Research Article

AM1 Study on the Conformational Analyses of Tautomers in Phenethicillin

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ABSTRACT

The geometry, conformation, electronic structure of phenethicillin and its tautomers have been optimized and calculated in the gas phase by semi-empirical molecular orbital AM1 method usually considering an isolated molecule, which is surrounded by vacuum. The mechanism of tautomerism in phenethicillin has been studied by comparison of the different positions of net charges at hetero-atoms in the molecule. Further, the heats of formation (ΔH_f^o), dipole moment (μ), ionization potential (IP), full atomic charges and energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) have been performed and their stable conformations have also been evaluated.

Key words: AM1, lactam, lactim, enol, tautomerism, phenethicillin, induction effect.

INTRODUCTION

Phenethicillin (Broxil) has been used most widely against gram-positive bacteria and readily absorbed into the blood stream where it is partially bound to plasma proteins in both animals and humans[1,2,3,4]. It has a high order of selective toxicity to micro-organisms which are pathogenic to human beings without obvious side effects[5]. The significance of tautomeric equilibria is familiar in the biochemical processes[6,7]. The prediction of the relative salvation energies[8], single spherical solute cavity in the solution[9], the electrostatic salvation of conformationally flexible molecules in high dielectric constant media[10], S_N2 reaction path ways and transition states in solution[11] were reported. The tautomerism of organic compounds was reported extensively theoretical and statistical-physical approaches[12]. Theoretical models of the salvation energies of tautomers[13], variations in dispersion energy[14], cavity formation and solvent re-structuring effects[15] were reported. The stability of tautomers[16,17] and equilibrium constants in electrostatic reaction field for heterocyclic compounds in aqueous solution[18] was studied. It is assumed that dipolar character of the drug could improve oral absorption[19].

Austin Model-1 (AM1) is one of the semi-empirical quantum calculations based on the neglect of differential diatomic overlap integral approximation, which includes experimental parameters and extensive simplification of the Schrodinger's equation ($H\Psi=E\Psi$) to optimize molecules for prediction of various properties of molecules to solve chemical problems[20,21,22]. In this way quantum chemistry simulates chemical structure and reactions numerically and allows studying chemical phenomena by running calculations on computer rather than by examining reactions experimentally. In this connection, theoretical investigations on conformational analyses and electronic properties of phenethicillin using AM1 method have been carried out[23,24,25,26]. Hence, the observation of tautomerism in phenethicillin has been fascinated much to carry out optimization of its tautomeric forms with a view to investigate its polarity, which are an advantage for the penetration through the porin channels of cell membrane. All naturally available and microbiologically active synthetic and semi-synthetic penicillins[3] have the same absolute configuration about three chiral centres of C_4-, C_{11}-C_1- atoms and designated as 4S: 11R: 1R. In this context, the numbering of phenethicillin (**1**) is shown in Figure –1. Hence it is important to know the

conformational changes in the molecule and worthwhile for the prediction of reactivity and pharmacological action using AM1 method. The present study reveals on molecular conformation and electronic properties of phenethicillin (**1**) and its tautomers (**2** to **4**) in gas phase usually considering an isolated molecule surrounded by vacuum has been evaluated by AM1 method.

Computational methods[21]

Austin Model 1 (AM1) is one of the Semi-empirical molecular orbital calculations was performed on the molecules shown in Scheme-1 using the MOPAC93 in WinMOPAC ver 5.13 program by means of Intel Dualcore D102GGC2 DDR2 1GB SDRAM PC. The AM1 semi-empirical method is a modification of MNDO, offering more accurate parameterizations for polar systems and transition states. Geometry calculations in the ground state (keywords: PRECISE, equivalent to GNORM=5.0, CHARGE, GEO-OK, and MMOK to correct the increase in the barrier to rotation of the amide linkage) were completely optimized until the lowest energy conformation was found. The position of the atom in the molecule is mentioned as subscript. The initial molecular geometry was adopted as Pople's standard data[27], and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms[28] using *s* = syn, *a* = anti, *p* = peri-planar (0 ± 30^0 & 180 ± 30^0) and all other angles *c* = clinal.

RESULTS AND DISCUSSION

Electronic structure of phenethicillin (1) and its tautomers (2 to 4):

The optimized electronic structure of phenethicillin (**1**) and its tautomers; lactim-form (**2**) enol-form (**3**) and lactim-enol form (**4**) are shown in Scheme-1. In this context, the numbering of phenethicillin (**1**) is shown in Figure -1. The calculated heats of formation (ΔH_f^o), ionization potential (IP), dipole moment (μ), the energies of frontier molecular orbitals (E_{HOMO} and E_{LUMO}) and net charges on hetero atoms of the molecules (**1** to **4**) are presented in Table-I. It is observed that the net charges on N_7- and N_{12}- atoms are -0.2402 and -0.3507 respectively in the case of phenethicillin (**1**). It is indicated that net charges of nitrogen atoms in the order of $N_7 < N_{12}$ and at the time of tautomerism more negative charge is observed at N_{12}- atom in all tautomers of phenethicillin (**2** to **4**).

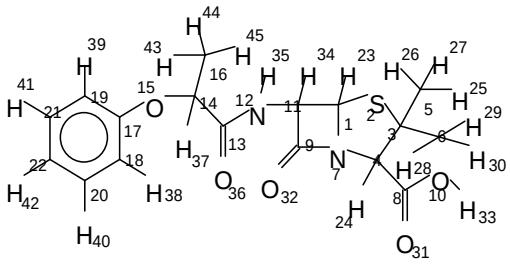


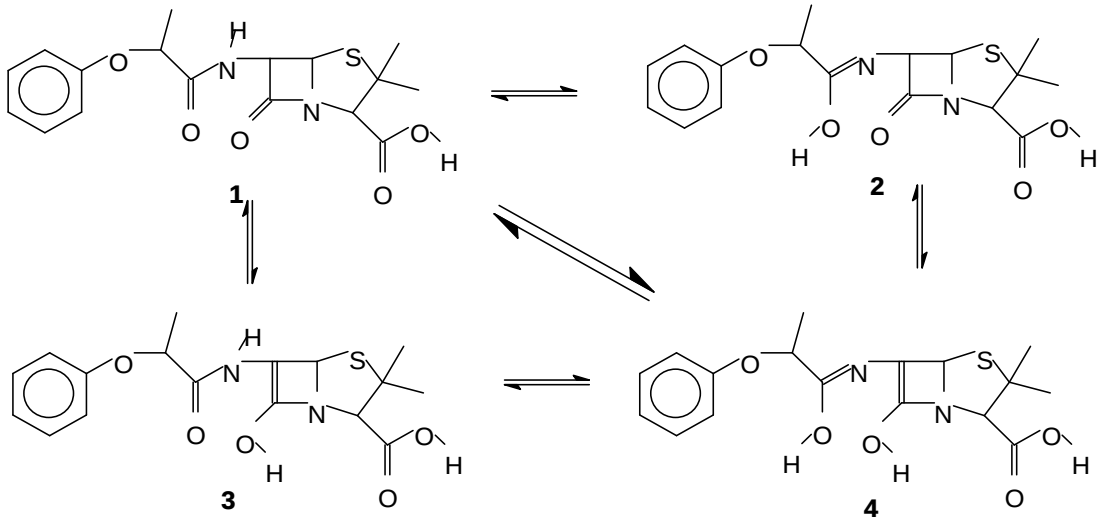
Figure - 1

The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **2** and **4** have more electron-donor character whereas other tautomers have electron-acceptor property. The results so obtained reveal that the electronic properties and reactivity of molecule depend on its conformational structure. The promotion of an electron from HOMO to LUMO, in a photochemical reaction, the supra-facial path way is allowed in the case of molecules **2** and **4**, due to the presence of same sign and other molecules undergo antara-facial path way is allowed due to the opposite sign[29,30]. The dipole moment of molecules depends on the nature of the atoms and bonds comprising the molecules and on their arrangement. The dipole moment is increasing in the order of molecules **4** < **2** < **3** < **1**. Phenethicillin (**1**) shows higher dipole moment. The electronegative heteroatoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[31] (μ_{ind}) of molecules can be estimated with respect to phenethicillin lactim-enol form (**4**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**2**) 0.2311 D < $\Delta\mu_{ind}$ (**3**) 0.6352 D < $\Delta\mu_{ind}$ (**1**) 1.0129 D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **4 < 3 < 2 < 1**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual tautomers. It can be assumed that the electronic properties and reactivity of the tautomer depend on its conformational structure.

Tautomeric equilibrium of phenethicillin

Equilibrium is normally established in polar solvents, in order to investigate the stable tautomer and it is observed the shifting of protons in phenethicillin (**1**) as per Scheme-1. Taking phenethicillin as a neutral molecule (**1**), the molecular geometry and conformations of its tautomers (**2** to **4**) have been determined by full optimization calculations. From the

obtained optimized electronic structure of phenethicillin, it is observed that the phenethicillin (**1**) predominates in its tautomers (**2** to **4**). At the time of tautomerism, the mechanism of proton shifting has been studied by comparison of the relative values of net charges at different atoms of the molecule and evaluated the predominated tautomer. Lactam-lactim tautomerism (**1** ↔ **2)** & **(3** ↔ **4)** of phenethicillin involves the shifting of hydrogen atom from nitrogen atom of lactam (-HN-C=O) group to the oxygen atom in the same molecule to form lactim (-N=C-O-H) group. Keto-enol tautomerism (**1** ↔ **3)** & **(2** ↔ **4)** of phenethicillin involves the shifting of hydrogen atom from α-carbon atom of keto (-HC-C=O) group to the oxygen atom in the same molecule to form enol (-C=C-O-H) group and both shifts (**1** ↔ **4)** involve as shown in Scheme-1. The stable tautomers of phenethicillin (**1**) are confirmed by the calculated heats of formation with full geometry optimization. Three tautomeric forms of phenethicillin (**1**) are possible, in the great majority of cases at chemical equilibrium under ordinary conditions. Instances are known when tautomeric forms are stable under ordinary conditions which are capable of inter-conversion at higher temperatures, often with the aid of catalyst. Fully optimized AM1 calculations scrutinize only the main data of bond lengths (Table-II) and dihedral angles (Table-IV) of tautomers (**2** to **4**) for the sake of simplicity. All tautomers are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium. As per electron excitation energies (ΔE) (in eV), it is observed the reactivity is decreased in the order of **4** > **3** > **2** > **1**. It is confirmed that phenethicillin (**1**) is more stable than its tautomers. The shifting of H_{35}-proton and H_{34}-proton of phenethicillin (**1**) to respective O_{36}-atom and O_{32}-atom are predicted for the formation of respective lactim-form (**2**) and enol-form (**3**). The simultaneous shifting of H_{35}-proton and H_{34}-proton of phenethicillin (**1**) to respective O_{36}-atom and O_{32}-atom is predicted for the formation of lactim-enol form (**4**) of phenethicillin.



Scheme -1: Tautomerism in Phenethicillin

1 - 2 & 3 - 4: lactam - lactim tautomerism.

1 - 3 & 2 - 4: keto - enol tautomerism.

1 - 4 : lactam - lactim & keto - enol tautomerism.

The tautomeric equilibrium constants $\log K_T$ was calculated[32,33] according to the equation (1) from AM1 calculated heat of formation :

$$\log K_T = \frac{\Delta G_T}{2.303\ R\ T} \approx \frac{\delta \Delta H_f^{o}}{2.303\ R\ T} \quad \text{- - - (1)}$$

Where ΔG_T is the free energy of the tautomeric equilibrium, $\delta\Delta H_f^o$ is the difference in the calculated heats of formation of the tautomeric species participating in this equilibrium. R is the gas constant and T is the absolute temperature. From this equation (1), $\log K_T$ values and the change of net charges were calculated and incorporated in Table - III. It is observed that the tautomeric equilibrium is increased in the order of $\log K_{T4} < \log K_{T3} < \log K_{T1} < \log K_{T2} < \log K_{T5}$, at the time of tautomeric conversion of **3** ↔ **4, 2** ↔ **4, 1** ↔ **2, 1** ↔ **3,** and **1** ↔ **4** respectively. The net charges are increased at O_{31}- for the conversion of **1** ↔ **2,** O_{10}-, O_{31}- for **1** ↔ **3,** N_{12}-, O_{10}-, O_{36}- for **2** ↔ **4,** N_{17}-, O_{15}- for **3** ↔ **4,** O_{10}- for **1** ↔ **4** and decreased at all other hetero-atoms.

From the Table-II, Table-III and Scheme - 1, it is observed that phenethicillin (**1**) may undergo lactam-lactim tautomerism for the formation of lactim form of phenethicillin (**2**) with increasing bond length of O_{36}-C_{13} (1.3802 Å) and decreasing bond length of C_{13}-N_{12} (1.2934 Å) with the formation of single bond length of H-O_{36} (0.9699 Å). It is also observed that phenethicillin (**1**) may undergo keto-enol tautomerism for the formation of enol form of phenethicillin (**3**) with increasing bond length of O_{32}-C_9 (1.3535 Å) and decreasing bond length at C_{11}-C_9 (1.3734 Å) with the formation of single bond length of H-O_{32} (0.9749 Å). But the formation of lactim-enol form of phenethicillin (**4**) is found with increasing bond lengths of O_{32}-C_9 (1.3479 Å), O_{36}-C_{13} (1.3772 Å) and decreasing bonds of C_{11}-C_9 (1.3799 Å), C_{13}-N_{12} (1.2992 Å) with the formation of single bonds of H-O_{32} (0.9745 Å), H-O_{36} (0.9772 Å).

The conformations of phenethicillin (1) and its tautomers (2 to 4)

The spatial arrangement of atoms in a molecule is considered to study the conformations of phenethicillin (**1**), and its lactim form (**2**), enol form (**3)** and lactim-enol form (**4**) of phenethicillin with a view to investigate molecular deformations. These can exist in *anti*- or *syn*- conformations, according to the position of atoms. Its conformation can be assigned by comparison of its geometry and electronic structure as per Scheme-1. In this context, the change in energy content of tautomerism may depend on the changes in the parameters of dihedral angles. Fully optimized AM1 calculations scrutinize only the main data of dihedral angles (Table-IV) of molecules (**1** to **4**) for the sake of simplicity.

It is observed as per Scheme - 1, the shifting of H_{35}- atom from N_{12}- atom of lactam (-HN-C=O) group to the O_{36}- atom in the same molecule to form lactim (-N=C-O-H) group in the case of phenethicillin lactim form (**2**). The conformations of $C_{13}N_{12}C_{11}C_9$, $O_{15}C_{14}C_{13}N_{12}$, $C_{16}C_{14}C_{13}N_{12}$, $C_{17}O_{15}C_{14}C_{13}$, $H_{33}O_{10}C_8C_4$, and $O_{36}C_{13}N_{12}C_{11}$ are changed respectively from -*ac* to +*ap, +sc* to +*ac, -sc* to +*sp, +ac* to +*sc, +ap* to –*ap,* and +*sp* to -*sp* conformations and all other conformations are moderately changed. It is observed that the shifting of proton from N_{12}-atom to O_{36}-atom in the formation of $HO_{36}C_{13}N_{12}$ is shown -*sp* conformation. Enol form of phenethicillin (**3**) is created with the shifting of H_{34}-atom from α-carbon atom (C_{11}-atom) of keto (-HC-C=O) group to the O_{32}-atom in the same molecule to form enol (-C=C-O-H) group in phenethicillin (**1**). At the time of tautomeric change, the conformation from –*ap* of $O_{10}C_8C_4C_3$, –*ac* of $C_{13}N_{12}C_{11}C_9$, +*ap* of $C_{14}C_{13}N_{12}C_{11}$, +*sc* of $O_{15}C_{14}C_{13}N_{12}$, -*sc* of $C_{16}C_{14}C_{13}N_{12}$, +*sp* of $O_{31}C_8C_4C_3$, +*ap* of $H_{33}O_{10}C_8C_4$ and +*sc* of $H_{35}N_{12}C_{11}C_9$, are observed respectively to –*ac,* +*ac, -ap, +ac, -sp, +sc, -ap* and -*sp* conformations. It is investigated that the shifting of proton from C_{11}-atom to O_{32}-atom in the case of $HO_{32}C_9N_7$ is shown +*sp* conformation and all other conformations are more or less changed. It is also observed that the shifting of H_{35}- atom from N_{12}- atom and H_{34}-atom from C_{11}-atom of phenethicillin (**1**) simultaneously to respective O_{36}- atom and O_{32}- atom is predicted for the formation of lactim-enol form of phenethicillin (**4**) with formation of –sp and +sp conformations in the case of $HO_{36}C_{13}N_{12}$ and $HO_{32}C_9N_7$ respectively. The change of dihedral angle of $O_{10}C_8C_4C_3$, $O_{15}C_{14}C_{13}N_{12}$, $C_{16}C_{14}C_{13}N_{12}$, $O_{31}C_8C_4C_3$ and $H_{33}O_{10}C_8C_4$ are converted from -*ap* to -*ac,* +*sc* to +*ac,* -*sc* to +*sp,* +*sp* to +*sc* and +*ap* to –*ap* conformations respectively and rest of positions have moderate changes.

CONCLUSION

AM1 calculations show that phenethicillin tautomers are nearly non-planar skeleton geometry, and all tautomeric forms are solvated to form hydrogen bonds with the polar solvents which would affect the position of the equilibrium. It is also observed that the phenethicillin predominates in its tautomers. The utility of theoretical predictions is important for evaluating the biochemical mechanism to prevent cell wall synthesis and binding to plasma protein. This study reveals about the stability of tautomers, conformations and molecular deformations.

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Table I: Heat of formation (ΔH_f^o in kcal/mol), ionization potential (eV), dipole moment (μ in Debye), energies of frontier molecular orbitals (in eV), electron excitation energies ($\Delta E = E_{LUMO} - E_{HOMO}$) (in eV) and the atomic charges on hetero-atoms of phenethicillin(1) and its tautomers lactim (2), enol (3) and lactim- enol (4) forms from AM1 calculations

Parameters	1	2	3	4
ΔH_f^o (kcal/mol)	-125.9658	-109.6539	-100.8945	-94.1242
Ionization potential (eV)	9.1224	9.1089	8.4589	8.6172
μ (Debye)	3.109	2.327	2.731	2.096
E_{HOMO} (eV)	-9.122	-9.109	-8.459	-8.617
E_{LUMO} (eV)	+0.099	-0.081	+0.083	-0.212
Electron excitation energies (eV)	9.221	9.028	8.542	8.405
S_2	+0.0526	+0.0281	+0.1012	+0.0578
N_7	-0.2402	-0.2249	-0.1449	-0.1624
N_{12}	-0.3507	-0.2517	-0.2760	-0.1877
O_{10}	-0.2865	-0.2807	-0.3232	-0.3227
O_{15}	-0.2261	-0.2020	-0.2014	-0.2096
O_{31}	-0.3519	-0.3561	-0.3535	-0.3511
O_{32}	-0.2363	-0.2280	-0.2243	-0.2000
O_{36}	-0.3522	-0.2751	-0.3510	-0.2945

Table II: Bond lengths of phenethicillin(1) and its tautomers lactim (2), enol (3) and lactim- enol (4) forms from AM1 calculations

Bond lengths (Å)	1	2	3	4
C_9-N_7	1.4491	1.4505	1.4617	1.4635
C_{11}-C_9	1.5696	1.5621	1.3744	1.3799
N_{12}-C_{11}	1.4125	1.4176	1.3727	1.3717
C_{13}-N_{12}	1.3831	1.2934	1.3862	1.2992
O_{32}-C_9	1.2176	1.2164	1.3535	1.3471
O_{36}-C_{13}	1.2443	1.3802	1.2450	1.3772
H-O_{32}	- -	- -	0.9741	0.9745
H-O_{36}	- -	0.9699	- -	0.9772
H-C_{11}	1.1257	1.1264	- -	- -

Table III: Tautomeric equilibrium in Phenethicillin (1) with its tautomers lactim (2), enol (3) and lactim- enol (4) forms from AM1 calculations

Equilibrium	$LogK_T$	$LogK_T$ - Values	Net charges on Hetero-atoms	
			Increasing	Decreasing
1 ↔ 2	$LogK_{T1}$	11.9558	O_{31}	N_7, N_{12}, O_{10}, O_{15}, O_{32}, O_{36}
1 ↔ 3	$LogK_{T2}$	18.3760	O_{10}, O_{31}	N_7, N_{12}, O_{15}, O_{32}, O_{36}
2 ↔ 4	$LogK_{T3}$	11.3824	N_{12}, O_{10}, O_{36}	N_7, O_{15}, O_{31}, O_{32}
3 ↔ 4	$LogK_{T4}$	4.9623	N_7, O_{15}	N_{12}, O_{10}, O_{31}, O_{32}, O_{36}
1 ↔ 4	$LogK_{T5}$	23.3380	O_{10}	N_7, N_{12}, O_{15}, O_{31}, O_{32}, O_{36}

Table IV: Dihedral angle (^{o}) of phenethicillin (1) and its tautomeric forms (2 to 4), from AM1 calculations

Dihedral angle (^{o})	1		2		3		4	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_8C_4C_3S_2$	+163.25	+*ap*	+163.15	+*ap*	+164.49	+*ap*	+162.70	+*ap*
$O_{10}C_8C_4C_3$	-173.78	-*ap*	-168.67	-*ap*	-137.39	-*ac*	-129.60	-*ac*
$C_{13}N_{12}C_{11}C_9$	-126.91	-*ac*	+160.93	+*ap*	+149.47	+*ac*	-139.40	-*ac*
$C_{14}C_{13}N_{12}C_{11}$	+179.33	+*ap*	+177.46	+*ap*	-179.49	-*ap*	+179.47	+*ap*
$O_{15}C_{14}C_{13}N_{12}$	+50.06	+*sc*	+146.27	+*ac*	+111.50	+*ac*	+124.99	+*ac*
$C_{16}C_{14}C_{13}N_{12}$	-67.73	-*sc*	+29.57	+*sp*	-7.67	-*sp*	+7.31	+*sp*
$C_{17}O_{15}C_{14}C_{13}$	+99.15	+*ac*	+85.90	+*sc*	+111.67	+*ac*	+112.27	+*ac*
$O_{31}C_8C_4C_3$	+11.66	+*sp*	+17.01	+*sp*	+46.35	+*sc*	+53.39	+*sc*
$O_{32}C_9N_7C_4$	+59.33	+*sc*	+58.15	+*sc*	+62.76	+*sc*	+65.41	+*sc*
$H_{33}O_{10}C_8C_4$	+179.98	+*ap*	-179.99	-*ap*	-177.75	-*ap*	-178.76	-*ap*
$O_{36}C_{13}N_{12}C_{11}$	+0.85	+*sp*	-0.70	-*sp*	+3.77	+*sp*	+2.04	+*sp*
$H_{35}N_{12}C_{11}C_9$	+57.52	+*sc*	- -	-	-19.99	-*sp*	- -	-
H-$O_{36}C_{13}N_{12}$	- -	-	-3.49	-*sp*	- -	-	-4.84	-*sp*
H-$O_{32}C_9N_7$	- -	-	- -	-	+27.47	+*sp*	+23.83	+*sp*

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs[28].

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