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Conformation Patterns in Penicillins and the Penicillin-Penicillinase Interaction

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SUMMARY

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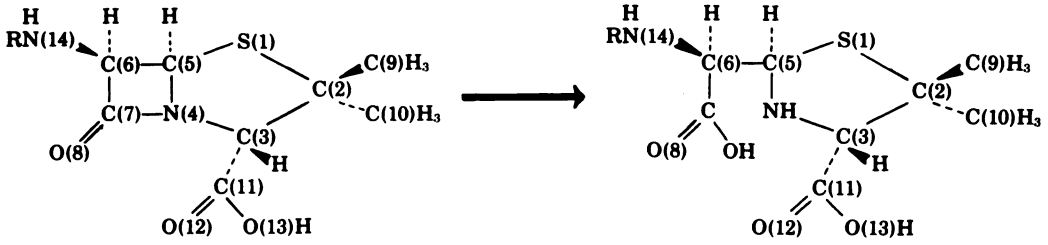
Conformational energy maps have been computed for three penicillin derivatives by the quantum mechanical method of perturbative configuration interaction using localized orbitals (PCILO). It was found that rotation about the C—N bond to the acyl side chain is quite hindered, with a preferred conformation that is practically independent of the side chain. However, the energetics of rotation about axes in the side chain depend greatly on its structure. The computed patterns permit two types of penicillin derivatives to be distinguished. This differentiation is parallel to the experimental classification of penicillins as S- or A-type by the mode of their interaction with penicillinase. An interpretation of this parallelism is proposed.

INTRODUCTION

The relationship between the chemical structure of penicillin antibiotics and their antibacterial activity has been studied extensively. 6-Aminopenicillanic acid (I, R = H) and those of its derivatives that carry a non-acyl substituent at N(14) are relatively inactive; acyl derivatives are much more active. All penicillins, including 6-aminopenicillanic acid, are susceptible to inactivation by penicillinase-producing bacteria. Penicillinases (penicillin β-lactamase, EC 3.5.2.6) catalyze the hydrolytic cleavage of the β-lactam ring [at N(4)—C(7)] to yield the biologically inactive penicilloic acids (II).



I *II*

Citri classified penicillins by the nature of their interaction with the penicillinase of *Bacillus cereus* 569/H (1, 2): S-type penicillins are "good" substrates that undergo hydrolysis more rapidly than 6-aminopenicillanic acid; A-type derivatives are "poor" and more resistant to enzymatic hydrolysis. The distinction between the two groups goes further: (a) A-type derivatives render the penicillinase highly susceptible to in-

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activating agents, while S-type penicillins stabilize the enzyme (1, 2); (b) the binding of homologous antibodies to penicillinase stimulates its activity toward A-type penicillins only (3); (c) the encounter of an A-type derivative with the enzyme triggers transient modifications of its catalytic parameters (K_m, k_{cat}), which are not observed with S-type substrates (4, 5); (d) numerous reports describe a progressive, irreversible inactivation of penicillinases from various sources upon mere incubation with an A-type penicillin (6–12). These contrasts in behavior have been interpreted as reflecting contrasting conformational changes induced in the enzyme by penicillins of the two groups (2, 5). By this interpretation, the penicillin side chain (R in Structure I), although remote from the bond undergoing cleavage, is decisive in determining the conformational response of the enzyme. It has been recognized that penicillins of type S induce in the enzyme a compact conformation, favorable to the catalytic reaction. By contrast, A-type penicillins induce an open conformation, which is unfavorable (2). It stands to reason that such a divergence is based on some inherent differences between the conformational characteristics of S- and A-type penicillins. Yet no conformational studies pertinent to this question have been described to date, and available data are not sufficient to link structural detail with the distinction between types.

We have studied theoretically conformational manifolds of representative compounds in the two groups. It seems that S-type penicillins are rather flexible, while the conformations of A-type derivatives are restricted to a number of deep potential wells.

In the present communication, we have sought to correlate the structural features of the side chain with the nature of the penicillin-penicillinase interaction. As both the substrate molecules and the enzymatic active site change in the course of binding and catalysis (induced-fit theory), one can visualize a reciprocal effect, wherein a slight change in one might require a considerable alteration in the other. We approach the problem through a conformational study of the substrates. Such an approach is justifiable in any enzyme-substrate system, but seems particularly appropriate here, because the induced change is appreciable (5) and the enzyme is "hysteretic" (13) (i.e., it responds only sluggishly to changes in its environment).

CALCULATION

Potential surfaces were computed by perturbative configuration interaction using localized orbitals (PCILO) (14), a rapid, semiempirical, "all-valence-electron" method that has been shown to perform reliably in the conformational analysis of biological molecules (15), including peptide models of penicillins (16). Calculations were performed on benzylpenicillin (S-type), oxacillin, and "difloxacillin" (both A-type) (see Table 1). The last of these molecules was taken to represent the halogenated isoxazolylpenicillins (e.g., cloxacillin, flucloxacillin, and dicloxacillin), which, because of computer program limitations, could not be used for calculation themselves. For similar reasons it was impossible to compute the energy of the whole molecule. Thus, to examine the energy dependence on the rotation about the N(14)—C(6) bond, the calculations were carried out on derivatives with much shorter side chains, where R = HCO— and CH_3CO— (see Table 1). To study the rotations about axes within the side chain, the thiazolidine ring in the penicillanic acid moiety had to be excluded in the structures investigated and replaced by 2 hydrogens, simulating C(5) and N(4), as in Fig. 1. The permissibility of this truncation will be discussed below.

The various conformations in each of the three penicillins were obtained by rotating the rigid parts of the molecule about the single bonds. An initial conformation was defined by placing in a plane the exocyclic amide bond, and the aromatic ring(s) in the side chain (see Fig. 1). Also, in the starting conformation the atoms C(15)—N(14)—C(6)—C(7) were placed in one plane. Geometrical data on penicillanic acid (17) and its derivatives (17–20) were supplemented, when necessary, by "standard" (21) interatomic distances and interbond angles. They were assumed not to change significantly on going from one conformation to another.

Also, the benzene ring was taken to be symmetrical, and substituents on unsaturated rings were assumed to bisect angles.

To span the conformational manifold, the terminal aromatic ring was locked in the initial plane, and internal rotations were performed about three axes (Fig. 1): *A*, terminal aromatic ring to residue (torsional angle α); *B*, residue to CO of the exocyclic amide group (torsional angle β); *C*, N(14)—C(6) bond (torsional angle γ). To visualize the sense of rotation, for instance, about axis *A*, the phenyl ring is held stationary while the second unit is rotated with the rest of the molecule, through angle α counterclockwisce, looking from the phenyl toward the rotating unit. Each conformation is thus definable in terms of three internal rotations: α, β, and γ. Rotations were initially performed in steps of 10°; a narrower grid was then taken, in the vicinity of energy minima and other features of interest.

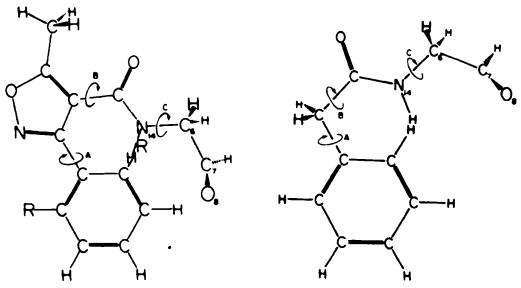


FIG. 1. *Initial conformation, rotational axes (A, B, C), and senses of rotation, counterclockwise when looking from the stationary toward the rotating moiety*

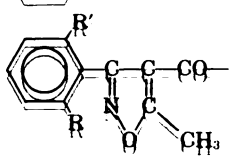
The aromatic ring(s) in the side chain and the exocyclic amide are coplanar, while C(7) is placed in the C(15)—N(14)—C(6) plane. The thiazolidine ring was omitted in the calculations, in which C(5) and N(4) were replaced by hydrogens. The starting conformation of the isoxazolylpenicillins (left) is obviously hypothetical, because N(14)—H practically overlaps the *ortho* substituents on the phenyl.

RESULTS AND DISCUSSION

The energy dependence on the rotational state about axis *C* (C—N bond to the acyl side chain) was calculated for methylpenicillin (see Table 1). Initially the C—N bond of the exocyclic amide and bond C(6)—C(7) of the β-lactam were placed in one plane. Then the amide group was locked in its plane while the twist angle γ was varied. In Fig. 2 the total conformational energy $E_T = E_N$ (core repulsion) + E_E (electronic stabilization) is plotted against the rotational angle γ. It shows that the energy barrier for rotation about axis *C*, as previously reported (22), is quite high; yet the allowed range is extensive, as for other penicillins (23). The energy profiles calculated for R ≡ $HCO-$ and CH_3CO- (methylpenicillin) were identical, and the energy barriers originated from hindrance by the carbonyl and NH of the exocyclic amide group. In terms of angle γ [torsional angle C(15)—

TABLE 1

Side chain structure and type of representative penicillins

Penicillin derivative	Side chain[a]	Type as substrate[b]
6-Aminopenicillanic acid	H	
Benzylpenicillin	$C_6H_5CH_2CO-$	S
Oxacillin (R ≡ H, R′ ≡ H)		A
Cloxacillin (R ≡ H, R′ ≡ Cl)		A
Dicloxacillin (R ≡ Cl, R′ ≡ Cl)		A
Flucloxacillin (R ≡ Cl, R′ ≡ F)		A
"Difloxacillin"[c] (R ≡ F, R′ ≡ F)		A
Methylpenicillin	CH_3CO-	S

[a] R in Structure I.

[b] By mode of interaction with penicillinase (see the text).

[c] Model compound, simulating the halogenated isoxazolylpenicillins in our calculation (see the text).

N(14)—C(6)—C(7)] the penicillin molecule is allowed to adopt almost any rotational conformation in the range $10° > \gamma > 130°$. The minima occur at 0°, 160°, and 250°. The last minimum is very close to that found for the S-type penicillins in the solid state (18), and the first minimum is similar to that observed ($\gamma = 5°$) for oxacillin (24). The different conformations observed for the various penicillins in the solid state [dicloxacillin itself adopts two different conformations (24)] may be due to crystal-packing forces, which were disregarded in our calculations. These results provide some support to our approximated model, in which the rotations inside the side chain, extending beyond the exocyclic amide bond, were taken as independent of rotations about the N(14)—C(6) bond.

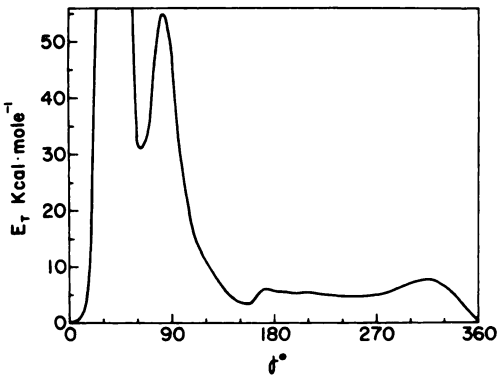


FIG. 2. *Rotation about axis C(C—N bond to the acyl side chain) in methylpenicillin*

$E_T = E_N$ (core repulsion) + E_E (all-valence-electron electronic energy) in kilocalories per mole above the global minimum.

The computed response of the energy toward rotation about axis *C* was found to be the same for benzylpenicillin, oxacillin, and "difloxacillin," and independent of the rotational state about axes *A* and *B*. Probably this particularity of all derivatives studied cannot have to do with the distinction between types S and A.

Conformational energy maps of benzylpenicillin (S-type) and oxacillin (A-type), for rotations about axes *A* and *B* at $\gamma = 250°$, are given in Fig. 3 (missing strips are derivable by mirror-imaging). E_T contours are plotted above the the global minimum which is taken as 0. It may be seen that the maps are quite dissimilar, which may reflect a fundamental difference between the two types of molecules.

In benzylpenicillin (Fig. 3, top), the preferred conformations ($\alpha = 0°$, $\beta = 90°$, and

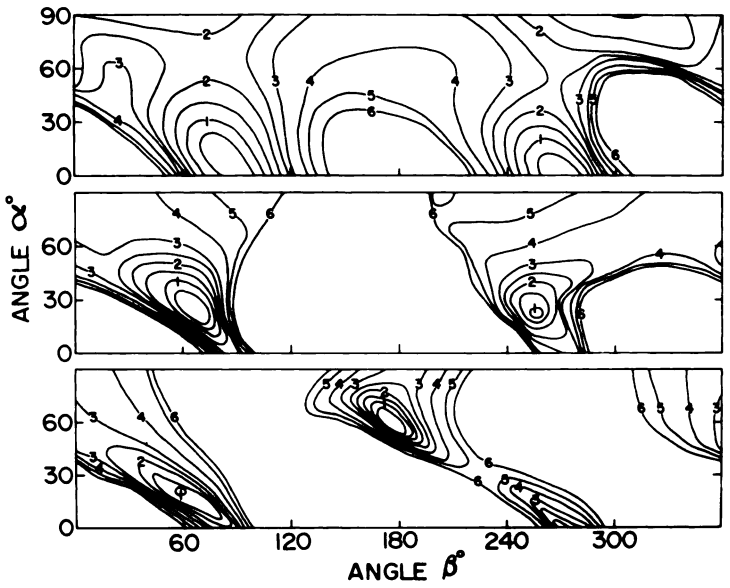


FIG. 3. *Conformational energy maps at $\gamma = 250°$ for $0° \leq \alpha \leq 90°$ and $0° \leq \beta \leq 360°$*

Energy contours (at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 kcal mole^{-1}) above the deepest minimum. Top, benzylpenicillin; center, oxacillin; bottom, "difloxacillin." The 0° angles for axes *A* and *B* are the planar structures depicted in Fig. 1.

the energetically almost equivalent $\alpha = 0°$, $\beta = 270°$) are such that the aromatic ring is perpendicular to the plane of the exocyclic amide. This perpendicularity has been observed in the crystal (18), where $\alpha = 90°$ and $\beta = 0°$; by our calculation, the energy difference between (0°, 90°) and (90°, 0°) is 2 kcal or less. With respect to axis *B*, the minima are deep. [We did not find it necessary to let the geometrical parameters relax (25), as our interest lies with the general pattern, not with the precise location and magnitude of the extrema.] However, rotations about *A* (at $\beta \sim 90°$ and $\beta \sim 270°$) are not predicted to be seriously restricted; rather, they correspond to two channels across the energy map, with a maximum height of about 2 kcal. These two channels are separated by a barrier of about 3 kcal.

In other words, apart from a small region of high energy (corresponding, perhaps, to the area delimited by the 6-kcal contour in Fig. 3, top), benzylpenicillin may be made to adopt any rotational state about axes *A* and *B*. Reciprocally, as the geometrical requirements of this molecule are not severe, its interaction with the enzyme is not expected to induce undue conformational changes in the latter.

The conformational energy map of oxacillin (Fig. 3, center), has two definite potential wells, with minima at $\alpha = 22°$ and $\beta = 65°$ and at $\alpha = 30°$ and $\beta = 250°$. These values can be compared with the recently reported solid-state structure of oxacillin (24), where $\alpha = 32°$ and $\beta = 58°$. In the calculated preferred conformation, the dihedral angle between the phenyl ring and the plane of the exocyclic amide is 61°—identical with the experimental value (24). However, and in contrast with benzylpenicillin, this disposition is achieved by rotation about both axes *A* and *B*. Transitions from one preferred conformation to another are restricted by barriers that are higher than before. Also, high-energy zones are extensive and, at room temperature, an appreciable conformational domain can be considered closed. Thus, although geometrical readjustments should not be ruled out, such processes require coordinated rotation about both axes, involve higher energies, and are confined to narrower ranges than in benzylpenicillin.

Cloxacillin, flucloxacillin, and dicloxacillin (A-type, Table 1) are known to affect the enzyme even more severely than oxacillin (5, 10, 12). Our theoretical counterpart is the energy map of "difloxacillin" (Fig. 3, bottom). In general form, it resembles the map for oxacillin, with optimal conformations at $\alpha = 60°$ and $\beta = 170°$ and at $\alpha = 20°$ and $\beta = 60°$; benzene rotation about *A* demands a concurrent rotation about axis *B*. Here, however, this requirement is more restrictive, for potential wells are steeper and crests more extensive. The preferred conformations can again be compared with the experimental values reported for the two structures of dicloxacillin in the single crystal, where $\alpha = 81°$ and $\beta = 195°$, $\alpha = 82°$ and $\beta = 186°$ (24).

In the course of enzyme-substrate interaction, the conformations of both enzyme and substrate are modified. A reciprocal effect may then be envisaged, wherein a lower flexibility in the substrate demands more pronounced changes in the enzyme. The conformational map of a given substrate should therefore furnish a clue to the extent of modifications imposed on the enzyme. Such an approach is particularly promising in the present case, in which the substrate-induced alteration of catalytic parameters is actually measurable with A-type penicillins: interaction brings about a hysteretic response of the enzyme (13), which acquires "new" values of affinity and catalytic activity toward its substrate (5). Oxacillin (A-type) is computed as relatively rigid; experimentally it exerts a large effect on the catalytic parameters. "Difloxacillin" is predicted to be even more rigid, in line with the extreme effect of the halogenated isoxazolylpenicillins. By contrast, in benzylpenicillin (a representative S-type), flexibility is high, and, indeed, the effect on the enzyme is barely detectable.

The difference in mode of behavior toward penicillinase between A- and S-type penicillins may be linked in part with the different sizes of the side chains. Yet it is not likely that the mere difference in size, between the 2 hydrogens in oxacillin and the 2 chlorines in dicloxacillin, would ac-

count for the marked accentuation of the A-type characteristics in the latter. Furthermore, A-type behavior is not limited to isoxazolylpenicillins. Methicillin [6-(2,6-dimethoxybenzamido)penicillanic acid] is an A-type (7, 26) derivative, although its side chain, which lacks the 5-membered heterocycle, is about the same size as that of benzylpenicillin. We therefore consider the size effect as partial at most. Apparently our results suggest that the considerations above are general, and can be extended to penicillins of other structures.

CONCLUSION

The penicillinase-penicillin system is particularly suitable for studying enzyme-substrate interactions, because of the simplicity of the enzyme (monomeric, single substrate, single active site), its "hysteretic" character, and the clear-cut experimental distinction between S- and A-type substrates.

Theoretically, energy maps distinguish benzylpenicillin, in which phenyl rotation is practically unhindered, from A-type compounds. In the latter there are definite potential wells, energy barriers are high, and conformational changes require simultaneous rotation about more than one axis. Also, the range of inaccessible structures is more extensive.

If this parallelism proves to be general for penicillins, the theoretical tool can become useful in predicting the type of a new derivative, that is, its mode of interaction with penicillinase. In any case, knowledge of the conformation patterns of penicillins should contribute to further elucidation of the dynamics of their recognition and interaction in enzyme-substrate systems.

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