Penicillin Biosynthesis: Stereochemical Dependence on Mode of Second Ring Closure

Jack E. Baldwin, Robert M. Adlington, Christopher J. Schofield, and Hong-Hoi Ting

The Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QY, U.K.

The stereochemical requirements for the conversion of tripeptides with unsaturated amino acids in the *C*-terminal position to bicyclic β-lactam products using isopenicillin N synthase was investigated using the diastereoisomeric peptides (**7**) and (**12**).

Mechanistic studies on the enzyme isopenicillin N synthase (IPNS) have led to the proposed reaction sequence shown in Scheme 1, which proceeds *via* an enzyme-bound intermediate β-lactam–iron oxene species.[1] This highly reactive entity mediates the second ring closure with retention of stereochemistry by way of a rotationally restricted free radical equivalent at the 3-valinyl site.[†] Replacement of the valine isopropyl group with an ethyl group, *i.e.* in the analogous α-aminobutyrate peptide, permitted rotation of the intermediate radical and resulted in both retention and inversion in the ring closure step, providing an epimeric mixture of norpenicillins.[1,2] Further characterisation of the reactivity of the proposed iron-oxene species was obtained through replacement of valine by the unsaturated amino acid allyl glycine, which gave both 'desaturase' as well as 'mono-oxygenase' pathways,[3] Scheme 2.

The oxygenase products (**1**), (**2**), and (**3**) were attributed to

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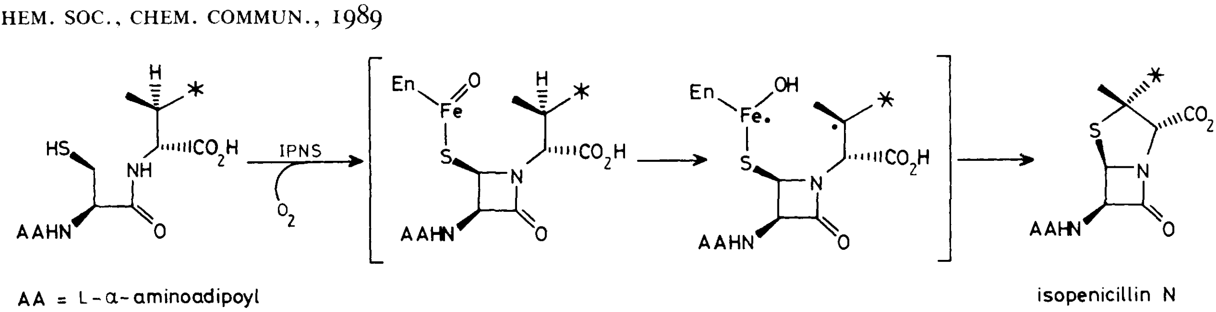
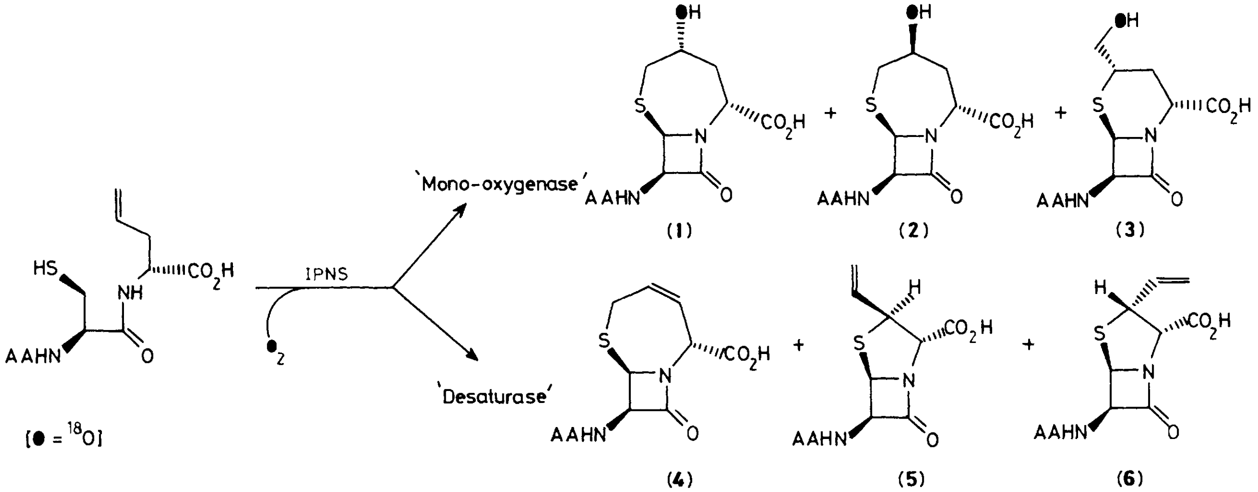
[†] The free radical may be preceded by an iron–carbon bonded species, *cf.* ref 1.



Scheme 1



Scheme 2

initial [2 + 2] cycloaddition of the iron–oxene moiety to the double bond,[4] the homocephem (4) to an 'ene' type reaction, and the penams (5) and (6) to the normal desaturative closure.[5] In order to establish whether stereochemical effects were responsible for the multiple pathways shown in Scheme 2 we devised the following experiments. Since chain branching at the β-position of the *C*-terminal amino acid apparently impeded rotation of this entity, *vide supra*, presumably a 3-methyl substituent in the allyl glycine unit would similarly restrict the active site conformations of the entity. Since such a substituent could exist in two distinct diastereotopic forms, each of which would present different groups to the reactive iron–oxene centre, then the products of these reactions might reveal the stereochemical requirements for the various possible reaction pathways. This was indeed observed as follows. The peptide (7),‡ with 3*S* stereochemistry, gave upon incubation with a highly purified form of the IPNS enzyme[9] from *Cephalosporium acremonium* CO 728, the 2-α-vinyl penam (11), δ_H§ 1.53—1.77 (4H, m, $CH_2CH_2CH_2CO$), 1.59 (3H, s, 2β-Me), 2.25—2.38 (2H, m, CH_2CO), 3.56—3.59 [1H, m, $CH(CH_2)_3$], 4.18 (1H, s, 3-H), 5.03 (1H, d, *J* 10.5 Hz, $CH{=}CH_2$), 5.22 (1H, d, *J* 17 Hz, $CH{=}CH_2$), 5.35, 5.45 (2H, ABq, *J* 4 Hz, 5,6-H), 5.91 (1H, dd, *J* 10.5, 17 Hz, $CH{=}CH_2$), *m/z* (positive argon fast atom bombardment) 372 (MH^+), as the only observed β-lactam containing product. Formulation as the 2-α-vinyl penam followed from nuclear Overhauser enhancement (n.O.e.) experiments in which irradiation of the 2β-methyl group, δ_H 1.59, gave enhancement of 3-H (12%) but not to 5-H, and irradiation of the $CH{=}CH_2$ proton, δ_H 5.91, gave enhancement to 5-H (3%). Compound (11) showed the expected penicillinase-sensitive antibacterial activity (*ca.* 85% at equivalent molar concentration to isopenicillin N) against *Staphylococcus aureus* N.C.T.C. 6571.

The second tripeptide (12)‡ gave, upon similar incubation with IPNS, the hydroxymethyl cepham (13) and the homoceph-3-em (14) without any detectable penam product. Formulation as (13) follows from n.m.r. and mass spectral data, δ_H§ 1.10 (3H, d, *J* 6 Hz, 3β-Me), 1.42—1.85 (4H, m, $CH_2CH_2CH_2CO$), 2.26—2.31 (2H, m, CH_2CO), 2.43—2.52 (1H, m, 3-H), 2.37—2.81 (1H, m, 2-H), 3.51—3.55 [1H, m, $CH(CH_2)_3$], 3.68 (1H, dd, *J* 6.5, 12 Hz, CH_2OH), 3.84 (1H,

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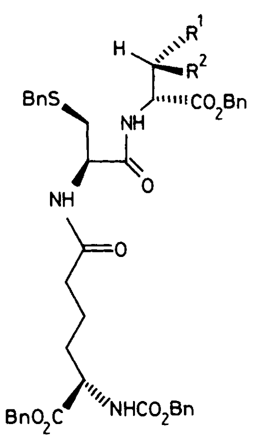
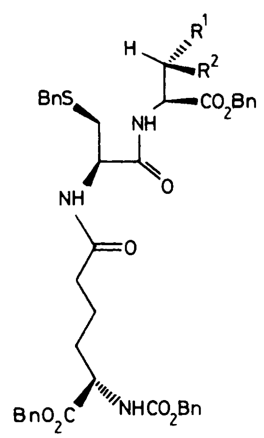
‡ Suitably protected forms of 2*R*,3*R*/2*S*,3*S* and 2*R*,3*S*/2*S*,3*R* 3-methylallylglycines were prepared from the appropriate *Z*- or *E*-crotonyl esters of *N*-protected glycines using the Ireland–Claisen rearrangement essentially as reported by Bartlett.[6] These acids were then separately coupled[7] to protected L-α-aminoadipoyl-L-cysteine to give the diastereoisomeric tripeptides (8a,b) and (9a,b) respectively, which were separated by chromatography ([*i.e.* (8a) from (8b) and (9a) from (9b)]. Confirmation of the stereochemistry of (8a) and (9a) followed from comparison, after reduction [H_2 (1 atm), $(Ph_3P)_3RhCl$] to suitably protected forms of the isoleucinyl (10a) and *allo*-isoleucinyl (10b) tripeptides respectively.[8] (8a) was deprotected to (12) and (9a) to give (7) (Na, NH_3).[7]

§ Chemical shifts are referenced to internal sodium (2,2,3,3-$^2H_4$)-3-trimethylsilylpropanoate.

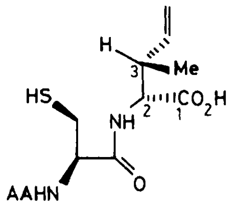


(8a) $R^1 = Me, R^2 = CH{=}CH_2$
(9a) $R^1 = CH{=}CH_2, R^2 = Me$
(10a) $R^1 = Me, R^2 = CH_2CH_3$
(10b) $R^1 = CH_2CH_3, R^2 = Me$

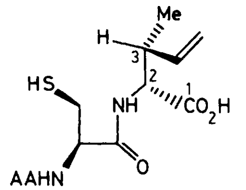


(8b) $R^1 = CH{=}CH_2, R^2 = Me$
(9b) $R^1 = Me, R^2 = CH{=}CH_2$

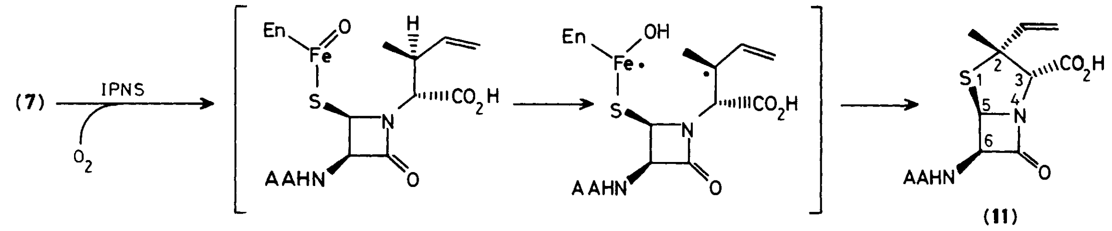
Bn = benzyl



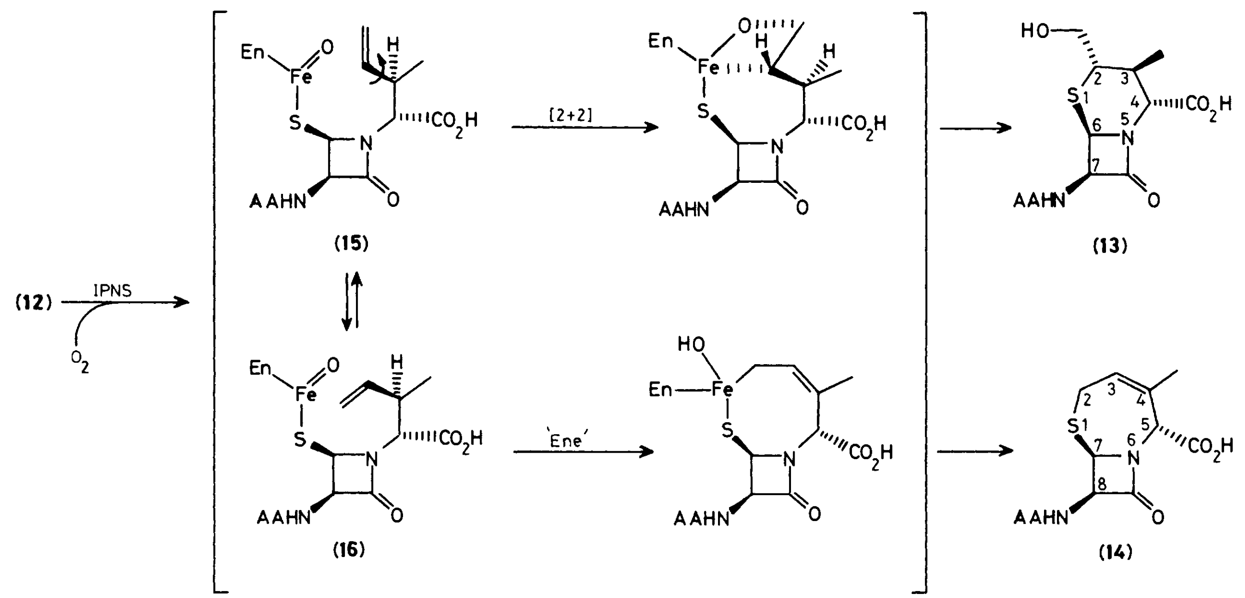
(7)



(12)



Scheme 3



Scheme 4

dd, J 8.5, 12 Hz, CH_2OH), 3.86 (1H, d, $J < 1$ Hz, 4-H), 5.00, 5.24 (2H, ABq, J 4 Hz, 6,7-H), m/z (positive argon fast atom bombardment) 390 (MH^+). A Jenner experiment was consistent with the reported structure.[10] The 2α-hydroxymethyl group was consistent with n.O.e. experiments. Thus irradiation of the CH_2OH group, δ_H 3.6—3.9, gave n.O.e. to 6-H (8%) and 3-H (2%) whilst irradiation of 2-H, δ_H 2.73—2.81, gave no n.O.e. to 6-H. A β-C-3 methyl group was also consistent with the C-3,C-4 coupling constant whose magnitude ($J < 1$ Hz) has precedent in related cephams.[8] Compound (**13**) showed no antibacterial activity against *S. aureus* N.C.T.C. 6571 at a concentration of 100 μg ml^{-1}. Formulation of the other product as **(14)** follows from n.m.r. and mass spectral data, δ_H§ 1.56—1.77 (4H, m, $CH_2CH_2CH_2CO$), 1.73 (3H, s, Me), 2.26—2.29 (2H, m, CH_2CO), 3.06 (1H, dd, *J* 7, 15.5 Hz, 2-H), 3.15 (1H, dd, *J* 7, 15.5 Hz, 2-H), 3.57—3.59 [1H, m, $CH(CH_2)_3$], 4.74 (1H, s, 5-H), 5.18, 5.40 (2H, ABq, *J* 4 Hz, 7, 8-H), 5.70 (1H, *ca.* t, *J* 7 Hz, 3-H), *m/z* (positive argon fast atom bombardment) 372 (MH^+). The connectivity S–CH_2–CH= was confirmed by proton decoupling experiments, and **(14)** showed no antibacterial activity against *S. aureus* N.C.T.C. 6571 at a concentration of 100 μg ml^{-1}.

The sharp contrast in products between these two substrates and the allylglycine case, Scheme 2, is most likely due to the stereochemical relationship between the putative iron–oxene and the methylallyl group. Thus the conformational restriction around C-2–C-3, in the 3*S* series **(7)** does not permit the [2 + 2] or the ene process since the vinyl group is remote from the reaction centre, Scheme 3. Consequently the 'normal' desaturative process follows yielding a penam **(11)** with retention of configuration. In the 3*R* series **(12)**, however, the vinyl function now is close to the iron–oxene unit and the two conformations about the C-3–C-4 bond, **(15)** and **(16)**, permit the [2 + 2] and the 'ene' reaction modes, respectively, Scheme 4, leading to the observed products **(13)** and **(14)**. These results support the concept of a monocyclic intermediate, bonded directly to the iron–oxene centre, and provide further information concerning the geometrical relationships between these entities.

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