expressed that the rate determining step involves the direct participation of the solvent in the reaction.[15,16] The results herein reported are compatible with this view.

We wish to thank Drs. J. Bigeleisen and M. Wolfsberg for their interest and helpful discussions.

UPTON, LONG ISLAND, NEW YORK

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(15) This point has been discussed in a paper by R. W. Dodson, presented at the New York meeting of the National Academy of Sciences, November, 1954.

(16) Hydrogen atom transport from ferrous ion *via* a water bridge also has been proposed by W. L. Reynolds and R. Lumry, 128th Meeting of the American Chemical Society, Minneapolis, 1955, Abstract 141-R.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Long-Lived States in Photochemical Reactions. I. Photoreduction of Eosin

BY GERALD OSTER AND ALBERT H. ADELMAN[1]

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A mechanism for the photoreduction of Eosin Y with allyl thiourea is presented which necessitates the postulation of a long-lived excited state of the dye. Extensive quenching of this metastable state by dye in the ground state is shown to occur. Kinetic and quenching data are used to evaluate the extent to which the unexcited dye, reducing agent, potassium iodide and *p*-phenylenediamine interact with the metastable excited state of the dye. Further, these data are used to calculate an approximate lifetime of the long-lived excited state which was found to be about fifty thousand times greater than that of the first excited singlet state.

Introduction

Long-lived or metastable states of excited dye molecules have been postulated to account for the phosphorescence of dyes dissolved in highly viscous media.[2] For fluorescein, at least, paramagnetic studies have identified this state to be the lowest triplet state.[3] Perrin and his students have performed semi-quantitative experiments whose results indicate that such long-lived states play a role in photochemical reactions.[4] These workers found that potassium iodide in small concentrations sufficient to quench the *phosphorescence* (of millisecond duration) in glycerol will strongly inhibit the photobleaching of the dye although the fluorescence is quenched to only a slight extent. Long-lived states of chlorophyll have been postulated to account for the decrease in over-all quantum yield of photobleaching with increase in concentration of this substance in methanol.[5] The kinetics of oxidation of allyl thiourea in acetone using ethyl chlorophyllide as the sensitizer[6] has been interpreted in terms of a long-lived state of the sensitizer.[7]

The present work is concerned with the photoreduction of eosin to the leuco form in aqueous medium using allyl thiourea as the hydrogen donor. As will be shown, the kinetics of this reaction requires the postulation of a long-lived species (probably free radical in nature) of the dye. Other work by us further substantiates this postulate.[8]

Experimental

Materials.—Eosin Y (tetrabromofluorescein-C.I.768) in the form of the disodium salt was obtained from Allied Chemical and Dye Corporation. The sample is 94% pure, the remaining 6% consisting mainly of salt additives. Allyl thiourea (Fisher C.P. grade) was recrystallized from acetone. Nitrogen (Airco prepurified) was further treated to remove traces of oxygen by passing over hot copper filings. The copper was periodically regenerated with hydrogen at elevated temperatures. Potassium iodide and *p*-phenylenediamine hydrochloride were Fisher C.P. grade.

Procedure.—All the solutions were made up in $^1/_{15}$ *M* phosphate buffer at *p*H 7.0. The solutions were placed in a cell 1 cm. in thickness and 5 × 5 cm. in cross-section and which was provided with a cover which allowed nitrogen to be passed through the solution to both stir the solution and to prevent contact with atmospheric oxygen. The solutions were flushed with nitrogen 15 min. prior to and during the irradiation. The cell was illuminated with a 500-watt tungsten lamp TDC slide projector (stabilized with a Sola constant voltage transformer) at a distance of 20 cm. An interference filter with maximum transmission at 518 mμ (band width 10 mμ) was placed between the light source and the cell. The transmittances of the solutions were measured with an Aminco photometer unit[9] (in conjunction with a highly dense neutral filter) and recorded continuously on a Leeds and Northrup Speedomax Type G recorder.

The absolute intensity of the light falling on the reaction cell was determined with a calibrated thermopile (Eppley Laboratories, Inc.) having a sensitivity of 0.039 microvolt per microwatt of radiant energy falling on one square cm. and the voltage was read on a galvanometer (Leeds and Northrup, type 2430-D with a sensitivity of 5×10^{-4} microamp. per mm.) in series with the thermopile and a 3200 ohm precision resistor.

Fluorescence measurements were carried out in an Aminco light scattering apparatus[9] using the 436 mμ line of mercury and placing a complementary yellow glass filter before the photomultiplier tube. Spectra of the dye solutions were determined in a Beckman model DU spectrophotometer.

Results and Discussion

The sodium salt of eosin in the phosphate buffer obeys Beer's law in the concentration range employed in the present work. The maximum in the spectrum is at 518 mμ with a molar extinction coefficient of $\epsilon_{\max}$ 7.25×10^4 liters per mole. The area $\int \epsilon d\nu$ of the spectral curve plotted against frequency in the visible range is 7.84×10^{18} liters per mole per sec.

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(1) Eastman Kodak Fellow.

(2) For review, see P. Pringsheim, "Fluorescence and Phosphorescence," Interscience Publishers, Inc., New York, N. Y., 1949, Ch. 5, Sec. E.

(3) G. N. Lewis, M. Calvin and M. Kasha, *J. Chem. Phys.*, **17**, 804 (1949); M. Kasha, *Chem. Revs.*, **41**, 401 (1947).

(4) F. Perrin, *Compt. rend.*, **184**, 1121 (1927); M. Privault, *ibid.*, **184**, 1120 (1927); S. Boudin, *J. chim. phys.*, **27**, 285 (1930).

(5) (a) J. D. Knight and R. Livingston, *J. Phys. Colloid Chem.*, **54**, 703 (1950); (b) R. Livingston, *Rec. Chem. Prog.*, **16**, 13 (1955). See also, E. I. Rabinowitch, "Photosynthesis" Interscience Publishers, New York, N. Y., 1945, Vol. I, Chapt. 18.

(6) H. Gaffron, *Biochem. Z.*, **264**, 251 (1933).

(7) J. Weiss, "Symp. Soc. Dyers and Colourists," Manchester, 1949, p. 135.

(8) Another paper in this series will be submitted for publication to THIS JOURNAL and will be presented in greater detail in the forthcoming Ph.D. thesis of A. H. Adelman.

(9) G. Oster, *Anal. Chem.*, **25**, 1165 (1953).

It was found for a given dye–allyl thiourea mixture that the transmission initially increased linearly with time. The initial slope of the fading curve is then proportional to the rate of fading for a given initial dye concentration. It was further found that the slope of the line increased directly with increase in light intensity which was varied over a tenfold range of intensities by inserting neutral filters between the light source and the reaction cell. We may therefore say that the time rate of photobleaching R (the rate of production of leuco eosin) is proportional to I, the intensity of light absorbed.

The reaction is retarded by oxygen but flushing with nitrogen for 15 min. was found sufficient to remove all measurable inhibition due to oxygen. In contrast to other systems which we have studied,[10] the present system does not show complete reversibility when exposed to oxygen in the absence of light. A solution faded in the absence of oxygen exhibits a very feeble turbidity probably due to the presence of insoluble polymerized allyl thiourea[11] to which some of the leuco form of the dye binds and is unable to revert to the normal form of the dye in the presence of oxygen.

The rate of fading R, as determined by the initial slope of the transmission *versus* time curve, as a function of allyl thiourea concentration (A) followed the empirical expression

$$R = \frac{I(\mathrm{A})}{\alpha + \beta(\mathrm{A})}$$

where α and β are constants. In Fig. 1 is illustrated the dependence of the rate, expressed in terms of the over-all quantum yield Φ, on allyl thiourea concentration. In conformity with the above expression, the reciprocals of these quantities should give a straight line. As is seen in the figure, this is the case except for very high concentrations of allyl thiourea.

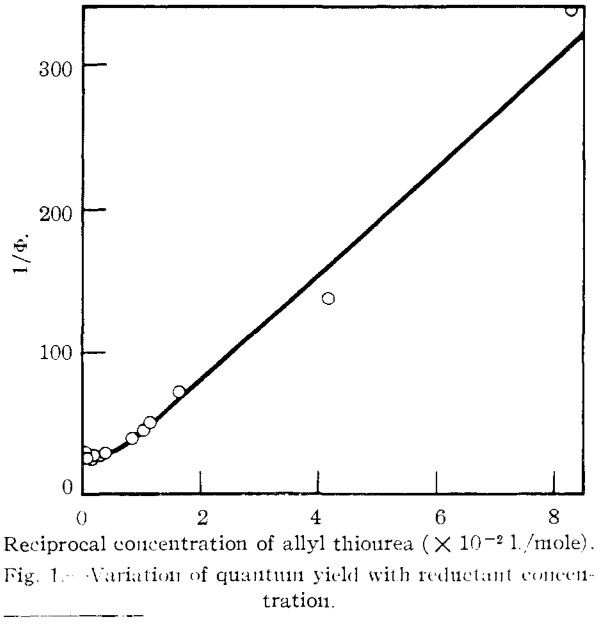

Fig. 1.—Variation of quantum yield with reductant concentration.

At first sight this empirical relation might appear to be compatible with the following photochemical scheme

(1) $\mathrm{D} + h\nu \rightarrow \mathrm{D}^*$ (absorption of light with excitation to first excited singlet state)

(2) $\mathrm{D}^* \rightarrow \mathrm{D}$ (reversion to ground state by a radiationless transition or by emission of fluorescence)

(3) $\mathrm{D}^* + \mathrm{A} \rightarrow$ products (reaction of dye in excited singlet state with allyl thiourea to give leuco eosin)

This scheme requires that allyl thiourea be a quencher of the fluorescence of eosin. We found that allyl thiourea quenches the fluorescence of eosin according to the Stern–Volmer expression with a quenching constant equal to 12.6 liters per mole. However, the change in rate with concentration of allyl thiourea in the concentration range of the linear portion of Fig. 1 is much greater than that expected from the fluorescence quenching constant. Even in the high allyl thiourea concentration range where the curve deviates from linearity step 3 is not operative. The falling off of the quantum yield at very high concentrations of allyl thiourea indicates quenching by the allyl thiourea according to the reaction

(4) $\mathrm{D}^* + \mathrm{A} \rightarrow \mathrm{D} + \mathrm{A}$ (fluorescence quenching by allyl thiourea)

Furthermore, extrapolation of the linear plot to infinite allyl thiourea concentration does not give a quantum yield of unity as required by the above scheme. The actual value is found to be $\Phi_{\max} = 0.092$ suggesting that there is a step which limits the availability of a reactive species.

We therefore postulate the existence of another (metastable) excited state D′ with which allyl thiourea reacts to give the leuco product. That is, we include three more steps, namely

(5) $\mathrm{D}^* \rightarrow \mathrm{D}'$ (transition of the first electronically excited singlet state to the metastable state)

(6) $\mathrm{D}' + \mathrm{A} \rightarrow$ products (reaction of allyl thiourea with the metastable state to give leuco eosin)

(7) $\mathrm{D}' \rightarrow \mathrm{D}$ (reversion to ground state by a radiationless transition or by the emission of a delayed fluorescence or phosphorescence)

The data of Fig. 1 as well as data for other dye concentrations (not shown) show that the overall quantum yield decreases with increasing dye concentration, the quantum yield decreasing linearly with increasing dye concentration. This result is compatible with the step

(8) $\mathrm{D}' + \mathrm{D} \rightarrow 2\mathrm{D}$ (concn. quenching of the metastable state)

The metastable state must be of very long life (compared with the life-time of the dye in the excited singlet state—of the order of 10^{-9} sec.) since the dye concentrations are very low (of the order of 10^{-6} molar) and since encounter frequencies of solvents in media of the same viscosity as that of water are of the order of 10^{10} moles^{-1} sec.$^{-1}$, then the life-time is seen to be of the order of 10^{-4} sec. (see below for more quantitative treatment). The existence of an exchange of energy between an excited molecule and an unexcited molecule has

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[10] G. Oster, *Photo. Engin.*, **4**, 173 (1953); G. Oster and N. Wotherspoon, *J. Chem. Phys.*, **22**, 157 (1954).

[11] Compare. G. Oster, *Nature*, **173**, 300 (1954).

a theoretical basis,[12] and the concept has been used to explain, among other things, the decrease in fluorescence with increasing dye concentration at concentrations exceeding the order of 10^{-3} molar (concentration quenching).[13]

In summary, then, at low concentrations of allyl thiourea the reaction consists of steps 1, 2, 5, 6, 7 and 8. Step 3 was shown not to exist and step 4 is important only at high concentrations of allyl thiourea. Assuming steady-state concentrations for the transient species D* and D′ we obtain for the rate of fading at low allyl thiourea concentrations (concentrations in the linear portion of the curves of Fig. 1) the expression

$$R = \frac{Ik_5k_6(\mathrm{A})}{[k_2 + k_5]\,[k_7 + k_6(\mathrm{A}) + k_8(\mathrm{D})]}$$

where the k's are the rate constants for the steps whose numbers are indicated by the subscripts. From the data of Fig. 1 and similar data obtained at other dye concentrations, we obtain the following values for the ratios of the rate constants

$$k_2/k_5 = 9.80,\ k_7/k_6 = 2.11 \times 10^{-2}\ \text{moles/l.}\ \frac{k_8}{k_6} = 1.03 \times 10^3$$

The value found for the ratio k_2/k_5 shows that on an average about one out of eleven molecules which are in the first excited state undergoes transition to the metastable state. Of these remaining ten excited molecules about 1.5 (on an average) revert to the ground state with the emission of fluorescence since the quantum yield of fluorescence of eosin is approximately 0.15[14] and the remainder fall to the ground state by a radiationless transition.

The molecule in the metastable state can fall to the ground state spontaneously (step 7) or by interaction with a dye molecule in the ground state (step 8) or can react with allyl thiourea to give the leuco dye (step 6). Step 8 seriously competes with the photoreduction process (step 6) and for highly concentrated dye solutions very little fading will occur. It has been noted that dyes in solution bound to high polymers readily undergo photoreduction[10,15] suggesting that step 8 is suppressed when the dyes are in the bound state. The retarding effect of the dye can be decreased by increasing the concentration of allyl thiourea, but then fluorescence quenching (step 4) will come into play. Step 4 is manifested by the non-linear portion of Fig. 1.

The ratio of the latter two quantities above gives $k_8/k_7 = 4.88 \times 10^4$ liters per mole. This is the concentration quenching constant for the metastable state of the dye. The mean life-time of the metastable state is given by $1/k_7$. We can calculate the life-time from the number of encounters per unit time and the efficiency of each encounter. The theory of diffusional collisions[16] gives for the number of collisions per mole per unit time $4\pi NDa$ where D is the sum of the diffusion constants of the two interacting species, a is the sum of their collision radii, and N is Avogadro's number. Taking the two species as being spherical, equation in size, and with encounter radii equal to their diffusional radii, then the number of collisions in one cc. per mole per second is $8RT/3\eta$ where R and η are the gas constant and the viscosity of the medium (in c.g.s. units), respectively, and T is the absolute temperature. For water at room temperature there are then 6.6×10^9 collisions per sec. in a liter of molar solution. If we assume that each collision is effective then k_8 is given by the collision frequency and hence we calculate that the mean lifetime of the metastable state is 7.4×10^{-6} sec. A similar computation applied to the data of the fluorescence quenching of eosin by allyl thiourea gives the life-time of the first excited state. We obtained a quenching constant of 12.6 liters per mole corresponding to a life-time for the first excited state of 1.9×10^{-9} sec. in good agreement with the result obtained by direct measurement of the decay of fluorescence.[17] The computed value for the life-time of the metastable state is nearly four thousand times greater than that for the first excited state. This is actually a lower limit for the lifetime of the metastable state since we have assumed that each encounter is effective (compare below).

p-Phenylenediamine hydrochloride (PPD) in small amounts retards the photoreduction of eosin (Fig. 2). The over-all quantum yield is reduced to one-half of its normal value in the presence of 2.55×10^{-6} mole per liter of PPD. To effect the same reduction in quantum yield by concentration quenching (step 8) would require a dye concentration of 25.3×10^{-6} mole per liter of eosin. It appears that PPD quenches the metastable state and does so more effectively than does the dye. We then postulate

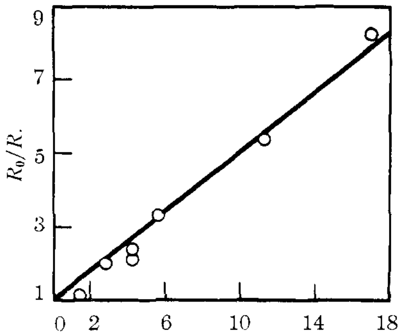

Fig. 2.—Retardation of the fading of eosin as a function of the concentration of p-phenylenediamine (PPD).

(9) $\mathrm{D'} + \mathrm{X} \rightarrow \mathrm{D} + \mathrm{X}$ (quenching of metastable state by substance X)

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[12] H. Kallmann and F. London, *Z. physik. Chem.*, **B2**, 207 (1928); F. Perrin, *Ann. phys.*, **17**, 283 (1932); T. Förster, *Ann. Physik*, **2**, 55 (1947).

[13] For reviews, see J. Franck and R. Livingston, *Rev. Mod. Phys.*, **21**, 505 (1949); S. I. Vavilov, "Mikrostruktura Sveta," Iz. Akad. Nauk SSSR, Moscow, 1950, Part 3, Ch. 3; T. Förster, "Fluoreszenz Organischer Verbindungen," Vandenhoeck und Ruprecht, Gottingen, 1951, Ch. 11.

[14] S. I. Wawilow, *Z. Physik*, **22**, 266 (1924).

[15] G. Oster, *Trans. Faraday Soc.*, **47**, 660 (1951).

[16] M. Smoluchowski, *Z. physik. Chem.*, **92**, 129 (1917); B. Sveshnikov, *Acta physicochim. U.S.S.R.*, **4**, 453 (1936); **7**, 755 (1937). More exact treatments of the diffusional problem are given by S. Chandrasakhar, *Rev. Mod. Phys.*, **15**, 1 (1943), and by J. Q. Umberger and V. K. LaMer, THIS JOURNAL, **67**, 1099 (1945).

[17] E. Gaviola, *Z. Physik*, **35**, 748 (1926).

Assuming that steps 1, 2, 4, 5, 6, 7, 8 and 9 are operative and that applying the steady-state hypothesis for the transient species D* and D′ we obtain for the ratio of the initial rates

$$\frac{R_0}{R} = 1 + \frac{k_9(\mathrm{X})}{k_7 + k_8(\mathrm{D}) + k_6(\mathrm{A})}$$

where R_0 is the rate in the absence of PPD. From the slope of Fig. 2 and the values for k_7/k_6, k_8/k_6, (D) and (A), we calculate $k_9/k_7 = 6.08 \times 10^5$ liters per mole. Again assuming that every encounter is effective we obtain a value 9.2×10^{-5} sec. for the life-time of the metastable state. With this assumption it follows that no more than one encounter in ten of step 8 is effective. Livingston[5] found that the rate constant for the inhibition of photobleaching of chlorophyll by oxygen was likewise ten times greater than the rate constants for the concentration quenching of the metastable state.

Eosin sensitizes the photoöxidation of PPD especially in the presence of oxygen with an intermediate free radical form of PPD.[18] It is probably the biradical nature of the metastable (triplet) state of eosin which causes it to be quenched so efficiently by the PPD radical intermediate. The fluorescence quenching of eosin by PPD was measured using fresh solutions of PPD–dye mixtures for each datum and the observations were carried out rapidly to avoid photoöxidation of the PPD. The results followed the Stern–Volmer expression from which a quenching constant of 49.1 liters per mole was obtained. This value is four times greater than that computed from the known life-time of the first excited state and the theoretical collision frequency suggesting that a non-diffusional quenching process is operative to some extent. At the concentrations of PPD employed in experiments on the retardation of fading the fluorescence quenching is negligible. That is, the reaction

(10) $\mathrm{D}^* + \mathrm{X} \rightarrow \mathrm{D} + \mathrm{X}$ (fluorescence quenching by substance X)

is unimportant at these concentrations.

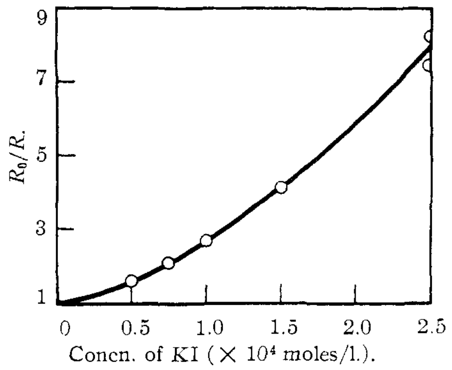

Fig. 3.—Retardation of the fading of eosin as a function of the concentration of potassium iodide.

Potassium iodide also retards the photoreduction of eosin but not as effectively as does PPD (Fig. 3). The fluorescence of eosin is quenched by potassium iodide according to the Stern–Volmer expression with a quenching constant of 4.0 liters per mole. The quadratic form of the curve of Fig. 3 can be explained by assuming that both the singlet and the metastable states are quenched by potassium iodide. Assuming that steps 1, 2, 4, 5, 6, 7, 8, 9 and 10 are operative and applying steady-state conditions, then we obtain an expression quadratic in (X), namely

$$\frac{R_0}{R} = 1 + (\mathrm{X})\,\frac{[\alpha k_9 + \beta k_{10}]}{\alpha\beta} + (\mathrm{X})^2\,\frac{k_9 k_{10}}{\alpha\beta}$$

where $\alpha = k_2 + k_5 + k_4(\mathrm{A})$ and $\beta = k_7 + k_6(\mathrm{A}) + k_8(\mathrm{D})$. If step 10 is negligible then we obtain the retardation formula given for PPD. The form of Fig. 3 indicates that the two quenching constants k_9 and k_{10} for potassium iodide are both significant. From Fig. 3 we calculate that k_9/k_7 is about 3×10^3 liters per mole. Thus, potassium iodide is about one-hundredth as effective as PPD in quenching the metastable state of eosin. Using the life-time of the metastable state as computed from Fig. 2, we find that potassium iodide is roughly one hundred times more effective per encounter as a quencher of the singlet excited state than that of the metastable state.

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(18) G. Oster and M. Schrader, to be published.