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The Ring Expansion of Penams to Cephams: a Possible Biomimetic Process

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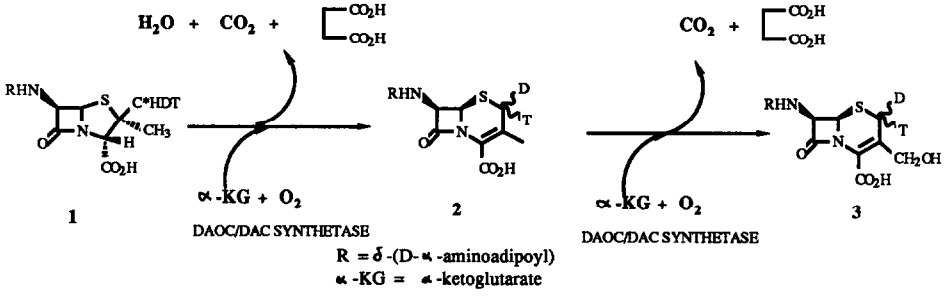
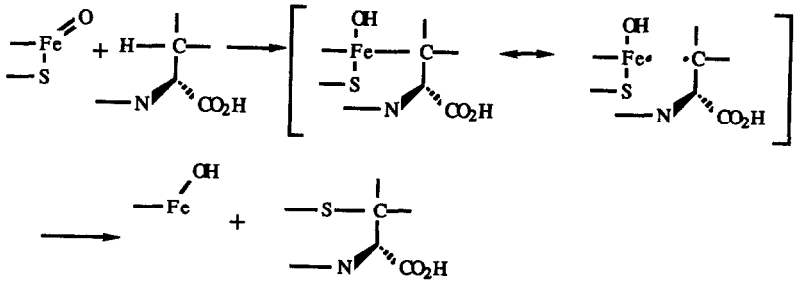
Abstract: Reaction of a 2β-bromomethyl penam with triphenyltin hydride gave, via ring expansion of the derived 2β-methyl radical, the corresponding cepham system; a similar process may be in operation during the biosynthetic ring expansion of penicillin N to deacetoxycephalasporin C.[1]

The ring expansion of penicillin N (1) to deacetoxycephalasporin C (DAOC, 2) and its subsequent hydroxylation to deacetylcephalosporin C (DAC, 3) are the key steps in the biosynthesis of cephalosporin C (4) (Scheme 1).[2] In Cephalosporium acremonium they are mediated by a single bifunctional enzyme (DAOC/DAC Synthetase)[3], and in Streptomyces by different proteins.[4] In both of these eukaryotic and prokaryotic organisms the conversions (1 → 2, and 2 → 3) are catalysed by iron dependent oxygenases, requiring α-ketoglutarate and dioxygen as cosubstrates.[3,5]



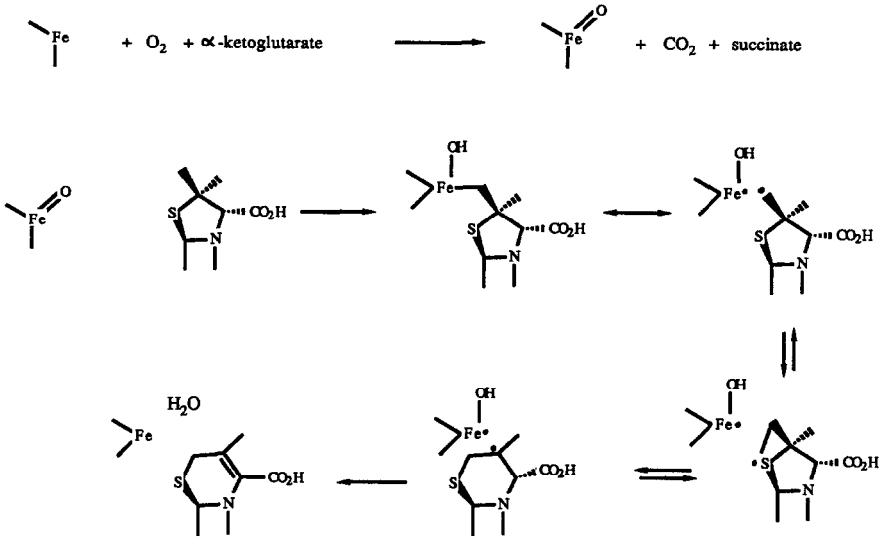
SCHEME 1

By feeding labelled valine to C. acremonium it has been shown that the β-methyl group of (1) is converted to the C-2 methylene of DAOC (2); moreover using valine stereospecifically labelled with H, D and T in the pro R methyl group that the ring expansion process occurs with complete loss of stereochemistry at the C-2 methylene of DAOC (2) (Scheme 1).[6]



Scheme 2

Our studies concerning Isopenicillin N Synthetase,[2a] which is responsible for the biosynthesis of the penicillin nucleus, have shown it to be an iron dependent desaturase of similar size to DAOC/DAC Synthetase. We have proposed that the crucial C-S bond forming step exhibits the characteristics of a free radical process or its equivalent, i.e. a weak iron-carbon bond, derived by insertion of an iron-oxo species into the valine 3-H bond (Scheme 2).[2a] It seemed reasonable to us that a similar process, involving insertion of an iron oxo species, derived from DAOC/DAC Synthetase and α-ketoglutarate and dioxygen, would account for the chiral methyl labelling experiments[6] for the ring expansion process, by dissociation of the weak iron carbon bond to form a freely rotating methylene radical, which could subsequently rearrange to the corresponding cepham radical (Scheme 3).



SCHEME 3

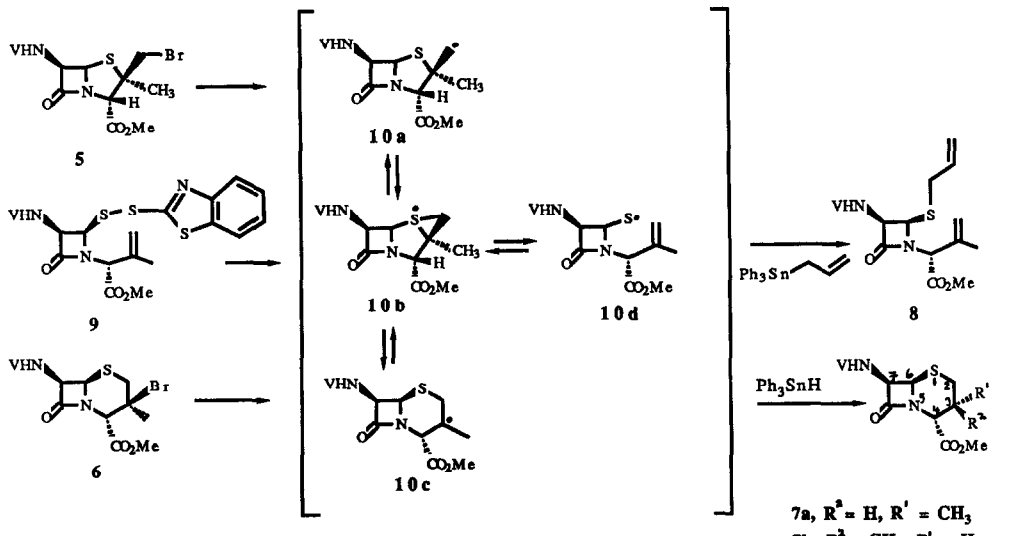
To test the chemical feasibility of such a process we have specifically generated a penicillin derived 8-methyl radical and examined its reactivity. The results are summarised in Table 1. Firstly the 2β-bromomethyl penicillin (5)[7] and the 3β-bromo-cepham[7] (6) were reacted with triphenyl tin hydride under radical chain conditions[8] [azobisisobutyronitrile (AIBN) (cat.), benzene, 80°C, 2h] to give cleanly the cephams (7a) and (7b)[9] in a similar ratio (Scheme 4). Under identical conditions, but in the absence of stannane, (5) was converted more slowly (approx. 30% of rate) into the more stable cepham (6) (itself stable to such conditions), demonstrating that the conversion of (5) into (7), does not require the intermediacy of (6). Reaction of (5) or (6) with triphenyl(allyl)stannane[10] [(2 equiv.),

AIBN (cat), benzene, 80°C] gave the S-allyl azetidinone (8) in high yield. The same interconverting radical manifold could also be entered via Kamiya's disulphide[7] (9), which with the allylstannane gave (8), and with triphenyltin hydride the same mixture of (7a) and (7b). In all cases substitution of AIBN by benzoquinone or hydroquinone completely inhibited reaction [by ^{1}H n.m.r. (500 MHz)].

Entry	Starting material	Reaction conditions	Product (%)
1	2β-Bromomethylpenam (5)	Ph_3SnH(2 equiv.),AIBN(cat.) benzene,reflux,2h	α-Methylcepham (7a) (40%); β-methylcepham (7b) (30%)
2	3β-Bromocepham (6)	Ph_3SnH(2 equiv.),AIBN(cat.) benzene,reflux,2h	α-Methylcepham (7a) (49%); β-methylcepham (7b) (39%)
3	2β-Bromomethylpenam (5)	Ph_3SnH(2 equiv.),benzoquinone (1 equiv.),benzene,reflux, 2h	Unchanged (5) (>95%); no (7a),(7b)
4	3β-Bromocepham (6)	Ph_3SnH(2 equiv.),benzoquinone (1 equiv.),benzene,reflux, 2h	Unchanged (6) (>95%); no (7a),(7b)
5	2β-Bromomethylpenam (5)	Ph_3Sn.allyl(2 equiv.),AIBN(cat.), benzene,reflux,3h	S-Allylazetidinone (8) (94%)
6	3β-Bromocepham (6)	Ph_3Sn.allyl(2 equiv.),AIBN(cat.) benzene,reflux,3h	S-Allylazetidinone (8)(91%)
7	Disulphide (9)	Ph_3SnH(2 equiv.),AIBN(cat.), benzene,reflux,24h	α-Methylcepham(7a)(35%); β-methylcepham (7b)(35%); (9)(10%)
8	Disulphide (9)	Ph_3Sn.allyl(2 equiv.),AIBN(cat.) benzene,reflux,16h	S-Allylazetidinone (8)(83%)
9	Disulphide (9)	Ph_3Sn.allyl(2 equiv.), benzoquinone(1 equiv.), benzene,reflux,16h	Unchanged (9) (>95%); no(8)
10	Disulphide (9)	Ph_3Sn allyl(2 equiv.), hydroquinone(0.2 equiv.), benzene,reflux,16h	Unchanged (9) (>95%); no(8)

TABLE 1

Our results imply the existence of a rapidly interconverting set of radicals (10a-d), Scheme 4, which can be generated from the precursors (5), (6) or (9). The sulphur-bridged radical species (10b) may be an energy minimum in the manifold (10a-d) as has been suggested for other heavy elements.[11] Thus it seems that the original hypothesis of the involvement of a β-methyl penam in the in vivo ring expansion of (1) → (2) has at least chemical validity. It is of interest to note that the involvement of free radical intermediates has been proposed for other α-Ketoglutarate dependent oxygenases.[12]



SCHEME 4

V = $PhOCH_2CO$

Acknowledgement: We thank Eli Lilly & Co., Indianapolis, Indiana 46206, U.S.A., for the generous gift of authentic (7a) and (7b) and for financial support.

Experimental

Standard experimental procedure as previously reported was carried out.[13] ^{1}H n.m.r. spectra were recorded on Bruker WH-300 (300 MHz) or AM 500 (500 MHz) spectrometers (calibrated to residual $CHCl_3$, δ = 7.27 for samples in $CDCl_3$). ^{13}C n.m.r. spectra were recorded on Bruker AM 250 instrument (62.9 MHz) or Varian Gemini 200 MHz instruments (50.3 MHz).

General Procedure for Radical Chain Reaction for the Preparation of Cephams (7a) and (7b). The radical precursor (1.0 equiv), AIBN (ca 5 mg) and triphenyl tin hydride (2.0 equiv) were dissolved in degassed benzene and refluxed under argon. The benzene was removed in vacuo and the residue partitioned between acetonitrile and petroleum ether (40-60°C). The acetonitrile layer was evaporated to dryness and the residue purified by chromatography on silica gel (diethyl ether).

Reaction of the Kamiya's Disulphide (9)[7] with Triphenyl Tin Hydride. The general procedure was followed; after chromatography: Yield [(7a):48 mg (35%), (7b):48 mg (35%), recovered (9):20 mg (10%); from (9) (200 mg, 0.38 mmol) and triphenyl tin hydride (266 mg, 0.76 mmol) and AIBN (5 mg)].

Reaction of 2β-Bromomethyl Penicillin V methyl Ester (5) with Triphenyl Tin Hydride. The general procedure was followed; after chromatography: Yield [(7a):27 mg (40%), (7b):20 mg (30%); from (5) (83 mg, 0.19 mmol) and triphenyl tin hydride (131 mg, 0.37 mmol) and AIBN (5 mg)].

Reaction of β-Bromo Cepham (6) with Triphenyl Tin Hydride. The general procedure was followed; after chromatography: Yield [(7a):16 mg (49%), (7b):13 mg (39%); from (6) (40 mg, 0.09 mmol) and triphenyl tin hydride (63 mg, 0.18 mmol) and AIBN (5 mg)].

Analytical Data for (7a) and (7b)[9]

For (7a):tlc (diethyl ether) R_f 0.45; m.p. 110-112°C; ν_{max} ($CHCl_3$) 1775s (β-lactam), 1745s (ester), 1690s (amide), and 1520s cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.03 (3H, d, J 7 Hz, CCH_3), 2.16 - 2.23 (1H, m, $CHCH_3$), 2.47 - 2.53 and 3.05 - 3.14 (2H, 8 lines ABX, S - CH_2), 3.77 (3H, s, OMe), 4.55 (2H, ca s, CH_2OPh), 4.63 (1H, d, J 7 Hz, 4-H), 5.37 (1H, d, J 4.5 Hz, 6-H), 5.71 (1H, dd, J 10 Hz, 4.5 Hz, 7-H), 6.93 - 7.4 (6H, m, Ar-H, NH); δ_C ($CDCl_3$) 17.6 (q,$CHCH_3$), 28.4 (d, $CHCH_3$), 29.4 (t, SCH_2), 52.7 (q, CO_2CH_3), 55.2, 55.4 59.1 (3 x d, HNCHCHS, $CHCO_2CH_3$), 67.4 (t, CH_2OPh), 114.9, 122.3, 129.7 (3 x d, 3 x ArC), 157.1 (s, O-C-Ar), 166.0, 168.3, 169.6 (3 x s, 3 x CO); m/z, (E.I.) 364 (M^+, 5%). For (7b): tlc (diethyl ether) R_f 0.4; m.p 88-90°C; ν_{max} ($CHCl_3$) 1775s (β-lactam), 1740s (ester), 1690m, 1520m cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.29 (3H, d, J 6.5 Hz, $CHCH_3$), 2.41 - 2.46 (1H, m, $CHCH_3$), 2.49 - 2.55 and 3.12 - 3.17 (2H, 8 lines ABX, S-CH_2), 3.79 (3H, s, OCH_3), 4.36 (d, J 2 Hz, 4-H), 4.57 (2H, ca s, CH_2OPh), 5.23 (1H, d, J 4.5 Hz, 6-H), 5.69 (1H, dd, J 9.5, 4.5, 7-H), 6.95 - 7.40 (5H, m, Ar-H), 7.61 (1H, d, J 9.5 Hz, NH). δ_C ($CDCl_3$) 17.6 (q, $CHCH_3$), 28.1 (d, $CHCH_3$), 32.5 (t, SCH_2), 52.1 (q, CO_2CH_3), 54.0, 54.1 54.7 (3 x d, HNCHCHS $CHCO_2CH_3$), 67.4 (t,CH_2OPh), 114.9, 122.4, 129.8 (3 x d, 3 x ArC), 151.1 (s, ipso Ar-C), 167.1, 168.3, (2 x s, 2 x CO); m/z (E.I.) 364 (M^+, 5%), 174 (100%)

Preparation of (2R,3R)-2-allylthio-1-[(1R)-2-(methylene)-1-(methoxycarbonyl)propyl)]-3-phenoxyacetyamido-azetidin-4-one (8). The general procedure was followed, except triphenyl allyl tin[16] was substituted for triphenyl tin hydride.

Reaction of Kamiya's Disulphide (9)[7] with Triphenyl Allyl Tin: Yield [(8):32 mg (83%); from (9) (50 mg, 0.096 mmol) and triphenyl allyl tin (74 mg, 0.19 mmol) and AIBN (5 mg)].

Reaction of 2β-Bromomethyl Penicillin V Methyl Ester (5) with Triphenyl Allyl Tin: Yield [(8):65 mg (94%), from (5) (75 mg, 0.17 mmol) and, triphenyl allyl tin (133 mg, 0.45 mmol) and AIBN (5 mg)].

Reaction of 2β-Bromo Cepham (6) with Triphenyl Allyl Tin: Yield [(8):33 mg (91%), from (6) (40 mg, 0.09 mmol) and triphenyl allyl tin (70.5 mg, 0.18 mmol) and AIBN (5mg)].

Analytical Data for (8):tlc (diethyl ether) R_f 0.6; $[\alpha]_D^{\circ}$ = -135° (C = 1.4, $CHCl_3$); ν_{max} ($CHCl_3$) 3415m, 1770s (β-lactam), 1745s, 1690s and 990w cm^{-1}; δ_H (300 MHz, $CDCl_3$) 1.92 (3H, s, C-CH_3), 3.06 - 3.10 (2H, m, SCH_2), 3.80 (3H, s, OCH_3), 4.50 (2H, s, CH_2OPh), 4.83, 5.01 (2H, 2 x s, C=CH_2), 5.01-5.17 (3H, m, C=CH_2, $CHCO_2$), 5.25 (1H, d, J 4.5 Hz, 2-H), 5.57 (1H, dd, J 9.5, 4.5 Hz, 3-H), 5.61 - 5.69 (1H, m, CH=CH_2), 6.95 - 7.41 (5H, m, Ar-H), 7.46 (1H, d, J 9.5 Hz, NH); δc ($CDCl_3$) 21.0 (q, CCH_3), 34.6 (t, SCH_2), 52.5 (q, OCH_3), 59.3, 59.5, 65.6 (3 x d, HNCHCHS, $CHCO_2CH_3$), 67.2 (t, CH_2OPh), 114.9, 122.3, 129.9, 133.4 (4 x d, x Ar-C, CH=CH_2), 117.3, 118.3 (2 x t, C=CH_2), 138.3 (s, C=C-CH_3), 157.3 (s,ipso Ar-C), 166.2, 168.8, 169.1 (3 x s, 3 x CO); m/z (desorption chemical ionisation, NH_3), 405 (100% MH^+), 331 (80%), 250 (60%), 214 (80%). [Analysis: Calculated: C, 59.39; H, 5.98; N, 6.93; S, 7.93%. Found: C, 59.50; H, 6.18; N, 6.55; S, 7.98%].

Inhibition of Reaction by p-Benzoquinone or Hydroquinone.

The general procedure was followed except that p-benzoquinone or hydroquinone was substituted for AIBN.

Reaction of (5) with Triphenyl Tin Hydride in the Presence of p-Benzoquinone.

Yield [recovered (5) 74 mg (95%); from (5) (78 mg, 0.18 mmol) and triphenyl tin hydride (123 mg, 0.35 mmol) and p-benzoquinone (19 mg, 0.18 mmol)].

Reaction of (6) with Triphenyl Tin Hydride in the presence of p-Benzoquinone.

Yield [recovered (6) 50 mg (96%); from (6) (52 mg, 0.12 mmol) and triphenyl tin hydride (83 mg, 0.24 mmol) and p-benzoquinone (12 mg, 0.11 mmol)].

Reaction of (9) with Allyl Triphenyl Tin in the presence of p-Benzoquinone.

Yield [recovered (9) 190 mg (95%); from (9) (200 mg, 0.38 mmol) and Allyl triphenyl tin (296 mg, 0.76 mmol) and p-benzoquinone (41 mg, 0.38 mmol)]. Isolation by chromatography flash silica (ethyl acetate/diethyl (ether, 3:2)].

Reaction of (9) with Allyl Triphenyl Tin in the presence of hydroquinone.

Yield [recovered (9) 189 mg (95%); from (9) (200 mg, 0.38 mmol) and allyl triphenyl tin (296 mg, 0.76 mmol) and hydroquinone (8.3 mg, 0.08 mmol, 0.2 equiv.).

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