Irreversible Photobleaching of the Solution of Fluorescent Dyes. II. Photobleaching of the Alcoholic Solution of Eosine in Vacuo

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Introduction

In the first paper of this series[1] kinetic studies on the photobleaching of eosine, erythrosine and uranine in aqueous solutions in the presence of oxygen were reported and some probable schemes were proposed for the primary processes of the reaction. In that paper the notable fact was found that the addition of ethyl alcohol to the aqueous solution of eosine caused a marked retardation on the rate of aerobic photobleaching. Thus in the mixture of water and ethyl alcohol 7: 3 in volume, the rate became practically negligible and in the pure alcoholic solution there was no photobleaching observed. But another remarkable fact then found was that if such solutions are deaerated, considerable rapid photobleaching takes place in contrast to the pure aqueous solution which shows no such photobleaching in vacuo at all.

Though it appears that the vacuum photobleaching (in alcohol) and the aerobic photobleaching (in aqueous solution) will proceed by different mechanisms, it is interesting to study about the primary processes of the former and about the retarding effect of oxygen. Moreover, it is not unexpected that the information obtained therefrom may be helpful for the further attack on the primary processes of the aerobic photobleaching in the pure aqueous solution.

The purpose of the present paper is to report some results obtained using methyl, ethyl, isopropyl and *n*-butyl alcohol as solvents and to make some discussions on the mechanism of the reaction.

Experimental

Procedure.—Eosine and the arrangements for irradiation were the same as those described previously[1,2]. Methanol, ethanol, isopropanol and *n*-butanol were purified by the method similar to that employed in the preceding paper for ethanol. Stock solutions of eosine in these alcohols were prepared at concentration of 10^{-3} mol./l. These solutions were diluted to the concentration of 10^{-5} mol./l. and were deaerated with much caution in view of the high solubility of oxygen in alcohols. The procedure was as follows. The solution was put into one of two ampoules attached to a reaction cell and was evacuated under cooling with use of a mercury diffusion pump. Then the alcohol was distilled into another ampoule and evacuated again. These processes were repeated four to six times.

Irradiated alcoholic solution of eosine in vacuo did not show any shift of the absorption maxima as compared with the initial solution, and the almost perfect absorption curve in visible region declined monotonously as the bleaching went on. Hence the change of the concentration of eosine could be followed by a photomultiplier and an interference filter in exactly the same way as described already[1,2].

Experiments were made on the oxygen effect on the rate, with use of the apparatus schematically shown in Fig. 1. At first, complete deaeration was accomplished by repeated vacuum distillation between E and F. Then a definite small amount of oxygen was taken between A and B manometrically and it was expanded to the right hand side of B, in which the solution F was being cooled with dry ice. (The volume of these two parts had been determined previously.) The oxygen pressure after expansion could easily be calculated. After C was fused off, oxygen was dissolved at room temperature into the solution, which was then poured into the cell, and D was fused off. Of course all the manipulations were made in the dark. The amount of oxygen in a reaction cell was calculated, employing the value

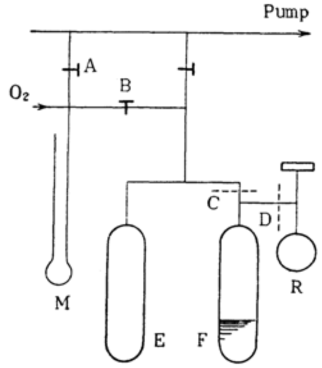

Fig. 1. Schematic diagram of the apparatus. M: manometer; R: reaction cell.

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[1] M. Imamura and M. Koizumi, This Bulletin, 28, 117 (1955).

[2] M. Imamura, *J. Inst. Polytech. Osaka City Univ.*, 6, No. 1, Series C, 85 (1956).

of 0.14 as a Bunsen coefficient3) for pure ethanol. and taking into account the volume of the solution and that of the gaseous phase.

Results

1. Photobleaching of the Alcoholic Solutions in Vacuo.—Investigations were carried out at 20°C with a filter Matsuda VGl. The intensity of light was varied with blackened metallic nets.

The rate of bleaching was relatively great compared with that of aerobic aqueous solution. Some examples of the bleaching curve are shown in Fig. 2, which includes a curve for the aerobic photobleaching of the aqueous solution for comparison. Absorption spectra of the irradiated solution are compared with the original one in Fig. 3.

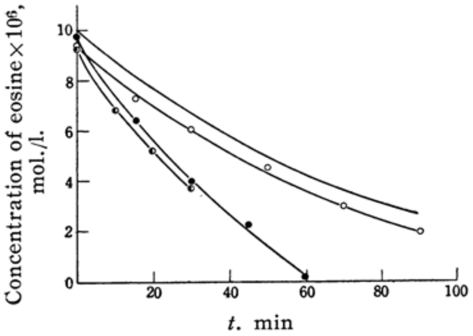

Fig. 2. Fading curves of eosine in vacuo at 20°C.

Full line: in aerobic aqueous solution, $I_0=1.74\times10^{16}\,h\bar{\nu}/\mathrm{cm}^2$. sec.; ○: in methanol, $I_0=1.18\times10^{16}\,h\mu/\mathrm{cm}^2$. sec., ●: in ethanol, $I_0=1.41\times10^{16}$, ◐: in isopropanol, $I_0=1.18\times10^{16}$. In all cases $d=15$ mm.

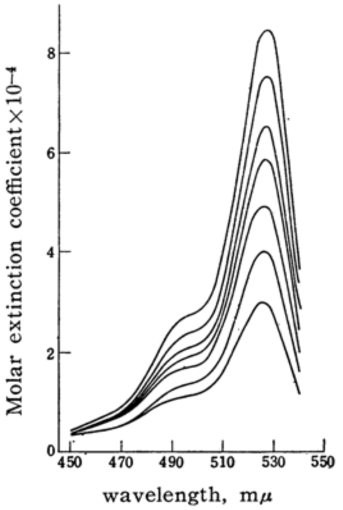

Fig. 3. Absorption spectra of irradiated eosine in EtOH in vacuo. At 34°C.

As anticipated from these figures, the experimental results obtained for every sort of alcohol except *n*-butanol could be represented satisfactorily by the same rate formula as that proposed in the previous paper1).

$$-\frac{dc}{dt}=\frac{k}{d}I_0(1-e^{-\alpha cd})\times1000^* \qquad (1)$$

$$S\equiv\ln(1-e^{-\alpha c_0 d})-\ln(1-e^{-\alpha cd})+\alpha d(c_0-c)=1000k\alpha I_0 t \qquad (2)$$

where c and c_0 are the concentrations of dye in mol./l. at time $t=t$ and $t=0$, α the mean molar absorption coefficient, d the thickness of the solution in cm., I_0 the intensity of light in moles per cm² per sec., and k is the usual overall rate constant and is also considered as a quantum yield of bleaching. Mean molar absorption coefficients of eosine (α's) in methanol, ethanol, isopropanol were determined by a thermopile to be 4.8×10^4, 6.6×10^4, 6.1×10^4 and 5.2×10^4, respectively.

Some examples of the application of eq. (2) to the experimental results are shown in Fig. 4 and

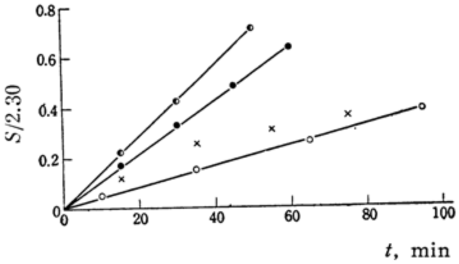

Fig. 4. Dependence of the left-hand side of eq. (2) divided with 2.30 ($S/2.30$) on irradiation time t. At 20°C.

○: in methanol, $I_0=0.69\times10^{16}\,h\bar{\nu}/^2$.cm sec.; ●: in ethanol, $I_0=0.83\times10^{16}$; ◐: in isopropanol, $I_0=0.69\times10^{16}$; ×: in *n*-butanol, $I_0=0.68\times10^{16}$.

TABLE I

QUANTUM YIELDS FOR PHOTOBLEACHING OF EOSINE IN MeOH, EtOH, AND i-PrOH AT 20°C

(k for aerobic photobleaching in aqueous solution is 1.8×10^4.)

Solvent	$I_0\times10^{16}$ $h\bar{\nu}$/cm² sec.	$k\times10^4$	Solvent	$I_0\times10^{16}$ $h\bar{\nu}$/cm² sec	$k\times10^4$
MeOH	0.69	5.3	EtOH	0.74	6.4
〃	〃	2.7	〃	0.83	4.4
〃	〃	4.0	〃	〃	4.6
〃	1.18	3.8	〃	1.25	5.4
〃	〃	3.7	〃	1.41	4.5
〃	〃	2.9	〃	〃	9.0
Average		(3.7)	Average		(5.6)
EtOH	0.35	4.2	*i*-PrOH	0.69	3.9
〃	0.39	4.3	〃	〃	7.4
〃	〃	4.5	〃	〃	7.8
〃	0.62	10.3	〃	1.18	4.6
〃	0.66	4.9	〃	〃	5.6
〃	〃	4.6	〃	〃	5.9
〃	0.74	6.4	Average		(5.9)
〃	0.83	4.4			

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3) "International Critical Table", McGraw Hill, New York (1929), 3, p. 262.

the values of k's calculated are summarized in Table I. Though it is certain that eq. (1) can be applied to ethanol, methanol, and isopropanol, some fluctuations exist among the k-values of different runs. It may perhaps be due to the slight difference of the pretreatment of alcohols in view of the existence of the very sensitive effect of a trace of oxygen described below. In spite of these fluctuations, it is evident that the order of the rate of photobleaching in vacuo is MeOH< EtOH<i-PrOH. From the measurements made on the quantum yield of the ethanolic solution of eosine at various temperatures, it was found that the activation energy for the reaction in ethanol in vacuo was computed as 5±1 kcal./mol., which is about the same as that obtained for aerobic aqueous solution of eosine1).

As to the reaction products, qualitative tests of aldehyde and acid were applied to the vacuum distillate from the irradiated ethanolic solution of eosine.

Acidity was examined by a glass electrode pH meter and also by the spectroscopic measurements with some ordinary pH indicators. There was no change observed in pH.

Aldehyde, on the other hand, could be detected by every test employed, i.e., by Tollen's reagent (NH_3—$AgNO_3$ solution), by Schiff's reagent (reduced *p*-fuchsine solution) and by the method which utilized the catalytic action of aldehyde on the oxidation of *p*-phenylene diamin by hydrogen peroxide4).

2. Effect of Small Amounts of Oxygen on the Photobleaching of Alcoholic Solution.—As mentioned above, aerobic pure alcoholic solution of eosine did not show any photobleaching. When a very small amount of oxygen was added to the completely evacuated solution, the photobleaching did not take place for a certain period which was almost proportional to the amounts of added oxygen, but after this induction period, the reaction began to proceed abruptly and with almost the same rate as that in the completely evacuated solutions.

Results are shown in Fig. 5, in which the number depicted along each curve is the oxygen pressure (mmHg) in the gaseous phase of a cell.

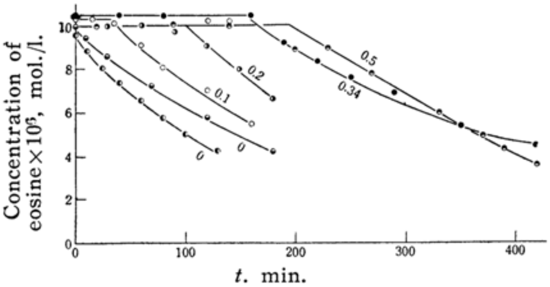

Fig. 5. Fading curves of eosine in EtOH in the presence of various amounts of oxygen. At 27°C.

The number depicted along each line is the oxygen pressure in mmHg. in the gaseous phase of a cell.

From the above results, there scarcely remains any doubt that a small amount of oxygen inhibits the photobleaching of eosine and this inhibiting action comes to its end when oxygen is used up by some unknown reaction. Thus it must be interpreted that during the induction period some photosensitized oxidation reaction is going on by virtue of eosine as a photosensitizer. As such a photosensitized oxidation, there will be no other possibility than that of the oxidation of alcohol to acid or aldehyde. Hence several tests for aldehyde and acid were tried for the irradiated aerobic alcoholic solution of eosine. The result was that the formation of aldehyde could not be detected by Tollens' and by Schiff's reagents, while the formation of acid was definitely established from the facts that reduction of pH could be observed by means of a glass electrode pH meter and by usual indicators spectroscopically.

Aldehyde would perhaps be oxidized to acid if it were produced, which was ascertained by an independent test that a small amount of aldehyde added to the aerobic alcohol solution was quickly converted to acid by irradiation. The acid formed would probably be acetic acid, but could not be identified because of its small quantity.

Another product confirmed in the irradiated aerobic alcoholic solution was hydrogen peroxide; it was detected by iodometry in nitrogen atmosphere and by titanium trichloride.

Assuming that the existing oxygen is exhausted during the induction period, the quantum yield for the photochemical disapperance of oxygen (γ) could be calculated from the data shown in Fig. 5. The ratio of γ to k (the quantum yield of photobleaching of eosine) is tabulated in Table II, which shows that the ratio is almost constant, independent of the quantity of oxygen; the value is about 3.

TABLE II

THE RATIO OF γ TO k

P_{O_2} (mmHg)	O_2 (10^{-3} mol.)	t_{ind} (min.)	γ/k
0.1	4.2	31	3.5
0.2	8.4	100	2.4
0.3	14.6	160	3.1
0.5	21.4	200	3.3
		Average	(3.1)

Discussion

Since the photobleaching of eosine in the evacuated alcoholic solution is accompanied by the production of aldehyde, it can be concluded that its primary process is the dehydrogenation of alcohol by the excited dye, most certainly in the triplet state;

$$D^t + RH \longrightarrow DH\cdot + R\cdot, \tag{i}$$

where D^t is the excited dye in the triplet state, RH is alcohol, DH· a semiquinone-type intermediate and R· a radical from alcohol. The process (i) can also be considered as an

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4) F. Feigl, "Spot Tests", Elsevier (1954), Vol. II, p. 146.

essential step of the photobleaching if one assumes, as is probable, that DH· is colorless or almost colorless. This is consistent with the experimental result that the rate of photobleaching depends only on the amounts of light absorbed; moreover, the fact that the rate increases in the order, $MeOH<EtOH<i\text{-}PrOH$, coincides nicely with the general trend of hydrogen releasing power of R· as observed, for example, in the oxidation of alcohol with Fenton's reagent[5]. Thus there scarcely remains any doubt about the primary process of the photobleaching in vacuo.

Turning to the inhibiting action of oxygen, although it is certain that oxygen oxidizes alcohol to acid by virtue of the excited dye, which acts as a sensitizer, there are two alternative mechanisms conceivable in respect to the step in which oxygen takes part. The one is

$$\left.\begin{aligned} &D^t+RH \longrightarrow DH\cdot+R\cdot && (a) \\ &DH\cdot+O_2 \longrightarrow D+HO_2\cdot && (b) \end{aligned}\right. \quad (ii)$$

and the other is

$$\left.\begin{aligned} &D^t+O_2 \longrightarrow D^t\cdots O_2 && (a) \\ &D^t\cdots O_2+RH \longrightarrow D+R\cdot+HO_2\cdot && (b) \end{aligned}\right. \quad (iii)$$

In general, two processes may occur simultaneously, but in the case of ethyl alcohol, the former is considered to be the principal process for the following reason.

As seen from Table II, the duration of the induction period during which photobleaching is completely suppressed, is roughly proportional to the amount of oxygen, and it reaches thirty minutes by the existence of as little oxygen as 4×10^{-8} mol., which corresponds to the oxygen concentration of 8×10^{-7} mol./l. in the solution. Thus it is almost certain that 10^{-8} mol./l. of oxygen or less is enough to completely prevent photobleaching.

This requires quite a long lifetime for the intermediate which is attacked by oxygen; it is estimated to be not less than 10^{-2}–10^{-3} sec. by the analogous argument performed in the previous paper[1]. Hence if the reactions (iii) are to occur, the lifetime of the triplet state must be of the same order. But in the case of the aerobic photobleaching in the aqueous solution, the lifetime of D^t is estimated to be 10^{-6} sec. or so if the reaction goes by (iii)**. Thus the process (iii) can be denied unless one recognizes a very unprobable assumption that the lifetime of the triplet state differs markedly in water and in ethyl alcohol.

Next, let us consider the processes which follow the reaction (ii). There were some investigations published on the photoöxidation of alcohols sensitized by some substances such as azo-compounds[6], benzoquinone[7] and benzophenone[8]. In particular, a recent work of Bolland and Cooper[9], on the photoöxidation of ethanol by anthraquinone 2,6-disodium-sulfonate as a sensitizer afforded some detailed mechanism of the reaction. Applying it to our case it was really found, as shown below, that our experimental results concerning the reaction products and particularly the fact that the ratio γ/k is about 3 can all be explained satisfactorily.

For the photobleaching in vacuo, the mechanism in question can be written as follows:

$$\begin{aligned} &D \xrightarrow{h\nu} D^* && (iv) \\ &D^* \longrightarrow D^t && (v) \\ &D^t+RH \longrightarrow DH\cdot+R\cdot && (vi)\ (\text{same as (i)}) \\ &DH\cdot+DH\cdot \longrightarrow DH_2+D && (vii) \\ &DH_2 \longrightarrow\cdots\text{products} && (viii) \\ &R\cdot+R\cdot \longrightarrow RH+\text{aldehyde} && (ix) \end{aligned}$$

In this scheme, (vi) is the main step of photobleaching and DH· has the lifetime of 10^{-2}–10^{-3} sec. as stated above. On the other hand, D is regenerated according to (vii). Hence the k-value experimentally obtained is just one half of the rate constant for the formation of DH· by (vi).

When oxygen is present, the following reactions[10] would occur producing hydrogen peroxide and acetic acid and regenerating the original dye molecule. (Aldehyde produced by (xiv) will be further oxidized to acid).

$$\begin{aligned} &R\cdot+O_2 \longrightarrow RO_2\cdot && (x) \\ &DH\cdot+O_2 \longrightarrow D+HO_2\cdot && (xi) \\ &RO_2\cdot+RO_2\cdot \longrightarrow 2\text{Acid}+H_2O_2 && (xii) \\ &HO_2\cdot+HO_2\cdot \longrightarrow H_2O_2+O_2 && (xiii) \\ &RO_2\cdot+HO_2\cdot \longrightarrow H_2O_2+\text{Aldehyde}+O_2 && (xiv) \end{aligned}$$

As regards the last three steps, the following three cases are possible:

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[5] J.H. Merz and W.A. Waters, *Faraday Soc. Discussion*, 2, 179 (1947); W.A. Waters, "The Chemistry of Free Radicals", Oxford (1946), p. 247.

* The rate formula $dc/dt=kI_0(1-e^{-\alpha cd})$ (3) written in the previous paper[1] must be corrected as above since all the evaluated values of k in that paper were the quantum yields and did not correspond to k in eq. (3). All the results and discussions given in it, however, are not affected by this modification.

** The proof that the primary process of the aerobic photobleaching of eosine in the aqueous solution is really $D^t+O_2\rightarrow D^t\cdots O_2$ will be concluded in the next paper. Cf. G. Oster and A.H. Adelman, *J. Am. Chem. Soc.*, 78, 913 (1956).

[6] B.E. Blaisdell, *J. Soc. Dyers & Colourists*, 65, 618 (1949).

[7] A. Berthoud and D. Parret, *Helv. Chim. Acta*, 17, 694 (1934).

[8] H.L.J. Bäckström, *The Suedberg (Mem. Vol.)*, 1944, 45; *C. A.*, 39, 1105 (1945); A. Berthoud, *Helv. Chim. Acta*, 16, 592 (1933).

[9] J.L. Bolland and H.R. Cooper, *Proc. Roy. Soc. (London)*, 225, 405 (1954).

[10] For the rapid reaction of radical R· with oxygen, see, for example, J. R. McNesby and C. A. Heller, Jr., *Chem. Rev.*, 54, 342 (1954).

(a) only (xii) and (xiii) take place;

(b) only (xiv) takes place;

(c) all the three processes take place simultaneously.

According to the above scheme the result, $\gamma/k=3$, can be accounted for as follows. For each DH· produced by (vi), two oxygen molecules are consumed in (x) and (xi), but in (xiii) and (xiv) one is regenerated. The total consumption of oxygen for one molecule of DH· thus becomes 3/2 for all the above three cases:

(a) $2-1/2=3/2$;

(b) $2-1+1/2=3/2$ (since 1/2 O_2 is necessary for the further oxidation of aldehyde);

(c) 3/2 (superposition of (a) and (b)).

In vacuo, on the other hand, for one DH· produced by (vi) 1/2 D should disappear since the process (vii) regenerates one D from two DH·.

Thus the ratio of the quantum yield for the oxygen consumption to that for the photobleaching should be $(3/2)/(1/2)=3$, which agrees very satisfactorily with the experimental values in Table II.

Here it is to be noted that from the above argument, the decomposition of dye through the interaction between DH· and R· can be practically neglected, for γ/k must otherwise be less than 3. Another fact to be added is that the reaction,

$$D^t+O_2 \longrightarrow D^t\cdots O_2,$$

is neglected in the above argument. But even if this reaction partially takes part, the products and the quantitative relation between γ and k are not affected, because, in this case, it is only necessary to replace (xi) by

$$D^t\cdots O_2+RH \longrightarrow D+R\cdot+HO_2\cdot \qquad (xv)$$

without any alterations for the succeeding steps.

Summary

Photobleaching of eosine in methanol, ethanol, isopropanol and *n*-butanol in vacuo was investigated and it was concluded that the primary process is the dehydrogenation of alcohols by the metastable excited eosine molecule. In the presence of oxygen, the photobleaching is suppressed completely, but the sensitized photoöxidation of alcohol takes place.

When a small amount of oxygen is added to the evacuated alcoholic solution of eosine, the induction period appears, after which the photobleaching proceeds with almost the same rate as that in the evacuated state. Both the quantum yield for the consumption of oxygen during the induction period (γ) and that of the photobleaching in vacuo (k) were determined and the ratio γ/k was found to be ca. 3. This result and the reaction products detected (aldehyde in vacuo, acid and hydrogen peroxide in the presence of oxygen) were satisfactorily explained by the mechanism proposed by Bolland and Cooper. Further results somewhat important from the general standpoint of photobleaching of dye are:

(1) The lifetime of the intermediate to be attacked by oxygen (which is most probably DH·) is not less than 10^{-2}–10^{-3} sec.

(2) The quantum yield for the primary process of the photobleaching $D \rightarrow D^* \rightarrow D^t(+RH) \rightarrow DH\cdot(+R\cdot)$ in pure ethanol is ca. 10×10^{-4} at 20°C.

(3) In pure ethanolic solution, the decomposition of dye by the interaction between DH· and R· practically does not occur.

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