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ORIGINAL PAPER

Activation Energy of Light Induced Isomerization of Resveratrol

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Abstract Isomerization of *trans*-stilbenes is known to be induced by light. The two isomers have distinct absorption, fluorescence excitation and emission spectra. Resveratrol, 3,4′,5-trihydroxystilbene, is a member of the stilbene family. The interest of the scientific community in resveratrol has increased over the last years due to its biomedical properties. Whereas there is a growing confidence that *trans*-resveratrol is non-toxic, very little is known about the pharmacology of *cis*-resveratrol. Of this very reason there is considerable interest in knowing the energetics of the *trans-cis* conversion. *Cis*-resveratrol is characterized by a large fluorescence quantum yield when compared to *trans*-resveratrol. In the present paper we report a detailed analysis of the spectral changes induced in *trans*-resveratrol upon 260 nm excitation for different time periods. Spectral changes have been monitored with UV-visible absorption and steady-state fluorescence spectroscopy at pH 4 at 20, 25, 30, 35, 40, 45 and 50 °C. Continuous 260 nm excitation induces a blue shift in the absorption and fluorescence excitation spectra of resveratrol and a 14 nm blue shift in its fluorescence emission. The photoisomerization yield is reported as a function of 260 nm excitation time. 330 min continuous excitation led to ~60% isomerization yield. The kinetics of *trans-cis* isomerization has been monitored following the increase in fluorescence quantum yield upon continuous 260 nm excitation of *trans*-resveratrol. The study was carried out at the above mentioned temperatures in order to obtain the Arrhenius activation energy of photoisomerization. Activation energy and pre-exponential factor were 3.7±0.3 kcal. mol^{-1} and 10.6±1.6 s^{-1}, respectively. The activation energy is comparable with previously reported values for the photoisomerization of other stilbenes.

Keywords Resveratrol · *Trans* · *Cis* · Isomerization · Photoisomerization · Activation energy

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Introduction

Phenolic compounds, especially flavonoids and stilbenes have been recognized as being responsible for several beneficial physiological effects owing to their potent antioxidant and anti-inflammatory properties [1–4]. Resveratrol (3,4′,5-trihydroxystilbene, Fig. 1) is a member of the stilbene family, a group of compounds that consist of 2 aromatic rings joined by a methylene bridge. It is produced by plants in response to fungal infection or abiotic stresses, e.g. induced by heavy metal ions. Resveratrol is the parent molecule of viniferins, a family of phytoalexin polymers

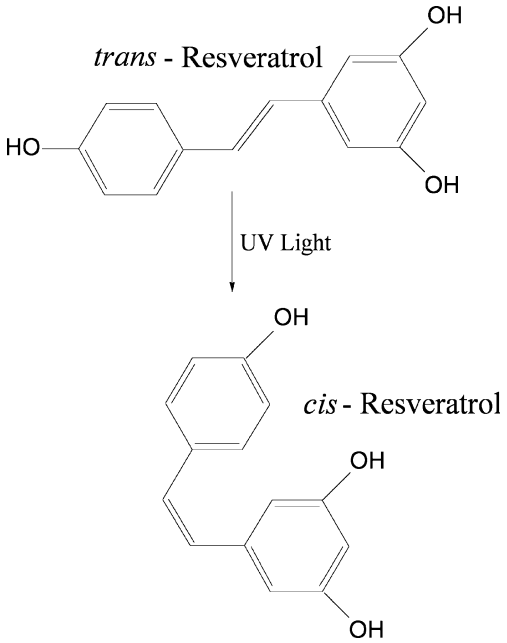

Fig. 1 Chemical structures of *trans* and *cis*-resveratrol

that prevent the progression of fungal infections [5, 6]. Resveratrol, an antioxidant, occurs naturally in mulberries, peanuts and grapes [7]. Grapes contain a large amount of different phenolic compounds in the skin and seeds that are partially extracted during winemaking [6]. The interest of the scientific community in resveratrol has increased over the last years and was originally sparked by studies indicating an inverse relationship between moderate wine consumption and risk of coronary heart disease, the so called "French Paradox" [8] and by the fact that cancer preventive properties of resveratrol were observed [9]. Numerous studies describe potential mechanisms by which *trans*-resveratrol could reduce heart disease, inhibiting platelet aggregation [10–13] and low density lipoprotein oxidation [14] and protecting the liver from lipid peroxidation [15]. *Trans*-resveratrol has received attention in recent years owing to its capacity to protect against global cerebral ischemic injury, to ameliorate oxidative damage and to inhibit cellular events associated with tumour initiation, promotion and progression [9]. Several reviews have been published on the major biological activities of resveratrol [16–21].

Resveratrol exists in two isoforms, *cis*- and *trans*-resveratrol (and their glycosidic forms), which may have different biological effects [22–25], the later being most widely studied. *Cis*-resveratrol may also have health promoting properties [26] although being less biologically active. Solar and UV irradiation of *trans*-resveratrol solutions induces partial isomeric conversion into the highly fluorescent *cis*-resveratrol [27–29], resulting in a mixture of *cis*- and *trans*-resveratrol [8, 28, 30–32]. *Trans*-resveratrol is reported to remain stable for several months (except in high pH buffers) when completely protected from light [31]. Previous studies were carried out at 254 nm and 366 nm excitation in order to obtain *cis*-resveratrol from the *trans* form [31]. *Trans*-resveratrol has a two absorption peaks centered at 306 nm and 319 nm in water, ethanol and hydroethanolic media at acid and neutral pH. Ethanol has no influence on the absorption signal [6]. *Trans*-resveratrol has a broad single emission peak in ethanol centered at 394 nm and excitation centered at 300 nm [33]. *Cis*-resveratrol has an excitation peak at 260 nm and two resolved emission peaks centered at 364 nm and 382 nm in 40% v/v hydroethanolic solutions [6].

According to Dugave et al. [34], there are different pathways leading to *trans-cis* isomerization: double bond breakage by radicals, direct photoisomerization or isomerization catalyzed by paramagnetic molecules and thermal isomerization. It is usually accepted that photoisomerization of ethylenes takes place *via* triplet excited state [35]. Several studies have been carried out in order to determine the activation energy of *trans-cis* photoisomerization of stilbenes [36–38]. It has been demonstrated that *cis*-resveratrol identified in certain wines can only result from a photochemical reaction and not from thermal equilibration [39].

In the present study, photo induced isomerization of *trans*-resveratrol has been carried out upon continuous excitation at 260 nm over 330 min. Spectral changes have been monitored with UV-Visible absorption and steady-state fluorescence spectroscopy at pH 4 at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. The kinetics of *trans-cis* photoisomerization has been obtained upon monitoring the increase in fluorescence quantum yield of resveratrol upon continuous excitation. The kinetic run was carried out at the above mentioned temperatures in order to obtain the Arrhenius activation energy of the photoisomerization. The obtained activation energy is compared with previously reported values for photoisomerization of other stilbenes.

Materials and Methods

Resveratrol Solution

Resveratrol was purchased from Sigma-Aldrich (99% purity, 038 K5202). A 5.7 μM stock solution was prepared in sodium acetate buffer (100 mM) at pH 4, using the absorption coefficient of *trans*-resveratrol at 304 nm of 30335 M^{-1} cm^{-1} [40]. Sodium acetate was purchased from Sigma. The stock solution was stored at 4 °C and protected from light.

Absorption and Fluorescence Spectroscopy

The absorption intensity measurements were carried out in a UV-Visible spectrophotometer (UV 1 VWR International – Thermo Electron Corporation). 1 cm path length quartz cuvette was used. Absorption measurements were carried out between 250 nm and 350 nm. 1 cm path length quartz cuvette was used.

Fluorescence Quantum Yield of Resveratrol as a Function of 260 nm Excitation Time

Steady-State Emission and Excitation Spectra, Absorption Measurements

The effects of 260 nm continuous illumination of resveratrol were probed by monitoring the time-dependent fluorescence emission intensity at 395 nm of resveratrol upon 260 nm excitation. Lamp power at the sample location was 81 nW. The run was carried out at seven different temperatures: 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. The fluorescent kinetic traces have been carried out at different temperature in order to obtain the Arrhenius activation energy of the 260 nm light induced *trans-cis* isomerization of resveratrol.

3 ml of 5.7 μM *trans*-resveratrol stock solution were placed in a quartz cuvette (1 cm path length) and excited at 260 nm. A fresh sample was used for each time based study. Fluorescence intensity measurements were carried out in a RTC 2000 Photon Technology International (PTI) fluorescence spectrometer with T-configuration, using a 75 W Xenon arc lamp coupled to a monochromator. Temperature was controlled externally by a Peltier element and kept constant until the end of the experiment. Samples were magnetically stirred at 900 rpm in order to secure homogeneous excitation. Real-time correction was enabled in order to correct for oscillations in lamp intensity (gain set at 2 V). Prior and after each illumination, emission and excitation spectra were recorded. Emission spectra were acquired upon 260 nm excitation. Emission was fixed at 396 nm while acquiring the excitation spectra. The same emission and excitation spectra were acquired for the buffer. Raman signal was removed by subtracting the buffer spectrum from each emission spectrum. All slits were kept at 5 nm during excitation, emission and time based measurements.

A second timebased experiment was carried out with the exact same settings described above, however not in a continuous illumination mode. The resveratrol sample was excited at 260 nm for discrete illumination times: 1 min, 2 min, 3 min, 4 min, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, 270 min, 300 min and 330 min. Absorption, fluorescence emission and excitation spectra were acquired after each illumination period, using the same settings described above.

Data Analysis

The ratios of isomers were calculated according to [40]:

$$C_{trans} = \frac{1}{R-1} \cdot \left(\frac{R \cdot A}{l \cdot \varepsilon_{trans}} - C_0 \right) \quad (1)$$

$$C_{cis} = \frac{1}{1-R} \cdot \left(\frac{A}{l \cdot \varepsilon_{cis}} - R \cdot C_0 \right) \quad (2)$$

where $R = \varepsilon_{\text{trans,304 nm}} / \varepsilon_{\text{cis,304 nm}} = 3.19$ at 304 nm, C_{trans} and C_{cis} are the concentrations of *trans*- and *cis*-resveratrol, respectively. ε_{trans} and ε_{cis} are the molar extinction coefficients of *trans* and *cis*-resveratrol, respectively. C_0 is the initial concentration of *trans*-resveratrol, A is the measured absorbance and l is the optical pathlength [40].

All absorption, fluorescence excitation and emission spectra were smoothed in Origin Pro 8 Lab with Adjacent-Averaging method using 7 points average. Normalized emission and excitation spectra were obtained by dividing each data point by the maximum intensity value in each spectrum. Normalized time-based fluorescence measurements were obtained by dividing each data point (emission intensity) by the time zero emission.

Each timebased fluorescence kinetic trace obtained at a specific temperature was fitted with a Boltzmann equation $F(t) = \frac{A_2 + (A_1 - A_2)}{1 + \exp((\chi - \chi_0)/d\chi)}$ in order to obtain the inflexion point of each curve. Normalized fluorescence kinetic traces obtained upon 260 nm excitation (396 nm emission) at different temperatures were fitted in MatLab R2009b using the Exponential Growth 1 model $F(t) = y_0 + A_1 e^{k \cdot t}$, where F (t) is the fluorescence intensity measured at time t, y_0 and A_1 are constants and k the rate constant of exponential increase. The first 1000 s were excluded while fitting all kinetic traces. Fittings were carried out from 1000 s till the time corresponding to 75% of the time correspondent to each curve inflection point, since in this region a clear linearity is observed between the log F (t) and time. Data fitting was done in MatLab and plotting was done in Origin 8.0. The logarithm of the each kinetic rate (ln k) obtained upon fitting each temperature specific timebased run has been plotted against T^{-1} (in Kelvin^{-1}) in order to determine the activation energy of the light induced *trans-cis* isomerization of resveratrol. The errors associated with ln k are also calculated and used as weighing instrumental factors while fitting the data with a linear model $\ln k = \ln A_0 - \frac{E_a}{R} \frac{1}{T}$.

Results

Figure 2a is displayed the absorption intensity spectra of resveratrol at 20 °C before (*trans*-resveratrol) and after 260 nm excitation for different illumination times: 1 min, 5 min, 10 min, 30 min, 90 min, 150 min, 210 min, 270 min, and 330 min. The absorption intensity of *trans*-resveratrol (non-illuminated sample) peaks at 316 nm. It can be observed that 330 min excitation at 260 nm leads to a 54% decrease in absorption intensity at 316 nm and to a 49.8% increase at 260 nm. In Fig. 2b are displayed the normalized absorption spectra of resveratrol before (*trans*-resveratrol) and after 260 nm excitation for different illumination times: 5 min, 30 min, 150 min, 270 min, and 330 min. It can be observed that 260 nm excitation of *trans*-resveratrol during 330 min leads to a 14 nm blue shift. A 10 nm blue shift is observed immediately after 1 min excitation (Fig. 2a, Table 1). In Fig. 2c is displayed the concentration of *trans* and *cis*-resveratrol present in solution after 260 nm excitation for different time periods. Prior to 260 nm illumination only *trans*-resveratrol is present. After 150 min of 260 nm illumination (81nW), 50.9% of the initial concentration of *trans*-resveratrol is converted into *cis*-resveratrol. After 330 min of illumination, the concentration of *cis*-resveratrol (3.4 μM) is larger than of trans-resveratrol (2.3 μM, Table 2), corresponding to a 59.6% isomerization of *trans*-resveratrol. No spectral shift is observed in the normalized absorption spectra of non-illuminated fresh *trans*-resveratrol samples acquired at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C (data not shown).

In Fig. 3a is displayed the excitation spectra at 20 °C (emission at 396 nm, characteristic emission peak of *trans*-resveratrol) of *trans*-resveratrol before and after 260 nm excitation for different illumination times: 15 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, and 330 min. *Trans*-resveratrol is characterized by a broad excitation single peak centered at 319 nm. UV illumination leads to a decrease of the intensity at 319 nm and to new excitation peaks centered at 261 nm, 341 nm, 359 nm. Excitation intensity between 278–310 nm has also increased with excitation time. In Fig. 3b is displayed a detailed evolution of the excitation spectra (emission at 396 nm) of resveratrol during the first 30 min of continuous 260 nm illumination. It can be observed that 260 nm illumination leads to a continuous decrease of the excitation

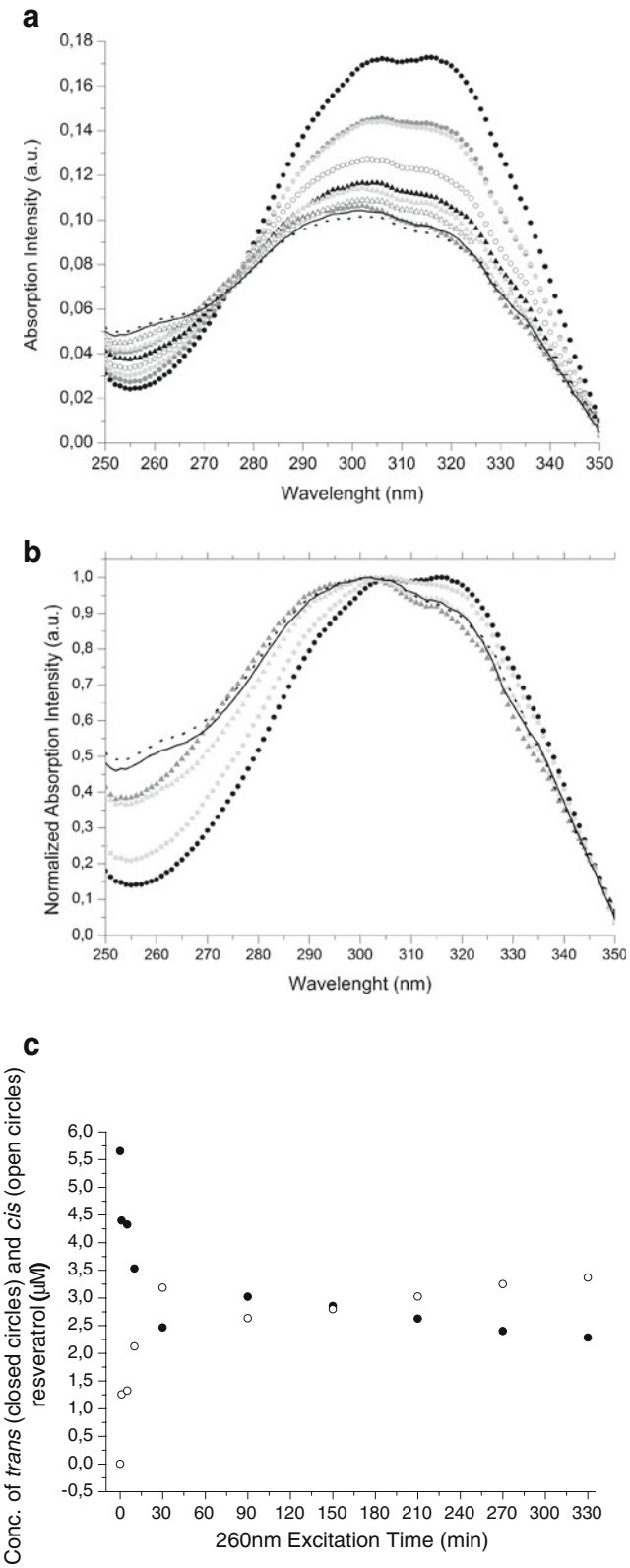

Fig. 2 **a** Absorption intensity spectra of resveratrol before (●, *trans*-resveratrol) and after 260 nm illumination for different illumination times: 1 min (●), 5 min (●), 10 min (○), 30 min (▲), 90 min (▲), 150 min (▲), 210 min (Δ), 270 min (−), 330 min (···). **b** Normalized absorption spectra of resveratrol before (●, *trans*-resveratrol) and after 260 nm illumination for different illumination times: 5 min (●), 30 min (▲), 150 min (▲), 270 min (−), 330 min (···). 260 nm illumination of *trans*-resveratrol leads to a 10 nm blue shift immediately after 1 min (see Table 1). **c** Concentration (μM) of *trans*-(closed circles) and *cis*- (open circles) resveratrol after 260 nm illumination for different time periods (see Table 2)

Table 1 Blue shift observed in the fluorescence emission of resveratrol after excitation at 260 nm for 0 min, 1 min, 5 min, 30 min, 150 min, 270 min and 330 min (Fig. 2b)

Time (min)	Δλ (nm)
0	0
1	10
5	10
30	14
150	14
270	14
330	14

intensity at 319 nm. Relative to time zero, excitation intensity at 319 nm has decreased 12.5%, 27.6%, 31.9%, 38.7%, 38.9%, and 43.8% after 5 min, 10 min, 15 min, 20 min, 25 min and 30 min excitation, respectively. The appearance of the characteristic *cis*-resveratrol excitation peak at 261 nm can be seen after 10–15 min excitation at 260 nm. No spectral shift is observed in the normalized fluorescence excitation spectra of non-illuminated fresh *trans*-resveratrol samples acquired at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C (data not shown).

In Fig. 4a is displayed the fluorescence emission spectra at 20 °C of resveratrol before (*trans*-resveratrol) and after 260 nm illumination for different time periods: 15 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, and 330 min. It can be observed that 260 nm illumination of resveratrol leads to the appearance of two new emission peaks centered at 364 nm and 381 nm, typical fluorescence emission peaks of *cis*-resveratrol [6]. As an insert, is shown the kinetic trace relative to the increase of the fluorescence at 364 nm and 381 nm as a function of 260 nm illumination time. It can be observed that the fluorescence intensity of both peaks intensity increases with the same rate. In Fig. 4b is displayed the evolution of the emission spectra (exc. 260 nm) of resveratrol during the first 30 min of continuous 260 nm excitation: 0 min, 5 min, 10 min; 15 min, 20 min, 25 min,

Table 2 Concentration of *trans*- and *cis*-resveratrol as a function of 260 nm illumination time. The concentrations have been calculated using formulas (1) and (2) described in the Methods section

Time (min)	Abs λ_{304nm}	C_{trans} (μM)	C_{cis} (μM)
	0,171	5,7	0,0
1	0,145	4,4	1,3
5	0,144	4,3	1,3
10	0,127	3,5	2,1
30	0,105	2,5	3,2
90	0,117	3,0	2,6
150	0,113	2,9	2,8
210	0,108	2,6	3,0
270	0,104	2,4	3,3
330	0,101	2,3	3,4

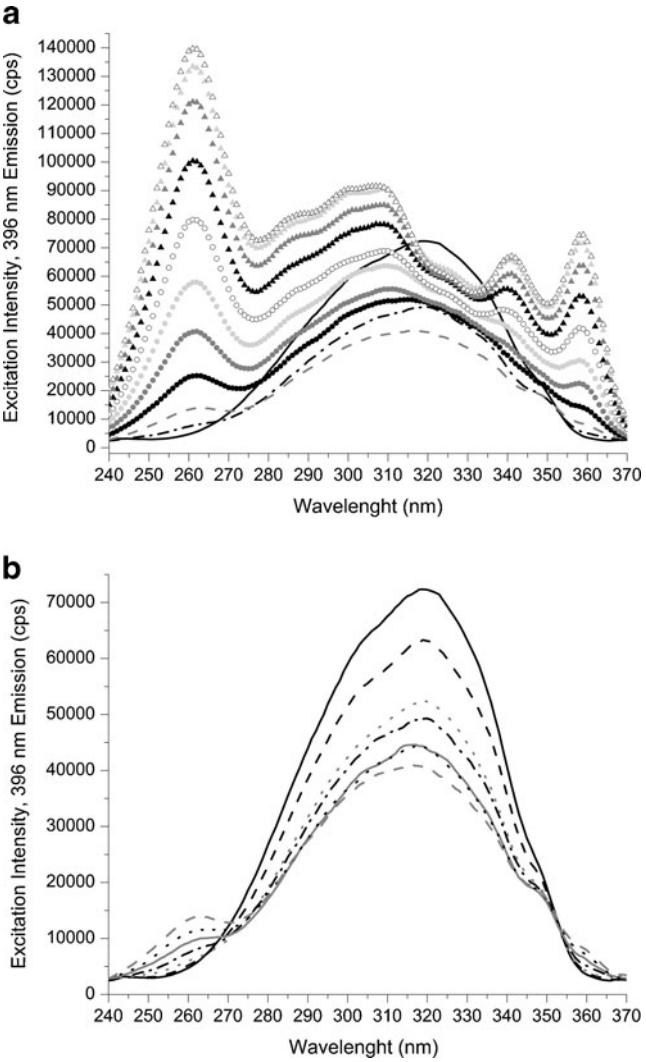

Fig. 3 **a** Excitation spectra (emission at 396 nm) of resveratrol before (–, *trans*-resveratrol) and after 260 nm illumination for different illumination times: 15 min (– · –), 30 min (–), 60 min (●), 90 min (●), 120 min (●), 150 min (○), 180 min (▲), 210 min (▲), 240 min (▲), 330 min (Δ). **b** Detailed evolution of the excitation spectra (emission at 396 nm) of resveratrol during the first 30 min of continuous 260 nm excitation: 0 min (–, trans-resveratrol), 5 min (– –), 10 min (···); 15 min (– · –), 20 min (–), 25 min (···), 30 min (– –)

and 30 min. *Trans*-resveratrol fluorescence emission is characterized by a single, low intensity broad peak centered at 395 nm. 260 nm illumination lead to the appearance of two emission peaks at 364 nm and 381 nm immediately after 5 min. The fluorescence intensity at 364 nm and 381 nm increases continuously during continuous 260 nm illumination. As an insert, is displayed the normalized fluorescence emission spectra (exc. 260 nm) before and after 30 min and 330 min illumination time. After illumination, the fluorescence peak is 14 nm blue shifted when compared to the non-illuminated sample. No spectral shift is observed in the normalized fluorescence emission spectra of non-illuminated fresh *trans*-resveratrol samples

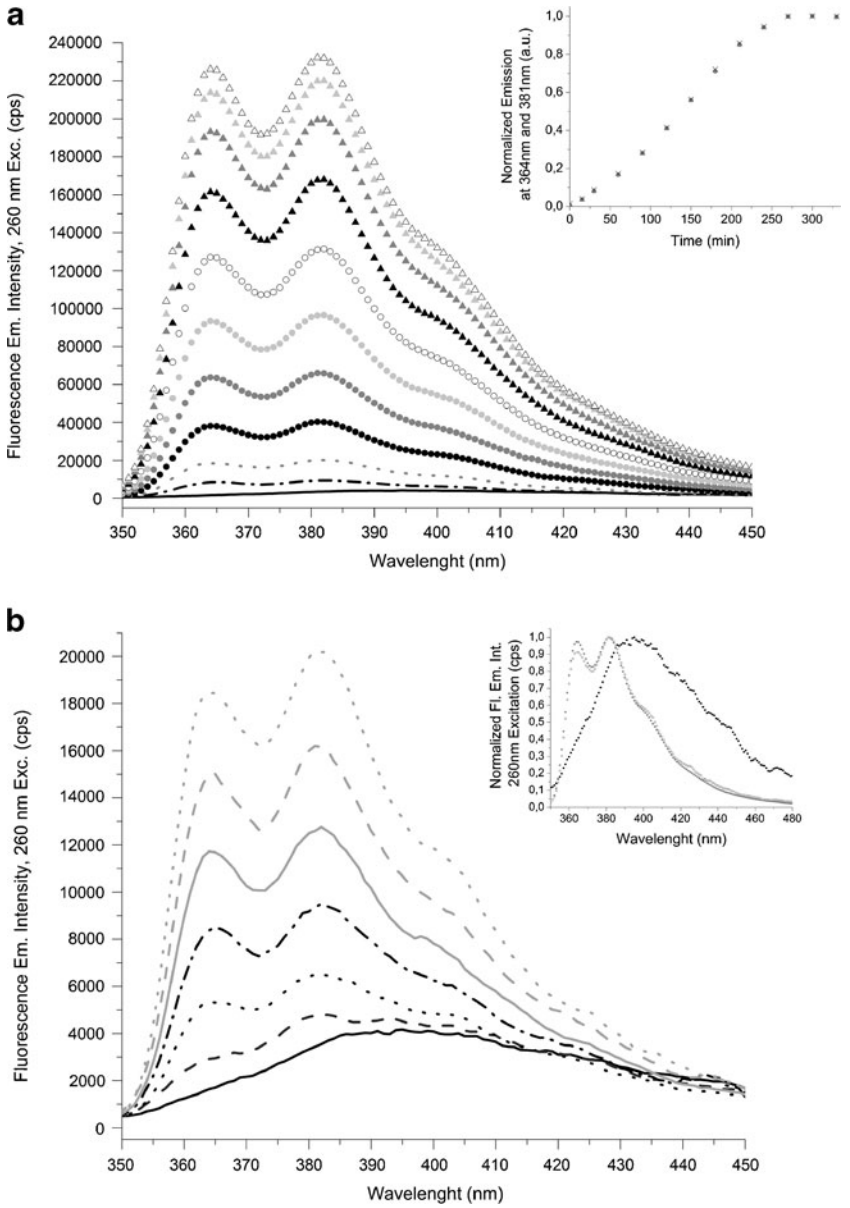

Fig. 4 **a** Emission spectra (excitation at 260 nm) of resveratrol before (−, *trans*-resveratrol) and after 260 nm illumination for different illumination times: 15 min (− · −), 30 min (···), 60 min (●), 90 min (●), 120 min (●), 150 min (○), 180 min (▲), 210 min (▲), 240 min (▲), 330 min (Δ). 260 nm illumination of resveratrol leads to the appearance of two new emission peaks centered at 364 nm and 381 nm, typical fluorescence emission peaks of *cis*-resveratrol [32]. As an insert is shown the kinetic trace relative to the increase of the fluorescence at 364 nm (●) and 381 nm (×) as a function of 260 nm excitation time. **b** Detailed evolution of the emission spectra (excitation at 260 nm) of resveratrol during the first 30 min of 260 nm illumination: 0 min (–, *trans*-resveratrol), 5 min (− −), 10 min (···); 15 min (− · −), 20 min (−), 25 min (− −), 30 min (···). As an insert is displayed a normalized fluorescence emission intensity (260 nm excitation) with a 14 nm of blue shift between before (●) and 30 min (○) and 330 min (●) of illumination

acquired at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C (data not shown).

In Fig. 5 is displayed the normalized fluorescence emission (exc. at 260 nm) and excitation spectra (em. at 396 nm) before and after 330 min illumination at 260 nm. A 79 nm Stokes shift is observed before illumination, compared to 120 nm after illumination (relative to the highest intensity peaks).

In Fig. 6a is displayed the 395 nm normalized fluorescence emission intensity of resveratrol as function of 260 nm illumination time at 7 different temperatures: 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C. An initial exponential increase in the fluorescence intensity is observed, followed by a plateau where the fluorescence yield is stable. After the plateau region, the fluorescence quantum yield was observed to drop if we further illuminate

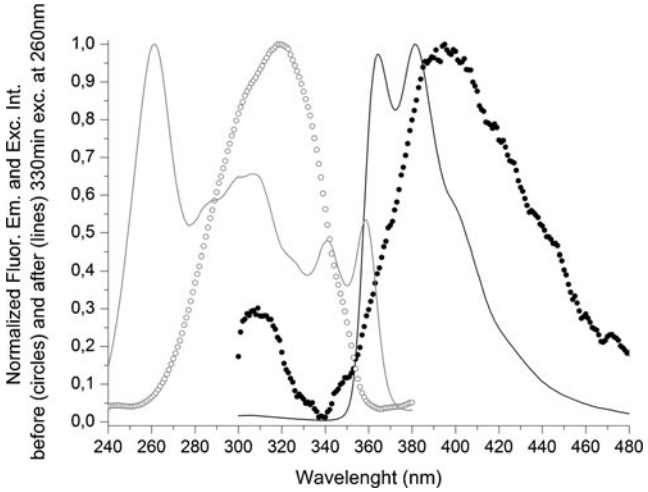

Fig. 5 Normalized fluorescence emission (black) and excitation (grey) intensity before (circles) and after (lines) 330 min illumination at 260 nm, with a Stoke shift of 79 nm and 120 nm, respectively

the sample (data not shown). The rate of fluorescence intensity increase observed is larger at higher temperatures. Fitted kinetic traces (from 1000 s till 75% of the inflection point of each curve, see Methods section) are displayed in Fig. 6b. Fitted parameters and root mean square deviation (R^2) are displayed in Table 3.

In Fig. 7 is displayed the Arrhenius plot of the experimental k values. The observed linear regression shows that k is temperature dependent and follows Arrhenius law (R^2=0.954). Activation energy (E_a) and pre-exponential factor A_0 calculated from the slope ($-Ea/R$) were respectively 3.7±0.3 kcal.mol^{-1} and 10.6±1.6 s^{-1}.

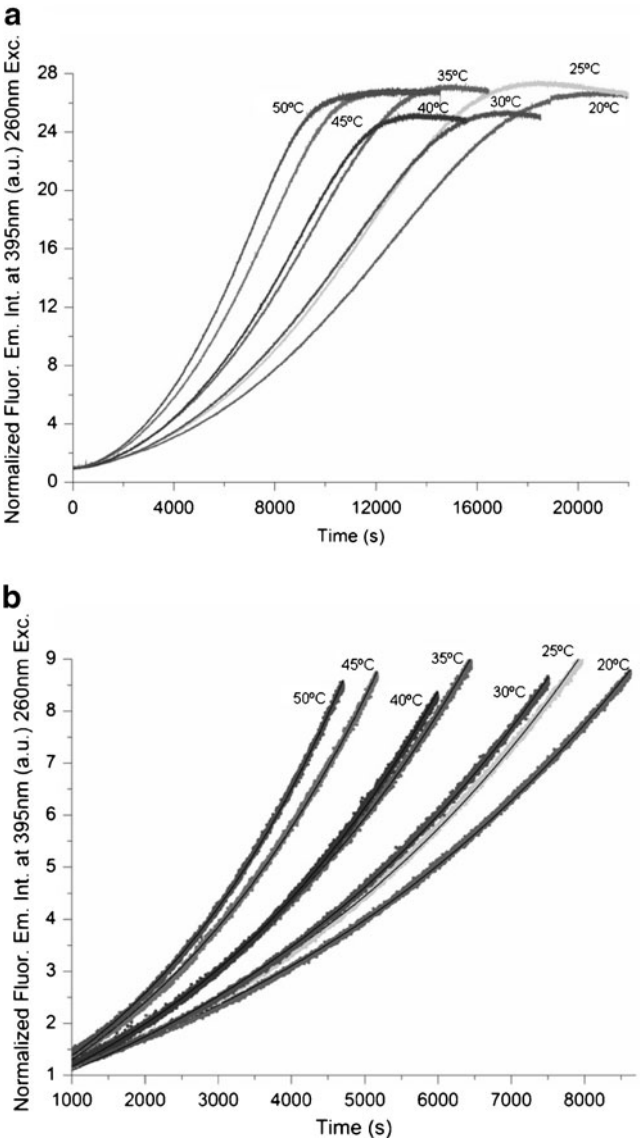

Fig. 6 **a** Normalized 395 nm fluorescence emission intensity of resveratrol as a function of 260 nm illumination time at different temperatures: 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, and 50 °C. **b** Fitting the normalized fluorescence kinetic traces from 1000 s till 75% of the time corresponding to the inflection point of each curve displayed in panel (A) with an exponential growth function $F(t) = y_0 + A_1 e^{x \cdot t}$. Fitted parameter values and correspondent errors and root mean square value obtained after fitting each temperature are displayed in Table 3

Discussion

The activation energy of photo induced isomerization of *trans*-resveratrol upon 260 nm illumination has been investigated and the isomerization yield as a function of excitation time has been quantified. Furthermore, absorption and fluorescence spectral changes have been quantified as a function of excitation time at different temperature. The concentration of resveratrol (5.7 μM) used in the studies carried out at pH 4 secured that no aggregation occurred between resveratrol molecules, in agreement with López-Nicolás et al. [41]. According to that study, aggregation has been observed to occur above 12.5 μM at pH 5.5 and above 37 μM at pH 10.5.

The absorption spectra shown in Fig. 2a reveals that non-illuminated *trans*-resveratrol has two absorption peaks at 304 nm and 316 nm, in agreement with Goldberg et al. [8], Trela et al. [31], Díaz et al. [6] and Liang et al. [42]. Upon 330 min of continuous 260 nm illumination the absorption intensity of resveratrol at 316 nm decreases 54%, while its absorption intensity at 260 nm increases 49.8%. This is due to photoisomerization of *trans*-resveratrol into *cis*-resveratrol. A 59% decrease in absorption intensity at 304 nm after 330 min excitation is hereby observed. Camont et al. reports a 45% decrease after 8 h sunlight exposure [40]. After 330 min illumination at 260 nm, a 14 nm blue shift is observed in the absorption intensity maximum of resveratrol (from 316 nm to 302 nm),

Table 3 Rate constant, pre-exponential factor and intercept recovered from fitting the kinetic traces showing the increase in the fluorescence emission intensity of resveratrol upon continuous 260 nm illumination at different temperatures

Temperature K	Intercept y_0	Pre-exponential factorA_1	Rate constant t_1	R^2
293	−1,77±0,02	2,49±0,00	1,7E-04±4,5E-07	0,9996
298	−1,80±0,02	2,45±0,02	1,9E-04±5,5E-07	0,9996
303	−2,01±0,02	2,57±0,02	1,9E-04±6,5E-07	0,9995
308	−2,09±0,03	2,62±0,02	2,2E-04±8,5E-07	0,9995
313	−1,71±0,03	2,24±0,02	2,5E-04±1,1E-06	0,9994
318	−2.23±0,04	2,68±0,03	2,7E-04±1,5E-06	0,9994
323	−1,83±0,03	2,34±0,03	3,2E-04±1,7E-06	0,9994

as shown in Fig. 2b. Camont et al. [40] reported a 18 nm shift, from 304 nm to 286 nm, upon 8 h exposure to sunlight, although no information is reported neither concerning illumination power levels nor wavelengths.

Equations (1) and (2) used in order to calculate the concentrations of *trans*- and *cis*- resveratrol present after different excitation times at 260 nm assumed that no *cis* isomer is present in the non-illuminated sample, as also reported by Lin et al. [43] and Camont et al. [40]. This assumption is correct since the absorption (Fig. 2a), fluorescence excitation (Fig. 3a) and emission (Fig. 4a) spectra of non-illuminated resveratrol do show the solely presence of *trans*-resveratrol.

Photoisomerization yield of *trans*-resveratrol was 59.6% after 330 min of illumination at 260 nm. Trela et al. [31] reports an isomerization yield of a pure 418 μM *trans*-resveratrol solution (in ethanol) after 366 nm illumination (180 μW) for 100 min and 10 h at 254 nm (750 μW) to be 90.6% and 63%, respectively. According to López-Nicolás et al. [41], resveratrol is already in the aggregation state at such concentrations. The experimental conditions used in our study secured that resveratrol was in its monomeric, non-aggregated form.

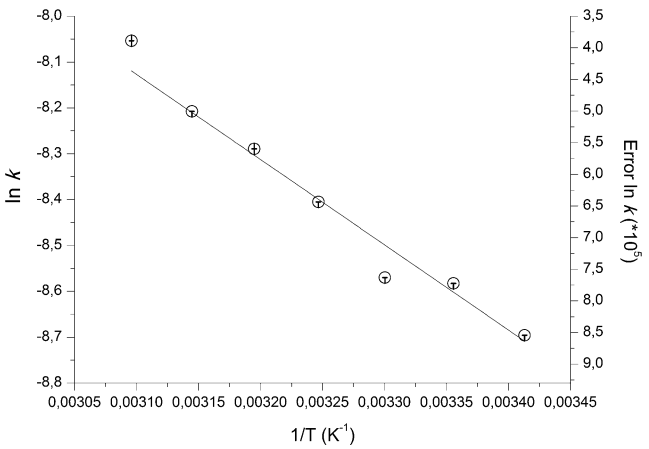

Fig. 7 Arrhenius plot: linear correlation between the logarithm of the rate constant ln k and the inverse of temperature $1/T$ (K^{-1}) (R^2=0.954). Calculated parameter: E_a=3.69±0.33 kcal.mol^{-1}. Uncertainty errors for ln k values are displayed with error bars

The fluorescence excitation spectra displayed in Fig. 3a shows that continuous 260 nm illumination leads to a fluorescence loss at 320 nm and to the appearance of a fluorescence excitation peak at 260 nm, the intensity of which increases upon continuous excitation, in accordance to Díaz et al. [6]. This new peak is the characteristic excitation peak of *cis*-resveratrol, while the single peak at 320 nm is the characteristic fluorescence excitation peak of *trans*-resveratrol in water. López-Nicolás et al. [41] report a single excitation peak of *trans*-resveratrol at 335 nm. Their 30 μM resveratrol solution was prepared in sodium phosphate at pH 4 from a stock solution in ethanol. Continuous excitation of resveratrol leads to a continuous increase in the fluorescence emission intensity at 364 nm and 381 nm (upon 260 nm exc, Fig. 4a and b) in agreement with Díaz et al. [6] who report the same two emission maxima centered at 364 and 382 nm.

The kinetics of fluorescence emission increase at 364 nm and 381 nm displayed as an inset in Fig. 4a shows that the intensity of the two peaks grows with the same kinetics, revealing that these peaks correspond to the same photoproduct, *cis*-resveratrol. The observed Stokes shift in Fig. 5 for *cis*-resveratrol (120 nm) is larger than for *trans*-resveratrol (79 nm). These values are higher than the values reported by Fischer et al. [44] for different stilbenes in a mixture of methylcyclohexane and 3-methylpentane (76 nm for *cis*-stilbene and 67 nm for *trans*-stilbene).

No spectral shifts were observed in the absorption, fluorescence emission and excitation spectra for the non-illuminated *trans*-resveratrol at all seven temperatures (20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C), proving that no thermal isomerization is happening at those temperatures in the dark. Fluorescence emission intensity at 395 nm increases continuously until the plateau, during the 330 min excitation at 260 nm at all temperatures, which is correlated with the fact that *cis*-resveratrol is more fluorescent than *trans*-resveratrol (Fig. 6a). The fluorescence quantum yield of *trans*-resveratrol increases 53.2% with a temperature decrease from 50 °C to 20 °C (data not shown). This expected since collisional quenching between the solvent molecules and resveratrol increases with temperature.

The 3.7±0.3 kcal/mol activation energy of *trans*-resveratrol photoisomerization into the *cis*-form calculated through the Arrhenius plot shown in Fig. 7, is comparable with the value of 3.4 kcal/mol reported by Sumitani et al. [45] for the photoisomerization energy of *trans*-stilbene in hexane. According to Wiemers at al. [36], *trans*-4-stilbenemethanol has a photoisomerization Arrhenius activation energy (exc. 298 nm) of 5.7 kcal/mol in ethanol, 7.9 kcal/mol in propanol and 5.2 kcal/mol in hexane. According to Garner et al. [37], the activation energy of photoisomerization (exc. 436 nm) of *trans*-4-nitro-4′-dimethylaminostilbene in toluene is 10.5 kcal/mol. Steiner et al. [38] showed that the activation energy for *cis-trans* thermal isomerization of 4-nitro-4′-methoxystilbene is 28.8 kcal/mol and 28.6 kcal/mol for 4′-hydroxy-1-methyl-stilbazolium betaine (in methanol). The Arrhenius activation barrier for the thermal isomerization of resveratrol has been reported to be ~280 kJ/mol or ~67 Kcal/mol [39]. Such high value implies that thermal equilibration cannot account for the *cis*-isomer found in nature [39], pointing at photoisomerization as the likely cause for the formation of *cis*-resveratrol.

It has been reported by Courtney and Fleming [46] that the pre-exponential factor, A_0, and the activation energy for the isomerization of stilbene, E_a, changes only slightly from solvent to solvent, concluding that they could extract the barrier height at constant viscosity. The activation energies have been calculated from linear Arrhenius plots obtained for different solvents. Therefore we anticipate that the activation energy for isomerization of our stilbene is only marginally influenced by a temperature induced viscosity change of water (the only solvent used in our study) in the temperature range from 20–50 °C.

Illumination of *trans*-resveratrol induces isomerization of the *trans*-resveratrol pool of molecules, which display very low fluorescence emission intensity, into their *cis*-conformation, which has high fluorescence emission intensity. This chemical conversion between the resveratrol molecules is correlated with an increase in resveratrol fluorescence emission intensity. Thus, the rate of fluorescence emission intensity increase k should be related to the kinetic rate of conversion between the two pools of molecules (Fig. 6b). The logarithm of the kinetic rates plotted as a function of 1/T (Arrhenius plot) resulted in a linear Arrhenius plot from where the E_a was extracted. A linear plot indicates that within experimental error there is only one process occurring. In the temperature range used in our study, Courtney and Fleming also obtained, within experimental error, a linear dependence of ln k vs 1/T [46].

We observed that the photoisomerization of *trans*- to *cis*-resveratrol at pH 4 was irreversible. However, Trela et al. [31] reports that *cis*-resveratrol under identical phosphoric acid buffer conditions is stable at pH 7, less stable at pH 3 and that at pH 1 50% of *cis*-form was isomerized to the *trans*-resveratrol. Whereas there is a growing confidence that *trans*-resveratrol is non-toxic, very little is known about the pharmacology of *cis*-resveratrol. The results presented in this paper can become an important component in analyzing and securing that resveratrol containing pharmaceuticals contains only the *trans* isomer.

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