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Theoretical studies on β-lactam antibiotics VI*: Conformational analysis and structure-activity relationships of penicillin sulfoxides and cephalosporins

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Abstract. Conformational energy calculations were carried out on penicillin α- and β-sulfoxides and Δ^2- and Δ^3- cephalosporins, in order to identify the structural features governing their biological activity.

Results on penicillin β-sulfoxide indicated that in its favoured conformation, the orientation of the aminoacyl group was different from the one required for biological activity. Penicillin α sulfoxide, like penicillin sulfide, favoured two conformations of nearly equal energies, but separated by a much higher energy barrier. The reduced activity of the sulfoxides despite the nonplanarity of their lactam peptide indicated that the orientations of the aminoacyl and carboxyl groups might also govern biological activity.

Δ^3- cephalosporins favoured two conformations of nearly equal energies, whereas Δ^2-cephalosporins favoured only one conformation. The lactam peptide was moderately nonplanär in the former, but nearly planar in the latter. The differences in the preferred orientations of the carboxyl group between penicillins and cephalosporins were correlated with the resistance of cephalosporins to penicillinases.

Keywords. Cephalosporins; penicillin sulfoxides; conformational analysis; structure activity relations.

Introduction

Penicillins, one of the most widely used anti-microbial agents, is known to act by inhibiting the enzyme(s) transpeptidase(s) and/or carboxypeptidase(s), which bring about the cross-linking reaction in peptidoglycan biosynthesis (Blumberg and Strominger, 1974). It has been suggested (Tipper and Strominger, 1965) and subsequently shown theoretically (Virudachalam and Rao, 1977) that penicillin is a structural analog of the natural substrate X-D-Ala-D-Ala.

The widespread use of penicillins has lead to the proliferation of penicillin resistant bacteria: these contain enzymes (penicillinases) which inactivate penicillins by hydrolysing the lactam peptide bond of the drug. Hence, a search has been on for developing drugs active against penicillin resistant bacteria. Cephalosporins, a class of compounds similar to penicillins, have been found to be

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resistant to the action of penicillinases, though their activity is low compared to penicillins. A detailed conformational analysis of cephalosporins, and their comparison with penicillin and its derivatives, is likely to throw light on the stereochemical basis of the resistance of cephalosporins towards penicillinases and their reduced antimicrobial activity.

It has also been observed that minor substitutions in the thiazolidine ring (as in penicillin α- and β- sulfoxides), can drastically reduce the biological activity: the reasons for this, however, are not clearly understood. In order to elucidate the structure-activity relationships in β-lactam antibiotics, conformational analysis of penicillin α- and β-sulfoxides has also been carried out, in addition to Δ^3- and Δ^2-cephalosporins.

Energy calculations

Choice of parameters

The molecule of penicillin sulfoxide is shown in figure 1, and the parameters used to describe it are shown in figure 2. Conformation of the thiazolidine ring is

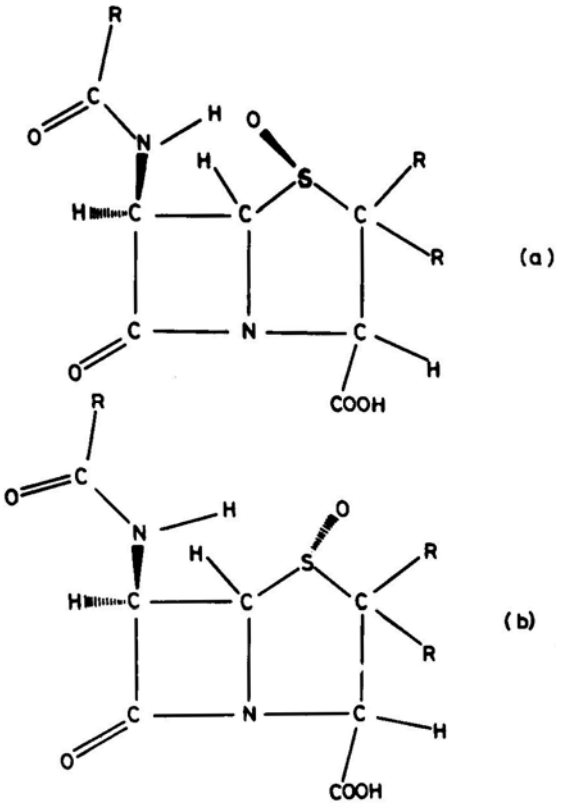

Figure 1. Structure of penicillin sulfoxide.

a .Penicillin β-sulfoxide.
b Penicillin α-sulfoxide. R denotes a methyl group.

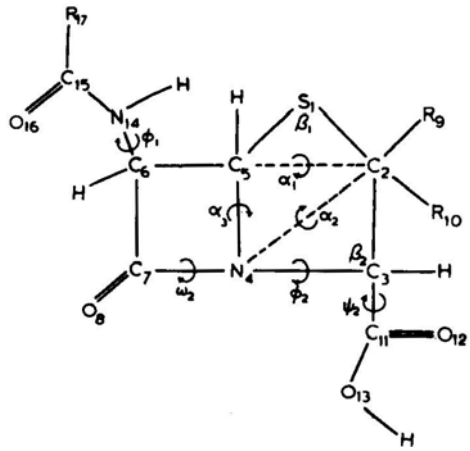

Figure 2. Numbering of atoms and conformational parameters for the thiazolidine ring. R denotes a methyl group.

described in terms of the two dihedral angles α_1 and α_2, denoting rotations about the virtual bonds C-5–C-2, C-2–N-4 respectively. The angle α_3 denotes the relative orientation of the lactam ring (assumed planar) with the C-5-C-2-N-4 plane. The aminoacyl group was fixed using the torsional angle θ_1(C-l5-N-14-C-6-C-7). Conformation of the sulfide molecule was thus completely specified by the parameters shown in figure 2. The bond lengths were kept constant throughout the calculations, using crystal structure values.

Figures 3 and 4 depict Δ^3- and Δ^2-cephalosporin respectively. The parameters used to describe the conformation of the six membered dihydrothiazine ring are

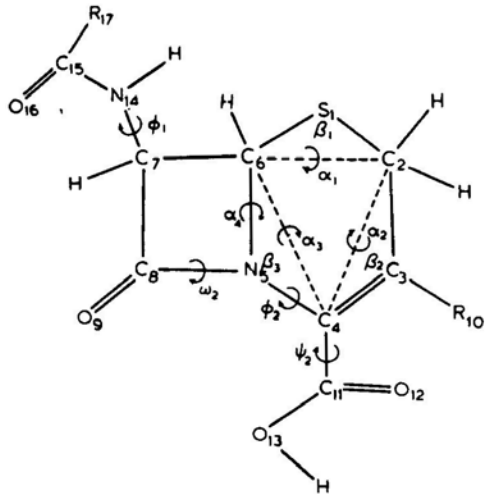

Figure 3. Conformational parameters for Δ^3-cephalosporin. R denotes a methyl group.

similar to those for the sulfoxide and are also shown in figures 3 and 4. Thus, α_l, α_2 and α_3 describe the six membered ring, while α_4 denotes the relative orientations between the lactam ring and the C-4-N-5-C-6 plane. The bond lengths were kept constant at the values obtained from crystal structure. The angle at the aminoacyl end, θ_1 was also kept constant at 160°, as it is energetically favoured, and also because it is unlikely to be affected by changes in the conformation of the dihydrothiazine ring. ψ_2 was kept constant at 30° as observed in simple peptides (Virudachalam and Rao, 1977).

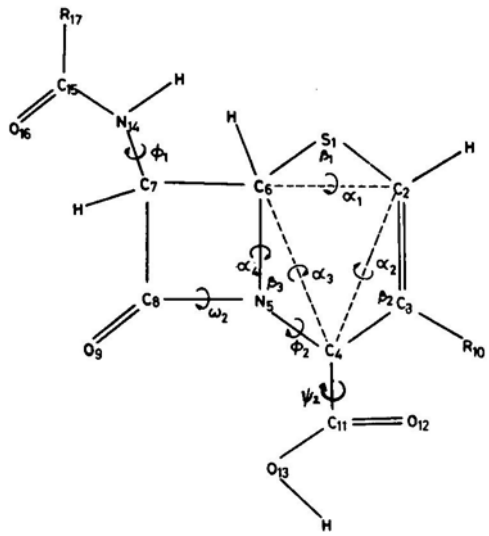

Figure 4. Conformational parameters for Δ^2-cephalosporin. R denotes a methyl group.

Conformational energy calculations

The total conformational energy was computed taking contributions from electrostatic and nonbonded interactions, as well as from bond angle and torsional angle distortions. The fractional charges on the atoms were taken to be the sum of the σ-*charges* (obtained by Del Re's (1958) method and π- charges (obtained using Huckel M O theory). Kitaigorodosky's (1961) functions were used to compute the nonbonded interaction energy. In the case of sulfoxides, to estimate the energy of hydrogen bond formation between the sulfoxide oxygen and aminoacyl N-H, the function proposed by Momany *et al.* (1975) was used. The other functions, as well as all the constants used in the present work, have been described earlier (Joshi and Rao, 1979; Joshi, 1980).

It is known from the earlier studies (Joshi *et al.*, 1978) that varying α_1 and α_2 over the range —70° to 70° is adequate for sampling all the sterically allowed conformations of the thiazolidine ring. Hence, in the present work, α_1 and α_2 were varied over this range at 10° intervals. At every grid point (α_1, α_2), conformational energy was minimised with respect to $ß_1$ β_2, α_3 and θ_1. Isoenergy contours were drawn in the α_1-α_2 plane.

Three variables (α_1 , α_2 and α_3) are required to specify the conformation of the six membered dihydrothiazine ring in cephalosporins. However, due to the double bond, for a given (α_1, α_3), the range of α_2 is considerably restricted. Hence, in the present work, α_1 and α_3 were varied over a range –70° to 70°. At every grid point (α_1, α_3), α_2 was varied over a restricted range, and at each point, the energy was minimised with respect to the bond β_1, β_2, β_3 and with respect to the angle between the rings, α_4. Conformational energy surface of the dihydrothiazine ring was represented by isoenergy contours in the α_1-α_3 plane. A point on such a map corresponds to a conformation whose energy has been minimized with respect to β_1, β_2, β_3, α_4 and α_2. To indicate the small but significant variations in α_2 for a few low energy conformation the values of α_1, α_2, α_3 and the conformational energies were shown in tables 2 and 3 for Δ^3- and Δ^2-cephalosporins respectively.

Results and discussions

The conformational energy map of penicillin α-sulfoxide is shown in figure 5a. There are two minima, the global minimum occurs at (α_1, α_2)=(40° , 15°) and a local minimum at (–20°, –25°), which is about 0.5 Kcal. mol^{-1} higher in energy than the former. The global minimum corresponds to the C_2 puckered conformation of the thiazolidine ring, and the local minimum to the C_3 puckered conformation. For penicillin α-sulfoxide, no hydrogen bond is possible between the sulfoxide oxygen and aminoacyl N-H.

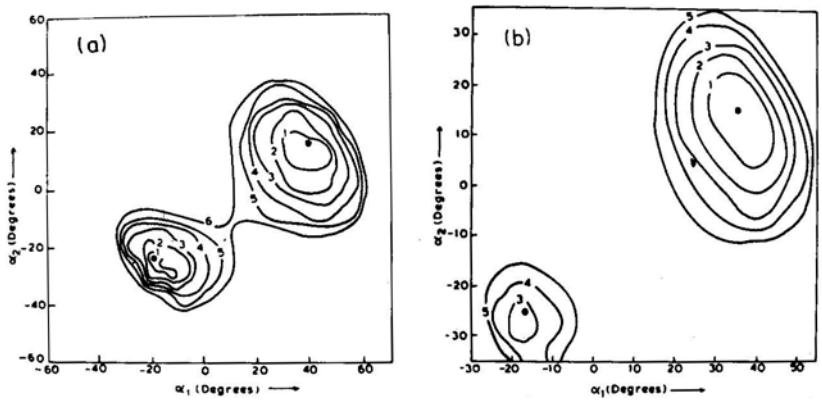

Figure 5. Conformational energy map.
a . Penicillin α sulfoxide,
b. Penicillin β sulfoxide.
Numbers on contours indicate energy in K cal mol^{-1}. Positions of the two minima are marked.

Table 1 shows the angles θ_1, θ_2 and ω_2 for penicillin sulfide, penicillin α-sulfoxide and penicillin β-sulfoxide. It is seen that for penicillin α-sulfoxide also, the lactam peptide is significantly nonplanar (ω_2 ~ 132°) as in penicillin sulfide. Sweet and Dahl (1970) have proposed that the nonplanarity of the lactam peptide bond plays a

Table 1. Calculated dihedral angles of the amino acyl group, carboxyl group and the lactam peptide at the minimum energy conformations for penicillin sulfide and sulfoxides.

Conformation	Favoured orientation of aminoacyl group θ_1 (degrees)	Favoured orientation of carboxyl group [a] θ_2 (degrees)	Non-planarity of the lactam peptide ω_2 (degrees)
Penicillin sulfide C_2 puckered [b]	170	111	133
Penicillin sulfide C_3 puckered [b]	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

[a] $\psi_{2=}-30°$ (see text.)
[b] (Joshi *et al*., 1978).

major role in governing the biological activity of these antibiotics. By this criterion, penicillin α-sulfoxide should be as active as penicillin G or V; on the contrary, the sulfoxides are known to be much less active compared to the sulfides (Gorman and Ryan, 1972).

As can be seen from figure 5a the energy barrier separating the two minima is about six Kcal. mol^{-1}, which is higher by about 2 Kcal. mol^{-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.

In penicillin β-sulfoxide the global minimum (figure 5b) occurs at (35°, 15°) as in penicillin α-sulfoxide and 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 groups. This is in agreement with X-ray crystal structure studies (Copper *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 energy barrier separating the two minima is about 7 Kcal. mol^{-1}.

Table 1 shows that the lactam peptide bond is nonplanar (ω_2=131°) in both the conformations. Thus, if nonplanarity of the lactam peptide alone is important for 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 ($\theta_1 \sim -90°$) different from the biologically active one ($\theta_1 \sim 180^o$). 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 difference in the activities of the two sulfoxides.

The conformational energy map of Δ^3-cephalosporin is shown in figure 6a. The positions of minimum energy conformations, and the solid state conformation as

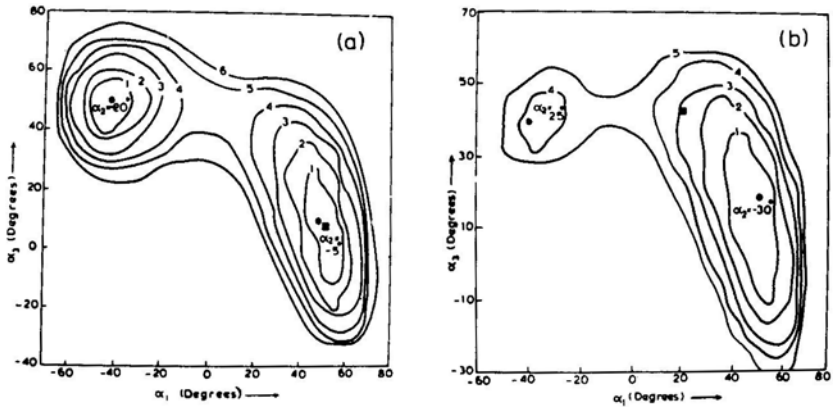

Figure 6. Conformational energy map.

a. Δ^3-cephalosporin and b. Δ^2-cephalosporin

Numbers on contours indicate energy in Kcal mol^{-1} . Positions of the two minima are marked. The value of α_2 (in degrees) at the minimum energy conformation is also shown in the figure. The solid state conformation is denoted by ■

observed in the crystal structure are also marked in the diagram. The global minimum occurs at (α_1, α_2, α_3) (–40°, –20°, 50°). The local minimum occurs at (50°, –5 ° , 10°) and is 0.4 Kcal, mole^{-1} higher in energy than the global minimum (table 2). Since this energy difference is small, the dihydrothiazine ring can

Table 2. 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. mol^{-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.

assume either of the puckered forms. The solid state conformation (Sweet and Dahl, 1970) corresponds to (47°, –8°, 8°) 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. mol^{-}), 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.

Recent molecular mechanics calculations of 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 latter conformation was about 2-4 Kcal. mol^{-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 more nonplanar at the global minimum (ω_2~161°). However, both these are much less nonplanar than that in the penicillins (ω_2~ 130°).

The orientations of the carboxyl group at the global and the local minimum are, respectively θ_2~90° and θ_2~35°. Interestingly, these are quite different from those observed for penicillins in either of the conformations (θ_2~ 160° and 110° for C_3 and C_2 puckered conformations respectively).

Conformational energy map of Δ^2-cephalosporin is shown in figure 6b. The position of the energy minima and the solid state conformation are marked in the diagram. Table 3 shows α_1, α_2 and α_3 for some of the low energy conformations.

Table 3. 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. mol^{-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 3, the global minimum occurs at (50°, –30°, 10°). The local minimum occurs at (−40°, 25°, 40°) and has about 3 Kcal. mol^{-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 (Sweet and Dahl, 1970) is similar to the one at the global minimum, but has about 2-3 Kcal. mol^{-1} higher energy. The value of θ_2 at the global minimum is ~90°, and at the local minimum is ~150°. At the global minimum, ω_2~170°, suggesting that the lactam peptide is less nonplanar, compared to the penicillins. At the local minimum, ω_2~143°, indicating considerable nonplanarity. However, due to its higher energy, this conformation is unlikely to be observed either in the solid state or in solution.

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 difference in the biological activities of these compounds.

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 θ_2 for Δ^3-cephalosporins (~35° and ~90°) are very much different from the one observed in the biologically active conformation of penicillin (~160°). Since Δ^3-cephalosporin is active (though less compared to

penicillin), the differences at the carboxyl group end suggest that the mode of binding of this drug with 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 groups are not unusual, e.g., binding of α and β anomers of N-acetyl glucosamine to lysozyme (Beddel *et al.,* 1970). However, it is not clear which of the conformations of the dihydrothiazine ring is associated with biological activity.

Our earlier studies (Joshi *et al*., 1978) on specificity of penicillinases have indicated that for binding to penicillinases, the orientation of the carboxyl group (θ_2) should be 150°; as in penicilin (C_3 puckered) or clavulanic acid. However, in neither Δ^3-nor Δ^2 cephalosporins, the energetically favoured conformations have carboxyl group orientations near this value. These differences in the orientations of the – COOH group perhaps account for the resistance of cephalosporins to penicillinases.

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