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Conformational Analogy between β-Lactam Antibiotics and Tetrahedral Transition States of a Dipeptide

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The three-dimensional structures of various penicillins and cephalosporins are compared to the spatial characteristics of glycylglycine and the tetrahedral adducts formed when a nucleophile attaches to the amide carbonyl carbon of this dipeptide. The dipeptide is taken to model the D-alanyl-D-alanine terminus of the precursors of bacterial cell-wall peptidoglycan cross-links. Least-squares fitting shows that the spatial match between the dipeptide and the antibiotic depends on the thiazolidine or dihydrothiazine ring conformation, as well as the conformation of the dipeptide. In general, the tetrahedral adducts fit somewhat better than the parent dipeptide. A previously unobserved 3-cephem conformer is found by molecular mechanics calculations to be less stable than the usual crystallographically observed conformer.

The final stage in peptidoglycan biosynthesis of bacterial cell walls involves cross-linking between the peptide side chains of glycan polymers.[1] The nascent pentapeptide side chains terminate in D-alanyl-D-alanine. Cross-linking in the peptidoglycan is regulated by various transpeptidases, carboxypeptidases, and endopeptidases. The former two enzymatic activities involve rupture of the D-Ala–D-Ala bond, presumably by a nucleophilic attack mechanism involving a tetrahedral adduct. The β-lactam antibiotics are known to upset the balance of the various cell-wall enzymatic activities[1] and thereby exert their antibacterial action.

Using molecular models, it was evident to earlier workers[2,3] that a structural analogy existed between the penicillins and what seemed to be possible transition-state (TS) structures for the scission of the peptide bond in D-Ala–D-Ala. Tipper and Strominger[2] described a TS structure only vaguely in terms of a nonplanar amide nitrogen. Lee[3] described a TS in terms of a severe twisting about the amide C–N bond so that the bond is weakened and the amide nitrogen is tetrahedrally hybridized.

Recently, some preliminary but detailed structural information on the transition states of a dipeptide became available from molecular-orbital calculations.[4] These calculations, for practical reasons, used glycylglycine as a model of D-Ala–D-Ala. A simple nucleophile, OH^-, was allowed to approach the peptide carbonyl carbon on either the α or β face. Produced were two transition intermediates with tetrahedral hybridization at the reaction center and slight pyramidal hybridization at the amide nitrogen. For convenience, they will be referred to as α-face TS and β-face TS. Although these structures probably represent energy minima on the gas-phase reaction surface, it is not known whether they are intermediates or TS's on a condensed-phase reaction surface.[4,5] It was visually apparent from molecular-structure drawings that for one pertinent dipeptide conformation the α-face TS was closer in certain spatial features to a penicillin G structure than were either the β-face TS or the Gly-Gly reactant.[4]

The purpose of this disquisition is to give a more quantitative and thorough comparison of Gly-Gly structures to β-lactam antibiotic structures. These include not only penicillin G but also penicillins with other thiazolidine conformations and cephalosporins with two possible conformations of the dihydrothiazine ring. In order to make the comparisons, a least-squares fitting of dipeptide

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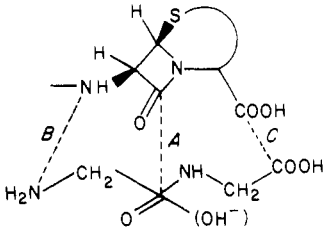
models and the antibiotics will be carried out, and several conformations of the dipeptide models will be calculated.

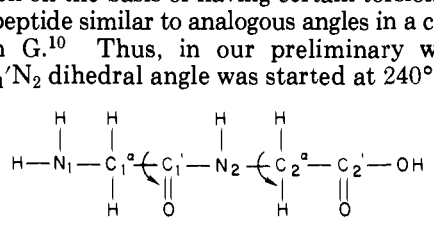
Three atoms of the dipeptide models will be superimposed as well as possible with comparable atoms in the antibiotics. The peptide carbonyl carbon of Gly-Gly will be matched with the β-lactam carbonyl carbon $C_{7(8)}$ of the penicillins (cephalosporins). These atoms are obviously important because they represent reaction centers when the substrate or antibiotic is bound to the cell-wall enzymes. The distance between these two atoms is called *A*. The second distance, *B*, is from the amino nitrogen of Gly-Gly to the 6(7)-amido nitrogen $N_{6\beta(7\beta)}$ of the penicillins (cephalosporins). The 6(7)-acylamino side chain of the antibiotics is no longer regarded as an absolutely essential feature for antibacterial activity. But in order to compensate for the lack of this structural feature, the β-lactam must be highly reactive, as in thienamycin and penem, or have other special structural features.[6] For regular penicillins and cephalosporins, the 6(7) side chain does seem to be necessary, and hence use of distance *B* is relevant. The third distance, *C*, is from the terminal carboxyl carbon to the carbon of the 3(4)-carboxyl group of penicillins (cephalosporins). The 3(4)-COOH group or a bioisosteric group[7] is essential for antibacterial activity, even in the newer β-lactams.[6] The carboxyl carbon $C_{3\alpha(4')}$ is used rather than one of the carboxyl oxygens because the latter atoms can be variously aligned for hydrogen bonding by rotation about the C_3–$C_{3\alpha}$ (C_4–$C_{4'}$) bond. Also, it has been pointed out before[8] that the carboxyl group position is quite variable between penicillins and cephalosporins (especially when penicillin G is used as the representative penicillin[8]), so presumably there is some freedom of movement of whatever hydrogen-bonding functionality exists in the receptor sites.

The three distances, *A*, *B*, and *C*, are shown in the drawing



The sum, $D^2 = A^2 + B^2 + C^2$, is minimized by moving the two molecules with respect to each other in terms of atomic coordinates in a computer.

In our earlier work,[4] the MINDO/3 molecular orbital method[9] was employed to arrive at the optimized molecular geometries (atomic coordinates) of Gly-Gly and its two TS's. The starting conformation for those MO calculations was chosen on the basis of having certain torsional angles in the dipeptide similar to analogous angles in a crystalline penicillin G.[10] Thus, in our preliminary work, the $N_1C_1^{\alpha}$–$C_1'N_2$ dihedral angle was started at 240°, and the



$C_1'N_2$–$C_2^{\alpha}C_2'$ dihedral angle was started at 60°. Optimization in the MINDO/3 framework led to a potential energy minimum at 236° for the former dihedral angle and 68° for the latter in Gly-Gly and to similar values in the TS's.[4] These angles represent a minimum on the multidimensional conformational energy surface that is closest to the starting conformation.

Table I. MINDO/3 Heats of Formation, in kcal/mol, of Optimized Molecular Geometries of Four Conformers with $C_1'N_2$–$C_2^{\alpha}C_2'$ Dihedral Angles[a] as Indicated

starting value	50°[c]	60°[b]	105°[c]	140°[c]
Gly-Gly	−155.3	−155.5	−154.8	−155.3
	53°	68°	105°	141°
α-face TS	−208.8	−209.5	−209.6	−209.7
	53°	84°	106°	131°
β-face TS	−209.6	−209.7	−210.0	−209.8
	63°	83°	107°	139°

[a] The dihedral angle is defined looking along the internuclear axis from N_2 to C_2^{α} and measuring clockwise from the $C_1'N_2C_2^{\alpha}$ plane to the $N_2C_2^{\alpha}C_2'$ plane. [b] Data from ref 4 and obtained using the procedure described therein. [c] Other input dihedral angles, as well as the input bond lengths and angles, were the optimized values of each of the three models from ref 4. All variables including the starting dihedral angle were optimized. Calculation of the nine new species took a total of about 59 CPU min on the IBM 370/168.

The NC–CN dihedral angle in the β-lactam antibiotics that is analogous to the $N_1C_1^{\alpha}$–$C_1'N_2$ is fairly well set by the rigidity of the β-lactam ring. However, the other torsional angle (analogous to $C_1'N_2$–$C_2^{\alpha}C_2'$) is relatively free to assume various values due to the various conformations either observed or conceivable for the thiazolidine and dihydrothiazine rings of penicillins and cephalosporins, respectively. The rotational potential about the N_2–C_2^{α} bond of the parent dipeptide nominally has a sixfold barrier because N_2 is sp^2 hybridized. In the TS's, an sp^3 nitrogen would correspond to a threefold barrier. In either case, rotation about the N_2–C_2^{α} bond is expected to be quite easy. Hence, it is imperative to examine at least several conformational possibilities. Taking the molecular geometries obtained previously,[4] Gly-Gly and its two TS's were reoptimized after setting the $C_1'N_2$–$C_2^{\alpha}C_2'$ torsional angle initially near 50°, 105°, and 140°. These three values were suggested by visually overlaying[11,12] Dreiding models of the three dipeptide structures and β-lactam antibiotic structures.

For the MO calculations, the MINDO/3 method was again used. Other more accurate MO methods capable of treating molecules as large as dipeptides now exist, such as PRDDO[13] and MNDO,[14] but the MINDO/3 computer program was selected because of its ready availability, its ability to automatically optimize all geometrical variables, and its greater speed.

Results and Discussion

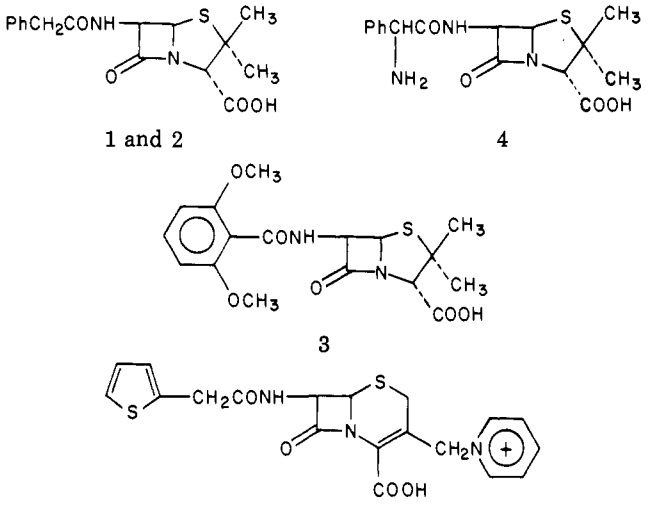
In Table I are the calculated energies of four conformers each of Gly-Gly, the α-face TS, and the β-face TS. Also tabulated are the optimized $C_1'N_2$–$C_2^{\alpha}C_2'$ torsional angles. It can be seen that the molecules are, indeed, predicted to be quite flexible. The relative energies of the four conformers differ by no more than 1 kcal/mol. Whereas MINDO/3 cannot be regarded as the best MO method for calculating rotational potentials, the data in Table I suffice to show that the dipeptide models can assume a wide range of conformations with respect to rotation about the N_2–C_2^{α} bond. Other calculations on other dipeptide models of glycine and alanine have also shown some freedom of rotation at this bond.[15]

The six β-lactam antibiotics used for comparison to the dipeptide models are shown in Chart I. The first is based on the refined X-ray diffraction coordinates[10] of potassium penicillin G (1). 2 is a hypothetical flipped form of penicillin G which has the same bond lengths and angles as 1 but has the thiazolidine ring in a 2β equatorial, 3α equatorial conformation,[16] rather than the 2β axial, 3α axial

Table II. Least-Squares Minimized Values of $D^2 = A^2 + B^2 + C^2$, in A^2, from Fitting β-Lactam Antibiotic Structures and Glycylglycine Structures with $C_1'N_2$–$C_2{}^{\alpha}C_2'$ Dihedral Angles as Indicated

	Gly-Gly				α-face TS				β-face TS			
β-lactam	53°	68°	105°	141°	53°	84°	106°	131°	63°	83°	107°	139°
1	0.94	1.04	0.59	0.26	0.05	0.01	0.00	0.02	0.18	0.15	0.15	0.24
2	0.45	0.53	0.25	0.09	0.12	0.05	0.11	0.20	0.01	0.02	0.05	0.13
3	0.43	0.48	0.18	0.03	0.22	0.08	0.13	0.23	0.03	0.01	0.01	0.05
4	0.48	0.54	0.23	0.06	0.14	0.04	0.09	0.17	0.02	0.01	0.02	0.08
5	0.13	0.19	0.16	0.22	0.44	0.37	0.52	0.69	0.10	0.16	0.26	0.37
6	0.26	0.34	0.22	0.18	0.24	0.20	0.32	0.45	0.04	0.09	0.18	0.30

Chart I

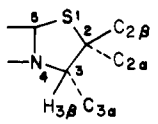


1 and 2 4

3

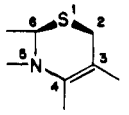
5 and 6

as in the crystal.[10] The methyl ester of methicillin (**3**), which has recently been reported in a careful, well executed crystallographic study,[17] has 2β-CH_3 and 3α-$COOCH_3$ both equatorial.

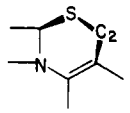


The final penicillin, **4**, is the anhydrate form of ampicillin, which has C_2 out of the plane of the other atoms in the five-membered thiazolidine ring.[18] The ring is more nearly 2β and 3α equatorial as in **2** and **3**, rather than axial as in **1**. The thiazolidine ring is thus observed in the crystalline state in two main conformations and many variations between these extremes. On the other hand, an NMR study[19] on several penicillins indicates that only a single conformation (2β equatorial, 3α equatorial) exists in solution. One is led to regard the thiazolidine ring as fairly flexible, so that it can easily adopt whatever conformation may be energetically advantageous under the conditions of the crystalline environment or, probably also, of the environment of the receptor sites in bacterial cell-wall enzymes.

Two cephalosporin structures are included. The well-known cephaloridine (**5**) X-ray study[20] showed that the dihydrothiazine ring has the sulfur atom out of the plane in which the other five atoms roughly lie.



All other crystallographic determinations of Δ^3-cephalosporins also indicate this conformation.[8,21] The N_5C_6–S_1C_2 dihedral angle in **5** is observed to be 55°. Dreiding models suggest the possibility that the dihydrothiazine ring can be flipped (with moderate difficulty) into a second conformation with C_2 "up" such that the N_5C_6–S_1C_2 dihedral angle is near −30°.



This new conformer was investigated using molecular mechanics calculations.[22] The new conformer is 2–3 kcal/mol less stable than the crystallographically observed one, and there is an energy barrier of approximately 6 kcal/mol over which the observed conformer must go in order to reach the new conformer. The relative instability of the new conformer and the high barrier to reaching it provide an explanation as to why it has not yet been observed. Atomic coordinates for a cephalosporin with the new conformation were obtained by building onto the molecular mechanics dihydrothiazine ring the other atoms using bond lengths and angles from **5**. Hence, the atoms for calculating distances *A* and *B* are identical to those in **5**, whereas the 4-carboxyl carbon is moved about 0.5 Å from its position in **5**. The new cephalosporin is designated **6**.

The least-squares fitting results are given in Table II. The fit achieved for the α-face and β-face TS's is somewhat better (lower D^2) than for Gly-Gly itself. Although further refinement of the dipeptide geometries would be desirable, the present findings lend support to the idea[2] that the β-lactam antibiotics can be acting as transition-state analogues. In other words, the antibiotics may be able to inhibit bacterial cell-wall enzymes by structurally mimicking one of the early geometries in the presumed reaction path for cleavage of the peptide bond of D-Ala-D-Ala.

The dimensions and functionalities of the receptor sites can apparently accommodate either the antibiotics or the natural pentapeptide. Since the antibiotics display a certain range of dimensions[8,23] involving some of the key atoms, the functionalities around the laniary pockets of the cell-wall enzymes may adjust position[24] in binding to substrate or inhibitor. In fact, the flexibility[25] of the dipeptide model (Table I) and the ability of the antibiotics to match the parent dipeptide not too poorly (Table II) suggest that quite a few different molecules that bind to D-Ala-D-Ala will also have an affinity for penicillins and cephalosporins and vice versa.

The data of Table II do not allow a generalization to be made as to whether the α or β face of the natural peptide substrate is approached by a nucleophilic group at the enzyme receptor sites. The α-face TS shows greater similarity than the β-face TS to penicillin G (**1**) as reported previously.[4] For penicillins with a thiazolidine conformation near 2β equatorial, 3α equatorial and for cephalosporins, the β-face TS model fits more closely. However, most of the available (albeit indirect) experimental evidence[26] has been interpretable in terms of the idea that

the α face of β-lactam antibiotics is the one toward which a nucleophile must approach in order to form a tetrahedral adduct and subsequently rupture the β-lactam ring.

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(11) The overlaying was done on the basis of matching the three pairs of atoms involved in distances *A*, *B*, and *C*. Other dihedral angles are suggested if the overlaying is done with other atoms of the backbone from N_1 to C_2' of the dipeptide and $N_{6\beta(7\beta)}$ to $C_{3\alpha(4')}$ of the penicillins (cephalosporins).

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(23) Intramolecular interatomic distances in **1**–**5** are in the following ranges: $N_{6\beta(7\beta)}\cdots C_{7(8)}$, 2.49–2.57 Å; $N_{6\beta(7\beta)}\cdots C_{3\alpha(4')}$,

5.47–5.80 Å; $C_{7(8)}\cdots C_{3\alpha(4')}$, 3.11–3.68 Å; $C_{7\beta(8\beta)}\cdots C_{3\alpha(4')}$, 3.20–4.26 Å; $N_{4(5)}\cdots C_{3\alpha(4')}$, 2.44–2.54 Å. The analogous distances in **6** are near the lower end of the quoted ranges.

(24) Bacterial β-lactamases are thought to undergo conformational changes upon binding to β-lactam antibiotics; see A. Samuni and A. Y. Meyer, *Mol. Pharmacol.*, **14**, 704 (1978), and references cited therein.

(25) The conformational freedom of the dipeptide may be one of the factors related to the diminished antibacterial activity of 6(7) α-substituted penicillins (cephalosporins). Even though D-Ala–D-Ala has a methyl group in the *topologically* analogous position, the *topographical* location can be different in the dipeptide compared to the β-lactam compounds. See D. B. Boyd, *J. Chem. Educ.*, **53**, 483 (1976), for references and further comment. Topology considers only the number of bonds and configuration of atoms, whereas topography pertains to the three-dimensional relationship between two molecules.

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Syntheses, Analgetic Activity, and Physical Dependence Capacity of 5-Phenyl-6,7-benzomorphan Derivatives[1,2]

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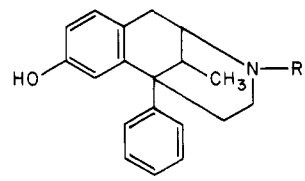
The synthesis, analgetic activity, and physical dependence capacity of a large number of 5-phenyl-6,7-benzomorphan derivatives are described. Observations made during the Stevens' rearrangement of 1-benzyl-1-methyl-Δ^3-piperidinium salt derivatives (V) under various conditions are discussed. The absolute configuration of the 9-demethyl series and the 2'-deoxy series is established by comparison of their ORD and CD spectra with those of **49**, whose absolute configuration was previously established by X-ray crystallography.[2] A convenient synthesis of ^{3}H-labeled phenols using $^3H_3PO_4$ is described, as well as the preparation of ^{14}C-labeled compounds by conventional methods.

l-β-2'-Hydroxy-2,9-dimethyl-5-phenyl-6,7-benzomorphan[2] (**49**; I, R = CH_3; GPA 1657) is a potent, orally effective analgetic.[2,4] **49** provides a structural link from the benzomorphans to the 4-phenylpiperidines[4-6] and the diphenylpropylamine analgetics.[5,7] **49** and its analogues display interesting pharmacological properties. For instance, **49** is an analgetic antagonist not only in reversal of morphine analgesia and precipitation of withdrawal symptoms in monkeys treated chronically with morphine[4] but also in opiate receptor binding studies;[8] **214** (the *N*-propargyl analogue of **49**) is a pure, long-acting antagonist in the guinea pig ileum assay[9] and binds more strongly to the opiate receptor in the presence of sodium ion than in its absence;[8] **207** and **219** [the *N*-(dimethylallyl) and *N*-(cyclobutylmethyl) analogues, respectively, of **49**] are both mixed agonist–antagonists;[4] and both optical isomers of the 9-demethyl and the 2'-deoxy-9-demethyl analogues of **49** are about equally potent as analgetics in the mouse phenylquinone writhing test.[5]

In this paper, we will describe the synthesis and summarize the pharmacology of the extensive series of 5-phenylbenzomorphans from which the above compounds were derived.

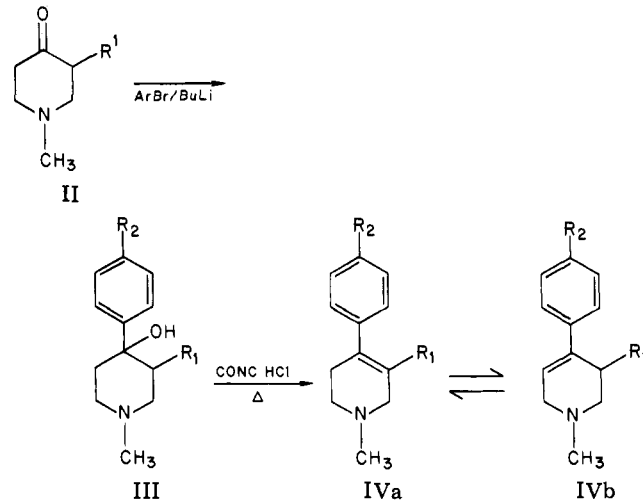
Chemistry. The synthesis of compounds related to **49** (I, R = CH_3) was similar to that described already for **49**[2] and its 9-demethyl analogue.[10] As shown in Scheme I, most of the required Δ^3-piperideine intermediates, IVa (see Table I), were prepared by reacting 4-piperidinones, II, with an aryllithium, generated in situ from the corresponding aryl bromide, followed by acid-catalyzed dehydration of the resulting 4-aryl-4-piperidinols, III. Compound **8** (Table I) was prepared by reacting 1-methyl-4-phenyl-3-piperidone[11] with ethyllithium and dehydrating the resulting 3-ethyl-3-piperidinol derivative. During the dehydration reaction, prolonged refluxing with

Scheme I[a]



I

49, R = CH_3
214 R = $CH_2C{\equiv}CH$
207 R = $CH_2CH{=}C(CH_3)_2$
219 R = CH_2-c-C_4H_7



[a] See Table I.

HCl usually provided a preponderance of the Δ^3-piperideines, IVa, which were separated by recrystalization of

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