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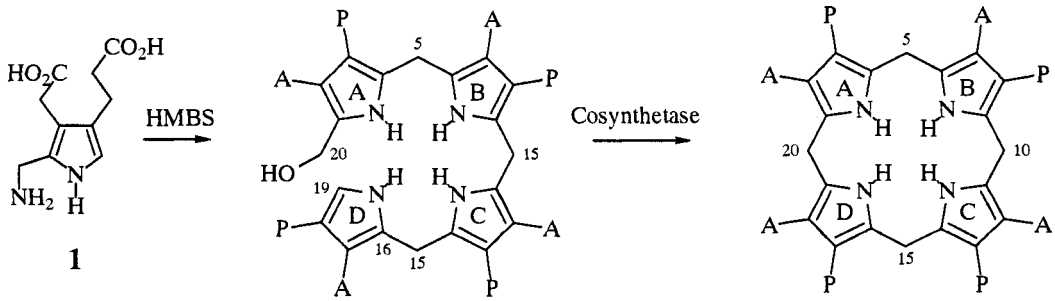
Biosynthesis of the pigments of life. The mystery of the cyclisation and ring D-inversion in the formation of uroporphyrinogen III

L. F. Tietze, H. Geissler and G. Schulz

Institut für Organische Chemie der Georg-August-Universität, Tammannstr. 2, D-37077 Göttingen, FRG

Abstract: Based on experiments and quantum mechanical calculations a new mechanism for the ring D inversion in the formation of uroporphyrinogen III **3** from hydroxymethylbilane and the mode of action of the enzyme cosynthetase is proposed. The easily occurring cyclisation of hydroxymethylbilane is traced back to the substitution pattern at the pyrrole rings forcing the acyclic molecule into a cyclic conformation due to an allylic 1,3-strain.

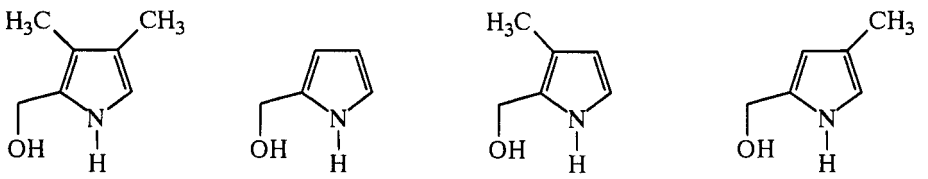
The pigments of life such as hem, the chlorophylls, the bacteriochlorophylls, sirohem, vitamin B_{12} and the factor 430 all derive in Nature from uroporphyrinogen III **3**. Uroporphyrinogen III is formed by enzymatic cyclotetramerisation from porphobilinogen **1** via the acyclic tetramer hydroxymethylbilane **2** by the action of hydroxymethylbilane synthase (HMBS) and uroporphyrinogen III synthase (cosynthetase) (1); in the absence of cosynthetase, uroporphyrinogen I is formed from **1** (2). It can be assumed that the uroporphyrinogens already existed in the premordial broth since the non-biotic acid catalysed transformation of porphobilinogen, which may be formed from two molecules of δ-aminolaevulinic acid, leads to a mixture of the four possible uroporphyrinogens I-IV in the ratio of 12.5 : 12.5 : 50 : 25 under equilibrium (3). Thus, Nature has chosen the main component uroporphyrinogen III **3** as the substrate for the synthesis of the absolutely essential cofactors for photosynthesis, respiration and electron transfer. In this respect, Nature had to develop an enzyme which allows the highly selective formation of uroporphyrinogen III **3** from hydroxymethylbilane **2** with inversion of ring D. In the last 30 years several



1 **2** **3**

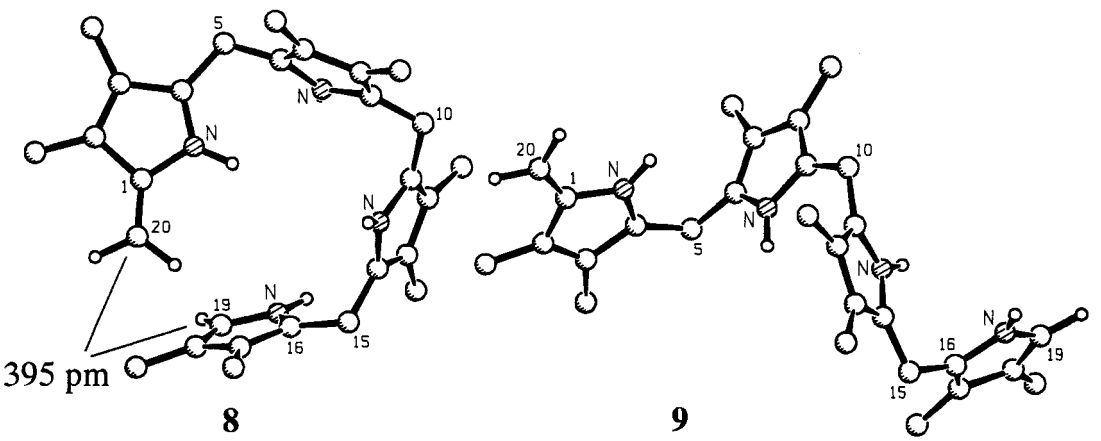
mechanisms have been proposed for this step of which most have been falsified. At the moment, the spiro-

mechanism (4), the fragmentation-recombination mechanism (1,5) and the lactone-mechanism (6) are under discussion. Although these three mechanisms match the known biosynthetic studies, no proof has been given and moreover they do not explain the selective formation of uroporhyrinogen III.



4 **5** **6** **7**

We have now addressed this question by calculations of the transition structures leading to uroporphyrinogen III. In addition, we have performed experiments and calculations using differently substituted hydroxymethylpyrroles **4-7** to explain why porphobilinogen **1** so easily cyclises to give the tetracyclic uroporphyrinogens (7). It was our assumption that the straight forward formation of the cyclic tetramer must be traced back to a conformational fixing of the stepwisely formed acyclic tetramer due to an allylic 1,3-strain (8) caused by the substituents at C-3 and C-4 of the pyrrole moieties. For the calculations the semiempirical AM1/RHF method (9) was used since the structures are too big for ab initio calculations. However, it was shown for the conformation of 2-ethyl-3-methylpyrrole that the AM1/RHF calculation matches well with that of the ab initio calculation (10). The hypersurface for the conformation of the acyclic tetrapyrrole was calculated by an iterative process starting from the lowest energy of the dipyrrole and stepwise adding of a third and fourth pyrrole moiety. It could be shown that the acyclic tetrapyrrole obtained from **4** is more stable in a cyclic conformation **8** by 5.5 kJ·mol^{-1} than in a linear conformation **9** which would lead to a polymeric compound (7). The distance between C-20 and C-19 in **8** is only 395 ppm which would explain the simple cyclotetramerisation. The acid-catalysed cyclization of the hydroxymethylpyrroles **4-7** clearly shows the importance of the substituents at C-3 and C-4 in porphobilinogen **1** since only the analogon **4** gives the cyclic tetramer in good yield, whereas with **5** the yield was below 5% and with **6** and **7** around 20%.

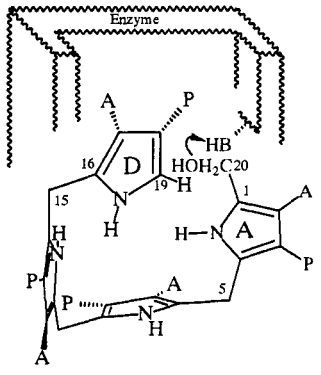


8 **9**

The calculation of the transition structures (11) shows that the formation of the uroporphyrinogen I-σ-complex **10** from **2** via transition state **13** is the most favoured step, whereas the transition structure **14**

leading to the spiro-σ-complex **11** is disfavoured by 47.6 kJ·mol^{-1} compared to **13**. We therefore assume that by the action of cosynthetase at first, hydroxymethylbilane **2** is transformed into the uroporphyrinogen I-σ-complex **10** which then, in an equilibrium, can give the spiro-σ-complex **11** either by several suprafacial 1,5-sigmatropic rearrangements or by a sterically controlled two step mechanism. From **11**, again either by 1,5-sigmatropic rearrangements or by a sterically controlled two step mechanism the uroporphyrinogen III-σ-complex **12** is formed, which gives uroporphyrinogen III **3** by the abstraction of a proton. The feasibility of such 1,5-sigmatropic rearrangements has been shown by calculations, though, for the dipyrrolomethane, the two step mechanism seems to be more favourable. The calculations also confirm that a migration of the alkyl-substituents (A and P) at the pyrroles is highly disfavoured (10).

In summary, according to the calculations the mode of action of cosynthetase can be traced back to a fixation of hydroxymethylbilane **2** into a cyclic conformation and by stabilisation of the primarily formed uroporphyrinogen I-σ-complex by lack of an appropriately situated base in the enzyme pocket which could remove a proton from the uroporphyrinogen I-σ-complex to give uroporphyrinogen I.



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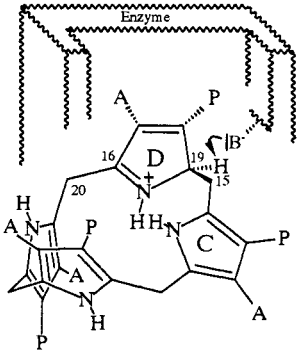

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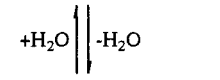

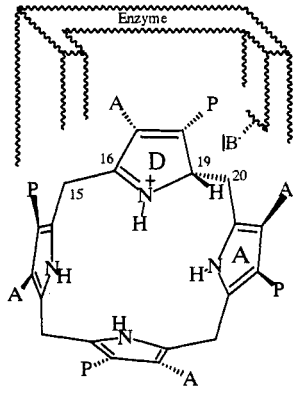

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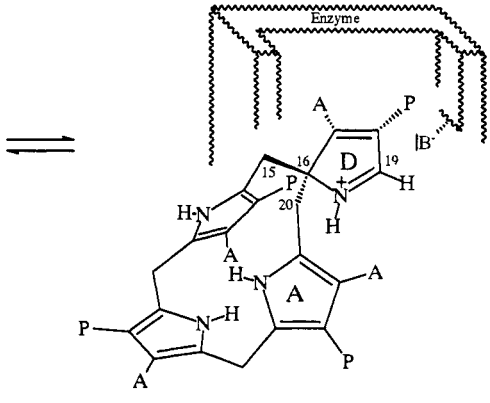

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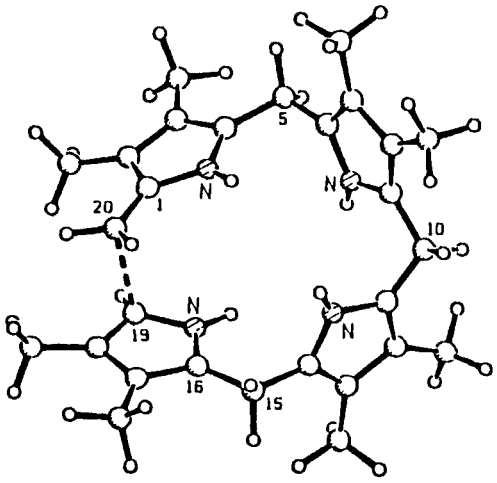

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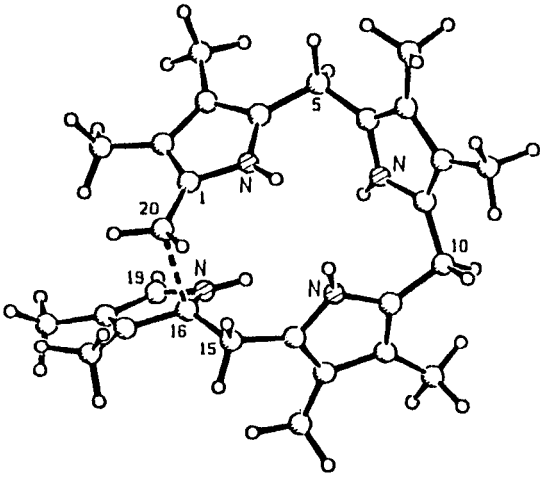

14

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