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Ab Initio Conformational Analysis of the (3S, 5R, 6R)-6 Acetylamidopenicillanic Acid

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Abstract: *A conformational analysis of the (3S,5R,6R)-6-acetylamidopenicillanic acid was performed using AM1, omega, and PM3 conformers. Their geometries were optimized at the ab initio HF/STO-3G level. The AM1 semiempirical MO method yields only eight conformers, the PM3 and Omega methods give 47 conformers, while the ab initio HF/STO-3G method leads to 28 conformers. For comparing the geometry of these conformers three geometrical properties were considered: the pseudo-chirality at N atom of the exocyclic amidic group, the anti/syn conformation of the O and H atoms from the exocyclic amidic group, and the three puckering classes of the thiazolidinic ring. The HF/STO-3G geometries do not differ significantly from the experimental ones or from those obtained by other authors who used basis sets of higher performance. However, unlike the high performant basis sets which tend to equalize the S1..C9 and S1..C10 distances, the HF/STO-3G basis set is able to distinguish between these bond lengths, namely the S1..C9 distance is larger than S1..C10. From this conformational analysis it resulted that the HF/STO-3G conformers belonging to the puckering classes (a) and (b) have the most favourable geometrical properties needed for the conversion of penicillins to cephalosporins.*

Keywords: *Geometric properties, Conformational analysis, (3S,5R,6R)-6-Acetylamidopenicillanic acid, ab initio HF/STO-3G method*

1. INTRODUCTION

Penicillins represent the first known class of antibmicrobian compounds [1]. Their molecular structure contains two fused rings: a four-membered β-lactamic ring and a five-membered thiazolidinic ring, with three chiral centers. These chiral centers generate 2^3 possible diastereoisomers [2]. According to Strominger's hypothesis the D-Ala-D-Ala sequence of the general structure of penicillins is responsible for their antibacterial activity [3-6]. In Fig. 1.1 the D-Ala-D-Ala sequence is formed by the following atoms: N14-C6(C5)-C7(O8)-N4-C3(C2)-C11(O12).

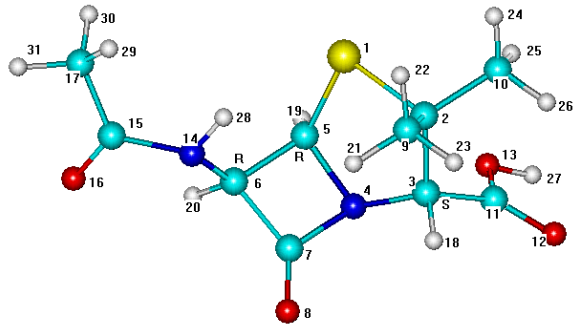


Fig 1.1. *Atom numbering in (3S,5R,6R)-6-acetylamidopenicillanic acid*

Even though all of the eight classes of diastereoisomers have been obtained by synthesis [7-9], geometric and electronic structures of only the (3S,5R,6R)-natural isomer of various penicillins have been extensively studied using different quanum mechanical methods [9-36].

The present study is aimed at analyzing the *ab initio* HF/STO-3G geometries by comparing 1) the N14 nitrogen atom pseudochirality, 2) *anti/syn* conformation of the exocyclic amidic group, and 3) the puckering type of the thiazolidinic ring.

In literature (organic chemistry, biochemistry and pharmaceutical chemistry) the amidic (peptidic) group O=C-N-H is considered planar [3,37-39]. In his studies on the hydrogen bonds in the alpha helix model of proteins Pauling admits that the amidic group is planar [40-42]. In 1968, Ramachandran states that hydrogen bonds can be formed in proteins without considering the amidic group planar [43-45]. Several semiempirical MO (CNDO/2 [43], and *ab initio* methods [46-48] have been used to study the structure of the amidic group in penicillins. The results of these studies suggested that the amidic group in penicillins can be unplanar due to the pyramidalization of the amidic nitrogen atom [46-48].

From all penicillins with exocyclic functions the (3S,5R,6R)-6-acetylamido-penicillanic acid in undissociated form was studied in this paper. The experimental data on penicillins shown in Tables 1, 2 and 3 from supplementary material 1 are for the sodium or potassium salts [49].

In previous papers [33-36] we used for conformational analysis the Conformational Search module implemented in HyperChem package [50], and the AM1 or PM3 semiempirical MO methods. From these methods resulted the non-coplanarity of the amidic group (O17=C15-N14-H28), and a certain pyramidalization of the nitrogen atom N14.

For consistency of data comparison, in this paper was used the same atom numbering as in previous papers [33-36]. Also, the same classification criteria were used: the pyramidalization degree of the N14 atom measured by its "conicity", the *syn* or *anti* conformation of the O17 and H28 atoms, and the three puckering classes (a, b, and c) of the thiazolidinic ring [33-36].

2. RESULTS AND DISCUSSIONS

The published studies on the quantum chemical methods used for the geometry investigation of the exocyclic amidic groups in penicillins indicate that the respective authors have not analyzed the anti/syn conformations of the exocyclic amidic group [3,10-32]. According to Pauling [40-42], the anti conformation imposes a strong tendency towards planarization of the amidic group, this conformation being the most energetically stable. In our previous works [33-36] we have used the dihedral angle O17=C15-N14-H28 to study the anti/syn conformation of the O17 and H28 atoms of the amidic group of penicillins. The conicity, respectively the pyramidalization of the N14 atom, is a measure of deviation from planarity of the exocyclic amidic group. Conicity can be determined by the value of the improper dihedral angle (C6.C15.H28.N14), which forms a trigonal pyramid with the basis C6, C15 and H28 atoms. This pyramidalization generates an R/S [2] pseudochirality at the N14 atom, similarly to the one from the quaternary ammonium salts.

The calculated data was compared with the experimental one obtained from the Cambridge Crystallographic Data Centre 2006-2010 [49]. Tables 1, 2 and 3 from supplementary material 1 present some experimental geometric data obtained by X-ray spectroscopy for 16 penicillins. On analyzing the geometrical data in these tables it results that from all these compounds, seven penicillins have the N14 atom pyramidalized and pseudochiral (six penicillins have R, and one S pseudochirality). However, there are nine structures with a planar amido group.

2.1. Analysis of available experimental and calculated geometrical data

Tables 1, 2 and 3, supplementary material 1 present structural properties experimentaly determined and calculated by other authors with ab initio HF, and DFT methods [14-24]. Because the experimental values of the bond lengths and angles are disperse, to analyze the calculated data we used the average value, and the standard deviation, SD for a 95% confidence interval. Analysing the data in these three tables it results that although the experimental data seems to be disperse, the standard deviations suggest that this dispersion is low, being around ± 2% of the experimental value, under the limit of measurement errors.

2.1.1. Bond lengths

The analysis of bond lengths in Table 1, supplementary material 1 indicates that for all penicillins the amidic C7-N4 bond length in the β-lactamic ring is longer than the exocyclic amidic N14-C15 bond length. This is in contradiction with Woodward's statement, who considered that due to a stronger conjugation in the β-lactamic ring the C7-N4 bond length should be shorter than N14-C15 [3a]. With one exception (MECILIN) for all the other penicillins the S1-C2 bond length is longer than that of the S1-C5 bond (Table 1 supplementary material 1). This also resulted from the calculated average values. The distances between the non-bonded atoms S1 and C9 (Cα), and S1 and C10 (Cβ) atoms were taken into consideration because they have an important role in the conversion mechanism of penicillins into cephalosporins. This mechanism is based on the enzymatic attack at the C2 atom of the thiazolidinic ring by the Fe2+ ion from the FeII/α-ketoglutarateoxygenase [51-56]. The structures in which the distance S1..C9 is larger than S1..C10 are favored in this conversion mechanism [34,35].

2.1.2. Bond angles

Analysing the data in Table 2 supplementary material 1 it results that the C2-S1-C5 bond angle has average values of 94.1º, which is close to 90º. This fact leads to the conclusion that the sulphur atom in the thiazolidinic ring is close to its fundamental state, i.e. it is not hybridized.

2.1.3. Dihedral angles

In Table 3 supplementary material 1 are presented the values of dihedral angles (C5-S1-C2-C3, and C5-N4-C3-C2) important for thiazolidinic ring puckering, and dihedral angle O17=C15-N14-H28 needed to appreciate the anti/syn conformation of the O17 and H28 atoms in the exocyclic amidic group. The dihedral angle N4-C5-C6-N7, and the improper dihedral angle C6.C15.H28.N14 allow to estimate the puckering (non-planarity) of the β-lactamic ring, and respectively, the conicity generating the R/S pseudochirality at the N14 atom. The negative values of the C6.C15.H28.N14 improper dihedral angle correspond to the R chirality, while the positive values to the S chirality.

The experimental structures of penicillins in Table 3 supplementary material 1 were superimposed in the HyperChem7.52 software using the N4, C5 and C6 atoms as a common fragment. This superimposition is shown in Fig. 2.1. From the docking studies and the analysis of the experimental values of the C5-S1-C2-C3 and C5-N4-C3-C2 dihedral angles measuring the thiazolidinic ring puckering (Fig. 2.1, and Table 3 supplementary material 1) it results the existence of three puckering classes:

a first – dominant – class (denoted by a), in which both dihedral angles are positive, but the C5-S1-C2-C3 dihedral angle has slightly lower values than the ones of the C5-N4-C3-C2 dihedral angle;

a second class (denoted by b), in which both dihedral angles have positive values, but the C5-S1-C2-C3 dihedral angle has a much lower value than the one of the C5-N4-C3-C2 dihedral angle;

a third class (denoted by c), in which both dihedral angles have negative values, and the C5-S1-C2-C3 dihedral angle is much higher than the C5-N4-C3-C2 dihedral angle in absolute terms.

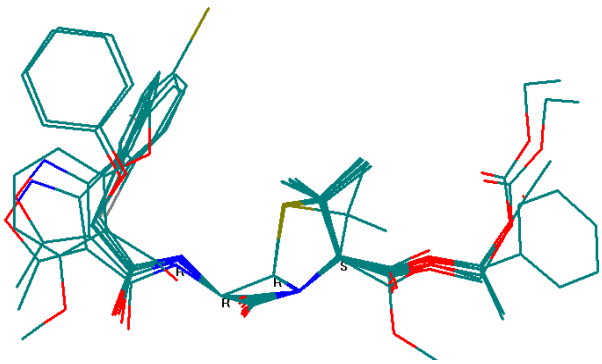


Fig2.1. *Superimposition of the experimental (X-ray) penicillin structures;for clarity hydrogen atoms are not shown*

Experimental values of the dihedral angle O17=C15-N14-H28 (Table 3 supplementary material 1) from the X-ray geometries show an *anti* conformation of the exocyclic amido group in all the penicillins.

2.2. Analysis of ab initio HF/STO-3G conformers

The conformational analysis of the 3S,5R,6R diastereoisomer performed with the Omega v.2.3.2 software (noted with ω) [57], led to 39 conformers with a planar structure, and an *anti* conformation of the exocyclic amido group.

The resulted ω conformers have an R/S pyramidalization at the N14 atom, and *anti* conformation. Geometries of these conformers have been optimized at the PM3 level and compared with PM3 conformers obtained from the Conformational search module implemented in HyperChem 7.52 software. Finally 47 PM3 conformers resulted.

Using the *ab initio* HF hamiltonian and the STO-3G basis set for the geometry optimization from eight AM1 and 47 PM3 conformers resulted 28 HF/STO-3G conformers. Of these, six conformers resulted starting from omega → PM3 conformers. The correspondence between the HF/STO-3G conformers and the starting AM1, ω-PM3, and PM3 conformers is presented in Table 1. After geometry optimization at the HF/STO-3G level a change in energy order resulted. Also, from Table 1 it can be seen that the AM1 method underestimates the number of conformers, while the PM3 and ω methods overestimate it. To prove that all the geometries are correctly optimized and they are not transition states, the energy of the lowest vibration, ν_0, was calculated, and its values are shown in Table 1.

The four methods of conformational search yield different global minimum conformers. In Fig. 2.2 is presented the superimposition of the four global minima conformers optimized with AM1, omega, PM3, and *ab initio* HF/STO-3G methods.

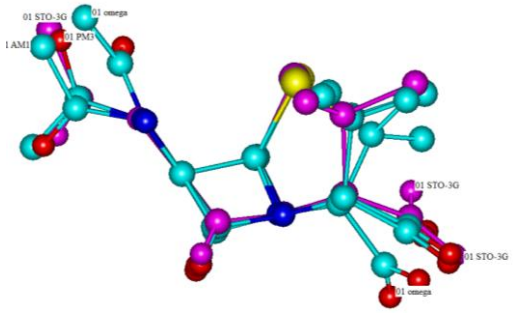


Fig2.2. *Superposition of the global minimum conformers of the (3S,5R,6R)-6-acetilamidopenicillanic acid obtained by AM1 and PM3, ω, and ab initio HF/STO-3G methods.*

The 01am1 conformer has an anti conformation of the exocyclic amido group, similarly to the HF/STO-3G global minimum conformer, while the 01pm3 conformer has a syn conformation. Even though the AM1 and PM3 global minima conformers have the C3-COOH group oriented in the same direction, by geometry optimization of the 01am1 conformer at the HF/STO-3G level, the C3-COOH group was rotated with 180º and led to the HF/STO-3G global minimum conformer, while the 01pm3 conformer generates the 13th HF/STO-3G conformer. The HF/STO-3G global minimum conformer is obtained also from the 04pm3, 11pm3 and 16ω, 17ω, 27ω, 28ω and 35ω conformers (Table 1).

From Fig. 2.2 one can see that the 01ω conformer is completely different from the others, because it has a completely different puckering of the thiazolidinic ring, and a different orientation of the acetamido and -COOH groups.

Between the 01STO-3G and the 28STO-3G conformer there is an energy gap of 8.585 kcal/mol (Table 1).

In Table 2 the RMS error fit between experimental and calculated geometries is presented. For calculating the RMS error fit values only heavy atoms of the two fused rings were considered. Three experimental geometries: BZPEN01 (class (a) of puckering, S pseudochirality), DCLOXL (class (a) of puckering, R pseudochirality) and AMCILL (class (c) of puckering, without pseudochirality) [49] were chosen for superpositions.

For BZPEN01 the best superposition was obtained for the following HF/STO-3G conformers: 01, 11, 13 and 14, which belong to the class (a) of puckering. 01 has anti, while the conformers 11, 13, and 14 have syn conformation, 01 and 14 have R, while 11 and 13 have S pseudochirality at the N14 atom.

For AMCILL the best superposition was obtained for conformers 12, 17, and 28, all of them belonging to class (c) of the thiazolidinic ring puckering, all have R pseudochirality, 12 and 17 have anti, while 28 has syn conformation of the exocyclic amido group.

For DCLOXL a good match have conformers 03 and 04. Both of them have anti conformation, S pseudochirality and they are members of the class (a) of puckering.

Conclusion resulted from these superpositions is that when the experimental and calculated geometries are from the same puckering class a good fit can be obtained.

2.3. Geometrical properties of the ab initio HF/STO-3G conformers

2.3.1. Bond lengths.

The bond lengths of both rings, the N14-C15 bond, as well as the distances from the S1 atom to the C9 (C_α) and C10 (C_β) atoms, in (3S, 5R, 6R)-6-acetylamidopenicillanic acid conformers resulted from the HF/STO-3G calculations are presented in Table 1 supplementary material 2.

Table1. *Correspondence between the starting PM3, AM1 and ω conformers and the resulted ab initio HF/STO-3G ones, their O17=C15-N14-H28 dihedral values* (*anti-syn* conformation), and lowest vibration energy.

Conformer ID	E_{tot}(HF/STO-3G) (kcal/mol)	Starting geometry AM1, PM3 and ω-PM3	O17=C15-N14-H28 dihedral (°)	*anti/syn* conformation	ν_0 vibration (cm^{-1})
01	-739367.228	01am1;04pm3;11pm3; 16ω;17ω;27ω;28ω;35ω;	151.848	*anti*	32.87
02	-739366.932	02am1;02pm3;03pm3;08pm3; 09ω;10ω;29ω;30ω;37ω;	151.846	*anti*	33.74
03	-739366.427	19pm3;25pm3;34pm3;	-157.144	*anti*	28.10
04	-739366.133	13pm3;22pm3;32pm3;	-157.062	*anti*	24.03
05	-739365.751	05ω;06ω;13ω;24ω;26ω;	-156.024	*anti*	27.16
06	-739365.743	45pm3;	-145.453	*anti*	35.13
07	-739365.718	01ω;03ω;20ω;21ω;31ω;34ω;	153.568	*anti*	30.75
08	-739365.478	44pm3;	-145.256	*anti*	37.04
09	-739365.153	07ω;08ω;14ω;	-156.193	*anti*	29.43
10	-739365.105	02ω;04ω;36ω;38ω;	153.469	*anti*	29.94
11	-739365.036	03am1;17pm3;23pm3;24pm3;26pm3	14.699	*syn*	28.58
12	-739364.958	05am1;35pm3	151.265	*anti*	30.03
13	-739364.768	04am1;09pm3;15pm3;16pm3;21pm3	14.550	*syn*	23.71
14	-739364.577	06pm3;12pm3;20pm3	12.512	*syn*	22.77
15	-739364.397	23ω;39ω;	-156.359	*anti*	35.02
16	-739364.332	01pm3;07pm3;14pm3	-12.517	*syn*	24.27
17	-739364.303	38pm3;40pm3	150.978	*anti*	30.40
18	-739363.958	39pm3;41pm3;47pm3	154.442	*anti*	32.31
19	-739363.747	36pm3	153.420	*anti*	14.89
20	-739363.733	25ω	-156.316	*anti*	30.54
21	-739363.673	30pm3;31pm3	-161.810	*anti*	28.70
22	-739363.517	5pm3;18pm3	15.360	*syn*	36.75
23	-739363.444	06am1; 27pm3;28pm3;46pm3	-161.640	*anti*	23.70
24	-739363.331	10pm3;	15.531	*syn*	36.65
25	-739360.010	07am1;29pm3	4.568	*syn*	36.17
26	-739359.367	08am1;33pm3	4.598	*syn*	33.08
27	-739359.284	37pm3	-7.998	*syn*	35.97
28	-739358.643	42pm3;43pm3	-8.002	*syn*	34.59

Because the variation of the calculated values of the bond lengths is very large, the mean values of the ring bond lengths have been calculated and presented in Table 2. These values are different but not statistically distinct, being close (within the standard deviation limit) to the average values of all conformers.

For the S1-C2, and S1-C5 bond lengths the calculated average values are with about 0.02-0.03 Å, and respectively 0.01-0.02Å lower than the experimental ones (Table 2). One can notice a slightly trend of the *ab initio* HF/STO-3G method to equalize the lengths of the two bond lengths. This equalization is much more pronounced when *ab initio* methods with more powerful basis sets are used. Moreover these bond lengths are in disagreement with the experimental data where the average value of the S1-C2 is larger than S1-C5 bond length [15-24]. The conclusion resulted from the average values of the S1-C2, and S1-C5 bond lengths in HF/STO-3G conformers is that the STO-3G basis set can distinguish between these two bond lengths and in the same time, this basis set gives values in agreement with the experimental trend.

Table2. *Average values of the bond lengths in rings, of the S1..C9, S1..C10, N14-C15 distances and the standard deviations (SD) for all conformers, with respect to the three criteria of classification (R/S pseudochirality, anti/syn conformation and the three classes of puckering (a, b, c)) and RMS error fit (with three experimental penicillins) values**

Number of conf.	Bond lengths (Å)									Distances (Å)		RMS error fit (Å)		
	1-2	2-3	3-4	4-5	5-1	5-6	6-7	7-4	14-15	1..9	1..10	BZPEN01	DCLOXL	AMCILL
28	**1.8268**	**1.5778**	**1.4871**	**1.4962**	**1.8038**	**1.5699**	**1.5617**	**1.4611**	**1.4419**	**2.7788**	**2.7696**	**0.1268**	**0.2025**	**0.1777**
SD	±0.0022	±0.0033	±0.0038	±0.0039	±0.0023	±0.0030	±0.0035	±0.0027	±0.0058	±0.0103	±0.0068	±0.1277	±0.0923	±0.1516
(12R)	**1.8270**	**1.5780**	**1.4874**	**1.4954**	**1.8032**	**1.5601**	**1.5629**	**1.4618**	**1.4423**	**2.7771**	**2.7709**	**0.1492**	**0.2130**	**0.1519**
SD	±0.0023	±0.0037	±0.0036	±0.0037	±0.0023	±0.0022	±0.0027	±0.0033	±0.0048	±0.0106	±0.0069	±0.1263	±0.0960	±0.1531
(16S)	**1.8266**	**1.5776**	**1.4869**	**1.4967**	**1.8034**	**1.5697**	**1.5608**	**1.4606**	**1.4416**	**2.7801**	**2.7686**	**0.1100**	**0.1947**	**0.1971**
SD	±0.0022	±0.0031	±0.0040	±0.0040	±0.0023	±0.0036	±0.0039	±0.0023	±0.0067	±0.0102	±0.0067	±0.1293	±0.0919	±0.1525
(10*syn*)	**1.8264**	**1.5773**	**1.4869**	**1.4966**	**1.8035**	**1.5709**	**1.5612**	**1.4602**	**1.4477**	**2.7807**	**2.7692**	**0.1087**	**0.1898**	**0.1975**
SD	±0.0009	±0.0005	±0.0023	±0.0016	±0.0022	±0.0025	±0.0037	±0.0024	±0.0019	±0.0106	±0.0073	±0.1329	±0.0880	±0.1630
(18*anti*)	**1.8270**	**1.5780**	**1.4873**	**1.4959**	**1.8032**	**1.5694**	**1.5620**	**1.4616**	**1.4384**	**2.7778**	**2.7698**	**0.1369**	**0.2096**	**0.1667**
SD	±0.0027	±0.0041	±0.0045	±0.0047	±0.0023	±0.0032	±0.0035	±0.0029	±0.0046	±0.0103	±0.0067	±0.1266	±0.0964	±0.1486
(12a)	**1.8263**	**1.5773**	**1.4857**	**1.4966**	**1.8050**	**1.5699**	**1.5609**	**1.4615**	**1.4448**	**2.7890**	**2.7638**	**0.0101**	**0.1245**	**0.3242**
SD	±0.0011	±0.0006	±0.0016	±0.0025	±0.0020	±0.0026	±0.0046	±0.0031	±0.0040	±0.0035	±0.0012	±0.0145	±0.0198	±0.0042
(4b)	**1.8310**	**1.5848**	**1.**4811	**1.4882**	**1.8037**	**1.5722**	**1.5644**	**1.4584**	**1.4332**	**2.7812**	**2.7644**	**0.0535**	**0.1319**	**0.2460**
SD	±0.0003	±0.0017	±0.0011	±0.0007	±0.0008	±0.0026	±0.0030	±0.0016	±0.0026	±0.0035	±0.0018	±0.0521	±0.0128	±0.0143
(12c)	**1.8256**	**1.5759**	**1.4906**	**1.4983**	**1.8015**	**1.5691**	**1.5617**	**1.4616**	**1.4419**	**2.7679**	**2.7771**	**0.2680**	**0.3042**	**0.0085**
SD	±0.0006	±0.0019	±0.0019	±0.0013	±0.0013	±0.0033	±0.0023	±0.0023	±0.0054	±0.0014	±0.0019	±0.0130	±0.0247	±0.0008
Average**	***1.8532***	***1.5686***	***1.4512***	***1.4721***	***1.8127***	***1.5564***	***1.5416***	***1.3932***	***1.3453***	***2.7691***	***2.7572***	-	-	-
SD**	***0.0119***	***0.0126***	***0.0147***	***0.0186***	***0.0197***	***0.0121***	***0.0128***	***0.0237***	***0.0264***	***0.0339***	***0.0224***	-	-	-

** BZPEN01=PENICILLIN G (class (a) of puckering, S pseudochirality); DCLOXL = DICLOXACILLIN (class (a) of puckering, R pseudochirality); AMCILL = AMPC = AMPICILLIN (class (c) of puckering, without pseudochirality) ** The average values and the standard deviations calculated from experimental data*

Because the variation of the calculated values of the bond lengths is very large, the mean values of the ring bond lengths have been calculated and presented in Table 2. These values are different but not statistically distinct, being close (within the standard deviation limit) to the average values of all conformers.

For the S1-C2, and S1-C5 bond lengths the calculated average values are with about 0.02-0.03 Å, and respectively 0.01-0.02Å lower than the experimental ones (Table 2). One can notice a slightly trend of the *ab initio* HF/STO-3G method to equalize the lengths of the two bond lengths. This equalization is much more pronounced when *ab initio* methods with more powerful basis sets are used. Moreover these bond lengths are in disagreement with the experimental data where the average value of the S1-C2 is larger than S1-C5 bond length [15-24]. The conclusion resulted from the average values of the S1-C2, and S1-C5 bond lengths in HF/STO-3G conformers is that the STO-3G basis set can distinguish between these two bond lengths and in the same time, this basis set gives values in agreement with the experimental trend.

The average value of the C3-N4 bond length is with about 0.04 Å larger than the experimental one. In case of N4-C5, C6-C7 bond lengths and S1..C10 distance the average value is with about 0.02 Å larger than the experimental one (Table 2). For C2-C3, C5-C6 bond lengths and S1..C9 distance the calculated average values are almost equal to the experimental ones, and in a good agreement with the *ab initio* HF and DFT (HF/6-31G* and B3LYP/6-31G**) methods [15-24].

In order to verify the Woodward theory regarding the amide resonance [3a] and the role played by the β-lactamic ring on the biological activity of penicillins, we compared the calculated and experimental average values of the N4-C7 and N14-C15 amido bond lengths for all the 28 conformers. According to Woodward the N4-C7 bond length should be shorter than the exocyclic amidic bond. It was found that the N4-C7 amido bond of the β-lactamic ring is longer with almost to 0.02Å than the exocyclic N14-C15 amido bond, (Table 2). The same order is also found in experimental values (Table 1 supplementary material 1), where the difference between these bond lengths is of 0.05 Å. In comparison with the PM3 method, in the HF/STO-3G method no reversal of the length of these two amido N4-C7 and N14-C15 bonds was found for any conformer [34].

For *ab initio* HF/STO-3G conformers from the classes (a) and (b) of the thiazolidinic ring puckering it was concluded that the average value of the S1..C9 distance is larger than the S1..C10 one (Table 2), while for the conformers from class (c) of puckering the S1..C9 distance is shorter than the S1.C10 one. This reversal order of distances in conformers from the class (c) of puckering was not remarked by any author who performed quantum chemical calculations. The reversed values of the S1..C9 and S1..C10 distances in conformers of class (c) show that these conformers can not compete with the

conformers from the classes (a) and (b) in conversion of penicillins into cephalosporins [51-56]. The same inversion was also observed in case of conformational analysis performed by the AM1 and PM3 semiempirical MO methods [34]. Taking into accout these distances one can suppose that in mechanism of the penicillin conversion into cephalosporin, especially the conformers from the classes (a) and (b) of puckering are favored.

2.3.2. Bond angles

The calculated ring bond angles, and the tilting angle between the β-lactamic and thiazolidinic rings (S1-C5-C6 and C3-N4-C7 planes) are presented in Table 2 Supplementary material 2. The calculated data reveals a large dispersion so the average values and standard deviations (SD) of the bond angles in rings are calculated and presented in Table 3.

One can see that the calculated values for the C2-S1-C5, N4-C5-C6, C5-C6-C7, C6-C7-N4 bond angles, including tilting angle for S1-C5-C6 plane are in the range of the experimental values with the exception of (c) class of puckering, in which the C2-S1-C5 angle is significantly lower than the experimental average values. The average values of the S1-C2-C3, C2-C3-N4, S1-C5-N4 bond angles are higher than the experimental ones, while the average values of the C3-N4-C5, C5-N4-C7 bond angles and tilting angle for C3-N4-C7 plane are lower than the experimental ones. Due to the large dispersion of experimental average values, these cover the calculated average values from the HF/STO-3G geometries (Table 3). The bond angle values obtained by semiempirical, ab initio HF and DFT methods by other authors are comparable with the values obtained in this paper (Table 2 Supplementary material 1).

A conclusion resulted from the values of the bond angles in rings and the tilting angles between the planes of the two rings is that these values are influenced by the thiazolidinic ring puckering (lowest rows on gray background in Table 3).

2.3.3. Dihedral angles

The improper dihedral angles measuring the pseudochirality at the N14 atom, the puckering of the β-lactamic ring, as well as the dihedral angles measuring the puckering of the thiazolidinic ring for all the 28 HF/STO-3G conformers are presented in Table 4.

It can be seen in Table 4 that 12 of the conformers have R pseudochirality and negative conicity at the N14 atom, while 16 conformers have S pseudochirality and positive conicity. Comparing the calculated conicity values (Table 4) with the experimental ones (Table 3 Supplementary material 1), it results that the calculated values are higher than the experimental ones.

Table 3*. Average values of the bond angles and the standard deviations (SD) for all 28 conformers, for R/S pseudochirality of the N14 atom, for anti/syn conformation of the exocyclic amidic group and for the three (a, b, c) puckering classes of the thiazolidinic ring.*

Calculation criterion	Average values and standard deviations (º)										
	2-1-5	1-2-3	2-3-4	3-4-5	1-5-4	5-4-7	4-5-6	5-6-7	6-7-4	1-5-6	3-4-7
(28*)	**93.390**	**106.176**	**108.402**	**113.674**	**108.852**	**91.884**	**89.479**	**85.523**	**91.097**	**118.650**	**120.037**
(SD)	±1.672	±0.888	±0.678	±0.561	±0.660	±0.467	±0.391	±0.125	±0.436	±0.922	±1.279
(12R*)	**93.182**	**106.087**	**108.360**	**113.658**	**108.775**	**91.794**	**89.542**	**85.525**	**91.061**	**118.286**	**119.928**
(SD)	±1.759	±0.914	±0.679	±0.531	±0.655	±0.607	±0.327	±0.148	±0.391	±0.792	±1.321
(16S*)	**93.182**	**106.244**	**108.433**	**113.687**	**108.909**	**91.961**	**89.433**	**85.521**	**91.118**	**118.923**	**120.119**
(SD)	±1.664	±0.892	±0.698	±0.599	±0.679	±0.335	±0.438	±0.111	±0.479	±0.941	±1.284
(10**syn*)	**93.479**	**106.160**	**108.370**	**113.720**	**108.778**	**91.963**	**89.394**	**85.515**	**91.064**	**118.729**	**120.056**
(SD)	±1.741	±0.841	±0.680	±0.680	±0.736	±0.231	±0.177	±0.163	±0.229	±0.509	±0.550
(18**anti*)	**93.393**	**106.186**	**108.420**	**113.649**	**108.893**	**91.840**	**89.527**	**85.527**	**91.110**	**118.606**	**120.698**
(SD)	±1.681	±0.936	±0.696	±0.502	±0.632	±0.559	±0.469	±0.104	±0.523	±0.686	±1.513
(12*a)	**94.647**	**106.484**	**108.592**	**113.371**	**109.380**	**91.930**	**89.539**	**85.529**	**91.149**	**118.695**	**120.166**
(SD)	±0.203	±0.553	±0.475	±0.613	±0.122	±0.223	±0.107	±0.102	±0.350	±0.801	±0.453
(4*b)	**95.233**	**107.662**	**109.535**	**114.375**	**109.043**	**92.520**	**88.812**	**85.490**	**90.346**	**119.086**	**122.649**
(SD)	±0.058	±0.221	±0.136	±0.318	±0.036	±0.057	±0.410	±0.070	±0.097	±0.315	±0.268
(12*c)	**91.519**	**105.374**	**107.834**	**113.748**	**108.259**	**91.625**	**89.642**	**85.528**	**91.287**	**118.459**	**119.037**
(SD)	±0.204	±0.085	±0.199	±0.291	±0.593	±0.513	±0.351	±0.162	±0.308	±1.144	±0.451
Average**	***94.135***	***104.608***	***105.949***	***117.403***	***104.586***	***93.353***	***88.401***	***84.677***	***91.941***	***118.909***	***126.286***
SD**	***2.261***	***0.867***	***1.006***	***1.579***	***1.444***	***1.674***	***1.169***	***0.726***	***1.345***	***1.674***	***1.406***

** Number of conformers with R or S chirality at the N14 atom or anti or syn conformation of the exocyclic amidic group*

*** Average values and standard deviations calculated from experimental data (Table 2 Supplementary material 01)*

Table 4. *Dihedral and improper dihedral angles, N14 pseudochirality, and puckering class of the thiazolidinic ring in HF/STO-3G conformers of the (3S,5R,6R)-6-acetylamidopenicillanic acid.*

ID	Dihedral angles (°)		Improper dihedral angle (°)		Pseudo-chirality At N14	Puckering class of the tiazolidinic ring
	C5-S1-C2-C3	C5-N4-C3-C2	N4-C5-C6-C7	C6.C5.H28.N14		
01	17.526	28.075	9.900	-28.202	R	a
02	18.434	28.850	9.777	-28.087	R	a
03	16.151	28.364	11.185	24.933	S	a
04	16.984	29.024	11.007	25.045	S	a
05	-34.590	-9.414	11.072	26.141	S	c
06	21.075	26.074	8.061	31.736	S	a
07	-33.609	-9.689	10.289	-26.775	R	c
08	22.248	26.826	7.834	31.820	S	a
09	-34.952	-9.537	11.173	26.139	S	c
10	-33.624	-9.541	10.309	-26.669	R	c
11	16.881	28.329	10.641	27.230	S	a
12	-33.714	-16.667	6.724	-27.145	R	c
13	17.832	29.169	10.494	27.160	S	a
14	16.915	28.234	10.502	-28.780	R	a
15	-32.909	-17.699	5.872	23.138	S	c
16	17.895	29.232	10.438	-28.842	R	a
17	-33.588	-16.029	6.943	-27.097	R	c
18	4.135	23.777	12.777	-24.392	R	b
19	-1.319	18.440	11.194	-25.301	R	b
20	-33.380	-18.200	5.795	23.192	S	c
21	4.356	23.393	11.994	21.462	S	b
22	20.299	26.730	9.030	30.090	S	a
23	3.512	22.783	11.865	21.460	S	b
24	21.739	27.549	8.700	30.027	S	a
25	-34.028	-9.588	11.285	26.573	S	c
26	-33.783	-9.054	11.410	26.524	S	c
27	-34.416	-9.837	11.308	-25.358	R	c
28	-35.049	-10.732	11.044	-25.385	R	c

The positive experimental (S) values are between 3.18º and 13.55º, while the calculated ones are between 21.460º and 31.820º. The negative experimental (R) values are between -6.58º and -3.42º while the calculated ones are between -28.842º and -24.392º (Table 4). The average value of the conicity for the R pseudochirality is -26.836 ±1.479º, and for S pseudochirality is 26.417±3.264º. From the comparison of the calculated versus experimental data one can conclude that the *ab initio* HF/STO-3G method leads to a more stronger calculated pyramidalization of the N14 atom compared to the experimental one.

2.4. Anti/syn rotation profiles.

Of 28 HF/STO-3G conformers in Table 1, 18 have anti and 10 syn conformation of the exocyclic amido group. The absolute values of the dihedral angles were taken into consideration such as to can compare the average value with the one of the individual conformers. The absolute average value of the O17=C15-N14-H28 dihedral angle for the anti conformation is of 154.115±4.437º, while for the syn conformation it is of 12.716±5.983º.

The global minimum conformer has an anti conformation, similarly to the experimental determined structure of conformers (Table 3 Supplementary material 1). The anti HF/STO-3G conformers are dominant. They are the first 10 from the total number of 18 anti conformers with the lowest energy (Table 1). From the 10 syn conformers, five of them occupy the last positions having the highest energies (Table 1).

To evaluate the energy variation to the rotation of the N14-C15 bond, which allows the interchange anti/syn and syn/anti conformations, two 1D profiles of the HF/STO-3G total energy were ploted for the global minimum conformer 01, and the highest energy conformer 28 (Fig. 2.3). HF/STO-3G single point calculations were performed for conformers obtained through the rotation of the O17=C15-N14-H28 dihedral angle between 0 and 360º with a 10º step.

The total energy profile of the anti conformer 01 shows a high local minimum around the dihedral angle value of 220º-230º. This minimum has an energy with 6.718 kcal/mol higher than the energy of the 01 conformer.

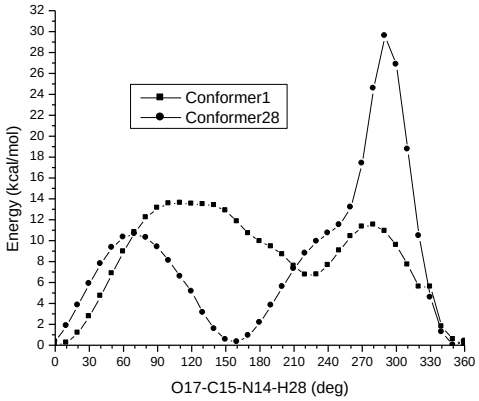


Fig2.3. *Total energy profiles for the internal rotation of the N14-C15 bond in amido groups resulted from the global minimum conformer (number 01) and the higest energy conformer (number 28) obtained with the HF/STO-3G method.*

The total energy profile of the syn conformer 28 has a local minimum at 160°, which corresponds to an anti conformation. This minimum has an energy with 0.299 kcal/mol higher than the energy of the syn conformer 28. For each of the two conformers the potential energy profile presents two maxima (Fig. 2.3). For the anti conformer 01, the two maxima correspond to a HF/STO-3G energy value of 13.586 kcal/mol (around a dihedral angle value of 110°), and 11.505 kcal/mol (around a dihedral angle value of 280°) respectively.

The energy profile of the anti conformer 01 has a relatively uniform shape. On the contrary, the shape of the energy profile for the syn conformer 28 is much different. The two maxima correspond to energy values with 10.632 kcal/mol (around a of 70°), respectively 29.568 kcal/mol (around a dihedral angle value of 290º) higher than the energy of the syn conformer 28.

2.5. Rotation of the COOH group around the C3-C11 bond

The C3-C11 flexible bond, to which the COOH group is attached, can have only two positions rotated each against the other by 180o. Taking into account the rotation of this group, an ensamble of paired conformers can be possible. The conformers of these pairs can have very close geometries in which only the COOH group is rotated by 180º. The conformers of these pairs are not energetically different. The pairs of conformers resulted from the HF/STO-3G calculations with the COOH group rotated around the C3-C11 bond, the relative energy (relative E) of conformers from different pairs, and values of the C2-C3-C11-O12 dihedral angle in both conformers (conformer 1, conformer 2) of all pairs are presented in Table 5.

Each pair has the same R or S pseudochirality of the N14 atom and same anti or syn conformation of the exocyclic amido group. The -COOH group rotation leads to relative energies between 0.211 and 0.664 kcal/mol (Table 5). For R, respectively S pseudochirality and syn, respectively anti conformations, the average values of the relative energies are

(R) 0.444±0.213 kcal/mol

(S) 0.397±0.200 kcal/mol

(*anti*) 0.425±0.199 kcal/mol

(*syn*) 0.402±0.220 kcal/mol

and the average values of the C2-C3-C11-O12 dihedral angle in both members of all pairs are:

	conformer 1	conformer 2
(R)	92.769±22.679º,	respectively -87.698±3.235º
(S)	98.087±21.762º,	respectively -86.578±2.185º
(*anti*)	94.909±21.548º,	respectively -87.658±2.938º
(*syn*)	97.425±23.712º,	respectively -85.977±1.726º

The above mentioned average values are different, but due to their large dispersion they can be considered statistically almost equal. Relative rotation energy of the COOH group in pairs of conformers is much smaller than the energy for the *anti*/*syn* rotation. These energies are about 1.0, and 3.0 kcal/mol, respectively (Figure 4).

Table5. *The pairs of the HF/STO-3G conformers with the COOH group rotated around the C3-C11 bond, the relative rotation energy (relative E) of conformers in different pairs, and the values of the C2-C3-C11-O12 dihedral angle measuring the COOH group rotation.*

Pair of conformers	Relative E (kcal/mol)	C2-C3-C11-O12 dihedral angle (º)*	
		Conformer1	Conformer2
01(R) – 02(S) (*anti*)	0.298	115.792 (01)	-84.094 (02)
03(R) – 04(S) (*anti*)	0.294	114.785 (03)	-84.675 (04)
05(S) – 09(R) (*anti*)	0.598	71.984 (05)	-87.321 (09)
06(S) – 08(R) (*anti*)	0.265	114.618 (06)	-84.786 (08)
07(S) – 10(S) (*anti*)	0.613	72.481 (07)	-87.112 (10)
11(R) – 14(R) (*anti*)	0.268	114.498 (11)	-84.981 (14)
12(R) – 17(S) (*syn*)	0.655	72.685 (12)	-88.700 (17)
13(R) – 16(R) (*syn*)	0.245	114.474 (13)	-84.888 (16)
15(R) – 20(S) (*syn*)	0.664	72.041 (15)	-89.107 (20)
18(R) – 19(S) (*syn*)	0.211	109.905 (18)	-93.159 (19)
21(R) – 24(S) (*syn*)	0.229	109.892 (21)	-89.972 (24)
22(S) – 23(S) (*anti*)	0.213	115.249 (22)	-84.370 (23)
25(S) – 26(S) (*anti*)	0.643	71.628 (25)	-87.410 (26)
27(S) – 28(S) (*syn*)	0.641	71.277 (27)	-88.235 (28)

** in paranthesis is given the conformer ID on which the measuring was made*

2.6. Puckering of the β-lactamic ring.

The four-membered β-lactamic ring is a strained ring, with a certain puckering as resulted from the experimental data (Table 3 Supplementary material 1 and Figure 2). As in case of AM1 and PM3 calculations [33-35], the *ab initio* HF/STO-3G calculations reveal a different puckering of the β-lactamic ring for each conformer (Table 4 and Fig. 5).

The average calculated value of the dihedral angle N4-C5-C6-C7, which measures the puckering of the β-lactamic ring, is of 9.951±1.877º and is statistically comparable with the experimental average value of 8.123±3.431º. The experimental average value was calculated without taking into account the two experimental negative puckering values (Table 3 Supplementary material 1). For the (3S,5R,6R)-6-acetylamidopenicillanic acid no negative values were obtained. Following average values were obtained for conformers with R, respectively S pseudochirality and *anti*, respectively *syn* conformation:

(R) 10.100 ± 1.721^{o}

(S) 9.838 ± 2.034^{o}

(*anti*) 9.654 ± 2.208^{o}

(*syn*) 10.485 ± 0.932^{o}

Even if the average values of the R/S pseudochirality are slightly different, because of their large dispersions they are not statistically distinct. The same conclsion resulted for the anti/syn conformations.

2.7. Puckering of the thiazolidinic ring.

The N4, C5 and C6 atoms of the β-lactamic ring were used as template for the superposition of all the 28 conformers obtained by the ab initio HF/STO-3G method. The resulted hyperstructure is given in Fig. 2.4. and 2.5. From the superposition presented in Fig. 2.4 one can observe the existence of three puckering classes of the thiazolidinic ring. This puckering is reflected, also, in the conformation of the -COOH group. The statistical analysis indicates the puckering classes as being defined by two dihedral angles: C5-S1-C2-C3 and C5-N4-C3-C2, as follows:

- Class (a) contains 12 HF/STO-3G conformers: 01, 02, 03, 04, 06, 08, 11, 13, 14, 16, 22, 24. In this class four conformers have R pseudochirality and eight S pseudochirality, six conformers have anti and six syn conformation. The C5-S1-C2-C3 dihedral angle varies between 16.151º and 22.248º, and the C5-N4-C3-C2 dihedral angle between 26.074º and 29.232º (Fig. 2.5.(a)). The relative rotation energies of the COOH group in conformers from the (a) puckering classs are in the range between 0.213 kcal/mol and 0.298 kcal/mol (Table 5).

- Class (b) contains 4 HF/STO-3G conformers, namely: 18, 19, 21, 23. In this class two conformers have R pseudochirality and two S pseudochirality, all conformers having anti conformation. The C5-S1-C2-C3 dihedral angle varies between -1.319º and 4.356º, and the C5-N4-C3-C2 dihedral angle has values between 18.440º and 23.777º (Fig. 2.5(b)). The relative rotation energies of the COOH group in conformers from the (b) puckering classs are in the range 0.211 kcal/mol and 0.229 kcal/mol (Table 5).

- Class (c) contains 12 HF/STO-3G conformers, namely: 05, 07, 09, 10, 12, 15, 17, 20, 25, 26, 27, 28. In this class six conformers have R pseudochirality and six S pseudochirality, eight conformers have anti and four syn conformation. The C5-S1-C2-C3 dihedral angle varies between -32.909º and -35.049º, the C5-N4-C3-C2 dihedral angle between -9.054º and -18.200º (Fig. 2.5.(c)). The relative rotation energies of the COOH group in conformers from the (c) puckering classs are in the range between 0.598 kcal/mol and 0.664 kcal/mol (Table 5).

By comparing the average values of the dihedral angles for the three puckering classes of the thiazolidinic ring, the classification of the conformers resulted from the HF/STO-3G calculations is obvious:

class (a): C5-S1-C2-C3 18.665±2.106º;respectively C5-N4-C3-C2 28.068±1.038º;

class (b): C5-S1-C2-C3 2.671±2.684º;respectively C5-N4-C3-C2 22.098±2.473º;

class (c): C5-S1-C2-C3 -33.970±0.653º;respectively C5-N4-C3-C2 -12.166±3.736º

One can notice that for the conformers from the class (b) of puckering, the C5-S1-C2-C3 dihedral angle has an average value which is smaller than the standard deviation.

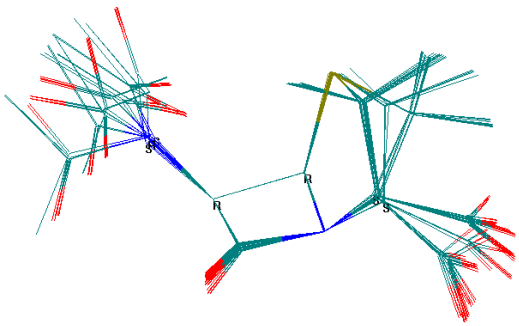
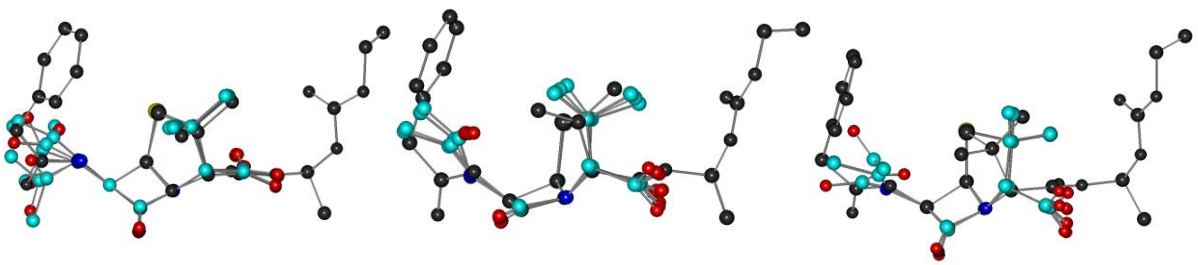


Fig2.4. *Superposition of 28 HF/STO-3G conformers of the (3S,5R,6R)-6-acetilamidopenicillanic acid (hydrogen atoms are omitted for clarity).*

The calculation of average values of the dihedral angles considering anti/syn conformation of the exocyclic amido group, and R/S pseudochiraly of the N14 atom does not make any sense because negative values are mixed with positive ones. As shown by the data presented in Tables 4, 5 and Fig. 2.4 the puckering of thiazolidinic ring influences the orientation of the C3-C11 bond and of the COOH adjacent group.

From Fig2.5. it can be seen that the experimental geometry of the BENPEN10 penicillin [49] is the best superposed on the conformers from the class (a) of puckering.



class a class b class c

Fig2.5. *Superpozition of the optimized HF/STO-3G conformers of the (3S,5R,6R)-6-acetilamidopenicillanic acid over the heavy atoms of the experimental geometry of BENPEN10 [49] (marked with black spheres) and the three puckering (a, b, c) classes.*

Taking into account the β-lactamic ring puckering with respect to the thiazolidinic ring puckering (see previous β-lactamic ring puckering discussions) together with the C7=O8 bond orientation, one can conclude that these two types of puckering are interrelated, as resulting from the large dispersions of the average values of two dihedral angles which define puckering classes.

The average values of the N4-C5-C6-C7 dihedral angle of the β-lactamic ring puckering (Table 4) are:

$$\text{N4-C5-C6-C7 (a)} = 9.768 \pm 1.057^{o}$$

$$\text{N4-C5-C6-C7 (b)} = 11.367 \pm 1.436^{o}$$

$$\text{N4-C5-C6-C7 (c)} = 9.786 \pm 2.291^{o}$$

The thiazolidinic ring puckering could have an influence over the β-lactamic ring puckering.

3. COMPUTATIONAL DETAILS

All the quantum chemical calculations were performed with the HyperChem 7.52 software. Eight AM1, and 47 PM3 conformers, previously obtained [33-36] were reoptimized using an ab initio HF hamiltonian with a STO-3G basis set for closed shell systems [50,58]. The SCF criterion was 10^{-8}, with a maximum number of SCF iterations of 150 [50]. For the initial calculation of the bicentric integrals the projected INDO method was used. For the bicentric integral calculation Raffenetti method was used, and a limit of precision of 10^{-10}, taking into consideration six d-orbitals. For the optimisation of the geometry, the Polak-Ribiere conjugate gradient algorithm was used, and a RMS gradient equal or less than 0.01 kcal/Åmol.

Along with the conformational analysis using the Conformational Search module of HyperChem 7.52 in this paper we have also used data obtained with a public program, i.e. the Omega v.2.3.2 (noted ω), which is based on the MMFF94 force field (Merck Molecular Force Field version 94) [57]. On using this program, the following working parameters were used: a maximum number of 200 conformers, an energy range above the minimum energy conformer of 10 kcal/mol, and a RMSD gradient of 0.5 kcal/Å·mol. Using these parameters all of the 8 chirality classes resulted [2], with the specification that for class 3S,5R,6R, 39 conformers were obtained. The geometries of these 39 conformers obtained with the ω program were optimized using the PM3 method and reoptimized at the ab initio HF/STO-3G level.

To find some functional dependencies between certain structural properties, namely: bond lengths, bond angles, dihedral angles and improper dihedral angles the data were statistically analyzed using different selection criteria. For this purpose the STATISTICA 5.0 software [59] was used.

4. CONCLUSION

Three criteria for clasfication of the geometry of the (3S,5R,6R)-6-acetylamidopenicillanic acid conformers were established: the R/S pseudochirality at the exocyclic amidic nitrogen N14 atom, the anti/syn conformation of the exocyclic amido group and three classes of puckering denoted by (a), (b) and (c). Statistical analysis was used to evidence some regularities and influences.

From the analysis of experimental geometries for 16 penicillins was established that: eight of them had R/S pseudochirality at the exocyclic amidic nitrogen atom, and eight had planar exocyclic amidic nitrogen. For all of experimental geometries the exocyclic amido group had anti conformation. Three classes of thiazolidinic ring puckering could be highlighted.

By optimizing the geometry of 47 PM3, and 8 AM1 conformers using the ab initio HF/STO-3G method 22 conformers resulted. By optimizing the geometry of 39 ω→PM3→STO-3G conformers 6 geometrically distinct conformers were obtained. Totally 28 HF/STO-3G conformers were obtained.

Between the lowest energy 01 and the highest energy 28 conformer there is a difference of 8.585 kcal/mol. Among the 28 HF/STO-3G conformers: 12 conformers have R pseudochirality and 16 have S pseudochirality; 18 conformers have anti and 10 syn conformation, 12 conformers are from (a) class of puckering, 4 from (b) class of puckering and 12 from (c) class of puckering.

The relative energy of the COOH group rotation around the C3-C11 bond are in the range 0.211 - 0.655 kcal/mol. It was demonstrated that neither anti nor syn exocyclic amido groups are flat. The anti structure is rotated with almost 30º with respect to the standard value of 180º, while the syn strucure is rotated with almost 13º with respect to the standard value of 0º.

It was established that the puckering of the thiazolidinic ring influences the COOH group orientation.

The puckering of the β-lactamic ring is influenced by the thiazolidinic ring puckering.

The average values of bond lengths are not statistically distinct. Therefore comparison of the calculated with the experimental bond lengths is based on the average values for all conformers. It should be noted that for the conformers from (a) and (b) classes of puckering the distance S1..C9 > S1..C10, while for the conformers from (c) class of puckering the distance S1..C9 < S1..C10. These results suggest that the geometry of conformers from the classes (a) and (b) of the thiazolidinic ring puckering favorizes this conversion.

The HF/STO-3G geometries do not differ significantly from those obtained by other authors who used basis sets of higher performance. The HF/STO-3G basis set is able to distinguish between the S1-C2 and S1-C5 bond lenghts, namely S1-C2 > S1-C5.

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