Mechanisms for the Autoxidation of Polyunsaturated Lipids

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Lipid peroxidation is a complex process in which molecular oxygen and lipid react by a free-radical chain sequence. This process, known as autoxidation in purely chemical systems, leads to the degradation of naturally occuring fats and oils, and has been of interest to the chemical and biological community since lipids were first purified and were shown to be reactive with oxygen. The first systematic study of lipid peroxidation was begun in the 1940's by Bateman and Bolland[1,2] at the British Rubber Producers Research Association. This effort was initiated to gain an understanding of processes which lead to degradation of natural compounds such as rubber, waxes, and other lipids. Commercial interest in the process and its inhibition grew and several laboratories in Europe, the U.S., and Canada contributed to the understanding of this problem.[3]

Over the past two decades, interest in lipid peroxidation has intensified. Reasons for this increase of interest include the following:

(1) It was discovered that active oxygen species derived from superoxide radical lead to lipid peroxidation[4] and that an enzyme, superoxide dismutase, for scavenging superoxide radicals[5] exists. Superoxide is thus viewed as an intermediate which is an important cause of oxygen toxicity and is also known to lead (via active oxygen species such as hydroxyl radicals) to lipid peroxidation.

(2) It was observed that products analogous to those expected from lipid peroxidation, i.e., lipid hydroperoxides, are formed enzymatically. An example of such products are the fatty acid hydroperoxides derived from arachidonic acid (5,8,11,14-eicosatetraenoic acid) via lipoxygenase enzymes.[6] These fatty acid hydroperoxides serve as key intermediates in the biosynthesis of the leukotrienes and lipoxins, compounds with activity in the immune response.[7]

Another example of important enzymatic products with peroxide functionality are the prostaglandin endoperoxides PGG_2 and PGH_2 (see Scheme I). Again, arachidonic acid is the precursor to these peroxide compounds and the products serve as key intermediates to the prostaglandin and thromboxane families.[8]

(3) It was suggested that lipid peroxidation may be involved in neoplastic transformations.[9] Superoxide dismutase, for example, inhibits neoplastic transformations induced by X-rays or bleomycin in conjunction with phorbol myristylacetate (used as a tumor promotor), and it has been speculated that events such as membrane lipid peroxidation are involved in these events.

Ned Porter was born in Marion, OH, on May 10, 1943. He graduated from Princeton in 1965 with a B.S. in Chemical Engineering and accepted a position as Assistant Professor at Duke University in 1969, after receiving his Ph.D. with Paul D. Bartlett at Harvard. He is now a James B. Duke Professor of Chemistry at Duke. His major research interests have involved studies of free radicals and radical pairs, lipids and bilayer membranes, and more recently, radical macrocyclization, hydrophobic stereoenforcement, and enzyme photoactivation.

Peroxidation has also been related to initiation of the oxidative metabolism of polynuclear aromatic hydrocarbons.[10] Some of the metabolites of these compounds are extremely mutagenic and may be the ultimate carcinogenic form of the parent hydrocarbon.

(4) It was proposed that lipid peroxidation leads to chemical debris that accumulates with age. Lipofuscin the "wear and tear" age pigment, may be such chemical debris and a significant effort has been directed towards developing an understanding of the nature of the age pigment and how it forms. Lipofuscin is apparently the result of amino acid and other amine residues reacting with malonaldehyde, an autoxidation product of polyunsaturated fatty acids.

Even though lipid peroxidation has been the focus of much recent research, details of the chemical mechanisms involved in the process have been scarce until recent years. Most biological studies of peroxidation have utilized colorometric assays such as the formation of conjugated dienes[11] or the reaction of a lipid oxidation product, malonaldehyde, with thiobarbituric acid to give a colored adduct[12] as a measure of autoxidation. Titrimetric methods have also been used to measure peroxide formed in the oxidations.[13] All these assays give only a crude indication of the extent of lipid peroxidation, and the nature of the chemical events involved in the autoxidation of fatty acids and unsaturated phospholipids have remained in a "black box". The basic aim of this article and the research described here is to open this "black box" and reveal the nature of the chemical processes involved in autoxidation of lipids.

Peroxidation and Inhibition

The classical mechanism for autoxidation[14] involves two propagation steps, a slow step (eq 1) involving

$$\text{R–H} + \text{ROO}^\bullet \xrightarrow{k_p} \text{R}^\bullet + \text{ROOH} \quad (1)$$

$$\text{R}^\bullet + \text{O}_2 \rightarrow \text{R–OO}^\bullet \quad (2)$$

(1) Bateman, L. *Q. Rev., Chem. Soc.* **1954**, *8*, 147.
(2) Bolland, J. L. *Q. Rev., Chem. Soc.* **1949**, *3*, 1.
(3) Ingold, K. U. *Acc. Chem. Res.* **1969**, *2*, 1.
(4) (a) Kellogg, E. W. I. I. I.; Fridovich, I. *J. Biol. Chem.* **1977**, *252*, 6721. (b) Lynch, R. E.; Fridovich, I. *J. Biol. Chem.* **1978**, *253*, 1838.
(5) (a) Fridovich, I. *Acc. Chem. Res.* **1972**, *5*, 321. (b) Diguiseppi, J.; Fridovich, I. *CRC Crit. Rev. Toxicol.* **1984**, *12*, 315.
(6) Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *70*, 899.
(7) Adams, J.; Fitzsimmons, B. J.; Girard, Y.; Leblanc, Y.; Evans, J. F.; Rokach, J. *J. Am. Chem. Soc.* **1985**, *107*, 464.
(8) Serhan, C. N.; Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*(17), 5335.
(9) *Free Radicals, Lipid Peroxidation, and Cancer*; McBrien, D., Slater, T., Eds.; Academic: London, 1982.
(10) Dix, T. A.; Marnett, L. J. *Science* **1983**, *221*, 77.
(11) Recknagel, R. O.; Choshal, A. K. *Exp. Mol. Pathol.* **1966**, *5*, 413.
(12) (a) Slater, T. Methods in Enzymology; Packer, L., Ed.; Academic: Orlando, FL, 1984; Vol. 105, p 283. (b) Nair, V.; Turner, G. A. *Lipids* **1984**, *19*, 804.
(13) Tagaki, T.; Mitsuno, Y.; Masumura, M. *Lipids* **1978**, *13*, 147.
(14) Howard, J. A. *Adv. Free-Radical Chem.* **1972**, *4*, 49.

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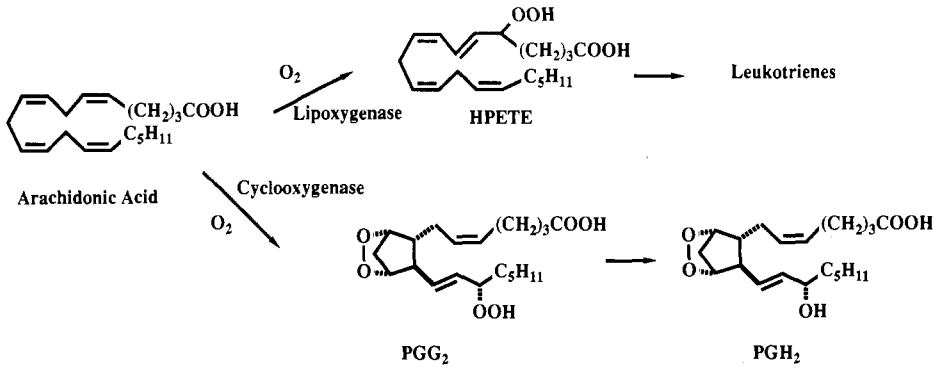
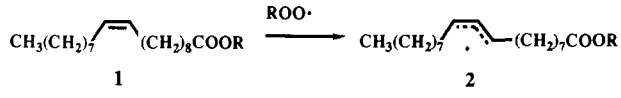
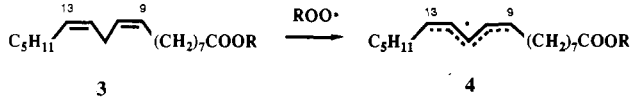
Scheme I



H-atom transfer with rate constant = k_p and a second step[15] in which oxygen adds to the intermediate alkyl radical (eq 2). The kinetics of autoxidation processes has been studied extensively by Ingold and Howard, and R–H which give stabilized radicals (R•) undergo rapid autoxidation.[16] This means that methylene-interrupted diene lipids such as linoleic acid, **3**, or arachidonic acid, **5**, undergo autoxidation more readily than monoolefin lipids such as oleic acid, **1**. Thus, oleic

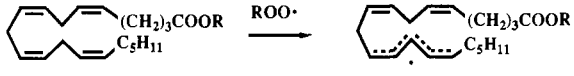


1 **2**



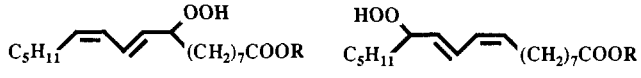
3 **4**

esters only undergo autoxidation at reasonable rates at elevated temperatures since simple allylic radicals like **2** are intermediates while linoleate esters autoxidize readily at room temperature via the more stabilized pentadienyl radicals, **4**. Arachidonic acid also leads to three different stabilized pentadienyl radicals of which **6** is but one example.

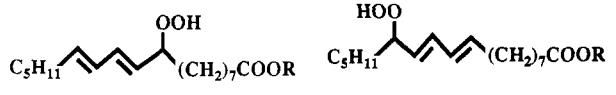


5 **6**

The primary products formed from linoleate oxidation[2] are hydroperoxides formed by oxygen addition at C-9 and C-13 of the pentadienyl radical **4**. Four of these products have been identified, and two of the products **7** and **8** have trans,cis-diene stereochemistry while the other two **9** and **10** have trans,trans stereochemistry.[17,18]



7 **8**



9 **10**

(15) Maillard, B.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 5095.
(16) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1967**, *45*, 785.

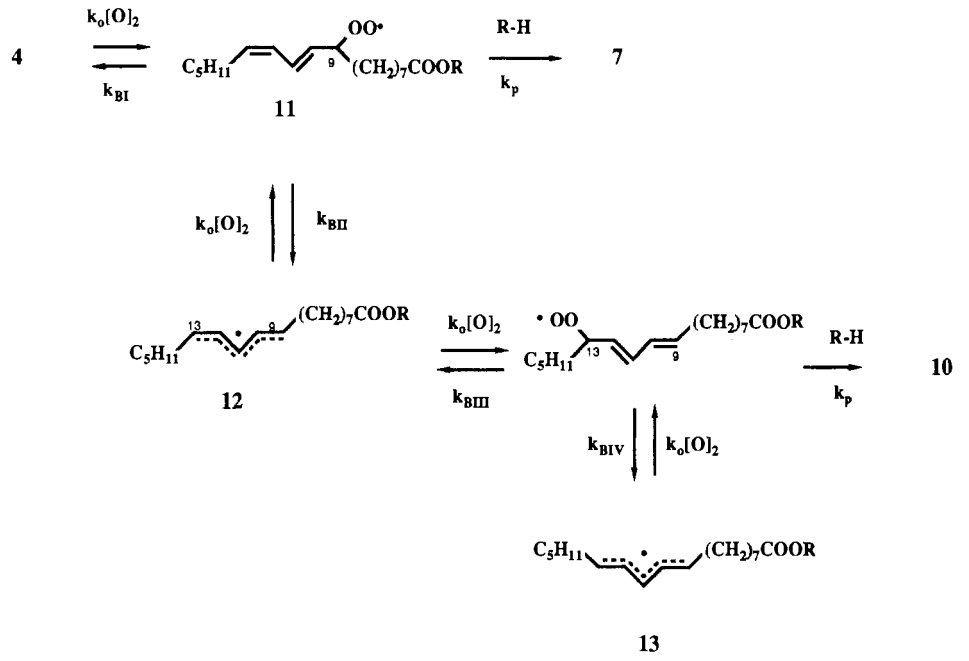
Other minor products are formed in the autoxidation of linoleate.[19] These minor products include nonconjugated diene hydroperoxides formed by abstraction of allylic hydrogens at C_8 and C_{14} of the linoleate precursor.

The distribution of the primary products **7–10**, depends on the conditions of the autoxidation,[18,19] and the following observations concerning the products formed are relevant to a consideration of the mechanism of the reaction: (1) The sum total of products formed from oxygen addition at C-9 (**7** and **9**) is the same as the products formed from oxygen addition at C-13 (**8** and **10**). (2) Higher autoxidation temperatures give rise to more trans,trans products. (3) Higher concentrations of linoleate give more trans,cis products. (4) Product distributions are independent of oxygen pressure between 10 and 1000 mm O_2. (5) The hydroperoxide **8** rearranges to a mixture of the four hydroperoxides by a free-radical mechanism and during this rearrangement atmospheric oxygen and the hydroperoxide oxygens exchange.[20] Based on these data, we proposed the mechanism shown in Scheme II to account for how products **7–10** are formed. The scheme shown here is simplified by consideration of initial oxygen addition at only one end (C-9) of the pentadienyl radical **4**, but it is understood that similar chemistry occurs at C-13.

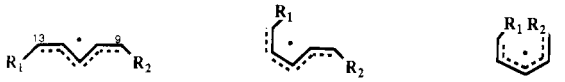
The first point to be considered in the mechanism described in Scheme II is the geometry of the radical **4**. There are three possible geometric isomers of the radical derived from linoleate (**4**, **14**, and **15**) that the Δ-9,10 and Δ-12,13 partial double bonds in the radical maintain the s-cis orientation of the linoleate precursor. We have suggested that, for linoleate, the radical **4** is favored since the Δ-10,11 and Δ-11,12 orientation is s-trans, thus minimizing unfavorable steric interactions such as those present in **14** and **15**. Bascetta, Gunstone, and Walton[21] have used EPR to study the radicals generated by *tert*-butoxy radical abstraction of the C-11 hydrogen of linoleate, and they conclude that the radical formed from linoleate under these conditions does indeed have the structure **4**. It is also worth noting that 1,4-pentadiene reacts with *tert*-butoxy radicals to give the parent pentadienyl radical in the *E,E* conforma-

(17) Porter, N. A.; Wolf, R. A.; Yarbro, E. M.; Weenen, H. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 1058.
(18) Porter, N. A.; Lehman, L. S.; Weber, B. A.; Smith, K. J. *J. Am. Chem. Soc.* **1981**, *103*, 6447.
(19) Haslbeck, F.; Grosch, W.; Firl, J. *Biochim. Biophys. Acta* **1983**, *750*, 185.
(20) Chan, H. W. S.; Levett, G.; Matthew, J. A. *Chem. Phys. Lipids* **1979**, *24*, 245.
(21) Bascetta, E.; Gunstone, F. D.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 1* **1983**, 603.

Scheme II



tion.[22] This supports the idea that 4 is the first-formed radical in the autoxidation of linoleate.



4 14 15

The second step of the sequence shown in Scheme II is the reaction of oxygen with the pentadienyl radical 4. The reaction of carbon radicals with oxygen is thought to be nearly diffusion controlled,[15] and the geometry of 4 requires that the initially formed peroxyl radical, 11, have trans,cis-conjugated diene geometry. That is, since the reaction of 4 and oxygen is rapid, other processes such as radical isomerization (vide infra) that cause 4 to lose geometry do not occur and the geometry of the initially formed carbon radical is translated to the geometry of the first-formed peroxyl radical.

Radical 11 has three mechanistic options available. First, abstraction of hydrogen from donors such as another linoleate converts 11 to the trans,cis product 7 and another radical 4. This is the normal formation of products 7 and 8 from linoleate. A second mechanistic pathway available to peroxyl radical 11 is loss of oxygen to give a pentadienyl radical, i.e., the reverse of oxygen addition. One mode of reversal of oxygen addition ($k_{\beta I}$) is stereochemically unproductive since no net change in stereochemistry attends oxygen loss from 11 leading back to 4. A second mode of oxygen loss ($k_{\beta II}$) from 11 leads to a new carbon radical 12 in which the stereochemistry of the Δ-9,10 partial double bond has been inverted from that in 4. The net effect of oxygen addition to 4 followed by loss of oxygen ($k_{\beta II}$) to give 12 is thus isomerization of the initially formed radical. Oxygen addition to 12 at the 13 position gives a peroxyl radical with trans,trans-diene geometry which ultimately gives hydroperoxide 10, a trans,trans-conjugated diene hydroperoxide. The mechanism outlined in Scheme II thus suggests that the trans,cis-conjugated dienes are products formed by autoxidation of linoleate under kinetic control while the trans,trans products can be considered to be the result of linoleate autoxidation under thermodynamic control.

The data concerning linoleate autoxidation are consistent with this mechanism. Thus, (1) products are formed from oxygen addition at either end of intermediate pentadienyl radicals and consequently the pseudosymmetry of the system leads to equal amounts of 9 and 13 substituted hydroperoxides. (2) At higher temperatures, β-fragmentation pathways become competitive with H-atom-transfer reactions and more trans,trans products are formed (thermodynamic control). (3) Higher concentrations of linoleate lead to more trans,cis products because increasing concentrations of the H-atom donor favor conversion of 11 to 7 (kinetic control). (4) Product distributions of hydroperoxide products are independent of $[O_2]$ pressure above 10 mm O_2 since all carbon radicals 4 are trapped at these concentrations of $[O_2]$ and the important stereochemical branch point in the sequence involves peroxyl radical 11 and not carbon radical 4. (5) The hydroperoxide, 8, rearranges to a mixture of the four hydroperoxides, and during this rearrangement atmospheric oxygen and the hydroperoxide oxygens exchange via β-fragmentation processes such as those outlined in Scheme II.[20]

Autoxidation Inhibitors

Phenolic autoxidants inhibit autoxidation of lipids by trapping intermediate peroxyl radicals in two ways.[23,24] First, peroxyl radicals are trapped by H-atom transfer giving hydroperoxide and aryloxy radicals (eq 3). Second, aryloxy radicals react by radical–radical

$$ROO^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet} \quad (3)$$

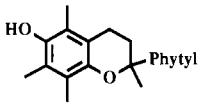
$$ROO^{\bullet} + ArO^{\bullet} \rightarrow ROO\text{–}ArO \quad (4)$$

(22) Griller, D.; Ingold, K. U.; Walton, J. C. *J. Am. Chem. Soc.* **1979**, *101*, 758.

(23) Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6472.

(24) Doba, T.; Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1983**, *105*, 6505.

coupling to give peroxide products. The intervention of ArOH in the autoxidation sequence slows autoxidative consumption of R–H, but also alters the stereochemistry of the hydroperoxide products formed during the period of inhibition.[25-27] Thus, autoxidation of linoleic acid or methyl linoleate in the presence of nature's antioxidant, α-tocopherol (vitamin E), leads



VITAMIN E

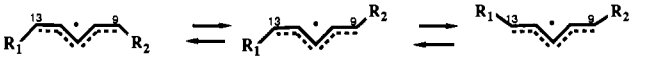
not only to the expected slowing of the rate of oxygen consumption, but also to a change in the product stereochemistry. The ratio of trans,cis-trans,trans-diene hydroperoxides formed during the lag-phase of inhibited autoxidation is >75/1 under conditions in which this product ratio would be .2/1 in the absence of α-tocopherol. The inhibitor clearly influences the product pathway and Scheme II provides a mechanism for understanding this product composition–inhibitor relationship. Inhibitors such as α-tocopherol transfer H atoms to intermediate peroxyl radicals with rates on the order of 10^5–10^6 M^{-1} s^{-1}, about four orders of magnitude faster than the normal propagation rates for atom transfer from hydrocarbons to peroxyl radicals. During inhibition, the hydroperoxide products are thus formed under kinetic control, the initial peroxyl radicals being efficiently converted to trans,cis products before unimolecular β-fragmentation processes can intervene.

The effect of inhibitors on lipid peroxidation products has not been previously recognized and is a logical manifestation of the modified peroxidation mechanism presented in Scheme II. It also has potentially important consequences in biological systems where inhibitors such as α-tocopherol act as first-line surveillance agents for free-radical processes. In biological systems with sufficient levels of protective α-tocopherol, the extent of lipid peroxidation is reduced. Furthermore, fatty acid or ester hydroperoxides formed during this period of inhibition would have only the trans,-cis-conjugated diene geometry. Only under conditions of free-radical overload would significant amounts of trans,trans products form. Said another way, if α-tocopherol is present to the extent that it is effective as an inhibitor of free-radical chains in a biological system, it will also be effective in directing the peroxidation to the kinetic products.

Our suggestions with regard to the role of Vitamin E in the autoxidation pathway of lipids have been used to account for products formed in carbon tetrachloride induced peroxidation of liver microsomes.[28] Thus, rats treated with CCl_4 formed significant amounts of trans,cis- and trans,trans-conjugated diene hydroperoxides, the trans,trans compounds being observed after extended exposure to the chlorocarbon. In rats that were pretreated with vitamin E, autoxidation was much reduced upon exposure to CCl_4, and the dominant products formed at all stages of peroxidation were the trans,cis-conjugated products, the kinetic products of autoxidation. Thus, our in vitro experiments appear to be good models for in vivo observations.

Alternate Mechanisms

The formation of four different hydroperoxides in the autoxidation of *cis,cis*-1,4-dienes like linoleate has been attributed to direct radical isomerization.[29] Thus interconversion of radicals **4**, **12**, and **13** in Scheme II by a simple bond rotation would account for the formation of the four products **7**–**10**. This mechanism is not



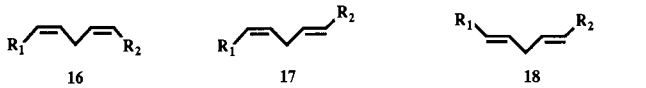
4 12 13

consistent with the data, however, since it does not account for the product composition behavior described above. The direct radical isomerization mechanism does not require oxygen for interconversion of **4**, **12**, and **13**, and product composition would not be independent of oxygen concentration and dependent on H donors present in the media in the observed manner.

The experiments with α-tocopherol illustrate the unimportance of direct radical isomerization. Tocopherol acts in the mechanism at the level of the peroxyl radicals, not the carbon radicals, **4**, **12**, and **13**. If loss of stereochemistry in the product hydroperoxides occurred by carbon radical isomerization, then α-tocopherol would have no effect on product composition. Since α-tocopherol does exert a dramatic product-directing effect, it is clear that the crucial product-determining intermediate is the peroxyl radical and not the carbon radical and that insignificant carbon radical isomerization occurs under normal conditions of autoxidation.

Geometric Isomers of Diene Fatty Acids

Isomeric dienes not having the cis,cis geometry of linoleate have been autoxidized and the products of such autoxidation analyzed. The simple dienes, **16**, **17**, and **18** lead to conjugated diene products analogous to **7**–**10**, and the mechanism for product formation from **16a** is identical with that described for linoleate.[30] The isomeric dienes **17a** and **18a** undergo autoxidation to give a more complex product mixture than **16a**. The



16 17 18

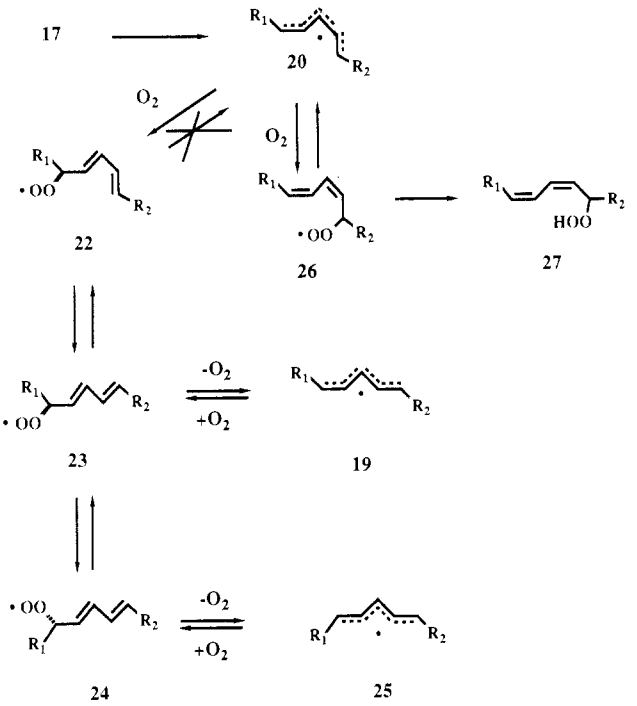
a, $R_1=R_2=CH_3$ a, $R_1=R_2=CH_3$ a, $R_1=R_2=CH_3$

b, $R_1=C_5H_{11}$, $R_2=(CH_2)_7COOR$ b, $R_1=C_5H_{11}$, $R_2=(CH_2)_7COOR$

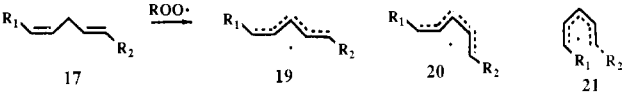
diene **17a**, for example, forms a product mixture (after reduction of hydroperoxides to alcohols) of 3,5-heptadien-2-ol that is made up of *cis*-3,*cis*-5-, *cis*-3,*trans*-5-, *trans*-3,*cis*-5-, and *trans*-3,*trans*-5-diene geometries. Dienes having the cis-3,cis-5 and cis-3,trans-5 geometries are not formed from **16a** or from linoleate, and they never comprise more than 6% of the product

(25) Weenen, H.; Porter, N. A. *J. Am. Chem. Soc.* **1982**, *104*, 5216.
(26) Peers, K. E.; Coxon, D. T.; Chan, H. W.-S. *J. Sci. Food Agric.* **1984**, *35*, 813.
(27) Koskas, J. P.; Cillard, J.; Cillard, P. *J. Am. Oil Chem. Soc.* **1984**, *61*, 1466.
(28) Corongiu, F. P.; Dessi, M. A.; Vargiolu, S.; Poli, G.; Cheeseman, K. H.; Dianzani, M. U.; Slater, T. F. In *Free Radicals in Liver Injury*; Poli, G., Cheeseman, K. H., Dianzani, M. U., Slater, T. F., Eds.; IRL: Oxford, 1985.
(29) Frankel, E. N. *Fatty Acids*; Pryde, E. H., Ed.; American Oil Chemical Society: Champaign, IL, AOCS, 1979; Vol. I, p 353.
(30) Frankel, E. N.; Garwood, R. F.; Vinson, J. R.; Weedon, B. C. L. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2707.

Scheme III



mixture from **17a**. These minor products apparently arise from formation of a mixture of carbon radicals (**19–21**) like those shown in Scheme III. The formation of cis-3,cis-5 and cis-3,trans-5 products can only occur from radicals **20** or **21**. The trans,cis double bond geometry of **17** is translated to these isomeric radicals.

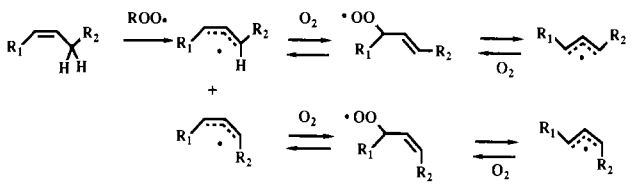


Autoxidation of the linoleate geometric isomers **17b** and **18b** also indicates the presence of small amounts of geometric isomers like those minor products formed from dienes **17a** and **18a**. The linoleate geometric isomers (.24 M) were autoxidized under conditions where the H-atom-donating ability of the medium was changed dramatically by addition of excellent donors such as α-tocopherol and 1,4-cyclohexadiene.[31] The trace cis,cis and cis,trans products were formed only under conditions where the H-donating potential of the medium was very high. At concentrations of cyclohexadiene lower than <1 M, none of the minor isomeric diene hydroperoxides were seen with only products **7–10** being formed. This indicates that **20** or **21** may be formed from the diene precursors **17a–17c**, but that these isomeric radicals are not reformed from peroxyl radicals by β-fragmentation pathways.

As an example of this, addition of oxygen to **20** gives **26** which, if trapped kinetically, would give the cis,cis-conjugated diene (Scheme III). Autoxidation with added H donors such as cyclohexadiene or with neat substrate thus provides measurable (~5%) quantities of **27** as the kinetic product. Autoxidation under thermodynamic control leads to no measurable **27** and suggests that the carbon radicals **19** and **25** are the dominant carbon radicals formed by β-fragmentation of the *trans,trans*-diene peroxyl radical (shown in three conformations in Scheme III as **22**, **23**, and **24**). Thus,

(31) Porter, N. A.; Wujek, D. G. *J. Am. Chem. Soc.* **1984**, *106*, 2626.

Scheme IV



β-fragmentation of **26** to **20** followed by readdition of oxygen and a second β-fragmentation results in the conversion of **20** to the more stable radicals **19** and **25**. In summary, radicals like **20** or **21** must be accessible to some extent from the diene precursor, but these carbon radicals are apparently not re-formed from peroxyl radicals by β-fragmentation processes.

Autoxidation of the linoleate geometric isomers **17b** and **18b** gives additional information about the mechanism of diene autoxidation. A detailed analysis of the systems was carried out and a kinetics scheme was developed that predicted the distribution of products **7–10** derived from each of the diene precursors as a function of H-donating ability of the medium.[31]

Monoene Substrates

The autoxidation of monoene fatty acid substrates such as oleic acid, **1**, or simple model substrates has been investigated, but the mechanistic picture in these systems is less certain than in the diene substrates.[32] Perhaps the most extensive study of simple monoene autoxidations is with the model substrate *cis*-hex-3-ene.[30] The allylic alcohols formed by autoxidation of neat *cis*-hex-3-ene at 25 °C (5–10% conversion) followed by reduction of the hydroperoxides to the corresponding alcohols are: *cis*-hex-3-en-2-ol (33%), *trans*-hex-3-en-2-ol (16%), *cis*-hex-2-en-4-ol (8%), and *trans*-hex-2-en-4-ol (43%). While a mechanism based on carbon radical isomerization has been proposed, a mechanism analogous to the diene mechanism (reversible oxygen addition) could account for the products formed. It is possible that loss of initial radical stereochemistry may involve reversible oxygen addition in this system (Scheme IV) and this proposal is testable by the use of external H-atom donors. An autoxidation study of monoene substrates with the medium of oxidation being changed from that of low H donation (substrate in benzene) to high donation (substrate in benzene and good H donor) would provide a more complete mechanistic picture in this system.

Tri- and Tetraene Substrates

The autoxidation of linolenic[26] and arachidonic acids[18] have been investigated. Arachidonic acid, **5**, leads to six major hydroperoxide products analogous to the linoleic hydroperoxides (**7–10**) with hydroperoxide substitution for the six products being at C-5, 8, 9, 11, 12, and 15. It is of interest that trans,cis-conjugated diene hydroperoxides are formed as major products from arachidonic acid and only small amounts of trans,trans-conjugated diene products can be detected. A detailed study of arachidonic acid autoxidation indicates that the mechanism differs from diene autoxidation in one respect. Peroxyl radicals derived from arachidonic acid that are homoallylic undergo cycliza-

(32) Frankel, E. N.; Garwood, R. F.; Khambay, B. P. S.; Moss, G. P. O.; Weedon, B. C. L. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2233.

tion reactions that lead to cyclic peroxide compounds.

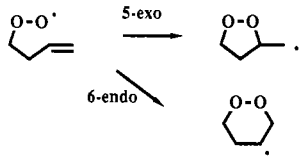
28 29

Thus, peroxyl radicals from arachidonic acid substituted at C-8, C-9, C-11, or C-12 can all undergo radical cyclization to give 1,2-dioxolane products. A cyclization from the 11-peroxyl radical is shown above.

The cyclization of peroxyl radicals has been the focus of much of our research and a host of products result from the initial cyclization. The following generalizations can be made about peroxyl radical cyclization:

(1) Peroxyl radical cyclization occurs only in the exo mode.[33] Products of 5-exo and 6-exo cyclization are formed to the exclusion of 6-endo or 7-endo products.

(2) The 5-exo and 6-exo cyclizations are stereoselective. Beckwith's guidelines[34] serve to predict products of the 5-*exo* mode of cyclization with 3,5-disubstituted



dioxolanes being formed with preferential cis substitution (cis:trans = 97:3). The preference for *cis* substitution can be understood by a transition state with diequatorial substitution **30**.[35] Similar arguments can be applied for 6-exo cyclization with diequatorial substituents giving trans-substituted 3,6-dialkyl-1,2-dioxanes via **31**.[36]

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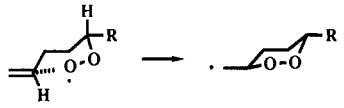


31

(3) Peroxyl radicals generated from 5-*exo* or 6-*exo* cyclization have a multitude of pathways available for further reaction. These pathways are illustrated for the peroxyl radical **29** and include S_Hi attack of the carbon radical on the peroxide bond,[37] entrapment of radical **29** with oxygen leading to monocyclic or polycyclic peroxides[38] or a second cyclization of radical **29** giving the bicyclic endoperoxide radical **32**.[39]

Peroxyl radical cyclization thus opens the door for lipid peroxyl radicals to form a host of different products and the complexity of lipid peroxidation results from these multiple pathways. Arachidonic acid, for example, gives six different trans,cis peroxyl radicals, four of which can undergo cyclization. Each of the cyclization pathways can lead to multiple products as is illustrated by the mixture formed in Scheme V for radical **29**. Furthermore, many of the products formed from cyclization are diastereomeric mixtures leading to an even greater product mixture complexity than is shown in Scheme V. The stereoselectivity of cyclization to form radical **32** has been studied in detail.[40-42]

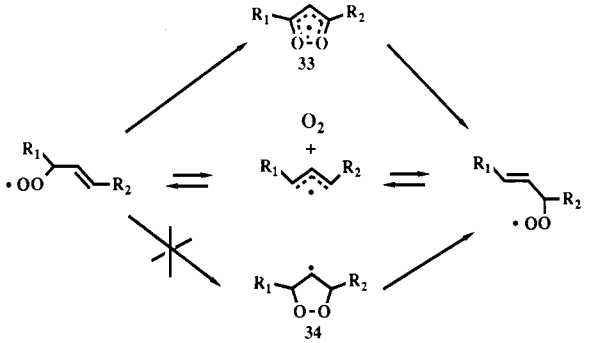
A final comment should be made concerning products from tetraene or triene substrates. Little trans,trans-substituted conjugated dienes are formed in these systems since radical cyclization occurs at a faster rate ($\sim$800 s^{-1}) than scission of intermediate peroxyl radicals (140 s^{-1}). The β-scission pathway leads to trans,trans products in linoleate autoxidation while this pathway is not competitive with cyclization of arachidonate peroxyl radicals.

The effect of vitamin E on arachidonate autoxidation is similar to its effect in linoleate. Vitamin E, if effective as an antioxidant, traps peroxyl radicals before cyclization can occur. The result in arachidonate autoxidation is to reduce or eliminate products from radical cyclization and divert the product mixture to the six simple diene trans,cis hydroperoxides.

Summary

Product studies of lipid peroxidation indicate that the classical mechanism for autoxidation must be modified to account for products formed. The product distribution obtained from peroxyl radicals depends on competition of β-scission, H-atom abstraction, and cyclization pathways. Antioxidants such as vitamin E exert a product distribution effect, directing peroxyl radicals to H-atom abstraction products.

While our investigations have opened the "black box" of lipid peroxidation to a degree, many questions remain to be answered. The autoxidation of monoene lipids such as oleic acid provides some unanswered questions as outlined in Scheme IV. Interconversion of allylic hydroperoxides clearly occurs in oleates and other simple hydroperoxides[43] and the mechanism may involve reversible oxygen addition as outlined in Scheme IV. Alternatively, a concerted 3,2-sigmatropic radical rearrangement as outlined below may account for interconversion of peroxyl radicals in monoene systems. The species **33** is a transition state and an intermediate



radical like **34** is not involved in the rearrangement

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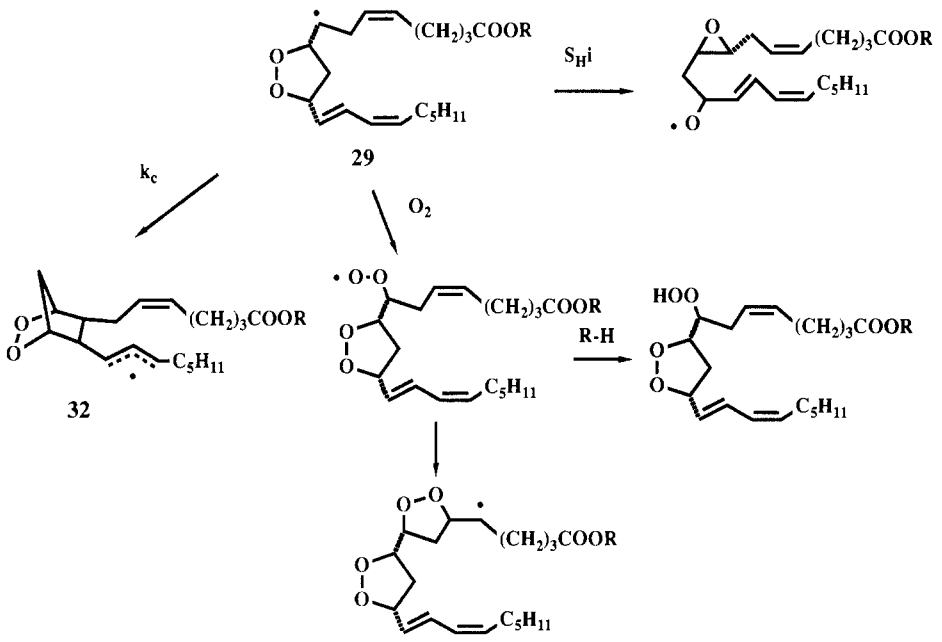
Scheme V



since authentic radicals like **34** add oxygen in competition with ring-opening and oxygen addition products derived from **34** are not observed.[44]

Other important problems in lipid peroxidation involve the transition of model studies like those reported in this account to in vivo systems. The early reports by Corongiu[28] are encouraging, but the analytical problems of studying in vivo lipid peroxidation are formidable. Natural targets of lipid peroxidation are fatty esters such as phospholipids present in membranes and these compounds are complex mixtures of different phospholipid classes and molecular species. We have directed some attention to this challenging problem,[25] but much remains to be done.

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Photoexcited States of Allyl Anions

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One of the triumphs of Hückel molecular orbital (HMO) theory has been its utility in representing the charge distribution and other ground-state properties of resonance-stabilized carbanions. Thus for odd-alternant hydrocarbon anions, the charge is localized on the odd-numbered ("starred" atoms in the Dewar formalism) and is zero on the even-numbered ("unstarred") atoms. Hückel theory predicts a considerably different charge distribution in the excited state, and we wondered if such a simple model could have any predictive power for carbanions.

One of the impediments to the development of carbanion photochemistry was recognition that it is usually characterized by electron ejection rather than adiabatic photochemistry.[1] This propensity was certainly taken advantage of when Bunnett and others recognized that the $S_{RN}1$ reaction could be photostimulated.[2] However, in the absence of suitable electron acceptors, redox chemistry was the common result.

For instance, irradiation of cyclopentadienyl anion produced a reductive dimer,[3a] irradiation of phenyllithium produced the oxidative dimer biphenyl,[3b] and irradiation of the 3-pentanone enolate produced oxidative and reductive dimers as the major reaction products (see Figure 1),[3c] along with a small amount of 2-pentene oxide **2**, the apparent product of cyclization/protonation. This latter product suggested that

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