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Why γ- and δ- Are Less Active than β-Lactams? An *ab Initio* Study

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Ab initio calculations (HF/6-31G** and MP2/6-31G**) were performed to investigate the intramolecular hydrogen bonding in the model β-, γ-, and δ-lactam molecules. It was found that the intramolecular (C=O)O–H...O=C hydrogen bond stabilizes much more the γ- and δ-lactam fused ring systems than the β-lactam penicillin and cephalosporin-like systems. This observation suggests that γ- and δ-lactams block themselves by the intramolecular hydrogen bond and therefore are less active toward receptor active site than β-lactams. It is also likely that this factor can discriminate the β-lactamase inhibitors.

Key words: *ab initio*, β-lactam, γ-lactam, δ-lactam intramolecular hydrogen bond

Penicillins and cephalosporins are large groups of drugs that share features of chemistry, mechanism of action, pharmacologic and chemical effects, and immunologic characteristics. These drugs are referred to as β-lactam antibiotics, because of their unique four-membered lactam ring. Both, penicillins and cephalosporins have two fused ring systems: one is the β-lactam (4-membered) ring and the other is the thiazolidine (5-membered) or dihydrothiazine (6-membered) ring for penicillins and cephalosporins, respectively. The two rings share a common amide nitrogen and a carbon atom. The strain along the ring bounding causes the amide torsion angle to be different from zero. To defend against β-lactam antibiotics, bacteria have developed β-lactamases. These enzymes break (hydrolize) the β-lactam ring and nullify the antibacterial effect of the drug.

β-Lactam antibiotics inhibit bacterial growth by interfering with a specific step in bacterial cell-wall synthesis [1]. Cell-wall is composed of peptidoglycan (murein, mucopeptide) consisting of polysaccharides (alternating aminosugars N-acetylglucosamine and N-acetylmuramic acid) and polypeptides (pentapeptide that terminates in D-alanyl-D-alanine). Penicillin binding proteins (PBP) catalyse the transpeptidase reaction that removes the terminal D-alanine, cross-link the peptides and gives the cell-wall rigidity. The β-lactam antibiotics mimic the D-alanyl-D-alanine tail and

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they are bound by PBP at the active site, the transpeptidation reaction is inhibited, the peptidoglycan synthesis is blocked, and the cell dies [1].

Resistance of β-lactams falls into several distinct categories [2]. Some are resistant because the β-lactam ring is protected by the side chain attached to 4-membered ring. Some, as carbapenems, have a different stereochemical configuration in the lactam ring, that apparently imparts resistance to β-lactamases. The discovery of the additional classes of β-lactams (as oxacephalosporins, carbapenems, penems) leads to the conclusion, that the structural elements, responsible for β-lactams activity, are simplified to the β-lactam ring and an acidic group. However, recently published results concerning γ-lactam analogues of carbapenems (two fused 5-membered rings) suggest that dogma of the β-lactam ring required for activity is not true. It was found that γ-lactam analogues of the penems and carbapenems show also a weak antimicrobial activity [3], while δ-lactams exhibit no activity [4].

Chemical reactivity of the β-lactam ring and its neutral or alkaline hydrolysis was the subject of molecular mechanics [5], semiempirical [6–10], and *ab initio* calculations [11–17]. In the recently published paper [17] the role of the 4-membered ring strain, reduced amide resonance, substituent and ring fusion effects on hydrolysis (methanololysis) have been studied. However, the role of the intramolecular hydrogen bonding between the lactam's C=O group and the COOH group attached to the C4 atom of the second ring has been very little looked into [18]. The recent IR and NMR study on the solvent effect on molecuar conformation of the diacetylocephalothin showed that in an inert environment or for concentration of DMSO up to 15% in acetone, the intramolecular hydrogen bond is present [18].

The aim of this work is therefore to present a study of the influence of the C=O···HO(O=C) intramolecular hydrogen bond on the stability of β-, γ-, and δ-lactams. Following the two above mentioned suggestions, we chose as a model of our study two groups of lactam systems (Figures 1 and 2). The first one corresponds to penicillins and cephalosporins and their analogs: 4-membered β-lactam ring is fused ether with 5- or with 6-membered ring. The second one represents γ- and δ-lactam systems, *i.e.*, either 5- or 6-membered lactam ring is fused with the other 5-membered ring. With the discovery of the additional classes of β-lactams, the β-lactam's pharmacophore is simplified to the β-lactam ring accompanied by an acidic group. This was the motivation to study of the simplified systems and to investigate also the influence of heteroatom X in the position 1 (X = S, O, NH(*a*), NH(*b*) and CH_2, where *a* and *b* denote the hydrogen atom of the NH group directed above and below the ring projection plane) on geometry and the hydrogen bond strength in the β-, γ-, and δ-lactam systems.

The present paper is organized as follows. First, we give computational methodology, next, the results are discussed concerning (1) the molecular structure and geometrical parameters; (2) the stability of the structures which contain different heteroatoms. Conclusions summarize our findings.

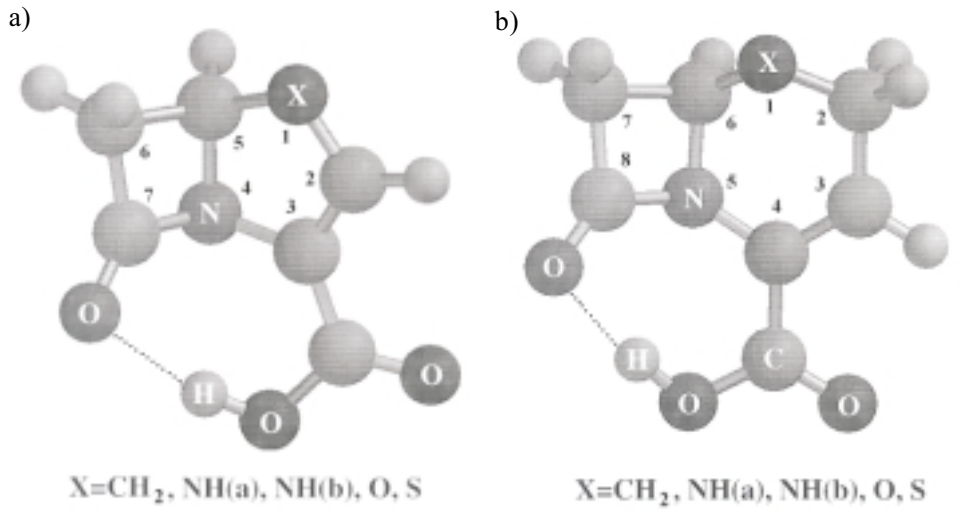

Figure 1. The scheme of β-lactam systems studied: a) penicillins, b) cephallosporins. NH(a) and NH(b) denote hydrogen atom directed respectively above and below the projection plane.

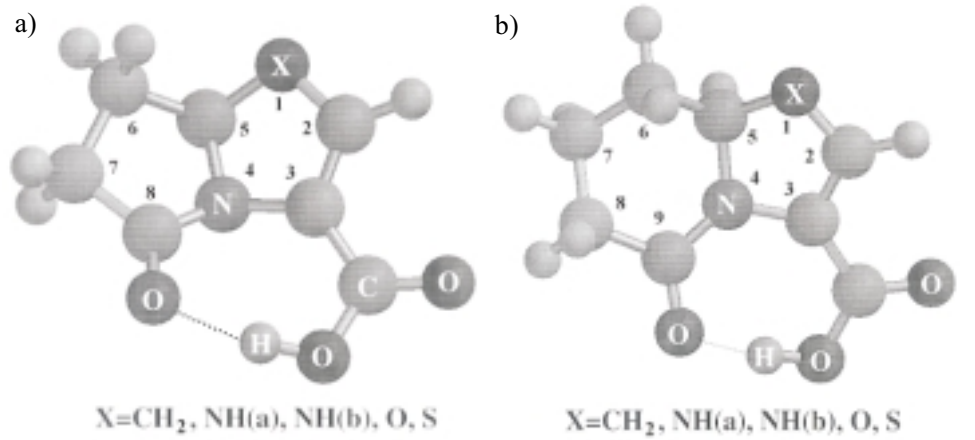

Figure 2. The scheme of a) γ- and b) δ-lactam systems studied. NH(a) and NH(b) denote hydrogen atom directed respectively above and below the projection plane.

CALCULATION METHODS

The *ab initio* calculations were carried out by using *Gaussian98* program [19]. Full geometry optimizations were carried out using both the Hartree-Fock (HF) and the second-order Møller-Plesset perturbation theory (MP2). Because of size of the calculated systems we decided to use the standard 6-31G** basis set [20,21]. Geometry optimization was carried out using redundant coordinate algorithm [22]. In many

papers it was suggested that the electron correlation is necessary to describe the structure and energetics of hydrogen-bonded systems [23]. Treatment of the correlation problem at the MP2 level was found to produce more accurate results for the structure and dipole moment as compared to SCF results [24]. There are two sources of possible errors in our calculations. The first is the treatment of the correlation energy and the second is the unsaturation of the basis set. The better representation of the basis set is difficult in the case of the molecules studied. Our calculations were performed at the HF and MP2 levels. Generally, both series of results are consistent, therefore only the MP2 results are discussed.

RESULTS AND DISCUSSION

The calculations were done for 20 structures with and 20 structures without intramolecular hydrogen bond. All results are presented in Tables 1–4. Each table presents selected parameters that characterize geometry and energetics of the intramolecular hydrogen bond and the influence of heteroatom X on the ring geometry. First, let us discuss the geometrical parameters. The hydrogen bond formation is accompanied by a significant shortening of the O...O distance. As a characteristic of the ring changes with the heteroatom X we chose two dihedral angles: τ_1 and τ_2, defined as follows: τ_1 = ∢C6C7N4C3, ∢C7CN5C4, ∢C7C8N4C3, and ∢C8C9N4C3 in penicillins, cephalosporins, γ- and β-lactams, respectively, and τ_2 = ∢C5C6C5X1, ∢C8C7C6X1, ∢C7C6C5X1, ∢C7C6C5X1 in in penicillins, cephalosporins, γ- and δ-lactams, respectively. For planar two condensed rings the dihedral angles should be equal 180 deg. All the investigated forms were found to be non-planar. One of the most striking features of the geometrical parameters is the observation that the β-lactams are much more distorted than γ and δ-lactams: τ_1 and τ_2 in β-lactams are much smaller than in γ and δ-lactams. Among the heteroatoms considered the S atom produces the smallest distortion which is due to the van der Waals radius of the atom. The largest dihedral angles run along with the longest C6...C2 or C5...C2 distance (both C-atoms are attached to the heteroatom X). The hydrogen bond geometry d(O...O) and a(OH..O) correlate with the hydrogen bond strength: the longer d(O...O) is, the weaker is the hydrogen bond. The hydrogen bond is more linear at the MP2 than at the HF level.

We continue with the discussion of the stabilization energy. As one can see from the tables, all but penicillins, are more stable in the H-bonded than the open forms at the HF as well as at the MP2 level. Moreover, the cephalosporin-like β-lactams form weaker hydrogen bonds than the γ- and δ-lactams. The sequence of the MP2 stabilization energies is: X = CH_2 > NH(*a*) ≈ NH(*b*) > S > O for β-lactams, and γ-lactams and X = NH > CH_2 > O > S for δ-lactams. Thus, the X = CH_2 favors the formation of the intramolecular hydrogen bonds, while in presence of the O and S heteroatoms the intramolecular hydrogen bonds are much weaker. Higher stabilization energy ΔE means that the carboxyl group is engaged in the intramolecular hydrogen bonding. In consequence the lactam N-atom is isolated from environment. Moreover, the car-

Table 1. Comparison of energies and selected geometrical parameters of the intramolecular hydrogen bond structures in penicillin β-lactams at the HF/6-31G** and MP2/6-31G** levels [ΔE = E(H-bonded) – E(open)].

Heteroatom X	Open Form		H-Bonded Form								
	d(O...O) Å	E hartree	d(O...O) Å	d(H...O) Å	a(O–H...O) deg	a(C–O–H) deg	τ_1 deg	τ_2 deg	d(C6–C2) deg	E hartree	ΔE kcal/mol
					HF/6-31G**						
CH_2	4.631	–548.183755	2.986	2.077	159.48	114.35	119.72	–101.77	2.385	–548.183389	0.29
NH(a)*	4.755	–564.173473	3.210	2.345	151.51	113.37	119.53	–98.02	2.291	–564.171242	1.40
NH(b)	4.755	–564.173473	3.210	2.345	151.51	113.37	119.53	–98.02	2.291	–564.171242	1.40
O	4.767	–583.994741	3.304	2.480	145.42	113.44	115.42	–101.56	2.227	–583.991336	2.14
S	4.640	–906.655983	2.996	2.084	160.33	114.54	128.19	–102.67	2.553	–906.653377	1.64
					MP2/6-31G**						
CH_2	4.667	–549.800036	2.917	1.957	166.35	111.96	120.38	–100.23	2.391	–549.800815	–0.50
NH(a)*	4.767	–565.814272	3.055	2.112	162.49	111.07	121.14	–96.01	2.307	–565.812272	1.26
NH(b)	4.767	–565.814272	3.055	2.112	162.49	111.07	121.14	–96.01	2.307	–565.812272	1.26
O	4.771	–585.641827	3.089	2.157	159.90	111.18	117.06	–100.42	2.253	–585.638622	2.01
S	6.690	–908.256829	2.944	1.983	167.07	112.02	128.65	–101.86	2.550	–908.255022	1.13

*This form converges to NH(b) form with the NH proton directed below the plane.

Table 2. Comparison of energies and selected geometrical parameters of the intramolecular hydrogen bond structures in β-lactams at the HF/6-31G** and MP2/6-31G** levels [ΔE = E(H-bonded) – E(open)].

Heteroatom X	Open Form		H-Bonded Form								
	d(O...O) Å	E hartree	d(O...O) Å	d(H...O) Å	a(O–H...O) deg	a(C–O–H) deg	τ_1 deg	τ_2 deg	d(C6–C2) deg	E hartree	ΔE kcal/mol
					HF/6-31G**						
CH_2	3.236	–587.240284	2.787	1.848	167.44	115.86	152.89	–108.12	2.490	–587.242219	–1.21
NH(a)	3.268	–603.222725	2.822	1.884	167.25	115.78	148.07	–110.84	2.376	–603.224159	–0.90
NH(b)	3.294	–603.221416	2.822	1.885	166.48	115.61	147.54	–104.76	2.405	–603.222845	–0.67
O	3.318	–623.048687	2.849	1.915	166.31	115.55	144.53	–107.44	2.327	–623.049387	–0.44
S	3.190	–945.704992	2.765	1.826	166.90	116.13	157.34	–110.19	2.697	–945.705642	–0.41
					MP2/6-31G**						
CH_2	3.153	–589.002323	2.753	1.775	172.78	113.85	152.22	–107.83	2.479	–589.005866	–2.22
NH(a)	3.182	–605.007579	2.787	1.810	172.58	113.74	146.59	–111.13	2.369	–605.010592	–1.89
NH(b)	3.202	–605.004897	2.783	1.806	172.09	113.45	147.20	–104.10	2.391	–605.007955	–1.92
O	3.230	–624.837218	2.810	1.836	171.91	113.46	143.67	–107.10	2.325	–624.839407	–1.37
S	3.094	–947.448681	2.735	1.758	172.15	114.00	155.25	–110.31	2.651	–947.450951	–1.42

Table 3. Comparison of energies and selected geometrical parameters of the intramolecular hydrogen bond structures in γ-lactams at the HF/6-31G** and MP2/6-31G** levels [ΔE = E(H-bonded) – E(open)].

Heteroatom X	Open Form		H-Bonded Form								
	d(O...O) Å	E hartree	d(O...O) Å	d(H...O) Å	a(O–H...O) deg	a(C–O–H) deg	τ_1 deg	τ_2 deg	d(C6–C2) deg	E hartree	ΔE kcal/mol
					HF/6-31G**						
CH_2	3.143	–587.257881	2.694	1.752	165.96	114.51	158.44	–141.37	2.371	–587.263551	–3.56
NH(a)	3.208	–603.245964	2.754	1.822	163.71	113.70	152.45	–135.25	2.284	–603.250961	–3.14
NH(b)	3.183	–603.242761	2.753	1.823	163.47	113.79	148.69	–140.85	2.293	–603.247407	–2.92
O	3.248	–623.069892	2.783	1.857	162.40	113.71	146.88	–134.88	2.221	–623.073047	–1.98
S	3.115	–945.728361	2.681	1.746	164.50	114.14	163.28	–140.77	2.528	–945.730917	–1.60
					MP2/6-31G**						
CH_2	3.080	–589.018463	2.658	1.672	172.02	112.45	162.02	–143.68	2.371	–589.026131	–4.81
NH(a)	3.130	–605.030488	2.709	1.730	170.29	111.53	155.96	–138.15	2.296	–605.036613	–3.84
NH(b)	3.106	–605.027475	2.714	1.738	169.87	111.57	150.44	–144.00	2.307	–605.033076	–3.52
O	3.180	–624.860243	2.737	1.763	169.19	111.40	149.54	–137.86	2.244	–624.864201	–2.48
S	3.043	–947.473218	2.653	1.671	170.79	111.90	166.43	–144.27	2.520	–947.477508	–2.69

Table 4. Comparison of energies and selected geometrical parameters of the intramolecular hydrogen bond structures in δ-lactams at the HF/6-31G** and MP2/6-31G** levels [ΔE = E(H-bonded) – E(open)].

Heteroatom X	Open Form		H-Bonded Form								
	d(O...O) Å	E hartree	d(O...O) Å	d(H...O) Å	a(O–H...O) deg	a(C–O–H) deg	τ_1 deg	τ_2 deg	d(C6–C2) deg	E hartree	ΔE kcal/mol
HF/6-31G**											
CH_2	3.735	–526.299703	2.582	1.644	163.63	112.47	171.18	–168.92	2.351	–626.303466	–2.36
NH(a)	3.948	–642.284334	2.619	1.684	163.29	112.52	166.28	–170.12	2.271	–642.289222	–3.07
NH(b)	3.989	–642.286247	2.625	1.693	162.79	112.21	167.95	–166.08	2.268	–642.291797	–3.48
O	4.007	–662.111268	2.645	1.714	162.47	112.46	164.55	–167.25	2.208	–662.115724	–2.80
S	3.779	–948.768604	2.606	1.683	160.15	111.93	173.59	–164.55	2.499	–984.768223	0.24[1)]
MP2/6-31G**											
CH_2	3.697	–628.207367	2.555	1.565	170.10	109.91	172.47	–168.84	2.346	–628.212848	–3.44
NH(a)	3.921	–644.215546	2.590	1.605	169.91	109.99	167.54	–170.29	2.274	–644.221369	–3.65
NH(b)	3.948	–644.217884	2.594	1.610	169.25	109.47	169.66	–165.82	2.274	–644.223863	–3.75
O	3.968	–664.048463	2.613	1.632	169.07	109.80	166.29	–167.49	2.222	–664.405323	–2.99
S	3.789	–986.660687	2.579	1.600	167.45	109.17	174.75	–164.88	2.483	–986.661951	–0.79[1)]

[1)]Note, that in this case ΔE is smaller than for β-lactams (Table 2).

boxyl group, which is supposed to be bound with the penicilline binding proteins, is engaged in competitive interactions. Thus, we can formulate the hypothesis that the stronger the intramolecular hydrogen bond is, the weaker is the possibility to bind with PBPs. This would mean that in the β-lactams the intermolecular hydrogen bond dominates over the intramolecular, whereas in the γ- and δ-lactams the intramolecular hydrogen bond is favored. The lactam activity against microorganisms is determined by several factors [1,2]: (1) specific step in which bacterial cell-wall synthesis is perturbed, transport through biomembrans, (2) cell-wall permeability, (3) resistance to β-lactamases, (4) antibiotic structure including: (i) interatomic distances defining pharmacophoric group, which must fit in the receptor site, (ii) the faces of the β-lactam ring which must be sterically accessible, (iii) substituents protecting β-lactam bond against hydrolysis: in the position C2 and C6 in penicillins or C3 and C7 in cephalosporins, (iv) the lactam bond which must be sufficiently reactive to acetylate serine, but not so reactive as to hydrolyze readily, (v) the scissile amide bond, which must be in the lactam ring so after the amide bonds open the fragments cannot easily dissociate, and many others. To be aware all these factors we suggest an additional one: lactam deactivation by intramolecular hydrogen bond. It is also likely that this factor can discriminate the β-lactamase inhibitors such as clavulanic acid.

CONCLUSIONS

In the present study we investigated the structural model forms of β-, γ-, and δ-lactams. Our findings can be summarized as follows:

1. The γ- and δ-lactams form stronger intramolecular O–H...O=C hydrogen bonds than the β-lactams.

2. The heteroatom X = CH_2 favors the formation of intramolecular hydrogen bonds, while in the presence of the O and S heteroatoms the intramolecular hydrogen bonds are much weaker.

3. Assuming, that many factors determine the activity of the lactam antibiotics against microorganisms, intramolecular bond formation may be important. Our results suggest that stronger intramolecular hydrogen bonds in γ- and δ-lactams may be the reason of their weaker antibacterial activity than that of the β-lactams.

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