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Received: 18/5/2011 Accepted: 21/6/2011

ABSTRACT

In this paper xanthine (C$_5$H$_4$N$_4$O$_2$, MW=152) drug (M$_1$) as a parent molecule and three of its dimethyl substituted isomers, namely, 3,7-dimethylxanthine (M$_2$), 1,3-dimethylxanthine (M$_3$) and 1,7-dimethylxanthine (M$_4$) of formula (C$_7$H$_8$N$_4$O$_2$, MW = 180), were investigated using electron ionization (EI) mass spectrometry (MS) at 70 eV. Semi-empirical MO-calculations, PM3 procedure, have been carried out on the drugs as neutral molecule and its corresponding molecular ion. These include molecular geometry (bond length), bond order, charge distribution on different atoms, heat of formation ($\Delta H_f$) and ionization energy. The mass spectral fragmentation pathways of the parent molecule (M$_1$) and the isomeric molecules (M$_2$-M$_4$) were proposed. All compounds dissociate in the gas phase by common pathways, initiated in the same site of bond rupture resulting a loss of imide group. Subsequently, a loss of CO followed by HCN, and formation of different fragment ions depends on the isomeric position of the methyl groups. The PM3 procedure provides information of initial bond cleavage and subsequent fragmentation of the molecules. Finally, the influence of CH$_3$ groups in different isomeric position via the two rings of xanthine caused by significant electron-donating of this group were discussed.

Key Words: Xanthine / Mass Spectrometry / Molecular Orbital Calculation / PM3.

INTRODUCTION

Xanthine drugs are a class of purine alkaloid caffeine, they play an important roles in biochemistry and pharmacology because they are metabolic intermediate products of purine metabolism formed by degradation of nucleic acid [1]. A few studies reported of ionization phenomena of Xanthine. There are mass spectrometric studies using electron impact at 70 eV [2]. Electrospray ionization followed by collisionally activated dissociation has been used to study the fragmentation pathways of Xanthine derivatives [3-4]. The structure formula and numbering system of xanthine (M$_1$) and its dimethyl isomers (M$_2$-M$_4$) are presented in Fig. (1).

![Fig. (1): Structure and numbering system of xanthine (M$_1$), 3,7-dimethylxanthine (M$_2$), 1,3-dimethylxanthine (M$_3$) and 1,7-dimethylxanthine (M$_4$)](image-url)
Rapid advances in biological sciences have led to an increased demand for chemical and structural information of biological systems. Mass spectrometry plays a pivotal role in the structural characterization and metabolism study of biological molecules. The technique is important because it provides a large amount of structural information with a little expenditure of sample. In electron ionization (EI) mass spectrum, the fragmentation consists of series of competitive and consecutive unimolecular fragmentation. At 70 eV the spectra are very complex; it is difficult to uncover all the competing and consecutive fragmentation reactions. On the other hand, computational quantum chemistry can provide additional information about the atoms and bonds, since the rupture takes place at weakest bond, which can be used successfully in an interpretation of experimental results. Combined the experimental results with the data obtained from theoretical calculations gives a valuable information about the atoms and bonds which helps in the description and prediction of the first site of bond rupture and subsequent one. Application of this combined techniques were discussed in many articles.

The main aim of the present work is to carry out experimental and theoretical investigation on Xanthine and three of its isomers namely 3,7-dimethylxanthine, 1,3-dimethylxanthine and 1,7-dimethylxanthine using electron impact (EI) mass spectral (MS) fragmentation at 70eV. Also, MO calculations are performed using PM3 procedure, on the neutral molecules and charged molecular ion to investigate the geometrical parameters (bond length), bond order, atomic charge distribution, ionization energy and heats of formation. PM3, calculations are correlated with results obtained of MS experimental technique about the stability of the drugs and prediction of the site of primary fragmentation steps and subsequent one. Also, the isomeric effect on different theoretical and experimental results is discussed.

**EXPERIMENTAL**

**Mass spectrometry (MS):**

Electron ionization (EI) mass spectra of xanthine and its isomers were obtained using Shimadzu GC-MS-Qp 1000 PX quadruple mass spectrometer with electron multiplier detector equipped with GC-MS data system. The direct probe (DP) for solid material was used in this study. The sample was put into a glass sample micro vial, by a needle (~ 1 µg max), the vial installed on the tip of the DP containing heating cable and inserted into the evacuated ion source. The sample was ionized by electron beam emitted from the filament, the generated ions being effectively introduced into the analyzer by the focusing and extractor lenses system. The MS was continuously scanned and the obtained spectra were stored. Electron ionization mass spectra were obtained at ionizing energy value of 70 eV, ionization current of 60 µA and vacuum is better that 10^{-6} torr.

**COMPUTATIONAL METHOD**

The MO calculations were performed using semi-empirical molecular orbital calculation. The method used in these computations is the parametric method (PM3) described by Stewart. The default criteria for terminating all optimizations were increased by a factor of 100 (keyword PRECISE). Vibrational frequencies were computed for the studied structures (keyword FORCE) so as to check whether the newly designed geometries are local minima. All the molecular orbital calculations were carried out at the restricted Hartree-Fock level (RHF) for the neutral molecule of pentoxifylline while the unrestricted Hartree-Fock level (UHF) were carried out for its cation by using PM-3 method followed by full optimization of all geometrical variables (bond lengths, bond angles, and dihedral angles), without any symmetry constraint. All structures were optimized to a gradient norm 0.01-0.05, using the eigenvector following (EF) routine. All the semi empirical MO calculations were performed with the MOPAC2000 software package implemented on an Intel Pentium IV 3.0 G Hz computer.
RESULTS AND DISCUSSION

It is important to compare the results obtained from xanthine as a parent molecule with its dimethyl isomers in both experimental and semi-empirical MO-calculations. This combination is very important to understand the following topics:

1- Primary site fragmentation process, and its major fragmentation pathway.
2- Stability of the drug as neutral molecule in solid state phase and molecular ion in gas phase.
3- Comparing the results obtained from xanthine as parent molecule with dimethyl isomeric drug.
4- Rationalized the primary site of bond rupture and subsequently one.
5- Substituent effect of two methyl groups on the fragmentation pathways and forming different ions.

Mass spectral behavior of xanthine and three of its di-methyl isomers.

Electron ionization (EI) mass spectra of xanthine (M₁) drug (as a parent molecule) and three of its dimethyl isomers (M₂-M₄) were recorded (as a bar graph) and investigated. The signal appear at m/z =152 (M₁) and at m/z =180 (M₂-M₄), is that due to the formation of molecular ion. All compounds exhibits the molecular ion as a base peak (R.I=100%), reflecting the high stability aromatic heterocyclic purine ring system toward fragmentation, due to the presence of four nitrogen and two oxygen hetero-atom in all compounds. The spectrum of xanthine was characterized by only two prominent fragment ions at m/z= 109 and m/z=64 (Fig.2) due to loss of imide molecule CONH and successive loss of CO+HCN molecule. On the other hand, the isomeric molecules are characterized by a wealth a moderate intensity fragment ion (Fig.3, a-c) including the loss of imide and CO+HCN and fragments related to two CH₃ addition.

Fig. (2) : Mass spectra of Xanthine at 70 Ev.

Computational:

Molecular orbital (MO) calculations gives a valuable information about the structure and reactivity of the molecules which actually be used to support the experimental data. The much important parameters calculated using MO-calculation include geometries, bond order, charge distribution, heat of formation and ionization energy.
Fig. (3) : Mass Spectra of 3,7-dimethylxanthine (a), 1,3-dimethylxanthine (b), and 1,7-dimethylxanthine (c).
In the present work the calculations have been carried out on Xanthine drug and its dimethyl isomers for neutral molecule and charged species. While are important for prediction of the weakest bond rupture to fragmentation pathways of the titled compounds.

Table (1) presents the values of bond length (Å), bond order from which one can concluded that:

1. Small differences in bond length in all compounds upon ionization, indicating no appreciable change in the geometries upon ionization.
2. No appreciable change in bond length due to ionization that no geometrical change

The charge distribution on different atoms (C, N and O) and heats of formation \( \Delta H \) (kJ mol\(^{-1}\)) for neutral and charged xanthine and its dimethyl isomers and the values of ionization energy (eV) for neutral are summarized in fig 4. Significant changes in the electron distribution with in a given system take place during the ionization process \^{18}. The greatest change occur in negative charge as the results of electron rupture is that observed in N\(_3\) for all the molecules (I.e. loss of negativity) are -0.320 (M\(_1\)), -0.334 (M\(_2\)), 0.349 (M\(_3\)) and -0.308 (M\(_4\)). The stability of drugs decrease upon ionization by a values is decreased by values; 607.77 (M\(_1\)), 574.19 (M\(_2\)), 588.00 (M\(_3\)) and 588.23 (M\(_4\)) kJ mol\(^{-1}\).

\[ \Delta H_f = -209.64 \text{ kJmol}^{-1} \]
\[ \Delta H_f = 607.75 \text{ kJmol}^{-1} \]
\[ \text{IE} = 9.22 \text{ eV} \]

\[ \Delta H_f = -214.24 \text{ kJmol}^{-1} \]
\[ \Delta H_f = 574.19 \text{ kJmol}^{-1} \]
\[ \text{IE} = 9.07 \text{ Ev} \]

\[ \Delta H_f = -256.06 \text{ kJmol}^{-1} \]
\[ \Delta H_f = 588.00 \text{ kJmol}^{-1} \]
\[ \text{IE} = 9.11 \text{ eV} \]

\[ \Delta H_f = -206.64 \text{ kJmol}^{-1} \]
\[ \Delta H_f = 588.23 \text{ kJmol}^{-1} \]
\[ \text{IE} = 9.04 \text{ eV} \]

Fig. (4): Charge distribution of xanthine (M\(_1\)), 3,7-dimethylxanthine (M\(_2\)) 1,3dimethylxanthine (M\(_3\)) and 1,7-dimethylxanthine (M\(_4\)) for neutral molecules (value in brackets are cation species) and heat of formation \( \Delta H \) (kJmol\(^{-1}\)).
Table (1): Computed bond length (in Å) and bond orders using PM3 method for neutral and cation species.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Xanthine (M1) Bond length (Å)</th>
<th>Bond order</th>
<th>3,7-dimethylxanthine (M2) Bond length (Å)</th>
<th>Bond order</th>
<th>1,3-dimethylxanthine (M3) Bond length (Å)</th>
<th>Bond order</th>
<th>1,7-dimethylxanthine (M4) Bond length (Å)</th>
<th>Bond order</th>
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<tr>
<td>N1-C2</td>
<td>1.431 1.421 1.038 1.070</td>
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<td>1.433 1.420 1.033 1.074</td>
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<td>1.447 1.427 1.011 1.067</td>
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<td>N1-C6</td>
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<td>1.429 1.440 1.046 1.006</td>
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<td>1.481 1.448 1.025 1.000</td>
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<td>1.445 1.449 1.019 0.994</td>
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<td>C2-N3</td>
<td>1.425 1.488 1.063 0.894</td>
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<td>1.441 1.497 1.031 0.882</td>
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<td>1.444 1.502 1.025 0.872</td>
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<td>1.435 1.486 1.046 0.896</td>
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<td>C2-O10</td>
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<td>N3-C4</td>
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<td>1.416 1.364 1.063 1.290</td>
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<td>1.407 1.351 1.078 1.347</td>
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<td>1.400 1.425 1.390 1.214</td>
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<td>1.400 1.430 1.392 1.189</td>
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<tr>
<td>C5-C6</td>
<td>1.450 1.472 0.990 0.928</td>
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<td>1.451 1.469 0.990 0.938</td>
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<td>1.222 1.210 1.780 1.887</td>
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<td>1.384 1.421 1.341 1.175</td>
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<td>1.361 1.335 1.476 1.656</td>
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<td>1.390 1.422 1.294 1.125</td>
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<tr>
<td>N1-CH3</td>
<td>--- --- --- ---</td>
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<td>--- --- --- ---</td>
<td>1.482 1.491 0.961 0.941</td>
<td>1.482 1.490 0.961 0.941</td>
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<tr>
<td>N3-CH3</td>
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<td>1.476 1.463 0.968 0.981</td>
<td>1.477 1.462 0.968 0.983</td>
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<tr>
<td>N7-CH3</td>
<td>--- --- --- ---</td>
<td></td>
<td>1.462 1.463 0.978 0.974</td>
<td>--- --- --- ---</td>
<td>1.462 1.463 0.978 0.975</td>
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</table>
Correlation of the mass spectral (MS) behavior and the MO-calculation for xanthine as apparent ion and its dimethyl isomers

The scope of the present investigation is restricted to a search for prediction discern features of initial bond ruptures during the course of fragmentation of studied compound and complex molecules, indicate that the course of subsequent fragmentation is determined to a large extent by the initial bond rupture of molecular ion in mass spectrometry[8]. It is quite acceptable to say that computational quantum chemistry can provide additional data which can be used successfully for interpretation of the MS experimental results. These theoretical data can, particularly, valuable for mass spectral scientists; they study in gas-phase species, which can be handled much more easily by quantum chemistry than those surrounded by solvent [18]. Mass spectra of the titled compounds reveal many competitive processes, including principal fragmentation pathways. In the present study the author focused on these principle pathways. Initial cleavage in xanthine and all its isomers is thought to involve rupture of C2-N3 and N1-C6 forming as CO-NR where R is hydrogen or methyl. To rationalized the main fragmentation pathway of the titled compounds, a correlation between experimental (MS) and computational (PM3) are carry out to determine the first site of bond rupture and subsequent one. Firstly, the above correlation was discussed for xanthine (M1) molecule as apparent one. Initial cleavage is though to involve a rupture of C2-N3 bond. PM3 procedure show that the C2-N3 bond has the smallest bond order (table 1) at a value = 0.894 with a large bond length 1.488Å, followed by N1-C6 bond rupture forming an stable ion (b) and rupture of imide molecule CO-NH2. On the other hand, two factors can help the faciliating this bond rupture.

1- Electrostatic repulsion between the positively charged localized on C3(0.160) and N3(0.477).
2- The weakness of the C2-N3 bond due to the electron rupture during the ionization process from N3 (IE =9.22 eV) Fig. 4. Since this rupture occur from N3 atom (Table 1) and the change of electron distribution occur at -0.321.

Imide loss from xanthine, forming a stable fragment (b) Scheme (1). i.e \([C_5H_4N_4O_2]^+\) (m/z= 109, R.I= 68.6%). The stabilization of this fragment is provided by the carbonyl group at C6, which forms an acyl ion conjugated with the imidazol ring. Subsequent loss of this CO fragment also can occur (bond order = 0.928). The remaining fragment ion (3-nitrogen atom can expelled one nitrogen atom by HCN loss forming a four member more stable ring.

The pathway of the main process can be rationalized as :

\[ \text{Xanthine} \quad [C_5H_4N_4O_2]^+ \quad m/z=152 \quad \text{R.I.} = 100\% \]

(a)  \[ \text{m/z}=109 \quad \text{R.I.} =68.6\% \]

(b)  \[ \text{m/z}=81 \quad \text{R.I.} =9.1\% \]

(c)  \[ \text{m/z}=54 \quad \text{R.I.} =82.8\% \]

(d)  \[ \text{Scheme (1): Fragmentation pathway of xanthine (1)} \]
The stability of neutral molecule is decreases upon ionization by 817.33 kJmol\(^{-1}\) \((\Delta H_2^\text{r} \Delta H_1^\text{r} = 574.19 \text{kJmol}^{-1})\). Secondly, the behavior of the three isomeric compounds toward the fragmentation and formation of different ions in comparison with xanthine itself is discussed. All the isomeric compounds can dissociate in the gas phase by common pathway due to imide rupture as xanthine. PM3 procedure (Table 1) show that C\(_2\)-N\(_3\) bonds for all isomeric compounds (M\(_2\)-M\(_4\)) have the smallest bond order; 0.882 (M\(_2\)), 0.872 (M\(_3\)) and 0.896 (M\(_4\)). These bonds are the first bond ruptures in the three molecules, subsequent one is due to rupture of N\(_1\)-C\(_6\) and loss of imide molecule; CO-NH (M\(_2\)) and Co-CH\(_3\) (M\(_3\) and M\(_4\)). Following imide rupture the behavior of the isomeric molecules obey common fragmentation pathways by successive loss of CO followed by HCN. These fragmentation pathways can illustrate in scheme (2).

Scheme (2): The fragmentation pathway of 3,7-dimethylxanthine (2), 1,3-dimethylxanthine (3), and 1,7-dimethylxanthine (4).
REFERENCES