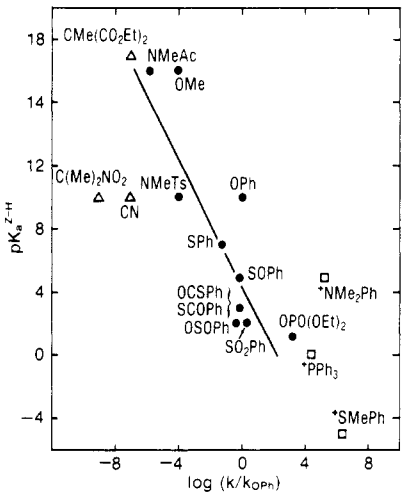
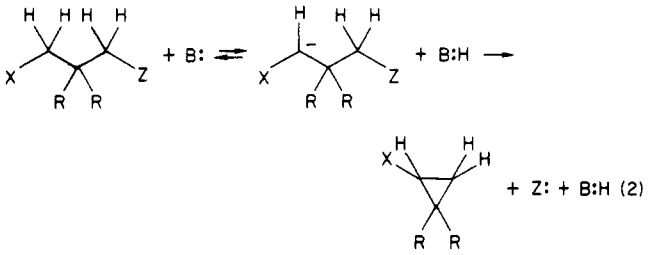


Figure 1. pK_a of the conjugate acid of the leaving group and relative reaction rates for 1,2-elimination. The regression line is fit to the data for eleven Z groups (solid circles). Two of these points coincide. The rates for the three Z groups linked through carbon (open triangles) were only determined to be *less* than the value plotted. The three onium Z groups are denoted by open squares.

lation between leaving-group ability and pK_a does exist for 1,3-eliminations (2) and that even in 1,2-eliminations good



correlations exist if the variation in the leaving group is small. In the present study, regression analysis is used to show that for a broad range of leaving groups in reaction 1 a relationship between acidity and reaction rate can exist.

Let us reexamine the data of Stirling et al., for the case of 1,2-elimination (1), which was the basis of their original conclusion.[2] Reaction rates relative to that for Z = OPh were reported for 19 Z groups. The data were incomplete or not exactly quantitative for five of the Z groups. Data were reported for only three onium groups (Z^+), which is too few to conclude anything statistically. For the remaining eleven Z groups, regression analysis can be done to find the following correlation with pK_a^{Z-H}. The sta-

$$\log (k/k_{OPh}) = -1.96pK_a^{Z-H} + 4.84 \qquad (3)$$

$$n = 11 \quad r = -0.85 \quad r^2 = 0.72 \quad s = 3.0$$
$$p = 0.0009$$

tistics clearly reflect the scatter of the data points as seen in Figure 1. The standard error of estimate of 3.0 is large. However, the variation in pK_a^{Z-H} is, nevertheless, able to explain 72% of the variance in the relative reaction rate, and the correlation is statistically significant with greater than 99% probability of not being fortuitous. Note that the regression includes a diverse set of Z groups linked through C–N, C–O, and C–S bonds. Qualitatively similar results are obtained by plotting nucleofugality ranks[3,6] against pK_a^{Z-H}.

Whereas Figure 1 shows that relative leaving-group ability (or nucleofugality[3,6]) does not vary monotonically with acidity of Z:H, the trend is apparent and in the direction expected. Even though not all data points shown in Figure 1 could be included in the regression, it is noteworthy that the excluded points roughly follow the trend. Of course, it is always possible to select a few isolated points from a figure like Figure 1 to claim there is no simple correlation.

Acids Z:H that dissociate more easily can, in some instances, correspond to leaving groups that promote the elimination reaction. It is true, based on the published data,[2] that the nucleophilicity of Z: is not related to rate of elimination. The important property of the leaving group is being able to stabilize the electron density transferred to it in the course of the reaction.[7-9] The leaving group must be able to act as an electron sink in the transition state for elimination. Thus, properties like inductive effect, electron affinity, and polarizability of Z can be expected to be related to leaving-group ability.

Acknowledgment. W. H. W. Lunn and C. J. M. Stirling provided helpful comments on the manuscript.

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Elucidating the Leaving Group Effect in the β-Lactam Ring Opening Mechanism of Cephalosporins

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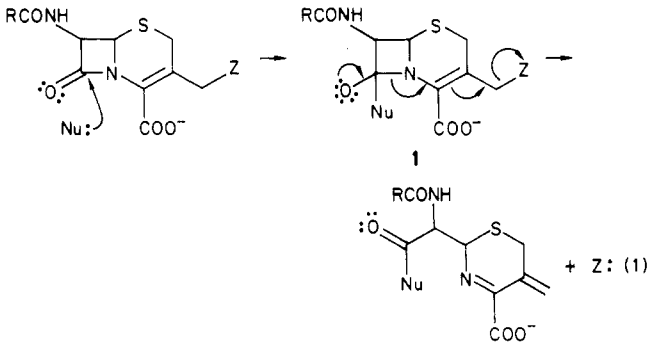
Antibacterial activity of cephalosporins against sensitive Gram-negative pathogens is observed to be better when there is a potential leaving group at the 3′ position.[1-4] One possible explanation of this fact is that when the serine hydroxyl group in the active site of the target enzymes is acylated by the β-lactam ring, acylation proceeds at a rate influenced by both the inductive effect and leaving group ability of Z. The neighboring-group participation of Z can be denoted summarily by 1. However, as has been care-



fully pointed out before,[1,2,4] mechanism 1 can take place

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with formation of a tetrahedral intermediate. The first step may be reversible.

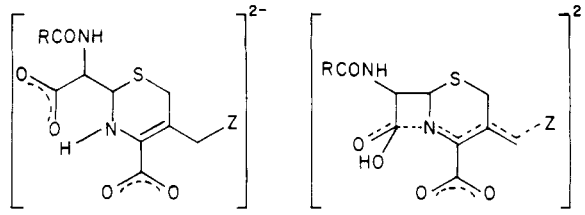
In the case of nonenzymatic hydrolysis of the β-lactam ring, it is known that the relative rate of ring opening is almost entirely explained by the inductive effect of Z for a series of closely related cephalosporins.[5] The Z group, in effect, acts as an electron sink whether or not it actually undergoes elimination. In contrast, at the active site of the bacterial cell wall enzymes inhibited by β-lactam antibiotics, the presence of a suitable leaving group in a cephalosporin may speed covalent binding and/or delay breakdown of the acylated enzyme. As far as interference with biosynthesis of cell wall components is concerned, the important thing is to form the covalently bound enzyme complex, EI*, and delay turnover of the free enzyme E.

$$E + I \overset{K}{\rightleftharpoons} E{\cdot}I \xrightarrow{k_3} EI^* \xrightarrow{k_4} E + P \qquad (2)$$

Within the context of the active site process, mechanism 1 is *concerted in the sense defined by Lowe*[6] (this is the only meaning used in prior papers[2,4] for the term "concerted" as was pointed out in those papers). Lowe described a reaction as being concerted if some bond undergoes breakage while a second bond is formed, so that the concertedness of a reaction relates to the variation in the geometrical parameters of the molecule along the reaction coordinate and does not say anything about the energy profile along the minimum energy pathway across of the reaction energy hypersurface. A reaction concerted by Lowe's definition can involve an intermediate.

It is not surprising that an intermediate in the hydrolysis or aminolysis of cephalosporins exists. In the nonenzymatic reaction, the presence of an intermediate is inferred from kinetic parameters.[7] In some β-lactamase catalyzed hydrolyses, an intermediate is also spectrophotometrically detectable, although not yet identified.[8] These experimental observations relate to the energy profile of the reaction.

How to best represent on paper the intermediate is a matter not resolved. One way would be **2**, but **3** may be



2 **3**

justifiable and has the same number of hydrogens. Quantum mechanical calculations by the MNDO molecular orbital method have shown that structures of the type **2** and **3** both correspond to minima on the gas-phase reaction energy surface.[9] After Z departs, the residual structure usually undergoes further rearrangements. Reports in the literature of experimental identification of the degradation products from the cephem nucleus have been infrequent.[10]

Returning to the matter of concertedness (in Lowe's sense) of β-lactam ring opening and the effect of a potential leaving group, it is of interest to examine crystallographic data on cephalosporins. Solid state data can sometimes be helpful in providing insight into dynamic chemical processes.[11] Mindful of our goal of understanding the mechanism in the enzymic environment, it is extremely doubtful whether other crystal lattices will provide a similar milieu.

There are 15 crystallographically independent molecular structures reported in the literature for the 10 cephalosporins with potential leaving groups (pyridinium, acetate, heterocyclic thiols) at the 3′-position.[12,13] The structures can be categorized as to whether or not short intermolecular contacts to the β-lactam carbonyl exist in the crystal. Four structures have close contacts to the β-lactam ring.[12] These contacts involve an electrophile 2.9–3.1 Å from the carbonyl oxygen. A similar type of electrostatic interaction in the active site of an enzyme might perturb the bonds in the 3-position side chain.

The published bond lengths for the chain of atoms connecting the β-lactam and the potential leaving group, O=CNCC=CCZ, were analyzed statistically and compared to standard bond lengths. Poorly determined bond lengths, judged by being more than one standard deviation (0.03–0.04 Å) away from the mean of the 15 values, were discarded. The remaining values[14] were averaged for the four[12] structures with short contacts and for the 11[13] structures without.

	with	without
$O_9=C_8$	1.208 ± 0.007	1.209 ± 0.013
C_8-N_5	1.365 ± 0.013	1.376 ± 0.015
N_5-C_4	1.416 ± 0.024	1.413 ± 0.014
$C_4=C_3$	1.352 ± 0.015	1.352 ± 0.025
$C_3-C_{3'}$	1.499 ± 0.003	1.515 ± 0.013
$C_{3'}-S$	1.836 ± 0.006	1.822 ± 0.019

No large differences between the two categories are seen. For the $C_{3'}$–S bond in thiol-substituted cephalosporins, the mean for the structures without a short contact is essentially the standard value for this bond,[15] whereas the bond is marginally longer when there is a close contact at the β-lactam. In the case of cephaloridine, which has a short contact of 2.87 Å between the β-lactam carbonyl oxygen and a pyridinium of an adjacent molecule,[12] the observed $C_{3'}$–N bond length of 1.49 Å is longer than the N–Me bond

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length (1.46 Å) in *N*-methylpyridinium.[16] Although small, these bond length differences are intriguing in that the C_3–$C_{3'}$ and $C_{3'}$–Z bond length variations seem to be inversely related and associated with short β-lactam contacts. The cause of the differences could be some thermal motion of the C_3 atom or a coupling akin to the leaving group mechanism.

The concept of leaving group effect takes on added significance in light of the recently reported X-ray diffraction investigation of an exocellular DD-carboxypeptidase-transpeptidase from Streptomyces R61.[17,18] Improving resolution of the X-ray data has shown that, when an antibiotic like cephalosporin C is in the active site cleft, the hydroxyl group of the enzyme's active serine is close enough to form a covalent bond to the β-lactam carbonyl carbon. With the 7-(acylamino) side chain of the antibiotic embedded into the deepest reaches of the cleft, the 3-position side chain projects back toward the opening and lies just below the surface of the enzyme. There is a lysine residue and a threonine residue in proximity to where the 3-(acetoxymethyl) side chain of cephalosporin C would be. The present interpretation of the Fourier electron density difference map is that the 3′ leaving group (acetate) has departed.[17,18] Hence, it has been proposed that these residues may direct the side chain's orientation and/or promote its elimination. This sort of phenomenon could help explain the Gram-negative biological activity data mentioned before.

A further clue about the synchronicity of ring opening and leaving group elimination comes in kinetics experiments. For nonenzymatic hydrolysis of cephalosporins, the rate of appearance of free acetate and pyridinium leaving groups has been confirmed to be comparable to that of β-lactam ring opening.[19] This fact means that ring opening and departure of the leaving group are simultaneous or that opening of the β-lactam ring is the rate-determining step.

In order to unambiguous about **1**, it should be pointed out that the placement of arrows in a drawing such as **1** is somewhat arbitrary. The placement should not be taken to imply that a particular pair of electrons is shifted to the potential leaving group. Indeed, it is impossible to identify particular pairs of electrons in a chemical reaction. The proper way to consider the electron movement is in terms of shifts in electron density.[20]

Previous papers have discussed the correlation between Gram-negative minimum inhibitory concentration (MIC) and transition-state energy (TSE), which is a theoretical measure of the ease of nucleophilic attack on the β-lactam carbonyl carbon of cephalosporins.[3,4] Such a correlation was discoverable because all the other factors affecting biological activity, such as penetration and β-lactamase resistance, were nearly constant for the set of closely related cephalosporins that was used. Hence the differences in MIC for the compounds in the set were related to their intrinsic inhibition of the bacterial enzymes, which, in turn, was related to the predicted acylating ability. A more diverse set of β-lactams would not have worked.

A final point that should not be overlooked is that β-lactam compounds like penicillins and monobactams are able to exert their antibiotic activity without any assistance from mechanism 1. Cephalosporins with "direct" 3-position side chains like methyl can exhibit excellent activity depending on the nature of the 7-(acylamino) side chain. Thus, the leaving-group mechanism enhances, rather than being essential to, the Gram-negative activity of certain cephalosporins.

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Registry No. Cephalosporin, 11111-12-9.

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(–)-Methyl 4,5-*O*-Benzylidene-4-*epi*-shikimate: An Intermediate for the Synthesis of (–)-Chorismic Acid and Analogues

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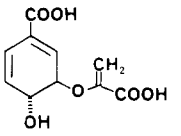
(–)-Chorismic acid (**1**) is the last common intermediate in the biosynthesis of aromatic substances through the



1

shikimate pathway in bacteria, fungi, and higher plants.[1] Previously we published the first synthesis of racemic **1**.[2] Shortly thereafter a synthesis was reported from Ganem's laboratory,[3] and more recently an improved synthesis was published from our laboratory.[4]

In view of our continuing interest in **1** and analogues for enzymatic studies, we desired a synthetic route to an enantiomerically pure intermediate for further transformation to (–)-**1** or analogues of (–)-**1** in which the enolpyruvyl side chain has been modified. The target intermediate selected was (–)-**2** since (±)-**2** is a convenient intermediate for the synthesis of (±)-**1**.[2] Described below is the synthesis of (–)-**2** from commercially available (–)-quinic acid (**3**).

Acid-catalyzed reaction of **3** and benzaldehyde with removal of H_2O gave **4**[5] as a ~3:1 mixture of diastereomers (75% yield) from which the major isomer could be obtained in crystalline form and, on the basis of steric arguments is assigned the *S* configuration at the acetal

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