On the Base Strength of Some Nitrogen Heterocycles in the Excited State

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Introduction

Some properties of a molecule such as basicity and acidity or its ability of dydrogen bonding etc., depend in general upon its electronic structure. Therefore, it may be expected that the above-mentioned properties in the excited electronic state are different as compared with the ground electronic state, and we really have such examples.

Th. Förster and A. Weller[1,2] have investigated the ionization tendency of naphthols, aminopyrene derivatives, etc. in their excited electronic states by measuring the fluorescene spectra and fluorescene intensities and confirmed that the degree of dissociation in the excited state is far greater than that in the ground state. Hence these compounds can be said to have a greater acidity in the excited state than in the ground state. As for hydrogen bonding, the present authors[3] have measured the absorption and fluorescence spectra and fluorescene intensities of *n*-hexane solutions of naphthols with addition of various proton acceptors, and found that the excited naphthols have a greater tendency to form hydrogen bond and that a new equilibrium is reached during the lifetime of the excited state.

Now in order to compare the basicity of a molecule in the ground and excited state, same nitrogen heterocycles such as quinoline, acridine and 2,8-diaminoacridine were chosen and their fluorescene and absorption spectra were measured over a wide pH range. In addition their spectra and fluorescene intensities in various organic solvents were compared. The present paper reports the results obtained with some discussions about the electronic structures of these molecules.

Experimental

Apparatus.—Fluorescene spectra were taken with Hilger E_2 type quartz spectrograph and small type glass prism spectrograph. For the excitation of fluorescene spectra, a high-pressure mercury lamp was used with appropriate filters for each samples.

Filters: for quinoline, K_2CrO_3 aq. solution (0.2 g./l.) which takes out 310 mμ line.

for acridine, Matsuda color filter UV-DI which takes out 360 mμ line.

for 2,8-diaminoacridine, Matsuda color filter UV-DI, which takes out 360 mμ or VC-I and IR-OI which together take out 405 and 435 mμ lines.

For the measurement of absorption spectra in solution, Beckman spectrophotometer model DU was used.

The apparatus used for the measurement of fluorescence intensity was the same as described elsewhere.* In the case of quinoline, the filters used were the same as before*, and in the case of acridine, Matsuda color filter UV-DI was used for exciting light and the solution of acridine in *n*-hexane, ca. 10^{-3} mol./l., was used as a filter for the fluorescent light.

Reagent.—Extra pure grade quinoline was dried over K_2CO_3 and distilled under the atmosphere of dry nitrogen before use.

Chemical pure grade acridine was recrystallized from alcohol.

A synthesized and purified sample of 2,8-diaminoacridine was kindly supplied by Mr. T. Tokuyama of this institute.

For the pH control of aqueous solution, citrate-phosphate-borate buffer was used. The reagents were of extra pure grade, and were used without further purification.

n-Hexane, CH_3CO_2Bu and CCl_4 were the same samples as described elswhere*. Extra pure grade benzene was recrystallized and distilled. Ethanol used was 99% in purity.

Extra pure grade *n*-butanol and pyridine were used without further purification.

Experimental Results

a) Dependence of the Absorption and Fluorescence on the Hydrogen Ion Concentration in Aqueous Solution

The absorption spectra of quinoline in aqueous solution of various pH values are shown in Fig. 2.

Quinoline being isoelectronic with naphthalene, its absorption spectrum is similar to that of the latter, with three absorption regions in the near ultraviolet; 295–320 mμ (band I), 250–295 mμ (band II) and the region

1) Th. Förster, *Z. Elektrochem.*, **54**, 42, 531 (1950).
2) A. Weller, *Z. Elektrochem.*, **56**, 662 (1952); *Z. physikal. Chem. N.F.*, 3, 238 (1955).
3) N. Mataga, Y. Kaifu and M. Koizumi; *Nature*, **175**, 731 (1955).

* N. Mataga, Y. Kaifu and M. Koizumi; to be published in this Bulletin.

near 220 mμ (band III). The absorption spectra in the pH range <1.4 and >8 coincide with the curve (1) and (6) respectively, and between ca. pH=7 and 2, they gradually change with isosbestic point at ca. 290 mμ. Therefore this spectral change can be attributed to the change of the concentration of free quinoline (A) and quinolinium ion (AH^+), according to the equilibrium relation $A+H^+ \rightleftarrows AH^+$. pK value is easily obtained from the inspection of these changes, and is depicted in Fig. 1 as a pH value at which [A]=[AH^+].

It is seen from Fig. 2 that in A the intensity of band I is rather smaller than that of band II, while in AH^+, the former becomes far greater, and the latter is diminished. It is noteworthy that λ_{max} of band I remains constant throughout the whole change, though in AH^+ there appears a slight hump in the neighbourhood of 330 mμ. The fluorescene spectrum shifts from 305–350 mμ band of A to 340–440 mμ band of AH^+ as indicated in Table I, but the change occurs at larger pH value compared with that of absorption spectrum. The line depicted in Fig. 1 is the pH value at about the middle point of the region in which the spectral change occurs.

TABLE I

RANGE OF FLUORESCENCE SPECTRA OF A, AH^+, AH_2^{++} AND AH_3^{+++} (in mμ)

	A	AH^+	AH_2^{++}	AH^{3+++}
Quinoline	305–350	340–440	——	——
Acridine	390–500	390–600	——	——
2,8-Diaminoacridine	——	450–610	505–610	420–610

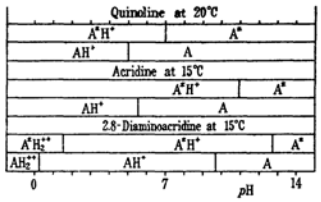

Fig. 1. Shift of the equilibrium in the excited state as revealed by the change of fluorescence and absorption spectra.

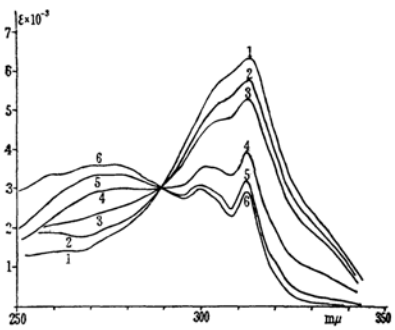

Fig. 2. pH Dependence of the absorption spectrum of quinoline in aqueous solution. at ca. 20°C
concentration of quinoline, 1.08 ×10⁻⁴ mol./l.
pH: 1. 1.42
2. 4.45
3. 4.89
4. 5.59
5. 6.47
6. 8.04

* The nomenclature in this case is rather conventional.

ii) Acridine.—The absorption spectrum of acridine is similar to that of isoelectronic anthracene, and there are 300–400 mμ band (Band I) and the band near 250 mμ (band II)*.

pK value or pH value at which [A]=[AH^+] is ca. 5 as shown in Fig. 3. The intensity of band I is stronger in AH^+ than in A and the position of maximum remains constant throughout the whole pH range. In the acidic solution, a new broad band appears in the region 380–440 mμ, and two isosbestic points are seen at 360 mμ and 377 mμ. λ_{max} of band II (249 mμ) in A shifts to 254 mμ in AH^+ with the intensity somewhat decreased.

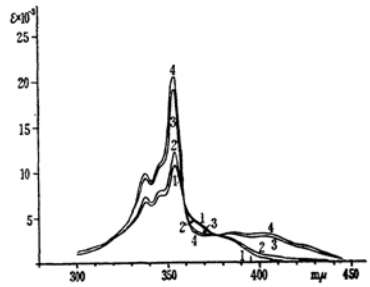

Fig. 3. pH Dependence of the absorption spectrum of acridine in aqueous solution. at ca. 15°C
concentration of acridine, 5.78 ×10⁻⁵ mol./l.
pH: 1. <8.04
2. 6.47
3. 4.88
4. >3.69

The fluorescence spectrum are shown in Fig. 4. At first sight, it appears as if there are two steps of change, i.e. at pH=ca. 10

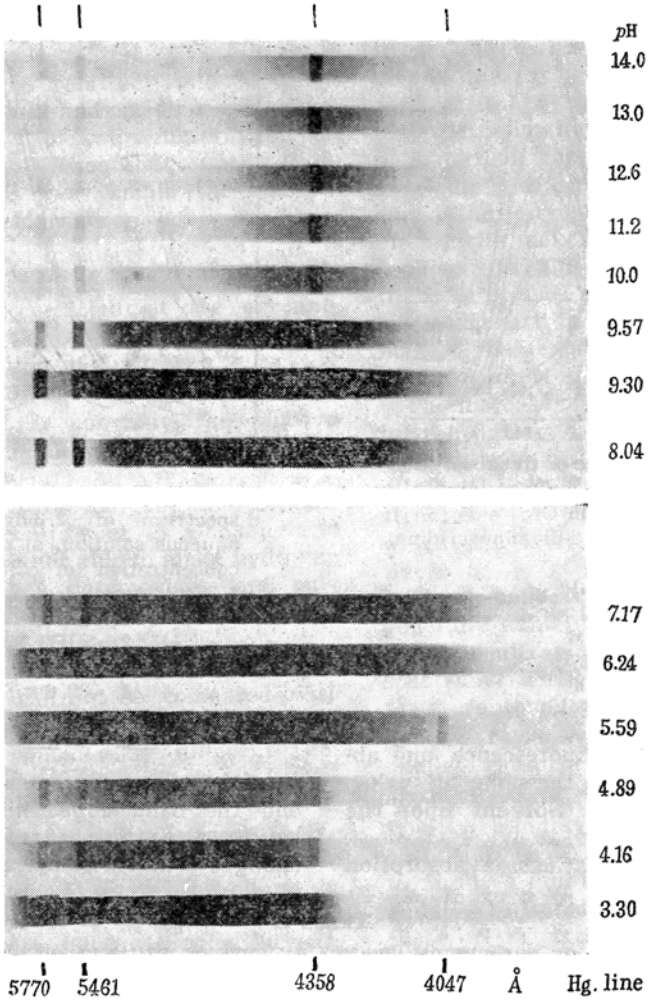

Fig. 4. pH Dependence of the fluorescence spectrum of acridine in aqueous solution, at ca. 15°C.

and pH=ca. 5. But the latter change is most probably attributed to the reabsorption of fluorescence by 380–440 mμ band, which is prominent when pH<5. It seems that the change of colour in the fluorescence of acridine cited in Pringsheims' book[4] corresponds to this apparent change. There scarcely remains any doubt that the real change of fluorescence spectrum occurs at far greater pH value compared with that of absorption spectrum.

iii) 2, 8-Diaminoacridine.—This molecule has three nitrogen atoms each of which may act as a proton acceptor. Accordingly, the spectral changes seem to occur in three stages. As indicated by Craig et al.[5], the first proton may attach to the ring nitrogen. In this first stage, the spectrum shows a marked change as shown in Fig. 5a. The spectrum shifts to longer wave length from λ_{max}=395 mμ to λ_{max}=443 mμ with the isosbestic point at 410 mμ, and the absorption intensity increase remarkably.

The change of fluorescence spectrum in the first stage occurs at far greater pH value than that of absorption spectrum as shown in Fig. 1. The two succeeding stages are not so clear-cut as the first one, but the following features may be observed from Fig. 5b. In the second stage, there appear two peaks at 455 mμ and 360 mμ with two isosbestic points at 470 mμ and 370 mμ, and the change of fluorescence spectrum occurs at larger pH value. In the third stage the band at 360 mμ and 455 mμ decline and a new band appears at 350 mμ. In this case,

4) P. Pringsheim, "Fluorescence and Phosphorescence" Interscience Publishers Inc., 1949, p. 416.

5) D.P. Craig and L.N. Short, *J. Chem. Soc.*, 1945, 419.

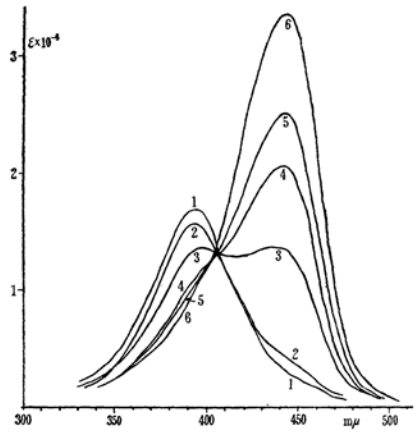

Fig 5a. pH Dependence of the absorption spectrum of 2,8-diaminoacridine in aqueous solution, at 15°C
concentration of 2,8-diaminoacridine, 1.28×10^{-5} mol./l.
pH: 1. 13~12
2. 11.24
3. 10.06
4. 9.57
5. 9.30
6. 4.89~1.92

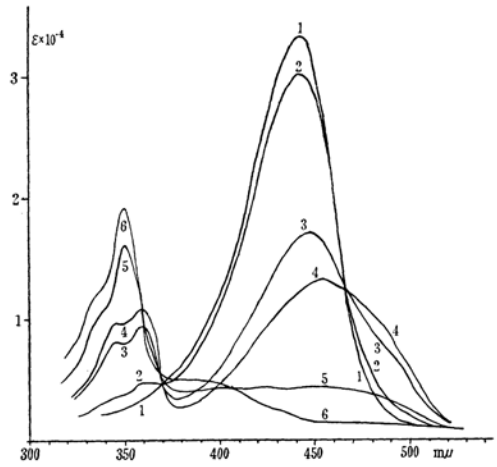

Fig. 5b. pH Dependence of the absorption spectrum of 2,8-diaminoacridine in aqueous solution, at ca. 15°C
concentration of 2,8 diaminoacridine, 1.28×10^{-5} mol./l.
1. pH 1.925
2. pH 1.038
3. pH O.
4. 3.6 N H_2SO_4
5. 12.6 N H_2SO_4
6. 18 N H_2SO_4

the spectral change of fluorescence and absorption occur almost at the same pH value.

b) Effect of Organic Solvent upon the Spectrum

In Table II the variations of absorption spectra in several solvents are indicated. When the solvent molecule increases in its proton-donating power from *n*-hexane to ethanol, band I grows and band II declines regularly in the case of quinoline, and in the case of acridine 373 mμ band becomes broader and the band edge shifts to longer wave length also in a regular manner. These changes conform completely to the already mentioned changes induced by the proton addition. Thus the parallelism exists between the spectral change induced by proton addition and that induced by the hydrogen bonding.

TABLE II

CHANGE OF ABSORPTION SPECTRA IN SEVERAL ORGANIC SOLVENTS

Quinoline

Solvent		*n*-Hexane	CCl_4	CH_3CO_2Bu	BuOH	EtOH
Extinction coeff. of abs. max. ($\times10^3$)	band I	2.1	2.3	2.1	2.8	3.0
	band II	3.6	4.1	3.6	3.4	3.3

Acridine

Solvent	*n*-Hexane	Benzene	Pyridine	BuOH	EtOH
Edge of the longest wave length band	392	393	395 (in mμ)	397	402

TABLE III

EFFECT OF SOLVENT ON THE FLUORESCENCE INTENSITY

Solvent	*n*-Hexane	Benzene	BuOH	EtOH	H_2O
Quinoline	$\lesssim10^{-3}$	$\sim10^{-3}$	$\sim3\times10^{-2}$	$\sim3\times10^{-2}$	~1.0 (pH 4.79)*
Acridine	——§	$\sim10^{-3}$	$\sim2\times10^{-2}$	$\sim3\times10^{-2}$	~1.0 (pH 6.30)*

* pH was not controlled by buffer, and measured by glass electrode pH meter.

§ Fluorescence was not detectable with our apparatus.

c) Effect of Solvent upon the Fluorescence Intensity

In Table III the relative yield of fluorescence in quinoline and acridine are given. The data in this Table were determined in the following way. When the concentration of a fluorescer is very small, the relation $I_f=\varepsilon c\Phi I_0$ must hold, where,

I_f ; Fluorescence intensity
Φ ; Quantum yield of fluorescence
ε ; Extinction coefficient at the exciting wave length
c ; Concentration of fluorescer
I_0 ; Intensity of exciting light

Hence when I_0 is constant, the relative yield is easily calculated from $\Phi'/\Phi=I'_f\varepsilon c/I_f\varepsilon' c'$.

As seen from Table III, the fluorescence yields in the solvents such as *n*-hexane and benzene which are not able to make hydrogen bond with nitrogen heterocycles, are only 10% or smaller compared with those in the solvents such as BuOH or EtOH.

The fluorescence intensity of AH^+ in aqueous solution can not be compared with those data, because the spectral range of fluorescence is considerably different (For quinoline, in *n*-hexane ; 305–350 mμ, in EtOH ; 305–370 mμ against 340–440 mμ of AH^+. For acridine in EtOH ; 360–480 mμ against 450–610 mμ of AH^+) and the exact calibration of the wave length sensitivity of the phototube was not undertaken. It is certain, however that the sensitivity falls with the increase of wave length*, and if one takes this into account, the fluorescence yield of AH^+ is expected to be far greater than the fluorescence yield of A in organic solvents. In the case of 2, 8-diaminoacridine, however, fairly strong fluorescence is observed even in the non-polar solvent such as benzene, and the hydrogen bond formation or proton addition is not necessarily indispensable for the fluorescent transition.

Discussion

I. The Increase of the Basicity in the Excited State

It can be concluded from the experimental results, that in these nitrogen heterocycles the excited electronic structure is more liable to proton addition than the ground state electronic structure. Thus the stabilization energy by proton addition is larger in the excited state than in the ground state. As Förster and his collaborators have confirmed, naphthols and some other compounds have greater acidity in the excited state, while in the present investigation, it has been confirmed that N-heterocycles have larger basicity in the excited state. Both effects will most certainly be attributed to the increase of polarity in the excited state. In the case of naphthol, for example, the migration of lone pair electrons of oxygen atom to the ring is larger in the excited state, the result of which makes the oxygen atom more repulsive toward proton. Nitrogen atom in the heterocycles on the contrary, will become more negative in the excited state and this makes the excited state more attractive toward proton.

The increase of electronegativity of N atom in the latter case will be interpreted in various ways but here the problem will be discussed on the basis of the electronic structures of cata-condensed hydrocarbons which are isoelectronic to their aza derivatives.

The so-called cata-condensed hydrocarbons generally exhibit three main band systems in the near ultraviolet or visible region of the spectrum. The prototypes of all these systems are to be found in benzene, which shows a weak band system in the 2500 Å region, exhibits absorption of moderate intensity around 2000 Å, and absorbs strongly near 1750 Å. Band systems of a similar type also occur for the higher homologs. According to Moffitt's notation[6], these are N→V, N→U and N→Y transitions, respectively. Among these, N→Y transition corresponds to the so-called "intramolecular charge transfer spectrum" of Mulliken[7]. From the standpoint of VB method, Y state is mainly contributed by ionic structures.

Now in the case of inductive substitution such as the replacement of —CH= by —N=, the effect of this perturbation is to mix the unperturbed V state to which transition from the ground state is virtually forbidden, with some contribution from Y state to which transition is strongly allowed. The result is that the partial transfer of intensity from N→Y transition to N→V transition occurs as is actually confirmed by the comparison of the absorption spectra of N-heterocycles and of their isoelectronic cata-condensed hydrocarbons. V state thus acquires considerable ionic character with negative charge at nitrogen atom, and this is the reason why the interaction energy with proton is larger in the excited state than in the ground state.

The intensification of the spectrum in the acidic region as observed in acridine, may

* The phototube used is ultraviolet sensitive one.

6) W. Moffitt, *J. Chem. Phys.*, 22, 320 (1954).
7) R.S. Mulliken, *J. Chem. Phys.*, 7, 20, 353 (1939).

be attributed to the further increase of such a polar structure since the electronegativity of N becomes larger by the attachment of a proton. But the conspicuous change of spectra in the case of quinoline and 2,8-diaminoacridine can not yet be interpreted.*

II. Solvent Effect on Fluorescence Intensity

It is almost certain that in the case of acridine and quinoline, the settlement of non-bonding electrons by proton addition or hydrogen bonding increases the fluorescence efficiency. The mechanism may presumably be interpreted in the following way.

In these compounds, $^1\Gamma_{\pi\to\pi^*}$ which is produced directly by the illumination has probably a large tendency of transfer to $^1\Gamma_{n\to\pi^*}$, because the authors have newly found the spectra of quinoline due to $n\to\pi^*$ transition and moreover, their positions are in the neighbourhood of $\pi\to\pi^*$ band (see Appendix).

If $^1\Gamma_{n\to\pi^*}\to{}^1\Gamma_{n\to\pi^*}$ transfer occurs, then it is followed by $^1\Gamma_{n\to\pi^*}\to{}^3\Gamma_{n\to\pi^*}$ with considerable ease[8]. Then owing to the long life of $^3\Gamma_{n\to\pi^*}$ state, emission is scarcely to be expected. This may be the reason why the fluorescence is not observed in non polar solvents. By proton addition or hydrogen bonding, however, the non-bonding character is lost almost completely or to some extent and the level of $^1\Gamma_{n\to\pi^*}$ state is raised remarkably. Accordingly, it becomes quite difficult for $^1\Gamma_{n\to\pi^*}\to{}^1\Gamma_{n\to\pi^*}$ transition to occur and the result is that the molecule emits more easily.

In the case of 2,8-diaminoacridine, the reasonance interaction between π-electron system and substituent-NH_2, makes $n\to\pi^*$ transition shift to blue, while $\pi\to\pi^*$ transition to red[9]. As the result of this perturbation, $^1\Gamma_{\pi\to^*}\to{}^1\Gamma_{n\to\pi^*}$ transition becomes energetically difficult. This will be the reason why the fluorescence of 2,8-diaminoacridine can be observed even in the non-polar solvent.

Appendix

$n\to\pi^*$ Spectrum of Quinoline Vapor

The absorption spectrum of quinoline vapor was photographed by Hilger E_2 type quartz spectrograph. The quartz absorption cell was 50 cm. in length with a side tube for reserving sample. As a light source an A. C. high voltage hydrogen discharge tube with water cooling was used. The temperature of bath was 90°C and that of absorption cell was 95°C. The slit width was 0.05 mm. and the time of exposure was about 2 min.

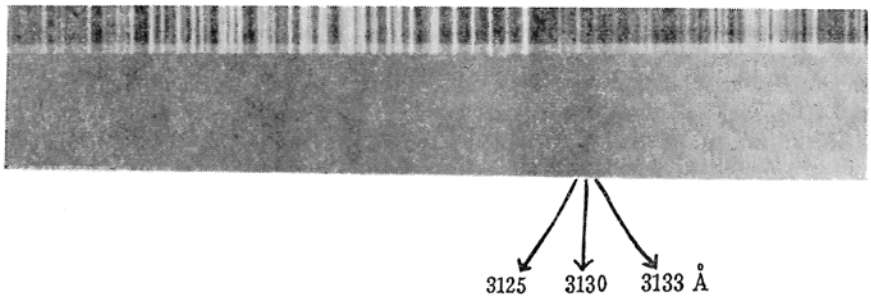

Fig. 6. Absorption spectrum of quinoline vapor.

As seen from Fig. 6, the line spectra at 3125, 3130 and 3133 Å are clearly observed, and these line spectra may most certainly be interpreted as due to the $n\to\pi^*$ transition.

In general, when molecule becomes larger, $\pi\to\pi^*$ transition becomes superposed by strong $\pi\to\pi^*$ transition[10], and moreover the oscillator strength of $n\to\pi$ transition becomes smaller[9]. Hence the observation of $n\to\pi^*$ transition is difficult in larger molecules. In fact the $n\to\pi^*$ transition of pyridine has been reported in literature, while that of quinoline has not been observed as yet, and we really had much difficulty in taking the line spectra in question.

In solution, the $n\to\pi^*$ spectra can never be observed even in n-hexane. It is certain from these line spectra, that $n\to\pi^*$ state of quinoline is nearly at the same energy level with $\pi\to\pi^*$ state.

* Note added in prof:
An alternative interpretations of these spectral changes are as follows. We divide the 300-400 mμ band of acridine into two parts, i.e., 300-370 mμ band (band I) and 370-400 mμ band (band II), and regard these bands as corresponding ones to N→V and N→U band, resqectively. In quinoline, the assignment is; band I←→N→V, band II←→N→U. By proton addition, band II of acridine shifts to red and somewhat strengthened, whereas band I does not shift although it is enhanced. The spectral changes of quinoline are similar to those of acridine, but in the case of quinoline, the shift of band II is very great, and the slight hump near 330 mμ may be attributable to this shifted band II. Moreover, the longest wave length band of 2,8-diaminoacridine may plausibly correspond to N→U transition, shifted to red by resonance interaction with substituent amino groups.

8) M. Kasha, *Discuss. Farad. Soc.*, No. 9, 14 (1950).

9) L. Goodman and H. Shull, *J. Chem. Phys.*, 22, 1138 (1954).

10) J.R. Platt, *J. Chem. Phys.*, 19, 101 (1951).

Summary

1. The fluorescence and absorption spectra of aqueous solutions of quinoline, acridine and 2,8-diaminoacridine were observed at various pH values, and it became clear that the stabilization energy by proton addition is larger in the excited state than in the ground state.

2. The effects of hydrogen bonding and proton addition upon the spectra were confirmed to be the same in tendency.

3. On the basis of the fluorescence intensities in various organic solvents, some discussions were made about the contribution of non-bonding electron to the inner quenching of fluorescence.

4. The vapor spectrum of quinoline was taken and line spectra were observed, which are most certainly attributable to $n \rightarrow \pi^*$ transition.

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