

Hydride stretch infrared spectra in the excited electronic states of indole and its derivatives: Direct evidence for the $^1\pi\sigma^*$ state

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The hydride stretch infrared spectra of indole, indole-H₂O, 3-methyl indole, 3-methyl indole-H₂O, the main conformer of tryptamine (TRA), two conformers of N-acetyl tryptophan amide (NATA), and three conformers of N-acetyl tryptophan methyl amide (NATMA), have been recorded in the electronically excited singlet states using excited-state fluorescence-dip infrared spectroscopy. NATA and NATMA are methyl-capped dipeptides of tryptophan that have conformational flexibility and exhibit sensitivity in their electronic spectra to the conformation of the dipeptide backbone. In the indole monomer, the indole NH stretch fundamental at the S_1 origin is shifted from its ground-state value (3525 cm⁻¹) to 3478 cm⁻¹. The corresponding band in the indole-H₂O complex appears at 3387 cm⁻¹, shifted by a similar amount from its ground-state position (3436 cm⁻¹). Higher vibronic levels within 1500 cm⁻¹ of the S_1 origin, which have been identified previously [B. J. Fender *et al.*, Chem. Phys. Lett. **239**, 31 (1995)] as being 1L_b or 1L_a in character, all show similar excited state indole NH stretch absorptions. The corresponding spectra in 3-methyl indole, 3-methyl indole-H₂O, TRA, and in the C5 conformers of NATA and NATMA all are missing the indole NH stretch absorption. In its place, a broad background absorption appears, spread over the entire 2800–3800 cm⁻¹ region. In these molecules, other CH stretch or amide NH stretch absorptions remain sharp, appearing in their expected frequency ranges. Finally, the C7 conformations of NATA and NATMA, which possess an intramolecular hydrogen bond in the dipeptide backbone, have all infrared transitions washed out, replaced by a stronger broad background absorption. The entire data set can be explained by the presence of an excited $^1\pi\sigma^*$ state which is dissociative along the indole NH stretch coordinate, as recently predicted by Sobolewski and Domcke [Chem. Phys. Lett. **315**, 293 (1999)]. In the weak coupling case (indole, indole-H₂O), the gap between the $^1\pi\sigma^*$ state and the S_1 origin is too large to be reached by infrared excitation. The selective loss of the indole NH stretch in the intermediate coupling case reflects the strong coupling of the 1L_b state NH stretch ($v=1$) level to the $^1\pi\sigma^*$ state, which is dissociative along the NH stretch coordinate. In the NATA and NATMA C7 conformers, an inversion of ordering of the electronic states occurs, pushing the 1L_a state below the 1L_b origin, and strengthening the coupling of all hydride stretch vibrational levels to the $^1\pi\sigma^*$ dissociative continuum. These results highlight the important influence of the conformation of the polypeptide backbone on the photophysics of tryptophan in polypeptides. © 2003 American Institute of Physics. [DOI: 10.1063/1.1536616]

I. INTRODUCTION

Tryptophan fluorescence is often used as an optical probe of the local structure and solvent accessibility in proteins.^{1,2} Its usefulness in this respect arises from several unique characteristics of tryptophan and its electronically excited states. First, tryptophan is responsible for the majority of the fluorescence from proteins. Second, tryptophan is a relatively uncommon amino acid, so that the observed fluorescence characteristics can be associated with particular tryptophan residues in the amino acid sequence. Third, as the largest aromatic side chain, the indole chromophore of tryptophan can be conveniently and selectively excited in the near-ultraviolet. Finally, both the emission wavelength and fluorescence time profile of tryptophan are unusually sensitive to the local environment of the indole chromophore.

The unfortunate corollary to the sensitivity of tryptophan's fluorescence to its local environment is that a proper interpretation of the observed fluorescence properties requires a firm knowledge of the molecular-scale properties of the local environment that lead to the observed changes. As a result, the biophysical literature is replete with studies of the photophysics and photochemistry of tryptophan and its derivatives under a wide range of solvent, pH, and temperature conditions, in the presence of quenchers of various kinds, in model polypeptide chains, and in the proteins themselves.¹⁻³

The model that has typically been used to explain much of these data focuses attention on two close-lying excited $^1\pi\pi^*$ states of the indole chromophore, historically labeled as 1L_b and 1L_a .^{1,4} In the indole monomer, the 1L_b state lies about 1400 cm⁻¹ below 1L_a ,⁵ and the S_0 - S_1 origin transition possesses a transition moment that is clearly ascribable to the 1L_b upper state.⁶ In indole derivatives, the splitting between the 1L_a and 1L_b states depends sensitively upon the

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position and type of substitution. The 3-position on indole is especially important, since it is here that the amino acid side chain connects to indole in tryptophan. In most instances, substitution at the C3 position reduces the splitting between 1L_a and 1L_b . For instance, in 3-methyl indole, the two states are nearly isoenergetic.⁴

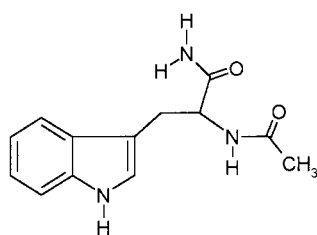
One of the important consequences of the presence of these two states is the sensitivity of the emission properties of tryptophan and indole derivatives to their solvent environment. The 1L_a state has a dipole moment several debye larger than either S_0 or 1L_b , and hence, in polar environments the 1L_a state is selectively stabilized relative to 1L_b . In circumstances where the two states are very close in energy, the coupling between the two states produces a redshift in the emission and a shortening in the fluorescence lifetime. Callis and co-workers have recently identified unique bands in the emission spectrum of 3-methyl indole ascribable to 1L_a character, and have used these bands, in conjunction with polarized two-photon fluorescence studies, to map out the degree of 1L_a - 1L_b mixing in a series of 3-methyl indole-solvent complexes with increasing solvent proton affinity.⁴

While this two-state picture has provided a coherent explanation of much of the observed photophysics of indole, tryptophan, and other indole derivatives, there are other pieces of evidence that point to the presence of one or more additional excited states that also play a role. In order to explain the anomalous increase in nonradiative decay of gas-phase indole with excess energy above the 1L_b origin, Glasser and Lami proposed that the 1L_a state undergoes efficient N-H bond fission under collision-free conditions.⁷ Wallace and co-workers carried out fluorescence lifetime measurements on a large number of indole derivatives and solvent-containing complexes in the gas phase.⁸ These stud-

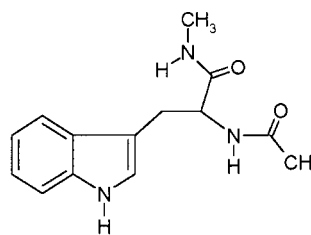
ies showed a surprising sensitivity of the fluorescence lifetime to deuterium substitution at the indole N-H position. These authors put forward a model in which 1L_a - 1L_b coupling mediated coupling to a dissociative state of unknown source. Finally, Jouvét and co-workers have recently probed the photochemistry of UV-excited indole-(NH₃)_n clusters, and have identified NH₄-(NH₃)_m photoproducts associated with an intracuster hydrogen atom transfer reaction.⁹

Recently, theoretical support for the existence of a third, dissociative excited state has come from the complete active space self-consistent field (CASSCF) calculations of Sobolewski and Domcke on indole.^{10,11} A $^1\pi\sigma^*$ excited state is calculated to lie about 0.5 eV above S_1 in the Franck-Condon region of indole. This state is calculated to be dissociative along the NH coordinate. It has an extremely large dipole moment in the Franck-Condon region (11.0 D), suggesting that this state may be even more sensitive to its environment than 1L_a , and could be pulled down into the region of the S_1 origin under certain circumstances. However, there is little direct experimental evidence for this state yet, apart from the recent photochemical study of Jouvét and co-workers.⁹ In particular, little is known about its spectroscopy or about its effects on the photophysical properties of the 1L_b and/or 1L_a states. Like the 1L_a state, one anticipates that the $^1\pi\sigma^*$ state will be sensitive to its solvent environment (particularly when bound to the solvent at the indole NH), to the nature of the substituent in indole derivatives, and potentially to the conformation in cases where the substitution involves a group with conformational flexibility.

As part of our ongoing study of the spectroscopy of flexible biomolecules, we have recently studied the ultraviolet and infrared spectroscopy of N-acetyl tryptophan amide (NATA) and N-acetyl tryptophan methyl amide (NATMA), shown below.¹²



N-acetyl tryptophan amide (NATA)



N-acetyl tryptophan methyl amide (NATMA)

These two molecules are dipeptides capped by either one (NATA) or two (NATMA) methyl groups. As such, they faithfully mimic the local polypeptide backbone of tryptophan in proteins. The low-energy conformers of NATA and NATMA fall into one of two families, labeled C5 and C7. The C5 structures contain an extended dipeptide backbone with no intramolecular H bond, while the C7 structures have a H bond joining the ψ -amide NH and the ϕ -amide carbonyl groups to form a seven-membered ring. Building on earlier work by Tubergen *et al.* on NATA,¹³ we have applied the

double-resonance methods¹⁴ of fluorescence-dip infrared spectroscopy and UV-UV hole-burning spectroscopy to determine the infrared and ultraviolet spectra of single conformations of these molecules.¹² Figure 1 shows the LIF excitation and UV-UV hole-burning spectra of NATA, while the corresponding spectra of NATMA are shown in Fig. 2. The hole-burning scans prove that all contributions to the LIF spectrum of NATA can be accounted for by two conformers, one of C5 type and one C7 conformer. In NATMA, there are two C5 conformers and one C7 conformer contributing to the

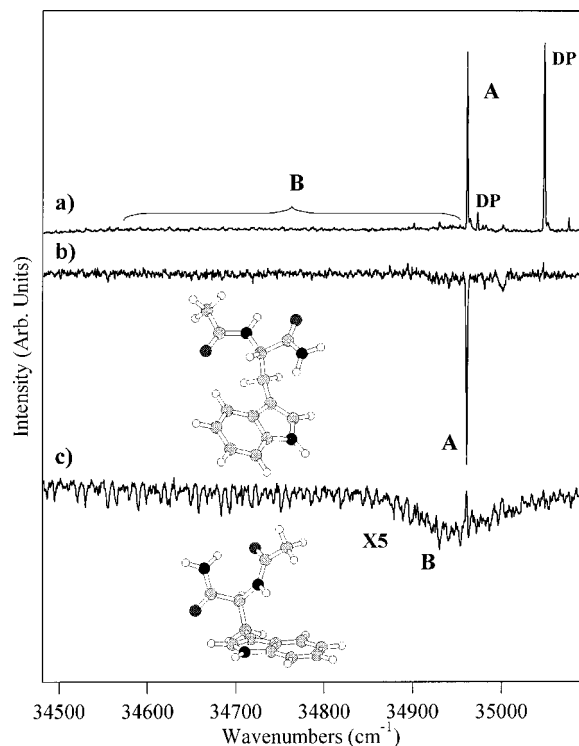


FIG. 1. (a) Laser-induced fluorescence excitation spectrum of N-acetyl tryptophan amide (NATA) in the region near the S_1-S_0 origin. The transition marked DP is due to a decomposition product (Ref. 12). (b) and (c): UV-UV hole-burning spectra of the C5 and C7_{eq} conformers of NATA assigned in Ref. 12 to the C5(AP) and C7_{eq}(Φ P) conformers, respectively, which are shown as insets.

spectrum. The assigned structures are shown as insets in Fig. 1 and 2. The intriguing result of this work is that in both molecules, the C7 conformers have LIF spectra that are very different from their C5 counterparts. The C5 spectra are sharp, with short Franck-Condon progressions involving one or more low-frequency vibrations. However, the C7 spectra exhibit a very dense and extended vibronic structure that appears essentially as a broad background to the sharp C5 transitions. As the figures show, the spectra of the C7 conformers have intensity maxima in the same region as the C5 S_1-S_0 origins. However, the spectra extend well below this origin region, suggesting the presence of another excited state. Based on the electronic- and ground-state vibrational spectroscopy, it is not clear whether this other state is the 1L_a state, the recently identified $^1\pi\sigma^*$ state, or some other state of as yet unidentified origin. The unmistakable conclusion of that work was that there is a dramatic effect of the conformation of the dipeptide backbone on the electronic spectroscopy of these molecules, pointing to the need for further studies of these excited states.¹²

In this paper, we report the results of a study whose goal is to further characterize the excited states of indole and its derivatives. The main contributions of the present work are the hydride stretch infrared spectra of these molecules in their excited electronic states. The double-resonance scheme used for this purpose involves pumping single vibronic level features in the ultraviolet spectrum with a pulsed ultraviolet laser, and then exciting out of these levels with an infrared

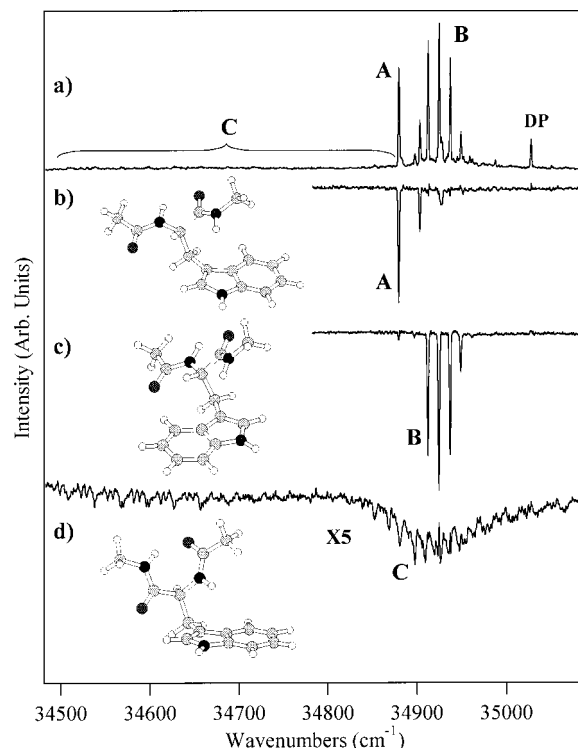


FIG. 2. (a) Laser-induced fluorescence excitation spectrum of N-acetyl tryptophan methyl amide (NATMA) in the region near the S_1-S_0 origin. The transition marked DP is due to a decomposition product (Ref. 12). (b)–(d): UV-UV hole-burning spectra of the two C5 and one C7_{eq} conformer of NATMA assigned in Ref. 12 to the C5(A Φ), C5(AP), and C7_{eq}(Φ P) conformers, respectively, which are shown as insets.

photon while the population is in the excited electronic state.¹⁵ The vibrational states reached by the infrared excitation are several thousand wave numbers above the electronic origin, and thus experience fast, nonradiative pathways that quench their fluorescence. It is thereby possible to detect the infrared absorption by the loss of fluorescence that accompanies infrared excitation. In so doing, we probe both the nature of the vibronic level out of which we are absorbing, and the nature of the states accessed by the infrared radiation, some 3500 cm⁻¹ higher in energy.

We have chosen to study a series of indole derivatives that is known to or suspected to vary the relative energies of the 1L_b , 1L_a , and (presumably) $^1\pi\sigma^*$ states. A starting point for our work is the indole monomer itself, with its S_1 origin that is known to be 1L_b in character based on the transition moment direction.¹⁶ Several vibronic transitions above the origin have been identified as having substantial 1L_a character, and we probe the infrared spectroscopy out of these levels as well. The indole-water complex has also been studied, building on the extensive body of work on this complex, including rotationally resolved ultraviolet spectroscopy⁶ and ground-state fluorescence-dip infrared spectroscopy.¹⁷ The water molecule in the indole-water complex is known to bind to indole as a H-bond acceptor at the indole NH. The indole derivatives that have been studied by excited state FDIR spectroscopy are all substituted at the 3-position on the ring, which is known to decrease the gap between 1L_b and 1L_a states. These include 3-methyl indole

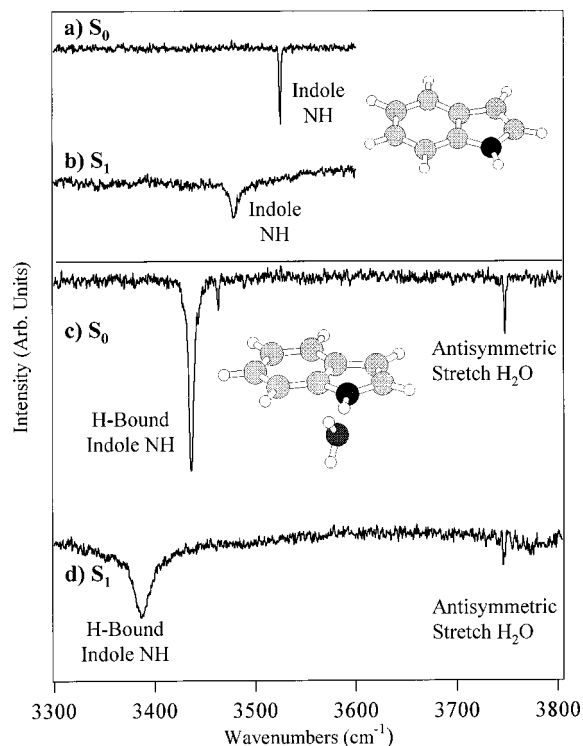


FIG. 3. (a) Ground-state and (b) excited-state fluorescence-dip infrared (FDIR) spectra of the indole monomer. (c) and (d): Analogous spectra of the indole-H₂O complex. The NH stretch fundamental of the indole monomer appears at 3478 cm⁻¹ in the S₁ state.

(3MI) monomer, the 3MI-water complex, the most stable conformer of tryptamine (TRA), and all the conformers of NATA and NATMA.

As we shall see, the infrared spectra provide striking, direct evidence for the existence of the $^1\pi\sigma^*$ state. In the most dramatic cases, the indole NH stretch fundamental is missing from the spectrum, even though other hydride stretch fundamentals in the same frequency region remain sharp and strong. In its place, a broad background absorption appears. Both these characteristics are ascribable to the $^1\pi\sigma^*$ state, to which the indole NH stretch fundamental is strongly coupled.

II. EXPERIMENT

The excited state FDIR spectra were recorded on a supersonic free-jet laser-induced fluorescence apparatus that has been described previously.¹⁸ The molecules of interest were entrained in several bar of helium by heating samples of the molecules to temperatures ranging from 25 °C (indole) to 150 °C (NATA and NATMA). A pulsed valve (0.8 mm diameter, General Valve) was used to produce the supersonic free jet. The cold molecules and molecular complexes are probed 6–10 nozzle diameters downstream using LIF detection.

Ground-state fluorescence-dip infrared (FDIR) spectra were often recorded for comparison with the excited-state spectra under identical conditions. In that case, the IR laser was fired about 150 ns before the UV probe. The difference

in fluorescence signal without and with the IR present was recorded in real time using active baseline subtraction on a gated integrator.

Several years ago, Huber and co-workers¹⁵ demonstrated the excited-state analog of fluorescence-dip infrared spectroscopy (FDIRS) on propynal. In excited-state FDIRS, the timing between UV and IR pulses is changed from that used for ground-state FDIRS simply by delaying the IR so that it occurs *after* rather than *before* ultraviolet excitation. If the infrared excitation occurs while population remains in the excited vibronic level, it promotes population out of the fluorescing level to vibrationally excited S_n(*v*) levels with much smaller fluorescence quantum yields, resulting in a depletion in the tail of the fluorescence decay signal. By gating the fluorescence detection on this trailing component of the fluorescence signal, an excited-state FDIR spectrum can be obtained. A prerequisite for the method is that the light sources used for the electronic excitation and infrared probe steps be short-lived by comparison to the excited-state lifetime, so that population can be promoted to the vibronic level of interest and probed prior to loss of the population from the excited state. All the indole derivatives of interest here have vibronic levels with fluorescence lifetimes of about 10 ns. Typical delays between UV and IR lasers were 5–10 ns, with a similar delay between the IR pulse and the beginning of the 10 ns gate used to detect the fluorescence signal.

III. RESULTS

A. Indole and indole-H₂O

The excited-state FDIR spectra of the indole monomer at the S₁ origin is shown in Fig. 3(b), while the corresponding ground-state FDIR spectrum is shown in Fig. 3(a) for comparison. The indole NH stretch fundamental appears at 3478 cm⁻¹ in the S₁ state, corresponding to a 47 cm⁻¹ shift down in frequency from its position in S₀ (3525 cm⁻¹).¹⁷ This modest decrease in frequency reflects the effect of the $\pi-\pi^*$ electronic excitation on the NH group. The transition is somewhat broadened relative to the ground-state transition, but the integrated intensity of the band is comparable to that transition.

One of the powerful capabilities of the excited-state FDIR spectroscopy is that it can be applied to any excited vibronic level with sufficiently long lifetime. In particular, Fender *et al.*⁵ have identified several vibronic bands above the S₁ origin that are of 1L_a character, and we can probe whether the fractional 1L_a character influences the appearance of the infrared spectrum. We have recorded excited-state FDIR spectra out of levels 479, 736, 988, and 1414 cm⁻¹ above the origin. Of these, the 736 and 1414 cm⁻¹ bands are most telling, since they represent strong bands that are predominantly 1L_b and 1L_a in character, respectively. The 736 cm⁻¹ band is a strong vibronic band built off the 1L_b origin, while the 1414 cm⁻¹ band is part of a clump of bands identifying the location of the 1L_a origin. These levels are sufficiently long-lived that excited-state FDIR spectra can be recorded, although at somewhat poorer signal-to-noise ratio than at the origin. Spectra out of the 736 and 1414 cm⁻¹ bands [Figs. 4(b) and 4(c), respectively] are compared with

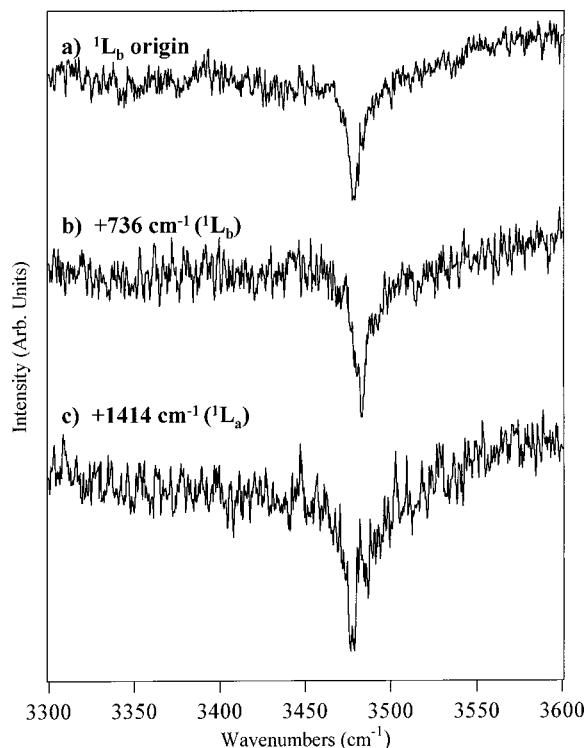


FIG. 4. Excited-state FDIR spectra out of single vibronic levels of the indole monomer at the (a) $S_1(^1L_b)$ origin; (b) the level 736 cm^{-1} above the S_1 origin, previously assigned (Ref. 5) as having predominant 1L_b character, and (c) the level 1414 cm^{-1} above the S_1 origin, previously assigned as predominantly 1L_a in character. The IR spectra focus on the region of the indole NH stretch fundamental.

the origin spectrum of Fig. 4(a). In all cases, the indole NH stretch fundamental is clearly observed, with a breadth and integrated intensity comparable to the origin. In all cases, it appears at a frequency within a few wave numbers of its position at the $S_1(^1L_b)$ origin. The IR spectrum out of the vibronic level 1414 cm^{-1} above the S_1 origin [Fig. 4(c)] does show a shoulder on the high-wave number side of the band, which might be indicative of the 1L_a character of this level.

The corresponding S_0 - and S_1 -state spectra for the indole- H_2O complex out of the S_1 origin are shown in Figs. 3(c) and 3(d), respectively. The ground-state FDIR spectrum has been reported previously, and has been used to determine the H-bonding structure of the complex, which is shown as an inset in the figure.¹⁷ The indole NH acts as a H-bond donor to the water molecule ($\text{NH}\cdots\text{OH}_2$), producing a shift in the indole NH stretch from 3525 to 3436 cm^{-1} , a substantial increase in intensity, and some broadening, all of which are signatures of an $\text{NH}\cdots\text{OH}_2$ H bond. In addition, the two OH stretch fundamentals of the water molecule appear within a few wave numbers of their positions in the H_2O monomer. Only the antisymmetric stretch fundamental is clearly observed in the FDIR spectra of Fig. 3(c), since it has an intensity several times that of the symmetric stretch. The high-resolution ultraviolet spectra of Korter *et al.* have provided a detailed structure for this complex, including the direction of the transition moment, proving that the S_1 origin of the complex is of 1L_b character, as it is in the monomer.⁶

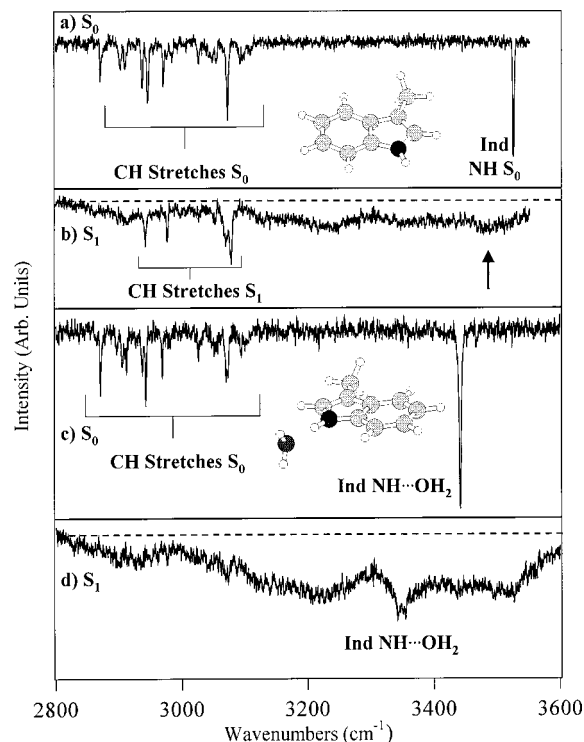


FIG. 5. (a) Ground-state and (b) excited-state fluorescence-dip infrared (FDIR) spectra of the 3-methyl indole (3MI) monomer following excitation to its S_1 zero-point level. (c) and (d): Analogous spectra of the 3MI- H_2O complex. Note that the indole NH stretch fundamental is missing from both spectra and is replaced by a broad absorption that extends throughout the region scanned in the infrared. The dashed lines indicate the baseline of the S_1 spectra. The arrow indicates where the indole-NH stretch is expected to appear based on the S_1 spectrum of indole (3478 cm^{-1}).

The S_1 -state spectrum of indole-water mirrors the same changes with electronic excitation that occurred in the indole monomer. The indole NH stretch fundamental is shifted from its ground-state frequency by -49 cm^{-1} , and spreads out its increased intensity over a somewhat broadened band.

In summary, the indole monomer and indole- H_2O complexes have indole NH stretch transitions that are in no way unusual, reflecting the modest changes associated with the $\pi-\pi^*$ excitation, and experiencing some broadening, but otherwise appearing much as one would expect based on past experience with other infrared spectra in excited electronic states.

B. 3-methyl indole and 3-methyl indole- H_2O

3-methyl indole and its solvent-containing complexes constitute first examples of the effects of substitution at the 3-position on the indole ring. 3MI has an S_1-S_0 origin that has recently been determined to be primarily 1L_b in character,¹⁹ although the $^1L_a-^1L_b$ gap is smaller than in indole.⁴ Figure 5(a) presents the ground-state FDIR spectrum of 3MI monomer. In the ground state, the indole NH stretch fundamental appears at 3526 cm^{-1} , almost unchanged from its value in indole monomer. The CH stretch region is composed of aromatic CH stretches between 3000 and 3100 cm^{-1} and the methyl CH stretch transitions in the $2850-3000\text{ cm}^{-1}$ region.

The corresponding spectrum out of the S_1 origin shown in Fig. 5(b) is dramatically different. The most obvious change is that the indole NH stretch fundamental is simply missing from the spectrum. We anticipate it to appear near its position in the S_1 -state indole monomer (3478 cm^{-1}), and based on the signal-to-noise ratio in the ground-state spectrum, there should be no difficulty in observing it if it is present. The disappearance of the indole NH stretch fundamental is accompanied by the appearance of a broad background absorption that spans the entire wave number region we have scanned ($3300\text{--}3800\text{ cm}^{-1}$). There is lumpiness to this background, but these may reflect changes in the infrared power of our parametric converter rather than real changes in the absorption intensity. There is no sign of a drop-off in intensity in this background near 3800 cm^{-1} , even though we are several hundred wave numbers above the highest frequency vibration in the molecule.

Despite these unusual aspects of the spectrum, the aromatic and alkyl CH stretch fundamentals remain sharp and are just as intense as in the ground state. This provides convincing evidence that the disappearance of the indole NH stretch fundamental is not an artifact of the conditions used in taking the spectrum. Rather, there is some mode-specific effect that appears to affect the indole NH stretch fundamental, but not the aromatic or alkyl CH stretch vibrations.

The corresponding spectra of the $3\text{MI-H}_2\text{O}$ complex are shown in Fig. 5(c) (ground state) and 5(d) (S_1 state). As with previous examples, the juxtaposition of the two spectra facilitates the direct comparison of the effects of electronic excitation on $3\text{MI-H}_2\text{O}$. The recent study of Short *et al.*⁴ deduced that the S_1 origin of $3\text{MI-H}_2\text{O}$ is of mixed $^1L_a\text{--}^1L_b$ character. The ground-state spectrum possesses a strong, sharp indole NH stretch fundamental with a frequency shift and intensity increase consistent with water binding at the indole NH site, as it is in indole- H_2O . The S_1 -state spectrum of the $3\text{MI-H}_2\text{O}$ complex shown in Fig. 5(d) shows a further degradation of the hydride stretch infrared spectrum in favor of an increased broad background. The remnant of the indole NH stretch may be present near 3340 cm^{-1} , as indicated in the spectrum, but if so, it is just barely recognizable in the spectrum. Furthermore, in the spectrum of Fig. 5(d), the CH stretch fundamentals are now also missing from the spectrum. The drop-off in the intensity of the background absorption near 3600 cm^{-1} is an artifact of the decreased infrared power at the chamber due to absorption of water vapor in the air.

C. The lowest-energy conformer of tryptamine

The ethylamine side chain of tryptamine produces several low-lying conformers that differ in the configuration of this side chain relative to the indole ring. Previous studies of tryptamine (TRA) have identified $S_1\text{--}S_0$ origin transitions due to seven conformations, leading to assignments of all seven.²⁰ The ultraviolet spectra of all seven species are sharp, and extend at least 1000 cm^{-1} above the origin with little loss in intensity or apparent broadening. The lowest energy conformer of TRA is shown as an inset to Fig. 6. As with all the conformers of TRA, the ethylamine side chain is roughly perpendicular to the indole ring. The lowest energy con-

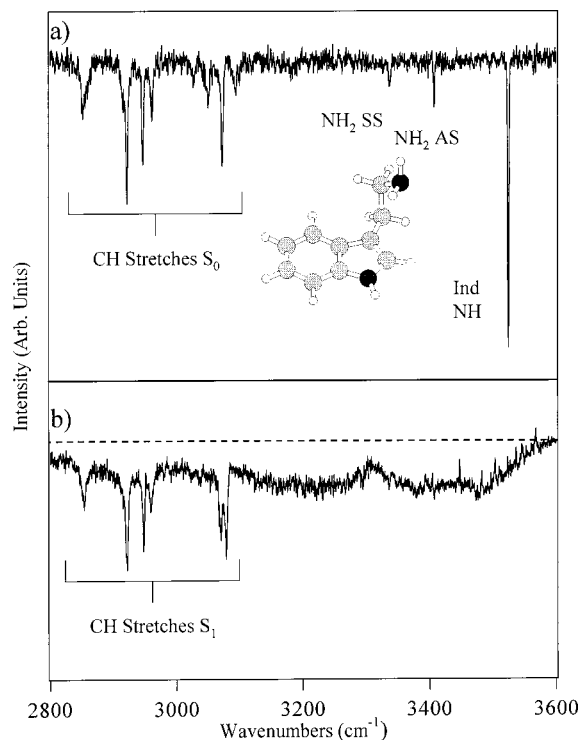


FIG. 6. (a) Ground-state and (b) excited-state fluorescence-dip infrared (FDIR) spectra of the most abundant conformer of tryptamine following excitation to its S_1 zero-point level. The conformation of tryptamine is shown as an inset. Note that the indole NH stretch fundamental is missing from both spectra, while the aromatic and alkyl CH stretch fundamentals remain sharp. The dashed lines indicate the baseline of the S_1 spectra. The NH stretch fundamental in the S_1 state is expected to appear at 3478 cm^{-1} , its position in the indole monomer.

former has the amino substituent in the *gauche* position on the pyrrole side of indole. The amino group is oriented so that the lone pair of the amino group points out away from the ring.

The infrared spectra of the lowest-energy conformer of TRA in its ground state and out of its S_1 -state zero-point level are shown in Figs. 6(a) and 6(b), respectively. The ground-state spectrum shows the indole NH stretch at an excellent signal-to-noise ratio commensurate with its strong fluorescence signal. The weak symmetric and antisymmetric stretch transitions of the NH_2 group are also observable, as are the aromatic CH stretches due to the indole ring and the alkyl CH stretches due to the ethylamine group.

The S_1 -state spectrum shares much in common with that of the 3MI monomer. Like 3MI , the spectrum of TRA(A) shows no remnant of the indole NH stretch, but instead possesses the broad background that covers the entire hydride stretch region. The amino NH stretch modes are so weak that it is hard to judge clearly whether they are present. However, the aromatic and alkyl CH stretch fundamentals are clearly observed and are sharp, providing another case of mode-selective loss of the indole NH stretch fundamental. As in 3MI , the decrease in the broad background near 3600 cm^{-1} is due to a decrease in infrared power accompanying absorption in this frequency region by water vapor in the air.

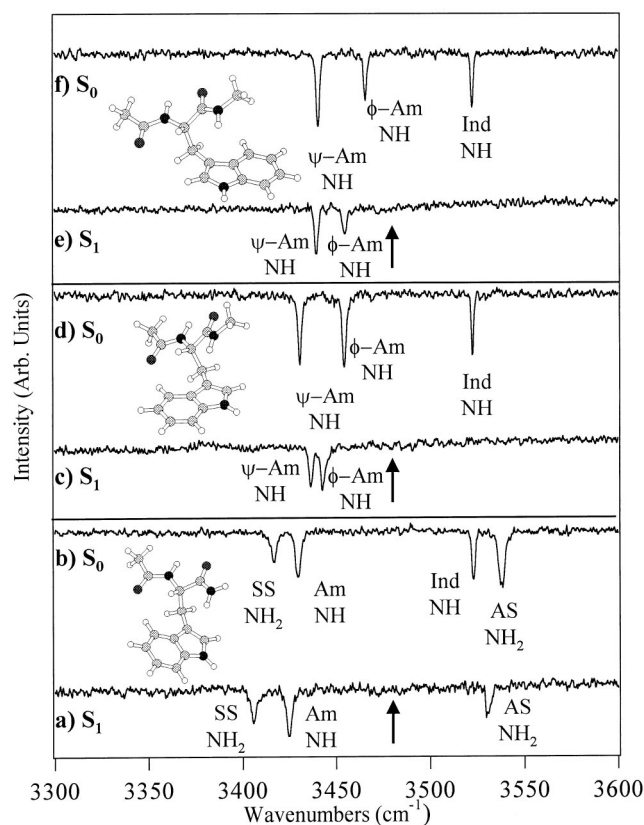


FIG. 7. Ground-state and excited-state fluorescence-dip infrared (FDIR) spectra of (a), (b) the C5(AP) conformer of NATA; (c), (d) the C5(AP) conformer of NATMA; and (e), (f) the C5(AΦ) conformer of NATMA. In all cases, there is mode-selective loss of the indole NH stretch fundamental in the S_1 spectra, while the other hydride stretch fundamentals are observable and sharp. The arrows indicate the expected position of the S_1 indole NH stretch fundamental (3478 cm^{-1}).

D. NATA and NATMA C5 conformers

The ground-state and excited-state FDIR spectra of the C5 conformers of NATA are shown in Figs. 7(a) and 7(b), respectively. Based on a comparison of the ground-state spectrum with calculated vibrational frequencies and infrared intensities, the assignment of the conformer responsible for this spectrum is firmly established as a C5 conformer, with its extended dipeptide backbone.¹² More specifically, the body of evidence pointed toward the C5(AP) conformer shown in the inset of the figure as the C5 conformer observed. The assignments of the amide NH stretch fundamental (Am–NH), the amino symmetric (NH_2 SS) and antisymmetric stretch fundamentals (NH_2 SS and AS), and the indole NH stretch are given in the figure. Once again, the indole NH stretch fundamental that is expected to appear at 3478 cm^{-1} (based on indole monomer) is missing from the excited-state spectrum, while all the other NH and CH stretch structure remains sharp and easily observed.

The analogous spectra of the two C5 conformers [C5(AP) and C5(AΦ)] of NATMA are shown in Figs. 7(c)–(f). In every case, the excited state spectra are missing the indole NH stretch fundamental, which is replaced by a broad background that spreads over the entire frequency range of the scans ($2800\text{--}3600\text{ cm}^{-1}$).

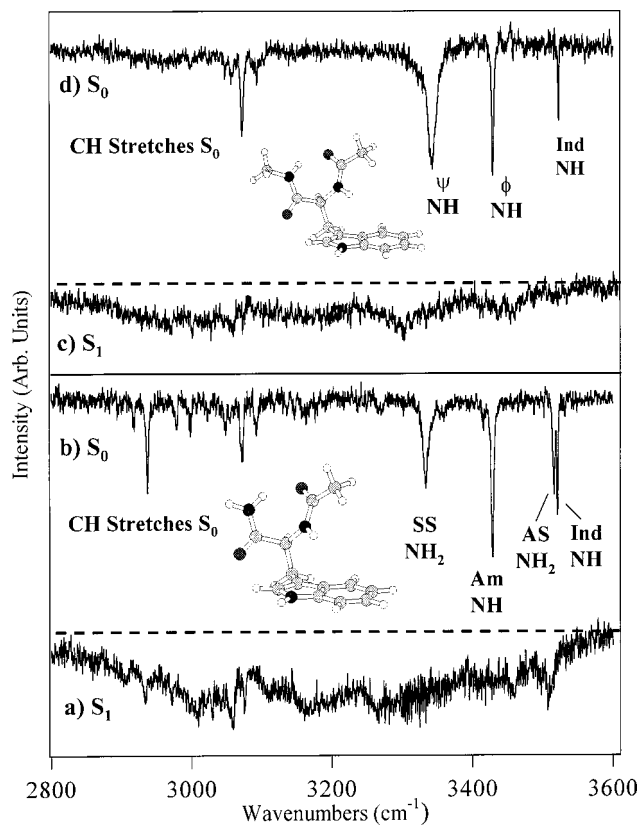


FIG. 8. Ground-state and excited-state fluorescence-dip infrared (FDIR) spectra of (a), (b) the $C7_{\text{eq}}(\Phi\text{P})$ conformer of NATA and (c), (d) the $C7_{\text{eq}}(\Phi\text{P})$ conformer of NATMA. The dashed lines indicate the baseline of the S_1 spectra.

E. NATA and NATMA $C7_{\text{eq}}$ conformers

Figure 8 contrasts the ground-state FDIR spectra of the $C7_{\text{eq}}(\Phi\text{P})$ conformers of NATA and NATMA [Figs. 8(a) and 8(c), respectively] with their excited-state counterparts [Figs. 8(b) and 8(d)]. In these conformers, all of the sharp infrared transitions are washed out, and are replaced by a broad background absorption that is somewhat more intense than in the C5 spectra of Fig. 7. Remnants of the vibrational transitions are apparent in the spectra, but the mode-selective loss of the indole NH stretch has now become a nearly complete loss of vibrational structure in these $C7_{\text{eq}}$ conformers. Clearly, the same conformation specificity that was the trademark of the $C7_{\text{eq}}$ conformers in their electronic spectroscopy [Figs. 1(c) and 2(d)] has carried over to their excited state infrared spectra as well.

IV. DISCUSSION

The excited-state FDIR spectra presented in this work were pursued out of a desire to understand and explain the unusual electronic spectroscopy of the C7 conformers of NATA and NATMA. The long tail present in the ultraviolet spectra of the C7 conformers, which extends well below the 1L_b origin of the C5 conformers [Figs. 1(c) and 2(d)], points to the presence of a second excited state that is brought down into the region of the 1L_b state when the dipeptide side chain adopts a C7 conformation. Until recently, the sole candidate for the state responsible for this behavior would have been

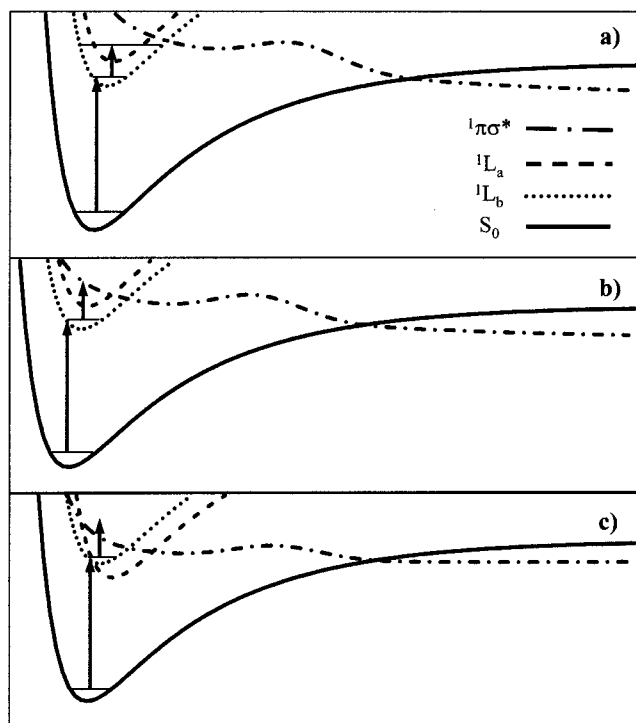


FIG. 9. Schematic potential energy curves illustrating the three categories of behavior observed in the present work, involving (a) a large separation/weak coupling between the 1L_b and $^1\pi\sigma^*$ states; (b) intermediate separation/mode-selective coupling; and (c) an inverted energy ordering/strong coupling. Arrows indicate UV excitation out of S_0 (long arrows) and IR excitation in the excited state (short arrows). See the text for further discussion.

the 1L_a state. However, the recent calculations of Sobolewski and Domcke^{10,11} have identified another excited state, the $^1\pi\sigma^*$ state, that is calculated to be nearby in energy and to have many attributes that make it a reasonable alternative candidate for the interacting state responsible for the unusual electronic spectroscopy of the C7 conformers. In particular, the σ^* molecular orbital accessed by a transition to this state is a Rydberg-type orbital localized outside the hydrogen atom of the indole NH group. As a result, in the region of vertical excitation from the ground state, the dipole moment of this state is very large (11.0 D), due to the effective transfer of an electron from the π cloud to this region outside the hydrogen atom. As the schematic potential energy curves shown in Fig. 9(a) portray, the $^1\pi\sigma^*$ state is dissociative along the indole NH coordinate, with electron transfer leading to proton transfer as the proton “follows” the electron away from the ring. According to Sobolewski and Domcke, a barrier exists on the S_1 surface due to the conical intersection of the S_1 and $^1\pi\sigma^*$ states.¹¹ At large N–H distances, the $^1\pi\sigma^*$ state is calculated to cross the ground state via a second conical intersection, providing a route for return to the ground-state surface.

The excited-state FDIR spectra reported in this work have provided a direct spectroscopic probe of the make-up of the vibronic states involved in the infrared transition in the excited state. The potential energy curves of Fig. 9 provide a unified explanation of the observed infrared and ultraviolet spectra of all species studied, which fall into one of three broad categories. These potential energy curves are adapted

from the calculations of Sobolewski and Domcke.^{10,11} The most important difference between the three sets of curves is the relative separation between the 1L_b state and the $^1L_a/^1\pi\sigma^*$ states, which varies with the nature of the substitution, complexation, or conformation of the indole derivative. The separation is large in Fig. 9(a), intermediate in Fig. 9(b), and small or inverted in Fig. 9(c).

A. Large separation/weak coupling to the $^1\pi\sigma^*$ state

The indole monomer and the indole–water complex are examples of species with a large 1L_b – $^1\pi\sigma^*$ energy separation [Fig. 9(a)], in which the $^1\pi\sigma^*$ state is well above the 1L_b origin. In this case, both the ultraviolet and infrared spectroscopy can be understood in terms of the 1L_b and 1L_a states without reference to the $^1\pi\sigma^*$ state. The S_1 origin of both the indole monomer and the indole–water complex has been proven to be 1L_b in character.¹⁶ Above this origin, the fluorescence quantum yield remains quite high for several thousand wave numbers, and individual vibronic transitions remain sharp. Vibronic transitions ascribable to the 1L_a state have been clearly identified in previous studies,⁵ and these transitions are also sharp and comparatively sparse in the first several hundred wave numbers above the 1L_a origin.

Infrared excitation out of the $S_1(^1L_b)$ zero-point level produces an indole NH stretch fundamental that reflects a transition localized within the $^1\pi\pi^*$ excited state, exhibiting a modest frequency shift (-48 cm^{-1}) from its position in the ground electronic state, and otherwise retaining a transition strength and breadth that make it easy to detect and identify in the infrared spectrum. We suggest, therefore, that in indole and indole–water, the NH($\nu=1$) level of the 1L_b state is still somewhat below the $^1\pi\sigma^*$ dissociative state, as shown in Fig. 9(a). Similarly, when the infrared spectra of vibronic levels above the S_1 origin are probed in the ultraviolet, the indole NH stretch fundamental undergoes only minor changes in frequency, intensity, and breadth, independent of whether the vibronic level that serves as lower level in the infrared transition is predominantly 1L_a or 1L_b in character. This demonstrates that both $\pi\pi^*$ states (1L_a and 1L_b) have similar indole NH stretch fundamentals, and neither has any apparent coupling to the $^1\pi\sigma^*$ state when the separation between them is large.

B. Intermediate separation/mode-specific coupling

The majority of the molecules studied in this work (3MI, TRA, and the C5 conformers of NATA and NATMA) belong to the second category, an intermediate case in which the 1L_b state remains the S_1 state, but the 1L_b – $^1\pi\sigma^*$ energy gap can be spanned by a single infrared photon in the hydride stretch region, as shown in Fig. 9(b). Substitution at the 3-position on the indole ring pulls down both the 1L_a state and the $^1\pi\sigma^*$ state relative to 1L_b , but retains the energy ordering from indole. Under these circumstances, the S_0 – S_1 electronic spectroscopy in the origin region can still be understood as a $\pi\pi^*$ transition to the 1L_b upper state. The ultraviolet spectrum is still sharp, and the majority of the vibronic structure mimics the strong Franck–Condon progressions present in indole. The potential presence of another excited

state is most evident in the sharp drop in fluorescence quantum yield that occurs reasonably close to the electronic origin. The energy at which this occurs varies from one derivative to the next, and is most obvious in the NATA and NATMA C5 cases, where the LIF spectrum drops off in intensity within a few hundred wave numbers of the electronic origin.¹²

While the $^1\pi\sigma^*$ state has only an indirect effect on the electronic spectroscopy of these intermediate case molecules, the infrared spectroscopy reflects its presence very directly and in a highly mode-specific fashion. Now, the infrared photon reaches up into the region where the $^1\pi\sigma^*$ state is present. Since the NH stretch vibration is essentially along the reaction coordinate for dissociation via the $^1\pi\sigma^*$ state, the indole NH($v=1$) level of the 1L_b state is strongly coupled to the $^1\pi\sigma^*$ dissociative continuum. This leads to a selective disappearance of the indole NH($v=1$) level of the 1L_b state, and its replacement by a broad absorption which we interpret as an electronic absorption to the dissociation continuum of the $^1\pi\sigma^*$ state. At the same time, other hydride stretch fundamentals in the same energy region, most notably the amide NH($v=1$) level of the NATA and NATMA C5 conformers and the aromatic or alkyl CH stretches of all the molecules in this category, remain sharp and are readily detected on top of the broad continuum. These vibrations, which are spatially far removed from the indole N–H bond where dissociation occurs, are not strongly coupled to this coordinate, and hence survive in the infrared spectrum, despite having sufficient energy to reach the dissociation continuum. This is a remarkable example of vibrationally mode-specific effects in electronic coupling, here to the dissociative $^1\pi\sigma^*$ state.

C. Inverted ordering/strong coupling

Finally, the C7 conformers of NATA and NATMA belong to a third category, in which the interfering state is brought down below the 1L_b origin, and is strongly coupled to it. Very dense, long progressions are observed in the LIF spectra of the C7 conformers, with a “lump” of intensity between 34 900 and 35 000 cm^{-1} in Figs. 1(c) and 2(d), right in the region where the 1L_b origin would be in the absence of the interfering state. The long tail in the spectrum that extends well below this 1L_b origin [Figs. 1(c) and 2(d)] is then ascribed to the interfering state itself, whose origin is several hundred wave numbers below the 1L_b origin.

Two scenarios are possible. First, it may be that this interfering state is the $^1\pi\sigma^*$ state itself. This is suggested by the fact that the excited-state FDIR spectra of the C7 conformers have the last remnants of their vibrational transitions washed out, including the amide NH and CH stretch fundamentals. This is indicative of strong coupling to the dissociative continuum of the $^1\pi\sigma^*$ state, as might occur if the vibronic states acting as the platform for infrared excitation were already of mixed $^1L_b/^1\pi\sigma^*$ character. The $^1\pi\sigma^*$ state, with its extremely large dipole moment (11.0 D),¹⁰ would be expected to cause a significant geometry change in the dipeptide backbone upon electronic excitation from the ground state, producing the long progressions in low-frequency

modes, with the accompanying congestion in the LIF spectrum.

One difficulty with this scenario is that the coupling to the $^1\pi\sigma^*$ state would be expected to change the fluorescence lifetime and quantum yield substantially. The low oscillator strength (3×10^{-5}) of the transition to $^1\pi\sigma^*$ would lengthen the lifetimes of the mixed $^1\pi\sigma^*/^1L_b$ levels,¹⁰ while coupling to the dissociative continuum would shorten it. However, the C7 conformers have excited-state lifetimes that are similar to those of the C5 conformers (11 ± 2 ns for C7 compared to 15 ± 2 ns for C5), still sufficiently long to support excited-state FDIR spectroscopy. Furthermore, the fluorescence quantum yield near the 1L_b origin still must be reasonably good for detection of the C7 conformers to occur despite the dilution of the oscillator strength over so many transitions. As shown in Fig. 9(c), it is possible that the low-energy region of the $^1\pi\sigma^*$ state accessed by UV excitation from the S_0 state is stable with respect to loss of the indole NH group, due to a small barrier to dissociation or weak coupling to the dissociative continuum in this region where the two states originate. Sobolewski and Domcke have ascribed such a barrier to the conical intersection associated with the $^1\pi\pi^*-^1\pi\sigma^*$ crossing.¹¹ As with the C5 conformers, this metastability would likely be overcome not far above the 1L_b origin region, accounting for the observed drop-off in fluorescence quantum yield that indicates that the strength of coupling to the continuum has increased at these higher energies, perhaps because the barrier to dissociation has been overcome.

An alternative explanation of the very different electronic spectroscopy of the C7 conformers involves the 1L_a state as the intermediate state that mixes with 1L_b at the 1L_b origin. Since both the 1L_a and $^1\pi\sigma^*$ states possess large dipole moments, one anticipates that both states will be very sensitive to the nature of the flexible substituent on the indole ring or its conformation. Based on the close proximity of the 1L_a state to 1L_b in 3MI ($\sim 200 \text{ cm}^{-1}$),⁴ one anticipates that a small preferential stabilization of 1L_a compared to 1L_b by the dipeptide backbone could bring the 1L_a origin below the 1L_b origin. Furthermore, the $S_0-^1L_a$ transition has a transition moment about 4 times larger than 1L_b , in keeping with the somewhat shorter excited state lifetime and reasonable fluorescence quantum yield observed for the C7 vibronic states in the origin region.

If both the 1L_a and $^1\pi\sigma^*$ states are stabilized by similar dipeptide backbone conformations, then the electronic spectroscopy could be reflecting coupling of the 1L_b state to the 1L_a state, with the stabilization of the $^1\pi\sigma^*$ state showing up in the washing out of the excited-state infrared spectrum. In this case, the 1L_a state would be acting as an intermediate, perhaps facilitating the coupling between 1L_b and $^1\pi\sigma^*$ states upon infrared excitation. This would require that the 1L_a state have very different Franck–Condon factors from the S_0 state than 1L_b , involving very long progressions in several low-frequency vibrational modes. Like the $^1\pi\sigma^*$ state, the large charge redistribution associated with excitation to the 1L_a state [$\mu(^1L_a) = 6.1$ D] (Ref. 10) may lead to a large perturbation in the preferred structure of the dipeptide backbone in the transition to 1L_a .

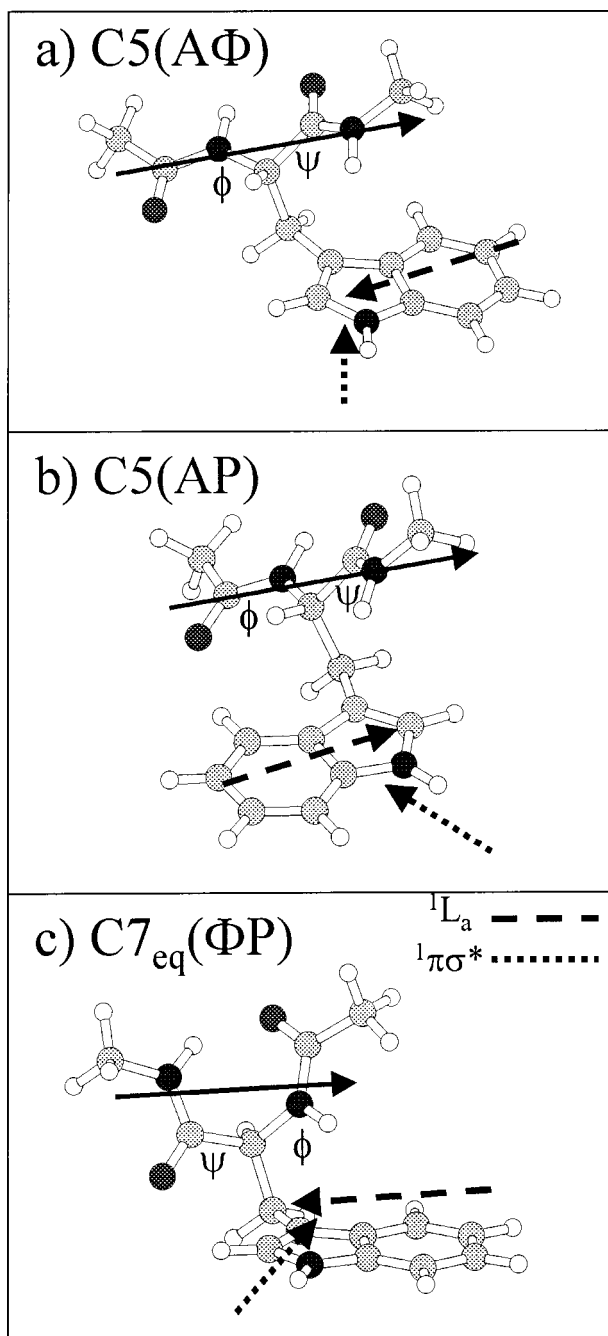


FIG. 10. Directions of the permanent dipole moments of the dipeptide backbone for each of the observed conformations of NATMA: (a) C5(AΦ); (b) C5(AP); and (c) C7_{eq}(ΦP). The directions of the dipole moments of the 1L_a and $^1\pi\sigma^*$ excited states are superimposed on the structures, taken from Ref. 10.

In order to distinguish which state (1L_a or $^1\pi\sigma^*$) is responsible for the unusual vibronic spectrum of the C7 conformers, one should consider the charge distributions formed by the C7 and C5 dipeptide backbones, and determine which state (1L_a or $^1\pi\sigma^*$) is selectively stabilized by the C7 conformation. A first level of distinction can be made simply by considering the dipole moments of the dipeptide backbones relative to the dipole moments on the indole ring in the 1L_a and $^1\pi\sigma^*$ states. Figure 10 displays the directions of the dipole moments of the dipeptide backbone for each of the observed conformations of NATMA, and of the 1L_a and

$^1\pi\sigma^*$ excited states calculated by Sobolewski and Domcke.¹⁰

The 1L_a state dipole moment points along the long axis of the indole ring, with the phenyl ring negative and the pyrrole ring positive. Because the $^1\pi\sigma^*$ state involves transfer of an electron from the π cloud of indole to a Rydberg-type orbital located outside the hydrogen atom of the indole NH group, the positive side of the dipole in the $^1\pi\sigma^*$ state is also located on the pyrrole ring, but the dipole is oriented along the short axis of indole. The Rydberg electron is easily polarized by an external electric field, and will likely be responsible for the majority of the effect of the dipeptide charge distribution on the $^1\pi\sigma^*$ state.

The closest point of contact between the dipeptide backbone and the indole ring in all three conformers of NATMA is the amide NH group, which forms a weak π H bond to the pyrrole ring (via the ψ -amide in the C5 conformers and the ϕ -amide in the C7 conformer). This NH is closest to the C3 carbon on the pyrrole ring in all three conformers, and this carbon (and the entire pyrrole ring) is positively charged in both the 1L_a and $^1\pi\sigma^*$ states. As a result, one might anticipate little stabilization via this interaction in all three conformations. The distinguishing feature of the C7 conformation is the position of the ψ -amide carbonyl group, which is in close proximity to the C2 hydrogen on the C2 carbon (2.52 angstroms) in C7_{eq}(ΦP). This negatively charged group would stabilize the positive charge on the pyrrole ring, thereby lowering both the 1L_a and $^1\pi\sigma^*$ states. The combination of amide NH and carbonyl oxygen are probably better positioned to stabilize 1L_a than $^1\pi\sigma^*$, and therefore provide another piece of evidence that it is the 1L_a state that is shifted below 1L_b in the C7 conformers of NATA and NATMA. However, a quantitative account of the relative energies of the three excited states (1L_b , 1L_a , and $^1\pi\sigma^*$) as a function of dipeptide conformation will need to await high-level calculations on these states.

V. CONCLUSIONS

The excited-state FDIR spectra presented here have led to an interpretation of the unusual ultraviolet spectroscopy of the C7_{eq} conformers of NATA and NATMA, further highlighting the dramatic changes that the conformation of the peptide backbone can have on the ultraviolet spectroscopy of the tryptophan residue. The NATA and NATMA molecules are methyl-capped dipeptides that incorporate the tryptophan side chain. By studying the isolated NATA and NATMA molecules using the double resonance methods of UV–UV hole-burning and FDIR spectroscopies, the infrared and ultraviolet spectra of individual conformations have been obtained free from interference from one another. The C5 conformers, with their extended dipeptide backbones, all have sharp LIF spectra, while the C7_{eq} conformations, with their intramolecular H bond, have characteristically broad, congested spectra indicative of a switch in the ordering of the 1L_b and $^1L_a/{}^1\pi\sigma^*$ states. The dipeptide backbones of these conformational families are similar to those adopted by beta sheet (C5) and γ turn (C7) structures in proteins. Clearly, the pres-

ence of solvent in aqueous solution may effect the relative energies and strengths of coupling of the 1L_b , 1L_a , and $^1\pi\sigma^*$ states still further. In fact, the excited state FDIR spectrum of 3MI-H₂O [Fig. 5(d)] shows greater coupling to the $^1\pi\sigma^*$ dissociative continuum than in the 3MI monomer [Fig. 5(b)]. It would be interesting to study a wider range of conformations of flexible indole derivatives and solvent-containing clusters in order to better understand the relationship between the chemical structure and conformation of the flexible side chain and the ultraviolet spectroscopy, single vibronic level photophysics, and the infrared spectroscopy in the excited state. By recording high-quality single vibronic level dispersed fluorescence spectra and excited-state FDIR spectra from the same upper state vibronic levels, it may be possible to locate and characterize the $^1\pi\sigma^*$ state in greater detail than has been done in the present work.

In light of the present data, it would appear that, in the gas phase, the $^1\pi\sigma^*$ state is nearby in energy to the 1L_b and 1L_a states, and likely plays an important role in both the spectroscopic and photophysical effects observed in indole derivatives. This realization may necessitate a reassessment of certain aspects of the condensed phase data on the spectroscopy and photophysics of tryptophan in proteins.

Finally, the results presented in this work further highlight the power of excited state FDIR spectroscopy for probing nonradiative pathways that are otherwise hard to access by electronic spectroscopy alone. There are whole classes of molecules with interesting nonradiative pathways that are amenable to such studies. Most aromatics, including benzene, possess fast nonradiative pathways within 3000 cm⁻¹ of the S_1 origin. If some component of these nonradiative pathways involves hydrogen loss, they may perturb the excited-state hydride stretch infrared spectrum in dramatic ways, as was demonstrated here for indole and its derivatives.

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