Irreversible Photobleaching of the Solutions of Fluorescent Dyes. IV. Photoreactions in Methanol, Isopropanol and Ketones, and the General Conclusion for the Photobleaching of Eosine

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Introduction

The present author and Koizumi have reported some investigations concerning the photoreaction of eosine in water, ethanol and water-ethanol in vacuo and in the presence of oxygen (air)[1-4]. In vacuo, no reaction occurs in water but in alcohol and in water-alcohol mixtures eosine is decolorized irreversively. In the presence of oxygen, eosine in alcohol acts as a sensitizer for the photoöxidation of alcohol, while in the aqueous solution the dye photobleaches irreversively. Our first plan was to gain some definite information on the primary processes of the photobleaching of eosine in water in the presence of oxygen, and the problem has already been partly cleared up; two possible mechanisms of the primary processes have been proposed in Part I[1] and a further discussion has been given in Part III[4].

In this paper, some results obtained in methanol, isopropanol, *n*-butanol, acetone and methyl ethyl ketone, and in their mixtures with water will be reported, and a conclusive discussion will be given of the photochemical primary process of eosine in the aqueous solution.

Experimental

Materials.—Merck Co. and Grübler's Eosin Yellow were used. These two species showed almost the same absorption spectra and similar photochemical behavior. Solvents were commercial products purified by the usual methods[5]. Apparatus and procedures were exactly as described in the previous papers[1-4].

1. Retarding Effects of Alcohols upon the Aerobic Photobleaching of the Aqueous Solution.—As already reported[1,2], ethanol shows a very marked retardation in the aerobic photobleaching of the aqueous solution of eosine and the quantum yield, k, is expressed, in the low concentration region of ethanol, by the equation:

$$\frac{1}{k}=\frac{1}{k^0}+K\frac{[\mathrm{EtOH}]}{[\mathrm{H_2O}]}, \qquad (1)$$

where k^0 is the quantum yield in the pure aqueous solution, [EtOH] and [H_2O] are molar concentrations, and K is a constant which gives a measure of the retarding effect of ethanol[1].

Now, it was established that other alcohols show similar behavior as ethanol[6] in the low concentration region, the rate of photobleaching

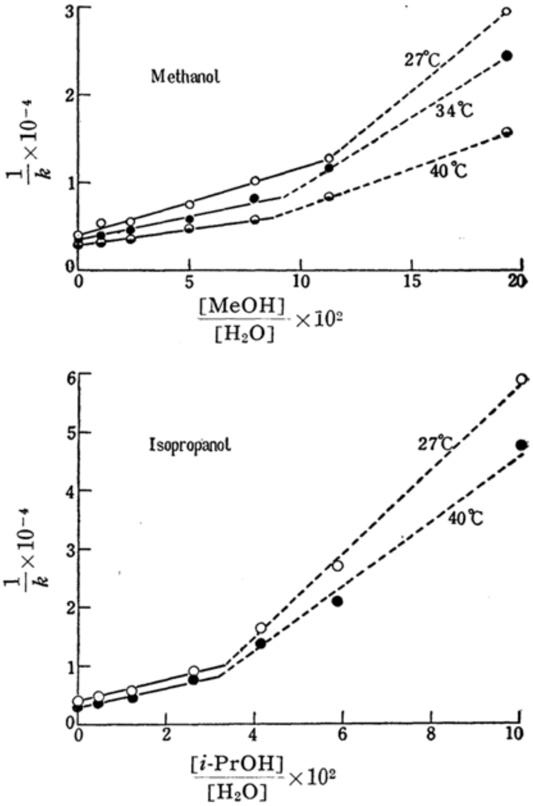

Fig. 1. Variation of the quantum yield with the mole ratio of alcohol to water. (Eq. (1))

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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, c, 85 (1956).

[3] M. Imamura and M. Koizumi, This Bulletin, **29**, 899 (1956).

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

[5] A. Weissberger and E. S. Proskauer, "Organic Solvents", (Revised by J. A. Riddick and E. E. Toops, Jr.), Interscience, New York (1955).

[6] Owing to the restriction of the solubility of *n*-butanol in water, experiment in a concentration higher than 8 vol. percent was impossible for it.

being expressed (Fig. 1) also by eq. (1). Values of K for these alcohols are listed with those for ethanol in the third column of Table I.

TABLE I

RH	Temp. °C	$K\times10^{-5}$	$k^0\times10^4$	k^0K	k^s_2/k^s_1
MeOH	27	0.77	2.5	19	—
	34	.58	2.9	17	9
	40	.37	3.6	13	—
EtOH	20	1.73	1.9	33	—
	27	1.62	2.5	41	19
	34	1.45	2.9	42	—
	40	1.33	3.6	48	—
i-PrOH	27	1.94	2.5	49	17
	40	1.83	2.9	53	—
n-BuOH	20	5.18	1.9	98	—
	27	3.41	2.5	85	—
Acetone	27	2.3	2.5	58	—
Methyl ethyl ketone	27	4.6	2.5	115	—

It is evident that K increases at constant temperature in the order, MeOH, EtOH, *i*-PrOH, *n*-BuOH, and the temperature coefficient is negative. As seen from Fig. 1, discontinuity appearing on each line is displaced towards the lower value of $[RH]/[H_2O]$ in the same order as above and with rising temperature.

2. Photoreactions of Eosine in Methanol- and Isopropanol-Water Mixtures.— As already described[4)], eosine in ethanol-water mixture is converted more or less to uranine by irradiation in vacuo; the initial rate of the reaction increases with the rising concentration of ethanol and reaches a limiting value in the neighborhood of ethanol concentration of 20 vol. percent.

In expectation of similar phenomena, the experiments were made on the methanol- and isopropanol-water solutions. Experimental procedures and the calculation of the quantum yields were exactly the same as before[4)].

The result with methanol at 34°C is shown in Fig. 2. Uranine production was also observed in this case but was less than with ethanol. Quantum yield reaches a limiting value in the neighborhood of concentration of 30 vol. percent methanol (in contrast to 20 vol. percent with ethanol). It is evident, from Fig. 2, that the participation of water in the primary process of the reaction can be neglected at a concentration of 30 vol. percent in vacuo, while, in the presence of oxygen (air), measurable amount of photobleaching is seen at this concentration (dotted line). The behavior is similar to that observed in the case of ethanol and this is another support in favor of the conclusion given in Part III[4)] that the primary process of the photobleaching of eosine is the attack of oxygen on D^f.

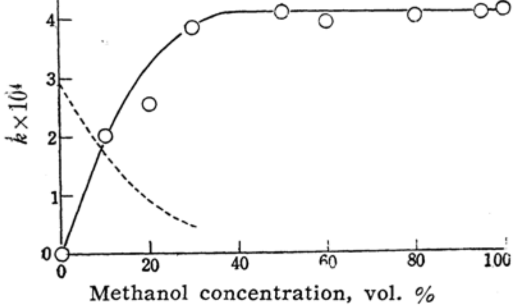

Fig. 2. Dependence of the quantum yield on methanol concentration. 34°C. Full line: in vacuo; Dotted line: aerobic.

On the other hand, quite a different result was obtained with isopropanol. In this case, uranine production was as much as with ethanol, and the difference between the quantum yields in the two pure alcohols in vacuo was not large. Nevertheless, as illustrated in Fig. 3, a prominent maximum in quantum yield was observed at a concentration of about 50 vol. percent isopropanol.

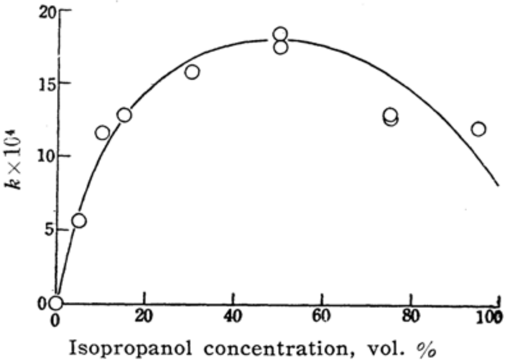

Fig. 3. Dependence of the quantum yield of the vacuum photobleaching on isopropanol concentration. 27°C.

3. Photobleaching in Acetone and Methyl Ethyl Ketone.— As organic solvents other than alcohols, it was attempted to use acetone and methyl ethyl ketone. Solubilities of eosine in such ketones are fairly low compared with those in alcohols. There were, in carrying out experiments with those ketones, some other difficulties since the absorption maximum gradually changes during storage in the dark. For instance, the peak at 534—536 mμ of acetone solution (10^{-5} mol./l. eosine) gradually shifts to the wavelength region of 540—542 mμ and the maximum optical density increases by a small percent after several days in the dark. Though the cause for the phenomenon is not clear, it may be attributed, partially at least, to the presence of oxygen, since the evacuated solution did not show any such displacements, but its absorption maximum was fixed at 540—542 mμ.

In view of the above fact, experiments were done with the solution which was maintained in the dark for a long time to attain the equilibrium condition. Rates of aerobic photobleaching of eosine both in acetone- and in methyl ethyl ketone-water mixtures were expressed well by the simple rate equation[3)]:

$$-\frac{dc}{dt}=1000\,k\frac{I_0}{d}(1-e^{-\alpha c d}). \qquad (2)$$

Quantum yield, k, decreases markedly with increasing concentration of acetone in the low concentration region as seen for alcohols. In the higher concentration region, however, k increases rapidly and reaches a high value, nearly five times that in the pure aqueous solution. This behavior is in sharp contrast with that of alcoholic solutions, in which such an increase in k was not observed but k was zero at 100 percent alcohols.

In methyl-ethyl-ketone, though the experiments between 20 and 95 vol. percent could not be done because of the restriction of its solubility in water, an analogous result was obtained. Though the rate at 100 percent methyl ethyl ketone does not obey eq. (2), the rate appears to be considerably large (Fig. 4).

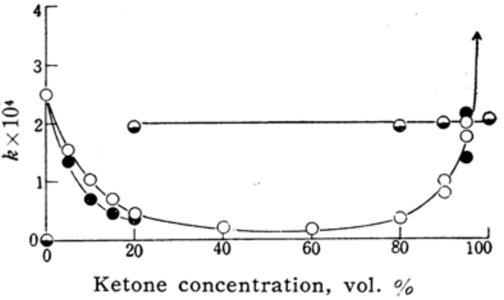

Fig. 4. Dependence of the quantum yield on the ketone concentration. 27°C.

○: acetone, aerobic
●: methyl ethyl ketone, aerobic
◒: acetone, in vacuo

Retarding effects of acetone and methyl ethyl ketone in the low concentration region can be expressed by eq. (1), values of K being listed in Table I.

In the vacuum photobleaching of acetone-water solution, no such spectral displacement as in alcohol-water mixtures[4] was observed and, therefore, uranine production was not realized.

At a concentration of acetone above 20 vol. percent the rate of photobleaching is expressed by eq. (2), while below this concentration deviation from eq. (2) is remarkable. Limiting quantum yield is 2×10^{-4} as illustrated in Fig. 4.

General Discussion

From the results so far obtained, some quantitative discussions will be given of the reaction scheme for the photobleaching of eosine in various conditions. For this purpose, the rate equation for the various cases must be given. It is to be noted that the rate equations proposed in Part I must be corrected in some respects.

1. Rate Equations.—In Part I the following two plausible schemes were proposed for the primary processes in the aerobic photobleaching of eosine in the water-alcohol solution.

(I) First attack of oxygen on the triplet state of dye:

$$D^t+O_2\rightarrow D\cdots O_2,\quad D\cdots O_2+H_2O\cdots\rightarrow\text{products}.$$

(II) First attack of water on the triplet state of dye:

$$D^t+H_2O\rightarrow D\cdots H_2O,\qquad D\cdots H_2O+O_2\cdots\rightarrow\text{products}.$$

The rate equations given for these two cases were, for (I)

$$k=\varphi_{S\rightarrow T}\cdot\frac{k_{O_2}}{k_{qi}+k_1[H_2O]+k_2[RH]+k_{O_2}}\times\frac{k_1'[H_2O]}{k'_{-1}+k_1'[H_2O]+k_2'[RH]} \qquad (3)$$

and for (II)

$$k=\varphi_{S\rightarrow T}\cdot\frac{k_1[H_2O]}{k_{qi}+k_1[H_2O]+k_2[RH]}\times\frac{k'_{O_2}}{k'_{-2}+k_1'[H_2O]+k_2'[RH]+k'_{O_2}} \qquad (4)$$

Both equations can be simplified into eqs. (9) and (10) to fit the experimental results, if $\varphi_{S\rightarrow T}$ is practically constant in various solvents and the following conditions are fulfilled:

$$\text{for (I)}\quad k_{O_2}\gg k_{qi}+k_1[H_2O]+k_2[RH] \qquad (5)$$

$$k'_{-1}\approx 0 \qquad (6)$$

$$\text{for (II)}\quad k'_{O_2}\gg k'_{-2}+k_1'[H_2O]+k_2'[RH] \qquad (7)$$

$$k_{qi}\approx 0 \qquad (8)$$

The conditions (5) and (7) actually hold in view of the experimental fact that the rate does not depend upon the pressure of oxygen under ordinary conditions.

Then from eq. (3)

$$k=\varphi_{S\rightarrow T}\frac{k_1'[H_2O]}{k_1'[H_2O]+k_2'[RH]} \qquad (9)$$

and from eq. (4)

$$k=\varphi_{S\rightarrow T}\frac{k_1[H_2O]}{k_1[H_2O]+k_2[RH]} \qquad (10)$$

If eqs. (9) and (10) were correct, the values of k in the pure aqueous solution should be $\varphi_{S\rightarrow T}$ in both cases. The value of $\varphi_{S\leftrightarrow T}$ is ca. 0.1 according to Oster and Adelman[7], while k is found to be of the order of 10^{-4}. This discrepancy has arisen from the neglect of the following processes. Taking eq. (3) for example, it was assumed in Part I that the attack of oxygen on the

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

triplet state leads solely to the reaction intermediate $D\cdots O_2$, which only gives reaction products by the attack of water. But, in reality, it is very plausible that oxygen may partially deactivate D^t without formation of $D\cdots O_2$ and the similar situation can exist in the attack of water on $D\cdots O_2$.

If one takes these deactivation processes into account, eq. (3) must be rewritten as

$$k=\varphi_{\mathrm{S}\to\mathrm{T}}\frac{k_{\mathrm{O}_2}[\mathrm{O}_2]}{k^s{}_{\mathrm{O}_2}[\mathrm{O}_2]}\cdot\frac{k_1{}'[\mathrm{H_2O}]}{k_1{}'^s[\mathrm{H_2O}]+k_2{}'^s[\mathrm{RH}]} \tag{11}$$

where the superscript *s* represents the summation of the mere deactivation and the effective process leading to the bleaching reaction.

From the similar reasoning, eq. (4) must be corrected as

$$k=\varphi_{\mathrm{S}\to\mathrm{T}}\frac{k_1[\mathrm{H_2O}]}{k_1{}^s[\mathrm{H_2O}]+k_2{}^s[\mathrm{RH}]}\cdot\frac{k'_{\mathrm{O}_2}[\mathrm{O}_2]}{k'^s{}_{\mathrm{O}_2}[\mathrm{O}_2]} \tag{12}$$

For the vacuum photobleaching in the aqueous alcoholic solution, the primary process is

$$\mathrm{D}^t+\mathrm{RH}\rightarrow\mathrm{DH}\cdot+\mathrm{R}\cdot$$

and the specific rate of this reaction gives certainly the rate constant of the overall bleaching reaction.

$$k=\varphi_{\mathrm{S}\to\mathrm{T}}\frac{k_2[\mathrm{RH}]}{k_1{}^s[\mathrm{H_2O}]+k_2{}^s[\mathrm{RH}]} \tag{13}$$

2. Further Confirmation of the Primary Processes in the Aerobic Photobleaching of the Aqueous Eosine Solution.— Though some evidences have already been presented in favor of the scheme I[4)], more definite discussions will be given here analyzing the experimental data on the basis of the rate equations (11), (12) and (13).

Now all the experimental results so far obtained for the aerobic photobleaching are expressed by eq. (1) which holds in the low concentration region of alcohols or ketones. Eqs. (11) and (12) can be written in the form of eq. (1). From eq. (11),

$$\frac{1}{k}=\frac{1}{\varphi_{\mathrm{S}\to\mathrm{T}}}\frac{k^s{}_{\mathrm{O}_2}}{k_{\mathrm{O}_2}}\frac{k_1{}'^s}{k_1{}'}\left(1+\frac{k_2{}'^s[\mathrm{RH}]}{k_1{}'^s[\mathrm{H_2O}]}\right), \tag{14}$$

$$\left.\begin{aligned}k^0&=\varphi_{\mathrm{S}\to\mathrm{T}}\frac{k_{\mathrm{O}_2}}{k^s{}_{\mathrm{O}_2}}\frac{k_1{}'}{k_1{}'^s}\\ K&=\frac{k_2{}'^s}{k_1{}'^s}\frac{1}{k^0}\ \text{or}\ \frac{k_2{}'^s}{k_1{}'^s}=k^0K\end{aligned}\right\} \tag{15}$$

From eq. (12),

$$\frac{1}{k}=\frac{1}{\varphi_{\mathrm{S}\to\mathrm{T}}}\frac{k'^s{}_{\mathrm{O}_2}}{k'_{\mathrm{O}_2}}\frac{k_1{}^s}{k_1}\left(1+\frac{k_2{}^s[\mathrm{RH}]}{k_1{}^s[\mathrm{H_2O}]}\right) \tag{16}$$

$$\left.\begin{aligned}k^0&=\varphi_{\mathrm{S}\to\mathrm{T}}\frac{k'_{\mathrm{O}_2}}{k'^s{}_{\mathrm{O}_2}}\frac{k_1}{k_1{}^s}\\ \frac{k_2{}^s}{k_1{}^s}&=k^0K\end{aligned}\right\} \tag{17}$$

While for the vacuum photobleaching, from eq. (13).

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

$$\text{or}\quad\frac{1}{k}\frac{]\mathrm{RH}]}{[\mathrm{H_2O}]}=\frac{1}{\varphi_{\mathrm{S}\to\mathrm{T}}}\frac{k_1{}^s}{k_2}+\frac{1}{\varphi_{\mathrm{S}\to\mathrm{T}}}\frac{k_2{}^s[\mathrm{RH}]}{k_2[\mathrm{H_2O}]} \tag{18}$$

According to eq. (18), a plot of $[\mathrm{RH}]/k[\mathrm{H_2O}]$ against $[\mathrm{RH}]/[\mathrm{H_2O}]$ should give a straight line of slope $k_2{}^s/\varphi_{\mathrm{S}\to\mathrm{T}}k_2$ and intercept $k_1{}^s/\varphi_{\mathrm{S}\to\mathrm{T}}k_2$; from these two values $k_2{}^s/k_1{}^s$ can be evaluated. An example of such plots is shown in Fig. 5, which is satisfactory at least in the low concentration region of alcohol. In the higher region of the concentration, the deviation from the linearity becomes appreciable, and this deviation will be due to the similar situations for the aerobic photobleaching. Values of $k_2{}^s/k_1{}^s$ thus obtained for methanol, ethanol and isopropanol are summarized in the sixth column of Table I.

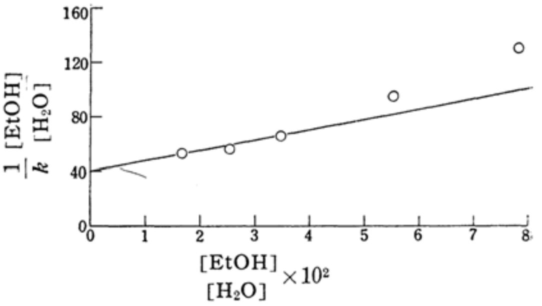

Fig. 5. Variation of the quantum yield of the vacuum photobleaching with ethanol concentration. 27°C. (Eq. (18))

Now if the scheme (II) be a real one, values of k^0K obtained from the aerobic photobleaching ought to be the same as those of $k_2{}^s/k_1{}^s$ obtained from the vacuum photobleaching, while this does not hold for the scheme (I) (see eqs. (15) and (17)). Values of k^0K calculated from the data are listed in the fifth column of Table I.

Comparing k^0K with $k_2{}^s/k_1{}^s$, it is clear that there is a large difference between them, which definitely exceeds the experimental errors. Thus it can be concluded that the primary processes of the photobleaching of eosine in the aerobic aqueous solution proceed via the mechanism (I). The values of k^0K in Table I are therefore interpreted as those of $k_2'^s/k_1'^s$ for the process (I).

Having established the reaction scheme, the values of $\varphi_{S\to T}\dfrac{k_2}{k_2^s}$ and $\varphi_{S\to T}\dfrac{k_2}{k_1^s}$ determined experimentally are tabulated in Table II.

TABLE II

RH	Temp. °C	$\varphi_{S\to T}\dfrac{k_2}{k_2^s}$	$\varphi_{S\to T}\dfrac{k_2}{k_1^s}$
MeOH	34	6.3×10^{-4}	5.6×10^{-3}
EtOH	27	13×10^{-4}	25×10^{-3}
i-PrOH	27	30×10^{-4}	53×10^{-3}

3. Reactivities of D^t and DH· with Oxygen.— In Part I the effect of oxygen pressure on the rate of photobleaching of eosine in the aqueous solution was examined, and it was found that the rate begins to decrease below ca. 10^{-5} mol./l. oxygen. Though the results are not very accurate, it is possible from the data to make a rough estimation about the reactivity of D^t towards oxygen and water. Since alcohol is absent in this case, the rate equation can be written as

$$k=\varphi_{S\to T}\frac{k_{O_2}[O_2]}{k^s{}_{O_2}[O_2]+k_1{}^s[H_2O]}\,\frac{k_1'[H_2O]}{k_1'^s[H_2O]}\,. \tag{19}$$

From eq. (19),

$$\frac{1}{k}\,\frac{[O_2]}{[H_2O]}=\frac{1}{k^0}\,\frac{[O_2]}{[H_2O]}+\frac{1}{k^0}\,\frac{k_1{}^s}{k^s{}_{O_2}}, \tag{20}$$

where $k^0=\varphi_{S\to T}\dfrac{k_{O_2}}{k^s{}_{O_2}}\,\dfrac{k_1'}{k_1'^s}$ is the specific rate when enough oxygen is present. A plot of $[O_2]/k[H_2O]$ against $[O_2]/[H_2O]$ gives a straight line as illustrated in Fig. 6. The values of $\varphi_{S\to T}\dfrac{k_{O_2}}{k^s{}_{O_2}}\,\dfrac{k_1'}{k_1'^s}$ and $\dfrac{k_1{}^s}{k^s{}_{O_2}}$ at 15, 30 and 40°C are summarized in Table III.

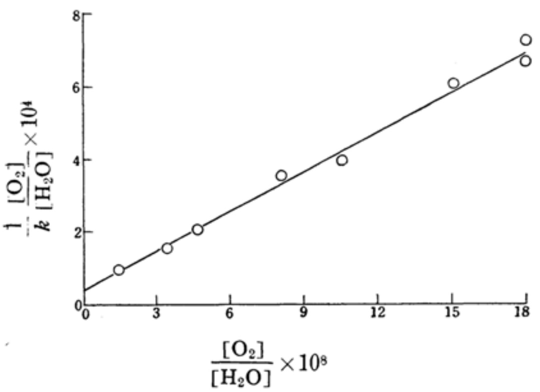

Fig. 6. Variation of the quantum yield with oxygen concentration in aqueous solution. 30°C. (Eq. (20))

TABLE III

Temp. °C	$\varphi_{S\to T}\dfrac{k_{O_2}}{k^s{}_{O_2}}\,\dfrac{k_1'}{k_1'^s}$	$\dfrac{k_1{}^s}{k^s{}_{O_2}}$
15	1.5×10^{-4}	2.1×10^{-8}
30	2.8×10^{-4}	1.3×10^{-8}
40	3.8×10^{-4}	3.4×10^{-8}

From the above results the ratio of the reactivity of D^t with oxygen to that with ethanol can be estimated as follows. Tentatively using the values of $k^s{}_2/k^s{}_1$ at 27°C for ethanol, it may be estimated that

$$\frac{k_2{}^s}{k^s{}_{O_2}}\approx 40\times10^{-8}$$

Hence, it is expected that the quantity of oxygen required for the reaction between D^t and oxygen to overwhelm that between D^t and alcohol, is larger than 10^{-5} mol./l., above which quantity the reaction between D^t and water can be neglected against the reaction between D^t and oxygen. In contrast to this expectation, the experimental result that 10^{-8} mol./l. or less oxygen is enough to inhibit the vacuum photobleaching in pure ethanol³⁾. This is certainly a strong evidence that oxygen in this system acts not on D^t but on DH·. The same conclusion has already been presented on the basis of different arguments in Part II.

4. The Deviation from the Linearity between $1/k$ and $[RH]/[H_2O]$ in the Higher Region of Alcohol Concentration. — In Part I this deviation was attributed to the change of environment around the dye ion. There is another thing, however, to be taken into account. When the concentration of alcohol is high, the term $k^s{}_2[RH]$ cannot be neglected against $k^s{}_{O_2}[O_2]$. The specific rate under this condition must be written as

$$k=\varphi_{S\to T}\frac{k_{O_2}[O_2]}{k^s{}_{O_2}[O_2]+k_2{}^s[RH]}\times\frac{k_1'[H_2O]}{k_1'^s[H_2O]+k_2'^s[RH]}$$

and eq. (14) must be replaced by

$$\frac{1}{k}=\frac{1}{\varphi_{S\to T}}\,\frac{k^s{}_{O_2}}{k_{O_2}}\,\frac{k_1'^s}{k_1'}\left(1+\frac{k_2'^s[RH]}{k_1'^s[H_2O]}\right)\times\left(1+\frac{k_2{}^s[RH]}{k^s{}_{O_2}[O_2]}\right) \tag{21}$$

Though eq. (21) can qualitatively explain the above deviation, it cannot be decided at the present stage what is the principal cause for the deviation.

5. Abnormalities Observed in Acetone and Isopropanol.— The experimental results obtained for the aerobic photobleaching of eosine in the acetone-rich aqueous solution and those for the vacuum photobleaching in isopropanol-water mixture are somewhat anomalous as compared with the results obtained with methanol and ethanol. Generally speaking, however, there is no reason why organic substances in the aqueous solution should behave in the same way irrespective of their concentration. From the experimental results that neither aldehyde nor acid was detected in the aerobic photobleaching of eosine in the aqueous solution containing a small amount of acetone, the retarding effect of acetone might be the mere deactivation of $D\cdots O_2$ in this concentration region, while in the higher region the fate of $D\cdots O_2$ would be quite different. The result that the vacuum photobleaching of eosine in isopropanol-water mixture has its maximum rate at a concentration of about 50 vol. percent alcohol would perhaps be ascribed to the similar change of environment around the dye ion. The elucidation of these phenomena must await further studies.

Summary

Studies on the photoreaction of eosine in the water-alcohols and -ketones have been made both in the presence and in the absence of oxygen. The corrected rate equations, giving the satisfactory explanation for the data obtained, have been presented. Analysis of the experimental results so far obtained, on the basis of these rate equations, has led to the conclusion unambigously that the primary process of the aerobic photobleaching of eosine in the aqueous solution is the attack of oxygen on the triplet state of dye.

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