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Review Article

OXYGEN RADICAL CHEMISTRY OF POLYUNSATURATED FATTY ACIDS

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Abstract—Polyunsaturated fatty acids (PUFA) are readily susceptible to autoxidation. A chain oxidation of PUFA is initiated by hydrogen abstraction from allylic or *bis*-allylic positions leading to oxygenation and subsequent formation of peroxyl radicals. In media of low hydrogen-donating capacity the peroxyl radical is free to react further by competitive pathways resulting in cyclic peroxides, double bond isomerization and formation of dimers and oligomers. In the presence of good hydrogen donators, such as α-tocopherol or PUFA themselves, the peroxyl radical abstracts hydrogen to furnish PUFA hydroperoxides. Given the proper conditions or catalysts, the hydroperoxides are prone to further transformations by free radical routes. Homolytic cleavage of the hydroperoxy group can afford either a peroxyl radical or an alkoxyl radical. The products of peroxyl radicals are identical to those obtained during autoxidation of PUFA; that is, it makes no difference whether the peroxyl radical is generated in the process of autoxidation or from a preformed hydroperoxide. Of particular interest is the intramolecular rearrangement of peroxyl radicals to furnish cyclic peroxides and prostaglandin-like bicyclo endoperoxides. Other principal peroxyl radical reactions are the β-scission of O_2, intermolecular addition and self-combination. Alkoxyl radicals of PUFA, contrary to popular belief, do not significantly abstract hydrogens, but rather are channeled into epoxide formation through intramolecular rearrangement. Other significant reactions of PUFA alkoxyl radicals are β-scission of the fatty chain and possibly the formation of ether-linked dimers and oligomers. Although homolytic reactions of PUFA hydroperoxides have received the most attention, hydroperoxides are also susceptible to heterolytic transformations, such as nucleophilic displacement and acid-catalyzed rearrangement.

Keywords—Autoxidation, Polyunsaturated fatty acids, Fatty acid hydroperoxides, Lipoxygenase, Peroxyl radical, Alkoxyl radical, α-Tocopherol, Cyclic peroxides, Epoxides, Bicyclo endoperoxides, Hock/Criegee rearrangement.

INTRODUCTION

Nearly a half century ago Farmer and coworkers laid the foundations for the chemistry of olefin autoxidation. In their classical communication Farmer et al.[1] reported that the esters of polyunsaturated fatty acids (PUFA), such as ethyl linolenate, autoxidized into conjugated diene hydroperoxides. Because triene conjugation also was observed, they postulated the existence of conjugated triene dihydroperoxides of linolenate almost 40 years before such products were actually isolated and characterized. On the basis of their findings, they recognized that the event preceding PUFA oxidation had to be hydrogen abstraction of the *bis*-allylic methylene to afford a pentadienyl radical.

Since these early beginnings, the study of PUFA autoxidation has expanded to include the competing reactions of the initial oxidation product, the peroxyl radical. And, more is known of the reaction pathways of the final oxidation products, the PUFA hydroperoxides. The diversity of reaction types occurring in autoxidation mixtures is largely dependent on the proximity of unsaturation to either the peroxyl radical or

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the hydroperoxide group permitting a variety of rearrangement reactions.

In order to maintain focus on reaction mechanisms occurring during autoxidation, this review selectively cites the extensive literature available. For example, oxidation of PUFA by singlet O_2 ($^1O_2$) is ignored, except as a method of obtaining structurally different hydroperoxides to study as reactants. Other recent reviews concerning autoxidation are available.[2-4]

1. AUTOXIDATION

The autoxidation of PUFA and monounsaturated fatty acids is comprised of three events: (1.1) initiation, (1.2) propagation, and (1.3) termination.

$$RH + X\cdot \xrightarrow{\text{activation}} R\cdot + XH \text{ (Ri = rate of initiation)} \qquad (1.1)$$

$$R\cdot + O_2 \xrightarrow{k_o} ROO\cdot$$
$$ROO\cdot + RH \xrightarrow{k_p} ROOH + R\cdot \qquad (1.2)$$

$$\begin{matrix} R\cdot + ROO\cdot \xrightarrow{k_t} \\ 2\,ROO\cdot \xrightarrow{k_t} \\ 2\,R\cdot \xrightarrow{k_t} \end{matrix} \left\{\text{Nonradical products}\right. \qquad (1.3)$$

In the presence of catalysts, particularly transition metal ions (M), chain branching theoretically can contribute by the formation of alkoxyl radicals.

$$ROOH + M^n \longrightarrow RO\cdot + OH^- + M^{n-1}$$
$$RO\cdot + ROOH \longrightarrow ROO\cdot + ROH \qquad (1.4)$$
$$RO\cdot + RH \longrightarrow ROH + R\cdot$$

Contrary to current dogma, hydrogen abstraction by alkoxyl radicals of PUFA per se is relatively unimportant, and any contribution of this species in maintaining a chain must arise from the rearranged alkoxyl radical as discussed later.

Although autoxidation of PUFA can be complex, the basic three steps (1.1) through (1.3) proves to be kinetically predictable, and the rate of O_2 uptake can be obtained from these reaction equations:

$$-d[O_2]/dt = k_p[RH]\left(\frac{R_i}{2k_t}\right)^{1/2} \qquad (1.5)$$

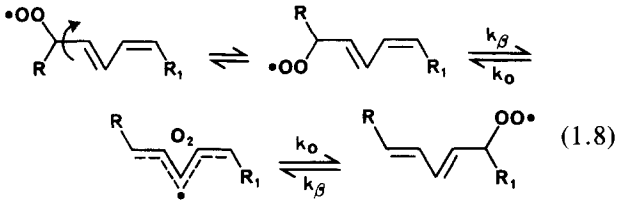
If the rate of initiation (Ri) is controlled with a predictable radical initiator, the "oxidizability" of various fatty acids can be derived from the expression above as follows:

$$\text{Oxidizability} = k_p/(2k_t)^{1/2} \qquad (1.6)$$

Using azo initiators, Cosgrove et al.[5] found that the oxidizability of PUFA is linearly dependent on the number of *bis*-allylic methylenes present in the fatty acid. Thus, it is reasonable that oxidizability is controlled by the initial event of hydrogen abstraction by radicals from this relatively weak C—H bond of the *bis*-allylic methylene with a bond dissociation energy (BDE) of about 75 kcal/mol. For monounsaturates, like oleic acid, oxidizability is much less because mono-allylic methylene hydrogens (C—H BDE = ≈88 kcal/mol) are more resistant to abstraction. Autoxidation of PUFA (RH) can be suppressed by introduction of compounds with even more readily abstractable hydrogens, such as certain antioxidants (AH):

$$ROO\bullet \begin{matrix} \xrightarrow{AH\ A\cdot} ROOH \\ \xrightarrow[RH\ R\cdot]{} ROOH \end{matrix} \qquad (1.7)$$

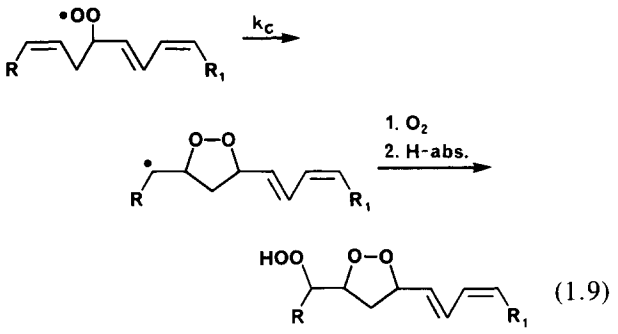
After hydrogen abstraction the pentadienyl radical combines with O^2 to generate a peroxyl radical, and this propagates another chain; reaction (1.2). Beside abstracting hydrogen from PUFA, peroxyl radicals are more kinetically involved in the outcome of autoxidation products than previously suspected. In other words, they are involved in competing reactions, such as β-scission and cyclization by intramolecular rearrangement. By β-scission of O_2 both the position of oxidation and the geometry of the diene conjugation can rearrange, and the released O_2 exchanges with gaseous O_2[6]:



(1.8)

Thus, it can be seen that rotation of the sp^3 carbon—carbon bond between the oxygenated carbon and the double bond can lead to isomerization of the *cis,trans* diene to *trans,trans*. Another competing reaction is cyclization by intramolecular rearrangement, which requires an "inner" peroxyl radical of PUFA with three or more double bonds. That is, the peroxyl

radical must be located β to an olefin:



(1.9)

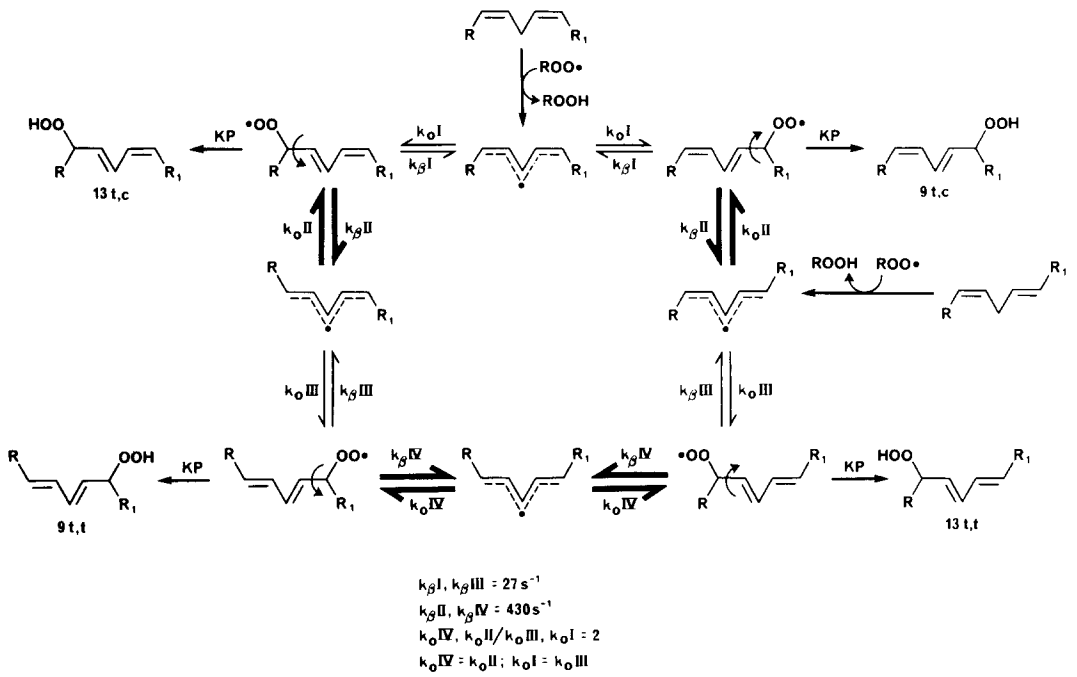
Roza and Francke[7] first described the product of reaction (1.9), and subsequently Chan et al.[8] defined the reaction mechanism. Other competitive pathways of the peroxyl radical may participate as well, such as intermolecular addition to double bonds. Such reactions, affording dimers and oligomers, might be important in the absence of organic solvent where peroxyl radicals are in proximity to double bonds of neighboring PUFA molecules.

The importance of competition between hydrogen abstraction, β-scission and intramolecular rearrangement of peroxyl radicals during autoxidation was emphasized by the elegant research of Porter and coworkers.[9-11] They found that the rate constants for β-scission (k_β), reoxygenation of the β-scission product (k_o) and cyclization by intramolecular rearrangement (k_c) were essentially invariable; however, the hydrogen donating parameter of the medium (KP) can be readily manipulated. The hydrogen donating parameter was defined as the sum of the hydrogen donating ability of all ingredients in an autoxidation mixture ($KP = \Sigma_{i=1}^{n}$ kpi $[R_iH]$). Porter and coworkers utilized p-methoxyphenol and 1,4-cyclohexadiene among others to increase KP, and the *bis*-allylic methylenes of PUFA are themselves significant contributors to KP.

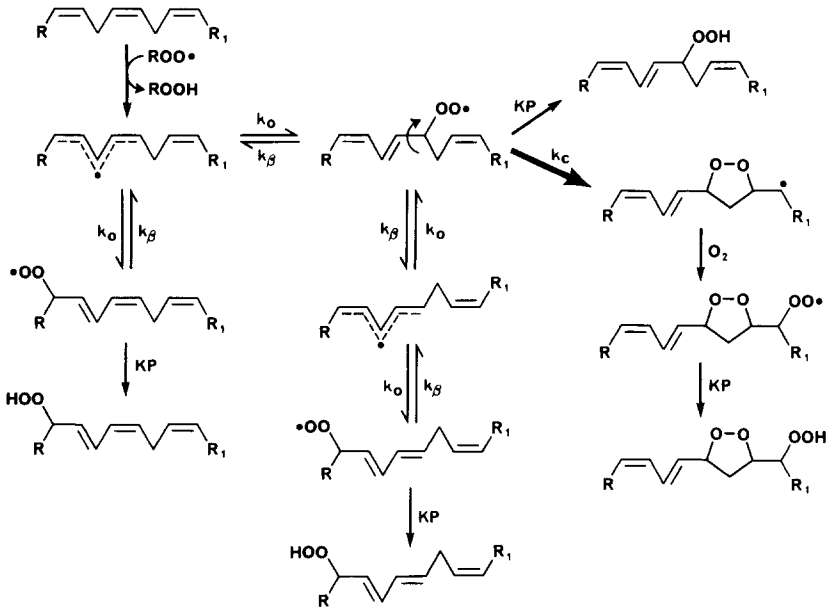
Since peroxyl radicals of linoleate can not cyclize by intramolecular rearrangement by reason of structural limitations, linoleate autoxidation constitutes only a competition between KP and β-scission[9,11] (Scheme 1). Accordingly, with the rate constants of β-scission (k_β) and rexoygenation (k_o) invariant all inceases in the hydrogen donating parameter (KP) linearly increased the ratio of *cis,trans* to *trans,trans* diene hydroperoxides. Although fatty acid hydroperoxides are themselves known to isomerize by β-scission after being transformed into a peroxyl radical,[6] it is important to recognize that the *cis,trans* to *trans,trans* ratio formed during autoxidation is under thermodynamic control, and this ratio, being dependent only on KP, is invariant during the initial stages of autoxidation.[9-10] In addition, it can be seen in Scheme 1 that the various β-scission and reoxygenation rates are not equivalent; that is, the transoid terminus of the pentadiene is more reactive to both β-scission and reoxygenation than a cisoid terminus.[11] Thus, k_βII and k_βIV are 16-fold greater than k_βI and k_βIII, and similarly, the ratio of k_oII or k_oIV to k_oI or k_oIII is 2. As a practical consequence, the autoxidation of various *cis,cis*, *cis,trans*, or *trans,trans* isomers of 9,12-octadecadienoic acid each afford a characteristic mixture of isomeric diene hydroperoxides at any given KP. For example, the entry of the 9-*trans*, 12-*cis* isomer into Scheme 1 (right center) at high KP results in mainly *trans,cis* 9-hydroperoxide and *trans,trans* 13-hydroperoxide with a preference for the former, but at low KP the main oxidation products become *trans,trans* 9- and 13-hydroperoxides. The *trans,cis* 13-hydroperoxide is insignificant at all KP values.

The kinetics become somewhat more complex with PUFA containing more than two double bonds as they are potential candidates for intramolecular cyclization of the peroxyl radical (reaction 1.9). As seen by Scheme 2, another kinetic parameter is introduced into autoxidation.[10] For example, in the autoxidation of linolenic acid only the "inner" 12- and 13-peroxyl radicals cyclize, and the "outer" 9- and 16-peroxyl radicals must be converted into inner peroxyl radicals by β-scission prior to cyclizing. Because the rate constants for cyclization (k_c) are 4.5 to 6.5 fold higher than β-scission (k_β), outer 9- and 16-hydroperoxides and cyclic peroxides (hydroperoxy-epidioxides) accumulate in media of low hydrogen donating capacity (KP), such as might be achieved without added antioxidants and the addition of a solvent with poor hydrogen donating capacity (e.g., benzene).[9] Minor quantities of *trans,trans* diene hydroperoxides are found at all values of KP, because cyclization outcompetes β-scission. The outer 9- or 16-peroxyl radicals, which cannot cyclize, are to some extent transformed into inner *trans,trans* 12- or 13-peroxyl radicals, which subsequently cyclize. Only in media of high KP are both β-scission and cyclization overwhelmed to afford a rather equal distribution of all four *cis,trans* hydroperoxides of linolenic acid. As expected, inclusion of 5% by weight of the excellent hydrogen donor, α-tocopherol, in autoxidation of methyl linolenate resulted in equal amounts of the four *cis,trans* 9-, 12-, 13- and 16-hydroperoxides with no observable hydroperoxy epidioxides.[12]

Surprisingly, a relatively large concentration of α-tocopherol (5% by wt) behaved like a prooxidant with methyl linolenate, compared to the antioxidant activity

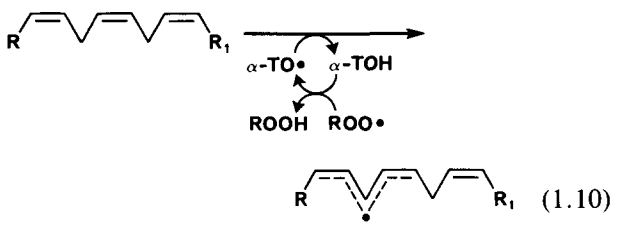


Scheme 1. The autoxidation of linoleic acid (top, center) and the *trans*-9, *cis*-12 isomer of linoleic acid (middle, right) into isomeric hydroperoxides. The bold arrows denote comparatively faster rates of reaction. k_β = rate of β-scission of O_2; k_o = rate of oxygenation; KP = the hydrogen-donating parameter of the reaction; R = $CH_3(CH_2)_4-$; R_1 = $-(CH_2)_7COOH$. Modified from Porter et al.[11]



Scheme 2. The autoxidation of linolenic acid (top, left) into isomeric hydroperoxides and hydroperoxy epidioxides. The bold arrow signifies a comparatively faster rate of reaction. k_c = rate of cyclization; k_β = rate of β-scission of O_2; k_o = rate of oxygenation; KP = hydrogen-donating parameter of the reaction; R = $CH_3CH_2^-$; R_1 = $-(CH_2)_7COOH$. Modified from Porter et al.[10]

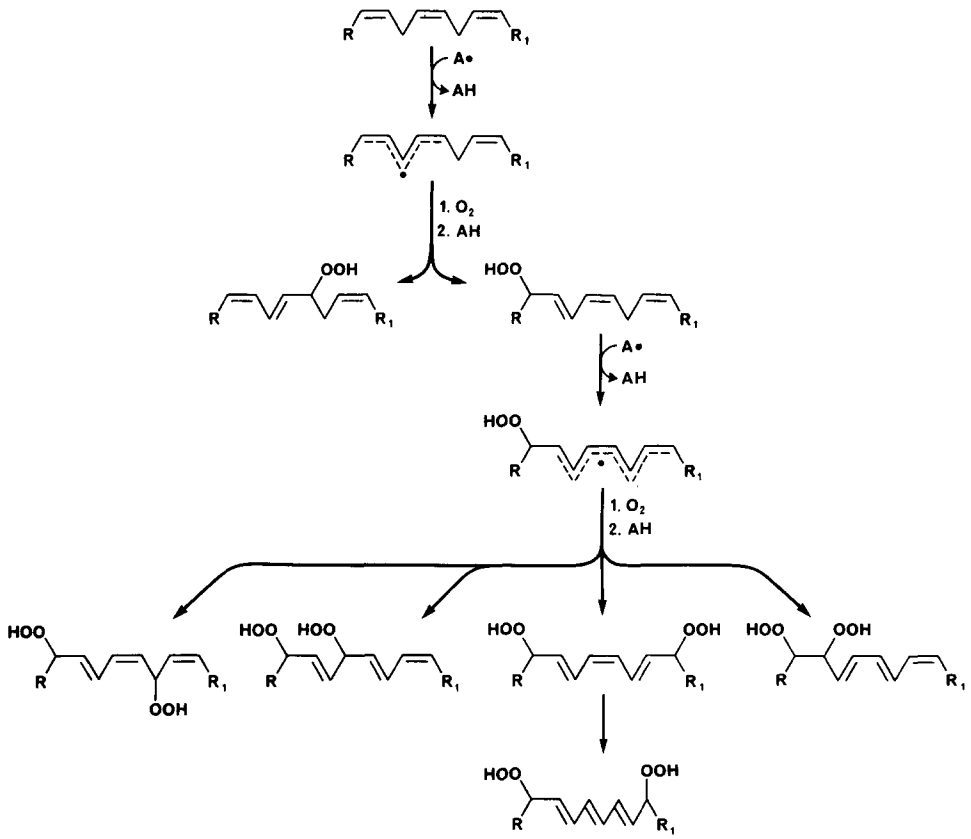
of a 100-fold smaller quantity of α-tocopherol.[12] Although large concentrations of α-tocopherol were effective hydrogen donators, evidently the α-tocopherol semiquinone was responsible for abstracting *bis*-allylic methylene hydrogens of methyl linolenate:



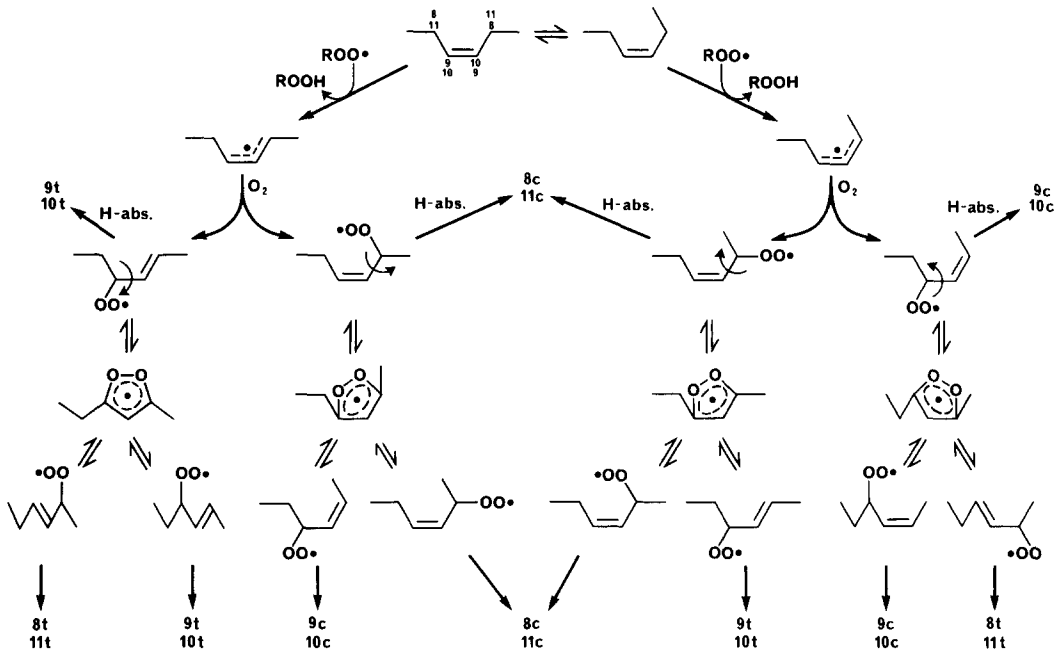
(1.10)

Thus, it was discovered that large concentrations of α-tocopherol (10% by wt) promoted the formation of 9,16-dihydroperoxides from methyl linolenate,[13] evidently owing to its dual ability to donate hydrogens to peroxyl radicals and abstract hydrogens from *bis*-allylic methylenes. In order for α-tocopherol to be an antioxidant, the hydrogen abstraction from *bis*-allylic methylenes cannot be very efficient. Four positional isomers of linolenate dihydroperoxides are theoretically possible (Scheme 3), but only the *trans*-10,*cis*-12,*trans*-14, or all-*trans* methyl 9,16-dihydroperoxy-10,12,14-octadecatrienoate isomers were detected with 10% α-tocopherol. In the absence of α-tocopherol both conjugated and non-conjugated dihydroperoxides reportedly form, but individual isomers were not isolated and characterized separately.[14]

The autoxidation of monounsaturates, which should represent the least complex example, actually is somewhat of an exception mechanistically. The autoxidation of oleic acid predicts the formation of 8- and 11-hydroperoxides with *cis* unsaturation and 9- and 10-hydroperoxides with either *cis* or *trans* unsaturation as shown in the top portion of Scheme 4. However, the observed formation of 8- and 11-hydroperoxides with *trans* unsaturation[15] cannot be explained, unless one invokes either a thermodynamically unfavorable rearrangement of a *cis* to a *trans* allylic radical or assume rearrangement through β-scission of the peroxyl radical. Recent evidence demonstrated that neither of these two alternatives is likely. Like the rearrangement of the linoleic acid peroxyl radical, the sp^3 carbon—carbon bond between the peroxyl radical and the



Scheme 3. Hypothetical dioxygenation of linolenic acid: R = CH_3CH_2-; R_1 = $-(CH_2)_7COOH$. Only two isomers have been specifically isolated and characterized, the *trans*-10, *cis*-12, *trans*-14 and all-*trans* 9,16-dihydro-peroxides.



Scheme 4. The autoxidation of oleic acid. Structures are abbreviated to specifically illustrate the configuration of carbons-8 through -11, and final products are designated by position of oxidation and configuration of double bond, e.g., 9t = 9-hydroperoxy-*trans*-10-octadecenoic acid. Scheme was constructed on the basis of research by Porter and Wujek.[16]

olefin rotates to give a new geometry in a thermodynamically feasible manner, but unlike the peroxyl radical of linoleic acid, experimental evidence indicates that O_2 transfer to another carbon occurs via a transition state involving a five-membered ring, rather than by β-scission of O_2.[16] As shown on the bottom of Scheme 4, the formation of 8- and 11-hydroperoxides with a *trans* double bond, as well as other isomers, originate from peroxyl radical rearrangement in the absence of O_2 exchange. If the kinetics of rearrangement prove to be similar to the β-scission model of linoleic peroxyl radicals, then it might be predicted that transoid configurations are more reactive to rearrangement, possibly explaining the prominence of *trans* unsaturated 8- and 11-hydroperoxides in autoxidation mixtures (cf. Frankel et al.[15]). Porter and Wujek[16] also noted that the transition state by virtue of its fixed ring structure probably permits transfer of hydroperoxide stereoconfiguration.

Similar to the oxidation of the allylic position of oleic acid, a minor oxidation also occurs at the allylic positions of methyl linoleate.[17,18] Because allylic hydrogens are more resistant to abstraction than *bis*-allylic hydrogens, it is not surprising that oxidation to 8- and 14-hydroperoxides with nonconjugated dienes amounts to only 1% of the total hydroperoxides produced,[17] and the nonconjugated diene 10- and 12-hydroperoxides were found in even smaller amounts.[18]

2. HYDROPEROXIDES AS REACTANTS

Although study of autoxidation products has revealed much of the free radical chemistry of PUFA, specific mechanisms for the reactions of PUFA hydroperoxides in forming secondary products is difficult to understand because several isomeric hydroperoxides are involved. Many workers have utilized regio- and stereo-chemically pure hydroperoxides from lipoxygenase oxygenation of PUFA to study mechanisms of reaction.

Soybean lipoxygenase has been used universally to generate isomerically pure hydroperoxides in relatively large amounts. The soybean enzyme primarily affords the (13-*S*)-13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid [13(*S*)-HPOD] from linoleic acid.[19] When the enzyme is incubated at high pH (≈10.5), the isomeric purity increases to give about 96% 13(*S*)-HPOD.[20] The soybean lipoxygenase-1 isoenzyme is responsible for the excellent specificity at high pH,[21] and the other isoenzymes do not interfere as they are inactive at alkaline pH's. Because soybean lipoxygenase oxygenates many PUFA substrates to (*S*) hydroperoxides without exception,[19] it has been assumed that linolenic and arachidonic acids are oxygenated all (*S*) to furnish (13*S*)-13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoic acid [13(*S*)-HPOT] and (15*S*)-15-hydroperoxy-*cis*-5,*cis*-8,*cis*-11,*trans*-13-eicosatetrae-

noic acid [15(*S*)-HPETE], respectively. In our laboratory it has been found that the anaerobic reaction of soybean lipoxygenase is the main factor to avoid in obtaining optimum yields of hydroperoxides; that is, too much enzyme and/or too little O_2 can cause formation of secondary products and incomplete reaction by anaerobic cycling of the enzyme. The hydroperoxides extracted from enzyme incubations can be readily purified in either gram quantities by column chromatography[22] or high purity by high performance liquid chromatography.[23,24]

Maize[25] and potato[26] lipoxygenases were the first characterized as oxygenating toward the carboxylic acid end of the pentadiene moiety of linoleic acid to afford (9*S*)-9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid [9(*S*)-HPOD]. For convenience most workers employ tomato lipoxygenase to obtain the 9(*S*)-HPOD from linoleic acid and the 9-hydroperoxide from linolenic acid [presumably 9(*S*)].[27] Although tomato lipoxygenase(s) also produce(s) some 13-HPOD, this isomer is removed by the presence of a hydroperoxide lyase in tomato that is specific for the 13-HPOD isomer.

At this juncture it should be emphasized that the stereochemistry of the 9(*S*)-HPOD often has been confused in the literature; that is, some authors have erroneously assigned this isomer as 9(*R*). A possible reason for the confusion lies in the difference in stereochemical rules between the Fischer and Cahn-Prelog-Ingold conventions. The 9(*S*)-HPOD is *D* by the Fischer convention; whereas, 13(*S*)-HPOD is *L* by the same rules. For the 13-HPOD the reference point is in the same direction using either convention; that is, the *trans*-11 double bond for Cahn-Prelog-Ingold and the carboxylic acid for Fischer. However, with the 9-HPOD the double bond and carboxylic acid reference points are in opposite directions. See Smith[28] for a more detailed explanation.

3. PEROXYL RADICAL REACTIONS

A. Formation of peroxyl radicals from hydroperoxides

As note previously, peroxyl radicals are an integral part of autoxidation, but they are relatively difficult to generate from preformed hydroperoxides; the O—H bond has a BDE of about 90 kcal/mol. Most commonly, peroxyl radicals form from hydroperoxides by hydrogen abstraction; whereby, a bond is formed for every bond broken. For example, the unstable tert-butylperoxyoxalate molecule decomposes spontaneously into tert-butyloxyl radicals, and in turn the tert-butyloxyl radical readily abstracts hydrogens:

$$\text{ROOH} + \text{t-BuO}\cdot \longrightarrow \text{ROO}\cdot + \text{t-BuOH} \quad (3.1)$$

Oxidation by higher oxidation states of transition metal ions is another pathway to peroxyl radicals:

$$\text{ROOH} + \text{M}^{n+1} \longrightarrow \text{ROO}\cdot + \text{H}^+ + \text{M}^n \quad (3.2)$$

Various metal ions have differing abilities to oxidize hydroperoxides, and their activity is also affected by a number of variables, such as pH, solvents and complexing ligands. As noted by previous workers, Fe^{3+} is not an effective oxidizer of hydroperoxides. This was recently confirmed by lack of a spin-trapped peroxyl radical signal by ESR measurements in the presence of Fe^{3+}.[29] Reviews are available concerning the specific topic of the effects of metal ions on oxygen radical reactions.[30,31]

Once generated, peroxyl radicals can in turn abstract hydrogens, and thereby initiate oxidation of another substrate as already discussed for the propagation stage of autoxidation, reaction (1.2). When the substance oxidized is other than PUFA, the process is sometimes called "cooxidation." Actually, little is known of how hydrogen abstraction by peroxyl radicals can directly affect the formation of products from lipid hydroperoxides. For example, oxooctadecadienoic acid (OOD) is an ubiquitous product of linoleic acid autoxidation noted by some of the earliest investigators.[32] Because this compound is usually found in the absence of hydroxyoctadecadienoic acid (HOD), it would appear to minimize its formation via the Russell Mechanism (see below). Whether OOD originates by either a peroxyl radical or alkoxyl radical mechanism is open to speculation.

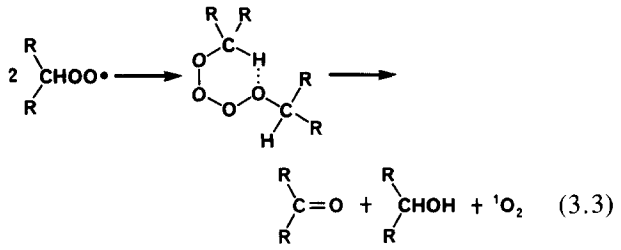
B. Peroxyl radical β-scission

The β-scission of O_2 from peroxyl radicals was detailed above in the autoxidation section (Schemes 1, 2). One must be cognizant that such transformations also occur as a result of generating peroxyl radicals from hydroperoxides.[6]

C. Peroxyl radical combination

While studying the kinetics of ethylbenzene autoxidation, Russell[33] obtained data suggestive of peroxyl radical combination followed by decomposition of product tetraoxide through a cyclic intermediate into

a molecule each of alcohol, ketone and O_2:

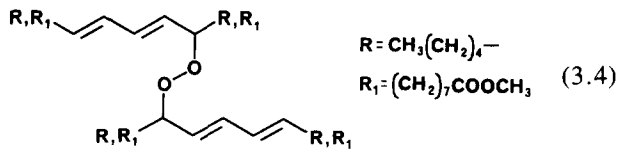


(3.3)

This "Russell Mechanism" would appear to be applicable to peroxyl radicals derived from lipid hydroperoxides because the secondary C—H bond necessary to complete the transition state is present. In order to conserve spin, cleavage of the tetraoxide must afford $^1O_2$ directly[34] or indirectly through exchange of ground-state O_2 with excited triplet carbonyl.[35] The formation of $^1O_2$ has been observed by chemiluminescence coincident with the production of peroxyl radicals from HPOD[36]; however, the yield of $^1O_2$ from such systems was only 12% of that predicted.[37] Enhanced opportunities for reaction by the Russell Mechanism may occur on active surfaces where radicals may interact on adjacent sites. Both the rate and intensity of chemiluminescence increased when the methyl ester of HPOD was absorbed on neutral alumina.[38]

Other than the detection of $^1O_2$, additional evidence for the Russell Mechanism has been variable. By inspection of reaction (3.3) it can be surmised that labeling hydroperoxides with mixtures of $^{16}O_2$ and $^{18}O_2$ should lead to isotope scrambling of the released O_2. Less than 1.5% isotope scrambling was observed with HPOD,[39] and none at all with the methyl ester of HPOD.[6] In the latter study the presence of peroxyl radicals was confirmed by the β-scission of O_2. In addition, the predicted products, a 1 to 1 ratio of the methyl esters of HOD and OOD, are not found when the methyl ester of HPOD was decomposed via peroxyl radicals by either tert-butylperoxyoxalate or Cu^{2+} ions.[40,41] On the other hand, peroxyl radicals of oleic acid do afford both hydroxyoctadecenoic and oxooctadecenoic acids.[16] The picture that seems to emerge is one in which peroxyl radicals of fatty acids containing two or more double bonds probably are diverted into more competitive pathways.

There is an additional caveat concerning the reaction of peroxyl radicals from the methyl ester of HPOD. Under anaerobic conditions a peroxide-linked dimer was obtained by Schieberle and Grosch[41]:

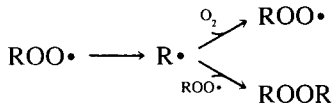


(3.4)

According to them, the dimer could form by a mechanism similar to the Russell Mechanism, except in the decomposition of the tetraoxide two alkoxyl radicals recombine in a solvent cage:

$$2\ ROO\bullet \longrightarrow ROOOOR \longrightarrow \underset{\text{solvent cage}}{[RO\bullet O_2\bullet OR]} \longrightarrow ROOR + O_2 \qquad (3.5)$$

If reaction (3.5) is the actual mechanism, it is difficult to rationalize why O_2 inhibited dimer formation.[41] A more attractive proposal in my view is peroxyl radical β-scission followed by pentadienyl-peroxyl radical combination,[42] and one would predict competition from O_2 in such a reaction:



(3.6)

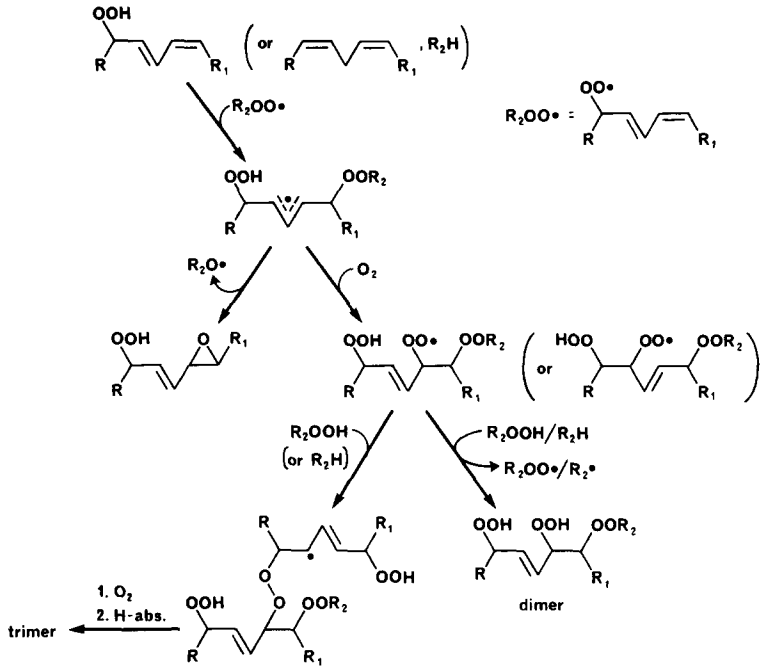
Reaction (3.6) is supported by the isolation of conjugated methyl octadecadienoate dimers from the anaerobic decomposition of HPOD methyl ester by a copper catalyst.[43] A similar pathway is indicated:

$$2\ ROO\cdot \xrightarrow{2O_2} 2\ R\cdot \longrightarrow RR \qquad (3.7)$$

Dimers structurally consistent with the pathway indicated by reaction (3.6) are produced during autoxidation of methyl linolenate, the methyl ester of HPOT, and other oxidation products of linolenate.[44] Interestingly, a variety of similar peroxide-linked dimers were produced regardless of the initial oxidation state of the reactant, probably because of multiple secondary reactions, such as oxidation and peroxyl radical β-scission and cyclization.

D. Intermolecular addition of peroxyl radicals

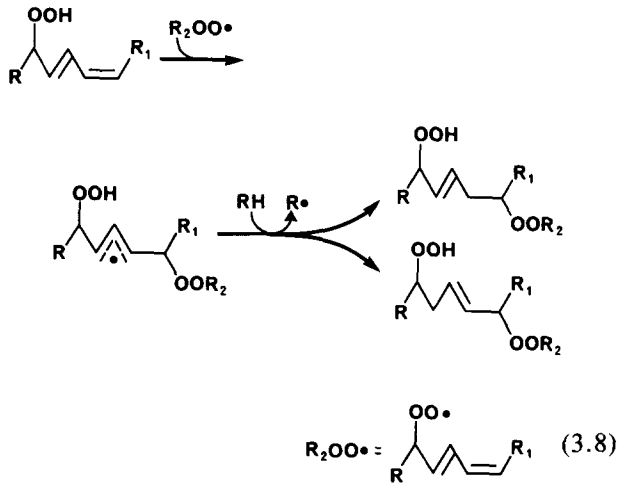
Intermolecular addition reactions of peroxyl radicals are difficult to review as information regarding them is sparse. Hypothetically, the peroxyl radical adds to the terminal olefinic carbon of a hydroperoxydiene or other olefinic carbons of PUFA. The carbon radical afforded by addition can either proceed on to dimers, trimers and oligomers or terminate as an epoxide in a competing pathway (Scheme 5). For the autoxidation of styrene the kinetics of the two pathways have been studied in detail.[45] One would surmise that such reactions would proliferate during the autoxidation of neat PUFA where intermolecular reactions would be



Scheme 5. Hypothetical mechanisms of intermolecular addition of peroxyl radicals to the 13-hydroperoxide of linoleic acid. R = $CH_3(CH_2)_4^-$; R_1 = $-(CH_2)_7COOH$.

favored, such as in the curing or polymerization of linseed oil. Indeed, PUFA oligomers appear to be cross-linked primarily by peroxide bonds.[46]

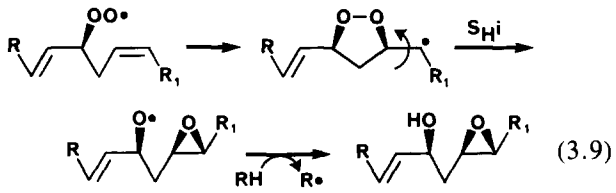
Recently, peroxide-linked dimers have been isolated from the neat autoxidation of HPOD methyl ester.[47] One type of dimer was structurally consistent with the mechanism shown in Scheme 5, and another could be derived from a similar pathway:



(3.8)

Such dimers constituted about 18% of products arising from the autoxidation of neat HPOD methyl ester,[47] and dimerization was observed even in the initial stage of autoxidizing neat methyl linoleate.[48]

Next, let us consider the evidence for epoxidation by peroxyl radicals (Scheme 5). Because there are ample heterolytic examples of epoxidations of olefins catalyzed by peracids and by hydroperoxides in the presence of metal ions, the homolytic route to epoxides is difficult to prove unequivocally. However, Mihelich[49] documented an intramolecular epoxidation which is comparable to the intermolecular pathway shown in Scheme 5. In his investigation the following reaction was characterized starting from a peroxyl radical generated from a nonconjugated hydroperoxide of methyl linoleate:



(3.9)

After cyclization of the peroxyl radical at the *cis* olefin, the sp^3 bond evidently rotates to a more favorable transoid conformer. Subsequent backside attack of the carbon radical on the peroxide bond resulted in formation of *trans* epoxide from the *cis* olefin. If the example reported by Mihelich[49] is instructive, this suggests that homolytic epoxidation may change the geometry of the reacting olefin; whereas, it is known that heterolytic epoxidation retains geometry. In autoxidation mixtures simple epoxy fatty acids containing no other oxygen-

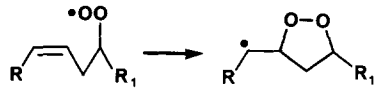
ated functionality are *cis* with linoleate[50] and linolenate,[51] and a mixture of *cis* and *trans* with oleate.[52] Does the epoxide geometry reflect their mechanism of formation? This would appear to be a question for further research. Inasmuch as 8,9- and 10,11-epoxides of oleate are found in addition to the expected 9,10-epoxide,[53] other free radical reactions may be involved with monounsaturates.

Epoxy fatty acids containing other oxygenated groups are usually isolated after peroxyl radical decomposition of PUFA hydroperoxides, and because alternative mechanisms exist, the origin of the epoxide is difficult to deduce. According to Schieberle et al,[40] the formation of methyl epoxyhydroxyoctadecenoate and methyl epoxyoxooctadecenoate from peroxyl radical decomposition of 13-HPOD methyl ester may have originated by either peroxyl radical epoxidation of the methyl esters of OOD and HOD or the alkoxyl radical rearrangement discussed below. Since 13-HPOD methyl ester was rapidly racemized both positionally and stereochemically by β-scission under their conditions, structrural analyses furnished no clues concerning the mechanism. Inasmuch as the presence of O_2 enhances the formation of these products, it is more likely that they do not originate via epoxidation by peroxyl radicals.

E. Intramolecular rearrangement of peroxyl radicals

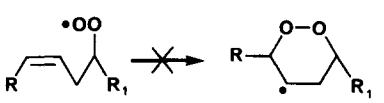
Intramolecular rearrangement of peroxyl radicals from PUFA hydroperoxides leads to both cyclic peroxides and bicyclo endoperoxides. The latter class of product received inordinate attention in recent years because it has similarities in structure to natural prostaglandins.

As discussed previously in the autoxidation section above, this rearrangement is initiated by cyclization through intramolecular addition of peroxyl radicals to the β,γ double bond:



(3.10)

Working with model compounds, Porter et al.[54] noted that only *exo* cyclization occurred [reaction (3.10)], and the *endo* cyclization was highly disfavored [reaction (3.11)].

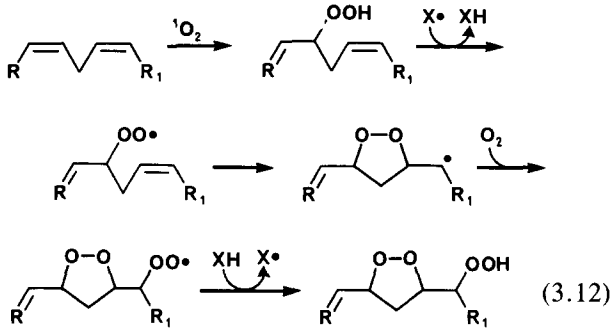


(3.11)

Organic chemists have known for years that *endo* cyclization by free radicals, is not very common.[55]

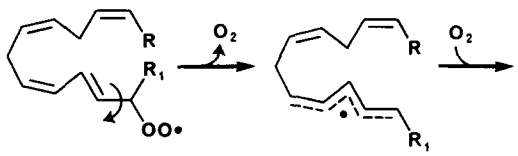
As seen by reaction (3.10), the structure necessary for rearrangement includes inner hydroperoxides derived from PUFA with three or more double bonds and non-conjugated hydroperoxides obtained by $^1O_2$ oxidation of PUFA with two or more double bonds. Despite certain proposals in the literature, the peroxyl radical from conjugated HPOD does not rearrange intramolecularly with either of the two double bonds; in particular, it should be noted that the α,β *trans*-olefin spatially separates the peroxyl radical from the γ,δ double bond.

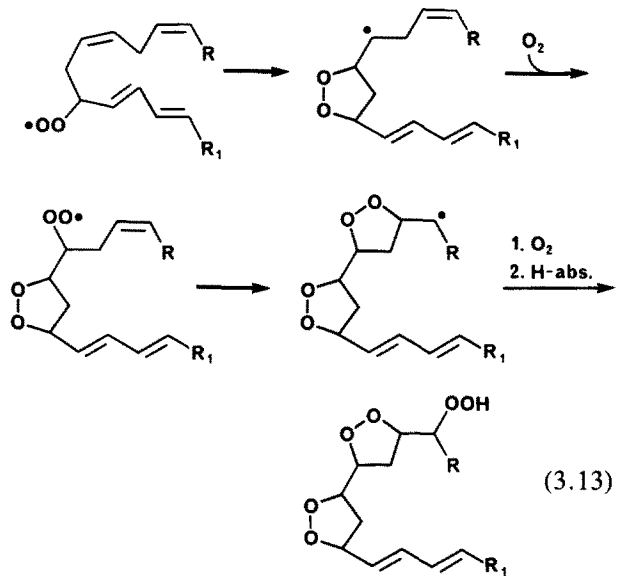
Chan, et al.[8] defined the mechanism of rearrangement by reacting the methyl ester of 13-HPOT to obtain methyl 13,15-epidioxy-16-hydroperoxy-*cis*-9,*trans*-11-octadecadienoate essentially by the pathway shown in reaction (1.9). Numerous examples of this pathway are now found in the literature, including the analogous reaction of 12-hydroperoxy-*cis*-9,*trans*-13,*cis*-15-octadecatrienoic acid (12-HPOT). Similar hydroperoxyepidioxides also are obtained from hydroperoxides of methyl arachidonate.[56] Mihelich[49] utilized nonconjugated hydroperoxides of methyl linoleate obtained by $^1O_2$ oxidation to produce hydroperoxy-epidioxides by peroxyl radical rearrangement:



(3.12)

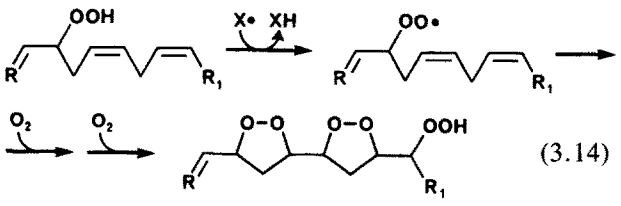
As might be surmised, PUFA with four or more double bonds are susceptible to serial cyclization. Starting with the methyl ester of 15-HPETE, Khan and Porter[57] demonstrated the formation of methyl 5-hydroperoxy-6,8,9,11-*bis*-epidioxy-*trans*-12,*trans*-14-eicosadienoate, which had to be transformed via sequential peroxyl radical reactions of β-scission and serial cyclization:





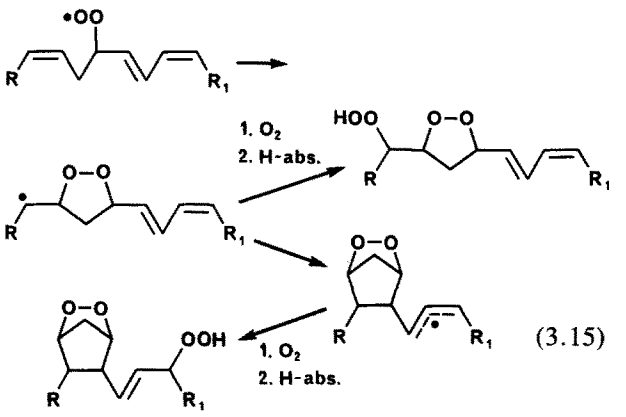
(3.13)

Similarly, unconjugated 10- and 15-hydroperoxides derived by $^1O_2$ oxidation of methyl linolenate also possess the requisite structures for serial cyclization[58]:



(3.14)

Discovery of the structures of prostaglandins led researchers to propose free radical approaches to its biosynthesis, and the possibilities for a wholly free radical pathway to prostaglandins from PUFA was not overlooked by investigators in the field. Early workers reported evidence for prostaglandin-like compounds from autoxidation of various triene fatty acids.[59-61] The pathway to prostaglandin-like bicyclo endoperoxides is competitive with the formation of hydroperoxy epidioxides as shown by the reaction of the 13-peroxyl radical of linolenic acid:

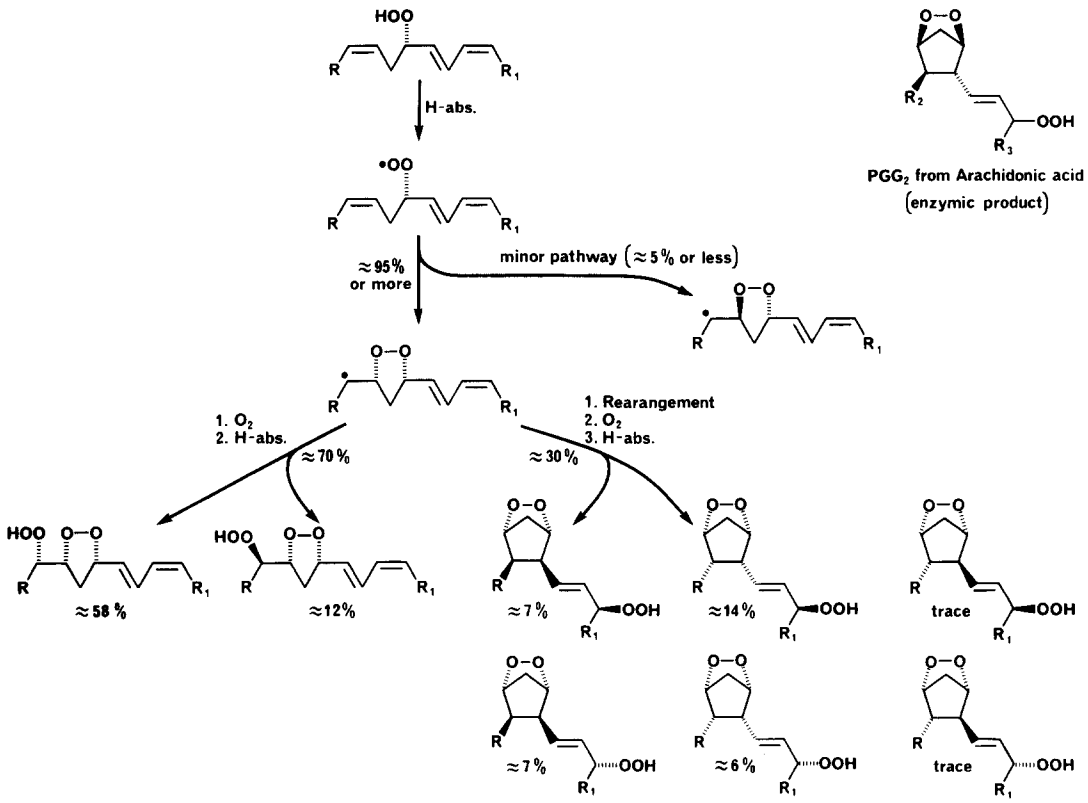


(3.15)

The structural requirements of the reactant hydroperoxides are the same as for the competing reaction producing hydroperoxy epidioxides. However, the formation of bicyclo endoperoxides has not yet been reported from reaction of $^1O_2$-produced nonconjugated hydroperoxides of linoleic acid even though the reaction appears theoretically possible. Since one pathway at the branch in reaction (3.15) requires O_2, lowering the partial pressure of O_2 decreased the yield of hydroperoxy epidioxide to favor bicyclo endoperoxides.[62,63] It also should be noted in reaction (3.15) that the final oxidation of the bicyclo endoperoxide to the hydroperoxide occurred only at the outer end of the allylic carbon radical.[62]

As summarized in Scheme 6, there were definite stereochemical preferences in both the cyclization of the peroxyl radical and the cyclopentane ring. With the initial cyclization of the peroxyl radical a decided preference for *cis* placement of the cyclic peroxide is observed. The *trans* conformation of the cyclic peroxide was only 5% of the total in the rearrangement of the nonconjugated hydroperoxides of methyl linoleate [reaction (3.12)],[49] and the *trans* was apparently so minor that it was not reported in the detailed study of isomeric hydroperoxy epidioxides and hydroperoxy bicyclo endoperoxides originating from hydroperoxides of linolenate.[62] In Scheme 6 it is also shown that there is preference for *cis* arrangement of the alkyl/alkenyl side chains in the formation of bicyclo endoperoxides; that is, the side chains are oriented either *exo,exo* or *endo,endo* with a preference for the latter.[62] A theoretical explanation for the *cis* orientation of the side chains was reported recently by Corey et al.[64] It is interesting to compare the stereochemistry of the free radical reactions with the related enzymic biosynthesis of prostaglandins. The *cis* placement of the cyclic peroxide is identical with prostaglandin synthetase, but the enzyme orients the side chains only in the *trans* configuration. As seen in Scheme 6, the formation of natural stereoisomers with this *trans* orientation of the side chains occurred only to a minor extent.[62]

In the generation of both hydroperoxy epidioxides and hydroperoxy bicyclo endoperoxides there is a stereochemical preference in the final oxygenation to hydroperoxides (Scheme 6). An *erythro* configuration between the hydroperoxide and the epidioxide is preferred 5 to 1 over the *threo* in the formation of hydroperoxy epidioxides from 13(*S*)-HPOT.[65,66] It should be noted that the first communication[65] reported a preference for *threo*, but further work by the same researchers[66] resulted in a correction in assignment to *erythro*. Formation of hydroperoxy epidioxides from nonconjugated hydroperoxides of linoleate resulted in a 58% preference for the *erythro* configuration between

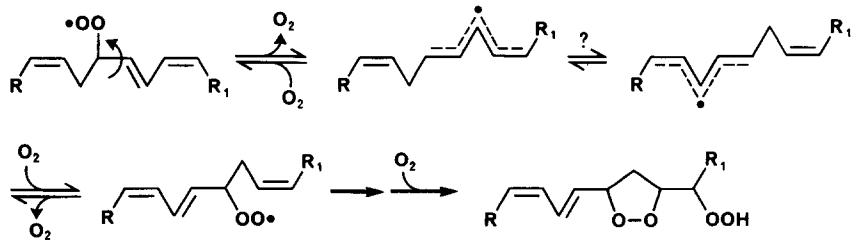


Scheme 6. Reaction of the peroxyl radical from the 13(*S*)-hydroperoxide of linolenic acid to afford isomeric hydroperoxy epidioxides[65,66] and hydroperoxy bicyclo endoperoxides.[62] R = $CH_3CH_2^-$, R_1 = $-(CH_2)_7COOH$.

the hydroperoxide and epidoxide.[49] In the final hydroperoxidation of bicyclo endoperoxides there also appears to be a decided stereochemical difference at least for the two *endo,endo* isomers (Scheme 6).

Since β-scission of peroxyl radicals tends to racemize the reactant hydroperoxide to some extent, the pathways shown in Scheme 6 are somewhat conceptual. Thus, starting with 13(*S*)-HPOT, the chirality of the hydroperoxide racemizes[62] and diene conjugation is converted from *cis,trans* to *trans,trans*[65] as the reaction progresses. Surprisingly, there also appears to be a pentadienyl radical system cross-over that permits the formation of a minor amount (12% of total hydroperoxy epidioxides) of methyl 11,12-epidioxy-9-hydroperoxy-*trans*-13,*cis*-15-octadecadienoate from the methyl ester of 13(*S*)-HPOT, but the exact mechanism is unknown[65]:

Although the methyl ester of 13(*S*)-HPOT partially racemizes by peroxyl radical β-scission, the *erythro* and *threo* isomers of the direct rearrangement products largely should be methyl (13*S*, 15*R*, 16*S*)-13,15-epidioxy-16-hydroperoxy-*cis*-9,*trans*-11-octadecadienoate and methyl (13*S*, 15*R*, 16*R*)-13,15-epidioxy-16-hydroperoxy-*cis*-9,*trans*-11-octadecadienoate, respectively (Scheme 6). The product of pentadienyl radical cross-over [reaction (3.16)] and the *trans,trans* isomers would undoubtedly be racemates from the prerequisite process of β-scission. The formation of hydroperoxy epidioxides from the methyl ester of (9*S*)-9-hydroperoxy-*trans*-10,*cis*-12,*cis*-15-octadecatrienoate [9(*S*)-HPOT][65] would by necessity have to racemize through β-scission of the peroxyl radical in order to cyclize. Similar racemizations undoubtedly



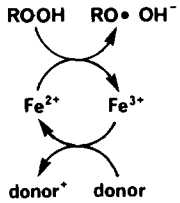
(3.16)

occur by the hydroperoxy bicyclo endoperoxide pathway as observed by O'Connor et al.[62]

4. ALKOXYL RADICAL REACTIONS

A. *Formation of alkoxyl radicals*

At a BDE of ≈44 kcal mol^{-1} the RO—OH bond does not spontaneously disrupt by homolytic fission. The most common route to alkoxyl radicals is from reduction of hydroperoxides by lower oxidation states of transition metal ions. In the presence of a molar equivalent of a suitable electron donor, only a catalytic quantity of metal ion is sufficient to permit redox cycling as shown for the Fe^{2+}/Fe^{3+} couple:



(4.1)

The reductive role of metal ions depends on several factors, such as the type of metal ion, complexing ligands, pH and solvent.[30,31] Non-metals are also known to reduce hydroperoxides to alkoxyl radicals, such as observed with bisulfite.[67] Convincing evidence has been presented that superoxide (O_2^-) reductively generates alkoxyl radicals from tert-butylhydroperoxide,[68] but O_2^- is unreactive with PUFA hydroperoxides.[69]

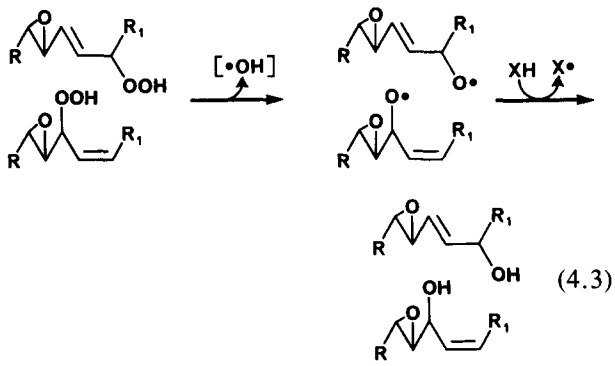
Photolysis of the peroxide bond by UV light is also very effective in generating alkoxyl radicals from lipid hydroperoxides.[70] Although heat homolyzes the peroxide bond, this method is too unspecific to exclude other homolytic and heterolytic reactions from interfering in the desired alkoxyl radical formation.

B. *Hydrogen abstraction by alkoxyl radicals*

Alkoxyl radicals generally are known for their ability to abstract hydrogens:

$$RO\cdot + XH \longrightarrow ROH + X\cdot \qquad (4.2)$$

Weakly bonded hydrogens are easily abstracted to form a stronger RO—H bond at about 104 kcal mol^{-1}. However, the facility by which organic alkoxyl radicals, like the tert-butyloxy radical, abstract hydrogens, apparently does not extend to alkoxyl radicals in PUFA. Because of the presence of unsaturation of PUFA hydroperoxides, other reactions are evidently more competitive than hydrogen abstraction. As discussed below, alkoxyl radicals of PUFA tend to rearrange into epoxyallylic radicals, even in the presence of compounds with a readily abstractable hydrogen, like 2,2,5,7,8-pentamethyl-6-hydroxychroman[71] and *N*-acetylcysteine.[72] As assessed by the formation of HOD (methyl ester) from HPOD (methyl ester), hydrogen abstraction by alkoxyl radicals generated from UV photolysis amounted to less than 10%.[70,73] On the other hand, the hydroperoxide group of epoxyhydroperoxyoctadecenoic acid (or ester), arising from O_2 scavenging of the epoxyallylic radical, degrades into either a hydroxy group[73] or hydroxy and oxo groups,[74] implying hydrogen abstraction:

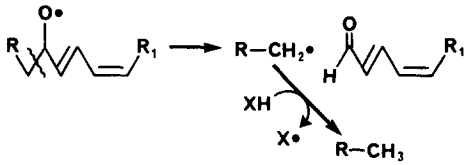


(4.3)

In summary, the putative hydrogen abstraction by primary PUFA alkoxyl radicals appears to be less important than originally thought, and the abstraction process may be restricted to alkoxyl radicals originating from secondary hydroperoxides as depicted in reaction (4.3).

C. β-*Scission of alkoxyl radicals*

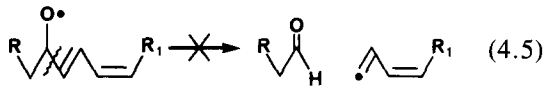
β-scission of alkoxyl radicals results in carbon-carbon chain cleavage:



(4.4)

For example, alkoxyl radical β-scission of 13-HPOD should afford pentane and 13-oxotridecadienoic acid which is observed. To explain the formation of certain other products from chain scission, like hexanal from 13-HPOD, cleavage between the alkoxyl-radical carbon and the double bond has been invoked; however, the heat of formation and the related BDE required for formation of a vinyl radical is larger than for an alkyl radical.[75] Thus reaction (4.5) is of doubtful signifi-

cance:



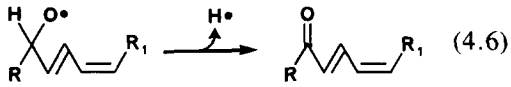
(4.5)

A heterolytic cleavage between the hydroperoxy carbon and the double bond, known to yield hexanal and 12-oxododecenoic acid from 13-HPOD, is discussed below. It is possible that such heterolytic reactions significantly contribute to cleavage products, but this hypothesis has not been tested.

β-Scission of alkoxyl radicals from PUFA hydroperoxides usually amounts to less than 10% of the total products. Oleate hydroperoxides afford 20% cleavage products.[76] Remarkably, the nonconjugated hydroperoxide of linoleic acid, 10-hydroperoxy-8,12-octadecadienoic acid, furnishes a 92% yield of the alkoxyl radical scission fragment, 10-oxo-8-decenoic acid.[76] This high yield from the latter hydroperoxide undoubtedly reflects the stability of the allylic radical cleavage product.

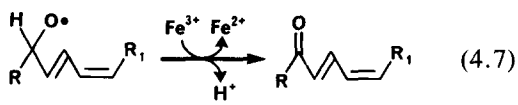
As revealed by recent reviews,[77-79] there is an extensive literature concerning cleavage of PUFA hydroperoxides into volatile fragments. Current research has focussed on scission products from hydroperoxides or peroxides of secondary oxidation products, such as hydroperoxy epidioxides,[80-81] dihydroperoxides,[81] hydroperoxy bicyclo endoperoxides,[82] epoxyhydroperoxides,[83] and dimers.[84]

In theory at least, oxodienes could arise from β-scission of hydrogen from the alkoxyl radical:



(4.6)

ODA and other oxodiene PUFA are often a significant portion of metal-catalyzed reactions; thus, it has been suggested that the alkoxyl radical may be oxidized to the ketone by a higher oxidation state of the metal.[76]



(4.7)

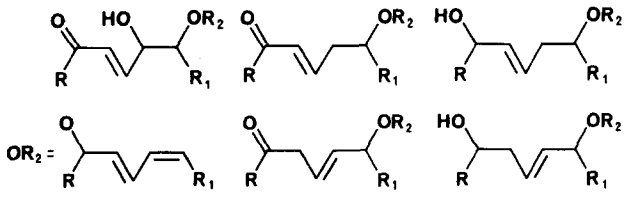
In any case, more definitive research is needed to understand the mechanistic origins of oxodiene PUFA.

D. Ether-linked dimers via alkoxyl radicals

Early investigators[85,86] found that oxidizing methyl oleate or triolein at high temperatures (65° or 185°C) led to dimers and oligomers linked by ether and/or carbon-carbon bonds. In theory, such ether-linked products should form by either combination or intermolecular addition of alkoxyl radicals. Considering the former reaction possibility, ether-linked dimers might form by combination:

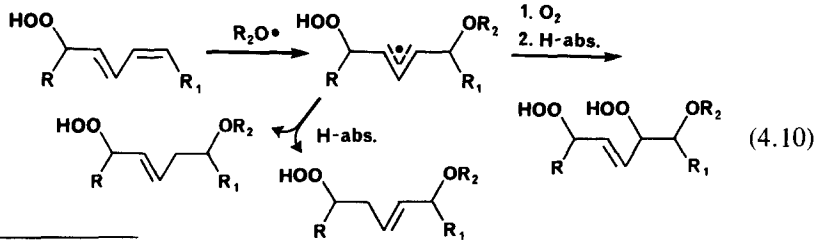
$$RO\cdot + R\cdot \longrightarrow ROR \qquad (4.8)$$

However, recent research has indicated that ether bonds are more likely to be formed by the latter mechanism of intermolecular addition of the alkoxyl radical. Dimers isolated from low temperature (30°C) autoxidation of the neat methyl ester of HPOD afforded structural information as follows[47]:



(4.9)

These structures permit construction of a hypothetic pathway by intermolecular addition (Eq. 4.10). Since it is conceivable that some of these dimers (4.9) also could arise by substitution at an epoxide, more research is required to ensure that the intermolecular addition mechanism is truly applicable.

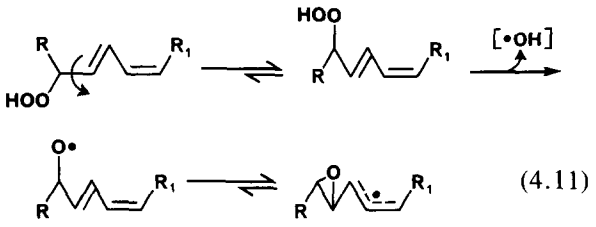


(4.10)

E. Intramolecular rearrangement of alkoxyl radicals

It has been known for several years that alkoxyl radicals of HPOD rearrange intramolecularly by addition to the α, β double bond to afford an epoxyallylic radical.[87-89] Because carbon-carbon bond rotation be-

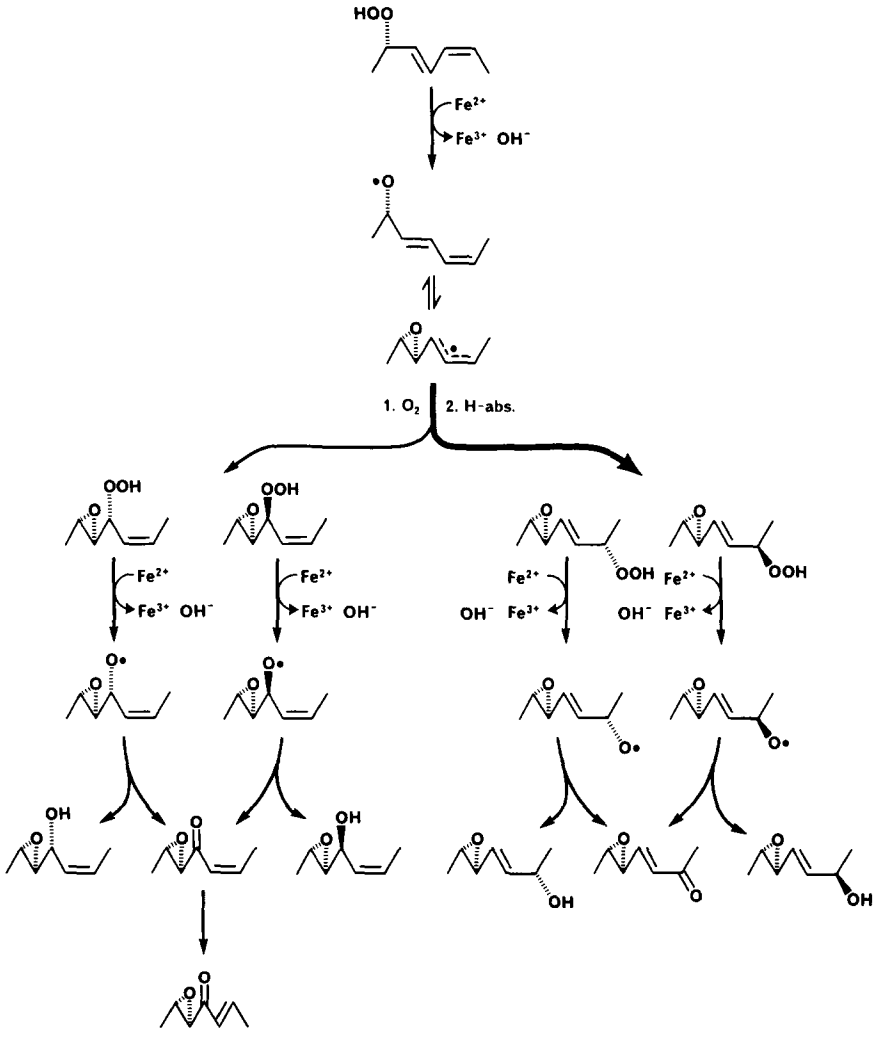
tween the hydroperoxide groups and the double bond gives the least hindered *transoid* rotamer, these epoxides are predominantly *trans*[74]:



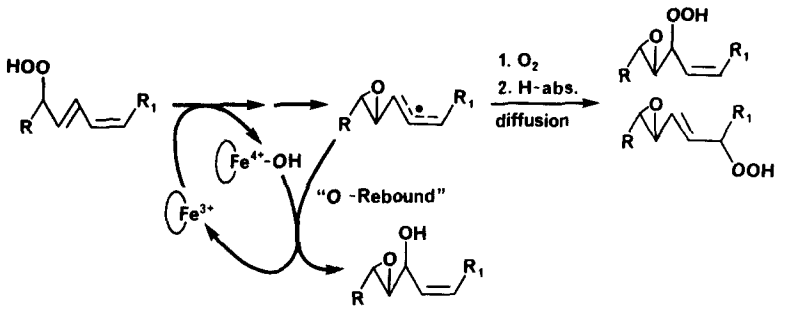
(4.11)

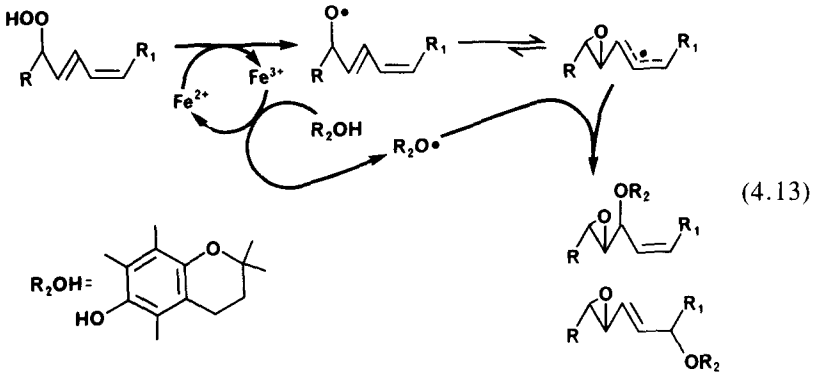
Recently, the epoxyallylic radical has been spin-trapped and characterized by electron spin resonance (ESR).[90] Autoxidation of methyl linolenate,[14] methyl oleate[52] and HPETE[56] also have produced epoxides of the type that could be attributed to this alkoxyl radical rearrangement.

After the epoxyallylic radical forms it combines with another available radical at either end of the allylic system, especially at the carbon γ to the epoxide.[72,74,89] Under aerobic conditions the epoxyallylic radical from 13(*S*)-HPOD is scavenged by O_2 to form isomeric epoxyhydroperoxyoctadecenoic acids,[91] and further homolysis of the hydroperoxide group by an Fe^{2+} catalyst results in isomeric epoxyhydroxyoctadecenoic and epoxyoxooctadecenoic acids,[74] (Scheme 7). Isomeric epoxyhydroxyoctadecenoic acids in the absence of epoxyoxooctadecenoic acid are obtained with UV photolysis of 13(*S*)-HPOD,[73] possibly indicating the intermediate alkoxyl radical in the former study is partially oxidized to the ketone by Fe^{3+} in a manner similar



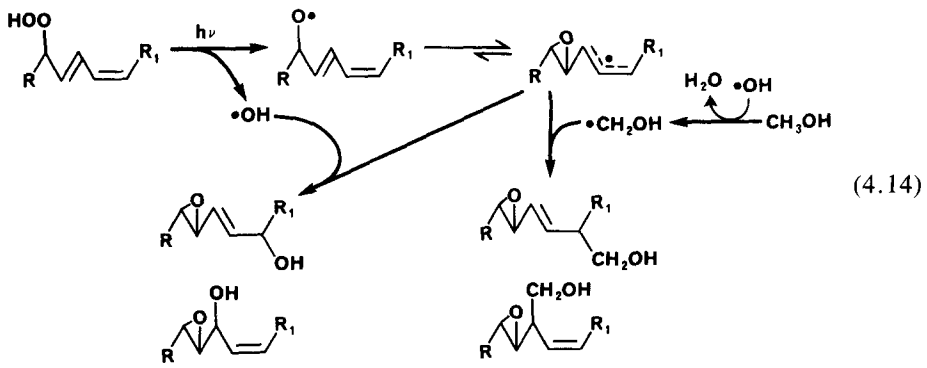
Scheme 7. Reaction of the alkoxyl radical from the 13 (*S*)-hydroperoxide of linoleic acid to afford isomeric epoxyhydroperoxy-, epoxyhydroxy- and epoxyoxo-octadecenoic acids.[74] Fatty acid structures are abbreviated to show only C-8 up to and including C-14.

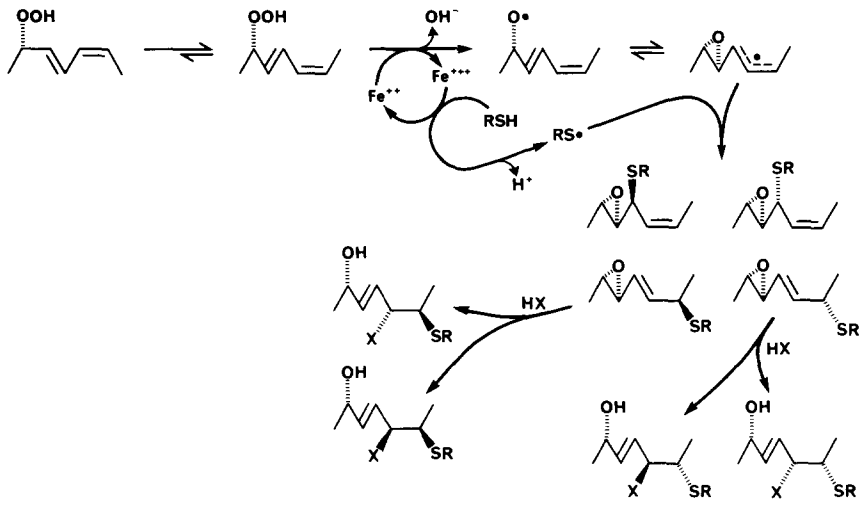




to reaction (4.7). With hematin as a catalyst a portion of the epoxyallylic radicals diffuse from the catalyst to combine with O_2, and the remainder react by transfer of ·OH from the hydroperoxide by "oxygen rebound" from the Fe^{4+}—OH oxidation state of hematin[92] (Eq. 4.12).

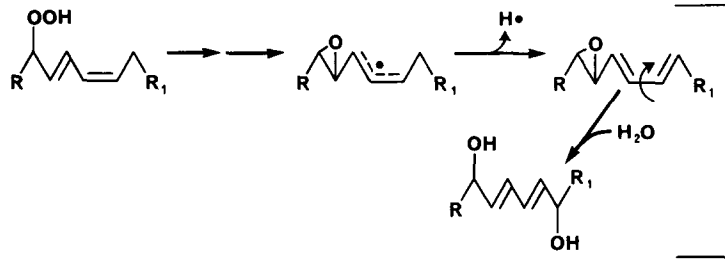
In the absence of O_2 the epoxyallylic radical traps the prevalent available radical. For example, an Fe^{2+}/Fe^{3+} redox cycle can generate epoxyallylic radicals and thiyl radicals from 13(*S*)-HPOD and *N*-acetylcysteine, respectively, and without competition from O_2 these radicals combine[72] (Scheme 8). Similar results are obtained in an anaerobic reaction of 13(*S*)-HPOD with the α-tocopherol analog, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC) as catalyzed by a Fe^{2+}/Fe^{3+} redox couple[87,93,94] (Eq. 4.13). Certain isomeric features of these PMHC-13(*S*)-HPOD combination products that were initially reported by our laboratory are incorrect,[87] but these features were corrected by subsequent unpublished research of ours[93] and by others[94] [a minor branch pathway to an isomer with a *trans*-9 double bond is not shown in reaction (4.13)]. In a final example, UV photolysis of 13(*S*)-HPOD in methanol with the absence of O_2 resulted in combination of the epoxyallylic radical with ·OH and $\cdot CH_2OH$.[70] Evidently, UV photolysis of the hydroperoxide generated ·OH which in turn abstracted hydrogen from methanol (Eq. 4.14).





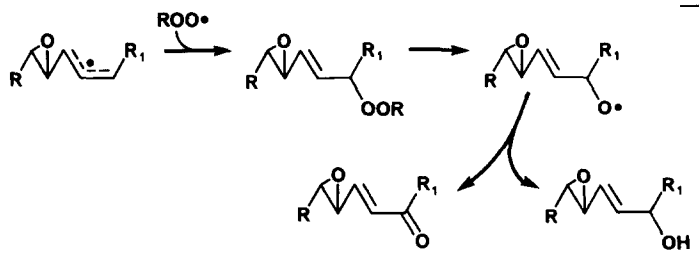
Scheme 8. Reaction of the alkoxyl radical from the 13 (*S*)-hydroperoxide of methyl linoleate under anaerobic conditions in the presence of *N*-acetylcysteine (RSH).[72] An Fe^{2+}/Fe^{3+} redox cycle generates alkoxyl radicals from the hydroperoxide and thiyl radicals from *N*-acetylcysteine; allylic epoxides are susceptible to solvolysis by protic solvent (HX). Fatty ester structures are abbreviated to show only C-8 up to and including C-14.

Low yields of leukotriene-like fatty acids occurring in the autoxidation of linoleic acid probably originate from the epoxyallylic radical. β-scission of H· radical reportedly leads to an unstable epoxydiene which solvolyzes[95]:



(4.15)

It is legitimate to ask whether alkoxyl radicals significantly contribute to reactions supposedly involving only peroxyl radicals. For example, in the tert-butylperoxyoxalate degradation of HPOD (methyl ester), do methyl epoxyhydroxyoctadecenoate and methyl epoxyoxooctadecenoate originate by peroxyl radical epoxidation or by alkoxyl radical rearrangement? Although these fatty esters form even anaerobically in the presence of tert-butylperoxyoxalate,[42] one plausible mixed alkoxyl/peroxyl radical mechanism does not require O_2:

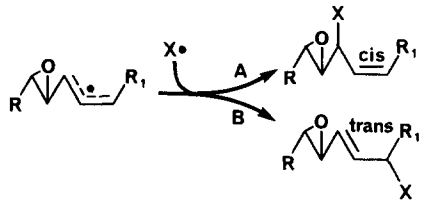


(4.16)

As shown in Schemes 7 or 8, the stereochemistry of alkoxyl radical rearrangement is straightforward being derived from the reactant 13(*S*)-HPOD. The stereochemistry of carbon-12 is based on the geometry of the epoxide, which is predominantly *trans* as shown in Schemes 7 and 8. Except for the nonchiral ketones, the functionality at carbons-9 and -11 are introduced *R* and *S* approximately in equivalent proportions, and these optical antipodes usually can be separated by chromatography because they are diastereomers.[72,74,96] Although the stereochemical projections in Scheme 7 are correct as shown, an incorrect Cahn-Prelog-Ingold designation was given to carbon-12 of the 12,13-epoxy-11-hydroxy-9-octadecenoic acid isomers.[74] Thus, both (11*S*,12*R*,13*S*)- and (11*R*,12*R*,13*S*)-12,13-epoxy-11-hydroxy-*cis*-9-octadecenoic acids should be changed

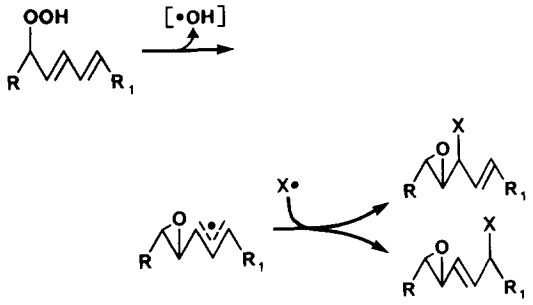
to (11*S*,12*S*,13*S*)- and (11*R*,12*S*,13*S*)-, respectively.

The stereochemistry of the olefin arising from the allylic radical usually conforms to the rules established for this type of reaction.[97] That is, allylic radicals are resistant to rotation, and the final products largely reflect the geometry of the original configuration. Thus, the position of combination determines the *cis* or *trans* geometry of the olefin by pathway A or B:



(4.17)

In the studies where a portion of the products obtained by pathway A are *trans* olefins instead, some contribution could arise from isomerization of the reactant hydroperoxide to *trans*, *trans* diene by β-scission of peroxyl radicals:



(4.18)

5. HETEROLYTIC REACTIONS

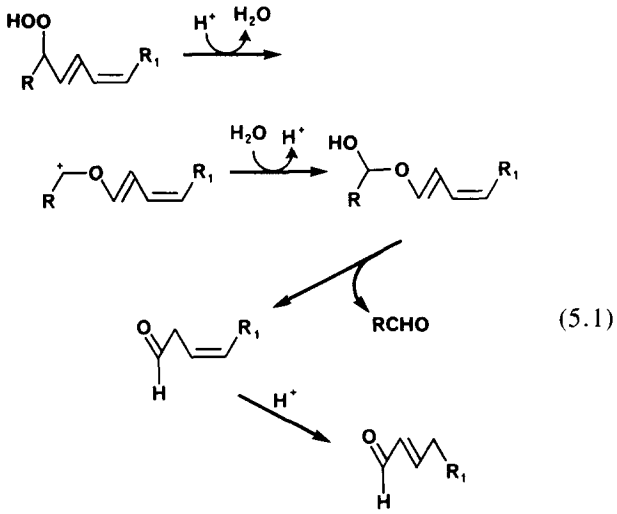
A common pitfall, occasionally ensnaring victims of free radical research, is the erroneous conclusion that hydroperoxides react only by homolytic mechanisms. Actually, hydroperoxides are fairly susceptible to heterolytic reactions often under mild conditions, and the resultant products can be similar to those obtained by homolysis. In addition, some of the products of homolytic reaction are transformed by heterolytic processes, such as epoxide solvolysis. Thus, it is appropriate to include a brief review of the non-radical character of hydroperoxides here.

A. Nucleophilic displacement

Hydroperoxides do not survive saponification[98] because OH^- as well as many other nucleophiles transform hydroperoxides into the corresponding hydroxyl group.[99] Whenever PUFA hydroperoxides are converted into corresponding hydroxy fatty acids, one should evaluate the nucleophilicity of the reaction and/or the methods of analysis. For example, below pH 7 cysteine is an ineffective nucleophile, but at pH's close to the pKa of the thiolate anion HPOD is readily transformed into HOD.[100]

B. Acid-catalyzed rearrangement

The acid-catalyzed carbon-to-oxygen rearrangement of hydroperoxides, the Hock/Criegee rearrangement, has been known for over a half century. In the example of acid treatment of the methyl ester of 13-HPOD, the expected products are hexanal and methyl 12-oxo-*cis*-9-dodecenoate, except that the *cis*-9-olefin isomerizes to *trans*-10 in acid[101]:

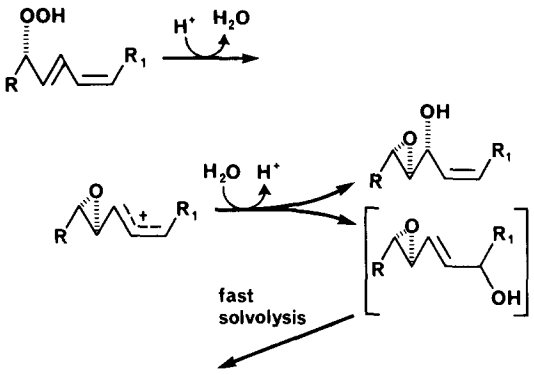


(5.1)

Although the cleavage into aldehydes occurs in protic solvents, aprotic solvent improves the yield of cleavage products.

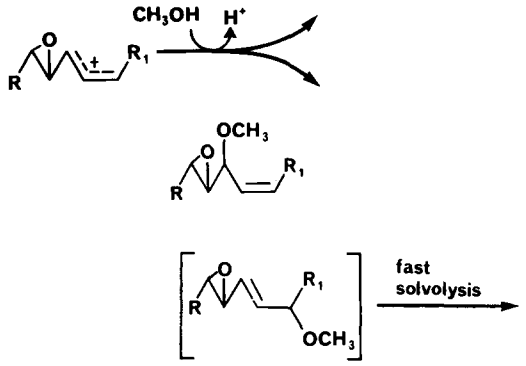
The role of hydroperoxide heterolysis in the production of volatiles could be more important than previously suspected. The unfavorable energetics involved in homolytic cleavage between the hydroperoxide and the double bond would appear to be circumvented by heterolysis. Future research is needed to address the question: is hydroperoxide heterolysis by action of incipient acids responsible for formation of certain volatiles, such as hexanal?

Acids in protic solvents shifts the reaction toward a modified rearrangement which results in epoxides[102]:



(5.2)

The stereochemistry of the heterolytic reaction is similar to the alkoxyl radical rearrangement described above, except the acid-catalyzed reaction affords selective substitution only *threo* at the carbon vicinal to the epoxide as shown in reaction (5.2). Thus, 13(*S*)-HPOD gives mainly (11*R*,12*S*,13*S*)-12,13-epoxy-11-hydroxy-*cis*-9-octadecenoic acid.† It is interesting to note that *threo*-12,13-epoxy-11-hydroxy-9-octadecenoic acid was obtained by heating 13-HPOD at 100°C in ethanol-water.[103] Inasmuch as $H_2{}^{18}O$-label was incorporated into the 11-hydroxyl, this reaction was undoubtedly a heterolysis of the same type. That the acid catalysis is strictly heterolytic was shown by its kinetic dependence on acidity and by substitution with methanol when it is the solvent[104]:



(5.3)

C. *Heterolytic epoxidation*

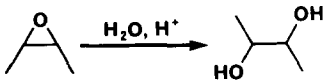
Hydroperoxides are known to epoxidize double bonds by heterolytic attack promoted by electrophilicity of the olefin. The stereochemistry of the double bond is retained:

(5.4)

Group IVa, Va and VIa transition metal ions, such as Mo, Cr, and V, sufficiently polarize the O—O bond of hydroperoxides by coordination to greatly facilitate epoxidation. Vanadium-catalyzed epoxidation of the hydroperoxides of methyl oleate produced methyl epoxyhydroxyoctadeconoates.[105] When vanadium catalyzed the reaction of 13(*S*)-HPOD, epoxidation occurred specifically at the *trans*-11 double bond affording two isomeric *trans*-epoxides; that is, 11*S*,12*S*- and 11*R*,12*R*-epoxides.[106] Transfer of hydroperoxide oxygen only occurred intermolecularly.

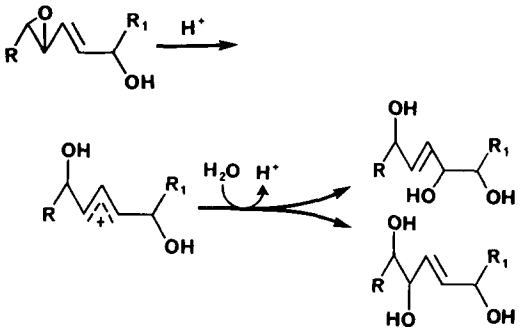
D. *Epoxide solvolysis*

Epoxides solvolyze by acid catalysis with substitution occurring from protic solvent, such as shown with H_2O:



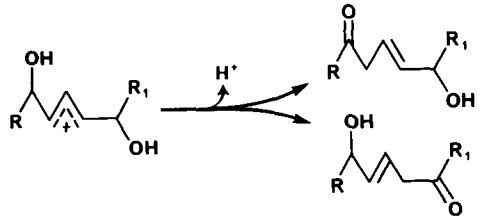
(5.5)

Allylic epoxides are particularly labile to solvolysis, even with very weak acids or protic solvent alone, and thus, methanol has been utilized as a trap to prove their existence.[107] Solvolysis of allylic epoxides permits substitution at either terminus of the allylic cation:[102,104,106,108]



(5.6)

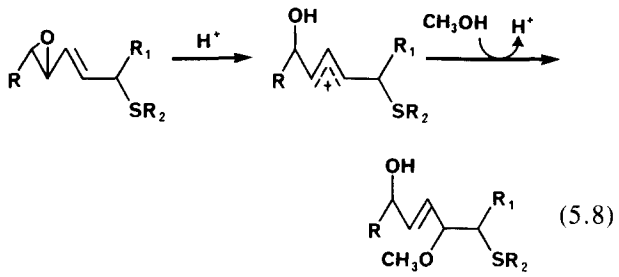
And, oxohydroxyoctadecenoic acid isomers apparently arise from the intermediacy of the allylic cation as well[109]:



(5.7)

†In the original report,[102] (11*R*,12*S*,13*S*)-12,13-epoxy-11-hydroxy-*cis*-9-octadecenoic acid was incorrectly designated (11*R*,12*R*,13*S*).

Certain substituents vicinal to one allylic terminus can direct substitution by anchimeric assistance, as for example, the thiyl ether group of the substituent, *N*-acetylcysteine[72]:



(5.8)

Nonallylic epoxides are considerably more stable even in acidic solution.[102,104] When they do solvolyze, usually one isomer is obtained with stereochemical inversion at the carbon being substituted.

Acknowledgments—Dr. Ned Porter kindly reviewed this manuscript. Dr. Mats Hamberg provided the correct Cahn-Prelog-Ingold nomenclature for the 12,13-epoxy-11-hydroxy-9-octadecenoic acids. Chuck Needham completed the art work.

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