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The Electronic Excited-States of Indole: Indirect Evidence for Excited 3h-Indole.

Cecil Mcclung Chopin
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THE ELECTRONIC EXCITED STATES OF INDOLE:
INDIRECT EVIDENCE FOR EXCITED 3H-INDOLE.

The Louisiana State University and Agricultural
and Mechanical College, Ph.D., 1972
Chemistry, physical

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THE ELECTRONIC EXCITED STATES OF INDOLE:
INDIRECT EVIDENCE FOR EXCITED 3H-INDOLE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Chemistry

by
Cecil McClung Chopin
B.S., Northwestern State University, 1964
August, 1972

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To my wife, Lydia

ACKNOWLEDGMENT

This research was suggested by Dr. J. H. Wharton and was carried out under his direction. The suggestions and untiring efforts of Dr. Wharton were indispensable to the success of this research and the author is deeply grateful for this association.

Research of any significance is generally not accomplished as an individual effort. The author expresses his appreciation to the research groups of Dr. R. V. Nauman and Dr. S. P. McGlynn for their technical assistance, to Dr. S. Monti for the sample of 2-ethoxyindole, to Dr. E. W. Berg and Dr. J. Chaing for assistance in compound purification, and to Dr. K. N. Houk for many helpful discussions.

The theoretical calculations and computer processed spectra would not have been possible without the dedicated efforts of Mr. Henry Streiffer. Special thanks are due Dr. Curtis Holmes not only for his aid in the interpretation of the computer programs but also for his general readiness to help in any phase of this work and especially for his encouragement and optimism.

The writer acknowledges the financial support of the National Aeronautics and Space Administration and the Department of Chemistry toward the preparation of this research and of the Dr. Charles E. Coates Memorial Fund of the L.S.U. Foundation toward the publication of this doctoral dissertation.

Finally, the author is grateful for the love and understanding shown him by his wife during this period. It is to her that this work is dedicated.

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Abstract

The anomalous emission properties of indole have been examined in terms of theory and experiment. Previous attempts to explain the excited-state chemistry of indole are classified and discussed in terms of four theories: 1) dual emission, 2) excited state complex, 3) solvent reorientation, and 4) excited-state proton transfer. A new theory, isomerization to 3H-indole, is proposed.

The absorption and emission spectra of indole, 1-methylindole (1MI), 2-methylindole (2MI), 3-methylindole (3MI), and 2-ethoxyindole (2EI) in polar and nonpolar solvents have been measured. Solvent effects on the rate of intersystem crossing in indole are presented as changes in the quantum yield of phosphorescence relative to fluorescence. These changes have been interpreted in terms of isomerization of indole to 3H-indole in the excited state. This model has been shown to be consistent with previous observations of anomalous fluorescence emission from indole.

Attempts to confirm this interpretation by photochemical rearrangement of 1-methylindole, nmr solvent studies of indole and 3-methylindole, laser-Raman spectroscopy of indole and fluorene and an examination of the absorption and emission properties of 2-ethoxyindole are presented. The laser-Raman spectrum of indole and the photoelectron spectra of indole, 1MI, 2MI and 3MI are included

CNDO/2 calculations are used to compare excited-state energy levels for indole and 3H-indole; 3H-indole is predicted to have an $S_{n\pi^*}$ state as the lowest excited singlet state. The predicted state orders and energy levels of indole and 3H-indole are compatible with the proposed theory.

Chapter I

Theory and Literature Survey

INDOLE

A. Introduction

In the last decade spectroscopists have shown considerable interest in the molecule indole. It is biologically important because it is the chromophore of tryptophan, one of the three amino acids exhibiting luminescence in the free state. It has been anticipated that an understanding of the excited state chemistry of indole would lead to an increased understanding of tryptophan and protein reactions.

Indole also has intrinsic spectroscopic interest. Several anomalous effects have been observed, primarily as a function of solvent variation: 1) Indole fluorescence was found to display a large shift to lower energy ("red shift") with increasing solvent polarity; 2) a solvent induced loss of vibration structure in the fluorescence spectrum was reported; 3) a change in the lifetime within the

fluorescence band was noted; 4) an abnormal fluorescence quenching has been observed on cooling to liquid nitrogen (LIN) temperature; 5) corresponding effects in the absorption spectrum have been minimal or completely absent. The effects listed above implied an excited state reaction induced by the solvent such that the ground state remained unaffected.

The present study was undertaken in an attempt to discern the nature of this reaction.

B. Absorption and Emission Spectroscopy

In earlier studies Van Duuren¹ observed pronounced shifts in the fluorescence/emission maxima of indole with a change in solvents. Since similar shifts did not occur in either the fluorescence excitation or the ultraviolet (UV) absorption spectra, he concluded there was an interaction between solvent and the activated (excited) indole molecule but not between solvent and the ground state molecule. To account for the interaction Van Duuren proposed that polar contributing mesomeric states of indole such as I and II made a larger contribution in the excited state than in the ground state.

Such polar structures would be more sensitive to changes in solvent dielectric constant and would account for the pronounced solvent shift observed in the fluorescence but not in the absorption. Hydrogen

bonding was eliminated because the solvent shifts observed in the case of 1,2-dimethylindole and 1-methyl-2-phenylindole were similar to the solvent shifts observed in indoles not substituted on the nitrogen atom.

About the same time, Zimmerman and Joop² measured the absorption and fluorescence polarization spectra and identified bands in terms of the Platt³ model. They reported that the 1L_b transition was polarized in the direction of the long molecular axis and that the 1L_a transition perpendicular to the 1L_b along the short molecular axis. From a careful comparison of the absorption and the fluorescence polarization spectra, they concluded that the fluorescence originated primarily from the 1L_a state but that it was possible a portion of the fluorescence also originated from the 1L_b state. Moreover, they found that the 0-0 bands of the 1L_a absorption and the 1L_b absorption were closely adjacent and nearly degenerate. It was also shown that the absorption and fluorescence polarization spectra behaved as image and mirror image in a region extending over several electronic states.

These relations are shown in figure 1 as taken from reference 2.

A, absorption; F, fluorescence;
AP, absorption polarization;
FP, fluorescence polarization.

They proposed that if it was valid to interpret the 1L_a and 1L_b as distinct electronic transitions then one must assume that the fluorescence resulted from both of these states.

The solvent effect on the fluorescence emission of carbazole led Mataga, *et al.*⁴ to conclude that the lowest excited state of carbazole has a much greater tendency toward ionic dissociation than does the ground state. The similar solvent effects observed in the case of indole, implied that, in indole, there is a greater tendency toward the ionic dissociation $>N-H \rightarrow N^- + H^+$ in the lowest excited singlet state than in the ground state.

For indole, they observed the solvent shift of the absorption spectrum was very small in going from cyclohexane to acetonitrile to water. In contrast, they observed the fluorescence emission greatly red shifted in going from cyclohexane to acetonitrile to water. This shift was attributed to a large increase of dipole moment in the excited state and a greater stabilization caused by solvent-solute interactions in the equilibrium fluorescent state than in the ground or Franck-Condon excited states.

More detailed studies showed that as the ethanol concentration increased in going from hexane up to a solvent that was 8.2 M ethanol in hexane, the 1L_b band decreased in intensity and a broad band arose at longer wavelength. Here there is evidence for one band disappearing and another appearing. This long wavelength broad band further shifted to the red as the concentration of alcohol was increased. To explain these observations Mataga proposed that the 1L_a state of indole has a

large dipole moment and is strongly stabilized by the interaction of solute with surrounding polar molecules. In this case it could become the lowest excited singlet state during the radiationless relaxation from the Franck-Condon to the equilibrium excited state. Evidence for a two component fluorescence decay curve and other examples of reversal of 1L_a and 1L_b states during the lifetime of the excited state were offered in support of this theory. Mataga did not indicate whether a Franck-Condon 1L_a state relaxed to an equilibrium 1L_a state or whether a Franck-Condon 1L_b state relaxed to an equilibrium 1L_a state. When solvent-solute equilibration is important to the ordering of the excited state energies, then excitation experiments are essential to determine the relaxation routes.

After the beginning of our work, an extensive discussion was made available by the translation from the Russian of a book by Konev.⁵ Konev pointed out that the absorption and fluorescence spectra of indole in hexane do not exhibit mirror symmetry. Carbazole, in which the 1L_a and 1L_b bands are well separated, was found to have perfectly symmetrical spectra. According to Konev, absence of such symmetry in indole, in which the 1L_a and 1L_b transitions are close together, indicates that two electronic transitions are involved in the long wavelength band of the absorption and fluorescence spectra.

When 1-butanol was gradually added to hexane solutions of indole, an isosbestic point was found in the series of absorption spectra. He suggested that this point arose from the spectra of two forms of indole, an unassociated form and a form bound to the alcohol

by a hydrogen bond. He found the bands of the first absorption transition to be shifted through different distances. The short wavelength maxima were red shifted through 700 cm^{-1} whereas the long-wave maxima were shifted only 150 cm^{-1} . The greater shift of the short-wave maxima (1L_a) indicated that the corresponding oscillators were oriented along the short molecular axis because the formation of a hydrogen bond has a greater effect on electronic transitions for which the direction of the moment passes through the substituent.

Similar observations in the case of the fluorescence emission spectrum confirmed that the fluorescence also involves two electronic transitions and not the single transition that is responsible for the fluorescence in the case of the majority of other organic molecules. Addition of alcohol reduced the intensity of the short-wave maximum and shifted it only slightly. The longer wavelength maxima became more pronounced and shifted greatly.

Organic molecules incapable of hydrogen bonding usually show the same fluorescence maxima in hydrocarbon or alcohol. In the case of substances capable of hydrogen bonding, the bond formation is generally accompanied by a shift in the total spectrum and no change in its shape. In the case of indole, Konev found this shift accompanied by an additional effect, the disappearance of the short-wave fluorescence band. He proposed that the hydrogen bond was apparently formed with two different electronically excited molecules and shifted their spectra by different amounts. Konev concluded that bond formation would have the greatest effect on the excited state involving

transition polarized along the nitrogen-hydrogen axis (1L_a). Thus, solvent stabilization results in the 0-0 level of the 1L_a state becoming the lowest excited level. In polar-protic solvents the energy difference becomes so great that indole ceases to be "peculiar" and begins to luminesce only from the lowest electronic level, the 1L_a . The decrease in the intensity of the 1L_b band is consistent with this model.

In addition to hydrogen bond formation, solvent relaxation effects were also implicated in the shift. According to Konev's rationale some orientation of polar molecules occurs around indole in the ground state. Since the dipole moment is altered in magnitude and direction upon conversion to the Franck-Condon excited state, the strength and geometry of interaction with nearest solvent molecules is changed. When the relaxation time is on the order of the lifetime of the excited state, emission can occur from a set of excited molecules caught in different stages of interaction with solvent molecules. This leads to a general broadening of the spectrum and asymmetric band shapes.

In addition to specific interactions between molecules of indole and one or several solvent molecules (hydrogen bonding and solvent relaxation), Konev considered long range interactions. In this consideration, the bulk properties of the solvent such as dielectric constant are important. The effects of long range interactions were observed by a red shift in the case of 1,3-dimethylindole which cannot form hydrogen bonds.

In a series of papers, Walker, et al.^{6,7,8,9} provided a different analysis. They were unable to relate the extent of fluorescence shift to solvent dipole moment by assuming any reasonable excited-state moment of indole. They found that the maximum shift occurred at an alcohol concentration so low that it had a negligible effect on the solvent dielectric constant. As a result they suggested that a specific solvent-solute excited-state complex ("exciplex") was responsible for the red shift in the emission.

Walker, et al. claim that the fluorescence emission from mixed solvents consists of a structured emission band characteristic of nonpolar solvents and a structureless band characteristic of emission in pure polar solvents. To them, this mixture of emissions implied that different ground states are associated with the two bands. The broad band arises from a complexed excited indole undergoing a transition to a ground state in which the complex is dissociative. The structured band arises from uncomplexed indole. The ratio of band intensities was predicted to be a function of solvent composition. Studies of the emission from two solvent systems were made; one system was one in which the polar solvent is self-associating and the other system was one in which the polar solvent is nonassociating. With associating solvents, results for indole, 1-methylindole and 1,3-dimethylindole were consistent with the formation of 1:2 excited state complexes. A 1:1 complex was indicated only in the case of nonassociating solvents.

The fact that similar results were observed in the case of the 1 substituted indoles and indole and the fact that the nitrogen atom of indole is electron deficient in the lowest excited state apparently excluded the possibility of hydrogen bonding. The authors found no correlation between the fluorescence shift and solvent dipole moment; the lack of correlation precludes specific dipole-dipole interaction.

From fluorescence lifetime and quantum-yield studies on indole and methylindoles, Walker, et al. report a temperature-independent and a temperature-dependent quenching process. The latter process has a large activation energy. Similar behavior of indole and 1-methylindole is used as evidence to exclude the possibility that quenching involves a change in protonation of the indole nitrogen atom. This conclusion is in disagreement with the conclusion of Stryer which will be presented below. The authors also do not consider possible protonation at other positions on the ring as being responsible for quenching, even though an increase in the charge density on the 2 and 3 positions in the first excited state has been noted.¹⁷

By comparing calculated and observed transition moments, Walker, et al. suggest that the ${}^1L_b \rightarrow {}^1A$ transition is responsible for fluorescence in non-polar solvents. Although they attribute the fluorescence in polar solvent to an emitting species that is no longer indole, a correlation of the emission with the calculated transition dipole moment suggests that the emitting state of the exciplex contains a greater proportion of 1L_a than 1L_b . They state that there may be a state involving mixing of the 1L_a and a charge

transfer state. Their proposal is that complex formation is due to charge-resonance stabilization in which excited indole serves primarily as donor and the polar solvent as acceptor. Although a quantitatively consistent description of the excited-state behavior of 1-methylindole is presented, it is significant to note that the authors feel that indole requires a more complicated interpretation.

In contrast to the conclusion by Walker, et al. that changes in protonation of the nitrogen are unimportant in the case of indole, Stryer¹⁰ has reached the opposite conclusion. His conclusions have been based on the deuterium isotope effects on the fluorescence yield of indole. Stryer has observed a large deuterium isotope effect on fluorescence emission spectra and quantum yields of chromophores which readily participate in proton-transfer reactions. Chromophores not involved in proton-transfer reactions during the excited state are expected by Stryer to show similar quantum yields and emission spectra in H₂O and D₂O. The D₂O/H₂O quantum yield of 1-methylindole is 1.09; of indole, 1.29. This large effect of D₂O on the fluorescence is interpreted in terms of an isotope effect on the rate of proton transfer during the excited-state lifetime. If the chromophore contains a proton-donor group and only the unionized species is fluorescent (indole), the isotope effect is expressed as a change in quantum yield. Emission from the un-ionized species competes with the quenching reaction (proton transfer) the rate of which is faster in H₂O than D₂O. Rates of proton-transfer reactions on the order of 10^8 sec^{-1} have been reported.¹⁰ It is likely that partial

proton transfer to some intermediate species during the lifetime of the excited state would cause similar quenching.

However, Förster and Rokos¹¹ pointed out examples in which a large isotope effect is observed when there is no mobile proton. They assume that a solute-solvent interaction involving a coupling between the electronic motion of the dissolved molecule and the nuclear motion of one or more solvent molecules is directly responsible for internal conversion.

Longworth¹² measured the fluorescence of 1,2-dimethylindole at a constant concentration (1×10^{-4} M) in 3-methylpentane (3MP) containing varying amounts of 2-propanol. At 298°K, an isoemissive point is observed in a series of fluorescence spectra as the alcohol concentration is increased. No change is observed in the absorption spectra of a similar series. He attributed the isoemissive point to a two component equilibrium. Further, he was unable to obtain reproducible results (isoemissive points of stoichiometries) unless the 2-propanol has been dried of water with CaH_2 . With dry 2-propanol a series of indoles was found to exhibit a 1:1 stoichiometry with the alcohol. This is in disagreement with the 1:2 exciplex concept reported by Walker.

Longworth states that increasing the viscosity and lowering the temperature should suppress the exciplex formation because a collision is required. The fluorescence of 1,2-dimethylindole was measured at 77°K in 3MP and in the presence of 0.1 M isopropanol in 3MP. Because these fluorescences are found to differ he concludes that

there must still be an interaction between the alcohol and the 1,2-dimethylindole at 77°K. He found that indole itself in pure 3MP at 77°K has absorption and fluorescence spectra identical with those in 2-propanol (0.1M) glass at 77°K. He attributes the fact that these spectra are identical to aggregation of the indole that does not occur when there is a methyl group on the nitrogen.

Eisinger and Navon¹³ have also questioned the interpretation of Walker, et al. and have suggested that the maximum shift observed at alcohol concentrations that are so low that they have negligible effect on the solvent dielectric constant could be explained if the concentration of alcohol in the indole solvent shell is much greater than that in the bulk solvent.

In their work a red shift was found when tryptophan and 1,2-dimethylindole in ethylene glycol:water (1:1) were studied as a function of temperature rather than of concentration. The shift is continuous and no isoemissive points were found; this observation contrasts the results of Longworth. In the case of indole, tryptophan, 1-methyltryptophan and 1,2-dimethylindole, three changes are noted as the temperature is lowered to 80°K. First, there is a blue shift and development of vibrational structure. Second the fluorescence quantum yield of each compound increases to about 0.6. Most of this increase occurs above the temperature at which the other spectral changes occur. Third, no isotope effect is found at or below the temperature at which the fluorescence quantum yield approaches a constant value. Further evidence was presented to show that the

nonradiative decay is not greatly changed as a result of solvent reorientation.

Eisinger and Navon suggested that the solvent shift was caused by a lowering of the excited-state energy that results from the reorientation of surrounding solvent molecules. This lowering in energy could be due to the excited-state dipole moment or could have subtler causes. The amount of energy lowering would depend on the number of solvent molecules that could reorient during the lifetime of the excited state and therefore on the temperature and viscosity of the solvent. Emission to a nonequilibrium ground state may also be involved.

According to their model, potential energy curves of the equilibrium ground state and the Franck-Condon excited state are well separated in energy. It is proposed that solvent reorientation would lower the equilibrium excited state energy. Fluorescence would be from this state to a nonequilibrium ground state. These two potential energy surfaces could either cross or become close enough to permit tunneling. If either case exists, then a more rapid nonradiative decay is expected. This scheme is fitted to their experimental data.

At low temperatures no isotope effect was noted although Stryer¹⁰ had reported a large effect at room temperature. This discrepancy was attributed to the necessity of solvent and/or solute motion for an isotope effect to be observed.

Lehrer¹⁴ considered the deuterium isotope effect from the opposite viewpoint. Whereas Stryer studied the effect on indole as a deuteron transfer from indole to the solvent, Lehrer presented the possibility that isotope effects could also be caused by the decreased rate of proton transfer from a quencher to indole. This transfer will have a similar effect in that the protonated form of indole appears to have reduced fluorescence.¹⁵

A later paper by Ricci¹⁶ further examined isotope effects on quantum yields and fluorescent lifetimes of indole, 1-, 3-, and 5-methylindoles, 2,3-dimethylindole, 2,3,6-trimethylindole, and tryptophan. The radiative rate constant for fluorescence emission was found to be the same ($4.5 \pm 0.5 \times 10^7 \text{ sec}^{-1}$) for all of the compounds. This consistency of rate constants led to the interpretation that the π -electronic systems undergoing excitation are equivalent and that emission occurs from comparable solvent equilibrated singlet excited states. The ratio of fluorescent lifetimes of each of the above compounds in D_2O and H_2O was found to be the same as the ratio of fluorescence yields. According to Ricci, the fact that these ratios are the same implies that the fluorescence rate constant is the same in H_2O as in D_2O . For this reason, the differences in fluorescence quantum yields and isotope effects must be attributed to variations in the rates of nonradiative deactivation.

Two distinct quenching mechanisms are suggested. A small isotope effect is noted in the case of 1-methylindole in which no isotopic substitution is possible on the nitrogen. The nonradiative

decay of 1-methylindole in H_2O is 1.19 times as efficient as that of 1-methylindole in D_2O . It is postulated that this mechanism involves tunneling in which CH stretching vibrations couple with the OH or OD vibrations of the solvent. Dissipation of energy in OD vibrations is less efficient than OH vibrations; therefore D_2O is a less effective quencher.

The other compounds examined have a mobile hydrogen and show a larger ratio of nonradiative rate constants ($k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$). This larger ratio observed in these compounds is attributed to a second decay path involving deprotonation that is competitive with the tunneling mode. As the number of methyl groups increase, the importance of OH coupling decreases. The isotope effect is then found to increase since deprotonation is more sensitive to isotope effects.

It should be noted that at pH 7, the isotope effect for tryptophan is one and one half times larger than that expected if 3-methylindole is used as a standard. Methylation of the indole nitrogen does not appreciably reduce the isotope effect in tryptophan; this lack of change in isotope effect of N-methyltryptophan indicates that the indole N-H bond is not involved. It is proposed that the quenching reaction involved is a transfer of a proton from the $-\text{NH}_3^+$ group to the 2-position on the indole ring. (This position is more electronegative in the first excited state than it is in the ground state¹⁷). The result is a nonradiative decay to the ground state via 2H-tryptophan.

A continuation of the discussion of the nonradiative deactivation process is provided by Kirby and Steiner.¹⁸ Their paper deals with the effects of temperature and solvent composition on quantum yields and lifetimes. In the case of indole in water, a five fold decrease in quantum yields is observed in going from 5 to 50°C. Analysis shows that the data cannot be rationalized on the basis of a single temperature-dependent quenching process. Assumption of a simultaneous temperature-independent process, however, allows data to be fitted to two exponential curves. It is suggested that in the case of indole and water, emission and the radiationless de-excitation by the temperature-independent process are about equally probable. The temperature-dependent process involves an activation energy of 12.5 kcal/mol and is approximately 1.6 times as probable as emission at 298°K.

The principal effect noted on substitution of D₂O for H₂O is a reduction in the magnitude of the frequency factor of the temperature-dependent process. In the case of indole in nonaqueous solutions the temperature dependence of quantum yields is much less than that of indole in water. In water:methanol mixtures, it appears that the addition of methanol decreases the probability of deactivation by the temperature-dependent process but does not affect the activation energy or alter the probability of the temperature-independent process. Similar results are obtained in mixtures of water with dioxane, dimethyl sulfoxide and propylene glycol; therefore, it is assumed that the observed effects are not due to alteration of the dielectric constant of the medium.

Weinryb¹⁹ has observed an enhanced fluorescence yield in the case of indole and tryptophan in propylene glycol compared with the yield in methanol. He attributes the variation to the higher viscosity of the propylene glycol because the solvents have similar functional groups and dielectric constants.

In a detailed article, Song and Kurtin²⁰ consider indole from experimental results based on polarization studies and theoretical results based on P P P SCF MO-CI calculations. They interpret their data to mean that dual emission from the 1L_a and 1L_b states definitely occurs in both polar (glycerol-methanol) and relatively nonpolar (EPA) solvents.

In contrast, DeLauder and Wahl²¹ recently studied decay times of indole in dioxane, water, methanol and cyclohexane and found no evidence of emission from two excited states. In a cyclohexane:dioxane mixture the decay consisted of a single exponential whereas in methanol:cyclohexane it did not. They were unable to fit the decay curve either to two fixed lifetimes or to two variable coefficients relating fractional contributions of two species to the total fluorescence decay. Basing their theory on exciplex formation, they conclude that in the cyclohexane:dioxane mixture the exciplex reaches equilibrium within the fluorescence lifetime. In methanol:cyclohexane, exciplex formation competes with emission.

Feitelson²² has reported that in ultraviolet irradiated solutions of indole in water at acidic pH, hydrogen atoms can be formed. These hydrogen atoms are produced in a reaction between H^+ and

solvated electrons formed from the excited-state indole. 2-Propanol is used as a scavenger, and the reaction is not observed in the absence of H^+ ions. It is proposed that without the scavenger, reactions like indole + $H \cdot$ would be likely. Arguments against quenching of fluorescence by electron transfer from indole to the quenching species are presented. Fluorescence quenching by H^+ is attributed to protonation of excited indole in the 3 position.

A delayed fluorescence emission of biphotonic nature with a triplet state intermediary has been observed by Aubailly, et al.²³ The process involves a recombination of the ejected electrons with the indole cations formed by photoionization of triplet indole.

There are two interesting papers that deal primarily with the absorption spectrum of indole. Hollas²⁴ interprets the absorption spectrum to consist only of allowed $\pi^* \leftarrow \pi$ transitions. Hot bands to the red of the 0-0 band were observed when a gaseous sample was heated to 100°C in the sidearm of a 1 m cell. The hotband ground state vibration that has a frequency of 760.6 cm^{-1} is identified to be the analog of the 717.6 cm^{-1} frequency in the excited state.

Strictland, et al.²⁵ compare the vapor absorption spectrum of indole at 70°C to the solution absorption spectra in methylcyclohexane and perfluorinated hexane. The same study was made in the case of 3-methylindole except that the vapor spectrum was measured at 100°C. They state that some low energy vibrations are excited extensively even in the ground electronic state. It is reported that the addition of butanol to 3-methylindole in methylcyclohexane shifts

the 0-0 1L_a band to the red side of the 0-0 1L_b . After vibrational analysis of the absorption spectra, they make assignments of bands in fluorescence excitation and emission spectra of indole in cyclohexane at 77°K. Their assignments include two vibrational progressions, one is associated with a 1L_a excited state and the other with a 1L_b excited state.

Although some of the preceding papers will be discussed in more detail later, two points should be mentioned now. First, there are four primary explanations advanced for the behavior of indole: a) dual emission from 1L_a and 1L_b states, b) the exciplex theory, c) a solvent reorientation effect, and d) proton transfer. In spite of the active research reported, the lack of agreement reached among authors and some apparently contradictory data imply that the excited-state chemistry of indole is complex in nature. Second, with the possible exceptions of Konev and Song, there is almost complete neglect of the involvement of the triplet excited state in the consideration of this problem. This failure to consider the triplet state is especially true in their discussions of solvent and temperature effects on fluorescence yields.

C. Indole Rearrangement

In the preceding section the anomalous effects observed in the luminescence of indole were discussed. At least four distinctly different explanations for these effects have appeared in the literature, and these explanations have been presented. A fifth

rationale²⁶ originated from our attempts to explain the observations of other researchers as well as our own results in terms of one internally consistent theory. Our interpretation of the anomalous effects in the luminescence of indole involves the possible rearrangement of indole to 2H- or 3H-indole. The rearrangement and its expected effect on the luminescence of indole is considered in detail in the chapter on experimental results and discussions. In this section the chemistry of indole that indicates that the proposed rearrangement is probable is reviewed. Two cases involving molecules that are known to undergo intramolecular proton transfer during the lifetime of the excited state are discussed for comparison.

In the ground state, delocalization of electron density from the nitrogen atom to the ring pi-electron system occurs. This delocalization gives rise to ten pi electrons on nine centers. Thus, the electron density at the nitrogen atom is diminished and indole is therefore a weak base.²⁷ The principal conjugate acid of indole in strong aqueous solutions has been determined to be the 3-protonated isomer.²⁸ In the nmr spectrum of 3-methylindole in strong acid, the methyl signal is a doublet and occurs at higher field (relative to the aromatic proton signal) than it occurs in in the case of the free base. The doublet results from the proton splitting of the 3 proton and the field shift from the change in hybridization of the 3 carbon. Acid catalyzed deuterium exchange has been noted at the 1, 2, and 3 positions.²⁹ It was not determined whether this exchange is direct with solvent or if it could involve tautomerism.

In other studies, a UV catalyzed rearrangement of 1,3,3-triphenylindene (indene is isoelectronic with indole) yields 1,2,3-triphenylindene.³⁰ It has also been shown that 2,3-dimethyl-3-phenyl-3H-indole and 3,3-dimethyl-2-phenyl-3H-indole are interconvertible under high temperature (150°C) acidic conditions.³¹

A case of indole-indolenine tautomerism has been presented.³² Oxindole treated with triethyloxonium fluoroborate yields II, a compound of mp 110°. Sublimation of II at 80°/0.1 torr produces an isomer shown to be I. Compound I melts at 63°C. Upon melting it resolidifies at 80° and melts again at 110°, interconversion of II having occurred.

I

II

The fact that both isomers are observed indicates their ground state energies are not widely separated; i.e., one isomer is not vastly more stable than the other.

Intramolecular proton transfer in excited fluorescent compounds has been reported. In crystalline 2-(2'-hydroxyphenyl)-benzothiazole and its derivatives, a proton is transferred during the excited state.³³ The fluorescence lifetime is less than 10^{-8} sec

and the fluorescence exhibits a Stokes shift of 7000 cm^{-1} . The explanation for this large Stokes shift is shown in the diagram below:

In this case the absorption is that of the O-H form, intramolecular proton transfer to the nitrogen occurs in the excited state and relaxation to an equilibrium excited state follows. Fluorescence occurs from the N-H form to a meta-stable ground state which reorients to the O-H form.

A similar explanation has been proposed for the red-shifted fluorescence of 3-hydroxy-2-naphthoic acid in solution, but in this case a ground-state equilibrium may be involved.³⁴

D. Theoretical Calculations

Calculations used in this study are based on the "complete neglect of differential overlap" (CNDO) approximation of Pople and Segal.^{35,36,37} This is a semiempirical method involving a linear combination of atomic orbitals, self-consistent field (LCAO-SCF) treatment extended to all valence electrons. In this approximation, the basic Hartree-Fock elements are given by:³⁴

the basic Hartree-Fock elements are given by:³⁴

$$F_{\mu\mu} = U_{\mu\mu} + (P_{AA} - \frac{1}{2}P_{\mu\mu})\gamma_{AA} + \sum_{B \neq A} (P_{BB}\gamma_{AB} - \gamma_{AB}), \quad (1)$$

$$F_{\mu\nu} = \beta_{AB}^0 S_{\mu\nu} - \frac{1}{2}P_{\mu\nu}\gamma_{AB} \quad (\mu \neq \nu) \quad (2)$$

in which atomic orbital ϕ_μ belongs to atom A and ϕ_ν to atom B. The orbital energies and LCAO coefficient are eigenvalues and eigenvectors of the matrix $F_{\mu\nu}$. $P_{\mu\nu}$ is the charge-density and bond-order matrix,

$$P_{\mu\nu} = 2 \sum_i^{\text{occ}} C_{i\mu} C_{i\nu} \quad (3)$$

in which the sum is over the occupied orbitals. P_{AA} is the total charge density on atom A,

$$P_{AA} = \sum_{\mu}^A P_{\mu\mu}. \quad (4)$$

In the above equations, $U_{\mu\mu}$ is the diagonal matrix element of the atomic orbital, ϕ_μ with respect to the core Hamiltonian of its own atom, A. It is essentially an atomic quantity and is a measure of the energy of the atomic orbital (the kinetic energy plus the potential energy in the field of the nucleus and inner shells). $P_{\mu\nu}$ is the diagonal matrix element corresponding to the electron populations of the atomic orbitals, ϕ_ν (the bond order). β_{AB}^0 is a bonding

parameter acting as a proportionality constant between the resonance integral and the overlap integral such that

$$K_{\mu\nu} = \beta_{AB}^0 S_{\mu\nu} \quad (5)$$

in which $S_{\mu\nu}$ is the overlap integral. γ_{AB} is the electron repulsion integral.

A discussion of the specification of the empirical parameters is found in reference 36. The actual calculations were performed by means of an IBM 360/65; a program written by Dobosh³⁸ was used. This program uses the CNDO/2 modification. This version neglects penetration integrals and uses electron affinity along with ionization potential to estimate the local core matrix element, $U_{\mu\mu}$. A discussion of these changes and their advantages is found in reference 37.

The calculations applied to the electronic spectra were based on the use of the CNDO method modified by Jaffé; and Del Bené.^{39,40,41,42,43} The basic equations are identical with those of Pople and Segal. Jaffé and Del Bené divide their calculation into two parts: 1) the determination of the CNDO-MO's by means of an SCF calculation, and 2) the generation of spectroscopic states by means of configuration interaction (CI). Their modifications involve changes in the parameterization of the electron repulsion integrals and the resonance integrals as documented in the series of papers referenced above. The program used was written by Del Bené, et al.⁴⁴

A detailed discussion of the theoretical basis for these calculations will not be given because the results will be used only in a qualitative manner. However, a brief consideration of the rationale in selecting these programs is in order. One of the difficulties which arises in the spectroscopic study of a heteroatomic molecule like indole is the identification or prediction of $n \rightarrow \pi^*$ transitions. Previously, theoretical studies involving electronic calculations have been based on variations of the Hückel method or the Pariser-Parr-Pople SCF-CI scheme. These methods can give very satisfactory accounts of the physical and chemical properties of conjugated hydrocarbons if the parameters are properly chosen.⁴⁵ These methods are deficient because the assumptions of the Hückel method break down in the case of molecules other than alternant aromatic hydrocarbons and the PPP method is limited to pi electrons and treats explicitly only those orbitals antisymmetric with respect to the molecular plane.³⁹ Therefore, neither of these methods is applicable to the study of compounds containing heteroatoms, which have polarizable orbitals in the molecular plane.

Because the CNDO method treats all valence electrons it can be extended to the study of $n \rightarrow \pi^*$ transitions. The conjunctive use of these programs is based in part on the effect of the empirical parameters chosen. Pople's program is parameterized to yield reasonable values for ground-state properties. On the other hand, the modifications by Jaffé were designed exclusively for the study of electronic spectra. The calculated ground-state transition energies

are refined by a configuration interaction treatment. This treatment allows states of the same symmetry that arise from different electronic configurations to interact. The result is that in this latter program properties involving a measure of the difference between energy levels are relatively accurate whereas the values of the levels themselves are not accurate.

Thus the attempt in this case is to use the ground-state results of Pople along with the spectral data obtained from the Jaffé program in order to calculate a more accurate energy level scheme.

Chapter I

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Chapter II

Methods and Materials

A. Solvents

1. Hydrocarbons

Phillips Petroleum Company pure grade isopentane (IP), 3-methylpentane (3MP), methylcyclohexane (MCH) and methylcyclopentane (MCP) were purified by a modification of the method reported by Potts.¹ The method is based on a fuming sulfuric acid wash to remove unsaturated impurities and has been previously used in these laboratories. (See Wharton² or Williamson,³ for example). Although careful examination of these solvents on a programmed temperature gas chromatograph showed presence of more than one component, absorbance measured in a 5 cm quartz cell vs air was less than 0.05 from visible wavelengths to 2400⁰Å and indicated that unsaturated compounds had been removed. It was necessary to examine each solvent after purification because the characteristic absorption of benzene could sometimes be found when different lots of the same compound underwent identical treatment. Solvents were considered to be satisfactory for absorption studies when no absorbance in the range of measurement were

observed. Emission solvents were tested under experimental conditions and were used when solvent emission was not observed.

2. Acetonitrile

Fisher Certified acetonitrile was dried over magnesium sulfate and distilled under vacuum.

3. Methyl Alcohol, Methylene Chloride and Mixed Alcohol

The above solvents are Hartman-Leddon Company Fluorimetric Grade solvents and were checked and used without further purification. Mixed Alcoholic solvent is a mixture of methyl, isopropyl and ethyl alcohols.

4. EPA Mixed Solvent

Matheson Coleman & Bell EPA (ethyl ether, isopentane and ethyl alcohol; 5:5:2) was used without further purification.

5. Ethyl Alcohol

Absolute ethyl alcohol from U. S. Industrial Chemicals Company was used without purification. Because this solvent was used only for double-beam absorption measurements, the slight trace of benzene usually present did not interfere with the measurements.

6. NMR Deuterated Solvents

Deuterium oxide (Stuart Oxygen Company), dimethyl sulfoxide- d_6 , chloroform- d , acetone- d_6 , benzene- d_6 , and acetonitrile- d_3 (all from Diaprep, Inc.) were used without purification.

B. Compounds

1. Indole

Indole from Matheson Coleman & Bell and Nutritional Biochemical Company was purified by means of one of the following methods.

a. Vacuum Sublimation: An all glass vacuum sublimation device containing a cold finger for liquid nitrogen was used. The sublimator containing indole was purged with nitrogen and evacuated to 5×10^{-5} torr while the indole was kept cold. The system was then sealed and heated gently with warm air from a hair dryer over a period of an hour. The apparatus was protected from light during this time. After the sublimation was complete, the vacuum was replaced with a nitrogen atmosphere and the sublimator moved to a nitrogen filled glove bag where the indole was transferred to storage vials and sealed under nitrogen.

b. Continuous Temperature Gradient Sublimation: A modified continuous temperature gradient fractional sublimator similar to that designed by Berg and Hartlage⁴ was used. As modified, the apparatus consisted of a forty-two inch length of $\frac{1}{2}$ " rigid copper pipe having an inside diameter of $\frac{3}{8}$ ". The pipe was wrapped with Scotch Brand electrical tape No. 27, a glass cloth that has thermosetting adhesive that will withstand temperatures of 150°C . The wrapped pipe was wound with nichrome wire with a gradual increase between windings to produce a temperature gradient. A 3' section of $\frac{1}{2}$ " mm pipe size fiberglass

insulation was applied. A similar design has been found to produce a linear temperature gradient along the cell.⁵

The sublimator may be operated in the vertical or horizontal position. The cell used was a piece of 9 mm OD glass tubing, the interior of which had been thoroughly cleaned and dried. A small aluminum foil sample boat was introduced into one end of the cell between two glass wool plugs. The cell was placed into the sublimator, and the sample boat was at the high temperature end. The cell was flushed with deoxygenated nitrogen before it was heated, and a slow nitrogen flow was maintained during heating. The tube outlet was either connected to a fume hood or a partial vacuum. The nitrogen was deoxygenated by bubbling it slowly through two solutions of vanadium (II) sulfate in dilute sulfuric acid in the presence of zinc amalgam according to the method of Meites and Meites.⁶ Temperature was controlled by a Variac transformer and was generally regulated so that a sublimation required twelve hours.

In the sublimation of indole both clear needle-like crystals and clear thin film crystals having the appearance of cellophane were formed. DTA-TGA analyses of these crystals showed identical behavior.

2. 1-Methylindole

1-Methylindole (1MI) obtained from Eastman Organic Chemicals was distilled under vacuum.

3. 2-Methylindole, 3-Methylindole

2-Methylindole (2MI) and 3-methylindole (3MI) from Matheson Coleman & Bell were purified by the method described for the purification of indole.

4. 2-Ethoxyindole

Samples of 2-ethoxyindole (2EI) were provided by Dr. Stephen Monti of the University of Texas at Austin. They had been purified in his laboratory by two sublimations (80°C/1.0mm) and one crystallization from diethyl ether. Samples were used in the condition received.

C. Measurements

1. Differential Thermal Analysis, Thermogravimetric Analysis

DTA-TGA measurements were performed by means of a Dupont 900 Thermal Analyzer having a Dupont 950 TGA attachment.

2. Gas Chromatography

GC solvent analysis was done by means of an F&M Scientific Corp. Model 810 Research Chromatograph; programmed temperature mode and flame ionization detection were used. The column was 1/8" aluminum 12' long, packed with SE 30.

3. Laser Raman

The laser raman spectrum of indole was measured by means of a Japan Electronics Optics Laboratory Co., Ltd. JRS-S1 instrument that is equipped with a Coherent Radiation Laboratory argon laser.

4. Nuclear Magnetic Resonance

NMR spectra at 100 M Hz were measured by means of a modified Varian HA60. The 60 M Hz spectra were measured by means of either a Varian A60A or a Perkin Elmer R12B equipped with an R112 double resonance accessory.

5. Photoelectron Spectroscopy

Photoelectron spectra (PES) were measured by means of a Perkin Elmer model PS18 photoelectron spectrometer.

6. Photochemistry

In the attempt to produce a molecular rearrangement in 1-methylindole, a Rayonet Type RS Preparative Photochemical Reactor RPR 204 was fitted with four #RUL 2537⁰ high intensity low pressure mercury lamps having a total output of approximately 60 watts of 2537⁰ UV.

Assuming a total absorbance of one watt and an arbitrary criterion of one reaction per twenty photons absorbed per molecule, a reaction time that was based on the concentration of lMI was calculated. Samples of 0.2 M lMI in 15 ml of methanol were degassed, sealed in quartz emission tubes and placed in the photoreactor for periods of from one to ninety hours. The sample was air cooled during irradiation. Each sample was then concentrated under vacuum and its nmr spectrum was measured.

7. Fluorescence Lifetimes

A TRW model 75A Decay Time Fluorometer system was used to measure fluorescence lifetimes. Lifetimes were measured in the case of degassed samples of the indoles in quartz tubes at concentrations of 10^{-5} M. The standard procedure for measuring decay times from 1.7 to 10 nanoseconds (ns) was used. The values that were obtained were reproducible to ± 0.5 ns and were found to be in reasonable agreement with literature values later published by Walker, et al.⁷

8. Ultraviolet-Visible Absorption

The absorption spectra of the molecules studied were measured by means of a Cary 14 Recording Spectrophotometer. According to the manufacturer, the resolving power of the monochromator is $1\text{\AA}$ throughout the range of $1900 - 30,000\text{\AA}$. This is equivalent to 25 cm^{-1} at $2000\text{\AA}$ and to 11.3 cm^{-1} at $3000\text{\AA}$.

The effect of temperature on the absorption of indole was examined. The concentration of indole in 3MP was adjusted so that the long wavelength portion of the spectrum had an absorbance of 0.2 at room temperature and then the 1 cm quartz cell was sealed. The top, bottom and sides of the sample cell compartment were insulated with styrofoam, and the hose from a hair dryer directed upon the cell through a hole in the top. Several spectra were measured during heating; the final compartment temperature was measured with a thermometer to be 48°C .

Low temperature absorption measurements of the spectra of indole in a 1:1 mixture of isopentane/3methylpentane (IP/3MP) were made at 77°K by means of the apparatus shown in Figure 2. A 1 cm Spectrosil fluorescence cell was fused to the base of a round quartz emission tube. A partially silvered quartz dewar flask was fitted into the sample compartment and the special cell was centered in the dewar by means of a vented Teflon plug that was fitted with an O-ring to hold it firmly in the top of the flask. The center of the plug was machined to fit a plastic insert that had a brass fitting that secured the top of the emission tube. This arrangement allowed the

Figure 2. Low temperature apparatus for
absorption and emission measurements.

- A- Teflon Plug
- B- O Ring
- C- Quartz Emission Tube
- D- Quartz Dewar
- E- Brass Fitting
- F- Plastic Insert
- G- Vent Holes

Side view

cell to be placed at any height and to be rotated while the cell remained rigidly centered. It also provided considerable advantage over other holders in that it involved no metal - liquid nitrogen (LIN) contact that caused bubbling in the optical path. The same Teflon holder was used extensively in the emission studies to be described later.

Observation of the solvent scatter of visible radiation was used to center the cell in the light path. The scattering and absorption due to the quartz layers and the LIN in the sample compartment were compensated by quartz plates and neutral density filters that were placed in the reference side along with a solvent filled 1 cm cell. A pure solvent spectrum was then taken over the range of interest to check the baseline. For comparison the room temperature spectrum of the same sample was measured on the same apparatus but without the LIN.

9. Emission Studies

a. Cary 14 Some emission studies were made by means of the Cary 14 as an emission monochromator-recorder unit coupled with a Cary 15 light source and monochromator as the excitation unit. This system has been adequately described by Holloway⁸ or Hughes.⁹

Samples for use with this instrumentation and that to be described were 10^{-5} M solutions contained in quartz emission tubes. In almost all cases, the samples were degassed with at least four freeze-pump-thaw cycles at 10^{-5} torr and the tubes were sealed while the sample was at this pressure.

In certain studies it was desirable to work with fresh samples in ultra-dry solvents. These were prepared by drying the solvent with lithium aluminum hydride and distilling it under vacuum directly into the emission tube containing the compound to be examined.

Variable temperature measurements were made by means of the apparatus shown in Figure 3. A Variac controlled nichrome coil heater was inserted through a glass "T" tube into a 5 l metal LIN storage dewar. The "T" tube was sealed on the Variac side. Heating the coil caused the nitrogen to boil and forced cold gas along the insulated copper tubing. An auxiliary Dewar containing a copper cooling coil in LIN could be added as required. The cold gas was directed on the emission tube through four small copper tubes designed to be inserted through the Teflon holder described previously. The cooling rate was controlled by varying the heater voltage; temperature was monitored with a thermocouple. The efficiency of the system was such that the Joule-Thompson effect at the openings of the small tubes would reliquify the nitrogen if a fast boil rate was used. The emission Dewar could then be easily filled with LIN if longer studies were desired at liquid nitrogen temperature. Warmer temperatures were also obtained by filling the auxiliary Dewar with hot water and using air in the system.

b. Component Emission Spectrophotometer, (CES) Other emission spectra were measured by means of a component system type emission spectrophotometer designed by Dr. J. H. Wharton and assembled by Dr. E. Hughes, Jr.¹⁰ and Dr. Wharton.

Figure 3. Apparatus for variable temperature measurements.

- A - Variac Transformer
 B - LIN Storage Dewar
 C - Nichrome Heater Coil
 D - Insulated Copper Tubing
 E - LIN Filled Dewar
 F - Quartz Emission Dewar

The excitation source of the CES consists of a 450 watt xenon arc lamp in a water-cooled, nitrogen-flushed housing. The lamp is coupled to a Cary 15 scanning monochromator having continuously adjustable slits to 3.0 mm. The output of the excitation monochromator is focused by a concave mirror onto the front surface of a quartz emission tube. Radiation from this surface is chopped with a Princeton Applied Research Corp. (PAR) Model 125 Mechanical Light Chopper. The chopper operates in conjunction with a PAR 220 Lock-In Amplifier, and its purpose is to interrupt the emission signal and to simultaneously generate a square-wave reference, synchronous with the chopping rate, which is fed to the amplifier.

The chopped emission signal is focused on the entrance slit of a Jarrell Ash 0.5 Meter Ebert Scanning Spectrometer. The manufacturer reports a resolution of at least $0.2 \text{ \AA}$ and a wavelength counter accuracy of $\pm 2 \text{ \AA}$. The monochromator is equipped with a dual unilateral entrance and exit slit assembly that provides openings between 0 and 3000μ . The exit slit is followed by a PAR 182 Photomultiplier Housing equipped with an E. M. I. 9558 QB end-on photomultiplier tube (PMT). The active area of the photocathode is limited by a magnetic lens assembly. This was necessary in order to reduce the current due to photoelectrons generated by Cerenkov radiation from cosmic rays.

The PMT is powered by a PAR 280 High Voltage Power Supply which provides a high quality regulated voltage. This unit and all following PAR components were packaged as Research Instrument Modules and were housed in and powered by a rack mounted PAR 200 NIM Bin.

The signal from the PMT was amplified by means of a PAR 221 AC Photometric Preamplifier. This unit is a high gain amplifier consisting of two physically separated units: A low leakage current amplifier mounted on a plug in circuit board located inside the photomultiplier housing and the remaining circuitry and operating controls mounted in the Bin. Location of the preamp input circuit in the PMT housing eliminates the need for long lines carrying low signal levels at high impedance.

Following the preamplifier is a PAR 211 amplifier. This amplifier is a low noise amplifier that has a 10^6 ohm impedance and flat frequency response from one Hz to 10^6 Hz. The signal is then fed to a PAR 220 Lock-In Amplifier, which is considered by the manufacturer to be the "heart" of a low-level signal recovery system. It enables an AC signal to be measured in the presence of noise by means of phase sensitive detection. The reference signal from the chopper is fed to the lock-in amplifier along with the input signal. The mixer component of the amplifier acts as a double pole, double throw switch and the synchronous frequency of the input is rectified as a DC signal; a diagram of the switch is shown in Figure 4. Non-synchronous components of the input such as noise are not rectified and are removed by the low pass filter. This results in a large signal-to-noise-ratio improvement.

Figure 4. The Mixer as a double-pole, double-throw switch.
(As taken from the PAR Model 220 Manual)

From the lock-in amplifier, the signal is fed to a Hewlett-Packard Model 7004A X-Y Recorder equipped with a H-P Model 17172A Time Base Generator to drive the X-axis. The amplifier also provides a signal monitor jack which is used to interface the amplifier with an analog to digital converter (ADC) that is connected to a computer. A block diagram of the instrumentation is shown in Figure 5.

c. Computer Processed Spectra During the course of the work, an on-line "mini-computer" was added to the system for the purpose of processing spectra. The computer was a Data General Corporation NOVA 1200 that has 8K of memory. Its peripheral equipment consisted of

Figure 5. Block diagram of the component emission spectrophotometer.

a 4033 Analog to Digital Converter (ADC), two 4037 Digital to Analog Converters (DAC), teletypewriter, slow speed paper tape punch and paper tape reader, high speed paper tape reader, a 4008 real-time clock and a Tektronix Type 611 Storage Display Oscilloscope.

All software for use with the CES was designed and programmed by Mr. Henry Streiffer and Mr. Cecil Reames of this Department.

The program, entitled "Spectrometer Driver," consisted of a versatile series of option choices that are briefly documented below:

RESTART-	Begins program; all parameters are initialized to previous values.
QUIT-	Ends program after run.
PARAM-	Questions user to allow entry of initial parameters such as chart length, chart units, scan speed, and number of points stored. When the full 8K of memory is used, the capacity is approximately 3000 points.
RUN-	In the Run mode, the CES is operated in the normal way; the NOVA receives and stores data points which duplicate the spectrum shown on the recorder. After the spectrum is measured and stored, the initial and final wavelengths are entered via the teletypewriter.
PLOT-	Any spectrum stored in the computer is replotted, either on the cathode ray tube (CRT) or the recorder or both.
PUNCH-	The spectrum in memory is punched out on paper tape for later processing.

EMISS-

Each stored point is recalled from memory, corrected and deposited back into memory. The process destroys the original spectrum. Corrections are required because the CES has a non linear response to radiation. The correction factors were determined by means of a modified method of Melhuish¹² that was confirmed by Argauer and White.¹³ The method involves measuring the energy output of the excitation monochromator as a function of wavelength by means of a quantum counter. A solution of 3g/l of Rhodamine B in ethylene glycol was used as a sample. The quantum counter was removed and replaced by Dupont Ludox solution and the scattered energy was measured. The calibration factor is:

$$S(\lambda) = \frac{Q(\lambda) M(\lambda)}{R(\lambda) B(\lambda) W(\lambda)}$$

in which $Q(\lambda)$ is the response of the quantum counter to energy leaving the monochromator; $R(\lambda)$ is the response of the PMT and emission monochromator to the reflected energy; $B(\lambda)$ is the bandwidth of the monochromator; $M(\lambda)$ is the transmittance of the quantum counter window; $W(\lambda)$ is the reflectance of the light scattering solution and $S(\lambda)$ is the factor by which the PMT readings must be multiplied to give the relative quanta per second.

- EXCIT- This subroutine corrects an excitation spectrum by the process described above.
- INVERT- The initial points are plotted as intensity vs wavelength. This subroutine changes the stored spectrum to a plot of intensity vs cm^{-1} .
- REVERSE- This entry will reverse the order of the points so that a spectrum originally taken from high to low energy may be replotted from low to high energy.
- ENTER POINTS- A previously punched spectrum on paper tape may be reentered for processing.
- INTEGRATE- This command sums the Y-axis values as the spectrum is scanned and the result is similar to the familiar integration of an NMR spectrum. Relative quantum yield values for fluorescence and phosphorescence may be determined by integrating corrected spectra.

There is also a series of subroutines that allows input from or output to the various peripheral devices.

10. Calculations

a. CNINDO: CNDO and INDO Molecular Orbital Program¹⁴

The program referred to above is a written for the IBM System 360/67 digital computer. It was used in the calculations of CNDO molecular orbitals and ground state energies of the indoles.

b. CNDO/2-CI Molecular Orbital Calculations with the Complete Neglect of Differential Overlap and Configuration Interaction¹⁵

This program performs molecular orbital calculations for molecules in closed shell configurations and uses the CNDO/2 method of Del Bené and Jaffé¹⁶ with the following revisions:

1. The two-center two-electron repulsion integrals are calculated by the Mataga approximations.
2. The CI matrix is symmetry blocked.

c. Cal-Comp Plotted Spectra

Some spectra included were corrected and plotted by means of the IBM 360/65 and a "Cal-Comp" plotter. The software for the corrections and plots was furnished by Dr. R. H. Hofeldt of this department.

Chapter II

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Chapter III

Results and Discussion

A. Absorption Spectra of Indoles

The absorption spectra of indole, 1-methylindole, 2-methylindole, and 3-methylindole in polar and nonpolar solvents at room temperature ($25 \pm 2^\circ\text{C}$) were measured. The spectra of the first two electronic transitions of indole in two hydrocarbons and three alcoholic solvents are shown in Figure 6. These spectra have been shifted along the wavelength axis so that the prominent long-wavelength absorption band coincides. This wavelength presentation was adopted in order to compare the bands with the results obtained by Strickland, et al.,¹ who used a similar presentation.

They observed that solvent perturbations on going from the vapor to perfluorinated hexane to hexane caused very little shift in the positions of the $^1\text{L}_b$ transitions. It was noted that the bands identified as $^1\text{L}_a$ transitions were much more sensitive to solvent perturbations. It appears from measurements made in these laboratories that this interpretation is valid because the change of solvent in the

Figure 6: Room temperature absorption spectra of Indole. Concentration: 1×10^{-4} M; 1) 3MP, 2) IP/3MP, 3) 1% EtOH in IP/3MP, 4) 15% EtOH in IP/3MP, 5) 100% EtOH.

case of indole produces very little shift in positions of the 1L_b transitions. If the identification of transitions provided by Strickland, et al. is used, the 1L_b 0-0, 0 + 930 and 0 + 1310 transitions are observed and coincide even in the wavelength plot. The 0 + 730 band is observed and coincides in the hydrocarbons.

The 0-0 1L_a transition is covered by the 1L_b bands.

According to Strickland, et al., it occurs around 274 nm in the vapor and 279 nm in hydrocarbon solvent and shifts to the red as the solvent perturbation increases. The unassigned bands in the short-wavelength portion of the spectra are primarily 1L_a transitions although there is probably an overlap of 1L_b transitions. These short wavelength bands show a greater solvent shift than do the 1L_b transitions. A change in solvent from pure hydrocarbon to absolute ethanol produces a maximum shift of the 1L_b 0-0 transition of approximately 150 cm^{-1} . Although there is obvious difficulty in identifying a given 1L_a transition, it appears that the maximum shift involved is about 800 cm^{-1} . These figures agree well with those of Konev² who found 150 and 700 cm^{-1} respectively. This shift is not unusual; under similar conditions carbazole shifts around 600 cm^{-1} .²

In Figure 7, a similar presentation is made. In Figure 7, spectrum of indole in the aprotic solvent, acetonitrile, is included along with those of indole in hydrocarbon and alcohol. Because similar behavior is noted in both polar-protic and polar-aprotic solvents, the shift appears to be due to solvent polarity rather than to any hydrogen bonding effect. This interpretation is in disagreement

Figure 7. Room temperature absorption spectra of indole. Concentration: 1×10^{-4} M; 1) IP/3MP, 2) Acetonitrile, 3) 5% EtOH in IP/3MP.

with Konev who presented hydrogen bonding in the ground state as the reason these two types of transitions are shifted through different absolute distances.

The absorption spectra of the first two transitions of the 1-, 2-, and 3-methylindoles are shown in Figures 8, 9, and 10. The red shift in the alcoholic solvent is greater in the case of 2- and 3-methyl derivatives than in that of indole itself. This greater red shift is only partially due to the fact that they are presented on a true wavelength scale instead of being shifted to superimpose the leading bands. A comparison of the original spectra shows that the greater red shift in alcoholic solvent of the methyl indoles absorptions compared with the indole absorption is a real effect. In the case of 3-methylindole in hydrocarbon, Strickland, *et al.*¹ place the 0-0 transition of the 1L_a band underneath the prominent long-wavelength region of the 1L_b band. According to their interpretation, addition of a drop of butanol shifts the 0-0 band of the 1L_a transition 5 to 50 nm to the red of that of the 1L_b transition. The absorption spectra shown in Figure 10 support their interpretation of the 3-methylindole spectra. It is also possible the 0-0 band of the 1L_a transition falls at lower energy than that of the 1L_b transition of 2-methylindole. In contrast the long wavelength band of 1-methylindole in alcoholic solvent shows only a slight shift and broadening.

A peculiarity in the spectrum of 1-methylindole is the slight shoulder in the long-wavelength portion of the hydrocarbon absorption.

Figure 8. RT absorption spectra of 1-methylindole in
IP (—) and 50% EtOH and IP (- - -).

1-Methylindole

Figure 9. RT absorption spectra of 2-methylindole in
IP (—) and 50% EtOH in IP (----).

2-Methylindole

Figure 10. RT absorption spectra of 3-methylindole in
IP (—) and 50% EtOH in IP (----).

An enhancement and redshift of this band in alcohol would have suggested that this band is the 0-0 1L_a transition; however, no enhancement and redshift occurred. In addition, the band is too weak to be part of the 1L_a transition.

Strickland, et al.¹ report hot bands in the vapor-phase spectra of indole at 70° and 3-methylindole at 100°. Hot bands were also reported in the case of 3-methylindole in perfluorinated hexane, but the authors did not list a temperature for this measurement. In these laboratories, the absorbance of indole was examined for temperature effects by the method described in the experimental section. For "high" temperature studies, the concentration in 3MP was adjusted until the leading band at 2867 Å had an absorbance of 2.3, and the sample compartment was heated. A comparison of the spectra of indole at room temperature (RT) and at 48°C showed only a very slight red shift of the entire leading edge of the 2867 Å band. No new bands of the energy reported for the hot bands were found under these conditions.

The absorption spectra of indole in 1:1 IP/3MP at RT and 77°K were measured under otherwise identical conditions. The results are shown in Figure 11. After the compound was cooled, the bands previously identified as 1L_b increased in absorbance from 0.51 to 1.65. The 1L_a portion of the spectrum at 2600 Å increased only from 1.08 to 1.14 absorbance units. No unusual shifts were observed.

It should be noted that in general the absorption spectroscopy of indole behaves like that of many other molecules. A previous comparison with carbazole indicated the similarity. Molecules

Figure 11. Absorption spectra of 5×10^{-4} M indole in 1:1 IP/3MP at room temperature (bottom trace) and 77°K (top trace).

like indole that are capable of forming hydrogen bonds will usually exhibit a slight solvent shift when the solvent is changed from nonpolar to associating solvents; the magnitude and nature of this shift is reasonably well understood and not considered related to the anomalous emission properties of indole.

B. Luminescence of Indoles

Some general observations will be presented prior to detailed consideration of individual spectra. For reference at this time, the total emission spectrum of indole in IP/3MP (isopentane and 3-methylpentane, 1:1) is presented in Figure 12. The phosphorescence extends from 19,000 to 25,000 cm^{-1} and is well separated from the fluorescence at 27,000 to 34,000 cm^{-1} . The normal excitation wavelength is 280 nm (35,800 cm^{-1}).

Solid indole as a solid and indole in concentrated solutions exposed to the air over a period of weeks will darken when exposed to light. The process will occur more rapidly when the sample is heated above 40°C. Although samples of indole used for luminescence studies were almost always prepared immediately prior to use, initial tests were made to ascertain the stability of indole under the experimental conditions. This testing was considered to be necessary because some experiments required that the sample be exposed to uv radiation for a period of hours.

The concentrations used in the luminescence studies were generally on the order of $1 \times 10^{-5} \text{ M}$. In most measurements, the sample was

Figure 12. Emission of indole in IP/3MP at 77°K.

INDOLE IN 1P/3MP
77 K EXC 280

sealed under vacuum after the solvent had been thoroughly degassed. On several occasions the same sample was measured on successive days after over 12 hr. total exposure to uv light before the final measurement. The emission was unchanged. One sample that was over two months old and had a light straw color, still exhibited the same characteristic fluorescence and phosphorescence; the stability of the emissions indicated that the possible oxidation products did not interfere with emissions excited at the wavelengths used in these studies. Thus indole under these conditions is sufficiently stable for routine use. It is emphasized that the stability referred to here is with respect to irreversible reactions; the theories to be examined in this work involve modifications of indole in the excited state which are considered to be of a reversible nature.

Several experiments were performed to determine the effect of the exciting light in general (as opposed to the specific excitation wavelength) on the emission. On three occasions the fluorescence of indole in IP/3MP was measured while the sample was cooled to 77°K and the emission monochromator was set at 300 nm ($33,300\text{ cm}^{-1}$). While the emission signal was being monitored, a shutter was opened and the exciting light was allowed to strike the sample tube. In two of these experiments there was immediate initial signal rise followed by a decrease of approximately 15 to 20% in the first three minutes. After this time the signal was constant. In the third experiment, no decrease was observed for over 15 min. after which time a gradual decrease of around 10% occurred over the next 30 minutes.

Some of the observed anomalies of indole and other compounds in glassy solvents might be the result of an excitation effect. If there is a reaction occurring in the excited state, it is possible that one set of results was observed when the solution is radiated during the freezing process and another set was observed when the solution is excited after the solution was solidified.

Because MCH/MCP (methylcyclohexane and methylcyclopentane, equimolar) forms a hard glass at 77°K, it was chosen for this experiment. A sample of indole was frozen and the emission was monitored at 300 nm. The exciting light was then allowed to strike the sample. An immediate initial emission signal was noted, and then a gradual increase in signal of approximately 20% occurred over the first 3 minutes. The signal then remained constant. The total emission spectrum was recorded. Similar monitoring of the fluorescence while freezing the sample during excitation could not be performed initially because of the vigorous bubbling. (An experiment using slow cooling with gaseous nitrogen will be described later.) However, the same sample was frozen while it was being irradiated, and the total emission again recorded. In order to measure relative quantum yields, the areas of the fluorescence and phosphorescence were measured with a planimeter in both cases. The ratio of phosphorescence to fluorescence yields, ϕ_P/ϕ_F , was 0.84 when the sample was frozen prior to excitation and 1.39 when excited during the solidification.

There appear to be two effects requiring explanation. First, there is an increase in fluorescence noted in MCH/MCP whereas a decrease was noted in the case of similar experiment involving IP/3MP. This increase in fluorescence may be due to an increased solvent quenching because of localized melting of the softer IP/3MP glass at the point of radiation. The second effect is the change in the relative quantum yields. The increase of ϕ_P/ϕ_F that occurs when the sample is irradiated during freezing supports the following interpretation. There is an excited state reaction occurring that requires some reorientation or rearrangement of indole which is favorable to an increase in the rate of intersystem crossing. When the solution is frozen prior to excitation, the geometry of indole is fixed and the reaction is inhibited.

Relative quantum yield data from other measurements were compared. The data were obtained from measuring the uncorrected areas of phosphorescence and fluorescence with a planimeter.* These data are shown in the left portion of Table 1. It became apparent upon examination of these ratios that samples prepared in the same solvent on separate occasions yielded different results. Although the emission

*Calibration data were available for correction of the excitation monochromator. From a correction curve based on these data and the manufacturers average response curve for the emission monochromator and photomultiplier tube, it is estimated that the corrected ϕ_P/ϕ_F ratio would be 1.3 to 1.5 times the uncorrected ratio. Because of the qualitative use of these data, the uncorrected spectra introduce no appreciable error.

Table 1

Regular Solvents	ϕ_P/ϕ_F	"Extra Dry" Solvents	ϕ_P/ϕ_F
EPA	0.6	IP/3MP, $1.0 \times 10^{-3} M$ Butanol	4.6
EPA	0.4	" $1.0 \times 10^{-2} M$ "	15
MCH/MCP	0.3	" $3.8 \times 10^{-2} M$ "	1.1
IP/3MP	0.8	" $7.0 \times 10^{-2} M$ "	0.8
IP/3MP	0.5	IP/3MP	40
IP/3MP	0.9	IP/3MP	40

grade solvents were considered to be dry (presence of water will cause clouding or cracking of the solvent glass when supercooled), the known sensitivity of indole emission to minute quantities of polar solvents led to the use of "extra dry" solvents. These solvents were distilled under vacuum from liquid containing lithium aluminum hydride through glass wool plugs directly into the sample tube. Such sample preparations will be referred to as "very dry" or "extra dry". Yield results using these solvents are listed in the right portion of Table 1. There is a very large increase in the yield of phosphorescence to fluorescence.

Consideration of the spectra from which these measurements were taken will make discussion of results more meaningful. The total emission of indole in very dry MCH/MCP is shown in Figure 13. The fluorescence at room temperature is essentially a mirror image of the lowest energy absorption in hydrocarbon solvent and is assigned to the $^1L_b \rightarrow A_1$ emission of the monomer indole. At $77^\circ K$, the fluorescence of

Figure 13. Total emission of indole in very dry MCH/MCP at RT, 77°K.

INDOLE IN MCH/MCP EMISS

○ RT SW 0.22 MM

▲ 77 K SW 0.36

the same sample shows a decrease in yield and more pronounced structure. There is a relatively strong phosphorescence.

In extra dry IP/3MP (Figure 14) a very unusual effect is observed. From room temperature down to the glass-point of the solvent, the fluorescence is that characteristic of indole in hydrocarbon. When the transition to supercooled liquid occurs, the fluorescence is quenched, and a very intense phosphorescence appears. Except for the increase in intensity, this phosphorescence is identical with that in MCH/MCP. This experiment was repeated several times. In most cases, if the slit width were tripled, a weak fluorescence was recorded. This weak fluorescence still possessed a Franck-Condon envelope with a shape typical of indole in hydrocarbon. On returning the sample to room temperature, the fluorescence was identical to that originally recorded.

Kasha³ has suggested the possibility that the enhanced phosphorescence is due to microcrystal formation. In his work with 7-azaindole in dry all-hydrocarbon solvents, he noted an apparent enhancement of phosphorescence accompanied by a change in the Franck-Condon envelope when solute micro-crystals were present.

The presence of micro-crystals is not believed to be the reason for the similar observation in the case of indole. Although a change in the Franck-Condon envelope is noted in one case, namely that of indole in hydrocarbon + butanol, there is no apparent change in the envelope in the case of a pure hydrocarbon solvent. It would be reasonable to assume that the micro-crystal effect would be more

The figure area is mostly blank, suggesting the plot content is not visible or has been redacted. Only a small label 'a. 14' is present at the top left of the figure area.

Figure 14. Total emission of indole in very dry IP/3MP at RT, 77°K.

pronounced in the all-hydrocarbon solvent. In order to investigate this matter further a sample of indole was placed in IP/3MP and the concentration increased to approximately 10^{-3}M so that the solution appeared opalescent on cooling to 77°K . In this case the fluorescence did disappear; however, no phosphorescence was observed either.

As earlier noted, Longworth⁴ reported micro-crystalline behavior of indole in very dry 3MP. He reported that the fluorescence was present but appeared structureless and identical with the spectrum of indole in a hydrocarbon-alcohol mixture. As stated above when a diminished fluorescence was observed in our studies, the fluorescence was characteristic of monomer indole. In support, Konev² noted that indole in a nonpolar solvent (hexane) exhibits hardly any fluorescence and gives intense phosphorescence. Possibly one of the better arguments against the micro-crystal interpretation results from the quantum yield studies previously discussed. The ϕ_P/ϕ_F ratio is different when the same sample is frozen before radiation in comparison with the ratio when the sample is frozen during radiation. Presumably the degree of micro-crystal formation would be the same in either case.

In very dry IP/3MP containing small to large quantities of 1-butanol, a variety of effects are observed. In approximately $5 \times 10^{-3}\text{M}$ 1-butanol the emissions at room temperature and 77°K are still similar to those of indole in hydrocarbon (Figure 15). The fluorescence yield apparently decreases at low temperature relative to that at room temperature. When the concentration is increased to $3.8 \times 10^{-2}\text{M}$ the fluorescence still shows structure at room temperature. At low

Figure 15. Total emission of indole in very dry IP/3MP, 5×10^{-3} M butanol
RT, 77°K .

INDOLE IN 0.005M 1-BUTANOL

○ 77 K SW 0.34

△ RT SW 0.46

temperature the measured area of fluorescence is similar to the area at room temperature for the same slit width (Figure 16).

At a concentration of $1 \times 10^{-1} \text{ M}$ (approximately 1%) 1-butanol, the room temperature fluorescence is now the broad structureless band characteristic of indole in alcohol. The maximum is redshifted approximately 2000 cm^{-1} in comparison with that of indole in IP/3MP. At low temperature the emission is similar to that at lower alcohol concentration.

This last measurement seemed to indicate that there is a temperature effect other than that associated with the normal increased resolution of vibrational structure at low temperature. For a more careful study, a solution of indole in $7 \times 10^{-2} \text{ M}$ 1-butanol was examined; the variable temperature device described in the experimental section was used. At 24°C , 35 to 36°C , and 44 to 45°C , the fluorescence band shape was unchanged; the integrated fluorescence decreased approximately 10% as the sample was heated from 24 to 45° . The sample was then slowly cooled. Some of the emission curves and the temperature range at which they were measured are shown in Figure 17. The fluorescence of indole at -63.5 to -64°C has the same spectral shape as that at room temperature and has a maximum at 325 nm . The measured area, however, has increased over 100%. As the solution was further cooled, the broad band began to show structure. The appearance of structure occurred between -100 and -140°C . The fluorescence yield appeared to increase until the point at which the curve from -164 to -165°C was measured. The only change noted from a comparison of this spectrum

Figure 16. Total emission of indole in very dry IP/3MP, 3.8×10^{-2} M butanol at RT, 77°K.

INDOLE IN 0.038M 1-BUTANOL
EMISS (EXC 280NM)

○ 77 K 9W 0.56
△ RT 9W 0.56

Figure 17. Emission-temperature study of indole
in 7×10^{-2} M 1-butanol in IP/3MP.

with one measured from -188 to -190° was a slight decrease in yield. As the measured temperature decreased beyond -192° , the nitrogen gas began to liquify. The third spectrum shown (-195 to -196°C) was taken after the Dewar had filled with liquid nitrogen. In this spectrum the phosphorescence is present and there is an apparent further decrease in fluorescence intensity.*

This behavior of indole in alcoholic solvent at different temperatures is confirmed by the emission of indole in Mixed Alcoholic solvent (Figure 18). At room temperature, there is a broad structureless fluorescence with a maximum at $30,300\text{ cm}^{-1}$ compared to the fluorescence in very dry hydrocarbon around $33,500\text{ cm}^{-1}$. At liquid nitrogen temperature the fluorescence in alcohol is structured and blue shifted to a maximum of $32,900\text{ cm}^{-1}$. There is a weak phosphorescence evident even though this sample was not degassed. A similar series of results is found in the case of indole in EPA.

A slight temperature effect on the corrected excitation spectra of indole in Mixed Alcohol is observed when the fluorescence is monitored at the respective emission maxima. At room temperature the excitation spectrum shows a maximum at $35,700\text{ cm}^{-1}$. At 77°K , the relative intensities of the two peaks are reversed, and the maximum excitation occurs at $34,800\text{ cm}^{-1}$. This effect and the broadening at

*It is possible that a portion of this decrease is the result of signal attenuation by the liquid nitrogen. However, a decrease in the fluorescence intensity of samples at higher temperature was observed, and in other cases an increase in intensity was observed. Because of these observations, the magnitude of this attenuation is presumably negligible.

Figure 18. Total emission of indole in Mixed Alcohol at RT, 77^oK.

INDOLE IN MIXED ALCOHOL

EXC 280

○ 77 K

△ RT

room temperature are attributed to the normal intensification at low temperature due to a narrowing of the band width and solvent contraction at lower temperatures (Figure 19).

The temperature study and the related differences in indole fluorescence in alcoholic solvent at room temperature and 77°K appear to be in agreement with the two quenching processes previously discussed.^{5,6} Our results are confirmed by the temperature studies of Eisinger and Navon⁷ who reported a blue shift and development of vibrational structure on cooling. There is also agreement in that they note that most of the observed increase in fluorescence quantum yield takes place above the temperature at which these spectral changes occur. In our case it is also noted that the shift and structure development are relatively complete at temperatures above those at which the phosphorescence first appears. The temperature dependent quenching process is evidently dominant until a temperature near -160°C is reached because the fluorescence yield increases with decreasing temperature until this temperature is attained.

The room temperature emission spectrum of indole in acetonitrile, Figure 20, is included to demonstrate that the broad structureless fluorescence observed is not specific to hydrogen-bonding solvents.

Preliminary studies of the luminescence of the methylindoles have been completed. The excitation and emission spectra of 1-methylindole (1MI), 2-methylindole (2MI), and 3-methylindole (3MI)

Figure 19. Fluorescence excitation spectra of indole in Mixed Alcohol
at RT, 77^oK.

INDOLE IN MIXED ALCOHOL
EXCITATION SPECTRA
○ 77 K MON 305 NM
△ RT MON 330 NM

Figure 20. Fluorescence of indole in acetonitrile at RT.

INDOLE IN ACETONITRILE
RT EXC 2785

in alcohol and very dry hydrocarbon solvents were measured. Figure 21 shows the room temperature at 77°K emission of lMI in very dry 3MP. Like that of indole, the fluorescence of lMI in hydrocarbon bears a mirror-image relation to the low energy absorption spectrum. The low temperature spectrum is similar to that at room temperature but has the expected sharper vibrational structure. A weak phosphorescence is recorded.

In Mixed Alcohol (MA) the room temperature fluorescence of lMI is a broad band which is centered at $30,300\text{ cm}^{-1}$. At 77°K the fluorescence is structured and has an envelope similar to that of lMI in hydrocarbon, and a phosphorescence like that of indole is seen (Figure 22).

A low temperature excitation spectrum of lMI in 3MP monitored in the long-wavelength portion of the fluorescence showed a band at $32,900\text{ cm}^{-1}$. This band, seen also at room temperature (Figure 23), is analogous to the long-wavelength shoulder noted in the absorption spectrum. Excitation at this wavelength produced an apparent enhancement of the low energy fluorescence; the phosphorescence was not observed. The spectra were normalized so that the intensities at $31,800\text{ cm}^{-1}$ were equal (Figure 24). This normalization could not be accomplished electronically without overloading the amplifier; thus changes in slit widths, as noted on the legend of the figure, were made. Measurements of other spectra at similar slit widths suggested that it is probable that this apparent enhancement of the fluorescence is due to exciting light scatter underlying the

Figure 21. Total emission of 1-methylindole in 3MP at RT, 77°K.

1-METHYLINDOLE IN 3MP
EMISS (EXC 282 SW 2.5)

○ 77 K MON SW 0.7
△ RT MON SW 0.8

Figure 22. Total emission of 1-methylindole in Mixed Alcohol at RT, 77°K.

Figure 23. Fluorescence excitation spectra of 1-methylindole at RT, 77^oK.

Figure 24. Total emission of 1-methylindole in 3MP at RT, 77^oK.

1 METHYLINDOLE IN 3MP
77 K EMISSION

○ EXC 282 SW 2.5 MON SW 0.7
△ EXC 305 SW 2.0 MON SW 1.8

spectrum and should be discounted. Insufficient work has been performed on this compound to ascertain whether the "bump" in the absorption and excitation spectra is a peculiarity of 1MI or is the result of an impurity effect. Neither this band nor similar excitation effects were noted in 2MI or 3MI.

The fluorescence emission of 2MI in dry hydrocarbon (Figure 25) shows less structure than the fluorescence of 1MI. Unlike the fluorescence of other indoles, the fluorescence is shifted to lower energies at low temperature. There is observed a stronger phosphorescence relative to either that of 1MI or that of 3MI. The characteristic broad fluorescence of 2MI at room temperature in alcohol is observed. The maximum occurs at $29,200\text{ cm}^{-1}$. The excitation spectra of 2MI in alcohol and hydrocarbon are similar to each other and to that of 1MI in alcohol.

Emission spectra of 3MI in dry hydrocarbon are shown in Figure 26. In comparison with the 2MI results, the maximum shifts less as the temperature is lowered. In 3MI the sharp band on high energy edge of the room temperature fluorescence becomes less prominent at 77°K . This behavior is confirmed in the fluorescence excitation spectra obtained from 3MI the same temperatures; it is noted that the structure present in the room temperature spectrum is less prominent at 77°K . In alcohol at room temperature, the broad fluorescence is at $28,600\text{ cm}^{-1}$. The excitation spectra of 3MI in alcohol and hydrocarbon show no unusual effects.

Figure 25. Total emission of 2-methylindole in dry IP at **RT**, 77°K.

2-METHYLINDOLE IN JP
EMISS. (EXC 2750 SW2.5)

○ 77 K MON SW 0.7
▲ RT MON SW 0.6

Figure 26. Total emission of 3-methylindole in dry IP at RT, 77°K.

Although there are certain irregular effects noted for the methylindoles, their spectroscopic properties in general parallel those of indole. In summary, 1) each shows a structured mirror-image type fluorescence in hydrocarbon solvents and the lower energy structureless fluorescence in alcoholic solvent; 2) in the cases measured, the low temperature fluorescence in alcohol is similar to that in hydrocarbon; and 3) the fluorescence excitation and absorption spectra in alcohol and hydrocarbon are similar. In addition, although the complete quenching of the fluorescence in dry hydrocarbon has not been observed, the ϕ_P/ϕ_F for 2MI is approximately four times that for 1MI or 3MI.

C. Discussion of Four Theories

It is necessary at this point to examine some of the experimental data in terms of the four models proposed. There is general agreement that the lowest energy absorption is a ${}^1L_b \leftarrow A_1$ transition and that there is a higher energy ${}^1L_a \leftarrow A_1$ transition in the first band. According to the dual emission theory, there is simultaneous emission from both states of the molecule in nonpolar solvents. Mataga, et al.⁸ proposed a reversal of the 1L_a and 1L_b states of indole in the equilibrium excited state so that the 1L_a state becomes the only emitting state.

The general dual emission scheme could then be represented by the two cases shown in Figure 27. The absorption is represented by paths 1 and 2 and 1' and 2' and is the same in either case. The

Figure 27. State levels in the dual emission theory.

CASE I: NONPOLAR

CASE II: POLAR

electronic transition in absorption occurs in approximately 10^{-15} sec.⁹ The equilibrium excited state is reached by a process which requires several vibrational periods (10^{-13} sec) as far as adjustment for size and a time of the order of 10^{-11} sec if solvent reorientation is involved.¹⁰ The lifetime of indole in hydrocarbon was measured in these laboratories, is shown in Table 2, and is compared with data from Walker, et al.¹¹ for indoles in H₂O.

Table 2

Compound	τ_F Hydrocarbon (nsec)	τ_F H ₂ O (nsec)
Indole	2.5 ± 0.6	4.1
1-Methylindole	6.7 ± 0.5	8.5
2-Methylindole	3.2 ± 0.4	2.0
3-Methylindole	3.0 ± 0.5	9.1

If simultaneous dual emission from indole in nonpolar solvents occurs, it requires that the two states be nearly degenerate and that either the radiationless decay (internal conversion) from the 1L_a to 1L_b states be of the order of the excited state lifetime or that thermal repopulation of the 1L_a state occur via path 3. If slow internal conversion were true, then selective excitation to the 1L_b state should produce a different emission. This experiment was tried in the case of indole and produced negative results. Our observation of a temperature effect does indicate that an activation

energy is involved and that there is a possibility that a different state or different species emits in nonpolar solvents.

In considering the reversal of the states in polar solvents, Mataga did not suggest a path of relaxation by which the 1L_a state becomes the lowest equilibrium excited state. Presumably this path could involve the Franck-Condon 1L_a state either decaying rapidly to the Franck-Condon 1L_b state followed by relaxation to the equilibrium 1L_a state or a relaxation directly to the equilibrium 1L_a state. If direct relaxation were to predominate than a change in the wavelength of excitation should produce a difference in the emission. This result has not been observed. If the state-reversal theory is true, the fact that the $^1L_b \rightarrow A_1$ type transition is observed at low temperature in polar solvents implies that the solvent is prevented from reorienting and the emission is from a Franck-Condon-like state. We consider this explanation unlikely because the structured fluorescence appears at a temperature at which the solvent is relatively fluid.

A second theory involves an explanation of results solely in terms of a solvent reorientation effect. Using primarily their observations of a continuous shift and the lack of an isoemissive point in studying the fluorescence shift as a function of temperature, Eisinger and Navon⁷ suggested that the solvent shift was a result of reorientation of solvent molecules. The specific decrease in energy would be related to the number of molecules that could reorient during the lifetime of the excited state.

Many workers, however, have described the change in fluorescence from nonpolar to polar solvents as a disappearance of one band, the appearance of a new band, and the shift of this new band. For example, Konev² writes in terms of a shift accompanied by the disappearance of the short-wavelength fluorescence (1L_b) band due to hydrogen bonding of two species. Mataga⁸ states that the relative intensity of the 1L_b transition decreases and a broad band arises at longer wavelength; it is this broad band that further shifts to the red as the alcohol concentration increases. Walker, et al.⁵ interpret their data in terms of a chemical reaction to form a new species at low alcoholic concentration. As the concentration becomes greater than 1 M, it is the fluorescence due to this new species that shifts to the red..

In addition, Longworth,⁴ in what we judge to be a more careful series of measurements (dry solvents, exact control of concentration), was able to obtain reproducible isoemissive points and a 1:1 stoichiometry for compounds between a series of indoles and alcohol. Each of the above experiments implies that there are two emitting species; one has a structured fluorescence and the other has a lower energy, broad structureless fluorescence.

Although reorientation of solvent is obviously involved in reaching the equilibrium excited state, the predominant experimental data suggest that this reorientation is neither the sole nor major factor in explaining the anomalous behavior of indole.

In proposing the theory of exciplex formation, Walker, et al.^{5,11} observed the appearance of a different fluorescence at a concentration low enough to have a negligible effect on bulk solvent properties. They proposed a reaction of excited-state indole with alcohol to form an excited-state complex. Their results in the case of indole, 1-methylindole and 1,3-dimethylindole in associating solvents were consistent with a 1:2 complex of the indole with solvent. In nonassociating polar solvents there was a 1:1 exciplex. The fact that the 1-substituted indoles and indole itself formed similar 1:2 complexes was used to eliminate the possibility of an effect due to hydrogen bonding.

These authors have shown rather conclusively that the solvent-induced loss in vibrational structure and apparent red shift are the result of two superimposed emissions; one of which is the $^1L_b \rightarrow A_1$ emission of monomer indole and the other an emission due to a complex between an excited solute molecule and a ground-state solvent molecule. By subtracting the monomer fluorescence from the total fluorescence and calculating the integrated intensity of each with respect to wavelength, they found the intensity ratio to obey the relation,

$$I_E/I_M = K[S_M]^n, \quad (1)$$

in which I_E is the intensity of exciplex emission, I_M that of monomer indole, $[S_M]$ is the monomer solvent concentration and K is a constant

at photostationary conditions and depends on the nature of the solvent. Values of K obtained by Walker, et al. are reproduced in Table 3.

Table 3

Complex formation constants*		
Solvent	Indole K	1-Methylindole K
Diethyl ether	0.20	0.14
1,4-dioxane	0.28	0.20
Acetonitrile	1.21	0.79
1-butanol	109.60	12.03
Methyl alcohol	141.30	9.55

* Reproduced from ref. [5].

As previously noted, Longworth⁴ disputed the 1:2 exciplex on the basis of his finding of a 1:1 stoichiometry for a series of indoles in alcohol. With the exception of the evidence used to propose a 1:2 complex (which depends on the successful integration of intensities of overlapping fluorescence bands), the data of Walker, et al. is not questioned. The interpretation of the forces involved, however, seems inconsistent with their data and totally inconsistent with the results obtained in these laboratories. They propose a charge resonance stabilization of the exciplex resulting from charge transfer out of the lowest excited state of indole to the lowest unfilled orbital of the solvent. Because the degree of stability should then only be related to the interaction of the electronic states, this

model does not account for the differences observed in the associating and nonassociating solvents. For example, the K for polar-protic solvents is 100-600 times as large as the K for polar-nonprotic solvents. Similarly, if the indole nitrogen atom is not involved in some respect, there is no explanation for the fact that the K values for 1-methylindole are only 1/15 - 1/10 those for indole in the same alcoholic mixture. In addition, it would be exceedingly difficult to explain the temperature effect on the basis of a charge transfer complex. The rate of complex formation should be diffusion controlled and should show little or no activation energy in concentrated alcoholic solutions.

The theory concerning an excited state proton transfer reaction has been presented by its authors to explain the fluorescence quenching rather than the fluorescence shift, although in Chapter I (Sec. C) an example of intramolecular proton transfer which produced a large fluorescence red shift was presented. Stryer¹² proposed a proton transfer from indole to explain the decrease in fluorescence yield of indole in H_2O as compared to that of indole in D_2O . He interpreted the quenching to be due to a proton transfer during the excited-state lifetime; the effect of the D_2O is to inhibit this transfer. He noted no isotope effect in the case for 1-methylindole although others have noted an isotope effect on the fluorescence lifetime of this compound.¹³ This point will be considered later. The possibility that the opposite reaction (excited-state transfer of a proton from the solvent to the indole) has the same fluorescence quenching effect

was discussed by Lehrer.¹⁴ Both the —NH_2^+ and —N^- forms of indole are relatively nonfluorescent.¹⁵

The great diversity in the observed luminescences of indole indicate that the excited states of indole are more involved than any of these theories imply. Furthermore the vast change in relative phosphorescence to fluorescence yields of indole in different solvents suggests that the intersystem crossing rate must be an important consideration in any theory proposed. The yield changes also suggest that n, π^* states are involved in the intersystem crossing process. The following discussion adapted from Lower and El-Sayed¹⁶ will illustrate this point.

The probability of radiationless transition from the singlet to triplet state is proportional to the square of the expectation value of the perturbing operator connecting these states. Because this transition is between states of different multiplicities, the perturbing operator is the spin-orbit coupling part of the total Hamiltonian. From several articles referenced in the Lower and El-Sayed article, it is shown that to the first order, spin-orbit coupling is forbidden between states of the same configuration. Spin-orbit coupling between two n, π^* states of different configurations is also forbidden, and interaction between two π, π^* states is forbidden. A summary of these selection rules follows:

$$\langle S_{n, \pi^*} | \mathcal{H}_{so} | T_{n, \pi^*} \rangle \approx \langle S_{\pi, \pi^*} | \mathcal{H}_{so} | T_{n, \pi^*} \rangle \approx 0 \quad (2)$$

and

$$\langle S_{n, \pi^*} | \mathcal{H}_{so} | T_{\pi, \pi^*} \rangle \neq 0$$

or in other words, $n, \pi^* \leftrightarrow \pi, \pi^*$ intercombinations are allowed and $\pi, \pi^* \leftrightarrow \pi, \pi^*$ and $n, \pi^* \leftrightarrow n, \pi^*$ are forbidden between states of different configurations. These selection rules and the effect of the order and types of states on the rate of radiationless and radiative processes are shown in Figure 28 which is adapted from Reference 16.

In case I, the lowest excited singlet state is n, π^* . The rate of fluorescence is slow and similar to the rate of intersystem crossing. Both fluorescence and phosphorescence can occur in this case. In case II, the lowest excited singlet is still n, π^* , however, there is a lower triplet π, π^* between the singlet and the lowest triplet π, π^* state. In this case the intersystem crossing process is very efficient, and there is no fluorescence. Discussing cases I and II, the authors point out that the only fluorescent molecules having an n, π^* state as the lowest excited singlet also have the ordering of triplet states shown in case I.

For cases III and IV in which the lowest singlet is a π, π^* state, there is a more rapid fluorescence. The greater ratio of phosphorescence to fluorescence in quinoline compared to its hydrocarbon analog, naphthalene, is due to the presence of an (n, π^*) triplet between the lowest $^1(\pi, \pi^*)$ and $^3(\pi, \pi^*)$ levels in quinoline. The presence of this triplet leads to an enhanced intersystem crossing process in quinoline and a greater phosphorescence yield.

Further discussion will be based on several of the facts presented in the figure, namely: 1) $S_{n, \pi^*} \rightarrow S_0$ fluorescence is slow compared to $S_{\pi, \pi^*} \rightarrow S_0$ fluorescence; 2) the rate of intersystem

Figure 28.* Selection rules: Effect of order and type of states on the rate of radiationless and emission processes in N-heterocyclic molecules.

————→ Radiative

~~~~~• Radiationless

Time given in seconds

*As adapted from Lower and El-Sayed, ref. [16].

I

II

III

IV

crossing for $S_{n, \pi^*} \rightarrow T_{\pi, \pi^*}$ or $S_{\pi, \pi^*} \rightarrow T_{n, \pi^*}$ is rapid; 3) the rate of $S_{n, \pi^*} \rightarrow S_{\pi, \pi^*}$ or $S_{\pi, \pi^*} \rightarrow S_{n, \pi^*}$ is rapid; and 4) Phosphorescence from $T_{n, \pi^*} \rightarrow S_0$ is of the order of 0.01 sec whereas phosphorescence from $T_{\pi, \pi^*} \rightarrow S_0$ is on the order of seconds.

D. Proposed New Theory: 3H-indole

Consideration of the experimental data in terms of these selection rules leads to the proposal of a new theory to explain the

behavior of indoles. This theory involves the formation of either 2H- or 3H-indole in the excited state. The theory was first presented by Chopin and Wharton¹⁷ in terms of 2H-indole. The structures of 2H- and 3H-indole are shown below. The later modification presented in terms of 3H-indole¹⁸ was made for the following reasons that are summarized from Chapter I and referenced therein:

- 1) although the charge density on both the 2 and 3 positions increases in the first excited state, the increase on the 3 position is greatest;
- 2) 3-methylindole in acid media undergoes protonation in the 3 position;
- 4) perturbation (substitution or hydrogen bonding) in the direction of the ^1L_a transition is expected to have the greatest effect on this transition and 5) the observation of the 3H-form in the ground state of 2-ethoxyindole. The scheme and the state-order model presented at that time is discussed below. Theoretical calculations later suggested a slightly different state-order model which will be discussed when these results are mentioned.

2H-INDOLE

3H-INDOLE

An interpretation of the experimental results presented herein is outlined in Figure 29: The absorption spectrum of indole involves only the indole species; however the emission spectrum is believed to involve indole and 3H-indole as well as complexed (solvated) 3H-indole.

The proposed scheme depends upon the near degeneracy of the lowest $S_{\pi\pi^*}$ state of indole and the lowest $S_{\pi\pi^*}$ state and the $T_{n\pi^*}$ state of 3H-indole. It is also necessary that different solvents stabilize these three states to different extents. The first assumption may be reasonable because of the small change involved in the molecular geometry. The second assumption is consistent with the expected change in stability of the short-axis transition that was stated by Konev² and with the fact that indole has no non-bonding electrons, the 3H-triplet state has one, and the 3H-singlet state has two. Furthermore, an extension of this same reasoning predicts that

Figure 29. Proposed energy level diagram for the electronic energy states of indole, 3H-indole and complexed 3H-indole.

the excited states of 3H-indole should be more highly stabilized by solvent interactions than are those of indole. This greater stability of the lowest excited singlet state of 3H-indole should be especially true when the solvent is polar-protic and thus can complex 3H-indole via hydrogen bonding. From these considerations, the degree of solvent stabilization should follow the order: $S_{\pi\pi^*}$ indole $\rightarrow T_{n\pi^*}$ 3H-indole $\rightarrow S_{\pi\pi^*}$ 3H-indole. In order to agree with experimental results the order of energies should be $S_{\pi\pi^*}$ indole $> S_{\pi\pi^*}$ 3H-indole $\rightarrow T_{n\pi^*}$ 3H-indole in non-polar solvents and $S_{\pi\pi^*}$ indole $> T_{n\pi^*}$ 3H-indole $\rightarrow S_{\pi\pi^*}$ 3H-indole in highly polar and especially polar protic solvents. This order is shown in Figure 29.

The normal fluorescence and phosphorescence of indole can be interpreted in terms of arrows 1, 2, and 3. In very dry IP/3MP the fluorescence is quenched and an intense phosphorescence is observed. This quenching and phosphorescence can be interpreted in terms of arrows 2 and 3; 4, 5, 6, 7, and 3; or 4, 5, 6, 8 and 9. These mechanisms require that the lowest $S_{\pi\pi^*}$ state of 3H-indole be lower in energy than the lowest $S_{\pi\pi^*}$ state of indole and higher in energy than the $T_{n\pi^*}$ state of 3H-indole. This order may well be the case in some solvents. It is also required that the $S_{\pi\pi^*} \rightarrow T_{n\pi^*}$ intersystem crossing be considerably faster than the $S_{\pi\pi^*} \rightarrow$ G.S. emission of 3H-indole in this solvent. Since the selection rules discussed predict the crossing period to be 10^{-9} sec, it may be possible that this fluorescence quenching mode predominates. (It shall be shown later that theoretical calculations predict the lowest singlet in 3H-indole to be a $S_{n\pi^*}$ state from which no fluorescence is expected.)

Because the presence of 3H-indole is assumed and because this species not directly indicated by an emission process in hydrocarbon solvents, 3H-indole is assumed not to emit. This lack of emission for the 3H-form is consistent with the knowledge that the protonated and nonprotonated (in terms of the nitrogen proton) forms of "indole" are found not to fluoresce. The vast change of the fluorescence intensity of indole in different solvents can be interpreted to be the result of thermal isomerization of excited-state indole to 3H-indole. In the general case the fluorescence yield would depend on the relative proportions of indole and 3H-indole, and the proportions would vary from solvent to solvent.

In mixed solvent systems such as alcohols in hydrocarbons, the chemistry becomes considerably more complex. In addition to the indole isomerization to 3H-indole, one must also consider the attack of the minor solvent constituent on indole or 3H-indole to form complexed 3H-indole. The relative proportions of each species under photostationary conditions would depend on a number of rate constants which would in turn depend on bulk solvent properties as well as the stability of the complex. In the discussion of complexed 3H-indole it is again assumed that the uncomplexed species does not emit and hence only influences relative yields. It is also assumed that the lowest $S_{\pi\pi^*}$ state of 3H-indole is stabilized relative to the lowest singlet states of indole and the uncomplexed 3H-indole. In addition, if the assumption is made that the lowest $S_{\pi\pi^*}$ state of complexed 3H-indole is sufficiently stabilized so that it is lower in energy

than the $T_{n\pi^*}$ state of the complexed species, then this is an example of case IV of Figure 28 in which the fluorescence emission can compete with the intersystem crossing process.

Considering this model in terms of some of the data presented, the fluorescence from complexed 3H-indole is believed to be the broad structureless emission observed at lower energies relative to the indole fluorescence. This scheme would be consistent with the 1:1 complex observed by Longworth, but would not be consistent with the 2:1 exciplex reported by Walker, et al. The fluorescence quenching and associated activation energy can be interpreted in terms of arrow 12 as well as changes in rate constants among the three species. The model is consistent with the large K for indole complexation compared with that of 1-methylindole.

There are other examples such as the isotope effect in which the anomalous effects are noted in the case of 1-methylindole but to a lesser degree than in the case of indole. In keeping with the model proposed here, this behavior can be explained in two ways: Either a methyl group migration to the three position can occur or the 1-methylindole can be protonated by transfer of a proton from the solvent. In either case, rate constants analogous to k_1 , k_2 , and k_2 in Figure 28 would be expected to decrease. The result would be observed as a decreased sensitivity to solvent perturbations. The model will not explain the reported 1:1 solvent complex with 1-methylindole unless complexation at the three position is postulated instead of actual protonation or methyl migration.

E. Experimental Attempts to Confirm 3H-Indole.

In the original paper by Chopin and Wharton¹⁷ written in terms of 2H-indole we proposed that if methyl migration occurs, then 2-methylindole can be photochemically prepared from 1-methylindole. A sample of 1MI was prepared and treated in a preparative photochemical reactor in the manner described in Chapter II, Sec. C.6. Various samples were reacted from 1 to 90 hours. After the reaction the alcoholic solvent was completely removed from the sample by vacuum distillation. The sample was diluted with carbon tetrachloride, and its spectrum was measured by means of the Varian A60A nmr instrument. No evidence for the presence of 2-methylindole was observed. After our theory was modified in terms of 3H-indole these spectra were reexamined. Although the presence of 3-methylindole was indicated, it was also found present as an impurity in an untreated sample of 1-methylindole. The difference in relative areas of these peaks before and after radiation did not appear to be significant.

The possibility that the ground-state energies of indole and 3H-indole could be similar was considered, and several experiments were performed in attempts to discover this species (3H-indole) in the ground state. One of these experiments involved the use of laser-Raman techniques to compare the spectra of indole and fluorene. Fluorene has a —CH_2 group as part of a five carbon ring joining two benzene rings. It was felt that identification of the CH_2 vibrations in the spectra of fluorene in various solvents could be compared with spectra of indole in the same solvents. If unassigned vibrations in

the indole spectra were located at frequencies similar to the known CH_2 vibrations in the fluorene spectra, this would be a positive identification of ground-state 3H- or 2H-indole. Samples of each compound were prepared as solids and as solutions in CCl_4 and methanol in capillary tubes. The samples were excited with the 5145 Å line of an argon laser. The fluorene samples all exhibited a strong fluorescence (presumably due to a two photon excitation process), and useful spectra were impossible to obtain. The liquid samples of indole decomposed immediately in the laser beam; this decomposition was indicated by the presence of a well defined dark region approximately 3 mm in height and the presence of perfectly clear solution above and below this band. In the solid indole sample, an initial problem of fluorescence was overcome and an excellent spectrum was obtained. This spectrum is included in the appendix for reference purposes. It may be possible to repeat this experiment under different conditions with dilute deoxygenated indole solutions and with a different compound other than fluorene for comparison.

A different experiment based on nmr was performed for a similar purpose. It was again reasoned that in some solvents the ground-state energy of 3H- or 2H-indole might be stabilized enough so that this species could exist at room temperature in the ground state. Although nmr is not considered to be a sensitive detection method, the possibility existed that a sufficient population of this species

could be seen directly as a proton signal in the region expected for a --CH_2 group. Later these spectra were examined for indirect evidence which should be seen as a splitting of the methyl proton signal due to the 3-proton in 3-methyl-3H-indole. The results are summarized in Table 4. Chemical shifts are expressed in parts per million on the " δ " scale with the internal standard, tetramethylsilane, taken as 0.00.

Some observations based on these measurements are that the resonance of the proton attached to the nitrogen is so broad that the absorption in non-polar solvents is difficult to locate the absorption in nonpolar solvents. The range of chemical shifts in CCl_4 listed for this proton was estimated from the difference in the spectra before and after indole in CCl_4 was heated with D_2O and agrees with a range of chemical shifts for this proton found in Sadtlers Standard NMR Spectra of indole and the methylindoles. It is also noted that the chemical shift of this proton is extremely sensitive to solvent perturbations because it changes from δ 7.1 in CCl_4 to δ 11.1 in DMSO-d_6 . The 2-proton shift is more sensitive than is the 3-proton shift under similar conditions.

The methyl-proton signal of 3MI is a doublet. When a double resonance technique is used to irradiate the spectrum at the frequency of the 2-proton signal (δ 7.1), the methyl proton signal becomes a singlet. When the reverse decoupling is performed, no change is observed in the region of the 2-proton signal; however, this lack of observable change is probably due to the fact that the 2-proton signal

Table 4.

Compound	Solvent	Chemical Shift (ppm, δ)		
		N-H	2-Proton	3-Proton
Indole	CCl ₄	(7.3-7.1)	6.8	6.3
	CDCl ₃	—	7.0	6.5
	CCl ₄ (dry)	—	6.7	6.4
	CHCl ₃	—	6.9	6.5
	CD ₃ CN	9.2	(7.1)	6.5
	DMSO-d ₆	11.1	7.2	6.3
3-Methylindole	CCl ₄	—	6.6	2.25
	CDCl ₃	—	6.9	2.32
	CD ₃ CN	8.8	(7.1)	2.30
	Acetone-d ₆	8.9	7.1	2.32
	DMSO-d ₆	10.65	7.2	2.29
2-Methylindole	CCl ₄	—	2.03	6.10
	CD ₃ CN	8.85	2.37	6.15

is masked by the aromatic-proton signal. The value of the coupling constant of the methyl-proton doublet (1.5 Hz) and the indicated coupling to the 2-proton are consistent with allylic coupling.

It has been reported that the methyl-proton line is a doublet at δ 1.0 in the case of 3MI protonated at the 3-position in 18 M sulfuric acid and that the additional 3-proton appears as a weak quartet at δ 3.75. Neither of these signals was evident in the nmr measurements of 3MI. In addition, Cohen, et al.²⁰ reported that no evidence of 3H-tautomerism was observed for the ground state of 3-methylindole or any other indoles studied.

Confirming experimental evidence for the 3H-indole model was sought from another source. The compound, 2-ethoxyindole (2EI) has been reported to undergo tautomerism from the indole form to the indolenine (3H) form when it is sublimed at 80°C/0.1 torr. A small sample of this compound was obtained, and its absorption and emission spectra were measured.

The absorption spectra of 2EI in several solvents are presented in Figure 30. With the exception of the spectrum measured in IP + 1% 1-butanol, the absorption properties are similar to those of indole and the methylindoles that were discussed. The broadening of bands and slight red shift noted in EPA is analogous to the behavior of the other indoles. Hinman and Whipple¹⁹ in their work with 3-protonated indoles report that in neutral solution indole and its mono-, di- and trialkyl derivatives exhibit a strong maximum near 220 nm and a maximum at lower intensity around 280 nm that is

Figure 30. Absorption spectra of 2-ethoxyindole in EPA (—); MCH/MCP (----); 3MP (·-·-·-·-); and 3MP + 1% 1-butanol (·····).

usually flanked by two shoulders. When these compounds are dissolved in acid and completely protonated, the peak near 220 nm is replaced by two red shifted peaks of lower intensity near 230 and 237 nm. When considered along with the effect mentioned below, the appearance of these new peaks may be a possible explanation for the absorption in the IP/3MP mixture.

One other anomalous absorption effect was noted. The sample was in two small sealed vials when it was received; each containing a few milligrams. One was marked "pure", and the other was marked "less pure". The spectra shown in Figure 30 were made from solutions of the pure sample. Other spectra of the "less pure" sample in EPA and 3MP were very different. The low energy maxima at 293 and 295 nm and the maximum at 265 nm were still present. However, instead of a decrease in absorbance from 265 nm to a minimum at 240 nm, the absorption intensity continued to increase. The spectrum of the "less pure" showed strong maxima around 257 and 247 nm like those in the butanol spectrum of the "pure" fraction. Due to the small amount of sample available it is likely that inhomogeneous samples were prepared and that the butanol solution contained more "impurity" than the samples prepared for the other spectra. Because the solution behavior of 2EI has not been reported, the possibility can only be suggested that the absorption bands at 257 and 247 nm are due to the 3H-tautomer.

Figure 31 shows the room temperature fluorescence spectrum of 2EI in 3MP and the spectrum of the same sample to which 1% butanol

Figure 31. Fluorescence spectra of the same sample of 2-ethoxyindole in 3MP and in 3MP + 1% 1-butanol.

was added. The intensities shown are not relative; the amplifier gain settings required to produce the spectra indicate that there is at least a two-fold increase in fluorescence yield in changing the solution from 3MP to 3MP + 1% 1-butanol. The slight shoulder on the low energy side of the fluorescence in 3MP appears to be the maximum in the alcoholic mixture.

Figure 32 shows the fluorescence of 2EI in EPA at RT and 77°K. There is a broad fluorescence at $30,000\text{ cm}^{-1}$ which reverts to a more structured emission at higher energy at low temperature. Although not shown, a weak phosphorescence, similar in shape and wavelength to that of indole, was noted.

In summary, the emission characteristics of 2EI are similar to those of indole. There is the characteristic broadening and red shift of the fluorescence on going from hydrocarbon to alcoholic solvent. In alcoholic solvent at 77°K the emission is like the fluorescence of the compound in hydrocarbon.

Except for the absorption anomaly, there is no direct indication for the presence of the 3H form of 2-ethoxyindole in solution. However, there is direct evidence for the ground-state indole—3H-indole tautomerism of this compound.²¹ A search has been made of Science Citation Index using Reference [21] as a key. There are many articles which reference this work of Harley-Mason and Leeney but none was found in which their findings are disputed. Because of the reported existence of the 3H-form of 2ethoxy-3H-indole in the ground state, it is proposed that the excited state properties

Figure 32. Fluorescence spectra of 2-ethoxyindole in EPA at RT, 77°K.

2-CHLOROETHANOL IN CCl₄
FLUORESCENCE, EXC 2875
RT MON SW=0.5
Δ 77 K MON 1.5

of this compound are partially due to the presence of the 3H- form in the excited state. Further, because similar excited-state behavior is observed for this compound and for indole, this experimental evidence is offered in support of the 3H-indole model.

Stronger experimental support for the 3H-indole model exists in the esr studies of Pailthorpe and Nicholls.²² They measured the esr spectra of tryptophan and 3-methylindole radicals prepared at 100°K in ethanol solution by irradiation with 300 - 360 nm radiation. A spectrum characteristic of the ethanol radical:

was obtained with either compound. Prolonged irradiation of pure ethanol with an intense 310 nm source produced no ethanol radical. The authors therefore attribute the ethanol radical produced in the tryptophan and 3MI solutions to photosensitization of the ethanol by the indole nucleus of the two molecules.

When 1-methyltryptophan and 1,3-dimethylindole were irradiated under conditions identical to those above, no detectable free radical spectra were observed. Because replacement of the hydrogen attached to the indole nitrogen by a methyl group prevented the formation of detectable radicals, the authors conclude that a radical formation in tryptophan and 3-MI is due initially to the rupture of the N-H bond of the indole nucleus. The atomic hydrogen formed then interacts with the surrounding solvent molecules to produce the ethanol radical.

Pailthorpe and Nicholls found that the free radical formed in the indole ring by N—H bond rupture is a singlet having a line width characteristic of an unpaired electron associated with a carbon atom. They did not observe the triplet expected from an unpaired electron on a nitrogen atom. This behavior is explained in terms of a bond rearrangement occurring immediately after the fission of the N—H bond. The free radical formed would initially have the unpaired electron associated with the indole nitrogen as shown in the following structure:

The proposed bond rearrangement would produce the following tryptophan free radical:

From esr measurements on tryptophan, 3MI, 1-methyltryptophan and 1,3-dimethylindole in aqueous solution the authors observe spectra

indicating the presence of photoejected electrons. The authors conclude that photoejection of electrons is independent of the bond fission because this reaction occurs even if there is a methyl group attached to the indole nitrogen.

In summary the authors state that one of the primary photochemical reactions that occurs when tryptophan is irradiated with ultraviolet light is the breakdown of the indole N—H bond; that this reaction occurs in addition to the photoejection of electrons; and that immediately following N—H bond fission, rearrangement of the indole nucleus occurs so that the free electron resides on carbon 3.

Based on the experiment cited above, a recombination of atomic hydrogen with the free electron on carbon 3 would result in the formation of 3H-tryptophan which is similar to the proposed 3H-indole.

F. Theoretical Calculations

In order to determine if the 2H- or 3H-indole model is theoretically possible, a series of calculations was performed; the programs previously described were used. To a certain degree the calculations are based on an energy minimization with respect to molecular geometry. In this respect the output of the programs depends on the correct input of atomic coordinates for the molecule under consideration.

The initial estimate of bond lengths and bond angles of indole was based on X-ray crystallographic data for molecules containing an indole nucleus. Bond lengths and angles for L-tryptophan hydrochloride,²³ 3-indolylacetic acid,²⁴ glycyl-L-tryptophan dihydrate,²⁶ and the 1:1 complex of indole with s-trinitrobenzene were averaged, and these averages were used to calculate the atomic coordinates used in the initial data input. The bond length averages and the final calculated bond lengths used in the case of indole are shown in Table 5, where they are compared with the calculated values obtained from two other treatments by Dewar and Gleicher.²⁷ Also shown for reference are the final bond lengths calculated for 2H and 3H-indole; the bond angles used were the same in all cases and are shown in Table 6.

The final values were calculated by means of the following iterative process. The initial bond lengths and angles of indole were used to calculate atomic coordinates. These data were used as input for the CNDO/2 program used to calculate the molecular ground-state energy (henceforth referred to as the "Pople" program). The density matrix generated by this program yields bond orders between carbon atoms. It has been shown that a linear relation between bond order and bond length should exist.²⁸ Therefore, these bond orders were plotted against bond lengths by means of a multiple regression least squares analysis program. The estimates for new bond lengths were obtained from the program and used to calculate a new set of coordinates, and the process was repeated.

Table 5.

Bond ^a	Average ^b	INDOLE		2H-INDOLE		3H-INDOLE
		Calculated ^c	PPP ^d	SPO ^d	Calculated ^c	Calculated ^c
1-2	1.402	1.403	1.404	1.388	1.336	1.335
2-3	1.341	1.350	1.351	1.356	1.401	1.416
3-4	1.457	1.447	1.456	1.451	1.396	1.420
4-5	1.405	1.411	1.409	1.419	1.401	1.393
5-6	1.397	1.377	1.389	1.379	1.394	1.390
6-7	1.389	1.407	1.404	1.415	1.400	1.392
7-8	1.397	1.377	1.390	1.380	1.395	1.390
8-9	1.405	1.411	1.405	1.418	1.400	1.393
9-1	1.366	1.360	1.407	1.390	1.381	1.415
9-4	1.392	1.405	1.400	1.394	1.401	1.415

^a See page 1 for numbering system; all values in Å.

^b Averages from X-ray data.

^c Calculated from bond order iteration.

^d Reference [27].

Table 6.

Angle ^a	Degrees
9-1-2	108.2
1-2-3	109.9
2-3-4	106.7
3-4-9	106.7
4-9-1	108.5
9-4-5	120.7
4-5-6	116.2
5-6-7	122.8
6-7-8	121.2
7-8-9	115.8
8-9-4	123.0

^a See "a", Table 5.

There were two tedious processes involved in these calculations. One was the calculation of atomic coordinates once all of the bond angle and bond length data were obtained. The other was the calculation of the carbon-nitrogen bond lengths because the linear bond-order, bond length relation does not hold for these bonds. Because the bond angles and all of the carbon-carbon bonds are fixed, the coordinates of the nitrogen atom are unique. However, to obtain these coordinates, and thus the bond lengths, an intricate series of stepwise trigonometric calculations is required. With the assistance of Dr. Holmes, there was written a program that uses the BASIC programming capability of the NOVA computer. Requiring only carbon-carbon bond lengths for input data, the program printed out the carbon-nitrogen bond lengths as well as the coordinates of each atom in the molecules. The use of this program reduced the computational time from several hours to a few minutes.

The bond lengths were considered to be correct when the correlation coefficient for bond length vs bond order was 0.999 ± 0.001 . This generally took less than five cycles even when the model for 3H-indole was begun with the coordinates for indole. It is interesting to note that the method was internally consistent in that each correction of bond lengths produced a lower ground-state molecular energy on the next iteration. It can also be noted from the table that the program correctly reversed the position of the "essential" double bond from the 2-3 bond in indole to the 1-2 bond in 3H-indole.

The sensitivity of both programs to changes in bond lengths was examined. The Pople program was checked by increasing the bond lengths symmetrically as in a breathing vibration. The change in ground state energy was little different from an estimate based on the breathing vibration of benzene (approximately 1000 cm^{-1}). The Jaffé-Del Bene ("Jaffe") program was much more sensitive to coordinate input data. Before the bond-order method of estimates was used, a set of coordinates based on intuitive estimates was used. When these guessed coordinates were used, convergence could not be attained in the program subroutine that diagonalizes the matrix. After that, the normal procedure was to calculate the coordinates by means of the Pople program and then to use the Jaffé program when the final geometry had been determined. This method was also required less computer time. The actual run time (CPU) for the indole or 3H-indole calculation with the Pople program was less than two minutes whereas the Jaffé program requires about 10 min. in the case of indole and 20 min. in the case of the 3H-indole.

The basic question always involved in a quantum mechanical calculations is the accuracy and reliability of the results. Prior to a discussion of the state-order and energy levels predicted by these programs, some of the results calculated for indole will be compared with experimental values. The Pople program, parameterized to yield accurate ground-state properties, predicts a dipole moment of indole of 1.97 D which is in good agreement with measured values of 2.05 D²⁹ and 2.08 D.³⁰ The photoelectron spectra of indole,

1-methylindole, 2-methylindole and 3-methylindole were measured and are included in the Appendix. The measured lowest ionization potential of indole is 8 eV whereas the calculation predicts a value of 9 eV.

The Jaffé program parameterized in terms of accurate spectra values predicts the three lowest energy singlet transitions in indole to be 292.8, 252.3, and 220.4 nm. These are in excellent agreement with the values of 287 and 220 nm for the well defined ^1L_b and ^1B_b transitions respectively. The ^1L_a transition predicted at 252.3 nm is in the correct spectral region of the most intense ^1L_a transitions in indole but is somewhat to the blue of the 0-0 transition. The predicted polarizations are correct, and the oscillator strengths predicted for these three transitions are correct in order of magnitude with respect to those predicted by Platt³¹ as well as similar to those actually observed. Copies of these calculated transitions are included at Tables 7-9 in the Appendix.

The state-order and energy level diagram predicted by the programs is shown in Figure 33. The relative energies of indole and 3H-indole are those predicted by the calculations; those of complexed 3H-indole are assumed in the fitting of experimental data. It should now be obvious why the use of two programs was necessary in order to relate all excited state energy levels to the energy of the indole ground state. For example, the differences between ground and excited state energy levels of 2H-indole were similar to the

Figure 33. State order and energy level diagram as predicted by theoretical calculations.

spacings in 3H-indole; the predicted ground state, however, was several eV higher than that of indole, thus eliminating excited state conversion to the 2H species.

One of the most significant predictions of these calculations is that the lowest singlet state of 3H-indole (3HI) is an $S_{n\pi^*}$ state (the lowest predicted singlet for 2HI is an $S_{\pi\pi^*}$). Considering the diagram, the normal fluorescence and phosphorescence of indole in hydrocarbon solvent can be interpreted in terms of arrows 1, 2 and 3. The intense phosphorescence and the associated fluorescence quenching can be interpreted in terms of arrows 2 and 3: or 4, 5, 6 and 3; or 4, 5, 7, 8 and 9. Note that if formation of 3HI in the excited state is efficient, the fact that this species has a lowest singlet $n\pi^*$ state would explain the complete lack of fluorescence in dry hydrocarbon solvent. (cf. selection rules).

The broad fluorescence in associating solvents is explained if one assumes that solvent stabilization results in the reversal of the lowest $S_{n\pi^*}$ and $S_{\pi\pi^*}$ states in complexed 3HI. Emission from this species would then occur in terms of arrows 10 and 11. The fluorescence quenching and associated activation energy can be interpreted in terms of arrow 12 as well as in terms of the rate constants between the various forms of indole, 3HI and complexed 3HI.

One experimental observation for which an interpretation is still required is the fact that even in pure alcoholic solvent at low temperature (but not necessarily the solvent glass point) the fluorescence reverts to a structured emission at higher energy. It

may well be that this structured emission is also the emission of complexed 3HI and that (in the rationale of Koney and Eisinger and Navon) the broadening and red shift are due to different numbers of solvent molecules that are able to reorient during the excited-state lifetime.

In summary, the 3H-indole model provides an adequate interpretation for the excited-state chemistry of indole. The predicted red shift for the 3H-form is $3,700\text{ cm}^{-1}$, in the exact range of the shift noted. This model accounts for the loss in vibrational structure in the fluorescence from indole in polar solvents. The similarity of effect (and the lack of a similarity in the degree of this effect) of associating and nonassociating polar solvents could be due to trace amounts of water or to a greater stabilization of the $S_{\pi\pi^*}$ state because of dipole-dipole interaction. The change in fluorescence polarization which has been observed could be due to the emission from both indole and 3H-indole because the emission from an $S_{n\pi^*}$ state would be perpendicularly polarized.

If we assume that the data of Longworth are correct, we have presented a model consistent with measured solvent-indole stoichiometry at low alcohol concentrations. The observable deuterium isotope effects are consistent with the 3H-indole model since it is based on the excited-state proton transfer proposed in these theories. We have also presented an example of a red-shifted emission based on intramolecular proton transfer. The fact that 1-methylindole exhibits the same behavior as indole but that the magnitude of this

behavior is greatly reduced in some cases (cf. K's of Walker, et al. and the reduced deuterium isotope effect) can be explained either in terms of a proton transfer from the solvent to the 3 position of formation of a solvent hydrogen bond at this point.

And finally, because this model provides for $n\pi^*$ type states and is dependent on relative excited-state energy levels, it offers an interpretation for the vast changes in relative quantum yields, accounts for the extreme sensitivity of measurements to trace amounts of water, and explains the complete fluorescence quenching in dry hydrocarbon solvents.

Chapter III

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Chapter IV

Conclusion

In drawing conclusions based on the use of experimental data, one expects to encounter the problem of resolving different interpretations of the same experimental results. One of the basic difficulties in this study of indole, however, is that in similar experiments there is apparent disagreement in the results themselves.

For example, Longworth found isoemissive points and noted that one fluorescence increased while another decreased; whereas, Eisinger and Navon found no isoemissive points and a gradual shift of a single fluorescence. Walker, et al. found an isotope effect for 1-methylindole; Stryer found none. Longworth found only 1:1 complexes; Walker, et al. found 1:2 complexes. Walker, et al. found an activation energy of 8.7 Kcal/mole for the quenching process in H_2O and noted that there was a marked change in the activation energy measured in D_2O . By the same procedure, Kirby and Steiner determined the activation energy to be 12.5 Kcal/mole in both H_2O and D_2O .

At first encounter, the 3H-indole model that has been proposed may appear complicated. The reason is that the choice was made to interpret the above contradictory data not in terms of

careless experimentation but in terms of the sensitivity of the reactions to experimental conditions. With this in mind, we presented a model that has sufficient flexibility to account for what may be termed the irreproducible chemistry of indole.

When examined thoroughly, this model is both conceptually acceptable and compatible with presently available data. It is not implied that this is a perfect model. There is much more experimental study required in order to test its validity in basis and detail. We do offer the 3H-indole concept as a working hypothesis and suggest that all further investigations of indole be undertaken with the exacting techniques one usually associates with the classical analytical chemist.

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APPENDICES

Table 7. Indole: Calculated singlet states after configuration interaction.

Indole

Output for singlet states after configuration interaction

CI STATE	ENERGY	FREQUENCY	WAVELENGTH	OSCILLATOR STRENGTH	POLARIZATION		
	eV	cm ⁻¹	nm		X	Y	Z
1	4.2341	34151.07	292.816	0.009038	-0.715737	-0.69837	0.0
2	4.9124	39622.21	252.384	0.007362	0.869362	-0.49405	0.0
3	5.6249	45369.09	220.414	0.367439	-0.857071	-0.51519	0.0

Table 8. 3H-indole: Calculated singlets after configuration interaction.

3H-Indole

Output for singlet states after configuration interaction

CI STATE	ENERGY eV	FREQUENCY cm ⁻¹	WAVELENGTH nm	OSCILLATOR STRENGTH	POLARIZATION		
					X	Y	Z
1	3.9621	31957.28	312.918	0.000258			
2	4.3367	34978.83	285.887	0.005272	0.961668	0.274217	0.000060
3	4.8432	39467.39	253.374	0.197089	-0.996958	-0.077940	0.000012

Table 9. Indole and 3H-indole: Calculated triplet states after configuration interaction.

INDOLE

OUTPUT FOR TRIPLET STATES AFTER CONFIGURATION INTERACTION

CI STATE	ENERGY EV	FREQUENCY CM-1	WAVELENGTH MMU
1	2.4213	19553.65	511.413
2	3.0059	24244.96	412.457
3	3.4538	27458.02	359.963
4	3.6201	29199.64	342.477
5	4.2050	33717.13	294.836
6	4.3704	40093.64	249.416
7	5.4544	43754.40	227.372
8	5.8514	47236.49	211.701
9	6.2799	50652.25	197.425
10	6.3453	51180.23	195.388
11	6.5397	52748.41	189.579
12	6.6035	53262.97	187.748
13	6.6749	53836.40	185.741
14	7.1302	57510.92	173.880
15	7.3706	59449.93	168.209
16	7.6047	61337.77	163.032
17	7.6509	61710.77	162.046
18	7.7277	62733.33	159.405
19	7.9390	64034.14	156.167
20	7.9773	64347.23	155.407
21	8.3569	67465.25	148.356
22	8.4009	67760.31	147.579
23	8.4786	68386.56	146.228
24	8.7802	70819.56	141.204
25	8.7856	70870.88	141.102
26	8.9789	71615.69	139.634
27	8.9689	72434.50	138.247
28	9.0253	72796.50	137.369
29	9.1846	74081.31	134.987
30	9.3072	75069.81	133.209

3H-INDOLE

OUTPUT FOR TRIPLET STATES AFTER CONFIGURATION INTERACTION

CI STATE	ENERGY EV	FREQUENCY CM-1	WAVELENGTH MMU
1	2.2356	18031.70	554.579
2	3.1238	25155.71	396.593
3	3.4839	28100.38	355.867
4	3.6993	29538.10	335.142
5	3.9545	31896.03	313.519
6	4.4067	35543.30	281.347
7	5.5539	44913.20	223.149
8	5.7783	46606.68	214.562
9	5.8802	47428.31	210.845
10	6.1518	49519.55	201.513
11	6.4802	52266.43	191.320
12	6.5456	52795.40	189.410
13	6.6515	53972.43	185.280
14	6.7771	54662.59	182.939
15	7.2376	58377.52	171.279
16	7.4952	60459.74	165.400
17	7.7559	62557.77	159.852
18	7.7675	62654.83	159.805
19	7.8022	62931.45	158.903
20	8.1424	65675.13	152.265
21	8.1793	65973.06	151.577
22	8.1909	66086.00	151.364
23	8.3462	67319.19	146.346
24	8.5138	68670.50	145.023
25	8.7133	70279.69	142.289
26	8.7525	70918.69	141.007
27	8.9491	72182.00	136.539
28	9.1742	73957.12	135.140
29	9.4153	75942.13	131.679
30	9.8483	79434.81	125.859

Figure 34. Laser-raman spectrum of crystalline indole.

Figure 35. Photoelectron spectrum of indole.

Figure 36. Photoelectron spectrum of 1-methylindole.

Figure 37. Photoelectron spectrum of 2-methylindole.

Figure 38. Photoelectron spectrum of 3-methylindole.

VITA

Cecil McClung Chopin was born January 5, 1942, in Natchitoches, Louisiana. He attended St. Mary's Academy and St. Mary's High School in Natchitoches and graduated from that high school in 1960. In September of 1960 he entered Northwestern State University in Natchitoches from which he received the Bachelor of Science degree in Chemistry in May, 1964.

In the fall of 1964 he entered the Graduate School of Louisiana State University at Baton Rouge, Louisiana. In 1968, he entered the United States Army as an Officer of the Chemical Corps. He was released from active duty as a Captain and returned to Louisiana State University in 1970. While in attendance at L.S.U. he was the recipient of a National Aeronautics and Space Administration Traineeship and the DuPont Teaching Award. He is presently a Candidate for the degree of Doctor of Philosophy in Physical Chemistry.

He is married to the former Lydia Ohlmeyer of Plaquemine, Louisiana and has two sons, Lamy Jean, three, and Charles Edouard, one year old.

EXAMINATION AND THESIS REPORT

Candidate: Cecil McClung Chopin

Major Field: Chemistry

Title of Thesis: The Electronic Excited States of Indole: Indirect Evidence
for Excited ^3H -Indole

Approved:

James H. Marton
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

July 13, 1971