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Substituent effects on the electronic spectroscopy of tryptophan derivatives in jet expansions

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Electronic excitation spectra of seven tryptophan derivatives entrained in a supersonic expansion have been recorded using both resonantly enhanced two-photon ionization and laser induced fluorescence. Two derivatives, tryptophan amide and tryptophan methyl amide, were found to have substantial low frequency vibrational progressions in their excitation spectra, yet in both compounds this behavior was apparent in only one conformer. Other derivatives did not display as much vibronic activity. Conformers which had vibrational progressions were found to emit in a broad band far to the red of excitation. All other conformers were found to fluoresce most strongly in resonance with excitation. The presence of low frequency vibrational activity and red shifted fluorescence correlates well with the ability of the derivative to form an intramolecular hydrogen bond between the amine and the carboxylic acid. Backbone conformers that contain an intramolecular hydrogen bond are expected to have large dipole moments, which may strongly perturb the electronic structure of the indole chromophore.

I. INTRODUCTION

Indole is a conjugated, heterocyclic ring system found in the sidechain of the amino acid tryptophan. The possibility of using the indole chromophore as an optical probe of biological systems has generated great interest in its ultraviolet spectroscopy. Indole fluorescence has a very large Stokes shift in polar solvents, an effect which is greatly diminished in nonpolar environments. One explanation for this behavior postulates the formation of exciplexes between electronically excited indole and the solvent.[1] Other models of indole photophysics involve solvent reorientation about the excited state to explain the red shifted emission.[2] The matter is complicated, however, by indole's two nearly degenerate excited states, the ^1L_a and the ^1L_b. The relative energies of these two states are solvent dependent, with the ^1L_a being stabilized by polar solvents. Vibronically resolved spectra of indole and 3-methylindole have been recorded using molecular beam techniques in an attempt to locate these two electronic states in the absence of solvent perturbations.[3,4] These studies were consistent with the usual ^1L_b assignment of the lowest excited singlet state and no strong evidence for assigning a ^1L_a transition was found. Thus the nature of the ^1L_a state in the isolated molecule remains unknown.

When tryptophan is cooled in a molecular beam, specific features in its excitation spectrum can be assigned to different ground state conformers.[5] One conformer, for example, is found to have a 26 cm^{-1} vibrational progression, while features arising from other conformers do not display as much low frequency vibronic activity. The dispersed fluorescence of these two types of conformers differ as well.[6] The progression forming conformer has a strong, unstructured fluorescence band far to the red of the excitation frequency, whereas the other features exhibit sharp, resonant fluorescence. As in indole, tryptophan's progression and broad, red shifted fluorescence were explained by invoking a perturbation of the excited state to form an intramolecular exciplex in that one particular conformer.[6]

The molecular beam spectroscopy of several tryptophan containing peptides has also been studied recently.[7,8] The spectra of these peptides have several similarities to the spectrum of tryptophan. The most striking similarity was the observation of long, low frequency progressions in the excitation spectra of the peptides. One conformer of Trp-Gly, for example, was found to have an 11 cm^{-1} progression extending for 18 members. Other conformers were also observed which did not have progressions associated with them. Dispersed fluorescence from the progression-forming conformers was found to contain a broad, red shifted band while fluorescence from the other conformers was sharp and resonant.[8] These similarities indicate that the indole chromophore of these peptides experiences a conformation dependent excited state interaction similar to tryptophan.

Exciplex like behavior has not been observed in the molecular beam studies of indole and 3-methyl indole. In tryptophan and the peptides, then, the backbone must somehow perturb the excited state. Park *et al.* undertook an initial study of several tryptophan derivatives and determined that both the amine and acid groups are necessary for exciplex formation.[9] When either group was removed or when they were both chemically blocked, progressions and red shifted emission were absent. To explain this behavior, a model was developed which associated the broad red shifted emission with zwitterion formation in the excited state.[6] The current work goes further by investigating tryptophan derivatives which are more closely related to peptides. Peptides have longer backbones than tryptophan and contain at least one amide group. Most of the derivatives we have studied contain amide groups which chemically block either the amine or the carboxylic acid. Finally, we have studied a tryptophan derivative with unmodified amine and acid groups but which has the backbone locked into a restricted geometry.

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II. EXPERIMENTAL

The spectra we present were recorded using both fluorescence excitation and resonantly enhanced two-photon ionization techniques. Detailed descriptions of these experimental techniques can be found elsewhere,[10,11] so only a brief summary will be given here. Since our samples were solids at room temperature, they were heated to approximately 180 °C to increase their vapor pressures. 2 atm of helium carrier gas was passed over the hot sample and expanded through a 0.1 mm pinhole to form the free jet. The sample of locked tryptophan was incorporated into a pulsed jet using the laser desorption apparatus described previously.[7,8]

The ionization experiments were performed by skimming the free expansion and passing the resulting molecular beam between the extraction grids of a time of flight mass spectrometer. The doubled output of a Nd:YAG pumped dye laser crossed the molecular beam in this region. The sample was ionized by a resonantly enhanced two photon ionization process, and spectra were obtained by detecting the ion signal of the parent mass as the excitation/ionization wavelength was varied. Mass selected photoionization spectra were initially recorded for all samples studied. The resolution of the mass spectrometer was sufficient to identify mass peaks separated by 1 amu and to ensure that thermal decomposition was not occurring under our typical conditions.

In the fluorescence experiments, the time of flight mass spectrometer was replaced by fluorescence collection optics. Total fluorescence was collected by $f/1.0$ optics and imaged onto a slit before an RCA 8575 photomultiplier tube. Fluorescence in the opposite direction was also collected by $f/1.0$ optics, imaged onto the entrance slit of a Spex 1.0 meter monochromator and detected by an RCA 4501 photomultiplier tube. The reciprocal linear dispersion of the monochromator was 4 Å/mm. Both total fluorescence and dispersed fluorescence could be collected at the same time. Excitation of the free jet occurred 5 mm down stream from the nozzle in the fluorescence configuration.

Samples of *N*-acetyl tryptophan, tryptophan methyl ester, tryptophan amide, and *N*-acetyl tryptophan amide were used as purchased from Sigma Chemical Company. The locked form of tryptophan was kindly provided by Dr. Maurice Eftink and Mr. Yi-Weh Jai. Tryptophan methyl amide and tryptophan dimethyl amide were prepared using active esters of carbobenzoxytryptophan and the corresponding amine.[12] The carbobenzoxy protecting group was then removed by catalytic transfer hydrogenation[13] to yield the substituted tryptophan amide. *N*-acetyl tryptophan dimethyl amide was prepared from *N*-acetyl tryptophan and dimethyl amine using the coupling reagent dicyclohexylcarbodiimide.[12] Purity of the synthesized samples was confirmed using a 500 MHz FTNMR and by resonantly enhanced multiphoton ionization mass spectrometry.

III. RESULTS

The goal of this work is to understand the effect of peptide functional groups on the spectroscopy of tryptophan and tryptophan containing peptides. Assignment of transitions to different conformers is necessary because the excited state perturbation of tryptophan is dependent on the conformation of the backbone. In this section we attempt to determine the number of different conformers observed in the spectra, but absolute assignments are not crucial. The important aspect of our spectra is that conformers display two different types of behavior one type has extensive low frequency vibrational progressions and broad red shifted fluorescence, the other type has weak vibronic activity and sharp, resonant emission.

A. Tryptophan amides

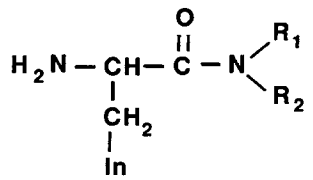
The carboxylic acid of tryptophan is replaced by an amide group in *N*-terminal tryptophan peptides, while the amine group remains unchanged. Tryptophan amide derivatives, therefore, contain the same functional groups as the *N*-terminal tryptophan dipeptides, with the exception of the carboxylic acid on the second amino acid residue. The effect of the amide protons was investigated by substituting the amide with methyl groups. The methyl amide has one free amide proton, like the peptides, and the dimethyl amide has no amide protons. Tryptophan methyl amide is very similar to the peptide Trp-Gly. The peptide backbone is the same for both samples, only the peptide has the additional acid group on the glycine residue. The structures of the various tryptophan amides are shown in Fig. 1.

The photoionization spectrum of tryptophan amide is shown in Fig. 2. The lowest energy transition observed occurs at 34 726 cm^{-1}. This feature is part of a harmonic progression containing seven members. The spacing is 31 cm^{-1} between the first and second members, but decreases to 26 cm^{-1} between the sixth and seventh members. Extensive vibrational progressions usually arise from a displacement of the excited state potential energy surface. We cannot assign the lowest energy feature as the transition to the vibrationless level of the excited state, since its strength depends on the extent of the displacement of the two potential energy surfaces. The spectrum of tryptophan amide becomes more complex at higher frequencies. A strong transition occurs at 34 944 cm^{-1} and appears to be a separate conformer origin. This feature is surrounded by many smaller peaks which may be either separate conformer origins or vibrations built on either the progression forming conformer or the strong transition.

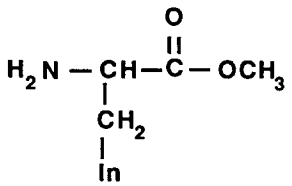
The total fluorescence excitation spectrum of tryptophan amide is presented in Fig. 3(b). This spectrum contains the same features shown in the ionization spectrum (Fig. 2). Compared to the photoionization spectrum, the intensity of the three marked peaks in Fig. 3(b) has become greater with respect to the remaining peaks. The large feature at 34 944 cm^{-1} and two peaks to the red appear enhanced in fluorescence excitation.

The enhancement of the intensities of these three peaks is even greater in the upper trace of Fig. 3. Figure 3(a) is a fluorescence excitation spectrum of tryptophan amide monitored through a monochromator with a bandpass of 145 cm^{-1} and centered on the excitation frequency. Both the monochromator and the dye laser were scanned so that the monochromator would remain resonant with the excitation

a)



b)



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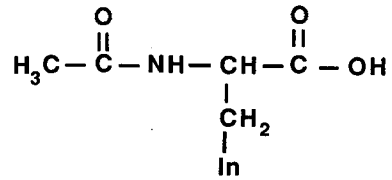

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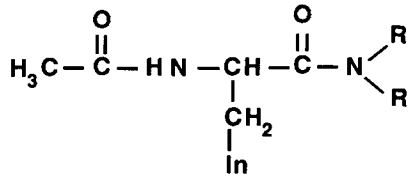

FIG. 1. Structure of tryptophan derivatives. Indole is abbreviated In. (a) Tryptophan amide $R_1 = R_2 = H$; tryptophan methyl amide $R_1 = CH_3$, $R_2 = H$; tryptophan dimethyl amide $R_1 = R_2 = CH_3$; (b) tryptophanmethyl ester; (c) *N*-acetyl tryptophan; (d) *N*-acetyl tryptophan amide (NATA) R = H; *N*-acetyl tryptophan dimethyl amide (NATDMA) $R = CH_3$.

frequency. The fluorescence excitation scan generated in this way is, therefore, biased towards features which have strong resonance fluorescence. Features which have strong fluorescence far to the red of the excitation frequency, such as the broad emitting conformer of tryptophan, appear much weaker.[6]

The excitation spectrum obtained by collecting resonance fluorescence looks very different from the total fluorescence excitation and photoionization spectra. Only three peaks in Fig. 3(b) can also be found in Fig. 3(a). We assign these three peaks as arising from three separate, resonantly emitting conformers. The remainder of the features do not have strong resonance fluorescence and hence do not appear in Fig. 3(a). The missing features could arise from other vibrational modes of the progression forming conformer or other conformers which emit far to the red of the excitation frequency.

The different emission characteristics of the two types of features can also be examined by comparing dispersed fluorescence spectra. Dispersed fluorescence spectra of the large feature at 34 994 cm^{-1} and the largest progression member (34 816 cm^{-1}) are compared in Fig. 4. The lower trace presents the dispersed fluorescence spectrum of the 34 944 cm^{-1} peak taken with 36 cm^{-1} resolution. The strong resonance line is primarily due to fluorescence. Sharp peaks are also observed 750 cm^{-1} and further to the red. No sharp features, however, are observed in the dispersed fluorescence spectrum of the progression member (upper trace). Scattered light dominates the resonance peak; fluorescence is very broad and shifted far to the red. Since the progression members have such weak resonance fluorescence, they are not observed in the excitation spectrum [Fig. 3(a)] filtered through the monochromator.

Figure 5 compares the total and resonance fluorescence excitation spectra of tryptophan methyl amide. Again, the total fluorescence excitation spectrum appears very complex [Fig. 5(b)]. Built on the lowest energy transition is a 26 cm^{-1} progression containing at least seven members. A second progression with the same spacing and at least five members begins 36 cm^{-1} above the lowest energy transition. Each member of this progression appears to be a doublet, split by 2 cm^{-1}. Two other combinations with this progression can be found 71 and 91 cm^{-1} above the lowest energy transition. These additional combinations also contain at least five members each and have the same 26 cm^{-1} spacing. A series of four peaks separated by 25 cm^{-1} intervals can be found starting at 34 940 cm^{-1}. These features are split, but

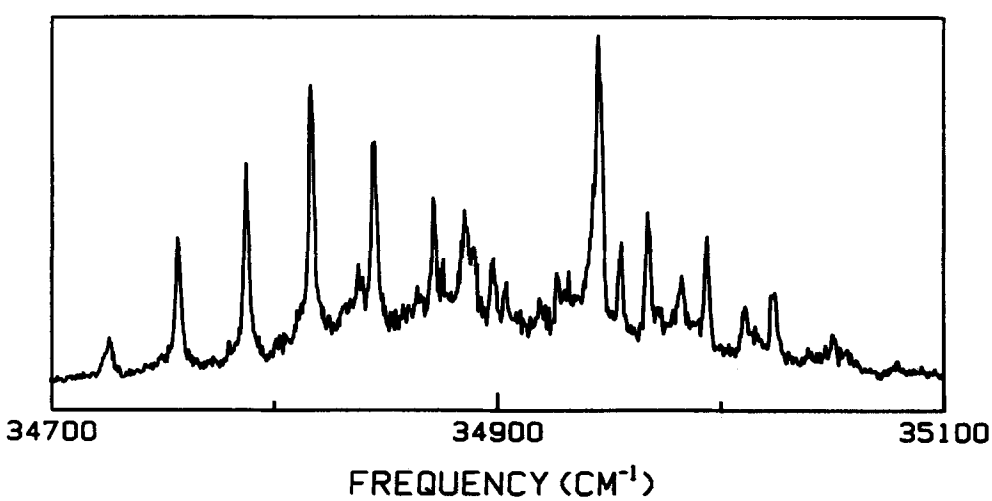

FIG. 2. Resonantly enhanced two photon ionization spectrum of tryptophan amide. This excitation spectrum was recorded by monitoring the parent mass at 203 amu as a function of excitation wavelength.

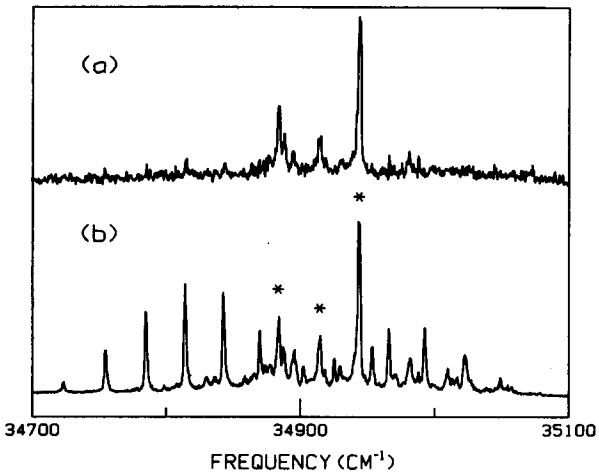

FIG. 3. Fluorescence excitation spectra of tryptophan amide recorded by (a) monitoring fluorescence through a monochromator centered on the excitation and scanned at the same rate as the excitation laser, (b) monitoring total fluorescence. The features marked with asterisks in the total fluorescence excitation scan appear enhanced compared to the ionization spectrum. The monochromator slits were 3 mm, resulting in a monochromator bandpass of 145 cm^{-1}.

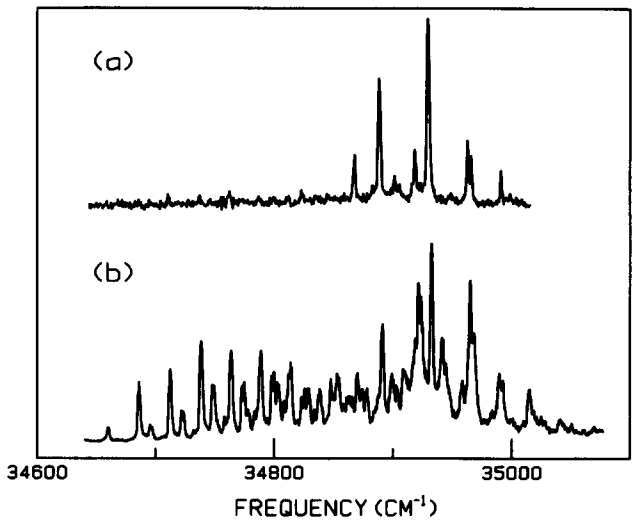

FIG. 5. Fluorescence excitation spectra of tryptophan methyl amide recorded by monitoring (a) resonance fluorescence monitored through a monochromator with a 145 cm^{-1} bandpass, and (b) total fluorescence.

both the intensity pattern and the amount of splitting differ for different peaks. The splittings, therefore, are thought to be due to coincidental overlap of spectral features. The strongest transition occurs at 34 932 cm^{-1} and is assigned as a separate conformer origin.

Comparison of the total and resonance fluorescence excitation spectra shows that the progression and its combinations have weak resonance fluorescence. The pattern of four 25 cm^{-1} spaced peaks at higher energy also changes in the resonance fluorescence spectrum. Both the first and the fourth peaks disappear completely. The second peak remains, but is weaker compared to its shoulder. One half of the doublet which makes up the third member also disappears. These observations support our conclusion that this series cannot be attributed to a single conformer. The strong 34 932 cm^{-1} peak and several other features remain in the resonance fluorescence spectrum and are due to other sharply emitting conformers.

The total and resonance fluorescence excitation spectra of tryptophan dimethyl amide are compared in Fig. 6. The total fluorescence spectrum is shown in the lower trace. Unlike the spectra of the other amides, it does not contain an extended low frequency vibrational progression. The strongest transition is at 34 828 cm^{-1}. Dispersed fluorescence spectra of this peak and the peak at 34 856 cm^{-1} show the presence of a weak 30 cm^{-1} ground state vibrational mode.

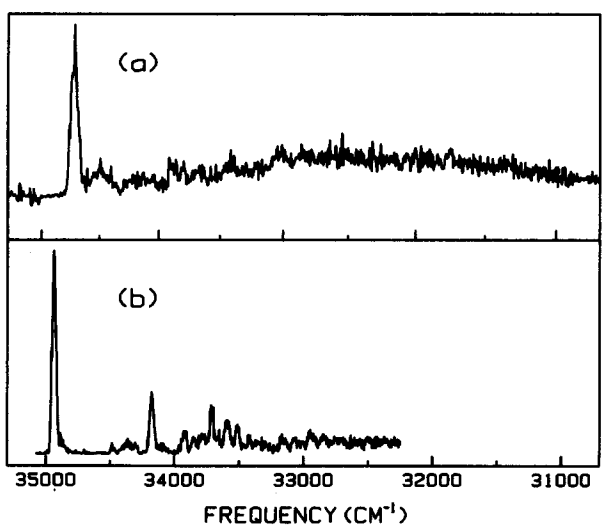

FIG. 4. Dispersed fluorescence spectra of tryptophan amide exciting (a) the fourth progression member at 34 816 cm^{-1} and (b) the largest conformer origin at 34 944 cm^{-1}. The resolution of the monochromator was 75 cm^{-1} for the upper spectrum and 36 cm^{-1} for the lower trace. Roughly 60% of the large feature at the excitation frequency is due to scattered light in (a), but the contribution from scatter is less than 40% in (b).

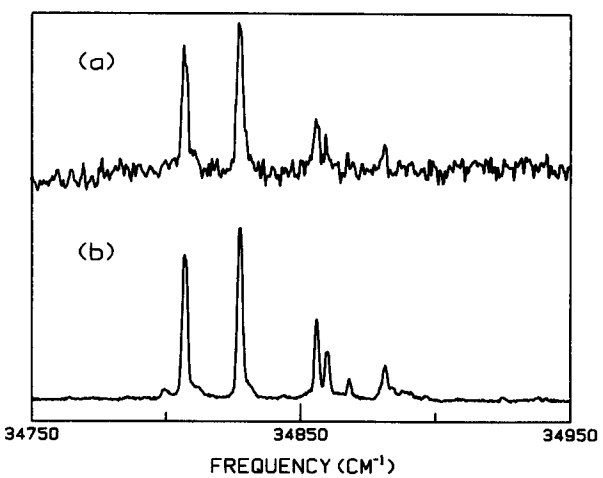

FIG. 6. Fluorescence excitation spectra of tryptophan dimethyl amide recorded by (a) detecting fluorescence through a monochromator with a 145 cm^{-1} bandpass centered at the excitation frequency, and (b) by detecting total fluorescence.

Therefore these two peaks, along with the peak at 34 882 cm^{-1}, arise from the same conformer. The intensity pattern suggests that the 30 cm^{-1} mode is only weakly allowed and built on the conformer origin at 34 828 cm^{-1}. The remaining features are assigned as separate conformer origins. The upper trace of Fig. 6 displays the resonance fluorescence excitation spectrum. This spectrum contains the same features found in the total fluorescence excitation spectrum. Features characteristic of the excited state perturbation, such as strong vibrational progressions and red shifted emission, are not observed for tryptophan dimethyl amide.

B. Tryptophan methyl ester

The acid proton of tryptophan is replaced by a methyl group in tryptophan methyl ester (see Fig. 1). This substitution blocks only the acid proton; the amine and the indole system are unaffected. Tryptophan methyl ester, therefore, should clarify the role of the acid proton in tryptophan exciplex formation.

The total fluorescence excitation spectrum of tryptophan methyl ester is shown in Fig. 7(b). The strongest transition occurs at 34 925 cm^{-1}. Further to the blue, there is a group of five closely spaced peaks. The spacing between the five peaks is irregular, suggesting that this group of peaks is not a vibrational progression. The spacing of between the first two peaks is 6 cm^{-1} but only 3 cm^{-1} between the last two features. The intensity pattern of the group members, moreover, is uncharacteristic of a vibrational progression. The set both begins and ends very abruptly, suggesting that they do not arise from a single conformer vibration. We consider the most intense feature, the members of the group of five, and the redmost feature at 34 878 cm^{-1} all to be origins to the first excited state of seven different conformations of tryptophan methyl ester. Starting at 34 975 cm^{-1} there is another group of three peaks, with spacing similar to the group at 34 933 cm^{-1}. It seems likely that these arise as a common 42 cm^{-1} vibration for three of the seven conformers.

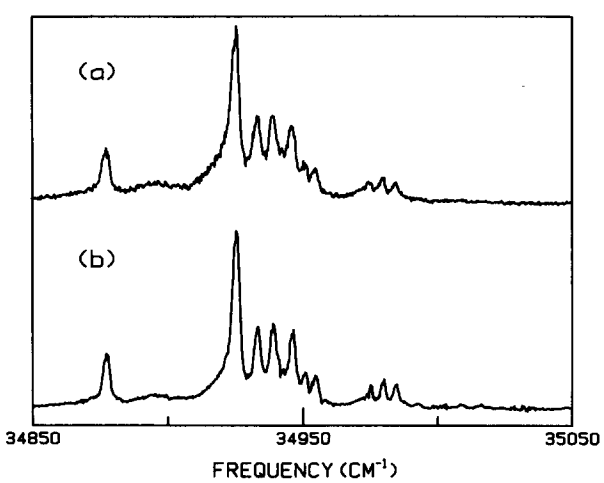

FIG. 7. Excitation spectra of tryptophan methyl ester recorded by monitoring (a) fluorescence passed through a monochromator with a 145 cm^{-1} bandpass centered on the excitation frequency, and (b) total fluorescence.

Figure 7(a) presents the resonance fluorescence excitation spectrum of tryptophan methyl ester. The intensity patterns of the spectra in Fig. 7 are the same, so all the features in the excitation spectrum must have similarly strong resonance fluorescence. The dispersed fluorescence spectrum of the feature at 34 925 cm^{-1} is shown in Fig. 8. This conformer displays sharp fluorescence and a large 750 cm^{-1} indole ring vibration. Fluorescence from the other conformers also contained sharp vibrations instead of broad emission. Tryptophan methyl ester, then, does not experience the same excited state perturbation that tryptophan, the peptides, and the amides experience.

C. *N*-acetyl tryptophan

N-acetyl tryptophan can be viewed as a derivative of both tryptophan and the dipeptide Gly-Trp (see Fig. 1). The free amine of tryptophan is now blocked by an acetyl group, which prevents the nitrogen atom from acting as a proton acceptor. The carboxylic acid group is not changed. The acetyl group contains an amide bond, so *N*-acetyl tryptophan is also related to a dipeptide. Only the free amine of Gly-Trp is missing in *N*-acetyl tryptophan. *N*-acetyl tryptophan should determine the effect of the free amine on the excited state perturbation of both tryptophan and Gly-Trp.

Figure 9 presents the fluorescence excitation spectrum of *N*-acetyl tryptophan. Only one strong peak is observed at 34 928 cm^{-1}. We assign this peak as the origin transition to the first excited state of the predominant conformer of acetyl tryptophan. To the blue are features much weaker in intensity. We assign them as weak vibrations of the main conformer. The dispersed fluorescence spectrum of the 34 928 cm^{-1} peak was found to be similar to the dispersed spectrum of tryptophan methyl ester displayed in Fig. 8. Sharp features were again observed in the dispersed fluorescence spectrum, and the 750 cm^{-1} indole ring vibration was easily identified.

D. *N*-acetyl tryptophan amide

The tryptophan derivative *N*-acetyl tryptophan amide (NATA) (see Fig. 1) contains two types of amide groups. One amide blocks the amine and is similar to the amide in *N*-acetyl tryptophan. The other amide replaces the carboxylic acid, like the tryptophan amides. When tryptophan is a residue in a peptide or protein, both its amine and acid are connected to the other residues through amides. Since NATA has these two types of amides, it is similar to tryptophan incorporated into the interior of a peptide.

The total fluorescence excitation spectrum of NATA is displayed in Fig. 10(b). There is a large, sharp feature at 34 955 cm^{-1}. Immediately to the red of this peak is a broad hump. Still further red is a series of very small peaks with a 10 cm^{-1} spacing. This series extends to 34 375 cm^{-1} and contains roughly 30 members. The relative intensities of all these features is the same as in an ionization spectrum, indicating that the fluorescence quantum yields of these features are similar. The upper half of Fig. 10 presents the resonance fluorescence excitation spectrum of NATA. The weak series of peaks and the hump are greatly reduced in intensity compared to the feature at 34 955 cm^{-1}. The series and the

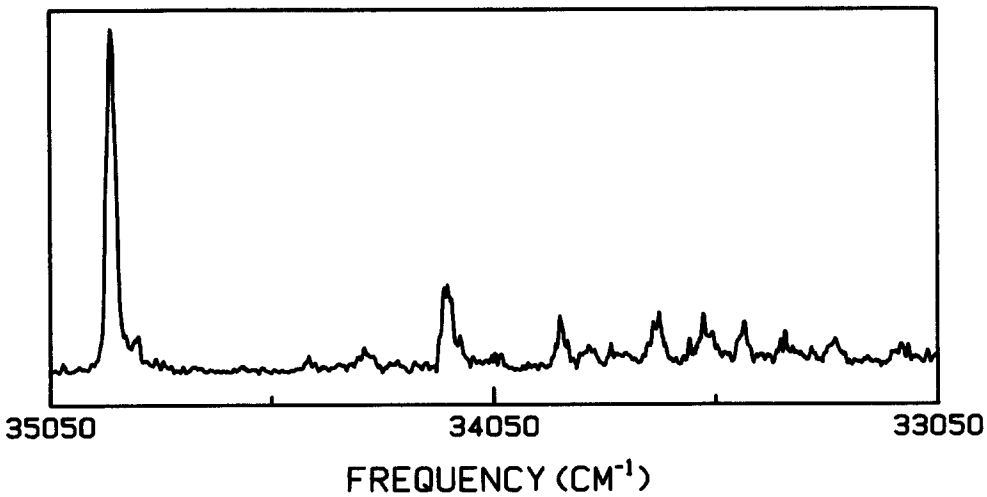

FIG. 8. Dispersed fluorescence spectrum of the strongest transition of tryptophan methyl ester at 34 925 cm^{-1}. The resolution of the monochromator was 24 cm^{-1}. The large feature at the excitation frequency is primarily composed of fluorescence.

hump, therefore, arise from conformers with weak resonance fluorescence. The predominant conformer, however, is similar to *N*-acetyl tryptophan and has strong resonance fluorescence.

E. *N*-acetyl tryptophan dimethyl amide

N-acetyl tryptophan dimethyl amide (NATDMA) is similar to NATA except the amide protons have been replaced by methyl groups (see Fig. 1). Figure 11 diplays the total fluorescence excitation spectrum [Fig. 11(b)] and the resonance fluorescence excitation spectrum [Fig. 11(a)]. The two spectra are similar, indicating that the features all have similar emission characteristics with strong resonance fluorescence. The excitation spectra contain four large peaks. The lowest energy transition is at 34 848 cm^{-1} and is followed by the strongest transition at 34 860 cm^{-1}. Two smaller peaks appear at 34 886 and 34 902 cm^{-1}. Dispersed fluorescence spectra of these peaks indicate that they are vibrations of 38 and 32 cm^{-1} built on the two lower energy features. The spectrum of NATDMA contains two conformer origins, each with a weak vibration. Unlike NATA, no long progression of weak features is observed in the spectrum of NATDMA.

F. Locked tryptophan

The backbone of locked tryptophan is attached to the indole ring in two positions. The β-carbon is attached to the indole three position, and the amine is attached through a methylene group to the indole two position (see Fig. 12). In this configuration, the backbone forms a six member ring attached to the five member ring of indole. The carboxylic acid remains unaltered.

Figure 12 presents the total fluorescence excitation spectrum of the locked tryptophan. There are three closely spaced peaks in the origin region of this spectrum. Both the intensity pattern of these peaks and the slight irregularity of their spacing suggest that they are not part of a progression. An additional feature is observed at 35 030 cm^{-1}. Since the side chain is no longer free to rotate about bonds to the ring, we assign these four features as arising from different conformations of the side chain ring and of the carboxylic acid with respect to the aromatic rings.

Dispersed fluorescence from the transition at 35 028 cm^{-1} is shown in Fig. 13. The 750 cm^{-1} indole ring vibration is evident, as well as other sharp vibrations. There is little broad, red shifted background in this spectrum, indicating that the excited state perturbation is prevented in this

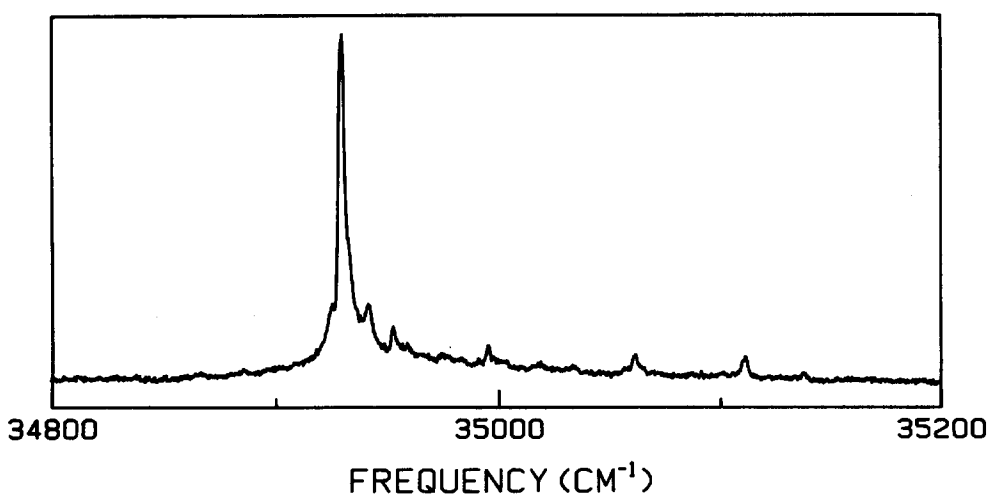

FIG. 9. Total fluorescence excitation spectrum of *N*-acetyl tryptophan.

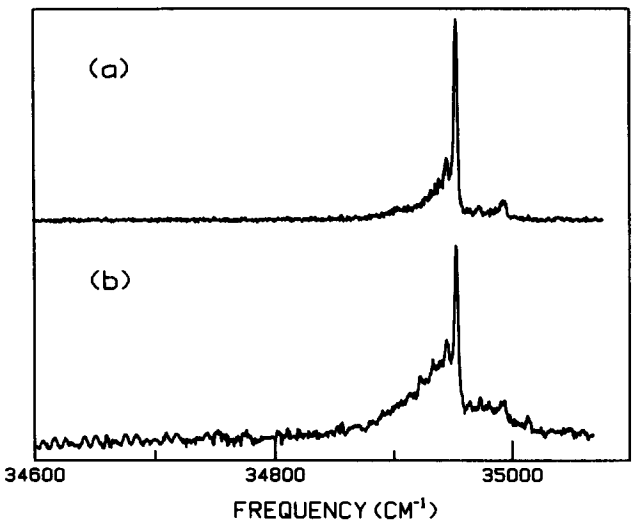

FIG. 10. Fluorescence excitation spectra of *N*-acetyl tryptophan amide (NATA) obtained by monitoring (a) fluorescence passed through a monochromator with a 192 cm^{-1} bandpass centered on the excitation frequency and (b) total fluorescence. The small, regularly spaced features at lower frequencies extend to 34 375 cm^{-1} and at least 30 peaks are discernible.

little broad, red shifted background in this spectrum, indicating that the excited state perturbation is prevented in this conformer of locked tryptophan. Other conformers have similar dispersed fluorescence spectra.

IV. DISCUSSION

A comparison of the spectra of the derivatives is given in Table I. The spectra of three derivatives differ greatly from the others. The lowest energy features of tryptophan amide, tryptophan methyl amide, and NATA are shifted further red, like the peptides, than the remainder of the derivatives. These samples have extensive vibrational progressions in their excitation spectra. Additionally, these three compounds are the only samples reported in this paper which display broad, red shifted emission.

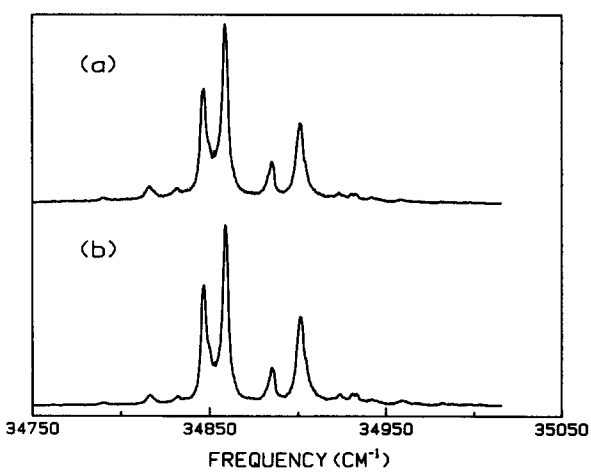

FIG. 11. Fluorescence excitation spectra of *N*-acetyl tryptophan dimethyl amide (NATDMA) obtained by monitoring (a) fluorescence passed through a monochromator with a 240 cm^{-1} bandpass centered on the excitation frequency and (b) total fluorescence.

The initial work on tryptophan derivatives was carried out by Park *et al.*[9] as part of the study of tryptophan. That study presented spectra of derivatives which were lacking either the carboxylic acid or the amine of tryptophan, such as 3-indole propionic acid, tryptamine, and 3-indole acetic acid. *N*-acetyl tryptophan ethyl ester (NATE), in which both the acid and the amine are chemically blocked, was also studied. Although each spectrum contained origin transitions for several different conformers, no extensive vibronic activity was observed. Dispersed fluorescence from these samples also indicated that all the conformers had sharp fluorescence spectra. The conclusion of this work was that both the amine and the carboxylic acid need to be present for the excited state interaction to occur. The early model for tryptophan photophysics was, therefore, that the amine and acid interact in the excited state to form a zwitterion, which stabilizes the nearby excited indole ring.

Some of the results in the present paper extend the observations of the previous work and are consistent with the zwitterion model. The spectra of tryptophan methyl ester and *N*-acetyl tryptophan are extensions of the previous work on NATE and indicate that blocking with either an *N*-acetyl group or an ester is sufficient to turn off the exciplex interaction. The presence of both blocking groups is not necessary to prevent the exciplex interaction.

However, some of the results of the present paper indicate that the zwitterion model for tryptophan photophysics must be modified. Although both sets of spectra indicate that a free amine is necessary for the excited state to be perturbed (with the one exception of NATA which will be discussed later), the spectra of tryptophan amide and tryptophan methyl amide indicate that a free carboxyl group is not necessary. However, the lack of an exciplex interaction in tryptophan dimethyl amide indicates that a hydrogen atom on the carboxyl end of the molecule in either the acid or amide form is necessary.

Observation of exciplex-like spectral behavior for the tryptophan amides is not consistent with excited state zwitterion formation. Since creation of a zwitterion is not normally energetically allowed in the gas phase, some interaction between the backbone and the excited indole rings (such as dipole–dipole or charge transfer) would have to stabilize the zwitterion. The amount of energy required to form the zwitterion is expected to depend largely on the acidity of the proton donating group. The gas phase acidities of several biological proton donors have been investigated recently.[14] Removal of a proton from amides was found to cost up to 4725 cm^{-1} more energy than proton removal from acetic acid. Amides, therefore, would not be expected to form an excited state zwitterion without a correspondingly large increase in the stabilizing interaction with the indole rings.

An excited state perturbation could alternatively arise from a simple dipole–dipole interaction between the amino acid backbone and the excited ring system. In several of the derivatives reported here, the backbone mimics the amino acid glycine. The *A*-axis component of the dipole moment of one conformer of glycine has been measured by microwave spectroscopy[15] as 4.5 D, and accompanying theoretical stud-

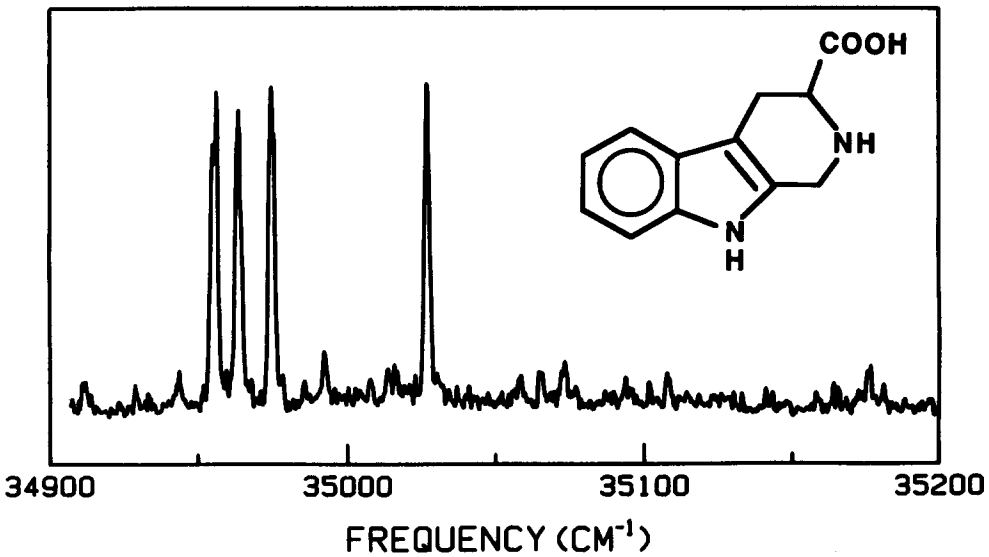

FIG. 12. Total fluorescence excitation spectrum of locked tryptophan. Locked tryptophan was incorporated into the jet expansion by means of the laser desorption method described in Ref. 7. The structure of locked tryptophan is also presented.

ies[16] suggest that the total dipole moment of this glycine conformer may be as large as 6.5 D. A second conformer of glycine was found to have a much smaller dipole moment. The polar conformer of glycine has an intramolecular hydrogen bond between the acid and the armine. While it is unlikely that backbones containing amides can form zwitterions, both acids and amides can hydrogen bond to amines, and therefore tryptophan, tryptophan amide, and tryptophan methyl amide could achieve the polar conformation containing an intramolecular hydrogen bond, but the hydrogen bond would be absent in tryptophan dimethyl amide and tryptophan methyl ester.

The spectra presented above are all due to excitation of the indole chromophore in tryptophan. Tryptophan has two low lying excited states, ^1L_a and ^1L_b. The transitions in the origin region of supersonically cooled tryptophan are all assigned as transitions to the ^1L_b state.[5] The lowest energy transitions of the samples we studied are listed in Table I. They all lie within 220 cm^{-1} of the tryptophan origin, suggesting that the spectra of these compounds also arise from excitation to the ^1L_b state.

Comparison of the solution spectra of indole and its derivatives with the gas phase spectra reported here allow some parallels to be drawn and suggest that similar interactions are present in both environments. In polar solvents, the fluorescence of indole and its derivatives shows a large Stokes shift, the magnitude of the shift depending on the polarity of the solvent. In nonpolar media the ^1L_b state is believed to be lower in energy than the ^1L_a state, but the energy of the ^1L_a state is much more strongly affected by interactions with polar solvents and is thought to fall below the energy of the ^1L_b state in a polar environment.[17] This solvent mediated energy shift of the ^1L_a state is thought to be responsible for the fluorescence Stokes shift in polar solvents.

Indole in the gas phase is, of course, in a nonpolar environment, so in analogy with the solution spectra the fluorescence spectrum of indole should not be Stokes shifted, as observed.[3(b)] However in some substituted indoles, the substituent itself could be polar. In these cases, the intramolecu-

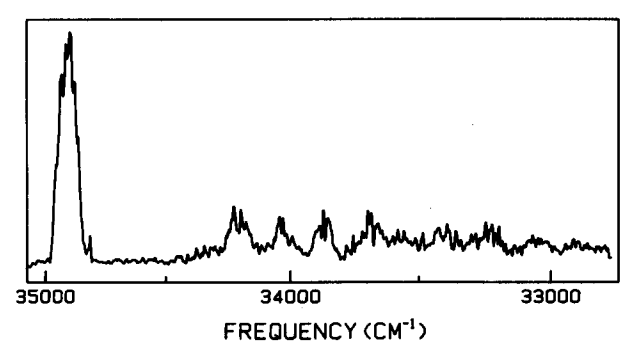

FIG. 13. Dispersed fluorescence spectrum of the locked tryptophan peak at 35 028 cm^{-1}. The resolution of the monochromator was 75 cm^{-1}. Scattered light accounts for only 20% of the intensity of the excitation resonance.

TABLE I. Summary and comparison of derivative spectra.

Sample	Lowest energy feature, cm^{-1}	$\nu_0 - \nu_0^{Trp}$, cm^{-1}	Progression frequency,[a] cm^{-1}
Tryptophan[b]			
(Trp)	34873	0	26 (4)
Trp amide	34724	− 149	31 (7)
Trp methyl amide	34660	− 213	26 (7), (5)
Trp dimethyl amide	34807	− 66	···
Trp methyl ester	34878	5	···
N - acetyl Trp	34928	55	···
NATA	34955[c]	82	···
	34375	− 498	10 (30)
NATDMA	34848	− 25	···
Locked Trp	34958	85	···
Peptides[d]			
Trp-Gly	34519	− 354	11 (18)
Gly-Trp	34712	− 161	42 (5)
Trp-Phe	34553	− 320	27 (5), 29 (4)
Phe-Trp	34720	− 153	24 (6)

[a]Number of progression members given in parentheses.
[b]From Ref. 5.
[c]Origin of the strongest feature.
[d]From Ref. 7.

lar interaction between the indole chromophore and the polar substituent could give rise to a Stokes shifted fluorescence spectrum. Therefore the existence of a broad, red-shifted feature in the jet emission spectrum could depend on whether a particular conformer of a particular substituent had a sufficient dipole moment to perturb the ^1L_a state to the degree that it is perturbed by polar solvents.

The ^1L_b state of indole is thought to have a dipole moment that is almost the same as the ground electronic state,[18] and therefore excitation to the ^1L_b state would not be expected to produce any significant increase in the dipole–dipole interaction between the chromophore and the backbone. Lami and Glasser, however, infer from the solution phase emission spectrum of the ^1L_a state that this state has a dipole moment 3 D larger than that of the ground state.[17] A change of 3 D in the indole dipole moment would result in a large change in the dipole–dipole interaction. The magnitude of the dipole–dipole interaction is a strong function of both the distance and orientation between the two dipoles. Assuming the most favorable orientation and a minimum distance of 3.5 Å between the dipoles, an increase of 3 D in a chromophore interacting with a 6 D backbone would produce a decrease in energy of 4200 cm^{-1}.

The ^1L_a state of indole could therefore be responsible for the perturbation of the ^1L_b state. As shown in Fig. 14, if the ^1L_a and ^1L_b are brought close together in energy by a dipole–dipole interaction, mixing between the two states could produce the double well excited state potential characteristic of an exciplex. Low frequency vibrations involving backbone motion would also be active in this conformer's excitation spectrum if the ^1L_b also has a small change in its dipole moment. The change in the dipole moment of the ^1L_b state would slightly shift the excited state potential, thereby making a vibrational progression Franck–Condon allowed.

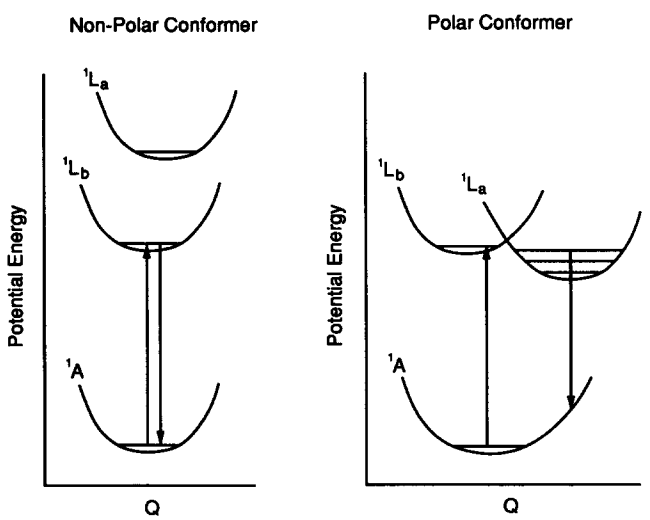

FIG. 14. Potential energy diagrams describing the relative energies of the ^1L_a and ^1L_b states as a function of Q, a parameter describing the relative orientation of the indole chromophore with respect to the backbone. The left diagram is for a nonpolar conformer where the dipole–dipole interaction is relatively weak. The right-hand diagram is for the polar conformer stabilized by a hydrogen bond between the acid and amine ends of the backbone. This conformer has a relatively strong dipole–dipole interaction.

Franck–Condon allowed fluorescence from the more stable state would be broad and red shifted.

Since the interaction between two dipoles is inversely proportional to the cube of the distance between the dipole centers, it will be important only for conformations which can bring the polar backbone close to the indole ring. Locked tryptophan can form a conformer with a large backbone dipole moment and an intramolecular hydrogen bond from the acid to the amine. However, this dipole is held rigidly away from the indole ring. The dipole–dipole interaction for locked tryptophan, therefore, is expected to be weak. Our spectra of rigid tryptophan show no signs of the excited state perturbation.

The excitation spectrum of NATA contains a weak vibrational progression of red-shifted emitting features in addition to its sharply emitting conformer. This behavior initially seems to contradict our new model. Although the acetyl group prevents hydrogen bonding to its nitrogen, a new intramolecular hydrogen bond can form to the acetyl oxygen atom. Such a conformation would form a seven member ring which is predicted to be a stable form of many model dipeptides.[19] In *N*-acetyl tryptophan a similar backbone conformer is also feasible although at the expense of a trans carboxylic acid conformation which in formic acid is measured to be 4.09 kcal/mol more stable than a *cis* geometry and which the tryptophan derivative would need to adopt to H bond to the amide carbonyl group.[20] NATDMA would not be expected to form this conformation since it has two methyl groups substituted onto the proton donating amide. Neither *N*-acetyl tryptophan nor NATDMA display much vibronic activity in their excitation spectra or have broad red shifted emission.

Several investigators have suggested that the ^1L_a excited state is dissociative along the N–H bond for indole isolated in the vapor phase.[21,22] Hagar *et al.* measured peak widths and fluorescence lifetimes for 2,3-dimethylindole and 1,2,3,4-hydrocarbazole and determined that peaks blue of the origin display significantly shorter lifetimes.[22] They explained their results by proposing that the ^1L_b state predissociatively couples to the ^1L_a state. Glasser and Lami have also proposed that the ^1L_a states of indole and several indole derivatives have a very efficient nonradiative decay process.[21] If the ^1L_a state is dissociative, it cannot account for the large amount of red shifted emission seen in tryptophan, tryptophan peptides, and tryptophan amides.

V. CONCLUSIONS

Spectra from five tryptophan derivatives in which either the amide or the amine group was blocked show that the excited state perturbation of tryptophan requires the presence of both a free amine and an unblocked acid or amide. Spectral features from this perturbation are observed in the spectra of tryptophan amide and tryptophan methyl amide, indicating that amide protons can replace the acid proton without preventing the perturbation. These results suggest that an intramolecular hydrogen bond between the amine and the acid may play an important role in the perturbation of the excited state of tryptophan.

Results from a tryptophan derivative in which the amino acid backbone is fixed to the indole ring in two positions are also presented. The spectra of this compound show no sign of the excited state perturbation, even though an intramolecular hydrogen bond can still form between the amine and the acid. The perturbation must, therefore, be a sensitive function of distance and orientation. For this reason, we propose that the amino acid backbone interacts with the ^1L_a state of the excited indole rings through a dipole–dipole interaction.

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