Biosynthesis of Type-III Porphyrins: Nature of the Rearrangement Process

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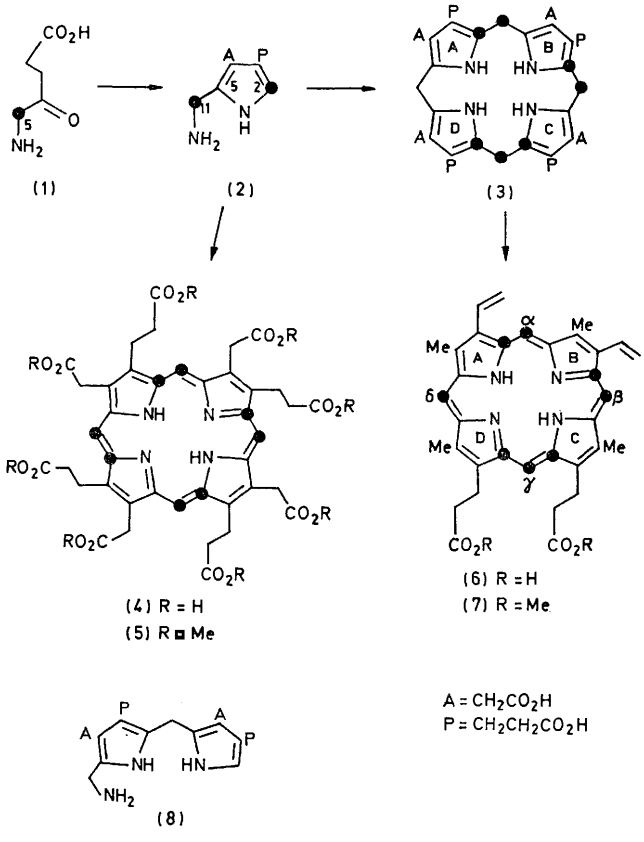
Summary During the biosynthesis of the macrocycle of natural porphyrins (type-III isomer), the porphobilinogen (PBG) unit forming ring D, and no other PBG unit, is found to undergo *intramolecular* rearrangement; ^{13}C-n.m.r. measurements using double labelled [2,11-$^{13}C_2$] PBG were used.

THE vitally important porphyrin derivatives haem, the cytochromes, and the chlorophylls are all based on the type-III isomer [see (**3**) and (**6**)] where the side chains on ring D are of reversed sequence. There has been intense speculation[1] about the way porphobilinogen, PBG [as (**2**)], is converted by the co-operative action of synthetase and cosynthetase enzyme systems into uroporphyrinogen-III (**3**) which is the precursor[2] of the key substance protoporphyrin-IX (**6**). It has been proved[3] recently that the type-III macrocycle once formed does not undergo rearrangement over the biochemical stages from (**3**) to (**6**). Our studies of the mechanism of type-III isomer formation using ^{13}C-n.m.r. spectroscopy are now outlined.

5-Amino[5-^{13}C]laevulinic acid[4] (**1**, 90 atom% ^{13}C) was converted by ALA-dehydratase[5] into [2,11-$^{13}C_2$]porphobilinogen (**2**). The mass spectrum of a derivative of this product confirmed the presence of two sites with 90 atom% ^{13}C† as indicated in (**2**). The size and complexity of ^{13}C–^{13}C couplings in the porphyrin series were then determined as follows. Uroporphyrinogen-I synthetase[6] transformed the ^{13}C-PBG (**2**) into uroporphyrinogen-I which was aromatised to form the *symmetrical* uroporphyrin-I (**4**) isolated as its ester (**5**). The ^{13}C-signal‡ from the four



† *Ca.* 81% of the porphobilinogen molecules carry two ^{13}C atoms.

‡ Chemical shifts, δ, in p.p.m. downfield from Me_4Si for solutions in $CDCl_3$.

equivalent *meso*-carbons appeared§ with *ca.* 90% as a double doublet (*J* 72 and 5 Hz) centred on a broad singlet, *ca.* 10%, at δ 97·5; the small splitting arises from longer range coupling (^{13}C–C–N–^{13}C). The four equivalent labelled positions in the pyrrolic rings gave rise to a similar pattern at δ 143·5.

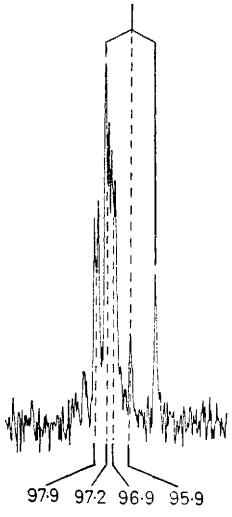


FIGURE. ^{13}C-*N.m.r.* *spectrum* ($CDCl_3$) *of protoporphyrin-IX dimethyl ester derived enzymically from diluted* [2,11-$^{13}C_2$]PBG.

Dilution of one part of ^{13}C-PBG (**2**) with four parts of unlabelled PBG gave a product which was converted with an enzyme preparation[7] from avian erythrocytes into uroporphyrinogen-III (**3**) labelled at the sites shown (90 × 0·2 = 18 atom% ^{13}C); *importantly, the majority of single molecules in the assembly contain only two* ^{13}C-*labels*. Product (**3**) was further converted enzymically, without biochemical scrambling,[3] into protoporphyrin-IX (**6**) isolated as its ester¶ (**7**), again labelled as shown. In the following argument, PBG units are designated A, B, C, or D depending on which ring of (**7**) a given unit provides.

If the α-*meso*-carbon and the adjacent carbon in ring A of (**7**) are derived from different PBG molecules, then the ^{13}C signal from the α-*meso* carbon will appear as 82% singlet and 18% doublet (72 Hz) since the probability is 18% that a ^{13}C-atom forming the α-*meso* position will be directly bonded to a second ^{13}C-atom in PBG unit-A. Also, if and only if, the α-*meso* carbon and ring B arise intact from PGB unit-B will the 5 Hz splitting recognised above be observed (81% of the labelled PBG molecules contain ^{13}C at both C-11 and C-2). In that event, the α-*meso* signal will appear as 82% fine doublet (5 Hz) and 18% double doublet (72 and 5 Hz). Conversely, if the α-*meso* carbon originates by *intramolecular* rearrangement of PBG unit-A, the α-*meso* signal will be 90% doublet (72 Hz) and 10% singlet and it will not show the 5 Hz coupling (no ^{13}C–C–N–^{13}C residue in the majority of molecules). These considerations apply equally to the other three *meso* positions.

Each *meso*-carbon of protoporphyrin-IX dimethyl ester (**7**) gives at natural abundance a sharp distinguishable singlet in the ^{13}C-spectrum[8] and these have been rigorously assigned[9] to the α-, β-, δ-, and γ-positions respectively 'reading' from low to high field. The ^{13}C-enriched sample of (**7**) obtained above from the diluted [2,11-$^{13}C_2$]PBG gave the spectrum shown in the Figure. The main signals for the α-, β-, and δ-*meso*-carbons appear as fine doublets (5 Hz) whereas that from the γ-*meso*-carbon is a doublet (72 Hz), without the 5 Hz coupling, centred on a singlet. A closely similar pattern to that in the Figure was obtained from (**7**) formed¶ from the diluted [2,11-$^{13}C_2$]PBG by an enzyme system from *Euglena gracilis*.[10] Our conclusion is that PBG unit-D undergoes *intramolecular* rearrangement during the biosynthesis of the type-III macrocycle in avian blood and in *Euglena* and the results are in agreement with PBG units A, B, and C being incorporated intact.

These findings eliminate most of the speculative mechanisms[1] for the rearrangement process. The aim of current work with labelled pyrromethanes[11] is to pinpoint the stage at which rearrangement occurs.

We thank the Nuffield Foundation and the S.R.C. for financial support.

(*Received, 19th April* 1973; *Com.* 561.)

§ The fine details of coupling have been omitted here and for later spectra since they do not affect the argument and are barely observable at the resolution available. The signal here should be made up of 81% double doublet (72 and 5 Hz) superimposed on 9% doublet (72 Hz) centred on 9% fine doublet (5 Hz) superimposed on 1% singlet; the observed pattern agrees well.

¶ A blank run with boiled enzyme afforded total porphyrin corresponding to <1% of (**7**) isolated in the active run.

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[11] For related work see B. Frydman, S. Reil, A. Valasinas, R. B. Frydman, and H. Rapoport, *J. Amer. Chem. Soc.*, 1971, **93**, 2738; R. B. Frydman, A. Valasinas, H. Rapoport, and B. Frydman, *FEBS Letters*, 1972, **25**, 309. Our results with the four labelled pyrromethanes [(**8**) and three isomers] will be reported when specificity of incorporation and site(s) of labelling have been established.