Ab Initio Calculations on Neutral and Alkaline Hydrolyses of β-Lactam Antibiotics. A Theoretical Study Including Solvent Effects

Jesús Pitarch,[†,‡] Manuel F. Ruiz-López,*[,‡] Juan-Luis Pascual-Ahuir,[†] Estanislao Silla,*[,†] and Iñaki Tuñón[†]

Departamento de Química Física, Universidad de Valencia, 46100 Burjassot (Valencia), Spain, and Laboratoire de Chimie Théorique, URA CNRS 510, Université Henri Poincaré-Nancy I, 54506 Vandoeuvre-les-Nancy, France

Received: October 30, 1996; In Final Form: January 29, 1997[⊗]

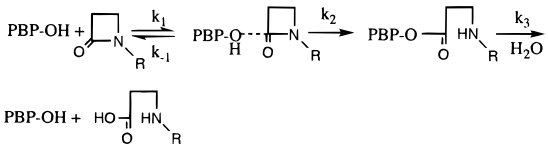
In this work we present a theoretical study of neutral and alkaline hydrolyses of *N*-methylazetidinone as a model of biological hydrolysis of β-lactam antibiotics. Calculations have been carried out at the HF and MP2 levels using the 3-21G, 6-31G*, and 6-31+G* basis sets. Solvent effects have been included by means of a polarizable continuum model. Our results indicate two possible reaction mechanisms for the β-lactam hydrolysis: concerted and stepwise. In both the neutral and alkaline hydrolyses the concerted mechanism presents a free energy barrier lower than that of the stepwise mechanism.

1. Introduction

β-Lactam antibiotics activity is due to the covalent bonding to the active site serine residue of a group of enzymes named penicillin-binding proteins (PBP).[1] In this way, the antibiotic inhibits the synthesis of the peptidoglycan component of the bacterial cell walls.[2] However, some bacteria are resistant to the action of β-lactam antibiotics because of their ability to produce β-lactamase enzymes that catalyze the hydrolysis of the antibiotic to its amino acid.[3] Most of the β-lactamases (classes A and C) also act by using an active site serine.[4] The general β-lactam antibiotics reaction mechanism with serine-containing bacterial enzymes can be represented by the kinetic scheme shown in Scheme 1. In the first stage, the antibiotic and the enzyme form the enzyme−substrate complex. Then, the hydroxyl group of the serine residue of the active site is acylated by the β-lactam carbonyl group and finally the acyl−enzyme is hydrolyzed, regenerating the enzyme and producing the degradation of the antibiotic. When k_3 is small, the enzyme activity inhibition is produced, interrupting the biosynthesis of bacterial cell walls. On the other hand, when k_3 is large, the PBP enzyme is considered to be a β-lactamase.[5]

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 the last years several theoretical studies employing molecular orbital calculations have been devoted to this subject.[6−11] To model the hydrolysis of the β-lactam antibiotics, the azetidinone molecule or some substituted derivative is used, although full antibiotic molecules have also been studied in semiempirical calculations.[8,9] The nucleophile agent is normally represented by a hydroxide anion,[6−9] a water molecule,[10] or methanol.[10] The alkaline β-lactam hydrolysis has been studied at the HF/STO-3G[6,7] and semiempirical levels.[8,9] The potential energy surface (PES) of the neutral hydrolysis with water or methanol has been explored carrying out geometry optimizations at the HF/3-21G or 3-21G* levels and single-point calculations at higher theoretical levels.[10] The hydrolysis of β-lactam antibiotics can be seen as a special case of the hydrolysis of amides.

SCHEME 1



Because of the importance of this reaction as a model for the cleavage of peptide bonds in living systems, there are several theoretical studies devoted to the study of the amide hydrolysis mechanism. Neutral, acid, base-promoted, and water-assisted hydrolyses of formamide and some derivatives have been investigated.[12−14]

From these previous theoretical works, it can be assessed that the general β-lactam hydrolysis mechanism usually begins with the formation of a substrate−nucleophile agent intermediate. This intermediate subsequently undergoes the cleavage of the C−N bond and the transfer of an hydrogen atom to the β-lactam nitrogen. All these steps can proceed consecutively (stepwise hydrolysis mechanism) or simultaneously (concerted mechanism) as shown in the Scheme 2.

Our purpose in this paper is to give a more complete and unified description of the possible mechanisms of β-lactam hydrolysis. The *N*-methyl-substituted β-lactam (*N*-methylazetidinone) has been choose as the substrate, while for the nucleophilic agent both hydroxyl anion and water molecule have been considered. The influence of the calculation level (basis set and correlation energy) is analyzed for the neutral and alkaline hydrolyses. Bulk solvent effects are also considered by means of a polarizable continuum model. The catalytic effect of solvent molecules in water-assisted hydrolysis will be the subject of a forthcoming paper.

2. Methodology

Preliminary exploration of the PES for the β-lactam hydrolysis has been carried out at the HF/3-21G level.[15] Stationary points have been relocated at the HF/6-31G*[16] level for both neutral and alkaline hydrolyses and also at the HF/6-31+G*[17] level only for alkaline hydrolysis. Geometry optimizations have been carried out using Berny's algorithm[18] as implemented in the GAUSSIAN92[19] package of programs and the redundant coordinates algorithm[20] implemented in GAUSSIAN94.[21] The

* To whom correspondence should be addressed.
† Universidad de Valencia.
‡ Université Henri Poincaré-Nancy I.
⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1997.

S1089-5647(96)03387-1 CCC: $14.00

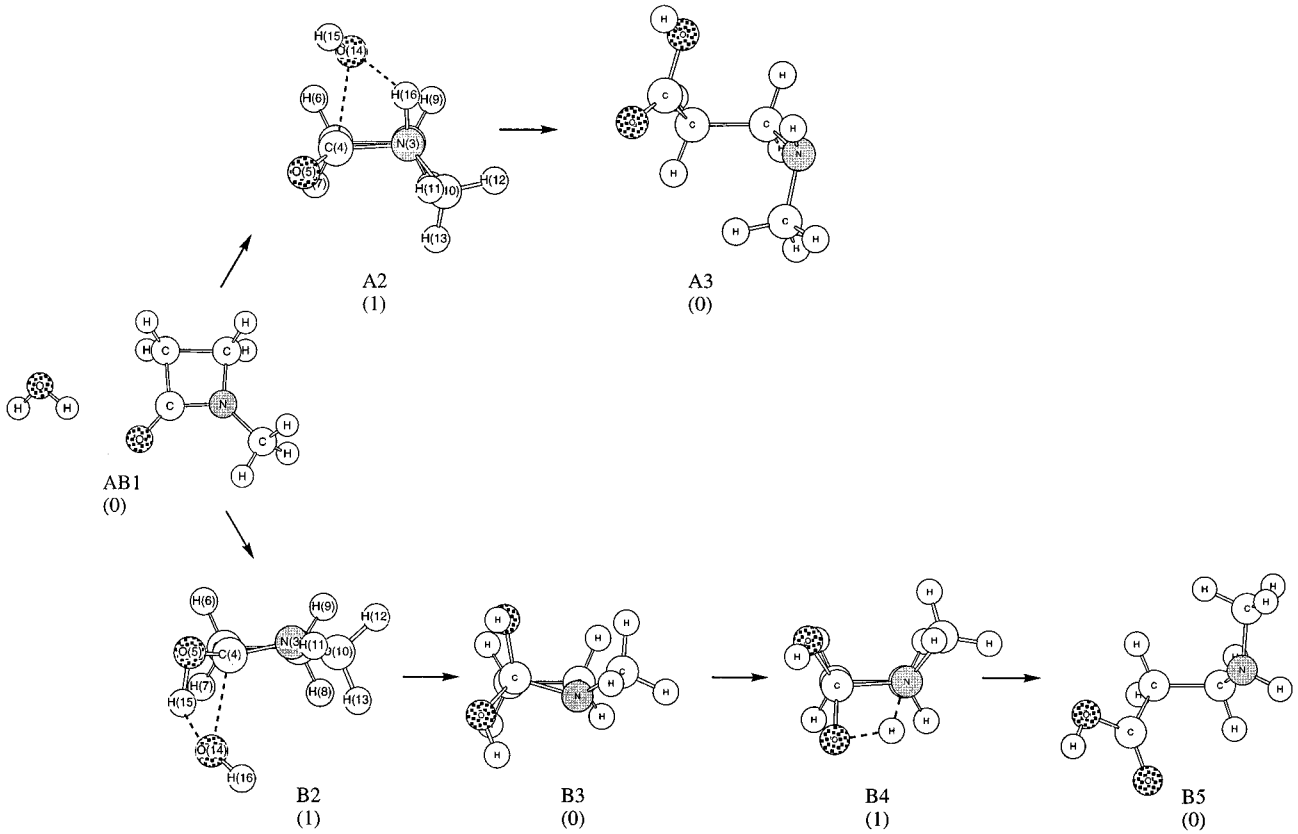
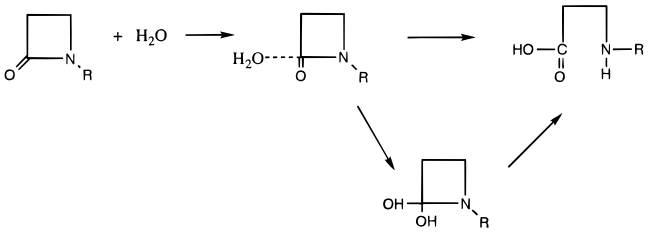


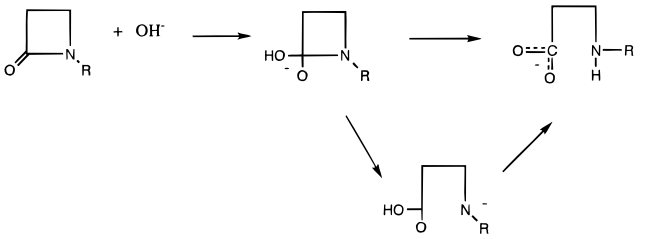
Figure 1. Stationary structures of the neutral hydrolysis. Number in parentheses indicates the number of imaginary frequencies.

SCHEME 2

Neutral hydrolysis



Alkaline hydrolysis



nature of the stationary points has been determined by analytical frequency calculation and verification that they had no imaginary frequencies in the case of minima or only one in the case of transition structures. The intrinsic reaction coordinate pathways (IRC)[22] from the transition structures down to the two lower energy structures have been followed using a second-order integration method.[23] Single-point calculations on the HF/6-31G* geometries have been carried out at the MP2/6-31G*[24] level to take into account correlation effects. Contributions to the free energy have been obtained using standard procedures.[25] Vibrational contributions have been always estimated from HF calculations in the gas phase.

The effects of the bulk solvent on hydrolysis mechanisms have been incorporated using a cavity model.[26−28] In this model the liquid is assimilated to a continuum characterized by its dielectric constant (78.4 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.[29] The analytical derivatives of the electrostatic term have been obtained[30] leading to an efficient geometry optimization procedure.[31] Solvent effects calculations have been carried out using the SCRFPAC package[32] added to the GAUSSIAN program.

3. Results and Discussion

3.1. Neutral Hydrolysis. The important stationary structures for the concerted and stepwise mechanisms of the β-lactam neutral hydrolysis are shown in Figure 1. The most interesting geometrical parameters obtained at the HF/6-31G* level are gathered in Table 1, and the relative energies and free energies of all the stationary points at the HF/6-31G* and MP2//HF levels are given in Table 2. In agreement with previous studies on similar molecules, the *N*-methyl-substituted β-lactam has an essentially planar structure with C_s symmetry[8−11,33] Thus, the nucleophilic attack yields the same result whether it happens on the upper or lower side of the β-lactam. In solution the *N*-methylazetidinone is also planar, and the most important change with respect to the gas phase geometry is the shortening of the C−N bond length, increasing its double-bond character.[13]

The concerted mechanism takes place through a single transition structure (A2). In this structure, the ring is still closed but the C−N bond length has increased from 1.355 up to 1.530 Å. A water hydrogen atom is nearly transferred to the nitrogen atom with a N3−H15 distance of only 1.081 Å, while the distance to the water oxygen (O14) is 1.485 Å. The ring

TABLE 1: Selected Geometrical Parameters (in Å and deg) Obtained at the HF/6-31G* Level for the Stationary Structures of the Neutral Hydrolysis

		β-lactam	AB1	A2	A3	B2	B3	B4	B5	product[a]
C4−N3	$\epsilon = 1$	1.355	1.347	1.530	3.047	1.323	1.449	1.564	2.991	3.056
	ϵ =78.4	1.340		1.525	3.141	1.304	1.448	1.538	2.981	3.000
C4−O5	$\epsilon = 1$	1.189	1.197	1.175	1.188	1.289	1.379	1.359	1.329	1.329
	$\epsilon = 78.4$	1.204		1.180	1.198	1.316	1.385	1.360	1.320	1.316
C4−O14	$\epsilon = 1$		3.337	2.071	1.334	2.018	1.381	1.326	1.188	1.190
	$\epsilon = 78.4$			2.148	1.318	2.105	1.381	1.348	1.197	1.200
O14−H15	$\epsilon = 1$		0.953	1.485	3.054	0.949	0.948	1.293	3.760	2.398
	$\epsilon = 78.4$			1.793	2.744	0.947	0.952	1.265	3.769	2.981
O14−H16	$\epsilon = 1$		0.947	0.948	0.952	1.437	2.200	2.234	2.259	2.261
	$\epsilon = 78.4$			0.948	0.959	1.833	2.267	2.317	2.337	2.349
N3−H15	$\epsilon = 1$		5.235	1.081	1.000	3.042	2.363	1.244	1.000	1.000
	$\epsilon = 78.4$			1.020	1.000	2.734	2.417	1.264	1.000	1.000
O5−H16	$\epsilon = 1$		2.008	2.889	2.261	1.058	0.949	0.951	0.952	0.952
	$\epsilon = 78.4$			2.586	2.338	0.968	0.951	0.952	0.958	0.959
C4−C1−C2−N3	$\epsilon = 1$	0.0	0.1	−3.32	55.3	−10.1	15.7	−3.5	−65.5	−67.1
	$\epsilon = 78.4$	0.0		−7.2	56.5	−5.7	14.3	0.0	−60.5	−62.4
C10−N3−C2−C1	$\epsilon = 1$	179.9	−179.4	129.0	59.0	165.4	−144.8	−126.9	−84.8	−172.9
	$\epsilon = 78.4$	180.0		121.4	62.7	178.6	−145.2	−132.2	−85.7	−173.5

[a] This is the lowest energy reaction product. It can be reached from A3 and B5 rotating around single bonds. It exists in another isoenergetic structure with the sign of all the dihedral angles changed.

TABLE 2: Energies and Free Energies for the Neutral β-Lactam Hydrolysis in the Gas Phase and in Aqueous Solution (in kcal/mol)[a]

	$\epsilon = 1$				$\epsilon = 78.4$			
	HF/6-31G*		MP2//HF/6-31G*		HF/6-31G*		MP2//HF/6-31G*	
	ΔE	ΔG	ΔE	ΔG	ΔE	ΔG	ΔE	ΔG
reactants	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AB1	−7.10	2.03	−9.05	0.08				
A2	57.12	68.60	39.19	50.67	55.04	66.52	38.43	49.91
A3	−21.61	−8.38	−19.53	−6.30	−16.98	−3.75	−14.53	−1.30
B2	55.29	65.61	43.55	53.87	52.66	62.98	45.65	55.97
B3	6.28	21.16	3.74	18.62	13.57	28.45	10.27	25.15
B4	51.82	63.77	35.29	47.24	55.29	67.24	34.29	46.24
B5	−22.51	−9.03	−19.87	−6.39	−17.93	−4.45	−15.31	−1.83
product[a]	−24.89	−10.89	−22.04	−8.03	−18.72	−4.71	−15.89	−1.88

[a] This is the lowest energy product.

remains planar, but the methyl group is now out of the ring plane because of the pyramidalization of the nitrogen atom. In aqueous solution, the proton transfer from water to the nitrogen atom is still more advanced, the N3−H15 and O14−H15 distances being equal to 1.020 and 1.793 Å respectively. In spite of the increased double-bond character of the C−N bond in solution, solvent effects favor nitrogen pyramidalization as can be seen comparing the values of the C10−N3−C2−C1 torsional angle in the gas phase and in solution. This result was also found and analyzed in formamide in ref 13. The free energy needed to reach the transition structure A2 is quite high, about 50 kcal/mol both in the gas phase and in solution at the MP2//HF level. The reaction product, A3, has a free energy lower than that of the reactants, −6.30 kcal/mol in the gas phase and −1.30 kcal/mol in aqueous solution at the MP2//HF level. It should be noted that several structural isomers can be found for the hydrolysis product by rotation around the C4−C1, C1−C2, and C2−N3 single bonds. The structure given in Figure 1 is obtained from the IRC calculation. The lowest energy product found in our exploration of the PES is 1.73 and 0.58 kcal/mol more stable than A3 in the gas phase and in solution respectively. This product is stabilized because a intramolecular hydrogen bond between the oxygen carbonyl and the hydrogen transferred to the nitrogen atom. The two oxygen atoms of the acid group lie in the C4−C2−C1 plane, whereas in A3 they are perpendicular.

Let us now consider the stepwise mechanism of the neutral hydrolysis. The first transition structure (B2) corresponds to the addition of the water molecule to the carbonyl group to form the diol. This transition structure presents some similarities with that of the hydration of formaldehyde.[34,35] The main difference is that in B2 the proton transfer from the water molecule to the oxygen atom of the carbonyl group is much more advanced than in the case of formaldehyde. Indeed, in our case, the distance of the transferred proton (H16) to the water oxygen (O14) (1.437 Å) is substantially larger than the distance to the carbonyl oxygen (O5) (1.058 Å), while the opposite is found in the transition structure of formaldehyde hydration.[34,35] As in the transition structure of the concerted mechanism, the proton transfer is enhanced through bulk solvent effects. The free energy barrier for carbonyl hydration in the β-lactam is somewhat larger than the corresponding quantity in formaldehyde hydration.[34,35] Note also that the solvent effect on the activation barrier is quite small. In the diol intermediate (B3), the β-lactam ring is still closed although the C−N bond has been considerably lengthened (1.449 Å). Pyramidalization of the nitrogen atom is also advanced. The final opening of the ring takes place simultaneously to the proton transfer from O14 to the nitrogen atom through the B4 transition structure. This process has a free energy barrier much smaller than the first step of the reaction. To our knowledge, this is the first time that the structures B3 and B4, and consequently the full reaction path, are reported. The structures A2 and B2 were reported by Wolfe et al. at the HF/3-21G level in previous theoretical studies on the neutral hydrolysis.[11] The reaction product obtained by IRC calculation, B5, is slightly more stable than A3. The relative free energies with respect to the lowest energy product

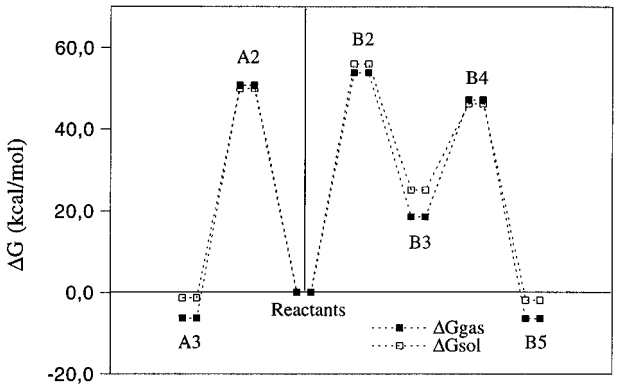


Figure 2. MP2//HF/6-31G* free energy profiles in the gas phase and in solution for the neutral hydrolysis.

are 1.64 kcal/mol in the gas phase and 0.05 kcal/mol in aqueous solution at the MP2//HF level.

It must be pointed out that in the exploration of the PES we have also located a *N*-methylazetidinone−water reactant complex, AB1, with a C4−O14 distance of 3.34 Å at the HF/6-31G* level and a water hydrogen oriented toward the carbonyl oxygen atom. The energy of this complex with respect to the separated reactants is −7.1 kcal/mol at the HF/6-31G* level and −9.1 kcal/mol at the MP2//HF level. However, when thermal and vibrational contributions are considered, this complex has a free energy of 2.0 kcal/mol at the HF/6-31G* level and 0.1 kcal/mol at the MP2//HF level, above the separated reactants. In our exploration using the 3-21G basis set we also located another reactant complex where a water hydrogen was oriented toward the nitrogen atom. However, this second complex was not found with the 6-31G* basis set, which is in agreement with the previously reported trend of the 3-21G basis set to overestimate hydrogen bonds.[36] The HF/6-31G* IRC started from B2 leads to the AB1 complex, while our attempts to follow the IRC started from A2 diverge in a zone of the PES close to AB1. Optimization of the last converged structure also led to AB1. A similar reactant complex has been not located in solution. Geometry optimization of the AB1 structure in a cavity leads to dissociation into water molecule plus β-lactam.

The MP2//HF free energy profiles for the concerted and stepwise mechanisms of neutral hydrolysis in the gas phase and in solution are compared in Figure 2. In general, the consideration of correlation energy at the MP2 level reduces the energy barriers with respect to the HF values. HF/3-21G energy profiles (not shown here) do not differ too much from those presented in Figure 2. It can be seen that in both mechanisms the free energy barrier is quite high, the concerted mechanism being slightly favored with respect to the stepwise one. Solvent effects on reaction energetics are quite moderate. Nevertheless the exothermicity of the reaction is reduced from 8.03 kcal/mol in the gas phase to only 1.88 kcal/mol in aqueous solution.

3.2. Alkaline Hydrolysis. The most important stationary geometries of the alkaline β-lactam hydrolysis are shown in Figure 3. Selected geometrical parameters for all of these structures at the HF/6-31G* level are shown in Table 3. Relative energies and free energies at the HF/6-31G*, HF/6-31+G*, and MP2//HF/6-31G* levels are given in Table 4.

Binding of hydroxyl anion to β-lactam carbonyl presents no energy barrier in the gas phase. The energy always decreases until the formation of a tetrahedral intermediate. This fact is already know from previous experimental[37] as well theoretical studies.[7,9,38] However, in solution we have located a transition structure for this process, with a C4−O14 distance of 1.933 Å and an energy 2.2 kcal/mol higher than that of the tetrahedral complex at the MP2//HF/6-31G* level. The presence of an activation energy in solution is probably a consequence of the hydroxyl ion desolvation process.[8,39] A similar transition structure has also been found in a semiempirical study of the alkaline hydrolysis of a cephalosporin where solvation with

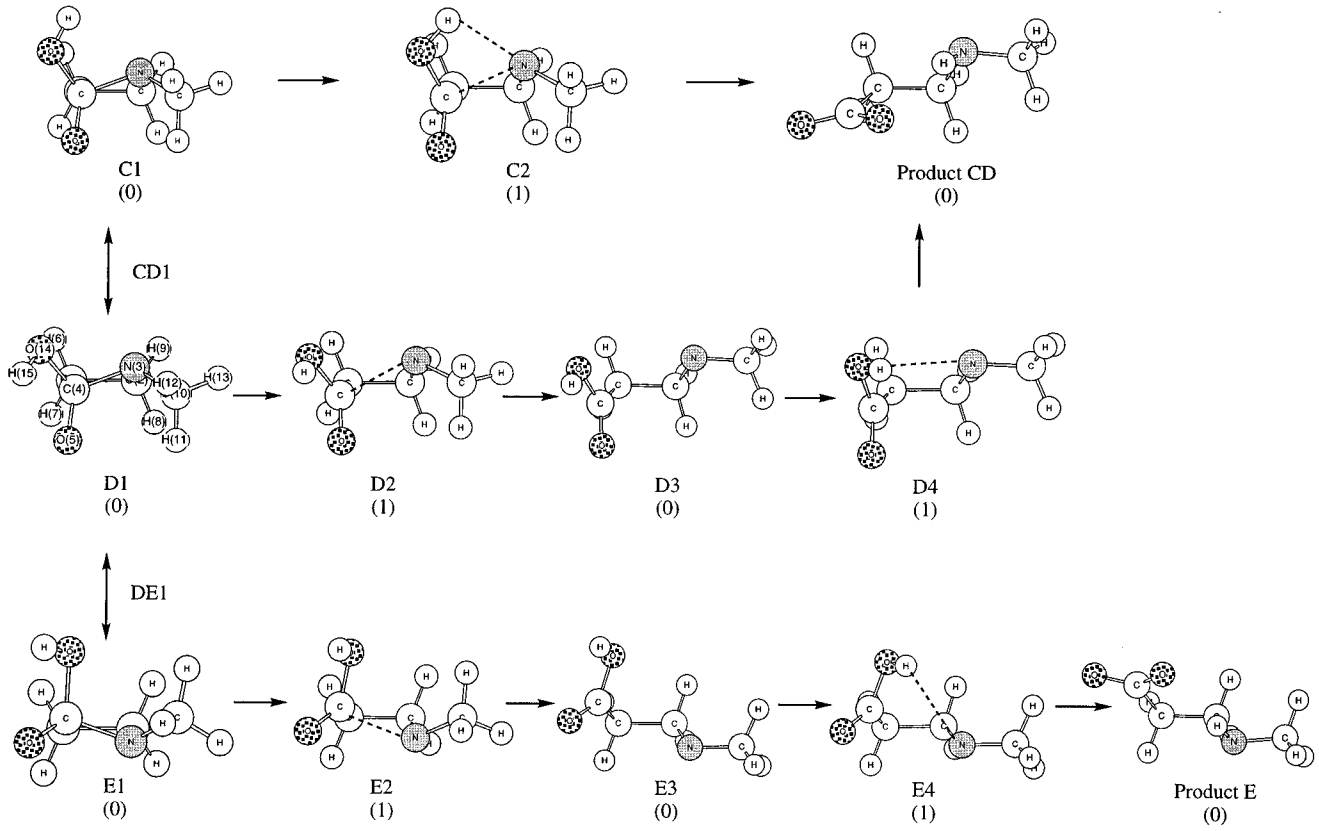


Figure 3. Stationary structures of the alkaline hydrolysis. Number in parentheses indicates the number of imaginary frequencies.

TABLE 3: Selected Geometrical Parameters (in Å and deg) Obtained at the HF/6-31G* Level for the Stationary Structures[a]

		C1	C2	D1	D2	D3	D4	product CD
C4−N3	$\epsilon = 1$	1.549	1.944	1.523	2.055	2.811	2.655	3.118
	ϵ =78.4	1.512	2.022	1.500	2.057	2.944	2.653	3.052
C4−O5	$\epsilon = 1$	1.264	1.217	1.282	1.220	1.196	1.192	1.230
	$\epsilon = 78.4$	1.289	1.227	1.298	1.232	1.205	1.200	1.248
C4−O14	$\epsilon = 1$	1.433	1.386	1.429	1.366	1.338	1.352	1.241
	$\epsilon = 78.4$	1.437	1.369	1.439	1.357	1.320	1.348	1.242
O14−H15	$\epsilon = 1$	0.947	0.949	0.948	0.949	0.950	0.950	2.075
	$\epsilon = 78.4$	0.948	0.952	0.947	0.951	0.957	0.954	2.747
N3−H15	$\epsilon = 1$	2.278	2.166	3.010	3.207	3.857	2.844	1.004
	$\epsilon = 78.4$	2.313	2.186	3.017	3.234	4.090	2.710	0.998
C4−C1−C2−N3	$\epsilon = 1$	−20.4	−28.3	−20.2	−33.5	−56.7	−45.9	−66.4
	$\epsilon = 78.4$	−20.3	−29.4	−19.5	−31.8	−61.2	−44.9	−62.6
C10−N3−C2−C1	$\epsilon = 1$	140.7	142.0	141.9	144.6	177.4	176.6	171.8
	$\epsilon = 78.4$	149.8	159.2	147.6	149.9	167.7	169.0	166.9
H15−O14−C4−O5	$\epsilon = 1$	158.8	143.3	1.6	18.0	8.7	56.6	−158.9
	$\epsilon = 78.4$	164.6	148.0	0.9	17.8	5.3	67.9	113.4

		CD1	DE1	E1	E2	E3	E4	product E
C4−N3	$\epsilon = 1$	1.548	1.486	1.519	2.018	2.808	2.626	3.118
	ϵ =78.4	1.511	1.471	1.504	2.080	2.881	2.568	3.052
C4−O5	$\epsilon = 1$	1.264	1.275	1.267	1.217	1.195	1.189	1.230
	$\epsilon = 78.4$	1.290	1.298	1.289	1.226	1.203	1.195	1.248
C4−O14	$\epsilon = 1$	1.447	1.453	1.462	1.381	1.341	1.359	1.241
	$\epsilon = 78.4$	1.451	1.455	1.453	1.358	1.322	1.358	1.242
O14−H15	$\epsilon = 1$	0.945	0.947	0.947	0.948	0.950	0.950	2.078
	$\epsilon = 78.4$	0.947	0.946	0.947	0.952	0.958	0.954	2.747
N3−H15	$\epsilon = 1$	2.281	3.004	2.988	3.137	3.872	2.867	1.004
	$\epsilon = 78.4$	2.321	3.017	2.918	3.226	4.111	2.714	0.998
C4−C1−C2−N3	$\epsilon = 1$	−15.0	−3.9	−18.8	−29.1	−53.2	−40.5	−66.5
	$\epsilon = 78.4$	−18.7	−3.5	−14.2	−24.0	−54.0	−35.8	−62.6
C10−N3−C2−C1	$\epsilon = 1$	134.8	172.6	−146.6	−148.0	−177.3	−178.4	−171.8
	$\epsilon = 78.4$	147.0	−172.2	−144.3	−147.0	−169.4	−169.3	−166.9
H15−O14−C4−O5	$\epsilon = 1$	100.7	−0.9	3.5	20.2	8.8	60.0	158.5
	$\epsilon = 78.4$	97.8	0.4	18.9	19.7	4.3	70.2	−113.3

TABLE 4: 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	ΔE	ΔG	ΔE	ΔE
reactants	41.63	28.11	22.50	9.30	52.49	38.97	6.07	−7.46	15.61	2.08
C1	4.04	3.87	4.17	4.02	3.08	2.91	1.33	1.16	1.36	1.19
C2	10.04	8.62	11.84	10.30	6.97	5.55	9.45	8.04	7.61	6.20
D1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D2	11.31	9.67	13.42	11.58	(9.17) 10.06	(7.53) 8.41	13.43	11.79	(10.82) 11.90	(9.18) 10.25
D3	3.31	0.76	4.00	1.27	(7.78) 10.45	(5.23) 7.90	3.00	0.45	(7.63) 9.91	(5.08) 7.36
D4	6.47	3.55	7.36	4.28	(10.74) 13.42	(7.83) 10.51	11.38	8.47	(15.03) 17.46	(12.12) 14.54
E1	1.47	1.34	1.31	1.15	0.78	0.64	−0.06	−0.20	−0.03	−0.17
E2	11.26	9.70	13.47	11.70	(8.61) 9.53	(7.05) 7.97	10.69	9.13	(9.74) 10.39	(8.18) 8.83
E3	3.05	0.46	3.70	0.93	(7.48) 10.24	(4.89) 7.66	2.22	−0.36	(7.59) 9.00	(5.00) 6.41
E4	6.42	3.40	7.37	4.16	(10.74) 13.53	(7.72) 10.51	10.68	7.67	(15.44) 16.52	(12.42) 13.51
CD1	5.97	5.14	5.87	5.10	5.53	4.70	3.84	3.00	4.12	3.29
DE1	7.85	6.78	7.49	6.31	9.66	8.59	5.19	4.12	7.23	6.16
products										
CD, E	−51.03	−51.21	−49.82	−50.17	−43.09	−43.27	−63.34	−62.51	−52.47	−52.65

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

discrete water molecules was considered.[8] In a recent work,[40] Frau et al. have studied the nucleophilic attack of the OH^- ion on the β-lactam carbonyl group with HF ab initio methods in vacuo and semiempirical methods in solution. Stereoelectronic aspects in the selective cleavage of tetrahedral intermediates in ester and amide hydrolysis have been also investigated.[41−43]

The tetrahedral complex is more stable than the separated reactants in the gas phase. In solution, the stability is smaller; it is somewhat more stable than the reactants when considering free energies at the MP2//HF/6-31G* level, although the opposite is found at the HF/6-31G* level. In the tetrahedral reactant complex, the planarity of the β-lactam ring is not preserved and the nitrogen atom presents an important pyramidalization. The C−N bond length is considerably longer than in the β-lactam. This intermediate can present different conformations. In the E1 structure the methyl group and the hydroxyl are found in the same side of the β-lactam ring. In D1 they are found in opposite sides. Also, 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, we have the C1 conformer. We have studied the interconversion among all these structures, and we show the energy profile obtained in Figure 4. In the gas phase D1 is the more stable conformer. DE1 is the transition

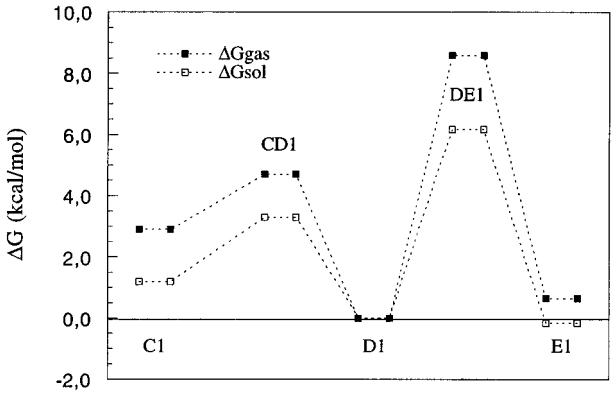


Figure 4. MP2//HF/6-31G* free energy profiles in the gas phase and in solution for the interconversion process among C1, D1, and E1 tetrahedral intermediates.

structure connecting D1 and E1 conformers, its free energy being about 8 kcal/mol higher than that of D1 at the MP2//HF/6-31G* level. The transition structure CD1 connects C1 and D1, and this step requires about 5 kcal/mol from D1 at the same level. In solution the free energy barriers are decreased, reducing also the energy differences among the three conformers, E1 becoming slightly more stable than D1.

The tetrahedral intermediate evolves with ring opening and hydrogen transfer from hydroxyl oxygen to β-lactam nitrogen. These two processes can occur simultaneously (concerted mechanism) or sequentially (stepwise mechanism). The only conformer adapted for the concerted process is C1, since the hydroxyl hydrogen is correctly oriented toward the nitrogen atom. In the concerted transition structure, C2, the C4−N3 bond length is 1.944 Å and the N3−H15 bond length is 2.166 Å. Solvent effects do not produce large geometrical changes with respect to the gas phase values. The IRC calculation show that in fact the C2 transition structure is directly reached from C1. At the MP2//HF/6-31G* level the free energy of the C2 transition structure with respect to C1 is only 2.64 kcal/mol in the gas phase and 5.01 kcal/mol in solution. Alkaline hydrolysis of the β-lactam is very exothermic, the free energy of the product CD being 40.36 kcal/mol lower than that of C1 in the gas phase, and 51.46 kcal/mol in aqueous solution at the MP2//HF/6-31G* level. In the gas phase, carboxyl group oxygen atoms of the CD product lie in the C4−C2−C1 plane, but, in solution, because of electrostatics interactions with the continuum, they lie in a perpendicular plane.

In the stepwise mechanism of alkaline hydrolysis, ring opening precedes the proton transfer. There are two analogous reaction paths, D and E. They have the D1 and E1 conformers as starting points respectively. The first transition structures, D2 and E2, correspond to the ring opening. They are geometrically close to the tetrahedral complexes D1 and E1, except for the partial cleavage of the amidic C−N bond. The length of this bond is 2.055 Å in D2 and 2.018 Å in E2 in the gas phase. The deviation from planarity also increases when going from the tetrahedral intermediate to D2 and E2. Thus, it seems that both the rotational motion of the torsional angle C4−C1−C2−N3 and the C−N bond stretching contribute to the ring opening. D2 and E2 transition structures lead to the D3 and E3 intermediates. This is accompanied by a substantial increase of the C−N bond length and torsional angle. The next step in the stepwise mechanism is the proton transfer from hydroxyl oxygen to β-lactam nitrogen. This proton transfer takes place through the transition structures D4 and E4 respectively. In these structures the proton is still completely bonded to the oxygen atom (the bond length is 0.950 Å in the gas phase and 0.954 Å in aqueous solution, in both transition structures), but the N3−H15 distance is considerably reduced with respect to its value in D3 and E3 by means of a rotation around the O14−C4 bond. Different from the results obtained with some semiempirical methods,[9] this transition structure directly leads to the hydrolysis products without any further step. Once the hydroxyl hydrogen atom (H15) is correctly oriented toward the nitrogen atom (N3), the transfer is not hindered by any energy barrier. Note that, path D leads to the product CD, the same as that in the concerted mechanism, while path E leads to product E. The products CD and E have the same energy, and the only difference between them is the sign of their dihedral angles. Contrary to the neutral hydrolysis reaction path, these products, which are obtained following the IRC started at C2, D4, and E4 respectively, are the lowest energy products found in our exploration of the PES at the HF/6-31G* level.

On the other hand, in the gas phase, the energy of the D3 and E3 intermediates at the MP2//HF/6-31G* level is larger than the energy of the D2 and E2 transition structures respectively. Such a result points toward a reaction mechanism that could be one-step (through D4 and E4) when correlation energy is taken into account and two-step (through D2, E2 and D4, E4) otherwise. Obviously, the relaxation of the geometry may be crucial in this case, and for that reason we have carried out geometry optimizations at the MP2 level for D2, E2, D3, E3, D4, and E4 structures. MP2//MP2/6-31G* results (in parentheses in Table 4) show that D3 and E3 are local minima, i.e., reaction intermediates of their respective paths.

Because of the presence of a negative charge, further HF calculations have been carried out with the 6-31+G* basis set, which includes diffuse functions on heavy atoms. SCF convergence problems prevented the use of diffuse functions in our continuum calculations so that only gas phase results are presented here. In fact, the inclusion of diffuse functions does not produce important changes in the description of the alkaline hydrolysis, either energetically nor geometrically. The energy values obtained with this basis set are also given in Table 4. The only noticeable change is the lower stabilization of the tetrahedral intermediate relative to the separated reactants, the free energy difference being 28.11 kcal/mol at the HF/6-31G* level and only 9.30 kcal/mol at the HF/6-31+G* level. This result is not surprising since the effect of the diffuse function is expected to be particularly large in the case of the small anion OH^-. On the other hand, the relative energies of all the other stationary points of the alkaline hydrolysis remain essentially unchanged upon inclusion of diffuse functions. Inclusion of correlation energy has a more decisive influence, leading to an important destabilization of D3, E3, D4, E4, and hydrolysis product with respect to the tetrahedral complex. The MP2//HF/6-31G* free energy profiles for the concerted and stepwise mechanisms of alkaline hydrolysis in the gas phase and in solution are compared in Figure 5. Because of the larger nucleophilic character of the hydroxyl anion relative to the water molecule, the free energy barriers of the concerted and stepwise alkaline hydrolysis mechanisms are considerably lower than in the neutral hydrolysis. The concerted mechanism presents a free energy barrier lower than the stepwise mechanism. Electrostatic solvent effects do not essentially affect the reaction activation energies. The same conclusion was reached in a semiempirical study with discrete water molecules.[8] However, solvent effects are quite important in the relative energy of the products with respect to the tetrahedral complex and that of the tetrahedral complex with respect to the separated reactants.

4. Conclusions

In this work we have presented a theoretical study of neutral and alkaline hydrolyses of *N*-methylazetidinone as a model of

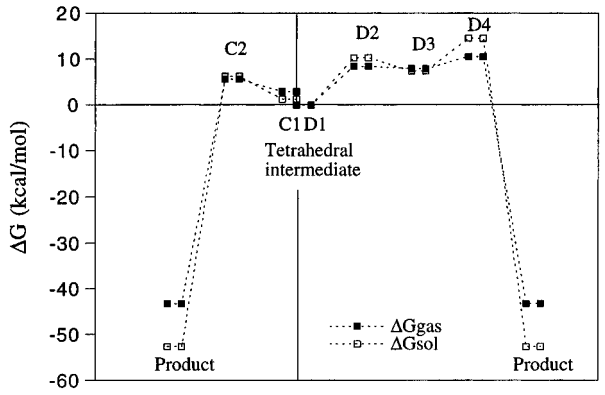


Figure 5. MP2//HF/6-31G* free energy profiles in the gas phase and in solution for the alkaline hydrolysis. The values corresponding to the E path are not shown, beacause they overlap with those of the D path. The energies are given in Table 4.

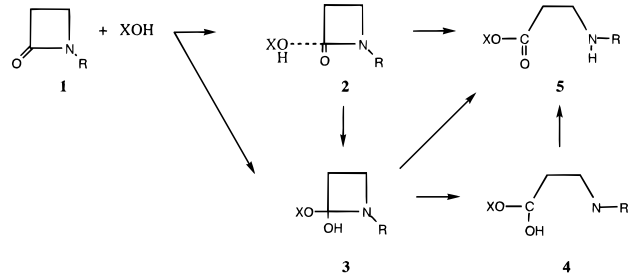
biological hydrolysis of β-lactam antibiotics. Basis sets, correlation energy, and solvent effects have been considered in this study. For both neutral and alkaline hydrolyses two possible reaction mechanisms (concerted and stepwise) have been analyzed.

In our calculations for the neutral hydrolysis, reactant complexes have been found to have a free energy larger than that of the separated reactants. Depending on the water molecule orientation, two possible transition structures (A2 and B2) can be reached from the reactants. In A2, a water hydrogen atom is already pointing toward the nitrogen atom and thus ring opening and proton transfer take place simultaneously. Alternatively, B2 leads to the corresponding diol, from which the hydrolysis reaction product is reached after passing through the B4 transition structure. Both mechanisms present high energy barriers. The concerted mechanism is favored with respect to the stepwise one by 3.2 kcal/mol in the gas phase and by 5.1 kcal/mol in aqueous solution at the MP2//HF/6-31G* level.

In the alkaline hydrolysis a tetrahedral intermediate arising from the hydroxyl binding to the β-lactam carbonyl group is found to be more stable than the separated reactants. This stabilization is diminished when diffuse functions or solvent effects are included in the calculations. From the tetrahedral complex two possible reaction pathways can be followed. In the first one, C−N bond cleavage and proton transfer take place simultaneously to directly give the reaction product through a single transition structure (C2). In the second one, the ring opening is initially produced (D2, E2) resulting in a reaction intermediate D3, E3. These structures undergo a subsequent proton transfer to yield the hydrolysis products. As in the case of neutral hydrolysis, the concerted mechanism has a free energy barrier lower than that of the stepwise mechanism, presenting lower energy barriers.

It is important to note that in the alkaline hydrolysis the expressions concerted mechanism and stepwise mechanism allude to the ring opening and hydrogen transfer processes. Both processes can occur by means of a single transition structure (concerted mechanism) or, alternatively, by two steps with the ring opening preceding the hydrogen transfer (stepwise mechanism). In the neutral hydrolysis we have found that these two processes always take place simultaneously. In this case, the expression stepwise mechanism refers to the existence of an intermediate, the B3 diol structure, in the reaction path. This B3 structure is formally equivalent to the tetrahedral intermediate of the alkaline hydrolysis, but B3 does not evolve with the ring opening preceding the hydrogen transfer. With these considerations in mind, both neutral and alkaline mechanisms can be gathered in only one reaction scheme (Scheme 3), where X is

SCHEME 3



a hydrogen atom in the neutral hydrolysis and a negative charge in alkaline hydrolysis. Structure **2**, corresponding to the reactant complex AB1 in the neutral hydrolysis, does not seem to exist for the alkaline mechanism in the gas phase. Structure **3** is the tetrahedral intermediate in the alkaline hydrolysis and the B3 diol in the neutral hydrolysis. As previously commented, structure **4**, corresponding to D3 and E3, has been not found for the neutral mechanism.

In both neutral and alkaline hydrolyses, bulk solvent effects produce only small changes in the reaction mechanisms. The catalytic effects of the solvent could be more important. The reliability of water-assisted mechanisms will be analyzed in a future work.

Acknowledgment. Authors are grateful for financial support from Picasso program (Action's 267B and 96121). I.T. acknowledges a postdoctoral contract of the Ministerio de Educación y Ciencia (Spain) and the Universitat de València. J.P. acknowledges a doctoral fellowship of M.E.C. (Spain). The authors also thank the reviewers for their constructive comments and fruitful suggestions. This work has been partially supported by DGICYT Project PB93-0699.

References and Notes

(1) Waxman, D. J.; Strominger J. L. *Annu. Rev. Biochem.* **1983**, *52*, 825.
(2) Flyn, E. H. In *Cephalosporins and Penicillins: Chemistry and Biology*; Academic Press: New York, 1972.
(3) Page, M. I.; Laws, A. P.; Slater, M. J.; Stone, J. R. *Pure Appl. Chem.* **1995**, *67*, 711.
(4) Page, M. I. *Adv. Phys. Org. Chem.* **1987**, *23*, 165.
(5) Avendaño, M. C. In *Introduccion a la Quimica Farmaceutica*; Interamericana (McGraw-Hill): New York, 1993.
(6) Petrongolo, C.; Ranghino, G. *Theor. Chim. Acta* **1980**, *54*, 239.
(7) Petrongolo, C.; Ranghino, G.; Scordamaglia, R. *Chem. Phys.* **1980**, *45*, 279.
(8) Frau, J., Donoso, J., Muñoz, F.; García Blanco, F. *J. Comput. Chem.* **1993**, *14*, 1545.
(9) Frau, J., Donoso, J., Muñoz, F.; García Blanco, F. *J. Comput. Chem.* **1992**, *13*, 681.
(10) Wolfe, S.; H. Jin, Yang, K.; Kim, C.; McEarchern, E. *Can. J. Chem.* **1994**, *72*, 1051.
(11) Wolfe, S.; Kim, C.; Yang, K. *Can. J. Chem.* **1994**, *72*, 1033.
(12) Krug, J. P.; Popelier, P. L. A.; Bader, R. F. W. *J. Phys. Chem.* **1992**, *96*, 7604.
(13) Antonczak, S.; Ruiz-López, M. F.; Rivail, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 3912.
(14) O'Brien, J. F.; Pranata, J. *J. Phys. Chem.* **1995**, *99*, 12759.
(15) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257.
(16) Hariharan, P. C.; Pople, J. A., *Theor. Chim. Acta* **1973**, *28*, 213.
(17) Clark, T.; Chandrasekhar, J.; Spitznagel, G. W.; Schleyer, P. v. R. *J. Comput. Chem.* **1983**, *4*, 294.
(18) Schlegel, H. B. *J. Comput. Chem.* **1982**, *3*, 214.
(19) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wrong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Reprogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *GAUSSIAN92*; Carnegie-Mellon Quantum Chemistry Publishing Unit: Pittsburgh, PA, 1992.
(20) Peng, C.; Ayala, P. Y.; Schlegel, H. B.; Frisch, M. J. *J. Comput. Chem.* **1996**, *17*, 49.

(21) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Wrong, M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R., Petersson, G. A.; Montgomery, J. A.; Raghavachari, Al-Laham, M. A.; Zakrzwski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Reprogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *GAUSSIAN94*; Carnegie-Mellon Quantum Chemistry Publishing Unit, Pittsburgh, PA, 1994.

(22) Fukui, K. *Acc. Chem. Res.* **1981**, *14*, 363.

(23) Gonzalez, C.; Schlegel, H. B. *J. Phys. Chem.* **1990**, *94*, 5523.

(24) See, for instance: Szabo, A.; Ostlund, N. S. In *Modern Quantum Chemistry: Introduction to Advanced Electronic Structure Theory*; Macmillan: New York, 1986; Chapter 6.

(25) Hehre, W. J.; Radom, L.; Schleyer, P. v., R.; Pople, J. A. In *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986.

(26) Rinaldi, D.; Rivail, J. L. *Theor. Chim. Acta* **1973**, *32*, 57.

(27) Rivail, J. L.; Rinaldi, D. *Chem. Phys.* **1976**, *18*, 233.

(28) Rinaldi, D.; Ruiz-López, M. F.; Rivail, J. L. *J. Chem. Phys.* **1983**, *78*, 834.

(29) Rivail, J. L., Rinaldi, D.; Ruiz-López, M. F. In *Theoretical and Computational Models for Organic Chemistry*; Formosinho, S. J.; Arnaut, L.; Csizmadia, I.; Eds.; Kluwer: Dordrecht, 1991; pp 79−92.

(30) Rinaldi, D. Rivail, J. L.; Rguini, N. *J. Comput. Chem.* **1992**, *13*, 675.

(31) Bertrán, J.; Ruiz-López, M. F.; Rinaldi, D.; Rivail, J. L. *Theoret. Chim. Acta* **1992**, *84*, 181.

(32) Rinaldi, D.; Pappalardo, R. R. *SCFRPAC*, QCPE; Indiana University: Bloomington, IN, 1992; Program number 622.

(33) Palafox, M. A.; Núñez, J. L.; Gil, M. *J. Phys. Chem.* **1995**, *99*, 1124.

(34) Ventura, O. N.; Coitiño, E. L.; Lledós, A.; Bertrán, J. *J. Comput. Chem.* **1992**, *13*, 1037.

(35) Wolfe, S.; Kim, C.; Yang, K.; Weinberg, N.; Shi, Z. *J. Am. Chem. Soc.* **1995**, *117*, 4240.

(36) (a) Williams, I. H. *J. Am. Chem. Soc.* **1987**, *109*, 6299. (b) Williams, I. H.; Spangler, D.; Femec, D. A.; Maggiora, G. M.; Schowen, R. L. *J. Am. Chem. Soc.* **1983**, *105*, 31.

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

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

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

(40) Frau, J.; Donoso, J.; Muñoz, F.; Garcia-Blanco, F. *Helv. Chim. Acta* **1996**, 79, 353.

(41) Deslongchamps, P.; Lebreux, C.; Taillefer, R. *Can. J. Chem.* **1973**, *51*, 1665.

(42) Lehn, J. M.; Wipff, G. *J. Am. Chem. Soc.* **1974**, *96*, 4048.

(43) Alagona, G.; Scrocco, E.; Tomasi, J. *J. Am. Chem. Soc.* **1975,** *97*, 6976.