Theoretical Study of the Alkaline Hydrolysis of an Oxo-β-Lactam Structure

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Received: June 14, 1999; In Final Form: September 2, 1999

Various potential mechanisms for the alkaline hydrolysis of an oxo-β-lactam in the gas phase (Scheme 1) were examined in the light of ab initio data obtained at the RHF/6-31+G*//RHF/6-31+G* and MP2/6-31+G*//MP2/6-31+G* levels. The influence of the solvent was also examined from IPCM (isodensity polarizable continuum model) computations at the RHF/6-31+G* level. In penicillins and cephalosporins, alkaline hydrolysis begins with a nucleophilic attack on the carbonyl group of the β-lactam ring, which is followed by cleavage of the C_7-N_4 bond. In the oxo-β-lactam studied, the process additionally involves cleavage of the C_7-O_6 bond in the ring. In fact, this cleavage is subject to a very small activation energy, as little as 0.21 kcal/mol versus the 14.15 kcal/mol for the typical cleavage energy (based on MP2/6-31+G*//MP2/6-31+G* calculations) for the C_7-N_4 bond. In addition, the hydrolysis end products are more stable than those resulting from the typical cleavage. Consequently, the alkaline hydrolysis involving cleavage of the C_7-O_6 bonds is kinetically and thermodynamically more favorable than the classical hydrolysis mechanism for penicillins and cephalosporins. This suggests that oxo-β-lactams might act as β-lactamase inhibitors.

Introduction

β-Lactam antibiotics are widely used against bacterial diseases on account of their high antibacterial power and low toxicity.[1] Their bactericidal action originates from the ability to interfere with the formation of bacterial walls by inhibiting transpeptidase[2] through acylation of a serine residue at the active site of the enzyme.[3,4]

The inception in recent years of resistant bacterial strains has promoted renewed efforts aimed at finding new molecules with antibacterial activity. Thus, penicillins and cephalosporins have been joined by carbapenems, cephamycins, oxacephamycins, and monobactams for this purpose. Each type of structure differs from the rest in its antibacterial spectrum.

The principal defense mechanism of bacteria against the action of these antibiotics is the presence of β-lactamases; these are enzymes that catalyze the hydrolysis of β-lactam antibiotics, which opens the ring and yields inert β-amino acids.[5] Like transpeptidases, most β-lactamases are serine enzimes.[6]

Attempts at solving the problems posed by bacterial resistance to β-lactam antibiotics have relied on two different approaches, viz. developing antibiotics stable against enzymatic hydrolysis (e.g., γ-lactams[7]) and designing substances capable of inactivating β-lactamases and use them in conjunction with antibiotics of proven antibacterial power (e.g., the combination of clavulanic acid,[8] an inhibitor for β-lactamases, and amoxycillin).

Nangia et al.[9−11] used semiempirical computation methods to determine various structural parameters for aza-β-lactams and concluded that the lactams must possess both antibacterial activity and inhibitory activity against β-lactamases.[9] The latter was recently confirmed in experimental terms.[12] The inhibitory properties of aza-β-lactams must arise from the formation of a carbamoyl−enzyme complex (Scheme 2) of substantially greater stability than the penicilloyl−serine complex. This idea was previously conceived by Ghosez et al.[13−15] in studying aza-γ-lactams (bicyclic imidazolidinones). However, this type of compound was subsequently found to exhibit little antibacterial action[14,15] as a result of the decreased chemical reactivity and topological changes introduced in replacing a four-member ring (azethidinone) with a five-member one (imidazolidinone). These effects were negligible in the structures examined by Nangia et al., which must possess a high chemical reactivity. Therefore, any structure preserving an intact C_7-N_4 bond upon acylation would in principle be a good candidate on account of its ability to form carbamoyl−enzyme compounds. Hence, the interest in studying β-lactam-related compounds bearing a different heteroatom at position 6.

Structural parameters are known to be inadequate to predict the chemical and antibacterial properties of these compounds. On the basis of the similarity between enzymatic acylation and alkaline hydrolysis,[16,17] some authors have suggested that the latter might be a reliable indicator for antibacterial activity in β-lactams.[18,19] It is therefore of interest to study the alkaline hydrolysis of compounds potentially capable of forming carbamoyl−enzyme intermediates with β-lactamases and rendering them inactive as a result. However, theoretical studies on this type of structure are virtually nonexistent.[20]

In this work, various potential mechanisms for the alkaline hydrolysis of an oxo-β-lactam (Scheme 1) where the CHR group at position 6 (next to the carbonyl group of the β-lactam ring) was isoelectrically and isosterically replaced with an oxygen atom was subject to a comprehensive theoretical study. Two different hydrolysis mechanisms were considered, namely: (a) the classical mechanism involving the attack of hydroxyl ion on the β-lactam carbonyl, with formation of the tetrahedral

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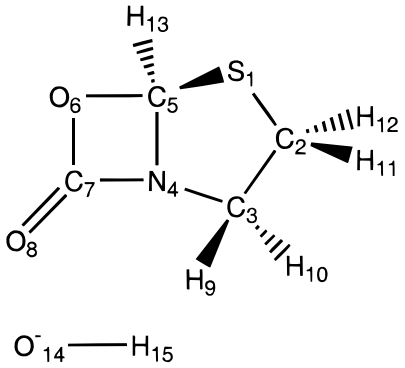
10.1021/jp991958n CCC: $18.00
Published on Web 10/21/1999

SCHEME 1



intermediate and subsequent cleavage of the C_7-N_4 bond (the typical mechanism for β-lactam antibiotics)[21−23] and (b) an alternative mechanism involving cleavage of the C_7-O_6 bond in the tetrahedral intermediate.

Methodology. The ab initio calculations on the ring together with the structures yielded in the studied reactions were initially carried out at the RHF/6-31+G*//RHF/6-31+G* level, which includes polarized and diffuse functions on heavy atoms. The incorporation of diffuse functions is especially relevant in the calculations of anionic systems.[24]

All the structures have also been optimized using Møller−Plesset's perturbation theory,[25] as implemented by Pople et al.,[26] at the MP2 level (MP2/6-31+G*//MP2/6-31+G*). Henceforward, RHF and MP2 stand for RHF/6-31+G*//RHF/6-31+G* and MP2/6-31+G*//MP2/6-31+G*, respectively. All the energies in the text include the ZPE correction. For MP2 energies, we use the HF ZPEs scaled by 0.8929 as recommended by Pople et al.[27]

Energies in solution were computed at the RHF/6-31+G* level using the isodensity polarizable continuum model (IPCM).[28] In this model, the solute is placed inside a cavity surrounded by a continuous medium (the solvent) with a bulk dielectric constant. The volume of the cavity surrounding the solute is computed by an isosurface of electron density obtained from standard quantum chemistry calculations (in this work, RHF). In this paper, IPCM calculations have been performed without geometry optimizations.

All the transition states are characterized by exhibiting just one imaginary frequency, greater than $100i$ cm^{-1} in all cases. IRC calculations of the former transition states were performed to confirm all the intermediates proposed in this study.

The calculations were performed on a SGI Origin 2000 and a SGI Origin 200 computers running the Gaussian 94 program.[29]

Results and Discussion

The structure studied (Scheme 1) was identical with that of a penicillin except for the oxygen atom replacing the carbon at position 6 in these antibiotics. To simplify computations, the carboxyl group in the β-lactams studied was omitted from the structure examined. The principal geometric parameters for this compound (**a**, Figure 1) and its energy are given in Tables 1 (RHF and IPCM calculations) and 2 (MP2 calculations).

The geometric parameters for the oxo-β-lactam ring are very similar to those provided by ab initio calculations for the β-lactam ring.[30] The C_7-N_4 distance in the oxo-β-lactam is slightly longer with the RHF calculations (1.399 Å against 1.356 Å in the β-lactam ring) and somewhat shorter with the MP2 calculations (1.345 Å). As in the oxo compound, the β-lactam ring is virtually planar; the $C_7N_4-C_5O_6$ dihedral is −5.0° with RHF and somewhat greater (28.2°) with MP2.

The alkaline hydrolysis of β-lactams involves the nucleophilic attack of hydroxyl ion on the carbonyl group to form a tetrahedral intermediate, followed by cleavage of the C_7-N_4 bond (viz. a B_{AC2} mechanism).[31] However, the structure studied here can additionally undergo cleavage of the C_7-O_6 bond in the tetrahedral intermediate to give different reaction products. A similar reaction mechanism was previously considered by Ghosez et al.[13] Figure 1 shows the different structures studied, and Figures 2 and 3 the RHF and MP2 profiles, respectively, for their reactions in the gas phase.

The nucleophilic attack on the carbonyl group was only examined on the α side of the ring, which was the energetically more favorable choice.[32] As in the alkaline hydrolysis of most β-lactams in the gas phase, the process was found to be subject to no activation energy.[21,33,34] The result of the nucleophilic attack is tetrahedral intermediate **b** (Figure 1, Tables 1 and 2), which is much more stable than the reactants (−42.83 kcal/mol with RHF and −47.02 kcal/mol with MP2). These values are greater in absolute terms than those for the azethidin-2-one ring[30] (−20.61 kcal/mol) provided by RHF/6-31+G*//RHF/6-31+G* calculations, which suggests an increased reactivity in the oxo-β-lactam structure.

Tetrahedral intermediate **b** can evolve with cleavage of the C_7-N_4 bond (subject to an activation energy of 12.62 kcal/mol with RHF and 14.15 kcal/mol with MP2), through transition state **c** (Figure 1). The activation energy for this process is similar to that for the azethidin-2-one ring (11.39 kcal/mol with RHF)[30] and to that calculated by Pitarch et al. for *N*-methylazethidin-2-one (12.16 kcal/mol with RHF).[34] On the other hand, the cleavage of the C_7-O_6 bond in the tetrahedral intermediate is much more favorable. In fact, the activation energy for this process is only 1.94 kcal/mol with RHF and 0.21 kcal/mol with MP2. Therefore, the alkaline hydrolysis of this compound must take place via transition state **i** instead of the classical pathway. This would allow the formation of carbamoyl−enzyme intermediates in its enzymatic hydrolysis counterpart (Scheme 2).

IRC computations on transition state **c** led to tetrahedral intermediate **b** on one hand and, directly, to product **d** on the other. This structure undergoes not only opening of the four-member ring at the C_7-N_4 bond but also cleavage of the C_7-O_6 bond, with release of carbonate ion. End product **d** is especially stable (−86.38 kcal/mol with RHF, and −79.02 kcal/mol with MP2) relative to the reactants.

If in transition state **c** the C_7-O_{14} bond is rotated to bring H_{15} nearer to N_4 and the structure is allowed to evolve freely, the cleavage of the C_7-N_4 bond is found to be accompanied by the transfer of the proton from O_{14} to N_4. This mechanism yields end product **e** and is identical with that for the alkaline hydrolysis of penicillins and cephalosporins.[35−37]

A third reaction pathway arises if the C_5-O_6 bond in transition state **c** is fixed to avoid its cleavage. If the transition state is then allowed to evolve freely, intermediate **f** is reached. This structure can be used to examine the opening of the five-member ring by cleavage of the C_5-S_1 bond. Thus, RHF calculations lead to end product **h** via transition state **g**. On the other hand, MP2 calculations reveal no intermediate structure between **c** and **h**. Although end product **h** is stabilized by the presence of an intramolecular hydrogen bond between N_{13} and H_{15}, it is the least stable structure. The activation energy for the formation of **h** is very small (0.26 kcal/mol with RHF). A similar process takes place in the alkaline hydrolysis of

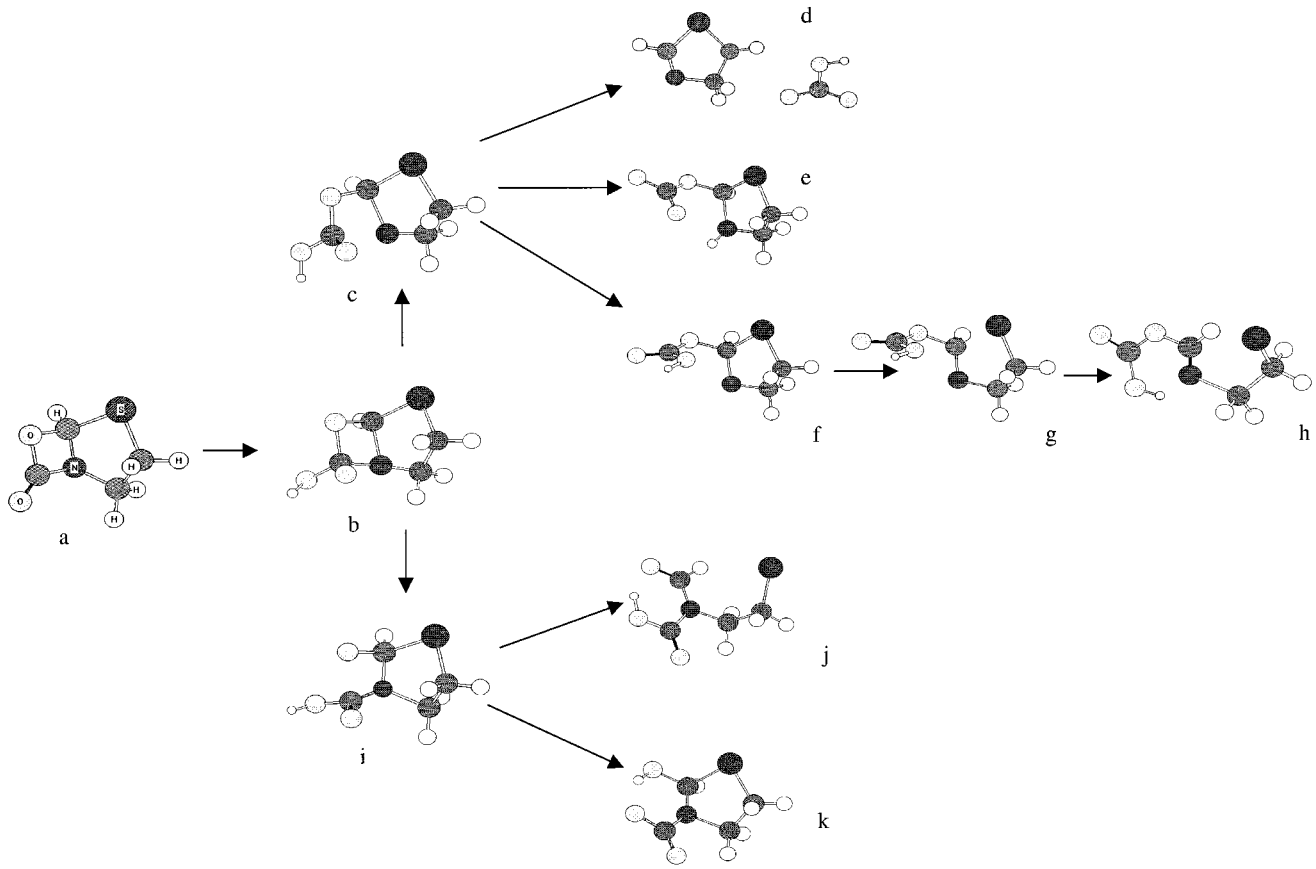


Figure 1. Structures corresponding to the different intermediate and final states of the reaction pathway of alkaline hydrolysis of the oxo-β-lactam compound in the gas phase at the RHF/6-31+G*//RHF/6-31+G* level.

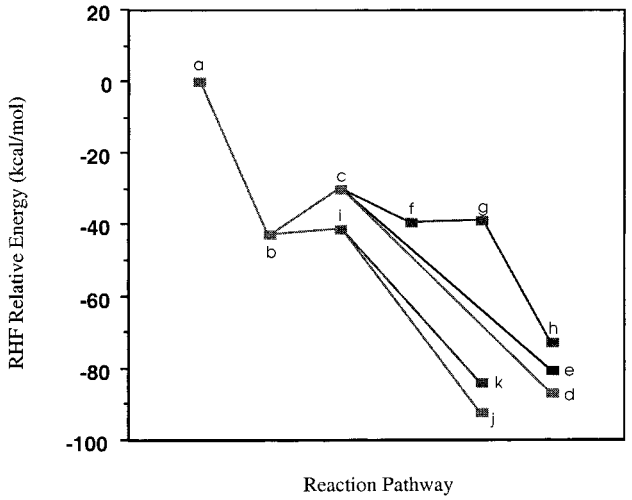


Figure 2. Reaction pathway for the alkaline hydrolysis of the oxo-β-lactam compound in gas phase at the RHF/6-31+G*//RHF/6-31+G* level.

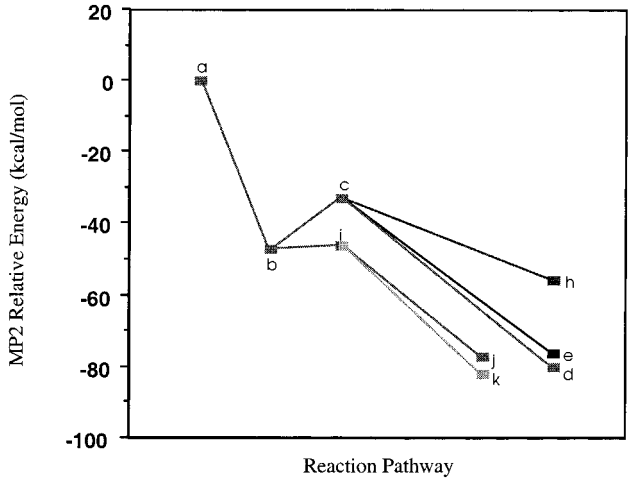


Figure 3. Reaction pathway for the alkaline hydrolysis of the oxo-β-lactam compound in the gas phase at the MP2/6-31+G*//MP2/6-31+G* level.

clavulanic acid,[33] also with a very low activation energy (3.9 kcal/mol with AM1 semiempirical calculations).

Transition state **i** can also yield various products. Thus, IRC calculations of this structure led both to tetrahedral intermediate **b** and end product **j**. This process involves the opening of the four-member ring (by cleavage of the C_7-O_6 bond) and also of the five-member ring (via the S_1-C_5 bond). Structure **j** is especially stable (-92.52 kcal/mol with RHF and -76.63 kcal/mol with MP2). This is partly the result of the intramolecular hydrogen bond between atom O_6 and H_{15}, with a distance of 1.714 and 1.680 Å with RHF and MP2, respectively.

If in transition state **i** the C_7-O_{14} bond is rotated to bring H_{15} nearer to O_6 and the structure is allowed to evolve freely, the proton is transferred from O_{14} in the acid group to O_6 to give an alcohol (product **k**). This structure also presents a hydrogen bond (the $O_{14}-H_{15}$ distance is 1.669 Å with RHF and 1.587 Å with MP2). On the basis of MP2 calculations, this is the most stable end product (-82.65 kcal/mol). In any case, the end products of the nonclassical hydrolysis are all more stable than those resulting from the alkaline hydrolysis.

The solvent is known to markedly alter the potential energy surfaces of many reactions, particularly those involving charged reactants or intermediates. Solvent effects can be approached

TABLE 1: Main Geometric Parameters, Energy (in the Gas Phase and in Aqueous Solution) and Imaginary Frequences at the RHF/6-31+G*//RHF/6-31+G* Level of the Reactants, Intermediates, Transition States, and Final Products of the Alkaline Hydrolysis of the Oxo-β-lactam Compound[a]

	C_7-N_4	C_7-O_6	N_4-H_{15}	O_6-H_{15}	C_5-S_1	$O_8C_7N_4$	$C_7N_4-C_5O_6$	RHF energy	ZPE	rel. energy	im. freq.	IPCM energy	IPCM rel. energy
a	1.399	1.347			1.801	134.6	−5.0	−831.44182[b]	0.11252[b]	0	0	−831.59228[b]	0
b	1.350	1.498	2.971	2.910	1.854	103.5	8.8	−831.51526	0.11771	−42.83	0	−831.62115	−18.12
c	1.882	1.370	3.071	3.018	1.860	96.3	6.4	−831.49278	0.11534	−30.21	1(−421.1)	−831.60019	−4.96
d	5.510	1.224	1.476	2.955	1.772	163.2	−5.4	−831.58181	0.11486	−86.38	0	−831.68166	−56.09
e	2.852	1.426	1.426	2.386	1.890	171.1	−14.8	−831.57698	0.11896	−80.77	0	−831.68188	−56.22
f	2.793	2.793	3.555	2.999	1.976	136.7	32.6	−831.50753	0.11556	−39.33	0	−831.60823	−10.01
g	2.834	1.303	3.685	2.999	2.050	138.9	38.1	−831.50747	0.11592	−39.07	1(−149.5)	−831.60326	−6.89
h	2.4.34	1.349	1.476	2.277	3.328	169.8	0.3	−831.55985	0.11410	−73.07	0	−831.65539	−39.60
i	1.098	1.780	2.942	2.901	1.903	98.4	42.9	−831.51098	0.11651	−40.89	1(−446.6)	−831.61255	−12.72
j	1.428	2.850	2.296	1.714	3.297	123.8	−3.3	−831.50515	0.11841	−92.52	0	−831.69038	−61.56
k	1.595	2.896	2.167	0.971	1.909	124.3	−40.2	−831.58237	0.11896	−84.16	0	−831.68225	−56.46

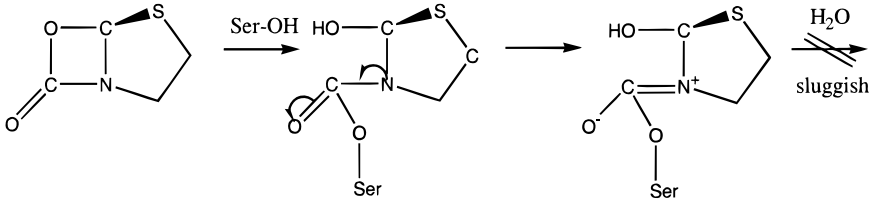
[a] Bond length in angstroms. Bond and dihedral angles in degrees. Energy and ZPE in hartrees and relative energies in kcal/mol. Frequences in cm^{-1}. [b] Addition of oxo-β-lactam structure and OH^-.

TABLE 2: Main Geometric Parameters and Energy at MP2/6-31+G*//MP2/6-31+G* Level of the Reactants, Intermediates, Transition States, and Final Products of the Alkaline Hydrolysis of the Oxo-β-lactam Compound[a]

	C_7-N_4	C_7-O_6	N_4-H_{15}	O_6-H_{15}	C_5-S_1	$O_8C_7N_4$	$C_7N_4-C_5O_6$	MP2 energy	ZPE	rel. energy
a	1.345	1.391			1.796	125.6	28.2	−832.81670[b]	0.10047	0
b	1.347	1.596	3.016	2.962	1.872	104.4	8.8	−832.89627	0.10510	−47.02
c	2.048	1.395	3.231	3.057	1.919	95.3	−2.4	−832.87160	0.10299	−32.87
d	5.214	1.258	5.797	2.187	1.769	169.7	−1.3	−832.94480	0.10256	−79.07
e	2.843	1.503	0.736	2.373	1.913	172.9	−17.2	−832.94359	0.10622	−76.02
h	2.423	1.405	1.315	2.290	3.222	170.7	−0.8	−832.90796	0.10188	−56.38
i	1.270	1.780	3.038	2.972	1.911	104.0	14.7	−832.89485	0.10403	−46.81
j	1.184	2.882	2.007	1.630	2.936	126.9	53.9	−832.94408	0.10573	−76.63
k	1.127	2.900	2.131	1.019	1.941	116.8	36.1	−832.95415	0.10622	−82.64

[a] Bond length in angstroms. Bond and dihedral angles in degrees. Energy and ZPE in hartrees and relative energies in kcal/mol. ZPE correction is the HF ZPE correction scaled by 0.8929. [b] Addition of oxo-β-lactam structure and OH^-.

SCHEME 2



in various ways, prominent among which are continuum quantum mechanical methods based on the Onsager reaction field model.[38] In such methods, the solvent is considered a continuum structure with a specific dielectric constant but no definite structure, and the solute is assumed to be in a cavity of such a continuum. A multipolar molecule located within a cavity will undergo electrostatic interactions with the medium and be stabilized as a result. The different methods used in this context differ essentially in the shape of the cavity, which can be spherical,[39,40] bounded by the van der Waals surface for the molecule (PCM model),[41−43] or based on an electron isodensity surface (IPCM model).[28] The last method provides results similar to those of PCM but converges somewhat better than this. According to Cramer and Truhlar,[44,45] most of the free energy changes in aqueous solutions are due to electron relaxation. Consequently, the results of IPCM calculations based on gas-phase geometries (RHF/6-31+G*//RHF/6-31+G*) should be suitable for a qualitative analysis of solvent effects.

The principal effect of the solvent is stabilizing the reactants with no substantial alteration of the activation energies for the different possible reaction pathways; as a result, the most favorable process in the gas phase (viz. cleavage of the C_7-O_6 bond) is also the most favorable in solution (Table 1, Figure 4). It should be noted that, in theoretical studies based on optimized geometries in the presence of a solvent, the nucleophilic attack of a hydroxyl ion on a carbonyl group to give a tetrahedral intermediate was found to be subject to an activation energy, both in β-lactams[21,46,47] and in other types of compounds.[24,48,49] Such an energy, 16 kcal/mol, arises from the

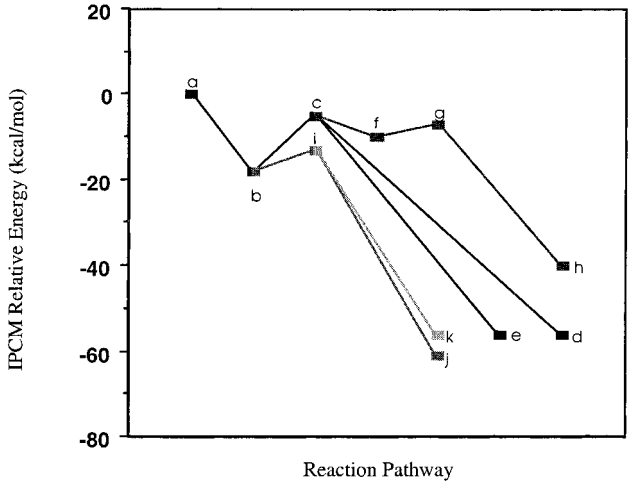


Figure 4. Reaction pathway for the alkaline hydrolysis of the oxo-β-lactam compound in aqueous phase by the IPCM method.

solvation of hydroxyl ion and is virtually independent of the particular β-lactam. On the basis of the similarity of the oxo-β-lactam to classical β-lactams, the former should exhibit an identical behavior in the aqueous phase.

The formation of the tetrahedral intermediate is not so exothermal as in the gas phase (−18.12 kcal/mol) as a result of the reactants being stabilized by the solvent. This value is somewhat greater than that obtained by Pitarch et al.[34] using ab initio calculations and a polarizable continuum solvent model for *N*-methylazethidin-2-one. It also exceeds that for *N*-methylazethidin-2-one provided by semiempirical calculations based on the AMSOL continuum method (−8.63 kcal/mol), that for cephalothin[37] based on solvation by five water molecules (−13.6 kcal/mol), and that for penicillin G[33] obtained with AMSOL (−13.1 kcal/mol). As in the gas phase, the formation of the tetrahedral intermediate must therefore be more favorable than in β-lactams.

A comparison of the activation energies of the different reaction pathways for the tetrahedral intermediate reveals that the cleavage of the C_7-O_6 bond is subject to a much lower barrier (5.4 kcal/mol) than is that of the C_7-N_4 bond in the classical process (13.16 kcal/mol). Also, the activation energy for the classical cleavage is similar to that for the opening of the tetrahedral intermediate of *N*-methylazethidin-2-one (13.43 kcal/mol).[34]

On the basis of these results, the alkaline hydrolysis of the oxo-β-lactam will yield preferentially product **j**, through cleavage of the C_7-O_6 bond and opening of the five-member ring fused to the β-lactam ring.

Solvent effects can play a role in the proton-transfer steps through a bifunctional catalysis mechanism. This possibility has been studied in nucleophilic additions to carbonyl[50−53] and, specifically, in alkaline hydrolysis of β-lactam antibiotics[37,54] and γ-lactams.[55] It leads generally to a diminution of the energy barriers without modifying the reaction mechanism. Therefore, it could be expected that solvent will diminish the energy of activation of the ring-opening process. However, this effect must happen in the two studied processes, and it would not affect the general conclusions above stated.

β-Lactamases, which degrade β-lactam antibiotics to cancel their bactericidal effect, act by forming an acyl−enzyme complex with the target antibiotic. Subsequently, the complex is attacked by a water molecule and forms the corresponding acid, which lacks antibacterial activity.

Because of the chemical reactivity of the oxo-β-lactams, it is expected that their acyl−enzyme complex does not form the acid but rather evolves with cleavage of the C_7-O_6 bond to an especially stable carbamoyl−enzyme complex (equivalent to structure **j** in Figure 1). As noted earlier, this compound results from the opening of the five-member ring fused to the β-lactam ring.

The inhibitory activity of clavulanic acid has been shown to arise from the formation of an especially stable acyl−enzyme complex where the five-member ring fused to the β-lactam ring is open.

Theoretical studies[33] on the chemical reactivity of clavulanic acid have shown it to depart from the typical behavior of penicillins and cephalosporins; in fact, its hydrolysis yields end products with an open five-member ring, similarly to the experimental results.

The chemical reactivity of clavulanic acid and oxo-β-lactams is obviously similar in some respects. Consequently, the latter might also be powerful inhibitors for β-lactamases.

Ghosez et al.[14,15] found bicyclic imidazolidinones to exhibit virtually no antibacterial activity. This may have been the result of (a) topological changes caused by the replacement of a four-member ring with a five-member ring and (b) the urea group in the imidazolidinone being much less prone to nucleophilic attack of the serine residue at the active site of a β-lactamase than is the carbonyl group in the classical process for β-lactams.[6] However, the structure studied here preserves the four-member ring, albeit in a highly stressed state; also, the three-dimensional structure of the molecule is consistent with the presence of antibacterial activity.[9,20]

The formation of the tetrahedral intermediate is highly favored, more than in classical β-lactams. Therefore, those factors that diminish the inhibitory and antibacterial activity of bicyclic imidazolidinones are absent from this structure, which might thus possess pharmacological interest as an inhibitor for serine enzymes.

A broad range of interesting structures can be obtained by replacing the O_6 atom with various heteroatoms.[20] The chemical reactivity and interactions with serine enzymes will be examined in future works.

Acknowledgment. The authors thank the Centre de Supercomputació de Catalunya (CESCA) for access to its computer facilities. The support of the Spanish DGICYT is gratefully acknowledged (Project PB96-0596).

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