Photoelectron spectroscopy of two pesticides: Diazinon and Dicamba

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Abstract

The electronic structures of pesticides diazinon and dicamba have been studied by UV photoelectron spectroscopy (UPS) and high-level Green’s function (GF) calculations. Our UPS data reveal the electronic structures of title compounds which is then used to discuss some aspects of biological activity of these pesticides.

Keywords: Pesticides; Photoelectron spectroscopy; Biological activity

1. Introduction

The importance of electronic structure of toxic substances for the development of quantitative structure-activity relationship models (QSAR) has been recognized for some time \cite{1}. However, the electronic structure is usually evaluated using computational methods rather than more accurate spectroscopic methods. In this work we present two toxic substances (pesticides): diazinon and dicamba and investigate their electronic structures within the framework of their biological activity/toxicity. Diazinon is organophosphate insecticide (triester) which acts by blocking acetylcholinesterase enzyme which leads to the accumulation of acetylcholine in synaptic clefts and over-excitation of nerves. Diazinon is more toxic to invertebrates than to mammals which is the basis of its current use. It is nonetheless toxic to both which leads to problems with its use \cite{2}. Dicamba is a herbicide which mimics the action of auxins (plant growth hormones) causing abnormal, rapid cell division and rapid plant growth \cite{2} so that plant outgrows its resources and dies.

We used UV photoelectron spectroscopy as the method for our study since it had been applied successfully to pesticides before as indicated in Scheme 1 \cite{3-5}.

2. Experimental and Theoretical Methods

Samples of diazinon (I) and dicamba (II) compounds studied in this work were obtained from Sigma-Aldrich. The selection of compounds was governed not only by their biological activity, but also by their thermal stability and volatility. These two properties are essential when using the UPS method since the sample needs to be vaporized without decomposition. Unfortunately, many pesticide compounds do not satisfy these criteria. The photoelectron spectra of I and II (Figures 1,2) were recorded on a Vacuum Generators UV-G3 spectrometer and calibrated with small amounts of Xe gas which was added to the sample flow. The spectral resolution in HeI and HeII spectra was 25 meV and 70 meV, respectively when measured as FWHM of the 3p\textsuperscript{1} \textsuperscript{1}P\textsubscript{3/2} Ar\textsuperscript{+} ← Ar (\textsuperscript{1}S\textsubscript{0}) line. The vertical ionization energy values have been determined from intensity maxima. The samples were recorded at 160, 150°C, respectively. The sample vapour pressures were maintained at constant value for each sample. The measured spectra were reproducible and showed no signs of decomposition e.g. no sharp peaks corresponding to small molecules-decomposition products were observed. The spectra were reproducible over long time intervals. After the measurements the sample residues were inspected and showed no discoloration or charring.

DFT calculations were performed with GAUSSIAN 03 software \cite{6} at the PBEPBE/6-31G(d,p) level and included full geometry optimization for each molecule. The optimized structures corresponded to the minima on the potential energy surface as was inferred from the absence of imaginary harmonic vibrational frequencies. The vertical ionization energies were calculated for the optimized structures using outer valence Green's function (OVGF) method \cite{7} at the 6-
31+G(d,p) level. The OVGF method used (using 4th order electron propagator level as coded in Gaussian 03 program) gives vertical ionization energies which are within 0.4 eV of the experimental values.

3. Results and Discussion

3.1. Photoelectron spectra

The photoelectron spectra of I-II are shown in Figures 1, 2 and their assignments are summarized in Table 1. We shall briefly discuss the assignment of each spectrum in turn based on the comparison with the previously reported spectra of pyrimidine, 1,4-dichlorobenzene and benzoic acid [8] and with OVGF calculations.

In the UPS of diazinon the unresolved band in the low energy region 9.0-9.35 eV corresponds to three ionizations. The corresponding three orbitals comprise in-plane and out-of-plane sulphur lone pairs (of σ and π type) and the antibonding combination of nitrogen lone pair from the pyrimidine ring. The band at 10.4 eV can be attributed to two ionizations from π-orbitals localized on the pyrimidine ring. In order to supplement the band assignments we have measured spectra at HeI and HeII photon energies. Knowing the ratios of HeII/HeI atomic orbital photoionization cross-sections [9] for C2p, N2p, O2p, S3p and Cl3p are 0.31, 0.45, 0.64, 0.14 and 0.05, respectively we can interpret decrease in relative band intensities on going from HeI to HeII radiation. The relative intensity of bands at 9.0-9.35 eV decreases significantly compared to the rest so we can unambiguously attribute ionizations from orbitals containing significant S3p character to this manifold. This assignment is consistent with the one obtained by comparison with spectra of related compounds and with OVGF calculations (Table 1).

In the spectrum of dicamba, the bands at 9.05 and 9.55 eV correspond to two ionizations from the ring π-orbital and the oxygen lone pair of the carboxylic group, respectively. Bands at 10.6, 10.9 and 11.4 eV correspond to ionizations from π-type oxygen lone pair of carboxylic acid group, oxygen lone pair of the methoxy group and ring π-orbital, respectively. This assignment is consistent with the observed increase in relative intensity of 10.6 and 10.9 eV bands (whose ionized orbitals have O2p characters) vs. the 11.4 eV band (C2p character). The relative intensity of the band manifold in 11.6-12.2 eV region decreases very prominently and can therefore be attributed to three chlorine lone pair ionizations.

3.2. Biological activity

The relationship of electronic structure to biological activity is often not easy to discern because many other molecular and thermodynamic properties of compounds contribute to the net, observed biological activity. Nevertheless, we have combined the results of UPS study and the reported biological activity together with ligand-receptor binding when such information is available. Orbital energies of pesticides (or any other molecules) deduced from UPS do not depend on solution environment. The receptor cavity is also often water free so UPS data (which pertain to free molecules) can be used to rationalize the specific details of bonding between ligand and receptor at the molecular level especially when some information about the structure of the receptor’s active site is known. Knowledge of electronic structure is not useful for correlation type studies e.g. QSAR,
Table 1. Experimental band maxima (Ei) and vertical ionization energies, calculated vertical ionization energies (OVGF), band assignments for the studied pesticides*. 

<table>
<thead>
<tr>
<th>Compound</th>
<th>Band</th>
<th>$E_i$±0.2/eV</th>
<th>OVGF/eV</th>
<th>MO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>X-B</td>
<td>9.05-9.35</td>
<td>9.10, 9.11, 9.38</td>
<td>π(σN, σS, π)</td>
</tr>
<tr>
<td></td>
<td>C-D</td>
<td>10.4</td>
<td>10.15, 10.33</td>
<td>π, π</td>
</tr>
<tr>
<td>Dicamba</td>
<td>X</td>
<td>9.05</td>
<td>8.90</td>
<td>π</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>9.55</td>
<td>9.68</td>
<td>π(πCO)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>10.6</td>
<td>10.55</td>
<td>π(πCOOH, πðOCH3)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>10.9</td>
<td>11.30</td>
<td>π(πC═O, πC═N)</td>
</tr>
<tr>
<td></td>
<td>E-H</td>
<td>11.4-12.2</td>
<td>11.34, 11.68, 11.71, 11.90</td>
<td>π(πC═O, πC═N)</td>
</tr>
<tr>
<td>Indoleb</td>
<td>X</td>
<td>7.93</td>
<td>7.69</td>
<td>π</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>8.38</td>
<td>8.01</td>
<td>π</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9.82</td>
<td>9.73</td>
<td>π</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>11.08</td>
<td>11.08</td>
<td>π(πN)</td>
</tr>
</tbody>
</table>

* molecules I-III have conformational flexibility (i.e. the UPS spectra may represent admixtures of different conformers) which should be born in mind when comparing OVGF and $E_i$ values.

Table 2. Comparison of experimentally determined toxicities LD$_{50}$ (mg/kg) and three lowest vertical ionization energies ($E_i$) for three organophosphorus pesticides studied by UPS$^{ab}$. 

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD$_{50}$</th>
<th>$E_1$/eV</th>
<th>$E_2$/eV</th>
<th>$E_3$/eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>250</td>
<td>9.0</td>
<td>9.35</td>
<td>10.4</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>80</td>
<td>9.4</td>
<td>12.3</td>
<td>13.4</td>
</tr>
<tr>
<td>Disulfoton</td>
<td>2</td>
<td>9.0</td>
<td>9.3</td>
<td>11.5</td>
</tr>
</tbody>
</table>

The ionization energies of dichlorvos and disulfoton are from ref. $^3$.

Toxicities from ref. $^{10}$.

hence no such correlations are described in this work. This work also demonstrates how scarce the information about pesticides is at the molecular level in contrast to the abundance of such molecular information regarding biologically active molecules in health sciences field. For neither pesticide studied here was the structure of ligand-receptor complex reported and hence no direct data exist about modes or relative strengths of binding in the receptor cavity. UPS data provide information and rationalize possible details of ligand binding on the basis of explored ligands’ electronic structures. The biological activity cannot be determined or explored from UPS data alone.

**Diazinon** is a pesticide which acts by blocking the activity of AChE. It is metabolized in the body into diazoxon by oxidation with NADH in the presence of oxygen (Scheme 2) [2]. In diazoxon the sulphur of the thioester group is replaced by oxygen. In order to gain some insight into the mechanism of action of these triesters we note that they have very different molecular structures (Scheme 1) and also widely different biological activities (Table 2). What is then the influence of electronic structure on this activity? In Table 2 we have represented their electronic structures by the three lowest vertical ionization energies and compared these energies to the measured biological activities. The activity of dichlorvos differs from the other two insecticides due to the presence of polar chlorovinyl group attached to the triester moiety. However, when we compare diazinon and disulfoton we notice that the difference between their biological activities is even larger even though the first two vertical ionization energies of diazinon and disulfoton are almost identical. These first two ionization energies can be attributed to sulphur localized orbitals. We can then conclude (on the basis of UPS data) that sulphur lone pairs are not crucial to biological activity. This comment is consistent with the independent observation (Scheme 2) that diazoxon rather than diazinon is the active compound supporting toxicity. Therefore, significant differences in electronic structures and biological activity in these triesters seem to be related to orbitals/function groups with ionization energies above 10 eV (Table 2). This observation (obtained from UPS) suggests that in disulfoton and dichlorvos it is the hydrogen bonding to oxygen lone pairs that are important in binding insecticide to AChE while in diazinon the sulphur lone pairs, or nitrogen lone pairs (in pyrimidine moiety) do not form binding interactions with the receptor residues. The strength of such interactions will govern the biological activity since it would enhance the stability of insecticide-AChE complex. This lack of binding interactions may be due to the size and rigidity of heterocyclic ring which prevents it from fitting snugly into the AChE cavity. The data in Table 2 do not represent correlation because the number of data points would be too small considering the number of variables governing biological activity. Hence UPS data serve explanatory purpose to rationalize the mode of binding.

This analysis is an example of how UPS data may be used to infer about the modes of binding between pesticide and receptor. The analysis is useful when the structure of ligand-receptor complex has not been elucidated via e.g. X-ray diffraction measurements as is the case for the two pesticides described in this work. One also needs to note that orbital ionization energies do not depend on the solution environment. On the other hand interactions between ligand (pesticide) and corresponding receptor depend on both the electronic structure and solution environment.

**Dicamba** is herbicide which mimics auxin (substituted indole) plant growth regulators (Scheme 3). Both the molecular structures and electronic structures of these compounds differ considerably; the only common structural feature being the presence of the aromatic ring. Comparison of the ionization energies of e.g. indole and dicamba (Table 1) highlights widely different electronic structures of the two...
compounds. This can be seen from the π-ionization energies in indole which span the range of 7.93, 8.38 and 9.82 eV while in dicamba they are at 9.05 and 9.55 eV. Yet both compounds act as plant regulators. This mimicking ability of auxin type regulators was explained by the specific shape of the auxin binding pocket of TIR1 protein receptor [12]. The “promiscuity” of this binding pocket suggests that the binding of aromatic ring of the regulator molecule to the bottom of box-like pocket is predominantly via hydrophobic interactions [12]. This is consistent with the difference in electronic structures (based on UPS data) of indole and dicamba as mentioned above.

4. Conclusions

We have used the UPS method to determine the electronic structure of two pesticides and shown how this data on electronic structure can be incorporated in the explanations of their mechanisms of biological action.

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