γ-Lactam Formation from Tripeptides with Isopenicillin N Synthase

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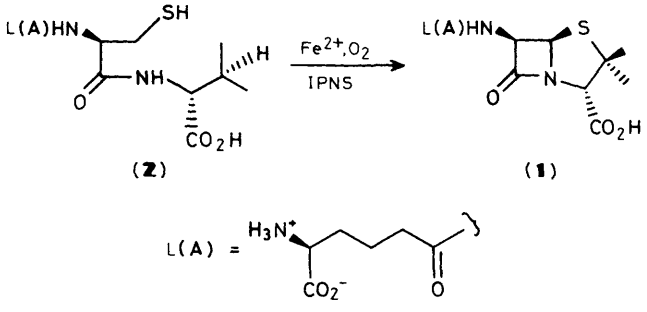
Incubation of isopenicillin N synthase (IPNS) with analogues of the natural substrate [(5*S*)-5-amino-5-carboxypentanoyl]-L-cysteinyl-D-valine (**2**) in which the cysteinyl residue was replaced by homocysteine gave epimeric 5-hydroxy γ-lactams (**10**), with no evidence for the formation of bicyclic products.

It is now well established that the biosynthesis of isopenicillin N (**1**) proceeds *via* oxidative enzymic cyclisation of [(5*S*)-5-amino-5-carboxypentanoyl]-L-cysteinyl-D-valine (L,L,D-ACV) (**2**) (Scheme 1).[1] We have reported that incubation of isopenicillin N synthase (IPNS) with substrates modified in the valinyl position can result in the formation of a range of alternative β-lactam metabolites,[1—3] in addition to novel penams. We have rationalised these results in terms of an enzyme-bound monocyclic intermediate, involving an oxo-iron moiety (**3**) which subsequently forms the second ring.[1] Evidence for such a monocyclic intermediate also follows from kinetic isotope studies[4] and the isolation of a 'shunt' metabolite.[5]

In contrast to the aminocarboxypentanoyl and valinyl residues, informative substrate analogues of the cysteinyl residues have been few. No products were detected when tripeptide (**4**) or (**5**), containing L-serine or L-aminobutyrate, was incubated with IPNS.[6] Recent work has involved more subtle changes;[7] thus, for example L,L,D-A-[(3*R*)-methylcysteinyl]-V (**6**) gave the penam (**7**), but the corresponding (3*S*)-methylcysteinyl peptide (**8**) was not a substrate for IPNS.

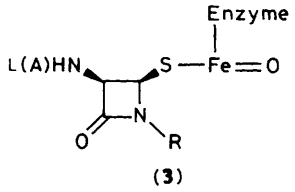
In order further to probe the substrate specificity of IPNS with regard to the central residue, and in the hope of shedding light on the nature of any intermediate, we synthesised the homocysteine-containing tripeptide (**9**). Incubation of (**9**) with IPNS gave after protein precipitation and h.p.l.c. purification [octadecylsilane (25 mM NH_4HCO_3)] the epimeric alcohols (**10a** and **b**). The ^1H n.m.r. spectrum (500 MHz) of the crude incubation mixture indicated the alcohols were present in *ca.* 1 : 1 ratio, of unknown hydroxy configuration. However, after purification one of them (**10a**) epimerised in aqueous solution (pH 7.5) to give the other (**10b**).† A control incubation using boiled enzyme did not give rise to (**10a**) or (**10b**).

The structural assignment of the enzymic products (**10**) was confirmed by independent synthesis. Thus L,L,D-A-(allylgly-

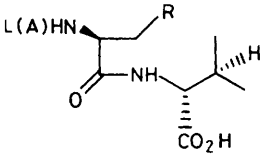


Scheme 1

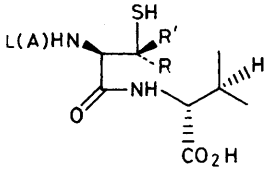
† *Spectral data* for (**10a**): δ_H (500 MHz; D_2O; ref. sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate) 0.90 and 0.97 [2 × 3H, 2 × d, *J* 7 Hz, $CH(CH_3)_2$], 1.62—1.76 and 1.80—1.91 (5H, 2 × m, 4-H and $CH_2CH_2CH_2CO$), 2.06—2.37 [3H, m, CH_2CO, $CH(CH_3)_2$], 2.78—2.85 (1H, m, 4-H), 3.60—3.73 (1H, m, $HNCHCO_2$), 3.96 [1H, d, *J* 10 Hz, $CHCH(CH_3)_2$], 4.33—4.38 (1H, m, 3-H), and 5.36—5.40 (1H, m, 5-H). For (**10b**): δ_H 0.88 and 1.01 [2 × 3H, 2 × d, *J* 7 Hz, $CH(CH_3)_2$], 1.65—1.73 and 1.84—1.91 (4H, 2 × m, $CH_2CH_2CH_2CO$), 2.20—2.38 (5H, m, CH_2CO, 2 × 4-H, $CH(CH_3)_2$], 3.73—3.75 (1H, m, $HNCHCO_2$), 4.09 [1H, d, *J* 10.5 Hz, $CHCH(CH_3)_2$], 4.43—4.47 (1H, m, 3-H), and 5.57 (1H, *ca.* d, *J* 5.5 Hz, 5-H); the connectivity as assigned was established by a 2D-Jeener experiment; m.s. *m/z* (positive argon fast atom bombardment) 360 (MH^+, 100%), 361(20), 362(6), and 363(5).



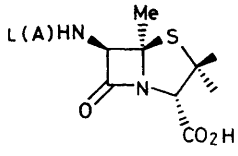
(3)



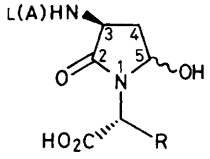
(4) R = OH
(5) R = Me
(9) R = CH_2SH
(11) R = $CH{=}CH_2$
(12) R = CHO
(15)a; R = (*R*)—CHDSH
b; R = (*S*)—CHDSH



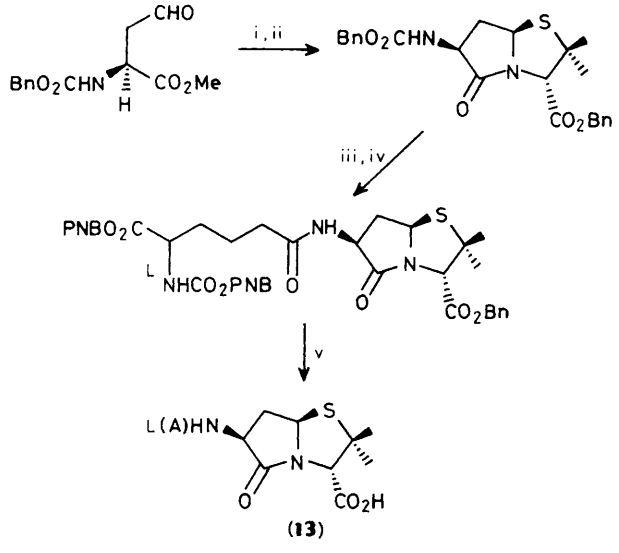
(6) R = Me, R' = H
(8) R = H, R' = Me



(7)



(10 a, b) R = Pr^i
(18) R = $CH_2CH{=}CH_2$



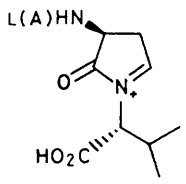
(13)

PNB = *p*-$NO_2C_6H_4CH_2$

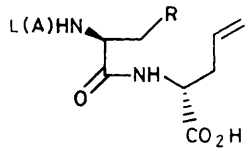
Scheme 2. *Reagents*: i, penicillamine, pyridine, reflux; ii, $PhCH_2Br$, dimethyl formamide, $NaHCO_3$, NaI(cat.); iii, HBr–AcOH, then $NaHCO_3$ (iv) L-$(PNBO_2C)(PNBO_2CHN)CHCH_2CH_2CH_2CO_2H$, CH_2Cl_2, ethyl-1,2-dihydro-2-ethoxy-1-quinoline carboxylate (EEDQ); v, H_2/Pd/C.

cine)-valine (**11**) was treated with ozone followed by dimethyl sulphide to give (**10b**), *via* the aldehyde (**12**).

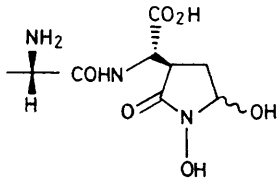
We had considered the bicyclic γ-lactam (**13**) as a potential product of incubation of (**9**) with IPNS; consequently the γ-lactam (**13**) was synthesised (Scheme 2), by methodology previously developed in the synthesis of a γ-lactam analogue of the penems.[8] However, examination of the crude incubation mixture by ^{1}H n.m.r. indicated no signals corresponding to (**13**).



(14)



(16) R = SH
(17) R = CH_2SH



(20)

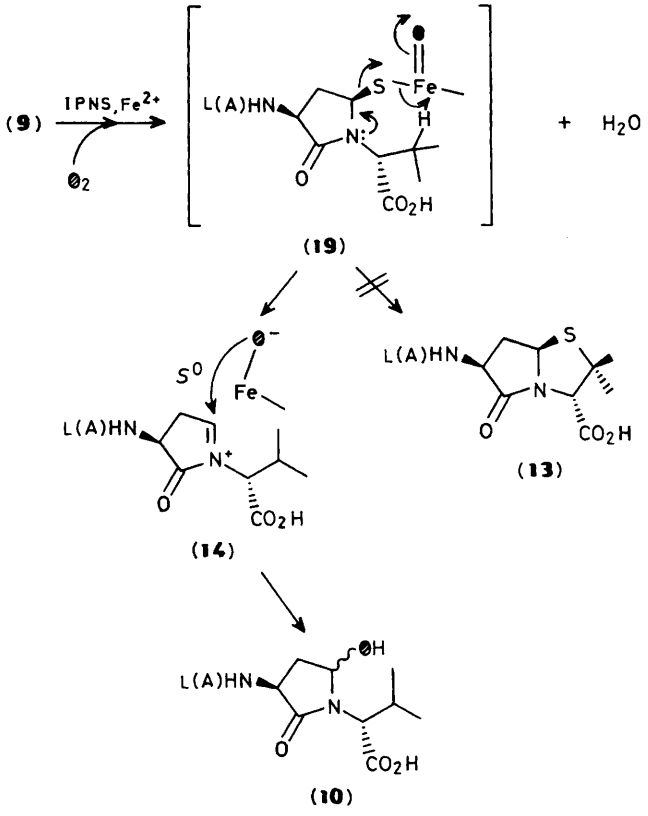
We also examined the incubation of (**9**) under an atmosphere of $^{18}O_2$ gas. For so-derived (**10b**) we observed *m/z* (positive argon fast atom bombardment) 360(100%), 361(24), 362(34), and 363(11); for (**10a**) 360(100%), 361(37), 362(40), and 363(22). These figures indicate a significant level of incorporation of ^{18}O into the products (**10**). The fact that the levels were lower than previously achieved for hydroxylated products derived from IPNS incubations is probably due to exchange of the 5-OH, *via* the acyl iminium ion (**14**).

Incubation of a 1:1 mixture of the (4*R*)- and (4*S*)-monodeuteriated homocysteinyl peptides (**15a** and **15b**) with an excess of IPNS gave the partially 5-deuteriated γ-lactam (**10b**), shown by ^{1}H n.m.r. (500 MHz) and mass spectrometry (fast atom bombardment) to have undergone *ca.* 80% of the maximum theoretical deuterium loss expected for a fully stereospecific event at the 4-position of the homocysteinyl residue of (**15**). This is consistent with the degree of stereospecificity observed during isopenicillin N (**1**) formation from (**2**).[9]

As L,L,D-[(5*S*)-5-amino-5-carboxypentanoyl]cysteinyl-allylglycine (**16**) has been shown to give five different bicyclic products with IPNS,[1] thus indicating a relaxed specificity for this substrate, we also synthesised and incubated L,L,D-(5-amino-5-carboxypentanoyl)-homocysteinyl-allylglycine (**17**) with IPNS in the hope of obtaining a bicyclic structure. However, no bicyclic products were detected, the only isolated materials being the epimeric alcohols (**18**), analogous to those obtained from (**9**).

In summary, these studies further support previous evidence for the initial closure of the lactam ring in penicillin biosynthesis as catalysed by IPNS. In our view the lack of bicyclic products from the homocysteinyl substrates (**9**) and (**17**) arises from the more rapid collapse of the monocyclic intermediate (**19**) to atomic sulphur and the iminium ion (**14**), which is trapped by the iron-bound hydroxyl (^{18}O) (Scheme 3). Subsequent release as hydroxy lactams (**10**) restores the enzyme's oxidation state for the next catalytic cycle.‡ A similar process, but dependent on a deuterium kinetic isotope effect, has been proposed to explain release of shunt metabolites from IPNS.[5] It is of interest that the lactams

‡ An alternative possible mechanism is one that is analogous to that for the substrate uncoupled catalytic turnover of α-ketoglutarate by prolyl hydroxylase.[10]



Scheme 3

produced by the incubation of (**9**) and (**17**) with IPNS bear a strong structural similarity to alahopcin (**20**), a novel dipeptide antibiotic isolated from *Streptomyces*.[11]

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