Free Rad. Res., Vol. 35, pp. 85–91
Reprints available directly from the publisher
Photocopying permitted by license only

Structure of the Radical from One-electron Oxidation of 4-Hydroxycinnamate

ROGER H. BISBY[a,*] and ANTHONY W. PARKER[b]

[a]*Division of Biological Sciences, School of Environmental and Life Sciences, University of Salford, Salford, M5 4WT, UK;*
[b]*Lasers for Science Facility, Rutherford Appleton Laboratory, Chilton, Oxfordshire, OX11 0QX, UK*

Accepted by Prof. C. Rice-Evans

(Received 25 September 2000; In revised form 9 October 2000)

Radicals from one-electron oxidation of 4-hydroxycinnamate, ferulate and 3,4-dihydroxycinnamate have been formed by reaction with the oxidising triplet state of duroquinone. All three compounds react with triplet duroquinone with second order rate constants close to the diffusion-controlled limit. The identity of the resulting radicals is confirmed by observation of their characteristic visible absorption spectra. Time-resolved resonance Raman (TR^3) spectra of the radical from 4-hydroxycinnamate were measured using a probe laser wavelength of 600 nm, to be in resonance with the long wavelength absorption band of the radical. The TR^3 spectra contain prominent bands ascribed to the C—O and ring C—C stretching vibrations. The spectra are interpreted as indicating strong delocalisation of the radical site to the double bond in conjugation with the aromatic ring in 4-hydroxycinnamate. This contributes to the low reduction potential of the radical and the antioxidant properties of hydroxycinnamates.

Keywords: Radical, hydroxycinnamate, time-resolved, resonance Raman, spectroscopy, oxidation

Abbreviations: 4-HC, 4-hydroxycinnamate; 3,4-DHC, 3,4-dihydroxycinnamate; FA, ferulic acid; DQ, duroquinone; TR^3, time-resolved resonance Raman

INTRODUCTION

Chain breaking antioxidants are responsible for the repair of lipid peroxyl radicals ($LOO^\bullet$) in membranes and lipoproteins, preventing deleterious damage to these structures. The most important of these is α-tocopherol (vitamin E, T-OH).[1] The favourable stereoelectronic properties of the α-tocopheroxyl radical ($T\text{-}O^\bullet$) which is formed in the chain-breaking reaction (1)

$$LOO^\bullet + T\text{-}OH \rightarrow LOOH + T\text{-}O^\bullet \qquad (1)$$

*Corresponding author. Tel.: (0161) 295 4912. Fax: (0161) 295 5210. E-mail: r.h.bisby@salford.ac.uk.

contribute to the stability and low reactivity of T-O$^{\bullet}$, enhancing the effectiveness of α-tocopherol as an antioxidant.[2] There is growing interest in other naturally occurring phenolic compounds that display biological antioxidant properties. These include the hydroxycinnamates of which the most important are 4-hydroxycinnamic acid (4-HC, *p*-coumaric acid), ferulic acid (FA, 4-hydroxy-3-methoxycinnamic acid) and 3,4-dihydroxycinnamic acid (3,4-DHC, caffeic acid). These compounds appear as components of many plant-derived foods.[3–5] Such compounds have been demonstrated to react with aqueous free radicals,[6] to inhibit lipoprotein oxidation,[7] and to react with oxidising $^{\bullet}$OH adducts of pyrimidines.[8,9] Foley *et al.*[10] have also shown that these compounds are also modest singlet oxygen quenchers (second order quenching rate constant, $k_q = 4 \times 10^6$ to 4×10^7 dm^3 mol^{-1} s^{-1}) compared with the water-soluble α-tocopherol analogue Trolox C ($k_q = 4.4 \times 10^8$ dm^3 mol^{-1} s^{-1}).[11] The same authors have also measured the one-electron reduction potentials of the radicals from these hydroxycinnamates and found them to be between 50 to 100 mV more positive that of the Trolox C radical, confirming them to be rather weaker antioxidants than Trolox C on this basis.

Time-resolved resonance Raman spectroscopy enables vibrational spectra of short-lived free radicals to be obtained using nanosecond pulsed lasers[12] and provides insights into the structural details of the intermediates not readily available through other time-resolved techniques. The resonance Raman spectra of phenoxyl radicals have been studied in detail.[13–16] In the case of several phenoxyl radicals having 4-substituents (-OCH_3, F, CH_3 etc),[15] the spectra indicate a structure approaching that of the semiquinone radical, indicating substantial involvement of the pπ electrons of the substituent with the π systems of the phenoxyl radical. Similar effects have been noted in the resonance Raman spectra of the tocopheroxyl[17] and probucol[18] radicals. In effect these observations substantiate the conclusions of Burton and Ingold,[2] based partly on ESR spectra. These suggest that the high effectiveness of α-tocopherol as an antioxidant stems from the delocalisation of the electrons from the 2pπ orbital of the chroman oxygen atom to the phenoxyl site in the tocopheroxyl radical, promoted by the rigid structure of the chromanol system.

MATERIALS AND METHODS

All chemicals used in these experiments were used as received from Sigma. Solvents were either HPLC or Spectroscopic grade. Water was obtained from a MilliQ purification unit.

Laser flash photolysis experiments were undertaken using the output of a dye laser (360 nm, ~1 mJ pulse^{-1}, 10 ns pulse width) pumped by a XeCl excimer laser. Transient absorption spectra were determined with a spectrometer system containing a tungsten lamp and photodiode detector. Solutions were thoroughly degassed by bubbling with argon. Time-resolved resonance Raman experiments were undertaken using the apparatus as described previously.[18] Briefly, the excitation (pump) laser pulse was the same as in the flash photolysis experiments, giving a pulse energy of ca 0.1 mJ pulse^{-1} (10 Hz) at the sample, which was circulated in a quartz tube (2 mm internal diameter). The probe laser beam (0.1 mJ pulse^{-1} at the sample) was obtained from a Continuum Sunlight OPO system. The scattered Raman signal was dispersed by a Spex Triplemate and detected using a thinned back-illuminated CCD detector. Frequency calibration was obtained from the known Raman spectrum of toluene and is expected to be ± 2 cm^{-1}.

RESULTS AND DISCUSSION

Oxidation of Hydroxycinnamates by Triplet Duroquinone

The radicals from 4-HC, 3,4-DHC and FA were generated by laser flash photolysis (360 nm) of a

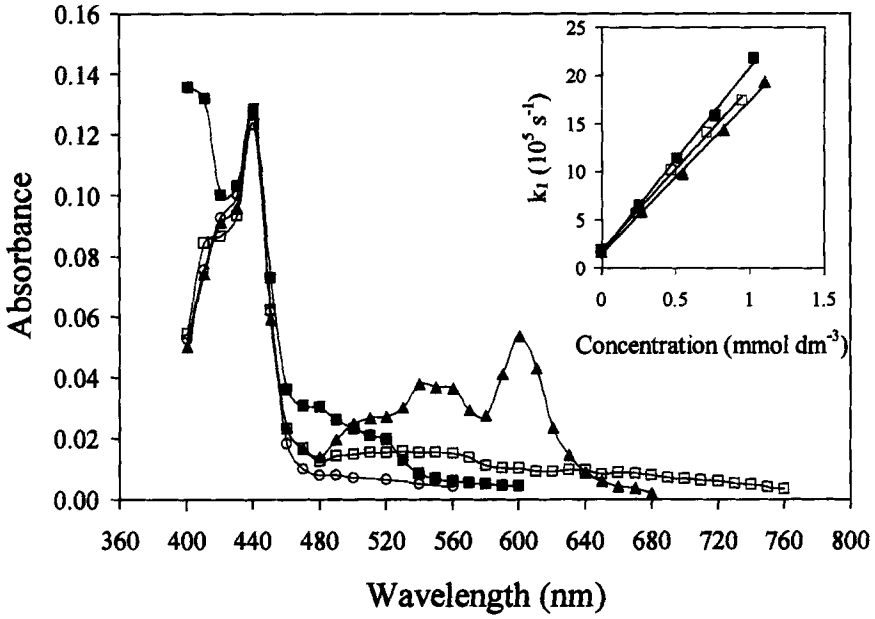


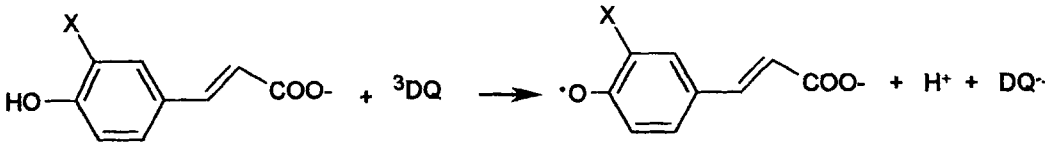
FIGURE 1 Transient absorption spectra of free radicals from one-electron oxidation of hydroxycinnamates by triplet duroquinone measured by laser flash photolysis. The solutions in ethanol/water (50/50 v/v) contained phosphate buffer (25 mmol dm^{-3}, pH 7.0), duroquinone (2.5 mmol dm^{-3}) and hydroxycinnamate (1 mmol dm^{-3}). The spectra are from solutions of 4-HC (▲), FA (□) and 3,4-DHC (■). Also shown for comparison is the spectrum from a similar solution containing ascorbate (○). Inset: First order rate constants (k_1) for decay of the ^{3}DQ absorption at 490 nm and 25 °C as a function of solute concentration, symbols as in the main figure.

solution of the appropriate solute and duroquinone (DQ). Excitation of DQ leads initially to the singlet state which relaxes on a picosecond timescale to the oxidising triplet state $E_o(^3DQ/DQ^{-\bullet} = 2.17\,V)$.[19] Rates of reaction of ^{3}DQ with the solutes were determined from measurements of the decay of the ^{3}DQ absorption at 490 nm in deaerated (argon purged) solutions containing phosphate buffer (25 mmol dm^{-3}, pH 7.0) in ethanol/water (50/50 v/v). The inset to Figure 1 shows the first order rate constants plotted against solute concentrations. The intercepts indicate the lifetime of ^{3}DQ to be ~5 μs in deaerated solution in the absence of reactive solute. The slopes of these plots give the second order rate constants for reaction of ^{3}DQ with 4-HC, 3,4-DHC and FA shown in Table I to be in the range (1.5–2.0) × 10^9 dm^3 mol^{-1} s^{-1}. These values are approaching the diffusion-controlled limit in accord with the high oxidation potential for ^{3}DQ. The increase in reactivity 4-HC < FA < 3,4-DHC appears significant considering the experimental error and follows the same trend established for reaction of singlet oxygen and the azidyl radical with these same compounds[10] (see Table I). Whilst the radicals from one-electron oxidation of 4-HC and FA have similar reduction potentials, the lower value for 3,4-DHC appears to be correlated with its higher reactivity.

In the presence of 1 mmol dm^{-3} of one of the hydroxycinnamates, the lifetime of ^{3}DQ will be of the order of 500 ns according to the second order rate constants described above. The transient absorption spectra in Figure 1 measured 10 μs after the laser pulse therefore represent the spectra of the product hydroxycinnamate and durosemiquinone radicals. The spectra at $\lambda > 480$ nm are very similar to those measured previously from pulse radiolysis experiments[10,20] in which reaction occurs by one-electron oxidation. This therefore confirms that the ^{3}DQ reacts by electron transfer to give the corresponding

TABLE I Second order rate constants (units $dm^3 mol^{-1} s^{-1}$) for reaction of triplet duroquinone (^3DQ), singlet oxygen ($^1O_2$) and azidyl radical ($N_3^\bullet$) with hydroxycinnamates, and one electron reduction potentials (E_1) of the radicals at pH 7. Marked values (#) are from reference[10]

Compound (X)	k ($^3DQ + X$)	k ($^1O_2 + X$)#	k ($N_3^\bullet + X$)#	E_1 (pH 7, mV)#
4HC	$(1.59 \pm 0.04) \times 10^9$	6×10^6	1.0×10^9	590
FA	$(1.69 \pm 0.05) \times 10^9$	20×10^6	4.3×10^9	595
3,4-DHC	$(1.93 \pm 0.06) \times 10^9$	40×10^6	3.9×10^9	534



X= -H, 4-hydroxycinnamate (4HC)
-OMe, ferulic acid (FA)
-OH, 3,4-dihydroxycinnamate (DHC)

SCHEME 1 Oxidation of hydroxycinnamates by triplet duroquinone (^3DQ) at pH 7.

phenoxyl radical (Scheme 1). The peak at 440 nm is due to the simultaneous formation of the durosemiquinone radical anion ($DQ^{-\bullet}$) as shown by the spectrum generated by reduction of ^3DQ with ascorbate. Whilst radicals from 3,4-DHC and FA lack strong absorption peaks in the visible region, that from 4-HC (the "cinnam-4-oxyl" radical) has a characteristic long wavelength peak at 600 nm with $\varepsilon \sim 3{,}200\ dm^3 mol^{-1} cm^{-1}$ by comparison with the intensity of the $DQ^{-\bullet}$ absorbance at 440 nm (ε, 7,600 $dm^3 mol^{-1} cm^{-1}$).[21]

Time-resolved Resonance Raman Spectra

Of the three hydroxycinnamates investigated, time-resolved resonance Raman (TR^3) spectra could be obtained only from the radical of 4-HC. The TR^3 spectrum of the cinnam-4-oxyl radical in a methanol/water (50/50 v/v) solution at pH 7 was observed at a pump-probe delay of 2 μs and is shown in Figure 2. The radical was generated by oxidation of 4-HC by ^3DQ as described above. The probe laser wavelength was tuned to 600 nm so as to be in resonance with the long wavelength absorption of the radical as shown in Figure 1. The durosemiquinone radical does not absorb at 600 nm, and therefore does not contribute to the observed TR^3 spectrum. The TR^3 spectrum contains two dominant bands at 1543 and 1593 cm^{-1}, in addition to some weaker bands at 1280–1320 cm^{-1} which are less well resolved. Aryloxyl radicals generally show strong resonance Raman bands assigned to the C—O (Wilson ν_{7a}) stretching vibration and the ring C—C (Wilson ν_{8a}) stretching mode.[13–16] When probed within its long wavelength absorption band at 400 nm, the C—O band of phenoxyl radical is observed[14] at 1505 cm^{-1}. At this excitation wavelength the C—C band of phenoxyl is not observed, but has been reported at 1552 cm^{-1} on probing within the higher energy transition corresponding to the shorter wavelength absorption band at 245 nm.[22] The resonance Raman spectrum of the cinnam-4-oxyl radical displaying the predominant C—O and C—C bands on long wavelength excitation is more similar to other reported phenoxyl radicals with electron donating *para* substituents, such the neutral hydrosemiquinone and 4-methoxyphenoxyl radicals.[15]

Consideration of these data clearly identifies the cinnamoxyl radical resonance Raman band

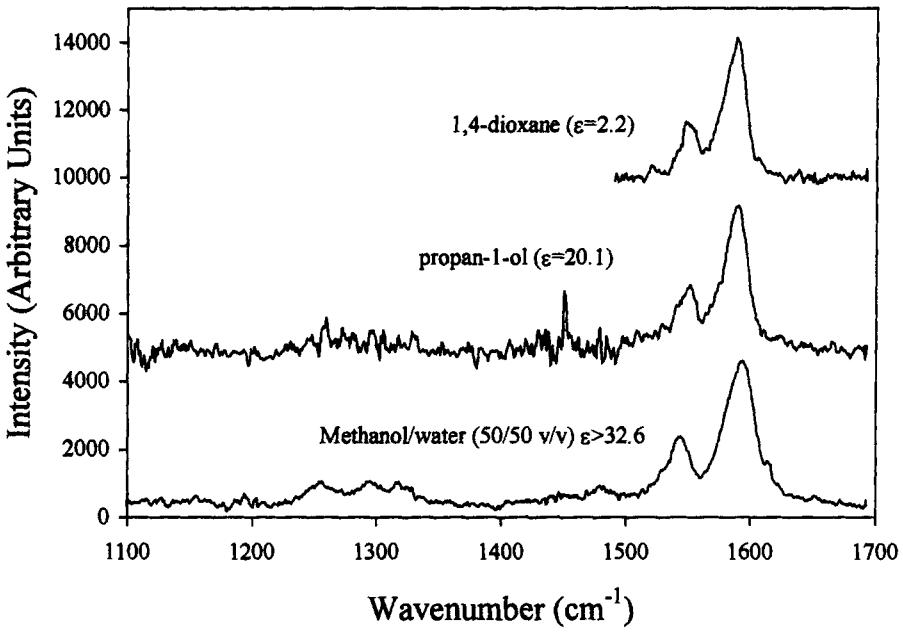
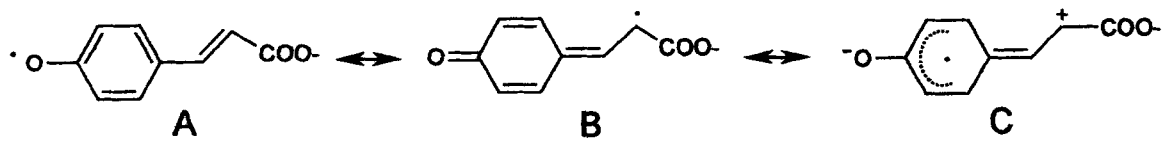


FIGURE 2 Time-resolved resonance Raman spectra of cinnam-4-oxyl radicals in solvents of varying dielectric constant. The spectra were measured in solutions containing DQ (2.5 mmol dm^{-3}) and 4-HC (1 mmol dm^{-3}) excited at 360 nm and probed by a second laser pulse (600 nm) delayed by 2 μs. Probe-only solvent spectra and sloping baselines have been subtracted.

at 1543 cm^{-1} to be the C—O stretching vibration. This may be compared[16] with the vibrational frequencies of a typical C=O double bond (bond order 2, at 1700 cm^{-1}), a C—O single bond (ca. 1200 cm^{-1} in phenols) and the symmetric C—O stretching vibration at in carboxylate ions (bond order 1.5, 1360–1450 cm^{-1}). This suggests that the C—O bond in the cinnam-4-oxyl radical has some single bond character. Nevertheless the band at 1543 cm^{-1} is at significantly higher frequency than in the phenoxyl radical (1505 cm^{-1})[14] and the 4-methoxyphenoxyl radical (1518 cm^{-1})[15] indicating that the resonance form B of the cinnam-4-oxyl radical (Scheme 2) contributes more in the present example. The charge separated form (C) is probably not so significant in this case, although it has been proposed for such as the 4-aminophenoxyl radical where the polar substituents stabilise the charge separation.[16]

The strong band at 1592 cm^{-1} may be assigned to the C—C ring stretching vibration by analogy with previous examples of phenoxyl radical spectra.[15,16] In the α-tocopheroxyl radical and analogues in which there is significant electron delocalisation from the 2p orbital of the *para* oxygen atom in the chroman ring to the phenoxyl radical site, the C—C band appears at ca 1600 cm^{-1} and is resonantly enhanced to an extent comparable with that of the C—O band.[17]



SCHEME 2 Possible resonance valence forms of the cinnam-4-oxyl radical.

In contrast, in the 4-methoxy,2,3,5,6-tetramethylphenoxyl radical steric interactions between methyl and methoxyl groups force the methoxy group out of the plane of the aromatic ring. The 2p orbital of the *para* oxygen atom is forced towards becoming parallel to the aromatic ring, reducing overlap between the methoxyl oxygen 2p orbital and the aromatic π orbitals. Consequently radical delocalisation is diminished, 4-methoxy,2,3,5,6-tetramethylphenol is a much weaker antioxidant than 4-methoxylphenol[2] and the C—C band is not observed in the TR^3 spectrum of 4-methoxy,2,3,5,6-tetramethylphenoxyl.[17] The observation of the C—C band in the cinnamoxyl radical therefore also implies substantial delocalisation from the 4-substituent, in this case the ethylenic bond, to the phenoxyl radical site.

Figure 2 also shows that the cinnam-4-oxyl radical may be observed on oxidation of 4-HC by 3DQ in less polar solvents. In contrast to the solvent effect on the TR^3 spectra of the Trolox C radical where both the position and enhancement of the C—C band and are fairly solvent-sensitive,[17] for the cinnam-4-oxyl radical there is no significant solvent effect on the enhancement of this band intensity. There are also fairly small changes in the positions of the C—O band (from 1543 cm^{-1} in methanol to ca. 1550 cm^{-1} in 1,4-dioxane) and the C—C band (from 1592 cm^{-1} in methanol to 1588 cm^{-1} in 1,4-dioxane). The lack of solvent effect is due to the lower polarity of the 4-substituted double bond in cinnam-4-oxyl compared with the chroman oxygen atom in α-tocopheroxyl and similar radicals.

In conclusion, the TR^3 spectra show that in the cinnam-4-oxyl radical the conjugated double bond serves the same purpose as the chroman oxygen atom in α-tocopheroxyl in permitting delocalisation from the *para* substituent to the radical site. The consequent increased stability of the radical is a major factor in reducing the one-electron reduction potential of the radical, thereby contributing to the increased antioxidant activity of these compounds.

Acknowledgements

This work was undertaken at the Lasers for Science Facility (LSF) at the Rutherford Appleton Laboratory (Council of the Central Laboratory of the Research Councils, CCLRC). We acknowledge the support of EPSRC and BBSRC during the course of this work.

References

[1] R. Brigelius-Flohe and M.G. Traber (1999) Vitamin E: function and metabolism. *FASEB Journal*, **13**, 1145–1155.
[2] G.W. Burton and K.U. Ingold (1986) Vitamin E – Application of the principles of physical organic chemistry to the exploration of its structure and function. *Accounts of Chemical Research*, **19**, 194–201.
[3] K. Herrmann (1989) Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in food. *Critical Reviews in Food Science and Nutrition*, **28**, 315–347.
[4] J.H. Chen and C.T. Ho (1997) Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *Journal of Agricultural and Food Chemistry*, **45**, 2374–2378.
[5] K.I. Priyadarsini, S.N. Guha and M.N.A. Rao (1998) Physico-chemical properties and antioxidant activities of methoxy phenols. *Free Radical Biology and Medicine*, **24**, 933–941.
[6] C.A. Rice-Evans, N.J. Miller and G. Paganga (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, **20**, 933–956.
[7] M. Nardini, M. Daquino, G. Tomassi, V. Gentili, M. Difelice and C. Scaccini (1995) Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biology and Medicine*, **19**, 541–552.
[8] W. Wenfeng, L. Jian, Y. Side, L. Zhirui, Z. Zhihua, Z. Jiashan and L. Nianyun (1995) Pulse radiolysis studies of the interaction of hydroxycinnamic acid derivatives with oxidising OH adducts of pyrimidine. *Radiation Chemistry and Physics*, **46**, 41–45.
[9] N.Y. Lin (1996) Excitation transfer, charge transfer and hydrogen atom abstraction reaction kinetic studies of modification of DNA components. *Radiation Physics and Chemistry*, **47**, 209–212.
[10] S. Foley, S. Navaratnum, D.J. McGarvey, E.J. Land, T.G. Truscott and C.A. Rice-Evans (1999) Singlet oxygen quenching and redox properties of hydroxycinnamic acids. *Free Radical Biology and Medicine*, **26**, 1202–1208.
[11] R.H. Bisby, C.G. Morgan, I. Hamblett and A.A. Gorman (1999) Quenching of singlet oxygen by Trolox C, ascorbate and amino acids. *Journal of Physical Chemistry*, **103**, 7454–7459.
[12] S.E.J. Bell (1996) Time-resolved resonance Raman spectroscopy. *Analyst*, **121**, R107–R120.
[13] G.N.R. Tripathi (1989) Time-resolved resonance Raman spectroscopy of chemical reaction intermediates in solution. In: *Time-resolved Spectroscopy* (eds. R.J.H. Clark and R.E. Hester), Wiley, London, pp. 157–218.

[14] G.N.R. Tripathi and R.H. Schuler (1984) The resonance Raman spectrum of phenoxyl radical. *Journal of Chemical Physics*, **81**, 113–121.
[15] G.N.R. Tripathi and R.H. Schuler (1988) Resonance Raman studies of substituent effects on the electronic structure of phenoxyl radicals. *Journal of Physical Chemistry*, **92**, 5129–5133.
[16] G.N.R. Tripathi (1998) Proton reactivity and electronic structure of phenoxyl radicals in water. *Journal of Physical Chemistry A*, **102**, 2388–2397.
[17] A.W. Parker and R.H. Bisby (1993) Time-resolved resonance Raman spectroscopy of α-tocopheroxyl and related radicals in solvent, micellar and membrane systems. *Journal of the Chemical Society, Faraday Transactions*, **89**, 2873–2878.
[18] R.H. Bisby, S.A. Johnson and A.W. Parker (1996) Quenching of reactive oxidative species by probucol and comparison with other antioxidants. *Free Radical Biology and Medicine*, **20**, 411–420.
[19] R. Scheerer and M. Gratzel (1977) Laser photolysis studies of duroquinone triplet state electron transfer reactions. *Journal of the American Chemical Society*, **99**, 865–871.
[20] P. Happiot, A. Neudeck, J. Pinson, H. Fulcrand, P. Neta and C. Rolando (1996) Oxidation of caffeic acid and related hydroxycinnamic acids. *Journal of Electroanalytical Chemistry*, **405**, 169–176.
[21] K.B. Patel and R.L. Willson (1973) Semiquinone radicals and oxygen. *Journal of the Chemical Society, Faraday Transactions I*, **69**, 814–825.
[22] C.R. Johnson, M. Ludwig and S.A. Asher (1986) Ultraviolet resonance Raman characterisation of photochemical transients of phenol, tyrosine and tryptophan. *Journal of the American Chemical Society*, **108**, 905–912.