Use of the Cyclopropylcarbinyl Test to detect a Radical-like Intermediate in Penicillin Biosynthesis

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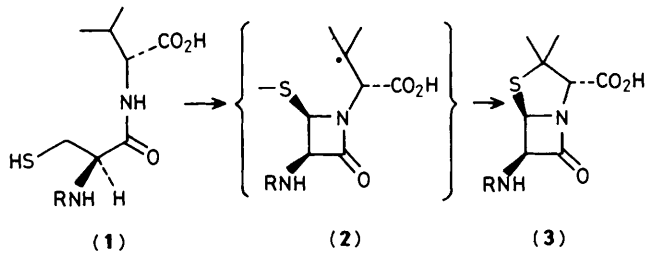
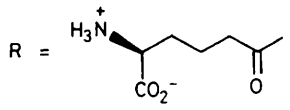
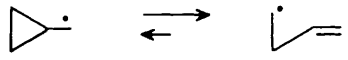
Cyclisation of the cyclopropyl-containing substrates (**6**) and (**7**) by the enzyme isopenicillin N synthetase (IPNS) gave the bicyclic β-lactams (**9**) and (**10**), respectively; these products support the hypothesis that a carbon-centred free radical or equivalent iron–carbon bonded intermediate is involved in the carbon–sulphur bond formation.

Previous results from these laboratories on the mechanism of cyclisation of the precursor (**1**) to the penicillin (**3**), catalysed by the enzyme isopenicillin N synthetase (IPNS) have led to the hypothesis that the carbon–sulphur bond formation to give the second ring may involve a free radical[1,2] (**2**) or equivalent weak iron–carbon bond.[3] As cyclopropylcarbinyl radicals are well known to rearrange to the but-3-enyl system[4] with a rate constant of 10^8 s^{-1} (25 °C)[5] (Scheme 1), we decided to apply this probe for radical behaviour in the C–S bond-forming step in penicillin biosynthesis. Thus two appropriately protected amino acids (**4**) and (**5**) were prepared by cyclopropanation [cat. $Pd(OAc)_2$, CH_2N_2, Et_2O, 0 °C] of protected (*R*)-2-allylglycine and by the route of Scheme 2, respectively. Coupling[6] of (**4**) to produce the fully benzyl-protected tripeptide gave, after deprotection (Na/NH_3) the substrate (**6**). Additionally, racemic (**5**) was coupled[6] to protected L-α-aminoadipoyl-L-cysteine to yield a mixture of diastereoisomeric tripeptides, which were separated (silica gel) and

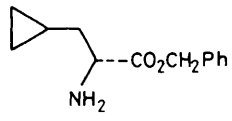


(1) (2) (3)

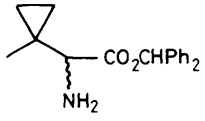




Scheme 1



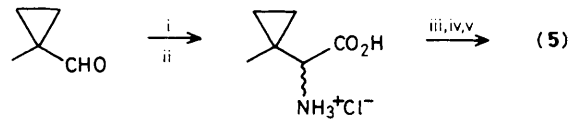
(4)



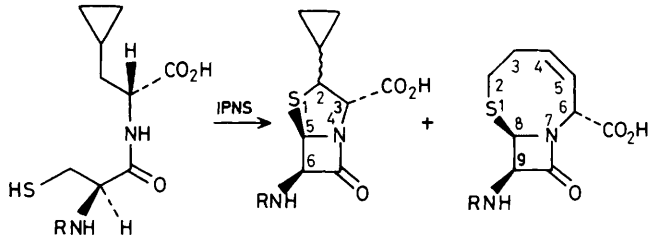
(5)

deprotected (CF_3CO_2H, anisole, reflux 30 min.) to give the required L,L,D-substrate (**7**).† Incubation of (**6**) with IPNS, under standard conditions, gave as a minor component a penicillin (**8**) (destroyed by β-lactamase I from *Bacillus cereus*), δ_H (D_2O; 500 MHz)‡ 0.08—0.18, 0.27—0.36, 0.43—0.55, and 0.57—0.66 (4H, 4 × m, CH_2–CH_2CH), 0.67—0.73 (1H, m, CH_2CH_2CH), 1.7—1.9 (4H, 2 × m, $[CH_2]_2CH_2CO$), 2.40—2.48 (2H, m, CH_2CO), 3.6—3.7 (1H, m, $CH[CH_2]_3$), 4.48 (1H, m, $CHCO_2H$), 5.43 and 5.47 (2H, 2 × d, *J* 3.5 Hz, 5 and 6-H), of unknown C-2 stereochemistry; and a major component shown to be (**9**) [ratio (**9**) : (**8**) >3 : 1], δ_H (D_2O, 500 MHz)‡ 1.72—1.97 (4H, 2 × m, $[CH_2]_2CO$), 2.40 (2H, t, *J* 7 Hz, CH_2CO), 2.52—2.64 (2H, m, 3-H), 2.76—2.82 and 3.07—3.12 (2H, 2 × m, 2-H), 3.67 (1H, *ca.* t, *J* 5 Hz, $CH[CH_2]_3$), 5.36 and 5.49 (2H, 2 × d, *J* 4.5 Hz, 8 and 9-H), 5.87—5.94 (1H, m, 4-H), and 6.02 (1H, *ca.* t, *J* 10 Hz, 5-H) (6-H obscured by HOD); *m/z* (positive argon fast atom bombardment) 372 (MH^+) and 394 (MNa^+); $\nu_{max.}$ (CaF_2 cells, D_2O) 1740 cm^{-1}. A Jeener two-dimensional n.m.r. experiment[7] confirmed the connectivity S–CH_2–CH_2–CH=CH–CH– and (**9**) showed no antibacterial activity against *Staphylococcus aureus* N.C.T.C. 6571 at a concentration of 500 μg ml^{-1}.§

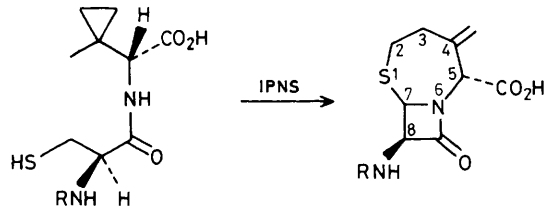
The second cyclopropyl substrate (**7**) gave only one detectable product (**10**) when incubated with the enzyme as before. The structure of (**10**) follows from n.m.r. {δ_H (D_2O; 500 MHz)‡ 1.68—1.75 and 1.80—1.90 (4H, 2 × m, $[CH_2]_2CH_2CO$), 2.41 (2H, *ca.* t, *J* 7.5 Hz, CH_2CO), 2.56—2.62 (1H, m, 3-H), 2.82—2.94 (3H, m, 2- and 3-H), 3.62 (1H, t, *J* 6 Hz, $CH[CH_2]_3$), 5.20 (1H, *ca.* s, CH_2=), 5.26 (1H, *ca.* s, CH_2=), 5.325 (1H, d, *J* 4.5 Hz, 8-H), and 5.39 (1H, d, *J* 4.5 Hz, 7-H) (5-H obscured by HOD) and mass spectral data, [*m/z* (positive argon fast atom bombardment) 372 (MH^+) and 394 (MNa^+)]; $\nu_{max.}$ (CaF_2 cells, D_2O) 1740 cm^{-1}. A two-dimensional n.m.r. experiment[7] confirmed the connectivity S–CH_2–CH_2 and indicated an allylic coupling between the olefinic hydrogen, δ_H 5.26, and the high-field component of the three-proton multiplet at δ_H 2.82—2.94. Irradiation at the



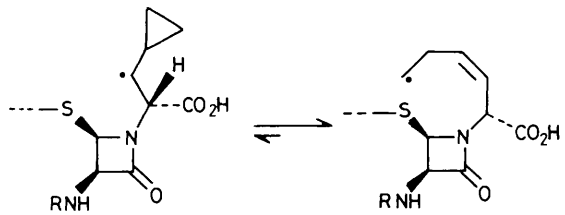
Scheme 2. *Reagents:* i, $(4\text{-}MeOC_6H_4)_2CHNH_2$, mol. sieves; ii, Me_3SiCN, then 6 M HCl, reflux; iii, 2-(t-butoxycarbonyloxyimino)-2-phenylacetonitrile, pH 8; iv, Ph_2CN_2, H^+; v, $C_7H_7SO_3H$ (1 equiv.), then $NaHCO_3$.



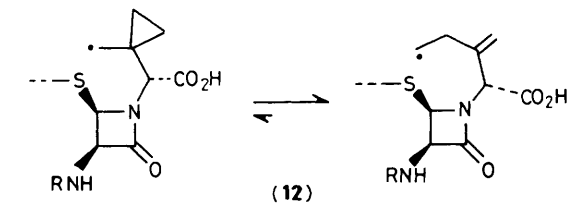
(6) (8) (9)



(7) (10)



(11)

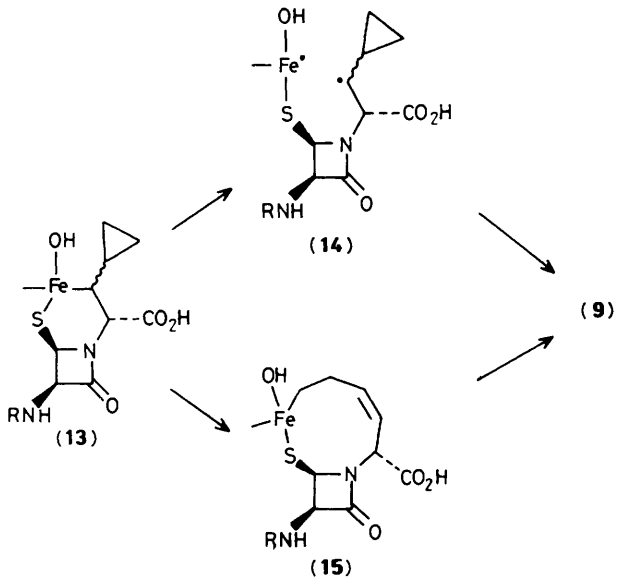


(12)

† The assignment of configuration as L,L,D is based on successful conversion of (**7**) with the enzyme IPNS. To date, of more than 100 synthetic variants on the natural substrate (**1**) which we have tested, no L,L,L-tripeptide was transformed in the enzymic reaction. The other epimer of (**7**) from the chromatography was inactive.

‡ Chemical shifts, referenced to internal sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propanoate, refer to material purified by h.p.l.c. (reverse phase octadecyl silane column).

§ Volume for bioassay 100 μl.



Scheme 3

(16)

frequency of this three-proton multiplet (containing 2α-H) gave a nuclear Overhauser enhancement (5%) at 7-H, δ_H 5.39. No antibacterial activity of (**10**) was observed against *S. aureus* N.C.T.C. 6571 (500 μg ml^{-1}).§

The formation in the enzymic reaction of the major bicyclic products (**9**) and (**10**) is certainly in accord with the intermediacy, during C–S bond formation, of the cyclopropylcarbinyl radicals (**11**) and (**12**), respectively.[1,2] The alternative formation of a labile Fe–C intermediate, *e.g.* (**13**), as previously suggested,[3] could also account for these observations *via* collapse to a radical pair (**14**), or by direct sigmatropy to (**15**) (Scheme 3).¶ Experiments to distinguish these alternatives are in progress.

The absence of cyclobutyl products in these reactions, as might have been expected of a cyclopropylcarbinyl *cation* [⇌ ion (**16**)[9]] is consistent with the intermediacy of the radical, as it has been reported that cyclopropylcarbinyl radicals show no evidence for non-classical behaviour.[10]

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¶ In this connection it is interesting that (η^5-cyclopentadienyl)(η^1-cyclopropylmethyl) dicarbonyl iron has been prepared and shown not to rearrange to the homoallyl derivative at 0 °C.[8]