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Preparative Singlet Oxygenation of Linoleate Provides Doubly Allylic Dihydroperoxides: Putative Intermediates in the Generation of Biologically Active Aldehydes in Vivo

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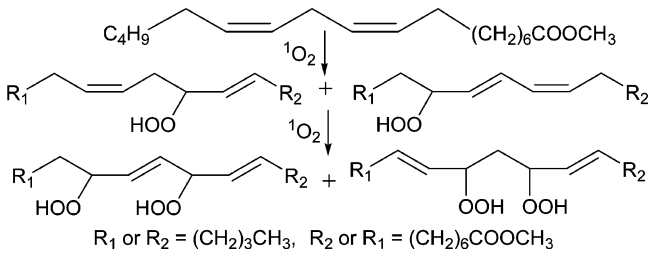

Photoinduced oxygenation generates biologically active, oxidatively truncated lipids in the retina. Previously, doubly allylic dihydroperoxides, 9,12-dihydroperoxyoctadeca-10,13-dienoic acid (9,12-diHPODE) and 10,13-dihydroperoxyoctadeca-8,11-dienoic acid (10,13-diHPODE), were postulated as key intermediates in the free radical-promoted oxidative fragmentation of linoleate that generates aldehydes, such as the cytotoxic γ-hydroxyalkenal 4-hydroxy-2-nonenal (HNE), in vivo. We now report an efficient preparation of regioisomerically pure 9,12- and 10,13-diHPODE, devised to enable studies of their fragmentation reactions. Free radical-induced oxygenation of linoleate initially generates conjugated monohydroperoxy octadecadienoates (HPODEs) that are then converted into diHPODEs. In contrast, we found that singlet oxygenation of conjugated HPODEs does not produce diHPODEs. Unconjugated HPODEs are unique products of singlet oxygenation of linoleate that are coproduced with conjugated HPODEs. Preparative separation of the mixture of regioisomeric mono and diHPODEs generated by singlet oxygenation of linloeate is impractical. However, a simple tactic circumvented the problem. Thus, selective conversion of the undesired conjugated HPODEs into Diels−Alder adducts could be accomplished under mild conditions by reaction with *N*-phenyltriazolinedione. These adducts were readily removed, and the two remaining unconjugated HPODEs could then be easily isolated regioisomerically pure. Each of these was subsequently converted into a different, regioisomerically pure, diHPODE through further singlet oxygenation.

Introduction

There is much current interest in determining the lipidome, the entire spectrum of lipids in biological systems.[1] Our research focuses on elucidating nonenzymatic biochemistry that generates a vast array of oxidized lipids. Peroxidation of polyunsaturated lipids in vivo is involved in many disease processes.[2] The initial products, lipid hydroperoxides, readily decompose, especially in the presence of transition metal ions, generating various small molecules including saturated and unsaturated aldehydes.[3] Many of the pathological effects of lipid peroxidation are mediated by such oxidatively truncated lipids. Oxidative fragmentation of polyunsaturated phospholipids generates biologically active truncated phospholipids that are abundant in atherosclerotic plaques.[4,5] Through recognition by receptors on macrophage cells, they promote the formation of foam cells[6] (precursors of atherosclerotic plaques) by fostering ingestion of oxidatively

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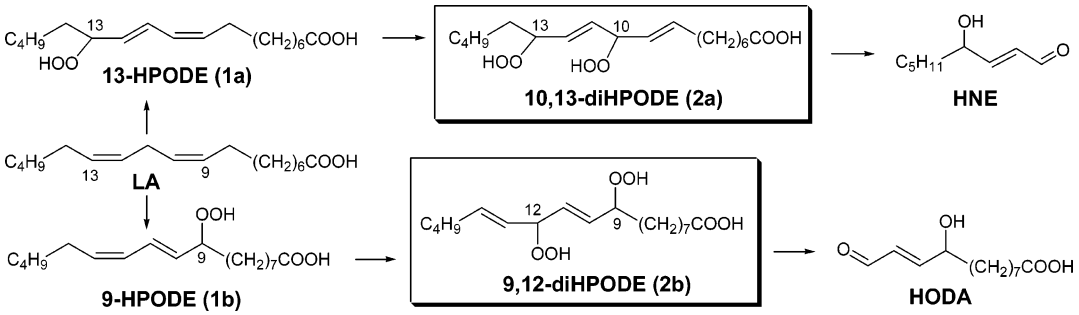
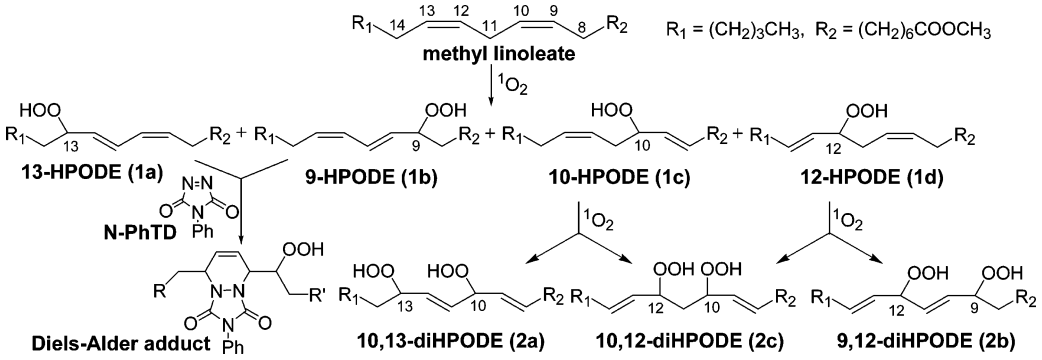
SCHEME 1



SCHEME 2



damaged low-density lipoproteins. They also activate human aortic endothelial cells to bind monocytes and express chemokines, thus promoting monocyte entry into chronic lesions where they become foam cells.[7] We recently identified several oxidatively truncated phosphatidylethanolamines[8] and phosphatidylcholines[9] in retina. Some of them serve as ligands for the scavenger receptor CD36, promoting phagocytosis of oxidatively damaged photoreceptors by retinal pigmented endothelial (RPE) cells. Because they are rich in highly polyunsaturated fatty acyls (PUFAs), photoreceptor cell membranes are especially susceptible to oxidative damage.[10] The formation of oxidized phospholipids (oxPLs) in the retina is promoted by light. For example, exposure of rats to intense light fosters the production of oxPLs and the consumption of PUFAs.[9]

A fundamental understanding of the mechanism by which light induces lipid oxidation is of considerable interest. Photosensitized oxidation reactions damage tissue through the formation of oxyradicals and singlet oxygen.[1] Ample evidence supports the premise that photoinduced singlet oxygenation contributes to oxidative damage in the retina.[11−15] Furthermore, lipofuscin, a pigment that accumulates with age in RPE cells, can promote the photogeneration of reactive oxygen species including singlet oxygen.[15] Thus, because it is rich in PUFAs, is continuously exposed to light and high oxygen tension, and contains photosensitizers, the environment in the retina is conducive to oxidative injury involving singlet oxygenation of PUFAs.

The conjugated hydroperoxyoctadecadienoates 9- and 13-HPODE are major primary products of both enzymatic and free radical-induced oxidation of linoleate. Further free radical-induced oxygenation of these conjugated HPODEs is believed to generate diHPODEs. The 10,13- and 9,12-dihydroperoxyoctadecadienoates (diHPODEs) were postulated as key intermediates in the autoxidative generation of 4-hydroxy-2-nonenal (HNE) and 9-hydroxy-12-oxo-10-dodecenoic acid (HODA) from LA (Scheme 1).

Unconjugated hydroperoxyoctadecadienoates, 10- and 12-HPODE, are produced exclusively through photoinduced oxidation of linoleate (Scheme 2).[16] To enable studies of their fragmentation reactions and investigations of their possible intermediacy in the photogeneration of truncated oxPL in the retina, we devised an efficient preparation of the unconjugated hydroperoxyoctadecadienoates, 10- and 12-HPODE, and the dihydroperoxyoctadecadienoates, 10,13- and 9,12-diHPODE.

Results and Discussion

Singlet Oxygenation of Methyl Linoleate. In a pilot study, photosensitized oxygenation of methyl linoleate at −60 °C delivered mainly monohydroperoxides. The experiment also afforded three dihydroperoxides, **2a**−**c** (Scheme 2), in 4−8% conversion. They could be clearly visualized on TLC plates with ferrous thiocyanate reagent.[17] With 30% ethyl acetate in hexanes,

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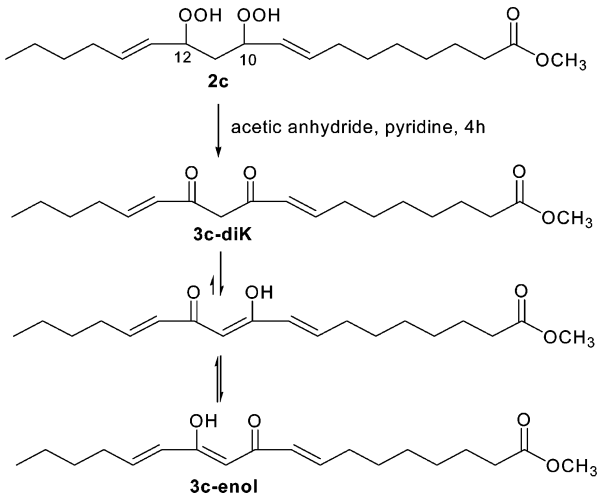
2c is well separated (R_f 0.31) from **2a** and **2b** (R_f 0.24). The ^{1}H NMR spectrum of the mixture of **2a** and **2b** exhibited resonances, centered at δ 4.72 and 4.25 ppm, presumed to be those of hydrogens α to hydroperoxy groups. The δ 4.72 resonance was especially encouraging as it corresponded well with that observed (δ 4.78) for hydrogen α to a doubly allylic hydroperoxide in a dihydroperoxide prepared from 2,6-dimethyl-2,5-hepta-diene.[18] The ^{13}C NMR spectrum of that dihydroperoxide exhibits resonances at δ 89 and 82 ppm. Our putative mixture of **2a** and **2b** exhibited pairs of resonances centered at δ 87 and 86 ppm consistent with a mixture of positional isomers. Because chromatographic separation of regioisomerically pure diHPODE **2a** or **2b** from this mixture is not a practical preparative method (vide infra), we explored the possibility of generating single pure diHPODE regioisomers through singlet oxygenation of individual pure HPODEs **1a**−**d**.

Free radical-promoted allylic oxygenation of conjugated monohydroperoxy dienes **1a** and **1b** is believed to generate diHPODEs. To test whether singlet oxygenation of these conjugated monohydroperoxides could be used to prepare doubly allylic dihydroperoxides under conditions that would allow their isolation, we applied photosensitized oxygenation to pure 9- or 13-hydroperoxyoctadienes (HPODE) that are readily available through enzymatic oxygenation of linoleic acid promoted by tomato lipoxygenase or soybean lipoxygenase, respectively. These photooxygenations did not produce any doubly allylic dihydroperoxides. This suggested that singlet oxygenation of the unconjugated hydroperoxydienes **1c** and **1d**, and not the conjugated hydroperoxy dienes **1a** or **lb**, generates **2a**−**c** (Scheme 2). Presumably **1a** or **lb** is preferentially converted into 3,6-dihydro-1,2-dioxenes through $2\pi + 4\pi$ cycloaddition of singlet oxygen.

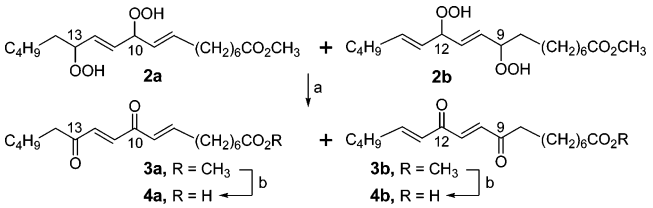
Isolation of Regioisomerically Pure 9,12- and 10,13-diHPODEs. The regioisomeric 10,13- and 9,12-diHPODE methyl esters **2a** and **2b** are not readily separable by normal or reverse[19] phase HPLC. Because each unconjugated monohydroperoxide **1c** and **1d** was presumed to be a precursor of a different doubly allylic dihydroperoxide, that is, **2a** and **2b**, respectively, we sought an efficient method for isolating isomerically pure 10-HPODE and 12-HPODE methyl esters **1c** and **1d** from the mixture of monohydroperoxides **1a**−**d**. Treatment of this mixture with 4-phenyl-1,2,4-triazoline-3,5-dione, a red dienophile, resulted in rapid formation of colorless Diels−Alder adducts from the conjugated HPODEs (Scheme 2). The desired 10-HPODE and 12-HPODE methyl esters **1c** and **1d** were then readily obtained isomerically pure from the reaction mixture by preparative HPLC. As expected, singlet oxygenation of 12-HPODE ester **1d** delivered 9,12-diHPODE ester **2b** together with 10,12-diHPODE ester **2c**, which were readily separable by flash chromatography. Similarly, 10,13-diHPODE ester **2a** was produced from 10-HPODE ester **1c** together with 10,12-diHPODE ester **2c**, which were readily separable by flash chromatography.

The ^{1}H NMR spectra of the individual 9,12- and 10,13-diHPODE esters are virtually identical. In contrast, their ^{13}C NMR spectra are very distinctive. For the individual diHPODE regioisomers, the signals for vinyl, methylene, and terminal methyl carbons all appear as single resonances instead of pairs

SCHEME 3



SCHEME 4 [a]



[a] Reagents and conditions: (a) acetic anhydride, pyridine, CH_2Cl_2, 4 h, 60%; (b) porcine pancreatic lipase (PPL), 5 h, 100%.

observed for the mixture of regioisomers. Thus, the vinyl carbon resonances appear at δ 128.74, 132.87, 134.66, 136.99 for the 9,12-diHPODE ester **2b** and at δ 128.88, 132.81, 134.72, 136.86 for the 10,13-diHPODE ester **2a**.

Characterization of Methyl 10,12-Bis-hydroperoxyoctadeca-8,13-dienoate (2c). The ^{1}H and ^{13}C NMR and 2D C−H correlation spectra for **2c** (Table 1, see Supporting Information) are consistent with the methyl 10,12-bis-hydroperoxyoctadeca-8,13-dienoate (10,12-diHPODE) structure. As expected, this dihydroperoxide is generated through singlet oxygenation of both **1c** and **1d** (Scheme 2). Further structural characterization of **2c** was accomplished by dehydration (Scheme 3). Treatment with acetic anhydride and pyridine produced **3c**. Rather than a β-dicarbonyl structure **3c-diK**, this compound exists predominantly as the enol tautomer **3c-enol**. **3c-enol** exhibits a characteristic ^{1}H NMR singlet absorption at 5.47 ppm and a ^{13}C NMR carbon resonance at δ 99.34, which is typical for an enol. Both HMQC and HMBC data (Table 2, see Supporting Information) strongly support an enol structure for **3c**. The HMQC of **3c** clearly shows a correlation between the hydrogen absorption at δ 5.47 and the carbon at δ 99.34. Thus, the HMBC spectrum clearly shows that the hydrogen at δ 5.47 is next to the carbonyl carbon at δ 183.66 ppm.

Synthesis and MS Analysis of Diketones from 2a and 2b. Dehydration of the diHPODEs **2a** and **2b** with acetic anhydride and pyridine delivered diketodienes **3a** and **3b** (Scheme 4). These methyl esters can be easily separated. They appear as two close, sharp peaks of equal height in a normal phase HPLC chromatogram eluting with 5% 2-propanol in hexanes. Positive ion ESI-MS/MS analysis produced diagnostic fragments *m*/*z* 193, 175, 99 for **3a** (Figure S25) and *m*/*z* 171, 165, 111 for **3b** (Figure S26).

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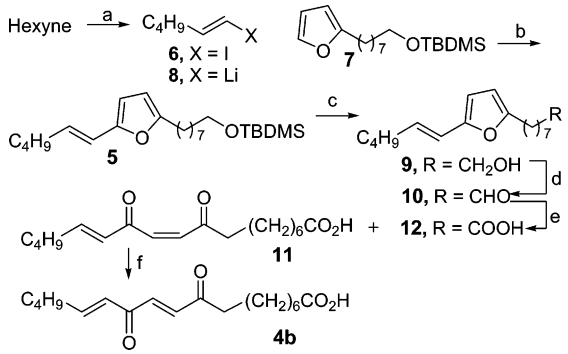
SCHEME 5 [a]



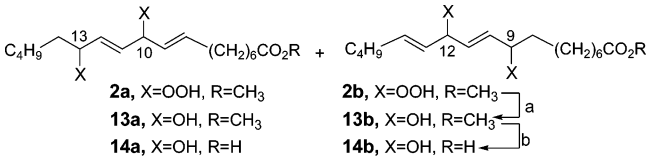
[a] Reagents and conditions: (a) (1) DIBAL, hexane, −40 °C; (2) I_2, 47%. (b) (1) *n*-BuLi, THF, −60 °C; (2) $ZnCl_2$, THF, rt; (3) $Pd(PPh_3)_4$, **6**, 75%. (c) TBAF, THF, 96%. (d) TPAP, NMO, CH_2Cl_2, 78%. (e) $NaClO_2$, *t*-BuOH/H_2O, 2-methyl-2-butene, **11** (58%) + **12** (24%). (f) Pyridine, THF/acetone/water, 2 h, 80%.

The diketodiene free acids **4a** and **4b** were obtained by treatment of the methyl esters **3a** and **3b** with porcine pancreatic lipase (PPL)[20] (Scheme 4). Many carbon absorptions in the ^{13}C NMR spectrum of the mixture of **4a** and **4b** appear in pairs, including the carbonyls, the double bonds, and many CH_2 groups. Electrospray tandem mass spectroscopic analysis (ESI-MS/MS) of **4a** and **4b**, which can be easily separated by normal phase HPLC with 1% 2-propanol in hexanes, confirmed the structures of these 9,12-diketodienoic and 10,13-diketodienoic acids. The spectra exhibit distinctive fragments, *m*/*z* 113, 153, 165, 191 for **4a** (Figure S28) and *m*/*z* 125, 137, 163, 185 for **4b** (Figure S27). The fragmentation pattern of the 9,12-diketodienoic acid **4b** was identical to that of an authentic sample prepared by an unambiguous total synthesis that is described below.

Unambiguous Total Synthesis of Diketone 4b. The synthesis of **4b** was accomplished by exploring the oxidative ring opening of a vinyl furan intermediate.

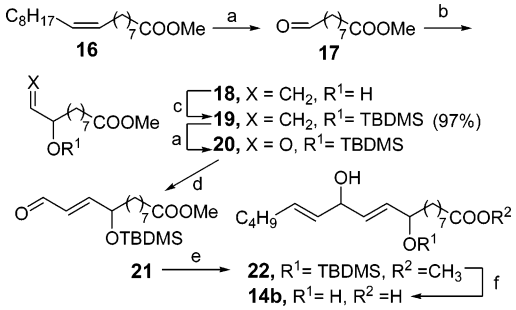
The electrophilic synthon **6** was prepared from 1-hexyne (Scheme 5).[21] A nucleophilic synthon, vinyllithium **8**,[22] is also readily available from **6**. Vinyl furan **5** was obtained by a Pd(0)-catalyzed coupling reaction between a furyl zinc intermediate and the vinyl iodide **6**.[23] Thus, furan **7**, which was prepared as described previously,[24] was first lithiated in THF by addition of 1 equiv of *n*-BuLi and then transmetalated to a furyl zinc by addition of zinc chloride. Subsequent slow addition of the furyl zinc reagent to a THF solution of the vinyl iodide **6** containing $Pd(PPh_3)_4$ (5 mol %) gave **5**. Desilylation of **5** produced alcohol **9** that was oxidized to aldehyde **10** by the mild TPAP/NMO method.[25] Further oxidation, by $NaClO_2/NaH_2PO_4$ in the presence of 2-methyl-2-butene, delivered the anticipated furyl acid **12** as well as an unexpected product, the diketodienoic acid **11**. This novel oxidative ring opening of the furan ring preferentially generated the 10-cis,13-trans isomer. Conversion to the all-trans isomer **4b** was readily achieved by treatment of the cis,trans isomer **11** with pyridine in aqueous acetone/THF.[26]

SCHEME 6 [a]



[a] Reagents and conditions: (a) Ph_3P, CH_2Cl_2, 1 h, 32%. (b) PPl, 6 h, 100%.

SCHEME 7 [a]



[a] Reagents and conditions: (a) (1) O_3, methanol, −60 °C; (2) Me_2S, −60 °C to rt, 81%. (b) CH_2═CHMgBr, THF, −78 °C, 66%. (c) TBDMSCl, imidazole, DMF, 98%. (d) Ph_3P═CHCHO, toluene, reflux, 4 h, 56%. (e) Vinyllithium **8**, THF, −78 °C, 48%. (f) (1) PPL; (2) TBAF, THF, 73%.

Synthesis of Diols Derived from 2a and 2b. Reduction of the dihydroperoxydienes **2a** and **2b** by reaction with triphenylphosphine delivered dihydroxydienoic esters **13a** and **13b**. These were converted to the dihydroxydienoic acids **14a** and **14b** by hydrolysis promoted by porcine pancreatic lipase (PPL) (Scheme 6).

To confirm its structure, dihydroxydienoic acid **14b** was prepared by an unambiguous total synthesis according to Scheme 7. Although **14b** and **22** are acid sensitive, they could be purified by chromatography using triethylamine-neutralized silica gel columns. The ^1H NMR spectrum of **14b** and that of the mixture of **14a** and **14b** are virtually identical. In contrast, many carbon absorptions in the ^{13}C NMR spectrum of the mixture of 1**4a** and **14b** appear in pairs, including the carbons α to the hydroxyl groups, the vinyl carbons, and many CH_2 groups.

Definitive Structural Characterization of the Regioisomeric Dihydroperoxides 2a and 2b. To determine the position of hydroperoxide groups on the fatty acid carbon chain, GC−MS analysis was performed on the corresponding bistrimethylsilyl ether derivatives **23a** and **23b** obtained from **2a** and **2b**, respectively, by reduction with borohydride and hydrogenation, as shown in Scheme 8 for **23a**. Chemical ionization with ammonia (negative ion mode) afforded an M−H ion at *m*/*z* 473 for both the 10,13- and the 9,12-bisTMS ether derivatives **23a** and **23b** (Figure S61). With electron ionization, the 10,13-bisTMS derivative **23a** showed characteristic ions at *m*/*z* 313, 173, indicating a 13-hydroxyl group, and *m*/*z* 273, 213, indicative of a 10-hydroxyl group. In contrast, the 9,12-bisTMS derivative **23b** gave characteristic ions at *m*/*z* 299, 187, which indicates the 12-hydroxyl group, and *m*/*z* 259, 227, indicative of a 9-hydroxyl group (Figure S62).

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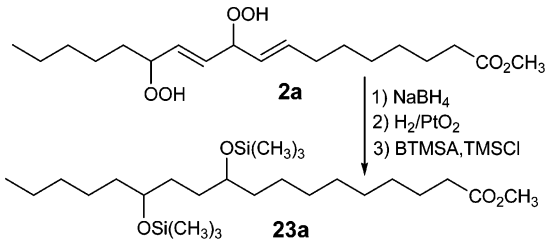
SCHEME 8



Decomposition of diHPODEs. It remains to be determined whether and by what mechanism(s) the dihydroperoxides **2a** and **2b** undergo fragmentation reactions. Two mechanistic possibilities involve alkoxy radical intermediates generated by homolysis of the doubly allylic hydroperoxide (Scheme 9). Fragmentation might then occur through β-scission of the alkoxy radical[27] or through an alkoxy radical cyclization followed by epoxycarbinyl radical cleavage.[8] A non-free radical alternative was proposed that accomplishes fragmentation through a polar (Hock) rearrangement of the doubly allylic hydroperoxide.[28] In a previous study, we established that oxidative fragmentation of the hydroxydiene 13-HODE occurs as readily as that of the hydroperoxydiene 13-HPODE, but did not distinguish between alkoxy radical and polar fragmentation pathways.[29] A recent study found that fragmentation can be promoted by strong acid, presumably through a polar rearrangement.[19] However, the operation of a polar fragmentation mechanism under physiological conditions remains to be established.

In a pilot study, we found that UV irradiation of diHPODEs generates 9-keto-10-octadecenoic acid (9-KODA), presumably through dehydration of 9-HPODA (Scheme 9). Unexpectedly, we also found that, rather than preventing fragmentation of 9,12-diHPODE by trapping intermediate alkoxy radicals, α-tocopherol (vitamin E) stoichiometrically promotes its decomposition to give 9-KODA and α-tocopheryl quinone. This reaction of α-tocopherol with diHPODE was completely inhibited by the transition metal ion chelator, diethylenetriaminepentaacetic acid. These observations do not rule out the involvement of alkoxy radical intermediates in the fragmentation reactions because alkoxy radical cyclization is expected to be a fast reaction that might compete with hydrogen atom transfer from α-tocopherol. Mechanistic studies of these and other reactions of 10,13- and 9,12-dihydroperoxyoctadecadienoates (diHPODEs) are in progress. Details will be reported in due course.

Conclusions

We addressed the challenge of devising a practical method for obtaining regioisomerically pure linoleate dihydroperoxides **2a** and **2b** to enable investigations testing their postulated intermediacy and determining environmental factors that favor their fragmentation to toxic aldehydes in vivo. The dihydroperoxides **2a** and **2b** are readily available by singlet oxygenation of linoleate at low temperatures under conditions that permit their isolation. The key step in our practical route for obtaining pure regioisomers of these doubly allylic dihydroperoxides is the efficient removal of conjugated isomers from the mixture of monohydroperoxydienes generated initially by singlet oxygenation of linoleate. The success of our approach also depends on the finding that the unconjugated monohydroperoxydienes **1c** and **1d**, and not conjugated monohydroperoxydienes **1a** and **1b**, are the precursors for the generation of **2a** and **2b** through singlet oxygenation of linoleate.

The fact that doubly allylic hydroperoxides can be generated by singlet oxygenation of linoleates suggests the interesting possibility that oxidative fragmentation of polyunsaturated fatty acyl derivatives in vivo can occur through an entirely non-free radical process involving photosensitized oxygenation followed by Hock rearrangement. This may be especially pertinent in the eye because photosensitizers present in the retina can promote the generation of singlet oxygen,[30,31] and oxidative fragmentation of PUFAs may occur through photoinduced singlet oxygenation even under conditions that block free radical-induced oxidation.

Experimental Procedures

Generation of Dihydroperoxides 2a, 2b, and 2c from Methyl Linoleate. Photosensitized oxidation was carried out in a coldfinger-cooled internally irradiated photoreaction vessel. A solution containing methyl linoleate (0.5 g) and tetraphenylporphine (1.5 mg) in CH_2Cl_2 (50 mL) was cooled in the apparatus to −60 °C. Oxygen was passed through the solution while the sample was illuminated by a tungsten lamp (250 W) for 3 h. The reaction afforded the four known monohydroperoxides **1a**−**d** (70% yield) and more polar dihydroperoxides **2a**+**2b** as an inseparable mixture (4% yield) and **2c** (8% yield). Immediately following evaporation of the solvent, the crude reaction product mixture was loaded onto a triethylamine preconditioned column. Eluting with a gradient from 10% to 30% ethyl acetate in hexanes, dihydroperoxides **2a**+**2b** (22.1 mg) and **2c** (47.2 mg) were collected separately.

Doubly Allylic Dihydroperoxides 2a and 2b. ^{1}H NMR (300 MHz, CD_3OD): δ 5.76 (m, 2H), 5.68 (dd, J = 15.60, 7.48, 1H), 5.55 (dd, J = 15.55, 7.09, 1H), 4.72 (dd, J = 7.58, 6.95, 1H), 4.25 (td, J = 7.14, 6.63, 1H), 3.64 (s, 3H), 2.30 (t, J = 7.38, 2H), 2.07 (dd, J = 9.43, 7.17, 2H), 1.59 (m, 2H), 1.20−1.50 (m, 12H), 0.90 (t, J = 7.29, 3H). HRMS (EI): m/z calcd for $C_{19}H_{32}O_6$ (M^+) − OH − OH, 324.2301; found, 324.2315.

Methyl 10,12-diHPODE (2c). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.13 (s, 1H), 8.05 (s, 1H), 5.83 (ddt, J = 14.45, 14.45, 6.98, 2H), 5.46 (dd, J = 15.58, 8.08, 2H), 4.45 (dd, J = 15.96, 7.46, 2H), 3.65 (s, 3H), 2.30 (t, J = 7.39, 2H), 2.08 (dt, J = 19.07, 7.19, 4H), 1.60 (m, 4H), 1.20−1.56 (m, 10H), 0.90 (t, J = 7.19, 3H). ^{13}C NMR (50 MHz, CD_3OD, APT): δ 176.1 (+), 136.5 (−), 136.5 (−), 130.6 (−), 130.4 (−), 52.0 (−), 37.5 (+), 34.8 (+), 33.3 (+), 33.2 (+), 32.5 (+), 30.1 (+), 30.0 (+), 29.8 (+), 26.0 (+), 23.3 (+), 14.3 (−). HRMS (EI): m/z calcd for $C_{19}H_{32}O_6$ (M^+) − $2H_2O$, 322.2144; found, 322.2150.

Treatment of Monohydroperoxides 1a−d with 4-Phenyl-1,2,4-triazoline-3,5-dione. A mixture of monohydroperoxides **1a**−**d** (1.7 g) in CH_2Cl_2 (140 mL) was titrated with a solution of 4-phenyl-1,2,4-triazoline-3,5-dione in CH_2Cl_2 under room temperature until the red color of the dienophile persisted. The reaction mixture was then allowed to stand for another hour before evaporation of solvent. Flash chromatography (15% ethyl acetate in hexanes) delivered a mixture (570 mg) of methyl 10-HPODE (**1c**) and methyl 12-HPODE (**1d**). Normal phase HPLC with 1% 2-propanol in hexanes provided isomerically pure methyl 10-HPODE (**1c**) and methyl 12-HPODE (**1d**).

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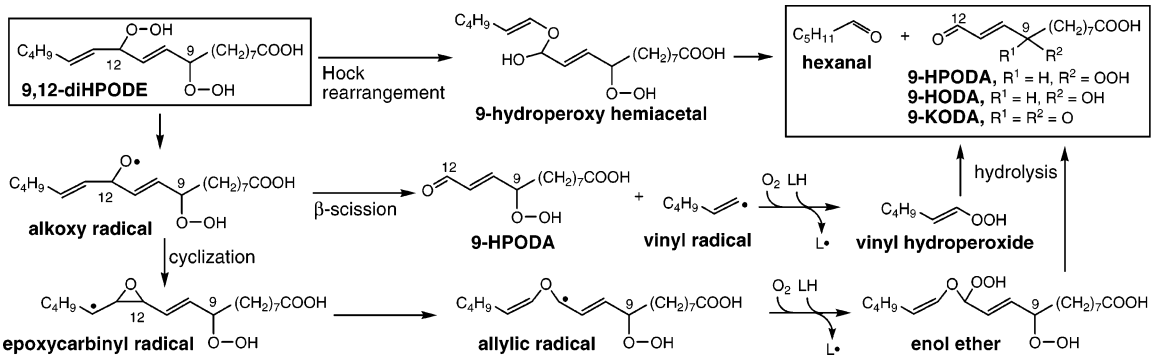

Methyl 10-HPODE (1c). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (br, 1H), 5.73 (dt, J = 15.43, 6.71, 1H), 5.26−5.50 (m, 3H), 4.28 (td, J = 7.51, 6.92, 1H), 3.64 (s, 3H), 2.40 (ddd, J = 14.98, 7.49, 6.51, 1H), 2.27 (t, J = 7.57, 2H), 2.19 (m, 1H), 2.04 (td, J = 7.22, 6.88, 2H), 1.99 (td, J = 7.57, 7.39, 2H), 1.58 (m, 2H), 1.19−1.42 (m, 12H), 0.85 (t, J = 7.20, 3H). ^{13}C NMR (50 MHz, $CDCl_3$, APT): δ 174.4 (+), 136.8 (−), 132.6 (−), 128.1 (−), 124.0 (−), 86.5 (−), 51.5 (−), 34.1 (+), 32.2 (+), 31.6 (+), 30.7 (+), 29.3 (+), 29.1 (+), 29.0 (+), 28.7 (+), 28.7 (+), 27.4 (+), 24.9 (+), 22.6 (+), 14.1 (−). MS: calcd for $C_{19}H_{34}O_4$, 326.2457; HREI found, 326.2381.

Methyl 12-HPODE (1d). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 5.76 (dt, J = 15.47, 6.85, 1H), 5.27−5.50 (m, 3H), 4.29 (td, J = 7.78, 7.01, 1H), 3.65 (s, 3H), 2.42 (ddd, J = 14.33, 7.49, 6.84, 1H), 2.28 (t, J = 7.45, 2H), 2.22 (m, 1H), 2.10 (td, J = 7.43, 6.85, 2H), 2.05 (td, J = 7.63, 6.94, 2H), 1.60 (m, 2H), 1.21−1.42 (m, 12H), 0.88 (t, J = 7.15, 3H). ^{13}C NMR (50 MHz, $CDCl_3$, APT): δ 174.5 (+), 137.1 (−), 132.4 (−), 127.9 (−), 124.1 (−), 86.5 (−), 51.5 (−), 34.1 (+), 32.1 (+), 31.2 (+), 30.7 (+), 29.4 (+), 29.3 (+), 29.1 (+), 29.1 (+), 27.4 (+), 25.0 (+), 22.2 (+), 14.0 (−). MS: calcd for $C_{19}H_{34}O_4$, 326.2457; HREI found, 326.2446.

Generation of the Individual 10,13-diHPODE and 9,12-diHPODE. They are generated from 10-HPODE and 12-HPODE the same way as the mixture of dihydroperoxides.

Methyl 10,13-DiHPODE (2a). ^{1}H NMR (400 MHz, CD_3OD): δ 5.6−5.8 (m, 3H), 5.46 (ddt, J = 15.10, 7.16, 1.45, 1H), 4.72 (dd, J = 7.34, 6.64, 1H), 4.25 (td, J = 7.02, 6.46, 1H), 3.64 (s, 3H), 2.30 (t, J = 7.40, 2H), 2.07 (td, J = 7.71, 7.16, 2H), 1.59 (m, 2H), 1.23−1.46 (m, 12H), 0.89 (t, J = 6.95, 3H). ^{13}C NMR (50 MHz, CD_3OD, APT): δ 176.0 (+), 136.9 (−), 134.7 (−), 132.8 (−), 128.9 (−), 87.4 (−), 86.8 (−), 52.0 (−), 34.8 (+), 33.7 (+), 33.4 (+), 32.9 (+), 30.0 (+), 29.9 (+), 29.8 (+), 26.0 (+), 26.0 (+), 23.7 (+), 14.4 (−). HRMS (EI): m/z calcd for $C_{19}H_{32}O_6$ (M^+) − OH − H_2O, 291.2325; found, 291.2323.

Methyl 9,12-DiHPODE (2b). ^{1}H NMR (400 MHz, CD_3OD): δ 5.76 (m, 2H), 5.68 (dd, J = 15.60, 7.48, 1H), 5.55 (dd, J = 15.55, 7.09, 1H), 4.72 (dd, J = 12.85, 6.33, 1H), 4.25 (dd, J = 14.35, 6.13, 1H), 3.64 (s, 3H), 2.30 (t, J = 7.38, 2H), 2.07 (dd, J = 9.43, 7.17, 2H), 1.59 (m, 2H), 1.20−1.50 (m, 12H), 0.90 (t, J = 7.29, 3H). ^{13}C NMR (50 MHz, CD_3OD, APT): δ 176.0 (+), 137.0 (−), 134.7 (−), 132.9 (−), 128.7 (−), 87.4 (−), 86.8 (−), 52.0 (−), 34.8 (+), 33.7 (+), 33.2 (+), 32.4 (+), 30.5 (+), 30.3 (+), 30.1 (+), 26.6 (+), 26.0 (+), 23.2 (+), 14.3 (−).HRMS (EI): m/z calcd for (M^+) − OH − H_2O, 291.2325; found, 291.2306.

Dehydration of Dihydroperoxides 2a and 2b. Acetic anhydride (30 μL) and pyridine (120 μL) were added to a solution of **2a** and **2b** (20 mg) in dry CH_2Cl_2 (2 mL). The resulting mixture was allowed to stand at room temperature for 4 h until all of the dihydroperoxides were consumed. Flash chromatography (15% ethyl acetate in hexanes, TLC, R_f = 0.28) afforded a mixture of **3a** and **3b** (10.7 mg, 59%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18 (dd, J = 15.85, 1.07, 1H), 7.02 (dd, J = 15.85, 2.08, 1H), 6.93 (dd, J = 15.69, 2.09, 1H), 6.33 (dd, J = 15.77, 0.92, 1H), 3.65 (s, 3H), 2.63 (t, J = 7.36, 2H), 2.22 (dt, J = 7.44, 2.75, 2H), 1.10−1.90 (m, 16H), 0.86 (t, J = 7.34, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.7, 200.5, 189.4, 174.3, 174.2, 151.3, 151.0, 136.6, 136.6, 134.9, 134.9, 129.6, 129.5, 51.6, 42.2, 42.1, 34.1, 34.0, 32.8, 32.6, 31.4, 30.1, 29.1, 29.0, 28.9, 27.8, 24.9, 24.8, 23.7, 23.5, 22.5, 22.3, 14.0, 13.9. MS: calcd for $C_{19}H_{32}O_4$, 322.2144; HREI found, 322.2147.

Methyl 10,12-dioxo-ocatadeca-8,13-dienoate (3c) was prepared by dehydration of **2c** by the same procedure as that used to prepare **3a** and **3b**. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.80 (m, 2H), 5.87 (ddt, J = 15.65, 4.86, 1.54, 2H), 5.47 (s, 1H), 3.60 (s, 3H), 2.24 (t, J = 7.54, 2H), 2.16 (dt, J = 13.96, 7.26, 4H), 1.56 (t, J = 7.28, 2H), 1.35−1.43 (m, 4H), 1.23−1.32 (m, 6H), 0.84 (t, J = 7.28, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.9, 183.6, 174.2, 145.4, 145.0, 127.3, 127.2, 99.1, 51.5, 34.1, 32.6, 32.4, 30.4, 28.9, 28.8, 28.1, 24.8, 22.3, 13.9. MS: calcd for $C_{19}H_{30}O_4$, 322.2144; HREI found, 322.2155.

4a+4b. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (d, J = 15.93, 1H), 7.04 (dd, J = 14.52, 6.94, 1H), 6.96 (dd, J = 15.73, 2.34, 1H), 6.36 (d, J = 15.85, 1H), 2.65 (t, J = 7.32, 2H), 2.2−2.4 (m, 4H), 1.1−1.8 (m, 14H), 0.89 (t, J = 7.31, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.7, *200.5*, *189.4*, *179.0*, 178.6, *151.3*, 150.9, 136.6, *136.5*, *134.9*, 134.8, 129.5, *129.4*, 42.1, *42.0*, *33.8*, 33.7, 32.7, *32.6*, 31.3, *30.1*, 29.7, *29.0*, *28.9*, *28.8*, 27.7, *24.6*, 24.5, *23.6*, 23.4, 22.4, *22.3*, 13.9, *13.8*. Those italicized values are for **4b**, 9,12-diketo. MS: calcd for $C_{18}H_{28}O_4$, 308.1988; HREI found, 308.1994.

Conversion of Dihydroperoxides 2a and 2b into Diols 14a and 14b. Immediately after their purification, the mixture of dihydroperoxides **2a** and **2b** was treated with triphenylphosphine in CH_2Cl_2 at room temperature for 1 h to generate a mixture of methyl dihydroxydienoates **13a** and **13b** that was purified by flash chromatography on a column that was preconditioned with triethylamine. The product (R_f = 0.17, 6.5 mg, 32%) eluted with 40% ethyl acetate in hexanes. ^{1}H NMR (300 MHz, CD_3OD): δ 5.62 (m, 3H), 5.42 (dd, J = 15.62, 6.59, 1H), 4.50 (dd, J = 6.37, 5.55, 1H), 4.01 (td, J = 12.72, 6.15, 1H), 3.64 (s, 3H), 2.30 (t, J = 7.51, 2H), 2.04 (dd, J = 14.40, 7.14, 2H), 1.59 (t, J = 6.76, 2H), 1.20−1.53 (m, 14H), 0.90 (t, J = 7.09, 3H). ^{13}C NMR (CD_3OD, 50 MHz, APT): δ 176.1 (+), 176.0 (+), 134.7 (−), 133.6 (−), 133.1 (−), 132.8 (−), 73.9 (−), 73.1 (−), 52.0 (−), 38.4 (+), 34.8 (+), 33.2 (+), 33.1 (+), 33.0 (+), 32.6 (+), 30.5 (+), 30.4 (+), 30.2 (+), 30.0 (+), 29.8 (+), 26.5 (+), 26.3 (+), 26.1 (+), 26.0 (+), 23.8 (+), 23.3 (+), 14.4 (−), 14.3 (−). MS: calcd for $C_{19}H_{32}O_4$−OH, 309.2430; HREI found, 309.2439; for $C_{19}H_{32}O_4$−OH−H_2O, 291.2324; HREI found, 291.2311. After hydrolysis with PPL, a mixture of the dihydroxydienoic acids **14a** and **14b** was obtained. ^{1}H NMR (300 MHz, CD_3OD): δ 5.62 (m, 3H), 5.49 (ddt, J = 15.65, 6.57, 1.47 Hz, 1H), 4.53 (dd, J = 7.00, 5.77 Hz, 1H), 4.04 (1H), 2.27 (t, J = 7.60 Hz, 2H), 2.08 (td, J = 8.07, 6.87 Hz, 2H), 1.62 (m, 2H), 1.27−1.59 (m, 14H), 0.93 (t, J = 7.09 Hz, 3H). ^{13}C NMR (50 MHz, CD_3OD, APT): δ 134.7 (−), 134.6 (−), 133.6 (−), 133.6 (−), 133.2 (−), 133.1 (−), 132.9 (−), 132.8 (−), 73.9 (−), 73.2 (−), 73.1 (−), 38.4 (+), 35.2 (+), 33.2 (+), 33.1 (+),

33.0 (+), 32.6 (+), 30.3 (+), 30.2 (+), 30.1 (+), 29.9 (+), 26.6 (+), 26.3 (+), 26.2 (+), 26.1 (+), 23.8 (+), 23.3 (+),14.5 (−), 14.3 (−). MS: calcd for $C_{18}H_{30}O_4$−OH, 295.2273; HREI found, 295.2278.

(*E*)1-Iodohex-1-ene (6). To a solution of 1-hexyne (2 g, 24.6 mmol) in hexanes was added DIBAL (1.0 M in cyclohexane, 24.6 mL, 24.6 mmol) at −40 °C. The solution was stirred for 20 min and then slowly warmed to room temperature over 1.5 h. After being stirred for an additional 2 h at room temperature, the solution was heated at 50 °C for 4 h, and then cooled to −40 °C. To the cooled solution was added dropwise a THF (15 mL) solution of I_2 (6.3 g, 24.6 mmol). To the resulting mixture after stirring for 12 h was added 10 mL of H_2SO_4 (20% in water) dropwise over 30 min. The product was extracted with hexanes (3 × 20 mL). The combined organic layer was sequentially washed with $Na_2S_2O_3$ (10 mL, 1 M), saturated $NaHCO_3$ (10 mL), and brine (10 mL). The hexane solution was dried with $MgSO_4$ and passed through a silica gel flash column with hexanes to afford **6** as a colorless oil (2.4 g, 11.4 mmol, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.52 (dt, J = 14.3, 7.2 Hz, 1H), 5.97 (dt, J = 14.3, 1.4 Hz, 1H), 2.06 (m, 2H), 1.2−1.5 (4H), 0.89 (t, J = 6.9 Hz, 3H). The ^{1}H NMR spectrum is consistent with a previous report.[32]

***tert*-Butyl-[8-(5-hex-1-enylfuran-2-yl)-octyloxy]dimethylsilane (5).** *n*-BuLi in hexane (3.9 mL, 6.3 mmol) was added dropwise to furan **7** (1.5 g, 4.83 mmol) in dry THF (15 mL) at −60 °C. The resulting mixture was stirred for 1 h after warming up to room temperature, and then cooled again to −60 °C. To the mixture was added dropwise a solution of $ZnCl_2$ (857 mg, 6.3 mmol) in THF (5 mL). The resulting solution was warmed to room temperature immediately, and stirred for 0.5 h at room temperature. A solution of iodide **6** (1.2 g, 7 mmol) and $Pd(PPh_3)_4$ (200 mg, 5%) in THF (5 mL) was added to the mixture over 1 h. After being stirred for 12 h, the mixture was poured into saturated aqueous NH_4Cl (10 mL). The compound was extracted with diethyl ether and concentrated by rotary evaporation. The residue was purified on a silica gel column (10% CH_2Cl_2 in hexanes, TLC: R_f = 0.25) to afford **5** (1.42 g, 75%) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.1−6.2 (2H), 6.00 (d, J = 3.1 Hz, 1H), 5.92 (d, J = 3.1 Hz, 1H), 3.60 (t, J = 6.4 Hz, 2H), 2.59 (t, J = 7.5 Hz, 2H), 2.1−2.2 (m, 2H), 1.2−1.7 (16H), 0.9 (12H), 0.06 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 155.6, 151.7, 128.6, 118.7, 106.7, 106.2, 63.4, 32.9, 32.5, 31.6, 29.4, 29.2, 28.2, 28.1, 26.1, 25.8, 22.3, 18.4, 14.0, −5.2. HRMS (EI): *m*/*z* calcd for $C_{24}H_{44}O_2Si$ (M^+), 392.3111; found, 392.3100.

8-(5-Hex-1-enylfuran-2-yl) Octan-1-ol (9). To a solution of vinyl furan **5** (540 mg, 1.38 mmol) in THF (5 mL) was added TBAF (4 mL, 1 M in THF, 4 mmol) dropwise under argon, and the solution was stirred overnight. Water (4 mL) was then added. The suspension was extracted with diethyl ether. The combined organic layers were dried with sodium sulfate and concentrated by rotary evaporation. The residue was then purified by flash chromatography on a silica gel column (30% ethyl acetate in hexanes, TLC: R_f = 0.36) to afford **9** (370 mg, 1.33 mmol, 96%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.07−6.2 (m, 2H), 6.00 (d, J = 3.1 Hz, 1H), 5.92 (d, J = 3.1 Hz, 1H), 3.64 (t, J = 6.0 Hz, 2H), 2.59 (t, J = 7.7 Hz, 2H), 2.1−2.2 (m, 2H), 1.2−1.7 (m, 16H), 0.91 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 155.6, 151.7, 128.6, 118.7, 106.7, 106.2, 63.1, 32.8, 32.5, 31.6, 29.4, 29.2, 28.2, 28.1, 26.4, 25.8, 22.3, 14.0. HRMS (EI): *m*/*z* calcd for $C_{18}H_{30}O_2$ (M^+), 278.2246; found, 278.2247.

8-(5-Hex-1-enyl-furan-2-yl)-octanal (10). Alcohol **9** (53 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (5 mL) containing both 4 Å molecular sieves and *N*-methyl morpholine *N*-oxide (NMO, 35 mg, 0.26 mmol).[33] After the mixture was stirred for 10 min, tetra-*n*-propylammonium perruthenate (TPAP, 5 mg, 0.01 mmol) was added and the reaction was monitored by TLC until complete. When complete, the mixture was diluted with CH_2Cl_2 (50 mL) and then washed with aqueous sodium sulfite (10 mL), brine (10 mL), and saturated aqueous $CuSO_4$ solution (10 mL) and dried with $MgSO_4$. After the solvent was rotary evaporated, the residue was purified by flash chromatography on a silica gel column (10% ethyl acetate in hexanes, TLC: R_f = 0.3) to afford **10** (39 mg, 78%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.77 (t, J = 1.8 Hz, 1H), 6.07−6.2 (2H), 6.00 (d, J = 3.1 Hz, 1H), 5.92 (d, J = 3.1 Hz, 1H), 2.60 (t, J = 7.2 Hz, 2H), 2.42 (dt, J = 7.1, 1.7 Hz, 2H), 1.5−1.8 (4H), 1.2−1.5 (10H), 0.91 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 220.9, 155.4, 151.7, 128.6, 118.7, 106.7, 106.3, 43.9, 32.5, 31.6, 29.2, 29.1, 29.0, 28.14, 28.0, 22.3, 22.1,14.0. HRMS (EI): *m*/*z* calcd for $C_{18}H_{28}O_3$ (M^+), 276.2089; found, 276.2099.

9,12-Dioxooctadeca-10(*Z*),13(*E*)-dienoic Acid (11). To a magnetically stirred solution of aldehyde **10** (39 mg, 0.14 mmol) in *t*-BuOH−H_2O (5:1, v/v, 0.3 mL) and 2-methyl-2-butene (1.44 mmol, 720 μL, 2 M in THF) were added NaH_2PO_4 (30 mg, 0.22 mmol) and $NaClO_2$ (40 mg, 0.4 mmol). The mixture was stirred at room temperature for about 25 min (monitored by TLC). The solvent was then removed by rotary evaporation. The residue was purified by flash chromatography on a silica gel column (first eluted with 25% ethyl acetate in hexanes and then ethyl acetate), affording **11** (25 mg, 58%) as yellowish crystals. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.83 (dt, J = 16.0, 6.8 Hz, 1H), 6.48 (d, J = 12.0 Hz, 1H), 6.38 (d, J = 12.0 Hz, 1H), 6.18 (dt, J = 16.0, 1.5 Hz, 1H), 2.52 (t, J = 7.2 Hz, 2H), 2.1−2.4 (4H), 1.1−1.8 (14H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 202.9, 192.6, 179.6, 150.8, 136.9, 134.2, 130.2, 42.6, 34.0, 32.5, 30.1, 29.1, 28.9, 24.6, 23.4, 22.3, 13.9. HRMS (EI): *m*/*z* calcd for $C_{18}H_{28}O_4$ (M^+), 308.1988; found, 308.1978.

8-(5-Hex-1-enylfuran-2-yl)octanoic Acid (12). Furan carboxylic acid **12** was coproduced with **11** during the oxidation of aldehyde **10** as described above. This compound was purified by flash chromatography (25% ethyl acetate in hexanes, TLC: R_f = 0.23), affording **12** (10 mg, 24%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.07−6.2 (m, 2H), 6.00 (d, J = 3.1 Hz, 1H), 5.92 (d, J = 3.1 Hz, 1H), 2.59 (t, J = 7.4 Hz, 2H), 2.35 (d, J = 7.5 Hz, 2H), 2.1−2.2 (2H), 1.5−1.8 (4H), 1.2−1.5 (10H), 0.91 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 179.6, 155.5, 151.7, 128.6, 118.7, 106.7, 106.2, 34.0, 32.5, 31.6, 29.0, 29.10, 28.2, 28.1, 22.3, 14.0. HRMS (EI): *m*/*z* calcd for $C_{18}H_{28}O_3$ (M^+), 292.2038; found, 292.2079.

9,12-Dioxo-octadeca-10(*E*),13(*E*)-dienoic Acid (4b). Freshly distilled pyridine (100 μL) was added to a solution of **11** (25 mg) in THF/acetone/water (5/4/1, v/v, 5 mL). The mixture was stirred for 2 h at room temperature. Solvents were removed on a rotary evaporator and a mechanical vacuum pump. The residue was chromatographed on a silica gel flash column (30% ethyl acetate in hexanes, TLC: R_f = 0.29), affording **4b** (20 mg, 80%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.21 (d, J = 15.9 Hz, 1H), 7.04 (dt, J = 15.7, 7.11 Hz, 1H), 6.96 (d, J = 15.9 Hz, 1H), 6.36 (d, J = 15.7 Hz, 1H), 2.64 (t, J = 7.1 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 2.2−2.3 (m, 2H), 1.1−1.8 (14H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.6, 189.5, 179.6, 151.4, 136.6, 135.0, 129.4, 42.1, 34.0, 32.6, 30.1, 29.0, 28.9, 28.9, 24.6, 23.7, 22.3, 13.9. HRMS (EI): *m*/*z* calcd for $C_{18}H_{28}O_4$ (M^+), 308.1988; found, 308.1978.

Methyl-9-oxononanoate (17). With stirring and cooling to −60 °C, ozone was bubbled through a solution of methyl oleate (1.7 g, 5.7 mmol) in dry methanol (50 mL). When the stirred solution turned blue after a short period, nitrogen was passed through to remove excess ozone. While still at −60 °C, dimethyl sulfide (1 mL) was added, and the resulting mixture was brought to room temperature over 4 h. After evaporation of the solvent under reduced pressure, the crude product was purified by flash chromatography on a silica gel column (20% ethyl acetate in hexanes, TLC: R_f = 0.5), affording **17** (866 mg, 81%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.77 (t, J = 1.8 Hz, 1H), 3.67 (s, 3H), 2.43 (dt, J = 7.2, 1.8 Hz,

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2H), 2.31 (t, J = 7.4 Hz, 2H), 1.55−1.7 (m, 4H), 1.25−1.4 (m, 6H). The spectrum agrees with that reported previously.[34]

9-Hydroxyundec-10-enoic Acid Methyl Ester (18). To a solution of aldehyde **17** (860 mg, 4.62 mmol) in THF (50 mL) was added vinylmagnesium bromide (5.07 mL, 1 M in THF) dropwise over 30 min at −78 °C under argon. The resulting solution was stirred for 5 h at −78 °C and then warmed to room temperature over another 2 h. The reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with diethyl ether. The solvent of the combined organic layers was removed by rotary evaporation, and the residue was purified by flash chromatography on a silica gel column (20% ethyl acetate in hexanes, TLC: R_f = 0.24), affording **18** (658 mg, 66%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.86 (ddd, J = 17.0, 10.5, 6.8 Hz, 1H), 5.21 (dt, J = 17.0, 1.6, 1.6, 1H), 5.09 (ddd, J = 10.4, 1.1, 1.1, 1H), 4.0−4.2 (m, 1H), 3.66 (s, 3H), 2.29 (t, J = 7.3 Hz, 2H), 1.1−1.7 (m, 10H). ^{13}C NMR ($CDCl_3$, 50 MHz, APT): δ 171.4 (+), 141.4 (−), 114.6 (+), 73.3 (−), 51.5 (−), 37.0 (+), 34.1 (+), 29.4 (+), 29.2 (+), 29.1 (+), 25.3 (+), 25.0 (+). The spectrum agrees with that reported previously.[32]

9-(*tert*-Butyl-dimethylsilanyloxy)undec-10-enoic Acid Methyl Ester (19). TBDMSCl (675 mg, 4.5 mmol) was added under argon to DMF (5 mL) containing both alcohol (**16**, 658 mg, 3.07 mmol) and imidazole (612 mg, 9 mmol).[33] The resulting solution was stirred for 24 h. After the disappearance of the alcohol monitored by TLC, the reaction solution was poured into a mixture of hexanes and aqueous sodium bicarbonate. The aqueous layer was extracted with hexanes. The combined organic layer was dried over magnesium sulfate and concentrated by rotary evaporation to afford an oil residue, which was purified by flash chromatography to produce **19** (988 mg, 98%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.79 (ddd, J = 17.2, 10.3, 6.0 Hz, 1H), 5.12 (ddd, J = 17.2. 2.0, 1.2, 1H), 5.01 (ddd, J = 10.3, 2.0, 1.2 Hz, 1H), 4.0−4.2 (m, 1H), 3.66 (s, 3H), 2.30 (t, J = 7.3 Hz, 2H), 1.1−1.7 (10H), 0.89 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz, APT): δ 174.3 (+), 141.9 (−), 113.5 (+), 73.9 (−), 51.5 (−), 38.1 (+), 34.2 (+), 29.5 (+), 29.3 (+), 29.2 (+), 25.9 (−), 25.2 (+), 25.0 (+), 18.3 (+), −4.3 (−), −4.8 (−). The spectrum agrees with that reported previously.[34]

9-(*tert*-Butyl-dimethylsilanyloxy)-10-oxo-decanoic Acid Methyl Ester (20). Ozonolysis of **19** (300 mg, 0.92 mmol) in 10 mL of dry methanol was performed by the same method as that of methyl oleate (vide supra). Purification by flash chromatography on a silica gel column (10% ethyl acetate in hexanes, TLC: R_f = 0.34) afforded **20** (292 mg, 97%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.59 (d, J = 1.7 Hz, 1H), 3.9−4.0 (m, 1H), 3.66 (s, 3H), 2.30 (t, J = 7.6 Hz, 2H), 1.1−1.7 (10H), 0.92 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz, APT): δ 204.5 (−), 174.3 (+), 51.5 (−), 34.1 (+), 32.6 (+), 29.3 (+), 29.1 (+), 25.8 (−), 24.9 (+), 24.6 (+), 18.2 (+), 4.9 (−), 4.6 (−). HRMS (EI): m/z calcd for $C_{16}H_{33}O_3Si$ (M^+ − CHO), 301.2199; found, 301.2196.

9-(*tert*-Butyl-dimethylsilanyloxy)-12-oxo-dodec-10-enoic Acid Methyl Ester (21). A toluene solution (20 mL) of (triphenylphosphoranylidene)acetaldehyde (267 mg, 0.88 mmol) and aldehyde **20** (292 mg, 0.88 mmol) was heated under reflux for 4 h under argon. The solvent was then removed under reduced pressure by rotary evaporation. The residue was purified by flash chromatography on a silica gel column (10% ethyl acetate in hexanes, TLC: R_f = 0.22), affording **21** (186 mg, 56%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.58 (d, J = 8.0 Hz, 1H), 6.80 (dd, J =15.5, 4.5 Hz, 1H), 6.26 (ddd, J =15.5, 8.0, 1.3, 1H), 4.3−4.5 (m, 1H), 3.67 (s, 3H), 2.31 (t, J = 7.5 Hz, 2H), 1.5−1.7 (4H), 1.2−1.4 (6H), 0.91 (s, 9H), 0.07 (s, 3H), 0.03 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 193.5, 174.1, 160.2, 130.6, 71.6, 51.4, 37.1, 34.0, 29.3, 29.1, 29.0, 25.8, 24.8, 24.7, 18.1, −4.7, −4.9. HRMS (EI): m/z calcd for $C_{18}H_{35}O_3Si$ (M^+ − CHO), 327.2355; found, 327.2358. m/z calcd for $C_{19}H_{36}O_4Si$ (M^+), 356.2383; found, 356.2354.

9-(*tert*-Butyl-dimethylsilanyloxy)-12-hydroxyoctadeca-10(*E*),-13(*E*)-dienoic Acid Methyl Ester (22). *n*-Butyllithium (0.47 mmol, 1.6 in THF, 0.29 mL) was added dropwise over 20 min to the iodide **6** (97.8 mg, 0.47 mmol) in anhydrous ether (10 mL) at −78 °C under argon. The solution was stirred for 1 h and then transferred into a solution of alkenal **21** (166 mg, 0.47 mmol) in 10 mL of ether using a cannula. After being stirred for 4 h, the mixture was brought to room temperature and stirred for 0.5 h. The reaction was quenched with aqueous sodium bicarbonate (10 mL) and extracted with ether. The combined organic layer was dried with magnesium sulfate and concentrated under reduced pressure by rotary evaporation, affording colorless oil, which was purified by flash chromatography on a triethylamine-treated silica gel column (15% ethyl acetate in hexnaes, R_f = 0.24 and 0.3) to afford **22** (48%). ^{1}H NMR (CD_3OD, 200 MHz): δ 5.55−5.8 (3H), 5.45 (dd, J = 15.4, 6.4 Hz, 1H), 4.45−4.55 (m, 1H), 4.1−4.2 (m, 1H), 3.66 (s, 3H), 2.32 (t, J = 7.5 Hz, 2H), 1.98−2.15 (m, 2H), 1.0−1.7 (m, 16H), 0.91 (s, 12H), 0.07 (s, 3H), 0.04 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz, APT): δ 176.1 (+), 134.9 (−), 134.8 (−), 133.2 (−), 133.2 (−), 133.2 (−), 133.1 (−), 132.8 (−), 132.7 (−), 74.5 (−), 74.3 (−), 74.0 (−), 73.8 (−), 52.0 (−), 39.4 (+), 34.8 (+), 33.0 (+), 32.6 (+), 30.5 (+), 30.4 (+), 30.1 (+), 26.5 (−), 26.3 (−), 26.1 (+), 23.2 (+), 19.1 (+), 14.3 (−), −3.9 (−), −4.5 (−). HRMS (EI): m/z calcd for $C_{25}H_{46}O_3Si$ (M^+ − H_2O), 422.3216; found, 422.3215.

9,12-Dihydroxyoctadeca-10,13-dienoic Acid (14b). Ester **22** (30 mg, 0.068 mmol) in THF (0.5 mL) was added to an aqueous solution (5 mL, pH 7.1−7.2) containing NaCl (1.7 mg), $CaCl_2$ (5 mg), and porcine pancreas lipase (20 mg, type II, crude). The resulting mixture was stirred at room temperature, and the pH was maintained between 7 and 7.2 by addition of NaOH solution (0.1 N). After 24 h, pH of the solution became constant, and solvents were removed with a rotary evaporator. The residue was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried with magnesium sulfate, and concentrated to obtain 20 mg of crude acid. The acid was dissolved in anhydrous THF (0.7 mL), and then TBAF (0.15 mL, 1 M in THF, 0.15 mmol) was added dropwise over 5 min. After 12 h, the reaction was quenched with water (5 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine and dried with magnesium sulfate. After concentration, the residue was purified on a triethylamine-treated silica gel column first with 2% methanol in chloroform, and then eluted with 100% methanol to afford **14b** (15 mg, 73%). ^{1}H NMR (CD_3OD, 600 MHz): δ 5.56−5.8 (m, 3H), 5.49 (ddt, J = 15.37, 6.61, 1.45 Hz, 1H), 4.53 (dd, J = 6.68, 5.79 Hz, 1H), 4.05 (td, J = 12.45, 6.08 Hz, 1H), 2.23 (t, J = 7.79 Hz, 2H), 2.08 (td, J = 7.72, 6.81 Hz, 2H), 1.62 (2H), 1.30−1.58 (14H), 0.94 (t, J = 7.17 Hz, 3H). ^{13}C NMR (CD_3OD, 50 MHz, APT): δ 181.8 (+), 134.7 (−), 134.6 (−), 133.6 (−), 133.5(−), 133.1 (−), 133.0 (−), 132.9 (−), 132.8 (−), 73.9 (−), 73.8 (−), 73.1 (−), 73.0 (−), 38.5 (+), 33.1 (+), 32.6 (+), 30.7 (+), 30.6 (+), 27.3 (+), 26.6 (+), 26.6 (+), 23.3 (+), 14.3 (−). HRMS (EI): m/z calcd for $C_{18}H_{30}O_3$ (M^+ − H_2O), 294.2195; found, 294.2213.

Derivatization of Individual diHPODE Methyl Esters for GC−MS Analysis. To a methanol solution containing 500 μg of 9,12-diHPODE methyl ester (**2a**) or 10,13-diHPODE methyl ester (**2b**) was added $NaBH_4$ (500 μg). The reaction temperature was kept below 15 °C. After the mixture was stirred 15 min, water was added and the product was extracted into CH_2Cl_2. The dried extract dissolved in ethanol was hydrogenated using PtO_2 as catalyst. The reaction was complete in 5 min. After filtration and evaporation, the residue was converted into TMS ether **23** by treatment with 500 μL of 10% trimethylchlorosilane in bis(trimethylsilyl)trifluoroacetamide at 65 °C for 45 min immediately prior to GC−MS analysis.

Decomposition of diHPODE. For UV-induced decomposition, a solution of DiHPODEs (20 μg) in methanol (100 μL) was evaporated under a stream of dry nitrogen to a film in the bottom

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of a 2 mL screw cap glass vial. The tube was capped under air and placed in the center of a Rayonet photochemical reactor and irradiated with three 80 W low-pressure mercury UV (350 nm) Rayonet lamps. The temperature was maintained at about 28 °C. After various times of irradiation, the tubes were uncapped and 20 μL of 200 μM BHT in methanol and 20 μL of 200 μM diethylenetriaminepentaacetic acid (DTPA) in methanol were added to quench the reaction. To examine the effect of α-tocopherol on the decomposition of diHPODE, samples of diHPODEs (40 μg) and 0−300 wt % (relative to diHPODE) of α-tocopherol were incubated in a 2 mL vial as a dry film (see above) at 37 °C for 2 h. The samples were recapped under a blanket of argon and stored under −80 °C until analysis by LC-ESI-MS/MS. To examine the effect of DTPA on the decomposition of diHPODE, DTPA (10 μg) was added to each vial before incubation. For LC-MS/MS analysis, the sample was dissolved in methanol (200 μL) containing internal standard, 9-(2-oxanyloxy)-11-(3,3-dimethyl-2,4-dioxolanyl)undec-10-enoic acid (40 ng). For MRM experiments, the mass transitions *m*/*z* 343→137, 225→109, 227→84, 383→101 were used to monitor diHPODE, KODA, HODA, and internal standard, respectively. Calibration curves were built by injecting various amounts of diHPODE, HODA, and KODA and 10 ng of internal standard into the LC/MS/MS.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of new compounds and ESI-MS/MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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