Photoelectron spectroscopic study of the negative ions of 4-thiouracil and 2,4-dithiouracil

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We report the photoelectron spectra of the negative ions of 4-thiouracil $(4\text{-TU})^-$ and 2,4-dithiouracil $(2,4\text{-DTU})^-$. Both of these spectra are indicative of valence anions, and they are each dominated by a single broad band with vertical detachment energies of 1.05 and 1.4 eV, respectively. Complementary calculations by Dolgounitcheva, Zakrzewski, and Ortiz (see companion paper) are in accord with our experimental results and conclude that the $(4\text{-TU})^-$ and $(2,4\text{-DTU})^-$ anions, reported herein, are valence anions of canonical 4-thiouracil and canonical dithiouracil. Comparisons among the anions and corresponding neutrals of 4-thiouracil, 2,4-dithiouracil, 5-chlorouracil, 5-fluorouracil, and uracil itself show that both sulfur and halogen modifications of uracil give rise to significant changes in the electronic structure. The electron affinities of the first four are all substantially larger than that of the canonical uracil. © *2011 American Institute of Physics*. [doi:10.1063/1.3555177]

I. INTRODUCTION

Modified nucleic acid bases have attracted significant attention due to their use as therapeutic agents.[1–14] The halo-nucleobases may have roles as radiosensitizers in the treatment of cancer.[15–20] Substituting sulfur for oxygen in nucleobases yields a variety of thio-nucleobases, and these also have interesting biological and pharmacological properties. The thiouracils, for example, have been detected in natural transfer-RNA and are used as anticancer and thyroid drugs.[1,2,11] Specifically, 4-thiouracil $(4\text{-TU})^-$ is known to possess cytostatic properties, and 2,4-dithiouracil $(2,4\text{-DTU})^-$ is used as a melanoma-seeking agent, owing to its specific incorporation into nascent melanin.[13,14] Experimental and theoretical studies of 4-thiouracil and 2,4-dithiouracil have revealed their structural, chemical, and spectroscopic properties.[5,21–45] In particular, the oxothione and dithione tautomers of 4-thiouracil and 2,4-dithiouracil, respectively, were found to be their most stable forms in the gas phase, in solution, and in their crystals. These are their canonical forms.

While neutral thiouracils have been studied extensively, their anions have received little attention, even though the interaction of thiouracils with electrons may be relevant to their therapeutic roles. In the present paper, we study the parent anions of $(4\text{-TU})^-$ and $(2,4,\text{-DTU})^-$, measuring their anion photoelectron spectra. We then compare these photoelectron spectra with those of the parent anions of uracil and of two halo-uracils.

II. EXPERIMENTAL

Negative ion photoelectron spectroscopy is conducted by crossing a mass-selected beam of negative ions with a fixed frequency photon source and energy analyzing the resultant photodetached electrons. This technique is governed by the energy-conserving relationship $h\upsilon = \text{EKE} + \text{EBE}$, where $h\upsilon$ is the photon energy, EKE is the measured electron kinetic energy, and EBE is the electron binding energy.

The negative ions of 4-thiouracil and 2,4-dithiouracil were generated in a source which consists of a rotating, translating sample-coated metal rod (Cu or Ag), a laser beam entrance port, a pulsed gas valve for feeding helium into the laser–sample interaction region, and a gas expansion exit nozzle. Typically, helium gas at 4 bars was expanded in synchronization with laser ablation pulses. The sample-coated rods were prepared by pressing 4-TU or 2,4,-DTU powder directly onto the metal rod to form a thin layer on its surface. The coating was then ablated at very low laser power with the second harmonic (532 nm) of a Nd:YAG laser. Upon generating the anions of interest, they were extracted into a linear time-of-flight mass spectrometer, mass-selected, and photodetached with the third harmonic frequency (355 nm or 3.49 eV/photon) of another Nd:YAG laser. The resulting photodetached electrons were then energy analyzed with a magnetic bottle, electron energy analyzer. Our anion photoelectron spectrometer has been described in detail previously.[46]

III. RESULTS

Figure 1 presents the anion photoelectron spectra of $(4\text{-TU})^-$ and $(2,4,\text{-DTU})^-$ along with two photoelectron spectra of the uracil anion, both of which are displayed in the same frame. The low EBE peak in the uracil anion spectrum is due to the photodetachment of its dipole-bound state; the broad band centered at EBE = 2.4 eV [its vertical detachment energy (VDE)] is due to the rare tautomer (N3-to-C5 proton transfer), valence anion of uracil. While the dipole-bound uracil anion was formed in a different source environment, the rare-tautomer uracil anion, $(4\text{-TU})^-$, and $(2,4,\text{-DTU})^-$ were all formed in the same source environment, i.e., the one described above. The photoelectron spectra of these three were recorded with 3.493 eV photons. The $(4\text{-TU})^-$ spectrum

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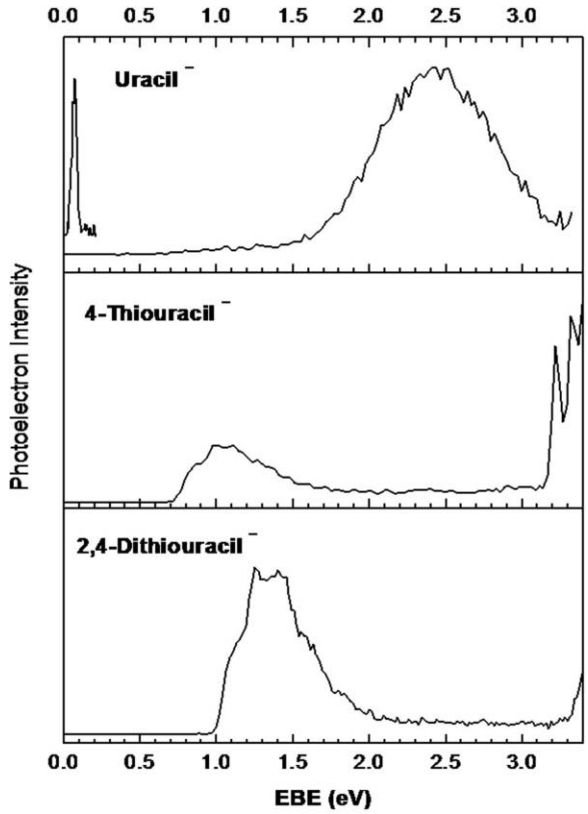

FIG. 1. The photoelectron spectra of $(4\text{-TU})^-$ and $(2,4\text{-DTU})^-$ anions both recorded with 3.493 eV photon. The photoelectron spectra of two forms of the uracil anion are also presented for comparison.

exhibits a broad peak centered at EBE = 1.05 eV (its VDE), with its width stretching from EBE = 0.7 to 1.5 eV. Two stronger features are located at EBE = 3.21 and 3.32 eV. The $(2,4,\text{-DTU})^-$ spectrum exhibits a broad band centered at 1.4 eV (its VDE), with its width stretching from EBE = 1.0 to 1.9 eV.

IV. DISCUSSION

A major objective in studying $(4\text{-TU})^-$ and $(2,4,\text{-DTU})^-$ was to compare the electrophilic properties of 4-thiouracil and 2,4-dithiouracil with those of uracil, where the replacement of its oxygen atom(s) by one or two sulfur atoms is the only difference between them. For present purposes, uracil is an example of a nucleic acid base, while 4-thiouracil and 2,4-dithiouracil are examples of the corresponding sulfur-substituted nucleobase. Before proceeding, we provide some background on the parent anions of (unsubstituted) nucleobases in general and of uracil in particular.

The interaction of nucleobases with electrons is of interest, in part because very low energy electrons, such as the secondary electrons produced by ionizing radiation, have been shown to induce single and double strand breaks in DNA.[47–49] While the mechanism of electron-induced strand breaks is not well understood, it is generally thought to involve electron attachment to nucleobases to form their negative ions. There are three categories of parent, nucleobase negative ions, and these are valence anions of canonical nucleobases, dipole-bound anions of canonical nucleobases, and valence anions of rare-tautomer (N-to-C proton transfer) nucleobases. Valence anions of canonical nucleobases are known through electron paramagnetic resonance studies to exist in irradiated condensed phases. However, in gas phase studies, parent valence, molecular anions of canonical nucleobases are elusive, and except via Rydberg electron transfer,[50] they have not been formed and observed as isolated species in mass spectra. The reason may have to do with the low electron affinity (EA) values of canonical nucleobases. Virtually everyone agrees that the electron affinity values of canonical nucleobases are small and that some of them are likely to be negative. The preponderance of evidence suggests that their electron affinity ordering is probably U > T > C > A > G (or perhaps, G > A), where at least the first two have positive EA values.[51–55] Lastly, temporary anion (electron scattering) states of nucleobases can also be viewed as valence anions, since the electrons go into empty valence orbitals, albeit briefly. Nevertheless, the valence anions of canonical nucleobases are not the only parent anions of nucleobases. The first parent anions of nucleobases to be observed in mass spectra were dipole-bound anions. In dipole-bound anions, excess electrons are weakly held by the dipolar fields of polar molecules, e.g., nucleic acid bases. Dipole-bound anions of nucleobases were initially predicted theoretically[56] and then observed and identified through their distinctive signatures in both anion photoelectron and Rydberg electron transfer spectra.[57,58] Valence anions of rare-tautomer nucleobases are another major category of parent nucleobase anions, and all five of them have been studied by photoelectron spectroscopy.[59] These robust valence anions of nucleobases come about through electron-induced, N-to-C proton transfers, and of course their structures differ from those of canonical nucleobases.

Uracil is unique among the nucleobases, in that it forms anions belonging to all of these categories. Its temporary anions have been studied by electron transmission spectroscopy.[60] In our own work, we have formed its dipole-bound anion using a nozzle-ion source (the sharp peak at low EBE in Fig. 1),[57] and we have also formed its rare tautomer, parent anion using the source described in the present paper (the broad peak in Fig. 1).[59] While we have not observed its parent, canonical valence anion with our sources, it has been formed and studied by Rydberg electron transfer.[50] High level theoretical calculations by Gutowski and co-workers[54,55] found the most stable valence anion of uracil to be its N1-to-C5 rare tautomer and *not its canonical form*, although the predicted VDE value of its N3-to-C5 rare tautomer agrees well with the observed VDE of the broad band in Fig. 1. Thus, while there are some lingering issues, for present purposes the important point is that the valence anion of canonical uracil is not believed to be the most stable parent anion of uracil.

By comparing the spectra of $(4\text{-TU})^-$ and $(2,4,\text{-DTU})^-$ with that of the dipole-bound form of uracil anion in Fig. 1, it is clear that neither $(4\text{-TU})^-$ nor $(2,4,\text{-DTU})^-$ are dipole-bound anions; they do not exhibit the characteristic signature of dipole-bound anions. The more pertinent question is whether the broad bands exhibited in the spectra of $(4\text{-TU})^-$ and $(2,4,\text{-DTU})^-$ are indicative of canonical or rare tautomer

anions. To answer this, we rely on the theoretical calculations of Dolgounitcheva, Zakrzewski, and Ortiz, which are presented in the companion paper.[61] These investigators found the valence anion of canonical 4-thiouracil to be its most stable parent anion. With a predicted VDE of 0.8 eV, this is consistent with the observed spectral band in the photoelectron spectrum of $(4\text{-TU})^-$. In addition, they found transitions at EBE ~3 eV, which corresponded to photodetachment from the ground electronic state of the canonical $(4\text{-TU})^-$ anion to electronically excited states of neutral 4-thiouracil. They also examined the N1-to-C5 rare tautomer of $(4\text{-TU})^-$, finding it to lie ≥0.5 eV higher in energy than its canonical anion with a predicted VDE of 1.30 eV. While this value is also consistent with the observed band in the spectrum of $(4\text{-TU})^-$, the N1-to-C5 anionic tautomer was ruled out as a contributor to the spectrum because of its significantly higher energy relative to the canonical anion. The N3-to-C5 anionic tautomer, which was found to have a similarly high energy, was predicted to have a VDE of 2.68 eV, a region of our spectrum where no significant signal was observed. Dolgounitcheva, Zakrzewski, and Ortiz concluded that only canonical, valence $(4\text{-TU})^-$ anions are responsible for the observed bands in our spectra.

The situation for the 2,4,-dithiouracil anion is similar. The same authors found the valence anion of canonical 2,4-dithiouracil to be its most stable parent anion. With a predicted VDE of 1.06 eV, this is consistent with the observed spectral band in the photoelectron spectrum of $(2,4\text{-DTU})^-$. They also investigated the N1-to-C5 rare tautomer of $(2,4\text{-DTU})^-$, finding it to lie ~0.25 eV higher in energy than its canonical anion with a predicted VDE of 1.77 eV. While this value is also numerically consistent with the observed band in the spectrum of $(2,4\text{-DTU})^-$, this anionic tautomer was again excluded as a contributor to the spectrum because of its higher energy relative to the canonical anion. The N3-to-C5 anionic tautomer, which was found to have an even higher energy relative to the canonical anion, was predicted to have a VDE of ~2.5 eV, again a region of our spectrum where no significant signal was observed. The authors again concluded that only canonical, valence anions are responsible for the observed bands in our spectra of $(2,4\text{-DTU})^-$.

Thus, based on our spectral results and complementary calculations by Dolgounitcheva, Zakrzewski, and Ortiz, we conclude that the valence anions of canonical 4-thiouracil and canonical 2,4-dithiouracil are their most stable anions. This is in contrast to the case of the uracil anion which had been generated in the same source environment. There, the most stable anion was found to be its N3-to-C5 rare tautomer, not its canonical form. Clearly, the replacement of oxygen atom(s) with sulfur atom(s) causes a significant change to the electronic structures of these two thiouracil anions relative to that of the uracil anion, switching the order of stability between canonical and rare tautomer forms. Thus, one should not assume that the valence anions of these two thiouracils are simply sulfur-substituted uracil valence anions. In reality, they might be expected to behave quite differently in physiological environments.

Lastly, we compare the photoelectron spectra of the 5-chlorouracil and 5-fluorouracil, halo-substituted uracil, anions with those of the 4-thiouracil and 2,4-dithiouracil, thio-substituted uracil, anions. The VDE values of 5-chlorouracil and 5-fluorouracil anions are 1.01 and 0.88 eV, respectively,[62] while those of $(4\text{-TU})^-$ and $(2,4\text{-DTU})^-$ are 1.05 and 1.4 eV, respectively. The electron affinities of 5-chlorouracil and 5-fluorouracil anions were measured to be ~0.5 and ~0.4 eV, respectively, while the electron affinities of 4-thiouracil and 2,4-dithiouracil were calculated to be 0.61 and 0.87 eV, respectively.[61] All four anions exhibit somewhat comparable VDE and EA values, and all four have spectral characteristics which identify them as valence anions. As described above, the electron affinity of uracil is thought to be positive but quite small. (The VDE of the valence anion of the canonical tautomer of uracil and the EA of its corresponding neutral have been computed to be 0.60 and 0.021 eV, respectively.[55]) Thus, both the thio-substituted uracils studied here and the halo-substituted uracils studied previously have electron affinities which are substantially larger than those of unsubstituted, canonical uracil.

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