Theoretical Study of the Alkaline Hydrolysis of a Bicyclic Aza-β-lactam

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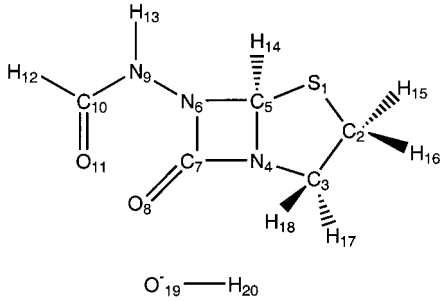
Various mechanisms for the alkaline hydrolysis of an aza-β-lactam in the gas phase were studied by ab initio calculations at the RHF/6-31+G*//RHF/6-31+G*, MP2/6-31+G*//MP2/6-31+G*, and B3LYP/6-31+G*//B3LYP/6-31+G* levels. Solvent effects were considered via IPCM (isodensity polarizable continuum model) calculations at the IPCM/6-31+G*//RHF/6-31+G* level. The alkaline hydrolysis of β-lactams begins with a nucleophilic attack of the hydroxyl ion on the carbonyl of the β-lactam ring. The tetrahedral intermediate thus formed undergoes cleavage of the C_7-N_4 bond to give the reaction end products. In addition to the typical cleavage reaction, the β-lactam studied can undergo opening at the C_7-N_6 bond (Scheme 1). Both processes have a similar activation energy that varies slightly depending on the particular computation method used. The most stable end products are those formed via the typical mechanism. In any case, both mechanisms yield products possessing a carbamate group, which suggests that the starting aza-β-lactam might be an effective inhibitor for β-lactamases.

Introduction

No doubt, antibiotics constitute one of the crucial landmarks in the history of medicine. Ever since penicillin was discovered in 1928 and subsequently introduced in clinical practice, β-lactam antibiotics have helped dramatically decrease mortality rates. The antibacterial action of β-lactams relies on their ability to inhibit PBPs and hence the formation of the bacterial wall.[1] Such inhibition results from the antibiotic acylating a serine residue at the active site of the enzyme.[2,3] However, bacteria possess an extremely high ability to adjust to attempts at eliminating them. After half a century of use, many survival bacteria are resistant to a greater or lesser degree to the antibiotics used at present; this has promoted the search for new, effective antibacterial substances. The principal defense mechanism of bacteria against antibiotics is the production of β-lactamases. These enzymes catalyze the hydrolysis of β-lactam antibiotics to products that are harmless to bacteria.[4] Like PBPs, many β-lactamases are serine enzymes.[4]

Attempts at overcoming bacterial resistance have been aimed in two directions as regards the development of new antibiotics. One approach has involved the development of molecules such as aztreonam[5] and imipenem,[6] which possess antibacterial power and are resistant to the action of β-lactamases. The other has addressed the problem by synthesizing molecules of little or no antibacterial power but capable of inactivating β-lactamases, for use in combination with other antibiotics possessing antibacterial action (e.g., clavulanic acid[7] or sulbactam,[8] which are used jointly with ampicillin, and tazobactam,[9] which is administered together with piperacillin). These inhibitory antibiotics can act via various mechanisms; thus, they can bind to β-lactamases in an irreversible (e.g., so-called "suicidal substrates" such as clavulanic acid) or reversible manner with low turnover rates (e.g., third-generation cephalosporins).[10]

SCHEME 1



Ghosez and coworkers[11−13] studied aza-γ-lactams (bicyclic imidazolidinones) with a view to identifying those with antibacterial power capable of inactivating β-lactamases. The inhibitory potential of these compounds possibly stems from their ability to form especially stable carbamoyl-enzyme complexes. However, the substances studied exhibited little antibacterial activity,[12,13] probably as a result of the low reactivity of the five-member ring of γ-lactams. Subsequently, Nangia and coworkers[14−16] used semiempirical methods to determine various structural parameters for aza-β-lactams including the pyramidality of the lactam nitrogen and the charges on the atoms in the diazethidin-2-one ring; they concluded that the substances studied must possess both antibacterial and inhibitory activity, due to the formation of carbamoyl-enzyme complexes with β-lactamases (Scheme 2). However, the semiempirical structural parameters (distances and charges) are known to be inadequate for describing the chemical and antibacterial properties of β-lactams. The results of recent experiments suggest that some aza-β-lactams possess antibacterial activity;[17] neither their chemical reactivity nor their ability to inhibit β-lactamases has so far been examined, however.

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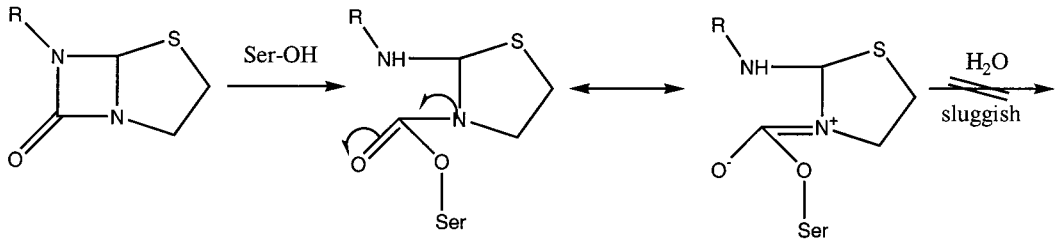
SCHEME 2



Studies of the alkaline hydrolysis of β-lactams using theoretical methods have provided useful information about their antibacterial properties on account of its similarity to enzyme hydrolysis.[18−21] It is therefore of interest to use theoretical methods to investigate the alkaline hydrolysis of compounds capable of forming carbamoyl-enzyme intermediates with a view to determining their antibacterial properties. In previous work,[22] semiempirical calculations revealed that substitution of the carbon atom adjacent to the carbonyl group in the β-lactam ring by various heteroatoms (nitrogen, oxygen, and sulfur) provides a pathway for the formation of carbamoyl-enzyme complexes; these molecules might thus act as β-lactamase inhibitors. Deeper studies confirmed that oxo-β-lactam compounds must possess such properties.[23,24]

In this work, potential mechanisms for the alkaline hydrolysis of an aza-β-lactam (Scheme 1) were examined. The compound studied is analogous to penicillins and results from substitution of the CH−NH−COR group at position 6 by an N−NH−COH group. Two different reaction pathways were considered, namely, (*a*) the typical mechanism,[25,26] which involves the nucleophilic attack of the hydroxyl group on the β-lactam carbonyl and the subsequent cleavage of the C_7-N_4 bond, and (*b*) one involving cleavage of the C_7-N_6 following the nucleophilic attack.

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.[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).

All the structures have also been optimized using Møller−Plesset's perturbation theory,[29] as implemented by Pople et al.,[30] at MP2 level (MP2/6-31+G*//MP2/6-31+G*). Also, calculations have been carried out with Becke's three parameters hybrid functional method,[31] using the LYP correlation functional,[32,33] with the 6-31+G* basis set (B3LYP/6-31+G*//B3LYP/6-31+G*). Henceforward, RHF, IPCM, MP2, and B3LYP stand for RHF/6-31+G*//RHF/6-31+G*, IPCM/6-31+G*//RHF/6-31+G*, MP2/6-31+G*//MP2/6-31+G*, and B3LYP/6-31+G*//B3LYP/6-31+G*, respectively. All the energies in the text include the ZPE correction. For IPCM energies we use the HF ZPEs. For MP2 energies we use the HF ZPEs scaled by 0.8929 as recommended by Pople et al.[34] For the B3LYP energies we use the HF ZPEs scaled by 0.9334.[35]

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.[36]

Results and Discussion

The structure examined in this study (Scheme 1) is similar to that of penicillins except for the fact that the carbon atom adjacent to the carbonyl group of the azethidin-2-one ring was replaced with a nitrogen atom, i.e., an azethidin-2-one ring was substituted by a 1,3-diazethidin-2-one ring. The carboxyl group present in penicillins was omitted in order to simplify calculations. The principal geometric parameters for this compound (**a** in Figure 1) are given in Tables 1 (RHF and IPCM calculations), 2 (MP2 calculations), and 3 (B3LYP calculations).

The length of the C_7-N_4 bond was slightly greater in the aza-β-lactam than in the β-lactam ring with the three computation methods used (viz., 1.400, 1.422, and 1.420 Å with RHF, MP2, and B3LYP versus 1.356 Å for the azethidin-2-one rings as calculated by the RHF/6-31+G* method[37]) and very similar to that of the oxo-β-lactam (1.399 Å with RHF/6-31+G*).[24] Like the azethidin-2-one and 3-oxa-azethidin-2-one ring, the 1,3-diazethidin-2-one ring is virtually planar. As expected, the MP2 calculations provided bond lengths similar to those of the B3LYP calculations and greater than those of the RHF calculations. The B3LYP method was that giving the longest C−S distances. These trends were consistent among the structures studied.

The alkaline hydrolysis of β-lactams begins with the nucleophilic attack of the hydroxyl group on the carbonyl group, followed by cleavage of the C_7-N_4 bond (a B_{AC2} mechanism).[38] The compound studied in this work can additionally undergo cleavage at the C_7-N_6 bond. Figure 1 shows the different structures studied and reaction pathways considered. The corresponding structural and energy data are given in Tables 1 (RHF and IPCM calculations), 2 (MP2 calculations), and 3 (B3LYP calculations). Figure 2 shows the reaction profiles obtained from the IPCM and B3LYP calculations, which were similar to those provided by the other two computation methods.

The charge on the carbon atom in the carbonyl group is directly related to the reactivity of this compound. Such a charge is 0.98 (RHF) in the aza-β-lactam and thus similar to that in the oxo-β-lactam (1.04). These values are much greater than those for penicillin G (0.48) and clavulanic acid (0.62) owing to the high electronegativity of the heteroatoms present in the oxo- and aza-β-lactams (Figure 3). Therefore, substituting the carbon adjacent to the carbonyl group in β-lactams by a nitrogen or oxygen atom must result in significantly increased reactivity. This effect was not observed by Nangia and coworkers[15] in their structural study of various aza-β-lactams, owing to the inherent shortcomings of the computation method used (semiempirical AM1).

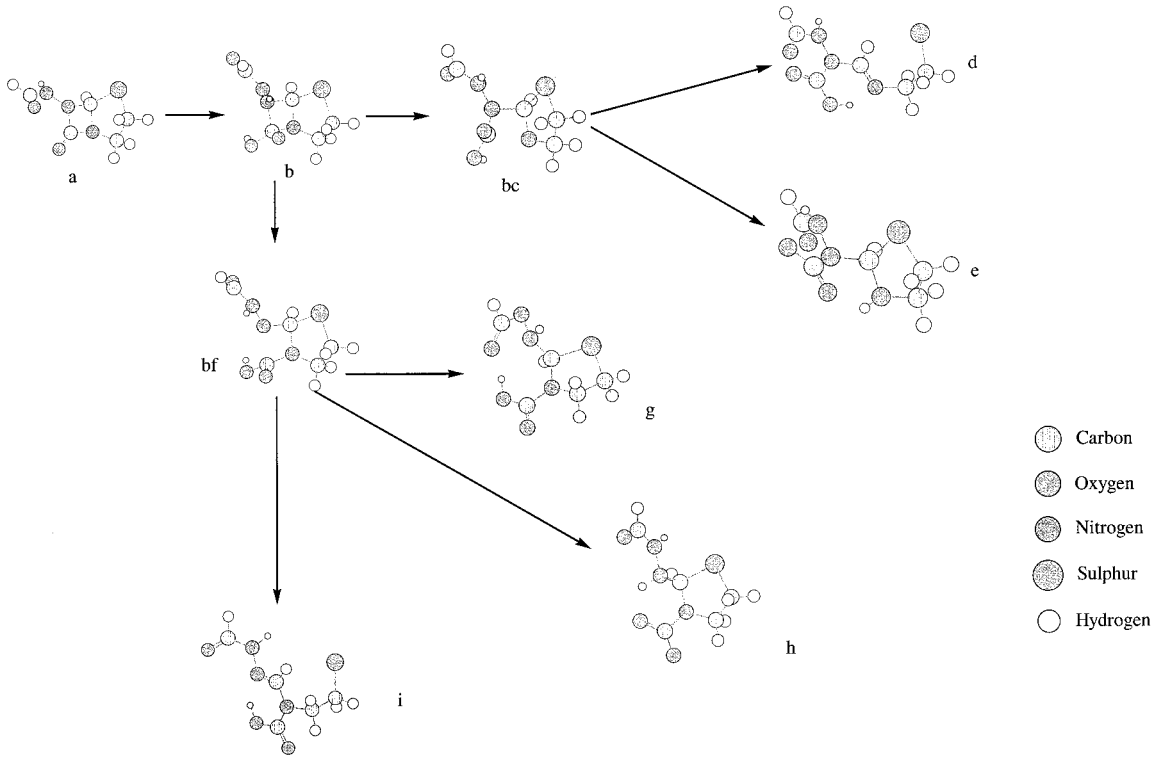


Figure 1. Structures corresponding to the different intermediate and final states of the reaction pathway of alkaline hydrolysis of the aza-β-lactam compound in the gas phase at the RHF/6-31+G*//RHF/6-31+G* level.

TABLE 1: Main Geometric Parameters, Energy (in gas phase and in aqueous solution) and Imaginary Frequencies at RHF/6-31+G*//RHF/6-31+G* Level of the Reactants, Intermediates, Transition States and Final Products of the Alkaline Hydrolysis of the Aza-β-lactam Compound[a]

	C_7-N_4	C_7-N_6	N_4-H_{20}	N_6-H_{20}	C_5-S_1	$O_8C_7N_4$	$C_7N_4-C_5N_6$	RHF energy	ZPE	rel energy	im freq.	IPCM energy	IPCM rel energy
a	1.400	1.393			1.812	134.2	−2.6	−979.351 30[b]	0.154 38[b]	0	0	−979.512 87[b]	0
b	1.525	1.528	2.434	2.437	1.848	118.5	16.8	−979.426 81	0.160 71	−43.41	0	−979.530 37	−7.01
bc	2.018	1.468	2.349	2.392	1.890	118.8	17.8	−979.408 26	0.158 15	−33.88	1(−322.1)	−979.508 42	5.16
d	2.916	1.397	1.748	2.286	3.257	171.4	2.9	−979.480 68	0.159 76	−77.81	0	−979.582 36	−40.23
e	3.046	1.463	1.003	2.381	1.911	164.2	25.9	−979.494 96	0.161 63	−85.60	0	−979.602 65	−51.79
bf	1.527	1.941	2.512	2.515	1.908	126.0	7.4	−979.410 44	0.159 34	−34.00	1(−288.3)	−979.499 38	11.58
g	1.374	3.018	2.491	2.434	1.863	120.6	−60.7	−979.481 94	0.161 70	−77.38	0	−979.583 89	−39.47
h	1.432	3.057	2.540	1.006	1.836	115.2	−72.9	−979.488 65	0.160 89	−82.10	0	−979.597 04	−48.73
i	1.392	2.893	2.358	1.782	3.163	121.7	−2.9	−979.486 36	0.160 23	−81.08	0	−979.583 10	−40.40

[a] Bond length in angstroms. Bond and dihedral angles in degrees. Energy and ZPE in hartree and relative energies in kcal/mol. Relative energies include ZPE correction. IPCM relative energies include RHF ZPE correction. Frequencies in cm^{-1}. [b] Addition of aza-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 Aza-β-lactam Compound[a]

	C_7-N_4	C_7-N_6	N_4-H_{20}	N_6-H_{20}	C_5-S_1	$O_8C_7N_4$	$C_7N_4-C_5N_6$	MP2 energy	ZPE	rel energy
a	1.422	1.423			1.808	134.4	−3.4	−981.182 15[b]	0.137 85[b]	0
b	1.558	1.583	2.417	2.452	1.848	71.8	19.1	−981.270 10	0.143 50	−51.64
bc	2.174	1.493	2.187	2.395	1.940	123.6	21.1	−981.251 66	0.141 21	−41.51
d	2.890	1.441	1.564	2.271	2.603	169.2	15.6	−981.291 01	0.142 65	−65.30
e	3.038	1.501	1.029	2.351	1.931	114.4	28.9	−981.323 99	0.144 32	−84.95
bf	1.511	1.878	2.413	2.541	1.891	121.1	−7.0	−981.257 42	0.142 28	−44.45
g	1.400	2.967	2.524	2.426	1.869	120.1	−55.7	−981.312 05	0.144 38	−77.42
h	1.464	3.078	2.534	1.032	1.837	114.9	−78.1	−981.311 05	0.143 66	−77.24
i	1.414	2.895	2.357	1.676	2.852	121.4	4.0	−981.295 79	0.143 07	−68.03

[a] Bond length in angstroms. Bond and dihedral angles in degrees. Energy and ZPE in hartree and relative energies in kcal/mol. Relative energies include ZPE correction. ZPE correction is the RHF ZPE correction scaled by 0.8929.[34] [b] Addition of aza-β-lactam structure and OH^-.

The nucleophilic attack takes place on the α side of the diazethidin-2-one ring, which is the energetically more favorable choice.[39] This process is subject to no activation energy, as in the alkaline hydrolysis of carbonyl compounds in the gas phase.[25,40−42] The tetrahedral intermediate formed (**b** in Figure 1) is much more stable than the reactants (−43.41, −51.64, and −46.26 kcal/mol with RHF, MP2, and B3LYP, respectively) (Tables 1−3). The absolute values of these energies are much greater than those for azethidin-2-one[37] as calculated by using the RHF/6-31+G*//RHF/6-31+G* method (−20.61 kcal/mol)

TABLE 3: Main Geometric Parameters and Energy at B3LYP/6-31+G*//B3LYP/6-31+G* Level of the Reactants, Intermediates, Transition States, and Final Products of the Alkaline Hydrolysis of the Aza-β-lactam Compound[a]

	C_7-N_4	C_7-N_6	N_4-H_{20}	N_6-H_{20}	C_5-S_1	$O_8C_7N_4$	$C_7N_4-C_5N_6$	B3LYP energy	ZPE	rel energy
a	1.420	1.421			1.833	134.2	−2.3	−983.413 89[b]	0.144 09[b]	0
b	1.567	1.596	2.430	2.470	1.889	120.0	17.4	−983.493 44	0.150 01	−46.26
bc	2.102	1.508	2.333	2.403	1.996	121.8	19.0	−983.481 70	0.147 62	−40.39
d	2.897	1.432	1.568	2.269	2.982	171.9	6.3	−983.534 89	0.149 12	−72.83
e	3.031	1.500	1.028	2.392	2.034	163.2	24.8	−983.556 42	0.150 87	−85.24
bf	1.465	2.220	2.388	2.556	1.923	123.4	8.0	−983.479 52	0.148 72	−38.33
g	1.395	3.102	2.541	2.518	1.919	118.1	−66.4	−983.543 17	0.150 93	−76.89
h	1.510	3.663	2.625	1.023	1.920	113.9	139.2	−983.547 77	0.150 17	−80.25
i	1.413	2.895	2.353	1.679	3.015	116.3	−1.4	−983.544 29	0.149 56	−78.45

[a] Bond length in angstroms. Bond and dihedral angles in degrees. Energy and ZPE in hartree and relative energies in kcal/mol. Relative energies include ZPE correction. ZPE correction is the RHF ZPE correction scaled by 0.9334.[35] [b] Addition of aza-β-lactam structure and OH^-.

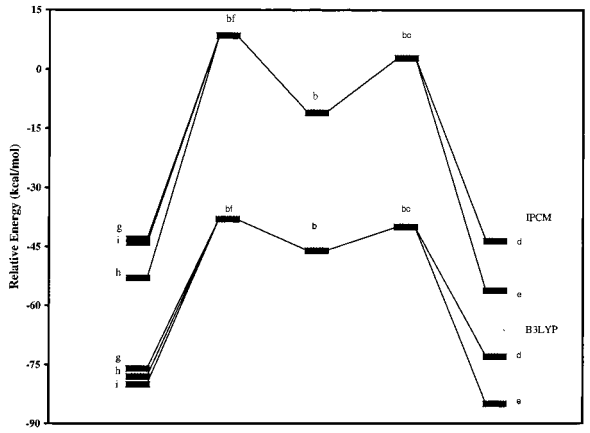


Figure 2. Reaction pathways for the alkaline hydrolysis of the aza-β-lactam compound in the aqueous phase at the IPCM/6-31+G*//RHF/6-31+G* level and in the gas phase at the B3LYP/6-31+G*//B3LYP/6-31+G* level.

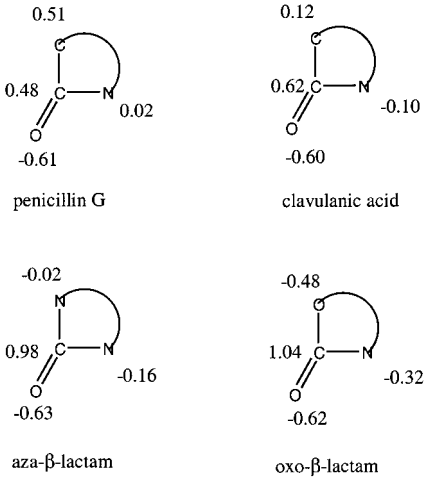


Figure 3. Mulliken charges (RHF/6-31+G*//RHF/6-31+G*) on the carbonyl group and nearest atoms in the lactam ring for different compounds.

and similar to those obtained for the oxo-β-lactam[24] from the RHF (−42.83 kcal/mol) and MP2 methods (−47.02 kcal/mol). The formation of the tetrahedral intermediate produces strong relaxation of the C_7-N_4 and C_7-N_6 bonds (0.12−0.13 Å, depending on the particular computation method); by contrast, only the C_7-N_4 bond changes (increases in length) in classical β-lactams (the C_7-C_6 bond length is hardly altered). This suggests that the aza-β-lactam ring can undergo both types of cleavage (via the C_7-N_4 or the C_7-N_6 bond).

The presence of a solvent is known to be able to substantially alter the potential energy surfaces of many reactions, particularly those involving charged species. The IPCM method,[28] used in this work, is based on Onsager's reaction field model,[43] which regards the solvent as a continuum with a given dielectric constant and assumes that the solute occupies a cavity within the continuum. In the IPCM method, the cavity is defined by the electronic isodensity surface of the molecule.

The principal effects of the solvent are stabilizing the reactants and creating an activation energy for the initial nucleophilic attack that yields the tetrahedral intermediate. This effect has been observed both in β-lactams[25,44,45] and in other carbonyl compounds.[46,47] The barrier arises from desolvation of hydroxyl ion and is about 16 kcal/mol, whichever the β-lactam. Based on the similarity between the aza-β-lactam studied here and β-lactams, the formation of the tetrahedral intermediate **b** in the aqueous phase must be subject to an activation energy in this region.

The formation of the tetrahedral intermediate in the aqueous phase is not so exothermal as in the gas phase (−7.01 kcal/mol with IPCM), owing to the stabilizing effect of the solvent on the reactants. This value is smaller than that obtained for the oxo-β-lactam compound (−18.12 kcal/mol, also with IPCM) and similar to those for β-lactams provided by various methods. Using ab initio calculations and a polarizable continuum model (PCM) for the solvent, Pitarch et al.[40] obtained a stabilization energy of −8.62 kcal/mol in the formation of the tetrahedral intermediate of *N*-methylazethidin-2-one. In previous work, a stabilization energy of −13.6 kcal/mol was obtained in a semiempirical study of the hydrolysis of cephalothin solvated by five water molecules[48] and one of −12.0 kcal/mol (calculated using the AMSOL method) for clavulanic acid.[41]

The tetrahedral intermediate, **b**, can evolve with cleavage of the C_7-N_4 bond to yield various products depending on the orientation of H_{20}, all via transition state **bc** (Figure 1, Tables 1−3). The activation energy for this process in the gas phase is 9.53 kcal/mol with RHF, 10.13 kcal/mol with MP2, and only 5.87 kcal/mol with B3LYP. The RHF and MP2 values are similar to those obtained in the gas phase for the alkaline hydrolysis of an oxo-β-lactam (14.15 kcal/mol with MP2)[24] and the azethidin-2-one ring (10.00 kcal/mol with MP2).[37] They are also similar to that obtained by Pitarch et al. for *N*-methylazethidin-2-one (10.06 kcal/mol with MP2).[40] The IPCM activation energy in the aqueous phase is 12.17 kcal/mol and also similar to those for azethidin-2-one (12.43 kcal/mol)[37] and *N*-methylazethidin-2-one (11.90 kcal/mol).[40]

An IRC calculation for transition state **bc** yielded tetrahedral intermediate **b** on one hand and end product **d** on the other (Figure 1, Table 1). Such a product is the result of the cleavage of the azethidin-2-one ring at the C_7-N_4 bond and of the opening of the thiazolidine ring at the C_5-S_1 bond. End products similar to **d** were previously obtained in the alkaline hydrolysis of the oxo-β-lactam[24] and clavulanic acid;[41] reaching the last

two, however, entails overcoming a low activation energy (0.26 kcal/mol for the oxo-β-lactam and 3.9 kcal/mol for clavulanic acid, both with RHF). Nevertheless, most lactams preserved the five-member ring fused to the azethidin-2-one ring upon alkaline hydrolysis.

Previous semiempirical calculations revealed that if the C_5-S_1 bond length is fixed and H_{20} is placed at an appropriate position, then this proton is transferred from the carboxyl group to N_4 with no potential barrier. This yields end product **e** (Figure 1, Tables 1−3), which is much more stable than the tetrahedral intermediate as the negative charge is transferred from N_4 to the carboxylate group. Similar end products were obtained in the alkaline hydrolysis of penicillins and cephalosporins.[41]

The cleavage of the C_7-N_6 bond gives rise to different products. The activation energy of this process (via transition state **bf**) is 9.41, 18.59, 7.19, and 7.93 kcal/mol with RHF, IPCM, MP2, and B3LYP, respectively. These values are slightly lower than those for the typical cleavage at C_7-N_4 provided by RHF and MP2 calculations and greater than those obtained from IPCM and B3LYP calculations. It is therefore impossible to determine which cleavage pathway is subject to the lower activation energy; in any case, both activation barriers are very similar.

IRC (RHF) calculations for transition state **bf** yielded the tetrahedral intermediate **b**, on one hand, and end product **i** (a result of cleavage at the C_7-N_6 and C_5-S_1 bonds) on the other. In previous work, semiempirical calculations led to other end products.[22] The previous structures were also optimized. Thus, end product **h** was a result of the transfer of H_{20} to N_6, which helped stabilize the negative charge on the nitrogen. In **g**, H_{13} was transferred to N_6.

All the end products are markedly stabilized in relation to the reactants (between −65 and −85 kcal/mol with RHF, MP2, and B3LYP), similarly to the products of the hydrolysis of the azethidin-2-one ring (−72.73 kcal/mol with RHF)[37] and an oxo-β-lactam (between −70 and −90 kcal/mol with RHF).[24] The differences are not so marked with IPCM calculations owing to the increased stability of the reactants in the aqueous phase. The energy difference between the end products and the reactants as per IPCM calculations ranges from −40 to −52 kcal/mol. Again, these values are similar to those for the azethidin-2-one ring (−56.88 kcal/mol with RHF/6-31+G* SCRF)[37] and the oxo-β-lactam (between −56 and −61 kcal/mol with IPCM).[24] The most stable end products, whichever the computation method used, are **e** and **h**. The high stability of these compounds is a result of their negative charge being delocalized over the carboxylate group formed in the process.

Both the geometric parameters for the aza-β-lactam and its chemical reactivity (viz., the stability of the geometric intermediate and subsequent activation energies) are similar to those obtained for classical β-lactam antibiotics. One can therefore expect this compound, and similar others, to possess antibacterial activity, as recently suggested elsewhere.[17] One additional factor that increases the interest of this structure and its analogues is its potential ability to inhibit β-lactamases. The mechanism by which β-lactamases inactivate β-lactam antibiotics involves the formation of an acyl-enzyme intermediate, the hydrolysis of the antibiotic, and the release of the acid formed, which is harmless to bacteria. Because of its peculiar structure, the aza-β-lactam yields end products possessing the carbamate group, whether through the typical cleavage pathway (viz., that at the C_7-N_4 bond) or through that at C_7-N_6. One can therefore expect the enzyme hydrolysis process not to involve the release of the corresponding acid; rather, a carbamoyl-enzyme complex will be formed, release from which of the hydrolysis product will be very slow.

On the other hand, reaction products **d** and **i**, yielded by the IRC calculations from the transition states **bc** and **bf**, are the result of opening of the thiazolidine ring fused to the diazethidin-2-one ring. This type of product tends to remain bound to β-lactamases and cause their irreversible inactivation in a manner similar to clavulanic acid.[41] The combination of the inhibitory ability resulting from the formation of the carbamoyl-enzyme complex and opening of the thiazolidine ring, together with its antibacterial property, make the aza-β-lactam and its analogues extremely promising substances as antibiotics.

A wide variety of potentially active substances against bacteria can be obtained by replacing C_6 in the azethidin-2-one ring with various heteroatoms[22] or by altering the side chains bonded to the heteroatom at position 6.[17] The chemical reactivity of these structures and their interactions with serine enzymes will be examined in future work.

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