PAPER

NJC
www.rsc.org/njc

Hydrogen atom abstraction from resveratrol and two lipophilic derivatives by *tert*-butoxyl radicals. A laser flash photolysis study

Salvatore Petralia,[a] Carmela Spatafora,[a] Corrado Tringali,[a] Mario C. Foti[b] and Salvatore Sortino*[a]

[a] *Dipartimento di Scienze Chimiche, Università degli Studi di Catania, Viale Andrea Doria 8, I-95125 Catania, Italy. E-mail: ssortino@unict.it*
[b] *Istituto di Chimica Biomolecolare del CNR – Sez. di Catania, Via del Santuario 110, I-95028 Valverde, (CT), Italy*

Received (in Montpellier, France) 14th July 2004, Accepted 24th August 2004
First published as an Advance Article on the web 10th November 2004

The reactions of *tert*-butoxyl radicals with resveratrol (**1**) and two acetylated derivatives (**2** and **3**) have been investigated by laser flash photolysis techniques in 1 : 2 (v/v) benzene–di-*tert*-butyl peroxide at room temperature. The transient absorption spectra of the phenoxyl radicals generated upon H atom abstraction by *tert*-butoxyl radicals from the phenols have been detected and assigned. The absolute rate constants for these reactions have been evaluated to be 45×10^7, 25×10^7 and 4×10^7 M^{-1} s^{-1} for **1**, **2** and **3**, respectively. The order of reactivity **1** $\geq$ **2** $\gg$ **3** has been rationalized in terms of the position and effect of the acetyl groups on the aromatic rings. Of the three OH groups present in resveratrol, the one in position 4′ appears to be the most reactive due to the large stability of the corresponding phenoxyl radical by conjugation with the rings. However, in our system, the H-atom-donating ability of resveratrol turns out to be inferior to that of α-tocopherol by *ca.* one order of magnitude.

Introduction

Resveratrol (**1**, see Chart 1) is a natural stilbene polyphenol contained in grape berries, red wines (0.1–10 mg L^{-1}), as well as in a number of plant species.[1] Lately, this phenol has been the subject of a massive investigation for the conventional wisdom holds that **1** is the key to the so-called "French Paradox", that is the fact that regular consumers of red wine in certain regions of France have a low risk of heart disease despite a diet overabundant in fat.[2]

Compound **1** has, therefore, been accredited with antioxidant properties[3,4] as well as other biological activities, for example, inhibition of the lipoxygenases,[5] protection against ischemia reperfusion injury[6] and cancer chemoprevention in humans.[7] However, the most surprising fact emerging from these investigations is the scarce amount of spectral and kinetic data on the reactions of this polyphenol with the most relevant radical species when such information could help in the comprehension of the aforementioned biological properties. In this concern, only recently, Stojanović and Brede have reported the first direct evidence of the reactivity of **1** and its analogues with $HO^{\bullet}$ radicals.[8] Interesting insight into the antioxidant potential of **1** has also been reported by Amorati *et al.* who determined the rate constant of H atom abstraction (k_H) from **1** by peroxy radicals in homogeneous solutions.[9] In this work it has been pointed out that, in contrary to the general belief, **1** is only a modest antioxidant. The reported value of k_H relative to **1** is in fact *ca.* one order of magnitude smaller than that of α-tocopherol as determined under the same experimental conditions. However, the antioxidant properties of **1** were previously claimed to be comparable to those of α-tocopherol.[3] On the other hand, Liu and co-workers have recently demonstrated that **1** is an effective antioxidant in microheterogeneous media such as micellar systems.[10] This overall scenario suggests that further work is required to shed light into the mechanisms of action and the biological properties of **1**.

The present study aims to provide a contribution to the spectroscopic and kinetic data regarding the reactivity of **1** towards active radicals. In particular, we consider of interest the investigation of the hydrogen atom abstraction from **1** and two more lipophilic derivatives, **2** and **3** (see Chart 1), by *tert*-butoxyl radicals produced *in situ* by laser flash photolysis.

Experimental

Chemicals

trans-Resveratrol was purchased from Sigma and used as received. Compounds **2** and **3** were obtained *via* regioselective enzymatic reactions, as previously reported by some of us.[11] Di-*tert*-butyl peroxide was purchased from Aldrich and was passed through alumina before use. Benzene was of spectrophotometric grade.

Nanosecond laser flash photolysis setup

All samples were excited with the third harmonic of a Nd-YAG Continuum Surelite II-10 laser system (pulse width 6 ns FWHM) and analyzed at a right angle geometry using a mini mLFP-111 apparatus developed by Luzchem Research. The monitoring beam was supplied by a ceramic xenon lamp and

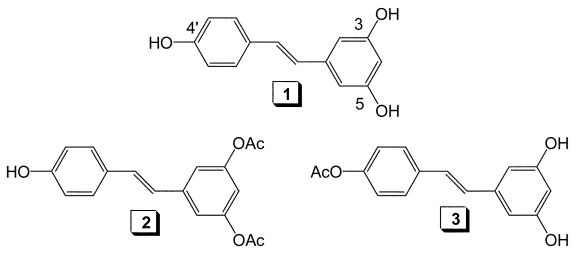


Chart 1

DOI: 10.1039/b410802g

delivered through quartz fibre optical cables. The laser pulse was probed by a fibre that synchronized the mLFP system with a Tektronix TDS 3032 digitizer operating in the pre-trigger mode. The signals from a compact Hamamatsu photomultiplier were initially captured by the digitizer and then transferred to a personal computer that controlled the experiments with Luzchem software developed in the LabView 5.1 environment, from National Instruments. The laser pulse energy was measured at each laser shot by a SPHD25 Scientech pyroelectric energy monitor. All samples were purged by bubbling through a vigorous and constant flow of pure argon (previously saturated with the solvent). For all experiments the solutions were renewed after each laser shot (in a flow cell of 1 cm optical path). The sample temperature was 295 ± 2 K

Results and discussion

The experiments described hereafter were carried out in a 1 : 2 (v/v) solvent mixture of benzene–di-*tert*-butyl peroxide. *tert*-Butoxyl radicals were generated, eqn. (1), *in situ* by fast laser flash photolysis of di-*tert*-butyl peroxide (within the duration of the laser pulse of *ca.* 6 ns), using the third harmonic of a Nd-YAG laser (λ_{exc} = 355 nm) as excitation light source:

$$tert\text{-BuOOBu-}tert \xrightarrow{h\nu} 2tert\text{-BuO}^{\bullet} \qquad (1)$$

tert-Butoxyl radicals may decay by β-cleavage and reaction with solvent, eqn. (2).[12] However, in the presence of hydrogen atom donors, such as compounds **1–3** (ArOH), the *tert*-butoxyl radicals are capable of abstracting a hydrogen atom from the phenolic OH group, thus generating a phenoxyl radical ArO$^{\bullet}$, eqn. (3).

$$tert\text{-BuO}^{\bullet} \xrightarrow{k_2} \text{decay} \qquad (2)$$

$$tert\text{-BuO}^{\bullet} + \text{ArOH} \xrightarrow{k_3} \text{ArO}^{\bullet} + tert\text{-BuOH} \qquad (3)$$

Laser flash photolysis with a nanosecond time-resolution is a powerful tool for obtaining spectroscopic and kinetic features of phenoxyl radicals. Indeed, these species are generally characterized by lifetimes in the microsecond timescale and spectral absorptions in the UV/Vis region.[14] For instance, Fig. 1 shows a typical buildup trace (following laser excitation) at 400 nm as observed during the growth of phenoxyl radicals from **1** as described in eqn. (3).

The absorption spectra (which were normalized for the sake of clarity) of the phenoxyl radicals generated from **1–3** are

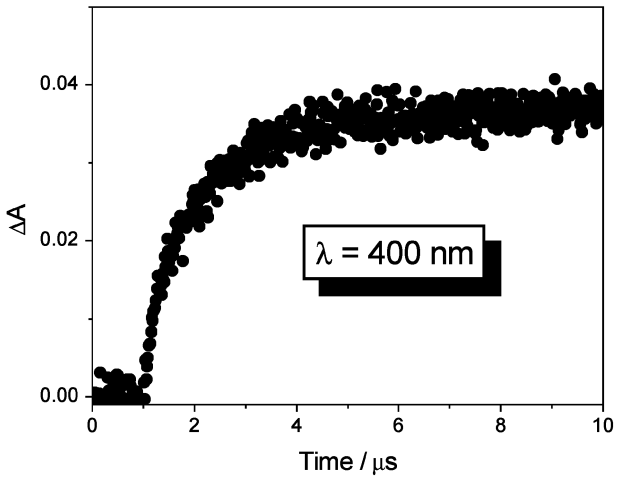


Fig. 1 Representative experimental trace showing the buildup of phenoxyl radicals at 400 nm observed after a 355 nm laser shot of a 1 : 2 benzene–di-*tert*-butyl peroxide solution of **1**. $E_{355} \simeq 5$ mJ pulse^{-1}; [**1**] = 1 mM.

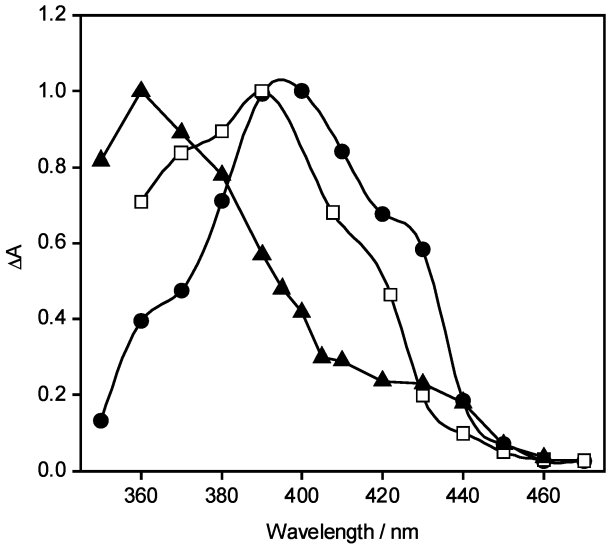


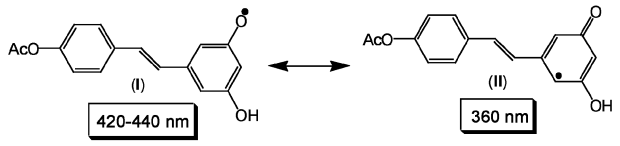
Fig. 2 Normalized time-resolved absorption spectra observed after 355 nm laser excitation of argon-saturated 1 : 2 benzene–di-*tert*-butyl peroxide solutions containing compounds **1** (●), **2** (□) and **3** (▲). Each spectrum was recorded at a delay time at which the formation of phenoxyl radicals had reached completion (plateau region in Fig. 1).

shown in Fig. 2.[15] Of the three different hydroxyl groups present in resveratrol, the one in position 4′ is certainly the most reactive towards radicals. The corresponding phenoxyl radical is, in fact, largely stabilized by spin-delocalization from both aromatic rings.[9] The bond dissociation enthalpy of the OH in 4′ is therefore significantly lower than that of the OH's in position 3 and 5, hence the H atom abstraction at position 4′ is faster. On these grounds, the transient spectrum observed when ArOH = **1** and reported in Fig. 2 can safely be assigned to a phenoxyl radical derived from the 4′-hydroxyl group. This spectrum is also very similar, except for a small blue shift,[16] to the one obtained in the reaction of **1** with HO$^{\bullet}$ radicals produced by pulse radiolysis in aqueous solution.[8]

Undoubtedly, 4′-phenoxyl radicals are also generated in the case of the reaction of **2** with *tert*-BuO$^{\bullet}$. The spectral features of this phenoxyl radical, see Fig. 2, are similar to those of the phenoxyl radical derived from **1**, except for a blue-shift attributable to the presence of electron-withdrawing acetyl groups in positions 3 and 5.

In the case of compound **3**, the selective acetylation of the hydroxyl in position 4′ makes the H atom abstraction reaction possible only from one of the two equivalent *meta*-OH's in positions 3 and 5. Indeed, the absorption spectrum of the phenoxyl radical obtained in this case is quite different from the others, see Fig. 2. This spectrum is characterized by a major band at around 360 nm and a weaker broad band in the range 420–440 nm. Since substitution at the *meta* position of a phenol generally leads to a red-shift in the spectrum of the corresponding phenoxyl radical,[14] we can reasonably attribute the 420–440 nm absorption to either 3- or 5-phenoxyl radicals generated from **3** (see structure I in Scheme 1). Regarding the 360 nm band, we believe that it may be attributed to a mesomeric quinoid contribution II to the electronic configuration of the above radical (see Scheme 1). This is in agreement with the assignment recently proposed by Stojanović and Brede of the spectrum of phenoxyl radicals obtained by pulse radiolysis of 3,5-dihydroxystilbene aqueous solutions.[8]

In order to determine the absolute rate constants k_3 we used a low laser pulse energy (<3.5 mJ per pulse). Such a weak laser impulse ensures that (i) the initial concentration of *tert*-BuO$^{\bullet}$ radicals is small, and (ii) the heating of the solution is negligible. Under these experimental conditions, the loss of *tert*-butoxyl radicals by the reverse of eqn. (1) is negligible and the observed rate constant, k_{obs}, for the growth of ArO$^{\bullet}$, eqn. (3),



Scheme 1

is related to the concentration of ArOH *via* the simple linear eqn. (4):

$$k_{obs} = k_2 + k_3[ArOH] \quad (4)$$

Fig. 3 reports the plots of k_{obs} *vs.* [ArOH] obtained by changing the concentration of **1–3** and determining the corresponding values of k_{obs} for the growth of ArO$^{\bullet}$.[17] Eqn. (4) appeared to be valid under all the experimental conditions employed.[17] It is worth noting that the *y*-intercept obtained under our conditions, $k_2 \simeq 5.4 \times 10^5$ s^{-1}, is in excellent agreement with other earlier values (5.7×10^5 s^{-1}) recorded in the same solvent mixture.[13a] The reciprocal of k_2 represents the lifetime of *tert*-BuO$^{\bullet}$, $\tau \simeq 1.8$ μs.

The slopes of the straight lines afforded the values of k_3 for all three compounds. In the case of **1**, we found that k_3 was *ca.* 4.5×10^8 M^{-1} s^{-1}. This value is almost one order of magnitude smaller than that reported for H atom abstraction from α-tocopherol by *tert*-BuO$^{\bullet}$ radicals in the same solvent mixture.[18] This relatively large difference is close to the difference recently found by Amorati *et al.* by using ROO$^{\bullet}$ as H-atom-abstracting radicals.[9] Indeed, this is not surprising since the relative reactivity of phenols is independent of the abstracting radical.[19]

As expected, the reactivity of compound **2** in comparison to **1** is only slightly decreased by the presence of the two *meta*-acetyl groups since the value of k_3 was found to be *ca.* 2.5×10^8 M^{-1} s^{-1}. This decrease in reactivity can most likely be attributed to the destabilization of the phenoxyl radical issued from **2** due to the presence of two electron-withdrawing acetyl substituents. Indeed, this trend is typically observed in a large variety of phenol derivatives.[14] In contrast, the reactivity of the mono-acetylated **3** towards *tert*-BuO$^{\bullet}$ radicals was much lower than those of the two other compounds. The value of k_3 dropped in this case to *ca.* 4×10^7 M^{-1} s^{-1}, that is, *ca.* one order of magnitude below that of **1**. We believe that this may reflect both the low intrinsic reactivity of the *meta*-hydroxyl groups, with respect to the 4′-OH group, and the additional strong deactivating effect of the 4′-acetyl group on the ring bearing the reactive OH groups.

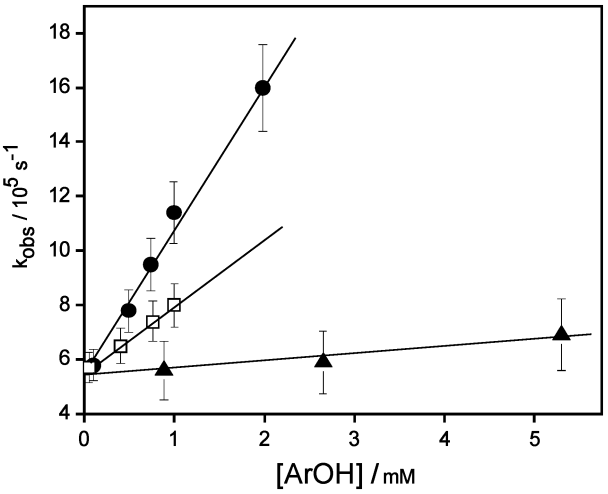


Fig. 3 Plots of k_{obs} according to eqn. (4) for compounds **1** (●), **2** (□) and **3** (▲).

In conclusion, we have detected and assigned the UV-Vis spectra of transient phenoxyl radicals generated by the reactions of **1–3** with *tert*-BuO$^{\bullet}$ radicals in benzene–*tert*-butyl peroxide solution at room temperature. The rate constants determined for these reactions show that the order of reactivity of **1–3** towards *tert*-BuO$^{\bullet}$ is **1** ≥ **2** ≫ **3**. Regioselective acetylation of the OH groups in positions 3 and 5 decreases only very slightly the reactivity of the remaining 4′-OH group. Conversely, acetylation of the 4′-OH group causes a reduction of *ca.* 10-fold in the reactivity of **3** with respect to **1**. Furthermore, our results confirm that the hydrogen-atom-donating ability of resveratrol is significantly lower than that of α-tocopherol. We finally want to point out that the diacetyl derivative **2** represents a suitable and appealing candidate for an investigation in micellar systems where resveratrol proved to be an excellent antioxidant.[10] Compound **2** is indeed a hydrogen atom donor comparable to resveratrol but characterized by a higher lipophilicity, which might favour solubility in such microenvironments. The results of this forthcoming investigation will be reported in due course.

Acknowledgements

We thank MIUR (PRIN, Rome, Italy, research projects) for their financial support.

References

1 (*a*) L. Fremont, *Life Sci.*, 2000, **66**, 663; (*b*) D. M. Goldberg, A. Karumanchiri, E. Ng, J. Yan, E. P. Diamantis and G. J. Soleas, *J. Agric. Food Chem.*, 1995, **43**, 1245.
2 See, for instance: (*a*) R. Corder, J. A. Douthwaite, D. M. Lees, N. Q. Khan, A. C. V. D. Santos, E. G. Wood and M. J. Carrier, *Nature (London)*, 2001, **414**, 863; (*b*) G. J. Soleas, E. P. Diamandis and D. M. Goldberg, *Clin. Biochem.*, 1997, **30**, 91; (*c*) S. Renaud and M. de Lorgelil, *Lancet*, 1992, **339**, 1523.
3 (*a*) B. Fauconneau, P. Waffo-Teguo, F. Huguet, L. Barrier, A. Decentit and J.-M. Merillon, *Life Sci.*, 1997, **61**, 2103; (*b*) D. G. Soares, A. C. Andreazza and M. J. Salvador, *J. Agric. Food Chem.*, 2003, **51**, 1077.
4 A. M. Alonso, C. Dominigues, D. A. Guillen and C. G. Barroso, *J. Agric. Food Chem.*, 2002, **50**, 3112.
5 (*a*) M. C. Pinto, J. A. García-Barrado and P. Macias, *J. Agric. Food Chem.*, 1999, **47**, 4842; (*b*) B. Tadolini, C. Juliano, L. Piu, F. Franconi and L. Cabrini, *Free Radical Res.*, 2000, **331**, 105–114.
6 L. M. Hung, J. K. Chen, S. S. Huang, R. S. Lee and M. J. Su, *Cardiovasc. Res.*, 2000, **47**, 549.
7 (*a*) M. S. Jang, E. N. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. Beecher, H. H. S. Fong, N. R. Rarnworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon and J. M. Pezzuto, *Science*, 1997, **275**, 218; (*b*) T. M. A. ElAttar and A. S. Virji, *Anti-Cancer Drugs*, 1999, **10**, 187.
8 S. Stojanović and O. Brede, *Phys. Chem. Chem. Phys.*, 2002, **4**, 757 and references therein.
9 R. Amorati, F. Ferroni, G. F. Pedulli and L. Valgimigli, *J. Org. Chem.*, 2003, **68**, 9654.
10 J.-G. Fang, M. Lu, Z.-H. Chen, H.-H. Zhu, Y. Li, L. Yang, L.-M. Wu and Z.-L. Liu, *Chem. Eur. J.*, 2002, **8**, 4191.
11 G. Nicolosi, C. Spatafora and C. Tringali, *J. Mol. Catal. B: Enzymol.*, 2002, **16**, 223.
12 Detailed descriptions of the hydrogen-atom abstraction reactions by *tert*-butoxyl radicals are given in milestone papers by Scaiano and co-workers[13].
13 (*a*) R. D. Small and J. C. Scaiano, *J. Am. Chem. Soc.*, 1978, **100**, 296; (*b*) H. Paul, R. D. Small and J. C. Scaiano, *J. Am. Chem. Soc.*, 1978, **100**, 4520.
14 P. K. Das, M. V. Encinas, S. Steenken and J. C. Scaiano, *J. Am. Chem. Soc.*, 1981, **103**, 4162.
15 Exploration of the spectral window below 350 nm was precluded because of the strong absorption of the ground-state of the solution components (benzene and **1–3**).
16 A small blue-shift of the absorption maxima when changing from water to benzene/di-*tert*-butyl peroxide solutions is characteristic of phenoxyl radicals[14].

17 Higher phenol concentrations than those reported in Fig. 3 could not be explored because of the significant ground-state absorption of compounds **1–3** at the excitation wavelength.

18 C. Evans, J. C. Scaiano and K. U. Ingold, *J. Am. Chem. Soc.*, 1992, **114**, 4589.

19 D. W. Snelgrove, J. Lusztyk, J. T. Banks, P. Mulder and K. U. Ingold, *J. Am. Chem. Soc.*, 2001, **123**, 469 and references therein.