Synthetic Studies on the Proposed Spiro Intermediate for Biosynthesis of the Natural Porphyrins: Inhibition of Cosynthetase

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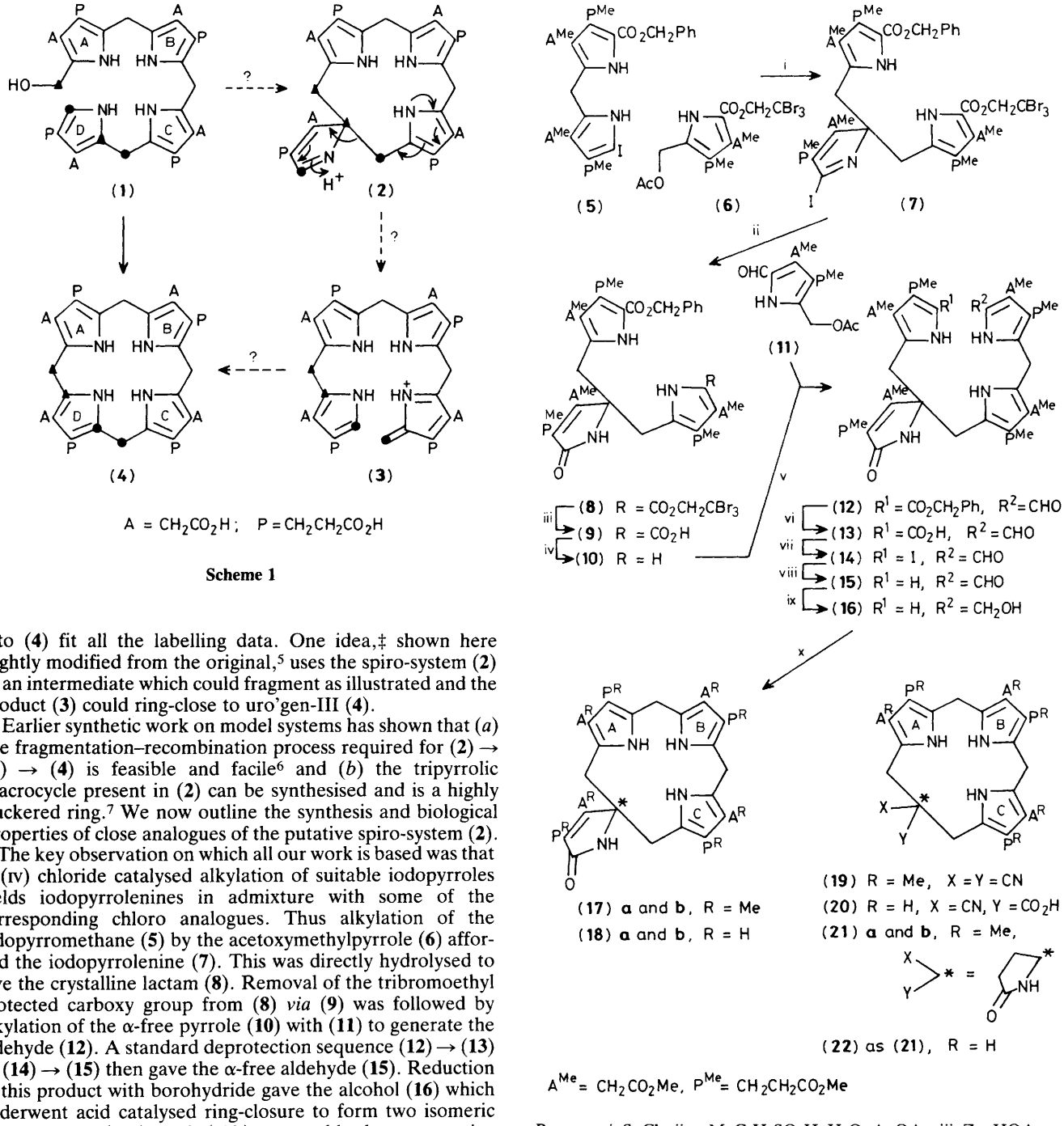
A macrocyclic spiro-lactam is synthesised which closely resembles the spiro system proposed as a biosynthetic intermediate for uro'gen-III; this spiro-lactam powerfully inhibits cosynthetase, the enzyme responsible for forming uro'gen-III.

Cosynthetase† is the enzyme which acts at the last stage in the construction of uroporphyrinogen-III (**4**), shortened to uro'gen-III; this macrocycle is the parent of all the pigments of life such as protohaem, chlorophyll, and vitamin B_{12}.[1] The role of cosynthetase is to convert the hydroxymethylbilane[2] (**1**) into uro'gen-III (**4**), Scheme 1, a process which was shown to involve a single intramolecular rearrangement of ring-D.[3,4] Scheme 1 also illustrates with ● and ▲ some of the ^{13}C labelling experiments[4] which probed the rearrangement process.

Only two mechanistic hypotheses for the conversion of (**1**)

† EC 4.2.1.75, systematic name uroporphyrinogen-III synthase.



Scheme 1

Reagents: i, $SnCl_4$; ii, *p*-$MeC_6H_4SO_3H$, H_2O, AgOAc; iii, Zn–HOAc; iv, CF_3CO_2H; v, $SnCl_4$; vi, H_2, Pd–C; vii, KI_3; viii, H_2, Pt, NaOAc; ix, $NaBH_4$; x, *p*-$MeC_6H_4SO_3H{\cdot}H_2O$, CH_2Cl_2.

into (**4**) fit all the labelling data. One idea,‡ shown here slightly modified from the original,[5] uses the spiro-system (**2**) as an intermediate which could fragment as illustrated and the product (**3**) could ring-close to uro'gen-III (**4**).

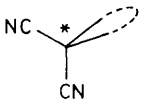
Earlier synthetic work on model systems has shown that (*a*) the fragmentation–recombination process required for (**2**) → (**3**) → (**4**) is feasible and facile[6] and (*b*) the tripyrrolic macrocycle present in (**2**) can be synthesised and is a highly puckered ring.[7] We now outline the synthesis and biological properties of close analogues of the putative spiro-system (**2**).

The key observation on which all our work is based was that tin(IV) chloride catalysed alkylation of suitable iodopyrroles yields iodopyrrolenines in admixture with some of the corresponding chloro analogues. Thus alkylation of the iodopyrromethane (**5**) by the acetoxymethylpyrrole (**6**) afforded the iodopyrrolenine (**7**). This was directly hydrolysed to give the crystalline lactam (**8**). Removal of the tribromoethyl protected carboxy group from (**8**) *via* (**9**) was followed by alkylation of the α-free pyrrole (**10**) with (**11**) to generate the aldehyde (**12**). A standard deprotection sequence (**12**) → (**13**) → (**14**) → (**15**) then gave the α-free aldehyde (**15**). Reduction of this product with borohydride gave the alcohol (**16**) which underwent acid catalysed ring-closure to form two isomeric spiro-lactams (**17a**) and (**17b**), separable by preparative h.p.l.c. The former was crystalline, the latter not, and both were homogeneous by all spectroscopic and chromatographic methods (^1H and ^{13}C n.m.r. and t.l.c.). Field-desorption mass spectrometry proved that both spiro-lactams had mol.wt. 964. Electron impact mass spectrometry showed (fortuitously) *m/z* 964.3946 for both (**17a**) and (**17b**); structure (**17**), $C_{48}H_{60}N_4O_{17}$, requires 964.3953.

The ^1H and especially the ^{13}C n.m.r. spectra of (**17a**) and (**17b**) confirmed that these products are closely related spiro macrocycles. The ^{13}C-signal (in $CDCl_3$) from the asterisked quaternary centre appeared at δ 67.8 and 65.5 for (**17a**) and (**17b**), respectively; *cf.* δ 64.1 and 63.6 for the corresponding carbon in the lactams[7] (**21a**) and (**21b**), see later.

All the evidence supports the view that the isomers (**17a**) and (**17b**) arise because of the locked conformation of the macrocyclic part of their structures. *X*-Ray analysis has shown[7] that rings A and C of the dinitrile (**19**) are strongly tilted and they point in the opposite direction to ring B; also, one cyano group is 'equatorial' and one is 'axial', see (**23**). In this case, only one structure is possible because the asterisked centre carries identical groups and only one was found.

‡ See ref. 2 for the other proposal.



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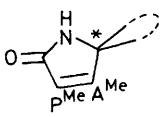

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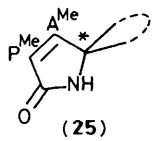

(25)

However, taking it that the macrocycle of the lactams (**17a**) and (**17b**) exists in the same locked conformation as in the dinitrile (**19**), two forms are possible as shown in part structures (**24**) and (**25**); both forms are racemic. The production of two isomeric forms was also observed for the simpler model[7] (**21a**) and (**21b**) and the same reasons apply.

Mild alkaline hydrolysis of the spiro-lactams (**17a**) and (**17b**) gave the salts of the corresponding octa-acids (**18a**) and (**18b**). The hydrolysis product from (**18b**) gave a ^{1}H n.m.r. spectrum (in D_2O) which was consistent with both the lactam and macrocyclic parts of the molecule having remained intact. Assays for cosynthetase activity[8] were then run using the hydroxymethylbilane§ (**1**) as substrate in the presence and absence of the hydrolysed spiro-lactams§ (**18a**) and (**18b**). The former (**18a**) had essentially no effect on the rate at which cosythetase converted hydroxymethylbilane (**1**) into uro'gen-III (**4**) but the latter (**18b**) was a powerful inhibitor. Double reciprocal plots of the kinetic results were consistent with the inhibition by (**18b**) being competitive with respect to the hydroxymethylbilane (**1**) with a K_i of *ca.* 1 μM. Since the lactam (**18b**) is racemic, the K_i for the enantiomer which binds tightly could approach 0.5 μM.

The strong inhibition of cosynthetase by the spiro-lactam (**18b**) is very significant and this view is strengthened (*a*) by the lack of inhibition by the isomer (**18a**); if one isomer (**18b**) fits the enzyme well, the other (**18a**) should not, (*b*) by the finding that none of the simpler systems[7] (**20**), (**22a**), and (**22b**) acts as a significant inhibitor of the enzyme;[9] these molecules all contain the tripyrrolic macrocycle but lack part or all of the substituted spiro-lactam ring present in (**18b**). Evidently an inhibitor must carry essentially all the correct functions in the right orientation for there to be tight binding, and (*c*) by recognising that the inhibitor (**18b**) is radically different in structure from both the substrate (**1**) for cosynthetase and the product (**4**) from the enzyme.

These results give indirect support to the idea that the spiro-system (**2**) is in fact the intermediate between (**1**) and (**4**); final proof requires the synthesis of (**2**) from (**17b**) or earlier intermediates.

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§ These materials are illustrated for simplicity as the acids but in the enzymic assay at pH 8.25 they are obviously largely ionised.

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