7-(Thiophene-2-acetamido)-3-methoxy-3-cephem-4-carboxylic Acid (13a). **12a**, 490 mg (1 mmol), was hydrogenolized as in the preparation of **8a**. The product was crystallized by trituration from ether. A first crop weighed 156 mg (28%): mp 168–171°; nmr (τ, A-60 MHz, DMSO-d_6) 6.35 (s, 2 H, C_2-H_2), 6.24 (s, 5 H, C_3-OCH_3 and α-CH_2), 4.94 (d, 1 H, C_6-H), 4.55 (q, 1 H, C_7-H), 3.10–2.55 (m, 3 H, aromatic H), and 1.10 (d, 1 H, C_7-NH). *Anal.* ($C_{14}H_{14}N_2O_5S_2$) C, H, N.

7-(D-Mandelamido)-3-methoxy-3-cephem-4-carboxylic Acid (13b). **12b** (1 mmol) was hydrogenolized as for **10a**: yield 115 mg (31%); mp 161–164°; nmr (τ, A-60 MHz, DMSO-d_6), 6.36 (s, 2 H, C_2-H_2), 6.23 (s, 3 H, C_3-OCH_3), 4.98–4.80 (m, 2 H, C_6-H and α-CH), 4.55 (q, 1 H, C_7-H), 3.8 (s, 1 H, α-OH), 2.78–2.38 (m, 5 H, aromatic H), and 1.45 (d, 1 H, C_7-NH). *Anal.* ($C_{16}H_{16}N_2O_6S$) C, H, N.

7-(D-2-Amino-2-phenylacetamido)-3-methoxy-3-cephem-4-carboxylic Acid (13c). **12c**, 500 mg (0.83 mmol), in 30 ml of CH_3CN and 15 ml of H_2O was acidified to pH 1 momentarily and then back to 2.5. The solvents were evaporated to dryness *in vacuo*. The residue was hydrogenolized in the manner already described. After removal of solvents of hydrogenolysis, the residue was dissolved in H_2O and adjusted to pH 4.5 for extractions with EtOAc. The aqueous phase was evaporated to dryness *in vacuo*. The residue crystallized from 2 ml of H_2O and 1 ml of CH_3CN: yield 122 mg (37%); mp 148–180° dec; nmr (τ, A-60 MHz, D_2O–DCl) 6.58 (AB q, 2 H, C_2-H_2), 6.10 (s, 3 H, C_3-OCH_3), 4.87 (d, 1 H, C_6-H), 4.70 (s, 1 H, α-CH), 4.54 (d, 1 H, C_7-H), and 2.41 (s, 5 H, aromatic H). *Anal.* ($C_{16}H_{17}N_3O_5S \cdot 2H_O$) C, H,.

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Electronic Structures of Cephalosporins and Penicillins. 4. Modeling Acylation by the β-Lactam Ring†

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Molecular orbital calculations by the CNDO/2 method are used to study the molecular and electronic details involved in the initial phases of the opening of the β-lactam ring of a model cephalosporin structure, 7-amino-3-acetoxymethyl-3-cephem. The effect of a simple nucleophile, OH^-, approaching the carbonyl carbon center of the β-lactam ring is monitored by following the charge redistributions that occur in the bicyclic system and in the 3 side chain. A migration of electron density to the ester oxygen of the CH_2OAc group is observed with concomitant weakening of the CH_2–OAc bond. The results are discussed in relation to the mechanism of acylation of bacterial cell wall enzymes by β-lactam antibiotics and in relation to the hydrolysis of these molecules. The results indicate that the ability of the 3′ substituent of cephalosporins to stabilize electron density transferred to it, *i.e.*, the leavability of the 3′ moiety, can be an important factor in activating the β-lactam toward nucleophilic attack.

The mode of action of the cephalosporins and penicillins has been unraveled to a considerable degree at the molecular level.[2,3] These antibiotics are thought to inhibit the final cross-linking reaction in bacterial cell wall synthesis[4] by mimicking some part of the substrate peptidoglycan, presumably a D-alanyl-D-alanine dipeptide.[5] An irreversible chemical binding mechanism, which is believed to be an acylation by the β-lactam, results in the inactivation of at least one transpeptidase enzyme which knits the peptidoglycan into a huge macromolecule.

A number of theoretical papers based on the correctness of the above mode of action, but also providing support for its feasibility, have been addressed to (a) just how the antibiotic mimics the transpeptidase substrate in terms of the geometric and electronic similarity of the antibiotic to the natural substrate in its ground or transition state,[6] (b) the chemical mechanism of the acylation reaction leading to destruction of enzyme activity,[7] and (c) the influence of chemical modification of the antibiotic on the above chemical mechanism.[7-9] This paper is concerned to a certain extent with all three of the above details but mainly with the second. We accordingly explore relevant parts of the reaction path (or, more generally, surface) that would be traversed when a nucleophile forms a transition state complex with a representative cephalosporin. Changes calculated in electronic structure are used in making generalizations (in terms of familiar, chemical concepts) about what types of molecular modifications may be desirable in promoting biological activity of cephalosporins.

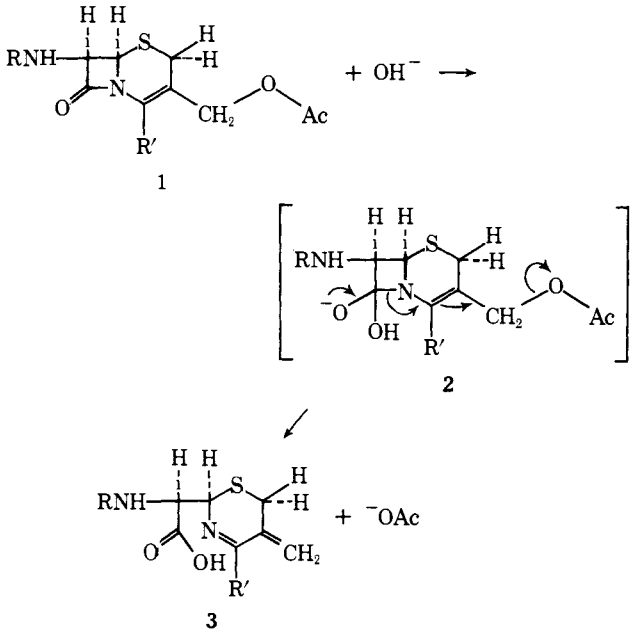
The acylation of the cell wall enzyme is believed to proceed *via* the attack at the carbonyl carbon of the antibiotic's β-lactam ring by a thiol group of the enzyme.[2,3,10-12]

† For paper 3 of this series, see ref 1.

Topp and Christensen[7] have treated this reaction as the usual sum of two steps: (1) reversible complexation of the antibiotic and enzyme, and (2) the formation of a covalent bond between the antibiotic and the enzyme. With respect to the second step, they hypothesized the formation of a transition state analog that could lead to this bond formation and proceeded to use a reparameterized CNDO/2 molecular orbital (MO) method to calculate so-called activation energies which result from placing an HS^- group at a fixed distance of 1.82 Å away from the α face of the carbonyl carbon atom in the β-lactam ring of penicillin and cephalosporin model structures. This type of complex is supposed to mimic the true transition state, the geometry of which, of course, is not yet known in any detail. The distance of 1.82 Å is a typical S–C bond length, so the thiol group is at a covalent bonding distance, which is probably closer than in the true transition state. Besides using nonstandard CNDO/2 parameters for sulfur, Topp and Christensen also chose to neglect the S 3d atomic orbitals (AO's) of this atom (the latter approximation renders their model nucleophile electronically more similar to OH^-).

In this paper, we study in considerably more detail a similar model of the transition state but one in which the attack on the carbonyl carbon of a cephalosporin antibiotic (**1**, R = acyl; R′ = COOH) is by a hydroxyl ion. The OH^- should serve adequately as a model nucleophile because its effect on the cephalosporin should be similar to that resulting from SH^- attack and conclusions should be just as transferable to the reaction with the true enzyme. Moreover, the hydroxyl ion is itself the attacking species in base-catalyzed hydrolysis of the β-lactam ring, a reaction which has been investigated kinetically.[13-15] We consider the acylation or hydrolysis reaction to be modeled by the mechanism illustrated in Scheme I when the 3 substituent of the cephalosporin is an acetoxymethyl group (CH_2OAc).

Scheme I



By quantum mechanical calculations, we study the approach of the reactant OH^- to the β-lactam carbonyl carbon of a model of 1 (R = H; R′ = H) up to the formation of some transition state analogs (**2**). Of the several possible modes of hydrolysis leading to rupture of the amide C–N bond the one involving attack at the carbonyl carbon is believed to be most representative of the reaction under *in vivo* conditions.[15-20] Thus, the transition state will involve

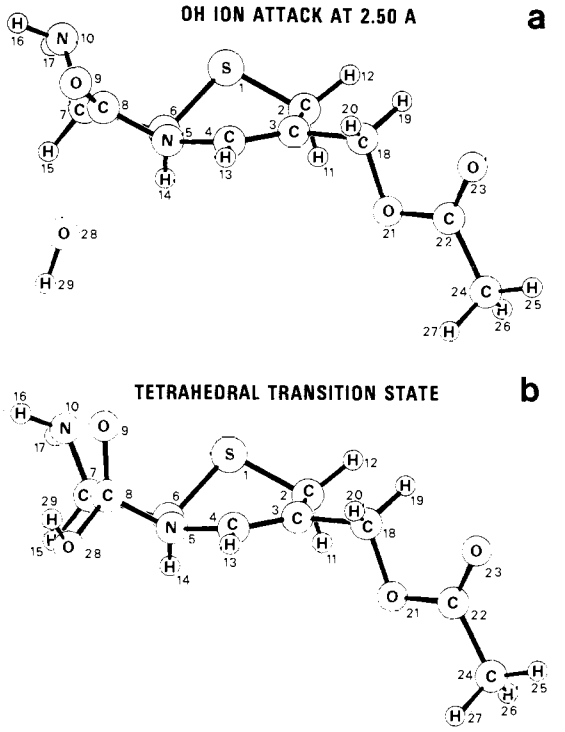


Figure 1. Structure and numbering system of (a) 7-amino-3-acetoxymethyl-3-cephem and OH^- at one position in the approach to the α face of the planar transition state model (**2a** has $C_8 \cdots O_{28}$ 1.50 Å), and (b) the tetrahedral transition state model (**2b**). The molecules are viewed with the β-lactam ring edgeways.

a tetracoordinated carbonyl carbon. Two choices of the transition state are investiaged here: "planar" (**2a**) and "tetrahedral" (**2b**). These geometries are shown in Figure 1. Our choices for representative transition state geometries are rather arbitrary since it is really not known what the various bond lengths are at the saddle point in the reaction surface. Presumably as the OH^- approaches there is a smooth structural reorganization with various bonds lengthening, such as the β-lactam C=O bond, and others becoming shorter, and perhaps groups of atoms rotating into more stable positions. Therefore, our choice of planar transition state model describes only one bond length change and our tetrahedral transition state model attempts to account for changes in bond lengths and angles at only one center.

In the cephem 1 chosen for this investigation, we pay particular attention to changes in electronic structure brought about by nucleophilic attack. Certain changes in the strengths of bonds connecting the site of nucleophilic attack and the 3-acetoxymethyl side chain, as well as of bonds within the side chain, are associated with the energetically favorable lengthening of the CH_2–OAc bond and the possibility of the departure of acetate from the methylene bridge. This rearrangement of the bonds associated with a leaving group[21,22] (acetoxy in our example) is influenced by the leavability of that group. We define leavability as a measure of the relative ease of various molecular fragments to dissociate with an extra electron from some common chemical structure in a given reaction system. Leavability reflects how well such a molecular fragment can exist with that additional electron density. The leaving group is able to stabilize the charge by dispersing it over the atom(s) of the group away from the site of incipient bond rupture. A good leaving group often has polarizable atom(s) or group(s) and/or a formal positive charge either in its normal covalent arrangement or after a protonation

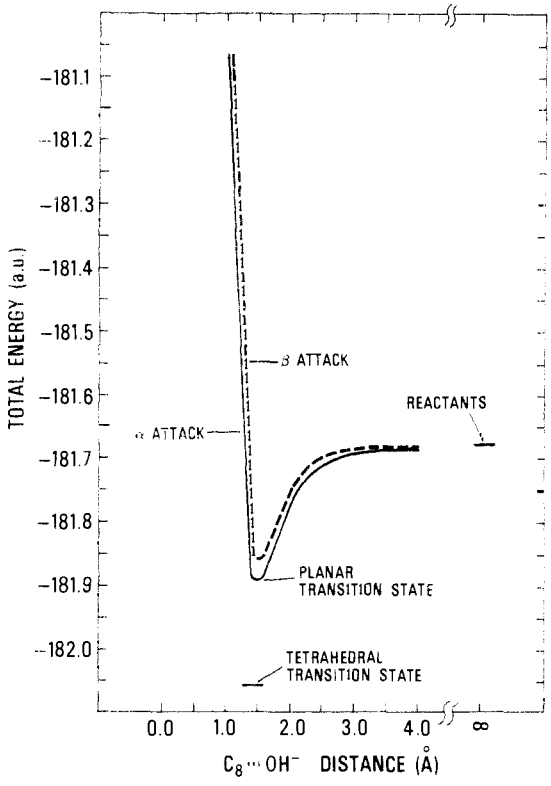
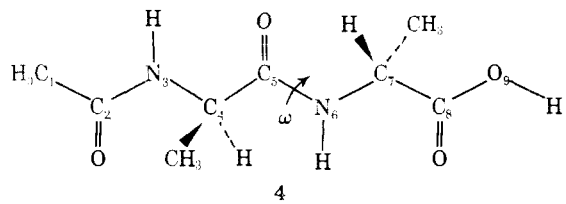


Figure 2. CNDO/2 total energy of 7-amino-3-acetoxymethyl-3-cephem and OH^- (reactants) as a function of approach of OH^- toward the α and β faces of C_8 in the β-lactam ring. A parabolic fit to the curve for α face attack in the region of the minimum indicates that the energy is lowest at a distance of 1.48 Å. This is sufficiently close to 1.50 Å (where a calculation was done) that the 1.50-Å results may be treated as corresponding to the saddle point (**2a**). The CNDO/2 energies of the transition state models (**2a** and **2b**) and the sum of the CNDO/2 energies of the infinitely separated reactants are also indicated. 1 atomic unit = 627.54 kcal/mol.

step. We show that leavability can be a factor in lowering the energy of our model transition states, **2a** and **2b**, relative to that of the ground state for the acylation and hydrolysis reactions. Our results are in some respects at variance to conclusions published by Topp and Christensen[7] in that we are able to show clearly an incipient CH_2–OAc bond rupture during nucleophilic attack on the β-lactam ring. Other factors which affect the electrophilicity of the β-lactam carbonyl carbon atom, such as the purely inductive effect[8] of a group attached to the 3 position of the cephem ring, will also be examined here.

In the last section we determine the preferred conformation of a model of the presumed natural substrate of bacterial cell wall transpeptidase using several different quantum mechanical calculational procedures. The model selected is *N*-acetyl-D-alanyl-D-alanine (**4**). Internal rotation



4

about the C_5–N_6 peptide bond, which in Strominger's hypothesis[2,5] is analogous to the β-lactam C–N bond of the antibiotics, is investigated by varying the C_4–C_5–N_6–C_7 dihedral angle ω. We have not attempted to investigate the more complex problem of general distortions of the amide unit, such as toward tetrahedral hybridization at N_6, which could render the three-dimensional structure of **4** closer to that of penicillins or cephalosporins. The cost in energy to go from the preferred conformation of **4** to one resembling penicillins *via* internal rotation is compared to values estimated from classical force field calculations.[6,23] We show that such a conformation change has the effect of activating the peptide bond toward nucleophilic attack, such as in hydrolysis or acylation. A distortion that weakens the C_5–N_6 bond seems like a reasonable occurrence in the formation of a transition state because this is the bond broken in the final cross-linking of the peptidoglycan chains.

Experimental Section

In this section we give the procedural details involved in the quantum mechanical computer experiments. The principal MO method that we use is CNDO/2 (complete neglect of differential overlap).[24] This method was selected because it is the best, presently available theory for the types of problems of interest here and because its strengths and weaknesses are fairly well known.[24,25] Standard CNDO/2 parameters are employed, including the spd basis set for sulfur.[24] To obtain the Mulliken population analyses,[26] a deorthogonalization[27] of the CNDO/2 MO's is first necessary. This so-called CNDO/2D (deorthogonalized) method, as well as the CNDO/2 method, is carried out by a double precision FORTRAN computer program called BNDO (Big NDO).[1] We also report a few results from the EH (extended Hückel) MO method[28,29] and the PCILO (perturbative configuration interaction using localized orbitals) procedure.[30] The EH computer program is a double precision one developed[29] and used[1,28] in earlier studies. The total molecular energy in EH theory is related to half the sum of the one-electron orbital energies, and this quantity is used for computing relative energies as in rotational barriers.[28,31,32] The PCILO program is a modified version of the one available from the Quantum Chemistry Program Exchange.‡ Like the QCPE version, ours is in single precision, optimizes bond polarities, and uses the same parameters. All calculations were carried out on an IBM 370/158 computer.

The atomic coordinates of the 3-cephem nucleus of 7-amino-3-acetoxymethyl-3-cephem (**1**) are from the X-ray crystallographic study of cephaloridine.[3,33] The other atoms of the model structure are added with standard bond lengths and angles.[24,28] The hydroxyl ion is taken in its singlet ground state with a bond length of 0.95 Å. The structures of the transition state models are seen in Figure 1. The hydroxyl group O_{28}–H_{29} is allowed to approach in small increments along an axis perpendicular to the carbonyl center on either the α or β faces of **1** while the latter's geometry is held fixed until the planar transition state model is reached. The perpendicular path of attack only approximately describes the less direct path of attack at carbonyl centers suggested by X-ray crystallographic[18] and quantum mechanical[19] model studies. The planar transition state model (**2a**) has the hydroxyl oxygen O_{28} 1.50 Å away from C_8. The tetrahedral transition state model (**2b**) is assumed to have the same geometry as **2a**, except the following bond lengths and angles are adopted: C_8–O 1.43 Å, O_{28}–H_{29} 0.95 Å, O_9–C_8–O_{28} 109.4712°, C_8–O_{28}–H_{29} 104°; the plane defined by O_9–C_8–O_{28} bisects the C_7–C_8–N_5 bond angle and vice versa; and H_{29} is cis with respect to O_9.

Apparent in our model structures is the fact that the 4-carboxyl group of the cephalosporins is replaced by a hydrogen (Scheme I, R′ = H instead of COOH). This carboxyl group is indeed essential for antibiotic activity apparently through its role of helping the antibiotic to bind at the proper receptor site. That is, the reversible binding step in the enzyme-antibiotic complexation is promoted by the carboxyl group. Our neglect of the 4-COOH group stems from the desire to reduce the size of the calculations and from the expectation that its effects on the molecular charge distribution will be more or less constant all along the reaction path. There is no evidence that the carboxyl group participates in the rearrangement shown in Scheme I.[3] Uv and CD spectral studies[3,28] indicate little electronic interaction between the dihydrothiazine ring and carboxyl moiety. For similar reasons, we have also replaced the 7-acylamido side chain of cephalosporins with an amino group in our model compounds (Scheme I, R = H instead of acyl). The 7 side chain influences the spectrum of antibiotic activity.[3] Also, certain 7 side chains have been implicated in intramolecular nucleophilic

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Table I. CNDO/2 Net Atomic Charges of 7-Amino-3-acetoxymethyl-3-cephem Complexed with a Hydroxyl Ion[a]

Atom	Tetrahedral transition state (**2b**)	$C_8 \cdots O_{28}$ distance from α face (ångstroms)			
		1.50 (**2a**)	2.50	3.50	∞
S_1	−0.1660	−0.1988	−0.1680	−0.1487	−0.1059
C_2	+0.0205	+0.0226	+0.0151	+0.0119	+0.0037
C_3	−0.1016	−0.1065	−0.0757	−0.0610	−0.0390
C_4	+0.1418	+0.1243	+0.1259	+0.1185	+0.0964
N_5	−0.1637	−0.1461	−0.0995	−0.1147	−0.1408
C_6	+0.0943	+0.0932	+0.0935	+0.0870	+0.0739
C_7	+0.0613	+0.0329	+0.0468	+0.0512	+0.0594
C_8	+0.3373	+0.5232	+0.4084	+0.3698	+0.3405
O_9	−0.6439	−0.5546	−0.3320	−0.3091	−0.3054
N_{10}	−0.1864	−0.2127	−0.2060	−0.2021	−0.1950
H_{11}	−0.0172	−0.0135	+0.0052	+0.0127	+0.0215
H_{12}	−0.0167	−0.0182	−0.0056	+0.0012	+0.0201
H_{13}	−0.0228	−0.0149	+0.0065	+0.0101	+0.0039
H_{14}	−0.0299	−0.0156	+0.0174	+0.0240	+0.0114
H_{15}	−0.0639	−0.0093	+0.0497	+0.0365	−0.0065
H_{16}	+0.0320	+0.0382	+0.0619	+0.0707	+0.0830
H_{17}	+0.0688	+0.0618	+0.0757	+0.0809	+0.0880
C_{18}	+0.1755	+0.1759	+0.1740	+0.1726	+0.1673
H_{19}	−0.0331	−0.0351	−0.0288	−0.0256	−0.0132
H_{20}	−0.0299	−0.0316	−0.0238	−0.0204	−0.0137
O_{21}	−0.2455	−0.2435	−0.2366	−0.2345	−0.2389
C_{22}	+0.3853	+0.3858	+0.3880	+0.3889	+0.3876
O_{23}	−0.3315	−0.3329	−0.3304	−0.3289	−0.3178
C_{24}	−0.0871	−0.0871	−0.0868	−0.0868	−0.0866
H_{25}	+0.0218	+0.0216	+0.0247	+0.0265	+0.0359
H_{26}	+0.0268	+0.0270	+0.0298	+0.0313	+0.0351
H_{27}	+0.0283	+0.0294	+0.0347	+0.0372	+0.0352
O_{28}	−0.3671	−0.5775	−0.8270	−0.8395	−0.8352
H_{29}	+0.1128	+0.0620	−0.1374	−0.1598	−0.1648

[a] Charges are obtained by subtracting the sum of the diagonal density matrix elements for each atom from the atomic core charges; units of e. A negative charge corresponds to an excess of electron density on an atom in a molecule compared to the free atom; positive charge corresponds to a deficiency. See Figure 1 for numbering system.

attack on the β-lactam ring.[13,34] For our purposes the hydroxyl ion serves adequately as a nucleophile for either an intermolecular or intramolecular reaction and regardless of whether a thiol, amino,[35] or other similar functionality is acylated.

The atomic coordinates of *N*-acetyl-D-alanyl-D-alanine (4) are generated from standard bond lengths and angles.[24] Dihedral angles determining the conformation are taken from the refined crystal structure coordinates of benzylpenicillin.[36] The total energy of 4 is obtained at values of the C_4–C_5–N_6–C_7 dihedral angle ω ranging from 0 to 360° in 15° increments and at ω = 135.7°, the analogous value in benzylpenicillin.[36]

Solvent effects on the reactions and conformations were neglected on grounds of expediency and the fact that the extent to which the reactants and products are solvated at the receptor site of transpeptidase (or whatever other killing sites are available to the antibiotics) is, of course, not known.

Results and Discussion

Nucleophilic Attack. In Figure 2 the molecular energy for the perpendicular attack at the carbonyl center of our model cephem is plotted. The lower curve for attack on the α face (Figure 1a) means that this reaction path is energetically preferred over attack on the β face. This difference arises mainly from the proximity of the 7-amino group to the reaction path for β-face attack. The optimum $C_8 \cdots OH^-$ distance (at a fixed β-lactam geometry) comes at a very reasonable value of about 1.50 Å for both paths of attack. We designate the structure at the minimum in the lower (α face) curve as **2a**. If the hydroxyl group gets closer than 1.5 Å, the energy rises steeply indicating a repulsive potential. The CNDO/2 method predicts the complex to be highly bound; that is, the transition state models are at much lower energies than the infinitely separated reactants. One also notes that CNDO/2 fails to predict an energy hill to go over in the approach of the nucleophile. Without a finite activation energy, the hydrolysis of the β-lactam ring would be too facile, and the antibiotic would be so unstable that its chances of reaching the transpeptidase receptor site intact would be unreasonably low. In fact, the failures to predict our transition state models at higher energies and a finite activation energy barrier to nucleophilic attack are due in part to the lack of solvation of the hydroxyl ion, which would be more highly solvated (and hence more stable) in the reactant stage than in the transition state stage, and in part to the fact that CNDO/2 overestimates bond energies, such as for the incipient $C_8 \cdots O_{28}$ bond (Figure 1). More sophisticated *ab initio* MO calculations with configuration interaction and some way of estimating solvation energies would be needed to more satisfactorily predict an activation energy (*vide infra*).

The shortcoming of CNDO/2 in overestimating bond strengths also means that the energy difference of *ca.* 21 kcal/mol between the α-face and β-face paths of attack at 1.50 Å cannot be related to relative rates of hydrolysis of sterically hindered cephalosporins or penicillins. Those experimental hydrolysis rates[14] imply an energy difference of no more than a few kcal/mol for the two modes of attack. In the bacterial cell wall enzyme system the geometrical constraints at the receptor sites probably dictate which face of the antibiotic is attacked.

Actually, both the CNDO/2 calculations and chemical intuition suggest that a planar transition state model is not,

Table II. CNDO/2D Net Atomic Charges of 7-Amino-3-acetoxymethyl-3-cephem Complexed with a Hydroxyl Ion[a]

Atom	Tetrahedral transition state (2b)	$C_8 \cdots O_{28}$ distance from α face (ångstroms)			
		1.50 (2a)	2.50	3.50	∞
S_1	−0.0483	−0.0872	−0.0521	−0.0278	+0.0271
C_2	−0.0852	−0.0825	−0.0935	−0.0983	−0.1100
C_3	−0.0647	−0.0706	−0.0462	−0.0334	−0.0160
C_4	+0.1443	+0.1230	+0.1296	+0.1246	+0.1061
N_5	−0.2848	−0.2572	−0.2175	−0.2364	−0.2669
C_6	+0.0330	+0.0332	+0.0287	+0.0187	+0.0005
C_7	+0.0452	+0.0090	+0.0229	+0.0298	+0.0446
C_8	+0.4589	+0.6583	+0.5101	+0.4627	+0.4263
O_9	−0.6080	−0.5586	−0.3753	−0.3529	−0.3483
N_{10}	−0.2955	−0.3263	−0.3222	−0.3180	−0.3097
H_{11}	+0.0007	+0.0058	+0.0291	+0.0385	+0.0491
H_{12}	−0.0004	−0.0023	+0.0139	+0.0227	+0.0471
H_{13}	−0.0042	+0.0061	+0.0353	+0.0401	+0.0319
H_{14}	−0.0126	+0.0061	+0.0504	+0.0592	+0.0426
H_{15}	−0.0530	+0.0188	+0.0931	+0.0759	+0.0194
H_{16}	+0.0689	+0.0774	+0.1067	+0.1178	+0.1332
H_{17}	+0.1149	+0.1063	+0.1235	+0.1300	+0.1391
C_{18}	+0.1486	+0.1498	+0.1484	+0.1471	+0.1407
H_{19}	−0.0120	−0.0147	−0.0065	−0.0022	+0.0142
H_{20}	−0.0075	−0.0097	+0.0005	+0.0049	+0.0137
O_{21}	−0.3324	−0.3300	−0.3227	−0.3206	−0.3262
C_{22}	+0.4825	+0.4828	+0.4847	+0.4854	+0.4838
O_{23}	−0.3782	−0.3796	−0.3772	−0.3757	−0.3641
C_{24}	−0.1842	−0.1842	−0.1845	−0.1848	−0.1852
H_{25}	+0.0533	+0.0530	+0.0570	+0.0593	+0.0719
H_{26}	+0.0564	+0.0567	+0.0604	+0.0624	+0.0674
H_{27}	+0.0585	+0.0599	+0.0671	+0.0704	+0.0676
O_{28}	−0.4574	−0.6430	−0.8283	−0.8373	−0.8320
H_{29}	+0.1631	+0.0998	−0.1356	−0.1621	−0.1680

[a] These are obtained from the usual Mulliken population analysis of the CNDO/2D MO's; units of e. Mulliken net charges from CNDO/2D MO's represent an alternative partitioning of electron density in a molecule among its constituent atoms, which is equally valid conceptually as that used in the plain CNDO/2 case.

in fact, a species with a meaningful lifetime. As the hydroxyl ion gets closer to C_8, other molecular distortions, including development of tetrahedral-like hybridization at C_8, would occur as indicated by the lower energy of **2b** in Figure 2. Because there are many possible paths to the tetrahedral transition state, we did not adjust the geometry or optimize the energy with respect to geometry at increments in the approach of the hydroxyl ion. Such adjustments would more thoroughly delineate the reaction surface but would not change the important charge redistribution effects described in the next section. Other skeletal rearrangements occurring up to the formation of the tetrahedral transition state cannot be investigated without a major extension of this work, but at least the tendency of the molecule to distort *toward* these rearrangements will be seen in the changes in the electron density distributions.

Charge Redistributions. CNDO/2 (Table I) and CNDO/2D (Table II) net atomic charges are given for 7-amino-3-acetoxymethyl-3-cephem at a few of the many points along the reaction path for α face attack. Corresponding overlap populations between bonded atoms in the model structures are given in Table III. Results are similar for β-face attack and so are not included. What is noteworthy in the tabulated net atomic charges and overlap populations is not so much the absolute values but the trends manifested by the geometrical changes. We expect the MO theories to be fairly reliable in showing these trends.

It is worthwhile here to explain a minor discontinuity in the charge distributions noticeable in Tables I–III which occurs when the nucleophile is brought in from infinity to a position where the complex is loosely associated (3–4 Å). The artifact arises because of the discontinuous change in basis set size: there are more AO's available for expansion of the MO's in the complex than in the infinitely separated reactants. *Ab initio* MO calculations are known to give energies and charge distributions which are basis set dependent.[37] A similar thing occurs in these CNDO/2 calculations, but fortunately the spurious charge distribution changes are minimized by the fact that the infinitely separated reactants are closed-shell systems. More complex MO calculations are capable of producing more smoothly varying wave functions.

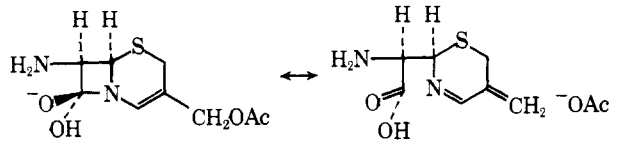
The most important observation to make about the charge distributions by either of the two methods (Tables I and II) is that the acetoxy group, including O_{21}, becomes *more* negative as OH^- approaches the β-lactam ring on either the α or β faces and as the structures adjust from the planar transition state model **2a** to the tetrahedral one **2b**. There is the initial readjustment in charges as the hydroxyl ion is brought in from infinity to 3.50 Å where C_8 is under imminent nucleophilic attack. Thereafter, from 3.50 Å on down to the transition state models, the OAc^- group as a whole gains 0.05–0.06 e. Some of this charge accumulates on the methyl hydrogens. Atom O_{21} and O_{23} each gain about 0.01 e. Whereas these figures are small, we believe they are significant within the context of CNDO/2 or CNDO/2D theory. In fact, the effect on the charge distribution on the acetoxy oxygens is sizable when one considers

Table III. Mulliken Overlap Populations for Selected Bonds in 7-Amino-3-acetoxymethyl-3-cephem Complexed with a Hydroxyl Ion[a]

Bond	Tetrahedral transition state (2b)	$C_8 \cdots O_{28}$ distance from α face (ångstroms)			
		1.50 (2a)	2.50	3.50	∞
$O_{28}-C_8$	0.5108	0.3664	0.0205	0.0002	0.0
O_9-C_8	0.4265	0.6577	0.7961	0.7977	0.7922
C_8-N_5	0.5654	0.5423	0.6959	0.7054	0.7083
N_5-C_4	0.7898	0.7304	0.7784	0.7713	0.7575
C_4-C_3	1.2403	1.2296	1.2512	1.2559	1.2608
C_3-C_{18}	0.8352	0.8340	0.8365	0.8364	0.8345
$C_{18}-O_{21}$	0.5215	0.5219	0.5287	0.5308	0.5331
$O_{21}-C_{22}$	0.5762	0.5761	0.5740	0.5725	0.5669
$C_{22}-O_{23}$	0.8101	0.8099	0.8111	0.8117	0.8146
$C_{22}-C_{24}$	0.8183	0.8185	0.8210	0.8221	0.8238
C_8-C_7	0.7316	0.7558	0.7989	0.7973	0.7944
C_7-N_{10}	0.6998	0.6659	0.6800	0.6833	0.6869
C_7-C_6	0.7279	0.7332	0.7410	0.7440	0.7476
C_6-N_5	0.6630	0.6356	0.6772	0.6764	0.6725
C_6-S_1	0.4767	0.4657	0.4842	0.4832	0.4738
S_1-C_2	0.5155	0.5173	0.5206	0.5221	0.5234
C_2-C_3	0.8670	0.8686	0.8618	0.8599	0.8569

[a] Since overlap populations are computed with overlap integrals between atomic orbitals included, only the CNDO/2D MO's are appropriate for evaluating them and are so used in obtaining this tabulation. Overlap populations measure the number of electrons between two atoms and, for a given pair of atoms, are proportional to the relative strengths of that type of bond; units of e. The C_8-O_{21} bond length is 1.43 Å.

that C_8 and O_{21} are separated by five bonds or that none or almost none of the excess negative charge introduced by the hydroxyl ion appears on the atoms adjacent to O_{21} (C_{18} and C_{22}). The charge redistribution on O_{21} is consistent with **2c** ↔ **2d** resonance in the transition state. Of course, the excess charge on the acetoxy group is spread among its atoms in the familiar pattern.



2c **2d**

The overlap populations (Table III) afford additional evidence for resonance structure **2d**. As the hydroxyl ion begins to approach the carbonyl center ($C_8 \cdots O_{28}$ decreases from ∞ to 2.50 Å), the bond strengths as reflected by the overlap populations vary in an alternating fashion just as suggested by the increasing importance of **2d**: C_8-N_5 weakens, N_5-C_4 strengthens, C_4-C_3 weakens, C_3-C_{18} strengthens, and $C_{18}-O_{21}$ weakens. After the hydroxyl oxygen is covalently bonded to C_8 (as indicated by the C_8-O_{28} overlap population of **2b**), some of these trends are no longer monotonic, but the CH_2-OAc bond is consistently weakened. We will apply one other criterion of bond strength in the next section, but it too will support the idea that the leavability of the 3′ substituent can play a role in activating the β-lactam ring toward nucleophilic attack. In other words, a 3′ substituent which can stabilize the electron density shifted out to it by a nucleophile approaching the β-lactam ring will favor the progress of that approach.

Before considering the CH_2-OAc bond strength, it is interesting to ascertain what other conclusions can be drawn from the charge distributions in Tables I–III. One can see that the CNDO/2 and CNDO/2D charges generally parallel each other, so it does not matter which we use in our discussion. As the hydroxyl ion approaches our model structure, electron density is seen to generally increase on S_1 and O_9. The former finding suggests that the electron accepting ability of sulfur (which is related to the availability of S 3d AO's) could conceivably promote reactivity of the β-lactam. The increase in charge on O_9 correlates with the sharp decrease in the O_9-C_8 overlap population. The approaching nucleophile is reducing the amount of double-bond character in the carbonyl bond. Of no little importance is the increase in electron density on C_3 as OH^- approaches the model structure. Substituents directly attached to C_3 that can withdraw electrons inductively are expected to stabilize that charge accumulation in the transition state and thereby promote the reactivity of the β-lactam ring and improve the antibiotic activity of a cephalosporin.[3,8,38] Besides the resonance effect (**2c** ↔ **2d**) discussed above, simple inductive pulling of electron density will also make the β-lactam carbonyl carbon more deficient in electrons. The higher the positive net atomic charge on the atom, the more electrophilic that center will be.[39] Thus, electron-withdrawing groups at either the 3 or 3′ positions

Table IV. CNDO/2 Total and Relative Energies of 7-Amino-3-acetoxymethyl-3-cephem with a Hydroxyl Ion as a Function of CH_2-OAc Bond Length[a]

Model[b]	$C_{18}-O_{21}$ distance (ångstroms)				
	1.43		1.53		1.63
1[c]	−181.6791		−181.6427		−181.5856
1, Δ		22.84		35.83	
2a	−181.8912		−181.8564		−181.8012
2a, Δ		21.84		34.64	
2b	−182.0580		−182.0233		−181.9682
2b, Δ		21.78		34.58	

[a] Total energies are in atomic units (1 au = 627.54 kcal/mol). Energy differences (Δ) for stretching the CH_2-OAc bond 0.1 Å are in kcal/mol. [b] The structures in Scheme I have R = H, R′ = H. [c] The total energies in this row include that of the hydroxyl ion at infinity (−18.8996).

Table V. Changes in Mulliken Overlap Populations for Selected Bonds in 7-Amino-3-acetoxymethyl-3-cephem with a Hydroxyl Ion as a Function of C_{18}–O_{21} Distance[a]

	2b		2a		1	
Bond	1.43–1.53 Å	1.53–1.63 Å	1.43–1.53 Å	1.53–1.63 Å	1.43–1.53 Å	1.53–1.63 Å
O_{28}–C_8	0.0004	0.0005	0.0006	0.0008		
O_8–C_8	−0.0002	−0.0003	0.0002	0.0	0.0003	0.0004
C_8–N_5	−0.0019	−0.0026	−0.0035	−0.0035	−0.0011	−0.0012
N_5–C_4	0.0019	0.0024	0.0009	0.0018	0.0014	0.0016
C_4–C_3	−0.0036	−0.0050	−0.0034	−0.0052	−0.0019	−0.0029
C_3–C_{18}	0.0017	0.0041	0.0017	0.0041	0.0017	0.0030
C_{18}–O_{21}	−0.0369	−0.0449	−0.0370	−0.0451	−0.0373	−0.0439
O_{21}–C_{22}	0.0034	0.0031	0.0035	0.0031	0.0024	0.0023
C_{22}–O_{23}	−0.0042	−0.0041	−0.0043	−0.0040	−0.0037	−0.0034
C_{22}–C_{24}	−0.0012	−0.0021	−0.0012	−0.0021	−0.0009	−0.0016
C_8–C_7	0.0005	0.0007	0.0008	0.0009	0.0002	0.0003
C_7–N_{10}	0.0002	0.0002	0.0003	0.0004	0.0	0.0001
C_7–C_6	0.0	−0.0001	0.0	−0.0001	0.0	0.0001
C_6–N_5	−0.0005	−0.0007	−0.0008	−0.0007	−0.0004	−0.0005
C_6–S_1	0.0001	0.0	0.0002	0.0003	−0.0002	−0.0002
S_1–C_2	0.0004	0.0003	0.0005	0.0005	0.0004	0.0003
C_2–C_3	0.0	−0.0002	0.0001	−0.0002	0.0002	0.0

[a] Entries represent overlap populations for each bond in the model with the longer C_{18}–O_{21} bond minus that in the model with the shorter one. Positive (negative) entries correspond to stronger (weaker) bonds in the stretched species. Changes in the fourth decimal place are without particular chemical significance.

can increase the reactivity of the β-lactam carbonyl carbon. A final interesting observation from Tables I and II is that electron density accumulates on the peripheral hydrogens in the transition state structures. This effect results from mutual electrostatic repulsions of the excess negative charge. A similar phenomenon has been observed in other systems[25,40] and has been used very recently as a rationale for the design of some new cephalosporins.[41]

Leavability of 3′ Substituent. We now spotlight another small portion of the reaction surface corresponding to the 3′ substituent physically moving away from the 3-cephem nucleus. Such a mechanism as in Scheme I results in expulsion of acetate and production of a structure with an exocyclic methylene group. Experimental evidence[3] shows that certain 3′ substituents do leave under conditions which lead to the cleavage of the β-lactam C–N bond. There are no experimental observations on whether the β-lactam ring opens before or after the departure of these 3′ groups or if, as seems likely, the processes proceed simultaneously. It also has not been determined whether the actual expulsion of the 3′ substituent is more or less important than simply the tendency of that substituent to pull or accept electrons from the 3-cephem nucleus, thus activating the β-lactam ring toward nucleophilic attack and, in particular, making a cephalosporin a stronger acylating agent of bacterial cell wall transpeptidase. Some cephalosporins,[3] such as cephalothin or cephaloglycin with their 3′-acetoxy side chain or cephaloridine with its 3′-pyridinium side chain, could function through a mechanism as in Scheme I, but other potent antibiotics, such as the new directly substituted 3-chloro- or 3-bromocephalosporins,[38] could not. Cephalexin is a highly useful antibiotic even though its acaudal 3-methyl side chain can neither leave nor withdraw electron density. The latter statement simply recalls the fact that factors[3,9] other than reactivity of the β-lactam ring must be given consideration in the selection of antibiotics for *in vivo* application.

Whether MO calculations, such as by CNDO/2, would predict an actual leaving of the 3′ substituent to be facile is doubtful for two reasons. First, the MO calculations refer to the unsolvated molecules in a vacuum. There is no solvent in the calculational model to stabilize the departing fragment. Second, CNDO/2 overestimates bond strengths and may therefore predict a seemingly insurmountable energy barrier to departure. Thus, the question we can and will address ourselves to is whether the theory predicts any *tendency* for the 3′ substituent to leave.

We have already seen two effects consistent with a concerted reaction in which the acetoxy group of our model structure 1 leaves as a nucleophile approaches the β-lactam ring. In going from 1 to 2 the charge redistributes toward a higher electron density on the ester oxygen and a lower overlap population for the CH_2–OAc bond. Still another measure of the strength of the CH_2–OAc bond is the energy required to stretch this bond before there is a nucleophile in the presence of the β-lactam carbonyl carbon compared to the energy afterward as in one of the transition state analogs. The CNDO/2 total energies of **1**, **2a**, and **2b** with a standard C_{18}–O_{21} bond length of 1.43 Å and with elongated values of 1.53 and 1.63 Å are given in Table IV. The standard bond length of 1.43 Å is very near the equilibrium C_{18}–O_{21} distance of *ca.* 1.39 Å given by CNDO/2 calculations for all three model structures. Obvious is the serious overestimation of energy to stretch the bond. The important point to note in Table IV, however, is that relative to **1**, it is easier to stretch the C_{18}–O_{21} bond in **2a** and **2b**. This result is consistent with the electron migration in **2c** to yield **2d** as an intermediate transition state in nucleophilic attack at the β-lactam ring. Further evidence of **2c** ↔ **2d** resonance is obtained from the data in Table V. Stretching the CH_2–OAc bond expectedly weakens this bond as indicated by the decrease in its overlap population. For the other bonds linking the acetyl carbonyl and the β-lactam carbonyl, there is an interesting alternation effect. These bonds are sequentially strengthened or weakened in an attenuated oscillatory manner, just as suggested by **2c** ↔ **2d**.

Relation to Earlier Calculations. Before considering the final topic of substrate conformation, we should consid-

er the fact that in the CNDO study of thiol complexed to a 7-amino-3-acetoxymethyl-3-cephem model, Topp and Christensen[7] found no indication of an expulsion of the acetoxy group during acylation. This contrasts with our evidence. A possible explanation may lie in the fact that they considered only one C_8–SH^- distance (1.82 Å), happening to pick one where the charge on O_{21} (our numbering system) was equal to that in the reactant. Note in Tables I and II how the charge on O_{21} in the infinitely separated reactant equals that in the approach to the planar transition state model at some point in the vicinity of 2.0 Å due to the artifact of the MO method mentioned earlier. Also, they did not compute overlap populations or energies at different C_{18}–O_{21} bond lengths.

Topp and Christensen[7] reported what were referred to as CNDO activation energies for forming transition state models with thiol attached to the β-lactam carbonyl carbon of a 3-cephem structure and also 6-aminopenicillanic acid. Whereas we plan to discuss our own CNDO/2 calculations on a penicillin model at greater length in a subsequent publication, it is appropriate here to add some comments on transition state model calculations and also on the comparison of penicillins and cephalosporins.

We find that when the reaction surface is more thoroughly explored (at least to the extent of Figure 2) the transition state structures of both 3-cephem and penam are not separated from the isolated reactants by any activation energy barrier according to the standard CNDO/2 method. On the other hand, Topp and Christensen reported activation energies of 19–36 kcal/mol. The reason for this apparent diversity of findings is obscured by the fact that their prescription for computing activation energies would require values with signs opposite to those they report. Nevertheless, the reason may be due to our use of standard CNDO/2 parameters for sulfur, whereas the other authors modified theirs on the basis of bond length and angle predictions for some simple sulfides. Also in connection with the previously reported activation energies, the authors[7] did not make clear if the presumed transition state structures represent saddle points on the reaction surface. As mentioned previously, the 1.82-Å distance chosen for the thiol–carbonyl carbon separation seems somewhat short, especially in light of the fact that the minima in the curves of Figure 2 occur at distances *ca.* 0.1 Å *longer* than normal covalent, single bond lengths. Minor modifications of the thiol position assumed by Topp and Christensen[7] could possibly change their apparent activation energies considerably or even make them disappear completely. The need for a more thorough search of the reaction surface, for a computational procedure more reliable than CNDO/2, and for the inclusion of solvation energies is obvious, if one hopes to predict meaningful activation energies.

The CNDO activation energies of Topp and Christensen were used to draw an interesting comparison of the reactivities of the β-lactam ring of penicillins and cephalosporins.[7] Perhaps surprisingly, the calculations indicated that the 3-cephem nucleus forms a complex with thiol more easily than does penam. This finding was viewed as in apparent contradiction with the intrinsically greater biological activity of penicillins over cephalosporins.[7] The opening of the β-lactam ring in penam was also predicted to be much more exothermic than in 3-cephem. Since their transition state model did not allow the molecular geometry to relax as would occur in the actual transition state, they suggested the possibility of partial compensation of the CNDO activation energies with the energies released by opening the β-lactam ring.[7] Indeed, strain can affect reactivities.[42] However, further studies are needed to prove that such a compensation is appropriate and necessary in the transition state analog approach to structure–activity relationships.

The CNDO prediction that the penam ring system should be more reactive than 3-cephem is in accord with what we encountered previously[9] in EH MO calculations of carbonyl carbon net atomic charges and C–N overlap populations. The apparent anomaly between predicted *reactivities* and observed biological *activities* is somewhat ameliorated by recent hydrolysis rate data.[13] The reactivities of penicillins toward hydrolysis are now known to be quite similar to those of cephalosporins. The relative susceptibility to nucleophilic attack depends on the side chains in the two types of antibiotics. Whether the quantum mechanical results for molecules in a vacuum should better reflect a direct measure of reactivity in an aqueous environment (hydrolysis rates) or an indirect measure in a heterogeneous, partly nonpolar environment (*in vivo* biological activity) is unknown. A reasonable conclusion to draw from the various MO studies[7,9] is that the differences in electronic properties of the β-lactam ring may not be the major determining factor in differentiating penicillin and cephalosporin activities. This conclusion then implicates the reversible, noncovalent transpeptidase-antibiotic binding step,[7,43] or the solubility and permeability in the cell wall membranes,[43–46] or β-lactamase resistance,[2,3] or some combination of various factors as differentiating these two related types of antibiotics. Thus, at least one other factor to consider is how well the antibiotics mimic the natural substrate and are able to compete for the enzyme receptor sites. This brings us to the final topic.

Conformation of *N*-Acetyl-D-alanyl-D-alanine. There has been a variety of proposals on what parts of peptidoglycan is a β-lactam antibiotic mimicking.[4,5,47] Some of these proposals have been discounted or criticized.[4,5,23,48] The one receiving the most current attention is by Strominger,[2,3,5] who pointed out a conformational analogy between penicillin and a D-Ala-D-Ala portion of a polypeptide which cross-links adjacent peptidoglycan polymers. This is reasonable from the point of view that the D-Ala-D-Ala peptide bond is the one that is cleaved, and D-Ala is observed to be liberated in cell wall transpeptidation. Lee[6] refined the conformational analogy by noting that internal rotation about the peptide C–N bond from its normal trans arrangement ($\omega = 180°$) to the dihedral angle present in benzylpenicillin[36] ($\omega = 135.7°$) rendered the structural similarity even more striking. Stereodrawings in the paper by Lee[6] show the effect of changing ω quite clearly. Of great value would be X-ray crystal structure analyses on the relevant bacterial cell wall components to establish the geometry of the penicillin binding sites. Also theoretical studies of the detailed mechanism of peptide bond hydrolysis and transpeptidation would be very desirable. In the meantime, we examine here the electronic changes occurring when a model of D-Ala-D-Ala, **4**, is subjected to internal rotation.

The conformational energy for rotation about the C_5–N_6 bond of **4** as computed by the PCILO method is plotted in Figure 3. PCILO is a very useful quantum mechanical procedure for studying conformational problems[49,50] and is seen in Figure 3 to satisfactorily predict the trans, planar conformation to be most stable. The barrier heights involved in cis–trans isomerization are on the order of 17–18 kcal/mol and are in reasonable agreement with the 20–23 kcal/mol obtained for simple, substituted formamides by *ab initio* calculations,[51] with the 20 kcal/mol adopted in force field calculations,[6,52] and with experimental estimates in the range 17–21 kcal/mol for a variety of substituted amides.[51,53,54] Of primary interest is the fact that PCILO predicts that 8.5 kcal/mol is required to change the conformation of 4 to one resembling penicillin. For this same confor-

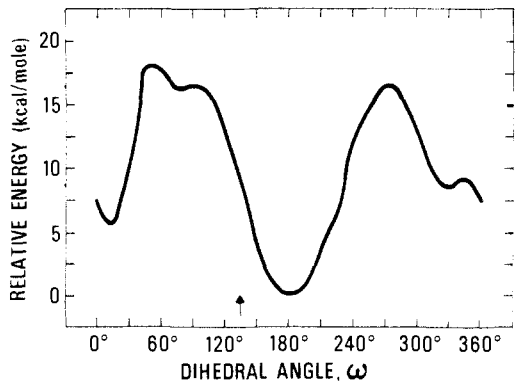


Figure 3. PCILO energy of *N*-acetyl-D-alanyl-D-alanine 4 as a function of internal rotation about C_5–N_6 bond. The energy is relative to that for the most stable conformation, which is the normal planar trans (ω 180°) arrangement. The dihedral angle corresponding to the conformation of benzylpenicillin is indicated by an arrow at 135.7°. The dihedral angle corresponding to the conformation of cephaloridine is 156.8°.

mational change we obtain figures of 9.3 and 7.5 kcal/mol by the CNDO/2 and EH MO methods, respectively. Estimates from classical force field calculations[6,23] have been slightly higher: 10–14 kcal/mol. To twist the dihedral angle from 180 to 156.8°, such as occurs in a Δ^3-cephalosporin cephaloridine, costs only about 2.5 kcal/mol according to the PCILO calculations.

The significance of the above figures is that they show how much energy is required to twist the peptide bond to what may be a transition state in the transpeptidation reaction. As pointed out by Lee,[6] the antibiotic molecules would have an advantage energetically over D-Ala-D-Ala in acylating a transpeptidase enzyme, if the natural substrate must suffer a distortion of the peptide bond before it is broken. The figures also give some indication of the amount of strain which is imposed upon the β-lactam C–N bond by the ring systems of the antibiotics. However, strain within the β-lactam ring itself is expected to make it even easier than in simple amides[55] to bend the exocyclic N_4–C_3 bond of penicillins (N_5–C_4 bond of cephalosporins) out of the plane of the amide group. This arises because the nearly 90° bond angle at the β-lactam nitrogen employs more 2p atomic orbital character in the four-membered ring bonds, leaving more 2s character in the exocyclic bond. A hybrid atomic orbital with considerable s character is less directed and the bond formed by it is more flexible angularly.[56]

That the distortion resulting from the change in ω from 180 to 135.7° in 4 does result in making the peptide bond more reactive is evidenced in Table VI. In terms of electrophilicity of C_5, which is the atom analogous to the β-lactam carbonyl carbon in Strominger's hypothesis,[5] this atom becomes more positive and, hence, more susceptible to nucleophilic attack in the penicillin-like conformation. In terms of the strength of the C–N bond analogous to the β-lactam C–N bond, the overlap population for the C_5–N_6 peptide bond decreases, corresponding to the expected weakening of this bond due to forcing the amide unit into a nonplanar arrangement. Any change in dihedral angle ω would probably be accompanied by certain bond length and bond angle changes when the natural substrate approaches its transition state in the presence of a transpeptidase. More studies are needed in this area, but we anticipate that the transition state of the polypeptide will be found to have a weaker peptide C–N bond than the reactant. Such a weakening is provided by distortions away from planarity, such as are present in the β-lactam antibiotics. The energy required to distort the substrate to a penicillin-like conformation could be derived by the stabilizations achieved by the interaction of the enzyme and substrate at the receptor site. Our present calculational results are at least compatible with a mode of penicillin or cephalosporin action requiring a structural analogy between them and D-Ala-D-Ala.

Table VI. Charge Distributions in *N*-Acetyl-D-alanyl-D-alanine 4 at Two Values of Dihedral Angle ω

	180°	135.7°
Net atomic charge on C_5		
CNDO/2	+0.3413	+0.3438
CNDO/2D	+0.4380	+0.4390
EH	+0.7934	+0.8623
C_5–N_6 overlap population		
CNDO/2D	0.8394	0.8178
EH	1.0752	1.0093

[a] EH values are from a Mulliken population analysis.

Conclusion

Our primary focus in this paper has been on the chemical mechanism of the *in vivo* acylation reaction leading to inactivation of the enzymes essential to bacteria in constructing their cell walls. The picture we obtain of the reaction is somewhat as follows. The nucleophile, such as a thiol group in the enzyme to be acylated (or a hydroxyl ion in base hydrolysis), approaches the β-lactam carbonyl carbon from a direction more or less perpendicular to the β-lactam ring. A negative charge develops on the β-lactam carbonyl oxygen, on C_3 in the dihydrothiazine ring, and on the ester oxygen of the acetoxy group at the 3′ position. The system moves through a tetrahedral transition state complex in which certain bonds are strengthened, notably the C_8–OH^- incipient bond, and other bonds are weakened, notably the β-lactam C_8–N_5 bond and the CH_2–OAc bond. The highly strained transition state complex then relaxes as the β-lactam ring rapidly opens to finalize the acylation or hydrolysis step. The reaction rate should be enhanced if the 3′ substituent is a good leaving group because our quantum mechanical results give much evidence of a concerted reaction linking the departure of a suitable 3′ group with the approach of a nucleophile to the β-lactam ring. Leavability is thus an important attribute in activating the β-lactam ring and may be as important as the inductive ability of the 3 or 3′ substituent. This conclusion, which arises out of CNDO/2 MO calculations on one model 3-cephem, fulfills another goal outlined at the beginning of this paper, namely, to consider what types of chemical modifications on the antibiotics would increase their intrinsic biological activity. Finally, we have briefly examined the structural and electronic analogy between the β-lactam antibiotics and a natural substrate of cell wall transpeptidase to show how certain distortions are consistent with the formation of a transition state of the substrate.

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Pseudosymmetry in the Structure of Luteinizing Hormone-Releasing Hormone Studies on a Series of Novel Analogs

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Pseudosymmetry in the LH-RH structure is described. Eleven analogs of LH-RH (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) have been synthesized by the fragment condensation method and the repetitive excess mixed anhydride method. Multiple substitutions have been made in the LH-RH sequence, which retain the pseudosymmetry of the LH-RH molecule, while presenting fewer problems of synthesis than the corresponding residues in the natural decapeptide. Thus Trp^3, Ser^4, Tyr^5, Gly^6, Leu^7, and Arg^8 residues were replaced by amino acids having similar properties to the residues that they replace. In all but one of the peptides the Gly^{10}-NH_2 residue was replaced by ethylamide, while in the remaining peptide, 1-methyl-5-aminomethyltetrazole (AMT-Me) was substituted at position 10. The compounds were assayed *in vitro* and *in vivo*. The following analogs had *in vivo* and *in vitro* activities in the range 1–28% relative to LH-RH: I, <Glu-His-Phe-Ala-Tyr-Gly-Leu-Arg-Pro-NHEt; II, <Glu-His-Phe-Gly-Tyr-Gly-Leu-Arg-Pro-NHEt; VII, <Glu-His-Phe-Ala-Tyr-Gly-Phe-Arg-Pro-NHEt; IX, <Glu-His-Phe-Ala-Tyr-D-Ala-Leu-Arg-Pro-NHEt; XI, <Glu-His-Phe-Gly-Tyr-Gly-Leu-Arg-Pro-AMT-Me.

Following the isolation[1] and characterization[2] of luteinizing hormone-releasing hormone (LH-RH) as the decapeptide amide, <Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, many analogs have been synthesized and assayed to evaluate structure–activity relationships.[3] Recently we have observed a pseudosymmetry apparent in the LH-RH