

Electronic Spectra of the Protonated Indole Chromophore in the Gas Phase

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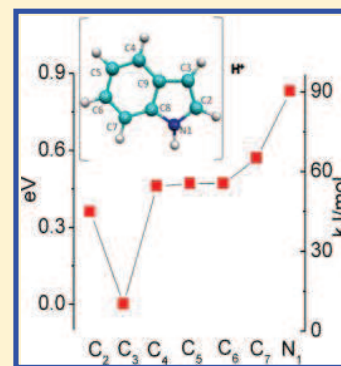
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Supporting Information

ABSTRACT: The electronic spectroscopy of cold protonated indole was investigated experimentally and theoretically. Two isomers were observed by experiment: The first isomer corresponds to the lowest-energy isomer in the calculations, absorbing at ~ 350 nm and protonated on the C₃ atom of the pyrrole ring. According to our calculations, the absorptions of the other isomers protonated on carbon atoms (C₂, C₄, C₅, C₆, and C₇) are in the visible region. Indeed, the absorption of the second observed isomer starts at 488 nm and was assigned to protonation on the C₂ carbon of the pyrrole ring. Because good agreement was obtained between the calculated and experimental transitions for the observed isomers, reasonable ab initio transition energies can also be expected for the higher-energy isomers protonated on other carbon atoms, which should also absorb in the visible region. Protonation on the nitrogen atom leads to a transition that is blue-shifted with respect to that of the most stable isomer.



INTRODUCTION

The spectroscopic properties of indole chromophores have attracted much interest over the past several decades¹ because these molecules can be used as model systems to study the complex photophysics of the amino acid tryptophan (Trp). Photochemical reactions of Trp and Trp residues in proteins play a fundamental role in nature. In particular, fluorescence and phosphorescence of tryptophan residues in proteins are widely used as tools to monitor a variety of biological processes, giving rise to structural and dynamic information.^{2–4} However, improvement of these optical probes requires an understanding of the fundamental photochemical processes occurring in the bare Trp molecule.^{5–7} Of particular interest is the strong pH dependence observed in the aqueous Trp fluorescence lifetime, which decreases from nanoseconds to femtoseconds when the molecule is protonated.⁸ The multiexponential decay observed for Trp in aqueous solution and in proteins has been tentatively explained by the rotamer model,^{2,6,9,10} which states that the lifetime is governed by C_α–C_β bond torsion.

The quenching of the Trp fluorescence has been suggested to result from an intramolecular hydrogen/proton exchange occurring in the Trp zwitterion, where the alanyl chain curves back over the indole moiety and a proton from the ammonium group is transferred to the C₂⁸ or C₄¹¹ carbon (see Figure 1 for atom numbering). This mechanism is corroborated by the fact

that the fluorescence of indole compounds is quenched by the occurrence of an intermolecular excited-state proton transfer involving protonation at different sites (C₄ or C₇).^{12,13}

Even though the lifetime of neutral Trp in the gas phase is long,^{14,15} the protonated species exhibits a very short lifetime^{16–19} that has been assigned to the excited-state dynamics of the proton. Further evidence that supports the occurrence of a proton-transfer mechanism can also be deduced from the fragmentation pattern in which the appearance of a fragment at *m/z* 132 (protonated methylindole fragment) can be explained only by a proton transfer from the terminal ammonium group C–NH₃⁺ to the indole ring and a second H transfer^{20,21} as the fragments separate; this process is corroborated by ab initio calculations.^{22,23}

Recently, femtosecond pump/probe experiments on tryptophan in solution have assigned the appearance of a transient absorption band in the visible region (425 nm) to the protonated indole photoproduct, on the basis of previous studies.²⁴ The validity of this assumption can be tested by investigating the excited states of protonated indole both theoretically and experimentally.

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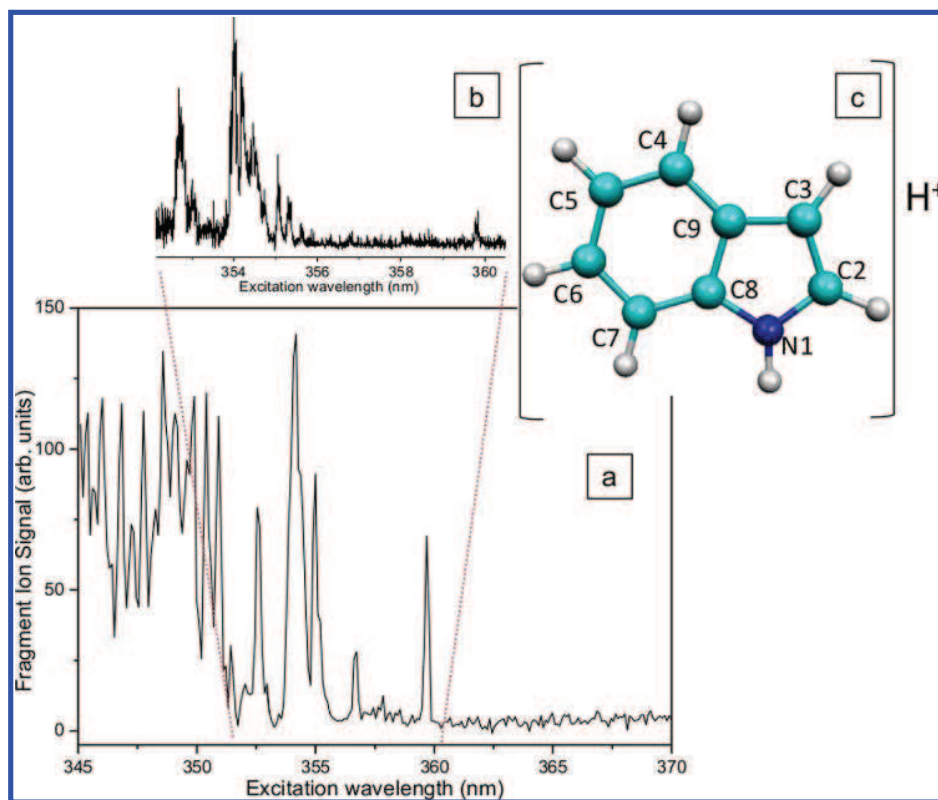


Figure 1. Two-photon photofragmentation spectrum of the C_3H^+ isomer of protonated indole recorded at m/z 91: (a) spectrum recorded at 8 cm^{-1} resolution, (b) part of the spectrum recorded at higher resolution (0.2 cm^{-1}), (c) structure of the indole molecule with numbering of the carbon atoms. The wavelengths were calibrated to vacuum.

We have measured the electronic spectrum of protonated indole in the gas phase by photofragmentation spectroscopy. Two isomers were detected: One is easily observed in the UV region and the second one appears in the visible region with less intensity. Based on *ab initio* calculations, the UV spectrum is assigned to the most stable isomer with protonation on the C_3 carbon (see Figure 1 for the atom numbering in indole; the numbering starts on the N_1 nitrogen atom and moves anticlockwise from the C_2 to the C_7 carbons, jumping the C_8 and C_9 carbons common to both rings). The second spectrum in the visible region is assigned to the isomer protonated on C_2 carbon. The different isomers are named in this work according to the protonation site: NH^+ if the proton is attached to the nitrogen atom and C_nH^+ if it is attached to the C_n carbon atom.

Because comparison between theory and experiment is quite good for the observed isomers (C_2H^+ and C_3H^+), it seems reasonable to trust *ab initio* calculations to predict the transitions of the other unobserved isomers.

METHODS

Experiments. The electronic spectrum of protonated indole was obtained by photofragment spectroscopy of the parent ion in a cryogenic cold quadrupole ion trap (Paul ion trap from Jordan TOF Products, Inc.). The setup is quite similar to the one developed by Wang and Wang.^{25–27} The protonated species are produced by an electrospray ionization source built at Aarhus University.²⁸ At the exit of the capillary, ions are trapped in an octopole trap for 90 ms. They are extracted by applying a negative pulse of 50 V and are further accelerated to 100/200 V by a second pulsed voltage just after

the exit electrode. This time sequence of pulsed voltages produces ion packets with a duration of between 500 ns and 1 μ s. Ions are driven by a pair of electrostatic lenses toward the Paul trap biased at 100/200 V so that the ions enter the trap gently. A mass gate placed at the entrance of the trap allows selection of the parent ion. The Paul trap is mounted on the cold head of a cryostat (Coolpak Oerlikon) connected to a water-cooled He compressor. Helium as the buffer gas is injected into the trap using a pulsed valve (General Valve) triggered 1 ms before the ions enter the trap. The ions are trapped and thermalized at a temperature of around 50 K through collisions with buffer gas. The ions are kept in the trap for 90 ms before the photodissociation laser is triggered; this delay is necessary to ensure thermalization of the ions and evacuation of the He buffer gas from the trap. Under these conditions, ions are not fragmented by collisions when they are extracted and accelerated for analysis in a 1.5-m-long linear time-of-flight mass spectrometer. Ions are fragmented in the trap, and the delay between laser and extraction is varied to measure the fragmentation time. The time resolution that can be obtained is around 100 ns. For protonated indole, the fragmentation time is faster than this resolution. Photofragmentation spectra are obtained by recording the fragment-ion signal on a microchannel plate (MCP) detector as a function of laser wavelength with a digitizing storage oscilloscope interfaced to a personal computer.

The photodissociation laser is an optical parametric oscillator (OPO) laser from EKSPILA with a repetition rate of 10 Hz, a resolution of 8 cm^{-1} , and a scanning step of 0.1 nm. The laser is mildly focused in the trap with a 1-m focal lens and the laser power is around 20 mW in the UV region and 40 mW in the

visible region. Part of the UV spectrum was recorded at the CLUPS facility in Orsay, France, on a similar setup, but using a Nd:YAG-pumped dye laser with a higher spectral resolution (0.2 cm^{-1}).

Calculations. Ab initio calculations were performed with the TURBOMOLE program package,²⁹ making use of the resolution-of-the-identity (RI) approximation for the evaluation of the electron-repulsion integrals.³⁰ The equilibrium geometry of protonated indole in its ground electronic state (S_0) was determined at the MP2 (Møller–Plesset second-order perturbation theory) level. Excitation energies and the equilibrium geometry of the lowest excited singlet state (S_1) were determined at the RI-CC2 level. Calculations were performed with two basis sets: the correlation-consistent polarized valence double- ζ (cc-pVDZ and aug-cc-pVDZ) basis sets. In addition, for the C_3H^+ isomer, which exhibits a quite complex vibrational structure, a triple- ζ basis set with diffuse orbital (aug-cc-pVTZ) was used.³¹

The vibrations in the ground and excited states were calculated and the electronic spectra were simulated using PGOPHER software³² for the Franck–Condon analysis.

RESULTS

Experiments. Clear electronic spectra of protonated indole were obtained in two spectral regions, namely, the visible ($\sim 480 \text{ nm}$) and UV ($\sim 360 \text{ nm}$) regions. The UV spectrum had much greater intensity. These vibronic spectra were assigned with the help of ab initio calculations to two different isomers. The UV photofragmentation spectrum recorded at m/z 91 is presented in Figure 1; its first band at 359.8 nm (3.446 eV) was assigned to the 0–0 transition of the C_3H^+ isomer (vide infra). Because the results of Yang et al.³³ showed that, in the collision-induced dissociation (CID) of protonated indole with xenon, a collision energy of at least 5 eV in the center-of-mass frame is required to induce fragmentation (mainly through the loss of HCN), the spectrum recorded here corresponds to two-photon absorption. The laser power dependence of the signal was recorded and it showed signs of saturation (see the Supporting Information). The bandwidth of the band origin is ca. 5 cm^{-1} (Figure 1b), indicating that the lower limit of the excited-state lifetime is 1 ps .

The spectrum recorded at m/z 91 in the visible region is presented in Figure 2. In this spectrum, the first band at 488 nm (2.54 eV) is assigned to the 0–0 band origin, as no transition

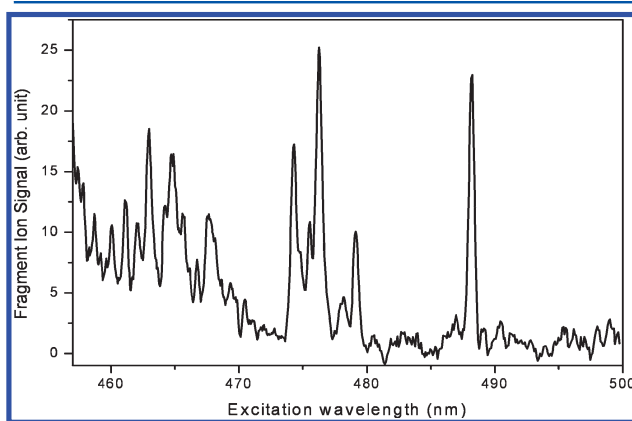


Figure 2. Two-photon photofragmentation spectrum of the C_2H^+ isomer of protonated indole recorded at m/z 91.

has been observed farther to the red. As in the previous case, the signal likely comes from a two-photon process. We show in the next section that this spectrum can be assigned to the C_2H^+ isomer.

Between these two spectral regions, where intense narrow transitions are observed, a signal is observed in the 460 – 410 nm spectral region but cannot be resolved. It can be assigned to the absorption of high vibrational levels of the C_2H^+ isomer or to other isomers. The spectral signatures of other isomers were sought in scanning from 600 to 230 nm , but none could be identified.

The fragmentation channels for the isomers absorbing in the UV and visible regions are presented in Figure 3. The

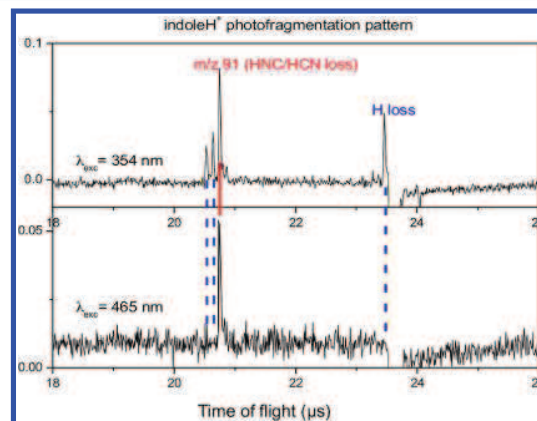


Figure 3. Difference time-of-flight mass spectra (with and without laser excitation) recorded for the two isomers of protonated indole at 354 and 465 nm .

fragmentation channels are not the same for the two isomers. The isomer excited in the visible region exhibits only the HCN/HNC loss channel as in CID experiments.^{33,34} For the isomer excited in the UV region, in addition to the HCN/HNC loss channel, the H loss channel is also observed, as well as the loss of $H + HCN/HNC$ and $H + H + HCN/HNC$, which are not observed in CID at a collision energy of 5 eV .³³ The two latter channels are the main fragments obtained from the fragmentation of the indole radical cation,³⁵ which strongly suggests that the radical cation produced after H loss undergoes further fragmentation, losing HCN/HNC and $H + HCN/HNC$.

Calculations. Ground-state calculations at the RI-MP2 and RI-CC2 (cc-pVDZ) levels are in good agreement with previous calculations giving the C_3H^+ isomer as by far the most stable (see Table 1).^{36,37} The different isomers are named according to the protonation site: NH^+ if the proton is attached to the nitrogen atom or C_nH^+ if it is attached to the C_n carbon atom (see Figure 1 for the atom numbering in indole). The calculated proton affinity is 9.53 eV (919.8 kJ/mol) at the CC2 level, in good agreement with previous calculations^{36,37} and with the experimental value of 933.4 kJ/mol .³⁸ The other isomers protonated on external carbon atoms (C_2 , C_4 , C_5 , C_6 , C_7) are less stable (stabilization energies in the range of 0.36 – 0.57 eV).

The vertical and adiabatic transitions calculated at the RI-CC2 (cc-pVDZ) level of theory for the different isomers (except C_8H^+ and C_9H^+) are given in Table 1. The isomers protonated on the C_8 and C_9 inner carbon atoms are very high in energy as compared to the most stable isomer (more than 1

Table 1. Ground-State Relative Stabilization Energies and Excited-State Transition Energies of the Protonated Indole Isomers^a

isomer	ground state ^b	excited-state transition energy				experimental
		S ₁ ← S ₀ vertical ^c	S ₁ ← S ₀ adiabatic ^d	S ₁ ← S ₀ adiabatic with δ ZPE ^e	S ₂ ← S ₀ vertical ^c	
C ₂ H ⁺	0.36	3.24	2.76	2.62	4.98	2.54
C ₃ H ⁺	0.00	4.09	3.49	3.33	4.25	3.45
C ₄ H ⁺	0.46	3.37	2.86	2.73	3.82	
C ₅ H ⁺	0.47	3.50	2.86	2.75	4.09	
C ₆ H ⁺	0.47	3.26	2.96	2.83	4.84	
C ₇ H ⁺	0.57	3.25	2.40	2.31	3.70	
NH ⁺	0.83	4.86	4.66	—	5.40	
C ₈ H ⁺	1.16	—	—	—	—	
C ₉ H ⁺	1.19	—	—	—	—	

^aAll values are in eV. The boldface text indicates the most stable isomer. ^bGround-state energy relative to the C₃H⁺ isomer calculated at the RI-MP2/cc-pVDZ level. ^cVertical excited-state transition energy at the RI-CC2/cc-pVDZ level. ^dAdiabatic excited-state transition energy at the RI-CC2/cc-pVDZ level. ^eAdiabatic excited-state transition energy corrected for the difference in zero-point energy, δ ZPE. For the C₃H⁺ isomer, the calculations were also done with the aug-cc-pVTZ basis set, in which case the adiabatic transition energy corrected for δ ZPE decreases to 3.28 eV.

eV higher), so these isomers are not expected to be present in the experiments and their transition energies were not calculated.

As in many aromatic molecules, the difference between the vertical and the adiabatic transition energies obtained by excited-state optimization is quite large, ranging from 0.2 eV for the NH⁺ isomer to 0.85 eV for C₇H⁺ and 0.6 eV for the most stable C₃H⁺ isomer. This clearly shows that comparison between calculations and experiments requires excited-state optimization, which is too seldom done. The difference in zero-point energy between the excited and ground states is not negligible (δ ZPE = -0.17 eV for the C₃H⁺ isomer) and is typical of many aromatic molecules. It has to be taken into account, but its evaluation is quite costly in terms of computer time and requires good facilities. When δ ZPE is taken into account, the transition energy for the C₃H⁺ isomer decreases to 3.33 and 3.28 eV with the cc-pVDZ and aug-cc-pVTZ basis sets, respectively. This is not too far from the experimental value for the band origin observed at 3.45 eV. C₃H⁺ is the only isomer having its calculated transition energy in the near-UV region. The other isomers with the proton located on a carbon atom have their calculated transition energies in the visible region.

Among the isomers protonated on carbon atoms, the C₂H⁺ isomer is the second lowest in energy (0.36 eV), and its calculated S₁-S₀ electronic transition is the closest from the origin of the spectrum observed in the visible region. The calculated transition energy is 2.62 eV (cc-pVDZ basis set with correction for δ ZPE), and the observed visible spectrum starts at 2.54 eV. The calculated S₁-S₀ electronic transitions for the C_nH⁺ isomers with $n = 4-6$ are in the 2.70-2.90 eV (420-450 nm) energy range, and their ground-state stabilization energy is 0.46-0.47 eV. The C₇H⁺ isomer has a higher stabilization energy (0.57 eV) and an even lower transition energy (2.31 eV). For all of these isomers, the transition energies are far to the red of the neutral indole S₁-S₀ transition (4.38 eV).^{39,40} Protonation on the nitrogen atom is unfavorable: According to our calculations, the ground state of the NH⁺ isomer is 0.83 eV higher in energy than the C₃H⁺ ground state. This is probably due to a loss of aromaticity occurring when the lone pair becomes involved in proton binding. Moreover, the NH⁺ isomer is the only one to have a calculated transition energy shifted to the blue (4.66 eV) from the neutral indole S₁-S₀ transition.

Because the indole chromophore in tryptophan is attached to the peptidic chain by a C-C bond at the C₃ position, complementary calculations were performed on the protonated 3-methylindole to test the influence of the C-C bond on the electronic properties. The relative ground-state stabilities and the transition energies of protonated 3-methylindole compared to protonated indole are presented in Figures 4 and 5,

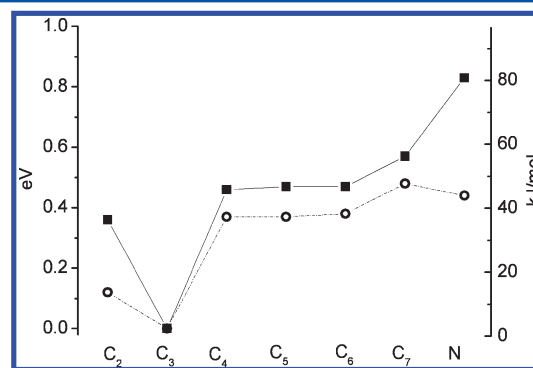


Figure 4. Comparison of the ground-state stabilities of the different isomers of protonated 3-methylindole (open circles) and protonated indole (solid squares).

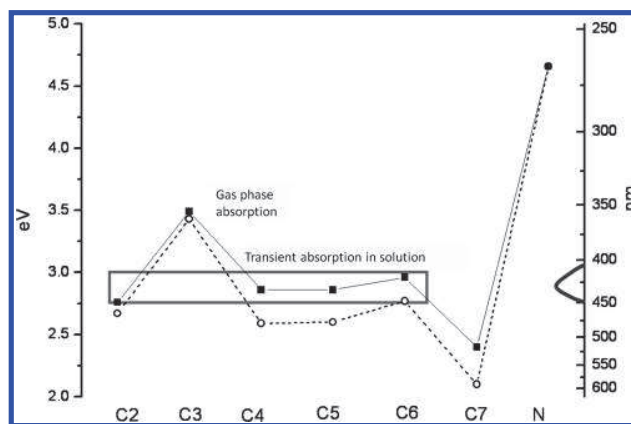


Figure 5. Comparison of the adiabatic transition energies in 3-methylindole (open circles) and indole (solid squares). The rectangle around 425 nm represents the transient absorption observed for tryptophan in solution, which can be assigned to the C₄H⁺, C₅H⁺, C₆H⁺, or C₂H⁺ isomers.

respectively. The most stable protonation site of 3-methylindole is on the C₃ atom as for protonated indole, and for this position, the calculated proton affinity is 9.43 eV = 909.8 kJ/mol, slightly smaller than for indole. The main difference between these compounds is that the NH⁺ isomer is more stable in 3-methylindole than in indole, being as stable as the isomers protonated on C₆ or C₇ (see Figure 4).

In the excited state, the transition energies are very similar in protonated 3-methylindole and in protonated indole (Figure 5). The C₃H⁺ isomer has a transition energy of around 3.5 eV (350 nm); the C₂H⁺, C₄H⁺, C₅H⁺, and C₆H⁺ all have lower transition energies of 2.6–3.0 eV (400–475 nm); the C₇H⁺ isomers have the lowest transition energies at 2.4 and 2.1 eV; and protonation on the nitrogen atom leads to a very high transition energy of 4.7 eV.

As can be seen, the transient absorption observed at 425 nm in solution (represented by the rectangle in Figure 5) and assigned to a primary photofragment of tryptophan, can be assigned to isomers of protonated indole, either C₄H⁺, C₅H⁺, C₆H⁺, or C₂H⁺.

DISCUSSION

a. C₂H⁺ Isomer. As mentioned in the preceding section, the spectrum observed in the visible region is assigned to the C₂H⁺ isomer of protonated indole on the basis of the comparison between the calculated (2.62 eV) and observed (2.54 eV) transition origins. The assignment is based strongly on the accuracy of ab initio calculations for the excited-state transition energy. In previous studies on protonated aromatic molecules^{41,42} and also on simple closed-shell aromatic molecules and clusters (phenol and substituted phenols with ammonia,⁴³ 7-azaindole with water⁴⁴) using the same method (RI-CC2/cc-pVDZ basis set), the comparison between theoretical and experimental results is surprisingly good. We did not find systematic deviations, but instead observed a dispersion between the calculated and the experimental values of about 0.15 eV. Moreover, a recent benchmark study was performed by Send et al.⁴⁵ for a set of different molecules showing that the mean error with the RI-CC2 method is typically 0.1 eV with a dispersion $\sigma = 0.15$ eV.

Additionally, the ground-state energy of the C₂H⁺ isomer was calculated to be 0.36 eV above that of the most stable isomer, with the other C_nH⁺ isomers ($n = 4–7$) having ground-state energies between 0.46 and 0.57 eV. The C₂H⁺ isomer is thus more likely to be observed at low temperature.

The assignment can be corroborated in comparing the observed spectrum to a spectrum simulated by convoluting the calculated Franck–Condon factors with a 10 cm⁻¹ Gaussian function as implemented in the PGOPHER software. As shown in Figure 6, the comparison between simulation and experiment is quite good for the band position, without any scaling factor. The relative intensities are better reproduced if one takes into account the saturation effect by multiplying the calculated intensity by $[1 - \exp(-aI)]$, where a is a constant chosen to fit the intensity (I) of the main bands. Because the ground-state and S₁-state optimized geometries of the C₂H⁺ isomer are both planar, the spectrum is built on one strongly active vibration at 515 cm⁻¹, which corresponds to a six-membered-ring in-plane deformation similar to the 6a vibration of benzene.⁴⁶ The increase of the baseline from 475 nm to the blue could be due to the spectral congestion, to saturation, or possibly to the presence of other isomers. The electronic transitions of C₄H⁺, C₅H⁺, and C₆H⁺ were calculated to fall in this spectral region.

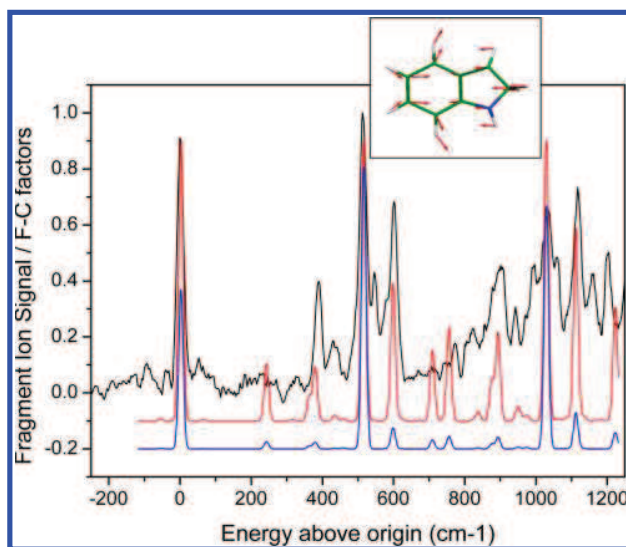


Figure 6. Comparison between the experimental and simulated spectra of the C₂H⁺ isomer of protonated indole. Black, experimental spectrum; blue, calculated spectrum at 50 K without saturation of the bands; red, simulated spectrum at 50 K with addition of some saturation in the transitions (see text). Inset: Scheme of the most active mode involved in the transition.

For the C₂H⁺ isomer (see Figure 3), only one fragmentation channel corresponding to HNC/HCN loss, which leads to m/z 91, is observed.

b. C₃H⁺ Isomer. For the most stable C₃H⁺ isomer, the calculated optical transition is also in good agreement (within 0.15 eV) with the observed band origin, but in this case, the S₁ excited-state optimized geometry is not planar, as the C₂ atom lies out of the molecular plane defined by the benzene ring (see the Supporting Information). For this isomer, the first two excited states are very close in energy, and the electronic nature of the S₁ state is changing during the optimization process, being {55% [(HOMO - 1) - LUMO] + 40% (HOMO - LUMO)} at the ground-state geometry and 90% (HOMO - LUMO) at the S₁ optimized geometry. Using PGOPHER software, we performed Franck–Condon simulations with several basis sets (cc-pVDZ, aug-cc-pVDZ, and aug-cc-pVTZ) at the RI-CC2 level of theory. Unlike for the C₂H⁺ isomer, the simulated spectrum did not fit the experimental spectrum well. The simulation obtained with the aug-cc-pVTZ basis set is shown in the lower panel of Figure 7. The weak 0–0 transition and the complexity of the spectrum reflect the large change in geometry between the ground and excited states, for which the calculated geometry shows strong out-of-plane distortions mainly in the pyrrole ring.

Better agreement between experiment and calculations was obtained when the calculated excited-state frequencies were scaled by a factor 0.8 (Figure 7, upper panel), which is greater than the scaling factor generally used for the ground state. Note that this scaling factor was not needed to simulate either the spectrum of the C₂H⁺ isomer or the spectra of other previously studied aromatic molecules.^{41,42,47} This discrepancy could be due to the energetic proximity of the S₁ and S₂ states. Starting from the ground-state optimized geometry for which the S₂ state is 0.16 eV higher than S₁, optimization of the S₂ state leads to a crossing with the S₁ state at an energy of 3.69 eV, that is, 0.2 eV higher than the optimized S₁ energy. Further optimization leads back to the S₁-state optimized geometry,

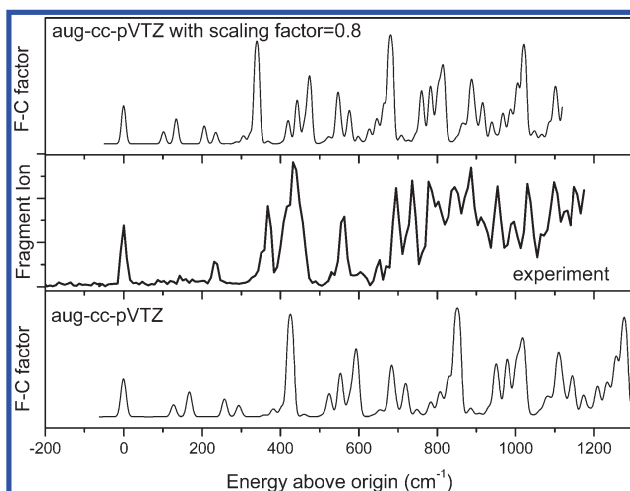


Figure 7. Comparison between experimental and simulated spectra for the C_3H^+ isomer. Lower trace: Spectrum simulated using the RI-MP2/CC2 ab initio method with the aug-cc-pVTZ basis set and PGOPHER software for the Franck–Condon simulation. Middle trace: Experimental spectrum recorded in the 362–345 nm range. Upper trace: Spectrum simulated using the same method as for the lower trace, with a scaling factor of 0.8 for the excited-state frequencies (see text).

and we were not able to calculate the S_2 -state minimum. The S_1 – S_2 Jahn–Teller interaction will strongly change the potential energy surfaces of both states and, consequently, the vibrational frequencies. Under these assumptions, the spectrum seems to be mainly built on the ν_8 vibrational mode calculated at 425 cm^{-1} (aug-cc-pVTZ). The 8_0^1 band in the simulated spectrum is the first intense band and can be assigned to the first intense band in the experimental spectrum. The ν_8 frequency is mainly due to N–H out-of-plane bending, and its overestimation is probably due to the coupling between S_1 and S_2 .

The fragmentation pattern of this isomer is somewhat surprising (Figure 3). In addition to the HNC/HCN loss as in CID experiments, the H loss channel leading to the radical cation and its fragments is observed. This can be rationalized if one considers that two photons are required for the fragmentation. The $\pi\sigma^*$ state, well-known to lead to excited-state H loss in many aromatic molecules,^{48,49} is calculated at 7.9 eV in the C_3H^+ isomer (at the ground-state geometry) and can be reached through a two-photon process. This state cannot be reached when exciting the C_2H^+ isomer because the available energy with two 2.54 eV photons is not sufficient.

c. Other Isomers. We also searched for other isomers, by scanning more to the red, between 600 and 490 nm, to find the C_7H^+ isomer calculated at around 2.3 eV (537 nm), and by scanning in the UV region between 290 and 230 nm for the isomer protonated on the nitrogen atom, without success. The transition energies of the C_4H^+ , C_5H^+ , and C_6H^+ isomers were calculated to be slightly higher than the transition energy of the C_2H^+ isomer, in a spectral region where the spectrum of the C_2H^+ isomer becomes congested. The 420–450 nm spectral region was scanned, but only an unresolved signal could be observed, which can be assigned to high vibrational levels of C_2H^+ . However, the signal-to-noise ratio of the experiments did not allow us to completely exclude the presence of these isomers.

All of these high-energy isomers were not expected to be observed in the experiment. Considering the relative stabilities

of isomers C_nH^+ in the initial room-temperature solution, the populations $N(C_nH^+)$ of the different isomers, apart from the C_3H^+ isomer, should be negligible [$N(C_nH^+)/N(C_3H^+) < 10^{-6}$]. Because the solution passes through a heated capillary (400 K), the initial population ratio increases to $N(C_nH^+)/N(C_3H^+) < 10^{-5}$. Based on the population estimates, the observation of the C_2H^+ isomer is questionable. It can be argued that the recorded signal depends on a number of parameters: the population, the oscillator strength of the transition, the Franck–Condon factors, the laser power, the fragmentation efficiency, and so on. Among these parameters, some can be measured or calculated, but the fragmentation efficiency is unknown.

However, if one assumes that the fragmentation efficiencies are the same for the different isomers (which might be completely wrong), because the Franck–Condon factors for the C_2H^+ isomer are nearly 30 times larger than those for the C_3H^+ isomer and the oscillator strength is 4 times greater for the C_2H^+ isomer than for the C_3H^+ isomer, the observed $N(C_2H^+)/N(C_3H^+)$ population ratio would correspond to a temperature of about $550 \pm 100\text{ K}$, not too far from the temperature of the heated capillary of the electrospray ionization apparatus.

Another tentative explanation for the observation of the high-energy C_2H^+ isomer relies on the possible activation of the ions during transfer from the octopole guide to the Paul trap. First, the ions are accelerated at 100/200 V, and any collision with the residual gas ($P = 10^{-5}$ mbar) would increase their internal energy. Second, although the bias voltage of the Paul trap is adjusted to match the kinetic energy of the incoming ions to prevent fragmentation, the possibility that activation occurs through collisions with the He buffer gas cannot be excluded. In any case, it seems that, under our experimental conditions, the cooling process is fast enough to relax the vibrational energy and freeze the ions in local minima of the potential energy surface.

The C_7H^+ isomer was calculated to be in a spectral region (around 530 nm) that is not obscured by the presence of other isomers, but it was not observed. This isomer has a ground-state energy of 0.57 eV relative to that of the C_3H^+ isomer and assuming the same temperature as for the C_2H^+ isomer in the formation process, its population would be 50 times smaller than the C_2H^+ population, and because the Franck–Condon factors are similar for these two isomers, the C_7H^+ isomer would not be detected with our signal-to-noise ratio.

d. Relation to Solution Studies. This study has shed light on the optical properties of protonated indole and can help to determine whether the transient absorption observed at 425 nm after the UV excitation of tryptophan in solution is compatible with the formation of protonated indole through excited-state proton transfer from the ammonium group toward the C_4 or C_5 atom of the indole chromophore. We found good agreement between experiment and calculations for transition energies of the isomers of protonated indole observed in this study (C_2H^+ and C_3H^+). From this starting point, our calculations can be used to predict (within $\sim 0.15\text{ eV}$) the electronic absorptions of the other isomers. The calculations give transitions around 425 nm for the C_4H^+ and C_5H^+ isomers of protonated indole and around 480 nm for the same isomers of protonated 3-methylindole, values that compare quite favorably with the transient spectrum observed in solution after photoexcitation of tryptophan. However, one should bear in mind that the calculations correspond to isolated molecules

in the gas phase whereas the Trp photoexcitation was a solution-phase study for which strong solvatochromic effects can be expected, whereas such effects cannot be taken into account in the present calculations.

Previous calculations on possible photoinduced reactions in the excited state of protonated tryptophan showed that a proton-transfer reaction could occur toward the C₄ or C₂ sites.²³ Protonation at the C₂ site is also compatible with a transient absorption at around 425 nm because the gas-phase absorption of the C₂H⁺ isomer starts at 488 nm in the gas phase and extends to the blue.

CONCLUSIONS

Among the protonated indole isomers, the most stable is the C₃H⁺ isomer. Its adiabatic transition energy was calculated at 3.33 eV (or 3.28 eV, depending on the basis set), which compares rather well with the experimental spectrum starting at 3.45 eV. The other isomers protonated on external carbon atoms (C₂, C₄, C₅, C₆) have higher stabilization energies and their transition energies were calculated in the 2.62–2.83 eV (440–470 nm) energy range, significantly different from the absorption region of the C₃H⁺ isomer. Surprisingly, the C₂H⁺ isomer, which has the second lowest ground-state stabilization energy (0.36 eV higher than the C₃H⁺ isomer), was clearly observed, with a transition starting at 2.54 eV and a vibronic spectrum that becomes crowded above 2.70 eV, where the transitions of the isomers C₄H⁺, C₅H⁺, and C₆H⁺ should appear. We thus cannot determine whether these isomers are present under our experimental conditions. The absorption of the C₇H⁺ isomer corresponds to an even lower transition energy (2.40 eV) and a higher ground-state stabilization energy (0.57 eV), and it was not observed. For all of these isomers, the transition energies are far to the red of the S₁–S₀ transition of neutral indole. Protonation on the nitrogen atom also leads to a high stabilization energy (0.83 eV higher than the C₃H⁺ isomer) and this is the only isomer having a transition energy calculated to the blue of that of the most stable isomer.

The hypothesis of a proton-transfer reaction from the ammonium group to the indole chromophore in photoexcited tryptophan has been postulated in a number of experimental and theoretical studies. The present calculations are in good agreement with the experimental data for the indole isomers protonated on C₃ and C₂, so that, on the basis of such calculations, the assignment of the 425 nm transient absorption to the indole photofragment protonated on C₄, C₅, or C₂ seems reasonable.

ASSOCIATED CONTENT

Supporting Information

Experimental setup, laser power dependence of the photofragmentation signal, calculated geometries for the C₃H⁺ and C₂H⁺ isomers, complementary calculations on 3-methylindole. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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