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Theoretical calculations of β-lactam antibiotics

Part 4. AM1, MNDO, and MINDO/3 calculations of hydrolysis of bicyclic system of penicillins

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Summary. We carried out a comprehensive theoretical study on the alkaline hydrolysis of the bicyclic system of penicillins (a four-member ring fused to a thiazolidine ring) on the basis of a B_{AC2} mechanism. We assayed the MINDO/3, MNDO, and AM1 semi-empirical calculation methods in order to determine their suitability for studying β-lactam rings.

Both the geometric and the energetic results obtained for the different intermediate states were compared with literature values – chiefly those determined by *ab initio* methods – with which they proved to be very consistent.

The conformation of the carboxyl group at position 3 was found to be rather significant to the determination of the energy of the different reaction maxima and minima.

Key words: AM1 – MNDO – MINDO/3 – Hydrolysis penicillins – β-Lactam

1. Introduction

The basic hydrolysis of β-lactam rings is not only of chemical, but also of biological significance inasmuch as the pathway involving a nucleophilic attack by the hydroxyl ion is very similar to the mechanisms of action of β-lactamases and other related enzymes on β-lactam antibiotics [1]. The thiazolidine ring of the penicillins is flexible and it can adopt two extreme conformations: the 2β-(CH_3)-3α-(COOH) axial conformation, where the C_3 carbon is out the plane formed by the other four atoms of the ring, and the 2β-(CH_3)-3α-(COOH) equatorial conformation, where the sulphur atom is out of the plane [2].

Few theoretical studies on this topic have so far been reported [3, 5]. In recent work [6] we addressed this issue by studying the azetidin-2-one ring using semiempirical theoretical calculation methods and assuming a B_{AC2} mechanism for the attack of the hydroxyl ion on the carbon atom of the β-lactam carbonyl group.

In the present work we extended the aforementioned study to the hydrolysis of penicillin nuclei, in which the azetidin-2-one ring is fused to a thiazolidine ring, which endows the bicyclic system with distinct features compared to the monocyclic system.

2. Methodology

All calculations were carried out by using the MINDO/3 [7], MNDO[8] and AM1 [9] semi-empirical calculation methods with the aid of a modified version [10] of the MOPAC integrated software package [11]. In addition, the AM1 method was used with a new parametrization of the sulphur atom developed by Dewar's group [12]. All calculations were done on VAX 8820 and 9210 VP computers.

The procedure used to determine and refine the different transition states and energy minima was described in detail elsewhere [6]. All calculations throughout the reaction development were derived from a single-determinant wavefunction (RHF) with no configuration interaction, and were based on the DFP (Davidon–Fletcher–Powell) optimization algorithm [13]. As a further control test for diradical character, single point calculations were performed with the UHF formalism [14] on selected RHF calculated transition states, essentially zero expectation values for the $\langle S^2 \rangle$ operator and enthalpies of formation essentially equal to the RHF enthalpies recorded in every case.

The geometries for stationary points were re-determined by minimizing the energy with respect to all geometric parameters using the DFP algorithm with the keyword PRECISE in order to diminish the convergence criterion by a factor of 100.

The geometry and energy of the transition states detected along the reaction coordinate were refined by minimizing the gradient norm using the NLLSQ algorithm [15].

Transition states were characterized by the fact that the matrix of the second-derivative of the energy with respect to the position (i.e. the Hessian matrix), has one and only one, negative eigenvalue. This test was applied to every case.

3. Results

Figure 1 shows the nucleus of the penicillin molecule and the numbering conventions used, as well as some of the different reaction intermediates involved

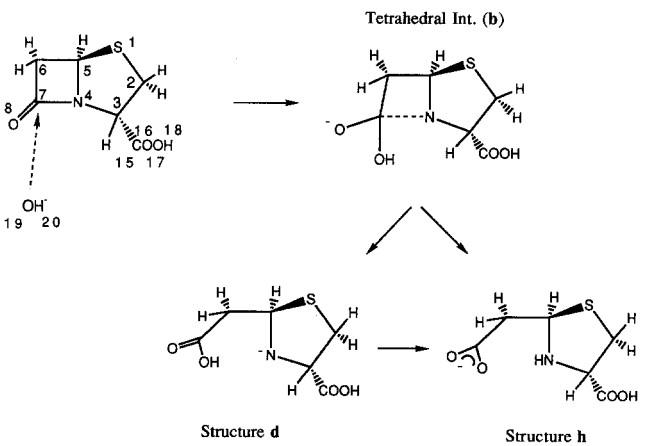

Fig. 1. β-lactam (bicyclic system of penicillins) + OH^- reaction B_{AC2} mechanism. Numeration of the bicyclic system

in the basic hydrolysis of β-lactam antibiotics via a B_{AC2} mechanism. Only the nucleophilic attack on the α (lower) side of the β-lactam ring – the most favoured energetically [6, 16] – was considered.

3.1 MINDO/3

Table 1 lists the values of the most significant geometric and energetic parameters, and net charge on the atoms of the thiazolidine ring obtained by using the MINDO/3 method. Also, Fig. 2 shows the most relevant structures involved in the reaction pathway and Fig. 3 shows the three different reaction profiles provided by the MINDO/3, MNDO, and AM1 methods.

In the gas state, the protonated acid group can occur in two different conformations with a 0° (pseudo-*cis*) and a 180° (pseudo-*trans*) $H_{18}O_{17}$–$C_{15}O_{16}$ dihedral angle, respectively. In the ground states, the pseudo-*cis* conformation is more stable than its pseudo-*trans* counterpart [9]. The MINDO/3 results are

Table 1. Optimized energetic, geometric parameters (bond distance, bond angle, and dihedral angle) and net charges for the different structures of the pathway reaction by the MINDO/3 method

Param.	Structures							
	a	***b***	***c***	***d***	***e***	***f***	***g***	***h***
$d(C_7–N_4)$	1.398	1.568	1.963	3.209	3.171	3.217	2.937	3.364
$d(C_7–O_8)$	1.199	1.261	1.232	1.220	1.217	1.221	1.227	1.244
$d(O_{19}–C_7)$	—	1.393	1.357	1.342	1.359	1.343	1.324	1.246
$d(O_{19}–H_{20})$	—	0.951	0.952	0.952	0.946	0.948	1.003	2.983
$d(H_{20}–N_4)$	—	2.922	3.072	4.453	3.020	3.104	1.560	1.028
$d(S_1–C_5)$	1.771	1.817	1.842	1.872	1.865	1.868	1.846	1.826
$C_5C_6C_7$	87.6	91.0	99.6	121.0	122.6	123.4	119.6	120.9
$N_4C_5C_6$	88.0	91.3	98.2	122.4	121.4	121.5	116.2	122.6
$C_2S_1C_5$	100.1	98.2	96.8	97.3	97.4	97.3	97.9	102.8
$H_{20}O_{19}–C_7O_8$	—	−22.1	−20.4	−4.4	−99.4	−174.4	175.4	−99.4
$H_{18}O_{17}–C_{15}O_{16}$	−1.6	−3.1	−2.0	−2.6	−3.0	−2.1	−2.1	−1.4
$N_4C_5–S_1C_2$	20.0	17.3	17.4	1.6	3.2	2.8	9.9	2.4
$C_7N_4–C_5C_6$	2.4	0.3	−0.1	22.6	18.8	20.6	12.7	28.5
$C_5C_6–C_7O_8$	−177.7	116.6	104.7	108.8	140.3	140.1	−161.6	−87.1
$N_4C_3–C_{15}O_{16}$	32.9	50.3	49.8	59.2	74.0	55.7	55.1	43.5
$Q(S_1)$	−0.249	−0.392	−0.444	−0.476	−0.465	−0.469	−0.436	−0.349
$Q(C_2)$	−0.173	0.161	0.155	0.139	0.139	0.139	0.142	0.141
$Q(C_3)$	0.040	0.067	0.107	0.162	0.168	0.164	0.154	0.091
$Q(N_4)$	−0.260	−0.344	−0.461	−0.498	−0.521	−0.508	−0.518	−0.217
$Q(C_5)$	0.282	0.373	0.421	0.459	0.456	0.464	0.446	0.408
$Q(C_6)$	−0.102	−0.103	−0.078	−0.094	−0.124	−0.140	−0.138	−0.144
$Q(C_7)$	0.641	0.844	0.859	0.827	0.839	0.831	0.833	0.872
$Q(O_8)$	−0.517	−0.776	−0.673	−0.621	−0.595	−0.593	−0.632	−0.752
$Q(O_{19})$	—	−0.610	−0.561	−0.542	−0.584	−0.543	−0.581	−0.758
ΔH_f°	−145.76 −139.91	−220.74 −216.66	−216.08 −212.07	−229.31 −226.02	−222.54 −218.94	−225.68 −222.24	−221.45 −217.48	−232.31 −227.90
S°	116.23	122.30	122.80	133.33	131.99	132.94	125.92	133.81

Bond distances are in angstroms and bond angles and dihedral angles in degrees.
Entropies are in cal/K mol and enthalpies in kcal/mol at 25°C.

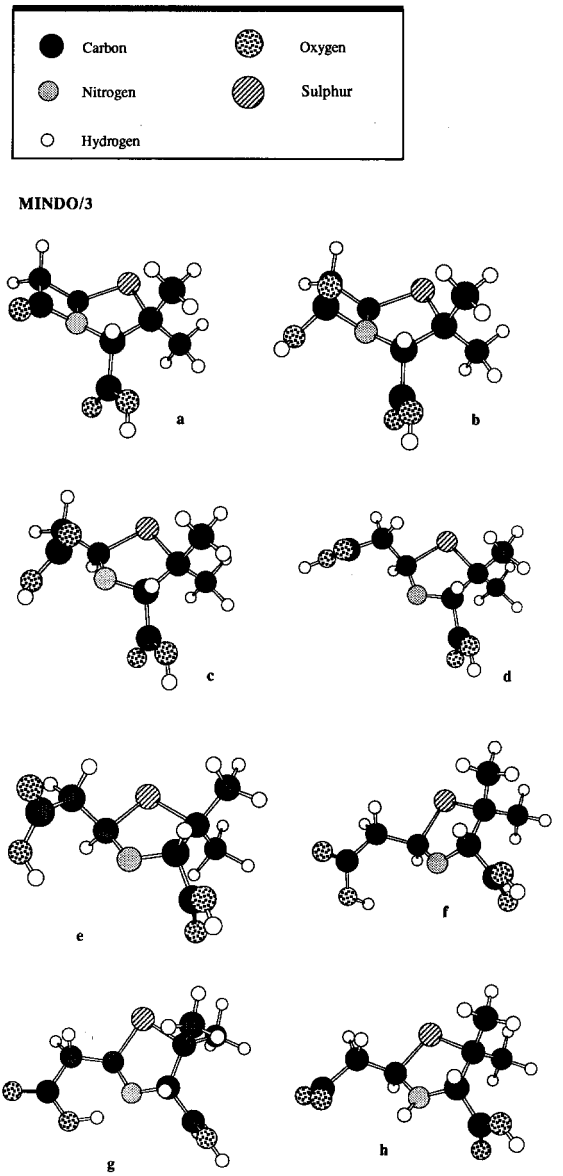

Fig. 2. Structures corresponding to the different intermediate and final states of the reaction pathway by MINDO/3 method

consistent in this respect since the enthalpy of formation provided for the pseudo-*cis* conformer is smaller than it is for the pseudo-*trans* conformer in all the intermediates. The most significant bond lengths and angles and dihedral angles of this initial structure (***a***) are very similar to those provided by this method for other penicillins [17].

The nucleophile approaches the carbon atom in the β-lactam carbonyl group along a line perpendicular to the amide plane under the constrain of no energy barrier, as expected for a gas-phase addition reaction [18].

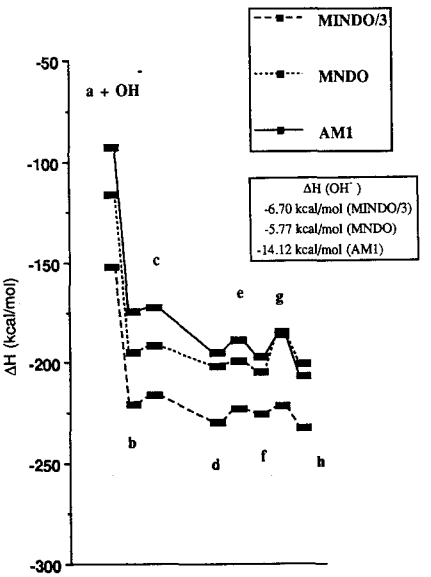

Fig. 3. Schematic representation of the reaction profile provided by the MINDO/3, MNDO, and AM1 methods

The tetrahedral intermediate formed (***b***) is ca. 75 kcal/mol more stable than the initial structure. The most salient geometric features of the β-lactam ring of this intermediate are consistent with those of the azetidin-2-one ring [6] with the exception of the C_7–N_4 bond length, which is somewhat longer in this case and virtually identical with those reported by Alagona et al. [19] and Petrongolo et al. [3]. The conformation of the thiazolidine ring in this intermediate remains 2β-3α equatorial (see N_4C_5–S_1C_2 in Table 1). This intermediate evolves to product ***d*** – with H_{20} bonded to the O_{19} atom – through transition state (***c***), which features a markedly cleft β-lactam. The geometric analysis of structure ***d*** shows the β-lactam ring to be fully open [$d(C_7$–$N_4) = 3.209$ Å].

It should be noted that the S_1–C_5 bond distance increases along the reaction profile up to 1.871 Å in structure ***d***. This is a result of the changes in the net charges of the atoms throughout the reaction (see Table 1). As can be seen, the excess negative charge supplied by the nucleophile is not taken up exclusively by the nitrogen atom, but is rather delocalized on the thiazolidine ring and on the sulphur atom in particular. Such delocalization is ultimately responsible for the increase in the S_1–C_5 bond distance to its present value.

Product ***d*** evolves to product ***h*** by hydrogen transfer to the β-lactam nitrogen. A direct transfer is hindered by the excessively long distance between the two atoms and the inappropriate orientation of the H_{20} atom. The reaction pathway, similar to that involved in the hydrolysis of azetidin-2-one, is characterized by a turn about the O_{19}–C_7 bond and a slight rotation of the acid group to structure ***f*** through an intermediate maximal-energy structure (***e***). The H_{20} atom in structure ***f*** lies at a distance of 3.127 Å and in the appropriate orientation (C_7O_{19}–$H_{20}N_4 = 37.0°$) for the transference.

Structure ***f*** evolves through a maximum structure (***g***), which the hydrogen atom bonded to the oxygen, as can be seen on comparing the H_{20}–O_{19} distances in structures ***f*** and ***g*** (0.948 and 1.003 Å, respectively). Therefore, the decrease in the H_{20}–N_4 bond length can only be attributed to a turn of the acid group by about 50° (see value of C_5C_6–C_7C_8 dihedral angle in Table 1).

3.2 MNDO

The reaction pathway determined by the MNDO method is analogous to that obtained by using the MINDO/3 method up to structure ***h***. The most salient geometric and energetic features of the most significant structures involved in the reaction are given in Table 2. Such structures are very similar to those obtained by AM1 method, and the reaction profile is shown in Fig. 3.

Unlike the MINDO/3 structures, those of MNDO with the acid group ($H_{18}O_{17}$–$C_{15}O_{16}$) in the pseudo-*cis* conformation are less stable than those in the pseudo-*trans* conformation, with the exception of the initial compound (***a***). However, we should note that the energy differences between the two conformers are very small for some structures.

As usual for MNDO calculations, C_7–N_4 distances were overestimated with respect to X-ray measurements [17].

Table 2. Optimized energetic, geometric parameters (bond distance, bond angle, and dihedral angle) and net charges for the different structures of the pathway reaction by the MNDO method

Param.	Structures							
	a	***b***	***c***	***d***	***e***	***f***	***g***	***h***
$d(C_7$–$N_4)$	1.452	1.615	1.973	3.007	2.890	3.120	2.865	3.179
$d(C_7$–$O_8)$	1.208	1.277	1.248	1.234	1.229	1.235	1.247	1.257
$d(O_{19}$–$C_7)$	—	1.407	1.380	1.364	1.378	1.358	1.299	1.260
$d(O_{19}$–$H_{20})$	—	0.946	0.947	0.948	0.948	0.949	1.294	2.663
$d(H_{20}$–$N_4)$	—	2.940	2.960	4.159	3.018	2.477	1.200	1.009
$d(S_1$–$C_5)$	1.734	1.741	1.752	1.776	1.771	1.770	1.757	1.760
$C_5C_6C_7$	86.5	90.4	98.5	114.2	113.9	115.2	114.9	114.8
$N_4C_5C_6$	89.5	92.3	97.9	115.1	114.2	113.5	113.9	116.6
$C_2S_1C_5$	97.5	97.0	96.5	96.4	96.5	96.7	97.7	98.5
$H_{20}O_{19}$–C_7O_8	—	−23.9	−32.4	−12.8	−84.7	169.8	172.2	127.5
$H_{18}O_{17}$–$C_{15}O_{16}$	4.2	177.3	177.4	177.6	176.7	176.7	175.6	176.4
N_4C_5–S_1C_2	8.8	6.2	7.2	−7.0	−5.5	−1.6	−5.4	−14.9
C_7N_4–C_5C_6	−3.0	−2.4	−2.4	23.9	18.6	28.6	13.6	28.3
C_5C_6–C_7O_8	179.5	115.1	103.7	115.8	125.8	−120.5	−158.5	−86.8
N_4C_3–$C_{15}O_{16}$	−123.8	176.2	179.7	−174.5	−168.2	−169.0	−167.0	−153.6
$Q(S_1)$	0.085	−0.022	−0.059	−0.109	−0.095	−0.098	−0.028	0.011
$Q(C_2)$	−0.108	−0.076	−0.076	−0.082	−0.083	−0.078	−0.082	−0.089
$Q(C_3)$	0.130	0.098	0.120	0.139	0.141	0.141	0.098	0.057
$Q(N_4)$	−0.393	−0.506	−0.668	−0.726	−0.744	−0.738	−0.566	−0.359
$Q(C_5)$	−0.026	0.028	0.044	0.128	0.118	0.109	0.052	0.035
$Q(C_6)$	−0.023	−0.038	0.019	0.012	−0.018	−0.005	−0.048	−0.072
$Q(C_7)$	0.322	0.444	0.447	0.377	0.387	0.350	0.349	0.348
$Q(O_8)$	−0.255	−0.673	−0.525	−0.411	−0.366	−0.388	−0.500	−0.580
$Q(O_{19})$	—	−0.380	−0.342	−0.314	−0.359	−0.305	−0.512	−0.599
ΔH_f°	−110.75 −107.39	−193.22 −195.83	−186.59 −191.87	−198.18 −201.59	−192.73 −198.96	−201.71 −204.49	−179.88 −184.71	−199.15 −200.53
S°	98.38	103.45	103.82	112.13	111.16	112.71	106.55	111.75

Bond distances are in angstroms and bond angles and dihedral angles in degrees.
Entropies are in cal/K mol and enthalpies in kcal/mol at 25°C.

The system evolves via tetrahedral intermediate ***b*** and transition state ***c*** to the first reaction product (***d***). The S_1–C_5 bond distance in this structure is longer than in the initial structure, though not to the extent predicted by the MINDO/3 method. The H_{20} atom is very far from the β-lactam nitrogen atom (4.159 Å), so a direct transfer is hindered. By a similar procedure to that used by MINDO/3 we found the most favoured pathway for structure ***d*** to involve a maximum (***e***) characterized by a turn about the C_7–O_{19} bond to a minimum (***f***) of pseudo-*trans* conformation in the H_{20}–O_{19} bond with respect to the C_7–C_8 bond (see value of $H_{20}O_{19}$–C_7O_8 dihedral angle in Table 2). This structure ***f*** shows an important rotation of acid group with respect to the structure ***e***, 115° approximately (see dihedral angle C_5C_6–C_7C_8 in Table 2). On analysing the rotation of the acid group in ***e*** it is seen that it it marginally more stable (ca. 0.035 kcal/mol) than in ***f***, in which the C_5C_6–C_7C_8 dihedral angle is positive.

This new structure, which is 3 kcal/mol more stable than ***d***, has the H_{20} atom at a distance of 2.479 Å from the N_4 atom and in the appropriate orientation, so it can indeed undergo the transfer, which takes place via a maximum (***g***) with partial transfer of H_{20} to the β-lactam nitrogen. The transfer yields product ***h***, which is less stable than ***d*** and ***f***.

Table 2 lists the net charges of the atoms of the β-lactam ring for the different reaction points. As can be seen, the net charge on the sulphur atom increases slightly from the initial structure to product ***d***. This slight increase results in a difference of only 0.035 Å in the S_1–C_5 bond distance between the two structures.

3.3 AM1

As noted earlier, the AM1 method was applied by using a new parametrization for the sulphur atom, so S–C bond distances and the bond and dihedral angles involving this atom were closer to their experimental counterparts [17, 20]. The procedure used was analogous to those employed in applying the MINDO/3 and MNDO method, viz. approach of the nucleophile and evolution of the system with cleavage of the β-lactam ring and subsequent transfer of hydrogen to this ring. Table 3 lists the most significant bond distances and angles, and the dihedral angles and the main net charges on the atoms of thiazolidine ring obtained for the differrent transition states and energy minima. Also, Fig. 4 shows the most relevant structures involved in the reaction pathway.

As with the other semi-empirical methods, the initial structure (***a***) in which the acid group is in a pseudo-*cis* conformation ($H_{18}O_{17}$–$C_{15}O_{16}$ dihedral angle = 0°) is ca. 3 kcal/mol more stable than in its pseudo-*trans* counterpart ($H_{18}O_{17}$–$C_{15}O_{16}$ dihedral angle = 180°). The system evolves via a tetrahedral intermediate (***b***) and an energy maximum (***c***) to product ***d***. Structures ***b*** and ***c*** are very similar geometrically to those provided by the MINDO/3 and MNDO methods. Also, as with the MNDO method, the structure with the acid group in the pseudo-*trans* conformation is more stable than its pseudo-*cis* counterpart.

The evolution of energetic maximum ***c*** yield an increase of the bond length S–C. The reaction coordinate drives to a structure which the bond length S_1–C_5 have been increased very much, specially if the acid group in C_3 is in the pseudo-*cis* conformation. In this conformation the N_4–H_{18} hydrogen bond is not permitted and the negative charge on the β-lactam nitrogen is delocalized on the sulphur atom of the thiazolidine ring.

Table 3. Optimized energetic, geometric parameters (bond distance, bond angle, and dihedral angle) and net charges for the different structures of the pathway reaction by the AM1 method

Param.	Structures							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
$d(C_7–N_4)$	1.453	1.614	1.859	3.041	2.944	3.000	2.808	2.955
$d(C_7–O_8)$	1.219	1.288	1.266	1.238	1.231	1.239	1.248	1.258
$d(O_{19}–C_7)$	—	1.423	1.399	1.368	1.388	1.358	1.314	1.268
$d(O_{19}–H_{20})$	—	0.964	0.965	0.969	0.966	0.979	1.274	2.030
$d(H_{20}–N_4)$	—	3.016	3.047	4.138	2.985	2.062	1.271	1.015
$d(S_1–C_5)$	1.785	1.809	1.825	2.042	1.922	1.941	1.888	1.845
$C_5C_6C_7$	86.6	89.8	94.8	112.3	110.0	114.0	111.6	112.6
$N_4C_5C_6$	89.5	93.0	97.6	117.5	115.1	115.7	113.6	116.4
$C_2S_1C_5$	94.6	94.2	93.7	89.0	91.7	91.6	92.4	94.6
$H_{20}O_{19}–C_7O_8$	—	−18.5	−24.3	−5.0	−81.5	172.2	166.7	141.4
$H_{18}O_{17}–C_{15}O_{16}$	−3.0	−177.0	−177.3	−179.9	−178.8	−179.7	−179.0	−179.1
$N_4C_5–S_1C_2$	6.8	−3.9	−5.2	19.3	12.1	13.3	16.3	−9.7
$C_7N_4–C_5C_6$	−4.7	−2.2	−2.6	33.4	29.3	26.6	20.9	23.7
$C_5C_6–C_7O_8$	176.4	117.7	108.8	137.1	135.2	−132.0	−143.4	−113.3
$N_4C_3–C_{15}O_{16}$	94.2	157.0	164.7	−174.0	176.7	−179.2	173.0	−179.3
$Q(S_1)$	0.082	−0.077	−0.116	−0.405	−0.272	−0.283	−0.179	−0.066
$Q(C_2)$	−0.290	−0.239	−0.235	−0.204	−0.226	−0.225	−0.240	−0.260
$Q(C_3)$	−0.001	−0.056	−0.028	0.014	0.031	0.032	0.041	−0.041
$Q(N_4)$	−0.257	−0.352	−0.469	−0.526	−0.589	−0.607	−0.568	−0.340
$Q(C_5)$	−0.149	−0.091	−0.054	0.096	0.058	0.059	0.023	−0.023
$Q(C_6)$	−0.200	−0.212	−0.176	−0.133	−0.168	−0.191	−0.208	−0.256
$Q(C_7)$	0.285	0.358	0.376	0.331	0.345	0.318	0.324	0.333
$Q(O_8)$	−0.245	−0.654	−0.572	−0.405	−0.352	−0.384	−0.465	−0.546
$Q(O_{19})$	—	−0.404	−0.375	−0.322	−0.374	−0.323	−0.486	−0.593
ΔH_f°	−78.89 −75.89	−171.57 −174.35	−168.57 −172.46	−195.08 −194.71	−186.95 −188.73	−197.03 −197.33	−184.41 −186.01	−206.40 −206.80
S°	100.85	104.12	104.27	116.39	115.48	112.70	108.05	112.21

Bond distances are in angstroms and bond angles and dihedral angles in degrees.
Entropies are in cal/K mol and enthalpies in kcal/mol at 25°C.

In gaseous phase the stabilization of structure ***d*** is generated through the transference the hydrogen to β-lactam nitrogen (evolution of system toward the intermediates ***e***, ***f***, and ***g***).

The increase of bond length S–C can be justified by the fact that, above pH = 8, the 5*R*, 6*R* benzylpenicilloate is very stable in aqueous solution, however, finally it can be observed an epimerisation at C_5, followed by a much slower epimerisation at C_6 [21]. Epimerisation at C_5 through a base catalysed elimination process to form the neutral imine by hydroxide ion abstraction of the hydrogen on the nitrogen, is a mechanism very similar to the delocalization of the negative charge toward the sulphur atom. The formed imine evolves through a re-closure of the thiazolidine ring and formation of the (5*R*, 6*R*) penicilloate or (5*S*, 6*R*) penicilloate. The enamine (***j***) could either be formed directly from the penicilloate or from the iminium ion or its conjugate base.

Figure 5 shows a general scheme of the products that can be obtained by alkaline hydrolysis of the penicillins.

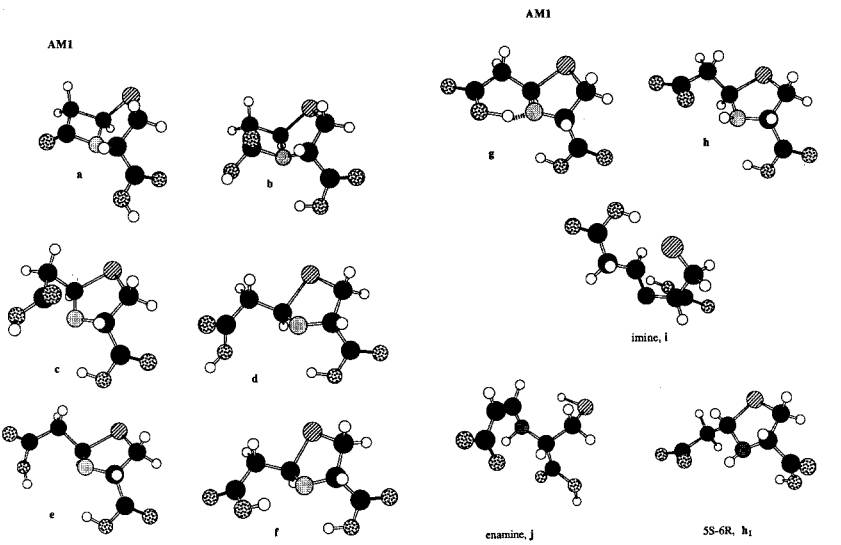

Fig. 4. Structures corresponding to the different intermediate and final states of the reaction pathway by AM1 method

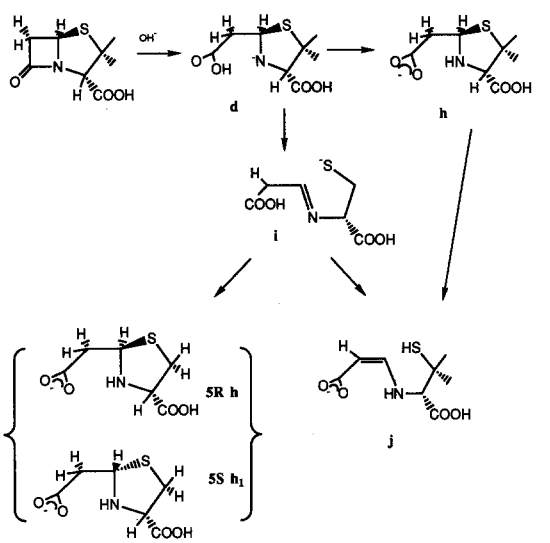

Fig. 5. Scheme of the products obtained by alkaline hydrolysis of penicillins

The evolution of structure ***d*** through the transference of hydrogen to β-lactam nitrogen is very similar to those obtained by MINDO/3 and MNDO methods. The H_{20} atom in structure ***d*** is still very far from the β-lactam nitrogen and in an inappropriate orientation for a direct transfer. By following a similar procedure to that described above is obtained a structure of maximal energy (***e***), the most salient feature of which is a turn about the O_{19}–C_7 bond (variation in the $H_{20}O_{19}$–C_7O_8 dihedral angle from $-5°$ to $-81.5°$).

The system evolves to structure ***f***, which is somewhat more stable than product ***d***. As with MNDO, the orientation of the acid group ($H_{18}O_{17}$–$C_{15}O_{16}$)

in structure ***b*** is markedly different. The S_1–C_5 bond distance, though still very long (1.941 Å) is shorter than in product ***d*** and obviously, the net charge on the sulphur atom is smaller. This, together with the low energy barrier (6.0 kcal/mol) resulting from the rotation about the O_{19}–C_7 bond, leads us to believe that the system may evolve directly to product ***f*** as the first reaction product.

Structure ***f*** can transfer hydrogen directly. The transfer involves a maximum ***g*** which, like that provided by the MNDO method, is characterized by a partial transfer of hydrogen [$d(O_{19}$–$H_{20} = 1.274$ Å)]. Finally, the end product ***h***, with the hydrogen atom bonded to the β-lactam nitrogen, is obtained. As can be seen in Fig. 3, which shows the reaction profile provided by the AM1 method, product ***h*** is the most stable of all involved in the reaction.

4. Discussion

In previous work we used semi-empirical calculation methods to study the nucleophilic attack of hydroxyl ion on the azetidin-2-one ring. In the present work we carried out a similar study on the same β-lactam ring fused with a thiazolidine ring, which make up the basic nucleus of penicillins. It should be emphasized that, even though the basic conformation of the tetrahedral intermediate and the other reaction intermediates is analogous with that determined for the monocyclic system, the presence of the thiazolidine ring introduces some differences. Thus, the amide bond in the tetrahedral intermediate of the bicyclic system is longer than in the monocyclic system and closer to those reported by other authors [3, 19].

Also, intermediates ***d*** and ***f*** have lower energies than the tetrahedral intermediate (***b***), which was not the case with our earlier findings, and the overall energy difference between the final (***h***) and initial products (***a***) is also much greater. Thus, the AM1 method provides a difference of ca. −103 kcal/mol for the monocyclic system of −130 kcal/mol for the bicyclic system.

One other major difference with the hydrolysis of azetidin-2-one lies in the fact that structure ***g*** is in this case an energy maximum with a higher energy than ***e***. Also, the hydrogen transfer is already under way in ***g*** – except with the MINDO/3 method.

It is well known that a carboxyl group is energetically more stable in its *cis* than its *trans* conformation. This is consistent with the MINDO/3 results for all the structures determined throughout the reaction pathway. On the other hand, according to the MNDO and AM1 methods, the *trans* conformation is the more stable – with the exception of the initial products (***a***), even though the energy differences are virtually negligible. This can be attributed to the overestimation of potential hydrogen bonds by the AM1 method [22, 23] – one between the H_{18} atom of the carboxyl group and the β-lactam nitrogen, which can indeed be formed if the former is in its *trans* conformation.

All three methods, particularly AM1, predict a lengthening of the S_1–C_5 bond as the reaction develops to a maximum value for intermediate ***d***. This is highly significant inasmuch as the chemical and enzymatic hydrolyses of β-lactam antibiotics yield reaction products with a cleft S_1–C_5 bond. The explanation for this phenomenon lies in the delocalization of the negative charge of the hydroxyl ion on the β-lactam nitrogen and the sulphur atom (see Tables 1, 2, and 3).

Once more, it should be stressed that, according to the results obtained in this work, the AM1 method appears to be the most suitable for studying the basic hydrolysis of β-lactam compounds, both in geometric and in energy terms.

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