Dyes and Pigments **22** (1993) 199–205

Evidence of Non-Emissive Protonated Forms in Methyl Esters of Rose Bengal and Eosin Y in Acidic Medium

J. C. del Valle, N. A. García*

Departamento de Química Física Aplicada, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

&

F. Amat-Guerri‡

Instituto de Química Orgánica, C. S. I. C., Juan de la Cierva, 3, 28006 Madrid, Spain

(Received 5 February 1993; accepted 12 March 1993)

ABSTRACT

Differences between the shapes of the absorption and fluorescence excitation spectra of the neutral forms of the methyl ester of Eosin Y, the methyl ester–methyl ethers of the same dye and of Rose Bengal (MeEO, DMeEO and DMeRB, respectively) in 10^{-6} M solutions in 1:1 v/v dioxane–pH 0·5 aqueous buffer, is explained by the existence in the ground state of small proportions of the corresponding protonated forms, which are only detectable through these differences.

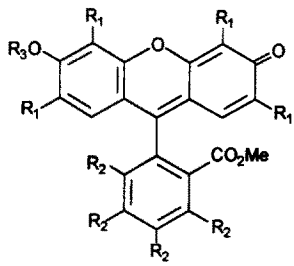
INTRODUCTION

The halogenated xanthene dyes Rose Bengal (RB) and Eosin Y (EO) are of interest because of their high efficiencies for the generation of singlet molecular oxygen, O_2 ($^1\Delta g$), and of their potential use in modern phototherapy.[1] In previous papers we have reported on the synthesis,[2] the

* Permanent address: Departamento de Química y Física, Universidad Nacional de Rio Cuarto, 5800 Rio Cuarto, Argentina.

‡ To whom correspondence should be addressed.

Dyes and Pigments 0143-7208/93/$06.00



	R_1	R_2	R_3
MeEO	Br	H	H
DMeEO	Br	H	Me
DMeRB	I	Cl	Me

Scheme 1

evaluation as O_2 ($^1\Delta g$) generators,[3] and other photophysical data[4] of a series of RB and EO derivatives, including the methyl ester of EO (MeEO), the methyl ether of this methyl ester (DMeEO), and the dimethylated derivative of RB (DMeRB) (see Scheme 1). All of them exhibit an unusual characteristic, namely, they behave as good O_2 ($^1\Delta g$) generators even in acidic media,[3] where the parent dyes lose the visible absorption bands because of the formation of lactonic structures.

In a photophysical study on these derivatives[4] we observed differences between the shapes of the absorption and fluorescence excitation spectra in 1:1 v/v dioxane–pH 0·5 aqueous buffer, which were provisionally assigned to the presence of aggregates in the medium, despite the low dye concentrations used (2×10^{-6} M). This solvent mixture guarantees the absence of anionic forms, when they are possible. In 1:1 dioxane–water both neutral and anionic forms are soluble. Aggregation of RB ethyl ester in aqueous solutions with 2–5% ethanol has been observed by other authors,[5,6] but at concentrations higher than 2×10^{-5} M, noting that the process is favoured by the presence of electrolytes. In the case of the parent dyes, the formation or aggregates significantly decreases the fluorescence and the ability to photogenerate O_2 ($^1\Delta g$).

In this communication, we report experimental results on MeEO, DMeEO and DMeRB which indicate that the spectral differences are a consequence of the presence in the acidic medium of ground-state protonated forms, only detectable by fluorescence excitation spectroscopy.

EXPERIMENTAL

The synthesis and purification of MeEO, DMeEO and DMeRB have been previously described.[2] Freshly-prepared solutions of the neutral forms of the dyes in 1:1 v/v dioxane–pH 0·5 aqueous buffer (3 : 1 v/v 0·2 M HCl/0·2 M KCl, plus drops of conc. HCl until pH 0·5) were analysed in 1 cm optical pathlength cells. The anionic form of MeEO

was analysed in a similar mixture with pH 8 aqueous buffer (0·033 M Na_2HPO_4/0·033 M KH_2PO_4).

UV–visible absorption spectra were recorded on a Shimadzu UV-2100 spectrophotometer at 298K. Fluorescence spectra, also at 298K, of solutions with optical densities in the excitation range of about 0·05, were recorded on a SLM 48 000s spectrofluorometer with a cooled wide band RF housing for the Hamamatzu R928 photomultiplier tube, measuring at right angles with respect to the exciting beam, and correcting for instrumental sensitivity. Corrected fluorescence excitation spectra were obtained at constant excitation intensity controlled by a Rhodamine B quantum counter. Artifacts due to polarisation effects were avoided by polarisers in magic angle arrangement.

RESULTS AND DISCUSSION

Absorption and emission spectra of MeEO, DMeEO and DMeRB, at pH 0·5, show good mirror-image symmetry (Figs. 1(a), 2(a) and 3(a)) and their shapes do not depend on the concentration under 10^{-5} M, nor on the presence of electrolytes such as 1 M KNO_3 or 3 M LiCl. These two salts favour dye aggregation.[5,7] In addition, the emission spectra are independent of the excitation wavelength. On the other hand, absorption and fluorescence excitation spectra of the dyes in the same medium clearly

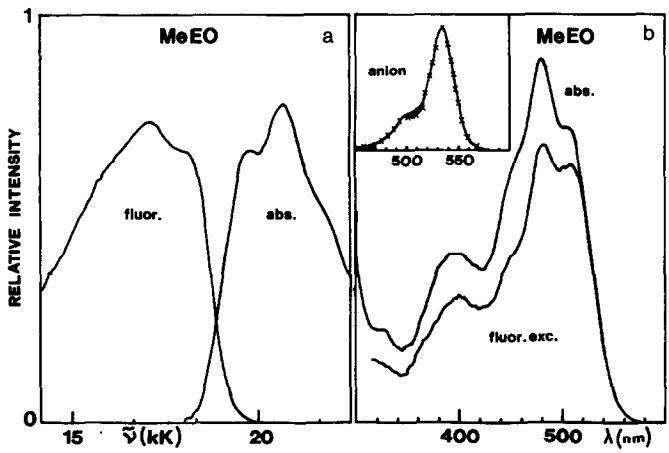


Fig. 1. (a) Absorption ($\varepsilon(\tilde{\nu})/\tilde{\nu}$) and emission corrected ($F(\tilde{\nu})/\tilde{\nu}^3$)[8] spectra of the methyl ester MeEO in 1:1 dioxane-pH 0·5 aqueous buffer, normalised at 19 700 cm^{-1} and at 17 560 cm^{-1}, respectively; and (b) absorption and fluorescence excitation spectra in the same solvent, normalised at 536 nm (0–0 position). Inset: absorption and fluorescence excitation spectra in 1:1 dioxane–pH 8 aqueous buffer, normalised at their maxima.

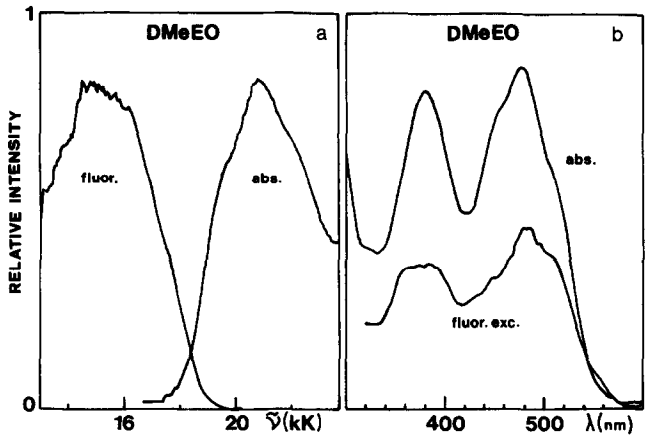


Fig. 2. (a) Absorption ($\varepsilon(\tilde{\nu})/\tilde{\nu}$) and emission corrected ($F(\tilde{\nu})/\tilde{\nu}^3$)[9] spectra of the dimethyl derivative DMeEO in 1:1 dioxane–pH 0·5 aqueous buffer, normalised at 20 812 cm^{-1} and at 14 502 cm^{-1}, respectively; and (b) absorption and fluorescence excitation spectra in the same solvent, normalised at 544 nm (0–0 position).

show the already cited differences, also observed in 10^{-6} M solutions. For a suitable comparison of these spectra in the pH 0·5 medium, normalisation at the wavelength of the 0–0 band must be performed. Assuming that the state involved in absorption and emission is the same for each dye (mirror-image spectral symmetry), each 0–0 band is found[8,9] at the wavelength where both spectra intersect (536 nm, 544 nm and 571 nm, for MeEO, DMeEO and DMeRB, respectively, Figs. 1(a), 2(a) and 3(a)).

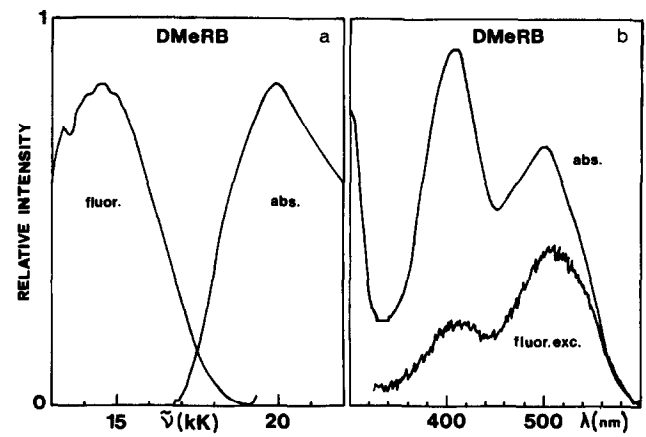
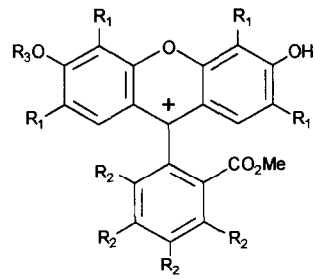


Fig. 3. (a) Absorption ($\varepsilon(\tilde{\nu})/\tilde{\nu}$) and emission corrected ($F(\tilde{\nu})/\tilde{\nu}^3$)[8] spectra of the dimethyl derivative DMeRB in 1:1 dioxane–pH 0·5 aqueous buffer, normalised at 19 922 cm^{-1} and at 14 612 cm^{-1}, respectively; and b) absorption and fluorescence excitation spectra in the same solvent, normalised at 571 nm (0–0 position).



Scheme 2

Comparison of the spectra normalised in this way (Figs. 1(b), 2(b) and 3(b)) indicates that each absorption spectrum always covers the corresponding excitation spectrum, with higher differences at lower wavelengths. The differences do not change with time and are not observed in 1:1 dioxane–pH 8 aqueous buffer.[4] The inset in Fig. 1(b) shows the absorption and fluorescence excitation spectra of MeEO in this solvent, in which this dye forms the highly coloured anion.

In a previous paper, the observed differences between absorption and fluorescence excitation spectra were assigned to the presence in the solution of non-emissive ground-state species.[4] In order to explain these findings, the first species to be taken into account are aggregates. However, aggregate formation can be conclusively ruled out, since the shapes of the absorption, fluorescence and fluorescence excitation spectra do not change when the dye concentration changes from 10^{-6} M to 10^{-5} M. Aggregation has never been observed at the low concentrations used herein.

A much better explanation is the presence of ground-state cationic (protonated) forms of the dyes (see Scheme 2). This explanation is supported by comparison of the spectra of the cationic forms of the dyes produced in 1:1 v/v dioxane-6 M HCl solution, with the corresponding differential spectra obtained by subtracting each fluorescence excitation spectrum from the respective absorption spectrum in the same solvent. As can be seen in Fig. 4, for each dye both spectra have similar patterns, with a common absorption maximum. In the case of DMeEO, a maximum at about 480 nm is reasonably assigned to the presence of traces of MeEO, most likely formed as a result of partial hydrolysis. The proportions of cationic forms in the pH 0·5 medium have to be very small, because they have not been detected by absorption[10] or emission spectroscopy. In addition, the pK_a values of the cation$\rightleftarrows$neutral-form equilibria in the parent dyes EO and Erythrosin in a similar solvent (2:3 v/v dioxane–aqueous buffer) are $-1{\cdot}68$ and $-1{\cdot}4$, respectively,[11] about two units lower than the pH of the solvent used here. Moreover, the life-

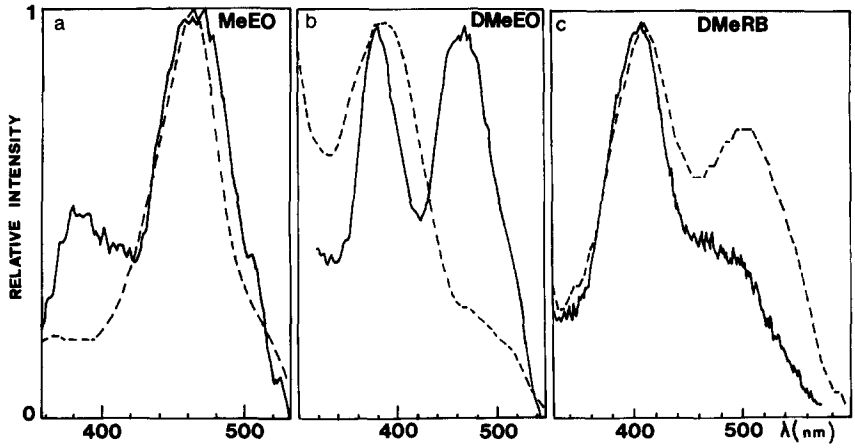


Fig. 4. Comparison of absorption spectra (dashed lines) of MeEO, DMeEO, and DMeRB, in 1:1 dioxane–6 M HCl, with the corresponding differential spectra (absorption minus fluorescence excitation) in 1:1 dioxane–pH 0·5 aqueous buffer.

times of MeEO and DMeEO fit very well with only one value (0·64 and 0·08 ns, respectively, the lifetime of DMeRB could not be measured),[4] and the absorption spectra are identical in 1:1 dioxane–pH 0·5 buffer and in 1:1 dioxane–pure water. The methyl ester of RB was not analysed because previous results indicate that in the pH 0·5 medium small proportions of the anionic form are present.[4] However, in accordance with the results obtained with the other dyes, it is expected that the cationic form of this dye must be also present in the medium.

In conclusion, if correct normalisation criteria are applied to the solutions of these dyes in the pH 0·5 medium, according to a good mirror-image relation, the presence of small proportions of the corresponding cationic species can be detected by fluorescence excitation spectroscopy, because these cations must be non-emissive or, at least, less emissive than the neutral molecule. To the best of our knowledge, the observed results herein shown are described for the first time in xanthene photochemistry.

ACKNOWLEDGEMENTS

Thanks to CICYT of Spain for financial support (Projects MAT89-0403 and PB90-0226-C02-01)); to the Comunidad Autónoma de Madrid for a F.P.I. grant (to J.C.V.); to the Ministerio de Educación y Ciencia of Spain for a sabbatical fellowship (to N.A.G.); and to Prof. J. Catalán for measuring facilities.

REFERENCES

1. Jori, G., *J. Photochem. Photobiol., A: Chem.*, **62** (1992) 371.
2. Amat-Guerri, F., López-González, M. M. C., Martínez-Utrilla, R. & Sastre, R., *Dyes and Pigments*, **12** (1990) 249.
3. Amat-Guerri, F., López-González, M. M. C., Martínez-Utrilla, R. & Sastre, R., *J. Photochem. Photobiol., A*, **53** (1990) 199.
4. del Valle, J. C., Catalán, J. & Amat-Guerri, F., *J. Photochem. Photobiol., A, Chem.*, **72** (1993) 49.
5. Valdés-Aguilera, O., & Neckers, D. C., *J. Phys. Chem.*, **92** (1988) 4286.
6. Xu, D. & Neckers, D. C., *J. Photochem. Photobiol., A, Chem.*, **40** (1987) 361.
7. Chambers, R. W., Kajiwara, T. & Kearns, D.R., *J. Phys. Chem.*, **78** (1974) 380.
8. Birks, J. B., *Photophysics of Aromatic Molecules*. Wiley-Interscience, New York, USA, 1970, p. 86.
9. Grawowski, Z. R. & Grawowska, A., *Zeit. Phys. Chem. Neue Folge*, **101** (1976) 197.
10. Amat-Guerri, F., López-González, M. M. C., Sastre, R. & Martínez-Utrilla, R., *Dyes and Pigments*, **13** (1990) 219.
11. Shibata, M., Nakamizo, M. & Kakiyama, H., *Nippon Kagaku Kaishi*, (1972) 681.