Degradation of β-lactam antibiotics

A. D. Deshpande, K. G. Baheti and N. R. Chatterjee*

Padmashree Dr D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411 018, India

Recent developments in the chemistry and biology of β-lactam antibiotics which culminated with the introduction of several clinically useful classical and non-classical β-lactams have been most thrilling and highly rewarding. While this review touches upon the historical development of β-lactams and their reactivity in a nutshell, it provides an overview of various aspects of chemical and enzymatic degradation of β-lactams, pointing to various routes of degradation, degradation products and allergenicity.

PENICILLIN is a unique molecule having a fused β-lactam–thiazolidine ring system, wherein the strained β-lactam ring is susceptible to cleavage by a variety of reagents as well as some enzymes[1,2]. The labile β-lactam ring of penicillins and other β-lactam antibiotics is characterized by its pronounced susceptibility to various nucleophiles, acid-base reagents, metal ions, oxidizing agents or even solvents like water and alcohol. While some of these are reported in the literature[3–7] in a scattered manner, there are scanty reviews on chemical and biological degradation of β-lactam. Hence it appeared worthwhile to present a brief overview on the stability and degradation of β-lactam antibiotics.

The instability of β-lactam antibiotics in solution was observed to be a major hurdle in the development of penicillin and other useful β-lactam antibiotics. Therefore, degradation or stability study of β-lactam antibiotics has been of paramount importance not only for their market availability, but also to evaluate their pharmacokinetic properties and adverse reactions. It is also interesting to note that stability or rate of degradation of different members of β-lactam antibiotics *in vivo* as well as *in vitro* has been quite different. However, the major pathways of their degradation have remained similar, leading to various breakdown products in a majority of the β-lactams. Furthermore, various antigenic determinants of penicillin responsible for its allergenicity have also been demonstrated to occur from many of their degradation products or through formation of penicilloyl proteins[8].

The discovery of cephalosporin from the culture of *C. acremonium* by Brotzu[9] and demonstration of its remarkable stability towards aqueous solution even at pH 2 as well as its excellent *in vitro* activity against penicillin-resistant organisms by Abraham and Newton[10], were major breakthroughs in the history of β-lactam antibiotics. The realization that fungi might be a good source for novel antibiotics spurred microbiologists to develop novel soil-screening programmes for the investigation of microbial culture leading to the discovery of several non-classical β-lactams like norcardicins[11], monobactams[12–14], carbapenem[15] and oxacephems[16]. Many of these agents are elaborated as chemical defences by moulds and actinomyces. It was also evident from their structure–activity relationship[17–20] studies that the reactivity of β-lactam antibiotics is fundamentally linked to antimicrobial activity and bacterial resistance, which led Woodward to design and synthesize carbapenem group of compounds[21,22] for evaluation of their antibacterial activity much before the actual discovery of this group of compounds through systematic screening of the soil microorganism. Many of these compounds did show broad-spectrum antimicrobial activity as predicted earlier and this was further substantiated by the subsequent discovery of thienamycin. Although its instability was an acute problem for market introduction, this was finally overcome by Merck group through its derivatization leading to introduction of imipenem, which is being regarded as one of the most effective drugs among the β-lactam antibiotics. The present review attempts to describe the various aspects of chemical and bio-degradation of the β-lactam antibiotics with particular reference to penicillin and cephalosporins, while focusing briefly on the relationship between β-lactam reactivity and bioactivity and the cause for allergenicity. Some other aspects like SAR of β-lactams, their metabolism and pharmacokinetics have been kept out of the purview of this article because of several limitations.

Historical development of β-lactams

Even before the actual discovery of penicillin from *Penicillium notatum* by Alexander Fleming in 1927, it is claimed that Lister and Sanderson at Oxford indicated the penicillin-producing mould way back in 1911. They used the fungal culture extract for treatment of localized wounds instead of carbolic acid. However, demonstration of the excellent antibacterial activity of penicillin coupled with its low toxicity by Chain, Florey and co-workers[23] in 1940 was the real beginning of the penicillin era. The phenomenal growth in the chemistry and biology of penicillin, including its industrial application was due to the joint Anglo-British venture during the Second World War[24]. Soon after the successful clinical introduction of penicillin in 1941 and its wide use during the Second World War, many other antibiotics like streptomycin (1943), chloramphenicol (1947), chlortetracycline (1948), neomycin (1949) and erythromy-

*For correspondence. (e-mail: nitharc@yahoo.com)

cin (1952) were discovered in succession through deliberate research of the metabolites isolated from a wide range of soil samples. A second spurt in the development of newer penicillins took place during 1958–60 with isolation of 6-amino penicillanic acid (6-APA); a key biosynthetic intermediate of penicillin isolated by Beecham group, which was also one of the key intermediates in Sheehan's synthetic route[25,26] of penicillin (1957). It was found to be the key nucleus of penicillin, while itself being devoid of biological activity. However, it offered an opportunity for the synthesis of a large number of penicillin analogues by acylating 6-APA with a range of acid chlorides. This could be regarded as the beginning of the semi-synthetic penicillin era.

The cheap availability of 6-APA through continuous development of fermentation technology has made it today a bulk chemical; as it is prepared through selective removal of the acyl side chain from potassium penicillin G or V (**1**) either by enzymatic or chemical process. While the enzymatic process takes advantage of amidases (acyalses) isolated from a number of bacteria for selective hydrolysis of penicillin G or V to 6-APA (**2**), the chemical method converts the amide to imino chloride intermediate, which through imino ether is hydrolysed to yield 6-APA[27] (**2**) (Figure 1). It may be worth mentioning that the enzymatic process has virtually replaced the chemical method of 6-APA production[28,29] due to the cleanliness of the process as well as improved product quality, including the recovery of the by-product (**4**), which is recycled.

Although the search for newer semi-synthetic penicillins is still being continued, much of the current highlighting of β-lactams came with the revelation that modified cephalosporins have much wider activity than penicillins with less toxicity and better stability. Cephalosporin C[30], isolated not too long after widespread use of penicillin, turned out to be a congener of penicillin-N and was found to contain a six-membered dihydrothiazine ring instead of thiazolidine ring of the penicillins. Although cephalosporin C showed superior resistance to hydrolysis by *S. aureus* β-lactamase, its clinical use was precluded due to its low potency and poor biopharmaceutical properties compared to penicillin-N[31]. Hence, the process of development for efficient removal of amino adipate side chain by chemical and enzymatic method was undertaken for providing 7-amino cephalosporanic acid (7-ACA), which served the same role in the series as 6-APA did in penicillin. Although removal of α-amino adipoyl side chain directly from cephalosporin C either by chemical or enzymatic method was problematic due to the presence of amino group, it was overcome by conversion to either imino lactone or imino ether intermediate (**6**) by chemical route, which was subsequently hydrolysed to knockout the side chain to give 7-ACA (**7**) in good yield[32,33] (Figure 2). Attempts to remove the side chain directly by chemical route or by penicillin acylase as was achieved in the case of penicillin G or V yielded less than 1% of 7-ACA due to interference of α-amino group in the side chain.

The chemical route for production of 7-ACA was accepted as the commercial process, since it was found that the amidase, which is capable of removing the side chain from penicillin G or V, was unsuccessful in cleaving the cephalosporin side chain. However, biotransformations leading to the removal of α-amino group using D-amino oxidase enzyme followed by removal of carbon dioxide through oxidation to obtain glutaryl-7-ACA and finally to cleave the side chain by glutaryl amidase have been successfully achieved for production of 7-ACA with reasonably good cost-benefit ratio. Hence the enzymatic process being green could compete well with the chemical method of 7-ACA production in the foreseeable future. It is worth mentioning here that continued thrust was also made to modify the penicillin ring system into cephalosporin structure through the ring expansion of fused thiazolidine, since cephalosporin C fermentation was costly. Success in chemical interconversion of penicillin into cephalosporin became a major landmark in the historical development of β-lactam antibiotics. This led to easy and cheap availability of de-

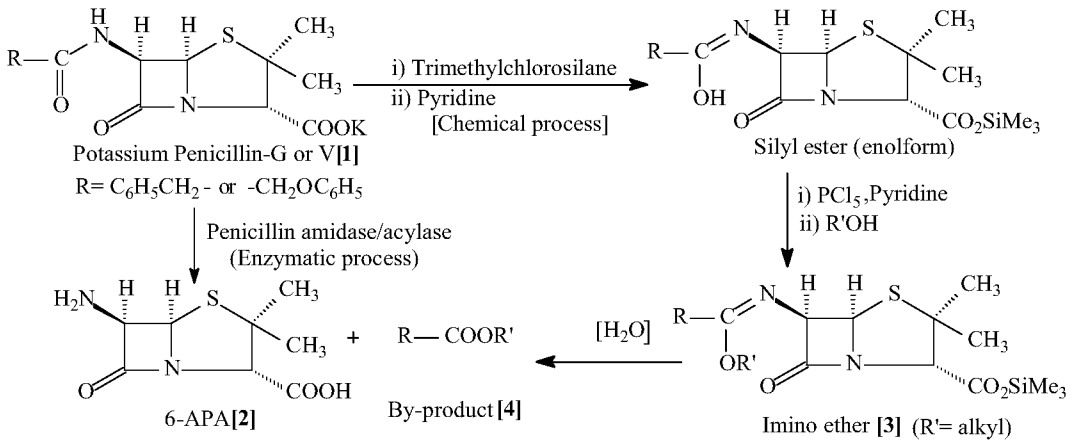


Figure 1. 6-APA production by chemical and enzymatic degradation of penicillin.

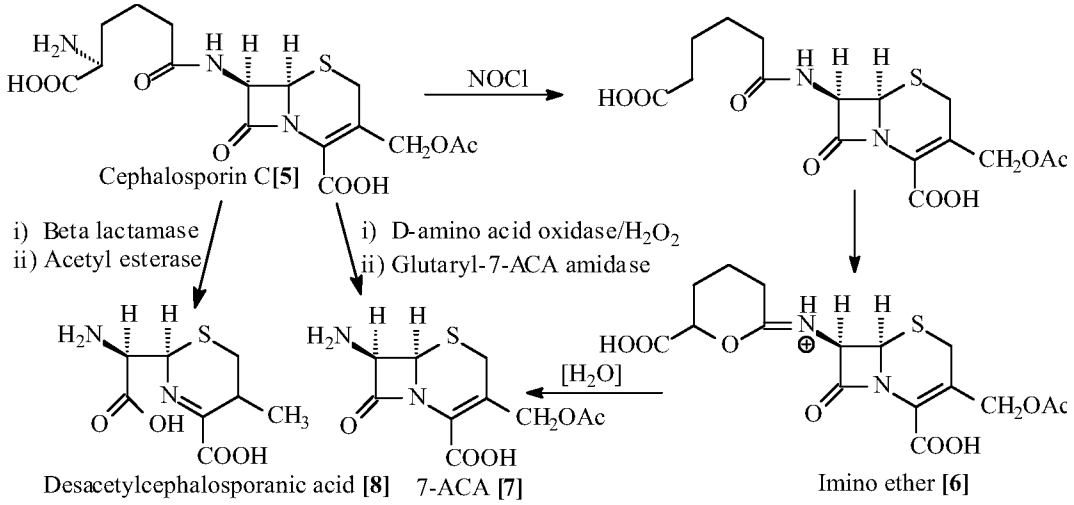


Figure 2. 7-ACA production by enzymatic and chemical degradation of cephalosporin C.

sacetyl-7-amino cephalosporanic acid (7-ADCA)[34–36] (**8**), which was found to be the backbone for oral cephalosporins. Majority of today's oral cephalosporins like cephalexin, cephachlor, etc. were commercially produced from natural penicillins because of their economic feasibility. The key intermediate of cephalosporins, i.e. 7-ACA and 7-ADCA thus being available in plenty through concerted efforts of dedicated microbiologists, medicinal chemists and technologists, opened the floodgate for semi-synthetic cephalosporins[37] following almost an identical route as done earlier in the case of semi-synthetic penicillins. The presence of an additional reactive allylic acetate group position 3 of natural cephalosporins provided an additional centre for modification. This was found to play a major role in improving the physico-chemical properties of the drug molecule. Therefore, both types of modification at position 7 and 3 in the cephalosporin nucleus have resulted in providing hundreds of novel cephalosporin analogues. It may be worth noting that out of a few lakhs of semi-synthetic penicillins and cephalosporins thus prepared for obtaining more potent and useful drugs, only about a hundred or so could have seen the light of the day due to some advantages over existing ones; only a few of them have gained prominence in the present antibacterial market in their own right. Some older generations of β-lactam have been rendered obsolete due to the emergence of better choice drugs. While the search for newer β-lactams has reached a point of no return with the availability of a wide range of antibacterial drugs, it may be surprising that medicinal chemists and microbiologist are still seeking new and improved antibacterial agents. The reason for this is primarily due to the fact that 60% of streptococcus pneumoniae strains have become resistant to frequently used (abused) β-lactams.

The rapid progress of the search for new β-lactams remained unabated till the end of 1980 with success as evidenced from the introduction of several non-classical β-lactams, viz. thienamycin, meropenem, moxalactam, aztreonam, all having broad-spectrum activity against certain key pathogens, e.g. *P. aeruginosa* and/or MRSA with improved pharmacokinetic properties and reduced undesirable side effects. Thus the major success achieved by the combined efforts of medicinal chemists and technologists in introducing several new β-lactams into the market gave further impetus to the continued search for the 'ideal peni
i.e. possessing all the desirable properties such as broad-spectrum activity against resistant strains, more stability with low toxicity and allergenicity, better absorption, distribution and metabolic properties[2] in a single entity. The combined share of semi-synthetic penicillins, cephalosporins and other β-lactams in the world market of antibacterials will now be around 50%. These drugs have virtually taken the lion's share by replacing other antibacterial agents, primarily due to their potent activity as a single agent or in combination with β-lactamase inhibitors as well as lesser toxicity and adverse reactions.

β-Lactam reactivity and biological activity

The remarkable properties of penicillin molecule, including its antibacterial activity could possibly be explained due to the fusion of the β-lactam ring with the thiazolidine ring. The strain developed the fusion of the five-membered thiazolidine ring with the four-membered β-lactam ring leads to nonplanarity of the molecule, which results in a large angle and torsional rotation[38,39]. These factors make it enormously labile to any kind of nucleophilic attack as well as in the presence of acid, alkali or even neutral molecules like water and alcohol. This also results in more pronounced susceptibility to hydrolysis of the penicillin β-lactam bond than amides in general or other lesser or non-strained β-lactams, e.g. cephalosporins, moxolactam, monobactam, etc. wherein partial stabilization of the C=O group could occur through delocalization of the lone pair of electrons

on the adjoining N-atom. This greater instability of penicillin molecule was mainly responsible for hindering early progress and development of the chemistry and biology of penicillins, as is evident from the fact that synthesis of benzyl penicillin by Sheehan *et al.*[26] took almost a decade after the establishment of its structure, in spite of their untiring efforts as well as those of many other medicinal chemists for synthesizing the wonder drug. Furthermore, the other source of β-lactam reactivity in cephalosporins being associated with the double bond in Δ^3-position interacting with the lone pair of nitrogen electrons and thus rendering the β-lactam ring less nucleophilic susceptible to β-lactamase attack than that of penicillins.

The acute penicillin lability could be rationalized for the bioactivity of the molecule to some extent, as evident also in case of Δ^3-cephalosporins. The cephalosporins possess a β-lactam ring fused with a dihydrothiazine ring structure and therefore do not lead to any substantial out-of-plane distortion, i.e. cephem amide system being essentially planner. However, Δ^2-cephalosporins are more stable than their Δ^3-isomers, but not biologically active[38]. Based on the subsequent discovery of various non-classical β-lactams, it became clear that the essential pharmacophoric requirement for biological action in this group was the reactive β-lactam function. It leads to irreversible blockade and thus inhibits the formation of peptidoglycan linkages during cell-wall synthesis required for bacterial replication, thereby inhibiting bacterial growth and generally possessing bactericidal activity.

Carbapenems like thienamycin and meropenem, panipenem have shown exceptionally high broad-spectrum activity as well as ability to inactivate β-lactamases[40,41], since they combine the functional feature of the best of β-lactams as well as β-lactamase inhibitors. Replacement of sulphur atom in penicillin by methylene moiety along with introduction of double bond, as it exists in carbapenems, has made the ring system highly strained and susceptible to reactions leading to β-lactam cleavage. The main area of modification in this class of compounds has been the C-2 side chain of carbapenem nucleus. Presence of terminal free amino group in the C-2 side chain in thienamycin has made the molecule unstable and difficult for isolation and purification, which was finally overcome by converting the free $-NH_2$ group into less nucleophilic *N*-formimidoyl moiety by semi-synthetic route for its market introduction as imipenem (**9**)[42]. Meropenem (**10**) is another successful example of a carbapenem antibiotic.

The discovery of monocyclic β-lactams, e.g. sulfazecin, norcardins, etc. from saprophytic soil bacteria in Japan and USA around 1980 unravelled the existence of a novel class of β-lactams, which contain a single β-lactam not fused to any other ring system. Although this group showed moderate Gram-negative activity, it was difficult to account for the antibacterial activity since the presence of a second fused ring to strained β-lactam ring has always been thought to be essential for bioactivity. However, it served as an inspiration to synthesize and evaluate many other members of this group through acylation of their key nucleus, 3-amino mobactamic acid (3-AMA)[43] in a similar fashion to semi-synthetic penicillins and cephalosporins. Concerted efforts towards this direction led to the development of clinically useful antibiotics like aztreonam (**11**) and norcardicin (**12**) (Figure 3). Majority of this class of compounds showed wide range of Gram-negative activity associated with strong β-lactamase inhibitory activity[44]. The molecular mode of (**11**) is similar to penicillins and cephalosporins and indicates that antibacterial activity may not be much linked to the fused β-lactam ring. However, the sulfamic acid moiety attached to β-lactam nitrogen possibly resembling C_2-carboxyl of the precedent β-lactams and the principal side chain attached to the amino group at position 3 along with α-oriented methyl group at C_2, are the source of their antibacterial and β-lactamase inhibitory activity. The strong electron-withdrawing character of the sulfamic acid group, however, makes the β-lactam more vulnerable to hydrolysis, thereby leading to instability of the compound[45].

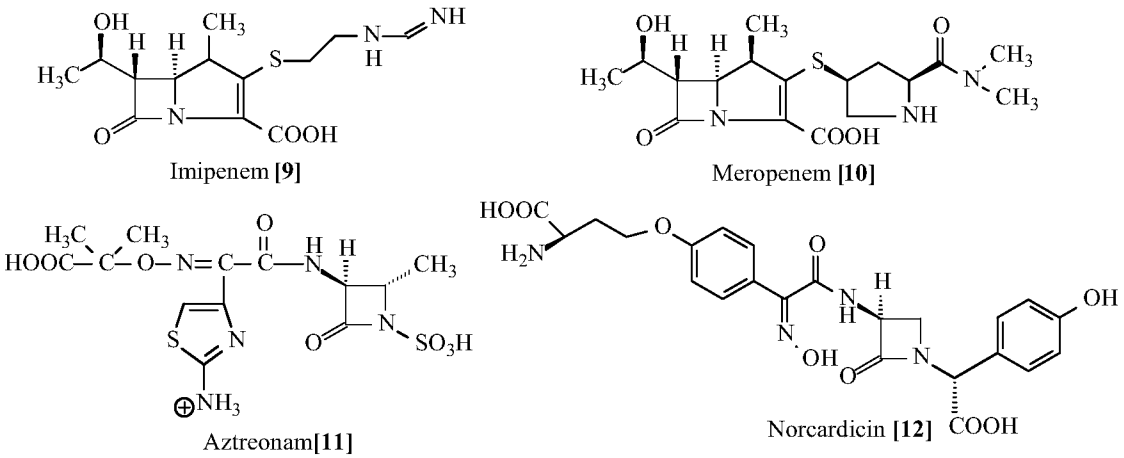


Figure 3. Some non-classical β-lactam antibiotics.

Hydrolytic stability versus ring size in lactams, e.g. β-propiolactam, γ-butyrolactam and δ-valerolactam has been considered for future development of lactam antibiotics; it has been observed that medium-sized lactams are least susceptible to hydrolysis, whereas the six-membered lactam also appeared to be a promising candidate for developing a newer class of anti-infective and other serine protease inhibitors[46].

Degradation of penicillin

Penicillin is a unique molecule containing unstable, highly strained and reactive β-lactam amide bond. The degradation of penicillin takes place in various conditions, viz. alkaline or acidic, in the presence of enzyme β-lactamase or treatment of weak nucleophiles like water and metal ions. The strained bicyclic system in penicillin is supposed to remain in equilibrium with pseudopenicillin (**14**) under neutral condition, which contains an oxazolone structure leading to various degradation products under a variety of conditions as detailed herein (Figure 4). It undergoes further isomerization on aging to yield penicillenic acid (**17**), as characterized by its strong UV absorption at 322 nm and has been regarded as haptenic component for penicillin allergenicity. Hence most of the oral and parenteral β-lactam preparations are generally kept below 1% moisture level during their shelf life.

The highly strained β-lactam ring and its amide bond break open in the presence of acid[47,48] giving an array of complex products, including penilloic acid, penicillamine and penilloaldehyde through the highly unstable intermediate, viz. penillic acid, penicilloic acid and penicillenic acid. Penicilloic acid (**16**) exists in its isomeric form, i.e. penamaldic acid (**19**) by opening of the thiazolidine ring which has been characterized by its UV peak around 320 nm under strong acid condition. It further degrades to give the ultimate decomposed products, viz. penilloaldehyde (**22**) through its parent acid i.e. penaldic acid (**20**), and penicillamine (**21**); possibly through its *N*-formyl penicillamine. It may also undergo ready decarboxylation to give penilloic acid (**18**). In strong acidic medium[49] (pH 2 or less), it undergoes rearrangement through oxazoline formation giving rise to penillic acid (**15**). It was thought worthwhile to protect the degradation of penicillins under acidic conditions by putting some electron-withdrawing groups in the α-position of the *N*-acyl side chain, which would decrease the electron density on the side chain carbonyl group and reduce its tendency to act as nucleophile and thus protect these penicillins. Such modifications resulted in the introduction of some acid-resistant penicillins, viz. ampicillin, amoxicillin, carbenicillin, ticarcillin, etc. in the market.

Penicillins undergo degradation rapidly in alkaline conditions[47–49] (pH 7.5–9.0), wherein the amide bond gets open to give penicilloic acid (**16**). The carboxyl group present on penicilloic acid after bond opening, undergoes decarboxylation giving rise to penilloic acid (**18**). The formation of these degradation products of penicillin leads to the total loss of activity (Figure 4). The extensive degradation of ampicillin was studied under alkaline conditions[50]. The first degradation product in alkaline condition was 5R-penicilloic acid, which subsequently undergoes epimerization at C-5 to form the 5S-isomer via the imine tautomer. A recent study indicated that semi-synthetic β-lactam undergoes hydrolysis with the help of penicillin acylase via formation of a covalent acyl enzyme intermediate[51].

Penicillin undergoes degradation by an enzyme β-lactamase or penicillinase, which is produced by many penicillin-resistant bacteria[52,53]. The enzyme opens the ring in the same way as acid hydrolysis does. In order to overcome the problem of β-lactamase sensitivity[54], it was thought that some bulky group could be placed in the penicillin side chain. This can act as a shield towards β-lactamase and thus prevent its binding. This rationale has led to the development of several β-lactamase-resistant penicillins like oxacillin, cloxacillin and flucloxacillin, which have wide clinical acceptance. It is also reported that cleavage of β-lactam amide bond takes place in water when it is heated, but this cleavage is slower than β-lactamase inhibitors.

Carbenicillin, a benzyl penicillin analogue having an α-carboxyl group in the acyl amido side chain at C-6, can readily undergo decarboxylation to produce benzyl penicillin. The degradation product thus formed being an antibiotic, the purpose of its use is lost since it has no activity against *P. aeruginosa* or *Proteus vulgaris* for which it is indicated. Hence ticarcillin, a bioisostere of carbenicillin, possessing a sulfonic acid group in place of carboxyl group in carbenicillin, is found to be more potent against *Pseudomonas*, since its degradation through decarboxylation is avoided.

Other factors that also contribute towards instability of penicillin are β-lactam carbonyl group and acyl side chain, which undergo neighbouring group participation leading to opening of β-lactam ring. Thus penicillin suffers from a built-in self-destructing mechanism (Figure 5).

Chemical and enzymatic degradation of cephalosporins

Although cephalosporins are more stable to hydrolytic degradation reactions than penicillins, they experience a variety of chemical and enzymatic transformations, whose specific nature depends on the side chain at C-7 and the substituent on C-3 atom[55]. The presence of a good leaving group at C-3 facilitates spontaneous expulsion of the 3′-substituent by concerted event due to hydrolysis of C–N bond of β-lactam nucleus by any general nucleophile or β-lactamase. Thus, desacetyl cefotoxime is more stable to hydrolysis in comparison to cefotoxime.

The absence of a leaving group at position 3 of cephalosporins makes them more acid-stable, thus rendering them suitable for oral consumption. Hence cephalexin posses-

sing methyl group at position 3 is much better absorbed than cephaloglycin having acetoxymethyl group at position 3, while both have identical phenylglycyl side chain at the position 7. The nature of substituents at C-7 of cephalosporins plays an important role in determining the facility with which the reactive β-lactam bond is hydro-

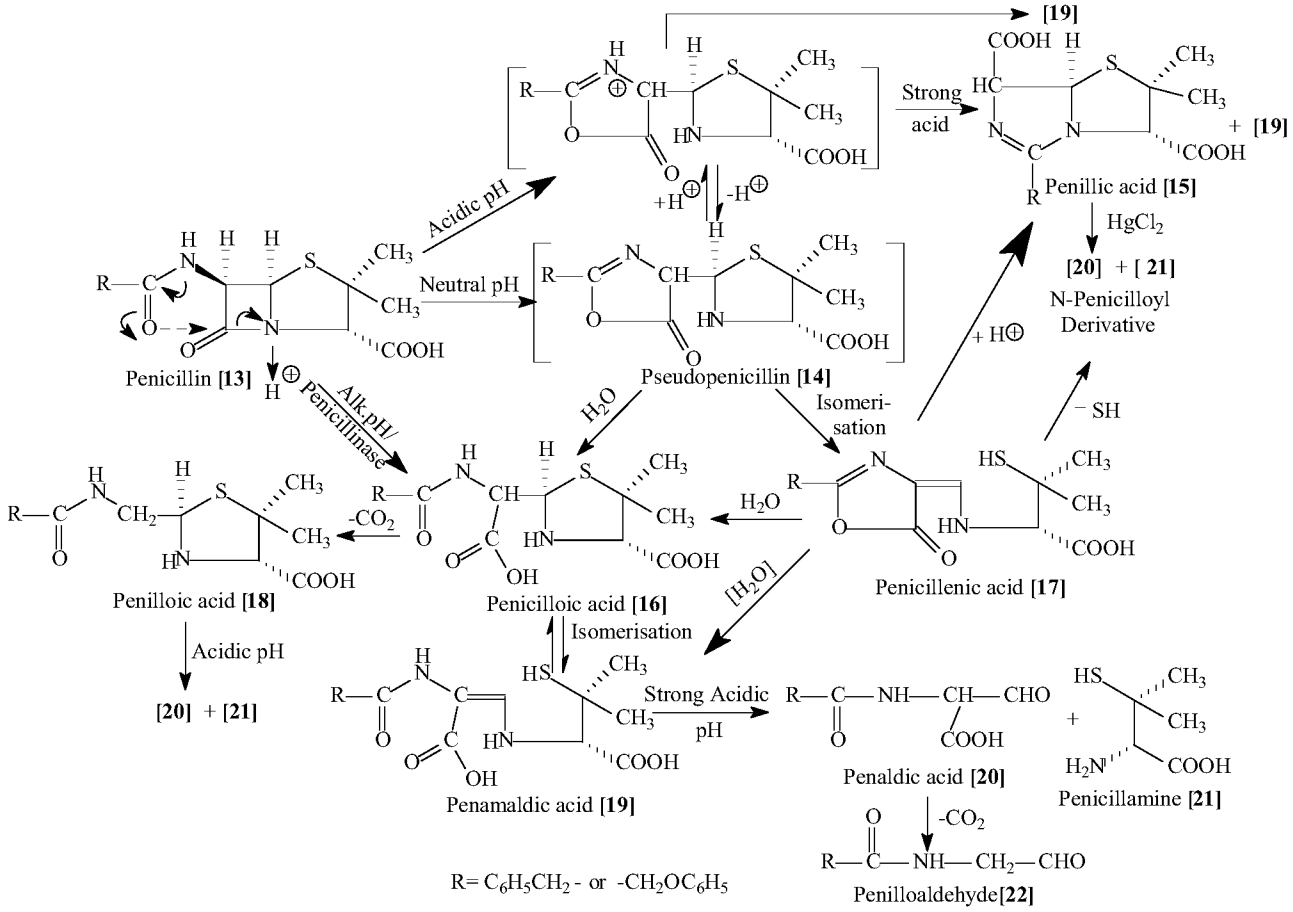


Figure 4. Pathways of degradation of penicillin in acidic and alkaline conditions.

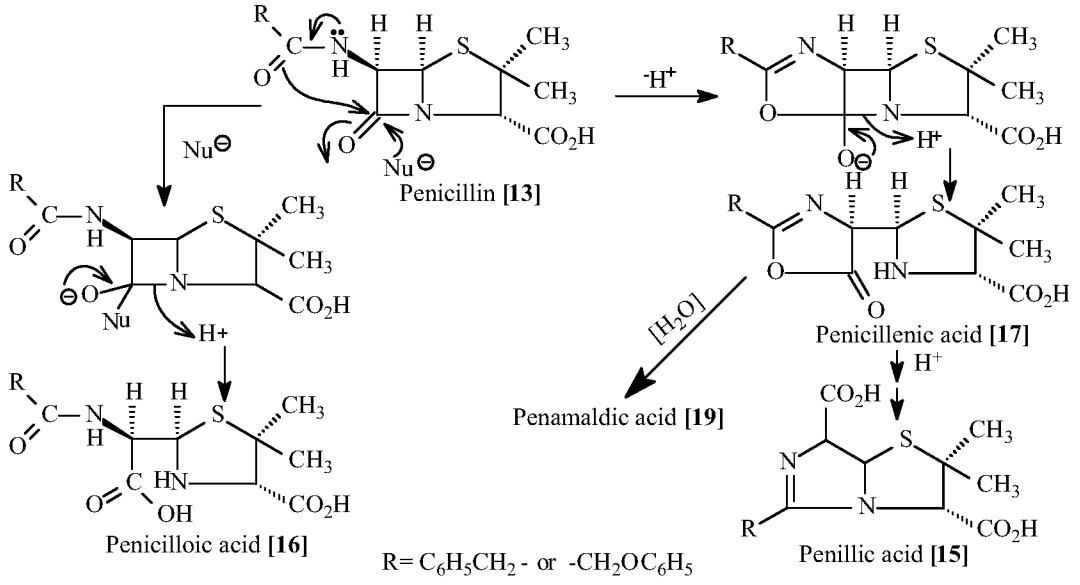


Figure 5. Nucleophilic ring opening and acid sensitivity of penicillin due to the influence of acyl side chain.

lysed or broken either by chemical or enzymatic means of degradation of cephalosporin C (Figure 6). Isomerization of Δ^3-cephalosporin to its Δ^2-isomer occurs with great loss of antibacterial activity, but it leads to more stability. Cephalosporins are significantly less sensitive to hydrolysis by various β-lactamases[56] of different origin than penicillins, which appears due to the intrinsic property of bicyclic ring system as discussed earlier in β-lactam reactivity. However, the rate of hydrolysis by β-lacatmase varies considerably with different cephalosporins[57] due to the nature of substituents at C-7 and C-3 as well as steric factor involved therein; although no empirical rule for the substituents exists. It is interesting to note that the acyl functionalities imparting β-lactamase resistance in penicillins unfortunately render cephalosporins virtually inactive against most Gram-positive bacteria. Like penicillins, cephalosporins are comparatively less soluble and unstable in aqueous solution and therefore are used generally as sodium salts. The stability of aqueous solution of cephalosporins as sodium or potassium salt increases at low temperature and therefore, it is mostly reconstituted before their use as observed in penicillins.

On enzymatic degradation in the presence of acylase cephalosporin C (**23**) gives 7-amino cephalosporanic acid (**24**), which in the presence of acid undergoes lactonization to give des acetyl-7-amino cephalosporanic acid lactone (**25**). In the presence of esterase, cephalosporin-C gave desacetyl cephalosporin (**26**) and then desacetyl cephalosporin lactone (**27**). The enzyme β-lactamase or cephalosporanase degraded cephalosporin C into cephalosporoic acid (**28**), anhydrodesacetyl cephalosporoic acid (**29**) and desacetyl cephalosporoic acid (**30**). Further breakdown of these acidic products leads to many other fragmented and rearranged products (Figure 6).

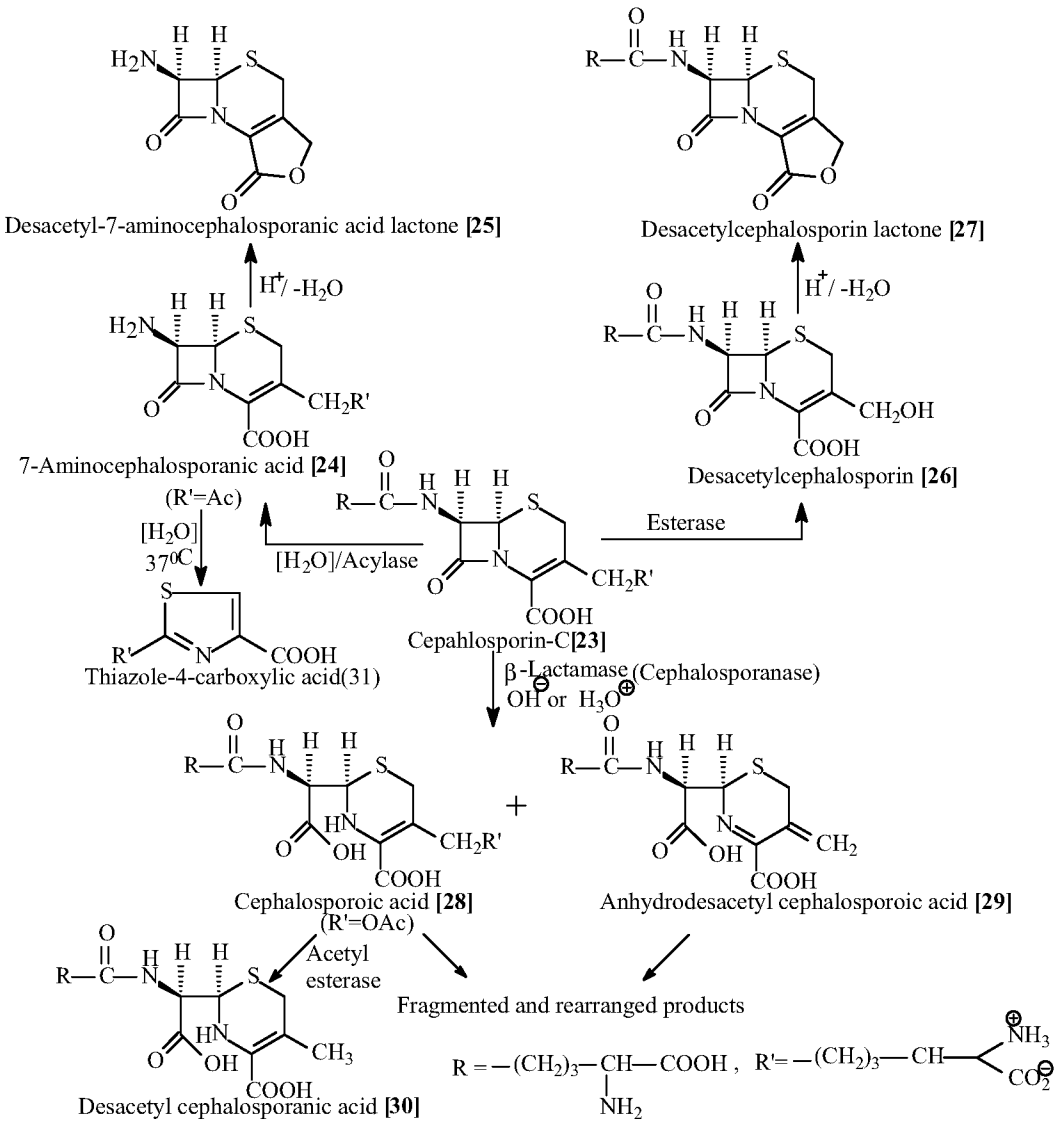


Figure 6. Possible pathways of degradation of cephalosporin C.

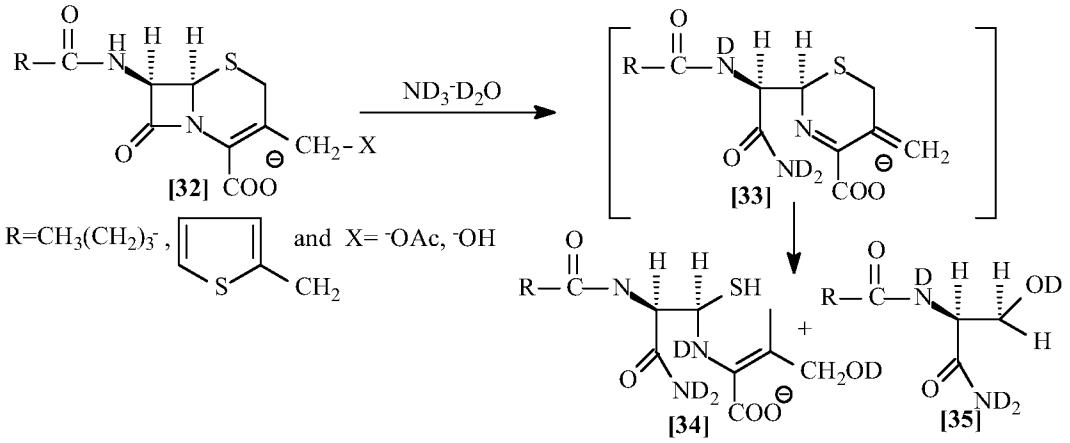


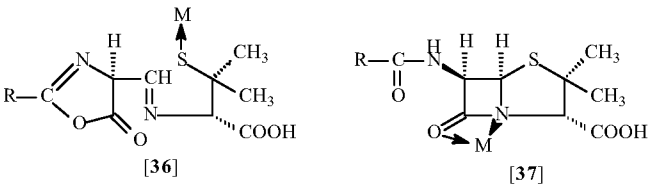
Figure 7. Enzymatic hydrolysis and aminolysis of cephalosporin C.

Most of the β-lactamases elaborated from various species of bacilli, hydrolyse natural penicillins or even ampicillin faster than cephalosporins. Some β-lactamases, like cephalosporanase, however, hydrolyse cephalosporins more rapidly and inactivation by β-lactamases plays a prominent role in determining resistance to cephalosporins in numerous strains of Gram-negative bacteria. Base or β-lactamase-catalysed degradation of cephalosporins having a good leaving group at C-3 is generally accompanied by elimination of leaving group to form anhydrodesacetyl cephalosporoic acid[58], which does not give stable degradation products like penicillin at the end. Cephalosprin C, on hydrolysis at 37°C in neutral aqueous solution, yields small amount of thiazole derivatives resulting from β-lactam cleavage followed by attack of sulphur on the acyl moiety (Figure 6).

Like in penicillin chemistry, the prodrug approach of converting cephalosporins to more potent drugs has been adopted with considerable success to confer acid stability (or oral activity) by conversion to lipophilic ester, which undergoes hydrolysis catalysed by hepatic esterases to release the parent drug. Based on this concept cefruoxime axetil, cefodoxime proxeitil and a few more orally active ester derivatives of β-lactamase-resistant cephalosporins[59] have been introduced and are well accepted in market. Enzymatic hydrolysis and aminolysis have thrown considerable light on the complex breakdown pattern of the cephalosporins, as monitored by NMR and UV spectra[60] using ND_4OH, suggesting that the intermediate exo-methylene compound (**33**) formation takes place through expulsion of the acetate ion at position 3; further degradation of (**33**) gives fragmented products (**34**) and (**35**) (Figure 7).

Degradation of β-lactams in the presence of metal ions

Degradation of penicillins and cephalosporins has been studied extensively in the presence of various metal ions, viz. mercury[61], zinc[62,63], cadmium[64], cobalt[65] and copper[66–68]. It was found that these ions catalysed the rate of inactivation or hydrolytic opening of β-lactams. Such metal ion-triggered assisted degradation was supposed to happen through the formation of a single intermediate substrate–metal complex or two intermediate substrate–metal complex[62]. It has been established that the metal ions catalyse penicillin degradation following two routes, i.e. first by interacting with sulphur atom of the penicillenic acid intermediate to form metal mercaptide (**36**) and second by complexing the penicillin with metal ion to form chelate (**37**). It has been observed that the former pathway plays a major role during penicillin degradation by metal catalysis.



Penicillin degradation in neutral solution in the presence of Hg^{+2} and Cu^{+2} ions was observed to be sufficiently strong and could be monitored by following UV absorption at 320 nm, which is characterized by penicillenic acid formation. The catalytic effect of Zn^{+2} in the degradation of penicillin was found to be much weaker than the that of Cd^{+2} ion, owing to the lesser ionic radius of the former, and the corresponding penamaldic acid derivative of the antibiotic was obtained as the degradation product in the form of complex with metal ions.

Degradation of some non-classical β-lactam

Imipenem (**9**) is a crystalline derivative of thienamycin[69] and it is generally less stable than penicillin and cephalosporin in the presence of acids and phosphate anion[70]. Imipenem does not chelate with Ca^{++}, Mg^{++}, Zn^{++} or Fe^{++}

but with Cu^{++} and Pb^{++}, which accelerate its decomposition. The major product of the first-order degradation of imipenem (**9**) is an open ring structure (**38**), which exists at neutral or low acidic pH and under highly acidic condition as (**39**) in the form of a mixture of isomers[71] (Figure 8).

Aztreonam was found to be present in two forms, i.e. α and β crystalline forms. The α-form is not very stable in aqueous ethanol or methanol[72], whereas the β-form is stable. It undergoes hydrolysis of β-lactam ring under the whole acidic or alkaline pH range[73]. In weakly acidic solution (pH 2–5), hydrolysis is preceded by isomerization of the side chain leading to the formation of E (anti) isomer[74] (Figure 9). In aqueous solution at pH 4–7, aztreonam is more stable than most of penicillins and cephalosporins. Further studies showed that aztreonam is highly stable in the pH range 5–7, undergoing only 10% degradation in 300–500 h. The non-classical β-lactam antibiotics, viz. imipenem, aztreonam and nocardicin A undergo hydrolysis and aminolysis at very fast rate in the presence of metal ions like zinc and cadmium[75].

β-Lactam antibiotic allergy

β-Lactam antibiotics (penicillin in particular) are known to cause allergic reaction in 3 to 5% of patients who take the drug by parenteral route. All β-lactam antibiotics ranging from benzyl penicillin to the more recently introduced β-lactams such as aztreonam or even the related β-lactamase inhibitors, like clavulanic acid[76] may induce allergic reactions to some extent depending on their degradation products. The great diversity of chemical structure available among the β-lactams has resulted in the generation of a large number of hapten-carrier conjugates, which can be recognized by the immunological system[77,78]. The allergic reactions usually appear within a maximum interval of 1 h after drug intake and are mediated by IgE antibodies, while the symptoms are produced by rapid release of histamine and other vasoactive inflammatory mediators immediately after hapten–antibody interaction.

The causative factors for eliciting allergic reactions have been studied extensively for penicillin which undergoes

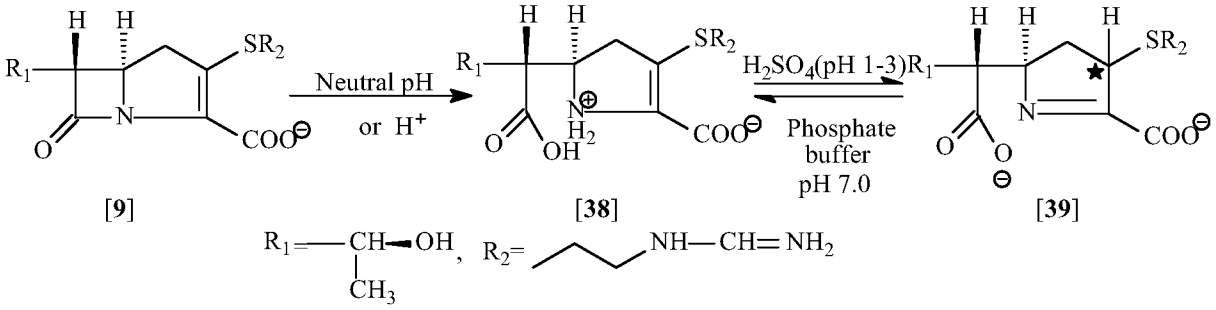


Figure 8. Degradation of imipenem.

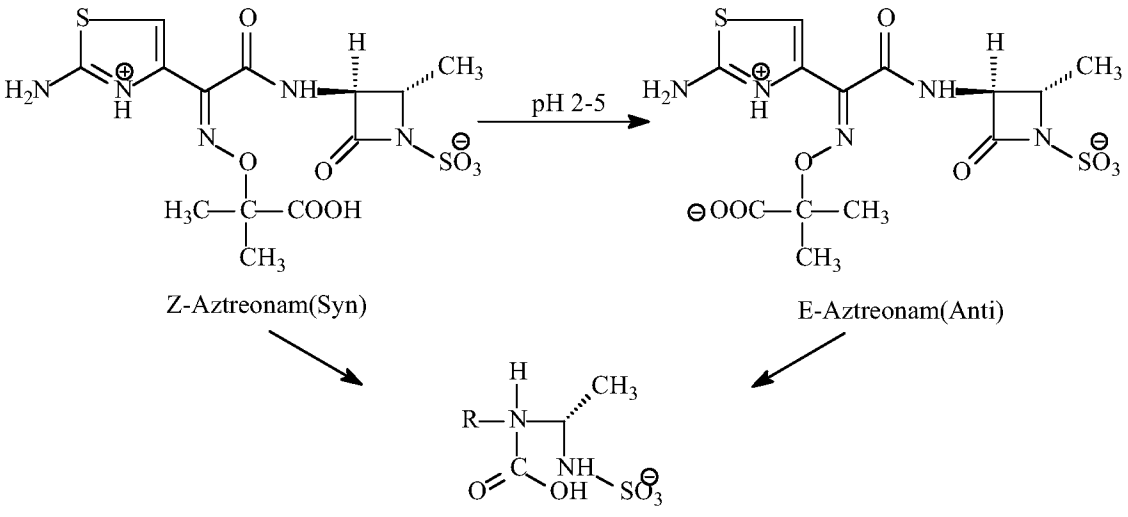


Figure 9. Degradation of aztreonam.

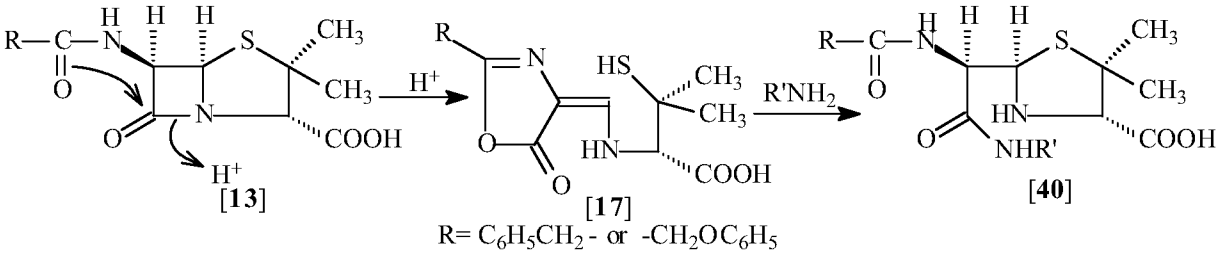
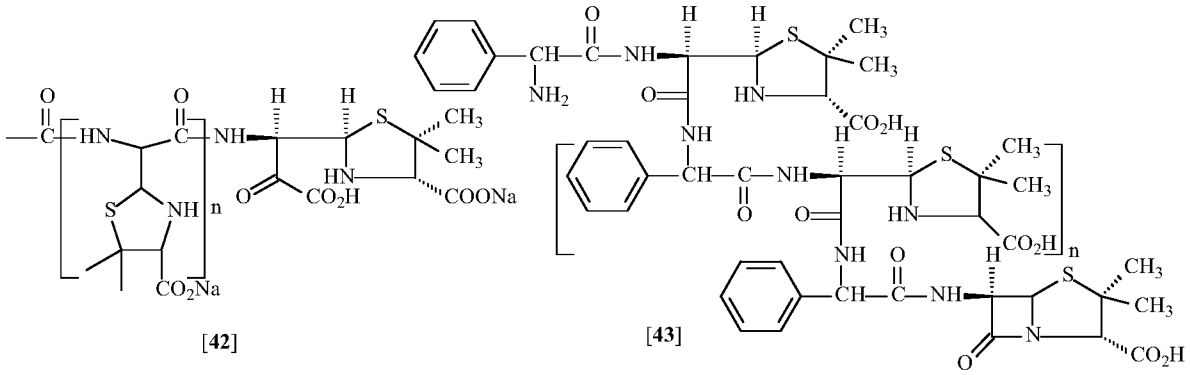


Figure 10. Pathway for formation of haptens of antigens.



in vivo degradation leading to various reactive low molecular weight degradation products and other derivatives like penicillionates, penamaldate, penaldate, penilloaldehyde, penicillenate, penillate and oligomers, which are immunogenic and thus elicit penicillin allergy. These degradation products may bind covalently to macromolecular carriers of the body giving rise to sensitizing conjugates. It has been thought since long that one of the major degradation products of penicillin (**13**), i.e. penicillenic acid[79] (**17**) and some of its derivatives (penicillinates) react with amino group of the protein to form haptens of antigens[80] (**40**) (Figure 10), which are responsible for sensitization[81,82]; this has been implicated as a factor in penicillin allergy.

Another characteristic reaction of penicillin is that it acts as acylating (penicilloylating) agent with reactivity comparable to carboxylic acid anhydrides. This factor leads to many difficulties in isolation, manipulation as well as polymer formation causing allergenicity of the penicillins. This also provides the possible mechanism for their ability to inhibit transpeptidase enzyme. This facet of penicillin chemistry has been exhaustively studied in order to provide a possible mechanism for the penicillin allergenicity due to the formation of irreversible protein conjugates by penicilloylation of free protein functional groups. The formation of high molecular weight polymeric substances during storage solution of potassium benzyl pencillin, i.e. polymeric 6-APA (**42**) and polymeric compound (**43**) arising from aqueous sodium ampicillin solution has been demonstrated by isolation of these polymeric products. These are formed by a chain reaction process involving intermolecular attack of $-NH_2$ group on the β-lactam group of another molecule. It has been observed that these well-defined polymers could be separated by anion exchange chromatography of degraded penicillin solution and are responsible for penicillin allergenicity.

Cephalosporins (although immunochemically as complex as penicillin) possess a less reactive β-lactam ring and therefore the cephalosporyl amide, the equivalent of penicilloyl antigenic determinants is of low stability and may decompose to a penaldate-type derivative[83,84].

Conclusion

The knowledge and understanding of the degradation of β-lactams as well as the nature of their breakdown products under a variety of conditions has not only helped in their isolation, purification and manipulation for therapeutic use, but also provided thrust for searching and developing newer β-lactams possessing increased bioactivity and antibacterial activity towards the future resistant strains. Several methods for quantitative estimation of penicillin have been based on the measurement of colour reaction of their degradation products and are used as well-accepted methods of assay[61,85]. It is also noteworthy that out of an array of penicillin degradation products, penicillamine[86–88] has been used as an established drug for metal poisoning and possesses non-steroidal anti-inflammatory activity.

Extensive study on the mechanism of β-lactam destruction or its inactivation by newly emerging resistant strains has not yet been seriously undertaken. The emergence of active efflux pump inhibitors[89] as a causative factor in combating antibiotic resistance has been observed in several cephalosporins, carbapenems and aztreonam against *P. aeruginosa*. Although third and fourth generation cephalosporins and some non-classical β-lactams, e.g. carbapenem, monobactam, etc. are being widely used in clinics singly or in combination with other classes of compounds for combating increasing antibacterial resistance, work towards their stability, longer duration of action, metabolic degradation and bioavailability has not received enough attention. In-depth investigation towards this direction will be of significant help in designing newer analogues possessing a good overall spectrum of antibacterial activity and less resistance against *P. aeruginosa*, MRSA and/or enterobacteria and in overcoming limitations of the existing β-lactams. Efforts for future development of carbapenems have to address their stability towards 'carbapenemases' that hydrolyse imipenem as well as hydrolytic cleavage catalysed[90,91] by DHP-I.

In addition to these developments, recent progress in designing dual action antibiotics by joining β-lactams with fluoroquinolones[92] through suitable linkages has shown promise in overcoming the problem of β-lactamase resistance produced by a wide range of Gram-negative microorganisms. A few among this class of compounds may soon become clinically useful, as they are found to be more potent and safe, possessing the desired stability. However, with major successes already achieved in addressing the increasing bacterial resistance and potency, it appears that future designing of β-lactams will depend on application of recent revolutionary techniques, e.g. application of genomics in antibacterial target discovery[93,94], combinatorial biosynthesis[95,96] and high throughput screening and/or computer-aided drug design[97,98] based on SAR. Thus, a blend of some of these techniques may lead to a more potent, specific and less allergenic antibacterial agent, i.e. an ideal β-lactam which has been the need of the medical community for treating multi-drug resistance bacterial infection.

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