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Intramolecular Interaction between the Phenol and the Indole Chromophores

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The singlet-energy transfer from the phenol to the indole chromophores occurs intramolecularly, with a high efficiency, in the compounds containing these chromophores joined by the methylene and the amide groups. The total emission spectra of the compounds consisted only of those from the indole chromophore, neither the fluorescence nor the phosphorescence of the phenol chromophore contributing to them, and the excitation spectra of the fluorescence were similar to the absorption spectra. The quantitative analysis of the fluorescence-polarization spectra suggested that the ^1L_b transition of the indole chromophore was excited in the energy transfer. The intramolecular interactions between these chromophores in the ground and excited states are discussed.

The fluorescence yield of the protein is very low compared with the contribution to be expected from the constituents of the aromatic amino acids.[1-4] Weber and Rosenheck[5] have suggested that the phenolic hydroxide of the tyrosine residue is hydrogen-bonded to the neighboring carboxylate moieties in the excited state, thus resulting in the non-fluorescent phenolate ion. Cowgill[6] has proposed the hypothesis that the peptide bond enhances the vibrational deactivation of the aromatic ring. The combined action of these effects may be responsible for the reduction of the fluorescence quantum yield of the protein.[7]

The fluorescence of the protein containing the tryptophane residue is the only one attributable to the residue, neither the phenylalanine residue nor the tyrosine residue having any detectable fluorescence. The excitation spectra of the fluorescence indicate the excitation-energy transfer from the tyrosine to the tryptophane residues in some proteins,[8-10] though no such sensitization of any tryptophanyl fluorescence by the tyrosine residue has been observed in other proteins.[1,11] The excitation-energy transfer seems reasonable from the viewpoint of the energy-level sequence of the aromatic amino acids: Phe<Tyr<Trp<Tyr$^-$; it is primarily due to the long-range dipole-dipole interaction, the theory of which has been developed by Förster.[12]

The measurements of the fluorescence of the compounds which contain the phenol and/or the indole chromophores provide convenient information for studying the fluorescence of the protein. A number of reports have been published concerning the quantum yield,[13-17] the lifetime,[18,19] and the peak position[20-23] of the fluorescence and the intramolecular[10] and intermolecular energy transfer.[24]

In this experiment, a series of compounds, I—VI, containing the phenol or its methyl ether, and the indole chromophores joined by the methylene groups and the amide group were prepared, and the intramolecular interaction between these chromophores was studied in

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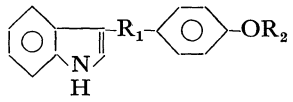
some detail on the basis of the fluorescence and the phosphorescence measurements.



I; $R_1=CH_2CH_2$, $R_2=CH_3$
II; $R_1=CH_2CH_2CH_2$, $R_2=CH_3$
III; $R_1=CH_2CH_2CH_2$, $R_2=H$
IV; $R_1=CH_2CONHCH_2$, $R_2=CH_3$
V; $R_1=CH_2CONHCH_2$, $R_2=H$
VI; $R_1=CH_2CH_2NHCOCH_2CH_2$, $R_2=CH_3$

Recently, the intramolecular energy transfer has been investigated in several donor-acceptor pairs combined by methylene groups.[25] Schnepp and Levy[26] have observed that the efficiency of the singlet-energy transfer from the naphthalene to the anthracene groups is comparable to that of the donor fluorescence, and they have considered a Förster-type mechanism for the transfer. Lamola *et al.*[27] have found that the singlet excitation is transferred from the naphthalene to the benzophenone chromophores with a high, but not total, efficiency, and that the triplet-energy transfer from the latter to the former chromophores occurs with a total efficiency, which the exchange mechanism can account for. The fluorescence from the intramolecular exciplex formed in this pair has also been reported.[28]

Experimental

*1-(3-Indolyl)-2-(*p*-methoxyphenyl)ethane (I).* The ethereal solution of indolylmagnesium iodide was prepared according to the procedure described by Baker.[29] Into 16.5 g of *p*-methoxyphenylethylbromide in ether, we stirred a solution of the Grignard compound of indole (9 g of indole). The reaction mixture was then gently refluxed for about 3 hr. The Grignard compound was decomposed with ice and ammonium chloride, and the compound was extracted with ether. The residue from the ether extract was recrystallized from benzene; mp 113—114 °C.

Found: C, 81.06; H, 6.47; N, 5.24%. Calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57%.

*1-(3-Indolyl)-3-(*p*-methoxyphenyl)propane (II).* This compound was prepared by a procedure analogous to that of 1-(3-indolyl)-2-(*p*-methoxyphenyl)ethane, using a Grignard compound of indole and 3-(*p*-methoxyphenyl)propylbromide in ether. The compound was recrystallized from benzene; mp 80—82 °C.

Found: C, 81.51; H, 7.51; N, 5.14%. Calcd for $C_{18}H_{19}NO$: C, 81.48; H, 7.22; N, 5.28%.

β-(3-Indolyl)vinyl p*-methoxyphenyl Ketone.* To 5.5 g of indole-3-aldehyde and 18 g of sodium hydroxide in water-ethanol (20—15 ml), we added 7.1 g of *p*-methoxyacetophenone. The reaction mixture was then stirred for 8 hr at 60 °C to give a red solution. The solution was concentrated, and the residue was solidified. The compound was subsequently washed with water and recrystallized from benzene; mp 169—173 °C.

Found: C, 78.46; H, 5.31; N, 4.59%. Calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05%.

2-(3-Indolyl)ethyl p*-methoxyphenyl Ketone.* The ethanol solution of 5 g of β-(3-indolyl)vinyl *p*-methoxyphenyl ketone was reduced with hydrogen at 10 atmospheres in the presence of Raney nickel at 55 °C for 30 min.[30] The product was collected and recrystallized from ethanol; mp 132 °C.

Found: C, 77.79; H, 6.10; N, 5.17%. Calcd for $C_{18}H_{17}NO_2$: C, 77.39; H, 6.13; N, 5.01%.

*1-(3-Indolyl)-3-(*p*-hydroxyphenyl)propane (III).* According to a modification of the Wolf-Kishner reduction,[31] the carbonyl group of 2-(3-indolyl)ethyl *p*-methoxyphenyl ketone was reduced and its methoxy group was simultaneously demethylated. A paste product was thus obtained, and the compound was repeatedly recrystallized from a water-acetone mixture; mp 108—110 °C.

Found: C, 82.15; H, 6.51; N, 5.34%. Calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57%.

N-p-*Methoxybenzyl-3-indolylacetamide (IV),* N-p-*Hydroxybenzyl-3-indolylacetamide (V), and* N-*2-(3-Indolyl)ethyl-2-(*p*-methoxyphenyl)propionamide (VI).* These compounds were prepared by the method using carbodiimide.[32] The reaction mixture including the corresponding acid and amide components, and *N,N*-dicyclohexylcarbodiimide in acetonitrile was stirred overnight over ice. The filtered solution was evaporated, and the products were recrystallized from benzene.

IV: mp 107—109 °C.

Found: C, 73.02; H, 6.18; N, 9.31%. Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52%.

V: mp 184—186 °C.

Found: C, 73.02; H, 5.58; N, 9.67%. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99%.

VI: mp 90—91 °C.

Found: C, 74.93; H, 6.70; N, 8.28%. Calcd for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69%.

Measurements. The emission and the fluorescence excitation spectra were measured by means of a Hitachi spectrophotofluorometer, Model MPF-2A. The excitation spectra were obtained at 20 per cent of the absorption of the peak maxima, so they could be compared with the absorption spectra.

The efficiency of the energy transfer of the singlet excitation from the phenol or its methyl ether to the indole chromophores, Φ_t, was calculated according to the following considerations.

The fluorescence intensity, $F_N(\lambda)$, of the compounds on excitation at λ nm is represented by this equation:

$$F_N(\lambda) = Q_N \cdot [A_{In}(\lambda) + A_{Ph}(\lambda) \cdot \Phi_t] \quad (1)$$

where Q_N is the fluorescence quantum yield and where A_{In} and A_{Ph} are the quanta absorbed by the indole moiety and the phenol or its methyl ether moiety respectively. It seems reasonable that A_{In} is equal to that of the model compound containing only the indole chromophore: 3-methylindole for I, II, and III; indole-3-acetamide for IV and V, and *N*-acetyltryptamine for VI; it also seems reasonable that A_{Ph} is equal to that of the model compound containing only the phenol or its methyl ether chromophore: *p*-methylanisole for I and II; *p*-cresol for III; *N*-acetyl-*p*-methoxyphenylethylamine for IV; *p*-hydroxyphenylethylamine for V, and *p*-methoxyphenylpropionamide for VI. On these assump-

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tions, the ratio of $F_N(\lambda)$ to the fluorescence intensity of the model compound, $F_{In}(\lambda)$, may be represented by an equation in which

$$F_N(\lambda)/F_{In}(\lambda) = \beta \cdot [\{\Phi_t \cdot A_{Ph}(\lambda)/A_{In}(\lambda)\} + 1] \quad (2)$$

β is the ratio of the fluorescence quantum yields. In Eq. (2), the value of Φ_t can be calculated from the measurements of $F(\lambda)$, $A(\lambda)$, and β.

In the study of the temperature dependence of the fluorescence intensity, a quartz tube (3 mm in diameter) containing the sample in EPA was cooled in the temperature of liquid nitrogen and then left in a Dewar flask at room temperature during the course of the measurement. The temperature was monitored by means of a copper-constantan thermocouple injected into the sample tube; it increased monotonously at the average rate of 2.5 °C per minute from −120 to 20 °C.

In order to avoid the depolarization due to the Brownian movement, the fluorescence-polarization spectra were measured at −70 °C in a propylene glycol solution, which forms a rigid glass at that temperature.

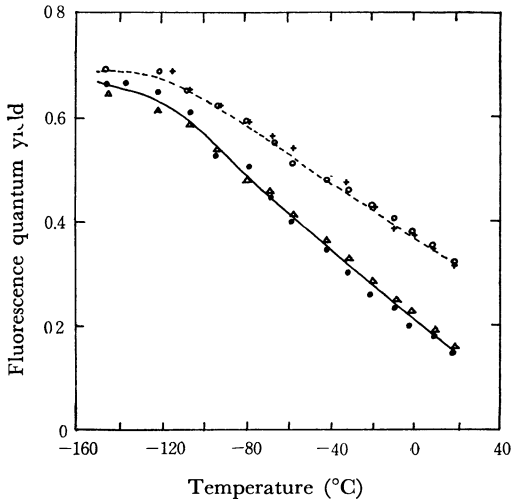

Fig. 2. The temperature dependence of the fluorescence intensities of (+) 3-methylindole, I (○), II (●), and III (△) in EPA.

Results

The total emission spectra of 3-methylindole, *p*-cresol, and an equimolar mixture of them in EPA at 77 K are shown in Fig. 1a. The 0–0 fluorescent emission band of 3-methylindole is located at a longer wavelength than that of *p*-cresol, as in the case of the phosphorescence. The emission spectrum of *p*-methylanisole was analogous to that of *p*-cresol. The excitation wavelength is 280 nm, at which the ratio of the number of the quanta absorbed by 3-methylindole and *p*-cresol is about 1 : 0.3. The emission spectrum of the equimolar (2×10^{-5} M) mixture is superimposed on those of the constituents, as is seen in Fig. 1a.

The total emission spectrum of I at 77 K is shown in Fig. 1b. The spectra of both the fluorescence and the phosphorescence are very similar to those of 3-methylindole. None of the emission from the anisole moiety can be seen in Fig. 1b. This confirms that the excitation energy is transferred from the phenol chromophore to the indole chromophore with a high efficiency. The shapes of the emission spectra of the other compounds were also very similar to that of 3-methylindole, except for the slight blue shift in the spectra of IV, V, and VI, which contain amide groups. The ratio of the intensities of the fluorescence to the phosphorescence of these compounds was little different from that of 3-methylindole.

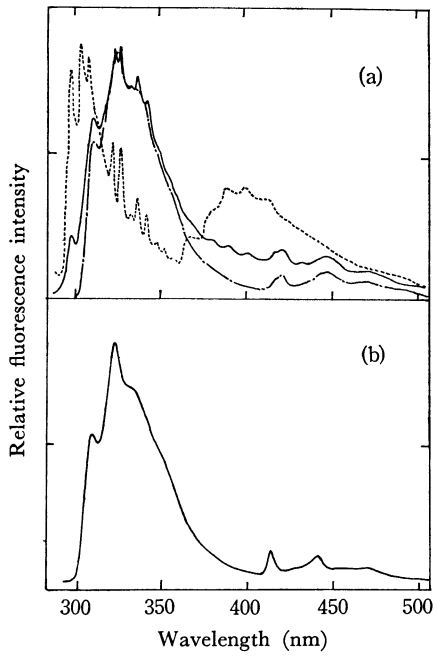

Fig. 1. The total emission spectra in EPA at 77 K, (a): (dotted line) *p*-cresol, (broken line) 3-methylindole, and (solid line) their equimolar (2×10^{-5} M) mixture. (b): I.

The fluorescence characteristics at room temperature in ethanol and in dioxane are listed in Table 1. The positions of the fluorescence peaks are shifted to longer wavelengths in ethanol, the absorption spectra undergoing a very small shift. The pronounced red shift of the fluorescence of indole and its derivatives induced by the polar solvent has been suggested as resulting from the reorientation of the surrounding solvent molecules in the excited state,[20,21] or, alternatively, from the formation of the exciplex with the solvent molecules.[22,23]

The fluorescence spectra of the compounds containing the amide groups (IV, V, and VI) are shifted to somewhat shorter wavelengths than that of 3-methylindole, as is shown in Table 1. These compounds also indicate a slight blue shift in the absorption spectra; they have the same Stokes shift as 3-methylindole. The fluorescence yields of these compounds are low in ethanol, whereas they approach the normal level in dioxane (Table 1). Cowgill[16] has also observed the low fluorescence yields of the tryptophanyl and the tyrosyl derivatives in a polar solvent that can solvate the carbonyl group by hydrogen-bonding.

The fluorescence yields of II and III are about half as high as those of 3-methylindole and I at room temperature, irrespective of the existence of oxygen molecules and of the polarizability of the solvent, as is demonstrated in Table 1. However, the former recover to the same level as the latter at low temperatures (Fig. 2).

Table 1. Relative quantum yields and wavelength maxima of the fluorescence in ethanol and dioxane which are in air and flushed with argon gas

Compound	in Ethanol			in Dioxane		
	Fluorescence Maximum (nm)	Relative Quantum yield		Fluorescence Maxmium (nm)	Relative Quantum yield	
		in Air	Ar		in Air	Ar
3-Methylindole	350	3.3_5	4.8	335	3.4	4.0
I	350	3.4	4.8	335	3.6	4.3
II	350	1.6	2.0	335	1.6	2.0
III	350	2.0_5	2.6	335	1.8_5	2.3
3-Acetamidoindole	345	2.7	3.4_5	333	3.4	4.0
IV	345	2.8_5	3.6	333	3.4_5	4.2
V	345	2.8	3.8	333	3.6	4.3
VI	348	3.0	4.2_5	335	3.1_5	3.9

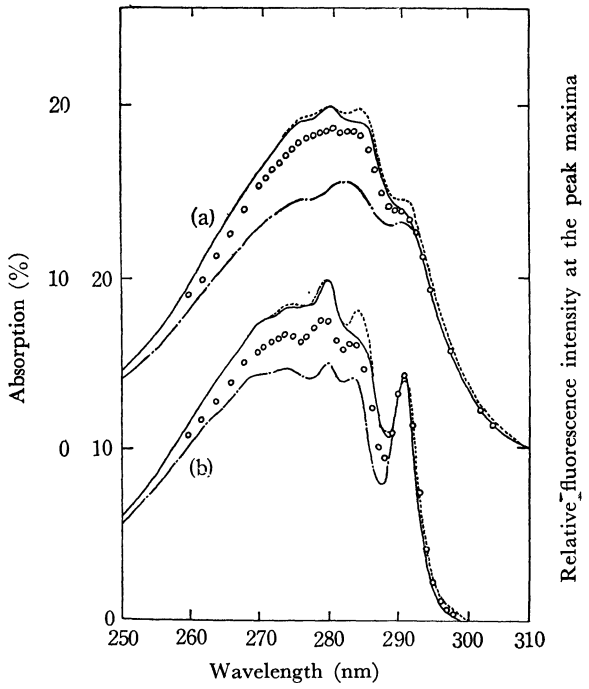

Fig. 3. The absorption and the fluorescence excitation spectra of I (a) in ethanol, and (b) in methylcyclohexane: (dotted line) the absorption spectrum, and (○) the excitation spectrum. The absorption spectra of (broken line) 3-methylindole, and (solid line) the equimolar mixture of 3-methylindole and *p*-methylanisole. The spectra except for that of 3-methylindole were obtained at 20 per cent of the absorption of the peak maxima.

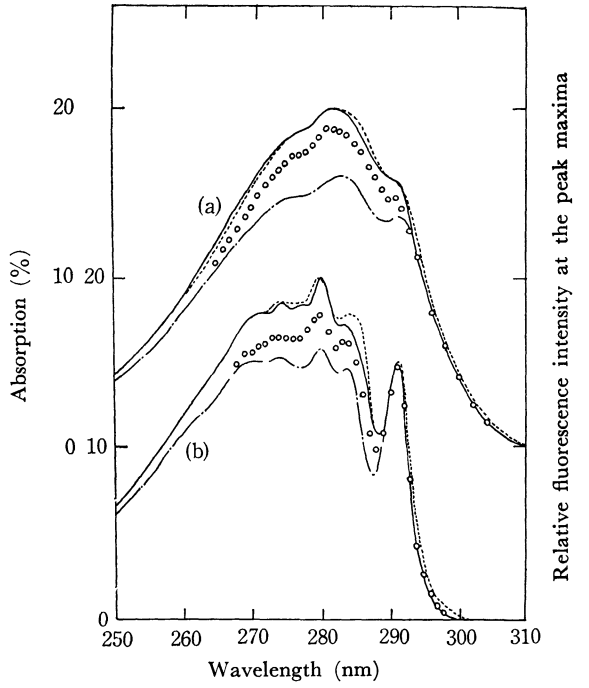

Fig. 4. The absorption and the fluorescence excitation spectra of III (a) in ethanol, and (b) in methylcyclohexane: (dotted line) the absorption spectrum, and (○) the excitation spectrum. The absorption spectra of (broken line) 3-methylindole, and (solid line) the equimolar mixture of 3-methylindole and *p*-cresol. The conditions are the same as those in Fig. 3.

As may be seen in Fig. 2, the fluorescence yields decrease upon an increase in the temperature, the shapes of the fluorescence spectra remaining unaltered. It is known that the nonradiative deactivation process of the indole derivatives is temperature-dependent.[3] From the slope of the straight line obtained by plotting $\log(1/Q_N-1)$ *vs.* the reciprocal of the absolute temperature,[15] the value of the activation energy of the process in I was evaluated to be 2.5 kcal/mol, which was equal to that of 3-methylindole. The fluorescence yields of II and III depend more strongly upon the temperature than do those of 3-methylindole and I (Fig. 2).

The absorption spectra of the compounds and the equimolar mixtures of the model compounds containing the indole chromophore or the phenol (anisole) chromophore alone are shown in Figs. 3—7. The absorption spectra of the compounds show a distinct spectral distortion compared with those of an equimolar mixture of the model compounds, as may be seen in the figures. The spectral difference is more predominant in methylcyclohexane than in ethanol. The absorption spectra of IV, V, and VI in methylcyclohexane were not available because of the poor solubility. The results obtained for II were very similar to those for I.

The fluorescence-excitation spectra are also illu-

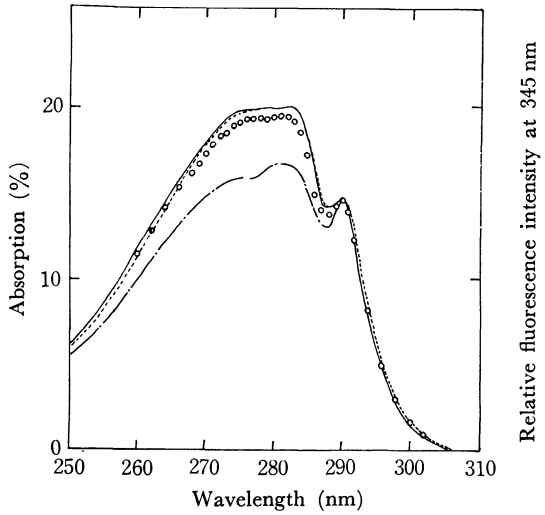

Fig. 5. The absorption spectra of (dotted line) IV, (broken line) 3-acetamidoindole, and (solid line) the equimolar mixture of 3-acetamidoindole and *N*-acety-*p*-methoxyphenethylamine, and (○) the fluorescence excitation spectrum of IV in ethanol. The conditions are the same as those in Fig. 3.

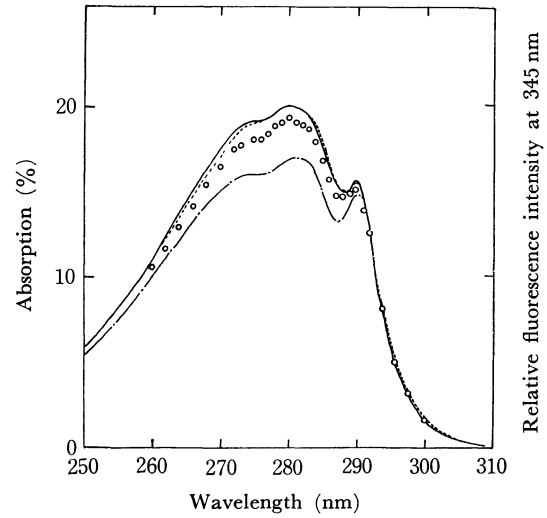

Fig. 6. The absorption spectra of (dotted line) V, (broken line) 3-acetamidoindole, and (solid line) the equimolar mixture of 3-acetamidoindole and *p*-hydroxyphenethylamine, and (○) the florescence excitation spectrum of V in ethanol. The conditions are the same as those in Fig. 3.

strated in Figs. 3—7. They are normalized to the absorption spectra of the equimolar mixtures of the model compounds on the long wavelength edge, in which the incident light is absorbed only by the indole chromophore. They are similar to the absorption spectra of the compounds rather than to those of the equimolar mixtures of the model compounds. However, the excitation spectra never reach the same level as the absorption spectra at wavelengths shorter than 290 nm, where the absorption spectra of the two chromophores overlap.

The values of the efficiencies of the singlet-excitation-energy transfer, Φ_t, which vary from 0.36 to 0.78, are listed in Table 2; they were calculated using Eq. (2). They seem to be subject to experimental error pri-

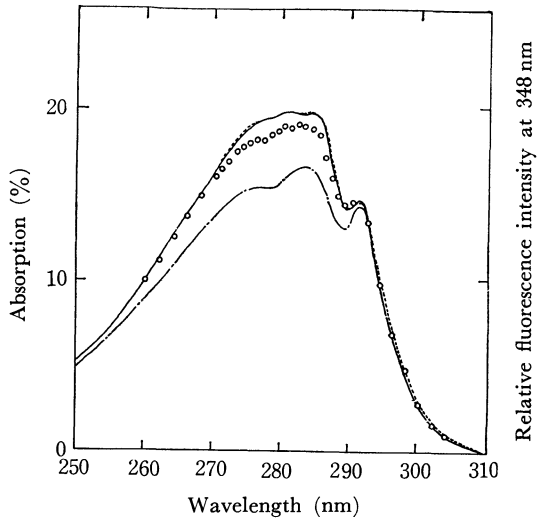

Fig. 7. The absorption spectra of (dotted line) VI, broken line) *N*-acetyltryptamine, and (solid line) the equimolar mixture of *N*-acetyltryptamine and *p*-methoxyphenylpropionamide, and (○) the fluorescence excitation spectrum of VI in ethanol. The conditions are the same as those in Fig. 3.

Table 2. Efficiencies of the intramolecular energy transfer from the phenol to the indole chromophores

Compound	Φ_t in Ethanol	Φ_t in Methylcyclohexane
I	0.63	0.46
II	0.60	0.49
III	0.48	0.36
IV	0.69	—
V	0.56	—
VI	0.78	—

marily because of our neglect of the small spectral differences between the absorption spectra of the compounds and the equimolar mixtures of the model compounds in the long-wavelength band.

The spectra of the fluorescence-excitation polarization in propylene glycol at −70 °C are shown in

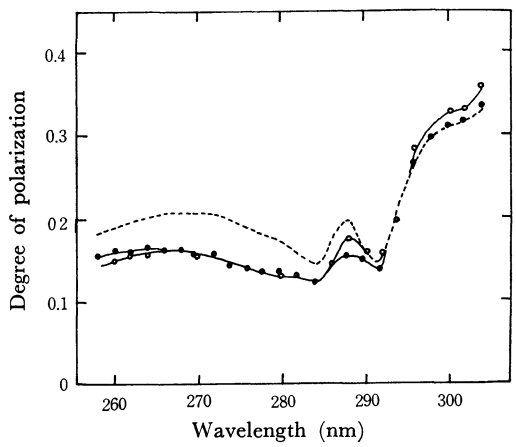

Fig. 8. The fluorescence-polarization spectra in propylene glycol at −70 °C: (dotted line) 3-methylindole, (-○-) I, and (-●-) III. The fluorescence was measured at 360 nm.

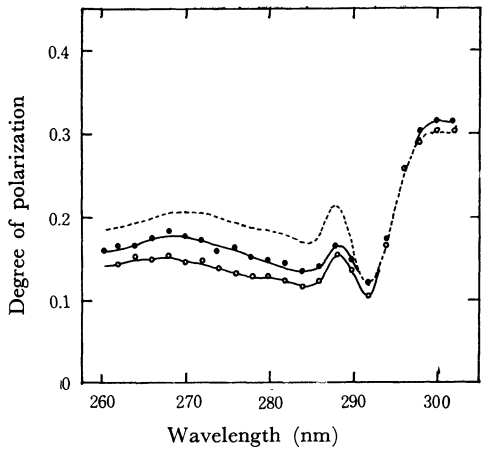

Fig. 9. The fluorescence-polarization spectra in propylene glycol at −70 °C: (dotted line) 3-acetamidoindole, (-○-) IV, and (-●-) V. The fluorescence was measured at 340 nm.

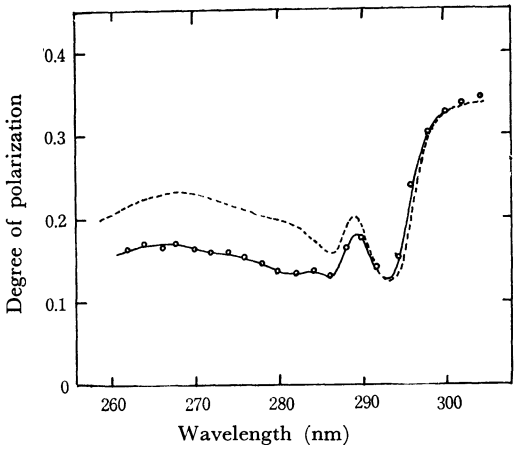

Fig. 10. The fluorescence-polarization spectra in propylene glycol at −70 °C: (dotted line) *N*-acetyltryptamine, and (-○-) VI. The fluorescence was measured at 360 nm.

Figs. 8—10. The spectrum of I exhibits an intense depolarization in wavelengths shorter than 290 nm, while it agrees well with the spectrum of 3-methylindole above 290 nm, as is shown in Fig. 8. The results for II were the same as those for I, and similar results were also obtained for the other compounds (Fig. 8—10).

Discussion

In the near ultraviolet spectra of indole and its derivatives, the ^1L_a and the ^1L_b electronic transition bands overlap strongly.[33-35] It is observed that the ^1L_a transition band is more sensitive to perturbation by solute-solvent interaction than is the ^1L_b transition band. Konev[7] and Strickland *et al.*[34] presented evidence that, on the addition of a drop of butanol to indole and 3-methylindole in hexane, the ^1L_a bands undergo a large red shift and broadening, while the ^1L_b bands undergo only a minor change. The spectral changes are believed to be due to the formation of a hydrogen bond, which severely affects the ^1L_a transition, the moment of which passes through the imino group, but which scarcely at all affects the ^1L_b transition, which is almost perpendicular to the ^1L_a transition.

From the facts that the distortion of the absorption spectra of I—VI is pronounced in the bands to which the absorption of the phenol group contributes little, and is obviously predominant in a non-polar solvent (Fig. 3–7), it may be said to be due to the change in the ^1L_a band of the indole chromophore, a change which is induced by weak intramolecular hydrogen bonding to the phenolic oxygen or the π orbitals of the phenol ring.[36,37] On the other hand, it has been pointed out that the absorption spectra of the adjacent chromophores close to each other differ from the sum of those of the constituents in the peak position and the intensity, mainly because of long-range exciton interaction.[27] The ^1L_b band as well as the ^1L_a band may be expected to change as a result of such interaction.

The low fluorescence yields of II and III at room temperature are anomalous (Table 1 and Fig. 2). The reduction of the population of the singlet state in II and III due to the low probability of the intramolecular energy transfer, due to the enhancement of the intersystem crossing, and due to the photochemical reaction may be excluded, since the efficiencies of the intramolecular energy transfer in II and III were as high as those in other compounds (Table 2), since the ratios of the fluorescence to the phosphorescence of II and III were the same as that of 3-methylindole, and since no detectable absorption change could be observed after irradiation for the fluorescence measurement. Therefore, it may be inferred that the molecular configuration of II and III, in which the two aromatic rings are joined by three methylene groups, is responsible for the fluorescence quenching.

Hirayama's $n=3$ rule states that the aromatic ring pairs combined with three methylene groups have a face-to-face parallel configuration and are likely to form an intramolecular excimer or exciplex as a result of the strong overlap of the two π orbitals.[38] Recently, the temperature dependence of the excimer formation in 1,3-dinaphthylpropane was reported.[39] The disappearance of the excimer emission at low temperature was interpreted in terms of rotation barriers in the methylene chain joining the naphthalene nuclei.

From the fact that the fluorescence yields of II and III approach that of 3-methylindole at low temperatures (Fig. 2), it may be suggested that the quenching of the monomer fluorescence at room temperature has its origin in the formation of the intramolecular com-

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plex between the indole group in the excited state and the phenol group in the ground state.

The efficiencies of the energy transfer from the phenol to the indole chromophores in the compounds containing the amide group (IV, V, and VI) remain as high as in the other compounds (Table 2), whereas it has been suggested that the peptide group enhances the internal conversion of the phenol chromophore.[16] On the other hand, there is no evidence that *N*,*N*-dimethylacetamide reduces the efficiencies of the fluorescence or of the intramolecular energy transfer in the compounds containing the phenol chromophore (III and V), although the amide compound intermolecularly quenches the fluorescence of phenol by forming a hydrogen bond.[17] These results indicate that the rate constant of the energy transfer is much greater than those of the radiationless deactivation and of the diffusion-controlled bimolecular encounter.

According to the theory of resonance-energy transfer developed by Förster,[12] the critical radius, R_0, that is, the distance at which the probability of the energy transfer is 50 percent, can be calculated from the experimental parameters. The values of R_0 calculated for the energy transfer from *p*-cresol or *p*-methylanisole to 3-methylindole both in ethanol and in methylcyclohexane are listed in Table 3. The results that the value of R_0 are larger in ethanol (26 and 29 Å for *p*-cresol, and *p*-methylanisole respectively) than in methylcyclohexane (22 and 25 Å) are mainly the result of the more intense overlap integrals and fluorescence yields of the donor molecules in ethanol than in methylcyclohexane. They may agree with the results that the efficiencies of the intramolecular energy transfer are larger in ethanol than in methylcyclohexane, as is shown in Table 2.

Table 3. Calculated critical radii for the resonance energy transfer from *p*-cresol or its methyl ether to 3-methylindole in ethanol and in methylcyclohexane

Donor	Solvent	R_0[a] (Å)
p-Cresol	Ethanol	26
p-Cresol	Methylcyclohexane	22
p-Methylanisole	Ethanol	29
p-Methylanisole	Methylcyclohexane	25

a) The quantum yields of the donor fluorescence were evaluated using that of *p*-cresol which is 0.23 in water.[16] The random orientation of the donor and the acceptor molecules was assumed.

However, it has been suggested that the intramolecular singlet-excitation transfer must be totally efficient in a compound in which the donor-acceptor chromophores are less than ten angstroms apart.[40] The experimental difficulties are responsible for the low transfer efficiencies, as is calculated in Table 2. Higher values might be obtained if the slight spectral distortion in the long-wavelength band could be taken into account.

The depolarization of the fluorescence of the compounds in propylene glycol (Fig. 8—10) may be quantitatively analyzed using the equation formulated by Weber:[41]

$$1/P - 1/3 = [\sum f_i/(1/P_i - 1/3)]^{-1} \quad (3)$$

where P is the degree of the polarization, P_i is that of the ith molecule, and f_i is the fraction of the fluorescence intensity of the ith molecule to the total fluorescence intensity. Now, two types of fluorescence are considered: one ($i=0$) is the fluorescence from the molecule in which the incident quanta are absorbed directly by the indole chromophore, and the other ($i=1$) is the fluorescence from the molecule in which the phenol chromophore is initially excited and the excitation energy is intramolecularly transferred to the indole chromophore. The further sequence of i can be disregarded, since the probability of the reverse transfer of the excitation energy is very low because of the small overlap integral.

Therefore, f_0 and f_1 are represented by the equation: $f_0=A_{\mathrm{In}}/(A_{\mathrm{In}}+\Phi_t\cdot A_{\mathrm{Ph}})$; $f_1=\Phi_t\cdot A_{\mathrm{Ph}}/(A_{\mathrm{In}}+\Phi_t\cdot A_{\mathrm{Ph}})$. The values of P_1, as calculated assuming that Φ_t is 100 percent and substituting the values of P of the model compounds containing only the indole chromophore for the values of P_0, are listed in Table 4. It is remarkable that P_1 indicates the negative polarization on the excitation near 270 nm. This can be expected if the emitting oscillator differs from the exciting one and if $\cos^2\theta<1/3$ is satisfied, where θ is the relative orientation angle.[42]

Table 4. Polarization of the fluorescence on excitation at 270 nm in propylene glycol at −70 °C

Compound	f_0	f_1	P	P_0	P_1[d]
I	0.76	0.24	0.15	0.20[a]	−0.02
II	0.76	0.24	0.16	0.20[a]	0.00
III	0.82	0.18	0.15_5	0.20[a]	-0.07_5
IV	0.80	0.20	0.15	0.21[b]	−0.13
V	0.84	0.16	0.17	0.21[b]	-0.02_5
VI	0.81	0.19	0.16	0.22[c]	−0.12

a) 3-Methylindole. b) 3-Acetamidoindole. c) *N*-Acetyltryptamine. d) Calculated using Eq. (3).

Since the relative orientation of the exciting oscillator in the phenol ring and the emitting one in the indole ring cannot remain unaltered because of the flexibility of the methylene groups combining the two chromophores, the negative polarization cannot be caused by intramolecular energy transfer, which may induce the depolarization of the fluorescence. Therefore, the result may be interpreted on the assumption that the excitation energy absorbed by the phenol group is transferred to the ^1L_b transition oscillator of the indole group, and that it is internally converted to that of the ^1L_a, which is perpendicular to the former and which is known to emit fluorescence in the polar solvent.[33]

These conclusions agree well with the results described by Weber,[24] who observed a small but doubtless negative polarization of indole in the presence of a

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large amount of phenol.

The overlap integral of the donor fluorescence and the acceptor absorption spectra, and the relative orientation of the two electronic transition moments, are critical in the intermolecular and intramolecular energy transfers. It is difficult to expect that the ^1L_b transition moment would take an especially favorable orientation in such energy transfers. On the other hand, the ^1L_b band may overlap more strongly with the phenolic fluorescence than the ^1L_a band, since the former is located in the long-wavelength absorption bands of the indole moiety. Thus, the activity of the ^1L_b transition in the energy transfer may be interpreted in terms of the overlap integral factor rather than in terms of the orientation factor.