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Spectrophotometric Determination of Ionization and Isomerization Constants of Rose Bengal, Eosin Y and Some Derivatives

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ABSTRACT

The experimental pK values corresponding to the acid forms of Rose Bengal, Eosin Y, their methyl esters, their methyl ethers, and the decarboxylated compound derived from the first dye, all of them in solution in 1:1 mixtures of dioxane with aqueous buffers, have been determined by applying graphical methods to visible spectrophotometric data. Assuming the same visible absorption spectra for molecules with similar chromophoric groups, the isomerization constants of the possible equilibria between lactonic and quinonoid tautomeric structures, and the pK values of the ionizations of the molecular forms which can be present, have been estimated. In the quinonoid molecular forms of Rose Bengal and Eosin Y, their phenol groups are ionized first, with estimated pK values of 2·57 and 2·69 respectively, while their carboxylic groups show much less acidity, and pK values of 5·01 and 6·25.

INTRODUCTION

The xanthenic salts Rose Bengal and, to a lesser extent, Eosin Y are two halogenated dyes of constant photochemical interest due to their high efficiencies as singlet oxygen photogenerators. The easy and selective esterification of their carboxylate group with alkyl halides gives rise to free or polymer-bound derivatives which are also good photo-oxygenation

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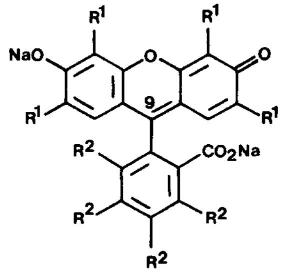
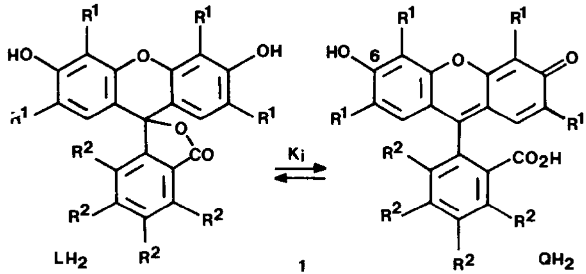
sensitizers for homogeneous or heterogeneous media.[1-4] The strong colour of the solutions of the parent dyes is mainly due to the phenol-ionized xanthenic moiety, the carboxylate group having a much lower influence on their visible absorption.[5] The corresponding pure molecular forms **RB.1** and **EO.1** are colourless and slightly yellow solids respectively, indicating prevailing lactonic structures LH_2.[3] However, their solutions can also be coloured due to two possible processes: first, the isomerization to the quinonoid tautomers QH_2, with a distinctly different and less intense colour than the respective salts, and second, the ionization of the phenol group in these QH_2 forms, a process favoured in polar or protic solvents or, in aqueous media, in the presence of bases.



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



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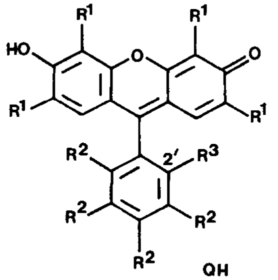


QH

	R^3
2	CO_2Me
3	H

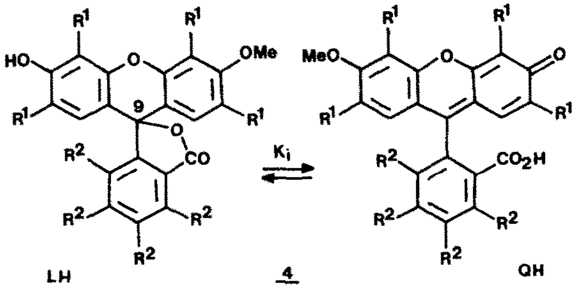
The values of the isomerization constant K_i of **EO.1** in several protic and aprotic solvents, containing enough strong acid to assure the absence of ionized forms, have been estimated assuming that in each solvent the QH_2 tautomer has the same molar extinction coefficient in its visible maximum as its ethyl ester.[6] In all the acidic media studied the equilibrium is strongly displaced towards the lactonic form.

The ionization equilibria of **RB.1** and **EO.1** have usually been studied by spectrophotometry in water[7–9] or in mixtures of water with miscible organic solvents.[7,10,11] The pK values thus found for the ionization constants depend not only on the medium but on the experimental method followed (Table 1). The first experimental ionization has been assigned in **EO.1** to the phenolic group in the QH_2 form, since its acidity is enhanced by the presence of the four halogens.[7] The second ionization is difficult to observe by spectrophotometry due to its overlapping with the first one, and to its low contribution to the visible spectrum. In fact, in some cases only one ionization could be determined in water.[12–14] A maximum observed in the variation of the absorbance readings of **EO.1** solutions with pH[7,10] has allowed the determination of the two constants by the method of Ang.[15] The different values shown in Table 1 for a given dye in water can be a consequence of the different experimental conditions and of the low solubility of the LH_2 forms, the precipitation of which on acidification can be inadvertent.[8]

In the present paper we continue our current interest on the analysis of the structures of xanthene dyes in solution,[16,17] reporting the results of a study directed to determine the values of the macroscopic (experimental) and microscopic (molecular)[18] pK values of the acid forms of Rose Bengal and Eosin Y, and to estimate the possible isomerization constants involved, not taken into account in former papers. To attain these aims the pK values of the methyl esters **RB.2** and **EO.2**, the decarboxylated compound **RB.3** and the methyl ethers **RB.4** and **EO.4** have been also determined. In order to

TABLE 1
Some pK Values Described for the Molecular Forms of Rose Bengal and Eosin Y, Determined by Spectrophotometry in Different Media

Compound	*Medium* (*v*:*v*)	pK_1	pK_2	*Ref.*
RB.1	H_2O	3·90	4·70	8
	H_2O	3·51	4·05	9
	Ethanol–H_2O (1:9)	4·26	5·25	11
EO.1	H_2O	2·85	4·37	7
	H_2O	2·60	3·40	8
	Dioxane–H_2O (4:6)	4·45	6·02	10
	Methanol–H_2O (5:5)	3·16	5·40	7
	Methanol–H_2O (9:1)	6·70	8·60	7
	Acetone–H_2O (5:5)	3·50	6·00	7



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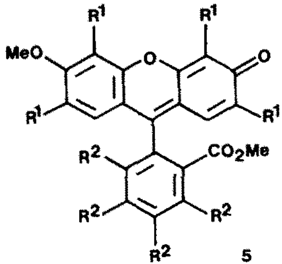

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preclude the precipitation of the corresponding molecular forms, solutions in 1:1 (v/v) mixtures of dioxane with aqueous buffers have been employed.

METHODS

The pK_1 value of a monoprotic acid with ionization constant K_1 can be determined by spectrophotometry if the molecular form AH has different electronic absorption spectra than the ionized form A^-. Assuming that both forms obey the Beer law, the ratio $[AH]/[A^-]$ in eqn (1) can be deduced from the absorbances a_{AH}, a_{A^-} and a, of solutions of AH, A^-, and their mixtures, respectively, measured in comparative conditions, at the analytical wavelength, and at extreme acidic, extreme basic and intermediate pH values. If $a_{A^-} > a_{AH}$, the intercept with the pH axis of the plot of pH vs the logarithmic term of eqn (2) gives the pK_1 value.[19]

$$\mathrm{p}K_1 = \mathrm{pH} + \log\frac{[\mathrm{AH}]}{[\mathrm{A}^-]} \tag{1}$$

$$\mathrm{pH} = \mathrm{p}K_1 + \log\frac{a_{\mathrm{A}^-} - a}{a - a_{\mathrm{AH}}} \tag{2}$$

In the case of a diprotic acid with ionization constants $K_1 = [AH^-][H^+]/[AH_2]$ and $K_2 = [A^{2-}][H^+]/[AH^-]$, the corresponding p$K_1$ and pK_2 values can also be easily determined by spectroscopy if the spectra of the three species involved, AH_2, AH^- and A^{2-}, are different, and the two equilibria $AH_2 \rightleftharpoons AH^- + H^+$ and $AH^- \rightleftharpoons A^{2-} + H^+$ do not overlap. If they overlap, i.e. if the pK values lie within three pK units, several procedures are available.[15,19–21] When the three species absorb at the analytical wavelength, the observed molar extinction coefficient ε can be expressed as a function of the respective coefficients ε_{AH_2}, ε_{AH^-} and $\varepsilon_{A^{2-}}$, and the molar fractions f:

$$\varepsilon = \varepsilon_{AH_2} \cdot f_{AH_2} + \varepsilon_{AH^-} \cdot f_{AH^-} + \varepsilon_{A^{2-}} \cdot f_{A^{2-}} \tag{3}$$

Substituting in eqn (3) the f values deduced from the expressions giving K_1 and K_2 results in:[15]

$$[H^+]^2(\varepsilon - \varepsilon_{AH_2}) + K_1[H^+](\varepsilon - \varepsilon_{AH^-}) + K_1 . K_2(\varepsilon - \varepsilon_{A^{2-}}) = 0 \tag{4}$$

This equation has three unknowns, K_1, K_2 and ε_{AH^-}, because ε_{AH_2} and ε_A can be determined at extreme pH values. Its resolution is only possible if the plot ε vs $[H^+]$ has a maximum or a minimum. In both cases there are equal ε values at two different proton concentrations, $[H^+]_1$ and $[H^+]_2$. Substituting each $[H^+]$ value in eqn (4) and solving the system gives:[15,20]

$$\varepsilon = \varepsilon_{AH^-} - K_2 . P \qquad \text{where } P = \frac{(\varepsilon - \varepsilon_{A^{2-}})([H^+]_1 + [H^+]_2)}{[H^+]_1[H^+]_2} \tag{5}$$

$$\varepsilon = \varepsilon_{AH^-} - \frac{S}{K_1} \qquad \text{where } S = (\varepsilon - \varepsilon_{AH_2})([H^+]_1 + [H^+]_2) \tag{6}$$

Thus, K_1 and K_2 can be deduced graphically from the slopes of the plots ε vs P and ε vs S, respectively. Of course, when optical pathlength and concentration are kept constant throughout the experiment, ε values can be substituted by absorbances.

RESULTS

The former methods have been applied in this work to the determination of macroscopic (experimental) ionization constants. Thus, the methyl esters **RB.2** and **EO.2** and the decarboxylated derivative **RB.3**, all with fixed quinonoid structures QH, have only one ionizable group, with ionization constant $K_P^Q = [Q^-][H^+]/[QH]$, so that eqn (2), with pK_P^Q instead of pK_1, can be directly applied to the absorbance data of solutions of the same dye concentration and different pH values. The results are shown in Table 2. The cases of the methyl ethers **RB.4** and **EO.4** are different, because it is necessary to take into account the tautomeric equilibria between lactonic and

TABLE 2
Experimental pK Values of the Molecular Forms of Rose Bengal, **RB.1**, Eosin Y, **EO.1**, and Some Derivatives, in Dioxane–Aqueous Buffers (1:1)

Compound	*Analytical* λ *(nm)*	pK_1	pK_2
RB.2	568	2·57	
RB.3	561	2·63	
RB.4	406	6·42	
RB.1	575	4·22	5·01
EO.2	535	2·69	
EO.4	474	6·51	
EO.1	540	3·75	6·25

quinonoid forms, $LH \rightleftharpoons QH$ and $L^- \rightleftharpoons Q^-$ (Scheme 1), with constants $K_i = [QH]/[LH]$ and $K_i' = [Q^-]/[L^-]$, respectively. Only the quinonoid forms absorb in the visible region, with characteristic spectral shapes: a maximum flanked by two shoulders.[3] Assuming that in the same solvent the spectra of QH and Q^- must be very similar to those of the model compounds, with the same chromophoric group, ether-esters **RB.5** (for **RB.4** in quinonoid form) and **EO.5** (for **EO.4** in the same form),[3] the proportions of quinonoid forms for each ether, at extreme pH values, must be approximately equal to the ratio of the corresponding molar extinction coefficients in the same visible maxima, ε(ether)/ε(ether-ester). These ratios allow the easy estimation of K_i and K_i' (Table 3).

$$\begin{tikzcd}
LH \arrow[rr, shift left, "K_{p1}^{L}"] \arrow[dd, shift right, "K_i"'] & & L^- \arrow[ll, shift left] \arrow[dd, shift right, "K_i'"'] \\
& \overset{K_1}{\rightleftarrows} & \\
QH \arrow[uu, shift right] \arrow[rr, shift left, "K_{c}^{Q}"] & & Q^- \arrow[uu, shift right] \arrow[ll, shift left]
\end{tikzcd}$$

Scheme 1. Ionization and isomerization equilibria in methyl ethers **RB.4** and **EQ.4**.

If the four equilibria shown in Scheme 1 are treated as only one equilibrium, $AH \rightleftharpoons A^- + H^+$, with macroscopic constant K_1, eqns (1) and (2) can be applied, being $[AH] = [LH] + [QH]$ and $[A^-] = [L^-] + [Q^-]$. K_1 is now defined as

$$K_1 = \frac{([Q^-] + [L^-])[H^+]}{[QH] + [LH]} \tag{7}$$

TABLE 3

Estimated Values for the Isomerization Constants K_i and K'_i of the Molecular Forms of Rose Bengal, Eosin Y, and their Methyl Ethers, in Dioxane–Aqueous Buffers (1:1)

Compound	K_i	K'_i
RB.4	~0[a]	0·919[b]
RB.1	0·022[c]	305[c,d]
EO.4	0·010[b]	0·067[b]
EO.1	0·084[b]	
	0·087[c]	610[c,d]

[a] Colourless solution.

[b] Based on percentage of quinonoid form, as estimated from model compounds.

[c] Deduced from eqn (14).

[d] The same value is estimated for K''_i.

Assuming that constants K_i and K'_i do not change with pH, the microscopic (molecular) ionization constants of the phenol group in the lactone form LH, $K^{L}_{p1} = [L^-][H^+]/[LH]$, and of the carboxylic group in the quinonoid form QH, $K^{Q}_{c} = [Q^-][H^+]/[QH]$, are related with K_1, K_i and K'_i by the easily deducible eqns (8) and (9), allowing in this way the estimation of the said microscopic constants (Table 4).

$$K^{L}_{p1} = K_1 \frac{1 + K_i}{1 + K'_i} \tag{8}$$

$$K^{Q}_{c} = K^{L}_{p1} \frac{K'_i}{K_i} \tag{9}$$

In the cases of the diprotic acids **RB.1** and **EO.1**, the careful analysis of the variation of the visible spectrum of each dye with pH allowed an analytical wavelength to be selected at which the absorbance goes through a maximum: 575 nm and 540 nm, respectively (Figs 1 and 2). In each case eqns (5) and (6) were applied to the absorbance ranges shown, and the two macroscopic constants K_1 and K_2 could be obtained for each acid (Figs 3 and 4 and Table 2).

However, the actual ionization system is more complex because, if zwitterionic structures are discharged, the seven equilibria shown in Scheme 2 can be present. Three equilibria can be established between isomeric lactonic and quinoid forms, with constants K_i, K'_i and K''_i, and the other four between forms with the same lactonic or quinoid structure but non-ionized, mono-ionized, and di-ionized, with microscopic ionization

$$\begin{tikzcd}
LH_2 \arrow[r, shift left, "K^L_{p1}"] \arrow[d, shift left] & LH^- \arrow[l, shift left] \arrow[r, shift left, "K^L_{p2}"] \arrow[d, shift left] & L^{2-} \arrow[l, shift left] \arrow[d, shift left] \\
{} \arrow[r, shift left, "K_1"] & {} \arrow[l, shift left] \arrow[r, shift left, "K_2"] & {} \arrow[l, shift left] \\
QH_2 \arrow[u, shift left, "K_i"] \arrow[r, shift left, "K^Q_p"] & QH^- \arrow[u, shift left, "K_i'"] \arrow[l, shift left] \arrow[r, shift left, "K^Q_c"] & Q^{2-} \arrow[u, shift left, "K_i''"] \arrow[l, shift left]
\end{tikzcd}$$

Scheme 2. Ionization and isomerization equilibria in the molecular forms of Rose Bengal, **RB.1** and Eosin Y, **EO.1**.

constants K^L_{p1}, K^L_{p2}, K^Q_p and K^Q_c. Similarly to the cases of the methyl ethers, the system can be regarded as formed by only two equilibria, with macroscopic ionization constants K_1 and K_2, which are related with the microscopic ones by the expressions:

$$K_1 = K^L_{p1}\frac{1+K_i'}{1+K_i} \tag{10}$$

$$K_1 = K^Q_p\frac{K_i}{K_i'}\cdot\frac{1+K_i'}{1+K_i} \tag{11}$$

$$K_2 = K^L_{p2}\frac{1+K_i''}{1+K_i'} \tag{12}$$

$$K_2 = K^Q_c\frac{K_i'}{K_i''}\cdot\frac{1+K_i''}{1+K_i'} \tag{13}$$

The system admits other simplifications: (a) at extreme acidic pH values, Rose Bengal solutions are colourless, and those of Eosin Y show only a weak

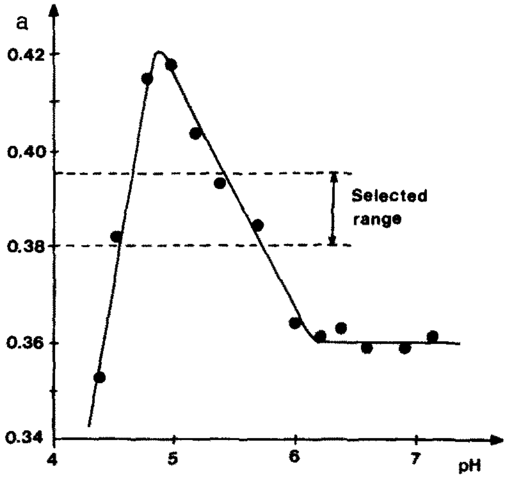

Fig. 1. Absorbance (a) variation at 575 nm with pH of $1{\cdot}25 \times 10^{-5}$ M solutions in

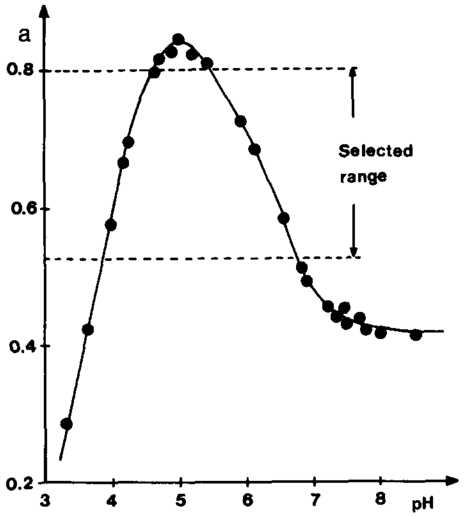

Fig. 2. Absorbance (*a*) variation at 540 nm with pH of $1{\cdot}33 \times 10^{-5}$ M solutions in dioxane–aqueous buffers (1:1) of the molecular form of Eosin Y, **EO.1**.

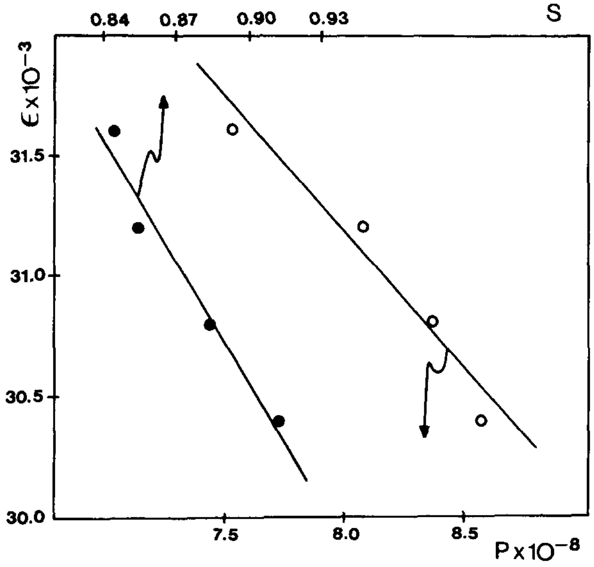

Fig. 3. Determination of pK_1 and pK_2 of **RB.1** applying eqns (5) and (6) to the absorbance range shown in Fig. 1.

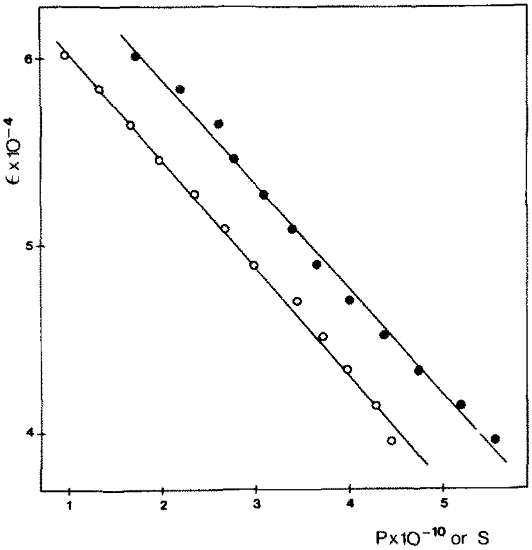

Fig. 4. Determination of pK_1 and pK_2 of **EO.1** applying eqns (5) and (6) to the absorbance range shown in Fig. 2.

visible absorption, with the shape of a quinonoid form, attributed to the presence of low proportions of the form QH_2, indicating that the equilibria $LH_2 \rightleftharpoons QH_2$ are strongly displaced towards the first member, i.e. K_i must be much lower than 1; (b) on the contrary, K_i' must be much higher than 1, because QH^- is more stable than LH^- due to the higher delocalization of the negative charge in the former; (c) for the same reason, K_i'' must also be much higher than 1, i.e. the corresponding equilibrium, the only one possible in extreme basic media, must be displaced far towards Q^{2-}. The doubly charged form L^{2-} has never been detected in either of the two dyes; if it exists, its proportion in the medium ought to be very low, because the ε values found for the visible maxima of both dyes in these basic media are close to those of the corresponding methyl esters **RB.2** and **EO.2**, two compounds where lactonic forms cannot exist. In accordance with these estimations, and assuming that K_i'' is likely to be similar to K_i', eqns (10)–(13) can be reduced to:

$$K_1 \approx K_{p1}^{L} \cdot K_i' \approx K_p^{Q} \cdot K_i \tag{14}$$

$$K_2 \approx K_{p2}^{L} \approx K_c^{Q} \tag{15}$$

There are no model compounds to estimate K_i and K_i' in the case of **RB.1**, so that K_{p1}^{L} and K_p^{Q} cannot be deduced from the experimental value K_1. In the case of **EO.1** only K_i can be estimated, comparing its spectrum at extreme acidic pH with that of the model quinonoid compound **EO.2** in the same medium (Table 3).

TABLE 4
Estimated Microscopic pK Values of the Molecular Forms of Rose Bengal, Eosin Y, and Some Derivatives in Dioxane–Aqueous Buffers (1:1)

Compound	pK_{p1}^{L}	pK_{p}^{Q}	pK_{c}^{Q}
RB.2		2·57[a]	
RB.3		2·63[a]	
RB.4	6·70		<5·71
RB.1	6·70	2·57	5·01[b]
EO.2		2·69[a]	
EO.4	6·53		5·71
EO.1	6·53	2·69	6·25[b]

[a] Coincident with the experimental values.
[b] The same value is estimated for pK_{p2}^{L}.

However, with the aid of eqns (14) the constants K_i and K_i' can be estimated for both dyes, taking into account that the values of K_p^Q and K_{p1}^L must be very similar to those of the corresponding constants of the respective methyl esters **RB.2** and **EO.2**, and methyl ethers **RB.4** and **EO.4** (Table 4). It is worth noting that the value so obtained for the constant K_i of **EO.1**, 0·087 (Table 3), is close to the one deduced by the former method, 0·084.

DISCUSSION

The microscopic (or molecular) pK values deduced for the seven compounds studied here have been collected in Table 4. The quinonoid esters **RB.2** and **EO.2** have ionization constants with pK_p^Q values of 2·57 and 2·69, respectively, confirming the high acidity of the phenol group in the halogenated xanthene moiety. The decarboxylated compound **RB.3** has a similar pK_p^Q, indicating that the C-2′ substituent has a negligible influence on the ionization of the phenol group.

Ethers **RB.4** and **EO.4** have two ionizations each, due to the presence of equilibria between lactonic and quinonoid species. The pK_{p1}^L values deduced for the ionization of the phenol group in the lactone isomers are also similar, 6·70 and 6·53, and are in accordance with the values expected for a phenol in an isolated phenyl group. On the contrary, the corresponding pK_c^Q values must be substantially different. Whilst ether **EO.4** has a value of 5·71, in accordance with what is expected for an aromatic carboxylic acid, ether **RB.4** must have a lower value due to the presence of four chlorine atoms in the phenolic nucleus. Unfortunately, this value could not be estimated.

With respect to the dyes Rose Bengal and Eosin Y, with molecular forms **RB.1** and **EO.1**, the p*K* values shown in Table 4 are estimations that probably coincide with the true values. Anyway, it is deduced that the ionization of the phenol group in the quinonoid forms must precede that of the carboxylic group, in accordance with previous results on Eosin Y.[7] In the case of the non-halogenated parent compound fluorescein, these two ionizations are almost overlapped, with experimental p*K* values in dioxane–water (4:6, v/v) of 6.97 and 7·43,[10] i.e. both groups are weak acids. The first value, corresponding mainly to the ionization of the carboxylic group,[7] is similar to our estimated pK_c^Q value for Eosin Y in dioxane–water (1:1), 6·25. The lower value for this pK_c^Q in Rose Bengal must be attributed, as in the case of the methyl ether **RB.4**, to the presence of the four chlorine atoms in the aromatic nucleus.

EXPERIMENTAL

The molecular forms of the dyes, obtained and purified as described,[3] were dissolved in 1:1 (v/v) mixtures of dioxane with various aqueous buffers with pH values separated about 0·3 or 0·5 pH units,[22] depending on the zone to be studied. The buffers were adjusted to ionic strength 0·1 M with KCl, in order to preclude possible absorbance variations due to the presence of different cation concentrations.[23] Dye concentrations were about 10^{-5} M or, in the case of ether **EO.2**, 10^{-3} M. For pH values between 0·5 and 2·0, enough conc. HCl was added to 0·1 M-KCl aqueous solutions. The analytical wavelength for each dye was the position of maximum absorption in extreme basic medium or, for **RB.1** and **EO.1**, the wavelength where the absorbance variations with pH showed a higher maximum. Absorbances were measured in 1-cm pathlength cuvettes thermostated at 20 ± 1°C, using Perkin–Elmer spectrophotometers, models 402 (monoprotic acids) and digital recording 554 (diprotic acids); the latter was equipped with a microprocessor for automatic electronic differentiation. The apparent pH value of each dye solution was measured at the same temperature with a Crison 501 pH-meter calibrated with standard aqueous buffers, without correcting for solvent effects.

Experimental pK_1 values of monoprotic acids were obtained graphically (duplicated assays, maximum deviations ± 0·05 p*K* units) with the aid of eqn (2), fitting the points by a least-squares approach. pK_1 and pK_2 values of diprotic acids were obtained by applying eqns (5) and (6) to the well-defined absorbance ranges shown in Figs 1 and 2. The straight lines representing ε vs P and ε vs S were also fitted by a least-squares approach. Molar extinction coefficients (litre mol^{-1} cm^{-1}) used in the calculations were: in dioxane–pH 8 aqueous buffer (1:1), **RB.4** 5010 at 498 nm, **RB.5** 10470 at 505 nm, **EO.4** 1000 at 474 nm, **EO.5** 15850 at 480 nm; in dioxane–pH 0·5 aqueous buffer (1:1), **EO.1** 1740 at 479 nm, **EO.2** 22390 at 481 nm, **EO.4** 160 at 480 nm, **EO.5** 15850 at 480 nm.

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