Penicillin Biosynthesis: Stereochemistry of Desaturative and Hydroxylative Pathways from L-α-Aminoadipoyl-L-cysteinyl-D-isodehydrovaline with Isopenicillin N Synthase

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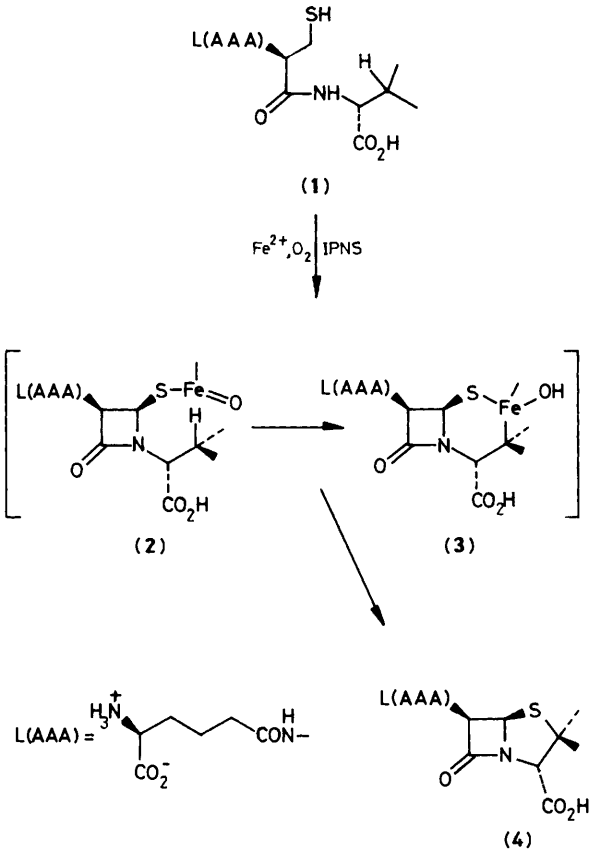
Transformation of L-α-aminoadipoyl-L-cysteinyl-D-(*Z*)-[4-^{2}H]isodehydrovaline (**5b**) and L-α-aminoadipoyl-L-cysteinyl-D-(*E*)-[4-^{2}H]isodehydrovaline (**5c**) with isopenicillin N synthase gave rise to specifically deuteriated exomethylene cepham (**6**) and α-hydroxymethyl penam (**7**) products *via* the concomitant operation of stereospecific desaturative and hydroxylative ring closure pathways. The stereochemistries observed are in accord with competing 'Ene' and [2π + 2π]cycloaddition of the proposed iron-oxo intermediate.

On the basis of studies on the mechanism of cyclisation of tripeptides by the enzyme isopenicillin N synthase (IPNS) we have proposed, as a working hypothesis, the intermediacy of an iron-oxo species (**2**), which mediates the second ring closure through an iron–carbon bonded species (**3**), as shown in Scheme 1, from the natural substrate (**1**).[1] When the valine moiety of (**1**) was replaced by unsaturated amino acids the enzymatic reaction produced products of desaturative (−4H) and hydroxylative (−2H + 1O) pathways,[2,3] in which the hydroxyl group of the latter pathway was derived from the cosubstrate dioxygen.[4] This hydroxylative pathway was suggested to arise from a competing [2π + 2π]cycloaddition of the iron-oxo species to the double bond followed by reductive elimination to the bicyclic product, Scheme 2. In accord with this the balance of desaturative *vs.* hydroxylative ring closure was critically dependent on the position of the alkene moiety in the C-terminal amino acid.[3] In the case of the isodehydrovaline peptide (**5**) both desaturative, to (**6**), and hydroxylative paths, to (**7**), were observed, Scheme 3.[3,4] We have now determined the stereochemical course of these two competing processes.

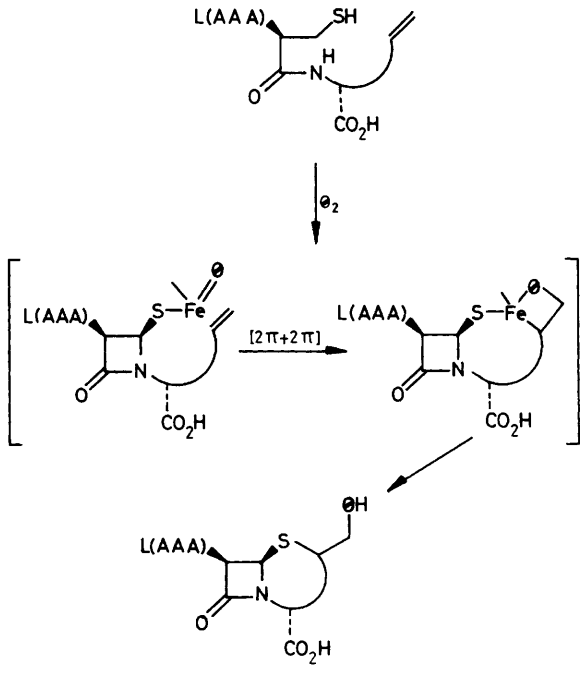
Stereoselectively labelled (*Z*)- and (*E*)-[4-^{2}H$_1$]isodehydrovalines† were prepared according to the method of Crout *et al.*[5] and incorporated into L-α-aminoadipoyl-L-cysteinyl-D-[4-^{2}H$_1$]isodehydrovaline tripeptides (**5b**) and (**5c**), respectively.‡ The unlabelled tripeptide (**5a**) was likewise prepared.

† The purity of the (*Z*)- and (*E*)-[4-^{2}H$_1$]isodehydrovaline isotopomers was 90% and 92%, respectively. The remainder of the material was composed of unlabelled isodehydrovaline [10% in the (*Z*)-, 4% in the (*E*)-isotopomer] and, in the case of the (*E*)-isotopomer, the opposite isotopomer, *i.e.* (*Z*)-, (4%).

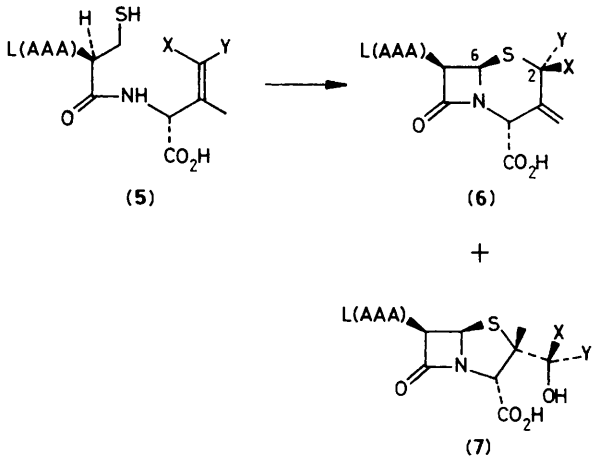
‡ Prepared by methods analogous to AC-(D)-vinylglycine synthesis, see ref. 3.



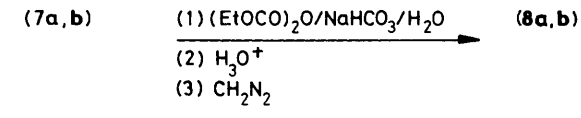
Scheme 1



Scheme 2



Scheme 3. a; X = Y = ^{1}H
b; X = ^{2}H, Y = ^{1}H
c; X = ^{1}H, Y = ^{2}H



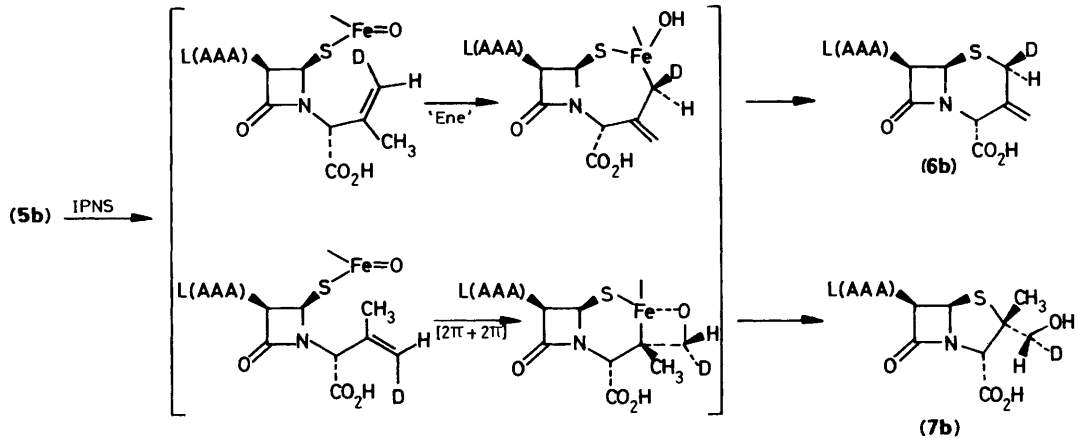
Scheme 4. a; X = Y = H
b; X = ^{2}H, Y = ^{1}H

As previously described,[3] incubation of the unlabelled tripeptide (**5a**) with IPNS affords an α-hydroxymethyl penam (**7a**) and an exomethylene cepham (**6a**) in a ratio of 3:1. Repeating the incubation with deuterium labelled tripeptides, (**5b** or **c**), revealed the formation of stereoselectively deuterium labelled§ penam, (**7b** or **c**), and cepham, (**6b** or **c**), products.

The stereochemistry of labelling of the cepham products (**6b** and **c**) was established by comparison of the chemical shift of the proton at the labelled methylene centre in the 500 MHz ^{1}H n.m.r. spectrum with the corresponding resonances in the unlabelled cepham (**6a**). The chemical shifts of the 2α- and 2β-proton resonances of the unlabelled cepham (**6a**) were assigned on the observation of a nuclear Overhauser effect (n.O.e.) enhancement (5%) between the low field 2α-methylene proton resonance and the H-6 β-lactam resonance. Furthermore, a similar n.O.e. enhancement (6%) was observed between the 2α-methylene proton resonance and the H-6 β-lactam resonance of the 2β-deuteriated cepham (**6b**).

The assignment of the stereochemistry of the deuterium labelled hydroxymethyl penams (**7b** and **c**) required further derivatisation of the incubation products. Thus the unlabelled (**7a**) and one of the labelled (**7b**) penams were each converted to their lactone derivatives (**8**). This was accomplished (Scheme 4) via initial *N*-protection of the penam side chain amine using diethylpyrocarbonate followed by acidification (pH 2), extraction into ethyl acetate and treatment with diazomethane.[6] Each crude product was purified by h.p.l.c. to afford lactones (**8a**) and (**8b**), respectively. The observation of an n.O.e. enhancement (5%) between the lactone high field

§ The ratio of deuterium incorporation at the two possible diastereotopic sites of the labelled methylene group in the β-lactam incubation products (**6b,c**) and (**7b,c**) was at least 9:1 in each case.



Scheme 5

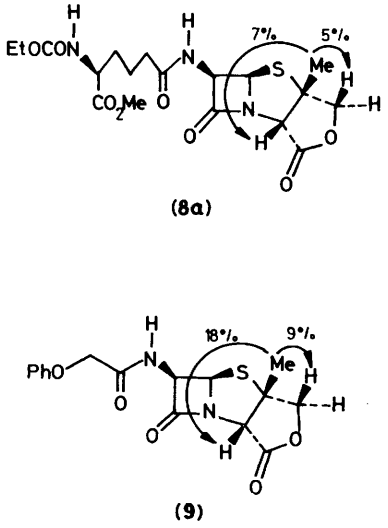


Figure 1. N.O.e. enhancements.

methylene β-proton resonance and the β-methyl group[3] of the unlabelled lactone (**8a**) enabled assignment of the lactone methylene proton resonances in the ^{1}H n.m.r. spectrum Figure 1. The model penam lactone (**9**), derived from penicillin V, showed a similar n.O.e. enhancement (9%) between the corresponding lactone methylene proton resonance and the β-methyl group (Figure 1).

The assignment of the absolute configuration of the deuterium labelled lactone methylene centre of (**8b**) as (*R*) then followed from chemical shift comparison. By implication the configuration of the labelled hydroxymethyl group of the hydroxymethyl penam (**7b**) is also (*R*) and that of the isomeric penam (**7c**) is (*S*). Thus the tripeptide containing the (*Z*)-[4-^{2}H$_1$]isodehydrovaline residue (**5b**) afforded a penam product (**7b**) with a deuterium labelled hydroxymethyl group of (*R*)-configuration and a (2*S*)-[2-^{2}H$_1$]exomethylene cepham (**6b**) while the tripeptide containing the (*E*)-[4-^{2}H$_1$]isodehydrovaline residue (**5c**) gave rise to the selectively deuterium labelled hydroxymethyl penam (**7c**) and exomethylene cepham (**6c**) products of opposite configuration at the labelled centres.

The observed stereochemistry is in accord with a branched pathway, emanating from the two conformers of the labelled isopropenyl group in the monocyclic iron-oxo intermediate, Scheme 5. A concerted 'Ene' reaction, followed by reductive elimination with retention, provides the exomethylene cephem. Alternatively and competitively the other rotamer provides, by way of a *syn* [2π + 2π]cycloaddition followed by reductive elimination with retention, the α-hydroxymethyl penam.¶ These results provide further support to our contention that the iron-oxygen entity is in intimate contact with the cysteinyl–valine part of the tripeptide precursor.

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¶ An alternate pathway *via* intermediate epoxide formation, as observed in related systems,[7] can be excluded as S_N2 opening of the epoxide by sulphur would result in *anti* addition to the isodehydrovalinyl double bond.