PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Synthesis of Probable and Improbable Precursors for Porphyrin Biosynthesis [and Discussion]

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Synthesis of probable and improbable precursors for porphyrin biosynthesis

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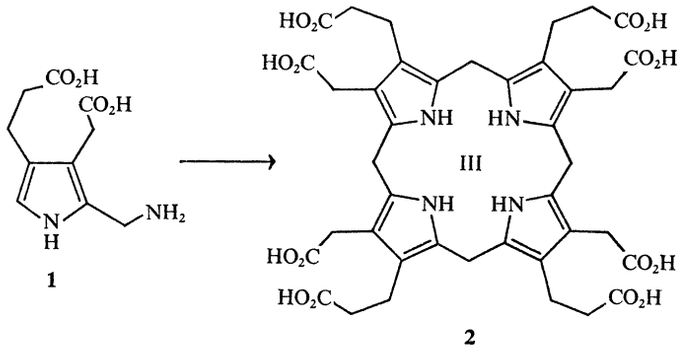
Insights into details of the biomechanism by which porphobilinogen (**1**) cyclotetramerizes to uroporphyrinogen III (**2**) as well as promising synthetic applications are provided by investigation of this reaction *in vitro*. The cyclotetramerization of newly prepared norporphobilinogen (**5**) proved to be extremely specific due to strong conformational control. Advantage was taken of this finding by preparing a *N*,*N*,*N*,*N*-tetramethyl-porphyrinogen (**13**a) for the first time. Protected derivatives of the linear tetramer of porphobilinogen (**20**c) which is regarded as an intermediate of the cyclotetramerization were gained by total synthesis and their transformations investigated.

Introduction

The biosynthesis of biologically active porphyrins is now clarified in its essential parts. Building units and kind of formation of these important natural products are largely known. Among the reactions which take part in porphyrin biosynthesis the construction of uroporphyrinogen III (**2**) from four molecules of the monopyrrole porphobilinogen (**1**) is of special interest to chemists and biochemists. As Cookson & Rimington (1954) had demonstrated, this cyclotetramerization of porphobilinogen (**1**) can be performed *in vitro*, acid or base catalysed. However in contrast to



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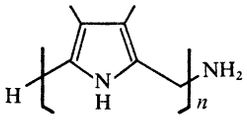

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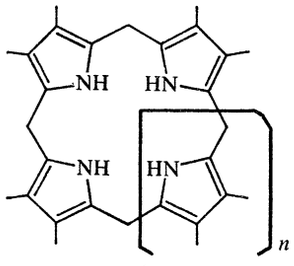


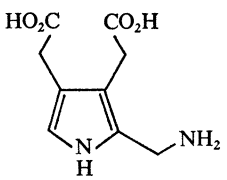
n > 1

4

the specific porphyrin biosynthesis this chemical condensation yields a mixture of four uroporphyrinogen isomers in which uroporphyrinogen III (**2**) predominates (Mauzerall 1960). This condensation reaction differs extremely from other syntheses of macrocyclic ring systems in that besides cyclic tetrapyrroles no other condensation products could be detected or trapped until now. Linear oligomers and polymers of porphobilinogen (**3**) as well as cyclic compounds containing more than four pyrrole units (**4**) might also be expected. According to these considerations it is appropriate to investigate the scope, variability and limitation of this promising reaction. Therefore several monopyrroles as well as di-, tri- and tetrapyrroles derived from porphobilinogen (**1**) were synthesized in order to study their reactivity. Some preliminary results of this work are described below.

Norporphobilinogen (5)

The first aim of the investigation was the question if, in contrast to existing knowledge, other products besides uroporphyrins could be detected during or after cyclotetramerization of porphobilinogen. Such investigations are impeded by the fact that a mixture of uroporphyrinogen isomers is formed from porphobilinogen (**1**). This drawback can be avoided by using *nor*porphobilinogen (**5**) with two identical acetic acid side-chains. So this hitherto unknown monopyrrole was prepared by approved methods of pyrrole chemistry and subjected to acid catalysed condensation with 0·5 M hydrochloric acid. On subsequent evaporation of the solution a deeply coloured porphyrin formed by oxidation of the primary condensation product



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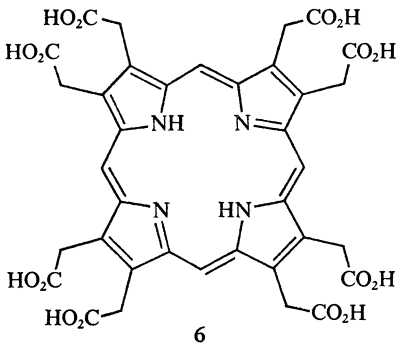

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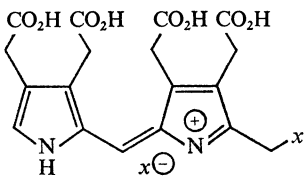

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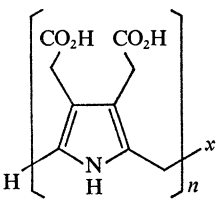

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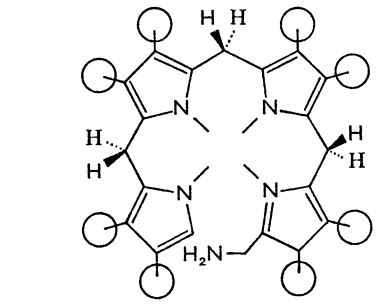
separated in crystalline form with 72 % yield. It was pure and homogenous and proved to be the tetranoruroporphyrin (**6**). Any other condensation products of norporphobilinogen (**5**) derived from noncyclic oligomers (**7**, **8**) could not be traced by intensive chromatographic search.

It might have been possible to trap a dimer of norporphobilinogen by dehyrogenating it to the dipyrrylmethene (**7**), which will not undergo condensation with itself or with the monomer.

However, oxygen as well as different oxidants did not influence the condensation of norporphobilinogen (**5**) to the porphyrinogen. Thus the dehydrogenation of intermediates can apparently not compete with the very fast condensation.

Some information on the reason for the extreme preference of cyclotetramerization compared with other reactions can be gained from an inspection of possible conformations of the linear tetrapyrrole, which may be the direct precursor of the porphyrin. The cyclic conformation (**9**) necessary for the porphyrin ring closure is favoured by relatively high stability. It shows little steric interaction between the side-chains and leaves the hydrogen atoms of the methylene groups in an optimum staggered arrangement to the adjacent pyrrol nuclei. In order to form a conformation (**10**) with the pyrrole units in a line, these have to be rotated by 180° whereby the sidechains in the β-positions become strongly compressed. Although numerous additional nonplanar conformations could also be discussed, this consideration shows clearly the steric preference of the porphyrin-forming conformation. This might be changed by substituting the nitrogen with voluminous groups or by removing the side-chains. With the second alternative the area of biogenetic type reactions would be left. In order to check the first possibility *N*-methylnorporphobilinogen (**11**) was synthesized and its acid catalysed condensation investigated.



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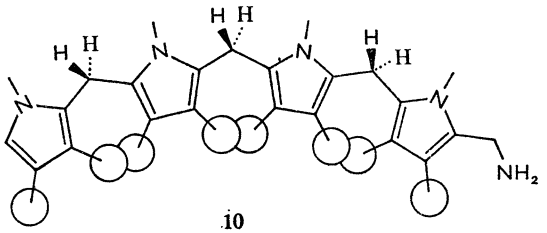

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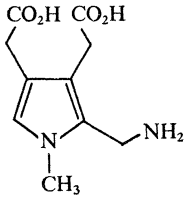
N-Methylnorporphobilinogen (**11**)

A number of research groups had tried without success to prepare porphyrinogens and porphyrins with four *N*-methyl goups (Corwin & Quattlebaum 1936; Jackson & Dearden 1973). Thus it was assumed that such porphyrin derivatives can not exist. Therefore the condensation of *N*-methylnorporphobilinogen should give other, possibly non-cyclic products. In a notable investigation Jackson & Dearden (1973) demonstrated that on condensation of a mixture of an acetoxymethylenpyrrole (**12**a) and its *N*-methyl derivative (**12**b) a low yield of a porphyrin without *N*-methyl groups was formed exclusively. Condensation of the *N*-methylpyrrol did not occur. Efforts to obtain *N*-methylporphyrins by methylation at the complete ground structure did not proceed beyond a *N*,*N*,*N*-trimethylporphyrin even on application of the very strong



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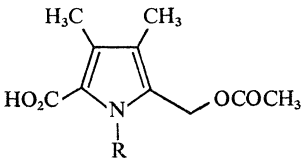

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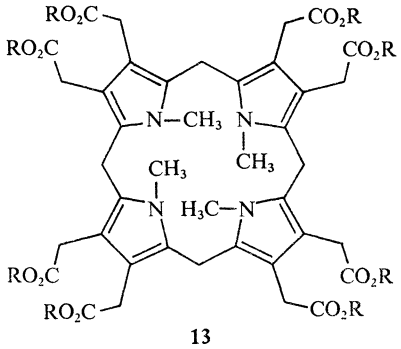
a: R=H
b: R=CH_3

methylating reagent methyl fluorosulphonate (Grigg, Sweeney, Dearden, Jackson & Johnson 1970).

On basis of this knowledge the result of the acid catalysed condensation of *N*-methyl-norporphobilinogen (**11**) was surprising. After treatment with 0.5 M hydrochloric acid during 30 min at 100 °C a colourless product crystallized from the reaction mixture in 17 % yield on cooling. This compound shows a negative Ehrlich colour reaction, it forms an octamethylester (**13**b), and proved itself to be a *N*,*N*,*N*,*N*-tetramethyl-porphyrinogen (**13**a) by its analytical and spectroscopic properties (Franck & Wegner 1975). A compound specially desired in porphyrin chemistry was thus prepared for the first time and quite easily. Interesting information on stereochemistry, aromaticity, and structure–biological activity relationships of porphyrinogens and porphyrins can be expected from its investigation. On the other hand it became apparent from this that larger substituents at the nitrogen than *N*-methyl are necessary for preventing the formation of cyclic tetrapyrroles during condensation of porphobilinogen.

The *N*,*N*,*N*,*N*-tetramethyl-porphyrinogen differs drastically in its chemical properties from



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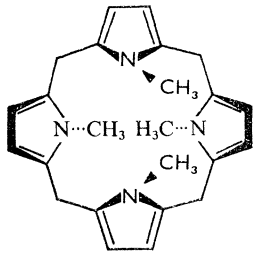

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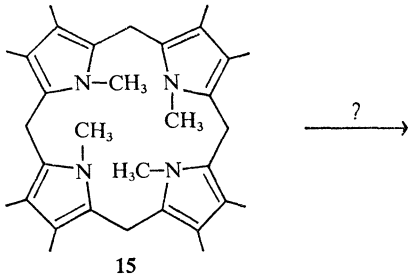
a: R=H
b: R=CH_3



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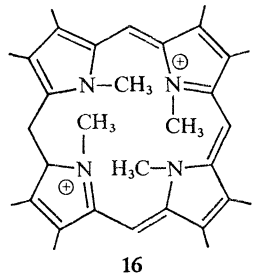

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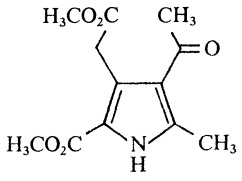
normal porphyrinogens with secondary nitrogen atoms. While porphyrinogens are usually dehydrogenated quickly by oxygen or iodine to form the deeply red coloured aromatic porphyrins, the *N*,*N*,*N*,*N*-tetramethyl-porphyrinogen (**15**) does not form a corresponding bis-quaternary porphyrin (**16**) under these conditions. Dehydrogenation experiments with stronger reagents and conditions are in progress. According to stereomodels the *N*,*N*,*N*,*N*-tetramethyl-porphyrinogen has a conformation (**14**) in which the pyrrole nuclei are strongly rotated against each other. Making it planar as is necessary for formation of aromatic porphyrins would induce heavy strain to the molecule. Starting material for the synthesis of *N*-methylnorporphobilinogen (**11**) was a acetylpyrrole (**17**) obtained by Knorr condensation. It was converted to the protected end-product (**19**) in nine steps with an average yield of 70 %. Transformation of the 3-acetylgroup of **17** with thallium (III) nitrate according to Kenner, Smith & Unsworth (1973) was especially useful herewith. The *N*-methylation could best be performed by warming the isolated and dried potassium salt of the intermediate pyrrole aldehyde (**18**) with methyl iodide.



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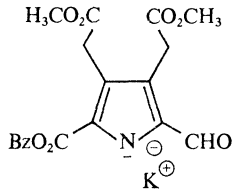

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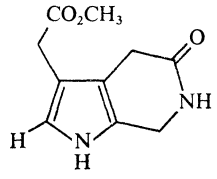

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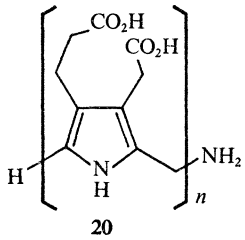
Linear porphobilinogen oligomers

As linear oligomers (**20**a–**20**c) of porphobilinogen (**1**) could not be gained by trapping them during acid catalysed condensation of the monopyrrole it was necessary to prepare the dimers, trimers and tetramers by synthesis, in order to investigate their reactivity. Independently from our efforts, which were started some years ago, several research groups, particularly those of MacDonald and of Frydman succeeded meanwhile in synthesizing dimers and trimers of porphobilinogen (**20**a, **20**b) (MacDonald *et al.* 1972; Frydman *et al.* 1974). Therefore only a tetramer (**20**c) which is of interest as a precursor of uroporphyrinogens will be described here.



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a: $n=2$
b: $n=3$
c: $n=4$

For the linear tetramer (**20**c) of porphobilinogen a high reactivity and condensation tendency had to be expected, as could be extrapolated from the observation (Franck & Eckert 1973) that the dimer (**20**a) condensates 100 times faster than the monopyrrole porphobilinogen (**1**) (see figure 1).

Thus a well-protected derivative (**24**) of the tetramer was chosen as the aim of the synthesis. In this compound the terminal α-position and the aminomethylene group are protected by a carbobenzoxy group and lactam ring closure respectively. One central pyrrole nucleus is desactivated by dehydrogenation, according to MacDonald (1972). From this stable depot compound the tetramer can easily be liberated as its further use demands. The protected

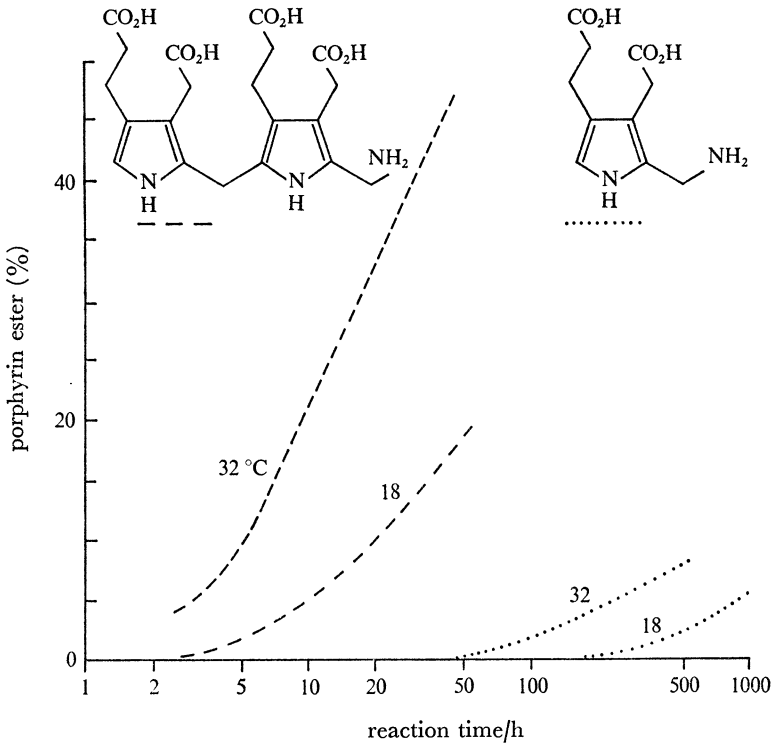


FIGURE 1. Formation of porphyrinester mixtures from a diphyrrylmethane (– – –) and from porphobilinogen (·····) at 32 and 18 °C.

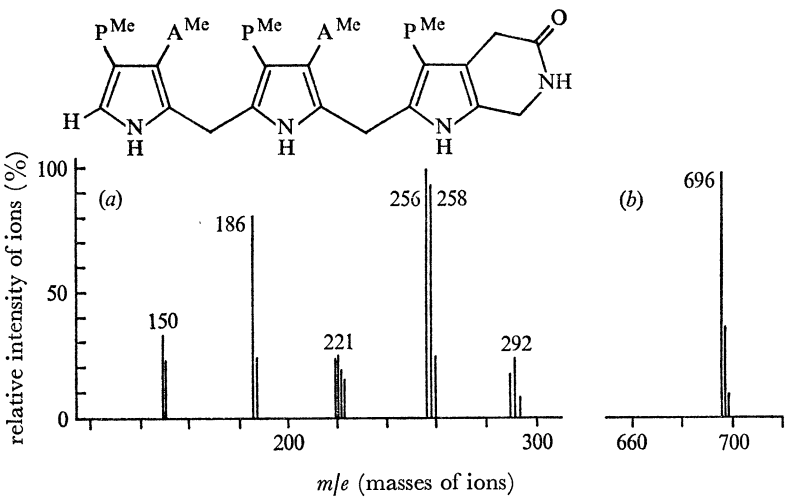
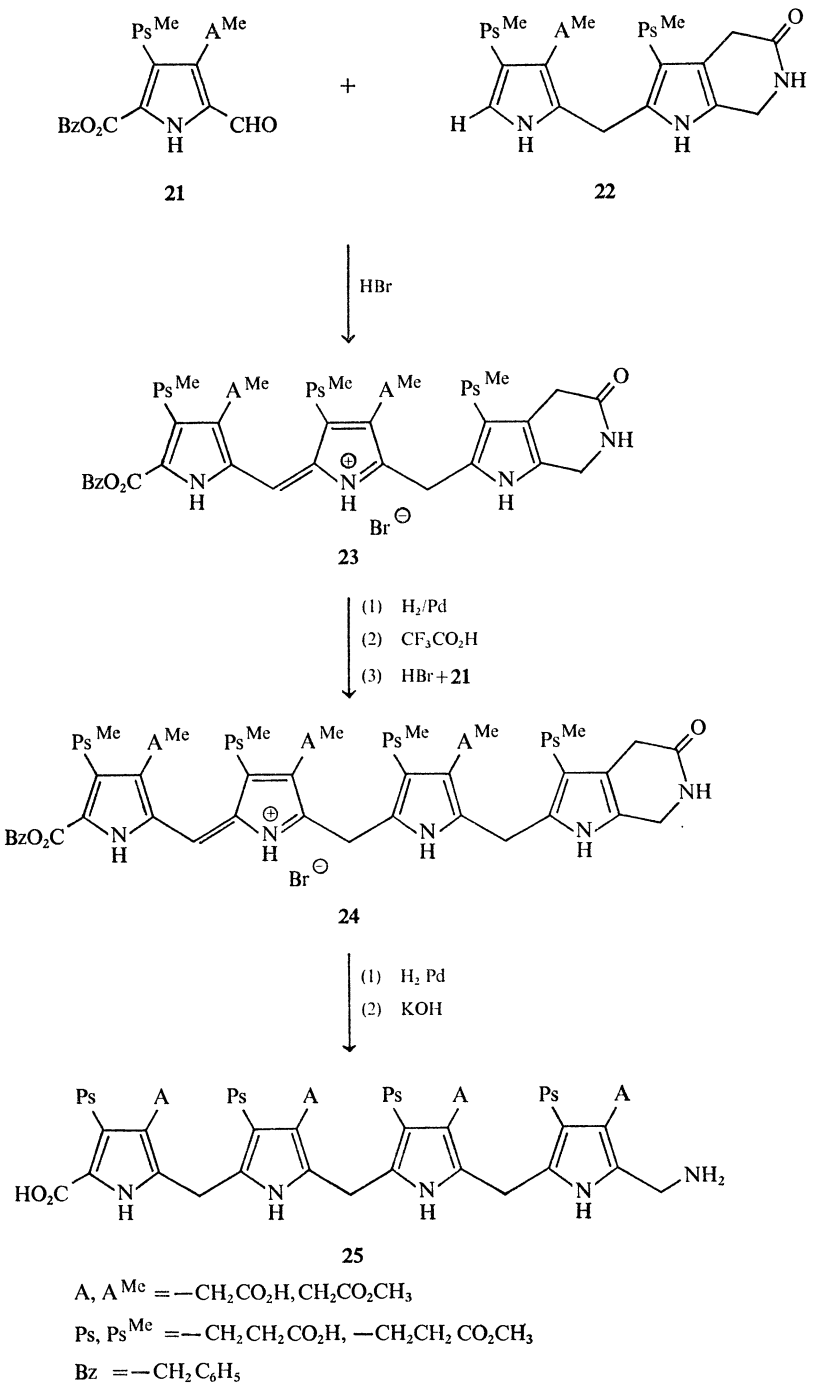


FIGURE 2. Electron impact (*a*) and field desorption mass spectrum (*b*) of the same tripyrrole derivative. The latter shows only the molecular ions.

tetrapyrrole (**24**) was obtained by the reactions shown in the formula scheme in fine red crystals of melting point 168–170 °C. The characterization of **24** as well as of the equally decomposable intermediary products was effectively supported by mass spectrometry with field desorption ionization (Beckey 1969). The superiority of field desorption mass spectrometry compared with the normal procedure in the case of fragile oligopyrroles is demonstrated in figure 2. The electron impact mass spectrum shows besides extensively degraded fragment ions of masses below the half



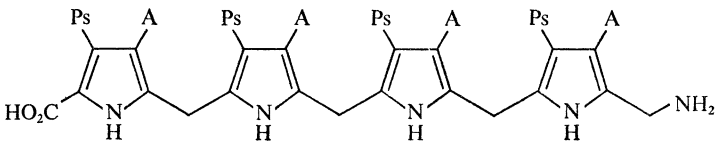
A, A^{Me} = —CH_2CO_2H, $CH_2CO_2CH_3$

Ps, Ps^{Me} = —$CH_2CH_2CO_2H$, —$CH_2CH_2CO_2CH_3$

Bz = —$CH_2C_6H_5$

molecular mass no molecular ion at all. In a remarkable contrast to this the f.d. mass spectrum gives the molecular ion as a single base peak.

The tetrapyrrole (**20**c) liberated from the depot compound (**24**) could not be isolated because of its high reactivity. This was in accord with observations of Radmer & Bogorad (1972), who had studied the same tetrapyrrole formed by enzymic transformation of porphobilinogen (**1**) under inhibition with ammonium ions. Therefore it was characterized by condensation of its weakly protected carboxy derivative (**25**) to porphyrinogen and subsequent oxidation to the red porphyrin. Analysis of the reaction product by the procedure described by Edmondson & Schwartz (1953) revealed a content of 80 % uroporphyrinogen I (**26**). Thus it was shown that this linear tetrapyrrole condenses *in vitro* almost without isomerization.



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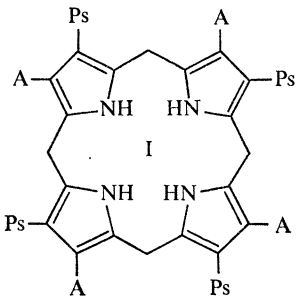

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Conclusions

Further evidence for the striking similarity of porphyrin formation from porphobilinogen (**1**) *in vivo* and *in vitro* is provided by the results described. The efficiency of the *in vitro* reaction for preparative purposes exceeds the best known biogenetic type alkaloid syntheses. Thus the enzymes forming uroporphyrinogen III (**2**) from porphobilinogen (**1**) act probably more in product control of the reaction than in bringing it about. As the linear tetrapyrrole (**25**) cyclizes under mild conditions with very little isomerization one can assume that the pyrrole inversion in uroporphyrinogen III (**2**) formation *in vitro* occurs already during the first condensation steps. To prove this by trapping a dipyrrylmethane formed by head to head condensation would give valuable support to the understanding of porphyrin biosynthesis. Another interesting aspect of this reaction is the possibility to develop procedures by which the ratio of the biologically important uroporphyrinogen III in the reaction mixture is increased.

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Discussion

A. H. Jackson (*University College, P.O. Box* 78, *Cardiff, CF*1 1*XL*). In relation to the synthesis of the tetra *N*-substituted porphyrinogen, Mr H. M. G. Al-Hazimi in Cardiff has synthesized both *N*-methyloctaethylporphyrin and *N*-methylaetioporphyrin in moderate yield by acid catalysed polymerization of appropriately substituted *N*-methyl-α-acetoxymethylpyrroles with the corresponding NH compounds. We had tried originally to synthesize *N*-methyloctamethylporphyrin in this way (because of the insolubility and relative difficulty of preparing the octamethylporphyrin, and methylating it directly).

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