

*Dedicated to the memory of
Professor Cristofor I. Simionescu (1920–2007)*

PM3 CONFORMATIONAL ANALYSIS OF THE (3S,5R,6R)-6 ACETYLAMIDOPENICILLANIC ACID. I. GEOMETRICAL PROPERTIES

Mihaela SCHULZ, Maria MRACEC, Eugen ŞIŞU, Ludovic KURUNCZI and Mircea MRACEC*

Institute of Chemistry Timișoara of Roumanian Academy, Bd. Mihai Viteazul Nr. 24, RO-300223 Timișoara, Roumania

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A conformational analysis with the PM3 semiempirical MO method was performed for the (3S,5R,6R)-6-acetylamidopenicillanic acid. Because the N atom of the exocyclic amidic group is pyramidalized by the PM3 method, a larger number of conformers (47) than the number of theoretically possible conformers have been obtained. The conformer that fit the best with the experimental geometry was not the lowest energy conformer, but the fourth conformer in the energy ascending order. The difference between the lowest energy and highest energy conformer is 11.36 kcal/mol. The calculated average values of various bond lengths, bond angles and dihedrals helped to observe some regularities in the conformers' geometries that otherwise could not be observed. The conformers could be divided in three distinct classes of puckering of the thiazolidinic ring. Ring puckering is not influenced by the rotation of the carboxylic group.

INTRODUCTION

The (3S,5R,6R)-6-acetylamidopenicillanic acid is one of the simplest antibacterial compounds of the penicillin class. Its molecular structure, as that of all penicillins, contains two fused rings (a four-member β -lactamic ring and a five-member thiazolidinic ring) and three chiral centers,¹ marked with an asterisk in Fig. 1. These chiral centers generate $2^3=8$ diastereoisomers: 3R,5R,6R, 3S,5R,6R, 3R,5S,6R, 3R,5R,6S, 3S,5S,6R, 3S,5R,6S, 3R,5S,6S, 3S,5S,6S.² The 3S,5R,6R diastereoisomer is the natural product. Its two-fused rings represent the general structure of all antibacterials forming the penicillin class.³

Regarding the action mechanism of the penicillins, the experimental data confirmed the hypothesis proposed by Strominger stating that the

D-Ala-D-Ala sequence in the general structure of penicillins is responsible for their antibacterial activity.³⁻⁸ The thick line in Fig. 1 shows the key atoms forming the D-Ala-D-Ala sequence of the penicillin backbone.

Only the molecular and electronic structures of the (3S,5R,6R)-natural diastereoisomer of penicillins have been studied extensively with different quantum-chemical methods,⁸⁻¹⁶ but in none of the studies is mentioned that the N14 nitrogen atom (Fig. 1) is pyramidalized. This effect influences the results of the quantum chemical methods applied to structures with rotatable bonds.¹⁷ This work presents a study of the influence of substituents on the geometry of the (3S,5R,6R)-6-acetylamidopenicillanic acid conformers resulted from a conformational search using the PM3 semiempirical MO method.¹⁸⁻²⁰

* Corresponding author: mracec@acad-icht.tm.edu.ro

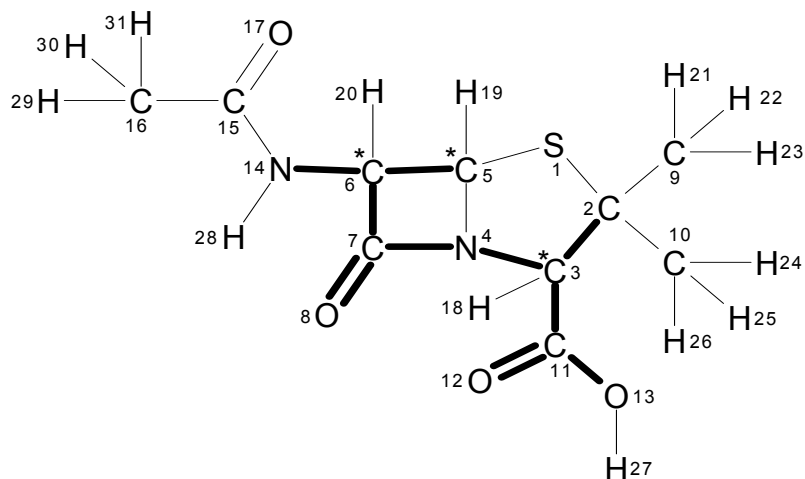


Fig. 1 – Atom numbering in the (3S,5R,6R)-6-acetylamidopenicillanic acid (* marks the three chiral atoms: C3, C5 and C6).

METHODS

The gas phase equilibrium geometry of the conformers was obtained by PM3 calculations with an SCF convergence of 10^{-5} , and a RMS gradient of 10^{-2} kcal/Å·mol.¹⁷⁻²⁰ Conformational search was performed by varying the C2-C3-C11-C12, C5-C6-N14-C15 and C6-N14-C15-C16 dihedrals in the range $0 \pm 180^\circ$ with steps of 15° . The energy criterion was set to 30 kcal/mol above the lowest energy conformer. Calculations have been performed with the Conformational Search module from the HyperChem7.52 package.^{19,20} To obtain all conformers, including the minimum energy conformer for structures with more than two flexible bonds, the conformational search is generally performed through the grid method.^{17,19,21-23} The general strategy for finding over 99% of the possible conformers was described in the precedent paper.²² Unlike the AM1 method that gave 8 conformers after ~300 starting conformers, the PM3 method gave 47 distinct conformers only after around 1000 starting conformers.^{22,23}

RESULTS AND DISCUSSION

To analyze the results given by the PM3 method, the geometry of the conformers was compared with the X-ray structure of the (2S,5R,6R)-3,3-dimethyl-7-oxo-6-phenylacetamino-4-thia-1-aza-bicyclo [3.2.0]heptane-carboxylic acid ethoxycarbonyloxyethyl ester (BENPEN10) by measuring the ring and the exocyclic bond lengths, the ring bond angles including those measuring the fused rings bendings, and some proper and

improper dihedrals.^{24,25} By selecting in the same order the corresponding atoms in the X ray geometry and those from each conformer the structures have been superposed and the RMS error fit, were calculated (Table 1, Fig. 2).

As other compounds containing amidic groups, the amidic nitrogen atom in the penicillanic acid is also pyramidalized by the PM3 method.^{23,26} This generates a pseudochiral configuration for the N14 atom in the (3S,5R,6R)-6-acetylamidopenicillanic acid. The C6-C15-H28-N14 improper dihedral measures the “conicity” of the out-of-plane substituents at the N14 nitrogen atom. This can be considered a measure of the pyramidalization of the N14 atom.

Although the most stable form of the amido group is the *anti* form, the conformer with the lowest energy resulted from PM3 calculations is *syn*. The numbering of the conformers, their ranking in increasing order of the formation enthalpies and some geometric data are presented in Table 1. As it can be seen in Fig. 2 and Table 1 the PM3 method leads to 47 distinct conformers. Between the formation enthalpy of the lowest energy conformer (01pm3) and the highest energy conformer (47pm3) is a difference of 11.356 kcal/mol. The calculated geometry data is dispersed and does not show any correlation. To find some rules the average values and their standard deviations have been calculated for the main angles and bond lengths of the rings and of the exocyclic distances between the sulphur atom and the 9 and 10 methylic carbon atoms (Fig. 1), taking into account all conformers, the pseudochiral structures, the *syn* or *anti* arrangements of the O17 and H28 atoms, as well as the three observed puckering classes (Table 1).²³

Table 1

Some geometric features of the conformers resulted from PM3 calculations for the (3*S*,5*R*,6*R*)-6-acetylamidopenicillanic acid arranged by increasing order of the calculated formation enthalpies

No	ΔH_{form} (kcal/mol)	Dihedral angles ($^{\circ}$)		Conicity ($^{\circ}$)		Pseudo- chirality of N14	Position of O17, H28	RMS Error Fit (\AA)
		5-1-2-3	5-2-3-4	6-15-28-14	5-2-3-4			
01 pm3	-143.995	10.932	19.077	-23.774	R	Syn	0.874	
02 pm3	-143.978	10.189	19.385	-24.677	R	Anti	0.903	
03 pm3	-143.977	10.144	19.362	-24.652	R	Anti	0.308	
04 pm3	-143.501	9.722	18.533	-24.639	R	Anti	0.290	
05 pm3	-143.501	9.843	18.575	-24.622	R	Anti	0.797	
06 pm3	-143.430	10.541	18.192	-23.755	R	Syn	1.139	
07 pm3	-143.368	17.897	17.557	-22.953	R	Syn	0.890	
08 pm3	-143.363	17.565	18.422	-24.332	R	Anti	0.401	
09 pm3	-143.117	10.719	19.386	20.191	S	Syn	1.266	
10 pm3	-143.116	10.581	19.332	20.173	S	Syn	0.936	
11 pm3	-142.921	17.291	17.623	-24.325	R	Anti	0.839	
12 pm3	-142.831	14.561	16.733	-22.986	R	Syn	1.153	
13 pm3	-142.756	9.473	19.575	20.755	S	Anti	0.453	
14 pm3	-142.746	18.062	17.942	-22.950	R	Syn	0.402	
15 pm3	-142.718	10.251	19.268	21.318	S	Syn	0.970	
16 pm3	-142.712	18.742	18.294	21.759	S	Syn	0.934	
17 pm3	-142.564	10.099	18.505	20.207	S	Syn	1.181	
18 pm3	-142.563	10.190	18.493	20.302	S	Syn	1.179	
19 pm3	-142.289	9.331	18.924	20.770	S	Anti	0.846	
20 pm3	-142.200	17.694	17.047	-22.904	R	Syn	1.153	
21 pm3	-142.194	18.435	18.016	22.460	S	Syn	1.283	
22 pm3	-142.192	18.527	18.875	21.507	S	Anti	0.493	
23 pm3	-142.176	18.391	17.451	21.750	S	Syn	1.175	
24 pm3	-142.165	9.884	18.434	21.348	S	Syn	1.204	
25 pm3	-141.747	18.246	18.130	21.389	S	Anti	0.868	
26 pm3	-141.661	18.120	17.188	22.403	S	Syn	1.465	
27 pm3	-139.455	-1.763	8.145	22.809	S	Anti	1.099	
28 pm3	-139.384	8.856	15.161	22.342	S	Anti	1.089	
29 pm3	-139.008	-1.139	12.549	21.734	S	Syn	1.106	
30 pm3	-138.880	-2.207	7.163	22.848	S	Anti	1.296	
31 pm3	-138.802	8.673	14.511	22.293	S	Anti	1.291	
32 pm3	-138.765	15.583	18.654	29.490	S	Anti	0.854	
33 pm3	-138.495	-1.089	12.062	21.757	S	Syn	1.306	
34 pm3	-138.285	15.143	17.676	29.453	S	Anti	1.100	
35 pm3	-138.209	-2.630	6.600	-19.998	R	Anti	1.117	
36 pm3	-138.157	9.372	14.393	-19.033	R	Anti	1.096	
37 pm3	-137.962	-7.231	7.551	-20.011	R	Syn	1.069	
38 pm3	-137.641	-3.032	5.640	-20.115	R	Anti	1.309	
39 pm3	-137.640	-2.874	5.860	-20.134	R	Anti	1.307	

Table 1 (continued)

40 pm3	-137.638	-2.961	5.718	-20.123	R	<i>Anti</i>	1.308
41 pm3	-137.597	9.193	13.824	-19.198	R	<i>Anti</i>	1.294
42 pm3	-137.450	-7.334	7.030	-19.976	R	<i>Syn</i>	1.266
43 pm3	-136.642	-19.035	-17.102	-21.566	R	<i>Syn</i>	1.533
44 pm3	-135.386	21.122	15.277	30.267	S	<i>Anti</i>	0.733
45 pm3	-134.953	20.824	14.614	30.206	S	<i>Syn</i>	1.037
46 pm3	-133.185	12.550	18.578	-30.570	R	<i>Syn</i>	1.036
47 pm3	-132.639	12.356	17.848	-30.499	R	<i>Syn</i>	1.253

In Table 2 are presented the average values of the bond angles of the two cycles (β -lactamic and thiazolidinic) and the conicity for the β -lactamic cycle (measured as 4-5-6-7) calculated for all conformers (47), for conformers with (R) (23), respectively (S) (24) pseudo-chirality at the N14 atom, for the conformers with *syn* (24), respectively *anti* (23) arrangement of the H28-N14 atoms and for the three puckering classes of the thiazolidinic cycle: class a (20 conformers), class b (16 conformers) and class c (11 conformers), as well as the standard deviations, compared to the experimental data for some penicilins.^{3,24,25,27-30} The bond lengths of the cycles and the average distances between the sulphur atom and the C9 and C10, compared to some experimental values are presented in Table 3. The notation codes of the penicilins are according to the CSD (Cambridge Structural Database)²⁵ notations. The primary

geometric data obtained through the PM3 semiempirical method from which average values were calculated are available as supplementary material.

Dihedral and improper dihedral angles

Following the data in Table 1, one can see that 23 conformers have (R) pseudo-chirality and negative conicity at the N14 atom (improper dihedral C5-C15-H28-N14), while 24 conformers have (S) pseudo-chirality and positive conicity. The negative conicity has an average value of $-22.948 \pm 3.113^\circ$. The positive conicity has an average value of $22.90 \pm 3.28^\circ$. Regarding the *syn-anti* arrangement of the O17 and H28 atoms, from the 47 conformers 24 are *syn* and 23 are *anti*.

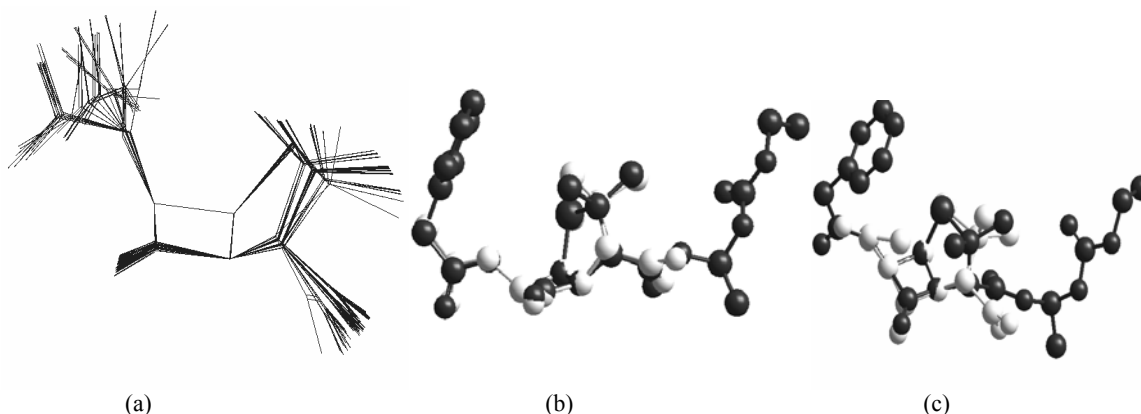


Fig. 2 – a) Superposition of the 47 conformers given by the PM3 method; b) and c) superposition of heavy atoms of the experimental geometry (black spheres)²³ over the heavy atoms of the PM3 conformers (white spheres) with the best concordance (lowest RMS error fit) and with the worst concordance (highest RMS error fit).

The structures with very close formation enthalpies and similar geometries form groups of two, three or four conformers. The energy differences between the valleys of potential energy on rotating the N14-C15 bond have values that vary between the minimum 1.0 kcal/mol and maximum 3.0 kcal/mol. On rotating the COOH function around the C3-C11 bond the energy differences have values between 0.2 kcal/mol and 0.5 kcal/mol.

The N4, C5 and C6 atoms of the β -lactamic ring were used as a base for the superposition of all distinct conformers given by PM3 method. The resulted hyperstructure is given in Fig. 2(a).

From the superposition presented in Fig. 2 one can observe the existence of three puckering classes of the thiazolidinic ring. The puckering of

this ring was defined by two dihedral angles: 5-1-2-3 and 5-2-3-4. The average values of these dihedral angles are:

$$\begin{aligned} 5-1-2-3(\textit{syn}) &= 9.792 \pm 10.020 \\ 5-2-3-4(\textit{syn}) &= 15.167 \pm 7.681 \\ 5-1-2-3(\textit{anti}) &= 8.818 \pm 7.850 \\ 5-2-3-4(\textit{anti}) &= 14.610 \pm 5.216 \\ 5-1-2-3(\textit{R}) &= 7.079 \pm 10.089 \\ 5-2-3-4(\textit{R}) &= 13.234 \pm 8.420 \\ 5-1-2-3(\textit{S}) &= 11.458 \pm 7.257 \\ 5-2-3-4(\textit{S}) &= 16.487 \pm 3.460 \end{aligned}$$

The average and SD values demonstrate that the puckering of the thiazolidinic ring is independent of the (R or S) pseudochirality, the *syn-anti* arrangement of the O17 and H28 atoms, or the rotation of the COOH group.

The conformers belonging to the three puckering classes (Fig. 3) are as follows:

Class (a) contains 20 conformers: 10 with a (R) and 10 with a (S) pseudo-chiral N14 atom. In this class 10 conformers have *anti* and 10 conformers have *syn* disposal arrangement of the O17 and H28

amidic atoms. The dihedral angle 5-1-2-3 varies between 8.673° and 12.550°. The dihedral angle 5-4-3-2 varies between 13.824° and 19.575°. The geometries' dispersion can be observed in Fig. 3 (a).

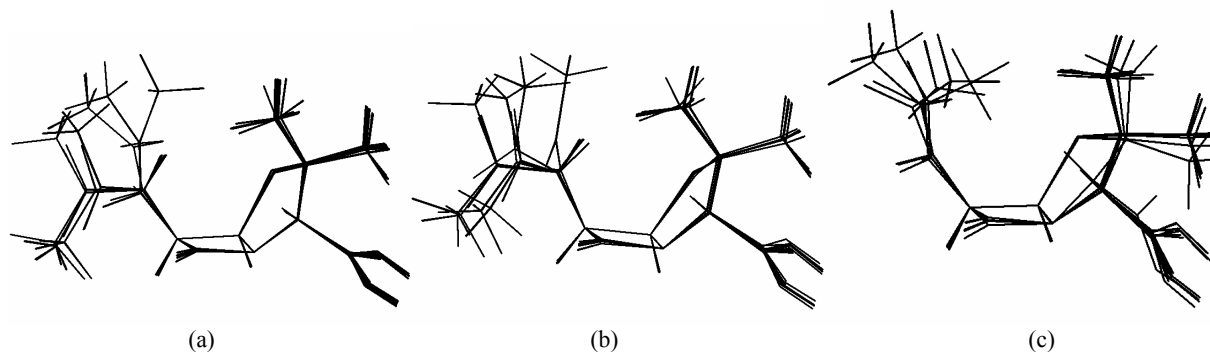


Fig. 3 – The three puckering classes of the thiazolidinic ring with the dispersion specific to each class, for the conformers resulted from PM3 calculations.

Class (b) contains 16 conformers: 6 with (R) pseudo-chirality and 10 with (S) pseudo-chirality at the N14 atom. Of the 16 conformers 7 have *anti*, while 9 have *syn* arrangement of the O17 and H28 amidic atoms. In this class the dihedral angle 5-1-2-3 varies between 14.561° and 21.122°. The dihedral angle 5-4-3-2 varies between 14.614° and 18.742°. The geometries' dispersion can be observed in Fig. 3 (b).

Class (c) contains 11 conformers: 7 with a (R) pseudo-chiral N14 atom and 4 with a (S)

$$5-1-2-3(a) = 10.145 \pm 0.994;$$

$$5-1-2-3(b) = 17.888 \pm 1.740;$$

$$5-1-2-3(c) = -3.226 \pm 2.251;$$

pseudo-chiral N14 atom. Of the 11 conformers 6 have *anti* and 5 have *syn* arrangement of the O17 and H28 amidic atoms. The 5-1-2-3 dihedral angle varies between -1.089° and -7.334°. The 5-4-3-2 dihedral angle varies between 5.640° and 12.549°. The geometries' dispersion can be observed in Fig. 3 (c).

By comparing the average values of the dihedral angles for the three puckering classes of the thiazolidinic ring:

$$\text{respectively } 5-4-3-2(a) = 17.968 \pm 1.863;$$

$$\text{respectively } 5-4-3-2(b) = 17.469 \pm 1.148;$$

$$\text{respectively } 5-4-3-2(c) = 7.832 \pm 2.496$$

This classification of the conformers resulted from PM3 calculation is evident.

By dividing the conformers in four groups (R,*anti*) – (R,*syn*) and (S,*anti*) – (S,*syn*), (Fig. 4) the three puckering groups are maintained.

$$5-1-2-3(a, \textit{syn}) = 10.810 \pm 0.920$$

$$5-1-2-3(a, \textit{anti}) = 9.480 \pm 0.505$$

$$5-1-2-3(b, \textit{syn}) = 18.081 \pm 1.611$$

$$5-1-2-3(b, \textit{anti}) = 17.640 \pm 1.995$$

$$5-1-2-3(c, \textit{syn}) = -4.198 \pm 3.562$$

$$5-1-2-3(c, \textit{anti}) = -2.578 \pm 0.449$$

$$5-1-2-3(a, R) = 10.484 \pm 1.518$$

$$5-1-2-3(a, S) = 9.806 \pm 0.699$$

$$5-1-2-3(b, R) = 17.178 \pm 1.310$$

$$5-2-3-4(a, \textit{syn}) = 18.711 \pm 0.525$$

$$5-2-3-4(a, \textit{anti}) = 17.224 \pm 2.413$$

$$5-2-3-4(b, \textit{syn}) = 17.205 \pm 1.092$$

$$5-2-3-4(b, \textit{anti}) = 17.808 \pm 1.211$$

$$5-2-3-4(c, \textit{syn}) = 9.798 \pm 2.910$$

$$5-2-3-4(c, \textit{anti}) = 6.521 \pm 0.991$$

$$5-2-3-4(a, R) = 17.777 \pm 1.997$$

$$5-2-3-4(a, S) = 18.159 \pm 1.804$$

$$5-2-3-4(b, R) = 17.554 \pm 0.607$$

The average values of the dihedral angles for the three puckering classes (a), (b) and (c), grouped by their *syn-anti* arrangement and by the (R-S) pseudo-chirality at the N14 atom, are as follows:

$$5-1-2-3(b,S) = 18.313 \pm 1.885$$

$$5-1-2-3(c,R) = -4.344 \pm 2.281$$

$$5-1-2-3(c,S) = -1.550 \pm 0.535$$

$$5-2-3-4(b,S) = 17.418 \pm 1.408$$

$$5-2-3-4(c,R) = 6.400 \pm 0.787$$

$$5-2-3-4(c,S) = 9.980 \pm 2.723$$

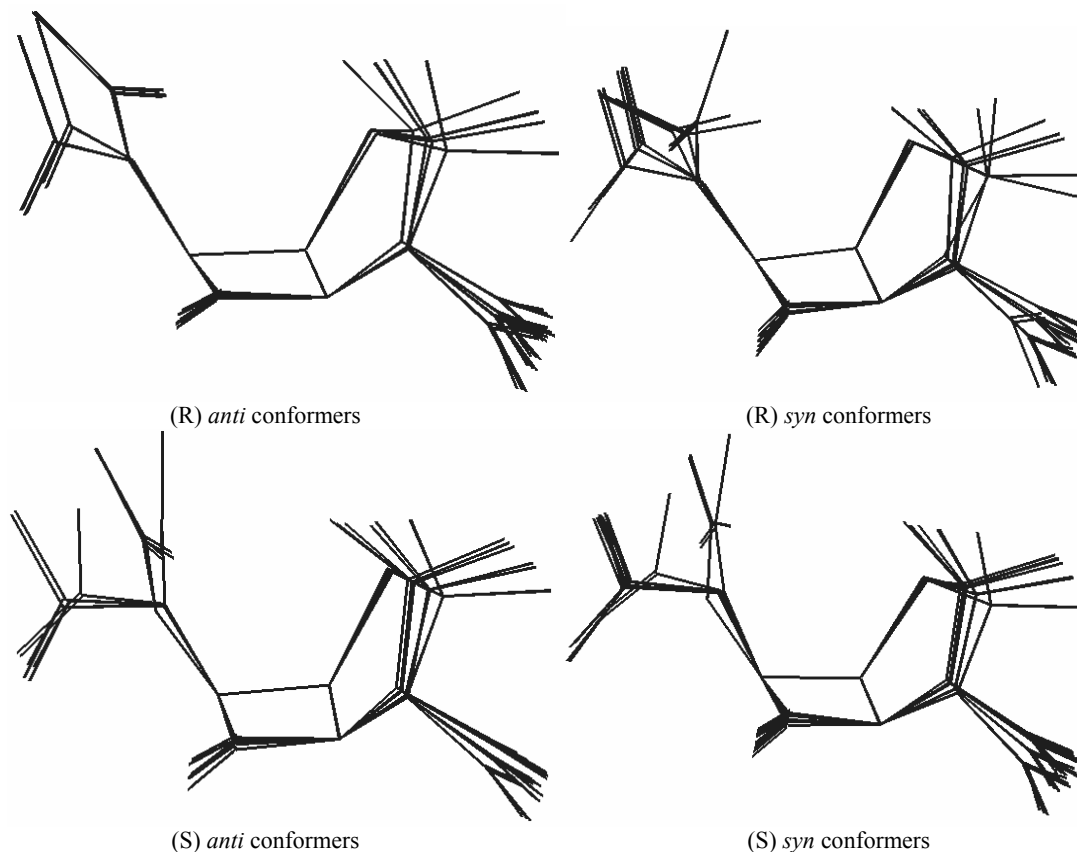


Fig. 4 – The four conformers groups resulted from PM3 calculations, with pseudochirality (R) and (S) at the N14 atom and with atoms' 17 and 28 arranged *anti*, or *syn*, respectively.

Analysing the average values one can observe that the dihedral angles for the *syn* arrangement are generally greater than those of the *anti* arrangement, while for (R) pseudochirality the average values are greater than of the (S) pseudochirality. However, this rule is not valid for both angles in all puckering classes. The average values of the two dihedrals suggest that the ring puckering classes are independent of the N14 atom pseudochirality. The puckering of the thiazolidinic ring is a real fact proven by experimental values. For BENPEN10 the values are $5-1-2-3 = 17.851^\circ$, $5-2-3-4 = 40.257^\circ$; for BPENCE10 they are $5-1-2-3 = 21.079^\circ$, $5-2-3-4 = 39.801^\circ$; for JOTLAF they are $5-1-2-3 = 22.209^\circ$, $5-2-3-4 = 37.518^\circ$; and for PMEPEN they are $5-1-2-3 = 23.540^\circ$, $5-2-3-4 = 35.582^\circ$.^{24,25,27-29} Comparing these experimental data with the calculated values with the semiempirical PM3 method one can observe that

the theoretical results obtained for the puckering of the thiazolidinic ring are lower than the experimental values.

Valence angles in the rings and the plication of the β -lactamic ring

To compare the calculated values with the experimental values, in Table 2 are shown the calculated average values of the angles between the ring bonds, the calculated inclination angles between the β -lactamic ring and the thiazolidinic ring (S1-C5-C6 and C3-N4-C7) and the improper N4-C5-C6-C7 angle by which the planarity of the β -lactamic ring can be determined both for (R) and (S) pseudochirality of the N14 atom. To evaluate if the average values are statistically distinct, in Table 2 are also presented the standard deviations (SD).

Table 2

Average values of the bond angles and of the improper angle of the β -lactamic cycle and the standard deviations (SD) of all conformers (47), for pseudochirality (23(R), 24(S)), for arrangement (24syn)(23anti) and for the three puckering classes (20a)(16b)(11c), compared to experimental data

Calculation criteria	Average value of the angle (°)												Conicity (°)	
	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	4-5-6-7	4-5-6-7	4-5-6-7
(47)	94.101	107.881	110.464	115.058	109.328	91.938	88.920	87.365	91.353	122.282	122.354	122.282	122.354	4.086
(SD)	0.374	0.446	0.370	0.512	0.296	0.247	0.234	0.094	0.246	1.122	1.191	1.122	1.191	1.996
(23R)	94.112	107.933	110.540	115.101	109.395	91.960	88.887	87.360	91.337	122.374	122.443	122.374	122.443	4.110
(SD)	0.369	0.472	0.355	0.578	0.317	0.310	0.267	0.048	0.208	1.349	1.230	1.349	1.230	1.836
(24S)	94.090	107.831	110.391	115.017	109.263	91.918	88.952	87.369	91.367	122.193	122.269	122.193	122.269	4.064
(SD)	0.387	0.424	0.377	0.449	0.265	0.170	0.199	0.124	0.281	0.872	1.172	0.872	1.172	2.177
(24syn)	94.026	107.814	110.328	114.974	109.290	91.931	88.896	87.323	91.396	122.334	122.246	122.334	122.246	4.110
(SD)	0.391	0.437	0.259	0.547	0.345	0.257	0.300	0.048	0.258	0.992	1.316	0.992	1.316	2.373
(23anti)	94.179	107.950	110.605	115.146	109.367	91.946	88.946	87.408	91.308	122.227	122.467	122.227	122.467	4.061
(SD)	0.347	0.455	0.419	0.469	0.235	0.241	0.139	0.111	0.229	1.264	1.062	1.264	1.062	1.562
(20a)	94.328	107.960	110.428	115.154	109.178	91.999	88.855	87.374	91.207	121.962	122.876	121.962	122.876	5.346
(SD)	0.018	0.100	0.224	0.106	0.232	0.187	0.210	0.102	0.057	1.277	0.300	1.277	0.300	0.764
(16b)	93.646	107.411	110.158	114.529	109.523	91.811	89.114	87.340	91.645	122.059	121.022	122.059	121.022	1.908
(SD)	0.174	0.163	0.129	0.171	0.204	0.084	0.046	0.044	0.104	0.846	0.136	0.846	0.136	1.069
(11c)	94.349	108.420	110.974	115.654	109.316	92.013	88.757	87.384	91.192	123.186	123.344	123.186	123.344	4.964
(SD)	0.311	0.413	0.268	0.534	0.362	0.403	0.259	0.128	0.217	0.662	1.364	0.662	1.364	1.972

Experimental data	Angle (°)												Conicity (°)	
	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	4-5-6-7	4-5-6-7	4-5-6-7
Exp.BENPEN ^{24,25}	95.19	104.17	105.89	116.13	105.26	94.40	88.13	84.69	91.54	118.20	127.63	118.20	127.63	7.82
Exp.BPENCE ^{10⁵,27}	95.21	103.95	105.62	115.76	105.49	94.21	87.75	84.42	92.58	118.88	126.71	118.88	126.71	7.10
Exp.JOTLAF ^{25,28}	94.55	104.37	105.80	116.14	106.00	94.15	88.61	84.94	91.48	119.97	126.14	119.97	126.14	6.38
Exp.PMEPEN ^{25,29}	96.19	104.97	104.17	119.68	102.73	88.19	92.35	82.86	96.02	121.82	129.28	121.82	129.28	-5.54
Exp.AMPC ³⁰	90.4	103.1	106.0	117.9	103.8	93.7	88.5	85.7	91.6	118.9	126.1	118.9	126.1	4.3
Exp.PN39 ³⁰	94.9	105.0	106.0	117.2	105.8	93.8	88.8	84.2	92.0	119.3	126.7	119.3	126.7	7.0
Exp.PenV ³	96.0	105.0	104.0	120.0	103.0	88.0	92.0	83.0	96.0	122.0	129.0	122.0	129.0	-
Exp.6APA ^{3,30}	95.6	105.7	106.0	118.0	103.7	93.5	88.1	84.6	91.7	120.2	132.5	120.2	132.5	-

The average values have been calculated for the following categories: for all 47 conformers, for 23 conformers with (R) pseudo-chirality, respectively for 24 conformers with (S) pseudo-chirality, for 24 *syn* conformers, respectively for 23 *anti* conformers and for the three puckering classes: 20 conformers in class (a), 16 in class (b), respectively 11 in class (c).

Comparing the calculated average values with experimental data (Table 2) one can see that the calculated values for angles 2-1-5, 3-4-5, 4-5-6, and 6-7-4 are in the range of the experimental values, while those for angles 1-2-3, 2-3-4, 1-5-4, 5-6-7 are greater than the experimental ones, and the 5-4-7 angle is lower than the experimental value. The 1-5-6 inclination angle is lower than the experimental determined value, while for 3-4-7 it is greater than the experimental values.

The values calculated by semiempirical, *ab initio* and DFT methods by other authors are comparable with the values obtained in this paper.⁸⁻¹⁶ Taking the SD into account there are no statistically significant differences for the average value of the 47 conformers and the average values for the three classification criteria. The only angle depending on the three puckering classes of the thiazolidinic ring is the improper dihedral 4-5-6-7. Graphically, the influence of the puckering of the thiazolidinic ring can be observed in Fig. 4. It can be best observed for the (S)-*anti* conformers (Fig. 4). The calculated values for the improper angle 4-5-6-7 show that the β -lactamic ring is not plane (Table 2). In Table 2 there are three distinctive average values of the plication of the β -lactamic ring: 5.35°, 1.91°, and 4.96°. The β -lactamic ring has a positively dominant plication with the exception of two conformers, which have a slight negative plication -0.45°, respectively -0.60° (see the supplementary material 44pm3, 45pm3). The average values of the 4-5-6-7 dihedral are situated around 4.09°±2.00. By comparing the experimental values of the conicity with the calculated values one can observe that the average values are lower than the experimental values. However, conformers 29pm3, 33pm3, 37pm3 and 42pm3 have the 4-5-6-7 conicity comparable to experimental values (see the supplementary material). The negative calculated values of the 4-5-6-7 conicity are not an isolated case, because for PMEPEN the experimental value of the 4-5-6-7 conicity is negative (Table 2).

The bond lengths in the rings

The average values of the bond lengths calculated with the PM3 method for the conformers of the (3S,5R,6R)-6-acetylamidopenicillanic acid: bond lengths of the thiazolidinic and the β -lactamic rings and the exocyclic distances from the sulphur atom to the C9 and C10 atoms of the methyl groups for the (R) and (S) pseudo-chirality at the nitrogen N14 atom are presented in Table 3. For comparison, in Table 3 are collected some available experimental data.^{3,24,25,27-30}

The estimation of the fitting level of the calculated with experimental geometries,^{24,25} has been done by comparing the RMS error fit values (in Å) resulted from superposition of the atoms 1,2,...,9,10 (Table 1).

From the RMS error fit values (Table 1) it results that the best fit is obtained for the 04pm3 conformer whose formation enthalpy is $\Delta H_{\text{form}} = -143.501$ kcal/mol (RMS fit = 0.29 Å). Although the calculated geometry of the 4pm3 conformer is in good accord with the experimental geometry, the conicity at the N14 atom (the 6-15-28-14 improper angle) is -24.64°, while the experimental angle is -1.15° (Table 1). This conformer is actually the fourth according to its formation enthalpy. The arrangement of the O17 and H28 atoms is *anti*. For all the other calculated conformers the fitting is lower. The 43pm3 conformer has the worst RMS fit (1.533 Å) and a *syn* arrangement of the O17 and H28 atoms. Its ΔH_{form} is -136.642 kcal/mol). Between the two conformers – the one with the best fit and the one with the worst fit – there is an energy difference of 6.859 kcal/mol.

The bond lengths calculated with the PM3 method for all the 47 conformers are unevenly distributed compared to the experimental values.^{3,24,25,27-30} Therefore, the average calculated values (Table 3) have been used as a comparison basis between the calculated values and the experimental data (Table 3).^{3,24,25,27-30} From this table it results that the average calculated values for bonds S1-C2 and C5-C6 are with ~0.02 Å higher than the experimental ones, the average calculated value for the C3-N4 bond is with ~0.03 Å higher than the experimental one, the average calculated value for the N4-C5 bond is with ~0.05 Å higher than the experimental value, and finally for the C7-N4 bond between the average calculated values and the experimental value is a difference of ~0.1 Å.

Table 3

Average values of the bond lengths and of the exocyclical distances from the sulphur atom of the methyl groups for conformers and the standard deviations (SD) for all conformers (47), for pseudochirality (23(R), 24(S)), for arrangement (24syn)(23anti) and for the three puckering classes (20a)(16b)(11c) compared to experimental data

Calculation criteria	Average value of the bond lengths (Å)										Average value of the distance (Å)		
	S1-C2	C2-C3	C3-N4	N4-C5	C5-S1	C5-C6	C6-C7	C7-N4	S1-C9	S1-C10	S1-C9	S1-C10	S1-C10
(47)	1.8716	1.5631	1.4800	1.5192	1.8186	1.5725	1.5487	1.4784	2.7712	2.7562			
(SD)	0.0026	0.0009	0.0020	0.0030	0.0033	0.0022	0.0021	0.0029	0.0065	0.0070			
(23R)	1.8722	1.5632	1.4799	1.5183	1.8178	1.5737	1.5483	1.4792	2.7699	2.7561			
(SD)	0.0020	0.0010	0.0020	0.0020	0.0029	0.0014	0.0013	0.0031	0.0067	0.0078			
(24S)	1.8711	1.5631	1.4800	1.5201	1.8194	1.5713	1.5490	1.4777	2.7724	2.7564			
(SD)	0.0031	0.0008	0.0021	0.0036	0.0022	0.0026	0.0025	0.0025	0.0062	0.0063			
(24syn)	1.8710	1.5629	1.4803	1.5201	1.8184	1.5723	1.5491	1.4774	2.7686	2.7582			
(SD)	0.0021	0.0010	0.0023	0.0028	0.0027	0.0029	0.0025	0.0027	0.0058	0.0056			
(23anti)	1.8723	1.5634	1.4796	1.5183	1.8188	1.5727	1.5483	1.4795	2.7739	2.7542			
(SD)	0.0030	0.0008	0.0017	0.0030	0.0039	0.0014	0.0015	0.0027	0.0062	0.0077			
(20a)	1.8725	1.5629	1.4787	1.5187	1.8204	1.5710	1.5496	1.4783	2.7744	2.7555			
(SD)	0.0017	0.0006	0.0005	0.0013	0.0031	0.0022	0.0018	0.0029	0.0019	0.0019			
(16b)	1.8687	1.5629	1.4819	1.5224	1.8182	1.5733	1.5489	1.4792	2.7669	2.7623			
(SD)	0.0009	0.0004	0.0013	0.0019	0.0032	0.0018	0.0014	0.0023	0.0075	0.0057			
(11c)	1.8744	1.5640	1.4795	1.5157	1.8160	1.5740	1.5466	1.4775	2.7716	2.7488			
(SD)	0.0009	0.0013	0.0026	0.0019	0.0015	0.0012	0.0020	0.0034	0.0074	0.0068			

Experimental data	Bond lengths (Å)										Distance (Å)		
	S1-C2	C2-C3	C3-N4	N4-C5	C5-S1	C5-C6	C6-C7	C7-N4	S1-C9	S1-C10	S1-C9	S1-C10	S1-C10
Exp.BENPEN10 ^{24,25}	1.861	1.573	1.458	1.466	1.824	1.550	1.547	1.377	2.780	2.750			
Exp.BPENCE10 ^{25,27}	1.859	1.564	1.468	1.476	1.815	1.561	1.541	1.367	2.777	2.750			
Exp.JOTLAF ^{25,28}	1.848	1.562	1.448	1.474	1.810	1.555	1.556	1.394	2.779	2.749			
Exp.PMEPEN ^{25,29}	1.868	1.574	1.460	1.522	1.824	1.578	1.554	1.455	2.790	2.765			
Exp.AMPC ³⁰	1.855	1.573	1.463	1.470	1.791	1.554	1.540	1.360	-	-			
Exp.PN39 ³⁰	1.857	1.567	1.450	1.457	1.838	1.546	1.542	1.378	-	-			
Exp.PenV ³	1.87	1.57	1.46	1.52	1.82	1.58	1.55	1.46	-	-			
Exp.6APA ^{3,30}	1.859	1.575	1.445	1.450	1.822	1.554	1.520	1.392	-	-			

The average calculated values for the bonds C2-C3, C5-S1, C6-C7, S1-C9 and S1-C10 are almost equal with the experimental values and in good concordance with the values calculated with the *ab initio* and DFT (HF/6-31G* and B3LYP/6-31G**) methods.¹⁰ Comparing the values calculated with the semiempirical methods (AM1, PM3) for other penicillins,⁹ one can observe that the differences between the average calculated values and the experimental values are of the same order of magnitude. The *ab initio* and DFT calculations on various penicillins,¹⁰ give the closest values to experimental data. Evaluating the results obtained with the PM3 method an interesting observation resulted namely, the average PM3 value of the S1-C2 bond length is of $\approx 1.872 \text{ \AA}$ which is higher than the PM3 value of the S1-C5 bond length of $\approx 1.818 \text{ \AA}$. This difference between the two lengths is also seen experimentally ($\approx 1.860 \text{ \AA}$, respectively $\approx 1.820 \text{ \AA}$). The advanced *ab initio* and DFT (HF/6-31G* and B3LYP/6-31G**) methods do not find this difference.¹⁰

For the distances between the sulphur and C9 (C_α) atoms, respectively between sulphur and C10 (C_β) atoms the calculated values are close to the experimental values (Table 3).^{3,24,25,27-30} Even if for the majority of conformers the distances between the sulphur and C9 atoms are with $\sim 0.01 \text{ \AA}$ larger than the distances between S1 and C10 atoms, there are 9 conformers with slight inversions, the inversion being better observable for conformer 43pm3 (see the supplementary material). These inversions are not observed among the conformers resulted from the AM1 method.²³

CONCLUSION

From the conformational analysis of the 6-acetylamidopenicillanic acid using the PM3 method have resulted 47 distinct conformers. Between the lowest energy conformer and the highest energy conformer there is an energy difference of 11.36 kcal/mol.

The existence of some regularities could be evidenced only by statistical analysis of the geometric data. There have been evidenced three distinctive classes of inclination of the thiazolidinic ring towards the β -lactamic cycle. The pseudochirality at the N14 atom, the *syn-anti* positions of the O17, H28 amidic atoms, and the rotation of the COOH group bound at C3 do not influence significantly the three puckering classes.

It resulted that there is at least one conformer (04pm3) with a pretty good concordance with the experimental geometry (RMS error fit = 0.290), even if this is not the conformer with the lowest PM3 energy.

From the statistical analysis (RMS error fit) two energetically degenerated conformers (04pm3 and 05pm3, supplementary data) with distinct geometries (RMS_{04pm3} = 0.290 compared to RMS_{05pm3} = 0.797, Table 1) could be evidenced.

For the penicillins there are no major differences between the results obtained with the semiempirical PM3 method and the *ab initio* or DFT methods.

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