Evidence for an Insertion–Homolysis Mechanism for Carbon–Sulphur Bond Formation in Penicillin Biosynthesis

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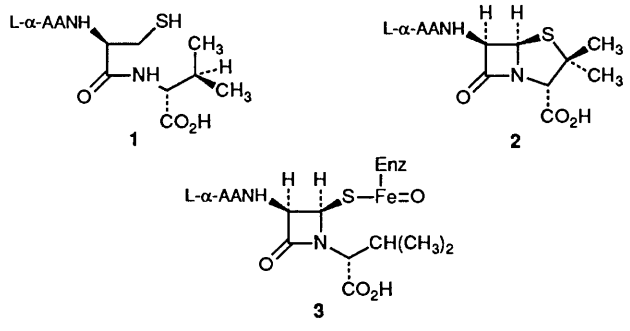
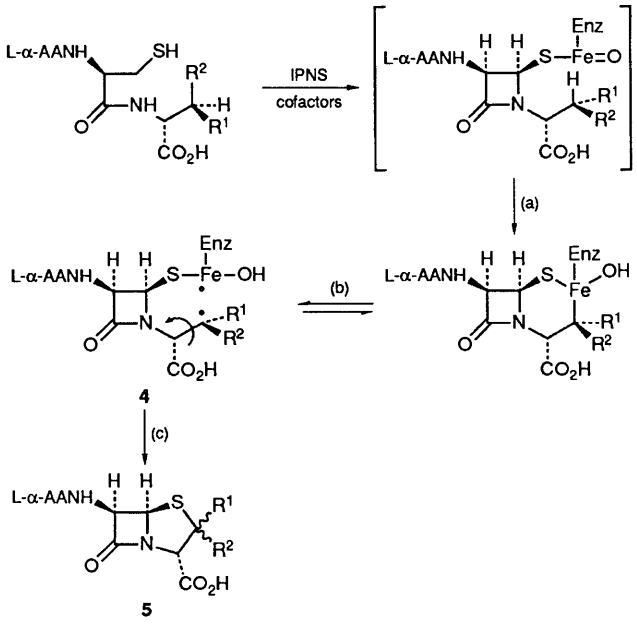
Evidence to support an insertion–homolysis mechanism for the event leading to carbon–sulphur bond formation in penicillin biosynthesis has been obtained by a stereochemical probe.

As a result of a wide range of studies on the mechanism of isopenicillin N synthase (IPNS), which is responsible for the desaturative ring closure of an acyclic tripeptide LLD-ACV **1** into isopenicillin N **2**, a stepwise mechanism[1a] has been proposed in which an intermediate, enzyme-bound, iron oxene species **3** mediates formation of the carbon–sulphur bond. From the study of various analogues of **1** it has further been proposed[1b] that the second step of the desaturase mode proceeds in three stages: (*a*) stereospecific insertion into a C–H bond forming an iron–carbon bond; (*b*) reversible homolytic dissociation to a diradical; (*c*) coupling of the carbon–sulphur bond.

The stereochemistry of the so-formed carbon–sulphur bond of penicllin **5** is dictated by competition between the rate of coupling and the rate of bond rotation in the diradical **4** (Scheme 1). Experiments that support this view have been reported,[2] in particular, the conversion of **6a** into **7a**, which suggested the intermediacy of cyclopropylcarbinyl radicals but did not rule out concerted mechanisms.[3] In order to investigate this conversion in greater detail we synthesised the two stereospecifically labelled isotopomers **6b** and **6c**.† We

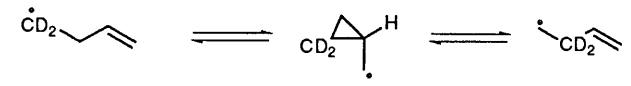


AA = $HO_2CC(NH_2)CH_2CH_2CH_2C(:O)$–

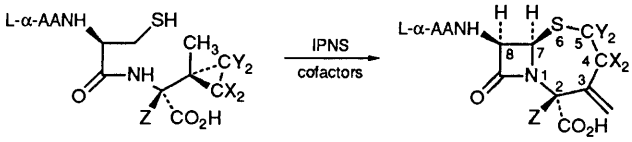


Scheme 1

† Full details of the synthesis of these tripeptides will be reported later.



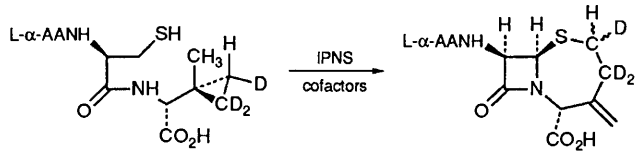
Scheme 2



6: a; X = Y = Z = H
b; X = H, Y = D, Z = D
c; X = D, Y = H, Z = H

7: a; X = Y = Z = H
b; X = H, Y = D, Z = D
c; X = D, Y = H, Z = H

Scheme 3



8 9

Scheme 4

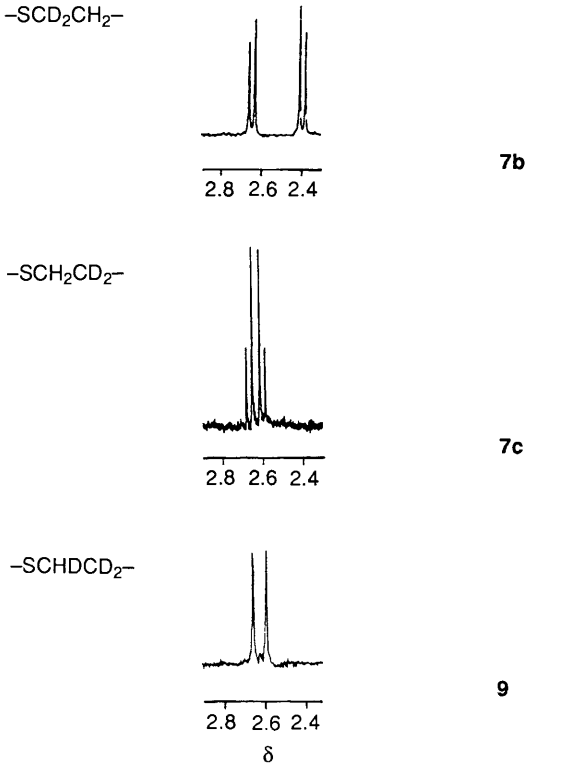
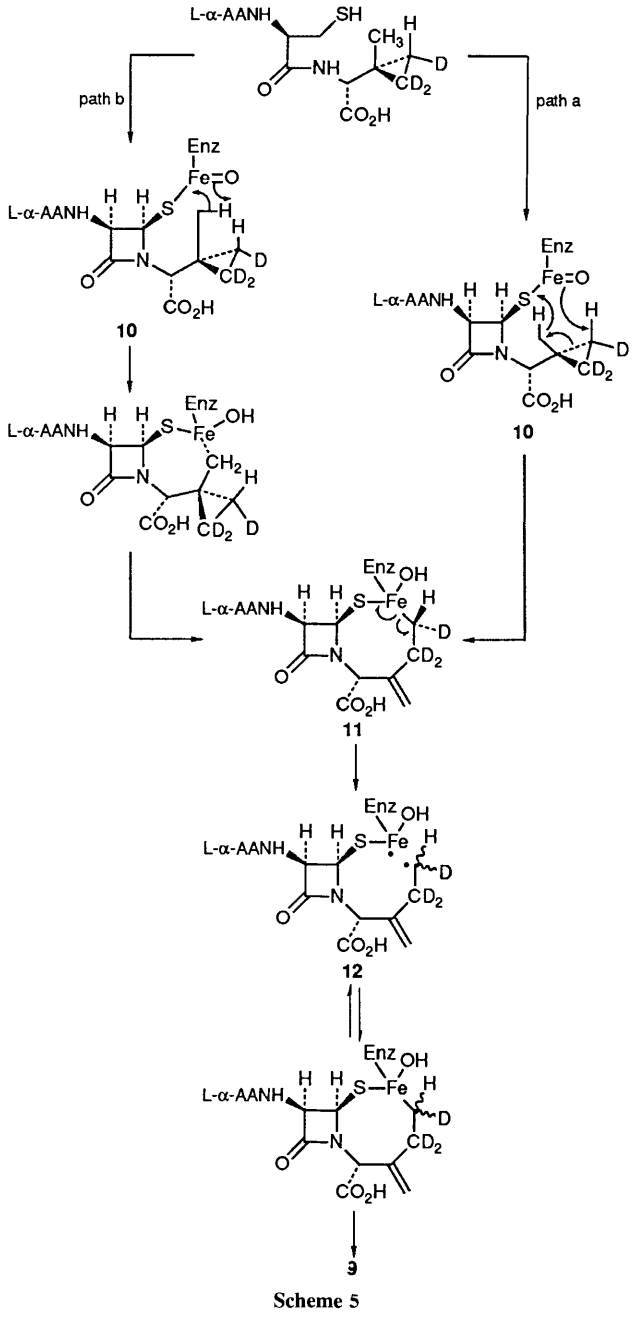


Fig. 1 ^{1}H NMR (500 MHz) spectra

reasoned that if the lifetimes of the postulated homoallyl radicals derived from **6b** or **6c** were sufficiently long,‡ we should observe scrambling of the label between C-4 and C-5 of the 3-exomethylene homocepham metabolite. It is known from studies carried out on deuterium-labelled substrates that rearrangement of cyclopropyl carbinyl radicals is reversible and at room temperature the label is fully scrambled (Scheme 2).[4]

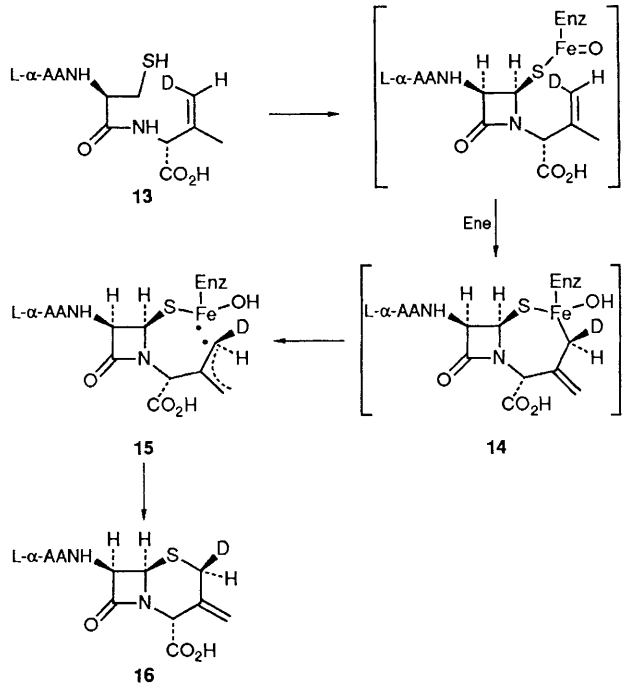
‡ The rate of ring opening[5] the cyclopropylcarbinyl radical to the homoallyl radical is 1.3×10^8 s^{-1}, the rate of the reverse reaction is 4.9×10^3 s^{-1}.



Scheme 5

Incubation of **6b** and **6c** with IPNS gave the labelled 3-exomethylene homocephams **7b** and **7c**. Examination of the region corresponding to $-SCY_2CX_2-$ in the ^{1}H NMR (500 MHz, D_2O) (Fig. 1) of the metabolites demonstrated that each isotopomer delivered a single regiospecifically labelled 3-exomethylene homocepham (Scheme 3). Confirmation of the $-SCY_2CX_2-$ connectivity in the 3-exomethylene homocepham was achieved from the observation of an allylic coupling (J 0.8 Hz) in **7b** between the low-field proton at C-4 and one of the alkenic protons. No equivalent coupling was observed in **7c**. Likewise, analysis of nuclear Overhauser experiments provided results consistent with this assignment. Thus, irradiation of $-SCH_2-$ in **7c** gave NOE to 7-H (12%) and 2-H (6%), whilst irradiation of either $-SCD_2CH_2-$ hydrogen in **7b** gave only a strong geminal NOE without detectable NOE to 7-H.

We next investigated a significantly faster radical clock, tripeptide **8**. In this mechanistic probe scrambling of the label would require only a bond rotation.



Scheme 6

Incubation of **8** with IPNS gave the labelled 3-exomethylene homocepham **9**. Examination of the region corresponding to $-SCHDCD_2-$ in the ^1H NMR (500 MHz, D_2O)(Fig. 1) of the metabolite demonstrated that the regiochemistry of the isotope labels was as anticipated. However, the single deuteron at C-5 was present as a 1 : 1 mixture of epimers **9** (Scheme 4).

From these results an ordered progression of a concerted, followed by a free radical pathway, consistent with the proposed stages of Scheme 1, appears to operate during the conversion by IPNS of the deuteriated cyclopropyl substrates to their corresponding 3-exomethylene homocepham products. Thus, by first assuming that a β-lactam bound iron oxene **10** is generated, it is necessary§ to assume that either a cyclopropyl-ene reaction (path a), or a C–H insertion (path b) followed by a concerted [1,3] shift, opens the 'pro(*S*)' cyclopropane C–C bond stereospecifically. If homolytic dissociation to a diradical preceded cyclopropyl cleavage we would not have expected to observe the complete regiochemical control during the conversion **6b** to **7b**, **6c** to **7c**. The so-formed homoallylic iron–carbon bonded intermediate **11** then equilibrates with a free radical form **12** which, after rotation, closes to provide a stereorandomised C-5 monodeuteriated product **9** (Scheme 5).

It is implicit in this analysis of these results that free radical character is only manifested *after* cyclopropane ring cleavage, when it is revealed by stereorandomisation of the methylene group. It is of interest to compare this situation with that previously reported[6] for substrate **13** in which the carbon–sulphur bond in the product **16** is formed stereospecifically. In this case the metallocycle **14**, formed by a stereospecific ene reaction of the iron oxene intermediate, may homolyse to an *allylic* radical **15**. As the barrier to rotation in such a radical is likely to be larger than that in a primary alkyl radical the stereospecificity observed becomes understandable (Scheme 6).

In conclusion these experiments support the stepwise nature of the carbon–sulphur bond formation process in the second step of the IPNS mechanism.

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§ A homolytic cyclopropane ring cleavage, regiochemically controlled by the IPNS active site topology, cannot be dismissed although we consider it less likely.

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