ESR Study on Fluorescein Semiquinone Radical

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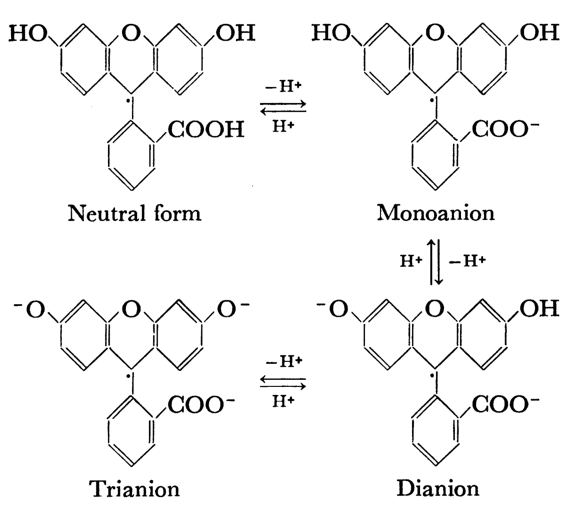
The ESR spectrum of the fluorescein semiquinone radical (dianion form) produced by illumination of an alkaline aqueous solution of uranine and leuco-uranine at pH between 11 and 14 was observed and its hfs constants were determined by comparing the spectrum with those of the mono- and trianion forms of fluorescein semiquinone and those of eosin semiquinone reported by Leaver. Six protons in the xanthene ring were found to be comprised of three pairs of magnetically equivalent protons, which suggests a certain dynamic mechanism to average the coupling constants. From a temperature dependence of the line width, protonation to the $-O^-$ group followed by deprotonation from the $-OH$ group was proposed as a plausible mechanism.

When alkaline water–ethanol and water–2-propanol solutions of uranine (disodium salt of fluorescein) are illuminated with visible light, uranine is reduced to leuco-uranine *via* a half-reduced form (semiquinone radical).1) The semiquinone, which is relatively stable in the alkaline solution, is also produced by uranine-sensitized photo-oxidation of leuco-uranine.2) According to the investigations using the steady light as well as the flash illuminations, it was established that the radical is in ionic equilibrium as shown in scheme (I). The pK_a values of the mono- and dianions are reported to be 9.5 and >13,3) respectively.



Scheme (I)

Therefore at the pH between 11 and 13, the semiquinone exists as the dianion form. From the kinetic study of the ESR signal of this radical, it was clarified that the signal intensity change after making the illumination off shows a typical second-order decay and the activation energy of disproportionation or recombination for this radical was found to be 10 to 12 kcal/mol.4) Though a highly resolved ESR spectrum has ever been observed,5,6) the analysis of hfs has not yet been performed sufficiently.

As to radicals containing one or more phenyl rings, for example benzophenone ketyl7) and α-phenylbenzilidene malononitrile,8) a steric and an electronic effect on rotation of the phenyl rings are recently treated as interesting problems. From this point of view, it will be interesting to analyse the hfs of the fluorescein semiquinone radical in detail in order to obtain the information about the rotation of the phenyl ring in this molecule.

By using the improved light source system, the more resolved spectrum than that in the previous report4) has been detected. The results of the analysis of hfs and the signal behavior give the following information; (1) The radical species obtained at pH between 11 and 14 was the only one and was identified as a dianion. At the pH range from 9.5 to 10.5, the asymmetric spectra were obtained, which means in general that signals of two kinds of radicals superpose each other. No further analysis of these spectra was made. (2) The ESR spectrum of the radical identified as a dianion revealed that six protons in the xanthene frame consist of three pairs of magnetically equivalent protons. Therefore it is suggested that this molecule has a certain mechanism to make some pairs of protons equivalent magnetically. (3) Line broadening and narrowing occur reversibly with the temperature decrease and increase. The interpretation of these results was attempted. Two conceivable mechanisms are (a) the hindered rotation of the phenyl ring and (b) protonation to the $-O^-$ group followed by deprotonation from the $-OH$ group. The latter is more plausible.

Experimental

Materials. Uranine was prepared by treating fluorescein (Hikotaro Shudzui & Co., G. R. grade) with sodium carbonate in water and was recrystallized three times from ethanol. Leuco-uranine was obtained by reducing the above uranine with sodium amalgam in water and was used without further purification.

Procedures. The radical dianion was produced mainly by illuminating the aqueous solution of uranine (10^{-5}—10^{-4} mol/l) and leuco-uranine (10^{-3}—10^{-2} mol/l) mixture in an

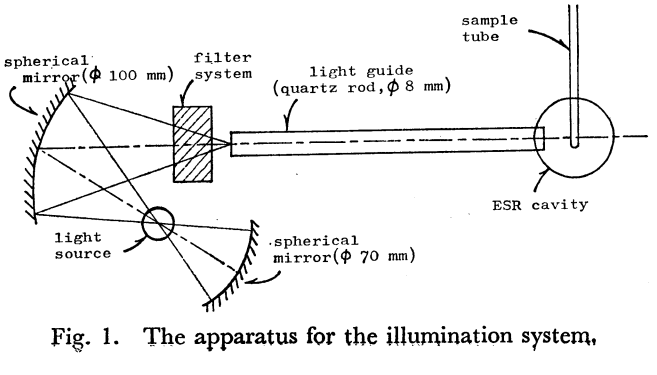


Fig. 1. The apparatus for the illumination system.

ESR sample tube placed in a cavity. pH of the solution was controlled with sodium hydroxide concentration. An Ushio super high-pressure mercury lamp (USH 500D) was used as a light source. The illuminating apparatus is shown in Fig. 1. Light was collected by two spherical mirrors, larger one (10 cm in diameter) being set to have a correct angle between the axis of incident light and that of reflected light and to focus the light on the front surface of a quartz light-guide. A filter system which consists of 1 cm water, a 1 cm cupric sulfate aqueous solution and a Toshiba VY-43 glass filter was placed between the mirror and the light-guide. This filter system transmits the visible light in the range from 430 to 600 nm. The wavelength of absorption maximum of uranine in alkaline aqueous solutions is 495 nm.

The temperature of the sample was controlled by inserting the doubly sealed quartz tube into a cavity and introducing cold nitrogen gas or hot air through the tube. A thermocouple was used to monitor the temperature in the tube.

Hyperfine splittings were determined using the signal of seven lines of Mn^{2+} in MgO as a standard. The separation between the third and fourth lines is 87.5 G.

A JEOL P-10 type ESR spectrometer (X band, 100 kHz modulation) was used. A NEAC 2200 MODEL 700 Electronic Computer in the Calculation Center of Tohoku University was used for the calculation of unpaired spin densities. For a simulation of ESR spectra, a JEOL JEC-6 Spectrum Computer was used.

Results and Discussion

ESR Spectra Obtained in the Solutions of Different pH Values. The ESR spectra obtained during photolysis of solutions of various pH values higher than 9.5 are shown in Fig. 2. The signals observed at pH values lower than 10.5 have a small S/N ratio because of a short lifetime of the radical and have an asymmetric line shape, which may be due to the superposition of signals from the mono- and dianions. At the pH range from 11 to 13, rather highly resolved spectra were detected. The di- and trianions might coexist at pH values greater than 14, but in fact similar spectra to those at pH range from 11 to 13 were observed with smaller intensity.

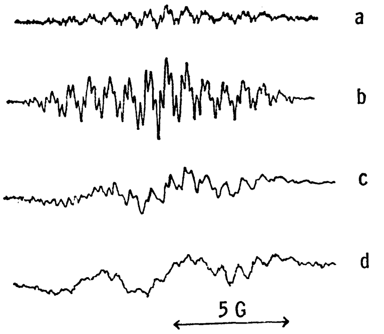


Fig. 2. ESR spectra of fluorescein semiquinone at pH$>$ 14 (a), 12.3 (b), 10.5 (c), and 9.5 (d).

Simulation of ESR Spectrum and Determination of Coupling-Constants and Unpaired Spin Densities on Carbons. Figure 3(1) shows a typical spectrum of the dianion which is approximately the same as that obtained by Kimura *et al.*[5] in the γ-irradiated methanol solution of uranine. The arrow marked a indicates the center of the spectrum. When the modulation width is wide, the signal is a 1 : 2 : 1 triplet. As the modulation width becomes narrow, it splits into fifteen lines as a whole. Each component of the fifteen lines comprises seven or eight lines which are not well-resolved. The line shape of components marked a and b in Fig. 3(1) resembles each other: moreover both sides of marks a and b have a similar shape over a few gauss. The same relation hold between marks a and b′. (b′ is the point at lower field half and not given in the figure.) Therefore the largest coupling exhibiting the triplet is taken as 3.287 G from the distance between a and b (or a and b′). The spectrum of a (or b) component (Fig. 4(1)) can be simulated satisfactorily by using four coupling constants of 0.220, 0.190, 0.170, and 0.090 G (Fig. 4(2)). This pattern is similar to the component of the triplet signal which has been obtained by photolysis of eosin in methanol-d_1[6] by Leaver (Fig. 4(3)). He reconstructed this pattern by using three protons of

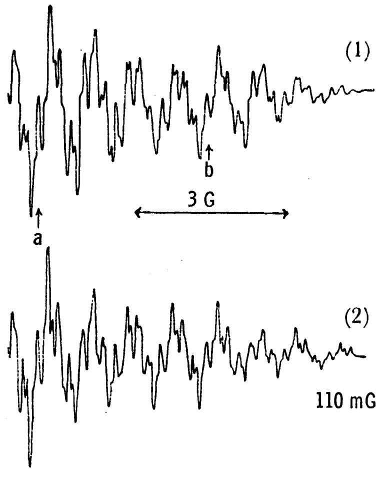


Fig. 3. ESR spectrum of the dianion in ethanol–aqueous solution (20 volume percent ethanol) at 44 °C (1) and its simulation (2).

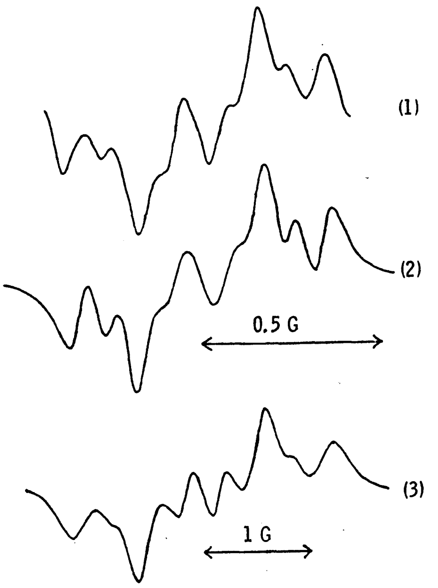


Fig. 4. a (or b) component of the fifteen lines (1), its simulation (2) and one component of the triplet spectrum of eosin semiquinone (3).

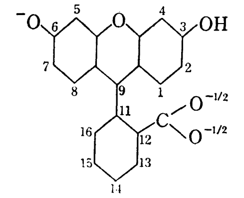
0.61 G and one proton of 0.29 G. The shape in Fig. 4(3) was assigned to that constructed with the coupling of four protons in the phenyl ring of eosin semiquinone.

The whole spectrum consisting of fifteen lines in Fig. 3(1) can be simulated by combining the four coupling constants, which produce the line shape in Fig. 4(1) with those of six protons in the xanthene ring, two of which are already estimated to be 3.287 G. As for the rest of the couplings, two of them are estimated to be 1.510 G and the other two 0.889 G. The coupling constants determined in this way are given in Table 1. The spectrum simulated with the aid of parameters in Table 1 is shown in Fig. 3(2).

Table 1. Coupling constants and unpaired spin densities

Position	Coupling const. Obsd(G)	Unpaired spin density		
		Obsd[a]	Calcd	Average
1	3.287	0.1133	0.1124	0.1120
8			0.1116	
2	1.510	0.0521	−0.0332	−0.0326
7			−0.0322	
4	0.889	0.0307	−0.0304	−0.0290
5			−0.0276	
13	0.220	0.0076	0.0098	
14	0.190	0.0066	0.0078	
15	0.170	0.0059	0.0028	
16	0.090	0.0031	0.0010	

a) $Q=29$ G was used.



The unpaired spin densities calculated by McLachlan's method based on simple LCAO MO method are listed in the fourth column of Table 1. The following parameters were used in the calculation:

$$
\begin{aligned}
\alpha_{-\mathrm{O}-} &= \alpha + 2.0\beta & \beta_{\mathrm{C-O-}} &= 0.6\beta \\
\alpha_{-\mathrm{OH}} &= \alpha + 2.5\beta & \beta_{\mathrm{C-OH}} &= 0.7\beta \\
\alpha_{-\mathrm{O}^{-1/2}} &= \alpha + 2.25\beta & \beta_{\mathrm{C-O}^{-1/2}} &= 0.8\beta \\
\alpha_{-\mathrm{O}^{-}} &= \alpha + 2.0\beta & \beta_{\mathrm{C-O}^{-}} &= 0.8\beta
\end{aligned}
$$

where α_i and $\beta_{\mathrm{C-R}}$ are respectively the Coulomb integral of C–i atom and exchange integral of C–R bond. As for McLachlan's parameter, $\lambda=1.0$ was used. Because of steric hindrance, the xanthene and phenyl rings cannot be in the same plane but incline to a considerable to extent each other. $\beta_{\mathrm{C_9-C_{11}}}=\beta\cos^2\theta$ was added to the parameter for calculation, where θ is the angle between the axis of the p-orbital of $\mathrm{C_9}$ and that of $\mathrm{C_{11}}$. The calculated values listed in Table 1 are for $\theta\approx55°$. The agreement between the observed and the calculated values is rather good.

It is obvious from the pK values of the mono- and dianions determined by Lindqvist that the radical in question has the dianion form. This is also supported by the fact that the observed coupling value of the protons at the 1- and 8-positions (3.287 G) lies between those of the monoanion (3.39 G) and the trianion (2.89 G) reported by Leaver for methanol solutions. The tendency of decreasing coupling constants of the 1- and 8-positions with increasing negative charge of the radical implies that the unpaired electron migrates from the xanthene into the phenyl ring as the –OH groups at the 3- and 6-positions are replaced by the $-\mathrm{O}^-$ groups.

If only the asymmetry of the molecule is taken into consideration, the coupling constant of the proton at the 1-position should differ slightly from that at the 8-position. A pair of protons at the 2- and 7-positions as well as a pair of those at the 4- and 5-positions should also have the different coupling constants due to the same reason. The differences in the calculated coupling constants of these pairs of protons are obviously beyond the simulation error. Therefore, a certain dynamic mechanism should be considered to explain the observed spectrum which shows the existence of three pairs of equivalent proton coupling constants.

Temperature Dependence of ESR Spectrum. The dynamic mechanisms conceivable are (a) the rapid hindered internal rotation of the phenyl ring about the $\mathrm{C_9}$–$\mathrm{C_{11}}$ bond and (b) the isomerization reaction by protonation to the $-\mathrm{O}^-$ group followed by deprotonation from the –OH group. The former is based on the assumption that the difference between the influences of $-\mathrm{O}^-$ and –OH on the unpaired electron distribution in the xanthene ring is negligible and that although the substituted phenyl ring lowers symmetry of the spin distribution in the xanthene ring, the $\mathrm{C_{2v}}$ symmetry may be restored by a rapid rotation of the phenyl ring. At any rate, as the ESR spectrum of this radical suggests the existence of dynamic mechanism, the temperature effect on the ESR spectrum was investigated.

The low-field half of the ESR spectra measured at various temperatures using the mixture of ethanol and water (volume percent of water is 20) are given in Fig. 5(1). The line broadening is seen with the decrease of temperature and this change is reversible. Figure 5(2) shows the simulation of the above spectra obtained

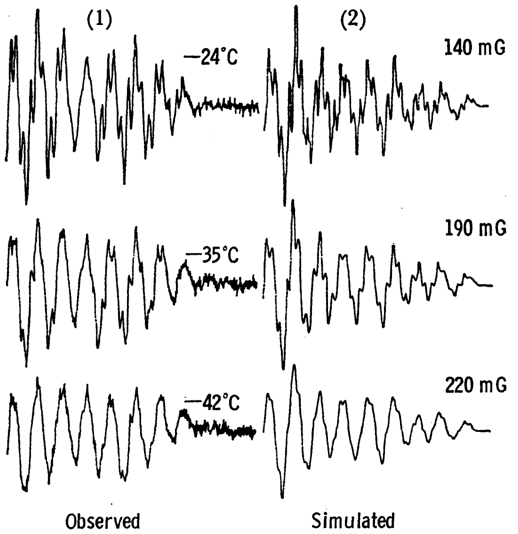


Fig. 5. ESR spectra of the dianion in an ethanol–aqueous solution (80 volume percent ethanol) at three different temperatures. Numbers in mG units are the line widths used for simulation.

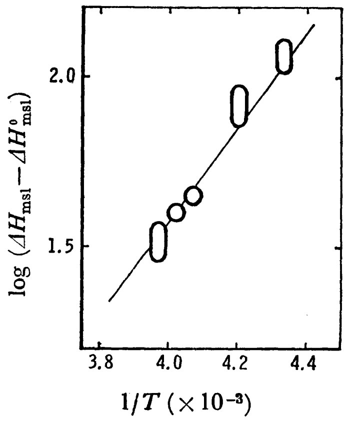


Fig. 6. Arrhenius plots for the increased portion of the line width used for simulation of the ESR spectra of the dianions in an ethanol–aqueous solution (80 volume percent ethanol).

from the same coupling constants as those used in simulating the spectrum of Fig. 3(1) and from the various line widths corresponding to various temperatures. Further, since the areas under the integrated curves of the ESR spectra at various temperatures are nearly constant, the change of the radical concentration is negligible.

Strictly speaking, the line width alternation ought to be detected, if the mechanism (a) or (b) is involved. But practically the line width broadening might be seen as a whole, because the semiquinone has three pairs of protons to commute their coupling constants each other and the differences in coupling constants of each pair are not so large. At the temperatures higher than room temperature, the line width converges to a constant value (100—110 mG; this is the half-width of Lorentzian line shape used for simulation). Therefore, as the observed line width, $1/T_{2,\mathrm{obsd}}$ is given by Eq. (1),[9] the reciprocal of the increased portion of the line width associated with the temperature decrease was plotted against the reciprocal of the absolute temperature,

$$\frac{1}{T_{2,\mathrm{obsd}}} = \frac{1}{T_2} + \frac{1}{\tau} \tag{1}$$

where τ is the life time of one of the isomers in the mechanism (a) or (b) and T_2 is the transverse relaxation time when neither (a) nor (b) is involved. A straight line was obtained as shown in Fig. 6, which means Eq. (2) holds. From the slope of the line in

$$\frac{1}{\tau} = \frac{1}{\tau_0}\exp\left[-E/kT\right] \tag{2}$$

Fig. 6, 6.3 kcal/mol is obtained as the activation energy E of the mechanism that brings the line broadening. The E values obtained for the various mixtures are listed in Table 2.

Table 2. Activation energy of the isomerization reaction

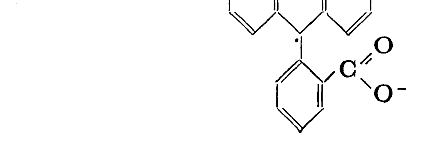
Solvent (volume ratio)	Activation energy (kcal/mol)
EtOH: H_2O (4: 1)	6.3
EtOH: H_2O (1: 4)	6.5
EtOH: D_2O (4: 1)	6.2
EtOH: D_2O (1: 4)	7.2

Discussion on Coupling Constant Averaging Mechanism. If the hindered rotation of the $-COO^-$ substituted phenyl ring has any effect on the unpaired electron distribution in the xanthene ring, the interaction must be the steric hindrance between the $-COO^-$ group and the xanthene ring plane. As fluorescein and eosine molecules can change their structure from normal to lactone form under a suitable condition, the $-COO^-$ group might have a large influence on the distribution of the odd electron in the xanthene ring.

Next, considered was the line broadening by the isomerization reaction of protonation to the $-O^-$ group followed by deprotonation from the $-OH$ group. The linewidth alternation due to a similar mechanism was found, for instance, in the ESR spectra of *p*-benzosemiquinone radical[9] and *trans*-biacetyl semidione radical.[10] If that is the case in our system, the life time required to average a pair of coupling constants can be estimated from the values in Table 1 and from the relation $\tau \ll 1/\gamma|a_i-a_j|$, where γ is a gyromagnetic ratio and a_i and a_j are a pair of coupling constants to be exchanged. The estimated life times are *ca.* 2.5×10^{-6} s, 2.0×10^{-6} s, and 6.7×10^{-6} s for the coupling constants of protons at the 1- and 8-positions, those at the 2- and 7-positions and those at the 4- and 5-positions, respectively. Therefore, the averaging may occur in order of 4- and 5-positions, 1- and 8-positions and 2- and 7-positions with the decrease of temperature. But the differences in the critical life time are not so large that the temperature range where the line broadening begins to appear may superpose each other.

According to Leaver, the proton coupling of the $-OH$ groups was observed in the monoanion form while in our case the same ESR signal as that in H_2O was detected in D_2O. This fact means that the signal does not contain the coupling of the proton (or deuteron) in the $-OH$ (or $-OD$) group. Moreover, taking the fact into account that the activation energy is too large for the rotational barrier of the phenyl ring, it seems reasonable to consider the protonation and deprotonation as a mechanism of averaging the proton coupling constants in the xanthene ring.

The proton concentration in the high pH solution where the fluorescein semiquinone radical has the stable form is extremely small. Therefore, the isomerization (protonation and deprotonation) of semiquinone must be altered as follows; at first semiquinone reacts with water molecule and then a proton transfers from water to the $-O^-$ group of the xanthene ring and immediately after that the $-OH$ group on another side of the xanthene ring releases a proton. The activation energy of 6—7 kcal/mol may be responsible for the transfer of a proton from water to the radical.



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