Journal of Photochemistry & Photobiology A: Chemistry 367 (2018) 327–331

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology A: Chemistry

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

Clearing up the photochemistry of resveratrol: Effect of the solvent

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ARTICLE INFO

Keywords:
Resveratrol
Photochemistry
UV radiation
Photoproducts

ABSTRACT

Polyphenolic substances synthesized by plants are generally involved in protection against UV radiation and the attack of pathogenic microorganisms. Resveratrol (3,5,4′-trihydroxystilbene, RSV) is synthesized in its *trans*-form (*trans*-RSV) in plants under stress conditions like infections or UV exposure and has attracted attention as an antioxidant agent. *Trans*-RSV was irradiated with both UV-A (λ_{MAX} = 365 nm) and UV-B (λ_{MAX} = 300 nm) radiation in aqueous and ethanolic solutions at room temperature. The reactions were followed by UV–Vis spectrophotometry, HPLC with UV and fluorescence detection, and UPLC coupled to mass spectrometry detection. In both solvents the irradiation caused the fast isomerization of *trans*-RSV to *cis*-RSV. In ethanolic solutions, a strong fluorescent compound, identified as resveratrone (RSVT) was detected independently on the irradiation wavelength. In aqueous solutions, RSVT was not detected in both irradiation conditions. However, in aqueous/ethanol mixtures the amount RSVT was found to be proportional to the amount of ethanol in the solution. Under UV-B irradiation, both in ethanolic or water solutions other products were detected. Our results demonstrated that RSV is photosensitive and its photochemistry depends on the solvent nature and on the irradiation wavelength.

1. Introduction

Numerous natural compounds isolated from plants with the common characteristic to bear hydroxyl groups on aromatic rings, have been identified as secondary metabolic products. Nowadays there are more than 5000 natural polyphenolic compounds identified [1], and the diversity goes from small molecules to polymerized substances. Plant phenolic compounds are generally involved in protection against UV radiation and the attack of pathogenic microorganisms, and also in giving colors and organoleptic properties to the plants [2]. One important class of polyphenolic substances are the polyhydroxystilbenes, being the best known of them 3,5,4′-trihydroxystilbene or resveratrol (RSV). During the last decades, RSV has attracted attention for its potential properties in cancer chemoprevention and as an anti-inflammatory, antiviral and antioxidant agent [3]. RSV is present in grapes, peanuts and berries [4], and it has been observed that it is synthesized in plants under stress conditions like infections or UV exposure [5].

RSV is naturally synthesized by plants as *trans*-RSV, but under UV radiation rapidly isomerizes to *cis*-RSV (Fig. 1). Isomerization of stilbenes has been exhaustively characterized, and usually this process occurs after UV radiation absorption, being *trans-cis* isomerization and fluorescence the principal pathways of deactivation [6,7]. Additionally, it was determined that the *trans-cis* isomerization cannot occur from thermal equilibration at room temperature [8]. Solvent properties, such as polarity and polarizability, pressure and temperature can change the properties of the excited states, thus altering not only the fluorescence properties, but also changing the photochemical processes [6].

RSV is present in red wines [9,10], and has attracted attention for its potential properties due to the “French paradox”, which implies low cardiovascular accidents with a high saturated fat diet [11]. Controversially, although RSV is supposed to act as antioxidant, it has also shown to exhibit prooxidant properties. In a previous article, it was determined that *trans*-RSV, expose to UV radiation in ethanolic solution is able to oxidized ergosterol to peroxide of ergosterol, suggesting the production of singlet oxygen ($^1O_2$) [12]. These results are contradictory to those that established that the RSV is an efficient antioxidant, and, therefore, the consumption of RSV, for cosmetic or dietary purposes, should be further evaluated. Additionally, a new yellow fluorescent product of the photodegradation of RSV was isolated and identified as resveratrone ((E)-4-(6,8-dihydroxynaphthalen-2-yl)but-3-en-2-one, denoted hereafter as RSVT, Fig. 1) [13], and has been proposed for bio-

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https://doi.org/10.1016/j.jphotochem.2018.08.050
Received 4 June 2018; Received in revised form 23 August 2018; Accepted 31 August 2018
Available online 31 August 2018

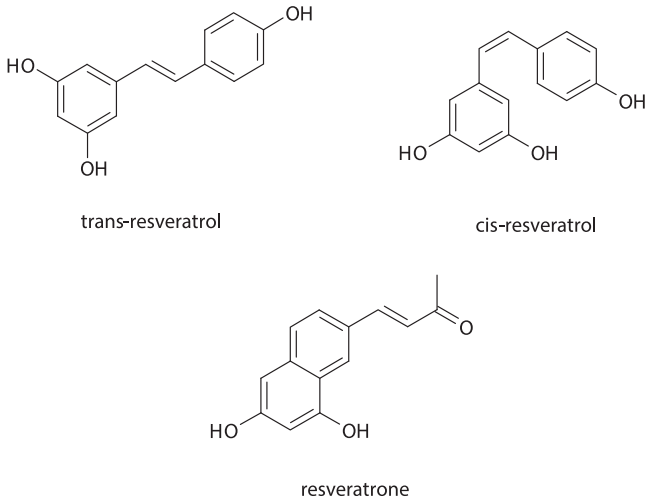

Fig. 1. Molecular structure of RSV, *trans*- and *cis*- isomers, and resveratrone.

imaging microscopy.

RSV is highly soluble in ethanol and slightly soluble in water, and for that reason most of the preparations of RSV use ethanol or ethanol/water as solvents. It was previously reported that solubility is 61 μg/mL in water at pH 6.8 and 37 °C [14] and 98 mg/mL in ethanol at 20 °C [15]. The stability of RSV strongly depends on the nature of the solvent; e.g. in H_2O, *trans*-RSV stability is highly dependent on the pH of the solution, being stable for days in neutral or slightly acidic media, but at pH 9 the degradation of *trans*-RSV occurs in a few minutes [14]. Although the fact that RSV is unstable under UV radiation is well known, the photochemical reactions involved in the photodegradation remain unclear and seem to be strongly depending on both the irradiation wavelength and the nature of the solvent [16–19]. In particular, it was recently proposed that extracts of red wines containing RSV were able to act as weak photosensitizers in the photo-oxidation of ergosterol indicating the possible generation of $^1O_2$ [20].

Therefore in the work reported here, we have studied the photochemistry of *trans*-RSV when exposed to UV radiation of different wavelengths (UV-A and UV-B), in water or ethanol solutions. In particular, the main photoproducts detected under different experimental conditions have been characterized and the influence of the composition of the solvent (water or ethanol) in photodegradation was elucidated.

2. Materials and methods

See Supplementary material

3. Results and discussion

3.1. Steady state UV-A irradiation

Air-equilibrated aqueous and ethanolic solutions containing *trans*-RSV were exposed to UV-A radiation, at a maximum wavelength of 365 nm. *Trans*-RSV concentration was determined by High-performance liquid chromatography (HPLC) with photodiode array (PDA) and the fluorescence (RF) detectors. as a function of irradiation time. In addition, several products were detected and characterized by Ultra performance liquid chromatography with a quadrupole time-of-flight mass spectrometry detector (UPLC-QTof-MS), and when possible their concentrations were determined. Additional experimental details and mass spectra of the molecules mentioned in the manuscript are shown in Fig. S2 (Supplementary material).

3.1.1. Aqueous solutions of trans-RSV (pH = 6.0)

Immediately after starting the irradiation, changes in the absorption spectrum of the solution were observed up to around 20 min and after that time the spectrum remained invariable and quite similar to that reported for *cis*-RSV [21]. These changes are shown in Fig. 2a, where the isosbestic point at 275 nm can be observed. No further changes were detected in irradiated solutions when stored afterwards in the dark for several hours, indicating the thermal stability of these solutions.

Solutions were analyzed by HPLC, registering both absorbance (PDA) and fluorescence emission (RF). Chromatograms recorded at different irradiation times showed that the concentration of *trans*-RSV decreased, while a product with higher retention time (t_R) was formed. The absorption spectrum of this product, registered during the HPLC runs with the PDA detector, was equal to that reported for *cis*-RSV. No further peaks were detected, indicating that isomerization was the only process. The concentration profiles of *trans*-RSV and *cis*-RSV (Fig. 2b) showed that under these conditions, the total concentration of RSV ([*trans*-RSV] + [*cis*-RSV]) was constant, within the experimental error, thus indicating that, in agreement with spectral analysis, the only photoproduct was *cis*-RSV.

In the UPLC-QTof-MS analysis, non irradiated solutions showed a signal corresponding to the intact molecular ion of RSV, $[M-H]^-$ specie at *m/z* 227.0712. The resolution was much better in ESI^- than in ESI^+ mode. Irradiated solutions were analyzed and a unique chromatographic peak, apart from that of the reactant was observed. The product showed a t_R value higher than those corresponding to *trans*-RSV and its mass spectrum showed a signal at *m/z* 227.0710, identical to the reactant. These results confirmed that in aqueous solutions, UV-A irradiation *trans*-RSV, only result in the isomerization to the *cis*-form of the compound.

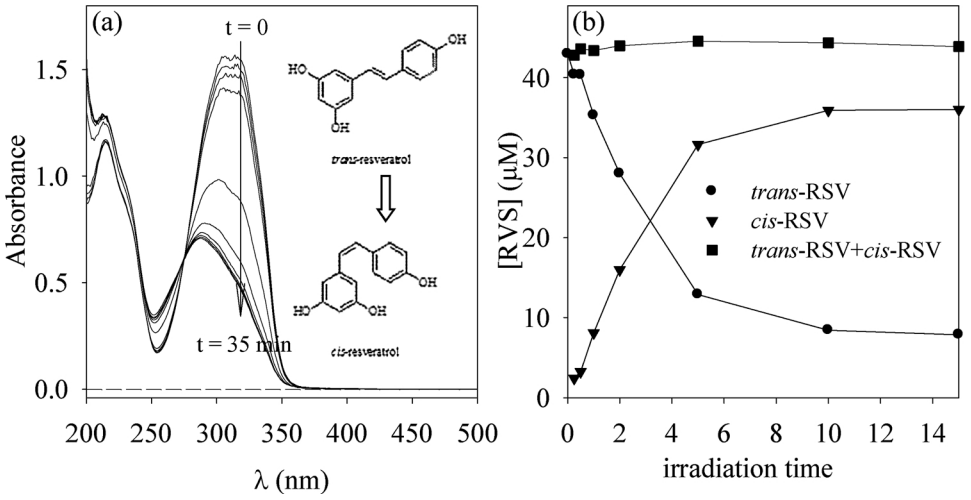

Fig. 2. UV-A irradiation (λ_{max} 365 nm) of *trans*-RSV in air-equilibrated aqueous solution ([*trans*-RSV]$_0$ = 45 μM, pH = 6.0). a) Time evolution of the absorption spectra. Spectra were recorded at 0, 0.25, 0.50, 1, 5, 10, 15, 20 25, 30 and 35 min; optical path length = 10 mm. Arrows indicate the changes observed. b) Evolution of *trans*-RSV (•) and *cis*-RSV (▼) concentrations as a function of irradiation time. Squares (■) represents the algebraic addition of *trans*-RSV and *cis*-RSV concentrations for each time.

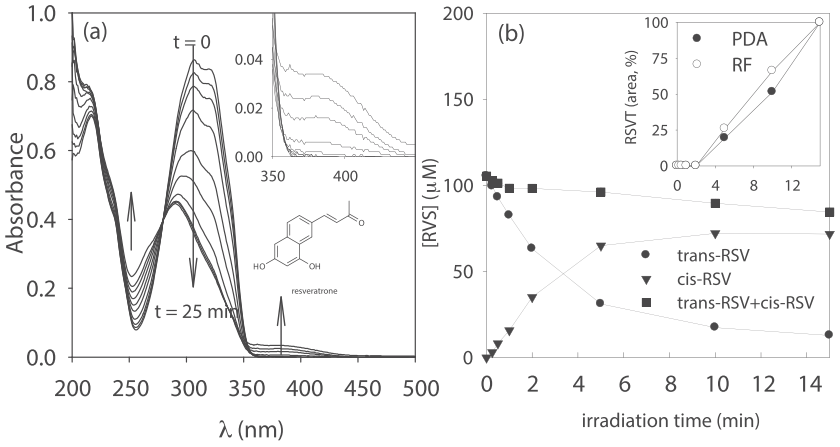

Fig. 3. UV-A irradiation (λ_{max} 365 nm) of *trans*-RSV in air-equilibrated ethanolic solution. a) Time evolution of the absorption spectra. Spectra were recorded at 0, 0.25, 0.50, 1, 5, 10, 15, 20 and 25 min. [*trans*-RSV]$_0$ = 50 μM, optical path length = 4 mm. Arrows indicate the changes observed. b) Evolution of: a) *trans*-RSV (●), *cis*-RSV (▼) concentrations as a function of irradiation time. Squares (■) represents the algebraic addition of *trans*-RSV + *cis*-RSV (■) concentrations for each time; [*trans*-RSV]$_0$ = 50 μM. Inset: Time evolution of the area of the peak corresponding to RSVT. RSVT was detected both in absorption (●,λ = 400 nm) and emission (○,λ_{EXC} = 380 nm, λ_{EM} = 550 nm) mode.

3.1.2. Ethanolic solutions of trans-RSV

Identical experiments were performed in ethanolic solutions. Spectral changes similar to those observed for the photolysis in H_2O were observed, but after some few seconds an increment of the absorbance in the range 350–450 nm was also observed (Fig. 3a), indicating that in this case isomerization was not the exclusive process. The concentration profiles of both *trans*-RSV and *cis*-RSV were determined by HPLC and revealed that the total concentration of RSV ([*trans*-RSV] + [*cis*-RSV]) decreased as a function of irradiation time (Fig. 3b).

In agreement with this fact, after a few minutes of irradiation, a new product with a t_R value longer than those corresponding to *trans*-RSV and *cis*-RSV was observed. This compound has an absorption spectrum with a lower energy absorption band centred on 400 nm, which explains the absorbance above 350 nm observed in the irradiated solutions (Fig. 3a). In addition, this compound presents fluorescence and was detected with the RF detector upon excitation at 380 nm. Other minor peaks were also detected. From kinetic analyses of the integrated area of the chromatographic peak of the new fluorescent compound, registered using both PDA and RF detectors, it was observed that its production started after some few minutes, when *cis*-RSV was accumulated in the solution, indicating that this product is generated from *cis*-RSV.

In the UPLC-MS analyses, non irradiated solutions showed the signal corresponding to the intact molecular ion of RSV. In irradiated solutions, three peaks presented the molecular weight corresponding to RSV (*m/z* = 227), two of them at t_R corresponding to *trans*-RSV and *cis*-RSV. In accordance with HPLC analysis the other product presented a t_R value higher than those corresponding to *trans*-RSV and *cis*-RSV.

Taking into account all these characteristics observed for the fluorescent product (absorption and emission spectra and the molecular weight), this compound was identified as (*E*)-4-(6,8-dihydroxynaphthalen-2-yl)but-3-en-2-one or resveratrone (RSVT), which has been previously characterized [13]. Our results point out that UV-A irradiation of *cis*-RSV leads to the formation of RSVT, and that this photochemical reaction takes place in ethanol, but not in water.

3.2. Steady state UV-B irradiation

3.2.1. Aqueous solutions of trans-RSV

During UV-B irradiation of *trans*-RSV aqueous solution, spectral absorption changes different from those corresponding to isomerization were observed (Fig. 4a) and a slightly increase in the absorption above 360 nm was detected. Accordingly, apart from those corresponding to *trans*-RSV and *cis*-RSV, additional peaks were observed in the HPLC chromatograms of irradiated solutions. The concentration of RSV isomeric forms were measured by HPLC, and it was observed that, as expected, *trans*-RSV concentration decreases and *cis*-RSV increased. However, after a few seconds this compound also decreases (Inset Fig. 4b).

These results clearly indicates that under UV−B radiation *trans*-RSV isomerization is faster than under UV−A radiation, due to higher absorption coefficients, and is also clear that *cis*-RSV is unstable. In addition, it was also observed that RSVT, with the typical band centred at around 400 nm formed in ethanolic solutions exposed to UV−A radiation, it is not formed in *trans*-RSV aqueous solution under UV−B radiation (higher energy). Additionally, no fluorescence emission was detected when irradiated solutions were excited at wavelengths higher to 380 nm.

In the UPLC-MS analyses, non irradiated solutions showed the peak corresponding to the intact molecular ion of RSV. In irradiated solutions, four main peaks were registered, only two of them having molecular weights of RSV, at t_R corresponding to *trans*-RSV and *cis*-RSV. The other two peaks, at higher t_R, showed $[M-H]^-$ signals at *m/z* 239.0345 and 225.0558. These compounds are consistent with the molecular compositions $C_{14}H_{10}O_3$ and $C_{14}H_{10}O_5$, respectively, and were previously reported [19].

3.2.2. Ethanolic solutions of trans-RSV

When an ethanolic solution of *trans*-RSV was irradiated with UV-B, changes in the absorption spectra were much faster than those observed in the all other conditions (Fig. 5a), and clearly the absorption above 400 nm increased with irradiation time (Fig. 5a, inset). HPLC analysis showed that indeed *trans*-RSV consumption was very fast, and no more *trans*-RSV was detected after 5 min of irradiation (Fig. 5b). Moreover, *cis*-RSV was formed in the first seconds, and immediately started its degradation, and it was almost completely consumed at 5 min of irradiation (Fig. 5b). In addition, it was observed that RVST was generated after the accumulation of *cis*-RSV (Fig. 5, inset). Other minor peaks were detected at higher t_R values.

In the UPLC-MS analyses, non irradiated solutions showed again the signal corresponding to the intact molecular ion of RSV. In irradiated solutions, four main peaks were registered, three of them having molecular weights of RSV (m.w. 228), at t_R corresponding to *trans*-RSV, *cis*-RSV and to the previous compound characterized as RSVT. The other peaks, at higher t_R, showed $[M-H]^-$ signal at *m/z* 225.0555 and 239.0345, respectively.

3.3. Fluorescence of irradiated solutions

3.3.1. Fluorescence characterization

UV irradiated ethanolic solutions of *trans*-RSV presented strong fluorescence emission when were exposed to radiation of wavelengths higher than 380 nm. This fluorescence increased with the irradiation time, and the fluorescence emission spectrum was registered,

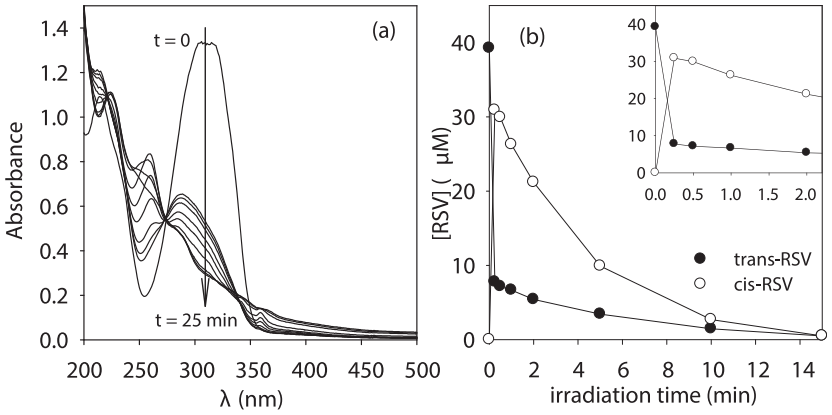

Fig. 4. UV-B irradiation (λ_{max} 300 nm) of *trans*-RSV in air-equilibrated aqueous solution (pH = 6.0). a) Time evolution of the absorption spectra. Spectra were recorded at 0, 0.25, 0.50, 1, 5, 10, 15, 20 and 25 min. [*trans*-RSV]$_0$ = 45 μM, optical path length = 10 mm. Arrows indicate the changes observed. b) Evolution of *trans*-RSV and *cis*-RSV concentrations as a function of irradiation time, [*trans*-RSV]$_0$ = 45 μM. Inset: detail of the first 2 min of irradiation.

presenting a maximum at 535 nm (Fig. 6a). All registered fluorescence spectra were normalized relative to the maximum emission value for comparative purposes (Fig. S1, Supplementary material), and they remained unchanged, suggesting that only one excited state contributes to the fluorescence.

3.3.2. Water-ethanol solutions

The exposure of RSV to UV-A wavelengths, which is the most abundant UV radiation in the solar electromagnetic spectrum, generates RSVT. These compound is a chromophore with absorption in the UV-A and visible region and presents strong fluorescent emission. These characteristics make RSVT a potential photosensitizing agent. The formation of RSVT was evaluated in solutions prepared with a mixture of water-ethanol at different ratio of both solvents. The observed increment in the fluorescence during irradiation is always lower than in 100% ethanolic solutions being the rate in the fluorescence generation proportional to the amount of EtOH, and negligible when the solvent is 100% H_2O.

4. Conclusion

Under UV irradiation *trans*-RSV isomerizes to *cis*-RSV independently on the wavelength and on the solvent (water or ethanol). Under UV-A radiation, isomerization is the unique reaction in aqueous solution, but in ethanol solutions a product of *cis*-RSV photodegradation was detected and identified as RSVT. Under UV-B radiation, photodegradation of RSV was observed besides photoisomerization, both in aqueous and ethanolic solutions, but the nature of the products depends on the solvent. In aqueous solution, two main products were detected with molecular weights of 240 Da and 226 Da. In ethanolic solutions, at least three products were detected, one a strong fluorescent product with molecular weight of 228 Da, which was previously identified as RSVT, and the products observed in water solutions (240 Da and 226 Da). These products that were detected both in UV-B irradiated aqueous and ethanolic solutions, are produce with higher efficiency in ethanolic solutions.

These results demonstrated that RSV is photosensitive and, depending on the solvent and on the irradiation wavelength, a product with absorption in the visible region is formed, that may be a potential photosensitizer and responsible of RSV prooxidant properties previously observed.

Acknowledgements

The present work was partially supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Grant PIP 2013-0304), Agencia de Promoción Científica y Tecnológica (ANPCyT-Grant PICT-2012–0508 and PICT-2015–1988), Universidad Nacional de La Plata (UNLP-Grant X712). The authors thank the Centre National de la Recherche Scientifique (CNRS) and CONICET for supporting their collaboration through a Programme de Coopération Scientifique (CONICET-CNRS/PICS No. 05920). M.G.T. thanks CONICET for postdoctoral research fellowships. A.H.T. and C.L. are research members of CONICET. The authors also thank Nathalie Martins-Froment of the Service Commun de Spectrométrie de Masse (FR2599), Universitè de Toulouse III (Paul Sabatier) for their valuable help with the mass spectrometry measurements.

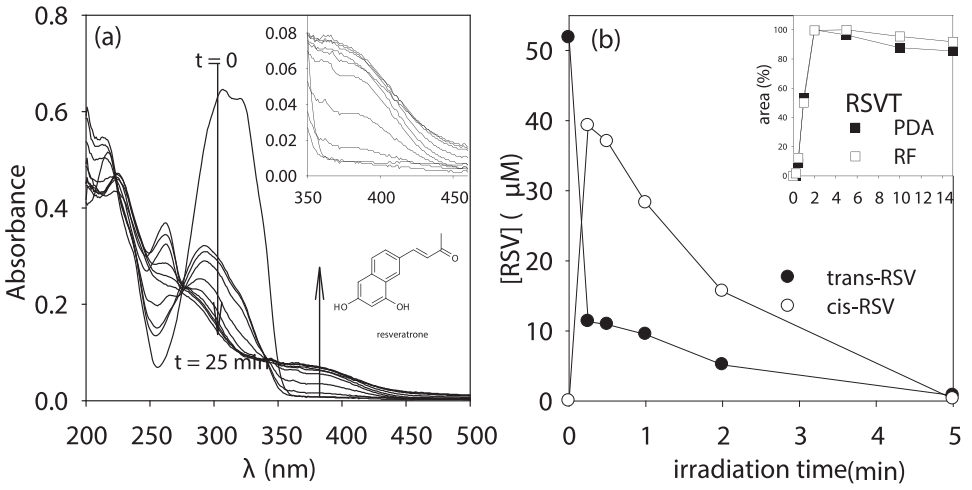

Fig. 5. UV-B irradiation (λ_{max} 300 nm) of *trans*-RSV in air-equilibrated ethanolic solution ([*trans*-RSV]$_0$ = 54 μM). a) Time evolution of the absorption spectra.Spectra were recorded at 0, 0.25, 0.50, 1, 5, 10, 15, 20 and 25 min. Optical path length = 4 mm. Arrows indicate the changes observed. b) Evolution of: *trans*-RSV (●) and *cis*-RSV (▼) concentrations, as a function of irradiation time. Inset: : Time evolution of the area of the peak corresponding to RSVT. RSVT was detected both in absorption (λ = 400 nm) and emission (λ_{EXC} = 380 nm, λ_{EM} = 550 nm) mode.

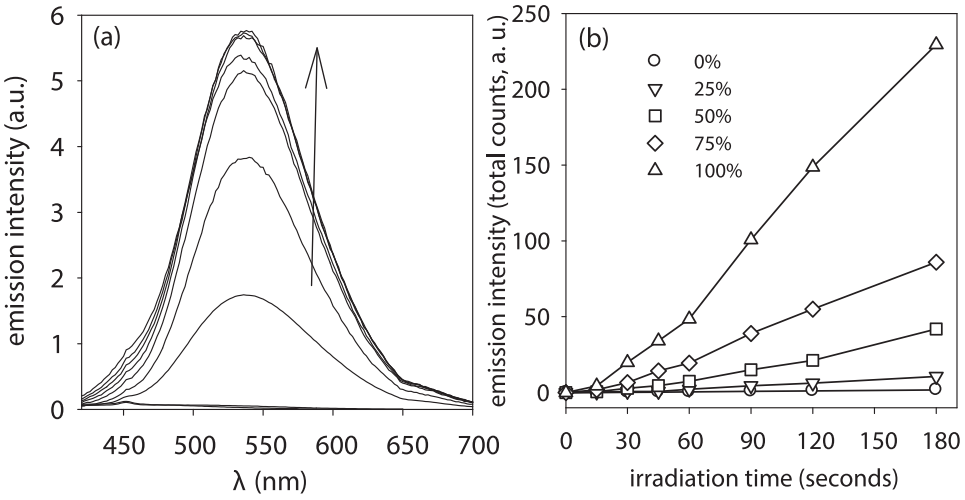

Fig. 6. a) Evolution of fluorescence spectra with irradiation at 300 nm. (b) Evolution of integrated fluorescence intensities as a function of irradiation time of solutions containing different proportion of ethanol (fluorescence intensities were calculated integrating the corresponding spectra; spectra were registered between 400 and 700 nm, λ_{EXC} = 390 nm).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jphotochem.2018.08.050.

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