*Irreversible Photobleaching of the Solution of Fluorescent Dyes. V. Photobleaching of Erythrosin**

By Masashi IMAMURA

(Received October 2, 1957)

In the first paper of this series[1], photobleaching of uranin (sodium fluoresceinate) and its halogenated derivatives, eosin and erythrosin, was investigated in the aqueous solutions; further studies were made on the photobleaching of eosin in alcoholic solutions in the presence of air[2] and in vacuo[3,4]. For eosin, its long-lived species (in the excited triplet state) participates in the primary acts: formation of labile complex with oxygen in the presence of air and dehydrogenation from alcohol in vacuo.

Recently, Oster and Adelman[5,6] declared that, in the photoreduction of fluorescein-type dyes with ascorbic acid, these dyes behave similarly. But the data on the effect of oxygen pressure upon the rate of photobleaching in the aqueous solution[1]

* Presented at the 10th Annual Meeting of the Chemical Society of Japan, Tokyo, April 5, 1957.

1) M. Imamura and M. Koizumi, This Bulletin, **28**, 117 (1955); M. Imamura, *J. Inst. Polytech. Osaka City Univ.*, **5C**, 85 (1956).

2) M. Imamura, This Bulletin, **30**, 249 (1957).

3) M. Imamura and M. Koizumi, ibid., **29**, 899 (1956).

4) M. Imamura and M. Koizumi, ibid., **29**, 913 (1956).

5) G. Oster and A. H. Adelman, *J. Am. Chem. Soc.*, **78**, 913 (1956).

6) A. H. Adelman and G. Oster, ibid., **78**, 3977 (1956).

suggests that, although erythrosin exhibits behavior similar to eosin, uranin is not quite similar[7]. This paper comprises a study of photobleaching reactions of erythrosin taking place in the aqueous and in the aqueous alcoholic solutions.

Experimental

Materials.—E. Merck Co. Erythrosin was used as in paper I[1]. Alcohols were purified by the usual methods[8]. Doubly distilled water was used in all mixtures.

Procedures.—The methods and the procedures were almost identical with those described previously[1,3,4]. A reaction cell was a cylindrical one to which an absorption cell (1 cm×1 cm×5 cm) was attached. Cells were made of glass. Dye concentration was determined from the absorption spectra or colorimetrically by using an interference filter or a gelatin filter of a narrow band. Absorption spectra were measured with the Beckman Spectrophotometer DU. Concentration of erythrosin used was 10^{-5} mole/l. throughout the experiments.

Analysis was made on aldehyde with Tollens' reagent and with fuchsin-sulfurous acid[9]. Changes in pH were examined spectrophotometrically by using some usual acid-base indicators. Acetone was examined either spectrophotometrically or with sodium nitroprussate[9].

Results

1. Photobleaching of Aerobic Aqueous Solutions.—All the data on the photobleaching of aerobic aqueous erythrosin solutions were reported in paper I. The quantum yields of photobleaching of erythrosin were much greater than those of eosin. For example, the quantum yield, k, was 3.7×10^{-4} for erythrosin, whereas 1.8×10^{-4} for eosin, at 20°C; the energies of activation were 5 and 6 kcal/mole, respectively.

2. Retarding Effect of Alcohols on the Aerobic Photobleaching.—As in the case of eosin[1,2], methanol, ethanol and isopropanol retarded the aerobic photobleaching of the aqueous erythrosin solutions. The absorption spectrum of erythrosin in the aqueous solution had its maximum optical density at the wavelength of 524 mμ at room temperature. By addition of alcohol to the system, the peak wavelength shifted for the longer wavelengths without appreciable change in the shape of the absorption curve. Limiting peak wavelengths in absolute alcohols were 529, 534 and 542 mμ, for methanol, ethanol and isopropanol, respectively. The rates of photobleaching of the aqueous alcoholic solutions were also expressed by the same equation as the one for eosin[3],

$$-\frac{dc}{dt}=1000\frac{k}{d}I_o(1-e^{-\alpha cd})$$

in which k is the quantum yield. The mean molar absorption coefficient, α, varies with the shift of the peak wavelength following the change in alcohol concentration, so its value was determined for each solution experimentally and/or by calculation with the same method as that described previously[4]. Quantum yields obtained at various alcohol concentrations are listed in Table I.

Table I
Quantum yields of aerobic photobleaching of the aqueous alcoholic solutions
$k\times10^4$ 27°C

Concn. of alcohols in vol. %	0	5	10	15	20	25	30	40	50
MeOH	5.0	3.8	3.1	2.3	1.9	1.3	1.1	0.6	0.4
EtOH	5.0	3.6	2.3	1.9	1.4	0.9	0.8	0.5	0.4
i-PrOH	5.0	3.5	2.3	1.6	1.1	0.7	0.4	0.3	0.3

The experimental formula which had been applied to eosin[1] held well for erythrosin in the low concentration ranges of alcohols (Fig. 1.):

$$\frac{1}{k}=\frac{1}{k^\circ}+K\frac{[RH]}{[H_2O]}$$

k° and k are the quantum yeilds for the pure aqueous solution and for the aqueous alcoholic solution, respectively. RH represents alcohol and K is a constant

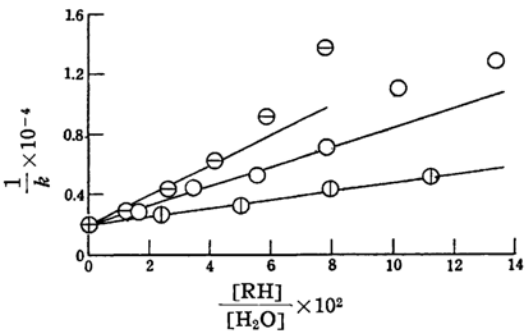

Fig. 1. Retarding effects of alcohols on the photobleaching of aerobic aqueous erythrosin solution at 27°C.
①: methanol; ○: ethanol; ⊖: *iso*-propanol.

7) To be published.
8) A. Weissberger and E. S. Proskauer, "Organic Solvents," Interscience, New York (1955).
9) F. Feigl, "Spot Tests," Vol. II, Elsevier Publ. Co. (1954).

TABLE II
VALUES OF K AND $k°K$. 27°C

	$K\times10^{-5}$	$k°K$
MeOH	0.29	15
EtOH	0.56	28
i-PrOH	0.97	49

experimentally determined. Values of K calculated from the slopes of these straight lines are tabulated in Table II.

With increasing concentration of alcohol, the rate of reaction more and more decreased, but it did not become zero even in absolute alcohol, showing a slight uniform reduction of the absorption curve after irradiation.

3. Photobleaching of the Aqueous Alcoholic Solutions in vacuo.—The rate of photobleaching of aqueous erythrosin solution, below a certain concentration of dissolved oxygen, decreased with decreasing concentration of oxygen[1], and at the complete deaeration photobleaching was not observed within the experimental errors. Addition of alcohol to the aqueous solution caused an increased rate of photobleaching in vacuo (Fig. 2). The rates of the reactions in this system were expressed also by the rate formula described above.

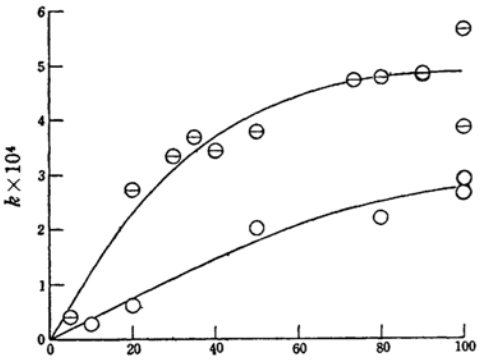

Fig. 2. Variation of the quantum yield with the alcohol concentration in vacuo at 27°C.
○: ethanol; ⊖: isopropanol.

In the case of eosin, when the alcohol concentration was relatively low, a greater part of the dye was converted into uranin by irradiation, which exhibited a strong fluorescence and had the peak wavelength at 490 mμ[4]; in absolute alcohol, however, no formation of uranin was observed[3]. In contrast with eosin, irradiated aqueous alcoholic erythrosin solutions, even at low concentration of alcohol, did not exhibit any detectable shift of the peak wavelength under the present conditions. The result seems to be very interesting at first sight, but it was found later that the formation of uranin from eosin and erythrosin (photochemical dehalogenation) is very sensitive to pH of the irradiating solutions. In the present investigation pH of the solutions was not controlled. In alkaline aqueous alcoholic solutions in vacuo, appreciable formation of uranin from erythrosin has been observed; pH-dependency of this reaction will be reported elsewhere in near future.

Apart from the peak wavelength, the solutions exhibited by irradiation slight increases in the optical densities of the absorption spectra at the wavelengths near 400 mμ, which was outside the spectral range of the incident light used. Photobleached alcoholic dye solutions, after introduction of air to the reaction cell and violent shaking for some minutes, and even after standing in the dark for several days, did not show any detectable changes in the shape of the absorption spectra in the near ultraviolet or in the visible region. Thus, it is concluded that the photobleaching of alcoholic erythrosin solution in vacuo is irreversible under these conditions.

The energy of activation of the photobleaching reaction of erythrosin in the absolute ethanolic solution was determined to be 8±1 kcal./mole, which is much larger than that for eosin (5±1 kcal/mole).

As in the case of eosin, erythrosin in alcoholic solution is believed to be photoreduced by alcohol in vacuo. Qualitative analysis of the irradiated methanolic and ethanolic solutions revealed the formation of aldehyde but not of acid. Acetone could not be detected from the irradiated *iso*-propanolic solution.

4. Influence of a Small Amount of Oxygen upon the Photobleaching of Alcoholic Solutions.—As described in sec. 2, the rate of photobleaching of erythrosin in aerobic absolute alcoholic solution was slight but detectable for each alcohol. When a very small amount of oxygen was added to the completely evacuated absolute *iso*-propanolic solution, an induction period appeared, after which the reaction proceeded at a rate equal to that of the oxygen-free system. The induction period was proportional to the amount of oxygen added. This phenomenon is similar to that observed in the case of ethanolic eosin solutions. During

this induction period, however, a slight decrease in concentration of erythrosin, which was approximately of the same degree as observed in the aerobic system, was observed. Assuming that the oxygen added was exhausted completely during the induction period, the ratio of γ (quantum yield for the photochemical disappearance of oxygen) to k (quantum yield for the photobleaching in vacuo) was obtained to be approximately 5. (3 for eosin in ethanol[3])

In the case of the absolute ethanolic solution, however, results were not reproducible. Though an induction period actually appeared, its duration was not dependent on the amount of oxygen added and the rates of photobleaching proceeded after the induction period were not always constant.

Discussion

Erythrosin is tetraiodo-uranin and a very weak fluorescer in water, while eosin is tetrabromo-derivative and a medium fluorescer: fluorescence yields are 0.02 and 0.15 in water for erythrosin and eosin, respectively[10]. From the results reported in paper I and in this paper, an analogous reaction scheme to that for eosin could be applied to erythrosin as well. This may be suppported by the works of Oster and co-workers[6,11].

Concerning the primary process in aerobic aqueous solution, it has been confirmed, for the case of eosin, that the dye participating in the process is in the long-lived excited state[1]. A similar conclusion is given also in the case of erythrosin as follows. The lifetime of the excited erythrosin attacked by oxygen was estimated in the same way as that for eosin[1] to be of the order of 10^{-7} sec., which is long enough as compared with that of the excited singlet state, 0.08×10^{-9} sec.[12]. The accuracy of the data is not sufficient for the precise value of the lifetime of the excited erythrosin to be obtained. It appears, however, that the lifetime of erythrosin is somewhat shorter than that of eosin from the data in paper I. These results are consistent with the facts that such substances as organic halogen-compounds readily transfer to the triplet levels[13] and that increasing atomic weight of the substituted atoms causes a decrease in the lifetime of the triplet state[14].

Alternative schemes for the primary process in the aerobic aqueous solutions in general were proposed in paper I: namely, $D^t+O_2\rightarrow D\cdots\cdots O_2$ (I) or $D^t+H_2O\rightarrow D\cdots\cdots H_2O$ (II); and in paper IV it was concluded that the process (I) is the correct one for eosin. It will be shown below that this is also true for erythrosin. Photobleaching of the aqueous alcoholic or the absolute alcoholic erythrosin solutions in vacuo should de due to the photoreduction of the dye by alcohol molecules, $D^t+RH\rightarrow DH\cdot+R\cdot$, hence the same equation as eq. 18 in paper IV[2] can be employed,

$$\frac{1}{k}\frac{[RH]}{[H_2O]}=\frac{1}{\varphi_{S\rightarrow T}}\frac{k_1^s}{k_2}+\frac{1}{\varphi_{S\rightarrow T}}\frac{k_2^s}{k_2}\frac{[RH]}{[H_2O]}$$ [15]

Fig. 3 illustrates a plot of $[RH]/k[H_2O]$ vs. $[RH]/[H_2O]$ for *iso*-propanolic solutions. A plot for ethanolic solutions also yielded a straight line. Values obtained from the slopes and the intercepts of the straight lines for ethanolic and *iso*-propanolic solutions are summarized in Table III.

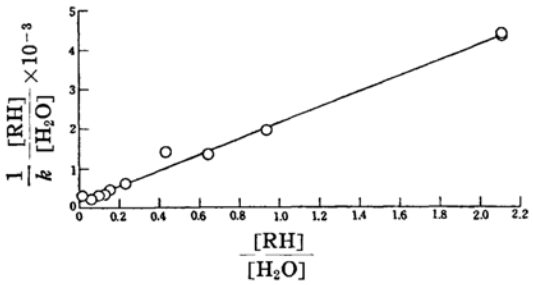

Fig. 3. The dependence of $[RH]/k[H_2O]$ upon $[RH]/[H_2O]$ for the isopropanolic erythrosin solution in vacuo at 27°C.

TABLE III
(at 27°C)

	$\varphi_{S\rightarrow T}\frac{k_2}{k_2^s}\cdot10^4$	$\varphi_{S\rightarrow T}\frac{k_2}{k_1^s}\cdot10^3$	$\frac{k_2^s}{k_1^s}$
EtOH	3	0.9	3
i-PrOH	5	6.7	13

10) P.Pringsheim, "Fluorescence and Phosphorescence." p. 316, Interscience, New York (1949).
11) J. S. Bellin and G. Oster, *J. Am. Chem. Soc.*, **79**, 2461 (1957).
12) E. Gaviola, *Z. Physik*, **42**, 853 (1927).
13) See, for example, E. J. Bowen and F. Workes, "Fluorescence of Solutions," p. 27. Longmans, Green and Co., London, (1953).
14) D. S. McClure, N. W. Blake and P. L. Hanst, *J. Chem. Phys.*, **22**, 255 (1954); D. S. McClure, ibid., **17**, 905 (1949).
15) Here, $\varphi_{S\rightarrow T}$ is the $D^*\rightarrow D^t$ transition probability, and k_1 and k_2 are the specific rates of the reactions D^t+H_2O and D^t+RH, respectively. The superscript s represents the summation of the mere deactivation and the effective process leading to the photobleaching.

On the basis of the similar arguments to those given in paper IV, the following two relations are derived from the schemes (I) and (II). If the primary process of the aerobic photobleaching follows (I),

$$\frac{k_2'^s}{k_1'^s}=k^\circ K, \text{ (eq. 15 in paper IV)}$$

and if it follows (II),

$$\frac{k_2^s}{k_1^s}=k^\circ K. \text{ (eq. 17 in paper IV)}$$

Values of $k^\circ K$ are given in Table II. Comparing these values with those of k_2^s/k_1^s in Table III, it is apparent that the differences between them are far beyond the errors, and the scheme (II) must be rejected. Thus, it can be concluded that the primary process of the photobleaching of erythrosin in the aerobic aqueous solution proceeds via the scheme (I). The values of $k^\circ K$ in Table II therefore are interpreted as those of $k_2'^s/k_1'^s$ for the process (I).

Further, from the data on the effect of oxygen pressure on the rate of the photobleaching of aqueous erythrosin solution given in paper I, the value of $k_1^s/k_{O_2}^s$ at 30°C is calculated as 8×10^{-8}, according to eq. 20 in paper IV:

$$\frac{1}{k}\frac{[O_2]}{[H_2O]}=\frac{1}{k^\circ}\frac{k_1}{k_{O_2}^s}+\frac{1}{k^\circ}\frac{[O_2]}{[H_2O]}$$

A Plot of $[O_2]/k[H_2O]$ against $[O_2]/[H_2O]$ is illustrated in Fig. 4.

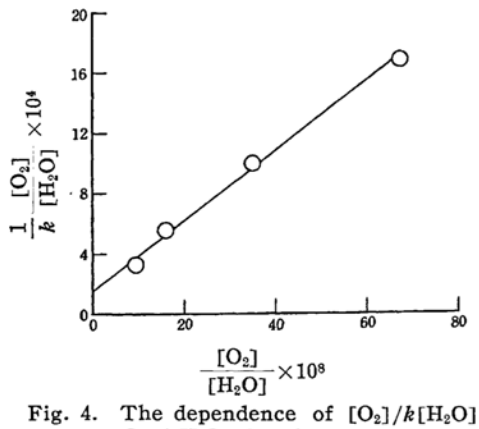

Fig. 4. The dependence of $[O_2]/k[H_2O]$ upon $[O_2]/[H_2O]$ for the aqueous erythrosin solution at 30°C.

The quantum yield, k°, for erythrosin in the aerobic aqueous solution is about twice that for eosin. In the presence of sufficient oxygen, k° is given by

$$k^\circ=\frac{k_{O_2}}{k_{O_2}^s}\frac{k_1'}{k_1'^s}$$

in which k_{O_2} and k_1' are the specific rates of the reaction between D′ and O_2 (dissolved), and between D·····O_2 and H_2O, respectively. Assuming that the rate of reaction between peroxy-dye-radical, D·····O_2, and H_2O is not so affected by the nature of dyes, k° depends mainly on $\varphi_{S\to T}(k_{O_2}/k_{O_2}^s)$. It seems probable to assume, therefore, that an increase in k° of erythrosin is due to the increases both in $\varphi_{S\to T}$ and in $k_{O_2}/k_{O_2}^s$. On the other hand, values of $\varphi_{S\to T}(k_2/k_2^s)$, $\varphi_{S\to T}(k_2^s/k_1)$ and k_2^s/k_1^s exhibit appreciable differences between eosin and erythrosin, while the values of $k_2'^s/k_1'^s(=k^\circ K)$ are nearly the same for both. It is concluded, therefore, that the reactivity of D′ towards alcohols is relatively smaller for erythrosin than for eosin.

Irregular results observed for the ethanolic erythrosin solutions in the presence of a small amount of oxygen may perhaps be related to the smaller reactivity of this dye towards ethanol. It is seen, from Table III, that *iso*-propanol is more photo-oxidizable than ethanol[16], and a clear-cut result was obtained for this solution.

Summary

Photobleaching of the aqueous alcoholic erythrosin solutions in air and in vacuo was investigated. In general, erythrosin showed behavior similar to eosin. An excited dye molecule which participates in the primary processes is the long-lived species, having a lifetime of the order of 10^{-7} sec. It was concluded that the primary process in aerobic aqueous erythrosin solution proceeds via the process, D′+O_2→D·····O_2, as in the case of eosin. It was found that excited erythrosin is somewhat more reactive than excited eosin towards dissolved oxygen, while it is less reactive towards alcohols in vacuo. Some quantitative values on the reactivities were given.

The author wishes to express his appreciation to Professor M. Koizumi of the Tohoku University for his support of this work and criticism of this paper.

Institute of Polytechnics,
Osaka City University,
Kita-ku, Osaka

16) It is probably true that $\varphi_{S\to T}$ in ethanol is not so different from that in *iso*-propanol.