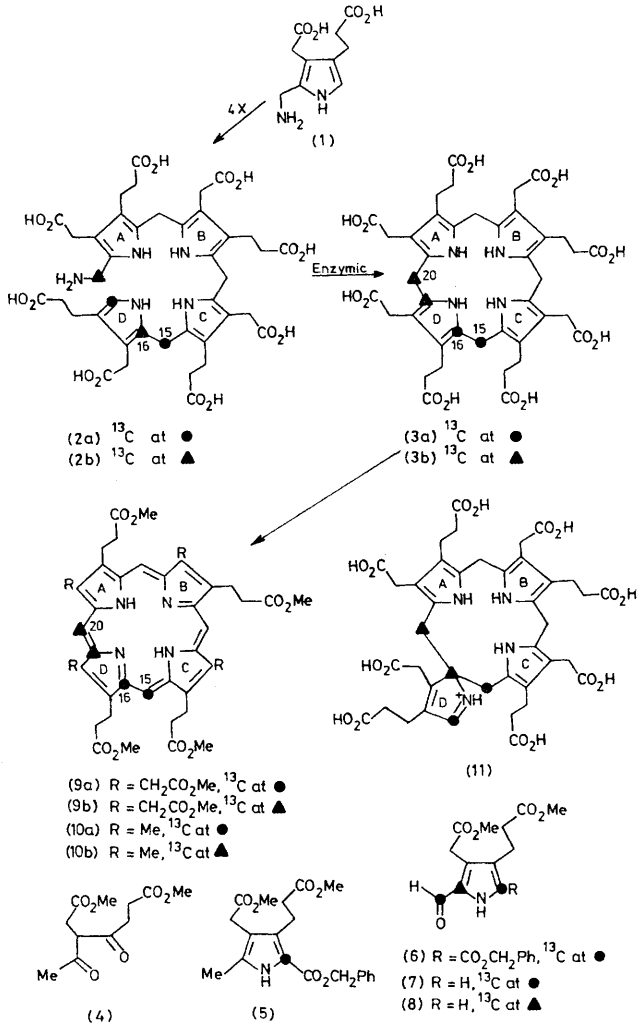
The relative sizes of the split and unsplit n.m.r. signals from C-15 of (**10a**) provide a sensitive check on the proportion of doubly and singly labelled species; those found corresponded within experimental error to there having been no change in the content of either during the enzymic conversion of bilane (**2a**), *diluted with unlabelled bilane*, into

† All ^{13}C-n.m.r. spectra were run using proton noise-decoupling and the signals discussed are those solely from the '*meso*' bridges.

‡ There is strong evidence that C-15 of coproporphyrin-III ester (**10**) is preferentially affected by shift reagents (see refs. 1 and 8) but rigorous proof came from unambiguous synthesis of [15-^{13}C]coproporphyrin-III ester (as **10**) and appropriate n.m.r. study.

uro'gen-III (**3a**). This was confirmed by analysis of the molecular ion group in the mass spectrum of (**10a**); found 21 ± 2% doubly labelled.

Thus no chemical cleavage occurs between C-15 and ring-D of the bilane (**2a**) before its enzymic conversion into uro'gen-III (**3a**) and importantly, the inversion of ring-D is proved to occur by an intramolecular process, exactly as found[1] for PBG.



Chemical cyclisation (no enzyme) of (**2a**) gave a uroporphyrin ester (47% yield) which was converted into coproporphyrin ester and shown to be essentially pure type-I (96%). The low field (*meso*) region of its ^{13}C-n.m.r. spectrum was, as expected, a 6 Hz doublet, corresponding[6] to unchanged 3-bond separation of the two ^{13}C-labels.

To prepare the second bilane (**2b**), the pyrrole (**8**) was synthesised largely by published methods[7] starting from [1-^{13}C]acetic acid (90 atom %). This and [11-^{13}C]PBG lactam[9] (90 atom %) as earlier[2] gave the bilane (**2b**) which was diluted with unlabelled bilane as before and incubated with the *Euglena* enzyme system. The mixed uroporphyrins produced (35% yield) were decarboxylated and shown to comprise type-III (**10b**) (81%) and type-I (19%); a parallel chemical cyclisation yielded only the type-I isomer.

The ^{13}C-n.m.r. spectrum of the separated coproporphyrin-III ester (**10b**) showed in the '*meso*' region, a strong 72 Hz doublet centred on a smaller singlet which, in agreement with the label being at C-20, were virtually unaffected by 1 equiv. Pr(fod)$_3$. Again the relative sizes of these split and unsplit signals proved that the entire bilane (**2b**) had been converted enzymically into uro'gen-III (**3b**) without detectable breakdown; mass spectrometry as above gave confirmation.

Rigorous proof that (**10b**) above was specifically labelled at C-20 was provided by rational synthesis of [20-^{13}C]coproporphyrin-III ester (as **10**) followed by appropriate comparison by n.m.r. spectroscopy, using shift reagents, with the biosynthetic product (**10b**).

All the foregoing results interlock perfectly and also with earlier findings.[1,2,10] They prove beyond doubt that the biosynthesis of natural porphyrins involves head-to-tail assembly of 4 PBG units (**1**) to form the unrearranged bilane (**2**), or its enzyme bound equivalent, which is converted *intact* into uro'gen-III (**3**), by an intramolecular process, ready for construction of haem, the chlorophylls, cytochromes, and vitamin B_{12}. Finally, these results limit to *two* the processes which are possible for the rearrangement of (**2**) → (**3**); that involving the spiro-intermediate[11] (**11**) is preferred§ and work is in hand on this last remaining facet of the problem.

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§ The second possibility involves fission of the C-15–C-16 bond of (**2**) whilst it is bound to the enzyme, followed by inversion of ring-D without exchange of the now separated fragment with the medium.

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