J. Org. Chem. 1989, 54, 5242–5246

Transient Phenomena in the Laser Flash Photolysis of Rose Bengal C-2′ Ethyl Ester C-6 Sodium Salt[1]

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Received April 3, 1989

The photochemistry of the title compound (RBEt) has been examined by laser flash photolysis in organic solvents at room temperature. The decay of the triplet state of RBEt, generated by either 308- or 337-nm excitation, involves extensive contributions from self-quenching and from triplet–triplet annihilation. It has been conclusively shown that both processes lead to electron transfer and the generation of the radical cation and radical anion from RBEt, but the efficiency is slightly higher in the case of triplet–triplet annihilation, reflecting the more favorable energetics. The various transient intermediates involved in the reaction have been characterized: the triplet state absorbs at 370, 470, and 605 nm (methanol), while the radical anion and cation absorb at 420 and 450 nm, respectively.

Among the different xanthene dyes, Rose Bengal and its derivatives are among the most frequently used in photooxidations mediated by singlet oxygen. The intersystem crossing yield of the disodium salt, which is the derivative invariably used in previous applications involving dye-sensitized photooxidations, is 0.76 in methanol, and rapid quenching of Rose Bengal triplets by dioxygen is responsible for the formation of singlet oxygen.[4]

Studies of Rose Bengal photochemistry have pervaded the literature since it was first used as a photodynamic sensitizer in 1904.[5] However, Rose Bengal has many applications other than its use as a source of singlet oxygen and has been the subject of at least two general reviews.[6,7] While other dyes in the xanthene series, particularly fluorescein and Eosin, have been the subject of a number of detailed flash photolysis studies,[8–14] these studies have frequently been applied to Rose Bengal merely by analogy.[15,16] There are few studies of transient phenomena involving Rose Bengal in the current literature.[4,15–20]

RBEt (Rose Bengal C-2′ ethyl ester C-6 monosodium salt)[21] differs from the disodium salt of Rose Bengal (RB) in at least two important ways. Esterification at C-2′ prevents at least one oxidative mode of bleaching that is available to RB, namely, oxidative lactonization,[22] and the ester is more easily reduced while less easily oxidized than is RB.[23] Further, the ethyl ester is insoluble in pure water. These differences are contrasted to many fundamental similarities between RBEt and RB in Table I.

Table I. Properties of Rose Bengal (RB) and Rose Bengal C-2′ Ethyl Ester (RBEt)

	RB	RBEt
λ_{max} (H_2O) [ϵ]^{23}	549 nm (100 000)	
λ_{max} (EtOH) [ϵ]^{23}	557 nm (112 400)	564 (105 600)
λ_{max}fl (MeOH)^{23} [τ]	569 nm (0.6 ns)	573 nm^a
E°_{red} vs SCE^{23}	−1.03 v^b	−.905 v^{b,c}
E°_{ox} vs SCE^{23}	+0.86 v^d	+0.92 v^{7,d}
λ_{max} phos (EtOH 77 °C)^7	716–717 nm	718–720 nm

[a] For the methyl ester. [b] Versus SCE in methanol; supporting electrolyte tetra-*n*-butylammonium perchlorate. [c] For benzyl ester, sodium salt. [d] Same conditions as reduction; see ref 23.

We have examined the transient phenomena following laser excitation of RBEt, mostly in methanol solution. Excitation was carried out in the near-UV region (337 nm). Our studies confirm some of the reported transient assignments but also provide new spectroscopic information on the contribution of various transients to the observed spectrum and for the first time establish unequivocally the role of electron transfer in the self-quenching and triplet–triplet annihilation processes. Our data also corroborate earlier unpublished solid-sample millisecond observations of radical ion formation.[24] These observations have mechanistic implications on a number of reported phenomena, in particular on the mechanism of reductive bleaching of RB[25] as well as on the formation of superoxide radical anion from RB in aqueous solution.[15,26]

Experimental Section

RBEt was synthesized according to the method of Lamberts and Neckers[21] and its purity confirmed by TLC.

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(1) Issued as NRCC-30580; Contribution No. 64 from the Center for Photochemical Sciences.

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(3) Bowling Green State University.

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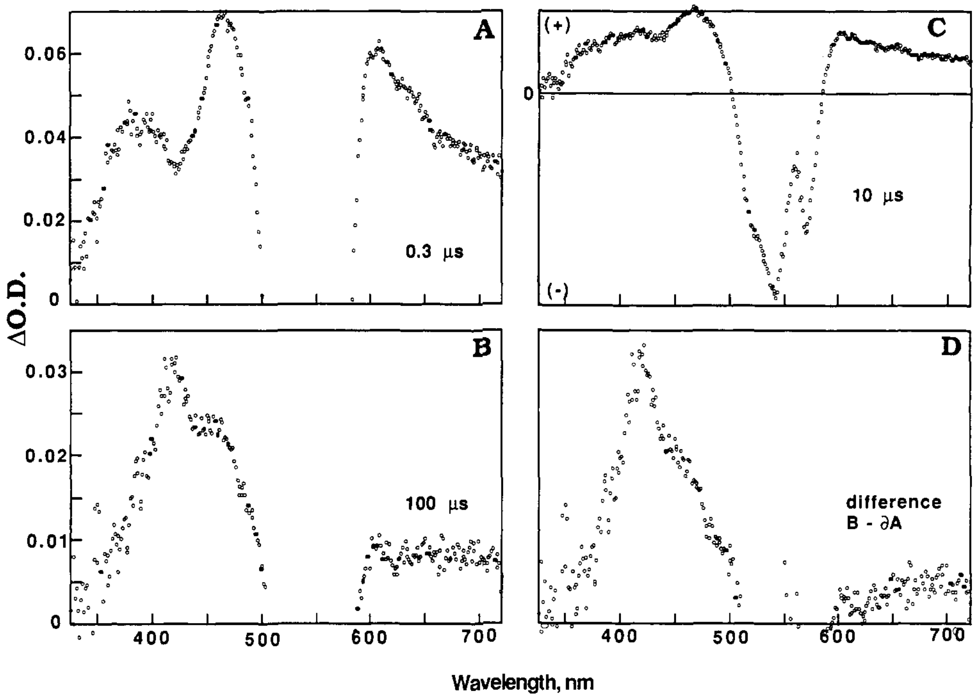
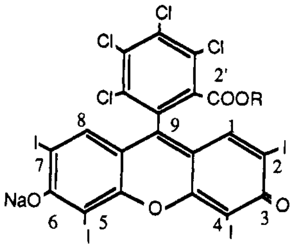


Figure 1. Transient absorption spectra recorded from a 4 × 10^{-5} M RBEt solution in methanol. Spectra A, B, and C obtained at delays of 0.3, 100, and 10 μs, respectivley; only C shows the bleaching part of the spectrum, which is present in A and B as well. Spectrum D shows the difference B − δA, where δ is the contribution of the triplet state (about 11%) to spectrum B. See text for further details on the bleaching component in spectrum C.

The various electron donors and acceptors were purchased from Aldrich and were distilled or recrystallized before use. The solutions of RBEt (10^{-5}–4 × 10^{-4} M) in methanol or acetonitrile (both Aldrich, spectroscopic grade) were deaerated by bubbling with a fine stream of oxygen-free nitrogen. Ground-state absorption spectra were recorded on a Hewlett-Packard 8451 A diode array spectrometer.



RB; R = Na
RBEt; R = C_2H_5

Laser flash photolysis experiments employed the pulses from either a Lumonics TE860-2 excimer laser (308 nm, ~5 ns, <40 mJ/pulse) or a Molectron UV-24 nitrogen laser (337.1 nm, ~8 ns, <10 mJ/pulse) for excitation. The transient signals from an RCA-4840 photomultiplier, initially captured by a Tektronix R7912 transient digitizer were then transmitted to a PDP-11/23+ computer that controls the experiment and provides processing, storage, and hard-copy facilities. Further details on our experimental set up for kinetic studies have been reported elsewhere.[27] Transient spectra were recorded by using an E.G.&G. gated intensified optical multichannel analyzer (OMA) equipped with a Model 1420 detector and interfaced to a PDP11/73 computer via an E.G.&G. Model 1461 interface.

Results and Discussion

Laser flash photolysis of a deaerated solution of RBEt in methanol leads to the transient spectra of Figure 1. All the experiments in this work have been carried out under conditions where the UV–vis spectrum reveals no evidence of the formation of aggregates,[28] although the experiments conducted at the higher concentrations are very close to the point where aggregation becomes important. Spectrum A in Figure 1, recorded 0.3 μs after excitation, shows three main peaks at 380, 465, and 605 nm. As these peaks decay, the spectrum after several hundred microseconds is dominated by a peak at ~420 nm with a clear shoulder at ~450 nm. Spectra A and B in Figure 1 depict only the positive-going signal; however, these signals are accompanied by extensive ground-state bleaching,[30] as illustrated in spectrum C, Figure 1. It should be noted that the strong absorption from the sample in the 520–590-nm region frequently prevents accurate transient measurements in this region as a result of extensive attenuation of the monitoring light beam; the spike at ca. 560 nm in Figure 1C is an artifact resulting from these problems. From the absorption in the 600–650-nm region it is clear that spectrum B incorporates some absorption due to the triplet state. The signals in this region are normally attributed to the triplet state,[15–17,19] and our own work supports this assignment. The triplet signals can be readily subtracted from spectrum B, and this is shown in spectrum D for δ = 0.11. This value for δ was determined from the known lifetime of the triplet, and the knowledge that the absorption cannot be negative at λ >600 nm.

Analysis of the transient absorption traces at different wavelengths indicated the simultaneous occurrence of several processes. At long wavelengths (>600 nm) and

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(28) See, for example: Valdes-Aguilera, O.; Neckers, D. C. *J. Phys. Chem.* **1988**, *92*, 4286.

(29) Aggregation is much more significant for Rose Bengal in water than it is in methanol (see: Xu, D.; Neckers, D. C. *J. Photochem.* **1987**, *40*, 361). However, high salt concentration causes changes in the spectra that drive the xanthanes toward aggregation. This will be the subject of a future publication (Valdes-Aguilera, O.; Neckers, D. C., *J. Am. Chem. Soc.*, submitted).

(30) Ground-state bleaching occurs both oxidatively[22] and reductively.[24] The products in the case of RBEt are the so-called protonated form of the dye appearing in both instances but by completely different mechanisms. See ref 23.

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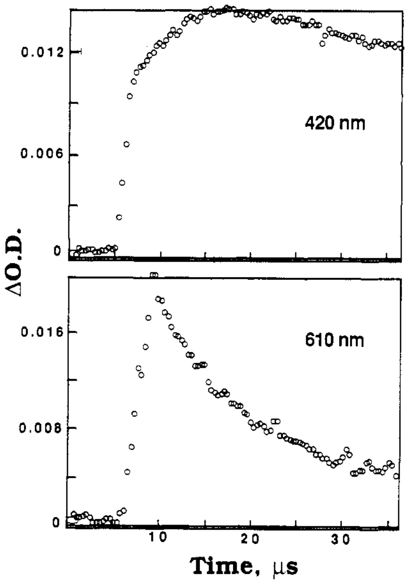
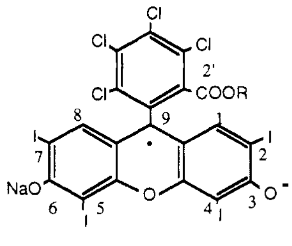


Figure 2. Transient absorption profiles obtained for a 4 × 10^{-5} M solution of RBEt in methanol at the highest laser dose.

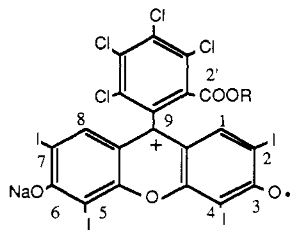
relatively low laser powers, the decay is almost monoexponential (e.g., $\tau_T \sim 16$ µs for [RBEt] = 4 × 10^{-5} M). The triplet lifetime (τ_T) is strongly dependent on the concentration of RBEt, indicating that self-quenching plays an important role, as is common in the case of other xanthene dyes. A plot of τ_T–1 vs [RBEt] in the (1–10) × 10^{-5} M concentration range leads to $k_{sq} = 7.8 \times 10^7$ M^{-1} s^{-1} as the rate constant for self-quenching at room temperature in methanol. The triplet lifetime was also dependent on the laser dose (i.e., on the concentration of triplets generated), and at high laser doses the decay traces incorporated an important second-order component; this is not uncommon under conditions of laser excitation and should be attributed to triplet–triplet annihilation (vide infra).

At shorter wavelengths (i.e., to the blue of the visible absorption peak) the kinetics are more complex than in the 600-nm region but can normally be fitted adequately by a biexponential decay. The short component resulting from such a fit is always in good agreement with the triplet lifetime measured at 610 nm, suggesting that the triplet also absorbs in this region; this was, for example, the case at 470 nm and at $\lambda < 380$ nm. The long component of the decay typically has a lifetime in excess of 50 µs.

It is interesting to note that at 420 nm we observe a growth in the absorption following laser excitation. Figure 2 shows a comparison of the growth trace at 420 nm with a triplet decay trace monitored at 610 nm. Visual inspection clearly shows that the growth part of the 420-nm trace occurs concurrently with triplet decay. The "jump" part of the 420-nm trace should be attributed to triplet absorption in this spectral region. In the case of other xanthene dyes it has been proposed that the two peaks in the 400–500-nm region are due to the semioxidized and semireduced dye,[10-12] shown below.



semi-reduced RB, or RBEt



semi-oxidized RB, or RBEt

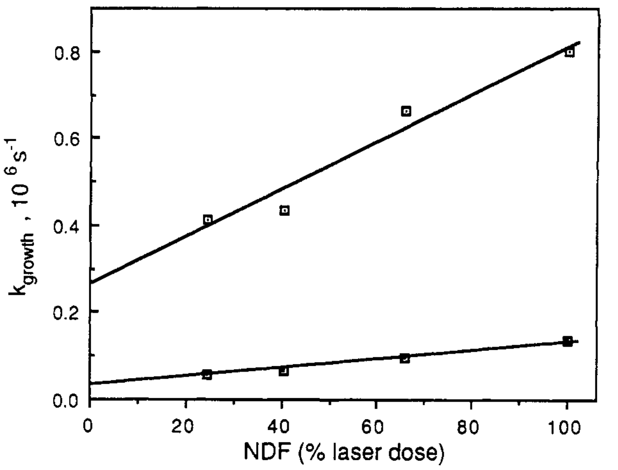


Figure 3. Laser dose dependence of the growth kinetics monitored at 420 nm for 4 × 10^{-4} (□) and 5 × 10^{-5} M (■) solution of RBEt in methanol, where 100% corresponds to 9 mJ/pulse at the laser wavelength (337 nm).

This original proposition by Lindqvist[12] has received support from a variety of experiments, but, to the best of our knowledge, it has never been directly observed that these transient products form concurrently with triplet decay. Scheme I shows the processes that could be anticipated in the case of RBEt on the basis of reactions proposed for Rose Bengal, one of its derivatives, or for other xanthene dyes.[10-12,14,16,17] Intersystem crossing is known to be a highly efficient process in RB,[4] and the production of at least some $^1RBEt^*$ in reaction 6 could be expected since RB is known to show delayed fluorescence. $RB^{\bullet+}$ and the semioxidized forms of other xanthene dyes (e.g., Eosin) normally absorb in the 450–470-nm region, and it is likely that the 450-nm shoulder in Figure 1 (spectra B and D) is due to this species. No transient assignments have been reported for $RB^{\bullet-}$, but in the case of Eosin in the semireduced form is know to absorb at 410 nm.[10-13] Since other transients are very similar for both Rose Bengal and Eosin, it would be reasonable to expect the semireduced form of RBEt in the same spectral region. We therefore assign the 420-nm species of Figures 1 and 2 to the semireduced form, $RBEt^{\bullet-}$; other experiments in our work (vide infra) support this assignment.

Scheme I

$$RB \rightarrow {}^1RB^* \rightarrow {}^3RB^* \qquad (1)$$

$${}^3RB^* \rightarrow RB \qquad (2)$$

$${}^3RB^* + RB \rightarrow RB^{\bullet-} + RB^{\bullet+} \qquad (3)$$

$${}^3RB^* + RB \rightarrow RB + RB \qquad (4)$$

$${}^3RB^* + {}^3RB^* \rightarrow RB^{\bullet-} + RB^{\bullet+} \qquad (5)$$

$${}^3RB^* + {}^3RB^* \rightarrow RB + RB \text{ (or } {}^1RB^*\text{)} \qquad (6)$$

The rate constant for the growth at 420 nm shows a dependence on dose and concentration of RBEt. If, for convenience, we approximate a first-order (i.e., monoexponential) fit to this growth at 420 nm, the resulting rate constants show dependence with both the laser dose and the concentration of RBEt. The dependence on laser dose is illustrated in Figure 3. The difference between the plot for 4 × 10^{-5} and 4 × 10^{-4} M RBEt clearly show the importance of ground-state concentration (i.e., self-quenching). Note, for example, that the extrapolated triplet lifetimes at zero laser dose are 3.8 and 40 µs for 4 × 10^{-4} and 4 × 10^{-5} M, respectively. The decay times at 610 nm

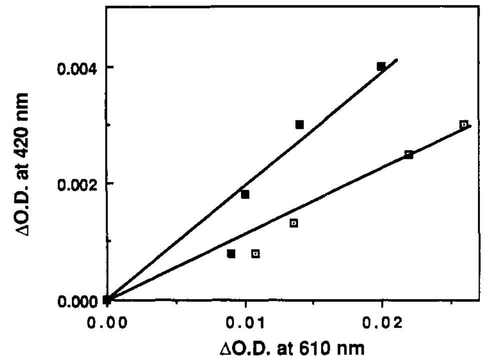


Figure 4. Laser dose dependence of the changes in optical density observed at 420 nm a function of the changes at 610 nm; the latter were adjusted by attenuating the laser dose with neutral density filters and are a measure of the triplet concentration: RBEt concentrations of 4×10^{-4} (□) and 5×10^{-5} M (■).

and the growth kinetics at 420 nm were in excellent agreement.

A rather interesting observation is that as the growth rate changes as a result of changes in laser dose and RBEt concentration, the ratio of the growth-to-jump of the 420-nm traces show only a modest change. For example, Figure 4 shows the dependence of the growth component of the signal at 420 nm with the initial signal at 610 nm (triplet). Note that the slope is about twice as high for the lower RBEt concentration. This suggests that $k_5/(k_5 + k_6)$ is slightly higher than $(k_3/(k_3 + k_4)$, where the denominators correspond to the experimental rate constants for T–T annihilation (k_{TT}) and for self-quenching (k_{sq}), i.e.

$$k_{sq} = k_3 + k_4$$

$$k_{TT} = k_5 + k_6$$

It is, in fact, remarkable that the yields of ions would be similar for both processes, since the formation of radical ions is about thermoneutral in the case of reaction 3 but much more favorable ($\Delta G \sim -41$ kcal/mol) in the case of reaction 5.

The value of k_{TT} can be estimated from a second-order analysis of the decay traces at higher laser dose and low RBEt concentration. Using the reported triplet extinction coefficient of 4300 M^{-1} cm^{-1},[16] we estimate $k_{TT} \sim 5 \times 10^9$ M^{-1} s^{-1}. This value, which is fast but not diffusion controlled, is consistent with the fact that spin statistics determines that four-ninths of the encounters could lead to quenching involving either neutral species or singlet or triplet radical ion pairs.

Further support for the generation of $RBEt^{\bullet -}$ and $RBEt^{\bullet +}$ in reactions 3 and 5 was obtained from the absence of the growth component in the 420-nm traces in micellar solution. Thus, in 0.05 M dodecyltrimethylammonium chloride (DTAC) the same band structure is observed for the triplet state as in methanol solution, but now no growth at 420 nm for $RBEt^{\bullet -}$ is observed. Under these conditions the triplet lifetime exceeds 50 μs. We attribute this observation to the isolation of individual RBEt molecules in micelles, which therefore cannot interact with the necessary reaction partners (see reactions 3–6). The detection of transient absorption below 500 nm in these experiments also confirms that the triplet state does indeed absorb in this wavelength range.

A few experiments were also carried out to determine if some of the $RBEt^{\bullet -}$ could result from electron photoejection followed by recapture by RBEt. No evidence for electron absorption (337-nm excitation) could be obtained in the 630–700-nm region, where the electron would be readily detectable in methanol.

Table II. Quenching of Triplet RBEt by Various Substrates

substrate	$k_q(CH_3OH)^a$	$k_q(CH_3CN)^a$	redox properties[b]
tetramethyl-*p*-phenylenediamine	9.6×10^9		$E_{ox} = 0.04$ V $\Delta G = -20$
N,N'-diphenyl-*p*-phenylenediamine	5.7×10^9	1.2×10^{10}	$E_{ox} = 0.29$ V $\Delta G = -15$
N,N-diethylaniline	2.0×10^9		$E_{ox} = 0.70$ V $\Delta G = -5$
triethylamine	8.5×10^5	4×10^5	$E_{ox} = 0.90$ V $\Delta G = -1$
chloranil	5.8×10^9	1.2×10^{10}	$E_{red} = -0.04$ V $\Delta G = -20$
tetracyanoethylene	*c*	3.2×10^9	$E_{red} = -0.30$ V $\Delta G = -14$
$Fe(CN)_6^{3-}$	2.9×10^8		

[a] Units of M^{-1} s^{-1}. [b] Redox properties in methanol; ΔG in kcal/mol, calculated using data from Table I for RBEt. Substrate data, originally in acetonitrile, corrected for methanol as suggested in ref 31. [c] Ground-state reaction; see text.

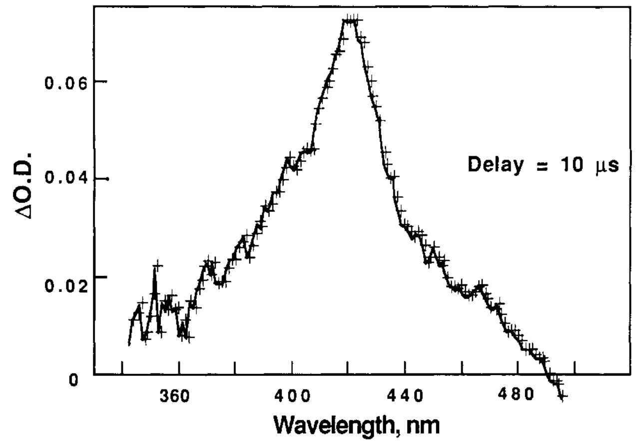


Figure 5. Spectrum obtained from 4×10^{-5} M RBEt in methanol in the presence of 3 mM sodium hydroxide and 0.01 M phenol. Recorded 10 μs after laser excitation.

To further establish the nature of the long-lived species and to probe the reactivity of RBEt, we carried out several experiments using electron donors and acceptors as quenchers for $^3RBEt^*$. The results have been summarized in Table II. Several of these experiments were performed in attempts to detect directly $RBEt^{\bullet -}$ or $RBEt^{\bullet +}$; in the paragraphs that follow we comment briefly on some of these experiments.

It has been reported that Eosin triplets react with phenol in basic medium to generate the semireduced form of the dye, while in acidic medium it yields the protonated semireduced dye.[13] In the case of RBEt triplets we find that in the absence of added base, phenol up to 0.01 M leads to no detectable change in the transient spectrum. By contrast, in the presence of 2 mM NaOH addition of phenol leads to the rapid formation transient spectrum of Figure 5; presumably, phenol acts as an excellent electron donor under these conditions. The position of the λ_{max}, 420 nm, is in excellent agreement with that assigned earlier for $RBEt^{\bullet -}$. We note that while phenoxyl radicals may underlay the signals observed, these cannot be entirely explained by this radical; quite simply, the absorption is at too long a wavelength and is too broad to be solely due to phenoxyl radicals.[32]

Experiments with various amines generally did not lead to readily detectable signals from $RBEt^{\bullet -}$. To some extent this may be due to the fact that RBEt is only a modest

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(32) Schuler, R. H.; Buzzard, G. K. *Int. J. Radiat. Phys. Chem.* **1976**, *8*, 563.

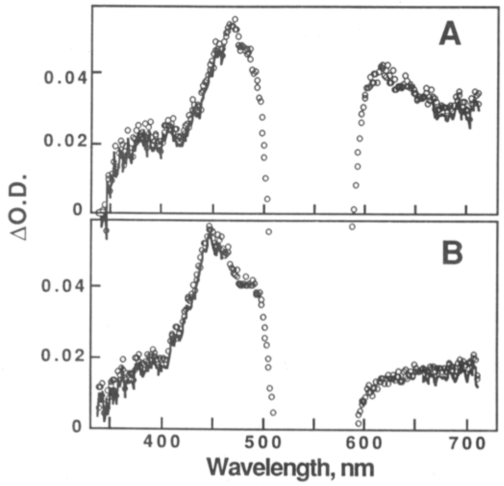
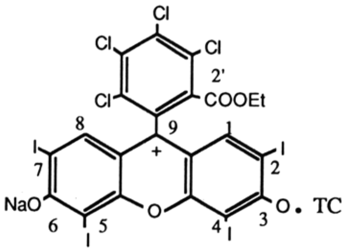


Figure 6. Transient spectra obtained from a 4×10^{-5} M solution of RBEt in acetonitrile and monitored 0.3 μs following laser excitation. Spectrum A (in the absence of TCNE) corresponds predominantly to the triplet, while spectrum B ([TCNE] ~ 1 mM) shows the 450-nm peak characteristic of $RBEt^{•+}$.

electron acceptor, as judged by the low rate constant for reaction with triethylamine. Alternatively $^1RBEt^*$ may be quenched by the amine in a reaction that does not involve electron transfer; however, that has been shown not to occur in previous experiments[10] and is also indicated by our work, as judged by the fact that only relatively small changes in the initial triplet concentration were caused by amine addition. Quite clearly the rate constant for quenching depends on the oxidation potential of the electron donors.[33] In the case of tetramethylparaphenylenediamine the absorption from its radical cation is readily detectable and interferes with the detection of $RBEt^{•-}$. With *N,N*-diethylaniline or trimethylamine no spectral changes are detectable. With *N,N′*-diphenylparaphenylenediamine (10^{-4} M) we observe a new transient at 390 nm, possibly due to the protonated semireduced form, $RBH^•$;[34] similar conclusions have been drawn by using CIDNP techniques.[35]

With electron acceptors in methanolic solution it was not possible to detect $RBEt^{•+}$. While strong electron acceptors are known to intercept the singlet[22] (e.g. diphenyliodonium), this is unlikely to be the reason for the failure to detect $RBEt^{•+}$, since the concentrations required to quench the triplet state are much lower than those needed to quench the fluorescence from RBEt. Again, only minor changes in the initial (i.e., immediately after laser excitation) triplet concentration were caused by the acceptors. With chloranil (see Table II) it was straightforward to measure the rate of quenching, but the transient spectra were dominated by intense transient absorption signals from chloranil in the 400–500-nm region. In the case of $Fe(CN)_6^{3-}$, where we observed signals in the 450-nm region, conclusive characterization was precluded by extensive screening by $Fe(CN)_6^{3-}$.

Addition of tetracyanoethylene (TCNE) in methanol leads to a rapid dark reaction that bleaches RBEt and produces new absorptions at 417 and 498 nm, likely the absorptions of the ground-state "protonated form" charge-transfer complex: This dark reaction does not appear to be important in acetonitrile, where we were able to measure a quenching rate constant (see Table II) and to detect $RBEt^{•+}$, which has a strong absorption at 450 nm (see Figure 6). We note that the spectrum of triplet RBEt is somewhat different in acetonitrile as compared with methanol (compare Figures 1 and 6).



Conclusion

Rose Bengal ethyl ester monosodium salt (RBEt) in methanol shows a triplet–triplet absorption spectrum with peaks around 370, 470, and 605 nm. The absorptions below 500 nm are due not only to the triplet state but also to the semireduced, $RBEt^{•-}$, and the semioxidized, $RBEt^{•+}$, forms of the dye; the various species show extensive overlap in this spectral region. The absorption maxima for $RBEt^{•-}$ and $RBEt^{•+}$ in methanol are 420 and 450 nm, respectively, and are in line with those reported for other halogenated xanthene dyes such as Eosin. The triplet state is best monitored in the >600-nm region where interference from other species is minimum. In methanol solution the semireduced form, $RBEt^{•-}$, is the longest lived species and after a few hundred microseconds the only one observed in deaerated solutions.

In deaerated methanol the origin of $RBEt^{•+}$ and $RBEt^{•-}$ has been conclusively shown for the first time to arise from self-quenching and triplet–triplet annihilation; the latter is somewhat more efficient than self-quenching reflecting a more favorable free energy change. Our experiments demonstrate unequivocally that the production of $RBEt^{•-}$ from two RBEt molecules is concurrent with triplet decay, and studies of the concentration and dose dependence show that reactions 3 and 5 are responsible for the generation of the semireduced form of $RBEt^{•-}$.

Acknowledgment. The work at Bowling Green State University was supported by grants from the National Science Foundation (CHE-850451). We are most grateful for this support. V.W. thanks the NRC (Canada)/CNRS (France) bilateral agreement for support.

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[33] This is clearly evident from those cases in which isolable bleached products have been obtained by reduction of Eosin (Phillips, K.; Read, G. *J. Chem. Soc., Perkin Trans. 1* **1986**, 671) and Rose Bengal.[24]

[34] Reduced Rose Bengal ethyl ester, the so-called protonated form, absorbs at 494 and 405 nm with an extinction coefficient some 10 times smaller than does RBEt. This spectral shift is consistent with this observation (see ref 21).

[35] Muzhat, K. A.; Khait, I. *Bull. Soc. Chim. Belg.* **1979**, *88*, 933.