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Photophysical and photochemical properties of resveratrol

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ABSTRACT

Photophysical and photochemical properties of *trans*-resveratrol (*trans*-3,5,4′-trihydroxystilbene, ArOH) were investigated in water and acetonitrile (ACN). S_1–S_n transient absorption spectrum with a peak around 530 nm was observed by a femtosecond transient absorption technique for ArOH in ACN. The excited singlet state lifetime was determined to be 28.6 ps. The radical cation of resveratrol ($ArOH^{\bullet}$+) was generated by resonant two photon ionization in ACN using nanosecond Nd:YAG laser pulses at 355 nm with an energy of 50 mJ. The molar absorption coefficient of $ArOH^{\bullet}$+ was determined to be, ε($ArOH^{\bullet}$+, 500 nm, ACN) = 33400 $M^{-1}cm^{-1}$. The transient $ArOH^{\bullet}$+ deprotonated yielding the long lived phenoxyl radical ($ArO^{\bullet}$) with a peak at 390 nm. This deprotonation was found to occur rapidly in the presence of water. The rate constant for the deprotonation of $ArOH^{\bullet}$+ in ACN, by the addition of small amounts of water (0.10–0.75%), was estimated to be around 1×10^8 M^{-1} s^{-1}. In comparison with molar absorption coefficients of the other phenoxyl radicals, an unexpectedly large value of the molar absorption coefficient of phenoxyl radicals of resveratrol was determined, ε($ArO^{\bullet}$, 390 nm, ACN) = 15200 M^{-1} cm^{-1}. The spectral properties of $ArO^{\bullet}$ were confirmed with pulse radiolysis, and the value of the molar absorption coefficient obtained by laser flash photolysis was found to be in a good agreement with the value obtained by pulse radiolysis, ε($ArO^{\bullet}$, 410 nm, H_2O) = 14600 M^{-1} s^{-1}. These spectral and kinetic data of the transients could contribute to the understanding of mechanisms of resveratrol reactions with biologically relevant radical species.

1. Introduction

trans-Resveratrol (*trans*-3,5,4′-trihydroxystilbene, ArOH)is a natural compound present in plant species such as grapes, peanuts and berries [1]. ArOH is produced in response to stressor agents like fungi so it can be classified as a phytoalexin [2]. In the last several years ArOH has been widely investigated because of its various beneficial biological activities like: anticancer, anti-inflammatory, cardioprotective and antioxidative activity [1,3,4]. The antioxidant activity of resveratrol is related to the ability of its stilbenic and polyphenolic structure to scavenge free radicals by different mechanisms: (1) a one-step hydrogen atom transfer from resveratrol to active radicals, (2) a sequential proton loss electron transfer process from the resveratrol anionic form (phenoxide anion), (3) an electron-transfer process from resveratrol to active radical followed by proton transfer, (4) the addition of an appropriate radical on the double bond of resveratrol [5,6]. Despite previous extensive studies of its biological activity, it is somewhat surprising that some spectral and kinetic data of resveratrol and its transients are still unknown. Also mechanisms of resveratrol reactions with most relevant radical species are scarce. In order to investigate the mechanism of its antioxidative action, it is important to fully characterize all ArOH transients in excited states and intermediates which can be formed in the reactions with different free radicals. The aim of this study was to investigate systematically properties of the ArOH intermediates. We determined that ArOH is unexpectedly a very reactive compound photochemically which undergoes a biphotonic process with the formation of $ArOH^{\bullet}$+. In order to avoid misinterpretation with the ArOH products of monophotonic reactions, we have characterized ArOH excited states and determined ArOH photophysical properties. This is, to our knowledge, the first comprehensive characterization of the resveratrol-derived transients (both qualitative and quantitative). The findings collected in this paper should help in better understanding of the mechanisms of antioxidative action of resveratrol and its other beneficial activities in living organisms.

2. Experimental

2.1. Materials

Resveratrol, sodium azide (NaN_3), 1,4-dicyanoanthracene (1,4-DCA), biphenyl (Bph), di-phenyl methanol ($(C_6H_5)_2CHOH$),

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stilbene and valerophenone were purchased from Sigma–Aldrich at highest purity available and used as received. Di-*tert*-butyl peroxide ((*t*-BuO)$_2$) from Sigma–Aldrich was passed through an alumina column to remove *tert*-butyl hydroperoxide before use. Acetonitrile (ACN) from Sigma–Aldrich was of the CHROMASOLV Plus purity and used as received. Water was purified through a Millipore (Milli-Q) system. High purity gases Ar, N_2O and O_2 were used to purge the freshly prepared reagent solutions for photolysis and radiolysis.

2.2. Nanosecond laser flash photolysis

Samples for time-resolved photolysis were excited using 355 nm or 266 nm Nd:YAG laser pulses (Spectra Physics Mountain View, CA, USA, model INDI 40–10) of 6–8 ns duration. The monitoring system consisted of a 150 W pulsed Xe lamp with the lamp pulser (Applied Photophysics, Surrey, UK), a monochromator (Princeton Instruments, model Spectra Pro SP-2357, Acton, MA, USA), and a R955 model photomultiplier (Hamamatsu, Japan), powered by PS-310 Power Supply (Stanford Research System, Sunnyvale, CA, USA). Data processing system consisted of the real-time acquisition using digital oscilloscope (WaveRunner 6100A, LeCroy, Chestnut Ridge, New York, USA) which was triggered by a fast photodiode (Thorlabs, DET10M, ca. 1 ns rise time) and transferred to the computer equipped with software based on LabView 8.0 (National Instruments, Austin, Texas, USA) which controls the timing and acquisition functions of the system [7]. Data aquired on the nanosecond laser setup were analyzed using Origin 8.0 fitting functions.

2.3. Femtosecond transient absorption

Femtosecond transient absorption spectra and kinetics were recorded on a commercial set-up from Spectra-Physics (USA) and Ultrafast Systems (USA). The laser system consists of a one-box short-pulse titanium–sapphire oscillator with a high-energy titanium–sapphire regenerative amplifier (Solstice, Spectra-Physics, USA, 80 fs at 1 kHz). The 800 nm beam is split into two beams to generate pump and probe pulses. 95% of the beam is directed to the automated optical parametric amplifier (TOPAS Prime, Spectra-Physics) and allows for generation of pump pulses in the wavelength range of 280 nm–2.5 μm. The remaining 5% of the beam is used for white-light continuum generation in a CaF_2 plate (HELIOS, Ultrafast Systems, USA). The two beams are combined in the spectrometer (HELIOS) equipped with a computer-controlled delay line (up to 3 ns). The set-up allows for measurements of events as short as 200 fs. The pump pulse energy in the experiment was set to about 10 μJ. The absorbance was set to about 0.5 at the excitation wavelength (typically 340 nm) in a 2 mm quartz flow-through cell. Transient absorption kinetic traces were analyzed and fitted using a commercial software from Ultrafast Systems (Surface Xplorer 2.2). The transient absorption experiments were carried out at room temperature.

2.4. Pulse radiolysis

The pulse radiolysis experiments were performed with the nanosecond INCT LAE 10 linear accelerator (10 MeV, 7–10 ns pulse duration, Institute of Nuclear Chemistry and Technology, Warsaw, Poland) [8].

The optical detection system consisted of a xenon lamp, monochromator (Spectra Pro-275), a 5-stage photomultiplier tube (Hamamatsu R955) with a wide spectral response (160–900 nm) powered with the HV Power Supply PS310 (Stanford Research Systems) and a PC computer with a software which was written using Delphi 3 (Borland) within Windows 9x/NT/ME [8]. The program controls most of the peripherals over the GPIB (IEEE488) and RS-232 and RS-485 lines. The data acquisition system allows for kinetic traces to be displayed on multiple time scales from a few nanoseconds to tens, or even hundreds of microseconds.

2.5. Preparation of samples

Laser flash photolysis and pulse radiolysis experiments were carried out in quartz cell (1 cm monitoring light path length for nanosecond and 2 mm path length for femtosecond measurements). Flow systems were used in all experiments with freshly prepared solutions of ArOH. All samples were purged with high purity Ar for 20 min before photolysis and with the N_2O 20 min before radiolysis. Actinometry was based on laser pulse generation of a transient triplet state, ^{3}BP* (benzophenone) in ACN measured at λ_{max} = 520 nm, $\varepsilon(^3BP^*, 520\,nm, ACN) = 6500\,M^{-1}\,cm^{-1}$ [9]. Dosimetry in pulse radiolysis experiments at typically 10 Gy per pulse was obtained using potassium thiocyanate. Absorption spectra of compounds were taken by a UV–vis Cary 300 Bio spectrophotometer and Cary 2200 (Varian, Mulgrave, Victoria, Australia). All measurements were carried out at room temperature.

2.6. UV-photolysis

Determination of the quantum yield of isomerisation of resveratrol was carried out in 15 ml quartz cuvettes using a Luzchem reactor equipped with 2 lamps with an output at 254 nm. Valerophenone was used as the standard, with its known quantum yield of isomerization in water, $\Phi_{iso} = 0.65$ [10]. ArOH in ACN solutions and valerophenone were freshly prepared and purged with N_2 for 20 min, and their concentrations adjusted to the same absorbances at 254 nm. Solutions were then irradiated in the Luzchem reactor for the same time of 15, 30, and 60 s. After each irradiation the isomerisation of resveratrol as well as conversion of valerophenone were analyzed by HPLC. Using stilbene as an actinometer, $\Phi_{iso} = 0.56$ [11], the quantum yield for the isomerisation of ArOH at 254 nm was confirmed spectroscopically.

3. Results and discussion

3.1. Photophysical properties of ArOH

Transient absorption spectroscopy with sub-picosecond time resolution was used for measurements of singlet–singlet absorption spectra and the excited state kinetics of ArOH. After 360 nm femtosecond laser excitation of ArOH in ACN, the S_1–S_n absorption spectrum was observed with a peak around 530 nm (Fig. 1).

The small blue shift of the spectra with the time was assigned to the vibrational cooling of the excited S_1 state. The short lifetime of ArOH excited state (ArOH*) is due to all unimolecular decay processes. The rate constant of the overall deactivation of the excited singlet state of ArOH is the sum of the rate constants of the processes:

$$k_d = (k_f + k_{IC} + k_{ISC} + k_{ISO})s^{-1} \quad (1)$$

where *f* stands for fluorescence, IC for internal conversion, ISC for intersystem crossing leading to the formation of the ArOH triplet state, ^{3}ArOH, and ISO for *trans-cis* isomerisation of ArOH. By fitting data of kinetic trace of ArOH at 501 nm to the first order kinetic, decay rate constant k_d was determined to be $k_d = 1/28.6$ ps, from which the decay rate constant is derived, $3.5 \times 10^{10}\,s^{-1}$.

The ArOH* fluorescence was measured using anthracene as a reference compound with a known quantum yield in EtOH, $\Phi_f = 0.27$ [12]. The fluorescence quantum yield of ArOH was