Irreversible Photobleaching of the Solution of Fluorescent Dyes. III. Photoreaction of Eosine in the Aqueous Alcoholic Solutions under Evacuated State

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Introduction

In the preceding paper¹⁾, the vacuum photobleaching of eosine in pure methanol, ethanol and isopropanol was investigated, and it was found that the absorption spectra decline steadily without any shift and that the rate depends only on the light absorbed.

Now as a natural extension of our research, the effect of water added on the vacuum photobleaching was examined, and a remarkable result was obtained; this was that the addition of water does cause a gradual displacement of the absorption spectra towards shorter wavelengths in the course of reaction and at the same time the solution becomes more and more fluorescent. The reaction is completely irreversible and the strongly fluo-

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1) M. Imamura and M. Koizumi, This Bulletin, 29, 900 (1956).

rescent product is very stable. Analyzing the absorption spectra carefully, this ultimate product was definitely confirmed to be uranine.

Kinetic studies were also undertaken, and from the results obtained, a conclusion was given as to the primary process of the aerobic photobleaching of eosine in the aqueous solution, which had remained undecided in the first paper²⁾ of this series.

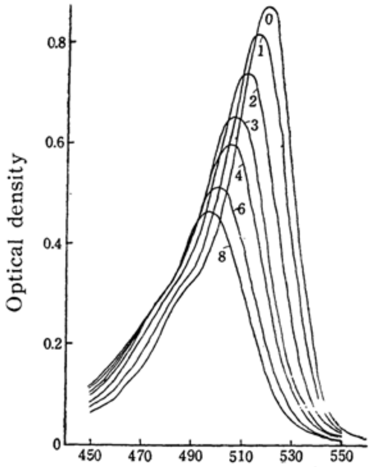
Experimental Procedures and Results

1. Photobleaching in the Aqueous Alcoholic Solutions.—Materials were the same as before¹,²⁾. Concentrated aqueous solution of eosine was diluted with water and ethanol to a desired concentration of alcohol, the resulting concentration of eosine being kept constant as 10^{-5} mol./l.

Evacuation was done under repeated cooling with dry ice and melting for several times with the use of a mercury diffusion pump. Reaction was carried out in a cylindrical glass cell to which a cuvett (path length: 1 cm.) for Beckman spectrophotometer was attached. Evacuated samples were irradiated with a tungsten lamp and Matsuda color filter VG1 as before.

At some minutes' intervals the cell was taken out of a thermostat and the absorption spectra were measured by Beckman spectrophotometer model DU.

An example of a series of measurements is shown in Fig. 1. From Fig. 1, it is seen that the optical density gradually decreases and, at the same time, absorption maximum is displaced towards shorter wavelengths; the irradiated solution becomes more greenly fluorescent than the initial solution.



Wavelength, mμ

Fig. 1. Absorption spectra of the irradiated eosine in 10 vol. % EtOH solution in vacuo.

No.'s depicted indicate the order of measurement.

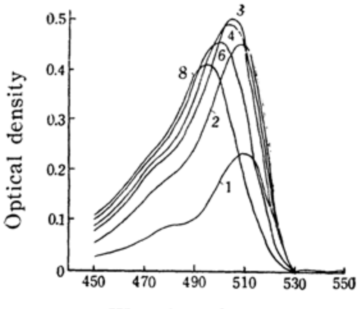
2. Analysis of the Absorption Spectra.—Although a gradual shift of the absorption maximum was observed during the reaction, its limiting position in every case was in the neighborhood of 490 mμ. Since the above results strongly suggest the formation of a coloring product from eosine, identification of the product was attempted from the analysis of absorption spectra. The method is essentially the same as reported by Blaisdell³⁾.

At various wavelengths, the ratio of the optical density of each irradiated solution to that of the initial solution was calculated, and the value of the minimum ratio was decided. For all the curves tried, the minimum points fell alike between 530 and 540 mμ. Minimum ratios thus obtained may be considered as relative measures of the concentration of eosine remaining.

Multiplying these values to the optical densities of the initial solution at each wavelength, the absorption curves could be drawn which may approximately be considered as the curves of eosine remaining in solution at each time.

Substracting these curves from the absorption curves of the original solutions, one can get the curves which may be attributed to intermediate products.

An example of such curves obtained is given in Fig. 2. It is seen from Fig. 2 that with decreasing concentration of eosine, optical density of the products increases moderately at the beginning, then more and more slowly, and at last begins to decrease very slowly. At the same time the position of the maximum is gradually displaced towards 490 mμ, where the displacement comes to a halt.



Wavelength, mμ

Fig. 2. Absorption spectra of the intermediates produced in 10 vol. % EtOH solution in vacuo.

No.'s depicted indicate the order of measurement. (cf. Fig. 1).

3. Identification of an Ultimate Intermediate Product.—The coloring product intermediately produced by the irradiation of the aqueous alcoholic solution of eosine has a strong green fluorescence and has its absorption maximum near 490 mμ as mentioned above.

From these facts, it seemed very plausible to suspect that this product would be fluoresceine-Na.

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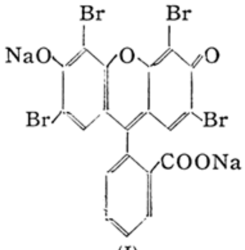
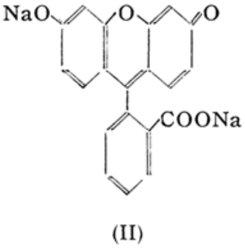
2) M. Imamura and M. Koizumi, This Bulletin, 28, 117 (1955).

3) B. E. Blaisdell, *J. Soc. Dyers & Colourists*, 65, 619 (1949).

(uranine) (II) which is a debrominated product of eosine (I).



(I)



(II)

To confirm this conjecture, the following methods were adopted.

(*a*) *Comparison of Absorption Spectra.*—If the coloring material were uranine as supposed, then the separated absorption curves in the final stage of reaction should be the same as that of uranine, or of uranine which is partly photobleached in vacuo in the same aqueous alcoholic solution. Uranine in alcoholic or in aqueous alcoholic solution also decolorizes in vacuo, but no shift of spectrum such as for eosine is observed.

The comparison of the separated curves with that of uranine partly photobleached in vacuo is shown in Fig. 3. In Fig. 3 suitable normalization is done to make all the curves coincide at 495 mμ.

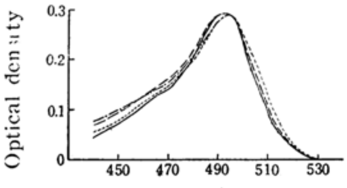


Fig. 3. Comparison of the absorption spectra.

———: Uranine partly photobleached in 10 vol. % EtOH solution;
- - - -: Separated curve for 5 vol. % EtOH solution;
·········: For 10 vol. % EtOH solution;
·—·—: For 20 vol. % EtOH solution;
(normalized at 495 mμ)

Absorption curves of an ultimate intermediate were measured in vacuo for the solutions in which the concentrations of alcohol were 5, 10 and 20 vol. per cent while the uranine partly photobleached was also measured in vacuo for 10 vol. per cent alcoholic solution. Agreement of all curves is satisfactory in view of the approximate nature of the analytical procedures.

(*b*) *Bromination.*—It is known that fluoresceine can easily be brominated by bromine into tetrabromofluoresceine[4,5]. In fact, uranine in aqueous alcoholic solution could be converted to eosine by adding few amounts of bromine and of sodium hydroxide. Hence similar bromination was applied to the 10 vol. per cent alcoholic solution in which eosine had almost completely disappeared in vacuo and the products were examined spectroscopically. The result is illustrated in Fig. 4.

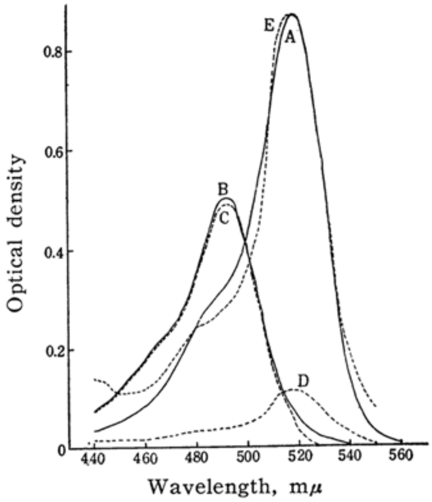


Fig. 4. Bromination of the intermediate. See sec. 3-b of procedures and results.

Curve A in Fig. 4 is an initial absorption curve of eosine in 10 vol. per cent alcoholic solution, B is the one after almost complete photobleaching of eosine in vacuo. Applying the analytical method mentioned in Sec. 2 to curve B, the resulting curve for intermediate is C. One drop of bromine and of concentrated sodium hydroxide solution were added to the irradiated solution of 10 ml.; then yellow color with strong green fluorescence instantly became reddish with weak fluorescence. Curve D indicates this color change. Levelling the curve D up to A at 520 mμ, the resultant is E.

If the intermediate, which is supposed to be uranine (curve C), be converted quantitatively to eosine by bromination, the maximum optical density of the curve D must be higher than the maximum optical density of the curve C*. The reason why the curve D is lower than the curve C in the present case, may plausibly be attributed to the simultaneous oxidative decomposition of uranine by bromine. When compared with the curve A, there scarcely remains any doubt that the curve E represents the absorption curve of eosine, although in the shorter wavelength region discrepancy is appreciable; this discrepancy may be due to the decomposition products.

(*c*) *Photobleaching of Uranine in Vacuo.*—When uranine in aqueous alcoholic solution was irradiated in vacuo under the same conditions as for eosine, it bleached in the same way but with a very slow rate and without any shift of its spectrum.

This behavior is qualitatively similar to that of the ultimate intermediate in eosine solution which was shown in Fig. 2.

From the facts mentioned above it can be concluded that the intermediate produced in the course of photobleaching reaction of eosine in aqueous alcoholic solution in vacuo is the debrominated eosine, that is, uranine.

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* Maximum molar extinction coefficient of uranine is lower than that of eosine in aqueous or alcoholic solution.

[4] K. Venkataraman, "The Chemistry of Synthetic Dyes", (1952) II, p. 747.

[5] G. Schultz, "Farbstofftabellen", Leipzig (1931), I-2, p. 374.

4. Rate of the Conversion of Eosine.—The real rate of disappearance of eosine can be found by the method described in Sec. 2. It seems very natural to expect that the rate thus obtained be expressed, if I_{abs} is the light absorbed and is expressed in mol. per 1. per sec., by the following equation:

$$-\frac{\mathrm{d}c}{\mathrm{d}t}=kI_{\text{abs}}, \tag{1}$$

since, as reported already[1], the photobleaching of eosine both in aerobic aqueous solution and in deaerated alcoholic solution obeys the following formula which is only a different form of Eq. (1).

$$-\frac{\mathrm{d}c}{\mathrm{d}t}=k\frac{I_0}{d}(1-e^{-\alpha cd})\times 1000. \tag{2}$$

It is to be noted, however, that in the present case there are more than two light absorbing species, eosine and intermediates, because the transmission of the filter covered the main absorption regions of them all. Hence, I_{abs} in Eq. (1) must be put as the portion of light actually absorbed by eosine, and this must be calculated as an integrated sum over the necessary wavelength regions, since the inner filter effect of the intermediates depends on the wavelength λ. Taking into account this fact, Eq. (1) is to be written as follows:

$$-\frac{\mathrm{d}c_1}{\mathrm{d}t}=1000k\int_{\lambda_1}^{\lambda_2}I_{0\lambda}\frac{\varepsilon_{1\lambda}c_1}{D_\lambda}(1-10^{-D_\lambda})\mathrm{d}\lambda, \tag{3}$$

where $\varepsilon_{1\lambda}$, D_λ and $I_{0\lambda}$ are the molar extinction coefficient of eosine, the optical density of the solution and the incident light intensity all at λ, respectively. In making the computation of Eq. (3), values of $I_{0\lambda}$'s are necessary and these can be given as follows:

$$I_{0\lambda}=L_\lambda T_{f\lambda}=L_{\lambda\max}T_{f\lambda}f_\lambda, \tag{4}$$

where,

L_λ: the light intensity per unit band of wavelength for a light source at λ,

$T_{f\lambda}$: transmission of the filter at λ.

f_λ: the intensity distribution fraction of the light source at λ.

Then I_0, the light intensity, is expressed as follows

$$I_0=\int_{\lambda_1}^{\lambda_2}I_{0\lambda}\mathrm{d}\lambda=\int_{\lambda_1}^{\lambda_2}L_\lambda T_{f\lambda}\mathrm{d}\lambda=L_{\lambda\max}\int_{\lambda_1}^{\lambda_2}f_\lambda T_{f\lambda}\mathrm{d}\lambda. \tag{5}$$

From Eqs. (3), (4) and (5),

$$-\frac{\mathrm{d}c_1}{\mathrm{d}t}=1000kI_0\frac{\int_{\lambda_1}^{\lambda_2}f_\lambda T_{f\lambda}\frac{\varepsilon_{1\lambda}c_1}{D_\lambda}(1-10^{-D_\lambda})\mathrm{d}\lambda}{\int_{\lambda_1}^{\lambda_2}f_\lambda Tf_\lambda\mathrm{d}\lambda} \tag{6}$$

$$=1000kI_0f_{\text{abs}}.$$

f_λ's were calculated assuming that the temperature of the light source was 2500°K,[6] which gave just the same value of the mean molar absorption coefficient, α, of pure eosine as that determined experimentally by a thermopile.

The values of f_{abs} were calculated at several points of each run using the value of D_λ of the solution. The rate of the disappearance of eosine at the corresponding time-point was determined directly from the concentration-time curve, and dividing this by $1000I_0f_{\text{abs}}$, one could get the value of k. Values of k, which ought to be constant if Eq. (1) holds, are plotted against f_{abs}. Some examples are shown in Fig. 5. The constancy of k does not hold except for the solution in which alcohol concentration is high. In general, k decreases more and more as the reaction proceeds, in other words, as the quantity of coloring intermediate increases. But in the earlier stage of the reaction k does not change practically.

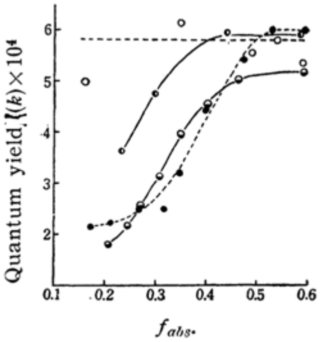

Fig. 5. Variation of the quantum yield with f_{abs}.

◒: 10 vol. %; ●: 20 vol. %;
◐: 50 vol. %; ○: 90 vol. %.

Although the above calculations are not altogether free from any ambiguity, there is no doubt about the general tendency that k-values decrease with the accumulation of the intermediate. Hence it was attempted to make clear some reasons for this behavior.

Supposedly Eq. (1) holds in the present case. One possible reason for this decrease might be the non-homogeneity of the irradiated solution; under the condition of non-stirring, intermediate coloring substances will become concentrated in the front part of the solution and this may cause a larger filter effect than that under the homogeneous condition which was assumed in the above calculation.

If this be the case, it should be possible to make this effect smaller with proper choice of experimental conditions. Some experiments were conducted for this purpose. Thus the initial concentration of eosine selected was half that of the ordinary one using another cell of 10 mm. path length (5 mm. shorter than ordinary cells) and the measurements of absorption spectra were made at shorter intervals. But the result is that there was no noticeable difference.

Another conceivable reason is some sort of retarding effect of the reaction products on the rate of photobleaching of eosine. The following experiments were conducted to examine this possibility. A definite amount of uranine was added previously to the pure ethanol solution of eosine and the rate of the photobleaching of the latter was measured by the usual method. It was definitely established by several experiments that

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[6] A. N. Lowan and G. Blanch, *J. Opt. Soc. Am.*, **30**, 79 (1940).

the addition of uranine causes a marked retarding effect and that the effect increases greatly with the increase of added uranine. Some results are shown in Table I, where k values are calculated by the method analogous to that stated above, taking the filter effect of uranine into account.

TABLE I

QUANTUM YIELDS FOR THE VACUUM PHOTOBLEACHING OF EOSINE IN EtOH IN THE PRESENCE OF URANINE

$I_0=1.4\times10^{16}h\bar{\nu}/\text{cm}^2.\ \text{sec.}\ 27^\circ\text{C}$

Conc. $\times10^6$ mol./l.		Quantum Yield, $k\times10^4$
Eosine	Uranine	
10.0	—	6.0
10.0	2.5	3.4
10.0	5.0	1.0
10.0	10.0	0.5

From the above tests it is certain that the decrease of k is mainly due to the retarding effect of intermediates including uranine, although it is impossible, in the present stage of investigation, to interpret the matter quantitatively on the basis of the above data, in view of the facts that the experimental conditions of the above tests differ from those in the ethanol-water solutions which contain some other unknown intermediate products preceding final uranine production.

Thus the k-value extrapolated to $t=0$ can be accepted as a true one. Plotting the quantum yield thus obtained, against the concentration of ethanol, a curve is obtained which is shown in Fig. 6. As seen from Fig. 6, the quantum yield increases with increasing concentration of ethanol up to ca. 20 vol. per cent and then it becomes constant, the value being the same as that obtained in pure ethanol in vacuo.

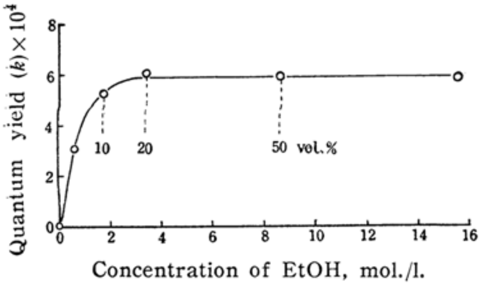

Fig. 6. Variation of the quantum yield with the concentration of EtOH.

Discussion

1. First the reliability of the evaluation of k-values and of the interpretation of the absorption spectra of intermediates will be discussed briefly. There is some suspicion that Blaisdell's method by which the minimum ratio of the optical density of the irradiated solution to that of the original one was taken for a measure of the concentration of the remaining eosine, might have given a smaller value than the real one, in view of the fact that the absorption curve of the intermediate is superposed on that of eosine in the whole range of wavelengths. But the superposition is negligible in the longer wavelength region above 530 mμ and the minimum ratio is exactly between 530 and 540 mμ without a single exception. Furthermore, the k-values in Fig. 6 are obtained from the initial rate which is scarcely affected by the reaction products. From these facts it is considered that the evaluation of k-values is very reliable.

As to the absorption spectra of the intermediates, there is no doubt that they are related only to the reaction products which are specific in the water-alcohol mixture. For, although there is another type of reaction which takes place in pure alcohol, the product of this reaction gives only a very small absorption band near ultraviolet and this band can not affect the present band even if the reaction of the latter type participates in wateralcohol mixture. Hence, the interpretation of the intermediates is considered to be fairly trustworthy.

2. From the fact that the quantum yield, k, is independent of the concentration of ethanol above 20 vol. per cent, it can be concluded that the primary processes in water-alcohol mixture are just the same as those in pure alcohol. They are

$$\text{D} \xrightarrow{h\nu} \text{D}^* \longrightarrow \text{D}^t \tag{i}$$

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

where D^t is eosine in the triplet state, RH is alcohol, and DH· is a colorless or almost colorless semiquinoid radical. This conclusion is further supported by the following preliminary experiments. Thus, similar experiments using methanol- and isopropanol-water mixtures were attempted and analogous spectral shifts due to the production of coloring intermediates were observed, the extent of which was less in methanol and was nearly equal or a little greater in isopropanol than in ethanol. This order is exactly the same as for the rate of photobleaching in pure alcohols as reported already¹⁾, and the result is quite reasonable if the two types of reaction proceed via the common primary processes.

It is significant that water molecules act as retarders only below 20 vol. per cent of alcohol. This result shows evidently a strong reactivity of ethanol with D^t, while the reaction of water with D^t is negligible, if it exist, at least when the content of alcohol exceeds ca 20 vol. per cent.

The production of uranine can be interpreted as a result of further interaction of

DH· with water.

3. On the basis of the above results concerning the variation of k with the concentration of ethanol, a definite conclusion can be drawn about the primary processes of the aerobic photobleaching of eosine in aqueous solution which remained undecided in the first paper[2] of this series. The two schemes then proposed were

$$D^t + O_2 \longrightarrow D\cdots O_2 \xrightarrow{H_2O} \cdots\cdots \text{prod.} \qquad \text{(iii)}$$

$$D^t + H_2O \longrightarrow D\cdots HOH \xrightarrow{O_2} \cdots\cdots \text{prod.} \qquad \text{(iv)}$$

and it can now be said that the former is suitable. The reason for it is as follows. As reported previously[2], ethanol causes a marked retardation on the aerobic photobleaching, but, at the concentration of ethanol of 20 vol. per cent there is observed photobleaching to some extent with a quantum yield about 1/5 of that in the pure aqueous solution. Hence if aerobic photobleaching in the aqueous solution goes via (iv), water ought to show a retarding effect just as great or greater than this value on the photobleaching in vacuo. But the present investigation shows that it is not the case.

Thus it can be concluded that the primary process in the aerobic photobleaching in the pure aqueous solution is (iii), in which D^t primarily reacts with oxygen molecule to form a labile complex, $D\cdots O_2$. It is to be noted, however, that the data for k-values are rather few and it is desirable to accumulate them or to find some other facts in support of this mechanism. Further confirmations and somewhat quantitative discussions will be given in the next paper.

4. Though the detailed mechanism of the uranine-producing reaction is not yet clear, it is certain that uranine is not directly formed from eosine but is formed via some preceding intermediates, may be dibromo-compound, in view of the fact that the absorption for intermediates (Fig. 2) shows a gradual displacement of their maxima towards shorter wavelengths.

When the quantity of intermediates produced per unit quantity of decomposed eosine is evaluated from the absorption data and is plotted against time, then it is found from the value extrapolated to time zero that the reaction which produces coloring materials decreases with increasing concentration of alcohol. The molar extinction coefficient of the intermediates can be estimated to be roughly 10^5 from the data for the solution of low alcohol content. Hence, in the region of low concentration of alcohol (~20 vol. per cent), the principal reaction is an uranine-producing one, while, with rising concentration of ethanol, the reaction in pure ethanol becomes predominant.

It is to be added here that, according to our supplementary experiments, an uranine-producing reaction is sensitive to pH; the addition of a very small amount of acetic acid causes no shift of spectrum. Further, in the case of erythrosine, uranine is not so much produced as in the case of eosine under analogous conditions.

At any rate, the reaction of this type seems to be exceedingly curious from the standpoint of organic reactions and may be worthy of further research from this point of view, as well as from the view point of photochemistry.

Summary

The photoreaction of eosine has been studied in ethanol-water mixture in vacuo. Irradiated solution showed a gradual spectral shift towards shorter wavelengths. Analyzing the spectra and applying the chemical method, it was found that the spectral shift is due to the production of uranine.

The rate of disappearance of eosine increases with rising concentration of ethanol until 20 vol. per cent, at which it reaches its maximum and constant value, which is the same as that in pure ethanol.

The primary processes of the aerobic photobleaching of eosine in aqueous solution were discussed and it was concluded that the process must be

$$D^t + O_2 \longrightarrow D\cdots O_2 \xrightarrow{H_2O} \cdots \text{prod.} \qquad \text{(iii)}$$

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