Stereospecificity of Hydrogen Removal from the Four Methylene Bridges in Haem Biosynthesis: Specific Incorporation of the 11 *pro-S* Hydrogen of Porphobilinogen into Haem

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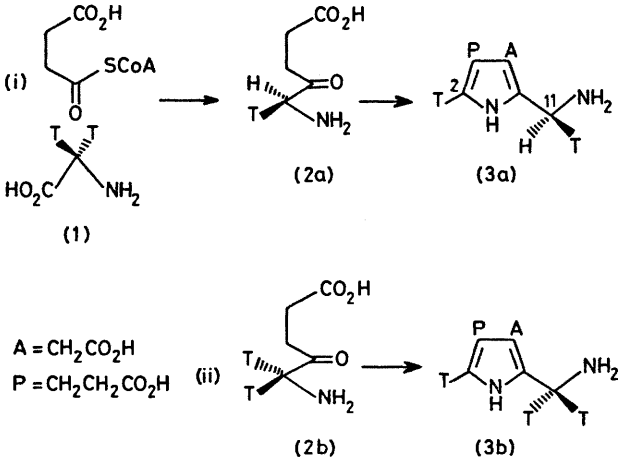
Summary Incorporation of 11*S* tritiated porphobilinogen into haem results in retention of tritium at only the C-10 position.

OUR investigations into the stereochemistry of the enzymic reactions of the early stages of porphyrin biosynthesis[1] has led us to a method for the preparation of porphobilinogen (PBG) (**3a**) stereospecifically labelled with tritium at C-11. We hoped that the incorporation of this molecule into a porphyrin might provide stereochemical information about the mechanism of condensation of four molecules of PBG (**3**, T replaced by H) to give uroporphyrinogen III (**5**) and also the subsequent oxidation of the resulting *meso* bridges in the transformation of protoporphyrinogen IX to produce protoporphyrin IX (**6**, Fe replaced by 2H).

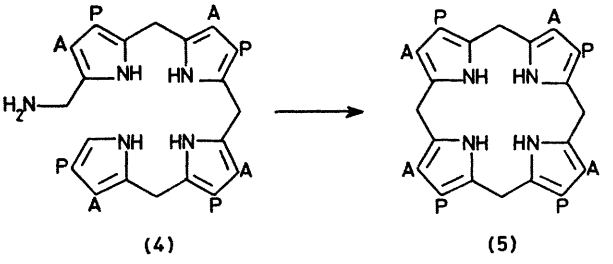
[2-$^3H_2$, 2-^{14}C]Glycine (**1**, $^3H/^{14}C = 43{\cdot}9$) and unlabelled succinyl CoA were incubated in a coupled enzyme system of *R. spheroides* ALA-synthetase and bovine liver ALA-dehydratase to give [2,11*S*-$^3H_2$; 2,11-$^{14}C_2$]PBG (**3a**, $^3H/^{14}C = 21{\cdot}7$) in about 30% yield. Acid treatment of the biosynthetic material resulted in loss of ^{3}H present at C-2 to give [11*S*-$^3H_1$; 2,11-$^{14}C_2$]PBG ($^3H/^{14}C = 10{\cdot}1$). The degradation of the PBG to glycine and stereochemical analysis of the latter showed that about 91% of ^{3}H at C-11 of the biosynthetic PBG (**3a**) was present in the *S* configuration.[2] These results confirm our previously published observation and allow the status of hydrogen atoms in the conversion of glycine into PBG to be defined as shown in Scheme 1 (i).



SCHEME 1. Preparation of PBG samples.

For comparison, a sample of PBG containing tritium randomly distributed between the two C-11 hydrogen atoms of PBG was required. This was accomplished by the incubation of [5*RS*-$^3H_2$; 5-$^{14}C_1$]ALA (**2b**) with ALA-dehydratase when the resulting PBG (**3b**) was shown to contain 30% and 70% of the ^{3}H at C-2 and C-11 corresponding to one and two tritium atoms, respectively [Scheme 1 (ii)].

PBG samples, prepared as above, were incubated aerobically in a cell-free system from chicken erythrocytes to produce haem (25—30% yield) which was isolated as haemin (**6**) and recrystallised after the method of Labbé.[3] Tritium present in haemin was not labile under these recrystallisation conditions. With the sample of racemic



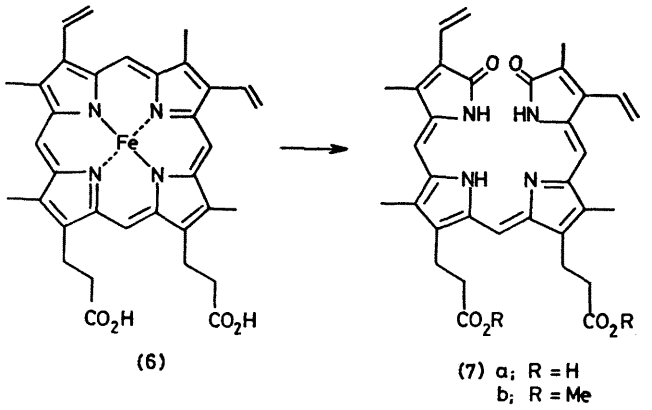
PBG (**3b**) 50% of the tritium originally present at C-11[4] was retained in the biosynthetic haem (expt. 1), whereas the chiral sample (**3a**) showed only 25% retention of tritium, suggesting the presence of tritium at only one of the four *meso* positions (Table 1, expts. 1 and 2). Incubation of [2,11*S*-$^3H_2$]- or [11*S*-$^3H_1$]-PBG resulted in the same incorporation of tritium into haem (Table 1, expts. 2 and 3), showing that the tritium present at C-2 is not incorporated as anticipated.

TABLE 1.

Expt.	Haemin biosynthesised from	$^3H/^{14}C$ Ratios in PBG	$^3H/^{14}C$ Ratios in Haemin	% Retention of the C-11 ^{3}H of PBG in haemin
1	[2, 11 *RS*-$^3H_3$; 2, 11-$^{14}C_2$]PBG (**3b**)[a]	4·94	1·71	49·5
2	[2, 11*S*-$^3H_2$; 2, 11-$^{14}C_2$] PBG (**3a**)[b]	15·58	1·99	25·4
3	[11*S*-^{3}H; 2, 11-$^{14}C_2$] PBG	8·87	2·12	24·0

[a] Containing 69·9% of tritium at C-11. [b] Containing 50·3% of tritium at C-11.

The position of the tritium in haem was determined by oxidation with oxygen and ascorbic acid to give the four biliverdin IX isomers [*e.g.* IXα, (**7a**)] which were methylated



(6) (7) a; R = H b; R = Me

SCHEME 2. For convenience only the formation of the biliverdin IXα is shown.

(BF_3–MeOH) and purified by[5,6] separation on t.l.c. in two solvent systems. Oxidation of a bridge position will result in loss of any tritium bond to it. If all the tritium is located at only one of the positions the biliverdin derived from the elimination of the atoms at this position will have a $^3H/^{14}C$ ratio of zero. Degradation of the haem derived from the racemic PBG resulted in biliverdin ester isomers (*e.g.* **7b**) whose $^3H/^{14}C$ ratios were comparable, indicating a random distribution of tritium on all bridges (Table 2). Degradation of the haemin derived from chiral PBG (**3a**) showed the β isomer had a ^3H ratio approximately 30% that of the other isomers, indicating that 73% of the tritium is localised at the C-10 position and an average of 9% at each of C-5, C-15, and C-20.

TABLE 2. $^3H/^{14}C$ Ratios of the biliverdin isomers originating from haemin biosynthesised either from the racemic (**3b**) or chiral (**3a**) PBG.

		Biliverdin			
	Haemin	α	β	γ	δ
Racemic PBG (**3b**)	1·72	1·54	1·64	1·69	1·57
Chiral PBG (**3a**)	1·99	2·16	0·57	1·91	1·78

The recent work by two groups[7,8] has established that a tetrapyrrole derivative (**4** or its equivalent) is involved in the biosynthesis of uroporphyrinogen III (**5**), and we suggest that in the formation of such an intermediate the relative stereochemistry of the methylene bridges destined to become C-5 and C-10 of the uroporphyrinogen III and hence of protoporphyrinogen IX will be the same. In the light of this consideration, the isotope results above suggest that in the protoporphyrinogen IX oxidase catalysed removal of hydrogen atoms from the four *meso* carbon atoms, those at the α and β positions are manipulated by two different mechanisms.† A mechanism accommodating this feature assumes that the overall transformation consists of three successive dehydrogenation reactions each involving pyrrole N–H and *meso* C–H bonds, followed by a prototropic shift removing a proton from the fourth methylene bridge.†

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† Two possibilities exist: (a) β position is handled differently from α, γ, and δ positions, and (b) α position is handled differently from β, γ, and δ positions.

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