

Oxidation of Ethanol Induced by Simple Polyphenols: Prooxidant Property of Polyphenols

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The aerobic oxidation of ethanol to acetaldehyde in water is induced by simple polyphenols, such as pyrogallol or catechol, in the presence of $FeSO_4$–DTPA (*N*,*N*,*N*′,*N*″,*N*″-diethylenetriaminepentaacetic acid) catalyst. The amount of acetaldehyde formed becomes an indicator of their "prooxidant" ability in terms of the activation of O_2. The "prooxidant" ability of pyrogallol is higher than that of catechol. Electron-withdrawing substituents decrease the ability, whereas electron-donating ones enhance it. The "prooxidant" property is exhibited by the total consequence of two processes: hydroxyl radical ($^{\bullet}OH$) generation from O_2 and its capture by phenolic compounds.

Polyphenolic compounds have been shown to have potential effects on human health.[1] In particular, tea catechins, such as those shown below, are implicated to exert antitumor, anticarcinogenic, and antiallergic activities.[2,3] These effects are considered to be mainly responsible for their "antioxidant" ability to scavenge oxyl radicals such as $^{\bullet}OH$ generated from O_2. Among extensive studies of polyphenolic compounds as "antioxidant",[4–7] some plant polyphenols have been shown to inversely exhibit "prooxidant" properties.[8,9] For instance, the green, pouching, and oolong tea extracts accelerate the oxidation of deoxyribose with H_2O_2 in the presence of Fe(III) catalyst.[9a] Furthermore, using a technique of trapping hydroxyl radicals ($^{\bullet}OH$) generated by a Fenton system ($FeSO_4$–H_2O_2), a variety of naturally occurring polyphenols and hydroxylated flavonoids have been classified to have effects of suppressing or enhancing $^{\bullet}OH$ generation.[9b]

Recently, catechins (Chart 1) have been reported to activate O_2 to produce H_2O_2 in aqueous solution.[10] Simple polyphenols, such as pyrogallol (**1**) or catechol (**2**) (Chart 2), which correspond to the essential unit of polyphenols, also induces the formation of H_2O_2 from O_2.[11] These studies have been made from

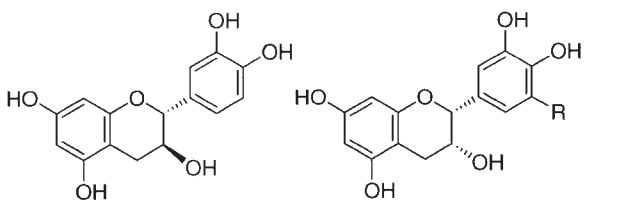

Chart 1.

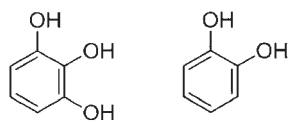

Chart 2.

biochemical viewpoints, and no straightforward chemistry for elucidating and evaluating this phenomenon as the "prooxidant" ability of polyphenols has been available. In our effort to develop such a chemistry, we have found that phenolic compound **1** or **2** aerobically oxidizes CH_3CH_2OH into CH_3CHO in the presence of $FeSO_4$ catalyst in aqueous solution. In light of this finding, we describe here that the "prooxidant" ability of simple polyphenols in terms of the activation of O_2 can be estimated by the extent of CH_3CHO formed. In addition, we report that the present oxidation unambiguously involves the formation of hydroxyl radials ($^{\bullet}OH$) formed from O_2.

Results

Figure 1a shows the time profile for the aerobic oxidation of CH_3CH_2OH to CH_3CHO induced by **1** in the presence of catalytic amounts of $FeSO_4$ and DTPA (*N*,*N*,*N*′,*N*″,*N*″-diethylenetriaminepentaacetic acid)[12] (Scheme 1). Thus, when 30% aqueous ethanol was allowed to react with 100 μM of **1** under air in a phosphate buffer (pH 7.5, 100 mM) (1 M = 1 mol dm^{-3}) by using $FeSO_4$–DTPA (4 μM) catalyst at 60 °C, 84 μM of CH_3CHO was produced in 80 min. As shown in Fig. 1a, the amount of CH_3CHO formed decreased with decreasing the amount of **1** used. This result unequivocally indicates that **1** acts as a "prooxidant". No acetaldehyde was formed in the absence

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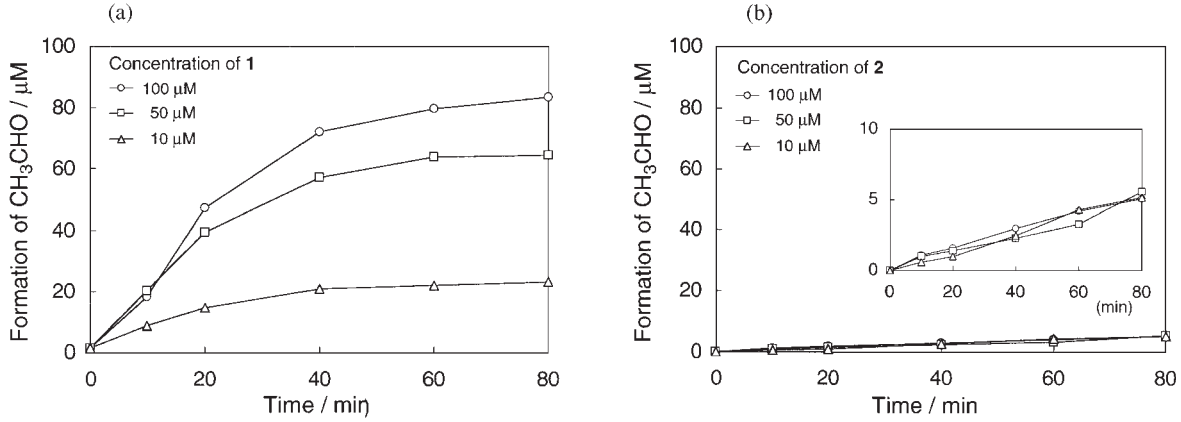


Fig. 1. Time course for the oxidation of ethanol to acetaldehyde under air in the presence of Fe(II) catalyst and pyrogallol (**1**) (a) or catechol (**2**) (b). Into 5 mL of a phosphate buffer solution (100 mM, pH 7.5) of 30% aqueous EtOH was added 20 μL of a solution containing $FeSO_4 \cdot 7H_2O$ (1 mM) and DTPA (1 mM) in H_2O. Subsequently, 5 (△), 25 (□), or 50 (○) μL of a solution containing pyrogallol (**1**) or catechol (**2**) (10 mM) in H_2O–EtOH (1:1) was added in each experiment, respectively, and the solution was stirred at 60 °C under air. Acetaldehyde formed was analyzed by the method using phthalaldehyde as a fluorescence labeling reagent for the analysis of acetaldehyde–hydrogensulfite adduct (Ref. 26).

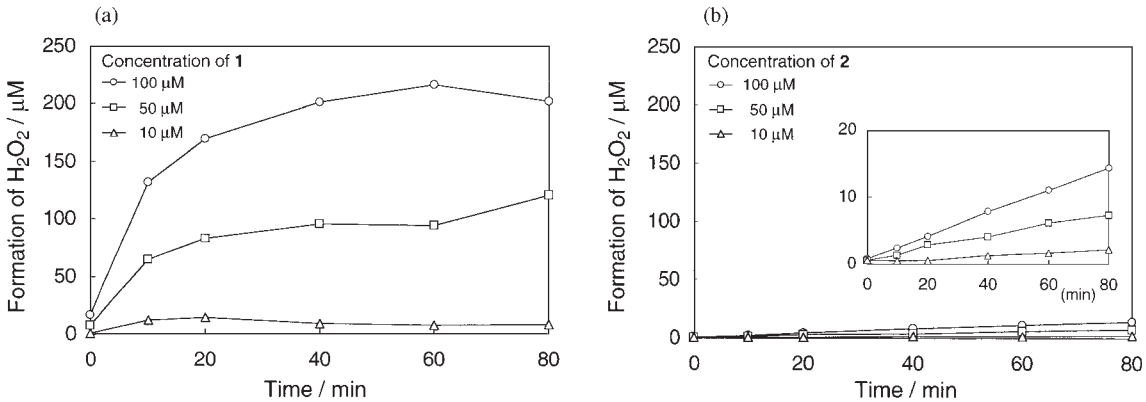
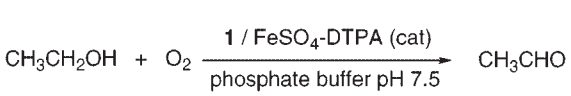
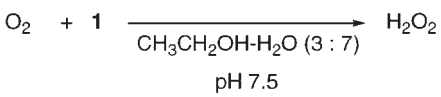


Fig. 2. Time course for the formation of hydrogen peroxide in the reaction of pyrogallol (**1**) (a) or catechol (**2**) (b) with air. Into 5 mL of a phosphate buffer solution (100 mM, pH 7.5) of 30% aqueous EtOH was added 5 (△), 25 (□), or 50 (○) μL of a solution containing pyrogallol (**1**) or catechol (**2**) (10 mM) in H_2O–EtOH (1:1) in each experiment, respectively, and the solution was stirred at 60 °C under air. Hydrogen peroxide formed was analyzed by the flow-injection method using a titanium(IV)–porphyrine complex (Ref. 27).



Scheme 1.



Scheme 2.

of either **1** or Fe(II)–DTPA catalyst.[13] The use of **2** (100 μM), instead of **1**, did not induce effective oxidation, and in fact smaller amounts of CH_3CHO were formed (5.1 μM, 80 min) (Fig. 1b). The relative ratio of CH_3CHO formation with **2**/**1** was 0.06.

In the absence of Fe(II)–DTPA catalyst, pyrogallol (**1**) itself reacts with O_2 in air to give H_2O_2 (Scheme 2). Thus, a stirring solution of **1** (100 μM) in aqueous ethanol (pH 7.5) reacts with O_2 in air to produce ~200 μM of H_2O_2 (80 min, 60 °C) (see Experimental). As shown in Fig. 2a, the formation of H_2O_2 decreased with decreasing the amount of **1** used. When catechol (**2**) (100 μM) was used in place of **1**, H_2O_2 was similarly formed (Fig. 2b). However, as compared to the case of **1**, its effectiveness was not significant (14 μM, 80 min).

The presence of Fe(II) in the reaction of Scheme 2 could induce the generation of hydroxyl radicals (•OH). Indeed, the reaction of **1** and O_2 in the presence of $FeSO_4$–DTPA catalyst produced •OH, which could be detected by ESR as the adduct of either *N*-*t*-butylbenzylideneamine *N*-oxide (PBN)[14] or 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO).[15] Since the DMPO adduct of •OH was quite unstable compared to that of PBN, the time profile of •OH generation was monitored by using PBN. The extent of PBN-OH signal was measured as the ratio of the signal intensity at the lowest magnetic field to that of MnO used as an internal standard. As can be seen in Fig. 3a, the progress of •OH formation is similar to that of CH_3CHO formation (Fig. 1a), suggesting that the oxidation of CH_3CH_2OH is induced by •OH generated in situ. The use of

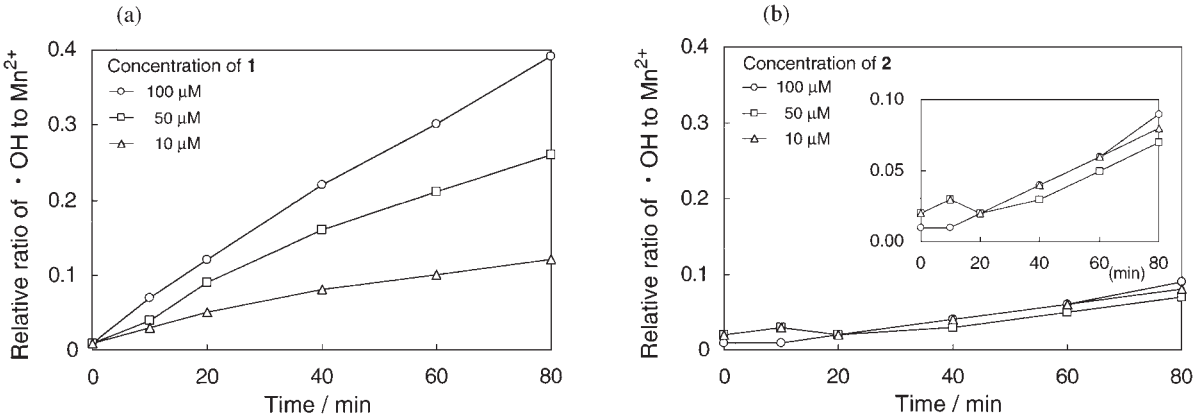


Fig. 3. Time course for the generation of $^{\bullet}$OH in the reaction of pyrogallol (**1**) (a) or catechol (**2**) (b) with air in the presence of Fe(II) catalyst. Into 1 mL of a phosphate buffer solution (100 mM, pH 7.5) of 30% aqueous EtOH was added 30 μL of a solution containing *N*-*t*-butylbenzylydeneamine *N*-oxide (PBN) (4.1 M) in EtOH. After stirring the solution for a few minutes, 4 μL of a solution of $FeSO_4 \cdot 7H_2O$ (1 mM) and DTPA (1 mM) in H_2O was added. Finally, 1 (△), 5 (□), or 10 (○) μL of a solution containing pyrogallol (**1**) (10 mM) in H_2O–EtOH (1:1) was added, respectively, in each experiment. Aliquot samples were analyzed by ESR using a hematocrit capillary tube (75 m/m, ϕ1.45–1.65 m/m). The peak height of $^{\bullet}$OH is indicated by the ratio relative to Mn marker (S/M).

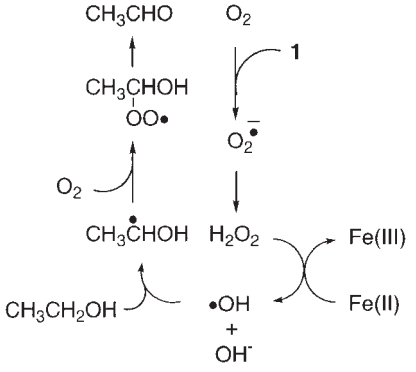
catechol (**2**) also produced $^{\bullet}$OH (Fig. 3b), but its formation was again not significantly effective as that from **1**. The relative ratio of $^{\bullet}$OH formation with **2**/**1** was 0.23.

Discussion

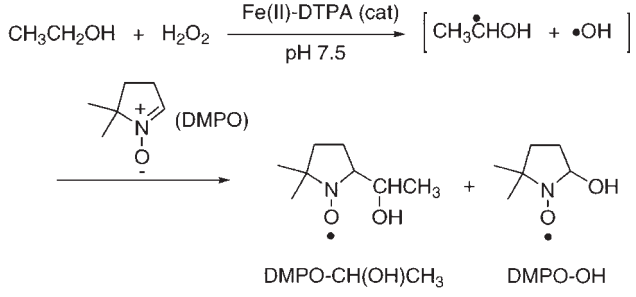
The role of "antioxidant" has been implicated in the prevention of oxy radicals generated in living organism.[16] Among oxy radicals, such as superoxyl, peroxyl, hydroxyl, and phenoxyl radicals, the most reactive species is the hydroxyl radical ($^{\bullet}$OH), and any hydroxyl radical produced in vivo has been thought to react at, or close to, its site of formation. In relation to disease and pathological disorders, the generation of $^{\bullet}$OH and its fate in aerobic organisms have been reviewed by Halliwell.[17] In such a context, a straightforward property of $^{\bullet}$OH in terms of its generation and capture by polyphenols is provided by the aerobic oxidation of CH_3CH_2OH to CH_3CHO described here.

The reaction pathways for the present oxidation are *conceptually* and *schematically* illustrated as shown in Scheme 3. One electron transfer takes place from pyrogallol (**1**) to O_2, affording superoxide anions ($O_2^{\bullet -}$) which result in the formation of H_2O_2 in an aqueous medium (pH 7.4).[10,11,18] Subsequently, Fenton reaction[17a,19,20] produces hydroxyl radicals ($^{\bullet}$OH), which abstract an α-hydrogen of CH_3CH_2OH.[21] The resulting 1-hydroxyethyl radical ($CH_3\dot{C}HOH$) reacts with O_2,[22] resulting in the formation of CH_3CHO. The validity of such pathways is supported by the references cited herein. In addition, the following experiment proves α-hydrogen abstraction from CH_3CH_2OH by $^{\bullet}$OH. Namely, when H_2O_2 was decomposed by $FeSO_4$–DTPA in CH_3CH_2OH, both $^{\bullet}$OH and $CH_3\dot{C}HOH$ could be detected by ESR as DMPO-adducts (Scheme 4) (see Experimental).

The "prooxidant" property of **1** and **2**, which is observed as the formation of CH_3CHO, thus corresponds to the in situ formation of $^{\bullet}$OH from O_2. The attack of $^{\bullet}$OH toward CH_3CH_2OH results in the formation of CH_3CHO. This attack is, however, competitively inhibited by phenolic compounds, such as **1** and **2**, because of their "antioxidant" ability to capture $^{\bullet}$OH. In the present study, we also evaluated this ability by the Fenton reaction (Eq. 1). Thus, when 30% aqueous ethanol was treated with H_2O_2 (176 μM) in phosphate buffer (100 mM) containing $FeSO_4$–DTPA (40 μM) under *argon* (60 °C, 60 min), 39 μM of CH_3CHO was formed (98% yield based on $FeSO_4$). The addition of **1** or **2** (5 μM each) decreased its formation, as given in Eq. 1. The extent of its decrease corresponded to 12% for **1** and 63% for **2**. Since Eq. 1 equivocally involves the $^{\bullet}$OH radical (Scheme 4), catechol (**2**) acts as a more effective "antioxidant" than **1** in terms of capturing $^{\bullet}$OH.



Scheme 3.



Scheme 4.

$$CH_3CH_2OH + H_2O_2\ (176\ \mu M) \xrightarrow[Ar,\ pH\ 7.5]{Fe(II)\text{-}DTPA\ (cat)\ (40\ \mu M)} CH_3CHO \quad (1)$$

additive (5 μM)	μM[a)]	%[b)]
—	39	98
1	34	86
2	15	36

a) The amount of CH_3CHO formed.
b) Yield based on Fe(II).

Accordingly, it can be said that pyrogallol (**1**), which *can* effectively promote •OH generation from O_2, does not significantly capture the hydroxyl radical (•OH). Inversely, catechol (**2**), which is a poor mediator of •OH generation, acts as a better scavenger of •OH. Although both generating and trapping •OH are involved in the aerobic oxidation of CH_3CH_2OH shown in Scheme 1, it is obvious that the extent of CH_3CHO formed serves as an indicator of the "prooxidant" ability of **1** and **2** as the whole consequence.

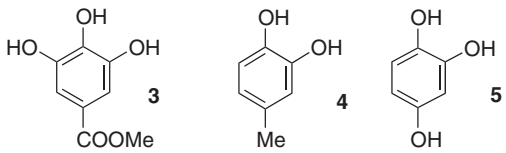
Table 1 shows such a "prooxidant" ability of simple polyphenols **1**–**5**, among which pyrogallol (**1**) is highly active. The introduction of methoxycarbonyl at 4-position (i.e., **3**) substantially reduces the formation of CH_3CHO (entries 1 and 2). In the series of catechol (**2**), the introduction of 4-methyl substituent (i.e., **4**) increases its amount by ~5 fold (entries 3 and 4). 1,2,4-Benzentriol (**5**) further increases its amount up to ~12 fold (entries 3 and 5). Generally, it appears that electron-withdrawing substituents retard the oxidation, while electron-donating ones accelerate it. This fact agrees with the idea that the "prooxidant" property of polyphenols is substantially governed by electron transfer to O_2.

Relevant to be noted here is the generation of H_2O_2 from O_2 by using **3**–**5**. Under the conditions given in Fig. 2, the amount (μM) of H_2O_2 formed was as follows: **1** (200) > **3** (140); **2** (14) < **5** (25) < **4** (36) (concentration of **1**–**5**, 100 μM; reaction time, 80 min). Namely, in the case of **5**, smaller amounts of H_2O_2 were produced, compared to **4**. In contrast, the reverse result was obtained for the formation of CH_3CHO (Table 1). In the case of **5**, the time profile for the formation of H_2O_2 was also different from those with others. Thus, the formation of H_2O_2 induced by **5** gradually decreased with time, while in other cases it increased, such as shown in Fig. 2. These results indicate that in the case of **5**, even if H_2O_2 is once produced, it readily reacts with **5**. Accordingly, the amount of H_2O_2 formed does not become an indicator of the "prooxidant" ability. However, when Fe(II) is present in the reaction system, H_2O_2 rapidly decomposes to •OH, which must react preferentially with CH_3CH_2OH present in large excess, resulting in the formation of CH_3CHO.[23]

Table 1. Amount of Acetaldehyde Formed by Aerobic Oxidation of Ethanol by Using Simple Polyphenols **1**–**5**[a)]

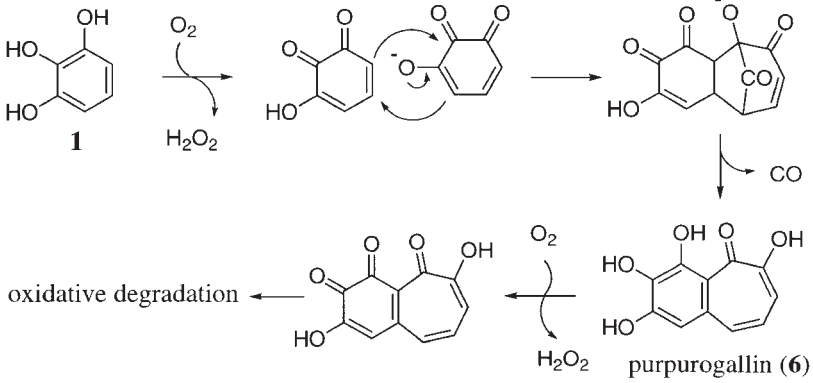
Entry	Compound	Amount of CH_3CHO/μM
1	**1**	84
2	**3**	7.8
3	**2**	5.1
4	**4**	25
5	**5**	61

a) Into 5 mL of a phosphate buffer solution (100 mM, pH 7.5) of H_2O–EtOH (7:3) was added 20 mL of a solution containing $FeSO_4$ (1 μM) and DTPA (1 μM) in H_2O. Subsequently, 50 μL of a solution containing polyphenol compound (10 mM) in H_2O–EtOH (1:1) was added, and the solution was stirred at 60 °C for 80 min under air.



Pyrogallol (**1**), when exposed to air, has been known to be transformed into purpurogallin (**6**) via the dimerization of 3-hydroxy-3,5-cyclohexadiene-1,2-dione (Scheme 5).[24,25] In the present study, we also confirmed the formation of **6** together with H_2O_2. This experiment was carried out by using 1 mmol of **1** in order to isolate **6**. The concentration of **1** was thus higher (0.01 M in 30% aq EtOH). Under this condition, the transformation did not proceed effectively, but the amount (0.58 mmol) of H_2O_2 formed was found to nearly coincide with the expected value (see Experimental). Since the diffusion of O_2 into the solution may be associated with the formation of H_2O_2, the analysis of "prooxidant" ability described here is recommended to be performed under a dilute condition (100 μM).

In summary, the aerobic oxidation of ethanol to acetaldehyde is induced by simple polyphenols and Fe(II) catalyst. The generation of hydroxyl radicals (•OH) from O_2 and its capture by phenolic compounds are involved in the oxidation. The extent



Scheme 5.

of acetaldehyde formed serves as the indicator of the "prooxidant" ability of phenolic compounds. The assessment of catechins or flavonoids by the present method will be the subject of further study.

Experimental

Materials. The commercial sources of reagents used were as follows: pyrogallol, catechol, iron(II) sulfate ($FeSO_4 \cdot 7H_2O$), sodium hydrogen sulfite ($NaHSO_3$), and 60% perchloric acid from Nacalai Tesque, Inc. (Kyoto, Japan); *N*,*N*,*N*′,*N*″,*N*″-diethylenetriaminepentaacetic acid (DTPA) from Dojindo Laboratories (Kumamoto, Japan); hydrogen peroxide (30%, v/v) from Santoku Chemical Industry; phthalaldehyde (OPA) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); oxo[5,10,15,20-tetra(4-pyridyl)porphinato]titanium(IV) from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan); *N*-*t*-butylbenzylideneamine *N*-oxide (PBN) from Aldrich Chemical Co. (Milwaukee).

Aerobic Oxidation of Ethanol to Acetaldehyde with Pyrogallol (1) or Catecol (2) in the Presence of Fe(II)–DTPA Catalyst. Into 5 mL of a phosphate buffer solution (100 mM, pH 7.5) containing EtOH (1.5 mL) was added 20 μL of a solution of $FeSO_4 \cdot 7H_2O$ (1 mM) and DTPA (1 mM) in H_2O. Subsequently, 5, 25, or 50 μL of a solution of pyrogallol (**1**) or catechol (**2**) (10 mM) in EtOH–H_2O (1:1) was added in each experiment, respectively. The resulting solution was stirred under air at 60 °C. The analysis of acetaldehyde formed was made by transforming it into hydrogensulfite adduct.[26] Thus, into 500 μL of an aliquot solution taken at an appropriate time was added 500 μL of 5 mM $NaHSO_3$ in 10 mM citrate buffer solution (pH 4.4). The solution was left standing at room temperature for 20 min. Note that because of no generation of H_2O_2 from O_2 at pH 4.4, the citrate buffer solution stopped the CH_3CHO formation. The acetaldehyde–hydrogensulfite adduct formed by this treatment was subjected into reversed-phase HPLC at pH 3.2 (eluting solution, 10 mM ammonium acetate and 20 mM acetic acid; flow rate, 0.5 mL/min; column, Waters Puresil 5μ C18, 4.6 × 250 mm; temperature, 20 °C). The 1-hydroxyethanesulfonic acid thus formed was passed through a post-column at >pH 7.0 to produce H_2SO_3, which was then allowed to react with phthalaldehyde in the presence of ammonium acetate. The resulting 2*H*-isoindole-1-sulfonic acid, a fluorescent compound, was analyzed by HPLC equipped with fluorescence detector ($\lambda_{ex.} = 320$ nm and $\lambda_{em.} = 390$ nm). This manipulation was carried out under the following HPLC conditions: eluting solution, 10 mM ammonium acetate; reaction solution, 10 mM methanol solution of phthalaldehyde in 100 mM borate buffer (pH 9.8) (v/v, 1/4); sample injection, 20 μL; flow rate, 0.2 mL/min; reaction temperature, 50 °C. Time-profile for the formation of CH_3CHO is given in Fig. 1.

Formation of Hydrogen Peroxide from O_2 and Pyrogallol (1) or Catechol (2). Into 5 mL of a phosphate buffer solution (100 mM, pH 7.5) containing EtOH (1.5 mL) was added 5, 25, or 50 μL of a solution of pyrogallol (**1**) or catechol (**2**) (10 mM) in EtOH–H_2O (1:1) in each experiment, respectively. The resulting solution was stirred under air at 60 °C. The analysis of H_2O_2 formed was performed by a sensitive flow-injection method[27] using oxo[5,10,15,20-tetra(4-pyridyl)porphinato]titanium(IV) [Ti(IV)O(tpypH$_4$)]. This compound reacted with H_2O_2 to form a monoperoxo complex [Ti(IV)O_2(tpypH$_4$)], and the absorption maximum at 450 nm of the monoperoxo complex was used for the analysis. Thus, into 50 μL of an aliquot of the solution taken at an appropriate time was diluted with 450 μL of H_2O, and 100 μL of the diluted solution was mixed with 1 mL of 30 μM [Ti(IV)O(tpypH$_4$)] solution of 5 M hydrochloric acid by a flow-injection analysis system, which consisted of two channels (mobile phase: flow rate of distilled water, 1.0 mL/min; flow rate of reagent solution, 1.0 mL/min). The sample solution containing H_2O_2 was allowed to react with [Ti(IV)O(tpypH$_4$)] in the mixing coil (15 m, 75 °C) to form [Ti(IV)O_2(tpypH$_4$)]. The time-profile for the formation of H_2O_2 is given in Fig. 2.

Measurement of Hydroxyl Radicals by ESR. Into 1 mL of a phosphate buffer solution (100 mM, pH 7.5) containing EtOH (0.3 mL) was added 30 μL of a solution containing *N*-*t*-butylbenzylideneamine *N*-oxide (PBN) (4.1 M) in EtOH. After stirring the solution for a few minutes at room temperature, 4 μL of a solution of $FeSO_4 \cdot 7H_2O$ (1 mM) and DTPA (1 mM) in H_2O was added. Finally, 1, 5, or 10 μL of a solution containing pyrogallol (**1**) or catechol (**2**) (10 mM) in H_2O–EtOH (1:1) was added, respectively, in each experiment. The concentration of **1** or **2** corresponds to 10, 50, or 100 μM, respectively, in each experiment. Aliquot samples were analyzed by ESR using a hematocrit capillary tube (75 m/m, ϕ 1.45–1.65 m/m). ESR spectra were measured with a JES-RE-2X ESR spectrometer (JEOL Ltd., Japan). The ESR analytical conditions were as follows: magnetic field, 335 ± 10 mT; power, 5 mW; response, 1 s; modulation, 0.1 mT; temperature, room temperature; sweep time, 8 min. The quantity of generated free radicals was expressed as the peak height of PBN spin adduct[14] obtained by measuring the peak height of the outermost peak at the left end of the spin adduct spectrum. The relative peak height of the adduct was calculated with respect to the signal intensity of the Mn^{2+} peak. The time-profiles for the formation of $^{\bullet}OH$ are given in Fig. 3.

Detection of Hydroxyl Radicals and α-Hydroxyethyl Radicals by ESR. Into 68 μL of a phosphate buffer solution (100 mM, pH 7.5) containing EtOH (0.3 mL) was added 73 μL of a solution of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) (8.9 M) in EtOH. After stirring the solution for a few minutes, 15 μL of a solution of $FeSO_4 \cdot 7H_2O$ (10 mM) and DTPA (10 mM) in H_2O was added. Finally, 127.5 μL of H_2O_2 (5.88 mM) in H_2O was added to the above solution at room temperature. After one minute of stirring, an ESR measurement was carried out under the following conditions: magnetic field, 335 ± 10 mT; power, 5 mW; response, 0.1 s; modulation, 0.1 mT; temperature, room temperature; sweep time, 0.5 min. The result is given in Fig. 4.

Oxidation of Ethanol with H_2O_2 Using Fenton Catalyst in the Presence or Absence of Pyrogallol (1) or Catechol (2). Into 3.5 mL of a phosphate buffer solution (100 mM, pH 7.5) was add-

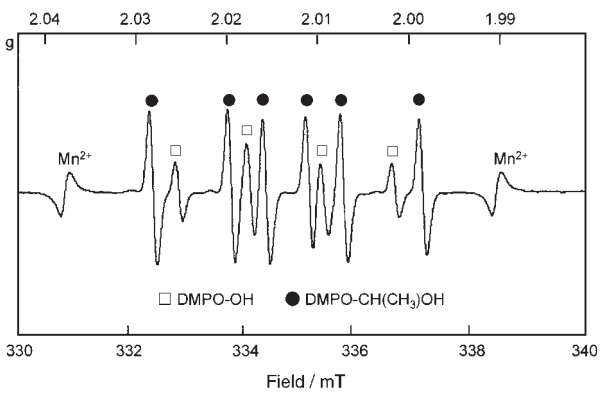


Fig. 4. Detection of hydroxyl radical and 1-hydroxyethyl radical by ESR as DMPO adducts.

Table 2. Formation of CH_3CHO from Fenton Reaction of CH_3CH_2OH

Additive	Amount of CH_3CHO formed/μM			Yield/%
	With H_2O_2	Without H_2O_2	Corrected value	Based on Fe(II)
—	46.8	7.7	39.1	98
Pyrogallol (**1**)	43.2	8.9	34.3	86
Catechol (**2**)	22.7	8.2	14.5	36

ed 200 μL of a solution of $FeSO_4 \cdot 7H_2O$ (1 mM) and DTPA (1 mM) in H_2O, and subsequently 150 μL of 5.88 mM H_2O_2 was added at room temperature. Into this solution was then added 2.5 μL of 10 mM pyrogallol (**1**) or catechol (**2**) in EtOH–H_2O (1:1) together with 1.5 mL of EtOH. The amount of **1** (or **2**) and Fe(II) used corresponded to 5 μM and 40 μM, respectively. The resulting solution was stirred at 60 °C *under argon* for 60 min. As a reference, the same reaction was performed in the absence of **1** or **2**. An analysis of CH_3CHO was carried out by the same procedure as mentioned above. Even without adding H_2O_2, the oxidation was found to slightly take place, because air was not rigorously excluded. Consequently, the amount of CH_3CHO formed in the Fenton reaction was corrected by using the amount of CH_3CHO formed in the absence of H_2O_2. The results are given in Table 2.

Formation of Purpurogallin (6) from Pyrogallol (1) and O_2. Into a 100 mL of phosphate buffer solution (100 mM, pH 7.5) containing CH_3CH_2OH (30 mL) was added **1** (1 mmol, 72.02 mg), and the solution (0.01 M of **1**) was stirred under O_2 (balloon) at 60 °C for 40 min. The products were immediately and rigorously extracted with ether (200 mL), and dried over Na_2SO_4. The evaporation of ether gave a light brown powder which contained purpurogallin (**6**) (22.88 mg, 0.10 mmol, 20% based on **1**) and **1** (72.02 mg, 0.57 mmol, 57%) by NMR analysis. From an analysis of the H_2O_2 formed, its amount was found to be 0.58 mmol. The authentic purpurogallin (**6**) was prepared from sodium iodate and **1** by the reported procedure.[28] ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.73 (dd, 1H, $J = 11.47$ and 9.43 Hz), 6.90 (br s, 1H), 7.07 (d, 1H, $J = 9.43$ Hz), 7.34 (d, 1H, $J = 11.47$ Hz); ^{13}C{^{1}H} NMR (100.5 MHz, DMSO-d_6) δ 111.7 (C-9), 116.3 (C-4a), 118.0 (C-1), 125.0 (C-8), 134.5 (C-3), 135.8 (C-7), 136.2 (C-9a), 153.0 (C-4), 153.2 (C-2), 156.1 (C-6), 183.7 (C-5).

If the following assumptions (1)–(3) based on Scheme 5 are valid, the amount of H_2O_2 formed is nearly consistent with that expected: (1) The pyrogallol (**1**) consumed (1.0 mmol − 0.57 mmol = 0.43 mmol) produces an equimolar amount of H_2O_2 (0.43 mmol). (2) The consumed **1** is quantitatively transformed into purpurogallin (**6**) (0.43 mmol/2 = 0.21 mmol). (3) The amount of **6** isolated is 0.10 mmol, and therefore a part of **6** formed (0.21 mmol − 0.10 mmol) is oxidized[29] by O_2 to give 0.11 mmol of H_2O_2. From these assumptions, the expected amount of H_2O_2 formed becomes 0.54 mmol (0.43 mmol + 0.11 mmol), which is nearly equal to the observed value of 0.58 mmol. Note that based on Scheme 5, the expected amount of H_2O_2 is to be 1.5 equivalent of **1** used. However, under the conditions using phosphate buffer (Fig. 2a), 100 μM of **1** produced 200 μM of H_2O_2. This amount exceeded the expected value of 150 μM. Therefore, unknown processes for the production of H_2O_2 must be involved, in which the phosphate buffer may take part. Since the phosphate buffer acts as a reductant,[30] it could induce the regeneration of **1** from the intermediate quinone (hydroxyl-3,5-cyclohexadiene-1,2-dione) (Scheme 5). The regenerated **1** again produces H_2O_2. Involvement of such a process explains 200 μM production of H_2O_2 from 100 μM of **1**. In place of the phosphate buffer, the use of MOPS buffer (3-(*N*-morpholino)propanesulfonic acid) decreased the amount of H_2O_2 down to 110 μM. This fact supports the above consideration.

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