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Bleaching Products of Rose Bengal Under Reducing Conditions

by

Andrzej Zakrzewski[†] and D.C. Neckers[*]
Center for Photochemical Sciences
Bowling Green State University
Bowling Green, OH 43403

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Abstract: The bleaching behavior of Rose Bengal under reducing conditions has been elucidated by determining the products of chemical and photochemical reduction of Rose Bengal.

Rose Bengal is a xanthene dye the structure of which has been well known since the 19th Century[1]. Named for the so-called "Indian Happiness Wart"[2], this red dye related to fluorescein has been important in photochemistry since Schenck and his co-workers popularized that one could use it conveniently in the "dye sensitized" photooxygenation[3] of organic substrates. Rose Bengal is only useful in polar, most generally protic solvents - a condition under which it bleaches in just a few hours when used in synthetic applications for the formation of singlet oxygen.

As we have recently shown[4], Rose Bengal bleaching occurs either under conditions in which the dye is oxidized by a partner ion (or by oxygen) or reduced. Whether oxidation or reduction occurs depends on the relative electrochemical potentials of the partner molecule or ion. Iodonium salts and diazonium salts are oxidizing, for example, whereas tertiary amines are reducing. In the latter context, it has been well known since the work of Oster and coworkers[5] that various amines accelerate this bleaching behavior. In their study Oster, Oster, and Kang suggested that the bleaching of Rose Bengal was accompanied by deiodination and that the ultimate product was (tetrachloro)fluorescein. Eaton, in an extensive review[6], reports that many xanthene dye - reducing agent combinations can be used as photoinitiators for vinyl polymerization. Thus it seems likely to assume that bleaching is accompanied by free radical formation if reduction of the dye occurs.

This is the first in a series of reports on the products from, and mechanisms by which, Rose Bengal undergoes photobleaching. In this report we describe the products of chemical reduction of Rose Bengal and identify the photoproduct of Rose Bengal and triethanolamine. Based on the earlier work of Davidson and Tretheway[7], as well as our own[8], the photochemical reduction of Rose Bengal in the presence of a tertiary amine is a triplet process and dye reduction either by oxygen or by the partner amine competes with energy transfer to acceptor oxygen. In view of the electrochemical behavior of the related xanthene, Erythrosin, the photochemical reduction of Rose Bengal must proceed by single electron transfer steps at the quinomethine unit of the dye.[9] Oxidative transfer of an electron from the dye to a partner reducing ion is orders of magnitude faster than reduction from the triplet state of the dye and must be a singlet state process.[9] The oxidative products of Rose Bengal will be reported in the subsequent publication.[10]

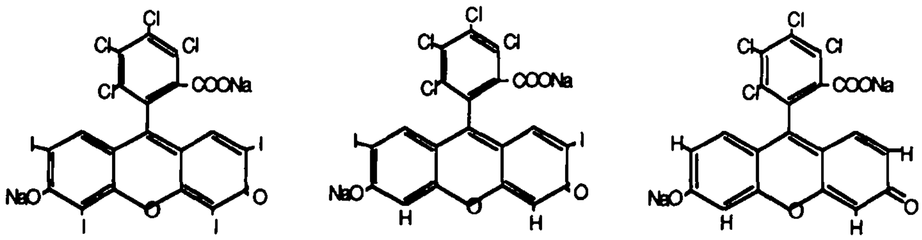
Photoreduction of Rose Bengal:

According to Oster, the photoreduction of Rose Bengal (1.8×10^{-5} M) in water, under oxygen with triethanolamine and white light ultimately produces "fluorescein"[5]. Irradiation of Rose Bengal with green light under the same conditions produces a compound which, from the spectrum, must be 2,7-diiodo-tetrachlorofluorescein[††], Eq 1.

[†] Visiting Scholar from the Department of Chemistry and Chemical Engineering, Technical and Agricultural University, Bydgoszcz, Bydgoszcz, Poland 1986-87.

[*] To Whom Inquiries Should be Addressed

[††] In the older literature, 3,9-dihydrofluorescein is called "fluorescin". On standing in air, fluorescin is known to oxidize to fluorescein.



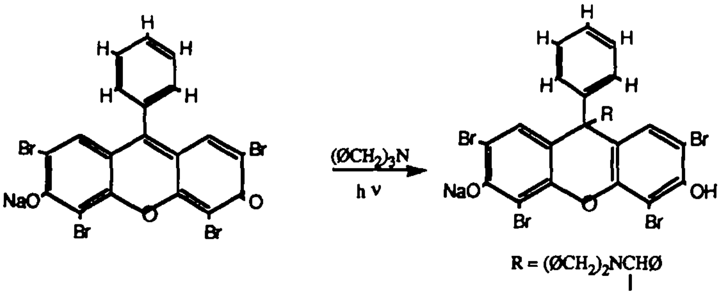
Rose Bengal, C-6, C-2' disodium salt: 2,4,5,7-tetraiodotetrachlorofluorescein

4,5-dihydro - 2,7-diiodo-tetrachlorofluorescein

Tetrachlorofluroescein

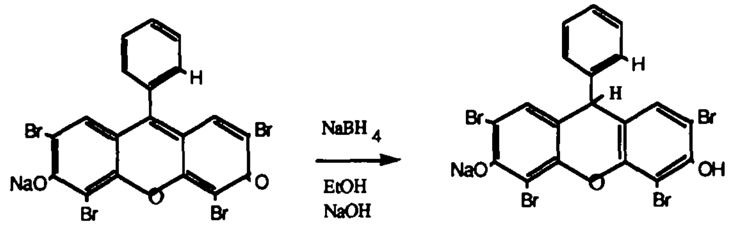
Eq 1

English workers[11] recently reported that decarboxylated Eosin, when irradiated in tribenzylamine, gave a reduced triarylmethane, Eq 2.



Eq2

Chemical reduction of decarboxylated Eosin, with sodium borohydride, produces 3,9-dihydroeosin† , Eq 3.



Eosin, monosodium salt C-2' decarboxyalte

Eosin, disodium salt 2',3,9 trihydro

Eq 3

The electrochemical reduction in a standard polarograph at pH 4.7 of Erythrosin occurs in steps the first of which is a 2 electron reduction occurring at a half wave potential of -.53v. The product reportedly is 2,7-diiodofluorescein[9]. Though no products were reported, the electrochemical reduction of Rose Bengal under similar conditions occurs at a half wave potential of -.60 v. No products were reported in this study.[12].

We have studied the chemical reduction of Rose Bengal, disodium salt (Ia) of Rose Bengal, C-2' methyl ester, C-6 molecular form (II-a) and of Rose Bengal, C-6 methyl ether, C-2' methyl ester (III-a)under a variety of reducing conditions.

Reduction of Rose Bengal with zinc and acetic acid under a variety of experimental conditions led to reduction of the quinomethine, and the accompanying deiodination, first at carbons 4 and 5, and then at carbons 2 and 7. Three

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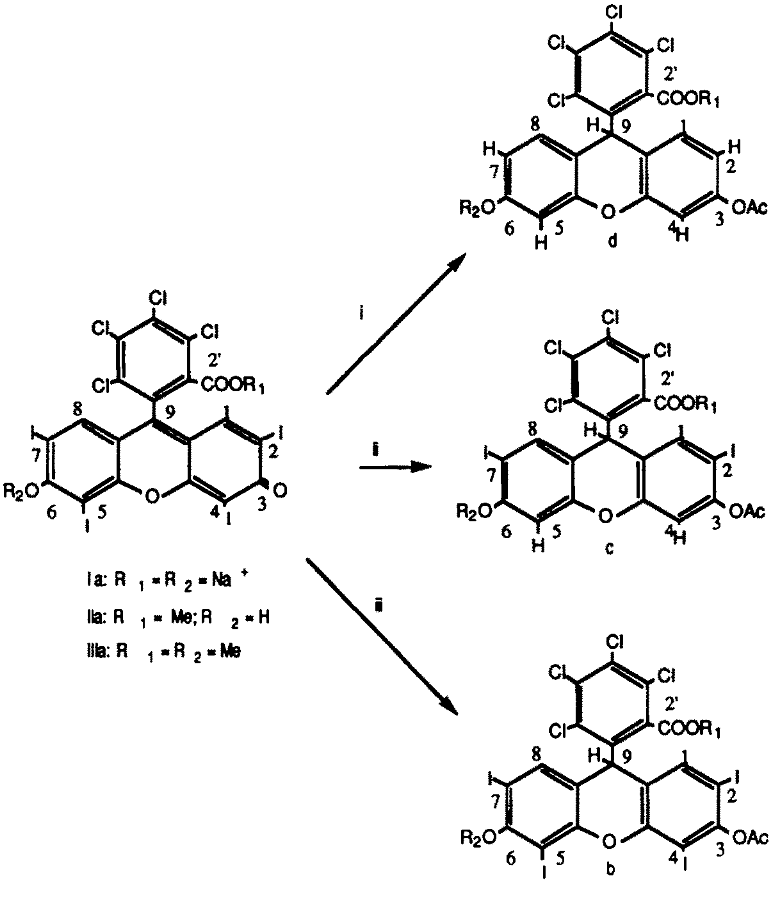
† Eosin, like Rose Bengal and Erythrosin are trade names. Because it is so much simpler to name derivatives of these compounds by referring to the structure of the parent, trade-named molecule rather than by their IUPAC name, we do this throughout our papers without apology.

reduction products could thus be obtained; C-9 hydro; the partially deiodinated product C-4,5,9 trihydro; and the totally deiodinated C-2,4,5,7,9 pentahydro. The products were isolated after acetylation, Equation 4. The accompanying lactone diacetate (I-a) was observed as the product of no reduction. The conditions under which each were obtained are given in Table II. As expected, the degree of deiodination increases with the higher reaction temperature and larger quantities of Zn used. For example, the total deiodination compound I-d was the only compound obtained in Run 1 (130°) while the half deiodination I-c was the sole product in Run 3 (60°).

The results confirm the greater lability of the 4,5 iodines to chemical reduction and that total deiodination derives under forcing conditions (130°, 24 h). It is also clear from the results that photochemical reduction under neutral conditions - which produces (Ib) exclusively - likely proceeds through a different mechanism.



Eq. 4: Ia i; (1) Zn, HOAc, 24 h, reflux; (2) Ac_2O, HOAc
ii; (1) Zn, HOAc, 60°, 24 h; (2) Ac_2O, HOAc
iii; (1) $(HOC_2H_4)_3N$, DME, 4 h hv; (2) Ac_2O

Eq. 5: IIa i; (1) Zn, HOAc, 24 h, reflux; (2) Ac_2O, HOAc
ii; (1) Zn, HOAc, 60°, 2 h; (2) Ac_2O, HOAc
iii; (1) $(HOC_2H_4)_3N$, DME, 4 h hv; (2) Ac_2O

Eq. 6: IIIa i; (1) Zn, HOAc, 24 h, reflux; (2) Ac_2O, HOAc
ii; (1) Zn, HOAc, 60°, 30 m; (2) Ac_2O, HOAc
iii; (1) $(HOC_2H_4)_3N$, DME, 4 h hv; (2) Ac_2O

Chemical reduction under less acidic conditions produces similar results but with even more extensive xanthene ring deiodination, Table III. Thus under conditions of reduction of rose bengal disodium salt with Zn/NH_4Cl virtually no quinomethine product (Ib) is formed and the reduction invariably proceeds with the removal of the 4,5 iodines. These are the only chemical conditions (Zn,HOAc and Zn/NH_4Cl) which - in our hands - reduce Rose Bengal cleanly to tractable products.

Chemical reduction of the C-2' methyl ester (II-a), and the C-2' methyl ester, C-6 methyl ether (III-a) also led to deiodination at the positions 2,4,5, and 7 depending on conditions; Equations 5 and 6.

The photochemical reduction of Rose Bengal, di sodium salt, in triethanolamine/DME when irradiated for four hours with light (>500 nm), produces an intermediate which, upon treatment with acetic anhydride, yields C-3,C-6 diacetyl, C-9 hydro, Rose Bengal C-2' carboxylic acid. Thus, whereas the chemical reduction even under the mildest conditions always results in extensive deiodination of the xanthene ring, Tables II and III, photochemical reduction leads exclusively to the two electron quinomethine product when the irradiation is directly into the long wavelength absorption bands of the dye. This suggests that Oster's report[5] of photochemical dehalogenation is the result of the broad band polychromatic light source. The likely explanation is that π–π^* dehalogenation of the aromatic nucleus leads to iodine atoms and eventually HI. There are many examples of such processes in the older literature[13] but these reactions virtually all derive from radical processes and involving carbon-halogen cleavage of the excited state.

The C-9 reduced product is susceptible to air, and care must be taken to prevent its reoxidation in the absence of reducing agents.

Mechanism of Photoreduction of Rose Bengal in the Presence of Triethanolamine

The oxidation potential of Rose Bengal is +.85 v, the reduction potential -1.20 v. Depending on solvent, the triplet energy of Rose Bengal is about 39.5 kcal/mol. With triethanolamine, the only electron transfer process which is exothermic is that of reduction;

$$\mathrm{Dye}^{*3} + R_3N\!: \dashrightarrow \mathrm{Dye}^{-\cdot} + R_3N^{+\cdot}$$

In the next step, the triethanolamine radical cation loses a proton;

$$(HOCH_2CH_2)_3N^{+\cdot} \dashrightarrow H^+ + (HOCH_2CH_2)_2N\dot{C}HCH_2OH$$

$$(HOCH_2CH_2)_2NCHCH_2OH + \mathrm{Dye}^{-\cdot} \dashrightarrow \text{Dihydro Dye} + [(HOCH_2CH_2)_2NCH{=}CHOH]$$

$$[(HOCH_2CH_2)_2NCH{=}CHOH] \dashrightarrow (HOCH_2CH_2)_2NCH_2CHO$$

On the other hand, chemical reduction under acid conditions causes extensive deiodination first at C-4,5 and subsequently at C-2,7.

Experimental

Rose bengal (Dye Tel) and all other reagents were used as received. Authentic samples of 3.6-0.0'-diacetyl rose bengal (I-a) and rose bengal, C-2' methyl ester, C-6 methyl ether (III-a) were synthesized using the methods published previously.[14,15] Infrared spectra were recorded on a Nicolet 20DX FT infrared spectrometer and nmr spectra were taken using a Varian XL-200 nuclear magnetic resonance spectrometer. The photoreductions were carried out with > 500 nm radiation source in a merry-go-round apparatus equipped with a medium pressure mercury-vapor lamp (Hanovia, 450 W) and sharp cut-off filters (Corning 3486). All new compounds gave satisfactory elemental analyses.

Synthesis of I-b

A solution of Rose Bengal (Ia) (0.1 g) and triethanolamine (1.49 g) in DME (100 ml) was irradiated for 4 hours with >500 nm. After irradiation 2ml of concentrated HCl was added to the solution and the solvent removed in vacuo. The precipitate was washed with water, and subsequently refluxed with 25 ml of acetic anhydride overnight. After cooling the solution was poured into 600 ml water and precipitate was filtered.

T.L.C. on 20 x 20 cm silica gel PKSF plates (Whatman) (C_6H_6 : EtOAc : AcOH = 70 : 29 : 1) separated the main component. Yield 0.07g (67.2%).

IR (KBr) ν cm^{-1} : 1771 (Acetyl), 1735 (COOH)

NMR ($CDCl_3$) δ : 7.49 (S,2H,$H_{1,8}$), 5.74 (S,1H,H_9), 2.92 (S,6H,CH_3CO)

Synthesis of I-c

A sample of Rose Bengal (2 g) was reduced with Zn-dust in (2 g) glacial acetic acid (100 ml) at 60° C for 24 hour. After decantation, to the solution was added 50 ml acetic anhydride and the solution was refluxed overnight. After cooling, the solution was poured into 800 ml of water and the precipitate was filtered. Yield 1.16g (71.8%).

IR (KBr) ν cm^{-1} : 1771 (Acetyl) 1735 (COOH)

NMR ($CDCl_3$) δ : 7.34 (S, 2H,$H_{1,8}$), 6.83 (S,2H,$H_{4,5}$), 5.54 (S,1H,H_9), 2.34 (S,6H,CH_3CO)

Synthesis of I-d

A sample of Rose Bengal (2 g) was reduced with Zn-dust (2 g) in glacial acetic acid (100 ml) under reflux for 24 hours. After decantation, to the solution was added 50 ml acetic anhydride and the solution was refluxed overnight. After cooling, the solution was poured into 800 ml of water and the precipitate was filtered. Yield 0.81g (79%).

IR (KBr) νcm^{-1} : 1771 (Acetyl) 1735 (COOH)

NMR ($CDCl_3$) δ : 6.96 (d ,2H,$H_{1,8}$) , 6.83 (s ,2H,$H_{4,5}$,), 6.74 (d ,2H,$H_{2,7}$), 5.62 (s ,1H,H_9), 2.26 (s, 6H,CH_3CO)

Synthesis of II-a

Rose Bengal (2.04 g) was dissolved in THF (20 ml) and iodomethane (5.5 g) and tetrabutyl ammonium chloride (0.1 g) were added. The resulting solution was refluxed for 12 hours. After cooling, the solution was poured into 180 ml of water and the precipitate was filtered. After drying the orange-red powder was dissolved in a mixture of DME-acetic acid (1:1) and poured into 5% HCl. The resulting slurry was filtered and thoroughly washed with water to remove all the excess HCl. The residue was dried at 40° in vacuum. Yield 9.02 (82.8%).

IR (KBr) ν cm^{-1} : 3430 (phenolic OH), 1735 ($COOCH_3$)

NMR ($CDCl_3$) δ :7.41 (s, 2H,$H_{1,8}$), 3.58 (s, 3H,$COOCH_3$)

Synthesis of II-b

A solution of Rose Bengal, methyl ester molecular form (II-a,.01 g) and triethanolamine (1.40 g) in DME (100 ml) was irradiated for 4 hours with >500 nm. After irradiation, 2 ml concentrated HCl was added to the solution and the solvent removed in vacuo. The precipitate was washed with water and subsequently was refluxed with 25 ml of acetic anhydride overnight. After cooling the solution was poured into 600 ml of water and the precipitate was filtered. Yield 0.079 (72.3%).

TLC on a 20 x 20 cm PKSF silica gel (Whatman) plate separated the product.

IR (KBr) ν cm^{-1} : 1771 (Acetyl), 1735 ($COOCH_3$)

NMR $CDCl_3$ δ : 7.37 (s, 2H,$H_{1,8}$), 5.41 (s, 1H,H_9),4.04 (s, 3H,$COOCH_3$), 2.42 (s,6H,CH_3CO)

Synthesis of II-c

Rose Bengal, methyl ester molecular form (0.2 g), and Zn-dust (0.2g) in glacial acetic acid (20 ml) thermostated at 60° C was mixed for two hours. After separation, the precipitate was added to a solution of 20 ml acetic anhydride and refluxed overnight. After cooling, the solution was poured into 240 ml of water and the precipitate was filtered. Yield 0.72g (72.2%).

IR (KBr) ν cm^{-1} : 1771 (CH_3CO) 1735 ($COOCH_3$)

NMR ($CDCl_3$) δ : 7.34 (s , 2H,$H_{1,8}$), 6.86 (s, 2H,$H_{4,5}$), 5.39 (s, 1H,H_9), 4.04 (s, 3H,$COOCH_3$),
2.34 (s, 6H,CH_3CO)

Synthesis of II-d

Rose Bengal, methyl ester molecular form (0.2 g) and Zn-dust (0.2 g) in glacial acetic acid (20 ml) was refluxed for 24 hours. After separation of the precipitate acetic anhydride (20 ml) was added to the solution and it was refluxed overnight. After cooling, the solution was poured into 240 ml of water and the precipitate was filtered. Yield 0.095g (82.6%).

IR (KBr) ν cm^{-1} : 1771 (CH_3CO) 1735 ($COOCH_3$)

NMR ($CDCl_3$) δ : 7.02 (d, 2H,$H_{1,8}$), 6.83 (s, 2H,$H_{4,5}$), 6.74 (d, 2H,$H_{2,7}$), 5.39 (s, 1H,H_9),
4.04 (s, 3H,$COOCH_3$), 2.34 (s, 6H,CH_3CO)

Synthesis of III-b

A solution of Rose Bengal methyl ester-methyl ether (0.1 g) and triethanolamine (1.49 g) in DME (100 ml) was irradiated for four hours at >500 nm. After irradiation, 2 ml of concentrated HCl was added to the solution and the solvent removed in vacuo. The precipitate was washed with water and then refluxed with 25 ml of acetic anhydride overnight. After cooling, the solution was poured into 600 ml of water and the precipitate was filtered. After TLC on a 20 x 20 cm PKSF silica gel (Whatman) plate the following products was separated. Yield 0.07g (69.2%).

IR (KBr) νcm^{-1} : 1771 (CH_3CO), 1735 ($COOCH_3$), 1130, 1052 (OCH_3)

NMR ($CDCl_3$) δ : 7.39 (s, 1H,H_8), 7.32 (s, 1H,H_1), 5.41 (s, 1H,H_9,), 4.04 (s, 3H,-OCH_3)
3.86 (s, 3H,$COOCH_3$), 2.42 (s, 3H,CH_3CO)

Synthesis of III-c

Rose Bengal methyl ester, methyl ether (0.2 g) and Zn-dust (0.2 g) in glacial acetic acid (20 ml) was mixed at 60° for 30 minutes. After separation of the precipitate 20 ml of acetic anhydride was added to the solution and it was refluxed overnight. After cooling, the solution was poured into 240 ml of water and the precipitate was filtered. Yield 0.102g (64.8%).

IR(KBr) ν cm^{-1} : 1771 (CH_3CO), 1735 ($COOCH_3$), 1130,1052 (OCH_3)

NMR ($CDCl_3$) δ : 7.39 (s,1H,H_8), 7.29 (s, 1H,H_1), 6.83 (s, 1H,H_5), 6.48 (s, 1H,H_4),
5.34 (s,1H,H_9), 4.04 (s, 3H,-OCH_3), 3.86 (s, 3H,$COOCH_3$), 2.34 (s, 3H,CH_3CO)

Synthesis of III-d

Rose Bengal methyl ester, methyl ether (0.2 g) and Zn-dust (0.2 g) in glacial acetic acid (20 ml) at 60° was stirred for six hours. After separation of the precipitate to the solution was added 20 ml of acetic anhydride and it was refluxed overnight. After cooling, the solution was poured into 240 ml of water and the precipitate was filtered. Yield 0.08g (74.5%).

IR (KBr) ν cm^{-1} : 1771 (CH_3CO), 17345 ($COOCH_3$) 1130, 1052 (OCH_3)

NMR ($CDCl_3$) δ : 7.00 (d, 1H,H_8), 6.95 (d, 1H,H_1), 6.85 (s, 1H,H $_5$), 6.83 (s, 1H,H_4), 6.71 (d,1H,H_7),
6.55 (d, 1H,H_2), 5.41 (s, 1H,H_9), 4.02 (s, 3H,-OCH_3), 3.79 (s, 3H,$COOCH_3$),
2.26 (s, 3H,CH_3CO)

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