Electronic Structures of Cephalosporins and Penicillins. 9. Departure of a Leaving Group in Cephalosporins[1]

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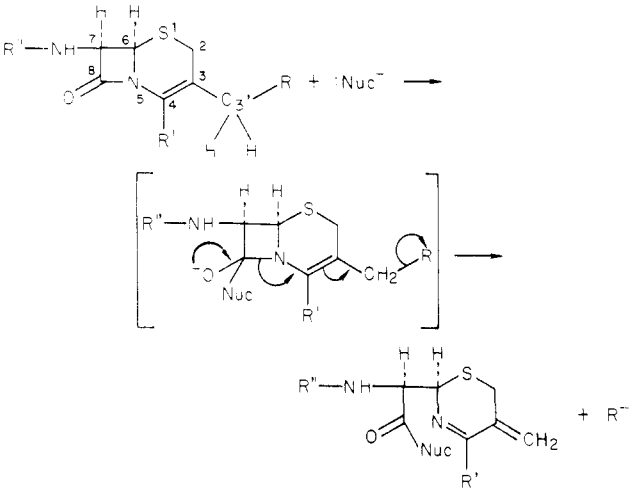
Molecular orbital calculations by the CNDO/2 method are used to study the potential energy surface for the stretching and rupturing of the CH_2–OAc bond in a model cephalosporin structure, 7-amino-3-(acetoxymethyl)-3-cephem. The bond is easier to stretch and break when a nucleophilic group is in the vicinity of or attached to the β-lactam carbonyl carbon (C_8). The rate of acylation by a β-lactam antibiotic at the receptor sites in bacterial cell-wall enzymes will be enhanced by a suitable leaving group at the 3′ position. An orientational specificity is predicted for the direction of departure of the leaving group. Regardless of the direction the nucleophile approaches C_8, the CH_2–OAc bond is easiest to break when the acetate group departs from the α face of the molecule.

It is well established that opening of the β-lactam ring of cephalosporins can be associated with release of a suitable leaving group on the side chain at position 3.[2-15] The reaction with a nucleophile (:Nuc) shown in Scheme I can take place both in vitro (as in base-catalyzed hydrolysis) and in vivo (as in acylation of the carboxypeptidases and transpeptidases regulating peptidoglycan synthesis[16]). Also, in the action of β-lactamases on, for instance, cephalothin, cephaloridine, and a 3-(azidomethyl)cephalosporin the liberation of acetate, pyridine, and azide, respectively, is demonstrable.[3,4,7,8] Even as poor a leaving group as hydroxyl ion is expelled when the β-lactam ring is hydrolyzed *enzymatically*.[4] In fact, because the leaving group R in Scheme I can be liberated inside a bacterial cell wall, it can be selected to have antibacterial properties of its own.[5,13] Thus, not only can the cephalosporin itself act as an antibacterial agent, but it also can be a carrier for another antibacterial agent. Another application of the mechanism in Scheme I is to assay β-lactamase activity with a cephalosporin that upon hydrolysis liberates a leaving group which further decomposes to a fluorescent product.[12]

Electronic details of the departure of the leaving group have not been fully elucidated. It is known that upon ring opening the departure is spontaneous, and therefore it is believed that the two processes occur simultaneously in a concerted mechanism.[3,4,10] Quantum mechanical computer calculations have been brought to bear on this question of concertedness,[17] and several indications were obtained that the mechanism is concerted.[15] These calculations involved using a model nucleophile (OH^-) and a 3-CH_2OAc side chain. When the nucleophile approaches the β-lactam carbonyl carbon (Scheme I), three noteworthy changes occur: (1) the negative charge on the acetate group increases; (2) the CH_2–OAc covalent bond strength decreases; and (3) the difficulty of stretching the CH_2–OAc bond over short distances decreases.[15] Thus, careful quantum mechanical studies are fully consistent with the overwhelming experimental evidence[2-13] that the acetate or other leaving group can be expelled as the β-lactam ring is opened.

The purpose of this paper is to examine more fully the departure of the leaving group in cephalosporins. Molecular orbital calculations will be used to study the stretching of the CH_2–OAc bond in our model cephalosporin structure (Figure 1) not only over short distances but also to complete rupture. The effect of a nucleophile in the vicinity of the β-lactam carbonyl carbon (C_8) on the characteristics of the CH_2–OAc bond will be investigated. Corroboration is found for the idea that the approach of the nucleophile and departure of the leaving group are interrelated. In addition, an interesting specificity for the direction of departure is found which may have implications for the spatial requirements of the receptor site in bacterial cell-wall enzymes.

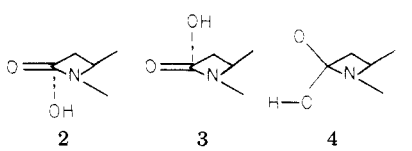
Scheme I



Experimental Section

The calculational experiments use the same methodology as described previously.[15] Potential-energy curves are constructed from CNDO/2 total energies.[18] This MO method is known to exaggerate certain dissociation energies and force constants for bond stretching,[15,19,20] but it should work satisfactorily for obtaining *relative* energies of the various modes to be investigated.

Molecular geometries and related assumptions for the model cephalosporin structures are the same as described before.[15] Besides the parent structure 1 (Figure 1), three hypothetical model transition-state forms will be used. (The transition states to which we refer are the intermediate structures involved in the acylation by or hydrolysis of the β-lactam ring.) Two of these involve a nucleophile situated perpendicularly above C_8, which is held fixed with its sp^2 hybridization. Depending on whether the nucleophile is 1.50 Å away from the α or β face of C_8, the structures are labeled



2 3 4

2 or 3, respectively. A third transition state has roughly sp^3 hybridization at C_8 and is labeled 4. The remainder of structures 2–4 not shown is the same as that for 1.[15]

The model nucleophile used in the calculations is OH^-. Although early studies[21] led to the idea that a sulfhydryl may be the nucleophile in the receptor sites of peptidoglycan-regulating enzymes, there are now several studies[22] pointing toward serine as the residue in the active sites (laniary pockets) which binds to the β-lactam antibiotics. Hence, use of hydroxyl ion is an appropriate model for both the enzymatic and nonenzymatic base-catalyzed processes.

In this paper, we will not discuss the mechanism of hydrolysis of cephalosporins with position 3 side chains lacking leaving

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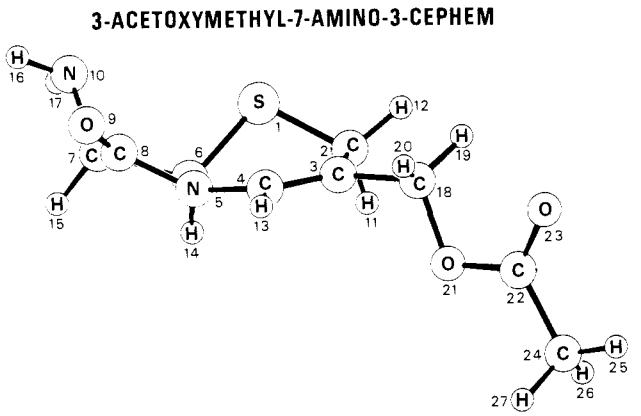


Figure 1. Structure and numbering system used for 7-amino-3-(acetoxymethyl)-3-cephem (1). As shown, the α face of the molecule is down and the β face is up.

groups. Compounds such as cephalexin (3-CH_3), cephradine (3-CH_3), and cefaclor (3-Cl) undergo β-lactam hydrolysis by interesting mechanisms, some leading to intact dihydrothiazine rings and others leading *eventually* to substitution of the group at position 3 by hydrogen.[23-25] Also, outside the scope of this paper is the reaction mechanism wherein a 3′-acetoxy group is displaced by various sulfur and nitrogen nucleophiles. The displacement mechanism being S_N1 involves an allylic carbonium ion which is stabilized enough within the dihydrothiazine ring such that β-lactam ring opening is not prevalent.[26]

Results and Discussion

Energetics of Expulsion. Potential-energy curves for stretching the CH_2–OAc bond of **1**, **2**, and **4** are shown in Figure 2. The curve for **3** is very similar to that for **2**, except for a vertical displacement (of less than 0.04 au). The energy minimum in each curve occurs at 1.39 Å, which is about 0.04 Å shorter than a standard[27] C–O bond length. This agreement is about as good as one anticipates from CNDO/2.[28] Not unexpectedly, the energy of each model rises steeply until the curves asymptotically approach the sum of the energies of the infinitely separated fragments, the acaudal 3-cephem (with or without OH^- near C_8) and OAc^-. Also as expected, the cephem fragment with a planar 3-CH_2 geometry is predicted to be more stable (by 15–35 kcal/mol) than the geometry with fixed tetrahedral C–C–H bond angles in the 3-methylene moiety. All other geometrical variables were held constant as in earlier calculations.[15] The net reaction in Scheme I is predicted by CNDO/2 to be endothermic in the gas phase because the sum of the total energies of the infinitely separated reactants (i.e., the minimum in the top curve in Figure 2) is below the lowest sum of the energies of the infinitely separated fragments treated here. Relaxation of the bond lengths and angles in the "carboxyl" portion of **4** to more appropriate values for a ring-opened structure might lower the total energy of the final products to a point below that of the reactant molecules.

The gap between the energy minimum and the total energy at infinite separation is seen to be greater for the uncomplexed 3-cephem **1** than for the transition-state models **2**–**4**. The gap amounts to 400–500 kcal/mol for **1** and only 300–400 kcal/mol for **2**–**4**. These gaps are obviously greatly overestimated (by a factor of at least three) because of the known characteristics of the CNDO/2 method and because of the neglect of solvation effects.[15] Also, use of more realistic molecular geometries for the transition-state species **2**–**4** would tend to lower the gaps for these still further. The important point is that on a *relative* basis the CH_2–OAc linkage is much easier to stretch and requires less energy to break when a nucleophile is impinging on the β-lactam carbonyl carbon.

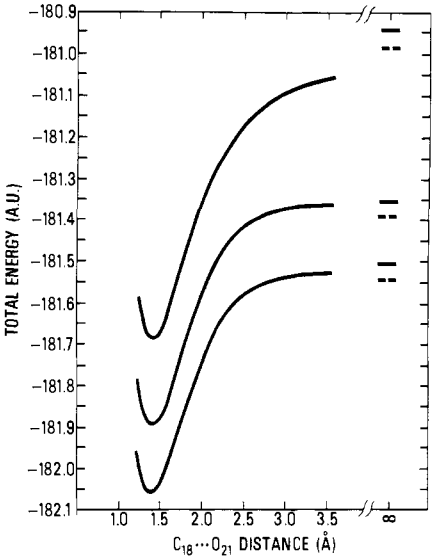


Figure 2. CNDO/2 total energy (in atomic units) of 7-amino-3-(acetoxymethyl)-3-cephem structures as a function of CH_2–OAc (C_{18}–O_{21}) bond length. The top curve is for **1** and has the energy of an infinitely separated OH^- (−18.8996 au) added on so as to be comparable to the other curves. The middle curve is for transition state **2** (OH^- on the α face of C_8). The bottom curve is for the tetrahedral transition state **4**. To the right is shown the sum of the energies of the infinitely separated fragments for each of the three structures. The energy of OAc^- is −53.1566 au. The solid line segments at ∞ correspond to the cephem fragment with the 3-CH_2 stub in a fixed sp^3 geometry. The broken-line segments correspond to the cephem fragment with the 3-CH_2 stub relaxed to a planar sp^2 geometry. 1 au = 627.54 kcal/mol.

Regardless of the direction from which the nucleophile approaches[29] and regardless of the hybridization at C_8 in the transition-state species, the feeding of electrons into the 3-cephem system at the β-lactam ring makes it easier for a leaving group at the 3′ position to depart with a negative charge. Besides having the orbitals necessary for transmitting the electron density between the β-lactam, enamine, and 3-CH_2R groups, another reason for the relative ease of breaking the CH_2–OAc bond in **2**–**4** is related to electrostatics. It is easier to break OAc^- away from an anionic parent (**2**–**4**) than from a neutral parent (**1**) because the latter would leave behind a cation. In Figure 2, the vertical gap of 0.07–0.11 au between the top curve (for **1**) at 3.5 Å and the curve segments at infinite separation is related to the contribution from electrostatic attraction. Solvation effects, if they were includable in the calculations, would modulate somewhat the attraction.

Correlation diagrams have been constructed by plotting the one-electron energies (eigenvalues) of the individual MO's as a function of CH_2–OAc stretch.[30] These show that the filled MO's stay filled and the empty MO's remain empty throughout the 1.2–3.5 Å range. There is no crossing of the filled and empty MO levels, which means that the process is allowed in the Woodward–Hoffmann sense.[31]

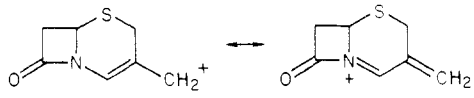
It may be noticed that the 4-carboxyl group of cephalosporin antibiotics is replaced by hydrogen in our model structures **1**–**4**. It has been ascertained[32] that such a change does not affect our conclusions because the energetics, as well as the charge distributions (which are discussed next), are affected about equally in all four models **1**–**4**.

Charge Redistributions during Departure. The trends in net atomic charges and bond-overlap populations that were apparent in limited earlier work[15] are verified.

Representative results are given in Tables I–IV (see paragraph at end of paper regarding supplementary material). For all structures 1–4, the acetate oxygens O_{21} and O_{23} (Figure 1) become more negatively charged as the CH_2–OAc bond is stretched. At 3.5 Å, the CNDO/2D[15] net charge on the acetate group as a whole is about −0.57 in 1 and −0.99 in 2–4. Thus, in the stretched transition-state species most of the anionic charge resides on the leaving group. As a whole, the acetate group gains about 0.8 e in going from 1.39 Å to infinite C_{18}--O_{21} separation in 1–4.

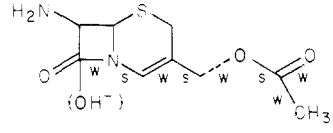
Attention should be called to the fact that the β-lactam carbonyl carbon C_8 of 1 becomes more positive as the acetate group pulls away (Table I). Thus, interactions between a cephalosporin and the functionalities of the receptor sites of transpeptidases and carboxypeptidases, which would tend to stretch the 3-CH_2–R bond, would tend to make C_8 more electrophilic and hence promote the ability of the β-lactam to act as an acylating agent.

Another interesting finding in the charge distribution is that C_{18} (Figure 1) gains, rather than loses, electron density as the CH_2–OAc bond is stretched. Upon complete removal of the acetate group, C_{18} is nearly neutral in 1 and bears a small negative net atomic charge in 2–4. Thus, the following resonance structure on the left is not important.

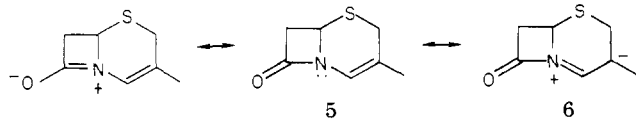


However, the cationic charge does not reside solely on N_5 either but rather disperses over the entire structure in accord with electrostatic principles.[15] For instance, in 1 both N_5 and O_9 become less negatively charged and S_1 and C_3 lose electron density as the CH_2–OAc bond is stretched. In 2–4, N_5, O_9, S_1,[33] and C_3 also lose electron population as the acetate group is pulled away.

Mulliken overlap populations computed from the CNDO/2D MO's are a measure of covalent bond strength.[15,34] The principal trends in the overlap populations for each bond are shown below. Bonds that



strengthen as the CH_2–OAc bond is stretched in 1–4 are marked with "s" and those that weaken with "w". Because the CH_2–OAc bond is the one being ruptured, the overlap population for the C_{18}–O_{21} bond shows the largest change, decreasing to zero at infinite separation. In addition, C_8–N_5 and C_4–C_3 weaken by relatively large amounts (ca. 15–30%), and C_3–C_{18} strengthens by a relatively large amount (ca. 15%). These changes fit into an alternating pattern of weakening and strengthening along the backbone from C_8 to the acetate group. The changes will be recognized as consistent with amide 5 and enamine 6



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resonance, which have the effect of making certain single bonds stronger and the C_3=C_4 double bond weaker. The redistributions are also in the same direction as the changes that occur when a nucleophile approaches the β-lactam carbonyl carbon.[15] Thus, the overlap populations, as well as the net atomic charges and the energetics, are consistent with a coupling of the departure of the leaving group and the opening of the β-lactam ring.

Experimental Evidence Related to Leavability. Because of the coupling of electron shifts, one may expect that the leavability[35] of the 3′ substituent in cephalosporins will influence somewhat the reactivity of the β-lactam ring and hence also the intrinsic antibacterial activity.[15] On the other hand, the logarithms of experimental nonenzymatic hydrolysis and aminolysis rates of four cephalosporins have been found to correlate roughly linearly with inductive substituent constants, σ_1, for their 3′ substituents.[10,36] At first sight, these last-mentioned experiments make it look like factors other than induction, such as leavability, contribute negligibly to reactivity. However, there are several factors which raise questions about this surmise.

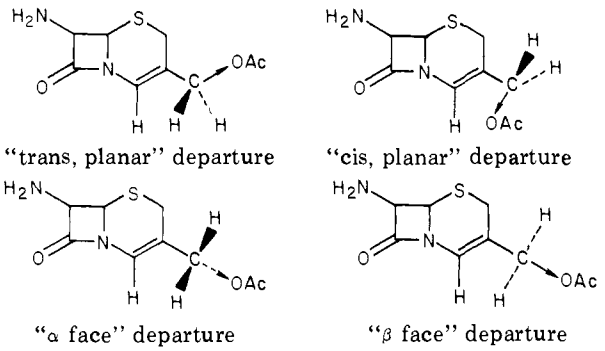
First, those studies[10,36] included only two cephalosporins having position 3 side chains containing leaving groups (acetoxymethyl and pyridiniummethyl) and only two not having good leaving groups (3-CH_3 and 3-CH_2OH). With such a small sample of compounds, it is possible that the occurrence of a single correlation equation may be merely fortuitous. Testing more cephalosporins in the future may reveal that the two types (those with and without good leaving groups) fit lines of different slope when rates are plotted against σ_1 values (or other measures of charge distribution). Already, the recently reported hydrolysis rates of 3-chloro-3-cephems[25] suggest that the slope originally published for the four cephalosporins[36] needs at least some revision or, more likely, that separate lines need to be drawn through the data points for leaving and nonleaving groups. After taking into account the fact the 3-chlorocephalosporins do not have an insulating[37] methylene bridge at the 3′ position, the rates of hydrolysis of the 3-Cl compounds are considerably slower than expected from the published σ_1 vs. log rate plot.[25,36] In other words, those cephalosporins *with* a 3′ leaving group appear to be more reactive than anticipated from the inductive effect alone.

A second factor is that the σ_1 values have a rather large uncertainty. For instance, the unavailability of a published σ_1 value for pyridinium led both Bundgaard[10] and Indelicato et al.,[36] to turn to $N(CH_3)_3^+$ as an approximation. However, in one case 0.73 was selected and in the other case 0.92 was used. Both values are in various tabulations.[37] Thus, the σ_1 value used for pyridinium has an uncertainty of at least 20%. Futher uncertainty can arise when σ_1 values for 3′ substituents are compared to σ_1 values for direct 3 substituents.

A third factor is that the hydrolysis rates themselves[10,24,36] have rather large uncertainties both in absolute and relative terms. The rates depend on the experimental technique used to measure them[24] and on what account is taken of minor side reactions. For instance, the ratio of the hydrolysis rates at about pH 10 of cephaloridine and cephalothin is variously reported as 1.9:1 (UV[36]), 5.4:1 (UV[10]), 4.9:1 (high-pressure liquid chromatography[24]), 3.6:1 (UV[24]), and 7.4:1 (iodometry[24]). These two antibiotics have the same 7-thiopheneacetamido side chain and differ only in the 3′ substituent, pyridinium or acetoxy. Similar uncertainties can also be seen to exist in the hydrolysis rates[24] of 7-(phenylglycyl)cephalosporins.

The importance of the inductive effect of the 3′ substituent should also be recognized. In fact, the significant role of induction was pointed out in the earlier study.[15] Induction and leavability are not two mutually exclusive effects. Induction should be important in the initial stage of reaction when the electrophilicity of the β-lactam

Scheme II



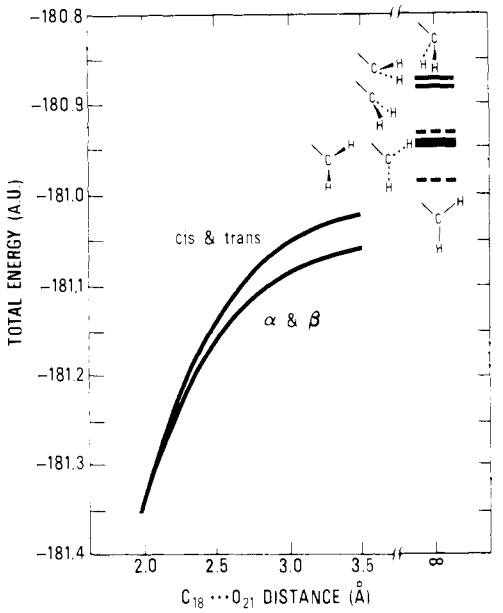


Figure 3. CNDO/2 total energy (in atomic units) of 7-amino-3-(acetoxymethyl)-3-cephem (1) plotted as a function of CH_2–OAc bond length for four modes of departure. As in Figure 2, the energy of infinitely separated OH^- is included to make the ordinate comparable to that of the other figures. On the right is plotted the sum of the energies of the infinitely separated cephem and OAc^- fragments, with solid lines for a fixed, tetrahedrally hybridized 3-methylene geometry and dashed lines for a planar 3-methylene. Extra-thick line segments correspond to two geometries with identical or similar energies. The sketches show the geometries of the 3-CH_2 stub on the cephem fragments.

carbonyl carbon attracts at long range the nucleophile involved in hydrolysis or acylation. Leavability should be important as soon as the mutual interaction between the nucleophile and the β-lactam is significant and the electrons begin shifting through the 3-cephem nucleus. Both effects will serve to lower the activation energy for the rate-determining step, which is presumably the addition of the nucleophile to the β-lactam carbonyl carbon. The relative importance of the two effects in lowering the energy of activation may be different for nonenzymatic hydrolysis, for β-lactamase-catalyzed hydrolysis, and for acylation of sensitive bacterial enzymes.

Obviously, in order to resolve experimentally the question of how much leavability contributes to β-lactam reactivity, more experimental data are needed than heretofore published. Also, highly accurate data are required. In order to sort out the effects of leavability and induction, it may be necessary to work with a series of closely related 3′ structures[38] that have similar inductive effects. Then any differences in reactivity would presumably be due to leavability.

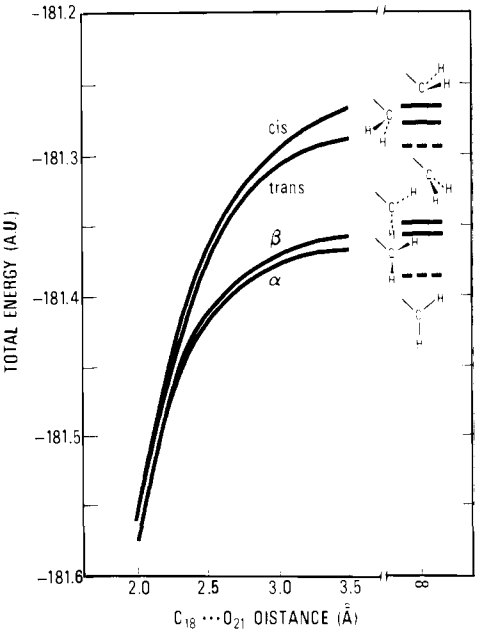


Figure 4. CNDO/2 total energy (in atomic units) of 7-amino-3-(acetoxymethyl)-3-cephem with OH^- on the α face of C_8 (2) plotted as a function of CH_2–OAc bond length for four modes of departure.

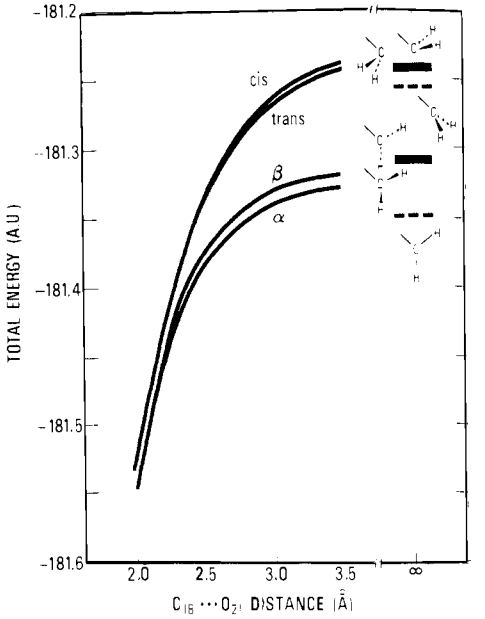


Figure 5. CNDO/2 total energy (in atomic units) of 7-amino-3-(acetoxymethyl)-3-cephem with OH^- on the β face of C_8 (3) plotted as a function of CH_2–OAc bond length for four modes of departure. As denoted by the extra-thick lines on the right, there are two cases of two cephem fragment geometries having the same energy.

Orientation of Departure. So far in our calculations and discussion, the conformation of the position 3 side chain is as shown in Figure 1. That is, the acetate group is directed "down" from the α face of the bicyclic nucleus. We have done additional CNDO/2 MO calculations which show that this is the preferred orientation for departure of the leaving group. These calculations are described next.

Four modes of departure of the acetate group of **1**–**4** were investigated. These modes are shown in Scheme II. Depending on the rotation about the C_3–$C_{3'}$ bond, the C_4C_3–$C_{3'}$–O dihedral angle is 180° (trans, planar), 0° (cis, planar), 90° (α face), or 270° (β face) in the four modes of departure.

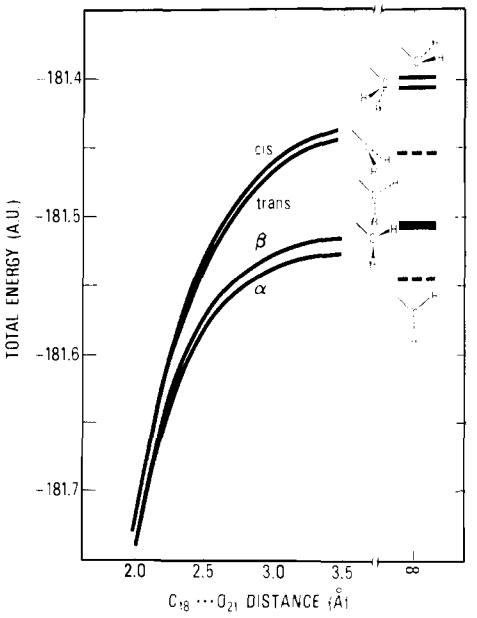


Figure 6. CNDO/2 total energy (in atomic units) of 7-amino-3-(acetoxymethyl)-3-cephem with OH^- attached at C_8 with tetrahedral hybridization (**4**) plotted as a function of CH_2–OAc bond length for four modes of departure.

The C_{18}–O_{21} distance (numbering system in Figure 1) is varied from 1.2 Å to infinity. As in our previous calculations, all geometrical variables are held fixed, unless otherwise apparent. Plotted in Figures 3–6 are the CNDO/2 total energies[18] of structures **1–4**, respectively. Except for the fact that the cis, planar conformation is 1–2 kcal/mol more stable at the equilibrium CH_2–OAc distance near 1.4 Å, the curves for all structures below 2.0 Å are practically identical and so they are not shown. On the right-hand side of each figure is the sum of the energies of the infinitely separated cephem and OAc^- fragments. Also shown are the orientations and geometries of the 3-CH_2 stubs of the cephem fragments. In all cases, the planar sp^2 geometry with 120° C–C–H bond angles (dashed line segments) is more stable than the sp^3 geometry with 109.4712° C–C–H bond angles (solid line segments).

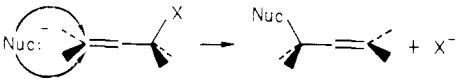
The curves for the reactant model **1** (Figure 3) show that departure of the 3′ group from the α and β faces are essentially equally difficult out to 3.5 Å. More difficult are the cis and trans planar modes, which are about equal in terms of energy cost to stretch the CH_2–OAc bond. Thus, when there is no nucleophile, such as OH^-, attacking the β-lactam ring, the α mode is almost no more difficult than the β mode, and the cis mode is almost no more difficult than the trans mode.

Figures 4–6 show that when there *is* a nucleophile near or attached to C_8 of the β-lactam ring, the α and β modes are differentiated; likewise, the cis and trans modes are differentiated. Departure from the α face is easiest; departure from the β face is a close second easiest; and cis, planar departure is most difficult. Thus, the breaking of the CH_2–OAc bond is predicted by CNDO/2 to be under stereoelectronic control when the β-lactam ring is opening in a concerted manner. As seen in Figures 4–6, differentiation of the four modes of departure in the transition-state structures **2–4** becomes noticeable in the 2.5–3.5 Å range. The overlap populations in Tables III and IV (supplementary material) show that only vestiges of covalent C–O bonding remain at these separations. The energetics of depature from the α and β faces are so close that they may be essentially equal in an in vivo situation where extensive enzyme interactions are operable. The key point is that perpendicular departure (α or β) is considerably easier than the trans or cis planar cases.

To understand the reason for the orientational specificity of departure, one can examine the orbitals of the cephem fragment. In all cases (Figures 3–6), the methylene stub prefers to be coplanar with the enamine atoms. Thus, the empty p orbital on the methylene carbon is roughly perpendicular to the six-membered ring and can overlap with and accept electrons from the enamine orbitals, as well as the β-lactam orbitals. The ability of the system to feed electrons from the β-lactam ring into the developing empty p orbital makes the α and β modes of departure preferred over the cis and trans planar modes. Because the β-lactam and enamine moieties are not exactly coplanar,[15,39] it is not possible to differentiate the σ and π electrons involved in the transfer. However, that additional electron density which does appear on C_{18} is π type. This is shown by electron density maps[32,40] computed from the CNDO/2D wave functions of the acaudal fragments from **2–4** compared to that for the cephem fragment of **1**.

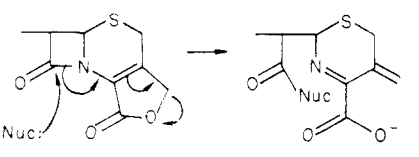
In the cephem system the leaving group experiences a *slight* preference to depart from the α face rather than the β face of the molecule regardless of the exact geometry at the β-lactam carbonyl in transition-state structures **2–4** (see Figures 4–6). One can think of **2–4** (but not **1**) as having a distorted carboxyl carbon C_8 impinging at a severe nonbonded distance on N_5 of the dihydrothiazine–exomethylene system. The C_8–N_5 interaction is transmitted to the C_{18}–O_{21} region. Based on calculations on a simplified model,[41] electron density between C_{18} and O_{21} would be reduced more when O_{21} is on the α face than when it is on the β face. Lowered electron density means less electron repulsions and, hence, greater stability for the transient species while OAc^- is departing from the α face. Because this particular stereoelectronic effect arises from the proximity of a carboxyl-like carbon (C_8) near N_5, one would expect the effect to hold only in the early stages of the reaction of Scheme I.

Although vaguely reminiscent of the S_N2' displacement reaction,[42] the mechanism in Scheme I is not the same. In the abnormal bimolecular substitution reaction, the nucleophile (Nuc:) can approach either the α or β face of the



allylic system. Originally, it was believed that the leaving group (X) departed from the same side that the nucleophile approaches, i.e., a syn relationship. Now, of course, it is known that either the syn or anti mechanism can hold depending on the nature of the displacing and departing groups.[42]

A case where the influence of the orientational specificity may be felt is in the cephem lactones. In these, the leaving group is, in effect, constrained by the geometry[43] of the molecule to leave in the cis, planar mode of Scheme II as shown below. The stability[4,9,24,36] of these γ-lactones and



their diminished antibacterial activity[44] may in part be related to the stereoelectronic effect. The stereoelectronic effect appears to more than compensate for the ring strain which would tend to favor opening of the lactone.

An orientational specificity for the mechanism in Scheme I would have some possible implications in regard

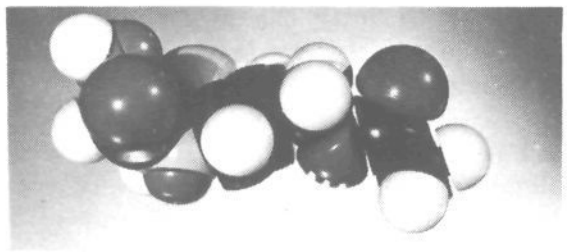


Figure 7. Space-filling model of 7-amino-3-(acetoxymethyl)-3-cephem (**1**) viewed on the side with the β-lactam carbonyl oxygen (seen at the left). The position 3 side chain on the right is oriented for greatest ease of breakage of the CH_2–OAc bond according to the CNDO/2 calculations. A 4-carboxyl group, which is replaced by hydrogen in the model structure and in the calculations, would not preclude the conformation shown.

to biological activity of cephalosporins. For instance, in the receptor sites of the enzymes regulating peptidoglycan biosynthesis, a residue or residues of the protein might interact with the 3′ substituent, orient it toward the α or β face, and make the bond to the leaving group easier to break. With the space-filling[45] model of **1** shown in Figure 7, one can envision various intermolecular interactions around the acetate group which would lead to the optimal conformation for release. By having the position 3 side chain suitably oriented, the reactivity[15,46] of the β-lactam ring would be enhanced for acylation of the bacterial enzymes. Moreover, if those interactions had the effect of slightly stretching the 3-CH_2–R bond, such as by electrostatic attraction to R, then the 3-cephem would be primed for reaction at the β-lactam ring.

One test for manifestations of the predicted orientational specificity, as well as the leaving-group mechanism, will come when the receptor sites of carboxypeptidase, transpeptidase, and/or β-lactamase are elucidated by X-ray crystallography.[47] It will be of interest to see if there are protein functionalities in the receptor sites which can serve to complement the inherent electronic characteristics of cephalosporin antibiotics.

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Supplementary Material Available: Tables I–IV of net atomic charges and overlap populations in **1** and **2** (4 pages). Ordering information is given on any current masthead page.

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(30) The lowest empty MO has S–C σ* character for the unstretched species (**1**–**4**) and acquires π* character delocalized over the enamine and 3-CH_2 moieties by the time the CH_2–OAc is stretched to 3.5 Å. The highest two or three occupied MO's in the unstretched species are enamine and amide (and, for **2**–**4**, hydroxyl) orbitals somewhat like the extended Hückel MO's for 3-cephem described by D. B. Boyd, *J. Am. Chem. Soc.*, **94**, 6513 (1972). At 3.5 Å, the high-lying filled MO's have become localized to various extents on the acetate group.
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(33) The redistribution at S_1 is particularly interesting because when OH^- attaches to **1** to form **2**–**4**, electron density at S_1 increases (changing the net atomic charge at S_1 from weakly positive to weakly negative; see ref 15). Thus, some of the electron density from OH^- which could go to the position 3 side chain, in fact, ends up on sulfur (which is a relatively good electron acceptor). It is found (ref 32) that when the sulfur of the 3-cephem nucleus is replaced in the calculational models by first-row atoms (or their hybrides), then somewhat more electron density can be shifted to the position 3 side chain upon attachment of the OH^- nucleophile to the β-lactam carbonyl carbon.

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(41) The conjugated N_5=C_4=C_3=C_{18} portion of **2**–**4** can be *rudimentarily* modeled by *trans*-1,3-butadiene. CNDO/2 calculations were done on butadiene with CO_2 near the β face of C_1 and OH^- near either the α or β face of C_4. CO_2 (representing the COOH part of **2**–**4**) was placed in the plane bisecting the H–C_1–H bond angle such that the carbon of CO_2 is only 2.0 Å from C_1 and C--C_1=C_2 equals 135° and O=C--C_1 equals 90°. The OH^- (representing OAc^-) was placed in the plane bisecting the H–C_4–H bond angle such that C_4--O is 3.5 Å and C_3=C_4--O equals 109.4712°. C_4 of butadiene was taken to be either planar or pyramidal. With this simplified model, the essential features of the cephem system are isolated for scrutiny and computer times are reduced. Significantly, the most stable arrangement has CO_2 and OH^- on opposite sides of the C_1=C_2–C_3=C_4 plane. This configuration corresponds to the cephem situation where OAc^- is departing from the α face of C_{18} and, hence, anti to C_8 near N_5. Electron-density maps of the butadiene complexes and its substructures were obtained from CNDO/2D wave functions (ref 32 and 40). Difference density maps were calculated by subtracting from the valence-electron density of each complex the sum of the valence-electron densities of the noninteracting substructures using the same atomic coordinates as in that complex. These plots show that perturbation of the electron density in the C_3=C_4 π region due to CO_2 is relatively small but that due to OH^- is large. Interelectron repulsion is the dominant effect when OH^- is present: electron density is significantly reduced on whichever face of C_4 is closest to OH^-. The reduction in the C_4--OH^- region is a little greater when OH^- is on the α face, which would be anti to the CO_2 on the β face of C_1. Thus, electron density between OH^- and butadiene is lower when CO_2 and OH^- are on opposite faces. Lowered electron repulsions are consistent with the anti configuration being more stable.
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Mathematical Considerations in Series Design

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It is proposed that a series designed to explore the potential of a "lead" should have the following characteristics: (1) the analogues should be synthetically feasible, (2) the series should contain enough variation in the properties which may influence potency, (3) these properties should be varied independently of each other, and (4) the series should be the minimum acceptable size, i.e., each analogue should contribute unique information. Point 2 is evaluated by a consideration of the definition of R^2. As a rule of thumb, the standard deviation of a property should usually be ≥ 1.0. Point 3 is evaluated by analyzing the correlation matrix of properties. If it has fewer significant eigenvalues than properties, then factor analysis reveals which properties are artificially correlated. Point 4 is evaluated by distance between analogues in property space. In order to be certain that the proposed molecular descriptors are independent, a large data set of possible substituents was analyzed. Factor analysis of the physicochemical properties of 78 aromatic substituents revealed that π, S, F, and MR are orthogonal descriptors. The proposed criteria have been applied to series designed by cluster analysis, multidimensional nonlinear mapping, Topliss batch methods, and to two Abbott series. The other mathematical methods of series design suffer from their lack of attention to all four points simultaneously.

If one accepts the premise that the biological properties of organic molecules are a direct consequence of their chemical and physical properties, then it becomes possible to propose strategies to make the process of drug discovery more efficient. This report suggests a method of evaluating the suitability of a set of analogues which have been proposed to follow up a lead. Synthesis and testing of this set of analogues will adequately explore the effect of variations in those properties used in its design. Hence, if it is found that this series does not possess or suggest analogues of sufficient potency, one can confidently decide not to explore further variations of these properties. Thus, the method is useful in both initial series design and in the decision to terminate further synthesis.

The proposed criteria for a suitable set are as follows: (1) It will be the smallest size consistent with the objectives of the synthetic program. (2) Those chemical and physical properties which are hypothesized to determine biological potency or profile will be varied widely enough that it is theoretically possible to find a useful relationship. (3) The series will exhibit variation in these molecular properties in such a way that the variation of each property is independent of the variation in all other properties. The two latter characteristics assure that the data space has been adequately explored. (4) It will contain only analogues which are not too difficult to synthesize. This point will

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