

## Biomimetic Synthesis of Penicillin

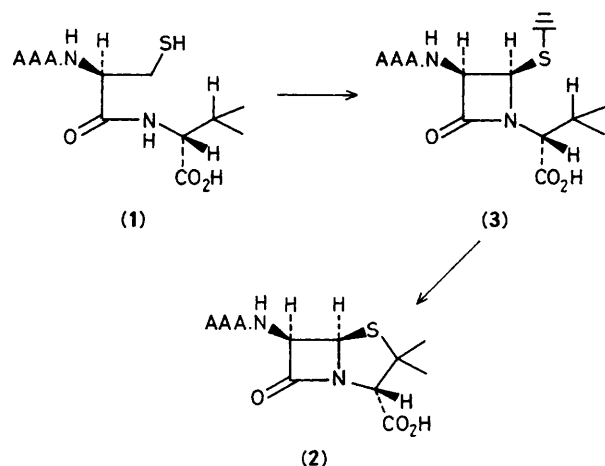
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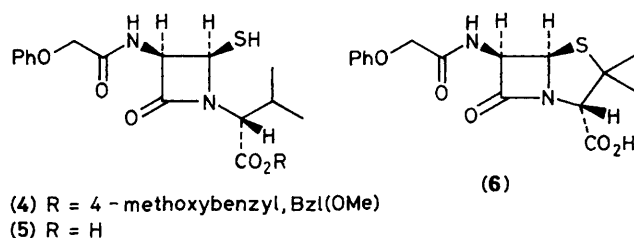
A working chemical model for the carbon–sulphur bond formation step involved in penicillin biosynthesis is presented: thus, oxidation of a monocyclic  $\beta$ -lactam thiol, or its disulphide form, with dioxygen or hydrogen peroxide, catalysed by iron(III) ion, ascorbic acid, and ethylenediaminetetra-acetic acid, results in direct ring closure to penicillins as well as cepham, in a process similar to the enzymatic synthesis.

For many years the chemical and biological details of penicillin's biosynthesis have remained obscure. Recently, a single enzyme, called isopenicillin N synthetase has been isolated from producing organisms, and shown to catalyse the conversion of the tripeptide (**1**) into isopenicillin N (**2**).<sup>1</sup> This enzymatic reaction requires iron(II) ions, ascorbic acid,

dithiothreitol [maintains thiol-form of (**1**)], and one molecule of dioxygen.<sup>2</sup> By the use of isotope effects this reaction has recently been shown to involve two separate carbon–hydrogen bond-breaking steps, the first of which occurs at the cysteinyl- $\beta$ -position and the second at the valinyl- $\beta$ -position.<sup>3</sup> This result, along with results of studies on modified substrates,<sup>4</sup>



**Scheme 1.** AAA =  $\delta$ -(L- $\alpha$ -aminoadipoyl) = 5-(5S)-amino-5-carboxypentanoyl.



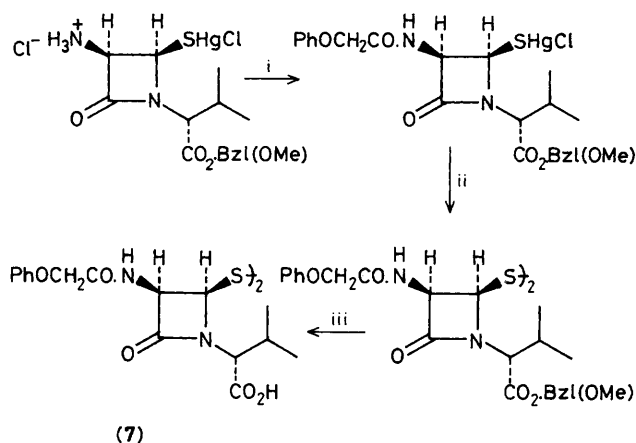
(4) R = 4-methoxybenzyl, Bzl(OMe)

(5) R = H

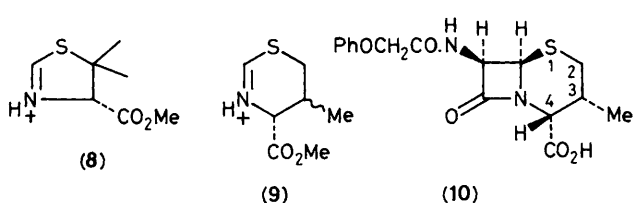
has allowed the postulation<sup>3,4</sup> of an intermediate, enzyme-bound  $\beta$ -lactam (3), Scheme 1.

The second step of carbon-sulphur bond formation occurs with retention of configuration<sup>5</sup> through the sole removal of the valinyl- $\beta$ -hydrogen.<sup>6</sup> Presumably, from the dioxygen stoichiometry (1:1) this last step is concomitant with the reduction of hydrogen peroxide to water and has been suggested<sup>7</sup> to proceed *via* a carbon radical (or iron-carbon bond) at C-3 of valine. In an attempt to achieve the biomimetic equivalent of this cyclisation process we have oxidised a suitable monocyclic azetidinone with the cofactors of the enzymatic reaction, but without the enzyme. A suitable substrate for these studies was provided by the monocyclic ester (4), prepared by methods previously described,<sup>8</sup> which on deprotection in benzene-anisole-trifluoroacetic acid (20:1:3, 1 h, 20 °C) gave the unstable free acid (5)<sup>†</sup> [half life at pH 4.4 (D<sub>2</sub>O) at 20 °C is *ca.* 9 min (by n.m.r.)]. This thiol (5) [39 mg in CH<sub>2</sub>Cl<sub>2</sub> (2 ml)] was added to 4.2 ml of freshly prepared Udenfriend's reagent [iron(II) sulphate, ascorbic acid, ethylenediaminetetra-acetic acid (EDTA) as described<sup>9</sup>] at pH 4.4 and shaken in the presence of oxygen (2 h, 37 °C). After acidification to pH 3 there was obtained from the CH<sub>2</sub>Cl<sub>2</sub> extract a bioactive material (*Staphylococcus aureus* N.C.T.C. 6571, hole plate assay), which was destroyed by addition of  $\beta$ -lactamase I (*Bacillus cereus*). Omission of the FeSO<sub>4</sub> in this experiment gave no bioactivity. The material responsible (equivalent to 0.4  $\mu$ g penicillin V) comigrated on h.p.l.c. (reverse phase octadecylsilane column, 50 mM pH 7 ammonium hydrogencarbonate-methanol as eluant) with penicillin V (6).

A better conversion was obtained from the disulphide (7) prepared as in Scheme 2, which from 5 mg of (7) gave under the above conditions 9.5  $\mu$ g (conversion 0.8%) of penicillin V (6) (bioassay, h.p.l.c., n.m.r.) as well as much unchanged



**Scheme 2.** Reagents: i, NEt<sub>3</sub>, ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate, PhOCH<sub>2</sub>CO<sub>2</sub>H, 24 h; ii, I<sub>2</sub> (0.5 equiv.); iii, PhH, PhOMe, trifluoroacetic acid.

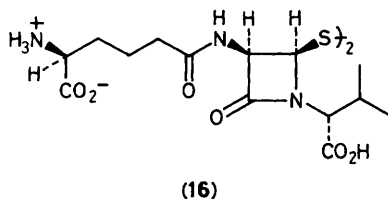
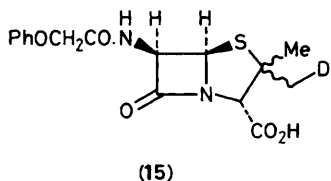
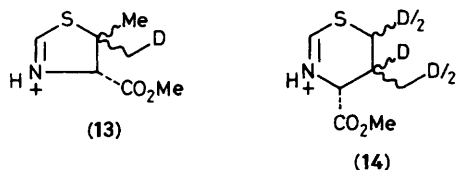
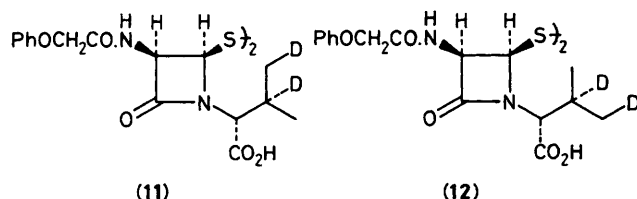


starting material. However g.c.-mass spectroscopy of the derived methyl esters from the crude product indicated that *three* species isomeric with pen V (6) were present from the observation of three g.c. separable entities giving rise to *m/z* 174, which is consistent with the presence of pen V, as ion (8) and, also, cepham, as ion (9). One of them was shown (h.p.l.c., n.m.r.) to be identical to (10) which was synthesised by reduction (H<sub>2</sub>, Pd/C, dioxane, H<sub>2</sub>O) of 7-phenoxyacetamido-3-*exo*-methylene cepham<sup>10</sup> whose n.m.r. spectrum was consistent<sup>†</sup> with the configuration indicated [*J*(H<sup>3</sup>, H<sup>4</sup>) 6 Hz]. The other is most likely the C-3 epimer of (10) but this has not yet been rigorously proven. After a survey of different reaction conditions we found that the use of a Fenton's type reagent<sup>11</sup> gave better conversions. Thus oxidation of (7) at pH 6.7 with FeSO<sub>4</sub>, EDTA, ascorbic acid with slow addition of hydrogen peroxide (0.06% by vol.) gave pen V (6) (conversion, 1.3%) again with unchanged starting material, and also now the reaction mixture contained hydroxylation products of the aromatic ring (h.p.l.c., n.m.r.) of penicillin V.

In order to establish that none of these products were derived from impurities of pen V in our starting material, (5) or (7), we prepared the dideuterated species as a mixture of epimers (11) and (12) [(11): (12), 2:1] by methods previously described.<sup>12</sup> When exposed to the Udenfriend reagent, as before, this gave a smaller yield (*ca.* 0.08%) of pen V, probably as the result of an isotope effect. Using the more potent Fenton's reagent we obtained the penam-cepham mixture and analysed this by g.c.-mass spectroscopy (methyl esters) which showed the three species, having the same retention times as in the undeuterated experiment, but now with *m/z* 175 from the pen V peak corresponding to ion (13) (D = <sup>2</sup>H) and *m/z* 176 from each of the two cepham peaks, corresponding to ion (14). This experiment proves rigorously that these bicyclic  $\beta$ -lactams originated from the monocyclic

<sup>†</sup> All new compounds have given satisfactory spectral data.

<sup>†</sup> A smaller coupling constant *J*(H<sup>3</sup>, H<sup>4</sup>) 1.5 or 2.5 Hz was observed for two similar 3- $\beta$ -methylcephams, see ref. 4(b).



precursors. Furthermore, isolation of the pen V from this deuteriated experiment (h.p.l.c.) gave a substance whose n.m.r. spectrum showed that the single deuterium atom in the product (15) was equally distributed between the two methyl groups (500 MHz D<sub>2</sub>O,  $\delta_{\beta}$  1.525 and  $\delta_{\alpha}$  1.501). Thus this biomimetic ring closure, unlike the enzymatic reaction, proceeds with complete loss of stereochemistry. Finally, we have also oxidised the  $\alpha$ -amino adipoyl- $\beta$ -lactam (16)<sup>8b</sup> and shown that it is converted into the normal enzymatic product, isopenicillin N (2) (500 MHz n.m.r.), but in this case the conversion is much lower, probably reflecting the greater instability of this penicillin.

In summary we have successfully closed the thiazolidine ring of penicillins, from the postulated<sup>3,4</sup> monocyclic inter-

mediate, by oxidative means using the cofactors, Fe<sup>II</sup> ion and ascorbic acid, common to the enzymatic reaction. This result supports our previous contention<sup>7</sup> that a free radical (or iron-carbon bond) is involved in the enzymatic reaction but this biomimetic route lacks the stereospecificity (retention of configuration) and regiospecificity (five-membered ring closure from the valinyl substrate only) of the enzyme catalysed process.

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