Monocyclic β-Lactam Tripeptide, 1-(D-Carboxy-2-methylpropyl)-3-L-(δ-L-2-aminoadipamido)-4-L-mercaptoazetidin-2-one†, a Putative Intermediate in Penicillin Biosynthesis

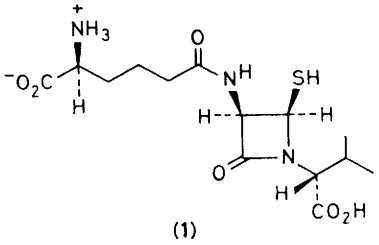
Sir Edward P. Abraham,*[b] Robert M. Adlington,[a] Jack E. Baldwin,*[a] Michael J. Crimmin,[a] Leslie D. Field,[a] Gamini S. Jayatilake,[b] and Robert L. White[b]

[a] *Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QY, U.K.*
[b] *Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, U.K.*

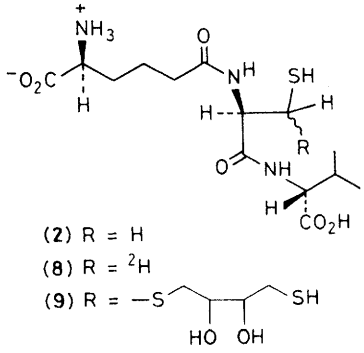
The disulphide corresponding to the above thiol has been synthesised, but all attempts to reduce this substance to the thiol have been unsuccessful, although an alternative procedure, *via* a thiomercury intermediate, enabled the thiol to be generated *in situ*; the properties of this thiol, however, are not in accord with those described for a putative intermediate in penicillin biosynthesis.[1]

Recently it was claimed[1] that the monocyclic β-lactam (**1**) was formed from δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine, (**2**),[2] by incubation with a protoplast lysate from *Penicillium chrysogenum*. The evidence supporting this claim was based on the isolation of labelled compounds believed to be (**1**), from [(3-^{3}H)Cys, (1-^{14}C)Val]-(**2**), [(3,4-$^3H_2$, 1-^{14}C)Val]-(**2**), and [(2-^{3}H, 1-^{14}C)Val]-(**2**). The loss of 49.6% of ^{3}H in the first case, coupled with the retention of all ^{3}H in the last two examples, was adduced to support the structure proposed for (**1**). Furthermore, the biosynthetic sample of (**1**) was claimed to be identical with a synthetic sample, as judged by cation-exchange chromatography.‡ The compound (**1**) appeared to be relatively stable since it was stored with an excess of dithiothreitol overnight at room temperature (pH 8.2) and was subsequently subjected to cation-exchange column chromatography. These results were all the more striking to us since, prior to the appearance of the publication, we had synthesised the disulphide of (**1**) but had been unable to reduce it to the thiol (**1**).

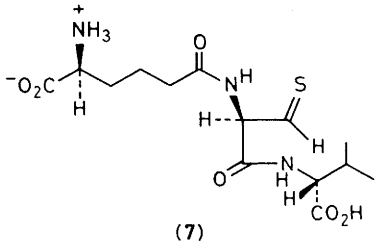
The synthetic route to the dimer, (**6**), from the thiadiazabicycloheptane, (**3**),[3] Scheme 1, involved the final deprotection of the azetidinone (**5**) followed by sequential ion-exchange and Sephadex-gel filtration chromatography to yield pure (**6**).§ Reductive cleavage of the disulphide (**6**) (1 μmol) with dithiothreitol (10 μmol) at pH 6.90 and pH 7.10 (phosphate buffer) at 21 °C led to the rapid opening of its β-lactam ring. Monitoring these reactions by i.r. and n.m.r. spectroscopy¶ it was possible to show that no β-



(**1**)



(**2**) R = H
(**8**) R = ^{2}H
(**9**) R = —S(HO)(OH)SH



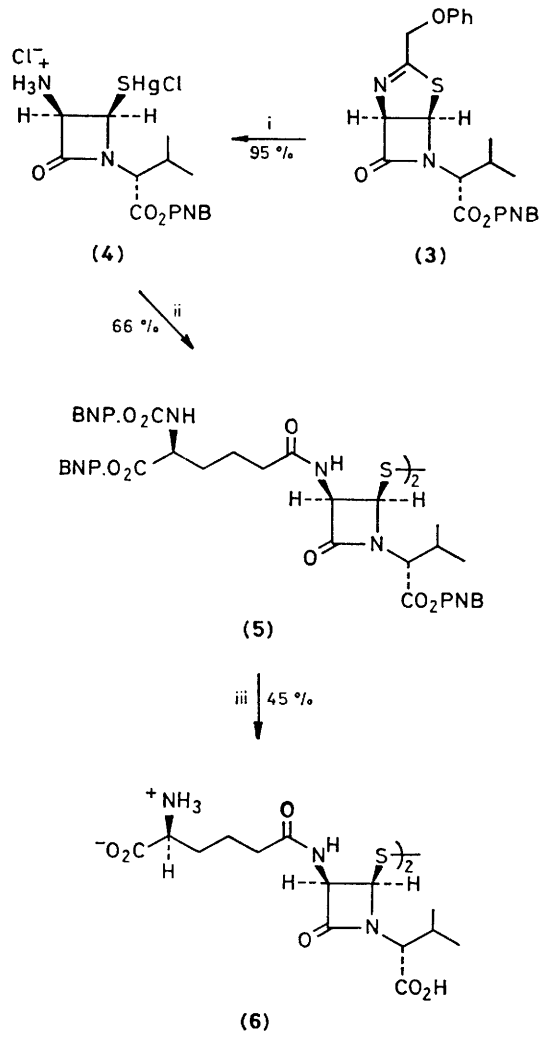
(**7**)

† This system of nomenclature has been retained to be consistent with ref. 1. According to the I.U.P.A.C. system the stereochemistry should be designated as *R* and *S*, *i.e.* 1-[(1*R*)-carboxy-2-methylpropyl]-(3*R*)-[(5*S*)-5-amino-5-carboxypentanamido]-(4*R*)-mercaptoazetidin-2-one.

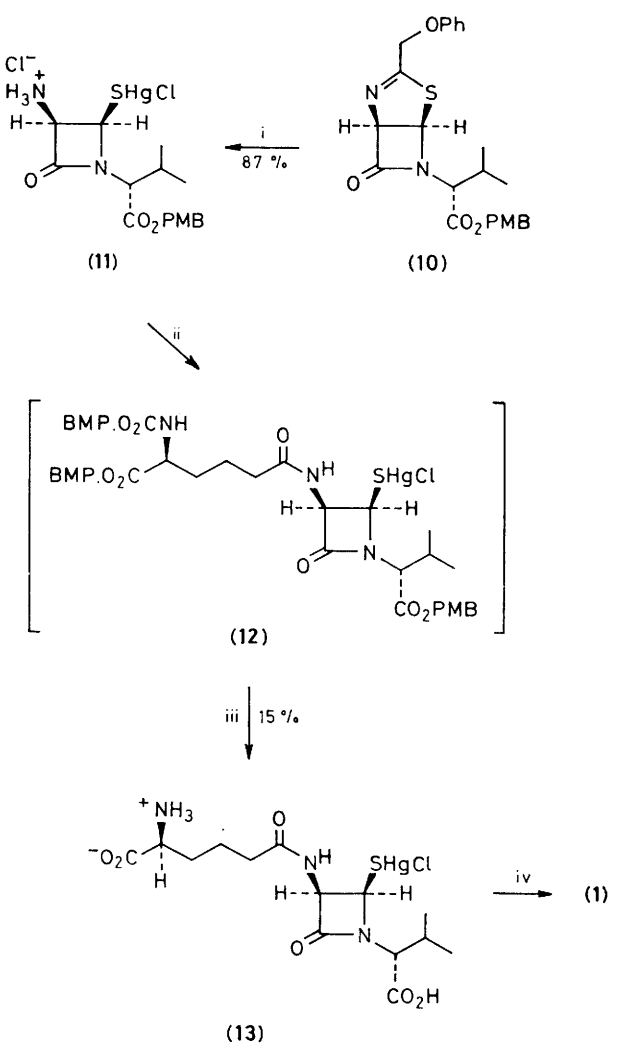
‡ In ref. 1 it was stated that the synthetic sample (**1**) was prepared by 'Roets *et al.* (to be published).' Unfortunately we have been unable to obtain a comparison sample, nor any preparative or spectroscopic data on this synthetic material from the authors.

§ The spectral data for (**6**) is completely in accord with its formulation: ν_{max}(nujol) 3200br. (*OH*, N*H*), 1750s (β-lactam C=O), 1640s (C=O, $-CO_2^-$), 1250m, 1160m, and 1075m cm^{-1}; δ(^{1}H, D_2O) 0.858 (3H, d, *J* 6.6 Hz, –CHCH*Me*), 0.915 (3H, d, *J* 6.6 Hz, –CHCH*Me*), 1.4—1.9 (4H, m, $-CH_2CH_2CH_2CO-$), 2.1—2.4 (3H, m, –CHC*H*Me$_2$ and $-CH_2CO-$), 3.716 (1H, t, *J* 6.1 Hz, $NH_3^+CHCO_2^-$), 3.768 (1H, d, –C*H*CHMe$_2$), 5.012 (1H, d, *J* 4.0 Hz, β-lactam ring H), and 5.094 (1H, d, *J* 4.0 Hz, β-lactam ring H); δ (^{13}C, D_2O) 19.58 (q, –CH*Me*), 19.83 (q, –CH*Me*), 21.81 (t, $-CH_2CH_2CH_2-$), 29.97 (d, –CHMe$_2$), 30.81 and 35.63 (2 × t, $-CH_2CH_2CH_2-$), 55.15 (d, $NH_3^+CHCO_2^-$), 60.94 and 66.20 (2 × d, –HNCHCON– and Me$_2$CHCH–), 77.76 (d, –C*H*S–), and 169.21, 175.05, and 177.49 p.p.m. (3 × s, carbonyls, one obscured). Derivatisation (see P. B. Loder and E. P. Abraham, *Biochem. J.*, 1971, **127**, 471) gave the bisethoxycarbonyl dimethyl ester of (**6**); *m/z*, *M*H$^+$ (weak) 921.362 ($C_{38}H_{61}N_6S_2O_{16}$ requires 921.359), *M*$^+$/2 — H (base) 459.168 ($C_{19}H_{29}N_3SO_8$ requires 459.166). The disulphide (**6**) contained less than 0.2 mol% cysteinyl impurity (amino-acid analysis after oxidation to cysteic acid with HCO_3H, 0 °C, 5 h and hydrolysis with 6 M HCl, 24 h, reflux).

¶ (a) I.r. (CaF_2 cells) ν_{max} (**6**), 1740 cm^{-1} (β-lactam). Within the time for mixing and scanning (*ca.* 30 s) there was no absorption above 1600 cm^{-1}. (b) N.m.r. (D_2O, buffer): β-lactam ring protons of (**6**) were monitored δ 5.012 and 5.094. Within the time for mixing and scanning (*ca.* 130 s) there were no β-lactam resonances. Compound (**8**) was obtained as a mixture of isomers at C-3 of the cysteine residue in 80% yield. The β-cysteinyl protons in the n.m.r. spectrum of (**8**) (D_2O solvent, ^{2}H irradiated) appear as two doublets at δ 3.11 (*J* 5.2 Hz) and 2.79 (*J* 8.7 Hz) of relative area 45:55 respectively. For (**9**): ν_{max}(nujol) 3300m and 1630m cm^{-1}; δ (^{1}H, D_2O) 0.7—1.0 (6H, m, –CH*Me*$_2$), 1.5—1.9 (4H, m, $-CH_2CH_2CH_2CO-$), 2.1—2.3 (1H, m, –C*H*Me$_2$), 2.4—2.5 (2H, m, $-CH_2CO-$), 2.6—2.7 and 2.8—3.0 (2 × 2H, 2 × m, 2 × $-CH_2S-$), 3.5—3.9 (3H, m, 2 × –C*H*O– and $NH_3^+CHCO_2^-$), and 4.1 (1H, d, *J* 8 Hz, –C*H*CHMe$_2$), other resonances not assigned. Compound (**9**) gave an *N*,*S*,*S*-triethoxycarbonyl dimethyl ester derivative *m/z*, *M*$^+$ = 760.246 ($C_{29}H_{50}N_3S_3O_4$ requires 760.245).



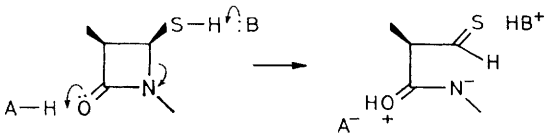
Scheme 1. *Reagents:* i, $HgCl_2$, $HOCH_2C(Me)_2CH_2OH$; ii, NEt_3, *N*-(4-nitrobenzoyl)-L-α-aminoadipic acid α-(4-nitrobenzyl) ester, ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate (EEDQ), 24 h; then $\frac{1}{2}I_2$; iii, Pd–C–H_2–HOAc(1 M)–$Hg(OAc)_2$–tetrahydrofuran. PNB = 4-nitrobenzyl.



Scheme 2. *Reagents:* i $HgCl_2$, $HOCH_2C(Me)_2CH_2OH$; ii, NEt_3, *N*-(4-methoxybenzoyl)-L-α-aminoadipic acid α-(4-methoxybenzyl) ester,[6] EEDQ, 24 h; iii, PhH,PhOMe, trifluoroacetic acid; iv, H_2S. PMB = 4-methoxybenzyl.

lactam-containing products were formed with lifetimes greater than *ca.* 100 s. The intermediacy of the thioaldehyde peptide, (**7**), in these reductions was demonstrated by trapping it by reduction with sodium borodeuteride to (**8**)¶ and by the formation of (**9**) on reduction with dithiothreitol. Compound (**9**) showed spectral data in accord with its structure but could not be isolated in a pure form.¶ These results are in accord with previous observations on the ring-opening reactions of organic-soluble mercaptoazetidinones.[4]

Since we had been unsuccessful in obtaining the crucial intermediate monocyclic β-lactam, (**1**), we resorted to an alternative strategy. Thus, we argued that the chloromercury derivative (**13**), which was readily prepared as in Scheme 2, would represent a precursor which, with hydrogen sulphide, would liberate the thiol, (**1**), under potentially very mild and controllable conditions. Compound (**13**) obtained in an unprotected form was purified on Sephadex G-10 and obtained as a foam [ν_{max}(D_2O,CaF_2 cells) 1735s (β-lactam C=O) and 1595s cm^{-1} ($-CO_2^-$); δ(^1H, D_2O) 0.718 (3H, d, *J* 6.4 Hz, –CH*Me*), 0.813 (3H, d, *J* 6.4 Hz, –CH*Me*), 1.5—1.9 (4H, m, $-CH_2CH_2CH_2CO-$), 2.2—2.4 (3H, m, $-CHMe_2$ and $-CH_2CO-$), 3.466 (1H, d, *J* 10.6 Hz, $-CHCHMe_2$), 3.526 (1H, t, *J* 6.2 Hz, $NH_3^+CHCO^-$), 4.980 (1H, d, *J* 4.4 Hz, β-lactam ring H), and 5.412 (1H, d, *J* 4.4 Hz, β-lactam ring H)] which was cleanly oxidised (KI,I_2,D_2O) to the previously characterised disulphide, (**6**). At pH 1.5 (DCl,D_2O) (**13**) was converted into the desired thiol (**1**) by treatment with hydrogen sulphide followed by removal of the precipitated HgS and excess of H_2S. At this pH, the thiol (**1**) [δ(^1H, D_2O–DCl) 0.790 (3H, d, *J* 6.6 Hz, –CH*Me*), 0.860 (3H, d, *J* 6.6 Hz, –CH*Me*), 1.4—1.9 (4H, m, $-CH_2CH_2CH_2CO-$), 2.2—2.4 (3H, m, $-CHMe_2$ and $-CH_2CO-$), 3.736 (1H, d, *J* 9.2 Hz, $-CHCHMe_2$), 3.850 (1H, t, *J* 6.2 Hz, $NH_3^+CHCO_2^-$), 4.987 (1H, d, *J* 4.6 Hz, β-lactam ring H), and 5.140 (1H, d, *J* 4.6 Hz, β-lactam ring H)] was relatively stable ($t_{\frac{1}{2}}$ *ca.* 25 min at 20 °C) and could be reconverted into the stable compound, (**13**), by treatment with mercury(II) chloride. However as the pH was raised, the lifetime of (**1**) rapidly decreased [pH 5 *ca.* 5 min, pH 6.95 <3 min. No β-lactam absorption could be detected after 3 min, the minimum time required to generate the thiol (**1**) and record the i.r. spectrum.] as monitored by i.r. and n.m.r. spectroscopy. We conclude that the thiol, (**1**), which we have generated *in situ* and characterised by spectral and chemical means, undergoes a facile ring opening reaction, probably of the type (**14**) → (**15**) at or above neutral pH in aqueous solution. Whereas (**1**) can be obtained at low pH,



(14) (15)

the decomposition is so fast at higher pH so as to preclude normal isolation procedures. On the other hand, the dimer of (**1**), as (**6**) is a perfectly normal tripeptide disulphide.

For the above reasons, we cannot accept that the substance described in the previous report[1] is the thiol, (**1**). Its properties, as far as they were described are completely at variance with our own observations on (**1**) and until further evidence appears to substantiate the earlier claim, the question of a monocyclic β-lactam intermediate in penicillin biosynthesis is still unresolved.

Incubation of the disulphide (**6**) (1 mM) with cell-free extracts of *C. acremonium* C-91 or *P. chrysogenum* (SC 6140)[5] in the presence or absence of dithiothreitol (0—2 mM) gave no isopenicillin N.** In separate experiments there was no inhibition by (**6**) of the conversion of LLD-ACV (**2**) into isopenicillin N by these cell-free systems.

We thank the BTG for financial support.

Received, 21st June 1982; Com. 709

** The incubation mixture was assayed against *Staph. aureas* NCTC 6571 which gave a lower detection limit of 15 μg ml^{-1} of isopenicillin N.

References

1 B. Meesschaert, P. Adriaens, and H. Eyssen, *J. Antibiot.*, 1980, 722.
2 This peptide (**2**) has been previously shown to be the precursor of isopenicillin N in *C. acremonium*, *cf.* J. O'Sullivan, R. C. Bleaney, J. A. Huddleston, and E. P. Abraham, *Biochem. J.*, 1979, **184**, 421; T. Konomi, S. Herchen, J. E. Baldwin, M. Yoshida, N. A. Hunt, and A. L. Demain, *ibid.*, p. 427.
3 Analogous to the dideuterio-compound, D. H. G. Crout, M. Lutstorf, P. J. Morgan, R. M. Adlington, J. E. Baldwin, and M. J. Crimmin, *J. Chem. Soc., Chem. Commun.*, 1981, 1175.
4 J. E. Baldwin and M. Jung, *J. Chem. Soc., Chem. Commun.*, 1978, 605.
5 E. P. Abraham, J. A. Huddleston, G. S. Jayatilake, J. O'Sullivan, and R. L. White, in 'Recent Advances in Chemistry of β-lactam Antibiotics (2nd International Symposium),' ed. G. I. Gregory, R. Soc. Chem., Spec. Publ., 1981, No. 38, p. 125.
6 J. J. Usher, B. Loder, and E. P. Abraham, *Biochem. J.*, 1975, **151**, 729.