Penicillin Biosynthesis: A Model for the Oxidative Cyclisation of a Peptide to a β-Lactam

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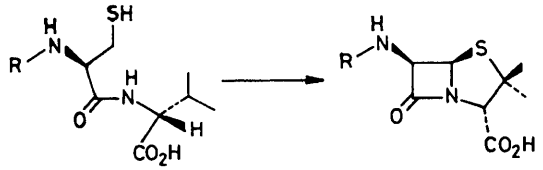
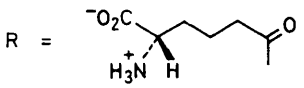
Summary An *in vitro* model for the biosynthetic formation of the β-lactam ring system of penicillin is described in which reaction of *N*-t-butylhydrocinnamamide with di-t-butyl peroxide and Cu(*o*-phenanthroline)$_2$Cl$_2$ at 140 °C

gave a small yield of the corresponding β-lactam; control experiments established that this did not derive from the corresponding β-chloroamide.

EVIDENCE presently available indicates that isopenicillin N (**2**) is derived by oxidative cyclisation of tripeptide (**1**) by a mechanism which is as yet unknown but which involves retention of configuration at carbons 2 and 5 of (**2**).[1] Attempts to demonstrate analogous reactions in organic chemistry have been unsuccessful[2] except when applied to artificially reactive systems.[3] In this report we describe the first case of a direct dehydrogenative cyclisation to a β-lactam of an acylic amide, which, although proceeding in low yield, does establish the chemical validity of such processes.



(1) (2)



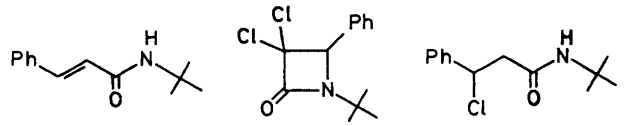
Evnin and Lam have reported that the treatment of cycloheptane and benzamide with $Cu(o\text{-phen})_2Cl_2$ (*o*-phen = *o*-phenanthroline) and di-t-butyl peroxide at high temperature gives *N*-cycloheptylbenzamide.[4] We felt that an intramolecular version of this reaction might yield a β-lactam, *i.e.* (**3**) to (**4**). Accordingly the amide (**3**) (2 mmol) was treated with 1·1 equiv. of di-t-butyl peroxide and 0·01 equiv. of $Cu(o\text{-phen})_2Cl_2$ in chlorobenzene at 140 °C for 48 h in a sealed tube. Most of the (**3**) was unchanged but a small amount of the β-lactam (**4**) was detected by g.l.c. and n.m.r. spectroscopy (yield, estimated by n.m.r. spectroscopy, *ca.* 1·7%). Smaller amounts of other products were detected by g.l.c., the most significant of which was *N*-t-butylcinnamamide (**5**) [53% of the level of (**4**)]. The identity of (**4**) was proved by comparison (n.m.r. and i.r. spectroscopy, g.l.c., m.p. 62·5—63·5 °C, mixed m.p.) with an authentic sample synthesized by reduction (Zn, aq. NH_3) of the dichloroazetidinone (**6**),[5] obtained by addition of dichloroketen to *N*-benzylidene-t-butylamine.

TABLE

Entry[a]	Substrate	Reagents[b]	Products[c] (4)	(5)	Ratio (4)/(5)[d]
(i)	(**3**)	peroxide, catalyst	+	+	1·9
(ii)	(**3**)	peroxide	−	+	
(iii)	(**5**)	peroxide, catalyst	−	+	
(iv)	(**7**)	—	−	+	
(v)	(**7**)	catalyst	−	+	
(vi)	(**7**)	peroxide	−	+	
(vii)	(**7**)	peroxide, catalyst	+	+	0·36

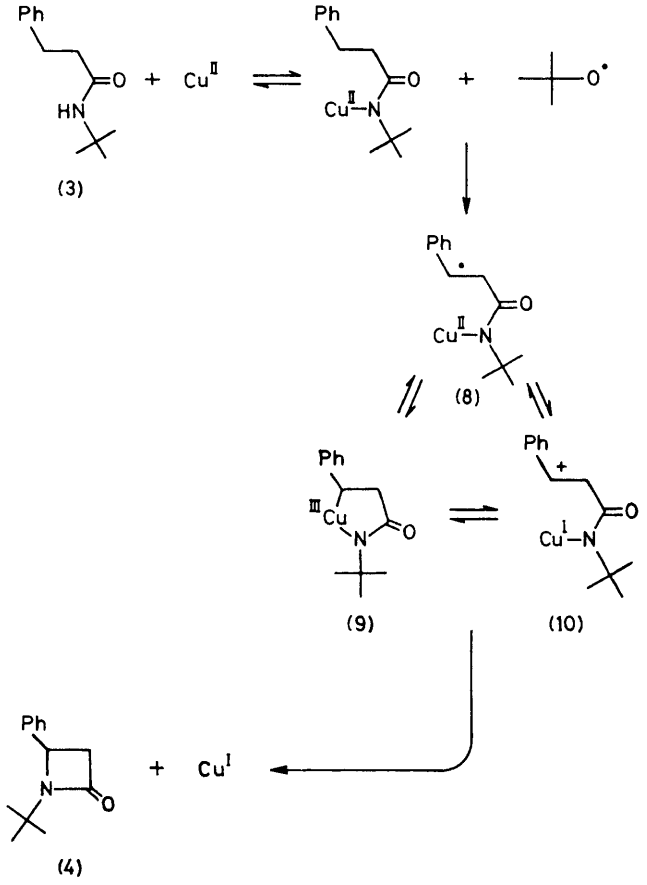
[a] All experiments performed in chlorobenzene at 140 °C for 48 h in a tube sealed under a vacuum. [b] Peroxide ≡ di-t-butyl peroxide; catalyst ≡ $Cu(o\text{-phen})_2Cl_2$. [c] Identified by g.l.c. and n.m.r. spectroscopy or g.l.c./m.s. + = detected, − = not detected. In entries (ii)—(vi) the β-lactam (**4**) could have been detected at levels down to 3% of the concentration of (**5**) (considerably better in most cases). Additional, unidentified products were always observed by g.l.c. [d] By quantitative g.l.c.

The mechanism of this reaction was investigated by the control experiments in the Table. Entry (iii) excludes the cinnamamide (**5**) as an intermediate while entries (iv) to



(5) (6) (7)

(vii) relate to the possible involvement of the β-chlorohydrocinnamamide (**7**) in the mechanism. This substance (**7**) was converted into the β-lactam (**4**) when both peroxide and catalyst were present but the relatively low ratio (**4**) : (**5**) obtained under these conditions suggests that (**7**) can only be an intermediate on a minor path between (**3**) and (**4**).



SCHEME

We suggest that the major pathway from (**3**) to the β-lactam (**4**) is as described in the Scheme. The investigations of Kochi on related systems[6] suggest that direct conversion of (**8**) into (**4**) or, alternatively, a sequence involving (**9**) followed by (**10**) should be favoured. Since it is known[1] that the penicillin synthetase enzyme is dependent on Fe^{2+}

and oxygen, a mechanism similar, but involving organoiron intermediates, might be involved in the biological synthesis. This would perhaps accommodate the predominant retention of configuration in the bond-forming steps.

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