Stereochemical Studies on the Proposed Spiro Intermediate for the Biosynthesis of the Natural Porphyrins: Determination by a Novel X-Ray Method of the Absolute Configuration of the Spirolactam which Inhibits Cosynthetase

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A novel X-ray analysis, combined with correlations by circular dichroism, has been used to establish the (*R*)-configuration at the stereocentre in that enantiomer of the spirolactam **4** which strongly inhibits uroporphyrinogen III synthase.

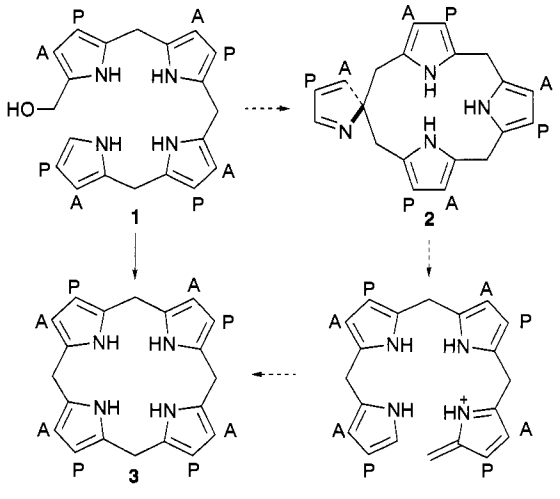
Uroporphyrinogen (uro'gen) III **3** is the first macrocyclic intermediate for the biosynthesis of the natural tetrapyrrole pigments (*e.g.* chlorophyll, haem, vitamin B_{12}) and also the last intermediate common to all these pathways.[1] It is formed by ring-closure of hydroxymethylbilane **1**, intriguingly with concomitant inversion of ring *D*, a process catalysed by the enzyme uro'gen III synthase (cosynthetase). ^{13}C Labelling experiments showed that this rearrangement is intramolecular[2] and that it only affects ring *D*.[3] The ^{13}C studies eliminated almost all of the proposed mechanisms for the rearrangement but the most economical and mechanistically attractive proposal, illustrated in Scheme 1, is consistent with the results. This simplified form of the original proposal[4] invokes the spiro-pyrrolenine **2** as a key intermediate which could undergo fragmentation (as indicated) and recombination to yield uro'gen III **3**. This idea was tested by synthesis[5] of the racemic spirolactam **4**, which differs from the putative pyrrolenine **2** only around the nitrogen of the five-membered spiro ring, the rest of the two molecules being identical.

The racemic lactam† **4** acted as a potent inhibitor of cosynthetase[5] thus giving strong support for the spiro-intermediate **2**. Furthermore, the two enantiomers† of **4** were individually synthesised from the separate enantiomers of dipyrrolic lactam **5** and one enantiomer of **4** was found to be *ca.* 20 times more powerful an inhibitor than the other.[6] This indicates that the binding of **4** to cosynthetase is highly specific.

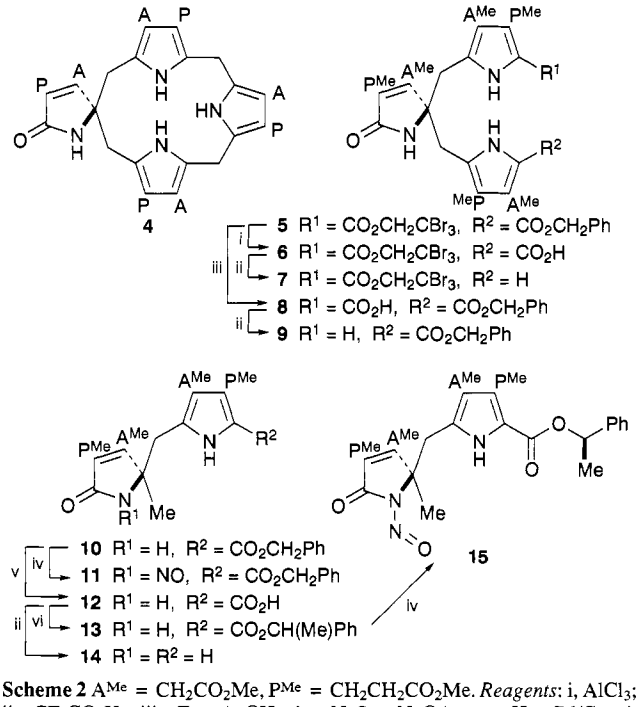
In this paper we describe the determination of the absolute configuration of a monopyrrolic *N*-nitrosolactam **15** by a novel use of X-ray crystallography and correlation of the configuration of **15** with that of the strongly inhibiting enantiomer of **4** by a combination of circular dichroism (CD) and chemical synthesis.

After exhaustive but unsuccessful attempts to crystallise derivatives of the resolved dipyrrolic lactam **5**, with or without chiral auxiliaries attached, we turned to monopyrrolic lactams such as **10** (Scheme 2). It was found that the racemic *N*-nitrosolactam **11** crystallised as rods suitable for X-ray analysis but the resolved‡ enantiomers of **11** had totally different solubility properties and were amorphous. It appeared that some crucial interactions between opposite enantiomers allowed strong lattice formation leading to good crystals for the racemic lactam derivative but not for the enantiomerically pure one. We therefore decided to generate a *racemic* diastereoisomeric derivative of **11** with a chiral auxiliary such that we knew which resolved sample of **11** was attached to which enantiomer of the chiral auxiliary.

The separate enantiomers† of lactam **10** (**10a** and **10b**) were each hydrogenated to give acids **12** and one enantiomer **12a** was esterified with (*R*)-1-phenylethanol ($[\alpha]_D^{23}$ +43.8) of proven absolute configuration[7] while the other enantiomer **12b** was esterified with (*S*)-1-phenylethanol. The resulting products **13a** and **b** are, therefore, enantiomers of the same diastereoisomer of lactam **13**. The two samples, **13a** and **b**, were mixed in equal proportions to give the racemic material and *N*-nitrosated. This racemic product crystallised well§ and the X-ray crystal structure (Fig. 1) showed it to have structure **15** (plus its enantiomer), in which the *N*-nitrosolactam with the (*R*)-configuration is coupled to the 1-phenylethyl moiety of (*R*)-configuration.¶ Thus the enantiomer **12a**, which was the one esterified with (*R*)-1-phenylethanol, has the (*R*)-configuration (as shown in Scheme 2), and so has enantiomer **10a** from which **12a** was derived. We are not aware of any other determination



Scheme 1 Proposed mechanism of action of cosynthetase. A = CH_2CO_2H, P = $CH_2CH_2CO_2H$



Scheme 2 A^{Me} = CH_2CO_2Me, P^{Me} = $CH_2CH_2CO_2Me$. *Reagents*: i, $AlCl_3$; ii, CF_3CO_2H; iii, Zn, AcOH; iv, N_2O_4, NaOAc; v, H_2, Pd/C; vi, PhCHOHMe, 1-benzotriazolyl-O-P(NMe_2)$_3^+$ PF_6^-, Pr^i_2NEt.

of the absolute configuration of a compound which has been achieved by obtaining the crystal structure of a racemate in this manner. It should be a valuable technique in any series in which the racemic compounds are more crystalline than the pure enantiomers.

With the absolute configuration of the monopyrrolic lactam **10** secured, CD was used to correlate its stereochemistry with that of the dipyrrolic lactam **5**. The CD spectrum of (*R*)-**10** showed a negative peak (Cotton effect) at 285 nm.‖ To prove that this peak is due to the pyrrolic ester chromophore (which has an absorption maximum in this region) and its interaction with the unsaturated lactam, (*R*)-**10** was converted into the α-free pyrrole (*R*)-**14**; as expected, this showed no peak above 220 nm in its CD spectrum.

Starting with **5x**, the enantiomer of **5** corresponding to the strongly inhibitory enantiomer of **4** (enantiomer X in ref. 6), either of the two pyrrolyl ester chromophores can be removed by deprotection of the appropriate carboxy group followed by decarboxylation. Thus, in one experiment the benzyl group of **5x** was removed to give acid **6x**, which was decarboxylated to give α-free pyrrole **7x**, and in another experiment the tribromoethyl group was removed to give acid **8x**, which was decarboxylated to give α-free pyrrole **9x**. The CD spectra of **7x** and **9x** were almost exact mirror images of each other, which is to be expected as they are enantiomeric apart from the difference in side chains on the pyrrolic rings. Compound **7x** had a negative peak at 285 nm whereas **9x** showed a positive peak.** As (*R*)-**10** showed a negative CD peak, it can be concluded that it is **7x** which has the same configuration with respect to the pyrrolyl ester chromophore as (*R*)-**10** and thus that the configurations of enantiomers **5x–9x** are as shown in Scheme 2.

The enantiomer of spirolactam **4** which strongly inhibits cosynthetase was derived from dipyrrolyl lactam enantiomer **5x** and so it also has the configuration shown in Scheme 2, which is the (*R*)-configuration at the chiral centre.

The sum of all the evidence outlined here and earlier[5,6] strongly supports the intermediacy of the spiro-pyrrolenine **2** in the biosynthesis of uro'gen III **3** and points to its absolute configuration being as shown in Scheme 1.

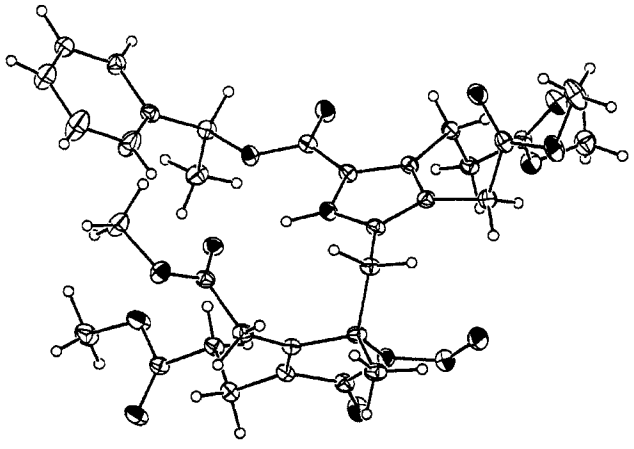


Fig. 1 Crystal structure of the racemic *N*-nitrosolactam **15**

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Footnotes

† The structures throughout show only one of the two mixed (racemate) or separated enantiomers.

‡ The synthesis of the resolved enantiomers of **10** will be described in our full paper.

§ The alternative racemic diastereoisomer of **15** was also made by esterification of **12a** with (*S*)-1-phenylethanol and **12b** with (*R*)-1-phenylethanol but it failed to crystallise.

¶ *Crystal data* for racemic **15**; $C_{33}H_{39}N_3O_{12}$, M = 669.67, monoclinic, space group $P2_1/n$, a = 15.142(3), b = 14.636(3), c = 15.795(3) Å, β = 108.605(10)°, V = 3317.3(10) Å^3 [from 25 centred reflections (109.47 ≤ 2θ ≤ 110.13°), Cu-Kα radiation, λ = 1.54178 Å], Z = 4, D_c = 1.341 Mg m^{-3}, T = 123(1) K, μ = 0.863 mm^{-1}, $F(000)$ = 1416. The data were collected on a Rigaku AFC7R diffractometer, graphite-monochromated Cu-Kα radiation, ω-2θ scans, max. 2θ = 140° at low temperature. After data reduction, the structure was solved by direct methods and refined on F^2 for all 5998 reflections, anisotropic displacement parameters for C, O, N atoms. H atoms located in the difference Fourier map were included in the model but not refined. At convergence, $wR^2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$ = 0.1848 for all data, conventional R [on F values for 5072 reflections with $F_o > 4\sigma F_o$] = 0.0616, S = 1.088 on F^2 for 434 parameters. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

‖ Another simpler example of a monopyrrolic lactam related to **10**, which shows a negative Cotton effect, has also recently been assigned the (*R*)-configuration by X-ray analysis.[8]

** As a check, the enantiomeric compounds **7y** and **9y** were also prepared, starting with the opposite enantiomer **5y** and, as required, they had the mirror-image CD spectra to those described here for **7x** and **9x**.

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