Research Article

AM1 Study on the Conformational Analyses of Tautomers In Methicillin

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ABSTRACT
The mechanism of tautomerism in methicillin has been studied by comparison of the different positions of net charges at hetero-atoms in the molecule. The geometry, conformation, electronic structure of methicillin 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. 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.

Keywords: AM1, lactam, lactim, enol, tautomerism, methicillin, induction effect.

INTRODUCTION

Methicillin is useful for treatment of severe staphylococcal infections, such as septicaemia, endocarditis, pneumonia, meningitis, osteomyelitis and septic arthritis[1,2,3]. It inhibits the growth of both penicillin susceptible and penicillinase-producing staphylococci[4]. The significance of tautomeric equilibria is familiar in the biochemical processes[5]. The tautomerism of organic compounds was reported extensively theoretical and statistical-physical approaches[6]. The predictions of the relative salvation energies[7], single spherical solute cavity in the solution[8] and the electrostatic salvation of conformationally flexible molecules in high dielectric constant media[9] were reported. Theoretical models of the salvation energies of tautomers[10], variations in dispersion energy[11], cavity formation and solvent re-structuring effects[12] were reported. The stability of tautomers[13] and equilibrium constants in electrostatic reaction field for heterocyclic compounds in aqueous solution[14] was studied. It is assumed that dipolar character of the drug could improve oral absorption[15]. It is important to know the conformational changes in the molecule for the prediction of its reactivity and pharmacological action.

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 (HΨ=EΨ) to optimize molecules for prediction of various properties of molecules to solve chemical problems[16]. 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 of AM1 study on conformational analyses have been carried out[17,18]. Hence, the observation of tautomerism in methicillin 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[2] 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 methicillin (**1**) is shown in Figure –1. In order to gain insight into the structure of methicillin, it is desirable to perform rigorous theoretical analysis on the different geometries of methicillin and its tautomers, so as to

predict the possibilities of existence of syn-, anti-, clinal- and peri-planar forms using AM1 method.

The present study reveals on molecular conformation and electronic properties of methicillin (**1**) and its tautomers (**2** to **4**) in gas phase usually considering an isolated molecule surrounded by vacuum has been evaluated by AM1 method. From the obtained optimized electronic structure of methicillin, it is observed that the methicillin (**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 methicillin 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 methicillin 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 in the formation of lactim-enol form of methicillin (**4**) as shown in Scheme-1. Taking methicillin as a neutral molecule (**1)**, the molecular geometry and conformations of its tautomers (**2** to **4**) have been determined by full optimization calculations.

Computational methods[16]

Austin Model 1 (AM1) Semi-empirical molecular orbital calculations were 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[19], and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms[20] 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 methicillin (1) and its tautomers (2 to 4)

The optimized electronic structure of methicillin (**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 methicillin (**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 -0.2440 and -0.3571 respectively in the case of methicillin (**1**). It is indicated that net charges of nitrogen atoms in the order of $N_7 < N_{12}$. At the time of tautomerism more negative charge is observed at N_7- atom in the case of tautomers (**2** and **4**) of methicillin.

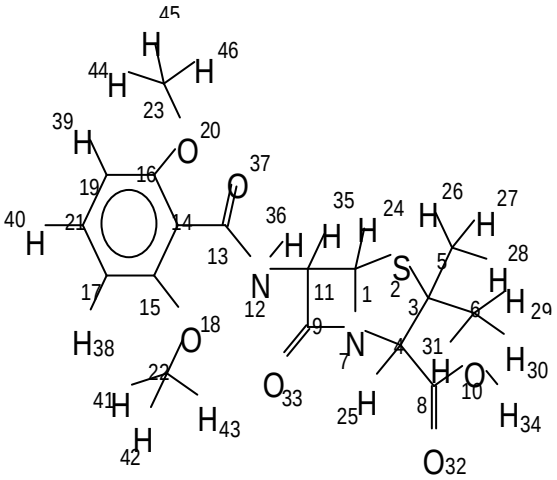


Figure - 1

The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that all tautomers have more electron-donor character. 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 all tautomers, due to the presence of same sign[21]. 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** < **3** < **1** < **2**. Lactim form of methicillin (**2**) 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[22] (μ_{ind}) of molecules can be estimated with respect to lactim-enol form of methicillin (**4**).

It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**3**) 2.806 D < $\Delta\mu_{ind}$ (**1**) 4.822 D < $\Delta\mu_{ind}$ (**2**) 5.082 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.

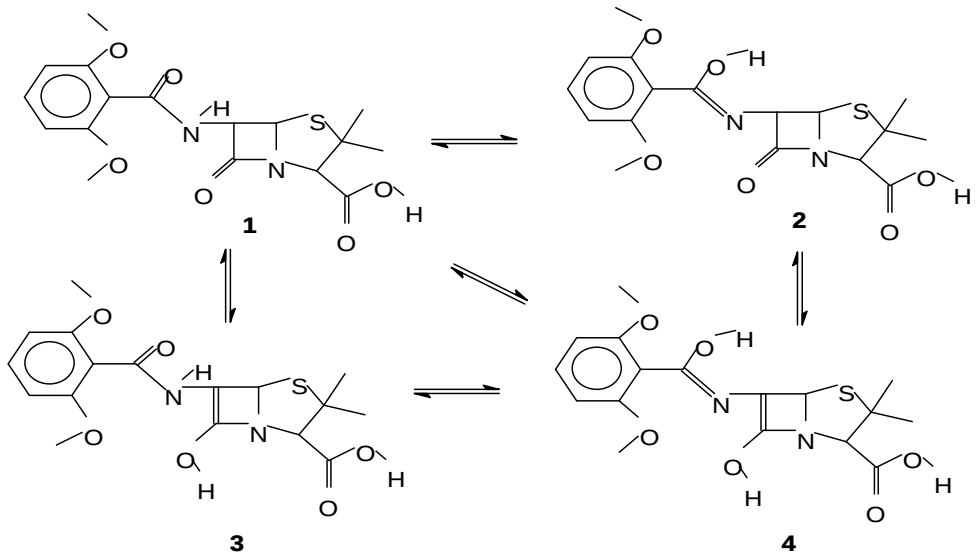
Tautomeric equilibrium of methicillin

Equilibrium is normally established in polar solvents, in order to investigate the stable tautomer and it is found out the shifts of protons of methicillin (**1**) as per Scheme-1. The stable tautomers of methicillin (**1**) are confirmed by the calculated heats of formation with full geometry optimization. These tautomers can exist in *anti*- or *syn*-conformations. Its conformation can be assigned by comparison of its geometry and electronic structure as per Scheme-1. Three tautomeric forms of methicillin (**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 **3** > **4** > **2** > **1**. It is confirmed that methicillin (**1**) is more stable than its tautomers. The shifting of H_{36}-proton and H_{35}-proton of methicillin (**1**) to respective O_{37}-atom and O_{33}-atom are predicted for the formation of respective lactim-form (**2**) and enol-form (**3**). The simultaneous shifting of H_{36}-proton and H_{33}-proton of methicillin (**1**) to respective O_{37}-atom and O_{33}-atom is predicted for the formation of lactim-enol form (**4**) of methicillin.

The AM1 calculated heat of formation, and the tautomeric equilibrium constants $\log K_T$ was calculated[23] according to the equation (1):

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

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 $\log K_{T4} < \log K_{T1} < \log K_{T3} < \log K_{T2} < \log K_{T5}$, at the time of tautomeric conversion of **3** ↔ **4, 1** ↔ **2, 2** ↔ **4, 1** ↔ **3,** and **1** ↔ **4** respectively. The net charges are increased at O_{18}-, O_{33}- for the conversion of **1** ↔ **2,** O_{10}-, O_{32}-, O_{37}- for **1** ↔ **3,** O_{10}-, O_{20}-, O_{32}- for **2** ↔ **4,** N_7-, O_{18}-, O_{20}- for **3** ↔ **4,** O_{10}-, O_{18}-, O_{32}-, O_{37}- for **1** ↔ **4** and decreased at all other hetero-atoms.



Scheme - 1:Tautomerism in Methicillin
1- 2 & 3 - 4 : lactam-lactim tautomerism
1 -3 & 2 - 4 : keto-enol tautomerism
1 - 4 : lactam-lactim & keto-enol tautomerism

From the Table-II, Table-III and Scheme - 1, it is observed that methicillin (**1**) may undergo lactam-lactim tautomerism for the formation of lactim form of methicillin (**2**) with increasing bond length of O_{37}-C_{13} (1.3854 Å) with the formation of double bond length of C_{13}-N_{12} (1.2972 Å) and single bond length of H-O_{37} (0.9681 Å). It is also observed that methicillin (**1**) may undergo keto-enol tautomerism for the formation of enol form of methicillin (**3**) with increasing bond length of O_{33}-C_9 (1.3535 Å) with the formation of double bond length at C_{11}-C_9 (1.3778 Å) and single bond length of H-O_{32} (0.9736 Å). But the formation of lactim-enol form of methicillin (**4**) is found with increasing bond lengths of O_{33}-C_9 (1.3435 Å), O_{37}-C_{13} (1.3912 Å) with the formation of double bonds of C_{11}-C_9 (1.3835 Å), C_{13}-N_{12} (1.2994 Å) and single bonds of H-O_{33} (0.9748 Å), H-O_{37} (0.9674 Å).

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

The spatial arrangement of atoms in a molecule is considered to study the conformations of methicillin (**1**), and its lactim form (**2**), enol form (**3**) and lactim-enol form (**4**) of methicillin with a view to investigate molecular deformations. These can exist in *anti-* or *syn-* conformation, according to the position of atoms. 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 tautomers (**1** to **4**) for the sake of simplicity. It is observed as per Scheme - 1, the shifting of H_{36}- atom from N_{12}-atom of lactam (-HN-C=O) group to the O_{37}-atom in the same molecule to form lactim (-N=C-O-H) group in the case of lactim form of methicillin (**2**). The conformations of $C_{14}C_{13}N_{12}C_{11}$, $O_{15}C_{14}C_{13}N_{12}$ and $O_{37}C_{13}N_{12}C_{11}$ are changed respectively from *+ap* to *+sp, -sc* to *–ac* 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_{37}-atom in the formation of $HO_{37}C_{13}N_{12}$ is shown *+ap* conformation. Enol form of methicillin (**3**) is created with the shifting of H_{35}-atom from α-carbon atom (C_{11}-atom) of keto (-HC-C=O) group to the O_{33}-atom in the same molecule to form enol (-C=C-O-H) group in methicillin (**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 $C_{14}C_{13}N_{12}C_{11}$, *+sp* of $O_{32}C_8C_4C_3$ and *+sc* of $H_{36}N_{12}C_{11}C_9$ are observed respectively to *+sc, +ac, -ap, -ac* and *-sc* conformations. It is investigated that the shifting of proton from C_{11}-atom to O_{33}-atom in the case of $HO_{33}C_9N_7$ is shown *-sp* conformation and all other conformations are more or less changed. It is also observed that the shifting of H_{36}- atom from N_{12}- atom and H_{35}-atom from C_{11}-atom of methicillin (**1**) simultaneously to respective O_{37}- atom and O_{33}- atom is predicted for the formation of lactim-enol form of methicillin (**4**)

with formation of +ap and -sp conformations in the case of $HO_{37}C_{13}N_{12}$ and $HO_{33}C_9N_7$ respectively. The change of dihedral angle of $O_{10}C_8C_4C_3$, $C_{13}N_{12}C_{11}C_9$, $C_{14}C_{13}N_{12}C_{11}$, $C_{15}C_{14}C_{13}N_{12}$ and $O_{32}C_8C_4C_3$ are converted from *-ap* to *+sc, -ac* to *+ap, +ap* to *-ap, -sc* to *-ac* and *+sp* to *–ac* conformations respectively and rest of positions have moderate changes.

CONCLUSION

AM1 calculations show that methicillin 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. The utility of theoretical predictions is important for evaluating the biochemical mechanism to inhibit 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 methicillin(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)	-157.5533	-145.9056	-136.0981	-126.1847
Ionization potential (eV)	8.914	8.794	8.002	8.090
μ (Debye)	5.428	5.688	3.412	0.606
E_{HOMO} (eV)	-8.914	-8.794	-8.002	-8.090
E_{LUMO} (eV)	-0.204	-0.312	-0.051	-0.120
Electron excitation energies (eV)	8.706	8.482	7.951	7.970
S_2	+0.0544	+0.1321	+0.0949	+0.0739
N_7	-0.2440	-0.2348	-0.1523	-0.1734
N_{12}	-0.3571	-0.2113	-0.2763	-0.1156
O_{10}	-0.2851	-0.2840	-0.3072	-0.3056
O_{18}	-0.2126	-0.2211	-0.2103	-0.2238
O_{20}	-0.1920	-0.1881	-0.1890	-0.1936
O_{32}	-0.3570	-0.3588	-0.3745	-0.3731
O_{33}	-0.2410	-0.2503	-0.2192	-0.1942
O_{37}	-0.3335	-0.2934	-0.3407	-0.2966

Table II: Bond lengths of methicillin(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	1.4480	1.4469	1.4624	1.4635
C_{11}-C_9	1.5685	1.5643	1.3778	1.3835
N_{12}-C_{11}	1.4102	1.4251	1.3701	1.3664
C_{13}-N_{12}	1.3937	1.2972	1.3912	1.2994
O_{33}-C_9	1.2184	1.2187	1.3502	1.3435
O_{37}-C_{13}	1.2421	1.3854	1.2431	1.3912
H-O_{33}	- -	- -	0.9736	0.9748
H-O_{37}	- -	0.9681	- -	0.9674
H_{35}-C_{11}	1.1259	1.1247	- -	- -
H_{36}-N_{12}	0.9932	- -	0.9955	- -

Table III: Tautomeric equilibrium in methicillin (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	$LogK_{T1}$	8.5371	O_{18}, O_{33}	N_7, N_{12}, O_{20}, O_{37}
1 ↔ 3	$LogK_{T2}$	15.7255	O_{10}, O_{32},O_{37}	N_7, N_{12}, O_{20}, O_{33}
2 ↔ 4	$LogK_{T3}$	14.4631	O_{10}, O_{20}, O_{32}	N_7, N_{12}, O_{33}
3 ↔ 4	$LogK_{T4}$	7.2660	N_7, O_{18}, O_{20}	N_{12}, O_{33}, O_{37}
1 ↔ 4	$LogK_{T5}$	22.9915	O_{10}, O_{18}, O_{32}, O_{37}	N_7, N_{12}, O_{33}

Table IV: Dihedral angle (°) of methicillin (1) and its tautomeric forms (2 to 4), from AM1 calculations

Dihedral angle (°)	1		2		3		4	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$O_{10}C_8C_4C_3$	-167.36	*-ap*	-172.91	*-ap*	+68.46	*+sc*	+68.47	*+sc*
$C_{13}N_{12}C_{11}C_9$	-124.04	*-ac*	-108.54	*-ac*	+144.67	*+ac*	+161.07	*+ap*
$C_{14}C_{13}N_{12}C_{11}$	+178.79	*+ap*	+0.57	*+sp*	-179.92	*-ap*	-179.84	*-ap*
$C_{15}C_{14}C_{13}N_{12}$	-59.83	*-sc*	-123.76	*-ac*	-61.95	*-sc*	-109.34	*-ac*
$O_{32}C_8C_4C_3$	+18.48	*+sp*	+12.87	*+sp*	-113.51	*-ac*	-113.71	*-ac*
$O_{33}C_9N_7C_4$	+58.22	*+sc*	+58.84	*+sc*	+65.88	*+sc*	+69.85	*+sc*
$O_{37}C_{13}N_{12}C_{11}$	-2.71	*-sp*	+0.57	*+sp*	-0.84	*-sp*	-1.14	*-sp*
$H_{36}N_{12}C_{11}C_9$	+45.99	*+sc*	--	- -	-39.89	*-sc*	- -	- -
$H_{35}C_{11}C_9N_7$	+115.94	*+ac*	+114.49	*+ac*	--	--	--	-
H-$O_{37}C_{13}N_{12}$	--	--	+168.81	*+ap*	--	--	+179.22	*+ap*
H-$O_{33}C_9N_7$	--	--	--	-	-2.35	*-sp*	-4.92	*-sp*

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs[20].

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