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PAPER

Theoretical and experimental exploration of the photochemistry of resveratrol: beyond the simple double bond isomerization†

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The photochemical isomerization of resveratrol has been the subject of recent studies in which contradictory results were reported. The photoproduct mixture of this reaction needs to be considered more complex than the coexistence of *cis* and *trans* isomers. An unidentified third product, at least, has been detected in various studies although its nature was unknown. In this work, we aim to provide a thorough description of the photochemical course of this reaction through experimental and computational approaches working in a synergetic association.

Introduction

Resveratrol (3,4′,5-trihydroxystilbene, see Fig. 1) is a natural product that is present in a wide variety of natural or processed foods such as grapes, wine, chocolate, peanuts, tea, soy, *etc.*[1] This biphenolic compound has captivated the attention of the scientific community due to its biological activity and the association of its moderate human consumption with healthy effects.[2]

Resveratrol is mainly produced in the seeds and pulp of plants in response to stressor agents like fungi and bacteria, or environmental pressure (excessive radiation or sudden changes in temperature among other factors). In this sense it can be classified as a phytoalexin.[3] The biological activity of resveratrol is twofold: it is involved in processes related to oxidative stress[4] and has also been reported as a sirtuin activator (*vide infra*).[5]

The biphenolic structure of resveratrol furnishes this natural product with anti-oxidative properties. This activity is however somehow controversial,[6] some studies conclude that resveratrol's exceptional properties are Sir2 dependent[5c] but actually only moderate capabilities can be found experimentally or computationally for resveratrol when compared to other compounds.[6b]

The main mechanism involved in oxidative stress is associated with very reactive oxygen species formed in the lipid peroxidation chain. Resveratrol is capable of stopping this chain reaction *via* the scavenging of peroxyl radicals, although at a lower rate than commonly thought ($k_{inh} = 2.0 \times 10^5$ M^{-1} s^{-1} in chlorobenzene at 303 K).[7] The peroxyl radical formally abstracts a H atom from one of the hydroxyl groups in resveratrol and forms stable radical species.[8] The OH group in the *para* position has been suggested as the most active[9] due to its lower O–H bond dissociation energy. This is also supported by the efficient redistribution of the spin density over the entire molecule once the *para* OH has lost a hydrogen atom to form a radical species.[10] Two main mechanisms have been described for this radical scavenging process that we have summarized in Fig. 2: one consisting of a hydrogen-atom transfer (HAT)[7,8b] and the other being based on a single electron transfer (SET) preceded or followed by a proton loss.[11]

On the other hand, resveratrol, as a sirtuin activator,[5] has been the focus of an important number of investigations due to the relation between sirtuins and lifespan.[12] Sirtuins are a family of proteins present in eukaryotic cells, which use genes to silence regions of DNA. Resveratrol, as an activator of sirtuins, is related to several experiments in lifespan,[13] cell repair and

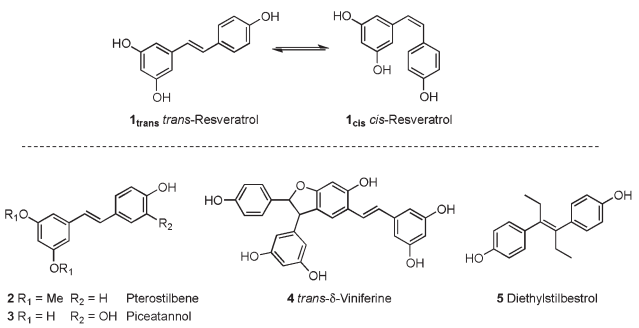

Fig. 1 Resveratrol, *cis* and *trans* isomers, together with several other natural occurring hydroxystilbene derivatives.

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†Electronic supplementary information (ESI) available: Cartesian coordinates, SCF energies and the number of imaginary frequencies for all stationary points computed in this work. NMR data for compounds **8a**, **8b**, **9a**, **9b** and **10**; also Fig. 1S showing the instability of **10** and Fig. 2S depicting the ^{1}H NMR of the oxygen-free photochemical reaction of EOM-**8** after 7 h of irradiation. See DOI: 10.1039/c1ce05336a

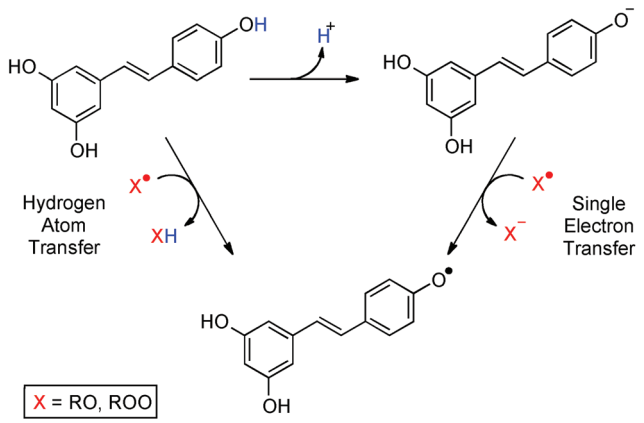


Fig. 2 Hydrogen atom transfer and electron transfer mechanisms proposed for the antioxidative activity of resveratrol.

tumoral cell apoptosis.[14] Nevertheless the capability of resveratrol to activate sirtuins has also been questioned in recent research.[6a,15]

Since resveratrol occurs in two isomeric forms (*cis*/*trans*, see Fig. 1), it is important to determine which of them is more biologically active. Of the two stereoisomers of resveratrol, the *trans* isomer is the most stable and the most abundant in nature, but both coexist in different proportions in wine and other foods. A number of recent studies have therefore been carried out to measure the absolute and relative amount of the two isomers in food products.

trans-Resveratrol, $\mathbf{1}_{trans}$, undergoes isomerization to the *cis* form $\mathbf{1}_{cis}$ when exposed to UV-vis light. The photostationary equilibrium is highly dependent on the specific spectrum of light reaching the sample and, at 350 nm, this equilibrium is characterized by the following kinetic parameters: k_{cis} = 0.0472 ± 0.0021 s^{-1} and $t_{1/2}$ = 14.7 min.[16] The chemical behavior of the *cis* isomer depends on the reaction conditions, which sometimes leads to contradictory results when quantification of resveratrol is pursued. Many analytical reports base their data on the aforementioned *cis–trans* equilibrium and do not mention the presence of a third compound in the reaction mixture. However, Roggero *et al.* admitted the possible presence of a phenanthrene derivative as a photoproduct of *trans*-resveratrol on the basis of its UV spectra and in analogy to the photochemical reactivity of other stilbenes.[17] Furthermore, a chromatographic analysis in wine samples has been reported that takes advantage of the photoreactivity of *cis*-resveratrol leading to the corresponding fluorescent phenanthrene, even if this phenanthrene derivative was neither isolated nor characterized.[18]

Experimental studies on resveratrol photoisomerization using HPLC-MS methods lead to contradictory conclusions. Mark *et al.* studied the experimental photoisomerization of resveratrol at different irradiation times. At short irradiation times they found two peaks in the UV detector with the characteristic mass of resveratrol (229.2 *m*/*z*), and they were assigned to the *trans* and *cis* isomers, respectively. At longer irradiation times a third peak appeared showing an MS fragmentation pattern that departed significantly from that of *cis* or *trans*-resveratrol. The MS spectrum of this unknown third photoproduct also showed a peak at 229.2 *m*/*z*, but Mark *et al.* pointed at another peak at 227.2 *m*/*z* as the compound responsible for the rather different fragmentation pattern observed in this case. This interpretation implies a reaction involving the loss of two hydrogen atoms from resveratrol. Mark suggested that this unknown compound is a diphenylacetylene derivative that could be formed through an oxidative reaction of the exocyclic double bond.[19a] In another report, López-Hernández *et al.* obtained an MS spectrum for this unknown third compound with the same features as that recorded by Mark. However, they considered that the mass of the unknown compound was indeed the same mass of resveratrol. Following this logic, they proposed that the third photoproduct should be a resveratrol isomer.[19b] Both studies, therefore, reported the detection of an unidentified product of resveratrol under photochemical conditions, but such a compound was never properly characterized and the structures proposed for it differ. Very recent results unveiled further complexity to this chemistry, based on the studies carried out by Ho, who proposed a reaction cascade pathway for the acid-assisted photoisomerization of stilbene,[20] Kim, Park and coworkers have assigned the structure of the fluorescent compound to a naphthalenylbuten-2-one. This arylbuten-2-one is the result of several isomerization reactions starting with the pericyclic ring closing of *cis*-resveratrol to the corresponding dihydrophenanthrene.[21]

This situation suggests that the photochemical reactivity of resveratrol is much more complicated than a simple *cis–trans* isomerization. For this reason, and due to our ongoing interest in the reactivity of food antioxidants and their derivatives,[22] we decided to explore this chemistry and unambiguously identify the unknown photoproduct isolated by the groups of Mark and López-Hernández.

Results and discussion

The hydroxyl substitution pattern of resveratrol might affect its thermal stability. We therefore started our exploration of the isomerization reactivity of resveratrol by analyzing computationally and experimentally its stability under radiationless conditions. Commercial resveratrol is very stable in the dark, which is in good agreement with a computed barrier for double bond isomerization of 43.6 kcal mol^{-1}. Accordingly, the computed barrier for stilbene is 43.9 kcal mol^{-1}, which is also in good agreement with its observed barrier of 42.8 kcal mol^{-1}. This suggests that the presence of three hydroxyl groups in resveratrol does not affect its thermal stability.[23] Once the thermal stability of the substrate was confirmed, we decided to also computationally characterize the photochemical isomerization process and compare it with the well studied photoisomerization of the parent stilbene.[24] A multireference methodology similar to that employed by Martinez *et al.* to describe the photochemical isomerization of ethylene and stilbene was employed.[24a] In short, this implies a state-averaged CASSCF(2,2)/6-31G(d) level of theory (see the methods section for further details). As expected, the ground state potential energy surface shows two minima for the *cis* and *trans* isomers and a high energy transition state (corresponding to the previously described thermal reaction). In the first excited state, near the Frank–Condon region, two very shallow minima can be observed (see Fig. 3). Another relevant stationary point on this excited state surface is the global

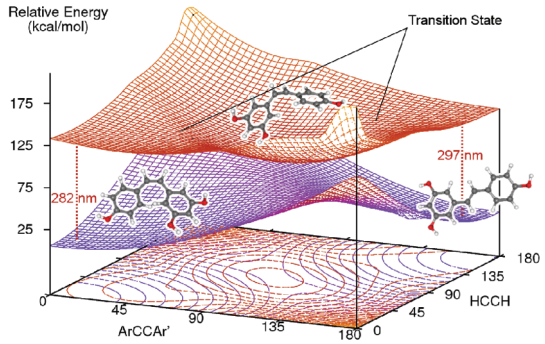
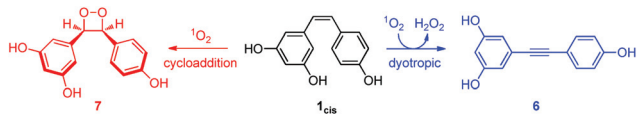


Fig. 3 Ground and first excited state potential energy surfaces for the photochemical and thermal isomerization of resveratrol at the CASSCF (2,2)/6-31G(d) level of theory.[25] The two reaction coordinates chosen correspond to the H–C–C–H and Ar–C–C–Ar dihedrals, which concentrate the changes along the reaction path.



Scheme 1 Proposed reactions for the dehydrogenation of resveratrol with singlet oxygen under photochemical conditions.

minimum just above the thermal transition state. Both minima at the Frank–Condon region are kinetically very unstable since a very low barrier separates them from the global minimum. In analogy to stilbene, and also in good agreement with the experimental data, the vertical excitation energy of *cis*-resveratrol is slightly larger than in the *trans* isomer. *trans*-Resveratrol, therefore, absorbs UV light at 308 nm and reaches the shallow minima at the first excited state surface. Surmounting a barrier of less than 0.5 kcal mol^{-1} the excited *trans*-resveratrol reaches the global minimum associated with a 90° distortion of the exocyclic double bond dihedral angle. Vibrational relaxation to the ground state makes the isomerization transition state accessible and, from there, both isomers can be formed completing either a globally unproductive reaction or a double bond isomerization process (see Fig. 3).

At this stage we decided to explore the possibility of oxidative photocyclization to yield a compound weighting 2 Da less than resveratrol. Oxidation under photochemical conditions calls for the intervention of singlet oxygen. Two different oxidation routes were considered for the reaction of resveratrol with this highly reactive oxidant: (1) the route proposed by Mark, in which the resveratrol is oxidized through the loss of the two hydrogen atoms attached to the exocyclic double bond yielding diphenylacetylene, and (2) the [π2 + π2] cycloaddition of singlet oxygen to the exocyclic double bond to furnish a dioxetane derivative (see Scheme 1).

The dehydrogenation of the exocyclic double bond of *cis*-resveratrol to form a diphenylacetylene derivative occurs *via* a concerted transition state. Both hydrogen atoms shift synchronously from the double bond to the singlet oxygen molecule in a type-II dyotropic reaction, a sort of chemical transformation that has gathered significant attention recently.[26] The energy barrier associated with the formation of the diphenylacetylene derivative

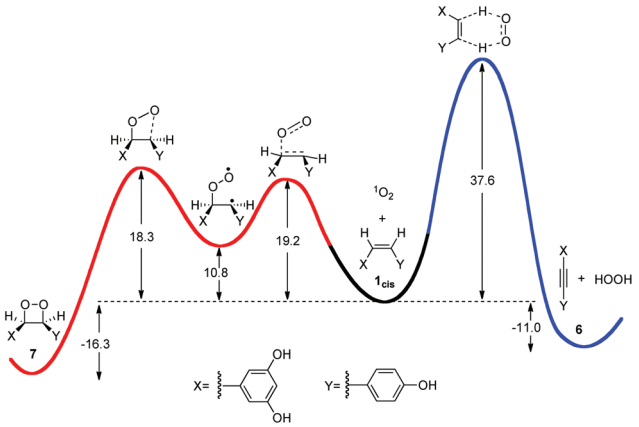


Fig. 4 Energy profile for the oxidation of *cis*-resveratrol, $\mathbf{1}_{cis}$, to furnish the derivative **7** *via* a type-II dyotropic reaction (in blue) and the dioxetane **7** *via* singlet oxygen addition to the exocyclic double bond (in red).

6 is ~38 kcal mol^{-1} and the products lie 11 kcal mol^{-1} lower in energy than the reactants (see Fig. 4). The alternative oxidation to yield a dioxetane ring is a formal [π2 + π2] cycloaddition, which is symmetry forbidden according to the Woodward–Hoffmann rules.[27] Not surprisingly, therefore, the reaction occurs in a stepwise manner through a diradical intermediate. Apparently, the energy penalty associated with the formation of a four membered ring is spread along the multi-step reaction coordinate, and the rate limiting transition state imposes a barrier of only 19.2 kcal mol^{-1}. The dioxetane formed is also rather stable with respect to the reactants (−16.3 kcal mol^{-1}). This energy profile shows that the formation of dioxetane **7** is more favorable both kinetically and thermodynamically than the formation of the diphenylacetylene derivative **6**. This suggests that, in the event of a reaction occurring between singlet oxygen and resveratrol, it is much more likely that this reaction will involve the addition of oxygen to furnish the dioxetane **7**, assuming that other reactions not examined here do not occur (see Fig. 4).

In summary, computational exploration suggests that: (1) the chemistry of resveratrol should not depart far away from the general reactivity of stilbene and (2) in the event of an oxidative process with photochemically activated oxygen, the expected course of the reaction is the formal addition to furnish a dioxetane derivative. Under these circumstances we turned to experimental work aiming to either confirm or refute such guidelines.

Following the close similarity found between the energetic barriers observed for resveratrol and stilbene under both thermal and photochemical conditions, we decided to find out whether the oxidative process *via* oxygen addition could also be experimentally confirmed. To accomplish this, we employed different irradiation conditions and we observed that the outcome of the photochemistry of a solution of *trans*-resveratrol is highly dependent on the specific reaction conditions: energy source, concentration, time of irradiation, *etc*.[28] For instance, the irradiation of a 0.2 mM aqueous ethanolic solution of *trans*-resveratrol $\mathbf{1}_{trans}$ with a 200 W lamp placed 5 cm from the reaction flask for 15 min led to a mixture of *trans*/*cis* resveratrol 1 : 0.7. Whereas if a 450 W hanovia lamp was used at the same distance for only 5 min, the ratio was 1 : 4 *trans*/*cis*. When it was irradiated for

15 min more a *trans*/*cis* mixture was also obtained, but along with other compounds.[29] The ^{1}H-NMR spectrum of these reaction mixtures showed a proton signal resonating at 9.1 ppm; the presence of which prompted us to assume that a phenanthrene derivative could be involved, since the chemical shift of H5 in this structural motif usually appears at such displacement.[30]

HPLC[31] of the mixture was then performed. Two main signals at 6.98 and 13.75 min were observed with UV detection, which were assigned to *trans*- and *cis*-resveratrol, as in previous studies,[19] along with signals at higher retention times. Furthermore, with fluorescence detection (λ_{exc} = 260 nm, λ_{em} = 364 nm) a peak at 14.95 min appeared as the most intense one suggesting an increased aromatic character of this unknown compound with respect to resveratrol (Fig. 5, top panel).

HPLC-APCI$^+$-MS performed under the conditions reported by Galeano-Díaz and coworkers[18,32] provided a chromatogram showing three peaks in close analogy to those obtained both by Mark and López-Hernández.[19] Unfortunately, this means that the fraction of the third unknown compound overlaps with *cis*-resveratrol and, therefore, the resulting MS spectrum is unreliable. By slightly modifying the elution conditions (reducing the flux from 0.8 ml min^{-1} to 0.3 ml min^{-1}) total separation of the three peaks was achieved with retention times of 4.22, 7.06 and 7.74 min (see Fig. 5, bottom panel). The mass spectra of these fractions reported molecular peaks of 229.2 Da for the first two fractions whereas the mass of the third compound was 227.2 Da. These results suggest that the controversy arising from the nature of the third compound in the photochemistry of resveratrol was likely due to traces of *cis*-resveratrol present in the HPLC fraction of the unknown compound. Under these circumstances two peaks with *m*/*z* ratios of 229.2 and 227.2 appear in the MS spectrum. Mark interpreted that the molecular peak of the unknown compound was 227.2 whereas López-Hernández opted for not interpreting the observations and assumed that the unknown compound was an isomer of resveratrol. Our results clearly demonstrate that the third compound found by these authors is formed through the loss of two hydrogen atoms from resveratrol.

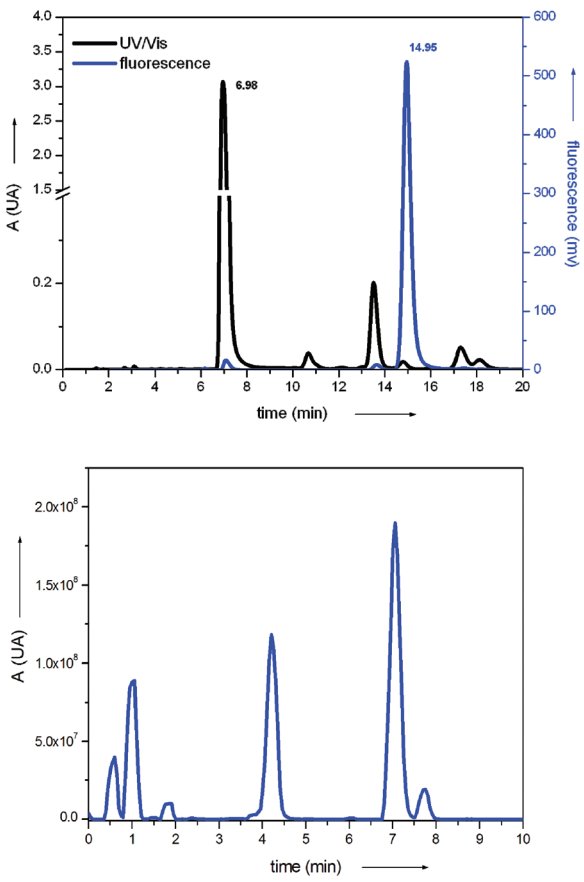
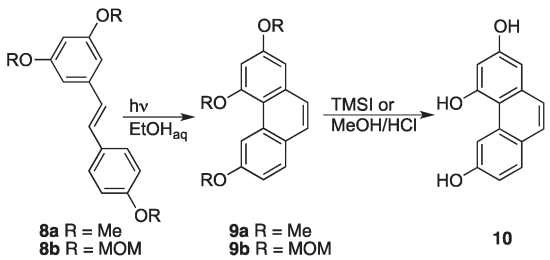


Fig. 5 HPLC chromatograms corresponding to the reaction mixture after irradiating commercial *trans*-resveratrol for 20 min at 5 cm with a 450 W lamp (top) and an APCI$^+$-MS chromatogram of the same reaction mixture (bottom).

All the efforts aimed to isolate this compound in amounts large enough for complete characterization were unsuccessful. Therefore, we decided to synthesize this phenanthrene *via* an indirect route to confirm this hypothesis. Thus, trimethyl-protected resveratrol **8a**,[33] prepared in good yields by nucleophilic substitution of **1** with methyl iodide, was irradiated with a 450 W lamp to furnish the phenanthrene derivative **9a** in 37% yield.[34] The methyl derivative **9a** was demethylated with TMSI in dichloromethane at 50 °C or MeOH/HCl 10% at 25 °C to give trihydroxyphenanthrene **10**[35] that proved to be highly unstable under these reaction conditions. The same results were obtained when the methoxymethoxy derivatives **8b** and **9b** were used (Scheme 2).[36]

A comparative ^{1}H NMR study is shown in Fig. 6. Spectrum 1 corresponds to a mixture of $\mathbf{1}_{trans}$ and $\mathbf{1}_{cis}$ obtained after irradiation with a 450 W lamp placed 25 cm from the sample for 5 min; spectrum 2 corresponds to the reaction mixture when irradiated at 5 cm for 20 min. and 3 corresponds to the isolated phenanthrene. With these results, we can conclude that the signal at 9.1 ppm corresponds to phenanthrene **10**. Moreover, when a sample of **10** was injected into the HPLC column under the conditions of this study, we could unequivocally assign compound **10** to the aforementioned third compound. Interestingly, we could identify the same ^{1}H-NMR signals of **10** in a spectrum of the reaction mixture that Prof. Lopez-Hernández kindly sent us.

In summary, the computational and experimental evidence gathered clearly suggests that the photochemistry of resveratrol is similar to that found in the parent system stilbene. Under photochemical activation, resveratrol undergoes rapid *cis*–*trans* isomerization and, under longer irradiation times, a six electron electrocyclic reaction followed by oxidation furnishes 2,4,6-trihydroxyphenanthrene. The formation of the trihydroxyphenanthrene derivative from resveratrol requires intense irradiation and therefore this compound is not expected to participate in the



Scheme 2 Synthetic route followed in the preparation of trihydroxyphenanthrene **10**.

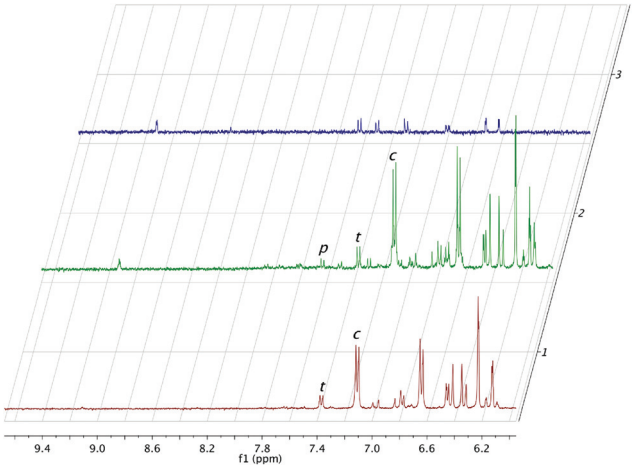


Fig. 6 ^{1}H-NMR spectra: (1) reaction of resveratrol at 25 cm for 5 min; (2) reaction at 5 cm for 20 min; (3) isolated phenanthrene (*c*: *cis*-resveratrol; *t*: *trans*-resveratrol; *p*: 2,4,6-trihydroxyphenanthrene).

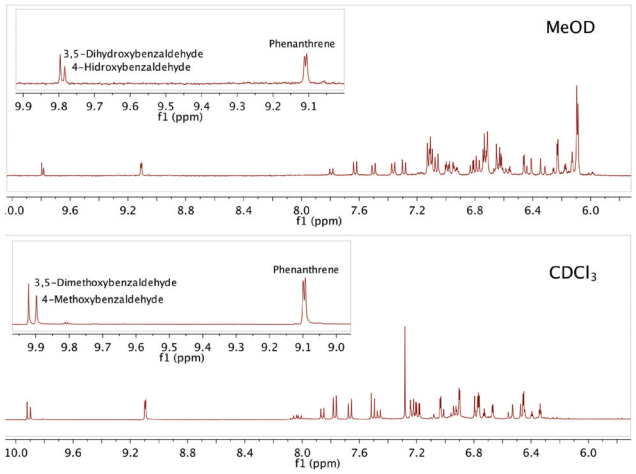


Fig. 7 ^{1}H-NMR spectra of the reaction mixture for the photoactivation of *trans*-resveratrol $\mathbf{1}_{trans}$ (top) and trimethyl-protected *trans*-resveratrol **8a** (bottom).

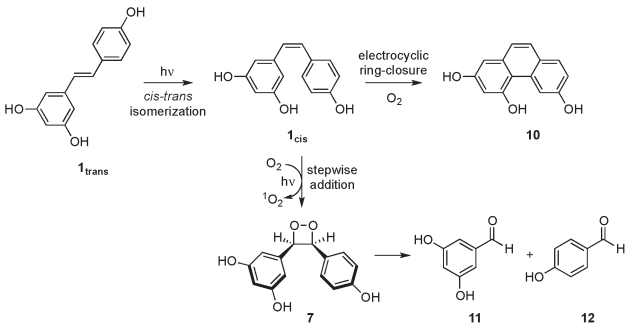


Fig. 8 Summary of the observed photochemistry of *trans*-resveratrol $\mathbf{1}_{trans}$.

phytochemistry of the plant. However, due to its high fluorescence it could be valuable when developing accurate detection protocols for resveratrol from natural resources.

The preliminary computational work performed to explore the possibility of the formation of diphenylacetylene derivative **6** suggested that, under photochemical conditions, singlet oxygen could readily react with resveratrol to give a dioxetane derivative. The energy barrier associated with this reaction was only 19.2 kcal mol^{-1}, which renders this process very facile. Guided by these results we decided to carefully analyze the ^{1}H-NMR spectrum of the reaction mixture to look for evidence of this reaction pathway being operative. Dioxetane species are very unstable and they spontaneously decompose yielding two carbonyl species.[37] To further confirm this reactivity a photoactivated reaction was performed while bubbling oxygen through the mixture. Such conditions favoured the formation of the transient dioxetane intermediate and we identified 4-hydroxy-benzaldehyde and 3,5-dihydroxy-benzaldehyde or 4-methoxy-benzaldehyde and 3,5-dimethoxy-benzaldehyde as products of the reaction when resveratrol **1** or the trimethoxy derivative **9a** were irradiated, respectively (Fig. 7).[38]

The complete picture of the photochemistry of resveratrol is therefore considerably more complicated than a simple *cis–trans* interconversion. Not only oxidative processes are operative and competitive under regular conditions, furnishing phenanthrene derivatives, but also fragmentation of fleeting dioxetane intermediates has been observed (see Fig. 8).

Conclusions

Under photochemical activation *trans*-resveratrol undergoes not only a double bond isomerization. The third compound observed in previous experimental studies has been characterized as 2,4,6-trihydroxyphenanthrene, formed *via* a 6-electron electrocyclic ring closure followed by dehydrogenation. The dyotropic reaction of singlet oxygen to afford a diphenylacetylene derivative has an associated activation energy of 37.6 kcal mol^{-1} and can be discarded as a competitive route. However, computational exploration uncovered the feasibility of singlet oxygen addition to *cis* or *trans*-resveratrol to furnish a dioxetane intermediate. Such prediction was confirmed experimentally through the detection of the products of dioxetane by spontaneous cycloreversion (benzaldehydes **11** and **12**), thus reinforcing the idea of the synergetic relationship between theoretical and experimental work.

Computational methods

Density functional theory[39] employed throughout this work was based on the Kohn–Sham formulation[40] and all calculations were performed with the Gaussian 09 suite.[41] The three-parameter hybrid B3LYP[42] functional was used in all DFT calculations along with the double-ζ basis set 6-31G(d,p). Due to significant high spin contamination occurring in singlet oxygen and compounds related to its chemistry the projection scheme of Yamaguchi[43] has been employed for all the structures illustrated in Fig. 4. This methodology has been successfully employed to study singlet oxygen reactivity very similar to that described in the current work.[44] Harmonic analysis has been applied to the second derivatives of the energy with respect to the nuclear motion for all structures. Through this analysis the nature

(minimum or transition structure) of the stationary points was identified and the zero point vibrational energies and the contributions to the free energy were obtained.

Due to the potential diradical character of some of the structures considered in this work, the internal and external stability of the wavefunctions was computed *via* the Hermitian stability matrices A and B in all cases.[45] For all the structures exhibiting unstable restricted wavefunctions, the spin-symmetry constraint of the wavefunction was released (*i.e.* expanding the SCF calculation to an unrestricted space, UB3LYP) leading to stable unrestricted wavefunctions.

Complete active space SCF (CASSCF) calculations were performed with the 6-31G(d) basis set to accurately describe the diradical character of several key intermediates. These calculations were performed with the GAMESS package.[46] The active space in the CASSCF calculations presented in this work includes the π and π* orbitals of the aliphatic double bond in resveratrol, resulting in two electrons in two orbitals active space, usually noted CASSCF(2,2).

Experimental methods

(*E*)-1,3-Dimethoxy-5-(4-methoxystyryl)benzene (8a)

Sodium hydride (134 mg, 3.5 mmol, 60% in mineral oil) was suspended in DMF (12 mL) at 0 °C in a round-bottomed flask under an argon atmosphere. Then a solution of *trans*-resveratrol (250 mg, 1.095 mmol) in DMF (10 mL) was added slowly, and the ice bath was removed. A solution of iodomethane (1.140 g, 8 mmol) in DMF (20 mL) was added slowly into the mixture from a dropping funnel with a pressure equalizing arm. After 5 h at 25 °C, the reaction mixture was plunged into crushed ice containing 5% HCl, and the reaction product was extracted with ethyl acetate. The combined organic layers were washed with brine and water, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (hexane–ethyl acetate 70 : 30) afforded 27 mg (46%) of (*E*)-1,3-dimethoxy-5-(4-methoxystyryl) benzene.[33]

(*E*)-1,3-Bis(methoxymethoxy)-5-(4-methoxymethoxy)styryl benzene (8b)

Sodium hydride (302 mg, 2.9 mmol, 60% in mineral oil) was suspended in THF (9 mL) at 0 °C in a round-bottomed flask under an argon atmosphere. Then a solution of *trans*-resveratrol (200 mg, 0.88 mmol) in THF (9 mL) was added slowly, and the ice bath was removed. After 30 min, a solution of chloromethyl methyl ether (922 mg, 11.45 mmol) was added *via* a syringe. After 15 h at 25 °C, the reaction mixture was partitioned between water and ethyl acetate. The aqueous phase was extracted with ethyl acetate, the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography on silica gel (hexane–ethyl acetate 70 : 30) afforded 73 mg (23%) of **8b**. Data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 8.7, 2H), 7.05 (m, 3H), 6.93 (d, J = 16.3, 1H), 6.87 (d, J = 2.2, 2H), 6.66 (t, J = 2.2, 1H), 5.22 (s, 6H), 3.52 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 157.2, 140.0, 131.2, 129.0, 128.0, 126.9, 116.6, 107.8, 104.26, 94.7, 94.6, 56.3, 56.2. EM (EI^+) m/z (%) 361 (8), 360 ([M^+], 66), 167 (79), 149 (100). HRMS (EI^+) m/z calc. for $C_{20}H_{24}O_6$ 360.1573; found 360.1584.

General procedure for the oxidative photocyclization: 2,4,6-trimethoxyphenanthrene (9a)

A 4 mM solution of the (*E*)-1,3-dimethoxy-5-(4-methoxystyryl)-benzene (27 mg, 0.1 mmol) in ethanol, under an air atmosphere, was irradiated using a 450 W medium pressure mercury lamp in a Pyrex immersion well. RMN was used to follow the irradiation progress at regular intervals. When the reaction was complete (6 h), the reaction mixture was concentrated *in vacuo* in the dark and purification by flash column chromatography on silica gel (hexane–DCM 70 : 30) afforded 10 mg (37%) of 2,4,6-trimethoxyphenanthrene.[34]

2,4,6-Tris(methoxymethoxy)phenanthrene (9b)

A 4 mM solution of **8b** (59 mg, 0.22 mmol) afforded 13 mg (16%) of **9b**. Data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (d, J = 2.4, 1H), 7.75 (d, J = 8.7, 1H), 7.62 (d, J = 8.7, 1H), 7.48 (d, J = 8.7, 1H), 7.26 (dd, J = 2.4, 8.7, 1H), 7.14 (d, J = 2.4, 1H), 7.06 (d, J = 2.4, 1H), 5.48 (s, 2H), 5.33 (s, 2H), 5.29 (s, 2H), 3.59 (s, 3H), 3.54 (s, 3H), 3.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 156.1, 155.8, 135.9, 131.5, 129.6, 128.1, 127.7, 125.3, 116.5, 116.3, 113.3, 106.3, 103.4, 95.2, 95.1, 94.8, 56.7, 56.4, 56.2. EM (EI^+) m/z (%) 359 (17), 358 ([M^+], 88), 327 (17), 326 (100), 280 (50), 265 (48), 250 (50), 223 (23), 196 (17), 195 (93), 165 (32), 151 (30). HRMS (EI^+) m/z calc. for $C_{20}H_{22}O_6$ 358.1416; found 358.1420.

Phenanthrene-2,4,6-triol (10)

From **9a**: Trimethylsilyl iodide (281 mg, 1.4 mmol) was added to a solution of **9a** (13 mg, 0.05 mol) in DCM (2.5 mL). After 24 h at 50 °C, under an argon atmosphere, an additional amount of Me_3SiI (141 mg, 0.7 mmol) was added. The reaction was complete after 23 h more. Then, MeOH (1 mL) was added and the solvent was removed to yield the trihydroxyphenanthrene **10**.[35]

From **9b**: HCl(c) (30 μL) was added to a solution of **9b** (3 mg, 0.01 mmol) in MeOH (0.3 mL) and the resulting reaction mixture was stirred under a drying tube at 25 °C for 24 h. Elimination of the solvent under reduced pressure rendered compound **10**.

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