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Synopsis of the thesis titled

"CONFORMATIONAL STUDIES ON SOME SIX-MEMBERED AND BICYCLIC RING SYSTEMS"

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This thesis deals with the conformational analysis of some pyranose sugars and β-lactam antibiotics. The former occur widely in nature, in mono-, oligo- and polymeric forms and are associated with many important biological functions in plants and animals. They are also covalently linked to other biopolymers as in glycoproteins. In fact, elucidation of the structure-activity relationship of simple and complex polysaccharides is one of the most interesting and challenging contemporary problems.

Sugars and their derivatives have been extensively studied by many investigators, using diverse experimental and theoretical techniques. One of the most promising aspects amongst the theoretical studies is molecular model building using the principles of conformational analysis. In recent years, this has led to a number of interesting and important results in the field of biopolymers. The starting point of polymer model building studies is the geometry of the monomer unit, generally obtained from X-ray crystal structures of related compounds. Recently X-ray crystal structure studies have indicated that the geometry of the pyranose ring is slightly different in

different derivatives. These small differences in the conformations of the monomer unit may lead to pronounced changes in the conformations, and hence in the properties, of the polymer. Since most of the naturally occurring polysaccharides have pyranose sugars as their repeating units, a detailed understanding of the possible conformations of the pyranose ring is very necessary for studies on polysaccharides. Hence in the present work, the different conformations which a pyranose ring can assume have been investigated in detail, using semiempirical potential energy functions.

The approach used for the study of six-membered rings has been extended to bicyclic systems, namely the β-lactam antibiotics (penicillins and cephalosporins). These drugs have been one of the most successful and widely used antimicrobial agents. However, their widespread use has led to the preponderance of penicillin resistant bacteria, which produce enzymes (penicillinases) which inactivate penicillin. Hence, there is a constant need for designing better drugs active against the resistant bacteria. A detailed knowledge of the mechanism of action of penicillin, and of penicillin-penicillinase interaction, is thus vital for drug design. Hence, in the present work, conformational analysis of penicillins, cephalosporins and clavulanic acid (an inhibitor of penicillinases) has also been included.

This thesis contains six chapters. A brief review of the earlier theoretical work on pyranose sugars and on penicillins

and cephalosporins, is presented in the first chapter. A summary of the principles of conformational analysis is included, where the various potential energy functions, used in the present work, are discussed. A brief discussion of the solvent accessibility calculations is also included.

Conformational analysis of eight D-pentopyranoses has been presented in Chapter II. The results indicate that the $^4C_1$(D) and $^1C_4$(D) chair conformations are the minimum energy conformations. The solid state conformations of the pentopyranoses, as observed in the crystal structures lie in the minimum energy regions. The calculated potential energy difference between the $^4C_1$ and $^1C_4$ chairs is small for all the molecules studied, suggesting that solvent interactions may play a dominant role in influencing the favoured conformations in solution. On the basis of both potential energy and solvent accessibility criteria, the results suggest the $^4C_1$ as the favoured conformation for β-D-lyxose, β-D-ribose and α- and β-D-xylase; the $^1C_4$ chair for α-D-arabinose; and a $^4C_1 \rightleftharpoons {}^1C_4$ equilibrium for β-D-arabinose, α-D-lyxose and α-D-ribose, in reasonable agreement with NMR studies. The present work also suggests that the $^4C_1$ and $^1C_4$ conformations of the pyranose ring are quite flexible, being able to accommodate distortions of upto 10° in torsional angles and 3° in bond angles, without much (< 0.5 Kcal.mole^{-1}) change in the potential energy. The boat and twist-boat conformations are 4-5 Kcal.mole^{-1} higher in energy than either of the chairs,

suggesting that the former are unlikely to be present either in the solid state, or in solution, or in a polymeric chain to any significant extent.

Similar studies carried out on the eight important D-hexopyranoses; α- and β-galactoses, glucoses, idoses and mannoses, are presented in Chapter III. On the basis of both potential energy and solvent accessibility calculations, the $^4C_1$ chair conformation is favoured for α- and β-galactoses, glucoses and mannoses both in solid state and solution. The solid state conformations of these sugars are also seen to lie in the minimum energy region near the $^4C_1$ chair. However, for both α- and β-idoses, potential energy calculations favour $^4C_1$ while solvent accessibility criteria favour $^1C_4$, suggesting a $^4C_1 \rightleftharpoons {}^1C_4$ equilibrium in solution. The $^4C_1$ chair conformation of the pyranose ring of the hexoses is also flexible, and it is possible to obtain a family of slightly distorted chair conformations, differing significantly (upto 10°) in the ring torsional angles, with only a small change in the potential energy. The boats and twist boats are more than 5 Kcal.mole^{-1} higher in energy than the $^4C_1$ chair, and are unlikely to be present in the solid state, solution or polymeric chains. These observations may be useful for refinement of X-ray fibre diffraction studies, and in understanding the various properties of carbohydrates.

Chapter IV deals with the conformational analysis of penicillin sulfide, penicillin α-sulfoxide and penicillin β-

sulfoxide. The thiazolidine ring shows two minimum energy conformations (C_3 puckered and C_2 puckered) in all the three molecules. For penicillin sulfide, the two minima are of comparable energies, and are separated by a low energy barrier. In penicillin α-sulfoxide also, the two minima are of comparable energies, but the barrier separating the two is higher than that of the sulfide. On the other hand, C_2 puckered conformation of the thiazolidine ring of β-sulfoxide has about 2.5 Kcal.mole^{-1} lower energy than the C_3 puckered form. The aminoacyl group also favours a different conformation in penicillin β-sulfoxide compared to the sulfide or α-sulfoxide. These differences have been attributed to the differences in their activities.

Conformational studies on Δ^3 and Δ^2 cephalosporins have been discussed in Chapter V. The dihydrothiazine ring of both these molecules show two minimum energy conformations. The two minima of Δ^3 cephalosporin are of comparable energies, and the lactam peptide bond is moderately nonplanar in both the conformations. For Δ^2 cephalosporin, in the energetically favoured form, the lactam peptide bond is nearly planar, which may account for its inactivity. The present studies also suggest that the mode of binding of cephalosporins with the cross-linking enzymes is likely to be different from that of penicillins.

Chapter VI discusses the conformational analysis of clavulanic acid, an inhibitor of penicillinases. The

oxazolidine ring shows only one minimum energy conformation, suggesting that the five-membered ring in clavulanic acid is more rigid compared to that in penicillins. A comparison of the conformations of penicillin and clavulanic acid suggests that the C_3 puckered conformation of penicillin is likely to be the one which binds with penicillinases.

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I. INTRODUCTION

1.1. Importance of the conformational analysis of six-membered (pyranose sugars) and bicyclic (β-lactam antibiotics) ring systems:

Conformations are the different shapes of a molecule obtained by rotations about one or more bonds, without any bonds being broken; and conformational analysis deals with the implications of these shapes on the physical and chemical properties of the molecule.

The influence of conformation on the chemical and physical properties of small and large molecules is well-known. Recently, conformational energy calculations have led to a number of interesting and important results in the field of biopolymers (Ramachandran, 1967; Flory, 1969; Chiurdoglu, 1971; Hopfinger, 1973; Brant, 1976). The polymer conformation is influenced to a great extent by the conformation of the monomer unit from which it is built. Hence, to understand the conformations of polysaccharides, a thorough understanding of the conformations of simple sugars is a necessity.

The term 'conformation' was first used by Howarth (Howarth, 1929) to describe the various shapes of carbohydrates. He also suggested that the pyranose sugar can assume two chair, six boat and six twist boat conformations. It was

subsequently established by single crystal X-ray diffraction studies (Cox and Jeffrey, 1939; McDonald and Beevers, 1953; Jeffrey, 1973) and nuclear magnetic resonance studies (Lemieux et al., 1958; Lenz and Heeschen, 1961; Rao and Foster, 1965; Angyal, 1969; Durette and Horton, 1971) that the pyranose sugars exist in the chair conformation in the solid state as well as in solution.

The geometry of the sugar ring forms the starting point of various theoretical studies on polysaccharides, e.g., molecular model building, X-ray fibre diffraction analysis, Monte Carlo simulations of polymer chains etc. Generally, the geometry of the sugar residue for such calculations is obtained using average values of internal coordinates (bond lengths, bond angles and torsional angles) derived from the crystal structure data of a large number of related compounds (Arnott and Scott, 1972). However, recent analysis indicated that the bond lengths are nearly constant for these molecules, but the ring bond angles vary over a small range, causing appreciable variations (upto 10°) in the ring torsional angles (French and Murphy, 1973, 1977). The pyranose ring thus seems to be somewhat flexible, and these small changes in the conformations of the sugar residue may have a pronounced effect on the conformations of polymers.

Also, during interactions with enzymes, the pyranose sugars are known to undergo considerable distortions (Blake

et al., 1967). A knowledge of the energy losses involved in such such deformations is important in understanding the substrate-enzyme binding interactions.

Hence, in the present work, conformational flexibility of the pyranose ring has been investigated in detail, using semi-empirical potential energy functions.

Moreover, if the energy difference between two conformations is small, a polar solvent may preferentially stabilize the one whose polar groups are so oriented as to interact favourably with the solvent. Hence, to estimate the solvent interaction, solvent accessibility maps have also been constructed for the sugars that have been studied in the present work.

The approach used for analysing the conformations of the six membered ring has been extended to the fused ring systems of β-lactam antibiotics such as penicillins, cephalosporins etc., which contain a lactam ring fused to a five-membered or six-membered ring.

Despite spectacular results in chemotherapy in the earlier stages, the efficacy of penicillins soon declined due to the emergence of penicillin resistant bacteria. These strains achieve their resistance by producing penicillinases, which cleave the lactam peptide bond and inactivate penicillins. Cephalosporins, though less active than penicillins, are stable against penicillinases. These are, however, susceptible

to cephalosporinases. There is thus a perpetual need for synthesizing better drugs.

In the present work, the different possible conformations of the bicyclic ring systems of these antibiotics have been analysed with a view to correlate the shapes of these molecules with their biological activity. Such information is vital for designing improved drugs, active against β-lactamase producing strains.

1.2. Nomenclature of Sugars:

1.2.1. Configurational Nomenclature:

Hexose and pentose sugars can adopt a six-membered ring form (pyranose), a five-membered ring form (furanose) or an open chain (acyclic) form. These have been shown in Fig. 1.1, for a hexose sugar.

In solution, these sugars exist in the ring forms in equilibrium with small amounts of the open chain forms. The pyranose ring is generally more stable than the furanose ring. In most of the naturally occurring polysaccharides, the sugar residues exist in the pyranose form. Hence in the present work, the pyranose sugars alone have been studied.

The sugars have been classified into D and L series, according to the configuration at the C-5 and C-4 atoms for hexoses and pentoses respectively. The D sugars predominate in nature.

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Depending on the orientations of the hydroxyl group at the C-1 atom, the sugars are further classified into α and β forms (α and β anomers).

The different orientations of the hydroxyl group at the remaining carbon atoms (C-2, C-3 and C-4 for hexoses and C-2 and C-3 for pentoses) give rise to eight hexose sugars and four pentose sugars.

1.2.2. Conformational Nomenclature:

As mentioned earlier, for pyranose sugars, two chair ($^4C_1$ and $^1C_4$), six boat ($^{1,4}B$, $^{2,5}B$, $^{3,O}B$, $B_{1,4}$, $B_{2,5}$ and $B_{3,O}$) and six twist-boat ($^1S_3$, $^1S_5$, $^3S_5$, $^3S_1$, $^5S_1$ and $^5S_3$) conformations are considered as most probable. These are shown in Figs. 1.2, 1.3 and 1.4 respectively. The nomenclature used here is adopted from Stoddart (Stoddart, 1971).

For the chair conformations, it is seen that C-2, C-3, C-5 and O-5 lie approximately in a plane. The substituents having orientations approximately parallel to this plane are termed as equatorial substituents, while those approximately perpendicular to this plane are called axial substituents. Thus, for β-D-glucopyranose in the $^4C_1$ combination, all the hydroxyl groups are in the equatorial orientation. The axially and equatorially oriented bonds interchange when the pyranose ring changes from the $^4C_1$ to $^1C_4$.conformation.

1.3. Brief Review of the Earlier Theoretical Studies:

1.3.1. Conformational Energy Calculations on Pyranoses:

The first attempts towards the conformational analysis of carbohydrates using classical potential functions were made by Rao and coworkers (V.S.R. Rao et al., 1967). Sundararajan and Rao (Sundararajan and Rao, 1968) predicted the favoured conformations of aldohexopyranoses and aldopentopyranoses by computing the nonbonded interactions using ideal geometries of these molecules. These predictions generally showed qualitative agreement with experimental data. However, there were discrepancies in the case of α-D-altropyranose and α-D-idopyranose. This is due to the neglect of polar interactions and failure to include distortions of the ring. More detailed analysis of the hexo- and pentopyranoses was carried out by Vijayalakshmi and Rao (Rao et al., 1971; Vijayalakshmi and Rao, 1972, 1973, 1974; Vijayalakshmi et al., 1973), in which the nonbonded and electrostatic interactions, as well as the bond angle strain energies were taken into account. Conformational energy was minimised by tilting the axially oriented hydroxymethyl and hydroxyl groups. The free energy differences between the two chair conformations were obtained by including conformational entropy contributions. These studies indicated that small tilts in the orientations of the axial substituent released the steric strain to a considerable extent. The $^{4}C_{1}(D)$ chair conformation was generally the most

favoured one. In some cases, especially in pentopyranoses, the energy difference between the two chair conformations was small, indicating that these would exist in equilibrium in solution. Thesp predictions are in good agreement with experimental observations (Rudrum and Shaw, 1966; Angyal, 1968). Vijayalakshmi and Rao have successfully extended these studies to methyl hexo- and pentopyranoses, acetylated sugars and cyclitols (Vijayalakshmi and Rao, 1972, 1973; Vijayalakshmi, 1972), where the predicted favoured conformations of these molecules show good agreement with experimental data.

Rees and Smith (Rees and Smith, 1975a, 1975b) have investigated the conformations of pentopyranoses in the $^{4}C_{1}(D)$ and $^{1}C_{4}(D)$ conformations. The ring was assumed to have a rigid geometry, obtained by using averaged bond lengths, bond angles and torsional angles. Ring geometry obtained from crystal structure was also used. Monte Carlo calculations were performed to investigate the populations of different conformations, obtained by allowing the hydroxyl hydrogens to rotate about the O-H bonds. The predictions based on these results do not explain the experimental results satisfactorily.

Virudachalam and Rao (Virudachalam and Rao, 1976) have recently studied conformations of 2-acetamido-2-deoxy-aldohexopyranoses and N-acetyl-D-hexosamines, the constituents of peptidoglycan. The favoured conformations assigned and the percentages of α and β anomers in the equilibrium mixture

predicted from these studies are in agreement with the available experimental data.

When the present work was completed, Melberg and coworkers (Kilderby et al., 1977, 1978) also published detailed conformational energy calculations on α- and β-D-glucopyranoses. In these studies, all the degrees of freedom (bond lengths, bond angles, and torsional angles in the ring as well as of the substituents) were allowed to vary while minimizing the energy. The results indicated the $^4C_1$ chair form to be the minimum energy conformation. A large number of closely spaced minima were observed, differing mainly only in the orientations of the hydroxyl hydrogens.

1.3.2. Studies on Non-Chair Conformations:

Rao and coworkers (Rao et al., 1967) on the basis of conformational energy calculations have shown that the boat conformations would be energetically highly unfavourable. Recently, Goebel and coworkers (Goebel et al., 1976) have also investigated the conformational energies of β-D-glucopyranose in chair, boat and twist-boat conformations, using ideal ring geometries, with slight deviations in the bond angles to ensure ring closure. These authors indicated that the boat and twist-boat forms have energies intermediate between the $^4C_1$(D) and $^1C_4$(D) forms. Melberg and coworkers (Kilderby et al., 1977) have also shown that the twist-boats have considerably higher energies than the $^4C_1$(D) chair conformations of α-D-glucopyranose.

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1.3.3. Penicillins:

Though extensive experimental studies have been carried out on penicillins, theoretical studies have been few. Earlier theoretical studies on penicillins were based on model building (Tipper and Strominger, 1965; Lee, 1971). Tipper and Strominger suggested that the action of penicillin is due to its structural similarity with the natural substrate (X-D-ala-D-ala) of the cross linking enzyme. Lee indicated that the similarity may be with the transition state of X-D-ala-D-ala. These hypotheses have also been supported by quantum chemical calculations (Topp and Christensen, 1974; Boyd et al., 1975). Recently, Boyd (Boyd, 1979) has tried to obtain a quantitative estimate of the structural similarity of β-lactam antibiotics and the transition state of glycyl dipeptide by comparing the interatomic distances between some of the atoms important for binding to the enzyme.

Virudachalam and Rao have carried out detailed studies on β-lactam antibiotics using semiempirical potential energy functions (Virudachalam and Rao, 1977; 1978; Virudachalam, 1978). They have established the conformational similarity of penicillin and the D-ala-D-ala fragment. They have also indicated that the orientation of the lactam ring relative to the thiazolidine ring, and specific orientations of aminoacyl and carboxyl groups are important for biological activity. These authors have also extended these studies to X-L-ala-D-ala,

X-D-butyl-D-ala and X-D-val-D-ala fragments of the pentapeptide moiety and have predicted, in agreement with experimental observations, that the changes in the pentapeptide moiety have significant effect on the cross-linking reaction during peptidoglycan biosynthesis.

1.3.4. Cephalosporins:

As indicated earlier, interest in cephalosporins stems from their stability against penicillinases. Their activity is much lower than the penicillins, and several chemical modifications have been made, with the hope of increasing their activity. Of these, the compound with a double bond in the dihydrothiazine ring between C-3 and C-4 (Δ^3 cephalosporin) is biologically active, whereas the one with the double bond between C-2 and C-3 (Δ^2 cephalosporin) has much reduced activity.

Several workers have attempted to unravel the structural basis of the differences in the activities of cephalosporins. Sweet and Dahl (Sweet and Dahl, 1970) tried to correlate the biological activity with the resonance properties of the β-lactam ring. X-ray crystal structure studies have shown that in the solid state, the β-lactam nitrogen of Δ^3 cephalosporins assumes a pyramidal character (Sweet and Dahl, 1970), while that of Δ^2 cephalosporin is nearly planar. The lactam C-N bond is also significantly shortened in the later compound. Sweet and Dahl have postulated that the cross-linking enzymes

would 'recognise' (bind to) both the molecules. However, the lactam peptide bond of the Δ^3 cephalosporins could be easily cleaved due to the nonplanarity, and the enzyme would be acylated and inactivated. The Δ^2 compound, due to the planarity of the peptide and the shorter C-N bond would be more stable, and hence would be inactive. Quantum chemical (CNDO/2) calculations on cephalosporins (Topp and Christensen, 1974) have indicated that the transition state energy of Δ^2 cephalosporin is 4-5 Kcal.mole^{-1} higher than the Δ^3 cephalosporin, suggesting this to be the cause of the differences in their activities. Recent studies of Virudachalam and Rao (Virudachalam and Rao, 1977, 1978) have indicated that specific orientations of aminoacyl and carboxyl groups are also important for biological activity. However, the preferred orientations of the carboxyl group in the cephalosporins are different from those in penicillins. These differences may lead to a different mode of binding of cephalosporins from that of penicillins with the cross-linking enzymes.

1.3.5. Specificity of Penicillinases:

Penicillin resistant strains achieve their resistance by producing penicillinases, which inactivate the drug by hydrolysing the lactam peptide bond. However, the binding specificity of penicillinases is not known. Different derivatives of penicillin show different modes of binding to penicillinase (Citri, 1972, 1973). Samuni and Meyer have done

quantum chemical calculations (Samuni and Meyer, 1978) on some of the derivatives of penicillin, and have tried to obtain a correlation between conformation and mode of binding to penicillinases. These authors have considered only the side groups in their calculations and have completely neglected the fused ring system. However, since fused ring systems do play a major role in affecting the stability of these drugs against penicillinases, the neglect of the fused ring system in the above theoretical study is not fully justified.

A recently isolated β-lactam compound, clavulanic acid, is a potent inhibitor of penicillin β-lactamases. Though biochemical (Fisher et al., 1978) and crystallographic (Howarth et al., 1976) studies have been carried out on this molecule, the possible range of conformations which its oxazolidine ring can assume has not been investigated by theoretical methods. Such information may be useful for understanding its interaction with penicillinases, and also the binding requirements of the enzyme.

1.4. Conformational Energy Calculations:

Conformational analysis of biomolecules using classical potential energy functions has been discussed at great lengths in several reviews and books (Westheimer, 1956; Kitaigorodsky, 1961; Hendrickson, 1962; Eliel et al., 1965; Scott and Scheraga, 1966a, 1966b; Ramachandran and Sasisekharan, 1968; Williams et al., 1968; Flory, 1969; Scheraga, 1971;

Schellman and Lifson, 1973; Altona and Faber, 1974; ~~Hopfinger, 1976a~~; Brant, 1976). Hence, only the potential functions used in the present work have been briefly ~~discussed~~ described.

The total conformational energy (V_{TOT}) of a molecule can be expressed as

$$V_{TOT} = V_{NB} + V_{ES} + V_{HB} + V_{TOR} + V_{\theta} + V_{L} \qquad .. 1.1$$

where

V_{NB} is the energy due to nonbonded interactions,

V_{ES} is the energy due to electrostatic interactions,

V_{HB} is the energy due to the formation of hydrogen bonds,

V_{TOR} is the energy due to torsion about single or double bonds,

V_{θ} is the energy due to bond angle distortion, and

V_{L} is the energy due to changes in the bond lengths.

Since the bond lengths were kept constant throughout, contributions due to V_{L} was not considered in the present work. The other functions are discussed below.

1.4.1. Nonbonded Interactions:

Two of the most commonly used potential functions to evaluate the nonbonded interaction energies are due to Buckingham and Lennard-Jones, and are given by equations 1.2 and 1.3 respectively.

$$V_{NB} = - \frac{A}{r_{ij}^6} + B \, Exp(-\mu r_{ij}) \qquad .. 1.2$$

$$V_{NB} = - \frac{A}{r_{ij}^6} + \frac{C}{r_{ij}^{12}} \qquad .. 1.3$$

where r_{ij} is the distance between the interacting atoms and A, B, C, μ are constants, specific for that pair. The first term denotes attraction due to London dispersion forces. The constant A is evaluated from the Slater-Kirkwood equation (Pitzer, 1959) using the atomic polarizabilities and the effective number of valence shell electrons. The second term corresponds to repulsion due to overlap of electronic clouds. This term dominates at short distances. The constants B and C are so chosen as to make the potential energy minimum at $r_{ij} = r_o$, the sum of the van der Waal (VDW) radii of the interacting atoms. A constant value of about 4.6 has been used for μ in the studies on biopolymer conformations. Both these functions have been extensively used in the studies on biopolymers (Ramachandran and Sasisekharan, 1968; Go and Scheraga, 1969; Yathindra and Rao, 1970; Olson and Flory, 1972; Engler et al., 1973; Yathindra and Sundaralingam, 1973; De Santis et al., 1974; Pincus et al., 1976; Brant, 1976; Tosi et al., 1978).

A slightly modified form of Buckingham function was proposed by Kitaigorodsky (Kitaigorodsky, 1961).

$$V_{NB} = 3.5[\frac{-0.04}{Z^6} + 8600 \, Exp(-13 \, Z)] \qquad .. (1.4$$

where $Z = r_{ij} / r_o$ ..

Here, r_o is the distance between the interacting atoms corresponding to minimum energy. The values of r_o for different pairs of atoms are given in Table 1.1. This function is computationally simpler to evaluate, since it has only one parameter. This function has been widely used in the studies on carbohydrates (Rao et al., 1967; Rees and Skerrett, 1968, 1970; Sundararajan and Rao, 1969a, 1969b, 1970; Rees, 1969; Yathindra and Rao, 1970, 1971, 1973; Sathyanarayana and Rao, 1970, 1971, 1972; Whittington, 1971; Vijayalakshmi and Rao, 1972, 1973; Whittington and Glover, 1973; Virudachalam and Rao, 1976, 1977; Nagarajan and Rao, 1978). Hence, in the present study, Kitaigorodsky's function has been used for evaluating the nonbonded interaction energy.

1.4.2. Electrostatic Interactions:

The electrostatic interaction energy can be expressed as

$$V_{ES} = \frac{332\, q_1 q_2}{\varepsilon' r_{ij}} \qquad .. 1.5$$

where q_i, q_j are the fractional charges of the atoms i and j, r_{ij} is the distance between them, ε' is the effective dielectric constant of the medium and 332 is the numerical factor for converting energies to Kcal.mole^{-1}.

In the present study, the fractional charges used in the above expression were obtained following Del Re's (Del Re, 1958) method for σ charges and Huckel's method (Pullman and Pullman, 1963) for π charges.

Table 1.1: Constants used for the Kitaigorodsky's function.

Atom pair	r_o (Å)	Atom pair	r_o (Å)
H ... H	2.67	C ... N	3.55
H ... O	2.90	C ... S	3.94
H ... C	3.22	C ... OH	3.55
H ... N	3.00	C ... CH_3	4.00
H ... S	3.39	C ... CH_2OH	4.11
H ... OH	3.00	N ... N	3.33
H ... CH_3	3.44	N ... S	3.72
H ... CH_2OH	3.56	N ... CH_3	3.78
O ... O	3.11	N ... CH_2OH	3.90
O ... C	3.44	N ... OH	3.33
O ... N	3.22	S ... OH	3.72
O ... S	3.61	S ... CH_3	4.17
O ... OH	3.22	OH ... OH	3.33
O ... CH_3	3.67	OH ... CH_3	3.78
O ... CH_2OH	3.80	OH ... CH_2OH	3.90
C ... C	3.80	CH_3 ... CH_3	4.22

The effective dielectric constant has been shown to have a much lower value than the actual dielectric constant of the medium (Ramachandran and Srinivasan, 1970). Generally, values between 2-5 are used in conformational analysis (Flory, 1967; Vijayalakshmi, 1972; Hopfinger, 1973). In the present work, a value of 3.5 has been used. It is known that small changes in the values of ε' do not affect the relative energies of the different conformations significantly.

1.4.3. Hydrogen Bond Energy:

For a hydrogen bond of the type X-H...Y to form, X and Y should be electronegative elements, and the distance between them should be around 3.0 Å. Hydrogen bond energy is essentially due to the electrostatic interaction between the partially polarised hydrogen atom covalently bonded to X and the negative charge density on Y. The attraction energy is between 3-8 Kcal.mole^{-1} and has to be explicitly included in the energy calculations.

Numerous empirical functions of varying degrees of complexity have been proposed to describe the hydrogen bond (Schroeder and Lippincott, 1957; Chidambaram and Sikka, 1968; Blackwell et al., 1969; Ramachandran, 1973). These include contributions due to X-H, Y···H, X···Y interactions.

Recently, Scheraga and coworkers (McGuire et al., 1972) have proposed a computationally simple function for the

hydrogen bond

$$V_{HB} = \frac{A_k}{r^{12}} - \frac{B_k}{r^{10}} \qquad .. (1.6)$$

where r is the distance between the hydrogen atom the the atom with which it forms the hydrogen bond, and A_k, B_k are constants, which have different values for OH...O<, OH...O=, NH...O<, NH...O=, etc. types of interactions. For NH...O= type of hydrogen bond, A_k = 7649.3 Kcal.mole^{-1}.Å^{12} and B_k = 3458.8 Kcal.mole^{-1}.Å^{10}.

1.4.4. Torsional Potential Energy:

The energy due to torsion about single or double bond is given by, respectively,

$$V_{TOR} = \frac{V_o}{2}(1 + \cos 3\chi) \qquad .. 1.7$$

$$V_{TOR} = \frac{V_o}{2}(1 + \cos 2\chi) \qquad .. 1.8$$

where V_o is the barrier height and χ is the torsional angle about the bond.

The equation 1.7 is valid when the substituents at the carbon atoms of the C-C bond about which rotations are made, make equal angles. Since in the present work, the bond angles are varied independently, the above equation is not strictly valid. Hence, a method proposed by Allinger (Allinger et al., 1971) is used in the present study. In this

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method the torsional energy of each of the nine (say X-C-C-Y) fragments is calculated separately using the expression

$$V_{TOR} = \frac{V_o}{2}(1 + \cos 3\chi), \quad 0^\circ \leq \chi \leq 60^\circ$$

$$= 0 \qquad 60^\circ < \chi \qquad \text{.. 1.9}$$

The value of V_o for C-C bond is taken to be 0.8 Kcal.mole^{-1}. This gives a total of 2.4 Kcal.mole^{-1} for the torsional barrier across the C-C bond, in agreement with earlier values (Scott and Scheraga, 1965).

Following Momany et al., for rotations about N-C$^\alpha$ and C$^\alpha$-C bonds in peptides (torsional angles φ and ψ) the torsional potential energy contribution is neglected (Momany et al., 1975).

1.4.5. Bond Angle Deformation:

The bond angle deformation energy can be expressed as

$$V_\theta = \frac{K_\theta}{2}(\theta - \theta_o)^2 \qquad \text{.. 1.10}$$

where θ is the bond angle, θ_o is the equilibrium (strainless) bond angle and K_θ is the force constant. Table 1.2 gives the values of θ_o and K_θ used for the different angles encountered in the present work.

1.5 Solvent Accessibility:

Favoured conformations of a molecule in solution are

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Table 1.2: The values of $K_\theta/2$ and θ_o for computing the bond angle distortion energy*

Bond Angle	$K_\theta / 2$ (Kcal.mole^{-1}/rad^2)	θ_o (Degrees)
HCH HCC HCO HCN	40.0	109.47
CCC OCC	58.0	109.47
OCO	50.0	109.47
COC	70.0	112.50
HCS	21.6	108.20
CCS NCS	30.20	107.80
CSC	36.0	94.30
CNC	80.0	120.0
SC $_{sp^2}$C	80.0	120.0

* Vijayalakshmi (1972); Allinger et al.,(1975).

influenced to a great extent by interactions with the solvent, if the energy difference between them is small. Since explicit computation of the solute-solvent interaction would require unrealistic amount of computer time, a number of approximate methods have been proposed to estimate these interactions.

The hydration shell model (Moelwyn-Hughes, 1959; Gibson and Scheraga, 1967) considered contributions only from those solvent molecules which were the nearest neighbours of the solute molecule, i.e., those within the first hydration shell. The Krimm-Venkatachalam model considered only the interactions between potential hydrogen bonding sites (Krimm and Venkatachalam, 1971). The exposure coefficient model (Brown et al., 1972) estimates the extent to which various atoms in the molecule are exposed to the solvent. These models have been reviewed by Hopfinger (Hopfinger, 1976b) and Scheraga (Scheraga, 1977).

Lee and Richards introduced the concept of solvent accessibility (Lee and Richards, 1971), or the accessible surface area of an atom in the solute molecule. This approach has been used successfully by several workers to correlate accessibility with physical properties of macromolecules, e.g., hydrophobicity, aqueous solubility of proteins (Chothia, 1974; Amiden et al., 1976). It has also been applied to study the conformational stabilities of dipeptides (Ponnuswamy and Manavalan, 1976, 1977; Manavalan et al., 1977) and dinucleotides (Ponnuswamy and Thiagarajan, 1978).

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An atom in the solute molecule is said to be accessible to the solvent molecule if the solvent can be brought into a van der Waal contact with the solute molecule, without penetrating the VDW spheres of any of the other atoms in the solute molecule. The accessible surface area of an atom is an area A on the surface of the sphere of radius R, such that at every point in A, the above condition is satisfied. The radius R is the sum of the VDW radii of the atom and the solvent molecule. Accessibility S is given by

$$S = \frac{100\ A}{4\pi R^2} \qquad .. 1.11$$

The accessibility of an atom is the measure of the surface area of the atom available.for interaction with the solvent. Though it may be possible to have a good hydrogen bond (and hence a strong interaction) with the solvent despite a low accessibility value, generally a higher solvent accessibility indicates a higher probability of interaction with the solvent. The value of S depends on the conformation of the solute molecule, since the positions of various groups accessible to the solvent vary significantly with changes in the conformation. Conformations with higher solvent accessibility may be regarded as favoured by solvent interaction.

It should be emphasized that a high value for solvent accessibility does not necessarily indicate a good hydrogen

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bond. Nor can the accessibility values be directly converted into free energy values. They serve only as qualitative estimates of solvent interaction.

In the present work, the solvent molecule (water) was assumed to be a sphere, and the accessibility of each atom in a pyranose sugar molecule was computed. For each of the conformations studied, the sum of the accessibilities of the polar groups is taken as a qualitative estimate of the non-specific solute-solvent interaction. Solvent accessibilities have been computed for all the pentapyranoses and eight hexapyranoses, over the entire range of conformations of the pyranose ring.

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II. ALDOPENTOPYRANOSES

2.1. Introduction:

As mentioned in Chapter I, conformational analysis of aldopentopyranoses has drawn the attention of a number of theoretical and experimental workers. Though the theory could successfully explain the favoured conformations of penta-pyranoses in solution, some discrepancies have been observed in the solid state. Though the theory predicts $^4C_1$ to be the favoured conformation for β-D-ribose and α-D-xylose, the former exists in the $^1C_4$ conformation, while the later exists in both the $^4C_1$ and $^1C_4$ conformations in the solid state. These discrepancies may be due to the fact that a rigid geometry was used for the pyranose ring in the earlier studies. In fact, it can be seen from recent X-ray data on sugars that though the bond lengths are reasonably constant from structure to structure, the ring bond angles and torsional angles vary over a small but significant range. This suggests that the use of a rigid ring geometry for pyranoses is not fully justified. However, no information is available about the energies involved in producing these distortions in the pyranose ring. Secondly, these sugars are a class of stereo-isomers which differ only in the orientations of the hydroxyl groups, and it is of interest to study the effects of various steric arrangements of these groups on the geometry of the ring.

Earlier theoretical (Vijalakshmi and Rao, 1972; Vijayalakshmi, 1972; Rees and Smith, 1975a, 1975b) and experimental (Rudrum and Shaw, 1965, Lemieux and Stevens, 1966) studies have indicated that the energy differences between the $^4C_1$ and $^1C_4$ conformations are small for some of the pentopyranoses and both the forms exist in equilibrium in aqueous solutions. Under these circumstances, interaction with the solvent may play an important role in determining the favoured conformations in solution.

Hence, in the present study, an attempt has been made to construct the potential energy and solvent accessibility surfaces of the α and β anomers of all the D-pentopyranoses.

2.2. Conformational Parameters:

For describing a molecule containing six atoms 12 parameters (3N-6, where N is the number of atoms) are necessary. For a six-membered ring, if the six bond lengths are assumed to be constant, six angles (three bond angles and three torsional angles) would be necessary to specify its conformation. Pickett and Strauss (Picket and Strauss, 1970) have showed that a six-membered ring can be completely specified by three bond angles (β_1, β_2 and β_3) and three 'conformational' angles (α_1, α_2 and α_3) which denote rotations about virtual bonds O_5-C_2, C_2-C_4 and C_4-O_5 (Fig. 2.1). All the possible shapes of the ring can be described by varying the α^s over a wide range.

The spherical polar coordinate system proposed by Pickett and Strauss provides a nearly ideal description of the shapes of the six membered ring. In this system, the α^s are rotated to the spherical polar coordinates r, θ and φ by the following expression

$$\alpha_i = r[\cos\theta + 2\sin\theta\cos(\frac{4\pi i}{3} - \frac{2\pi}{3} + \varphi)]$$

$$i = 1,2,3 \qquad .. 2.1$$

Studies on cyclohexane and related oxanes have indicated that the entire range of conformations of the six membered ring could be scanned by varying θ and φ over ranges 0°-180° and 0°-360° respectively. Since the variations in r and β^s are found to be comparatively small, the conformation of the ring can be represented by the other two parameters θ and φ.

This description is very convenient for constructing the conformational energy maps of a six membered ring, since the energy surface and solvent accessibility surface can be depicted respectively in the form of isoenergy and iso-accessibility contours in the θ - φ plane. The boat and twist-boat conformations, and the process of $^4C_1 \rightleftharpoons {}^1C_4$ interconversion can also be described using this notation. Hence the r, θ, φ coordinate system of Pickett and Strauss has been adopted in the present work.

Table 2.1 shows the approximate values of α_1, α_2, α_3, θ and φ for various chair, boat and twist-boat conformations.

Table 2.1: Values (approximate) of the conformational parameters α_1, α_2, α_3, θ and φ for the chair, boat and twist-boat conformations of a pyranose ring.

Conformation	α_1 (Degrees)	α_2 (Degrees)	α_3 (Degrees)	θ (Degrees)	φ (Degrees)
$^4C_1$	-35	-35	-35	+ 0	0-360
$^1C_4$	35	35	35	180	0-360
$B_{3,0}$	30	-60	30	90	0
$^1S_3$	60	-60	0	90	30
$1,4_B$	60	-30	-30	90	60
$^1S_5$	60	0	-60	90	90
$B_{2,5}$	30	30	-60	90	150 120
$^3S_5$	0	60	-60	90	150
$3,0_B$	-30	60	-30	90	180
$^3S_1$	-60	60	0	90	210
$B_{1,4}$	-60	30	30	90	240
$^5S_1$	-60	0	60	90	270
$2,5_B$	-30	-30	60	90	300
$^5S_3$	0	-60	60	90	330

2.3. Conformational Energy Calculations:

2.3.1. Generation of coordinates:

For a set of values of φ, θ and r, the angles of rotation (α_1, α_2, α_3) about the virtual bonds were calculated using Eqn. 2.1. These, together with the bond angles β_1, β_2 and β_3 were used to generate the coordinates of the ring atoms. The hydroxyl groups were not fixed, such that the C-O bonds made equal (tetrahedral) angles with the two adjacent ring bonds. The hydrogens attached to the ring carbons were fixed such that the C-H bonds make equal angles with the remaining three bonds at the ring carbon atom. If β is the bond angle in the ring, τ is the angle made by the C-O bond of the substituent with the two ring bonds, and ψ is the angle between the C-H bond and the other three bonds at the ring carbon, then ψ is given by the expression:

$$\tan \psi = \frac{2(1 - \cos \tau)}{[4 \cos^2 \tau - 2(1 + \cos \beta)]^{1/2}} \qquad .. 2.2$$

Coordinates of hydroxyl hydrogens were not calculated since the hydroxyl groups were treated as single united atoms with higher VDW radius. The bond lengths used to fix the various atoms are: C-C = 1.52 Å, C-O = 1.42 Å and C-H = 1.0 Å.

2.3.2. Energy Minimization:

The total conformational energy of the molecule was

calculated taking into account nonbonded, electrostatic, torsional and bond angle distortion energies, using expression 1.1. The fractional charges used to calculate electrostatic energy were adopted from Vijayalakshmi (Vijayalakshmi, 1972) and are shown in Fig. 2.2. As the hydroxyl groups were treated as single united atoms the charges on them were taken to be the sum of the charges on the constituent atoms. The values of r_o used for calculating nonbonded energies involving hydroxyl groups are given in Table 1.1. Torsional and bond angle distortion energies were computed using expressions 1.9 and 1.10 respectively, with the constants given in Chapter I.

The conformational energy of the molecule is a function of φ, θ, r, β_1, β_2 and β_3. For each set of (θ,φ), the energy was minimized with respect to r, β_1, β_2 and β_3 (denoted as X_1, X_2, X_3 and X_4). For a starting conformation (θ,φ) and a starting set of values of X_i^s, conformational energy E and its partial derivatives $\partial E/\partial X_i$ were calculated. These derivatives were used to calculate the increments (ΔX_i) for the variables $\underline{u}$ delete U.

$$\Delta X_i = -0.5\left(\frac{\partial E}{\partial X_i}\right) \Big/ \left|\frac{\partial E}{\partial X_i}\right| \qquad .. 2.3$$

and the new values for the variables were obtained from

$$X_i' = X_i + \Delta X_i \qquad .. 2.4$$

where X_i', X_i and ΔX_i are expressed in degrees.

This iterative process was continued till there was no further decrease in energy, indicating the proximity of a minimum. The region near the minimum was scanned at 0.2° intervals, i.e.,

$$\Delta X_i = -0.2 \left(\frac{\partial E}{\partial X_i}\right) \Big/ \left|\frac{\partial E}{\partial X_i}\right| \qquad \ldots 2.5$$

to determine the positions of the minimum more accurately. The reliability of this algorithm was tested by comparing its results with those obtained by using the two minimization algorithms available from the Scientific Subroutine Package of IBM (the Conjugate Gradient method used by the subroutine FMCG and Fletcher-Powell method used by subroutine FMFP). The minimum energy values obtained using these subroutines were within ± 0.2 Kcal.mole^{-1}, and the values of the variables were within $\pm 0.5^\circ$ from those obtained using the algorithm described here. The present algorithm was found to be computationally more economical and hence was adopted throughout the present work.

2.3.3. Conformational Energy Maps:

The θ-φ plane was scanned at intervals of 5° in θ and 15° in φ. For a fixed value of φ, θ was initially fixed at 0°, and the starting values of β^s and r were taken as 109.47° and -35.26° respectively. The energy was minimized

with respect to β_1, β_2, β_3 and r. Nex, θ was increased Next by 5° and the values of β^s and r corresponding to the minimum energy of the previous conformation were used as starting values for the next minimization. This procedure was repeated to cover the entire range of concentrations Conformations ($\theta = 0\text{-}180^\circ$, $\varphi = 0\text{-}360^\circ$) which pyranose ring can theoretically assume.

The conformational energies thus obtained as functions of θ and φ are displayed in Figs. 2.3-2.10. The crystal structures of a number of pentopyranoses and their derivatives have been solved in the past few years. The solid state conformations of these molecules are also indicated on the conformational maps.

2.4. Solvent Accessibility:

The method of Lee and Richards (Lee and Richards, 1971) was used to calculate the solvent accessibilities of different conformations. An atom or a group of atoms in a molecule is said to be accessible to a solvent molecule if its distance from the centre of the solvent molecule is equal to the sum of the VDW radii of that atom and the solvent molecule, and if the distance of the solvent molecule in that position from any other atom in the solute molecule is greater than the sum of their VDW radii. Accessibility is defined as the ratio of the accessible area to the total area of the atom.

To compute the accessible area, the soluble molecule is represented as a set of interlocking spheres, and is divided into equidistant planar sections. The solvent molecule is moved along the periphery of each of these sections, and the positions for which different atoms in the molecule are accessible are determined. If, for a given atom, L_i denotes the arc length along the periphery in the ith section for which it is accessible to the solvent molecule, then the total accessible area of that atom is given by

Solute

$$A = \sum_i \frac{R.D.L_i}{\sqrt{R^2 - Z_i^2}} \qquad .. 2.6$$

where R is the VDW radius of the atom, Z_i is the distance of the section from the centre of the atom and D is the distance between two consecutive sections. The accessibility, S, expressed as percentage, is given by

$$S = \frac{100\ A}{4\ \pi\ R^2} \qquad .. 2.7$$

Solvent accessibility calculations were performed for the entire range ($\theta = 0^\circ$ to 180°, $\varphi = 0^\circ$ to 360°) at 10° intervals in θ and 30° intervals in φ. The geometries used in these calculations were those corresponding to the minimum energy conformation at that point in the $\theta - \varphi$ plane. The solvent (water) molecule was assumed to be a sphere of radius 1.4 Å.

For a polar solvent like water, interactions with the

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polar groups would be of more significance than the nonpolar groups. Hence, accessibilities of the polar groups were added to give the total solvent accessibility for that conformation. Isoaccessibility contours were drawn in the Θ-φ plane, and are shown in Figs. 2.11 to 2.18.

2.5 Results and Discussion

2.5.1. Flexibility of the pyranose ring

The conformational energy maps of all the pentoses (Figs. 2.3-2.10) have some common features. There are two prominent minima, one along $\Theta \simeq 0^\circ$ ($^4C_1$(D) chair) and the other along $\Theta \simeq 180^\circ$ ($^1C_4$(D) chair). There are a few local maxima and minima near the $\Theta = 90^\circ$ line (Table 2.1) which correspond to the boat and twist-boat conformations, respectively. The energy barriers (transition states) occur near the lines $\Theta = 55^\circ$ and $\Theta = 135^\circ$, and correspond to the half-chair conformations. Figures 2.3-2.10 indicate that the two maxima corresponding to the $^4C_1$ and $^1C_4$ conformations have comparable energies. The solid state conformations of α- and β-D arabinose, β-D-lyxose, β-D-ribose and α- and β-D-xyloses are seen to lie in these minimum energy regions.

The calculated energy differences between the two chair conformations are shown in Table 2.2, along with the energy differences reported in the earlier work (Vijayalakshmi and Rao, 1972). It is seen that the calculated energy differences

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Table 2.2: Calculated potential energies and solvent accessibilities of pentopyranoses.

Pentopyranose	Calculated energy differences $^4C_1 - {}^1C_4$ (Kcal.mole^{-1})		Solvent accessibility difference (ΔS) $^4C_1 - {}^1C_4$	Conformation in solid state[b]	Conformation in solution from NMR[c]
	Present work	Earlier work[a]			
α-D-Arabinose	0.20	0.88	-8	$^1C_4$	$^1C_4$
β-D-Arabinose	0.00	-0.14	5	$^1C_4$	$^4C_1$
α-D-Lyxose	0.20	0.51	1	-	$^1C_4 \rightleftharpoons {}^4C_1$
β-D-Lyxose	0.10	-0.68	14	$^4C_1$	$^4C_1$
α-D-Ribose	-0.40	-0.39	1	-	$^1C_4$
β-D-Ribose	-0.60	-1.28	13	$^1C_4$	$^4C_1 \rightleftharpoons {}^1C_4$
α-D-Xylose	-0.50	-0.91	11	$^4C_1$, $^1C_4$	$^4C_1$
β-D-Xylose	-0.60	-1.86	25	$^4C_1$	$^4C_1$

[a] Vijayalakshmi and Rao (1972); Vijayalakshmi (1972). [b] Takagi and Jeffry (1978a); Takagi and Jeffy (1977b); Hordvik, (1966); Hordvik (1974); Hordvik (1971); Takagi and Jeffry (1978c); Harata (1976). [c] Rudrum and Shaw (1965); Lemieux and Stevens (1966).

in the present work are small compared to the earlier values. This is due to the use of a flexible ring geometry in the present work. The table also shows that the energy difference between the two chair conformations is $\leq$ 0.6 kcal.mole^{-1}, suggesting that the crystal field forces may exercise a dominating influence on the molecular conformation in the solid state. In fact, the solid state conformations of β-D-ribose ($^1C_4$) has about 0.6 kcal.mole^{-1} higher energy than the $^4C_1$ conformation. Similarly, α-D-xylose seems to favour both the chair conformations in the solid state, though they differ in energy by about 0.5 kcal.mole^{-1}.

The minimum energy region near the chair conformations (θ = 0° and 180°) is seen to be broad and shallow. As θ changes from 0° - 5° and φ from 0° - 360° (near the $^4C_1$ chair) the change in energy is small ($<$ 0.5 kcal.mole^{-1}) for all the pentoses. For α- and β-D-arabinoses the change in energy is small near the $^1C_4$ chair conformation also (θ = 175°-180°, φ = 0°-360°). To illustrate the flexibility of the pyranose ring, variations in the ring bond angles and torsional angles have been shown as functions of φ for a fixed value of θ (θ = 5°) near the $^4C_1$ chair conformations for all the pentapyranoses. Similar plots are also shown for α- and β-D-arabinoses and α- and β-D-lyxoses near the $^1C_4$ conformation (θ = 175°). In these regions, the bond angles vary over a range of 2°-4° (Figs. 2.19-2.30) and the torsional angles over 8°-10° (Figs. 2.31-2.42). These results suggest that

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the ring torsional angles in the chair conformation can vary significantly without much change in the conformational energy, indicating that the chair conformation is quite flexible. Hence, small contributions from the lattice energy may significantly affect the geometry of the pyranose ring in the solid state. This alsc suggests that the use of a rigid geometry for the sugar residue in model building and other studies is not fully justified. Though such a procedure can form a starting point of the model building studies, interpretation of X-ray diffraction data of fibres etc., the flexibility of the ring and the possibility of alternative chair forms should be included in more detailed analysis or refinements.

2.5.2. Favoured conformations of pentopyranoses in solution:

Table 2.2 shows potential energy and solvent accessibility differences between the $^4C_1$ and $^1C_4$ conformations of the pentopyranoses. Potential energy differences of $<$ 0.6 kcal. 0.6 kcal.mole^{-1} for all the pentopyranoses suggest that interactions with the solvent may play an important role in influencing the favoured conformations in solution.

For α-D-arabinose, the $^1C_4$ conformation has lower energy and higher solvent accessibility than the $^4C_1$ chair, indicating that in aqueous solutions, the former would be favoured. For β-D-arabinose, α-D-lyxose and α-D-ribose, both the chair combinations have nearly equal potential energies and also

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nearly equal solvent accessibilities, suggesting an equilibrium between the two conformations in aqueous solutions. For β-D-lyxose, though both the chairs have comparable energies, the $^4C_1$ chair has a much higher solvent accessibility indicating that in polar solvents like water, $^4C_1$ conformation is favoured over $^1C_4$. The $^4C_1$ chair is seen to be favoured for α and β-D-xyloses, by both potential energy and solvent accessibility criteria. All these predictions are in agreement with experimental observations in solution (Lemieux and Stevens, 1966; Rudrum and Shaw, 1965; Durette and Horton, 1971). However, for β-D-ribose NMR studies indicate that both the chair conformations would be present in solution in appreciable proportions, whereas the present study indicates the $^4C_1$ chair to be favoured by both the potential energy and solvent accessibility criteria. The reasons for this discrepancy are not very clear.

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2.5.3. Effect of axial substituents on the ring geometry:

Normally, in an ideal chair conformation, the ring torsional angles should be near 60°, making the sum of the six torsional angles ($\sum_i |\nu_i|$) to be close to 360°. Hence, the deviations of ($\sum_i |\nu_i|$) from 360° indicate the extent of distortion in the chair conformation.

Table 2.3 shows that the $^4C_1$ conformations of all the pentopyranoses have $\sum_i |\nu_i|$ less than 360°, indicating that the chair is flattened to some extent, even in the case of

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Table 2.3: Hydroxyl group orientations at various ring atoms and the sum of the calculated ring torsional angles in the $^4C_1$ conformations for the pentopyranoses.

Pentopyranose	Orientation of the hydroxyl group*				$\sum_{i=1}^{6} \lvert \nu_i \rvert$
	C-1	C-2	C-3	C-4	(Degrees)
α-D-Arabinose	A	A	A	E	334
β-D-Arabinose	E	A	A	E	342
α-D-Lyxose	A	A	E	E	338
β-D-Lyxose	E	A	E	E	344
α-D-Ribose	A	E	A	E	337
β-D-Ribose	E	E	A	E	344
α-D-Xylose	A	E	E	E	342
β-D-Xylose	E	E	E	E	345

* A - Axial orientation

E - Equatorial orientation.

β-D-xylose, where all the hydroxyl groups are in the equatorial orientation. It is interesting to note that $\sum_i |\nu_i|$ is least (suggesting a greater distortion in the ring geometry) for those cases where two hydroxyl groups are placed on the same side of the ring in axial orientations. Table 2.3 shows that two adjacent hydroxyl groups, or even a single axial hydroxyl group causes significant deviations in the ring geometry, from that of β-D-xylose. These results are in agreement with the findings on cyclitols (Jeffrey and Kim, 1970). Comparison of the calculated $\sum_i |\nu_i|$ values of α-D-xylose, β-D-ribose and β-D-lyxose in $^4C_1$ conformation suggests that an axial hydroxyl group at C-1 causes more distortions in the ring than that at C-2 or C-3.

2.5.4. Non-chair conformations:

i) Boat and Twist-Boat conformations:

As mentioned earlier, the boat conformations correspond to local maxima (Figs. 2.3-2.10, $\theta \simeq 90^\circ$, $\varphi \simeq 0^\circ$, 60°, 120°, 180°, 240° and 300°) on the energy surface and are unlikely to be present either in the solid state or in solution.

The twist-boat fonromations correspond to local minima (Figs. 2.3-2.10, $\theta \simeq 90^\circ$, $\varphi \simeq 30^\circ$, 90°, 150°, 210°, 270° and 330°). However, their energies are at least 4-5 kcal.mole^{-1}. higher than those of the chair conformations, indicating that they are unlikely to be present in the solid state. The

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solvent accessibilities of some of the twist-boats are seen to be high, e.g., for α-D-arabinose, the $^{1}S_{3}$ and $^{1}S_{5}$ twist boats are higher in accessibility (by about 10) than the most favoured chair conformations. However, this may not be sufficient to offset the 4-5 kcal.mole^{-1} differences in the potential energies, and hence the twist boats are also unlikely to be present, even in solution, to any significant extent.

iii) <u>The half chair conformations</u>:

These conformations occur along $\theta = 55^{\circ}$ and $\theta = 135^{\circ}$. They range in energies from 9-15 kcal.mole^{-1}. The lowest amongst them corresponds to the transition state for $^{4}C_{1} \rightleftharpoons {}^{1}C_{4}$ interconversion. The energy barriers for this interconversion are seen to be about 9-10 kcal.mole^{-1} for pentopyranoses. The solvent accessibilities for some of these conformations are seen to be quite high, suggesting that the energy barrier for interconversion may be lowered due to the favourable solvent interactions of these conformations.

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III ALDOHEXOPYRANOSES

3.1. INTRODUCTION

Several workers have investigated the favoured conformations of aldohexopyranoses, both by theory and experiment. The solid state conformations of a number of free sugars, pyranosides and oligosaccharides have been determined by X-ray crystallography.(Jeffrey and Sundaralingam, 1974, 1975, 1976; Marchessault and Sundararajan, 1976). The solution conformations of these molecules have been investigated by many physical techniques such as NMR (Lemieux et al., 1958; Rao and Foster, 1963; 1965; Rudrum and Shaw, 1966; Angyal and Pickler, 1967; Durette and Horton, 1971), infrared spectroscopy (Tipson and Isbell, 1960; Tipson, 1968), optical rotation studies (Kauzmann et al., 1961; Lemieux and Martin, 1970) etc. As mentioned earlier in the theoretical investigations (Rao et al., 1967) potential energies of aldohexopyranoses in $^{4}C_{1}$ and $^{1}C_{4}$ conformations were computed by assuming a rigid geometry for the pyranose ring. The energy was minimised by tilting only the axial substituents (Vijayalakshmi and Rao, 1972; Vijayalakshmi, 1972).

A careful examination of the X-ray crystal structure data reveals that for pyranoses, the ring torsional angles deviate considerably from their average values. These deviations lead to significant variations from 4.1 Å to 4.8 Å in the length of the α-D-glucopyranose residue (French and Murphy, 1973, 1977). Such differences have also been noted for β-D-glucopyranoses

(Arnott and Scott, 1972). In view of these observations, the assumption of a rigid ring geometry and the use of 'averaged' ring parameters for the pyranose ring in studies on simple hexose sugars, their derivatives, oligosaccharides and polysaccharides does not seem to be fully justified when comparing the theoretical values of end to end dimensions of polysaccharides with experiment, interpreting the fibre diffraction data or other comparisons. Kilderby et al. have (Kilderby et al., 1977) minimised the conformational energies of α and β-D-glucopyranoses with respect to bond lengths, bond angles and torsional angles. But these studies indicate neither the possible distortions in the ring, nor the energies involved in producing such distortions.

Amongst the various hexose polysaccharides, cellulose, amylose, β-1,4-mannan and pectic acids and their constituents have been widely studied, both theoretically and experimentally. Hence an attempt has been made to construct the conformational energy and solvent accessibility surfaces of α and β anomers of D-glucopyranose, D-galactopyranose and D-mannopyranose. D-Idopyranose is also included in the present study since its $^{4}C_{1}$ conformation has more axial substituents than the $^{1}C_{4}$ unlike the other hexoses. These studies make it possible to estimate the flexibility of the pyranose ring in hexoses, and also to arrive at the best parameters to be used in conformational studies. A comparison of these results

with crystal structure data also indicates the effect of crystal field forces on the pyranose ring conformation.

3.2 Conformational Energy Calculations

The parameters and method used for calculating conformational energies have been described in Chapter II. The fractional charges used in computing the electrostatic energy are adopted from Vijayalakshmi (Vijayalakshmi, 1972) and are shown in Fig. 3.1. In the hexopyranoses, the hydroxymethyl group was also treated as a single group, with VDW radius 2.0 Å. Conformational energy maps have been drawn and conformations corresponding to those observed in the solid state have been marked in the maps.

3.3 Solvent Accessibility Calculations

Solvent accessibility for the hexopyranoses was calculated using the method described in Chapter II.

For each conformation of the ring (in the θ-φ plane), the hydroxyl group of the hydroxymethyl group was fixed in all the three staggered orientations and the solvent accessibility in each case was calculated separately. Of the three orientations, the one leading to the highest solvent accessibility was used for plotting the isoaccessibility contours. In almost all the cases, the trans orientation (O_6-C_6-C_5-C_4 = 180°) was found to have the highest solvent accessibility.

Solvent accessibility maps are shown in Figs. 3.10 to 3.17.

3.4 RESULTS AND DISCUSSION

3.4.1. Conformational Energy Maps:

The calculated conformational energy contours for the aldohexopyranoses are shown in Figs. 3.2 to 3.9. These maps are very similar in appearance to those for the pentopyranoses. The global minimum occurs near $\theta = 0^{\circ}$ and this corresponds to the $^{4}C_{1}$(D) chair conformation. The local minimum, corresponding to the $^{1}C_{4}$(D) chair, occurs near $\theta = 175^{\circ}$ unlike pentoses ($\theta = 180^{\circ}$), indicating that the $^{1}C_{4}$(D) chair conformation for hexoses is slightly more flattened. Moreover, it is also seen from Figs. 3.2, 3.3, 3.8 and 3.9 that for glucose, and mannose, the energy difference between the two regions ($\theta \simeq 180^{\circ}$, $\varphi = 0^{\circ}$-360° and $\theta \simeq 0^{\circ}$, $\varphi = 0^{\circ}$-360°) increases significantly compared to the corresponding pentoses (xylose and lyxose) suggesting that the presence of the hydroxymethyl group in the hexoses increases the difference in energy between the two chair forms. In other words, the hydroxymethyl group destabilises the $^{1}C_{4}$(D) conformation.

A series of local maxima and minima occur along $\theta \simeq 90^{\circ}$, and these correspond to boat and twist-boat conformations respectively. The half chair conformations occur along $\theta \simeq 55^{\circ}$ and $\theta \simeq 135^{\circ}$.

3.4.2. Favoured conformations of the Aldohexopyranoses:

For α- and β-D-glucopyranose, α- and β-D-galactopyranose

and α- and β-D-mannopyranose, the maps (Figs. 3.2-3.5, 3.8, 3.9) show the $^4C_1$(D) chair to be at least 2.5 Kcal.mole^{-1} lower in energy than the $^1C_4$(D) chair conformation, and about 5 Kcal.mole^{-1} lower than the nearest twist-boat conformation. These figures also show that the observed solid state conformations of these sugars occur in the minimum energy region, suggesting that the lattice energy has a minor effect on the pyranose ring conformations of these sugars. For α- and β-D-idopyranoses, although $^4C_1$(D) chair has the minimum energy, the energy difference between the two chair conformations is small, while the twist-boats are still about 5 Kcal.mole^{-1} higher in energy than the $^4C_1$(D) chair conformations. It is also interesting to note that the global minimum of α- and β-D-idopyranoses occurs at $\theta = 3^\circ$ and $\theta = 1.5^\circ$ respectively, unlike the other hexopyranoses, indicating that the pyranose ring in the $^4C_1$(D) chair conformation of idopyranoses is more distorted compared to the other hexopyranoses.

The $^4C_1$(D) conformations of α- and β-D-glucopyranose, β-D-galactopyranose and β-D-mannopyranose are favoured both by potential energy ($>$ 2.5 Kcal.mole^{-1}) and by solvent accessibility (Table 3.1) considerations. Both the chair conformations of α-D-galactopyranose and α-D-mannopyranose have about the same solvent accessibility, but the $^4C_1$(D) conformation is favoured by about 2.5 Kcal.mole^{-1} over the $^1C_4$(D)

Table 3.1: Potential energy and solvent accessibility differences between the $^4C_1(D)$ and $^1C_4(D)$ conformations of hexopyranoses.

Hexpyranose	Potential energy difference $^4C_1 - {}^1C_4$ Kcal.mole^{-1}	Solvent accessibility difference (ΔS) $^4C_1 - {}^1C_4$
α-D-Galactose	2.70	- 1
β-D-Galactose	3.29	20
α-D-Glucose	3.19	7
β-D-Glucose	3.74	27
α-D-Idose	1.59	-27
β-D-Idose	2.16	-28
α-D-Mannose	2.66	- 2
β-D-Mannose	3.25	15

conformation. These results suggest that all these hexopyranoses occur preponderantly in the $^4C_1$(D) conformation, in agreement with the NMR studies in solution (Durette and Horton, 1971). On the other hand, for α- and β-D-idopyranoses, the $^4C_1$ conformation is favoured by potential energy (1.6-2.2 Kcal.mole^{-1}) considerations, while $^1C_4$ conformation is favoured by high solvent accessibility (27-28). This suggests that in solutions, both the chair conformations of these molecules exist in considerable proportions.

3.4.3. Flexibility of the pyranose ring:

The global minimum for all the sugars studied (Figs. 3.2-3.9) is seen to be quite diffuse. As θ varies from 0°-5° and φ from 0°-360°, there is only a slight change (< 0.5 Kcal.mole^{-1}) in the conformational energy.

To illustrate the possible distortions in the chair conformations, the calculated endocyclic torsional angles and bond angles are plotted as functions of φ for θ = 5° (since most of the experimental points lie close to this line) in Figs. 3.18-3.27 and 3.28-3.37, respectively. It is interesting to note from these diagrams that variations of 8°-12° in torsional angles and of 2°-4° in bond angles can take place without much change in energy. This indicates that a large number of distorted chair conformations, differing significantly in both torsional and bond angles, can occur both in solid state and solution, suggesting that the

ring is quite flexible and not rigid as previously believed.

As shown in Table 3.2, for glucose, galactose and mannose, the calculated values for the ring torsional angles C-C-C-C, C-C-C-O and C-C-O-C at the global minimum are 55°, 56° and 60° respectively. The theory thus predicts the qualitative behaviour of these angles (C-C-C-C < C-C-C-O < C-C-O-C) in agreement with the experimental observations. In fact, these values for α-D-glucose show quantitative agreement with the values of these angles (53°, 56° and 62°) for α-D-glucose residues reported by French and Murphy (French and Murphy, 1973).

These results thus justify the use of average parameters, obtained from crystal structure data, as a first approximation in the conformational studies on polysaccharides. However, calculations with flexible $^4C_1$(D) chair conformations may offer a measure of refinement in comparing theory with experiment, since the ring torsional angles can vary as much as 10° without much change in the conformational energy.

3.4.4. <u>The Twist-boat conformations</u>:

The twist-boat conformations correspond to the local minima near $\theta = 90°$. The solvent accessibilities in this region vary over a wide range. Generally, the $^1S_5$ conformation ($\varphi \simeq 90°$) is seen to have the lowest energy amongst the twist-boat conformations. It also has higher solvent accessibility amongst the twist-boat conformations and is thus

Table 3.2: Ring torsional angles (in degrees) at the minimum energy conformation for the hexapyranoses.

Hexa Hexopyranose	O_5-C_1-C_2-C_3	C_1-C_2-C_3-C_4	C_2-C_3-C_4-C_5	C_3-C_4-C_5-O_5	C_4-C_5-O_5-C_1	C_5-O_5-C_1-C_3
α-D-Galactose	56.0	-55.2	55.0	-55.8	59.7	-59.4
β-D-Galactose	56.7	-55.9	55.9	-56.7	60.6	-60.6
α-D-Glucose	55.8	-55.0	55.0	-55.7	59.6	-59.6
β-D-Glucose	56.3	-55.5	55.5	-56.2	60.1	-60.1
α-D-Idose	52.0	-51.2	53.5	-57.1	61.0	-58.1
β-D-Idose	55.2	-53.3	53.6	-55.9	61.0	-60.6
α-D-Mannose	55.6	-54.8	54.8	-55.6	59.5	-59.5
β-D-Mannose	56.7	-55.8	55.8	-56.6	60.6	-60.6

likely to be the most favoured of the boat/twist-boat conformations. However, it is about 5 Kcal.mole^{-1} higher in energy than the $^{4}C_{1}$(D) chair conformation, suggesting that the pyranose ring of these sugars in mono-, oligo- or polysaccharides is unlikely to exist in the twist-boat conformation. This is in disagreement with the assumption of Goebel and co-workers (Goebel et al., 1976) that 6% of the glucose residues in cellulosic chains exist in twist-boat conformations. The present study also indicates that the $^{1}C_{4}$(D) conformation of α- and β-D-glucoses have energy intermediate between the $^{4}C_{1}$(D) and the boat/twist-boat conformations. This is in disagreement with the results of Brant (Brant, 1976) that the boat and twist-boats have energies intermediate between the $^{4}C_{1}$(D) and $^{1}C_{4}$(D) chairs. This discrepancy arises mainly due to the fact that the earlier results were based on ideal models, whereas in the present calculations, the energy was minimised with respect to all the ring bond angles and ring torsional angles.

3.4.5. The Half-chair conformations:

These occur along the lines $\theta \simeq 55^{\circ}$ and $\theta \simeq 135^{\circ}$, and correspond to the transition states. These ranges in energies between 9-17 Kcal.mole^{-1}. Some of these half chairs have very high solvent accessibilities. Hence, the height of the barrier between the global and the local minima, which is 10-11 Kcal.mole^{-1}, may be lowered due to favourable interactions with the solvent, as in the case of pentopyranoses.

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IV. PENICILLINS

4.1 INTRODUCTION

Penicillin is one of the most widely used antibiotics to combat many bacterial diseses. It interferes with the biosynthesis of peptidoglycan at the cross-linking stage by inactivating the cross linking enzymes (trans-peptidases, carboxypeptidases etc.). As a result, the cell wall of the bacterium weakens and finally bursts leading to the death of the bacterium. As indicated in Chapter I, the clinical importance of penicillins has drawn the attention of a number of workers. Several derivatives of penicillin have been synthesized, either by modifying the R group (Fig. 4.1) or the five membered ring (Gorman and Ryan, 1972). These show a wide range of biological activity. Thus, oxidation of the sulfur atom in penicillin is known to reduce significantly its activity (Garman and Ryan, 1972). The α-sulfoxide of penicillin is five times more active than the β-sulfoxide. Several attempts have been made to correlate the biological properties of these molecules with their structural features. Morin and coworkers (Morin et al., 1969) tried to correlate the infrared stretching frequencies of the β-lactam carboxyl group with biological activity; the higher the infrared frequency, the higher would be the activity. Sweet and Dahl (Sweet and Dahl, 1970), from an examination of X-ray crystal structure data, suggested that the nonplanarity of the

β-lactam nitrogen is important for biological activity. Virudachalam and Rao (Virudachalam and Rao, 1977; Virudachalam, 1978) from a detailed conformational analysis of penicillins, have shown that for biological activity, specific orientations of the aminoacyl and carboxyl groups, besides the lactam ring are necessary. Orientations of these groups depends on the conformation of the thiazolidine ring.

Hence, in the present study, a detailed analysis of the conformations of the thiazolidine ring of penicillins has been undertaken, using semiempirical potential energy functions. Such studies may throw light on the relative activities of penicillin sulfoxides and sulfides.

4.2 METHOD OF CALCULATION

4.2.1. Nomenclature:

The numbering of the atoms and the dihedral angles in penicillin are shown in Fig. 4.1. The torsional angles α_1, α_2 and α_3 specify the relative orientations of the planes (C_5,S_1,C_2), (C_2,C_3,N_4) and (N_4,C_5,C_6,C_7) respectively, with respect to the reference plane (C_2,N_4,C_5). Hence, in this notation, α_1 and α_2 define the conformation of the five membered ring and α_3 defines its orientation with respect to the lactam ring. In the initial conformation ($\alpha_1 = \alpha_2 = \alpha_3 = 0^\circ$), all the atoms ($S_1,C_2,C_3,N_4,C_5,C_6$ and C_7) of the bicyclic ring system are in one plane, as in Fig. 4.1.

Clockwise rotation about the virtual or real bonds (indicated by arrows looking along C_5-C_2, C_2-N_4 and N_4-C_5 is considered as positive for the dihedral angles α_1, α_2 and α_3. The definition of the torsional angles ($\beta\varphi_2$, ψ_2) which define the orientation of the carboxyl group is according to the IUPAC-IUB nomenclature.

4.2.2. Energy calculations:

The bond lengths and bond angles used for generating the coordinates of the molecule were obtained from X-ray crystal structure data (Abrahamsson et al., 1963; James et al., 1968). The lactam ring was fixed in a planar conformation, as observed in a number of crystal structures. The value of ψ_2 was fixed at 30°, as observed in simple peptides (Virudachalam and Rao, 1977). A value of 1.47 Å was used for the S=O bond length for fixing the sulfoxide oxygen, and the angles C_2-S=O and C_5-S=O were kept constant at 107°. The procedure used for generating the co-ordinates of the molecule was similar to that used for sugars.

The total conformational energy of the molecule was computed by taking into account the nonbonded, electrostatic, torsional and bond angle distortion energies. In the case of penicillin sulfoxides, contribution due to the hydrogen bond between the sulfoxide oxygen and N-H of the aminoacyl group was also considered. The relevant potential functions and the constants used have been described in Chapter I.

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The fractional charges for penicillin used in computing the electrostatic interaction energy are adopted from Virudachalam (Virudachalam, 1978) and are shown in Fig. 4.2. For computational simplicity, the R group (Fig. 4.1) was treated as a methyl group. This and the other methyl groups in the molecule were treated as single (united) atoms, with appropriately higher van der Waal radii. The energy of the molecule was calculated as a function of (α_1, α_2). These dihedral angles were varied in the range -60° to $+60^\circ$ at 10° intervals, and at each grid point, the energy was minimised with respect to α_3, β_1, β_2 and φ_1. The minimization algorithm was the same as described in Chapter II.

4.3 RESULTS AND DISCUSSION

4.3.1. Penicillin sulfide:

Conformational energy map obtained for penicillin sulfide is shown in Fig. 4.43. The energy minima, and the conformations obtained from the crystal structures are also marked in the diagram. 4.3.

Figure 4.3 shows two regions of minimum energy. The global minimum occurs at $(\alpha_1, \alpha_2) = (40^\circ, 10^\circ)$ in region I, and the local minimum at $(-10^\circ, -25^\circ)$ in region II. The solid state conformations of ampicillin (Boles and Griven, 1976; Girven James et al., 1968) fall in region I, while those of penicillin G or V (Pitt, 1952; Abrahamsson et al., 1963)

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fall in region II. The thiazolidine ring assumes C_2 puckered and C_3 puckered conformations in regions I and II respectively. Depending on the mode of puckering, the relative orientations of the bicyclic ring system ($180^\circ - \alpha_3$) and the orientation of the carboxyl group (φ_2) vary. These are shown in Table 4.1. The table also shows, the nonplanarity of the lactam peptide (ω_2). For both the local and the global minima, the value of ω_2 is around 132°, indicating that the lactam peptide bond is significantly nonpolar.

The global minimum (C_2 puckered conformation) is about 0.5 Kcal.mole^{-1} lower in energy than the C_3 puckered conformation. The barrier between the two minima is small ($\simeq$4 Kcal.mole^{-1}), and the ring can easily flip over from one conformation to the other. This suggests that in solution, both C_2 and C_3 puckered forms would exist in equilibrium and the former would predominate. This agrees with the NMR observations (Dobson et al., 1975).

Recent studies on penicillin (Virudachalam and Rao, 1977, 1978) have indicated that for optimal binding with the cross-linking enzymes, the orientation of the carboxyl group should correspond to $\varphi_2 \simeq 160^\circ$. Table 4.1 shows that in the C_3 puckered conformation, $\varphi_2 \simeq 160^\circ$ which is close to the required angle for biological activity. Though the thiazolidine ring of ampicillin exists in the C_2 puckered conformation in the solid state, a considerable amount of the C_3 puckered

Table 4.1: Calculated dihedral angles of the aminoacyl group, carboxyl group and the lactam peptide at the minimum energy conformations for penicillin sulfide and sulfoxides.

Conformation	Orientation of		Nonplanarity of the ~~peptide~~ lactam peptide
	aminoacyl group φ_1 (Degrees)	carboxyl group φ_2 (Degrees)	ω_2 (Degrees)
Penicillin sulfide C_2 puckered	170	111	133
Penicillin sulfide C_3 puckered	170	161	131
Penicillin α-sulfoxide C_2 puckered	170	108	131
Penicillin α-sulfoxide C_3 puckered	170	157	132
Penicillin β-sulfoxide C_2 puckered	-120	110	131
Penicillin β-sulfoxide C_3 puckered	- 75	157	133

conformation may also be present in solution, since the energy difference between these two forms and the energy barrier separating them is low. This accounts for the biological activity of ampicillin.

delete The orientation of the aminoacyl group is not altered significantly by the puckering of the thiazolidine ring, and φ_1 assumes a value near 170°. Interestingly, this is close to the orientation required for binding with the cross linking enzymes ($\varphi_1 \simeq 180°$).

4.3.2. Penicillin α-sulfoxide:

Conformational energy map for penicillin α-sulfoxide is shown in Fig. 4.44. The map is very similar to that of penicillin sulfide. The global minimum occurs at (α_1, α_2) (40°,15°) and a local minimum at (-10°,-25°) which is about 0.5 Kcal.mole^{-1} higher in energy than the former. No hydrogen bond is possible between the sulfoxide oxygen and the aminoacyl N-H. 4.4

Table 4.1 shows that in penicillin α-sulfoxide also, the lactam peptide is significantly nonplanar ($\omega_2 \simeq 132°$) at the local and global minima. If nonplanarity of the peptide unit is important for biological activity (Sweet and Dahl's hypothesis), then penicillin α-sulfoxide should have about the same biological activity as penicillin G or V. But it is known that the sulfoxides are much less active compared to the sulfides.

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The energy barrier separating the two minima is about 6 Kcal.mole^{-1}, which is higher by about 2 Kcal.mole^{-1}, which is higher by about 2 Kcal.mole^{-1} than that obtained for penicillin sulfide. As a result, the rate of interconversion between the C_2 and C_3 puckered forms is about 20 times slower for the sulfoxides compared to the sulfides. This suggests that in solution, as the population of the biologically active C_3 puckered conformation decreases due to the interaction with the cross-linking enzymes, the rate at which it is restored due to the conversion from C_2 to C_3 puckered conformation will be much slower than that for the sulfide. This may account for the reduced activity of penicillin α-sulfoxide.

4.3.3. Penicillin β-sulfoxide:

The conformational energy map of penicillin β-sulfoxide also shows two minima (Fig. 4.5). The global minimum occurs at (35°,15°) as in penicillin sulfide, and corresponds to the C_2 puckered conformation. There is a possibility of hydrogen bond formation between the sulfoxide oxygen and the aminoacyl N-H group. This is in agreement with X-ray crystal structure studies (Cooper et al., 1969).

The local minimum occurs at (-15°,-25°), and corresponds to the C_3 puckered conformation. In this conformation also, a hydrogen bond between sulfoxide oxygen and the aminoacyl N-H group is possible. However, this conformation has about 2.5 Kcal.mole^{-1} higher energy than the global minimum. The

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energy barrier separating the two minima is about 7 Kcal.mole^{-1}.

Table 4.1 shows that the lactam peptide bond is nonplanar ($\omega_2 = 131^\circ$) in both the conformations. Thus, if nonplanarity of the lactam peptide were to play a dominant role in biological activity, penicillin sulfide and both the sulfoxides should show more or less the same degree of biological activity. The fact that the activity of β-sulfoxide is considerably lower than that of α-sulfoxide suggests that other conformational features (such as orientations of aminoacyl and carboxyl groups) in the molecule may also have an important role to play in the biological activity.

Since the C_2 puckered conformation of β-sulfoxide is 2.5 Kcal.mole^{-1} lower in energy than the C_3 puckered conformation, in solution the population of the C_3 puckered conformation would be negligible. In fact, from NOE studies (Cooper et al., 1969) it has been shown that penicillin β-sulfoxide exists in solution in the C_2 puckered conformation, which is different from the one required for biological activity. In addition to this, in the minimum energy conformation, the aminoacyl group favours a conformation ($\varphi_1 \simeq -90^\circ$) different from the biologically active one ($\varphi_1 \simeq 180^\circ$). This explains the greatly reduced activity of penicillin β-sulfoxide compared to the α-sulfoxide.

Thus, on the basis of a detailed conformational analysis of the thiazolidine ring, the present study consistently

explains both, the reduced biological activity of penicillin sulfoxides relative to the sulfides, and also the differences in the activities of the two sulfoxides.

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V. CEPHALOSPORINS

5.1. Introduction:

Cephalosporins are similar to penicillins in the presence of the lactam ring, but the fivemembered thiazolidine ring of penicillin is replaced by a six membered dihydrothiazine ring in cephalosporins. The Δ^3 and Δ^2 cephalosporins are shown in Fig. 5.1 and Fig. 5.2 respectively.

Cephalosporins are active against both gram positive and gram negative bacteria, but their activity is much lower than that of penicillins. In fact, penicillin, Δ^3 cephalosporin and Δ^2 cephalosporin have activities in the ratio 180:15:1. Cephalosporins have attracted attention due to their stability against penicillinases. They have been extensively investigated by various physical techniques such as infrared spectroscopy, nuclear Overhauser effect, X-ray crystallography, etc. (Gorman and Ryan, 1973), to throw light on their structure-activity relationship. These have already been briefly described in Chapter I.

Recent theoretical studies by Virudachalam and Rao (Virudachalam and Rao, 1977) have indicated that the conformation of the dihydrothiazine ring may not affect the orientation of the aminoacyl group. However, the orientation of the carboxyl group is very sensitive to the ring conformation. Since the orientations of these groups are important

for biological activity, in the present work, an attempt has been made to study the conformations of the bicyclic ring system in cephalosporins. Such an analysis may also elucidate the conformational features responsible for the differences in the activities of the Δ^3 and Δ^2 cephalosporins, and also for their stability against penicillinases.

5.2 Method of Calculation:

5.2.1. Nomenclature:

Figures 5.1 and 5.2 show the numbering of atoms and conformational parameters for Δ^3 and Δ^2 cephalosporins respectively.

The conformation of the six membered dihydrothiazine ring can be described by the three conformational angles α_1, α_2 and α_3, which denote the relative orientations of the planes (C_6, S_1, C_2), (C_2, C_3, C_4) and (C_4, N_5, C_6) with respect to the reference plane consisting of atoms C_6, C_2 and C_4. Similarly, α_4 denotes the orientation of the planar lactam ring with respect to the plane (C_4, N_5, C_6). Clockwise rotations are taken as positive. In the initial conformation ($\alpha_1 = \alpha_2 \ll \alpha_3 = \alpha_4 = 0^\circ$), all the atoms of the bicyclic ring system lie in a plane, as shown in the figures.

5.2.2. Energy Calculation:

The total conformational energy of the molecule was obtained by considering contributions from electrostatic and

monbonded interactions and from torsional angle and bond angle distortions. The relevant potential functions have been described in Chapter I. The fractional charges on the various atoms were obtained as a sum of the σ and π charges, computed separately as described in the earlier chapters. These are shown in Fig. 533 and 5.4 for Δ^3 and Δ^2 cephalosporins [5.3 respectively.

The bond lengths and angles used for fixing the co-ordinates of the bicyclic system were adopted from X-ray crystal structures (Sweet and Dahl, 1970; Sweet, 1972). The geometry of the aminoacyl and the carboxyl group was the same as used for penicillins. The R groups in both these molecules were treated as single (united) atoms. The lactam ring was fixed in a planar conformation, since its deviation from planarity as observed in crystal structures is very small.

As mentioned earlier, preliminary model building studies have indicated that the aminoacyl group is quite distant from the dihydrothiazine ring, and that its orientation is likely to be unaffected by the changes in the ring. Hence, it was fixed with $\varphi_1 \simeq 170^\circ$, which corresponds to the minimum energy conformation in penicillins. The angle ψ_2 at the carboxyl end was fixed at -30°, a value observed in simple peptides. The procedure used for fixing the co-ordinates is the same as used in the earlier chapters.

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5.2.3. Energy Minimization:

To scan the entire range of conformations of the dihydrothiazine ring, all the three conformational angles (α_1, α_2, α_3) should be varied independently over the range -60° to $+60^\circ$. However, the presence of the double bond in the ring implies that all the three angles are not completely independent of each other. Due to the high barrier across the C=C double bond ($\gtrsim$20 Kcal.mole^{-1}), it is unlikely that at the minimum energy conformation, the torsional angle across the double bond would be outside the range -25° to $+25^\circ$. Hence, for a conformation specified by α_1 and α_3, the range of α_2 is considerably restricted. In the present work, α_1 and α_3 were varied over a range -70° to $+70^\circ$. At every grid point (α_1,α_3), α_2 was varied over its restricted range, and at every point, the energy was minimised with respect to the bond angles β_1, β_2, β_3 and with respect to the angle between the rings, α_4. The energy minimisation procedure followed here is the same as described in Chapter II.

Since the bond angles β_1, β_2 and β_3 vary over a small range, and since α_2 also varies over a limited range due to the presence of the double bond, the conformational energy surface of the dihydrothiazine ring is represented as isoenergy contours in the (α_1,α_3) plane. A point on such a map corresponds to a conformation whose energy has been minimised with respect to β_1, β_2, β_3, α_4 and α_2. To indicate the small but

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significant variations in α_2, for a few low energy conformations in the minimum energy region, the values of α_1, α_2, α_3 and the conformational energies are shown in Tables 5.1 and 5.2 for Δ^3 and Δ^2 cephalosporins respectively. The values of α_2 at the minimum energy are also marked in the Figs. 5.5 and 5.6.

5.3 Results and Discussion:

5.3.1. Δ^3 Cephalosporins:

The conformational energy map of Δ^3 cephalosporin is shown in Fig. 5.5. The positions of minimum energy conformations, and the solid state conformation as observed in the crystal structure are also marked in the diagram. The global minimum occurs at $(\alpha_1, \alpha_2, \alpha_3) \simeq (-40^\circ, -20^\circ, \pm 50^\circ)$. The local minimum occurs at $(50^\circ, -5^\circ, 10^\circ)$ and is 0.4 Kcal.mole^{-1} higher in energy than the global minimum (Table 5.1). Since this energy difference is small, the dihydrothiazine ring can assume either of the puckered forms. The solid state conformation (Sweet and Dahl, 1970) corresponds to $(47^\circ, -8^\circ, 8^\circ)$ and lies near the local minimum, indicating that the small energy difference may have been offset by lattice energy. The energy barrier separating the two minima is low (4-5 Kcal.mole^{-1}), suggesting that the ring can easily flip over from one conformation to the other. Hence, in solution, both the conformations may exist in considerable proportions.

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Table 5.1: Conformational angles and energies of some of the low energy conformations of Δ^3 cephalosporin.

No.	α_1 (degrees)	α_2 (degrees)	α_3 (degrees)	E Kcal.mole^{-1}
1	-40	-20	50	0.00[a]
2	-50	-20	50	0.70
3	-40	-25	60	0.90
4	-30	-20	50	0.90
5	-40	-15	40	1.00
6	50	- 5	10	0.40[b]
7	50	- 5	20	0.40
8	50	- 5	0	0.70
9	60	- 5	0	0.70
10	60	0	-10	0.90
11	60	0	10	1.10

[a] Global minimum

[b] Local minimum.

Recent molecular mechanics calculations of Boyd (Boyd, 1979) have also indicated that Δ^3 cephalosporin can exist in two minimum energy conformations, with the N_5-C_6-S_1-C_2 angle having values 51° and -39°. The present study shows that the values at the local and the global minima are 56° and -21° respectively. However, according to Boyd's study, the later conformation was about 2-3 Kcal.mole^{-1} higher in energy than the former, whereas the present study indicates that both have nearly equal energies. Since details of the molecular mechanics calculations were not reported, the reasons for the discrepancy between these two results cannot be discussed here.

The present study shows that the lactam peptide is much more nonplanar at the global minimum (ω_2 = 147°) than at the local minimum ($\omega_2 \simeq 161°$). However, both these are much less nonplanar than that in the penicillins ($\omega_2 \simeq 130°$).

The orientations of the carboxyl group at the global and the local minimum are, respectively $\varphi_2 \simeq 90°$ and $\varphi_2 \simeq 35°$. Interestingly, these are quite different from those observed for penicillins in either of the conformations ($\varphi_2 \simeq 160°$ and 110° for C_3 and C_2 puckered conformations respectively).

5.3.2. Δ^2 Cephalosporins:

Conformational energy map of Δ^2 cephalosporin is shown in Fig. 5.6. The position of the energy minima and the solid state conformation are marked in the diagram. Table 5.2 shows α_1, α_2 and α_3 for some of the low energy conformations.

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Table 5.2: Conformational angles and energies for some of the low energy conformations of Δ^2 cephalosporin.

No.	α_1 (degrees)	α_2 (degrees)	α_3 (degrees)	E (Kcal.mole^{-1})
1	50	-30	10	0.00[a]
2	50	-30	20	0.00
3	60	-35	10	0.40
4	60	-35	0	0.40
5	50	-30	30	0.40
6	40	-25	30	0.50
7	40	-25	20	0.70
8	60	-35	20	0.90
9	-40	25	40	3.10[b]
10	-30	20	40	3.40
11	-30	20	50	3.80

[a] Global minimum

[b] Local minimum.

The dihydrothiazine ring of Δ^2 cephalosporin shows two minimum energy conformations. As seen from Table 5.2, the global minimum occurs at (50°, -30°, 0°). The local minimum occurs at (-40°, 25°, 40°) and has about 3 Kcal.mole^{-1} higher energy than the global minimum, indicating that the former would be favoured both in solid state and in solution. The conformation observed in the solid state is similar to the one at the global minimum, but has about 2-3 Kcal.mole^{-1} higher energy (Sweet and Dahl, 1970). The value of Φ_2 at the global minimum is $\simeq 90^\circ$, and at the local minimum is $\simeq 150^\circ$. At the global minimum, $\omega_2 \simeq 170^\circ$, suggesting that the lactam peptide is less nonplanar, compared to the penicillins. At the local minimum, $\omega_2 \simeq 143^\circ$, indicating considerable nonplanarity. However, due to its higher energy, this conformation is unlikely to be observed either in the solid state or in solution.

5.3.3. Biological Activity of Cephalosporins:

As mentioned earlier, for the Δ^3 cephalosporins, in both the puckered conformations of the dihydrothiazine ring, the lactam peptide bond will assume appreciable nonplanarity. On the other hand, in the preferred conformation of Δ^2 cephalosporin, the lactam peptide bond is less nonplanar. This can account for the observed differences in the biological activities of these compounds.

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For Δ^3 cephalosporins, the aminoacyl group assumes approximately the same orientation as in penicillins, but the carboxyl group orientation is different. In fact, the values of φ_2 for Δ^3 cephalosporins ($\simeq 35^\circ$ and $\simeq 90^\circ$) are very much different from the one observed in the biologically active conformation of penicillin ($\simeq 160^\circ$). Since Δ^3 cephalosporin is active (though less compared to penicillin), the differences at the carboxyl group suggest that the mode of binding of this drugwwith the cross linking enzymes may differ slightly from that of penicillins. Such differences in the mode of binding of molecules which differ in the orientations of some of the group groups are not unusual, e.g., binding of α and β anomers of N-acetyl glucosamine to lysozyme (Beddel et al., 1972). [1970 However, it is not clear which of the conformations of the dihydrothiazine ring is associated with biological activity.

These studies suggest that the reduced activity of cephalosporins compared to penicillins may be due to (a) the differences in the nonplanarity of the lactam peptide bonds, and (b) differences in the orientations of the carboxyl group.

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VI. CLAVULANIC ACID

6.1 Introduction:

As mentioned in Chapter I, efficacy of penicillins has declined due to the emergence of penicillin resistant bacteria, which produce penicillinases. However, a recently isolated β-lactam antibiotic, clavulanic acid, has been found to be a potent inhibitor of penicillinases (Howarth et al., 1976; Reading and Cole, 1977). The structure of clavulanic acid is shown in Fig. 6.1. The thiazolidine ring of penicillins is replaced by the oxazolidine ring in clavulanic acid. Also, the aminoacyl group is absent, indicating that it is not as essential for binding with penicillinases as the lactam ring and the carboxyl group are.

Despite extensive experimental work (Citri, 1977) as 1972 penicillinases, the details about the binding requirements of these enzymes are not known. Penicillinases show different reactivities against penicillins, cephalosporins and clavulanic acid. As mentioned earlier, the enzymes can cleave penicillins, but not cephalosporins. On the other hand, they are strongly inhibited by clavulanic acid. Hence, a comparison of the conformations of these molecules may yield information about the binding requirements of the enzyme. With this view in mind, conformations of the oxazolidine ring have been investigated in detail, using classical potential energy functions.

A knowledge of the binding specificity thus obtained is essential for designing better drugs against penicillinase producing bacteria.

6.2 Conformational Energy Calculations:

The same procedure and parameters as adopted for penicillins were used for fixing the atoms initially in this case also. The C-O bond length was taken to be 1.42 Å. The methods used for calculating and minimizing the conformational energy, and for drawing the conformation energy maps are the same as described in Chapter IV. The fractional charges used for calculating the electrostatic energy are shown in Fig. 6.2.

6.3 Results and Discussion:

6.3.1. Clavulanic Acid:

Conformational energy map of clavulanic acid is shown in Fig. 6.3. The oxazolidine ring shows a single minimum at $(\alpha_1, \alpha_2) \simeq (10^\circ, -10^\circ)$. This indicates that the ring is highly rigid, and favours only one type of conformation. The conformation observed in the solid state (Howarth and King, 1976) et al is around $(-10^\circ, -20^\circ)$ and is very near to the minimum energy conformation. However, the solid state conformation has about 2 Kcal.mole^{-1} higher energy than the minimum energy conformation, which may be compensated for by the lattice energy in the crystal.

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The relative orientations of the two rings ($180^{\circ}-\alpha_3$) and the orientation of the carboxyl group (φ_2) predicted by the theory are 122° and 148°, respectively. These are in good agreement with the experimental values of 120° and 161°. The calculated value of the nonplanarity of the lactam peptide bond ($\omega_2 \simeq 121^{\circ}$) is also in close agreement with that observed in the solid ~~state~~ ($\omega_2 \simeq 124^{\circ}$). It is seen that lactam peptide bond in clavulanic acid is significantly more nonplanar than the penicillins ($\simeq 130^{\circ}$) or cephalosporins ($\gtrsim 160^{\circ}$).

6.3.2. Binding specificity of penicillin β-lactamases:

Unlike penicillins (Fig. 4.3), clavulanic acid favours only one type of conformation (Fig. 6.3). Since a (competitive) inhibitor has the highest probability of existing in the right conformation for interacting with the enzymes, clavulanic acid, the inhibitor would have the orientation of its carboxyl group and the relative orientation of its bicyclic ring system very close to the binding requirements of penicillinases.

As mentioned earlier, for clavulanic acid, $\varphi_2 \simeq 148^{\circ}$ and the angle between the two rings is $\simeq 122^{\circ}$. These values are very close to those of the C_3 puckered conformation of penicillin sulfide, where $\varphi_2 \simeq 161^{\circ}$ and the angle between the two rings is $\simeq 113^{\circ}$ (Table 4.1). The projection diagrams of penicillin and clavulanic acid (Fig. 6.4a and 6.4b) also bring out the conformational similarity between

The relative orientations of the two rings ($180^{\circ}-\alpha_3$) and the orientation of the carboxyl group (φ_2) predicted by the theory are 122° and 148°, respectively. These are in good agreement with the experimental values of 120° and 161°. The calculated value of the nonplanarity of the lactam peptide bond ($_2$ 121°) is also in close agreement with that observed in the solid state ($_2$ 124°). It is seen that lactam peptide bond in clavulanic acid is significantly more nonplanar than the penicillins (130°) or cephalosporins (160°).

6.3.2. Binding specificity of penicillin β-lactamases:

Unlike penicillins (Fig. 4.3), clavulanic acid favours only one type of conformation (Fig. 6.3). Since a (competitive) inhibitor has the highest probability of existing in the right conformation for interacting with the enzymes, clavulanic acid, the inhibitor would have the orientation of its carboxyl group and the relative orientation of its bicyclic ring system very close to the binding requirements of penicillinases.

As mentioned earlier, for clavulanic acid, φ_2 148° and the angle between the two rings is 122°. These values are very close to those of the C_3 puckered conformation of penicillin sulfide, where φ_2 161°) and the angle between the two rings is 113° (Table 4.1). The projection diagrams of penicillin and clavulanic acid (Fig. 6.4a and 6.4b) also bring out the conformational similarity between

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clavulanic acid and the C_3 puckered conformation of penicillin. This suggests that for binding with penicillinases, the C_3 puckered conformation of penicillin is required.

As indicated in Chapter IV, the population of penicillin molecules in this conformation would be small in an equilibrium mixture. This accounts for the competitive inhibitory property of clavulanic acid.

As shown in Chapter V, neither Δ^3 nor Δ^2 cephalosporin can exist in a conformation with $\varphi_2 \simeq 150^\circ$, due to the rigidity of the dihydrothiazine ring. This may explain the resistance of the cephalosporins to penicillinases.

It is interesting to note (Chapter IV) that C_3 puckered conformation of penicillins is the one which binds to the cross linking enzymes is nearly the same as the one required for binding to penicillin β lactamases.

Experimental studies indicate that the rate of hydrolysis of the lactam peptide bond by β-lactamases is very slow in clavulanic acid compared to that in penicillin. It is not clear at this stage whether the presence of aminoacyl group or the flexibility of the five membered ring is required for this reaction.

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Figurs 2.3 to 2.10:
Figures 2.3 to 2.10:

Conformational enery maps for ~~pentop~~pyranoses. Numbers on contours indicate relative energies in Kcal.mole^{-1}. The conformations of the pyranose ring observed in the crystal structures are indicated for α-D-arabinose, β-D-arbinose, β-D-lyxose, β-D-ribose, α-D-xylose and β-D-xylose. The co-ordinates of L sugars, reported in literature, have been converted into those of D sugars for plotting.

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Molecule	α_1	α_2	α_3	Angle between Axis	Non Planarity	Angle at the carbonyl end		
Cephalosporin active crystal structure	+47	−8	+8	158	−18	54		
Cephalosporin Active I	+50	0	10	162	−18	36		
Cephalosporin Active II	−40	−20	+50	146	−32	89		
Cephalosporin Inactive crystal structure	~~+50~~ +17	~~−30~~ −12	+43	183°	−4	110°		
Cephalosporin I	+50	−30	+10	167	−13	82		
Cephalosporin II	−40	+25	40	149	−30	156		

3K

S_1	0	0	0
C_2	1.790	0	0
C_3	1.330	131.0	
C_4	1.530	121.9	−1
N_5	1.449	110.6	−6
C_6	1.453	127.0	41

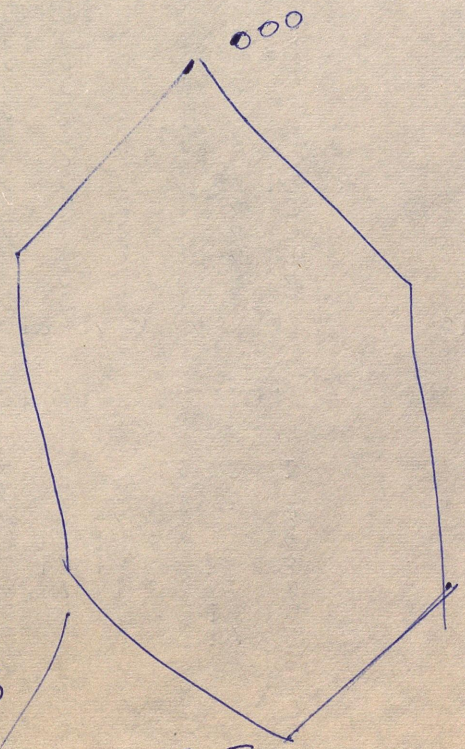
Check $C_6 - S_1$ 1.795

N_5 C_6 S_1 110.4

Z^S . −60

.72
.1
.2

−0.8396
.3446
.4513
−0.8777
0.3567
0.0274



1.790, 0, 0

1.1743, 1.3509
+1.7900
1.9643

~~−0.4228~~, ~~1.5833~~,
~~−0.5622~~
−0.4659 1.5965
−0.1845

0.7797, 2.31895, ~~0.2266~~ −0.0227

LIST OF PUBLICATIONS

1. Studies on the conformational flexibility in the pyranose ring of α- and β-D-glucose
N.V. Joshi and V.S.R. Rao (1978)
~~Presented at the~~ "International Symposium on Biomolecular Structure, Conformation, Function and Evolution" (Madras, India)

2. Theoretical studies on β-lactam antibiotics III
Conformational flexibility of the five membered ring in clavulanic acid and penicillin and the binding specificity of β-lactamases
N.V. Joshi, R. Virudachalam and V.S.R. Rao (1978)
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3. Flexibility of the pyranose ring in α- and β-D-glucoses
N.V. Joshi and V.S.R. Rao II
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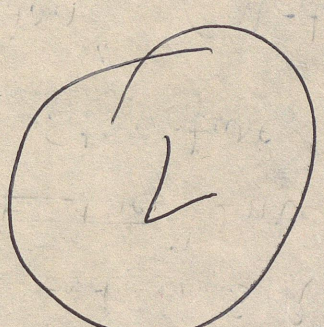

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VI Conformational Analysis of Clavulanic Acid and Binding Specificity of Penicillin β-lactamases

6.1 Introduction.

6.2 Conformational Energy Calculations.

6.3 Results and Discussion

6.3.1 Clavulanic Acid

6.3.2 Binding specificity of Penicillin β lactamases.

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000      22222      88888
       2      2    8     8
       0      2    8     8
         2222      88888
        2          8     8
       2           8     8
000    2222222     88888
```

* Penicillinases show different reactivities against ~~react differently to~~ penicillins, cephalosporins and clavulanic acid. As mentioned earlier, the enzymes can cleave penicillins, ~~while cephalosporins are stable against~~ but not cephalosporins. On the other hand, they are strongly inhibited by ~~clavulanic~~ clavulanic acid.

~~8.4~~

~~8.3~~

= ',I5)

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N	SYMBOL	LOCATION	SYMBOL	LOCATION
N	SYMBOL	LOCATION	SYMBOL	LOCATION
	K	0001C4	L	0001EC
N	SYMBOL	LOCATION	SYMBOL	LOCATION
N	LABEL	LOCATION	LABEL	LOCATION
	10	000328	4	000388
	6	0004EE	11	000556

VI Conformational Analysis of Clavulanic Acid and Binding Specificity of Penicillin β-lactamases

6.1 Introduction

As mentioned in chapter I, efficacy of penicillins has declined due to the emergence of penicillin resistant bacteria, which produce penicillinases. However, a recently isolated β-lactam antibiotic, clavulanic acid, has been found to be a potent inhibitor of penicillinases (Howarth et.al., 1976; Reading and Cole, 1977). The structure of clavulanic acid is shown in Fig. 6.1. The thiazolidine ring of penicillins is replaced by the oxazolidine ring in clavulanic acid. Also, the aminoacyl group is absent, indicating that it is not as essential for binding with penicillinases as the lactam ring and the carboxyl group are.

Despite extensive experimental work (Citri, 1977) on penicillinases, the details about the binding requirements of these enzymes are not known. Since penicillinases can cleave penicillins, cannot cleave cephalosporins, and are strongly inhibited by clavulanic acid, a comparison of the conformations of these molecules may yield information about the binding requirements of the enzyme. With this view in mind, conformations of the oxazolidine ring have been investigated in detail, using classical potential energy functions. A knowledge of the binding specificity, thus obtained is essential for designing better drugs against penicillinase producing bacteria.

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For generating the co-ordinates of the atoms, the same procedure which was used for penicillins was used.

* The bond lengths used for fixing the co-ordinates of the molecule were also the same, ~~except in the case of~~ A C–O bond length of 1.42 Å was used for fixing the ring oxygen.

The same procedure and parameters as adopted for penicillins were used for fixing the atoms initially in this case also the C–O bond length was taken to be 1.42 Å.

6.2 Conformational Energy Calculations

The parameters used to describe the oxazolidine ring are similar to those described in chapter IV, for used for the thiazolidine ring of penicillin.

* The methods used for calculating and minimizing the conformational energy, and for drawing the conformational energy maps are the same as described in chapter IV. The fractional charges used for calculating the electrostatic energy are shown in Fig. 6.2

6.3 Results and Discussion

6.3.1 Clavulanic Acid

Conformational energy map of clavulanic acid is shown in Fig. 6.3. The oxazolidine ring shows a single minimum at $(\alpha_1, \alpha_2) \simeq (10°, -10°)$. This indicates that the ring is highly rigid, and favours only one type of conformation. The conformation observed in the solid state (Howarth and King, 1976) is around $(-10°, -20°)$ and is very near to the minimum energy conformation. However, the solid state conformation has about 2 Kal.mole^{-1} higher energy than the minimum energy conformation, which may be compensated for by the lattice energy in the crystal.

The relative orientations of the two rings $(180° - \alpha_3)$ and the orientation of the carboxyl group (ϕ_2) predicted by the theory are 122° and 148°, respectively. These are in good agreement with the experimental values of 120° and 161°.

The calculated value of the nonplanarity of the lactam peptide ~~bond~~ bond ($\omega_2 \simeq 121°$) is also in close agreement with that observed in the solid state ($\omega_2 \simeq 124°$). It is seen that ~~clavulanic acid~~ ~~is~~ lactam peptide bond in clavulanic acid is significantly more nonplanar than the penicillins ($\simeq 130°$) or cephalosporins ($> 160°$). This may partially account for the ~~higher~~ reactivity of clavulanic acid compared to penicillins or cephalosporins.

6.3.2 Binding Specificity of penicillin β lactamases

Unlike penicillins (Fig. 4.3), clavulanic acid favours only one type of conformation (Fig. 6.3). Since ~~an~~ ~~the~~ inhibitor ~~has~~ a (competitive) inhibitor has the highest probability of existing in the right conformation for interacting with the enzymes, clavulanic acid, the inhibitor, (would?) have ~~its~~ the orientations of ~~the~~ its carboxyl group and the relative orientation of its bicyclic ring system very close to the binding requirements of penicillinases.

As mentioned earlier, for clavulanic acid, $\phi_2 \simeq 148°$ and the angle between the two rings is $\simeq 122°$. These values are very close to those of the C_3 puckered conformation of penicillin sulfide, where $\phi_2 \simeq 161°$ and the angle between the two rings is $\simeq 113°$ (Table 4.1). The projection diagrams of penicillin and clavulanic acid (Fig 6.4a

* Experimental studies ~~have~~ indicated that the rate of hydrolysis of the lactam peptide bond by β lactamases is very slow in clavulanic acid compared to that in penicillin. It is not clear at this stage whether the presence of the aminoacyl group or the flexibility of the five membered ring is required for this reaction ~~to take place~~.

and 6.4(b)) also brings out the conformational similarity between clavulanic acid, and the C_3 puckered conformation of penicillin. This suggests that for binding with penicillinases, the C_3 puckered conformation of penicillin is required.

As indicated in chapter IV, the population of penicillin molecules in this conformation would be small in an equilibrium mixture. This accounts for the competitive inhibitory property of clavulanic acid.

As shown in chapter V, neither Δ^3 nor Δ^2 cephalosporin can exist in a conformation with $\phi_2 \simeq 150°$, due to the rigidity of the dihydro thiazine ring. This may explain the resistance of the cephalosporins to penicillinases, ~~for these compounds are unlikely to bind to the enzyme~~.

It is interesting to note (chapter IV) that C_3 puckered conformation of penicillins is the one which binds to the cross-linking enzymes [also. Thus, the ~~conformations~~ orientation of the carboxyl group of penicillin required for ~~binding~~ binding with the cross linking enzymes] is nearly the same as the one required for binding to penicillin β lactamases.

* The present study thus predicts that modifications made at the C terminal of penicillins are unlikely to lead to drugs with reasonable activity against penicillinase producing bacteria.

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Fig. 2.4: β-D-Arabinose

Solid state conformations of

○ Methyl β-D-arabinose (Takagi and Jeffrey, 1978a).

⊙ β-D-Arabinose (Takagi and Jeffrey, 1978b).

⊡ β-D-L-Arabinose (Kim and Jeffrey, 1967)

are shown in the diagram.

Fig. 2.3: α-D-Arabinose

Solid state conformation of

⊙ Methyl α-[illegible]-arabinose (Takagi and Jeffrey, 1978a)

is shown in the diagram.

Fig. 2.6: β-D-Lyxose

Solid state conformation of

β-D-Lyxose (Hordvik, 1966)

is shown in the diagram.