Molecular Structure-Reactivity Study of Para-Methyl Maleanilic Acid Using Mass Spectrometry, Thermal Analyses and Semi-Empirical Molecular Orbital Calculation

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ABSTRACT

The chemistry and reactivity of maleanilic acid (MA) derivatives have always been of great interest because of their importance and biological activities. In the present work p-methyl maleanilic acid (C_{11}H_{11}NO_{3}) compound (P-MMA) was investigated using thermal analyses (TA) measurements (TG/DTA) in comparison with EI mass spectral (MS) fragmentation at 70 and at some different low energies 10,11,12eV. Semi-empirical MO calculations using PM3 procedure have been carried out on P-MMA both as neutral molecule and the corresponding positively charged molecular ion. These include molecular geometry, bond order, and charge distribution, heats of formation and ionization energy. It is concluded that this compound passed through two steps of fragmentation via rupture of the weakest bond C1-C2 to give CO_{2} gas molecule by decomposition of COOH functional group. As a result of this rupture active ingredient of mole mass 160 or fragment ion m/z=160 is formed. It is followed by the rupture of the second weak bond C4-N8 which leads to thermal fragment of mole mass 106 and competitive active mass fragment ions of m/z=106 or 107. This study is thought to be helpful in establishing a quantitative and qualitative structure—activity relationship for the given compound, which is the main objective of the present work.

Keywords: p-methymaleanilic acid (P-MMA) / MS / TA / MO Calculation / Structure-reactivity relationship.

INTRODUCTION

The chemistry and reactivity of maleanilic acid (MA) derivatives have always been of great interest because of their importance and biological activities. They have many synonymous and IUPAC names. Maleanilic itself has a systematic name (2Z)-4-Anilino-4-oxo-2-butenoic acid, 4-anilino-4-oxo-2-butenoic acid; 2-butenoic acid, 4-oxo-4-(phenyl amino)- and another alternative name N-phenyl-maleamic acid. Many alternative procedures are reported in the literature (1-6) for their preparation, but little bit about their chemical and biological properties (7, 8). However, studies about their decomposition or fragmentation in relation to their molecular structure stabilities are scanty in the literature. Therefore this paper is mainly focused on studying molecular structure-reactivity relationship of p-methyl-maleanilic acid (P-MMA) via investigation of its decomposition mechanisms using thermal (TA) and mass (MS) techniques in comparison with the theoretical molecular orbital calculations.

The mass spectrometry has become a powerful tool for studying decomposition mechanism of medical and biological compounds (9). The technique is important because it provides a large amount of structural information with little expenditure of sample. In electron impact (EI) mass spectrum, the fragmentation consists of series of competitive and consecutive uni-molecular fragmentation (10). The fragmentation of ionized molecule depends mainly on their internals energy (11). At 70 eV the spectra are very complex; it is difficult to uncover all the competing and consecutive fragmentation reactions. Lowering the energy of the ionized electron beam is possible to make the spectra simpler and the high energy processes are thus suppressed (12).

Thermogravimetric (TG/DTG) analysis is used to provide quantitative information on weight losses due to decomposition and/or evaporation of low molecular material as a function of time and temperature. In conjunction with mass spectrometric analysis (13-15), the nature of the released volatile components may be deduced, thus greatly facilitating the interpretation of thermal degradation processes.

On the other hand, computational quantum chemistry can provide additional information, which can be successfully used for the interpretation of experimental results (16) which may use in the description and prediction of primary fragmentation processes and subsequent one.
The fragmentation in both EI-MS and TA techniques (17) are similar in that rupture takes place at weak bonds. In TA, the molecules are continuously energized and deactivated by a gas evolution, and the distribution of energy can be described by a temperature. In EI-MS, the ion produced is formed with a specific amount of internal energy, which is conserved independently in all subsequent dissociations. During the ionization process, transitions to various states are possible leading to a collection of ions with a distribution of internal energies. This will be quite different from the thermal distribution, and will differ with electron energy and nature of the molecular species. In MS, the dissociation does not immediately follow ionization, but it is slow enough to permit the transfer of energy into the various degree of freedom involved in the observed dissociation(18).

The main aim of the present work is to carry out experimental and theoretical investigation on P-MMA using thermal analyses (TA) and EI mass spectrometry (MS) fragmentation at 70eV and at low energies of 10,11,12eV. Also, MO calculations (19-20) are performed using PM3 procedure on the neutral molecules and charged molecular ion to investigate the geometrical parameters, heat of formation, bond orders and the atomic charge distribution. PM3 calculations are then correlated with the obtained experimental results of both TA and MS experimental techniques about the stability of the P-MMA and predication of the site of primary fragmentation step and subsequent ones. This study is thought to be helpful in establishing a quantitative and qualitative structure—activity relationship for the given compound, which is the main objective of the present work. It is worth mentioning here that, the literature about such comparative study is scanty.

EXPERIMENTAL

Mass Spectrometry (MS)

Electron ionization (EI) mass spectra of P-MMA were obtained using Thermo Finnegan TRACE DSQ quadrupol mass spectrometer with electron multiplier detector equipped with a GC–MS data system. The direct probe for solid material was used in this study. EI mass spectra were obtained at ionizing energy values of 10, 11, 12 and 70eV, the vacuum is better than 10⁻⁶torr.

Thermal Analyses (TA)

The TA studies of P-MMA were made using conventional thermal analyzer (Shimadzu system of DSC-50 and 30 series thermal analyses instrument TG-50) at the Microanalytical center Cairo University. The mass losses of a 5 mg sample and heat response of the changes in the sample were measured from room temperature up to 600 °C. The heating rate was 10°C min⁻¹ in an inert argon atmosphere. These instruments were calibrated using indium metal as a thermally stable material. The reproducibility of the instrument readings was determined by repeating each experiment more than twice.

Quantum-chemical Calculations (MOC) of the P-MMA Structural Data

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 (21). All the molecular orbital calculations were carried out at the restricted Hartree-Fock level (RHF) for the neutral molecule of P-MMA while calculations for the unrestricted Hartree-Fock level (UHF) were carried out for its cation by using PM-3 method followed by optimization of geometrical variables (bond lengths and bond order), without any symmetry constraint. All structures were optimized to a gradient norm 0.01-0.05, using the eigenvector following (EF) routine (22). All the semi empirical MO calculations were performed with the MOPAC2012 software package (23) implemented on an Intel Pentium IV 3.0 G Hz computer.

RESULTS AND DISCUSSION

The chemistry and reactivity of P-MMA compound have always been of great interest because of its importance due to its biological activities. Knowledge of the thermal decomposition mechanism of P-MMA and other related compounds is very important in order to understand the chemical processes shared in biological systems. It is difficult to establish the exact major fragmentation pathway in EI using conventional MS. Combination of the experimental techniques (MS and TA) and MO calculation is very important to understand the following topics:

1- The stability of the given compound as neutral and ionized forms.
2- The primary sites of fragmentation mechanism of this compound.
3- The major fragmentation pathways in both techniques.
4- The selection of the most probable decomposition pathway of P-MMA by both TA and MS.

Para-methyl maleanilic acid (C₁₁H₁₁NO₂) compound is of an amino acid character. It has a geometrical structure as shown in Fig. 1. This geometrical structure and numbering system of P-MMA will
be used to discuss all data obtained by thermal (neutral molecule), mass (charged molecule) and molecular orbital calculations (MOCs) in relation to structure-reactivity of the given compound.

Fig. (1): The geometrical structure and numbering system of P-MMA.

**Thermal Analyses (TA)**

The TG/DTA curves of P-MMA (Fig.2a) were displayed between 25 to 600°C. The compound has the general formula C_{11}H_{11}NO_3 in solid-state phase with molecular weight MW=250 and melting point (m.p.) = 180°C.

It is clear from the thermal analyses results that this compound decomposed in one step as indicated by the TG/DTG curve (Fig 2a), but there are three steps of decomposition of this compound are detected by DTA data (Fig 2b). The first one appeared at 150 -220°C with mass loss% 21.46 may be attributed to the loss of CO_2 (calculated mass loss%=21.5). The second mass loss is approximately = 32.5% and occurs at 250 - 350°C which may be due to the loss of OCH-C≡CH part. The third one occurred at 410- 520°C which may be attributed to the loss of the remainder part of this compound. These mass losses were observed at the three main peaks at values of 171, 354 and 464.6°C in DTA respectively. The appearance of the first endothermic peak may be attributed to the loss of carboxylic group as CO_2 gas molecule and the other two exothermic peaks may be attributed to the possible rupture of bonds (C1-C2 and C4-N8 respectively) and other chemical rearrangements occur in a molecule during its thermal degradation as explained by scheme 1. The obtained fragments coming from thermal degradation of neutral molecule P-MMA will be confirmed by both mass (MS) and molecular orbital calculations (MOCS).

Fig. (2a): TG/DTG curve of P-MMA.

Fig. (2b): DTA curve of P-MMA
Scheme (1): Thermal decomposition pathways of P-MMA

Mass Spectral Fragmentation of P-MMA

A typical mass spectrum (bar-graph) of the compound P-MMA at 70 eV is shown in Fig.3. The main fragmentation pathways following electron ionization of P-MMA may be rationalized to eliminate of the molecular ion \([\text{C}_{11}\text{H}_{11}\text{NO}_3]^+, \text{M}^+\) of \(m/z = 205\) as represented by scheme 2. The low relative intensity (RI\% = 20\%) of the main molecular ion of P-MMA at 70 eV may be attributed to this high energy which leads to its parallel fragmentation through pathways (1-4).

Fig. (3): Mass spectrum of P-MMA molecules at 70 eV

Scheme (2): Mass fragmentation pathways of P-MMA at 70 eV
The appearance of the fragment ions at \( m/z = 160 \) (RI\% = 16), at \( m/z = 107 \) (RI = 100) as a base peak and at \( m/z = 105.9 \) (RI\% = 68) confirm the fragments obtained in thermal decomposition (TA) (scheme 1) of mole masses 160, 107 and 106 respectively. The proposed fragmentation pathway reveals four fragmentation pathways that can be rationalized in scheme 2. Process 1 reveals the formation of the fragment ion at \( m/z=106 \) \([\text{CH}_2\text{C}_6\text{H}_5-\text{NH}_2]^+\) by direct C-C bond rupture i.e. NH-CO bond rupture, this fragment ion was formed at threshold with benzyl structure \((\Delta H_f=204.59\text{ kcal mol}^{-1})\), which need small energy to transformed to tropylium structure \((\Delta H_f=207.50 \text{ kcal mol}^{-1})\). The second fragment ion may be given by \([\text{C}_7\text{H}_8\text{N}]^+\) of \( m/z=106 \) or \( m/z=107 \) (p-toluidine, process 2). The fragment ion \([\text{C}_7\text{H}_8\text{N}]^+\) cab be obtained by C3-N4 bond rupture which can rearranged to benzylic ion or to tropylium fragment ion. The formation of the fragment ion \([\text{C}_7\text{H}_9\text{N}]^+\) at \( m/z=107 \) may be obtained by direct rupture of C4-N8 bond and H-migration.

Electron ionization mass spectrum (EI) of P-MMA at low energies (10, 11 and 12 eV) were recorded (Fig. 4) and investigated aiming to show how the RI% of fragment ion is varied with the variation of eV power. The signal observed as \( m/z=205 \) at 70 eV and at low energies is surly due to the appearance of molecular ion \([\text{C}_{11}\text{H}_{11}\text{NO}_3]^+\). Generally, as seen from the inspection of the given mass spectra (Fig. 4), it clear that RI % of each fragment ion even main molecular ion is varied with the variation of electron beam power (10-12 eV). The signal of the main molecular ion is small (RI = 10%) at low energy (10 eV), one expect that the ionization energy of the molecule is less than 9.0 eV. Theoretically it is calculated at 8.80 eV, experimentally not be observed at 9 eV. Increasing the ionizing energy by 1 eV steps i.e. at 11eV (RI = 75%) and 12 eV (RI = 100%), i.e. the RI% increases and it becomes the base peak at 12eV. The fragment ion at \( m/z=106 \) \([\text{CH}_2\text{C}_6\text{H}_5-\text{NH}_2]^+\) has RI = 100%, at both 10 and 11 eV, but it decreases at 12 eV to RI = 25%. On the other hand the fragment ion \([\text{C}_7\text{H}_8\text{N}]^+\) of \( m/z=107 \) (p-toluidine, process 2), has RI = 27% at 10 eV, RI = 35% at 11 eV and RI= 45% at 12 eV. These data refer to the possible inter exchange between fragment ions during variation of electron power as explained by different possible parallel pathways given by the proposed scheme 2. This phenomenon may be attributed to the distribution of the electron power energy given to each fragment ion depending upon its stability and consequently its relative intensity (RI %).

**Fig. (4)**: Mass spectrum of P-MMA molecules at 12 eV (a), 11 eV (b) and at 10 eV(c).
All molecular structure changes during thermal and mass fragmentation of P-MMA molecule can easily be expected, confirmed and explained by theoretical molecular orbital calculation of geometrical parameters of the given molecule either in its neutral (TA) and/or its positively charged cationic form (MS).

**Theoretical Calculations**

Molecular orbital (MO) calculations give valuable information about the structure of the molecules, which are actually used to support the experimental evidences (TA and MS). The most important parameters calculated using MO calculation include geometries, bond orders, charge distribution and heat of formation. Fig. 1 shows the numbering system of P-MMA skeleton that helps in ordering of charge distribution (Fig. 5), heat of formation and bond orders. Geometrical parameters (bond length and bond order) for both neutral molecules and charged molecular ions are presented in Table 1. The calculated data showed that little differences are predicted in geometries and bond order between neutral (used in TA) and charged species (used in MS) and can briefly summarized in the following main points: The charge distribution on different atoms (C, N and O) and heats of formation ΔHf (kcal mol⁻¹) for neutral form is given in (Fig 5a), while the charged P-MMA species of cationic form is given in (Fig. 5b). Significant changes in the electron distribution within a given system often take place during the ionization of P-MMA process (24). The greatest change occurs in negative charges as a result of electron rupture, while no appreciable change occurs in the positive charges.

![Image of molecular structure](image_url)

**Fig. (5):** Distribution of charge on different atoms in neutral (a) and charged species (b), the figure includes the values of heats of formation and ionization energies (IE).

**Table (1):** Comparison between computed bond length (in Å) and bond order using PM3 method for neutral and molecular cation of P-MMA compound.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Bond length (Å)</th>
<th>Bond order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral</td>
<td>Cation</td>
</tr>
<tr>
<td>C1-C2</td>
<td>1.479</td>
<td>1.489</td>
</tr>
<tr>
<td>C2-C3</td>
<td>1.336</td>
<td>1.336</td>
</tr>
<tr>
<td>C3-C4</td>
<td>1.497</td>
<td>1.482</td>
</tr>
<tr>
<td>C4-N8</td>
<td>1.409</td>
<td>1.485</td>
</tr>
<tr>
<td>N8-C9</td>
<td>1.434</td>
<td>1.367</td>
</tr>
<tr>
<td>C9-C10</td>
<td>1.400</td>
<td>1.434</td>
</tr>
<tr>
<td>C9-C14</td>
<td>1.405</td>
<td>1.441</td>
</tr>
<tr>
<td>C10-C11</td>
<td>1.389</td>
<td>1.376</td>
</tr>
<tr>
<td>C12-C15</td>
<td>1.485</td>
<td>1.474</td>
</tr>
<tr>
<td>C13-C14</td>
<td>1.387</td>
<td>1.373</td>
</tr>
</tbody>
</table>

The bonds are ordered in increasing its stability as a function of bond length and bond order values as: C1-C2 < C4-N8 < C3-C4 < C12-C15 < N8-C9 < C2-C3.
It is clear from the values of the bond length and bond order of the P-MMA compound in Table(1) that; the weakest bond (large bond length and less bond order) is C1-C2 which is the first bond ruptured on thermal degradation and mass fragmentation leading to the loss of COOH as CO2 gas molecule (Schemes 1 and 2). This bond rupture gives thermal fragment of mole mass 160 and fragment ion of m/z = 160 at different electron power energy values in MS. This is followed by the C4-N8 bond rupture in TA leading to the formation of fragment of mole mass 106 after the loss of C2H4O (mole mass = 54). The same bond rupture in MS occurs for P-MMA at m/z = 106 [CH2=C6H5-NH2]+, it has RI% = 100, at both 10 and 11 eV, but it decreases at 12 eV to RI = 25%. On the other hand the fragment ion [C6H5N]+ of m/z=107 (p-toluidine, process 2), has RI = 27% at 10 eV, RI = 35% at 11 eV and RI= 45% at 12 eV as a result of formation of various fragment ions of different relative intensities at different electron power energy. From this discussion it is clear that, one can expect molecular structure changes of the compound obtained by experimental tools TA and MS using MOCS before doing this experimental work. The theoretical calculation can predict and confirm the TA and MS results.

CONCLUSION

In this research the molecular structure of p-methyl-maleanilic acid (P-MMA) was critically investigated by both TA and MS experimental work and main fragmentation pathways were proposed depending upon MOCS. It is concluded that this compound passed through two steps of fragmentation via rupture of the weakest bond C1-C2 to give CO2 gas molecule by decomposition of COOH functional group. As a result of this rupture active ingredient of a mole mass 160 or fragment ion m/z=160 is formed. It is followed by the rupture of the second weak bond C4-N8 which leads to thermal fragment of mole mass 106 and competitive active mass fragment ions of m/z=106 or 107. The difficult thermal fragmentation of the compound P-MMA and its consequent stability is related to the highly negative value [ΔHf]=-90.80 kCalmol-1. The more easy mass fragmentation of cationic form of P-MMA may be related to its activity as a result of positive heat of formation [ΔHf]= 92.84 kCalmol-1. It is also related to recharge distribution on atoms of a molecule as a result of its ionization. It is generally concluded that TA, MS and MOCS confirm each other during studies of molecular structure –reactivity relationship of P-MMA.

REFERENCES