REVIEWS

The Molecular Basis for the Mode of Action of Beta-Lactam Antibiotics and Mechanisms of Resistance

Barrie W. Bycroft[1,2] and Richard E. Shute[1]

Abstract: This review on the molecular basis for the mode of action of β-lactam antibiotics and mechanisms of resistance is divided into three main sections. Firstly, a brief introduction to the β-lactam antibiotic family is presented from the standpoint of their natural product origins. The second section is concerned with bacterial cell wall structure and biosynthesis, and the mode of action of β-lactam antibiotics. It includes an attempted rationalization of the multiple enzyme targets of penicillin, the so-called "penicillin binding proteins", into one or two lethal sites of action and the interaction of these enzymes with β-lactams in terms of their analogy to the natural substrate and to the substrate-enzyme transition state. The final part covers the phenomenon of bacterial resistance to β-lactam antibiotic therapy and deals with the two most important manifestations of resistance; permeability and the production of β-lactamases. This latter more crucial factor is then expanded with particular reference to the irreversible inhibition of these enzymes by suicide inactivators; a general theory for irreversible β-lactamase inhibition is discussed and the future prospects within this whole area are briefly overviewed.

Since Fleming's initial observations on penicillins were published more than half a century ago (1), the β-lactam antibiotics have continued to be one of the most intensely investigated areas of pharmaceutical research. The past decade has witnessed the isolation from natural sources of several new types of highly active β-lactam structures, and seen considerable advances in the biochemistry associated with the target and deactivating enzymes, the so-called penicillin binding proteins (PBP's) and β-lactamases, respectively (2). The following article illustrates the various structural types of β-lactam antibiotics and attempts to review critically and concisely the current state of knowledge concerning the molecular basis for their mode of action.

Structural Types of β-Lactam Antibiotics

From the late fifties to the present time numbers of semi-synthetic penams and cephems[3] have been, and continue to be, prepared by attaching a wide variety of side-chains at the 6-position of 6-aminopenicillanic acid (6-APA, **3**) and the 7- and 3-positions of 7-aminocephalosporanic acid (7-ACA, **11**). This semi-empirical approach has been successful in generating not only compounds with enhanced and broader antibacterial activity, particularly against Gram negative organisms, but also compounds with improved pharmacokinetic properties, and with greater stability to a wide range of β-lactamases, the major cause of resistance in an increasing variety of organisms. Some of the more notable examples are illustrated in Fig. 1 and 2.

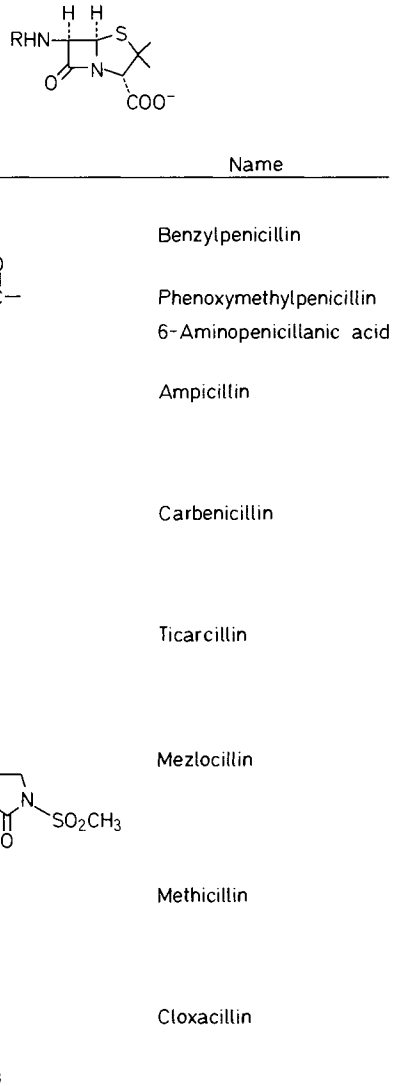
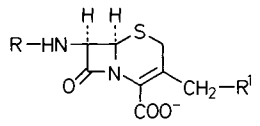


Fig. 1 Penicillin structural types.

However, attempts to modify the basic bicyclic system with retention of notable activity were unsuccessful until the discovery of the naturally occurring 7-methoxycephem cephamycin C (**18**) (4). This compound presented a modified cephem with stability to a wide spectrum of Gram negative β-lactamases and led to the development of a number of semi-synthetic 7-meth-

[1] Department of Pharmacy, University of Nottingham, U.K.
[2] Correspondence to be addressed to Professor B. W. Bycroft, Department of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, U.K.
[3] The basic bicyclic ring systems of the penicillins and cephalosporins are referred to as penams and cephems respectively (3).



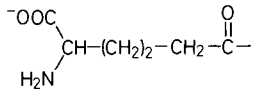
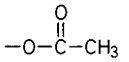
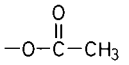
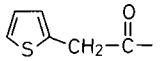
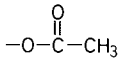
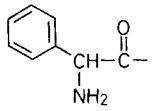
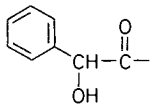
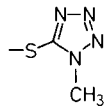
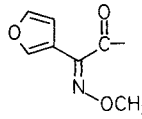
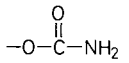
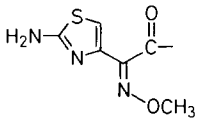
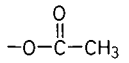
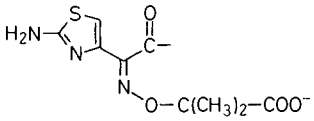
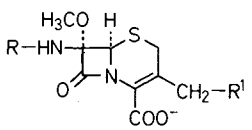
	Side Chain Group R	C-3 Substituent R^1	Name
10			Cephalosporin C
11	H—		7-Aminocephalosporanic acid
12			Cephalothin
13		—H	Cephalexin
14			Cefamandole
15			Cefuroxime
16			Cefotaxime
17			Ceftazidime

Fig. 2 Cephalosporin structural types.



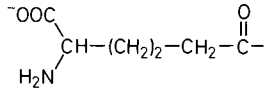
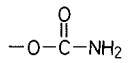
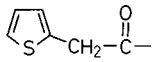
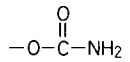
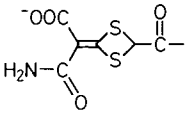
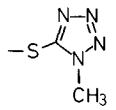
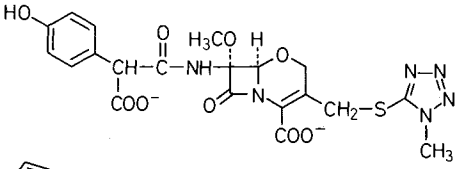
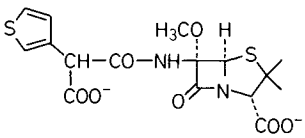
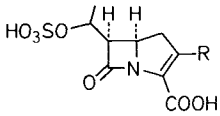
	Side Chain R	C-3 Substituent R^1	Name
18			Cephamycin C
19			Cefoxitin
20			Cefotetan
21			Moxalactam
22			Temocillin

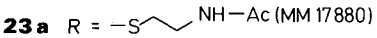
Fig. 3 Cephamycin structural types and other methoxylated β-lactam antibiotcs.

oxycephems, 7-methoxyoxacephems (5) and 6-methoxypenams (6) (Fig. 3). In addition, this discovery provided the impetus for a renewed interest in micro-organisms as sources of novel β-lactams. Streptomycetes have proved particularly fruitful in this respect, yielding thienamycin (**24**) (7) and the olivanic acids (**23**) (8) which are the most prominent members of the now well-established carbapenem family (Fig. 4) (7–10). These antibiotics are currently the most significant in terms of breadth of antibacterial spectrum, potency and stability to β-lactamases. Unfortunately, owing to their inactivation by mammalian renal dehydropeptidases (11), the clinical potential of the carbapenems has been somewhat compromised. Recently, considerable effort has been directed towards the development of inhibitors of the dehydropeptidases with the objective of enhancing their activity by combination therapy *in vivo* (12). The modified thienamycin, Imipenem (MK 0787) (**28**) (13) in combination with a non β-lactam inhibitor is currently a strong clinical candidate (14) but because of the low titres of the parent compound thienamycin from all the producing strains, MK 0787 is presently manufactured by total chemical synthesis (15).

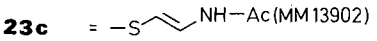
Further sophistication in screening methods and an expansion of types of micro-organisms investigated led to the discovery of β-lactam containing antibacterial agents not only

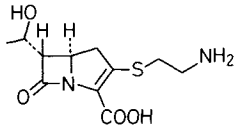


23 Olivanic Acids

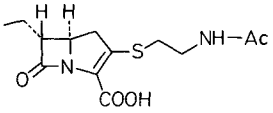
23a R = 

23b = 

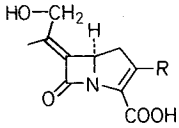
23c = 

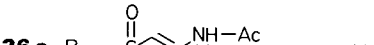


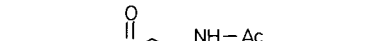
24 Thienamycin

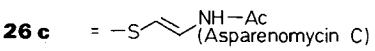


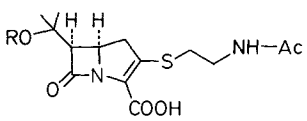
25 PS-5

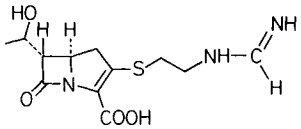


26a R = 

26b = 

26c = 





28 (MK 0787)

27a R = —H (Carpetimycin A)
27b = —SO_3H (Carpetimycin B)

Fig. 4 The carbapenem family.

in fungi and streptomycetes, but also from bacteria themselves. Novel structures that have been isolated include two new classes of monocyclic β-lactam antibiotics the nocardicins (16), eg. Nocardicin A (**29**), from *Nocardia*, another actinomycete, and the monobactams (17, 18), eg. Sulfazecin (**30**), from *Pseudomonas spp.*, *Gluconobacter* and *Acetobacter* (Fig. 5).

Both classes possess features already encountered in semi-synthetic penicillins and cephalosporins, eg. a *syn*-oxime in Nocardicin A and an α-methoxyl group in Sulfazecin, but it is the structural differences, their origins and their unusual antibacterial properties that make these compounds of particular interest. Clinically useful monobactams have been produced by utilizing the semi-synthetic experience of penicillins and cephalosporins and the more advanced chemical procedures now available and has resulted in the derivative Aztreonam (**31**) (19). This compound possesses a high degree of stability to β-lactamases and low activity against Gram positive organisms but exhibits potent activity against Gram negative organisms (20).

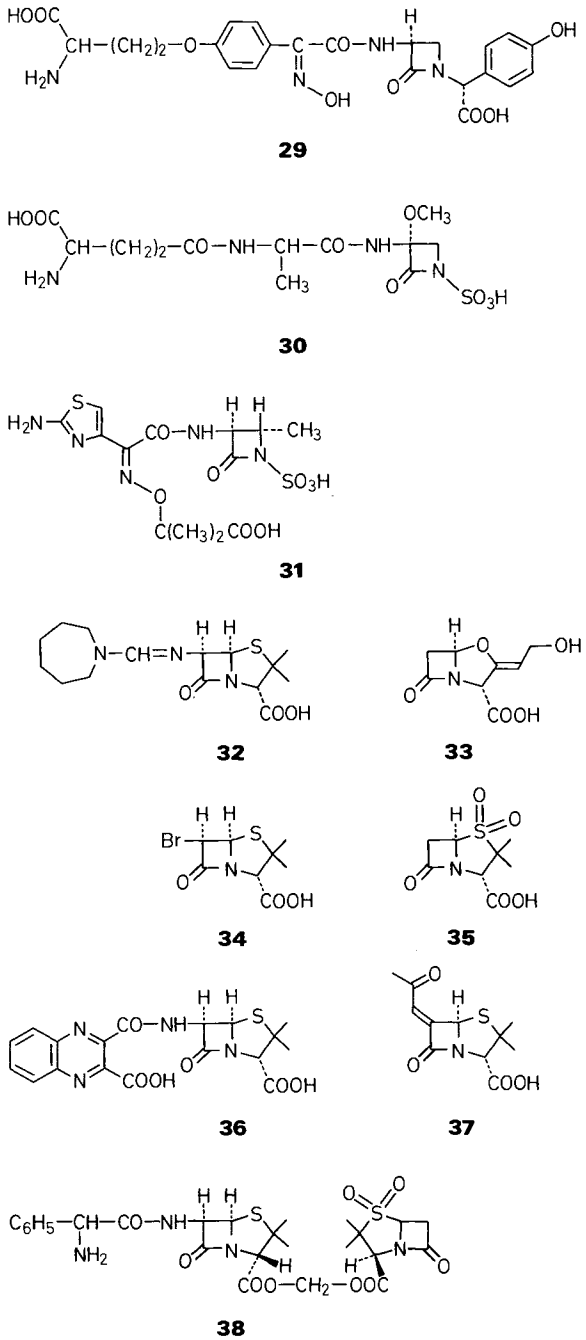


Fig. 5 Monocyclic β-lactam antibiotics.

It is thus apparent that nature has, to date, provided four major structural types of β-lactam antibiotic, the penams, the cephems, the carbapenems and the monocyclic β-lactams. Despite these structural variations, microbiological and biochemical studies have established a common mode of action and deactivation mechanisms.

The Mode of Action of β-Lactam Antibiotics

Early work on the morphological and lytic effects of penicillin (21, 22) led to the conclusion that β-lactams selectively inhibit bacterial cell wall biosynthesis. The penicillin-induced accumulation of several novel uridine nucleotides (23, 24) related to those known to be involved in cell wall synthesis (25) provided support for this theory. In all bacteria it is the cell wall which serves to define the characteristic shape of the organism and to provide protection against disturbances in osmotic pressure and other potentially fatal environmental changes. Its detailed structure and biosynthesis have been exhaustively reviewed (26–28), and only a very brief résumé will be presented here.

Peptidoglycan Structure and Biosynthesis

The cell wall is a macromolecule which envelops the bacterial cytoplasm. Within it are long linear polysaccharide or "glycan" strands that are cross-linked by branched peptide chains, and this structure is termed "peptidoglycan". The glycan strands are made up of alternating *N*-acetyl-glucosamine and *N*-acetyl-muramic acid, connected by β-1,4 glycosidic linkages. The short peptide cross-links are attached to the carboxyl groups on the *N*-acetyl-muramic acid residues. They vary in length and composition depending on the particular bacteria, but often have the sequence:

L-Alanyl-D-Glutamyl-γ-L-X-D-Alanyl

"X" is most often L-lysine (eg. in *Staph. aureus*) or *meso*-diaminopimelic acid (eg. in *E. coli* and *B. subtilis*).

The peptide units on adjacent glycan strands are linked together, and the interpeptide bridges in the majority of bacteria extend between the C-terminal D-alanine of one tetrapeptide and the free amino group of the L-X moiety of an adjacent tetrapeptide (see Fig. 6). The actual extent of cross-linking varies with the bacterial species and growth conditions. The range can be as low as 25 % in *E. coli* to greater than 90 % in *Staph. aureus*; therefore, by no means all the *N*-acetyl-muramyl-peptide residues are interlinked.

The early stages in the biosynthesis of peptidoglycan involve the formation of an uncrosslinked nascent glycan polymer on the outer face of the cell membrane. These reactions are not penicillin sensitive, whereas the primary transpeptidase process leading to the insolubilization of the new peptidoglycan by covalent linkage to the pre-existing peptidoglycan network is highly penicillin sensitive. It is this "anchoring reaction" coupled with the subsequent cross-linking and further modifications which control all aspects of cell shape and growth and which are essential for survival. These reactions are catalyzed by a range of peptidases which can be divided into three broad categories; DD-transpeptidases catalyze the cross-linking of adjacent *N*-acetylmuramyl-*penta*peptide strands with the loss

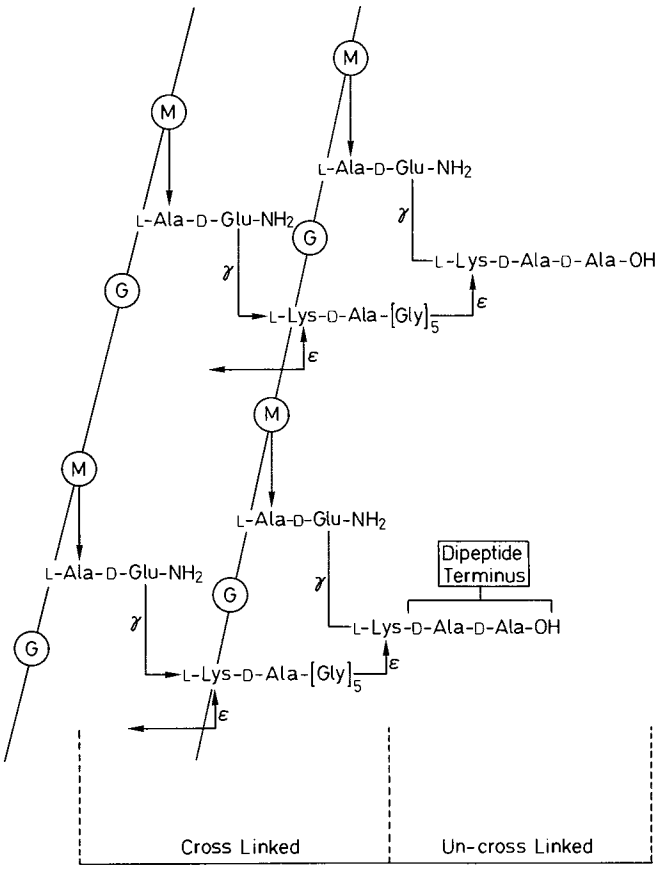


Fig. 6 Monolayer representation of *Staph. aureus* Copenhagen peptidoglycan showing cross-linked peptide bridges and uncrosslinked "nascent" peptidoglycan with terminal dipeptide D-alanyl-D-alanine.

of a terminal D-alanine residue, DD-carboxypeptidases mediate the cleavage of the terminal D-alanine from pentapeptide side chains without concomitant transpeptidation, and endopeptidase activity catalyzes the hydrolysis of the interpeptide link formed in the transpeptidation reaction.

The observation that several proteins in the bacterial membrane bind penicillin covalently (29), led to the introduction of the term "Penicillin Binding Protein" (PBP), and first suggested that a range of β-lactam sensitive enzymes were involved in bacterial cell wall biosynthesis.

Penicillin Binding Proteins

The full spectrum of PBP's are most easily observed by first incubating bacterial membranes with radiolabelled penicillin followed by gel electrophoresis and fluorography (30). The most widely studied organism is *E. coli* (30-32) and, because of the conservation of peptidoglycan structure amongst Gram negative bacteria the observations can be applied generally. For *E. coli* seven separable PBP's have been isolated (30) with apparent molecular weights ranging from 40,000 daltons (PBP 6) to 90,000 daltons (PBP 1A). The properties of these proteins and their proposed functions are listed in Table I. The enzymes also differ significantly in their ability to bind various other β-lactam antibiotics, in their relative abundance, activities and importance for cell survival.

In general penicillins, particularly benzylpenicillin (**1**) have a broad affinity for all the PBP's. Cephalosporins, however, have a considerably greater affinity for the high molecular weight PBP's 1-4 with very poor affinity for the low molecular weight proteins 5 and 6. However, the most interesting observations result from the binding of non-classical β-lactam antibiotics, particularly the amidinopenicillin Mecillinam (**32**) (33) which binds exclusively to PBP 2 (34). The morphological consequence in *E. coli* is the formation of large osmotically stable round cells which lyse after several hours of growth. Thienamycin (**24**) (7) and the natural β-lactamase inhibitor clavulanic acid (**33**) (35) also have a high affinity for this protein (30).

Table I. PBP's of *E. coli* – Properties and Roles in Cell-Wall Metabolism (37)

PBP	Apparent M.W. (daltons)	Abundance (% total PBP's)	Morphological effects of inactivation (where known)	Activities (where known)	Cell Survival/ Viability	Proposed in vivo function
1A	90,000	6	–	–	Non-essential	Minor transpeptidase, can compensate for PBP 1B's
1B	87,000	2	Rapid lysis	Transpeptidase and possible trans-glycosylase	Essential	Major trans-peptidase of cell elongation
2	66,000	0.7	Ovoid cell formation	–	Essential	Cell shape determination
3	60,000	2	Filamentation	–	Essential	Implicated in cell division and specifically in cross-wall formation
4	49,000	4	–	Carboxypeptidase Transpeptidase Endopeptidase	Non-essential	Secondary trans-peptidase to increase cross-linking
5	42,000	65	–	Carboxypeptidase (transpeptidase)	Non-essential	Regulation of cross-linking
6	40,000	21	–	Carboxypeptidase (transpeptidase)	Non-essential	

Classical β-lactam antibiotics generally have a high affinity for PBP 3. The morphological changes associated with binding to this protein involve filamentation and inhibition of cell septation and cell division (31).

The low molecular weight PBP's 5 and 6 are the major PBP's, in *E. coli* accounting for 90 % of the binding capacity (30). By comparison the high molecular weight PBP 1 accounts for 8 % and PBP 2 less than 1 % (30). In the case of PBP 2 in *E. coli*, this may amount to only 15 to 20 molecules per cell.

By the technique of β-lactam affinity chromatography (36) specific PBP's can be isolated and their activities investigated. This has only really been successful for the low molecular weight PBP's that have been shown to have primarily DD-carboxypeptidase activity (32). The actual functions *in vivo* of the various PBP's have been investigated in two ways (31); either by analysis of genetic mutants with altered PBP patterns, or by correlation between the *in vitro* and *in vivo* effects of β-lactams. However, any proposed relationship between *in vitro* and *in vivo* activity should be treated with caution. It should be noted that desired single point mutations to delete one specific protein are rare and deletion of a particular protein is usually accompanied by other mutations leading to very weak strains whose viability is already compromised. The second approach is exemplified by mecillinam and its specific binding to PBP 2; unfortunately there is a dearth of agents which bind specifically to other PBP's and this offers a direction of pharmaceutical research which is yet to be fully exploited.

Extensive binding and genetic studies (30–34, 36) suggest that the high molecular weight PBP's 1, 2 and 3 are essential for cell survival (see Table I). On the other hand the low MW PBP's can be inactivated or deleted without killing the organism (32). Thus the observation that cephalosporins have low affinity for the low MW PBP's, yet retain their lethal effects, further implies that these proteins are non-essential.

In *E. coli*, PBP 1 is a complex of enzymes that can be further resolved into two groups 1A and 1B and appears to be involved in cell elongation. Deletion of either 1A or 1B can lead to viable cells, but deletion of both leads to lysis (37). The role of PBP 2 in determining cell shape is particularly well studied owing to the action of mecillinam (34). Inhibition of PBP 3, particularly by classical β-lactam antibiotics, leads to filamentation and prevention of cross wall formation and causes inhibition of cell division (37). It should be noted that specific β-lactams can kill by inactivation of a single PBP (eg. mecillinam, PBP 2 and cephalexin (**13**), PBP 3); however, most β-lactams have affinity for several target enzymes.

PBP 1B is likely to be the most crucial PBP because inhibition or deletion by genetic mutation leads to cell lysis (31); nevertheless, viable PBP 1B-lacking mutants have been claimed (37). Also, the fact that β-lactams can kill by binding to PBP 2 or PBP 3 exclusively indicates that inactivation of the major transpeptidase, possibly PBP 1B, is not the only means by which these agents kill bacteria.

In summary, therefore, β-lactam antibiotics appear to exert their lethal effects by binding to, and thereby inactivating the "penicillin-sensitive enzymes" that in *E. coli*, correspond, to PBP 1B, 2 and 3 (31).

The situation in Gram positive bacteria is rather more complex owing to the wide variation in peptidoglycan structure (26). From the studies of various Gram positive PBP's a few general observations can be made (38). The PBP patterns show wide variation and multiple morphological effects when inhibited by β-lactams. Mecillinam does not show specificity for any one PBP nor does it produce ovoid cells. Finally, the major PBP is a low molecular weight DD-carboxypeptidase (cf. PBP's 5 and 6 of *E. coli*) in all Gram positive bacteria but in none is it the "killing target" (39). For some specific Gram positive bacteria this target has been suggested (36) but no general rule can be proposed.

The final consideration in this section involves the question of whether penicillin-induced lysis is the direct result of the inactivation of an essential PBP or whether this primary action leads to a secondary irreversible effect. The implication of peptidoglycan hydrolases or "autolysins" in the killing action of penicillin is based on the work of Tomasz (40) who has shown that in some pneumococci, inhibition of the autolytic activity protects the organism from the lytic effects of cell wall inhibitors. In the case of penicillin, this "tolerance" is not accompanied by a change in growth sensitivity suggesting that the primary targets, i. e. the PBP's have not been altered. Thus in a normal organism there is a balance between peptidoglycan biosynthesis and autolytic activity and that in certain organisms, any disruption of cell wall metabolism upsets that balance in favor of autolysis resulting in destruction of peptidoglycan and eventual cell lysis.

The Interaction of Penicillin Binding Proteins with β-Lactams

In the late 1960's Tipper and Strominger proposed (41) that penicillin behaved as a substrate analogue for the dipeptide terminus (acyl-D-Ala-D-Ala) of nascent peptidoglycan (see Fig. 6). A comparison of one particular conformation of acyl-D-Ala-D-Ala and the rigid penicillin molecule is shown in Fig. 7, illustrating the coincidence of the highly reactive β-lactam peptide bond with the peptide bond of the dipeptide.

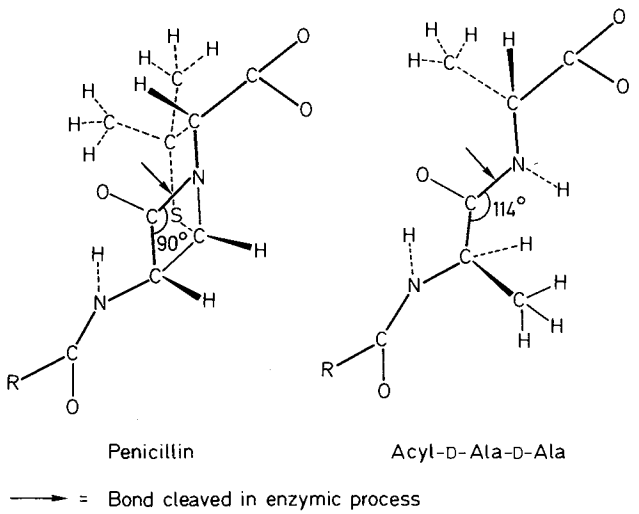


Fig. 7 Conformational representation of Penicillin and Acyl-D-Ala-D-Ala showing proposed structural similarity and indicating the coincidence of the peptide bond cleaved by the enzymes of the peptidoglycan biosynthetic machinery with the highly reactive β-lactam bond.

This hypothesis led initially to the suggestion that the primary effect of β-lactam antibiotics was the facile acylation of some functionality in the active site of essential PBP's, and that therefore, penicillin could be considered as an active site-directed acylating agent (42).

Studies subsequently showed that relatively stable stoichiometric penicillin-PBP complexes could be isolated (42), and it

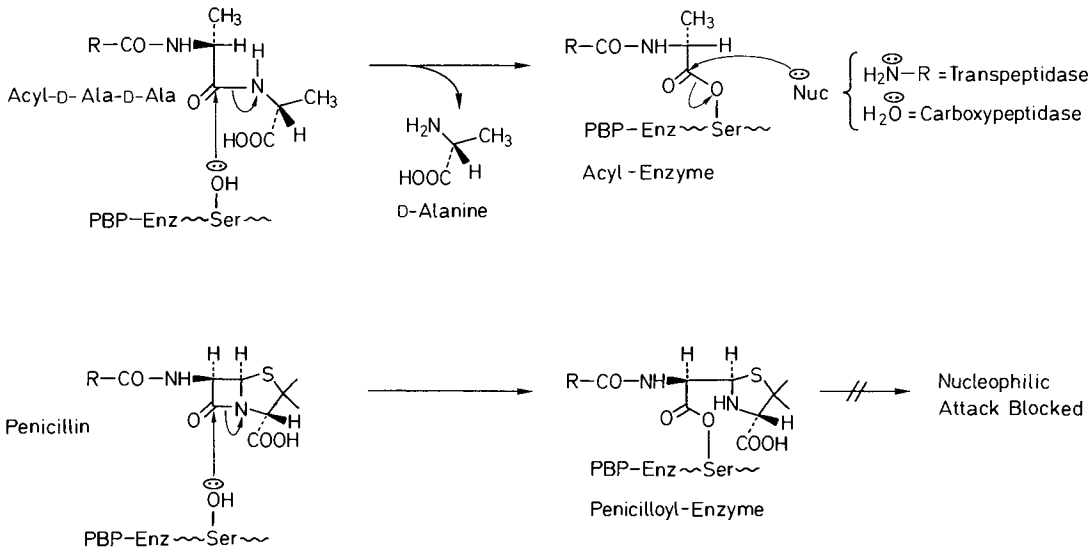


Fig. 8 Proposed mechanism for interaction of Acyl-D-Ala-D-Ala (top) and penicillin (bottom) with PBP's showing participation of an active site serine residue, involvement of an acyl- or penicilloyl-enzyme intermediate and possible subsequent reactions.

is now established that the covalently-bound penicillin is in the form of a penicilloyl moiety bound via an ester linkage (43) to a serine residue which is present in the several enzymes investigated (42, 44) (see Fig. 8). This residue can also be shown to bind D-Ala-derived peptides in an acyl-enzyme complex (42).

Criticism of the theory has centered around discrepancies based on important *differences* between β-lactams and acyl-D-Ala-D-Ala.

As can be seen from Fig. 9 the fused bicyclic systems of most β-lactam antibiotics force the β-lactam nitrogen to adopt a pyramidal geometry, whereas the analogous nitrogen within the peptide bond of acyl-D-Ala-D-Ala is, as expected, planar. In the course of the enzymic process the elimination of the terminal D-alanine will inevitably involve a change in geometry from planar to pyramidal at this nitrogen (cf. Serine proteases (45)). Consequently, penicillin can be regarded as a "Transition-State Analogue" rather than a Substrate Analogue (46).

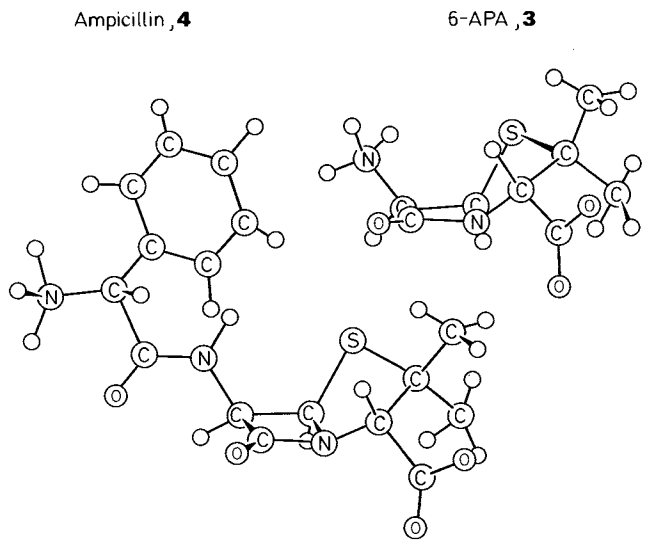


Fig. 9 Computer-modelled molecular graphics representation of ampicillin (**4**, left) and 6-aminopenicillanic acid (**3**, right) showing near-planarity of β-lactam ring and "wedge shape" associated with tetrahedral ring junction nitrogen.

The fused bicyclic system of penicillins and cephalosporins also causes reduced amide character of the β-lactam bond thus labilizing the system towards nucleophilic attack. This is reflected in a longer C-N and shorter C-O bond lengths and a greater C-O stretching frequency in the IR spectrum (47). In cephalosporins, enamine resonance acts further to decrease amide character (48). The non-planarity of the bicyclic system favors penicillin recognition by the enzyme in terms of the tetrahedral transition state. Once within the active site, the combination of four-membered ring strain and the proposed loss of amide resonance confers considerable reactivity on the molecule and facilitates the acylation of the nucleophilic serine residue to form the observed enzyme-penicilloyl complex associated with penicillin binding to the enzyme (43). In the cephalosporins the situation is more complex owing to the presence of a leaving group at C-3, and thus the potential to form an exocyclic double bond at this position further increases β-lactam reactivity and biological activity (48).

Secondly, a further conformational comparison between β-lactam antibiotics and acyl-D-Ala-D-Ala (see Fig. 7) suggested that 6α-methylpenicillins and 7α-methyl-cephalosporins should be closer structural analogues and should have enhanced activity. Subsequent synthesis of these compounds (49, 50) demonstrated their considerably reduced activity, compared to the parent molecules.

The isolation (4) and semi-synthetic derivatization (see Fig. 3) of 7α-methoxycephalosporins followed by the synthesis of 6α-methoxypenicillins [e.g. Temocillin (**22**) (5)] and the observation of their increased activity illustrate the difficulties in drawing conclusions from a comparison of rigid analogues with a conformationally mobile substrate. Retrospectively, attempts have been made to account for these anomalies in terms of more detailed conformational analysis (51). However, until detailed molecular data become available on the nature of the substrate-PBP binding and hence the relevant conformation of acyl-D-Ala-D-Ala, the prospects of reliable structure-activity predictions will remain unlikely. This point is

further illustrated by the pronounced activity of compounds belonging to both the thienamycin and the olivanic acid families which differ only in the configuration of their 6-hydroxyethyl side chain (7, 8).

Until relatively recently it was believed that monocyclic β-lactams were not sufficiently susceptible to nucleophilic attack to act as enzyme acylating agents. The recent isolation of the highly active monobactams (17, 18) and extensive synthesis of analogues (19) has dispelled this view. Even though X-ray data indicate planar geometry for the ring nitrogen (52), they are potent acylating agents and possess high β-lactam carbonyl stretching frequencies. These properties have been attributed to the powerful electron-withdrawing effect of the sulphonic acid residue reducing the amide character of the β-lactam bond.

Thus, in conclusion, the high potency and specific inhibitory properties of β-lactam antibiotics are derived from two important features. Firstly, recognition by the enzyme, and all the specific steric and geometrical requirements that that entails; and secondly, a strong acylating ability to form a covalent inhibitor-enzyme complex at the active site.

The Resistance of Bacteria to β-Lactam Antibiotics

It has been known for the past 40 years that bacteria which may be initially susceptible to β-lactam antibiotics can suddenly or progressively develop resistance (53). The possession or acquisition of resistance to drug therapy provides a further crucial factor in the understanding and treatment of infection.

A number of factors have been advanced to account for bacterial resistance to β-lactam antibiotics (37, 38). Those most significant clinically and which have been most studied are permeability and the production of β-lactamases.

The inability of antibiotics to penetrate the bacterial cell wall is of particular concern in relation to Gram negative bacteria and is associated with their outer cell wall. The outer membrane consists of a complex lipopolysaccharide-phospholipid-protein structure stabilized by Mg^{2+} ions (54). It presents a significant permeability barrier to many types of antibiotics and is responsible for the high resistance of the problem pathogens *Ps. aeruginosa* and *Klebsiella spp.* to β-lactams. This can be contrasted with the high sensitivity of *E. coli* to β-lactams even though their PBP profiles are essentially the same (55).

The outer cell membrane acts as a molecular sieve excluding compounds of molecular weight greater than approximately 600 daltons. These sieving properties are a function of transmembrane proteins called "porins" (56), which are usually made up of three sub-units enclosing a water-filled channel. Studies (57, 58) have indicated several types of porins which can undergo conformational changes to "open" or "close" a channel (59), thus providing a further barrier, even to molecules of molecular weight less than 600 daltons. Uptake is also affected by the physical properties of the compound, particularly electrical charge and lipophilicity. In general, increasing the anionic character or lipophilicity leads to significantly slower permeation (37, 38). Consequently the penetration of β-lactam antibiotics (60, 61) into Gram negative bacteria is constrained both within defined MW limits and the balance between ionic and lipophilic character.

The more crucial factor in bacterial resistance is the ability to produce β-lactamases. Since their discovery (53) these enzymes have increased in importance, and they now represent the most serious threat to the continuing therapeutic utility of β-lactam antibiotics.

The chemical, biochemical and genetic aspects, and clinical consequences of this group of enzymes, their classification, distribution and properties have all been extensively reviewed (62–64), and it is to these texts that the reader is referred for more detailed information.

β-Lactamases catalyze the hydrolysis of the β-lactam ring of a variety of antibiotics, most particularly penicillins and cephalosporins, thereby leading to inactive products. They are widely distributed throughout lower micro-organisms (65), and the different types have been classified on the basis of their so-called "substrate profiles" (The Richmond-Sykes-Matthew classification system, see Table II) (63, 66). Although extensive physical and chemical studies have been conducted on a number of these enzymes (62) providing considerable information, a fully refined X-ray analysis is still eagerly awaited. To date the aforementioned studies indicate the intermediacy of an acyl-enzyme complex involving the hydroxyl group of a serine residue within the active site (67–69). Thus it would appear that β-lactamases are a sub-class of serine proteases and have probably evolved from enzymes of the peptidoglycan biosynthetic machinery (41).

Table II. Richmond-Sykes-Matthew Classification System (63, 66)

Enzyme class	Properties
I	Predominantly cephalosporinases. Inhibited by Cloxacillin (**9**). Resistant to *p*-chloromercuribenzoate (*p*-CMB).
II	Predominantly penicillinases. Inhibited by Cloxacillin. Resistant to *p*-CMB.
III	Broad spectrum penicillinase and cephalosporinase. Inhibited by Cloxacillin. Resistant to *p*-CMB.
IV	Broad spectrum β-lactamase. Not inhibited by Cloxacillin, which is a poor substrate. Sensitive to *p*-CMB.
V	Broad spectrum β-lactamase. Not inhibited by Cloxacillin, which is an excellent substrate. Resistant to *p*-CMB.

NB: – Gram positive β-lactamases can be classed as Richmond type II enzymes.

A number of reasons have emerged to account for the stability of particular β-lactam antibiotics to certain β-lactamases. Firstly, the drug may be a poor substrate, i. e low affinity for the active site (high K_m values), e. g. Cefoxitin (**19**) and thienamycin-type compounds (70). Alternatively, the antibiotic may have a high affinity for the enzyme active site (low K_m) but the overall rate of hydrolysis is extremely slow; such drugs are behaving as competitive inhibitors, e. g. cephalosporins and the staphylococcal β-lactamase (64). Similar inhibition can occur with antibiotics which have a high affinity for the active site but for which only the deacylation step is slow, thus leading to accumulation of an acyl-enzyme intermediate which slowly hydrolyzes (71). Penicillin and cephalosporin antibiotics which are good competitive inhibitors of β-lactamases have, in general, reduced antibacterial activitiy (72); however, combination of such an inhibitor with a potent, but susceptible, β-lactam has found some clinical utility, e. g. the isoxazolypenicillins/ampicillin (62, 73).

The Irreversible Inhibition of β-Lactamases

In the early 1970's it was recognized that the irreversible inactivation of β-lactamases by agents which themselves might possess only minimal antibacterial activity, could extend the concept of combination therapy. The break-through in this area occurred with the isolation from a streptomycete of clavulanic acid (**33**) (35), which was shown to be a potent irreversible inhibitor of many β-lactamases (74–76) and which was found to restore the antibacterial activity of ampicillin against resistant bacteria (77). Subsequently, it was noted that other, semi-synthetic reagents e.g. 6β-bromopenicillanic acid (**34**) (78, 79) and penicillanic acid sulphone (Sulbactam) (**35**) (80, 81) also irreversibly inhibited β-lactamases (82).

Irreversible enzyme inhibition by substrate analogues has since received considerable attention. This type of inactivation has been termed both "Active site-directed" or "Suicide" inhibition, the latter because the enzyme, essentially, catalyzes its own inactivation (83, 84). Extensive studies on the mode of action of clavulanic acid (74–76) and the semi-synthetic inhibitors (79, 80) have been interpreted in terms of a general theory of irreversible β-lactamase inhibition (82).

The sequence of transformations outlined in Fig. 10 summarizes the proposals for a general β-lactamase inhibitor and is largely based on detailed kinetic data obtained from characteristic UV spectral changes associated with the intermediates indicated. It was evident from this data that the inhibitors are good substrates for the enzymes and indeed are turned over to the ring-opened compounds in varying degrees (Pathway (i) "Deacylation"). However, competing elimination reactions (Pathways (ii) and (iii)) of the acyl-enzyme complex ("E. C.") lead to reactive intermediates that can further interact with other nucleophiles, as yet undefined, affording a covalently bound fragment within the active site, and thus irreversibly inactivating the enzyme.

The rate of reaction for the three pathways (i), (ii) and (iii) shown in Fig. 10 determines the effectiveness of the particular vnhibitors. The most potent are ones forming stable, long-lived acyl-enzyme complexes, which then fragment preferentially to the reactive Michael acceptor species. Consequently, to be an irreversible inactivator, a compound should (82) have a high affinity for the enzyme active site and form a relatively stable acyl-enzyme intermediate. Furthermore, it would appear essential that the molecule possess a proton at C-6 with the correct configuration and sufficiently acidic, as determined by the groups X and Y to allow the β-elimination (iii) across C-5 and C-6 to take place; and in addition possess a suitable leaving group, Y, to facilitate this elimination.

These findings were successfully applied to the design of new *in vitro* β-lactamase inhibitors. Methicillin (**8**) and Quinacillin (**36**), which were known to form long-lived acyl enzyme complexes, on oxidation to the corresponding sulphone yielded potent irreversible β-lactamase inhibitors (81). This strategy of oxidation of the sulphur of a penam has since been extensively utilized for the synthesis of novel inhibitors (85–87).

On the basis of more recent work on the novel inhibitor 6-acetylmethylene penicillanic acid (**37**) (88), it has been proposed that its mechanism of inhibition deviates at the acyl-enzyme stage from that outlined in Fig. 10 and involves a complex series of rearrangements (89, 90). Similarly, the mechanism of irreversible inhibition by some sulphated carbapenems involves a complex rearrangement at the acyl-enzyme stage (91, 92) as opposed to a more simple elimination originally proposed (82) (see Fig. 11).

It is noteworthy that all the above studies have been conducted on the so-called Class A serine β-lactamases. There presently exists a further class of these enzymes, namely the Class C (93). They occur in two bacteria, a mutant *Ps. aeruginosa* and *E. coli* K12, and they differ from the Class A type in that they are not inhibited by clavulanic acid (94, 95) and can utilize lower alcohols as the nucleophile in the deacylation of the acyl-enzyme complex, as well as water. The clinical significance of these enzymes has yet to be fully evaluated.

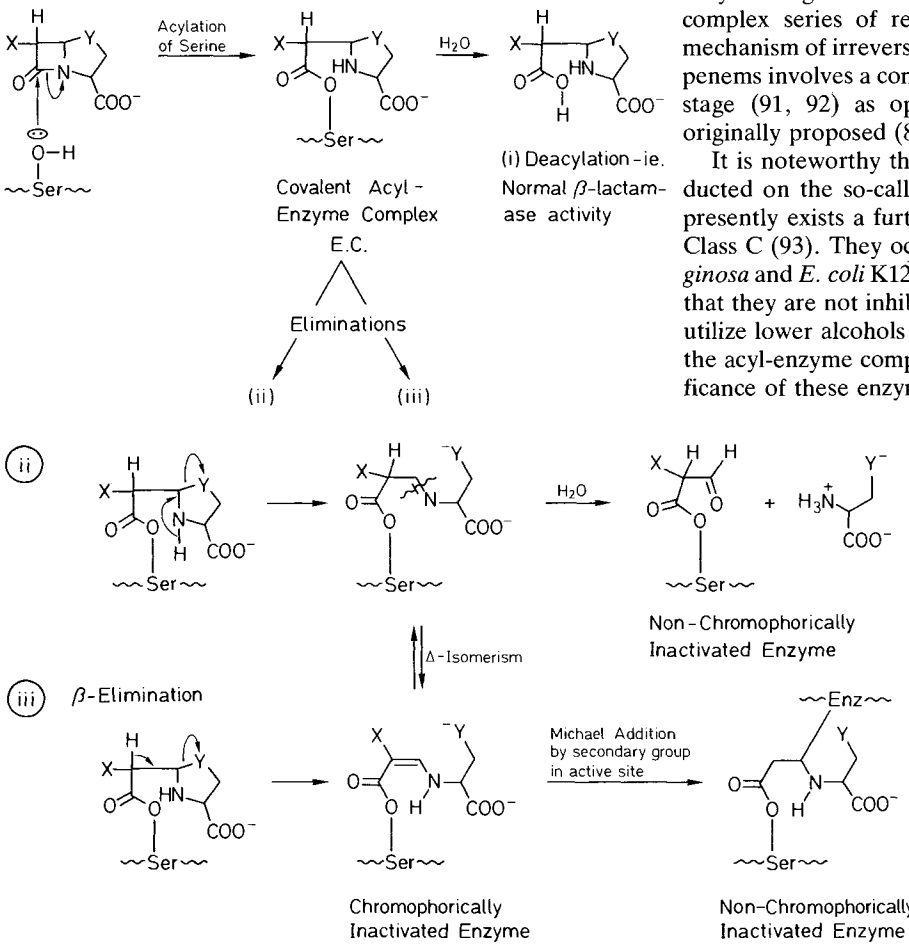


Fig. 10 Interaction of hypothetical β-lactamase inhibitor with serine β-lactamase. X = electronegative group or hydrogen, Y = leaving group. Diagram indicates (i) normal β-lactamase deacylating activity; (ii) and (iii) elimination rearrangements, fragmentations and further nucleophilic entrapments leading to chromophoric or non-chromophoric inactivated enzyme.

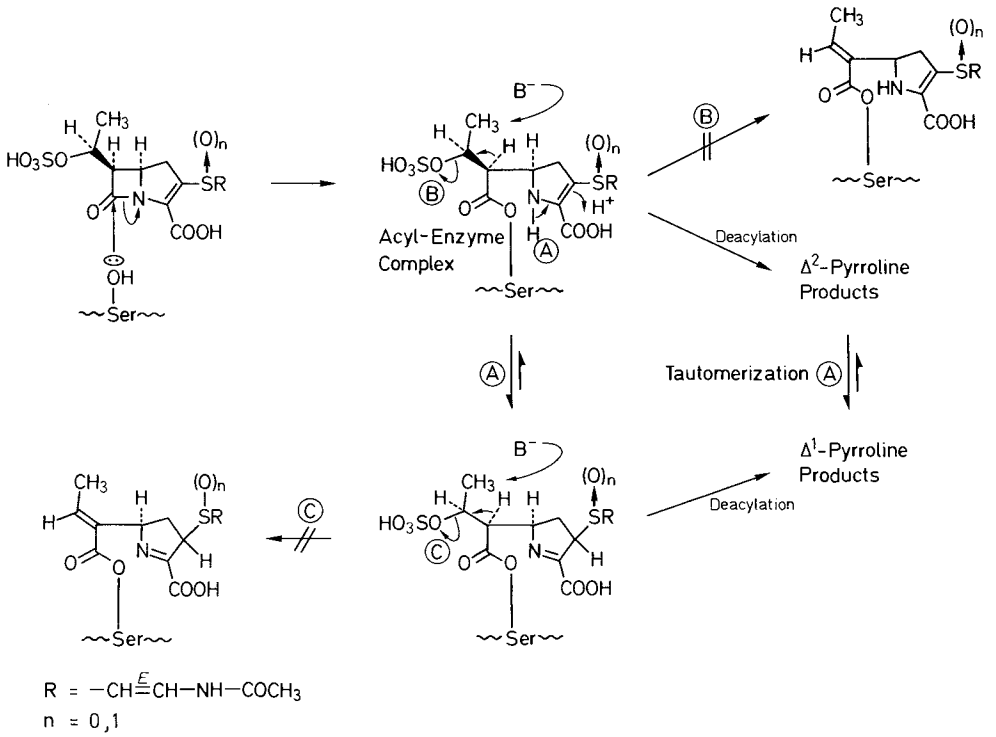


Fig. 11 β-Lactamase inhibition by sulfated carbapenem showing acyl-enzyme intermediate, "A" preferred $\triangle^2$-pyrroline tautomerization to more tightly bound $\triangle^1$-pyrroline adduct "B" and "C" originally proposed for β-elimination of side-chain sulfate to putative inactivating species, an α, β-unsaturated acyl-enzyme; now disproved.

Finally, there is one other β-lactamase enzyme which does not operate by means of an active site serine. This is the Class B metallo-β-lactamase II from *B. cereus* (62, 93). It has a broad specificity for penicillins, cephalosporins and other β-lactams, and none of the current inhibitors is effective against it. Structural and biochemical studies to date indicate a molecular weight of approximately 22,000 daltons, a requirement for a Zn (II) cofactor and a cysteine and three histidine residues as active site groups. The detailed mechanism of action remains to be unravelled but does not involve a covalent acyl-enzyme intermediate.

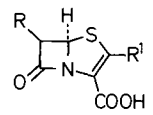
The extensive work on irreversible inhibitors of the serine β-lactamases has been prompted by the realization of their considerable clinical potential. This is based on early observations of notable *in vitro* and, more importantly, *in vivo* synergism between clavulanic acid and susceptible β-lactams against β-lactamase producing bacteria (77) and has resulted in the introduction into the clinic of an amoxycillin/clavulanic acid combination (96). Although similar pharmacokinetic properties have meant that a combined formulation of the two components has been possible (96) an alternative has recently been advanced in place of combination therapy. This involves the chemical attachment of the inhibitor to the antibiotic via an ester linkage which is broken down by non-specific esterases *in vivo* regenerating the two active components. Such compounds have been termed 'mutual prodrugs' (97, 98) and an example currently under development is sultamicillin (**38**) which comprises sulbactam (**35**) and ampicillin (99).

β-Lactamase inhibitors have, therefore, come of age over the last five years not only as clinical candidates but also as biochemical tools. In these respects, such compounds are likely to assume increasing importance in the future firstly in the treatment of resistant bacterial infection and secondly as aids towards a greater understanding of the nature of bacterial resistance due to β-lactamase production. Thus, it appears that a rich future for agents of this type is guaranteed.

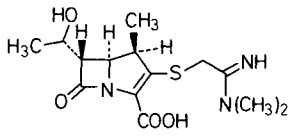
Future Prospects

Since almost all the fundamental advances have to date stemmed from investigations on the secondary metabolites of micro-organisms, it is reasonable to suppose that detailed examination of nature's resources may well provide further quantum leaps in terms of new β-lactam systems which will then require fine tuning by medicinal chemists. Considerable ingenuity and effort has already been, and undoubtedly will continue to be, expended on the modification of the known natural β-lactam systems to improve their antibacterial spectrum or to render them stable to the various hydrolytic enzymes. Efforts in this direction regarding the susceptibility of the potently active carbapenems to the renal dehydropeptidase are already well advanced. The totally synthetic derivative of thienamycin (**45**) which retains almost full biological activity but is stable to the dehydropeptidase is illustrative of the growing sophistication of the synthetic chemistry now available in this area (100).

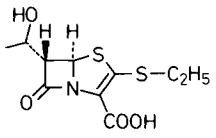
The design and synthesis of potentially useful compounds based on systems not found in nature has been, on the whole, unsuccessful. The two principal exceptions are the oxacephems, e.g. moxalactam (**21**), and the penems (Fig. 12). The latter were first reported in the mid 1970's (101) and are currently attracting considerable attention (102). These molecules can be regarded as synthetic hybrids of the penicillins, cephalosporins and thienamycin structures and appear to combine the advantages and disadvantages of all three systems in



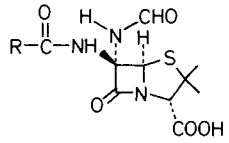
	Side Chain R (+ Configuration)		R^1
39	(β)-$C_6H_5O-CH_2-CO-NH-$		$-CH_3$
40		H–	$-S-CH_2CH_3$
41	(α)-	CH_3O-	$-CH_3$
42	(α)-	$HOCH_2-$	$-S-CH_2CH_2-NH_2$
43	(α)-		$-H$
44		H–	$-COOCH_3$



45



46 SCH 29482



47

Fig. 12 Penem structural types.

terms of activity and stability (103). The orally active penem Sch 29 482 (**46**) (104) is presently the derivative that has been investigated in the most depth (105). Preliminary reports on the properties of derivatives based on a novel 6-substituted penicillin skeleton (**47**), as yet not encountered in nature,[4] could represent another important advance in this area (106). However, more detailed clinical evaluation is awaited with anticipation.

So far all these impressive advances have been achieved without any detailed knowledge of the structures of the target enzymes or the nature of the interaction with their natural substrates or inhibitors. These deficiencies will undoubtedly be overcome within the next few years. An X-ray crystallographic analysis aimed at establishing the structure and the binding site of penicillins and cephalosporins for *Streptomyces* R61 D-Alanyl-carboxypeptidase-transpeptidase is well underway (107) and crystallographic structure analyses of β-lactamases, though less advanced, are progressing. It is to be hoped that such information, coupled with the advances in the computer modelling of molecular interactions, will enable the more rational design of novel β-lactams and perhaps even non-β-lactam systems with both antibacterial and β-lactamase inhibitory activity.

[4] Subsequent to the preparation of this manuscript, the natural occurrence of two 7-X-formamidocephalosporins from a *Flavobacterium sp.* have been described [Singh, P. D., Young, M. G., Johnson, J. H., Cimarusti, C. M., Sykes, R. B., (1984) J. Antibiotics 37, 773–780].

References

(1) Fleming, A. (1929). Br. J. Exp. Path. 10, 226–236.
(2) Morin, R. B., Gorman, M., eds. (1982) "Chemistry and Biology of Beta-Lactam Antibiotics" Academic Press, London.
(3) Brown, A. G. (1982) J. Antimicrob. Chemother. 10, 365–368.
(4) Nagarayan, R., Boeck, L. D., Gorman, M., Hamill, R. L., Higgens, C. E., Hoehn, M. M., Stark, W. W., Whitney, J. G. (1971) J. Am. Chem. Soc. 93, 2308–2310.
(5) Narisada, M., Yoshida, T., Onoue, H., Ohtani, M., Okada, T., Tsuji, T., Kikkawa, I., Haga, N., Satoh, H., Itani, H., Nagata, W. (1979) J. Med. Chem. 22, 757.
(6) Slocombe, B., Basker, M. J., Bentley, P. H., Clayton, J. P., Cole, M., Comber, K. R., Dixon, R. A., Edmondson, R. A., Jackson, D., Merrikin, D. J., Sutherland, R. (1981) Antimicrob. Agents Chemother. 20, 38–46.
(7) Albers-Schoenberg, G., Arison, B. H., Hensens, O. D., Hirshfield, J., Hoogsteen, K., Kaczka, E. A., Rhodes, R. E., Kahan, J. S., Kahan, F. M., Ratcliffe, R. W., Walton, E., Ruswinkle, L. J., Morin, R. B., Christensen, B. G. (1978) J. Am. Chem. Soc. 100, 6491–6499.
(8) Brown, A. G., Corbett, D. F., Eglington, A. J., Howarth, T. T. (1977) J. Chem. Soc. Chem. Commun. 523–525.
(9) Okamura, K., Hirata, S., Okumura, Y., Fukagawa, Y., Shimauchi, Y., Kouno, K., Ishikura, T., Lein, J. (1978) J. Antibiotics 31, 480–482.
(10) Tanaka, K., Shoji, J., Terui, Y., Tsuji, N., Kondo, E., Mayama, M., Kawamura, Y., Hattori, T., Matsumoto, K., Yoshida, T. (1981) J. Antibiotics 34, 909–911.
(11) Kropp, H., Sundelof, J. G., Hadju, R., Kahan, F. M. (1982) Antimicrob. Agents Chemother. 22, 62–70.
(12) Kahan, F. M., Kropp, H.; Sundelof, J. G.; Birnbaum, J. (1983) J. Antimicrob. Chemother. 12, Supplement D, 1–36.
(13) Cama, L. D., Christensen, B. G. (1978) J. Am. Chem. Soc. 100, 8006–8007.
(14) Marier, R. L., McCloskey, R. V., Dickenson, G., Sanders, C. V., Aldridge, K. E., Hoffman, T., Gutterman, D.; Janney, A. (1983) J. Antimicrob. Chemother. 12, Supplement D, 133–140.
(15) Ratcliffe, R. W., Albers-Schoenberg, G. (1982) Reference 2, Vol. 2, 276–310.
(16) Aoki, H.; Sakai; H., Kohsaka, M., Konomi, T., Hosoda, J., Kubochi, Y., Iguchi, E., Imanaka, H. (1976) J. Antibiotics 29, 492–500.
(17) Imada, A., Kitano, K., Kintaka, K., Muroi, M., Asai, M. (1981) Nature 289, 590-591.
(18) Sykes, R. B., Cimarusti, C. M., Bonner, D. P., Bush, K., Floyd, D. M., Georgopapadakou, N. H., Koster, W. H., Liu, W. C., Parker, W. L., Principe, P. A., Rathnum, M. L., Slusarchyk, W. A., Trejo, W. H., Wells, J. S. (1981) Nature 291, 489–491.
(19) Sykes, R. B., Bonner, D. P., Bush, K., Georgopapadakou, N. H. (1982) Antimicrob. Agents Chemother. 21, 85–92.
(20) Koster, W. H., Cimarusti, C. M., Sykes, R. B. (1982) Ref. 2, Vol. 3, 357–368.
(21) Gardner, A. D. (1940) Nature 146, 837–838.
(22) Duguid, J. P. (1946) Edinburgh Med. J. 53, 401–412.
(23) Park, J. T., Johnson, M. J. (1948) J. Biol. Chem. 179, 585–592.
(24) Park, J. T. (1952) J. Biol. Chem. 194, 897–904.
(25) Park, J. T., Strominger, J. L. (1957) Science 125, 99–101.
(26) Ghuysen, J.-M. (1977) Cell Surfaces Rev. 4, 463–595.
(27) Tipper, D. J., Wright, A. (1979) in The Bacteria (Sokatch, J. R., Ornston, L. N. eds.), Vol. 7, 291–426, Academic Press, N. Y.
(28) Mirelman, D. (1979) in Bacterial Outer Membranes: Biosynthesis and Functions (Inouye, M., ed.) 115–166, Wiley and Sons, N.Y.
(29) Suginaka, H., Blumberg, P. M., Strominger, J. L. (1972) J. Biol. Chem. 247, 5279–5288.
(30) Spratt, B. G. (1977) Eur. J. Biochem. 72, 341–352.
(31) Spratt, B. G. (1980) Phil. Trans. R. Soc. Lond. Ser. B. 289, 273–288.

(32) Amanuma, H., Strominger, J. L. (1980) J. Biol. Chem. 255, 1173–1180.
(33) Lund, F., Tybring, L. (1972) Nature 236, 135–137.
(34) Tybring, L., Melchior, N. H. (1975) Antimicrob. Agents Chemother. 8, 271–276.
(35) Howarth, T. T., Brown, A. G., King, T. J. (1976) J. Chem. Soc. Chem. Commun. 266–267.
(36) Blumberg, P. M., Strominger, J. L. (1974) Bacteriol. Rev. 38, 291–335.
(37) Waxman, D. S., Strominger, J. L. (1982) Reference 2, Vol. 3, 209–285.
(38) Ghuysen, J.-M. (1980) in Topics in Antibiotic Chemistry (Sammes, P. G., ed.), Vol. 5, 9–117, Wiley and Sons, N. Y.
(39) Blumberg, P. M., Strominger, J. L. (1971) Proc. Nat. Acad. Sci. USA 68, 2814–2817.
(40) Tomasz, A. (1979) Ann. Rev. Microbiol. 33, 113–137.
(41) Tipper, D. J., Strominger, J. L. (1965) Proc. Nat. Acad. Sci. USA 54, 1133.
(42) Waxman, D. J., Strominger, J. L. (1980) J. Biol. Chem. 255, 3964–3976.
(43) Kozarich, J. W., Nishino, T.; Willoughby, E., Strominger, J. L. (1977) J. Biol. Chem. 252, 7525–7529.
(44) Georgopapadakou, N., Hammarstrom, S., Strominger, J. L. (1977) Proc. Nat. Acad. Sci. USA 74, 1009–1012.
(45) Stietz, T. A., Shulmann, R. G. (1982) Ann. Review. Biophys. Bioeng. 11, (Mullins, L. J., ed.), 419–444, Annual Reviews Inc. Palo Alto.
(46) Boyd, D. B. (1981) Reference 2, Vol. 1, 437–545.
(47) Sweet, R. (1972) in Cephalosporins and Penicillins: Chemistry and Biology (Flynn, E. H., ed.), 280–309, Academic Press, London.
(48) Morin, R. B., Jackson, B. G., Mueller, R. A., Lavagnino, E. R., Scanlon, W. B., Andrews, S. L. (1969), J. Am. Chem. Soc. 91, 1401–1407.
(49) Boehme, E. H. W., Applegate, H. E., Toeplitz, B., Dolfini, J. E., Gougoutas, J. Z. (1971) J. Am. Chem. Soc. 93, 4324–4326.
(50) Firestone, R. A., Schelechow, N., Johnston, D. B., Christensen, B. G. (1972) Tetrahedron Letts. 375–378.
(51) Virudachalan, R., Rao, V. R. S. (1977) Int. J. Pept. Protein. Res. 10, 51–59.
(52) Cohen, N. C. (1983) J. Med. Chem. 26, 259–264.
(53) Abraham, E. P., Chain, E. B. (1940) Nature 146, 837.
(54) Inouye, M., ed. (1979) Bacterial Outer Membranes: Biosynthesis and Functions, John Wiley and Sons, N. Y.
(55) Noguchi, H., Matsuhashi, M., Nikaido, T., Itoh, J., Matsubara, N., Takaoka, M., Mitsuhashi, S. (1979) Microb. Drug Resis. 2, 361–387.
(56) Nakae, T., Nikaido, H. (1975) J. Biol. Chem. 250, 7359–7365.
(57) Nakae, T. (1976) J. Biol. Chem. 251, 2176–2178.
(58) Nakae, T. (1976) Biochem. Biophys. Res. Comm. 71, 877–884.
(59) Schindler, H., Rosenbusch, J. P. (1981) Proc. Natl. Acad. Sci. 78, 2302–2306.
(60) Zimmerman, W., Rosselet, A. (1977) Antimicrob. Agents Chemother. 12, 368–372.
(61) Nikaido, H., Rosenberg, E. Y., Foulds, J. (1983) J. Bacteriol. 153, 232–240.
(62) Hamilton-Miller, J. M. T., Smith, J. T., eds, (1979) Beta-Lactamases, Academic Press, London.
(63) Sykes, R. B., Matthew, M. (1976) J. Antimicrob. Chemother. 2, 115–157.
(64) Hamilton-Miller, J. M. T. (1982) J. Antimicrob. Chemother. 9, Supplement B, 11–19.
(65) Matthew, M., Harris, A. M. (1976) J. Gen. Microb. 94, 55–67.
(66) Richmond, M. H., Sykes, R. B. (1973) Adv. Microb. Physiol. 9, 31–88.
(67) Fisher, J., Belasco, J. G., Khosla, S., Knowles, J. R. (1980) Biochemistry 19, 2895–2901.
(68) Knott-Hunziker, V., Redhead, K., Petursson, S., Waley, S. G. (1980) FEBS Letts. 121, 8–10.
(69) Anderson, E. G., Pratt R. F. (1981) J. Biol. Chem. 256, 1401–1404.
(70) Richmond, M. H. (1981) J. Antimicrob. Chemother. 7, 279–285.
(71) Bush, K., Sykes, R. B. (1983) J. Antimicrob. Chemother. 11, 97–107.
(72) O'Callaghan, C. H. (1980) Phil. Trans. R. Soc. Lond. Ser. B. 289, 197–205.
(73) Cole, M. (1980) Phil. Trans. R. Soc. Lond. Ser. B. 289, 207–223.
(74) Fisher, J., Charnas, R. L., Knowles, J. R. (1978) Biochemistry 17, 2180–2184.
(75) Charnas, R. L., Fisher, J., Knowles, J. R. (1978) Biochemistry 17, 2185–2189.
(76) Charnas, R. L., Knowles, J. R. (1981) Biochemistry 20, 3214–3219.
(77) Reading, C., Cole, M. (1977) Antimicrob. Agents Chemother. 11, 852–857.
(78) Pratt, R. F., Loosemore, M. J. (1978) Proc. Nat. Acad. Sci. USA 75, 4145–4149.
(79) Knott-Hunziker, V., Orlek, B. S., Sammes, P. G., Waley, S. G. (1979) Biochem. J. 177, 365–367.
(80) English, A. R., Retsema, J. A., Girard, A. E., Lynch, J. E., Barth, W. E. (1978) Antimicrob. Agents Chemother. 14, 414–419.
(81) Fisher, J., Charnas, R. L., Bradley, S. M., Knowles, J. R. (1981) Biochemistry 20, 2726–2731.
(82) Fisher, J., Belasco, J. G., Charnas, R. L., Khosla, S., Knowles, J. R. (1980) Phil. Trans. R. Soc. Lond. Ser. B. 289, 309–319.
(83) Abeles, R. H., Maycock, A. L. (1976) Acc. Chem. Res. 9, 313–319.
(84) Walsh, C. (1982) Tetrahedron 38, 871–901.
(85) Cartwright, S. J., Coulson, A. F. W. (1979) Nature 278, 360–361.
(86) Mezes, P. S. F., Clarke, A. J., Dmitrienko, G. I., Viswanatha, T. (1982) FEBS Letts. 143, 265–267.
(87) Gottstein, W. J., Crast, L. B., Graham, R. G., Hayes, U. J., McGregor, D. M. (1981) J. Med. Chem. 24, 1531–1534.
(88) Arisawa, M., Then, R. L. (1982) J. Antibiotics 35, 1578–1583.
(89) Arisawa, M., Then, R. L. (1983) Biochem. J. 209, 609–615.
(90) Arisawa, M., Adam, S. (1983) Biochem. J. 211, 447–454.
(91) Charnas, R. L., Knowles, J. R. (1981) Biochemistry 20, 2732–2737.
(92) Easton, C. J., Knowles, J. R. (1982) Biochemistry 21, 2857–2862.
(93) Ambler, R. P. (1980) Phil. Trans. R. Soc. Lond. Ser. B. 289, 321–331.
(94) Jaurin, B., Grundstrom, T. (1981) Proc. Nat. Acad. Sci. USA 78, 4897–4901.
(95) Knott-Hunziker, V., Petursson, S., Waley, S. G., Jaurin, B., Grundstrom, T. (1982) Biochem. J. 207, 315–322.
(96) Leigh, D. A., Robinson, O. P. W., eds. (1982), Augmentin, Excerpta Medica, Amsterdam.
(97) Baltzer, B., Binderup, E., Vandaehn, W., Godtfried, W. O., Hansen, K., Nielson, B., Sorensen, H., Vangedal, S. (1980) J. Antibiotics 33, 1183–1192.
(98) Wise, R. (1981) J. Antimicrob. Chemother. 7, 503–504.
(99) Hartley, S., Wise, R. (1982) J. Antimicrob. Chemother. 10, 49–55.
(100) Christensen, B. G. (1984), in Recent Advances in the Chemistry of Beta-Lactam Antibiotics (Brown, A. G., Roberts, S. M., eds.), The Royal Society of Chemistry, Special Publication, London (in press).
(101) Woodward, R. B. (1976) in Recent Advances in the Chemistry of Beta-Lactam Antibiotics (Elks, J., ed.) pp 167–180, The Chemical Society, Special Publication No 28, London.
(102) Ernest, I. (1982) Reference 2, Vol. 2. pp 315–360.
(103) Mitsuhashi, S., Inoue, M., Saino, Y., Ogashiwa, M. (1982) J. Antimicrob. Chemother. 9, Supplement C, 91–95.
(104) Ganguly, A. K., Girijavallabhan, J. M., McCombie, S., Pinto, P., Rizvi, R., Jeffrey, P. D., Lin, S. (1982). J. Antimicrob. Chemother. 9, Supplement C, 1–5.
(105) Kayser, F. H., Kuhn, I. (1982) J. Antimicrob. Chemother. 9, Supplement C, 181–188.

(106) Ponsford, R. J. (1984) in Recent Advances in the Chemistry of Beta-Lactam Antibiotics (Brown, A. G., Roberts, S. M., eds.), The Royal Society of Chemistry, Special Publication, London (in press).

(107) Knox, J. R. (1984) in Recent Advances in the Chemistry Beta-Lactam Antibiotics (Brown, A. G., Roberts, S. M., eds.), The Royal Society of Chemistry, Special Publication, London (in press).

Properties of Film-Formers and Their Use in Aqueous Systems

Hermann P. Osterwald[1]

Received: July 1984

Abstract: For the manufacture of film tablets a variety of polymers with different properties are used in organic and aqueous systems. In the present review, the most important film-forming agents and application systems are discussed with regard to their chemical qualities and physical formulation properties. Furthermore, general phenomena and differences among these agents are illuminated by means of models from macromolecular chemistry.

Film Formation

Film-formers are polymers capable of hardening to coherent films. In addition, they require chemical structures in their molecules that provide a given solubility in certain media. The physical property of these polymers essential for coating is the ability to form films. How does this kind of film formation occur?

All coating materials used so far in the pharmaceutical industry are physically drying preparations. During the drying procedure either the solvent evaporates from the dissolution or the water evaporates from the dispersion or dissolution. Here the polymers first are present as isolated coils. If the solvent evaporates slowly, the coils approach until, at a certain polymer concentration, they begin to penetrate each other. This concentration is identical with the reciprocal value of intrinsic viscosity.

$$\lim_{c \to o} \left[\frac{\Delta \eta}{\eta_o} \right] = [\eta]$$

A film is allowed to form provided the individual sprayed droplets are able to coalesce in any one flowable state to permit the macromolecular coils to penetrate each other. For this it is necessary that at least segments of the coils be mobile, i. e. they must be present above the glass transition temperature.

Like the polymer coils the particles of a dispersion may also approach each other. Depending on the temperature, the latex particles either aggregate through additives (emulsifying agents, protective colloids) or they adhere directly to each other.

The penetration of the macromolecular chains and the resulting properties of the film are best illuminated by the differentiated network model shown in Fig. 1.

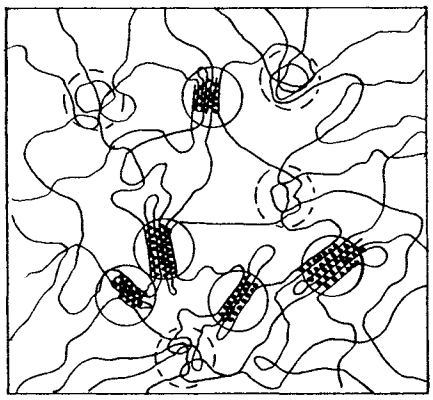


Fig. 1 Differentiated network model of polymers (1).

Here, the polymer or the film, respectively, is thought to be composed of an uninterrupted entanglement of coiled molecular chains. In this network two areas can be distinguished:

1. ordered, crystalline areas;
2. amorphous areas in which several chains form a loose coil stabilized by weak forces only. If a mechanical strain is applied above the glass transition temperature, i. e. at a temperature at which segments of chains become mobile, these coils will loosen slowly, the chains gliding past each other. Penetration is thus rendered possible. These amorphous areas show more or less appreciable variations in the distribution of free volume, which are characterized by a lack of major intermolecular forces.

The overall behavior of the film depends on the mesh size of the network and on bond strength, but above all on the degree of entanglements. If no amorphous areas form but only ordered structures, this will very easily result in domain formation and thus cracking, orange peel, etc.

Besides the capacity of penetration, film-formers must present a certain adhesiveness to the material to be coated. The adhesion to the dosage form and, in the further course of the coating process, to the polymers themselves, depends on the chemical and physical interactions between polymers, plasticizer, solvent and surface as well as on diffusion phenomena. To put it more simply, the surface properties and energies of dosage form and coating material, their interfacial tension, geometry of the cores, viscosity of the solution, substances absorbed in the dosage form, such as water, etc.,

[1] Pharmazeutische Entwicklungsabteilung, A. Nattermann & Cie GmbH, Postfach 35 01 20, D-5000 Köln 30