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ROSE BENGAL DERIVATIVES AS SINGLET OXYGEN SENSITIZERS

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Abstract—Derivatives of Rose Bengal are reported and the relationships between subtle structural and dramatic changes in photophysical properties pointed out.

Rose Bengal was discovered little over a century ago by Gnehm shortly after Baeyer's[1] elegant papers describing the syntheses of fluorescein, rhodamine and phenolphthalein. Recognized by organic chemistry students of a former day as structurally related dyes of the triphenylmethane series, fluorescein, rhodamine and phenolphthalein, and many of their derivatives, played a critical role in the development of synthesis and structure determination in organic chemistry.

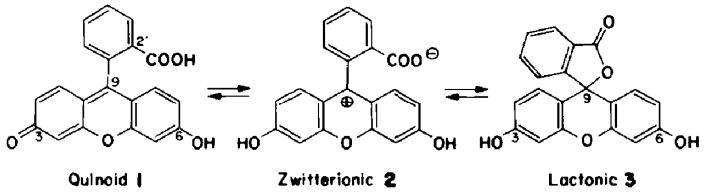
So too, Rose Bengal (3-, 4-, 5-, 6-tetrachloro-2,4,5,7-tetraiodouranine). Though its major recognition is by photochemists as a premier sensitizer for singlet oxygen formation, it was one of those very important early dyes on which much of the original chemical industry was based.

Raab,[2] in 1900, observed that paramecia, when exposed to acridine, were killed only in the presence of light but not in the dark, and this process was later termed the 'photodynamic effect'.[3] In 1931 Kautsky and DeBruijn[4] first proposed that singlet oxygen was the reactive intermediate in dye-sensitized oxygenations though Windaus and Brunken[5] had earlier reported dye-sensitized photo-oxygenation yielding an isolatable peroxide. Subsequently, Kautsky's singlet oxygen was discounted in favour of a mechanism in which the dyestuff sensitizer was excited to a metastable state having biradical character, the latter reacting with oxygen to form a labile sensitizer–oxygen complex. This complex was then suggested to transfer oxygen to the substrate giving the photo-oxygenation product.[6,7] The first detailed kinetic investigation of dye-sensitized oxygenation was reported by Schenck[8] in 1951, and this was followed by the development of a method for determining the quantum yield of triplet formation of the sensitizer.[9] A procedure based on quenching all dye triplets with oxygen and the subsequent trapping of all the singlet oxygen with a very reactive acceptor was developed by Schenck and Gollnick with the reaction rates thus obtained being independent of acceptor concentration. Presently, it is generally accepted that singlet oxygen is the reactive intermediate in dye-sensitized photo-oxygenation reactions.[10–16] Apart from their action as singlet oxygen sensitizers (type II mechanism) the n,π^* triplets of the halofluorescein dyes may also interact directly with substrates (type I mechanism).[17–23] This often leads to hydrogen transfer or electron transfer, especially with easily oxidizable (phenols, amines) or reducible substrates (quinones).[20–22] A concomitant dehalogenation of the dye may take place.[19–21] Recently, the photodimerization of 2-acyl-1,4-benzoquinones in the presence of Rose Bengal has been reported.[24] The purity of halofluorescein dyes has always presented a major problem. Even recently published chromatographic purification methods are unsatisfactory from a preparative point of view.[25,26] Other groups have resorted to preparation of purer dyes through carefully controlled halogenation conditions in the final synthetic step.[27]

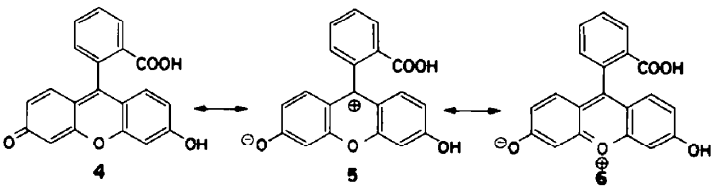
In addition to their wide application in textile colouring[28] and biological staining,[29] the number of studies devoted to the interaction of dyes and biopolymers or living cells is enormous[30] and a fair portion of these deal with the influence of fluorescein dyes, most often Rose Bengal (**10**), on proteins,[31] intact cells[32,33] and membranes.[34–40] Erythrosin and Rose Bengal have also been used successfully in insect control.[41–48] Rose Bengal has potential applications in the photochemical treatment of excessive algal growth in water[49] and the degradation of organic phosphate pesticides in waste water for which model studies have been carried out.[50,51] In recent years, photodynamic destruction of tumours has experienced a revival with promising results which justify increasing research efforts in the biological activity of the photosensitizing dyes.[30]

The structure of fluorescein and its derivatives

The relationships between the colour and spectral properties of fluorescein (**1–3**, Scheme 1) and its molecular structure has been studied for many decades.[52–70] Although many contradictory inter-



Scheme 1. The three modifications of fluorescein.

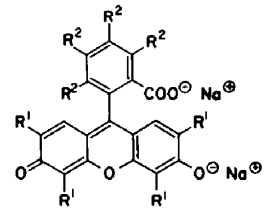


Scheme 2. Some mesomeric structures of fluorescein.

pretations have emerged from spectral studies, authors generally agree on a quinoid structure, **1**, for the red modification and a lactonic structure, **3**, for the colourless modification of fluorescein. Quinoid fluorescein (**1**) consists of a 6-hydroxyxanthen-3-one chromophore substituted at C-9 with a 2'-carboxyphenyl group and lactonic fluorescein (**3**) of a 3,6-dihydroxyxanthene substituted at C-9 with an aromatic lactone. Recently, a third, yellow modification has been isolated to which a zwitterionic structure, **2**, has been assigned.[64] Henceforth, the fused three-ring part of the molecule will be referred to as the xanthene moiety. Structures **1**–**3** show that there is an intimate relationship between the 2'-carboxyl group and the quinone of **1**. Formation of the lactone is always accompanied by destruction of the quinone and, consequently, the chromophore forming the phenol. Consequently, **3** has limited reactivity towards nucleophiles. The nucleophile will not attack the carbonyl group of the lactone, but act as a base and abstract one of the very acidic phenolic protons of **3** which leads to formation of the carboxylate monoanion of the quinoid modification. Scheme 2 shows three mesomeric structures of quinoid fluorescein. The xanthene core of the molecule and, in particular, C-9 is positively polarized explaining the special reactivity of the carboxyl group towards C-9 and the particular chemistry of fluorescein and related compounds.

X-ray studies have been reported for the lactonic and quinoid modification.[65,66] The aromatic ring of the lactone was found to be almost perpendicular to the xanthene moiety, the two outer rings of which are inclined at 9.4°. The bond between C-9 of the xanthene and the lactone-oxygen was exceptionally long (1.525 Å) and this evident weakness explains the ready cleavage of this bond in solution. In the quinoid modification the 2'-carboxyphenyl group is also perpendicular to the xanthene moiety, the three fused rings of which are coplanar which corresponds well with the xanthene being an extensive delocalized system. A number of studies have focused on the influence of pH and molecular aggregation on the absorption and fluorescence spectra of fluorescein.[54–58] The 2'-carboxy group is very important to the fluorescence quantum yield; its absence leads to an increase of the radiationless deactivation of the excited singlet state and, consequently, to a decreased quantum yield of fluorescence.[59,60]

Substitution with halogens has a large influence on the photophysical properties of fluorescein (Scheme 3).[61–63] The absorption maxima are moved to longer wavelength and the quantum yield of intersystem crossing increases (heavy atom effect). This is important since the triplet state of fluorescein dyes can sensitize



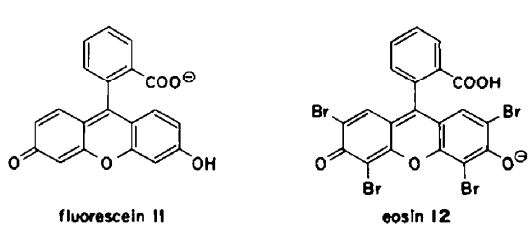
		R'	R^2	ϕ_F	ϕ_{ST}	$\phi_{^1O_2}$	λ_{max} (nm)
7	uranine*	H	H	0.93	0.03	0.1	491
8	eosin	Br	H	0.63	0.3	0.4	514
9	erythrosin	I	H	0.08	0.6	0.6	525
10	rose bengal	I	Cl	0.08	0.76	0.76	548

* Uranine is the disodium salt of fluorescein

Scheme 3. The photophysical properties of fluorescein and its halo-derivatives.[20,21]

the formation of singlet oxygen. Therefore, the quantum yield of singlet oxygen formation increases dramatically with the increasing number and atomic mass of the halogens that are introduced.[69,71]

Recent work by Fompeydie *et al.*[68] shows an interesting difference in the evolution of the structure with the pH of the solution between fluorescein and its tetrabromo derivative eosin (**8**). In fluorescein the carboxylic acid group is more acidic than the phenol of the xanthene moiety. This is reversed in eosin so the monoanionic species of fluorescein and eosin look like **11** and **12**, respectively (Scheme 4). The four electron attracting bromine atoms in eosin stabilize the negative charge of the phenolate. Dissociations of the phenol and carboxylic acid groups in eosin were visible in the electronic absorption spectrum as a large red-shift and a small blue-shift, respectively. Obviously, a change in the charge distribution in the chromophore will have the larger influence on the spectrum. The difference in acidity between the carboxylic acid and phenol groups of the halogenated fluoresceins and the change of the electronic absorption spectrum on dissociation of these groups is of crucial importance in the Rose Bengal chemistry and photophysics described below.



fluorescein 11 eosin 12

Scheme 4. The monoanionic species of fluorescein and eosin.

The development of new Rose Bengal type singlet oxygen sensitizers

Rose Bengal is a widely used and very efficient singlet oxygen sensitizer. It is commercially available as the disodium salt (**10**) which is essentially insoluble in non-polar solvents. Singlet oxygen is, therefore, not formed effectively in these solvents from **10**.

Almost 10 years ago, a way round this solubility problem was designed by immobilizing Rose Bengal to lightly crosslinked polystyrene beads.[72] The resulting polymer-supported Rose Bengal is compatible with most non-polar solvents because of the non-polar polystyrene backbone. One of ®-Rose Bengal's important limitations, however, is that the quantum yield of singlet oxygen formation decreases to 0.43 as compared to 0.76 for Rose Bengal in solution.

A second major disadvantage of commercial Rose Bengal is its purity, which is often only *ca* 90%, the remainder being mainly inorganic contaminants. Elaborate small-scale purification attempts have been reported, but these provide only quantities of reasonably pure material that are far too small to be attractive as a starting point for further synthesis.[25,26] In a sense, therefore, many elegant photophysical and photochemical experiments have been performed with Rose Bengal of 90% purity or less.

We describe herein the development, in our laboratories, of new Rose Bengal type singlet oxygen sensitizers, Schemes 5 and 6. Attention will be drawn to relationships between subtle structural modifications and sometimes dramatic changes in photophysical properties. In the syntheses to be described the difference in nucleophilicity between the carboxylate and phenolate group of Rose Bengal is a structural feature which is advantageously used to develop a versatile method for forming derivatives of Rose Bengal. The syntheses presented here also constitute a high-yield purification method for Rose Bengal and avoid using less satisfactory, complicated elaborate chromatographic procedures.

Phase 1. *Solubility in non-polar solvents by elimination of the negative charges* (*Scheme* 5, *Table* 1)

Initially, the synthesis of Rose Bengal derivatives soluble in non-polar solvents was attempted by converting the carboxylate and phenolate groups of Rose Bengal disodium salt (**10**) to an ester and an ether function, respectively, by treatment with an excess of alkyl halide. Reaction of the purple compound (**10**) with 2.5 equivalents of benzyl chloride in DMF yielded a purple product that was insoluble in dichloromethane. The lack of colour change indicated that no substitution had taken place at the phenolate site and that the charge distribution in the chromophore was the same as in **10**. The IR spectrum of the new compound showed an absorption band at 1730 cm^{-1} corresponding to an ester C=O. The electronic absorption spectrum in methanol had the same shape as that of **10** in methanol, but was shifted to the red by 6 nm. These data indicated that the product was Rose Bengal benzyl ester, monosodium salt (**23**). Hurd and Schmerling had succeeded in preparing fluorescein allyl ester allyl ether in practically quantitative yield by refluxing fluorescein with a 25% excess of allyl bromide in an acetone–water mixture. We repeated the reaction of **10** with benzyl chloride in acetone–water (50%) and obtained a product with an orange–red colour. This compound was soluble in dichloromethane and produced a solution of the same colour. The electronic absorption spectrum in dichloromethane showed maxima at 496 and 407 nm indicating that a change in the chromophore had taken place during the reaction. On the other hand, a solution of the product in methanol had a purple colour and the electronic absorption spectrum in this solvent was the same as that of **23**. In addition to an ester C=O band at 1730 cm^{-1} the IR spectrum showed an O—H absorption band at 3410 cm^{-1}. From these data it was concluded that the orange–red compound was Rose Bengal benzyl ester, molecular form (**17**). In the acetone–water mixture in which the reaction was carried out the alkyl halide was partially hydrolysed. This led to an acidic solution in which the phenolate was protonated. The phenol group of **17** was so acidic that it dissociated in the more polar solvent methanol. In dichloromethane this was not possible so explaining the observed differences in colour and in the electronic absorption spectrum of **17** in both solvents.

Based on these results we sought to derivatize the phenolate function with a non-dissociable function in order to obtain a Rose Bengal derivative with a fixed structure soluble in dichloromethane. In an attempt to prepare the 6-O-acetyl derivative of Rose Bengal benzyl ester (**18**), **17** was refluxed with acetic anhydride. Surprisingly, the expected orange–red **18** was not obtained, but instead the colourless diacetate of Rose Bengal lactone (**16**) resulted, as was indicated by disappearance in the IR spectrum of the ester C=O band at 1730 cm^{-1} and appearance of a lactone C=O band at 1780 cm^{-1}. This was confirmed by independent synthesis of **16** via Rose Bengal lactone (**15**). The formation of **16** can be explained by cleavage of the methylene C—O bond in the benzyl ester. This bond, weakened by the ease of benzyl oxygenation cleavage, is attached by the carbonyl oxygen of the xanthene moiety, which is nucleophilic enough to attack acetic anhydride when aided by attack of the ester oxygen on the slightly positively charged C-9, two atoms which, by virtue of the structure, are always at close distance as in fluorescein. The benzyl cation is eliminated in this process. This again draws attention to the intimate relationship between the carboxyl group and C-9.

A way round this problem was found by repeating this reaction using an ester with a less polarized C—O bond, viz. Rose Bengal ethyl ester (**19**) which yielded the bright-red coloured 6-O-acetyl Rose Bengal ethyl ester (**20**) showing IR absorption bands at 1780 and 1730

Table 1. Spectral data of some Rose Bengal derivatives

	IR $\nu_{C=O}$ (cm^{-1})	IR ν_{O-H} (cm^{-1})	UV λ_{max} (nm)	Solvent
10	<1600	—	558, 519	MeOH
23	1730	—	564, 524	MeOH
17 (**19**)	1730	3410	564, 524	MeOH
			496, 407	CH_2Cl_2
20	1780, 1730	—	494, 400	MeOH
			494, 395	CH_2Cl_2
15	1770	3430	558, 519	MeOH
			<300	CH_2Cl_2
16	1780	—	<300	MeOH
			<300	CH_2Cl_2

Table 2. Visible absorption spectra of Rose Bengal derivatives in methanol

	R^1	R^2	UV λ_{max} (nm)	ε	log ε	$\varepsilon_1:\varepsilon_2$[a]	Concentration (M)
10	Na	Na	558	104 713	5.02	3.24	4.91×10^{-6}
			518	32 359	4.51		
23	$PhCH_2$	Na	564	102 329	5.01	3.24	4.81×10^{-6}
			524	31 623	4.50		
13	Et_3NH	Et_3NH	557	109 500	5.04	3.19	5.53×10^{-6}
			519	34 341	4.54		
24	$PhCH_2$	Et_3NH	563	91 109	4.96	2.96	5.02×10^{-6}
			524	30 808	4.49		
25	Et	Et_3NH	563	86 351	4.94	2.89	5.58×10^{-6}
			524	29 914	4.48		
26	Octyl	Bu_3NH	564	103 226	5.01	3.13	4.33×10^{-6}
			525	32 960	4.52		
14	Bu_4N	Bu_4N	557	109 244	5.04	3.18	1.43×10^{-6}
			518	34 314	4.54		
27	Et	Bu_4N	563	93 549	4.97	2.92	3.22×10^{-6}
			523	32 012	4.15		

[a] $\varepsilon_1:\varepsilon_2$ is the ratio of the molar absorptivities of the longest wavelength absorption maximum and the secondary maximum.

Table 3. Visible absorption spectra of Rose Bengal derivatives in dichloromethane

	R^1	R^2	UV λ_{max} (nm)	ε	log ε	$\varepsilon_1:\varepsilon_2$[a]	Concentration (M)
17	$PhCH_2$	H	496	15 488	4.19	—	—
			407	15 488	4.19		
20	Et	Ac	494	10 715	4.03	—	—
			395	16 596	4.22		
13	Et_3NH	Et_3NH	556	76 394	4.88	2.35	4.97×10^{-6}
			518	32 568	4.51		
25	Et	Et_3NH	563	73 705	4.87	2.15	4.71×10^{-6}
			525	34 254	4.53		
24	$PhCH_2$	Et_3NH	569	61 735	4.79	2.24	5.02×10^{-6}
			528	27 582	4.44		
14	Bu_4N	Bu_4N	565	90 856	4.96	3.65	2.77×10^{-6}
			525	24 877	4.40		
27	Et	Bu_4N	574	102 379	5.01	3.39	3.28×10^{-6}
			532	30 165	4.48		

[a] $\varepsilon_1:\varepsilon_2$ is the ratio of the molar absorptivities of the longest wavelength absorption maximum and the secondary maximum.

Table 4. Visible absorption spectrum of Rose Bengal derivatives in toluene

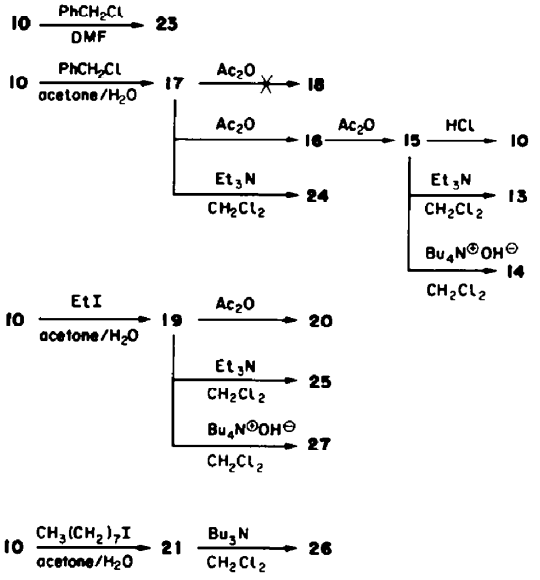
	R^1	R^2	UV λ_{max} (nm)	ε	log ε	$\varepsilon_1:\varepsilon_2$[a]	Concentration (M)
26	Octyl	Bu_3NH	563	49 406	4.69	0.86	4.64×10^{-6}
			536	57 529	4.76		
27	Et	Bu_4N	572	68 671	4.84	2.23	3.38×10^{-6}
			533	30 783	4.49		
27	Et	Bu_4N	570	65 711	4.82	1.98	3.38×10^{-5}
			537	33 181	4.52		

[a] $\varepsilon_1:\varepsilon_2$ is the ratio of the molar absorptivities of the longest wavelength absorption maximum and the secondary maximum.

cm^{-1} for the acetyl and ethyl ester C=O functions, respectively. The electronic absorption spectrum was about the same in methanol and dichloromethane, with maxima at 494 and *ca* 400 nm. The electronic absorption spectra for Rose Bengal in its various dissociation states and the parameters calculated from these spectra are given in Tables 2 and 3. The dianionic (e.g. **10**) and monoanionic (e.g. **23**) species have similar spectra and about the same molar absorptivity. The spectrum of the neutral species is entirely different (e.g. **20**): maxima are at shorter wavelengths and the molar absorptivity is much smaller than for the other two species. Table 5 shows the quantum yields of singlet oxygen formation of the Rose Bengal derivatives. The figure for **20** is large enough compared to that of Rose Bengal (**10**) itself to make it a useful singlet oxygen sensitizer, but its low molecular absorptivity is a serious drawback, especially in applications in which the light intensity should be as low as possible.

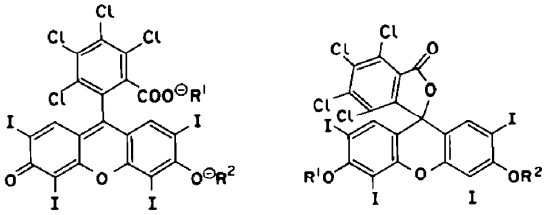
Phase 2. Improving the absorptive properties

Figure 1 shows that, in order to preserve a high molar absorptivity and a longer wavelength absorption maximum, the xanthene system must remain as a monoanion. Based on the knowledge that triethylammonium hydrochloride is soluble in dichloromethane, we tried to obtain the desired monoanions soluble in this solvent by treating Rose Bengal lactone (**15**) or the molecular form of Rose Bengal esters (**17**, **19** or **21**), which are all strong acids, with trialkylamines. The solubility of the products (**13** and **24–26**) in dichloromethane is excellent. Rose Bengal octyl ester tributylammonium salt (**26**) is soluble even in toluene



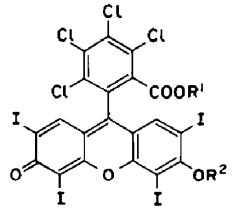
Scheme 6. Synthesis of the Rose Bengal derivatives.

by virtue of its long alkyl chains. Tables 2–4, in which the electronic absorption parameters of several Rose Bengal derivatives are tabulated, show that the molar absorptivity and the ratio $\varepsilon_1 : \varepsilon_2$ of the molar extinction coefficients of the two absorption maxima of **13** and **24–26** are considerably smaller in dichloromethane than in methanol. The same goes for their quantum yields of singlet oxygen formation (Table 5). Both effects are even more pronounced for **26** in toluene which has its

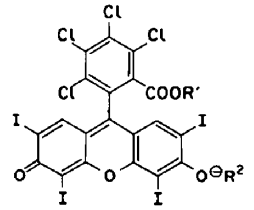


10 $R^1 = R^2 = Na^{\oplus}$
13 $R^1 = R^2 = Et_3NH^{\oplus}$
14 $R^1 = R^2 = Bu_4N^{\oplus}$

15 $R^1 = R^2 = H$
16 $R^1 = R^2 = Ac$



17 $R^1 = CH_2Ph$; $R^2 = H$
18 $R^1 = CH_2Ph$; $R^2 = Ac$
19 $R^1 = Et$; $R^2 = H$
20 $R^1 = Et$; $R^2 = Ac$
21 $R^1 = (CH_2)_7CH_3$; $R^2 = H$
22 $R^1 = H.Et_3N$; $R^2 = H.Et_3N$



23 $R^1 = CH_2Ph$; $R^2 = Na^{\oplus}$
24 $R^1 = CH_2Ph$; $R^2 = Et_3\overset{\oplus}{N}H$
25 $R^1 = Et$; $R^2 = Et_3\overset{\oplus}{N}H$
26 $R^1 = (CH_2)_7CH_3$; $R^2 = Bu_3\overset{\oplus}{N}H$
27 $R^1 = Et$; $R^2 = Bu_4\overset{\oplus}{N}$

Scheme 5. The structures of the Rose Bengal derivatives.

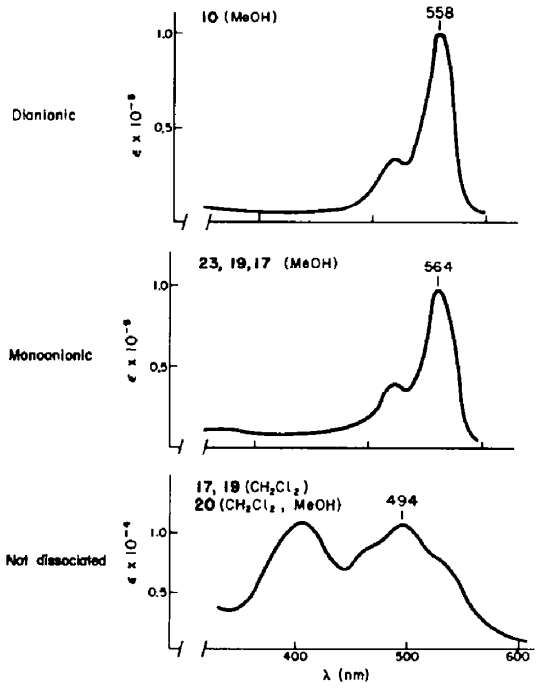


Fig. 1. The absorption spectra of the Rose Bengal derivatives in the visible region as a function of the dissociation state.

maxima shifted and has a shape that is entirely different from that of a regular Rose Bengal spectrum (Table 4, Fig. 2). The data in dichloromethane and toluene suggest that association of the dye molecules occurs in non-polar solvents, because the $\varepsilon_1 : \varepsilon_2$ ratio was found

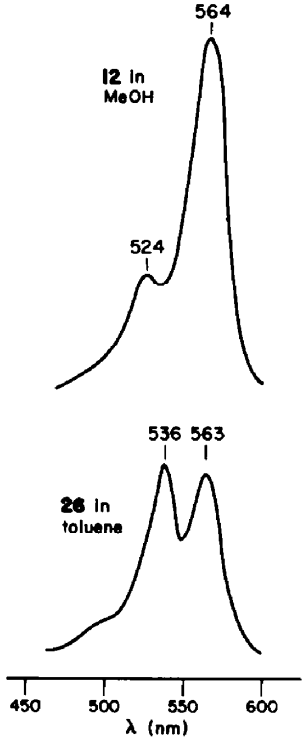


Fig. 2. The electronic absorption spectrum of Rose Bengal octyl ester, tributylammonium salt (**26**) in toluene.

Table 5. Quantum yields of singlet oxygen formation

10	Na	Na	0.76[a]	MeOH
19	Et	H	0.73	MeOH
13	Et_3NH	Et_3NH	0.72	MeOH
24	$PhCH_2$	Et_3NH	0.74	MeOH
25	Et	Et_3NH	0.74	MeOH
19	Et	H	0.61	CH_2Cl_2
20	Et	Ac	0.61	CH_2Cl_2
13	Et_3NH	Et_3NH	0.48	CH_2Cl_2
24	$PhCH_2$	Et_3NH	0.67	CH_2Cl_2
25	Et	Et_3NH	0.71	CH_2Cl_2
14	Bu_4N	Bu_4N	—	CH_2Cl_2
27	Et	Bu_4N	—	CH_2Cl_2
26	Octyl	Bu_4N	0.40	Toluene
27	Et	Bu_4N	—	Toluene

[a] See Ref. 9.

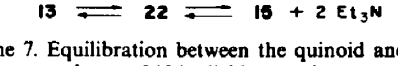
to decrease with increasing concentration. Also, an equilibrium between the quinoid and the lactonic form of the dye or between tight ion pairs and solvent separated ion pairs may be involved (Scheme 7). In these equilibria small amounts of free triethylamine will be released, which explains the large decrease in quantum yield in the case of **13**, since amines are efficient quenchers of singlet oxygen. The FAB mass spectra also suggest that the ionic bonds in **13** and **25** are not very strong. The observed $[M+1]^+$ peak is two triethylamine units too low for **13** and one triethylamine unit too low for **25**, indicating loss of triethylamine.

Phase 3. *Locking the dye into the quinoid modification*

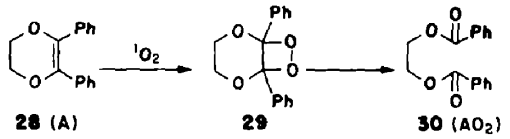
The equilibria responsible for the release of triethylamine are a consequence of the presence of a proton in the ammonium salts **13** and **24–26**. An obvious remedy is the introduction of a quaternary instead of a tertiary ammonium counter ion in the dye which should make it possible to lock the dye into the quinoid modification while retaining the solubility in dichloromethane. Two exemplary reactions were carried out that are again based on the high acidity of Rose Bengal lactone (**15**) and the Rose Bengal ester, molecular form (**19**). Compounds **15** and **19** were treated with tetrabutylammonium hydroxide leading to the tetrabutylammonium salts **14** and **27**. Tables 2–4 show that both compounds have excellent absorptive properties compared to the tertiary ammonium salts **13** and **24–26** and a quantum yield in dichloromethane that is almost equal to that of Rose Bengal itself in methanol (Table 5). Compound **27** is even soluble in toluene, but here the molecular aggregation effect is shown again by a low $\varepsilon_1 : \varepsilon_2$ ratio, although less so than for **26**.

CONCLUSION

It has been shown that derivatization (Scheme 6) of the carboxylate group does not negatively affect the photophysical properties of Rose Bengal and that a quaternary ammonium counter ion, while ensuring a good solubility in non-polar solvents, preserves the



Scheme 7. Equilibration between the quinoid and lactonic forms of **13** in dichloromethane.



Scheme 8. The photo-oxygenation of 2,3-diphenyl-*p*-dioxene (**28**).

monoanionic form that is essential for a high molar absorptivity. It has also been shown by the elemental analyses that, without resorting to chromatographic methods, dye samples of excellent purity can be obtained in large quantities. This opens up new opportunities for the design of dye derivatives for applications in which a low radiation intensity is essential.

EXPERIMENTAL[13]

Rose Bengal (**10**), dye content 92%, was purchased from Aldrich. It was used in synthesis without prior purification. Rose Bengal (98%) and several of the derivatives reported herein can be obtained from Dye Tel, Inc., Box 23, Perrysburg, OH 43551, U.S.A. ^{1}H-NMR spectra were measured on a Varian CFT-20 79.6 MHz or a Bruker WH-90 ^{1}H-NMR spectrometer in $CDCl_3$ with TMS as internal standard. Chemical shifts are reported in δ and J-values in Hz. IR spectra were obtained using a Perkin–Elmer 337 or 397 IR spectrometer and electronic absorption spectra using a Varian Cary 219 or Perkin–Elmer 555 instrument. Quantum yields were measured with a Bausch and Lomb high-intensity monochromator fitted with an Osram HBO 200W-L2 super-pressure mercury lamp. GLC analysis was performed on a Hewlett–Packard gas chromatograph fitted with a glass capillary column (0.20 mm i.d., length 12 m) containing crosslinked methyl silicone film (film thickness 0.33 m) and a flame ionization detector with 2,3-diphenyldioxene as the singlet oxygen trap (Scheme 8). Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee, U.S.A., or by Mr J. Diersmann of the University of Nijmegen, The Netherlands. M.ps were measured on a Thomas–Hoover capillary m.p. apparatus or on a Leitz m.p. microscope and were uncorrected. Mass spectra were obtained using a VG 7070E mass spectrometer equipped with a fast atom bombardment facility. Samples were measured in suspension in glycerol using xenon as carrier gas. Data were processed with a PDP 11-24 data system. The chlorine cluster patterns corresponded to four chlorine atoms. Isolated yields of syntheses were *ca* 80% except where another figure is mentioned.

Rose Bengal benzyl ester, monosodium salt (**23**). A soln of **10** (1.58 g; 1.55 mmol) and benzyl chloride (0.28 g; 2.22 mmol) in dry DMF (60 ml) was heated overnight at 80° while stirring. The excess benzyl chloride and DMF were distilled off *in vacuo* and the residue was stirred with ether for 1 hr. After filtration and thoroughly washing with ether a purple powder was isolated which had no distinct m.p.

Rose Bengal benzyl ester, molecular form (**17**). Rose Bengal (1 g; 3 mmol) was dissolved in 10 ml of water and a soln of benzyl chloride (0.32 g; 2.5 mmol) in 10 ml of acetone was added. The resulting soln was refluxed overnight while stirring. After cooling the orange–red ppt was filtered off and dried at 80° in a vacuum oven. The sample was then washed with ether and dried again. There was no distinct m.p. but at 220° the compound was transformed into a dark oil. ^{1}H-NMR ($CDCl_3$) δ 5.03 (2H, s, benzyl CH_2), 6.82–7.49 (7H, m, arom.).

Rose Bengal ethyl ester molecular form (**19**). Synthesis analogous to **17**, using 5 equivalents of EtI.

6-O-*Acetyl Rose Bengal ethyl ester* (**20**). A soln of Rose Bengal ethyl ester, molecular form (**19**) (0.5 g; 0.50 mmol) in 2.5 g of Ac_2O was refluxed overnight and the solvent was distilled off *in vacuo*. The residue was stirred for 1 hr in ether and filtered off, washed again with ether and dried in a vacuum oven at 80°. The resulting bright-red powder had no distinct m.p. Its colour became gradually brown upon approaching 250°, while the powder stayed dry and could easily be removed from the capillary tube. The electronic absorption spectrum of this heated sample in MeOH and CH_2Cl_2 showed it had partially been deacetylated to **19**. The sample was charred when heated above 300°. ^{1}H-NMR ($CDCl_3$) δ 0.98 (3H, t, J = 7.1 Hz, $—CH_3$), 2.48 (3H, s, $CH_3C{=}O$), 4.01 (2H, q, J = 7.1 Hz, $—CH_2—$), 7.41 (1H, s, xanthene-H), 7.65 (1H, s, xanthene-H).

Rose Bengal bis(triethylammonium) salt (**13**). Rose Bengal lactone (**15**) was suspended in CH_2Cl_2 and treated with excess Et_3N yielding a clear red soln which was evaporated *in vacuo*. The resulting purple–red powder was placed on a filter, thoroughly washed with ether to remove residual Et_3N and dried, m.p. 194–195°. ^{1}H-NMR δ 1.18 (18H, t, amine-CH_3), 2.98 (12H, q, $—CH_2—N$), 7.57 (2H, s, xanthene-H). MS [M + 1]$^+$ = 972 corresponding to loss of two molecules of Et_3N. (Found: C, 32.72; H, 3.00; Cl, 11.92; I, 42.96%. Calc: C, 32.68; H, 2.91; Cl, 12.06; I, 43.16%.)

Rose Bengal benzyl ester, triethylammonium salt (**24**). Rose Bengal benzyl ester, molecular form (**17**) was dissolved in CH_2Cl_2 yielding an orange soln. Upon addition of excess Et_3N the soln turned deep red and was evaporated *in vacuo*. The resulting purple–red powder was thoroughly washed with ether to remove residual Et_3N and then dried. M.p. *ca* 160°. ^{1}H-NMR ($CDCl_3$) δ 1.33 (9H, t, amine-CH_3), 3.10 (6H, q, $CH_2—N$), 4.97 (2H, s, benzyl-CH_2), 6.9–7.5 (5H, m, arom.-H), 7.29 (2H, s, xanthene-H).

Rose Bengal ethyl ester, triethylammonium salt (**25**). From Rose Bengal ethyl ester, molecular form (**19**) analogous to previous procedure. M.p. *ca* 200°. ^{1}H-NMR ($CDCl_3$) δ 0.92 (3H, t, ester-CH_3), 1.38 (9H, t, amine-CH_3), 3.18 (6H, q, $CH_2—N$), 4.00 (2H, q, ester-CH_2), 7.40 (2H, s, xanthene-H). MS [M + 1]$^+$ = 1000 corresponding to loss of one molecule of Et_3N. (Found: C, 30.28; H, 2.17; Cl, 12.69; I, 45.77%. Calc: C, 30.49; H, 2.10; Cl, 12.86; I, 46.02%.)

Rose Bengal octyl ester, tri-n-butylammonium salt (**26**). Rose Bengal octyl ester, moelcular form (**21**) was synthesized by refluxing **10** in acetone–water (1 : 1) with 5 equivalents of 1-iodooctane analogous to the syntheses of **17** and **19**. The ester was converted to **26** by addition of excess n-Bu_3N to a soln of **21** in CH_2Cl_2, evaporating to dryness and washing with hexane. ^{1}H-NMR ($CDCl_3$) δ 0.5–2.3 (36H, m, alkyl-H of octyl and butyl groups), 3.1 (6H, t, $CH_2—N$), 3.9 (2H, t, $CH_2—O$), 7.45 (2H, s, xanthene-H).

Rose Bengal bis(tetrabutylammonium) salt (**14**). A 40% soln of tetrabutylammonium hydroxide in water (2.4 g) was diluted with 25 ml of water and added to a suspension of Rose Bengal lactone (0.98 g; 1.00 mmol) in 25 ml of CH_2Cl_2. After stirring overnight, a red CH_2Cl_2 layer and a colourless water layer had been obtained. The CH_2Cl_2 layer was thoroughly extracted with water and dried over $CaCl_2$. After filtration and evaporation *in vacuo* a dark red powder was obtained. Isolated yield: 0.64 g (44%). M.p. 100–120°. ^{1}H-NMR ($CDCl_3$) δ 0.7–2.0 (28H, m, butyl $CH_3—(CH_2)_2—$), 2.9–3.3 (8H, m, $—CH_2—N$), 7.54 (2H, s, xanthene-H). MS could not be obtained. (Found: C, 42.77; H, 5.18; N, 1.95%. Calc: C, 42.88; H, 5.12; N, 1.92%.)

Rose Bengal ethyl ester tetrabutylammonium salt (**27**). Synthesis from Rose Bengal ethyl ester, molecular form (**19**) analogous to previous one. Isolated yield: 0.76 g (61%). M.p. 130–135°. ^{1}H-NMR ($CDCl_3$) δ 0.8–2.0 (31H, m, ethyl-CH_3 and butyl $CH_3—(CH_2)_2—$), 3.0–3.5 (8H, m, $—CH_2—N$), 4.01 (2H, q, $CH_2—O$), 7.34 (2H, s, xanthene-H). MS could not be obtained. (Found: C, 36.66; H, 3.39; N, 1.08%. Calc: C, 36.71; H, 3.49; N, 1.13%.)

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