Mechanistic Study of the Enzymic Incorporation of Unrearranged AP·AP Pyrromethane into Uro'gen-III

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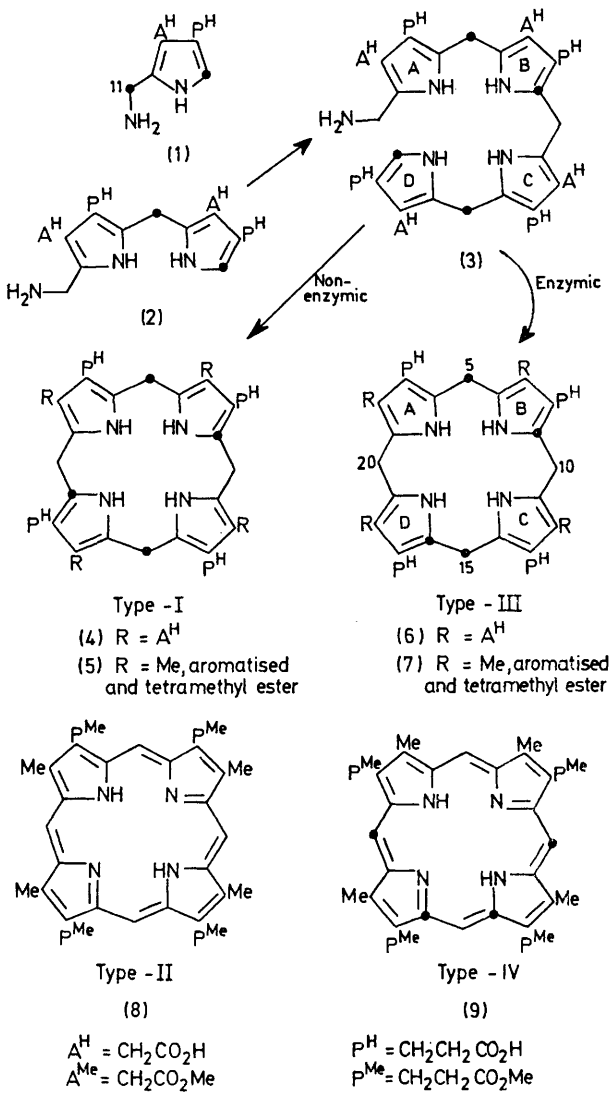
Summary Experiments with $^{13}C_2$-labelled materials prove that the formation of uro'gen-III (**6**) from unrearranged AP·AP pyrromethane (**2**) by deaminase-cosynthetase is mechanistically equivalent to what was discovered earlier for porphobilinogen.

STUDIES of the enzymic conversion of [2,11-$^{13}C_2$]PBG (**1**) with rearrangement into uro'gen-III (**6**) uncovered the three characteristic features of this vital process[1] (summarised in ref. 2). In particular, the PBG unit forming ring-D of (**6**) undergoes rearrangement which is intramolecular with respect to that unit. If it can be shown that the same holds true for the residue which provides ring-D when the AP·AP pyrromethane (**2**) is built enzymically[3] into uro'gen-III (**6**) then interlocking strength is provided. The necessary experiments are outlined here.

The synthesis of [$^{13}C_2$]-AP·AP pyrromethane (**2**) was largely as earlier[1,4,5] to give material in which 81% *of the labelled molecules carried two* ^{13}C-*atoms*. Dilution with *ca.* 3 parts of unlabelled material reduced the ^{13}C-enrichment at the labelled sites to *ca.* 20 atoms %.

Incubation of this product with deaminase–cosynthetase as previously[2,3] followed by the same work-up gave a mixture of coproporphyrin 4-Me esters of type-I (**5**, 15%), type-III (**7**, 57%), and type-IV (**9**, 28%).

The crucial carbon atom to study is C-15 of the type-III isomer (**7**) in this mixture and the ^{13}C-n.m.r. analysis was focussed on it as follows; for simplicity, only the signals from the *meso*-bridges (C-5, C-10, C-15, and/or C-20) will be considered. (a) The spectrum of the total product was determined and it showed a sharp doublet (70 Hz) centred on a broad signal; these represent the sum of signals from the enriched *meso*-carbons of the three coproporphyrin esters (**5**), (**7**), and (**9**). (b) The spectrum was re-run in the presence of Pr([$^2H_9$]fod)$_3$ and it then showed the 70 Hz doublet moved massively upfield[2] and the previously broad signal separated into three clear doublets (*J ca.* 3—3·5 Hz); see Figure. (c) The content of type-I (**5**) was halved



(h.p.l.c. analysis) by chromatography on cellulose and the spectrum run as for (b) allowed assignment of the signal for type-I (**5**). (d) The shifted spectrum was re-determined after addition of sufficient unlabelled type-III ester (**7**) to enhance the signal in the centre of the 70 Hz doublet, so confirming the assignment of this signal to C-15 of the type-III isomer (**7**).

The ^{13}C-signals in the Figure are in full agreement[1,7] with the expected labelling patterns illustrated for the type-I (**5**) and type-IV (**9**) systems produced *chemically* from the AP·AP pyrromethane (**2**). More importantly, the 70 Hz doublet which is strongly shifted upfield together with the above data establish that the enzymic formation of (**6**) from (**2**) involves ^{13}C at C-15 becoming directly bonded to the ^{13}C-atom of the pyrrole to which it was originally attached; *i.e. intramolecular rearrangement* occurs with respect to the ring-D unit. Thus, the incorporation of the AP·AP pyrromethane (**2**) *via* the bilane (**3**) enzymically into uro'gen-III (**6**) is mechanistically equivalent to what was discovered for [2,11-$^{13}C_2$]PBG (**1**).

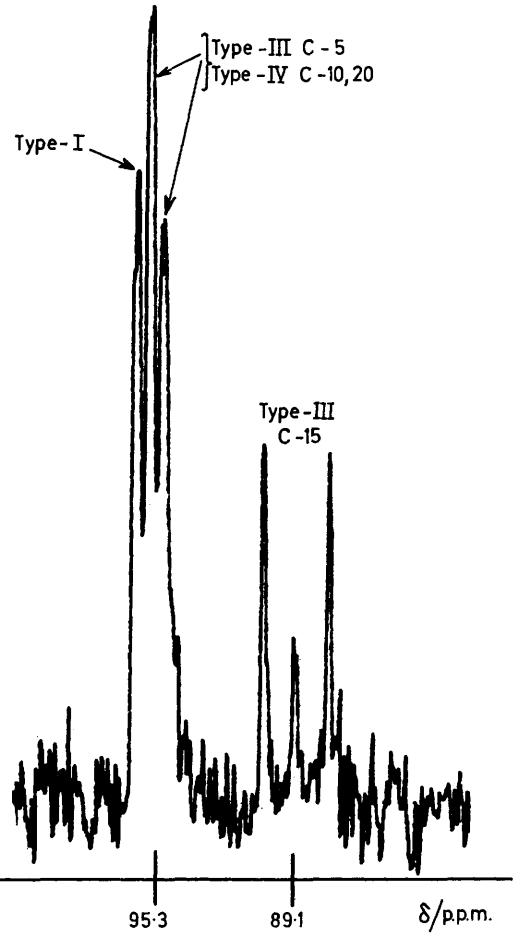


FIGURE. ^{13}C-N.m.r. spectrum from *meso* bridges of [^{13}C]-coproporphyrin esters determined in $CDCl_3$ in presence of Pr([$^2H_9$]fod)$_3$; chemical shifts downfield from Me_4Si.

The findings reported here and in the preceding communication[3] confirm, and, further, allow understanding of, all the results previously reported from Cambridge[6] on the incorporation of ^{14}C and ^{13}C labelled AP·AP pyrromethane (as **2**) into type-III porphyrins. They also add strength from a different approach to the conclusion of the accompanying communications that the *unrearranged* bilane (**3**, or with NH_2 replaced by enzyme) is the precursor of uro'gen-III (**6**).

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