Ring Expansion of Penams to Cephams: a Possible Biomimetic Process

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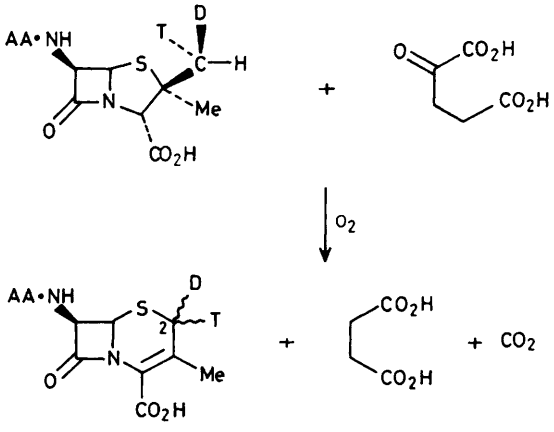
Homolytic reductive debromination of a 2β-bromomethyl penam by triphenyltin hydride provides, *via* ring expansion of the derived 2β-methyl radical, the corresponding cepham system; a similar process may explain the biosynthetic ring expansion of penicillins to cephalosporins.

The biosynthetic conversion of penicillin N to deacetoxycephalosporin C is the first step in the biosynthesis of the cephalosporin group of antibiotics.[1] This conversion is mediated by an iron-dependent desaturase, which utilises α-ketoglutarate and oxygen as cosubstrates,[2] and effects the oxidative ring expansion by conversion of the β-methyl group of penicillin N into the methylene group (C-2) of the product.[3] By feeding valine stereospecifically labelled with hydrogen, deuterium, and tritium in the *pro R* methyl group to the fungus *Cephalosporium acremonium* it has been shown[4] that the



Scheme 1. AA = D-α-aminoadipoyl.

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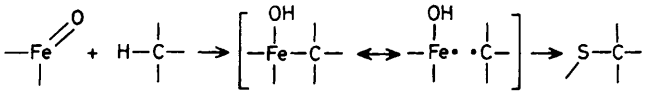
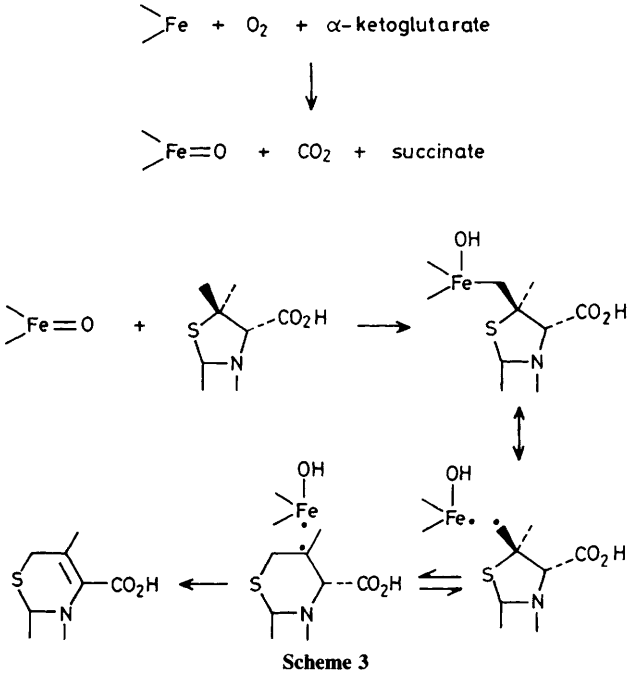
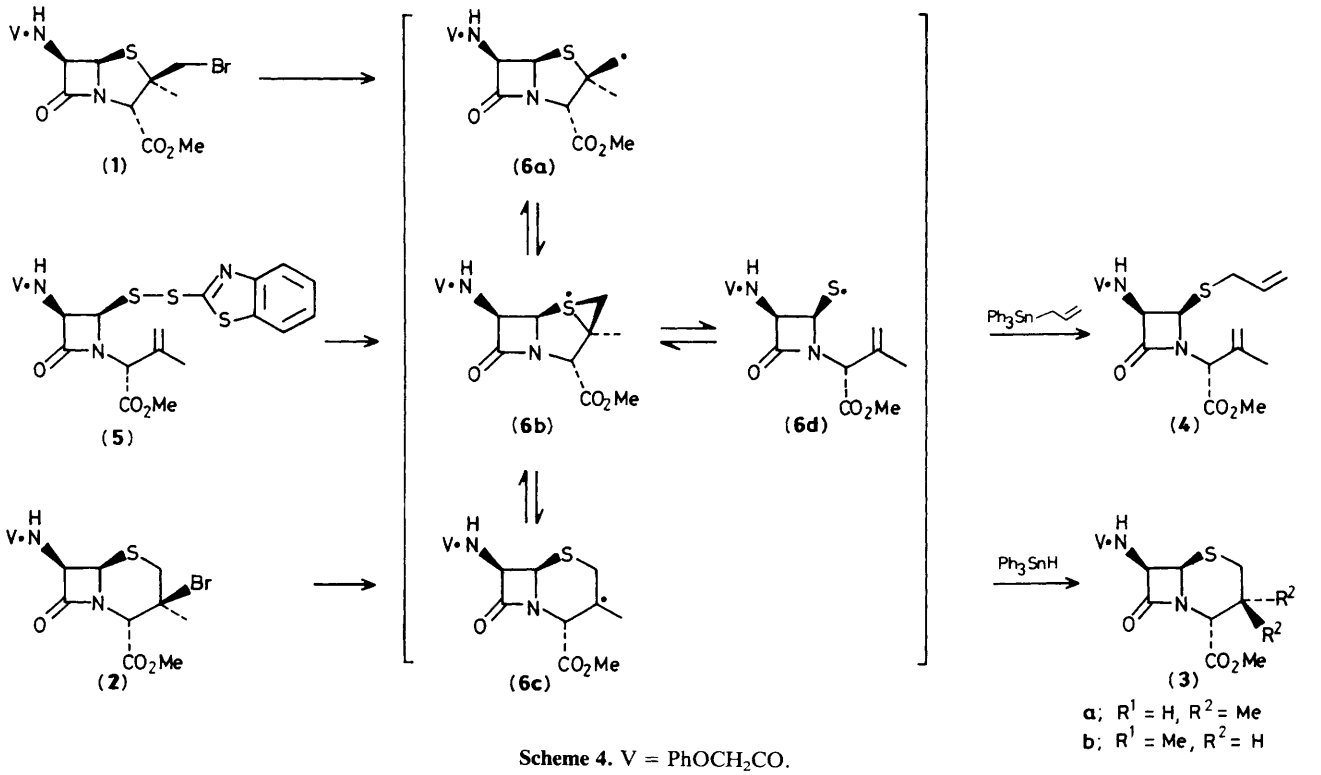


Scheme 2



Scheme 3



Scheme 4. V = $PhOCH_2CO$.

Table 1.

Entry	Starting material	Reaction conditions	Product (%)
1	2β-Bromomethylpenam (**1**)	Ph_3SnH (2 equiv.), AIBN (cat.) benzene, reflux, 2 h	α-Methylcepham (**3a**) (40%); β-methylcepham (**3b**) (35%)
2	3β-Bromocepham (**2**)	Ph_3SnH (2 equiv.), AIBN (cat.) benzene, reflux, 2 h	α-Methylcepham (**3a**) (49%); β-methylcepham (**3b**) (39%)
3	2β-Bromomethylpenam (**1**)	Ph_3SnH (2 equiv.), benzoquinone (1 equiv.), benzene, reflux 2 h	Unchanged (**2**) (>95%); no (**3a**), (**3b**)
4	3β-Bromocepham (**2**)	Ph_3SnH (2 equiv.), benzoquinone (1 equiv.), benzene, reflux, 2 h	Unchanged (**2**) (>95%); no (**3a**),(**3b**)
5	2β-Bromomethylpenam(**1**)	Ph_3Sn allyl (2 equiv.), AIBN (cat.), benzene, reflux, 3 h	*S*-Allylazetidinone (**4**) (94%)
6	3β-Bromocepham (**3**)	Ph_3Sn allyl (2 equiv.), AIBN (cat.) benzene, reflux, 3 h	*S*-Allylazetidinone (**4**) (92%)
7	Disulphide (**5**)	Ph_3SnH (2 equiv.), AIBN (cat.), benzene, reflux, 24 h	α-Methylcepham (**3a**) (35%); β-methylcepham (**3b**) (35%); (**5**) (10%)
8	Disulphide (**5**)	Ph_3Sn allyl (2 equiv.), AIBN (cat.) benzene, reflux, 16 h	*S*-Allylazetidinone (**4**) (85%)
9	Disulphide (**5**)	Ph_3Sn allyl (2 equiv.), benzoquinone (1 equiv.), benzene, reflux, 16 h	Unchanged (**5**) (>95%); no (**4**)
10	Disulphide (**5**)	Ph_3Sn allyl (2 equiv.), hydroquinone (0.2 equiv.), benzene, reflux, 16 h	Unchanged (**5**) (>95%); no (**4**)

methyl to methylene conversion occurs with complete loss of stereochemistry, Scheme 1. From our work on the enzyme isopenicillin N synthase from *C. acremonium*, which is responsible for the synthesis of the penicillin nucleus and is also an iron-dependent desaturase of similar molecular size, we have suggested that the crucial C–S bond forming step exhibits the characteristics of a free radical process or its equivalent, *i.e.* a very weak iron–carbon bond, derived by insertion of an iron–oxo species into the unactivated valine β-CH bond, Scheme 2.[5] It seemed reasonable that a similar process, involving an insertion of an iron–oxo species, derived from the ring-expansion enzyme and its cosubstrates oxygen and α-ketoglutarate, into the penicillin β-methyl group would explain the above stereochemical result through dissociation of the iron–carbon bond to form a freely rotating methylene radical, which could rearrange to the corresponding cepham radical, Scheme 3.[4]

In order to test the chemical feasibility of such a scheme we have generated a penicillin derived β-methyl radical with the following results. First the 2β-bromomethyl penicillin (**1**) and the 3β-bromocepham (**2**)[6] with triphenylstannane (2 equiv.) under radical chain conditions[7] [azobisisobutyronitrile

(AIBN) (cat.), benzene, 80 °C, 2 h] were each cleanly converted into the same mixture of cephams, (**3a**),(**3b**),[8] in an approximately equal ratio. Under the same conditions, but in the absence of stannane, (**1**) was more slowly converted (approx. 30% of rate) into the more stable cepham (**2**) (which itself was stable to such conditions), proving that the conversion of (**1**) into (**3a**),(**3b**) does not require the intermediacy of (**2**). When triphenyl(allyl)stannane was used [triphenyl(allyl)stannane (2 equiv.), AIBN (cat.), benzene, 80 °C, 3 h] both (**1**) and (**2**) gave cleanly the *S*-allylazetidinone (**4**),‡ *via* trapping on sulphur. The same manifold of interconverting radicals could be entered from the monocyclic disulphide (**5**), which with the allylstannane gave (**4**) and with triphenylstannane the same mixture of (**3a**),(**3b**), although in this case both reactions were slower than those derived from the bromides (**1**) and (**2**). These results are summarised in Table 1. In all these cases the substitution of AIBN by benzoquinone or hydroquinone completely inhibited the reaction as assayed by n.m.r. spectroscopy (300 MHz).

Our findings are in accord with the existence of a rapidly interconverting set of radicals (**6a—d**), Scheme 4, which can be generated from the three possible precursors (**1**), (**2**), and (**5**), and can be intercepted on carbon to give (**3a**),(**3b**) or, alternatively, in the allyl transfer case on sulphur, to give (**4**).[9] Thus in regard to the original question of cephalosporin biosynthesis it follows that the hypothetical interaction of a β-methyl penam radical on the biosynthetic path to the cephalosporins has at least chemical validity. In this connection, it is interesting to note that the involvment of free radical intermediates has been suggested for other α-ketoglutarate dependent oxygenases, *e.g.* prolyl hydroxylase[10] and γ-butyrobetaine hydroxylase.[11]

‡ For (**4**): δ_H (300 MHz, $CDCl_3$) 1.93 (3H, s, vinyl Me), 3.0—3.15 (2H, AB part of ABX, SCH_2) 3.80 (3H, s, CO_2Me), 4.58 (2H, s, $PhOCH_2$), 4.83, 5.01, and 5.16 (3H, 3 × s, $NCHC{=}CH_2$), 5.08—5.17 (2H, 2 × d, obscured, $SCH_2CH{=}CH_2$), 5.25 (1H, d, *J* 5 Hz, NHCHCHS), 5.565 (1H, dd, *J* 5, 9 Hz, NHCHCHS), 5.6—5.75 (1H, m, X part of ABX, SCH_2CH), and 6.95—7.46 (6H, m, aryl H, NH); *m/z* (NH_3 desorption chemical ionisation) 405 (MH^+, 100%); $\nu_{max.}$ ($CHCl_3$) 3415m, 1770s, 1745s, 1690s, and 990w cm^{-1}; $[\alpha]_D^{20}$ −135° (*c* 1.4, $CHCl_3$).

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