Synthetic Studies on the Proposed Spiro Intermediate for Biosynthesis of the Natural Porphyrins: The Stereochemical Probe

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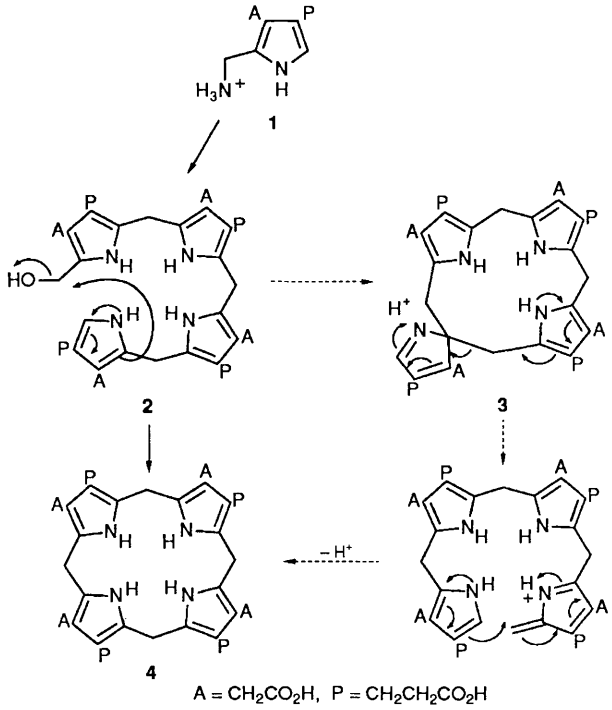
The intermediacy of the spiro-pyrrolenine for biosynthesis of uro'gen III is supported by synthesis of both enantiomers of the spiro-lactam **6** followed by demonstration that cosynthetase, the enzyme which forms uro'gen III, is inhibited over twenty times more strongly by one enantiomer than by the other.

Uro'gen III **4** is the biosynthetic parent of all the pigments of life (haem, chlorophyll, vitamin B_{12}) and is built from porphobilinogen, PBG **1**, by the cooperative action of two enzymes, deaminase† and cosynthetase.‡ The most intriguing feature of the structure of uro'gen III is the reversal of ring-D with respect to rings A to C. This feature stimulated over 20 mechanistic proposals for possible sequences to account for the conversion of PBG **1** into uro'gen III **4**. Experiments involving double labelling[1,2] with ^{13}C eliminated almost all of these proposals but one proposed mechanism, shown in Scheme 1, remained fully consistent with the labelling studies. This invoked the spiro-pyrrolenine **3** as an intermediate in the formation of uro'gen III by the action of cosynthetase on hydroxymethylbilane **2**, which is the product[3] formed by deaminase from PBG **1**. Fragmentation of **3** and recombination, for which there is sound precedent,[4] leads to intramolecular inversion of ring-D. When the spiro-system was originally proposed,[5] a heavily C-protonated form of it was used to give the flexibility thought necessary to allow formation of the macrocycle. In fact, the parent tripyrrolic macrocycle present in **3** can be built, it exists in a conformation which is markedly puckered and on spacing filling models is locked.[6] Synthesis of the spiro-lactam system **5** led to two separable isomers interpreted on the above basis as atropisomers[7] **5a** and **5b**. The octaacid§ **6** derived from one of these isomers strongly inhibited cosynthetase in carrying out its normal function of catalysing the conversion of hydroxymethylbilane **2** into uro'gen III **4**. Importantly, the octaacid derived from the other isomer had no detectable effect on cosynthetase.

This lack of inhibition of cosynthetase by one isomer of the spiro-lactam **6** but strong inhibition by the other, whose structure resembles neither substrate **2** nor product **4** but only matches the proposed spiro-intermediate **3**, gave strong support[7] to the intermediacy of the spiro-pyrrolenine **3** *en route* to uro'gen III **4**.

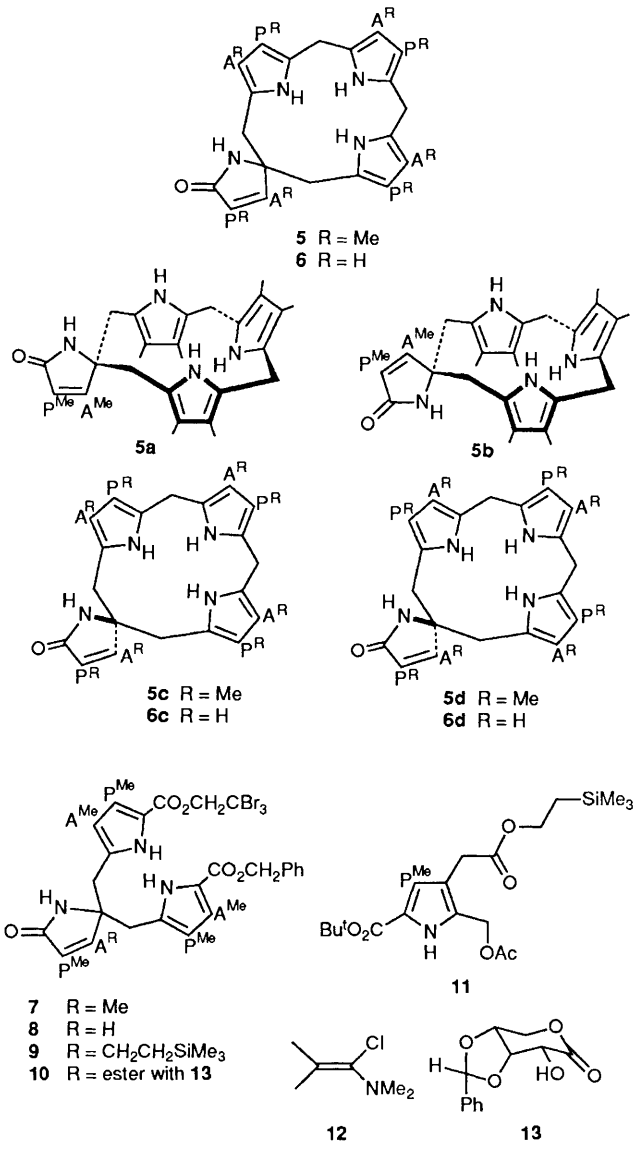


Scheme 1

† EC 4.3.1.8, systematic name hydroxymethylbilane synthase.

‡ EC 4.2.1.75, systematic name uroporphyrinogen III synthase.

§ For simplicity, the acids are illustrated throughout as neutral species but in the enzymic assays at pH 8.25, they are obviously largely anionic.



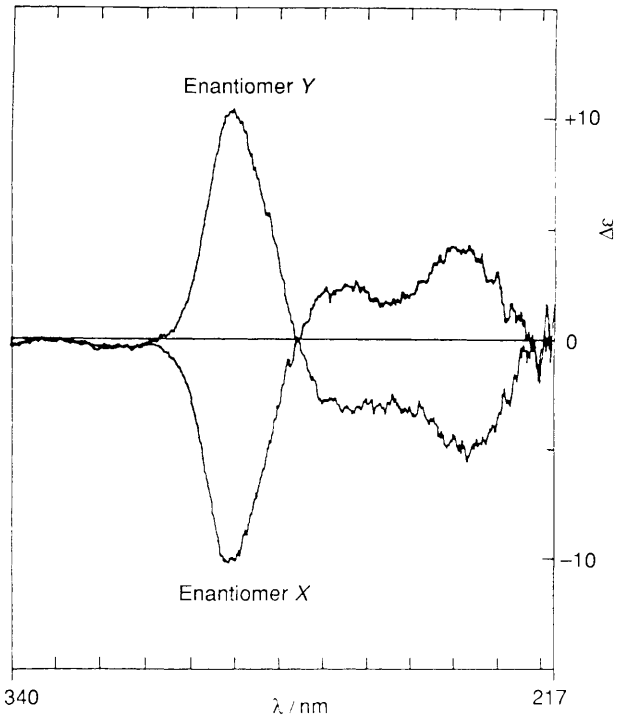


Fig. 1 Circular dichroism curves for resolved lactams **7** (see the footnote to Table 1 for definition of enantiomers *X* and *Y*)

Table 1 Inhibition of cosynthetase by resolved spiro-lactams **6c** and **d**[a]

		Inhibitory effect/ $\times 10^{-6}$ mol dm^{-3}
Enantiomer *X*	K_I	1.8
Racemate *X* + *Y*	K_I	2.5
Enantiomer *Y*	K_I	38
Substrate **2**	K_M	37

[a] Enantiomer *X* was derived from the diastereoisomer of **10** having the shorter retention time on HPLC (Spherisorb S5CN; diethyl ether–ethyl acetate, 3 : 1) and enantiomer *Y* from the other diastereoisomer. Analytical HPLC and NMR spectroscopy indicated that complete (>98%) separation of the diastereoisomers had been achieved.

The foregoing spiro-lactams **5a** and **b** are both racemic but only one enantiomer of the inhibitory spiro-lactam will match the putative spiro-pyrrolenine **3**. Therefore, it should be possible to add further strength to the inhibition experiments if both enantiomers can be prepared of the spiro-lactam which, as the racemate, blocks cosynthetase. One difficulty must be considered: the mirror image of enantiomer **5c** is **5d**, which differs only by having each acetate (A) on a pyrrolic ring replaced by a propionate (P) and each propionate (P) by an acetate (A). So though the active site which binds the putative spiro-intermediate **3** should perfectly fit one enantiomer of the inhibitory spiro-lactam, *e.g.* **6c**, when it accepts the other enantiomer, **6d**, it will still be presented with a set of six acidic side chains in the correct locations but of the wrong size (A for P and P for A) on rings A to C. The likely effect of this will be considered later.

The resolution studies were made on various mono-carboxylic acids related to **7** which were prepared by suitable cleavage of a single ester function. Only one of a number of such approaches involving many resolving agents was successful and this was based on the acid **8**. The protected form **9** of this acid was synthesised by chemistry analogous to that used originally[7] but now starting with the pyrrole **11**. Fluoride ion removed the silyl ethyl group from **9** and the acid **8** was activated using the α-chloroenamine[8] **12** ready for esterification with the δ-ribonolactone derivative **13**. The resultant diastereoisomers **10** were just separable by HPLC and the chiral auxiliary was removed from each one by ester exchange using sodium methoxide in methanol. Fig. 1 shows the circular dichroism curves for the resultant enantiomers of **7**. Each enantiomer was then converted by a series of synthetic steps analogous to those used originally in the racemic series,[7] into the resolved spiro-lactams, **5c** and **d**. Two separable atropisomers were obtained from each enantiomer of **7** to provide the two enantiomers corresponding to the inhibiting racemate and the two enantiomers which make up the non-inhibiting racemate. Attention focused on the former pair.

These two enantiomeric spiro-lactams were treated with alkali under conditions known to hydrolyse only the ester groups[7] to yield the corresponding octaacids **6c** and **d**. Assays for the activity of cosynthetase from *Euglena gracilis*[9] were then run using a range of concentrations of the hydroxymethylbilane **2** as substrate in the presence and absence of each enantiomer of the spiro-lactam acid, **6c** and **d**, in turn. Kinetic runs with the racemic inhibitory spiro-lactam **6** were included in the set of experiments as standards. The K_M value for **2** obtained from these experiments and the K_I values for the enantiomers **6c** and **d** are given in Table 1. They show a striking difference between the two enantiomers in their effectiveness as inhibitors of cosynthetase with enantiomer *X* being *ca.* 20 times more inhibitory than enantiomer *Y*. Moreover, the K_I for the strongly inhibiting enantiomer *X* is

more than an order of magnitude lower than the K_M for the substrate (hydroxymethylbilane) of cosynthetase. The fact that enantiomer *Y* is a weak inhibitor rather than having no effect is not unexpected since, as noted earlier, the two enantiomers of the spiro-lactam **6c** and **d** are related more closely than most. Hence, it is not surprising that the wrong enantiomer does bind to some extent to cosynthetase, albeit *ca.* 20 times less strongly than the correct enantiomer.

The foregoing stereochemical studies add further support to the view that the spiro-pyrrolenine **3** is the intermediate involved in the conversion of **2** into uro'gen III **4** as shown in Scheme 1.

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