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Bleaching Studies of Rose Bengal Onium Salts[†]

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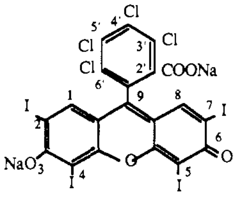
Abstract: The bleaching behavior of Rose Bengal onium salts containing one or two iodonium, sulfonium, and phosphonium ions in different solvents has been investigated. Fluorescence quenching of Rose Bengal sodium salts (Ia, IIa) by cationic onium salts (IIIa–d) and by the corresponding neutral species suggests a parallel exists between the rates of bleaching of the onium salts of Rose Bengal (Ia–c, IIa–d) and the fluorescence quenching of the sodium salts by the corresponding cationic salt, but not the neutral species. Quenching data combined with redox potentials measured by cyclic voltammetry, as well as preliminary product studies, indicate that the dye singlet is oxidized during the photobleaching process in cases where the gegen ion is strongly reducing. Nonreducing gegen ions cause photobleaching of the dye too, but at a much slower rate. In the latter case the triplet of the dye must be responsible for photobleaching since limited or no fluorescence quenching is observed.

Background

Rose Bengal is a xanthene dye that first appeared in Schultz's tables in 1881.[1] It was originally synthesized by Rudolf Gnehm, later to become the President of ETH in Zurich, as part of a program seeking "acid dyes"[2] for wools.[3-5] Other familiar xanthene dyes, such as fluorescein, Eosin, and Erythrosin, differ from Rose Bengal only in aromatic ring substitution. Fluorescein is substituted at positions 2, 4, 5, 7, and 3′–6′ with hydrogens. Eosin has hydrogens at positions 3′–6′, but bromines at positions 2, 4, 5, and 7, while Erythrosin has iodines at these positions. The history and the chemistry of the xanthenes have been reviewed in detail in other literature.[6]

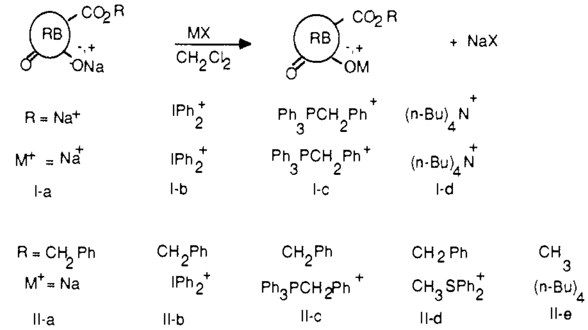


Rose Bengal

The color and spectroscopy of the xanthenes is almost entirely a function of xanthene ring substitution. Fluorescein, which is unsubstituted, fluoresces with very high quantum efficiency. Eosin, Erythrosin, and Rose Bengal intersystem-cross to the triplet state and have a higher population of triplet relative to singlet. Hence, they also show a lower quantum yield of fluorescence. All of the spectroscopic properties of Rose Bengal depend on the ionization state at C-3 and on the solvent.[7a] The state diagram for Rose Bengal can be derived from its absorption and emission spectra and from short-lifetime spectroscopic measurements.[7b] Because it is so strongly absorbing, Rose Bengal plays an active role in many areas of photochemistry. Its spectral properties are of interest because one can use them as a molecular probe to study chemical environment, for example in solution, or within biological molecules. In electron-transfer processes, Rose Bengal can be either oxidized or reduced depending on the gegen ion.

The electrochemical potentials of a series of onium salts of Rose Bengal and other factors effecting photochemical bleaching processes are outlined in this paper. In general we have studied the effect of the gegen ion on the bleaching rate and correlated this with the electrochemical potentials of the compounds measured under identical conditions by cyclic voltametry. These data clearly show for the first time that bleaching can occur in specific instances by oxidative electron transfer, and fluorescence quenching data show that fast oxidative bleaching may often be a singlet process. Later papers will deal with the products of bleaching as well as more detailed lifetime studies.

Scheme I



Results and Discussion

The synthesis of Rose Bengal onium salts is generalized in Scheme I.[8] The visible absorption spectra of these salts in EtOH, CH_2Cl_2, toluene and DME are summarized in Table I. The absorption spectrum of the Rose Bengal moiety is that of the loose ion pair in all cases.[8] Photobleaching rate studies for these salts, followed from the decrease of the 560–570-nm peak in each of the above solvents, are summarized in Table II. In general, solutions of the disalts Ib and Ic bleached from pink to colorless, while the monosalts IIb–d changed from pink to yellow. Isosbestic points appeared at 508 nm in the case of each monosalt IIb–d. The bleaching rates for these salts decrease as a function of the counterion in the order diiodonium > monoiodonium > monosulfonium > diphosphonium > monophosphonium.

There have been many literature reports on the photobleaching of xanthene dyes in most of which the xanthene is reduced in the process.[9] Thus, likely by means of single-electron transfer, the quinone moiety of the xanthene ring undergoes photochemical reduction. This is shown in Scheme II. But the measured electrochemical potentials indicate reduction is not the only possible route for bleaching Rose Bengal onium salts. Oxidative electron transfer is also predicted for the xanthene on the basis of the Rehm–Weller equation,[10] particularly in the presence of an readily reduced gegen ion. We have observed oxidative bleaching of the

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[†] Fundamental Properties of Rose Bengal 25.

(1) See: Rose, R. E. *J. Chem. Educ.* **1926**, *3*, 973.

(2) Acid dyes are those used to dye wool from an acid bath.

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Table I. Visible Absorption Spectra of the Salts in Different Solvents[a]

	EtOH		CH_2Cl_2		DME		toluene	
compd	λ_{max}, nm	ϵ ($\times 10^{-4}$)	λ_{max}, nm	ϵ ($\times 10^{-4}$)	λ_{max}, nm	ϵ ($\times 10^{-4}$)	λ_{max}, nm	ϵ ($\times 10^{-4}$)
Ia	557	11.24			557	8.22		
	517	3.29			518	3.12		
	(490)*	0.86			(490)*	0.89		
	(400)*	0.16			(400)*	0.23		
Ib	558	11.84	566	10.22	565	8.43		
	519	3.56	526	3.75	524	3.30		
	(490)*	0.93	(490)*	0.85	(490)*	0.86		
	(400)*	0.17	(410)*	0.22	(400)*	0.53		
Ic	559	11.59	564	11.42	563	11.07		
	518	3.41	523	2.97	520	2.95		
	(490)*	0.73	(490)*	0.71	(490)*	0.49		
	(400)*		(410)*	0.41	(400)*	0.12		
IIa	564	10.56			569	9.94		
	526	3.29			528	3.93		
	(490)*	0.33			(490)*	0.87		
	(410)*	0.07			(400)*	0.31		
IIb	568	11.94	572	9.26	572	9.23	569	4.34
	526	3.83	530	3.81	530	3.73	540	3.96
	(490)*	0.85	(494)*	1.29	(490)*	0.88	(492)*	1.63
	(410)*	0.44	410	0.88	(400)*	0.37	405	1.07
IIc	567	11.82	573	11.82	572	9.65	574	5.94
	525	3.61	530	3.27	528	2.84	535	2.57
	(490)*	0.65	(490)*	0.51	(490)*	0.51	(494)*	0.64
	(400)*		(410)*	0.32	(400)*	0.37	(400)*	0.23
IId	568	8.64	572	5.26	572	1.78	(570)*	0.21
	527	2.75	528	2.08	525	1.52	(526)*	0.96
	(494)*	0.66	494	1.17	490	1.56	494	1.35
	(410)*	0.20	410	2.71	406	1.51	408	1.69
IIe	566	8.02	572	9.20	573	5.04	568	0.60
	525	2.63	529	2.76	529	1.95	530	0.73
	(490)*	0.97	(492)*	0.68	(490)*	0.89	496	0.71
	(400)*	0.38	404	0.42	(400)*	0.96	400	0.93

[a] Asterisk indicates shoulder.

Table II. Bleaching Rates in Different Solvents[a]

compd	EtOH	DME	CH_2Cl_2
Ia		6.2×10^{-4}	
Ib	6.7×10^{-1}	9.3×10^{-1}	3.8
Ic		2.7×10^{-4}	5.7×10^{-3}
IIa		8.0×10^{-4}	
IIb	1.0×10^{-3}	9.0×10^{-1}	1.6
IIc			3.5×10^{-4}
IId	1.7×10^{-4}	3.4×10^{-3}	7.3×10^{-3}

[a] Bleaching rates were calculated from $[(A_0 - A)/A_0]$/time (min).

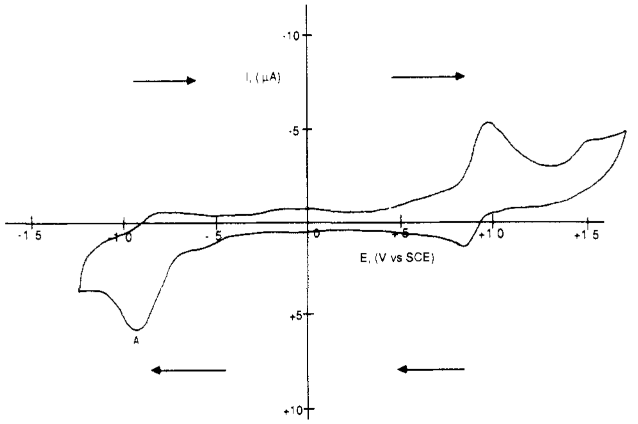

Figure 1. Cyclic voltammogram of IIa in MeOH.

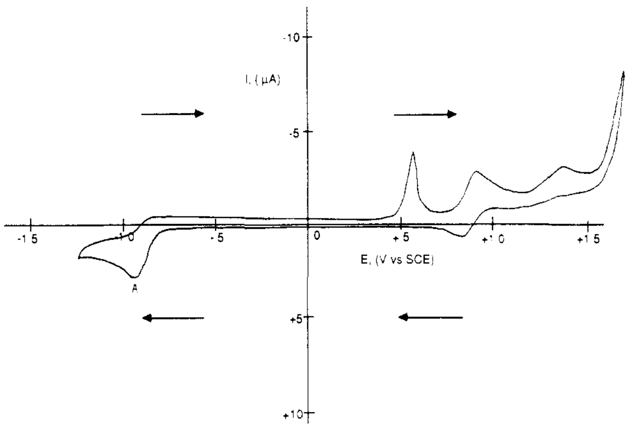

Figure 2. Cyclic voltammogram of IIc in MeOH.

xanthenes and suspect it will be even more important than reductive bleaching in applications of these xanthene systems.

Scheme II

$$\text{dye} \rightarrow \rightarrow [\text{dye}]^{*3}$$

$$[\text{dye}]^{*3} + R_nM^+ \rightarrow \text{dye}^{\bullet} + R_nM^{\bullet 2+}$$

$$\text{dye}^{\bullet} \rightarrow \text{products}$$

$$R_nM^{\bullet 2+} \rightarrow \text{radicals} + R_{n-1}M$$

Table III. Electrochemical Potentials of Rose Bengal Derivatives

compd	E_{red}[a]	E_{ox}[b]	comments[c] 11
Ia	−1.03	+0.86	one-step quasi-reversible reduction
Ib	−1.00	+0.86	two-step irreversible reduction
Ic	−1.05	+0.85	multistep reversible reduction
Id	−1.01	+0.90	two-step reversible reduction
IIa	−0.905	+0.92	one-step reversible reduction
IIb	−0.895	+0.95	multistep irreversible reduction
IIc	−0.875	+0.93	multistep reversible reduction
IId	−0.865	+0.94	one-step irreversible reduction
IIe	−0.86	+0.98	multistep reversible reduction

[a] $E_{red} = (E_{pa} + E_{pc})/2$, where E_{pa} and E_{pc} are the anodic and cathodic peak potentials, respectively; voltage vs SCE. [b] E_{ox} is determined from the peak (A) located at the bottom left corner of the cyclic voltammogram (see Figures 1 and 2); voltage vs SCE. [c] These comments apply only to the Rose Bengal peaks.

The oxidation potential (E_{ox}) of the dye and the reduction potential (E_{red}) of the countercation will clearly determine the bleaching rate. If the reduction potential of the countercation

Table IV. Fluorescence Quenching of Ia and IIa by Ionic Species

compd	E_{ox}	quencher	E_{red}	k_q,[a] M^{-1} s^{-1}
Ia	+0.86	$Ph_2I^+Cl^-$, IIIa	−0.2	2.3×10^{10}
Ia		$Ph_2S^+CH_3BF_4^-$, IIIb	−1.8	4.5×10^9
Ia		$Ph_3P^+CH_2PhCl^-$, IIIc	−2.1	2.7×10^9
Ia		$(n\text{-}Bu)_4N^+Cl^-$, IIId	−2.6	no quenching
IIa	+0.92	$Ph_2I^+Cl^-$		1.26×10^{10}
IIa		$Ph_2S^+CH_3BF_4^-$		3.78×10^9
IIa		$Ph_3P^+CH_2PhCl^-$		no quenching
IIa		$(n\text{-}Bu)_4N^+Cl^-$		no quenching

[a] Calculated from the slope of Stern–Volmer plots as $k_q\tau$, where $\tau = 0.66 \times 10^{-9}$ s is used for Rose Bengal's fluorescence lifetime.[16]

Table V. Fluorescence Quenching of Ia and IIa by Neutral Compounds

compd	quencher	$10^{-9}k_q$,[a] M^{-1} s^{-1}	compd	quencher	$10^{-9}k_q$,[a] M^{-1} s^{-1}
Ia	Ph_3P	6.1	IIa	Ph_3P	3.8
Ia	$PhSCH_3$	2.0	IIa	$PhSCH_3$	2.0
Ia	Ph_2S	1.3	IIa	Ph_2S	1
Ia	PhI	2.1	IIa	PhI	2.1
Ia	Et_3N	1	IIa	Et_3N	no quenching

[a] Calculated from the slope of Stern–Volmer plots as $k_q\tau$, where $\tau = 0.66 \times 10^{-9}$ s is used for Rose Bengal's fluorescence lifetime.[6]

is small, bleaching can occur by means of a rapid process in which the dye is oxidized. The electrode potential at which a compound undergoes reduction or oxidation can be located by cyclic voltammetry.[11] Two examples of cyclic voltammograms for Rose Bengal salts are shown in Figures 1 and 2, and the E_{ox} and E_{red} for each of the Rose Bengal derivatives are summarized in Table III. As shown in Table III, the countercations have little effect on the electrochemical potentials of the dye moiety. Thus, the E_{ox} is +0.855 ± 0.005 V for the Rose Bengal derivatives in the dianionic form (Ia–c) and +0.935 ± 0.015 V for its comparable monoionic structures (IIa–d). All other things being equal, the dye in its dianionic form is more easily oxidized than in its monosalt form. This suggests a faster bleaching for the disalt, and this is shown by our result. For the countercation component E_{red} decreases from −0.200 V for diphenyliodonium,[12] to −1.77 V for diphenylmethylsulfonium,[13] to −2.150 V for benzyltriphenylphosphonium relative to SCE.[14] Based on these data, bleaching rates (k_b) should decrease in the order iodonium salt > sulfonium salt > phosophonium salt. This is precisely what we have observed. On the basis of the observed order of salt bleaching, an oxidative electron-transfer mechanism is proposed for the bleaching of some of the onium salts and illustrated in Scheme III.

Scheme III

$$\text{dye} + h\nu \rightarrow \text{dye}^{*1}$$

$$\text{dye}^{*1} + \text{gegen ion} \rightarrow \text{dye}^{\bullet+} + \text{gegen ion}^\bullet$$

$$\text{gegen ion}^\bullet \rightarrow \text{rad}^\bullet + \text{neutral species}$$

$$\text{dye}^{\bullet+} \rightarrow \text{rad}^\bullet + \text{neutral species}$$

Fluorescence quenching experiments were carried out in CH_3OH at room temperature. In these experiments, the quenching of the fluorescence of 1×10^{-5} M solutions of Ia and IIa by the corresponding iodonium (IIIa), sulfonium (IIIb), phosphonium (IIIc), or ammonium (IIId) salts deriving from unreactive anions was studied. In a related experiment, the fluorescence of the dye was also quenched with corresponding neutral species, i.e., iodobenzene, diphenyl sulfide, triphenylphosphine, and triethylamine. The Stern–Volmer quenching plots of Ia and IIa with ionic and neutral species are summarized in Tables IV and V (see example, Figure 3). The effect of the ionic salt quencher on the fluorescence quenching intensity decreases in the order of iodonium > sulfonium > phosphonium > ammonium. This is the same sequence in which the bleaching rates decrease. The same ionic quencher has a larger effect on the fluorescence intensity of the disalt Ia than on the monosalt IIa, and this also parallels the bleaching rate data. Diphenyliodonium chloride is a very effective singlet quencher. This implies that the photobleaching of Rose Bengal iodonium salt occurs predominantly from the singlet state. In view of the known rate constants of the photophysical events of the Rose Bengal disodium salt, significant (>60%) singlet-state quenching—relative to intersystem crossing—results only when $k_q > 2 \times 10^{10}$ M^{-1} s^{-1} for the quenching species. Assuming a fluorescence lifetime of 0.66 ns, k_q values of (2–5) $\times 10^9$ M^{-1} s^{-1} approximate the known singlet–triplet distribution derived from the disodium salt in polar solvents. The phosphonium and ammonium salts, in contrast to the iodonium salts, react principally from the triplet state. Among the comparable neutral species, triphenylphosphine is the only one that significantly quenches Rose Bengal fluorescence, while the fluorescence quenching of the others is too small to differentiate. This reversed trend in k_q between comparable ionic and neutral quenchers suggests that the xanthenes are capable of undergoing bleaching by different mechanisms with different partner molecules. We suggest that very rapid singlet-state bleaching occurs to produce oxidized dye products in the case of easily reduced partner ions; while much shower triplet-state bleaching producing reduced dye products occurs in the case of neutral species. In the case of the nonreducible counterions, reduction via hydrogen abstraction rather than electron transfer is likely.[9]

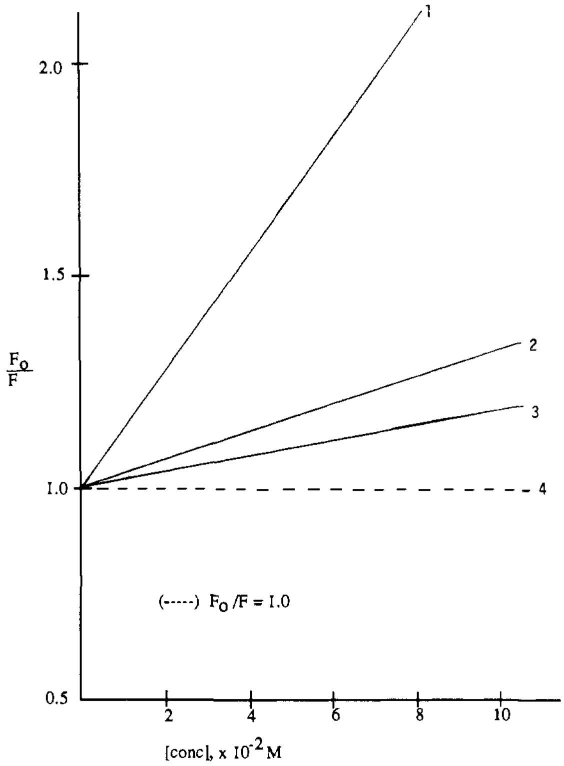

Figure 3. Stern–Volmer plots of Ia quenched by ionic species: (1) Ph_2ICl, (2) $Ph_2SCH_3BF_4$, (3) Ph_3PCH_2PhCl, (4) $(n\text{-}Bu)_4NCl$.

Bleaching rates for both the mono- and disalts are dramatically different in nonpolar and polar solvents (Table II). The relative rates for the monoiodonium salt (IIb), for example, in methylene chloride and in ethanol differ by 3 orders of magnitude. This

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(11) (a) Mabbot, G. A. *J. Chem. Educ.* **1983**, *60*, 697. (b) Kissinger, P. T. *J. Chem. Educ.* **1983**, *60*, 705.

(12) (a) Bachofner, H. E.; Beringer, F. M.; Meites, L. *J. Am. Chem. Soc.* **1958**, *80*, 4269. (b) Mann, C. K.; Barnes, K. K. *Electrochemical Reactions in Non-Aqueous Systems*; Marcel Dekker: New York 1970; pp 221–223.

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(14) (a) Grimshaw, J.; Ramsey, J. S. *J. Chem. Soc., B*, **1968**, *68*, 63. (b) Saveant, J. M.; Binh, S. K. *Bull. Soc. Chem. Fr.* **1972**, 3549.

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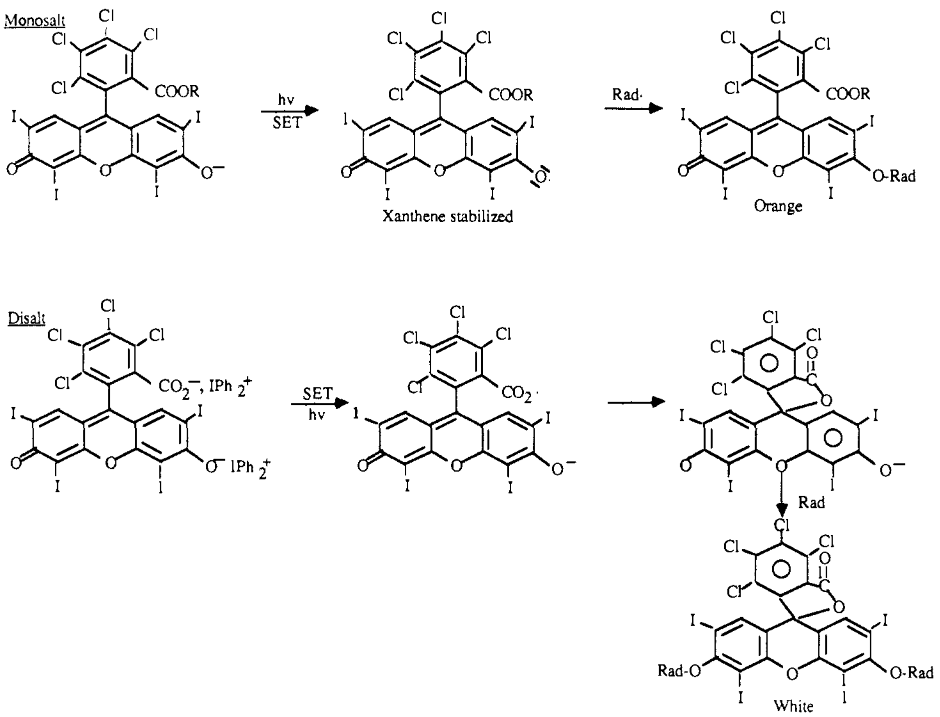
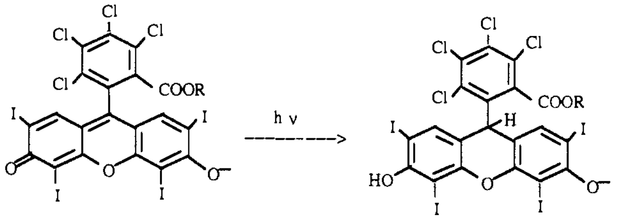
Scheme IV. Oxidative Electron Transfer



Scheme V. Reductive Electron Transfer



result, which derives from the proximity of the to be reduced gegen ion to the xanthene nucleus in the nonpolar solvent, is predicted by the Rehm–Weller equation wherein rate of electron transfer is inversely proportional to the dielectric constant of the solvent.[10]

Chemical Events in the Oxidative Bleaching of Rose Bengal Onium Salts. The rates of bleaching for similar onium salts (Ib,c, IIb–d) are significantly different under similar conditions of light intensity and solvent. Color changes and product composition suggest that the bleaching mechanisms differ in the two cases and that different products derive from the dye in the initial bleaching step. The disalt Ib produces Rose Bengal lactone, which is colorless and has a characteristic lactone carbonyl stretching frequency at 1771 cm^{-1}, upon bleaching. The monosalt IIb, on the other hand, gives rise to a yellow product with visible absorption spectrum similar to the C-3 molecular form. The 1735-cm^{-1} IR peak remains as evidence that the C-2′ ester function also still exists in the product. Thus, in this instance, oxidation most likely yields a C-3 covalent product. Iodobenzene is the partner product in both processes. The chemical events in the oxidative process are shown in Scheme IV; those in the reductive process, in Scheme V.

No quenching of fluorescence of IIa is observed in the case of either ammonium salt IIId or phosphonium salt IIIc, nor is the fluorescence of Ia quenched by the ammonium salt IIId. Reductive bleaching of the xanthenes (Scheme V), which is the process previously recognized in the literature,[9] must be several orders of magnitude slower than the oxidative process, the outer limits of which are placed by the cases of the iodonium salts studied here.

In summary, bleaching rates of Rose Bengal salts are effected by the gegen ion and the solvent. Oxidizing gegen ions intercept the singlet, and this leads to oxidized products of the dye and radical products form the gegen ion. Disalts reduce more rapidly than do monosalts in every case compared. Though detailed product studies of the dye are in progress, it seems clear that the recognition of oxidative routes to photobleaching the xanthenes opens a number of new opportunities for applications of these systems.

Experimental Section

Rose Bengal (92%), tetra-*n*-butylammonium perchlorate (GFS) and all other reagents (Alfa) were used as received. Rose Bengal onium salts (Ib,c, IIb–d) were prepared according to the syntheses outlined in our previous paper.[8] Spectrophotometric grade solvents (Aldrich) were used in all irradiation runs and for the cyclic voltammetric measurements. Absorption spectra were recorded on a Varian Cary 219 UV-vis instrument. The photobleaching studies were conducted with a Bausch and Lomb high-intensity monochromator (560 nm) with an Osram SP-200 mercury lamp. Fluorescence quenching was measured at room temperature on a Perkin Elmer (MPF-44A) fluorescence spectrophotometer.

CV (cyclovoltammetric) measurements were made on an EG and PAR Model 175 universal programmer with Houston Omnigraphic 2000 XY recorder utilizing three electrode systems. A glassy carbon disk served as the working electrode, a platinum disk served as the counter electrode, and a commercial saturated calomel electrode (SCE) served as the reference electrode. The SCE was separated from the bulk of solution with a fritted glass bridge filled with solvent and supporting electrolyte. All electrolyte solutions were degassed with argon for at least 10 min before experiment. An electrolyte solution of 0.1 M tetra-*n*-butylammonium perchlorate (TBAP) in MeOH was used to measure the redox potentials of dyes. The reference standards were 0.1 M TBAP–MeOH. It was frequently necessary to use ultrasonics to clean the adsorbed dye on the working glassy carbon disk and auxillary platinum disk electrode, but this proved no problem after practice. Fast scan rates (100 mV s^{-1}) were used to limit the possibility of a secondary chemical reaction interfering with the reversal current magnitude, thus preserving a reversible wave shape. Slow scan rates (50 mV s^{-1}) were used to stretch the criterion of electrochemical reversibility as far as practical.

Acknowledgment. This work has been supported by the National Science Foundation. We are grateful for their support. We thank Prof. V. S. Srinivasan for making his cyclic voltammetric instrumentation available for our use, to Eliane Chesneau for helpful discussions and Manachi Bagchi for carrying out the CV measurements and the fluorescence quenching data.

Registry No. Ia, 74198-03-1; Ib, 112374-53-5; Ic, 112374-54-6; Id, 97816-39-2; IIa, 88157-09-9; IIb, 112374-56-8; IIc, 112374-57-9; IId, 112374-58-0; IIe, 112374-60-4; IIIa, 1483-72-3; IIIb, 10504-60-6; IIIc, 1100-88-5; IIId, 1112-67-0; Ph_3P, 603-35-0; $PhSCH_3$, 100-68-5; Ph_2S, 139-66-2; PhI, 591-50-4; Et_3N, 121-44-8.