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Tryptophol Cation Conformations Studied with ZEKE Spectroscopy

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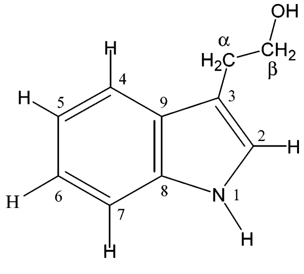
The relative energies of several conformations of the tryptophol cation are determined by zero kinetic energy (ZEKE) photoelectron spectroscopy and photoionization efficiency measurements. Recently published high-resolution electronic spectroscopy on the neutral species determined the absolute configuration of the different conformers in the S_1 spectrum. These assignments are utilized in the photoelectron experiments by pumping through conformer specific S_1 resonances yielding ZEKE spectra of the specific, assigned conformations. The adiabatic ionization of one specific conformation is definitively determined, and two others are estimated. The photoelectron spectra, coupled with calculations, reveal that structural changes upon ionization are dominated by interactions of the hydroxyl group with the changes of electronic structure in the aromatic system.

Introduction

Detailed studies of the gas-phase spectroscopy of tryptophan and tryptophan analogues have been ongoing for a number of years and substantial progress has been made in understanding the structure and dynamics of these molecules.[1−5] Much of this interest derives from the widespread use of tryptophan as a fluorescence probe of protein structure and dynamics.[6] One of the important properties is the significant variability in the photophysical behavior of tryptophan as a function of molecular conformation.[7−11] Time-resolved fluorescence of tryptophan in proteins typically yields two to four fluorescence lifetimes. This phenomenon has been well studied and is ascribed to the presence of multiple conformations which have starkly different fluorescence properties.[12]

As more information has been reported on the electronic spectroscopy of substituted indoles they have come to constitute important molecules for the general understanding of conformational effects on excited-state properties. Thus independent of the biological ramifications this is an important class of molecules. Several developments have made a particularly important impact on elucidating the molecular behavior. The first is the contribution of ab initio calculations, particularly the ability to do excited-state calculations.[13] The second is the application of high-resolution electronic spectroscopy, which has allowed definitive conformational assignments to particular bands in the electronic spectrum.[14−16] The third is the important recent experiments of Zwier et al.,[17,18] which probe not only the conformational minima but give information on the transition states for conformational interconversion and add immensely to the mapping of the conformational landscape.

In this context we have undertaken the high-resolution photoelectron spectroscopy of tryptophol, one member of this class of compounds (Figure 1). An important motivation for this work was the recently published work of Pratt et al.,[14] which gave a definitive conformational assignment to the observed S_1 bands in the jet-cooled spectrum of this molecule. (The predominant conformer ground-state structure was also determined recently using millimeter wave spectroscopy.[19]) In the two color−two photon zero kinetic energy (ZEKE) spectroscopy these assigned S_1 transitions are used as intermediate states, thus providing resolved photoelectron spectra of two of the three conformers, with knowledge of the absolute configuration. As will be shown below, these spectra offer interesting information on the relative energetics of the conformations in the cation. The presence of a positive charge on the aromatic π system is seen to have important consequences for the orientation of the hydroxyl group and the overall structure of the conformationally flexible side chain.



Tryptophol

Figure 1. Tryptophol molecule with atomic numbering scheme.

Experimental Section

Several types of spectroscopy have been applied to study the conformations of tryptophol in the ground state S_0, first excited S_1 state and the cation ground state, D_0, which is the primary focus of this work. The S_1 spectrum was measured using mass resolved resonance-enhanced multiphoton ionization (REMPI) spectroscopy. The ionic ground state of the parent was studied using ZEKE photoelectron spectroscopy,[20] and the neutral ground state was studied, in a very limited way, using IR−UV double resonance ion dip spectroscopy. The following briefly describes the experimental apparatus, which is presented more completely in previous publications.[21−23]

Tryptophol was obtained from Aldrich and used without further purification. It was heated to 140 °C in the sample

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container of a pulsed nozzle (General Valve). This nozzle, located in a differentially pumped molecular beam apparatus, produces a supersonic expansion using He buffer gas at a total pressure of 2 Bar. The resulting molecular beam is skimmed before entering the second chamber, where REMPI and ZEKE spectroscopy take place. A set of microchannel plates (MCP) (Galileo Electro-optic Corp.) located perpendicular to and below the interaction region are used as electron detectors. Located perpendicular and above the interaction region is a linear time-of-flight mass spectrometer with a separate MCP detector. The appropriate electron and ion optics are located in the second chamber for extraction of the resulting electrons and ions to the detectors.

Two independently tunable ultraviolet laser pulses are used in the experiments. One laser is a pulsed nanosecond Nd:YAG operating at 20 Hz (Lumonics NY-61) pumping a tunable dye laser (Lumonics HD500) with a visible bandwidth of 0.05 cm^{-1}. The visible output of this laser is frequency doubled (Spectra-Physics WEX) and provides approximately 1 mJ per pulse in the UV. The second laser system is also a Nd:YAG (Quanta-Ray GCR-3) pumping a tunable dye laser (Quanta-Ray PDL) equipped with frequency doubling capabilities (Spectra-Physics WEX). These two lasers are spatially overlapped in the interaction region, one functioning as the pump laser and the other as the probe. The temporal overlap of the lasers is controlled by a digital pulse generator (Stanford Research Systems, DG535) and adjusted to optimize the two-color dependent signal.

REMPI spectra were acquired using a single laser providing both the excitation and ionization photons. The ZEKE spectra were obtained by fixing the pump to a desired resonance in S$_1$ and scanning the probe through the spectral region of the ionization potential. For ZEKE spectra the pump and probe excitation occurs in a field-free environment, and then, following a delay of 1−5 μs, a −200-V pulse was applied to a grid in the interaction region to field ionize the highly excited Rydberg states and direct the resulting electrons to the detector. The rather large extraction pulse voltage was used to increase the sensitivity of collection and results in only a modest broadening to ~6 cm^{-1}.[20,24] Peak shifts from the true ionization limits are related to residual stray fields and are likely less than several wavenumbers under the conditions utilized.

Several conformer specific infrared spectra were measured in the OH and NH stretching region of the spectrum. These spectra were obtained using the ionization depletion method described in detail in a previous publication.[21] For these experiments a UV pump laser was tuned to the S$_1$ spectral feature assigned to a particular conformer. The ion signal resulting from the REMPI process was then monitored while a second infrared laser was scanned in the region of 3500 cm^{-1}. The infrared spectrum is then recorded as an ion dip. The infrared pulses were generated by difference-frequency mixing of the Nd:YAG fundamental with the tunable dye laser output near 770 nm.

Results and Discussion

Review of Structure and Nomenclature. The molecular conformations arise from different orientations of the hydroxyethyl side chain with respect to the indole plane. A shorthand nomenclature describing these structures has been developed and is in widespread use.[13,14,25,26] The side chain is either anti or gauche. The gauche forms can have the side group oriented toward the pyrrole ring and thus described as GPy or toward the phenyl, GPh. Within each of the structures the hydroxyl group can be pointing toward the indole moiety (in), away (out), or directed upward (up). For instance the most stable conformation is designated GPy-in. The anti form can have the hydroxyl pointed either up (anti-up), toward pyrrole (anti-Py), or toward phenyl (anti-Ph). Calculations reveal a total of seven stable conformers, although only four are observed experimentally in the jet cooled spectra.[14]

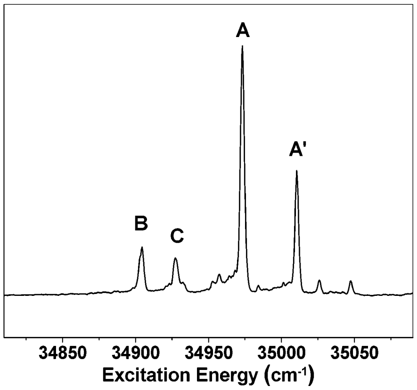

Figure 2. REMPI spectrum of tryptophol in the region of the S$_1$ origin. The three assigned conformers origins are labeled as A (GPy-in), B (GPh-in), and C (Anti-up) as defined in ref 14. A′ is a vibration of GPy-in.

ZEKE Spectra. The S$_1$ spectrum of tryptophol was obtained using mass resolved one color 1 + 1 REMPI. The spectrum obtained agreed with the previously published work of Pratt et al.[14] and Sulkes et al.[27,28] As shown in Figure 2 the three bands labeled A, B, and C are assigned to different conformer origins. By use of the nomenclature discussed above, band A has been assigned[14] as GPy-in, band B as GPh-in, and band C as anti. Pratt[14] has identified two distinct anti conformations that contribute to band C and have a band origin separation of only ~0.5 cm^{-1}. The rotational contours of these two bands overlap extensively and thus appear as a single band in our low-resolution spectrum. Selective excitation of each of the components was thus not possible. The rotationally resolved experiments unequivocally determine that band C is anti but cannot determine the position of the hydroxyl group. On the basis of the calculated energetics they assigned the most intense band as anti-up and the other is left indeterminate as anti-Py or anti-Ph.

Photoelectron spectra were successfully recorded using bands B and C as an intermediate state in the 1 + 1 photoionization. Band A did not yield a spectrum as discussed below. As shown in Figure 3, band C led to the most intense and well-defined spectrum even though this was the weakest S$_1$ band of the three. There is an observed vibrational progression; however it is quite short, and one can confidently identify the first peak as the cation origin and thus determine the ionization potential of this conformer to be 61 028 ± 5 cm^{-1} (34 918.8 cm^{-1} S$_1$ energy plus the 26 109 cm^{-1} appearance energy of the first band. The error is estimated based on the width of the ZEKE peak and the uncharacterized shift of the ionization potential due to stray fields.). This spectrum shows no evidence of multiple conformations contributing to the absorption of band C, but this does not exclude the possibility since the other contributing ZEKE spectra could be overlapping or shifted significantly and thus not observed or so weak as to not be a substantial contributor. A more complete analysis of the observed vibrational structure in the cation will be presented below.

Band B, assigned as the GPh-in conformation, led to a measurable ZEKE spectrum, which was, however, quite weak and exhibited an extended vibrational progression as shown in

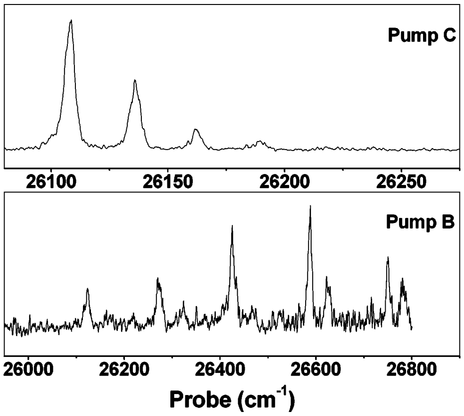

Figure 3. ZEKE photoelectron spectrum obtained by pumping through the origins of (a) band C assigned as anti-up and (b) band B assigned as GPh-in. The spectrum for GPh-in was quite weak and appeared to not have any structure to lower energy. Note the much wider spectral range shown in b.

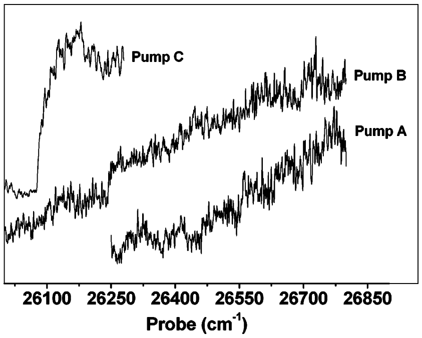

Figure 4. PIE curves pumping through the three origins as indicated. The sharp onset for anti-up is consistent with the ZEKE spectrum of this conformer. The broad appearance curves for the other two origins indicate a significant change in geometry upon ionization.

Figure 3. Identifying the first observed member of the progression, and thus determining the adiabatic ionization potential, cannot be done with confidence, but in the absence of any other information we will assume that the first member of the progression, the band at 26123 cm^{-1}, represents the adiabatic ionization threshold. Thus the ionization energy of conformer B is estimated to be 61 020 cm^{-1} (34 897 + 26 123 cm^{-1}). It will be shown below that the actual ionization energy is likely significantly lower.

The strongest observed peak in the S$_1$ spectrum was due to peak A, the Gpy-in conformer. However, a resolvable ZEKE spectrum pumping through this state could not be obtained. When tuned significantly to the blue the probe laser did yield an enhanced, two-color-dependent, ionization signal, but when the extraction field was delayed no ZEKE signal was observed. In the absence of a true ZEKE signal, a photoionization efficiency (PIE) curve was scanned to try and locate the ionization threshold. The PIE curve, shown in Figure 4, was obtained using ion detection but with a delayed, pulsed extraction field so that the shift of the IP due to the presence of an electric field was minimized. For comparison the PIE data of conformers B and C are included. As expected the onset for ionization was quite broad, extending from the first observed signal at 26 400 cm^{-1} to greater than 26 800 cm^{-1}. The ionization energy is poorly characterized by this curve, but by use of the onset of the ionization signal, we estimate it as ~61 370 cm^{-1} (34 966 + 26 400 cm^{-1}). The observation of a broad ionization threshold and lack of observed ZEKE spectrum is the signature of a significant geometry change between the S$_1$ state and the cation. Quantum chemical calculations have been used to probe these structural changes and these are presented below. The calculations suggest that the PIE is likely not probing the region near the adiabatic ionization threshold.

It should be noted that, in assessing the Franck−Condon activity in the ZEKE spectra, most of the discussion will focus on comparing the geometry of the neutral ground state and the cation rather than detailed consideration of the S$_1$ to cation transition. This approach is taken because we have detailed calculations on the neutral ground state and cation states of the molecule including structures of the minima and vibrational frequencies. Excited-state calculations are becoming more tractable,[13] but we have not yet pursued their application to this problem. The approximation in the analysis is that the S$_1$ potential is quite similar to that of the ground state. This is supported by several experimental observables. First, the lack of substantial vibrational progressions in the S$_0$ to S$_1$ spectrum suggests that the transition is quite vertical and that the geometry of the excited state is similar to that of the ground state and so using the ground state geometry is a good approximation. The shape of the potential could change in S$_1$, but this has less of an effect on the Franck−Condon factors for the transition to the ion than a change in geometry does. Also, the observed S$_1$ transitions for the various conformers fall within a 100-cm^{-1} region. The S$_1$ shifts directly give the change in stability upon electronic excitation and so the relative energetics of the conformers are not changing dramatically.

Calculations. Extensive calculations have been presented in the literature on the ground-state structures of tryptophol using a number of theoretical methods and basis sets.[14,19] We have repeated the calculations of all the minima in the neutral and have also included calculations of the corresponding minima in the cation. The calculations used the Gaussian 03 program[29] and applied the B3LYP density functional method with a 6-31G(d,p) basis set using the default convergence criteria. Zero-point energy corrections were made to the minimum energy of each conformation. These results are summarized in Table 1. The relative merits and shortcomings of density functional theory (DFT) compared to other methods for similar cations[30] and neutral molecules[31] have been documented.

As reported previously[14] the observed relative S$_1$ spectral intensities of bands A, B, and C are in qualitative agreement with the calculated relative stability of the conformers in the ground state. Here we are more interested in the characterization of the energetics and structure in the cation and the ionization energies. In Table 1, we list the calculated absolute ionization potentials including adiabatic IP and vertical IP, as well as the relative energies of the cation conformations. It is well-known that the calculations underestimate the absolute value of the ionization potential,[12] but it is expected that the relative energies would be more accurate. We also include the difference between the calculated vertical and adiabatic IPs, which should reflect the amount of vibrational structure we expect in the ZEKE spectra. Figure 5 gives a graphical representation of the calculated conformer structures.

The most striking result of the calculations is that the two lowest-energy neutral minima, GPy-in (peak A) and GPh-in (peak B), do not have corresponding minimum structures in the cation. For instance, doing a minimization of the cation starting with the GPy-in structure leads to a minimum structure, which is characterized as GPy-up. The hydroxyl group in the neutral has the hydrogen pointing toward the aromatic π system. In the ion this structure is not a minimum but rather leads to a rotation about the C−O bond until a minimum is found with

TABLE 1: Calculated Energies of the Optimized Neutral and Cation Conformations of Tryptophol Using Gaussian 03 with B3LYP Method and 6-31G Basis Set**

conformation	S_0[a,b]	cation[a,b]	adiabatic IP (cm^{-1})[b]	vertical IP	difference (IP)
GPy-in(A)	0	converged to Gpy-up	55 886	59 559	3 673
GPh-in(B)	13	converged to Gph-up	56 188	58 747	2 559
Anti-up (C_{red})	622	1618	56 882	58 286	1 404
Anti-Ph	519	1607	56 973	58 420	1 446
Anti-Py (C_{blue})	529	1521	56 878	58 378	1 500
GPy-up	632	0	55 253	56 782	1 529
GPh-up	730	316	55 471	56 709	1 238

[a] Energies are reported relative to the lowest conformation of the electronic state in question. [b] Zero-point energy corrections included.

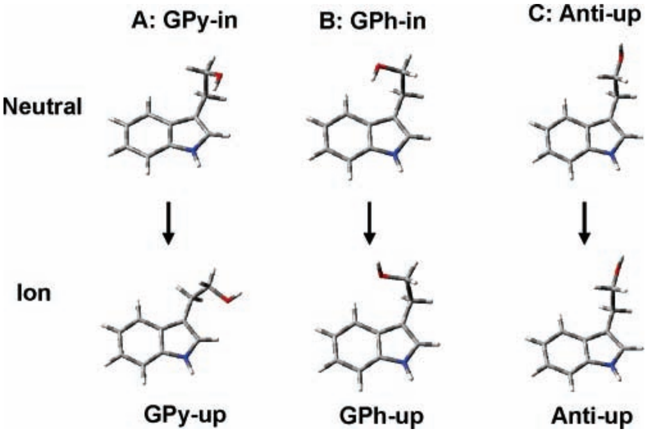

Figure 5. Calculated minimum structures of the neutral and cation of the three conformers observed in the spectrum. Note that the GPy-in and GPh-in neutral structures are not stable in the cation but converge to GPy-out and GPh-out by rotation of the hydroxyl group away for the indole π-system.

the H in the "up" position. The structure is similar to the GPy-up neutral structure (Figure 5). In the neutral molecule this is at significantly higher energy (632 cm^{-1}) than GPy-in, but in the ion it is the lowest-energy structure. Upon ionization the positive charge is located on the atoms comprising the π system but in particular the pyrrole moiety. Atom C2 (Figure 1) and the hydrogen at this position carry a significant positive charge, and it is likely that this interaction destabilizes the "in" hydroxyl orientation in the cation but similarly stabilizes the "up" conformation due to interaction of the hydroxyl oxygen with the positive charge. This can be seen in Figure 5. A similar effect can be seen for the GPh-in neutral and GPh-up cation but with a less pronounced change of positive charge on the phenyl aromatic atoms. It should also be noted that in the cation the GPy-up structure has the entire side chain rotated away from the vertical, with respect to the indole plane, toward the pyrrole. In the neutral the side chain has a dihedral (C2−C3−Cα−Cβ) angle of 96° relative to the pyrrole side of the indole plane, tipping 6° toward the phenyl side from the vertical. In the cation the angle decreases to 53°, now tipping 37° toward the pyrrole. This may be driven by the favorable interaction between the hydroxyl oxygen and the acidic hydrogen at C2.

The calculated energies of the anti-up conformations also have a significant change in relative stability from the neutral to the cation. In the neutral the anti-up conformation is ∼620 cm^{-1} above the lowest-energy conformer (GPy-in); however, in the ion it is ∼1620 cm^{-1} above the lowest-energy conformation. An interpretation, based on the discussion above, is that in the cation the interaction of the hydroxyl oxygen with the positively charged aromatic ring is quite favorable thus lowering the GPh-up and GPy-up conformations relative to the anti conformation. The anti conformation is still stable however, as evidenced by the Band C ZEKE spectrum, because the barrier to rearrangement is significant.

Interpretation of Ionization Potentials. The lack of minima found in the cation calculations for GPy-in and GPh-in is consistent with the appearance of the ZEKE spectra for these two conformations. GPy-in was devoid of any sharp structure and exhibited a PIE spectrum with a very broad onset. GPh-in has some sharp structure in the ZEKE spectrum, but it is quite weak, and it could also be significantly above the adiabatic threshold.

As mentioned above, the calculations of the absolute ionization energies are known to be inaccurate and to underestimate the actual value.[12] An alternative approach is to use the relative calculations between the various conformers in the neutral and cation, and then combine these with the one experimentally measured IP, which is conformer C with a value of 61 028 cm^{-1}. The error in the calculated IPs will then be related to the error in the relative energies of the various conformers. With reference to Table 1 one can predict the adiabatic ionization potential for GPy-in (going to GPy-up in the cation) to be ∼60 030 cm^{-1}. The first observed ionization signal in the PIE appears significantly higher at ∼1 340 cm^{-1}. The vertical ionization energy can be estimated, again by combining the relative ground state and cation conformer energies (including the cation energy of GPy-in calculated using the neutral ground state geometry) with the observed ionization energy of anti-up. This calculation yields a value of 63 600 cm^{-1}. This is greater than the observed blue limit of the PIE that we scanned, which was still increasing. Therefore the observed photoionization, as measured by the PIE, appears between the predicted adiabatic and vertical ionization values expected.

The first observed ZEKE peak for GPh-in yields an ionization energy of 61 020 cm^{-1} (S_1 energy of 34 897 cm^{-1} plus energy of first ZEKE peak at 26 123 cm^{-1}). It is much less clear in this case whether this is a measure of the adiabatic ionization potential. An analysis such as that performed above, using the measured ionization energy of anti-up and calculations of the relative energetics in the neutral and cation, yields an estimate of the adiabatic IP of 60 340 cm^{-1}. In this case the predicted and measured values are closer but still suggest that the first observed ZEKE peak is well above (∼700 cm^{-1}) the adiabatic ionization threshold. The observation of a ZEKE spectrum for GPh-in but not for GPy-in is consistent with different changes in geometry upon ionization. GPy-in and GPh-in both undergo reorientation of the hydroxyl group to the up position, but in addition GPy-in also undergoes a significant change in the torsional angle for the entire side chain as discussed above.

Cation Torsional Potential of Anti-Up. The anti-up conformer has a clearly defined and assignable ZEKE spectrum. The adiabatic ionization potential of this conformer is measured to be 61 028 cm^{-1} with one simple vibrational progression observed consisting of four equally spaced bands with a

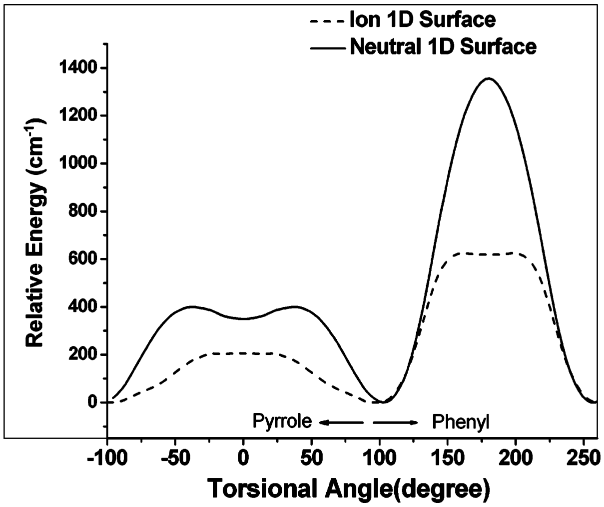

Figure 6. Calculated neutral and cation torsional potentials for rotation of the hydroxyethyl side chain relative to the indole moiety. 0 and 180° represent the planar molecule with the side group oriented toward pyrrole and phenyl, respectively.

vibrational spacing of 27 cm^{-1}. Vibrational frequency calculations on the cation using the same basis set (using a 0.96 correction factor) show the lowest frequency vibration to be 30 cm^{-1} and to consist almost entirely of a torsion of the hydroxyethyl side group relative to the indole plane. Since this motion appears to be primarily one-dimensional we pursued a Franck−Condon simulation[22,32] using the calculated neutral and cation ground state potentials.

The calculated neutral and cation potentials are shown in Figure 6 plotted as a function of the torsional angle. The torsional angle was scanned in 3° increments, and at each point a geometry optimization was performed in all the remaining coordinates. In this coordinate system, 90° reflects a perpendicular orientation with respect to the indole plane and 0 and 180° refer to the group being in the plane and oriented toward the pyrrole and phenyl sides, respectively. The vibrational frequencies and wavefunctions were calculated for each potential by numerically solving the one-dimensional Schrödinger equation using the torsional angle and the appropriate reduced moment of inertia determined from the calculated structural parameters (9.35×10^{-39} g cm^2). We used a matrix approach described previously[22,32] and calculated the Franck−Condon factors based on the overlap of the neutral ground state wavefunction with the cation wavefunctions. The spacing of the cation vibrational eigenvalues are 21.4, 19.5, and 20 cm^{-1}. This is in fair agreement with the measured cation mode spacing of 27 cm^{-1}, but the calculation involved no adjustable parameters and the relative intensities, as shown in Figure 7, are excellent. The agreement between calculation and experiment validates the assumption that the motion is primarily one-dimensional and that the major change upon ionization is an opening up of the torsional potential with respect to rotation toward pyrrole. The planar barriers are also calculated to decrease, particularly for the phenyl side.

As noted above the specific assignment of band C to the anti-up conformation is not definitive and in fact there could be contributions from several anti conformations in the S_1 band pumped. We performed similar calculation on the anti-py and anti-ph conformers and the potentials and Franck−Condon factors were found to be similar to the anti-up results presented above. In this regard the photoelectron results do not give further information on which anti conformations are contributing to the observed S_1 resonance.

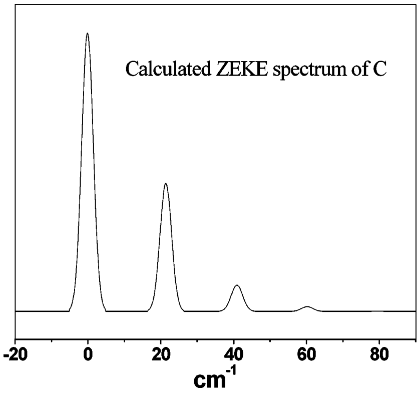

Figure 7. Simulated ZEKE spectrum for anti-up using the one-dimensional potentials shown in Figure 5. The intensity pattern is in excellent agreement with the experimental spectrum, Figure 2.

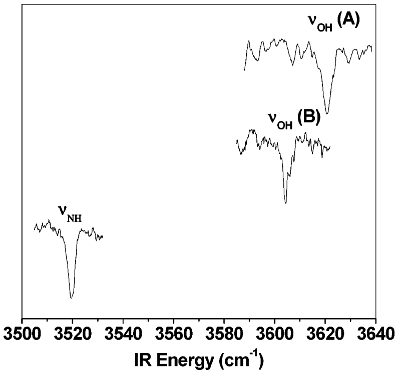

Figure 8. Conformer specific IR ion-dip spectrum in the OH and NH stretching region. The NH band was identical in both conformers, but the OH bands show a 16-cm^{-1} shift.

Conformer Specific IR Spectra. During some earlier work in our group[21] we had measured the conformer specific infrared spectrum of a number of jet-cooled molecules in the OH stretching region. Tryptophol had been measured, but the results were not published, however they have a bearing on the discussion herein. As mentioned in the experimental section the conformer specific IR spectra are obtained by tuning a UV laser to a specific conformer resonance, thus producing an ionization signal. A tunable IR pulsed laser is then introduced and tuned in the region of the expected OH and NH stretching region. When the IR is resonant with a vibration the ground state of the particular conformer is depleted and a loss of ionization signal is detected. Figure 8 shows three such spectra for tryptophol with the top spectra showing the OH stretch of conformer GPh-in, the middle trace showing the OH stretch of conformer GPy-in, and the lower trace showing the NH stretch for both conformers. (For the NH stretch the band did not shift when monitoring either of the conformers). The OH stretching frequency exhibits a shift of 17 cm^{-1} going from 3621 cm^{-1} in GPh-in to 3604 cm^{-1} in GPy-in. This shift is consistent with the arguments above that in the neutral the hydroxyl hydrogen is interacting with the aromatic system more strongly in GPy-in than in GPh-in. It would be useful to compare the value for the anti-up conformer where no interaction is expected, but unfortunately the signal strength for the weaker C band prevented a measurement from being made.

Conclusions

Photoelectron spectroscopy combined with DFT calculations have revealed that the ethanol side chain in tryptophol can undergo significant structural changes as a result of the charge redistribution in the molecule upon ionization. In particular the neutral molecule favors structures in which the hydroxyl hydrogen interacts favorably with the π system. This is evidenced by the fact that the two lowest-energy structures of the neutral both have this interaction (GPy-in interaction with pyrrole and GPh-in interaction with phenyl). Upon ionization the situation is quite different. Significant structural changes upon ionization are revealed by the weak or absent ZEKE spectra and the broad onset for ionization in the PIE curves. Calculations show that the structural minima of the neutral molecule are not stable minima upon ionization but rather the cation has minima in which the hydroxyl hydrogen points away from the π system and the oxygen lone pair interacts with the now positively charged ring system. This is particularly evident in GPy-in where not only does the hydroxyl group flip around but the entire side chain rotates to facilitate the interaction of oxygen with C2 and its hydrogen, which is now likely significantly more acidic in the cation.

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