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Electronic structure and biological activity: barbiturates vs. thiobarbiturates

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Abstract: The electronic structure of the derivatives of thiobarbituric acid: 1,3-diethyl-2-thiobarbituric acid (I) and 1,3-dibutyl-2-thiobarbituric acid (II) has been investigated by HeI and HeII UV photoelectron spectroscopy (UPS) and quantum chemical calculations. We discuss their electronic structures and compare them with barbituric acid. We also relate the difference in electronic structure between barbituric and thiobarbituric acids to difference in biological activity of their derivatives.

Keywords: photoelectron spectroscopy; thiobarbituric acid
1. Introduction

Thiobarbituric acid (TBA) is the parent compound of thiobarbiturate family which includes some very interesting substances. We shall mention only two points of interest. Some thiobarbiturates are sedatives and anaesthetics and include the fastest acting anaesthetic thiopental [1]. 2-thiobarbituric acid family also exhibits the largest number of tautomeric polymorphs reported to date [2]. In view of such unusual properties it is surprising that only a single study of electronic structure of these compounds has been made. Barbituric acid (BA) had been studied by UV photoelectron spectroscopy (UPS) [3]. In this work we aim to integrate and correlate the results pertaining to electronic structure, thermodynamic stability and biological activity of BA and TBA derivatives.

2. Experimental and computational methods

The sample compounds 1,3-diethyl-2-thiobarbituric acid (I) and 1,3-dibutyl-2-thiobarbituric acid (II) were purchased from Aldrich and used without further purification after checking its identity and purity by NMR spectroscopy. The dialkyl derivatives were selected instead of the parent thiobarbituric acid because the derivatives are more volatile and hence more readily measured in the spectrometer.

The HeI/HeII photoelectron spectra (UPS) were recorded on a Vacuum Generators UV-G3 spectrometer and calibrated with small amounts of Xe or Ar gas being added to the sample flow. The spectral resolution of the HeI and HeII spectra was 25 meV and 70 meV, respectively being measured as FWHM of the 3p^{-1}{^2}P_{3/2} \text{Ar}^{+} ← \text{Ar} (^{1}S_{0}) line. The samples were studied with the inlet probe heated to 90^\circ C-130^\circ C. The spectra obtained were reproducible and showed no signs of decomposition. Decomposition is usually discernible from the appearance of sharp intense peaks which are due to the presence of small molecules (decomposition products) in the
spectrometer's ionization chamber. Quantum chemical calculations were performed with the Gaussian 03 program [4] to aid in the assignment of photoelectron spectra. We used the Green's functions method (GF) and 6-31G* basis set for the calculation of ionization energies [5a] and the G3MP2/B3LYP method for calculating total energies of tautomers of I and II [5b]. The method has root-mean-square deviation of approximately 4 kJ/mol and includes full geometry optimization at the B3LYP/6-31G* level followed by single point QCISD type calculations. The calculated molecular geometries, fully optimized at the B3LYP/6-31G* level were similar to the experimentally determined structure of I [6] (Table 1). The NCO angle is an exception and this discrepancy between measured and calculated angle is due to the hydrogen bonding in the solid state [6]. The optimized structures corresponded to the minima on potential energy surfaces as was inferred from the absence of imaginary vibrational frequencies.

3. Results and Discussion

The photoelectron spectra of I are shown in Figs. 1-2. The spectral assignments are summarized in Table 2 and are based on HeI/HeII intensity variations, GF calculations and comparison with the assigned spectra of related molecules [3]. The assignments obtained are unambiguous.

*Photoelectron spectra*

In the ionization energy region below 12 eV we observe bands at 8.6, 9.05, 9.85 and 10.7 eV for I and at 8.4, 8.85, 9.6, 9.9 and 10.55 eV for II. These regions comprise a total of five ionizations each. Bands at 8.6, 9.05 eV in I and at 8.4, 8.85 eV in II, show pronounced decrease in relative band intensity on going from HeI to HeII radiation. The calculated partial photoionization cross-section ratios (HeII/HeI) for C2p, N2p, O2p and S3p are: 0.31, 0.45, 0.64 and 0.14, respectively [7]. This
suggests that the bands whose intensity decreases have significant S3p character which is the case for 8.6, 9.05 eV and 8.4, 8.85 eV bands (Figs.1-2 and Table 2). The bands whose relative intensity increases on going from HeI to HeII photon energy (at 9.85, 10.7 eV in I and 9.6-10.55eV in II) correspond to oxygen and nitrogen lone pair ionizations. The reported photoelectron spectrum of barbituric acid (BA) was assigned by the semiempirical method and was not detailed [3]. We therefore present in Table 2 its reassessment using GF method. The spectra of I and II on the one hand and BA on the other are very different which reflects the underlying difference in their electronic structures. For example, HOMO and SHOMO in I and II are at significantly lower energies (by 1.7-1.8 eV) compared to HOMO and SHOMO in BA. This signifies that TBA derivatives will be more polar and more polarisable molecules than BA analogues. They will also be expected to be better Lewis bases, better nucleophiles and better hydrogen bond acceptors (by CS group). The difference between the spectra of I and II is in the uniform 0.2 eV shift towards lower ionization energies on going from I to II which can be attributed to the inductive effect of larger alkyl groups in the latter.

Tautomerism

BA and TBA exist as single molecular species in the gas phase with trioxo and dioxo tautomers being the most stable. This conclusion was reached on the basis of calculated tautomer energies and FT-IR spectra [8]. We have used G3MP2B3 method to calculate Gibbs free energies of the two possible tautomers of I and II (Scheme 1) and established that keto tautomers are more stable than enols by 36.8 kJ/mol and 36.7 kJ/mol, respectively.
Scheme 1 TBA tautomers and ring atom numbering scheme

This result entails that our photoelectron spectra do represent single, keto tautomers rather than the mixture of tautomers.

*Biological activity of thiobarbiturates*

The barbiturates and thiobarbiturates can be used as anaesthetics, sedatives or anti-convulsive agents. They bind to specific regions of various receptors e.g. to GABA, nicotinic-acetylcholine (nAChR) or BK channel receptors which are all ligand-gated ion channels [9]. The binding to the GABA receptor requires that C5 atom be substituted by alkyl or aryl groups i.e. the BA and TBA are not biologically active themselves. The substitution enhances lipid solubility and facilitates transport of BA and TBA towards their enzyme targets.

The biological activities of TBA and BA derivatives are different as already noted. For example, replacement of one keto oxygen with sulphur atom leads to pronounced shortening of the onset and duration of anaesthetic activity; recall that thiopental in Scheme 1 is the fastest acting anaesthetic. The molecular mechanism of action of barbiturates is not completely understood, but recent quantitative-structure-activity-relationship (QSAR) study has suggested that electronic factors are more important for biological activity than geometrical factors [10]. Our study describes electronic structure which should therefore help in explaining the mechanism of action of
barbiturates and thiobarbiturates. We propose that the reason why TBA derivatives are more powerful anaesthetics than BA derivatives is due not only to better lipid solubility of TBA derivatives but is also due to TBA derivatives being better hydrogen bond acceptors. This latter property facilitates binding to the enzyme receptor. It is a rather rare case where the replacement of a single atom in a biologically active molecule by atom of another element (congener) leads to such drastic change in activity. Evidence for our assertion follows. Sulfur atom in C=S group can be considered a poor hydrogen bond acceptor, but it does form hydrogen bonds with enzymes (e.g. uridine phosphorylase) and biologically important thioureas as determined experimentally [11]. The strength of sulphur based hydrogen bonds has been estimated as being approximately 28 kJ/mol weaker than the standard hydrogen bonds involving oxygen [12]. Significantly lower ionization energies and subsequent better electron donating abilities of sulphur in TBA vs. oxygen in BA derivatives turn sulphur into a viable hydrogen bond acceptor which can compete with oxygen. TBA and BA derivatives act as BK channel openers where BK channel regulates the amount of Ca$^{2+}$ in various cells and electrical properties of cell membranes [9b]. Interestingly, the measured activities of TBA and BA analogues have shown the former to be more potent BK channel openers than the latter. This can be explained by our results which suggest that sulphur in C=S group is a good electron donor and hydrogen bond acceptor.

An interesting biotechnological application of TBA derivatives which also stems from their propensity to form hydrogen bonds, has been suggested recently [13]. TBA derivatives form hydrogen bonds with protein residues when the former are adsorbed on gold nanoparticles.
4. Conclusion

We have compared the electronic structures of BA and TBA and found them to be significantly different especially with respect to HOMO, SHOMO energies. This difference is reflected in their different biological activities and may lead to new biotechnological applications.

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Table 1. Selected geometry parameters (bond angles and bond lengths) of I and II a

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-S</th>
<th>C-O</th>
<th>NCS</th>
<th>NCO</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.670Å</td>
<td>1.216Å</td>
<td>121.4°</td>
<td>121.8°</td>
</tr>
<tr>
<td>II</td>
<td>1.670Å</td>
<td>1.216Å</td>
<td>121.3°</td>
<td>121.8°</td>
</tr>
<tr>
<td>Ref. 6</td>
<td>1.658Å</td>
<td>1.265Å</td>
<td>122.6°</td>
<td>117.3°</td>
</tr>
</tbody>
</table>

a the parameters for I are from DFT calculations, those from Refs. 6 and are from X-ray and neutron diffraction studies of I. There are no reported experimental structure studies of II.

Table 2. Experimental ($E_i$/eV) vertical ionization energies, orbital assignments for TBA and BA ab

<table>
<thead>
<tr>
<th>Compound</th>
<th>Band</th>
<th>$E_i$/eV</th>
<th>GF/eV</th>
<th>Assignment</th>
<th>HeII/HeI intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>X</td>
<td>8.6</td>
<td>8.39</td>
<td>nS</td>
<td>1.0</td>
</tr>
<tr>
<td>A</td>
<td>9.05</td>
<td>8.62</td>
<td></td>
<td>$\pi_{CS}$</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>9.85</td>
<td>9.92</td>
<td></td>
<td>nO</td>
<td>3.43</td>
</tr>
<tr>
<td>C</td>
<td>10.7</td>
<td>10.85</td>
<td></td>
<td>nN$^-$</td>
<td>2.38</td>
</tr>
<tr>
<td>D</td>
<td>12.1</td>
<td>11.49</td>
<td></td>
<td>nO$^+$</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>X</td>
<td>8.4</td>
<td>8.32</td>
<td>nS</td>
<td>1.0</td>
</tr>
<tr>
<td>A</td>
<td>8.85</td>
<td>8.55</td>
<td></td>
<td>$\pi_{CS}$</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>9.6  (9.9)</td>
<td>9.74</td>
<td>nO$^-$</td>
<td>2.66</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10.55</td>
<td>10.77</td>
<td></td>
<td>nN$^-$</td>
<td>1.86</td>
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<tr>
<td>D</td>
<td>11.45</td>
<td>11.37</td>
<td></td>
<td>nO$^+$</td>
<td></td>
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<tr>
<td>BA (ref. 3)</td>
<td>X</td>
<td>10.40</td>
<td>10.85</td>
<td>nO</td>
<td></td>
</tr>
<tr>
<td>A-B</td>
<td>11.05</td>
<td>10.96, 11.02</td>
<td>$\pi_{CO}$ + n$^-$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-D</td>
<td>11.45</td>
<td>12.07, 12.39</td>
<td>$\pi$, nO , nO</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a n$^-$ and n$^+$ designate out-of-phase and in-phase linear combinations of nitrogen or oxygen lone pairs
b Numbers in brackets indicate shoulders
References


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Fig. 1 HeI and HeII photoelectron spectra of I
Fig. 2 HeI and HeII photoelectron spectra of II