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Infrared and Ultraviolet Spectral Signatures and Conformational Preferences of Jet-Cooled Serotonin

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Abstract: The ultraviolet and infrared spectroscopy of single conformations of neutral serotonin (5-hydroxytryptamine) have been studied in the gas phase using a combination of methods including laser-induced fluorescence, resonance-enhanced two-photon ionization, UV−UV hole-burning spectroscopy, and resonant ion-dip infrared spectroscopy. By comparison to its close analogue tryptamine, for which firm assignments to seven low-energy conformations have been made, UV and IR transitions due to eight conformations of serotonin are observed and assigned. The ultraviolet spectrum divides into two subsets of transitions separated from one another by ∼230 cm^{-1} ascribable to *syn* and *anti* conformations of the 5-OH group. These two subsets are also distinguishable via their 5-OH stretch fundamentals, with the *anti*-OH subset shifted by ∼4−5 cm^{-1} to lower frequency than those due to *syn*-OH conformers. The existing firm assignments for tryptamine play a decisive role in assignments in serotonin, where the alkyl CH stretch infrared spectrum is diagnostic of the conformation of the ethylamine side chain. Conformer A of serotonin (SERO(A)), with $S_1 \leftarrow S_0$ origin transition at 32584 cm^{-1}, is assigned to Gpy(out)/*anti*-OH, SERO(B) at 32548 cm^{-1} to Gpy(up)/*anti*, SERO(C) at 32545 cm^{-1} to Gph(out)/*anti*, SERO(D) at 32560 cm^{-1} to Anti(py)/*anti*, SERO(E) at 32537 cm^{-1} to Anti(up)/*anti*, SERO(F) at 32353 cm^{-1} to Gpy(out)/*syn*, SERO(G) at 32313 cm^{-1} to Gpy(up)/*syn*, and SERO(H) at 32282 cm^{-1} to Gph(out)/*syn*. The conformational preferences of serotonin differ from those of tryptamine most notably in the selective stabilization observed for the Gph-(out)/*anti*-OH conformer SERO(C), which makes it the second-most intense transition in the ultraviolet spectrum, surpassed only by the Gpy(out)/*anti*-OH conformer SERO(A).

I. Introduction

Serotonin is a neurotransmitter of enormous biological importance. Despite its simple molecular structure (Figure 1), serotonin plays a significant role in a number of fundamental biological processes, including the regulation of mood,[1−3] stress,[4] sleep and eating cycles,[2,5] and muscle and gastrointestinal function.[2,3] More recently it has been shown that serotonin also plays key roles in heart disease and asthma.[2] At physiological pH, serotonin is protonated,[3,6] and not surprisingly, there are a variety of receptor sites involved in these diverse circumstances.[2] Serotonin's versatility is likely influenced by its ability to reorient the flexible ethylamine side chain and 5-OH groups in matching up with differences in these receptor sites. By studying the conformational preferences inherent to the isolated molecule serotonin, we hope to contribute to a better understanding of how serotonin might interact with these receptor sites to carry out its many biological functions.

The inherent conformational preferences of serotonin are also interesting from a fundamental viewpoint as a prototypical flexible biomolecule. A long-term goal of studies of isolated gas-phase serotonin would be to map out the potential energy surface for isomerization in as complete a fashion as possible. This would include determining the spectroscopic signatures of the low-energy conformational isomers and their fractional abundances, measuring the relative energies of the minima and the energy barriers separating those minima. In so doing, one could learn about the efficient isomerization pathways on the multidimensional potential energy surface.

Serotonin (SERO) is a close analogue of tryptamine, differing only in its hydroxy substituent in the 5-position on the indole ring (Figure 1). Tryptamine (TRA) is a molecule that itself has played a foundational role as a prototypical flexible biomolecule.[7−20] One of the particular challenges to the spectroscopic study of tryptamine is that it spreads its population out over seven conformational isomers, even under expansion

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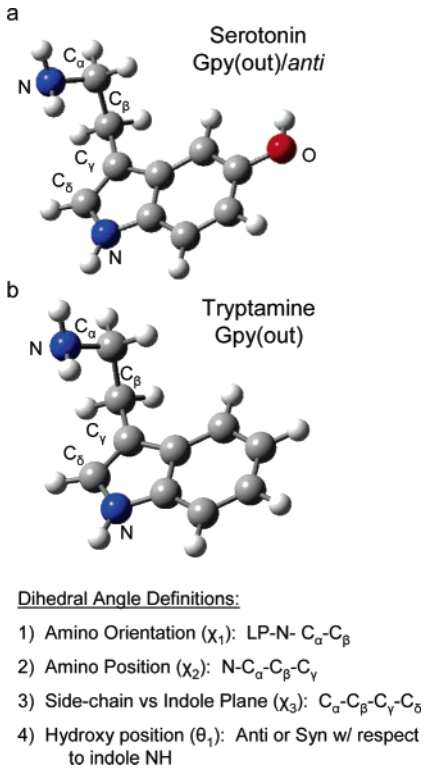

Figure 1. Picture of the (a) serotonin and (b) tryptamine molecules. Atom labels and dihedral angles are also shown for clarity.

cooling. This large number of conformational isomers occurs despite the comparative simplicity of its flexible ethylamine side chain. There are three torsional coordinates to consider, involving hindered rotation about the C(α)−C(*β*), C(*β*)−C(*γ*), and C−N bonds associated with changes in angles $\chi_1-\chi_3$ (Figure 1). The NH_2 inversion coordinate adds a fourth flexible coordinate. The seven conformers observed in jet-cooled spectra of TRA have structures that can conveniently be placed on a two-dimensional surface involving torsion about the C(α)−C(*β*) and C−N bonds. This has led to a short-hand notation for the isomers, in which the *position* of the NH_2 group is given (Gpy = *gauche* on the pyrrole side of indole, Gph = *gauche* on the phenyl side, and *anti*), followed in parentheses by a description of the *orientation* of the NH_2 group relative to the indole ring (up, out, in). The 3-fold barriers along each of these coordinates produce nine minima. Of these nine, only the two minima that orient the nitrogen lone pair on the amino group toward the π cloud of indole (Gpy(in) and Gph(in)) do not hold a measurable population.

Many of the spectroscopic studies of TRA have taken advantage of the indole ultraviolet chromophore that it shares with tryptophan.[7−20] The seven conformational isomers have $S_1 \leftarrow S_0$ origin transitions that are spread over about 100 cm^{-1}, a small percentage of the total photon energy, but sufficient to resolve all but two of the conformer origin transitions under jet-cooled conditions.[7,12,21] High-resolution studies of the rotational structure of the $S_1 \leftarrow S_0$ origin transitions of the conformational isomers led to first assignments,[7] which were subsequently refined on the basis of single-conformation UV and IR spectra by Carney and Zwier.[12] Microwave spectra of Caminati et al.[14] have provided very accurate ground-state rotational constants of the two most highly populated conformers of tryptamine. Recently, Nguyen et al.[19] and Schmitt et al.[15] have recorded high-resolution ultraviolet spectra of the rotational structure present in the $S_1 \leftarrow S_0$ origin transitions of the seven conformers of TRA, confirming and strengthening the earlier assignments. Finally, stimulated emission pumping population transfer spectroscopy (SEP-PTS) has been used to map out the energy barriers separating several of the ground-state minima.[20,22]

In approaching the study of the spectroscopy of serotonin, one wonders whether its conformational preferences can be understood simply as the sum of its parts due to the 5-OH and ethylamine substituents. The 5-OH group in 5-hydroxyindole produces two conformational isomers in which the OH group points *syn* (toward) or *anti* (away from) with respect to the pyrrole ring of indole (θ_1, Figure 1). The ultraviolet spectrum of 5-hydroxyindole exhibits transitions due to two isomers, with $S_1 \leftarrow S_0$ origins at 32685 and 32914 cm^{-1}, separated by 229 cm^{-1}.[23] These transitions have been tentatively assigned to the *syn* and *anti* isomers of the 5-OH group, respectively, on the basis of their relative intensities and the calculated energy ordering.

The obvious prediction for serotonin based on independent flexible substituents is that, under jet cooling, it will spread its population over 2 groups of 7 transitions, leading to a total of 14 conformational isomers. In fact, in the limit that the 5-OH group does not perturb any other aspect of the spectroscopy of the molecule beyond its electronic frequency shift, one should be able to read off the conformational assignments of the 14 isomers based on the positions and intensities of each conformational origin. On the other hand, the conformational preferences of flexible molecules are at times surprisingly sensitive to seemingly innocuous differences in chemical structure. For instance, while *N*-acetyltryptophan methylamide (NATMA) spreads its population over three conformational isomers, the entire spectrum of 5-methoxy-NATMA (differing from NATMA by virtue of a methoxy group in the 5-position on the indole ring) is due to a single conformer.[24−26]

Recently, van Mourik and Emson have carried out a computational study of the conformational minima of neutral serotonin. They identified a total of 23 conformational minima at the B3LYP/6-31+G* level of theory. This includes the full set of 18 conformers (2 × 9) anticipated, plus an additional 5 in which C(α) is nearly in-plane. They predict that, in every case, the *anti* configuration for the 5-OH group is lower in energy than its *syn* counterpart. The calculations also predict

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that the Gpy(out) conformer (which is the global minimum in TRA) retains that status in SERO as well.[27]

In the present paper, we report the resonant two-photon ionization (R2PI), laser-induced fluorescence (LIF), UV−UV hole-burning, resonant ion-dip infrared (RIDIR), and fluorescence-dip infrared (FDIR) spectra of isolated serotonin cooled in a supersonic expansion. As we shall see, the ultraviolet spectrum divides into two sets of transitions split by about 230 cm^{-1}, ascribable to subsets of conformers due to *syn*- and *anti*-5-OH orientations. Transitions due to a total of eight conformational isomers have been identified and spectroscopically characterized. The alkyl CH stretch infrared transitions are diagnostic of the conformations, leading to firm assignments for all eight isomers by comparison with the spectra of the conformers of tryptamine. As in TRA, transitions due to the gauche isomers, in which the amino group positions itself back over the indole ring, dominate the spectrum. However, there are some surprises in the relative energy ordering that indicate that the 5-OH substituent is playing an active role in reshaping the potential energy surface for the ethylamine group.

II. Methods

A. Experimental Methods. The experimental methods used in the present study have been described in detail elsewhere.[28] Those aspects particularly relevant to the present work are described here. Serotonin was commercially obtained (Wako) and used without further purification. Jet-cooled serotonin was prepared by flowing a 70% neon/30% helium mixture at a pressure of 0.5−1.5 bar over the sample, heated to 170 °C. Once picked up by the carrier gas, the sample was pulsed into the vacuum chamber at 20 Hz, using a high-temperature pulsed valve (General Valve, Series 9, 0.4 mm diameter). For R2PI spectroscopy, the expansion is skimmed ∼3 cm downstream from the nozzle orifice before being ionized by an ultraviolet laser in the ion source region of a linear time-of-flight mass spectrometer. For LIF, helium was used as the carrier gas at 0.5−1.5 bar. The resulting expansion is intersected with the UV excitation laser ∼4 mm downstream from the nozzle orifice, with the fluorescence collected through an *f*:1 lens, and then imaged onto a photomultiplier tube.

In both R2PI and LIF schemes, the collimated, doubled output of a Nd:YAG-pumped dye laser (20 Hz) is used as the ultraviolet light source. A rhodamine 610/sulforhodamine 640 dye mixture is used in the dye laser to cover the wavelength range from 297.5 to 310 nm.

The R2PI and LIF spectra possess transitions due to all conformational isomers present in the expansion-cooled mixture. UV−UV hole-burning spectroscopy is used to determine the number of conformational isomers present and their individual UV spectral signatures. Conformational selection is achieved by using one UV source as a hole-burn laser, operating at 10 Hz, with its wavelength fixed on a specific transition in the spectrum. A second UV source, operating at 20 Hz, is spatially overlapped with the hole-burn laser and time-delayed by 200 ns from it. This probe laser is wavelength tuned through the R2PI spectrum. The hole-burn spectrum is recorded by monitoring the difference in ion signal intensity with and without the hole-burn source present. All vibronic transitions that arise out of the same ground-state level involved in the hole-burn transition appear as depletions in the hole-burn spectrum.

The infrared spectrum of each conformer was obtained by the double-resonance techniques of RIDIRS and FDIRS. In either case, the infrared output of a seeded Nd:YAG-pumped optical parametric converter operating at 10 Hz is spatially overlapped with the ultraviolet laser (20 Hz), preceding it by 200 ns. The wavelength of the ultraviolet laser is fixed on the $S_1 \leftarrow S_0$ origin transition of a particular conformer, producing a constant ion or fluorescence signal in the monomer mass channel that is due only to that conformer. As the IR source is tuned through the IR wavelength region of interest, IR transitions of the conformer being probed result in partial depletion in the ion signal due to that conformer. Active baseline subtraction is used to monitor the difference between the ion signal with and without the IR source present.

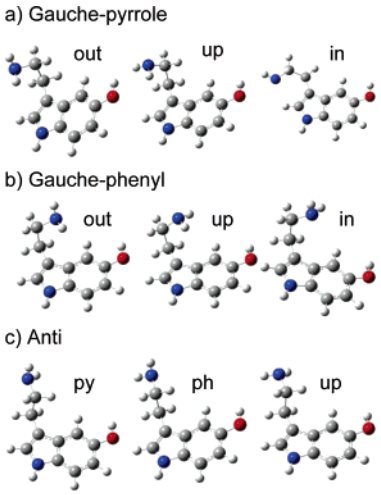

Figure 2. Picture of the nine calculated *anti* conformations of serotonin.

B. Computational Methods. Density functional theory calculations of the structures and relative energies of serotonin's conformational minima, harmonic vibrational frequencies, and infrared intensities were carried out in Gaussian 03[29] using the Becke3LYP functional[30,31] with a 6-31+G* basis set[32] and *ultrafine* gradient as well as MP2[33] with the same basis set for the 18 lowest energy conformational minima. The starting structures were determined by using the nine lowest energy conformational minima of tryptamine and adding the 5-hydroxy group to each in both the *anti* and *syn* positions relative to the indole NH.

III. Results and Analysis

A. Computational Results. Given the previous calculations of van Mourik and Emson,[27] our goal in the present work is to provide a foundation for comparison of SERO and TRA at identical levels of theory to understand any differences observed. As a result, calculations were only carried out on the subset of structures most relevant to this comparison, namely, the "core" set of nine ethylamine side chain conformers Gpy(out, up, in), Gph(out, up, in), and Anti(up, ph, py) with the 5-OH group in either the *syn* or the *anti* configuration. The nine *anti*-OH structures are shown in Figure 2.[34]

Not surprisingly, all of the anticipated 18 minima are indeed minima, on the basis of the lack of imaginary frequencies found by vibrational frequency calculations that followed optimization. Table 1 lists the ZPE-corrected relative energies of these 18 conformational minima for the B3LYP/6-31+G* and MP2/6-31+G* levels of theory, where they are compared with the corresponding calculations in TRA. The DFT results

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Table 1. Comparison of the *anti*/*syn* Conformational Pairs of Serotonin Relative Zero-Point Energies (kcal/mol) with Those of Tryptamine at the DFT B3LYP/6-31+G* with Ultrafine Grid and MP2/6-31+G* Levels of Theory[b]

description of the ethlyamine side chain	tryptamine,[a] DFT/6-31+G*	serotonin, DFT/6-31+G*		serotonin, MP2/6-31+G*	
		anti-5-OH	*syn*-5-OH	*anti*-5-OH	*syn*-5-OH
Gpy(out)	0.000	0.000	0.223	0.103	0.536
Gpy(up)	0.186	0.164	0.426	0.600	1.090
Anti(up)	0.448	0.432	0.621	1.822	2.230
Gph(out)	0.553	0.206	0.998	0	1.122
Anti(ph)	0.562	0.454	0.837	1.620	2.229
Anti(py)	0.620	0.614	0.752	1.798	2.130
Gph(up)	0.798	0.869	0.985	1.197	1.520
Gpy(in)	1.174	1.172	1.330	–	–
Gph(in)	2.502	2.076	2.845	–	–

[a] Tryptamine calculations from Carney and Zwier,[18] calculated at the DFT B3LYP/6-31+G* level of theory, but without the ultrafine grid used in the present study of SERO. [b] Zero-point corrections use the DFT B3LYP/6-31+G* harmonic frequencies.

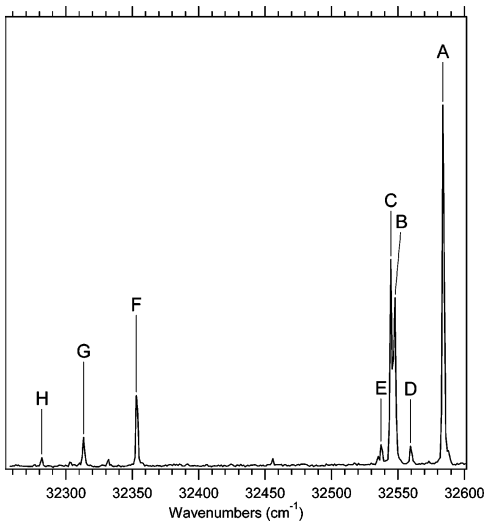

Figure 3. LIF spectrum of the $S_1 \leftarrow S_0$ origin region of serotonin.

are in close agreement with the results of van Mourik and Emson,[27] apart from small changes in the structures and zero-point energy corrections due to the tight convergence criteria used in this study. This led to a switch in the zero-point-corrected energy ordering between Gpy(up)/*syn* and Gpy(up)/*anti*, with the present study predicting the former to be higher in energy than the latter, while van Mourik and Emson predict the reverse.[27] The reason for this difference is most likely because van Mourik and Emson used Gaussian98[29] for their calculations, while we used Gaussian03.[29]

In our previous study of TRA, the relative energies of the minima were calculated at a range of levels of theory, including full optimizations at RIMP2/aug-cc-pVDZ, B3LYP/6-31+G*, and B3LYP/aug-cc-pVDZ and single-point calculations employing RIMP2/aug-cc-pVTZ and CCSD(T)/6-31+G*.[20] The main conclusions drawn from this comparison were that (i) DFT provided relative energies intermediate between those of HF and CCSD(T), (ii) the Gpy and Gph ethylamine side chain conformations were more sensitive than the *anti* conformations to the level of theory used, (iii) MP2, RIMP2, and CCSD(T) calculations amplify differences between the conformers relative to those from DFT and lower the energies of the *gauche* conformers relative to the *anti* conformers, and (iv) the MP2, RIMP2, and CCSD(T) results preferentially stabilize the Gph-(out) conformer relative to Gpy(out) and Gpy(up).

The results in Table 1 exhibit the same general trends with level of theory in SERO, with the MP2 results predicting that Gph(out) is lower in energy than Gpy(out) or Gpy(up). As in TRA, the MP2 calculations seem to overestimate the stabilization in Gph(out) relative to experiment, where transition A due to Gpy(out) is twice as intense as other transitions (Figure 3). As a result, we will use the DFT calculations as our primary point of comparison with experiment, recognizing that the same general trends are present in the MP2 calculations.

The DFT calculations predict that, as in tryptamine, the Gpy-(out) and Gpy(up) structures of SERO are the most stable conformations, while the Gph(in) and Gpy(in) structures are the least stable. In every case, the nine pairs of structures that share the same ethylamine side chain conformation but differ in 5-OH orientation are energy ordered, with the *anti*-OH structures lower in energy than their *syn*-OH counterparts. The relative energy difference between the *anti* and *syn* conformations is typically in the range 0.100−0.400 kcal/mol, with the exception of the Gph(out) pair, which has an energy difference of ∼0.800 kcal/mol. By comparison with TRA, it appears that this larger separation in Gph(out) is due to a stabilization of Gph(out)/*anti* rather than a destabilization of Gph(out)/*syn*. The combination of small energy differences and modest level of theory used here argue against a too-detailed analysis of the source of these energy differences. However, the MP2 calculations also reveal the same trends, with a *syn*-OH/*anti*-OH energy separation about twice as large in Gph(out) as in other conformers (Table 1). As we shall see shortly, the Gph(out)/*anti* conformer is indeed responsible for one of the most intense $S_1 \leftarrow S_0$ origin transitions in the spectrum, indicating that the calculations correctly predict the selective stabilization of this structure. As a result, we will return to consider possible reasons for this stabilization in the Discussion.

B. LIF, R2PI, and UV−UV Hole-Burning Spectra. Figure 3 presents an LIF spectrum of the $S_1 \leftarrow S_0$ origin region of serotonin cooled in a supersonic expansion. Transitions A−H fall into two groups, with five transitions (labeled A−E) about 200 cm^{-1} to the blue of the other three (labeled F−H). The spectrum is dominated by transitions A (32584 cm^{-1}), B (32548 cm^{-1}), and C (32545 cm^{-1}), with two much less intense transitions at 32560 cm^{-1} (D) and 32537 cm^{-1} (E). The set of three transitions shifted to the red appear at 32353 cm^{-1} (F), 32313 cm^{-1} (G), and 32282 cm^{-1} (H).

Figure 4a presents an overview LIF scan of the first 1200 cm^{-1} of the $S_1 \leftarrow S_0$ spectrum of serotonin. A series of six UV−UV hole-burning spectra are shown below it for comparison, with the hole-burn laser fixed on transitions A−C and F−H. These spectra confirm that these six transitions are due to six distinct conformations of SERO. Since these spectra are recorded as difference signals (with and without the HB laser present), the spectra of the smaller intensity conformers show evidence of incomplete subtraction when the probe laser scans through a large transition due to another conformer. For this reason, hole-burning spectra for conformers D and E were not taken; however, the infrared spectra in the CH stretch region will show

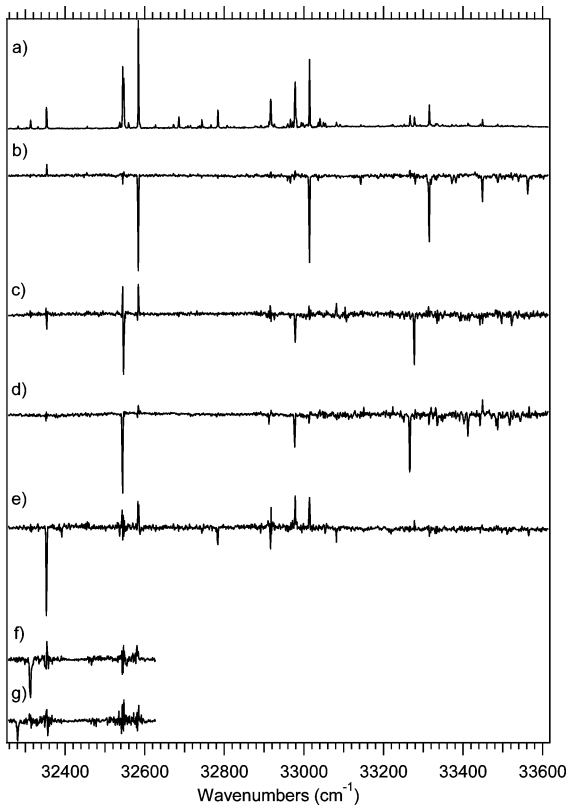

Figure 4. (a) LIF spectrum and (b−g) UV−UV hole-burning spectra of serotonin conformers A−C and F−H, respectively.

Table 2. Summary of the Transition Frequencies and Relative Intensities Deduced from the R2PI, LIF, and UV−UV Hole-Burning Spectra

conformer[a]	$S_1 \leftarrow S_0$ origin[b]	relative intensity[c]	conformer[a]	$S_1 \leftarrow S_0$ origin[b]	relative intensity[c]
A	32584	100	E	32537	10
B	32548	44	F	32353	27
C	32545	53	G	32313	15
D	32560	10	H	32282	8

[a] $S_1 \leftarrow S_0$ origin transition selected in the hole-burning scan (see Figure 3). [b] Wavenumber position (cm^{-1}) of the indicated transition. [c] Intensities determined by peak area, intensity relative to that of conformer A (100).

each to be a distinct conformation. Several small transitions in Figure 4a that are not accounted for in the hole-burning scans are likely due to conformer D, E, G, or H for which hole-burning spectra at higher frequency were not taken.

Previous studies have shown that the *anti* and *syn* conformations of 5-hydroxyindole are separated by 229 cm^{-1}.[23] If the $S_0 \leftarrow S_1$ origin transitions of SERO were to retain this same spacing, we would anticipate that the spectrum would be composed of pairs of transitions due to a given ethylamine side chain conformation separated by 229 cm^{-1}. On the basis of this simple argument, a preliminary assignment would be to pair transitions A and F ($\Delta\nu = 231$ cm^{-1} separation) and B and G (235 cm^{-1} separation).

The frequencies and relative intensities of the origins of all eight conformations are summarized in Table 2. The UV spectra of each conformation show comparatively little activity in the low-frequency region, where the torsional bands due to the ethylamine side chain should appear. Major transitions appear about 430 and 730 cm^{-1} above each origin. These transitions

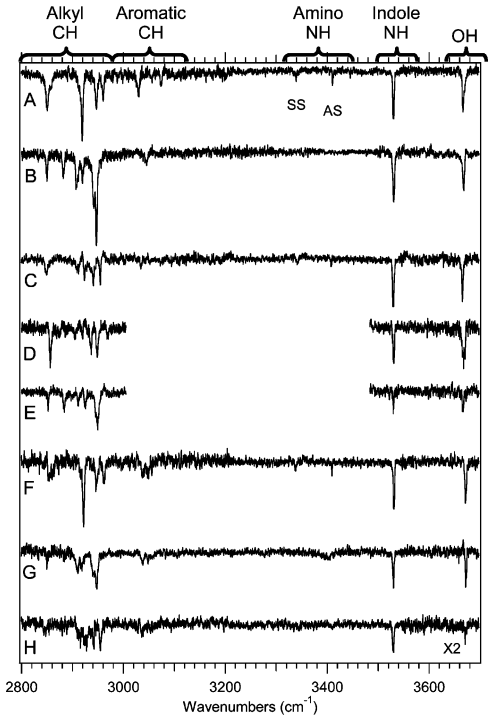

Figure 5. Resonant ion-dip infrared (RIDIR) spectra of conformers A−H. For each RIDIR spectrum, the UV laser was fixed on the $S_1 \leftarrow S_0$ origin transitions of each conformer. The alkyl CH stretch regions of conformers B, D, and E were taken using fluorescence-dip infrared spectroscopy.

have close analogues in the ultraviolet spectrum of indole, ascribable to in-plane distortions of the indole ring. In particular, the 730 cm^{-1} vibronic band is associated with a ring-breathing mode involving the expansion of the aromatic rings in response to the $\pi-\pi^*$ excitation.[35−37]

C. Infrared Spectroscopy. Figure 5 presents a series of infrared spectra recorded in the hydride stretch region of the infrared, taken in IR depletion using either fluorescence (FDIRS) or R2PI (RIDIRS) as a detection scheme. LIF detection provided a better signal-to-noise ratio for the weak transitions, while R2PI detection was useful in ensuring that no interference from clusters was present.

Transitions due to the 5-OH, indole NH, amino NH, aromatic CH, and alkyl CH stretches are evident in the spectra. Not surprisingly, the indole NH stretch fundamentals of the eight conformers are virtually unchanged from one conformer to the next, as they were in TRA. As a result, we set the scale factor (0.962) for the calculated harmonic vibrational frequencies to bring experimental and calculated indole NH stretch fundamentals into agreement with one another.

The preliminary assignment of the set of ultraviolet transitions to the blue (A−E) as due to one 5-OH orientation and those shifted to the red (F−H) as due to another (Figure 3) is confirmed from the OH stretch fundamentals. As Table 3 summarizes, all transitions in the former category have OH stretch fundamentals in the 3666−3668 cm^{-1} region, while those in the latter category appear at 3671−3672 cm^{-1}. This shift is

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Table 3. Experimental Transition Frequencies (cm^{-1}) for the Amino NH, Indole NH, and OH Stretches in the Eight Observed Conformers of Serotonin

	anti					*syn*		
	A	B[a]	C	D[a]	E[a]	F	G[a]	H[a]
amino NH (SS)	3339		3342			3338		
amino NH (AS)	3410		3407			3409		
indole NH	3530	3531	3530	3531	3530	3531	3530	3530
OH	3667	3668	3666	3668	3667	3671	3672	3671

[a] The amino NH stretches could not to be determined in conformers B, D, E, G, and H.

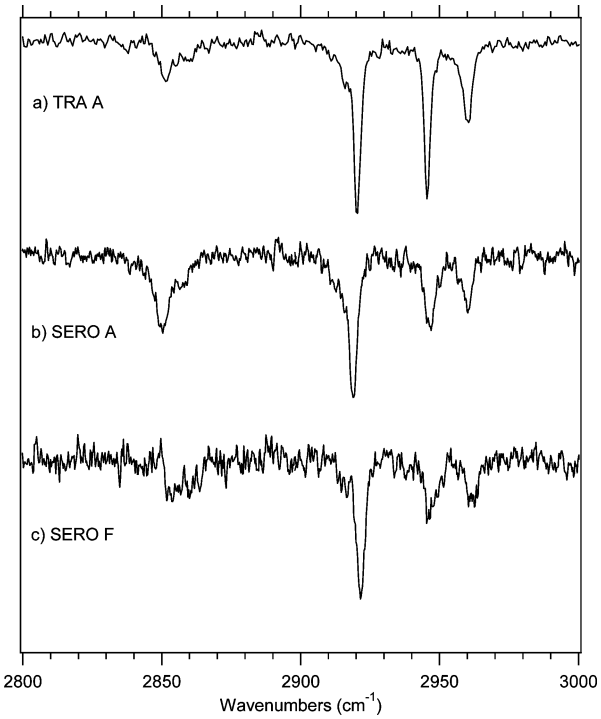

Figure 6. Comparison of the single-conformation infrared spectra of (a) tryptamine A and serotonin (b) A and (c) F. The spectrum of TRA(A) is reprinted from ref 12. Copyright 2000 American Chemical Society.

also present in the calculated OH stretch frequencies, with the *anti*-OH stretch fundamentals lower in frequency than the *syn* fundamentals by 4−5 cm^{-1}. This suggests that A−E are *anti*-OH conformers and F−H *syn*-OH. Such an assignment is also consistent with the greater stability calculated for *anti* over *syn* conformers, since the *anti* isomers have greater intensity than the *syn* isomers in the ultraviolet spectrum.

The amino NH stretch fundamentals are very weak and are only observable in conformers A, B, and F. Interestingly, the two *anti* isomers have different amino NH stretch fundamentals (3338/3409) than the *syn*-OH conformer F (3342/3407), despite the fact that the OH group is remote from the amino group in all conformers.

The alkyl CH stretch region provides the most useful diagnostic of the ethylamine side chain conformation. Since the ethylamine side chain in SERO is identical to that of TRA, for which we have firm conformational assignments, it seems plausible that a comparison between the alkyl CH stretch regions of the two molecules could be useful in making conformational assignments. To that end, Figure 6 compares the alkyl CH stretch region of the spectrum of conformer A of TRA with the corresponding spectra of conformers A and F of SERO. Figures 7−10 provide similar comparisons: TRA(B) with SERO(B,G), TRA(C2) with SERO(C,H), TRA(C1) with SERO(D), and TRA(E) with SERO(E). Table 4 provides the observed frequencies of the observed alkyl CH stretch transitions for the eight conformations of serotonin and their matching tryptamine conformers.

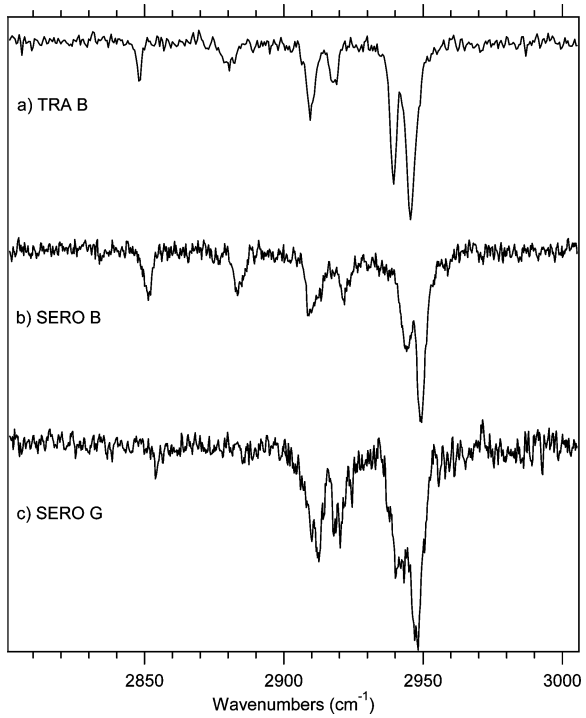

Figure 7. Comparison of the single-conformation infrared spectra of (a) tryptamine B and serotonin (b) B and (c) G. The spectrum of TRA(B) is reprinted from ref 12. Copyright 2000 American Chemical Society.

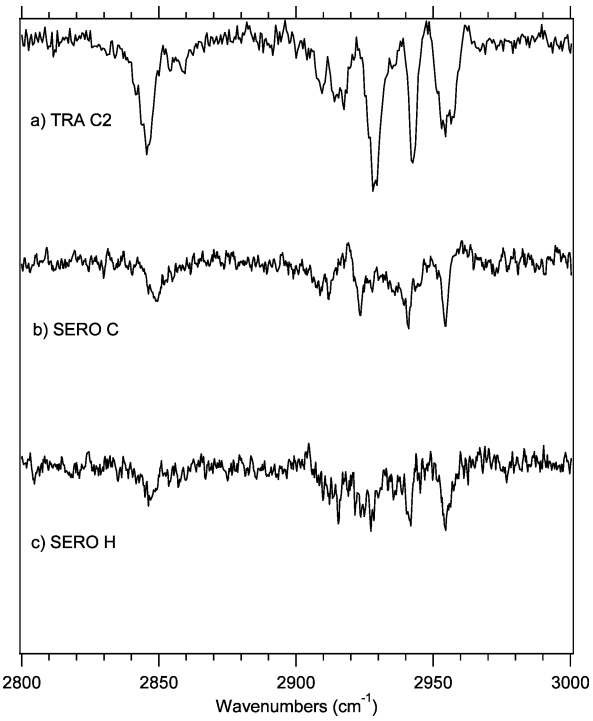

Figure 8. Comparison of the single-conformation infrared spectra of (a) tryptamine C2 and serotonin (b) C and (c) H. The spectrum of TRA(C2) is reprinted from ref 12. Copyright 2000 American Chemical Society.

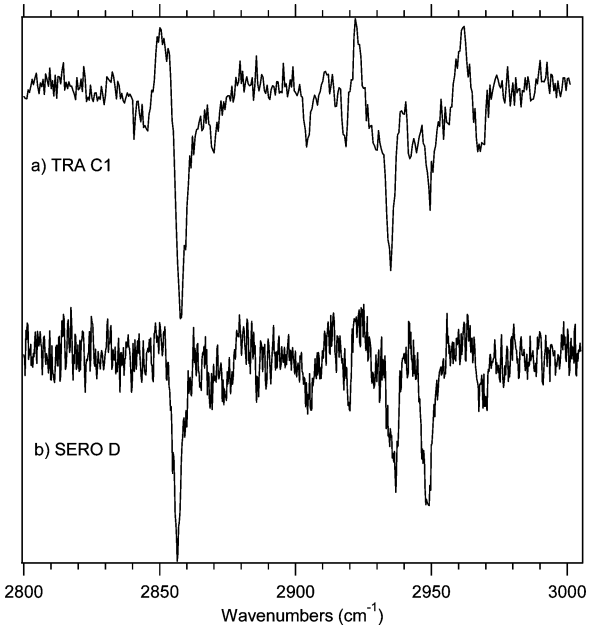

Figure 9. Comparison of the single-conformation infrared spectra of (a) tryptamine C1 and (b) serotonin D. The spectrum of TRA(C1) is reprinted from ref 12. Copyright 2000 American Chemical Society.

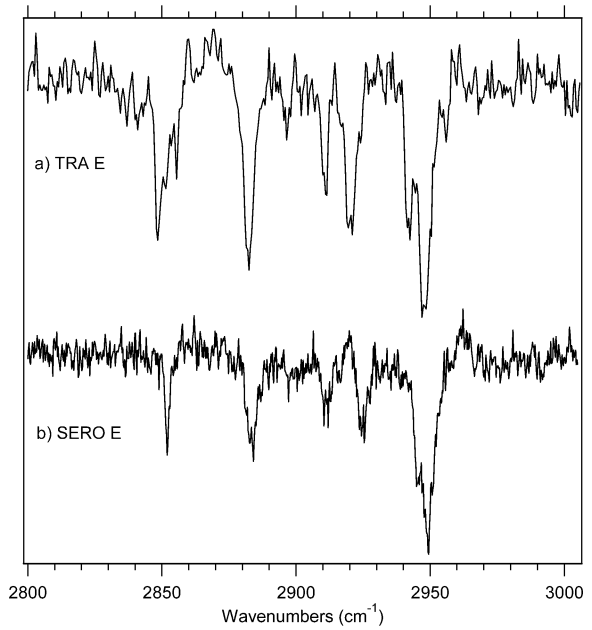

Figure 10. Comparison of the single-conformation infrared spectra of (a) tryptamine E and (b) serotonin E. The spectrum of TRA(E) is reprinted from ref 12. Copyright 2000 American Chemical Society.

The close correspondence in each case is striking. Not only the frequencies, but also the relative intensities, and hence the entire pattern of the spectrum, carry over from TRA to SERO. We can surmise on this basis that the alkyl CH stretch spectrum is a reliable diagnostic of the ethylamine side chain. Given the firm conformational assignments for TRA, the alkyl CH stretch infrared signatures lead to the assignments given in Table 5, which are also used to label the $S_1 \leftarrow S_0$ origin transitions in Figure 11b,c. For comparison, the corresponding assignments of the ultraviolet transitions in TRA are shown above it (Figure 11a).

Table 4. Comparison in the Alkyl Stretch Region of Tryptamine Conformers A, C2, B, C1, and E with Their Serotonin Conformer Counterparts

tryptamine[a]			serotonin		
conformer	frequency[c]	intensity[d]	conformer[b]	frequency[c]	intensity[d]
A	2852	w	A	2851	w
	2920	s		2919	m
	2945	s		2947	w
	2960	m		2960	w
			F	2854	w
				2922	m
				2945	w
				2963	w
B	2848	w	B	2851	w
	2880	w		2883	w
	2909	m		2909	w
	2919	w		2921	w
	2939	s		2944	m
	2945	s		2949	s
			G	2849	w
				2882	w
				2911	w
				2919	w
				2939	w
				2948	m
C2	2845	m	C	2849	w
	2929	s		2912	w
	2942	s		2923	m
	2954	m		2941	m
				2955	m
			H	2846	w
				2915	m
				2927	m
				2942	m
				2955	m
C1	2858	s	D	2856	s
	2935	m		2937	m
	2949	m		2949	m
	2968	w		2969	w
E	2848	m	E	2852	s
	2855	m		2854	w
	2882	s		2884	m
	2911	m		2912	m
	2921	s		2925	m
	2942	s		2945	s
	2947	s		2949	s

[a] Experimental data from Carney and Zwier.[12] [b] See Table 2 for the origin positions. [c] Wavenumber values from FDIR and RIDIR spectra in the alkyl CH stretch region. [d] s = strong, m = medium, and w = weak.

Table 5. Conformational Assignments for the Eight Observed Conformations of Serotonin

conformer	assignment	conformer	assignment
A	Gpy(out)/*anti*	E	Anti(up)/*anti*
B	Gpy(up)/*anti*	F	Gpy(out)/*syn*
C	Gph(out)/*anti*	G	Gpy(up)/*syn*
D	Anti(py)/*anti*	H	Gph(out)/*syn*

IV. Discussion

A. Conformational Preferences of Serotonin. The hydride stretch infrared spectra of SERO have provided firm assignments for the observed ultraviolet transitions to particular conformations of SERO. The $S_1 \leftarrow S_0$ origins and OH stretch IR transition frequencies both reflect a division into two subsets of conformers with *syn*- and *anti*-OH orientations. Comparison of the alkyl CH stretch spectra of single conformations of SERO with those of TRA determine the ethylamine side chain conformation, based on the previously known, firm assignments for TRA. The fact that such a procedure works so well provides a basis of

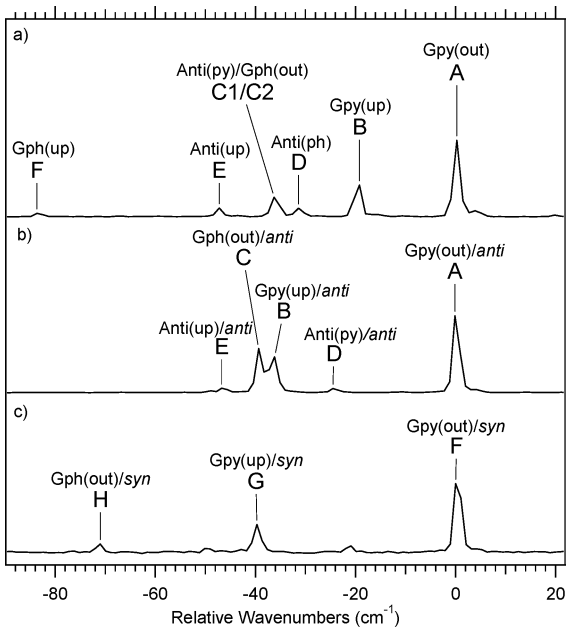

Figure 11. Comparison of the (a) tryptamine and serotonin (b) *anti* and (c) *syn* $S_1 \leftarrow S_0$ origin regions with conformational assignments.

attack for future studies of molecules that possess more than one independent flexible subgroup, as long as the subgroups do not interact strongly with one another.

1. Preference of *anti*-OH over *syn*-OH. The fact that the ultraviolet spectrum breaks up into two subsets due to *anti*-OH and *syn*-OH conformers (Figure 3) indicates that the position of the $S_1 \leftarrow S_0$ origin is more sensitive to the OH orientation than the conformation of the ethylamine side chain. The splitting between the three *anti*/*syn*-OH conformer pairs is 231 (A/F), 235 (B/G), and 263 (C/H) cm^{-1}, while the largest shift observed for the transitions that share the same OH orientation is 71 cm^{-1} (F vs H). This is not surprising, given that the OH reorientation involves hindered rotation about the C−O bond attached directly to the indole ring. By comparison, all of the observed ethylamine side chain conformers share the same out-of-plane orientation for the C(α)−C(*β*) bond and hence involve changes in the position and orientation of the NH_2 group, which is one bond further from the ring.

The separation of the ultraviolet spectrum into subgroups makes it readily apparent that the transitions tentatively assigned to the *syn*-OH conformers are 3−4 times smaller than their *anti*-OH counterparts. In fact, this intensity comparison is one of the factors used in assigning the red-shifted bands to the *syn*-OH conformers and blue-shifted bands to *anti*-OH. Of course, the observed intensities of the transitions could give a false impression about the relative populations if the oscillator strengths or nonradiative quantum yields were to change significantly with OH orientation. The fact that both LIF and R2PI spectra show a preponderance of intensity in the blue-shifted set of transitions probably means that the latter effect is small. In the end, it would be helpful to have independent confirmation of the *syn*/*anti*-OH assignment. High-resolution electronic spectroscopy[15,16,19] or microwave optical double resonance[38,39] has the potential to provide such data, although the change in rotational constants with OH orientation is very small.

2. Similarities and Differences with TRA. A primary goal of the present study is to determine the conformational assignments of jet-cooled SERO and to see how these compare with its close structural analogue TRA. Figure 11 provides such a comparison, presenting close-up views of the $S_1 \leftarrow S_0$ origin transitions of TRA, SERO(*anti*-OH), and SERO(*syn*-OH), labeled with their conformational assignments. Not surprisingly, the spectra of SERO bear an undeniable resemblance to that of TRA, but also show differences that suggest that the two substituents (5-OH and 3-ethylamine) do influence one another to some degree.

In the Introduction, we predicted that serotonin would spread its jet-cooled population over 14 conformational isomers associated with *syn*-OH and *anti*-OH versions of the seven conformers due to the ethylamine side chain observed in jet-cooled TRA. Instead, we have identified transitions due to eight conformers of serotonin: five *anti*-OH and three *syn*-OH conformers. Close inspection of Figure 11b,c reveals another 2−3 weak transitions that are likely due to additional conformers with a very small population. However, the comparison of the transitions in TRA (Figure 11a) with those in SERO suggests a funneling of the population into fewer ethylamine side chain conformers in SERO relative to TRA.

Having said this, the comparison between the ultraviolet spectra of SERO and TRA shown in Figure 11 shows a similar pattern of transitions that mirror similar overall ethylamine side chain preferences. For instance, the most intense transition in all three spectra is due to Gpy(out), indicating that this conformation is, in all probability, the most stable conformation in SERO, like it is in TRA. This transition is the furthest blue-shifted transition in each case.

The transitions assigned to Gpy(up) (SERO B and G) are about half the intensity of their Gpy(out) counterparts in both SERO(*anti*-OH) and SERO(*syn*-OH), as it is in TRA. The Gpy(up) transitions are shifted −36 and −40 cm^{-1} in SERO *anti*/*syn*, compared to −20 cm^{-1} in TRA. The *syn*/*anti*-OH splitting is 231 cm^{-1} in Gpy(out) and 235 cm^{-1} in Gpy(up), compared to 229 cm^{-1} in 5-hydroxyindole. On this basis, we surmise that the Gpy position for the amino group is sufficiently remote from the 5-OH substituent that it is not influenced greatly by the presence of the 5-OH group.

The Anti-ethylamine side chain conformers Anti(up) and Anti(py) (in which the amino group points away from the indole ring) are present and observed in SERO(*anti* OH) (transitions D and E), but are somewhat weaker than in TRA. The position of the Anti(up) transition (−47 cm^{-1}) is nearly identical to that in TRA (−47 cm^{-1}), and the Anti(py) transition is shifted to the high-frequency side of Anti(up), as it is in TRA.

Both the Anti(ph) and Gph(up) transitions have not been observed in the spectrum of either SERO(*anti*-OH) or SERO(*syn*-OH). Their absence may result from a destabilization associated with the 5-OH substitution or to a lowered barrier separating these minima from others, leading to removal of the preexpansion population during the cooling process in the expansion. However, we must be careful in ascribing the missing conformers to particular aspects of the SERO potential energy surface, because some of the reason that transitions due to certain

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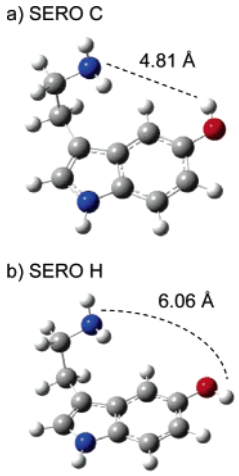

Figure 12. Comparison of the structures of serotonin Gph(out) (a) *anti* C and (b) *syn* H conformers.

conformers are "missing" may simply be that, in spreading the population out over more minima, the fractional abundance of any one conformer necessarily decreases. This may make it difficult to observe small population conformers. In the present study, we have identified transitions due to eight conformers of SERO, with the smallest intensity transition (H) about 8% of the most intense (A). A similar ratio exists between the largest and smallest transitions in TRA, where seven conformers are observed.

The most dramatic effect of 5-OH substitution is the significant additional intensity it induces in the Gph(out)/*anti* conformation of SERO (transition C) compared to TRA. In SERO, this transition is the second-most intense transition in the spectrum (Figure 11b), about 4 times the relative intensity of its counterpart (transition C2) in TRA (Figure 11a). In TRA, transition C is composed of three subbands, a red-shifted single band due to Gph(out) and a tunneling doublet shifted 0.6 cm^{-1} to the blue due to Anti(py).[19] A similar enhancement is not observed in transition H assigned to the Gph(out)/*syn*-OH conformer, indicating that the stabilization of Gph(out)/*anti* is indeed due to the *anti*-OH orientation. In addition, the electronic frequency shift of conformer H (-71 cm^{-1}) is nearly twice its value in SERO *anti*-OH (-39 cm^{-1}), leading to a splitting between the two *syn*/*anti*-OH partners of 263 cm^{-1}. The unusual intensity and frequency shifts both reflect a sensitivity of the Gph(out) ethylamine side chain to the presence and orientation of the OH group.

We noted in section III.A that the DFT calculations correctly predict the selective stabilization of the Gph(out)/*anti*-OH conformer (SERO(C)). It is intriguing that this structure (Figure 12a) is one in which the nitrogen lone pair on the amino group is oriented to face the *anti*-OH hydrogen. This is suggestive of a H-bonding interaction between the two groups, with the 5-OH group as a H-bond donor to the NH_2 acceptor. However, as Figure 12a summarizes, the distance separating the two groups is sufficiently large to argue against any real H-bonding interaction, consistent with a lack of any observed wavenumber shift in the Gph(out)/*anti*-OH stretch fundamental relative to other *anti*-OH conformers (Table 3). Instead, it is more likely that the selective stabilization is an electronic effect transmitted through the indole ring to the amino group. A comparison between the structures and charge distributions of SERO C and H shows such small differences between them that it is difficult to associate any definite structural change to the calculated energy difference. As a result, it is difficult to ascribe this selective stabilization to particular structural or electronic effects. A more detailed analysis of the conformational preferences of SERO using natural bond orbital (NBO) methods is presently being pursued.[40,41]

B. ^1L_a State in Neutral Serotonin. One of the ongoing, important challenges in the electronic spectroscopy of indole-containing molecules such as tryptophan, tryptamine, and serotonin is the close proximity and interactions between the lowest excited electronic states of the indole chromophore.[42−47] The fascinating and complicated interplay between these states is important for the excited-state lifetimes, fluorescence quantum yields, and photochemistry of these molecules.[37,42−47] In most circumstances, the lowest excited singlet state is the ^1L_b state, with a transition dipole moment (TDM) from the S_1 state polarized along the "*a*" axis of indole. Only slightly higher in energy is the ^1L_a state, with a much larger excited-state dipole moment and a TDM (from S_0) oriented along the "*b*" axis. There is also a $^1\pi\sigma^*$ state that is near in energy and is calculated to be repulsive along the indole N−H coordinate, leading to dissociation of the H atom.[42]

While calculations[42] have uncovered the presence of these states and are making predictions of increasing sophistication and accuracy regarding their structures and properties, there are still many fundamental aspects of the spectroscopic consequences of these states that are not understood. Even such fundamental information as the position of the ^1L_a origin relative to ^1L_b is still a matter of ongoing debate and study.

Recently, Schmitt et al.[16] carried out DFT/MRCI and TDDFT calculations on tryptamine that provided predictions for the structural changes and relative energies of the ^1L_a and ^1L_b states of tryptamine. While TDDFT calculations invert the order relative to experiment, DFT/MRCI results place the ^1L_b state below ^1L_a, with an energy difference of about 2000 cm^{-1} between them. However, recent high-resolution spectra of vibronic bands in TRA by Schmitt and co-workers[48] have identified a series of bands only 412, 467, 574, and 595 cm^{-1} above the ^1L_b origin that have rotational structure that reflects a TDM direction along the *b* axis, indicating ^1L_a character.

One of the striking effects of 5-OH substitution is that it lowers the $S_1 \leftarrow S_0$ origin by over 2000 cm^{-1}, from 34919 cm^{-1} for TRA(A) to 32584 cm^{-1} in SERO(A). A shift of this same magnitude was observed previously in the ultraviolet spectrum of melatonin, which has a 5-methoxy substitution on the indole ring.[49] The ultraviolet spectra (whether LIF or R2PI) of many

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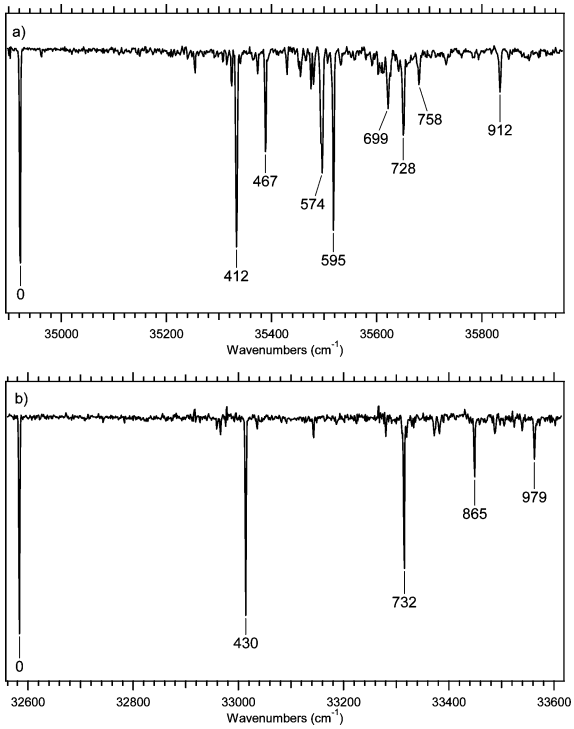

Figure 13. UV−UV hole-burning spectra of (a) tryptamine A and (b) serotonin A.

of the indole derivatives that lack the 5-OH or 5-methoxy substituent (e.g., tryptophan, *N*-acetyltryptophan methyl amide)[24] have rather sharp cutoffs in the observed vibronic structure that occur within a few hundred wavenumbers of the S_1 origin, indicative of some fast nonradiative process turning on there. In the case of melatonin, the spectrum extended much further above the S_1 origin.[49] It seems likely that the 5-methoxy substitution could be responsible for the difference in photophysical behavior, selectively moving the ^1L_b state lower in energy relative to ^1L_a, thereby creating a larger S_1-S_2 energy gap.

In the present study of SERO, 5-OH substitution has a similar effect on the $S_1 \leftarrow S_0$ transition energy. As a result, it is worthwhile to compare the single-conformation ultraviolet spectra of SERO(A) and TRA(A) to see how the spectrum changes with 5-OH substitution. Figure 13 makes such a comparison between the UVHB spectra of TRA(A) (Figure 13a) and SERO(A) (Figure 13b). Not surprisingly, the two spectra show a similarity in the positions and relative intensities of many of the main vibronic bands (e.g., near 400, 700, and 900 cm^{-1}). However, the spectrum of SERO(A) is more sparse, with only five vibronic bands with significant intensity in the first 1000 cm^{-1} (at 0, 430, 732, 865, and 979 cm^{-1}), compared to about twice that number in TRA(A). While it is possible that this difference reflects a more complicated geometry change or Fermi resonances present in TRA, it is notable that most of the extra transitions in TRA derive their intensity from ^1L_a rather than ^1L_b.[48] It will be important to carry out high-resolution spectroscopy on these vibronic transitions of SERO to see whether the observed bands are all ^1L_b. If they are, then the likely cause for the less congested spectrum in SERO is smaller vibronic coupling to ^1L_a arising from either a larger energy separation or smaller coupling matrix elements.

V. Conclusions

Single-conformation infrared and ultraviolet spectroscopy of jet-cooled serotonin has been used to provide firm conformational assignments for the eight observed conformations of neutral serotonin. These assignments build off the known assignments for TRA. Single-conformation infrared spectra of SERO in the alkyl CH stretch region are near-identical matches with their TRA counterparts, making it possible to deduce the conformational assignment for the ethylamine side chain by inspection based on the comparison.

While aspects of the ultraviolet spectrum bear a striking resemblance to those of its close analogue tryptamine, substantial differences are also observed. The ultraviolet spectrum divides into two subsets due to the two distinct OH orientations, *syn* and *anti*. Within each subset, the Gpy(out), Gpy(up), and Gph-(out) conformations dominate. The most dramatic change induced by the 5-OH substitution is the selective stabilization of the Gph(out)/*anti* conformation relative to all others. This stabilization has its most likely cause in electronic effects transmitted through the fused-ring system to the ethylamine side chain. A more detailed understanding of these effects must await proper theoretical treatment via NBO analysis.[40,41]

The results of the present work also point the way for a number of follow-up studies of significance. The present study of the conformational preferences of neutral serotonin begs the question of their relevance to the conformational preferences of serotonin under biologically relevant conditions. As was pointed out in the Introduction, at physiological pH, serotonin exists predominantly in its protonated rather than neutral form.[3,6] Nevertheless, given the large variety of biological functions played by serotonin, it likely explores a variety of environments ranging from fully solvent-exposed to hydrophobic in nature. While the former circumstances will favor the protonated form, the latter will shift equilibrium toward the neutral. It is likely that the flexibility of the ethylamine side chain and 5-OH groups, and the ability to take up or remove a proton, contributes to the versatility of serotonin's biological functions. Clearly, it will be important to complement the present studies with analogous work on the conformational preferences of protonated serotonin. Such studies are currently being pursued using infrared photodissociation in a cold trap.[50]

The present study of single-conformation spectroscopy also provides a basis for stimulated emission pumping population transfer spectroscopy of conformational isomerization in SERO.[20,22,26,51] The application of these methods to SERO will provide an intriguing comparison with the benchmark studies of TRA which have preceded it.[20,22] It should be possible to directly determine energy thresholds for isomerization between individual X → Y reactant−product conformer pairs in SERO and bracket the relative energies of minima from a combination of X → Y and Y → X threshold measurements. The threshold measurements will be particularly intriguing in SERO because isomerization pathways can involve isomerization within the 5-OH or 3-ethylamine side chains alone or in combination with one another.

Finally, it will be worth pursuing studies of the spectroscopy of $SERO(H_2O)_n$ clusters to understand the ways in which bound

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water molecules change the conformational preferences of neutral serotonin. Since the 5-OH group is a good H-bond donor and the NH_2 group a good H-bond acceptor, it seems likely that water molecule(s) will form bridges between the two, thereby locking in conformations that facilitate these bridges.

Acknowledgment. We gratefully acknowledge the National Science Foundation (Grant CHE-0551075) for support of this research. T.A.L. is grateful to the U.S. Department of Education for a Graduate Assistance in Areas of National Need Fellowship. We thank M. Schmitt and K. Kleinermanns for providing the results of their high-resolution spectroscopy of vibronic bands of TRA prior to publication.

Supporting Information Available: Full author list for ref 29. This material is available free of charge via the Internet at http://pubs.acs.org.

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