



Title	DPPH (=2,2-diphenyl-1-picrylhydrazyl=2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) radical-scavenging reaction of protocatechuic acid esters (=3,4-dihydroxybenzoates) in alcohols : Formation of bis-alcohol adduct
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# **DPPH Radical Scavenging Reaction of Protocatechuic Esters**

## **in Alcohols: Formation of Bis-alcohol Adduct**

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Protocatechuic esters scavenge approximately five equivalents of radical in alcoholic solvents, whereas they consume only two equivalents of radical in non-alcoholic solvents. We have previously reported that the high radical scavenging activity of protocatechuic esters in alcoholic solvents compared to that in non-alcoholic solvents is due to a nucleophilic addition of an alcohol molecule at C(2) of *o*-quinones, which leads to a regeneration of a catechol structure. However, it is still unclear why protocatechuic esters scavenge more than four equivalents of radical. Therefore, to elucidate the oxidation mechanism beyond the formation of C(2) alcohol adduct, 3,4-dihydroxy-2-methoxybenzoic acid methyl ester (C(2) methanol adduct), an oxidation product of methyl protocatechuate in methanol, was oxidized by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical or *o*-chloranil in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone, (3:1) and its reaction mixture was directly analyzed by NMR. Oxidation with both DPPH radical and *o*-chloranil produced C(2), C(6) bis-methanol adduct, which could scavenge additional two equivalents of radical. Calculations of LUMO electron densities of *o*-quinones corroborated the regioselective nucleophilic addition of alcohol molecules on *o*-quinones. Our results strongly suggest that the regeneration of a catechol structure *via* a nucleophilic addition of alcohol molecules on *o*-quinone is a key reaction for the high radical scavenging activity of protocatechuic esters in alcoholic solvents.

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Keywords: Antioxidant DPPH Radical Protocatechuic esters Radical-scavenging mechanism Alcohol adduct
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**Introduction.** Hydroxybenzoic acids such as protocatechuic acid (3,4-dihydroxybenzoic acid) and gallic acid (3,4,5-trihydroxybenzoic acid) are ubiquitously found in vegetables and fruits [1][2]. These compounds are known to exhibit potent antioxidant activities [3 – 5], and could prevent carcinogenesis and cardiovascular diseases that are associated with free radicals [6 – 8]. Recently, numerous studies have been reported on radical scavenging activity of phenolic compounds. It is well-known that the radical scavenging activity of phenolic acids largely depends on the number and arrangement of phenolic hydroxyl groups in the molecule [9 – 11]. Among them, *o*- or *p*-diphenols such as protocatechuic acid exhibit high radical scavenging activity, since they would be converted to the corresponding *o*- or *p*-quinones, respectively [9]. In addition, higher radical scavenging activity of polyphenols can be, in part, ascribed to dimerization of *o*-quinones or semiquinone radicals, since the resultant dimers could scavenge additional radicals if they possess catechol structures [12]. However, more detailed studies are needed to understand the radical scavenging mechanism beyond the formation of quinones.

We have recently reported the DPPH radical scavenging mechanism of

protocatechuic esters in non-alcoholic and alcoholic solvents [13]. In non-alcoholic acetone or acetonitrile, protocatechuic acid and its esters scavenge two equivalents of radical to yield the corresponding *o*-quinones. In contrast, in alcoholic solvents, protocatechuic esters rapidly react with approximately five equivalents of radical with a concomitant conversion to the corresponding *o*-quinones, 3-hemiacetals [14], and their alcohol adducts at C(2) [13]. It was suggested that the higher radical scavenging activity of protocatechuic esters in alcoholic solvents than in non-alcoholic solvents is due to a regeneration of catechol structures *via* a nucleophilic addition of an alcohol molecule at C(2) of *o*-quinones [13]. Moreover, we found that catechols possessing strong electron-withdrawing substituents at C(1) exhibit high DPPH radical scavenging activity in alcoholic solvents, since electron-withdrawing substituents enhance the electrophilicity of *o*-quinones, and hence facilitate a nucleophilic addition of an alcohol molecule on *o*-quinones [15]. However, the reason why protocatechuic esters scavenge more than four equivalents of radical in alcoholic solvents remained to be clarified. Therefore, the aim of this study is to elucidate the radical scavenging mechanism beyond the formation of C(2) alcohol-adduct. We hypothesized that a second nucleophilic addition of an alcohol molecule occur on *o*-quinone of the C(2) adduct. In the present study, protocatechuic esters and authentic bis-alcohol adducts were oxidized by DPPH radical or *o*-chloranil, and their reaction mixtures were directly analyzed by NMR. In addition, to substantiate the regioselective nucleophilic addition, LUMO

energy and electron density of *o*-quinones were calculated by a semi-empirical method.

We propose the whole picture of the radical scavenging reaction of protocatechuic esters in alcoholic solvents.

**Result and Discussion.** Methyl protocatechuate (**1**) scavenges approximately five equivalents of DPPH radical in methanol [13]. Previously, we reported that **1** scavenges two equivalents of radical to yield the corresponding *o*-quinone (**2**) and its acetal (**3**) [14], and subsequent nucleophilic addition of methanol molecule at C(2) of **2** produces **4**, which scavenges additional two equivalents of radical to give *o*-quinone (**5**) and its acetal (**6**) [13]. However, taking into account that **1** scavenges more than four equivalents of radical, **5** and **6** should undergo further oxidation. Therefore, to elucidate the oxidation mechanism beyond the formation of C(2) adduct, the reaction mixture of **4** with DPPH radical in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1) was directly analyzed by NMR. (D<sub>6</sub>)acetone was added as a cosolvent for enhancing a solubility of DPPH radical. In the spectrum of the reaction mixture after 10 min, the signals of **4** completely disappeared and a new singlet signal at  $\delta$  5.75 was observed, together with the doublet signals of *o*-quinone (**5**) at  $\delta_{\text{H}}$  6.19 (*d*,  $J=10.3$ , H-C(5));  $\delta_{\text{H}}$  7.38 (*d*,  $J=10.3$ , H-C(6)), and its 3-hemiacetal (**6**) at  $\delta_{\text{H}}$  5.83 (*d*,  $J=10.3$ , H-C(5));  $\delta_{\text{H}}$  7.53 (*d*,  $J=10.3$ , H-C(6)) (Fig. 1(a)) [13]. Since further 2D-NMR analyses of the reaction mixture was restricted by low solubility of DPPH radical in methanol, *o*-chloranil was used as an oxidizing reagent.

The  $^1\text{H-NMR}$  spectrum of the reaction mixture of **4** and *o*-chloranil in  $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1) after 10 min showed, together with the signals of unreacted **4**, the signals of the corresponding *o*-quinone (**5**) and its 3-hemiacetal (**6**), which were identical to those of the oxidation products of **4** by DPPH radical (Fig. 2(a)). The result indicates that the reaction of **1** with *o*-chloranil proceeds in the similar manner as that with DPPH radical. After 30 min, two new singlet signals at  $\delta_{\text{H}}$  5.37 and  $\delta_{\text{H}}$  5.75 appeared, and the intensities of these two peaks gradually increased as those of peaks of **4**, **5** and **6** decreased (Fig. 2(b)). The *in situ* HMBC analysis of the reaction mixture showed the correlation of  $\delta_{\text{H}}$  5.37 and acetal carbon at  $\delta_{\text{C}}$  91.5, and  $\delta_{\text{H}}$  5.75 and carbonyl carbon at  $\delta_{\text{C}}$  178.2. In addition, the  $^1\text{H-NMR}$  spectrum of the mixture of **1** and *o*-chloranil after 6 h also showed the two singlet signals at  $\delta_{\text{H}}$  5.37 and  $\delta_{\text{H}}$  5.75, indicating that oxidation of **1** also gives the same products. We assumed that these singlet signals are the signals of bis-methanol adducts.

To confirm the formation of the bis-methanol adduct, 2,6-dimethoxy and 2,5-dimethoxyprotocatechuic acid methyl esters (**7** and **10**, respectively) were prepared, and the chemical shifts of their oxidation products were compared with those of the unknown products of **1** and **4**. Compound **7** and **10** were synthesized according to the procedure of scheme 1.

The reaction mixture of **7** and DPPH radical in  $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1) was directly analyzed by NMR. In the  $^1\text{H-NMR}$  spectrum after 10 min, the signals of **7**

disappeared and two new singlet signals at  $\delta_{\text{H}}$  5.37 and  $\delta_{\text{H}}$  5.75 were observed (Fig. 1(b)). The HMBC spectrum of the reaction mixture of **7** and DPPH radical was similar to that of the mixture of **4** and *o*-chloranil, and  $\delta_{\text{H}}$  5.75 showed cross peak with quinone carbonyl carbon at  $\delta_{\text{C}}$  178.2, and  $\delta_{\text{H}}$  5.37 with acetal carbon at  $\delta_{\text{C}}$  91.5. Thus, the signals at  $\delta_{\text{H}}$  5.75 and  $\delta_{\text{H}}$  5.37 were assigned to H-C(5) of *o*-quinone (**8**) and its 3-hemiacetal (**9**), respectively (Fig. 3). In addition, the signals of **8** and **9** remained unchanged for more than 5 h, indicating that **8** and **9** are more stable than the parents **2** and **3**, which disappeared within 1 h [13]. Hence, further nucleophilic addition of an alcohol molecule on **8** seems to be unlikely to occur. Moreover, oxidation of **7** with *o*-chloranil in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1) also formed the corresponding *o*-quinone (**8**) and its 3-hemiacetal (**9**).

In the <sup>1</sup>H-NMR spectrum of the reaction mixture of **10** and DPPH radical in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1), two singlet signals at  $\delta_{\text{H}}$  5.34 and  $\delta_{\text{H}}$  6.22 were observed. HMBC spectrum showed correlations between  $\delta_{\text{H}}$  6.22 and quinone carbonyl carbon at  $\delta_{\text{C}}$  174.5, and  $\delta_{\text{H}}$  5.34 and acetal carbon at  $\delta_{\text{C}}$  92.5. Thus, the  $\delta_{\text{H}}$  6.22 and  $\delta_{\text{H}}$  5.34 were assigned to H-C(6) of *o*-quinone (**11**) and its 4-hemiacetal (**12**), respectively (Fig. 3). Oxidation of **10** with *o*-chloranil in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1) also produced **11** and **12**. Since the singlet signals at  $\delta_{\text{H}}$  5.34 and  $\delta_{\text{H}}$  6.22 were not detected in the <sup>1</sup>H-NMR spectra of neither **1** nor **4** reacted with DPPH radical (or *o*-chloranil) in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1), it strongly indicates that C(6) of **5** is the preferred position

for the second nucleophilic attack of a methanol molecule.

We previously reported that oxidation of **1** in the presence of cysteine forms C(2) adduct, and subsequent oxidation of the resultant C(2) adduct leads to the corresponding *o*-quinone, which undergoes a second nucleophilic attack by cysteine at C(5) to produce C(2), C(5) bis-adduct instead of C(2), C(6) bis-adduct [16]. *Cheynier et al.* also reported that quinone of caffeoyltartaric acid undergoes a nucleophilic attack by glutathione at C(2), and further oxidation of the resultant C(2) adduct in the presence of glutathione yield C(2), C(5) bis-adduct [17]. To understand the regioselectivities of the nucleophilic attacks, LUMO energies and electron densities of *o*-quinone were calculated by semi-empirical method (Table 1). The result demonstrates that the LUMO electron densities of C(3) of *o*-quinones (**2**, **5** and **8**) are higher than those of C(4), indicating that an alcohol molecule preferentially attacks C(3) quinone carbonyl, rather than C(4), to form 3-hemiacetals (**3**, **6** and **9**). In addition, LUMO energies of *o*-quinones (**2**, **5** and **8**) are much lower than those of the corresponding 3-hemiacetals (**3**, **6** and **9**), suggesting that *o*-quinones exclusively undergo a nucleophilic attack by an alcohol molecule. In the case of compound **5**, C(6) has higher LUMO electron density compared to C(5). This result revealed that an alcohol molecule regioselectively attacks C(6). However, the presence of methyl sulfanyl group on C(2) produces a modification of LUMO parameters. The LUMO electron density of C(5) of **13** is, in fact, higher than C(6), and thus be the preferred center for a second nucleophilic attack. These results

support the regioselective nucleophilic additions in our experiments.

Time course of DPPH radical scavenging activity of **1** and its oxidation products (**4** and **7**) in methanol is shown in Fig. 1. The DPPH radical scavenging equivalence was expressed as the values relative to that of DL- $\alpha$ -tocopherol as 2.0. The result shows that compounds **1**, **4** and **7** rapidly reacted with DPPH radical and reached plateau within 20 min. The relative radical scavenging equivalences of each compound after 30 min were **1**, 5.0; **4**, 3.1; **7**, 1.9. Considering that **1** needs to scavenge six equivalents of radical to produce **8**, a second methanol addition on **5** to yield **7** might be limited. Furthermore, since **7** scavenged only two equivalents of radical, nucleophilic addition of an alcohol molecule on **8** would be unlikely to occur. This was supported by the result of LUMO energies of *o*-quinones which increased in the order of **2** < **5** < **8** (Table 1). In addition, steric hindrance due to bis-methanol addition may also explain the observed lack of reactivity of **8** toward a nucleophilic attack.

A plausible radical scavenging mechanism of **1** in methanol is shown in Scheme 2. First, **1** scavenges two radicals and is converted to the corresponding *o*-quinone (**2**). The resultant *o*-quinone (**2**) undergoes a nucleophilic attack by a methanol molecule at C(3) quinone carbonyl to yield its 3-hemiacetal (**3**) [14], which is equilibrated with **2** in the reaction solution. Then, a regeneration of a catechol structure occurs *via* a nucleophilic addition of an alcohol molecule at C(2) of *o*-quinone (**2**) to give **4**, which scavenges another two radicals to yield the corresponding *o*-quinone (**5**)

and its 3-hemiacetal (**6**). The resultant *o*-quinone (**5**) undergoes a second nucleophilic addition of an alcohol molecule at C(6), leading to a regeneration of a catechol structure to form **7**, which could consume additional two radicals to give *o*-quinone (**8**) and its 3-hemiacetal (**9**). Hence, the formation of the bis-alcohol adduct contribute to the high radical scavenging activity of **1** in alcoholic solvents.

### **Conclusions.**

In this study, we showed that oxidation of methyl protocatechuate in alcohol form C(2), C(6) bis-alcohol adduct, together with C(2) adduct. Our results strongly suggest that the regeneration of a catechol structure *via* a nucleophilic addition of an alcohol molecule on *o*-quinone is a key reaction for the high radical scavenging activity of protocatechuic esters in alcoholic solvents. Further study is needed to examine whether the oxidation mechanism shown in this paper also occur in biological aqueous system.

### **Experimental Part**

*Chemicals.* Protocatechuic acid was obtained from Sigma Chemical Co., and *o*-chloranil (tetrachloro-1,2-benzoquinone) from Aldrich Chemical Co. Methyl 2,4,6-trihydroxybenzoate was purchased from Alfa Aesar. Methyl protocatechuate (**1**) and methyl 3,4-dihydroxy-2-methoxybenzoate (**4**) were prepared by the methods

described previously [13]. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical and other reagents were purchased from Wako Pure Chemical Industries. All solvents used were of reagent grade.

*Apparatus.* NMR spectra were recorded on a Bruker AMX-500 spectrometer ( $^1\text{H}$ , 500 MHz;  $^{13}\text{C}$ , 125 MHz); chemical shifts are expressed relative to the residual signals of chloroform-*d* ( $\delta_{\text{H}}$  7.24,  $\delta_{\text{C}}$  77.0),  $\text{CD}_3\text{OD}$  ( $\delta_{\text{H}}$  3.30,  $\delta_{\text{C}}$  49.0) and ( $\text{D}_6$ )acetone ( $\delta_{\text{H}}$  2.04,  $\delta_{\text{C}}$  29.8). Electron ionization mass spectra (EI-MS) and Field desorption mass spectra (FD-MS) were obtained with a JEOL JMS-AX-500 and JEOL JMS-SX102A instruments, respectively. Melting point data were measured with a hot-stage apparatus and are uncorrected. Preparative and analytical thin-layer chromatography was performed on silica gel plates Merck 60 F<sub>254</sub> (0.5 and 0.25 mm thickness, respectively). Ordinary phase column chromatography was performed with silica gel, Wakogel C-300 (Wako Pure Chemical Industries).

*4-Benzoyloxy-2,6-dimethoxybenzoic acid methyl ester (7a).* A mixture of methyl 2,4,6-trihydroxybenzoate (1.84 g, 10 mmol), benzyl bromide (1.2 ml, 10 mmol, 1.0 equiv) and potassium carbonate (1.4 g, 10 mmol, 1.0 equiv) in acetone (50 ml) was refluxed for 3 h. After cooling to room temperature, the mixture was filtered, and the filtrate was evaporated under reduced pressure. The resulting benzyl ether was dissolved in acetone (50 ml), and methyl iodide (1.2 ml, 20 mmol, 2.0 equiv) and potassium carbonate (2.8 g, 20 mmol, 2.0 equiv) were added. After refluxing for 6 h, the

reaction mixture was filtered. The filtrate was concentrated under reduced pressure, and the crude product was subjected to silica gel column chromatography (hexane/ethyl acetate 2:1) to yield **7a** (2.1 g, 70%). <sup>1</sup>H-NMR (methanol-*d*<sub>6</sub>): 3.73 (*s*, 3H, COOMe); 3.77 (*s*, 6H, OMe); 5.16 (*s*, 2H, CH<sub>2</sub>); 6.35 (*s*, 2H, H-C(3) and H-C(5)); 7.31–7.49 (*m*, 5H, *Bn*). HR-FD-MS: 302.1152 (*M*<sup>+</sup>, C<sub>17</sub>H<sub>18</sub>O<sub>5</sub>; calc. 302.1154).

*4-Hydroxy-2,6-dimethoxybenzoic acid methyl ester (7b)*. Compound **7a** (1.8 g, 6.0 mmol) was deprotected by hydrogenation at an atmospheric pressure with a catalytic amount of 10% palladium on carbon in acetone (50 ml). The reaction mixture was filtered through celite, and the filtrate was concentrated under reduced pressure to afford **7b** (1.1 g, 87%). White powder. <sup>1</sup>H-NMR (CD<sub>3</sub>OD): 3.71 (*s*, 3H, COOMe); 3.72 (*s*, 6H, OMe); 6.13 (*s*, 2H, H-C(3) and H-C(5)). HR-EI-MS: 212.0667 (*M*<sup>+</sup>, C<sub>10</sub>H<sub>12</sub>O<sub>5</sub>; calc. 212.0685).

*3,4-Dihydroxy-2,6-dimethoxybenzoic acid methyl ester (7)*. Compound **7** was prepared by the modified method of *Shaw et al.* [18]. To a stirred mixture of **7b** (1.0 g, 4.7 mmol) and sodium hydroxide (2.0 g, 50 mmol) in water (50 ml) was added dropwise a solution of potassium persulfate (2.7 g, 10 mmol) in water (50 ml) at room temperature. After stirring for 24 h, the solution was acidified to pH 4 with conc. HCl. The reaction mixture was filtered, and the filtrate was washed with diethyl ether to remove the unreacted material (**7b**). The aqueous phase was added conc. HCl (10 ml), and refluxed for 2 h. After cooling to room temperature, the reaction mixture was

extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed and the residue was subjected to preparative thin-layer chromatography (chloroform/methanol/formic acid 100:4:0.1) to give **7** ( $R_f$  = 0.34, 0.15 g, 14%). Yellow crystalline solid.  $^1\text{H-NMR}$  ( $(\text{D}_6)$ acetone): 3.68 (*s*, 3H, *Me*); 3.76 (*s*, 3H, *Me*); 3.78 (*s*, 3H, *Me*); 6.35 (*s*, 1H, H-C(5)).  $^{13}\text{C-NMR}$  ( $(\text{D}_6)$ acetone): 51.9; 56.6; 61.5; 96.8; 110.9; 132.3; 147.3; 148.9; 151.0; 166.8. HR-FD-MS: 228.0620 ( $M^+$ ,  $\text{C}_{10}\text{H}_{12}\text{O}_6$ ; calc. 228.0634).

*3,4-Dihydroxy-5-methoxybenzoic acid methyl ester (10a)*. Compound **10a** was prepared by the method of *Chang et al.* [19]. To a solution of sodium tetraborate decahydrate (38 g, 0.10 mol, 2.5 equiv) in water (500 ml) was added methyl gallate (7.5 g, 41 mmol) at room temperature. After stirring for 1 h, dimethyl sulfate (15 ml, 0.16 mmol, 3.9 equiv) and 6.5 M aq. sodium hydroxide (25 ml) were added dropwise. After stirring for an additional 12 h, the reaction mixture was acidified to pH 2 with conc. sulfuric acid. The mixture was poured into water and extracted with ethyl acetate. The organic layer was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate 1:1) to afford **10a** (5.9 g, 73%). White powder.  $^1\text{H-NMR}$  ( $(\text{D}_6)$ acetone): 3.80 (*s*, 3H, *Me*); 3.86 (*s*, 3H, *Me*); 7.15 (*d*,  $J=2.0$ , 1H); 7.21 (*d*,  $J=2.0$ , 1H). HR-EI-MS: 198.0529 ( $M^+$ ,  $\text{C}_9\text{H}_{10}\text{O}_5$ ; calc. 198.0528).

*2-Bromo-3,4-dihydroxy-5-methoxybenzoic acid methyl ester (10b)*.

Compound **10b** was prepared by the modified method of *Lai et al.* [20]. To a solution of

**10a** (3.0 g, 15 mmol) in acetic acid/acetonitrile (3:1, 20 ml) was added dropwise *N*-bromosuccinimide (2.7 g, 15 mmol, 1.0 equiv) in acetic acid/acetonitrile (3:1, 24 ml), and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was suspended in water and extracted with ethyl acetate. The organic layer was concentrated under reduced pressure, and the crude product was subjected to silica gel column chromatography (hexane/ethyl acetate 1:1) to afford **10b** (3.8 g, 91%). <sup>1</sup>H-NMR ((D<sub>6</sub>)acetone): 3.82 (*s*, 3H, COOMe); 3.86 (*s*, 3H, 5-OMe); 7.06 (*s*, 1H, H-C(6)). <sup>13</sup>C-NMR ((D<sub>6</sub>)acetone): 52.2 (*Me*); 56.7 (*Me*); 102.8 (C(2)); 107.0 (C(6)); 123.7 (C(1)); 138.5 (C(4)); 144.4 (C(3)); 147.3 (C(5)); 167.0 (C=O). HMBC: H-C(6)↔C(2), C(4), C=O; 5-OMe↔C(5); COOMe↔C=O. HR-EI-MS: 275.9651 (*M*<sup>+</sup>, C<sub>9</sub>H<sub>9</sub>O<sub>5</sub>Br; calc. 275.9633).

*3,4-Dibenzoyloxy-2-bromo-5-methoxybenzoic acid methyl ester (10c)*. A mixture of **10b** (3.8 g, 14 mmol), benzyl bromide (3.3 ml, 28 mmol, 2.0 equiv) and potassium carbonate (3.9 g, 28 mmol, 2.0 equiv) in acetone (50 ml) was refluxed for 3 h. After cooling to room temperature, the mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate 2:1) to yield **10c** (5.4 g, 86%). <sup>1</sup>H-NMR ((D<sub>6</sub>)acetone): 3.87 (*s*, 3H, *Me*); 3.93 (*s*, 3H, *Me*); 5.04 (*s*, 2H, *CH*<sub>2</sub>); 5.15 (*s*, 2H, *CH*<sub>2</sub>); 7.26 (*s*, 1H, H-C(6)); 7.32–7.52 (*m*, 10H, *Bn*). HR-FD-MS: 456.0563 (*M*<sup>+</sup>, C<sub>23</sub>H<sub>21</sub>O<sub>5</sub>Br; calc. 456.0572).

*3,4-Dibenzyloxy-2-hydroxy-5-methoxybenzoic acid (10d)*. Compound **10d** was prepared by the modified method of *Cakmak et al.* [21]. To a stirred solution of **10c** (5.5 g, 12 mmol) in DMF (40 ml) was added a suspension of sodium methoxide (6.5 g, 120 mmol, 10 equiv) and cuprous iodide (1.1 g, 6.0 mmol, 0.50 equiv) in DMF (80 ml). The reaction mixture was stirred for 2 d under a nitrogen gas atmosphere at 90°C. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was concentrated under reduced pressure, and the crude product was chromatographed on silica gel column (hexane/ethyl acetate 1:1) to give **10d** (1.9 g, 42%). <sup>1</sup>H-NMR ((D<sub>6</sub>)acetone): 3.82 (*s*, 3H, 5-OMe); 5.07 (*s*, 2H, CH<sub>2</sub>); 5.09 (*s*, 2H, CH<sub>2</sub>); 7.28–7.51 (*m*, 11H, Bn, H-C(6)). HR-FD-MS: 380.1236 (*M*<sup>+</sup>, C<sub>22</sub>H<sub>20</sub>O<sub>6</sub>; calc. 380.1260).

*3,4-Dibenzyloxy-2,5-dimethoxybenzoic acid methyl ester (10e)*. To a solution of **10d** (1.0 g, 2.6 mmol) in acetone (20 ml) were added methyl iodide (0.32 ml, 5.2 mmol, 2.0 equiv) and potassium carbonate (0.72 g, 5.2 mmol, 2.0 equiv), and refluxed for 5 h. After filtering, the filtrate was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography (chloroform/methanol 100:3) to yield **10e** (0.96 g, 90%). <sup>1</sup>H-NMR (chloroform-*d*): 3.84 (*s*, 3H, Me); 3.87 (*s*, 3H, Me); 3.90 (*s*, 3H, Me); 5.01 (*s*, 2H, CH<sub>2</sub>); 5.08 (*s*, 2H, CH<sub>2</sub>); 7.13 (*s*, 1H, H-C(6)); 7.28–7.44 (*m*, 10H, Bn). HR-FD-MS: 408.1581 (*M*<sup>+</sup>, C<sub>24</sub>H<sub>24</sub>O<sub>6</sub>; calc. 408.1573).

*3,4-Dihydroxy-2,5-dimethoxybenzoic acid methyl ester (10)*. Compound **10e** (0.50 g, 1.2 mmol) in methanol (15 ml) was deprotected by hydrogenation at an atmospheric pressure with a catalytic amount of 10% palladium on carbon. The reaction mixture was filtered through celite, and filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (chloroform/methanol 10:1) to afford **10** (0.26 g, 93%). Pale yellow powder. M.p. 126–128°. <sup>1</sup>H-NMR (CD<sub>3</sub>OD): 3.77 (*s*, 3H, *Me*); 3.82 (*s*, 3H, *Me*); 3.84 (*s*, 3H, *COOMe*); 6.95 (*s*, 1H, H-C(6)). <sup>13</sup>C-NMR (CD<sub>3</sub>OD): 52.3 (*COOMe*); 56.7 (*Me*); 62.0 (*Me*); 105.5 (C(6)); 114.6 (C(1)); 140.5 (C(3)); 141.3 (C(4)); 145.4 and 145.6 (C(2) and C(5)); 167.9 (C=O). HMBC: H-C(6)↔C(2), C(4), C=O; 2-*OMe*↔C(2); 5-*OMe*↔C(5); *COOMe*↔C=O. HR-EI-MS: 228.0669 (*M*<sup>+</sup>, C<sub>10</sub>H<sub>12</sub>O<sub>6</sub>; calc. 228.0634).

*NMR analysis. NMR measurements of the reaction mixtures of catechols (4, 7 and 10) and DPPH radical.* To a catechol (2.5 μmol) was added DPPH radical (3.0 mg, 7.6 μmol, 3.0 equiv) in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1, 0.4 ml). (D<sub>6</sub>)acetone was added as a cosolvent for enhancing a solubility of DPPH radical. The mixture was immediately transferred to a NMR tube and mixed vigorously. <sup>1</sup>H-NMR spectra were recorded at 10 min after mixing.

*Reaction of 4 and DPPH radical.* **5**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 6.19 (*d*, *J*=10.3, 1H, H-C(5)); 7.38 (*d*, *J*=10.3, 1H, H-C(6)). **6**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 5.83 (*d*, *J*=10.3, 1H, H-C(5)); 7.53 (*d*, *J*=10.3, 1H, H-C(6)).

**8:**  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 5.75 (*s*, 1H, H-C(5)).

*Reaction of 7 and DPPH radical. 8:*  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 3.85 (*s*, 3H, *Me*); 3.90 (*s*, 3H, *Me*); 3.98 (*s*, 3H, *Me*); 5.75 (*s*, 1H, H-C(5)). HMBC: H-C(5) $\leftrightarrow$ 123.5 (C(1)), 178.2 (C(3)). **9:**  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 3.79 (*s*, 3H, *Me*); 3.85 (*s*, 3H, *Me*); 4.04 (*s*, 3H, *Me*); 5.37 (*s*, 1H, H-C(5)). HMBC: H-C(5) $\leftrightarrow$ 91.5 (C(3)), 109.6 (C(1)).

*Reaction of 10 and DPPH radical. 11:*  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 3.75 (*s*, 3H, *Me*); 3.85 (*s*, 3H, *Me*); 3.87 (*s*, 3H, *Me*); 6.22 (*s*, 1H, H-C(6)). HMBC: H-C(6) $\leftrightarrow$ 150.2, 151.9, 167.1, 174.5. **12:**  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 3.72 (*s*, 3H, *Me*); 3.76 (*s*, 3H, *Me*); 3.84 (*s*, 3H, *Me*); 5.34 (*s*, 1H, H-C(6)). HMBC: H-C(6) $\leftrightarrow$ 92.5, 145.5, 161.0, 167.4.

*NMR measurements of the reaction mixtures of catechols (1, 4, 7 and 10)*

*and o-chloranil.* To a catechol (20  $\mu\text{mol}$ ) was added *o*-chloranil (4.9 mg, 20  $\mu\text{mol}$ , 1.0 equiv) in  $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (0.4 ml, 3:1). The mixture was immediately transferred to a NMR tube and mixed vigorously.  $^1\text{H}$  NMR spectra of **1** and **4** were recorded at 10, 30, 60, 120, 180, 360 min, and **7** and **10** at 10 min after mixing.

*Reaction of 1 and o-chloranil. 2:*  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 6.44 (*d*,  $J=10.3$ , 1H, H-C(5)); 6.93 (*d*,  $J=2.0$ , 1H, H-C(2)); 7.52 (*dd*,  $J=10.3, 2.0$ , 1H, H-C(6)). **3:**  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 6.11 (*d*,  $J=10.3$ , 1H, H-C(5)); 7.21 (*d*,  $J=2.0$ , 1H, H-C(2)). **5:**  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}/(\text{D}_6)\text{acetone}$  (3:1)): 6.18 (*d*,  $J=10.3$ , 1H, H-C(5)). **6:**

<sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 5.83 (1H, *d*, *J*=10.3, 1H, H-C(5)). **8**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 5.75 (*s*, 1H, H-C(5)). **9**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 5.37 (*s*, 1H, H-C(5)).

*Reaction of 4 and o-chloranil. 5*: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 6.19 (*d*, *J*=10.3, 1H, H-C(5)); 7.38 (*d*, *J*=10.3, 1H, H-C(6)). **6**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 5.83 (*d*, *J*=10.3, 1H, H-C(5)); 7.53 (*d*, *J*=10.3, 1H, H-C(6)). **8**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 5.75 (*s*, 1H, H-C(5)). **9**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 5.37 (*s*, 1H, H-C(5)).

*Reaction of 7 and o-chloranil. 8*: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 3.85 (*s*, 3H, *Me*); 3.90 (*s*, 3H, *Me*); 3.98 (*s*, 3H, *Me*); 5.75 (*s*, 1H, H-C(5)). **9**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 3.79 (*s*, 3H, *Me*); 3.84 (*s*, 3H, *Me*); 4.04 (*s*, 3H, *Me*); 5.37 (*s*, 1H, H-C(5)).

*Reaction of 10 and o-chloranil. 11*: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 3.76 (*s*, 3H, *Me*); 3.85 (*s*, 3H, *Me*); 3.87 (*s*, 3H, *Me*); 6.22 (*s*, 1H, H-C(6)). **12**: <sup>1</sup>H-NMR (CD<sub>3</sub>OD/(D<sub>6</sub>)acetone (3:1)): 3.72 (*s*, 3H, *Me*); 3.76 (*s*, 3H, *Me*); 3.84 (*s*, 3H, *Me*); 5.34 (*s*, 1H, H-C(6)).

*Molecular orbital calculations.* Electron density and energy of LUMO were calculated by AM1 method using MOPAC 2000 program combined in Chem3D package, CambridgeSoft Co.

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Table 1. *LUMO Energy and Electron Density at Each C-Atom of o-Quinones (2, 5, 8, and 13) and Their 3-Hemiacetals (3, 6, and 9)*

Compound	<b>2</b>	<b>3</b>	<b>5</b>	<b>6</b>	<b>8</b>	<b>9</b>	<b>13</b>
LUMO	-2.086	-1.336	-1.978	-1.171	-1.706	-1.368	-1.913
C(1)	0.30	0.25	0.20	0.11	0.21	0.23	0.25
C(2)	0.41	0.63	0.38	0.57	0.40	0.55	0.47
C(3)	0.20	0.02	0.29	0.02	0.24	0.01	0.20
C(4)	0.14	0.17	0.16	0.26	0.16	0.16	0.12
C(5)	0.21	0.29	0.14	0.21	0.13	0.32	0.17
C(6)	0.16	0.29	0.17	0.37	0.23	0.35	0.15

Scheme 1. *Synthesis of Compounds 7 and 10*

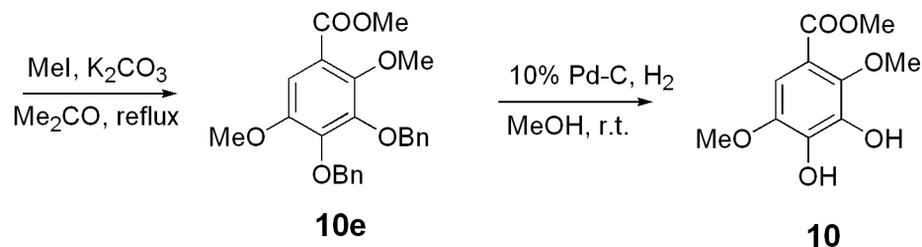
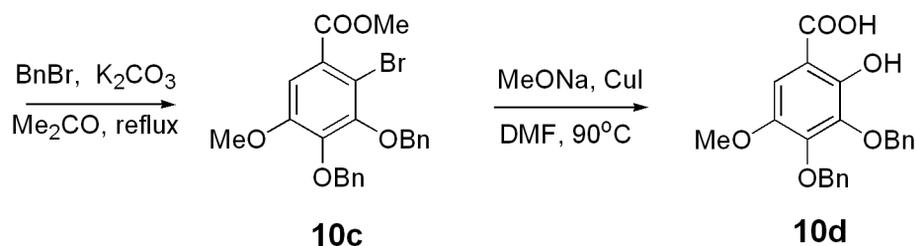
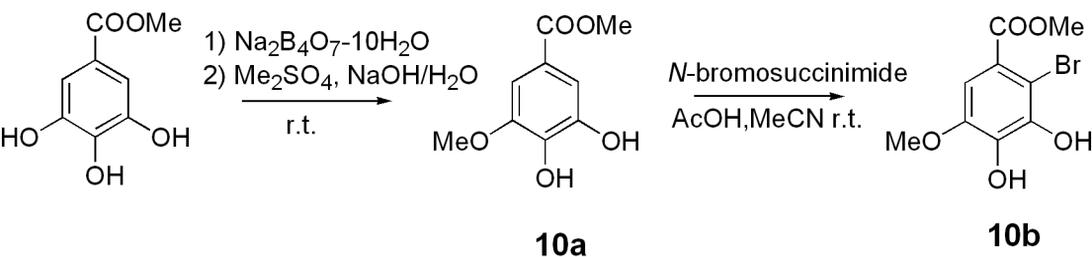
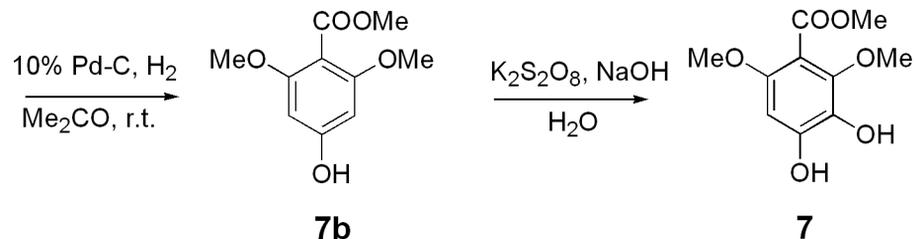
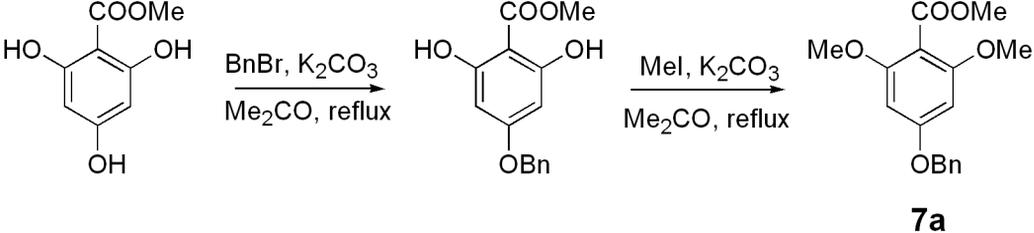
Scheme 2. *Plausible Radical Scavenging Mechanism of Methyl Protocatechuate (1) in Methanol*

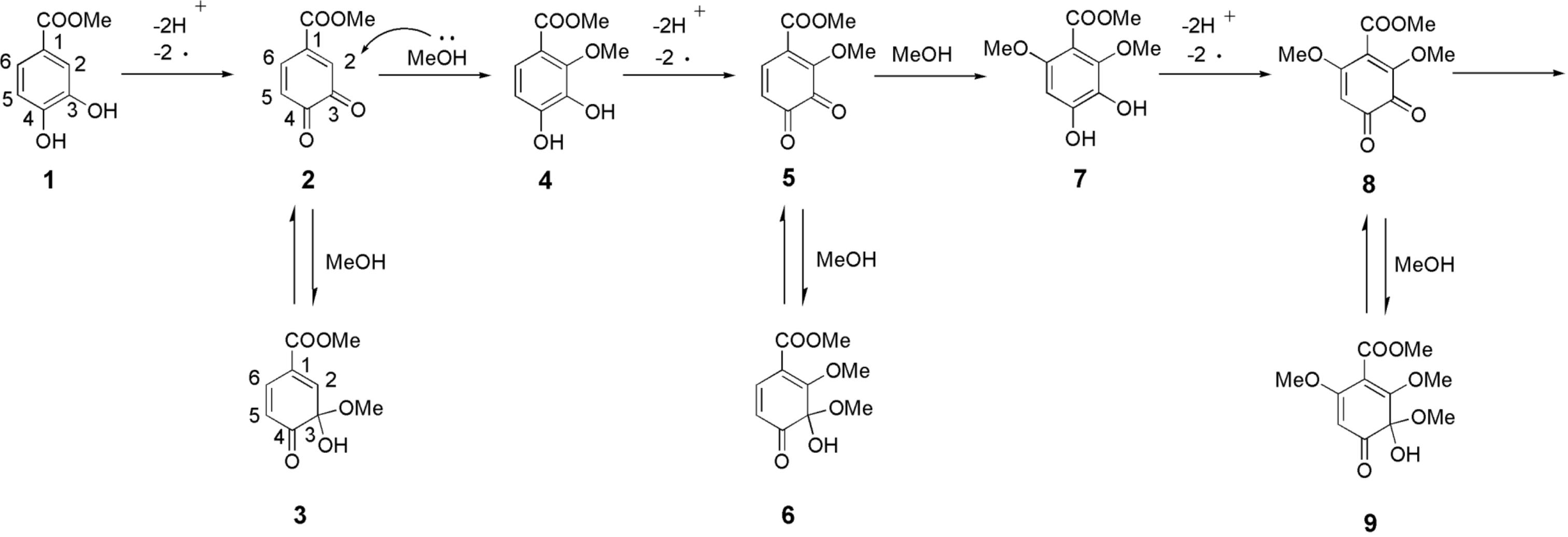
Fig. 1. *<sup>1</sup>H-NMR Spectra of 4 (a) and 7 (b) reacted with DPPH radical in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone 3:1 10 min after being mixed. The intense signals in the range of 7.1 – 7.4 ppm are due to 2,2-diphenyl-1-picrylhydrazine.*

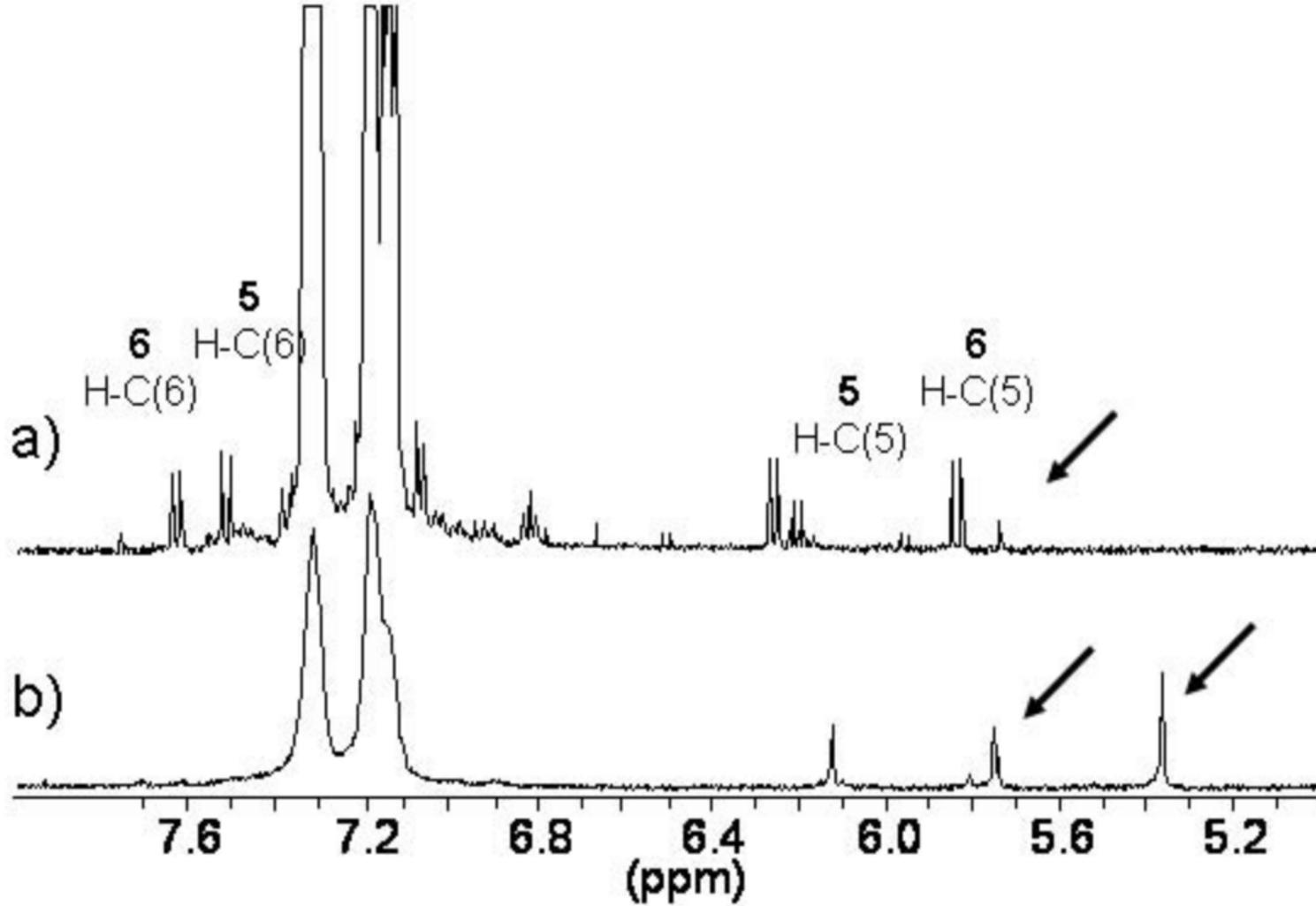
Fig. 2. *<sup>1</sup>H-NMR Spectra of 4 reacted with o-chloranil in CD<sub>3</sub>OD/(D<sub>6</sub>)acetone 3:1 after 10 min (a) and 60 min (b)*

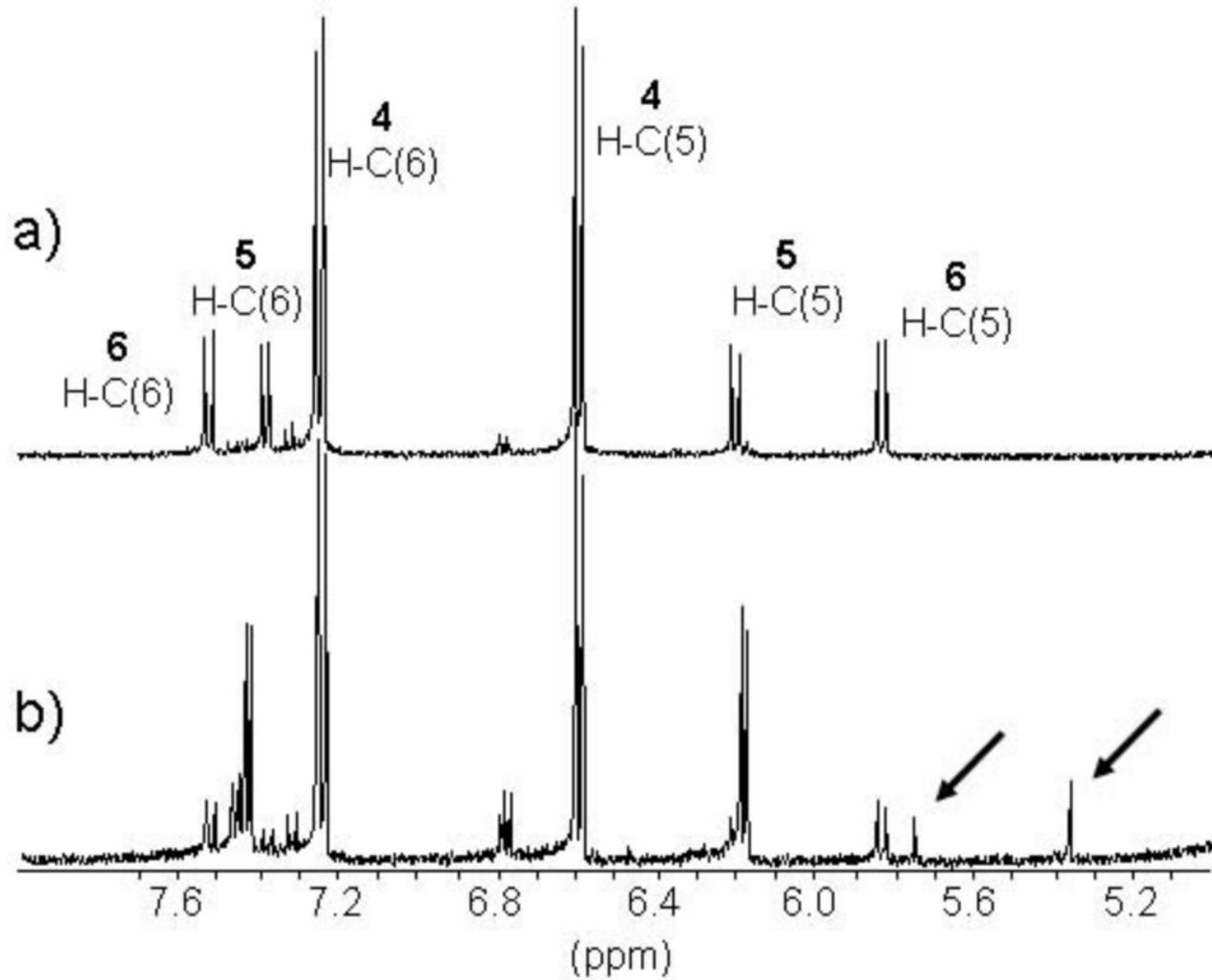
Fig. 3. *Compounds 7, 10, 13, and their oxidation products*

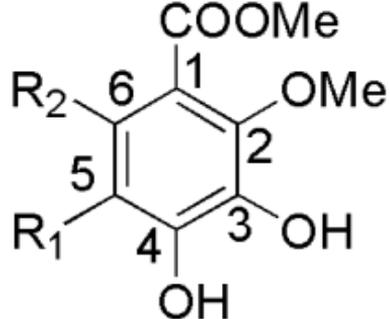
Fig. 4. *Time course of DPPH radical scavenging activity of 1 (●), 4 (○), and 7 (■) in methanol. The equivalence is expressed as the values relative to that of DL- $\alpha$ -tocopherol as 2.0.*





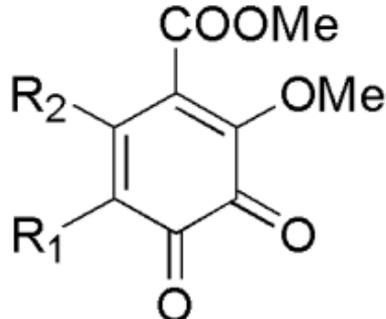






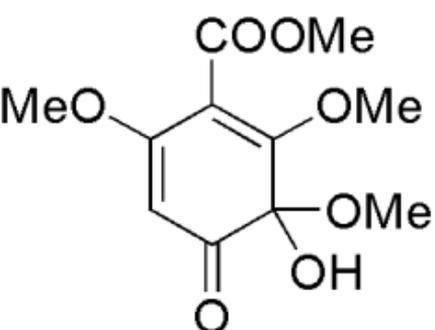
**7** R<sub>1</sub>=H, R<sub>2</sub>=OMe

**10** R<sub>1</sub>=OMe, R<sub>2</sub>=H

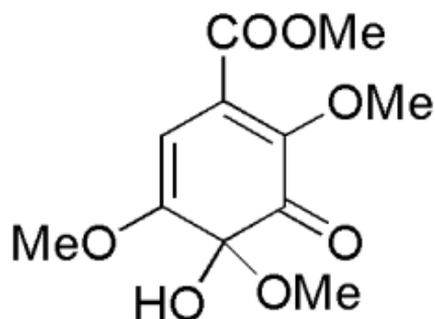


**8** R<sub>1</sub>=H, R<sub>2</sub>=OMe

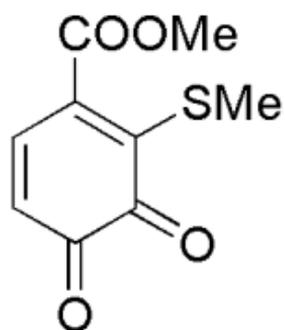
**11** R<sub>1</sub>=OMe, R<sub>2</sub>=H



**9**



**12**



**13**

