Journal of

The Chemical Society,

Chemical Communications

NUMBER 13/1975 2 JULY

Stereochemistry of Biosynthesis of the Vinyl Groups of Protoporphyrin-IX: A Short Synthesis of Porphobilinogen

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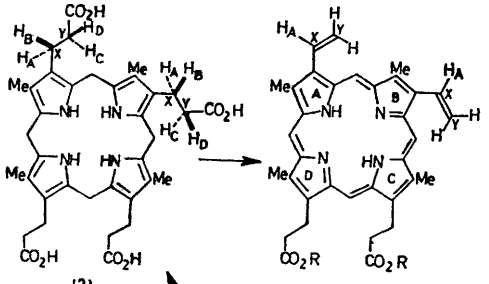
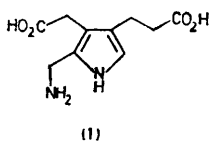
Summary Porphobilinogen (**1**), [^{2}H]-labelled in the propionic residue, is synthesised by a short route and is used to establish that both vinyl groups of protoporphyrin-IX are biosynthesised by overall antiperiplanar elimination of a proton and carbon dioxide.

The biosynthesis of protoporphyrin-IX (**3**) and thus also of haem involves oxidative conversion of the propionic acid groups on rings A and B of coproporphyrinogen-III (**2**) into vinyl groups by the enzyme coproporphyrinogenase.[1]



(2)

(3); R = H
(4); R = Me



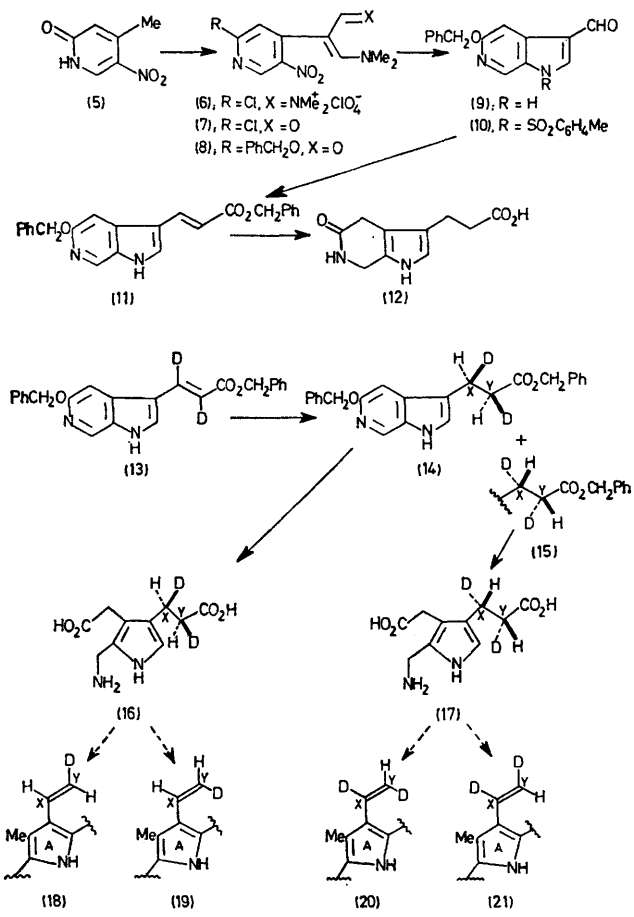
(1)

Coproporphyrinogen-III (**2**) is biosynthesised from four moles of porphobilinogen, PBG (**1**) by way of uroporphyrinogen-III (**2**, CH_2CO_2H in place of Me). Earlier studies from this laboratory[2] and elsewhere[3] showed that the conversion of (**2**) → (**3**) involved loss of only one hydrogen atom from each of the centres X and retention of both hydrogens at positions Y. Hydrogen removal from the centres X was found to be stereospecific[3] with loss of the *pro-S* hydrogens[3]. We now define the stereochemistry of formation of the vinyl groups.

We developed a synthesis of PBG based on Rapoport's azaindole approach[4] but new chemistry allowed considerable shortening and more than doubled the yield.

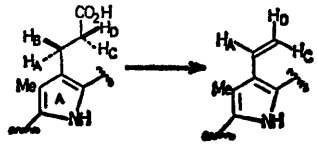
The pyridone[5] (**5**) with $POCl_3$–dimethylformamide (DMF)[6] in refluxing chloroform (24 h) gave the salt (**6**), 90% yield, m.p. 233° (decomp.), which was hydrolysed by sodium hydroxide in aqueous acetone to the aldehyde (**7**) m.p. 189—190° and then (**7**) with sodium benzyloxide in benzyl alcohol gave the ether (**8**), 80% yield from (**6**), m.p. 128—129°. Reduction of (**8**) with zinc dust and aqueous acetic acid yielded the azaindole (**9**), 60% yield, m.p. 194—195° which reacted with monobenzyl malonate in dry pyridine–piperidine to form the acrylate (**11**), 98% yield, m.p. 175—177°. Palladium and hydrogen then cleaved the benzyl groups, saturated the double bond and reduced the pyridone to give PBG lactam (**12**), 84% yield, characterised as its methyl ester, m.p. 245—247°. The yield of lactam (**12**) from the pyridone (**5**) was 35% overall.

The *N*-tosyl aldehyde (**10**) was deuteriated by the morpholinonitrile method[7] and cleavage with NaOD in D_2O–tetrahydrofuran gave, after exchange of >ND against water, the deuteriated aldehyde (**9**, CDO in place of CHO; no CHO detectable by n.m.r.). This condensed with deuteriated monobenzyl malonate to give the [$^2H_2$]-acrylate (**13**) which was reduced by diimide to form the racemate (**14**) + (**15**). In this product, the relative configuration at centres X and Y has been fixed by the established *syn*-stereospecificity of



diimide. Hydrogenation gave the corresponding PBG lactams and hydrolysis then yielded labelled PBG as a racemate (**16**) + (**17**). This was converted by our preparative cell-free system from *Euglena gracilis*[8] into protoporphyrin-IX isolated as its ester.

The ^{1}H n.m.r. signals from H_A of each vinyl group of unlabelled protoporphyrin-IX dimethyl ester (**4**) appear as a double doublet centred at *ca.* τ 2·8 (J_{trans} 18 Hz, J_{cis} 11 Hz) which can overlap, but at a suitable concentration eight separate signals can be observed corresponding to the two hydrogens H_A. In the enzymic conversion of labelled PBG (**16**) + (**17**) into protoporphyrin-IX, the (*R,R*)-enantiomer (**17**) will lead either to arrangement (**20**) or to (**21**) and neither of these can give rise to a signal at τ 2·8 (no ^{1}H at X centres). In contrast, the (*S,S*)-enantiomer (**16**) by elimination of the forward hydrogen (D in this case) antiperiplanar with the carboxyl group would lead to vinyl groups as in (**18**) whereas a synperiplanar process would form vinyl groups as in (**19**). The ^{1}H n.m.r. spectrum of the labelled protoporphyrin-IX dimethyl ester showed two slightly broadened doublets both with the *trans* coupling (J 18 Hz) for the two H_A hydrogens and so it was established that both vinyl groups had *trans* oriented hydrogen atoms (**18**). The ^{1}H n.m.r. signals from the centres Y will be analysed in full later; the signal pattern confirmed the observations for the centres X. Thus, biosynthesis of the two vinyl groups of protoporphyrin IX occurs by an overall antiperiplanar elimination of a proton and carbon dioxide (Scheme).



SCHEME

We thank Drs. D. H. Grayson and E. Hunt for their help and the British Council, the Nuffield Foundation and S.R.C. for financial support.

(*Received, 24th March* 1974; *Com.* 355.)

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