Research Article

AM1 study on the conformational analyses of tautomers in benzylpenicillin

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ABSTRACT

The geometry, conformation and electronic structure of tautomers in benzylpenicillin 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. In this connection, 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. The conformational analyses of stable tautomers have also been discussed.

Keywords: AM1, lactam, lactim, benzylpenicillin, induction effect, frontier molecular orbital.

INTRODUCTION

Benzylpenicillin has been recognized as first antibiotic in chemotherapy for the treatment of infections caused by most species of Gram-positive bacteria[1]. The importance of β-lactam antibiotics contributes a potent and rapid bactericidal action against the growth phase of bacteria and also very low toxic adverse reactions in the host[2]. Tautomeric equilibrium is predictable for the study of the processes of both organic chemistry and biochemistry[3] and it is reported extensively theoretical and statistical-physical approaches[4]. The stability of tautomers[5] and equilibrium constants in electrostatic reaction field for heterocyclic compounds in aqueous solution[6] was reported. It is assumed that dipolar character of the drug could improve oral absorption[7]. Austin Model-1 (AM1) is one of the semi-empirical quantum calculation methods, which is based on the neglect of differential diatomic overlap integral approximation, it includes experimental parameters and extensive simplification of the Schrodinger's equation (HΨ=EΨ) to optimize molecules for calculation of various properties and solve chemical problems[8]. In this way quantum chemistry simulates chemical structure and allows studying chemical phenomena by running calculations on computer rather than by examining reactions experimentally. In this connection, theoretical investigations of HMO study on the effect of methyl group perturbations[9] and AM1 study on conformational analyses[10], [1,3]sigmatropic hydrogen migration[11], electronic structure[12], correlation studies[13] and computational studies[14] were reported. It is worthwhile to study the tautomerism in benzylpenicillin with a view to investigate their polarities, which are an advantage for the penetration through the porin channels of cell membrane.

The present investigation reveals tautomerism of benzylpenicillin and it may involve either the shifting of hydrogen atom from α-carbon atom of β-lactam (-HC-C=O) group to the oxygen atom to form enol (-C=C-O-H) group in the case of **2** or shifting of hydrogen atom from nitrogen atom in lactam (-HN-C=O) group to the oxygen atom to form lactim (-N=C-O-H) group in the case of **3** or simultaneously shifting of both hydrogen atoms in the case of **4,** as shown in Scheme-1. Tautomeric equilibrium and electronic properties of benzyl penicillin (**1**) in gas phase usually considering isolated molecules which are surrounded by vacuum and it has been evaluated by AM1 method. From the obtained optimized electronic structure of benzyl penicillin tautomers, the mechanism of proton shifting has been studied by comparison of the relative values of net charges at different atoms of the

molecule and also observed the predominated tautomers. Taking benzylpenicillin as a neutral molecule (**1**), the molecular geometry and conformations of enol (**2**), lactim (**3**) and lactim-enol (**4**) systems have been determined by full optimization calculations using semi-empirical molecular orbital AM1 method.

Stereochemistry of penicillins[2]

All naturally occurring and microbiologically active synthetic and semi-synthetic penicillins have the same absolute configuration about three chiral centres of C_4- C_{11}- C_1- and designated as 4S: 11R: 1R. In this context, the numbering of benzylpenicillin (**1)** is shown in Figure -1. L-configuration in the case of the benzyl amino group bearing C_{11}- atom whereas the carboxyl group is attached to C_4-atom has the D-configuration. Thus, the benzyl amino and carboxyl groups are ***trans*** to each other with the former in α- and the later in the β-orientation relative to the β-lactam thiazolidine fused ring system. Hence, it is worthwhile to know the conformational changes in the molecule for the prediction of reactivity and pharmacological action using AM1 method.

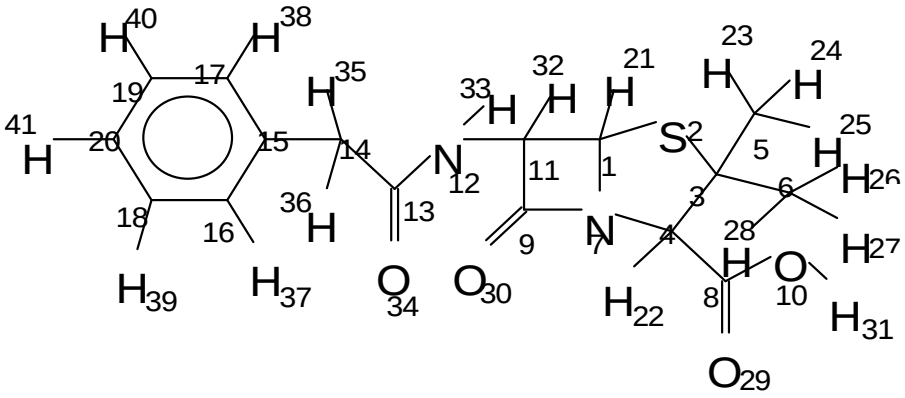


Figure - 1

Computational methods[8]

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[15], and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms[16] 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 benzylpenicillin (1) and its tautomers (2 to 4)

The optimized electronic structure of benzylpenicillin (**1**) and its tautomers (**2** to **4**) are shown in Scheme-1. The calculated heats of formation (ΔH_f^0), 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 -0.2584 and -0.3614 respectively in the case of benzylpenicillin (**1**). It is investigated 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 benzylpenicillin.

The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) reveal that molecules **1** 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 **1** and **4,** due to the presence of same sign and other molecules undergo antara-facial path way is allowed due to the opposite sign[17]. 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 **3** < **2** < **4** < **1**. Benzylpenicillin (**1**) shows higher dipole moment. The electronegative hetero-atoms cause displacement of electrons that induces an additional dipole moment in the molecule. The magnitude of the induction effect[18] (μ_{ind}) of molecules can be estimated with respect to benzylpenicillin lactim form (**3**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**2**) 0.591D < $\Delta\mu_{ind}$ (**4**) 1.178 D < $\Delta\mu_{ind}$ (**1**) 3.711 D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **4 < 2 < 3 < 1**. But geometry calculations in the ground state were completely optimized until the lowest energy conformation was found in the individual tautomer. It can be assumed that the electronic properties and reactivity of the tautomer depend on its conformational structure.

Tautomeric equilibrium of benzylpenicillin

Equilibrium is normally established in polar solvents, in order to investigate the stable tautomer and it is found out the shifts of protons of benzylpenicillin (**1**). The stable tautomers of benzylpenicillin (1) are confirmed by the calculated heats of formation with full geometry optimization. The 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 benzylpenicillin (**1**) are possible, in the great majority of cases the molecules 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** > **2** > **3** > **1**. It is confirmed that benzylpenicillin (**1**) is more stable than its tautomers.

The shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}-atom and O_{34}-atom are predicted for the formation of respective enol-form(**2**) and lactim-form(**3**). The simultaneous shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}-atom and O_{34}- atom is predicted for the formation of lactim-enol form **(4)** of benzylpenicillin.

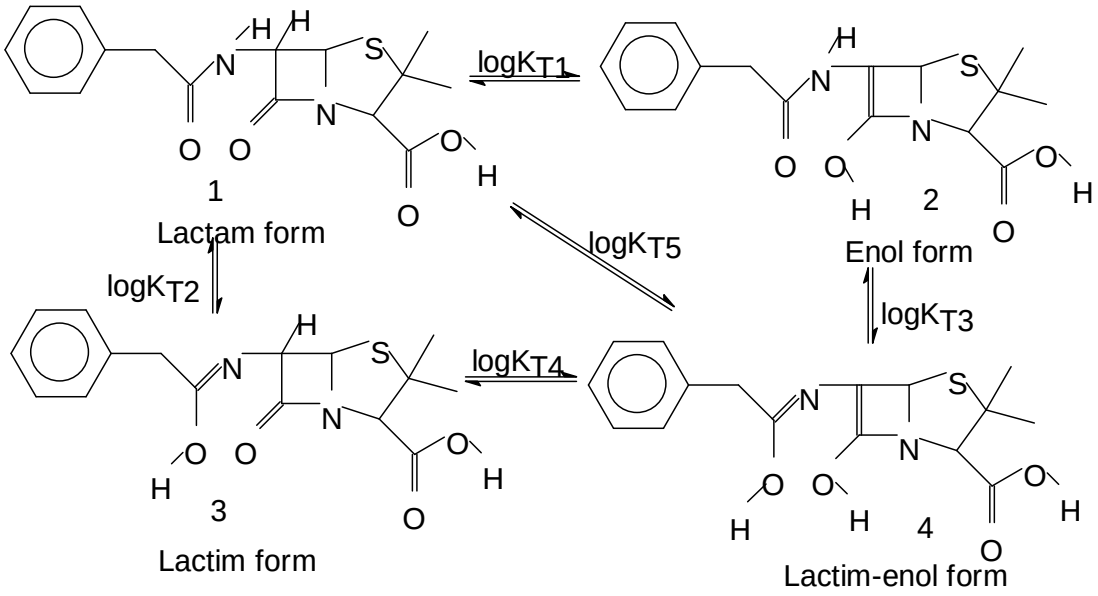
The AM1 calculated heat of formation, and the tautomeric equilibrium constants $\log K_T$ was calculated[19] according to the equation (1):

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

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 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 $\log K_{T3} < \log K_{T2} < \log K_{T4} < \log K_{T1} < \log K_{T5}$. At the time of tautomeric conversion of (**1**) to (**2**), (**1**) to ((**4**) and (**3**) to (**4**) the net charges are increased at O_{29}- atom and decreased at all other hetero atoms. In the case of tautomeric conversion of (**1**) to (**3**) the net charge is increased at O_{29}- atom, O_{30}-atom and decreased at all other hetero-atoms, but the tautomeric conversion of (**2**) to (**4**), the net charge is increased at N_7-atom, O_{29}-atom, O_{30}-atom and decreased at all other hetero-atoms.

From the Table-II, Table-III, and Scheme-1, it is observed that benzylpenicillin (**1**) would undergo lactam-enol tautomerism and form enol of benzylpenicillin (**2**) with increasing bond length of O_{30}-C_9 (1.3468 Å) and decreasing bond length of C_{11}-C_9 (1.3757 Å) with the formation of H_{32}-

O_{30} bond (0.9752Å). It is confirmed that benzylpenicillin (**1**) may undergo lactam-lactim tautomerism, and form benzylpenicillin lactim (**3**) with increasing bond length of O_{34}-C_{13} (1.3798 Å) and decreasing bond length of C_{13}-N_{12} (1.2940 Å) with the formation of H_{33}-O_{34} bond (0.9709 Å). But the formation of lactim-enol tautomerism (**4**) from benzyl penicillin (**1**) is observed with increasing bond lengths of O_{30}-C_9 (1.3486 Å) and O_{34}-C_{13} (1.3789 Å), decreasing bond lengths of C_{11}-C_9 (1.3831 Å) and C_{13}-N_{12} (1.3017 Å) with the formation of H_{32}-O_{30} bond (0.9751Å) and H_{33}-O_{34} bond (0.9690 Å).



Scheme - 1

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

The spatial arrangement of atoms in tautomers are considered to study the conformations of benzylpenicillin (**1**), and its enol form (**2**), lactim form (3) and lactim-enol form (**4**) of benzylpenicillin 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 the protonation may depend on the changes in the parameters of dihedral angles. Fully optimized AM1 calculations for the sake of simplicity, scrutinize only the main data of dihedral angles (Table-IV) of molecules (**1** to **4**).

As per Scheme-1, the H_{32}-proton shifting to O_{30}-atom in the benzylpenicillin **(1)** is predicted for the formation of enol-form(**2**). The conformations of ***–ac*** of $C_{13}N_{12}C_{11}C_9$, ***-ac*** of $O_{29}C_8C_4C_3$ and ***–sc*** of $O_{30}C_9N_7C_4$ are changed to conformation ***+sc***. Dihedral angle of $C_8C_4C_3S_2$, $O_{10}C_8C_4C_3$, $C_{15}C_{14}C_{13}N_{12}$, $H_{31}O_{10}C_8C_4$,$O_{34}C_{13}N_{12}C_{11}$ and $H_{33}N_{12}C_{11}C_9$ are changed to respectively from ***–ac*** to ***+ap***, ***+sc*** to ***–ac***, ***-sp*** to ***–sc***, ***+ap*** to ***–ap***, ***+sp*** to ***–sp*** and ***+sc*** to ***-ac*** conformations and all other conformations are moderately changed. After lactam-enol rearrangement, the enol form of benzylpenicillin (**2**) is formed with the ***+sp*** conformation in the case of dihedral angle of $H_{32}O_{30}C_9N_7$. If the H_{33}-proton shifting to O_{34}-atom in the benzylpenicillin **(1)** is predicted for the formation of lactim-form(**3**). The change of conformation from ***–ac*** of $C_8C_4C_3S_2$ and ***+sc*** of $O_{10}C_8C_4C_3$ are changed to ***+ac*** conformation. The conformation of ***–ac*** of $C_{13}N_{12}C_{11}C_9$, ***–sp*** of $C_{15}C_{14}C_{13}N_{12}$ and ***–ac*** of $O_{29}C_8C_4C_3$ are changed to ***–sc*** conformation. After lactam-lactim rearrangement, the lactim form of benzylpenicillin (**3**) is formed with the ***+sp*** conformation in the case of dihedral angle of $H_{33}O_{34}C_{13}N_{12}$.

The simultaneous shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}-atom and O_{34}- atom is predicted for the formation of lactim-enol form **(4)**. The conformations of ***–ac*** of $C_8C_4C_3S_2$ and $C_{13}N_{12}C_{11}C_9$ are changed to ***+ap*** and ***–sp*** conformations respectively to form stable conformation and rest of positions have moderate changes. Dihedral angle of $C_{15}C_{14}C_{13}N_{12}$ and $O_{34}C_{13}N_{12}C_{11}$ are changed respectively ***-sp*** to ***–sc*** and ***+sp*** to ***-sp*** conformation and all other conformations are moderately changed. It is observed that the shifting of H_{32}-proton and H_{33}-proton of benzylpenicillin **(1)** to respective O_{30}- atom and O_{34}- atom is predicted for the formation of lactim-enol form **(4)** with the formation of ***-sp*** and ***+sp*** conformations in the case of $H_{32}O_{30}C_9N_7$, and $H_{33}NO_{34}C_{13}N_{12}$ respectively.

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 benzyl penicillin(1) and its tautomers enol form(2 to 4) from AM1 calculations

Parameters	1	2	3	4
ΔH_f^o (kcal/mol)	-94.5278	-70.6933	-82.8829	-68.0049
Ionization potential (eV)	9.310	8.799	8.947	8.378
μ (Debye)	5.546	2.426	1.835	3.013
E_{HOMO} (eV)	-9.310	-8.799	-8.947	-8.379
E_{LUMO} (eV)	-0.062	+0.063	+0.086	-0.204
Electron excitation energies (eV)	9.248	8.862	9.033	8.175
S_2	+0.0366	+0.0685	+0.0935	+0.0974
N_7	-0.2584	-0.1588	-0.2572	-0.1606
N_{12}	-0.3614	-0.2917	-0.2701	-0.1957
O_{10}	-0.3225	-0.3200	-0.3178	-0.3050
O_{29}	-0.3282	-0.3540	-0.3342	-0.3755
O_{30}	-0.2396	-0.2025	-0.2740	-0.2346
O_{34}	-0.3570	-0.3412	-0.2892	-0.2875

Table –II : Bond lengths of benzyl penicillin(1) and its tautomeric forms (2 to 4) from AM1 calculations

Bond lengths (Å)	1	2	3	4
C_9-N_7	1.4512	1.4685	1.4342	1.4608
C_{11}-C_9	1.5689	1.3757	1.5659	1.3831
O_{30}-C_9	1.2176	1.3468	1.2225	1.3486
C_{13}-N_{12}	1.3873	1.3904	1.2940	1.3017
C_{14}-C_{13}	1.5176	1.5227	1.5192	1.5171
O_{34}-C_{13}	1.2452	1.2426	1.3798	1.3789
H_{32}-O_{30}	- -	0.9752	- -	0.9751
H_{33}-O_{34}	- -	- -	0.9709	0.9690

Table – III: Tautomeric Equilibrium of Benzylpenicillin

$logK_T$	Equilibrium	$logK_T$ - Values	Change of Net Charges on Hetero-atoms	
			Increasing	Decreasing
$logK_{T1}$	**1 ↔ 2**	17.47	O_{29}	S_2, N_7, N_{12}, O_{10}, O_{30}, O_{34}
$logK_{T2}$	**1 ↔ 3**	8.53	O_{29}, O_{30}	S_2, N_7, N_{12}, O_{10}, O_{34}
$logK_{T3}$	**2 ↔ 4**	1.97	N_7, O_{29}, O_{30}	S_2, N_{12}, O_{10}, O_{34}
$logK_{T4}$	**3 ↔ 4**	10.90	O_{29}	S_2, N_7, N_{12}, O_{10}, O_{30}, O_{34}
$logK_{T5}$	**1 ↔ 4**	19.44	O_{29}	S_2, N_7, N_{12}, O_{10}, O_{30}, O_{34}

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

Dihedral angle (°)	1		2		3		4	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-18.79	*-sp*	-22.38	*-sp*	-12.39	*-sp*	-18.81	*-sp*
$C_8C_4C_3S_2$	-137.46	*-ac*	+162.73	*+ap*	+129.36	*+ac*	+157.03	*+ap*
$O_{10}C_8C_4C_3$	+88.68	*+sc*	-136.12	*-ac*	+98.40	*+ac*	+64.85	*+sc*
$C_{13}N_{12}C_{11}C_9$	-127.62	*-ac*	+59.03	*+sc*	-52.15	*-sc*	-12.94	*-sp*
$C_{14}C_{13}N_{12}C_{11}$	-177.09	*-ap*	-178.79	*-ap*	-176.35	*-ap*	-179.34	*-ap*
$C_{15}C_{14}C_{13}N_{12}$	-26.76	*-sp*	-39.91	*-sc*	-84.33	*-sc*	-76.38	*-sc*
$O_{29}C_8C_4C_3$	-91.56	*-ac*	+47.29	*+sc*	-81.92	*-sc*	-117.61	*-ac*
$O_{30}C_9N_7C_4$	-52.24	*-sc*	+67.28	*+sc*	-51.95	*-sc*	+68.72	*+sc*
$H_{31}O_{10}C_8C_4$	+178.59	*+ap*	-178.41	*-ap*	+179.04	*+ap*	+179.01	*+ap*
$H_{32}O_{30}C_9N_7$	- -	- -	+26.05	*+sp*	--	- -	-0.29	*-sp*
$H_{33}O_{34}C_{13}N_{12}$	- -	- -	- -	- -	+8.21	*+sp*	+4.59	*+sp*
$O_{34}C_{13}N_{12}C_{11}$	+2.00	*+sp*	-1.27	*-sp*	+1.40	*+sp*	-1.08	*-sp*
$H_{33}N_{12}C_{11}C_9$	+56.65	*+sc*	-126.67	*-ac*	- -	- -	- -	- -

* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - Signs[16]

CONCLUSION

AM1 calculations show that benzylpenicillin tautomeres 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 ability to cross cell wall barriers, 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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