BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 39 1221—1227 (1966)

Studies of the Photoreduction of Acridine in Ethanol by the Flash Technique

By Akira KIRA, Shunji KATO*[1] and Masao KOIZUMI

Department of Chemistry, Faculty of Science, Tohoku University, Katahira-cho, Sendai

(Received October 6, 1965)

With the flash technique, the absorption spectra of the triplet state and of the semireduced form of acridine were observed, and the kinetics of the reactions involving these transient species was investigated. It was established that the photoreduction of acridine in the deaerated ethanol solution proceeds via two paths. One is a path via semireduced acridine; this path is strongly inhibited by the presence of oxygen, which efficiently oxidizes semireduced acridine. In the other path, the final product, acridan, is produced immediately after flashing, with no appreciable transient species being formed. The photoreduction by this path is not affected by the presence of oxygen. It may be presumed that this process is a molecular one between singlet acridine and alcohol. The reactive species in the former path also seems to be the singlet excited state instead of the lowest triplet state.

The photoreduction of acridine in alcohol has recently been studied by several workers,[1-5] but no attempt to elucidate the detailed mechanism seems to have been made except by Kellmann and Dubois[6]. They proposed the possibility of the n-π^* triplet being a reactive species. Here, however, we have paid attention only to the problem of whether the reactive species is the singlet excited state or the lowest triplet state. Our results, particularly those concerning the effect of oxygen on the reaction, have nevertheless revealed another important feature of the reaction mechanism which Kellmann and Dubois did not mention. Hence the authors would like to stress this point in the present stage, postponing a decision whether our "singlet excited state" is a true singlet excited state or an n-π^* triplet state or whether it consists of both these states.

In previous papers[4,5] the steady light photoreduction of acridine in ethanol, methanol and isopropanol was studied in both aerated and deaerated solutions. The quantum yields of the photoreduction for the degassed solutions are 0.13 (ethanol), 0.13 (methanol) and 0.09 (isopropanol), while for the air-saturated solutions, they are 0.043 (ethanol), 0.032 (methanol) and 0.032 (isopropanol). When the concentration of dissolved oxygen is low, the reaction first proceeds at a rate approximately equal to that in the air-saturated solution, but a sharp break becomes apparent at a certain timepoint in the decay curve, after which the rate becomes approximately equal to that of the degassed solution.

On the basis of the above results, a scheme was tentatively proposed in which half-hydrogenated acridine is attacked by oxygen and the resultant $AH\cdots O_2$ reacts bimolecularly in the following two, alternative ways:

$$2AH\cdots O_2 \rightarrow AH_2 + A + 2O_2$$

$$2AH\cdots O_2 \rightarrow 2A + H_2O_2 + O_2$$

The above scheme, however, had no definite experimental evidence; moreover, it was not decided whether the reactive species is the singlet excited state or the triplet state. The purpose of the present paper is to identify the transient species produced during the reaction and to study the kinetics of the elementary reactions which are related with the overall reaction. No transient absorption due to $AH\cdots O$ was observed; indeed, the analysis of the data led to quite a different scheme in which singlet excited acridine reacts with alcohol in two alternative ways. One produces acridan or its derivatives,*[2] presumably by a molecular mechanism, while the other produces semireduced acridine as a transient intermediate which, in the presence of oxygen, is easily reoxidized to acridine.

Experimental

Our flash photolysis apparatus is the same as that

---

*[1] Present address: Osaka University, Shibahara, Toyonaka, Osaka.

*[2] As to the final product, it is not certain whether it is acridan or its derivative with a fragment of an alcohol molecule attached to the 9-position. Cf. Ref. 3. Since our main interest is in elucidating the primary processes, we tentatively assume that the product is acridan.

1) V. Zanker and P. Schmid, *Z. physik. Chem.*, *N. F.*, **17**, 11 (1958).
2) A. Kellmann, *J. Chim. Phys.*, **57**, 1 (1960).
3) M. Giurgea, G. Mihai, V. Topa and M. Musa, ibid., **61**, 619 (1964).
4) S. Kato, S. Minagawa and M. Koizumi, This Bulletin, **34**, 1026 (1961).
5) S. Niizuma and M. Koizumi, ibid., **36**, 1629 (1963).
6) A. Kellmann and J. T. Dubois, *J. Chem. Phys.*, **42**, 2518 (1965).

previously described.[7,8] The cells used are 10 cm. long. The procedure for degassing is the same as that described in a previous paper.[4] The samples were illuminated by a 90—125 Joule flash through a ultaviolet filter, which transmits the light from 320 mμ to 400 mμ. The concentration range of acridine examined was from 1×10^{-5} to 1×10^{-4} M, but in most experiments a 3×10^{-5} M solution was used. Under the above conditions, 4—7% of acridine undergoes a permanent change after one flash.

The acridine was recrystallized three times from the water-ethanol mixture. The ethanol was fractionally distilled.

Results

The Quantity of Acridan Produced as a Function of the Oxygen Concentration.—Acridan has a prominent peak at 286 mμ[4]; therefore, the optical density at 290 mμ after flashing was used as a measure of the quantity of acridan produced. This is plotted against the oxygen concentration in Fig. 1. It is evident that above ca. 5×10^{-6} M of oxygen, the yield of acridan is about 1/2.8 of that in the degassed solution, independent of the oxygen concentration. This result agrees quite well with that for the steady-light experiment.[4] The concentrations of acridan in the two cases are 2.2×10^{-6} M and 0.8×10^{-6} M respectively, corresponding to 7 and 2.5% of the original acridine. These refer to the case of the 125 J. flash illumination with the ultraviolet filter.

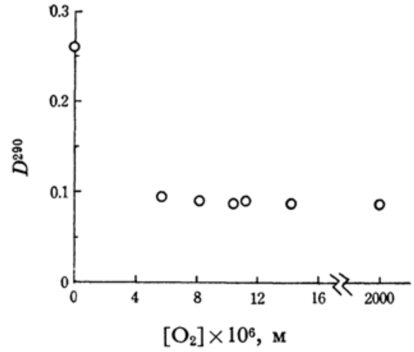


Fig. 1. Effect of oxygen concentration on the quantity of acridan produced by a flash illumination.

The Absorption Spectra of the Transient Intermediates.—The absorption spectra of the transient intermediates are shown in Figs. 2a and 2b. They are assigned as follows.

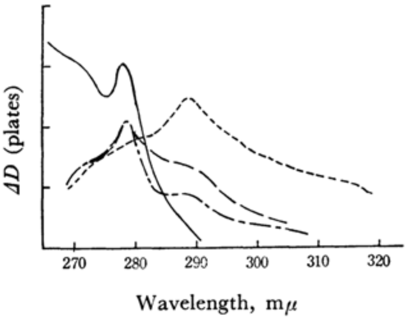


Fig. 2a. Transient absorption spectra in UV region.

ΔD(plates) is the difference in the density of two plates, one of which was measured at a given time after flashing and the other, before flashing.

—— 80 μsec — — — 1.42 msec
— — 3.5 msec - - - - 3 min

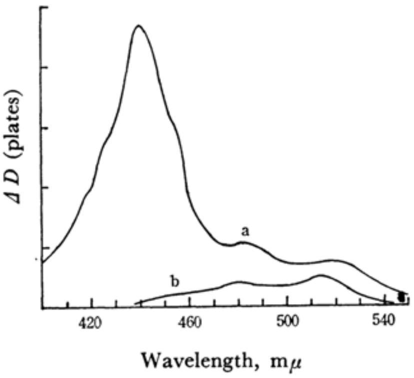


Fig. 2b. Transient absorption spectra in the visible region.

a, 80 μsec. b, 360 μsec.

The band at 440 mμ, with a rather short life time, is attributed to the triplet state A^T, as was done by Jackson and Porter.[9] The molar extinction coefficient of the triplet state, $\varepsilon_A T$, could not be determined since semireduced acridine has an appreciable absorption in the region of the acridine absorption band, as plate 1 shows. $\varepsilon_A T$ in a benzene solution is ca. 7×10^4 M^{-1} cm^{-1}, as will be reported later; this value will be used tentatively when necessary.

Jackson and Porter attributed the band at 520 mμ to the triplet state. However, this band, which clearly has a longer life time than that at 440 mμ, must be attributed to a species different from A^T.

The sharp peak at 278 mμ has a rather long life, and as it declines, the abosrption near 290 mμ due to acridan rises. Hence, it can safely be concluded that this is semireduced acridine. A superposing broad absorption band from aronud 290 mμ extending to a shorter wavelength region is assigned to A^T, since its duration is about the

---

[7] S. Kato, M. Morita and M. Koizumi, This Bulletin, **37**, 117 (1964).

[8] S. Kato, T. Ohno and M. Koizumi, ibid., **38**, 1023 (1965).

[9] G. Jackson and G. Porter, *Proc. Roy. Soc.*, **A260**, 13 (1961).

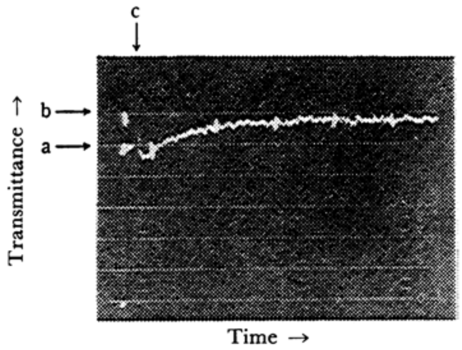


Plate 1. Change in the transmittance at 360 mμ, where the molar extinction coefficient of acridine is 6×10^3 M^{-1} cm^{-1}.
Time marker; 1 msec.
(a) Signal before flashing. This refers to the absorption of acridine.
(b) Signal at a long time after flashing. The decrease in the acridine absorption is due to the formation of acridan.
(c) The time when a flash was fired, The increase in transmittance is due to a flash, and immediately after the flash absorption exceeds that of the original acridine, This is due to the formation of semireduced acridine as described in the text.

same magnitude as that of 440 mμ.

The air-saturated solution (the oxygen concentration is 2.0×10^{-3} M) does not give any transient absorption spectrum. The solutions containing 10^{-6} M—10^{-5} M of oxygen give an absorption band quite similar to that in the degassed solution, but only to a small extent. No new absorption band appeared. Thus no evidence could be obtained for the formation of such intermediates as $A^T\cdots O_2$ and $AH\cdots O_2$.

The Decay of the Triplet State.—The decay of the triplet state was examined by measuring the optical density change at 440 mμ under vairous illuminating conditions. The results are shown in Fig. 3. Evidently the decay curves do not obey a first-order law. The decay at the initial stage becomes faster when the initial concentration of A^T is higher. Further, it is seen that the decay curves cross one another. The former finding may imply that a first-order process and a second-order process are superposed. If one plots the apparent first-order rate constant at the initial stage against the optical density, a fairly satisfactory linear relation is obtained. From the slope and the intercept, one gets for the first-order rate constant, $k_1\cong10^2$—10^3 sec^{-3}, and for the second-order rate constant, $2k_2\cong1\times10^{10}$ M$^{-1}\cdot$sec^{-1}. These are, of course, very rough values, since it is thought that other decay processes are also taking place. Other molecular species which may attack the triplet state of acridine are the ground state acridine, acridan, and semi-

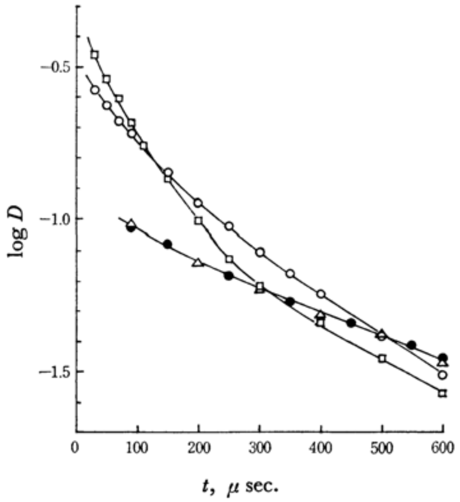


Fig. 3. Decay curves of the absorption at 440 mμ. The order of the flash intensity is

□ > ○ > △, ●.

reduced acridine. If $A^T+A\rightarrow$ occurs with about the equal rate constant as that of $A^T+A^T\rightarrow$, then the first-order decay curve should be obtained, but this is not the case. $A^T+AH_2\rightarrow$ is judged not to contribute so much, because of the following check. In Fig. 3, □, ○ and △ refer to the plots for similar samples illuminated with different intensities, the first with strong, the second with medium, and the third with weak intensities. Plot ● is the one obtained when the sample for ○ was again flashed with the same intensity as that used for △. It may be seen that the ● plot agrees quite well with the △ plot; this rules out the contribution of $A^T+AH_2\rightarrow$. Thus, it seems that acridan and the ground-state acridine do not deactivate triplet acridine very much. It may be inferred, however, that $A^T+AH\rightarrow$ occurs to some extent, because this process is now the only one left that gives rise to the crossing of the curves. At any rate, the exact analysis of the plots at the present stage of study is very difficult, and no further attempt was undertaken. The above values of k_1 and k_2 are tentative, but it is thought that they are correct in the order of magnitude.

When oxygen is added, the decay of triplet acridine becomes faster with an increase of the oxygen concentration. In the air-saturated solution the absorption at 440 mμ is not observed, even immediately after flashing. When the concentration of oxygen is $\geq1\times10^{-5}$ M, the decay satisfactorily obeys the first-order rate law. In Fig. 4, the first-order rate constants are plotted against the oxygen concentration. The data for 5.7×10^{-6} M of oxygen is somewhat doubtful. From the inclination of the line, the rate constant for $A^T+O_2\rightarrow$ can be calculated as $k=2.2\times10^9$ M$^{-1}\cdot$sec^{-1}.

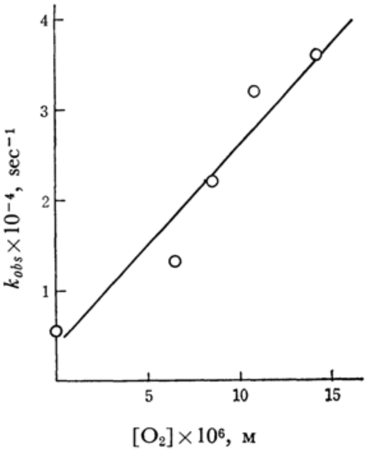


Fig. 4. Effect of oxygen concentration on the first-order rate constant of the triplet state, measured at 440 mμ.

The Decay of Semireduced Acridine and the Rate of the Formation of Acridan.—As Fig. 5 shows, the decline in the optical density at 278 mμ and the rise at 290 mμ proceed in parallel, supporting the view that the $2AH\rightarrow AH_2+A$ reaction is taking place. These curves will be analyzed later, since such an analysis requires some information concerning the reaction mechanism.

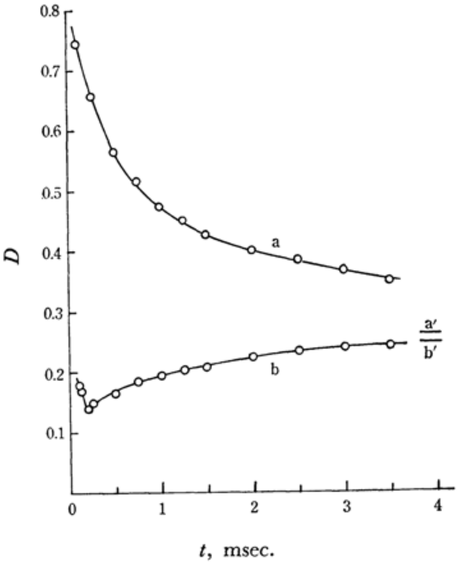


Fig. 5. Change in optical density at (a) 278 mμ and (b) 290 mμ. (a′) and (b′) refer to a long time after flashing.

When the concentration of oxygen is about 10^{-5} M, the disappearance of the 278 mμ peak is much faster, but this is not accompanied by the production of acridan. In other words, the optical density at 290 mμ remains constant, with

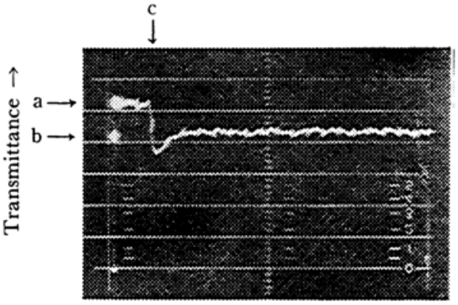


Plate 2. Change in the transmittance at 290 mμ in the presence of oxygen. $[O_2]=8.6\times10^{-6}$ M. Time marker 100 μsec.

(a) Signal before flashing.
(b) Signal at a long time after flashing.
(c) The time when a flash was fired. The increase in the absorption immediately after a flash is due to the formation of the triplet state and semireduced acridine.

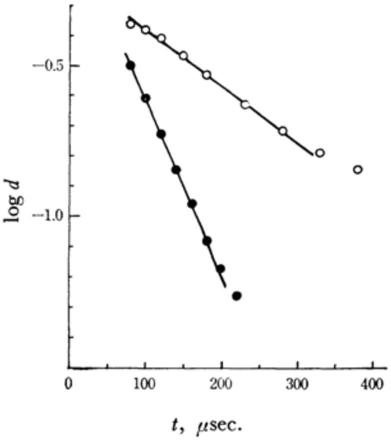


Fig. 6. Examples of decay curves of semireduced acridine in the presence of oxygen.

$[O_2]$ ○ 5.7×10^{-6} M, ● 1.1×10^{-5} M

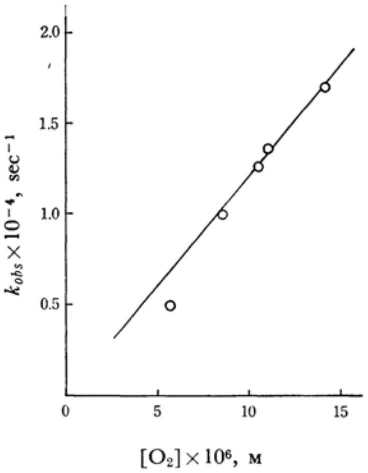


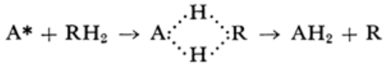
Fig. 7. Effect of oxygen concentration on the decay rate constant of semireduced acridine, measured at 278 mμ.

the same value as that for $\gtrsim 5\times10^{-6}$ M oxygen in Fig. 1 (see plate 2). The reaction is a first-order one. Some examples are shown in Fig. 6, where d is $D^{278}_{AH}-D^{278}_{AH_2\cdot\infty}$. In these experiments the concentration of acridine was 3×10^{-5} M and the input for the flash was 125 J.; an ultraviolet filter was used. In Fig. 7, the first-order rate constants are plotted against the oxygen concentration. From the slope one gets $k=1.2\times10^{9}$ M^{-1} sec^{-1}.

Discussion

In connection with the mechanism for the photoreduction in the presence of oxygen assumed in the previous paper, the following two findings are most important: 1) The deactivation of the triplet state by oxygen occurs very fast, with a rate constant of $k=2.2\times10^{9}$ M^{-1} sec^{-1}, and no transient absorption spectrum is observed in the air-saturated solution. 2) In the presence of about 10^{-5} M of oxygen, the reaction between semireduced acridine and oxygen is very rapid, with a rate constant of 1.2×10^{9} M^{-1} sec^{-1}, and there is no formation of acridan. The first finding strongly supports the view that the reactive species in the air-saturated solution is the singlet excited state. Because practically all the triplet state is annihilated by oxygen, there is no room to consider the occurrence of the $A^{T}+RH_2\rightarrow AH+RH$, $AH+O_2\rightarrow AH\cdots O_2$ reaction. A conceivable different mechanism which can be reconciled with the present kinetic data is the one involving the formation of $A^{T}\cdots O_2$ (a loose molecular complex which Schenck[10)] proposed), which abstracts the hydrogen atom from alcohol. However, this mechanism is also improbable because, according to our unpublished results, triplet acridine in the presence of oxygen at a high concentration does not react with acridan, which is a more powerful hydrogen donor.

Next, judging from the second finding, the singlet state of acridine cannot yield semireduced acridine, and so the only possible mechanism for the air-saturated solution is:



$$A^{*}+RH_2 \rightarrow A\!:\!\overset{H}{\underset{H}{\cdots}}\!:\!R \rightarrow AH_2+R$$

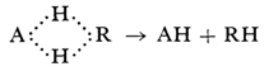
$$(A\cdots H_2R)$$

In the absence of oxygen, an additional reaction involving the formation of semireduced acridine occurs. Thus, the reaction occurs via the two paths.

The remainning problem is now to decide:

1) whether $A\cdots H_2R$, if it is formed, is partially attacked by oxygen or not;

2) whether $A\cdots H_2R$ partially produces half-hydrogenated acridine or not;



$$A\!:\!\overset{H}{\underset{H}{\cdots}}\!:\!R \rightarrow AH+RH$$

and in addition,

3) whether semireduced acridine is formed via the singlet or the triplet state, or both.

First, the results presented in Fig. 5 will be analyzed. Of all the acridan produced,[*3] a 1.8/2.8 part is considered to result from the $2AH\rightarrow AH_2+A$ reaction; it is convenient to discuss only this part. Since the absorption due to the triplet state (T–T absorption) is negligible during the time interval in question, one can write:

$$\overline{D}^{278}=l\{\varepsilon^{278}_{AH}[AH]+\varepsilon^{278}_{AH_2}[AH_2]\} \qquad (1)$$

$$\overline{D}^{290}=l\{\varepsilon^{290}_{AH}[AH]+\varepsilon^{290}_{AH_2}[AH_2]\} \qquad (2)$$

where l is the length of a cell and $[AH_2]$ is the concentration of acridan produced only via semireduced acridine; accordingly, both $\overline{D}$'s are the differences between the observed optical density and 1/2.8 of the optical density of acridan at a sufficiently long time after flashing, $D_{AH_2\cdot\infty}$. If one assumes the $2AH\rightarrow AH_2+A$ disproportionation reaction, the following relation:

$$[AH]_i=2[AH_2]_\infty$$

holds, where $[AH]_i$ is the initial concentration of semireduced acridine after flashing and $[AH_2]_\infty$ is the concentration of acridan a long time after flashing. This relation and the values of $D^{278}_{\infty}\equiv(D^{278}_{AH_2\cdot\infty})$, D^{290}_{∞} and $\varepsilon^{290}_{AH_2}=1.2\times10^{4}$ M^{-1} cm^{-1}, combined with Eqs. 1 and 2, enable us to estimate the values of ε^{278}_{AH} and ε^{290}_{AH} to be as follows:

$$\varepsilon^{278}_{AH}=2.4\times10^{4}\ \mathrm{M^{-1}\,cm^{-1}}$$

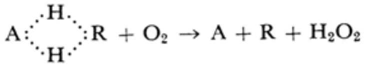
$$\varepsilon^{290}_{AH}=6\times10^{2}\ \mathrm{M^{-1}\,cm^{-1}}$$

$$[AH]_i=2.0\times10^{-6}\ \mathrm{M}$$

Since ε^{290}_{AH} is only 1/20 of $\varepsilon^{290}_{AH_2}$, the optical density in the region of 290 mμ is a measure of the quantity of acridan. The following table gives D_i and D_∞ at three wavelengths for a typical case.

λ, mμ	290	295	300
D_i	0.07	0.07	0.05—0.06
D_∞	0.21	0.19	0.16

Thus $[AH_2]_i$ is about one-third of $[AH_2]_\infty$. This agrees with the ratio of the quantity of acridan produced in the evacuated and in the aerated solutions, leading to the conclusion that one-third of the acridan is produced by the $A^{*}+RH_2\rightarrow AH_2+R$ reaction and not via semireduced acridine. One can, therefore, safely rule out the possibility of this process:



$$A\!:\!\overset{H}{\underset{H}{\cdots}}\!:\!R+O_2 \rightarrow A+R+H_2O_2$$

---

10) G. O. Schenck, *Naturwissenschaften*, **40**, 212 (1953).

[*3] In the flash experiment where a large quantity of semireduced acridine is formed, some of the product may be diacridan. However, to make the description simple, we assume that all the product is acridan. Some of the data must be modified if the above situations really hold.

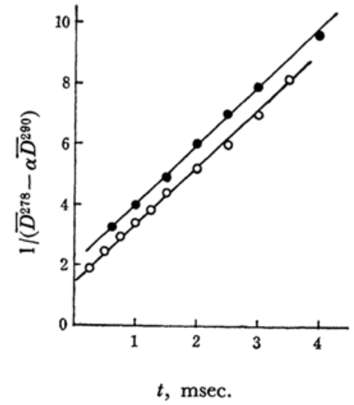


Fig. 8. Second-order decay of semireduced acridine calculated from the decrease of semireduced acridine.
$[A]_0$: ● 2×10^{-5} M, ○ 3×10^{-5} M

Now from Eqs. 1 and 2, one obtains:

$$\overline{D}^{278} - \alpha\overline{D}^{290} = l(\varepsilon^{278}_{AH} - \alpha\varepsilon^{290}_{AH})[AH]$$

where $\alpha \equiv \varepsilon^{278}_{AH_2}/\varepsilon^{290}_{AH_2}$ and where the value of α is approximately one. Hence, $1/(\overline{D}^{278}-\alpha\overline{D}^{290})$ should be linear with time. As Fig. 8 shows, this relation really holds, and the slope gives $2k/l(\varepsilon^{278}_{AH}-\alpha\varepsilon^{290}_{AH})$, where k is the true second-order rate constant for $2AH \rightarrow AH_2 + A$. The rate constant, k, obtained is 2.5×10^8 M^{-1} sec^{-1}. This rate constant can also be evaluated by the following independent method. From 1 and 2 one obtains:

$$\overline{D}^{278}\varepsilon^{290}_{AH} - \overline{D}^{290}\varepsilon^{278}_{AH} = l[AH_2]\{\alpha\varepsilon^{290}_{AH} - \varepsilon^{278}_{AH}\}\varepsilon^{290}_{AH_2}$$

By the $[AH]=2\{[AH_2]_\infty-[AH_2]\}$ relation and by taking into account that $\varepsilon^{290}_{AH}\simeq(1/40)\varepsilon^{278}_{AH}$, one can write:

$$[AH] = \frac{2}{l\varepsilon^{290}_{AH_2}}\left\{\overline{D}^{290}_{\infty} - \overline{D}^{290} + \overline{D}^{278}\frac{\varepsilon^{290}_{AH}}{\varepsilon^{278}_{AH}}\right\}$$

Hence, the rate constant is evaluated from the plot of $1/(\overline{D}^{290}_{\infty} - \overline{D}^{290} + \overline{D}^{278}\cdot\varepsilon^{290}_{AH}/\varepsilon^{278}_{AH})$ against time.

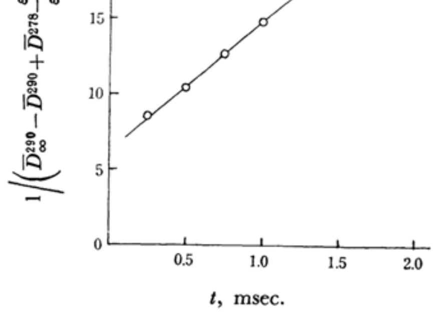


Fig. 9. Second-order decay of semireduced acridine calculated from the increase of acridan.

An example of the plot is given in Fig. 9; from the slope one gets $k=2.5\times10^8$ M^{-1} sec^{-1}, which is in good agreement with the above value.

The success of the above analysis eliminates the participation of the $AH+RH\rightarrow AH_2+R$ reaction, for the following reason. According to Taub and Dorfman,[11] the rate constant, $2k$, for $RH+RH\rightarrow$ is larger than 8×10^8 M^{-1} sec^{-1}; thus, if the above reaction occurs, the second-order rate formula should not hold.

The question whether semireduced acridine is produced in the singlet excited state or in the triplet state of acridine can not be answered clearly in the present stage of study, but the former seems to be more probable. The reason for this is as follows.

First, the value of $[AH]_1$ obtained above (2.0×10^{-6} M) is much larger than the value of $[A^T]_1$ obtained under similar conditions ($\sim9\times10^{-7}$ M). Moreover, it must be taken into account that most of the triplet acridine disappears, at least in the initial stage, according to the $A^T+A^T\rightarrow$ reaction. In agreement with this, the plot in Fig. 8 remains linear, even in the $t<1$ msec. region. Figure 6 also supports this view because, since triplet acridine is deactivated by oxygen very efficiently ($k=2.2\times10^9$ M^{-1} sec^{-1}), the initial concentration of semireduced acridine should largely decrease with the concentration of oxygen if semireduced acridine is really produced via the triplet state.

Concluding Remarks.—The elementary reactions substantiated by the present flash photolysis experiment may be summarized as follows:

$$A \xrightarrow{h\nu} A^*$$

$$A^* + RH_2 \rightarrow \left(A \begin{smallmatrix} \cdot H \cdot \\ \vdots \quad \vdots \\ \cdot H \cdot \end{smallmatrix} R\right) \rightarrow AH_2 + R \qquad (1)$$

$$\xrightarrow[\times]{O_2} A + R + H_2O_2 \qquad (2)$$

$$A^* + RH_2 \rightarrow AH + RH \qquad (3)$$

$$A^* \rightarrow A^T \qquad (4)$$

$$A^T \xrightarrow{k_5} A \qquad (5)$$

$$A^T + A^T \xrightarrow{k_6} (A + A) \qquad (6)$$

$$A^T + RH_2 \xrightarrow{\times} AH + RH \qquad (7)$$

$$A^T + O_2 \xrightarrow{k_8} A + O_2 \qquad (8)$$

$$2AH \xrightarrow{k_9} AH_2 + A \qquad (9)$$

$$AH + O_2 \xrightarrow{k_{10}} A + HO_2 \qquad (10)$$

$$AH + RH \xrightarrow{\times} AH_2 + R \qquad (11)$$

× denotes the non-occurrence of the reaction. The rate constants obtained are:

---

[11] I. A. Taub and L. M. Dorfman, *J. Am. Chem. Soc.*, **84**, 4053 (1962).

$k_5 \cong 10^2 - 10^3\ \mathrm{sec}^{-1}$

$k_6 \cong 5 \times 10^9\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$

$k_8 = 2.2 \times 10^9\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$

$k_9 = 2.5 \times 10^8\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$

$k_{10} = 1.2 \times 10^9\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$

The scheme for the reaction in the presence of oxygen is 1, this may be a one-step molecular process, with $A \cdots H_2R$ or the like being its transition state. In the absence of oxygen, the scheme consists of 3 and 9 in addition to 1. However, it can not yet be said certainly that 7 does not occur. It remains to be solved, as was mentioned at the beginning, whether our "singlet excited state" is really a true singlet excited state or an n-π^* triplet state, as Kellmann and Dubois(6) proposed; in any case, however, there is no doubt that the photoreduction of acridine in ethanol occurs via two different paths.