CONFORMATION AND ACTIVITY OF β-LACTAM ANTIBIOTICS

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I. INTRODUCTION

The β-lactam antibiotics are unique among antibacterial agents for their broad spectrum of activity and low toxicity. It is now well established[1-6] that these antibiotics exert their antibacterial action by interfering in the final stages of the biosynthesis of peptidoglycan, which is an important constituent of bacterial cell wall. However, in recent years the use of these antibiotics has been limited by the emergence of resistant bacteria. These strains achieve their resistance mainly by producing β-lactamase enzymes which inactivate the antibiotic molecules by hydrolyzing the lactam peptide bond.[7-9] This necessitates a constant need for the design of drugs active against resistant strains also. Hence, a knowledge of the conformational manifolds of the β-lactam drug molecules becomes important for uncovering their mode of action at the molecular level. Despite the various mechanisms proposed[1-6,10-13] for their mode of action, a complete understanding has not been reached yet. In recent years, a number of reviews[3-6] have appeared on this subject dealing with chemical and biochemical aspects. Hence this review is limited to the recent studies on the conformations of β-lactam antibiotics and their mode of action.

II. CHEMICAL STRUCTURE OF PEPTIDOGLYCAN

Peptidoglycan is a net-like macromolecule which occurs in almost all bacterial cell walls (Figure 1). It imparts rigidity to the cell wall and protects the organism from high internal osmotic pressure. Interference with the synthesis or hydrolysis of the peptidoglycan layers leads to the formation of a fragile network which fails to provide the necessary osmotic support. Information about the chemical structure and biosynthesis of peptidoglycan is available through the work of several investigators.[3,4]

Peptidoglycan, also known as murein or mucopeptide, is made up of β(1-4) linked repeating units of *N*-acetyl glucosamine (NAG) and *N*-acetyl muramic acid (NAM). The NAM units are linked to short peptide units having the general sequence L-alanyl^1-γ-D-glutamyl^2-L-R^3-D-alanyl^4-D-alanine^5. Wide variations (Figure 2) are observed at positions 1, 2, and 3, but not at 4 and 5. The most common residues at the L-R^3 position are L-lysine and meso- of LL-diaminopimelic acid. These peptide chains cross-link to give a large polymer of high tensile strength. The cross-linkages are formed between the carboxyl group of the penultimate D-alanine and an amino acceptor in a nearby chain, either directly or through bridging peptides whose composition may exhibit variations. During the cross-linking process, the terminal D-alanine is released. The rigidity of peptidoglycan depends upon the extent of such cross-linkages. The direction of transpeptidation (Figures 3 and 4) reaction itself is not uniform among bacteria. In *B.*

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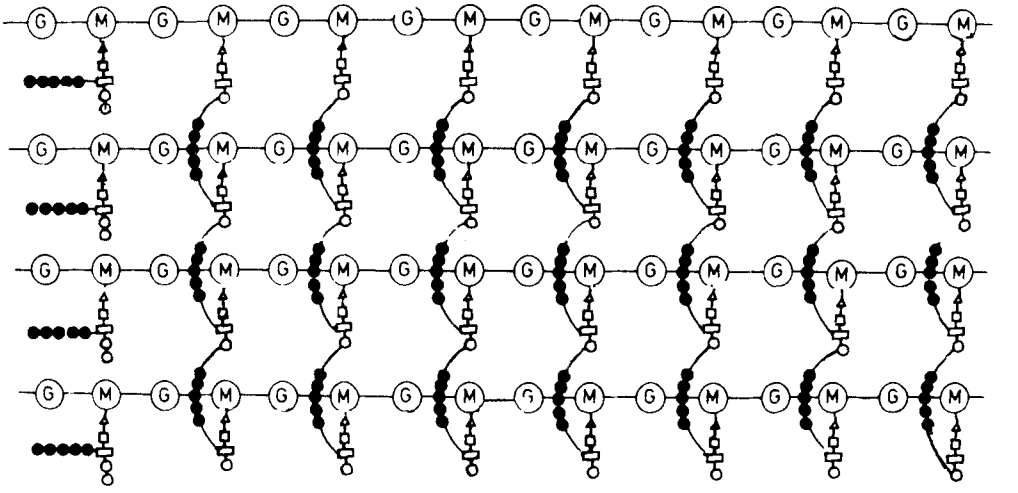
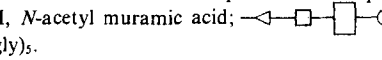
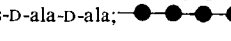


FIGURE 1. Schematic representation of peptidoglycan of *Staphylococcus aureus*. G, *N*-acetyl glucosamine; M, *N*-acetyl muramic acid;  = L-ala-γ-D-glu-L-lys-D-ala-D-ala;  , -(gly)$_5$.

licheniformis[5] for example, transpeptidation proceeds from the nascent peptidoglycan acting as carboxyl donor to the preformed peptidoglycan acting as amino acceptor (Figure 3). This is opposite to the direction of transpeptidation (Figure 4) observed recently in *Gaffkya homari*.[14-17] Transpeptidation can thus proceed in two possible directions.

III. ENZYMES INVOLVED IN BIOSYNTHESIS OF PEPTIDOGLYCAN

The cross-linking between different peptide chains of peptidoglycan is an indispensable process for the bacterial survival and it is carried out by the transpeptidase enzymes. In addition, DD-carboxypeptidases which catalyze the hydrolysis of the terminal D-alanine from the pentapeptide chains are also found to be present in many organisms. However, the role of these DD-carboxypeptidases seems to be different in different organisms. In *B. subtilis* and *B. stearothermophilus*,[18-19] the DD-carboxypeptidase activity appears to limit the extent of cross-linkages, since the resulting tetrapeptides will not be good substrates for the transpeptidase enzyme(s). Hence inhibition of the activity of DD-carboxypeptidase does not result in detectable damages to the cell. On the other hand, in *G. homari*,[14-17] the DD-carboxypeptidase activity is found to be essential for transpeptidation. In this organism, an LD-carboxypeptidase activity which leads to the hydrolytic removal of the penultimate D-alanine is also found to be essential for transpeptidation. This is because, the tripeptides (Figure 4) resulting from the sequential action of DD- and LD-carboxypeptidases are assumed to be substrates for the transpeptidase enzyme(s). Hence, inhibition of either of the carboxypeptidases leads to cell damages.

Besides these transpeptidases and carboxypeptidases, endopeptidases which catalyze the cleavage of the peptide bond formed in the transpeptidation reaction are also found to be present in many organisms.[20] However, the biological significance of these enzymes is not clear. Furthermore, peptidoglycan-degrading activities of autolytic enzymes have also been reported.[21]

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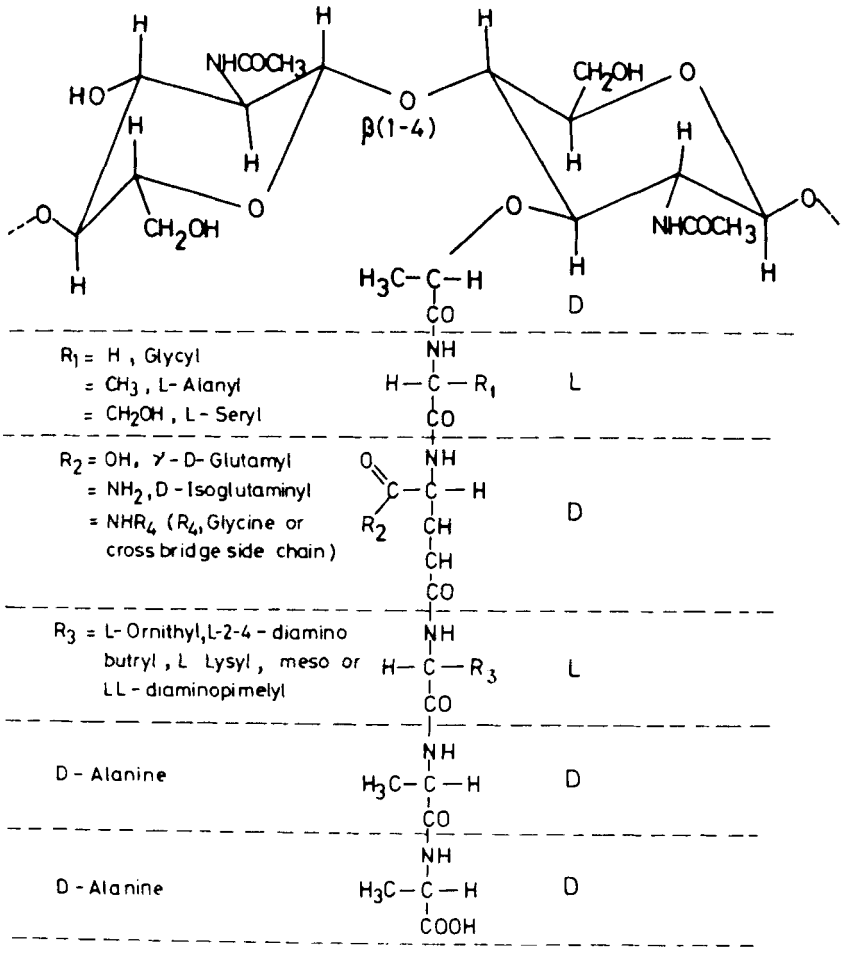

FIGURE 2. The disaccharide-pentapeptide subunit of peptidoglycan and observed variations in the peptide moiety.

IV. CONFORMATIONAL ANALYSIS OF β-LACTAM COMPOUNDS

The β-lactam compounds (Figures 5 to 8) are essentially bicyclic ring systems consisting of a β-lactam to which a five- or six-membered ring is fused. The atoms of the lactam ring essentially lie in a plane and the ring is fairly rigid.[22-33] The five- or six-membered ring fused to the lactam may assume one or more conformations.

A. Conformational Parameters and Their Definitions

The conformation of a five-membered ring (Figure 9) can be specified by two bond angles, β_1 and β_2, and two dihedral angles, α_1 and α_2, which denote rotations about the virtual bonds (C_5-C_2) and (C_2-N_4) provided the five bond distances are assumed to be constant. Since the variations in βs are comparatively small, α_1 and α_2 alone are generally used to specify the conformation of the five-membered ring. The dihedral angle α_3 specifies the orientation of the five-membered ring with respect to the lactam ring. Hence the conformation of the bicyclic ring system can be specified by the three parameters α_1, α_2, and α_3. When a six-membered ring is fused to the lactam ring (Figure 10), one more parameter is necessary to specify the conformations of the bicyclic ring system. In this

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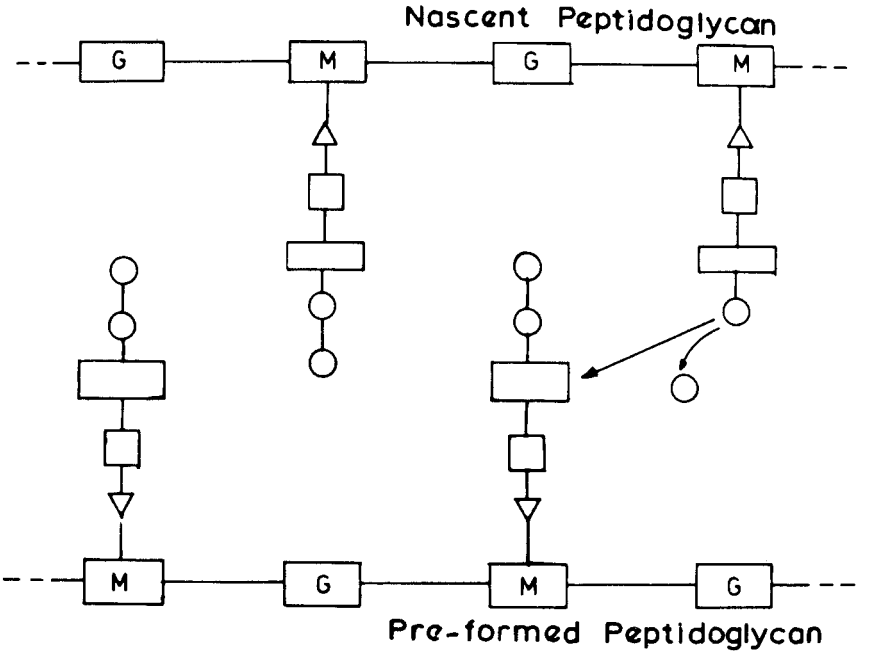

FIGURE 3. Schematic representation of cross-linking reaction in *B. subtilis*. G, *N*-acetyl glucosamine; M, *N*-acetyl muramic acid; —◁—□—□—○—○— = L-ala-γ-D-glu-meso-diaminopimelic acid-D-ala-D-ala.

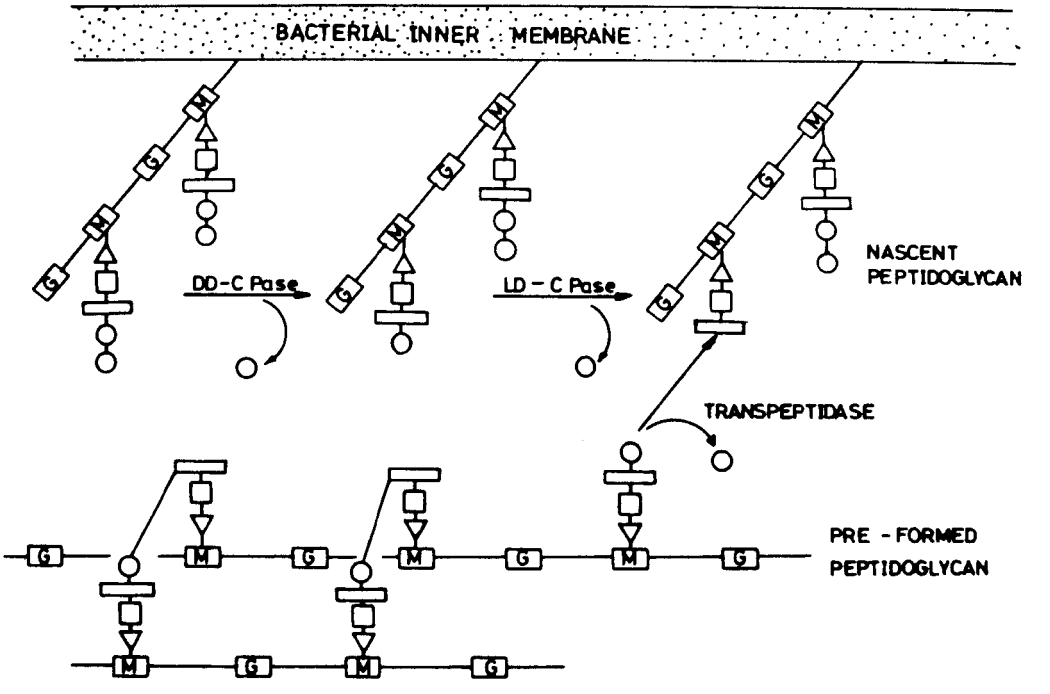

FIGURE 4. Schematic representation of cross-linking reaction in *Gaffkya homari*. G, *N*-acetyl glucosamine; M, *N*-acetyl muramic acid; —◁—□—□—○—○— = L-ala-γ-D-glu-L-lys-D-ala-D-ala; DD-CPase-DD carboxypeptidase; LD-CPase-LD carboxypeptidase.

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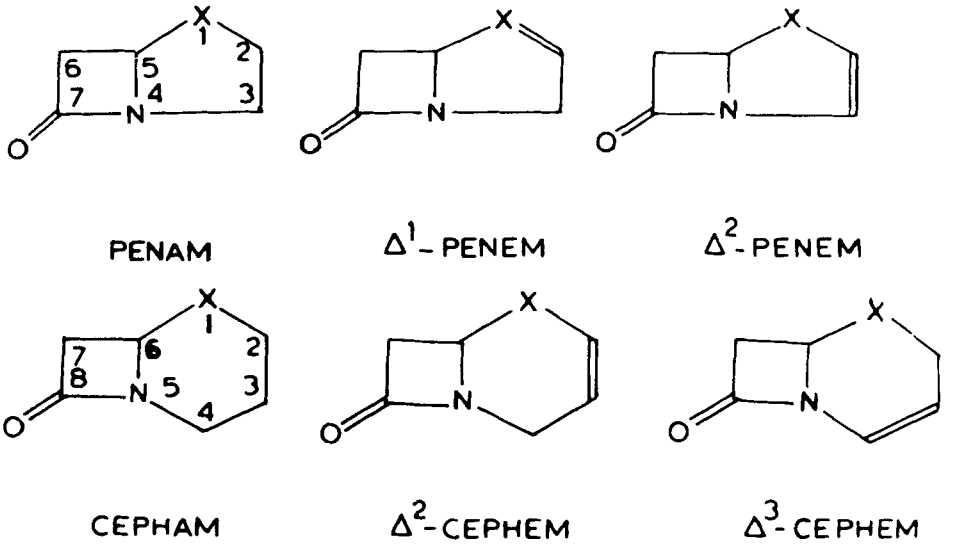

FIGURE 5. General structures of β-lactam compounds.

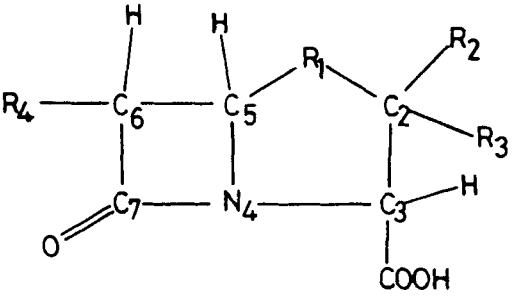

FIGURE 6. Chemical structures of some penicillins.

R_2=R_3=—CH_3; R_4=—H
1. R_1=—S— 2. R_1=—O—
3. R_1=—CH_2—
4. R_1=—SO_2—

R_2=R_3=—CH_3
5. R_1=—S—; R_4=—$NHCOCH_2Ph$
R_1=—S—; R_4=—$NHCOCH_2OPh$
6. R_1=—O—; R_4=—$NHCOCH_2OPh$
7. R_1=—CH_2—; R_4=—$NHCOCH_2Ph$
8. R_1=—SO_2—; R_4=—$NHCOCH_2Ph$

R_2=R_3=—H
9. R_1=—S—; R_4=—$NHCOCH_2OPh$

system, α_1, α_2, and α_3 define the conformation of the six-membered ring and α_4, its orientation with respect to the lactam ring.

The torsional angles ϕ_1, ω, and ϕ_2 represent the orientation of the aminoacyl group, the nonplanarity of the lactam peptide bond, and the orientation of the carboxyl group, respectively. In penicillins (Figure 9) these parameters are defined as follows:

- $\phi_1 = 0$ when the bond C_6-C_7 eclipses the bond N_{12}-C_{13}

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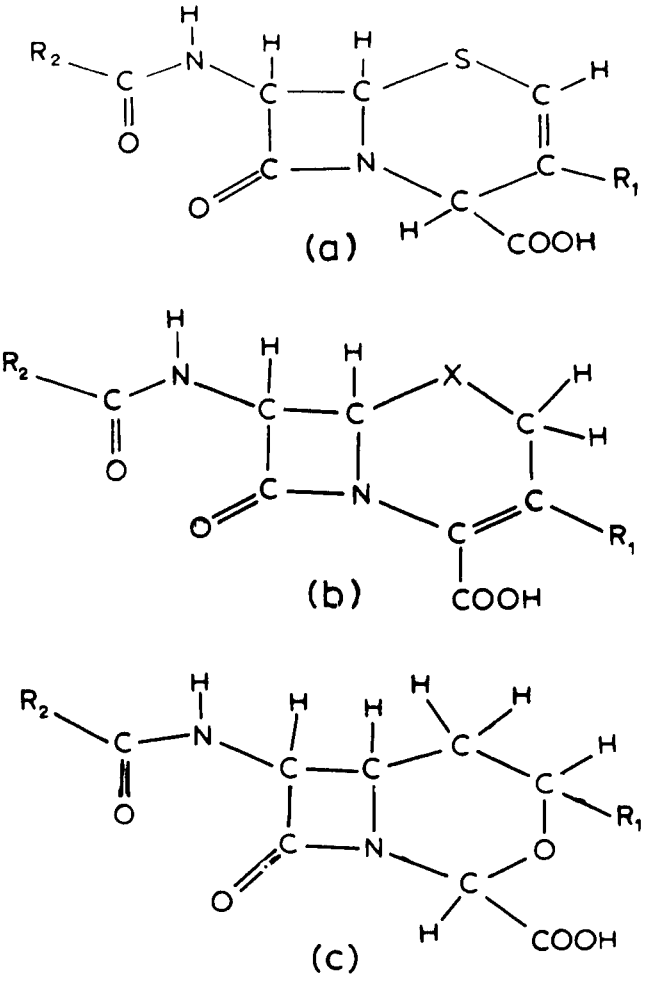

FIGURE 7. Chemical structures of (a) Δ^2-cephalosporin, (b) Δ^3 cephalosporin (X=S or O), and (c) 3-oca-1-dethia cephalosporins. The Rs represent variable groups.

- $\psi_1 = 0$ when the bond C_7-N_4 eclipses the bond C_6-N_{12}
- $\omega = 0$ when the bond N_4-C_3 eclipses the bond C_7-C_6
- $\phi_2 = 0$ when the bond C_3-C_9 eclipses the bond N_4-C_7
- $\psi_2 = 0$ when the bond C_9-O_{10} eclipses the bond C_3-N_4

Similar definitions hold good for the other systems of β-lactam compounds.

From extensive studies, Rao and co-workers[34-44] have assigned the favored conformations of a number of β-lactam compounds by computing their conformational energies using classical potential energy functions.

B. Method of Conformational Analysis

In the conformational analysis of a molecule, initially, the stereochemically allowed conformations are determined by the method of contact criteria developed by Ramachandran and co-workers.[45] Subsequently, potential energy calculations are carried out to find out the relative preferences of the various allowed conformations of

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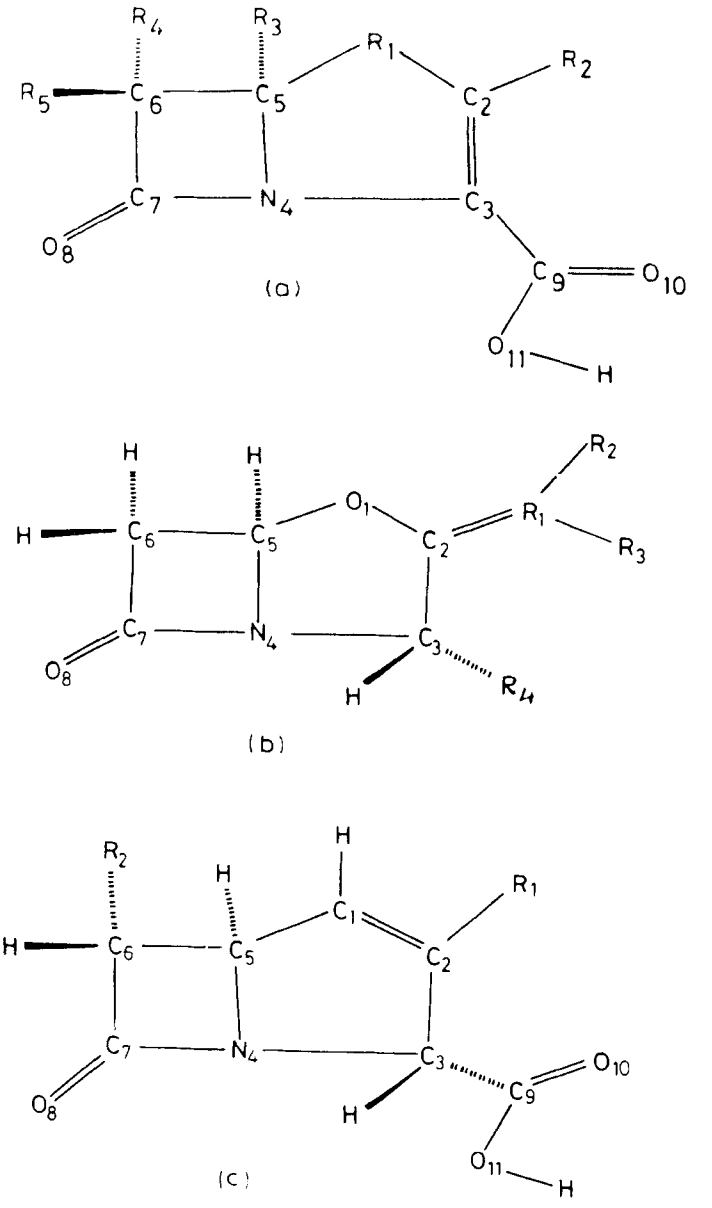

FIGURE 8. Chemical structures of **(a)** Thienamycin nucleus (R_1)=CH_2; R_2=R_3=R_4=R_5=H); Decysteaminyl thienamycin (R_1=CH_2; R_2=R_3=R_5=H; R_4=—CHOH—CH_3); Thienamycin (R_1=CH_2; R_2=—S—CH_2—CH_2—NH_2; R_3=R_5=H; R_4=—CHOH—CH_3); 6-epi-thienamycin (R_1=CH_2; R_2=—S—CH_2—CH_2—NH_2; R_3=R_4=H; R_5—CHOH—CH_3); PS-5 (R_1=CH_2; R_2=—S—CH_2—CH_2—$NHCOCH_3$; R_3=R_5=H; R_4=—CH_2—CH_3) Compound 6 (R_1=CH_2; R_2=—S—Ph; R_3=CH_3; R_4=R_5=H); Δ^2-Penem (R_1=S; R_2=CH_3; R_3=R_4=R_5=H); Compound 8 (R_1=—O—; R_2=—CH_2—CH_3; R_3=R_4=R_5=H); **(b)** Compound 9 (R_1=C; R_2=CH_3; R_3=R_4=H); Compound 10 (R_1=C; R_3=H; R_2=R_4=H); Clavulanic acid (R_1=C; R_2=CH_2OH; R_3=H; R_4=COOH) **(c)** Δ^1-thienamycin (R_1=—S—CH_2—NH_2; R_2=—CHOH—CH_3)

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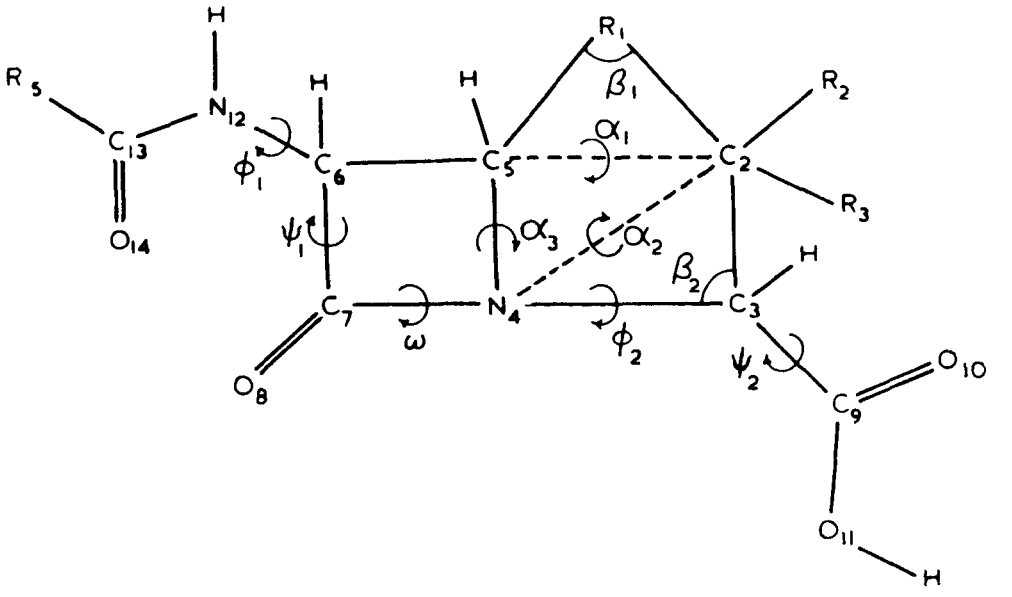

FIGURE 9. Schematic representation of the numbering of atoms and dihedral angles in the penicillin-like β-lactam compounds. The Rs represent variable groups.

the molecule. These energy calculations are based on the assumption that the intramolecular energy of a molecule may be represented as a sum of several energy terms which are functions of internal parameters (such as bond length, bond angle, and dihedral angle) and the nonbonded distances which describe the molecular conformation. Hence the total conformational energy of a molecule is computed by considering the nonbonded, electrostatic, and torsional contributions, and including the energies due to hydrogen bond formation and bond angle distortion. Several functions have been proposed to compute these energy contributions and they are described in detail in the literature.[45-49]

C. Conformational Features of Penams and Penems and Related Systems

A typical conformational energy map[36] obtained for the bicyclic ring system of penicillins is shown in Figure 11. The two regions of minimum energy centering around $(\alpha_1, \alpha_2) \approx (40°, 10°)$ [region I] and $(\alpha_1, \alpha_2) \approx (-10°, -25°)$ [region II] suggest that the five-membered ring favors two distinct conformations. If the conformations fall in region I, the C-2 atom of the ring deviate significantly from the mean plane defined by the other four atoms of the five-membered ring; hence they are termed as C_2 puckered conformations. In region II, the C-3 atom deviates from the mean plane passing through the remaining four atoms of the five-membered ring; hence conformations which fall in this region are termed as C_3 puckered conformations. Such differences in the ring puckering affect the orientation of the carboxyl group significantly (Figure 12).

Of the two puckered conformations, the C_2 puckered form is slightly more favored by about 0.2 kcal/mol compared to the C_3 puckered form and is separated by a barrier of about 3.5 kcal.mol. From these studies, it has been suggested that in solution both the C_2 and C_3 conformations would exist in equilibrium, in agreement with the results of NMR studies.[50] In the solid state, the energy difference between the two puckered forms can be offset easily by lattice energy. Hence it is also predicted from theory[36] that the five-

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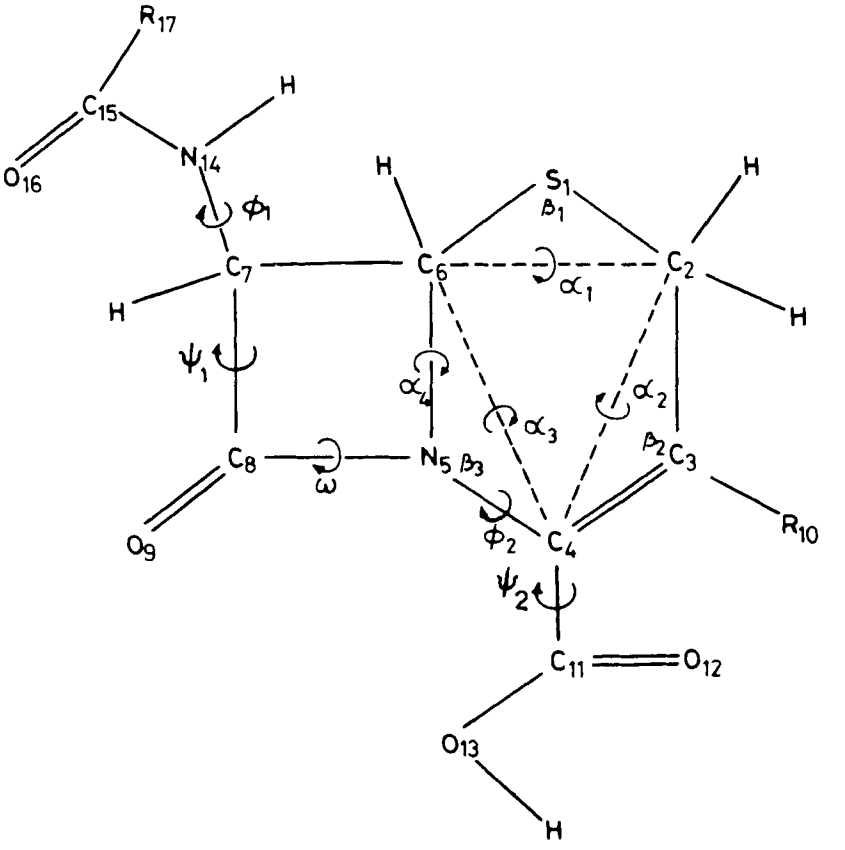

FIGURE 10. Schematic representation of the numbering of atoms, bond angles, and dihedral angles in the β-lactam compounds with a fused six-membered ring. The Rs represent variable groups.

membered ring can exist in either of the conformations in the solid state, which agrees with crystallographic studies.[25,27]

Detailed energy calculations carried out on a number of penicillins by Rao and co-workers[35,36,38-44] have shown that, in most of the penicillins, the five-membered ring favors the two puckered forms. From the spacings of the energy contours (Figures 11, 13-17), it is suggested that the five-membered ring in penicillin sulfides and 1-carba penicillins are markedly more flexible than in penicillin sulfoxides and sulfones. In 1-oxa penicillin, also, the five-membered ring is quite flexibile but favors a single conformation (Figure 18) which is close to the C_3 puckered form (Figure 11). In the new series of β-lactam compounds (Figure 8) such as thienamycin, PS-5, etc. where a double bond is introduced within or outside the five-membered ring at the C-2 atom, the five-membered ring favors[37] (Figure 19) a single conformation ($\alpha_1, \alpha_2 \approx 5°, -5°$), intermediate between the C_2 and C_3 puckered conformations of penicillins (Tables 1 and 2).

The relative orientation of the bicyclic ring system ($180° - \alpha_3$) are in the ranges 137 to 148° and 113 to 118° in the C_2 and C_3 puckered conformations, respectively, and 119 to 130° in the new β-lactam compounds.

The lactam peptide bond is highly nonplanar (ω) and deviates as much as 40 to 60° from planarity ($\omega \approx 180°$). The magnitude of nonplanarity of the lactam peptide bond

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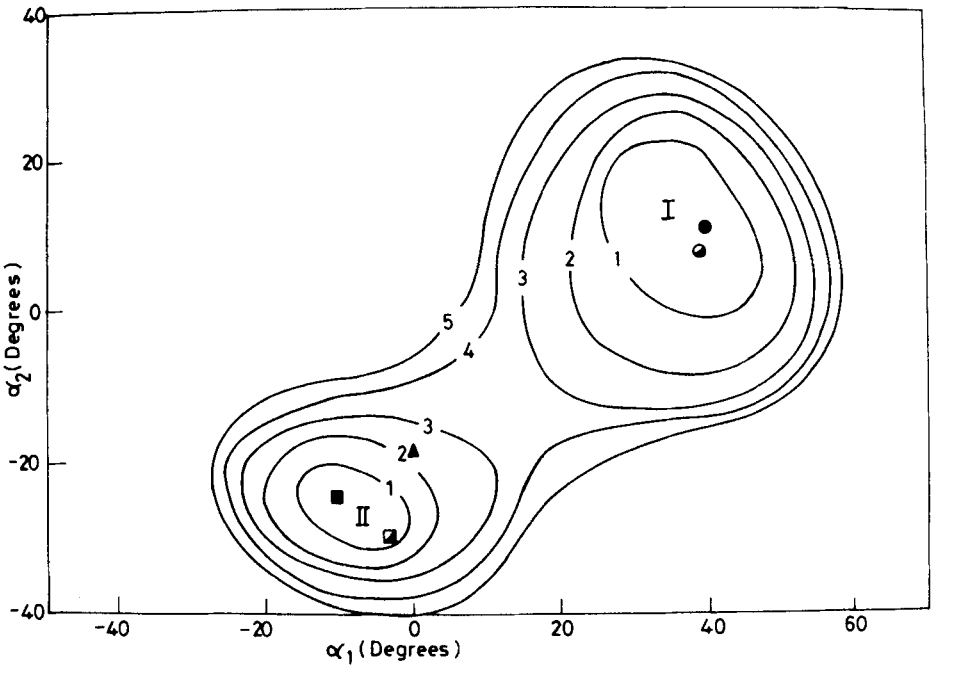

FIGURE 11. Conformational energy map of penicillin-G. Solid state conformations are marked: □;[22] ▲, ○.[27] The globlal (●) and local (■) minimum energy conformations are also shown. Numbers on contours indicate energy in kcal/mole.

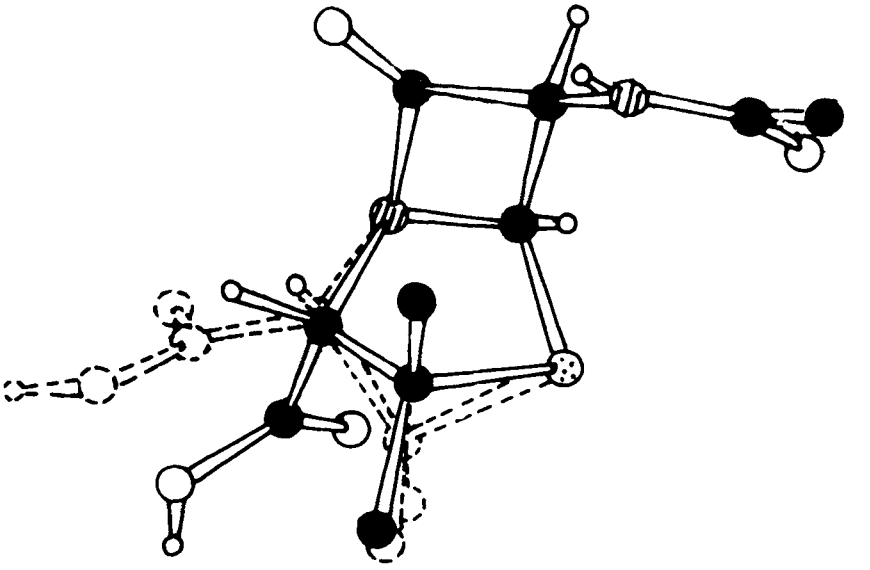

FIGURE 12. Diagram showing the (a) C_3 (solid lines) and (b) C_2 (dashed) puckered conformations of penicillins.

also seems to depend on the nature of the five-membered ring fused to the β-lactam. In general, the nonplanarity is more in the new β-lactam compounds ($\omega \approx 120°$) than in penicillins ($\omega \approx 135°$). Among penicillins, the lactam peptide is more nonplanar in 1-oxa

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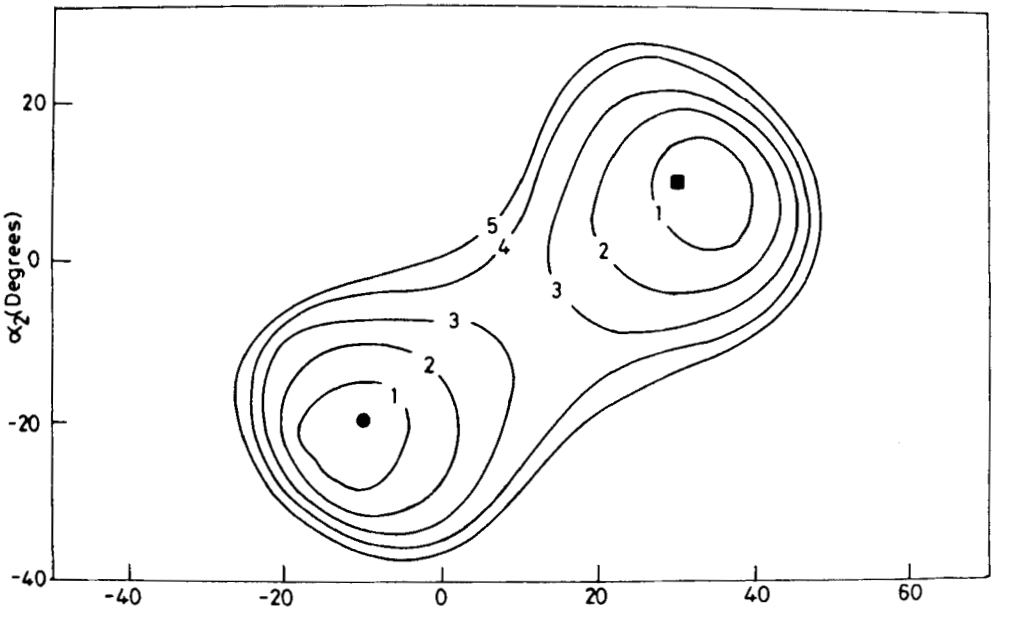

FIGURE 13. Conformational energy map of 1-carba-1-dethia penicillin-G. The global (●) and local (■) minimum energy conformations are also shown. Numbers on contours represent energy in kcal/mole.

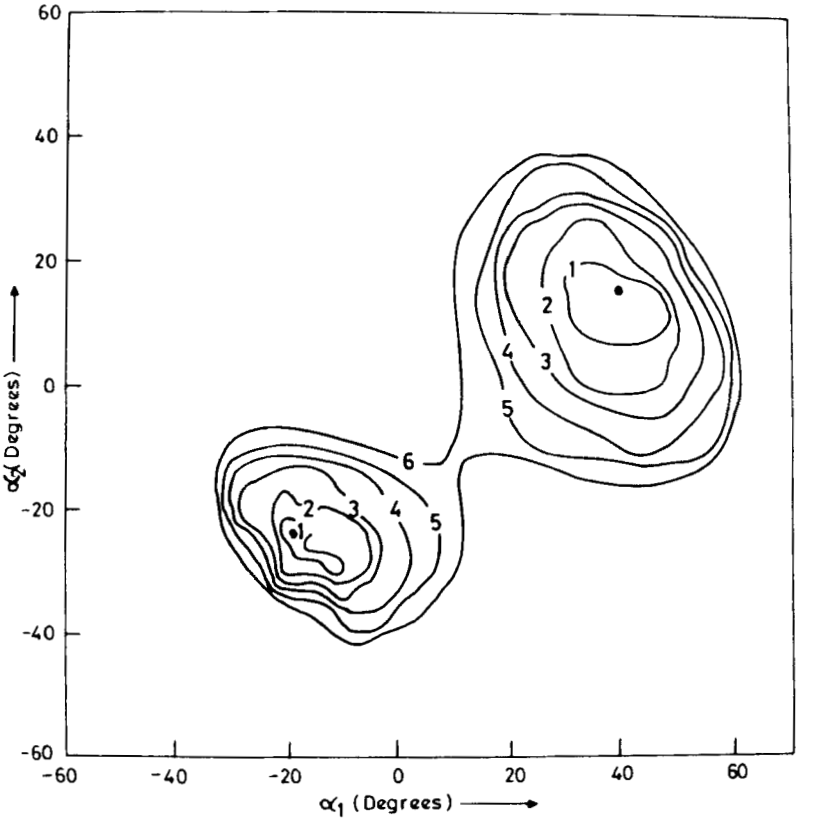

FIGURE 14. Conformational energy map of penicillin- α-sulfoxide. The minimum energy conformations are also marked. Numbers on contours represent energy in kcal/mole.

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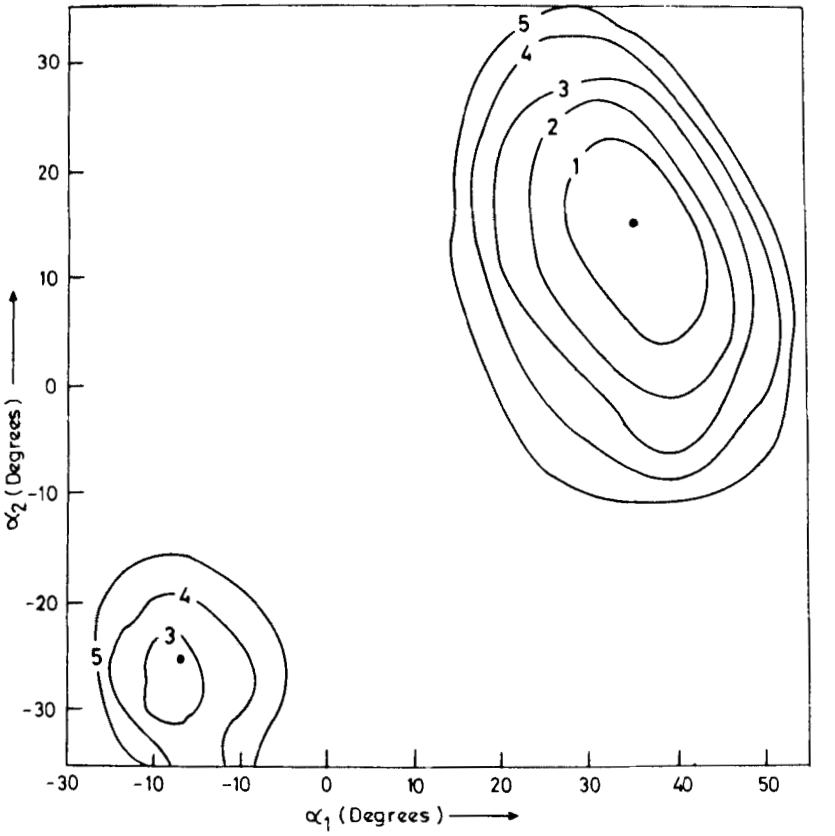

FIGURE 15. Conformational energy map of penicillin-β-sulfoxide. The minimum energy conformations are also marked. Numbers on contours represent energy in kcal/mole.

and 1-carba penicillins ($\omega \approx 125°$) than in penicillin sulfides, sulfoxides, and sulfones ($\omega \approx 135°$).

The orientation of the carboxyl group (ϕ_2) also seems to depend on the preferred conformation of the five-membered ring (Figure 12 and Table 2). In the C_2 puckered conformation, ϕ_2 favors a value between 103 and 119°, and in the C_3 puckered form, the favored values of ϕ_2 lie between 152 and 158°. In the new class of β-lactam compounds (Table 1) the carboxyl group[37] favors values around 75°, if the double bond is between the C-2 and C-3 atoms, and ≈ 146°, if otherwise. The latter values of ϕ_2 are close to that of the C_3 puckered conformation.

Vasudevan and Rao[40] have shown theoretically that the addition of 6 β side chain in penicillins slightly shifts the conformational equilibrium towards the C_3 puckered conformation (i.e., the energy difference between the two puckered conformations is reduced and the C_3 puckered form is more favored compared to the penicillins without the 6 β side chain). Theoretical studies also suggest that the side chain in the 6 β position favors a conformation with $\phi_1 \approx 170°$ in penicillins and 1-oxa and 1-carba penicillins. But in penicillin β-sulfoxide and sulfone, the 6 β side chain favors a conformation with $\phi_1 \approx -85°$ in which an intra molecular hydrogen bond of the of the type N-H . . . O = S is possible.

In the most favored conformations (Figure 20) of the antibacterially active penicillins[41] (such as penicillin-G and D-ampicillin), the 6 β side chain folds over the bicyclic ring

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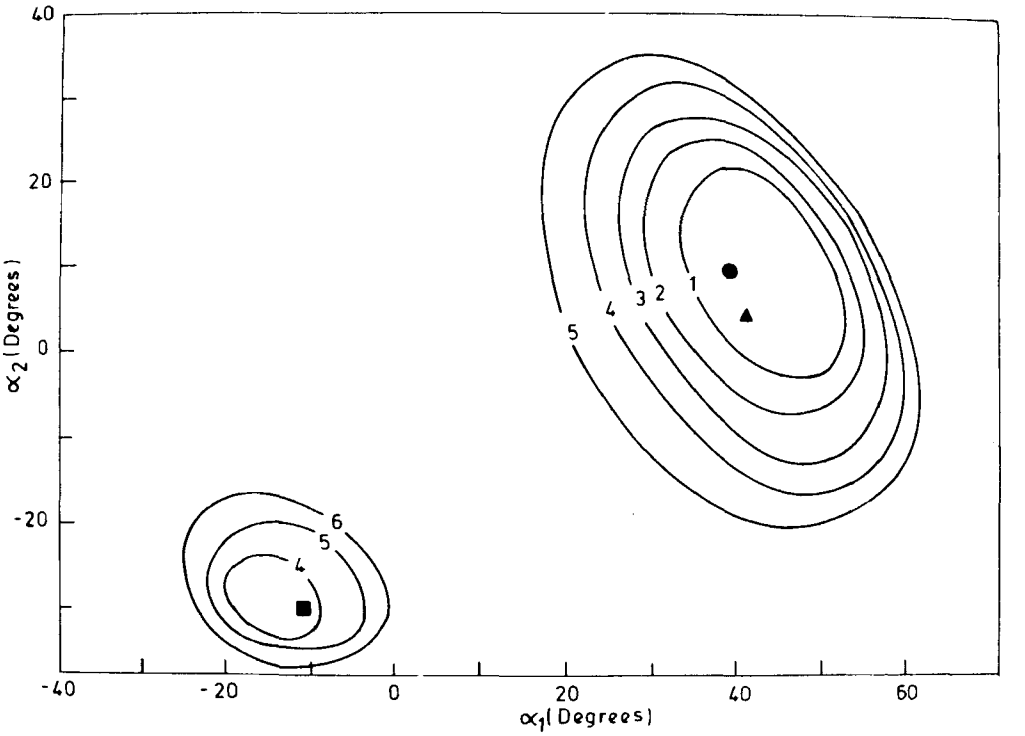

FIGURE 16. Conformational energy map of CP-45899 penicillanic acid sulfone. The solid-state conformation is marked (▲).[32] The global (●) and local (■) minimum energy conformations are also shown. Numbers on contours represent energy in kcal/mole.

system and the overall shapes of these molecules become compact. It is also suggested from conformational analysis[41,42] that the 6 β side chain of these β-lactamase-susceptible penicillins are more flexible compared to the β-lactamase-resistant phenyl and isoxazolyl penicillins.

Energy calculations[37] on the 5 S and 5 R isomers of Δ^2-penem, have shown that the change in configuration at C-5 atom affects drastically the favored conformations of the bicyclic ring system (Figure 21 and Table 1). The favored conformation of the 5 S-isomer of penem is the mirror image of its 5 R-isomer. This brings in significant changes in the α and β faces of these molecules.

D. Conformational Features of Cephems and Cephams

From conformational energy calculations, Rao and co-workers[39,44] predicted that the six-membered ring in cephalosporins also favors two puckered conformations. In Δ^3-cephalosporin, the conformation corresponding to the global minimum energy (α_1, α_2, α_3) $\approx$ (−40°, −20°, 50°) is about 0.4 kcal/mol lower in energy than the conformation at the local minimum (50°, −5°, 10°). In solid state, Δ^3-cephalosporin exists in a conformation (47°, −8°, 8°) close to the local minimum. However the small energy difference between the two possible conformations suggests that in solution, the six-membered ring can exist in both the conformations in considerable proportions. From molecular mechanics calculations, Boyd[51] has also indicated that Δ^3-cephalosporins exist in two minimum energy conformations. In 1-oxa-Δ^3-cephalosporin the six-membered ring also favors two conformations. However, the conformation with (α_1, α_2, $\alpha_3 \approx$ 50°, 0°, 0°) is energetically more favored compared to the other, viz. (−40°, −15°, 35°), by

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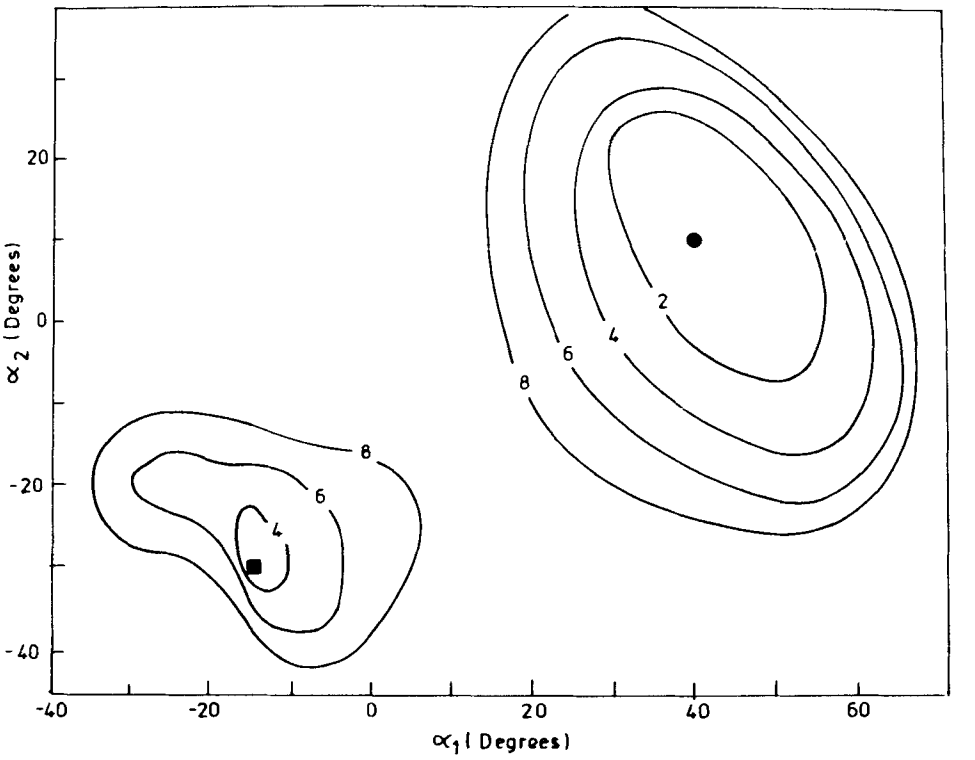

FIGURE 17. Conformational energy map of penicillin-G sulfone. The global (●) and local (■) minimum energy conformations are also shown. Numbers on contours represent energy in kcal/mole.

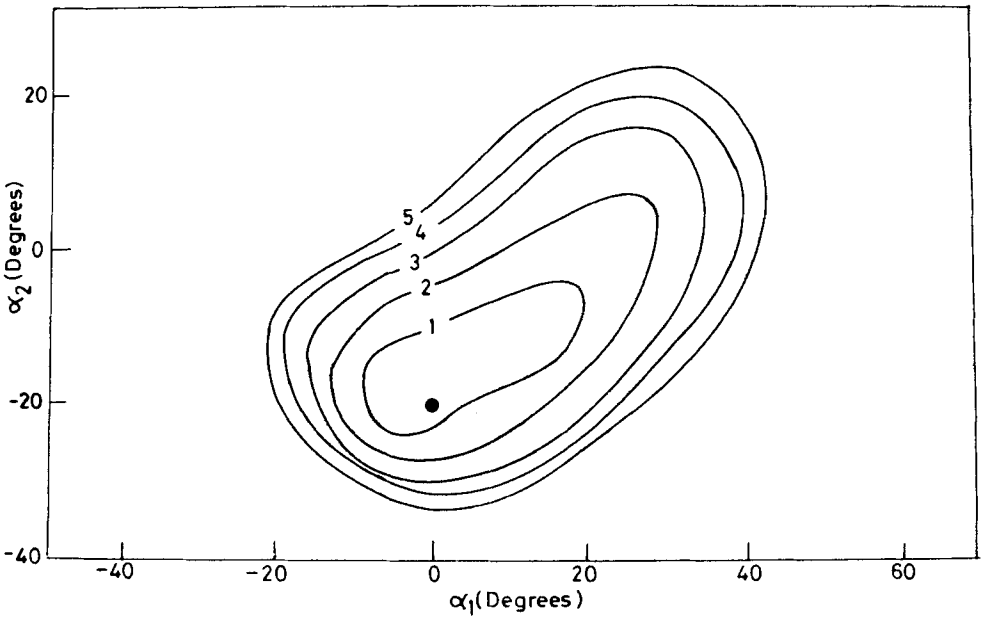

FIGURE 18. Conformational energy map of 1-oxa-1-dethia penicillin-V. The global minimum energy conformation (●) is also shown. Numbers on contours represent energy in kcal/mole.

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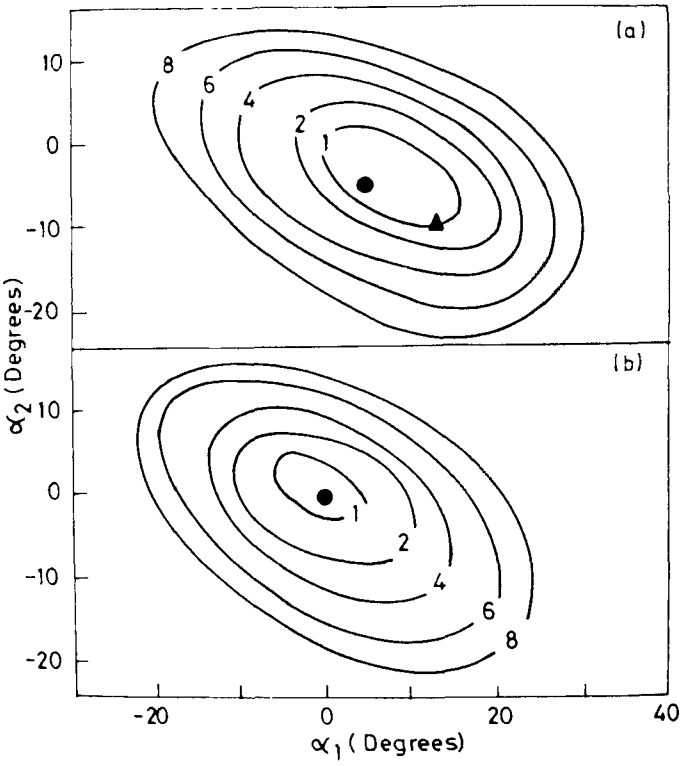

FIGURE 19. Conformational energy maps of (a) Thienamycin and (b) PS-5. Positions of minimum energy conformation is marked (●). Solid state conformation (▲)[29] is also shown. Numbers on contours represent energy in kcal/mole.

about 3 kcal/mol. The barrier of separation of the two puckered conformations in 1-oxa-Δ^3- cephalosporin is slightly higher than that obtained in Δ^3-cephalosporins. In Δ^2-cephalosporin, the conformation (α_1, α_2, $\alpha_3 \approx 50°$, $-30°$, $10°$) at global minimum has about 3 kcal/mol lower energy than the local minimum conformation ($-40°$, $25°$, $40°$). The conformation observed in the solid state is similar to the global minimum conformation.

The nature of the peptide bond in the lactam ring depends much on the position of the double bond in the six-membered ring. Theory also predicts[39] in agreement with experiments that the lactam peptide bond is more nonplanar ($\omega \approx 147°$) in Δ^3-cephalosporin than in Δ^2-cephalosporin ($\omega \approx 170°$). The nonplanarity is more in 1-oxa-Δ^3-cephalosporin ($\omega \approx 141°$) than in Δ^3-cephalosporin. However, the nonplanarity of the lactam peptide bond in these cephalosporins, is much less than the values of nonplanarity arrived theoretically and experimentally for penicillins and the penem family of β-lactam compounds.

The aminoacyl group at C-7 atom is unaffected by the nature of the fused ring systems and it favors approximately the same orientation ($\phi_1 \approx 170°$) as in active penicillins, but the preferred conformations of the carboxyl group (ϕ_2) seem to be affected by the nature of the fused ring systems. The preferred values of ϕ_2 in Δ^3-cephalosporins are in the range 35 to 50° and 85 to 90°, whereas in Δ^2-cephalosporin, ϕ_2 favors a value of $\approx 90°$.

In 3-oxa-1-dethia cephalosporins, also, the six-membered ring favors two conformations. The nonplanarity of the lactam peptide bond of these compounds ($\omega \approx 160°$) is

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Table 1
CALCULATED[36,37] CONFORMATIONAL PARAMETERS FOR VARIOUS NEW β-LACTAM ANTIBIOTICS IN THEIR MINIMUM ENERGY CONFORMATIONS[a]

Compound no.	β-lactam antibiotic	(α_1, α_2)	Non-planarity of lactal peptide bond (ω)	Orientation of carboxyl group (ϕ_2)	Relative orientation of bicyclic rings (180-α_3)	Activity[b]
1	Thienamycin nucleus	5, − 5 (13, − 6)	119 (118)	76 (85)	121 (128)	A
2	Decysteaminyl thienamycin	10, −10	120	79	123	A^+
3	Thienamycin	5, − 5	118	76	123	A^+
4	6-Epi thienamycin	5, − 5	120	74	124	A^+
5	PS-5	0, 0	117	75	124	A^+
6	Compound 6	10, − 5	119	76	122	W
7	Penem	5, − 5 (13, −10)	128 125	73 (80)	126 (130)	A^+
8	Compound 8	0, 0	112	79	119	I
9	Compound 9	10, −10	123	—	121	I
10	Compound 10	10, −10	123	—	122	I
11	Δ^1 thienamycin	10, −10 (8, −11)	126 (124)	146 (151)	121 (123)	W
12	Clavulanic acid	10, −10 (10, −20)	121 (124)	148 (161)	122 (120)	W, I
13	5S-isomer of penem	−10, 10	−128	−75	−126	Inactive

[a] Crystal structure[29,36] parameters are given in parenthesis. Columns 3 to 6 given in degrees.
[b] A, broad spectrum potent activity; A^+, broad spectrum potent activity including β-lactamase producing strains; W, weakly active; I, inhibitor of β-lactamases.

intermediate between those obtained for Δ^2- and Δ^3-cephalosporins. Here, too, the amino acyl group favors a conformation with $\phi_1 \approx 170°$ as in the other active cephalosporins and penicillins. However, the carboxyl group favors values of ϕ_2 around −25° or 105° depending upon whether the configuration at C-4 atom is L or D.

V. MECHANISM OF ACTION OF β-LACTAM ANTIBIOTICS

There have been many hypotheses[1-6,10-13,34,35] regarding the mode of action of β-lactam antibiotics. Collins and Richmond[10] suggested that penicillin might be a stereo-analog of *N*-acetyl muramic acid (NAM). However, it has been shown that there is little resemblance between these molecules in their low energy conformations.[52,53] The fact that penicillin specifically inhibits the last stages of peptidoglycan biosynthesis led to the emergence of the most widely accepted substrate analog hypotnesis.[1,2]

Wise and Park[1] and Tipper and Strominger[2] independently explained the mechanism of penicillin action by assuming the penicillin is analogous to the substrate of the transpeptidase enzyme. However, they differed in identifying the portion of the pentapeptide moiety of peptidoglycan that has structural similarity to penicillin (Figure 22). Since the backbone of the penicillin molecule, viewed as L-cysteinyl-D-valine (Figure 23), has LD sequence and a free carboxy group, Wise and Park hypothesized that penicillin is a structural analog of L-ala^1-γ-D-glu^2 portion of the pentapeptide segment (Figure 22). Tipper and Strominger later observed that the α-carboxyl group of D-glu^2 is not always free. This led these authors to postulate that penicillin bears resemblance to

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the D-ala[4]-D-ala[5] portion where the carboxyl group is free. Such a similarity would allow the antibiotic to mimic the substrate and to irreversibly inactivate the transpeptidase enzyme by acylation. However, to overcome the configurational difference (L in the antibiotic and D in the substrate) at the N-terminal side, these authors assumed that L and D amino acid residues could take up similar conformations. It was also felt that 6 α-methyl penicillin would be more similar to D-ala[4]-D-ala[5], and hence enhanced activity was expected of this compound. But the 6 α-methyl derivative[54] was found be much less active compared to the parent compound, in disagreement with predictions of Tipper and Strominger. Also, recent conformational analysis[55] of L and D amino acid residues does not justify the assumption that L and D residues can assume similar conformations.

From model building studies, Lee[12] modified the substrate analog hypothesis of Tipper and Strominger and suggested that penicillins and cephalosporins have structural similarity to the transition state of the substrate D-ala-D-ala. Using molecular orbital methods, Boyd[56] showed that such a similarity exists between the antibiotic and the substrate. He also estimated[51] the structural similarity by least squares fitting. Holtje,[53] by similar methods, determined the most stable conformations of NAM, *N*-acetyl-D-alanyl-D-alanine, and a penicillin, and confirmed the structural similarity between the last two. Though these studies lent support to the substrate analog hypothesis, no attempt was made to resolve the configurational paradox and to explain the biological inactivity of 6 α-methyl penicillin.

Ghuysen and co-workers,[11] from fluorescence and circular dichroism investigations of the interactions between penicillin-G and the enzyme *Streptomyces* R 61, suggested a conformational response model for the mode of action of penicillins. According to this model, the effect of penicillins is to impart to the catalytic center of the enzyme a conformation that is incorrect for enzymic action. Extensive studies[5,6,57,58] on the exocellular penicillin-sensitive enzymes (PSEs) from various actinomycetes led these authors to suggest that the antibiotic might be a "k_{cat}" inhibitor (i.e., an inhibitor rendered reactive upon binding to the target enzyme). Evidences[58] from kinetic studies appeared to suggest that the main cause of inhibition by the antibiotics is the rapid irreversible inactivation step rather than an especially good recognition of the antibiotic molecule by the receptor enzyme. The driving force in enzyme action is thought to be in the interaction between the structurally unrelated portion of the antibiotic with the target enzyme rather than the vague structural similarity between the substrate D-ala-D-ala and the nucleus of the antibiotic.

Virudachalam and Rao[34,35] undertook detailed conformational analysis of several penicillins, cephalosporins, and related dipeptides and showed that the allowed range of the dihedral angle (ϕ_1, ψ_1) of the antibiotic falls within the allowed regions of the (ϕ_4, ψ_4) map of D-ala-D-ala but not in the (ϕ_1, ψ_1) map of L-ala- γ-D-glu (Figure 24). These authors also pointed out that the lactam ring is mainly responsible for bringing out the conformational similarity of the antibiotic to the former rather than the latter. In other words, when the lactam ring is absent or when it is opened, the compound cannot assume conformations similar to D-ala-D-ala. Hence it was suggested that the β-lactam is responsible for achieving the conformational similarity between the antibiotic and the substrate D-ala-D-ala (Figure 25). These studies also provided a theoretical basis for the assumption of organic chemists that in all β-lactam antibiotics the lactam ring is vital for antibacterial activity. Recent experimental studies[59,60] on penicillin-sensitive carboxypeptidases provide direct evidence that both penicillin and substrate bind to identical amino acid residues in these enzymes, consistent with the substrate analog hypothesis.

By comparing the favored conformations of various penicillins, Virudachalam and Rao[35] suggested that besides the nonplanarity of the lactam peptide bond, specific orientations of the amino acyl ($\phi_1 \approx 180°$) and carboxyl ($\phi_2 \approx 160°$) groups are

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Table 2
CALCULATED CONFORMATIONAL PARAMETERS FOR VARIOUS PENICILLINS IN THEIR FAVORED CONFORMATIONS[40]

S1: Number	Antibiotic and mode of puckering	(α_1, α_2)	Non-planarity of the lactam peptide bond (ω)	Conformation of the amino-acyl group (ϕ_1)	Relative orientation of the bicyclic rings (180-α_3)	Orientation of the carboxyl (ϕ_2)	Relative energy of the two puckered forms	Barrier between two puckered conformations	Anti-bacterial activity	Activity against penicillinases
1	Penicillin nucleus									
	C_2	40, 10	136	—	146	106	0.00	3—4	Weak	Poor substrate
	C_3	−10, −30	136	—	114	157	1.29			
2	1-oxa-1-dethia penicillin nucleus	− 5, −20	123	—	115	154	—	—	**[a]	Inhibitor
3	1-carba-1-dethia penicillin nucleus									
	C_2	30, 10	125	—	137	119	0.07	3—4	**	**
	C_3	−10, −20	126	—	114	153	0.00			
4	Penicillanic acid sulfone CP-45899									
	C_2	40, 10	138	—	147	104	0.00	9	Weak	Inhibitor
	C_3	−10, −30	135	—	114	156	3.30			

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5	Penicillin-G									
	C_2	40, 10	135	172	144	108	0.00	3—4	Good	Good substrate
	C_3	−10, −25	136	166	115	156	0.20			
	Penicillin-V									
	C_2	40, 10	136	174	145	106	0.00	3—4	Good	Good substrate
	C_3	−10, −30	136	168	115	156	0.66			
6	1-oxa-1-dethia penicillin-V	0, −20	124	167	116	153	—	—	Moderate	Susceptible
7	1-carba-1-dethia penicillin-G									
	C_2	30, 10	125	170	137	119	0.55	3—4	**	**
	C_3	−10, −20	127	172	115	152	0.00			
8	Penicillin-G sulfone									
	C_2	40, 10	139	−83	148	103	0.00	9	Weak	Substrate
	C_3	−15, −30	137	178	115	156	3.63			
9	Bisnor penicillin-V									
	C_2	40, 5	137	174	146	106	0.00	2—3	Moderate	Substrate
	C_3	−10, −25	134	172	113	158	1.40			
10	Clavulanic acid	10, −10	121	—	122	148	—	—	Weak	Inhibitor
11	Thienamycin nucleus	5, − 5	119	—	121	76	—	—	Good	Susceptible

Note: Columns 3 through 7 are in degrees; columns 8 and 9 are in kilocalories per mole.

[a] **, not known

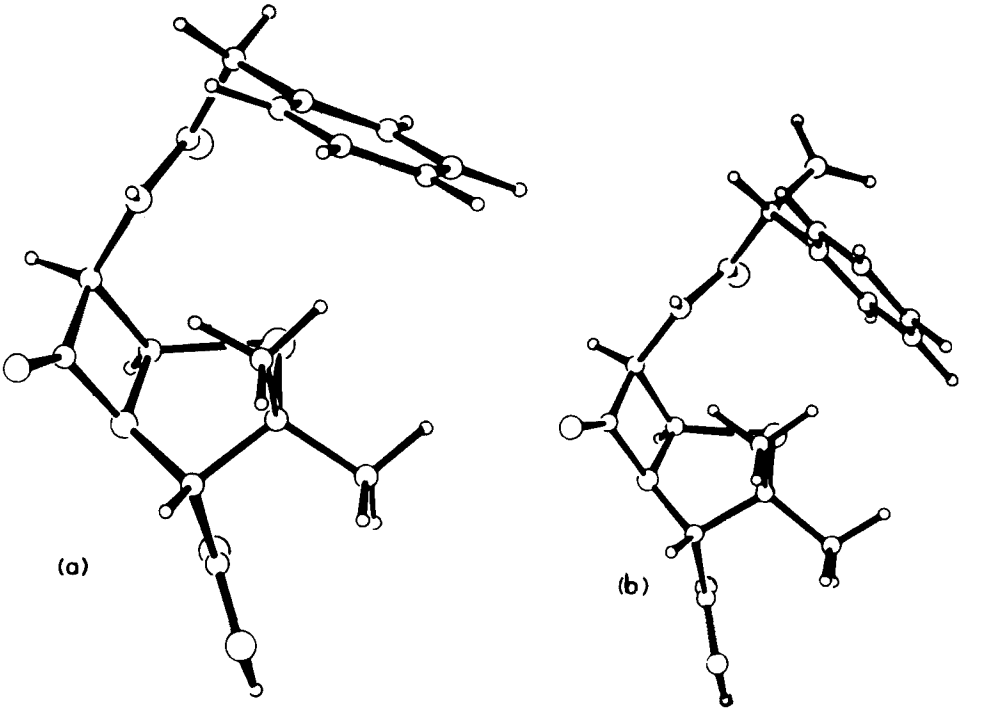

FIGURE 20. Projections of (a) penicillin-G and (b) D-ampicillin in their minimum energy (compact) conformations.

important for biological activity. Using this model, these authors successfully explained[61] the reduction in cross-linking in peptidoglycan biosynthesis[62,63] due to replacement of D-ala residues at the 4th and 5th positions of the pentapeptide moiety by L-ala, D-val and D-abu.

The experimental observation[17] that thienamycin which has D-configuration at C-6 atom is a very good inhibitor of an LD-carboxypeptidase from *G. homari,* is also in good agreement with the theoretical prediction that the β-lactam compounds (penems and penams) with a D-center at the C-6 atom would be conformationally similar to LD-dipeptides. This suggests that the conformational similarity of the drug molecules with the substrates plays a dominant role in drug-receptor interactions.

VI. STRUCTURE-ACTIVITY RELATIONSHIPS IN β-LACTAM COMPOUNDS

A. Nonplanarity of Lactam Peptide Bond

Crystal structure data[22,23] on several β-lactam compounds revealed that the geometry of the lactam peptide unit differs significantly from that of normal peptide unit (Table 3). The length of the C-N bond increases and the dihedral angle ω deviates significantly from 180°. The extent of these deviations seems to be dependent on the nature of the rings fused to the lactam. Recent *ab initio*[64] molecular orbital calculations on β-lactam unit also predicted an increase in C-N bond length with nonplanarity of the lactam nitrogen. These studies also suggested that the lactam ring is fairly rigid. Hence the role of the fused ring system is to provide the required nonplanarity to the lactam peptide bond and also to provide another site of attachment via the carboxyl group to receptor enzymes.

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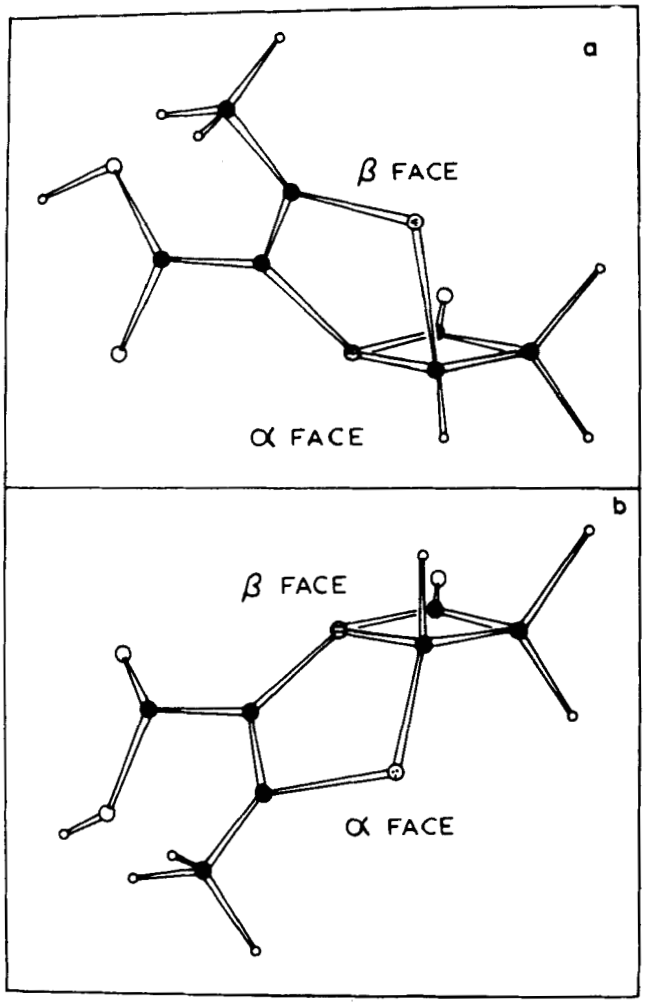

FIGURE 21. Projections of (a) Δ^2-penem and (b) 5 S-isomer of Δ^2-penem in their minimum energy conformations.

Sweet and Dahl,[24,25] from crystal structure analysis of active and inactive penicillins and cephalosporins, observed that the nonplanar character of the lactam nitrogen is high in active compounds compared to inactive ones. These authors also suggested that the stereochemical requirement of the carboxyl group in the antibiotic, for recognition by the transpeptidase enzyme(s) might not be very restrictive. Therefore, the degree of nonplanarity of the lactam nitrogen was thought to have a direct bearing on the biological activities of these compounds. The high reactivity of the active compounds was assumed to be the consequence of the hindered amide resonance in the lactam which arises from the nonplanar character of the lactam nitrogen. In Δ^3-cephalosporins and Δ^2-penems, enamine resonance resulting from the delocalization of the unshared electron pair of lactam nitrogen into the adjacent π system also was thought to activate the β-lactam.

From chemical studies of a number of β-lactam compounds, Woodward and co-workers[29,65] pointed out that there is no direct correlation between the chemical and biological activities of these compounds and that there is an optimal range of β-lactam

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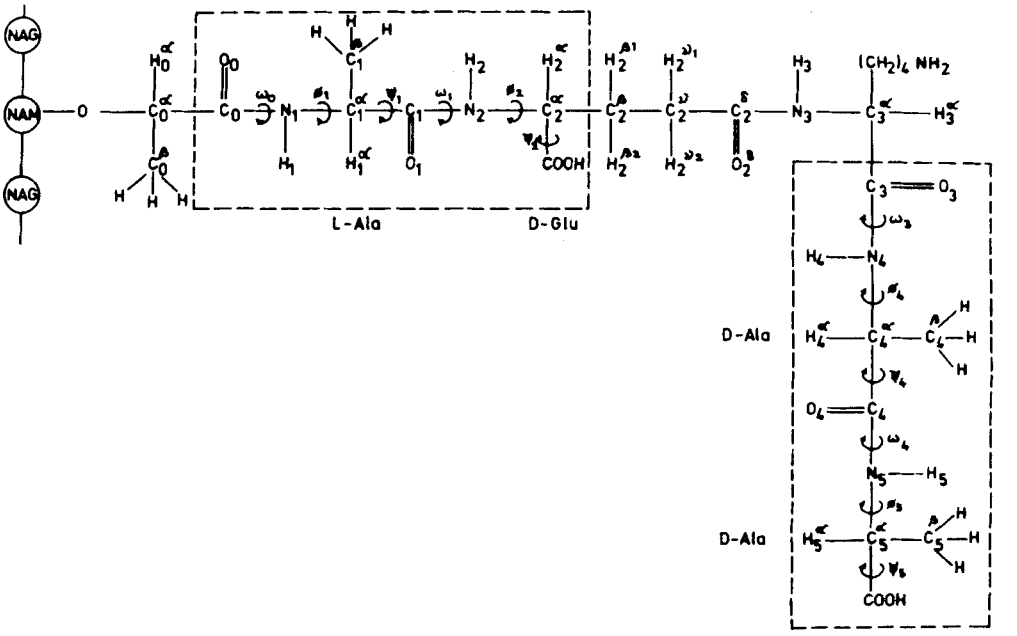

FIGURE 22. Diagram showing the pentapeptide moiety of peptidoglycan. The segments L-ala-γ-D-glu and D-ala-D-ala are enclosed within the dashed lines.

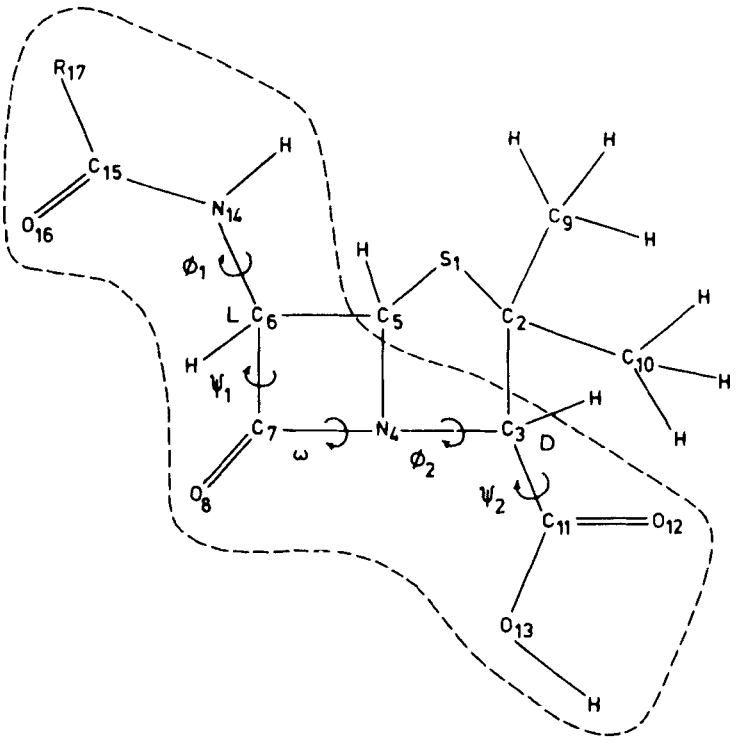

FIGURE 23. Schematic representation of a penicillin molecule. The backbone of the molecule is enclosed within the dashed lines.

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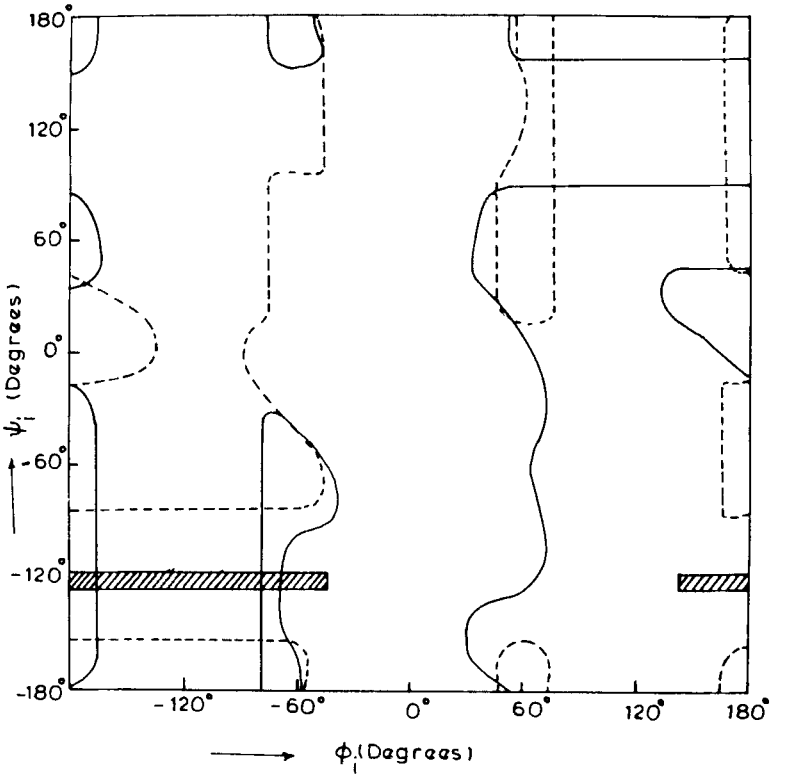

FIGURE 24. Ramachandran plots of (ϕ_i, ψ_i) for the allowed ranges of (1) X-D-ala-D-ala (enclosed by solid lines), and (2) X-L-ala-γ-D-glu (enclosed by dashed lines). Allowed ranges of (ϕ_i, ψ_i) for the antibiotic are shown as ▨.

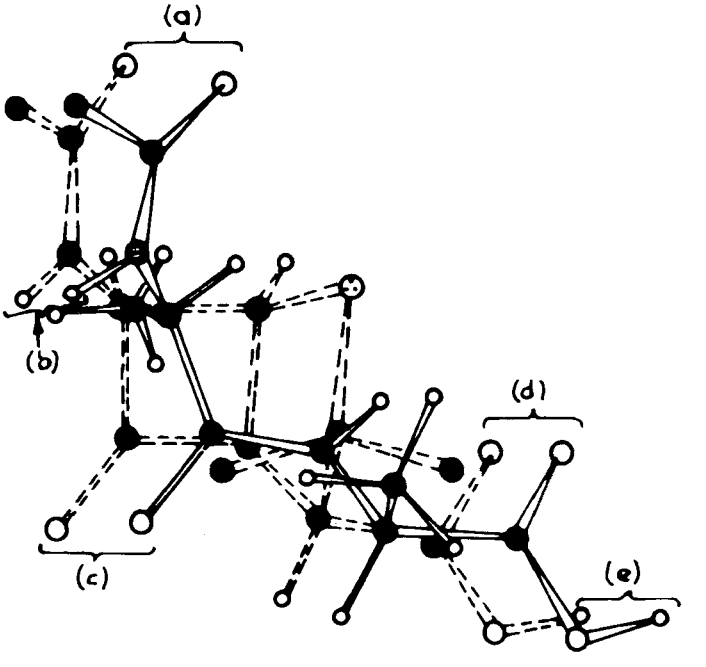

FIGURE 25. An overlay diagram of the dipeptide D-ala-D-ala (in solid lines) and penicillin (in dashed lines) which resembles the dipeptide.

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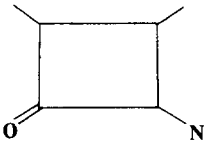
Table 3
CONFORMATIONAL PARAMETERS[6,22-33,38,65] OF β-LACTAM OBTAINED FROM X-RAY CRYSTAL STRUCTURE DATA



	C–N(A°)	C=O(A°)	W(°)	h*(A°)[a]
Normal amides	1.33	1.23	180	0
Δ^2-Cephems	1.351	1.207	176	0.06
Δ^3-Cephems	1.373	1.215	162	0.24—0.32
Penams	1.382	1.193	139	0.38—0.40
Δ^2-Penems	1.419	1.204	125	0.43—0.44
Δ^2-Carba penems	1.423	1.201	118	0.49—0.50
Δ^1-carba penems	1.402	1.210	124	0.54
Clavulanic acid	1.406	1.195	121	.053

[a] h*, distance of lactam nitrogen from the plane of its three substituents.

reactivity below and above which the antibacterial activity would be weak. However, correlating only the nonplanarity of lactam nitrogen to antibacterial activity of the drug seems to be taking an oversimplified view. This is because the antibiotic should have the proper conformation to fit in the active site of the receptor enzyme and also provide the necessary sites of attachment for the enzyme, before the cleavage of the lactam peptide bond. In fact, Vasudevan and Rao[37] were able to explain the weak antibacterial activity of a number of β-lactam compounds from the differences in their overall shapes and the orientations of the possible sites of attachment. From conformational studies, these authors concluded that both conformation and nonplanarity of the lactam peptide bond are important for antibacterial activity.

B. Conformation of the Bicyclic Ring System and the Orientation of the Carboxyl Group

Δ^2-penems (compounds 1 to 4 and 7 of Figure 8) are the simplest of β-lactam compounds which exhibit good antibacterial properties.[66-80] The conformational features of these compounds (Table 1) suggest that in these compounds, the bicyclic ring system and its functional groups are placed so that not only do they fit well in the active site of the cross-linking transpeptidase enzymes, but also provide the necessary sites of attachment to the enzymes. By comparing the favored conformations of these compounds, it was suggested that besides the nonplanarity of the lactam peptide bond (ω), the conformation of the carboxyl group (ϕ_2) is also important for antibacterial activity. The ideal conformation of the carboxyl groups seems to be $\approx$75 to 80°.

In the favored conformations[36,37] of clavulanic acid and Δ^1-thienamycin, the nonplanarity of the lactam peptide bond is close to those of Δ^2-penems (Table 1). However, the favored conformations of these compounds differ significantly in the orientations of the carboxyl group which might provide a site of attachment to the receptor enzyme. Hence, if clavulanic acid and Δ^1-thienamycin were to bind in the same

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Table 4
RELATIVE DISPOSITION OF THE CARBOXYLIC ACID GROUP WITH RESPECT TO THE β-LACTAM GROUP (IN THE C_3 PUCKERED FORM OF THE FIVE-MEMBERED RING)[40]

Number	Antibiotics	χ (6-7-4-11) (°)
1	Penicillins	172
2	1-oxa-1-dethia penicillins	157
3	1-carba-1-dethia penicillins	159

mode of binding as the Δ^2-penems, their carboxyl groups might encounter some short contacts with the atoms of the enzyme at the active site. Since the bicyclic ring systems are rigid, such contacts could be relieved either by a rotation of the molecules slightly out of the site, or by the movement of the amino acid residues in the active site in order to accommodate the changes in the orientation of the carboxyl groups. This may lead to a slightly different mode of binding and weak interactions which, in turn, may result in a reduced level of antibacterial activity.[65,81]

In the C_3 puckered conformation of penicillin nucleus (Tables 1 and 2) the relative orientation of the fused rings ($180°-\alpha_3$) is approximately the same as in Δ^2-penems, whereas the orientation of the carboxyl group (ϕ_2) differs. In the C_2 puckered form, both ($180°-\alpha_3$) and ϕ_2 differ from those of Δ^2-penems. As pointed out earlier, such changes will lead to weak activity.[82] However, the antibacterial activity of penicillin nucleus is shown to be improved by an addition of a suitable side group as in penicillin-G or -V. This suggests that once the bicyclic ring system is bound to the enzyme, the side group with appropriate conformation can function as a handle through which the enzyme can easily reorient the nuclear part of the molecule, not only to relieve possible bad contacts but also for proper positioning of the functional groups. Thus, the good antibacterial activity[83-84] of penicillins appears to be the consequence of the 6β-side chain. This brings out the necessity of a suitable side group in penicillins for imparting the desired biological activity.

Balsamo et al.,[85] from the analysis of crystal structure data of several penicillins, suggested that the C_2 puckered form is important for bioactivity, but the arguments of these authors do not explain the weak antibacterial activity of CP-45899 sulfone[86,87] and the reduced level of activity of bisnorpenicillin-V (compared to penicillin-V).[88] In the former, the C_2 puckered form is the most favored conformation (Figure 16); in the latter, the C_2 puckered form is much more favored than in penicillin-V. Similarly, penicillin-G sulfone and penicillin sulfoxides exhibit only poor antibacterial activities,[89,90] though the most favored conformation of their five-membered ring is the C_2 puckered form.[39,40]

In the case of 1-oxa-penicillin-V, the favored conformations[40] of the amino acyl and carboxyl groups are the same as in penicillin-V. The lactam peptide bond in the former is also more nonplanar (Table 2). Since the conformational parameters of the ring are not the same, the spatial relationships between the carboxyl group and the lactam carbonyl group are different in these compounds (Table 4). This appears to affect the efficiency of interaction of these drugs with the receptor enzymes. The reduced level of antibacterial activity[91] of 1-oxa penicillin-V compared to penicillin-V appears to be the consequence of such a conformational difference.

C. Conformation of Amino-Acyl Side Chain

From energy calculations,[41] the favored conformations of penicillins have been

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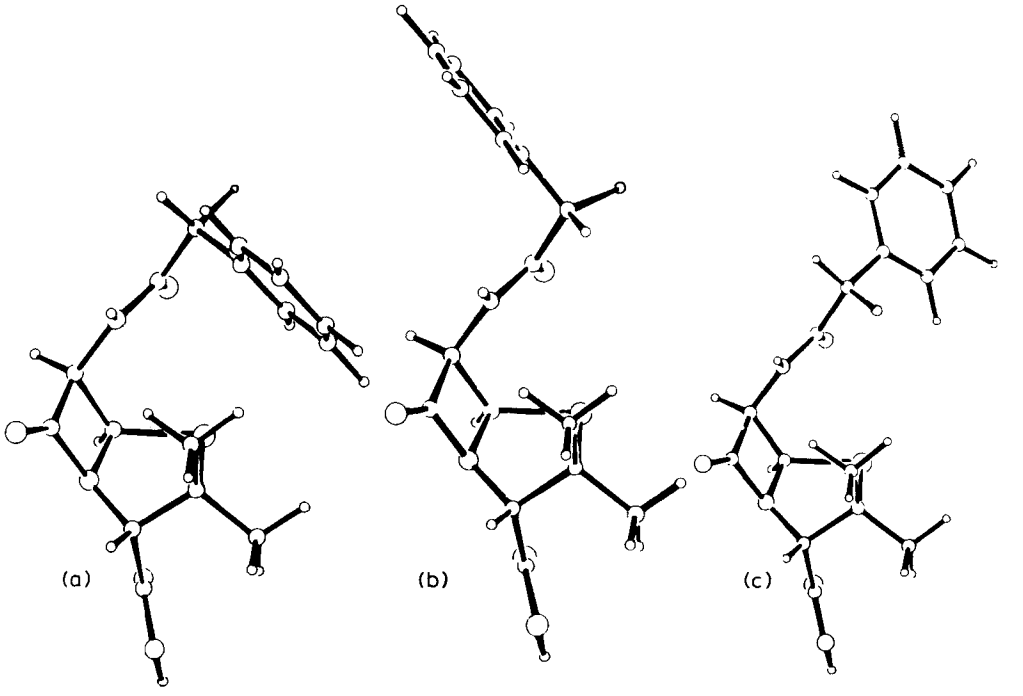

FIGURE 26. The (a) compact, (b) extended, and (c) fully extended conformations of penicillins.

broadly classified as (1) compact, (2) extended, and (3) fully extended, based on their overall shapes (Figure 26). The highly active penicillins such as penicillin-G and D-ampicillin, seem to favor the compact conformation (Figure 20). In the less active L-ampicillin, the extended conformation is most favored and the compact conformation of this molecule has about 3 kcal/mol higher energy than its global minimum. Further, the L-isomer in compact shape differs from the D-isomer in the orientation of the amino group at the asymmetric atom in the 6 β side chain. These differences[41] have been attributed to the reduced level of antibacterial activity[83,84] of the L-isomer. A higher degree of rotational freedom in the 6 β side chain of the active drugs suggests that such a flexibility might facilitate better drug-receptor interactions.

In penicillin β-sulfoxide and sulfone, the conformations of the 6 β side chain ($\phi_1 \approx 180°$) conducive for proper fit with the transpeptidase enzyme have about 2 kcal/mol higher energy over their most favored conformations ($\phi_1 \approx -85°$).[38-40] Hence, the difference in the preferred orientation of the 6 β substituents may prevent proper fitting and hence may lead to weak antibacterial activity.[89]

Joshi and Rao,[39] from energy calculations, showed that in the penicillin α-sulfoxide (though the 6 β side chain favors a conformation close to that of penicillin-G), the energy barrier separating the C_2 and C_3 puckered conformations is higher by about 2 kcal/mol. As a result, the rate of interconversion between the two puckered conformations is about 20 times slower in α-sulfoxide. This suggested that in solution, as the biologically active C_3 puckered conformation gets depleted due to interaction with the transpeptidase enzyme, the rate at which it is restored due to interconversion would be much slower. Thus, in the α-sulfoxide, the conducive effect of the 6 β side chain is reduced by the less flexible nature of the nuclear part of the molecule, making the molecule only weakly active.[89]

Theoretical studies[43] on 6-[α-(α'-ureido-acylamino) acylamino] penicillanic acids

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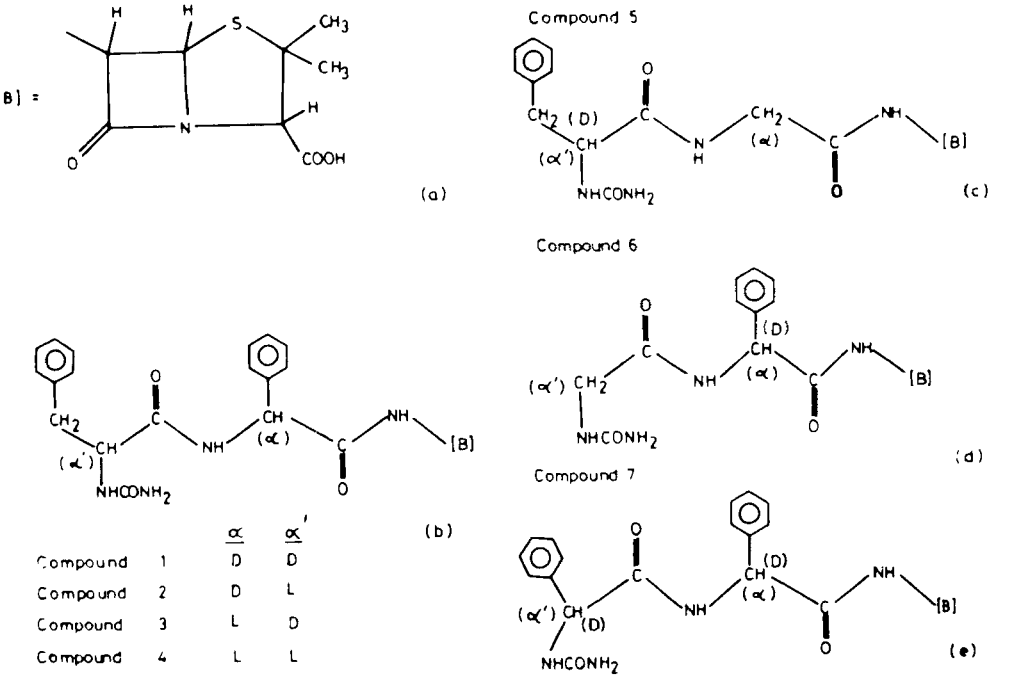

FIGURE 27. Chemical structures of 6-[α-(α[1]-ureido-acylamino) acylamino] penicillanic acids.

(Figure 27) with two chiral centers in the 6 β side chain showed that the nature and orientation of the substituents affect the overall shapes of the molecules significantly. The changes in the conformation that occur beyond the first chiral center appear to have little influence on the biological activity against Gram-positive bacteria. On the other hand, the antibacterial activity against Gram-negative organisms seems to be influenced to a great extent by the substitutional and, hence, the conformational environment at the second chiral center also, though it is remote from the reactive β-lactam peptide bond. The effect of lipophilic or electronic character of these compounds on their bioactivities has been found[93] to be less pronounced. Hence it has been suggested[43] that the cross-linking transpeptidase enzyme(s) of Gram-negative organisms are much more specific than those of Gram-positve bacteria. In the Gram-negative organisms, the permeability of the drug molecules through the outer membrane of the bacterial cell wall, also seems to affect the level of antibacterial properties.[41,94]

D. Importance of α-Face of β-lactam Compounds

The conformational parameters[37] obtained for the compound 6 (Figure 8) are very similar to those obtained for the other potent antibiotics of the penem family (Table 1). The nonplanarity of the lactam peptide bond is also very similar in these compounds. These should have made the compound 6 a potent drug. But, contrary to expectations, it is found to be only weakly active as an antibacterial agent.[95] In the minimum energy conformation (Figure 28), the methyl group at C-5 is protruding outside from the α-face of the molecule. Such an orientation of the bulky group will interfere with the fitting of the molecule with the active site of the target enzyme, if the enzyme approaches the β-lactam from the α-face. This will lead to unfavorable steric interactions with the functionalities in the active site of the enzyme, rendering the compound antibacterially ineffective.

The 5 S-isomer of Δ^2-penem favors a conformation which is a mirror image of

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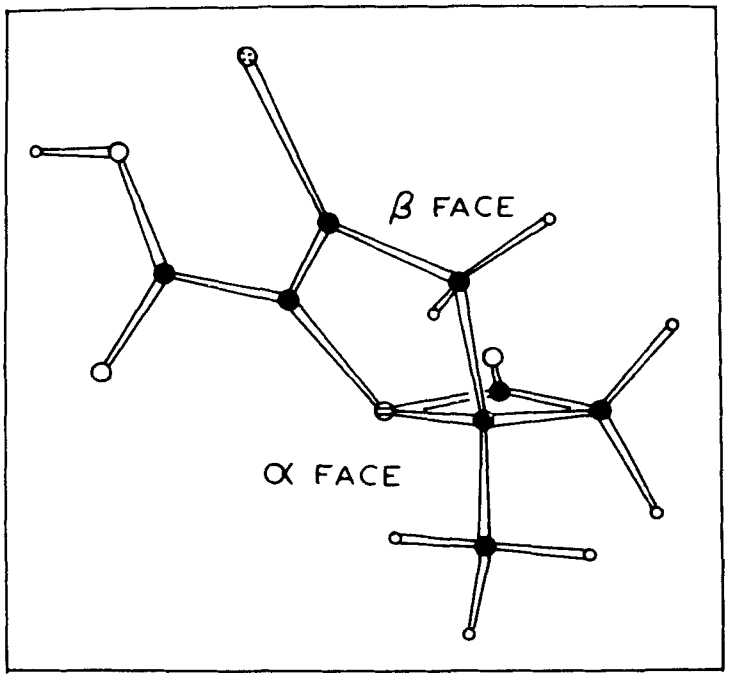

FIGURE 28. Projection of compound 6 in the minimum energy conformation.

Δ^2-penem (Figure 21). Hence the 5 S-isomer cannot provide the necessary structural complementarity required for steric fit with the enzyme, leading to total loss of antibacterial activity.[71,72]

E. Cephalosporins

Conformational data[39] on Δ^3- and Δ^2-cephalosporins suggest that the shifting of the double bond in the six-membered ring affects the orientation of the carboxyl group and the nonplanarity of the lactam peptide bond, but not the orientation of the amino acyl group. These differences affect the β-lactam reactivity and the structural complementarity to some extent; hence their antibacterial activities.[89]

Recently reported[96] 3-oxa-1-dethia-cephalosporins with a saturated 4:6 bicyclic ring system have been found to exhibit potent antibacterial properties, in spite of the L-configuration at C-4 atom, unlike in penicillins. Conformational studies[44] showed that the lactam peptide bond is sufficiently nonplanar and the spatial relationship between the β-lactam carbonyl group and the carboxyl group at C-4 remains nearly the same as in the other active Δ^3-cephalosporins. These perhaps account for the higher biological activities of the L-isomer of 3-oxa cephalosporins. From energy calculations, it has also been shown that the methoxyl group at C-2 atom does not increase the strain in the lactam ring. Perhaps such a group facilitates reactions leading to secondary reactive centers capable of interfering in the biosynthesis of peptidoglycan. These also suggest that the mode of interaction of cephalosporins with the enzymes of cell wall biosynthesis is more complex.

F. Monocyclic β-Lactam Compounds

Recently, monocylic β-lactam compounds such as nocardicin (Figure 29) have attracted attention because of their interesting in vivo antibacterial properties[66,97-99]

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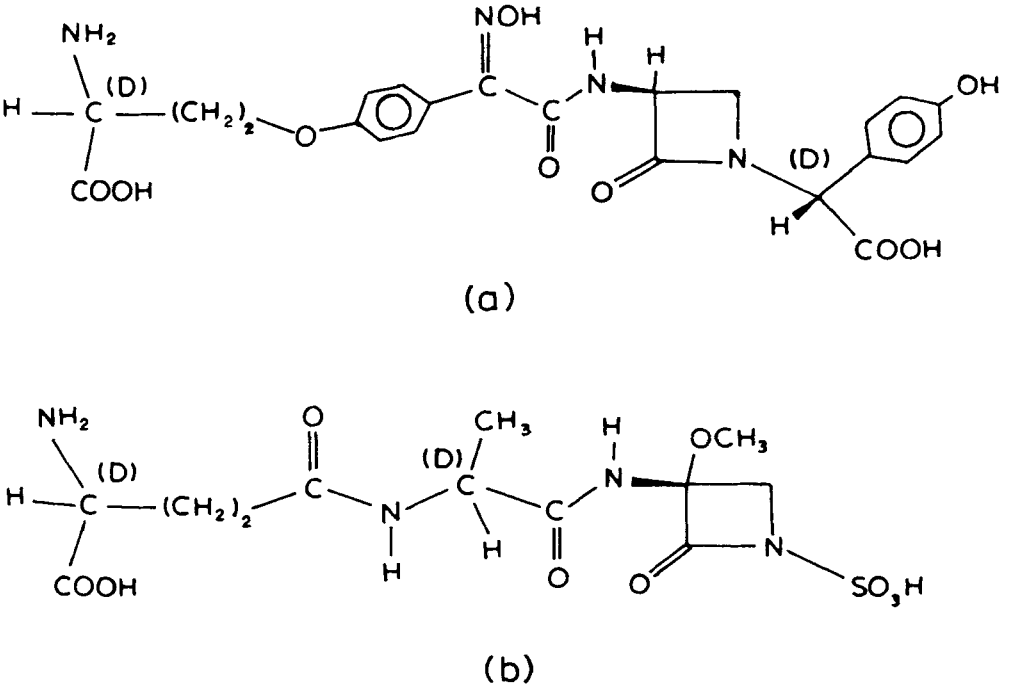

FIGURE 29. Chemical structures of the monocyclic β-lactam compounds: (a) nocardicin-A and (b) sulfazecin.

From molecular orbital (MINDO/3) calculations on model structures related to monocyclic β-lactams, Boyd[100] has shown that the conformation in which the exocyclic N-substituent lies in the lactam plane is the most stable state. He has estimated that about 1 to 2 kcal/mol and 5 to 7 kcal/mol would be required to twist the stable conformer of nocardicin into conformations as found in Δ^3-cephalosporins and penicillins, respectively. Recent *ab initio* calculations[64] also indicate that the conformation in which the exocyclic N-substitutuent lies in the lactam plane is more favored, and nonplanarity of the order observed in Δ^3-cephalosporins could be produced by a small increase in the energy (1 to 2 kcal/mol). This suggests that these compounds may also exhibit biological activity, if the substituent at lactam nitrogen does not cause steric problems.

VII. BINDING SPECIFICITIES OF β-LACTAMASES

Detailed conformational studies on compounds 2 to 5, 7 to 10, and 12 (Table 1) which are good inhibitors[81,86,87,101-103] of β-lactamases showed that the relative orientations of the bicyclic ring system ($180°-\alpha_3$) in these compounds are the same. But the orientations of the carboxyl groups (ϕ_2) are not the same in all these compounds. Moreover, some of the compounds (9 and 10), in fact, lack the carboxyl group at C-3 atom. These suggested that penicillin β-lactamases are not very specific for the presence and/or the orientation of the carboxyl group. However it is likely that different types of β-lactamases may differ slightly in their binding requirements. The difference in the binding specificities for penicillins and cephalosporins may lie mainly in the requirement of the orientation of the carboxyl group of the drug molecules.

It is interesting to see that in the favored conformations[36,40] of CP-45899 sulfone and clavulanic acid (the two potent β-lactamase inhibitors[81,86,87,103]), both the orientation of

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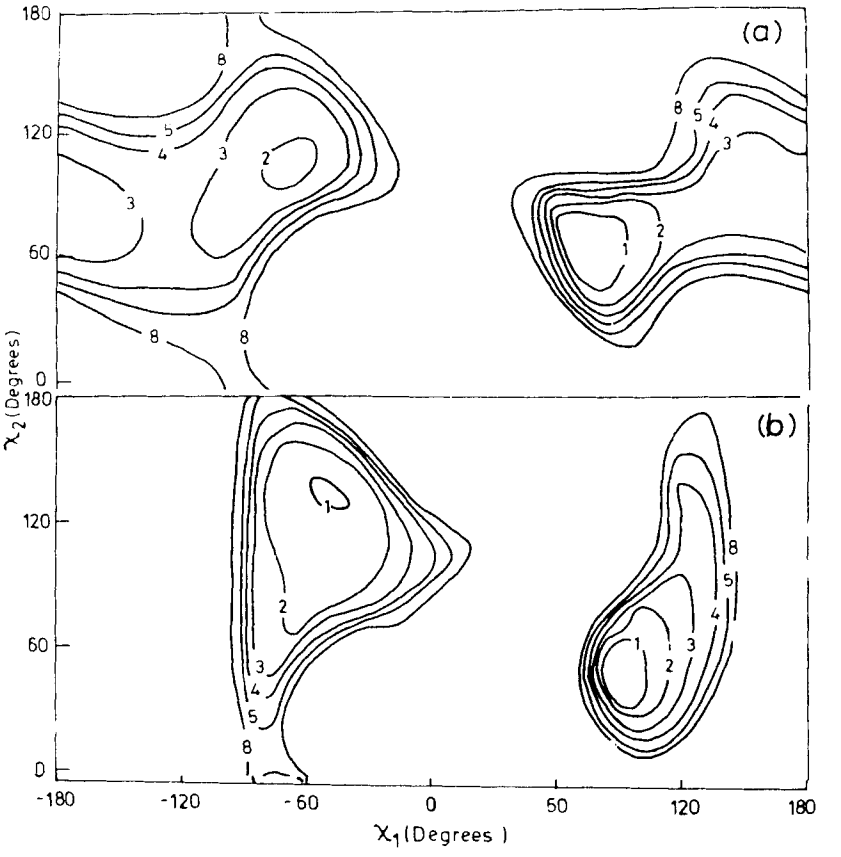

FIGURE 30. Conformational energy maps for the 6β-side chains of (a) penicillin-G and (b) oxacillin. Numbers on contours represent energy in kcal/mole.

the carboxyl group (ϕ_2) and the relative orientation of the bicyclic ring system (180-α_3) differ significantly. This suggests that penicillin β-lactamases can tolerate such conformational changes in the nuclear part of these β-lactamase inhibitors. Another point of interest is that the bicyclic ring systems of penicillin-G sulfone and CP-45899 sulfone are conformationally similar (Table 2).[40] However, they differ in the nature of the substituents at the C-6 atom. This makes the former a substrate for β-lactamases, and the latter a potent inhibitor.[86,87,90] Similarly, 1-oxa penicillin-V is unstable to β-lactamases whereas its nucleus is an inhibitor.[91,104] These suggest that, as in the case of transpeptidases, the 6 β side chain may affect the orientation of the nuclear part in the active site of β-lactamase enzymes such that the lactam peptide bond is suitably placed for cleavage. This explains why the modifications made in the 6 β side chain, with a view to extend drug activity against β-lactamase-producing strains, generally resulted in drugs with reduced level of antibacterial activity.[83,84]

From the extensive studies on penicillin-penicillinase interactions, Citri and co-workers[105–108] have classified penicillins as (1) S-type penicillins that are good substrates and undergo hydrolysis more rapidly than penicillin nucleus and (2) A-type derivatives which are poor substrates and are more resistant to enzymatic hydrolysis. Theoretical[28,41,42,109] studies showed that penicillins could be distinguished on the basis of the computed energy maps for the side chains. In general, S-type penicillins may adopt a variety of conformational states whereas the conformational domain of A-type penicillins may be considered as closed in narrow regions (Figure 30). A lower flexibility

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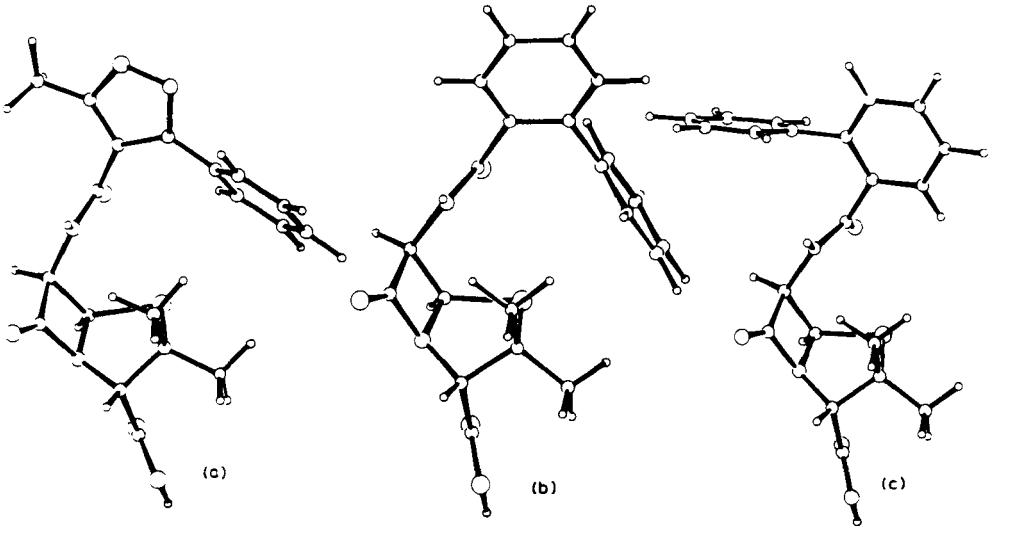

FIGURE 31. Projections of the minimum energy conformations of (a) oxacillin, (b) ancillin, and (c) the local minimum energy conformation of ancillin.

of the 6 β side chain of the A-type penicillins may demand more pronounced changes in the active site of the enzyme in the binding process. Such changes may lead either to a weak or a different mode of binding, unfavorable for enzyme action. On the other hand, since S-type penicillins exhibit greater conformational flexibility for their 6 β side chain, they could easily be aligned in their active site by the receptor enzymes without spending much of its binding energy.[42] Therefore, in the course of enzyme-penicillin interaction, these flexible molecules could easily be placed in the active site in proper orientation for the cleavage of the lactam peptide bond.

In addition to the conformational rigidity of the 6 β side chains in A-type penicillins, the steric hindrance brought about by the bulky substituents in the aromatic (or hetero-aromatic) ring systems also appear to contribute to the degree of resistance to β-lactamases. Thus, both the conformational rigidity and the nature and orientation of the substituents beyond the 6 β amide carbonyl group in the side chain appear to be important for manifesting the resistance to β-lactamases. This is exemplified by the conformational studies[42] on oxacillin and ancillin. Though both these β-lactam compounds exhibit conformational rigidity around 6 β-amide carbonyl, steric hindrance is manifested more effectively in the former than in the latter (Figure 31), which accounts for the slight increase in the rate of hydrolysis of the latter by β-lactamases from *S. aureus* and *B. cereus*.[108,110]

VIII. GENERAL CONCLUSIONS

Conformational studies carried out on several β-lactam compounds have shown that an intact lactam is essential to bring about the conformational similarity between the antibiotic molecule and the natural substrate. The five- or six-membered ring system fused to the lactam is important not only for imparting a nonplanar character for the lactam peptide bond (and hence the required chemical reactivity), but also for keeping the carboxyl group, a possible site of attachment with receptor enzymes, in an appropriate orientation. These chemical and stereochemical requirements for drug activity are effectively met in Δ^2-penems and related compounds, whereas in the classical penicillins and cephalosporins, potentiation by additional side chains appears to be necessary. The

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specific antibacterial properties of β-lactam drugs, thus, appear to be related to their conformational properties as well as the chemical nature of their lactam peptide bonds. It is of interest to note that the binding requirements of transpeptidases and β-lactamases are very close. The former enzymes appear to tolerate only certain conformational features of the drug molecules whereas the latter seem to have some conformational adaptability.

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