A TD-DFT Study of the Photochemistry of Urocanic Acid in Biologically Relevant Ionic, Rotameric, and Protomeric Forms

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Abstract: The photochemistry of Urocanic acid, a chromophore present in human skin and linked to photoimmunosuppression and skin cancer, is investigated theoretically by means of time-dependent density functional theory. Extensive calculations are carried out for different ionic, rotameric, and protomeric forms of both the trans and cis form. Inclusion of solvation effects, here accounted for by means of a continuum solvent model, are found to be crucial for the correct description of the biologically relevant zwitterionic forms of the molecule. For the trans zwitterionic form, it is found that the planar form usually assumed in the literature is not stable, and that a realistic charge separation cannot be achieved in the gas phase. Calculated vertical excitation energies are in excellent agreement with available experimental data, with a weakly absorbing $n \rightarrow \pi^*$ transition around 4.0 eV, and strongly absorbing $\pi \rightarrow \pi^*$ transitions at 4.5−4.9 eV. The debated intramolecular hydrogen bond is predicted to have a modest impact on the vertical spectra in solution, but improves agreement with experiment when included. In general, we also predict that different rotameric forms have very similar absorption spectra. In addition, we find a candidate absorbing state to link *trans*-urocanic acid to singlet oxygen production and subsequent photoaging of the skin.

Introduction

Urocanic acid (4-imidazoleacrylic acid), see Figure 1, is an epidermal chromophore normally present in human skin.[1] It is formed in its trans form by deamination of histidine by the enzyme histidine ammonia-lyase. Because the enzyme that degrades urocanic acid, urocanase, is not present in the human skin, urocanic acid accumulates in the outer *epidermis*. Since the absorption spectrum of urocanic acid strongly overlaps with that of DNA in the UVB region it is thought that it has a UV protective role.[2] However, through a groundbreaking paper in the 80's De Fabo and co-workers demonstrated that urocanic acid is a mediator of UV-induced immunosuppression in its cis form,[3] which occurs due to photoisomerization of urocanic acid in the UV range.[4] This immunosuppressive effect has also been linked to UV-induced skin cancer.[5]

These findings sparked a large interest in understanding the photochemistry of this chromophore, and a large number of spectroscopic studies have been reported. See the excellent review of Mohammad et al.[6] for many references. Soon it was determined that the photochemistry was quite complex and dependent on both wavelength[7] and protonation state.[8,9] Urocanic acid has three protonation sites, one located at the carboxylic group and two on the nitrogens of the imidazole ring (see Figure 1). The absorption spectrum of both *trans*- and *cis*-urocanic acid is broad and featureless and peaks at about 4.7 eV at the pH where urocanic acid is present in its zwitterionic form ($3.5 < pH < 5.8$). This is the form that dominates in the outer *epidermis* where pH ranges between 4 and 6. As pH is increased to neutral as it is in the inner, viable part of the *epidermis* the anionic form dominates and the absorption maximum is redshifted about 0.15 eV. On the other hand, the isomerization efficiency is highly wavelength dependent with the maximum efficiency in the red tail of the spectrum (around 4.0 eV), where the absorption is low. Early theoretical investiga-

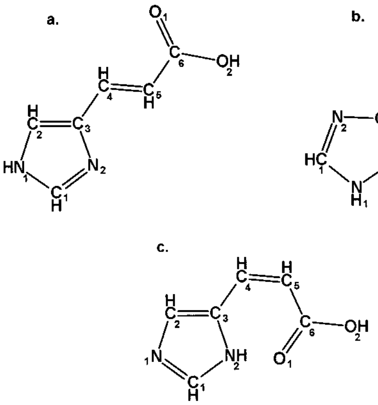

Figure 1. Structures of neutral trans (a and b) and cis (c) forms of urocanic acid. (a) The lowest energy rotamer of the trans isomer.

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tions by Shukla and Mishra using the complete neglect of differential overlap (CNDO/S) method[8] suggested that this could be due to population of different rotamers in solution, but experimental data from photoacoustic calorimetry by Hanson et al.[10,11] strongly indicate that the photochemistry can be explained in terms of the presence of several states with different photochemical behavior.

In a more recent study by Hanson et al,[12] the action spectrum of singlet oxygen production by urocanic acid was linked to the corresponding spectrum of skin photoaging. Although it is yet to be shown that this has biological significance, they concluded that the peak of the singlet oxygen production action at 3.6 eV comes from a triplet state that could be populated either directly or via a singlet state.

Very recently, supersonic jet spectroscopy was used to investigate the gas-phase photochemistry of the neutral form of *trans*-urocanic acid.[13] They concluded that the lowest lying excited state resulted from a quite strongly absorbing $\pi \rightarrow \pi^*$ transition close to the band origin that was placed at 4.04 eV, and also that the population of this state was responsible for the photoisomerization. This stands in contrast to the interpretation of the solution experiments, conducted with photoacoustic calorimetry, where the photoisomerization was assigned to a weakly absorbing $n \rightarrow \pi^*$ transition.[10]

There have also been two recent high-level quantum chemistry investigations of the low-lying states of urocanic acid in different protonation forms.[14,15] Although the method used, complete active space with second order perturbation (CASPT2), is considered as very reliable in predicting excited state photochemistry, the calculations were all carried out in the gas phase and with forced planar geometries, which could be a problem, especially when considering the zwitterionic forms. Also, the experimental investigation of the electronic spectroscopy of urocanic acid in a supersonic jet did not show any obvious assignment in terms of the CASPT2 results.[13]

This and the need to characterize the excited states in a biologically relevant solvent motivated us to combine correlated time-dependent density functional calculations with a polarizable continuum solvent model, and to perform calculations on a large number of possible structures to address the influence of ionic, rotameric, and protameric states of both trans and cis isomers of urocanic acid.

Computational Details

For all calculations, we have used Becke's three-parameter hybrid functional[16] for exchange combined with the correlation functional due to Lee, Yang, and Parr[17] (B3LYP). Both geometries and excitation energies were calculated by using the 6-311+(d,p) basis set, including both polarization functions and diffuse basis functions. For excitation energies we used the GAUSSIAN implementation of time-dependent density functional theory (TD-DFT),[18] which only allowed us to calculate vertical excitations, since gradients were not available. TD-DFT in combination with the B3LYP has been shown to give reliable excitation energies for low-lying states, except for Rydberg states, which require functionals with a better asymptotic behavior.[19] Therefore we will not report excitation energies of Rydberg states here. We feel safe in doing so, since earlier CASPT2 calculations[14] indicate that they have no significant impact on the relevant part of the vertical spectrum. Earlier studies[20] have shown that when problems with TD-DFT occur it is mostly due to extensive mixing between valence and Rydberg states. We see no such mixings in the calculations presented here, with one exception (see below).

Solvent effects were included in the calculations regarding the anionic and zwitterionic forms by using the Polarizable Continuum Model[21,22] (PCM). This model reproduces the polarization of the ground state induced by the solvent but cannot, of course, mimic the specific interactions of individual solvent molecules. We set the α-parameter to 1.3 and used 80 tessarae per sphere when defining the cavity. This gives a slightly bigger cavity than the default settings, but we found this necessary to minimize the problems of charge penetration into the continuum. The dielectric constant was set to that of bulk water (78.39) in all cases.

Geometry optimizations with the polarizable continuum model is sometimes difficult since the procedure may get stuck in a periodic behavior with oscillations on the order of 10^{-3} hartree. Although such oscillations are observed in some cases, test calculations showed that our results are not sensitive to details in the geometry.

All calculations were carried out with the GAUSSIAN 98[23] suite of programs.

Results and Discussion

Phenol and Phenolate: A Test of Our Approach. Since the approach we have chosen here, to combine TD-DFT with the polarizable continuum model method, is not a standard one, we find it appropriate to do some test calculations to estimate the accuracy of the method. Even without the polarizable continuum model, the use of TD-DFT to study ionic species is not yet standard, although some successful applications to biological systems have been reported.[24] As a test case we have chosen phenol and its anionic form, phenolate. These were recently subjects for a theoretical study on excited-state acidity in the gas phase and solution,[25] thus providing reference values from both experiment and high-level computations (CASPT2 for gas phase and state-to-state complete active space SCF calculations with a polarizable continuum model (CASSCF/PCM) for aqueous solution). We report here excitation energies calculated for the lowest lying $\pi \rightarrow \pi^*$ transition. From Table 1 we can see that all our excitation energies deviate less than 0.5 eV from the experimental value. It is especially encouraging that the accuracy is at least as good for the anionic form and for the calculations involving the polarizable continuum model

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Table 1. Gas-Phase and Solution Vertical Excitation Energies (in eV) for the Lowest $\pi \rightarrow \pi^*$ Transition of Phenol and Phenolate[a]

	gas phase			aqueous solution		
molecule	TD-DFT	CASPT2	exptl	TD-DFT	CASSCF	exptl
phenol	5.06	4.64	4.59	5.05	5.01	4.59
phenolate	3.92	4.38	3.5−3.65	4.21	4.40	4.32

[a] CASPT2, CASSCF and experimental values are from Granucci et al.[25]

Table 2. Gas-Phase Vertical Excitation Energies (in eV) and Oscillator Strengths in Parantheses[a]

state	trans (a)	trans (a) (CASPT2[14])	trans (b)
S_1 A′ ($\pi \rightarrow \pi^*$)	4.54 (0.0834)	4.93/4.73 (0.1203)	4.37 (0.0603)
S_2 A″ ($n_O \rightarrow \pi^*$)	4.58 (<0.0001)	5.12/4.88 (0.0005)	4.52 (<0.0001)
S_3 A″ ($n_N \rightarrow \pi^*$)	5.34 (0.0002)	6.86/6.76(<0.0001)	5.25 (<0.0001)
S_4 A′ ($\pi \rightarrow \pi^*$)	5.50 (0.0130)	5.40/5.09 (0.3427)	5.47 (0.0109)

state	cis (c)	cis (c) (CASPT2[14])
S_1 A′ ($\pi \rightarrow \pi^*$)	4.04 (0.0560)	4.16/4.15 (0.4568)
S_2 A″ ($n_O \rightarrow \pi^*$)	4.53 (<0.0001)	5.31/5.31 (0.0004)
S_3 A″ ($n_N \rightarrow \pi^*$)	4.88 (<0.0001)	5.37/5.36 (<0.0001)
S_4 A′ ($\pi \rightarrow \pi^*$)	5.10 (0.0017)	5.19/5.19 (0.0058)

[a] Notation of structures as in Figure 1. CASPT2 values are given for CASSCF and MP2 geometries.

method. These facts, and perhaps more importantly the nice overall agreement of the results presented below with experimental data, lead us to the conclusion that the accuracy in these calculations (<0.5 eV) is representative for our calculations.

Urocanic Acid: Neutral Form. First, we calculated the vertical excitation spectra of both the trans and cis forms of urocanic acid in the gas phase. The results are summarized in Table 2. Since the neutral form is expected to exist in the gas phase with the protonated carboxylic moiety and monoprotonated imidazole group (see Figure 1) this was the form we considered for these calculations.

For the trans form, there are in principle two different possible rotamers around the C_3-C_4 bond. The photoacoustic calorimetric studies of Hanson et al.[11] provided convincing evidence that the rotamers are not linked to the wavelength-dependent photochemistry of urocanic acid. That, however, does not exclude the possibility that there can be a population of rotamers. In fact, our B3LYP calculations only indicate an energy difference of 1.9 kcal$\cdot$mol^{-1} (the structure shown in Figure 1a being more stable), which given the accuracy of our method and the fact that this is not a free energy difference is too small to claim that any rotamer is dominant. For the sake of completeness we therefore consider both rotamers around this bond (a and b in Figure 1).

When it comes to the protonation state of the imidazole ring, we have selected to look at the lowest energy structures, that is the N_1-protonated for the trans form (Figure 1a,b) and the N_2-protonated form that preserves the intramolecular hydrogen bond for the cis form (c in Figure 1).

In both cases, our calculations predict that the lowest lying vertical excitation in the gas phase is a $\pi \rightarrow \pi^*$ transition, in good agreement with CASPT2 calculations[14] and also recent experiments.[13]

(a) Trans. For the trans forms (Figure 1a,b), the abovementioned transition was calculated to occur at an energy of 4.54 eV, which is lower than the one calculated by Page et al. (4.93 or 4.73 eV at CASSCF and MP2 geometry, respectively)[14] but higher than the one observed in recent supersonic spectroscopy experiments[13] where the band origin is at 4.04 eV and it is believed that this is also close to the vertical excitation energy. We note that the reported maximum absorption energy for the second $\pi \rightarrow \pi^*$ observed (4.58 eV) is much closer to our theoretical result.

Very close to this, at 4.58 eV, we have calculated a practically "silent" state which has $n \rightarrow \pi^*$ character, originating from the carboxyl oxygens. This is also in qualitative agreement with CASPT2, which places it at 5.12 or 4.88 eV. This state was not observed experimentally by Ryan and Levy[13] but can be understood by the vanishing oscillator strength.

The largest difference compared to the CASPT2 calculations occurs for the second $n \rightarrow \pi^*$ transition with a large weight on a transition from the N lone pairs, which is calculated at a considerably lower energy than the corresponding CASPT2 result (5.34 eV for our result and 6.86 eV for CASPT2). Since no experimental data are available, we cannot say that our result is in error, but it is possible that some valence-Rydberg mixing is responsible for this large deviation. In contrast, the second $\pi \rightarrow \pi^*$ is very close to the CASPT2 energy (5.50 eV for our result and 5.40 eV for CASPT2). However, none of these fits the assigned second $\pi \rightarrow \pi^*$ transition from the Ryan and Levy experiment of 4.58 eV particularly well.

For the second rotamer, no dramatic differences can be seen, which adds further weight to the experimental arguments that these play no crucial role in determining the photophysics, but also shows that few conclusions can be drawn about the population of rotamers from electronic absorption spectroscopy.

(b) Cis. In the case of the intramolecularly hydrogen bonded cis isomer (Figure 1c), we predict that the hydrogen bond has a large impact on the spectrum in the gas phase. The lowest lying $\pi \rightarrow \pi^*$ transition undergoes a major shift to 4.04 eV, the lowest $n \rightarrow \pi^*$ transition is more or less constant, the second $n \rightarrow \pi^*$ state is shifted down close to the first one, and the second $\pi \rightarrow \pi^*$ is also shifted to lower energy (5.10 eV). We note that similar trends were obtained in calculations with CASPT2.[14]

Urocanic Acid: Anionic Forms. Since a major part of the spectroscopic experiments in solution have been carried out at a pH of around 7,[10,11] which is also the pH of the viable part of the epidermis, we investigate here the anionic forms present at those pH values. To calculate the vertical spectra of the anionic species in solution we have considered a number of possibilities in terms of structures. To investigate both the influence of protomeric and rotameric forms we performed extensive calculations on 4 different trans and 2 different cis structures, as shown in Figure 2. The existence of the intramolecular hydrogen bond forming cis protomer (Figure 2e) is sometimes debated. It can be argued that competition with hydrogen bonding solvent destabilizes the intramolecular hydrogen bond. In fact, in earlier gas phase theoretical studies, good agreement with experiment was obtained although only the protomer lacking the intramolecular hydrogen bond was considered.[15] On the other hand, the intramolecular hydrogen bond has been shown to give substantial stabilization, also when solvent is accounted for.[26] Here we investigate if the form excluding the intramolecular hydrogen bond explains the absorption spectra in solution better than the intramolecular hydrogen bond-forming protomer.

The results are collected in Table 3 for trans results and Table 4 for cis results. All these calculations have been performed with the polarizable continuum model method to model the solvent effects.

(a) Trans. There are some major changes observed in comparison with the gas-phase spectra of the neutral form

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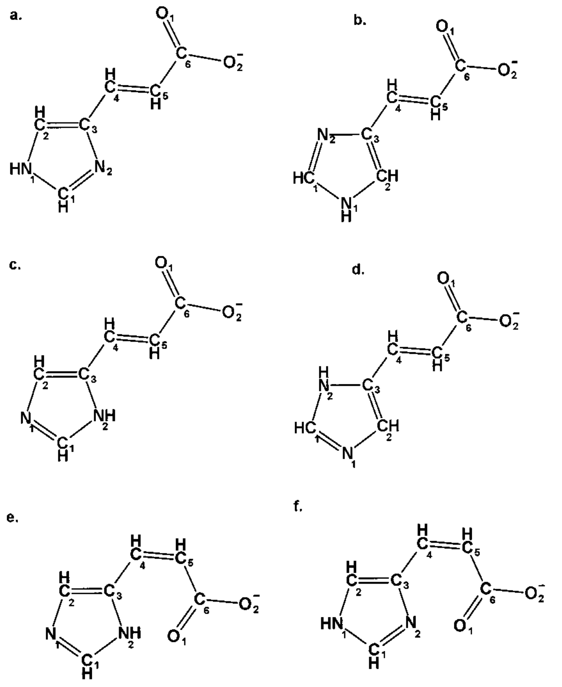

Figure 2. Structures of anionic trans (a−d) and cis (e and f) form: (a and c) the lowest energy rotamer form; (a and b) the lowest energy protomer form in the gas phase for the trans isomer. The intramolecular hydrogen bond is formed in structure e.

Table 3. Solution-Phase Vertical Excitation Energies (in eV) and Oscillator Strengths in Parantheses for Anionic *trans*-Urocanic Acid[a]

state	a	b	c	d
S_1 A''	3.93 (<0.0001)	3.96 (<0.0001)	3.62 (<0.0001)	3.62 (<0.0001)
S_2 A''	4.38 (<0.0001)	4.31 (0.0001)	4.00 (<0.0001)	4.01 (<0.0001)
S_3 A'	4.70 (0.0674)	4.59 (0.3834)	4.52 (0.0114)	4.42 (0.0468)
S_4 A'	5.00 (0.0226)	4.98 (0.0463)	4.72 (0.0882)	4.60 (0.0296)

[a] Structures as in Figure 2.

Table 4. Solution-Phase Vertical Excitation Energies (in eV) and Oscillator Strengths in Parantheses for Anionic *cis*-Urocanic Acid[a]

state	e	f
S_1 A''	3.87 (<0.0001)	3.83 (<0.0001)
S_2 A''	4.40 (0.0002)	4.36 (0.0001)
S_3 A'	4.43 (0.4965)	4.87 (0.5328)
S_4 A'	4.99 (0.0291)	5.08 (0.0607)

[a] Structures as in Figure 2.

Table 5. Solution-Phase Vertical Excitation Energies (in eV) and Oscillator Strengths in Parantheses for Zwitterionic Urocanic Acid[a]

state	a	b
S_1	3.62 (0.0166)	3.94 (0.0002)
S_2	3.89 (<0.0001)	4.43 (0.0002)
S_3	4.47 (0.0123)	4.89 (0.4393)
S_4	4.69 (0.1027)	4.99 (0.0006)
S_5	4.89 (0.3262)	5.35 (0.0014)

[a] Structures as in Figure 3.

obtained above. For the N_1-protonated trans species (Figure 2a,b; Table 3), the lowest excitation is now a $n \rightarrow \pi^*$ type of transition located at 3.9−4.0 eV with miniscule oscillator strength. Another weakly absorbing state of the same symmetry is predicted between 4.3 and 4.4 eV. The computed spectrum is dominated by a strongly absorbing $\pi \rightarrow \pi^*$ transition located at 4.6−4.7 eV, and another $\pi \rightarrow \pi^*$ transition with sizable oscillator strength is found at approximately 5.0 eV. These results agree very well with the experimental assignment, both that the absorption maximum occurs at 4.48 eV as a $\pi \rightarrow \pi^*$ transition and that the isomerization efficiency peak at 4.0 eV is a result of a $n \rightarrow \pi^*$ transition.[10] We could not see any low-lying $\pi \rightarrow \pi^*$ transitions, obtained in the gas phase by CASPT2.[15]

From our calculations it becomes clear that the different rotamers have very similar vertical excitation spectra in solution, so that it is the presence of different electronic states rather than rotamers that is the cause of the wavelength-dependent photochemical behavior. Quite the same cannot be said about the influence of the protomeric state. For the N_2-protonated structures (Figure 2c,d) as opposed to the N_1 protonated (Figure 2a,b), a noticeable redshift of about 0.3−0.4 eV is predicted for the lowest $n \rightarrow \pi^*$ state, placing this transition at about 3.6 eV. Note that this energy coincides with the peak of the singlet oxygen production action spectrum reported by Simon and Hanson.[11] Also the $\pi \rightarrow \pi^*$ states are shifted, but to a smaller degree.

(b) Cis. For the cis species, it has been predicted that the presence of the intramolecular hydrogen bond would cause a large redshift in the excitation energies in the gas phase.[15] In solution, the effect is expected to be much smaller due to the weakening of the hydrogen bond. This trend is, indeed, well reproduced by the continuum model used here. Actually, the only large shift induced by the intramolecular hydrogen bond is for the strongly absorbing $\pi \rightarrow \pi^*$ transition that is redshifted 0.44 eV when the intramolecular hydrogen bond is introduced, but the result with the cis structure that conserves the intramolecular hydrogen bond (Figure 2f) fits experimental data[9] much better than the other protomer (g), so the existence of the intramolecular hydrogen bond in solution cannot be ruled out for this reason.

We can see that the overall structure of the low-lying vertical excitations is similar as for the trans isomer, in accordance with experimental findings.[10]

Urocanic Acid: Zwitterionic Forms. The trans form of urocanic acid in human skin is mainly present in the *stratum corneum*, where pH gradually changes between 4 and 6,[27] making the zwitterionic form of urocanic acid predominant. This may therefore be the biologically most relevant form of urocanic acid to study. It presents problems, however, for theoretical gas-phase studies for reasons outlined below.

It is important to stress that it is strictly necessary to include solvent effects at some level to get a reasonable picture of the electronic structure of zwitterions. In the trans case, it is difficult to get a realistic charge separation such as will be present in aqueous solution through gas-phase calculations, as was discussed by Page et al.[15] Also, if a full geometry optimization without symmetry constraints is done in the gas phase, the geometry is largely distorted, with the carboxyl group twisted 52°. The cis isomer is not even stable in the gas phase; if the geometry is optimized, a proton transfers from N_2 to the carboxyl group and the resulting structure is that of a neutral molecule.

If solvent is accounted for, however, the zwitterionic forms are stable and the vertical spectra can be calculated. The results for both isomers are collected in Table 5.

In a paper by Tozer et al.[20] some problems were reported in describing charge transfer states of a dipeptide with the TD-DFT approach. The excitation energies from TD-DFT deviated significantly (>1.0 eV) from those of CASPT2. No experimental values were available for comparison. Since in

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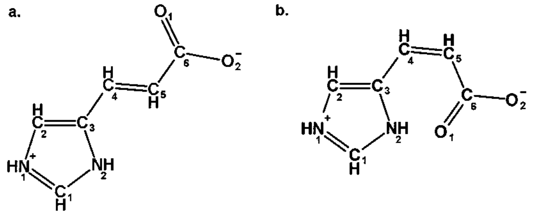

Figure 3. Structures of zwitterionic *trans*- (a) and *cis*-urocanic acid (b).

the gas-phase CASPT2 study of zwitterionic (protonated ionic) urocanic acid it was found that most low-lying excited states had charge-transfer character[15] one might be worried about the ability of our chosen approach to describe this form. However, any transition involving charge transfer in this molecule would lead to a large decrease in dipole moment and thus be strongly disfavored in a polar solvent. It is therefore no surprise that our results indicated that there is no strong charge-transfer character of the lowest lying states of zwitterionic urocanic acid in aqueous solution.

Since we have found no significant influence of the rotameric state in our investigations of the neutral and anionic form, we do not expect the situation to be any different for the zwitterionic forms. Thus we only consider the most stable rotamer in each case here. In addition, since both nitrogens are protonated, there are no protomers to be considered either. The structures considered can be viewed in Figure 3.

One thing should be noted. Previous theoretical studies used an unspoken assumption that all forms of urocanic acid adapt a planar conformation. However, for the trans zwitterionic form it turns out that the planar conformation is a saddle point, with one pure imaginary frequency. If the symmetry constraint is released, the carboxyl group twists moderately around the C_5-C_6 bond in solution, although not as dramatically as in the gas phase. Of course, since the symmetry of the molecule is broken, there are no symmetries of the transitions, but we can in most cases extrapolate from the calculations from the anionic state.

The calculated transitions in zwitterionic *trans*-urocanic acid are close to the ones obtained for the anionic form, and particularly the N_2 protomeric form. Our calculations suggest that the protonation of this site leads to the weak transition at 3.6 eV. The agreement between experimental absorption spectra and our theoretical predictions is encouraging. Most features observed in the anionic forms are not changed when considering the zwitterionic form. Most notably the weak transition at 4.0 eV and the strongly absorbing states between 4.6 and 4.9 eV persist. The most strongly absorbing state is found at 4.9 eV for both isomers while the experimental absorption maxima occurs at 4.7 eV.[9] This difference is clearly within the tolerance of the method used here. Just as in experimental spectra we can see that the absorption maxima coincides for the two isomers.

It is found experimentally that the absorption maximum is blueshifted about 0.15 eV when going from the anionic to the zwitterionic form.[9] For the trans form, we can see that this shift in maximum is reproduced not by shifting the transition energy but by shifting the relative oscillator strengths of two states. On the contrary, in the cis form, it seems that the same state is responsible for the strongest transition in both cases. The shift of the cis isomer is also significantly larger, 0.5 eV (vs 0.2 eV for the trans form). The size of these shifts is small, around the expected accuracy level of absolute excitation energies calculated with TD-DFT, but since TD-DFT is known to give the correct ordering of states, we think these trends are reliable.

Conclusions

We have performed extensive TD-DFT calculations for urocanic acid in several ionic forms, taking into account different rotamers and protomers.

Solvent effects were found crucial for a reasonable modeling of zwitterions and included in our model through a continuum model. Generally we find good agreement with experimental data in the condensed phase with a common $n \rightarrow \pi^*$ state as the most likely candidate for the photoisomerization in solution, although an excited-state potential energy surface mapping will be necessary to resolve this issue. The major features of the spectra are found to be very stable in solution and the absorption maximum is reproduced to good accuracy.

The calculated excitation energies are very similar for different rotamers, confirming the experimental conclusion that different rotamers are not the cause of the wavelength-dependent photochemistry of urocanic acid. It is predicted that the protonation state of the trans isomer has influence over the lowest transition in solution, with a redshift of 0.4 eV upon protonation of N_2. Based on our calculations we suggest that when this is protonated, which is the case in the *stratum corneum*, it gives rise to a transition that fits the singlet oxygen production action spectrum maximum very well, making it probable that this is an intermediate state that subsequently populates a triplet state.

The best agreement with calculated geometries for *cis*-urocanic acid is obtained when the protomer allowing the formation of a intramolecular hydrogen bond is considered, although the intramolecular hydrogen bond does not change the vertical spectrum qualitatively in solution.

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Supporting Information Available: Tables of experimental data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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