

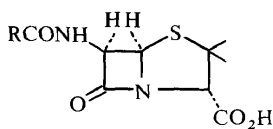
PENICILLINS AND CEPHALOSPORINS

E. P. ABRAHAM

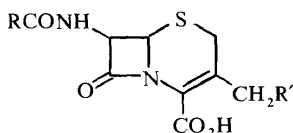
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ABSTRACT

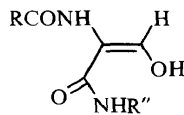
Resemblances and differences between the penicillins (I) and cephalosporins (II) are considered from a chemical and biochemical point of view.



I



II



III

Penicillin N and cephalosporin C [RCO = δ -(D- α -aminoadipyl)] are produced by a *Cephalosporium* sp. and isopenicillin N (RCO = δ -(L- α -aminoadipyl)) and benzylpenicillin (RCO = phenylacetyl) by *P. chrysogenum*. In a broken cell system from *P. chrysogenum* ^{14}C was incorporated into benzylpenicillin from [^{14}C] phenylacetyl CoA in the presence of either 6-APA or isopenicillin N. Thus isopenicillin N is a possible precursor of benzylpenicillin. δ -(L- α -Aminoadipyl)-L-cysteinyl-D-valine and a peptide containing β -hydroxyvaline have been isolated from the *Cephalosporium* sp. The possible role of such peptides in biosynthesis is discussed.

Opening of the labile β -lactam ring of penicillins and cephalosporins occurs during the action of β -lactamases, during inhibition of cell wall synthesis, and in the formation of conjugated protein antigens. X-ray crystallography and other studies support the view that the lability of the ring is associated with suppression of amide resonance.

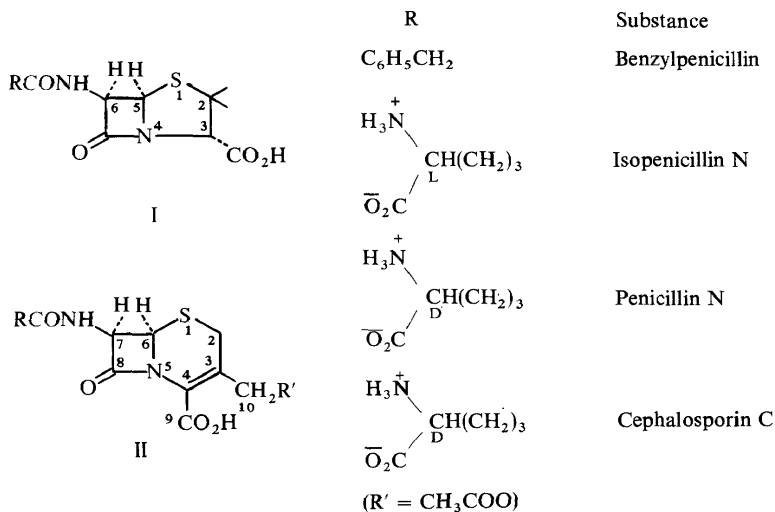
Many β -lactamases are now known whose active centres differ strikingly in their ability to form bonds with the ring systems and side-chains of particular β -lactam antibiotics and to catalyse hydrolysis of the β -lactam ring. There is no direct correlation between the rate of enzymic hydrolysis and the ease of cleavage by simple nucleophiles and a highly reactive β -lactam is a necessary but not sufficient condition for high biological activity.

Reaction of the cephalosporins with simple nucleophiles leads to labile products in which the sulphur-containing ring ultimately undergoes fission to yield a penaldate (X). If similar reactions occurred in protein conjugates, antibody-combining sites to cephalosporins might differ considerably from those to penicillins. With rabbit haemagglutinating antibodies to benzylpenicillin and different cephalosporins the only common major features of the combining sites appear to be those directed towards a common antibiotic side-chain (RCO in I and II) and adjacent atoms.

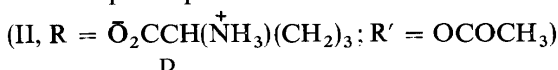
The place of the cephalosporins in medicine depends partly on properties which they share with penicillins, such as low toxicity and powerful bactericidal activity, and partly on ways in which they differ from the penicillins. I propose here to say something about the resemblances and the differences between these substances as they appear from a chemical or biochemical point of view.

BIOGENESIS

The penicillins and cephalosporins which have so far proved clinically useful have the general structures I and II respectively. The general similarity



in structure of the two fused ring systems is clearly due to common features in their biogenesis. Cephalosporin C

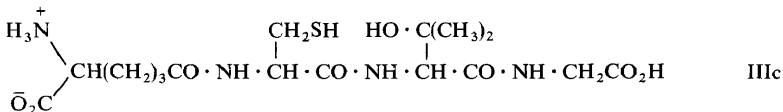
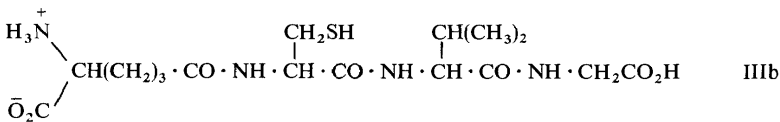
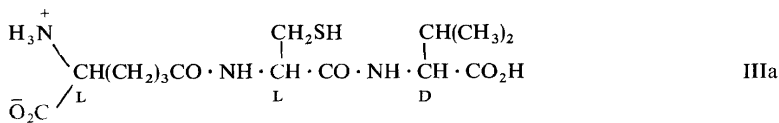


the only member of this family produced by a *Cephalosporium* sp., is formed from α -amino adipic acid, cysteine and valine. Penicillin N, which has the same δ -(D- α -aminoadipyl) side-chain as cephalosporin C, is also formed by the *Cephalosporium* sp. from the same amino acids. Isopenicillin N, with an L- α -aminoadipyl side-chain has been found in *Penicillium chrysogenum*. Dr P. B. Loder has shown in our laboratory that labelled benzylpenicillin (I, R = C₆H₅CH₂) can be formed in a broken cell system from *P. chrysogenum* to which [¹⁴C]phenylacetic acid, coenzyme A, and either 6-amino-penicillanic acid (6-APA:I, RCO = H) or synthetic isopenicillin N have been added¹. The occurrence of a reaction of this type with 6-APA has been reported previously². But the formation of benzylpenicillin in the presence of isopenicillin N is consistent with the suggestion that isopenicillin N is a precursor of the benzylpenicillin molecule³ and that these two substances have the following biosynthetic relationship to 6-APA: Isopenicillin

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N → benzylpenicillin \rightleftharpoons 6-APA. If this is so, the failure of the *Cephalosporium* sp. to make any penicillin or cephalosporin other than one with an α -aminoadipyl side-chain could be directly associated with the fact that the latter has the D-configuration in the extracellular antibiotics produced by this organism.

Only three substances appear to have been found so far in the mycelium of the *Cephalosporium* sp. which qualify for consideration as intermediates, or derivatives of intermediates, in penicillin N or cephalosporin C biosynthesis. These substances are present in only very small amounts, but they have been isolated by making use of the fact that they can be precipitated, like glutathione, as cuprous mercaptides, separated from each other as S-sulphonates and then reduced to the thiol form. The first, whose structure has been determined by mass spectrographic analysis of the N,S-ethoxy-carbonyl derivative of its methyl ester and by measurements of circular dichroism, is δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine (IIIa). The second may be δ -(α -aminoadipyl)cysteinylvalylglycine (IIIb). The third has a residue of β -hydroxyvaline in place of a valine residue and also a residue of glycine, and may be δ -(α -aminoadipyl)cysteinyl- β -hydroxyvalylglycine(IIIc)⁴. If the first peptide is indeed an intermediate it appears that the D-configuration of the α -aminoadipyl side-chain of penicillin N and cephalosporin C arises at a late stage and that intracellular isopenicillin N is a possible precursor of penicillin N. It also appears that the D-configuration of the penicillamine moiety of the penicillins arises before ring closure and not (or not only) during it, as has been suggested⁵, through addition of $-S^-$ to the double bond of an $\alpha\beta$ -dehydrovaline fragment. In this case a β -hydroxyvaline residue would be a possible intermediate.



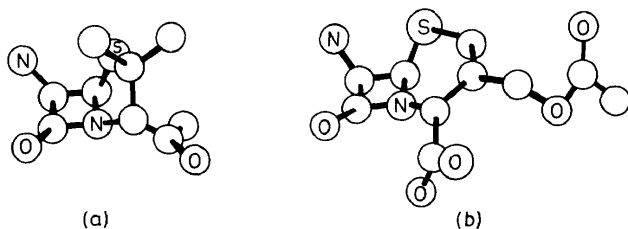
The stage at which the biosynthetic pathway to penicillin N branches towards cephalosporin C has not been determined. Outstanding work by Morin and Webber and their colleagues^{6,7} has established a chemical route from the penicillin to the cephalosporin ring system which depends on fission of the thiazolidine ring of a penicillin sulphoxide and subsequent reclosure to the six-membered dihydrothiazine ring. However, the course of labelling of penicillin N and cephalosporin C from [¹⁴C]L-valine added to a mycelial suspension provided no evidence for a precursor-product relationship and suggested the possibility that peptide precursors of the

two antibiotics are not synthesized at the same site in the cell⁸. It is of interest in this connection that the biosynthesis of δ -(α -aminoadipyl)cysteinylvaline from L- α -aminoadipyl-L-cysteine and [¹⁴C]DL-valine in a broken cell system is catalysed by the particulate fraction of the preparation, whereas the biosynthesis of the structurally analogous glutathione occurs in the supernatant⁴.

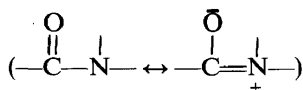
STRUCTURE AND REACTIVITY OF THE RING SYSTEMS

Reactions of the penicillins and cephalosporins which have particular medical importance and illustrate the similarities and differences between the two ring systems are those which result from a nucleophilic attack on the β -lactam carbonyl. One example is the reaction with β -lactamases, which open the β -lactam ring of penicillins or cephalosporins, or both, and release the products of the reaction. A second is the irreversible inactivation by penicillin of a Zn^{2+} -requiring D-alanine carboxypeptidase which appears to play a role in bacterial cell wall synthesis⁹. A third is the reaction of penicillins and cephalosporins with body proteins to form conjugates which are antigenic. In these reactions two factors are clearly important: the chemical reactivity of the β -lactam ring; and the nature and spatial relationship of atoms attached to this ring which help to determine whether it will be set in an appropriate position on an active site of a protein molecule.

In both the penicillins and cephalosporins the spatial relationship of the atoms in the β -lactam ring and those immediately attached to it are very similar, but there is some difference in the orientation of the carboxyl group at C2 and C3 respectively and considerable differences are obvious at the ends of the molecules furthest from the *N*-acyl side-chain (Figure 1).



The lability of the β -lactam of the penicillins, compared with that of monocyclic β -lactams or of normal peptide linkages was attributed, more than 20 years ago, to a suppression of amide resonance

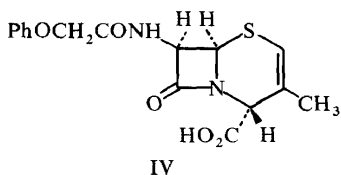


resulting from the fact that the atoms linked to the β -lactam C—N link in the fused ring system could not be coplanar¹⁰. In the absence of charge delocalization resulting from normal resonance the electronic properties of the C=O become closer to those of a ketone and increase its susceptibility to nucleophilic attack. This situation is reflected in the stretching frequency of the C=O in the infra-red spectra of the compounds concerned,

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which is about 1790 cm^{-1} in the penicillins compared with 1740 cm^{-1} in the relatively stable desthiopenicillin and 1660 cm^{-1} in normal amides.

A similar relationship is found with the cephalosporins and corresponding compounds in which the double bond is in the 2 position (IV). With a



cephalosporin containing a phenoxymethyl side-chain the β -lactam $\text{C}=\text{O}$ stretching frequency (1792 cm^{-1}) is very close to that with phenoxymethyl penicillin (Table 1). With the corresponding 2-cephalosporin, which is relatively stable and has little antibacterial activity, it is 8 cm^{-1} lower¹¹.

Table 1. Physical parameters of penicillin and cephalosporin β -lactams.

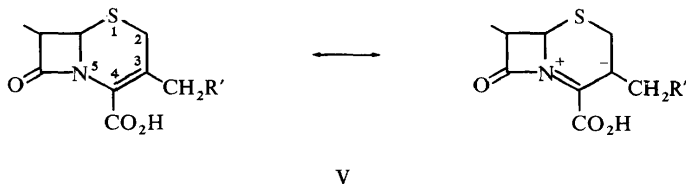
Compound	Distance of N from plane of substituents (Å)	Length of OC—N bond (Å)	C=O stretching frequency (cm^{-1})
Phenoxymethyl penicillin	0.40	1.46	1790
Phenoxymethyl cephalosporin			1792
Ampicillin	0.38	1.36	
Cephaloridine	0.24	1.38	
Phenoxymethyl deacetoxy cephalosporin			1785
Phenoxymethyl 2-cephalosporin			1784
Phenoxymethyl 2-deacetoxy cephalosporin	0.06	1.34	1780

Data from references 11 and 12–16.

Other evidence that the susceptibility of the β -lactam ring of penicillins and cephalosporins to cleavage is associated with a suppression of amide resonance has come from x-ray crystallographic analysis. Accurate structural studies have been made on phenoxymethylpenicillin¹², ampicillin¹³ (I, $\text{R} = \text{C}_6\text{H}_5\text{CHNH}_2$), cephaloridine¹⁴ (II, $\text{R} = 2\text{-thienylmethyl}$, $\text{R}' = \text{C}_5\text{H}_5\text{N}$), and phenoxymethyl 2-deacetoxycephalosporin¹⁵. An analysis of the data from these and other studies has been made by Dr R. M. Sweet¹⁶. He has pointed out that the relatively stable 2-deacetoxycephalosporin has a shorter β -lactam OC—N bond and a nitrogen that is more nearly planar than the other compounds (Table 1).

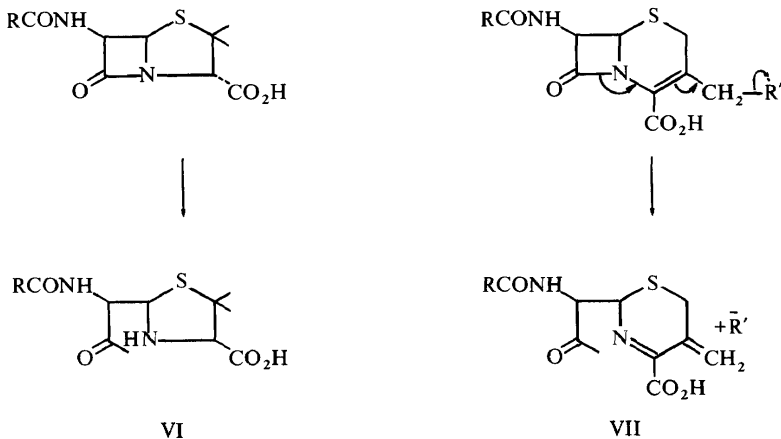
In addition to the non-planarity of N5, the nature of the group R' at C10 of a cephalosporin molecule may affect the electronic properties of its β -lactam carbonyl. X-ray crystallographic data show that the C3—C4 bond

in cephaloridine ($R' = C_5H_5N^+$) is slightly longer and the C4–N5 significantly shorter than would be expected for double and single bonds respectively and it has been suggested that enamine resonance occurs as shown



(V)¹⁶. Such enamine resonance, which would decrease amide resonance, could be stabilized by an electron withdrawing group at R' . This would account for the fact that the β -lactam ring in a deacetoxycephalosporin, such as cephalixin (II, $R = C_6H_5CHNH_2, R' = H$), is considerably more resistant to nucleophilic attack than is the ring of a cephalosporin in which R' is acetoxy, as in cephalothin, or pyridinium, as in cephaloridine.

The presence of a leaving group at C10 in the cephalosporin structure not only facilitates cleavage of the β -lactam ring but also affects the nature of the immediate product of the reaction. With a penicillin this is a D- α -penicilloate (VI). With a cephalosporin in which R' is acetoxy or pyridinium aminolysis with simple amino compounds is accompanied by the expulsion of R' and the formation of a labile compound of structure (VII) which shows λ_{max} at 230 nm instead of at 260 nm.



Products with structure VII are unstable and have not been isolated in the solid state, but the evidence for this structure includes the formation from the product of formaldehyde on ozonolysis and the changes observed in the NMR spectrum during the reaction. Among the latter are the disappearance of signals assigned to the original protons at C10, the appearance of two singlets which could be assigned to the protons of an exocyclic methylene group, the replacement of a peak due to acetoxy protons by one due to

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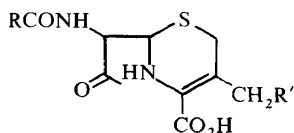
acetate protons and an upscale shift in the signals from the protons of the original β -lactam ring (Table 2)^{17, 18}.

Table 2. Changes in NMR spectrum of *n*-butylcephalosporin

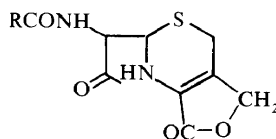
Signals from	Cephalosporin	τ values with Derivatives with λ_{\max} 230 nm
CH ₃ CO ₂	7.9 s	8.08 (s)
H10	5.09 d (12)	4.28 (s)
	5.29 d (12)	4.33 (s)
H6	4.9 d	5.18 d
H7	4.38 d	4.56 d
H2	6.31 d (17)	6.25 (d)
	6.63 d (17)	6.50 (d)

Data from reference 18.

In contrast to the behaviour of cephalosporin in which R' is a leaving group, the deacetoxycephalosporins, the deacetylcephalosporins, and the deacetylcephalosporin lactones yield labile products on aminolysis which show λ_{\max} near to 260 nm, but have a lower extinction than the parent compounds. These products have structures VIII (R' = H or OH) and IX respectively, containing the original dihydrothiazine ring^{17, 18}. However, although the β -lactam ring in deacetoxycephalosporins shows an increased resistance to cleavage, the β -lactam in deacetylcephalosporin C lactone is less resistant than that in cephalosporin C itself. X-ray crystallographic data have provided no indication that this can be associated in the lactone with a nitrogen which is less planar or an OC—N bond of greater length¹⁹. Possibly electron withdrawal from the nitrogen atom by the lactone grouping is important in this case.



VIII



IX

The question may now be asked whether the known differences in structure of penicillins and cephalosporins and in their behaviour to simple nucleophiles throw light on their highly specific reactions with the active centres of certain proteins.

BEHAVIOUR TO β -LACTAMASES

The behaviour of a penicillin or cephalosporin to a β -lactamase may vary strikingly not only with the structure of the antibiotic used but also with the source of the enzyme. This can be illustrated with data relating to four enzymes: two different β -lactamases (I and II) produced by the same strain

of *B. cereus*^{20, 21}, and β -lactamases from strains of *Staph. aureus* and *Ps. pyocyanea* respectively^{22, 23}. Enzyme kinetics provide an indication of the affinity of a substance for a β -lactamase (from values of $1/K_m$ and $1/K_i$ determined with substrates and competitive inhibitors respectively) and of the relative rates of hydrolysis when different substrates fully occupy the active site. With β -lactamase I from *B. cereus*, which is a penicillinase with negligible activity against most cephalosporins, the apparent affinities of a number of penicillins with very different *N*-acyl side-chains are similar and of the same order as that of cephalosporin C (Table 3). However, the value

Table 3. Binding of penicillins and cephalosporins to different β -lactamases.

Substance	Reciprocals of K_i or K_m † (mM) with enzyme from				
	I	<i>B. cereus</i>	II	<i>Staph. aureus</i>	<i>Pseudomonas pyocyanea</i>
Benzylpenicillin	(25)		(0.3)	(400)	(80)
Methicillin	10		(0.6)	(0.01)	6000
Penicillin N	20			(2.6)	
Cephalosporin C	5		(1.0)	4	(1.0)
Deacetyl C lactone	(1.0)		(0.3)	(0.2)	
Benzylcephalosporin	10			24000	(1.0)
Cephalothin			(1.0)	80000	(2.4)
Cephaloridine			(3.3)	90000	(8)

† Values for $1/K_m$ in parenthesis. Data from references 20-23 and 26.

for deacetylcephalosporin C lactone, which has no free carboxyl group attached to the ring system, is 25 times less than that of benzylpenicillin. A somewhat similar situation obtains with the Zn^{2+} -requiring β -lactamase II from the same organism, except that the calculated affinities are significantly lower than those for β -lactamase I. These values suggest that atoms in, or immediately attached to, the ring systems of both penicillins and cephalosporins, such as the lactam C=O, the NH attached to C6 and C7 respectively and the CO_2^- at C3 and C4 respectively, play a major role in the initial binding to the enzyme, and that little interaction occurs between the peripheral portions of the molecules and groups surrounding the active site.

With the staphylococcal β -lactamase the situation is entirely different (Table 3). The affinity of benzylpenicillin for this enzyme is more than 10 times that for β -lactamase I from *B. cereus*, and the affinities of benzylcephalosporin and of cephalosporins with a thienylacetyl side-chain (cephalothin and cephaloridine) are greater than that of benzylpenicillin. In contrast, the affinities of cephalosporin C and deacetylcephalosporin C lactone are at least two and three orders lower respectively and that of methicillin more than four orders lower. In this case a major contribution to the binding seems to be made by the phenylacetyl or thienylacetyl side-chain in both the penicillin and cephalosporin series. Presumably appropriate amino acid residues are so situated near the active site of the enzyme that hydrophobic

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bonds are established with these side-chains, particularly when the latter are linked to the cephalosporin ring system.

The side-chain also has a powerful influence on the affinity of penicillins and cephalosporins for the enzyme from *Ps. pyocyanea*. But with this enzyme side-chains such as the dimethoxybenzoyl in methicillin, which bring bulky groups near to the β -lactam ring, show the reverse effect of that observed with the staphylococcal enzyme, being associated with very strong binding (Table 3). It appears that such groups can form bonds with the β -lactamase from the *Pseudomonas* while steric factors hinder their approach to the active site of the enzyme from *Staph. aureus*.

As would be expected, there is no correlation between the affinity of a penicillin or cephalosporin for a β -lactamase and its maximum rate of hydrolysis. Penicillins with *N*-acyl side-chains which bring bulky groups near to the β -lactam ring, such as methicillin, are hydrolysed only very slowly by enzymes from *Staph. aureus* and a *Pseudomonas* for which they have a relatively very low and very high affinity respectively (Table 4). The

Table 4. Hydrolysis of penicillins and cephalosporins by different β -lactamases.

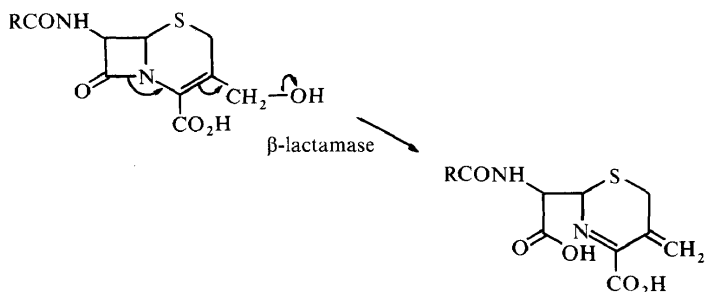
Substance	V_{\max} (relative) with enzyme from				
	I	<i>B. cereus</i>	II	<i>Staph. aureus</i>	<i>Pseudomonas pyocyanea</i>
Benzylpenicillin	100	100	100	100	100
Methicillin	2	89	2	2	0.1
Penicillin N	50		54	54	12
Benzylcephalosporin	0.06	80	0.1	0.1	600
Ampicillin	100	64	150	150	14
Cephalosporin C	0.01	60	0.1	0.1	600
Cephaloglycin	0.1	50			
Cephalexin		<0.5		0.1	
Deacetyl C	0.01	<0.5		0.1	120
Deacetyl C lactone	30	<0.5		15	380

Data from references 20-23 and 26.

relative values of V_{\max} for benzylpenicillin and benzylcephalosporin are inversely related to their affinities for each of these enzymes. Similarly, with the staphylococcal enzyme V_{\max} for a penicillin with the α -phenoxybutyryl side-chain is higher when this side-chain has the *L*-configuration than when it has the *D*-configuration, but the compound with the *D*-configuration has a higher affinity for the enzyme. With the *DL*-compound this leads to the preferential hydrolysis of the *D*-isomer in the early stages and an increase in the rate of reaction with time. It appears that the side-chain may cause a conformational disturbance in the enzyme or a change in the precise alignment of the substrate, which affects the ability of a nucleophilic centre to attack the carbon of the β -lactam $C=O^{22}$.

It is clear from these considerations that there will be no simple overall relationship between the maximum rates of hydrolysis of different penicillins and cephalosporins by β -lactamases and the ease with which their β -lactam rings are cleaved by simple nucleophilic reagents. In some cases an apparent

correlation is observed. For example, the deacetoxycephalosporin, cephalixin, is hydrolysed much more slowly than cephaloridine by a number of β -lactamases from gram negative bacteria²⁴. Deacetylcephalosporin C lactone is hydrolysed at a relatively significant rate by β -lactamase I from *B. cereus* and by a β -lactamase from *Staph. aureus* while other cephalosporins are not (Table 4). However, with β -lactamase II from *B. cereus* the only compounds resistant to hydrolysis among the penicillins and cephalosporins tested were deacetylcephalosporin C, the relatively stable deacetoxycephalosporin, cephalixin, and the relatively unstable deacetylcephalosporin C lactone. One property which these three compounds have in common is the absence of a leaving group (R') at C10. Thus, the possibility arises that the rapid hydrolysis of a cephalosporin β -lactam ring by this enzyme is dependent on an ability of the enzyme (which it does not share with β -lactamase I) to facilitate the removal of the leaving group, perhaps by the provision of a proton at the appropriate location. The nature of the product obtained when deacetylcephalosporin C ($R' = OH$) is hydrolysed by the β -lactamase from the *Pseudomonas* suggests a similar mechanism. This product appears to be identical with that from cephalosporin C itself and to have structure VII instead of VIII ($R' = OH$). Thus in the presence of this enzyme the hydroxyl at C10 behaves as a leaving group, whereas during simple aminolysis it does not.



With β -lactamase I from *B. cereus* and the staphylococcal enzyme many cephalosporins are much more resistant to hydrolysis than penicillins with the same *N*-acyl side-chains, even when they have a leaving group at C10 and a β -lactam ring which is highly susceptible to nucleophilic attack. In these cases it seems possible that the specific differences in the spatial arrangement of atoms in the two ring systems, such as in that of the carboxylate ion at C3 and C4 respectively, give rise to changes in the precise orientation of the molecule on the active site.

REACTION WITH PROTEINS AND ANTIBACTERIAL ACTIVITY

The variety of factors which affect the outcome of the interaction of a penicillin or cephalosporin with a β -lactamase are presumably similar to some of those which play a part in the inhibition of the transpeptidation step of cell wall biosynthesis.

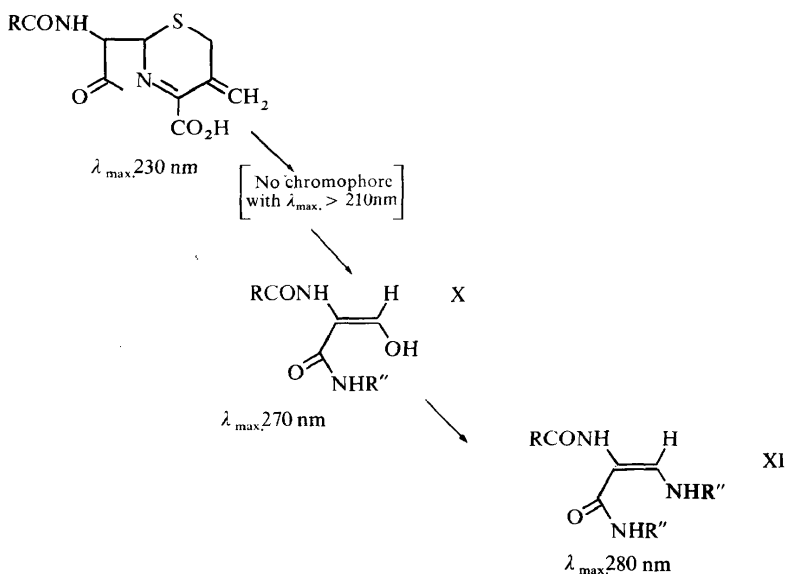
The Zn^{2+} -requiring *D*-alanine carboxypeptidase, which may be regarded as an uncoupled transpeptidase, has been found to contain a thiol group

which reacts with penicillin to yield an enzymically inactive thiol ester of penicilloic acid⁹. Steric factors may inhibit the reaction of the acyl enzyme carbonyl with the amino group of a mucopeptide monomer. Although several β -lactamases have now been reported to contain thiol groups and β -lactamase II from *B. cereus* requires Zn^{2+} , there is at present no reason to believe that a thiol ester intermediate is involved in any β -lactamase-catalysed hydrolysis. However, in each case both the affinity of a penicillin or cephalosporin for the active site and its maximum rate of reaction will influence its biological effect. With the β -lactamases a significant factor is the rate of hydrolysis of the antibiotic when the latter is present in a concentration just sufficient for it to show bactericidal activity. In the inhibition of transpeptidases the effective concentration of the antibiotic may be that at which it competes sufficiently well with the natural substrate for inactivation to occur more rapidly than resynthesis. Analogy with the β -lactamases suggests that a highly reactive β -lactam ring is a necessary but not a sufficient condition for high activity in this system and that variations in amino acid residues near the active sites of transpeptidases from different organisms could be responsible for many of the differences in sensitivity to a particular member of the penicillin or cephalosporin family.

CHEMICAL PROPERTIES AND IMMUNOLOGICAL SPECIFICITY

In addition to variations in the biological properties of the penicillins and cephalosporins which are a consequence of differences in the reactivities of β -lactam rings or in the precise geometrical pattern of the ring systems there are others which may depend partly on the fact that the products formed by nucleophilic attack on the β -lactam of the cephalosporins are less stable than the penicilloates formed from the penicillins. A dilute solution of compound VII, formed on hydrolysis of cephalosporin C by a β -lactamase, lost its ultra-violet absorption band (λ_{\max} 230 nm) within 7 hours at pH 7.0. The corresponding product of aminolysis of cephalosporin C decomposed similarly, at a rate which increased when its concentration was raised, or the pH of the solution was reduced below 8. The uncharacterized derivative which was present at this stage gave rise, within a few hours to a penaldate (X) (with λ_{\max} 270 nm in alkaline solution), which was slowly converted to a penamaldate (XI) with λ_{\max} 280 nm. A similar fission of the ring system occurred after aminolysis of deacetylcephalosporin C and deacetoxycephalosporin C^{17, 18}.

It would not be surprising if part of an antibody combining site which was complementary to the sulphur-containing ring of structures such as VII also combined with the thiazolidine ring of a penicilloyl group, although the difference in the orientation of the carboxyl group in these rings might weaken the cross reaction. But, if the dihydrothiazine ring underwent fission after acylation of protein with a cephalosporin, little structural resemblance to a thiazolidine ring would remain. Experiments to determine the extent of cross reactions in one antigen-antibody system which could be associated with similarities in the penicillin and cephalosporin ring systems have been carried out by Hamilton-Miller. Antibodies were obtained in rabbits to benzympenicillin and to members of the cephalosporin C family (containing



a δ -(D- α -aminoadipyl) side-chain) by injection of the antibiotics in the free state and as protein conjugates prepared by reaction with bovine γ -globulin (BGG). The specificities and strength of the antisera were then estimated from their ability to agglutinate red blood cells sensitized by reaction with various penicillins and cephalosporins.

In forming conjugates benzylpenicillin and cephalosporin C reacted with equal facility, presumably with lysine residues, but the less stable deacetylcephalosporin C lactone reacted more readily and the more stable deacetoxycephalosporin C less readily. Antisera to either free or conjugated benzylpenicillin agglutinated red cells sensitized with a variety of penicillins., including penicillin N, although the antibody titre varied with the nature of the side-chain. In contrast, these antisera showed substantial titres with red cells sensitized by the cephalosporins tested only when the latter contained an *N*-phenylacetyl side-chain or the related thienylacetyl side-chain of cephalothin (Table 5). Cells sensitized with cephaloglycin, which has the

Table 5. Cross reactions with antiserum to benzylpenicillin.

Red cells sensitized with	Titre of antiserum to benzylpenicillin	
	free	conjugated
Benzylpenicillin	128	512
Ampicillin	16	64
Penicillin N	>8	>16
Benzylcephalosporin	64	64
Cephalothin	16	64
Cephaloglycin	<2	<2
Cephalosporin C	2	2

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Table 6. Cross reactions with antisera to cephalosporins.

Red cells sensitized with	Cephalosporin C	Titre of antisera to Deacetyl ceph. C lactone	Deacetoxyceph. C
Cephalosporin C	128	128	32
Deacetylceph. C lactone	256	128	64
Deacetoxy ceph. C	128		
Penicillin N	32	64	8
Benzylcephalosporin	<2	<2	<2
Cephalothin	<2	<2	<2

same D-phenylglycyl side-chain as ampicillin, did not agglutinate. Similarly, antisera to members of the cephalosporin C family agglutinated cells sensitized with these cephalosporins or with a penicillin (penicillin N) containing the α -aminoadipyl side-chain, but not with cephalosporins whose side-chains were chemically unrelated to α -aminoadipic acid (Table 6)²⁵. Hapten inhibition studies showed that a benzylpenicilloate was a much more powerful inhibitor of the antipenicillin serum than was benzylpenicillin itself, but that the immediate product of aminolysis of cephalosporin C (VII) was no more effective as an inhibitor of the anti-cephalosporin C serum than cephalosporin C itself or the penaldate (X) derived from it (Table 7).

Table 7. Hapten inhibition of antisera to benzylpenicillin and cephalosporin C.

Hapten	Concn (μ M) to reduce titre to 1 of antiserum to Benzylpenicillin	Concn (μ M) to reduce titre to 1 of antiserum to Cephalosporin C
Benzylpenicillin	5600	
α -Amide of benzylpenicilloate	1.3	
Cephalosporin C		39
Amide of compound with λ_{\max} 230 nm (VII) from ceph. C		35
Penaldate from ceph. C		49

These results suggest that there are haemagglutinating antibodies in anti-cephalosporin sera whose binding sites are complementary only to the cephalosporin side-chain and part of its β -lactam ring, whereas binding sites exist in antipenicillin sera which are complementary to much or all of the penicilloyl group. However, whether fission of the dihydrothiazine ring has in fact occurred in antigens formed by the acylation of protein by a cephalosporin remains to be determined.

REFERENCES

- 1 P. B. Loder Proc. Symp. Biosynthesis Antibiotics, Warsaw (1971).
- 2 S. Gatenbeck and U. Brunsberg. *Acta Chem. Scand.* **22**, 1059 (1968); R. Brunner, M. Röhr and M. Zinner. *Z. Physiol. Chem.* **349**, 85 (1968); B. Spencer and C. Maung, *Biochem. J.* **118**, 29P (1970).
- 3 A. L. Demain. In *Biosynthesis of Antibiotics* Vol. 1, p. 29. Academic Press: New York (1966).
- 4 P. B. Loder and E. P. Abraham, *Biochem. J.* **123**, 471, 476 (1971).
- 5 H. R. V. Arnstein and J. C. Crawhall. *Biochem. J.* **67**, 180 (1957).
- 6 R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon and S. L. Andrews. *J. Am. Chem. Soc.* **85**, 1896 (1963).

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- ⁷ J. A. Webber, E. M. Van Heyningen and R. T. Vasileff. *J. Am. Chem. Soc.* **91**, 5674 (1969).
- ⁸ S. C. Warren, G. G. F. Newton and E. P. Abraham. *Biochem. J.* **103**, 902 (1967).
- ⁹ P. J. Lawrence and J. L. Strominger. *J. Biol. Chem.* **245**, 3660 (1970).
- ¹⁰ R. B. Woodward. In *The Chemistry of Penicillin* p. 443. Princeton University Press (1949).
- ¹¹ R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon and S. L. Andrews. *J. Am. Chem. Soc.* **91**, 1401 (1969).
- ¹² S. Abrahamsson, D. C. Hodgkin and E. N. Maslen. *Biochem. J.* **86**, 514 (1963).
- ¹³ M. N. G. James, D. Hall and D. C. Hodgkin. *Nature, London*, **220**, 168 (1968).
- ¹⁴ R. M. Sweet and L. F. Dahl. *Biochem. Biophys. Res. Commun.* **34**, 14 (1969).
- ¹⁵ R. M. Sweet and L. F. Dahl. *J. Am. Chem. Soc.* **92**, 5489 (1970).
- ¹⁶ R. M. Sweet. Personal communication.
- ¹⁷ J. M. T. Hamilton-Miller, G. G. F. Newton and E. P. Abraham. *Biochem. J.* **116**, 371 (1970).
- ¹⁸ J. M. T. Hamilton-Miller, E. Richards and E. P. Abraham. *Biochem. J.* **116**, 385 (1970).
- ¹⁹ R. D. Diamond. *D. Phil. Thesis*. University of Oxford (1963).
- ²⁰ L. D. Sabath and E. P. Abraham. *Biochem. J.* **98**, 11c (1966).
- ²¹ S. Kuwabara and E. P. Abraham. *Biochem. J.* **115**, 859 (1969).
- ²² B. Crompton, M. Jago, K. Crawford, G. G. F. Newton and E. P. Abraham. *Biochem. J.* **83**, 52 (1962).
- ²³ L. D. Sabath, M. Jago and E. P. Abraham. *Biochem. J.* **96**, 739 (1965).
- ²⁴ G. W. Jack, R. B. Sykes and M. H. Richmond. *Postgraduate Med. J. Supplement* Vol. **48**, p. 41 (1970).
- ²⁵ J. M. T. Hamilton-Miller and E. P. Abraham.
- ²⁶ G. G. F. Newton and J. M. T. Hamilton-Miller. *Postgraduate Med. J. Supplement* Vol. **43**, p. 10 (1967).