Irreversible Photobleaching of the Solutions of Fluorescent Dyes. VI. Photobleaching of Uranin in Alkaline Solutions[*]

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Previous papers[1-6] in this series have described the photobleaching of two halogenated xanthene dyes, eosin and erythrosin, in aerated and deaerated solutions. Photochemical primary processes of these dyes both in aqueous and in alcoholic solutions have been concluded. Reactivities of the triplet-excited states toward oxygen and alcohol molecules have also been discussed.

The present paper concerns with the photobleaching reactions of uranin, an unhalogenated xanthene dye, in water and in aqueous alcohols in the presence of alkali. Comparative studies on the photoreduction of uranin and its halogenated derivatives have been done by Adelman and Oster[7]. The present study has been performed under aerated and deaerated conditions, and some new facts have been revealed.

Experimental

Uranin (sodium fluoresceinate). — Ishizu Chemicals guaranteed uranin was employed without further purification. Its purity was stated to be greater than 98.5%. (*Anal.* Found: C, 63.02; H, 2.70. Calcd. for $C_{20}H_{10}O_5Na_2$: C, 63.83; H, 2.67.) Maximum molar extinction coefficient (at λ 490 mμ) was reported by several authors to be 7.85×10^4 [8], 7.82×10^4 [9], 7.62×10^4 [10] and 5.46×10^4(at pH 7)[7]. Under the consideration of a fluorescence error accompanying absorption measurements with Beckman Model DU Spectrophotometer[11-13], real molar extinction coefficient in an alkaline (pH more than 9) aqueous solution was measured to be 7.80×10^4 at $\lambda_{\max}$ 490 mμ.

Other Materials.—Commercial alcohols were purified by the usual methods[14] and were distilled with a fractionating column of a high efficiency. Takeda Chemicals sodium hydroxide (extra pure) and doubly distilled water were used in all mixtures.

Procedures.—Apparatus and technique were essentially identical with those described previously[2]. Matsuda V-B2-photographic Y1 combination, which transmits λ 450~570 mμ with maximum transmission 52% at λ 490 mμ, was used as a filter. Concentration was determined from the optical density at $\lambda_{\max}$ of the solution. Aqueous or aqueous alcoholic solution of uranin exhibits stronger fluorescence than that of eosin or erythrosin. Accordingly, measured optical densities would more or less include the fluorescence error mentioned above. This error was, however, evaluated experimentally to be less than 1.6%, which was within experimental error.

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[*] Presented in part before the 11th Annual Meeting of the Chemical Society of Japan, Tokyo, April 5, 1958.

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

2) M. Imamura, *J. Inst. Polytech. Osaka City Univ.*, **5C**, 85 (1956).

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

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

5) M. Imamura, ibid., **30**, 249 (1957).

6) M. Imamura, ibid., **31**, 62 (1958).

7) A. H. Adelman and G. Oster, *J. Am. Chem. Soc.*, **78**, 3977 (1956).

8) J. Q. Umberger and V. V. LaMer, ibid., **67**, 1107 (1945).

9) V. L. Levschin, *Z. Phys.*, **72**, 368 (1931).

10) W. R. Orndorff, R. C. Gibbs and C. V. Shapiro, *J. Am. Chem. Soc.*, **50**, 819 (1928).

11) K. S. Gibson and H. J. Keegan, *J. Opt. Soc. Am.*, **28**, 180 (1938); E. A. Braude, J. S. Fawcett and C. J. Timmons, *J. Chem. Soc.*, **1950**, 1019.

12) T. C. J. Ovenston, *P. S. G. Bull.*, No. 6, 132 (1953).

13) J. J. Moran and H. I. Stonehill, *J. Chem. Soc.* **1957**, 779.

14) A. Weissberger and E. S. Proskauer, "Organic Solvents", Interscience, New York (1955).

Observed values of optical density were therefore not calibrated except in the cases where higher precise values were needed. Deviation from Beer's law resulting from re-absorption of fluorescence by the solution[13] was not observed at λ's 450, 470, 490 and 500 mμ in the concentration range $0 \sim 10 \times 10^{-6}$ mole/l.

pH of the solution was determined with Horiba Glass Electrode pH-Meter Model P.

Results

Photobleaching of Aerobic Aqueous Solutions.—*Effect of pH.*—Data on the photobleaching of aerated aqueous solutions to which no alkali was added have been reported[1,2]. Absorption spectrum of aqueous uranin solution below pH 7.5 depends sensitively on pH of the solution, while above pH 7.5 it is independent[15] of it. Over the whole pH-range investigated the simple rate formula, which has been applied to the cases of eosin and erythrosin[3], is applicable:

$$-\frac{\mathrm{d}c}{\mathrm{d}t} = \frac{k}{d} I_0 (1 - \mathrm{e}^{-\alpha c d}) \times 10^3 \qquad (1)$$

Integrated form:

$$\alpha c d + \ln(1 - \mathrm{e}^{-\alpha c d}) = -k \alpha I_0 t \cdot 10^3 + [\alpha c_0 d + \ln(1 - \mathrm{e}^{-c \alpha_0 d})] \qquad (2)$$

The mean molar absorption coefficient, α, was determined experimentally with a thermopile or by calculation[4].

Quantum yields, k's, which were obtained with the solutions of various alkali concentrations or pH's are listed in Table I. It shows that the quantum yield is constant at least down to pH 7.17, where the absorption spectrum is affected to a certain extent; at pH 5.9, however, k is reduced considerably.

TABLE I.
QUANTUM YIELDS FOR AEROBIC AQUEOUS URANIN SOLUTION. EFFECT OF pH. 30°C

Mean molar absorption coeff. $\alpha \times 10^{-4}$	NaOH concn. N	pH	Quantum yield $k \times 10^4$
2.7	0.0242		1.6
2.7	〃		2.0
2.7	〃		1.7
2.7	0.0121		1.7
2.7	〃		1.7
2.7	0.0024		1.7
2.7	〃		1.7
2.3	—	7.17*	1.7
1.6	—	5.9*	0.8
1.6	—	5.9*	1.1

* Phosphate (1/15 N) buffer solutions.

Effect of Concentration of Oxygen Dissolved in the Aqueous Solution upon the Quantum Yield.—As already reported[1,2], pure aqueous uranin solution does not photobleach in vacuo and its quantum yield gradually increases with increasing concentration of oxygen dissolved in the solution; with eosin and erythrosin, at a considerably low oxygen concentration, their quantum yields reach the constant values which are equal to those obtained with the aerated solutions. An analogous phenomenon to that in the pure aqueous uranin solution is observed also in the presence of alkali (sodium hydroxide 0.0121 N).

Retarding Effect of Ethanol on the Aerobic Photobleaching.—On addition of ethanol to the aqueous alkaline (sodium hydroxide 0.0121N) solution, photobleaching of uranin is strongly retarded as in the cases of eosin and erythrosin. Rates of reaction for all solutions investigated can be reproduced by Eq. 1 or 2. Since absorption spectra show the shift of $\lambda_{\text{max.}}$ for longer wavelengths with increase in ethanol concentration, α was calculated for each solution.

Quantum yield decreases with increasing concentration of ethanol as shown in Fig. 1. Irradiated solutions do not show any shift of $\lambda_{\text{max.}}$ compared with the initial ones. In the low concentration range of ethanol, $0 \sim 20$ vol. %, the experimental formula[1,2]

$$\frac{1}{k} = \frac{1}{k^0} + K\frac{[\mathrm{EtOH}]}{[\mathrm{H_2O}]} \qquad (3)$$

holds well also for the present case (see Fig. 2). Here, k^0 and k are the quantum yields for aqueous and aqueous ethanolic solutions (ethanol concentration: [EtOH]), respectively. From the slope of the straight line of Fig. 2. one obtains

$$K = 1.0_9 \times 10^6 \quad (30^\circ\mathrm{C}) \qquad (4)$$

then

$$k^0 K = 185 \qquad (5)$$

Photobleaching of Alkaline Solutions in Aqueous Alcohol in Vacuo.—On the contrary to the aerobic system described above, alkaline (sodium hydroxide 0.0121 N) aqueous solution of uranin, in the absence of air, does not photobleach. With increase in ethanol concentration, however,

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[15] In contrast to uranin, absorption spectra of eosin and erythrosin are quite independent of pH at pH above 5.5 and 6.5, respectively.

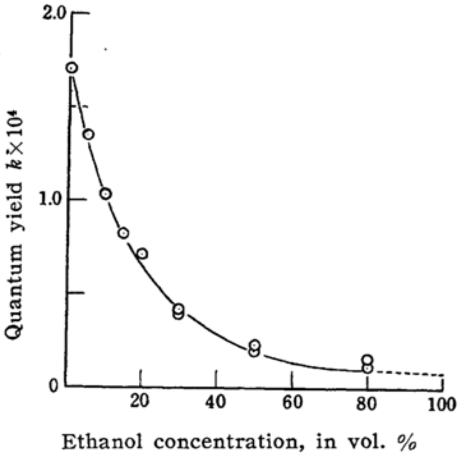

Fig. 1. Variation of k with ethanol concentration. Aerobic, 30°C.

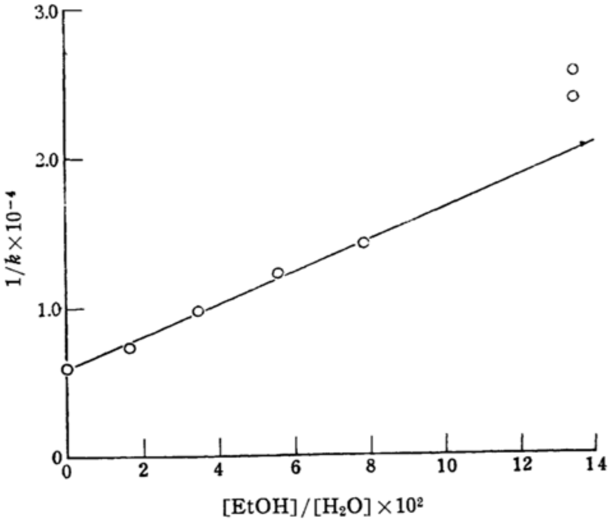

Fig. 2. Dependence of $1/k$ on $[EtOH]/[H_2O]$. (Eq. 3).

the rate of photobleaching becomes appreciable and reaches a constant value after passing through a maximum point, as shown in Fig. 3. An essentially analogous phenomenon is observed in the isopropanol system. In both cases the rates of photobleaching for the solutions of every alcohol concentration are expressed by Eq. 1 or 2.

Similar photobleaching in vacuo to that of alkaline uranin solutions is also observed in the case of eosin or erythrosin. A characteristic feature for the present case is that, at pH above a certain value, photobleaching of uranin is almost reversible. Photobleaching of eosin or erythrosin solution in the absence of alkali and of

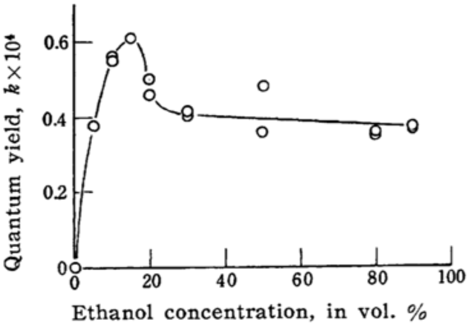

Fig. 3. Variation of the quantum yield with ethanol concentration in vacuo. NaOH, 0.0121 N, 30°C.

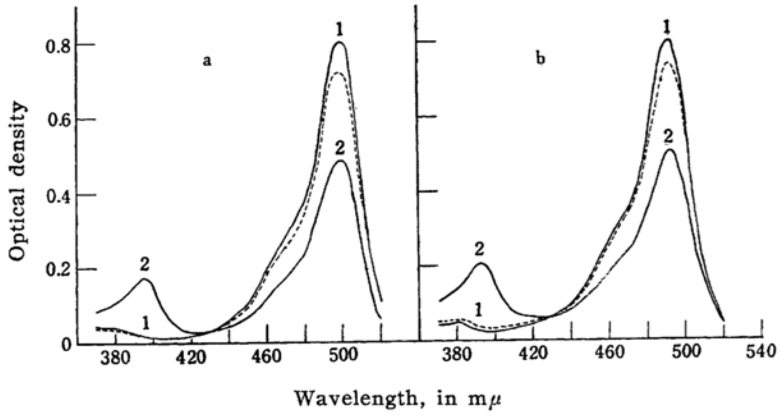

Fig. 4. Spectrum changes on irradiation in vacuo. NaOH, 0.0121 N, 30°C.

a: 80 vol. % aqueous ethanol: 1, initial; 2, 180 min.-irradiation.

b: 10 vol. % aqueous ethanol: 1, initial; 2, 123 min.-irradiation.

uranin solution of a lower alkali-concentration, are, on the other hand, irreversible. A few examples of the spectrum change of uranin resulting from irradiation are illustrated in Figs. 4 and 5. Fig. 4 shows the results obtained with alkaline solutions in aqueous ethanol, and Fig. 5 with an alkaline solution in aqueous isopropanol.

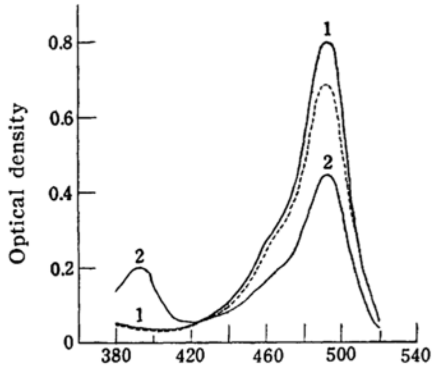

Fig. 5. Spectrum changes on irradiation in vacuo. NaOH, 0.0121 N, 30°C. 20 vol. % aqueous isopropanol: 1, initial; 2, 110 min.-irradiation.

In Figs. 4 and 5 full curves represent absorptions of the solutions unirradiated (1) and those irradiated for a while (2). On introduction of air into the irradiated solutions, their absorption curves 2 are immediately restored to a great extent, which are shown in Figs. 4 and 5 by dotted curves.

A striking feature is that, as shown in Figs. 4 and 5, a new absorption band appears at λ 390~400 mμ by irradiation, which is enhanced with decrease in the optical density at $\lambda_{\max.}$ near 490 mμ, and which disappears immediately by introducing air. A distinct isosbestic point is seen in each figure. A quite analogous but very slow restoration of the absorption curve is realized even in vacuo, when an irradiated solution is kept in the dark for a long time. Since it usually takes 20~40 hours to attain the same degree as air-restoration, the slight restoration during absorption measurement which should result in an error on determining concentration could be neglected.

Degree of air-restoration, which is almost 80~90% of the initial height, is almost independent of the alcohol concentration of the solution, no definite relationship between them being able to be found. Two or more cycles of photobleaching with new band formation and restoration in the dark and in vacuo, are possible without appreciable variation of the individual rates.

As mentioned above, such a reversible photobleaching is predominant in the higher pH range, while, in the lower alkali-concentration range, it is almost irreversible. In the case where irreversible photobleaching predominates, the new band formation is hardly observed at all. In Table II are given the results on the pH-dependency of the photobleaching of the alkaline uranin solutions in 20 vol. % aqueous isopropanol performed at 20°C.

In Table II, D^0 and D_b^V are the optical densities determined at $\lambda_{\max.}$ for the initial and the irradiated (in vacuo 80 min.) solutions, respectively. D_r^V and D_r^A are for the solutions which are, after irradiation, kept in vacuo and in the dark for 60 min., and for the solutions into which air is introduced (determined immediately after air-introduction), respectively. $\Delta D^V{}_{b395}$ is an increase in optical density at λ 395 mμ by irradiation. All the values of optical density correspond to the 10 mm-path length.

TABLE II

pH-DEPENDENCY OF THE PHOTOBLEACHING OF THE SOLUTIONS IN 20 VOL. % ISOPROPANOL IN VACUO, AT 20°C

$\alpha=2.7\times10^4$ $\lambda_{\max.}=492$ mμ

pH	D^0	D_b^V (80 min.)	D_r^V (60 min.)	D_r^A	$\frac{D_r^A-D_b^V}{D^0-D_b^V}$	$\frac{D_r^V-D_b^V}{D_r^A-D_b^V}$	$\frac{\Delta D^V{}_{b395}}{D_r^A-D_b^V}$	$k\times10^4$
9.24	0.812	0.500	0.538	0.721	0.708	0.172	0.629	0.57
8.70	.822	.536	.565	.768	.812	.125	.647	.57
8.34	.822	.532	.560	.770	.821	.118	.643	.51
8.00	.826	.545	.607	.785	.854	.258	.613	.54
7.92	.822	.542	.610	.753	.755	.322	.697	.52
7.42	.830	.615	.680	.692	.358	.845	.695	.31
7.30	.820	.645	.700	.702	.326	.965	.175	.27
7.04	.815	.600	.655	.660	.279	.834	.083	.31

Thus, $(D_r^A - D_b^V)/(D^0 - D_b^V)$ represents a fraction of the amount of an intermediate which can be restored by air to that of photobleached dye. It is seen that this fraction increases with increase in pH, having its highest value 0.8~0.9. On the other hand, $(D_r^V - D_b^V)/(D_r^A - D_b^V)$ may give a measure for the stability of the reversible intermediate in vacuo. This value increases with decrease in pH-value, indicating that the reversible intermediate, in vacuo, is relatively unstable at low pH.

In the last column of Table II are given the quantum yields, which may include slight errors resulting from a back reaction. Since the time required for determining optical densities of the irradiated solution is as short as possible, the errors from the dark back reaction may, even in a low pH solution, be considerably small or negligible. Essentially analogous results have been obtained with the solutions in 50 vol. % ethanol in vacuo at 20°C. It is presumable, from these data, that the completely reversible photobleaching would give a quantum yield which is twice that of the completely irreversible one. The molar extinction coefficient of the newly formed intermediate can be estimated from the limiting value of $\Delta D^V_{b395}/(D_r^A - D_b^V)$ to be 6.5×10^4 at λ 395 mμ in alkaline aqueous ethanol and isopropanol.

Discussion

Photobleaching of the Alkaline Solutions in Aqueous Alcohol in Vacuo.—As in the case of uranin at a lower pH, alcoholic eosin and erythrosin solutions into which no alkali is added show an irreversible photobleaching in vacuo[3-6]. It has been concluded experimentally that the primary process in these systems is the dehydrogenation from alcohol molecules by the excited (in the triplet state) dyes. No distinct absorption in the near ultraviolet region as the one observed for the irradiated uranin solutions at higher pH is detected. It has been assumed therefore that the succeeding process is the formation of leuco dye DH_2[16], which have no distinct absorption in near ultraviolet and may decompose into irreversible products under the experimental conditions.

Quite a different result obtained with alcoholic uranin solutions at higher pH in vacuo may be interpreted as assuming the formation of a carbinol base. It is known that triphenylmethane dyes, such as crystalviolet, form their carbinol bases in the presence of alkali[17]. Though it is not the case for xanthene dyes, it may probably be assumed that the presence of sufficient alkali in the reductive condition is preferable to form a carbinol base.

Carbinol bases should reverse to the original dyes not only by oxidation with oxygen (or air) but also by spontaneous separation of water from them. Their stabilities in vacuo and in the dark may probably be more or less higher at higher pH than at lower pH. These expectations are all realized in the present experimental results summarized in Table II, at least qualitatively.

Next, it is noted that the quantum yield at a higher pH is nearly twice those at lower pH. At lower pH, uranin photobleaches preferentially in an irreversible fashion. The situation will be the same as those for eosin and erythrosin, i.e.,

$$D^t + RH \rightarrow DH\cdot + R\cdot \qquad \text{(i)}$$

$$DH\cdot + DH\cdot \rightarrow DH_2 + D \qquad \text{(ii)}$$

$$DH_2 \rightarrow \text{Products.} \qquad \text{(iii)}$$

On the other hand, in the case of higher pH solutions, where the formation of carbinol base is assumed to occur as mentioned above, the succeeding process followed i should be quite a different one:

$$DH\cdot \xrightarrow{OH^-} DH(OH) \qquad \text{(iv)}$$

Here DH(OH) represents a carbinol base. From the above schemes it is shown that, in the former case, of one mole of DH· formed by i, one-half should return to the original dye by the disproportionation reaction ii, whereas, in the latter where the reaction ii is almost negligible, such a regeneration of a dye, on neglect of a slow dark back reaction, does not occur. Thus, for limiting conditions of these two cases, it is apparent that the quantum yield of the latter case may reach a value close to twice the value found in the former. Experimental data are, therefore, considered as good evidence for the formation of carbinol base of uranin at higher pH, and it follows therefrom that the appearence of a new absorption at λ 390~395 mμ may be due to this formation.

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16) Leuco forms of these dyes are obtained by reduction of their alkaline solutions with zinc powder, which exhibit no absorption in ultraviolet region.

17) a) L. Gattermann, "Die Praxis des organischen Chemikers", Walter de Gruyter, Berlin (1940), p. 328. b) K. Venkataraman, "The Chemistry of Synthetic Dyes", Vol. II, (1952), pp. 705—754.

Primary Process in Alcoholic Solutions and the Reactivity of the Excited Uranin toward Alcohol. — It is clear from the above discussion that the primary process conducted in the deaerated alcoholic solutions should, independently of pH, be given by mechanism i. Excited uranin is assumed to be in the triplet state, in analogy with the cases of eosin and erythrosin. So far as semileuco-dye DH· is assumed to be colorless, the specific rate (quantum yield) of the photobleaching of the deaerated alcoholic solutions is given as

$$k=\varphi_{S\to T}\frac{k_2[\mathrm{RH}]}{k_1{}^S[\mathrm{H_2O}]+k_2{}^S[\mathrm{RH}]} \qquad (6)$$

Here, transition probability $\varphi_{S\to T}$ for uranin without substituted halogen atoms may be considered to be lower than those for eosin and erythrosin with substituted halogen atoms[18]. From Eq. 6 one obtains

$$\frac{1}{k}\frac{[\mathrm{RH}]}{[\mathrm{H_2O}]}=\frac{1}{\varphi_{S\to T}}\frac{k_1{}^S}{k_2}+\frac{1}{\varphi_{S\to T}}\frac{k_2{}^S[\mathrm{RH}]}{k_2[\mathrm{H_2O}]} \qquad (7)$$

Plotting $[\mathrm{RH}]/k[\mathrm{H_2O}]$ against $[\mathrm{RH}]/[\mathrm{H_2O}]$ using the results obtained with the alkaline (sodium hydroxide 0.0121 N) solutions in aqueous ethanol at 30°C, one obtains a straight line in the relatively low alcohol concentration region, as shown in Fig. 6.

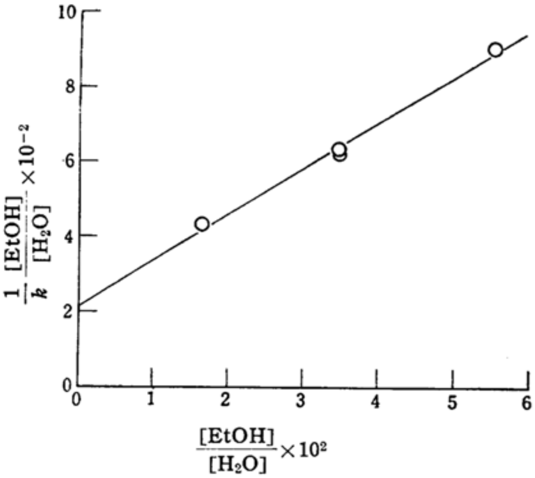

Fig. 6. Dependence of $[\mathrm{EtOH}]/k[\mathrm{H_2O}]$ on $[\mathrm{EtOH}]/[\mathrm{H_2O}]$ in vacuo, 30°C. (Eq. 7).

From the slope and the intercept of this straight line, respectively,

$$\varphi_{S\to T}\frac{k_2}{k_2{}^S}=0.81\times10^{-4} \qquad (8)$$

and

$$\varphi_{S\to T}\frac{k_2}{k_1{}^S}=4.7\times10^{-3} \qquad (9)$$

and from these two values

$$\frac{k_2{}^S}{k_1{}^S}=57 \qquad (10)$$

The last value gives a ratio of the interaction probability of $\mathrm{D^t}$ with alcohol to that with water molecules.

Primary Process in Aerobic Aqueous Solution and the Reactivity of the Excited Uranin toward Oxygen.—As to the primary process in the aerobic aqueous solutions, the following two possible mechanisms have been suggested[1,2]:

$$\mathrm{D^t+O_2\to D\cdots O_2\xrightarrow{H_2O}\cdots Prod.}$$

$$\mathrm{D^t+H_2O\to D\cdots HOH\xrightarrow{O_2}\cdots Prod.}$$

On the kinetic ground, it has been concluded[5] that, if the former mechanism is a correct one,

$$\frac{k_2{}'^S}{k_1{}'^S}=k^0K \qquad (11)$$

on the other hand, if the latter is a correct one,

$$\frac{k_2{}^S}{k_1{}^S}=k^0K \qquad (12)$$

Though it is difficult to obtain the value of $k_2{}'^S/k_1{}'^S$ experimentally in the present investigation, comparison of the value of k^0K (5) obtained in the aerobic systems with that of $k_2{}^S/k_1{}^S$ (10) in the deaerated systems leads to the conclusion that the primary process of the excited uranin in the aerobic aqueous solutions is expressed by the former mechanism, as in the cases of eosin and erythrosin[5,6].

According to the conclusion obtained above, the specific rate (quantum yield) for the photobleaching of the aqueous solutions should be given as

$$k=\varphi_{S\to T}\frac{k_{\mathrm{O_2}}[\mathrm{O_2}]}{k^S{}_{\mathrm{O_2}}[\mathrm{O_2}]+k_1{}^S[\mathrm{H_2O}]}\frac{k_1{}'[\mathrm{H_2O}]}{k_1{}'^S[\mathrm{H_2O}]} \qquad (13)$$

The fact that the quantum yields of photobleaching of eosin and erythrosin solutions reach, already at the relatively low oxygen concentrations, the corresponding limiting values which are equal to those for the aerobic solutions, indicates that, in Eq. 13 $k_1{}^S[\mathrm{H_2O}]$ can be neglected against $k_{\mathrm{O_2}}{}^S[\mathrm{O_2}]$, above a relatively low oxygen concentration, i. e.,

$$k_{\mathrm{O_2}}{}^S[\mathrm{O_2}]\gg k_1{}^S[\mathrm{H_2O}] \qquad (14)$$

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[18] D. S. McClure, N. W. Blake and P. L. Hanst, *J. Chem. Phys.*, **22**, 255 (1954).

In the case of uranin, however, such a limiting value is not attained, indicating that, even under the atmospheric air, neglect of $k_1{}^S[H_2O]$ is unreasonable for the present case. Then, from Eq. 13, one obtains

$$\frac{1}{k}\frac{[O_2]}{[H_2O]}=\frac{k_1{}'^S}{\varphi_{S\to T}k_1{}'}\frac{k_1{}^S}{k_{O_2}}+\frac{k_1{}'^S}{\varphi_{S\to T}k_1{}'}\frac{k^S_{O_2}}{k_{O_2}}\frac{[O_2]}{[H_2O]} \qquad (15)$$

Application of Eq. 15 to the results previously obtained with the aqueous uranin solutions at 30°C is illustrated in Fig. 7.

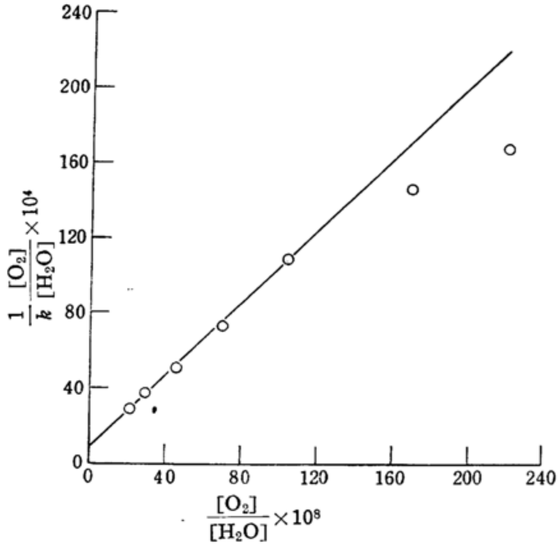

Fig. 7. Dependence of $[O_2]/k[H_2O]$ on $[O_2]/[H_2O]$, 30°C (Eq. 15).

It is shown in Fig. 7 that a linear relation is realized at the oxygen concentration up to 58×10^{-6} mole/l. The intercept and the slope of the line yield, respectively,

$$\frac{k_1{}'^S k_1{}^S}{\varphi_{S\to T}k_1{}'k_{O_2}}=9\times10^{-4} \qquad (16)$$

and

$$\frac{k_1{}'^S k^S_{O_2}}{\varphi_{S\to T}k_1{}'k_{O_2}}=0.96\times10^4 \qquad (17)$$

From these values, it follows that

$$\frac{k^S_{O_2}}{k_1{}^S}=1\times10^7 \qquad (18)$$

which gives the reactivity of D^t toward oxygen relative to that toward water molecules.

If a sufficient amount of oxygen is dissolved in the solution to justify the relation 14, the quantum yield should be given as the reciprocal of the relation 17:

$$\varphi_{S\to T}\frac{k_1{}'k_{O_2}}{k_1{}'^S k^S_{O_2}}=1.0\times10^{-4} \qquad (19)$$

Under the atmospheric air, aqueous uranin solution gives a rather higher value (1.3×10^{-4}, at 30°C) than that from Eq. 19. As to the nature of this enhancing effect of oxygen at higher concentrations, it is noteworthy to mind the recent Evans' work[19] on the perturbation of $S\to T$ transitions of organic molecules by oxygen. For eosin and erythrosin such an enhancing effect of oxyen is, at least under the atmospheric air, not observed, and, moreover, the quantum yields at the sufficient oxygen concentrations are quite consistent with those obtained from the slopes of the corresponding plots of Eq. 15. According to the Evans' results, these halogenated dyes may be affected by oxygen far less than the unhalogenated one is. In the case of uranin, the transition probability, $\varphi_{S\to T}$, should depend on oxygen pressure, resulting in an increase in quantum yield with increasing oxygen concentration. This is still in speculation; more quantitative studies must be performed to throw light on this problem.

Comparison between the Reactivities of Excited Xanthene Dyes toward Oxygen and Alcohol.—Reactive species of three xanthene dyes so far investigated are in the excited triplet states, and their primary processes are concluded to be the formation of labile complexes with oxygen molecules in the aerobic aqueous solutions, and to be the dehydrogenation from alcohol molecules in the alcoholic solutions. Relative reactivities of excited eosin and erythrosin have been reported previously[5,6]. In Table III are summarized the relative reactivities of these dyes.

TABLE III

RELATIVE REACTIVITIES OF EOSIN, ERYTHROSIN AND URANIN

	Eosin	Erythrosin	Uranin
$\frac{k_2{}^S}{k_1{}^S}$ (ethanol)	19 (27°C)	3 (27°C)	57 (30°C)
$\frac{k^S_{O_2}}{k_1{}^S}\times10^7$	7.7 (30°C)	1.3 (30°C)	1.0 (30°C)

The values in Table III are reactivities toward ethanol and oxygen (dissolved) relative to those toward water molecules. Though it is difficult to compare exactly their reactivities in absence of the numerical values of $k_1{}^S$, it appears possible

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[19] D. F. Evans, *J. Chem. Soc.*, **1957**, 1351; *Nature*, **178**, 534 (1956).

to say that, in general, halogenated dyes are more reactive toward oxygen than the unhalogenated ones, while toward alcohol vice versa. There seems to be a close relation between such facts and the characteristic pH-dependency of the absorption spectra mentioned before[15].

Summary

Investigation of the photobleaching of uranin solutions has led to the same conclusions as for eosin and erythrosin, concerning the primary processes both in aqueous and in alcoholic media. Strongly alkaline solutions of uranin in aqueous alcohol show an irreversible photobleaching in vacuo. On the reactivities of the excited uranin, and on the comparison of them with those of two other xanthene dyes previously investigated, some discussions have been given.

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