Near-Ultraviolet Absorption Bands of Tryptophan. Studies Using Indole and 3-Methylindole as Models*

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ABSTRACT: The fine structure characteristics of tryptophanyl absorption bands are examined by using indole derivatives dissolved in nonpolar solvents. Many of the vibronic transitions of indole and 3-methylindole have been identified by using solvent perturbation to differentially shift the ^1L_a and ^1L_b electronic transitions. Perfluorinated hexane solutions give absorption spectra which are sufficiently well resolved to permit comparison with vapor-phase absorption spectra. Methylcyclohexane causes greater red shifting of the ^1L_a transitions than does perfluorinated hexane, but the individual vibronic transitions are not as well resolved. The positions of the 0–0 ^1L_a transition have been identified in the spectra of both indole and 3-methylindole. In the case of 3-methylindole dissolved in perfluorinated hexane, the 0–0 ^1L_a band (285.2 nm) is well resolved from the ^1L_b transitions (0–0 at 288 nm). The remaining ^1L_a bands of 3-methylindole cannot be identified conclusively due to overlapping ^1L_b transitions. The spectra do, however, limit the possible positions of the remaining ^1L_a bands. In indole spectra, ^1L_a bands can be identified at 0–0, 0 + 1700, and 0 + 2450 cm^{-1}. ^1L_b bands of both indole and 3-methylindole are evident at 0–0, 0 + 730, 0 + 980, and 0 − 760 cm^{-1}. Additional ^1L_b bands at shorter wavelengths are unresolved. The vibronic transitions identified in 3-methylindole aid in analyzing the circular dichroism and absorption spectra of tryptophan residues in both polar and hydrophobic environments.

The indolyl chromophore of tryptophan is especially important in spectroscopic studies of proteins since it dominates the near-ultraviolet circular dichroism and absorption spectra of many proteins (Wetlaufer, 1962; Strickland *et al.*, 1969). The spectral characteristics of this chromophore differ among proteins, since the environments of tryptophanyl side chains vary from completely hydrophobic to hydrophilic. Although the absorption spectrum of exposed tryptophanyl side chains is well known, much less is known about the spectrum of buried tryptophanyl side chains (Wetlaufer, 1962; Herskovits and Sorensen, 1968; Bailey *et al.*, 1968; Donovan, 1969). The spectra of side chains buried in a hydrophobic environment may be especially important since the fine structure bands should be well resolved. Owing to the poor solubility of tryptophan in nonpolar solvents, indole has been used as a model for buried tryptophanyl side chains (Konev, 1967).

Unfortunately even the absorption bands of indole have been difficult to analyze; just as with tryptophan, both the ^1L_a and ^1L_b electronic transitions overlap extensively in the near-ultraviolet spectra. To date the most detailed interpretation of indole spectra has resulted from fluorescence techniques (Weber, 1960; Zimmermann and Joop, 1961; Konev, 1967). The most prominent ^1L_b bands and several regions of ^1L_a absorption have been identified. The 0–0 ^1L_a band, however, has yet to be observed, even though it is allowed by symmetry (Konev, 1967). A more complete analysis of the individual vibronic transitions has not been possible due to poor spectral resolution in solutions. Furthermore, a previous analysis of the indole vapor spectrum (Hollas, 1963) failed to consider the overlapping ^1L_a transitions.

In this article the ^1L_b and ^1L_a vibronic transitions are identified in the absorption spectra of indole and 3-methylindole. The latter compound is included because its spectra are essentially identical with those of tryptophan in a variety of solvents. Our approach to unscrambling the two overlapping electronic transitions is based upon the general observation that the position of the ^1L_a transition is more sensitive to perturbations than that of the ^1L_b transition (Platt, 1951). Thus the two electronic transitions may be distinguished by the differential shifting of these transitions when the indolyl chromophore is perturbed by solute–solvent interactions. The necessity of high-spectral resolution, however, requires using only nonpolar solvents to perturb the indolyl chromophore. A mild perturbation is introduced by perfluorinated hexane—a solvent noted for giving well-resolved absorption spectra even at room temperature (Lawson *et al.*, 1969). Methylcyclohexane is used to perturb the indolyl chromophore more severely. These solution-phase spectra are compared to each other and also to vapor-phase absorption spectra. The results of these studies on model compounds are applied to the circular dichroism and absorption spectra of tryptophan.

Experimental Section

Instrumentation. Absorption spectra of solutions were recorded on a Cary Model 15 spectrophotometer. Low-temperature spectra were obtained using a Dewar adapted for variable-temperature studies (Strickland *et al.*, 1970). These indole and 3-methylindole solutions were examined in the concentration range of 0.1–1 × 10^{-4} M. At 24° the concentration dependence of these absorption spectra followed Beer's law. The most concentrated solutions aggregated

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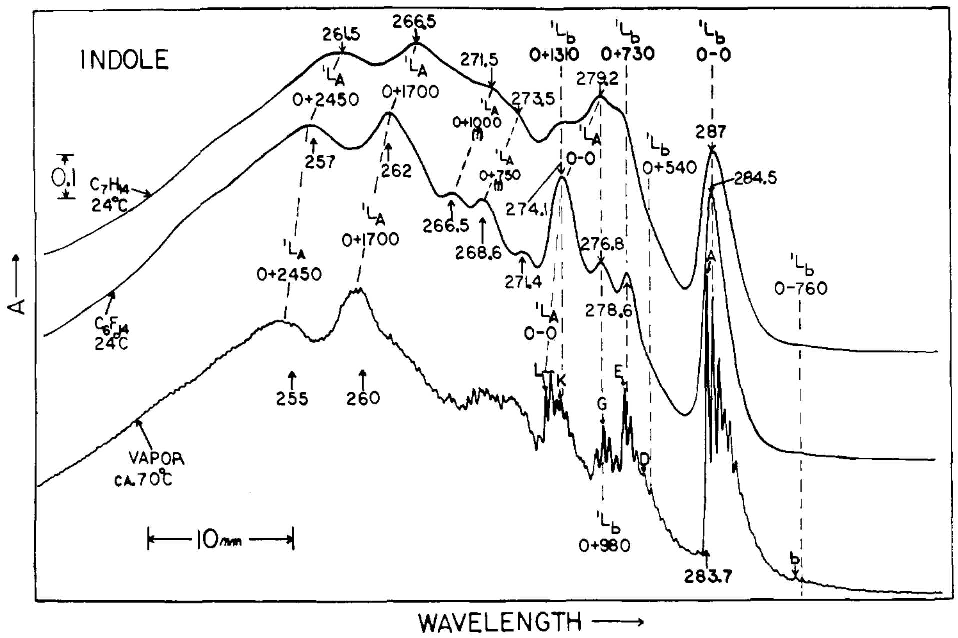

FIGURE 1: Absorption spectra of indole in the vapor phase (bottom trace), in perfluorinated hexane (middle trace), and in methylcyclohexane (top trace). These spectra have been shifted along the wavelength axis so that the prominent long-wavelength absorption band coincides in all records. Path length, 1.0 cm for solutions and 10 cm for vapor; spectral half-intensity band width, 0.2 nm for solutions and 0.08 nm for vapor. Letters designate principal bands in vapor spectrum (see Table I).

when the temperature was lowered below −20°. The aggregation was observed visually and also caused obvious blurring of the fine-structure absorption bands. At 10^{-5} M, however, the major fine-structure absorption bands continued to sharpen as the temperature was lowered (to −100° for methylcyclohexane and to −60° for the perfluorinated hexane).

Vapor-phase spectra at low resolution (*ca.* 0.1 nm) were obtained using a 10-cm path-length cuvet in the Cary 15 spectrophotometer. The cuvet containing a crystal of either indole or 3-methylindole was heated in an oven until all the material vaporized. Then the hot cuvet was placed in the spectrophotometer to record rapidly the absorption spectrum before condensation occurred. The temperatures listed for the vapor spectra are only approximate. A higher resolution absorption spectrum of 3-methylindole in the vapor state was obtained using a 3.4-m Jarrell-Ash spectrograph in the second order.[1] The spectrograph records were used to identify the existence of band heads. The wavelength positions of these band heads were determined mainly from the spectra recorded on the Cary 15 spectrophotometer.

Raman spectra were recorded on a Cary Model 81 spectrometer using a Spectra-Physics 125 He-Ne laser for excitation.[2]

Materials. Indole and one sample of 3-methylindole were obtained from K and K Laboratories (New York, N. Y.). Another sample of 3-methylindole (Givaudaun Co.), which had been recrystallized from petroleum ether, was kindly supplied by Professor Wayland Noland (University of Minnesota). The absorption spectra of both these samples of 3-methylindole were identical. Perfluorinated hexane was a gift from Dr. J. Donald LaZerte (3M Co., Saint Paul, Minn.). Methylcyclohexane was Spectral Grade from Matheson, Coleman and Bell.

Results

Indole. The vapor-phase spectrum (bottom trace in Figure 1) shows extensive fine structure. Some of these bands (principal bands or band heads) arise from transitions involving both electronic and vibrational excitation. Each principal band is accompanied by sequences of bands toward longer wavelengths. In the Cary spectrophotometer the observed sequence has a spacing of about 55 cm^{-1}. This sequence occurs because some low-energy vibrations are excited extensively even in the ground electronic state (Hollas, 1963). The energy of the vibrational mode changes somewhat due to electronic excitation. Thus the energy of a transition is influenced slightly by the extent of vibrational excitation existing before light absorption. The strong 55-cm^{-1} sequence can be used to locate the position of each principal band; *i.e.*, each sequence begins at a principal band and extends to longer wavelengths. In this way, it has been possible to locate most of the principal bands of indole (Hollas, 1963), even in our low-resolution vapor-phase spectrum. The positions of these bands are indicated by the letters in Figure 1, using the designations given by Hollas (1963) in his Table 2.

Placing indole in nonpolar solvents causes two major alterations in the absorption spectrum. The most obvious difference is that each principal band and its sequence bands are smeared into a single, relatively broad band. For example, the prominent long-wavelength band of indole in perfluo-

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[1] We are grateful to Professor Kyle Bayes of the Chemistry Department at UCLA for his aid in operating the spectrograph.

[2] Purchase of the Cary 81 Raman spectrometer was supported by National Science Foundation Grant No. GP-5240 to the UCLA Chemistry Department.

TABLE I: Comparison of Band Spacings for Indole Dissolved in Perfluorinated Hexane with Those in the Vapor State.[a]

Vapor			C_6F_{14}	
Band[b]	Int[c]	Assignment	Spacing Obsd	λ (nm)
b	w	$0 - 760\ ^1L_b$	$0 - 760\ ^1L_b$	290.6
	vw	$0 - 612\ ^1L_b$		NR[d]
A	vs	$0 - 0\ ^1L_b$	$0 - 0\ ^1L_b$	284.5
	vw	$0 + 354\ ^1L_b$		NR
	vvw	$0 + 369\ ^1L_b$		NR
D	w	$0 + 538\ ^1L_b$	$0 + 540\ ^1L_b$	280.3
E	s	$0 + 718\ ^1L_b$	$0 + 730\ ^1L_b$	278.6
	m	$0 + 736\ ^1L_b$		
G	ms	$0 + 907\ ^1L_b$	$0 + 980\ ^1L_b$	276.8
	m	$0 + 968\ ^1L_b$		
	m	$0 + 989\ ^1L_b$		
	vw	$0 + 1120\ ^1L_b$ (?)		NR
K	m	$0 + 1313\ ^1L_b$	$0 + 1310\ ^1L_b$	274.1[e]
L	s	0–0 ^1L_a (?)	0–0 ^1L_a	274.1[e]
			$0 + 1700\ ^1L_a$	262
			$0 + 2450\ ^1L_a$	257

[a] The band positions in the vapor spectrum are taken from the high-resolution spectrum of Hollas (1963). [b] Letters designate bands labeled in the vapor spectrum shown in Figure 1. [c] Int = intensity; w, weak; m, medium; s, strong; v, very. [d] NR, not resolved. [e] Two overlapping bands.

rinated hexane covers the fine structure bands evident in the vapor phase (Figure 1). The second difference is that some bands are shifted relative to each other.

To facilitate identifying the differential shift of the two electronic transitions, the small solvent-induced shift of the ^1L_b bands has been eliminated by aligning the prominent long-wavelength absorption band in all indole spectra.[3] This band is known to be the 0–0 ^1L_b transition[4] (Konev, 1967). Thus the remaining ^1L_b vibronic transitions should coincide in all records (vertical dashed lines in Figure 1). Bands that are differentially shifted relative to the 0–0 ^1L_b transition may be identified as ^1L_a bands (oblique dashed lines in Figure 1). In some cases, ambiguities may arise because an observed band may result from more than one transition. Sometimes the existence of overlapping ^1L_a and ^1L_b bands may be determined by comparing the intensities of the corresponding bands in all indole spectra. A consistent interpretation of the spectra can be obtained by making the plausible assumption that the relative intensities of the individual vibronic transitions are nearly constant *within each electronic band* (Baba and Suzuki, 1961).

In all three indole spectra (Figure 1), moderately strong bands coincide at 0 + 730 and 0 + 980 cm^{-1} and, hence, are ^1L_b transitions. These bands are almost as well resolved in perfluorinated hexane as in the vapor-phase spectrum (see Table I). The 0 + 730 and 0 + 980 cm^{-1} ^1L_b transitions of indole are least well resolved in methylcyclohexane. Another ^1L_b band occurs weakly at 0 + 540 cm^{-1} in all spectra. This band can be seen more clearly in the solution spectra after cooling to −60° or below. The minor band on the red side of the 0–0 ^1L_b band is the 0 − 760 cm^{-1} ^1L_b hot band. The three indole spectra also contain a band at + 1310 cm^{-1} from the 0–0 ^1L_b band. The relative intensity of this band, however, varies greatly, being most prominent in perfluorinated hexane (274.1 nm) and least prominent in methylcyclohexane. Apparently the indole band at 274.1 nm in perfluorinated hexane is partially a ^1L_b band and partially an overlapping ^1L_a band.

This question can be evaluated by a more detailed comparison of the three indole spectra in Figure 1. In methylcyclohexane the absorption near the 0 + 980 cm^{-1} ^1L_b band is much more prominent than is the case with indole in the vapor phase or dissolved in perfluorinated hexane. This intensification in methylcyclohexane is accompanied by a major loss of intensity near the 0 + 1310 cm^{-1} ^1L_b band relative to the spectrum of indole in perfluorinated hexane. Evidently there is an absorption band which is shifted from overlapping the 0 + 1310 cm^{-1} ^1L_b band in perfluorinated hexane to overlapping the 0 + 980 cm^{-1} ^1L_b band in methylcyclohexane. This red shift of 330 cm^{-1} results because the solvent influences the position of the ^1L_a electronic transition to a greater extent than is the case with the ^1L_b transition. The alternative explanation, a shift due to a perturbation of an indole vibrational frequency, seems highly improbable since solvent-induced changes of vibrational frequencies are relatively small in other aromatic compounds (Weigang and Dahl, 1961; Weigang and Robertson, 1959; Horwitz *et al.*, 1969, 1970).

This transition occurring at 274.1 nm in perfluorinated hexane and shifting to 279.2 nm in methylcyclohexane has the characteristics expected of the 0–0 ^1L_a transition: it is a moderately intense band, its intensity is not diminished by cooling, and it is the lowest energy ^1L_a band identified in the indole absorption spectra. It seems unlikely that any major ^1L_a bands were missed at longer wavelengths, since the indole spectra are well resolved in this region.

The approximate position of the 0–0 ^1L_a band in the vapor spectrum can also be identified by comparing the three indole spectra. The relative intensities of the bands in the vapor spectrum are not much different from those of indole dissolved in perfluorinated hexane. Apparently the 0–0 ^1L_a band must be located in about the same relative position in these two spectra. Band L may possibly be the 0–0 ^1L_a transition in the vapor spectrum, since this band and its sequence bands do not coincide with any band observed for indole dissolved in perfluorinated hexane (Figure 1).

Additional ^1L_a bands can also be observed at the short-wavelength end of all indole spectra. The bands at 0 + 1700 and 0 + 2450 cm^{-1} undergo about the same amount of solvent induced red shift as the 0–0 ^1L_a band (Figure 1). Neither of these bands, however, is a single transition, since their band widths are too broad. No additional ^1L_a bands can be identified with certainty, because the bands are too poorly resolved

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[3] The error introduced by this small wavelength shift, instead of a wave number shift, is negligible at our resolution.

[4] In spectroscopic terminology, the vibrational states involved in excitation are designated by numbers preceding the transition; *e.g.*, 0–0 refers to a transition from the 0 vibrational state in the ground electronic level to the 0 vibrational state in the excited electronic level; 0 + 730 cm^{-1} indicates a transition from the 0 vibrational state in the ground electronic level to the 730-cm^{-1} vibrational state in the excited electronic level (Suzuki, 1967).

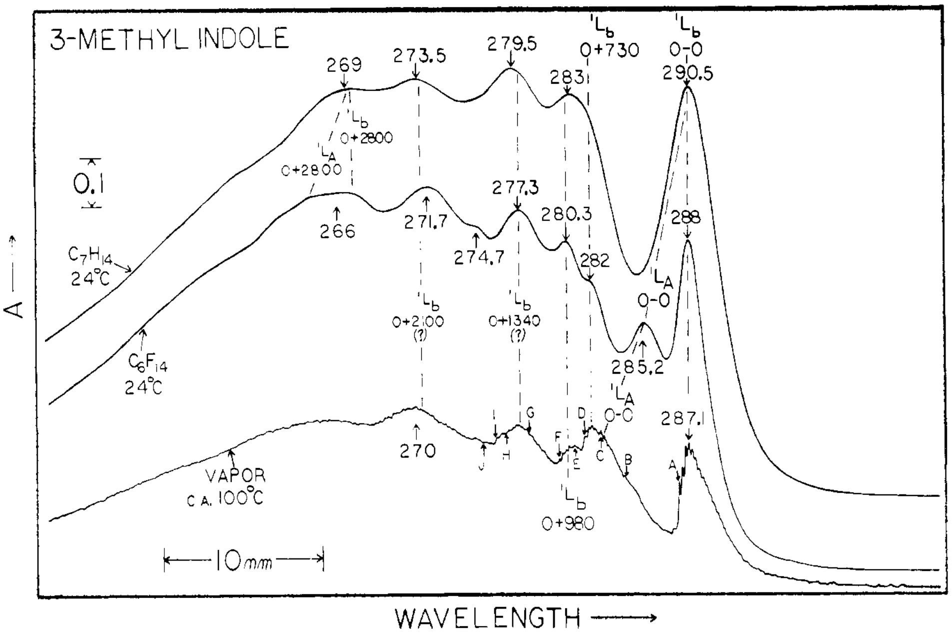

FIGURE 2: Absorption spectra of 3-methylindole in the vapor phase (bottom trace), in perfluorinated hexane (middle trace), and in methylcyclohexane (top trace). These spectra have been shifted along the wavelength axis so that the prominent long-wavelength absorption band coincides in all records. Path length, 1.0 cm for solutions and 10 cm for vapor; spectral half-intensity band width, 0.2 nm for solutions and 0.08 nm for vapor. Principal bands are designated by letters: A (34,900 cm^{-1}, s), B (very diffuse, w), C (35,510 cm^{-1}, m) D (35,630 cm^{-1}, m), E (35,710 cm^{-1}, w), F (35,850 cm^{-1}, m), G (36,080 cm^{-1}, w), H (36,290 cm^{-1}, m), I (36,380 cm^{-1}, w), J (36,460 cm^{-1}, w). The symbols following each position indicate intensities: w, weak; m, medium; s, strong.

in the region between the 0–0 and 0 + 1700 cm^{-1} ^1L_a bands. Nevertheless, the possibility exists that other ^1L_a bands may occur at about 0 + 750 and 0 + 1000 cm^{-1} (Figure 1).

3-Methylindole. The absorption spectra of 3-methylindole (Figure 2) are somewhat less resolved than those of indole. For example, compare the vapor-phase spectra of these two compounds (bottom traces in Figures 1 and 2). The principal bands of 3-methylindole vapor are more difficult to identify, partly because the sequence bands are too close together for optimal resolution in the Cary Model 15 spectrophotometer. At low resolution the sequence bands (25-cm^{-1} spacing) appear as weak fine structure superimposed upon broader absorption bands. At higher resolution the sequence bands are better resolved and identify the transitions designated in Figure 2. Even in the Jarrell-Ash spectrograph, no fine structure is resolved at short wavelengths (<274 nm).

A comparison of the 3-methylindole spectra in the vapor phase and in perfluorinated hexane indicates the existence of the 0–0, 0 + 730, and 0 + 980 cm^{-1} ^1L_b bands (vertical dashed lines in Figure 2). In the vapor spectrum the principal bands contributing to these transitions are labeled A, D, and F, respectively (Figure 2). Apparently these same ^1L_b transitions also occur in methylcyclohexane, although the 0 + 730 and 0 + 980 cm^{-1} ^1L_b bands are not resolved from each other, even after cooling to −100°. At higher absorbances than those in Figure 2, the 0 − 760 cm^{-1} ^1L_b band becomes evident in both the vapor phase and in the perfluorinated hexane. Cooling the perfluorinated hexane solution diminishes the intensity of this band, as is expected for a 0 − 760 cm^{-1} ^1L_b hot band. Other weak ^1L_b bands may also exist at about 0 + 1340, 0 + 2100, and 0 + 2800 cm^{-1}; but they are probably overlapped by ^1L_a bands in all 3-methylindole spectra (see below). Band B in the vapor spectrum may be a very weak ^1L_b band similar to that observed for indole between the 0–0 and 0 + 730 cm^{-1} ^1L_b bands (Figure 1).

The absorption spectrum of 3-methylindole in perfluorinated hexane permits identifying the 0–0 ^1L_a transition. The prominent band at 285.2 nm in perfluorinated hexane does not coincide with bands observed in either the vapor spectrum or in the methylcyclohexane spectrum. Thus it must be a ^1L_a band. The 285.2-nm band should be the 0–0 ^1L_a transition, since the spectrum in perfluorinated hexane is too well resolved to hide any other major ^1L_a transitions at longer wavelengths. As expected, the 285.2-nm band is not diminished by cooling to −60°.

The 0–0 ^1L_a transition occurs at different positions in the vapor phase and in methylcyclohexane (oblique dashed lines in Figure 2). Band C in the vapor spectrum seems to be the 0–0 ^1L_a transition. Both wavelength position and intensity considerations support this assignment. The spectrograph record revealed two series of moderately intense sequence bands beginning at +610 cm^{-1} (band C) and +730 cm^{-1} (band D) from the 0–0 ^1L_b band. Band D is the 0 + 730 cm^{-1} ^1L_b band and is not shifted by solvents. In contrast, band C appears to be red shifted in perfluorinated hexane by 280 cm^{-1} relative to the 0–0 ^1L_b band. Compare the relative intensities of the bottom two absorption spectra (Figure 2) in the regions around the 0–0 ^1L_b, 0 + 730 cm^{-1} ^1L_b, and 0 + 980 cm^{-1} ^1L_b bands. In the vapor spectrum the absorption around the 0 + 730 cm^{-1} ^1L_b band is more intense than that around either the 0–0 ^1L_b or the 0 + 980 cm^{-1} ^1L_b band. In perfluorinated hexane, however, the absorption around 0 + 730 cm^{-1} ^1L_b band is less than that around the other two major ^1L_b bands. Apparently the 0–0 ^1L_a band (C) nearly overlaps

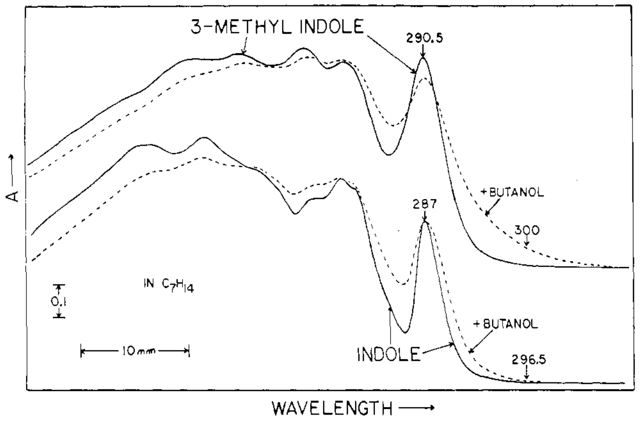

FIGURE 3: Effect of 1-butanol upon the absorption spectra of indole (bottom) and 3-methylindole (top) dissolved in methylcyclohexane. - - -, added 0.05 ml of 1-butanol to 2 ml of either indole or 3-methylindole solution (—). Temperature, 24°; path length, 1.0 cm.

the 0 + 730 cm^{-1} ^1L_b band in the vapor, giving rise to an intensification in this spectral region.

In methylcyclohexane the 0–0 ^1L_a band of 3-methylindole is not resolved. Nevertheless, several lines of evidence indicate that the 0–0 ^1L_a band is red shifted sufficiently to coincide approximately with the 0–0 ^1L_b band. (1) The deep trough at 287 nm indicates the absence of any major transitions between the two longest wavelength bands in methylcyclohexane. (2) The ratio of absorption around the 0–0 ^1L_b band to the total absorption of 3-methylindole is greater in methylcyclohexane than is the case in perfluorinated hexane (Figure 2). This intensity difference is even greater than it appears in Figure 2, because the 0–0 ^1L_b band should be shorter in methylcyclohexane than in perfluorinated hexane as a result of greater band broadening. (3) Adding a minute concentration of butanol distorts the long-wavelength absorption band of 3-methylindole much more than is the case with indole, where the 0–0 ^1L_a band does not overlap the 0–0 ^1L_b band.

The latter experiment is shown in Figure 3. Konev (1967) has previously presented evidence that adding a drop of butanol to indole causes only minor changes in the position and intensity of the 0–0 ^1L_b band, but causes a large red shift at the short-wavelength end of the spectrum. Due to the interaction with butanol, the ^1L_a bands are red shifted and also broadened greatly, which prevents resolving the exact positions of these bands. After the butanol addition, the absorption spectrum arises from a mixture of unaltered indole molecules (^1L_a and ^1L_b bands resolved) and indole molecules which have interacted with butanol (resolved ^1L_b bands superimposed upon broad ^1L_a bands). In contrast to the results with indole, adding butanol to 3-methylindole causes a reduction in the intensity of the prominent longest wavelength absorption band and greatly intensifies the absorption on the red side of this band (Figure 3). Apparently the butanol interaction shifts the 0–0 ^1L_a transition from under the 0–0 ^1L_b band to a position about 5 to 10 nm further to the red.

Turning again to Figure 2, it can be seen that the only well-resolved ^1L_a band of 3-methylindole is the 0–0 transition. No other ^1L_a transition can be identified with certainty, since solvent perturbation does not cause systematic shifting of any other well-resolved bands. A comparison of the 3-methylindole spectrum in perfluorinated hexane with that in methylcyclohexane indicates that some ^1L_b bands extend even to the short-wavelength region. In perfluorinated hexane a broad absorption band extends from 264 to 267 nm. This same band (269 nm) is much sharper in methylcyclohexane, a solvent which normally gives poorer band resolution than perfluorinated hexane. This difference in band shape is more evident in cooled solutions. Apparently this band contains both ^1L_a and ^1L_b components (0 + 2800 cm^{-1}) which coincide in methylcyclohexane and shift apart slightly in perfluorinated hexane. Although no other ^1L_a bands can be identified in these spectra (Figure 2), the peak locations do limit the spacings possible for other strong ^1L_a bands (0 + 850, 0 + 1350, and 0 + 2100 cm^{-1}). Each of these possible bands may be composed of two or more transitions lying close by each other.

Raman Spectrum of 3-Methylindole. Within the range of 200–1560 cm^{-1}, the following vibrational frequencies were observed: 231, 425, 463, 533, 565, 709, 759 (strong), 877, 983, 1010 (strong), 1077, 1128, 1232, 1252, 1303, 1347 (strong), 1422, 1458, 1558 (strong).

Discussion

The absorption spectra of 3-methylindole are essentially identical with those of tryptophan derivatives in solvents which dissolve both compounds. Apparently the spectrum of 3-methylindole in hydrocarbon solvents, *e.g.*, methylcyclohexane, is a reliable model for a tryptophanyl side chain buried in a completely hydrophobic region of a protein. If the tryptophanyl side chain is buried in a region containing polar groups, or if the NH group of the indolyl ring is hydrogen bonded, then the relative positions of the ^1L_a and ^1L_b band will be shifted. For example, 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 band (Figure 3). Thus, to understand the absorption and circular dichroism spectra of buried tryptophanyl side chains, it is necessary to characterize separately the ^1L_a and ^1L_b electronic absorption bands.

For this reason, we shall now give an evaluation of our findings based upon using nonpolar solvents to differentially shift the ^1L_b and ^1L_a electronic absorption bands of the indolyl chromophore. As expected from theoretical considerations (Platt, 1951), solvent perturbations cause the ^1L_a electronic transition to be red shifted by a much greater amount than the ^1L_b transition. This basis for differentiating between these two electronic transitions permits a more detailed understanding of the indolyl chromophore than has been heretofore possible.

In the case of indole, our assignments based on low-resolution spectra facilitate analyzing high-resolution spectra previously described by other workers. In Table I the absorption spectrum of indole dissolved in perfluorinated hexane is compared to the high-resolution vapor spectrum reported by Hollas (1963). As might be expected, several of the bands observed in perfluorinated hexane contain more than one transition. Nevertheless, the major ^1L_b transitions may be readily identified in the high-resolution vapor spectrum. The only suggested change from Hollas' original assignments (1963) is that band L may be the 0–0 ^1L_a band (see Results). The remaining ^1L_a bands cannot be assigned to any specific

TABLE II: Reanalysis of the Major Fluorescence Excitation and Emission Lines of Indole in Cyclohexane[a] at −196°.

Excitation $1/\lambda$ (cm^{-1})	Excitation Intensity[b]	Excitation Assignment ^1L_b	Excitation Assignment ^1L_a	Emission $1/\lambda$ (cm^{-1})	Emission Intensity[b]	Emission Assignment ^1L_b	Emission Assignment ^1L_a	Raman Frequency[c] (cm^{-1})
34,734	s	0–0		34,734	s	0–0		
35,150	m		0–0					
35,262	w	0 + 530		34,129	m	0 − 607[d]		607
35,461	s	0 + 730		33,987	s	0 − 760		757
35,638	m	0 + 900						
35,727	m	0 + 990		33,681	m	0 − 1006 0 − 1061		1006 1061
36,050	m	0 + 1310		33,389	s	0 − 1350[d]		1350
36,166	m		0 + 1000	34,129	w[e]		0 − 1020	1006 1061
36,430	m	0 + 1690 (?)						
36,900	m		0 + 1750	33,389	m[e]		0 − 1760	
37,594	m		0 + 2450	32,637	m		0 − 2510	

[a] Line positions and intensities are taken from work of Kembrovskii *et al.* (1966). [b] w, weak; m, medium; s, strong. [c] Values taken from reference Kohlrausch and Seka (1938). [d] Band is predominantely this transition, but it overlaps weaker ^1L_a transition. [e] Estimated contribution of ^1L_a transition overlapped by ^1L_b transition.

transitions reported by Hollas (1963). Although many transitions occur at wavelengths shorter than the position of the 0–0 ^1L_a band, none dominates the vapor spectrum. Some of this spectral broadening may result from vibronic interactions between overlapping ^1L_a and ^1L_b transition (Hochstrasser, 1968). For practical purposes, however, broad ^1L_a bands can be observed at about 0 + 1700 and 0 + 2450 cm^{-1} in indole. It is not certain that these spacings between ^1L_a bands correspond to excited-state vibrational energies, because vibronic coupling may cause some distortions (Fulton and Gouterman, 1961, 1964).

Additional support for our assignments can be obtained from the fluorescence excitation and emission spectra of indole in cyclohexane at −196° (Kembrovskii *et al.*, 1966). The position of the 0–0 ^1L_a band can be determined by comparing the fluorescence excitation spectrum with the vapor absorption spectrum of Hollas (1963). Kembrovskii *et al.* (1966) list a 0 + 420 cm^{-1} ^1L_b transition at 35,150 cm^{-1} having medium strength (see their Table I). No such ^1L_b spacing, however, is observed in the vapor (Hollas, 1963). Consequently, this band at 35,150 cm^{-1} cannot be a ^1L_b band; instead, it is the 0–0 ^1L_a transition of indole in cyclohexane[5] at −196°. Identification of the 0–0 ^1L_a band permits assigning the major indole transitions in the fluorescence excitation and emission spectra (Table II).

The fluorescence emission spectrum of indole in cyclohexane at −196° has the anticipated mirror image symmetry relative to the excitation spectrum, provided that the ^1L_a and ^1L_b bands are considered separately (Table II). As noted previously (Konev, 1967; Kembrovskii *et al.*, 1966), the fluorescence emission of indole occurs from both the ^1L_a and ^1L_b electronic states. Owing to its lower excited state energy, the ^1L_b electronic state fluorescence more strongly than does the ^1L_a state. Thus the ^1L_b emission bands are readily identified at 0–0, 0 − 607, 0 − 760, 0 − 1050, and 0 − 1350 cm^{-1} (Table II). Two of these emission bands, however, may contain both a ^1L_b and a ^1L_a component. The 0 − 2510 cm^{-1} ^1L_a emission band is also observed. Apparently the major ^1L_a transitions of indole can be detected in both the emission and excitation spectra.

The comparison between fluorescence excitation and emission of indole permits associating some of the excited-state vibrational modes with ground-state frequencies observed in Raman (Kohlrausch and Seka, 1938) and infrared (Kanoka *et al.*, 1960) spectra. Our Raman assignments (Table II) are similar to those of Konev (1967).

Having evaluated the vibronic characteristics of indole, we shall now consider the transitions of 3-methylindole. The individual vibronic transitions of 3-methylindole are more difficult to interpret, primarily because the ^1L_a and ^1L_b bands overlap throughout almost the entire near-ultraviolet band. Nevertheless, in perfluorinated hexane the resolution is excellent for the first several bands. The more obvious ^1L_b transitions of 3-methylindole occur at 0 − 760, 0–0, 0 + 730, and 0 + 980 cm^{-1}, just as is the case with indole. This similarity between indole and 3-methylindole is not surprising in view of the close correspondence between the relevant Raman frequencies of indole (757, 1006, and 1061 cm^{-1}) and of 3-methylindole (759, 1010, and 1077 cm^{-1}). Other ^1L_b bands are not well resolved, but may possibly occur around 0 + 1350, 0 + 2100, and 0 + 2800 cm^{-1}.

Unfortunately, with the exception of the 0–0 ^1L_a band the ^1L_a transitions cannot be identified from the solvent perturbation studies. The spectral bands of 3-methylindole in methyl-

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[5] The shift in position of the 0–0 ^1L_a band from that observed in methylcyclohexane at 24° is due to temperature effects on cyclohexane properties, not to the difference in solvent.

TABLE III: Analysis of the Absorption Spectrum of *N*-Acetyl-L-tryptophanamide in Methanol–Glycerol (9:1, v/v) at −196°.

λ[a] (nm)	$1/\lambda$ (cm^{-1})	Assignment[b]	
		^1L_b	^1L_a
Ca. 297	33,670		0–0
290[c]	34,480	0–0	0 + 850
286[d]	34,960		0 + 1350
283	35,340	0 + 730[e] 0 + 980	
280	35,710		0 + 2100
274[f]	36,500	0 + 2100	0 + 2800

[a] Data taken from Strickland *et al.* (1969). [b] ^1L_a bands are much broader than ^1L_b bands. [c] The fine structure in this band mainly arises from 0–0 ^1L_b band. [d] Band probably occurs, but not actually resolved in instrument trace. [e] These transitions are not sharp enough to be resolved as separate bands. [f] Band probably arises from overlap of intense, broad ^1L_a band and weak, sharp ^1L_b band.

cyclohexane and in perfluorinated hexane do, however, limit the possible locations of other major ^1L_a bands to a few value (0 + 850, 0 + 1350, 0 + 2100, and 0 + 2800 cm^{-1}).

The transitions observed in 3-methylindole provide a model for interpreting the circular dichroism and absorption spectra of tryptophan—a derivative of 3-methylindole. The spectra of tryptophan solutions are difficult to analyze directly because most tryptophan derivatives are not soluble in nonpolar solvents. At −196° in polar solvents, the circular dichroism spectra of some tryptophan derivatives (Strickland *et al.*, 1969) reveal prominent ^1L_b bands at 0–0 and 0 + 850 cm^{-1}. From our analysis of the 3-methylindole spectra, it appears that the 0 + 850 cm^{-1} ^1L_b band of tryptophan is composed of overlapping transitions at 0 + 730 and 0 + 980 cm^{-1}. This interpretation is supported by Raman spectra, which showed that tryptophan retains the indolyl vibrations at 759, 1010, and 1077 cm^{-1} (unpublished experiments).

Our band assignments for 3-methylindole also permit analyzing the major absorption bands of tryptophan in a polar glass (Table III). Apparently the poor resolution of these absorption bands at −196° results because the ^1L_a bands are greatly red shifted and blurred, just as occurs when butanol is added to 3-methylindole (Figure 3).

Previously an attempt was made to identify the major ^1L_a transitions by simultaneously curve fitting the circular dichroism and absorption spectra of tryptophan derivatives in polar glasses at −196° (Strickland *et al.*, 1969). This procedure suggested that major ^1L_a bands occur at 0–0, 0 + 400, and 0 + 1400 cm^{-1}. The present investigation shows that there is not a major ^1L_a band at 0 + 400 cm^{-1}, although a weak transition cannot be excluded. The curve-fitting approach failed because the ^1L_a spectrum is extremely sensitive to the effects of polar solvents. Apparently solvent–solute interactions produce multiple tryptophan species having somewhat different circular dichroism and absorption spectra. Such conditions can cause a mismatch between circular dichroism and absorption spectra (Moscowitz *et al.*, 1963), as has been recently observed with tyrosine (Horwitz *et al.*, 1970). In addition, the multiple tryptophan species cause extensive blurring of the ^1L_a absorption bands.

These difficulties do not necessarily occur in proteins containing a single tryptophan residue buried in a unique site. The circular dichroism and absorption spectra of two peroxidase isoenzymes, proteins having only a single buried tryptophan residue, provide support for the occurrence of a 0 + 850 cm^{-1} ^1L_a band in tryptophan and thereby also in 3-methylindole. In spectra recorded at −196°, the 0–0 ^1L_a and 0 + 850 cm^{-1} ^1L_a tryptophanyl bands are resolved on the long-wavelength side of the 0–0 ^1L_b tryptophanyl band (E. H. Strickland, J. Horwitz, E. Kay, and L. M. Shannon, 1969, unpublished data). These ^1L_a bands may be red shifted by virtue of the indolyl ring being hydrogen bonded in a hydrophobic pocket.

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Effect of Conformation on Isotopic Exchange in Synthetic Polypeptides*

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ABSTRACT: Calculated exchange rates are presented for amide isotopic exchange in synthetic polypeptide homopolymers and copolymers. The effects of pH, temperature, and ionic strength are considered. It is assumed that exchange does not occur from intramolecularly hydrogen-bonded (helical) amides and that the kinetics of the helix to random-coil conformation change is rapid compared with the isotopic exchange. The exchange rate law is considered to be a sum of independent parallel first-order terms with the apparent rate constants dependent on conformation as well as other factors. In homopolymers deviation from first-order behavior results from the high probability of residues near the end of a chain being in nonhelical conformation under all environmental conditions.

The calculated rates are shown to be consistent with known experimental data with respect to apparent reaction order, ionic strength effects, and conformational contribution to the exchange activation energy. The effects of composition and sequence on the exchange kinetics are investigated in copolymers where one of the monomers has an ionizable side chain. Decreasing the amount of ionizable monomer increases the deviation from simple first-order behavior. Comparison with experimental data indicates that only the conformational thermodynamics, and not the conformational kinetics, need to be considered to understand the exchange kinetics in copolymers.

Amide hydrogen–deuterium or hydrogen–tritium exchange in simple amides follows a first-order rate law although the reaction mechanism is complex, involving both acid and base catalysis (Berger *et al.*, 1959; Nielsen, 1960; Klotz and Frank, 1965). The pseudo-first-order rate constant at any pH varies somewhat with the amide. When amides are incorporated into a random-coil homopolymer the rate law is still simple first order (Ikegami *et al.*, 1965; Scarpa *et al.*, 1967; Englander and Poulsen, 1969). However, there may be differences between monomer and polymer exchange kinetics, one of the more striking examples being that of poly-*N*-isopropylacrylamide (Scarpa *et al.*, 1967; Klotz and Mueller, 1969).

Other than a simple first-order rate law might be expected in a polymer containing internal structure as a result of intramolecular amide hydrogen bonding, or in a copolymer if the exchange rate constant is different for each monomer. The rate law would be expected to be a sum of parallel first-order terms, or more complicated if the kinetics of conformational change must be included. It is thus interesting that Leichtling and Klotz (1966) and Ikegami and Kono (1967) found simple first-order behavior for polyglutamic acid irrespective of the degree of intramolecular hydrogen bonding. Deviation from first-order behavior has been observed, however, in copolymers of alanine and glutamic acid (Ikegami and Kono, 1967), which appears to depend in a complicated manner on the alanine content.

In many naturally occurring polymers the exchange kinetics are quite complicated. Exchange in proteins (Hvidt and Nielsen, 1966) and in nucleic acids (Printz and von Hippel, 1968) must be described frequently by a sum of first-order terms. Separation of copolymeric effects from effects of internal polymer structure are of importance but difficult.

In this communication calculated exchange curves are presented for polypeptide polymers whose residues exist either in disordered or in intramolecularly hydrogen-bonded helical conformations. The effects of pH, ionic strength, temperature, and monomer composition are considered. Comparison is made with the experimental values for polyglutamic acid and alanine–glutamic acid copolymers.

Basic Assumptions and Equations. An amide proton in a collection of polypeptide chains each containing $N + 1$ residues is classified as being in one of two states, according to whether or not it is intramolecularly hydrogen bonded. According to conformation, an intramolecularly H-bonded amide is assigned to a helical conformation, otherwise the amide is in a disordered or random-coil conformation.

The rate of isotopic exchange of a H-bonded or helical amide is taken to be negligible compared to the exchange rate

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