DPPH (=2,2-Diphenyl-1-picrylhydrazyl) Radical-Scavenging Reaction of Protocatechuic Acid (=3,4-Dihydroxybenzoic Acid): Difference in Reactivity between Acids and Their Esters

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Protocatechuic acid (=3,4-dihydroxybenzoic acid; **1**) exhibits a significantly slow DPPH (=2,2-diphenyl-1-picrylhydrazyl) radical-scavenging reaction compared to its esters in alcoholic solvents. The present study is aimed at the elucidation of the difference between the radical-scavenging mechanisms of protocatechuic acid and its esters in alcohol. Both protocatechuic acid (**1**) and its methyl ester **2** rapidly scavenged 2 equiv. of radical and were converted to the corresponding *o*-quinone structures **1a** and **2a**, respectively (*Scheme*). Then, a regeneration of catechol (=benzene-1,2-diol) structures occurred *via* a nucleophilic addition of a MeOH molecule to the *o*-quinones to yield alcohol adducts **1f** and **2c**, respectively, which can scavenge additional 2 equiv. of radical. However, the reaction of protocatechuic acid (**1**) beyond the formation of the *o*-quinone was much slower than that of its methyl ester **2**. The results suggest that the slower radical-scavenging reaction of **1** compared to its esters is due to a dissociation of the electron-withdrawing carboxylic acid function to the electron-donating carboxylate ion, which decreases the electrophilicity of the *o*-quinone, leading to a lower susceptibility towards a nucleophilic attack by an alcohol molecule.

Introduction. – Polyphenols are known to exhibit potent antioxidant activities. Recently, numerous studies have been reported on radical-scavenging activities of phenolic compounds. Particularly, structure–activity–relationship studies of polyphenols and kinetic studies on reactions of phenolic compounds with radicals, including substituent effects, have been extensively investigated [1–3]. It is well-known that the radical-scavenging activity of phenol-derived acids largely depends on the number and arrangement of phenolic OH groups in the molecule [4–6]. Among them, *o*- or *p*-benzenediol derivatives exhibit high radical-scavenging activity compared to *m*-benzenediol derivatives, since they could be converted to the corresponding stable *o*- or *p*-quinone derivatives [7].

Protocatechuic acid (=3,4-dihydroxybenzoic acid; **1**) and its esters are *o*-benzenediols, ubiquitously found in edible plants, vegetables, and fruits, and are known to exhibit potent antioxidant activities [4][8]. Additionally, it has been demonstrated that protocatechuic acid has preventive effects on carcinogenesis and cardiovascular diseases that are associated with free radicals [9][10].

Previously, we have found that the radical-scavenging reactions of protocatechuic acid (**1**) and its esters depend on the applied solvents [11]. In non-alcoholic solvents, **1** and its esters scavenge 2 equiv. of radical to yield the corresponding *o*-quinones. In alcoholic solvents, protocatechuic acid esters rapidly react with more than 4 equiv. of

radical with a concomitant conversion to the corresponding *o*-quinones, 3-hemiacetals [12], and their alcohol adducts at C(2) [11]. Interestingly, unlike protocatechuic esters, **1** scavenged only 2 equiv. of radical within 30 min at room temperature. The previous study has demonstrated that the higher radical-scavenging activity of protocatechuic acid esters in alcoholic solvents than in non-alcoholic solvents is due to a regeneration of catechol (=benzene-1,2-diol) structures *via* a nucleophilic addition of a solvent molecule to the *o*-quinones [11][13]. However, it is still unclear why sterically unhindered protocatechuquinone (**1a**) scarcely undergoes a nucleophilic attack by an alcohol molecule, in contrast to its methyl ester **2a**, which leads to low activity of **1**. *Kimura et al.* [14] have also shown the low activity of **1** compared to methyl protocatechuate (**2**) in EtOH. Furthermore, caffeic acid (=3,4-dihydroxycinnamic acid) is reported to exhibit lower radical-scavenging activity than its esters [15][16].

Therefore, the aim of this study was to elucidate why **1** exhibits low activity compared to its esters in alcoholic solvents. To confirm whether acids generally show lower activity than their esters or not, 3,4-dihydroxybenzenesulfonic acid (**4**), (3,4-dihydroxyphenyl)phosphonic acid (**6**), and their esters **5**, **7**, and **8** were synthesized (*Fig. 1*), and the DPPH and ABTS (diammonium salt of 2,2′-azinobis[(3-ethyl-2,3-dihydrobenzothiazole-6-sulfonic acid]) radical-scavenging activities of acids **1**, **4**, and **6** were compared with those of their corresponding esters **2**, **5**, **7**, and **8**. DPPH and ABTS radicals are strongly electrophilic radicals which act as a one-electron oxidant and hence, they can readily oxidize catechols into the corresponding quinones *via* semiquinone radicals. In addition, the radical-scavenging reactions of **1** and its sodium salt **3** were directly analyzed by NMR spectroscopy. Finally, isolation of an oxidation product of **1** from the reaction mixture was attempted, with the aim to explain the difference between the radical-scavenging mechanisms of protocatechuic acid and its esters in alcoholic solvents.

Results and Discussion. – *Radical-Scavenging Activity.* The DPPH-radical-scavenging activity of protocatechuic acid (**1**), 3,4-dihydroxybenzenesulfonic acid (**4**), and (3,4-dihydroxyphenyl)phosphonic acid (**6**) was compared with that of the corresponding esters **2**, **5**, **7**, and **8** in MeOH and MeCN. The relative radical-scavenging equivalences of compounds **1**, **2**, and **4**–**8**, when that of (2*RS*)-α-tocopherol as standard was designated as 2.0, are listed in *Table 1*. In inert MeCN, all test compounds scavenged approximately 2 equiv. of radical within 30 min at room temperature, and no significant difference in activity between acids and their esters was observed. In nucleophilic MeOH, the acids **1**, **4**, and **6** also consumed *ca.* 2 equiv. of radical, whereas their esters **2**, **5**, and **8** scavenged 5 equiv. of radical. The radical-scavenging activity of salt **3** was comparable to that of **1**. In addition, the activity of phosphonic acid **6** and its monoethyl ester **7** and diethyl ester **8** increased as the number of the Et groups increased. The results clearly show that not only protocatechuic [11][14] and caffeic [15][16] acids but also 3,4-dihydroxybenzenesulfonic acid (**4**) and (3,4-dihydroxyphenyl)phosphonic acid (**6**) have significantly lower DPPH-radical-scavenging activity in MeOH as compared to their esters.

Fig. 2 shows time courses of the DPPH-radical-scavenging activity of **1**–**3** in MeOH and MeCN over 6 h. In MeCN, acid **1** and ester **2** readily reacted with 2 equiv. of radical and reached steady states in 30 min. It is indicated that although dimerization of the

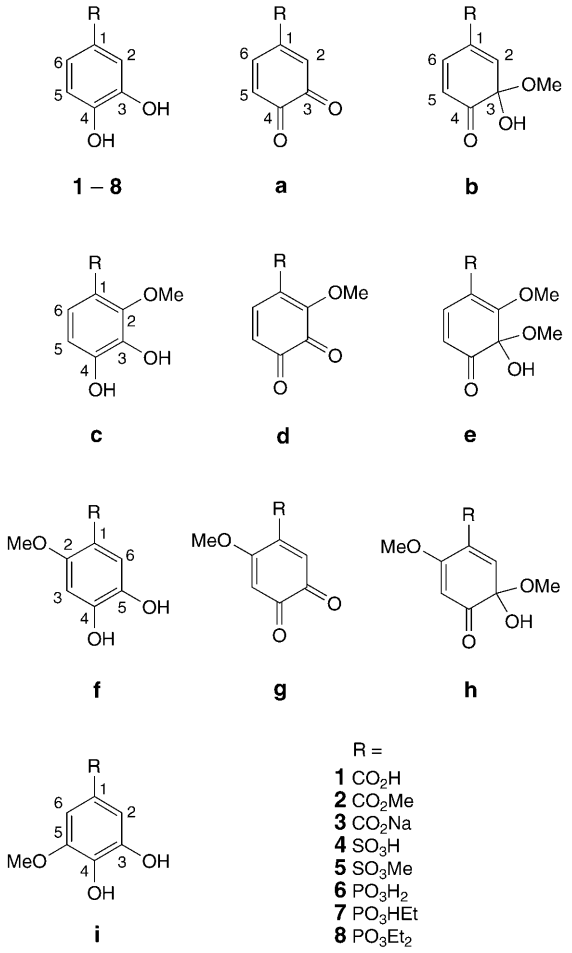


Fig. 1. *Structures of protocatechuic acid* (**1**) *and related compounds* **2**–**8**, *as well as their oxidation products*

formed *o*-quinones **1a** and **2a** might occur similarly to the reaction in acetone [17], the resultant dimers are unlikely to scavenge radicals. In MeOH, ester **2** rapidly scavenged 5 equiv. of radical and reached a plateau within 30 min. In contrast, acid **1** and salt **3** scavenged only 2 equiv. of radical in 30 min, but they gradually consumed another 3 equiv. of radical and reached steady states within 6 h. The DPPH-radical-scavenging equivalences of **1** and **3** in MeOH after 6 h were comparable to that of **2**. These results suggest that the radical-scavenging reactions beyond the formation of *o*-quinones **1a** and **3a** might proceed in a similar manner as that of **2a**, although the reaction rates are significantly slower.

To establish whether the difference in reactivity between acid and its esters also occurs in aqueous solution, the ABTS-radical-scavenging activity of **1**–**8** was evaluated in aqueous solution. The radical-scavenging equivalence is expressed as the values rel-

Table 1. *DPPH and ABTS Radical-Scavenging Equivalences of* **1**–**8** *after 30 min at Room Temperature*

	DPPH radical[a])		ABTS radical[b])
	MeOH[c])	MeCN[c])	1% EtOH/H_2O[c])
1	2.5	2.2	2.4
2	5.0	2.2	5.9
3	2.7	–[d])	2.2
4	2.3	2.0	2.8
5	5.2	2.8	5.6
6	1.6	–[d])	1.8
7	1.9	1.7	3.0
8	4.8	2.1	5.9

[a]) The equivalence is expressed as the values relative to that of (2*RS*)-α-tocopherol taken as 2.0. [b]) The equivalence is expressed as the values relative to that of *Trolox* taken as 2.0. [c]) Solvent. [d]) Not tested due to low solubility.

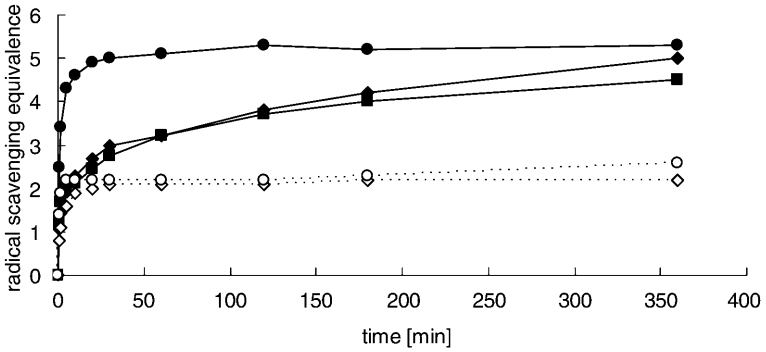


Fig. 2. *Time courses of DPPH-radical-scavenging activity at room temperature of* **1** (◆), **2** (●), *and* **3** (■) *in MeOH, and of* **1** (◇) *and* **2** (○) *in MeCN*. The equivalence is expressed as the values relative to that of (2*RS*)-α-tocopherol taken as 2.0.

ative to the standard value 2.0 of *Trolox*, and the results are shown in *Table 1*. Compounds **1**, **4**, and **6** consumed approximately 2 equiv. of radical, whereas their esters **2**, **5**, and **8** scavenged more than 5 equiv. of radical. In addition, **3** had an activity similar to that of **1**. Thus, the tendency of the ABTS-radical-scavenging activity in H_2O was similar to that of the DPPH-radical-scavenging activity in MeOH, suggesting that the radical-scavenging reaction in an aqueous solution is similar to that in an alcohol.

It is well-known that the pH of the solution affects the radical-scavenging activity of phenols [5]. Therefore, the pH of the reaction mixtures was measured. Since the pH of the mixtures was in the range of 6.63–6.77, the difference in radical-scavenging activity between acids and esters is not caused by the pH of the reaction mixtures.

Furthermore, the dissociation of the free carboxylic group of quinone **1a** to the carboxylate ion is expected to be facilitated by the strong electron-withdrawing property of the quinone carbonyl groups. This was supported by comparing the dissociation enthalpy of the carboxylic acid function of **1** and of protocatechuquinone (**1a**), which

was estimated from MOPAC calculations to be −27.9 and −39.7 kcal/mol, respectively. We have previously demonstrated that catechols bearing a strong electron-withdrawing substituent at C(1) exhibit high DPPH-radical-scavenging activity in alcoholic solvents, since electron-withdrawing substituents enhance the electrophilicity of the *o*-quinone intermediate [13]. Therefore, it is suggested that the low reactivity of **1** compared to its ester **2** in MeOH is due to the dissociation of the electron-withdrawing carboxylic acid function to the electron-donating carboxylate ion.

NMR Analyses of the Reaction Mixtures. To elucidate the radical-scavenging mechanism of **1** and **3**, the reaction mixture of **1** (or **3**) and DPPH was directly analyzed by NMR. The ^{1}H-NMR spectrum of the reaction mixture **1**/DPPH in CD_3OD/(D_6)acetone 3 : 1 after 10 min showed signals of the corresponding *o*-quinone **1a** and of its hemiacetal **1b** [12], similar to those observed for **2**/DPPH [11]. However, no signal due to a MeOH adduct at the benzene ring was observed. Even after 2 h, the signals of **1a** and **1b** remained unchanged. Apparently, **1a** was stable compared to **2a**, which disappeared within 1 h in CD_3OD/(D_6)acetone 3 : 1 [13]. Since an electron-donating substituent at C(1) enhances the stability of such an *o*-quinone [13], the reason that **1a** is stable compared to **2a** can be explained by the deprotonation of the carboxylic acid function to the carboxylate ion, which enhances the electron-donating property of the substituent.

The ^{1}H-NMR spectrum of the reaction mixture **3**/DPPH in CD_3OD/(D_6)acetone 3 : 1 after 10 min showed peaks of the *o*-quinone **3a** (*Fig. 3*, *a*), and the HMBC data confirmed the formation of the *o*-quinone **3a** (see *Exper. Part*). In contrast to **1** and **2**, no signal due to a hemiacetal **3b** was detected. After 2 h, the reaction mixture **3**/DPPH exhibited no *d* characteristic of a C(2) adduct. Instead, two new *s* appeared, which were assumed to be H−C(5) and H−C(2) of the C(6) MeOH adduct **3g** (*Fig. 3*, *b*). The formation of **3g** was confirmed by the ^{1}H-NMR spectrum of the mixture of authentic **3f** and DPPH radical (*Fig. 3*, *c*), and the H,C-HMBC data of this mixture (see *Exper. Part*) allowed to assign the two *s* to H−C(5) and H−C(2) of the C(6) adduct **3g**. These results revealed that unlike methyl protocatechuate (**2**), forming the C(2) adduct **2d**, protocatechuic acid sodium salt (**3**) preferentially formed a C(6) adduct.

Isolation of an Oxidation Product of **1**. Although no signal of an adduct at the benzene ring was observed in the ^{1}H-NMR spectrum of the mixture **1**/DPPH radical in CD_3OD/(D_6)acetone 3 : 1, it can be speculated that **1a** undergoes a nucleophilic attack similar to that of **3a**, since the radical-scavenging activity of **1** was comparable to that of **3** (*Fig. 2*). To substantiate the formation of a MeOH adduct, an attempt was made to isolate an oxidation product from the reaction mixture **1**/DPPH in MeOH. For that purpose sodium dithionite was added to the reaction mixture after 12 h to reduce *o*-quinones and 3-hemiacetals to their catechol forms. Purification of the reaction mixture afforded indeed the C(6) adduct **1f**, and neither the C(2) adduct **1c** nor the C(5) adduct **1i** was detected by HPLC analysis. The result suggests that **1a** preferentially undergoes a nucleophilic attack at C(6) to form **1f**, that the reaction mechanism of **1** is similar to that of **3**; and that **1a** is present in a dissociated form in the reaction solution, *i.e.*, as an anion identical to that from **3a**.

Molecular-Orbital Calculations. To substantiate the preference for the position of the nucleophilic attacks, the electron densities of the LUMO of *o*-quinones **1a**, **2a**, **4a**, and **5a** were calculated by a semiempirical method (*Table 2*). Like the esters **2a**

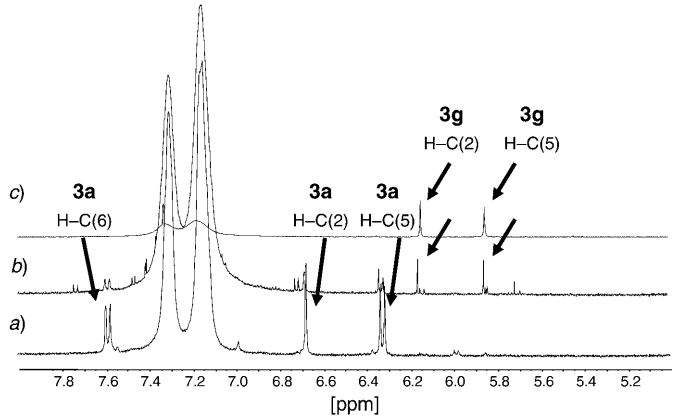


Fig. 3. *^{1}H-NMR spectra* (CD_3OD/(D_6)acetone 3:1) *of the reaction mixture* **3**/*DPPH after* a) *10 min and* b) *120 min, and* c) *of a mixture* **3f**/*DPPH after 10 min.* The intense signals in the range δ 7.1–7.4 are due to DPPH.

Table 2. *LUMO Energy and Electron Density at the Atoms C(1)–C(6) of the* o-*Quinone Derivatives* **1a**, **2a**, **4a**, *and* **5a**

		LUMO energy [eV]	Electron density					
			C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
1a	**1a**′ (CO_2H)	−2.339	0.34	0.41	0.19	0.14	0.22	0.16
	1a″ (CO_2^-)	1.656	0.21	0.17	0.26	0.29	0.18	0.31
2a		−2.086	0.30	0.41	0.20	0.14	0.21	0.16
4a	**4a**′ (SO_3H)	−2.494	0.34	0.55	0.20	0.11	0.18	0.12
	4a″ (SO_3^-)	1.423	0.22	0.23	0.27	0.26	0.17	0.27
5a		−2.423	0.33	0.55	0.20	0.11	0.18	0.12

and **5a**, the undissociated acids **1a**′ (−COOH) and **4a**′ ($-SO_3H$) had the largest LUMO electron density at C(2). On the other hand, the dissociated acids **1a**″ ($-COO^-$) and **4a**″ ($-SO_3^-$) had the largest LUMO electron density at C(6). The values predict that **2a**, **5a**, **1a**′ (−COOH), and **4a**′ ($-SO_3H$) undergo a nucleophilic attack at C(2), and **1a**″ ($-COO^-$) and **4a**″ ($-SO_3^-$) at C(6), in agreement with the results from the NMR experiments (*Fig. 3*). However, we have previously reported that both **1a** and **2a** underwent a nucleophilic attack by a thiol to yield C(2) adducts in acetone [18]. We suggest that the difference in the position of the nucleophilic attacks of **1a** by thiols and alcohols are due to the solvent used. That is, in the less polar acetone, deprotonation of the carboxylic acid group would be suppressed compared to that in alcoholic solvents, and hence **1a**′ undergoes a nucleophilic attack by a thiol at C(2), which is the position of the highest LUMO electron density. In addition, the LUMO energies of **1a**″ ($-COO^-$) and **4a**″ ($-SO_3^-$) were higher than those of **2a**, **5a**, **1a**′ (−COOH), and **4a**′ ($-SO_3H$), suggesting that the lower reactivity of the acids **1**, **4**, and **6** as compared to their esters toward a nucleophilic attack is due to the increase of LUMO energy of the *o*-quinones by deprotonation of the free acid functions.

Radical-Scavenging Activity of the Oxidation Products. The DPPH radical-scavenging activity of **1f**, **2c**, and **3f**, which are the oxidation products of **1**, **2**, and **3**, was evaluated in MeOH (*Fig. 4*). Compound **2c** readily scavenged more than 3 equiv. of DPPH. In contrast, **1f** and **3f** rapidly reacted with 2 equiv. of DPPH, and then slowly consumed more of it. The radical-scavenging equivalences of **1f**, **2c**, and **3f** after 6 h were approximately 2 equiv. lower than those of **1**, **2**, and **3**. Considering that **1f**, **2c**, and **3f** are derived from an addition of a MeOH molecule to the *o*-quinones **1a**, **2a**, and **3a**, which were produced from **1**, **2**, and **3** by reaction with 2 equiv. of DPPH, **1f**, **2c**, and **3f** could largely contribute to the total radical-scavenging activity of **1**, **2**, and **3**.

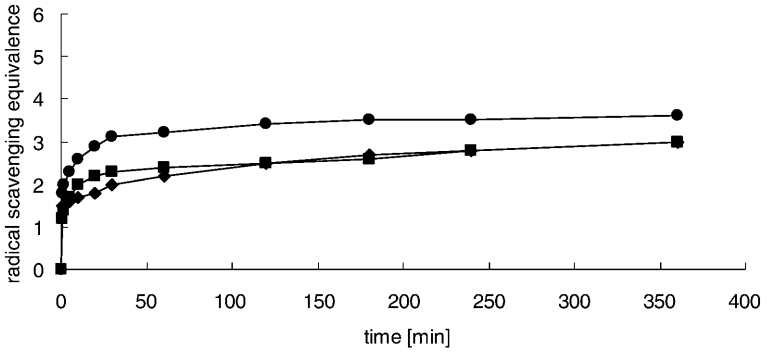
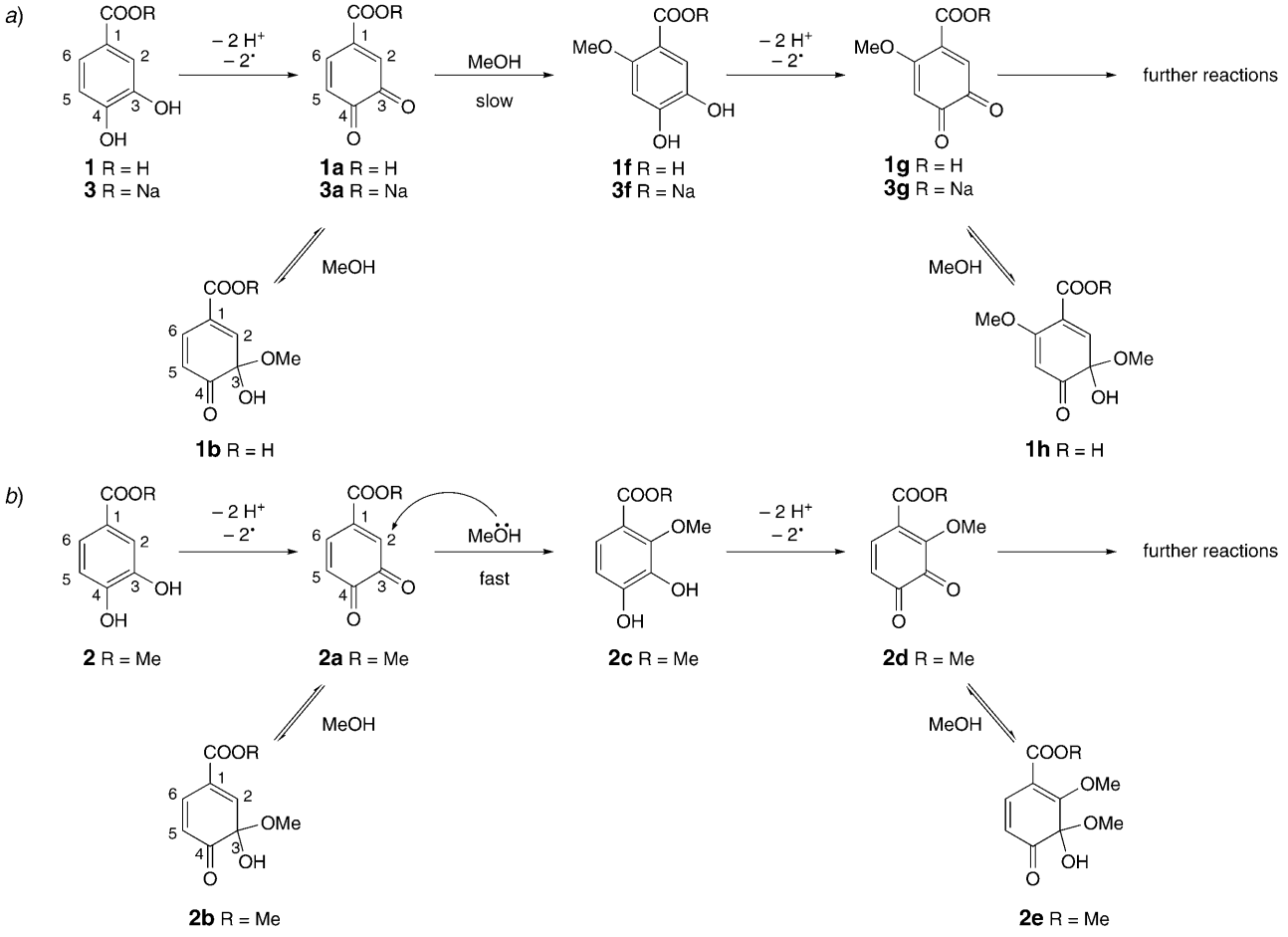


Fig. 4. *Time courses of DPPH-radical-scavenging activity at room temperature of* **1f** (◆), **2c** (●), *and* **3f** (■) *in MeOH*. The equivalence is expressed as the values relative to that of (2*RS*)-α-tocopherol taken as 2.0.

Plausible Radical-Scavenging Mechanism. The difference in radical-scavenging mechanisms of protocatechuic acid (**1**) and its ester **2** in MeOH are proposed in the *Scheme*. Both acid **1** and its ester **2** react with 2 equiv. of radical and are converted to their *o*-quinones and 3-hemiacetals. Then, in the case of ester **2**, a MeOH molecule readily attacks C(2) of the *o*-quinone **2a**, which leads to a regeneration of a catechol structure, *i.e.*, **2c** (*Scheme*, *b*). The MeOH adduct **2c** scavenges additional 2 equiv. of radical to yield *o*-quinone **2d** and its 3-hemiacetal **2e**. In contrast, *o*-quinones **1a** and **3a** slowly undergo a nucleophilic attack by a MeOH molecule at C(6), leading to a regeneration of the catechol structures **1f** and **3f**, respectively, which could scavenge additional 2 equiv. of radical (*Scheme*, *a*).

Conclusion. – In conclusion, the overall results suggest that the slow radical-scavenging reaction of protocatechuic acid (**1**) compared to its esters is due to the dissociation of the carboxylic acid function, since it decreases the electron-withdrawing property of the substituent, and thus decreases the susceptibility of the first formed *o*-quinone towards nucleophilic attack by a solvent molecule. The difference in reactivity between protocatechuic acid (**1**) and its ester **2** described in this paper might also play a role in an aqueous biological system.

Scheme. *Plausible Radical-Scavenging Mechanisms in MeOH of* a) *Protocatechuic Acid* (**1**) *and its Sodium Salt* (**3**), *and* b) *Protocatechuic Ester* **2**



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Experimental Part

1. *General.* Protocatechuic acid (**1**) and diammonium 2,2′-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulfonate] (ABTS) were obtained from *Sigma Chemical Co.* The 2,4,5-trimethoxybenzoic acid and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (*Trolox*) were purchased from *Aldrich Chemical Co.* Methyl protocatechuate (**2**), methyl 3,4-dihydroxy-2-methoxybenzoate (**2c**), and methyl 3,4-bis(benzyloxy)-2-methoxybenzoate were prepared by the methods described previously [11]. The 2,2-diphenyl-1-picrylhydrazyl (=2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; DPPH) and other reagents were purchased from *Wako Pure Chemical Industries.* All solvents used were of reagent grade. Anal. TLC, silica gel plates *Merck 60* F_{254} (0.25 mm thickness). Column chromatography (CC): silica gel, *Wakogel C-300* (*Wako Pure Chemical Industries*); reversed phase CC with *Cosmosil 75*C_{18}*-OPN* (*Nacalai Tesque Inc.*). Anal. HPLC: *LC-10AD-vp* pump and *SPD-10A-vp* detector (*Shimadzu*). M.p.: hot-stage apparatus; uncorrected. UV/VIS Spectra: *Hitachi U-3210* spectrophotometer. 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 CD_3OD (δ(H) 3.30, δ(C) 49.0) and (D_6)acetone (δ(H) 2.04, δ(C) 29.8). MS: *Jeol-JMS-AX-500* (for EI) and *Jeol-JMS-SX-102A* (for FD) instruments.

2. *Syntheses. Sodium 3,4-Dihydroxybenzoate* (**3**). To a suspension of **1** (0.50 g, 3.2 mmol) in H_2O (10 ml) was added dropwise Na_2CO_3 (0.17 g, 1.6 mmol, 0.50 equiv.) in H_2O (5 ml). The resultant colorless soln. was concentrated *i.v.* to afford **3** (0.56 g, 98%). White powder. ^{1}H-NMR (CD_3OD): 6.71 (*d*, *J*=8.1, H–C(5)); 7.34 (*dd*, *J*=8.1, 2.0, H–C(6)); 7.41 (*d*, *J*=2.0, H–C(2)).

3,4-Dihydroxy-2-methoxybenzoic Acid (**1c**). To a suspension of methyl 3,4-bis(benzyloxy)-2-methoxybenzoate [11] (0.76 g, 2 mmol) in EtOH (40 ml) was added 20% aq. KOH soln. (8 ml), and stirred for 6 h at 50°. After cooling, the mixture was concentrated *i.v.* The residue was diluted with H_2O and washed with AcOEt. The aq. phase was acidified with 1M HCl to pH 2, and the mixture was then extracted with AcOEt and the org. phase concentrated *i.v.* to afford 3,4-bis(benzyloxy)-2-methoxybenzoic acid (0.63 g, 86%). This acid (0.63 g, 1.7 mmol) was deprotected by hydrogenation under 1 atm H_2 in the presence of a catalytic amount of 10% Pd/C in MeOH (15 ml). The mixture was filtered through *Celite*, the filtrate concentrated *i.v.*, and the crude product subjected to CC (silica gel, $CHCl_3$/MeOH 10:1): **1c** (0.26 g, 83%). Yellow powder. M.p. 183–185°. ^{1}H-NMR (CD_3OD): 3.85 (*s*, MeO); 6.58 (*d*, *J*=8.6, H–C(5)); 7.24 (*d*, *J*=8.6, H–C(6)). HR-EI-MS: 184.0396 (M^+, $C_8H_8O_5^+$; calc. 184.0372).

4,5-Dihydroxy-2-methoxybenzoic Acid (**1f**). To a soln. of 2,4,5-trimethoxybenzoic acid (4.2 g, 20 mmol) in CH_2Cl_2 (30 ml) at −80° was added 1M BBr_3 in CH_2Cl_2 (100 ml, 5.0 equiv.), and stirred for 1 h. The mixture was kept for another 12 h at r.t. The mixture was poured into ice-water and extracted with AcOEt. The org. layer was washed with H_2O and then concentrated *i.v.* to give 2,4,5-trihydroxybenzoic acid (3.2 g, 19 mmol, 95%). Then **1f** was prepared by the same method as described for **2c** [11]: A mixture of 2,4,5-trihydroxybenzoic acid (1.70 g, 10 mmol), benzyl bromide (3.2 ml, 27 mmol, 2.7 equiv.), and potassium carbonate (3.7 g, 27 mmol, 2.7 equiv.) in acetone (30 ml) was refluxed for 6 h. After cooling to r.t., the mixture was filtered and the filtrate concentrated *i.v.* The residue was subjected to CC (silica gel, hexane/AcOEt 3:1): 4,5-bis(benzyloxy)-2-hydroxybenzoic acid benzyl ester (3.1 g, 70%). To a suspension of this ester (3.1 g, 7.0 mmol) in acetone (50 ml) were added MeI (0.48 ml, 7.7 mmol, 1.1 equiv.) and potassium carbonate (1.1 g, 7.7 mmol, 1.1 equiv.), and the mixture was refluxed for 6 h. The mixture was filtered, the filtrate concentrated *i.v.*, and the residue subjected to CC (silica gel, hexane/AcOEt 3:1): 4,5-bis(benzyloxy)-2-methoxybenzoic acid benzyl ester (2.5 g, 79%). The methoxy ester (1.0 g, 2.2 mmol) was deprotected by hydrogenation under 1 atm H_2 in the presence of a cat. amount of 10% Pd/C in MeOH (15 ml). The mixture was filtered through *Celite*, the filtrate concentrated *i.v.*, and the crude product subjected to CC (silica gel, hexane/AcOEt 1:2): **1f** (0.36 g, 91%). Pale brown powder. M.p. 193–194°. ^{1}H-NMR (CD_3OD): 3.86 (*s*, MeO); 6.57 (*s*, H–C(3)); 7.37 (*s*, H–

C(6)). ^{13}C-NMR (CD_3OD): 57.2 (MeO); 101.4 (C(3)); 109.7 (C(1)); 119.4 (C(6)); 140.3 (C(5)); 153.2 (C(4)); 155.9 (C(2)); 169.0 (C=O). HMBC: H–C(3) ↔ C(1), C(5); H–C(6) ↔ C(2), C(4), C=O; MeO ↔ C(2). HR-EI-MS: 184.0391 (M^+, $C_8H_8O_5^+$; calc. 184.0372).

Sodium 4,5-Dihydroxy-2-methoxybenzoate (**3f**). As described for **3**, from **1f**: **3f** (97%). Brown powder. ^{1}H-NMR (CD_3OD): 3.72 (*s*, MeO); 6.42 (*s*, H–C(3)); 7.03 (*s*, H–C(6)).

3,4-Dihydroxy-5-methoxybenzoic Acid (**1i**). Methyl 3,4-dihydroxy-5-methoxybenzoate (**2i**) was prepared by the method of *Chang et al.* [19]. To a suspension of **2i** (0.99 g, 5.0 mmol) in EtOH (50 ml) was added 20% aq. KOH soln. (8 ml), and the mixture was stirred for 3 h at 50°. After cooling, the mixture was concentrated *i.v.* The residue was suspended in H_2O, the suspension washed with AcOEt, the aq. phase acidified with 1M HCl to pH 2, and the mixture extracted with AcOEt. The org. phase was concentrated *i.v.*, and the crude product recrystallized from H_2O: **1i** (0.79 g, 86%). Pale brown powder. M.p. 220–221°. ^{1}H-NMR (CD_3OD): 3.87 (*s*, MeO); 7.17, 7.18 (*d*, J=2.0, H–C(2), H–C(6)). HR-EI-MS: 184.0372 (M^+, $C_8H_8O_5^+$; calc. 184.0372).

3,4-Dihydroxybenzenesulfonic Acid (**4**). Compound **4** was prepared by the method of *Ban et al.* [20]: To a soln. of catechol (=benzene-1,2-diol; 1.1 g, 10 mmol) in dimethyl carbonate (5 ml) was added dropwise chlorosulfonic acid (=chlorosulfic acid; 0.66 ml, 10 mmol, 1.0 equiv.) in dimethyl carbonate (2.8 ml), and the mixture was stirred for 2 h at 0°. Then, the mixture was allowed to warm to r.t., stirred for 12 h, and concentrated *i.v.* The crude product was subjected to reversed phase CC (H_2O): **4** (1.87 g, 98%). Red oil. ^{1}H-NMR (CD_3OD): 6.78 (*d*, J=8.4, H–C(5)); 7.18 (*dd*, J=8.4, 2.2, H–C(6)); 7.25 (*d*, J=2.2, H–C(2)). HR-EI-MS: 189.9933 (M^+, $C_6H_6O_5S^+$; calc. 189.9936).

3,4-Dihydroxybenzenesulfonic Acid Methyl Ester (**5**). To a soln. of catechol (1.1 g, 10 mmol) in dimethyl carbonate (5 ml) was added fluorosulfonic acid methyl ester (=fluorosulfuric acid methyl ester; 0.80 ml, 10 mmol, 1.0 equiv.), and the mixture was refluxed for 12 h. After cooling, the mixture was poured into ice-water and extracted with Et_2O. The org. phase was concentrated *i.v.* and the crude product subjected to CC (silica gel, hexane/AcOEt 1:1): **5** (1.01 g, 50%). Red oil. ^{1}H-NMR (CD_3OD): 3.67 (*s*, MeO); 6.91 (*d*, J=8.3, H–C(5)); 7.23 (*d*, J=2.2, H–C(2)); 7.25 (*dd*, J=8.3, 2.2, H–C(6)). HR-EI-MS: 204.0092 (M^+, $C_7H_8O_5S^+$; calc. 204.0092).

[3,4-Bis(benzyloxy)phenyl]phosphonic Acid Diethyl Ester. To a soln. of 3,4-bis(benzyloxy)phenol (3.83 g, 12.5 mmol) in pyridine (10 ml) at 0° was added trifluoromethanesulfonic anhydride (2.5 ml, 15 mmol, 1.2 equiv.), and the mixture was stirred at 0° for 1 h, allowed to warm to r.t., and stirred for further 3 h. The resulting mixture was adjusted to pH 2 with 2M HCl and extracted with AcOEt. The org. phase was concentrated *i.v.* to afford trifluoromethanesulfonic acid 3,4-bis(benzyloxy)phenyl ester (4.8 g, 88%) which was used without further purification.

[3,4-Bis(benzyloxy)phenyl]phosphonic acid was prepared by the method of *Thurieau et al.* [21]: To a soln. of trifluoromethanesulfonic acid 3,4-bis(benzyloxy)phenyl ester (2.5 g, 5.7 mmol) in MeCN (20 ml) were added *N*-methylmorpholine (0.95 ml, 8.6 mmol, 1.5 equiv.), tetrakis(triphenylphosphine)palladium (330 mg, 0.29 mmol, 0.05 equiv.) and phosphonic acid diethyl ester (1.1 ml, 8.6 mmol, 1.5 equiv.). The mixture was stirred at 70° for 24 h and then the reaction stopped by an addition of AcOEt. The mixture was washed successively with 5% citric acid and 5% aq. $NaHCO_3$ solns., the org. phase concentrated *i.v.*, and the residue subjected to CC (silica gel, hexane/AcOEt 1:1): 3,4-bis(benzyloxy)phenylphosphonic acid diethyl ester (1.9 g, 78%). Yellow oil. ^{1}H-NMR ((D$_6$)acetone): 1.22 (*t*, J(HH)=6.9, 2 *Me*CH_2); 3.97 (*m*, 2 MeC*H*$_2$); 5.21 (*s*, PhC*H*$_2$); 5.25 (*s*, PhC*H*$_2$); 7.20 (*dd*, J(HH)=8.6, J(HP)=4.4, H–C(5)); 7.29–7.53 (*m*, 2 *Ph*CH$_2$, H–C(2), H–C(6)). HR-FD-MS: 426.1589 (M^+, $C_{24}H_{27}O_5P^+$; calc. 426.1596).

(3,4-Dihydroxyphenyl)phosphonic Acid (**6**). A soln. of [3,4-bis(benzyloxy)phenyl]phosphonic acid diethyl ester (200 mg, 0.47 mmol) in conc. HCl soln. (10 ml) was refluxed for 3 h. After cooling, the mixture was poured into H_2O, and washed with AcOEt, the aq. phase concentrated *i.v.*, and the crude product subjected to reversed-phase CC (H_2O): **6** (73 mg, 82%). Colorless oil. ^{1}H-NMR (CD_3OD): 6.82 (*dd*, J(HH)=8.1, J(HP)=4.4, H–C(5)); 7.14 (*ddd*, J(HH)=8.1, 1.5, J(HP)=13.3, H–C(6)); 7.20 (*dd*, J(HH)=1.5, J(HP)=14.0, H–C(2)). HR-FD-MS: 191.0113 (M^+, $C_6H_8O_5P^+$; calc. 191.0109).

(3,4-Dihydroxyphenyl)phosphonic Acid Monoethyl Ester (**7**). To a suspension of [3,4-bis(benzyloxy)-phenyl]phosphonic acid diethyl ester (0.43 g, 1.0 mmol) in EtOH (50 ml) was added 20% aq. KOH soln. (4 ml), and the mixture was stirred at 50° for 12 h. After cooling, the mixture was adjusted to pH 2 with 2M HCl and extracted with AcOEt. The org. phase was washed with H_2O and concentrated *i.v.*, and the

residue subjected to CC (silica gel, hexane/AcOEt 1:5): [3,4-bis(benzyloxy)phenyl]phosphonic acid monoethyl ester (0.30 g, 75%). The resultant monoethyl ester (0.30 g, 0.75 mmol) was deprotected by hydrogenation under 1 atm H_2 in the presence of a cat. amount of 10% Pd/C in MeOH (15 ml). The mixture was filtered through *Celite*, the filtrate concentrated *i.v.*, and the crude product subjected to reversed-phase CC (H_2O): **7** (0.14 g, 86%). White powder. M.p. 179–181°. ^{1}H-NMR ((D$_6$)acetone): 1.23 (*t*, *J*(HH) = 7.1, *Me*CH$_2$); 3.90 (*qd*, *J*(HH) = 7.1, *J*(HP) = 7.1, MeC*H*$_2$); 6.83 (*dd*, *J*(HH) = 8.1, *J*(HP) = 4.4, H–C(5)); 7.12 (*ddd*, *J*(HH) = 8.1, 1.5, *J*(HP) = 13.0, H–C(6)); 7.17 (*dd*, *J*(HH) = 1.5, *J*(HP) = 13.8, H–C(2)). HR-EI-MS: 218.0319 (M^+, $C_8H_{11}O_5P^+$; calc. 218.0344).

(3,4-Dihydroxyphenyl)phosphonic Acid Diethyl Ester (**8**). To a soln. of [3,4-bis(benzyloxy)phenyl]-phosphonic acid diethyl ester (0.21 g, 0.5 mmol) in MeOH (10 ml) was added a cat. amount of 10% Pd/C, and the mixture was stirred under 1 atm of H_2 for 24 h. The mixture was filtered through *Celite*, the filtrate concentrated *i.v.*, and the residue subjected to CC (silica gel, hexane/AcOEt 1:2): **8** (0.10 g, 81%). Yellow oil. ^{1}H-NMR ((D$_6$)acetone): 1.26 (*t*, *J*(HH) = 7.1, 2 *Me*CH$_2$); 4.04 (*m*, 2 MeC*H*$_2$); 6.94 (*dd*, *J*(HH) = 8.1, *J*(HP) = 4.7, H–C(5)); 7.14 (*ddd*, *J*(HH) = 8.1, 1.7, *J*(HP) = 13.0, H–C(6)); 7.38 (*dd*, *J*(HH) = 1.7, *J*(HP) = 13.8, H–C(2)). HR-EI-MS: 246.0684 (M^+, $C_{10}H_{15}O_5P^+$; calc. 246.0657).

3. *Colorimetric Radical-Scavenging Tests.* 3.1. *DPPH-Scavenging Test.* To a soln. of a test compound (12.5 μM, 4 ml) in a test tube, 1 ml of DPPH (500 μM), was added. The soln. was immediately mixed vigorously for 10 s by a *Vortex* mixer and transferred to a cuvette. The absorbance reading at 517 nm was taken 1, 2, 5, 10, and 30 min, then 1, 2, 4, and 6 h after initial mixing. MeCN and MeOH were chosen as inert non-alcoholic and nucleophilic alcoholic solvents, resp. A soln. of (2*RS*)-α-tocopherol of the same concentration was measured as a positive control. A reduction of the absorbance, 0.228, by the positive control was regarded as corresponding to the consumption of 2 equiv. of DPPH. All experiments were performed in triplicate at 23–25°.

3.2. *ABTS-Radical-Scavenging Test.* ABTS-Radical-scavenging activity was measured by the method of *Re et al.* [22] with a modification. The ABTS radical cation (ABTS$^{\bullet+}$) was produced by treating a 7mM aq. ABTS soln. with 2.45mM potassium persulfate (final concentration). The mixture was allowed to stand in the dark at r.t. for 12–16 h prior to use. The ABTS$^{\bullet+}$ soln. was diluted with dist. H_2O to an absorbance of 0.70 (±0.02) at 734 nm. To a diluted ABTS$^{\bullet+}$ soln. (2.97 ml) was added an EtOH soln. of a test compound (0.5mM, 0.03 ml). The soln. was immediately mixed vigorously for 10 s by a *Vortex* mixer and transferred to a cuvette. The absorbance reading was taken 30 min after initial mixing. EtOH was used in place of an antioxidant soln. as a control. An EtOH soln. of *Trolox* was measured as a positive control. A reduction of the absorbance by the positive control was regarded as corresponding to the consumption of 2 equiv. of ABTS$^{\bullet+}$. The pH of the reaction solns. was measured 10 min after mixing. All experiments were performed in triplicate at 23–25°.

4. *NMR Analyses.* 4.1. *NMR Measurements of the Reaction Mixtures* **1**/*DPPH and* **3**/*DPPH.* To a catechol derivative **1** or **3** (2.5 μmol) was added DPPH (5.0 mg, 13 μmol, 5.0 equiv.) in CD$_3$OD/(D$_6$)acetone 3:1, (0.4 ml). (D$_6$)acetone was added as a cosolvent to enhance the solubility of the DPPH radical. The mixture was immediately transferred to an NMR tube and mixed vigorously. ^{1}H-NMR spectra were recorded 10, 60, and 120 min after mixing.

4.1.1. *Reaction Mixture* **1**/*DPPH: 3,4-Dioxocyclohexa-1,5-diene-1-carboxylic Acid* (**1a**) *and 3-Hydroxy-3-methoxy-4-oxocyclohexa-1,5-dienecarboxylic Acid* (**1b**). *Quinone* **1a**: ^{1}H-NMR (CD$_3$OD/(D$_6$)acetone 3:1): 6.43 (*d*, *J* = 10.3, H–C(5)); 6.91 (*s*, H–C(2)); 7.53 (*d*, *J* = 10.3, H–C(6)). ^{13}C-NMR (CD$_3$OD/(D$_6$)acetone 3:1): 131.5 (C(5)); 133.3 (C(2)); 138.8 (C(6)); 141.7 (C(1)); 167.2 (COOH); 181.2 (C(4)); 182.3 (C(3)). HMBC: H–C(2) ↔ C(4), C(6), COOH; H–C(5) ↔ C(1), C(3); H–C(6) ↔ C(2), C(4).

Quinone Hemiacetal **1b**: ^{1}H-NMR (CD$_3$OD/(D$_6$)acetone 3:1): 6.10 (*d*, *J* = 10.3, H–C(5)); 7.21 (*s*, H–C(2)); 7.43 (*d*, *J* = 10.3, H–C(6)). ^{13}C-NMR (CD$_3$OD/(D$_6$)acetone 3:1): 89.5 (C(3)); 126.1 (C(5)); 128.6 (C(1)); 139.5 (C(6)); 145.1 (C(2)); 166.5 (COOH); 198.0 (C(4)). HMBC: H–C(2) ↔ C(4), C(6), COOH; H–C(5) ↔ C(1), C(3); H–C(6) ↔ C(2), C(4).

4.1.2. *Reaction Mixture* **3**/*DPPH: Sodium 3,4-Dioxocyclohexa-1,5-diene-1-carboxylate* (**3a**) *and Sodium 6-Methoxy-3,4-dioxocyclohexa-1,5-diene-1-carboxylate* (**3g**). *Quinone* **3a**: ^{1}H-NMR (CD$_3$OD/(D$_6$)acetone 3:1): 6.33 (*d*, *J* = 10.1, H–C(5)); 6.68 (*d*, *J* = 2.0, H–C(2)); 7.61 (*dd*, *J* = 10.1, 2.0, H–

C(6)). ^{13}C-NMR (CD_3OD/(D_6)acetone 3:1): 129.3 (C(2)); 130.0 (C(5)); 142.4 (C(6)); 148.7 (C(1)); 170.6 (COONa); 181.0 (C(4)); 183.1 (C(3)). HMBC: H–C(2) ↔ C(4), C(6), COONa; H–C(5) ↔ C(1), C(3); H–C(6) ↔ C(2), C(4).

6-Methoxyquinone **3g**: ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): 5.85 (*s*, H–C(5)); 6.14 (*s*, H–C(2)). ^{13}C-NMR (CD_3OD/(D_6)acetone 3:1): 103.8 (C(5)); 123.5 (C(2)); 151.6 (C(1)); 170.1 (C(6)); 170.1 (COONa); 179.6 (C(4)); 182.9 (C(3)). HMBC: H–C(2) ↔ C(4), C(6), COONa; H–C(5) ↔ C(1), C(3); MeO ↔ C(6).

4.2. *NMR Measurements of the Reaction Mixtures* **1f**/*DPPH* and **3f**/*DPPH*. To a catechol derivative **1f** or **3f** (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). The mixture was immediately transferred to an NMR tube and mixed vigorously. ^{1}H-NMR spectra were recorded 10 min after mixing.

4.2.1. *Reaction Mixture* **1f**/*DPPH: 6-Methoxy-3,4-dioxocyclohexa-1,5-diene-1-carboxylic Acid* (**1g**) *and 3-Hydroxy-3,6-dimethoxy-4-oxocyclohexa-1,5-diene-1-carboxylic Acid* (**1h**). *6-Methoxyquinone* **1g**: ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): 3.95 (*s*, MeO); 5.94 (*s*, H–C(5)); 6.53 (*s*, H–C(2)). ^{13}C-NMR (CD_3OD/(D_6)acetone 3:1): 58.2 (MeO); 105.9 (C(5)); 129.7 (C(2)); 145.2 (C(1)); 166.4 (COOH); 168.1 (C(6)); 179.1 (C(4)); 180.8 (C(3)). HMBC: H–C(2) ↔ C(4), COOH; H–C(5) ↔ C(1), C(3); MeO ↔ C(6).

6-Methoxyquinone Hemiacetal **1h**: ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): 3.88 (*s*, MeO); 5.53 (*s*, H–C(5)); 6.83 (*s*, H–C(2)). ^{13}C-NMR (CD_3OD/(D_6)acetone 3:1): 57.6 (MeO); 89.7 (C(3)); 99.4 (C(5)); 129.6 (C(1)); 142.9 (C(2)); 166.7 (COOH); 168.4 (C(6)); 195.6 (C(4)). HMBC: H–C(2) ↔ C(4), COOH; H–C(5) ↔ C(1), C(3); MeO ↔ C(6).

4.2.2. *Reaction Mixture* **3f**/*DPPH. 6-Methoxyquinone* **3g**: ^{1}H-NMR (CD_3OD/(D_6)acetone 3:1): 3.90 (*s*, MeO); 5.85 (*s*, H–C(5)); 6.14 (*s*, H–C(2)). ^{13}C-NMR (CD_3OD/(D_6)acetone 3:1): 57.8 (MeO); 103.8 (C(5)); 123.5 (C(2)); 151.6 (C(1)); 170.1 (C(6)); 170.1 (COONa); 179.6 (C(4)); 182.9 (C(3)). HMBC: H–C(2) ↔ C(4), C(6), COONa; H–C(5) ↔ C(1), C(3); MeO ↔ C(6).

5. *Isolation of an Oxidation Product of* **1** *after Reaction with DPPH in MeOH*. To a soln. of **1** (77 mg, 0.50 mmol) in MeOH/acetone 3:1 (50 ml) was added DPPH (788 mg, 2.0 mmol, 4.0 equiv.), and the mixture was stirred for 12 h at r.t. Then sodium dithionite (348 mg, 2.0 mmol, 4.0 equiv.) in H_2O (15 ml) was added and the mixture stirred for 30 min. The mixture was concentrated *i.v.*, and the residue subjected to CC (silica gel, $CHCl_3$/MeOH 100:7). The crude product was further purified by prep. TLC ($CHCl_3$/MeOH/formic acid 100:7:0.1; R_f 0.27): **1f** (1.9 mg, 2.1%).

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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