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Visible light-promoted interactions between riboflavin and 3-hydroxypyridine in aqueous solution

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Dedicated to the memory of Professor Juanjo Cosa

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Abstract

Visible light-promoted interactions between the natural pigment Riboflavin (Rf) and 3hydroxypyridine (3-OHP) have been studied in air equilibrated aqueous solution, as a model system for the natural photodegradation of some aquatic contaminants. Results from static and dynamic fluorescence, laser flash photolysis, and polarography of oxygen consumption support the existence of competitive oxidation processes that involve singlet molecular oxygen and/or superoxide anion, with their relative importance depending on the 3-OHP concentration. Both the excited singlet and triplet states of Rf are quenched by 3-OHP, with respective rate constants of $5.9\times10^9\,M^{-1}\,s^{-1}$—close to the diffusion rate constant—and $8.9\times10^8\,M^{-1}\,s^{-1}$. The final result of the interactions is the photooxidation of 3-OHP and the partial photostabilization of Rf. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Pesticides constitute one of the most frequent sources of natural water contamination because of their broad employment for agricultural purposes. Under natural conditions, their spontaneous photodegradation is an important way of elimination from the aqueous environment. Since most common pesticides are transparent to natural daylight, the sensitized photooxidation in the presence of air appears as a plausible alternative for their degradation, because the only requirement for the reaction is the coincidence in the polluted medium of dissolved oxygen, daylight, and a day-light absorbing compound, a scheme that is frequently fulfilled in surface waters.

In the course of our studies on the photooxidation of compounds of environmental interest, in a recent paper [1] we have reported the kinetics of the dye-sensitized photooxidation of several

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hydroxypyridines, hydroxyquinolines and hydroxypyrimidines, all with the basic molecular structure of known *N*-heteroaromatic pesticides [2]. The main goal of these studies was to determine the most efficient experimental conditions for the dye-promoted photooxidation of the pesticides. Employing Rose Bengal—the synthetic xanthene dye most frequently used as a sensitizer—a singlet molecular oxygen [$O_2(^1\Delta_g)$]-mediated mechanism exclusively operates [3]. Photooxygenation quantum efficiencies for the reactive heteroaromatic compounds were in the range 0.01–0.50, being the hydroxypyridines in general, and 3-hydroxypyridine (3-OHP) in particular, the most susceptible compounds towards photooxidation. These results indicate that the sensitized photooxidation is an alternative pathway for the programmed degradation of some of these colourless compounds.

In the presence of oxygen, flavins photosensitize the decomposition of a wide variety of biologically relevant substances [4,5]. In particular, Riboflavin (Rf)-sensitized photodecomposition has been postulated as an alternative route for the natural degradation of herbicides and related compounds [6,7], because Rf is a well known natural pigment present in waters of rivers, lakes and sea [8]. Nevertheless, the role of Rf in the visible-light promoted decomposition of hydroxy-*N*-heteroaromatic pesticides has not been studied, although this information is particularly important for the prediction of the photolysis rates of these contaminants in natural waters. For this reason, the purpose of the present work was to investigate the behaviour of Rf as a sensitizer for the photodecomposition of 3-OHP, a model compound for some of said pesticides, as well as to get information on the physical and chemical interactions taking place in this and similar systems.

2. Experimental

2.1. Materials and methods

Eosin, furfuryl alcohol (FFA), 3-OHP, Rf (all from Sigma Chem. Co.) and sodium azide (from Aldrich) were used as received. Water was distilled three times.

The stationary quenching of the Rf fluorescence was studied with a RF 5301-PC Shimadzu spectrofluorimeter at 25 ± 1°C in air equilibrated solutions. Fluorescence lifetimes were measured with a time-correlated single photon counting technique on an Edinburgh FL-9000CD instrument. In both cases, excitation and emission wavelengths were 445 and 515 nm, respectively. Ground state absorption spectra were registered in a Hewlett Packard 8452A diode array spectrophotometer.

Nitrogen-saturated 0.01 mM Rf aqueous solutions were photolysed using a flash photolysis apparatus with the frequency-doubled output of a Nd:YAG laser (Spectron) at 532 nm as excitation wavelength, employing a 150 W xenon lamp as analysing light. The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666). The signal, acquired and averaged by a digital osciloscope (Hewlett Packard 54504A), was transferred to a PC via a HPIB parallel interface, where it was analysed and stored.

2.2. Sensitized photooxidations

Static photolysis were carried out in a PTI unit, provided with a high pass monochromator and 150 W Xe lamp, irradiating with 480 ± 10 nm. The home-made photolyser for non-monochromatic irradiation (150 W quartz-halogen lamp and 400 nm cut-off filter) and the specific oxygen electrode (Oriol 97-08) have been described elsewhere [9].

The photooxygenation rate constant (k_r) of the chemical reaction of 3-OHP with $O_2(^1\Delta_g)$ was determined by a modification of an already described comparative method [10], using as reference compound FFA ($k_{rFFA} = 1.2\times10^8\,M^{-1}\,s^{-1}$ [11]). Experiments of oxygen consumption vs irradiation time were run in solutions containing Eosin, with absorbance at 480 nm ca. 0.4, plus either 3-OHP (0.4 mM) or FFA (0.4 mM). Assuming that the reaction of $O_2(^1\Delta_g)$ with the substrate is the only way for oxygen consumption, the ratio of the slopes of the first order plots of oxygen uptake by substrate and reference compound (both at identical concentrations) yields k_r/k_{rFFA} and, hence, k_r.

3. Results

3.1. Sensitized photooxidation of 3-OHP

The spectral evolution of 3-OHP upon eosin-sensitized irradiation in air-equilibrated aqueous solution is shown in Fig. 1. In this medium 3-OHP reacts with $O_2(^1\Delta_g)$, generated by Rose Bengal sensitization, with a k_r value of $(1.9\times0.2)\times10^7\,M^{-1}\,s^{-1}$ [1], and a similar value was obtained using eosin, as comparative experiments with both dyes showed. The *pseudo*-first order plots for oxygen uptake by 3-OHP and furfuryl alcohol (FFA, the reference) exhibit an excellent linearity (Fig. 2, inset), from which a k_r value of $(2.0\times0.2)\times10^7\,M^{-1}\,s^{-1}$ can be deduced for 3-OHP.

Working with identical absorbance values at the irradiation wavelength (480 ± 10 nm), and using the same reactor but with Rf as a sensitizer, the photooxidation of 3-OHP gives rise to the spectral changes shown in Fig. 3, and the first order plot for oxygen uptake is not linear (Fig. 2, main), at least with 0.01–0.10 mM 3-OHP. Moreover, the rate of oxygen uptake was nil, or greatly reduced, in the comparative irradiations of: (i) a solution of either eosin or Rf, but in the absence of 3-OHP; (ii) a solution of Rf, but with >5 mM 3-OHP; and (iii) the mixture eosin + 3-OHP or Rf + 3-OHP, with 0.01–0.10 mM 3-OHP and 1 mM sodium azide, a selective physical quencher of $O_2(^1\Delta_g)$ [11].

Photodegradation of Rf in water under visible light irradiation predominantly proceeds through the triplet state [12], and the rate of the process can be estimated by absorption spectroscopy. Competitive irradiations of nitrogen-saturated solutions of Rf in the absence and in the presence of 0.10 mM 3-OHP showed that this rate is drastically

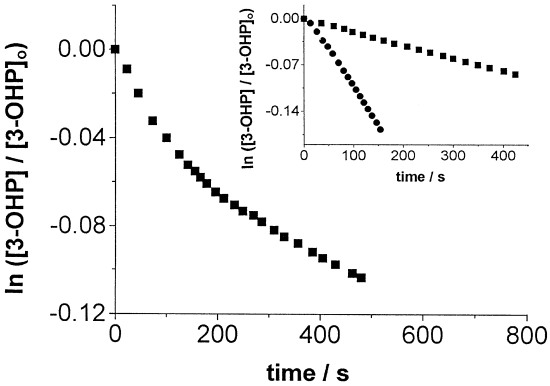


Fig. 2. *Pseudo*-first order plots for oxygen uptake of 0.4 mM 3-hydroxypyridine in air-equilibrated aqueous solution, upon irradiation in the presence of Riboflavin. Inset: *pseudo*-first order plot for oxygen uptake of 0.4 mM 3-hydroxypyridine (squares) and 0.4 mM furfuryl alcohol (circles) in air-equilibrated aqueous solution, upon eosin-sensitized photooxidation. Irradiation wavelength 480 ± 10 nm in all the cases. $Abs_{480} = 0.4$.

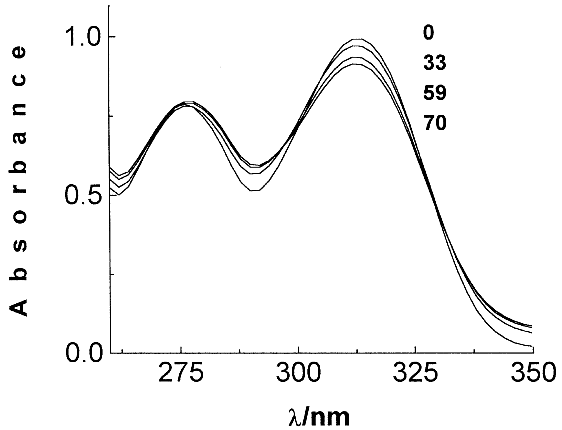


Fig. 1. Spectral changes in the eosin-sensitized photooxidation of 3-hydroxypyridine in air-equilibrated aqueous solution. $Abs_{530} = 0.6$. Numbers on the spectra represent irradiation time in seconds.

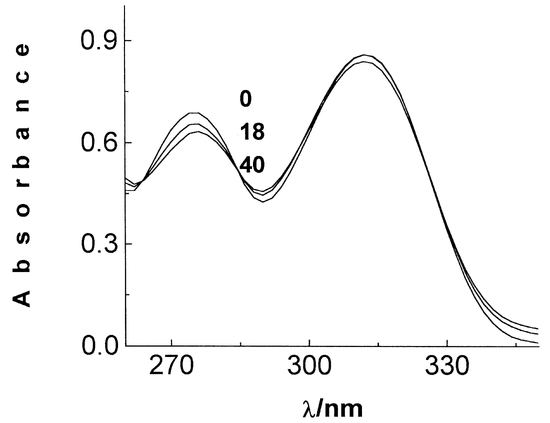


Fig. 3. Spectral changes in the Riboflavin-sensitized photooxidation of 3-hydroxypyridine. $Abs_{445} = 0.6$. Numbers on the spectra represent irradiation time in minutes.

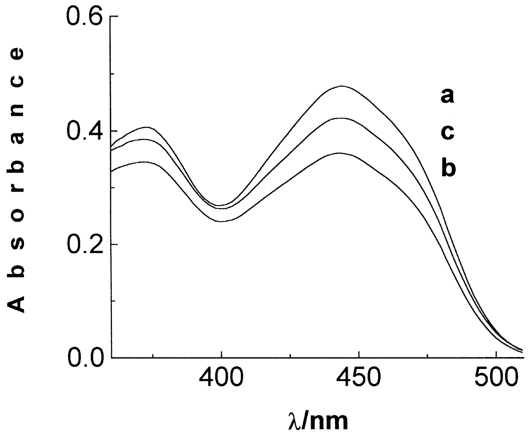


Fig. 4. Absorption spectra of Riboflavin in nitrogen-saturated aqueous solution: (a) non photolysed; (b) 5 min photolysis; (c) 5 min photolysis in the presence of 0.10 mM 3-hydroxypiridine. Irradiation wavelength >400 nm.

diminished in the latter case (Fig. 4), and the same effect was observed in air-equilibrated solutions, although much longer irradiation times were necessary in order to obtain measurable absorption changes.

No association between Rf ground state and 3-OHP was detected by spectroscopy. The difference absorption spectrum of 0.05 mM Rf in the 380–600 nm range was not affected by the presence of up to 1 mM 3-OHP (results not shown).

3.2. *Quenching of excited singlet Rf by 3-OHP*

In air-equilibrated aqueous solution Rf exhibits an intense fluorescence emission centred at 515 nm, with a reported quantum yield of 0.25 [13]. In the presence of >4 mM 3-OHP, the quenching of the fluorescence from excited singlet Rf (^{1}Rf*) is detectable as a decrease in the stationary emission intensity, but the shape of the emission spectrum does not change. Monitoring the fluorescence intensity of Rf in the absence (I_o) and in the presence (I) of different 3-OHP concentrations, the classical Stern–Volmer treatment ($I_o/I = 1 + 1K_{sv}[3\text{-OHP}]$) allows the determination of the Stern–Volmer constant ($^1K_{SV}$) (Fig. 5). Assuming that the Rf fluorescence inhibition is a consequence of the quenching of ^{1}Rf* by 3-OHP, the $^1K_{SV}$ value so obtained (31 M^{-1}) will be equal to the ratio $^1k_q/^1k_d$, where ^1k_q and ^1k_d are the respective rate constants for the quenching of ^{1}Rf* by 3-OHP, and for the thermal deactivation of ^{1}Rf* (Scheme 1, processes 3 and 2, respectively). Time-resolved fluorescence measurements in the absence of 3-OHP yielded a value for ^1k_d of $1.9\times10^8\,M^{-1}\,s^{-1}$ (corresponding to a Rf fluorescence lifetime, $^1\tau_o$, of 5.2 ns, in accord with published data [14]) and, consequently, it could be calculated a ^1k_q value of $5.9\times10^9\,M^{-1}\,s^{-1}$, close to the diffusion rate constant in aqueous solution, $6.4\times10^9\,M^{-1}\,s^{-1}$ [15]. The former value is practically the same as that obtained by direct application of the Stern–Volmer equation ($^1\tau_o/^1\tau = 1 + [3 - \text{OHP}]^1k_q/^1k_d$, see Fig. 5) to time resolved fluorescence lifetimes of Rf in the absence ($^1\tau_o$) and in the presence ($^1\tau$) of 3-OHP.

3.3. *Quenching of triplet Rf by 3-OHP*

The disappearance of Rf triplet state (^{3}Rf*), generated by a 532 nm laser pulse, was followed from the first-order decay of the absorbance at 680 nm, a zone where the interference from other possible species is negligible. In this way it was observed that the lifetime of ^{3}Rf* appreciably decreases in the presence of 3-OHP in concentrations up to 0.15 mM. As before, a Stern-Volmer treatment of the triplet quenching (Fig. 5, inset), using the expression $1/^3\tau = (1/^3\tau_o) +^3 k_q$ [3-OHP] (where $^3\tau_o$ and $^3\tau$ are the experimentally determined triplet lifetimes of Rf in the absence and in the presence of 3-OHP, respectively), yielded a bimolecular rate constant value for the quenching of ^{3}Rf by 3-OHP (^3k_q, Scheme 1, process 5) of $9.7\times10^8\,M^{-1}\,s^{-1}$.

4. Discussion

As the former results show, Rf sensitizes the photooxidation of 3-OHP (Figs. 2 and 3) by a mechanism that involves $O_2(^1\Delta_g)$ processes, as well as other competitive reactions that include 3-OHP and Rf photoconsumption. In this section we will discuss, in kinetic terms, the importance of all these processes on the prediction of the chemical fate of 3-OHP—a compound taken as a model of contaminants with related structure—and of the

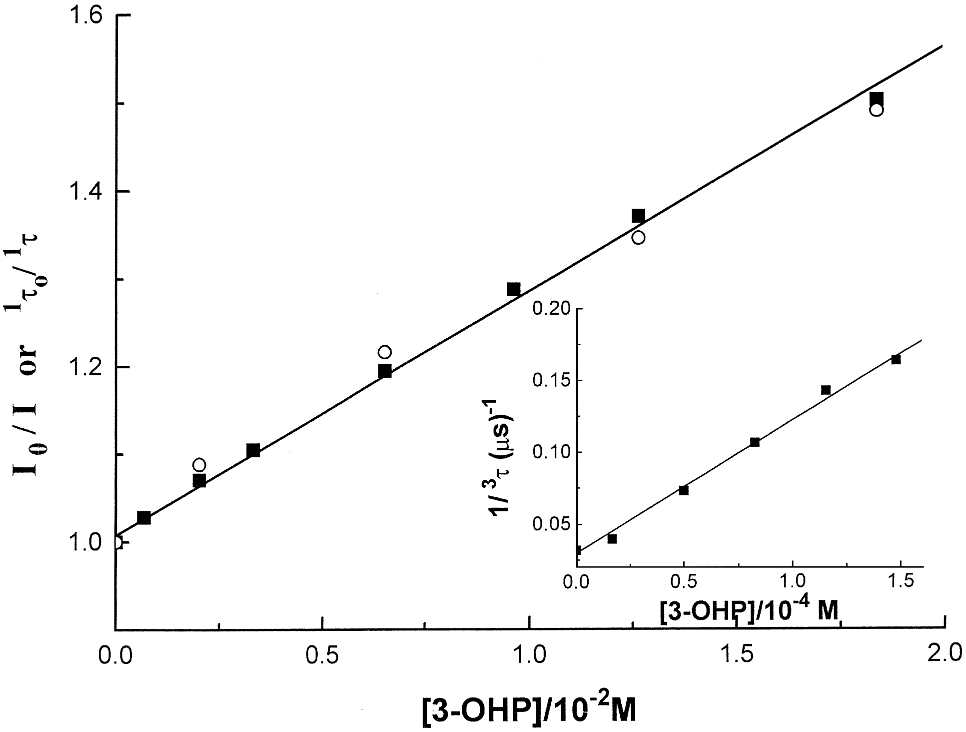
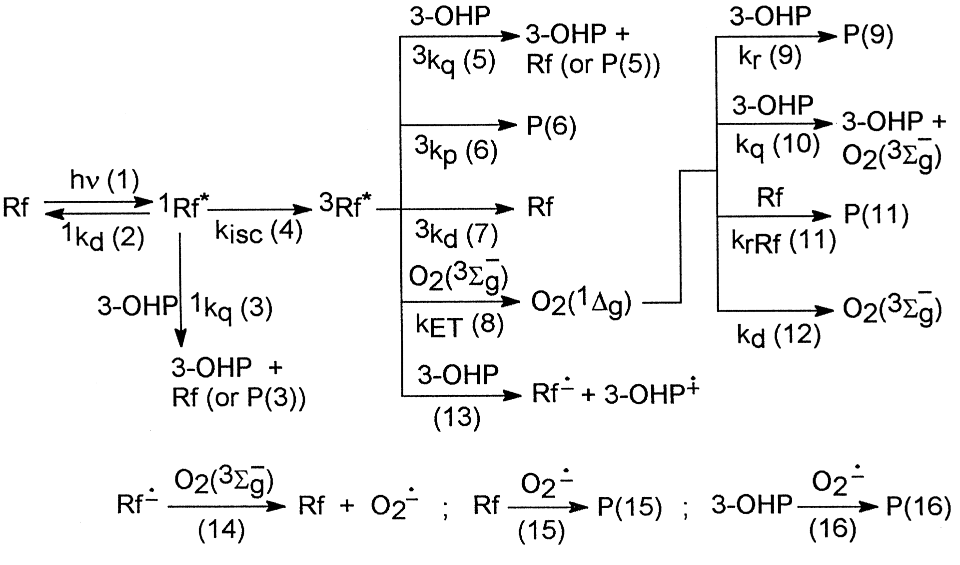


Fig. 5. Stern–Volmer plot for the quenching of Riboflavin fluorescence by 3-hydroxypyridine in air-equilibrated aqueous solution. ${}^1\tau_0/{}^1\tau$ and I_0/I correspond to time-resolved (squares) and static (circles) experiments, respectively. Inset: Stern–Volmer plot for the quenching of Riboflavin triplet state by 3-hydroxypyridine in nitrogen-saturated aqueous solution. ${}^3\tau$ is the triplet lifetime of Riboflavin.



Scheme 1. Possible interactions in the visible light irradiation of 3-hydroxypyridine in the presence of Riboflavin. P denotes a reaction product.

isoalloxazine pigment Rf, under experimental conditions that mimic the natural aqueous environment.

The general scheme for sensitized photooxidations includes two major pathways [16]. In type II processes the triplet sensitizer transfers energy to ground state oxygen (Scheme sc1, process 8), generating the reactive species $O_2(^1\Delta_g)$, that can decay by chemical or physical interaction with a quencher, 3-OHP in the present case (processes 9 and 10, respectively), or by collision with solvent molecules (process 12). In other processes, superoxide anion ($O_2^{\cdot -}$) is formed by electron transfer from the sensitizer.

The two sensitizers herein used, Eosin and Rf, are efficient $O_2(^1\Delta_g)$ generators, with reported quantum yields in water of 0.57 and 0.49, respectively [17]. As we have previously demonstrated employing Rose Bengal as sensitizer [1], and as is also shown in the present work employing eosin, the first order plots of oxygen uptake upon visible-light irradiation of the systems (Fig. 2, inset) indicate a predominant reaction with $O_2(^1\Delta_g)$. In the system Rf + 3-OHP, the reaction with $O_2(^1\Delta_g)$ must also be important, as it is partially inhibited by sodium azide. Direct comparison of the photooxdation rate of the substrate with that of a reference is, however, only valid in the absence of specific interactions between sensitizer and substrate. The evident curvature in the *pseudo*-first order plot shown in Fig. 2, suggests that, besides the $O_2(^1\Delta_g)$-mediated pathway (Scheme sc1, process 9), other interactions between ground state 3-OHP and ground or excited state Rf could be present. Although flavins, including Rf, are probably the most extensively studied biomolecules with respect to their complexation ability with other molecules of biological and environmental relevance, including hydroxyaromatic derivatives [18], ground state interactions between 3-OHP and Rf have not been detected by absorption spectroscopy (see above). On the contrary, the present experimental results show that both $^1Rf^*$ and $^3Rf^*$ interact with ground state 3-OHP.

The excellent concordance between static and time-resolved experiments on the quenching of the fluorescence of Rf by 3-OHP (Fig. 5) demonstrates that the only process involved is an interaction between $^1Rf^*$ and 3-OHP, and the linearity of the plots corroborates the absence of associations between the corresponding ground states. Moreover, the kinetic data indicate that the singlet quenching process does not affect, to a considerable extent, the population of ^3Rf (Scheme 1, process 4), because $^1Rf^*$, with a lifetime of 5.2 ns, can be hardly quenched by 3-OHP in the concentrations herein employed (ca. 0.10 mM). For > 1 mM 3-OHP, the massive quenching of ^1Rf prevents the formation of ^3Rf (process 4) and, hence, of $O_2(^1\Delta_g)$ (process 8), as the oxygen uptake experiments indicate.

Triplet Rf is quenched by 3-OHP with a ^3k_q value of $9.7\times10^8\,M^{-1}\,s^{-1}$ (Scheme 1, process 5), as determined by flash photolysis (see Results). Hence, in the absence of oxygen and in the presence of 0.1 mM 3-OHP, Rf photodecomposition via triplet state (process 6) is prevented (Fig. 4). In the presence of oxygen the deactivation process 5 could compete with the generation of $O_2(^1\Delta_g)$ (process 8). For a rate constant $k_{ET}\approx 7\times10^8\,M^{-1}\,s^{-1}$ [15,19], 0.1 mM 3-OHP, and 0.26 mM oxygen concentration—the value in air equilibrated aqueous solution [20]—the deduced reaction rates of processes 5 and 8, $^3k_q\times[3OHP]=9.7\times10^4\,s^{-1}$, and $k_{ET}\times[O_2(^3\Sigma_g)]=18\times10^4\,s^{-1}$, respectively, are comparable.

In air saturated solutions, Rf decomposes via its triplet state by the processes 6 and 11 shown in Scheme 1 [6,21,22]. Nevertheless, with typical Rf concentrations (ca. 0.01 mM), and considering a k_{rRf} value of $6\times10^7\,M^{-1}\,s^{-1}$ [5], the $O_2(^1\Delta_g)$-mediated degradation of Rf should be inhibited in the presence of $\geqslant 0.10$ mM 3-OHP, because (k_q+k_r) [3-OHP] $\approx 3\times k_{rRf}$ [Rf], being $(k_q+k_r) = 2.2\times10^7\,M^{-1}\,s^{-1}$ [1].

As already said, the possible implication of superoxide anion ($O_2^{\cdot -}$) should also be considered within this complex reaction mechanism, because it is known that in oxygenated aqueous solutions, and in the presence of hydroxy substituted aromatic compounds such as phenols, $^3Rf^*$ generates $O_2^{\cdot -}$ through an electron transfer process where the hydroxyaromatic substrate acts as a donor [23,24]. If, as it would be expected, these processes were also operative in the presence of 3-OHP, the corresponding reactions should consume oxygen, Rf, and/or 3-OHP (Scheme 1, reactions 13–16), as experimentally observed, and their relative contributions to the total result should depend on the concentration of the reactants, i.e. the process could switch from a mechanism via $O_2(^1\Delta_g)$ (type II) to another one via $O_2^{\cdot -}$.

5. Conclusions

From the results herein shown it can be concluded that visible-light irradiation of 3-OHP in air-equilibrated aqueous solution and in the presence of Rf triggers competitive physical and chemical processes that depend on the relative concentrations of Rf and 3-OHP. With ca. 0.01 mM Rf and $\leqslant$0.01 mM 3-OHP, the dominant pathway is the generation of $O_2(^1\Delta_g)$ and the concomitant photooxygenation of 3-OHP (Scheme 1, processes 8 and 9). Under similar conditions, but with ca. 0.10 mM 3-OHP, competitive reactions of $^3Rf^*$ with both $O_2(^3\Sigma_g^-)$ and 3-OHP (processes 8 and 13, respectively) are operative, giving rise to: (i) the inhibition of the Rf photodegradation (process 6); (ii) the generation of both $O_2(^1\Delta_g)$ (process 8) and $O_2^{\cdot -}$ (process 14); and (iii) the subsequent photooxygenation of both 3-OHP (process 9) and Rf (process 11). In the presence of much higher 3OHP concentrations ($\geqslant$1 mM), the quenching of $^1Rf^*$ (process 3) predominates, and the photodegradation of Rf is thus prevented.

Acknowledgements

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