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Photo-induced chemical reaction of *trans*-resveratrol

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ABSTRACT

Photo-induced chemical reaction of *trans*-resveratrol has been studied. UV B, liquid state and sufficient exposure time are essential conditions to the photochemical change of *trans*-resveratrol. Three principal compounds, *cis*-resveratrol, 2,4,6-phenanthrenetriol and 2-(4-hydroxyphenyl)-5,6-benzofurandione, were successively generated in the reaction solution of *trans*-resveratrol (0.25 mM, 100% ethanol) under 100 μW cm^{-2} UV B radiation for 4 h. *cis*-Resveratrol, originated from isomerization of *trans*-resveratrol, resulted in 2,4,6-phenanthrenetriol through photocyclisation reaction meanwhile loss of 2 H. 2,4,6-Phenanthrenetriol played a role of photosensitizer producing singlet oxygen in the reaction pathway. The singlet oxygen triggered [4+2] cycloaddition reaction of *trans*-resveratrol, and then resulted in the generation of 2-(4-hydroxyphenyl)-5,6-benzofurandione through photorearrangement and oxidation reaction. The singlet oxygen reaction was closely related to the substrate concentration of *trans*-resveratrol in solution.

1. Introduction

trans-Resveratrol (*trans*-3,4′,5-trihydroxystilbene, Fig. 1) is a naturally occurring phytoalexin found in foods such as red grapes, wines, cranberries and peanuts. *trans*-Resveratrol has drawn great attention due to its various health benefits and physiological functionalities, including antioxidative, anti-inflammatory, cardioprotective and anti-tumour properties (Larrosa et al., 2009; Peritore, Ho, Yamamoto, & Schaus, 2012; Tosun & Inkaya, 2010). There is growing interest in its use as an ingredient for human health supplement and medicine (Lu, Cheng, Hu, Zhang, & Zou, 2009; López-Nicolás, Núñez-Delicado, Pérez-López, Barrachina, & Cuadra-Crespo, 2006), hence it is important to understand chemical change of *trans*-resveratrol.

trans-Resveratrol has an isomer *cis*-resveratrol with lower bioactivities (Fig. 1). Isomerization from *trans*- to *cis*-form is thought to be facilitated by UV/sunlight radiation (Vian, Tomao, Gallet, Coulomb, & Lacombe, 2005; Wang, Catana, Yang, Roderick, & van Breemen, 2002), which results in a mixture of only *trans*- and *cis*- forms of resveratrol as universally assumed (Goldberg et al., 1995; Jeandet et al., 1995). However, several studies reported a new product with strong fluorescence in conversion of *trans*- to *cis*-resveratrol triggered by UV/sunlight radiation (López-Hernández, Paseiro-Losada, Sanches-Silva, & Lage-Yusty, 2007; Rodríguez, Lahoz, Faza, Cid, & Lopez, 2012; Wang et al., 2002; Yang et al., 2012), but no consistent conclusion was obtained. Rodríguez et al. (2012) assigned the new compound to trihydroxyphenanthrene but Yang et al. (2012) identified the new compound as (E)-4-(6,8-dihydroxy-naphthalen-2-yl)but-3-en-2-one, and many others had no essential information for structural identification of the detected new compound. In addition, the generation of an oxidative dimer of resveratrol—viniferin and its isomers has been reported through oxidation reaction by peroxidases or metallic oxidants *in vivo* and *in vitro* (Pezet et al., 2003; Sako, Hosokawa, Ito, & Iinuma, 2004; Song et al., 2014). These make the chemical transformation of *trans*-resveratrol much opaque.

In the present study, the photostabilities of *trans*-resveratrol in different conditions were evaluated. A kinetic study was carried out to investigate the chemical transformation of *trans*-resveratrol under UV B radiation. The principal photo-product of *trans*-resveratrol was isolated and further identified by MS, UV absorption, FTIR and NMR spectra analysis, and the possible reaction mechanism was elucidated.

2. Materials and methods

2.1. Materials

trans-Resveratrol (R5010, 99%) was purchased from Sigma–Aldrich (China). *cis*-Resveratrol (5 mg, >98%) from Cayman Chemical Supplier was purchased from Sapphire Bioscience Pty., Ltd. (Australia). Chemical purity grade ethanol was purchased from Sinopharm Chemical Reagent CO., Ltd. (Beijing, China). The other chemical

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reagents used were of HPLC grade (Jinmei Biotech Corporation, Tianjin, China). The water was prepared by an EASYPure II UV Ultra Pure Water System (Barnstead International, Dubuque, IA, USA).

2.2. Assessment of photostabilities of trans-resveratrol in different mediums

An ethanol solution of *trans*-resveratrol (0.25 mM, 100% ethanol) and an aqueous solution of *trans*-resveratrol (0.038 mM, water) were prepared for irradiation tests. The *trans*-resveratrol solutions and *trans*-resveratrol powder were respectively exposed to sunlight (75000 lux), laboratory light (35000 lux) and UV B for 4 h. The intensity of UV B was achieved at 100 μW cm^{-2} by using six UV lamps (SPECTRONICS BLE-1T158 Tube 15 watt, main output at312 nm) according to the UV B intensity in the sunlight. The *trans*-resveratrol solutions with and without irradiation treatments were sampled for HPLC analysis. The *trans*-resveratrol powder was dissolved by 100% ethanol for HPLC analysis.

2.3. Study on the transformation of trans-resveratrol under UV B radiation

Based on the HPLC profiles of the reaction mixture of *trans*-resveratrol in liquids, Peaks 1–4 are assigned to *trans*-resveratrol, *cis*-resveratrol, compound A and compound B respectively. A kinetic study was carried out to investigate the evolution of *trans*-resveratrol under UV B radiation. UV lamps were used to achieve a constant UV B radiation at 100 μW cm^{-2}. 60 mL ethanol solution of *trans*-resveratrol (0.25 mM, 100% ethanol) was UV B irradiated and sampled every 30 min for HPLC analysis.

2.4. HPLC analysis

The HPLC conditions were: Injection volume 10 μL, Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm), column temperature 28 °C, mobile phase A = acetonitrile/acetic acid/water (6:1:193, v), mobile phase B = acetonitrile, linear gradient elution: from 75% (v) A and 25% (v) B to 55% (v) A and 45% (v) B during early 30 min and then 75% (v) A and 25% (v) B till 35 min, flow rate 1 mL min^{-1}, Shimadzu SPD ultraviolet detector at 285 nm.

2.5. Structural identification of the photoproducts

2.5.1. Preparation of the compound B

The reaction mixture of *trans*-resveratrol (0.25 mM, 100% ethanol) under UV B radiation was submitted to semi-preparative HPLC (Varian Proster 218, USA). The fractions of compound B were collected and combined. The compound B was obtained by freeze drying.

2.5.2. Structural identification

2.5.2.1. UPLC–UV-MS/MS analysis. UPLC–UV-MS/MS (Waters Corporation, Milford, USA) was employed to provide the molecular mass information of compounds A and B. The UPLC conditions were: acquity UPLC HSST3 column (2.1 mm × 150 mm, 1.8 μm), column temperature 35°C, injection volume 10 μL, mobile phase A = 0.1% formic acid + 99.9% water (v/v), mobile phase B = 0.1% formic acid + 99.9% acetonitrile (v/v), linear gradient elution: from 99.9% (v) A/0.1% (v) B to 10% (v) A/90% (v) B during 38 min, flow rate 1 mL min^{-1}. An electrospray ionisation (ESI) technique in a negative ion mode was employed for MS/MS analysis. The ion source conditions were set as follows: capillary voltage 3000 V, cone voltage 30 V, extractor 3.0 V and RF lens 0.2 V, ion source temperature 150 °C, desolvation gas nitrogen at a flow rate of 600 L h^{-1} and temperature at 300 °C. Argon was used as the collision gas at a flow rate of 0.13 mL min^{-1} and the collision energy was 22 eV. Full scan ranging from 100 to 400 atomic mass unit (amu) and daughter ion scan with the mass of parent ion at 227 *m*/*z*, 225 *m*/*z* and 239 *m*/*z* were recorded. UV–visible spectrum of each compound was recorded in the range 190–400 nm by a photodiode detector.

2.5.2.2. Fourier transforms infrared (FTIR) spectroscopic analysis. A Nicolet AVATAR 370 FTIR Spectrometer (Thermo Fisher, Massachusetts, USA) was used to determine the FTIR spectrum of the compound B in KBr pellets in the wavelength ranging from 400 to 4000 cm^{-1} according to method described by Yang, Li, He, Ren, and Wang (2009).

2.5.2.3. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) measurement. ^{1}H and ^{13}C NMR spectra were obtained from ~20 mg of the freeze dried compound B suspended in 0.8 mL acetone-d 6. The spectra were recorded on a Bruker AVANCE III spectrometer at 600 MHz with a 5 mm DCH cryoprobe (Bruker, Wissembourg, France). Integration of the spectra was performed with Bruker Topsin 3.2 software.

3. Results and discussion

3.1. Photostability of trans-resveratrol

Table 1 shows the HPLC profiles of *trans*-resveratrol in different mediums with and without irradiation treatments. For liquid state, the trace levels of *cis*-resveratrol in the aqueous and ethanol solution of *trans*-resveratrol without irradiation indicated that *trans* to *cis* conversion of resveratrol naturally occurred in liquid state, and laboratory light exerted no obvious effect on the isomerization of *trans*-resveratrol according to the HPLC profiles in Table 1. Two remarkable new peaks were observed in the HPLC profiles of sunlight/UV B irradiated *trans*-resveratrol in aqueous and ethanol solutions (Table 1), suggesting that two new compounds are abundantly generated. For solid state, no new compounds were detected in all the irradiated *trans*-resveratrol in solids (Table 1). The level of *cis*-resveratrol slightly increased after exposure of *trans*-resveratrol powder to sunlight/UV B for 4 h, and accordingly the *trans*-resveratrol powder turned reddish under 4 h sunlight/UV B radiation while remained white in the laboratory light.

Sunlight basically consists of visible, UV and infrared light whereas laboratory light mainly consists of visible light. From above results, UV B spectrum in the sunlight may play an important role in generating new photoproducts of *trans*-resveratrol, which is in agreement with the conclusion demonstrated by Nour, Trandafir, and Muntean (2012). Besides, the occurrence of photo-induced chemical reaction of *trans*-resveratrol requires a liquid medium. In our study, two principal photoproducts were observed, which is inconsistent with the previous studies that only one photoproduct of *trans*-resveratrol formed under UV B radiation (López-Hernández et al., 2007; Wang et al., 2002; Yang et al., 2012). This may be attributed to different HPLC separation systems and irradiation treatments employed. Exceptionally, ethanol solutions of *trans*-resveratrol with high concentrations ranging from 20 mM to 100 mM were also irradiated by UV B/sunlight for 4 h in our study, but no new compounds were found (data not shown), indicating that the concentration of *trans*-resveratrol significantly affects the photochemical reaction of *trans*-resveratrol.

3.2. Photo-induced transformation of trans-resveratrol

Fig. 1 shows the chemical transformation of *trans*-resveratrol in 100% ethanol as UV B exposure time increased. At 30 min of UV B radiation, most of *trans*-resveratrol (Peak 1) converted to

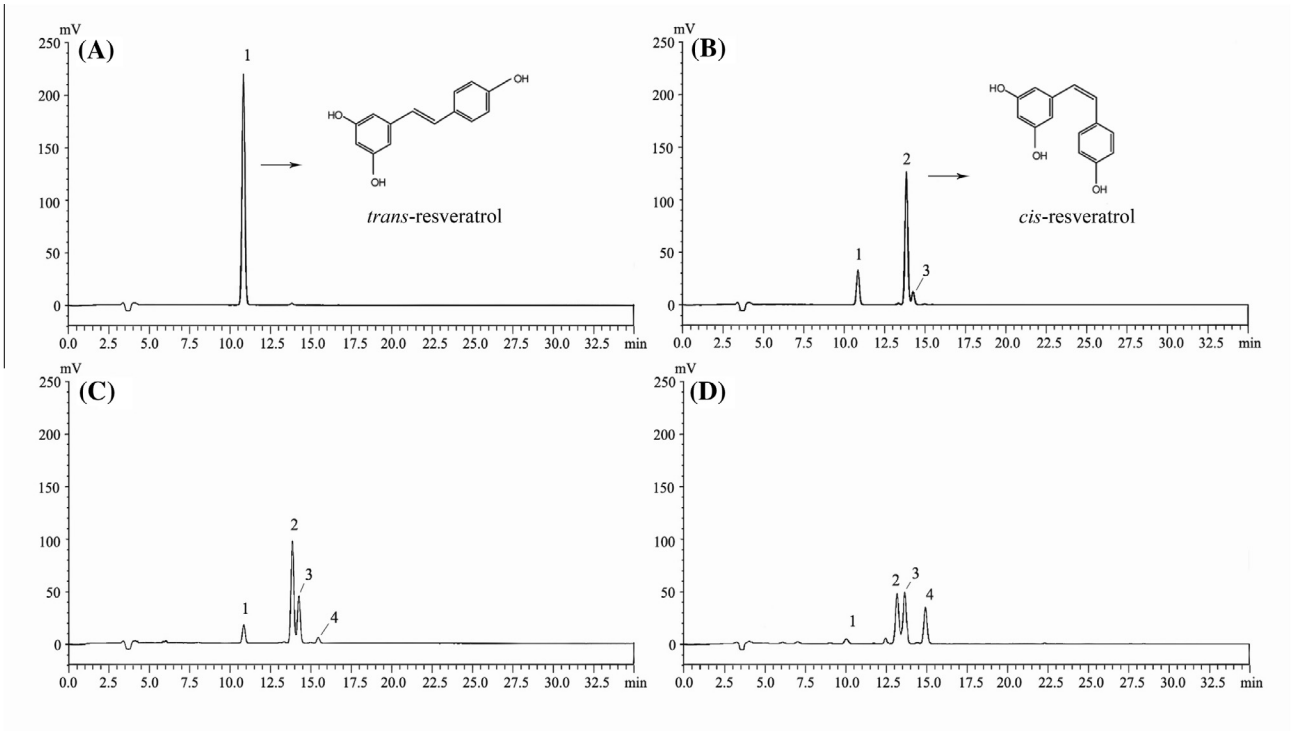
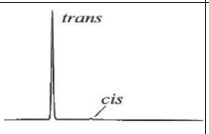
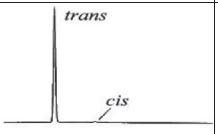
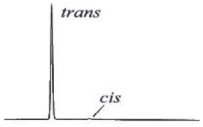
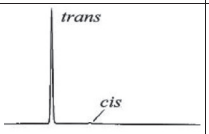
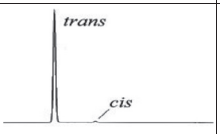
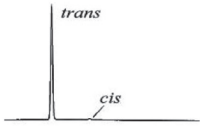
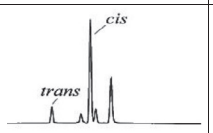
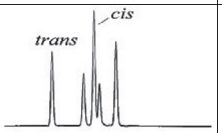
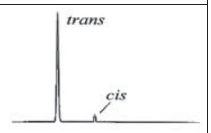
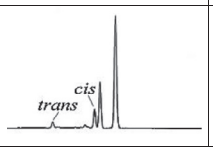
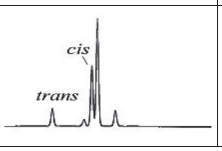
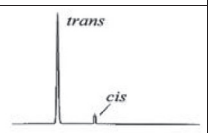


Fig. 1. HPLC profiles of *trans*-resveratrol (0.25 mM, 100% ethanol) under UV-B radiation for 0 min (A), 30 min (B), 90 min (C) and 240 min (D). Peak 1: *trans*-resveratrol; Peak 2: *cis*-resveratrol; Peak 3: compound A; Peak 4: compound B.

cis-resveratrol (Peak 2) meanwhile a small peak 3 appeared (Fig. 1B). Upon 90 min, the height of Peak 3 (compound A) increased significantly, the height of Peak 1 (*trans*-resveratrol) and Peak 2 (*cis*-resveratrol) further decreased and the second new Peak 4 (compound B) appeared (Fig. 1C). As exposure time increased up to 240 min, the height of Peak 4 (compound B) increased remarkably, and the height of Peaks 1 and 2 (*trans*- and *cis*-resveratrol) decreased while Peak 3 corresponding to compound A sustained a high level (Fig. 1D). These results indicated that most *trans*-resveratrol converted to *cis*-resveratrol within 30 min of UV B radiation, and new photoproducts were successively generated as the exposure time to UV B increased. This also explains why different results have been reported regarding photostabilities of *trans*-resveratrol. Difference in exposure time

Table 1
Photostability of *trans*-resveratrol in different mediums.

Irradiation treatment	*trans*-Resveratrol in Water	*trans*-Resveratrol in 100% ethanol	*trans*-Resveratrol in solids
Without irradiation			
Laboratory light [a]			
Sunlight [a]			
UV B [a]			

[a]The *trans*-resveratrol solutions (0.038 mM, water and 0.25 mM, 100% ethanol) and *trans*-resveratrol powder were exposed to laboratory light, sunlight and UV B for 4 h, respectively. Figures in the table are the HPLC profiles of samples.

might be one of plausible reasons. The transformation of *trans*-resveratrol (0.25 mM, 100% ethanol) under 20 min of UV B radiation was investigated to better understand the characteristics of compound A. Three peaks corresponding to *trans*- and *cis*-resveratrol and compound A were observed in the HPLC chromatograms, and compound A presented extremely strong fluorescence (Supplement 1). This is in line with the characteristics of the new compound respectively reported by Rodríguez et al. (2012) and Yang et al. (2012) after UV B irradiating *trans*-resveratrol for 20 min and 1.5 min.

3.3. Structural identification of photoproducts

Fig. 2 shows the mass spectra of *trans*-resveratrol, *cis*-resveratrol, compounds A and B in daughter ion scan mode after MS prescan. Mass spectra of *trans*-resveratrol and *cis*-resveratrol presented similar fragmentation pattern with main peaks being at 143, 159, 185 and 227 *m*/*z* (Fig. 2A and B), which is in line with the reported MS data of *trans*/*cis*-resveratrol (Camont et al., 2009). Compounds A and B had 225 Da and 239 Da molecular mass respectively (Fig. 2C and D). As we known, stilbene-like molecules undergo a reversible cyclisation reaction induced by UV B radiation and convert to phenanthrene derivatives meanwhile lose 2 H (Irie & Mohri, 1988; Jørgensen, 2010; Rodríguez et al., 2012). Phenanthrene derivatives exhibit extremely strong fluorescence due to the highly conjugated structure of phenanthrene skeleton. In our study, *trans*-resveratrol is a derivative of stilbene. The 2 Da decrement of molecular mass and strong fluorescence suggested that compound A might be a phenanthrene derivative originated from *trans*-resveratrol through isomerization and photocyclisation reaction, namely 2,4,6-phenanthrenetriol. Fig. 3A showed the proposed fragmentation pathway of compound A with the molecular mass at *m*/*z* 225. The phenanthrene skeleton of compound A was also testified by its UV spectrum with maximum absorbance at 261 nm that showed the characteristic shape of phenanthrene (Fig. 4C). Phenanthrene has maximum absorbance at 251 nm (Kibbey & Hayes, 1993). The 10 nm difference might be attributed to the substitution of three hydroxyl groups of the phenanthrene nucleus, which leads a shift of λ_{max} value to longer wavelength (bathochromic effect) because of the electron donation of hydroxyls. In Fig. 4A and B, *trans*- and *cis*-resveratrol exhibited their characteristic UV spectra with maximum absorbance at 305 nm and 285 nm as reported (Camont et al., 2009; Nour et al., 2012).

The principal photoproduct compound B was isolated by semi-preparative HPLC for structural identification. The FTIR and NMR spectra data of compound B are as follows: FTIR cm^{-1}: 3351 (OH), 2926 (–CH str), 1650 (>C=O str), 1620, 1584, 1447 (Benzene skeleton vibration), 1055 (C–O–C); ^{1}H NMR (600 MHz, Acetone-d6) δ ppm: 9.10 (s, 1H, ArO–H), 8.20–8.19 (s, 2H, ArH), 7.97–7.92 (d, 2H, ArH, J = 8.34 Hz), 7.34–7.32 (d, 2H, ArH, J = 8.71 Hz), 6.182 (s, 1H, benzofuran H); ^{13}C NMR (600 MHz, Acetone-d6) δ ppm:110.7, 113.4, 119.6, 122.1, 126.8, 131.5, 132.0, 132.9, 134.9, 150.8, 158.4, 160.0, 189.7, 198.6 (Ar–C). Based on the previous works (Aruna Kumar, Nivedita, Krishnaswamy, Sreenivasa, & Mahadevan, 2014; Kumar & Karvekar, 2010; Roaiah et al., 2010; Sako et al., 2004), we consider the unknown compound B as 2-(4-hydroxyphenyl)-5,6-benzofurandione with a benzofuran skeleton, and no CAS number of the new compound has been registered. This was testified by its UV spectrum with a characteristic weak absorption band of furan at ~239 nm (Fig. 4D). Viniferin, the oxidative dimer of resveratrol with a benzofuran skeleton, has been synthetised from *trans*-resveratrol through a visible-light protocatalytic protocol by using mesoporous graphitic carbon nitride and molecular oxygen at room temperature (Song et al., 2014), which suggests a feasible way of generating a benzofuran skeleton from resveratrol. Figs. 2D and 3B showed the MS spectrum and proposed fragmentation pathway of 2-(4-hydroxyphenyl)-5,6-benzofurandione. The ethanol solution of compound B turned from red to blue at pH value >7 (picture not shown), indicating that more conjugated structures are formed in alkaline condition.

3.4. Proposed reaction mechanisms

The photochemistry of phenolics has been an important subject of producing new products in an energy-efficient and environment-friendly way (Bach & Hehn, 2011). Generation of singlet

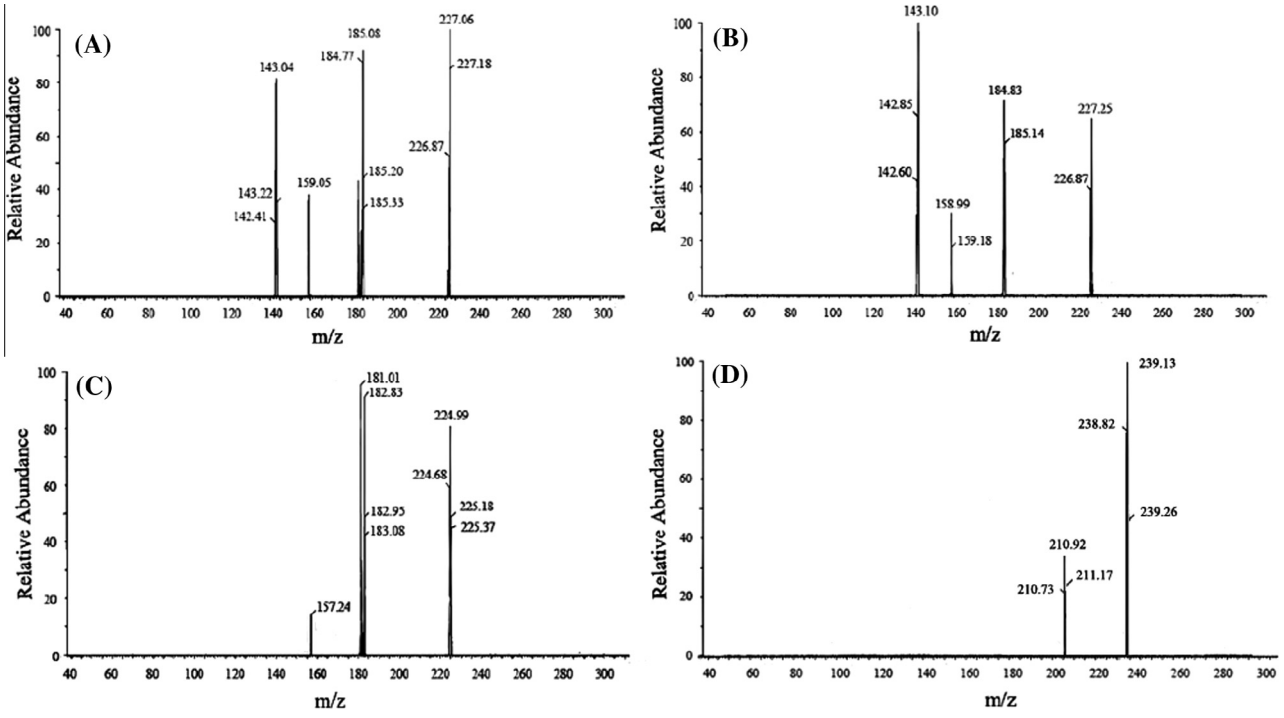


Fig. 2. Mass spectra of *trans*-resveratrol (A), *cis*-resveratrol (B), compound A (C) and compound B (D) in daughter ion scan mode.

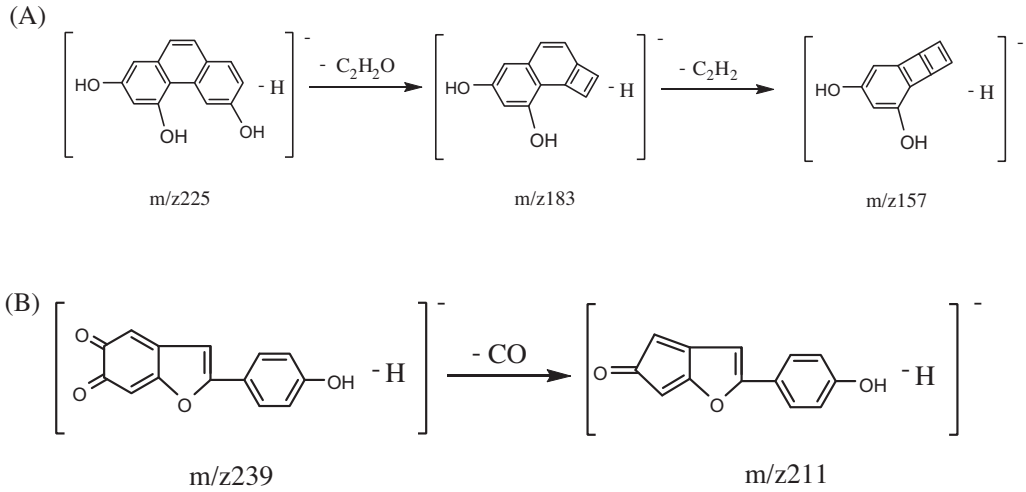


Fig. 3. Proposed fragmentation pathway of the ion at *m*/*z* 225 and 239.

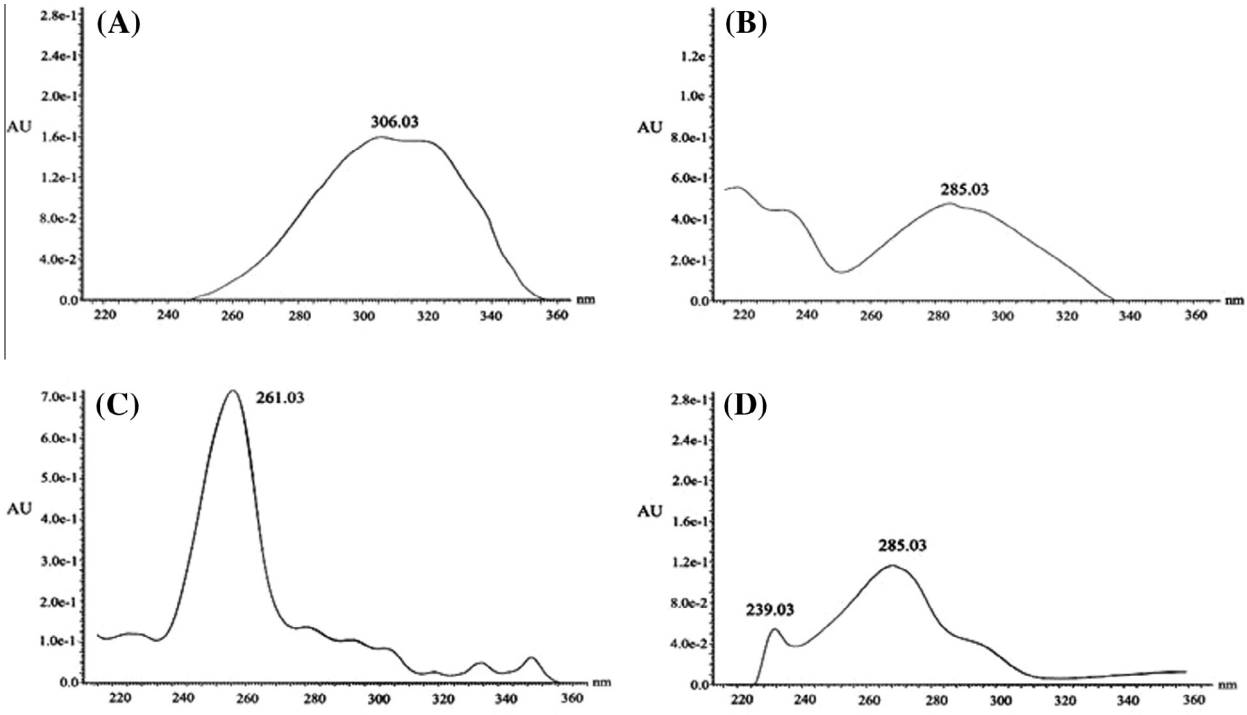


Fig. 4. UV absorption spectra of *trans*-resveratrol (A), *cis*-resveratrol (B), compound A (C) and compound B (D).

oxygen by photosensitized reactions is a pervasive reaction in case of photochemical reactions in solution, and light provides the activation energy. Two major classes of photosensitized oxygenations have been designated as Type I (radical reaction) and Type II (singlet oxygen reaction) (DeRosa & Crutchley, 2002). Singlet oxygen, an electronically excited state of molecular oxygen (O_2), is less stable than the normal triplet oxygen and usually involved in many kinds of reactions, such as [4+2] and [2+2] cycloaddition reactions, ene reactions and organometallic complex oxidation reactions (Hoffmann, 2008). The phenanthrene-like photoproduct has been reported as a photosensitizer to generate singletoxygen under UV radiation due to its planar conjugated structure of phenanthrene (Montanaro, Lhiaubet-Vallet, Iesce, Previtera, & Miranda, 2009). In our case, *trans*-resveratrol is not a photosensitizer pigment that can't generate singlet oxygen, but under UV B radiation *trans*-resveratrol converts to *cis*-resveratrol and then results in the generation of 2,4,6-phenanthrenetriol through photocyclisation reaction meanwhile loss of 2 H (Fig. 5). 2,4,6-Phenanthrenetriol is a phenanthrene-like photoproduct that plays a role of photosensitizer in the photochemical reaction of *trans*-resveratrol by producing singlet oxygen. The singlet oxygen triggers [4+2] cycloaddition reaction of *trans*-resveratrol resulted in the formation of a benzofuran skeleton. After photorearrangement of phenolic groups and oxidation, the new compound 2-(4-hydroxyphenyl)-5,6-benzofurandione is generated (Fig. 5). Analogously, the chemical reaction of *trans*-resveratrol with extrinsic singlet oxygen has been reported, and two types of products were generated through two pathways: Moracin M with a benzofuran skeleton as well as aldehydes (Celaje, Zhang, Guerrero, & Selke, 2011). In our study, 2-(4-hydroxyphenyl)-5,6-benzofurandione is

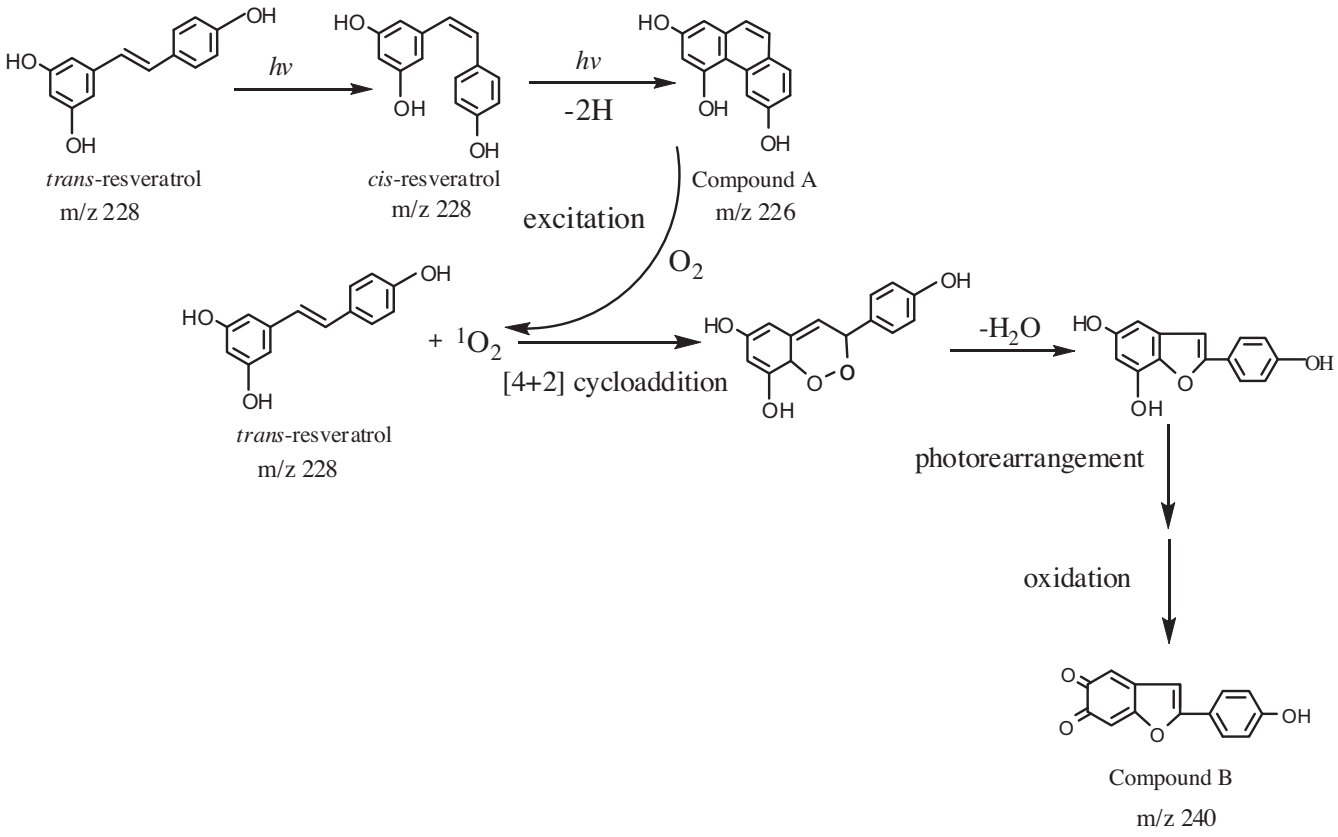


Fig. 5. A proposed mechanism of the generation of compound B.

also a derivative of quinone, which is different from Moracin M with no quinoid structure (Celaje et al., 2011). This may be ascribed to different reaction environments.

However, *trans*-resveratrol has been reported as an efficient quencher of singlet oxygen (Rimando et al., 2002; Sparrow et al., 2003), which seems inconsistent with our conclusion. In fact, no significant change occurred to the high concentration of *trans*-resveratrol (>20 mM, 100% ethanol) under the same UV B radiation (100 μW cm^{-2}, 4 h) as demonstrated before, indicating that the photochemical change of *trans*-resveratrol triggered by singlet oxygen is be closely related to the substrate concentration of *trans*-resveratrol. Singlet oxygen reaction is usually preferred at low substrate concentration, high oxygen concentration and liquid environment. Hence, in our study *trans*-resveratrol preferentially reacts with singlet oxygen at low concentration of *trans*-resveratrol but quenches singlet oxygen at high concentration, and no singlet oxygen reaction occurred to the UV-B irradiated *trans*-resveratrol in solids. Further experiments to elucidate the effects of reaction environments, such as reaction solvent, pH value and spectrum of radiation, on the singlet oxygen reaction of *trans*-resveratrol are in progress.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.08.130.

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