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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Protocatechuic acid esters (=3,4-dihydroxybenzoates) scavenge *ca.* 5 equiv. of radical in alcoholic solvents, whereas they consume only 2 equiv. of radical in nonalcoholic solvents. While the high radical-scavenging activity of protocatechuic acid esters in alcoholic solvents as compared to that in nonalcoholic solvents is due to a nucleophilic addition of an alcohol molecule at C(2) of an intermediate *o*-quinone structure, thus regenerating a catechol (=benzene-1,2-diol) structure, it is still unclear why protocatechuic acid esters scavenge more than 4 equiv. of radical (C(2) refers to the protocatechuic acid numbering). Therefore, to elucidate the oxidation mechanism beyond the formation of the C(2) alcohol adduct, 3,4-dihydroxy-2-methoxybenzoic acid methyl ester (**4**), the C(2) MeOH adduct, which is an oxidation product of methyl protocatechuate (**1**) in MeOH, was oxidized by the DPPH radical (=2,2-diphenyl-1-picrylhydrazyl) or *o*-chloranil (=3,4,5,6-tetrachlorocyclohexa-3,5-diene-1,2-dione) in CD_3OD/(D_6)acetone 3:1). The oxidation mixtures were directly analyzed by NMR. Oxidation with both the DPPH radical and *o*-chloranil produced a C(2),C(6) bis-methanol adduct (**7**), which could scavenge additional 2 equiv. of radical. Calculations of LUMO electron densities of *o*-quinones corroborated the regioselective nucleophilic addition of alcohol molecules with *o*-quinones. Our results strongly suggest that the regeneration of a catechol structure *via* a nucleophilic addition of an alcohol molecule with a *o*-quinone is a key reaction for the high radical-scavenging activity of protocatechuic acid esters in alcoholic solvents.

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 radicals [6–8]. Recently, numerous studies have been reported on the 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 OH groups in the molecule [9–11]. Among them, *o*- or *p*-dihydroxy compounds (='*o*- or *p*-diphenols') such as protocatechuic acid exhibit a high radical-scavenging activity, since they would be converted to the corresponding *o*- or *p*-quinone derivatives, respectively [9]. In addition, the higher radical-scavenging activity of benzenepolyols ('polyphenols') can be, in part, ascribed to the dimerization of *o*-quinones or semiquinone radicals, since the resultant dimers could scavenge additional radicals if they possess a catechol (=benzene-1,2-diol) structure [12]. However, more

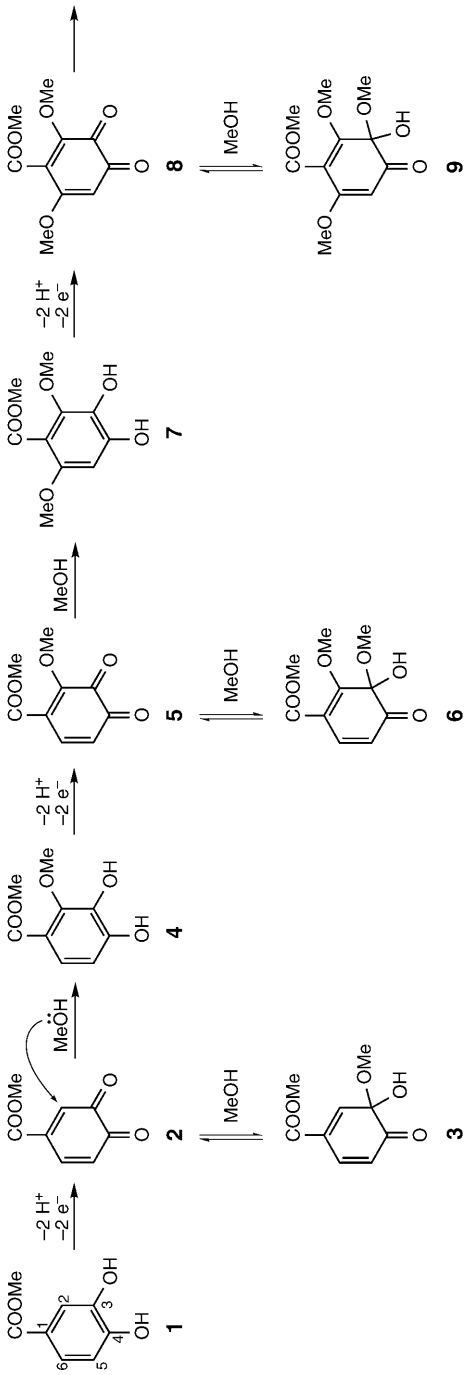
detailed studies are needed to understand the radical-scavenging mechanism beyond the formation of quinones.

We have recently reported the DPPH (=2,2-diphenyl-1-picrylhydrazyl=Ph_2-NN(Picr)$^{\bullet}$) radical-scavenging mechanism of protocatechuic acid esters (=protocatechuates) in nonalcoholic and alcoholic solvents [13]. In nonalcoholic acetone or acetonitrile, protocatechuic acid and its esters scavenge 2 equiv. of radical to yield the corresponding *o*-quinones. In contrast, in alcoholic solvents, protocatechuates rapidly react with *ca.* 5 equiv. of radical with a concomitant conversion to the corresponding *o*-quinone derivatives, 3-hemiacetals [14], and their alcohol adducts at C(2) [13]. It was suggested that the higher radical-scavenging activity of protocatechuates in alcoholic solvents as compared to that in nonalcoholic solvents is due to a regeneration of a corresponding catechol structure *via* a nucleophilic addition of an alcohol molecule at C(2) of an *o*-quinone intermediate (C(2) refers to the protocatechuic acid numbering) [13]. Moreover, we found that catechols possessing strong electron-withdrawing substituents in *p*-position C(1) exhibit a 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 with an *o*-quinone [15]. However, the reason why protocatechuates scavenge more than 4 equiv. 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 the C(2) alcohol adduct of protocatechuate. We hypothesized that a second nucleophilic addition of an alcohol molecule occurs with an *o*-quinone intermediate generated from the C(2) adduct. In the present study, protocatechuic acid esters and authentic bis-alcohol adducts were oxidized by the DPPH radical or by *o*-chloranil, and the oxidation mixtures were directly analyzed by NMR. In addition, to substantiate the regioselective nucleophilic addition, the LUMO energy and electron densities of the *o*-quinone intermediates were calculated by a semi-empirical method. Finally, we propose a scheme with the entire radical-scavenging reaction pattern of protocatechuic acid esters in alcoholic solvents.

Result and Discussion. – Methyl protocatechuate (**1**) scavenges *ca.* 5 equiv. of DPPH radical in MeOH [13]. Previously, we reported that **1** scavenges 2 equiv. of radical to yield the corresponding *o*-quinone derivative **2** and its hemiacetal **3** [14], and subsequent nucleophilic addition of a MeOH molecule at C(2) of **2** produces **4**, which scavenges additional 2 equiv. of radical to give *o*-quinone derivative **5** and its hemiacetal **6** [13] (*Scheme 1*). However, taking into account that **1** scavenges more than 4 equiv. of radical, **5** and **6** should undergo further oxidation.

Therefore, to elucidate the oxidation mechanism beyond the formation of the C(2) adduct **4**, the reaction mixture of **4** and the DPPH radical in CD_3OD/(D_6)Acetone 3:1 was directly analyzed by ^{1}H-NMR. (D_6)Acetone was added as a cosolvent to enhance the solubility of the DPPH radical. In the ^{1}H-NMR spectrum of the reaction mixture after 10 min, the signals of **4** had completely disappeared, and a new *s* at δ 5.75 was observed, besides the *d* of the *o*-quinone derivative **5** at δ 6.19 (*d*, J=10.3 Hz, H–C(5)) and 7.38 (*d*, J=10.3 Hz, H–C(6)), and the signals of the 3-hemiacetal **6** at δ 5.83 (*d*, J=10.3 Hz, H–C(5)) and 7.53 (*d*, J=10.3 Hz, H–C(6)) (*Fig. 1*,*a*) [13]. Since further 2D-NMR analyses of the reaction mixture was hampered by the low solubility

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



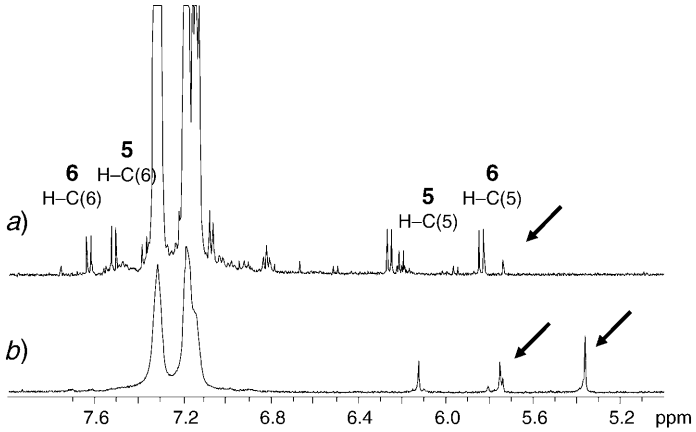


Fig. 1. *^{1}H-NMR Spectra of the reaction mixture of* a) **4** *and* b) **7** *with the DPPH radical in CD_3OD/(D_6)acetone 3:1, 10 min after being mixed.* The intense signals in the range δ 7.1–7.4 are due to DPPH.

of the DPPH radical in MeOH, *o*-chloranil (=3,4,5,6-tetrachlorocyclohexa-3,5-diene-1,2-dione) was used as an oxidizing reagent. The ^{1}H-NMR spectrum of the reaction mixture of **4** and *o*-chloranil in CD_3OD/(D_6)acetone 3:1 after 10 min showed, besides the signals of unreacted **4**, the signals of the corresponding *o*-quinone derivative **5** and the 3-hemiacetal **6**, which were identical to those of the oxidation products of **4** by DPPH (*Fig. 2,a*). This result indicates that the reaction of **1** with *o*-chloranil proceeds similarly to that with DPPH. After 30 min, two new *s* at δ 5.37 and 5.75 appeared the intensities of which gradually increased as those of the signals of **4**, **5**, and **6** decreased (*Fig. 2,b*). The *in situ* HMBC analysis of the reaction mixture **4**/*o*-chloranil showed the correlation of δ(H) 5.37 with the acetal C-atom at δ(C) 91.5, and of δ(H) 5.75 with the carbonyl C-atom at δ(C) 178.2. In addition, the ^{1}H-NMR spectrum of the mixture **1**/*o*-chloranil, after 6 h, also showed the two *s* at δ 5.37 and δ 5.75, indicating that oxidation of **1** also gives the same product as **4**. We assumed that these *s* 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**, resp.) were prepared according to the procedure of *Scheme 2*, and the chemical shifts of their oxidation products were compared with those of the unknown products obtained from **1** and **4**.

The reaction mixture of **7** and DPPH in CD_3OD/(D_6)acetone 3:1 was directly analyzed by NMR. In the ^{1}H-NMR spectrum after 10 min, the signals of **7** disappeared and two new *s* at δ 5.37 and 5.75 were observed (*Fig. 1,b*). The HMBC plot of the reaction mixture **7**/DPPH was similar to that of the mixture **4**/*o*-chloranil, and δ(H) 5.75 showed a cross peak with the quinone carbonyl C-atom at δ(C) 178.2, and δ(H) 5.37 with the acetal C-atom at δ(C) 91.5. Thus, the signals at δ 5.75 and δ 5.37 were assigned to H−C(5) of the *o*-quinone derivative **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 with **8** seems to be

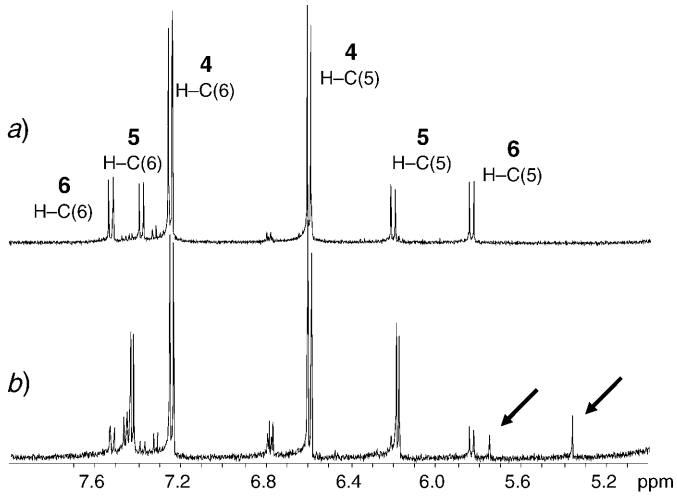
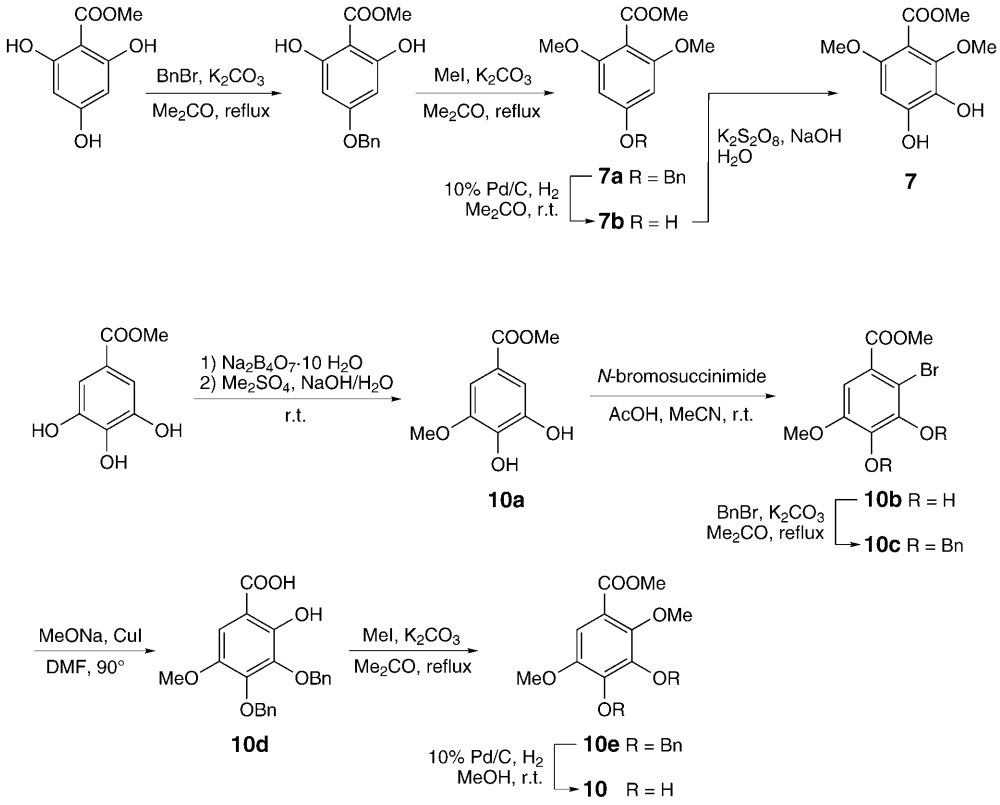
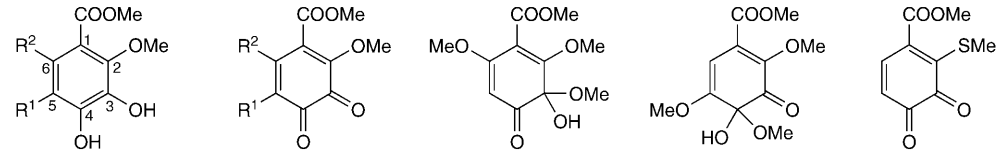


Fig. 2. *^{1}H-NMR Spectra of the reaction mixture of* **4** *with* o-*chloranil in $CD_3OD/(D_6)$acetone 3:1, after* a) *10 min and* b) *60 min*

Scheme 2. *Synthesis of Compounds* **7** *and* **10**



unlikely to occur. Moreover, oxidation of **7** with *o*-chloranil in $CD_3OD/(D_6)$acetone 3 : 1 also formed the corresponding *o*-quinone derivative **8** and its 3-hemiacetal **9**.



7 R^1 = H, R^2 = MeO
10 R^1 = MeO, R^2 = H
8 R^1 = H, R^2 = MeO
11 R^1 = MeO, R^2 = H
9
12
13

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

In the ^{1}H-NMR spectrum of the reaction mixture of **10** and DPPH in $CD_3OD/(D_6)$acetone 3 : 1, two *s* at δ 5.34 and 6.22 were observed. The HMBC spectrum showed correlations between δ(H) 6.22 and the quinone carbonyl C-atom at δ(C) 174.5, and between δ(H) 5.34 and the acetal C-atom at δ(C) 92.5. Thus, δ 6.22 and 5.34 were assigned to H–C(6) of the *o*-quinone derivative **11** and its 4-hemiacetal **12**, respectively (*Fig. 3*). Oxidation of **10** with *o*-chloranil in $CD_3OD/(D_6)$acetone 3 : 1 also produced **11** and **12**. The absence of the *s* at δ 5.34 and 6.22 in the ^{1}H-NMR spectra of the reaction mixtures of **1** or **4** with DPPH (or *o*-chloranil) in $CD_3OD/(D_6)$acetone 3 : 1 strongly indicates that C(6) of **5** is the preferred position for the second nucleophilic attack of a MeOH molecule.

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

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

		2	**3**	**5**	**6**	**8**	**9**	**13**
	LUMO	−2.086	−1.336	−1.978	−1.171	−1.706	−1.368	−1.913
Electron density at	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

The time course of the DPPH radical-scavenging activity of **1** and its oxidation products **4** and **7** in MeOH is shown in *Fig. 4*. The DPPH-radical-scavenging equivalence is expressed as a value relative to that of DL-α-tocopherol taken as 2.0. The result shows that compounds **1**, **4**, and **7** rapidly reacted with the DPPH radical and reached a plateau within 20 min. The relative radical-scavenging equivalences of each compound after 30 min were 5.0 for **1**, 3.1 for **4**, and 1.9 for **7**. Considering that **1** needs to scavenge 6 equiv. of radical to produce **8**, a second MeOH addition with **5** to yield **7** might be limited. Furthermore, since **7** scavenged only 2 equiv. of radical, the nucleophilic addition of an alcohol molecule with **8** would be unlikely to occur. This was supported by the calculated values of the LUMO energies of the *o*-quinone derivatives which increased in the order of **2** < **5** < **8** (*Table*). In addition, steric hindrance due to bis-methanol addition may also explain the observed lack of reactivity of **8** toward a nucleophilic attack.

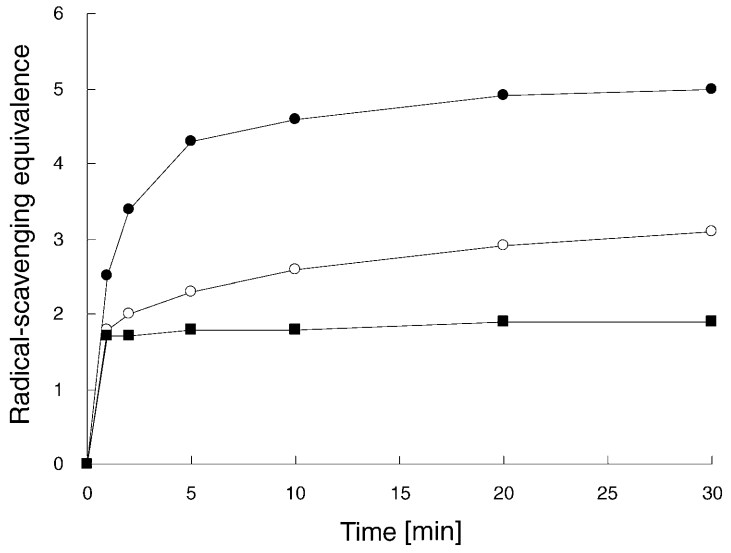


Fig. 4. *Time course of DPPH-radical-scavenging activity of* **1** (●), **4** (○), *and* **7** (■) *in methanol.* The equivalence is expressed as the values relative to that of DL-α-tocopherol taken as 2.0.

A plausible radical-scavenging mechanism of **1** in MeOH is shown in *Scheme 1*. First, **1** scavenges two radicals and is converted to the corresponding *o*-quinone derivative **2**. The latter undergoes a nucleophilic attack by a MeOH molecule at C(3) to yield the 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 a MeOH molecule at C(2) of **2** to give **4**, which scavenges another two radicals to yield the corresponding *o*-quinone derivative **5** and its 3-hemiacetal **6**. The *o*-quinone derivative **5** undergoes a second nucleophilic addition of a MeOH molecule at C(6), leading to **7**, *i.e.*, to a regeneration of a catechol structure which can consume additional 2 equiv. of radical to give *o*-quinone derivative **8** and its 3-hemiacetal **9**. Hence, the formation of a bis-alcohol adduct contributes to the high radical-scavenging activity of **1** in alcoholic solvents.

Conclusions. – In this study, we showed that oxidation of methyl protocatechuate (**1**) in MeOH leads to a C(2),C(6) bis-methanol adduct, besides a C(2) adduct. Our results strongly suggest that the regeneration of a catechol structure *via* a nucleophilic addition of an alcohol molecule with an *o*-quinone derivative is a key reaction for the high radical-scavenging activity of protocatechuic acid esters in alcoholic solvents. Further study is needed to examine whether the oxidation mechanism described above also occurs in a biological aqueous system.

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Experimental Part

General. Protocatechuic acid was obtained from *Sigma Chemical Co.*, and *o*-chloranil (=3,4,5,6-tetrachlorocyclohexa-3,5-diene-1,2-dione) 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]. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and other reagents were purchased from *Wako Pure Chemical Industries*. All solvents used were of reagent grade.

Prep. and anal. TLC: silica gel plates *Merck 60* F_{254} (0.5 and 0.25 mm thickness, resp.). Column chromatography (CC): silica gel, *Wakogel C-300* (*Wako Pure Chemical Industries*). M.p.: hot-stage apparatus; uncorrected. NMR Spectra: *Bruker-AMX-500* spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz); chemical shifts δ in ppm rel. to the residual signals of $CDCl_3$ (δ(H) 7.24, δ(C) 77.0), CD_3OD (δ(H) 3.30, δ(C) 49.0) and (D_6)acetone (δ(H) 2.04, δ(C) 29.8). EI-MS and FD-MS: *Jeol-JMS-AX-500* and *Jeol-JMS-SX102A* instruments, resp.; in *m*/*z* (rel.%).

4-(Benzyloxy)-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 K_2CO_3 (1.4 g, 10 mmol, 1.0 equiv.) in acetone (50 ml) was refluxed for 3 h. After cooling to r.t., the mixture was filtered and the filterate evaporated. The resulting benzyl ether was dissolved in acetone (50 ml), and MeI (1.2 ml, 20 mmol, 2.0 equiv.) and K_2CO_3 (2.8 g, 20 mmol, 2.0 equiv.) were added. After refluxing for 6 h, the mixture was filtered, the filterate evaporated, and the crude product subjected to CC silica gel, hexane/AcOEt 2:1: **7a** (2.1 g, 70%). ^{1}H-NMR (CD_3OD): 3.73 (*s*, COOMe); 3.77 (*s*, 2 MeO); 5.16 (*s*, PhCH_2); 6.35 (*s*, H–C(3), H–C(5)); 7.31–7.49 (*m*, *Ph*CH_2). HR-FD-MS: 302.1152 (M^+, $C_{17}H_{18}O_5^+$; 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 1 atm. in the presence of a catalytic amount of 10% Pd/C in acetone

(50 ml). The mixture was filtered through *Celite*, and the filtrate was evaporated: **7b** (1.1 g, 87%). White powder. ^{1}H-NMR (CD_3OD): 3.71 (*s*, COOMe); 3.72 (*s*, 2 MeO); 6.13 (*s*, H–C(3), H–C(5)). HR-EI-MS: 212.0667 (M^+, $C_{10}H_{12}O_5^+$; calc. 212.0685).

3,4-Dihydroxy-2,6-dimethoxybenzoic Acid Methyl Ester (**7**). According to the modified method of *Shaw et al.* [18]: To a stirred mixture of **7b** (1.0 g, 4.7 mmol) and NaOH (2.0 g, 50 mmol) in H_2O (50 ml) was added dropwise a soln. of potassium persulfate (2.7 g, 10 mmol) in H_2O (50 ml) at r.t. After stirring for 24 h, the soln. was acidified to pH 4 with conc. HCl soln. The mixture was filtered, and the filtrate was washed with Et_2O to remove unreacted **7b**. To the aq. phase was added conc. HCl soln. (10 ml), and the mixture was refluxed for 2 h. After cooling to r.t., the mixture was extracted with AcOEt, the org. layer dried (Na_2SO_4) and evaporated and the residue subjected to prep. TLC ($CHCl_3$/MeOH/HCOOH 100 : 4 : 0.1): **7** (0.15 g, 14%). R_f = 0.34. Yellow crystalline solid. ^{1}H-NMR ((D$_6$)acetone): 3.68 (*s*, 1 Me); 3.76 (*s*, 1 Me); 3.78 (*s*, 1 Me); 6.35 (*s*, H–C(5)). ^{13}C-NMR ((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^+, $C_{10}H_{12}O_6^+$; calc. 228.0634).

3,4-Dihydroxy-5-methoxybenzoic Acid Methyl Ester (**10a**). According to the method of *Chang et al.* [19]: To a soln. of sodium tetraborate decahydrate (38 g, 0.10 mol, 2.5 equiv.) in H_2O (500 ml) was added methyl gallate (= methyl 3,4,5-trihydroxybenzoate; 7.5 g, 41 mmol) at r.t. After stirring for 1 h, dimethyl sulfate (15 ml, 0.16 mmol, 3.9 equiv.) and 6.5M aq. NaOH (25 ml) were added dropwise. After stirring for an additional 12 h, the mixture was acidified to pH 2 with conc. H_2SO_4 soln. The mixture was poured into H_2O and extracted with AcOEt. The org. layer was evaporated and the residue was purified by CC (silica gel, hexane/AcOEt 1 : 1): **10a** (5.9 g, 73%). White powder. ^{1}H-NMR ((D$_6$)acetone): 3.80 (*s*, Me); 3.86 (*s*, Me); 7.15 (*d*, *J* = 2.0, 1 H); 7.21 (*d*, *J* = 2.0, 1 H). HR-EI-MS: 198.0529 (M^+, $C_9H_{10}O_5^+$; calc. 198.0528).

2-Bromo-3,4-dihydroxy-5-methoxybenzoic Acid Methyl Ester (**10b**). According to the modified method of *Lai et al.* [20]: To a soln. of **10a** (3.0 g, 15 mmol) in AcOH/MeCN 3 : 1 (20 ml) was added dropwise *N*-bromosuccinimide (2.7 g, 15 mmol, 1.0 equiv.) in AcOH/MeCN 3 : 1 (24 ml), and the mixture was stirred at r.t. for 4 h. The mixture was evaporated, the residue suspended in H_2O and extracted with AcOEt, the org. layer evaporated, and the crude product subjected to CC (silica gel, hexane/AcOEt 1 : 1): **10b** (3.8 g, 91%). ^{1}H-NMR ((D$_6$)acetone): 3.82 (*s*, COOMe); 3.86 (*s*, MeO–C(5)); 7.06 (*s*, H–C(6)). ^{13}C-NMR ((D$_6$)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). ^{1}H,^{13}C-HMBC: H–C(6) ↔ C(2), C(4), C=O; MeO–C(5) ↔ C(5); COOMe ↔ C=O. HR-EI-MS: 275.9651 (M^+, $C_9H_9BrO_5^+$; calc. 275.9633).

3,4-Bis(benzyloxy)-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 r.t., the mixture was filtered, the filtrate evaporated and the crude product purified by CC (silica gel, hexane/AcOEt 2 : 1): **10c** (5.4 g, 86%). ^{1}H-NMR ((D$_6$)acetone): 3.87 (*s*, 1 Me); 3.93 (*s*, 1 Me); 5.04 (*s*, 1 PhC*H*$_2$); 5.15 (*s*, 1 PhC*H*$_2$); 7.26 (*s*, H–C(6)); 7.32–7.52 (*m*, 2 *Ph*CH$_2$). HR-FD-MS: 456.0563 (M^+, $C_{23}H_{21}BrO_5^+$; calc. 456.0572).

3,4-Bis(benzyloxy)-2-hydroxy-5-methoxybenzoic Acid (**10d**). According to the modified method of *Cakmak et al.* [21]: To a stirred soln. of **10c** (5.5 g, 12 mmol) in DMF (40 ml) was added a suspension of NaOMe (6.5 g, 120 mmol, 10 equiv.) and CuI (1.1 g, 6.0 mmol, 0.50 equiv.) in DMF (80 ml). The mixture was stirred for 2 d under N_2 at 90°. After cooling to r.t., the mixture was poured into H_2O and extracted with AcOEt. The org. layer was evaporated, and the crude product subjected to CC (silica gel, hexane/AcOEt 1 : 1): **10d** (1.9 g, 42%). ^{1}H-NMR ((D$_6$)acetone): 3.82 (*s*, MeO–C(5)); 5.07 (*s*, PhC*H*$_2$); 5.09 (*s*, PhC*H*$_2$); 7.28–7.51 (*m*, 2 *Ph*CH$_2$, H–C(6)). HR-FD-MS: 380.1236 (M^+, $C_{22}H_{20}O_6^+$; calc. 380.1260).

3,4-Bis(benzyloxy)-2,5-dimethoxybenzoic Acid Methyl Ester (**10e**). To a soln. of **10d** (1.0 g, 2.6 mmol) in acetone (20 ml) were added MeI (0.32 ml, 5.2 mmol, 2.0 equiv.) and K_2CO_3 (0.72 g, 5.2 mmol, 2.0 equiv.). The mixture was refluxed for 5 h and then filtered. The filtrate was evaporated and the residue subjected to CC (silica gel, $CHCl_3$/MeOH 100 : 3): **10e** (0.96 g, 90%). ^{1}H-NMR ($CDCl_3$): 3.84 (*s*, 1 Me); 3.87 (*s*, 1 Me); 3.90 (*s*, 1 Me); 5.01 (*s*, PhC*H*$_2$); 5.08 (*s*, PhC*H*$_2$); 7.13 (*s*, H–C(6)); 7.28–7.44 (*m*, 2 *Ph*CH$_2$). HR-FD-MS: 408.1581 (M^+, $C_{24}H_{24}O_6^+$; calc. 408.1573).

3,4-Dihydroxy-2,5-dimethoxybenzoic Acid Methyl Ester (**10**). Compound **10e** (0.50 g, 1.2 mmol) in MeOH (15 ml) was deprotected by hydrogenation at 1 atm. in the presence of a catalytic amount of 10% Pd/C. The mixture was filtered through *Celite*, the filtrate evaporated, and the crude product puri-

fied by CC (silica gel, $CHCl_3$/MeOH 10:1): **10** (0.26 g, 93%). Pale yellow powder. M.p. 126–128°. ^{1}H-NMR (CD_3OD): 3.77 (*s*, 1 Me); 3.82 (*s*, 1 Me); 3.84 (*s*, COOMe); 6.95 (*s*, H–C(6)). ^{13}C-NMR (CD_3OD): 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, 145.6 (C(2), C(5)); 167.9 (C=O). ^{1}H,^{13}C-HMBC: H–C(6) ↔ C(2), C(4), C=O; MeO–C(2) ↔ C(2); MeO–C(5) ↔ C(5); COOMe ↔ C=O. HR-EI-MS: 228.0669 (M^+, $C_{10}H_{12}O_6^+$; calc. 228.0634).

NMR Analyses. NMR Measurements of the Reaction Mixtures of Catechol Derivative **4***,* **7***, or* **10** *with the DPPH Radical.* To a catechol derivative (2.5 μmol) was added DPPH (3.0 mg, 7.6 μmol, 3.0 equiv.) in CD_3OD/(D_6)acetone 3:1 (0.4 ml). (D_6)acetone was added as a cosolvent to enhance the solubility of DPPH. The mixture was immediately transferred to a NMR tube and mixed vigorously. ^{1}H-NMR spectra were recorded 10 min after mixing.

Reaction of **4** *with DPPH.* ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): **5**: 6.19 (*d*, *J* = 10.3, H–C(5)); 7.38 (*d*, *J* = 10.3, H–C(6)); **6**: 5.83 (*d*, *J* = 10.3, H–C(5)); 7.53 (*d*, *J* = 10.3, H–C(6)); **8**: 5.75 (*s*, H–C(5)).

Reaction of **7** *with DPPH.* ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): **8**: 3.85 (*s*, 1 Me); 3.90 (*s*, 1 Me); 3.98 (*s*, 1 Me); 5.75 (*s*, H–C(5)); ^{1}H,^{13}C-HMBC: H–C(5) ↔ 123.5 (C(1)), 178.2 (C(3)); **9**: 3.79 (*s*, 1 Me); 3.85 (*s*, 1 Me); 4.04 (*s*, 1 Me); 5.37 (*s*, H–C(5)); HMBC: H–C(5) ↔ 91.5 (C(3)), 109.6 (C(1)).

Reaction of **10** *with DPPH.* ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): **11**: 3.75 (*s*, 1 Me); 3.85 (*s*, 1 Me); 3.87 (*s*, 1 Me); 6.22 (*s*, H–C(6)); HMBC: H–C(6) ↔ 150.2, 151.9, 167.1, 174.5; **12**: 3.72 (*s*, 1 Me); 3.76 (*s*, 1 Me); 3.84 (*s*, 1 Me); 5.34 (*s*, H–C(6)); HMBC: H–C(6) ↔ 92.5, 145.5, 161.0, 167.4.

NMR Measurements of the Reaction Mixtures of Catechol Derivatives **1***,* **4***,* **7***, or* **10** *with* o*-Chloranil.* To a catechol derivative (20 μmol) was added *o*-chloranil (4.9 mg, 20 μmol, 1.0 equiv.) in CD_3OD/(D_6)acetone 3:1 (0.4 ml). The mixture was immediately transferred to a NMR tube and mixed vigorously. ^{1}H-NMR spectra of **1** and **4** were recorded 10, 30, 60, 120, 180, and 360 min after mixing, and those of **7** and **10** 10 min after mixing.

Reaction of **1** *and* o*-Chloranil.* ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): **2**: 6.44 (*d*, *J* = 10.3, H–C(5)); 6.93 (*d*, *J* = 2.0, H–C(2)); 7.52 (*dd*, *J* = 10.3, 2.0, H–C(6)); **3**: 6.11 (*d*, *J* = 10.3, H–C(5)); 7.21 (*d*, *J* = 2.0, H–C(2)); **5**: 6.18 (*d*, *J* = 10.3, H–C(5)); **6**: 5.83 (*d*, *J* = 10.3, H–C(5)); **8**: 5.75 (*s*, H–C(5)); **9**: 5.37 (*s*, H–C(5)).

Reaction of **4** *and* o*-Chloranil.* ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): **5**: 6.19 (*d*, *J* = 10.3, H–C(5)); 7.38 (*d*, *J* = 10.3, H–C(6)); **6**: 5.83 (*d*, *J* = 10.3, H–C(5)); 7.53 (*d*, *J* = 10.3, H–C(6)); **8**: 5.75 (*s*, H–C(5)). **9**: 5.37 (*s*, H–C(5)).

Reaction of **7** *and* o*-Chloranil.* ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): **8**: 3.85 (*s*, 1 Me); 3.90 (*s*, 1 Me); 3.98 (*s*, 1 Me); 5.75 (*s*, H–C(5)); **9**: 3.79 (*s*, 3 H, 1 Me); 3.84 (*s*, 1 Me); 4.04 (*s*, 1 Me); 5.37 (*s*, H–C(5)).

Reaction of **10** *and* o*-Chloranil.* ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): **11**: 3.76 (*s*, Me); 3.85 (*s*, Me); 3.87 (*s*, Me); 6.22 (*s*, H–C(6)); **12**: 3.72 (*s*, Me); 3.76 (*s*, Me); 3.84 (*s*, Me); 5.34 (*s*, H–C(6)).

Molecular-Orbital Calculations. The electron densities and energies of LUMOs were calculated by the AM1 method with the MOPAC 2000 program included in the Chem3D package (*CambridgeSoft Co*).

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