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Synthesis of Six Epoxyketooctadecenoic Acid (EKODE) Isomers, Their Generation from Nonenzymatic Oxidation of Linoleic Acid, and Their Reactivity with Imidazole Nucleophiles

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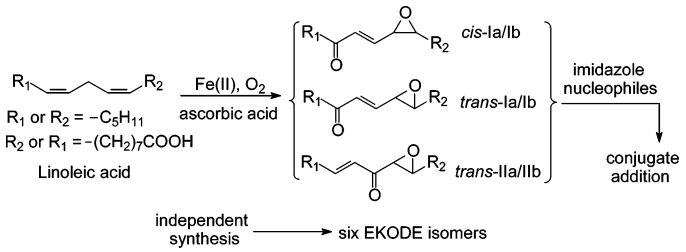

As a class of linoleic acid oxidation products, epoxyketooctadecenoic acids (EKODEs), are formed *in vivo* and *in vitro* by a free radical mechanism initiated by either enzymatic or nonenzymatic pathways. They have so far been made available in small-scale quantities, often as isomeric mixtures, from reductive decomposition of linoleic acid-derived hydroperoxides. There is major interest in these compounds owing to their highly potent biological activities and their ability to covalently modify proteins. The synthesis of six EKODE regio- and stereoisomers, two trans α',β'-epoxy-α,β-enones, and two trans and the two cis γ,δ,-epoxy-α,β-enones was accomplished, with the key steps being Wittig-type reactions and aldol condensations. All six EKODE isomers were confirmed by HPLC to be generated in the autoxidation of linoleic acid promoted by Fe(II)/ascorbic acid through spiking in of authentic samples. On the basis of evidence for EKODE modification of protein His residues, the reactions of N^{α}-benzoyl-L-histidine with autoxidizing linoleic acid and with the individual EKODE isomers were compared, as were the kinetics of the various EKODE reactions with imidazole nucleophiles. The structures of His-EKODE-(*E*)-I adducts were confirmed to reflect conjugate addition (epoxide ring remains intact) through an NMR study of the reaction of imidazole with a generic EKODE-(*E*)-I analog. The synthesis of the EKODE isomers makes these important molecules available for further chemical and biological evaluation.

Introduction

Polyunsaturated fatty acids (PUFAs) may undergo both enzymatic and nonenzymatic lipid peroxidation leading to unsaturated lipid hydroperoxides (LOOHs). The former process contributes to physiological generation of bioactive mediators such as the inflammatory agents leukotrienes,[1] whereas the latter process can reflect a cellular state of oxidative stress.[2] The initially formed hydroperoxides can undergo a variety of secondary reactions, some leading to stable oxygenated and polyoxygenated acyl chains and others leading to chain cleavage and production of metabolites containing either the methyl or carboxy terminus, including reactive aldehydes such as 4-hydroxy-2-nonenal.[3,4] Our laboratory has been interested in the modification of protein side chains by electrophilic products of lipid peroxidation. Many such adducts have been characterized through conduct of model studies using amino acyl side-chain surrogates and individual synthetic lipoxidation-derived aldehydes,[5] and the generation of such adducts on proteins has been confirmed through mass spectrometric studies.[6]

Linoleic acid is the most abundant polyunsaturated fatty acid in living systems. As part of our efforts to determine the

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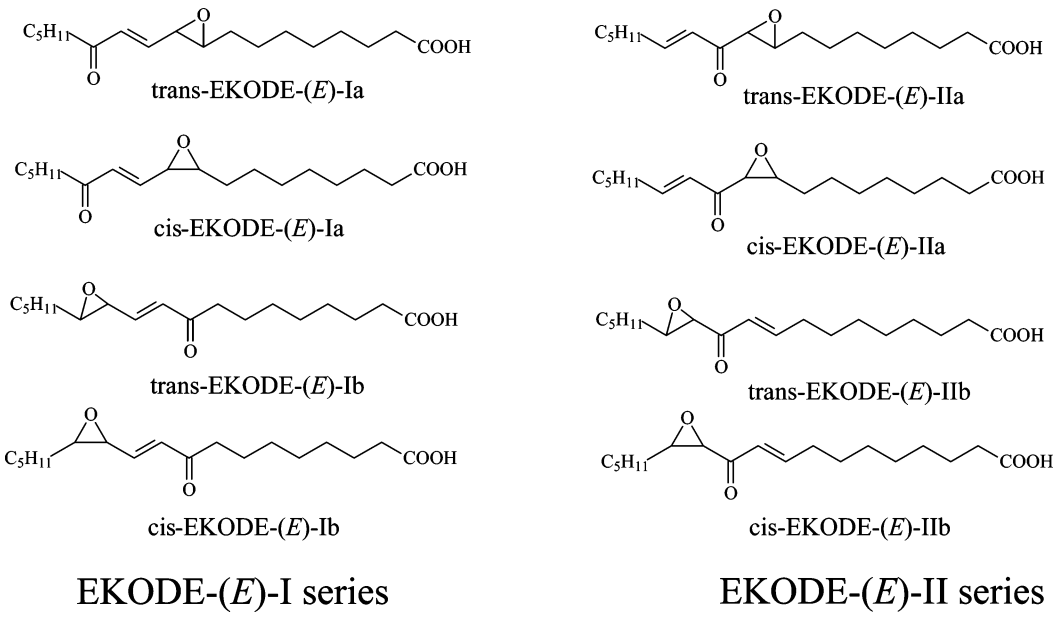
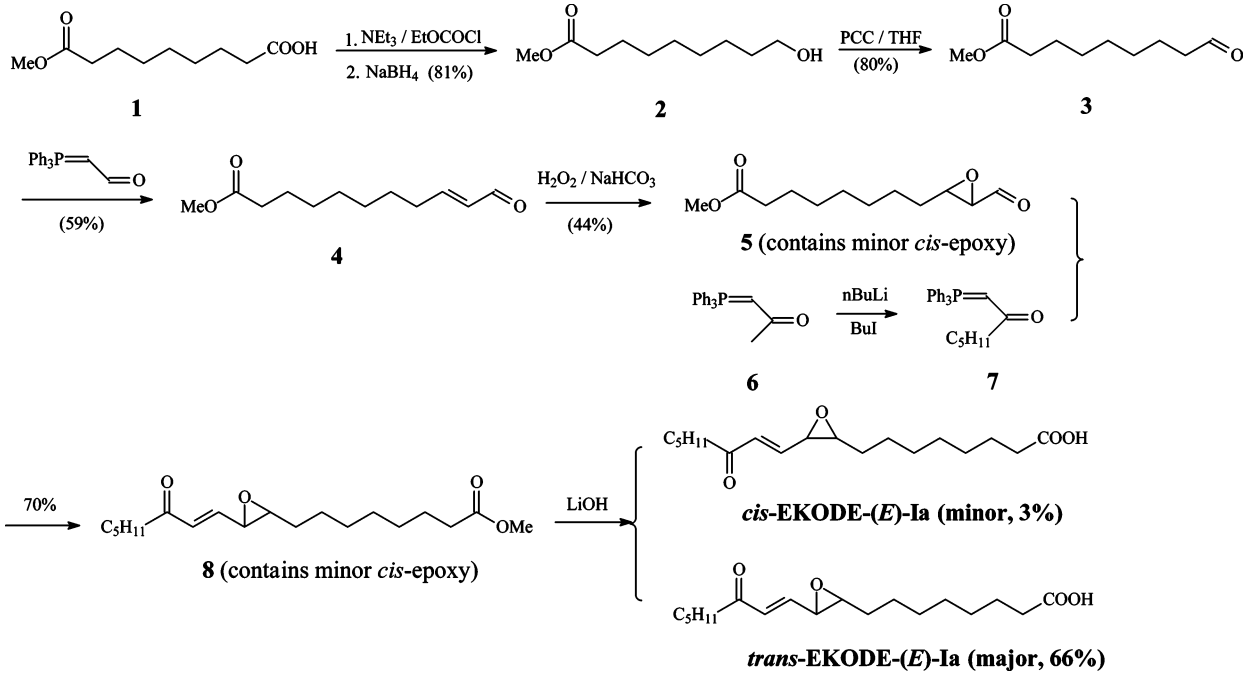


FIGURE 1. Proposed common nomenclature of EKODE-(*E*) isomers.

SCHEME 1. Synthetic Route to *trans*/*cis*-EKODE-(*E*)-Ia



principal protein-modifications that would be generated when a protein is exposed to linoleic acid undergoing oxidation, we obtained evidence using mild oxidation conditions (Fe(II) + ascorbate) of selective His imidazole adduction by one or more isomers of an electrophile with MW = 310 Da, corresponding to a linoleic acid derivative with two oxygen atoms and one

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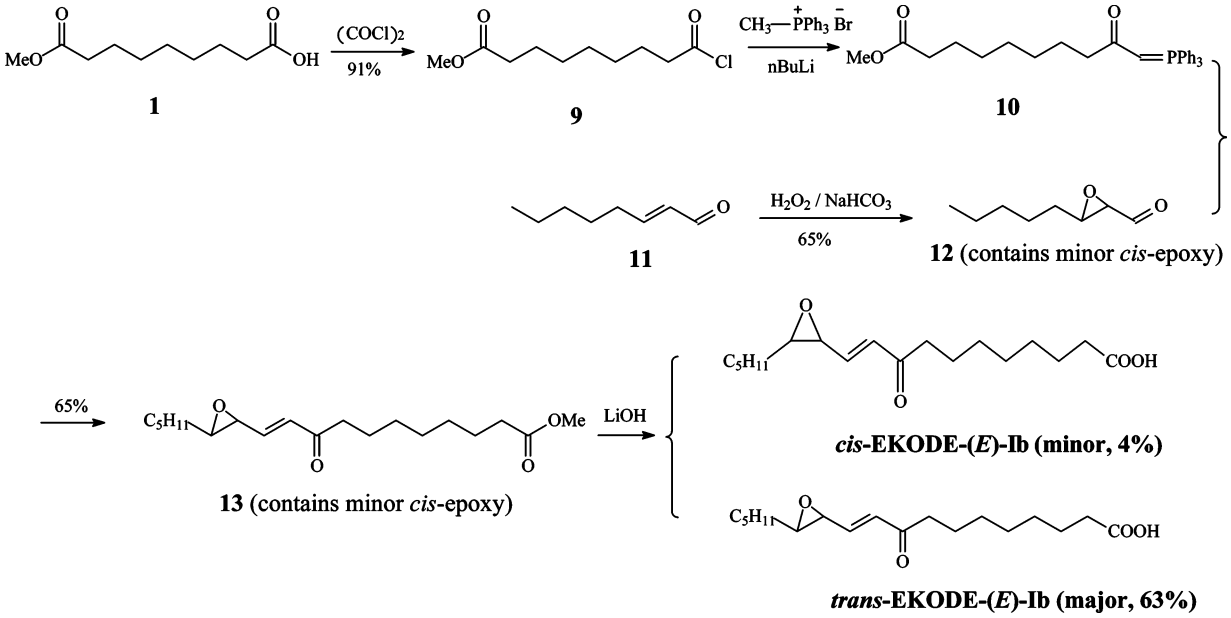
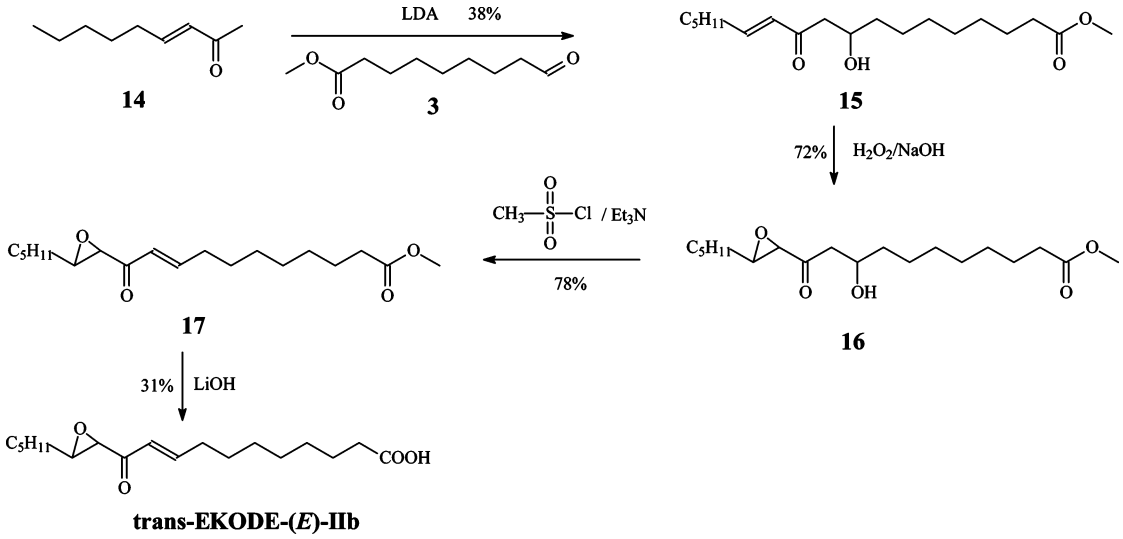
SCHEME 2. Synthetic Route to *trans*/*cis*-EKODE-(*E*)-Ib



SCHEME 3. Synthetic Route to *trans*-EKODE-(*E*)-IIb



additional degree of unsaturation.[6b] A search of the literature revealed that various isomers of α,*β*-unsaturated epoxyketooctadecenoic acid (EKODE)[7] with this molecular weight are generated naturally from linoleic acid oxidation, and we suspected they would be capable of undergoing reaction with protein-based His nucleophiles. Although no indication of such covalent binding potential is evident in the literature, these EKODE isomers have been shown, in a series of studies, to have pronounced biological activity in stimulating steroid hormone production and secretion[8] and in increasing cellular calcium.[9] Thus, regardless of whether any EKODE biological activity is associated with their covalent binding capacities, access to the individual isomers is crucial to advancing structure−activity knowledge on these highly potent bioactive molecules.

The chemical nature of mild linoleic acid oxidation without chain cleavage was studied principally by the Gardner group in the 1970s and early 1980s.[7] In these studies, the researchers chose to isolate and purify the linoleic acid-derived hydroperoxides and then effected their homolytic decomposition by a cysteine•$FeCl_3$ catalyst in the presence of air or O_2. The first study started from the mixture of 13-hydroperoxy-9*Z*,11*E*-octadecadienoic acid (13-HPODE) and 9-hydroperoxy-10*E*,12*Z*-octadecadienoic acid (13-HPODE) and yielded the two *trans*-*γ*,*δ*-epoxy-α,*β*-enone EKODE isomers as a mixture of regioisomers. Characterization of the mixture of two corresponding *cis*-epoxy regioisomers required esterification with diazomethane, which achieved nearly complete separation from contaminating hydroxyoctadecadienoic acids.[7a] The regioisomeric composition in neither case could be discerned by NMR on account of the apparent identity of the spectra at the low field available at the time. Seven years later, these workers succeeded in isolating the individual regioisomers as methyl esters, starting

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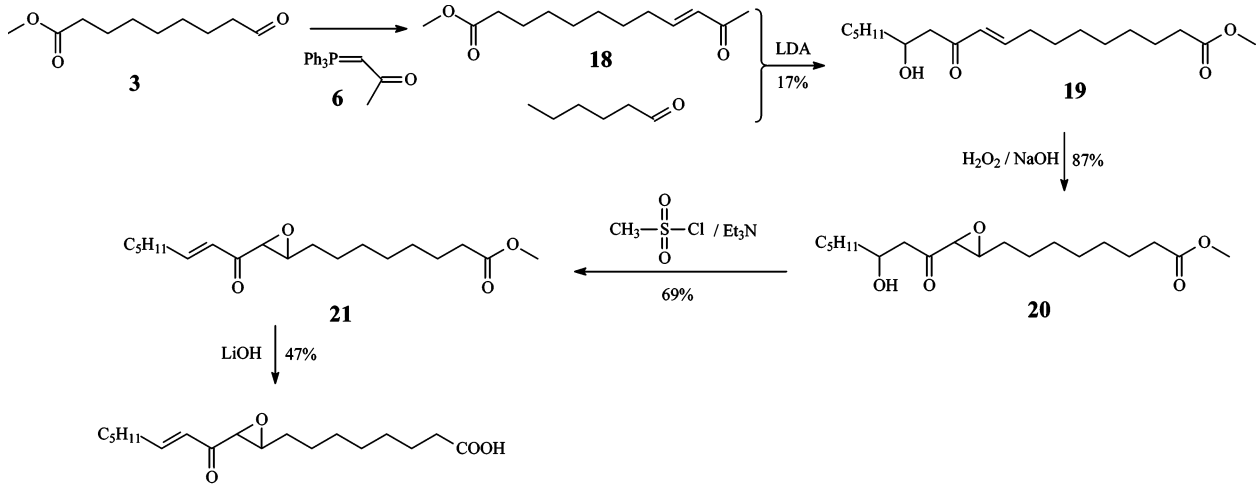
SCHEME 4. Synthetic Route to *trans*-EKODE-(*E*)-IIa



with either pure 9-HPODE or pure 13-HPODE, but no additional NMR characterization was provided.[7b] In that same year, α′,β′-epoxy-α,β-enone EKODE isomers were also observed to form under similar reaction conditions for degradation of the initially formed hydroperoxides.[7c,d]

To provide access to individual EKODE isomers needed for probing the structural basis of their highly potent biological activities, and to determine if certain isomers are more reactive than others in modifying protein His residues, it was desirable to devise methods for total synthesis of the various individual EKODE regioisomers and geometrical diastereomers. We chose to prepare the six isomers suspected of being produced in greatest amount from mild oxidation of linoleic acid under physiomimetic conditions, based on the earlier work by the Gardner group. In general, the preparative procedures and purification techniques used were straightforward and the yields were satisfactory. Although the current work describes the synthesis of EKODE isomers as racemic mixtures, modifications of the synthetic routes chosen could provide access to homochiral versions if it were desirable to study the individual optical antipodes.

With the six authentic isomers in hand, we first verified by analytical HPLC that these six compounds are indeed formed in the Fe(II)/ascorbate-mediated oxidation of linoleic acid, and their relative yields were estimated through knowledge of the respective extinction coefficients. We then went on to show that exposure of N^{α}-benzoyl-L-histidine to autoxidizing linoleic acid does indeed result in adducts, which are the same as those formed in reaction with the individual synthetic EKODE isomers and which represent conjugate addition with retention of the epoxide ring. The relative reactivities of the various isomers toward imidazole nucleophiles were then determined. These results provide the chemical background for interpreting a subsequent study (to be published elsewhere) that describes protein modification by the EKODE isomers and by autoxidizing linoleic acid.

Proposed Nomenclature of EKODE Isomers. Because of the variety of structural EKODE isomers that can be formed in the nonenzymatic oxidation of linoleic acid, we felt it was worthwhile to propose a common (non-IUPAC) nomenclature for this series of compounds. As Figure 1 shows, there are two different families, one with the epoxy function adjacent to the C═C of the enone moiety and the other with the epoxy function adjacent to the C═O of the enone moiety, which we assigned as the EKODE-(*E*)-I and EKODE-(*E*)-II series, respectively, where (*E*) refers to the C═C stereochemistry. Because the C═C bond is formed in free radical steps leading to formation of the precursor hydroperoxides, it would be mainly in the trans form, so that the EKODE-(*Z*)-I/II forms would be expected to be minor. We used the "trans/cis" identifier to define the stereochemistry of the epoxy group, which apparently is more likely to be generated physiologically in both stereochemical forms than is the C═C (the trans epoxy forms are more stable and should thus be more prominent). Last, "a" and "b" were used to define whether the epoxy function is near the carboxy or methyl terminus, respectively. In this way, the proposed nomenclature and structures of the eight possible EKODE-(*E*) isomers are shown in Figure 1. Of the eight isomers shown, we omitted synthesis of the two *cis*-EKODE-(*E*)-II isomers, on the basis of their not being observed in any report by Gardner and co-workers.

Results and Discussion

Preparation of *trans*/*cis*-EKODE-(*E*)-Ia. The synthetic procedure for *trans*/*cis*-EKODE-(*E*)-Ia was based on the preparation of methyl 11-oxoundec-9-enoate followed by epoxidation to give methyl 11-oxo-9,10-epoxyundecanoate and subsequent chain elongation by a Wittig-type reaction, followed by demethylation (Scheme 1). Commercially available azelaic acid monomethyl ester **1** was converted in two steps to methyl 9-oxononanoate **3** in 64% overall yield. Elongation of **3** by a Wittig-type reaction led to α,β-unsaturated aldehyde **4** in 59% yield. Epoxidation of **4** with alkaline H_2O_2 proceeded in 44% yield to give *trans*-α,β-epoxy aldehyde **5**. However, as the literature on epoxidation of *trans*-α,β-unsaturated aldehydes by alkaline H_2O_2 suggests the expectation of production of a minor amount of the *cis*-α,β-epoxy product,[10] **5** was assumed to contain a minor cis isomer contaminant. A Wittig-type reaction

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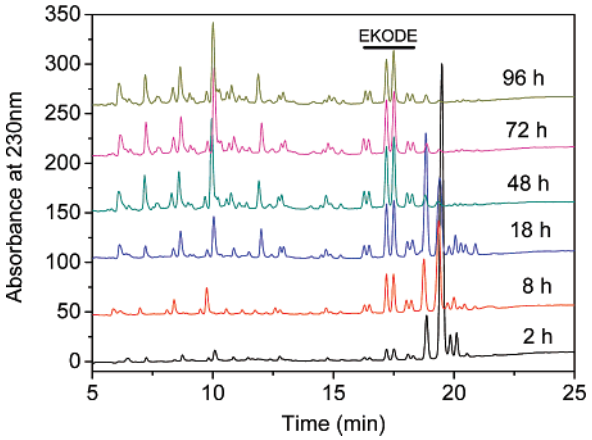


FIGURE 2. HPLC chromatogram (230 nm) for the incubation of linoleic acid (5 mM), $Fe(NH_4)_2(SO_4)_2$ (0.5 mM), and ascorbic acid (1.0 mM) in 50 mM pH 7.4 HEPES buffer containing 20% ethanol at 37 °C for times ranging from 2 to 96 h.

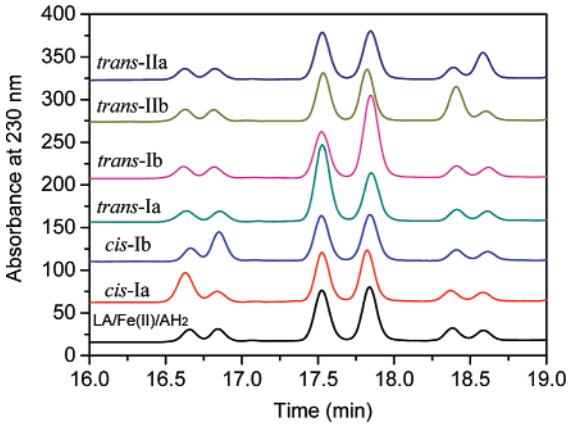


FIGURE 3. HPLC chromatogram (230 nm) of the incubation of linoleic acid (5 mM), $Fe(NH_4)_2(SO_4)_2$ (0.5 mM), and ascorbic acid (1.0 mM) in 50 mM pH 7.4 HEPES buffer containing 20% ethanol at 37 °C for 48 h (lower trace), with spiking with independently synthesized EKODE isomers (six upper traces).

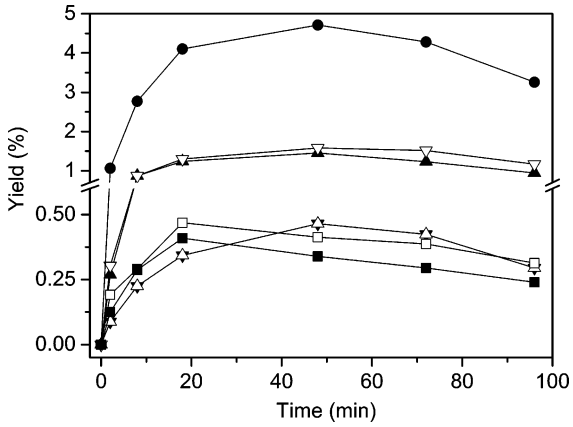


FIGURE 4. Time profile of the yield of EKODE isomers generated from the incubation of linoleic acid (5 mM), $Fe(NH_4)_2(SO_4)_2$ (0.5 mM), and ascorbic acid (1.0 mM) in 50 mM pH 7.4 HEPES buffer containing 20% ethanol at 37 °C. ▲, *trans*-EKODE-(*E*)-Ia; ▽, *trans*-EKODE-(*E*)-Ib; ■, *trans*-EKODE-(*E*)-IIa; □, *trans*-EKODE-(*E*)-IIb; ▼, *cis*-EKODE-(*E*)-Ia; △, *cis*-EKODE-(*E*)-Ib; ●, total of six EKODE isomers.

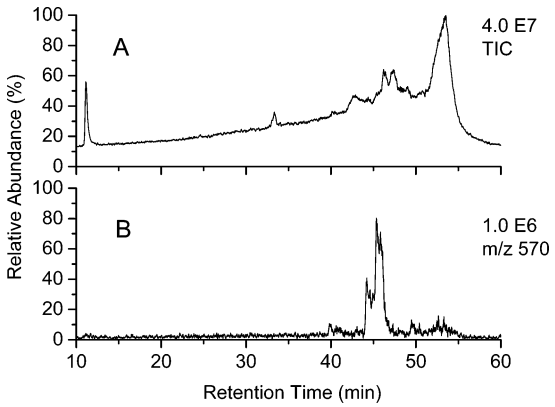


FIGURE 5. Reversed phase LC-MS analysis of products derived from Fe(II)-mediated autoxidation of linoleic acid in the presence of N^{α}-benzoyl-L-histidine. (A) TIC; (B) SIC for MH^+ (*m*/*z* 570) expected for EKODE (M_w = 310) Michael adducts.

of **5** with **7**, prepared from 1-triphenylphosphoranylidene-2-propanone **6**, gave rise to methyl 13-oxo-9,10-*trans*-epoxy-11-(*E*)-octadecenoate (**8**) in 70% yield, which was also assumed to contain the *cis*-epoxy isomer as a minor contaminant. The final hydrolytic demethylation step was accomplished by LiOH and allowed isolation (after preparative TLC separation) of 66% *trans*-EKODE-(*E*)-Ia and 3% *cis*-EKODE-(*E*)-Ia, with stereochemistry being verified by ^{1}H NMR spin−spin couplings.

Preparation of *trans*/*cis*-EKODE-(*E*)-Ib. The synthetic procedure for *trans*/*cis*-EKODE-(*E*)-Ib was similar to that for *trans*/*cis*-EKODE-(*E*)-Ia except for the use of a reverse Wittig coupling strategy, as shown in Scheme 2. Commercially available azelaic acid monomethyl ester **1** was converted to its acid chloride **9** in 91% yield. The latter was converted to Wittig reagent **10** by reaction with the ylide resulting from stoichiometric *n*-BuLi-mediated deprotonation of methyltriphenylphosphonium bromide. The epoxidation of commercially available 2-octenal (**11**) with alkaline H_2O_2 afforded in 65% yield the α,β-epoxyaldehyde **12**, which was presumed to contain a mixture of the major trans and minor cis isomers. Elongation of **12** by a Wittig-type reaction with **10** gave rise to a mixture of methyl 13-oxo-9,10-(*cis*/*trans*)-epoxy-11(*E*)-octadecenoate **13** in 65% yield. The final demethylation step was performed by LiOH and allowed isolation (after preparative TLC separation) of 63% *trans*-EKODE-(*E*)-Ib and 4% *cis*-EKODE-(*E*)-Ib.

Preparation of *trans*-EKODE-(*E*)-IIb. The synthetic procedure for *trans*-EKODE-(*E*)-IIb was based on the preparation of methyl 9-hydroxy-11-oxooctadec-12(*E*)-enoate **15**, subsequent epoxidation to give methyl *trans*-12,13-epoxy-9-hydroxy-11-oxooctadecanoate **16**, and subsequent dehydration and then demethylation (Scheme 3). Aldol condensation of commercially available 3-nonen-2-one **14** with **3** afforded **15** in 38% yield. Epoxidation of **15** with alkaline H_2O_2 afforded *trans*-epoxy ketol **16** in 72% yield after chromatographic isolation, as a mixture of diastereomers. The dehydration of **16** by methanesulfonyl chloride afforded **17** in 78% yield as a single diastereomer. The final demethylation step was accomplished by LiOH to give *trans*-EKODE-(*E*)-IIb in 31% yield.

Preparation of *trans*-EKODE-(*E*)-IIa. The synthetic procedure for *trans*-EKODE-(*E*)-IIa was similar to that for *trans*-EKODE-(*E*)-IIb. As shown in Scheme 4, elongation of **3** by Wittig reagent **6** led to α,β-unsaturated aldehyde **18** in 75% yield. Aldol condensation of **18** with hexanal resulted in methyl 13-hydroxy-11-oxooctadec-9-enoate **19** in 17% yield. The

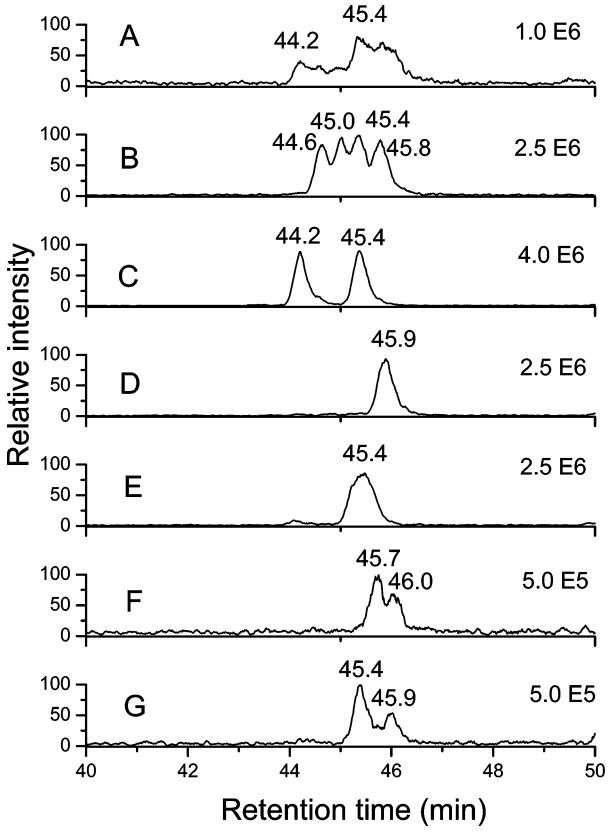


FIGURE 6. Reversed phase LC selected ion chromatograms for MH^+ (*m*/*z* 570) of the N^{α}-benzoyl-L-histidine adducts of (A) EKODE isomers derived from autoxidation of linoleic acid ($Fe(II)/AH_2$); (B) *trans*-EKODE-IIb; (C) *trans*-EKODE-IIa; (D) *trans*-EKODE-Ib; (E) *trans*-EKODE-Ia; (F) *cis*-EKODE-Ib; and (G) *cis*-EKODE-Ia.

epoxidation of **19** with alkaline H_2O_2 gave, after chromatographic isolation, methyl *trans*-9,10-epoxy-13-hydroxy-11-oxooctadecanoate **20** (87% yield) as a mixture of two diastereomers, which was dehydrated with methanesulfonyl chloride to afford methyl *trans*-9,10-epoxy-11-oxooctadec-12-enoate **21** in 69% yield as a single diastereomer. The final hydrolytic demethylation step was performed by LiOH and gave *trans*-EKODE-(*E*)-Ia in 47% yield.

Relative Yield of EKODEs from Nonenzymatic Autoxidation of Linoleic Acid. With six isomers of EKODE in hand, the next goal was to determine the yield of each of the various EKODE isomers arising from nonenzymatic oxidation of linoleic acid under the conditions, we had used to achieve modification of protein side-chains by linoleic acid oxidation products. Thus, incubation of linoleic acid with 0.1 equiv of Fe(II) and 0.2 equiv of ascorbic acid resulted in a product mixture (Figure 2), which was shown by analytical HPLC to contain six EKODE isomers (four EKODE-(*E*)-I isomers and two *trans*-EKODE-(*E*)-II isomers), identified by their diode array absorbance spectra (not shown) and by spiking with the authentic samples (Figure 3) prepared above. The yields of the six EKODE isomers after 18 h of oxidation were determined to be 0.34% (*cis*-EKODE-(*E*)-Ia), 0.34% (*cis*-EKODE-(*E*)-Ib), 1.24% (*trans*-EKODE-(*E*)-Ia), 1.30% (*trans*-EKODE-(*E*)-Ib), 0.41% (*trans*-EKODE-(*E*)-IIa), and 0.47% (*trans*-EKODE-(*E*)-IIb). The combined yield of these six EKODE isomers was 4.1%, increasing to 4.7% at the 48 h time point of autoxidation (Figure 4), after which point the yield decreased, probably because of further oxidation of the initially formed EKODE isomers.

LC/MS Comparison of the Products of the Reactions of N^{α}-Bz-His either with Autoxidizing Linoleic Acid or with Individual EKODE Isomers. After confirming the presence of six EKODE isomers in the linoleic acid autoxidation mixture, the latter was also analyzed by LS-MS/MS, with selected ion monitoring at *m*/*z* 311 (MH^+) to determine if there are additional oxidation products isomeric with the six identified EKODE products. Six small peaks in the selected ion chromatogram (SIC) were observed in the elution time range of 17−25 min (Figure S45), which were not apparent as discrete peaks in the total ion chromatogram (TIC). In contrast, the six major peaks seen in the range of 28−35 min were also the only prominent peaks seen in the TIC in this elution time range (Figure S46), and the elution pattern and intensities matched that for the EKODE isomers in the HPLC-UV chromatogram (Figure 3) (though the elution times were longer due to the different gradient eluent used). MS^3 analysis of the four faster eluting peaks (*cis*-EKODE-(*E*)-Ia/b and *trans*-EKODE-(*E*)-Ia/b) each exhibited a prominent granddaughter fragment at *m*/*z* 293 (example in Figure S46), occasionally accompanied by a weak peak at *m*/*z* 275, whereas the late eluting peaks (*trans*-EKODE-(*E*)-IIa,b) exhibited a prominent granddaughter fragment at *m*/*z* 275, with an occasional weak peak at *m*/*z* 293. As these fragments appear to represent the loss of one or two water molecules, it appears that the EKODE-I and EKODE-II series can exhibit distinctive collision-induced dissociation chemistry.

The above results provide presumptive evidence that the protein modifications corresponding to adduction of 310 Da to His residues in the presence of autoxidizing linoleic acid[6b] represent adduction of one or more EKODE isomers. To confirm the ability of His side-chains to undergo adduction by EKODEs, N^{α}-benzoyl-L-histidine (BzHis) was used as a surrogate nucleophile for protein-based His to react both with autoxidizing linoleic acid and with authentic EKODE isomers. Thus, 2 mM BzHis was incubated with 5 mM linoleic acid, 1 mM ascorbic acid, and 0.5 mM $Fe(NH_4)_2(SO_4)_2$ or with 0.2 mM each EKODE isomer in 50 mM, pH 7.4 HEPES buffer at 37 °C for 18 h. LC/MS analysis of the reaction mixture of BzHis with autoxidizing linoleic acid (TIC shown in Figure 5A) showed a broad stretch of peaks eluting at 40−55 min, including peaks with retention time from 44 to 46 min that corresponding to MH^+ ion at *m*/*z* 570 (SIC shown in Figure 5B). The latter peaks were assigned to a mixture of isomeric EKODE BzHis adducts. MH^+ ions at *m*/*z* values corresponding to BzHis modification by other possible linoleic acid-derived reactive species, including 4-hydroxy-2-nonenal (HNE), were not observed (data not shown), a result consistent with the apparently quite selective generation of the EKODE isomers relative to chain-cleavage products, under these apparently rather mild linoleic acid oxidation conditions.

For the LC-MS analysis of the reactions of BzHis with each of the six synthetic EKODE isomers (Figure 6), the selected ion chromatograms (SICs) at *m*/*z* 570 in all cases were consistent with the expected EKODE-His adducts. However, the adducts from the different EKODE isomers showed different retention time and peak patterns. For example, the chromatogram for adduction of *trans*-EKODE-IIb to BzHis revealed the presence of four peaks with retention times of 44.6, 45.0, 45.4, and 45.8 min (Figure 6B). This finding is consistent with the formation of all four possible diastereomers that would be produced upon conjugate addition of BzHis to *trans*-EKODE-IIb, which would create a new chiral center (*R* or *S*) superimposed on the existing

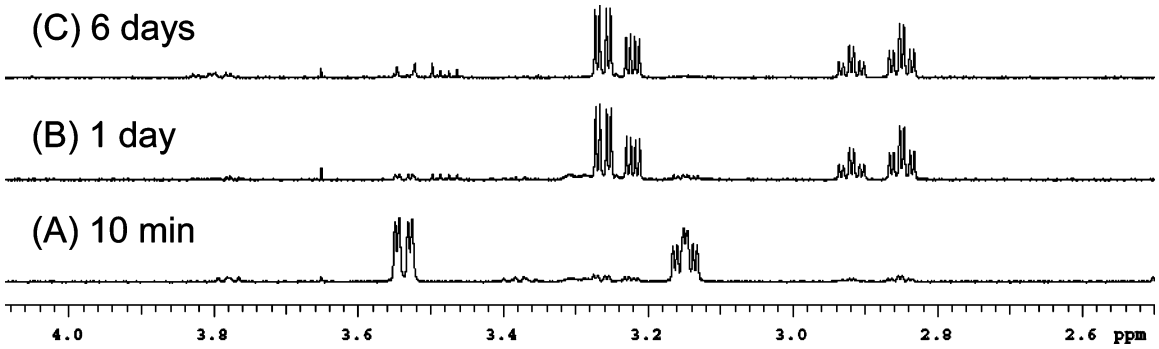
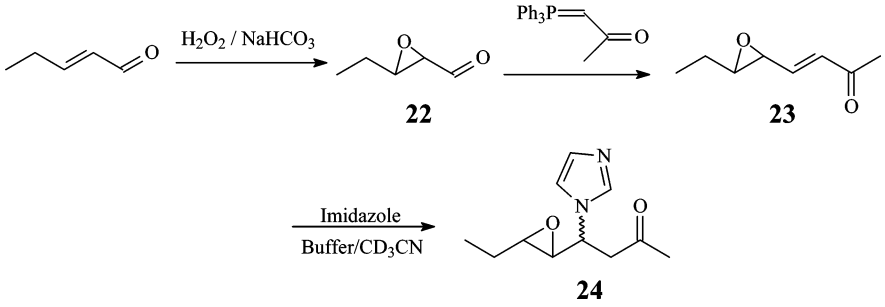


FIGURE 7. Expanded NMR spectra (2.6−4.0 ppm region) for the conversion of *trans*-5,6-epoxy-3(*E*)-octen-2-one (**23**) to its imidazole Michael adduct(s) **24** in 100 mM, pH 7.4 deuterated sodium phosphate buffer containing 20% CD_3CN, 25 °C.

SCHEME 5. Synthesis of *trans*-5,6-Epoxy-3(*E*)-octen-2-one (23) and Its Reaction with Imidazole



epoxide chirality (*R,R* or *S,S*) and the fixed His α-carbon chiral center. In contrast, epoxide ring-opening, also consistent with a +310 Da mass addition, would be regio- and likely also stereospecific and, thus, should give only two diastereomers. Curiously, BzHis adducts of other EKODE isomers showed only one or two peaks in their chromatograms (Figure 6C−G). However, since it is unlikely that there would be a changeover to epoxide ring-opening for these isomers, the latter observation most likely represents coelution of Michael adduct diastereomers in these cases, though we cannot exclude the possible occurrence of diastereoselective adduction chemistry.

Comparing the SICs at *m*/*z* 570 for the reaction of BzHis with autoxidizing linoleic acid (Figure 6A) with the same *m*/*z* 570 SICs obtained for reaction of BzHis with the different EKODE isomers, it is possible to estimate the extents to which adduction by the various EKODE isomers contribute to the adduct profile generated from autoxidizing linoleic acid. For example, the most intense peak in Figure 6A near 45.4 min appears to represent some combination of contributions from one of four peaks seen for *trans*-EKODE-IIb and one of the two peaks seen for *trans*-EKODE-IIa, *trans*-EKODE-Ia, and/or *cis*-EKODE-Ia. The other large peak in Figure 6A with retention time 44.2 min can only come from one of the two peaks of *trans*-EKODE-IIa. On the basis of the similarity in the structure of all the isomeric EKODE BzHis Michael adducts, and the assumption that their ESI sensitivities are thus not significantly different, the above qualitative analysis suggests that *trans*-EKODE-IIa/b would represent the major EKODE isomers that reacted with BzHis. Although the yield of EKODE-II was lower than of EKODE-I, the former is shown in kinetic studies described below to be much more histidine-reactive than the latter, which appears to play a key factor in the modifications that are observed during the 18 h linoleic acid autoxidation reaction.

Preparation EKODE Analog *trans*-5,6-Epoxy-3(*E*)-octen-2-one (23) and NMR Spectroscopic Study of its Imidazole Adduction Chemistry. To confirm our tentative mass spectrometric conclusion that the adduction of EKODE isomers by protein His residues under physiomimetic conditions represents Michael addition rather than epoxide ring-opening chemistry, we carried out an NMR spectroscopic study on the reaction of imidazole with the low molecular weight EKODE-I analog *trans*-5,6-epoxy-3(*E*)-octen-2-one (**23**). Compound **23** was prepared by epoxidation of 2(*E*)-pentenal to give **22** and subsequent chain elongation by a Wittig reaction (Scheme 5).

The reaction of **23** with 3 equiv of imidazole in 100 mM, pH 7.4 deuterated sodium phosphate buffer in D_2O containing 20% CD_3CN was monitored by ^1H NMR over time (Figure 7). Although the starting material could be seen after 10 min, more than 95% of **23** was transformed over 24 h with loss of the olefinic signals, confirming a conjugate addition. The epoxide methine region of the spectrum exhibited a change (upfield shift of the two ^1H signals) consistent with the formation of a diastereomeric mixture of imidazole Michael adducts **24** (Scheme 5), where integration indicated an isomer ratio of 1.7:1 (Figure 7B). A trace amount of Michael adduct **24** deteriorated in 6 days under these reaction conditions (possibly to the diol coming from epoxide hydrolysis), but the His-EKODE Michael adducts should clearly qualify as stable protein adducts at physiological pH conditions.

Although the above model study confirms the occurrence of conjugate addition chemistry under physiological conditions, the conduct of the reaction in deuterated solvent and the consequential exchange next to C═O precluded a full structural characterization of the adduct by NMR. To accomplish the latter, the reaction of imidazole (in excess) with **23** was repeated using $CDCl_3$ as solvent at room temperature. Monitoring the ^1H NMR spectrum over time showed disappearance of the olefinic

TABLE 1. Reaction Rate Constants for the Reaction of EKODE Isomers with Imidazole Nucleophiles[a]

EKODE	reaction with imidazole ($M^{-1}s^{-1}$)	reaction with N^{ω}-Ac-histamine ($M^{-1}s^{-1}$)
cis-Ia	$1.29 \pm 0.02 \times 10^{-3}$	$1.35 \pm 0.02 \times 10^{-3}$
cis-Ib	$1.28 \pm 0.02 \times 10^{-3}$	$1.40 \pm 0.07 \times 10^{-3}$
trans-Ia	$2.66 \pm 0.04 \times 10^{-3}$	$2.24 \pm 0.04 \times 10^{-3}$
trans-Ib	$2.64 \pm 0.07 \times 10^{-3}$	$2.22 \pm 0.10 \times 10^{-3}$
trans-IIa	$19.7 \pm 0.8 \times 10^{-3}$	$13.9 \pm 0.3 \times 10^{-3}$
trans-IIb	$19.5 \pm 1.2 \times 10^{-3}$	$13.8 \pm 0.4 \times 10^{-3}$

[a] Reactions were conducted at 30 °C in 50 mM, pH 7.4, sodium phosphate buffer containing 20% ethanol. [Imidazole nucleophile] = 5 mM, [EKODE] = 0.2 mM.

resonances after 18−36 h (Figure S47), confirming Michael adduct formation, and the appearance of two peaks at 4.85 and 4.75 ppm in a ratio of 4:1 (methine position bonded to imidazole), consistent with the formation of two diastereomeric Michael adducts with a diastereoselectivity somewhat different than in buffered aqueous CH_3CN solvent.

Relative Reactivity of EKODE Isomers with Histidine Analogs. From the foregoing conclusion that covalent adduction of His imidazole to EKODE isomers involves Michael addition, the disappearance of the α,β-unsaturated carbonyl chromophore should allow for monitoring the rates of the Michael addition reactions spectrophotometrically. Thus, each of the six EKODE isomers was allowed to react with an excess of either imidazole or N^{ω}-Ac-histamine, as surrogates of the protein His side-chain, under physiomimetic conditions (pH 7.4 sodium phosphate buffer containing 20% ethanol). The derived pseudo first-order rate constants are listed in Table 1. Whereas there were no significant rate differences observed within each regioisomeric pair of EKODEs, the different EKODE types showed significantly different reactivities. Thus, the two EKODEs in the EKODE-II series (*trans*-IIa and *trans*-IIb) were found to be 7−15 times more reactive than the four EKODE-I isomers, whereas the *trans*-Ia/Ib isomers were a little more than twice as reactive as the *cis*-Ia/Ib isomers. The low reactivity of the latter two isomers is also apparent from the lower adduct ion intensities seen for the mixture of adducts generated from autoxidizing linoleic acid (Figure 6).

In reality, the conclusions on adduct structure that we drew using EKODE model **23** pertain only to the EKODE-(*E*)-I series of isomers. Nonetheless, although we did not conduct an analogous model study for the EKODE-(*E*)-II series, the fact that the reactions with imidazole nucleophiles for all six EKODE isomers could be monitored by disappearance of the characteristic α,β-unsaturated carbonyl UV chromophore suggests that all reactions represent conjugate addition chemistry rather than epoxide opening. The much higher reactivity of the EKODE-II series over EKODE-I series may then reflect a steric slowing of Michael addition to the electrophilic position of the reactive C═C by the adjacent epoxide ring, though we cannot rule out a contribution by an electronic effect. The more modest difference in reactivity between *trans*-EKODE-Ia/b and *cis*-EKODE-Ia/b is also likely to reflect a steric issue.

Conclusion

EKODE isomers are important molecules because of their highly potent biological activities[8,9] and our recent finding that they selectively modify protein histidine side-chains.[6b] Several EKODE isomers were first isolated and characterized from cysteine•$FeCl_3$-mediated homolytic decomposition of linoleic acid-derived hydroperoxides (9- and 13-HPODE) by Gardner and co-workers.[7] Starting with purified 13-HPODE as starting material, *trans*-EKODE-(*E*)-Ib and *cis*-EKODE-(*E*)-Ib were isolated in 18 and 5% yield, respectively, based on hydroperoxide consumed.[7b] This work required initial silica gel fractional column chromatography followed by diazomethane derivatization and purification of small amounts of the methyl esters by TLC. Our synthetic routes now provide a means to obtain not only the EKODE-I series but also the EKODE-II series in preparative amounts. Access to the *cis*-epoxy isomers in the EKODE-I series arose from the lack of complete stereospecificity in the base-mediated H_2O_2-dependent epoxidation of the α,β-unsaturated *aldehyde* intermediates. On the other hand, in the synthetic routes to the EKODE-II series, the same reaction of α,β-unsaturated *ketone* intermediates appeared to yield exclusively *trans*-epoxy products.

With the six individual EKODE isomers in hand, we were able to quantitate their yields in the direct mild oxidation of linoleic acid. Although the EKODEs are generated in overall lower yields than when they are formed from pre-synthesized HPODEs,[7] the EKODE isomers were still major products as observed by HPLC chromatography. It seems clear that the major hydroperoxide decomposition route affords the *trans*-EKODE-(*E*)-Ia/b regioisomers, with the *trans*-EKODE-(*E*)-IIa/b and *cis*-EKODE-(*E*)-Ia/b regioisomers being formed in about 3 and 4 times lower amounts, respectively.

Our current finding that EDOKE isomers are the predominant products generated from oxidizing linoleic acid with a mass of 310 Da suggests that the protein His imidazole adducts with a mass gain of 310 Da[6b] indeed represent adduction by EKODE isomers. However, until now, we had no information on which of the EKODE isomers are likely to be involved or on the structure of the adducts. The relative reactivity studies using multiple imidazole electrophiles showed that the EKODE-(*E*)-II isomers are substantially more reactive than the EKODE-(*E*)-I isomers, though they are generated in lower amounts from linoleic acid. Overall, this would mean that any given protein His adduct arising from oxidizing linoleic acid is likely to represent a spectrum of adduction by various EKODE isomers. In any event, our results further demonstrate that these adducts would reflect conjugate addition of His imidazole to the EKODEs rather than the isomeric scenario involving nucleophilic epoxide ring opening. These results establish the chemical precedent for interpreting the reaction of proteins with the individual EKODE isomers or their mixture generated from autoxidizing linoleic acid. The knowledge of the EKODE electrophilic reactivities also provides an underpinning for the eventual interpretation of various types of biological activities that are being observed for these important endogenously formed molecules.

Experimental Section

General experimental details and preparation of key intermediates can be found in the Supporting Information.

Methyl *trans(cis)*-9,10-Epoxy-11-oxoundecanoate (5). A 30% aqueous H_2O_2 solution (708 μL, 6.9 mmol) was added to a solution of methyl 11-oxoundec-9(*E*)-enoate **4** (500 mg, 2.36 mmol) in MeOH (10 mL) at 0 °C, and then $NaHCO_3$ (238 mg, 2.83 mmol) was added. The mixture was stirred vigorously for 3 h at room temperature; then brine (20 mL) was added, and the resulting suspension was extracted with ethyl ether. The combined organic extract was dried (Na_2SO_4), the solvent was evaporated, and the

crude product was purified by silica gel chromatography (eluent hexanes-ether 3:1), affording **5** as a yellow oil (236 mg, 44%): ^{1}H NMR (trans isomer, 200 MHz, $CDCl_3$) δ 1.22−1.52 (8H), 1.52−1.70 (4H), 2.31 (t, 2H, J = 7.3 Hz), 3.13 (dd, 1H, J = 6.3 and 2.0 Hz), 3.23 (td, 1H, J = 5.6 and 2.0 Hz), 3.67 (s, 3H), 9.02 (d, 1H, J = 6.3 Hz); ^{13}C NMR (trans isomer, 50 MHz, $CDCl_3$) δ 24.9 (+), 25.8 (+), 28.99 (+), 29.06 (+), 29.09 (+), 31.2 (+), 34.1 (+), 51.5 (−), 56.8 (−), 59.2 (−), 174.3 (+), 198.6 (−); HRMS (FAB) calcd for $C_{12}H_{21}O_4$ (MH^+) 229.1440, found 229.1436. NMR resonances for the minor cis isomer could not be readily ascertained.

Methyl 13-Oxo-*trans(cis)*-9,10-epoxy-11(*E*)-octadecenoate (8).[13] *n*-Butyllithium (1.6 mL, 2.5 M in hexane, 3.8 mmol) was added to a cooled (−70 °C) solution of 1-(triphenylphosphoranylidene)-2-propanone (**6**) (1.0 g, 3.2 mmol) in THF (40 mL), causing a deep-red color to develop. The mixture was stirred for 30 min, whereupon *n*-butyl iodide (0.83 g, 4.5 mmol) was added dropwise, and the mixture was then allowed to stir at room temperature for 18 h. After evaporation of THF, the resulting red oil was dissolved in EtOAc (20 mL) and washed with water. The dried (Na_2SO_4) organic layer was evaporated to afford crude 1-(triphenylphosphoranylidene)-2-heptanone[14] (**7**) as a viscous oil in essentially quantitative yield (1.35 g). A solution of crude **7** (213 mg, 0.57 mmol) in 3 mL of anhydrous CH_2Cl_2 was added to a solution of methyl 9,-10-epoxy-11-oxoundecanoate (**5**) (100 mg, 0.44 mmol) in 1 mL of anhydrous CH_2Cl_2 at 0 °C. After stirring for 1 h, the mixture was directly applied to a preparative silica gel TLC plate (0.5 mm), which was eluted with hexanes-EtOAc 3:2. The band at R_f = 0.59 was extracted with ether, and the extract was filtered and evaporated, affording **8** as a yellow oil (99 mg, 70%): ^{1}H NMR (trans isomer, 200 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 6.6 Hz), 1.22−1.70 (18H), 2.30 (t, 2H, J = 7.3 Hz), 2.53 (t, 2H, J = 7.2 Hz), 2.89 (td, 1H, J = 5.3 and 2.0 Hz), 3.20 (dd, 1H, J = 6.4 and 2.0 Hz), 3.66 (s, 3H), 6.38 (d, 1H, J = 15.9 Hz), 6.51 (dd, 1H, J = 15.9 and 6.4 Hz); ^{13}C NMR (trans isomer, 50 MHz, $CDCl_3$) δ 14.0 (-), 22.5 (+), 23.8 (+), 24.9 (+), 25.8 (+), 29.0 (+), 29.2 (2, +), 31.5 (+), 31.9 (+), 34.1 (+), 40.7 (+), 51.5 (−), 56.7 (−), 61.6 (−), 131.4 (−), 142.5 (−), 174.3 (+), 199.8 (+); HRMS (FAB) calcd for $C_{19}H_{33}O_4$ (MH^+) 325.2379, found 325.2378. NMR resonances for the minor cis isomer could not be readily ascertained.

13-Oxo-*cis/trans*-9,10-epoxy-11(*E*)-octadecenoic Acid (*cis*/*trans*-EKODE-(*E*)-Ia). A solution of **8** (20 mg, 0.062 mmol) in *i*-PrOH (1 mL) was treated with a solution of LiOH (2.7 mg, 0.11 mmol) in H_2O (0.5 mL). After the solution was stirred at room temperature for 1 h, saturated NH_4Cl (400 μL) was added, and the pH was adjusted to 4−5 with 0.1 N HCl. The aqueous layer was extracted with EtOAc, and the combined organic extract was dried (Na_2SO_4) and evaporated. The crude product was applied to a preparative silica gel TLC plate (0.5 mm), which was eluted with hexanes-ether-CH_3COOH 30:50:1. The band at R_f = 0.24 was extracted with CH_3OH, and the extract was filtered and evaporated, affording *trans*-EKODE-(*E*)-Ia as a white solid (13 mg, 66%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 6.4 Hz), 1.22−1.70 (18H), 2.35 (t, 2H, J = 7.2 Hz), 2.53 (t, 2H, J = 7.2 Hz), 2.89 (td, 1H, J = 5.4 and 2.0 Hz), 3.20 (dd, 1H, J = 6.4 and 2.0 Hz), 6.38 (d, 1H, J = 15.9 Hz), 6.51 (dd, 1H, J = 15.9 and 6.4 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 (−), 22.5 (+), 23.8 (+), 24.7 (+), 25.8 (+), 29.0 (+), 29.1 (+), 29.2 (+), 31.5 (+), 31.9 (+), 34.0 (+), 40.7 (+), 56.7 (−), 61.6 (−), 131.4 (−), 142.5 (−), 179.7 (+), 199.9 (+); UV (ethanol) λ_{max} = 232 nm, $\epsilon(\lambda_{max})$ = 13 700 L·mol^{-1}·cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{31}O_4$ (MH^+) 311.2222, found 311.2214.

A second band at R_f = 0.22 was extracted with CH_3OH, and the extract was filtered, purified by preparative HPLC, and evaporated, affording *cis*-EKODE-(*E*)-Ia as an yellow oil (0.8 mg, 3%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 6.7 Hz), 1.22−1.70 (18H), 2.36 (t, 2H, J = 7.3 Hz), 2.55 (t, 2H, J = 7.2 Hz), 3.20 (m, 1H), 3.53 (dd, 1H, J = 6.4 and 3.5 Hz), 6.42 (d, 1H, J = 15.9 Hz), 6.65 (dd, 1H, J = 15.9 and 6.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 13.9, 22.5, 23.8, 24.6, 26.2, 27.6, 28.8, 28.97, 29.03 (2), 31.4, 41.0, 55.5, 59.8, 132.9, 139.7, 199.7; UV (ethanol): λ_{max} = 232 nm; $\epsilon(\lambda_{max})$ = 11 000 L·mol^{-1}·cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{31}O_4$ (MH^+) 311.2222, found 311.2210.

9-Oxo-*cis*/*trans*-12,13-epoxy-10(*E*)-octadecenoic Acid (*cis*/*trans*-EKODE-(*E*)-Ib). A solution of methyl 13-oxo-*trans(cis)*-9,10-epoxy-11(*E*)-octadecenoate (**13**) (20 mg, 0.062 mmol) in *i*-PrOH (1 mL) was treated with a solution of LiOH (2.7 mg, 0.11 mmol) in H_2O (0.5 mL). After the solution was stirred at room temperature for 1 h, saturated NH_4Cl (400 μL) was added, and the pH was adjusted to 4−5 with 0.1 N HCl. The aqueous solution was extracted with EtOAc. The combined organic extract was dried (Na_2SO_4) and evaporated. The crude product was applied to a preparative silica gel TLC plate (0.5 mm), which was eluted with hexanes−ether−CH_3COOH 30:50:1. The band at R_f = 0.24 was extracted with CH_3OH, and the extract was filtered and evaporated, affording *trans*-EKODE-(*E*)-Ib as white solid (12 mg, 63%): mp 69 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 6.5 Hz), 1.22−1.70 (18H), 2.34 (t, 2H, J = 7.4 Hz), 2.53 (t, 2H, J = 7.4 Hz), 2.91 (td, 1H, J = 5.3 and 2.1 Hz), 3.20 (dd, 1H, J = 6.5 and 2.1 Hz), 6.38 (d, 1H, J = 16.0 Hz), 6.51 (dd, 1H, J = 16.0 and 6.5 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 (−), 22.6 (+), 24.0 (+), 24.6 (+), 25.6 (+), 28.9 (+), 29.0 (2, +), 31.6 (+), 32.0 (+), 34.0 (+), 40.6 (+), 56.7 (−), 61.7 (−), 131.4 (−), 142.7 (−), 179.5 (+), 199.7 (+); UV (ethanol): λ_{max} = 232 nm; $\epsilon(\lambda_{max})$ = 13 700 L·mol^{-1}·cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{31}O_4$ (MH^+) 311.2222, found 311.2227.

A second band at R_f = 0.22 was extracted CH_3OH, and the extract was filtered and evaporated, affording *cis*-EKODE-(*E*)-Ib as a yellow oil (0.8 mg, 4%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz), 1.22−1.70 (18H), 2.35 (t, 2H, J = 7.4 Hz), 2.54 (t, 2H, J = 7.2 Hz), 3.20 (m, 1H), 3.52 (ddd, 1H, J = 6.4, 4.4, and 0.8 Hz), 6.40 (dd, 1H, J = 16.0 and 0.8 Hz), 6.65 (dd, 1H, J = 16.0 and 6.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.2, 22.7, 24.2, 24.9, 26.2, 27.8, 29.1, 29.21, 29.24, 31.7, 41.1, 55.7, 60.1, 133.1, 140.0, 199.6 (one C signal missing and one apparently overlapped); UV (ethanol): λ_{max} = 232 nm; $\epsilon(\lambda_{max})$ = 11 000 L·mol^{-1}·cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{31}O_4$ (MH^+) 311.2222, found 311.2230.

Methyl 9-Hydroxy-11-oxooctadec-12(*E*)-enoate (15). To a precooled (−78 °C) solution of lithium diisopropylamide (2.0 mL, 2M in THF/*n*-heptane, 4.0 mmol) was added 3-nonen-2-one (**14**) (560 mg, 4.0 mmol) slowly. The resulting solution was stirred at −78 °C for 15 min before slowly adding methyl 9-oxononanoate (**3**) (368 mg, 2.0 mmol) in anhydrous THF (2 mL). The reaction mixture was stirred at −78 °C for a further 20 min and then quenched by the addition of saturated aqueous NH_4Cl (1 mL). The resulting reaction mixture was allowed to warm to room temperature. The mixture was further extracted with EtOAc and water, and then the combined organic layers were dried (Na_2SO_4) and evaporated. The crude product was purified by silica gel chromatography (eluent hexanes-ether 1:1), affording **15** as slightly yellow oil (246 mg, 38%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 7.2 Hz), 1.20−1.62 (18H), 2.19 (dtd, 2H, J = 6.8, 6.8, and 1.6 Hz), 2.26 (t, 2H, J = 7.6 Hz), 2.58 (dd, 1H, J = 17.6 and 9.2 Hz), 2.71 (dd, 1H, J = 17.6 and 2.8 Hz), 3.24 (d, 1H, J = 3.2 Hz), 3.63 (s, 3H), 3.98−4.04 (1H), 6.05 (dt, 1H, J = 16.0 and 1.6 Hz), 6.83 (dt, 1H, J = 16.0 and 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.6, 25.1, 25.6, 27.9, 29.3, 29.4, 29.6, 31.5, 32.7, 34.3, 36.7, 46.2, 51.7, 67.9, 130.8, 149.2, 174.5, 201.5; HRMS (FAB) calcd for $C_{19}H_{35}O_4$ (MH^+) 327.2535, found 327.2532.

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Methyl *trans*-12,13-Epoxy-11-oxo-9-hydroxyoctadecanoate (16). Hydrogen peroxide (30%, 0.3 mL, 2.9 mmol) was added slowly to a stirred solution of **15** (162 mg, 0.5 mmol) in methanol (2 mL) at 5 °C. Aqueous NaOH (1N, 0.5 mL) was added over 30 min, and the mixture was stirred at room temperature for 4 h. Saturated aqueous $Na_2S_2O_4$ (0.5 mL) was then added to destroy any remaining peroxide while maintaining the temperature below 40 °C. The mixture was diluted with water (5 mL) and extracted with ether (3 × 5 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The crude product was purified by silica gel chromatography (eluent hexanes-ether 1:1), affording **16** as colorless oil as a mixture of two isomers (ratio: 1.25/1) (122 mg, 72%):

^{1}H NMR (major isomer, 400 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 7.2 Hz), 1.20−1.62 (20H), 2.26 (t, 2H, J = 7.6 Hz), 2.34 (dd, 1H, J = 17.6 and 9.2 Hz), 2.50 (dd, 1H, J = 17.6 and 7.2 Hz), 2.84−2.88 (1H), 3.02 (ddd, 1H, J = 6.0, 4.8, and 2.0 Hz), 3.18 (d, 1H, J = 2.0 Hz), 3.62 (s, 3H), 3.98−4.04 (1H); ^{1}H NMR (minor isomer, 400 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 7.2 Hz), 1.20−1.62 (20H), 2.26 (t, 2H, J = 7.6 Hz), 2.42 (dd, 1H, J = 17.6 and 3.2 Hz), 2.51 (dd, 1H, J = 17.6 and 4.4 Hz), 2.76−2.80 (1H), 3.08 (ddd, 1H, J = 6.0, 4.8, and 2.0 Hz), 3.16 (d, 1H, J = 2.0 Hz), 3.62 (s, 3H), 3.98−4.04 (1H); ^{13}C NMR (isomeric mixture, 100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.1, 25.5, 25.6, 29.2, 29.3, 29.5, 31.6, 31.9, 34.2, 36.8, 43.8, 44.1, 51.6, 58.4, 58.8, 59.9, 60.2, 67.3, 67.6, 174.5, 208.9, 209.0; HRMS (FAB) calcd for $C_{19}H_{35}O_5$ (MH^+) 343.2484, found 343.2476.

Methyl *trans*-12,13-Epoxy-11-oxo-9(*E*)-octadecenoate (17).[7c] Methanesulfonyl chloride (42 μL, 0.55 mmol) was added dropwise to a stirred solution of **16** (170 mg, 0.5 mmol) and triethylamine (209 μL, 1.5 mmol) in CH_2Cl_2 (1 mL) at 0 °C. Upon complete addition, the reaction mixture was stirred for 30 min at 0 °C, allowed to warm to room temperature with stirring for 1 h, diluted with CH_2Cl_2 (10 mL), and washed with water (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated. The crude product was purified by silica gel chromatography (eluent hexanes−ether 5:1), affording **17** as a colorless oil (125 mg, 78%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, 3H, J = 7.2 Hz), 1.20−1.62 (18H), 2.17 (dtd, 2H, J = 6.8, 6.8, and 1.6 Hz), 2.25 (t, 2H, J = 7.6 Hz), 3.00 (ddd, 1H, J = 6.4, 5.2, and 2.0 Hz), 3.29 (d, 1H, J = 2.0 Hz), 3.62 (s, 3H), 6.18 (dt, 1H, J = 16.0 and 1.6 Hz), 7.02 (dt, 1H, J = 16.0 and 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.1, 25.7, 28.1(2), 29.2(2), 31.6, 32.0, 32.9, 34.2, 51.7, 58.6, 59.2, 124.2, 150.6, 174.4, 195.9; HRMS (FAB) calcd for $C_{19}H_{33}O_4$ (MH^+) 325.2379, found 325.2381.

***trans*-12,13-Epoxy-11-oxo-9(*E*)-octadecenoic Acid (*trans*-EKODE-(*E*)-IIb).** A solution of **17** (20 mg, 0.062 mmol) in *i*-PrOH (1 mL) was treated with a solution of LiOH (2.7 mg, 0.11 mmol) in H_2O (0.5 mL). After the solution was stirred at room temperature for 1 h, saturated aqueous NH_4Cl (400 μL) was added, and the pH was adjusted to 4−5 with 0.1 N HCl. The aqueous solution was extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) and evaporated, and the crude product was applied to a preparative silica gel TLC plate (0.5 mm), which was eluted with hexanes−ether−CH_3COOH 30:50:1. The band at R_f = 0.26 was extracted with CH_3OH, and the extract was filtered, purified by preparative HPLC, and evaporated, affording a slightly white oil (6 mg, 31%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, 3H, J = 7.2 Hz), 1.30−1.40 (10H), 1.42−1.56 (4H), 1.60−1.70 (4H), 2.22 (dtd, 2H, J = 7.2, 7.2, and 1.6 Hz), 2.34 (t, 2H, J = 7.2 Hz), 3.04 (ddd, 1H, J = 6.0, 4.8, and 2.0 Hz), 3.34 (d, 1H, J = 2.0 Hz), 6.23 (dt, 1H, J = 15.6 and 1.6 Hz), 7.02 (dt, 1H, J = 15.6 and 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.0, 22.5, 24.6, 25.5, 27.9, 28.9, 29.0, 31.4, 31.8, 32.7, 33.8, 58.4, 59.1, 124.0, 150.4, 178.8, 195.8 (one C signal is apparently overlapped); UV (ethanol): λ_{max} = 232 nm; $\epsilon(\lambda_{max})$ = 11 400 L·mol^{-1}·cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{31}O_4$ (MH^+) 311.2222, found 311.2236.

***trans*-9,10-Epoxy-11-oxo-12(*E*)-octadecenoic Acid (*trans*-EKODE-(*E*)-IIa).** A solution of methyl *trans*-12,13-epoxy-11-oxo-9(*E*)-octadecenoate (**21**) (20 mg, 0.062 mmol) in *i*-PrOH (1 mL) was treated with a solution of LiOH (2.7 mg, 0.11 mmol) in H_2O (0.5 mL). After the solution was stirred at room temperature for 1 h, saturated aqueous NH_4Cl (400 μL) was added, and the pH was adjusted to 4−5 with 0.1 N HCl. The aqueous solution was extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) and evaporated. The crude product was applied to a preparative silica gel TLC plate (0.5 mm), which was eluted with hexanes−ether−CH_3COOH 30:50:1. The band at R_f = 0.26 was extracted with CH_3OH, and the extract was filtered, purified by preparative HPLC, and evaporated, affording a slightly yellow oil (9 mg, 47%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.82 (t, 3H, J = 7.2 Hz), 1.25−1.41 (10H), 1.42−1.56 (4H), 1.60−1.72 (4H), 2.16 (dtd, 2H, J = 7.2, 7.2, and 1.6 Hz), 2.29 (t, 2H, J = 7.2 Hz), 2.98 (ddd, 1H, J = 6.0, 4.8, and 2.0 Hz), 3.28 (d, 1H, J = 2.0 Hz), 6.16 (dt, 1H, J = 15.6 and 1.6 Hz), 7.02 (dt, 1H, J = 15.6 and 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.0, 22.5, 24.6, 25.5, 27.9, 28.9, 29.0, 31.5, 31.8, 32.7, 33.4, 58.4, 59.1, 124.0, 150.4, 176.8, 195.7 (one C signal is apparently overlapped); UV (ethanol): λ_{max} = 232 nm; $\epsilon(\lambda_{max})$ = 11 400 L·mol^{-1}·cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{31}O_4$ (MH^+) 311.2222, found 311.2230.

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Supporting Information Available: General experimental procedures including HPLC and HPLC-MS conditions; preparation of compounds **2**−**4**, **9**, **12**, **13**, **18**−**21**, and **23**; establishment of purity of the EKODE isomers; linoleic acid oxidation and confirmation of EKODE isomers as products; kinetics of reaction of imidazole nucleophiles with EKODE isomers; incubation of BzHis with autoxidizing linoleic acid and with EKODE isomers; reactions of **23** with imidazole in different solvents; ^{1}H and ^{13}C NMR spectra for all compounds; LC-MS analysis of autoxidized linoleic acid; and ^{1}H NMR spectrum of the Michael adducts formed from **23** and imidazole. This material is available free of charge via the Internet at http://pubs.acs.org.

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