JMCDD

Journal of Medicinal Chemistry and Drug Discovery

Available online at http://www.jmcdd.com
November-December, 2016, Vol. 2, pp 1-12
ISSN: 2347-9027

Research Article

AM1 study on the electronic structure and conformations of lactim-enol tautomerism in benzylpenicillin

Mandla Venugopal[1], Papammagari Raveendra Reddy[2] and Bojja Rajeshwar Rao*
[1]Department of Chemistry, Govt. Degree College, Nandyal-518502, India
[2]Department of Chemistry, Sri Krishnadevaraya University, Anantapur-515 003, India
*Chemical Division, Kakatiya Thermal Power Project (O&M), Chelpur- 506 170, India
E-mail: rrbojja@yahoo.com; r_bojja@yahoo.com

Abstract

The geometry, conformation and electronic structure of lactim-enol tautomerism in benzylpenicillin have been optimized and calculated in the gas phase by semi-empirical molecular orbital AM1 method usually considering an isolated molecule surrounded by vacuum. In this connection, the mechanism of protonation in lactim-enol tautomer of benzylpenicillin has been studied by comparison of the different positions of net charges at nitrogen 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 discussed. The conformational analyses of mono-, di-protonated and anion of lactim-enol tautomer and their stable conformations have also been performed.

Key words: AM1, lactim-enol tautomerism, benzylpenicillin, induction effect, frontier molecular orbital.

Introduction

Benzylpenicillin is the first antibiotic for the treatment of infections caused by the most species of gram-positive bacteria[1,2,3]. The contribution of β-lactam antibiotics in chemotherapy is based on (a) a potent and rapid bacterial action against bacteria in growth phase and (b) a very low frequency of toxic and other adverse reactions in the host. The main cause of deterioration of penicillin is the reactivity of the strained β-lactam ring and the nature of degradation products are influenced by the pH of the solution. In strongly acidic solutions (pH < 3), penicillin undergoes a

complex series of reactions leading to a variety of inactive degradation products. Acid-catalysed degradation in the stomach contributes strongly to the poor oral absorption of penicillin[4]. It is readily absorbed into the blood stream where it is partially bound to plasma proteins in animals and human[5,6]. It has a high order of selective toxicity to micro-organisms which are pathogenic to human beings without side effects[7,8,9]. The importance of tautomeric equilibria has recognised for the study of the processes of both organic chemistry and biochemistry[10]. The tautomerism of organic compounds was reported extensively theoretical and statistical-physical approaches[11]. The stability of tautomers[12,13] 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].

Quantum chemistry is the field in which solutions to the Schrodinger's equation are used to predict the properties of molecules for solving chemical problems. Austin Model-1 (AM1) is one of the semi-empirical quantum chemical calculation methods 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 calculation of various properties[16,17,18]. It is important to know the conformational changes in the molecule for the prediction of its reactivity and pharmacological action. For this intention, 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 HMO study on the effect of methyl group perturbations[19,20] and AM1 study on conformational analyses[21], [1,3]sigmatropic hydrogen migration[22,23,24,25], electronic structure[26,27], correlation studies[28] and computational studies[29] were carried out. Hence, the observation of lactim-enol tautomerism in benzylpenicillin has been attracted much to carry out optimization of its anion and protonated forms with a view to investigate its polarity.

The present investigation reveals on molecular conformation and electronic properties of lactim-enol form of benzylpenicillin (**2**) and its anion and protonated forms in gas phase usually considering an isolated molecule surrounded by vacuum has been evaluated by AM1 method. It is also observed that the lactim-enol form (**2**) is less stable than benzylpenicillin (**1**). From the obtained optimized electronic structure of lactim-enol tautomerism of benzylpenicillin, the mechanism of protonation has been studied. Taking lactim-enol form of benzylpenicillin as a neutral molecule (**2**), the molecular geometry and conformations of mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) systems have been determined by full optimization calculations using semi-empirical molecular orbital AM1 method.

Computational methods[16,17]

The Austin Model 1 (AM1) semi-empirical method is a modification of MNDO, offering more accurate parameterizations for polar systems and transition states. 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. 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. In this context, the numbering of lactim-enol form of benzylpenicillin (**2)** is shown in Figure -1 and the position of the atoms in the molecule is used as subscript in the text. The initial molecular geometry was adopted as Pople's standard data[30,31], and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms[32] using *s* = syn, *a* = anti, *p* = peri-planar (0 ± 30^0 & 180 ± 30^0) and all other angles *c* = clinal.

Results & Discussion

Electronic structure of benzylpenicillin (1) and its lactim-enol tautomer (2) mono-protonated (3 & 4), di-protonated (5) and anion (6)

The optimized electronic structure of benzylpenicillin (**1**) and its lactim-enol tautomer (**2**) mono-protonated (**3** & **4**), di-protonated (**5**) and anion (**6**) are shown in Scheme-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 **6**) are presented in Table-I. It is observed that the net charges on N_7- and N_{13}- atoms are -0.1606 and -0.1957 respectively in the case of lactim-enol tautomer of benzylpenicillin (**2**). Usually, the nitrogen atom with larger negative value of net charge accepts proton more easily. It is also investigated that the sequence of protonation for nitrogen atoms of lactim-enol tautomer of benzylpenicillin (**2**) is increasing in the order of $N_7 < N_{13}$. Thus, N_{13}- atom is predicted to be main protonation site of lactim-enol tautomer of benzylpenicillin (**2**), according to the negative charge distribution on nitrogen atoms.

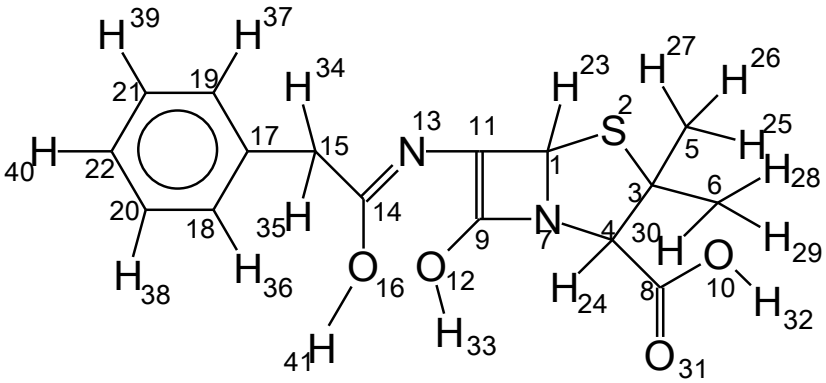


Figure - 1

The calculated values of frontier orbital energies (E_{HOMO} and E_{LUMO}) 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** to **5**, due to the presence of same sign and other molecules undergo antara-facial path way is allowed due to the opposite sign[33].

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** < **5** < **1** < **4** < **6**. Anion (**6**) 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[34] (μ_{ind}) of molecules can be estimated with respect to benzylpenicillin lactim-enol form (**2**). It is found that the induction effect is increasing in the order of $\Delta\mu_{ind}$ (**3**) -0.604 D < $\Delta\mu_{ind}$ (**5**) 1.824 D < $\Delta\mu_{ind}$ (**1**) 2.538 D < $\Delta\mu_{ind}$ (**4**) 4.880 D < $\Delta\mu_{ind}$ (**6**) 10.855 D. According to the heat of formation (ΔH_f^o) data, the stability of compounds have increased in the order of **5** < **4** < **3** < **2** < **1** < **6**. It can be assumed that the electronic properties and reactivity of the molecule depend on its conformational structure. It is predicted that the protonation would take place preferably at N_{13}-atom than N_7-atom in the case of lactim-enol form of benzylpenicillin (**2**). It is confirmed that the stability of mono-protonated enol form of benzylpenicillin **4** (ΔH_f^o, +89.2562 Kcal/mol) is less stable than **3** (ΔH_f^o, +73.3233 Kcal/mol). The lactim-enol form of di-protonated benzylpenicillin **(5)** is possible (with the heat of formation (ΔH_f^o) of +316.2056 Kcal/mol**)** from mono-protonated lactim-enol form of benzylpenicillins (**3** & **4**). However, negative atomic charges are also present on the other atoms of the molecule. The protonation at N_{13}-atom in the case of lactim-enol form of benzylpenicillin (**2**) to mono-protonated form (**3**) is considered by decreasing net atomic charges at N_7-, N_{13}-, O_{10}-, O_{12}-, and O_{16}- atoms and increased at O_{31}- atom. The protonation site of lactim-enol form of benzylpenicillin (**2**) at N_7- atom to mono-protonated form (**4**) is considered by decreasing net atomic charges at N_7-, O_{10}-, O_{12}-, O_{16}- and O_{31}-atoms and increasing at N_{13}-atom. In the case of di-protonated form (**5**), the negative atomic charges are decreased at all hetero atoms except at N_{13}-atom. Anion of lactim-enol form of benzylpenicillin (**6**) is formed by the removal of a proton on O_{10}-atom with increasing net charges at N_7-, O_{10}-, O_{12}-, O_{16}- and O_{31}-, and decreasing at N_{13}- atom.

Lactim-enol tautomerism of benzylpenicillin

Lactim-enol tautomerism of benzylpenicillin involves the shifting of hydrogen atom from α-carbon atom of keto (-HC-C=O) group of the oxygen atom for the formation of enol (-C=C-O-H) group and the shifting of hydrogen atom from α-nitrogen atom of lactam (-HN-C=O) group of the oxygen atom for the formation of lactim (-N=C-O-H) group at the same time with in the molecule as shown in Figure-**2**. In the great majority of cases the molecules at chemical

equilibrium under ordinary conditions, both forms of tautomers are possible. Instances are known when tautomeric forms which are stable under ordinary conditions 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-III) of molecules (**1** to **6**) for the sake of simplicity.

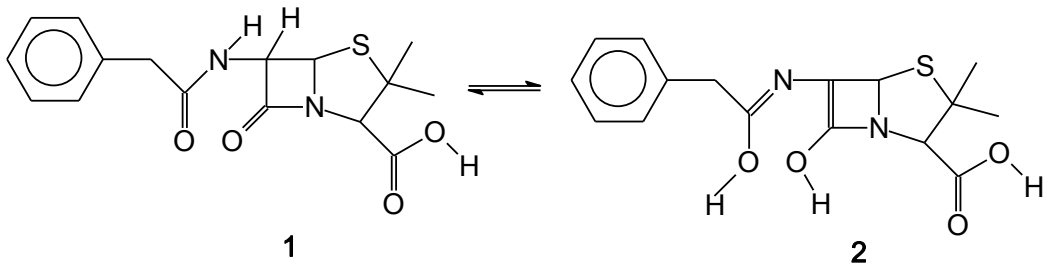


Figure - 2: Benzylpenicillin lactim-enol tautomerism

The AM1 calculated heat of formation, and the tautomeric equilibrium constants $\log K_T$ was calculated[35,36] according to the equation (2):

$$\log K_T = \frac{\Delta GT}{2.303\ RT} \approx \frac{\delta \Delta H_f^o}{2.303\ RT} \quad \text{- - - (2)}$$

Where ΔGT 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 (2), $\log K_T$ value was calculated as 19.44.

Equilibrium is normally established in polar solvents, in order to investigate the basicity and it is found out the main basic centre in accordance with the negative charge distribution on N-atoms. The exact protonation centre of lactim-enol form of benzylpenicillin (2) is determined using the proton affinities (PA) for the different nitrogen atoms of the molecule. The stable conformation of the cations formed by the protonation of each nitrogen atom of the molecule is determined from the data of heats of formation with full geometry optimization. The cations formed by the protonation at N_7- or N_{13}- atoms of lactim-enol form of benzylpenicillin (**2**) can exist in *anti*- or *syn*-conformations, according to the position of Nitrogen atoms as shown in Scheme-1. Its conformation can be assigned by comparison of its geometry and electronic structure. The proton affinity (PA)[37] values for the different nitrogen atoms of lactim-enol form of benzylpenicillin RH (**2**) were calculated by using the equation (1) and found to be 225.8718 kcal/mol and 209.9389 kcal/mol respectively in the case of mono-protonated benzylpenicillins (

3 and **4**). Di-protonated form (**5**) was formed from either of mono-protonated benzylpenicillins (**3** and **4**) respectively with PA 124.3177 kcal/mol and 140.2506 kcal/mol.

$$PA = \Delta H_f^o(H^+) + \Delta H_f^o(B) - \Delta H_f^o(BH^+) \quad \ldots (1).$$

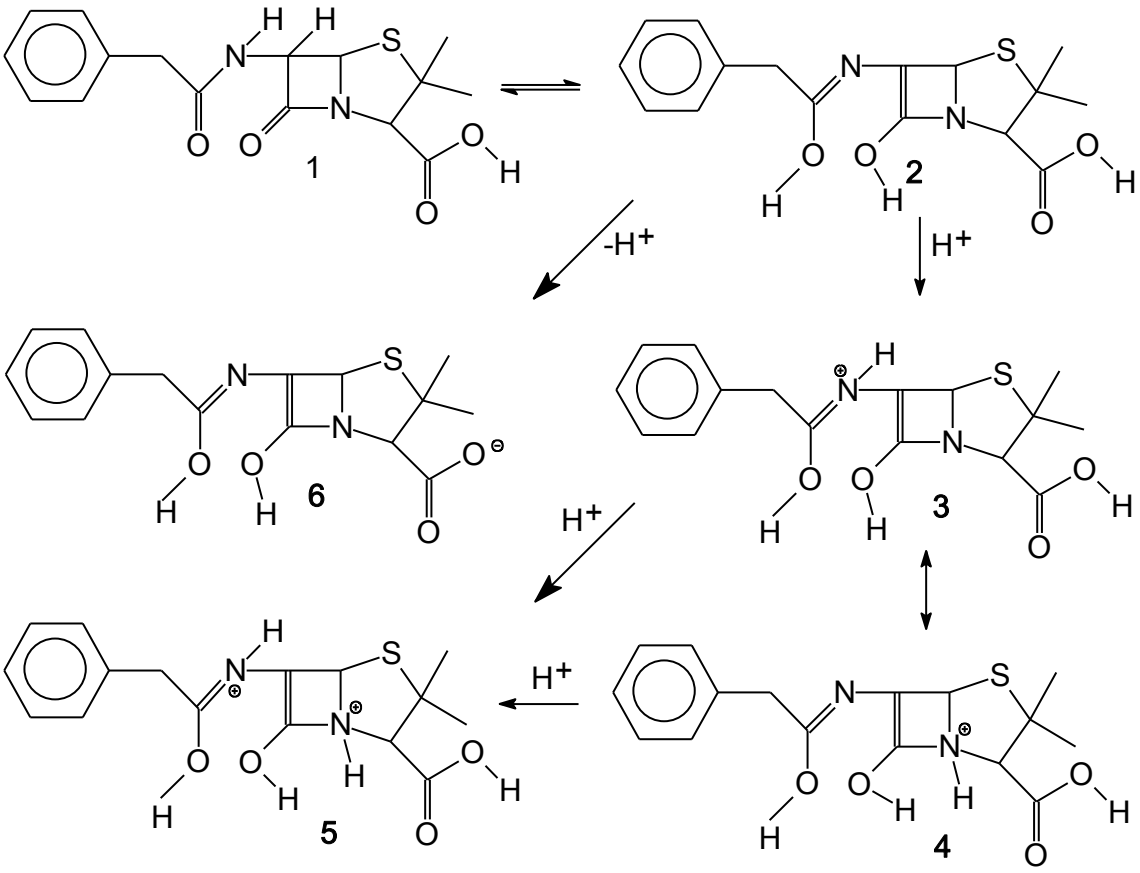
Where PA is the proton affinity, $\Delta H_f^o(B)$ is the heat of formation for enol form of benzylpenicillin (**2**), $\Delta H_f^o(BH^+)$ is the heat of formation for the cation, and $\Delta H_f^o(H^+)$ is heat of formation for the proton (367.2kcal/mol). The proton affinity is in the order of N_{13} (225.8718 kcal/mol) > N_7 (209.9389 kcal/mol) and mono-protonated benzylpenicillin lactim-enol form (**4**) appears to be more stable. All cations 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 increased in the order of **1** < **2** < **6** < **3** < **4** < **5**. It is confirmed that benzylpenicillin (**1**) is more stable than its lactim-enol form (**2**).

It is confirmed that benzylpenicillin (**1**) would undergo lactim-enol tautomerism of benzylpenicillin (**2**) with increasing bond lengths of O_{12}-C_9 (1.3486 Å), O_{16}-C_{14} (1.3789 Å) and decreasing bond lengths of C_{11}-C_9 (1.3831 Å), C_{14}-N_{13} (1.3017 Å), with the formation of H_{13}-O_{12} (0.9751 Å) and H_{41}-O_{16} (0.9690 Å) bonds.

The change of conformation from *–ac* of $C_8C_4C_3S_2$, $C_{14}N_{13}C_{11}C_9$ and $N_{13}C_{11}C_9N_7$, are changed respectively to *+ap*, *-sp* and *-ap* conformations. Dihedral angle of $O_{16}C_{14}N_{13}C_{11}$ and $C_{17}C_{15}C_{14}N_{13}$ are changed respectively *+sp* to *–sp* and *-sp* to *-sc* conformations. Lactim-enol form of benzylpenicillin (**2**) is formed with ***–sp*** and ***+sp*** conformations respectively in the case of dihedral angle of $H_{33}O_{12}C_9N_7$ and $H_{41}O_{16}C_{14}N_{13}$.

The conformations of lactim-enol form of benzylpenicillin (2) and its mono-protonated (3 & 4), di-protonated (5) and anion (6)

The spatial arrangement of atoms in a molecule is considered to study the conformations of benzylpenicillin (**1**), and its lactim-enol form of benzylpenicillin (**2**), mono-protonated forms (**3 & 4**), di-protonated form (**5**) and anion (**6**) 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 scrutinize only the main data of bond lengths (Table-II) and dihedral angles (Table-III) of molecules (**1** to **6**) for the sake of simplicity.



Scheme - 1

From the Table-II, and Table-III, it is observed that as per Scheme-1, mono-protonated lactim-enol form of benzylpenicillin (**3**) is formed by the addition of proton at N_{13}-atom of lactim-enol tautomer of benzylpenicillin (**2**), with increasing bond lengths at O_{12}-C_9, H_{33}-O_{12}, C_{14}-N_{13}, N_{13}-C_{11}, H_{41}-O_{16}, H_{32}-O_{10} and decreasing bond lengths at O_{16}-C_{14}, O_{10}-C_8. The conformations of $C_{14}N_{13}C_{11}C_9$, and $O_{16}C_{14}N_{13}C_{11}$ are changed from -*sp* to +*sp*, the conformation of $C_{15}C_{14}N_{13}C_{11}$ is changed from -*ap* to -*ac* and all other conformations are moderately changed. It is observed that the protonation at N_{13}-atom in the case of $HN_{13}C_{11}C_9$ is shown -*ap* conformation.

If the mono-protonated benzylpenicillin lactim-enol (**4**) is formed by the addition of proton at N_7- atom of benzylpenicillin lactim-enol tautomer (**2**), with increasing bond lengths at C_{14}-N_{13}, H_{33}-O_{12}, H_{41}-O_{16}, C_9-N_7 and decreasing bond lengths at C_{11}-C_9, O_{12}-C_9, N_{13}-C_{11}, O_{16}-C_{14}, O_{10}-C_8. The conformations of $C_{14}N_{13}C_{11}C_9$, and $O_{16}C_{14}N_{13}C_{11}$ are changed from -*sp* to +*sp*, the conformations of $H_{32}O_{10}C_8C_4$, $N_{13}C_{11}C_9N_7$, $H_{33}O_{12}C_9N_7$, and $C_{17}C_{15}C_{14}N_{13}$ are changed from +*ap* to –*ap*, -*ap* to +*ap*, -*sp* to -*sc* and –*sc* to –*ac* respectively and all other conformations are moderately changed. It is observed that the protonation at N_7-atom in the case of $HN_7C_4C_3$ is shown –*ac* conformation.

The formation of di-protonated benzylpenicillin lactim-enol (**5**) is observed by the addition of protons at N_7- and N_{13}-atoms of benzylpenicillin lactim-enol tautomer (**2**). It is evidenced with increasing bond lengths at C_{14}-N_{13}, H_{33}-O_{12}, H_{41}-O_{16}, C_9-N_7, N_{13}-C_{11}, C_{15}-C_{14}, H_{32}-O_{10} and decreasing bond lengths at C_{11}-C_9, O_{12}-C_9, O_{16}-C_{14}, O_{10}-C_8. It is found that the dihedral angle of $N_{13}C_{11}C_9N_7$, $H_{33}O_{12}C_9N_7$, $C_{14}N_{13}C_{11}C_9$, $C_{15}C_{14}N_{13}C_{11}$ and $C_{17}C_{15}C_{14}N_{13}$ are changed conformation, *-ap* to *-ap*, *-sp* to *-sc*, *-sp* to *+sp*, *-ap* to *+ap* and *-sc* to *-ac* conformations respectively and all other conformations are unaltered. It is also investigated that the protonation at N_7- atom and N_{13}-atom are shown respectively *-ac* and *-ap* conformations to form stable di-protonated lactim-enol benzylpenicillin (**5**).

It can be concluded that the anion (**6**) is formed with the removal of a proton on O_{10}-atom of benzylpenicillin lactim-enol tautomer (**2**), with increasing bond lengths at C_{11}-C_9, H_{33}-O_{12}, O_{16}-C_{14}, O_{31}-C_8 and decreasing bond lengths at C_9-N_7, O_{12}-C_9, O_{10}-C_8. The conformations of $O_{10}C_8C_4C_3$ and $O_{31}C_8C_4C_3$ are changed from *+sc* to *–ac* and *–ac* to *+sc* respectively to form stable anion (**6**) and rest of positions have moderate changes.

Conclusion

AM1 calculations show that lactim-enol tautomerism of benzylpenicillin and its protonated forms are nearly non-planar skeleton geometry, and the sequence of proton transfer at nitrogen atom is $N_{13} > N_7$. All protonated 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 hydrolysis and the nature of degradation products are influenced by the pH of the solution. In strongly acidic solutions (pH < 3), penicillin undergoes a complex series of reactions leading to a variety of inactive degradation products. Acid-catalysed degradation in the stomach contributes strongly to the poor oral absorption of penicillin. This study reveals about the stability of tautomers, conformations and molecular deformations.

Acknowledgements

This research project has been carried out by one of the authors (M Venugopal) with permission of Commissioner, Collegiate Education AP, Hyderabad and thanks for cooperation and encouragement.

References

1. Shechan JC, Henery-logan KR, *J Amer Chem Soc*,1962; **84:** 2983-90.
2. Stewart GT, *The Penicillin Group of Drugs*, Elsevier Publishing Co., Amsterdam-longon-NewYark, 1965, 46-64.
3. Kulikova DA, Radkewich TP, Rudzith EA, *Antibiotiki*, 1973; **7:**835.
4. Hou, J P, Poole J W, *J Pharm Sci*, 1971; **60**,503.
5. Bogan JA, *Pharmacological Basis of Large Animal Medicine*, Ed. Bogan JA, Lees P, Yoxall, Blackwell AT, 1983; 3-22.
6. John H Block, John M Beak Jr, *Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry*, 11th edn., (Lippincott Williams & Wilkins, NewYork), 2004.
7. Kaiser GV, Kukolja S, *Cephalosporins and Penicillins*, E H Flynn, Ed. Academic Press, New York & London, 1972; 74-131.
8. Perlman D, Eds, *Structure Activity Relationships among the semi-synthetic Antibiotics*, Academic press, New York, 1977.
9. Morin RB, Gorman M, Eds, *Chemistry and Biology of Beta-lactam Antibiotics*, Volumes 1-3, (Academic press, New York), 1982.
10. Elguero J, Marzin C, Katritzky AR, Linda P, *The Tautomerism of Heterocycles*, Academic Press, New York, 1976.
11. Kwiatkowski JS, Zielinski TJ, Rein R, *Adv Quant Chem*, 1986; **18:** 85.
12. Karelson MM, Katritzky AR, Szafran M, Zerner Mc, *J Org Chem*, 1989; **54:** 6030.
13. Karlson MM, Tamm T, Katritzky AR, Cato SJ, Zerner MC, *Tetrahedron Comput Methodol*, 1989; **2:** 295.
14. Karlson MM, Katritzky AR, Szafran M, Zerner MC, *J Chem Soc Perkin Trans-2*, 1990; 195.
15. Daehne WV, Frederiksen E, Gundersen E, Lund F, Morch P, Persen HJ, Roholt K, Tybring L, Godtfredsen WO, *J Med Chem*, 1970; **13(4):** 607-612..
16. Dewar MJS, Zeobisch EG, Healy EF, Stewart JJP, *J Am Chem Soc*, 1985; **107:** 3902-3909.
17. Stewart JJP, *MOPAC, A general molecular orbital package, QCPE 455*, 5th edn, 1988.
18. Coppola BP, *Chem Educator*, 1997; **2(2):** 1-8. Springer-verlag New York, INC.
19. Rajeshwar Rao B, *Indian J. Chem*, 2000; **39B:** 154-155.
20. Rajeshwar Rao Bojja, *Indian J. Chem*,2002; **41B(8):** 1694 -1696.
21. Rajeshwar Rao Bojja, *Indian J. Chem*.2002; **41B(8):** 1697-1701.
22. Rajeshwar Rao Bojja, *Indian J. Chem*, 2003; **42B(12):** 3081-3088.
23. Rajeshwar Rao Bojja, *Indian J. Chem*, **2006; 45B(9)**: 2083-2090.
24. Rajeshwar Rao Bojja, *Indian J. Chem*, 2009; **48B (10):**1411- 1415.
25. Rajeshwar Rao Bojja, *Int J Res Pharm and Biomed Sci*, 2011; **3(1):** Jan-Mar, 275-281. www.ijrpbsonline.com.
26. Rajeshwar Rao Bojja, Chandra Sekhar Rao D, Sanjeeva Reddy CH, *Indian J. Chem*, 2012; **51B (1):** 307-312.
27. Venugopal M, Raveendra Reddy P, Rajeshwar Rao B, *Int J Org Bioorg Chem*, 2012; **2(1):** 1-6. www.urpjournals.com.

28. Ranga Reddy S, Rajeshwar Rao B, Manikyamba P, *Indian J. Chem*, 2007; **46(A):** 436-439.
29. Ranga Reddy S, Kalyani P, Rajeswara Rao B, Manikyamba P, *Indian J Chem*, 2008; **47A(2):** 236-239.
30. Pople JA, Beveridge DL, *Approximate molecular orbital theory*, McGraw-Hill, New York, 1970.
31. Fleming I, *Frontier Orbital and Organic Chemical Reactions*, Wiley-Interscience, New York, 1976.
32. Klyne W, Prelog V, *Experientia*, 1960; **16:** 521.
33. Woodward RB, Hoffmann R, *The conservation of orbital symmetry*, Academic press, Inc, New York, 1970.
34. Paperno TYA, Pozdnyakov VP, Smirnova AA, Elagin LM, *Physico-Chemical Laboratory Techniques in Organic and Biological Chemistry* (Translated from Russian by Oleg Glebov), MIR Publishers, Moscow, 1979; 171.
35. Kresge J, *Acc Chem Res*, 1990; **23:** 43.
36. Lochmuller CH, Maldacker T, Cefola M, *Anal Chim Acta*, 1969; **48:** 139.
37. Saltek N, Abbasogulu R, Ikizler A, *Acta chim hung, Models in chemistry*, 1996; **133**: 43-51.

****

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 lactim-enol form(**2**), mono-protonated forms (**3** & **4**), di-protonated form (**5**), and anion (**6**) from AM1 calculations.

Parameters	**1**	**2**	**3**	**4**	**5**	**6**
ΔH_f^o (kcal/mol)	-94.5278	-68.0049	+73.3233	+89.2562	+316.2056	-106.9222
Ionization potential (eV)	9.310	8.378	12.138	11.865	14.799	5.116
μ (Debye)	5.546	3.013	2.409	7.893	4.837	13.868
E_{HOMO} (eV)	-9.310	-8.379	-12.138	-11.865	-14.800	-5.116
E_{LUMO} (eV)	-0.062	-0.204	-4.760	-4.603	-8.728	+2.300
Electron excitation energies (eV)	9.248	8.175	7.378	7.262	6.072	7.416
S_2 (atomic charge)	+0.0366	+0.0974	+0.1277	+0.2640	+0.3047	-0.0263
N_7 (atomic charge)	-0.2584	-0.1606	-0.1438	-0.0208	-0.0381	-0.1626
N_{13} (atomic charge)	-0.3614	-0.1957	-0.0993	-0.2617	-0.2023	-0.1507
O_{10} (atomic charge)	-0.3225	-0.3050	-0.2782	-0.2781	-0.2492	-0.5831
O_{12} (atomic charge)	-0.2396	-0.2346	-0.2300	-0.1917	-0.2096	-0.2673
O_{16} (atomic charge)	-0.3570	-0.2875	-0.1884	-0.2395	-0.1464	-0.3103
O_{31} (atomic charge)	-0.3282	-0.3755	-0.3874	-0.3438	-0.3678	-0.5303

Table –II : Bond lengths of benzyl penicillin(**1**) and its lactim-enol form(**2**), mono-protonated forms (**3** & **4**), di-protonated form (**5**), and anion (**6**) from AM1 calculations.

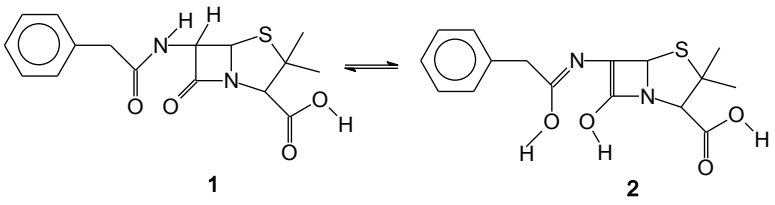
Bond lengths (Å)	**1**	**2**	**3**	**4**	**5**	**6**
C_9-N_7	1.4512	1.4608	1.4534	1.5088	1.5089	1.4486
C_{11}-C_9	1.5689	1.3831	1.3866	1.3789	1.3709	1.3922
O_{12}-C_9	1.2176	1.3486	1.3779	1.3378	1.3312	1.3377
H_{33}-O_{12}	- -	0.9751	0.9839	0.9827	0.9907	0.9888
C_{14}-N_{13}	1.3873	1.3017	1.3337	1.3200	1.3564	1.2984
N_{13}-C_{11}	1.4092	1.3601	1.3778	1.3446	1.3790	1.3586
O_{16}-C_{14}	1.2452	1.3789	1.3432	1.3589	1.3283	1.3873
C_{15}-C_{14}	1.5177	1.5171	1.5202	1.5206	1.5272	1.5141
H_{41}-O_{16}	- -	0.9690	0.9786	0.9746	0.9828	0.9689
O_{10}-C_8	1.3651	1.3617	1.3517	1.3523	1.3419	1.2573
O_{31}-C_8	1.2294	1.2351	1.2366	1.2316	1.2341	1.2645
H_{32}-O_{10}	0.9716	0.9731	0.9839	0.9776	0.9824	- -
H-N_{13}	- -	- -	1.0137	- -	1.0135	- -
H-N_7	- -	- -	- -	1.0197	1.0241	- -

Table – III Dihedral angle (^{o}) of benzyl penicillin (**1**) and its lactim-enol form (**2**), mono-protonated forms (**3** & **4**), di-protonated form (**5**), and anion (**6**) from AM1 calculations.

Dihedral angle (^{o})	**1**		**2**		**3**		**4**		**5**		**6**	
	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)	Angle	(*)
$C_4C_3S_2C_1$	-18.79	***-sp***	-18.81	***-sp***	-17.94	***-sp***	-24.18	***-sp***	-28.40	***-sp***	-18.51	***-sp***
$C_8C_4C_3S_2$	-137.46	***-ac***	+157.03	***+ap***	+155.52	***+ap***	+154.13	***+ap***	+158.47	***+ap***	+159.19	***+ap***
$O_{10}C_8C_4C_3$	+88.68	***+sc***	+64.85	***+sc***	+68.44	***+sc***	+76.94	***+sc***	+73.46	***+sc***	-105.15	***-ac***
$H_{32}O_{10}C_8C_4$	+178.60	***+ap***	+179.00	***+ap***	+178.75	***+ap***	-179.42	***-ap***	+178.94	***+ap***	- -	-
$O_{31}C_8C_4C_3$	-91.56	***-ac***	-117.61	***-ac***	-122.64	***-ac***	-103.27	***-ac***	-107.48	***-ac***	+74.41	***+sc***
$N_{13}C_{11}C_9N_7$	-125.24	***-ac***	-168.66	***-ap***	-175.38	***-ap***	+177.65	***+ap***	+174.99	***+ap***	-168.53	***-ap***
$H_{33}O_{12}C_9N_7$	- -	- -	-0.29	***-sp***	-13.09	***-sp***	-88.18	***-sc***	-66.22	***-sc***	-24.24	***-sp***
$C_{14}N_{13}C_{11}C_9$	-127.63	***-ac***	-12.94	***-sp***	+5.38	***+sp***	+12.03	***+sp***	+29.61	***+sp***	-12.56	***-sp***
$O_{16}C_{14}N_{13}C_{11}$	+2.00	***+sp***	-1.08	***-sp***	+0.18	***+sp***	+0.42	***+sp***	-2.74	***-sp***	-0.29	***-sp***
$H_{41}O_{16}C_{14}N_{13}$	- -	- -	+4.59	***+sp***	+3.11	***+sp***	+6.93	***+sp***	+5.91	***+sp***	+2.78	***+sp***

$C_{15}C_{14}N_{13}C_{11}$	-177.09	***-ap***	-179.34	***-ap***	-117.64	***-ac***	-179.02	***-ap***	+178.52	***+ap***	-178.61	***-ap***
$C_{17}C_{15}C_{14}N_{13}$	-26.76	***-sp***	-76.38	***-sc***	-49.37	***-sc***	-90.70	***-ac***	-100.29	***-ac***	-76.68	***-sc***
$HN_7C_4C_3$	- -	- -	- -	- -	- -	- -	-144.25	***-ac***	-147.93	***-ac***	- -	- -
$HN_{13}C_{11}C_9$	- -	- -	- -	- -	-176.22	***-ap***	- -	- -	-153.81	***-ap***	- -	-
* Conformational analyses using prefixes *a* = anti, *s* = syn, *p* = peri-planar, *c* = clinal, and + & - signs[32].												

Graphical Abstract

Page No	AM1 study on the electronic structure and conformations of lactim-enol tautomerism in benzylpenicillin Mandla Venugopal Papammagari Raveendra Reddy Bojja Rajeshwar Rao	The geometry, conformation and electronic structure of lactim-enol tautomerism in benzylpenicillin have been optimized and calculated in the gas phase by semi-empirical molecular orbital AM1 method usually considering an isolated molecule surrounded by vacuum. In this connection, the mechanism of protonation in lactim-enol tautomer of benzylpenicillin has been studied by comparison of the different positions of net charges at nitrogen 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 discussed. The conformational analyses of mono-, di-protonated and anion of lactim-enol tautomer and their stable conformations have also been performed.  1 2