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Dyes and Pigments 40 (1999) 95–98

DYES AND PIGMENTS

Photochemistry of fluorescein and eosin derivatives

P. Meallier [a,*], S. Guittonneau [a], C. Emmelin [a], T. Konstantinova [b]

[a] *Université Claude Bernard Lyon 1, LACE, Photochimie Industrielle, 69622 Villeurbanne Cedex, France*
[b] *University of Sofia, Départment of Organic Synthesis, 8 Kl. Ohridesky Bld., 1756 Sofia, Bulgaria*

Received 3 February 1998; accepted 8 April 1998

Abstract

Spectroscopic and photochemical properties of allyloxyfluorescein and allyloxyeosin have been studied and the fluorescence quantum yield and photodegradation quantum efficiency calculated. Whilst the photostability is the same for the two compounds, allyloxyfluorescein has the better fluorescence quantum yield.

Keywords: Allyloxyfluorescein; Allyloxyeosin; Fluorescence; Photochemistry

1. Introduction

Eosin and fluorescein have been reported as dyes for laser applications [1], as fluorescent tags for use in molecular biology [2] and for potential photosensitising biological activity [3].

We have now synthesized two derivatives, viz. allyloxyfllluorescein and allyloxyeosin, containing two allyloxy groups capable of polymerisable reactions. We report here the results of the photochemical analysis on these dyes and also of the polymers obtained with methylmethacrylate. The photochemistry of the dyes was studied by means of the software PHOTON which has been realised in the laboratory [4].

2. Experimental

In a previous paper [5] we studied the possibility of synthetising these compounds under phase-transfer catalysis conditions; the basic procedure was as follows: 0.01 mol of fluorescein dissolved in 200 ml acetonitrile was mixed with 0.025 mol of anhydr. KOH and 0.001 mol of 18-crown-6 as catalyst; 0.02 mol of allylbromide was then added dropwise and the mixture refluxed for 2 h. Allyloxyfluorescein was isolated with 80% yield after filtration and evaporation of the solvent and allyloxyeosin was similarly obtained in 65% yield. Improved yields using a modified procedure were developed [5].

Absorption spectra were recorded in ethanol on a UVIKON 930 spectrofluorimeter. The fluorescence and phosphorecence spectra were respectively recorded in ethanol and in 'E.P.A.' a mixture of i-pentane (18%), ethanol (41%) and diethylether (41%). The fluorescent standard was fluorescein.

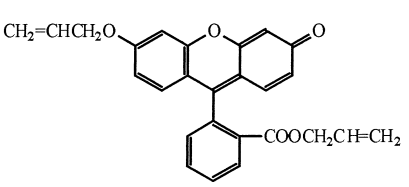
The photodegradation of the dyes was investigated using a Xenotest apparatus (HERAEUS).

Concentration was monitored during irradiation by H.P.L.C. using:

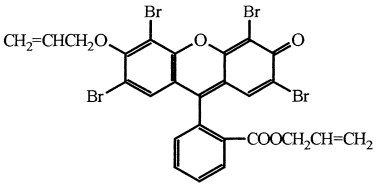
- a lichrosphere 100 DIOL (5μ) column and, as mobile phase, methanol/water (30:70) for allylloxyfluorescein.

* Corresponding author. Fax: +33-04-78-94-19-95; e-mail: lace@univ.lyonl.fr

0143-7208/98/$—see front matter
PII: S0143-7208(98)00040-0



Allyloxyfluorescein



Allyloxyeosin

- a Rsil C18 (10μm) column and, as mobile phase, methanol/water (50:50) for allyloxyeosin.

3. Results and discussion

3.1. Quantum efficiency

The software 'Photon' can be used to calculate the quantum efficiency Φ_d and the life time τ_d of molecule, parameters which are characteristic of their behaviour to light.

The quantum yield (ϕ) of a compound X is defined as number of moles transformed per mol of photons (einstein) absorbed by the substance (i.e. number of molecules reacting per number of a photons absorbed by the substance X). It is defined by:

$$\phi = \frac{dc/dt}{\Sigma P_{abs(t_1,\lambda)}.\Delta\lambda \times 10^3}$$

dc: variation of the concentration of the substance for a time t_1;

P: absorbed light for the same time in the studied range of wavelength;

10^3: converts (cm^3) to 1^{-1}.

The summation has to be performed at appropriate intervals $\Delta\lambda$ (2.5 nm) in the absorption range of the substance. The limits λ_1 and λ_2 have to be selected according to the absorption spectrum of X, of the actinometer and the irradiation spectrum.

The life time of a photodegradable compound under sunlight and in a solution of low absorbance (where the absorbed light intensity is proportional to the concentration) can be calculated from the quantum yield, the molar extinction coefficient and the source intensity. From this data it is possible to evaluate the photolytic lifetime in the environment in polychromatic light. Frank and Klöpfer have calculated the intensity of the solar spectrum in Eastern Europe [6] and Zepp and Cline in North America [7]. The lifetime is given by:

$$\tau = \frac{1}{\phi_r.k_a} \text{ with } k_a = \Sigma\ 2.303 \times 10^3.\text{Po}\ (\lambda)\Delta\lambda.\epsilon(\lambda)$$

where τ is the life time in s^{-1}.

k_a is the pseudo-first order rate constant of direct photolysis in s^{-1} in the studied range of wavelength.

ϕ_r is the quantum yield in polychromatic light between $\lambda 1$ and $\lambda 2$ in (mole.einstein).

Po (λ) is the solar intensity at the wavelength λ in (einstein $cm^2\ s^{-1}\ nm^{-1}$).

$\epsilon(\lambda)$ is the molar extinction coefficient at the wave length λ in ($1\ mol^{-1}\ cm^{-1}$).

For these calculations, it is necessary to know the spectroscopic parameters, for each wavelength, of the molecule, and of the actinometer, which is used to evaluate the number of photons emitted by the lamp, and the photodegradation kinetic constant. Table 1 shows the values for the absorption maxima of the dyes.

Table 1
Absorption spectroscopic parameters of allyloxyfluorescein and allylloxyosin

Dye	Solvent	Concentration $mol1^{-1}$	λ_{abs} in nm	ϵ ($1\ mol^{-1}\ cm^{-1}$)
Allyloxyfluorescein	EtOH	2.10^{-5}	458	25080
Allyloxyeosin	EtOH	2.10^{-5}	535	59252

The photodegradation kinetic constants are:

$k_a = 1.55\ 10^{-5}\ s^{-1}$ for allyloxyfluorescein

$k_a = 3.20\ 10^{-6}\ s^{-1}$ for allyloxyeosin

From these values the results shown in Table 2 are obtained.

3.2. Quantum yield of fluorescence

Fluorescence experiments were made in ethanol at room temperature, using dilute solutions in which absorption effects are negligible. Results are shown in Table 3, which show a considerable decrease in fluorescence quantum yield for the allyloxy derivatives.

3.3. Copolymerisation of dyes with methylmethacrylate [9]

To determine the influence of the polymer on the absorption range of the dyes and on their photostability, we studied a solution of the copolymers in chloroform. Photostability has been investigated by irradiation with the xenon lamp, using the same conditions as for the dyes alone. The values of the rate constants were:

for allyloxyfluorescein: $k_a = 6.5\ 10^{-6}\ s^{-1}$

for allyloxyeosin: $k_a = 5\ 10^{-6}\ s^{-1}$

Pertinent spectroscopic properties used for the calculation of the photodegradation quantum efficiency are shown in Table 4

Table 2
Quantum efficiency and photolytic lifetime data

	Allyloxyfluorescein	Allylloxyeosin
Φ_r	$2.8\ 10^{-5}$ molecule photon^{-1}	$8.9\ 10^{-7}$ molecule photon^{-1}
τ	$1.1\ 10^3$ s	$9.1\ 10^3$ s

Table 3
Wavelength λ_f and quantum yield Φ_f of fluorescence

Dye	Solvent	λ_f	ϕ_f
Fluorescein [8]	Ethanol	520	0.97
Allylloxyfluorescein	Ethanol	522	0.38
Eosin [8]	Ethanol	540	0.45
Allyloxyeosin	Ethanol	547	0.05

Table 4
Absorption maxima of the dyes in polymers

	λ nm	ϵ l mol^{-1} cm^{-1}
Copolymer with allyloxyfluorescein	451	28031
Copolymer with allyloxyeosin	369	65800

The resultant quantum efficiency values are given in Table 5

Table 5
Quantum efficiency photolytic lifetime of the dyes in polymers

	Allylofluorescein	Allyloxyeosin
ϕ_r	$1.2\ 10^{-5}$ molecule photon^{-1}	$9.4\ 10^{-6}$ molecule photon^{-1}
"τ	$2.41\ 10^3$ s	$1.6\ 10^3$ s

For allyloxyfluorescein, the fluorescence quantum yield is relatively high, and there is no significant difference between the photostability of the dye alone and for the dye which is copolymerised.

For allyloxyeosin, the fluorescence quantum yield is low, as would be expected for a brominated analogue substitution. The decrease of the photostability of the copolymerised allyloxyeosin is related to the difference in its absorption range and its molecular extinction coefficient between the solution of the dye alone and the solution with the polymer.

These two dyes are compounds which have a generally good stability in UV and visible light, and which can be used easily in a copolymerisation process. Allyloxyfluorescein has the advantage of a higher fluorescence quantum yield.

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