57. Study of the Alkaline Hydrolysis of the Azetidin-2-one Ring by *ab initio* Methods: Influence of the Solvent

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A comprehensive study of the alkaline hydrolysis of the β-lactam ring of azetidin-2-one was carried out using *ab initio* molecular-orbital calculations at the RHF/6-31 + G* level. The influence of the solvent on this reaction was investigated by using the reaction field method (SCRF); the solvent was found to suppress the interference of some gas-phase reactions and allow the presence of a transition state to be detected as the nucleophile approaches the β-lactam ring. The transition state corresponds to a structure where the OH$^-$ group lies at a distance of 1.927 Å from the C=O group of the β-lactam ring and exhibits a potential barrier of 13.6 kcal/mol.

Introduction. – Since their discovery in the 1920s, β-lactam antibiotics have played a prominent role in the fight against bacterial diseases. The early theoretical studies of *Boyd* [1], who related various structural parameters of β-lactam antibiotics to their chemical reactivity and antibacterial action, have been followed by much research on their structure and reactivity. Such research has substantially expanded available knowledge on the mechanisms of action of β-lactam antibiotics, which in turn has facilitated the determination of new structures with a similar chemical reactivity besides resistance to bacterial defense mechanisms.

Ab initio calculations were recently used to determine structural parameters for various β-lactams [2–7]. On the other hand, the chemical reactivity (basically alkaline hydrolysis) of β-lactam antibiotics has been studied preferentially by using semi-empirical methods [8–12], with the exception of early investigations of *Petrongolo et al.* [13] [14].

It should be noted that most of such studies have been carried out in the gas phase and were thus subject to interferences from substitution and elimination reactions [9] [15] [16].

Neither *ab initio* study of the alkaline hydrolysis of β-lactams using quality basis sets nor analysis of the influence of the solvent on the process has been reported to date.

This paper presents a comprehensive theoretical study of the alkaline hydrolysis of the azetidin-2-one ring. To elucidate the influence of the solvent, *ab initio* calculations were also performed by using the reaction-field method, which allows the solvent to be considered as a continuum with a given dielectric constant that surrounds the molecule. All calculations were based on a 6-31 + G* basis set including polarized and diffuse function on heavy atoms.

Methodology. – Azetidin-2-one was the model compound used to study the β-lactam ring. Previously elucidated semi-empirical structures [9] were used as the starting points for the *ab initio* calculations, which were done on the 6-31 + G* and MP2/6-31 + G*// 6-31 + G* basis set. Polarized and diffuse basis for heavy atoms were included to ensure reliable results for small charge-localized anions [17]. In those cases where the geometry derived from the semi-empirical calculations was inappropriate (particularly concerning saddle points), reaction coordinates were used with full optimization of every parameter until the desired steady-state point was reached.

Calculations were performed on an *ALPHA DEC 10620 AXP* computer running the program GAMESS [18] as modified by *Schmidt et al.* [19].

The influence of the solvent on the system studied was analyzed by using the reaction field method (SCRF) as implemented in the software package GAMESS. The cavity radius used, 3.26 Å, was calculated using the rules proposed by *Szafran et al.* [20]. Following the suggestions of *Schmidt* [21], a dielectric constant of 80.0 was employed. All structures were characterized by vibrational analysis.

Results. – *Gas-Phase Hydrolysis.* Both the geometric and energy parameters for the β-lactam ring obtained in this work are consistent with those recently reported by *Alcolea et al.* [5] for 6-31G** basis; however, we used diffuse functions since some calculations involved anionic structures as well (see *Tables 1* and *2*).

The *Scheme* shows the reaction mechanism studied and the numbering conventions used in this paper. *Fig. 1* depicts the reaction energy profile derived for the gas-phase reaction.

Scheme. *Reaction Mechanism for the Alkaline Hydrolysis of the Azetidin-2-one Ring in Aqueous Solution and the Numbering Convention Used in This Paper.* The structures of the different intermediates and transition states correspond to those obtained with the 6-31 + G* basis set plus the reaction-field method (SCRF).
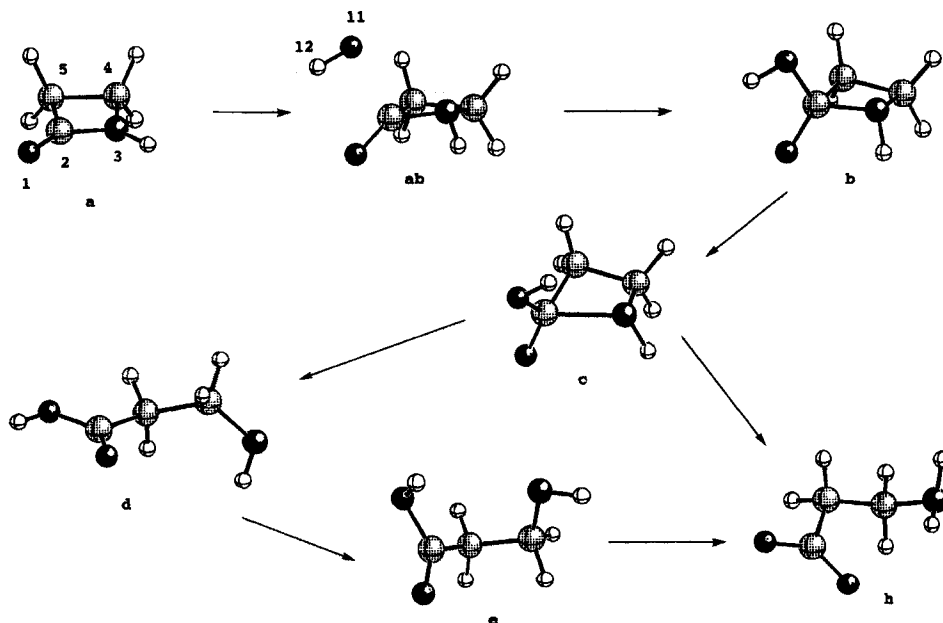
Table 1. *RHF/6-31-G* Energy and Selected Geometric Parameters for the Stationary Points*[a]

	a	b	c	d	e	f
O(1)–C(2)	1.1888	1.2955	1.2207	1.1986	1.1946	1.2352
C(2)–N(3)	1.3563	1.5093	1.9638	2.8372	2.7132	3.1618
N(3)–C(4)	1.4575	1.4662	1.4498	1.4234	1.4274	1.4604
C(4)–C(5)	1.5497	1.5353	1.5274	1.5429	1.5499	1.5276
C(2)–C(5)	1.5312	1.5642	1.5420	1.5019	1.5005	1.5521
C(2)–O(11)		1.4275	1.3845	1.3360	1.3496	1.2403
O(1)C(2)N(3)	132.91	115.68	118.20	114.54	117.23	69.30
O(1)C(2)C(5)	135.77	117.98	120.32	125.38	124.93	116.59
C(2)N(3)C(4)	96.12	90.68	81.64	61.51	63.97	54.98
N(3)C(4)C(5)	86.89	90.14	96.72	109.07	109.01	115.33
O(11)C(2)N(3)		110.22	97.88	98.37	88.79	69.30
O(1)C(2)–N(3)C(4)	180.06	102.29	96.78	85.40	87.86	121.88
C(2)N(3)–C(4)C(5)	0.06	18.06	21.12	28.06	26.60	28.34
H(12)O(11)–C(2)O(1)		3.16	141.55	11.66	56.98	211.52
Energy	−245.8175934[b] −75.3764240[c]	−321.2332213	−321.2128230	−321.2227768	−321.2182751	−321.3160937
ZPE	0.086835[b] 0.009042[c]	0.102226	0.099982	0.099441	0.098330	0.103176
Energy[d]	−246.5416494[b] −75.5877813[c]	−322.1748741	−322.1589375	−322.130935	−322.1490874	−322.2453364

[a] Distances in Å, energy in Hartree.   [b] β-Lactam ring.   [c] OH⁻ Ion.   [d] MP2/6-31+G*//RHF/6-31+G* Energy.

Table 2. *RHF/6-31-G* (SCRF) Energy and Selected Geometric Parameters for the Stationary Points*[a)]

	a	ab	b	c	d	e	h
O(1)–C(2)	1.2004	1.2334	1.3036	1.2230	1.1938	1.1898	1.2586
C(2)–N(3)	1.3449	1.4271	1.4679	1.9981	3.9470	2.8155	3.1888
N(3)–C(4)	1.4648	1.4766	1.4732	1.4529	1.4784	1.4397	1.4576
C(4)–C(5)	1.5492	1.5431	1.5366	1.5283	1.5510	1.5492	1.5272
C(2)–C(5)	1.5291	1.5382	1.5631	1.5394	1.5309	1.5008	1.5436
C(2)–O(11)		1.9274	1.4432	1.3821	1.3144	1.3560	1.2365
O(1)C(2)N(3)	132.86	124.10	116.42	119.28	102.95	116.73	149.22
O(1)C(2)C(5)	135.52	128.50	119.69	121.55	124.12	124.90	115.25
C(2)N(3)C(4)	96.25	92.05	91.96	81.56	19.92	60.95	55.00
N(3)C(4)C(5)	86.45	89.39	90.81	97.45	126.89	111.49	112.03
O(11)C(2)N(3)		104.98	109.33	96.11	132.73	113.84	76.04
O(1)C(2)–N(3)C(4)	179.96	130.18	114.66	100.53	12.40	86.55	105.38
C(2)N(3)–C(4)C(5)	359.98	10.03	8.38	18.63	1.82	27.53	331.23
H(12)O(11)–C(2)O(1)		353.71	358.07	145.06	0.42	64.78	149.95
Energy	−245.8260466[b)] 75.49376917[c)]	−321.2982094	−321.3179236	−321.2981137	−321.3593891	−321.3048353	−321.4174684
ZPE	0.087214[b)] 0.009042[c)]	0.099382	0.102243	0.100141	0.100208	0.100208	0.103355
Energy[d)]	−246.5496582[b)] −75.7053449[c)]	−322.2467974	−322.2589550	−322.2427426	−322.2939212	−322.2355325	−322.3456699

[a)] Distances in Å, energy in Hartree.   [b)] β-Lactam ring.   [c)] OH⁻ Ion.   [d)] MP2/6-31+G* RHF/6-31+G* Energy.

A nucleophilic attack of the OH⁻ ion on a C=O group in the gas phase is known to take place with no potential barrier between the reactants (**a**) and the tetrahedral intermediate formed (**b**) provided the attack pathway is roughly normal to the plane of the β-lactam ring – be it on the upper or the lower side. However, the situation is different if variations in the attack pathway are allowed for (see dotted line in *Fig. 1*). The low stability of the OH⁻ ion in the gas phase results in the reaction pathway deviating from verticality in order to stabilize the charge by interacting with the different protons of the β-lactam ring, even though it always remains at the same distance from the C=O group. The intermediate structures formed in the process (**a1** and **a2**) are difficult to characterize as they rapidly evolve to form a H_2O molecule by elimination and transfer the negative charge to the β-lactam ring. Structures of this type were previously observed for other systems [15] [17] [22].
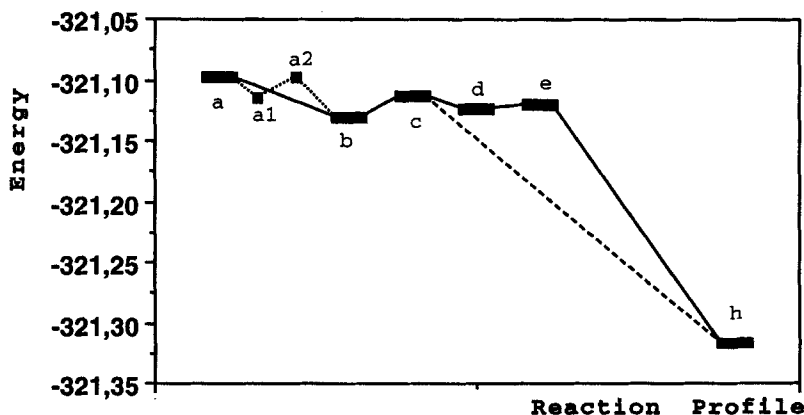

Fig. 1. *RHF/6-31 + G* Energy profile along the* $B_{AC}2$ *reaction pathway for the reaction between the OH⁻ ion and β-lactam ring.* Energy in Hartree.

The tetrahedral intermediate **b** can undergo ring opening to reach the transition state **c**, in which the C(2)–N(3) distance is 1.964 Å. This bond length is different from those obtained from *ab initio* calculations using 4-31 G [23] and 3-21 + G* basis [24] (*viz.* 2.224 and 2.088 Å, resp.). Therefore, ensuring reliable results for a system such as that studied in this work entails using quality basis, at least as good as those used here.

One other salient feature of transition state **c** is that OH⁻ ion is slightly rotated: its proton H(12) is 2.167 Å from N(3), which allows it to be readily transferred.

The vibrational analysis of this structure revealed a single negative constant involving the atoms C(2), N(3), and H(12). This confirms the hypothesis that point **c** may be the transition state for the opening of the β-lactam ring and for the transfer of a proton to the β-lactam N-atom. As a result, this structure can evolve to the end-point **h** (see broken line in *Fig. 1*).

On the other hand, the tetrahedral intermediate could also evolve through a pathway involving the cleavage of the C(2)–N(3) bond until point **d**, where the β-lactam ring is fully open and H(12) has not yet been transferred. Such a proton is 3.848 Å from the β-lactam ring and in an inappropriate spatial orientation for direct transfer. This prob-

lem was previously detected in studying the present reaction using semi-empirical methods [9]. For proton H(12) to be properly oriented, the acid group (or only the OH⁻) must rotate to a structure **f** where the proton can be more readily transferred. Semi-empirical methods have shown this alternative pathway to involve a lower energy. The last step would be the proton transfer (*via* a transition state **g**) to form compound **h**, the most stable in the whole reaction pathway.

The methodology used in this work was identical to that employed for the semi-empirical calculations. Thus, the OH⁻ group was rotated until the transition state **e** (with a single, clear-cut negative force constant) was reached. However, structure **f** could not be obtained, because the proton was immediately transferred to the N-atom and the reaction end-product (**h**) was directly formed. This behavior was previously observed in studying the alkaline hydrolysis of penicillin G using the AM1 method [25].

As can clearly be seen from *Fig. 1*, the rate-determining step of the process in the gas phase is the cleavage of the C—N bond (the step from **b** to **c**), which contradicts the experimental results obtained in solution [26], where the formation of the tetrahedral intermediate is the limiting step. The potential barrier obtained was 11.39 kcal/mol (0.01854 Hartree).

It is worth noting the high stability of the end-product **h** relative to the reactants and intermediates as the main result of it being the only structure where the negative charge can be delocalized over the carboxy group.

Influence of the Solvent. Including the reaction-field method, the *ab initio* calculations introduced some changes in the geometry of both the β-lactam ring and the reaction intermediates (see *Tables 1* and *2*). The most important change in the β-lactam ring was an increase in the C(2)—O(1) distance and a decrease in the C(2)—N(3) bond length. It suggests that the amide resonance is displaced to the zwitterion ($^-O-C_2=N^+$), which is logical inasmuch as polar solvents stabilize charged species.

Fig. 2 shows the reaction energy profile obtained by using the reaction-field method. Unlike the gas phase, the presence of the solvent allows for a transition state in the attack of the OH⁻ group on the β-lactam ring (point **ab** in *Fig. 2*). This intermediate was fully characterized by vibrational analysis and found to posses a single imaginary force constant involving primarily C(2) and O(11). In this transition state, the OH⁻ group is partly desolvated and 1.927 Å from the β-lactam C=O; such a distance is slightly shorter than those reported in other systems by *Weiner et al.* [27], and *Yu* and *Karplus* [28], and very similar to that derived by *Madura* and *Jørgensen* [17]. It should be noted that the solvent stabilizes the charge on OH⁻, so the attack always takes place *via* a pathway normal to the cycle and hence free of interferences from other gas-phase reactions, described previously.

It is worth noting the strong stabilizing effect of the solvent on the reactants (particularly OH⁻), which are made even more stable than the tetrahedral intermediate (**a** and **b** points in *Fig. 2*). Such behavior had never been described previously.

A comparison of the geometric parameters for the tetrahedral intermediate (**b**) in the aqueous and gas phase reveals the same effect observed for the β-lactam ring, *i.e.*, a lengthened C(2)—O(1) distance and a shortened C(2)—N(3) distance.

The reaction involves the cleavage of the β-lactam ring *via* the transition state called **c**. As in the gas phase, structure **c** has the H(12)-atom 1.853 Å from the β-lactam N-atom and its imaginary force constant involves atoms C(2), N(3), and H(12). This

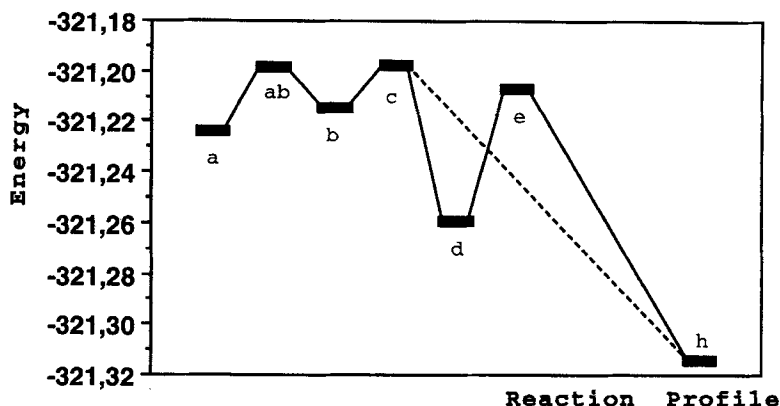Fig. 2. *RHF/6-31 + G* + SCRF Energy profile for the reaction between the OH^- ion and β-lactam ring. Energy in Hartree.*

suggests that **c** represents the transition state corresponding to the cleavage of the β-lactam ring and the proton transfer to yield the end structure **h** (the most stable in the whole reaction pathway) directly (see broken line in *Fig. 2*).

As in the previous case, the ring opening could reach the minimum **d**, which, by rotation about OH^-, can be transformed into the transition state **e**, from which the system evolves spontaneously by transferring H(12) until point **h** is reached.

As can be seen from *Fig. 2*, structure **d** is highly stable, and the energy barrier to the transition state **e** is very high. This is a result of structure **d** being fully unfolded; consequently, for the proton to be transferred, the conformation of the straight chain must previously be altered, and the OH^- group subsequently rearranged. However, it lacks practical significance since, as noted earlier, the system can evolve directly from **c** to **h**.

In presence of H_2O, a potential barrier arises from the partial desolvation of OH^- required to form the tetrahedral intermediate; such a barrier, 13.6 kcal/mol, is very similar to the experimental value (16.1 kcal/mol [26]).

It should be noted that the presence of the solvent reduces the energy difference between the products and reactants dramatically, *viz.* from 0.217954 Hartree (136.8 kcal/mol) in the gas phase to 0.090574 Hartree (56.8 kcal/mol) in solution.

Discussion. – Early theoretical studies on the attack of nucleophiles to various C=O groups in gas phase revealed the existence of various channels for the nucleophilic approach and the absence of a potential barrier to the formation of the tetrahedral intermediate [1] [15]. However, subsequent studies considering the influence of the solvent led to a potential barrier of 12–18 kcal/mol for the formation of such an intermediate [17] [27] [28].

Our *ab initio* calculations revealed that the nucleophilic attack of the OH^- group on the β-lactam ring in the gas phase takes place *via* a mechanism other than B_{AC2}, which is only feasible if the system is forced to adopt it. The presence of the solvent, simulated in this study by using the reaction-field method, stabilizes the OH^- group and hence

overcomes any interferences from substitution and/or elimination reactions. The same behavior can be found in the gas-phase and aqueous-phase hydrolysis of esters [29] [30].

There are various methods for assessing the influence of the solvent in theoretical terms. The most frequent choices for this purpose are the continuum method, which considers the solvent as a continuum with a given dielectric constant that involves the structures to be studied, and the supermolecular approach, which involves wrapping the structure with a given number of solvent molecules. In a recent work involving the use of semi-empirical methods to study the attack of the OH^- ion on a β-lactam $C=O$ group [6], we used both types of methods to assess the influence of the solvent. Application of the supermolecular approach involved wrapping the system with 20 H_2O molecules. The continuum method used was AMSOL [31]. The two methods led to very similar results; thus, both predicted the formation of a transition state from partial desolvation of OH^- ion at a distance of 2.247 and 2.037 Å from the $C=O$ group according to AMSOL and the supermolecular approach, respectively. Also, the two potential energy barriers were similar, *viz.* 20.7 kcal/mol for AMSOL and 17.5 kcal/mol for the supermolecular approach; both are very close to the experimental value [26].

The present state of development of computers compels one to consider the solvent in the light of continuum methods and to use high quality basis for the *ab initio* calculations. With semi-empirical calculations, the computational power of most available workstations enables the use of both types of methods. The supermolecular approach affords a wealth of information on the system, but computations are usually time-consuming and cumbersome owing to the large number of atoms of the system and the difficulty of minimizing the geometry of a system with a nearly planar potential-energy surface. Such difficulty is even greater for transition states, where vibrational analyses occasionally provide more than one imaginary force constant. It should be noted, however, that the additional imaginary constants usually have very small absolute values, and that they only involve rotations of some H_2O molecules.

On the other hand, continuum methods (*e.g.* AMSOL and the reaction-field method described above) afford acceptable evaluation of the solvent effect from much more expedient computations relative to the supermolecular approach.

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