

## AM1 study on the conformations of zwitterions of 6-aminopenicillanic acid (6-APA)

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The geometry and electronic structure of zwitterions of 6-aminopenicillanic acid (6-APA) have been optimized and calculated by semi-empirical molecular orbital AM1 method. The mechanism of formation of zwitterions of 6-APA has been studied by comparison of the different positions of net charges on nitrogen atoms in the molecule. In this connection, the heats of formation ( $\Delta H_f^\circ$ ), dipole moments ( $\mu$ ), ionization potentials (IP), full atomic charges, and energies of frontier molecular orbital ( $E_{HOMO}$  and  $E_{LUMO}$ ) have been performed and discussed. The effect of conformational changes and electronic properties of stable conformations have been determined.

**Keywords:** 6-APA, zwitterions, AM1, frontier molecular orbital

**IPC:** Int.Cl.<sup>8</sup> C 07 D

The significance of 6-aminopenicillanic acid (6-APA) has been recognized as the essential intermediate for the production of penicillin antibiotics<sup>1</sup>. Isolation<sup>2</sup>, synthesis<sup>3</sup> and identification<sup>4</sup> of new pencillins<sup>5,6</sup> have been reported in the medicinal use. The dipolar character<sup>7</sup> of the molecule has been expected to influence and improve the oral absorption deeply. As a part of ongoing research towards the molecular conformations and electronic properties of 6APA (**1**)<sup>8</sup>, we have studied the theoretical investigation on the zwitterions [ $RH^\pm$  (**2** and **3**)] of 6-APA. The mechanism of proton transfer in 6-APA has been studied by comparison of the different positions of net charges on nitrogen atoms in the molecule. It would be important to know the exact position of protonation centers<sup>9</sup>. Taking 6-APA, as a neutral molecule (RH) **1**, the molecular geometry and electronic structures of zwitterions  $RH^\pm$  (**2** and **3**) systems, in which are included ( $N_{12}H^\pm$ ) **2** and ( $N_7H^\pm$ ) **3** have been determined by full optimization calculations, using semi-empirical molecular orbital AM1 method.

### Computational methods<sup>9</sup>

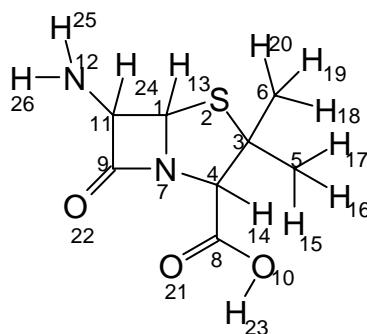
Semi-empirical molecular orbital calculations were performed using the AM1 (Austin Model 1) method

included in the MOPAC93 in WinMOPAC ver 5.13 program by means of Intel Celron-333MHz PC. Geometry calculations in the ground state (keywords: PRECISE, equivalent to GNORM=1.0, 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 were adopted as Pople's standard data<sup>10</sup>, and subsequently fully optimized using an energy gradient method. The conformations were designated by Klyne-Prelog terms<sup>11</sup> using *s* = syn, *a* = anti, *p* = peri-planar ( $0\pm30^\circ$  &  $180\pm30^\circ$ ) and all other angles *c* = clinal.

### Results and Discussion

#### The molecular geometry of zwitterions $RH^\pm$ ( $N_{12}H^\pm$ ) **2** and ( $N_7H^\pm$ ) **3**

The spatial arrangements of atoms in a molecule are considered to study the conformations of zwitterions  $RH^\pm$  ( $N_{12}H^\pm$ ) **2** and ( $N_7H^\pm$ ) **3** with a view to undergoing molecular deformations. In this context, the changes in energy content of the zwitterion may depend on the changes in the



6-Aminopenicillanic acid

Figure 1

**Table I**—Bond lengths (Å) of 6-APA and its zwitterions (**1-3**) from AM1 calculation

Bond lengths (Å)	RH <b>1</b>	RH <sup>±</sup> (N <sub>12</sub> H <sup>±</sup> ) <b>2</b>	RH <sup>±</sup> (N <sub>7</sub> H <sup>±</sup> ) <b>3</b>
C <sub>1</sub> -S <sub>2</sub>	1.7817	1.7619	1.7509
S <sub>2</sub> -C <sub>3</sub>	1.8226	1.8408	1.9192
C <sub>3</sub> -C <sub>4</sub>	1.5605	1.5491	1.5577
C <sub>3</sub> -C <sub>5</sub>	1.5090	1.5068	1.5031
C <sub>4</sub> -N <sub>7</sub>	1.4520	1.4653	1.4865
C <sub>4</sub> -C <sub>8</sub>	1.5140	1.5667	1.6144
N <sub>7</sub> -C <sub>9</sub>	1.4496	1.4518	1.5173
C <sub>8</sub> -O <sub>10</sub>	1.3576	1.2830	1.2542
C <sub>9</sub> -C <sub>11</sub>	1.5633	1.5858	1.5867
C <sub>11</sub> -N <sub>12</sub>	1.4145	1.4705	1.4033
C <sub>8</sub> -O <sub>21</sub>	1.2343	1.2432	1.2407
C <sub>9</sub> -O <sub>22</sub>	1.2173	1.2147	1.2018
O <sub>10</sub> -H <sub>22</sub>	0.9731	----	----
N <sub>7</sub> -H	----	----	1.0507
N <sub>12</sub> -H	----	1.0233	----

parameters of the bond lengths, bond angles and dihedral angles. **Figure 1** illustrates the atomic numbering of 6-APA (**1**). Fully optimized AM1 calculations scrutinize only the main data of bond lengths (**Table I**), bond angles (**Table II**), and dihedral angles (**Table III**) of molecules (**1** to **3**) for the sake of simplicity. It has been found that the bond length of N<sub>7</sub>-H (1.0507 Å) in (N<sub>7</sub>H<sup>±</sup>) **3** system is larger than that of N<sub>12</sub>-H bond (1.0233 Å) in (N<sub>12</sub>H<sup>±</sup>) **2** system. From the study it can be concluded that the sequence of proton transfer for nitrogen atoms in 6-APA (**1**), agrees well with the results attained.

From **Tables I** to **III**, it can be concluded that the zwitterion RH<sup>±</sup> (N<sub>12</sub>H<sup>±</sup>) **2** is formed by the proton transfer between O<sub>10</sub><sup>-</sup> atom to N<sub>12</sub><sup>-</sup> atom with

**Table II**—Bond angles (°) of 6-APA and its zwitterions (**1-3**) from AM1 calculation.

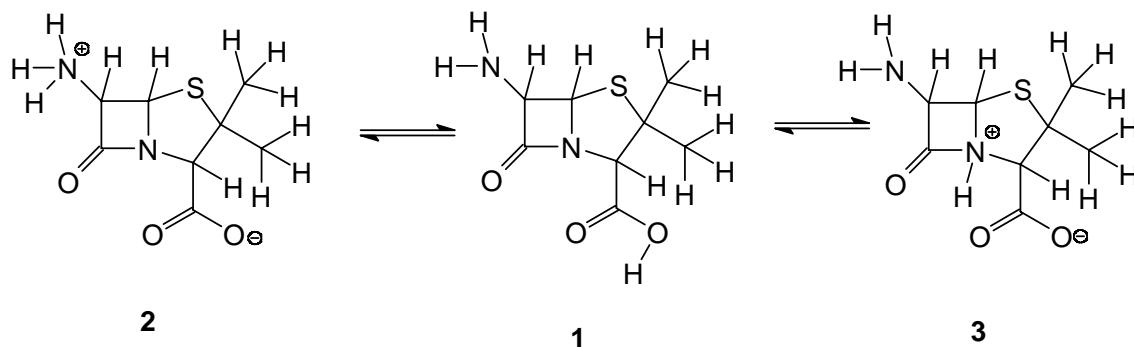
Bond angles (°)	RH <b>1</b>	RH <sup>±</sup> (N <sub>12</sub> H <sup>±</sup> ) <b>2</b>	RH <sup>±</sup> (N <sub>7</sub> H <sup>±</sup> ) <b>3</b>
C <sub>1</sub> S <sub>2</sub> C <sub>3</sub>	94.63	93.88	88.07
S <sub>2</sub> C <sub>3</sub> C <sub>4</sub>	104.34	106.20	107.83
S <sub>2</sub> C <sub>3</sub> C <sub>5</sub>	108.28	107.34	104.07
C <sub>3</sub> C <sub>4</sub> N <sub>7</sub>	110.60	111.23	105.48
C <sub>3</sub> C <sub>4</sub> C <sub>8</sub>	112.72	109.76	110.21
C <sub>4</sub> N <sub>7</sub> C <sub>9</sub>	123.84	116.86	139.84
C <sub>4</sub> C <sub>8</sub> O <sub>10</sub>	114.76	118.14	114.20
C <sub>9</sub> C <sub>11</sub> N <sub>12</sub>	116.30	112.34	114.76
S <sub>2</sub> C <sub>1</sub> H <sub>13</sub>	110.01	111.21	104.55
C <sub>3</sub> C <sub>4</sub> H <sub>14</sub>	107.18	108.72	111.59
C <sub>4</sub> C <sub>8</sub> O <sub>21</sub>	128.04	117.91	114.15
N <sub>7</sub> C <sub>9</sub> O <sub>22</sub>	132.43	136.60	130.15
C <sub>8</sub> O <sub>10</sub> H <sub>23</sub>	109.44	----	----
C <sub>9</sub> C <sub>11</sub> H <sub>24</sub>	110.81	116.17	109.13
C <sub>4</sub> N <sub>7</sub> H	----	----	101.66
C <sub>11</sub> N <sub>12</sub> H	----	109.78	----

**Table III**—Dihedral angles (°) of 6-APA and its zwitterions (**1-3**) from AM1 calculation.

Dihedral angles (°)	RH <b>1</b>		RH <sup>±</sup> (N <sub>12</sub> H <sup>±</sup> ) <b>2</b>		RH <sup>±</sup> (N <sub>7</sub> H <sup>±</sup> ) <b>3</b>	
	Angle	*	Angle	*	Angle	*
C <sub>1</sub> S <sub>2</sub> C <sub>3</sub> C <sub>4</sub>	-19.15	-sp	16.35	+sp	19.17	+sp
S <sub>2</sub> C <sub>3</sub> C <sub>4</sub> N <sub>7</sub>	30.21	+sp	-23.52	-sp	9.37	+sp
S <sub>2</sub> C <sub>3</sub> C <sub>4</sub> C <sub>8</sub>	161.80	+ap	104.04	+ac	128.28	+ac
C <sub>3</sub> C <sub>4</sub> N <sub>7</sub> C <sub>9</sub>	80.91	+sc	125.26	+ac	-151.03	-ap
C <sub>3</sub> C <sub>4</sub> C <sub>8</sub> O <sub>10</sub>	-166.8	-ap	-96.08	-ac	-92.94	-ac
C <sub>4</sub> N <sub>7</sub> C <sub>9</sub> C <sub>11</sub>	-123.5	-ac	-109.61	-ac	135.06	+ac
N <sub>7</sub> C <sub>9</sub> C <sub>11</sub> N <sub>12</sub>	117.40	+ac	107.41	+ac	-139.74	-ac
C <sub>3</sub> S <sub>2</sub> C <sub>1</sub> H <sub>13</sub>	129.3	+ac	119.40	+ac	72.35	+sc
C <sub>3</sub> C <sub>4</sub> C <sub>8</sub> O <sub>21</sub>	18.94	+sp	84.82	+sc	86.87	+sc
C <sub>4</sub> N <sub>7</sub> C <sub>9</sub> O <sub>22</sub>	57.36	+sc	82.57	+sc	-47.98	-sc
C <sub>4</sub> C <sub>8</sub> O <sub>10</sub> H <sub>23</sub>	179.9	+ap	----	----	----	----
C <sub>3</sub> C <sub>4</sub> N <sub>7</sub> H	----	--	----	--	78.56	+sc
C <sub>9</sub> C <sub>11</sub> N <sub>12</sub> H	----	--	-141.85	-ac	----	--

\*Conformational analyses<sup>11</sup> using prefixes *a* = *anti*, *s* = *syn*, *p* = *peri-planar*, *c* = *clinal*, and + & - signs.

decreasing bond lengths of C<sub>1</sub>-S<sub>2</sub>, C<sub>3</sub>-C<sub>4</sub> and C<sub>8</sub>-O<sub>10</sub> by 0.0198 Å, 0.0114 Å and 0.0746 Å respectively and increasing of S<sub>2</sub>-C<sub>3</sub>, C<sub>4</sub>-C<sub>8</sub>, C<sub>9</sub>-O<sub>11</sub>, C<sub>11</sub>-N<sub>12</sub> and C<sub>8</sub>-O<sub>21</sub> by 0.0182 Å, 0.0527 Å, 0.0225 Å, 0.056 Å and 0.0089 Å, respectively. Bond angles are decreased in the case of C<sub>3</sub>C<sub>4</sub>C<sub>8</sub>, C<sub>4</sub>N<sub>6</sub>C<sub>9</sub>, C<sub>9</sub>C<sub>11</sub>N<sub>12</sub> and



### Scheme I

**Table IV** — Ionization potential (IP), dipole moment ( $\mu$ ), energies of frontier molecular orbitals ( $E_{\text{HOMO}}$  and  $E_{\text{LUMO}}$ ), heats of formation ( $\Delta H_f^\circ$ ), the atomic charges on N<sub>7</sub> and N<sub>12</sub> atoms of 6-APA and its zwitterions (**1-3**) from AM1 calculation

Molecule	$\Delta H_f^\circ$ (kcal/mol)	IP (eV)	$\mu$ (Debye)	$E_{\text{HOMO}}$ (eV)	$E_{\text{LUMO}}$ (eV)	$N_7$ (atomic charge)	$N_{12}$ (atomic charge)
RH <b>1</b>	-81.1212	9.1066	2.9003	-9.107	-0.126	-0.2389	-0.3376
RH $^\pm$ (N <sub>12</sub> H $^\pm$ ) <b>2</b>	-40.5877	9.0704	11.1696	-9.070	-0.394	-0.1819	-0.0873
RH $^\pm$ (N <sub>7</sub> H $^\pm$ ) <b>3</b>	-7.2451	9.1368	12.2404	-9.137	-1.258	-0.0946	-0.3667

$C_4C_8O_{21}$  from  $10.13^\circ$  to  $2.96^\circ$  and increased in the case of  $S_2C_3C_4$ ,  $C_4C_8O_{10}$ ,  $S_2C_1H_{13}$ ,  $C_3C_4H_{14}$ ,  $N_7C_9O_{22}$  and  $C_9C_{11}H_{24}$  from  $1.2^\circ$  to  $5.36^\circ$ . Dihedral angle is changed conformation from  $-sp$  to  $+sp$ ,  $+sc$  to  $-sp$ ,  $+ap$  to  $+ac$ ,  $+sc$  to  $+ac$ ,  $-ap$  to  $-ac$ , and  $+sp$  to  $+sc$  in the case of  $C_1S_2C_3C_4$ ,  $S_2C_3C_4N_7$ ,  $S_2C_3C_4C_8$ ,  $C_3C_4N_7C_9$ ,  $C_3C_4C_8O_{10}$  and  $C_3C_4C_8O_{21}$  respectively and all other positions are altered insignificant.

If the transfer of proton between  $O_{10}^-$  atom to  $N_7^-$  atom forms the zwitterion  $RH^\pm (N_7H^\pm)$  **3**, the bond lengths are increased from 0.0234 Å to 0.1 Å in the case of  $S_2-C_3$ ,  $C_4-N_7$ ,  $C_4-C_8$ ,  $N_7-C_9$ , and  $C_9-C_{11}$  bonds. Bond lengths are decreased from 0.1 Å to 0.0112 Å in the case of  $S_2-C_1$ ,  $C_8-O_{10}$ , and  $C_{11}-N_{12}$  bonds. Bond angles of  $C_1S_2C_3$ ,  $S_2C_3C_5$ ,  $C_3C_4N_7$ ,  $C_3C_4C_8$ ,  $C_9C_{11}N_{12}$ ,  $S_2C_1H_{13}$ ,  $C_4C_8O_{21}$  and  $N_7C_9O_{22}$  are decreased from  $13.89^\circ$  to  $1.54^\circ$  and simultaneously increased in the case of  $S_2C_3C_4$ ,  $C_4N_7C_9$  and  $C_3C_4H_{14}$  by  $3.49^\circ$ ,  $16.0^\circ$  and  $4.41^\circ$ , respectively. Dihedral angle of  $C_1S_2C_3C_4$  of  $-sp$  and  $S_2C_3C_4N_7$  of  $+sc$  are changed to  $+sp$ . The change of conformations from  $+ap$  of  $S_2C_3C_4C_8$  and  $-ac$  of  $C_4N_7C_9C_{11}$  are changed to  $+ac$ .  $-ap$  of  $C_3C_4C_8O_{10}$  and  $+ac$  of  $N_7C_9C_{11}N_{12}$  are converted to  $-ac$  conformation.  $+ac$  of  $C_3S_2C_1H_{13}$  and  $+sp$  of  $C_3C_4N_7O_{21}$  are converted to  $+sc$  conformation.  $+sc$  of  $C_3C_4N_7C_9$  and  $C_4N_7C_9O_{22}$  dihedral angles are changed to  $-ap$  and  $-sc$ , respectively with the

formation dihedral angles of  $C_3C_4N_7H$  by  $+sc$  conformation.

## Electronic structure of zwitterions $\text{RH}^\pm$ ( $\text{N}_{12}\text{H}^\pm$ ) 2 and ( $\text{N}_7\text{H}^\pm$ ) 3

The optimised electronic structure of 6-APA **1**, and its zwitterions  $\text{RH}^\pm$  ( $\text{N}_{12}\text{H}^\pm$ ) **2** and ( $\text{N}_7\text{H}^\pm$ ) **3** are shown in **Scheme I**. Moreover, the calculated heats of formation ( $\Delta\text{H}_f^\circ$ ), ionization potentials (IP), dipole moments ( $\mu$ ), the energies of frontier molecular orbitals ( $E_{\text{HOMO}}$  and  $E_{\text{LUMO}}$ ), and net atomic charges at  $\text{N}_7$ - and  $\text{N}_{12}$ - atoms of the molecules (**1** to **3**) are presented in **Table IV**. The net charges on  $\text{N}_7$ - and  $\text{N}_{12}$ - atoms are -0.2380 and -0.3368, respectively in the case of neutral molecule 6-APA **1**. Usually, the nitrogen atom with larger value of net charge accepts proton more easily. Thus, the sequence of protonation for nitrogen atoms in 6-APA, which should be consistent with relative values of net charges on nitrogen atom is  $\text{N}_{12} > \text{N}_7$ . Zwitterion ( $\text{N}_7\text{H}^\pm$ ) **3** shows higher ionization potential and dipole moment. The calculated values of frontier orbital energies ( $E_{\text{HOMO}}$  and  $E_{\text{LUMO}}$ ) reveal that zwitterion  $\text{RH}^\pm$  ( $\text{N}_{12}\text{H}^\pm$ ) **2** has more electron donor character than ( $\text{N}_7\text{H}^\pm$ ) **3**, which has more electron-acceptor property. However, in a photochemical reaction, promotion of an electron from HOMO to LUMO, the suprafacial pathway is allowed due to the presence of same sign. In the case of HOMO, the electron density is highest

at N<sub>12</sub>-atom for molecules **1** and **3**. The results so obtained reveal that the electronic properties and reactivity of the molecule depend on its conformational structure. From the reactivity point of view, N<sub>12</sub>-atom is to be main protonation site of 6-APA molecule **1**, according to the negative charge distribution on nitrogen atoms. The stability of the formed zwitterion RH<sup>±</sup> (N<sub>12</sub>H<sup>±</sup>) **2** is relatively higher than that of zwitterion RH<sup>±</sup> (N<sub>7</sub>H<sup>±</sup>) **3**, due to its heats of formation is relatively lower.

In order to investigate the basicity and to find out the proton transfer of 6-APA molecule **1**, containing different N-atoms is important. N<sub>12</sub>-atom is predicted to be the main basic center in accordance with the negative charge distribution on N-atoms (**Table IV**). The stable conformations of zwitterions (**2** and **3**) are forming with the proton transfer to alternate nitrogen atom of the molecule. In addition, the heats of formation are calculated with full geometry optimization by AM1. The zwitterions can exist in *anti*- or *syn*- conformation, according to the position of N-atom. The conformational analyses of zwitterions reveal about molecular deformations.

### The equilibrium of zwitterions (**2** and **3**)

Equilibrium is normally established in polar solvents by rapid inter- or intra-molecular proton transfer between O<sub>11</sub>-atom and N<sub>7</sub>- and N<sub>12</sub>-atoms of 6-APA (**Scheme I**). When one zwitterion is formed predominantly in a polar solution, its conformation can be assigned by comparison of its geometry and electronic structure. The positions of proton transfer equilibrium can be affected by the nature of the solvent and concentration of the solution<sup>12</sup>. The proton affinities (PA)<sup>13</sup> for the different nitrogen atoms of 6-APA molecule were calculated by using the Equation 1.

$$PA = \Delta H_f^\circ (H^+) + \Delta H_f^\circ (B) - \Delta H_f^\circ (BH^+) \quad \dots (1)$$

Where PA is the proton affinity,  $\Delta H_f^\circ (B)$  is the heat of formation for the molecule,  $\Delta H_f^\circ (BH^+)$  is the heat of formation for the cation, and  $\Delta H_f^\circ (H^+)$  is the heat of formation for the proton (367.2 kcal/mol). It can be assumed that  $\Delta H_f^\circ (H^+)$  is to be neglected in polar medium, due to the inter- or intra-molecular proton transfer in the equilibrium as per the Equation



Thus, the Equation 1 becomes:

$$PA = \Delta H_f^\circ (RH) - \Delta H_f^\circ (RH^\pm) \quad \dots (3)$$

Where  $\Delta H_f^\circ (RH)$  is the heat of formation of 6-APA (**1**) and  $\Delta H_f^\circ (RH^\pm)$  is the heat of formation of zwitterions (**2** or **3**). A comparison of PA of zwitterion RH<sup>±</sup> (N<sub>12</sub>H<sup>±</sup>) **2** (-40.5334 kcal/mol) is higher than zwitterion RH<sup>±</sup> (N<sub>7</sub>H<sup>±</sup>) **3**. However, zwitterion **2** appears to be more stable. Polar solvents can effect the positions of the equilibrium, because the zwitterions are solvated in their ability to form hydrogen bonds with the solvent. Thus, the chemical reactions of the zwitterionic mixtures are related particularly to transfer protons rapidly between the atoms in the molecule. The isoelectric point<sup>2</sup> of 6-APA with minimum solubility was determined at pH 4.3.

### Conclusion

AM1 calculations show that zwitterions of 6-APA are nearly non-planar skeleton geometry, and the sequence of proton transfer at nitrogen atoms is N<sub>12</sub> > N<sub>7</sub>. Solubility of zwitterions (**2** and **3**) in polar solvents is more due to the dipole moment change. In view of the fact that the proton transfers sites of 6-APA molecule is predicted to be the main basic center in accordance with the negative charge distribution on N-atoms. All these studies reveal about the stability of zwitterions and their conformations.

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