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Synthesis and Spectroscopic Properties of New Rose Bengal and Eosin Y Derivatives

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ABSTRACT

Selective esterification of purified Rose Bengal and Eosin Y have been carried out by reaction with methyl iodide, benzyl chloride or p-*isopropylbenzyl chloride. The lactonic form of each dye reacts with diazomethane producing mixtures of the monomethyl ether and the dimethyl ether of the lactone, and the methyl ether of the methyl ester of the tautomeric quinonoid form. On heating, Rose Bengal decarboxylates in dimethylformamide solution, while Eosin Y loses a bromine atom in dimethylsulphoxide. The structures of all the products obtained have been studied by the usual spectroscopic techniques. HPLC data are also reported.*

INTRODUCTION

The halogenated xanthene dyes Rose Bengal and Eosin Y (disodium salts of the quinonoid forms 3′, 4′, 5′, 6′-tetrachloro-2,4,5,7-tetraiodofluorescein, and 2,4,5,7-tetrabromofluorescein, respectively) are widely applied as photo-oxidation sensitizers due to their high efficiencies as singlet oxygen generators.[1–3] Although they are currently employed in solution, their use in heterogeneous phase is also possible,[4,5] particularly as a consequence of the work of Schaap, Neckers *et al.*[6–8] on the chemical binding of Rose

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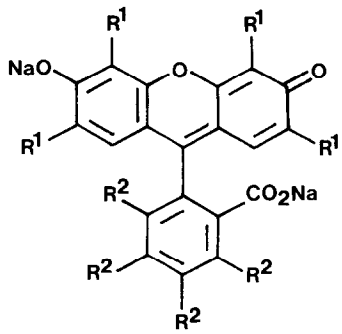
Bengal to cross-linked polystyrene beads, and the more recent work of Neckers *et al.*[9-12] and, independently, of our group[13] on its binding to linear polystyrene.

As part of a continuing study on ketone[14-19] and dye-based[13,20] linear polymeric photosensitizers, knowledge of the chemical reactivity of Rose Bengal and Eosin Y was needed to provide a solid basis for the preparation and characterization of linear polymeric sensitizers possessing any of these dyes as chromophore. Specifically, the authors were looking for selective reactions through the carboxylate group of each dye which would keep intact their xanthenic moiety, i.e. the structure responsible for the colour.



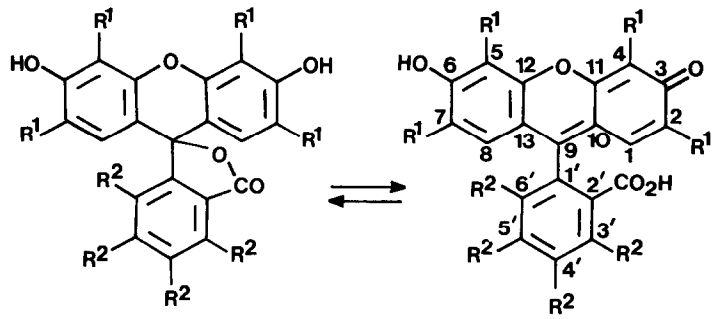
	R^1	R^2
Rose Bengal (RB)	I	Cl
Eosin Y (EO)	Br	H

Working in a parallel direction, and with similar purposes, the already cited workers have published several papers dealing with the particular chemistry of Rose Bengal and with the selective esterification of this dye at its C-2′ carboxylate group,[21-27] a reaction used to prepare new derivatives which have been employed for interesting studies on dye photochemistry.[28-30] Our own work also showed that the reactivity of this dye, as well as that of Eosin Y, was different from that of the parent, non-halogenated compound uranine (disodium salt of fluorescein). Two facile reactions were of special interest: their selective esterification with alkyl halides, allowing the synthesis of model esters of the corresponding polymeric dyes,[13] and their thermal decomposition in solution, producing decarboxylation in the case of Rose Bengal,[31] or debromination in the case of Eosin Y. Reported here are these reactions and other related chemistry of both dyes. Thus, the acid forms of the corresponding parent compounds, **RB.1** and **EO.1**, obtained with analytical purity from commercial samples, have been esterified and etherified, and the products have been studied by the usual spectroscopic techniques. Differing from the parent dyes, which are only soluble in polar solvents, the derivatives with quinonoid structures are soluble in both polar and non-polar solvents and have proved to be good singlet oxygen generators.[32]

RESULTS AND DISCUSSION

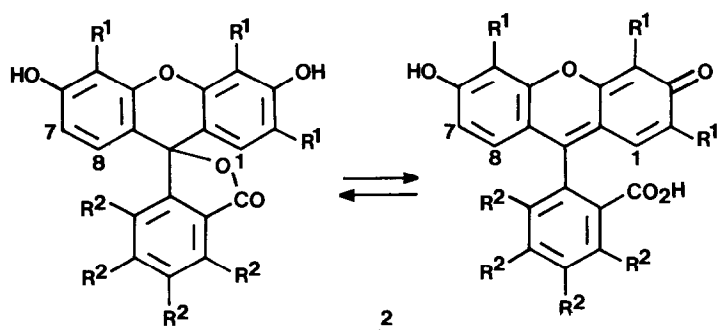
Purification and analysis of Rose Bengal, Eosin Y, and their monodehalogenated derivatives

Commercial samples of Rose Bengal and Eosin Y are complex mixtures with variable contents in each dye from which the pure compounds have been separated by various chromatographic methods,[33-40] usually working on the milligram scale. From two of these commercial mixtures, and by column chromatography on silica gel, the authors isolated grams of two pure compounds, characterized as lactones **RB.1** and **EO.1**. The main impurity in



lactone 1 quinonoid

each mixture could also be isolated as the acid form: the monodehalogenated compounds **RB.2** and **EO.2**, respectively. Compounds **RB.1**, **RB.2**, and **EO.1**, crystallized from dioxane–water, are in the form of lactones, as shown by their white or almost white colour and by the presence in their IR spectra of characteristic carbonyl stretching bands. The orange debrominated compound **EO.2**, crystallized from dioxane–hexane, must be a mixture of lactonic and quinonoid species, presenting four bands between



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1700 and 1769 cm^{-1}, probably due to different crystalline modifications of both tautomers.

Pure Rose Bengal and Eosin Y were obtained by neutralization of lactones **RB.1** and **EO.1** with sodium carbonate in aqueous methanol. The IR spectrum of the purified salt Rose Bengal showed strong bands at 1460 and 1550 cm^{-1}, formerly attributed[41] to vibrations of the carboxylate group. However, as both bands are also present in the IR spectrum of the sodium (phenoxide) quinonoid salt of the methyl ester **RB.3** (see later), these absorptions must be better assigned to stretching vibrations of the ionized xanthenic nucleus. Another band at 960 cm^{-1} is characteristic of all tetraiodinated quinonoid forms. The IR spectrum of pure Eosin Y also presents strong absorptions at 1465 and 1560 cm^{-1}.

In solution, the structures adopted by the acid forms of these four dyes depend on the solvent, as is usual in these compounds.[42,43] Thus, their solutions in dry dioxane are colourless (Table 1), indicating lactonic forms, whilst in methanol or dimethylformamide (DMF) the corresponding highly

TABLE 1
Electronic Absorption Spectral Data of Rose Bengal and Eosin Y Derivatives in Dioxane

Compound	λ_{max} (*nm*)[a] (*log* ε)			
Lactones				
RB.1	248 (4·78)	313[s](3·74)		
RB.2	243 (4·69)	308[s](3·66)		
RB.8	247 (4·78)	315[s](3·89)		
RB.9	248 (4·75)	315[s](3·91)		
EO.1	285 (3·85)	300[s](3·45)		
EO.2	280 (3·77)	300[s](3·36)		
EO.8	285 (3·78)	304 (3·48)		
EO.9	284[s](3·70)	307 (3·60)		
Quinonoids				
RB.3	412 (4·20)	470[s](4·15)	500 (4·22)	534[s](4·06)
RB.4	412 (4·19)	468[s](4·15)	497 (4·22)	530[s](4·05)
RB.5	412 (4·18)	468[s](4·16)	497 (4·23)	530[s](4·13)
RB.6	410 (4·19)	464[s](4·15)	495 (4·22)	530[s](4·05)
RB.7	404 (4·24)	474[s](4·02)	500 (4·07)	536[s](3·86)
RB.10	410 (4·19)	464[s](4·18)	496 (4·26)	532[s](4·11)
RB.11	396 (4·24)	464[s](4·07)	494 (4·11)	534[s](3·87)
EO.3	378 (4·08)	453 (4·22)	476 (4·27)	510[s](4·07)
EO.4	378 (4·09)	455 (4·22)	477 (4·31)	510[s](4·07)
EO.5	378 (4·08)	452 (4·21)	478 (4·27)	510[s](4·06)
EO.7	368 (4·18)	454 (4·13)	478 (4·15)	518[s](3·86)

[a] Superscript s indicates a shoulder.

coloured quinonoid species, ionized at the phenolic group, are formed. These ionizations must be total, because spectra of solutions of **RB.1** and **EO.1** are identical to those of the corresponding pure disodium salts in the same solvents.

In aqueous media the proportion of dye with di-ionized quinonoid form depends on the pH, so that at sufficiently high pH only this form is present. Visible spectral data for solutions in dioxane–aqueous pH 8 buffer, 1:1 (Table 2) support the trihalogenated xanthenic nature of **RB.2** and **EO.2**

TABLE 2

Visible Spectral Data of Rose Bengal and Eosin Y Derivatives with Quinonoid Structure, in Un-ionized Form (in Dioxane–Aqueous pH 0·5 Buffer, 1:1) or as Salts (in Dioxane–Aqueous pH 8 Buffer, 1:1)

Compound	λ_{max} (*nm*)[a] (*log* ε)			
Quinonoids				
RB.3	426 (4·10)	472[s](4·12)	505 (4·26)	536[s](4·15)
RB.4	426 (4·09)	472[s](4·12)	505 (4·25)	536[s](4·18)
RB.5	426 (4·08)	472[s](4·12)	505 (4·23)	536[s](4·14)
RB.6	424 (4·12)	470[s](4·14)	505 (4·27)	534[s](4·19)[b]
RB.7	410 (4·23)	477[s](4·05)	505 (4·09)[c]	543[s](3·88)
RB.10	424 (4·09)	468[s](4·10)	505 (4·30)	534[s](4·15)
EO.3	400 (4·02)	450[s](4·18)	481 (4·35)	511 (4·28)
EO.4	400 (4·03)	452[s](4·19)	484 (4·36)	516 (4·29)
EO.5	400 (3·99)	452[s](4·15)	484 (4·33)	516 (4·28)
EO.7	382 (4·16)	454[s](4·14)	480 (4·20)	518[s](3·97)
Quinonoid salts				
RB.1	522 (4·52)	559 (5·05)		
RB.2	515 (4·43)	550 (4·90)		
RB.3	529 (4·53)	568 (5·00)		
RB.4	529 (4·51)	570 (5·01)		
RB.5	527 (4·51)	568 (5·01)		
RB.6	526 (4·49)	566 (5·00)		
RB.8[d]	406 (3·80)	498 (3·70)	532[s](3·57)	
RB.10	523 (4·52)	561 (5·02)		
EO.1	494[s](4·46)	526 (4·99)		
EO.2	485[s](4·44)	518 (4·92)		
EO.3	502 (4·47)	535 (5·00)		
EO.4	502 (4·47)	537 (5·02)		
EO.5	504 (4·47)	538 (5·00)		
EO.8[d]	446[s](2·91)	474 (3·00)	510[s](2·80)	

[a] Superscript s indicates a shoulder.

[b] Another shoulder at 526 nm (log ε 4·07).

[c] log ε 4·02, in dioxane–aqueous pH 8 buffer, 1:1.

[d] Ionized in the carboxylic group.

because, in accordance with the empirical rule proposed by Marshall,[44] the position of their maximum absorptions are blue-shifted 9 and 8 nm with respect to the corresponding solutions of the tetrahalogenated compounds **RB.1** and **EO.1**. Solutions of **RB.1** and **RB.2** in dioxane–aqueous pH 0·5 buffer, 1:1, are colourless, indicating lactonic forms, whilst those of **EO.1** and **EO.2** have visible absorptions due to the presence of small amounts of

TABLE 3

^{1}H-NMR Spectral Data (90 MHz, DMSO-d_6) of Rose Bengal and Eosin Y Derivatives[a]

Compound	*Molecular forms*		*Salt forms*		*Other signals*[b]
	H-1	*H-8*	*H-1*	*H-8*	
	Lactones		*Quinonoid salts*		
RB.1	7·55		7·35		—
RB.2	7·63	6·95[c]	7·28	6·71[c]	[d]
RB.8	7·68	7·55	7·64	7·30	3·82 (CH_3)
RB.9	7·71		—		3·83 (CH_3)
EO.1	6·92		6·98		—
EO.2	6·92	6·81[e]	6·91	6·64[e]	[f]
EO.8	7·03	6·84	[g]		3·88 (CH_3)
EO.9[h]	6·95		—		3·93 (CH_3)
	Quinonoids		*Quinonoid salts*		
RB.3	7·72		7·31		3·48 (CH_3)
RB.4	7·67		7·53		5·00 (CH_2)[i]
RB.5	7·68		7·45		4·93 (CH_2)[i,j]
RB.6	7·77	7·78[k]	7·30	7·33[k]	[l]
RB.7	8·04	7·82	—		3·49, 3·90 ($2CH_3$)
RB.10	7·63		7·38		7·90 (H-2′)
RB.11	7·97	7·72	—		7·83 (H-2′), 3·90 (CH_3)
EO.3	7·13		7·05		3·62 (CH_3)
EO.4	7·09		6·92		4·95 (CH_2)[i]
EO.5	7·08		6·91		4·93 (CH_2)[i,j]
EO.7[h]	7·27	7·10	—		3·73, 4·01 ($2CH_3$)

[a] δ scale.

[b] Common signals for molecular and salt forms, except otherwise noted; in Eosin Y derivatives, protons H-3′ to H-6′ appear between 7·20 and 8·40 ppm.

[c] Doublet, $J = 7{\cdot}5$ Hz.

[d] H-7 at 6·72 (lactone) or 6·26 (salt) ppm.

[e] Doublet, $J = 8{\cdot}8$ Hz.

[f] H-7 at 6·63 (lactone) or 6·50 (salt) ppm.

[g] Complex spectrum.

[h] In $CDCl_3$.

[i] Aromatic protons of the benzyl or *p*-isopropylbenzyl group appear between 6·70 and 7·20 ppm.

[j] $CH(CH_3)_2$ at 2·88 and 1·16 ppm.

[k] Assignments can be reversed.

[l] Other signals in Experimental.

the corresponding non-ionized quinonoid forms in equilibrium with the lactonic species. The percentage of this coloured form in **EO.1** has been estimated as 7% on the basis of the visible spectrum of the methyl ester **EO.3** (see later), a compound that cannot lactonize and that has the same chromophore group. Both acid and salt forms of the four compounds have been analysed by ^{1}H- and ^{13}C-NMR spectroscopy in DMSO-d_6 solutions. Spectra of **RB.1** and **EO.1** are in accordance with symmetric lactones, with only one signal for protons in positions 1 and 8 (Table 3) and seven signals for carbons of the xanthenic nucleus (positions 1 to 13) (Tables 4 and 5), the aliphatic carbon at position 9 resonating at about 80 ppm. The corresponding salts, with a negative charge delocalized throughout each xanthenic nucleus, have non-fixed quinonoid structures and also show symmetry in this solvent. On the contrary, spectra of solutions of the acid forms **RB.2** and **EO.2** in the same medium indicate that both compounds are unsymmetric lactones without halogen in position 7 and with carbon 7 appearing at lower field than the same carbon in the corresponding tetrahalogenated lactones. These assignments agree with the known lower susceptibility to halogenation of positions 2 and 7 with respect to 4 and 5.[33,45]

Compounds **RB.1, EO.1** and **EO.2** showed enough volatility and stability to be studied by mass spectrometry in the electronic impact mode, although the molecular ions were of low intensity, as expected for substituted γ-lactones.[46] Main fragmentations were the loss of CO_2, X (halogen), HX and X_2. It is worth noting that in **EO.1** and **EO.2**, as well as in all the neutral (lactonic or quinonoid) forms of the Eosin Y derivatives studied in this work, a prominent fragment without bromine was always present at *m/z* 198, the composition (probably $C_{12}H_6O_3$) and origin of which could not be explained. In the parent debrominated compound fluorescein this peak is absent, although important signals at 143 and 202 have not been explained either.[46]

Formation of esters

Looking for selective and quantitative reactions which could be used to bind Rose Bengal or Eosin Y to a linear copoly(styrene-*p*-vinylbenzyl chloride) without producing cross-linking, the esterification of their carboxylate group with alkyl halides was tested. The parent non-halogenated disodium salt of fluorescein (uranine) reacts with alkyl halides yielding mixtures of derivatives alkylated in the phenoxide and/or in the carboxylate groups,[47] indicating that in this dye both groups have similar nucleophilicity. In Rose Bengal and Eosin Y, with tetrahalogenated xanthenic nuclei, the nucleophilicity of the phenoxide groups is strongly diminished, so that when acetone/DMF 2:1 solutions of each dye (or of each lactonic form **RB.1** or

TABLE 4
^{13}C-NMR Spectral Data of Rose Bengal Derivatives in DMSO-d_6[a]

Compound	*C-1* *C-8*	*C-2* *C-7*	*C-3* *C-6*	*C-4* *C-5*	*C-10* *C-13*	*C-11* *C-12*	*C-9*	*—CO_2—*	*Others*
Lactones									
RB.1	136·3	81·6	158·4	76·9	110·6	151·8	79·2	163·0	[b]
RB.2	136·6	81·5	158·5*	77·1	110·8	151·9 #	80·5	163·1	[b]
	128·3	111·8	160·1*	74·1	107·6	152·0 #	—	—	—
RB.8	137·0	86·0	161·3	83·2	115·0	152·0	78·8	162·9	60·3 ($\underline{C}H_3$)[b]
	136·4	82·0	158·6	76·9	110·3	151·6	—	—	—
RB.9	137·0	86·3	161·4	83·2	114·7	151·8	78·3	162·8	60·4 ($\underline{C}H_3$)[b]
Quinonoids									
RB.3	137·1	95·6	168·9	77·0	115·4	156·2	139·6	163·7	53·2 ($\underline{C}H_3$)[c]
RB.4	136·9	95·7	168·8	77·2	115·5	155·9	139·0	162·7	67·9 ($\underline{C}H_2$)[c,d]
RB.5	137·0	95·7	168·8	77·0	115·6	156·1	139·3	162·9	68·0 ($\underline{C}H_2$)[c,e]
RB.6	137·0*	[f]	168·6 #	76·6*	115·8 #	156·3*	139·6	161·5	[c,g]
	137·3*		169·1 #	76·7*	116·4 #	156·6*	—	—	—
RB.7	137·9	107·6	174·4	79·0	121·7	158·1	138·7	163·6	53·4, 60·8
	137·2	88·4	163·8	83·6	117·8	153·1	—	—	(2 × $\underline{C}H_3$)[c]

RB.10	137·1	95·5	168·7	76·9	116·1	156·4	142·0	—	[h]
RB.11	138·1*	107·3	174·4	78·4	121·6	158·6	139·0	—	60·8 ($\underline{CH}_3$)[h]
	137·1*	88·5	163·7	83·6	118·7	153·3	—	—	—
Quinonoid salts									
RB.1	136·8	96·4	171·5	75·3	110·6	157·2	143·1[i]	165·1	[j]
RB.3	136·3	96·7	171·2	76·2	111·3	156·7	139·4	163·7	[k]
RB.10	136·3	96·5	170·7	76·2	112·3	156·9	141·9	—	[l]

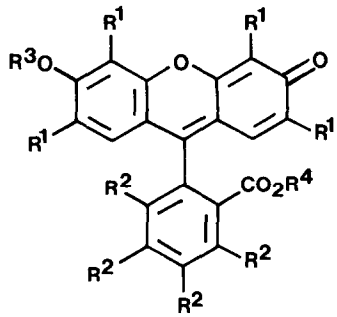
[a] δ scale with respect to TMS as the internal reference; the same symbols (* or #) indicate that assignments can be reversed.
[b] C-1′ to C-6′ at *c.* 146·7, 126·8, 135·2, 124·5, 138·7 and 131·0 ppm, respectively.
[c] C-1′ to C-6′ at *c.* 134·9 (C-1′), 128·7 and 129·1 (C-2′ and C-4′), 131·7 and 133·2 (C-3′ and C-6′) and 135·3 (C-5′) ppm.
[d] Aromatic carbons of the benzyl group at 124·7, 128·0, 128·2 and 133·5 ppm.
[e] ($\underline{CH}_3)_2\underline{CH}$ at 23·7 and 33·0 ppm; aromatic carbons of the *p*-isopropylbenzyl group at 126·0, 127·8, 131·1 and 148·4 ppm.
[f] At *c.* 96 ppm, with intensity similar to that of the noise.
[g] Other signals in Experimental.
[h] C-1′ to C-6′ at *c.* 134·0, 130·0, 132·2, 130·9, 131·4, 133·5 ppm, respectively.
[i] This assignment can be reversed with that of C-2′.
[j] C-1′ to C-6′ at 132·7, 144·6, 128·7, 127·3, 130·3 and 127·6 ppm, respectively.
[k] C-1′ to C-6′ at 134·4 (C-1′), 128·9 and 129·7 (C-2′ and C-3′), 131·8 and 133·5 (C-3′ and C-6′) and 135·0 (C-5′) ppm.
[l] C-1′ to C-6′ at 133·2, 130·0, 132·3, 130·8, 132·1 and 133·1 ppm, respectively.

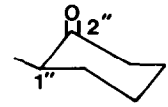
TABLE 5
^{13}C-NMR Spectral Data of Eosin Y Derivatives in DMSO-d_6[a]

Compound	*C-1* *C-8*	*C-2* *C-7*	*C-3* *C-6*	*C-4* *C-5*	*C-10* *C-13*	*C-11* *C-12*	*C-9*	*—CO_2—*	*Others*
Lactones									
EO.1	129·6	107·0	153·3	100·6	112·8	150·9	80·9	168·0	[b]
EO.2	129·6	106·6	153·1	100·5	112·9	151·4	81·7	168·1	[b]
	127·3	112·8	148·3	97·4	110·5	156·8	—	—	—
EO.8	130·2*	112·1	155·6	107·5 #	117·8	147·5[c]	80·5	167·8	60·6 ($\underline{C}H_3$)[b]
	129·6*	107·4 #	153·6	100·5	112·3	150·8	—	—	—
EO.9[d]	130·0	112·6	155·9	107·7	117·4	150·7	80·1	167·8	60·6 ($\underline{C}H_3$)[b]
Quinonoids									
EO.3	129·9	115·1	164·6	100·0	117·1	152·0	151·2	165·0	52·4 ($\underline{C}H_3$)[e]
EO.4	129·9	115·3	164·3	100·1	116·9	151·7	150·4	164·6	67·0 ($\underline{C}H_2$)[e,f]
EO.5	129·9	115·2	164·5	100·0	117·0	151·9	150·5	164·7	66·8 ($\underline{C}H_2$)[e,g]
EO.7[h]	130·4*	127·4	165·1	108·3	119·8	149·4 #	154·3	172·3	52·7, 61·3
	129·9*	114·4	158·6	102·6	119·7	148·6 #	—	—	(2 × $\underline{C}H_3$)[i]
Quinonoid salt									
EO.1	130·1	117·6	168·0*	109·7	98·9	152·9	157·5	168·1*	

[a] δ scale with respect to TMS as the internal reference; the same symbols (* or #) indicate that assignments can be reversed.
[b] C-1′ to C-6′ at *c.* 147·8, 124·1, 135·9, 130·7, 125·1 and 125·6 ppm respectively.
[c] This assignment can be reversed with that of C-1′.
[d] In DMSO-d_6–$CDCl_3$, 1:1.
[e] C-1′ to C-6′ at *c.* 132·2 (C-1′), 129·5 (C-2′), 130·5, 130·6 and 131·0 (C-3′, C-4′ and C-6′), and 133·3 (C-5′) ppm.
[f] Aromatic carbons of the benzyl group at 127·8, 128·0, 128·2 and 134·1 ppm.
[g] ($\underline{C}H_3$)$_2$$\underline{C}$H at 23·6 and 32·9 ppm; aromatic carbons of the *p*-isopropylbenzyl group at 126·0, 127·7, 132·0 and 148·3 ppm.
[h] In $CDCl_3$.
[i] C-1′ to C-6′ at 133·3 (C-1′), 129·8 (C-2′), 130·5, 130·7 and 131·8 (C-3′, C-4′ and C-6′) and 133·4 (C-5′) ppm.
[j] C-1′ to C-6′ at 140·9 (C-1′), 132·6 (C-2′), 128·5, 128·7 and 130·5 (C-3′ to C-6′) ppm.

EO.1 in the presence of excess of a base) were treated with methyl iodide, benzyl chloride or *p*-isopropylbenzyl chloride, the corresponding methyl esters **RB.3** and **EO.3**, benzyl esters **RB.4** and **EO.4**, or *p*-isopropylbenzyl esters **RB.5** and **EO.5**, were formed in near-quantitative yields. These six esters, specially the last two, can be regarded as model structures of the former linear copolymers possessing the corresponding dyes covalently bound through ester unions.



	R^3	R^4
3	H	Me
4	H	$CH_2C_6H_5$
5	H	$CH_2C_6H_4(p)CHMe_2$
6	H	
7	Me	Me

Spectroscopic data of these esters are in accord with their structures. Their IR spectra show carbonyl stretching bands at 1735–1740 cm^{-1} (**RB** esters) or at 1722–1730 cm^{-1} (**EO** esters). Esters of each dye have similar visible spectra in dioxane solution (Table 1), with four maxima at 412, 468–470, 497–500 and 530–534 nm (**RB** esters), or at 378, 452–455, 476–478, and 510 nm (**EO** esters). Spectra in dioxane–aqueous pH 0·5 1:1 solution (Table 2) have the same shape as in dioxane, indicating that the molecules cannot lactonize in acidic media, whilst in the mixture of dioxane–aqueous pH 8 buffer, 1:1, the ionization of their free phenolic group produces strong absorption at 568–570 nm, or at 535–538 nm, respectively. In each medium, esters of each dye have very similar molar extinction coefficients, revealing the small contribution of the carboxylate group to the absorption properties of these molecules. Their ^{1}H- and ^{13}C-NMR spectra in DMSO-d_6 (Tables 3, 4 and 5) are in accord with symmetric xanthenic structures, which are induced by the fast interchange of the phenolic proton between positions 3 and 6. It is interesting to note that when the ^{13}C-NMR spectrum of the methyl ester **EO.3** was registered in the coupling mode, a pseudo-quadruplet ($^3J = 4$ Hz) at δ 151·2 was observed for C-9, suggesting that H-1, H-8, and H-6′ have similar stereochemistry with respect to this carbon, and that the benzoate nucleus is not in the same plane as the quinonoid xanthenic moiety. ^{1}H-NMR spectra of the salts (phenoxides) of the six esters (Table 3) also indicate symmetric structures, with H-1 and H-8 appearing together at higher field than in the corresponding un-ionized forms. Useful mass spectra

of **RB** esters could not be obtained. On the contrary, spectra of **EO** esters show fragmentations due to the loss of the corresponding carboxyalkyl groups.

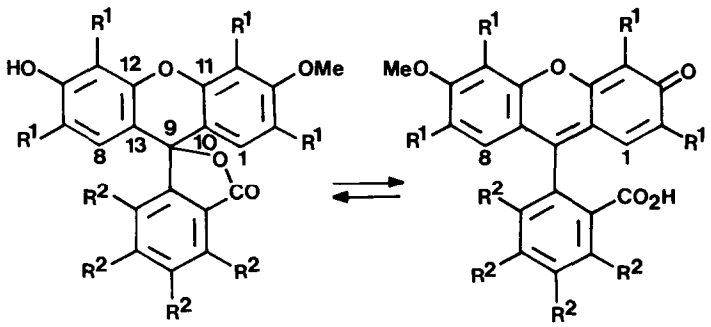
The esterification of Rose Bengal with benzyl chloride was also studied under other conditions. In refluxing acetone the reaction rate was similar, but it was increased by a factor of about two in the presence of potassium cations (potassium carbonate or potassium chloride) instead of sodium cations, or working with Rose Bengal in the form of the dipotassium salt. In refluxing cyclohexanone, an excellent solvent for polystyrene, the expected benzyl ester **RB.4** was also obtained, together with variable amounts of two other products which were also formed in the absence of benzyl chloride. They were identified as the 2-oxocyclohexyl ester **RB.6** and the decarboxylated derivative **RB.10** (see later).

As for other **RB** esters, aqueous alkaline hydrolysis of **RB.6** produced Rose Bengal. Its IR spectrum showed bands at 1725 (ketone CO), at 1740 (ester CO), and at *c.* 2900 cm^{-1} (aliphatic chain), and its visible spectral data were similar to those of other **RB** esters (Tables 1 and 2). The lack of symmetry of the molecule was reflected in both its ^{1}H- (Table 3) and ^{13}C- (Table 4) NMR spectra. Moreover, its ^{1}H-NMR spectrum had a double doublet at δ 5·14, assigned to the proton of the CO_2CH group (position 1″) with axial–axial and axial–equatorial couplings, showing that the cyclohexanone moiety is attached to the carboxylate group through an equatorial bond. Ester **RB.6** was obtained only when oxygen and visible light were simultaneously present, so that it could be formed via α-hydroperoxycyclohexanone, generated by the dye-sensitized photooxidation of cyclohexanone. No efforts were made to confirm this hypothesis.

Reaction with diazomethane

Contrary to the esterification, the reaction with diazomethane of the lactones **RB.1** and **EO.1** was not selective, producing a mixture of the respective ether-esters **RB.7** and **EO.7**, monoethers **RB.8** and **EO.8**, and diethers **RB.9** and **EO.9**, a result similar to that described for fluorescein.[48] Methyl esters **RB.3** and **EO.3** were not detected. With excess of diazomethane, mixtures of ether-esters and diethers were obtained, showing that the primary reaction is the formation of monoethers, followed by the formation of diethers, or of esters of the quinonoid tautomers of the monoethers. The compounds were separated by chromatography and identified by spectroscopy.

The visible spectrum of the ether-ester **RB.7** in dioxane (Table 1), or in its 1:1 mixture with aqueous pH 0·5 buffer (Table 2), had similar shape, although



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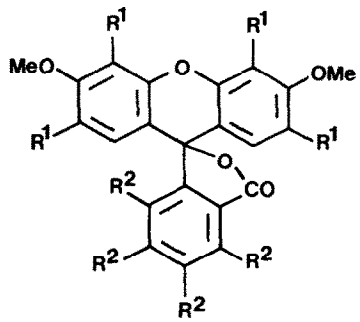
somewhat lower molar extinction coefficients, than the spectra of other **RB** esters with free phenolic groups, and it was very little influenced by the change of the medium pH, indicating that the molecule cannot lactonize or ionize. Its IR spectrum showed carbonyl absorption, and both its ^{1}H- and ^{13}C-NMR spectra (Tables 3 and 4) were in accordance with a fixed, non-symmetric xanthenic group. Similar comments are relevant to the ester **EO.7**, a compound which shows in its mass spectrum a molecular ion with unusually high intensity. Non-symmetric monoethers **RB.8** and **EO.8** were isolated as almost colourless solids showing characteristic carbonyl lactonic bands in their IR spectra. The lactonic form remained in dioxane solution (also colourless) or in DMSO-d_6, as reflected in their ^{1}H- and ^{13}C-NMR spectra. The visible spectrum of each compound in dioxane–aqueous pH 8 buffer, 1:1, indicated that the lactone was only partially opened, producing a mixture of two forms: the quinonoid form, ionized in the carboxylic group, and the lactonic form, ionized in the phenolic group. Percentages of ionized quinonoid forms of *c.* 48% and 6% respectively could be deduced from their visible spectra, assuming that they must be identical to those of the corresponding ether-esters **RB.7** and **EO.7** in the same medium. This assumption is founded in the previously noted small influence of the 2′-carboxylate group on the absorption properties of the quinonoid molecules.

The dimethyl ethers **RB.9** and **EO.9** were fixed lactones with symmetric structures, each one showing equivalent methyl groups in both the ^{1}H- and ^{13}C-NMR spectra. Comparison of all their spectral data with those of other **RB** and **EO** lactones confirmed their structures.

Thermal decomposition

As noted above, when Rose Bengal is heated in cyclohexanone it produces the decarboxylated derivative **RB.10**,[31] among other products. This compound also appeared, in almost quantitative yield, in refluxing anhydrous dimethylformamide. It displayed a visible spectrum very similar

to those of **RB** esters, with identical pH dependence, but its IR spectrum did not show carbonyl absorption. Its ^{1}H-NMR spectrum showed two singlets, assigned to the two equivalent protons in positions 1 and 8, and to the proton in position 2'. In its coupled ^{13}C-NMR spectrum, carbon in position 2' appeared as a doublet ($J_{CH} = 175$ Hz) at δ 130, and no carboxylic carbon was observed. Diazomethane treatment of **RB.10** afforded the methyl ether **RB.11**, an unsymmetric compound with fixed quinonoid structure, in accord with its spectral data.



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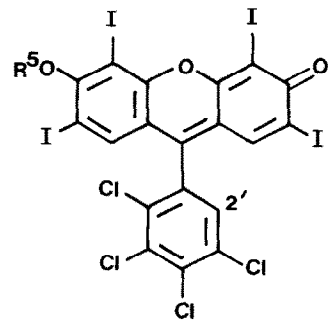

	R^5
RB.10	H
RB.11	Me

Neither uranine nor Eosin Y decarboxylates under the same conditions, so that the reaction must be related to the presence of chlorine atoms in the benzoate ring of Rose Bengal, because salts of pentachlorobenzoic acid[49] and of 3,5-dichloro-4-hydroxybenzoic acid[50,51] also decarboxylate on heating.

When Eosin Y was heated in DMF under similar conditions, complex mixtures of unidentified products were obtained. In contrast, good yields in the debrominated compound **EO.2** were observed after heating in DMSO.

HPLC analysis

All the compounds were analysed by HPLC in a reverse-phase column. In the acidic mobile phase used they adopt the corresponding non-ionized quinonoid or, if possible, lactonic structures.[52] Retention and response data, both relative to the corresponding parent compounds **RB.1** and **EO.1**, are shown in Table 6. Roughly speaking, the retention of a neutral compound in such a system depends essentially, and in this order, on its molecular volume, on its capacity for H-bonding with the mobile phase, and on its polarity (or polarizability).[53,54] Results presented here are in accord with these effects, and confirm the assigned structures. Molecules with higher volume are more

TABLE 6
HPLC Data for Rose Bengal and Eosin Y Derivatives[a]

Compound[b]	*Relative retention*	*Relative response*	*Compound*[b]	*Relative retention*	*Relative response*
RB.2 (l)	0·52	1·32	**EO.2** (l)	0·58	1·18
RB.1 (l)	1·00[c]	1·00[d]	**EO.3** (q)	0·75	0·86
RB.6 (q)	1·37	—	**EO.1** (l)	1·00[e]	1·00[f]
RB.3 (q)	1·43	1·98	**EO.8** (l)	1·09	0·89
RB.4 (q)	1·81	2·17	**EO.4** (q)	1·10	0·71
RB.8 (l)	1·84	1·16	**EO.7** (q)	1·18	0·83
RB.10 (q)	1·97	1·89	**EO.5** (q)	1·74	0·92
RB.7 (q)	2·02	1·86	**EO.9** (l)	2·13	1·04
RB.5 (q)	3·06	1·94			
RB.9 (l)	3·16	1·16			
RB.11 (q)	3·33	2·22			

[a] Operating conditions in Experimental section.
[b] Lactonic (l), or quinonoid (q) structures.
[c] k' 1·65.
[d] Absolute response $1{\cdot}28 \times 10^{-5}$ μmol mm^{-2}.
[e] k' 0·76.
[f] Absolute response $3{\cdot}18 \times 10^{-5}$ μmol mm^{-2}.

retained, as can be seen in the couples of lactones differing in the number of halogen atoms **RB.2/RB.1**, or **EO.2/EO.1**, or in the quinonoid esters differing in the volume of the ester group **RB.3/RB.4/RB.5**, or **EO.3/EO.4/EO.5**, or in derivatives of Eosin Y when compared with derivatives of Rose Bengal with the same structure. Moreover, H-bonding with the mobile phase can justify the increasing retention values observed for lactones with similar volume but with none, one or two methyl ether groups **RB.1/RB.8/RB.9**, or **EO.1/EO.8/EO.9**, or for the couples of quinonoid compounds differing in a methyl ether group **RB.3/RB.7**, **RB.10/RB.11**, and **EO.3/EO.7**. Esters **RB.3** and **RB.7**, with carboxymethyl groups in position 2′, are less retained than the corresponding compounds without this group, **RB.10** and **RB.11**, probably due to the higher H-bonding capacity and/or to the higher polarity of the former with respect to the latter. The same effects must be the cause of the somewhat lower retention of the non-symmetric 2-oxocyclohexyl ester **RB.6** with respect to that of the methyl ester **RB.3**, although the carboxylate group of the former has a bigger volume.

EXPERIMENTAL

General data

Melting points (m.p.) and softening points (s.p.) were obtained in a Reichert microscope and are not corrected. Microanalyses were performed in a

Perkin–Elmer (PE) 240C apparatus; solvent-free samples were obtained after selective treatments: dioxane was eliminated by heating in vacuum (0·5 torr, 100°C, 5 h); benzene and $CHCl_3$ were removed by stirring for some hours with hexane and diethyl ether, successively, and vacuum treatment at room temperature; water was withdrawn by refluxing with benzene for 2 h, and eliminating the occluded benzene as before. Electronic absorption spectra were recorded on a PE 402 spectrophotometer, in dioxane or in its 1:1 mixture with aqueous buffer of pH 0·5 (0·2 M-HCl/KCl), or of pH 8 (0·033 M-phosphate). IR spectra were recorded in a PE 681 apparatus. ^{1}H-NMR spectra were obtained in a Varian EM-390 spectrometer at 90 MHz, using 0·05–0·1 M-solutions in DMSO-d_6 and TMS as internal reference; spectra of compounds **EO.2** and **RB.6** were recorded in a Varian XL-300 at 300 MHz; ^{13}C-NMR spectra of similar solutions were recorded in this apparatus, at 75 MHz, and assignments were made with the aid of off-resonance experiments; the spectra of ionized forms, except those of Rose Bengal and Eosin Y, were recorded after adding a drop of 10% NaOD in D_2O. Mass spectra (MS) of Eosin Y derivatives were obtained in a RMU-6MG Hitachi-PE spectrometer in the electronic impact mode, at 75 eV, with direct injection and heating at 250–300°C; MS spectra of the lactone **RB.1**, and of the decarboxylated derivative **RB.10** were obtained in a Varian MAT-711 spectrometer, at 100 eV; other **RB** derivatives deiodinate in the probe, producing unsatisfactory spectra. Thin layer chromatography (TLC) was carried out on 0·2 mm plates of silica gel Merck 60 F_{254}. Column chromatography was performed with silica gel Merck 60G and the short column procedure;[55] after solvent elimination, compounds were usually submitted to treatment with aqueous 2 M-HCl and ethyl acetate (EtOAc) extraction. HPLC analyses were run with a Waters M45 pump, a 7125 Rheodyne sample injector (20 μl), a PE C18/10 reverse phase column (0·46 cm × 25 cm), a Philips PU 4020 UV detector fixed at 254 nm, and a PE 159 recorder working with a chart speed of 12·5 mm min^{-1}; the best mobile phase found was the mixture methanol–aqueous pH 1 buffer (0·2 M-HCl/KCl), 9:1 v/v, at 2 ml min^{-1}, although its use for long periods is not advisable; for quantitative analysis, response factors expressed as μmol per mm^2 of chart paper in attenuation unit (25 cm of chart paper per absorbance unit), were determined.

Purification of the main components of commercial Rose Bengal and Eosin Y

A solution of 1·5 g of commercial (Fluka) Rose Bengal (77% molar by HPLC) or Eosin Y (46% molar) in 100 ml water was acidified (pH 1, HCl), extracted with 3 × 80 ml EtOAc, and the organic layer was washed with water, dried and concentrated to about 5 ml. This solution was placed on the

top of a column (7·5 cm internal diam.) formed with 150 g silica gel and $CHCl_3$–acetic acid, 99:1 v/v, using as mobile phase the same solvent mixture. In each case, the higher halogenated lactone **RB.1** or **EO.1** was eluted first; yield of pure product 50–60%. Monodehalogenated compounds **RB.2** and **EO.2** were purified in a second column, prepared with EtOAc and eluted with the mixture EtOAc–EtOH, 9:1 v/v, or prepared with benzene and eluted with benzene–acetic acid, 99:1 v/v respectively. In all the cases the separated solids were stirred for 1 h with 5 ml $CHCl_3$, filtered and crystallized from dioxane–water (**RB.1**, **RB.2** and **EO.1**) or dioxane–hexane (**EO.2**). A solid with 96% content (HPLC) in lactone **RB.1** could also be obtained from commercial Rose Bengal by iodination in aqueous pH 8 buffer,[56] acidification, EtOAc extraction, solvent elimination, and crystallization as before.

Pure disodium salts Rose Bengal and Eosin Y were obtained by heating (reflux, stirring, 10 min) a solution of 1 g of the corresponding lactone and an equimolecular amount of sodium carbonate in 10 ml water–MeOH, 1:1.[57] After adding an equal volume of EtOH, the solution was refluxed (10 min), cooled and the solvent vacuum-evaporated. The isolated solids were crystallized from EtOH–hexane and handled as hygroscopic products. Yields of pure dried salts were 70% and 95%, respectively.

*Lactone **RB.1***

White crystals, m.p. 286–287°C, s.p. 242°C. (Analysis. Calculated for $C_{20}H_4Cl_4I_4O_5$: C, 24·65; H, 0·41; I, 52·14. Found: C, 24·34; H, 0·56; I, 52·42%). UV–vis., λ_{max} (log ε), DMF: 522 (4·54), 563 (5·13) nm. IR (KBr) ν_{max}: 3450s, 1773s, 1450m, 1410vs, 1380m, 1210s, 1170m, 875w cm^{-1}. MS *m/z* (%): 972 (M^+, 3), 928 (3), 892 (19), 800 (40), 765 (3), 254 (74), 128 (54), 127 (100).

Disodium quinonoid salt Rose Bengal

Red crystals, m.p. >300°C. (Analysis. Calculated for $C_{20}H_2Cl_4I_4Na_2O_5$: C, 23·58; H, 0·20; I, 49·88. Found: C, 23·28; H, 0·52; I, 50·25%). UV–vis., λ_{max} (log ε): water, 265 (4·59), 316 (4·16), 351 (3·68), 516 (4·57), 551 (5·03) nm; DMSO, 525 (4·49), 567 (5·07) nm. IR (KBr) ν_{max}: 1610m, 1550s, 1460vs, 1340s, 1235m, 960s cm^{-1}.

*Lactone **RB.2***

Pink crystals, m.p. >300°C, s.p. 260°C. (Analysis. Calculated for $C_{20}H_5Cl_4I_3O_5$: C, 28·31; H, 0·59; I, 44·91. Found: C, 28·02; H, 0·80; I, 44·70%). IR (KBr) ν_{max}: 3450b, 1775m, 1745vs (spectra of crystals with traces of solvents present only one carbonyl band, its position depending on the solvent, 1780 cm^{-1} with dioxane–water, or 1745 cm^{-1} with EtOAc–hexane), 1618m, 1595m, 1432s, 1415vs, 1385m, 1215s, 1170m cm^{-1}.

Lactone ***EO.1***
Yellowish crystals, m.p. 299–301°C, s.p. 250°C. (Analysis. Calculated for $C_{20}H_8Br_4O_5$: C, 37·06; H, 1·23; Br, 49·35. Found: C, 37·21; H, 1·39; Br, 49·72%). UV–vis., λ_{max} (log ε), MeOH: 494sh (4·48), 527 (5·00) nm; dioxane–aqueous pH 0·5 buffer, 1:1 v/v: 286 (3·86), 300sh (3·52), 400 (2·88), 450sh (3·05), 479 (3·24), 510 (3·15) nm. IR (KBr) ν_{max}: 3470s, 3240b, 1755vs, 1465s, 1425vs, 1331s, 1290s, 1260s, 1245s, 1210vs, 1115s, 985m cm^{-1}. MS *m/z* (%): 644 (M^+, 0·1), 600 (3), 599 (3), 521 (36), 512 (4), 442 (7), 198 (100).

Disodium quinonoid salt Eosin Y
Red crystals, m.p. >300°C. (Analysis. Calculated for $C_{20}H_6Br_4Na_2O_5$: C, 34·72; H, 0·87; Br, 46·19. Found: C, 34·27; H, 1·01; Br, 45·66%). UV–vis., λ_{max} (log ε), water: 255 (4·57), 303 (4·18), 345 (3·60), 486sh (4·50), 519 (4·98) nm. IR (KBr) ν_{max}: 1615m, 1560vs, 1505m, 1465vs, 1355vs, 1240s, 1175m, 1060m, 978s cm^{-1}.

Lactone-quinonoid ***EO.2***
Orange crystals, m.p. 256–257°C, s.p. 183°C. (Analysis. Calculated for $C_{20}H_9Br_3O_5$: C, 42·20; H, 1·58; Br, 42·15. Found: C, 42·37; H, 1·86; Br, 42·20%). UV–vis., λ_{max} (log ε), dioxane–aqueous pH 0·5 buffer 1:1 v/v: 282 (3·83), 300sh (3·48), 406 (3·16), 442sh (3·38), 472 (3·58), 502 (3·51) nm. IR (KBr) ν_{max}, 3430b, 1769w, 1749w, 1729w, 1700w (the spectrum in DMSO presents a sole carbonyl band at 1768 cm^{-1}), 1575m, 1515s, 1495s, 1469vs, 1422s, 1309vs, 1265m, 1215m, 974w cm^{-1}. MS *m/z* (%): 566 (M^+, 3), 522 (32), 521 (30), 486 (3), 458 (3), 443 (100), 414 (10), 364 (10), 336 (24), 200 (64), 198 (32).

Synthesis of esters

A mixture of 0·2 mmol of **RB.1** or **EO.1**, 30 ml acetone, 15 ml DMF, 0·3 mmol anhydrous sodium carbonate and 5 mmol methyl iodide, or 0·3 mmol benzyl chloride, or 0·3 mmol *p*-isopropylbenzyl chloride,[58] was stirred at room temperature for 2 h (for methyl esters) or at reflux temperature for 2 days (for benzyl or *p*-isopropylbenzyl esters). After vacuum-elimination of the volatile components, excess aqueous 2 M-HCl was added and each mixture was extracted with 3 × 100 ml EtOAc, the extract was washed with water and, without drying, the solvent was eliminated. Yield (HPLC) higher than 95% in all the cases. The esters were further purified by column chromatography (benzene–acetic acid, 90:10 v/v, for **EO** esters and the methyl ester of **RB.1**, or 99:1 v/v for other **RB** esters). Yield of isolated product > 70%. The ester **EO.5** so isolated still contained traces of a compound which were eliminated by preparative TLC. The purified esters were crystallized from $CHCl_3$–hexane.

Methyl ester ***RB.3***[27]
Red crystals, m.p. >300°C, s.p. 294°C. (Analysis. Calculated for $C_{21}H_6Cl_4I_4O_5$: C, 25·52; H, 0·61; I, 51·40. Found: C, 25·50; H, 0·86; I, 51·13%). IR (KBr) ν_{max}: 3450b, 1735s, 1607s, 1560vs, 1530s, 1510vs, 1400m, 1325s, 1295s, 1260s, 960s cm^{-1}.

The sodium salt of **RB.3** was obtained by neutralization with sodium carbonate in acetone. IR (KBr) ν_{max}: 1754m, 1618m, 1550s, 1460vs, 1348s, 1245m, 960s cm^{-1}.

Benzyl ester ***RB.4***[23]
Orange crystals, m.p. 184–185°C (decomp.). (Analysis. Calculated for $C_{27}H_{10}Cl_4I_4O_5$: C, 30·46; H, 0·94; I, 47·72. Found: C, 30·97; H, 1·06; I, 47·59%). IR (KBr) ν_{max}: 3450b, 1740m, 1605s, 1565vs, 1530m, 1505s, 1330m, 1300m, 1250m, 960m cm^{-1}.

p-*Isopropylbenzyl ester* ***RB.5***
Orange crystals, m.p. 246–247°C (decomp.). (Analysis. Calculated for $C_{30}H_{16}Cl_4I_4O_5$: C, 32·56; H, 1·45; I, 45·91. Found: C, 33·09; H, 1·45; I, 46·02%). IR (KBr) ν_{max}: 3380b, 1735s, 1610s, 1560vs, 1530s, 1505vs, 1410m, 1330s, 1265s, 1250s, 1180m, 960s cm^{-1}.

Methyl ester ***EO.3***
Reddish orange crystals, m.p. 291–292°C, s.p. 245°C. (Analysis. Calculated for $C_{21}H_{10}Br_4O_5$: C, 38·09; H, 1·51; Br, 48·13. Found: C, 38·06; H, 1·53; Br, 48·14%). IR (KBr) ν_{max}: 3420b, 1722m, 1705w, 1615m, 1575vs, 1515vs, 1470s, 1335m, 1300s, 1285s, 1180m, 1089m, 985s cm^{-1}. MS *m/z* (%): 658 (M^+, 20), 579 (6), 520 (2), 492 (8), 413 (20), 198 (100).

Benzyl ester ***EO.4***
Orange crystals, m.p. 264–265°C, s.p. 250°C. (Analysis. Calculated for $C_{27}H_{14}Br_4O_5$: C, 43·93; H, 1·90; Br, 43·33. Found: C, 43·66; H, 1·78; Br, 43·76%). IR (KBr) ν_{max}: 3450b, 1730s, 1572s, 1515vs, 1472m, 1433m, 1340m, 1288s, 1135s, 983s cm^{-1}. MS *m/z* (%): 734 (M^+, 6), 599 (4), 520 (6), 492 (4), 198 (100).

p-*Isopropylbenzyl ester* ***EO.5***
Orange crystals, m.p. 146–148°C (decomp.). (Analysis. Calculated for $C_{30}H_{20}Br_4O_5$: C, 46·18; H, 2·57; Br, 40·99. Found: C, 45·72; H, 2·54; Br, 41·16%). IR (KBr) ν_{max}: 3480b, 1725s, 1602m, 1575vs, 1505vs, 1465s, 1340m, 1300s, 1275s, 1183m, 980m cm^{-1}. MS *m/z* (%): 776 (M^+,5), 697(10), 599 (20), 521 (100), 414 (20), 198 (40).

2-Oxocyclohexyl ester ***RB.6***

This was obtained, together with variable amounts of the decarboxylated derivative **RB.10**, when a solution of 200 mg of pure Rose Bengal in 50 ml cyclohexanone was heated at reflux for two days in the presence of oxygen and under the normal illuminating conditions of the laboratory. Subsequent work-up, column separation (benzene–acetic acid, 95:5 v/v), and crystallization from dioxane–hexane yielded orange crystals, m.p. 259–261°C. Yield 8–15%. (Analysis. Calculated for $C_{26}H_{12}Cl_4I_4O_6$: C, 29·19; H, 1·13. Found: C, 29·50; H, 1·30%). IR (KBr) ν_{max}: 3410b, 2960m, 2935m, 2865m, 1740m (CO ester), 1725m (CO ketone), 1565vs, 1505s, 1250s, 955s cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1·10–2·00 (m, 6 H, H-4″ to H-6″), 2·07 (m, 1 H, H-3″ equatorial), 2·34 (dt, $J_{ax-ax} = 13$ Hz and $J_{ax-eq} = 6$ Hz, 1 H, H-3″ axial), 5·14 (dd, $J_{ax-ax} = 12$ Hz and $J_{ax-eq} = 6$ Hz, 1 H, H-1″), 7·77 and 7·78 (2 s, H-1 and H-8) ppm. ^{13}C-NMR (DMSO-d_6) (only the 2-oxocyclohexyl group is shown; other signals in Table 4) δ: 22·4 and 26·4 (C-5″ and C-4″), 32·0 (C-6″), 78·1 (C-1″), 202·4 (ketone CO) ppm; C-3″ can be under the solvent signal.

Reactions with diazomethane

A solution of diazomethane in ether (0·6 mmol ml^{-1}) was added dropwise to an ice-cooled solution of 0·4 mmol **RB.1** or **EO.1** in 80 ml dioxane. After the addition of 0·9 mmol diazomethane, each reaction mixture showed (HPLC) the absence of the starting lactone and the presence of the respective ether-ester **RB.7** or **EO.7**, monoether **RB.8** or **EO.8**, and diether **RB.9** or **EO.9**, in molar ratio of about 5:3:2 or 2:3:5. After the addition of more than 2 mmol diazomethane only ether-esters and diethers were detected, in respective molar ratios of about 6:4 and 2:8. Elimination of the solvents yielded mixtures which were subjected to column chromatography ($CHCl_3$). The purified products were crystallized from $CHCl_3$–hexane.

Methyl ether–methyl ester ***RB.7.***[25]

Reddish orange crystals with undefined m.p., s.p. 215°C. (Analysis. Calculated for $C_{22}H_8Cl_4I_4O_5$: C, 26·36; H, 0·80; I, 50·68. Found: C, 25·91; H, 0·62; I, 50·88%). IR (KBr) ν_{max}: 1735s, 1610s, 1565s, 1535s, 1510m, 1395m, 1375m, 1265vs, 1180m, 970s cm^{-1}.

Methyl ether ***RB.8.***[25]

Pale orange crystals, m.p. 306–307°C, s.p. 265°C. (Analysis. Calculated for $C_{21}H_6Cl_4I_4O_5$: C, 25·52; H, 0·61; I, 51·40. Found: C, 26·04; H, 0·74; I, 51·01%). IR (KBr) ν_{max}: 3450b, 1770s, 1455m, 1410vs, 1380s, 1235m, 1205s, 1170m, 870w cm^{-1}.

Dimethyl ether ***RB.9***
White crystals, m.p. >300°C. (Analysis. Calculated for $C_{22}H_8Cl_4I_4O_5$: C, 26·36; H, 0·80; I, 50·68. Found: C, 26·57; H, 0·71; I, 50·91%). IR (KBr) ν_{max}: 1780vs, 1460m, 1410vs, 1390vs, 1238s, 1200s, 1170m, 870w cm^{-1}.

Methyl ether–methyl ester ***EO.7***
Reddish orange crystals, m.p. > 300°C, s.p. 285°C. (Analysis. Calculated for $C_{22}H_{12}Br_4O_5$: C, 39·08; H, 1·78; Br, 74·31. Found: C, 38·75; H, 1·54; Br, 47·54%). IR (KBr) ν_{max}: 1729vs, 1619vs, 1575s, 1540s, 1515s, 1330m, 1270s, 1195m, 1089m, 990s cm^{-1}. MS *m/z* (%): 672 (M^+, 100), 657 (7), 613 (3), 593 (20), 524 (7), 509 (9), 428 (36), 198 (99).

Methyl ether ***EO.8***
Pale orange crystals, m.p. 298–300°C, s.p. 280°C. (Analysis. Calculated for $C_{21}H_{10}Br_4O_5$: C, 38·09; H, 1·51; Br, 48·31. Found: C, 37·79; H, 1·37; Br, 48·66%). IR (KBr) ν_{max}: 3460b, 1755vs, 1615m, 1605m, 1595m, 1550m, 1470s, 1420vs, 1405s, 1330m, 1259m, 1230m, 1210vs, 1085s, 1000m cm^{-1}. MS *m/z* (%): 658 (M^+, 4), 614(12), 613(8), 535(100), 520(16), 492(12), 278(20), 198(68).

Dimethyl ether ***EO.9***
White crystals, m.p. >300°C, s.p. 250°C. (Analysis. Calculated for $C_{22}H_{12}Br_4O_5$: C, 39·08; H, 1·78; Br, 47·31. Found: C, 39·08; H, 1·79; Br, 47·05%). IR (KBr) ν_{max}: 1785vs, 1610m, 1470s, 1420vs, 1402vs, 1255s, 1200s, 1085s, 1075s, 998m cm^{-1}. MS *m/z* (%): 672 (M^+, 5), 627 (7), 549 (100), 534 (15), 519 (6), 440 (10), 412 (5), 198 (28).

Decarboxylation of Rose Bengal

A mixture of 200 mg (0·2 mmol) of the lactone **RB.1**, 50 mg potassium carbonate and 20 ml DMF was heated at reflux, while stirring, for 1 h in the dark. After cooling, the solvent was vacuum-evaporated, aqueous 2 M-HCl was added to the residual solid and the mixture was extracted with 4 × 70 ml hot $CHCl_3$. Elimination of the solvent yielded the decarboxylated compound **RB.10** with 95% purity (HPLC). It could be further purified by column chromatography (benzene–acetic acid, 99:1 v/v) and crystallization from $CHCl_3$–hexane. Yield of purified product >70%. Similar yields were obtained when a mixture of 0·2 mmol **RB.1**, 6 ml aqueous 0·1 M-KOH and 20 ml DMF was heated for 1 h, or when the disodium or the dipotassium salt of **RB.1** was heated in DMF for the same time.

Decarboxylated compound ***RB.10.***[31]
Red crystals, m.p. >300°C, s.p. 280°C. (Analysis. Calculated for $C_{19}H_4Cl_4I_4O_3.0{\cdot}4(C_2H_5)_2O$: C, 25·57; H, 0·77; I, 53·24. Found: C, 25·43; H, 1·26; I, 52·85%). IR (KBr) ν_{max}: 3430b, 1608s, 1565vs, 1530s, 1510vs, 1417m, 1330s, 1300m, 1255m, 1190m, 960s cm^{-1}. MS *m/z* (%): 928 (M^+,3), 801(2), 674(3), 574(2), 254(27), 128(100), 127(63).

Methyl ether ***RB.11***
This was obtained by treating 0·2 g of **RB.10** in dioxane with excess of diazomethane in ether. The product was purified by column chromatography ($CHCl_3$), and by crystallization from dioxane–hexane. Orange crystals, m.p. >300°C. Yield 91%. (Analysis. Calculated for $C_{20}H_6Cl_4I_4O_3$: C, 25·43; H, 0·64; I, 53·79. Found: C, 25·74; H, 0·83; I, 53·93%). IR (KBr) ν_{max}: 1610vs, 1560s, 1533s, 1505s, 1410m, 1378s, 1267m, 1176m, 970s cm^{-1}.

Debromination of Eosin Y

A solution of 10 mg of purified Eosin Y in 20 ml DMSO was heated at reflux in the dark. After 10 h no initial product was observed (HPLC), while *c.* 90% of the debrominated compound **EO.2** was detected, among other products. It was identified by comparison with a pure sample.

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