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*REVIEW ARTICLE*

# β-Lactam Antibiotics: Their Physicochemical Properties and Biological Activities in Relation to Structure

J. P. HOU and J. W. POOLE

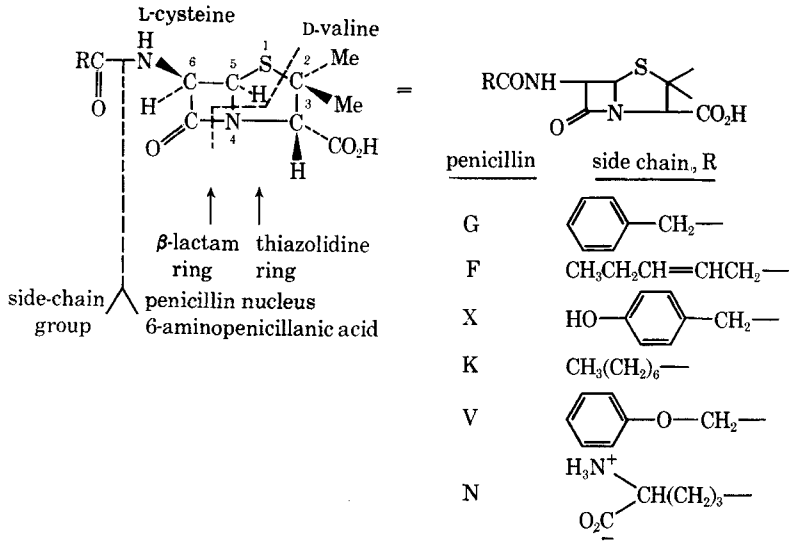
**Keyphrases** ☐ Antibiotics, β-lactams—review ☐ β-Lactam antibiotics—physical, chemical properties ☐ Biological activity–structure relationships—β-lactam antibiotics ☐ Penicillin, cephalosporins, inactivation—bacterial enzymes

CONTENTS



The laboratory and clinical study of antibiotics, particularly the β-lactam-containing group (penicillins and cephalosporins), has been pursued vigorously during the past 30 years. The molecular modification of the naturally occurring penicillins and cephalosporins, either by fermentation or chemical synthesis, has been remarkably successful. The availability of the β-lactam nucleus has made it possible to prepare semisynthetic penicillins and cephalosporins with superior clinical effectiveness. There have been many excellent reviews of these advances, such as those by Abraham (1–4), Doyle and Nayler (5), Van Heyningen (6), Klein and Finland (7), Price *et al.* (8), Price (9), and Smith *et al.* (10). The present review attempts to cover the most significant recent progress made in this very broad field. Special attention has been paid to the physicochemical and biological properties and structure–activity relationships of the clinically important β-lactam drugs. Also discussed are some of the latest developments in the field of bacterial enzymes, particularly the β-lactamases, and their relationships to the β-lactam antibiotics.

Fleming's discovery of penicillin in 1929 (11) and the characterization of the compound by Chain *et al.* (12) and Abraham *et al.* (13) marked the beginning of a new era in chemotherapy. Extensive chemical and physicochemical studies, particularly with the aid of X-ray crystallography, provided an unequivocal demonstration of the fused β-lactam–thiazolidine




*Basic structure of penicillin and the structures of naturally occurring penicillins G, F, X, K, and N and the biosynthetic penicillin V*

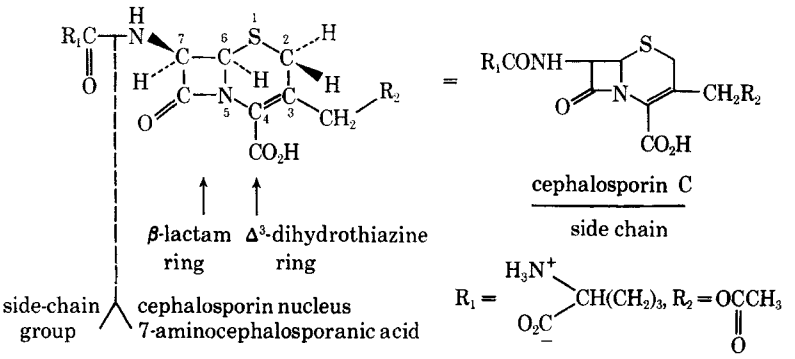
structure of penicillin (14). The penicillin molecule consists of a nucleus and a condensed side-chain group. The nucleus, known as 6-aminopenicillanic acid, is made of two amino acids, L-cysteine and D-valine, twisted together biogenetically into a cyclic dipeptide. The basic structure of the penicillin molecule and of natural and biosynthetic penicillins is shown above.

Since 1945, it has been realized that molds of the species of *Cephalosporium* produce antibiotics possessing some activity against Gram-positive and Gram-negative bacteria. These antibiotics were classified as cephalosporins N, C, and P (1, 2). Cephalosporin N was shown to be identical in structure to synnematin B, isolated by Gottshal *et al.* (15). Due to the identity of its nucleus, cephalosporin N should be called penicillin N (16). Cephalosporin P is known to be a group of tetracyclic compounds of steroid structure (17). Cephalosporin C resembles penicillin in possessing a β-lactam ring, but it is fused with a six-membered dihydrothiazine ring instead of a five-membered thiazolidine ring, and cephalosporin C also has a D-α-aminoadipoyl side chain (18). The full structure of cephalosporin C was elucidated by Abraham and Newton (19) and was confirmed by X-ray crystallographic analysis (20). The basic structure of cephalosporin and cephalosporin C is shown below.

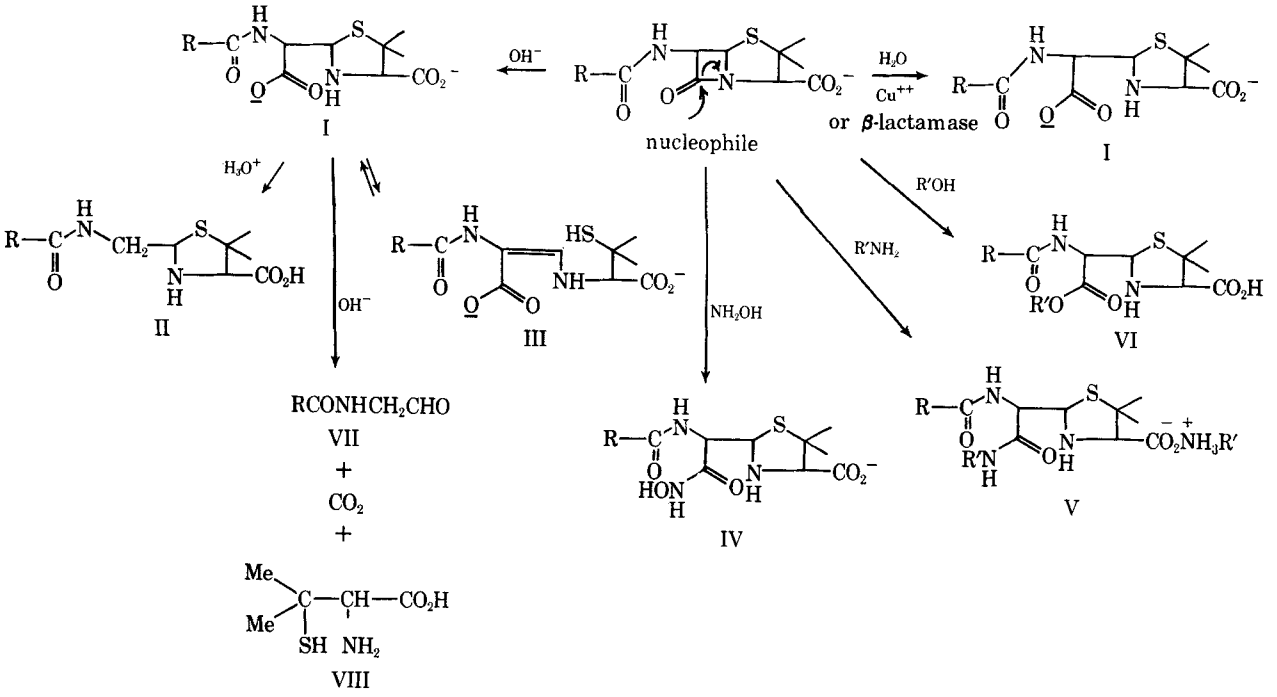
Early attempts to synthesize new penicillins were made to improve clinical usefulness of the antibiotic. Sheehan and his colleagues achieved the β-lactam synthesis (21, 22), which led to the formation of 5-phenylpenicillin (23, 24) and the sulfonyl analog of benzylpenicillin (25). This group also succeeded in the total synthesis of penicillin V (26, 27) and other penicillins (28). In 1959, Batchelor *et al.* (29) were successful in isolating 6-aminopenicillanic acid from penicillin G fermentation liquor. Several other research groups (30–39) independently reported that penicillins G and V could be hydrolyzed into 6-aminopenicillanic acid by amidase in good yield. Nonenzymic conversion of penicillin to 6-aminopenicillanic acid was also reported (40, 41).

Unlike natural penicillins, cephalosporin C and related analogs are relatively resistant to the enzyme penicillinase (42) and are acid stable. Attempts to prepare other cephalosporins by a fermentation process have been unsuccessful. Although the D-α-aminoadipoyl side chain of cephalosporin C can be removed by acid hydrolysis (43) to prepare 7-aminocephalosporanic acid, the yield is very poor. A vast improvement in the synthesis of 7-aminocephalosporanic acid was accomplished by Morin *et al.* (44). Woodward and his associates (45, 46) were successful in the total synthesis of cephalosporins.

The availability of 6-aminopenicillanic acid, 7-aminocephalosporanic acid, and the synthetic techniques has permitted the preparation of numerous new




*Basic structure of cephalosporin and the structure of naturally occurring cephalosporin C*



*Scheme I*

semisynthetic penicillins (47–71) and cephalosporins (72–80), varying in side-chain groups as well as derivatives, which were not previously available through fermentation[1]. Those of clinical importance have been listed in Tables I and II, respectively, with their names, side-chain structures, and special activities and properties.

## PHYSICAL AND CHEMICAL PROPERTIES

**Degradation Reactions in Solution**—The bicyclic β-lactam–thiazolidine structure is more sensitive than the simple β-lactam structure to nucleophiles, electrophiles, oxidizing agents, and even water molecules (14, 81). The initial β-lactam cleavage has been shown to be responsible for many succeeding degradation reactions of penicillins in solution. It has been proposed that the nonplanarity of the fused penicillin nucleus may cause a great deal of suppression of the usual amide resonance as compared with that caused by the dipolar stabilized forms in the normal β-lactam structure (81).

*Nucleophilic Attack Reactions*—All penicillins are extremely susceptible to nucleophilic attack (Scheme I) by hydroxyl ions, primary and secondary amines, *etc.* The initially hydrolyzed product of penicillin is biologically inactive penicilloic acid (I). Penicilloic acid is stable in the form of salts or esters in neutral solutions, but on acidification it readily loses one molecule of carbon dioxide, giving the corresponding penilloic acid (II) (81, 82). Under drastic conditions, penicilloic acid may transfer to its tautomeric penamaldic acid (III) (83). Hydroxylamine reacts with penicillins quantitatively to form hydroxamic acid (IV). Similarly, penicillins interact readily with two molecules of amines, such as the alkylamine, to form corresponding alkylammonium penicilloic alkylamide (V). Primary alcohols (81), sugars, carbohydrates, and glycols at a neutral pH (84) react with penicillins to form monoesters of α-penicilloate (VI). Penicillin β-lactamases (3, 4) and metal ions, such as copper (85), are powerful catalysts to hydrolyze penicillins to penicilloic acids or a penicilloyl–copper-ion complex. The end hydrolytic degradation products of penicillins are penicilloaldehyde (VII), carbon dioxide, and a sulfur-containing amino acid, penicillamine (VIII) (81).

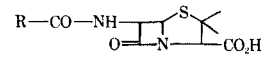
Cephalosporins are readily attacked similarly by nucleophilic reagents and cephalosporin β-lactamases (2, 6). The initial degradation product corresponding to penicilloic acid, however, is not stable and is readily fragmented in aqueous solution (86, 87).

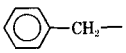
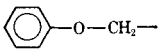
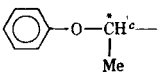
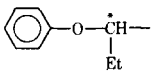
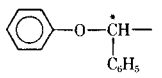
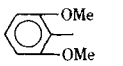
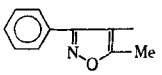
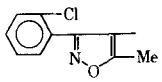
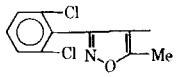
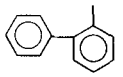
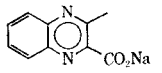
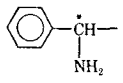
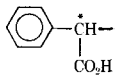
*Electrophilic Attack Reactions*—Penicillins are also sensitive to electrophilic attack both at the β-lactam nitrogen and the sulfur atom (Scheme II). In strong acid, penicillins are isomerized to penillic acid (IX) by a mechanism involving the transient oxazolone structure (X) (81). Benzylpenillic acid has a typical UV absorption band near 228 nm. (88). In weak acid or in neutral solution, penicillins inevitably undergo transformation to penicillenic acid (XI), which possesses a characteristic UV band near 320 nm. regardless of the type of penicillin used as the source (89, 90). Penicillenic acid is very unstable; it quickly isomerizes to either penicilloic or penillic acid, depending on the pH of the solution (90, 91). Metal ions are able to attack the sulfur atom of the thiazolidine ring, as is shown by the degradation of benzylpenicillin in neutral solution by $HgCl_2$ (90).

Evidently, the side-chain group of penicillins is directly involved in the penillic acid–penicillin transformation reactions. Substitution with a powerful electron-attracting group somewhere in the side chain, particularly in the α-position of benzylpenicillin, has been shown to stabilize the penicillin markedly in the acid solution (92, 93). Positive linear correlations

[1] Reports on the preparation of semisynthetic penicillins and cephalosporins are voluminous; only those for the preparation of compounds listed in Tables I, II, and XII and some of their derivatives are cited.

**Table I**—Name, Structure, Special Activity, and Properties of Several Clinically Useful Penicillins



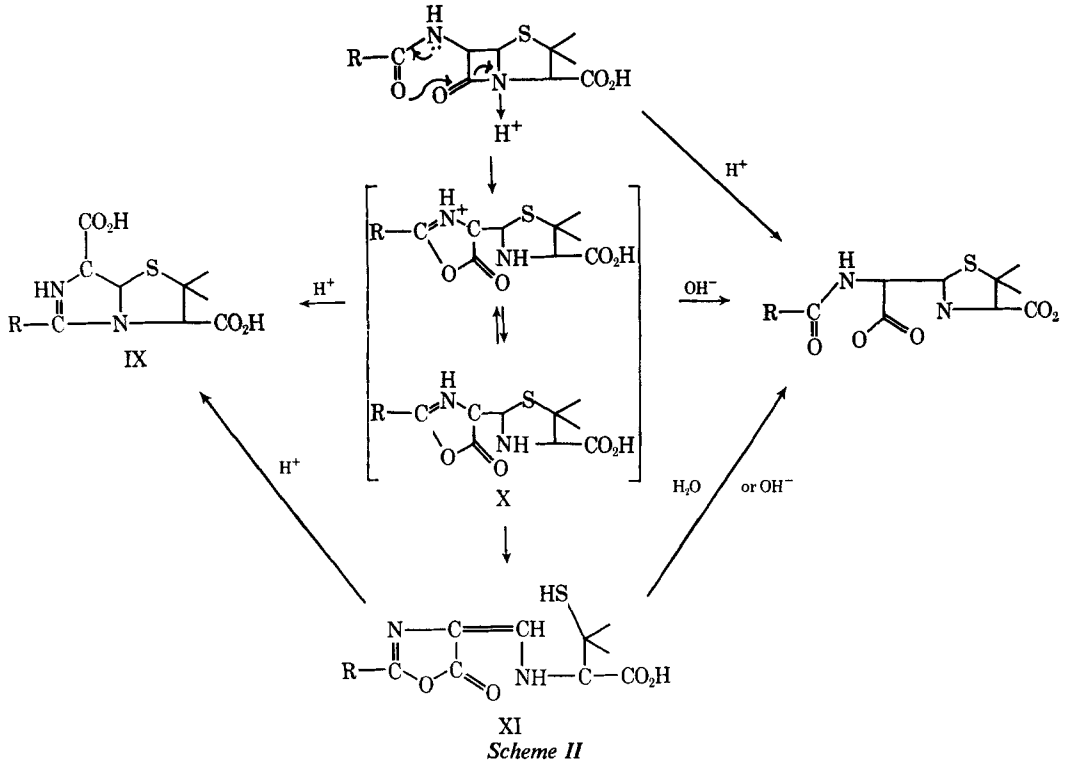
| Nonproprietary Name | Chemical Name | Structure[a], R | Activity and Properties[b] |
|---|---|---|---|
| Penicillin G | Benzylpenicillin |  | G(+)-effective, very acid unstable, penicillin β-lactamase sensitive |
| Penicillin V | Phenoxymethylpenicillin |  | |
| Phenethicillin | Phenoxyethylpenicillin |  | G(+)-effective, acid stable, sensitive to penicillin β-lactamase, well absorbed |
| Propicillin | Phenoxypropylpenicillin |  | |
| Phenbenicillin | Phenoxybenzylpenicillin |  | |
| Methicillin | 2,6-Dimethoxyphenylpenicillin |  | G(+)-effective, acid unstable, resistant to some penicillin β-lactamase |
| Oxacillin | 3-Phenyl-5-methyl-4-isoxazolylpenicillin |  | |
| Cloxacillin | 3-*o*-Chlorophenyl-5-methyl-4-isoxazolylpenicillin |  | |
| Dicloxacillin | 3-*o*,*o*-Dichlorophenyl-5-methyl-4-isoxazolylpenicillin |  | G(+)-effective, acid stable, resistant to most β-lactamase, highly serum bound, orally absorbed |
| Nafcillin | 2-Ethoxy-1-naphthylpenicillin |  | |
| Diphenicillin | 2-Biphenylpenicillin |  | |
| Quinacillin | 3-Carboxy-2-quinoxalinylpenicillin |  | |
| Ampicillin | D-α-(−)-Aminobenzylpenicillin |  | Broad spectrum, very acid stable, penicillin β-lactamase sensitive, well absorbed |
| Carbenicillin | α-Carboxybenzylpenicillin |  | Broad spectrum and *Ps. aeruginosa* effective, very acid unstable, orally ineffective |

[a] Penicillins are available in soluble or sparingly soluble salts or in acidic or zwitterionic forms. [b] G(+) and G(−) represent Gram-positive and Gram-negative organisms; broad spectrum means effective against both Gram-positive and Gram-negative organisms; β-lactamases refer to the β-lactam-inactivating enzymes from both Gram-positive and Gram-negative bacteria; penicillin β-lactamase refers to the enzyme preferentially attacking susceptible penicillins (*References 3, 7, 10*). [c] Penicillins carrying asymmetric carbon atom in the side chain (shown by asterisk) are stereospecific.

of the acid stability of the corresponding penicillins with the acidity of the side-chain acids were obtained. For example, substitution of the α-hydrogen atom with the amino or halogen atom in benzylpenicillin (92), or into the benzene ring in methicillin (94) or in phenylpenicillin (95), has been shown to stabilize the substituted penicillins drastically.

Cephalosporins are relatively stable in acid and do not undergo the penicillin–penillic acid type of rearrangement even when they have the same side chain as the penicillins (3). This is probably due to the lower anionoid reactivity of the nitrogen in the dihydrothiazine ring (2, 3).

**Reactions of the 3-Carboxyl Group in Penicillins**—In general, the penicillin molecule is a fairly strong organic acid with a pKa value of about 2.6–2.7 in water (96, 97). The C-3 carboxyl group rapidly forms crystalline soluble (inorganic) and sparingly soluble (organic) salts or esters (98). Sodium, potassium, and calcium salts are the most useful soluble salts, while procaine (99), benzathine (100–103), benethamine (104), and dehydroabietylethylenediamine (hydrabamine) (105, 106) salts are the most commonly used organic salts. Similarly, various sparingly soluble penicillin esters (107, 108), amides (109–111), and other derivatives such as methyl alcohol (112), nitrile (113),



*Scheme II*

and thiopenicillanic acid (114) have also been prepared by substitution at C-3 in place of the carboxyl group.

**Reactions of 3-Acetoxy and 4-Carboxyl Groups in Cephalosporins**—The most reactive group in cephalosporins is the 3-acetoxy group. The chemistry of cephalosporin C, as elucidated by Abraham and Newton (19, 115), has greatly aided later workers in the area of the semisynthetic cephalosporins.

Scheme III illustrates the general reactions of the cephalosporins. Treatment of cephalosporin C with dilute acid gives not only a small amount of 7-aminocephalosporanic acid but also 7-aminocephalosporanic acid lactone (XII) and cephalosporin C lactone, known as cephalosporin $C_e$ (XIII) (19, 43, 116). With the treatment of cephalosporin C with acetyl esterase from orange peel, or under mild acid hydrolysis, the acetoxy group is cleaved without lactonization and the corresponding 3-methyl alcohol (XIV) is obtained (116). This desacetylcephalosporin C is not readily acylated but, instead, is lactonized under acid and anhydrous conditions. In basic solution and by reacting with aromatic acid chloride, however, cyclization and esterification take place (XV) (73). Similarly, 3-*O*-carbamoyldesacetylcephalosporanic acid (XVI) is formed in a basic medium through a reaction with isocyanate (6).

Cephalosporin C absorbs hydrogen; if the hydrogenation is carried out in the presence of palladium on charcoal, the product will be desacetoxycephalosporin C (XVII) (19, 86, 116). Stedman *et al.* (117, 118)

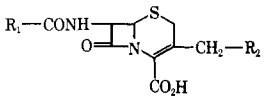
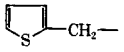
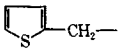
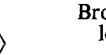
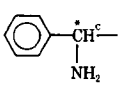
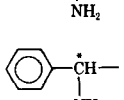
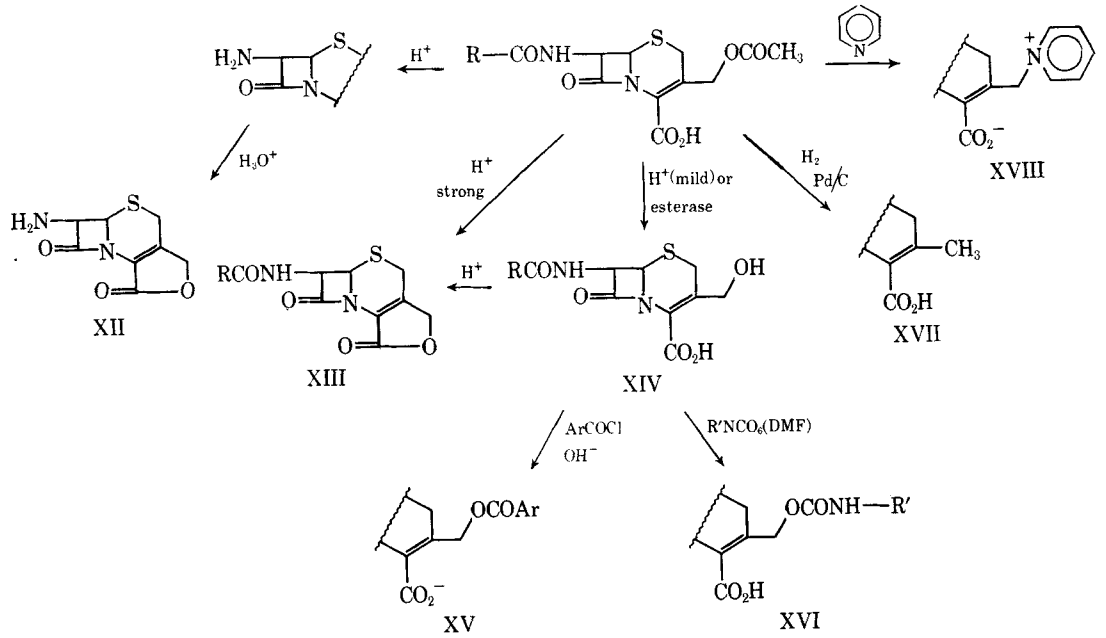


**Table II**—Name, Structure, Special Activity, and Properties of Several Clinically Useful Cephalosporins

| Nonproprietary Name | Chemical Name | Structure[a] $R_1$ | Structure[a] $R_2$ | Activity and Properties[b] |
|---|---|---|---|---|
| Cephalothin | 7-(Thiophene-2-acetamido)-cephalosporanic acid |  | $-OCOCH_3$ | Broad spectrum, poorly absorbed |
| Cephaloridine | 7-(2-Thienyl)acetamido-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine |  |  | Broad spectrum, poorly absorbed, less serum bound |
| Cephaloglycin | 7-(D-α-Aminophenylacetamido)-cephalosporanic acid |  | $-OCOCH_3$ | Broad spectrum, well absorbed |
| Cephalexin | 7-(D-α-Amino-α-phenyl-acetamido)-3-methyl-3-cephem-4-carboxylic acid |  | $-H$ | Broad spectrum, well absorbed |

[a] Most of the cephalosporins are available in soluble salts or in zwitterions. [b] Broad spectrum means effective against both Gram-positive and Gram-negative organisms. Cephalosporins are usually penicillin β-lactamase resistant but very susceptible toward cephalosporin β-lactamases (*References 1–3, 6, 10*). [c] Cephalosporins carrying asymmetric carbon atom (shown by asterisk) are stereospecific.



*Scheme III*

showed that this reaction can be carried out at low pressure by using a large amount of palladium as the catalyst.

In neutral solution, cephalosporin C reacts with pyridine to form cephalosporin $C_A$ (XVIII) and also becomes quaternized (119). It is now known that any agent of greater nucleophilicity than oxygen will displace the acetoxy group (6). Nucleophiles such as bivalent sulfur compounds, tertiary amines, azides, thiourea, thiocarbamates, xanthates, and pyridines have been used (74, 75, 119–121). The kinetics of the displacement reactions (122) have been reported. Cephalosporins such as cephalothin (123) and cephaloglycin (124) have been shown to have essentially the same chemical properties as cephalosporin C.

The 4-carboxyl group in cephalosporin, as in penicillin, can be modified to esters or amides. Many esters and amides of cephalothin (125) have been prepared, all of which have been shown to be much less active than the parent compound.

**Reactions of 6-Aminopenicillanic Acid**—Since 6-aminopenicillanic acid contains no side chain, it cannot undergo intramolecular rearrangement to form a penillic acid type of product. It is, therefore, relatively stable toward acids (29). However, 6-aminopenicillanic acid is readily attacked by carbon dioxide, to which penicillins are inert. The carbonation product of 6-aminopenicillanic acid is biologically inactive, having the structure of XIX (126, 127).

Grant *et al.* (128) showed that in neutral pH and at room temperature, a concentrated aqueous solution of 6-aminopenicillanic acid gave a high molecular weight polymer of poly-6-aminopenicillanic acid (XX). Such a product showed little antistaphylococcal activity.

Another interesting reaction of 6-aminopenicillanic acid is that of the 6-amino group with the hydroxyl group of a reducing sugar at room temperature and neutral pH, giving derivatives of *N*-glycosyl-6-aminopenicillanic acid (XXI). Though sodium salts of the glycosyl, maltoxyl, and lactosyl derivatives of 6-aminopenicillanic acid are stable toward both staphylococcal and bacillary $\beta$-lactamases, the antibacterial activity of these derivatives is much lower than that of 6-aminopenicillanic acid (129).

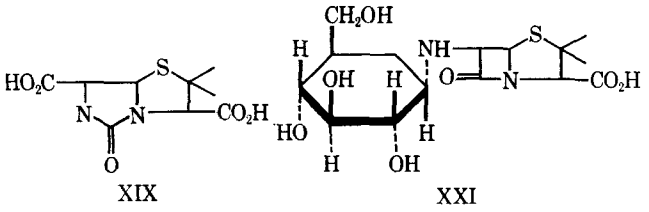
**Rate of Degradation in Solution and Stabilization**—*Kinetics*—The instability of penicillin in aqueous solution has been known since the time of its discovery. Abraham and Chain (130) were the first to report that the maximum stability range of barium penicillin in solution is between pH 5.5 and 7.5. Other workers (131–134) also investigated the decomposition of natural penicillins in aqueous media. They found that the destruction of penicillin in aqueous solution is a first-order irreversible reaction.

Brodersen (135–138) indicated that the rate of inactivation of penicillin G is largely dependent on hydrogen-ion concentration and temperature. The molecular and the ionized forms of penicillin interact at different rates with hydrogen ion. Within a pH range of 1–10 and at a total ionic strength of 0.5 at 30°, the rate of degradation of penicillin G follows pseudo-first-order kinetics and the pH of minimum degradation is at 6.5. The degradation reactions observe Arrhenius' law, and the apparent energies of activation vary with pH (17.57, 20.98, and 9.8 kcal./mole at a pH of 1.20, 4.54, and 9.57, respectively) but are independent of temperature (136–138).

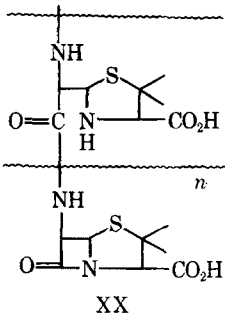
The rate constant for the overall degradation of penicillin G and the rate of formation of penicillenic acid were reported by Krejci (139).

Swintosky *et al.* (140, 141) demonstrated that the degradation of a procaine–penicillin G suspension is of zero order, since only the material in solution degrades, and that the decreased solubility could be utilized to stabilize the penicillins.

The degradation kinetics of phenethicillin in a pH range of 1–11.4, with a constant ionic strength of 0.5 and at 35°, was reported by Schwartz *et al.* (142). The apparent pH of minimum degradation occurred



XIX XXI



XX

at about 6.5. At a pH of 6.7, the energy of activation was 17.6 kcal./mole. The half-life of potassium phenethicillin at a pH of 1.5 and 35° is about 1 hr., in comparison with about 4 min. for penicillin G. In the acid region, the phenethicillin anion was shown to undergo degradation about 13 times faster than the molecular form.

Methicillin is very unstable in acid (143, 144), and its decomposition is roughly 2–3 times faster than that of phenethicillin. The reactions, in general, are very dependent on pH and have a much narrower stability zone near a pH of 6.5.

Finholt *et al.* (145) were the first to investigate the effect of buffer on the degradation of penicillin G. They found that the dihydrogen citrate ion, the monohydrogen phosphate ion, and the borate ion imposed significant catalytic effects on the penicillin ion, while acetic acid, the acetate ion, the monohydrogen citrate ion, the unprotonated citrate ion, the dihydrogen phosphate ion, and boric acid were noncatalytic or nearly so. No primary salt effect was observed at a pH of 4.5, but a significant salt effect was shown at 6.8 and 8.75. The log *k*–pH profile at zero buffer concentration gave a pH of minimum degradation at 6.65 at 60°. The penicillin G degradation due to nucleophilic attack is about 5000 times faster than in acid solution.

Stability studies on oxacillin and penicillin V in aqueous solution at several pH values and temperatures were reported by Kondrat'eva and Bruns (146). These penicillins are most stable at a pH of 6.0–7.0.

Saccani and Pansera (147) recently reported the apparent stabilities of ampicillin and hetacillin at 27° in several buffers (without controlling the ionic strength). The pH of minimum degradation occurred at 4.4 for both ampicillin and hetacillin.

Hou and Poole (148) investigated the kinetics of degradation of ampicillin in several buffer systems of pH 0.8–10, with a constant total ionic strength of 0.5 and at several temperatures. The observed rates were shown to follow pseudo-first-order kinetics and were significantly affected by general acid–base catalysis, particularly by the polycharged phosphate ions. At pH 4.94, no primary salt effect was observed in buffers of widely varied ionic strength. In 0.08 *N* HCl solution, however, the apparent rates were increased with the ionic strength but decreased with the dielectric constant of the solvent. The pH of minimum degradation (pH minimum) occurred at 4.85, which is the isoelectric point of ampicillin under the conditions used. In other words, the zwitterionic form of the ampicillin molecule is the most stable species toward both acids and bases. At zero buffer concentrations, however, the pH minimum shifts to a neutral value of 5.85, and degradation at this pH is mainly due to a spontaneous reaction. Inactivation of ampicillin in base is about 1400 times faster than in acid. The apparent energies of activation for the degradation of ampicillin are 16.4, 18.3, and 9.2 kcal./mole in buffers of pH 1.35, 4.93, and 9.78, respectively.

*Kinetic Assay Procedures*—The rate of degradation of a penicillin in solution can be determined by measuring the change in the amount of intact penicillin or, alternatively, by determining the amount of degradation product, either biologically or chemically (149, 150). Since the reaction mechanism of penicillins is complex, the quantitative determination of the initial degradation product (penicillenic or penicilloic acid) is most difficult. On the other hand, the microbiological, hydroxylamine (151), and iodometric (152–154) methods can rather accurately measure the residual intact penicillin and have been widely used.

Other methods, such as the polarimetric, spectrophotometric, manometric, acidimetric, or alkalinetitrimetric, are also available (150). These procedures have been chiefly used in studies on penicillinase.

*Stabilization of Penicillins*—The β-lactam antibiotics can be classified into the monobasic and the zwitterionic (ampicillin, cyclacillin, cephaloglycin, *etc.*). The optimum pH of stability for the former lies between 6 and 7; for the latter, it apparently coincides with the isoelectric point of the antibiotic. Accordingly, the pH of maximum stability of the zwitterionic antibiotics is at 4.85 for ampicillin (148, 155), 5.0 for cyclacillin (96), and 4.5 for cephaloglycin (72).

To date, no agent has been found to preserve or stabilize the β-lactam ring. However, numerous reports show that the degradation of penicillin in solution can be retarded in a particular type of buffer system or in the presence of certain chemical additives (156). Citric acid–citrate buffer has less catalyzing effect than phosphate buffer of similar buffer concentration (157–160). A combined citric–phosphate buffer is superior for the stabilization of ampicillin and hetacillin (147, 148).

The penicillins, cephalosporins, and their salts all have a strong tendency to form crystalline hydrates. For example, ampicillin (anhydrate) transforms to the long-needle crystalline trihydrate in a cold acidic solution (161). Grant and Alburn (162) reported that ampicillin monohydrate is less stable than its anhydrate analog when exposed to high humidity and elevated temperature. Similarly, ampicillin (anhydrate) has been shown to be more stable than ampicillin trihydrate in pharmaceutical preparations (163). On the other hand, sodium nafcillin monohydrate is more stable than its anhydrate in air (163). Normally, the anhydrous salt absorbs moisture relatively faster than the crystalline hydrate form. Accordingly, it was shown that sodium

Table III—Equilibrium Solubility (Milligrams per Milliliter) of β-Lactam Antibiotics and Their Salts in Several Solvents at Room Temperature[a]

| Compound | Solvent | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Water | Methanol | Ethyl Alcohol | Isopropanol | Acetone | *p*-Dioxane | Ethyl Ether | Chloroform | Carbon Tetrachloride | Ethyl Acetate | Isooctane | Pyridine | Carbon Disulfide |
| Sodium penicillin G | >20 | >20 | 10 | 0.75 | 0.19 | 1.9 | 0.06 | 0.05 | 0.042 | 0.4 | 0.03 | 1.15 | 0.08 |
| Potassium penicillin V | >20 | >20 | 1.35 | 0.11 | 0.22 | 0.56 | 0.84 | 0.45 | 0.04 | 0.65 | 0 | 0.13 | 0.07 |
| Penicillin V | 0.9 | >20 | >20 | >20 | >20 | 12.6 | 11.75 | >20 | 0.1 | >20 | 0.04 | >20 | 0.3 |
| Sodium methicillin | >20 | >20 | >20 | >6.54 | 0.26 | 0.52 | 0.02 | 0.61 | 0 | 0.05 | 0.22 | >20 | 0.04 |
| Sodium oxacillin | >20 | >20 | 11.7 | 3.58 | 0.16 | 0.66 | 0.01 | 0.05 | 0 | 0.03 | 0 | >20 | 0.02 |
| Sodium cloxacillin | >20 | >20 | >20 | 9.2 | 2.72 | 4.22 | 0.08 | 1.82 | 0.01 | 0.6 | 0 | >20 | 0.06 |
| Sodium dicloxacillin | >20 | >20 | >20 | 9.5 | 13.44 | 4.57 | 0.09 | 0.54 | 0 | 1.4 | 0 | 6.38 | 0.02 |
| Sodium nafcillin | >20 | >20 | >20 | >20 | 20 | 20 | 0.41 | >20 | 0 | >20 | 0 | >20 | 0.06 |
| Procaine penicillin G | 6.8 | >20 | >20 | 6.5 | 14.95 | 9.8 | 0.6 | >20 | 0.12 | 3.35 | 0 | >20 | 0.51 |
| Benzathine penicillin G | 0.32 | 16.9 | 15.4 | 3.65 | 3.0 | 2.4 | 0.4 | 2.1 | 0.57 | 1.2 | 0.09 | >20 | 0.5 |
| Benzathine penicillin V | 0.32 | >20 | 14.6 | 7.15 | >20 | >20 | 1.2 | 20 | 0.73 | 9.0 | 0.2 | >20 | 0.55 |
| Hydrabamine penicillin G | 0.075 | 7.3 | 5.2 | 1.7 | 3.4 | 14.65 | 0.7 | >20 | 0.5 | 1.65 | 0.5 | >20 | 1.4 |
| Ampicillin (anhydrous) | 10.1 | 2.97 | 0.39 | 0.05 | 0.12 | 0.60 | 0.02 | 0.09 | 0.01 | 0.025 | 0 | 2.1 | 0.015 |
| Ampicillin (trihydrate) | 7.5 | 6.65 | 2.54 | — | 8.95 | 2.77 | 0.03 | 0.07 | 0.025 | 0.225 | 0.02 | 12.13 | 0.02 |
| Cephaloridine | >20 | 2.91 | 1.95 | 0.21 | 0.18 | 0.46 | 0.01 | 0.24 | 0.05 | 0.04 | 0.05 | 0.74 | 0.02 |
| Sodium cephalothin | >20 | 11.93 | 1.77 | 0.08 | 0.06 | 0.52 | | 0.07 | 0.02 | 0.02 | 0.03 | 0.10 | 0.01 |

[a] Data from *References 165–167.*

oxacillin monohydrate and sodium methicillin monohydrate tended to absorb moisture much more slowly than the anhydrous sodium salts (164).

**Solubility Characteristics**—Nearly all sodium and potassium salts of penicillins and cephalosporins are: freely soluble or highly soluble (>20 mg./ml.) in water, 0.1 *N* NaOH, the lower alcohols, dimethylformamide, and formamide (some are also soluble in pyridine); sparingly soluble (1–20 mg./ml.) to slightly soluble (0.1–1 mg./ml.) in the higher alcohols, acetone, and ethyl acetate; and practically insoluble (<0.1 mg./ml.) in isooctane, cyclohexane, benzene, diethyl ether, petroleum ether, chloroform, carbon disulfide, and carbon tetrachloride. The amine salts, esters, and the free acid forms of the monobasic penicillins are generally more soluble in nonpolar than in polar solvents (165, 166).

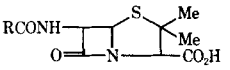
On the other hand, penicillin and cephalosporin zwitterions are freely soluble in formamide and dimethylformamide, slightly soluble in water or other polar solvents, and practically insoluble in nonpolar solvents such as ether or carbon tetrachloride (165–167). Since the zwitterionic penicillins and cephalosporins carry both ionic and nonpolar groupings, they behave like both ions and organic molecules; which is dominant depends on structural and environmental factors. Because of the dual functionality, significant effects of pH, salt, dielectric constant (96), and temperature (168) on the aqueous solubility of ampicillin have been observed.

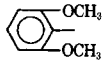
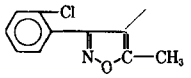
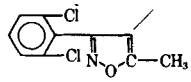
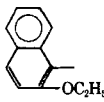
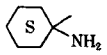
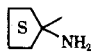
Penicillin salts were shown to be significantly more soluble in a nonpolar solvent in the presence of a small amount of water. Similarly, the water of crystallization in penicillin hydrates may help to increase the solubility in a nonpolar solvent. Ampicillin trihydrate is more soluble than its corresponding anhydrate in several organic solvents such as acetone, ethyl acetate, dioxane, and ether. Table III shows the solubilities of several penicillins, cephalosporins, and their salts in various commonly used solvents at room temperature.

**Acid Dissociation Constants**—Caution is required in determining the dissociation constants of penicillins and cephalosporins by conventional potentiometric titration methods. The difficulty arises from the fact that most of the monobasic penicillins precipitate when the pH drops or when a sufficient amount of penicillin-free acid is formed. This is particularly true for those penicillins with a bulky side-chain group. The problem is not usually encountered with amphoteric penicillins or cephalosporins.

The apparent pKa value of the carboxyl group of all penicillins ranges from 2.6–2.8 in water at 25° (88, 96, 97). The fairly strong acidity is believed to be due to the adjacent strong electronegative groups, namely the *S*-atom and β-lactam peptide linkage (88). The peptide and other groups in the side chain, because of the wide separation, probably do not contribute any inductive effect. Accordingly, the (nucleus) carboxyl group of all penicillins (except 6-aminopenicillanic acid) (97) has essentially the same acid dissociation constant (pKa) regardless of the side-chain group. On the other hand, penicillins carrying one or more ionizable groups in the side chain usually give rather varied pK

**Table IV**—Apparent Acid Dissociation Constants of Penicillins in Water



| Penicillin | Side Chain, R | Temperature | pKa | | References |
|---|---|---|---|---|---|
| | | | $pK_1$, $COO^-$ | $pK_2$, $NH_3^+$ | |
| Penicillin G | $C_6H_5CH_2$— | 25° | 2.76 | — | 88 |
| | | 25° | 2.72 | — | 97 |
| | | 23° | 2.71, 2.73 | — | 88 |
| Penicillin X | *p*-$HOC_6H_5CH_2$— | 23° | 2.62 | — | 88 |
| *p*-Heptylpenicillin | $C_7H_{13}C_6H_5$— | 23° | 2.66 | — | 88 |
| Penicillin V | $C_6H_5OCH_2$— | 25° | 2.73, 2.74 | — | 97 |
| Phenethicillin | $C_6H_5OCH(CH_3)$— | 25° | 2.72, 2.74 | — | 97 |
| Propicillin | $C_6H_5OCH(C_2H_5)$ | 25° | 2.72, 2.72 | — | 97 |
| Methicillin |  | 25° | 2.76, 2.78 | — | 97 |
| Cloxacillin |  | 25° | 2.73, 2.70 | — | 97 |
| Dicloxacillin |  | 25° | 2.67 | — | 96 |
| Nafcillin |  | 25° | 2.65 | — | 96 |
| *p*-Aminobenzylpenicillin | *p*-$NH_2$-$C_6H_5$-$CH_2$—$C_6H_5CH$(—$NH_2$)— | — | 2.6 | 4.6 | 169 |
| Ampicillin | | 25° | 2.66, 2.64 | 7.25, 7.24 | 96 |
| | | | 2.52, 2.53 | 7.24, 7.25 | 97 |
| Cyclacillin (WY-4508) |  | 25° | 2.68 | 7.50 | 96 |
| WY-7953 |  | 25° | 2.62, 2.61 | 7.60, 7.62 | 96 |
| 6-Aminopenicillanic acid | | 25° | 2.29, 2.30 | 4.90, 4.92 | 97 |

values as a result of the inductive effect of the adjacent groups (96). This is true, for instance, in the case of amphoteric penicillins such as penicillin N (16), ampicillin, cyclacillin, BL-P875, and 6-aminopenicillanic acid (97) but not *p*-aminobenzylpenicillin (169).

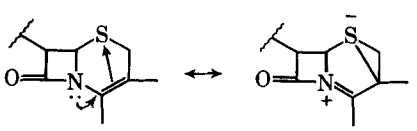
In the case of ampicillin (96), a change in the dielectric constant of the titration medium affects the $pK_1$ (carboxyl group) more than the $pK_2$ (amino group); a change in temperature exerts the opposite effect. The formation of ampicillin zwitterion from its uncharged species is an exothermic reaction, with a heat of formation of about 10 kcal./mole.

Tables IV and V list the acid dissociation constants of several penicillins and cephalosporins, respectively, in water and/or partially aqueous solvent at 25°.

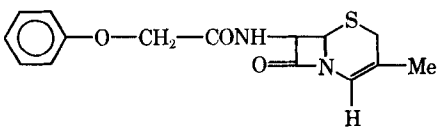
Because of the leveling effect, the acidic groups of penicillins and cephalosporins can be titrated in a nonaqueous or partially aqueous solvent. The pK value of penicillin G is increased by 2 pK units when the titration is carried out in 80% ethanol (88). Similarly, the $pK_1$ values of ampicillin and cyclacillin are increased by about 1.5 units, while the $pK_2$ values are decreased by 0.27 (ampicillin) and 0.46 (cyclacillin) in 50% *p*-dioxane (96). By varying the amount of the organic solvent, the pKa value in water for certain insoluble or sparingly soluble penicillins can be obtained by extrapolation. The pK values of nafcillin and dicloxacillin, thus obtained, were shown to be essentially identical to those of other penicillins determined in water (96).

**Other Physicochemical Properties**—*UV Absorption*—Pure 6-aminopenicillanic acid and cyclacillin, because they lack a chromophore group, have no absorption band in the UV region. Penicillin G, like other monoaromatic penicillins, has a weak end-absorption in water (88), with $\lambda_{max.}$ at 264 ($\epsilon$, 173, l/m./cm.), 257.5 ($\epsilon$, 256), 252 ($\epsilon$, 255), and 257 nm. ($\epsilon$, 285) (96). These bands are obviously typical absorption bands of the benzyl group. Sodium nafcillin, however, absorbs strongly in water at 332, 292, 280 ($\epsilon$, 5680), and 227 nm. ($\epsilon$, 6100). Clearly, these bands are from the conjugated naphthalene group.

Cephalosporins and their salts give a typical UV absorption band near 260 nm. in aqueous solution,



XXII



XXIII

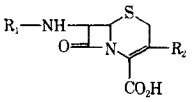
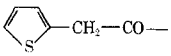
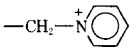
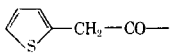


**Table V**—Acid Dissociation Constants of Cephalosporins at Room Temperature

| Cephalosporin | $R_1$ | $R_2$ | pKa: Side Chain $COO^-$ | pKa: Nucleus $COO^-$ | pKa: Side Chain $NH_3^+$ | References |
|---|---|---|---|---|---|---|
| Cephalosporin C | $H_3N^+—CH(CH_2)_3—CO—$ <br> $O_2C^-$ | $—CH_2OCOCH_3$ | <2.6 | 3.1 | 9.8 | 2 |
| Desacetyl-cephalosporin C | $H_3N^+—C(CH_2)_3—CO—$ <br> $O_2C^-$ | $—CH_2OH$ | <2.5 | 3.0 | 9.7 | 116 |
| D-Cephaloglycin | $C_6H_5—CH(NH_3^+)—CO—$ | $—CH_2OCOCH_3$ | — | 4.4 | 7.1 | 78 |
| L-Cephaloglycin | $C_6H_5—CH(NH_3^+)—CO—$ | $—CH_2OCOCH_3$ | — | 4.6 | 7.1 | 78 |
| | $C_6H_5—CH(NH_3^+)—CO—$ | $—CH_2OH$ | — | 4.9 | 7.6 | 124 |
| Cephaloridine |  |  | — | 3.4 | — | 75 |
| |  | $—CH_2SC(=S)—alkyl$ | — | 5.0 | — | 74 |
| | H | —H | — | 2.38 | 4.79 | 118 |
| 7-Aminocephalosporanic acid | H | $—CH_2OCOCH_3$ | — | 1.75 | 4.63 | 118 |

which is claimed to be due to the O=C—N—C=C— linkage (72), with possible participation of the nucleus sulfur atom (170), as shown in XXII. The 4-carboxyl group is not an essential part of the cephalosporin chromophore, since descarboxylated compounds (XXIII) also have a $\lambda_{max.}$ at 256 nm. (80). This particular band is dependent on the integrity of the β-lactam ring as well as on the C-3 and C-4 double-bond conjugation in the $\Delta^3$-cephalosporin system (125). The $\lambda_{max.}$ at 260 nm. for cephalosporin C and at 267 nm. for desacetylcephalosporin C disappears upon alkaline hydrolysis (86). New semisynthetic cephalosporins such as cephalothin (72, 73), cephaloglycin (78), desacetylcephaloglycin (124), and desacetylcephalothin methyl ester (123), as well as other cephalosporin derivatives (79), all have a typical $\lambda_{max.}$ at or near 260 nm. Cephaloridine and many pyridine-substituted cephaloridines, however, show absorption at 230–240 and 250–260 nm., respectively (75, 76). The cephalosporin C band ($\lambda_{max.}$ 260 nm.) is shifted at 257 nm. upon lactonization in dilute acid (2, 124). As a result of formation of $\Delta^2$-isomers of cephalosporin amides and esters, the original absorption at 260 nm. also disappears (125).

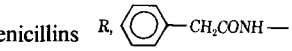
*IR Absorption*—Because of the fused β-lactam ring, both penicillins and cephalosporins exhibit a characteristic strong absorption band in the $\nu_{max.}$ 5.62–5.65-μ region (171, 172). This band disappears on hydrolysis by either base or β-lactamase, and a new band near 5.75 μ (the normal absorption band for penicilloyl ester or amide) forms. Similarly, bands at 5.69 μ (COOH), 3.00–3.05 μ (NH stretching), 6.0–6.1 μ (Amide I), and 6.5–6.7 μ (Amide II) of C=O stretching are also exhibited for all penicillins.

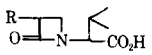
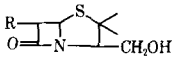
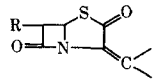
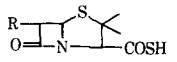
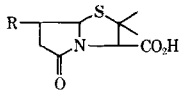
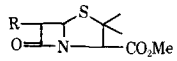
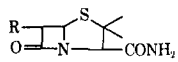
The IR absorption bands of cephalosporins and their esters, salts, and analogous derivatives have also been reported (72, 173); the new bands at 5.7 and 9.7 μ are due to the C-3 ester group (2, 72).

*NMR Spectroscopy*—NMR spectroscopy is another useful technique for characterization of the β-lactam drugs. Normally, the NMR spectra of the soluble salts of the penicillins and cephalosporins can be determined in $D_2O$. Penicillins usually exhibit a two-proton signal at τ 4.42 for the β-lactam hydrogen atoms, a sharp one-proton signal at τ 5.71 for the proton next to the carboxylate group, and sharp two- or three-proton signals at τ 8.38 and 8.47 for the gem-dimethyl group. In cephalosporins, the $\Delta^3$-cephalosporin methylene protons adjacent to the sulfur exhibit doublets centered near τ 6.4 and 6.7; these signals disappear upon transformation to the $\Delta^2$-isomer, and new single peaks appear near τ 3.6 and 5.0 (125). The centers of the single-proton quartet and the single-proton doublet, representing the β-lactam hydrogens at C-7 and C-6, are generally τ 0.8 unit apart in the $\Delta^3$-esters but more than τ 0.4 unit apart in the $\Delta^2$-isomers (125, 173). Upon acid hydrolysis of cephaloglycin, the acetyl group, which originally shows a singlet at τ 7.9, is lost and a new signal for acetate anion appears at τ 8.05 (124, 174).

## BIOLOGICAL ACTIVITY IN RELATION TO STRUCTURE

In the last decade, attempts to develop new penicillins through molecular modification were extensive and successful. The main efforts were directed toward finding the so-called "ideal penicillin" or "all-purpose penicillin" (5, 7–9, 175). The ideal penicillin should

Table VI—Inactive or Less Active Penicillins 

| Number | Compound | Structure | References |
|---|---|---|---|
| 1 | Desthiobenzylpenicillin |  | 176 |
| 2 | Benzylpenicillin alcohol |  | 112 |
| 3 | Anhydrobenzylpenicillin |  | 177 |
| 4 | Benzylthiopenicillin |  | 114 |
| 5 | Benzylhomopenicillin (γ-lactam) |  | 178 |
| 6 | Benzylpenicillin methyl ester |  | 110 |
| 7 | Benzylpenicillin amide |  | 109 |

be stable toward acids and β-lactamases, well absorbed, less serum bound, and nonallergenic, and it should have a broad spectrum and high antibacterial activity. Because of the inherent acid stability and staphylococcal β-lactamase-resistant character of the cephalosporin nucleus, the semisynthetic cephalosporins have received considerable attention in the search for the ideal antibiotic. The antibacterial activities and some of the most important biological properties of the β-lactam antibiotics in relation to their chemical structure are discussed in this section.

**Fundamental Structural Requirements**—The antibacterial activity of the β-lactam antibiotics is no doubt related directly to the fused β-lactam–thiazolidine ring (penicillin nucleus) or fused β-lactam–dihydrothiazine ring (cephalosporin nucleus), since a breakage at any point leads to complete loss of activity, irrespective of the side chain (3, 8). Table VI shows the structures of a number of inactive and less active penicillins. Desthiobenzylpenicillin (Compound 1) is inactive (176); consequently, the sulfur atom is considered necessary. The free carboxyl group in both the thiazolidine ring and the dihydrothiazine ring is also necessary, since all types of derivatives of this carboxyl group of penicillins (3, 8, 9, 107–109, 111) and cephalothin (125) have been shown to be much less active or inactive. Accordingly, penicillin alcohol (112) (Compound 2), anhydrobenzylpenicillin (177) (Compound 3), benzylpenicillin methyl ester (110) (Compound 6), and benzylpenicillin amide (109) (Compound 7) have only slight antibacterial activity. A thioacid of benzylpenicillin (Compound 4) (114) exhibits about 90% of the antistaphylococcal activity of the active penicillin G. The γ-lactam-containing homopenicillin (Compound 5), unlike the β-lactam compound, is also biologically inactive (3, 178).

X-ray analysis has shown that, stereochemically, the β-lactam rings of penicillin and cephalosporin are essentially identical (21, 179) but the exocyclic carboxyl groups are different (180). Therefore, the observed fundamental differences in activity, as well as in stability, between penicillins and cephalosporins may be due to differences in the stereospecificity of the carboxyl groups as well as to the geometry of the fused ring systems (175).

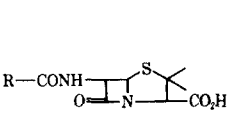
It was reported earlier that the penicillin β-lactam ring is energetic (81) and that the activity of the β-lactam antibiotics is related to the lability of their β-lactam ring structure (181). Correlations have been obtained between β-lactam ring stability and antibiotic activity and, similarly, between β-lactam stability and absorption in the IR spectrum (182). That is, with the β-lactam drugs, the more labile the β-lactam, the lower is the wavelength of absorption in the IR spectrum and, generally, the greater is the observed antibacterial activity. Conversely, the more resonant the β-lactam ring, the more stable is the ring toward base and, accordingly, the longer is the wavelength of absorption in the IR spectrum and, generally, the lower is the observed antibacterial activity (182).

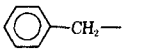
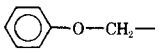
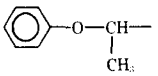
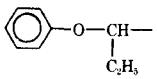
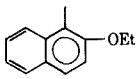
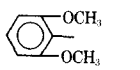
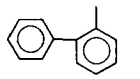
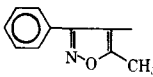
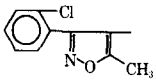
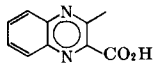
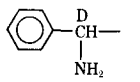
**Effect of Side Chain on Activity**—Although the penicillin or cephalosporin nucleus is essential for antibacterial activity, the potency of the various compounds is controlled to a great extent by the nature of the side chain (3, 8, 9). Since 6-chloro- and 6-bromopenicillanic acids are inactive (183), the amino groups of both 6-aminopenicillanic acid and 7-aminocephalosporanic acid are considered essential. Penicillins with benzylamino, phenylamino, and *N*-benzylphenylacetamido side chains (8) are also inactive compared with 6-aminopenicillanic acid. Penicillins with side chains having carboxamido, alkyl- and arylsulfonamido (8), and alkyl- and arylphosphinylacetamido groups (68) are much more active than 6-aminopenicillanic acid, but none is superior to penicillin G against staphylococci. The urido derivatives of 6-aminopenicillanic acid (69) have slightly lower antistaphylococcal activity than penicillin G but are better against penicillin-resistant strains. Penicillins with a phenylacetamido or thienylacetamido side-chain group appear to be the most active compounds. All compounds (both penicillins and cephalosporins) that have high antibacterial activity possess a continuous —C—CO—NH—C—CO—NH—C—COO— linkage, beginning in the side chain (—C—CO—NH—) and continuing along the β-lactam and the thiazolidine or dihydrothiazine nucleus.

The D-amino acid side chain of penicillin N and the phenylacetic acid side chain of penicillin G play a significant role in determining the biological capacities of these antibiotics (184). Penicillins with side chains having different physicochemical and spatial characteristics, such as methicillin (185) and the phenoxyalkylpenicillins (186), have properties significantly different from those of penicillin G.

**Intrinsic Antibacterial Activity–Structure Relationships**—*Gram-Positive Activity*—The first series of semisynthetic penicillins prepared for clinical use were the acid-stable α-phenoxyalkylpenicillins (186–190). α-Phenoxyalkylpenicillins are effective against infections caused by Gram-positive organisms. In this group of

**Table VII—Minimal Inhibitory Concentrations (MIC) of Some Penicillins against Sensitive and Resistant Staphylococci**



| Number | Penicillin | R | MIC (mcg./ml.), Range | | References |
|---|---|---|---|---|---|
| | | | Sensitive | Resistant | |
| 1 | Penicillin G |  | 0.005–0.05<br>0.03–0.06 | 5–125<br>1–6 | 5<br>189 |
| 2 | Penicillin V |  | 0.03–0.06 | 0.4–3.0 | 189 |
| 3 | Phenethicillin |  | 0.06–0.125 | 0.4–3.0 | 189 |
| 4 | Propicillin |  | 0.06–0.125 | 0.25–0.6 | 189 |
| 5 | Nafcillin |  | 0.12–0.5 | 0.12–1.0 | 204 |
| 6 | Methicillin |  | 0.6–1.25<br>0.5–2.0 | 1.25–2.5<br>0.5–4.0 | 5<br>204 |
| 7 | Biphenecillin |  | 0.25–<br>0.12–1.0 | 0.5–5.0<br>0.25–1.0 | 5<br>204 |
| 8 | Oxacillin |  | 0.05–0.5 | 0.25–1.25 | 5 |
| 9 | Cloxacillin |  | 0.05–0.25<br>0.06–0.25 | 0.25–0.5<br>0.06–0.5 | 5<br>204 |
| 10 | Quinacillin |  | 0.25–2.0 | 0.5–4.0 | 204 |
| 11 | Ampicillin |  | 0.1–0.8 | >100 | 7 |

compounds, as the $\alpha$-substitution of the side chain becomes longer, the antistaphylococcal activity decreases, as well as the activity against other Gram-positive organisms (186, 188, 189). However, some derivatives ($\alpha$-*tert*-isobutyl) show an increase in activity against $\beta$-lactamase-forming staphylococci (186, 188, 191). A series of analogous substitutions in the benzene ring of phenoxyethylpenicillin (phenethicillin) resulted in only a minor change in activity (188), and no direct relationship could be shown between antistaphylococcal activity and the electronic or steric effects obtained by the substitutions. However, one fact was apparent: the higher the molecular weight of the substituted side chain, the greater the serum binding and the lower the *in vivo* activity. Hansch and his coworkers (192, 193) demonstrated a correlation between the antistaphylococcal activity of 22 phenoxyalkylpenicillins and the negative lipophilic character of the side chain; the more hydrophilic the side chain, the greater was the antistaphylococcal activity of the penicillin. Similarly, among a series of substituted $\alpha$-methoxypenicillins, 3,4 - dichloro - $\alpha$ - methoxybenzylpenicillin (194) was shown to be acid stable and to give good clinical results (195).

The majority of clinically used penicillins are 6-aminopenicillanic acid acyl derivatives of substituted acetic acid. For example, benzylpenicillin is a monosubstituted (phenyl), ampicillin is a disubstituted (phenyl and $\alpha$-amino), and triphenylpenicillin is a trisubstituted (triphenyl) acetic acid derivative. Price *et al.* (8) showed that, in general, the disubstituted acetic acid penicillins have lower intrinsic antistaphylococcal activity than the monosubstituted derivatives, particularly if the substituents are bulky groups. The antistaphylococcal activity of the monosubstituted acetic acid derivatives was shown to be from 25 to 100% of that of penicillin G, with the majority in the range of 75–100%; the range for disubstituted derivatives was from 5 to 75%, with the majority in the 20–50% range; the trisubstituted derivatives were the least active, none being more than 10% as active as penicillin G.

The 6-aminopenicillanic acid derivatives of aromatic and heterocyclic acids, such as methicillin (196, 197), oxacillin (198, 199), cloxacillin (200), dicloxacillin (201), nafcillin (202), and quinacillin (203), also possess lower antistaphylococcal activity (about 20% of that of penicillin G), although they are all resistant to $\beta$-lactamase staphylococci (8, 9). In general (204), penicillin G is sufficiently active against non-$\beta$-lactamase-producing *Staphylococcus aureus* (Oxford), *Streptococcus pyogenes*, and *Listeria monocytogenes*, while methicillin, nafcillin, the isoxazolylpenicillins, quina-

cillin (203), and certain cephalosporins (204) are particularly active against the β-lactamase-producing staphylococci. As a result of both intrinsic resistance and enzymic inactivation (10), however, a few strains of *S. aureus* are resistant to methicillin, the isoxazolylpenicillins (205), and quinacillin (203).

Table VII shows that the penicillinase-resistant penicillins (Compounds 5–10) are about as active against resistant as against sensitive staphylococci [similar minimum inhibitory concentration (MIC) value], but penicillin G, the phenoxyalkylpenicillins, and ampicillin (Compounds 1, 2–4, and 11) are not.

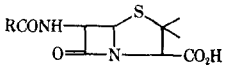
*Gram-Negative Activity and Antibacterial Spectrum*—Gram-negative activity here refers to activity against the Gram-negative bacteria. This activity was initially thought to be due to the presence of the free amino group in the penicillin or cephalosporin side chain (184, 206), this group being capable of attaining and maintaining close contact with susceptible sites on the Gram-negative bacteria (207). Penicillin N was the first penicillin used in the treatment of infections caused by Gram-negative bacteria, although it was considerably less active against the Gram-positive organisms than penicillin G (16, 184). *p*-Aminobenzylpenicillin (169) has a spectrum similar to that of penicillin N. The *ortho*- and *meta*-aminobenzylpenicillins (8, 9) have spectra similar to that of the *para*-isomer toward Gram-positive bacteria, but they are slightly less active against the Gram-negative organisms. Ampicillin (66, 206) has become one of the most effective broad spectrum antibiotics developed to date. It is effective against infections of the gastrointestinal, respiratory, and urinary tracts due to *Staphylococci, Streptococci, Haemophilus influenzae, Proteus mirabilis, Neisseria gonorrhoeae,* certain strains of *Escherichia coli,* and some species of *Salmonella, Shigella,* and *Moraxella.* The main disadvantage of ampicillin is that it is rather unstable toward penicillin-inactivating β-lactamases; its usefulness in infections caused by β-lactamase-forming *Staphylococcus, Proteus, Pseudomonas,* and *Aerobacter* is quite limited (208). Other amino group-containing penicillins, such as cyclacillin (WY-4508) (209) and BL-P875 (210), are also excellent broad spectrum penicillins but may be similarly sensitive to penicillinase.

The presence of a free amino group in the side chain is not the only requirement for Gram-negative activity; α-hydroxybenzylpenicillin (8, 9), azidobenzylpenicillin (211), pyridylmethyl derivatives (212) and isothiazolylmethyl derivatives of 6-aminopenicillanic acid and 7-aminocephalosporanic acid (213), and α-carboxybenzylpenicillin (carbenicillin) (214–216) are all broad spectrum β-lactam antibiotics, even though they do not contain such a free amino group. Table VIII lists the known penicillins with Gram-positive and Gram-negative activities. These compounds do have a side chain with sufficient polarity or hydrophilic property, and these characteristics are now thought to be essential for Gram-negative activity (71, 211–213, 217).

*N*-Acylation or condensation of penicillin N (3), *p*-aminobenzylpenicillin (169), ampicillin (218, 219), or cyclacillin (219) causes a drastic decrease in Gram-negative activity. The low activity of the phenoxyalkylpenicillins (220), methicillin, and the isoxazolylpenicillins (198–201, 221) against Gram-negative bacteria was shown not to be due to inactivation by β-lactamase (222). Nafcillin (223), quinacillin (224), diphenicillin (225), and penicillin dimer (226), none of which has the hydrophilic character, are also inactive against Gram-negative bacteria.

The mechanism responsible for the insusceptibility of the Gram-negative bacteria to penicillins is apparently more complex than that of the Gram-positive bacteria to penicillins. In general (10, 227), the resistance nature or insusceptibility of the coliform bacteria to penicillins may be chiefly due to three factors: (*a*) inherent resistance plus β-lactamase inactivation, as in the case of the resistance of β-lactamase-producing *Pseudomonas aeruginosa* (*pyocyanea*) and indole-positive *Proteus* and *Enterobacter* sp. to ampicillin (10); (*b*) inactivation due solely or primarily to β-lactamase, as in the case of the relative resistance of *Aerobacter aerogenes, Proteus vulgaris, Proteus morganii,* and *Proteus rettgeri* (10) to ampicillin, or the initial resistance of *S. aureus* to this drug (227); and (*c*) inherent resistance alone, as in the case of the *Salmonella* species and some strains of *Pr. mirabilis* and *Klebsiella,* which are sensitive to ampicillin and penicillin G

**Table VIII**—Broad Spectrum Penicillins



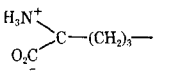
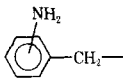
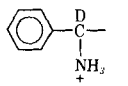
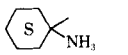
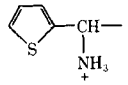
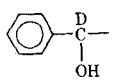
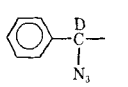
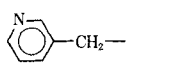
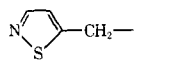
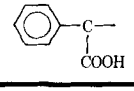
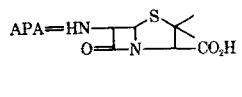
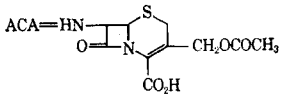
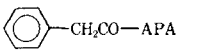
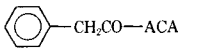
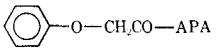
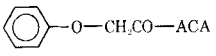
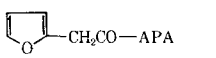
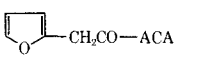
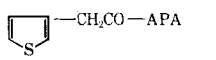
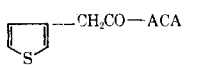
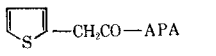
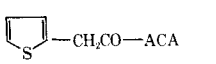
| Number | Compound: Name | Compound: Side Chain R | References |
|---|---|---|---|
| 1 | Penicillin N |  | 184 |
| 2 | *p*-Aminobenzylpenicillin |  | 169 |
| 3 | *o*-Amino- or *m*-aminobenzylpenicillin |  | 8, 9 |
| 4 | Ampicillin |  | 66, 206 |
| 5 | Cyclacillin |  | 209 |
| 6 | BL-P875 |  | 210 |
| 7 | α-Hydroxybenzylpenicillin |  | 8, 9 |
| 8 | Azidopenicillin |  | 211 |
| 9 | 3-Pyridylmethylpenicillin |  | 212 |
| 10 | Isothiazolylmethylpenicillin |  | 213 |
| 11 | Carbenicillin |  | 214–216 |

**Table IX**—Comparison of Minimal Inhibitory Concentrations (MIC) of Derivatives of 6-Aminopenicillanic Acid and 7-Aminocephalosporanic Acid in a Gradient Plate Test (mcg./ml.)[a]




| Number | Structure of Compound | *Shigella* sp. | *Escherichia coli* | *Klebsiella* sp. | *Aerobacter* sp. | *Shigella sonnei* |
|---|---|---|---|---|---|---|
| 1 |  | 32 | 42–48 | 13–109 | 42 | 44 |
| 2 |  | 33 | 24–37 | 2–27 | 15 | 27 |
| 3 |  | 112 | 110–144 | 138–>200 | >200 | 105 |
| 4 |  | 107 | 101–132 | 7–>200 | 110 | 102 |
| 5 |  | 31 | 41–59 | 13–>200 | 84 | 40 |
| 6 |  | 15 | 10–36 | 7–11 | 8 | 17 |
| 7 |  | 15 | 25–33 | 10–105 | 38 | 26 |
| 8 |  | 31 | 17–33 | 2–20 | 7 | 8 |
| 9 |  | 28 | 38–46 | 10–122 | 56 | 169 |
| 10 |  | 11 | 8–15 | 6–8 | 5 | 11 |

[a] Data from *Reference 72.*

but which, even though producing little or no β-lactamase, are generally resistant to the phenoxyalkylpenicillins, methicillin, and the isoxazolylpenicillins.

*Antibacterial Activity of Cephalosporin*—Cephalosporin C is much less active than penicillin G against both Gram-positive and Gram-negative bacteria (2), probably because of its D-α-aminoadipoyl side chain since substitution of a phenylacetyl, 2-furylacetyl, 2- or 3-thienylacetyl, aryl- or alkylmercaptoacetyl, or other similar side-chain group results in a drastic increase in activity. In general, derivatives of 7-aminocephalosporanic acid are slightly less active than the corresponding derivatives of 6-aminopenicillanic acid against staphylococci but, as shown in Table IX, are several times more active against most Gram-negative bacteria (72, 204, 228).

Cephalothin is essentially similar to the furan-2-acetamido derivatives in antibacterial activity. Clinically, cephalothin is effective against a wide variety of organisms, particularly against the β-lactamase-forming *S. aureus* and some Gram-positive cocci, but is ineffective against *Pseudomonas*, the indole-positive *Proteus*, some strains of the *Klebsiella-Aerobacter* group, and the enterococci (229–232).

Cephaloridine (79) is moderately to highly effective against *A. aerogenes*, pyogenic cocci, most strains of *E. coli*, *Klebsiella*, *Pr. mirabilis*, *Salmonella* sp., and *H. influenzae* (233, 234) and is equally active against both sensitive and resistant staphylococci. Its inhibitory capacity for staphylococci is about 8–26 times greater than that of methicillin (234). Although many other desacetoxy derivatives of cephalosporins have been prepared, none is more active than cephaloridine, particularly against Gram-negative bacteria (74–76, 230). Any substitution into the pyridine ring in cephaloridine results in a decrease in activity (75).

The α-aminocephalosporins (78, 79) are cephalosporanic acid derivatives having a broad spectrum of activity, stability to gastric acid, and effective gastrointestinal absorption. Examples of such compounds are the α-aminophenylacetamido derivative (known as cephaloglycin) and α-aminothienylacetamidocephalosporanic acid. Among cephalothin, cephaloridine, and cephaloglycin, cephaloridine is the most effective of the three against sensitive Gram-positive organisms, its activity approaching that of penicillin (235); but cephaloglycin is the most effective against the Gram-negative bacteria (235–238).

In a series of α-substituted phenylacetamidocephalosporanic acids, the α-aminophenylacetamido and α-chlorophenylacetamido derivatives were found to be most active against *E. coli*, while the α-phenoxyphenylacetamido derivative was most active against β-lactamase-forming staphylococci (239). Another series of cephalosporins, prepared from acids such as the monocarboxylic, aromatic and heterocyclic diacetic, and sulfur-containing aliphatic dicarboxylic (240, 241), showed promising activity *in vitro* but negligible activity *in vivo*.

Cephalexin differs from cephaloglycin only by the lack of an acetoxy group attached to the dihydrothiazine ring. The excellent oral absorption and lack of serum binding of cephalexin compensate for its lower *in vitro* activity compared to cephaloglycin (242–244). Although cephalexin is less active against many organisms and is relatively slow acting compared to ceph-

aloridine, it is more active than cephaloridine against *N. gonorrhoeae* and more active than ampicillin toward penicillin-resistant staphylococci. Its overall activity is about 20% that of cephaloridine or ampicillin (243). It is rapidly inactivated by most strains of enterococci that are generally susceptible to ampicillin (244).

Both cephalexin and cephaloglycin (245) are moderately to highly effective against strains of group A hemolytic streptococci, viridans streptococci, pneumococci, gonococci, meningococci, and penicillin-sensitive *S. aureus*, but cephaloglycin is about 2–8 times more active than cephalexin toward these strains. On the other hand, both are moderately inactive against *H. influenzae*, enterococci, and most of the common Gram-negative bacilli. The activity of cephalexin is not significantly affected by the pH of the medium within the clinical range (pH 5.5–8.5). In contrast, the activity of cephaloglycin was shown to be quickly destroyed at the higher pH.

Studies on therapy of staphylococcal infections in monkeys (246, 247) showed that cephaloridine is the most active compared to cephaloglycin, cephalexin, and cephalothin.

Cephalexin (248), an orally absorbed cephalosporin antibiotic, is basically bactericidal in action. *Strep. pyogenes*, *Strep. viridans*, *Diplococcus pneumoniae*, *N. gonorrhoeae*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, *Clostridium tetani*, and *Clostridium perfringens* were consistently sensitive (MIC about <3 mcg./ml.) to this agent; most staphylococci (both penicillin-sensitive and penicillin-resistant strains), many of the Gram-negative enteric bacilli, including *Klebsiella*, *E. coli*, *Pr. mirabilis*, *Salmonella*, and *Shigella*, were moderately sensitive (MIC about 7.8 to 15.6 mcg./ml.). The strains of *H. influenzae*, isolates of *Enterobacter*, *Serratia*, *Enterococcus*, indole-positive *Proteus*, and *Pseudomonas* were usually resistant to cephalexin.

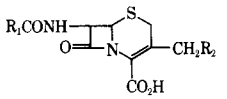
A cephalosporanic acid derivative, BL-P1322 [sodium 7-(pyrid-4-yl-thioacetamido)cephalosporanate], possesses antibacterial activity essentially the same as that of cephalothin (249). However, BL-P1322 was found to be significantly more effective against strains of *D. pneumoniae*, *Enterobacter* sp., and *Mycobacterium tuberculosis*.

Cefazolin (250, 251) is a new bactericidal cephalosporin active *in vitro* against most Gram-positive and Gram-negative bacteria except *Ps. aeruginosa;* it is also resistant to staphylococcal β-lactamase (250). The overall activity was shown to be parallel to that of cephaloridine; however, intramuscular administration of cefazolin resulted in blood levels twice as high as those observed with cephaloridine after similar administration (251).

A comparison of the antimicrobial activity of the various cephalosporanic acids and derivatives, the MIC's against both penicillin-sensitive and penicillin-resistant staphylococci and Gram-negative bacteria, are listed in Tables X and XI, respectively.

*Activity against Strains of* Pseudomonas *and* Proteus—*Ps. aeruginosa* (*pyocyanea*), as well as other strains of *Pseudomonas*, are inherently resistant to all forms of penicillins, whether or not assisted by inactivating β-lactamase (252). Carbenicillin was the first penicillin shown to be clinically efficacious against strains of *Proteus*, *Pseudomonas*, and *E. coli* (214, 215, 253–255). Carbenicillin has been used in patients with *Pseudomonas*-infected burns as well as in respiratory, urinary tract, and generalized *Pseudomonas* infections (255–257). It is relatively unstable toward staphylococcal β-lactamases. It is less active than ampicillin against *H. influenzae*, *E. coli*, *Salmonella* sp., *Shigella* sp., and *Pr. mirabilis*, as well as against staphylococci and streptococci (215, 253, 258–260), and it is ineffective against infections caused by *Klebsiella* sp. (215, 253) and *Serratia* sp. (258). The greatest disadvantage of carbenicillin is that it is extremely unstable toward acids and must be administered parenterally

**Table X**—Minimal Inhibitory Concentrations (MIC) of Some Cephalosporins against Sensitive and Resistant Strains of Staphylococci



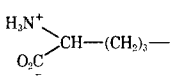
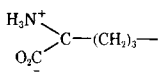
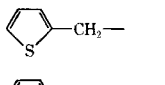
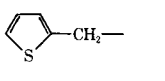
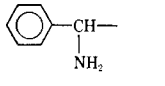
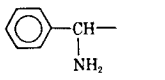
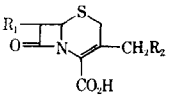
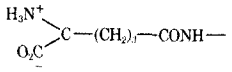
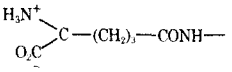
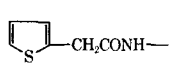
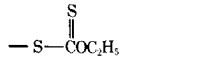
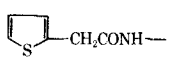
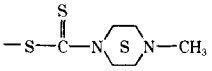
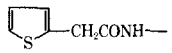
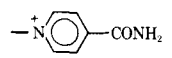
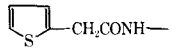
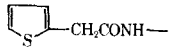
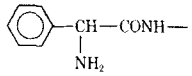
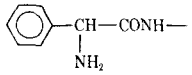
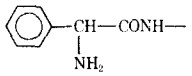
| Number | Cephalosporin | Side-Chain Structure $R_1$ | Side-Chain Structure $R_2$ | MIC (mcg./ml.), Range Sensitive | MIC (mcg./ml.), Range Resistant | References |
|---|---|---|---|---|---|---|
| 1 | Cephalosporin C |  |  | 60–125 | 60–125 | 5 |
| 2 | Cephalosporin $C_A$ |  |  | 5–20 | 5–20 | 5 |
| 3 | Cephalothin |  |  | 0.3–0.6<br>0.25 | 0.3–0.6<br>0.25–0.5 | 5<br>204 |
| 4 | Cephaloridine |  |  | 0.12 | 0.12–0.25 | 204 |
| 5 | Cephaloglycin |  |  | 0.78–3.12 | 6.25–12.25 | 235 |
| 6 | Cephalexin |  | —H | 1.6–3.12 | 6.25–8.0 | 242<br>243 |

**Table XI**—Minimal Inhibitory Concentrations (MIC) of 7-Aminocephalosporanic Acid and Derivatives in a Gradient Plate Test



| Number | Structure $R_1$ | Structure $R_2$ | *Shigella* sp. | *Escherichia coli* | *Klebsiella* sp. | *Aerobacter* sp. | *Shigella sonnei* | References |
|---|---|---|---|---|---|---|---|---|
| 1 |  |  | 75 | 79 | 38 | 48 | 83 | 230 |
| 2 |  |  | 22 | 26 | 23 | >50 | 23 | 230 |
| 3 |  |  | 78 | 83 | 6 | 67 | — | 230 |
| 4 |  |  | 2 | 3 | 2 | 4 | — | 230 |
| 5 |  |  | 6 | 2 | 3 | 2 | 2 | 75 |
| 6 |  |  | 5.6 | 2.4–2.8 | 4.2–4.5 | 3.8 | 3.8 | 75 |
| 7 |  |  | 11 | 8–15 | 6–8 | 5 | 11 | 72 |
| 8 |  |  | 9.1 | 12.6 | 9.7 | 8.2 | — | 124 |
| 9 |  |  | 11.6 | 16.3 | 14.4 | 16.4 | — | 124 |
| 10 |  |  | 2.1 | 3.6 | 3.6 | 3.9 | — | 124 |

(215). In addition, the emergence of resistant strains has occurred during therapy of *Pseudomonas* infections (259).

An α-sulfoamino derivative of ampicillin, BL-P1462 [6-(D-α-sulfoaminophenylacetamido)penicillanic acid and disodium salt] (261, 262), was shown to be active against a wide variety of Gram-negative bacilli, particularly *Ps. aeruginosa*. This drug was also shown to be slightly more active than carbenicillin against the same isolated strains of *Pseudomonas*, *Enterobacter*, and *Klebsiella* sp. However, it was not as active as carbenicillin against other Gram-negative bacilli, such as *E. coli* and *Proteus* sp. (262).

Table XII shows the antimicrobial spectrum and relative potency of several available penicillins and cephalosporins against both Gram-positive and Gram-negative organisms.

*Effect of Diastereoisomers on Activity*—Strikingly different antibacterial activity has been observed with the diastereoisomers of penicillins (187, 190, 211) and cephalosporins (78, 230). In a comparison of L-phenethicillin, D-phenethicillin, and DL-phenethicillin, McCarthy *et al.* (220) and English and McBride (263) pointed out that the L-epimer is the most active and the D-epimer the least active while the DL-form (phenethicillin) is intermediate in activity toward Gram-positive organisms.

Ampicillin [D-(−)-α-aminobenzylpenicillin], on the other hand, is about 2–6 times more active than its L-(+)-epimer and about 10 times more active than penicillin G against certain Gram-negative organisms (3). Similarly, the D-epimer of α-azidopenicillin is about 3–10 times more active than its L-epimer against both Gram-positive and Gram-negative organisms (211).

Spencer *et al.* (78) and Flynn (230) reported that L-cephaloglycin has about 5% of the antistaphylococcal activity (penicillin resistant) of its D-isomer; against Gram-negative organisms, D-cephaloglycin is at least 25 times more potent than its L-epimer.

**Absorption and Serum Concentrations**—Table XIII summarizes the peak serum concentrations of several penicillins and cephalosporins in human subjects.

The phenoxyalkylpenicillins are stable to gastric acid and well absorbed from the gastrointestinal tract (264, 267). The peak serum concentrations listed for these penicillins were reached at 1 hr. after oral administration in healthy volunteers, regardless of the dose. After oral administration of 250 mg. in solid dosage forms, the peak serum concentrations ranged as follows: penicillin V, 1–2 mcg./ml.; phenethicillin (189), 2–5.3 mcg./ml., or about twice that of penicillin V; and propicillin and phenoxyisopropylpenicillin, 2–7 mcg./ml., or about 2–3 times that of penicillin V (264–267). The L-isomer and the DL-isomer of the α-phenoxyethylpenicillins gave about twice the serum concentrations of the D-isomers (268). Similarly, the L-isomer of propicillin gave higher

penicillin levels than its D-isomer in dogs and rats (269). Food apparently interferes with the efficient absorption of these antibiotics, and the serum penicillin concentrations are decreased by about 30–60% when they are administered after a meal compared to those observed under fasting conditions (268, 270). There was no indication that a difference in the activity of human subjects (*i.e.*, as between ambulatory and bedridden patients) significantly influences the peak serum levels after oral administration of penicillin V (271). Water-soluble salts (potassium, sodium, and calcium) of penicillin generally give higher and earlier serum concentrations than the sparingly soluble salts (*i.e.*, benzathine penicillin) or the penicillin-free acid form (271, 272).

The peak serum levels of oxacillin, cloxacillin, and dicloxacillin (273–280) were also attained at 1 hr. after oral dosing in fasting volunteers. The serum concentrations of oxacillin were shown to be slightly lower than those attained after oral administration of phenethicillin or penicillin V (221, 275). After oral administration of a single dose of 1.0 g. of oxacillin, the peak serum concentrations were from 9 to 12 mcg./ml., which are well in excess of the MIC for resistant staphylococci (276, 278). The oral absorption characteristics of the three isoxazolylpenicillins were compared after a single dose of 500 mg. in fasting volunteers. The peak serum levels ranged from 5 to 7 mcg./ml. for oxacillin and from 7.5 to 14.4 mcg./ml. for cloxacillin, or about twice that of oxacillin (201, 277–279), and about 10–17 mcg./ml. for dicloxacillin (279, 280). The isoxazolylpenicillins are about 5–10 times more active than methicillin, while dicloxacillin is 2–3 times more active than cloxacillin and oxacillin against penicillinase-producing *S. aureus* (280). The observed therapeutic activity of the isoxazolylpenicillins seems to correlate well with their serum concentrations.

Methicillin (281, 282), nafcillin (282), diphenicillin (283, 284), and quinacillin (224) give relatively less serum levels. This may be due to either low stability or low aqueous solubility, or both of these factors may be operative for the higher molecular weight penicillins. Nafcillin was shown to be absorbed better than methicillin (281), but the absorption was quite irregular and significantly affected by food (202). However, the addition of buffer to nafcillin formulations results in an improvement in the oral absorption characteristics of this agent (202).

In dogs the peak serum level of ampicillin (7.2 mcg./ml.) is obtained 1 hr. after a single oral dose of 20 mg./kg., while those for phenethicillin and penicillin V (about 4.0 and 2.6 mcg./ml., respectively) are observed at only 30 min. after dosing. In dogs, as well as in humans, the ampicillin serum levels are more persistent than those of penicillin V and phenethicillin (285). After single oral doses of 250, 500, 750, and 1000 mg. of ampicillin in fasting volunteers, the mean peak serum concentrations were 1.9, 3.8, 5.1, and 6.8 mcg./ml., respectively (286). These peaks usually occurred at about 2 hr. after dosing in human subjects (compared to 1 hr. in dogs), and significant serum concentrations were maintained even at 6 hr. Doubling the dose virtually doubles the peak serum concentration. Probenecid delays the excretion of ampicillin (287). In rats, after either oral or intraduodenal administra-

**Table XII**—Antibacterial Spectra of Several β-Lactam Antibiotics[a]

| Organism | Penicillin G | Penicillin V, Phenethicillin Propicillin, Phenbenicillin | Methicillin, Nafcillin, Ancillin, Quinacillin | Oxacillin, Cloxacillin, Dicloxacillin | Ampicillin, Hetacillin, Cyclacillin, BL-P875, Azidopenicillin | Carbenicillin, α-Sulfonamidobenzylpenicillin (BL-P1462) | Cephalothin, Cephaloridine, Cephaloglycin, Cephalexin, Cefazolin |
|---|---|---|---|---|---|---|---|
| *S. aureus*, β-lactamase producing | ± | + | ++ | ++ | ± | + | ++ |
| *S. aureus*, non-β-lactamase producing | +++ | ++ | ++ | ++ | +++ | ++ | ++ |
| *Strep. pneumoniae* | +++ | ++ | ++ | ++ | +++ | + | ++ |
| *Strep. faecalis* | ++ | ++ | ++ | + | ++ | + | + |
| *Strep. pyogenes* | +++ | ++ | +++ | ++ | +++ | ++ | +++ |
| *Strep. viridans* | +++ | ++ | ++ | ++ | ++ | + | ++ |
| *N. meningitidis* | ++ | ++ | ++ | ++ | ++ | + | ++ |
| *H. influenzae* | ++ | + | + | + | ++ | ++ | + |
| *Salmonella* sp. | + | ± | − | − | + | + | + |
| *E. coli* | ± | − | − | − | + | + | ++ |
| *Klebsiella, Aerobacter* spp. | − | − | − | − | ± | ± | ++ |
| *Pr. mirabilis*, non-β-lactamase producing | − | − | − | − | + | ++ | + |
| *Pr. mirabilis*, β-lactamase producing | − | − | − | − | − | + | + |
| *Pr. rettgeri, morganii, vulgaris* | − | − | − | − | − | + | − |
| *Ps. aeruginosa* | − | − | − | − | − | + | − |
| References | 7, 10, 187, 189, 206 | 7, 10, 189 222, 225 | 7, 10, 196, 202, 204, 223, 224, 225 | 7, 10, 204, 221, 222 | 7, 10, 204, 206, 209, 210, 211, 222, 292 | 10, 214, 215 253, 259, 260, 261 | 7, 10, 72, 124, 204, 235, 250, 251 |

[a] Minimum inhibitory concentration (median): +++, active at <0.1 mcg./ml.; ++, active at 0.1–10 mcg./ml.; +, active at > 10 mcg./ml.; ±, active at 250 mcg./ml; and −, no activity at >250 mcg./ml.

Table XIII—Representative Peak Serum Concentrations of Several β-Lactam Antibiotics in Human Subjects after a Single Oral Dose[a]

| Antibiotics | Form | Dose, mg. | Dosage Form | Peak Serum Concn. (Range) mcg./ml. | Peak Time (hr.) | References |
|---|---|---|---|---|---|---|
| Penicillin V | Pot. salt | 250 | Unspecified | 1.6 | 1 | 189 |
| | Pot. salt | 500 | Unspecified | 6.0 | 1 | 265 |
| Phenethicillin | Pot. salt | 250 | Unspecified | 3.3 | 1 | 189 |
| | Pot. salt | 500 | Unspecified | 8.8 | 1 | 265 |
| Propicillin | Pot. salt | 250 | Unspecified | 6.2 | 1 | 189 |
| | Pot. salt | 500 | Unspecified | 12.2 | 1 | 265 |
| Phenbenicillin | Pot. salt | 125 | Capsules | 4.9 | 0.5 | 267 |
| | Pot. salt | 250 | | 7.5 | 1 | 267 |
| Nafcillin | Sod. salt | 1000 | Tablets | 6.4 | 1 | 202 |
| | | | Capsules | 6.6 | | |
| Oxacillin | Sod. salt | 250 | Capsules | 1.9 | 1 | 277 |
| | Sod. salt | 500 | | 6.0(5.0–7.0) | 1 | 277,278 |
| Cloxacillin | Sod. salt | 250 | Capsules | 3.5 | 1 | 277 |
| | Sod. salt | 500 | | 10.5(7.5–14.4) | | 277,278,279 |
| Dicloxacillin | Sod. salt | 250 | Capsules | 8.5 | 1 | 279 |
| | Sod. salt | 500 | | 17.0 | | |
| Ampicillin, anhydrous | Acid | 250 | Suspension | 2.2 | 1 | 291 |
| | | | Capsules | 2.0 | 2 | |
| Ampicillin, trihydrate | Acid | 250 | Suspension | 1.7 | 2 | |
| | | | Capsules | 1.9 | 2 | |
| Hetacillin | Acid | 250 | Capsules | 1.7 | 2 | 292 |
| | | 500 | | 2.5(2.3–2.8) | | |
| Cephaloglycin | Acid | 500 | Unspecified | 2.6 | 2 | 238 |
| Cephalexin | Acid | 250 | Capsules | 8.4 | 1 | 244 |
| | | 500 | | 15.0(11–18.4) | 1 | 243,244 |
| | | 1000 | | 23.0 | 1 | 243 |

[a] In fasting human volunteers.

tion, ampicillin and penicillin V are both poorly absorbed. In dogs and humans, however, ampicillin is well absorbed and gives serum levels about twice those of penicillin V (288). Ampicillin is destroyed in the stomach by acid and in the intestine by the β-lactamases present there, but it remains intact in the bile (286, 288–290); thus, an absorbed–excreted–reabsorbed cycle of ampicillin in the body may be partly responsible for the long-lasting serum levels of this agent compared to those of the phenoxyalkylpenicillins. Because of the cycle, very little intact ampicillin remains in the middle and lower part of the gut, and none is present in the feces (290).

Ampicillin is available in two crystalline forms, the anhydrate and the trihydrate, having different properties. For instance, the anhydrous form has greater aqueous solubility and a higher apparent rate of dissolution than the hydrated substance (168). This difference is probably responsible for the fact that, after oral dosing to both dogs and humans, the anhydrous form gives higher and generally earlier peak serum levels and an overall more efficient absorption (greater area under the time–serum level curve) than the hydrated form (291).

Hetacillin, a condensation product of ampicillin and acetone which is less soluble than either form of ampicillin, gives serum levels about one-half of those obtained with ampicillin in volunteers (292, 293). After an oral dose of 500 mg. of hetacillin or ampicillin to fasting human subjects, the peak serum levels attained with hetacillin are only about 1–3 mcg./ml. 2 hr. after dosing, in comparison with 3–5 mcg./ml. for ampicillin (293, 294).

Cephalosporins N and C are rather acid stable but are very poorly absorbed from the gastrointestinal tract in both humans and animals. The poor absorption, however, does not completely depend on the α-aminoadipoyl side chain, since cephalothin and cephaloridine are also poorly absorbed (232–234). The absorption of cephaloridine can be improved if the antibiotic is given in combination with sodium lauryl sulfate (295). Cefazolin gives serum levels about twice as high as cephaloridine after intramuscular injection of a single 500-mg. dose (251). Many of the derivatives of 7-aminocephalosporanic acid, such as *N*-phenylacetyl, *N*-phenoxyacetyl, and phenylglycol, are absorbed much less readily than are the corresponding derivatives of 6-aminopenicillanic acid.

Cephaloglycin is well absorbed in comparison with cephaloridine or penicillin V (235). Two hours after oral administration of 250- and 500-mg. doses to fasting volunteers, the mean peak serum concentrations were about 1.3 and 2.3–2.6 mcg./ml., respectively (238, 296), which were slightly lower than those obtained from ampicillin with equal doses. Cephalexin has been shown to be absorbed more efficiently; in fasting volunteers in a crossover study, the peak serum concentrations were 8.4 and 18.8 mcg./ml. after oral administration of 250- and 500-mg. doses, respectively, and were reached 1 hr. postadministration (244). The serum concentration obtained from a single dose of 0.5 g. of cephalexin is virtually equal to that of cephaloridine by intramuscular injection of the same size dose (244). The rather high serum concentrations and the nearly complete recoveries of cephaloglycin and cephalexin indicate that the human is able to absorb these antibiotics substantially better than the rat and the dog (243).

**Factors Influencing Oral Absorption**—It is well known that the acid stability of the penicillins and cephalosporins is an important factor in their oral absorption. However, acid stability is obviously not the only property governing the oral absorption of the β-lactam antibiotics. β-Lactamase inactivation may

play an important role in the relatively low serum levels attained with some of these agents. Solubility and dissolution rate are other factors that may contribute to the faster and more efficient absorption of such drugs, as was shown in the case of anhydrous ampicillin in comparison with ampicillin trihydrate (291) and sodium and potassium ampicillin in comparison with ampicillin trihydrate (297).

Well-documented reports (202, 286, 298) have shown that stomach conditions, such as occur with fasting or nonfasting, may affect absorption. In general, the presence of food retards the rate and extent of penicillin absorption.

Similarly, formulation factors play a significant role in oral absorption. For example, dicloxacillin was shown to be more efficiently absorbed in suspension than in gelatin capsules (298). The physical and chemical properties of the drug (*i.e.*, crystalline state, stability, and metabolism in the body) conceivably are other key factors governing the blood serum levels.

A relationship certainly exists between protein binding and serum penicillin levels. For instance, the serum binding of the isoxazolylpenicillins is about 92–94% for oxacillin, 94–96% for cloxacillin, and 98–99% for dicloxacillin, and the observed serum penicillin levels of these drugs correlate with their respective magnitudes of serum binding (299). Measurement of serum penicillin levels alone, however, does not accurately reflect efficiency of oral absorption, since penicillins differ in tissue distribution, rate of removal from the blood, and rate of inactivation and excretion; each of these factors may be important in determining the serum levels (299). Dicloxacillin, for example, shows slower and lower renal excretion and is less well distributed in the pericellular space than oxacillin or cloxacillin (295, 299). Nafcillin and oxacillin, on the other hand, are equally well absorbed, the lower serum levels of nafcillin compared to those from oxacillin or cloxacillin being mainly due to the facts that nafcillin is better distributed in the tissues and is less serum bound than the isoxazolylpenicillins (299).

## INACTIVATION OF PENICILLINS AND CEPHALOSPORINS BY BACTERIAL ENZYMES

The bacterial enzymes capable of attacking either the penicillins or the cephalosporins, or both, are the β-lactamases, the acylases (amidases), and the acetyl esterases (300, 301). β-Lactamases hydrolyze the β-lactam linkage, with production of the antibiotically inactive penicilloic or cephalosporoic acid. The acylases are enzymes which hydrolyze the *N*-acyl side chain (peptide bond) (300, 302). Acetyl esterases are of minor interest because they attack selectively the C-3 acetyl ester group in cephalosporins to desacetyl cephalosporins (300). β-Lactamases and acylases can both be produced by the same microbial strain, such as during incubation of penicillin G with strains of *E. coli*, *Streptococcus lavendulae*, or *Klebsiella aerogenes*, which usually produce more than one decomposition product (37, 300, 302, 303). In the cephalosporins, the opening of the β-lactam ring may be accompanied by the simultaneous expulsion of the C-3 ester or pyridine group due to strains of *Bacillus cereus* (β-lactamase II) or *Ps. aeruginosa* (304, 305). The distribution, specificity, and selective preparation of penicillin acylases (306–308) and the properties and sources of the known β-lactamases (309, 310) were reported. Comprehensive reviews on β-lactamase were recently published by Citri and Pollock (309), Pollock (301, 310), Abraham (311), and Rauenbusch (312).

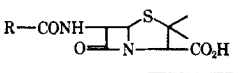
**β-Lactamases: Their Distribution and Properties**—Although β-lactamase was first discovered by Abraham and Chain in 1940 (311, 313), studies before 1960 were mostly confined to the β-lactamases of Gram-positive origin (314–319). The activity of enzymes from different sources and preparations was determined by various biological and chemical techniques, such as manometric, alkalititrimetric, hydroxylamine, optical rotatory, and iodometric (320). These studies were directed largely toward the production, purification, and elucidation of the enzymic properties of β-lactamase from *B. cereus* (316, 318, 319, 321), *Bacillus subtilis* (317, 322), *Bacillus licheniformis* (323), and *S. aureus* (324–328). Interest in the β-lactamases produced by the Gram-negative coliform bacteria arose only after the introduction of ampicillin (206) and other broad spectrum β-lactam antibiotics; bacteria such as *E. coli* (328–331), *K. aerogenes* (331, 332), *Ps. aeruginosa* (305, 333), *Proteus* sp. (331, 334–336), *Enterobacter cloacae* (337), and *E. coli* carrying the resistant (R) factor (338) gradually became resistant to the broad spectrum drugs. It is now known that β-lactamases are very widely distributed among bacteria of both Gram-positive and Gram-negative origin (324, 339), and the β-lactamase inactivation of penicillins and cephalosporins has been shown to be the major factor responsible for bacterial resistance to them (10, 300, 310).

Like other enzymes, β-lactamases are proteinoid macromolecules. Although the purified β-lactamases from Gram-positive organisms have a similar molecular weight (about 30,000) and an identical hydrolytic action toward penicillins, they differ greatly in other physicochemical properties, such as in electrophoretic mobility, adsorption and elution properties, optimal pH, isoelectric point, and overall amino acid composition (300, 301, 310, 312, 324, 340). β-Lactamases from *S. aureus* are rich in lysine; those from *B. licheniformis* are rich in aspartic acid (340, 341); and those from *B. cereus* are rich in alanine (310).

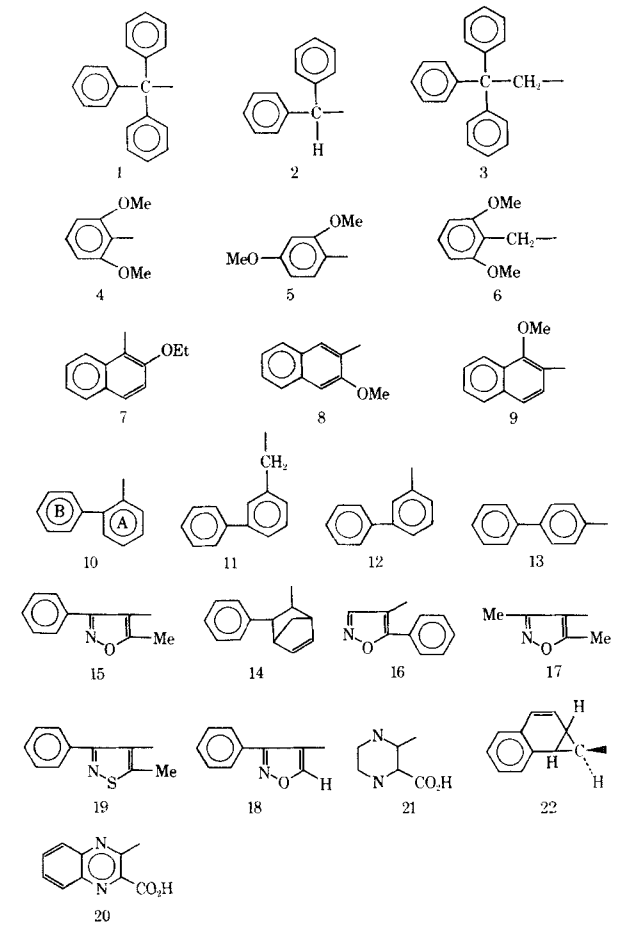
The β-lactamases from Gram-positive organisms are "extracellular" enzymes and are normally liberated in their growth media, while those from Gram-negative coliform bacteria are cell bound, or "endocellular," the enzymic activity appearing to take place inside the barrier of the cells. With the latter, liberation of the enzyme into the growth medium can be achieved only after disruption of the cell wall (300).

The β-lactamases of Gram-positive origin appear to be inducible (317); that is, they are formed in response to the presence of a substrate (such as methicillin), have greater enzymic activity, and have a higher energy of activation (about 7 kcal./mole or higher). The β-lactamases of Gram-negative bacteria cannot be induced; they are "constitutional," have a markedly varied differential permeability barrier, and have a rather low

**Table XIV**—Structural Comparison of Penicillins Resistant and Susceptible to β-Lactamase-Producing Staphylococci



| Resistant Penicillin, R | Susceptible Penicillin, R |
|---|---|



energy of activation (about 4–5 kcal./mole) (300, 342, 343).

In general, the β-lactamases from *S. aureus*, *B. cereus* (β-lactamase I), *B. licheniformis*, *Klebsiella* sp., *Pr. mirabilis*, and *Aerobacter cloacae* selectively attack the β-lactam of penicillins. These bacterial enzymes are often referred to as "penicillinases" or "penicillin β-lactamases" (310). On the other hand, bacterial enzymes from *B. cereus* (β-lactamase II), *E. coli*, *Enterobacter* sp., *Pr. vulgaris*, *Pr. rettgeri*, *Pr. morganii*, and *Ps. aeruginosa* are known to hydrolyze cephalosporins rapidly but to attack penicillins only of certain types (10, 300). These enzymes are often called "cephalosporinases" or "cephalosporin β-lactamases."

**β-Lactamase Resistance of Penicillins**—One of the most important organisms causing clinical resistance to penicillins is the β-lactamase-producing *S. aureus*. To overcome the effect of this enzyme, penicillins having appropriate *N*-acyl side chains have had to be used. Thus, in this case, the same feature of the side chain structure determines both the intrinsic activity and the stability toward β-lactamase (3). When the side chain of an α-phenoxyalkylpenicillin (186, 188, 191) is made longer, the activity against the penicillin-sensitive strains of *S. aureus* decreases but that against the resistant strains increases. Phenoxyisopropylpenicillins and phenoxyisobutylpenicillins show the highest stability toward β-lactamase-forming staphylococci. Such a sterically hindered group in the side chain may act by interfering with the enzyme attachment (8, 9).

Doyle *et al.* (92, 93) showed that marked antistaphylococcal β-lactamase activity is obtained when the penicillins are prepared from six-membered aromatic or heterocyclic carboxylic acids substituted in both *ortho*-positions with relatively bulky groups. Table XIV illustrates the structural requirements of the side-chain group of several types of penicillins for staphylococcal β-lactamase resistance. Triphenylmethylpenicillin (Compound 1) (344) was noted to have pronounced stability toward β-lactamase-forming staphylococci and *B. cereus*. Omission of one of the phenyl groups (Compound 2) or interpolation of a methyl group, as in β,β,-β-triphenylethylpenicillin (Compound 3), results in a β-lactamase-sensitive compound. 2,6-Dimethoxyphenylpenicillin, known as methicillin (Compound 4) (93), was the first of a series of penicillins proved to be effective and useful against staphylococcal infection, while 2,4-dimethoxyphenylpenicillin (Compound 5) and 2,6-dimethoxybenzylpenicillin (Compound 6) were found to be β-lactamase susceptible. Several methicillin analogs that possess a sterically hindered ring, such as 2-alkoxy-1-naphthylpenicillin (345), are stable toward β-lactamase. For example, 2-ethoxy-1-naphthylpenicillin (known as nafcillin) (Compound 7) and 2-methoxy-1-naphthylpenicillin (345, 346) are β-lactamase stable. Other similar forms, such as 2-methoxy-3-naphthylpenicillin (Compound 8) and 1-methoxy-2-naphthylpenicillin (Compound 9), are unstable toward the enzyme.

2-Biphenylpenicillin (Compound 10) has been shown to be 3–5 times more stable toward staphylococcal β-lactamase than methicillin. Substitution in either of the two aromatic rings results in a loss of activity (347). To exert maximal antistaphylococcal activity, the phenyl Ring A (proximal to the penicillin nucleus) must be directly attached to the side-chain peptide linkage, while Ring B (distal to the penicillin nucleus) must occupy the *ortho*-position of Ring A (Compound 10). Accordingly, the 2-biphenylmethylpenicillin, 3-biphenylpenicillin, and 4-biphenylpenicillin (Compounds 11, 12, and 13, respectively) are all unstable toward staphylococcal β-lactamase. If Ring A is changed to cyclohexyl or a bridged ring instead of an aromatic ring, the antistaphylococcal activity is lowered about 20–1000 times compared to that of 2-biphenylpenicillin (348); for example, *endo*-phenyl-*exo*-carboxamidopenicillin (Compound 14) and its analog are β-lactamase unstable. However, the penicillinase-resistant property will be retained if Ring A is replaced by a heterocyclic ring with sufficient size and aromaticity and provided there is a phenyl or an equivalent substituent adjacent to the side-chain peptide linkage. Ring B can be replaced by an aromatic heterocyclic system without losing activity. Also, replacement of the phenyl rings with α- or β-naphthyl groups results in no drastic loss of activity (349).

Some 3,5-disubstituted-4-isoxazolylpenicillins are also highly stable toward staphylococcal β-lactamase (93,

222). One example is 5-methyl-3-phenyl-4-isoxazolylpenicillin, known as oxacillin (Compound 15) (222), which was the first of several isoxazolylpenicillins used against Gram-positive penicillin-resistant organisms. Its 2-chlorophenyl and 2,6-dichlorophenyl analogs, known as cloxacillin and dicloxacillin, are particularly effective against β-lactamase-forming staphylococci. In contrast, 5-phenyl-4-isoxazolylpenicillin, 3,5-dimethyl-4-isoxazolylpenicillin, and 3-phenyl-4-isoxazolylpenicillin (Compounds 16, 17, and 18, respectively) are all unstable to staphylococcal β-lactamase (5, 8, 9).

Naito *et al.* (350) reported that some of the 3,5-disubstituted isothiazolylpenicillins have similar or improved activity compared to oxacillin. 5-Methyl-3-phenyl-4-isothiazolylpenicillin (Compound 19) and its 2,6-dichlorosubstituted compounds (not shown) have significantly greater stability toward staphylococcal β-lactamase than either oxacillin or dicloxacillin.

6-Aminopenicillanic acid derivatives prepared with vicinal dicarboxylic acids, such as quinoxacillin-2,3-dicarboxylic acid, known as quinacillin (Compound 20), are effective against penicillin-resistant staphylococci (203, 221). Other dicarboxylic acids, such as quinoline-2,3-dicarboxylic acid, have activity similar to quinacillin, but not the derivatives from pyrazin-2,3-dicarboxylic acid (Compound 21) or pyridine-2,3-dicarboxylic acids. Penicillins derived from cycloheptatriene carboxylic acid (351) and benznorcaradiene carboxylic acid (352) (Compound 22) show little β-lactamase resistance in comparison to methicillin or other β-lactamase-resistant penicillins.

**β-Lactamase Resistance of Cephalosporins**—Cephalosporin C and its derivatives, such as cephalothin and cephaloglycin (235), are effective against most β-lactamase-forming staphylococci and *B. cereus* and are moderately active against some coliform bacilli (3, 342, 353, 354). In addition, cephalosporin C is able to protect penicillin G from inactivation by these enzymes (3, 115). The resistance of cephalosporin C to staphylococcal penicillinases apparently is related more closely to the nucleus than to the nature of the side chain, since cephalosporin N (penicillin N) is very labile toward these β-lactamases (3, 115). Crompton *et al.* (42) showed that desacetylcephalosporin C and cephalosporin C itself are the most resistant toward staphylococcal β-lactamases, while the pyridine derivative (cephalosporin $C_A$) is less stable and the lactone derivative is the least stable. Apparently the nature of the side chain at C-3 in cephalosporin C as well as in other cephalosporins must also play an important role in staphylococcal β-lactamase stability.

Data obtained from the study of methicillin and its cephalosporin analogs against the β-lactamases from Gram-negative organisms indicate that the structure of the substitution in the 7-position of cephalosporin, like in penicillins, is the main determining factor in the resistance or inhibition of the β-lactamase (355). However, the structure of the group in the 3-position of cephalosporin modifies this character; it is suggested that a labile group in the C-3 position which can easily accept electrons from β-lactam conjugation gives the cephalosporin a higher affinity for the bacterial enzyme and thus increases its effectiveness as a competitive inhibitor to protect a susceptible penicillin from being inactivated.

**Kinetic Studies on β-Lactamase Inactivation**—The study of the bacterial β-lactamase inactivation of the β-lactam antibiotics from an enzymic kinetics approach provides valuable information concerning the magnitude of the interaction between enzyme and substrate, the possible mechanism of inactivation, and the inhibition of enzyme activity by substrate analog or competitive inhibitor (309, 356–360). The β-lactamases, like other biologically important enzymes, have been shown to be extremely specific and highly dependent on: (*a*) source and preparation, (*b*) experimental conditions, (*c*) accessibility of substrate, and (*d*) nature of the enzyme–substrate complex.

According to the conventional Michaelis–Menten expression (361), $V_{max.}$ is the maximum velocity in the presence of excess substrate, and $K_m$ is the Michaelis constant, representing the dissociation constant of the enzyme–substrate complex. This constant, $K_m$, is inversely proportional to the affinity of the enzyme for the substrate (penicillin or cephalosporin). The enzyme (β-lactamase) and the substrate react to form an intermediate enzyme–substrate complex, which dissociates to the product and the original enzyme. The initial rate is usually measured over a wide range of substrate concentrations. From steady-state treatment, the $V_{max.}$ and $K_m$ can normally be obtained by using Lineweaver–Burk (362) plots.

The efficiency of the β-lactamase can be expressed in terms of either the speed or the specificity of the reaction that they catalyze. The relative physiological efficiency of the activity of a β-lactamase can be indicated by the value of the product of its $V_{max.}$ and $K_m$ with any substrate (323). The effectiveness of a competitive inhibitor in preventing the activity of a particular β-lactamase is determined from the ratio of the inhibitor to the substrate required for 50% inhibition of the initial rate of hydrolysis (42, 363).

*β-Lactamase Kinetics*—The kinetics of the penicillinase from *B. cereus* and *S. aureus* have been studied in great detail. In 1946, Henry and Housewright (364) found that the β-lactamase *B. cereus* 569 inactivates penicillins G and X at an approximately equal rate and penicillins F and K at a somewhat slower rate. The reactions were pseudo zero order in the presence of saturating concentrations of the substrate. The apparent energy of activation for the hydrolysis of penicillin G was about 7.5 kcal./mole.

Abraham and Newton (357) reported their manometrically obtained data on the *B. cereus* NRRL569 hydrolysis of penicillins V, G, and N; synnematin B; and cephalosporin C at pH 7 and 30°. Compared to penicillin G, penicillin V was hydrolyzed 1.25 times as fast; penicillin N and synnematin B were hydrolyzed 0.54 times as fast. Under identical conditions, cephalosporin C underwent no hydrolysis. The energies of activation were 5.3 and 5.7 kcal./mole for penicillins G and N, respectively, compared to 14.5 kcal./mole for the nonenzymic hydrolysis of penicillin G in dilute sodium hydroxide solution. Cephalosporin C behaved as a competitive inhibitor of the activity of β-lactamase from *B. cereus*.

The interaction of *B. cereus* 569/H with several penicillins and cephalosporins was explored by Citri and coworkers (365–370). The conformation of this extracellular enzyme can be reversibly modified in various ways. A conformational change in its active site was reflected, for instance, in an increased susceptibility to iodination, urea, and heat. The activity of the enzyme was found to depend on a definite and precise orientation of the active site, which was distorted by competitive inhibitors (358). The relative rates of hydrolysis of the penicillins and cephalosporins at pH 6 and 30° were: ampicillin, 120; phenoxyalkylpenicillins, 60–130; penicillin G, 100; 6-aminopenicillanic acid, 40; ancillin, 28; nafcillin, 17; oxacillin, 5; cloxacillin, 3; cephaloridine, 2; and 7-aminocephalosporanic acid = cephalosporin C = benzylcephalosporin = cephalothin, <0.1. All the competitive inhibitors exerted a powerful effect on the conformation of the active site of the enzyme (369), an effect described as "conformative response" (370).

Kuwabara and Abraham (371) studied the behavior of purified enzyme from *B. cereus* 569/H with regard to several penicillins and cephalosporins. They were able to separate the β-lactamase I (penicillinase) from the β-lactamase II (cephalosporinase) in a crude enzyme culture.

The kinetics of interaction of the β-lactamase from *B. subtilis* and penicillin G were investigated by Banfield (372) by an alkalimetric titration technique at varied pH, temperature, and ionic strength. From Lineweaver–Burk (362) plots, both the $K_m$ and $V_{max.}$ values varied as the pH was increased from about 5.5 to 8.5. The heat of activation was approximately 5.7 kcal./mole at pH 7.14. Varying the ionic strength through the range 0.002–0.08 was found to have little effect on the reaction rate.

Novick (373) investigated the kinetics of several penicillins with both cell-bound and free staphylococcal β-lactamase. Measurements of the Michaelis constant revealed that the effectiveness of β-lactamase resistance is inversely proportional to the affinity of the β-lactam compounds for the enzyme. Methicillin, with a $K_m$ of $2.8 \times 10^{-2}$ *M*, is by far the most resistant. The $K_m$ of other penicillins ranges from $3.8 \times 10^{-6}$ *M* (penicillin V) to $17 \times 10^{-6}$ *M* (propicillin), and these penicillins are unstable toward the enzyme. Usually, higher $V_{max.}$ and lower $K_m$ values are obtained from the free than from the cell-bound form of penicillinase.

Gourevitch *et al.* (374) compared the $V_{max.}$ and $K_m$ values of 11 penicillins with their *in vitro* MIC values against both penicillin-sensitive and penicillin-resistant staphylococci. In accordance with their data, they classified the penicillins into three groups. The nonpenicillinase-resistant compound had very high $V_{max.}$ and low (about $4–10 \times 10^{-5}$ *M*) $K_m$ values and a large difference in MIC against the two types of staphylococci. Penicillin G and the phenoxyalkylpenicillins belong to this group. The moderately resistant penicillins have a $K_m$ 30–80 times larger (about $2–3 \times 10^{-3}$ *M*) than that of the nonresistant penicillins, although the rates of reaction are about 20–50% lower and the MIC values for the two groups are fairly close. α-Phenoxyisobutylpenicillin and 2-(*N*-ethyl-*N*-phenylcarbamoyl)-phenylpenicillin belong to this group. The resistant penicillins are characterized by very low $V_{max.}$ and very high (about $2–5.4 \times 10^{-2}$ *M*) $K_m$ values and a nearly identical MIC against sensitive and resistant staphylococci. Diphenicillin, methicillin, and the isoxazolylpenicillins belong to this group.

Knox and Smith (375) found that methicillin was the best inducer for staphylococcal penicillinase. Compared to the rate of hydrolysis of penicillin G, this enzyme hydrolyzed ampicillin about twice as fast; phenethicillin, penicillin V, and propicillin were hydrolyzed at about the same rate; 6-aminopenicillanic acid about 80 times slower; and methicillin about 120 times slower. The $K_m$ obtained in cell-free staphylococcal β-lactamase is usually about 20 μ*M* for penicillin G and 50,000 μ*M* for methicillin, but only one-fifth of these values is obtained with either penicillin in the case of the cell-bound enzyme (376). DePue *et al.* (377) pointed out that the Michaelis constants of the penicillins are relatively little affected by interchange of the phenyl, benzyl, or aliphatic side chains. However, substitution of a side chain having an amino group (as in 6-aminopenicillanic acid, ampicillin, or *p*-aminobenzylpenicillin) or a nitro group caused a marked increase in $K_m$ values or a lowering of the affinity for the enzyme. This drastic change in $K_m$ values suggests that the electron-attracting nature of the side chain plays an important role in the formation of the enzyme–substrate complex.

Staphylococcal exopenicillinase was further investigated by Dyke (378) at pH 7.0 and 35°. The Michaelis constants for several penicillins were found to be in the order of: penicillin G (2.5 μ*M*) < cloxacillin (0.35 m*M*) < quinacillin (1.5 m*M*) < methicillin (24 m*M*). The $V_{max.}$, in comparison to that of penicillin G (1.0), was methicillin (1.54) > quinacillin (0.85) > cloxacillin (0.43). No change in $K_m$ was observed for hydrolysis of quinacillin at a pH of 7.0, 5.9, or 4.8, but the maximum rate was about 10 times higher at pH 4.8 than at pH 7.0.

Studies on β-lactamase from Gram-negative bacteria opened another area of interesting enzymic kinetics. Jago *et al.* (333) reported that the enzyme *Ps. pyocyanea* NCYC-8023 hydrolyzes cephalosporin C faster than it does benzylpenicillin. Similarly, Sabath *et al.* (305) pointed out that the $V_{max.}$ of hydrolysis of cephalosporin C and several other cephalosporins by a crude extract of this enzyme was about 5 times as high as that of penicillin G. Methicillin, 6-aminopenicillanic acid, 7-aminocephalosporanic acid, and cloxacillin were all resistant to hydrolysis. Methicillin and cloxacillin were also powerful competitive inhibitors protecting cephalosporin C against inactivation.

Hamilton-Miller and coworkers reported broad studies (379, 380) on the β-lactamases from Gram-negative organisms. Toward the β-lactamases from several strains of *K. aerogenes*, penicillin G and ampicillin were more labile than cephaloridine and other cephalosporins, and cephaloridine was hydrolyzed at a relatively higher rate than cephaloram, cephalothin, or cephalosporin C. One strain of *A. cloacae* resembled *Klebsiella* in its activity toward cephalosporins. Strains of *E. coli* that do not contain an R-factor also had homogeneous specificity profiles; that is, all cephalo-

Table XV—Relative Rates of Hydrolysis of β-Lactam Antibiotics by Several β-Lactamases[a] (Maximum Rate, $V_{max.}$, of Hydrolysis of Penicillin G = 100)

| | β-Lactamase | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Penicillin β-Lactamase | | | | | | Cephalosporin β-Lactamase | | | |
| Antibiotics | *S. aureus* | *B. cereus* β-Lactamase I | *Klebsiella* sp. | *Pr. mirabilis* | *Aerobacter cloacae* | *B. cereus* β-Lactamase II | *E. coli* | *Pr. rettgeri* | *Pr. morganii* | *Ps. aeruginosa* |
| Penicillin V | 95–160 | 102–125 | 85–119 | — | 60–70 | — | **50–82** | — | **38–44** | **76–79** |
| Phenethicillin | 90–100 | 103 | — | — | 57–100 | — | **<10** | — | — | **<1.0** |
| Propicillin | 60–77 | 56 | — | — | 40–45 | — | **<10** | — | — | — |
| Phenbenicillin | 167 | 91 | — | — | 49–64 | — | **<10** | — | — | — |
| 6-APA | <0.1 | 40 | 31–34 | 4 | 14 | — | **5–22** | — | — | **<1.0** |
| Ampicillin | 120–200 | 100–120 | 182–287 | 9 | 100–120 | 64 | **3** | — | — | **2–14** |
| Methicillin | **0.02–5.0** | **<0.1–3** | **<10** | **<0.1** | **<0.1** | 89 | **7** | — | — | **<1.0** |
| Oxacillin | **<1.0–7.0** | **<0.1–5** | **<10** | **<0.1** | **20–40** | 89 | **2–<10** | — | — | **<1.0** |
| Cloxacillin | **0.1–3.5** | **<0.1–3** | **<10** | **0.1** | **<10** | 89 | **1.0** | — | — | **<1.0** |
| Nafcillin | — | **17** | — | — | — | — | — | — | — | — |
| 7-ACA | — | **<0.1** | — | — | — | — | — | — | — | <1.0 |
| Cephalosporin C | **<0.1–1.2** | **<0.1** | **2–14** | **<2.0** | **4** | 80 | 400–1250 | 6040 | 1010–1090 | 614 |
| Cephaloram | **0.1** | **<0.1** | **10–20** | **<2.0** | **15** | — | 360–560 | 1300 | 371–372 | 573 |
| Cephalothin | **0.01** | **<0.1** | **4–15** | **<2.0** | **15** | 89 | 350–515 | 2320 | 205–233 | 420–450 |
| Cephaloridine | **0.2** | **<0.1–2** | **45** | **<2.0** | **64** | 41 | 172–232 | 2280 | 300–350 | 700 |
| Cephaloglycin | — | **<0.1** | — | — | — | 50 | — | — | — | — |
| References | 3, 309, 312, 342, 374, 377, 378 | 3, 342, 357, 369, 371 | 3, 309, 379, 380 | 3, 309 | 342, 379, 380 | 3, 371 | 312, 342, 379, 380 | 379, 380 | 309, 379, 380 | 305, 309 |

[a] The maximum rate constant can be varied drastically in accordance with the temperature, pH, and buffer of the system used as well as the origin and preparation of the enzyme. The boldface numbers indicate the hydrolytic rates are very slow in comparison with that of penicillin G.

sporins tested were more labile than penicillin G toward these β-lactamases. *Pr. morganii* strains were found to hydrolyze cephalothin, cephaloridine, and cephaloram about 2–3 times faster than penicillin, while *Pr. rettgeri* hydrolyzed all cephalosporins about 13–60 times faster than penicillin G. The β-lactamases from the Gram-negative bacteria studied, *i.e.*, the *E. coli* and *Proteus* strain, preferentially attacked the cephalosporins, while those from *Klebsiella* sp. were found to inactivate the penicillins (380).

The maximum rates of inactivation of penicillins and cephalosporins by known β-lactamases of different origin are listed in Table XV. The wide variation in reported rates is due mainly to the great difference in origins and preparations of the enzymes and in assay techniques, as well as to the lack of uniformity of the experimental conditions. Nevertheless, the study of β-lactamase kinetics to date is far from complete. Particularly lacking is knowledge on the mechanism of β-lactamase inactivation of different penicillins and cephalosporins, the effect of pH on the affinity of a substrate for the enzyme, and the nature of the active site.

*Inhibition of β-Lactamase*—Methicillin and the isoxazolylpenicillins that are relatively resistant to hydrolysis by penicillinases from *B. cereus*, *S. aureus*, *B. licheniformis*, and *K. aerogenes* have been reported to be capable of inhibiting the inactivation of penicillin G by these enzymes (42, 340, 358, 359, 381, 382).

Hamilton-Miller and Smith (383) reported that neither methicillin, cephalosporin C, nor the isoxazolylpenicillins were found to exert any measurable inhibitory effect on the staphylococcal β-lactamase. However, methicillin did inhibit the hydrolysis of penicillin G, the phenoxyalkylpenicillins, phenbenicillin, and ampicillin by the penicillinase from *B. cereus* as well as that from *B. licheniformis*. Cephalosporin C had the same effect on *B. cereus* penicillinase. For a given amount of enzyme, the inhibition was proportional to the logarithm of the inhibitor concentration. The Gram-negative coliform enzymes are much more susceptible to inhibition than are those of Gram-positive origin (383). Compounds such as the isoxazolylpenicillins are very resistant to β-lactamases from *Pr. morganii* and *E. coli*. Against *K. aerogenes* β-lactamase, methicillin has a greater inhibitory effect than the isoxazolylpenicillins (383); against β-lactamase from *Ps. pyocyanea*, both methicillin and cloxacillin are powerful inhibitors (333, 384).

β-Lactamases from strains of *A. aerogenes*, *K. aerogenes*, *Pr. morganii*, *Pr. rettgeri*, *Pr. vulgaris*, and *Ps. aeruginosa* can readily inactivate all the cephalosporins (385). However, the inactivation, particularly that of cephaloridine, is inhibited in the presence of methicillin or cloxacillin. The magnitude of enzyme inhibition depends directly on the nature of the 7-acyl group. It was shown that the cephalosporin analog of methicillin is about 7 times more effective than methicillin itself in protecting cephaloridine against *A. aerogenes* (385).

The interaction of the competitive inhibitor with the enzyme (penicillin β-lactamase) leads to the formation of an inhibitor–enzyme complex. The bulky side-chain group or steric effect of these inhibitors causes repulsion, resulting in a conformational change in the enzyme, with a progressive loss of activity (309). Cephalosporins exert an inhibitory effect toward penicillin β-lactamase which may be due to the geometry

of their nucleus (7-aminocephalosporanic acid), which is considered to be unfavorable to attack by the penicillinases (3).

Reports have appeared in the literature (386–392) on the protection of penicillin G against penicillinase-catalyzed hydrolysis in the presence of certain kinds of compounds, including sodium sulfanilate, benzoic acid, imidazoles, 2-aminomethylthiazole, histamine, sodium benzoate, *p*-aminobenzoic acid, sulfanilic acid, salicylic acid, pyrogallol, quinine, chloroquine, quinacrine, proteolytic enzymes of trypsin and chymotrypsin, and some surface-active agents such as sodium tridecyl-2-sulfate and sodium diodecylbenzene sulfonate. Except at very high concentrations (about 1 m*M*), sodium or potassium chloride, nitrate, sulfate, phosphate, and acetate ions showed no measurable inhibitory effect on the activity of β-lactamases isolated from *E. coli* harboring transmissible R-factor, $R_{GN238}$ and $R_{GN14}$ (393). To date, no full investigations have been carried out on the magnitude and mechanism of inhibition of β-lactamases by these chemical agents and no attempt has apparently been made to compare their actions with those exerted by the resistant penicillins.

**Synergistic Activity and Increasing Usefulness of Antibiotics**—The combined use of antibiotics may sometimes result in a synergistic effect, as shown earlier in the case of cephalosporin C, which is a competitive inhibitor of *B. cereus* β-lactamase and protects penicillin G from being hydrolyzed by this enzyme (357). The penicillin G–cephalosporin C pair also acts synergistically against *S. aureus* (354).

Several studies have shown ampicillin to be synergistic with other antibiotics. In combination with methicillin, it had either an additive or a synergistic effect against a series of 17 methicillin-resistant staphylococcal strains (394). A mixture of ampicillin or penicillin G with methicillin or one of the isoxazolylpenicillins showed synergistic action against several Gram-negative bacteria, namely *E. coli*, *A. aerogenes*, *Proteus* sp., *Ps. aeruginosa*, and *K. pneumoniae*. For example, against *E. coli* R136, ampicillin alone was inhibitory only when the concentration reached 25 mcg./ml., and cloxacillin alone was without effect at a concentration of 10 mcg./ml.; together, however, 10 mcg./ml. of cloxacillin and only 2.5 mcg. of ampicillin were inhibitory (395). In the treatment of mice infected with *Shigella flexneri*, a combination of ampicillin and dicloxacillin showed significant synergism (396). This combination also showed significant synergism against strains of *E. coli* resistant to both of these penicillins (397).

Against *Pr. mirabilis*, the best antibiotic combination was found to be ampicillin and gentamicin; this pair was considerably more effective than gentamicin–penicillin G, ampicillin–kanamycin, or several others, all of which exhibited some synergistic action (398). Against *Pr. morganii*, ampicillin and cloxacillin were synergistic (399). *Ps. aeruginosa* was inhibited fairly well with a combination of ampicillin and oxacillin (400). In urinary tract infections where patients had a significant bacteriuria, a combination of ampicillin or penicillin G with nafcillin, methicillin, or one of the isoxazolylpenicillins produced a prolonged antibacterial activity against *Ps. aeruginosa*, *E. coli*, the *Enterobacter* group, and *Proteus* sp. (401, 402).

Other synergistic combinations include carbenicillin and gentamicin, which demonstrated a striking bactericidal effect against *Ps. aeruginosa*, whereas neither drug alone was effective in low dosage (403). Another such combination is penicillin G and dicloxacillin against *Sh. flexneri*. High levels of these antibiotics (200 and 250 mcg./ml., respectively) are required to inhibit this organism when the drugs are used alone, but in combination only 20 mcg./ml. of each demonstrated complete inhibition. For synergism against *Shigella* β-lactamase, it is essential that the inhibitor and substrate have a very different affinity for it (396).

The observed synergism through the combined use of antibiotics may be due to the following mechanisms (404): (*a*) a competitive inhibition of the inhibitor for the β-lactamases, involving either a tie-up of the active site or the rendering of a conformational change of the enzyme (366–370); (*b*) the effect of a favorable pH of the medium; and (*c*) a delayed excretion of the antibiotics; for example, probenecid has negligible antibacterial activity, but if given with a penicillin or cephalosporin, the serum levels of the antibiotic are notably increased and prolonged, thus indirectly resulting in a sustained antibacterial activity (405, 406).

Jawetz (407) objected to the combined use of antibiotics because: (*a*) adequate information is not available concerning the mechanism of synergy; (*b*) the use of a combination may encourage the emergence of drug-resistant organisms; and (*c*) adverse reactions may be lessened by using the individual drugs. Others have strongly favored synergistic combinations, arguing that the proper use of antibiotics in combination is not only scientifically sound but also often lifesaving (408). Several examples of the rational use of combinations have been reported. For instance, best results were obtained with a penicillin G–streptomycin combination in the treatment of bacteremia or subacute bacterial endocarditis (SBE) caused by either *Strep. viridans* or *Streptococcus faecalis*. The penicillin G–erythromycin pair was also very effective against strains of *S. aureus* highly resistant to both antibiotics. Cephalothin–streptomycin was shown to be effective for severe urinary tract infections and SBE due to *Strep. faecalis* (408, 409). A carbenicillin–gentamicin combination demonstrated synergistic activity against severe infections caused by *Ps. aeruginosa* (408).

To maximize the activity of the antibiotic combination, the pH of the medium should also be adjusted to the most favorable value (410). Ampicillin was shown to be about 10 times more active against *E. coli* and *Strep. faecalis* at a pH of 5.5 than at pH 8 (206). The MIC of streptomycin against *S. aureus* was about 100 times less at pH 5.2 than at pH 7.7 (411). Similarly, it was recently reported that penicillin G, ampicillin, cephalothin, cephaloridine, and novobiocin are considerably more active against strains of *Streptococcus* (*Enterococcus*) at pH 5.0 than in neutral or alkaline (pH 8.5) media (412). On the other hand, lincomycin, clindimycin, erythromycin, and gentamicin are moderately to markedly more active at pH 8.5 than in acidic (pH 5.0) media. This clearly indicates that

different molecular forms of an antibiotic (ionized, unionized, or zwitterion) may exert a drastically different antibacterial action (412, 413). Varying the pH may thus change the charge of the susceptible receptor on the bacterial cell, consequently altering its affinity for, or susceptibility to, the antimicrobial agent (413). Accordingly, for proper therapy of urinary tract infection, an adjunctive alkalinizing (410, 414) or acidizing medication may be required to maintain the urine at the optimal pH (412).

## CONCLUSION

The semisynthetic β-lactam antibiotics have provided outstanding examples of how naturally occurring medicinal agents can be improved by molecular modification. Although the art of semisynthesis has overshadowed that of total chemical synthesis, the latter certainly has great potential, because it may lead to the exploration of the fundamental structure of other novel antibiotics having an even broader spectrum than the presently available penicillins and cephalosporins (3, 175). Another important goal for drug research is the development of new antibiotics that are effective against infectious diseases caused by protozoa and viruses as well as by bacteria.

The fundamental structural requirements of the β-lactam antibiotics include: (*a*) the fused β-lactam structure; (*b*) a free carboxyl group; and (*c*) one or more properly substituted amino side-chain groups (3–9). The expansion of the thiazolidine ring (as in cephalosporins) does not destroy activity as long as a free carboxyl group is retained in the same position (415). Although the role of this free carboxyl group in the overall bactericidal activity is not clear, it is known that the nucleus of penicillin and cephalosporin must contain a free carboxylate ion. The *N*-condensation side chain apparently is the key group governing the hydrophilic hydrophobic character as well as the overall spatial relationship within the two-ring system, which in turn affects not only the antibacterial activity but also the stability of the compounds.

In general, penicillins kill Gram-positive bacteria more effectively than they do Gram-negative bacteria (416–418). They destroy the dividing rather than the resting cell and induce a morphological alteration in the treated culture (415–417). The particular lability of Gram-positive organisms is due to the fact that their cell wall structure is relatively simple and devoid of lipids in comparison with that of the Gram-negative bacteria. Penicillins directly interfere with the glycopeptide cross-linking reaction and thus inhibit the synthesis of the bacterial cell wall. The relative insensitivity of *E. coli* to penicillin G and similar penicillins is probably due to failure of the penicillin to reach the sensitive site of the organism. In contrast, the greater bactericidal effect of ampicillin and other broad-spectrum analogs toward the Gram-negative bacteria is due to the greater ability of these drugs to penetrate the cell wall (416, 417). A mechanistic explanation has also been advanced, *i.e.*, that the antimicrobial activity of the energetic β-lactam ring is directly related to the inactivation or inhibition, *via* acylation, of the cell wall-synthesizing enzyme (transpeptidase) of the bacteria (418).

In studies on the kinetics and mechanisms of action of the β-lactamases, the aim is to gain first-hand information on the chemical nature of the catalysis. The search for more effective β-lactamase inhibitors will no doubt continue.

Although many of the criteria for the "ideal" or "all-purpose" penicillin and/or cephalosporin have been attained, a greater problem—that of allergenicity—remains unsolved. From immunological studies on the mechanism of allergenicity, it has been shown that the penicilloylated and cephalosporoylated protein impurity in the natural penicillins and cephalosporins may be largely responsible for the induction of hypersensitivity and anaphylactic reactions in patients treated with these drugs (419–424). Recently it was demonstrated that some dosage forms of penicillins G and V contain a small amount of highly antigenic penicilloylated protein (423) and that removal of this impurity reduces the incidence of allergic responses in sensitive patients (422, 424–426).

Besides the proteinaceous contaminants, the β-lactam antibiotics contain polymers that are formed in solution by internal reactions between the intact and degraded molecules (427, 428). The polymerization normally takes place as a chain reaction, initiated by the degradation product and influenced by pH, temperature, oxygenation, and other factors known to increase conversion to penicillenic acid (429).

When purified, either by dialysis, molecular sieving, or chromatography, penicillins have been tolerated without adverse reaction by patients whose hypersensitivity to the unpurified compounds was proven. Thus, to eliminate penicillin and cephalosporin allergenicity, the final product must be pure and free of contaminants. Obviously, the pharmaceutical formulator must remain aware of the possible degradations of the β-lactam antibiotics and must exercise due care in the design of new dosage forms to ensure that no adjuvant is added that could interact to produce allergenic by-products (430).

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