Biosynthesis of the Natural (Type-III) Porphyrins: Proof that Rearrangement Occurs After Head-to-tail Bilane Formation

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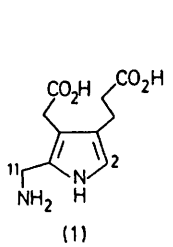
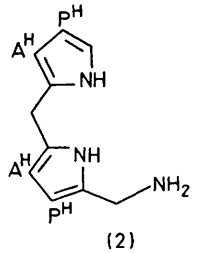
Summary The aminomethylbilane corresponding to head-to-tail combination of four porphobilinogen units is unambiguously synthesised and it is converted (rearranged) by the deaminase–cosynthetase enzyme system into uro'gen-III, the precursor of the natural porphyrins, chlorins, and corrins; ^{13}C-labelling establishes the specificity of the conversion.

HAEM, chlorophyll, and the cytochromes are built in living systems[1] from 4 units of porphobilinogen (**1**) (PBG) with elimination of 4 NH_3. Head-to-tail combination of the PBG units would generate uro'gen-I (**10**) but, in nature, the *unexpected* isomer uro'gen-III (**11**) is specifically formed by the co-operative action of two proteins, deaminase and cosynthetase.[2] Some 25 speculative mechanisms have been proposed (see ref. 1) to account for the rearrangement.



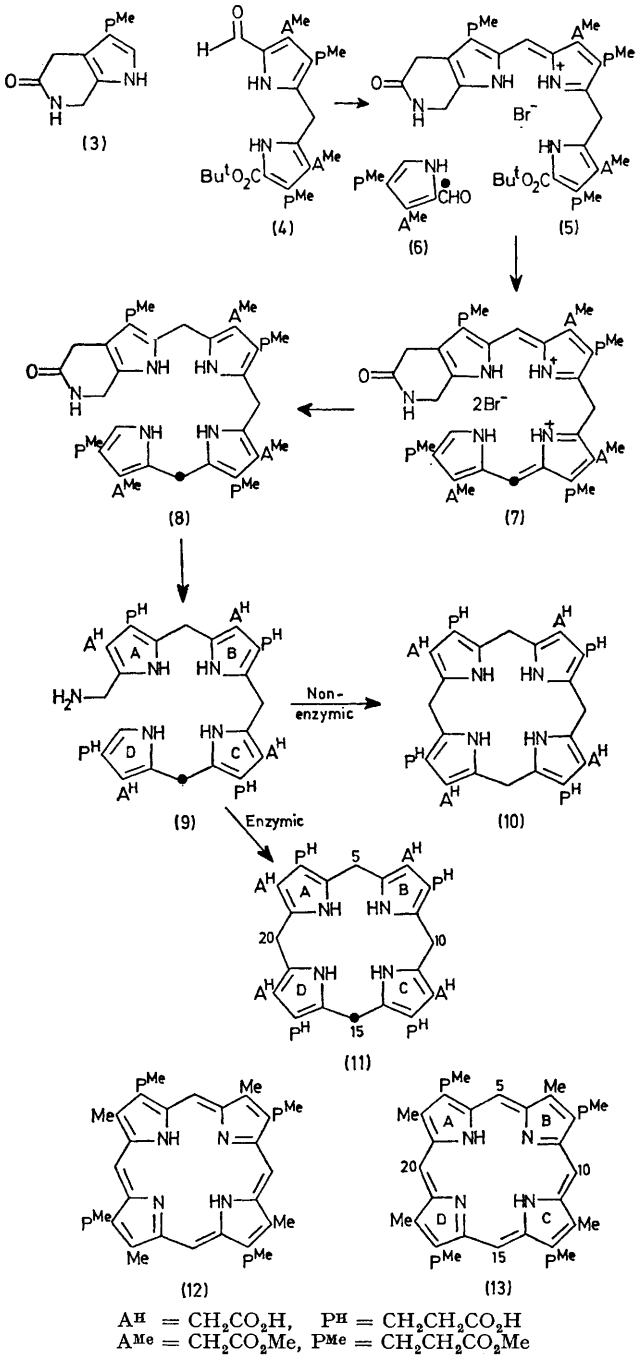
(1)

$A^H = CH_2CO_2H$



(2)

$P^H = CH_2CH_2CO_2H$



Studies[3] with [2,11-$^{13}C_2$] PBG (**1**) greatly reduced the viable possibilities by establishing (a) rings A, B, and C of uro'gen-III (**11**) are derived from intact unrearranged PBG units,† (b) the PBG unit which forms ring D undergoes rearrangement which is intramolecular with respect to that PBG unit, and (c) the rearranged carbon atom forms C-15 of (**11**). With the problem solved of what happens, attention is focussed on when and how.

Frydman[4] favoured rearrangement as the initial step with chain building continuing on the rearranged pyrromethane [(**2**), or enzyme-bound form]. Recently, Scott[5] claimed proof of initial rearrangement involving head-to-head reaction of the first 2 PBG units and enzymic transfer of a C_1 unit.

The studies outlined in these communications show in contrast that the key rearrangement step occurs *after the unrearranged bilane* [(**9**) or enzyme-bound form, replace H_2N by enzyme] has been built by head-to-tail reaction of 4 PBG units.

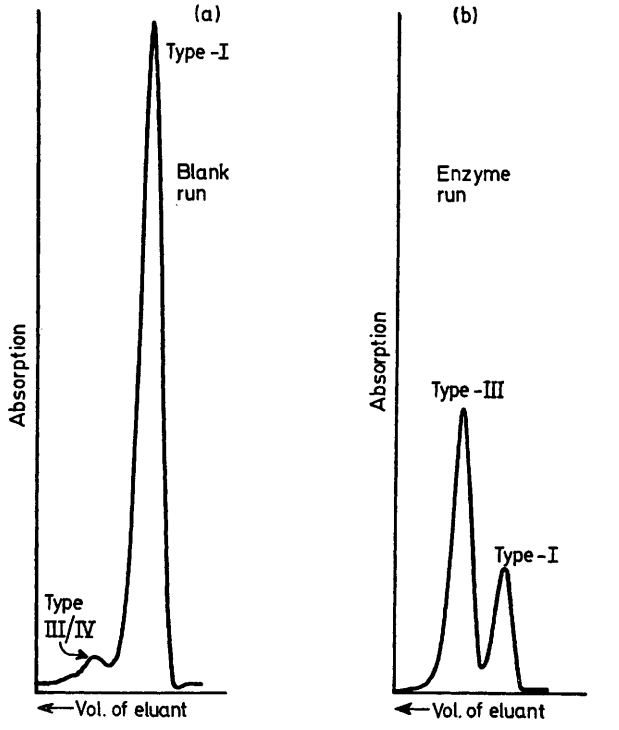


FIGURE. H.p.l.c. traces for analyses of coproporphyrin esters run on C_{18} reverse phase column; u.v. analyser set at *ca.* 390 nm. (a) Blank run; (b), enzyme run.

Interaction of the formylpyrromethane‡ (**4**) with PBG lactam ester (**3**) gave the hydrobromide‡ (**5**) (65%) which condensed with (**6**) to yield the biladiene-*a*,*c*‡ (**7**) (95%). Hydrogenation of the corresponding base over platinum gave the colourless bilane ester‡ (**8**); *cf.* refs. 6 and 7.

Alkaline hydrolysis of (**8**) gave (**9**) which cyclised[8] at 55 °C, pH 7·2, and the porphyrinogen formed was aromatised with iodine. Decarboxylation then gave the corresponding coproporphyrin whose ester was proved by h.p.l.c.[9] to be type-I (**12**). Thus the non-aromatised product from chemical ring-closure of (**9**) is essentially pure uro'gen-I (**10**). This confirms structure (**9**) and eliminates significant rearrangement at pH 7·2 during non-enzymic ring-closure to (**10**).

A further amount of the bilane (**9**) (4 mg) was incubated at pH 7·2, for 4 h at 37 °C with purified deaminase–cosyn-

† Treatment of PBG (**1**) with deaminase alone produces uro'gen-I (**10**). Dr. Roger Hollenstein of this laboratory has shown by the previous $^{13}C_2$-labelling method (ref. 3) that during this enzymic conversion all four PBG units remain intact.

‡ All new products have been characterised by analysis and/or accurate mass determination, n.m.r. and u.v.-visible spectroscopy, and mass spectrometry.

thetase from *Euglena gracilis* (*ca.* 40,000 units) and a parallel blank was run lacking the enzymes. Both reaction mixtures were quenched with iodine, then worked up and analysed as above. The product from the blank was essentially pure type-I isomer (**12**); see Figure (a). The striking result for the enzymic run§ (Figure, b) showed that 70% of the product was type-III¶ (**13**) the rest being type-I (**12**) formed in the competitive non-enzymic cyclisation. These results demonstrate a major enzymic conversion of the unrearranged bilane (**9**) into uro'gen-III (**11**) and the proportions of type-III and type-I produced show an enzymic rate enhancement for the formation of uro'gen-III (**11**).

The bilane (**9**) was then synthesised with ^{13}C at the position marked ● (90 atom % ^{13}C) and part (10·8 mg) was incubated as before (16 h) with deaminase–cosynthetase. The porphyrins formed (34% yield) were decarboxylated and analysed to show *ca.* 20% of type-I and *ca.* 80%** of type-III. The site of labelling of the latter (as coproporphyrin-III 4 Me ester) was determined by ^{13}C n.m.r. spectroscopy; *ca.* 75%** of the large signal (two almost overlapping singlets) from the fully relaxed ^{13}C enriched sites was moved massively upfield by $Pr([^2H_9]fod)_3$; the signal from the type-I isomer was essentially unaffected. The former result locates the label at C-15 of (**13**) since $Pr([^2H_9]fod)_3$ selectively affects[3,10] this carbon atom of (**13**). In addition to proving specific incorporation, this finding registers the bilane (**9**) with respect to uro'gen-III (**11**) and the corresponding rings are lettered.

At a late stage of our work, Müller *et al.*[11] reported that unlabelled bilane (**9**) (different synthesis) with deaminase–cosynthetase from *P. shermanii* gave 19—20% yields of uroporphyrinogen containing 14—18% of the type-III isomer and 82—86% of type-I isomer; our work and that at Stuttgart are mutually reinforcing.

The foregoing results, supported in accompanying communications, establish *when* rearrangement occurs by which the natural type-III porphyrins are formed; the *nature* of the rearrangement had previously been precisely defined[3] and current work on the intermediate(s) between (**9**) and (**11**) is aimed at understanding *how*.

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§ The yield of porphyrins was 12%; repetition of the enzymic run for 16 h raised the yield to 27%, again 70% type-III, 30% type-I

¶ The material from the left-hand peak was isolated and was re-run under conditions which separate coproporphyrin-III from coproporphyrin-IV 4 Me esters (ref. 9); the product was then shown to be type-III isomer containing 0 to <5% of type-IV isomer.

** Our experience is that the reproducibility of these analyses is *ca.* ±5%.

[1] For review up to September 1974 and leading references see A. R. Battersby and E. McDonald in 'Falk's Porphyrins and Metalloporphyrins,' 2nd edn., ed. K. M. Smith, Elsevier, Amsterdam, 1975.

[2] L. Bogorad and S. Granick, *Proc. Nat. Acad. Sci. U.S.A.*, 1953, **39**, 1176; L. Bogorad, *Science*, 1955, **121**, 878; *J. Biol. Chem.*, 1958, **233**, 501, 510.

[3] A. R. Battersby, E. Hunt, and E. McDonald, *J.C.S. Chem. Comm.*, 1973, 442; A. R. Battersby, G. L. Hodgson, E. Hunt, E. McDonald, and J. Saunders, *J.C.S. Perkin I*, 1976, 273.

[4] B. Frydman and R. B. Frydman, *Accounts Chem. Res.*, 1975, **8**, 201.

[5] A. I. Scott, K. S. Ho, M. Kajiwara, and T. Takahashi, *J. Amer. Chem. Soc.*, 1976, **98**, 1589.

[6] J. A. P. Baptista de Almeida, G. W. Kenner, K. M. Smith, and M. J. Sutton, *J.C.S. Chem. Comm.*, 1975, 111.

[7] J. Engel and A. Gossauer, *J.C.S. Chem. Comm.*, 1975, 570, 713.

[8] *Cf.* R. C. Davies and A. Neuberger, *Biochem. J.*, 1973, **133**, 471.

[9] A. R. Battersby, D. G. Buckley, G. L. Hodgson, R. E. Markwell, and E. McDonald, in 'High Pressure Liquid Chromatography in Clinical Chemistry,' eds. P. F. Nixon, C. H. Gray, C. K. Lim, and M. S. Stoll, Academic Press, London, 1976, p. 63.

[10] For the related case in ^1H n.m.r. spectroscopy, see M. S. Stoll, G. H. Elder, D. E. Games, P. O'Hanlon, D. S. Millington, and A. H. Jackson, *Biochem. J.*, 1973, **131**, 429.

[11] H.-O. Dauner, G. Gunzer, I. Heger, and G. Müller, *Z. physiol. Chem.*, 1976, **357**, 147.