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Fragmentation of β-Hydroxy Hydroperoxides

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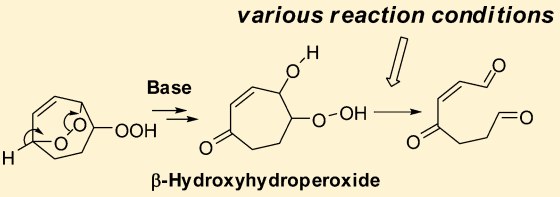
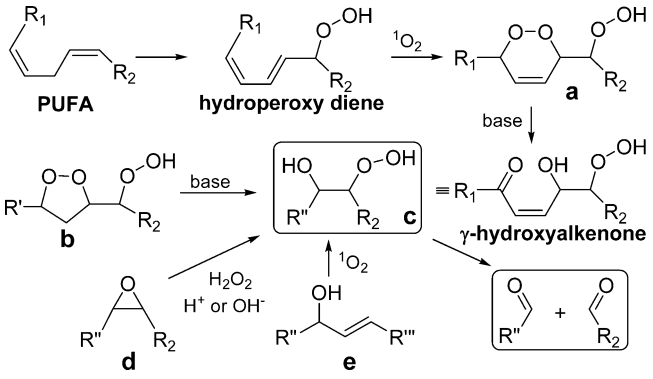
Supporting Information

ABSTRACT: A β-hydroxy hydroperoxide was obtained through base-catalyzed disproportionation of a hydroperoxy endoperoxide available by singlet oxygenation of cyclohepta-1,4-diene. Vitamins E and C induce fragmentation of this β-hydroxy hydroperoxide generating aldehydes, especially in the presence of redox active metal ions such as those present in vivo, e.g., under conditions of "iron overload". This chemistry may contribute to the oxidative cleavage of polyunsaturated fatty acyls that produces similar aldehydes, which damage proteins and DNA through covalent adduction resulting in "oxidative injury".



Oxidative cleavage of unsaturated fatty acyls produces aldehydes in vivo. Many of these aldehydes are toxic, causing "oxidative injury" owing to damage of proteins or DNA through covalent adduction. We postulated that β-hydroxy hydroperoxides (Scheme 1c) are one type of intermediate that

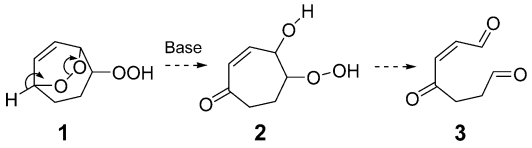
Scheme 1



may be susceptible to fragmentation under mild physiological conditions. Possible routes to β-hydroxy hydroperoxides include Kornblum–DeLaMare rearrangement[1] of β-hydroperoxy alkylperoxides (Scheme 1a,b). Singlet oxygenation of conjugated hydroperoxy dienes generates the requisite endoperoxides (Scheme 1a).[2,3] Hydroperoxy dienes, which are major[4] products of both enzymatic and free radical-promoted oxidation of polyunsaturated fatty acyls (PUFAs), accumulate in atherosclerotic lesions.[5] Singlet oxygen can be generated in vivo through photosensitization[6] by retinal[7] in the eye as well as nonphotochemically.[8−10] β-Hydroperoxy alkylperoxides (Scheme 1b) are also produced by free radical cyclization of unsaturated peroxy radicals[11−13] formed during the autoxidation of PUFAs.[14,15] β-Hydroxy hydroperoxides (Scheme 1c) are also available through addition of H_2O_2 to epoxides (Scheme 1d)[16−18] or through singlet oxygenation of allylic alcohols (Scheme 1e).[19,20]

We chose the hydroperoxy endoperoxide **1** (Scheme 2) as a simple model system that would deliver a single product **3** by

Scheme 2



fragmentation of an intermediate β-hydroxy hydroperoxide **2**. Notably, the electrophilic conjugated enedione functionality present in **3** is also found in certain, especially toxic, lipid oxidative fragmentation products, e.g., 4-oxo-2-nonenal, that damage proteins[21] and DNA[22] through covalent adduction.

The hydroperoxy endoperoxide **1** is readily available through singlet oxygenation of cyclohepta-1,4-diene.[3] Reaction conditions were defined that optimized the conversion of **1** into **2**, an analogue of the PUFA-derived γ-hydroxyalkenone of Scheme 1. This model β-hydroxy hydroperoxide was exploited to define reaction conditions that promote fragmentation.

Disproportionation of the endoperoxide **1** was expected to produce β- and γ-hydroxyhydroperoxides **2** and **5** (Scheme 3). However, attempted use of triethylamine to catalyze[23] the isomerization of endoperoxide **1** delivered a ketodiol **4** and a cyclic hydroxydione hemiacetal **7**, in isolated yields of 50 and 14%, respectively, and none of the desired β-hydroxy hydroperoxide **2**. The production of **7** undoubtedly involves dehydration of the α-hydroperoxy ketone array in **5**. Reduction of **2** by triethylamine presumably accounts for the generation of **4**. It is known that amines can reduce hydroperoxides to alcohols.[24] For example, *tert*-butyl hydroperoxide was reduced by tri-*n*-propylamine to give alcohol in good yield (~ 80%) and products generated through fragmentation of tripropylamine,

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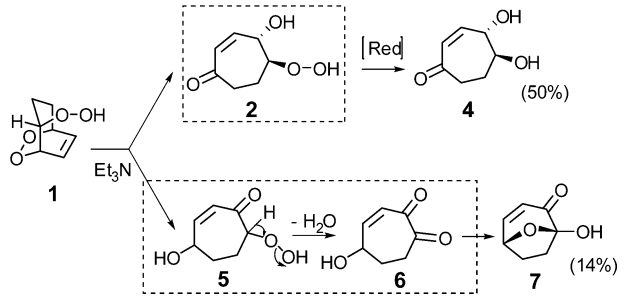
Scheme 3

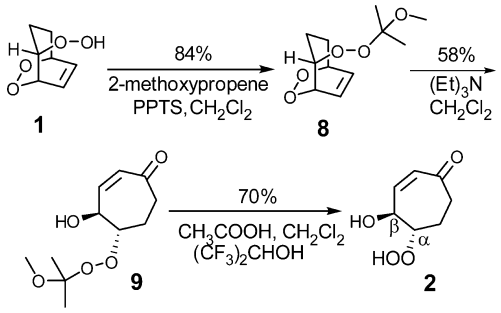


which included di-*n*-propylamine (32%) and carbonyl compounds (mainly 2-methyl-2-pentenal, 17%).

Other bases were also tested as catalysts for disproportionation. Proton sponge (*N*,*N*,*N*′,*N*′-tetramethyl-naphthalene-1,8-diamine), DABCO (1,4-diaza-bicyclo-[2.2.2]-octane), or pyridine were added to solutions of **1** in $CDCl_3$, and the reactions were monitored by NMR. Endoperoxide **1** is stable in the presence of pyridine. The main product generated through catalysis by proton sponge or DABCO is **7** (Scheme 3).

In an alternative route to **2** (Scheme 4), hydroperoxy endoperoxide **1** was first protected by treatment with 2-

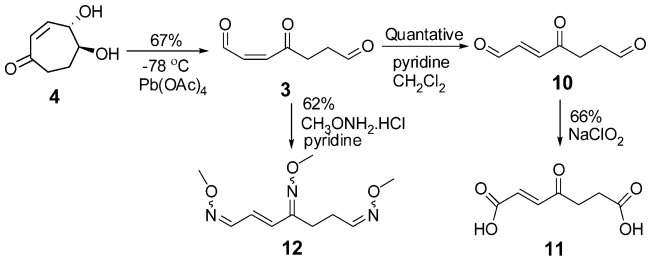
Scheme 4



methoxypropene to give **8**.[25] In contrast with **1**, triethylamine-catalyzed isomerization of the methoxyisopropyl derivative **8** of hydroperoxide **1** delivered **9** in 58% yield together with **7** in 13% yield. Deprotection of **9** under mild acidic conditions delivered the requisite *β*-hydroxy hydroperoxide **2** in 70% yield. Acetic acid was used to catalyze the deprotection of **9** in a mixed solvent system of hexafluoro-2-propanol and methylene chloride. The isolation of **2** was facilitated by the ease with which acetic acid can be removed by rotary evaporation into a dry ice-cooled trap under high vacuum. This simple route provided ready access to the *β*-hydroxy hydroperoxide **2** for the proposed fragmentation reaction studies. The contrasting behavior of the hydroperoxide **1** and 2-methoxypropyl derivative **8** is especially striking. It provides a useful protection strategy. The discovery that such derivatives are not reduced by amines, in contrast with the hydroperoxide precursor, is valuable information for those engaged in synthetic manipulation of hydroperoxides.

cis-Aldehyde **3**, the expected product from the fragmentation of *β*-hydroxy hydroperoxide **2**, was synthesized through the oxidative cleavage of diol **4** by lead tetraacetate (Scheme 5).[26] *trans*-Aldehyde **10** was readily obtained by treatment of **3** with pyridine.[27] *trans*-Aldehyde **10** can be selectively oxidized to 4-oxo-2-heptenedioic acid **11**, a known compound.[28] The *cis*-aldehyde **3** reacts with methoxyamine hydrochloride to form an

Scheme 5



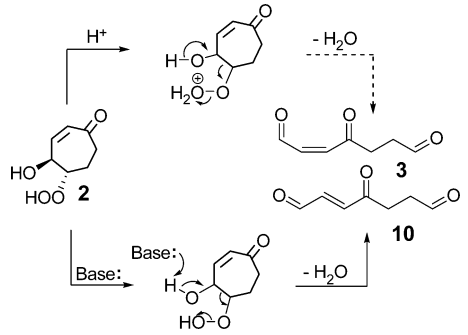
oxime derivative **12**,[29] which is stable and whose structure was further confirmed by mass spectroscopy. Thus, the structures of *cis*- and *trans*-aldehydes **3** and **10** were unambiguously confirmed.

Previous studies showed that *β*-hydroxy hydroperoxides are subject to acid-[16,17] or base-catalyzed[18,30] fragmentation to carbonyl compounds. We examined the ability of amine bases or carboxylic acids (Table 1) to catalyze the fragmentation of **2**

Table 1. Acid and Base Promoted Decomposition of 2

		unreacted (%)	product yields (%)		
catalyst (in CH_2Cl_2)	reaction time	**2**	**3**	**10**	**4**
pyridine	12 h			38	16
Et_3N	5 h				57
CF_3COOH	12 h	30	1		

Scheme 6



(Scheme 6). In the presence of pyridine, the *β*-hydroxy hydroperoxide **2** readily fragmented in CH_2Cl_2 solution to afford *trans*-aldehyde **10** (38%) and the reduction product **4** (16%). Treatment of **2** with triethylamine gave only diol **4** (57%) and no fragmentation products. The result supports the involvement of **2** in the mechanism postulated above for the conversion of **1** into **4** upon treatment with triethylamine and further confirms the fact that **2** can be easily reduced. *β*-Hydroxy hydroperoxide **2** was partly decomposed when it was incubated in trifluoroacetic acid overnight; however, the yield of aldehyde is very low.

Although the *β*-hydroxy hydroperoxide **2** is stable in $CHCl_3$ or CH_2Cl_2 solutions, it decomposed in D_2O upon incubating at 37 °C for 24 h to form aldehyde **3** (as a hydrate in water) and diol **4** (Table 2). It is tempting to speculate that the effect of a strongly polar protic solvent indicates a polar mechanism for the decomposition in water. However, hydroperoxides are known to undergo transition-metal-ion-mediated decomposi-

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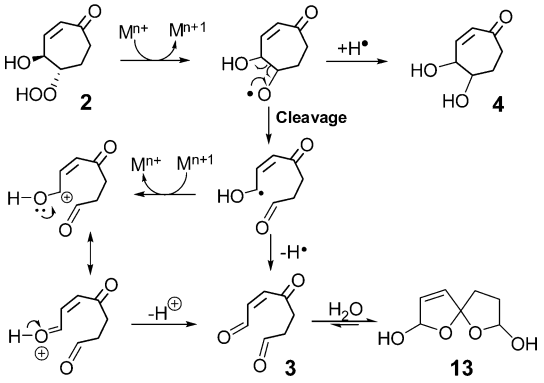
Table 2. Metal-Ion-Promoted Decomposition of 2[a]

catalyst (in D_2O)	reaction time	unreacted 2	product yields (%) 3[b]	4
	24 h		43	11
PBS buffer (200 mM)	0.5 h		15	30
Fe^{3+} (3.6 mM)	0.5 h		70	5
Cu^{2+} (3.6 mM)	0.5 h		80	9

[a]36 mM at 37 °C. [b]As the hydrate **13**.

tion through single electron transfer, leading to the formation of an alkoxy radical.[31,32] Possibly, there are traces of metal ions in water that are sufficient to promote the fragmentation (vide infra). The formation of diol **4** from the β-hydroxy hydroperoxide **2** may also involve homolytic cleavage of the hydroperoxide, promoted by trace metal ions, that generates an alkoxy radical that abstracts hydrogen or an alkoxide that abstracts a proton. Alternatively, β-scission of the alkoxy radical can result in the formation of *cis*-aldehyde **3** (Scheme 7). In aqueous solution, water adds to an aldehyde carbonyl in **3** to form a hydrate **13**.

Scheme 7



Decomposition of **2** was much faster in phosphate-buffered saline or in the presence of added metal ions (Cu^{2+} or Fe^{3+} 10 mol %) than in "pure" D_2O. Incubation of **2** with Cu^{2+} or Fe^{3+} (0.1 equiv) in aqueous solution at 37 °C for 0.5 h delivered aldehyde hydrate (~70% yield) and diol **4** (5%). Although pure *cis*-aldehdye **3** was not readily extracted from the aqueous solution even under acidic conditions, the aldehyde hydrate reacts with methoxyamine hydrochloride to form oxime derivatives, which were readily isolated and characterized.

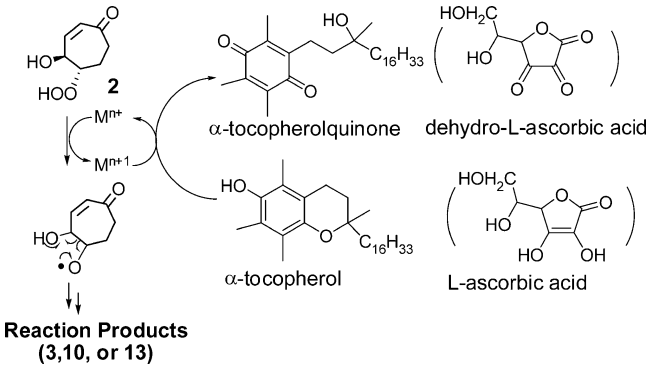
Vitamins E (α-tocopherol) and C (L-ascorbic acid) are generally considered to protect biological membranes from lipid peroxidation because of their ability to scavenge reactive oxygen species including peroxy radicals derived from unsaturated lipids.[33−35] Vitamins E and C efficiently transfer a hydrogen atom to a peroxyl radical, and hence, break the chain reaction of lipid peroxidation. However, their potential to serve as pro-oxidants rather than antioxidants in the presence of metal ions has also been recognized.[35,36] Ascorbic acid is known to accelerate the decomposition of lipid hydroperoxides by reducing Fe^{3+} to Fe^{2+}, which reduces the hydroperoxy group to generate alkoxy radicals and hydroxide.[37] We tested the effects of vitamins E and C on the decomposition of β-hydroxy hydroperoxide **2** (Table 3). Both vitamins E and C promoted the fragmentation of **2** in the presence of metal ions (Fe^{2+}, Cu^{2+}). When reacted at room temperature for 4 h, β-hydroxy hydroperoxide **2** with only vitamin E or catalytic amounts (0.1 equiv) of metal ions (Fe^{2+}, Cu^{2+}) gave very low yields (<5%) of fragmentation product (**3** or **10**), and most of **2** was unreacted under these conditions. But when both vitamin E and metal ions were added, the reaction was accelerated. Decomposition of **2** was complete in 4 h. Aldehydes **3** or **10** were produced in about 40% yield. A possible mechanism for this effect is presented in Scheme 8. Reduced metal ions induce the

Table 3. Vitamin E- and C-Promoted Fragmentation of 2

reagents (slovent)[a]	reaction time, temperature	unreacted (%) 2	product yields (%) 3[b]	10	4
Vit E (CH_3CN)	4 h, 25 °C	96	4		
Vit E, Fe^{2+}(CH_3CN)	4 h, 25 °C		26	14	
Vit E, Cu^{2+} (CH_3CN)	4 h, 25 °C		36		
Fe^{2+} (CH_3CN)	4 h, 25 °C	99	1		
Cu_2+ (CH_3CN)	4 h, 25 °C	98	2		
Vit C (D_2O)	3 h, 37 °C		35		13
Vit C, Fe^{3+} (D_2O)	5 min, 25 °C		65		15
Vit C, Cu^{2+} (D_2O)	5 min, 25 °C		66		13

[a]The concentration of vitamin E (or C)/**2**/metal ions = 1.5:1:0.1. [b]As the aldehyde hydrate **13**.

Scheme 8



homolysis of the β-hydroxy hydroperoxide **2** to form an alkoxy radical,[38] which undergoes β-scission to generate aldehydes. Vitamin E (α-tocopherol) is known to donate one electron to regenerate Fe^{2+} from Fe^{3+} and Cu^{1+} from Cu^{2+} and thus can promote the homolysis. The oxidation product of vitamin E is α-tocopheroquinone (α-TQ), which was also detected by NMR in the reaction product mixture. It is interesting that diol **4**, the product of hydroperoxide reduction, was not detected in this reaction of 2. Apparently β-scission of the putative alkoxy radical intermediate is much faster than the abstraction of a hydrogen atom to form diol **4**, even in an environment where a potential hydrogen atom donor (vitamin E) is abundant. This is a reasonable consequence of the stabilization of the radical produced upon fragmentation by conjugation with both a C=C bond and an α-oxygen. It also may reflect suppression of hydrogen atom transfer of the phenolic hydrogen owing to hydrogen bonding with the relatively strong hydrogen-bond-acceptor solvent CH_3CN.[39] Vitamin C also accelerated the fragmentation of **2** in water even without added redox active metal ions.[40] In a D_2O solution containing both vitamin C and

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Cu^{2+} or Fe^{3+}, the β-hydroxy hydroperoxide **2** was completely decomposed in 5 min. The main product was the aldehyde hydrate (~60% yield). These results clearly demonstrate the ability of vitamins E and C to promote the formation of toxic aldehydes from β-hydroxy hydroperoxides.

In conclusion, a β-hydroxy hydroperoxide, synthesized through base-catalyzed disproportionation of a hydroperoxy endoperoxide, undergoes acid- as well as base-promoted fragmentations. Under neutral aqueous biomimetic conditions, fragmentation can be induced by redox active metal ions. Vitamins C and E strongly *promote* fragmentation of β-hydroxy hydroperoxides by reducing catalytic metal ions. This chemistry may explain why vitamins E and C exhibit disappointingly low efficacy in some disease therapies that attempt to prevent oxidative injury mediated by toxic aldehydes generated through oxidative fragmentation of PUFAs. Thus, although these antioxidants may decrease the free-radical-induced production of intermediates such as hydroperoxy endoperoxides and β-hydroxy hydroperoxides, they can promote, especially in the presence of redox-active metal ions, the fragmentation reactions that convert such intermediates into toxic aldehydes, which damage proteins and DNA. Further studies to evaluate the biological significance of the fragmentation of β-hydroxy hydroperoxides, demonstrated in the present investigation, should focus on detecting lipid-derived β-hydroxy hydroperoxides (Scheme 1c) and their putative β-hydroperoxy alkylperoxide precursors (Scheme 1a,b) in vivo.

EXPERIMENTAL SECTION

Triethylamine-Catalyzed Isomerization of Exo-6,7-dioxabicyclo[3.2.2]non-8-en-2-yl-hydroperoxide (1). Endoperoxide **1** (100 mg, 0.64 mmol) was dissolved in dry CH_2Cl_2 (10 mL), and the solution was cooled with an ice bath. To this solution was added Et_3N (178 μL, 1.28 mmol) dropwise over 20 min. The mixture was stirred for 1 h at 0 °C and then 12 h at room temperature. Then the solution was neutralized with 1.0 M aqueous HCl. The organic layer was removed, and the water layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layer was washed with water and dried with $MgSO_4$. The crude product was chromatographed on silica gel by elution with ethyl acetate–hexanes (8:2) to afford 4,5-dihydroxy-cyclohept-2-enone (**4**) (46.1 mg, 0.32 mmol, yield = 50%, R_f = 0.20) and 1-hydroxy-8-oxa-bicyclo[3.2.1]oct-3-en-2-one (**7**) (12.5 mg, 0.09 mmol, yield = 14%, R_f = 0.51).

4,5-Dihydroxy-cyclohept-2-enone (4). Spectral data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (dd, J = 12.8, 3.2 Hz, 1H), 5.92 (ddd, J = 12.8, 2.6, 1.0 Hz, 1H), 4.44 (dt, J = 8.8, 2.6 Hz, 1H), 3.84 (ddd, J = 8.8, 6.4, 5.0 Hz, 1H), 3.12 (s, 1H), 2.85 (s, 1H), 2.75–2.61 (1H), 2.62–2.51 (1H), 2.20–2.10 (1H), 2.05–1.95 (1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1, 146.3, 130.3, 75.0, 73.8, 38.8, 28.5; HRMS (FAB) m/z calcd for $C_7H_{11}O_3$ (MH^+), 143.0708, found 143.0715.

1-Hydroxy-8-oxa-bicyclo[3.2.1]oct-3-en-2-one (7). Spectral data: ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (dd, J = 9.6, 4.4 Hz, 1H), 5.99 (d, J = 9.6 Hz, 1H), 4.91 (dd, J = 6.8, 4.4 Hz, 1H), 2.40–2.20 (1H), 2.05–1.95 (2H), 1.80–1.70 (1H); ^{13}C NMR (100 MHz, CD_3OD) δ 196.5, 154.8, 125.1, 104.6, 74.8, 29.6, 26.9.

2-(1-Methoxy-1-methyl-ethylperoxy)-6,7-dioxabicyclo-[3.2.2]-non-8-ene (8). Endoperoxide **1** (150 mg, 0.95 mmol) and pyridinium p-toluene sulfonate (15 mg) were dissolved in dry CH_2Cl_2 (15 mL) under argon. 2-Methoxypropene (200 μL, 2.09 mmol) was added dropwise. The resulting solution was stirred for 10 min at room temperature. After removal the solvent by rotary evaporation, the crude product was chromatographed on silica gel by elution with ethyl acetate–hexanes (3:7) to afford 2-(1-methoxy-1-methyl-ethylperoxy)-6,7-dioxabicyclo[3.2.2]non-8-ene (**8**) (184 mg, 0.80 mmol, yield = 84%, R_f = 0.28): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.42–6.30 (2H), 5.05–4.95 (1H), 4.70–4.60 (1H), 4.24 (ddd, J = 11.4, 5.0, 2.0 Hz, 1H), 3.22 (s, 3H), 1.90–1.70 (3H), 1.30 (d, J = 2.4 Hz, 6H), 1.30–1.10 (1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.0, 126.4, 105.0, 84.6, 76.6, 76.5, 49.4, 26.0, 23.0, 22.6, 22.0.

4-Hydroxy-5-(1-methoxy-1-methyl-ethylperoxy)-cyclohept-2-enone (9). An approximately 0.2 M solution of protected endoperoxide **8** (216 mg, 0.94 mmol) in dry CH_2Cl_2 was cooled with an ice bath. To this solution was added Et_3N (262 μL, 1.88 mmol, 2 equiv) dropwise over 20 min. The mixture was stirred at 0 °C for 3 h. Then the solution was neutralized with 1 M aqueous HCl. The organic layer was removed, and the water layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layer was dried with $MgSO_4$. The crude product was chromatographed on silica gel by elution with ethyl acetate–hexanes (4.5:5.5) to recover starting material **8** (93 mg, 0.40 mmol) and afford 4-hydroxy-5-(1-methoxy-1-methyl-ethylperoxy)-cyclohept-2-enone (**9**) (72 mg, 0.31 mmol, yield = 58%, R_f = 0.24) and **7** (10 mg, 0.07 mmol, yield = 13%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 (ddd, J = 12.4, 3.2, 0.8 Hz, 1H), 5.91 (ddd, J = 12.4, 2.8, 1.2 Hz, 1H), 4.82–4.70 (1H), 4.23–4.15 (1H), 3.62 (s, 1H), 3.30 (s, 3H), 2.70–2.50 (2H), 2.25–2.15 (1H), 2.15–2.00 (1H), 1.40 (d, J = 2.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.2, 146.5, 129.9, 105.8, 86.0, 71.6, 50.1, 38.6, 24.5, 23.1, 22.8.

5-Hydroperoxy-4-hydroxy-cyclohept-2-enone (2). The protected hydroperoxide **9** (180 mg, 0.78 mmol) was dissolved in a mixture of CH_2Cl_2 (3 mL) and hexfluoro-2-propanol (1 mL). Acetic acid (25 μL) was added to the reaction mixture as catalyst. The solution was stirred for 4 h under an argon atmosphere. After removal of the solvents by rotary evaporation, the crude product was separated on a silica gel column eluting with ethyl acetate–hexanes (1:1) to afford 5-hydroperoxy-4-hydroxy-cyclohept-2-enone (**2**) (52 mg, 0.33 mmol, yield = 70%, R_f = 0.14): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 6.54 (dd, J = 12.4, 3.2 Hz, 1H), 5.97 (ddd, J = 12.4, 2.8, 1.6 Hz, 1H), 4.86 (dm, J = 8.0 Hz, 1H), 4.30–4.20 (1H), 2.91 (s, 1H), 2.80–2.60 (2H), 2.30–2.00 (2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.0, 146.3, 130.1, 87.0, 71.8, 38.6, 24.0. This compound was further characterized by converting to the diol **4**: HRMS (FAB for diol **4**) m/z calcd for $C_7H_{11}O_3$ (MH^+), 143.0708, found 143.0715.

Oxidative Cleavage of 4,5-Dihydroxy-cyclohepta-2-enone (4). Diol **4** (9.5 mg, 0.067 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and was cooled with a dry ice–acetone bath (−78 °C). $Pb(OAc)_4$ (22 mg, 0.050 mmol) was also dissolved in dry CH_2Cl_2 (1 mL), and the solution was cooled to −78 °C. Then the $Pb(OAc)_4$ solution was added dropwise through a cannula to the solution of diol **4**. The reaction mixture was stirred for 30 min at −78 °C under an argon atmosphere. Then the solvent was removed, and the residue was purified by flash chromatography on a silica gel column (ethyl acetate/hexanes, 8:2) to give *cis*-4-oxo-hept-2-enedial (**3**) (4.8 mg, 0.034 mmol, yield = 67%, R_f = 0.40). The *cis*-aldehyde **3** was dissolved in CH_2Cl_2 (1 mL) with 50 μL of pyridine. The mixture was stirred overnight at room temperature. The solvent was removed to deliver the pure *trans*-4-oxo-hept-2-enedial (**10**).

***cis*-4-Oxo-hept-2-enedial (3).** Spectral data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.19 (d, J = 7.2 Hz, 1H), 9.84 (s, 1H), 6.99 (d, J = 11.6 Hz, 1H), 6.22 (dd, J = 11.6, 7.2 Hz, 1H), 2.80–3.00 (4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.8, 198.4, 192.7, 139.7, 138.5, 37.7, 35.7. This compound was further characterized by conversion into the methoxime **12**.

***trans*-4-Oxo-hept-2-enedial (10).** Spectral data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 9.80 (d, J = 7.2 Hz, 1H), 6.95–6.80 (2H), 3.10–3.00 (2H), 3.00–2.90 (2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.8, 197.8, 193.3, 144.4, 138.0, 37.5, 33.4. This compound was further characterized by conversion into the known acid **11**.

Synthesis of 4-Oxo-hept-2-enedioic acid (11). To a magnetically stirred solution of aldehyde **10** (10.0 mg, 0.07 mmol) in *t*-BuOH–H_2O (5:1, v/v, 1.5 mL) was added a solution containing $NaH_2PO_4{\cdot}H_2O$ (29 mg, 0.21 mmol), 2-methyl-2-butene (400 μL, 0.8 mmol, 2 M solution in THF), and $NaClO_2$ (40 mg, 0.42 mmol) in *t*-BuOH–H_2O (5:1, v/v, 1.5 mL). The resulting mixture was stirred for 2 h at room temperature under argon. Then the pH of the solution was adjusted to 3.0 by adding 1 M aqueous HCl. The mixture was extracted with CH_2Cl_2/CH_3OH (3:1) (3 × 15 mL). The combined organic layer was dried with $MgSO_4$. After removal of the solvents by

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rotary evaporation, 4-oxo-hept-2-enedioic acid (**11**, 8.0 mg, 0.046 mmol, yield = 66%, R_f = 0.25) was purified by through a silica gel column eluting with CH_2Cl_2/CH_3OH (3:1).

4-Oxo-hept-2-enedioic acid (11). Spectral data: ^{1}H NMR (400 MHz, CD_3OD) δ 7.02 (d, J = 16.0 Hz, 1H), 6.70 (d, J = 16.0 Hz, 1H), 2.98 (t, J = 6.4 Hz, 2H), 2.60 (t, J = 6.4 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 199.1, 175.0, 167.6, 139.0, 131.7, 35.4, 27.3. These spectra agree with those reported previously.[41]

Preparation of Oxime Derivatives of *cis*-4-Oxo-hept-2-enedial (3). A solution of aldehyde **3** (4.2 mg, 0.03 mmol) and methoxyamine hydrochloride (45 mg, 0.54 mmol) in anhydrous pyridine (500 μL) was stirred at room temperature for 15 h. Pyridine was then removed with a steam of argon. Then water (1 mL) was added to the solid residue. The aqueous layer was extracted with ethyl acetate (3 × 5 mL), and the combined organic extracts were dried with $MgSO_4$. The crude product was chromatographed on silica gel with 20% ethyl acetate in hexanes to afford 4-methoxyimino-hept-2-enedial bis-(*O*-methyl-oxime) (**12**) (4.2 mg, 0.018 mmol, yield = 62%, R_f = 0.38), which is mixture of eight isomers.

4-Methoxyimino-hept-2-enedial Bis-(*O*-methyl-oxime) (12). Spectral data: HRMS (FAB) m/z calcd for $C_{10}H_{18}N_3O_3$ (MH^+), 228.1348, found 228.1346; m/z calcd for $C_{10}H_{17}N_3O_3$ (M^+)−OCH, 198.1243, found 198.1227. See the Supporting Information for the ^{1}H NMR spectrum of **12**.

Acid- or Base-Catalyzed Decomposition of 5-Hydroperoxy-4-hydroxy-cyclohept-2-enone (2). β-Hydroxyhydroperoxide **2** (4 mg, 0.025 mmol) was dissolved in CH_2Cl_2 (1 mL). Then pyridine (100 μL), triethylamine (50 μL), CF_3COOH (50 μL), or HCl (20 μL, 37% in water) was added to catalyze the decomposition. The reaction mixtures were vortexed and then incubated at 37 °C under argon for various times. Then the solvents were removed with a steam of nitrogen. The residues were dissolved into 0.7 mL of $CDCl_3$ (with 0.01 M phenyltrimethylsilane as internal standard) and transferred into NMR tubes. From the ratio of the integral areas of NMR signals belonging to decomposition products **3**, **4**, **10**, and **13** to that for the internal standard (phenyltrimethylsilane), the yields of **3**, **4**, **10**, and **4** were determined.

Decomposition of 5-Hydroperoxy-4-hydroxy-cyclohept-2-enone (2) in Deuterium Oxide Solutions. *cis*-4-Oxo-hept-2-enedial (**3**, 3 mg, 0.021 mmol) was dissolved in D_2O (0.7 mL) and transferred into an NMR tube. The tube was mixed thoroughly by shaking, and the ^{1}H NMR spectrum was immediately recorded. The aldehyde peaks had totally disappeared, and the peaks belonging to aldehyde hydrates (δ 5.40−5.80) appeared. It is difficult to extract the aldehyde from aqueous solution even under acidic conditions. Methoxyamine hydrochloride (45 mg, 0.54 mmol) was added to the aqueous solution, and the resulting mixture was stirred overnight to deliver the methoxime derivative: HRMS (FAB) m/z calcd for $C_{10}H_{18}N_3O_3$ (MH^+), 228.1348, found 228.1348.

β-Hydroxyhydroperoxide **2** (4 mg, 0.025 mmol) was dissolved into D_2O (0.7 mL), phosphate-buffered saline (pH 7.4, 200 mM in D_2O), Cu^{2+} (3.6 mM $CuSO_4$ in D_2O), and Fe^{3+} (3.6 mM $FeCl_3$ in D_2O) solutions. *tert*-Butyl alcohol (5 μL) was added as an internal standard. The solutions were transferred into NMR tubes and incubated at 37 °C under an argon atmosphere for various times. From the ratio of the integral areas of NMR signals belonging to decomposition products to that for the internal standard (*tert*-butyl alcohol), the yields of decomposition products were determined. Because Fe^{3+} is paramagnetic, before obtaining the NMR spectrum, Fe^{3+} was removed by passing through chelex 100 resin (2 g) in a pipet.

Vitamin E- and C-Promoted Fragmentation of 5-Hydroperoxy-4-hydroxy-cyclohept-2-enone (2). β-Hydroxyhydroperoxide **2** (4 mg, 0.025 mmol) and vitamin E (16.4 mg, 0.038 mmol) were dissolved in CH_3CN (0.8 mL). $FeSO_4{\cdot}7H_2O$ (0.7 mg, 0.0025 mmol) or $CuSO_4$ (0.4 mg, 0.0025 mmol) in 20 μL of water was added to catalyze the reaction. The resulting solution was stirred for 4 h at room temperature under an argon atmosphere. Traces of water and metal salts were removed by passing the reaction mixture through a pipet containing anhydrous Na_2SO_4 (2 g). The solvent was removed, and the residue was dissolved into 0.7 mL of $CDCl_3$ (with 0.01 M phenyltrimethylsilane as internal standard) in an NMR tube. From the ratio of the integral areas of NMR signals belonging to decomposition products to that for the internal standard, the product yields were determined. For comparison, β-hydroxyhydroperoxide **2** with vitamin E or with Fe^{2+} or Cu^{2+} were reacted under the same conditions, and the product yields were also determined from the ratios of the integral areas of ^{1}H NMR signals.

β-Hydroxyhydroperoxide **2** (4 mg, 0.025 mmol) with vitamin C (0.038 mmol) or with vitamin C (0.038 mmol) and Cu^{2+} or Fe^{3+} (0.0025 mmol) was dissolved into D_2O (0.7 mL). *tert*-Butyl alcohol (5 μL) was added as an internal standard. Then the mixture was vortexed, transferred into NMR tubes, and incubated at 37 °C under an argon atmosphere for various times. From the ratio of the integral areas of NMR signals attributable to decomposition products to that for the internal standard, the yields of the products were determined.

■ ASSOCIATED CONTENT

Supporting Information

General experimental methods and NMR spectra of compounds **2**−**4** and **7**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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