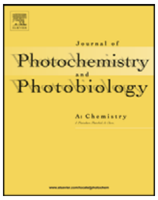
Journal of Photochemistry and Photobiology A: Chemistry 202 (2009) 221–227

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem



Visible-light-mediated photodegradation of 17β-estradiol: Kinetics, mechanism and photoproducts

Marta Díaz[a], Marta Luiz[a], Patricia Alegretti[b], Jorge Furlong[b], Francisco Amat-Guerri[c], Walter Massad[d], Susana Criado[d], Norman A. García[d,*]

[a] Departamento de Química, FCN, Universidad Nacional de la Patagonia San Juan Bosco, 9000 Comodoro Rivadavia, Argentine
[b] Laboratorio LADECOR, División Química Orgánica, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900 La Plata, Argentine
[c] Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain
[d] Departamento de Química, FCEFQyN, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentine

ARTICLE INFO

Article history:
Received 18 September 2008
Received in revised form 6 November 2008
Accepted 1 December 2008
Available online 11 December 2008

Keywords:
Estradiol
Riboflavin
Singlet molecular oxygen
Superoxide anion radical
5,6,7,8-Tetrahydro-2-naphthol

ABSTRACT

The endocrine disrupting compounds 17β-estradiol (ESD) and its derivatives – major constituents of contraceptives – are incorporated to waste waters mainly through human excretion and anthropogenic industrial activities. In this paper, the kinetic and mechanistic aspects of the Riboflavin (Rf)- and Rose Bengal (RB)-photosensitized degradation of ESD and the model compound 5,6,7,8-tetrahydro-2-naphthol (THN), irradiating with visible light (>400 nm), have been studied in methanolic solution by stationary photolysis, polarographic detection of dissolved oxygen, stationary and time-resolved fluorescence spectroscopy, and laser flash photolysis. Rf (Vitamin B_2) is a known human endogenous photosensitizer present as traces in most natural waters. The irradiation of Rf in the presence of ESD triggers complex competitive reactions that finally produce the photodegradation of both ESD and, to a lesser extent, Rf. ESD quenches singlet and triplet excited states of Rf, $^1Rf^*$ and $^3Rf^*$, respectively. The quenching of $^3Rf^*$ with ESD gives rise, through an electron transfer process, to the semireduced form of the pigment, $Rf^{\bullet -}$, a species that generates superoxide radical anion, $O_2^{\bullet -}$. Moreover, energy transfer from $^3Rf^*$ to dissolved molecular oxygen generates the oxidative species singlet molecular oxygen, $O_2(^1\Delta_g)$. Both $O_2^{\bullet -}$ and $O_2(^1\Delta_g)$ produce the photodegradation of ESD. The exclusively $O_2(^1\Delta_g)$-mediated photooxidation mechanism was studied employing the artificial dye RB as a sensitizer, a very efficient process especially in alkaline medium. Several photooxidation products, likely generated through the primary oxidation of the aromatic ring of ESD, have been detected under this experimental condition by means of HPLC/MS techniques.

1. Introduction

The presence of measurable amounts of estrogenic compounds in surface waters has been a subject of great concern during the last two decades [1]. Some of these compounds, such as 17β-estradiol (ESD, Scheme 1) and its derivatives – major constituents of contraceptives – are endocrine disrupting chemicals that are incorporated to waste waters mainly through human excretion and anthropogenic industrial activities [1–3]. It is known that these steroids show high physiological activity in natural aquatic fauna, even in very low concentrations [1,2]. These effects have generated a number of studies directed to investigate their natural or artificially induced degradation, in particular through photochemical methods. TiO_2-assisted photodegradation is an efficient method for the fast elimination of estrogenic compounds from contaminated waters, although it is highly dependent on the presence of dissolved organic matter and other additives such as H_2O_2 [2]. UV irradiation (254 nm), both in the presence of H_2O_2 [4] and in its absence [5], also induces the degradation of these compounds in solution, and it has been reported that the process with H_2O_2 is favored at pH values in the range 6–8. Nevertheless, these photochemical methods are energetically expensive and, in fact, could be only applicable to limited volumes of contaminated waters.

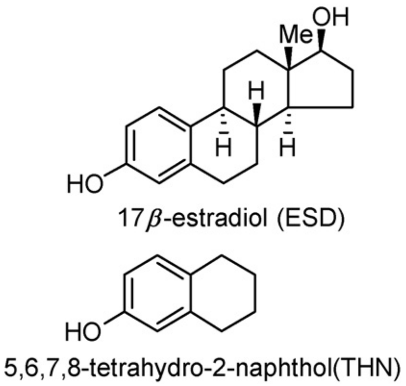
More promising, although slower, methods for the degradation of these environmental contaminants are based on the irradiation with sunlight under natural conditions [6] and in the presence of natural dye sensitizers, because ESD and most of its derivatives do not absorb visible light. Thus, the photodegradation under visible light irradiation, and in the presence of flavins or other unknown sensitizers, of ESD, estrone and estriol in ethanol solution [7], or of ESD and some of its derivatives in natural waters [1], has been studied, although limited information was reported about the kinetic aspects of the processes or the degradation products.

All these ESD derivatives possess a common phenol group in their structures. Photoprocesses involved in sensitized aerobic and anaerobic processes of phenolic compounds have been extensively

---

* Corresponding author.
E-mail address: ngarcia@exa.unrc.edu.ar (N.A. García).

1010-6030/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2008.12.008



Scheme 1. Structures of the studied compounds.

investigated [8–11]. In general, phenols are efficiently photooxidized in solution under irradiation with visible light in the presence of oxygen and a dye sensitizer, with the involvement of reactive oxygenated species (ROS) such as singlet molecular oxygen [$O_2(^1\Delta_g)$], superoxide radical anion ($O_2^{\bullet -}$) and hydroxy radical ($OH^\bullet$), among others [12]. ROS species are photochemically generated by interaction of dissolved molecular oxygen ($O_2(^3\Sigma_g^-)$) with the electronically excited states of the photosensitizers or of species generated from them.

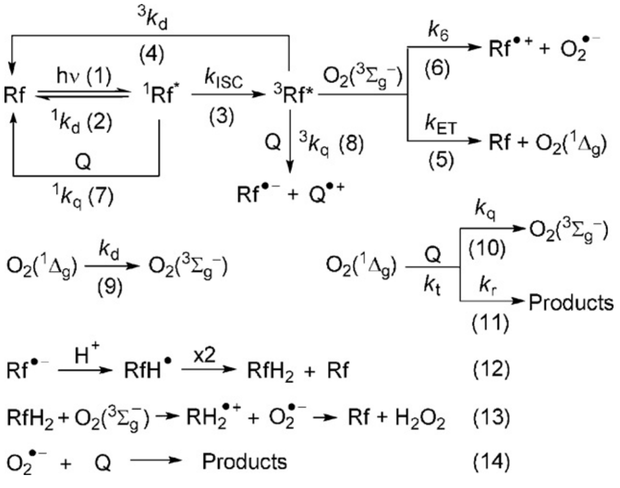
In the present paper, we study the photosensitized degradation of ESD and the model compound 5,6,7,8-tetrahydro-2-naphthol (THN), in MeOH, in the presence of Riboflavin (Rf) or of the synthetic dye Rose Bengal (RB), trying to find the experimental conditions that maximize the degradations. ESD is sparingly soluble in water (ca. 0.01 mM in buffered aqueous solutions [13]). In waste and natural waters, ESD is frequently found as aggregates or dissolved in micellar media [13,14], provided that surfactants are important contaminants of surface waters [15].

The mechanism of sensitization with Rf, a flavin frequently found in natural waters [16,17], is rather complex, in many cases with the concurrent involvement of the oxidative species $O_2(^1\Delta_g)$ and $O_2^{\bullet -}$, both generated with quantum yields of 0.49 and 0.009, respectively, in MeOH solution [18]. RB exclusively generates $O_2(^1\Delta_g)$ with quantum yield of 0.81 in the same solvent [19]. The knowledge of the kinetics and mechanism of the sensitized degradation of ESD and derivatives, and of the structure of the degradation products, could contribute to the prediction or programming of the natural decay of these contaminants.

2. Materials and methods

2.1. Materials

Riboflavin, 17β-estradiol (ESD), 5,6,7,8-tetrahydro-2-naphthol and superoxide dismutase (SOD) from boverythrocytes were purchased from Sigma Chem. Co. Rose Bengal, monodeuterated methanol (MeOD, 99.5 atom % D) and 9,10-dimethylanthrancene (DMA) were from Aldrich. Sodium azide (NaN_3) was from Merck. All these compounds were used as received. Methanol (MeOH) and acetonitrile (MeCN), both HPLC quality, were from Sintorgan, Argentina. All experiments were carried out at room temperature and with freshly prepared solutions. MeOH was employed as a solvent instead of water, the preferred medium for environmental studies, in order to avoid possible aggregation of ESD. Kinetic/photophysical experiments here performed required ESD concentrations higher than 0.1 mM.



Scheme 2. Main processes in the Riboflavin (Rf) photosensitized degradation of a substrate *Q* (ESD or THN in the present paper).

2.2. Methods

Ground state absorption spectra were registered in a Hewlett Packard 8452A diode array spectrophotometer. The overall quenching rate constant of $O_2(^1\Delta_g)$ by a quencher *Q*, k_t, was determined using time resolved phosphorescence detection (TRPD) in a system previously described [20]. Briefly, the $O_2(^1\Delta_g)$ phosphorescence at 1270 nm, generated by excitation with the 532-nm emission from a Nd:YAG laser (Spectron), was detected at right angles using an amplified Judson J16/8Sp Germanium detector, after filtering through a 1270-nm interference and a wratten filter. The output of the detector was coupled to a digital oscilloscope and to a personal computer for the signal processing. Usually, ten shots were needed for averaging so as to achieve a good signal-to-noise ratio, from which the decay curve was obtained. Air-equilibrated solutions were employed in all cases. In the dynamic determinations, solutions of RB in MeOD ($Abs_{532} = 0.2$) were used in order to enlarge the lifetime of $O_2(^1\Delta_g)$ [21]. $O_2(^1\Delta_g)$ lifetimes were evaluated in the presence (τ) and in the absence (τ_0) of the quencher, and the data were plotted as a function of ESD or THN concentration, according to a simple Stern–Volmer treatment: $\tau_0/\tau = 1 + k_t\ \tau_0[Q]$. Continuous aerobic photolysis of aqueous solutions of ESD or THN with RB or Rf as sensitizers was carried out in a home-made photolyser with the filtered light (cut-off filter, $\lambda > 350$ nm) from a 150-W quartz–halogen lamp. The rate constant k_r for the reaction $O_2(^1\Delta_g) + Q$ (process (11), see Scheme 2, later) was determined using a described method [22], for which the knowledge of the reactive rate constant k_{rR} for the photooxidation of a reference compound *R* is required, using the expression $\text{slope}/\text{slope}_R = k_r[Q]/k_{rR}[R]$, where slope and slope_R are the respective slopes of the first-order plots of *Q* and *R* consumption. DMA was employed as a reference compound, with a k_{rR} value of $4.7 \times 10^7\ M^{-1}\ s^{-1}$ determined in this work, in concordance with reported data [10].

The photodegradation rates of ESD, THN and DMA were determined from the absorbance decreases of the respective absorption maxima at ca. 200 nm (ESD and THN) or at 358 nm (DMA) in samples taken at conversions lower than 15%. The rates of oxygen consumption were determined with the specific oxygen electrode Orion 97-08 in MeOH–H_2O 1:1 (v/v), because this electrode must be used in media containing a high percentage of water.

Fluorescence lifetimes were determined with a time-correlated single photon counting technique (SPC) on an Edinburgh FL-9000CD instrument provided with a nF900 nanosecond flashlamp. Excitation and emission wavelengths for Rf were 445 and 515 nm, respectively. The physical quenching rate constant ${}^1k_q$ of the interaction of ${}^1Rf^*$ with *Q* (process (7)) was determined by a classical Stern–Volmer treatment with the expression ${}^1\tau_0/{}^1\tau = 1 + {}^1k_q\,{}^1\tau_0[Q]$, where ${}^1\tau$ and ${}^1\tau_0$ are the respective lifetimes of Rf fluorescence in the presence and in the absence of *Q*. The aerobic and anaerobic photodegradation rates of Rf were deduced from the decrease of the 445-nm absorption. Argon-saturated 0.04 mM Rf aqueous solutions were photolysed using a previously reported flash photolysis apparatus [23]. ${}^3Rf^*$ disappearance was monitored from the first-order decay of the absorbance at 670 nm, a zone where the interference from other possible species is negligible. To avoid self-quenching and triplet–triplet annihilation, the triplet decay was measured at low Rf concentration (typically 0.05 mM) and at low enough laser energy. The rate constant ${}^3k_q$ of the interaction ${}^3Rf^* + Q$ (process (8)) was deduced by the Stern–Volmer expression $1/{}^3\tau = (1/{}^3\tau_0) + {}^3k_q[Q]$, where ${}^3\tau_0$ and ${}^3\tau$ are the experimentally determined lifetimes of ${}^3Rf^*$ in the absence and in the presence of *Q*, respectively.

2.3. Analysis of photooxidation products

Steady-state photolysis ($\lambda > 420$ nm) of air-equilibrated solutions (25 mL) of ESD (1.15 mM) and RB ($Abs_{532} = 0.85$) in MeOH–H_2O 1:1 (v/v) with 1 mM KOH were performed at room temperature in the above described photoreactor until ca. 80% disappearance of ESD, as determined by absorbance measurements. The photolysed sample was analyzed by combined HPLC/MS, employing an Agilent 1100 LC-MSD modular system consisting on a binary pump, an autosampler, a thermostated Agilent RP-C18 aminoacid column at 25 °C, a diode array detector and a mass selective detector with electrospray ionization (API) and atmospheric pressure chemical ionization (APCI) interfaces. Conditions: MeOH–H_2O 1:1 as eluent at a flow rate of 1 mL min^{-1}, injection of 50 μL. MSD parameters: API and APCI interfaces, positive and negative modes; mass range 50–600 amu (0.5 amu mass resolution).

3. Results

The processes involved in the generation of reactive oxygenated species by visible-light irradiation of Rf solutions in the presence of a quencher *Q* are shown in Scheme 2. Upon absorption of a photon, Rf gives rise to its excited singlet state (${}^1Rf^*$) and, through intersystem crossing, to the triplet state (${}^3Rf^*$) (processes (1) and (3)). ${}^3Rf^*$ can transfer energy or an electron to ground state oxygen [$O_2({}^3\Sigma_g{}^-)$] in the aerated solution, generating $O_2({}^1\Delta_g)$ (process (5)) and superoxide radical anion ($O_2{}^{\bullet-}$) (process (6)), respectively. Typical electron donors, as phenols [24], can also transfer an electron to ${}^3Rf^*$, giving rise to $Rf^{\bullet-}$ (process (8)). The species $O_2({}^1\Delta_g)$ can decay by collision with solvent molecules (process (9)), and can interact physically (rate constant k_q) and/or chemically (rate constant k_r) with the substrate *Q* (processes (10) and (11), respectively), with overall rate constant $k_t = k_q + k_r$.

3.1. Phosensitization with Riboflavin

3.1.1. Stationary photolysis

The visible-light photoirradiation of the mixture ESD (0.8 mM) + Rf (0.02 mM) in MeOH induces changes in the absorption spectra of the solutions (Fig. 1A, inset) that can be attributed to transformations in both ESD and Rf. Similar spectral changes were observed in the irradiation of THN under the same conditions.

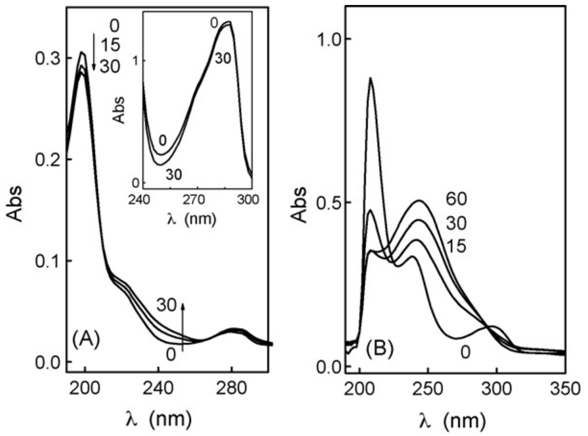


Fig. 1. UV–vis spectral changes during photosensitized irradiations with visible light (>400 nm) under air-saturated conditions. (Panel A) RB ($A_{549} = 0.40$) plus 0.27 mM 17β-estradiol in MeOH, taken vs. RB ($A_{549} = 0.40$) in MeOH; inset: 0.03 mM Rf plus 0.78 mM 17β-estradiol in MeOH, taken vs. 0.03 mM Rf in MeOH. (Panel B) RB ($A_{549} = 0.42$) plus 0.70 mM 17β-estradiol in MeOH with 1 mM KOH, taken vs. RB ($A_{549} = 0.42$) in the same solvent. Numbers on the spectra represent irradiation times in min.

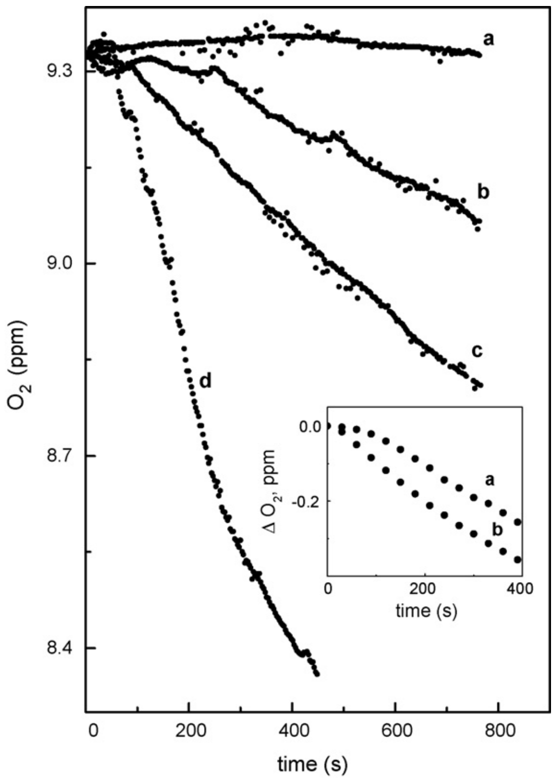


Fig. 2. Remaining oxygen vs. photoirradiation time in MeOH–H_2O 1:1 (v/v) solution. Solutions were irradiated with visible light (>400 nm) under air-saturated conditions in the presence of 0.04 mM Rf: (a) as such; (b) with 17β-estradiol (0.4 mM) plus NaN_3 (2 mM); (c) with 17β-estradiol (0.4 mM); (d) with 17β-estradiol (0.4 mM) plus SOD (14 μg/mL). Inset: consumed oxygen vs. photoirradiation time in the same solvent in the presence of RB ($A_{549} = 0.41$): (a) 17β-estradiol (0.4 mM) and (b) tetrahydronaphthol (0.4 mM).

The anaerobic photodegradation of Rf under visible-light irradiation predominantly proceeds through ${}^3Rf^*$ [25], and the rate of the process can be deduced from the absorbance decrease of the 445-nm band. Comparative irradiations of N_2-saturated aqueous solutions of Rf (ca. 0.02 mM) demonstrated that this rate decreases when working in the presence of 0.2 mM ESD or THN, strongly suggesting the occurrence of a quenching process of ${}^3Rf^*$ by the substrates (data not shown).

Under aerobic conditions, the photoirradiation of MeOH–H_2O 1:1 (v/v) solutions of ESD (0.5 mM) + Rf (0.04 mM) gave rise to oxygen consumption (Fig. 2, main). Relative values for ESD and THN

Table 1

Rate constants for overall (k_t) and reactive (k_r) quenching of $O_2(^1\Delta_g)$, rate constants for the quenching of singlet (^1k_q) and triplet (^3k_q) excited states of Rf by ESD and THN, and relative rates of oxygen consumption ($\Delta O_2/\Delta t$) upon RB- or Rf-sensitization. MeOH/KOH indicates 1 mM KOH in MeOH.

Compd.	Solvent	k_t ($\times 10^8$ M^{-1} s^{-1})	k_r ($\times 10^6$ M^{-1} s^{-1})	k_r/k_t	$\Delta O_2/\Delta t$ (RB)	$\Delta O_2/\Delta t$ (Rf)	^1k_q ($\times 10^9$ M^{-1} s^{-1})	^3k_q ($\times 10^9$ M^{-1} s^{-1})
ESD	MeCN	0.16						
	MeOH–H_2O 1:1				0.7	0.2		
	MeOH	0.18[a]	0.9	0.05			3.3	1.3
	MeOH/KOH	0.67[b]	8.0	0.12				
THN	MeCN	0.015						
	MeOH–H_2O 1:1				1	1		
	MeOH	0.02[a]	<0.2				4.6	6.2
	MeOH/KOH	1.3[b]	5.2	0.04				

[a] Determined in MeOD.

[b] Determined in 1 mM KOH in MeOD.

are shown in Table 1. In comparative irradiations, these rates were decreased, by ca. a factor 4, in the presence of 5 mM NaN_3 and incremented in the presence of SOD (14 μg/mL). Fig. 2, main, shows the corresponding runs for ESD. The enzyme SOD, which catalyzes the dismutation of $O_2^{\bullet -}$, has been used in similar concentrations in order to elucidate the participation of the species $O_2^{\bullet -}$ in a given photooxidation process [26,27]. In MeOD, the presence of the former concentration of SOD does not affect the lifetime of $O_2(^1\Delta_g)$, as TRPD experiments showed.

Three observations can be withdrawn from the experimental data in the photosensitized irradiations with Rf: (a) ESD, THN and Rf are degraded in the processes; (b) oxygenated species participate in these degradations; and (c) both ESD and THN interact with electronically excited states of Rf.

3.1.2. Quenching of ^{1}Rf* and ^{3}Rf*

In air-equilibrated MeOH solution, Rf shows a fluorescence emission band centered at 525 nm with a fluorescence quantum yield, Φ_f, of 0.25. In the presence of ≥10 mM ESD or THN, the quenching of the fluorescence from ^{1}Rf* is detectable as a decrease in the stationary emission intensity, but the shape of the emission spectrum does not change. The interaction was quantified through the determination of the rate constant ^1k_q (process (7)) (Table 1). The fluorescence decay in MeOH in the absence and in the presence of Q was monoexponential, with $^1\tau_0 = 5$ ns, in excellent agreement with previously published data [28]. According to the kinetic data in Table 1, less than 1% ^{1}Rf is quenched in the presence of any Q at 0.5 mM concentration, whereas a concentration of as much as 50 mM of any Q is necessary for the quenching of 50% ^{1}Rf*. Hence, the quenching of ^{1}Rf* is practically nonoperative under the experimental conditions employed in the photosensitization experiments, with [Q] values ≤0.5 mM.

The interaction ^{3}Rf*–Q (process (8)) was studied through laser flash photolysis experiments in the absence of oxygen. The monoexponential decay curves indicated that the ^{3}Rf* lifetime appreciably decreases in the presence of any Q in the mM concentration range. As before, a Stern–Volmer treatment of the ^{3}Rf* quenching yielded the value of the bimolecular rate constant ^3k_q for this interaction (Fig. 3, inset, and Table 1). Fig. 3, main, shows the known spectrum of ^{3}Rf* obtained after the laser pulse [24,29]. The shape of the long-life absorption, obtained in the presence of 1 mM ESD (ca. 95% ^{3}Rf* quenched by ESD) is in good agreement with that of the semiquinone radical, $RfH^{\bullet}$, previously reported [29,30]. This species is formed (process (12)) from the protonation of $Rf^{\bullet -}$, generated in process (8). The bimolecular decay of $RfH^{\bullet}$ is known to proceed through a disproportionation reaction yielding Rf and fully reduced Rf (RfH_2) (process (12)). In the presence of $O_2(^3\Sigma_g^-)$, RfH_2 is reoxidized, giving rise to the radicals $RfH_2^{\bullet +}$ and $O_2^{\bullet -}$ and, eventually, Rf and H_2O_2 [29,31] (process (13)). The $O_2^{\bullet -}$ generated through processes (6) and (13) can react with Q (process (14)) yielding oxidized products.

3.2. Photosensitization with Rose Bengal

The possible involvement of the species $O_2(^1\Delta_g)$ in the photodegradation of ESD and THN, was investigated through different experimental determinations employing RB as a dye sensitizer.

The visible-light irradiation of air-equilibrated MeOH solutions of ESD in the presence of RB induces changes in the absorption spectrum of the solutions (Fig. 1A, main). These changes were clearly higher in the presence of 1 mM KOH (Fig. 1B), i.e. with the phenol group of ESD fully ionized (pK_a of ESD = 10.1 [32]), and were totally suppressed when argon-saturated solutions were photoirradiated (result not shown).

Relative values of oxygen uptake rates in MeOH–H_2O 1:1 by ESD and THN (both 0.1 mM), sensitized by RB ($A_{549\,nm} = 0.40$) are shown in Table 1. In these systems, oxygen uptake was totally inhibited by the presence of 5 mM NaN_3, a known selective $O_2(^1\Delta_g)$ physical quencher [10], pointing to a type II ($O_2(^1\Delta_g)$-mediated) photooxidation as the main degradation reaction of ESD and THN.

Employing RB as a dye sensitizer, the rate constant k_t for ESD and THN was determined in MeCN, MeOH and MeOH/KOH

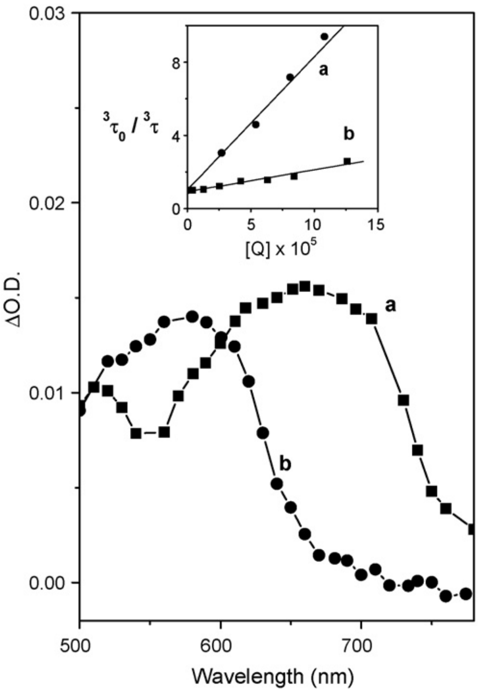


Fig. 3. Transient absorption spectra of 0.05 mM Riboflavin in argon-saturated MeOH–H_2O 1:1 (v/v) solution in the absence (a) and in the presence (b) of 17β-estradiol (0.40 mM), taken at 5 and 20 μs after the laser pulse, respectively. Inset: Stern–Volmer plot for the quenching of ^{3}Rf* by tetrahydronaphthol (a), and 17β-estradiol (b), in the same solvent.

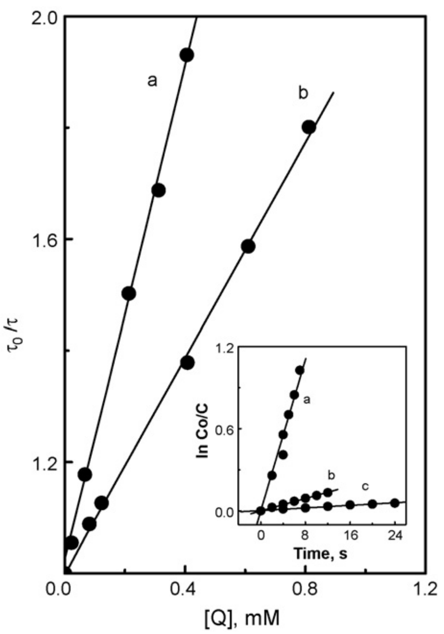


Fig. 4. Stern–Volmer plot for the quenching of $O_2(^1\Delta_g)$ by 17β-estradiol (a), and tetrahydronaphthol (b) in 1 mM KOH in MeOD, with RB ($A_{532} = 0.20$) as sensitizer. Inset: first-order plots of substrate consumption upon visible-light irradiation in the same solvent in the presence of RB ($A_{549} = 0.32$) plus: (a) DMA (0.5 mM); (b) 17β-estradiol (0.5 mM); (c) tetrahydronaphthol (0.5 mM).

1 mM (Table 1), by means of TRPD. Typical runs are shown in Fig. 4.

The k_r values in MeOH and in MeOH/KOH 1 mM for ESD and THN are shown in Table 1, and were obtained through experiments of substrate consumption, as previously described. Typical experimental results are shown in Fig. 4, inset. The k_r values determined in MeOH follow the same trend that the respective relative rate values of oxygen uptake obtained in MeOH–H_2O 1:1.

The efficiency of a $O_2(^1\Delta_g)$-mediated oxidation can be evaluated through the expression $\Phi_r = k_r[Q]/(k_d + k_t[Q])$, where k_d is the rate constant of the $O_2(^1\Delta_g)$ deactivation by interaction with solvent molecules (process (9)) [8]. This expression cannot be applied when the substrate concentration is unknown as in the cases of complex biological or natural environments. In these cases a useful and simpler approach is the evaluation of the k_r/k_t ratio (Table 1), i.e. the fraction of overall quenching that effectively leads to chemical reaction.

Prolonged RB-sensitized photoirradiation of a MeOH/KOH 1 mM ESD solution – a medium where the efficiency of the degradative process is higher – was performed up to ca. 80% ESD conversion and the photooxidation products were analyzed. Composition of the solution and analytical details were described in Section 2.

4. Discussion

4.1. Photosensitization with Rose Bengal

The interaction of both ESD and THN with $O_2(^1\Delta_g)$ generated with RB is corroborated by TRPD experiments. The increase of both k_t and k_r values with the ionization of the phenol group has been already observed in the photooxidation of phenols, hydroxypyridines, hydroxypyrimidines and hydroxyquinolines [8,33]. This effect has been explained as due to the formation of a charge-transfer encounter complex of the type [$O_2(^1\Delta_g)$-hydroxyaromatic compound], a formation that is favored by the higher electron–donor ability of the ionized species [8,34–37]. Both chemical reaction (generation of oxidation products) and physical quenching (regeneration of $O_2(^3\Sigma_g^-)$) can operate, and their relative importance is measured by the respective rate constants k_r (process (11)) and k_q (process (10)). ESD in MeOH appears as an excellent physical scavenger of $O_2(^1\Delta_g)$. Nevertheless, the photooxidation efficiency, defined as the ratio k_r/k_t, reaches a value of 0.12 in alkaline medium, a value relatively high if compared to the same ratios in other simpler phenols [8]. Apparently, and compared to THN as a structurally related reference, the presence of the cyclic aliphatic moiety in ESD enhances the susceptibility towards photooxidation with $O_2(^1\Delta_g)$.

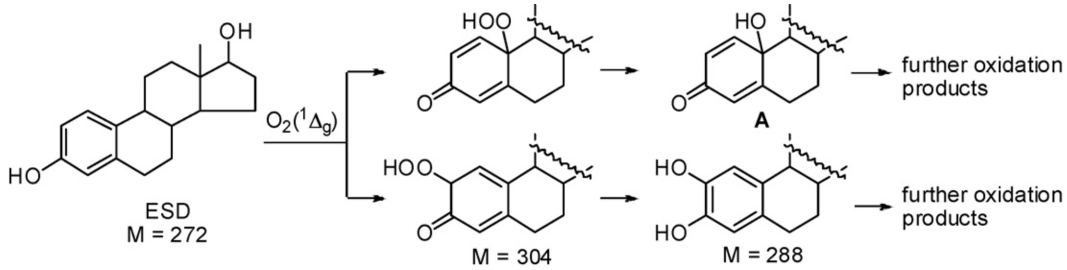
The mentioned charge-transfer encounter complex is affected by solvent polarity. This is in agreement with the fact that the respective k_t values for EDS determined in MeOD and MeCN (Table 1), two solvents of similar dielectric constant [38], are very close. The same is valid for THN.

4.2. Photosensitization with Riboflavin

The oxygen consumption results for the sensitization with Rf (Table 1) point to a different process for this consumption, if compared with the same results for the sensitization with RB, where an exclusively $O_2(^1\Delta_g)$-mediated mechanism operates. In the case of Rf, an additional source of oxygen consumption must be the existence of processes with the involvement of the species $O_2^{\bullet-}$, as supported by the SOD experiments. Although the direct generation of $O_2^{\bullet-}$ by electron transfer from $^3Rf^*$ to $O_2(^3\Sigma_g^-)$ (process (6)) must be negligible, due to the very low values of the quantum yield of the process, the species $O_2^{\bullet-}$ could also be formed via processes (8), (12) and (13). This sequence has been formerly proposed in the photooxidation of other phenols [39,36]. The high rate constant value obtained for the generation of $Rf^{\bullet-}$ through process (8), and the inhibitory effect of SOD in oxygen uptake experiments, strongly support that the oxidative species generated by process (13) are involved in the oxidation of ESD and THN (process (14)). The increase of the rate of oxygen uptake due to the presence of SOD (Fig. 2, main) supports the involvement of the species $O_2^{\bullet-}$ in the proposed mechanism. It is well known that SOD can either inhibit or promote the $O_2^{\bullet-}$-mediated oxidation of a given substrate [40,41]. An oxidative SOD stimulation effect has been reported in hydroxy-aromatic compounds such as hydroquinones and, recently, bisphenol A [42,43]. H_2O_2 is formed as a product of $O_2^{\bullet-}$ dismutation, constituting in this way an additional channel of substrate oxidation and oxygen consumption, a behavior that could be ascribed to the cases of ESD and THN.

In the visible-light irradiation of systems Rf-ESD in MeOH in the presence of 2 mM NaN_3, the $O_2(^1\Delta_g)$ lifetime should be reduced by a factor of ca. 15, a value calculated through a simple Stern–Volmer treatment using the described k_t value ($9.5 \times 10^8\ M^{-1}\ s^{-1}$) [10] and a $O_2(^1\Delta_g)$ lifetime, τ_0, in the same medium of 7 μs [10]. However, the rate of oxygen uptake by ESD under these conditions was only reduced by a factor of ca. 4 (Fig. 2, main). This fact strongly supports the existence of at least a second pathway for oxygen consumption different from that driven by $O_2(^1\Delta_g)$, likely represented by the $O_2^{\bullet-}$ pathway. The participation of the species $O_2^{\bullet-}$ in the sensitization process with Rf could be also supported by the close concordance between the ratio of relative rates of oxygen consumption $\Delta O_2/\Delta t(\mathrm{THN})/\Delta O_2/\Delta t(\mathrm{ESD}) = 5$, and the ratio of rate constants for the quenching of the electronically excited state for $O_2^{\bullet-}$ generation, $^3Rf^*$, $^3k_q(\mathrm{THN})/^3k_q(\mathrm{ESD}) = 4.8$, assuming similar efficiencies of ESD and THN in process (14).

Process (8), generating $Rf^{\bullet-}$ and, hence, $O_2^{\bullet-}$, is thermodynamically feasible for the system Rf–Q. This can be evaluated by means of the Gibbs free energy for electron transfer, $\Delta_{ET}G_0 = E_{(ESD/ESD+)} - E_{0(Rf/Rf-)} - E_{Rf^*} + C$, where $E_{(ESD/ESD+)}$ is the oxidation potential value of ESD (or of THN), ca. 1.25 V [44,45], similar to the redox potential values for other phenols [46], $E_{(Rf/Rf-)}$



Scheme 3. Proposed primary mechanism in the Rose Bengal-sensitized photooxidation of ESD.

is the redox potential of Rf (−0.80 V), E_{Rf^*} is the $^3Rf^*$ energy (2.17 eV), and *C* is the coulombic energy term (−0.06 V) [47]. The so-calculated $\Delta_{ET}G_0$ = −0.18 V indicate that process (8) may be thermodynamically feasible and that the species $O_2^{\bullet-}$ can be formed if processes (8) and (5) + (6) are kinetically competitive. Being $k_{ET} = 1.2 \times 10^9\ M^{-1}\ s^{-1}$ in MeOH (process (5)), and employing the corresponding values of ^3k_q for ESD and THN (Table 1), the kinetic balance for solutions with similar concentrations of dissolved oxygen and *Q* indicates that $Rf^{\bullet-}$ production via process (8) is the same or ca. five times faster than process (5) for ESD and THN, respectively, being process (5) the pathway responsible for $O_2(^1\Delta_g)$ generation. In synthesis, experimental evidence and quantitative kinetic evaluation strongly support the participation of the species $O_2^{\bullet-}$ in the Rf-sensitized process.

4.3. 17β-Estradiol photoproducts

The aerobic photodegradation of ESD has been formerly studied under several conditions. Thus, the direct UV photolysis of concentrated aqueous solutions of ESD gives rise to the oxidation and breaking of the phenol ring, yielding carbonyl compounds [48]. In water and in the presence of TiO_2, the irradiation with visible light of ESD also produces the primary oxidation of the phenol ring [49]. In ethanol and in the presence of Rf or lumichrome, the naturally occurring estrogens ESD, estriol and estrone are also oxidatively altered on exposure to daylight. The photooxidation is accompanied by a large loss in biological activity [7]. Other non-photochemical ESD degradations have also been studied, such as the enzymatic oxidation in organic solvents or in biphasic systems, yielding products that can be explained through the formation of carbon-located radicals [50], and the ozonization in aqueous media, giving rise to dicarboxilic acids via O_3 or $OH^\bullet$ addition to different positions of the phenol ring [51].

In our case, HPLC–MS analysis of a RB-sensitized irradiated solution of ESD in MeOH with 1 mM KOH indicates that the products result from the formal addition of oxygen atoms to ESD, in some cases with the concomitant addition of water (Table 2). The absence of ESD in the analyzed mixture indicates that the substrate is totally degraded under the used conditions and/or in the work-up.

According to the accepted mechanism of the reaction of phenols with $O_2(^1\Delta_g)$ [8], the primary step in the photooxidation of ESD under RB sensitization conditions must be an electron/proton transfer process from the phenol group to $O_2(^1\Delta_g)$ – a process favored in alkaline medium – giving rise to a phenoxy radical and to the subsequent formation of compounds with hydroperoxide group at the *orto* and (mainly) *para* OH-position (Scheme 3), with a molecular mass *M* = 304, as compound 6 in Table 2 [52]. A two-electron reduction in the reaction medium would give rise to the corresponding alcohols, with *M* = 288, as compound 7 in Table 2. A compound with the same *M* value (compound A in Scheme 3) has been formerly identified in the photodegradation of ESD in the presence of TiO_2 [49]. UV absorption spectra of the corresponding HPLC peaks were not of help in the structure elucidation of these compounds. Dimeric compounds or products from MeOH addition have not been detected. Under Rf sensitization, the same primary hydroperoxides should be generated by reaction with $O_2^{\bullet-}$ or H_2O_2, both species being mainly formed in process (13). With both sensitizers, subsequent similar oxidation/reduction reactions could give rise to more oxygenated species such as those found in the HPLC analysis, with the more oxygenated and, likely, more polar species eluting first. In these species, the probable breaking of the initial phenol ring, in special in the case of polyhydroxybenzenes [53], cannot be discarded.

Table 2
HPLC/MS (ESI⁻) analysis of the degradation products from the Rose Bengal-sensitized photooxidation of ESD in 1 mM KOH in MeOH. R_t, retention time. Molecular weight of ESD (M_{ESD}) = 272.

Compd.	R_t (min)	[M−H]−/M	Assignment of *M*
1	2.35 – 2.67	369/370	$M_{ESD} + 80 + 18$
2	2.67 – 2.90	335/336	$M_{ESD} + 64$
3	2.67 – 2.90	371/372	$M_{ESD} + 64 + 36$
4	3.18 – 3.23	335/336	$M_{ESD} + 64$
5	11.98	321/322	$M_{ESD} + 32 + 18$
6	14.29	303/304	$M_{ESD} + 32$
7	18.21	287/288	$M_{ESD} + 16$
8	18.21	323/324	$M_{ESD} + 16 + 36$
9	18.21	325/326[a]	$M_{ESD} + 54$
10	18.21	347/348[a]	$M_{ESD} + 80 - 4$

[a] Minor compound.

5. Conclusions

As the results show, the hormone ESD an the model compound THN are degraded in MeOH solution under visible-light irradiation in the presence of photosensitizers such as Rose Bengal or Riboflavin through the action of reactive oxygenated species generated in the medium. In the presence of Rose Bengal, both substrates are photooxidized by a $O_2(^1\Delta_g)$-mediated mechanism in a relatively efficient process, especially in alkaline medium, where the phenol group is ionized. In this case, HPLC/MS analysis of the reaction mixture indicates the generation of species likely formed through the primary oxidation of the phenol ring. With Riboflavin as a sensitizer, the photooxidations of ESD and THN take place through a combination of $O_2^{\bullet-}$ and $O_2(^1\Delta_g)$-mediated processes. Evaluation of the kinetic data indicates that in natural waters the contaminant hormone ESD undergoes a moderate environmental photodegradation induced by daylight and photosensitized by the naturally occurring pigment Rf.

Acknowledgements

Financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto (SECyT UNRC), Universidad Nacional de la Patagonia SJB (SECyT UNP-SJB), all from Argentine, and Consejo Superior de Investigaciones Científicas (CSIC), from Spain, is gratefully acknowledged.

References

[1] Y. Zuo, K. Zhang, Y. Deng, Chemosphere 63 (2006) 1583–1590.
[2] Y. Zhang, J.L. Zhou, B. Ning, Water Res. 41 (2007) 19–26.
[3] C. Wicks, C. Kelly, E. Paterson, Ground Water 42 (2004) 384–389.
[4] E.J. Rosenfelt, K. Linden, Environ. Sci. Technol. 38 (2004) 5476–5483.
[5] B. Liu, F. Wu, N.-S. Deng, J. Hazard. Mater. B 98 (2003) 311–316.
[6] Y. Zuo, R. Jones, Naturwissenchaften 8 (1995) 472–474.
[7] J.F. Nyc, H.B. Friedgood, J.B. Garst, D.M. Maron, J. Am. Chem. Soc. 75 (1953) 907–910.
[8] N.A. García, J. Photochem. Photobiol. B: Biol. 22 (1994) 185–196.
[9] D.O. Mártire, M.C. Gonzalez, in: S.G. Pandalai (Ed.), Recent Research Developments in Photochemistry and Photobiology, vol. 4, 2000, pp. 271–280 (Part II).
[10] F. Wilkinson, W.P. Helman, A. Ross, J. Phys. Chem. Ref. Data 24 (1995) 663–1021.
[11] Y. Zuo, R. Jones, Water Res. 31 (1997) 850–858.
[12] A.M. Braun, M.-T. Maurette, E. Oliveros, Photochemical Technology, John Wiley & Sons, Chichester, 1991, pp. 445–499.
[13] D. Ilardia Arana, H.C. Kristiansen, A. Müllertz, J. Pharm. Sci. 95 (2006) 248–255.
[14] O. Braga, G.A. Smythe, A.I. Schäfer, A.J. Feitz, Chemosphere 61 (2005) 827–833.
[15] A.M. Becker, S. Gerstmann, H. Frank, Chemosphere 72 (2008) 115–121.
[16] C.A. Benassi, E.S. coffone, G. Galiazzo, G. Jori, Photochem. Photobiol. 28 (1967) 857–862.
[17] J.N. Chacón, J. McLearie, R.S. Sinclair, Photochem. Photobiol. 47 (1988) 647–656.
[18] C.M. Krishna, S. Uppuluri, P. Riesz, J.S. Zigler, D. Balasubramanian, Photochem. Photobiol. 54 (1991) 51–56.
[19] F. Amat-Guerri, M.M.C. López-González, R. Martínez-Utrilla, R. Sastre, J. Photochem. Photobiol. A: Chem. 53 (1990) 199–210.
[20] M. Neumann, N.A. García, J. Agric. Food Chem. 40 (1992) 957–960.
[21] S. Nonell, L. Moncayo, F. Trull, F. Amat-Guerri, E.A. Lissi, A.T. Soltermann, S. Criado, N.A. García, J. Photochem. Photobiol. B: Biol. 29 (1995) 157–162.
[22] F.E. Scully, J. Hoigné, Chemosphere 16 (1987) 694–699.
[23] S. Criado, A. Pajares, J. Gianotti, G. Stettler, J.P. Escalada, S. Bertolotti, F. Amat-Guerri, N.A. García, J. Photochem. Photobiol. B: Biol. 71 (2003) 19–25.
[24] E. Haggi, S. Bertolotti, S. Miskoski, F. Amat-Guerri, N.A. García, Can. J. Chem. 80 (2002) 62–67.
[25] P.F. Heelis, Chem. Soc. Rev. 11 (1982) 15–39.
[26] R.M. Baxter, J.H. Carey, Nature 306 (1983) 575–576.
[27] S. Criado, S. Bertolotti, N.A. García, J. Photochem. Photobiol. B: Biol. 34 (1996) 79–86.
[28] S. Criado, N.A. García, Redox Report 9 (2004) 291–297.
[29] A. Pajares, J. Gianotti, G. Stettler, S. Bertolotti, S. Criado, A. Posadaz, F. Amat-Guerri, N.A. García, J. Photochem. Photobiol. A: Chem. 139 (2001) 199–204.
[30] C.-Y. Lu, W.-F. Wang, W.-Z. Lin, Z.-H. Han, S.-D. Yao, N.-Y. Lin, J. Photochem. Photobiol. B: Biol. 52 (1999) 111–116.
[31] C. Lu, G. Bucher, W. Sander, Chem. Phys. Chem. 5 (2004) 47–56.
[32] R.J. Kindani, J. Burgett, Arch. Biochem. Biophys. 118 (1967) 33–36.
[33] N.A. García, F. Amat-Guerri, Chemosphere 59 (2005) 1067–1082.
[34] A. Pajares, J. Gianotti, E. Haggi, G. Stettler, F. Amat-Guerri, S. Criado, S. Miskoski, N.A. García, J. Photochem. Photobiol. A: Chem. 119 (1998) 9–14.
[35] A. Pajares, J. Gianotti, G. Stettler, E. Haggi, S. Miskoski, S. Criado, F. Amat-Guerri, N.A. García, J. Photochem. Photobiol. A: Chem. 135 (2000) 207–212.
[36] E. Haggi, S. Bertolotti, N.A. García, Chemosphere 55 (2004) 1501–1507.
[37] G. Bosio, S. Criado, W. Massad, F.J. Rodríguez Nieto, M. González, N.A. García, D.O. Mártire, Photochem. Photobiol. Sci. 4 (2005) 840–846.
[38] Physical Chemistry of Organic Solvent Systems, Plenum Press, New York, 1973.
[39] W. Massad, S. Bertolotti, N.A. García, Photochem. Photobiol. 79 (2004) 428–433.
[40] I.B. Afanasǐev, Superoxide Ion: Chemistry and Biological Implications, vol. I, CRC Press, Inc., Boca Ratón, FL, 1989.
[41] B. Bandy, J. Moon, A.J. Davison, Free Radic. Biol. Med. 9 (1990) 143–148.
[42] D. Fatur. M.Sc. Thesis, Simon Fraser University, Burnaby, B.C., Canada, (1988) pp. 99–116.
[43] Y. Barbieri, W.A. Massad, D.J. Díaz, J. Sanz, F. Amat-Guerri, N.A. García, Chemosphere 73 (2008) 564–571.
[44] M. Murugananthan, S. Yoshihara, T. Rakuma, N. Uehara, T. Shirakashi, Electrochim. Acta 52 (2007) 3242–3249.
[45] T. Bhattacharya, T. Misra, M. Maiti, R.D. Saini, M. Chanda, S. Lahir, T. Ganguly, Spectrochim. Acta A 59 (2003) 525–535.
[46] M.M. Ngundi, A.O. Sadik, T. Yamaguchi, S.-I. Suye, Electrochem. Commun. 5 (2003) 61–67.
[47] S. Criado, C. Allevi, C. Cevallos, N.A. García, Redox Report 12 (2007) 282–288.
[48] B. Liu, X. Liu, Sci. Total Environ. 320 (2004) 269–274.
[49] Y. Ohko, K.-I. Luchi, C. Niswa, T. Tatsuma, T. Nakashima, T. Iguchi, Y. Kubota, A. Fujishima, Environ. Sci. Technol. 36 (2002) 4175–4181.
[50] S. Nicotra, A. Intra, G. Ottolina, S. Riva, B. Danieli, Tetrahedron: Asymmetry 15 (2004) 2927–2931.
[51] S. Irmak, O. Erbatur, A. Akgerman, J. Hazard. Mater. B 126 (2005) 54–62.
[52] M. Kępczyński, A. Czosnyka, M. Nowakowska, J. Photochem. Photobiol. A: Chem. 185 (2007) 198–205.
[53] M.I. Gutiérrez, A.T. Soltermann, F. Amat-Guerri, N.A. García, J. Photochem. Photobiol. A: Chem. 136 (2000) 67–71.