Some Spectral Studies of the Aqueous Solution of Pyronine G

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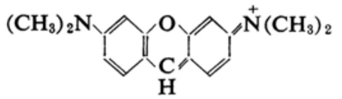
Pyronine G is a xanthene dye with a positive charge, as againt anionic eosine and uranine, and it has a simpler structure than the latter dye ions, as is shown below:



We have studied some of its optical properties in an aqueous solution as a preparatory step for studying its photochemical behavior; this paper concerns the following three phenomena: I) dimerization, II) formation of carbinol as studied from the absorption spectra (pH dependence of the absorption spectra), and III) pH dependence of the fluorescent behavior.

I. Dimerization

Not long ago, one of the present authors studied the dimerization of this dye in relation to metachromasy[1] and observed a typical spectral change due to the dimerization. In this paper, special attention has been paid to the interaction between the dye cation and inorganic anion.

Dimer bands, when observed, usually lie on the shorter wavelength side of monomer bands; this phenomenon has been interpreted on the basis of Förster's model[2]. Thus, when the dimer is formed from two monomeric ions lying close together, with their molecular planes opposite each other, the excited level is split into two, of which two only the higher level has an appreciable transition moment, causing the blue shift of the dimer-band. But it is not quite certain how, if at all, the anion takes part in the dimerization. Simpson et al.[3] observed that in the case of 1, 1′-diethyl-2, 2′-pyriodocyanine, iodide and perchlorate have different association constants and that, moreover, the position of the dimer band is different in the two cases; from these facts they concluded that anions are involved in the dimerization. They further inferred that the ionic pair is formed in both monomer- and dimer- forms, but in the former case, it can only occur at the expense of the resonance energy of dye ion (due to the electrical charge), while in the latter, the univalent anion is sandwiched between two dye ions, each with half a unit charge; this partly contributes to the stability of the dimer. Ramette[4] studied the association of rhodamine B, and from the finding that the dimerization constant is different for chloride, bromide and perchlorate (they studied in particular the variation of an orange-band which is due to a two-protonated species), they also surmised the participation of the ionic pair in the dimerization.

It may now be possible in principle to decide whether the molecular species involved in the dimerization are an ionic pair or free ions and what their ionic values are, if the effect of the ionic strength on the association constant is carefully investigated. Denoting monomer and dimer as M (or M′ if in a different state) and MM′, the thermodynamic dimerization constant for

$$\mathrm{M} + \mathrm{M}' \rightleftharpoons \mathrm{MM}'$$

would be given as follows:

$$K_a = K\frac{f_{\mathrm{MM}'}}{f_{\mathrm{M}}f_{\mathrm{M}'}}$$

$$\log K = \log K_a + \log f_{\mathrm{M}} + \log f_{\mathrm{M}'} - \log f_{\mathrm{MM}'} \tag{1}$$

in which K is the apparent association constant and f's, the activity coefficients.

If Debye-Hückel's theory in its simple form is applied to f's as an approximation, then

$$\log f_i = -aZ_i^2\sqrt{J} \tag{2}$$

The combination of 1 and 2 would enable us to predict how the apparent dimerization constant is affected by the ionic strength. Some of the conceivable cases will be cited below:

a) Participation of no anion

$$\log K = \log K_a + 2a\sqrt{J}$$

or approximately

$$\log K = \log K_a + \sqrt{J}$$

b) Participation of a univalent anion (X^-)

i) if $\mathrm{M} = \mathrm{DX}$ $\quad \mathrm{M}' = \mathrm{DX}$

$$\log K = \log K_a$$

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[1] M. Koizumi and N. Mataga, This Bulletin, 27, 194 (1954).

[2] Th. Förster and E. Konig, *Z. Elektrochem.*, 61, 344 (1957).

[3] G. S. Levinson, W. T. Simpson and W. Curtis, *J. Am. Chem. Soc.*, 79, 4314 (1957).

[4] R.W. Ramette and E. B. Sandell, ibid., 78, 4872 (1956).

ii) if $M=DX \qquad M'=D^+$

$$\log K=\log K_a$$

c) Participation of a bivalent anion (Y^{2-})

i) if $M=M'=DY^-$

$$\log K=\log K_a+\sqrt{J}$$

ii) if $M=DY^-, \qquad M'=D^+$

$$\log K=\log K_a-\sqrt{J}$$

In the above classification, case b ii) and case c ii) will perhaps correspond to the sandwich-model of Simpson, while b i) and c i) will be cases in which a 1 : 1 ion pair is formed between the dye and the anion, those perhaps occupying lateral position in both monomer and dimer.

From such a view, the effects of the addition of some salts such as potassium chloride, potassium nitrate, magnesium sulfate, potassium iodide, potassium perchlorate and sodim perchlorate were investigated. As a supplement, the effect of pH on the dimerization was also examined.

Experimental.—*Sample.*—Merck's reagent was used without further purification. $\varepsilon\approx1.2\times10^4$. Later, for the sample in parts II and III, purification was made by repeated crystallization, giving an appreicable increase of ε; this indicated the crudeness of above sample. Hence, the absolute value of the dimerization constant is not very reliable, but discussion on the salt effect will nevertheless be permissible since it is essentially based on the relative rather than the absolute quantity.

The Method of Determining the Association Constant.—The absorption spectra are measured by a Hitachi spectrophotometer EPU-2, using cells 1, 0.3 or 0.1 cm. in thickness according to the concentration of dye (from ca. 10^{-5} to 10^{-3} M). Putting the analytical concentration of dye as C_0 and the molar fraction of dimer as x in a monomer unit, the apparent association constant is given by;

$$K=\frac{x}{2C_0(1-x)^2}$$

If one measures the apparent molar absorbance ε and determines in a suitable way the molar absorbance of monomer and dimer, ε_m and ε_d, then K can be evaluated from the following equation[5]:

$$\log C_0(\varepsilon_m-\varepsilon)=\log\frac{2K}{(\varepsilon_m-\varepsilon_d/2)}+2\log C_0(\varepsilon-\varepsilon_d/2) \qquad (3)$$

In the case where further polymerization as well as dimerization occurs among three monomers and four monomers and so on, the following formula may hold if the absorbance of all the polymers can be disregarded:

$$C_0(\varepsilon_m-\varepsilon)=\frac{2K_1}{\varepsilon_m}(C_0\varepsilon)^2+\frac{3K_1K_2}{\varepsilon_m{}^2}(C_0\varepsilon)^3+\frac{4K_1K_2K_3}{\varepsilon_m{}^3}(C_0\varepsilon)^4+\cdots\cdots \qquad (4)$$

in which K_1, K_2, K_3,…… are respectively the equilibrium constants for each successive polymerization.

For most of the experimental results a linear relation holds between $C_0(\varepsilon_0-\varepsilon)$ and $(C_0\varepsilon)^2$, whence it can be concluded that $K_2=K_2=\cdots\cdots=0$, and, further, that $\varepsilon_d\approx0$.

This method, which is satisfactory for the majority of cases, gives $2K_1/\varepsilon_m$ as a solpe of the plot.

Results and Discussion.—The dependence of the absorption spectra of pyronine G on pH is shown in Fig. 1. It is evident that in the region $0<\text{pH}<3$, the maximum shifts several mμ towards red without any appreciable change in the entire feature. This would perhaps be due to the attachment of one proton to the $N(CH_3)_2$ group. A remarkable change was found to occur in the 5 N hydrochloric acid solution, the absorption spectra turning into a broad band with a maximum at 495 mμ. The exact assignment of this band was not undertaken, but in view of a large blue shift this would probably be due to the attachment of another proton to the C- or O- atom of the central ring. The case for $\text{pH}>7$ will be described in Part II. No particular attention was paid to the pH of the solution in studying the salt effect on the dimerization.

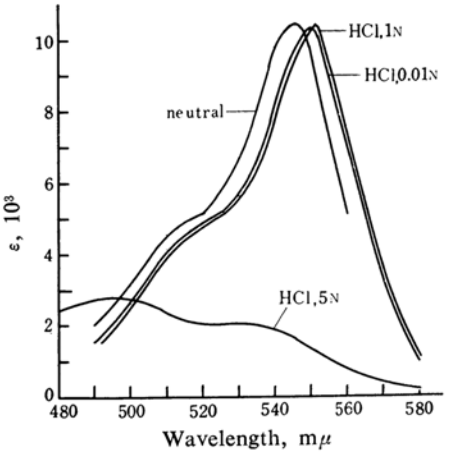

Fig. 1. pH dependence of the absorption spectra of pyronine G (2.12×10^{-5} M).

i) Potassium Chloride, Potassium Nitrate, Magnesium Sulfate.—In order to determine the salt effect on the dimerization constant, the absorption spectra of pyronine G in the aqueous solution were measured, adding various amounts of these salts and changing the dye concentration from ca. 2×10^{-5} to 9×10^{-4} M. Fig. 2 shows the case of 0.3 M potassium nitrate as an example. The position of the dimer band is practically the same for magnesium nitrate, potassium chloride or without any salt. The K-values evaluated from 3 or 4 (disregarding ε_d) are shown in Table I. In Fig. 3 the cases

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[5] N. Mataga, This Bulletin, 30, 375 (1957).

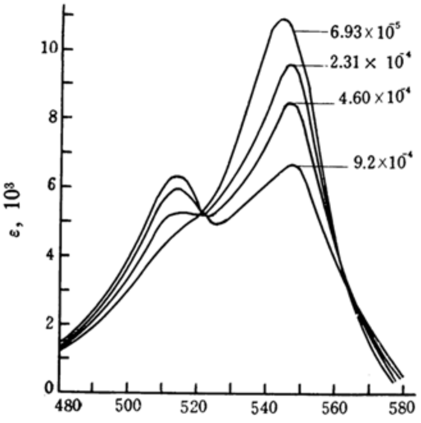


Fig. 2. The absorption spectra of pyronine G in the aqueous solution containing 0.3 M KNO_3.

TABLE I

Temp.	Salt	Concn., M	*K*	log *K*
20°C		0	720∼ 900 ∼ 510	2.86∼2.95 ∼2.71
	KCl	0.5	620∼ 710 580∼ 650	2.80∼2.85 2.76∼2.81
		1	1100∼1300	3.04∼3.11
		2.2	1900∼1950	3.28∼3.29
	KNO_3	0.3	450∼ 510	2.65∼2.71
		0.5	560∼ 620	2.75∼2.79
		1	680∼ 730 730∼ 780	2.83∼2.86 2.86∼2.89
	$MgSO_4$	0.25	700∼ 820	2.85∼2.91
		0.5	690∼ 750	2.84∼2.87
		1.0	1200∼1900	3.08∼3.28
		2.0	∼2980	∼3.47
30°C		0	600∼ 650	2.78∼2.81
	KCl	1	1200∼1320	3.08∼3.12
	$MgSO_3$	1	1560∼1700	3.19∼3.23

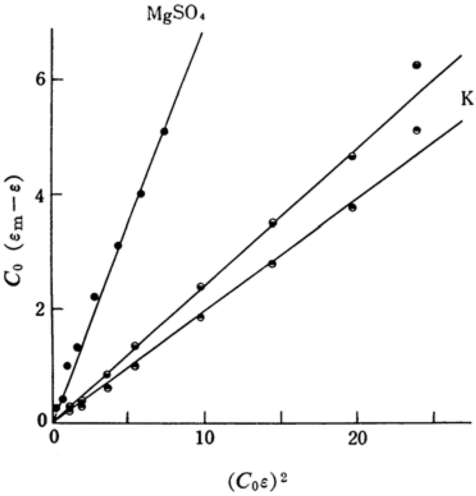


Fig. 3. Plot of $C_0(\varepsilon_m - \varepsilon)$ against $(C_0\varepsilon)^2$.
◒ $\varepsilon_m = 12.0\times10^3$, ◓ $\varepsilon_m = 10.9\times10^3$

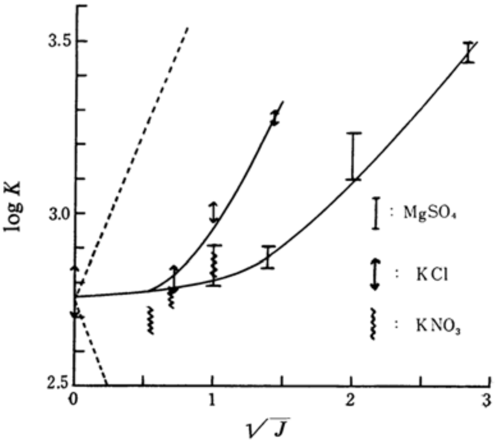


Fig. 4a. The dependence of log *K* on $\sqrt{J}$.

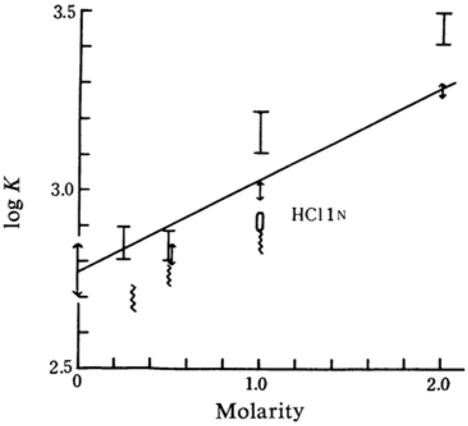


Fig. 4b. The dependence of log *K* on molarity.

of potassium chloride and magnesium sulfate are shown. These results are not satisfactorily accurate, the main cause of error perhaps lying in the fluctuation of ε_m. The *K*-value for the simple aqueous solution is quite smaller than that reported before¹⁾ (1.6×10^3 at 27°C). This is probably due to the circumstance that in the previous case, the ε_d-value evaluated from the extrapolation of the ε-value in the higher concentration region was used for calculating the *K*-value. The present results are considered more reliable in view of the fact that a disregard of ε_d can give a satisfactory analysis in most cases.

Figs. 4a and 4b give plots of the log *K*-value at 20°C against the ionic strength and the molarity of the solution respectively. These figures show clearly the specificity in the effect of anions. Apart from the region of great ionic strength where Debye-Hückel's formula does not hold, log K is almost independent of J (in the region $\sqrt{J} \ll 1$). The dotted lines in Fig. 4a are written with an inclination of ±1.

Thus, the results obtained obviate the possibility of a reaction between free ions (case a) in the case of a univalent anion, while in the case of bivalent anions both case c i) and case c ii) do not match the experimental results, not to speak of the case of free ions. If the sandwich-model is generally to be adopted, case c ii) should hold for the bivalent anion, but this proved not to be the case. Hence, by analogy this model may also be rejected for the univalent anion (case b ii). This conclusion is also supported by the constancy of the position of the dimer band irrespective of the various anions.

Taking all this into consideration, it is most plausible that the dimerization in this case takes place as shown in Fig. 5.

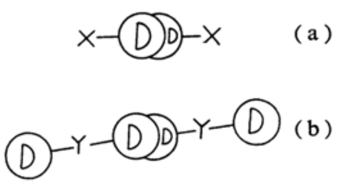


Fig. 5. Plausible models of a dimer.

Fig. 5a corresponds to the aforementioned case b i) of a univalent anion, but Fig. 5b for a bivalent anion Y^{2-} corresponds to neither c i) nor c ii).

ii) Potassium Iodide, Potassium Perchlorate, Sodium Perchlorate.—The specificity of the anion mentioned above is particularly prominent in the case of I^- and of ClO_4^-.

The measurements were made for 0.05, 0.1, 0.3, 0.5 and 1 M solutions of potassium iodide. In all cases the dimer band somewhat increases up to $(2.3\sim3.0)\times10^{-4}$ M in the dye concentration, but beyond this the entire absorption curve declines noticeably, accompaied by a slight red-shift of the maximum. No new band appears, at least in the region 400∼780 mμ. The increase of the salt concentration does not essentially affect the above behavior. Fig. 6 shows the result for 0.1 M potassium iodide.

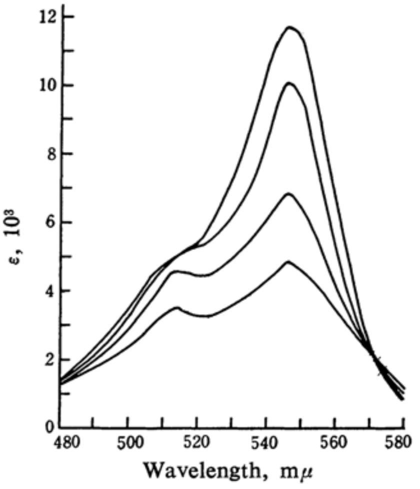


Fig. 6. The absorption spectra of pyronine G in the aqueous solution containing 0.1 M KI.

As for ClO_4^-, the measurements were made in 0.5 and 1 M. In both cases, the absorption band declines monotonously with the increase of the dye concentration from 2.11×10^{-5} to 9.2×10^{-4} M. Even a slight growth of the dimer band is not observable (Fig. 7). A similar change is observed when the dye concentration is kept constant and the quantity of sodium perchlorate is varied.

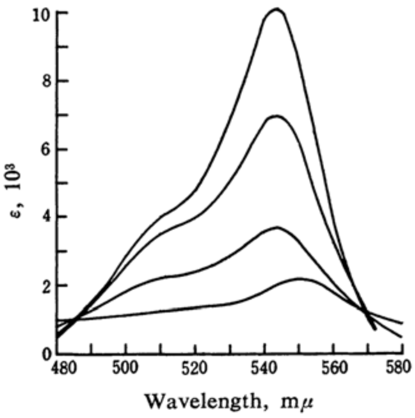


Fig. 7. The absorption spectra of pyronine G in the aqueous solution containing 0.5 M $NaClO_4$.

For the above two cases, perhaps polymers of higher association would be produced in view of the weak and broad absorption band.

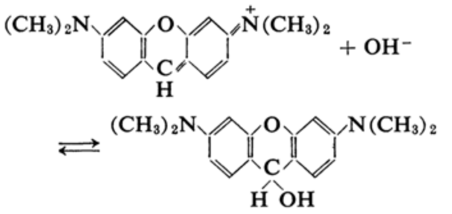
The reason that only dimerization occurs in the case of Cl^- and of SO_4^{2-} may lie in the bulky size of such ions due to the high degree of hydration, these ions laterally attached to the dimer cation probably prohibiting the further approach of other dye ions. In the case of I^- and of ClO_4^-, for which the hydration is small at most, the lateral attachment of these ions close to the dye cation might not inhibit the alignment of many dye ions face to face and thus allow the high polymer to be formed.

iii) Hydrochloric Acid.—Similar experiments were made using 5 N hydrochloric acid, 1 N hydrochloric acid (potassium chloride 1 M) and 0.001 N hydrochloric acid (potassium chloride 0.1 M) solutions. Qualitatively the dimer formation is quite the same in the case of 0.001 N and of 0.1 N hydrochloric acid as for the neutral solution. One notable fact is that in spite of a shift of the monomer band (toward red), the position of the dimer band completely coincides with that in the neutral solution. The K-values were found to be 785 and 1300 for 1 N (20°C) and 0.001 N respectively. Taking into account the somewhat larger error of these experiments, it may be said that the above values do not differ much from those in the neutral solution.

The result is quite different in the case of 5 N hydrochloric acid. The absorption spectra, which are shown in Fig. 1, never change through the variation of dye concentration from 2.8×10^{-5} to 9.2×10^{-4} M. The cause for this different behavior is not known at present.

II. The Equilibrium Constant of Carbinol Formation Evaluated from the pH Effect on the Absorption Spectra

The color of the acidic or neutral solution of pyronine G is red, while the alkaline solution is colorless; this change is reversible. As described below, the maximum absorption at 546 mμ in the neutral solution turns into a broader one with a new maximum at 390 mμ. A shift as large as 156 mμ clearly suggests the disappearance of the conjugation in the pyronine cation and may be inferred to be due to the formation of carbinol.



In xanthene dyes there seems to exist no detailed study about the formation of carbinol, but it is well known that triphenylmethane dyes easily form carbinols such as, the carbinol of phenolphthalein⁽⁶⁾ crystal violet⁽⁷⁾.

The reason for the easy formation of carbinol in the case of pyronine G and the rapid establishment of its equilibrium may perhaps be that it is a positive ion and has no steric hindrance, as judged from its rather simple structure.

In this paper, the equilibrium constant of the carbinol formation is determined at several temperatures from the variation of the absorption spectra due to pH change. From the results, some thermodynamic constants are estimated.

Experimental.—Pyronine G of Merck was dissolved in ethanol and recrystallised by evaporating the solution spontaneously. The crystal obtained was dried in a descicator. $\varepsilon_{\max}\approx70000$. Sodium hydroxide, G. R. was used without further purification. Borate and Na_2HPO_4, both G. R., were recrystallized. The buffer solutions employed were the $NaOH\text{-}Na_2B_4O_7$ system and the $NaOH\text{-}Na_2HPO_4$ system. The former was used for the preliminary experiment in the range pH 9.24∼12.25 and the latter, for more accurate measurements between pH 11.0 and 12.0. The pH of the solution was measured by a Hitachi pH meter prior to the measurement of the absorption spectra. The glass electrode used was GL-13 for the alkaline solution. Quartz cells were used for spectral measusement in order to minimize the adsorption of dye on the cell wall. The spectrophotometer used was a Hitachi EPU-2. The wavelength width was always maintained at 0.5 mμ. The temperature was controlled by circulating the water from the constant temperature bath.

Results and Discussion.—The pH change of absorption spectra is shown in Fig. 8. As seen from the figure, the peak at 546 mμ in the neutral solution declines with the increase of pH, and a broad band with a peak at 390 mμ gradually rises ($\varepsilon\approx10000$) with an isosbestic point at 445 mμ, which supports the existence of the equilibrium.

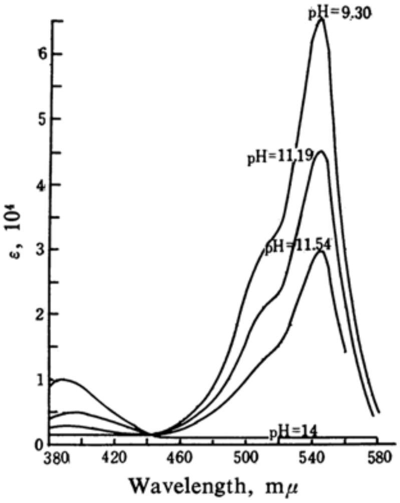


Fig. 8. pH change of absorption spectra of pyronine G.

From the data the equilibrium constant was determined by the following procedure⁽⁸⁾. Writing pyronine G cation as A^+ and carbinol as B, the reaction is represented by

$$A^+ + OH^- \rightleftharpoons B \tag{5}$$

From a mass action law

$$K=\frac{[B]}{[A^+][OH^-]}=\frac{[B][H^+]}{[A^+]K_w} \tag{6}$$

where K_w is the ionic product of water.

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6) Cf., e. g., M. D. Barnes and V. K. LaMer, *J. Am. Chem. Soc.*, **64**, 2312 (1942).

7) S. Hochberg and V. K. LaMer, ibid., **63**, 3110 (1941).

8) This is a slight modification of the method usually employed for the case of Base+H⁺⇄Acid. Cf., e. g., H. H. Perkampus and Th. Rössel, *Z. Elektrochem.*, **60**, 1102 (1956).

TABLE II. THE VARIATION OF THE OPTICAL DENSITY AT 546 mμ THROUGH pH-CHANGE (dye concentration: 2×10^{-5} M)

20°C	pH	(9.25)	11.38	11.65	11.83	12.00	(14.0)	
	O. D.	1.35	0.80	0.655	0.513	0.379	0.052	
22.5°C	pH	(9.25)	11.17	11.34	11.53	11.66	11.88	(14.0)
	O. D.	1.30	0.96	0.81	0.66	0.518	0.389	0.055
25°C	pH	(9.30)	10.94	11.19	11.34	11.54	(14.0)	
	O. D.	1.30	1.06	0.90	0.79	0.590	0.020	
27.5°C	pH	(9.30)	10.97	11.21	11.42	11.57	11.78	(14.0)
	O. D.	1.35	1.06	0.925	0.72	0.529	0.360	0.024
30°C	pH	(9.30)	10.93	11.15	11.43	11.60	11.81	(14.0)
(a)	O. D.	1.36	1.14	1.04	0.91	0.76	0.495	0.024
30°C	pH	(9.30)	11.07	11.19	11.39	11.58	11.75	(14.0)
(b)	O. D.	1.30	0.85	0.78	0.570	0.416	0.304	0.056

TABLE III. pK VALUES AT VARIOUS TEMPERATURES

Temp., °C.,	20	22.5	25	27.5	30
pK	-2.52 ± 0.02	-2.47 ± 0.02	-2.45 ± 0.02	-2.37 ± 0.02	-2.06 ± 0.04 -2.48 ± 0.02

Of course, K in 6 is not a rigorous equilibrium constant in the thermodynamical sense but only an apparent one in the phosphate buffer solution in the pH range 11∼12.

Taking logarithm of Eq. 6

$$\log K=\log[\mathrm{H}^+]-\log K_{\mathrm{w}}+\log([\mathrm{B}]/[\mathrm{A}^+])$$

or

$$\mathrm{p}K=\mathrm{pH}+\log K_{\mathrm{w}}-\log([\mathrm{B}]/[\mathrm{A}^+]) \qquad (7)$$

the application of Beer's law for each molecular species leads to Eq. 8 in which $\varepsilon_{\mathrm{A}^+}$, ε_{B} and ε are respectively, the molar absorbancy of A^+ and of B and the apparent molar absorbancy at the corresponding pH.

$$\mathrm{p}K=\mathrm{pH}+\log K_{\mathrm{w}}-\log\frac{\varepsilon_{\mathrm{A}^+}-\varepsilon}{\varepsilon-\varepsilon_{\mathrm{B}}} \qquad (8)$$

For $\varepsilon_{\mathrm{A}^+}$ and ε_{B}, one can use respectively the molar absorbancy in the neutral solution and in the strong alkaline solution. Plotting $\log(\varepsilon_{\mathrm{A}^+}-\varepsilon)/(\varepsilon-\varepsilon_{\mathrm{B}})$ against pH, one obtains a straight line with a slope of 45°, and the pH-value at intercept, combined with $\log K_{\mathrm{w}}$[9], gives the pK-value.

The experimental results in the phosphate buffer solution are given in Table II.

Using the above data and putting ε_{B} as 0.02, pK was calculated from 8. An example of the plot is shown in Fig. 9, and the results are given in Table III.

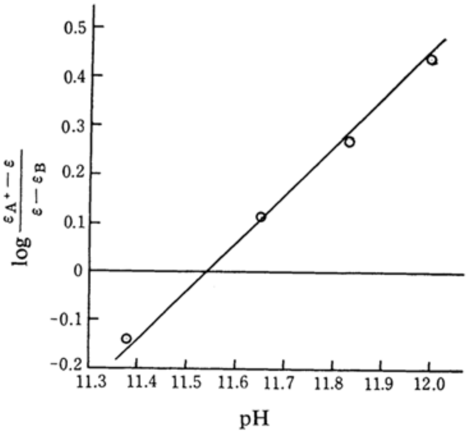

Fig. 9. Plot of $\log\frac{\varepsilon_{\mathrm{A}^+}-\varepsilon}{\varepsilon-\varepsilon_{\mathrm{B}}}$ against pH (20°C).

From these (the values at 30°C were omitted because they are less reliable), ΔG^0, ΔS^0 and ΔH^0 were calculated using the relations,

$$\Delta G=2.303\,\boldsymbol{RT}\,\mathrm{p}K,\quad \Delta S=-(\partial\Delta G/\partial T)$$

$$\Delta H=4.56\{\partial\mathrm{p}K/\partial(1/T)\}$$

The results are

ΔG(kcal.): -3.38(20°C) -3.35(22.5°C)
-3.34(25°C) -3.30(27.5°C)

$\Delta S=$ca. -12 cal./deg.mol.

$\Delta H=-8\pm3$ kcal./mol.

These values are not very accurate, but it is certain that the formation of carbinol in the phosphate buffer is an exothermic reaction with a small decrease of entropy.

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[9] Beilstein, International Critial Table, Vol. VI, (1929), p. 152.

III. The Fluorescent Behaviors of the Aqueous Solution of Pyronine G

The formation of cabinol, which was established by the pH variation of the absorption spectra would not occur so promptly as does the proton addition or its detachment. Hence, the same equilibrium constant is to be expected from the pH variation of fluorescence if it can be measured. This was confirmed by the present investigation; in addition, the excitation spectra were measured.

Experimental.—*Sample.*—Same as the absorption experiment. The concentration is 2×10^{-5} M unless otherwise described.

Procedure.—The fluorescence spectra were measured with the fluorescence accessory of a Hitachi spectrophotometer EPU-2; hence, they are not genuine fluorescence spectra. The measurements were made at room temperature. The exciting light is 365 mμ from a high pressure mercury lamp (100 W). In order to obtain the p*K*-value of the carbinol formation from the pH variation of the fluorescence intensity, a newly constructed fluorometer was used which had been designed on the principle of the optical zero-method and which enables one to measure the action spectra of the fluorescence[10]. As the light source, a 200W tungsten filament lamp (projection lamp) was used. Its intensity distribution was calculated by the use of Planck's formula for black body radiation[11], in

$$N_\lambda=\frac{2\pi c}{\lambda^4}\left\{\exp\left(\frac{hc}{k\lambda T}\right)-1\right\}^{-1}$$

which c, λ and T are light velocity, wavelength (μ), and absolute temperature respectivery; the dimension of N_λ is photons/cm² sec. μ. The color temperature of the tungsten lamp (100 V.) was determined in such a way as to make the action spectra of eosine and uranine, of which the quantum yield is known to be independent of wavelength, as similar to the absorption spectra as possible. It was found that $T=2800$°K gives a satisfactory result, as is shown in Fig. 10.

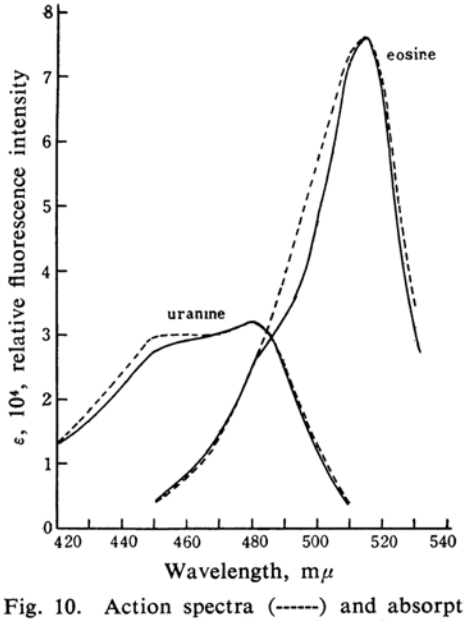

Fig. 10. Action spectra (------) and absorption spectra (——) of eosine and uranine.

The wavelength width was taken into account.

Results.—*pH Variation of the Fluorescence Spectra.*—An example is shown in Fig. 11. The maximum of the fluorescence spectra in the neutral solution lies at 566 mμ, which is displaced about 20 mμ from the absorption maximum. The result that there exists no shoulder corresponding to a dimer is naturally to be expected, since the dimer in most dyes has no power of emission. The alkaline solution, on the other hand, gives a fluorescence spectra with a maximum at 460 mμ corresponding to 390 mμ of the absorption spectra. Stokes' shift of 70 mμ is much larger than the 20 mμ of the

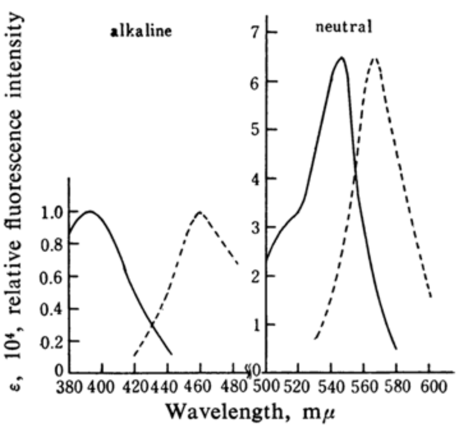

Fig. 11. Fluorescence spectra of pyronine G.

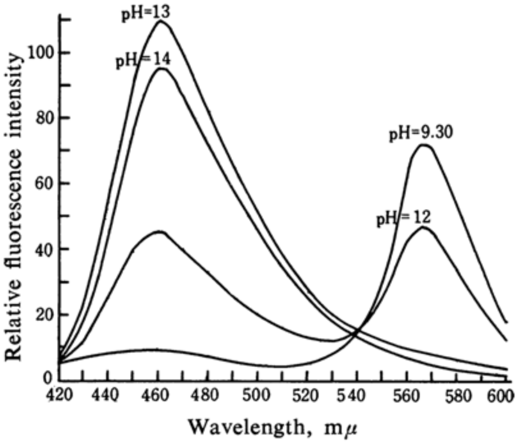

Fig. 12. The pH dependence of the flourescence spectra of pyronine G.

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[10] To be published elsewhere.

[11] A. N. Lowan and G. Blauch, *J. Opt. Soc. Am.*, **30**, 70 (1940).

TABLE IV. pH CHANGE OF THE FRUORESCENCE INTENSITY

20°C	pH	9.30	10.95	11.37	11.51	11.87	12.00	14
	I	563	380	355	251	174	112	1.00
25°C	pH	9.30	11.12	11.35	11.45	11.60	12.06	14
	I	38	25.12	17.8	16.6	7.58	6.45	1.00
30°C	pH	9.30	11.0	11.19	11.37	11.54	11.76	14
	I	44.7	30.2	24.0	17.4	10.5	7.08	1.00

neutral solution. The spectra at various pH cross at a point near 540 mμ, which indicates, analogous to the isosbestic point in the case of absorption spectra, that two molecular species existent in equilibrium give rise to an independent fluorescence. This is shown in Fig. 12. The fluorescence of carbinol declines somewhat at pH 13; this may perhaps be due to the quenching by the OH^- ion.

The Determination of pK from the pH Change of Fluorescence Intensity.—Since pyronine G cation and carbinol each emit a separate fluorescence, the quantitative investigation of the pH change of the fluorescence intensity of one component will make it possible to determine the equilibrium constant of the carbinol formation. To make the calculation feasible, λ of the exciting light was chosen at 445 mμ, which is an isosbestic point. A photographic filter (0–2) which cuts $\lambda<560$ mμ was used to remove the exciting light.

The calculation was made as follows, taking into account the fact that the fluorescence of the two molecular species slightly overlap. Putting the molar fraction of the cation and carbinol as X_{A^+} and X_B respectively, and denoting the relative intensity as I_{A^+} and I_B, the measured intensity can be expressed by

$$I=I_{A^+}X_{A^+}+I_BX_B$$
$$=I_{A^+}\frac{[A^+]}{[A^+]+[B]}+I_B\frac{[B]}{[A^+]+[B]}$$

Combining this with

$$K=\frac{[B]}{[A^+][OH^-]}$$

one gets

$$pK=-\log\frac{I_{A^+}-I}{I-I_B}+pH+\log K_w$$

This is quite analogous to the formula for the absorption spectra. The results are given in Table IV. An example is shown in Fig. 13. From the above data, pK and the related thermodynamic quantities were calculated as follows:

pK: -2.66 ± 0.03(20°C), -2.59 ± 0.05(25°C), -2.50 ± 0.02(30°C)

ΔG: -3.60(20°C), -3.52(25°C), -3.45(30°C)

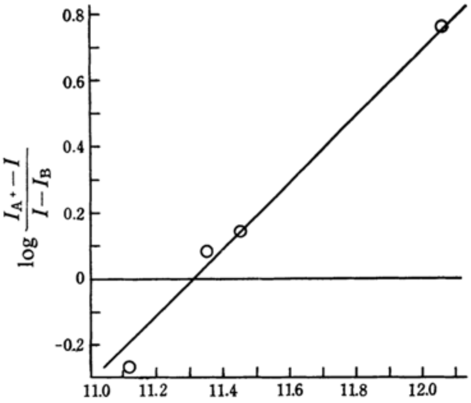

Fig. 13. Plot of $\log\frac{I_{A^+}-I}{I-I_B}$ against pH (25°C).

$\Delta H=-6.5\pm1.5$ kcal./mol.
$\Delta S=-14\pm3$ kcal./mol.

All the above data are nearly equal to those obtained from the absorption spectra. Above pH=13 the fluorescence intensity shows a decrease with the increase of $[OH^-]$, but in the above pH region this effect may perhaps be so small that it can be disregarded.

Action Spectra.—Excitation spectra of fluorsecence in the aqueous solution of pyronine G were measured by a newly constructed fluorometer. The result, as shown in Fig. 14, largely

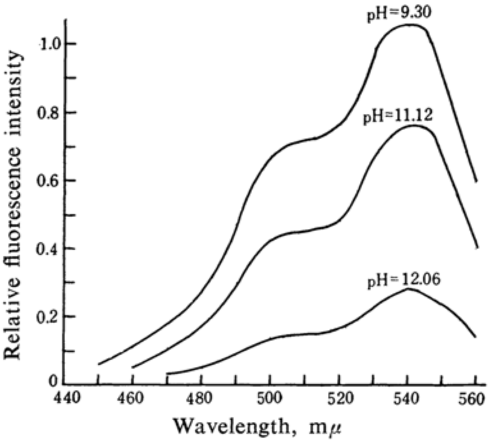

Fig. 14. Excitation spectra of fluorescence in the aqueous solution of pyronine G.

coincides with the absorption spectra, indicating that the quantum yield of fluorescence is independent of the wavelength. It is, however, worth while to note that a shoulder similar to that of the absorption is existent in the action spectra. This is somewhat curious in view of the fact that a dimer, which is the source of this shoulder, is commonly considered to be non-fluorescent, and there is indeed no shoulder in the fluorescence spectra. The reason for this is not yet certain ; it might be that the exciting energy is transferred to a neighboring monomer or that the excited dimer rapidly dissociates into monomers (in an excited and ground state) and then the emission takes place. Further studies are required.

Summary

Th effect of inorganic anions on the dimerization constant of pyronine G in an aqueous solution was examined. It was found that the effect is very specific and can not be explained simply by the effect of the ionic strength. The analysis led to the conclusion that a dimer exists as an ion-pair with the anions attached laterally to dye ions instead of being sandwiched between two dye ions.

The change of absorption spectra in the alkaline solution was interpreted to be due to the formation of carbinol, and from its analysis the formation constant of carbinol was determined.

Carbinol was found to have also its proper fluorescence, and from the pH change of fluorescence spectra the formation constant of carbinol was again decided, the value of which coincided well with that obtained from the absorption spectra.

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