The present strategy has been used, additionally to the syntheses noted above, in the syntheses of the heavily substituted benzene derivatives mycophenolic acid[4c], antibiotic DB-2073, a degradation product of the polyketide phomazarin[23], the biphenyl derivatives alternariol[24], and altenusin[24] and the macrocyclic lactone, lasiodiplodin[25]. The hydroxy-acid corresponding to the dimethyl ether of the medium ting lactone curvularian has been made[25] but so far without success in ring closure. Full details of this work will be published elsewhere.

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CONFORMATIONAL STUDIES OF PHENYL AND ISOXAZOLYL PENICILLINS

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ABSTRACT

Conformational energy calculations have been carried out on a few representative β-lactamase-resistant and susceptible, phenyl and isoxazolyl penicillins. These studies, in agreement with those of earlier workers, show that the 6 β-side chains of resistant penicillins are highly rigid as compared to those of susceptible penicillins. The present studies also suggest that the degree of resistance to β-lactamases depends not only on the rigidity of the side chain but also on the nature and orientation of the substituent, beyond the amide carbonyl group in the side chain. The overall shapes of these penicillins correlate well with their antibacterial properties.

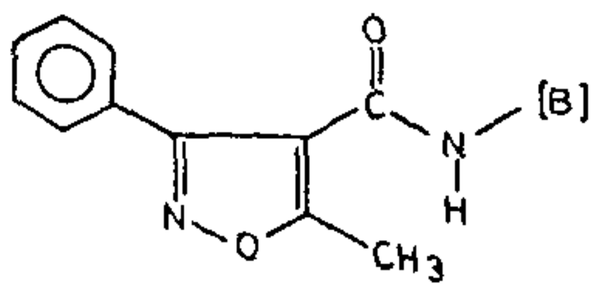
INTRODUCTION

THE search for β-lactam antibiotics resistant to β-lactamases has resulted in the synthesis of penicillins such as oxacillin, ancillin, etc (Figure 1) where the α-carbon atom in the 6 β-side-chain is incorporated in an aromatic or hetero-aromatic ring. In these penicillins, the resistance to β-lactamases has been attributed to the steric effects around the amide-carbonyl in their 6 β-side-chain[1,2].

From a number of chemical studies, it has been postulated that penicillins (A-type) which show resistance to β-lactamases bring about a conformation change in the active site of the enzyme that is unfavourable for catalytic reaction and hence lead to a lower, but constant level of enzymic activity. On the

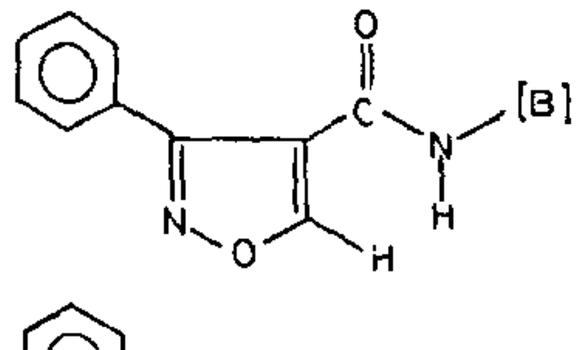
COMPOUND 1

5 - Methyl - 3 - Phenyl - 4 - Isoxazolyl Penicillin (Oxacillin)



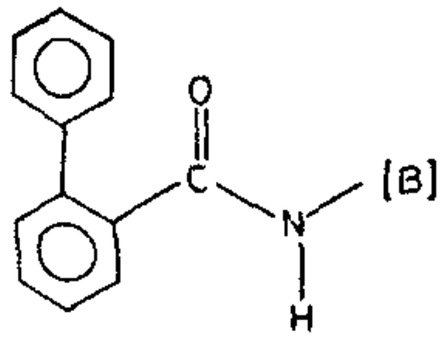
COMPOUND 2

3 - Phenyl - 4 - Isoxazolyl Penicillin



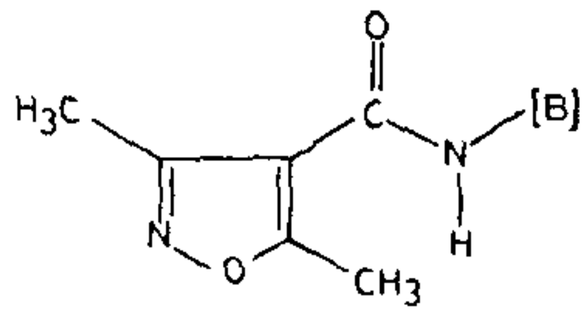
COMPOUND 3

2 - Biphenylyl Penicillin (Ancillin)



COMPOUND 4

3,5 - Dimethyl 4 - Isoxazolyl Penicillin



COMPOUND 5

2,6 - Dimethyl Phenyl Penicillin

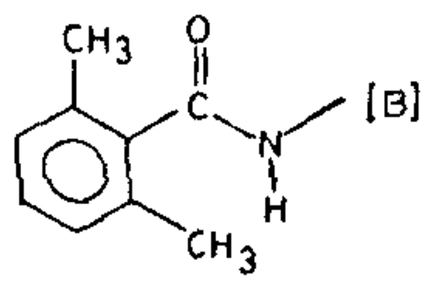


Figure 1. Chemical structures of isoxazolyl and phenyl penicillins. [B] represents bicyclic ring systems.

other hand, susceptible penicillins (S-type) induce a conformation which is favourable for the catalytic reaction of the enzyme. From these studies, it has also been concluded that the nature of such conformational changes (and hence the catalytic activity) is very much related to the nature of the 6 β-side-chain of penicillins[3-6].

Recent theoretical studies[7, 8] also indicate that the two types of penicillins could be distinguished on the basis of the computed energy maps of their side-chains. However, in these studies, the bicyclic ring system is either fixed at the crystallographic value or not at all considered. Therefore, to obtain a better picture, conformational energy calculations on a few representative S-type and A-type penicillins have been carried out using semi-empirical potential energy functions. These studies throw light on their different antibacterial properties.

METHODS OF CALCULATION

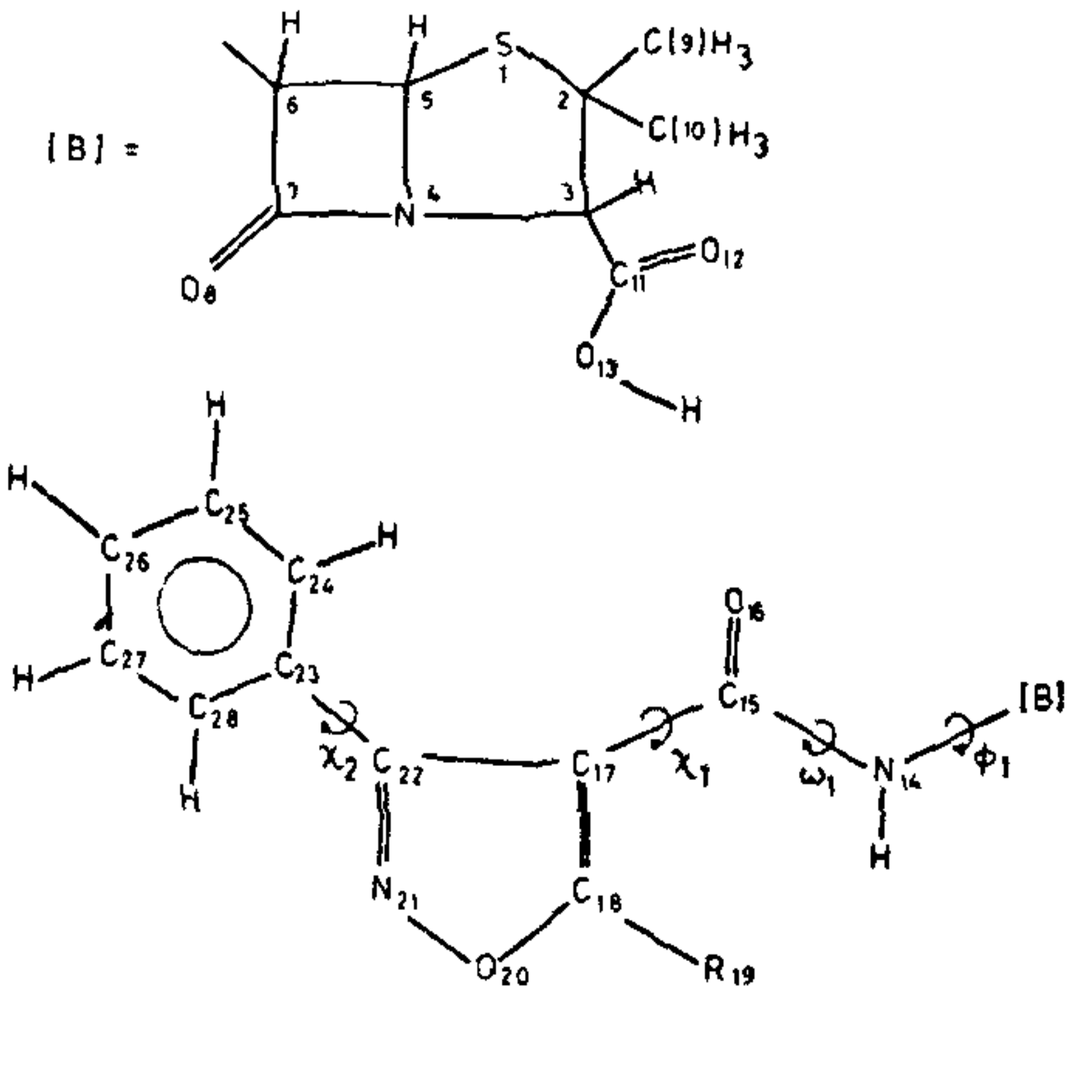
Nomenclature, geometry and fractional charges

The numbering of atoms and dihedral angles of the isoxazolyl penicillins are indicated in Figure 2. The definition of the backbone dihedral angles of the side-chain is as follows:

ϕ_1=0 when the bond C_6-C_7 eclipses the bond $N_{14}-C_{15}$
ω_1=0 when the bond $N_{14}-C_6$ eclipses the bond $C_{15}-C_{17}$
χ_1=0 when N_{14} is *cis* to C_{22}
χ_2=0 when C_{17} is *cis* to C_{24}.

For all the dihedral angles, clockwise rotations were considered as positive. A similar nomenclature was adopted for phenyl penicillins also.

The coordinates of the various penicillins were generated using the crystal structure parameters[7,9-13] and standard[14] bond lengths and bond angles wherever necessary. The nuclear part of these penicillins was kept in the C_3 puckered conformation. The peptide group in the 6 β-side chain was kept in *trans*-planar confirmation (ω_1=180°). The fractional charges on the various atoms of the molecules were obtained using molecular orbital methods [15,16]. The intrinsic torsional barrier for rotations (ϕ_1) about $N_{14}-C_6$ bond was neglected for the reasons cited by



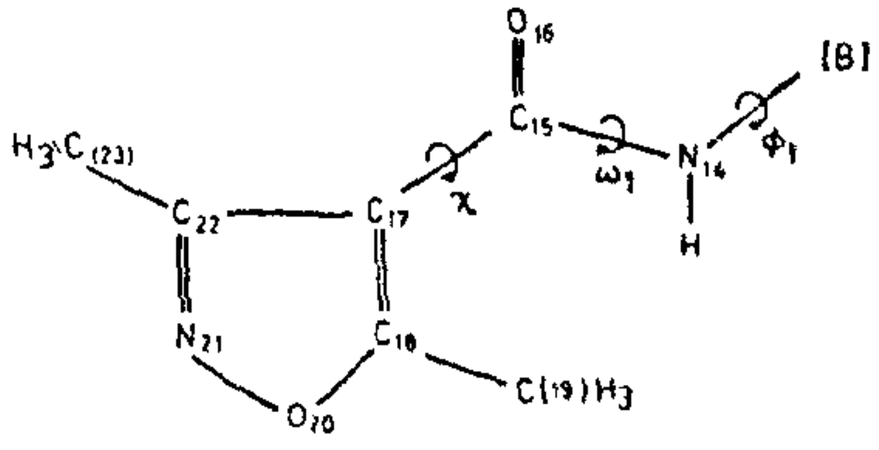


Figure 2. Numbering of atoms and dihedral angles in isoxazolyl penicillins. R_{19} represents a hydrogen atom or a methyl group.

Momany *et al.*[17]. For the determination of torsional energy contributions due to rotations (χ's) in the conjugated groups, the method proposed by Allinger and Sprague was used[18].

Energy calculations

The total potential energy of the molecules was calculated as described earlier[19]. In compounds 1–3, the total energy was minimized with respect to the parameters ϕ_1, χ_1 and χ_2, all being varied simultaneously. About 50 starting conformations were considered for energy minimization from the allowed ranges of χ_1 and χ_2 obtained using contact criteria[20]. For ϕ_1, two values (−90° and 160°) corresponding to the two minima were considered[21]. Combination of these sets of (ϕ_1, χ_1, χ_2) formed the starting conformations for energy minimization. In order to study the relative flexibility of these molecules near the amide-carbonyl in the 6β-side chain, (χ_1, χ_2) energy maps (Figure 3) were also constructed, keeping the dihedral angle ϕ_1 at its favoured value. In compounds 4 and 5, conformational energy maps were obtained as a function of ϕ_1 and χ (Figure 4).

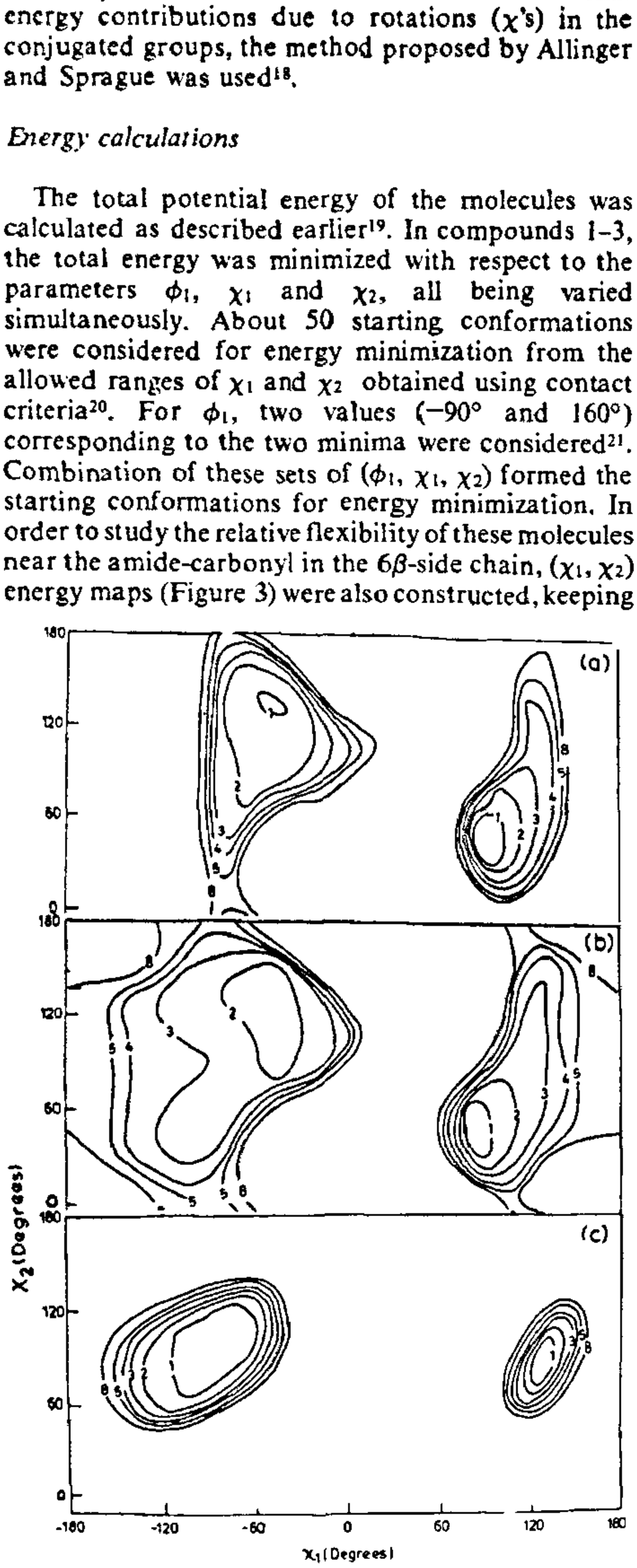


Figure 3. Conformational energy maps for the 6 β-side chain of (**a**) compound 1 (ϕ_1=160°), (**b**) compound 2 (ϕ_1=165°), (**c**) compound 3 (ϕ_1=163°). Numbers on contours indicate energy in kcal/mol. Missing portions are derivable by translation.

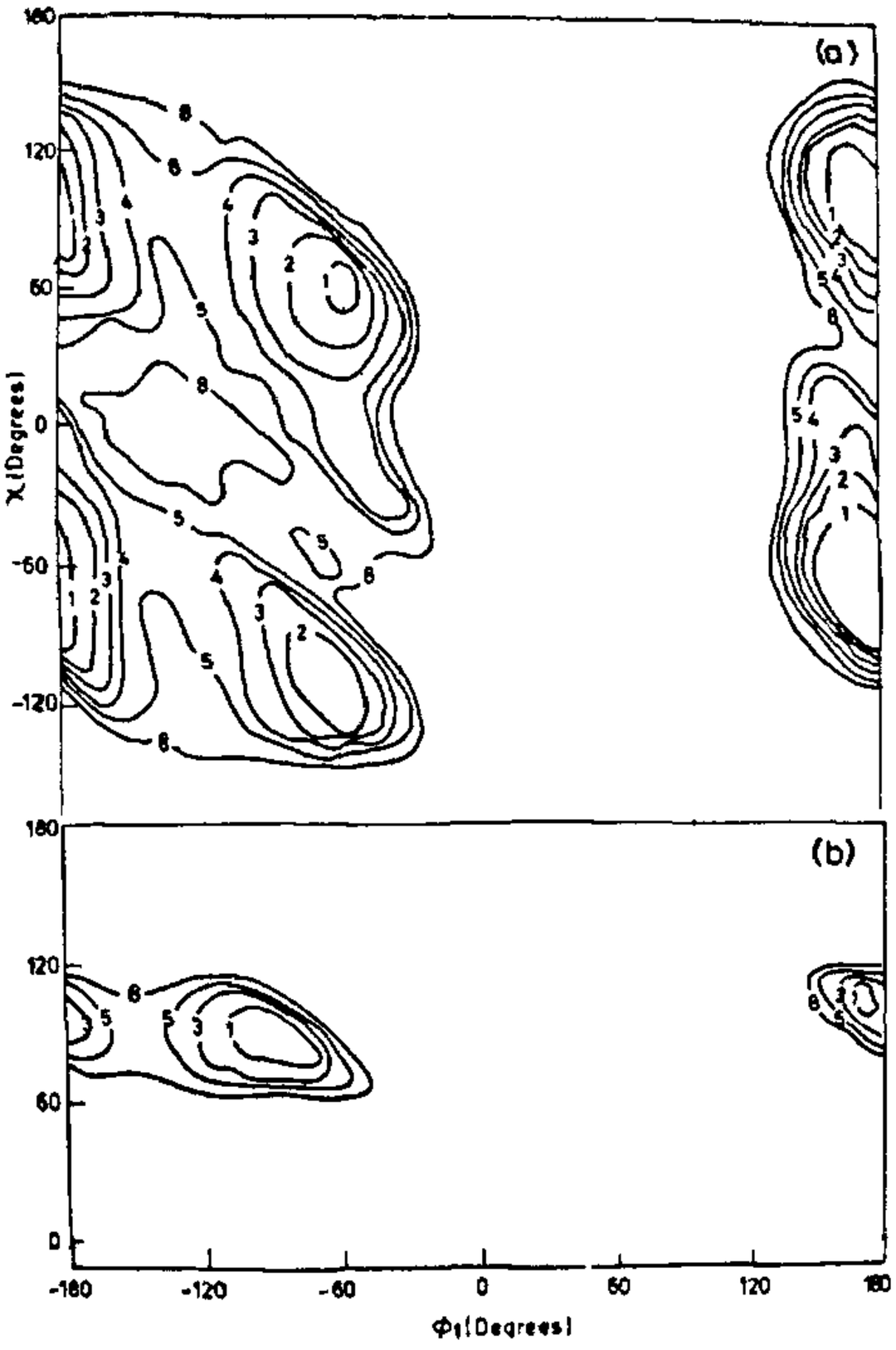


Figure 4. Conformational energy maps for the 6β-side chain of (**a**) compound 4 and (**b**) compound 5. Numbers on contours indicate energy in kcal/mole. in (**b**) missing portions are derivable by translation.

Results and Discussion

Tables 1 to 3 show that a number of conformations are possible for the compounds 1–3. Of these, in the most favoured conformations, the dihedral angle ϕ_1 assumes values around 160°. In the minimum energy conformation of the compounds 4 and 5 also, ϕ_1 favours a value around 160°. This is different from the values assumed by the earlier investigators[7,8] in their energy calculations.

The conformational energy maps(Figures 3a and 3c) obtained for compounds 1 and 3 exhibit well-defined low energy regions separated by relatively higher energy barrier (compared to those[22] of penicillin-G or D-ampicillin). This indicates that the rate of inter-conversion from one conformation to another will be

TABLE 1
Minimum energy conformations of compound 1

ϕ_1	χ_1	χ_2	Relative energy (kcal/mol)
in degrees			
160	100	48	0·00
162	−58	−46	0·90
−105	−56	−39	1·00
−102	44	42	2·04
−92	86	132	3·26
−100	−104	121	3·35
162	130	122	3·89

slow. On the other hand, the energy map (Figure3b) of compound 2 shows that the 6β-side chain of this molecule has relatively greater conformational flexibility. Figure 4a obtained for compound 4 shows that the energy barrier separating the minima is small, indicating greater flexibility for this side chain also. But in compound 5, the allowed (ϕ_1, χ) values are very much restricted (Figure 4b) suggesting that the side chain is highly rigid. These results suggest that the 6β-side chains of A-type penicillins (compounds 1, 3 and 5) are more rigid compared to S-type penicillins (compounds 2 and 4). This is in agreement with the results reported by the earlier investigators[7,8]. The higher flexibility of 6β-side chains of compounds 2 and 4 (S-type) suggest that β-lactamases can easily align them in the active site without spending much of the binding energy for hydrolytic cleavage of β-lactam peptide bond. Therefore in the course of enzyme-penicillin interaction, it is unlikely that these flexible molecules would induce undue conformational changes in the active site of the enzyme.

On the other hand, a lower flexibility (Figures3a, 3c and 4b) of the 6 β-side chain of A-type penicillins may demand more pronounced changes in the active site of the enzyme in the process of binding. Such changes may either lead to weak or a different mode of binding, resulting in a conformation unfavourable for enzymatic action. This may account for the reduced level of hydrolysis of the A-type penicillins by β-lactamases[3,23]. Thus, the rigidity of the 6 β-side chain near the amide carbonyl of these penicillins appears to be responsible for their greatly reduced susceptibility to β-lactamases.

It is also interesting to see from the minimum energy conformations of A-type penicillins (compounds 1 and 5) that the methyl substituent in the aromatic or hetero-aromatic ring protrudes to the left side of the projections (Figures 5a and 6c). In the conformer 2 (table 1) of compound 1, the positions of methyl and phenyl substituents get interchanged and the phenyl substituent protrudes to the left side. Thus, in both the favoured conformers of compound 1, a bulky group is placed on the left side. But in compound 3, there is no such group in its minimum energy conformation (*Figure 5b). However, in the conformer 2 (table 3) of* this compound which has ≈0·23 kcal/mole higher energy, the phenyl substituent protrudes out (figure 5c). These suggest that if hindering by these protruding groups is also important for manifesting the steric effect during binding with β-lactamases, it is done only less effectively in compound 3.

Comparison of the relative rates of hydrolysis of compounds 1 and 3 by β-lactamases from *S. aureus* and *B. cereus*[3,23] shows that compound 3 is hydrolyzed at a slightly higher rate. But the energy maps (Figures 3a and 3c) of these compounds show that the 6 β-side-chain of compound 3 is as rigid as that of compound 1. This suggests that the conformational rigidity of the 6 β-side chain of these compounds alone cannot explain such differences in the rates of hydrolysis. As indicated earlier, the effect of steric hindrance will be brought out more effectively in compound 1 than in compound

TABLE 2
Minimum energy conformations of compound 2

ϕ_1	χ_1	χ_2	Relative energy (kcal/mol)
in degrees			
165	81	52	0·00
−102	−60	−36	1·00
157	−45	−42	1·15
−110	33	55	1·91
156	−95	−139	2·41
160	128	129	2·47
−96	−107	−140	3·39
−109	66	30	3·45
−124	54	44	3·76
−96	111	135	4·00

TABLE 3
Minimum energy conformations of compound 3

ϕ_1	χ_1	χ_2	Relative energy (kcal/mol)
in degrees			
163	121	77	0·00
154	−73	−68	0·23
−103	−91	−79	0·97
−95	64	73	1·46
−82	104	108	1·83
−129	75	81	4·16

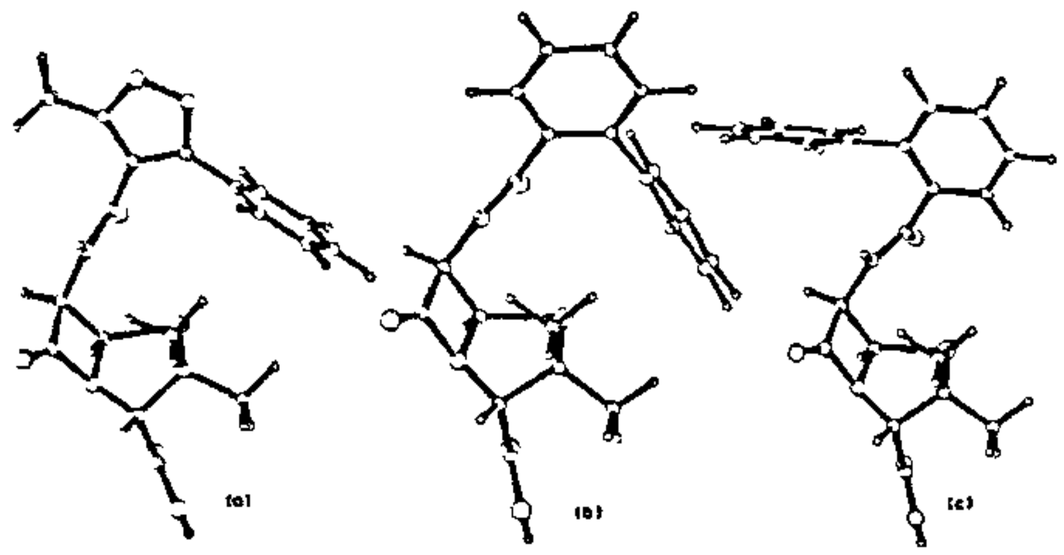


Figure 5. Projections of (**a**) the minimum energy conformers of compound 1 and (**b**) compound 3 and (**c**) the conformer 2 of compound 3.

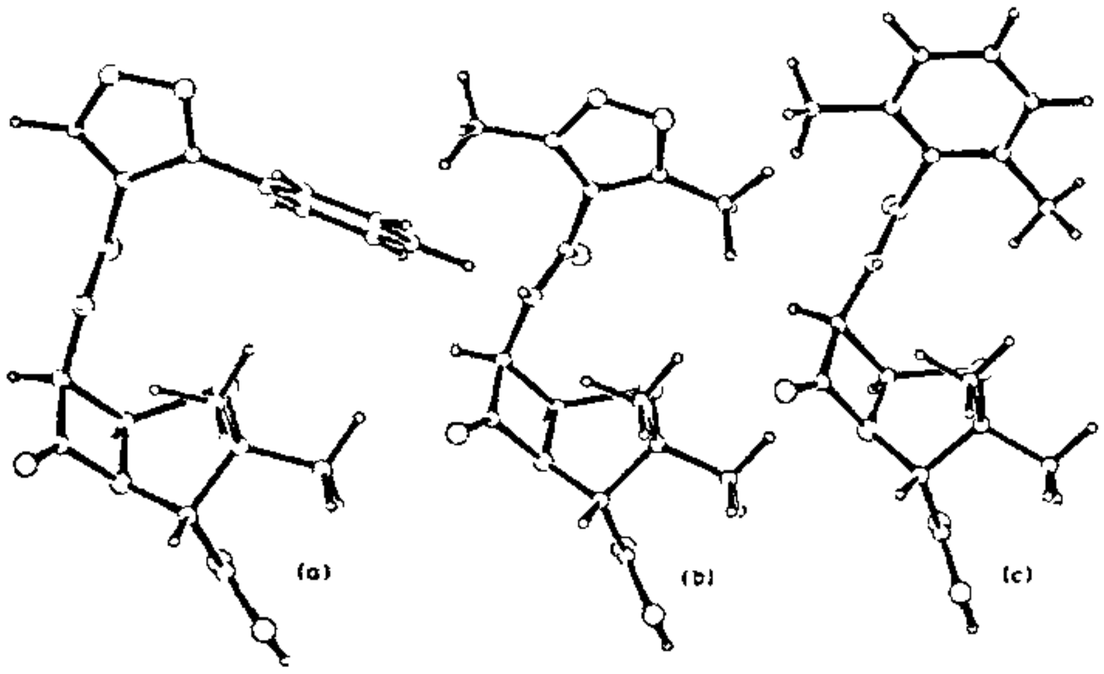


Figure 6. Projections of the minimum energy conformers of (**a**) compound 2, (**b**) compound 4 and (**c**) compound 5.

3. This may explain the differences in the rates of hydrolysis of these compounds by β-lactamases from *S. aureus* and *B. cereus*.

It is interesting to note that the favoured conformations of compounds 1-3 (Figures 5a, 5b and 6a) have close similarity to the compact conformation of penicillin-G[22]. In compounds 4 and 5 also (Figures 6b and 6c) a conformation similar to the compact one is favoured. However, in the latter compounds, a methyl group occupies the place of an orthophenyl group which folds over the bicyclic ring system to give a compact shape. That all these compounds exhibit antibacterial activity against Gram-positive bacteria is consistent with these observations. However, since the ring systems beyond the amide-carbonyl in the 6 β-side chain differ from that in penicillin-G, these differences may lead to lower activity[1]. In Gram-negative bacteria, on the other hand, the anti-bacterial activity depends on both the conformational features and the permeability of the drug molecules through the outer-membrane of the bacteria[22]. Since no information is available about the permeability of these antibiotics, it is not possible to conclude whether the weak antibacterial activity of these drugs against Gram-negative organisms is due to slight differences in the conformational features or permeability or both.

The present studies, in agreement with those of earlier workers, show that the.conformational energy maps of the 6 β-side chains can be used to distinguish β-lactamase-resistant and susceptible penicillins. These studies also suggest that in the resistant penicillins, the degree of resistance to β-lactamases depends both on the rigidity of the 6 β-side chains and the nature and orientation of the substituents beyond the 6 β-amide carbonyl group. The overall shapes of these penicillins also correlate well with their antibactierial properties.

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STEROIDOGENIC POTENTIAL OF THE TESTIS OF *COLUMBA LIVIA DURING* THE PREINCUBATION, INCUBATION AND SQUAB FEEDING PERIODS OF THE REPRODUCTIVE CYCLE

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ABSTRACT

Δ^5-3 β-hydroxysteroid dehydrogenase (Δ^5-3 β-HSDH), 17 β-hydroxysteroid dehydrogenase (17 β-HSDH), 11β-hydroxysteroid dehydrogenase (11β-HSDH), glucose-6-phosphate dehydrogenase (G-6-PDH) and NADH- diaphorase activity was observed in the Leydig cells and the seminiferous epithelium including Sertoli cells in the testis of the pigeon, *Columbia livia* during the preincubation period. During the incubation and squab feeding periods Δ^5-3 β-HSDH, G-6-PDH and NADH-diaphorase activity was observed and there was no activity of 17 β-HSDH and 11 β-HSDH in these cells. These results suggest that the testis of the pigeon during preincubation period actively synthesizes sex steroids while during incubation and squab feeding periods the testis might be relatively inactive in the synthesis of sex steroids as indicated by the absence of 17 β-HSDH and 11 β-HSDH enzyme activity.

INTRODUCTION

IN birds, as in a majority of seasonal breeding vertebrates, the interstitial cells undergo well-defined secretory cycle which involves a rhythmic accumulation and depletion of cholesterol-positive lipoidal material[1-4]. The presence of Δ^5—3β-hydroxysteroid dehydrogenase (Δ^5 — β-HSDH) and 17β-hydroxysteroid dehydrogenase (17β-HSDH) in the Leydig cells and the seminiferous epithelium including the Sertoli cells in the testis of fowl, crow-pheasant, pigeon and hawk has been reported [4-11]. The domestic pigeon, *Columba livia* is known to breed throughout the year[12]. The present work was designed to study whether there were any changes in the intensity of hydroxysteroid dehydrogenase activity during periods of preincubation, incubation and squab feeding to understand the steroidogenic potentiality of the testis of pigeon during the reproductive cycle by histochemical demonstration of Δ^5—3β-HSDH, 17 β-HSDH, 11β-HSDH, G-6-PDH and NADH-diaphorase activity.

MATERIALS AND METHODS

The pigeons were obtained from the pigeon colony maintained in this University. The testes of the adult pigeon during preincubation, incubation and squab feeding were used for this study. The histochemical procedures followed were as described earlier[13].

RESULTS AND DISCUSSION

The Leydig cells and the seminiferous epithelium including the Sertoli cells showed Δ^5—3 β-HSDH (Figure 1), G-6-PDH and NADH-diaphorase activity throughout the reproductive cycle. However, the intensity of enzyme activity was reduced to certain extent during incubation (Figure 2) and squab feeding period (table 1). Further, 17β-HSDH and 11β-HSDH activity was observed in the Leydig cells and seminiferous epithelium including the Sertoli cells during preincubation period only (Figure 3; table 1).

The presence of Δ^5-3 β-HSDH enzyme activity in the Leydig cells and the seminiferous epithelium including Sertoli cells suggests that the testis of *C. livia* is capable of converting Δ^5-3β-HSDH hydroxysteroids to Δ^5-3 ketosteroids. The enchanced Δ^5-3 β-HSDH activity during the preincubation period suggests that the testis is very active in steroidogenesis during this period.

It is well known that the testis of birds synthesizes sex steroids [14-20]. The synthesis of sex steroids involves another enzyme, 17 β-HSDH. This enzyme has been histochemically demonstrated in the testis of fowl[8], crow-pheasant[9] and hawk[11]. The presence of 17 β-HSDH in the Leydig cells and seminiferous epithelium including the Sertoli cells of the testis of pigeon during preincubation period provides an additional evidence for the synthesis of sex steroids. Chan and Pots[2] have reported maximum testosterone