BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 1337—1342 (1970)

The Radiolysis and Photolysis of Methanolic Solutions of Eosin. II. The Photo-Debromination of Eosin in an Alkaline Solution

Kazuie KIMURA, Takuji MIWA[*1] and Masashi IMAMURA

The Institute of Physical and Chemical Research, Yamato-machi, Saitama

(Received December 1, 1969)

Semireduced eosin was found to undergo photo-debromination and was identified with a precursor in the photo-debromination of eosin in alkaline methanolic solutions. Semireduced eosin was prepared by the γ-irradiation of an eosin solution. The photolysis of semireduced eosin was investigated by the use of continuous and flash light. From the absorption spectral change and a study of the decay, the mechanism of the photo-debromination was proposed. The debromination process of the photo-excited semireduced eosin was explained satisfactorily by assuming the transient formation of the debrominated-eosin σ-radical as an intermediate. In addition, we found an electron-transfer reaction from semireduced debrominated eosin to eosin, which results in the formation of semireduced eosin and debrominated eosin in the dark. A general conclusion as to the photo-dehalogenation of xanthene dyes is also given.

The photochemical debromination of eosin was found by Imamura and Koizumi in alkaline alcoholic solutions.[1] Upon irradiation by light, the absorption peak of eosin shifts gradually to shorter wavelengths; this shift proceeds until it attains the wavelength corresponding to the peak wavelength of uranin. Other halogenated xanthene dyes, such as eosin-blue (dinitro-dibromo-fluorescein) and erythrosin (tetraiodofluorescein), have also been observed to undergo similar photochemical dehalogenation.[2]

The debromination of eosin has also been reported for aqueous solutions containing phenol[3] and tertiary amines.[4] Zwicker and Grossweiner[3] assumed that the debromination takes place from the semireduced eosin on the basis of the marked dependence on the acidity. Oster *et al.*[4] postulated the same intermediate, which decomposes spontaneously to yield the partially-dehalogenated species as well. No concrete evidence has, however, yet been presented for the precursor of the dehalogenation or for its reaction mechanism.

In the photochemical system of eosin solutions, there appear several reactive intermediates, either one of which might be the precursor of debromination. These are the triplet state and semioxidized and leuco-eosin as well as the semireduced eosin. In fact, leuco-anthraquinonoid dyes in alkaline aqueous ethanolic solutions have been reported to undergo photo-dehalogenation.[5] In the preceding paper of this series,[6] we have reported on the γ-radiolysis of the neutral and alkaline methanolic solutions of eosin. In both solutions, eosin is reduced with the CH_2OH radical to form semireduced eosin. The semireduced eosin becomes leuco-eosin by means of the disproportionation reaction in the neutral solution, whereas it is stable in the dark in the alkaline solution.

No debromination has been observed in the radiolysis in the dark. The stable semireduced eosin has, however, been found to undergo photo-debromination. We have, therefore, investigated in detail the photo-debromination of the semireduced eosin which had been prepared by γ-irradiation in alkaline methanolic solutions of eosin. The results having been briefly communicated,[7] the accounts will be presented in detail in this paper.

Experimental

The materials and the experimental procedures, except for the photolysis, were largely the same as those reported previously.[6]

Irradiation was carried out with a projector lamp (300 W) with the aid of a lense and several filters.

[*1] Present address: Institute of Natural Color Technology, Chiba University, Yayoi-cho, Chiba.

1) M. Imamura and M. Koizumi, This Bulletin, **29**, 913 (1956).

2) M. Imamura, unpublished results.

3) E. F. Zwicker and L. I. Grossweiner, *J. Phys. Chem.*, **67**, 549 (1963).

4) G. Oster, G. K. Oster and G. Karg, *ibid.*, **66**, 2514 (1962).

5) J. J. Moran and H. I. Stonehill, *J. Chem. Soc.*, **1957**, 788; C. J. Cooper and H. I. Stonehill, *ibid.*, **1958**, 3353.

6) K. Kimura, T. Miwa and M. Imamura, This Bulletin, **43**, 1329 (6970).

7) K. Kimura, T. Miwa and M. Imamura, *Chem. Commun.*, **1968**, 1619.

Toshiba glass filters, VG-1, VY-450, and VV-40, and Bausch and Lomb filters, 7-3269-2, C, and C-1, were used independently or in combination. The absorption spectra were recorded on a Shimadzu SV-50A or a Cary 14R spectrophotometer.

Flash photolysis was carried out with semireduced eosin solutions by using an apparatus which has been described elsewhere.[8] Briefly, the photolysis flash consisted of two Ushio xenon lamps. The operating characteristics of the lamps were: energy per flash: 100 joule; flash duration, to 50% peak: 10 μsec. The reaction vessel was a quartz cylinder, 10 mm in *i.d.* and 10 cm in length, with flat plates sealed on either end. The apparatus had facilities for both flash spectroscopic and kinetic spectrophotometric measurements.

Results

Photo-Debromination of Eosin in Alkaline Solutions. The absorption spectral shift for eosin solutions in deaerated aqueous methanol containing alkali was observed when they were irradiated with white light or with light which had passed through a Toshiba glass filter VG-1[9] (Fig. 1). The spectral shift induced by light absorption is identical with that reported previously; it has been proved to be due to the debromination of eosin.[1] The absorption peak finall attains a wavelength corresponding to the peak wavelength (495 nm) of uranin (disodium salt of fluorescein), which has no bromine substitution. The absorption bands appearing between 521 and 495 nm are due to the formation of dyes in which there is a replacement of bromine by hydrogen; 4′,5′-dibromouranin has its absorption peak at 506 nm.[4]

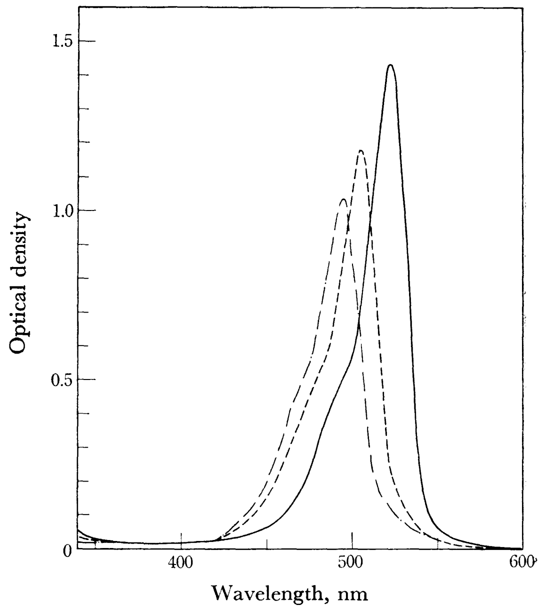


Fig. 1. Absorption spectra of 1.2×10^{-5}M eosin solution in 75% MeOH containing 1.2×10^{-2}M NaOH before and after light-irradiation.
—— before irradiation, ------, —·—·— after irradiation, filter VG-1.

Photolysis of Semireduced Eosin. If an interference filter which transmits a narrow band of light at 527 nm was used, irradiated eosin solutions showed merely the decrease at 521 nm and the increase at 408 nm (semireduced eosin[6]) in the intensity of the absorption peaks; they showed no

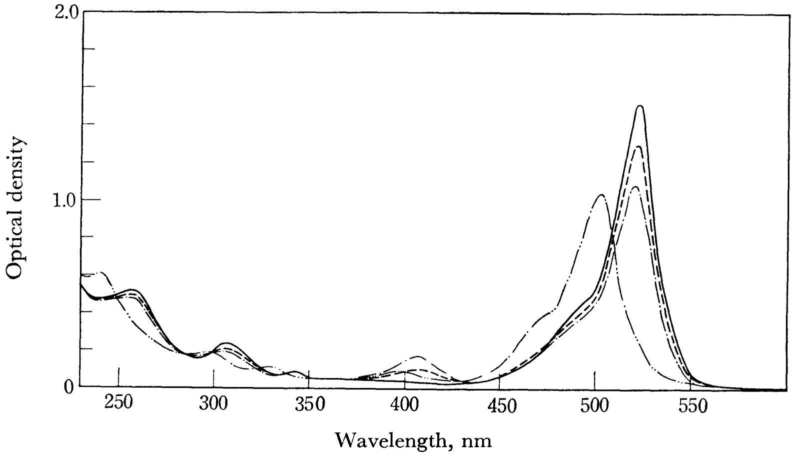


Fig. 2. Absorption spectra of 1.3×10^{-5}M eosin solution in 98% MeOH containing 0.2M NaOH before and after light-irradiation.
—— before irradiation, ------, —·—·— after irradiation by 527 nm light, filter combination C-1+7-3269-2+VY-450, —··—··— after irradiation of —·—·— by 402 nm light, filter combination 7-3269-2+C.

---

[8] A. Kira and K. Nishi, *Rep. Inst. Phys. Chem. Res.*, **44**, 56 (1968).

[9] The filter transmits light which is absorbed not only by eosin but also, to some extent, by semireduced eosin produced in alkaline solution.

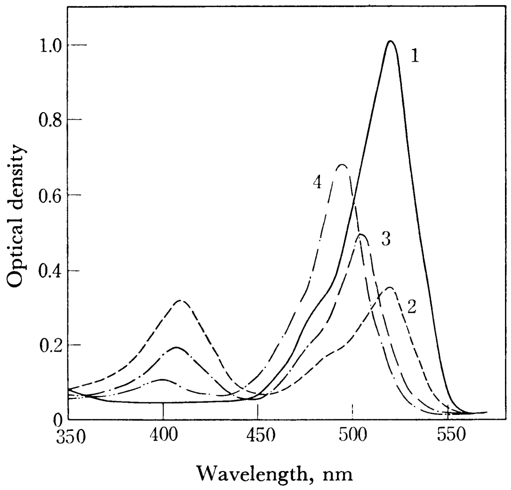


Fig. 3. Absorption spectra before and after irradiation.
1: 0.86×10^{-5}M eosin solution in 98% MeOH containing 0.2M NaOH before irradiation.
2: after γ-irradiation for 15 min at a dose rate of 1.13×10^{16} eV/ml min.
3 and 4: after irradiation of 2 by 402 nm light.

substantial spectral shift, as is shown in Fig. 2. These facts caused us promptly to carry out experiments to determine if the debromination would occur upon the photo-excitation of semireduced eosin. In fact, it took place, as is also shown in Fig. 2.

The photo-debromination of semireduced eosin was more clearly shown to take place for the solution of semireduced eosin which had been prepared by the γ-irradiation of an alkaline methanolic solution of eosin. In this solution, semireduced eosin is produced as quite a stable product, as has been reported previously.[6] Using an interference filter which transmits the light (402 nm) to be absorbed solely by semireduced eosin, we could observe the spectral change shown in Fig. 3; the change indicates that the photo-debromination of semireduced eosin is taking place.

As may be seen in Figs. 1, 2, and 3, the absorption bands developing in the course of the debromination process are as sharp as those for eosin or uranin, indicating that each intermediate absorption band may be due largely to a single compound and that, therefore, debromination may proceed in a step-by-step fashion. The above experimental results also indicate that the precursor of the photo-debromination of eosin is semireduced eosin.

ESR Spectrum Change Upon Light Irradiation. Figure 3 shows that the absorption peak of semireduced eosin also shifts to a shorter wavelength as its intensity is decreased. In a previous paper ESR spectra have been reported for semireduced eosin and uranin, prepared by the γ-irradiation of alkaline methanolic solutions of eosin and uranin respectively.[6] Figure 4 shows the successive change in the ESR spectra for the semireduced-eosin solution which was subjected to light-irradiation for different periods. The ESR spectrum of semireduced eosin grows more complex until ultimately it is superimposable on that of semireduced uranin. These results indicate that semireduced forms of debrominated eosins are also produced as intermediate products.

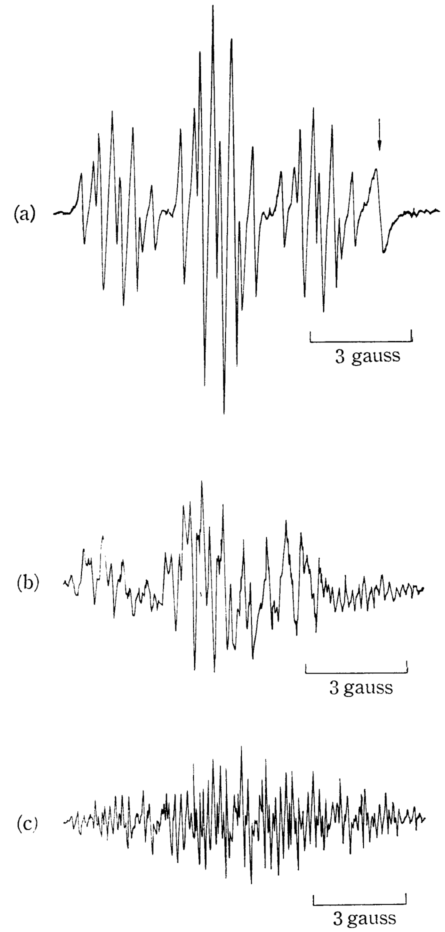


Fig. 4. Change of ESR spectrum of semireduced eosin caused by light-irradiation.
a: for semireduced eosin prepared by γ-irradiation of alkaline methanolic solution of eosin.
b and c: after irradiation by 402 nm light. ESR spectrum c is almost superimposable on that of semireduced uranin (see Ref. 6). The arrow indicates the standard signal of $K_2(SO_3)_2NO$.

Reaction of Eosin with Semireduced Uranin. Figure 3 implies another interesting feature. Upon the excitation of semireduced eosin (408 nm of the Absorption Spectrum 2), the absorption band of debrominated dye (505 nm of 3) developed and the original absorption band due to eosin (521 nm of 2) vanished. This result may be explained in terms of electron transfer from the semireduced form of dehalogenated dye to eosin, the latter having an electron affinity higher than the former. By this electron-transfer reaction, semireduced debrominated-eosin is oxidized to debrominated eosin and the remaining eosin is reduced to semireduced eosin.

This reaction was demonstrated to take place by following experiment shown in Fig. 5. When semireduced uranin (2 in Fig. 5) prepared by the γ-irradiation of uranin (1) in an alkaline methanolic solution was mixed with eosin *in vacuo* and in the dark, the absorption peak of semireduced uranin (394 nm) shifted to a longer wavelength, the absorption band of eosin (521 nm) disappeared, and that of uranin (495 nm) increased (3). The results clearly show that uranin and semireduced eosin (408 nm) were produced by the electron-transfer reaction between semireduced uranin and eosin; this is in agreement with the assumption mentioned above.

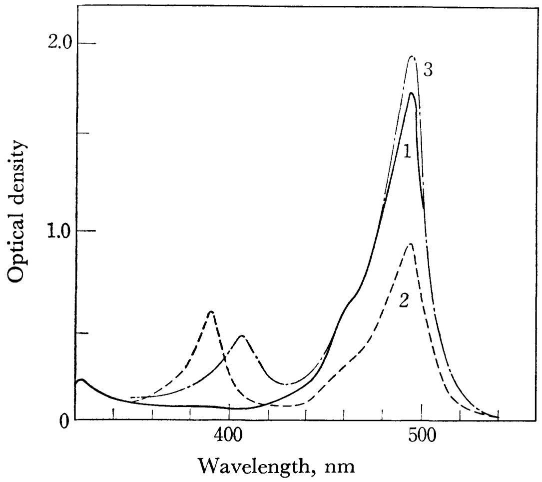


Fig. 5. Absorption spectra for (1) uranin, (2) semireduced uranin and (3) the mixed solution of semireduced uranin and eosin. All the solutions were in alkaline MeOH *in vacuo*. Note that the absorption of added eosin (521 nm) vanished.

Flash Photolysis of Semireduced Eosin. The primary processes of the photo-debromination of semireduced eosin were studied by flash photolysis. Semireduced eosin which had been prepared by the prolonged γ-irradiation of an alkaline methanolic solution of eosin, and which contained a negligible amount of unreacted eosin, was photolyzed by flash light through a VV-40 glass filter. The absorption spectra recorded 20 μsec and several minutes after the flash are given in Fig. 6. Upon the excitation of the semireduced eosin, a rapid development of the absorption band at 514 nm was observed; this development is due to the formation of partially-debrominated eosin. A decrease in the absorption intensity of the original semireduced eosin was observed at the same time. The absorption band at 514 nm then showed a decrease in intensity in the dark, along with a recovery of the absorption band at a shorter wavelength to an extent equivalent to the decrease in the intensity of the former band. The decay characteristics of semireduced eosin are given in Fig. 7, in which the oscillographic trace of the transmittance at 514 nm is also shown. These traces were recorded separately with semireduced eosin solutions which had been prepared in the same way by γ-irradiating solutions of equal eosin concentrations. Figure 7 shows that, upon flash-excitation, semireduced eosin rapidly converts during the duration of the flash to partially-debrominated eosin having its absorption peak at ~514 nm.

The rapid processes are followed in the dark by

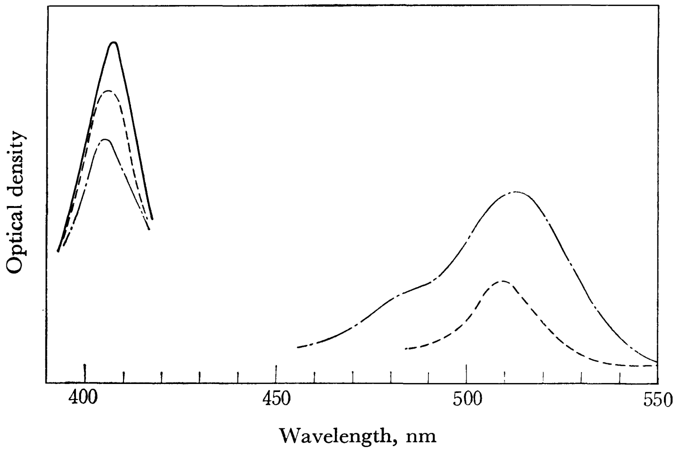


Fig. 6. Absorption spectra of semireduced eisoin solution after flash-irradiation.

—— before flash, practically no absorption of eosin (521 nm) is observed. -·-·- 20 μsec after flash, ----- several min after flash.

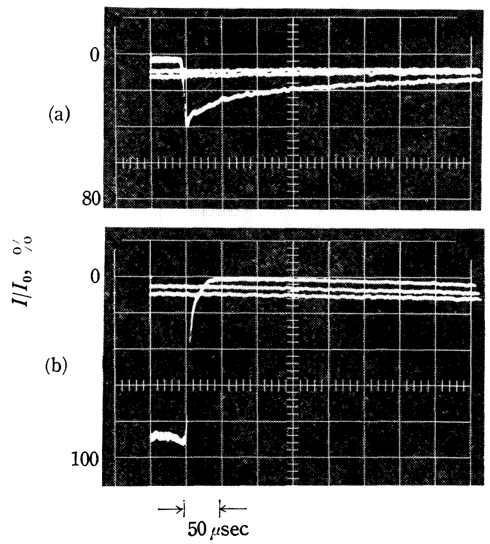


Fig. 7. Oscillographic traces of (a) semireduced eosin at 408 nm and (b) debrominated eosin at 514 nm.

the slow processes. Figure 8 shows the slow processes more clearly by plotting the concentrations of semireduced and partially-debrominated eosin as a function of the time from 0.1 msec after the flash. The concentrations of both species were calculated by taking ε (514 nm) $= 1.08 \times 10^5 \mathrm{M}^{-1}\,\mathrm{cm}^{-1}$ and ε (408 nm) $= 5.67 \times 10^4 \mathrm{M}^{-1}\,\mathrm{cm}^{-1}$.[10] The two curves are apparently mirror images of each other, indicating that partially-debrominated eosin is reduced solely to its semireduced form in the dark.

Discussion

From the experimental results presented above, the following conclusions can be immediately drawn:

(1) The precursor of the photo-debromination is semireduced eosin, which is quite stable in deaerated alkaline methanolic solutions.

(2) Semireduced eosin is debrominated and oxidized, upon photo-excitation, to debrominated eosin.

(3) The photo-debromination of semireduced eosin takes place in a step-by-step fashion.

(4) Semireduced forms of debrominated eosin are successively produced as intermediates in the debromination process.

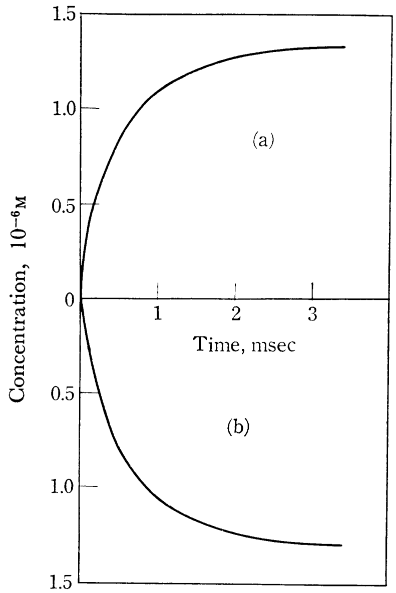


Fig. 8. Slow processes in the dark followed by flash photolysis of semireduced eosin.
(a) Increase in [semireduced eosin] at 408 nm.
(b) Decrease in [debrominated eosin] at 514 nm.

(5) The electron transfer from semireduced debrominated-eosin to eosin occurs in the dark.

Semireduced Eosin as a Precursor. The present results show clearly that the photo-debromination of eosin is actually initiated by the photo-excitation of semireduced eosin. Semireduced eosin is stable in alkaline media because of the symmetrical structure of its basic form.[6] In a neutral solution, however, semireduced eosin undergoes a disproportionation reaction to produce leuco-eosin and eosin.[6] These results provide an explanation of the fact that the photo-debromination of eosin is observed only in alkaline media in the presence of such reducing agents as alcohols and amines. This conclusion is in agreement with previous suggestions by other authors.[3,4]

All other reaction intermediates can be ruled out as the precursor of the photo-debromination. The experiment in which only eosin was photo-excited showed no debrominations, indicating that the excited eosin is not the precursor of the debromination. Semioxidized eosin, which has been proved to form in the radiolysis of aqueous solutions,[11-13] is not produced in alcoholic solutions.[6] Leuco-eosin, if any is present, might undergo debromina-

---

[10] The molar extinction coefficients of debrominated eosins were estimated by assuming the linear relationship between the maximum ε's and the peak wavelengths of these dyes, those for eosin, dibromoeosin and uranin being known. The ε's for semireduced forms with different numbers of bromine were determined from the estimated ε's for their corresponding oxidized forms as mentioned above, where each semireduced form is assumed to be oxidized quantitatively by oxygen.

[11] J. Chrysochoos, J. Ovadia and L. I. Grossweiner, *J. Phys. Chem.*, **71**, 1629 (1967).

[12] P. Cordier and L. I. Grossweiner, *ibid.*, **72**, 2018 (1968).

[13] A. F. Rodde, Jr., and L. I. Grossweiner, *ibid.*, **72**, 3337 (1968).

tion when it is oxidized, as has been observed for anthraquinonoid dyes,[5] because it probably produces semireduced eosin as a reaction intermediate. In the present system, however, no leuco-eosin was produced and no experiment has been carried out with leuco-eosin.

Debromination of Semireduced Eosin. The spectral change recorded in the flash photolysis of semireduced eosin (Fig. 6) seems to indicate that the replacement of bromine atoms by hydrogen atoms takes place one by one. Upon the flash irradiation of semireduced eosin, a new absorption band appeared with a peak at 514 nm. This absorption band is apparently due to the formation of debrominated eosin. The absorption peaks of eosin and uranin are at 521 and 495 nm respectively, while that of dibromouranin is between them (506 nm).[4] Insofar as the absorption band at 514 nm can be ascribed to any single debrominated product, this product (514 nm) may be assigned to the debrominated eosin, in which one bromine is replaced by one hydrogen. We have concluded in the "Results" section that each absorption band appearing in the course of the photo-debromination is due largely to a single debrominated product. The shape of the absorption spectra in Fig. 6 also supports this conclusion.

Figure 7 indicates that semireduced eosin is debrominated and simultaneously oxidized to debrominated eosin. The reaction is completed within the duration of the flash (10 μsec). Since no oxidizing species can be considered to be present in a concentration as to react rapidly with semireduced eosin, the reaction may reasonably be assumed to proceed by a first-order kinetics. No experimental evidence has been obtained for the reaction of excited semireduced eosin producing semireduced debrominated-eosin directly.

Proposed Mechanism of Debromination. The rapid formation of debrominated eosin caused by the photo-excitation of semireduced eosin and the ensuing slow process can be explained by assuming the following reactions:

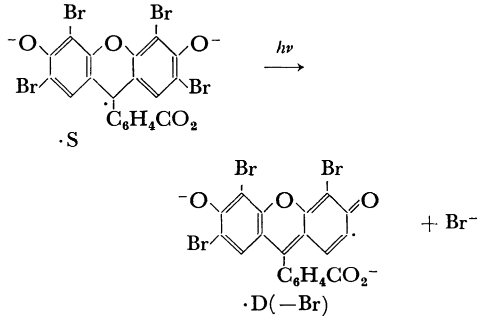
$$\cdot\mathrm{S} \xrightarrow{h\nu} \cdot\mathrm{S}^* \longrightarrow \cdot\mathrm{D}(-\mathrm{Br}) + \mathrm{Br}^- \qquad (1)$$

$$\cdot\mathrm{D}(-\mathrm{Br}) + \mathrm{CH_3OH} \longrightarrow \mathrm{D}(-\mathrm{Br}) + \cdot\mathrm{CH_2OH} \qquad (2)$$

$$\mathrm{D}(-\mathrm{Br}) + \cdot\mathrm{CH_2OH} \longrightarrow \cdot\mathrm{S}(-\mathrm{Br}) + \mathrm{CH_2O} \qquad (3)$$

where D and S stand for eosin and semireduced eosin respectively.

Semireduced eosin exists in an alkaline solution in the basic symmetrical form.[6] The electron-rich semireduced eosin may easily release a bromide ion upon photo-excitation, thus forming the debrominated-eosin σ-radical, ·D(−Br), Reaction (1). The exact position where the first bromine atom is released has not yet been established; ·D(−Br) is, in any case, a free radical and will rapidly abstract hydrogen from the neighboring methanol to form debrominated eosin, D(−Br), and the $\mathrm{CH_2OH}$ radical (Reaction (2)). The rapid process shown in Fig. 7 can thus be explained by the sequence of Reactions (1) and (2).



The ensuing slow process can be explained by Reaction (3), where debrominated eosin D(−Br) is reduced with the $\mathrm{CH_2OH}$ radical produced by Reaction (2). The semireduced form of D(−Br), ·S(−Br), then replaces its bromine with hydrogen by a sequence similar to that of Reactions (1) and (2), and so on. Thus photo-debromination will continue until all the bromines are replaced with hydrogens, final product being nothing but uranin.

This mechanism can also explain the fact that semireduced forms of various debrominated eosins are successively produced in the course of the debromination process. It may be speculated that such an electron-transfer reaction as that observed between eosin and semireduced debrominated-eosin plays a role in making the intermediate products a single component.

Photo-debromination of Eosin. The overall mechanism of the photo-debromination of eosin can be understood on the basis of the results presented above. The photo-debromination does not take place unless semireduced eosin is photo-excited. In previous experiments,[1-4] solutions were exposed to the light, which is absorbed not only by eosin, but also by its semireduced form. Furthermore, the debromination is observable only in deaerated alkaline media containing appropriate reducing agents; every role of those agents is also clear from the present investigation. The dehalogenation of other xanthene dyes can be explained largely in the same manner.

Recently Sherman[14] has found another type of radiation-induced dehalogenation for some halogenated benzenes as well as for alkyl halides. Alkaline alcoholic solutions of these compounds have been reported to show chain dehalogenation. The reaction, however, is initiated by dissociative electron attachment to the halogens of these compounds; no indication of such a reaction has been observed with eosin solutions in the present study.

The authors wish to thank Dr. Akira Kira for his helpful advice in the flash-photolysis experiments.

---

14) W. V. Sherman, *J. Phys. Chem.* **72**, 2287 (1968).