Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 62 (2006) 1273–1278

Oxidative chemistry of the natural antioxidant hydroxytyrosol: hydrogen peroxide-dependent hydroxylation and hydroxyquinone/*o*-quinone coupling pathways

Maria De Lucia, Lucia Panzella, Alessandro Pezzella, Alessandra Napolitano* and Marco d'Ischia

Department of Organic Chemistry and Biochemistry, University of Naples 'Federico II', Via Cinthia 4, I-80126 Naples, Italy

Received 25 July 2005; revised 5 October 2005; accepted 20 October 2005

Available online 10 November 2005

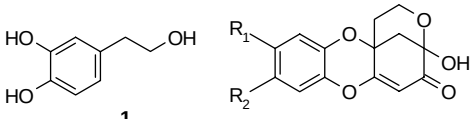
Abstract—Oxidation of the natural antioxidant hydroxytyrosol (**1**) with peroxidase/H_2O_2 in phosphate buffer at pH 7.4 led to the formation of two main ethyl acetate-extractable products. These could be isolated by preparative TLC after reduction and acetylation, and were identified as the tetraacetyl derivative of 2-(2,4,5-trihydroxyphenyl)ethanol (**3**) and the heptaacetyl derivative of the pentahydroxybiphenyl **4** by 2D NMR and MS analysis. Similar oxidation of 4-methylcatechol gave, after the same work-up, the acetylated derivatives of 1,2,4-trihydroxy-5-methylbenzene (**5**) and the pentahydroxybiphenyl **6**. Mechanistic experiments suggested that hydrogen peroxide affects the course of the oxidation of **1** by adding to the first formed *o*-quinone to give a hydroxyquinone intermediate. This could bring nucleophilic attack to the *o*-quinone of **1** to give the dimer **4**. These results disclose novel oxidative pathways of 4-alkylcatechols and provide an improved chemical basis to enquire into the mechanism of the antioxidant action of **1**.
© 2005 Elsevier Ltd. All rights reserved.

1. Introduction

Hydroxytyrosol (2-(3,4-dihydroxyphenyl)ethanol, **1**) and related polyphenolic constituents of extra virgin olive oil are endowed with several biological properties that may contribute to the reduced risk of cardiovascular diseases and malignant neoplasms in the populations of Mediterranean countries.[1–3] The beneficial role of olive phenols in cancer prevention is ascribed to their potent antioxidant properties enabling them to counteract the geno- and cytotoxic effects of reactive oxygen species generated in settings of oxidative stress.[4,5]

Despite extensive studies on the antioxidant and free radical scavenging properties of **1**, its mechanism of action is still far from being completely elucidated, especially as concerns the identity of the reactive oxygen species targeted and the nature of the products formed. Knowledge of the oxidative chemistry of **1** is of central relevance to an understanding of the fate of this *o*-diphenolic compound during its antioxidant action in vivo and for delineating the chemical processes underlying quality deterioration of olive oil.

The first insight came from a previous study[6] showing that autoxidation or tyrosinase-catalysed oxidation of **1** in phosphate buffer at pH 7.4 leads to the formation of two main regioisomeric products, which could be isolated and identified as the novel methanooxocinobenzodioxinone derivatives **2a**,**b**. These products were suggested to arise by catechol–quinone coupling routes in which the *ortho* hydroxyl groups of **1** added onto the corresponding quinone.



1

2a: R_1 = H, R_2 = -CH_2CH_2OH

2b: R_1 = -CH_2CH_2OH, R_2 = H

In another study[7] it was shown that **1** can act as an efficient scavenger of hydrogen peroxide produced by human neutrophils and may therefore provide a useful tool to analyse the role of hydrogen peroxide in pathological processes, although no product arising from the scavenging action was identified. More recently, a mass spectrometric analysis of the species formed by 2,2′-azo-bis(2-amidinopropane) (AAPH)-induced oxidation of **1** was reported,[8] suggesting the formation of dimers with concomitant incorporation of a water molecule. It was proposed that *o*-quinones derived from **1** or tautomeric *p*-quinone

Keywords: *o*-Diphenols; Oxidation; 2-Hydroxy-*p*-quinones; Dimerization.
* Corresponding author. Tel.: +39 081 674133; fax: +39 081 674393; e-mail: alesnapo@unina.it

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.10.055

methides undergo water addition before coupling with a second *o*-quinone molecule. The structures of the reaction products, however, were not supported by a complete spectral analysis.

The present study aimed to obtain a deeper insight into the mechanism of the antioxidant action of **1** by providing a structural characterisation of the products formed upon exposure to peroxidase/H_2O_2, as a representative H_2O_2-containing oxidising system. For comparative purposes, the oxidative chemistry of 4-methylcatechol as model compound was also investigated.

2. Results and discussion

In preliminary experiments a straightforward procedure for the preparation of **1** was developed. This involved oxidation of tyrosol with 2-iodoxybenzoic acid (IBX)[9] in methanol at −25 °C followed by dithionite reduction and column chromatographic fractionation on silica gel to give **1** in 30% yield.

Exposure of **1** (1 mM) to horseradish peroxidase (HRP, 3 U/mL) and 4 mM H_2O_2 in 0.1 M phosphate buffer, pH 7.4, led to the rapid consumption of the substrate (about 90% after 1 h) and the gradual development of a purple red colouration (absorption maximum at 490 nm). Attempts to isolate and characterise reaction products met with failure because of the apparent instability under conventional chromatographic conditions. Accordingly, a procedure for product analysis was developed, which involves reduction of the reaction mixture with $NaBH_4$, acidification to pH 3, extraction with ethyl acetate and acetylation with Ac_2O/pyridine at room temperature. TLC analysis of the mixture thus obtained showed the presence of two main species eluted at R_f 0.47 (I) and 0.32 (II) (eluent cyclohexane/ethyl acetate 1:1). Small amounts of the acetylated derivatives of **2a**,**b** were also detected. Products I and II could be isolated in pure form from a large scale reaction and were subjected to complete spectral characterisation.

The ^{1}H NMR spectrum of product I displayed two 1H singlets at δ 7.01 and 7.09 showing direct correlation with carbon signals resonating at δ 116.1 and 123.0, respectively. In addition, two 2H triplets at δ 4.21 and 2.83 and four singlets for acetyl groups were distinguishable in the high field region, suggesting a tetraacetyl derivative. This latter conclusion was confirmed by the positive ion electrospray ionization (ESI+)/MS spectrum showing a pseudomolecular ion peak $[M+H]^+$ at *m*/*z* 339. On this basis, product I was formulated as the tetraacetyl derivative of 2-(2,4,5-trihydroxyphenyl)ethanol (**3**).

The ESI+/MS spectrum of product II exhibited pseudomolecular ion peaks $[M+H]^+$ and $[M+Na]^+$ at *m*/*z* 617 and 639, respectively, indicating a fully acetylated dimer of **1** bearing an additional acetoxyl group on one of the aromatic rings. Consistent with this conclusion was the ^{1}H NMR spectrum, showing seven singlets for the acetyl groups and four 2H triplets in the high field region, and three 1H singlets at δ 6.89, 7.17 and 7.21 in the aromatic proton region. The compound was therefore identified as the heptaacetyl derivative of 2,3,3′,4′,6-pentahydroxy-5,6′-bis(2-hydroxyethyl)biphenyl (**4**), arising evidently by oxidative coupling of **1** with **3**. The mode of linkage of the units was deduced from the presence of distinct NOE contacts in the ^{1}H, ^{1}H ROESY spectrum between the triplet at δ 2.86 and the singlet at δ 7.21 indicating that the 6-position of the 2,4,5-trihydroxyphenylethanol unit was unsubstituted.

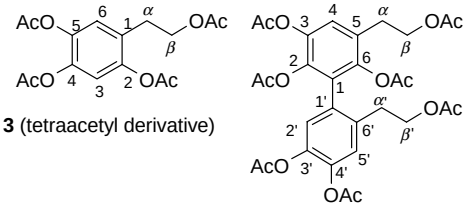
NMR assignments for the acetylated derivatives of **3** and **4** are reported in Table 1.

Table 1. NMR spectral data of the acetylated derivatives of **3** and **4** ($CDCl_3$)

	3 (Tetraacetylated)[a]		**4** (Heptaacetylated)[b]	
	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C
1[c]	—	126.4	—	128.4
2	—	144.8	—	140.8[d]
3	7.01 (s)	116.1	—	139.5
4	—	138.0[e]	7.21 (s)	124.3
5	—	139.1[e]	—	129.4
6	7.09 (s)	123.0	—	145.1
α	2.83 (t, 7.0)	27.6	2.86 (t, 6.8)	29.6
β	4.21 (t, 7.0)	61.5	4.27 (t, 6.8)	63.1
1′			—	129.7
2′			6.89 (s)	125.7
3′			—	140.2[d]
4′			—	142.1
5′			7.17 (s)	123.8
6′			—	135.9
α′			2.71 (t, 7.2)	31.4
β′			4.20 (t, 7.2)	63.3

[a] Acetyl groups: ^{1}H NMR δ (ppm) 2.02 (s, 3H), 2.26 (s, 3H), 2.27 (s, 3H), 2.32 (s, 3H); ^{13}C NMR δ (ppm) 18.9 (2×CH_3), 19.1 (CH_3), 19.2 (CH_3).
[b] Acetyl groups: ^{1}H NMR δ (ppm) 1.92 (s, 3H), 1.96 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.25 (s, 3H), 2.28 (s, 3H), 2.29 (s, 3H); ^{13}C NMR δ (ppm) 19.8 (CH_3), 19.9 (CH_3), 20.4 (CH_3), 20.6 (2×CH_3), 20.9 (2×CH_3).
[c] Numbering as shown in structural formulas **3** and **4**.
[d,e] Interchangeable.

The formation yields of the acetylated derivatives of **3** (t_r 28.5 min, eluent II) and **4** (t_r 39.9 min, eluent II) were 31 and 3%, respectively, as estimated by HPLC analysis of the reaction mixture after the acetylation treatment.



3 (tetraacetyl derivative) **4** (heptaacetyl derivative)

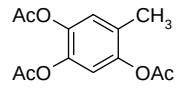
When the oxidation of **1** was carried out under an argon atmosphere no appreciable change in product distribution was observed, ruling out a significant role of oxygen in the oxidation process.

To test if the additional hydroxyl group of **3** derives from H_2O_2, the oxidation of **1** was carried out with equimolar amounts of $K_3Fe(CN)_6$ in 0.1 M phosphate buffer, pH 7.4, in the presence and in the absence of variable amounts of H_2O_2. As expected, no trace of **3** and **4** was found in the absence of H_2O_2, dimers **2a**,**b** being the main identifiable

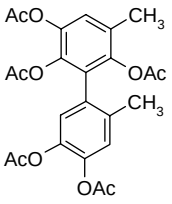
species. However, a pattern of products matching that formed in the HRP/H_2O_2 catalysed reaction was produced in the presence of H_2O_2.

To estimate the effect of H_2O_2 the reaction of **1** with HRP/H_2O_2 was carried out with increasing amounts of H_2O_2 (in the range 4–10 M equiv). Under these conditions the yields of **3** varied from 25 to 93%.

In another series of experiments, the mechanism of formation of **4** was investigated using 4-methylcatechol as a model compound. The oxidation reaction, carried out in 0.1 M phosphate buffer, pH 7.4, with the substrate at 1 mM concentration and HRP (3 U/mL)/H_2O_2 (4 mM), led to the formation of two main acetylated products eluting on TLC at R_f 0.46 and 0.61 (eluent cyclohexane/ethyl acetate 6:4). These were isolated and identified as the triacetyl derivative of 1,2,4-trihydroxy-5-methylbenzene (**5**) (14% yield) and the pentaacetyl derivative of 2,3,3′,4′,6-pentahydroxy-5,6′-dimethylbiphenyl (**6**) (21% yield), suggesting an oxidative pathway of 4-methylcatechol akin to that of **1**.



5 (triacetyl derivative)



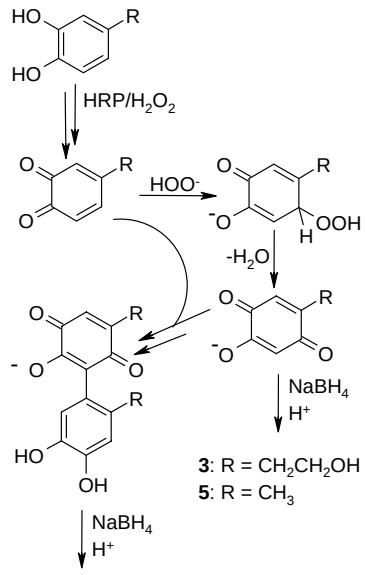
6 (pentaacetyl derivative)

Since formation of **6** was evidently the result of the oxidative coupling of 4-methylcatechol with **5**, it seemed of interest to inquire whether the two partners reacted both in their quinonoid forms or in different oxidative states. In an ad hoc experiment 2-hydroxy-5-methyl-1,4-benzoquinone, produced by periodate oxidation of **5**, was allowed to react with 4-methyl-1,2-benzoquinone[10] in phosphate buffer, pH 7.4, under an argon atmosphere and was found to give, after the usual work up, the acetylated dimer **6** in good yield.

A plausible mechanism to account for the formation of products **3/5** and **4/6** is depicted in Scheme 1.

In the proposed scheme, interaction of the catechol with the HRP/H_2O_2 system generates the corresponding *o*-quinone, which may be trapped by H_2O_2 to give hydroxyquinone intermediates via a carbonyl-forming fission of the hydroperoxy initial adduct.[11] Nucleophilic attack of H_2O_2 to *o*-quinones substituted at the 4-position is an efficient process, which proceeds regioselectively at the 6-position[12–14] and appears to compete favourably with alternative reaction routes, including nucleophilic attack by the *o*-diphenolic substrate to give benzodioxinone adducts, an event that becomes significant only when H_2O_2 is low or absent. The observation of a purple red colouration in the early phases of the oxidation provides support for the generation of hydroxyquinone species.[15]

Just formed, the hydroxyquinones, which are vinylogous carboxylic acids ($pK_a = 2.90$),[16] are expected to be partially ionised at neutral pH and are able to couple with the primary *o*-quinones to give the pentahydroxybiphenyl dimers.



Scheme 1.

The regiochemistry of the coupling reaction reflects the enolate-like character of the 3-position of hydroxyquinones. Consistent with this view, DFT analysis of 2-hydroxy-5-methyl-1,4-benzoquinone in the ionised form revealed a relatively large HOMO coefficient on the 3-position (Fig. 1).

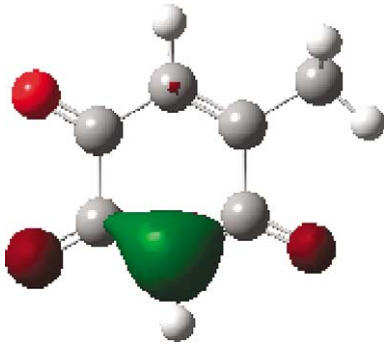


Figure 1. HOMO of 2-hydroxy-5-methyl-1,4-benzoquinone calculated at the HF/6-31+G(d,p) level using PCM to model the effects of the solvent medium.

Although the proposed quinone–quinone coupling mechanism might seem unusual, it is reminiscent of the early steps of purpurogallin formation by oxidation of pyrogallol[17] and of the previously reported generation of pentahydroxybiphenyl dimers by tyrosinase-catalysed oxidation of hydroquinone via hydroxybenzoquinone.[18]

In conclusion, the results of this study contribute to expand current knowledge of the oxidative chemistry of **1**, highlighting the peculiar effect of hydrogen peroxide on the normal course of the reaction and supporting the proposed role of olive phenols as hydrogen peroxide scavengers. Of particular interest is the demonstration of a specific H_2O_2-dependent oxidative pathway of 4-alkylcatechols that seems to have escaped the attention of previous workers.

3. Experimental

3.1. General methods

UV spectra were performed with a Beckmann DU 640 spectrophotometer. Positive ion electrospray ionization (ESI+)/MS spectra were obtained in methanol/2% formic acid 1:1 v/v using a Micromass ZQ Waters equipment. Main peaks are reported with their relative intensities (percent values are in parentheses). HR EI mass spectra were obtained with a Finnegan MAT 90 instrument. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with a Bruker WM 400 spectrometer at 400.1 and 100.6 MHz, respectively. The instrument was equipped with a 5 mm ^{1}H/broadband gradient probe with inverse geometry. ^{1}H, ^{1}H COSY, ^{1}H, ^{13}C heteronuclear multiple quantum coherence, ^{1}H, ^{13}C heteronuclear multiple bond correlation and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments were run using standard pulse programs from the Bruker library. Chemical shifts are reported in δ values (ppm) downfield from TMS.

Analytical and preparative TLC was carried out on silica gel plates (0.25 and 0.50 mm, respectively) from Merck. Column chromatography was performed using silica gel (0.063–0.200 mm). Analytical HPLC was carried out on a Gilson apparatus equipped with an UV detector set at 280 nm. The chromatographic separation was achieved on a Sphereclone ODS column (5μ, 250 mm×4.6 mm) using binary gradient elution conditions as follows: water–trifluoroacetic acid (97/3), solvent A; acetonitrile, solvent B; from 2 to 20% B, 0–40 min; from 20 to 55% B, 40–55 min; 55% B, 55–65 min; flow rate, 1 mL/min (eluent I); water, solvent A; acetonitrile, solvent B; from 2 to 30% B, 0–15 min; from 30 to 60% B, 15–45 min; 60% B, 45–55 min; flow rate, 1 mL/min (eluent II).

Tyrosol (2-(4-hydroxyphenyl)ethanol) was from Fluka; 4-methylcatechol, 2-iodobenzoic acid, oxone® ($2KHSO_5$–$KHSO_4$–K_2SO_4), $Na_2S_2O_4$, $NaIO_4$, $K_3Fe(CN)_6$ and $NaBH_4$ were from Aldrich; hydrogen peroxide (30% solution in water) was from Carlo Erba; horseradish peroxidase type II (HRP) (EC 1.11.1.7) was from Sigma. 4-Methyl-1,2-benzoquinone[10] was prepared as reported.

3.2. Preparation of hydroxytyrosol (2-(3,4-dihydroxyphenyl)ethanol) (1)

The reaction was carried out as reported[9] with modification. In summary, a solution of tyrosol (200 mg, 1.4 mmol) in methanol (20 mL) was treated with IBX (600 mg, 2.1 mmol) under vigorous stirring at −25 °C. Aliquots of the reaction mixture were periodically withdrawn and analyzed by HPLC, using eluent I as the mobile phase. After 1 h the reaction mixture was reduced with a solution of $Na_2S_2O_4$ (300 mg) in water (10 mL) and then extracted with ethyl acetate (3×15 mL). The combined organic layers were dried over sodium sulfate and taken to dryness. The residue obtained (640 mg) was fractionated on a silica gel column (30 cm×2 cm) using a gradient of $CHCl_3$–CH_3OH containing 0.5% acetic acid (from 98:2 to 90:10) as the eluent. Fractions eluted with $CHCl_3/CH_3OH$ 90:10 were collected and taken to dryness to afford pure **1** (66 mg, 0.43 mmol, 30% yield) as an oily solid. Spectral data were in agreement with that previously published.[19]

3.3. Oxidation of 1 and 4-methylcatechol: general procedure

A solution of **1** (10 mg, 65 μmol) or of 4-methylcatechol (8 mg, 65 μmol) in 0.1 M phosphate buffer, pH 7.4 (65 mL) was treated with HRP (3 U/mL) and H_2O_2 (4 M equiv) under vigorous stirring. After 1 h the reaction mixture was reduced with $NaBH_4$ (20 mg), acidified to pH 3 with 3 M HCl and extracted with ethyl acetate (3×10 mL). The combined organic layers were dried over sodium sulfate and taken to dryness. The residue was treated with acetic anhydride (500 μL) and pyridine (25 μL) at room temperature for 16 h. The mixture was taken to dryness and analyzed by TLC (eluent cyclohexane/ethyl acetate 1:1) and HPLC (eluent II). In the case of **1**, other experiments were performed (i) with H_2O_2 varying in the range of 4–10 M equiv; (ii) purging the solution with a stream of argon for at least 15 min prior to addition of the solution of the oxidant thoroughly purged with argon; (iii) with $K_3Fe(CN)_6$ (21 mg, 65 μmol) as the oxidant; after 20 min the reaction mixture was worked up and analyzed as above. When required oxidation of **1** with $K_3Fe(CN)_6$ was carried out in the presence of H_2O_2 varying in the range of 1–3 M equiv.

3.3.1. Isolation of the tetraacetyl derivative of 2-(2,4,5-trihydroxyphenyl)ethanol (3) and of the heptaacetyl derivative of 2,3,3′,4′,6-pentahydroxy-5,6′-bis(2-hydroxyethyl)biphenyl (4). For preparative purposes, the reaction of **1** with HRP/H_2O_2 was carried out as described above using 90 mg of the starting material. After work-up of the reaction mixture, the residue (50 mg) was treated with acetic anhydride (1 mL) and pyridine (50 μL) at room temperature for 16 h and then purified by preparative TLC (eluent cyclohexane/ethyl acetate 1:1) to give the acetylated derivatives of **3** (R_f 0.47, 42 mg, 21% yield, >95% pure by NMR analysis) and **4** (R_f 0.32, 4 mg, 2% yield, >95% pure by NMR analysis).

Compound **3** *(tetraacetyl derivative).* UV λ_{max} (CH_3OH): 267 nm; ESI+/MS: *m/z* 339 ($[M+H]^+$, 82), 361 ($[M+Na]^+$, 87), 377 ($[M+K]^+$, 36); EI/HRMS calculated mass for $C_{16}H_{18}O_8$ $[M]^+$338.1002, found *m/z* 338.0997.^{1}H and ^{13}C NMR data are reported in Table 1.

Compound **4** *(heptaacetyl derivative).* UV λ_{max} (CH_3OH): 268, 311 nm; ESI +/MS: *m/z* 617 ($[M+H]^+$, 100), 639 ($[M+Na]^+$, 30), 655 ($[M+K]^+$, 21); EI/HRMS calculated mass for $C_{30}H_{32}O_{14}$ $[M]^+$616.1792, found *m/z* 616.1789.^{1}H and ^{13}C NMR data are reported in Table 1.

3.3.2. Isolation of the triacetyl derivative of 1,2,4-trihydroxy-5-methylbenzene (5) and of the pentaacetyl derivative of 2,3,3′,4′,6-pentahydroxy-5,6′-dimethylbiphenyl (6). For preparative purposes, the reaction of 4-methylcatechol with HRP/H_2O_2 was carried out as described in the general procedure using 100 mg of the starting material. For isolation of **5** the reaction was carried out using 10 M equiv of H_2O_2. After work-up of the reaction

mixture, the residue was treated with acetic anhydride (1 mL) and pyridine (50 μL) at room temperature for 16 h and then purified by preparative TLC (eluent cyclohexane/ethyl acetate 6:4) to give the triacetyl derivative of **5** (R_f 0.61, 30 mg, 14% yield, >98% pure by NMR analysis) and the pentaacetyl derivative of **6** (R_f 0.46, 20 mg, 21% yield, >98% pure by NMR analysis).

Compound **5** *(triacetyl derivative)*. UV λ_{max} (CH_3OH): 268 nm; ^1H NMR δ (ppm): 2.15 (s, 3H), 2.25 (s, 3H), 2.26 (s, 3H), 2.29 (s, 3H), 6.95 (s, 1H), 7.04 (s, 1H); ^{13}C NMR δ (ppm): 15.9 (CH_3), 20.6 (2×CH_3), 20.7 (CH_3), 117.2 (CH), 124.9 (CH), 128.7 (C), 139.4 (C), 140.0 (C), 146.4 (C), 168.0 (C), 168.2 (C), 168.6 (C); ESI+/MS: *m/z* 267 ($[M+H]^+$, 100), 289 ($[M+Na]^+$, 57), 305 ($[M+K]^+$, 4); EI/HRMS calculated mass for $C_{13}H_{14}O_6$ $[M]^+$266.0790, found *m/z* 266.0795.

Compound **6** *(pentaacetyl derivative)*. UV λ_{max} (CH_3OH): 268 nm; ^1H NMR δ (ppm): 1.94 (s, 3H), 1.96 (s, 3H), 2.11 (s, 3H), 2.21 (s, 3H), 2.25 (s, 3H), 2.28 (s, 3H), 2.29 (s, 3H), 6.88 (s, 1H), 7.07 (s, 1H), 7.14 (s, 1H); ^{13}C NMR δ (ppm): 16.2 (CH_3), 19.1 (CH_3), 19.9 (2×CH_3), 20.6 (3×CH_3), 124.2 (2×CH), 125.1 (CH), 128.4 (C), 129.5 (C), 129.8 (C), 136.4 (C), 139.3 (C), 139.7 (C), 140.3 (C), 141.7 (C), 144.8 (C), 168.2 (5×C); ESI+/MS: *m/z* 473 ($[M+H]^+$, 100), 505 ($[M+Na]^+$, 23); EI/HRMS calculated mass for $C_{24}H_{24}O_{10}$ $[M]^+$472.1369, found *m/z* 472.1373

3.4. Reaction of 4-methyl-1,2-benzoquinone with 2-hydroxy-5-methyl-1,4-benzoquinone

A solution of the triacetyl derivative of **5** (6 mg, 23 μmol) in acetone (100 μL) was added to 0.025 M sodium phosphate buffer (pH 12) (12 mL) that had been previously purged with a stream of argon for 15 min. After 2 min, the solution was acidified to pH 7 with $NaH_2PO_4 \times H_2O$ (29 mg) and treated with $NaIO_4$ (5 mg, 23 μmol) predissolved in H_2O (8 mL). After 30 s, a solution of 4-methyl-1,2-benzoquinone[10] (3 mg, 23 μmol) in acetone (145 μL) was added and after 3 min the mixture was worked-up, acetylated and analyzed as reported under the general procedure.

4. Computational methods

Quantum-mechanical computations were carried out with the Gaussian03 revision B.05 program[20] using the PBE0 density functional.[21] The 6–311+G(d,p) basis set was used for geometry optimisations. Frontier orbitals were calculated at the HF/6-31+G(d,p) level. The most recent version[22] of the polarizable continuum model (PCM) was used to model the effects of the solvent medium.

Acknowledgements

This study was carried out in the frame of the MIUR projects 'Sostanze naturali ed analoghi sintetici con attività antitumorale' (PRIN 2003) and 'Tecniche Integrate di Decontaminazione' (TIDe). Computational analysis by professors Orlando Crescenzi and Vincenzo Barone is gratefully acknowledged. We thank the 'Centro Interdipartimentale di Metodologie Chimico-Fisiche' (CIMCF, University of Naples Federico II) for NMR, mass spectroscopy, and computational facilities and Mrs. Silvana Corsani for technical assistance.

References and notes

1. Keys, A. *Am. J. Clin. Nutr.* **1995**, *61*, 1321S–1323S.
2. Owen, R. W.; Haubner, R.; Wurtele, G.; Hull, E.; Spiegelhalder, B.; Bartsch, H. *Eur. J. Cancer Prev.* **2004**, *13*, 319–326.
3. Tuck, K. L.; Hayball, P. J. *J. Nutr. Biochem.* **2002**, *13*, 636–644.
4. Visioli, F.; Bellomo, G.; Galli, C. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 60–64.
5. Owen, R. W.; Mier, W.; Giacosa, A.; Hull, W. E.; Spiegelhalder, B.; Bartsch, H. *Food Chem. Toxicol.* **2000**, *38*, 647–659.
6. Vogna, D.; Pezzella, A.; Panzella, L.; Napolitano, A.; d'Ischia, M. *Tetrahedron Lett.* **2003**, *44*, 8289–8292.
7. O'Dowd, Y.; Driss, F.; My-Chan Dang, P.; Elbim, C.; Gougerout-Pocidalo, M.; Pasquier, C.; El-Benna, J. *Biochem. Pharmacol.* **2004**, *68*, 2003–2008.
8. Roche, M.; Dufour, C.; Mora, N.; Dangles, O. *Org. Biomol. Chem.* **2005**, *3*, 423–430.
9. Pezzella, A.; Lista, L.; Napolitano, A.; d'Ischia, M. *Tetrahedron Lett.* **2005**, *46*, 3541–3544.
10. Adler, E.; Magnusson, R. *Acta Chem. Scand.* **1959**, *13*, 505–519.
11. Plesnicar, B. In *The Chemistry of Peroxides*; Patai, S., Ed.; Wiley: New York, 1983; pp 573–578.
12. Napolitano, A.; Crescenzi, O.; Pezzella, A.; Prota, G. *J. Med. Chem.* **1995**, *38*, 917–922.
13. Teichner, H.; Weil, H. *Chem. Ber.* **1905**, *38*, 3376–3377.
14. Weitz, E.; Schobbert, H.; Seibert, H. *Chem. Ber.* **1935**, *68*, 1163–1168.
15. Wehrli, P. A.; Pigott, F.; Fischer, U.; Kaiser, A. *Helv. Chim. Acta* **1972**, *55*, 3057–3061.
16. Bailey, S. I.; Ritchie, I. M. *Electrochim. Acta* **1985**, *30*, 3–12.
17. Critchlow, A.; Haslam, E.; Haworth, R. D.; Tinker, P. B.; Waldron, N. M. *Tetrahedron* **1967**, *23*, 2829–2847.
18. Palumbo, A.; d'Ischia, M.; Misuraca, G.; Prota, G. *Pigment Cell Res.* **1992**, *2*, 299–303.
19. Capasso, R.; Evidente, A.; Avorio, S.; Solla, F. *J. Agric. Food Chem.* **1999**, *47*, 1745–1748.
20. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Scalmani; G.; Cossi, M.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.;

Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision B.05. Gaussian Inc.: Pittsburgh, PA, 2003.

21. Adamo, C.; Barone, V. *J. Chem. Phys.* **1999**, *110*, 6158–6170.
22. Cossi, M.; Scalmani, G.; Rega, N.; Barone, V. *J. Chem. Phys.* **2002**, *117*, 43–54.