BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 40 1332—1338 (1967)

ESR Studies on the Behavior of Fluorescein Semiquinone

Michio OKUDA, Yoshihiro MOMOSE, Shigeya NIIZUMA and Masao KOIZUMI

Department of Chemistry, Faculty of Science, Tohoku University, Katahira-cho, Sendai

(Received October 15, 1966)

The structure and reactivity of different types of fluorescein semiquinone were studied in an alkaline solution pH 11—14, by the ESR method. The hyperfine structures with more than fifty lines are semiquantitatively interpreted by the McLachlan method. The three lines with the largest splitting constant, 3.44G, were attributed to the protons at the next neighboring carbon atoms (on the outside benzene ring) from the central carbon atom. The decay of semiquinone obeys the second order, but the rate constant depends largely on the pH. This difference was attributed to the difference in the reactivities of semiquinone monoanion, dianion, and trianion. The activation energy, however, was found to be about 10—12 kcal/mol, regardless of the pH. In order to interpret the above results a mechanism was proposed in which the essential process is the recombination of two radical anions at the position of the central carbon atoms. Evidence for this mechanism was afforded by the calculation of the delocalization energy for the above process, which has proved to be almost constant for different species of semiquinone.

In a prevous paper,[1] the behavior of fluorescein semiquinone produced by the photoreduction of fluorescein by the use of several kinds of reducing agents has been reported on. Especially, the fluorescein semiquinone produced by using leuco-fluorescein as a reducing agent changed quantitatively to fluorescein in the dark. Fluorescein semiquinone was identified by the absorption band at 394 mμ, and its decay rate was determined by the spectrophotometric method.

Fluorescein semiqunione, which has an unpaired electron, may naturally be expected to show the ESR signal. In the present work, we have attempted to study, by means of the ESR method, the molecular structure and chemical reactivity of fluorescein semiquinone under experimental conditions almost identical with those of the previous study.

ESR spectra with similar hyperfine splittings were observed for the fluorescein semiquinone produced by using several different kinds of reducing agents (leuco-fluorescein, disodium ethylenediaminetetraacetate (EDTA), and ethanol). It

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[1] Y. Momose, K. Uchida and M. Koizumi, This Bulletin, **38**, 1601 (1965).

was established, on the basis of abundant evidence, that the ESR signal with a g-value of 2.00_2 and the absorption band at 394 mμ are due to the same species. The 2nd-order decay rate, which depends upon the pH of the solution, applies to the intensity change in the ESR signal as well as to that in the absorption band.

Lagercrantz[2] had reported a three-line structure for the ESR spectrum of eosine semiquinone; we could observe about a 50-line hyperfine structure of fluorescein, the splitting constants of which were nearly equal to those estimated from the spin density of the unpaired electron calculated by McLachlan's method.[3] This calculation has led to the conclusion that fluorescein semiquinone has an unpaired electron, located mainly at a central carbon atom. Although Lagercrantz had mentioned the difficulty of interpreting the three-line structure, our calculation clearly showed that these splittings can be ascribed to the protons attached to the carbon atoms of the 1- and 8- positions in the xanthene ring.[*1]

Monoanion, dianion, and trianion of fluorescein semiquinone in the aqueous solution are in equilibrium with each other over a wide pH range. From the analysis of the pH-dependence of the decay rate of the ESR signal, it was concluded that the decay rates of these ions decrease as their charge increases. The mechanism of the decay reaction of fluorescein semiquinones was discussed from the point of view of the similarity of electronic structures between triphenylmethyl radical and fluorescein semiquinone.

Experimental

Materials. All the reagents used have been purified in the way described in a previous paper.[1]

Procedure. Mixed solutions of fluorescein and a reducing agent were prepared in almost the same way as that described in the previous paper, but the concentration of fluorescein was always about 10^{-5} M. In the previous paper it was about 10^{-6} M.

The fluorescein solutions were degassed five times by using a mercury-diffusion pump, melting and freezing being repeated each time. After degassing, the solutions were poured into quartz tubes for ESR measurement; the diameters of the tubes were 1 mm for aqueous solutions and 4 mm for ethanol solutions. The quartz tubes were set in the ESR cavity and irradiated by light, for $\lambda > 430$ mμ. The light emitted from a tungsten lamp (500 W) was passed through a glass filter and through a water layer 1 cm thick, and then focused on the quartz tube by means of a lens.

The ESR spectra of the samples were recorded at a magnetic field of about 3300G by a Nippon Denshi (Japan Electron Optics), P-10-type ESR spectrometer (X band) with 100 kc field modulation. The temperature in the ESR cavity was controlled over the range between 2 and 65°C by having air of a constant temperature flow through the inside of the cavity.

Molecular Orbital Calculation. The wave functions and orbital energies of fluorescein semiquinone and other free radicals were calculated by the simple LCAO-MO method. The calculations were carried out on the NEAC-2230 Electronic Computer in the Calculation Center of Tohoku University.

Results and Discussions

Electronic Structure of Fluorescein Semiquinone. *ESR Spectra of Fluorescein Semiquinone.* When measured with a relatively wide field modulation, three line splittings were always observed, regardless of the sort of reducing agent.

However, as the field modulation becomes narrower, the number of h. f. s. lines increases to more than 50, though this occurs only in certain kinds of reducing agents. The h. f. s. differ according to the reducing agent; the details will be described below.

In the case of an aqueous solution containing leuco-fluorescein at pH 13.7, in which fluorescein semiquinone was stable during the measurement, the spectra at $g = 2.00_2$, observed with a relatively wide field modulation, consist of 3 lines whose separations, A_1, are 3.44 G. When the field modulattion width become narrower, each line splits into 5 lines, the separations of which, A_{II}, are 0.7—0.8 G, as is shown in Table 1. Further, each of these 15 lines splits into 3—4 lines (Fig. 1). The ESR spectra of fluorescein semiquinone in a saturated sodium hydroxide aqueous solution split into more than 13 lines; the separations, A_1, are 3.36 G (Fig. 2).

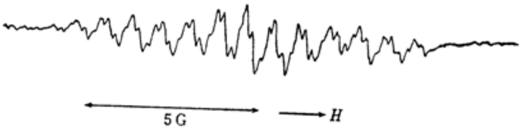

Fig. 1. ESR spectra from the irradiated solution of fluorescein, 10^{-5} M and leuco-fluorescein 7.5×10^{-4} M, 0.5 N, NaOH at 25°C.

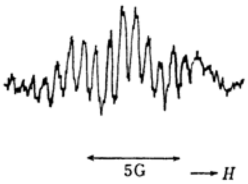

Fig. 2. ESR spectra from the irradiated solution of fluorescein and leuco-fluorescein. Strong alkaline.

When an aqueous solution of fluorescein containing EDTA is irradiated at pH 13.4 by visible light,

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[2] C. Lagercrantz and M. Yhland, *Acta Chim. Scand.*, **16**, 508 (1962).

[3] A. D. McLachlan, *Mol. Phys.*, **2**, 233 (1960).

[*1] 4 and 10 positions in Fig. 4.

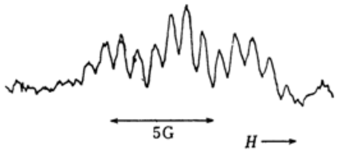

Fig. 3a. ESR spectra from the solution containing EDTA and fluorescein.

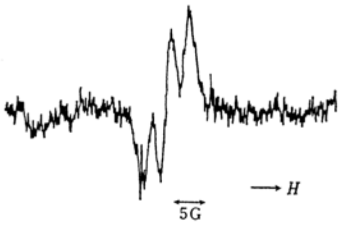

Fig. 3b. ESR spectra obtained by the irradiation of the ethanol solution of fluorescein, 1.1×10^{-3} M at 19°C.

the ESR spectra have 13 lines at $g=2.0034$ (Fig. 3a).

The fluorescein semiquinone produced in ethanol showed ESR spectra with 3 lines (Fig. 3b), lines which were independent of the width of field modulation. The coupling constant, A_I was 3.90G, and the g value was 2.0029. The reason why we could not observe h. f. s. more than 3 lines is that the exchange broadening occurs because of the short life of fluorescein semiquinone in ethanol. Lagercrantz had had a similar result for the ESR of fluorescein semiquinone produced by the photoreduction of fluorescein by the use of ascorbic acid in ethanol.[2)] When allylthiourea was used as a reducing agent, no ESR spectra could be observed because of the small amount of fluorescein semiquinone produced. This is consistent with the results in the previous paper.[1)]

Explanation of the h. f. s. of Fluorescein Semiquinone. The monoanion, dianion, and trianion of fluorescein semiquinone are in an acid-base equilibrium with each other. The pK_a's for the equilibria between the monoanion and the dianion, and between the dianion and the trianion, are, respectively, 9.5 and >13.[4)] Hence, there may be three kinds of fluorescein semiquinone present in the solution at pH 13. We have calculated the spin density of the unpaired electron of fluorescein semiquinone by the simple LCAO-MO method and by McLachlan's method in order to determine the species to which the ESR signal may be ascribed.

$$\text{monoanion} \rightleftharpoons \text{dianion} \rightleftharpoons \text{trianion}$$

In the calculation according to the simple LCAO-MO method, the values of the Coulombic integrals for oxygen atoms and of the resonance integral for the C-O bond are assumed to be as follows:

$$\alpha_{\mathrm{OH}} = \alpha_{\mathrm{C}} + 2.0\beta_{\mathrm{CC}}$$
$$\alpha_{\mathrm{O}^-} = \alpha_{\mathrm{C}} + 1.5\beta_{\mathrm{CC}}$$
$$\alpha_{\mathrm{O}^{-1/2}} = \alpha_{\mathrm{C}} + 1.75\beta_{\mathrm{CC}}$$
$$\beta_{\mathrm{CO}} = \beta_{\mathrm{CC}}$$

The angle between the xanthene ring and the benzoic acid group in a fluorescein semiquinone molecule was assumed to be about 45°. The value of the resonance integral of the C-C bond combining these two groups was set as:

$$\beta = 0.7\beta_{\mathrm{CC}}$$

Since we could not find any appreciable difference in the electron density among three semiquinone anions, the calculation of the spin density by McLachlan's method was carried out only for the dianion. The results are shown in Fig. 4. The ESR line separation was calculated from McConnell's equation:

$$a_{\mathrm{H}} = Q\rho$$

in which Q is set at 27 gauss instead of at the usual value of 23 gauss. Table 1 compares the observed and calculated line separations.

The value of Q for semiquinones containing an oxygen atom are usually larger than that for hydrocarbon radicals. Bolton, Carrington and

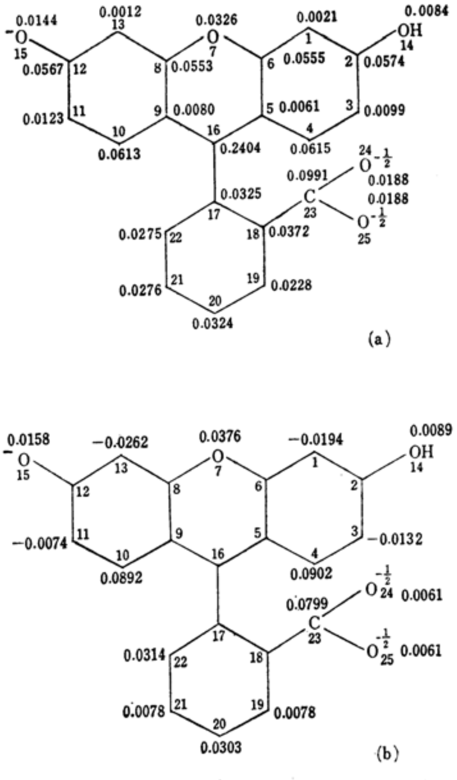

Fig. 4. Spin density of fluorescein semiquinone dianion as calculated (a) by simple LCAO and (b) by a McLachlan's method.

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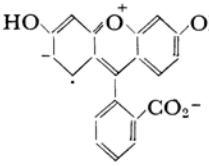
4) L. Lindqvist, *Arkiv för Kemi*, **16**, 79 (1960).

TABLE 1. LINE SEPARATION OF ESR SPECTRUM OF FLUORESCEIN SEMIQUINONE

Line number	Line separation	
	Obs.	Calc. (Q=27G)
1—2	0.83G	0.825G
2—3	0.77G	0.787G
3—4	0.83G	0.826G
4—5	0.87G	0.795G
5—6	0.83G	0.814G
6—7	0.89G	0.817G
7—8	0.81G	0.820G
8—9	0.81G	0.820G
9—10	0.87G	0.817G
10—11	0.83G	0.814G
11—12	0.79G	0.795G
12—13	0.79G	0.826G
13—14	0.77G	0.787G
14—15	0.75G	0.825G

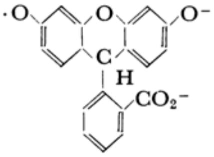
Line number was assigned successively from the line located in the lowest magnetic field.

Santos-Veiga⁵⁾ have chosen 25 gauss as the Q value of semiquinones. Vincow and Fraenkel⁶⁾ have found Q=20—27 gauss for *p*-semiquinones and Q=25—37 gauss for *o*-semiquinones on the basis of a comparison between the a_H's observed and calculated by the simple LCAO-MO method. A large Q value for fluorescein semiquinone might be ascribed to the contribution of the *o*-quinoid-type resonance structure:

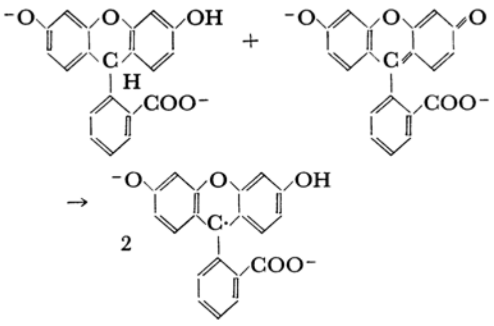


The a_H of the aqueous solution at pH 13.7 is larger than that of the saturated sodium hydroxide solution. The ratio of these a_H values is nearly equal to the ratio of spin density on the 1, 8-position carbon atoms of the dianion and the trianion. It is evident from this fact that the main component changes from the dianion to the trianion as the pH increases.

Another conceivable structure of fluorescein semiquinone:



can be rejected, because its spin density expected from the LCAO-MO calculation does not agree with the experimental value at all. Hence, it can be said that the formation of fluorescein semiquinone from fluorescein and leuco-fluorescein takes place as follows:



The ESR spectrum of eosine semiquinone had been observed by Bubnov *et al.*⁷⁾; the spectrum splits into 3 lines, like that of fluorescein, but the value of a_1 is equal to 4.6 gauss, larger than that of fluorescein. It may be concluded from the LCAO-MO calculation of the distribution of the unpaired electron that the separation is also due to the spin density on the 1, 8 carbon atoms of the xanthene ring.

Kinetic Behavior of Fluorescein Semiquinone. *Decay Rate of Fluorescein Semiquinone.* The ESR signal of fluorescein semiquinone produced by irradiating a solution of fluorescein and leuco-fluorescein, increased in intensity with the time; the build-up may be represented by the hyperbolic tangential function of the time (Fig. 5). Several minutes are required for the signal to reach its maximum value. The signal of eosine semiquinone, on the other hand, reaches its maximum value as soon as the irradiation starts.²⁾

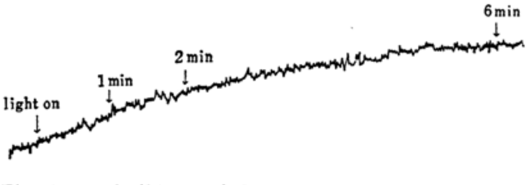


Fig. 5. A build-up of fluorescein semiquinone at 35°C, [leuco-fluorescein], 7.5×10^{-4} M, [fluorescein], $\sim10^{-5}$ M, 0.02 N, NaOH.

The decay rate of the fluorescein semiquinone produced by leuco-fluorescein or EDTA was evaluated from the change in the peak-to-peak distance on the differential curve of the ESR signal, which is proportional to the radical concentration. The change is of the second order,

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5) J. R. Bolton, A. Carrington and J. dos Santos-Veiga, *Mol. Phys.*, **5**, 465 (1962).

6) G. Vincow and G. K. Fraenkel, *J. Chem. Phys.*, **34**, 1333 (1961).

7) N. N. Bubnov, L. A. Kibalko, V. F. Tsepolov and V. Ya. Shliapintokh, *Optika i Spektroskopiya*, **7**, 117 (1959).

as Figs. 6 and 7 show. The relative decay-rate constant was obtained from the slope of the line in these figures. The absolute value of the spin concentration of fluorescein semiquinone could not be determined. The relative rate constant of the fluorescein semiquinone produced by leucofluorescein is 2.52×10^{-2} at pH 13.7, and that of that produced by EDTA is 3×10^{-2} at pH 13.4, the ratio of the two being 1.2. The same ratio in the spectrophotometric studies was 2. Although the agreement is thus not very good, it is evident that the band at 394 mμ and the ESR signal may be attributed to the same species, *i. e.*, semiquinone.

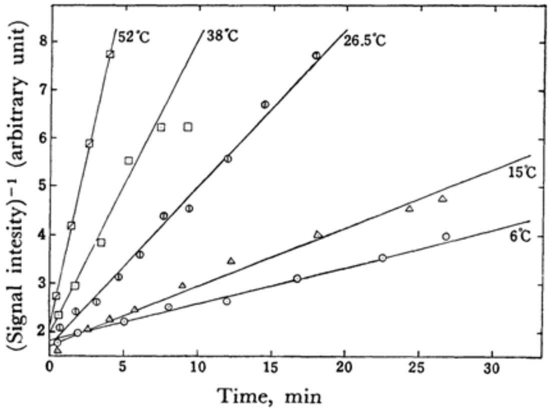

Fig. 6. Decay of fluorescein semiquinone, [leucofluorescein], 7.5×10^{-4} M, [fluorescein], $\sim10^{-5}$ M, 1×10^{-2} N, NaOH.

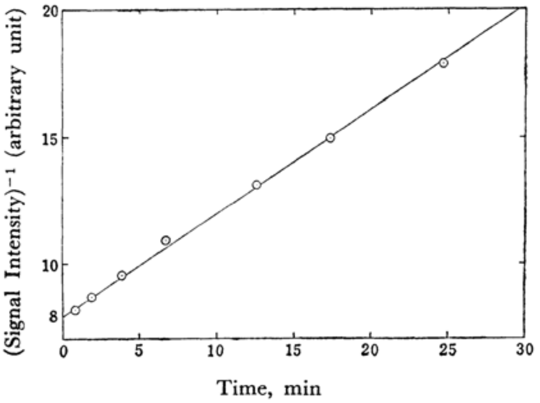

Fig. 7. Decay of fluorescein semiquinone at 24°C, [fluorescein], 1.1×10^{-3} M, [EDTA], 5.5×10^{-2} M, 2.2×10^{-1} N, NaOH.

The pH Dependence of the Decay Rate. We have reported in the previous paper that the decay rate of the semiquinone greatly depends upon the pH of solutions. In order to compare the reactivity of each semiquinone anion, we examined, by the ESR method, the pH dependence of the decay rate of the semiquinones over the pH range 11—14, a range which was chosen from a consideration of the pK_a values of monoanion (9.5)⁴⁾ and dianion (>13)⁴⁾.

The observed decay constants of semiquinone,

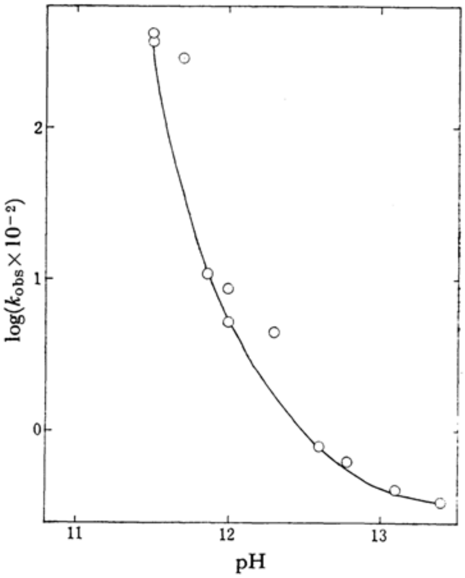

Fig. 8. PH dependence of the decay constant (k_{obs}) at 24—26°C, [leuco fluorescein], 7.5×10^{-4} M [fluorescein], $\sim10^{-5}$ M.

TABLE 2. RATE CONSTANTS OF DECAY REACTION OF FLUORESCEIN SEMIQUINONE

pH ([NaOH])	k_{obs}(cm^{-1}/10 min)$\times10^2$		
11.5(3.0×10^{-3} N)	134(12°C)	177(17°C)	360(26°C)
11.7(5.0×10^{-3} N)			288(26°C)
11.9(7.5×10^{-3} N)	5.6(15°C)	11.6(24°C)	$31._8$(37°C)
	14.6(44°C)	50(58°C)	
12.0(1.0×10^{-2} N)	4.7(12°C)	8.2(25°C)	9.6(35°C)
	21(46°C)	55(52°C)	
12.3(2.0×10^{-2} N)	2.7(13°C)	4.4(25°C)	7.9(35°C)
	$16._2$(45°C)	38(65°C)	
13.1(1.25×10^{-1} N)	0.30(24.5°C)	1.5_6(36°C)	2.4(48°C)
13.7(5.0×10^{-1} N)	0.25_2(25°C)	1.41(36°C)	1.1(49°C)

including the three kinds of anions, are shown in Table 2 and Fig. 8.

The second-order decay reaction of semiquinones is considered to consist of the following processes:

$$\mathrm{S_1} + \mathrm{S_1} \xrightarrow{k_1} \mathrm{P_1}$$

$$\mathrm{S_1} + \mathrm{S_2} \xrightarrow{k_2} \mathrm{P_2}$$

$$\mathrm{S_2} + \mathrm{S_2} \xrightarrow{k_3} \mathrm{P_3}$$

$$\mathrm{S_1} + \mathrm{S_3} \xrightarrow{k_4} \mathrm{P_4}$$

$$\mathrm{S_2} + \mathrm{S_3} \xrightarrow{k_5} \mathrm{P_5}$$

$$\mathrm{S_3} + \mathrm{S_3} \xrightarrow{k_6} \mathrm{P_6}$$

where S_i is fluorescein semiquinone, where the suffix i represents the number of the charge of the semiquinone anion, and where P is the product of each process. During these processes, an acid-base equilibrium between three kinds of anions may be assumed to be established at any time; that is,

$$\mathrm{S_1} \xrightleftharpoons{\overline{K_1}} \mathrm{S_2} + \mathrm{H^+}$$

$$\mathrm{S_2} \xrightleftharpoons{\overline{K_2}} \mathrm{S_3} + \mathrm{H^+}.$$

Since the observed ESR signal may be due to monoanion, dianion, and trianion, the observed intensity of the ESR signal is proportional to the sum of the concentration of these 3 anions, S:

$$S = \mathrm{S_1} + \mathrm{S_2} + \mathrm{S_3}.$$

The experimental decay rate is:

$$-\frac{\mathrm{d}S}{\mathrm{d}t} = k_{\mathrm{obs}} \cdot S^2$$

where:

$$k_{\mathrm{obs}} = \frac{2}{(C_{\mathrm{H}}^2 + K_1 C_{\mathrm{H}} + K_1 K_2)^2} \cdot \{k_1 C_{\mathrm{H}}^4 + k_2 K_1 C_{\mathrm{H}}^3 + (k_3 K_1^2 + k_4 K_1 K_2) C_{\mathrm{H}}^2 + k_5 K_1^2 K_2 C_{\mathrm{H}} + k_6 K_1^2 K_2^2\}$$

and where C_{H} is the hydrogen ion concentration. The relative rate constant of each process was evaluated from the above formula, neglecting the $\mathrm{S_3} + \mathrm{S_3} \rightarrow$ process because of a much lower concentration of $\mathrm{S_3}$ in the present experimental conditions.[*2]

$$k_1 : k_2 : k_3 : k_5 = 10^7 : 10^3 : 1 : 1.$$

It may be concluded that the main reactions in the pH ranges lower than 12, between 12 and 13, and higher than 13 are, respectively, process (1), process (2), and process (3). Since the rate constant observed at pH 14 by the spectroscopic method is about $10\,\mathrm{M^{-1}sec^{-1}}$ 1), k_5 may be assumed to equal $10\,\mathrm{M^{-1}sec^{-1}}$. Using this value, the value of the rate constant of each process may be estimated as follows:

$$k_1 \fallingdotseq 10^8,\ k_2 \fallingdotseq 10^4,\ k_3 \fallingdotseq k_5 \fallingdotseq 10\ (\mathrm{M^{-1}sec^{-1}})$$

The decay occasioned by the collision of two monoanions has a rate constant close to that of the diffusion-controlled process.

The activation energies of an over-all reaction in this pH range, as evaluated from the data at several temperatures between 5 and 55°C, are 11—13 kcal/mol, as Table 3 shows: they are not influenced significantly by the change in pH.

TABLE 3. ACTIVATION ENERGIES OF DECAY REACTION OF FLUORESCEIN SEMIQUINONE

pH	ΔE (kcal/mol)
13.7	12.3 ± 0.2
13.4	13.3 ± 0.6
12.8	11.2 ± 0.3
12.6	13.2 ± 0.4
12.3	10.0 ± 0.1
12.0	11.8 ± 0.7
11.5	11.0 ± 0.7

Evidently the value is much larger than that for a diffusion-controlled process if we assume, as is plausible, a small temperature dependence of K_1 and K_2. Using the value of the activation energy, the frequency factors for k_1, k_2 and k_3 are estimated to be, respectively, $\sim 10^{16}$, $\sim 10^{13}$, and $\sim 10^{10}$. The abnormally large value for k_1 suggests that the reaction in this case is not an elementary reaction. A dimerization equilibrium might be involved prior to the genuine decay process, for instance, as in the following scheme:

$$\mathrm{DH^-} + \mathrm{DH^-} \rightleftharpoons (\mathrm{DH^-})_2$$

$$(\mathrm{DH^-})_2 \rightarrow$$

At any rate, it can be concluded that the decay is an activation-requiring process, and yet the differences in the reactivities of different types of semiquinones arise mainly from the frequency factor.

Chemical Reactivity of Fluorescein Semiquinone. The decay of fluorescein semiquinone proceeds mainly through the process:

$$2\mathrm{DH^-} \rightarrow 2\mathrm{D^-} + \mathrm{H_2},$$

as has been reported in the previous paper.1) The stability of fluorescein trianion may be due partly to the absence of a hydrogen atom which can be eliminated.

In order to examine the mechanism of the above reaction, we have calculated the wave functions and molecular orbital energies for fluorescein semiquinones, for their halogen derivatives, such as eosine and erythrosine, and, further, for the triphenylmethyl radical, which may be regarded

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[*2] The derivation is straightforward. See, for example, This Bulletin, **35** 1875 (1962).

as a molecular skeleton of many similar substances. Such reactive indices of free radicals as the unpaired-electron distribution, the frontier-electron density, the localization energy, and the free valence were evaluated.

A comparison of the unpaired-electron distributions of the above substances showed that the electronic structures of all the above substances are in a zeroth approximation the same as that of the triphenylmethyl radical and are slightly perturbed by substituents. For fluorescein semiquinone and its derivatives, unpaired electron distribution in the xanthene ring show an alternation, but those in benzoic groups do not because of the weak conjugation between the xanthene and benzoic groups which are not on the same plane.

The density of unpaired electrons at the central carbon atom is the highest in all the above substances, much higher than those at other atoms. Therefore, the radical reaction of the above semiquinones probably occurs at the central carbon atom, as in the recombination of triphenylmethyl radicals.

The similarity in the mechanism of the decay reaction of fluorescein semiquinone and that of the recombination of triphenylmethyl radicals is also supported by the fact that the activation energies of the two reactions are very close to each other (10 kcal/mol for the triphenylmethyl radical[8]). The mechanism may be considered to be as follows: at first, two semiquinone radicals combine with each other at their central carbon atoms, transiently forming a dimer-like species. In the case of fluorescein semiquinone, the dimer changes to two fluorescein ions by the elimination of a hydrogen molecule from two hydroxy groups facing each other at the side of a dimer.

TABLE 4. REACTIVE INDICES OF FLUORESCEIN SEMIQUINONE

	$\delta_{14}=\delta_{15}$	Fr	Lr	$fr^{(R)}$
Monoanion	2.0	0.324	-1.678β	0.507
Dianion	1.5	0.323	-1.683β	0.491

Fr: free valence, Lr: localization energy
$fr^{(R)}$: frontier level density for radical reaction. (all at the position 16)

The transition state of the dimerization reaction is, then, probably approximated by the state in which the unpaired electron is localized on the central carbon atom, and the conjugation of this carbon atom with the surrounding atoms disappears completely. The localization energy of each fluorescein semiquinone anion, based on this transition state, is shown in Table 4.

The differences in the localization energies of different types of semiquinones are within the limits of experimental error. This explains the experimental finding that the activation energy of the decay process of fluorescein semiquinone is independent of the pH. The large difference in the rate constant and, hence, in the frequency factor between dianion and monoanion, arises whithout doubt, mainly from a difference in the ionic charge, but it is larger than predicted by Debye's theory.[9] The remaining part may be due partly to the number of OH groups from which a hydrogen molecule is to be detached.

Summary

1) The molecular and electronic structures of fluorescein semiquinone have been determined by an analysis of the hyperfine structure of the ESR spectrum. The unpaired electron is located mainly in the central carbon atom, which is sp^2 hybrid, not sp^3 hybrid.

2) The species showing the ESR signal has been identified, by a comparison of the kinetic behavior of the two species, as the one showing light absorption at 394 mμ. The decay in the semiquinone has been interpreted as consisting of several processes involving monoanion, dianion, and trianion. Each rate constant of these reactions has been estimated. The activation energies are about equal for all the processes, 10—12 kcal/mol, while the frequency factors differ in order of magnitude.

3) A plausible mechanism has been proposed in which an essential process is a recombination of two semiquinone radicals at the central atom. This mechanism was supported by our LCAO calculation of the delocalization energies for the process, which have proved almost the same for all the species of semiquinone.

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[8] K. Ziegler, A. Seib, F. Knoevenagel, P. Herte and F. Andrews, *Ann.*, **551**, 150 (1942).

[9] P. Debye, *Trans. Elecktrochem. Soc.*, **82**, 265 (1942).