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Photoelectron Spectra of Important Drug Molecules: Zidovudine and Artemisinine

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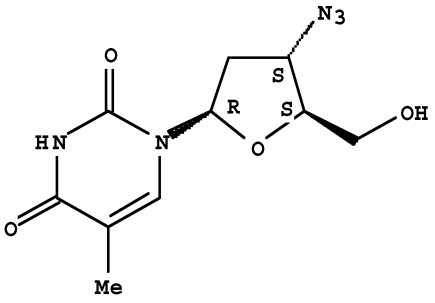
Abstract: The electronic structures of the anti-HIV drug zidovudine (AZT) and antimalarial drug artemisinine have been investigated by UV photoelectron spectroscopy (UPS), MO calculations, and comparison with the spectra of related molecules. The analysis of their electronic structure is correlated with known biological activities.

We are interested in investigating the role of electronic structure in the biological activity of important drugs (pharmaceuticals). One of the best methods to study the electronic structure of molecules is UV photoelectron spectroscopy (UPS) in combination with MO calculations. We have used such methodology previously[1,2] and were able to shed some light on the biological activity of steroids and thio-TEPA antitumor drugs. Here, we report the analysis of two important drug molecules: anti-HIV compound zidovudine and anti-malarial agent artemisinine.

Zidovudine. This compound (also called azidothymidine, AZT) is one of the most successful anti-HIV drugs used at present. The mode of action comprises interference with the mechanism of production of proviral DNA.[3] The molecule resembles normal nucleoside deoxythymidine, the only difference being the presence of azido instead of the hydroxyl group in the molecule.



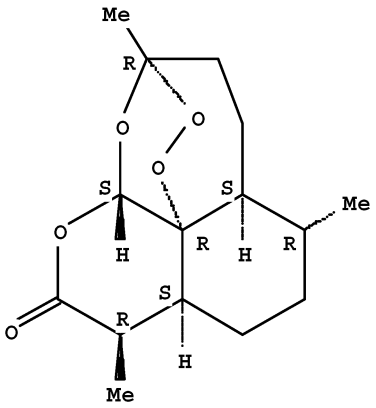
Zidovudine is metabolized into triphosphate derivative upon which the reverse DNA transcriptase acts. However, due to the presence of the azido group, the enzyme cannot proceed with viral DNA chain elongation.

TABLE 1. Measured Vertical Ionization Energies (E_i/eV), Bandwidths (fwhm), DFT Energies (ϵ/eV), and Final UPS Assignments for AZT and QHS Together with UPS of Related Molecules[a]

molecule	E_i (fwhm)	ϵ (MO)	final assignment
AZT	9.10 (1.2)	6.62 π_1 (thym)	π_1 (thym)
	(9.10)	7.23 π (N_1-N_3)	n_O (ribose)
	9.88 (0.3)	7.27 n_1 (thym)	π (N_1-N_3)
	10.03	7.81 n_O (ribose)	n_1 (thym)
	10.38	7.95 π_2 (thym)	π_2 (thym)
	(10.38)	8.03 n_{OH} (ribose)	n_{OH} (ribose)
	10.83	8.31 n_2 (thym)	n_2 (thym)
	(10.83)	8.52 π (N_2-N_3)	π (N_2-N_3)
	12.28	9.69 π_3 (thym)	π_3 (thym)
thymine	9.18 (0.5)	6.64	π_1 (thym)
	10.03	7.22	n_1 (thym)
	10.39	7.82	π_2 (thym)
	10.82	8.03	n_2 (thym)
	12.27	9.84	π_3 (thym)
EtN_3	10.02, 11.21 (0.3)	6.95, 8.18	π (N_1-N_3), π (N_2-N_3)
THF	9.77, 10.66 (0.4)	6.61, 7.38	n_O, n_{OH}
QHS	9.4	6.91 n_O^-	n_O^-
	9.75	7.25 n_o(CO)	n_o(CO)
BTL	10.27, 10.97		n_o(CO), n_o
DOBN	9.67, 11.07		n_O^-, n_O^+

[a] The values were obtained from DFT calculations assuming the validity of Koopmans approximation; the values in brackets indicate presence of several overlapping ionizations.

Artemisinine. Artemisinine (also called qinghaosu, QHS) is a very important anti-malarial agent to which malarial parasites have not yet developed resistance. Its mode of action relies on the presence of a reactive endoperoxide bond, accessible to electrophilic attack. The rupture of this bond, under the influence of the parasite's own biochemical agents, leads to the formation of highly reactive peroxide free radicals which can then participate in the killing of parasitic cells. However, the details of the mechanism of QHS action are still being debated.[4]



The results of our studies are presented in Table 1 and Figure 1. We shall discuss the electronic structure of each molecule in a separate paragraph.

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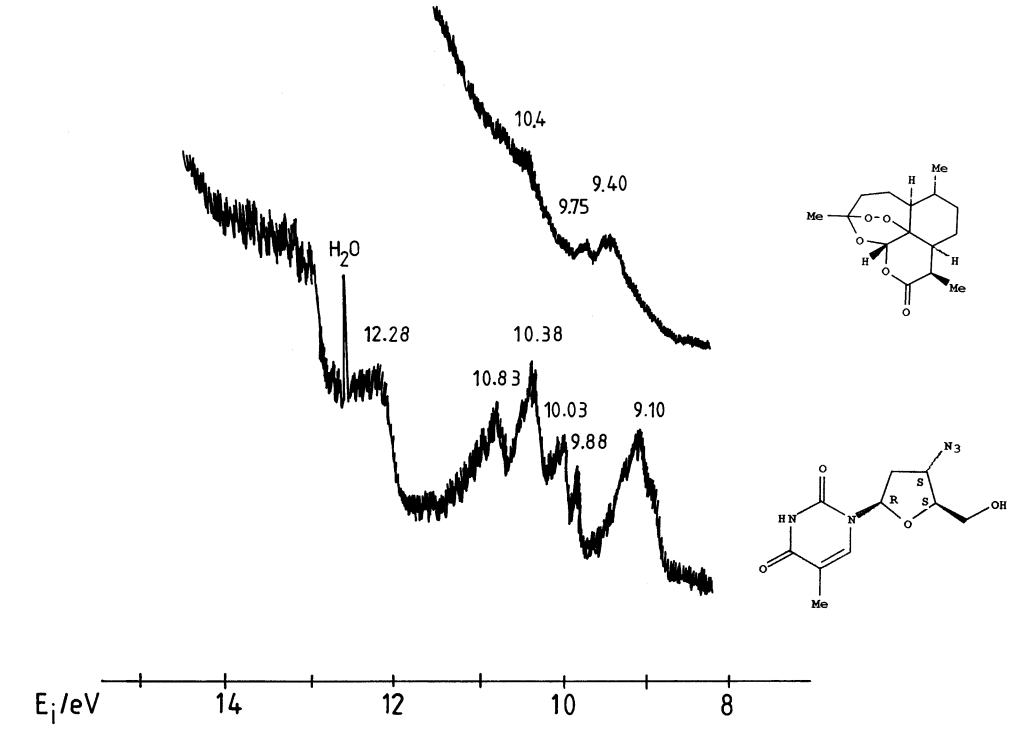

FIGURE 1. HeI photoelectron spectra of AZT and QHS.

Zidovudine (AZT). The molecule has a high density of ionic states, which results in many overlapping bands in the spectrum (Figure 1). In fact, the AZT spectrum looks like the thymine spectrum[6] with additional, superimposed bands. When overlapped bands exist, MO calculations based on Koopmans' approximation are not a sufficiently reliable guide to spectral interpretation, especially when calculated energy levels are less than 0.5 eV apart. We have therefore also taken into account relative band intensities, bandwidths, and the photoelectron spectra of "fragment molecules" which contain individual functional groups present in AZT. Only through the combination of empirical and theoretical arguments could the interpretation of the AZT spectrum be performed successfully.

The "fragments" whose UPS spectra are relevant for AZT are thymine,[6] 3-hydroxy-tetrahydrofurane[7] (THF) and ethyl azide.[8] THF was used to mimic the ribose unit. The UPS of azides available in the literature mostly describe inorganic azides; the ethyl azide[8] spectrum is the one relevant for AZT. The experimental ionization energies of "fragments" are also included in Table 1. The resolved band profiles (fwhm bandwidths) for AZT and related molecules are also given in Table 1 as an aid to band assignments.

The relative intensity of the first band manifold at around 9.1 eV suggests that it comprises two overlapping ionizations, while the manifold in 9.8−11.8 eV region contains six ionizations. Following the combined empirical and theoretical analysis, we conclude that the first manifold comprises ionizations from: π orbital of the thymine ring and the oxygen lone pair on the ribose ring. The cluster of bands between 9.88 and 10.83 eV contains six ionizations which were analyzed in the same manner; the assignment is given in Table 1. This cluster contains three ionizations from the thymine localized orbitals, two ionizations from orbitals on the azide moiety and the oxygen lone pair of the ribose's hydroxyl group. The 9.88 and 10.83 eV bands were attributed to azide ionizations. The 9.88 eV band is sharp which can be expected for ionization from the strongly localized azide group orbital. The band was therefore assigned to azide ionization. The second azide ionization was attributed to 10.83 eV band on the basis of comparison with the ethyl azide spectrum. The DFT calculations support this relative order of ionizations with the exception of ribose oxygen lone pair and the first azide orbital. However, in view of the deficiencies of Koopmans approximation and small energy difference between the calculated energy levels, such discrepancies can be expected.

The most interesting feature of the spectrum is the closeness of ionization energies and spectral resemblance between AZT and the thymine base. The introduction of azide group into deoxythymidine nucleoside does not change the electronic structure of thymine base, but instead inductively shifts the energy levels of ribose and azide groups. The AZT molecular conformation is such that ribose and thymine rings are not coplanar and thus any intramolecular interactions are limited to inductive shifts.

The presence of azide group shifts the orbitals of the thymine fragment only slightly (<0.1 eV) toward lower ionization energies.

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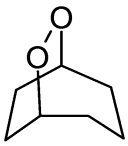
As a result, the introduction of azide group induces mainly changes in polarity and lipophilicity of the nucleoside. The "retention" of the basic electronic structure of the DNA base appears to be essential for biological activity of AZT and related drug molecules.

Artemisinine (QSH). QSH has been studied extensively by quantum chemical methods,[9−11] but no electronic structure information is currently available in the literature even though it's mechanism of biological activity involves free radicals and can thus be expected to depend on the electronic structure. QHS molecule, like AZT, has high density of ionic states which results in many overlapping bands in the spectrum (Figure 1). We have performed DFT calculations as an aid to the assignment. The calculated geometry matched the experimental geometry[9] well; e.g. the calculated and measured endo-peroxide bond lengths were 1.468 and 1.478 Å, respectively. The QHS spectrum has only two features which stand out from the broad, unresolved manifold and rising background: bands at 9.40 and 9.75 eV. The comparison with the spectra of related fragment molecules is therefore necessary for a reliable assignment. The photoelectron spectrum of the related molecule, 6,7-dioxabicyclo[3.2.2]nonane DOBN (shown below) has been reported[12] and may be used to simulate the endo-peroxide bond in QSH.



The spectrum of valerolactone, which can represent the electronic structure of lactone ring in QSH has not been reported, but the UPS of related γ-butyrolactone (BTL) has.[13] Taking into consideration the spectra of the two fragments (Table 1) and DFT results, we suggest the assignment for QSH spectrum as given in Table 1. The interesting point is that HOMO ionization corresponds to oxygen lone pairs of endo-peroxide bond rather than to the oxygen lone pair of the lactone ring's carbonyl group. The electron donating ability of endoperoxide moiety is therefore higher than keto moiety from the lactone ring. This observation is important, because artemisinine acts in the body via the mechanism which involves monoelectronic reduction of the peroxide bridge to generate alkoxyl radical. The reduction is heme catalyzed and produces radicals capable of alkylating vital biomolecules in the malaria parasite.[14] The alkylation disrupts important biochemical processes and kills the parasite. The biological activity of QSH thus rests on free radicals originating within peroxide moiety. It was established by UPS that substitution of methyl groups at bridgehead position in bicyclic peroxides destabilizes peroxide moiety (i.e. makes it more electron donating) by 0.4 eV or 38.5 kJ/mol.[12] This observation may be relevant for reactivity of the alkoxyl radical which is formed by heme-catalyzed cleavage of the endoperoxide bond. It is known that the nucleophilicity of the radical increases when it's ionization energy decreases.[15] The introduction of electron donating substituents at the bridgehead position of the seven member ring (which contains endoperoxide bond) may thus enhance radical reactivity and by inference, biological activity of QSH analogues.

Our work shows that further UPS studies of molecules which exhibit biological activity, may help to clarify such activity. This report also demonstrates that this research can be pursued profitably even though biologically active molecules are not generally suitable for UPS due to their thermal instability below or in the vicinity of the melting point.

Experimental Section

The HeI spectra were recorded on Vacuum Generators UV-G3 photoelectron spectrometer with spectral resolution of 25 meV when measured as the full width at half-maximum (fwhm) of the $Ar^+ {}^2P_{3/2}$ calibration line. The sample inlet temperatures required to generate sufficient vapor pressure were around 160 and 130 °C for AZT and QSH, respectively. We have paid special attention to the possibility of sample decomposition so the samples were carefully heated with slow increase in temperature. The absence of major sample decomposition was inferred from the absence of very intense peaks due to small molecules, e.g., CO, CO_2, and N_2. Furthermore, after the completion of spectral measurement, the identity of the sample remaining in the sample holder was again checked by MS to probe for possible thermal decomposition. There were no volatile impurities (e.g., solvents) present in the measured spectrum; they were not detected during the measurement and would at any rate have evaporated by the time the high temperatures (>100 °C) necessary for sample measurements were reached.

The electronic structure calculations were performed with Spartan 02 program package.[5] The calculations included full geometry optimization using B3LYP/6-31G* DFT method. DFT results were used in conjunction with the Koopmans approximation. The choice of the method and the basis set was governed by the molecules' sizes which precluded the use of more sophisticated, non-Koopmans-type calculations. The DFT-based Koopmans ionization energies for AZT show discrepancies vs experimental ionization energies. However, this is not unexpected. We have included the DFT Koopmans energies for related molecules, thymine, EtN_3, and THF in Table 1, in order to demonstrate that the energy discrepancies observed for AZT are of the same magnitude as for related molecules. Nonetheless, DFT calculations do give correct orbital sequences in thymine, EtN_3, and THF.

Zidovudine was synthesized, purified, and identified by the manufacturer, GlaxoSmithKline Research and Development Section (Greenford, UK), and used as received. Artemisinine sample was purchased from Aldrich and (after checking mass and NMR spectra) used without further purification.

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