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Formation Mechanism of *p*-Methylacetophenone from Citral via a *tert*-Alkoxy Radical Intermediate

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The aim of this study was to clarify the formation mechanism of a potent off-odorant, *p*-methylacetophenone, from citral under acidic aqueous conditions. An acidic aqueous solution (pH 3.0) containing 10 mg/L of citral was stored at 40 °C for 2 weeks. Among the compounds detected in the stored citral solution, 4-(2-hydroxy-2-propyl)benzaldehyde was identified for the first time as a degradation product from citral. The formation of *p*-methylacetophenone and 4-(2-hydroxy-2-propyl)-benzaldehyde behaved the same when antioxidants were added to the citral solution. In addition, both compounds were formed by the Fe^{2+}-induced decomposition of 8-hydroperoxy-*p*-cymene, another compound identified in the stored citral solution. These results suggested that both *p*-methylacetophenone and 4-(2-hydroxy-2-propyl)benzaldehyde can be formed via the same radical intermediate [$p\text{-}CH_3C_6H_4C(CH_3)_2O^{\bullet}$] that can be derived from the O−O bond homolysis of 8-hydroperoxy-*p*-cymene. On the other hand, the degradation of 8-hydroperoxy-*p*-cymene without Fe^{2+} under acidic aqueous conditions did not yield *p*-methylacetophenone and 4-(2-hydroxy-2-propyl)benzaldehyde, but the degradation of citral without Fe^{2+} did. Therefore, other than the decomposition of 8-hydroperoxy-*p*-cymene, a mechanism to generate the *tert*-alkoxy radical intermediate was proposed for the formation of *p*-methylacetophenone and 4-(2-hydroxy-2-propyl)benzaldehyde in the citral solution.

KEYWORDS: Citral; degradation; off-odors; *p*-methylacetophenone; formation mechanism; *tert*-alkoxy radical; 4-(2-hydroxy-2-propyl)benzaldehyde

INTRODUCTION

The stability of citral (a mixture of neral and geranial) under acidic aqueous conditions is a critical issue in the field of flavor chemistry. Under such conditions, citral easily degrades by a series of cyclization and oxidation reactions to form a variety of degradation products (*1*−*8*). Consequently, not only is the fresh lemon-like odor of citral lost, but undesirable off-odors develop. Among the degradation products from citral, *p*-cresol and *p*-methylacetophenone were reported to be the most potent off-odorants (*6*, *7*). The contribution of these two compounds to the off-odors formed in the acidic solution of citral was also confirmed in our experiments (*9*).

Despite the importance as off-odorants derived from citral, the formation mechanisms of *p*-cresol and *p*-methylacetophenone have not yet been fully clarified. **Scheme 1** shows the previously proposed formation pathways of the oxidation products including *p*-cresol and *p*-methylacetophenone from citral under acidic aqueous conditions. It has been established that citral in acidic solutions undergoes cyclization reactions leading to the formation of *p*-menthadien-8-ols (*1*−*5*, *8*). *p*-Cymen-8-ol and its dehydration product, α,*p*-dimethylstyrene, have been proposed to be formed from *p*-menthadien-8-ols by their oxidation with dissolved oxygen (*5*) or disproportionation reactions (*4*) producing α-terpineol as the reduction product. *p*-Methylacetophenone was then suggested to be formed by the oxidation of α,*p*-dimethylstyrene (*6*). In our previous studies, on the other hand, 8-hydroperoxy-*p*-cymene was detected in the stored acidic solution of citral, and it was demonstrated that this hydroperoxide decomposes to *p*-cresol, *p*-cymen-8-ol, and α,*p*-dimethylstyrene, and not to *p*-methylacetophenone (*9*) under the same acidic conditions as the citral solution. The formation mechanism of *p*-methylacetophenone from citral under our experimental conditions thus remains a question.

Therefore, the aim of this study was to clarify the formation mechanism of *p*-methylacetophenone from citral under acidic aqueous conditions.

MATERIALS AND METHODS

Chemicals. Citral was purchased from Polarome International (Jersey City, NJ). *p*-Cymen-8-ol and α,*p*-dimethylstyrene were purchased from Sigma-Aldrich Japan (Tokyo, Japan). (−)-Epicatechin (EC), (−)-epicatechin gallate (ECg), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCg), and (+)-catechin were purchased from Funakoshi (Tokyo, Japan). *p*-Cymene and quercitrin were purchased from Wako Pure Chemical Industries (Osaka, Japan). *p*-Cresol, *p*-

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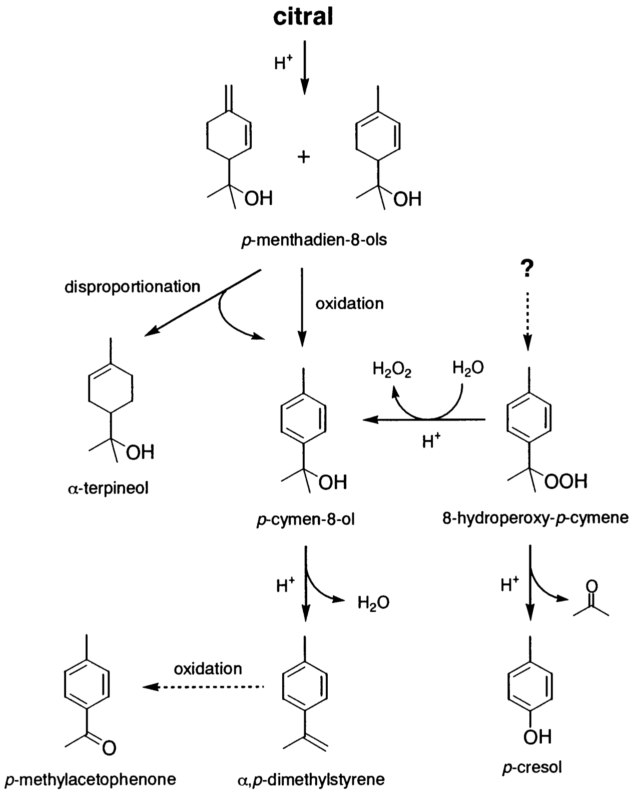
Scheme 1. Previously Proposed Formation Pathways of *p*-Cresol and *p*-Methylacetophenone and Other Oxidation Products from Citral under Acidic Aqueous Conditions (*4–6*, *9*)



methylacetophenone, and ascorbic acid were purchased from Nacalai Tesque (Kyoto, Japan).

Synthesis. 4-(2-Hydroxy-2-propyl)benzaldehyde was synthesized from *p*-bromobenzaldehyde dimethylacetal (Sigma-Aldrich Japan) according to the method described by Creary and Wang (*10*). A yield of 13 mg (31%) of 4-(2-hydroxy-2-propyl)benzaldehyde was obtained: ^{1}H MMR ($CDCl_3$) δ 10.00 (s, 1 H), 7.76 (AA′BB′ aromatic quartet, 4H), 2.16 (br s, 1H), 1.61 (s, 6H) [agreed with the literature (*10*)]; MS (EI), *m*/*z* (relative intensity) 164 (2), 150 (10), 149 (100), 145 (5), 133 (3), 121 (4), 115 (5), 107 (6), 105 (5), 91 (7), 77 (14), 74 (4), 65 (3), 59 (6), 51 (10), 43 (94).

8-Hydroperoxy-*p*-cymene was synthesized as follows: A solution of 150 mg of *p*-cymen-8-ol in 5 mL of ethanol was added dropwise to the stirred mixture of 150 mL of 30% hydrogen peroxide and 15 mL of 2.5% (w/v) sulfuric acid. After stirring for 1 h at room temperature, the reaction mixture was extracted with 250 mL of dichloromethane, washed with water (250 mL × 2), dried over sodium sulfate, and concentrated in vacuo to ∼1 mL. The crude product was purified by thin-layer chromatography on silica gel plates using a mixture of ethyl acetate and hexane (1:5, v/v) as the eluent to give 8-hydroperoxy-*p*-cymene (38 mg, yield 23%). The MS (EI) and ^{1}H NMR spectra were consistent with the literature values (*11*, *12*).

Model Reactions. A sample solution containing 10 mg/L of citral in an acidic buffer (0.1 M citric acid/0.2 M sodium hydrogen phosphate, pH 3.0) with or without 60 mg/L of an added antioxidant (**Table 2**) was prepared. One hundred milliliters of the prepared solution was transferred to a 100-mL glass bottle, and the sample bottle was sealed with a Teflon liner and a screw cap. The sample was stored in a dark incubator at 40 °C for 2 weeks. For studies of the metal-induced decomposition of 8-hydroperoxy-*p*-cymene, 1 mL of 5×10^{-2} M ferrous sulfate heptahydrate in water was added to 100 mL of the sample solution containing 2 mg/L of 8-hydroperoxy-*p*-cymene in the acidic buffer. The color of the solution immediately turned yellow. The sample bottle was shaken and then left at room temperature for 30 min.

Preparation of Analytical Samples. The degradation products of citral or those of 8-hydroperoxy-*p*-cymene were extracted with dichloromethane (30 mL × 2). One milliliter of 0.01% (w/v) *n*-pentadecane in dichloromethane was added to the extract as the internal standard. The extract was dried over sodium sulfate, concentrated in vacuo to ∼5 mL, and further concentrated under a stream of nitrogen to ∼200 μL.

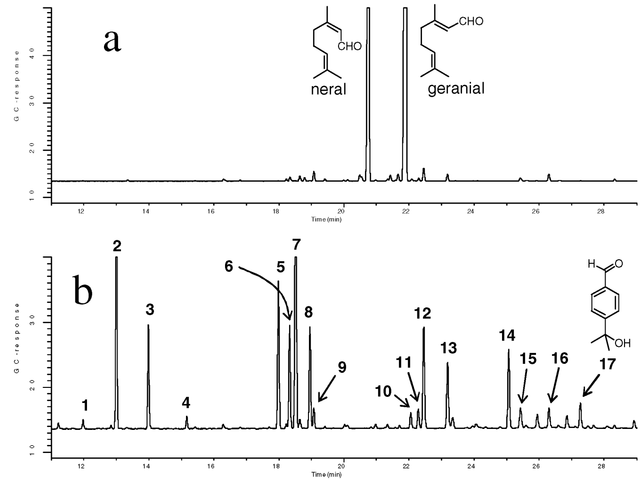


Figure 1. Gas chromatographic analysis of the acidic buffer solution (pH 3.0) of citral (10 mg/L) (**a**) before and (**b**) after storage at 40 °C for 2 weeks. Numbers correspond to those in **Table 1**.

Gas Chromatography (GC). An Agilent 6890 N gas chromatograph equipped with a flame ionization detector (FID) and a DB-1 fused silica capillary column (60 m × 0.25 mm i.d.; film thickness of 0.25 μm; J&W Scientific) was used. To minimize the decomposition of 8-hydroperoxy-*p*-cymene, an injector temperature of 100 °C was used. The other operating conditions were as follows: detector temperature, 250 °C; nitrogen carrier gas flow rate, 1 mL/min; oven temperature program, 80 °C, raised at 3 °C/min to 210 °C (15 min); 1 μL of sample was injected using a split ratio of 1:50. The amounts of the citral degradation products were estimated by computing the areas versus that of the internal standard (*n*-pentadecane). The response factors of all compounds to the FID were assumed to be the same.

Gas Chromatography−Mass Spectrometry (GC-MS). A Hewlett-Packard 5890 series II gas chromatograph equipped with an HP-5972 mass selective detector and a DB-1 fused silica capillary column (60 m × 0.25 mm i.d.; film thickness of 0.25 μm; J&W Scientific) was used. The operating conditions were as follows: injector temperature, 250 °C; helium carrier gas flow rate, 1 mL/min; oven temperature program, 60 °C, raised at 3 °C/min to 210 °C (40 min); 1 μL of sample was injected, using a split ratio of 1:50; ionization voltage, 70 eV; ion source temperature, 140 °C.

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker Avance 400 spectrometer (Bruker, Tsukuba, Japan) in $CDCl_3$ with TMS as internal standard (= 0 ppm).

RESULTS AND DISCUSSION

Degradation of Citral under Acidic Aqueous Conditions. During storage under acidic aqueous conditions, citral (neral and geranial) was completely degraded and almost totally converted to its cyclization products **1**−**17** (**Figure 1** and **Table 1**), among which 4-(2-hydroxy-2-propyl)benzaldehyde (**17**) was identified for the first time as a degradation product from citral. The structural estimation based on the MS spectra and subsequent synthesis according to the published method (*10*) led to the identification of **17**. The synthetic sample exhibited an odor character similar to that of benzaldehyde. Because of the weak odor, compound **17** was assumed not to contribute to the off-odors of the stored citral solution.

It has been accepted that compounds **1**, **2**, **5**, **8**, **12**, **13**, **15**, and **16** are formed from citral by a series of acid-catalyzed reactions (*1*−*5*, 8). In agreement with most published data,

Table 1. Degradation Products Detected in the Acidic Buffer Solution (pH 3.0) of Citral (10 mg/L) Stored at 40 °C for 2 Weeks

no.[a]	compound	RI[b]	ID[c] method
1	2,3-dehydro-1,8-cineole	985	B
2	*p*-cymene	1017	A
3	*p*-cresol	1044	A
4	α,*p*-dimethylstyrene	1077	A
5	*p*-mentha-1,5-dien-8-ol	1150	B
6	*p*-methylacetophenone	1158	A
7	*p*-cymen-8-ol	1163	A
8	*p*-mentha-1(7),2-dien-8-ol	1174	B
9	α-terpineol	1177	A
10	(2*R**,5*R**)-2-formylmethyl-2-methyl-5-(1-hydroxy-1-methylethyl)tetrahydrofuran	1249	C
11	(2*S**,5*R**)-2-formylmethyl-2-methyl-5-(1-hydroxy-1-methylethyl)tetrahydrofuran	1254	C
12	*trans*-*p*-menth-2-ene-1,8-diol	1259	B
13	*cis*-*p*-menth-2-ene-1,8-diol	1276	B
14	8-hydroperoxy-*p*-cymene	1321	A
15	*trans*-*p*-menth-1-ene-3,8-diol	1330	B
16	*cis*-*p*-menth-1-ene-3,8-diol	1351	B
17	4-(2-hydroxy-2-propyl)benzaldehyde	1365	A

[a] Numbers correspond to those in **Figure 1**. [b] Retention index on DB-1 (60 m). [c] Identification methods: A, mass spectrum and retention index agree with those of authentic compounds; B and C, compounds were tentatively identified on the basis of the following criteria: (B) mass spectrum agrees with that of the Wiley mass spectral database (Agilent Technologies, 2000) and RI agrees with literature value (*5*) or (C) mass spectrum agrees with literature spectrum (*13*).

p-menthadien-8-ols (**5** and **8**) and *p*-menth-2-ene-1,8-diols (**12** and **13**) were among the major acid-catalyzed reaction products in the citral solution. Unlike the studies conducted at room temperature (*1*, *2*, *5*), a dehydration product, *p*-cymene (**2**), was also one of the major products in our experiments, probably due to the relatively high temperature (40 °C) of our experimental conditions.

Oxidation reactions as well as the acid-catalyzed reactions would be involved in the formation of compounds **3**, **4**, **6**, **7**, **9**−**11**, **14**, and **17**. Compounds **10** and **11** were reported to be formed via the direct epoxidation of the 6,7-double bond of citral (*13*, *14*). Kimura et al., on the other hand, demonstrated the formation of α,*p*-dimethylstyrene (**4**), *p*-cymen-8-ol (**7**), and α-terpineol (**9**) using isolated *p*-menthadien-8-ols **5** and **8**, respectively, under acidic aqueous conditions (*4*). It was unclear, however, whether the other oxidation products, that is, *p*-cresol (**3**), *p*-methylacetophenone (**6**), 8-hydroperoxy-*p*-cymene (**14**), and the newly found **17**, would be formed via the major acid-catalyzed degradation products of citral, such as **5** and **8**, or would be directly formed from citral.

Figure 2 shows the concentration changes of citral (neral and geranial, **Figure 2a**), its major acid-catalyzed degradation products (**Figure 2b**), and the oxidation products (**Figure 2c**,**d**) in the citral solution during storage. Neral and geranial almost totally degraded during the first 3 days of storage (**Figure 2a**). The concentration of acid-catalyzed degradation products **5**, **8**, **12**, and **13** rapidly increased during this period (**Figure 2b**) and then started decreasing with almost the entire loss of citral, whereas the concentration of their dehydration product **2** kept increasing during the storage. On the other hand, the concentration of the oxidation products **3**, **6**, **7**, and **14** (**Figure 2c**) and **4** and **17** (**Figure 2d**) also kept increasing after almost the entire loss of citral, although compound **14** started to decrease after 7

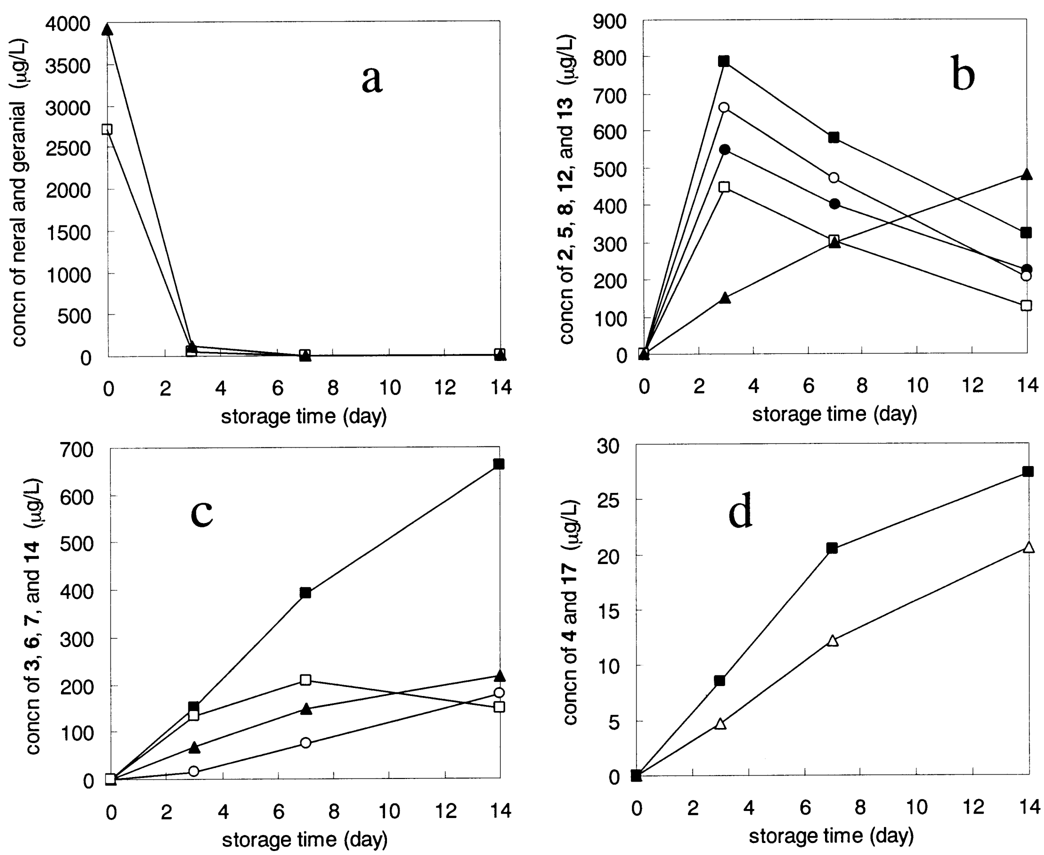


Figure 2. Concentration changes of (**a**) neral (□) and geranial (▲), of (**b**) acid-catalyzed degradation products **2** (▲), **5** (■), **8** (●), **12** (○), and **13** (□), of (**c**) oxidation products **3** (○), **6** (▲), **7** (■), and **14** (□), and of (**d**) oxidation products **4** (△) and **17** (■) during the storage of citral (10 mg/L) in an acidic buffer solution (pH 3.0) at 40 °C. Each point is the mean value of three experiments. Coefficient of variation for each point was <10%.

Table 2. Effects of Antioxidant Addition on the Formation of the Oxidation Products (**3**, **4**, **6**, **7**, **14**, and **17**) from Citral under Acidic Aqueous Conditions

antioxidant[b]	concn of compound[a] (μg/L)					
	3	4	6	7	14	17
control	197	24	209	677	171	29
(+)-catechin	170[c]	68	23[c]	1589	134	tr[d]
(−)-epicatechin (EC)	344	42	45	1018	278	3.2
(−)-epigallocatechin (EGC)	299	65	29	1402	263	tr
(−)-epicatechin gallate (ECg)	592	28	21	805	618	tr
(−)-epigallocatechin gallate (EGCg)	556	30	28[c]	835	565	tr
quercitrin	514	22	106	624	625	11
ascorbic acid	132	23	137	654	152	19

[a] Concentration was determined after the storage of the citral solution at 40 °C for 2 weeks. Each value is the mean of five experiments. Coefficient of variation for each value was <10% unless otherwise specified. [b] Sixty milligrams per liter of an antioxidant was added to an acidic buffer solution (0.1 M citric acid/0.2 M sodium hydrogen phosphate, pH 3.0) containing 10 mg/L of citral. [c] Coefficients of variation ranged from 10 to 20%. [d] Trace.

days of storage, probably due to its degradation to **3**, **4**, and **7** under the acidic aqueous conditions (*9*). These results indicate that the oxidation products **3**, **6**, **14**, and **17** as well as **4** and **7** would be formed not directly from citral but via the major acid-catalyzed cyclization products from citral.

Antioxidant Additions and the Resulting Formation Correlations among Oxidation Products from Citral. The use of antioxidants to inhibit the formation of off-odorants from citral in acidic solutions has already been attempted (*4*, *5*, *9*). It was reported (*5*) that the addition of isoascorbic acid to an acidic solution containing citral strongly inhibited the formation of α,*p*-dimethylstyrene (**4**) and *p*-cymen-8-ol (**7**). We previously reported that catechins from green tea showed strong inhibitory effects on the formation of *p*-methylacetophenone (**6**) from citral under acidic aqueous conditions (*9*). In the present study, information regarding such antioxidant additions was examined to investigate the formation pathways of oxidation products from citral.

Table 2 shows the effects of the added antioxidants (60 mg/L) including the catechins, quercitrin, and ascorbic acid on the formation of **3**, **4**, **6**, **7**, **14**, and **17** from citral under acidic aqueous conditions. Among these results, three pairs of oxidation products from citral, that is, **3** and **14**, **4** and **7**, and **6** and **17**, showed linear relationships between their concentrations (**Figure 3**). This means that the formation of the two oxidation products in each pair was influenced in the same manner by this series of antioxidant additions, reflecting the formation pathways of the oxidation products from citral. Therefore, it is not surprising that good correlations were obtained between the concentrations of **3** and **14** (correlation coefficient, $R = 0.975$) and between those of **4** and **7** ($R = 0.993$). These can be explained by their reported formation pathways, in which **3** is formed from **14** and **4** is formed from **7** by acid-catalyzed reactions (**Scheme 1**). It is more interesting to see a good correlation between the concentrations of **6** and **17** ($R = 0.995$, a trace amount of **17** was assumed to be zero), because this might suggest a possible connection between the potent off-odorant **6** and newly found product **17** during their formation from citral.

Fe^{2+}-Induced Decomposition of 8-Hydroperoxy-*p*-cymene (14). It is well-known that hydroperoxides can undergo homolytic O−O bond cleavage by accepting one electron from reducing metal ions such as ferrous ion (Fe^{2+}) to produce alkoxy radicals (*15*). In the case of the hydroperoxide **14**, the homolytic O−O bond cleavage would produce the corresponding *tert*-alkoxy radical [*p*-$CH_3C_6H_4C(CH_3)_2O^\bullet$], which can undergo further decomposition with the elimination of a methyl radical, that is, β-fragmentation (*16*), leading to the formation of *p*-methylacetophenone (**6**). To confirm the formation of **6** and other products resulting from the O−O bond homolysis of **14**, we conducted the Fe^{2+}-induced decomposition of **14** under acidic aqueous conditions. As shown in **Figure 4**, hydroperoxide **14** immediately degraded to **6** along with **7** and **17** by the addition of a large excess of ferrous sulfate ($FeSO_4$). The quantitative data for this experiment are given in **Table 3**.

As shown in **Scheme 2**, all of the products **6**, **7**, and **17** were interpreted to be formed via the *tert*-alkoxy radical **18** generated by the reduction of **14** with Fe^{2+}. *p*-Cymen-8-ol (**7**) could be formed by the further reduction of the alkoxy radical **18** by

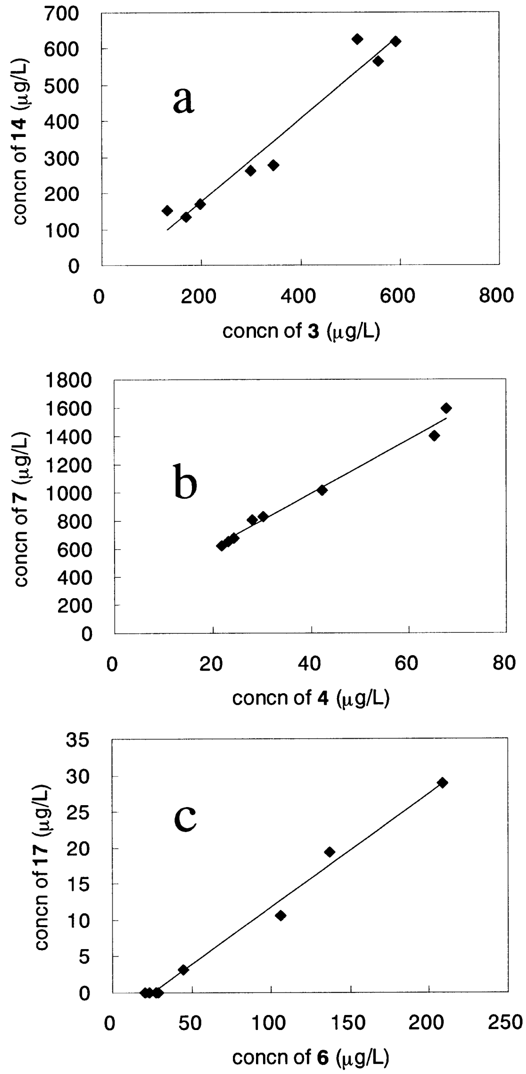


Figure 3. Linear relationships between the concentrations of (**a**) compounds **3** and **14**, (**b**) **4** and **7**, and (**c**) **6** and **17** in the citral solution stored with antioxidants (data from **Table 2**).

Table 3. Quantitative Data for the Fe^{2+}-Induced Decomposition of 8-Hydroperoxy-*p*-cymene (**14**) under Acidic Aqueous Conditions

sample[a]	concn of compound (μg/L)			
	6	7	14	17
before addition of Fe^{2+}	9	32	2085	nd[b]
after addition of Fe^{2+}	1378	273	nd	278

[a] $FeSO_4$ (5×10^{-4} M) was added to the acidic buffer solution (pH 3.0) of **14** (2 mg/L). [b] Not detected.

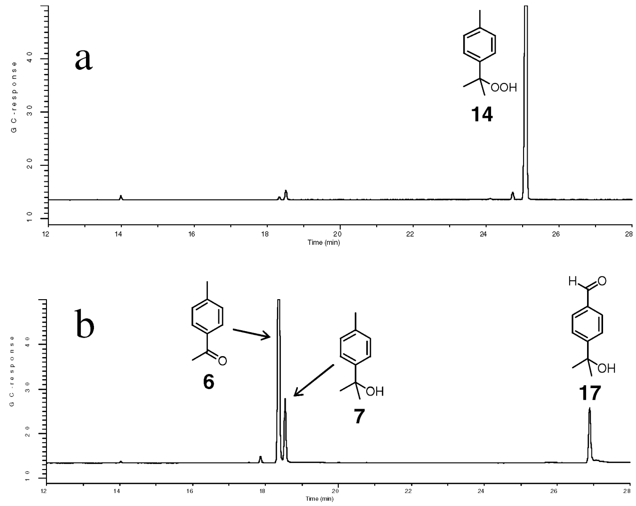
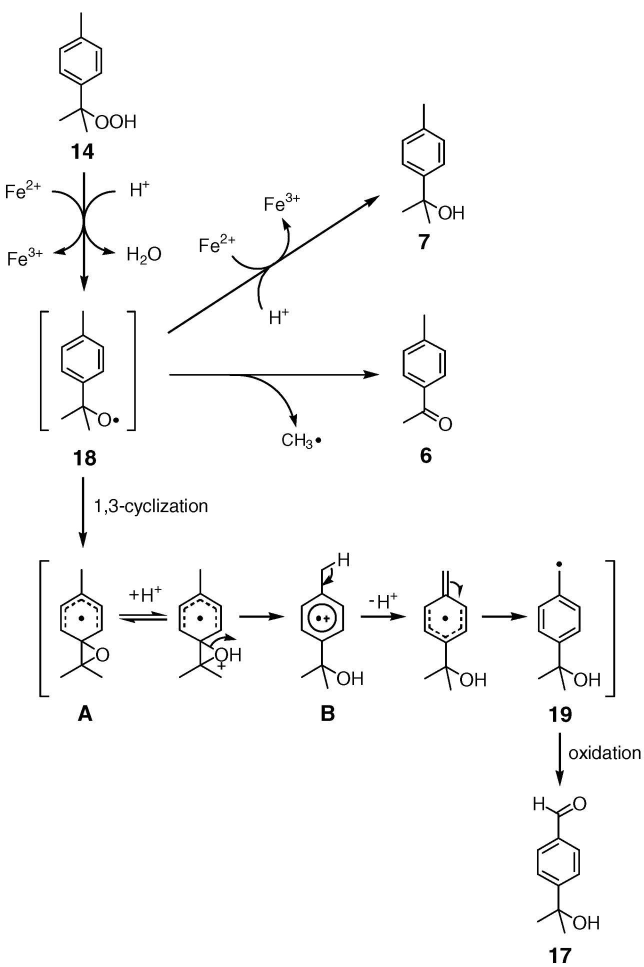


Figure 4. Gas chromatographic analysis of the acidic buffer solution (pH 3.0) of hydroperoxide **14** (2 mg/L) (**a**) before and (**b**) after the addition of Fe^{2+} (5×10^{-4} M).

Scheme 2. Possible Mechanism for the Fe^{2+}-Induced Decomposition of Hydroperoxide **14** under Acidic Aqueous Conditions



Fe^{2+} (*15*). Regarding the formation of **17** from **18**, the transformation of **18** to a benzylic radical **19** was postulated to be involved. A possible mechanism for this transformation is as follows. The alkoxy radical **18** could undergo 1,3-cyclization to form a cyclohexadienyl radical **A** (*17*), which might convert via the acid-catalyzed ring opening to an aryl radical cation **B**. The aryl radical cation **B** could typically undergo deprotonation at the benzylic position to form the benzylic radical **19** (*18*, *19*).

Table 4. Concentration of Degradation Products Obtained from the Acidic Buffer Solutions (pH 3.0) of 8-Hydroperoxy-*p*-cymene (**14**) (2 mg/L), Citral (10 mg/L), and Citral (10 mg/L) with **14** (2 mg/L) Stored at 40 °C for 2 Weeks

	concn of compound[a] (μg/L)					
sample	3	4	6	7	14	17
14	849[b]	25	17	685	127	nd[c]
citral	183	23[d]	224	678	153	27
citral with **14**	1017	48	317	1410	324	41

[a] Concentration was determined after the storage of the sample solutions at 40 °C for 2 weeks. Each value is the mean of three experiments. Coefficient of variation for each value was <10% unless otherwise specified. [b] Coefficient of variation was 13%. [c] Not detected. [d] Coefficient of variation was 23%.

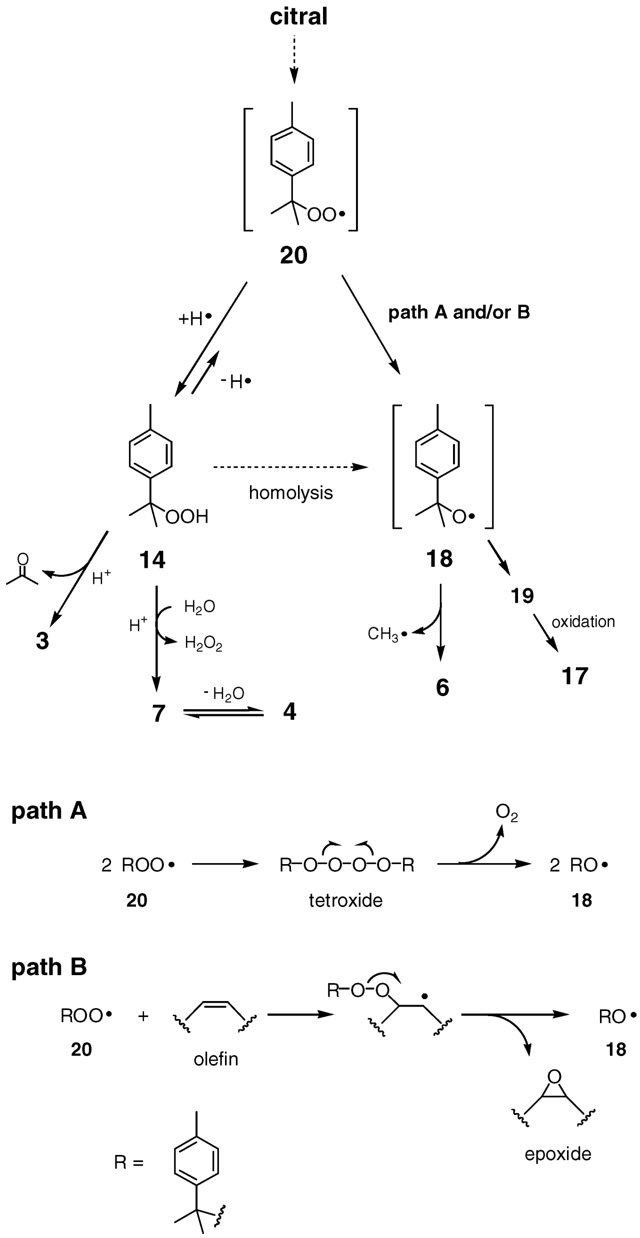
Another possibility for the formation of **19** might be H abstraction at the benzylic position of **7** with free radicals. However, we did not detect *p*-formylacetophenone, an oxidation product that could be obtained from H abstraction at the benzylic position of **6**. This observation suggested that H abstraction from **7** would also not occur. Therefore, we assumed that **19** would be formed from **18** as mentioned above.

Degradation of Hydroperoxide 14 under Acidic Aqueous Conditions without Fe^{2+}. In contrast to the Fe^{2+}-induced decomposition, there was little or no production of **6** and **17** during the storage of **14** under acidic aqueous conditions in the absence of Fe^{2+} (*9*). This result indicated that **14** without Fe^{2+} rarely undergoes the O−O bond homolysis under our experimental conditions. In this paper, we also report the degradation of **14** in the acidic aqueous solution of citral. **Table 4** shows quantitative data for the degradation products from **14** (2 mg/L), citral (10 mg/L), and citral (10 mg/L) with added **14** (2 mg/L) stored under acidic aqueous conditions. With the addition of **14** to the citral solution, the formation of **3** and **7** (and **4**) increased almost as much as expected from the data of the degradation of **14** by itself. The formation of **6** and **17** in the citral solution was also increased with the addition of **14**, but the increased amounts were much lower than those of **3** and **7**. These results suggested that the degradation of **14** would not be, at least, the main pathway for the formation of **6** and **17** in the citral solution.

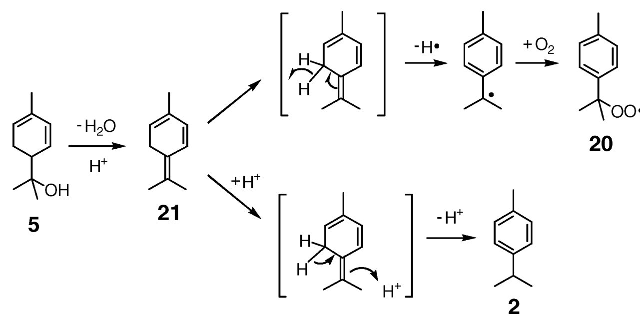
Formation Mechanisms of *p*-Methylacetophenone (6) and Compound 17 from Citral. On the basis of the presented data, we propose the formation mechanisms of **6** and **17** in the citral solution as shown in **Scheme 3**. Our results suggested that both **6** and **17** could be formed via the *tert*-alkoxy radical **18**, yet the decomposition of **14** would not be the main pathway for the formation of **6** and **17** in the citral solution. Therefore, it seems reasonable to assume that, other than the O−O bond homolysis of **14**, a mechanism to generate the radical **18** might be involved in the formation of **6** and **17** in the citral solution. In addition, the formation of **14** in the citral solution suggests the possible formation of the corresponding peroxy radical **20**. Therefore, we assumed that the radical **18** might be generated from the peroxy radical **20**, not via hydroperoxide **14**, in the citral solution.

It is generally accepted that alkoxy radicals can be formed directly from peroxy radicals, not via hydroperoxides (*20*−*25*). In the case of the formation of **18** from **20**, there seem to be two possible pathways: one is the bimolecular self-coupling of the peroxy radical **20** followed by the decomposition of the resulting tetroxide (*20*−*22*) (*path A*), and the other is the addition

Scheme 3. Possible Mechanism for the Formation of *p*-Methylacetophenone (**6**) and 4-(2-Hydroxy-2-propyl)benzaldehyde (**17**) from Citral under Acidic Aqueous Conditions



Scheme 4. Possible Mechanism for the Formation of Peroxy Radical **20** in the Acidic Solution of Citral



reaction of the peroxy radical **20** to olefins, such as *p*-menthadien-8-ols **5** and **8**, followed by the elimination of the alkoxy radical **18** (*23*–*25*) (*path B*).

Although further studies are needed for the elucidation of these mechanisms, the postulated formation of **18** from **20** can give a rational explanation for the formation of **6** and **17** from citral without Fe^{2+}. Also, on the basis of our proposed mechanism, the observed increase in **6** and **17** with the addition of **14** to the citral solution can be rationalized by the formation of **20** resulting from H abstraction from **14** with free radicals. This would probably be a minor reaction for **14**, which preferably undergoes acid-catalyzed degradation to **3** and **7** along with **4** under our experimental conditions.

A possible mechanism for the formation of the peroxy radical **20** in the citral solution is shown in **Scheme 4**. The oxidation of *p*-mentha-1,4(8),5-triene (**21**) was postulated to be involved in the mechanism for the following reasons. It appears that *p*-cymene (**2**) is formed from the major acid-catalyzed degradation products including *p*-menthadien-8-ols **5** and **8** (**Figure 2b**). This reaction might involve the acid-catalyzed dehydration of **5** and subsequent isomerization, during which **21** could be formed as an intermediate. Probably due to the difficulties in detection, the formation of **21** in the acidic citral solution has not been elucidated so far, but its possible formation as an intermediate during the formation of **2** has been postulated (*26*).

On the other hand, Andemichael et al. reported that **21** was rapidly oxidized to hydroperoxide **14** upon brief exposure to air (*27*). They also pointed out that the facile rate of oxidation of **21** can be reasonably attributed to the gain in aromaticity through a free radical chain mechanism, which suggests the formation of **20** as a radical intermediate. Although the oxidation of *p*-cymene (**2**) could also produce **20**, we observed that the addition of **2** (2 mg/L) to the citral (10 mg/L) solution did not affect the formation of any of the oxidation products **3**, **4**, **6**, **7**, **14**, and **17**.

Mechanisms of Antioxidant Effects. The observed antioxidant actions (**Table 2**) were interpreted on the basis of the proposed mechanism for the formation of oxidation products from citral. All of the tested catechins strongly inhibited the formation of both **6** and **17**. However, the formation of **3** and **14** was promoted by the catechins except for (+)-catechin, and the formation of **7** and **4** was also promoted by all of the tested catechins. Especially, the gallated catechins (ECg and EGCg) showed strong promoting effects on the formation of **3** and **14**, and the nongallated catechins [(+)-catechin, EC, and EGC] showed strong promoting effects on the formation of **7** and **4**.

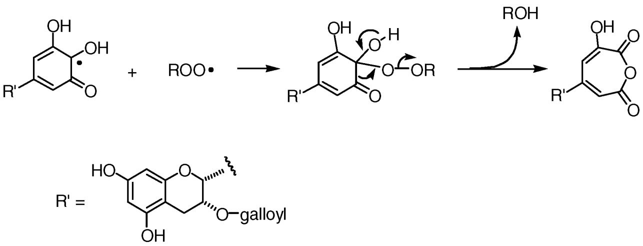
These promoting effects of the catechins on the formation of oxidation products from citral might be accounted for by the two different radical scavenging mechanisms against the peroxy radical **20**. It is generally accepted that phenolic antioxidants (ArOH) can scavenge two peroxy radicals ($ROO^•$) by means of H donation and the subsequent phenoxy–peroxy coupling reaction (eqs 1 and 2).

$$ROO^• + ArOH \rightarrow ROOH + ArO^• \quad (1)$$

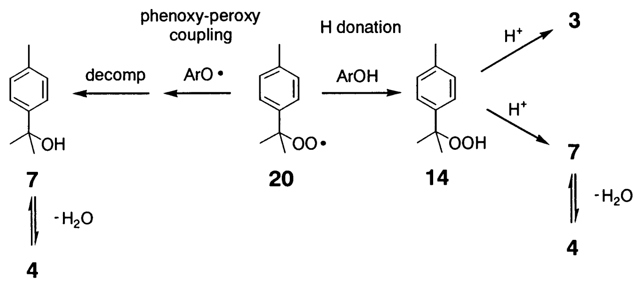
$$ROO^• + ArO^• \rightarrow \text{non-radical products} \quad (2)$$

Relatively stable coupling products were obtained when phenoxy radicals derived from 2,6-di-*tert*-butyl-4-methylphenol (*28*), α-tocopherol (*29*), and genistein (*30*) reacted with peroxy radicals. However, Valcic et al. (*31*) reported that phenoxy radicals derived from EGC and EGCg did not form stable coupling products with peroxy radicals and the decomposition of the unstable coupling products afforded the catechin oxidation products including the seven-membered B-ring anhydrides of EGC and EGCg (**Scheme 5**). In these processes, peroxy radicals ($ROO^•$) might be reduced to the corresponding alcohols (ROH) as suggested by their proposed mechanism.

Scheme 5. Coupling Reaction between the Phenoxy Radical Derived from EGCg and Peroxy Radicals (ROO•) Followed by the Decomposition of the Coupling Product (*31*)



Scheme 6. Possible Radical-Scavenging Mechanisms of Phenolic Antioxidants (ArOH) on the Formation of Oxidation Products from Citral



In the present case, as shown in **Scheme 6**, H donation from the catechins to the peroxy radical **20** can afford **14**, which subsequently degrades to **3** and **7** along with **4** under acidic aqueous conditions. On the other hand, coupling reactions between phenoxy radicals derived from the catechins and the peroxy radical **20** followed by the decomposition of the coupling products might produce **7** and **4**.

The observed promoting effects of the catechins seem to be the results of these competing reactions with the peroxy radical **20**. The phenoxy−peroxy coupling reactions that could afford **7** and **4** might be inhibited by the H donation to **20** from the catechins. Therefore, the H donor abilities of the catechins would contribute to the promotion of the formation of **3** and **14** rather than that of **7** and **4**. This might account for the strong promoting effects of the gallated catechins on the formation of **3** and **14**. The importance of the 3-galloyl moiety of the catechins on their H donor abilities has been established in the literature (*32*−*37*).

On the other hand, the phenoxy−peroxy coupling reactions might account for the strong promoting effects of the nongallated catechins on the formation of **7** and **4**. This assumption might also be supported by the work of Valcic et al. (*31*). They concluded that the B-ring rather than the galloyl moiety is the active site for the phenoxy−peroxy coupling reactions of the catechins, because no product resulting from the oxidation of the galloyl moiety was detected when EGCg reacted with peroxy radicals.

Both of the radical scavenging mechanisms of the catechins, that is, H donation and phenoxy−peroxy coupling reactions, against the peroxy radical **20** could contribute to the inhibition of the formation of **6** and **17** according to our proposed mechanism (**Scheme 3**). Further studies including structural elucidations for the resulting oxidation products of the catechins might provide some evidence to support the proposed mechanism for the radical scavenging actions of the catechins in the citral solution.

Quercitrin (quercetin 3-*O*-rhamnoside) showed strong promoting effects on the formation of **3** and **14**, as much as those of the gallated catechins, yet did not inhibit the formation of **6** and **17** as much as the catechins. This might be accounted for by the lack of a promoting effect of quercitrin on the formation of **7** and **4**. Because of the electron delocalization due to the 2,3-double bond of the C-ring of the quercetin moiety (*38*), phenoxy radicals derived from quercitrin might be less reactive to the peroxy radical **20** as compared to those derived from the catechins.

Ascorbic acid also did not inhibit the formation of **6** and **17** as much as the catechins, but the mechanism seems to be different from that of quercitrin. When we conducted the degradation of hydroperoxide **14** (2 mg/L) with added EGC, EGCg, and ascorbic acid (60 mg/L, respectively) under acidic aqueous conditions, only ascorbic acid induced the decomposition of **14** to **6** and **17**. This reduction of **14** by ascorbic acid might be accounted for by the low reduction potential of the ascorbate anion radical (*39*) compared to those of the catechin phenoxy radicals (*40*, *41*).

In conclusion, this study provided a specific insight into the formation mechanism of the potent off-odorant *p*-methylacetophenone (**6**) from citral under acidic aqueous conditions. Our data suggested that **6** is formed via the *tert*-alkoxy radical **18**, which is most likely derived from the corresponding peroxy radical **20**. Further studies including direct detection of the free radicals by electron spin resonance spectroscopy might provide unambiguous evidence to support our proposed mechanism for the formation of **6** from citral.

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