Neutral and Alkaline Hydrolyses of Model β-Lactam Antibiotics. An ab Initio Study of Water Catalysis

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Abstract: The effect of an ancillary water molecule on the neutral and alkaline hydrolysis mechanisms of a simple β-lactam molecule (*N*-methylazetidinone) has been studied at the Hartree−Fock and MP2 levels using the 6-31G* and 6-31+G* basis sets. The results have been compared with a nonassisted study carried out previously. Solvent effects have been also considered by means of a polarizable continuum model. In the neutral hydrolysis, the additional water molecule diminishes the free-energy barriers only when correlation energy is taken into account. Concerted and stepwise mechanisms have been described. The corresponding barriers are close, and the actual mechanism could be conditioned by the molecular environment, solution, protein, etc. Using the results of a molecular dynamics simulation of *N*-methylazetidinone in aqueous solution, it has been shown that the stepwise process is more likely to occur in such conditions. In the alkaline hydrolysis, the first reaction step consists of the formation of a tetrahedral intermediate which requires a desolvation of the hydroxyl anion difficult to reproduce by calculation. Afterward, the hydrolysis reaction proceeds through either concerted or stepwise mechanisms for ring opening and proton transfer. The concerted channel presents a very low energy barrier, and the species involved are dependent on the calculation level. The stepwise mechanism is virtually the same as that previously reported for the nonassisted hydrolysis, the relative energy of all the points along the path being diminished and the energy barriers remaining essentially unaltered.

1. Introduction

β-Lactam antibiotics activity is due to covalent bonding between the antibiotic and the active site serine residue of a group of enzymes named penicillin-binding proteins (PBP).[1] These enzymes serve to complete bacterial cell wall synthesis by a cross-linking of peptidoglycan chains, and they are essential to cells.[2] Because mammalian cells do not have this cell wall component, β-lactam antibiotics generally have very favorable ratios of therapeutic to toxic effect. However, some bacteria are resistant to the action of β-lactam antibiotics because of their ability to produce β-lactamase enzymes which catalyze the hydrolysis of the antibiotic to its inactive amino acid.[3] Most of the β-lactamases (classes A and C) also act by using an active site serine,[4] and it is generally accepted that these β-lactamases, as well as the PBP's, form acylenzyme intermediates with the antibiotic. Also, in both the solvolytic and enzyme catalyzed reactions of β-lactams, the approach of the nucleophile to the carbonyl carbon of the lactam group is expected to form a tetrahedral adduct.[4−6] Since the knowledge of the detailed molecular mechanism of β-lactam antibiotic hydrolysis can be of great importance to develop new and more efficient antibacterial drugs, in past years several studies employing molecular orbital calculations have been devoted to this subject.[7−12] Within this scope, we have presented in a previous work a theoretical study of neutral and alkaline hydrolyses of *N*-methylazetidinone as a model of biological hydrolysis of β-lactam antibiotics.[13]

Neutral and alkaline mechanisms can be gathered in a single reaction scheme (Scheme 1), where X is a hydrogen atom in the neutral hydrolysis and a negative charge in alkaline hydrolysis.

The continuous arrows correspond to reaction paths for neutral hydrolysis. There are two possible pathways. In the first one, the reactant complex **2** directly leads to the product **5** through a single transition structure. In this transition structure the participating water molecule orients so that one of its hydrogen atoms points toward the β-lactam nitrogen. This proton is transferred to the nitrogen atom while the water oxygen

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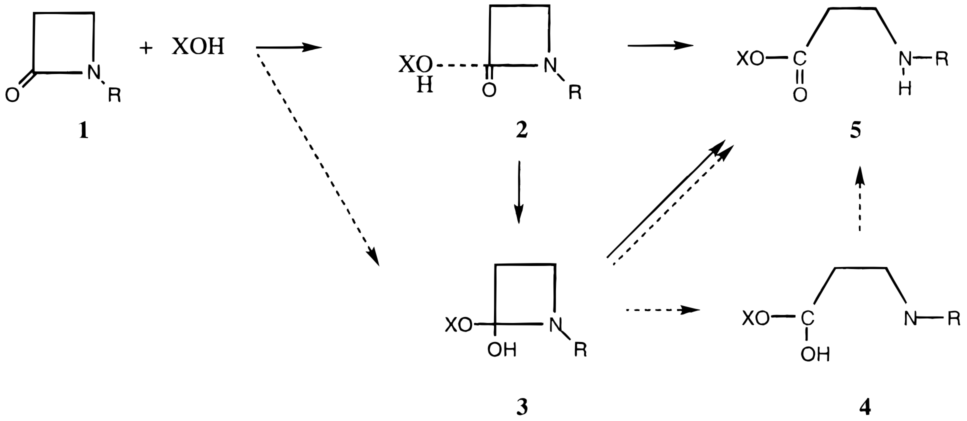
Scheme 1



simultaneously attacks the carbonyl carbon. In the second one, the proton transfer from XOH in **2** is produced toward the carbonyl oxygen instead of the nitrogen atom, leading to a diol (structure **3**). This diol evolves with simultaneous ring opening and proton transfer to the nitrogen atom yielding the product **5**. The dashed arrows correspond to possible paths for alkaline hydrolysis. Structure **2** seems unlikely in this case (in the gas phase), and the tetrahedral intermediate **3** is presumed to be directly formed. This intermediate can evolve toward the product in two different ways: a concerted mechanism, where the ring opening and proton transfer takes place simultaneously (step **3** → **5**), and stepwise, where the ring opening is produced first (step **3** → **4**) and the reaction is completed afterward by means of a proton transfer (**4** → **5**).

In our previous study, bulk solvent effects were also considered by means of a polarizable continuum model, giving rise to only small changes in the reaction mechanisms. However, one may expect specific solvent effects to play a role in the proton-transfer steps through a bifunctional catalysis mechanism. This possibility has been studied in nucleophilic additions to carbonyl[14−17] and in some other reactions[18−24] and leads generally to a noticeable diminution of the energy barriers. The ancillary water molecule acts as both proton donor and proton acceptor and serves as a bridge for proton relay, participating in the definition of the reaction coordinate. In the case of amide hydrolysis, such a water-assisted mechanism has been also considered[25] and the results show again an important catalytic effect.

It is our aim in this paper to explore this possibility for the alkaline and neutral hydrolyses of β-lactam compounds, by the inclusion of an ancillary water molecule in all the mechanisms mentioned. Indeed, the presence of water molecules in the vicinity of the serine active site of some penicillin-recognizing enzymes is known and has motived a previous study on water catalysis at the first stages of the reaction pathways for neutral hydrolysis, which exhibit a pronounced effect on the energy barrier amounting to over 10 kcal/mol.[11] In this work we extend such an analysis to the full reaction path in the neutral hydrolysis and alkaline hydrolysis. Note that water-assisted mechanisms have been rigorously studied for neutral or positively charged systems, but negatively charged systems have received less attention.

2. Methodology

Calculations have been carried out using the GAUSSIAN94 package of programs.[26] The geometries have been fully optimized at the HF/6-31G*[27] level. In the alkaline hydrolysis, because of the presence of a negative charge, the 6-31+G*[28] basis set, which includes diffuse functions on heavy atoms, has been also employed. Correlation energy has been taken into account by MP2 single point calculations at the HF geometry. When necessary, MP2 geometry optimization has been also carried out. Location of stationary points on the potential energy surface (PES) was done using the redundant coordinates algorithm[29] as implemented in the GAUSSIAN94. The nature of the stationary points was determined by analytical frequency calculation and verifying that they had no imaginary frequencies in the case of minima or only one in the case of transition structures. The intrinsic reaction coordinate (IRC) pathways[30] from the transition structures down to the two lower energy structures have been followed using a second-order integration method.[31] Contributions to the free energy have been obtained using standard procedures.[32] Vibrational contributions have been estimated from HF calculations in the gas phase, in all cases.

The effects of the bulk solvent on hydrolysis mechanisms have been incorporated using a cavity model.[33−35] In this model the liquid is assimilated to a continuum characterized by its dielectric constant (78.4

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for water). The quantum system is then placed in a cavity surrounded by this continuum. The electrostatic solvation free energy is obtained as the sum of multipole contributions (up to sixth order in our case). The electrostatic interaction can be included in the solute's Hamiltonian allowing the inclusion of polarization effects. The cavity is ellipsoidal and adapted to the shape of the solute.[36] Solvent effects calculations have been carried out using the SCRFPAC package[37] added to the GAUSSIAN program.

A molecular dynamics simulation in the microcanonical ensemble (NVE) was performed on a cubic box of edge 21.67 Å containing 336 water molecules and the *N*-methylazetidinone molecule. Periodic boundary conditions and a cutoff distance of 10.84 Å for the solute−solvent and solvent−solvent interactions were used. The SPC/E model potential[38] was selected for the water molecules. For the β-lactam we used the HF/6-31G* fixed geometry and Lennard-Jones parameters taken from reference.[39] Potential derived atomic charges were obtained using the GRID program[40] at the HF/6-31G* level. After the equilibration, the production run was carried out for a total of 112.5 ps, with a time step of 1.5 fs. For this task the MDpol program[41] was used.

3. Results and Discussion

3.1. Neutral Hydrolysis. In our previous theoretical study on the nonassisted hydrolysis of *N*-methylazetidinone, we obtained two possible reaction pathways for the neutral hydrolysis,[13] as explained in the Introduction. All the involved transition structures correspond to proton transfers, and it is likely that an additional water molecule could have a pronounced catalytic effect. The stationary structures obtained for both the concerted (path A) and the stepwise (path B) assisted mechanisms are shown in Figure 1. The path A corresponds to the structure sequence **1** → **2** → **5** in Scheme 1, and the path B corresponds to the sequence **1** → **2** → **3** → **5**. The most relevant geometrical variables of the stationary points are supplied as Supporting Information, and the relative energies and free energies at HF/6-31G* and MP2//HF/6-31G* levels are given in Table 1. In Figure 2, we show the free-energy profile for the two pathways (A and B) at the HF and MP2//HF levels, both for the assisted and nonassisted processes.

Essentially, **A1** and **B1** minima consist of a water dimer interacting with the β-lactam ring. These minima and the transition structures **A2** and **B2** were also obtained by Wolfe et al.[11] at the HF/3-21G level. One of the water dimer hydrogen atoms is directed toward the β-lactam nitrogen atom in **A1** and toward the carbonyl oxygen atom in **B1**. In **B1**, the β-lactam geometric structure is not affected by this interaction keeping the ring planarity. Conversely, in **A1**, the nitrogen atom is pyramidalized and the planarity of the β-lactam is lost. This is not favored energetically, the **B1** structure being 5.5 and 4.73 kcal/mol more stable than **A1** at the HF and MP2//HF levels, respectively. Both structures present a positive free energy at the HF level, although at the MP2//HF level **B1** is 1.24 kcal/mol more stable than the isolated reactants. For this reason we have chosen the separated reactants as the energy reference for the neutral hydrolysis process.

In the first transition structures of both reaction paths, **A2** and **B2**, the bifunctional role of the water molecule can be clearly seen. A water molecule is simultaneously accepting a proton (from the other water molecule) and donating another proton either to the nitrogen atom (in **A2**) or to the carbonyl oxygen atom (in **B2**). In both structures, the ring remains closed, as evidenced by the C−N bond lengths of 1.510 Å in **A2** and 1.439 Å in **B2**.

Let us consider first the energy results at the HF/6-31G* level. The free energy needed to reach the transition structure **A2** is quite high, 66.2 kcal/mol from isolated reactants. This value is very close to that obtained for the nonassisted process,[13] 68.60 kcal/mol at the same computational level, although it is slightly smaller, thereby predicting a minor catalytic effect. This result is reproduced for the **B2** structure, where the HF values are again very close, 64.48 and 65.61 kcal/mol for the assisted and nonassisted processes, respectively. Despite this small effect on free energies, the effect of the ancillary water molecule is quite large on the internal energy differences (ΔE). Indeed, the energy barrier for both A and B paths is about 13 kcal/mol lower in the assisted process than in the nonassisted one. However, the internal-energy decrease in the assisted process is compensated by a strong entropic decrease, with $T\Delta S$ values −22.10 and −23.24 kcal/mol for **A2** and **B2** in the assisted process and only −11.92 and −11.54 kcal/mol in the nonassisted one. This more unfavorable entropic contribution to the free energy of the catalyzed with respect to the uncatalyzed reaction seems to be a general feature of the bifunctional catalysis by water.[17] Consequently, more important catalytic effects are expected at lower temperatures. These trends are similar to those previously pointed out in the assisted and nonassisted free-energy profiles for the neutral hydrolysis of formamide when the 6-31G* basis set was used.[25] However, in other works using the 3-21G basis set, an important catalytic effect was predicted at 298 K.[11,15,16]

When we consider the description at the MP2//HF level, the free energy barriers are significantly lowered through water catalysis and especially in the case of the stepwise process: from 50.67 and 53.87 kcal/mol for the nonassisted A and B paths, to 44.32 and 40.98 kcal/mol for the corresponding assisted paths. Path B, which corresponds to the stepwise mechanism, becomes now the preferred one. Previously,[11,12] path A has been considered to be the most favorable mechanism in an enzymatic environment, on the basis of energetic (MP2/6-31G*//3-21G) and structural (how well the antibiotic fits into the active site) considerations. The results presented here stress that the energetic preference is not so clear and that the conclusions are intimately dependent on basis set and calculation level.

Structure **A2** leads directly to the hydrolysis product **A3**, which is more stable than the reactants ($\Delta G = -5.43$ kcal/mol, at MP2//HF level) while the **B2** structure leads to the diol **B3**. This reaction intermediate **B3** has been located by IRC calculations from **B2**. The water molecule remains attached to the system by means of hydrogen bonds with H18 and O14. We found another structure for the diol−water complex, **B4**, which presents nearly the same relative free energy as **B3** at the HF level and is only 1.73 kcal/mol above **B3** at the MP2//HF level. Although other hydrogen-bonded structures are conceivable, structure **B4** is particularly interesting since the water molecule presents an orientation which is appropriate to catalyze the subsequent reaction step, i.e., proton transfer from O14 to N3. The passage from **B3** to **B4** is expected to be

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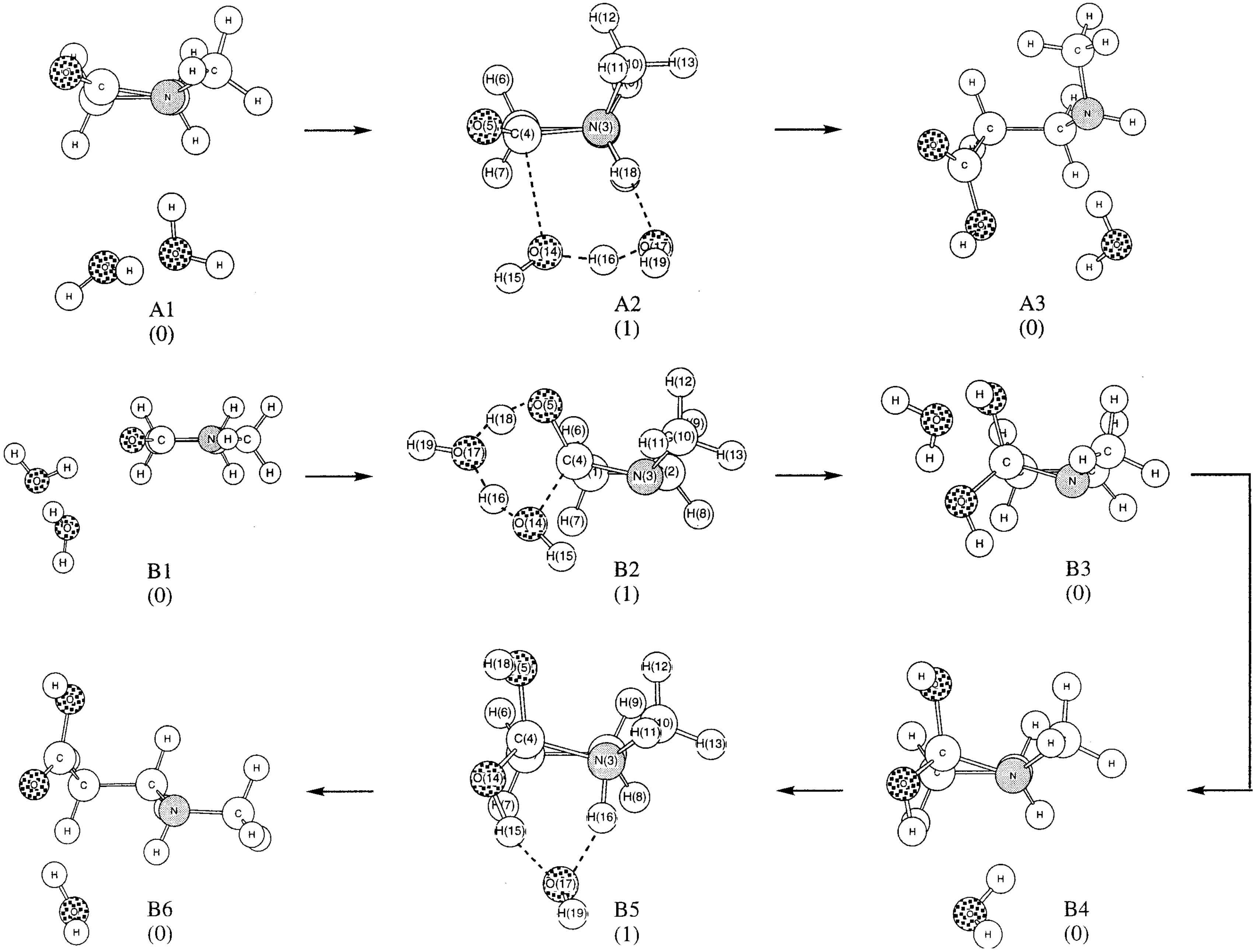

Figure 1. Stationary structures of the neutral hydrolysis. Numbers in parentheses indicate the number of imaginary frequencies.

Table 1. Energies and Free Energies for the Neutral β-Lactam Hydrolysis in the Gas Phase and in Aqueous Solution (in kcal/mol)

	$\epsilon = 1$				$\epsilon = 78.4$	
	HF/6-31G*		MP2//HF/6-31G*		HF/6-31G*	MP2//HF/6-31G*
	ΔE	ΔG	ΔE	ΔG	ΔG	ΔG
reactants	0.00	0.00	0.00	0.00	0.00	0.00
A1	−9.79	8.86	−15.17	3.49	4.29	0.50
A2	43.98	66.22	22.09	44.32	70.15	49.10
A3	−28.14	−4.27	−29.29	−5.43	−0.75	−2.05
B1	−16.45	3.36	−21.05	−1.24	5.77	0.04
B2	42.20	64.48	18.70	40.98	75.72	50.88
B3	−2.25	23.62	−8.77	17.10	35.46	27.26
B4	−2.56	23.51	−10.24	15.83	32.71	23.48
B5	25.05	50.20	5.96	31.11	58.68	38.72
B6	−31.63	−7.24	−32.02	−7.63	−5.42	−6.92

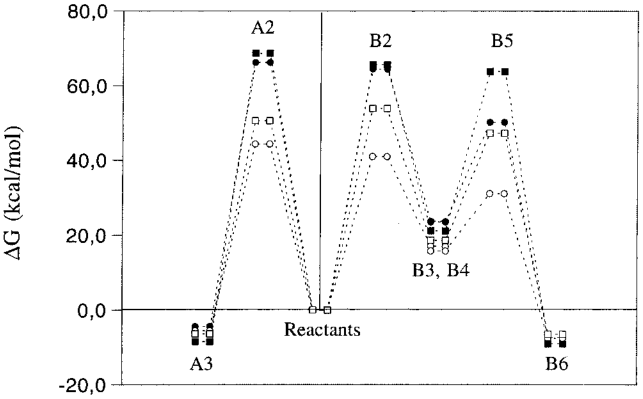

Figure 2. HF/6-31G* and MP2//HF/6-31G* free-energy profiles for the neutral hydrolysis: (●) HF assisted; (○) MP2//HF assisted; (■) HF nonassisted; (□) MP2//HF nonassisted.

favored, independent of the molecular environment, gas phase, aqueous solution, or protein. However, the detailed mechanism may differ resulting, for instance, from thermal agitation or from participation of neighboring water molecules. Whatever the mechanism, **B4** is expected to be the next configuration for the reaction to proceed through path B in the assisted process.

B4 evolves donating a water proton to the nitrogen atom and simultaneously accepting the H15 hydroxyl proton, regenerating the water molecule. This process is reflected in the **B5** transition structure in which the transferred water proton, H16, is already bonded to the nitrogen atom, with the N3−H16 distance 1.089 Å and the broken O17−H16 bond length 1.481 Å. The water molecule accepts the H15 proton from O14 but this process is only slightly initiated; the distances O14−H15 and H15−O17 are 1.069 and 1.390 Å, respectively.

As opposed to the reaction steps presented above, where HF free energies are very similar for the assisted and nonassisted processes, the activation to **B5** is quite favored in the assisted process, even at the HF level. The water-assisted HF free-energy barrier from **B4** to **B5** is 26.69 kcal/mol, whereas the free-energy barrier for the equivalent step in the nonassisted process is 42.61 kcal/mol.[13] At the MP2//HF level, these values are 15.28 and 28.62 kcal/mol, respectively. In the hydrolysis product **B6**, the formed water molecule presents two hydrogen bonds which involve the same atoms as in structure **B4**, before the double proton transfer. This **B6** structure is slightly more stable than **A3** (by 2.97 and 2.20 kcal/mol at the HF and MP2//HF levels, respectively), where the water molecule does not maintain such interactions.

At this point, we have a global perspective of the A and B paths. At the HF level, the assisted stepwise process is slightly favored, without large influence of bifunctional catalysis. At the MP2//HF level, the assisted stepwise process is still preferred, but the influence of bifunctional catalysis is here fundamental. Nevertheless, the difference between the energy barriers of the rate-limiting steps for concerted and stepwise reaction mechanisms is rather small (1.74 kcal/mol at the HF level, 3.34 kcal/mol at the MP2//HF level, in absolute values). Thus, the actual preference for one or another reaction channel can be determined by other factors such as the molecular environment. In particular, the probability distribution of water molecules around the β-lactam prior to the initial attack, which is related to the β-lactam ability to form hydrogen bonds through the carbonyl oxygen and nitrogen atoms, is certainly an important aspect.

With the aim of investigating the interaction of a β-lactam molecule with water, we have performed molecular dynamics simulations for *N*-methylazetidinone in aqueous solution. Details on these calculations will be presented elsewhere,[42] but here we outline the first initial conclusions which are relevant to the present study. The radial distribution functions obtained for the C4, N3, and O5 atoms of the β-lactam indicate the absence of a well-organized coordination shell around the N3 nitrogen atom, whereas it exists around the carbonyl O5 oxygen, and C4 carbon. The maxima of these distribution functions are found at distances in close agreement with those obtained in ab initio calculations for the complexes β-lactam−water dimer and β-lactam−water molecule (around 3.6 Å for the water oxygen−C4 and 1.7 Å for the water hydrogen−O5). We have analyzed 750 configurations homogeneously distributed along the trajectory searching for structures in which some water molecules lie close to the carbonyl carbon atom and are conveniently oriented to attack either the O5 or the N3 atoms. In other words, we have looked at configurations resembling the initial species in mechanisms A and B. Significantly, we have found 318 configurations with at least one water molecule presenting both a water oxygen−C4 distance smaller than 3.5 Å and a water hydrogen−O5 distance smaller than 2.5 Å. However, along the 750 configurations analyzed there are only two structures that fulfill similar requirements with respect to the β-lactam nitrogen, i.e., water oxygen−C4 distance smaller than 3.5 Å and a water hydrogen−N3 distance smaller than 2.5 Å.

The previous statements make reference to the interaction between the β-lactam molecule and water monomers. We can extend this analysis to the water dimers introducing some different search requirements. For each configuration we have obtained a set I of water molecules for which the oxygen−C4 distance is smaller than 3.5 Å. Independently, a set II where the hydrogen−O5 distance is smaller than 2.5 Å has been also

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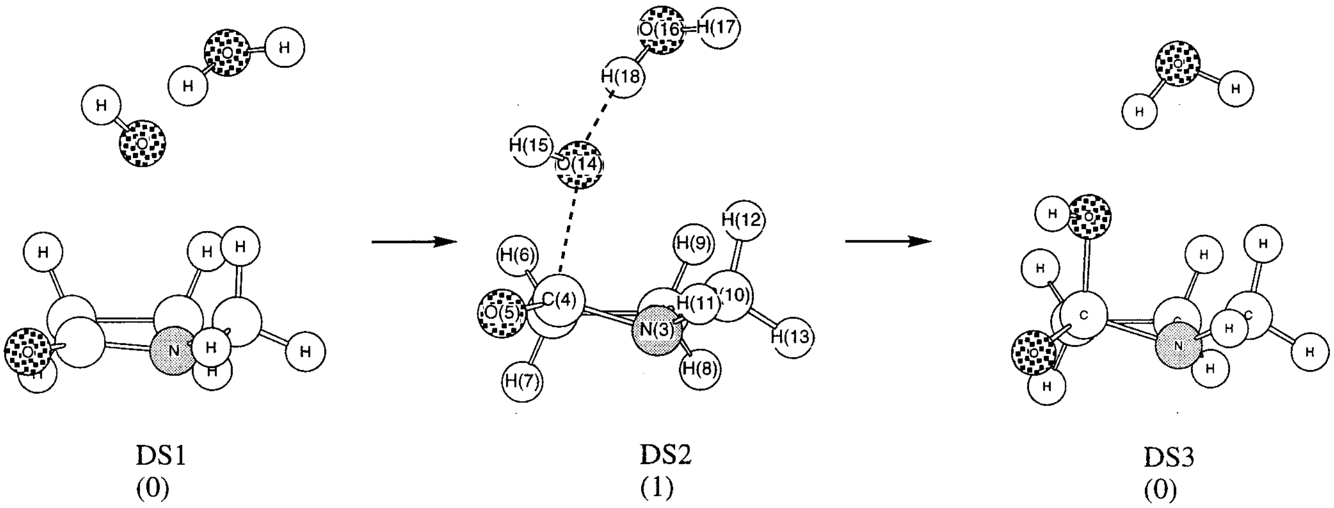
[42] Work in progress.



DS1
(0)

DS2
(1)

DS3
(0)

Figure 3. Stationary structures for the hydroxyl addition to the β-lactam. Numbers in parentheses indicate the number of imaginary frequencies.

obtained. The water dimers conveniently oriented to attack according to the B path correspond to those molecules of the I and II sets that mutually form hydrogen bonds. There are 39 configurations where a dimer fulfills these requirements. When we apply this analysis to the search of dimers interacting through the nitrogen atom (now the set II is selected according to the hydrogen−N3 distance; the set I is the same), no configurations satisfying the criteria were found. Thus, in aqueous solution, it is quite probable to find situations where the orientation and position of solvent water molecules are favorable for hydrolysis of the carbonyl group, i.e., the first step of path B. From these MD results, one could also question the adequacy of modeling solutions by solvent clusters of limited size. In fact, the **A1** minimum could be an artifact of the cluster model where the lack of interactions with other solvent molecules leads to relative orientations of the molecules forming the cluster especially adapted to optimize the interactions among them and that are different from those orientations found in the solution.

Bulk solvent effects have been also considered in this study by means of a polarizable continuum model.[37] In all the points along the gas-phase reaction paths, the electrostatic interaction energy with the solvent was evaluated. The resulting relative free energies of all the structures are given in Table 1. The height of the energy barriers is increased, and, as opposed to the gas-phase results, path A now has the lower energy barrier at the rate-limiting step.

3.2. Alkaline Hydrolysis. The binding of a hydroxyl anion to the β-lactam carbonyl group in the gas phase does not present any energy barrier. The energy always decreases along the reaction path until the formation of a tetrahedral intermediate. This fact is known both from experimental[43] and theoretical studies.[7,9,44,45] However, in solution, an activation energy exists probably as a consequence of the hydroxyl desolvation process.[10,46] In the assisted mechanism, the hydroxyl anion is initially bonded to a water molecule which stabilizes it. Transfer of OH^- from the initial hydroxyl−water complex to the β-lactam molecule models in a first approximation the desolvation effect. However, it is clear than the energetics of such a process in the gas phase must be quite different from the experimental free-energy barrier for addition of OH^- to the β-lactam molecule in solution. This is so because the solvation energy of the hydroxyl ion in water is substantially larger, in absolute value, than the free energy for complexation with a single water molecule.[47] We shall comment further on this point below.

In Figure 3, we show the structures related to the process of hydroxyl anion binding. In Figure 4, we represent the remaining structures found in the alkaline hydrolysis study for two possible mechanisms. The most relevant geometrical parameters for the stationary points are supplied as Supporting Information. Because of the presence of a negative charge, we have also optimized all the stationary points of the alkaline mechanisms with the 6-31+G* basis set, which includes diffuse functions on heavy atoms. The inclusion of diffuse functions stabilizes the separated reactants but does not produce important changes either in the structural description of the mechanisms or in the relative energies among the structures of the reaction path. Hence, in the discussion that follows, we will focus on the results of 6-31G* calculations, referring to the 6-31+G* calculations only as needed.

The **DS1** structure corresponds to a reactant complex between the β-lactam and the hydroxyl ion solvated by one water molecule. The distance from the hydroxyl oxygen to the carbonyl carbon atom is 3.0 Å. The hydroxyl ion has a charge of −0.78 au so that it has shared part of the negative charge with the water molecule (charge of −0.12 au) and the β-lactam (−0.10 au). The **DS2** structure is a transition structure, corresponding to the desolvation process and simultaneous addition of the hydroxyl to the β-lactam carbonyl. Here, the distance O14−C4 is reduced to 1.918 Å.[48] The hydroxyl addition process presents a free-energy barrier of 8.7 kcal/mol at the HF/6-31G* level. When diffuse functions are included on nonhydrogen atoms, the barrier is considerably increased, up to 16.2 kcal/mol, reflecting the large basis-set effect of the reactant complex. However, inclusion of correlation energy at

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(43) Olmstead, W. N.; Brauman, J. I. *J. Am. Chem. Soc.* **1977**, *99*, 4219 and references therein.

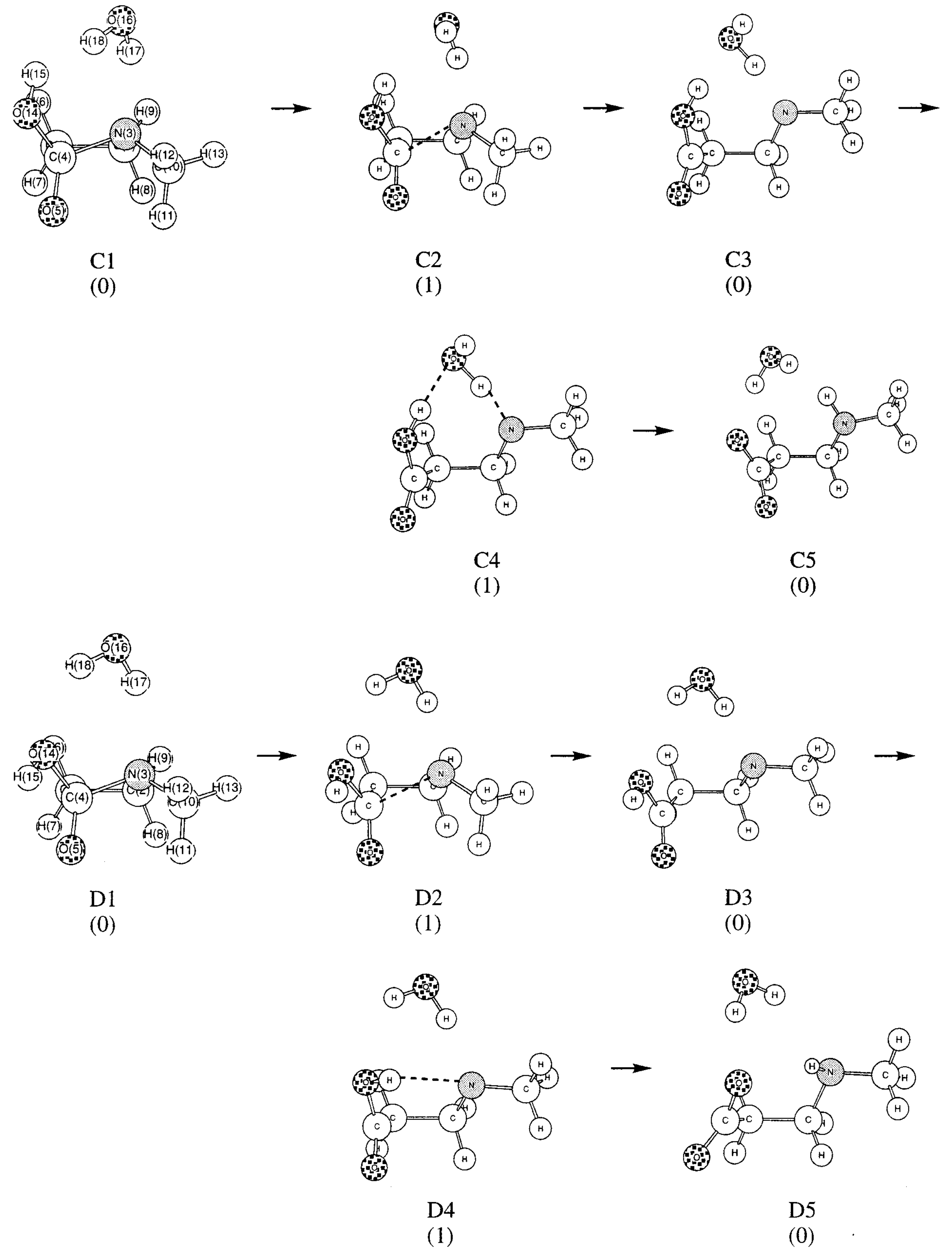
(44) Weiner, S. J.; Singh, U. C.; Kollman, P. A. *J. Am. Chem. Soc.* **1985**, *107*, 2219.

(45) Madura, J. D.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1986**, *108*, 2517−2527.

(46) Dewar, M. J. S.; Storch, D. M. *J. Chem. Soc., Chem. Commun.* **1985**, 94.

(47) Tuñón, I.; Rinaldi, D.; Ruiz-López, M. F.; Rivail, J. L. *J. Phys. Chem.* **1995**, *99*, 3798 and references therein.

(48) It is interesting to note that in the nonassisted process, we found a transition structure for the addition of $OH^-$to the β-lactam ring in a dielectric continuum medium with O14−C4 bond length equal 1.933 Å, at the same computational level. The two values are rather close which signifies that the single water molecule model enables an estimation of the geometry of the stationary points. This does not mean, as we said above and we show below, that the relative free energies are comparable to liquid-state results.



C1
(0)

C2
(1)

C3
(0)

C4
(1)

C5
(0)

D1
(0)

D2
(1)

D3
(0)

D4
(1)

D5
(0)

Figure 4. Stationary structures of the alkaline hydrolysis. Numbers in parentheses indicate the number of imaginary frequencies.

the MP2//HF/6-31G* level decreases the free-energy barrier up to 2.9 kcal/mol. Experimental studies have suggested that the addition of the hydroxyl ion is the rate-determining step in the alkaline hydrolysis of β-lactams,[49] yielding a value of 23.6 kcal/mol at 30 °C for the free-energy barrier in solution. As mentioned, the use of only one water molecule cannot reproduce the energetics of the desolvation process and gives, as expected, a much lower value for the free-energy barrier. Unfortunately, the accuracy to which this value may be determined is seriously limited by the solvent models available nowadays although the

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(49) Bowden, K.; Bromley, K. *J. Chem. Soc., Perkin Trans. 2* **1990**, 2111.

use of combined quantum mechanics and molecular mechanics potentials within molecular dynamics is promising.

The addition product, **DS3**, can be reached by IRC calculation from **DS2**. This product corresponds to a tetrahedral intermediate (structure **3** in Scheme 1) interacting with a water molecule by means of a hydrogen bond at O14. In analogy with the nonassisted process, this tetrahedral intermediate can achieve different conformations depending of the methyl group orientation and hydroxyl hydrogen dihedral angle. The structures located here are, apart from **DS3**, **C1** and **D1**. These two last are represented in the Figure 4. In the **DS3** structure, the methyl group and the hydroxyl lie in the same side of the β-lactam ring. In **C1** and **D1**, they are in opposite sides. In addition, in **D1**, the hydroxyl hydrogen H15 is oriented toward the carbonyl oxygen O5. If the C4−O14 bond is rotated so that hydroxyl hydrogen is oriented toward the nitrogen atom, the **C1** conformer is obtained. From the first stages of the hydroxyl addition, the nitrogen atom electron pair occupies the opposed side to the attacking group, the hydroxyl and the methyl group being at the same side. This means that **C1** and **D1** intermediates cannot be obtained directly in the hydroxyl addition process but must follow pyramidal inversion of the nitrogen atom in **DS3**. The interconversion process among all these structures was studied in a previous paper.[13]

The orientation of the water molecule in **C1** is suitable for double proton exchange. In the nonasssisted process, conformer **C1** was predicted to evolve with simultaneous ring opening and proton transfer through a single transition structure (step **3** → **5** in Scheme 1). Remarkably, in the assisted process, ring opening is made without any proton transfer. The **C2** transition structure corresponds to such a process, as reflected by its transition vector. The C−N distance contribution to that vector is the most important one (55%), and one may note a small participation of the H17−N3 distance (2%) as well as negligible contributions of the other geometrical parameters related to the water molecule. The distance C−N is lengthened from 1.568 Å in **C1** to 1.879 Å in **C2**. This is the only noticeable geometrical change. The water molecule is slightly closer to the hydroxyl hydrogen, having a H15−O16 distance 2.509 Å in **C1** and 2.244 Å in **C2**. The free energy of the **C2** transition structure is slightly higher than that of the energy minimum **C1** preceding it in the reaction path (2.31 kcal/mol at the HF/6-31G* level and 4.00 kcal/mol at the HF/6-31+G* level) but it is smaller (by 0.73 kcal/mol) at the MP2/6-31G* level. The nature of this transition structure therefore may be questioned. We shall come back to this point below.

By means of an IRC calculation, the subsequent minimum, **C3**, can be reached. In the **C3** structure, the distance C−N is 3.013 Å and the dihedral angle C1C2N3C4 is strongly incremented, up to −62.6°, so that the ring opening is concluded. This process is accompanied by a diminution of 20.94 kcal/mol with respect to **C2** (free energy, HF/6-31G* level). The water oxygen approaches the hydroxyl hydrogen atom to within 1.801 Å, and the water hydrogen H17 lies at 1.576 Å from the nitrogen atom. This arrangement is favorable for the next reaction step in which two protons are transferred: one from the water molecule to N3, another one from O14 to the water molecule. The **C4** transition structure corresponds to this concerted double proton transfer, as reflected in the transition vector in which the H15−O16 (9%), O16−H17 (21%), and H17−N3 (66%) distances participate.

Contrary to the neutral hydrolysis case, in the **C4** structure the O14−H15 and O16−H17 bonds have not yet begun to break. This is a common feature for transition structures found in the proton-transfer reaction steps of the alkaline hydrolysis. The transition structure generally has a reactant-like character, the original OH bonds being still quite strong. The **C4** structure does not present noticeable geometric differences with respect to the previous minimum, except for the decrease of the H15−O16 and H17−N3 distances (from 1.815 and 1.576 Å in **C3** to 1.744 and 1.443 Å in **C4**, respectively). As expected by Hammond's postulate,[50] this fact makes the potential energy of **C4** structure extremely close to that of **C3**, only 0.11 kcal/mol higher, and when free energies are considered, **C4** is lower than **C3** (by 0.76 and 2.39 kcal/mol at the HF/6-31G* and MP2//HF levels, for instance). Hence, as in the previous reaction step, we locate a transition structure in the HF potential energy surface which is lower than the preceding energy minimum in the MP2//HF free-energy profile. For this reason, we decided to carry out further calculations for this reaction path by optimizing the molecular geometries at the MP2/6-31G* level.

Results at the MP2//MP2/6-31G* level predict a single-step process which proceeds now through structures **C1**−**C2**−**C5**. However, some important changes hold for geometry parameters. In **C1**, the bond distance C4−N3 is increased from 1.568 to 1.738 Å. The water oxygen atom is considerably nearer to the hydroxyl hydrogen H15 (2.138 vs 2.509 Å at the HF level), and the water hydrogen atom is closer to the nitrogen atom (1.744 vs 2.021 Å at the HF level). All these changes favor the concerted proton relay mechanism. The transition structure **C2** has again a pronounced reactant-like character and its free energy is only 0.57 kcal/mol over **C1**. The C−N, H15−O16, and H17−N3 distances participate in the transition vector. Using IRC computations at the MP2 level, we have confirmed that, in fact, this transition structure leads directly to the hydrolysis product which is 39.07 kcal/mol more stable than **C1**.

Now we describe the hydrolysis process starting from the **D1** conformer. The **D2** transition structure corresponds to the ring-opening process (step **3** → **4** in Scheme 1), with the C−N distance 2.005 Å (1.535 Å in **D1**). Its free energy is 7.35 and 5.24 kcal/mol higher than that of **D1** at the HF/6-31G* and MP2//HF/6-31G* levels, respectively. The ring opening is achieved in the **D3** intermediate (2.834 Å for the C−N distance). Initially, in **D1**, the distance of the water molecule to the O14 and N3 atoms is similar. We can measure its value by means of the O14−H18 and N3−H17 distances, which are 2.164 and 2.115 Å, respectively, in **D1**. In **D3**, despite the larger O14−N3 distance caused by the ring opening, the water molecule keeps its hydrogen atoms oriented toward those atoms, though the interaction with N3 is preferred, with the O14−H18 and N3−H17 distances of 2.592 and 1.829 Å. **C3** and **D3** structures are formally equivalent, although **D3** is significantly less stable than **C3**. The differences are fundamentally the H15 dihedral angle and the water molecule orientation. In **C3**, the system structure is so that the water molecule plays the role of a proton relay in the transfer from hydroxyl to the nitrogen atom. However, in **D3** direct transfer of H15 to the nitrogen atom is likely to occur without active participation of the water molecule. Despite the larger H15−N3 distance (3.858 Å in **D3**), it is relatively simple to achieve the transition structure **D4**, which reflects this proton transfer with a transition vector dominated by the H15−N3 distance (47%) and the dihedral angle related to the H15 atom. In **D4** the distance between H15 and N3 is reduced to 2.778 Å, and this transition structure is only 3.66 and 3.42 kcal/mol higher in free energy than the intermediate **D3** at the HF/6-31G* and MP2//HF levels. There is a large

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(50) Hammond, G. S. *J. Am. Chem. Soc.* **1955**, *77*, 334.

Table 2. Energies and Free Energies for the Alkaline β-Lactam Hydrolysis in the Gas Phase and in Aqueous Solution (in kcal/mol)[a]

	$\epsilon = 1$						$\epsilon = 78.4$	
	HF/6-31G*		HF/6-31+G*		MP2//HF/6-31G*		HF/6-31G*	MP2//HF/6-31G*
	ΔE	ΔG	ΔE	ΔG	ΔE	ΔG	ΔG	ΔG
reactants	0.00	0.00	0.00	0.00	0.00 (0.0)	0.00 (0.0)	0.00	0.00 (0.00)
DS1	−54.78	−34.98	−40.97	−22.89	−66.89	−47.08	4.40	−7.54
DS2	−48.19	−26.28	−28.19	−6.70	−66.09	−44.18	13.36	−3.95
DS3	−53.70	−30.89	−33.32	−11.09	−68.87	−46.06	6.31	−8.18
C1	−52.30	−27.63	−30.78	−7.05	−69.50 (−71.66)	−44.83 (−47.55)	14.28	−5.53 (−7.23)
C2	−49.34	−25.32	−25.97	−3.05	−69.58 (−71.22)	−45.56 (−46.98)	17.16	−3.20 (−8.45)
C3	−69.50	−46.26	−45.98	−23.26	−81.44	−58.19	−1.98	−13.77
C4	−69.39	−47.02	−45.81	−24.06	−82.95	−60.58	−2.54	−15.89
C5	−103.71	−79.64	−81.70	−58.40	−110.03 (−111.25)	−85.96 (−86.62)	−50.82	−55.39 (−60.01)
D1	−59.22	−34.78	−38.63	−14.39	−75.04	−50.60	7.89	−8.43
D2	−50.93	−27.43	−28.03	−5.24	−68.86	−45.36	15.74	−2.75
D3	−61.06	−38.36	−38.49	−16.86	−69.86	−47.17	0.83	−8.39
D4	−56.45	−34.70	−34.34	−12.88	−66.13	−43.75	9.13	−0.26
D5	−108.28	−83.35	−85.70	−61.88	−115.42	−90.50	−53.75	−59.78

[a] Values in parentheses are calculated at the MP2 geometries.

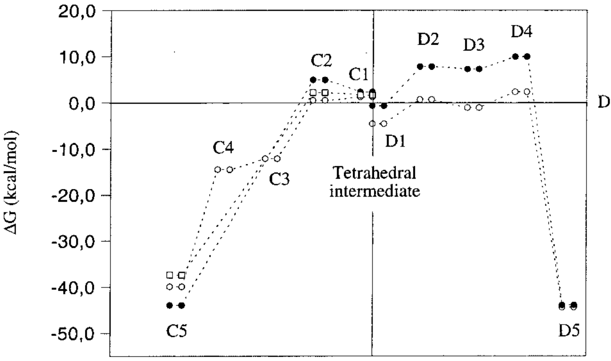

Figure 5. Free-energy profiles for the alkaline hydrolysis: (○) MP2//HF/6-31G* assisted; (□) MP2//MP2 assisted; (●) MP2//HF/6-31G* nonassisted.

energy release upon going from **D4** to the **D5** product, 48.65 and 46.75 kcal/mol at the HF and MP2//HF levels. The ring opening practically does not provide energy release, as can be seen in the free-energy difference between **D3** (ring opened intermediate) and **D1**. At the HF level **D3** is only 3.58 kcal/mol more stable than **D1**, and at the MP2//HF level it is even 3.43 kcal/mol higher in free energy than **D1**. The structure **D5** is a little more stable than **C5**, probably as a consequence of the intramolecular hydrogen bond between the transferred H15 and the proton donor O14, but both **C5** and **D5** are rotamers of the same product.

Further comments are needed with respect to the difference between HF and MP2//HF energies of the ring-opened and ring-closed structures. The MP2 structure of **D2** is 2.11 kcal/mol more stable than the HF structure with respect to **D1**. However, it is interesting to note that the ring-opened structures, **D3**, **D4**, and **D5**, are in MP2 7.01, 6.77, and 8.67 kcal/mol less stable than the HF structures with respect to the reference **D1**. This implies that in this system the HF description overestimates the stability of the ring-opened structures with respect to the ring-closed ones. When correlation is considered, this stabilization diminishes by about 7 kcal/mol. Apart from this, the HF and MP2//HF descriptions of the profile are similar when considering energy differences among ring-opened structures on one hand or among ring closed structures on the other hand.

Another aspect to analyze is related to the effect of the ancillary water in the free energy. Figure 5 shows the free energy profiles for paths C and D. The values of the corresponding nonassisted mechanisms are also shown for comparative purposes. In this figure we have chosen the **DS3** structure as the energy reference with the aim of collecting in the same graph the **C1**, **D1**, and **DS3** relative energies. From a global perspective, path D presents the same structures as the analogous nonassisted process, with addition of an electrostatic interaction between the water and β-lactam molecules. This interaction lowers the energy of all the stationary points along the path with respect to the nonassisted process, preserving in general the energy difference among the structures, with the exception of the first energy barrier from **D1** to **D2** which is decreased by 3.17 kcal/mol in the assisted mechanism. Although the energy of **C1** is slightly higher than that of **D1**, from that energy minimum structure the reaction may proceed practically barrierless; this mechanism is therefore predicted to be the most favorable.

We have also considered the reaction path starting from the **DS3** structure. In principle, this structure may evolve like **D1**. Actually, this is true for the ring-opening process but not for the following steps. We have searched for a transition structure similar to **D4**, where proton transfer from O14 to N3 takes place, the water molecule playing a passive role. However, we have not found such a structure because the −COOH group turns around the C4−C1 single bond and a transition structure identical with **C4** (but with the opposite dihedral angles) is found.

The electrostatic interaction energy with the solvent was also evaluated for all the points along the alkaline gas-phase reaction paths. The resulting relative free energies for all the structures are given in Table 2. The most pronounced effect is the stabilization of the separated reactants, due fundamentally to the large solvation energy of the hydroxyl ion. This fact has a strong effect on the relative free energies of the tetrahedral intermediates, **DS3**, **C1**, and **D1**, which go from −46.06, −44.83, and −50.60 kcal/mol in the gas phase to −8.18, −5.53, and −8.43 in the continuum, at the MP2//HF level. The same effect is presented at the HF level, where the free energies become positive. Apart from this, the relative free-energy profile remains essentially unaltered.

4. Conclusions

In this article we have examined the effect of an ancillary water molecule in the energetics and mechanism of neutral and alkaline hydrolysis of *N*-methylazetidinone. Solvent effects have been also considered by means of a polarizable continuum model. In the neutral hydrolysis at the HF/6-31G* level, we have determined that the free-energy profiles for the assisted and nonassisted processes are nearly identical. When correlation energy is considered at the MP2 level, the height of the free-energy barriers is considerably lowered. The energy difference between the two reaction pathways considered is small, and the preference for one or another reaction channel can be determined by the position and orientation of the water molecules in their attack on the β-lactam. Molecular dynamics simulation results indicate the absence of an organized coordination layer around the β-lactam nitrogen in aqueous solution. However, it exists around the β-lactam oxygen and carbon atoms. Hence, hydration of the carbonyl group appears to be the most probable event for initiating the β-lactam hydrolysis reaction, and the nonconcerted path B would be more likely to occur in such conditions. One must keep in mind that these considerations are valid in aqueous solution where participation of neighboring water molecules may render easier the passage from **B3** to **B4**. In an enzymatic environment, this conversion is expected to be energetically easy also, but the detailed mechanism may vary from system to system and could require the presence of two ancillary water molecules in the vicinity of the active site.

Water-assisted hydrolysis in basic media also has been studied. With respect to reactions examined before, the alkaline hydrolysis has the particularity of the presence of a negative charge. The first reaction step is the formation of a tetrahedral complex which requires hydroxyl desolvation. A transition structure for this process has been described although the free-energy barrier is difficult to compute. Starting from this complex, several mechanisms are possible to achieve the hydrolysis of the β-lactam. Bifunctional catalysis is predicted for one of the reaction pathways described here, the concerted mechanism C. It must be stressed that this process is not predicted to be concerted when studied at the HF level, contrary to the corresponding nonassisted reaction. The inclusion of correlation is therefore fundamental in this case. A stepwise mechanism has also been investigated, but here the water molecule plays a passive role. It stabilizes all the structures along the reaction path, which are similar to those found in the nonassisted hydrolysis, the height of the energy barriers being conserved generally. Comparison of the two assisted mechanisms for alkaline hydrolysis shows that the energy barrier in the concerted mechanism is significantly smaller than that in the stepwise one. Therefore, these results support the preference for the concerted mechanism, though one should consider that the energies of the tetrahedral intermediates, which constitute the starting points for concerted and stepwise paths (**C1** and **D1**, respectively), are different. Ultimately, the preferential mechanism may be determined by comparison of the barriers for interconversion among the **C1**, **D1**, and **DS3** intermediates. These barriers were determined in a previous study.[13]

Bulk solvent effects have been also considered by means of a polarizable continuum model. In the neutral hydrolysis the heights of the energy barriers are increased. Whereas in the gas phase, the energy barrier is smaller for path B, the reverse is found in solution, with path A having a smaller value. The energy difference between the two reaction paths remains small at the MP2//HF level. In the alkaline hydrolysis, the tetrahedral intermediates are less stabilized with respect to the reactants. At the HF level, their relative free energies become even positive. The relative free-energy profile remains essentially unaltered.

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Supporting Information Available: Tables of selected geometrical parameters for all structures studied in this work (2 pages). See any current masthead page for ordering information and Web access instructions.

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