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Photochemical generation of a new, highly fluorescent compound from non-fluorescent resveratrol

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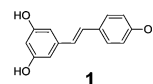
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UV irradiation of *trans*-resveratrol leads to its photochemical transformation to a new, highly fluorescent compound, whose chemical structure was unambiguously identified. The new compound has large values of fluorescence quantum yield, Stokes' shift, and two-photon absorption cross section, which make it suitable for bio-imaging, multi-color labeling, and two-photon microscopy.

Luminescence is a broad term used to describe the conversion of energy into light in matter. Different sources of energy such as chemical reactions, electrical potential, or light irradiation produce different types of light emission such as chemi-, electro-, or photoluminescence, respectively.¹ In this communication, we report a novel luminescence phenomenon resulting from the combination of photo- and chemi-luminescence of a non-emitting molecule (*trans*-resveratrol in this study) that is transformed into a highly fluorescent species by a photochemical reaction triggered by light. This is a distinctly different phenomenon from photocaging of fluorophores² or photoactivated switching^{3,4} as it involves only a single-moiety compound, with no release of a component species, no protected ("caged") molecules involved, no need for an activator or foreign agent such as thiol,³ or no shelving–deshelving process to achieve reversibility.⁴

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene), **1**, is a naturally occurring phytoalexin compound found mostly in berries and nuts, whose main function is protection against infection and other external stresses.⁵ Its lifespan-extending effect for simple forms of life such as nematodes and fruit flies has drawn considerable interest in recent years, along with its significant medicinal effects as an anti-oxidant, anti-carcinogenic, and cardio-protective agent.⁶ Chemically, the mechanism for photoisomerization from *trans*- to *cis*-resveratrol has been studied in view of its application in chemical content analysis,⁷ but chemical transformations along

the entire photochemical reaction pathway have been left virtually uninvestigated so far.



Several research groups have sporadically reported observing fluorescence from the solution of UV-irradiated *trans*-resveratrol.^{7,8} Roggero and Garciaparrilla found that intense UV irradiation of an alcoholic solution of *trans*-resveratrol first induces isomerization to the *cis*-form followed by formation of an unidentified, highly fluorescent compound.^{8a} Using a fluorometric technique to assess the local stilbene content in grapevine leaf, Poutaraud *et al.* reported a broad emission band of unknown origin from a UV-irradiated resveratrol solution.^{8b} In addition, Martínez-Ortega *et al.* confirmed the presence of a highly fluorescent compound in their quantitative analyses of resveratrol in red wine.^{7a} In all these previous studies, however, no structural information was given about the highly fluorescent species and its chemical identification has remained elusive to date.

In our experiment, we irradiated 125 μ M methanol solution of *trans*-resveratrol (R5010, Sigma) with UV light for 90 seconds, which is much shorter than in earlier studies ($\sim$ hours),⁸ using a 6 watt UV lamp ($\lambda_{\text{max}} = 305$ nm) with a photon intensity of 0.6 mW cm^{-2} at 295 K. The photoproducts were analyzed by high-performance liquid chromatography (HPLC). The wavelength of the UV lamp was chosen to match that of the maximum absorption for *trans*-resveratrol, ensuring the highest yield in the shortest time. In agreement with the earlier studies,⁸ we confirmed the existence of three major species using HPLC (middle panel of Fig. 1(a)): *trans*-resveratrol, *cis*-resveratrol, and an unknown fluorescent compound ("X" hereafter). Once photochemically produced, this compound remains quite stable in aqueous solution. Under prolonged exposure, however, it undergoes photodegradation (bottom panel of Fig. 1(a)). Fig. 1(b) shows that the absorption spectrum of X is rather different from that of *trans*- or *cis*-resveratrol, while its emission in the visible range is quite a distinct feature from the two resveratrol compounds, which are non-fluorescent. Fig. 1(c) compares the actual photographs of luminescence from the solution before and after UV irradiation.

In order to identify X chemically, we first isolated it from the other resveratrol compounds by using preparatory thin-layer chromatography where the mobile phase was composed of

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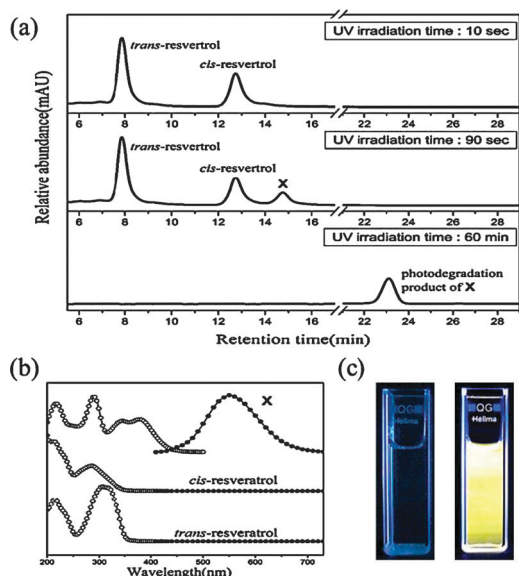


Fig. 1 (a) HPLC data for the reaction products obtained after different durations of exposure to UV irradiation. The unknown, highly fluorescent species is denoted by X. (b) Absorption (open circles) and emission (solid circles) spectra of *trans*-resveratrol, *cis*-resveratrol, and X. No emission is detected from *trans*- and *cis*-resveratrol whereas X gives a strong emission in the visible range when excited at 390 nm. (c) Photographs showing photoluminescence (or lack thereof) of the resveratrol solution before (left) and after (right) UV irradiation at 305 nm.

methanol and methylene chloride (1:20). A high-resolution mass spectrometer (LTQ Orbitrap, Thermo Scientific) showed that the molecular mass of HPLC-separated X is the same as that of resveratrol itself (Fig. 2(a)), suggesting an isomerization reaction as the main pathway for the photochemical generation of X. For structural identification of X, we employed ^1H NMR

and ^{13}C NMR with DEPT (distortionless enhanced polarization transfer), HSQC (heteronuclear single quantum correlation), and HMBC (heteronuclear multiple bond correlation) analysis along with FT-IR spectroscopy. The coupling constant between the two peaks of ^1H NMR (Fig. 2(b)) at δ 7.56 and at δ 6.71 was 13.5 Hz, indicating the presence of *trans*-olefin, whereas that between the two peaks at δ 7.56 and at δ 7.50 was 7.25 Hz, indicating the presence of two aromatic protons. The ^{13}C chemical shift of X at δ 201 (Fig. 2(c)) indicates the presence of an aldehyde or ketone carbonyl group ($\text{C}=\text{O}$). DEPT analysis (Fig. 2(d)) of the ^1H NMR peak at δ 2.3 suggests a methyl group adjacent to the carbonyl ($-\text{CO}-\text{CH}_3$). HSQC and HMBC analyses (Fig. 2(e)) suggest that the carbons of the *trans*-olefin are connected directly to the carbon of the acetyl group and indirectly to the carbons of the two coupled aromatic protons *via* one quaternary carbon. The presence of an acetyl group and an olefin moiety in X deduced above is also consistent with the FT-IR spectrum (Fig. 2(f)).

Considering all the results above, we propose that the structure of the unknown fluorescent compound X should be that of (*E*)-4-(6,8-dihydroxynaphthalen-2-yl)but-3-en-2-one (Fig. 3), which has never been registered in the CAS. It will be interesting to see if this relatively simple molecule can be synthesized without resorting to a photochemical reaction. For this structure of X, which for now we would like to name “resveratrone” for short, we performed density functional theory calculations with the Gaussian03 program package using the B3LYP/6-31G* basis set and found that its simulated IR spectrum (Fig. 2(g)) is in good agreement with the experimental FT-IR spectrum.

Our proposed structure and reaction pathway for X are somewhat similar to those suggested by Ho *et al.*,⁹ who found a series of photocyclization, hydrogen shift, and ring opening in their study of acid-assisted switching of the reaction pathway for stilbene analogues upon UV irradiation. However, as shown

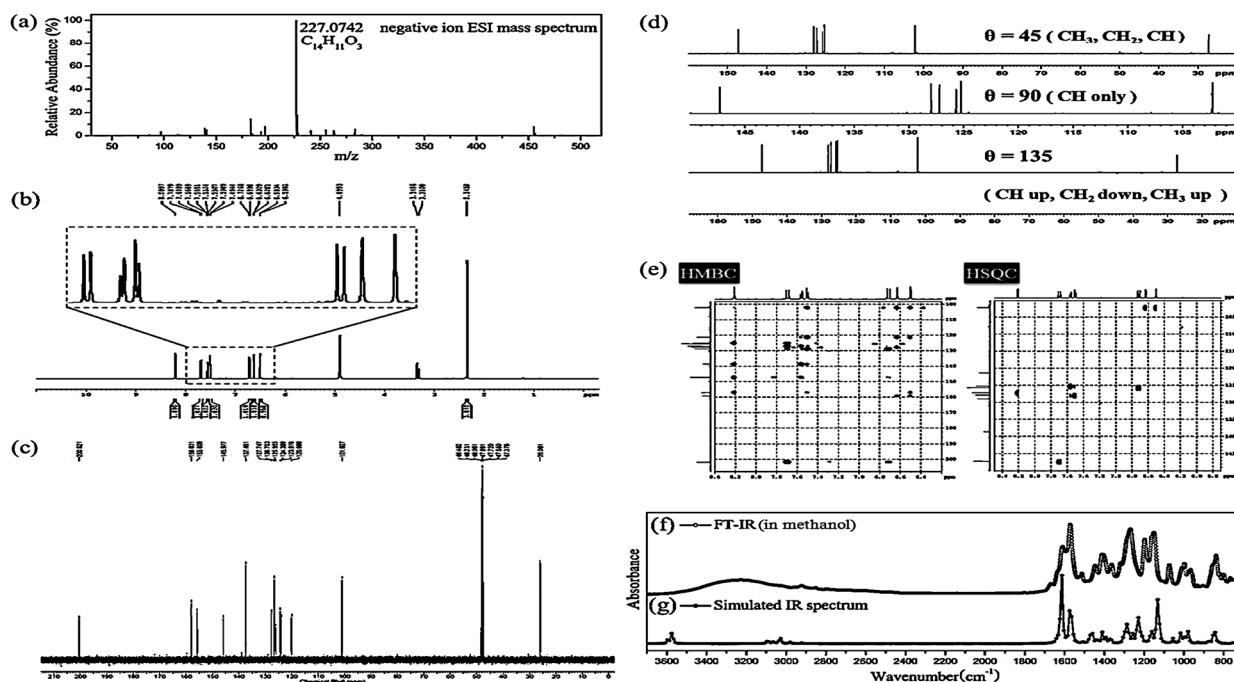


Fig. 2 (a) High-resolution mass, (b) ^1H NMR, (c) ^{13}C NMR, (d) DEPT NMR, (e) 2D NMR, (f) FT-IR, and (g) simulated IR spectrum of X.

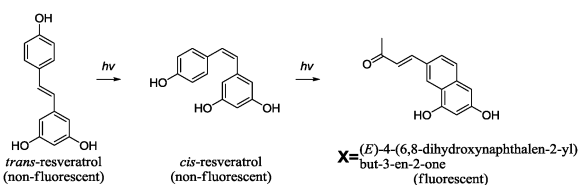


Fig. 3 Photochemical reaction pathway for the generation of the highly fluorescent compound X (“resveratrone”) by UV light.

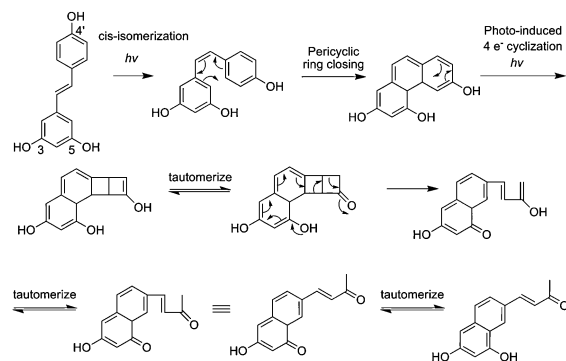


Fig. 4 Hypothetical photochemical reaction mechanism for the generation of resveratrone.

in the hypothetical mechanism we propose in Fig. 4, the photochemical transformation of resveratrol was achieved in the absence of acidic conditions. It is also essential to have the 4'-hydroxyl moiety, which was confirmed by the failure of 4'-methylated resveratrol to undergo the desired photochemical transformation.

To characterize some crucial optical properties of this newly identified compound, we measured its fluorescence quantum yield, excited-state lifetime, and two-photon absorption cross section. The quantum yield of resveratrone as measured by an absolute quantum yield measurement system (QE-1000, Otsuka Electronics) is 0.49 in DMSO and 0.31 in iso-propanol, which are comparable to those of many commonly-used Alexa family dyes. The Stokes' shift of resveratrone is very large (~ 150 nm, Fig. 1(b)), which makes it an ideal fluorophore in multicolor labeling and *in vivo* imaging. The excited-state lifetime was measured to

be ~ 250 ps in methanol by time-correlated single photon counting (TCSPC) (Fig. 5(a)), which is somewhat shorter than those of other dyes with similar quantum yields. In addition to the single-photon emission (Fig. 5(b)), we observed a two-photon fluorescence emission from resveratrone (Fig. 5(c) and (d)). The two-photon absorption cross section of resveratrone measured by the two-photon induced fluorescence method¹⁰ is ~ 132 GM ($1 \text{ GM} = 10^{-50} \text{ cm}^4 \text{ s}$) in ethanol, which is of comparable magnitude to other commonly used two-photon dyes.

In conclusion, we identified the highly fluorescent yet elusive compound that results from UV irradiation of *trans*-resveratrol. The reaction pathway toward the observed luminescence starts with photoisomerization of *trans*-resveratrol to *cis*-resveratrol, which then undergoes another isomerization reaction that leads to the product (named “resveratrone”), (E)-4-(6,8-dihydroxynaphthalen-2-yl)but-3-en-2-one, presumably through photoinduced ring opening and closing. This new molecule is thermally stable and highly fluorescent with a large quantum yield. It also has a large Stokes' shift appropriate for multicolor labeling and *in vivo* imaging, and is even suited for two-photon microscopy with its high two-photon absorption cross section.

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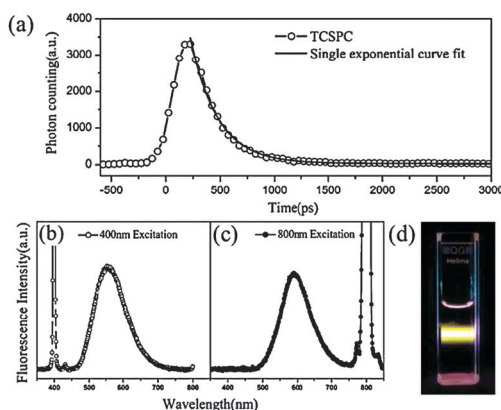


Fig. 5 (a) A single-exponential temporal decay in TCSPC measurement of the excited-state lifetime for resveratrone, (b) single-photon, and (c) two-photon emission spectrum of resveratrone photoexcited at 400 and 800 nm, respectively. (d) A photograph showing two-photon emission of resveratrone following photoexcitation at 800 nm.