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# TD-DFT Computational Insight into the Origin of Wavelength-Dependent *E*/*Z* Photoisomerization of Urocanic Acid

**Olga Dmitrenko,*,[†] Wolfgang Reischl,[‡] Robert D. Bach,[†] and Jens Spanget-Larsen[§]**

*Brown Laboratory, Department of Chemistry and Biochemistry, University of Delaware,*
*Newark, Delaware 19716, Institute of Organic Chemistry, University of Vienna, A-1090 Vienna, Austria,*
*and Department of Life Sciences and Chemistry, Roskilde University, DK-4000 Roskilde, Denmark*

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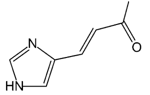
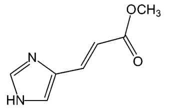
DFT and TD-DFT calculations in the gas phase and water have been performed for both anionic and zwitterionic forms of urocanic acid (UA) to gain an insight into the origin of its wavelength-dependent *E*/*Z* photoisomerization. Theoretical examination of the rotational flexibility of the carboxylic acid group by the B3LYP/6-31+G(d,p) method in conjunction with the COSMO solvent model supports the earlier suggestion (Danielsson, J.; Soderhall, J. A.; Laaksonen, A. *Mol. Phys.* **2002**, *100*, 1873) that in aqueous solution the carboxylic acid group may rotate. Our TD-B3LYP results on vertical excitation energies for the different C═C−COO$^-$ rotamers of zwitterionic UA indicate that deviation from planarity may lead to an alteration of the excited-state order. We suggest two possible singlet/triplet conical intersections (S2/T5 and S1/T3) located around 306 and 314 nm. A mechanistic discussion and simplified Jablonski-type diagrams are presented for three spectral regions where UA exhibits different photobehavior: (i) before S2/T5 ($\lambda$ < 306 nm), (ii) between two, S2/T5 and S1/T3, intersections (306 nm < $\lambda$ < 314 nm), and (iii) after S1/T3 (UV-A irradiation at $\lambda$ > 314 nm). *E*/*Z* photoisomerization occurs in the narrow spectral region ii on the n$\pi$* singlet excited surface. The comparison of molecular orbitals and an effective electronic potential analysis of anionic UA forms suggest that the excited state of parent N-anionic UA undergoes proton photoattachment, whereas T-anionic UA does not change its protonation state upon becoming excited. It is suggested that the formation of zwitterionic species from excited N-anionic UA is responsible for a similar photochemical behavior at different pH values (5.0 and 7.0) under the photostationary irradiation. The presence of the second tautomeric component can be one of the reasons for the slight differences for zwitterionic and anionic UA solutions observed experimentally in the transient absorption dynamics and fluorescence spectra.

## 1. Introduction

*trans*-Urocanic acid (*trans*-UA), produced by the enzyme histidase as a product of the metabolic deamination of histidine, is a major ultraviolet (UV) absorbing component in the epidermis with strong absorption in the UV-B region.[1] It is believed that its *Z*-isomer, *cis*-urocanic acid, formed upon UV irradiation via *E*/*Z* photoisomerization, possesses immunomodulating properties which may compromise the immune system.[1−3] Thus, the underlying photophysical/chemical processes are of significant interest for the understanding of urocanic acid's biological mode of action.

It has been found that *trans*-UA has very unusual wavelength-dependent photobehavior. *E*/*Z* isomerization has its maximum efficiency at the red edge of the absorption spectrum, whereas irradiation into the absorption band maximum results in very little *cis*-isomer formation.[4a,b] However, the methyl ester of UA (Me-UA) studied by Lewis et al.[5a,b] shows the opposite behavior (Table 1). Recent studies on some urocanic acid derivatives[5c] demonstrated that replacing the carboxylic acid hydrogen with $CH_3$, $C_2H_5$, and $C_{12}H_{25}$ does shift the *E*/*Z* isomerization efficiency to shorter wavelengths. *Thus, the nature of the carboxyl group, free acid or ester, in the UA molecule determines its specific wavelength-dependent behavior*. It is important to note that, in aqueous solution, *trans*-UA exhibits identical wavelength-dependent photochemistry regardless of pH (5.6−7.2).[4c] To our knowledge, this fact has not been addressed previously from a theoretical perspective. Here we consider both forms of *trans*-UA, two anionic tautomers and a zwitterion. The experimental studies indicate that at pH 5.6 UA exists in its zwitterionic form and becomes an anion at pH 7.2.[4d]

**TABLE 1: UA and Me-UA *E*/*Z* Isomerization Quantum Yields at Different Wavelengths**

| wavelength, nm | $f_{E/Z}$ (**UA**) [a]<br><br> | $f_{E/Z}$ (**Me-UA**)[b]<br><br> |
|---|---|---|
| 254 | 0.043; 0.058 | 0.94 |
| 266 | - | 0.84 |
| 290 | 0.079 | 0.78 |
| 313 | 0.49; 0.52 | 0.67 |

[a] Data are from ref 4, aqueous solutions. [b] Data are from ref 5a, acetonitrile solutions.

As evident by numerous experimental studies,[4,5] the mechanism of *trans*-UA photoconversion is complex and still unclear. Two typical models have been considered to explain the wavelength dependence: (i) ground-state conformational control;[6a]

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[*] To whom correspondence should be addressed. E-mail: odmitr@udel.edu.
[†] University of Delaware.
[‡] University of Vienna.
[§] Roskilde University.

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(ii) involvement of different excited states. Experimental studies established that unique photoreactivity does not arise from the presence of multiple ground-state conformers,[4c] but rather it is due to different electronic states.[6b] However, to date no general explanation for the existence of three spectral regions (see below) of the different photobehavior in aqueous solution has been put forth. Urocanic acid has also attracted much computational effort; a variety of theoretical methods have been used.[7] Despite the experimentally observed activity of the triplet excited state[4c−e] of *trans*-UA, its potential role in aqueous media has not been the focus of previous computational studies. In this connection, our study is aimed at the investigation of the overall activity of triplet and singlet excited states. It is important to note the following findings that stimulated our study: (a) Using MD simulations, Danielsson, Söderhäll, and Laaksonen have demonstrated that the carboxylic acid group rotates freely in an aqueous solution of the zwitterionic form.[8a] Thus, we investigate here the vertical excitation energy dependence on the carboxylic acid group torsion. (b) The order and character of the excited states are different in the gas phase and in water media. In aqueous solution, the photoizomerizing state does not absorb and has been assigned to the $n \rightarrow \pi^*$ transition,[4c] whereas in the gas phase, a study of *trans*-UA in a supersonic jet indicated that photoisomerization occurs after vertical excitation to a strongly absorbing state (S1) which the authors assigned to $\pi \rightarrow \pi^*$.[9] Theoretical studies predict that this is the lowest-lying vertical excited state for neutral *trans*-UA.[4f,8b]

Finally, three mechanistic pictures based on different *trans*-UA photoactivities experimentally observed in three spectral regions are proposed in the present paper: (i) Irradiation with high-energy photons (264 and 266 nm) results in intersystem crossing (ISC) and the population of triplet states. The studies were performed at pH 5.6 and 7.2, where UA exists in its zwitterionic and anionic forms, respectively.[4d] (ii) Regardless of pH (5.6 or 7.2), at 306 and 313 nm, an efficient *E*/*Z* photoisomerization dominates. No ISC has been observed.[1a,c,4d] (iii) Excitation in the UV-A region (320−400 nm) results in competing ISC and *E*/*Z* processes with the peak of triplet formation at ∼340 nm (pH 7.3).[1c−e,3b]

## 2. Computational Details

Quantum chemistry calculations were carried out using the Gaussian98 program system[10] utilizing gradient geometry optimization.[11] All geometries were fully optimized using the B3LYP functional[12,13] with the 6-31+G(d,p) basis set and the COSMO solvation model (polarizable conductor model)[14] with a dielectric constant $\epsilon = 78.9$. Vertical energies of the singlet- and triplet-excited-state transitions have been calculated using the time-dependent DFT method,[15] TD-B3LYP/6-311+G(d,p), which has been shown to be excellent for modeling ionic, rotameric, and protomeric forms of urocanic acid.[8b] TD-DFT provides good quantitative accuracy for small molecules ($NH_3$, $CH_4$, $H_2O$),[15d] and its computational economy makes it a good candidate for application to more complicated molecules.[15e] Corrections for water solvation in the excited states were made using COSMO model calculations (dielectric constant $\epsilon = 78.9$) in conjunction with the TD-B3LYP/6-311+G(d,p) method. The partial charges were calculated using an MP2 density, a 6-31+G(d,p) basis set, and the NBO method[16a] implemented in the Gaussian 98 program. For the prediction of effective electronic potentials $\delta W$ (see below),[16b] partial atomic charges for ground and excited electronic states were computed with the ZINDO/S[16c] method as implemented in the HyperChem[16d] molecular modeling package. The ZINDO/S $\sigma-\sigma$ and $\pi-\pi$ overlap weighting factors[16c,d] were taken as 1.267 and 0.585, and the excited state was obtained by selecting the SCF option "Next lowest state". For the anionic UA normal and tautomeric forms, the energy of this state ($\pi\pi^*$) is predicted to be 5.0 and 4.6 eV above that of the ground-state configuration. This is in good quantitative agreement with the TD-B3LYP results.

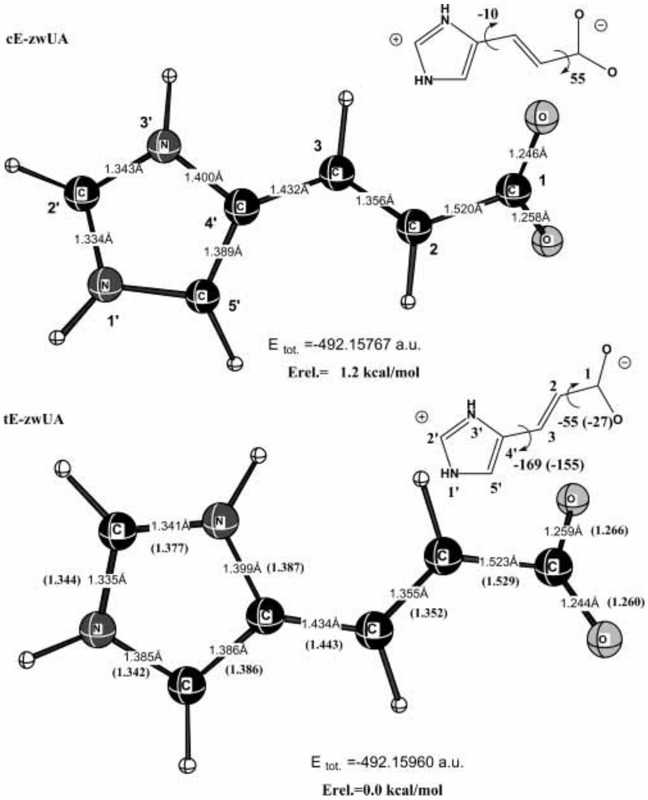


**Figure 1.** Zwitterionic structures of UA optimized at B3LYP/6-31+G(d,p) in the gas phase. Numbers in parentheses are at the MP2/6-31+G(d) level of theory.

The reliability of TD-B3LYP/6-311+G(d,p) solution-phase (water) calculations has been tested by comparing them with recent MS-CASPT2 results for the anionic form of urocanic acid.[7g] TD-DFT excitation energies for $1^3A'$, $1^3A''$, $2^3A'$, $1^1A''$, $2^1A'$, and $3^1A'$ excited states (Supporting Information) are in satisfactory agreement with the MS-CASPT2 energies from ref 7g as well as with similar TD-DFT calculations performed by Danielsson et al.[8b] As discussed by Page et al.[7g] and Danielsson et al.,[8b] there is a problem obtaining reasonable gas-phase results for zwitterionic UA (zwUA) because of charge separation; thus, one must account for solvent effects.

To describe the geometries of the *trans*-isomer of zwUA, we use the following abbreviations: "E" is equivalent to the above "*trans*" (*E*-isomer). "c" and "t" refer to *cis* and *trans* geometries, respectively, about the single C−C bond attached to the five-membered ring and determined by torsional angle −C=C−C=C.

In this study we focus on zwUA (section 3.1), since this form dominates under physiological conditions (pH ≈ 4−6). An anionic UA will be discussed in section 3.2.

## 3. Results and Discussion

**3.1. Zwitterionic Form of Urocanic Acid**. Both zwitterionic UA conformers, cE-zwUA and tE-zwUA, optimized in the gas phase are nonplanar (Figure 1) as also noted by Danielsson et al.[8b] The tE-zwUA conformer is preferred by 1.2 and 0.8 kcal/mol in the gas phase and in water. No significant conformational influence was found for the excited states of neutral and anionic

**TABLE 2: NBO Charges of the Functional Units of Neutral, Anionic, and Zwitterionic Urocanic Acid Optimized at the B3LYP/6-31+G(d,p) Level in the Gas Phase**

| unit | tEc-UA | tE-UA anion | planar tE-zwUA[a] | nonplanar tE-zwUA |
|---|---|---|---|---|
| five-membered ring | 0.026 | −0.085 | 0.792 | 0.774 |
| −CH═CH− | 0.026 | −0.070 | −0.038 | −0.056 |
| COO(H) | −0.052 | −0.845 | −0.754 | −0.719 |

[a] Transition structure ($\upsilon_i$=79.4i cm$^{-1}$)

forms of UA.[8b] Thus, we report here only the results for the tE conformation, which is the most stable, whereas almost identical results for cE-zwUA are given in the Supporting Information.

Planar tE-zwUA, located in the gas phase, represents a transition structure which is slightly higher in energy than the nonplanar ground-state minimum by 0.9 kcal/mol and has a small imaginary frequency ($\upsilon_i = 79.4i$ cm$^{-1}$) characterized by two torsional vibrations: vibration of the carboxylic acid group around the carbon−carbon bond and H−C═C−H torsion vibration. The rotamer with its −C3═C2−C1−O dihedral angle fixed to −60° is 0.7 kcal/mol higher in energy than the nonplanar minimum shown in Figure 1. Thus, in the gas phase, the ground-state potential energy surface (PES) is quite shallow.

The fact that the global minimum for zwitterionic UA in the gas phase is a nonplanar structure can be understood from a comparison of NBO charges of the functional units of tE-zwUA (Table 2). The charge separation is maximal in planar tE-zwUA, thus providing electrostatic destabilization for this structure. If solvent is accounted for, the charge separation is stabilized and a planar minimum of tE-zwUA is located. The rotamer with its −C3═C2−C1−O angle fixed to −60° is 2.2 kcal/mol higher in energy.

Such relatively small energy and geometry fluctuations upon phase alteration (gas phase/solvent phase) allow us to assume that in aqueous solution the carboxylic acid group may, at least, vibrate around a planar minimum in the range of dihedral angles −60°/+60°. Molecular dynamic studies have provided a strong suggestion that the carboxylic acid group rotates almost freely in an aqueous solution of the zwitterionic form.[8a]

The effect of carboxylic acid group rotation on the excited states of tE-zwUA was studied using TD-B3LYP calculations on the fully optimized planar structure in water and two rotamers partially optimized with their −C3═C2−C1−O torsional angles fixed to −30° and −60° (no other constraints). The results are summarized in Table 3. Our excitation energies for planar tE-zwUA agree very well with the TD-B3LYP/PCM results presented in ref 8b. We also found that there are two "dark" first singlet excited states of $n \rightarrow \pi^*$ character (S1, HOMO → LUMO [n(anti-OO) → $\pi^*$(C═C)], and S2, HOMO − 2 → LUMO [n(symm-OO) → $\pi^*$(C═C)], in planar zwUA) with almost zero oscillator strength followed by "bright" $\pi \rightarrow \pi^*$ transitions. The transition assignments were based upon detailed consideration of molecular orbitals shown in Table 4 and principal configurations from the TD-B3LYP calculations. A comparison of molecular orbitals in Table 4 indicates that there are pronounced changes upon carboxylic acid group rotation (from 0° to −60°), which affect the character of calculated excited-state transitions. In the twisted structures, HOMO orbitals possess some $\pi$ in addition to n character (antibonding combination of two in-plane oxygen lone pairs, Table 4). Thus, initially forbidden transitions to the excited singlet states S1 and S2 become allowed due to the planarity distortion, which is reflected by increased oscillator strengths (Table 3).

Using the simplified approach from our earlier study on previtamin D,[17a] we have simulated the UV absorption spectrum of zwUA (Figure 2). Our simulation is based upon the assumption that 0°, −30°, and −60° rotamers are equally populated and have a Gaussian band shape of half-width equal to 25 nm, whose maximum occurs at the Franck−Condon transition wavelength.[17b] The maximum of the simulated spectrum is around 255 nm, which differs from the experimental maximum ($\lambda_{\max}$ = 268 nm at pH 5.6).[4d,e] Nevertheless, qualitatively, it demonstrates that at the red edge of the absorption band twisted structures may be responsible for the low-energy photon absorption.

Analysis of the data in Table 3 indicates that, in the rotameric structures with a carboxylic acid group dihedral angle of −60°, the energetic order of the pairs of states (S1, T3) and (S2, T5) is the reverse of the one in the strictly planar structure.

In Figure 3, the excitation energies to these four states are plotted vs the torsion angle of the carboxylic acid group. Two intersection points, at 306 and 314 nm, suggest that three spectral regions should be considered (i−iii) representing different photobehavior. Almost identical TD-B3LYP results and energy at the intersection points (306 and 313 nm) are found for the cE-zwUA conformer (Figure 3S, Supporting Information).

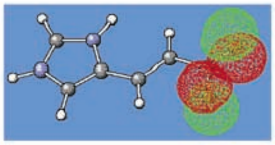
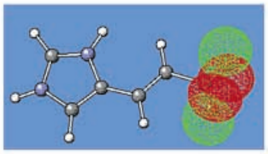
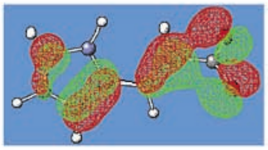
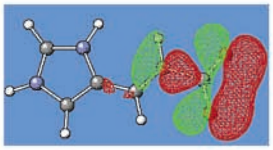
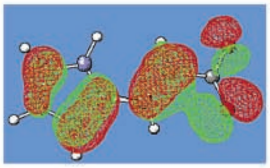
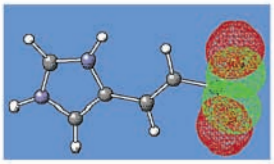
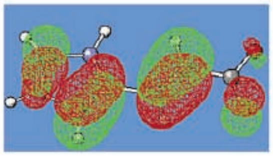
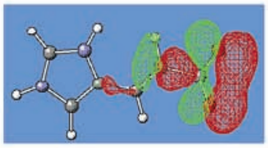
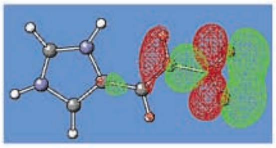
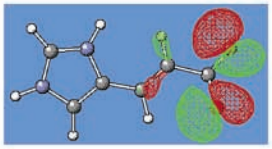
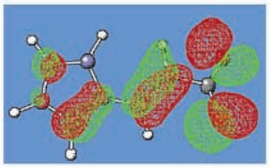
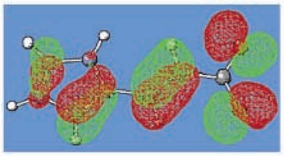
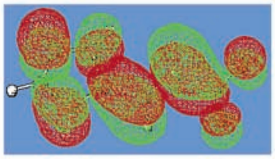
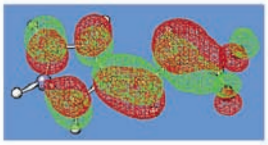
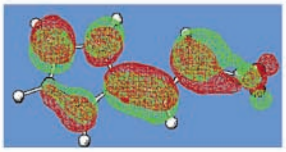
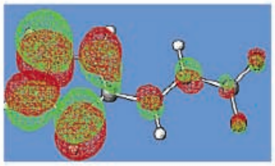
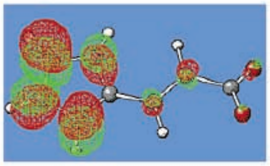
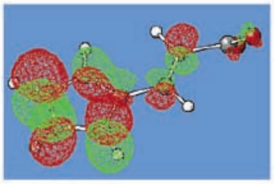
*(i) Excitation by UV Light at $\lambda$ < 306 nm.* In this relatively high energy region, higher $\pi \rightarrow \pi^*$ singlet states or vibrationly

**TABLE 3: Excitation Energies (*E*, eV) and Oscillator Strengths ($f_{osc}$) Calculated at the TD-B3LYP/6-311+G(d,p)+COSMO(Solvent=Water) Level of Theory for the Planar E-zwUA and Its Rotamers with the Twisted Carboxyl Group (−C3═C2− C1−O)[a]**

| | dihedral angle −C3═C2−C1−O = 0° | | dihedral angle −C3═C2−C1−O = −30° | | dihedral angle −C3═C2−C1−O = −60° | |
|---|---|---|---|---|---|---|
| state and description | E | $f_{osc}$ | E | $f_{osc}$ | E | $f_{osc}$ |
| T1 $\pi\pi^*$ [35 → 37][b] | 2.85 | 0.00 | 2.82 | 0.00 | 2.86 | 0.00 |
| T2 n$\pi^*$ [36 → 37] | 3.50 | 0.00 | 3.74 | 0.00 | 3.95 | 0.00 |
| T3 n$\pi^*$ [43 → 37] | 3.75 | 0.00 | 3.83 | 0.00 | 4.10 | 0.00 |
| T4 $\pi\pi^*$ [33 → 37] | 3.79 | 0.00 | 3.97 | 0.00 | 4.23 | 0.00 |
| T5 $\pi\pi^*$ [35 → 38] | 4.11 | 0.00 | 4.05 | 0.00 | 4.41 | 0.00 |
| T6 $\pi\pi^*$ [32 → 37] | 4.89 | 0.00 | 4.95 | 0.00 | 5.10 | 0.00 |
| S1 n$\pi^*$ [36 → 37] | 3.66 (3.62)[c] | 0.00 (0.02) | 3.81 | 0.05 | 4.13 | 0.18 |
| S2 n$\pi^*$ [34 → 37] | 4.03 (3.89) | 0.00 (0.00) | 4.15 | 0.00 | 4.45 | 0.02 |
| S3 $\pi\pi^*$ [33 → 37] | 4.57 (4.47) | 0.03 (0.01) | 4.66 | 0.01 | 4.82 | 0.01 |
| S4 $\pi\pi^*$ [35 → 37] | 4.79 (4.69) | 0.50 (0.10) | 4.79 | 0.24 | 4.84 | 0.02 |
| S5 n$\pi^*$ [36 → 38] | 5.02 (4.89) | 0.00 (0.33) | 5.06 | 0.28 | 5.24 | 0.35 |
| S6 n$\pi^*$ [34 → 38] | 5.04 | 0.00 | 5.10 | 0.00 | 5.28 | 0.00 |

[a] Structures were optimized at the B3LYP/6-31+G(d,p)+COSMO(Solvent=Water) level. [b] Characteristic transitions with coefficients larger than 0.53 are given in brackets; corresponding molecular orbitals and their numbering are reported in Table 4. [c] Solution-phase data given in parentheses are from ref 8b.

**TABLE 4: Selected Molecular Orbitals of Zwitterionic *trans*-Urocanic Acid, Planar tE-zwUA, and Its Rotamers with the Twisted Carboxyl Group [B3LYP/6-31+G(d,p),scrf=(COSMO, Solvent=Water)]**

| | Dihedral angle <C3=C2—C1-O | | |
|---|---|---|---|
| orbital | 0° | -30° | -60° |
| 33<br>HOMO-3 |  |  |  |
| 34<br>HOMO-2 |  |  |  |
| 35<br>HOMO-1 |  |  |  |
| 36<br>HOMO |  |  |  |
| 37<br>LUMO |  |  |  |
| 38<br>LUMO+1 |  |  |  |

hot S2 states are activated. The final point of the relaxation process is the intersection S2/T5 (306 nm), and then ISC (intersystem crossing) takes place and triplet states are produced (Figure 4).

*(ii) Excitation by UV Light at 306 nm < λ < 314 nm*. All the experimental studies show that there is only a very narrow spectral window of UVlight that may initiate efficient *E*/*Z* photoisomerization of urocanic acid in aqueous solutions. Our calculations suggest this region is the spectral interval between two intersection points [91.2 and 93.5 kcal/mol (306 and 314 nm)]. When the system is excited to its S2 state or to hot S1 (which becomes partially allowed in the twisted structure), it relaxes on the S1 surface. If the excited molecule possesses enough energy on the S1 surface to overcome a small barrier [the estimate of 1500 $\text{cm}^{-1}$ (4.3 kcal/mol) is given in ref 1d], it will undergo *E*/*Z* isomerization (Figure 5). Otherwise, the system relaxes through the ISC point S1/T3 and produces excited triplets (spectral region iii).

*(iii) Excitation by UV Light at λ > 314 nm.* In addition to the finding that photoisomerization occurs after excitation of *trans*-UA in UV-A (320−400 nm),[3b] recently, Simon and co-workers have also observed that, in the region around 320 nm and longer, ISC and *E*/*Z* isomerization processes compete.[1c,d] The peak of the action spectrum for triplet formation was found at $\lambda \approx 340$ nm (pH 7.2).[1c] In a more recent study, it was remeasured and found at $\lambda \approx 320$ nm.[1d] A plot of the efficiency spectrum for intersystem crossing for *trans*-UA (which is more relevant for the mechanism discussion than the action spectrum for triplet formation) has nonmonotonic character with a maximum at around 330 nm as indicated in Figure 8 given in ref 1d. In this connection, we suggest (Figure 6) that with a certain probability, a vibrationally hot excited S1 system may bypass the barrier for *E*/*Z* isomerization due to tunneling, or it may relax via the S1/T3 intersection and produce triplets. The competition between these two channels of relaxation depends on the tunneling efficiency, which in turn is determined by the

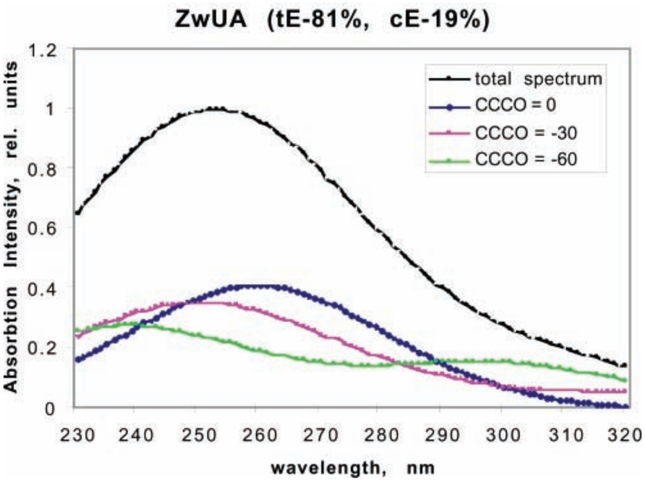


**Figure 2.** Calculated UV absorption spectrum of zwUA. The individual contributions from the planar and two twisted structures are shown by blue, pink, and green curves.

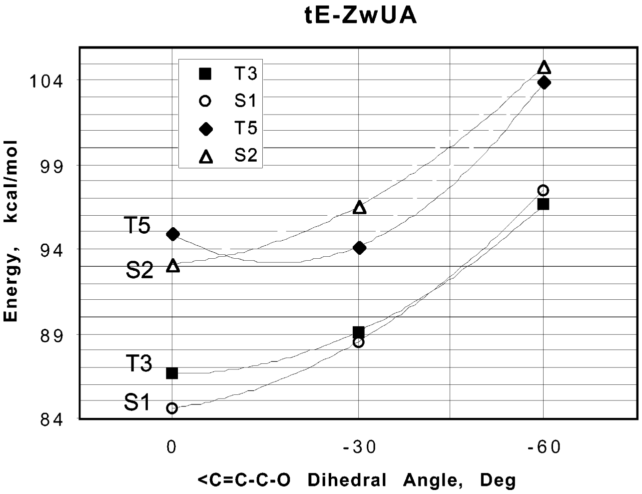


**Figure 3.** S1, S2, T3, and T5 excitation energies in tE-zwUA vs dihedral angle $-C3{=}C2-C1-O$ of the carboxylic group [TD-B3LYP/6-311+G(d,p)+COSMO(water)//B3LYP/6-31+G(d,p)+COSMO-(water)].

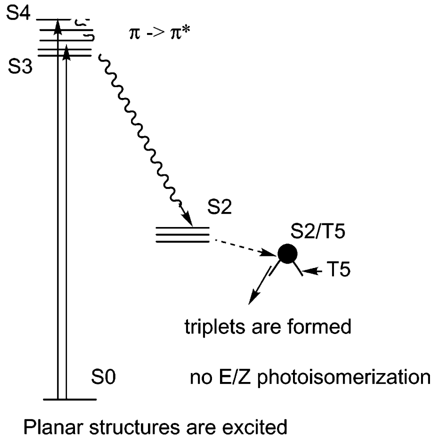


**Figure 4.** Simplified Jablonski-type diagram illustrating the mechanistic scenario proposed for high-energy irradiation at $\lambda < 306$ nm.

excess excitation energy. With the lowering of excitation energies, the system becomes unable to reach the S1/T3 intersection and triplet formation decreases (in accord with the probable tunneling contribution).

**3.2. Anionic Form of Urocanic Acid**. The photochemistry and photophysics of urocanic acid in aqueous media were found to be almost similar for both zwitterionic and anionic forms (at pH values around 5 and around 7).[4c,d,6a] To get an idea of why there is no or little pH effect, we have optimized anionic UA in its two forms, parent and tautomeric (N-anionic UA and T-anionic UA, Figure 7). In water, we found the N-anionic UA to be more stable than T-anionic UA by 0.4 kcal/mol. Additionally, supermolecules with the total charge "−1" consisting of anionic UA and two water molecules were optimized as well (Figure 7). In this case, the tautomeric form (T) is 0.3 kcal/mol higher in energy. This is a quite small energetic difference. It is suggestive that both anionic forms of urocanic acid exist in about 1:1 equilibrium which can be shifted in favor of either N-anionic or T-anionic UA by external microenvironment factors. Thus, we consider here both forms, starting from N-anionic UA.

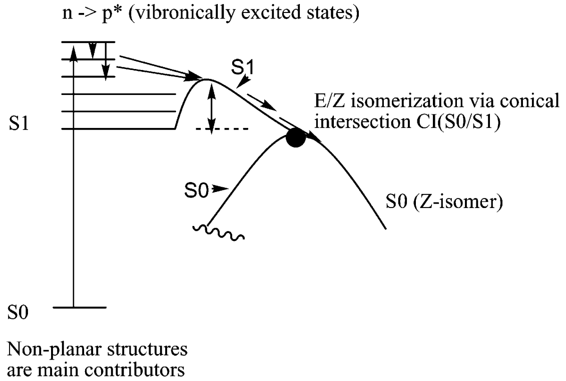


**Figure 5.** Simplified Jablonski-type diagrams illustrating the mechanistic scenario proposed for the spectral interval between 306 and 314 nm.

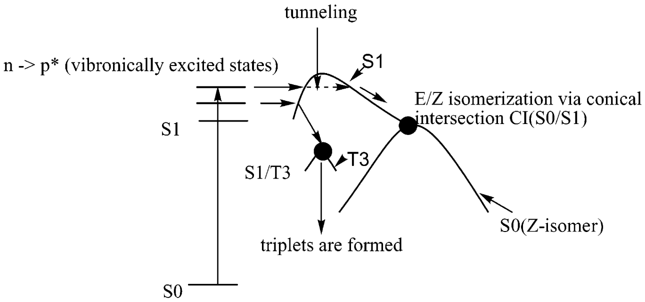


**Figure 6.** Simplified Jablonski-type diagrams illustrating the proposed mechanistic scenario for UV-A irradiation at $\lambda > 314$ nm.

On the basis of the fact that urocanic acid in its anionic and zwitterionic forms shows similar excitation and fluorescence spectra and photobehavior (regardless of pH), it was suggested earlier that the excitation of zwUA and anionic UA results in the population of the same anionic excited state due to imidazole proton transfer to the solvent.[4c,d,6a] Our data indicate that it might be the same excited state but not necessarily anionic. Recent photophysical studies by Chacón et al. questioned the statement that the fluorescence of urocanic acid does not depend on the initial protonation state and always occurs from the singlet excited state of the anionic form of UA.[18] Our analysis below suggests that, when excited, N-anionic UA may attach the proton to the N3′ nitrogen (which becomes more basic upon excitation) and produce the zwitterionic excited state.

The suggestion of a common anionic excited state in ref 4d is based upon the fact that the excitation spectrum (its 280 nm maximum is independent of the pH) agrees well with the absorption spectrum of the anion and the idea that the absorption transition must decrease the electron density on the imidazole ring, making the nitrogen more acidic. The authors refer to

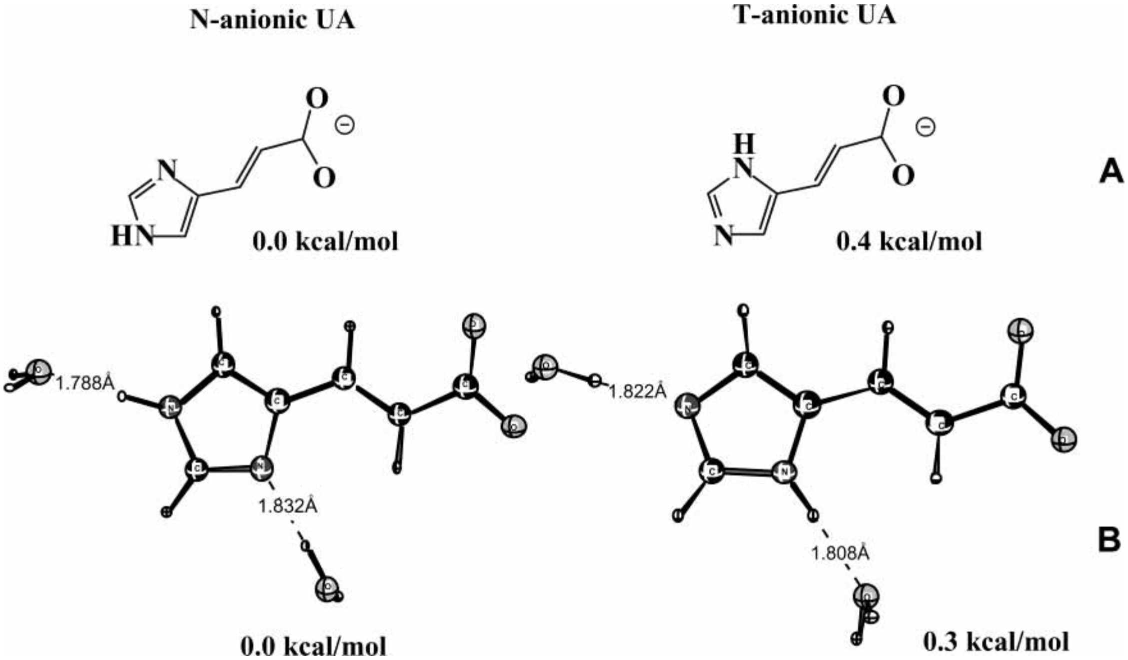


**Figure 7.** Relative energies of parent N-anionic UA and its tautomeric form, T-anionic UA, optimized in water (COSMO solvent model) without (A) and with (B) two additional explicit H-bonded water molecules at the B3LYP/6-31+G(d,p) level of theory. The total charge of the system is "−1".

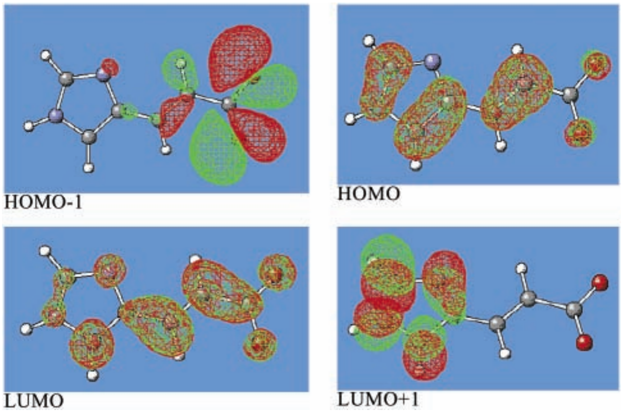


**Figure 8.** HOMO − 1, HOMO, LUMO, and LUMO + 1 orbitals of N-anionic UA.

studies on 2-naphthol, which is a weak acid in the ground state, but a much stronger acid upon excitation to its lowest excited singlet state.[19] To our knowledge, there is no experimental p$K_a$ trend data available for the urocanic acid, and comparison with 2-naphthol photobehavior is not supportive of the anionic character of the urocanic acid excited state, which has two possible protonated/deprotonated sites (OH and NH). In the literature, one may find closer bifunctional examples, where, for a molecule with the opposite p$K_a$ tendencies in its excited states, a photon absorption may initiate protonation and deprotonation to yield zwitterions. For instance, a kinetic study on 7-hydroxyquinoline indicates that a change in prototropic equilibrium can occur as a result of photon-triggered p$K$ values of OH and N atoms and thereby lead to the population of excited zwitterionic species.[20]

Since the idea on the protonation state of the excited UA was based upon the premise that the electron density on the imidazole ring is decreasing upon excitation, we compared the molecular orbitals for anionic UA. Both LUMO and LUMO + 1 orbitals of the N-anionic UA have a contribution from the N3′ atom orbital in contrast to the case of HOMO and HOMO − 1 (Figure 8). This indicates an increase of electron density at N3′ upon excitation (for $\pi\pi^*$ and n$\pi^*$ surfaces), and thus, one should expect an increase of atom basicity in the excited state.

However, it is well-known that, in general, there is no simple correlation between computed nitrogen charge densities and experimental p$K_a$ values (see refs 16b and 21 and references cited therein). To support the zwitterionic-excited-state suggestion, effective electronic potentials were computed by using the previously published semiempirical procedures.[16b,21] The effective potentials $W$ correspond to "local ionization energies" [21] and include electrostatic long-range contributions that have been shown to be important in the prediction of relative p$K_a$ values.[16b,21,22] The present predictions were based on the optimized molecular geometry for anionic UA in an aqueous medium computed with DFT (see above), using ZINDO/S[16c]-derived ground- and lowest-excited-state[16d] ($\pi\pi^*$) partial atomic charges. The resulting atomic $\delta W$ values (eV) are shown in Figure 9. $\delta W$ indicates the effective potential relative to the isolated neutral atom; the more positive the $\delta W$, the higher the basic character associated with the corresponding region of the molecule. As shown in Figure 9, $\delta W$ for the N3′ position is increased from 5.3 to 5.7 eV on excitation; this indicates a large increase in nitrogen basicity corresponding to several p$K$ units.[21,22] At the same time, a large decrease in basicity of the carboxylate group is predicted.

Thus, we suggest that N-anionic UA, due to the increase in basicity of the nitrogen atom upon excitation, will undergo rapid protonation, producing the excited state of zwitterionic character. As has been observed for 7-hydroxyquinoline, nitrogen excited-state protonation is fast and occurs on the picosecond time scale.[20]

There are no similar observations for T-anionic UA (molecular orbitals and $\delta W$ are given in the Supporting Information); thus, one may conclude that these species are not prone to excited-state protonation and remain in their anionic form.

The independence of the pH excitation spectrum[4d,6a] can be explained by the similar $\pi\pi^*$-excited-state energies for zwUA and both anionic forms (4.6−4.5 eV, Table 5). The absorption

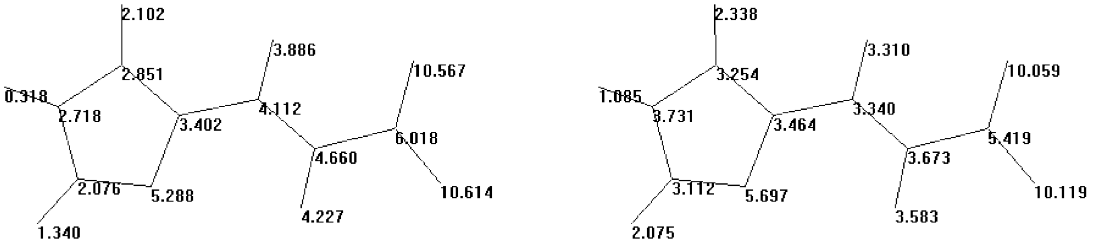


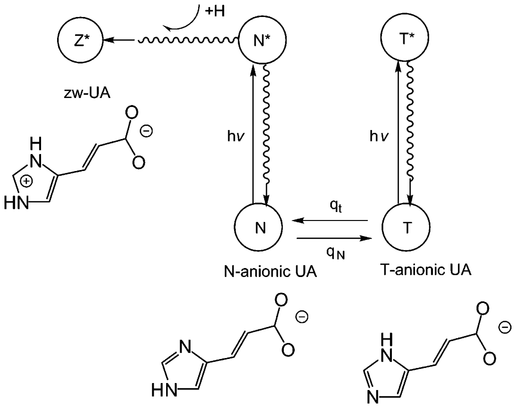
**Figure 9.** $\delta W$ values (eV) calculated for the ground state (left) and excited state (right) of N-anionic UA.

**TABLE 5: Singlet−Singlet Excitation Energies (*E*, eV) and Oscillator Strengths ($f_{osc}$) Calculated at the TD-B3LYP/6-311+G(d,p)+COSMO(Solvent=Water) Level of Theory for the tE-zwUA and Two Anionic Forms, N-anionic UA and T-anionic UA[a]**

| | zwUA | | N-anionic UA | | T-anionic UA | |
|---|---|---|---|---|---|---|
| state | E | $f_{osc}$ | E | $f_{osc}$ | E | $f_{osc}$ |
| S1 $n\pi^*$ | | | | | | |
| [n(anti-OO) → $\pi^*$(C=C)] | 3.66 | 0.00 | 4.23 | 0.00 | 4.11 | 0.00 |
| S2 $n\pi^*$ | | | | | | |
| [n(symm-OO) → $\pi^*$(C=C)] | 4.03 | 0.00 | 4.66 | 0.00 | 4.51 | 0.00 |
| S3 $\pi\pi^*$ | | | | | | |
| [$\pi$(anti-OO) → $\pi^*$(C=C)] | 4.57 | 0.03 | 5.33 | 0.04 | 5.15 | 0.04 |
| S4 $\pi\pi^*$ | | | | | | |
| [$\pi$(C=C) → $\pi^*$(C=C)] | 4.79 | 0.50 | 4.63 | 0.58 | 4.47 | 0.66 |

[a] Structures were optimized at the B3LYP/6-31+G(d,p)+COSMO(Solvent=Water) level.

**SCHEME 1**



spectrum of zwUA is blue-shifted due to the additional strongly allowed $\pi\pi^*$ excited state at 4.8 eV.

Simon and co-workers found a slight difference at pH 5.6 and 7.2 in the 266 nm pump/340 nm probe transient absorption dynamics. They observed an initial fast decay component (∼1 ps) at pH 7.2 which is absent at pH 5.6.[4d] It has been suggested that the excitation at 266 nm generates a molecule with more excess energy at pH 7.2 than at pH 5.6 since at pH 7.2 the electronic excited state of t-UA lies lower in energy than it does at pH 5.6.[4d] Our data given in Table 5 indicate that zwUA and N-anionic UA have the same energies for the lowest $\pi\pi^*$ transition (about 4.6 eV), whereas the T-anionic UA $\pi\pi^*$ transition is slightly lower, at 4.5 eV. This can be an additional potential source for the fast vibrational relaxation process characterized by that 1 ps decay component observed in the experiment.[4d]

On the basis of the results from Table 5 and experimental facts, one may suggest that excitation of the anionic UA solutions occurs as shown in Scheme 1. According to Scheme 1, under the conditions of photostationary irradiation, the presence of the tautomeric form, T-anionic UA, should not affect the overall photochemistry. Thus, the resulting *trans*-UA photochemistry is controlled by relaxation of the excited N-anionic UA state through the process of proton attachment and subsequent photoconversion of the zwitterion, zwUA.

Recent studies on another bifunctional molecule, 5*H*-pyrido[3,2-*b*]indole, have indicated that the existence of tautomeric forms in the ground state may lead to the wavelength dependence of the excitation and emission fluorescence spectra in acid media.[23] The presence of the second populated tautomer, T-anionic UA, can be a potential reason for the observed different emission maxima (400 nm at pH 7.0 and 360 nm at pH 4.0). More theoretical (similar to the study performed by Sobolewski, Domcke, and co-workers[24]) and experimental work is required to asses the contribution of both anionic tautomers vs the zwitterionic form.

## 4. Conclusions

In aqueous media, *E*/*Z* photoisomerization of urocanic acid occurs on the $n\pi^*$ singlet excited surface. This explains the unique photobehavior of *trans*-urocanic acid, which undergoes efficient *E*/*Z* isomerization only upon irradiation at the red edge of the absorption band. The mechanism responsible for the wavelength-dependent urocanic acid photochemistry in aqueous media may involve two conical intersections between excited singlet and triplet surfaces. These conical intersections are suggested to exist due to possible rotation of the carboxylic acid group in the zwitterionic form of UA. The high density of excited states (which in addition can be modulated by interactions with water molecules) makes it very possible that singlet/singlet and triplet/triplet intersections contribute to the complex UA photochemistry as well. In the present work we addressed only the possibility of singlet/triplet conical intersections using potential energy profiles of the excited states as a function of the carboxylic acid group torsion. The comparison of molecular orbitals and an effective electronic potential analysis of anionic UA suggest that the excited parent N-anionic UA accepts the proton from the solution and becomes a zwitterion. This can be a reason for a similar photochemical behavior at pH 4.0 and 7.0 under the photostationary irradiation. T-anionic UA does not change its protonation form upon excitation. The presence of the second tautomeric component can be responsible for the slight differences observed in the transient absorption dynamics experiment (266 nm pump/340 nm probe)[4d] and fluorescence spectra at $\lambda_{excitation} = 310$ nm.[18]

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**Supporting Information Available:** Total energies, Cartesian coordinates, molecular orbitals. and excitation energies for neutral, zwitterionic. and anionic forms of *trans*-UA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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