Autoxidation of Polyunsaturated Lipids. Factors Controlling the Stereochemistry of Product Hydroperoxides

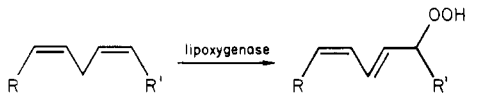
Ned A. Porter,* Bruce A. Weber, Hugo Weenen, and Jamil A. Khan

Contribution from the Paul M. Gross Chemical Laboratories, Duke University, Durham, North Carolina 27706. *Received March 24, 1980*

Abstract: The mechanism of the autoxidation of linoleic acid and phospholipid esters of this acid was investigated. The products of autoxidation, 13-hydroperoxy-9-*cis*,11-*trans*-octadecadienoic (**4**), 13-hydroperoxy-9-*trans*,11-*trans*-octadecadienoic (**5**), 9-hydroperoxy-10-*trans*,12-*cis*-octadecadienoic (**6**), and 9-hydroperoxy-10-*trans*,12-*trans*-octadecadienoic (**7**) acids, were analyzed by LC after reduction to the corresponding hydroxy fatty acids. The ratio of trans,cis/trans,trans products, (**4** + **6**/**5** + **7**), formed during the initial stages of oxidation (<2% for the free acids) was dependent on temperature and the concentration of linoleic acid. This trans,cis/trans,trans ratio varied from 4.2 (with neat linoleic acid oxidations at 10 °C) to 0.23 (0.24 M linoleic acid in benzene at 50 °C). A similar product distribution was found in emulsion oxidation of mixtures of 1,2-dilinoleoylglycerolphosphatidylcholine and 1,2-dipalmitoylglycerophosphatidylchlorine with the trans,cis/trans,trans product ratio depending on the ratio of dilinoleoyllecithin to dipalmitoyllecithin. Mixtures of linoleic acid and *p*-methoxyphenol give trans,cis/trans,trans product ratios dependent on the concentration of added phenol. A kinetic scheme consistent with these observations is presented.

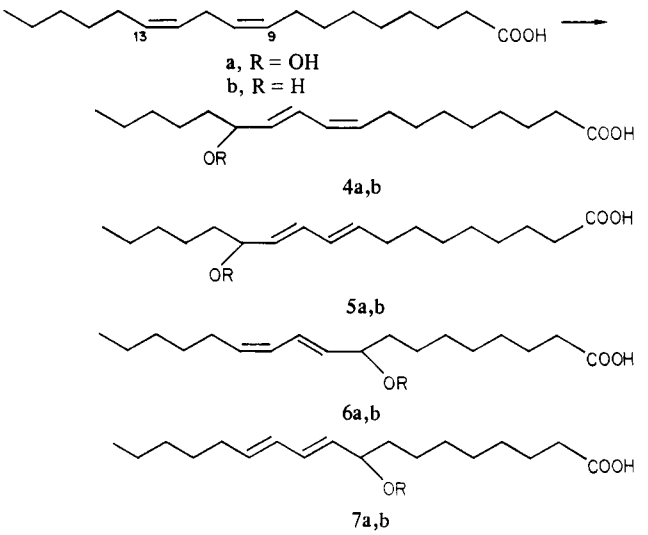
The autoxidation of polyunsaturated fatty acids and esters has attracted renewed research interest. Lipid peroxidation in diverse biological systems leads to peroxide products that have interesting and unique properties. Hydroperoxides derived from arachidonic acid, for example, serve as modulators[1,2] of the enzymes involved in prostaglandin biosynthesis and, because of the relationship of the prostaglandins and platelet functions,[3] it has been suggested[4] that heart attack and stroke are "essentially a lipid peroxidation disease". A lipid hydroperoxide derived from arachidonic acid, 5-hydroperoxyeicosatetraenoic acid (5-HPETE), is a proposed intermediate in the biosynthesis of leukotrienes,[5,6] and a lipoxygenase pathway leading to 5-HPETE in leukocytes has been demonstrated.[7] Lipid hydroperoxides thus are formed not only in random free radical lipid autoxidation[8] but also by enzymes (lipoxygenases) that lead to specific positional (and configurational) isomers.

Lipoxygenase enzymes[1,2] convert the 1,4-diene present in unsaturated fatty acid **1** to a conjugated diene hydroperoxide. The



1 **2**

diene formed, **2**, has trans,cis geometry with the double bond closest to the hydroperoxide being trans and the one remote from the peroxide group being cis. Free-radical autoxidation[9] of unsaturated fatty acids also leads to product hydroperoxides like **2**. In autoxidation, however, both trans,cis hydroperoxides like **2** and trans,trans isomers are formed. Thus, linoleic acid (**3**) leads to four different hydroperoxides, **4a**–**7a**. Two of these, **4a** and **6a**, have the trans,cis geometry while **5a** and **7a** are trans,trans diene isomers. These hydroperoxides all derive from oxygen entrapment of a pentadienyl-type radical (delocalized between carbons 9 and 13 of the fatty acid chain) but factors that control the stereochemical course of the oxidation have not yet been established. In fact, the kinetics of lipid oxidation and its inhibition have been studied extensively, but the study of products and particularly product stereochemistry has received little attention.



7a,b

With the development of LC methods for analysis of hydroperoxy fatty acids like **4a**–**7a**,[9-11] detailed product study of lipid peroxidation is possible. We report here a study of the autoxidation of unsaturated fatty acids and esters. A kinetic scheme that serves as a basis for understanding the proportions of trans,cis and trans,trans products formed in autoxidation is presented and, by choosing appropriate cosubstrates for lipid autoxidation, significant stereochemical control of the product distribution is possible.

Results

Linoleic acid was oxidized neat or in benzene solution at 10, 30, 37, and 50 °C. The oxidation was normally carried out under air and was initiated by di-*tert*-butyl hyponitrite.[12] The extent of oxidation was measured by UV spectroscopy, the diene products having absorption maxima in the 230–235-nm region.[9] Extent of oxidation did not exceed 2% in any of the linoleic acid oxidations reported here.

Products of oxidation were analyzed by LC after reduction of the hydroperoxides with triphenylphosphine. The hydroxy fatty acids formed from reduction of hydroperoxides **4a**–**7a** may be

(1) Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3400.
(2) Nugteren, D. H. *Biochim. Biophys. Acta* **1975**, *380*, 299.
(3) Salmon, J. A.; Smith, D. R.; Flower, R. J.; Moncada, S.; Vane, J. R. *Biochim. Biophys. Acta* **1978**, *523*, 250.
(4) Moncada, S.; Gryglewski, R. J.; Bunting, S.; Vane, J. R. *Prostaglandins* **1976**, *715*, 12.
(5) Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 2643.
(6) Borgeat, P.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2148.
(7) Borgeat, P.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3213.
(8) Howard, J. A. *Adv. Free Radical Chem.* **1972**, *4*, 49.
(9) Chan, H. W. S.; Levett, G. *Lipids* **1977**, *99*, 12.
(10) Funk, M. O.; Isaac, R.; Porter, N. A. *Lipids* **1976**, *11*, 113.
(11) Frankel, E. N. In "Fatty Acids", Pryde, E. H., Ed.; AOCS: Champaign, Ill., 1979; pp 353–378.
(12) Mendenhall, G. D. *J. Am. Chem. Soc.* **1974**, *96*, 5000.

0002-7863/80/1502-5597$01.00/0

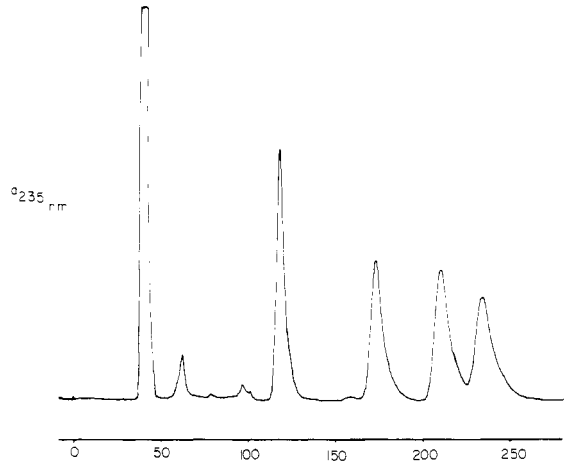


Figure 1. LC chromatogram for analysis of hydroxy fatty acids **4b–7b**, flow rate 4 mL/min, conditions as described in text.

Table I. Product Distribution of Linoleic Acid Autoxidation[a]

no.	concn, M	t, °C	4	5	6	7	(4 + 6)/(5 + 7)
1	3.2[b]	10	0.44	0.10	0.36	0.09	4.21
2	1.8	10	0.35	0.14	0.34	0.16	2.30
3	0.9	10	0.26	0.22	0.27	0.24	1.16
4	0.45	10	0.21	0.30	0.18	0.31	0.64
5	0.24	10	0.11	0.36	0.16	0.37	0.37
6	3.2	30	0.33	0.19	0.29	0.19	1.63
7	1.8	30	0.31	0.25	0.24	0.20	1.14
8	0.90	30	0.20	0.31	0.16	0.33	0.56
9	0.45	30	0.11	0.35	0.12	0.42	0.30
10	0.24	30	0.10	0.40	0.09	0.41	0.23
11	3.2	37	0.24	0.25	0.23	0.28	0.89
12	1.8	37	0.19	0.30	0.19	0.33	0.60
13	0.90	37	0.14	0.34	0.13	0.39	0.37
14	0.45	37	0.11	0.36	0.13	0.39	0.30
15	0.24	37	0.09	0.39	0.09	0.44	0.22
16	3.2	50	0.22	0.28	0.20	0.30	0.72
17	1.8	50	0.17	0.32	0.16	0.34	0.49
18	0.90	50	0.13	0.38	0.09	0.40	0.28
19	0.45	50	0.11	0.39	0.08	0.41	0.24
20	0.24	50	0.12	0.41	0.09	0.41	0.24

[a] Oxidations were initiated by di-*tert*-butyl hyponitrite. [b] The 3.2 M oxidations were with neat linoleic acid; other oxidations were carried out in benzene solvent.

conveniently separated by LC on μ-silica. A typical chromatogram of hydroxy fatty acids derived from linoleic acid is presented in Figure 1. The elution order is as follows: 120 mL, 13-hydroxy-9-*cis*,11-*trans*-octadecadienoic acid (**4b**); 175 mL, 13-hydroxy-9-*trans*,11-*trans*-octadecadienoic acid (**5b**); 210 mL, 9-hydroxy-10-*trans*,12-*cis*-octadecadienoic acid (**6b**); 240 mL, 9-hydroxy-10-*trans*,12-*trans*-octadecadienoic acid (**7b**). Product structures were determined by GC/MS and infrared spectroscopy as previously described.[9]

In Table I is presented the product distribution of hydroxy fatty acids **4b–7b**, obtained from oxidation of linoleic acid followed by reduction of the primary products, the hydroperoxides **4a–7a**. The product distribution was shown to be independent of the extent of oxidation, within the 2% total oxidation limit of this study. As an example, the product distribution of oxidation of 1.8 M linoleic acid in benzene at 30 °C was identical when monitored at 0.5, 1, 2, 4, and 8 h. Further, the product distribution was identical when the oxidation was carried out under 1000 mmHg or 80 mm oxygen. Linoleic acid (0.4 M) in benzene was oxidized at 30 °C with *p*-methoxyphenol, an excellent autoxidation inhibitor, present. Autoxidation did occur when *p*-methoxyphenol was added, albeit at a slower rate than with no added inhibitor. Products of autoxidations carried out in the presence of inhibitor were worked up in the normal way but an oxidation product of *p*-methoxyphenol complicates the LC analysis of **4b–7b**. In these studies, reproducible analysis of only **6b** and **7b**, the trans,cis and trans,trans 9-substituted isomers, could be obtained. In Table II is presented the product distribution of compounds **6** and **7** formed in oxidation as a function of added *p*-methoxyphenol.

Table II. Product Distribution of Linoleic Acid Autoxidation with Added *p*-Methoxyphenol[a]

no.	concn of *p*-methoxyphenol, M	6	7	6/7
1	0	18	82	0.23
2	0.02	58	42	1.4
3	0.04	72	28	2.6
4	0.06	82	18	4.0
5	0.08	88	12	7.7
6	0.20	95	5	18
7	0.40	97	3	35

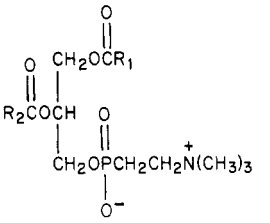
[a] Oxidations were initiated by di-*tert*-butyl hyponitrite at 30 °C in benzene solutions of 0.4 M linoleic acid.

Table III. Product Distribution of Lecithin Autoxidation at 37 °C

no.	exptl conditions	lecithins oxidized	4	5	6	7	(4 + 6)/(5 + 7)
1	emulsion[a]	8	0.21	0.28	0.20	0.31	0.69
2	neat	9	0.28	0.22	0.27	0.22	1.25
3	emulsion	9	0.30	0.24	0.26	0.20	1.26
4	emulsion	9:10 (5:1)[b]	0.26	0.24	0.25	0.25	1.04
5	emulsion	9:10 (4:2)	0.25	0.26	0.24	0.25	0.93
6	emulsion	9:10 (3:3)	0.23	0.27	0.21	0.29	0.77
7	emulsion	9:10 (2:4)	0.19	0.31	0.20	0.31	0.63
8	emulsion	9:10 (1:5)	0.17	0.31	0.18	0.34	0.54

[a] Lipid concentration was 15 mM in 50 mM KCl. [b] Ratio of 9:10 with total phospholipid concentration 15 mM.

Oxidations of phospholipids derived from linoleic acid were also investigated. Thus, 1-palmitic–2-linoleic–glycerophosphatidylcholine (**8**) and 1,2-dilinoleic–glycerophosphatidylcholine (**9**) were oxidized neat[13] or in aqueous emulsions at 37 °C. The dilinoleic lecithin, **9**, was oxidized in mixtures with varying proportions of 1,2-dipalmitoylglycerophosphatidylcholine (**10**). Products of the oxidation were reduced with triphenylphosphine, and the esters were then hydrolyzed[13] with either phospholipase A_2 (Sigma, *Crotalus adamanteus* snake venom) or with tetraethylammonium hydroxide, affording the hydroxy fatty acids **4b–7b**, which were analyzed by LC. Although the hydroperoxy fatty acids **4a–7a** appear to be the major oxidation products formed from autoxidation of the free fatty acid[9] and neat lecithins,[13] significant amounts of other as yet unidentified products are formed in lecithin emulsion oxidations. Product distributions of **4–7** obtained from lecithin oxidations are presented in Table III.



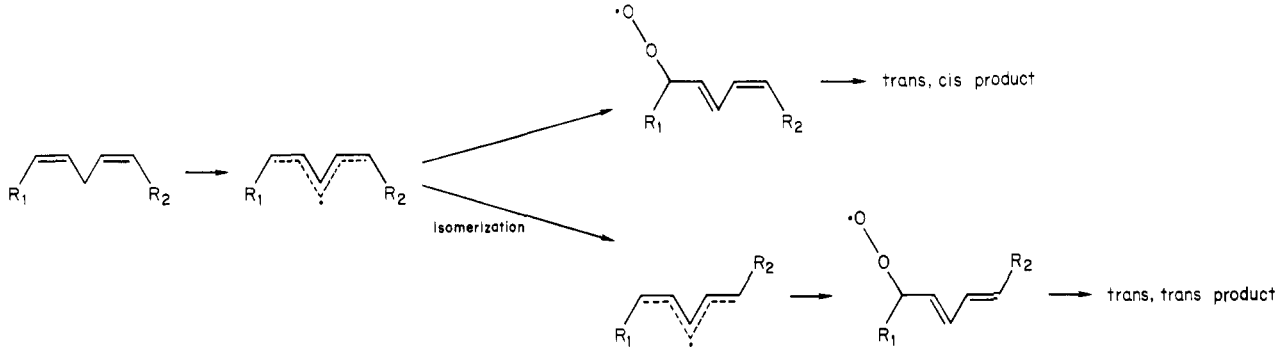
8, R_1 = palmitate (16:0); R_2 = linoleate (18:2)
9, $R_1 = R_2$ = linoleate (18:2)
10, $R_1 = R_2$ = palmitate (16:0)

Discussion

Free Fatty Acid Oxidation. The autoxidation of polyunsaturated fatty acids has been studied extensively and other workers[9] have isolated trans,cis and trans,trans conjugated diene hydroperoxides from linoleic acid (L-H) in these studies. Similarly, oleate oxidation[11,14] has been shown to lead to both cis and trans allylic

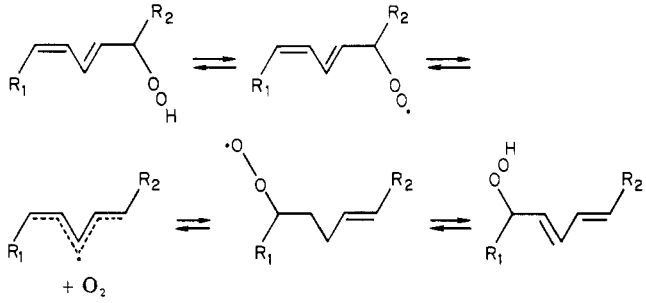
(13) Porter, N. A.; Wolf, R. A.; Weenan, H. *Lipids*, **1980**, *15*, 163.
(14) Garwood, R. F.; Khambay, B. B. S.; Weedon, B. C. L.; Frankel, E. N. *J. Chem. Soc., Chem. Commun.* **1977**, 364.

Scheme I



hydroperoxides and the trans to cis product ratio is observed to be temperature dependent. We note that the distribution of products derived from linoleic acid (L-H) is not only dependent on the temperature but also on the concentration of fatty acid undergoing oxidation. In Figure 2 is presented the ratio of trans,cis substituted products (**4** and **6**) to trans,trans products (**5** and **7**) formed in linoleic acid (L-H) oxidation as a function of temperature and concentration of fatty acid. This product ratio is dramatically dependent on concentration of linoleic acid as can be seen in the figure and, in fact, an excellent linear correlation of the trans,cis/trans,trans ratio with L-H concentration is observed at all temperatures.

Any consideration of a mechanism consistent with these data must be concerned with the fact that the product distribution observed does not change significantly during the time period (<2% conversion) of oxidation. This observation suggests that the product ratios reported here are kinetically controlled and do not represent any significant isomerization of hydroperoxides once they are formed. This is a particularly important point since it has been recently shown[15,16] that linoleate hydroperoxides interconvert thermally. This thermal isomerization has been shown to occur by β-scission of peroxy radicals formed from the hydroperoxides and the equilibration of hydroperoxides **4a–7a** has been demonstrated after thermolysis at 60 °C for 48 h. The



shorter reaction times and lower temperatures of our study apparently allow no significant isomerization of the product hydroperoxides.

A second mechanistic consideration is the lack of dependence of the product composition on oxygen concentration. It has been shown that the rate of autoxidation of linoleate is independent of oxygen pressure (between 100 and 760 mmHg).[17] These observations suggest that the chain-carrying radicals are primarily peroxy and not carbon radicals and that isomerization of carbon radicals as shown in Scheme I is not a tenable mechanism. Further, it is difficult to account for the dependence of the trans,cis/trans,trans product ratio on fatty acid concentration if this carbon radical isomerization scheme is used as a format for discussion.

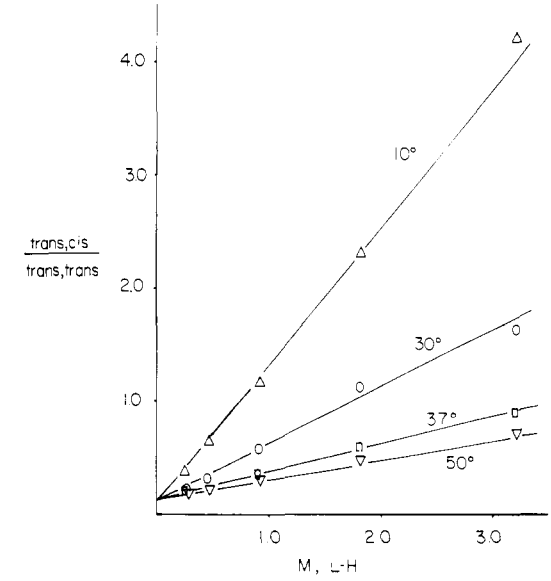
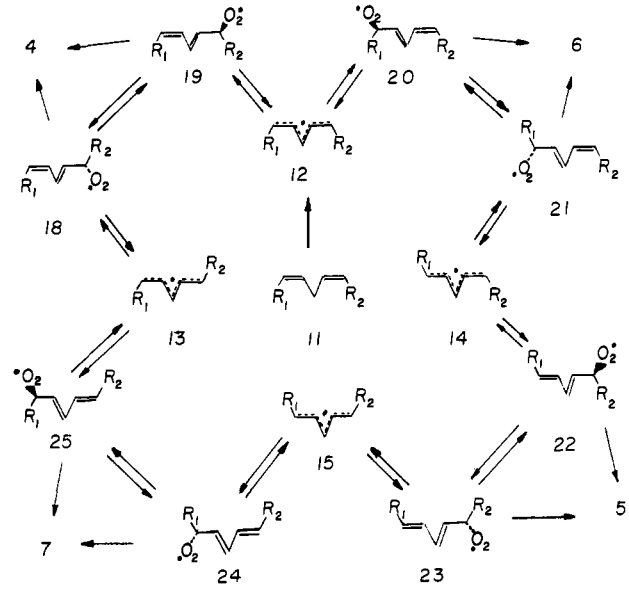


Figure 2. Product ratio of trans,cis/trans,trans hydroxy fatty acids formed in autoxidation of linoleic acid (L-H) in benzene solution.

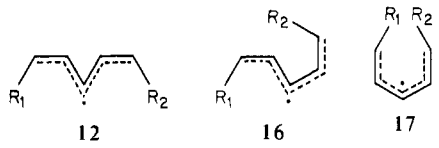
Scheme II



The mechanism presented graphically in Scheme II accounts for the formation of products **4–7** from linoleic acid and also may be used to explain the observed temperature and fatty acid concentration dependence of the trans,cis/trans,trans product ratios. In this mechanism, H-atom abstraction from fatty acid **11** leads to the W radical **12**.[8] Substitution about the Δ^1 and the Δ^4 partial double bonds of this substituted pentadienyl radical **12** must

(15) Chan, H. W. S.; Levett, G.; Matthew, J. A. *J. Chem. Soc., Chem. Commun.* **1978**, 756.
(16) Chan, H. S. W.; Levett, G.; Matthew, J. A. *Chem. Phys. Lipids* **1979**, *24*, 245.
(17) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1967**, *45*, 793.

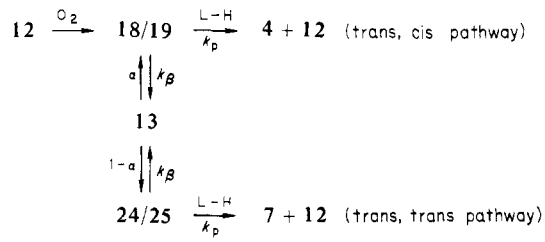
initially be cis since these bonds derive from the cis fixed fatty acid precursor. The radicals **16** and **17** (previously shown not to



12 **16** **17**

exist at ambient temperatures)[18] would appear to be unlikely intermediates owing to the additional strain involved in having three and four cis interactions, respectively, in the pentadienyl system.

Entrapment of **12** by oxygen gives peroxy radicals **18–21**. Note that the pairs **18/19** and **20/21** are merely conformers. These conformers are independently represented, however, since scission of **19** yields the carbon radical **12**, whereas scission from radical conformer **18** gives the new carbon radical **13**. Competing with scission of the radical **18/19** is H-atom abstraction from fatty acid leading to the product hydroperoxide, **4**. The crucial competition, then, that determines the trans,cis/trans,trans product ratio is (1) β-scission of peroxy radical **18/19** which ultimately leads to trans,trans product or (2) H-atom abstraction by **18/19** giving the trans,cis product. A simplified kinetic analysis based on Scheme II and radicals **18**, **13**, and **12** is shown below. (A similar



scheme based on the symmetry-related radicals **21**, **14**, and **12** may also, of course, be developed.) Only the important product-determining steps are included in this analysis and the terms α and $1 - \alpha$ are factors that represent the distribution of carbon radical **13** between the trans,cis peroxy radical (**18/19**) and trans,trans peroxy radical (**24/25**). In short, α and $1 - \alpha$ are inserted to account for equilibration of peroxy radicals subsequent to the initial β-scission of **18/19**. Steady-state assumptions lead to the equation

$$\frac{\text{trans,cis}}{\text{trans,trans}} = \frac{k_p[\text{L-H}]}{k_\beta(1-\alpha]} + \frac{\alpha}{(1-\alpha)} \qquad (1)$$

Equation 1 predicts a trans,cis/trans,trans product dependence that is directly related to L-H with the slope = $k_p/k_\beta(1 - \alpha)$ and intercept = $\alpha/(1 - \alpha)$. Analysis of the data presented in Figure 2 leads to $\alpha = 0.13$ and k_p/k_β (10 °C) = 1.5; (30 °C) = 0.56; (37 °C) = 0.25; (50 °C) = 0.20 M^{-1}.

The value of k_p for linoleic acid autoxidation at 30 °C is known to be 62 M^{-1} s^{-1} [17] and the k_β value may then be calculated and is found to be 111 s^{-1} (30 °C). A plot of log (k_p/k_β) vs. $1/T$ gives a good linear correlation ($r = 0.99$) and from this the values $\Delta H_\beta^\ddagger - \Delta H_p^\ddagger = 10.5$ kcal/mol and $\Delta S_\beta^\ddagger - \Delta S_p^\ddagger = 35$ eu are found. Although the activation enthalpy of linoleate autoxidation propagation has not been reported, $\Delta H_p^\ddagger$ for most comparable autoxidation[8] falls between 6 and 8 kcal/mol, giving an approximate $\Delta H_\beta^\ddagger$ of 16–18 kcal/mol.[19]

Autoxidation with Added *p*-Methoxyphenol. If the competition between β-scission and H-atom abstraction of peroxy radicals determines the trans,cis/trans,trans product ratio, then reagents other than L-H that serve as H-atom donors may be added to the reaction mixture and alter the observed trans,cis/trans,trans ratio. This effect is observed with added *p*-methoxyphenol (A-H), an excellent H atom transfer agent.[20] Thus, addition of A-H leads

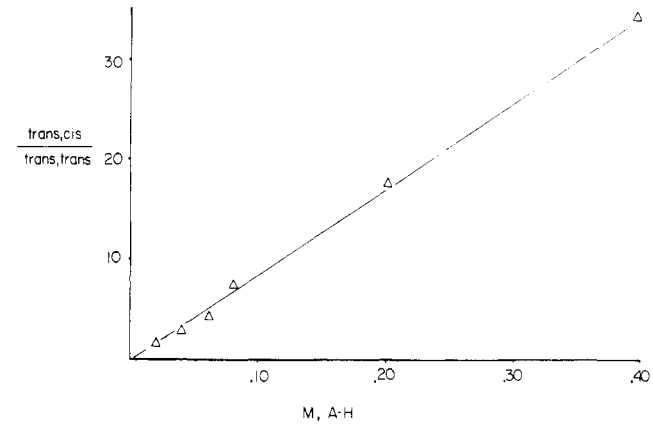


Figure 3. Product ratio of trans,cis/trans,trans hydroxy fatty acids formed in autoxidation of linoleic acid with added *p*-methoxyphenol (A-H).

to an increased trans,cis/trans,trans ratio with as little as 0.25 M *p*-methoxyphenol increasing the product ratio from 0.23 (no added phenol) to over 25. In Figure 3 is presented a plot of the product ratio as a function of added A-H. The slope of this plot corresponds to $k_{inh}/k_\beta(1 - \alpha)$ and, with k_β and α known from autoxidation of the free fatty acid alone, k_{inh}, the rate constant for H-atom transfer from A-H to the peroxy radical, may be calculated.

$$\text{L-OO}\cdot + \text{A-H} \xrightarrow[k_{inh}]{} \text{L-OOH} + \text{A}\cdot$$

The value of k_{inh} obtained in this way, 8.5×10^3 M^{-1} s^{-1} (30 °C), is over two orders of magnitude greater than k_p, the rate constant for H atom transfer from L-H to the peroxy radical, LOO·. Cooxidation of L-H with other substrates in a manner similar to that described here for A-H offers an alternate competition method for determining rates of inhibition of autoxidation. Any added reagent should affect the trans,cis/trans,trans product ratio of L-H autoxidation and this ratio may then be used as a measure of the overall H atom donating ability of solvent and solutes present in the autoxidation.

Autoxidation of Phospholipids. The autoxidation of the unsaturated lecithins **8** and **9** was carried out on the neat lipids or in 15 mM aqueous emulsion. We have earlier reported[13] on the isolation and characterization of lecithin hydroperoxides formed in the autoxidation of neat lecithin. In the work described here, lecithin hydroperoxides were not isolated and characterized as such. Rather, the crude oxidation product mixture was reduced with triphenylphosphine and the hydroxy fatty acids were analyzed by LC subsequent to base or phospholipase A_2 hydrolysis. The product distribution was found to be independent of the method of hydrolysis with phospholipase and tetrabutylammonium hydroxide giving the same results. We emphasize again that oxidation of lecithin emulsions is not as clean as the oxidation of free fatty acids. Other products may be generated depending upon the conditions of oxidation. Our concern in this study was, however, only with the stereochemistry of the hydroxy fatty acids formed.

Autoxidation (37 °C) of 15 mM emulsions of **9** (Table III) gives trans,cis/trans,trans product ratios (1.26) even greater than those obtained in the oxidation of neat fatty acid (0.89). Phospholipid oxidation was carried out to 10% conversion as measured by UV and the product composition did not vary with extent of oxidation. In aqueous emulsion at lecithin concentrations as low as 15 mM, there exists a hydrophobic region in which the linoleate moiety acts effectively as a neat ordered liquid.[21] The somewhat higher product ratios obtained from the lecithin as compared to the neat fatty acid might result from an increase of either k_p or α in the bilayer as compared with the fatty acid oxidation (vide infra).

(18) Sustman, R.; Schmidt, H. *Chem. Ber.* **1979**, *112*, 1440.
(19) Benson has estimated a standard heat of dissociation of an allyl peroxy radical to be 15 ± 2.5 kcal. Benson, S. W. *J. Am. Chem. Soc.* **1965**, *87*, 972.
(20) Mahoney, L. R. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 547.
(21) Huang, C.; Thompson, T. E. *Methods Enzymol.* **1974**, *32*, 485.

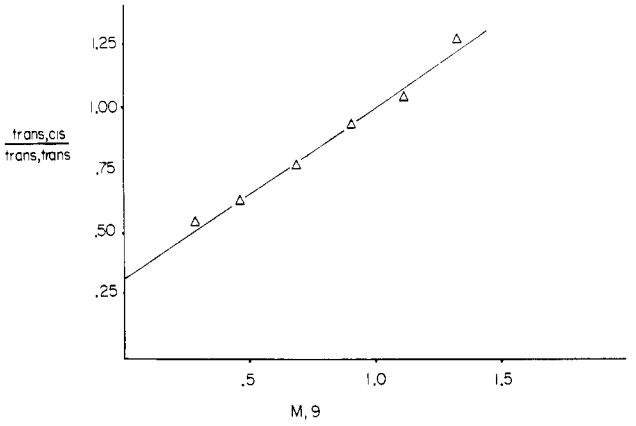


Figure 4. Product ratio of trans,cis/trans,trans hydroxy fatty acids formed in autoxidation of **9** in emulsion.

Dilution of the dilinoleic lecithin with dipalmitic lecithin (**10**) gives rise to a decrease in the trans,cis/trans,trans product ratio. Palmitate, being a saturated fatty acid is a poor H-atom donor and the β-scission pathway (leading ultimately to trans,trans products) competes more effectively with H-atom abstraction as the concentration of linoleate decreases and that of palmitate increases. It is interesting to note that a 1:1 mixture of **9** and **10** gives a product ratio (0.77) close to that observed in autoxidation of 1-palmitic-2-linoleic lecithin (0.69). There thus appears to be little difference between intra- and intermolecular linoleate with regard to the overall product stereochemistry observed.

One can calculate a molarity of **9** in the phospholipid bilayer phase. Note that this is not the molarity of **9** in the total solution, but rather it is the molarity of dilinoleic lecithin in the hydrophobic region of the emulsion. The trans,cis/trans,trans product ratio of the hydroxylinoleates **4**–**7** vs. the molarity of **9** in the bilayer phase is presented in Figure 4. Again, a good linear correlation obtains and the values of $\alpha = 0.24$ and $k_p/k_\beta = 0.52$ are found. Since there are two linoleate residues per molecule of **9**, the effective k_p/k_β ratio for each linoleate residue in the bilayer oxidation at 37 °C is 0.26. This compares to a free fatty acid value for k_p/k_β at 37 °C of 0.25. The major difference in linoleate oxidation in solution and emulsion is in the value of α (0.13 vs. 0.24) rather than in the values of k_p and/or $k\beta$ of the oxidation. Autoxidation in isotropic liquid appears to favor trans,trans products more ($\alpha = 0.13$) than in the ordered bilayer ($\alpha = 0.24$).

The oxidation of membrane phospholipid has been suggested to be the primary chemical reaction involved in a variety of pathological events. Membrane damage induced by radiation[22] and toxic agents such as carbon tetrachloride and ethanol[23,24] has been proposed to be the result of phospholipid destruction by molecular oxygen. Further, the effects of superoxide radical and hydrogen peroxide on fatty acids and model membranes are known to result in fatty acid oxidation and membrane disruption.[25,26] The studies reported here address the important question of the mechanism of membrane oxidation. The simple lecithin bilayers examined serve as models for oxidation in a membrane bilayer and an understanding of these simple systems may serve as a basis for further studies on more complex membrane models.

Experimental Section

High-Pressure Liquid Chromatography. A Whatman Magnum 9–10-μm silica column was used for separation of the hydroxy fatty acids **4b**–**7b**. The solvent used was acetic acid–2-propanol–hexane (1:16:983). A typical chromatogram is presented in Figure 1. Relative amounts of **4b**–**7b** present in the reaction mixture were determined by correcting the areas of each peak by the known molar absorptivity[9] of each of the hydroxy fatty acids.

Free Fatty Acid Oxidations. Linoleic acid (99+%, Nu-Chek-Prep, Elysian, Minn.) was used without further purification. No peroxides were detected in unoxidized fatty acid. Benzene solvent was distilled before use and a center cut retained for oxidation studies. A round-bottom flask was charged with 0.1 g of linoleic acid diluted to the desired molarity with benzene. The flask was equipped with a drying tube and placed in a constant-temperature bath and, after a brief period of equilibration, one crystal of di-*tert*-butyl hyponitrite was added and the solution was stirred magnetically. Oxidation was continued until enough peroxide products were generated for convenient analysis (50 °C, 0.5 h; 37 °C, 1.5 h; 30 °C, 3 h; 10 °C, 8 h). The product mixture was reduced with triphenylphosphine as previously described[27] and analyzed by LC. The procedure was essentially the same for oxidations with added *p*-methoxyphenol.

Emulsion Phospholipid Oxidations. The lecithins **8**–**10** were synthesized as previously described[28] and emulsions were prepared according to the procedure of Van Deenen et al.[29] Thus, 15 μmol total of lipid in $CHCl_3$ was freed of solvent under vacuum, 1 mL of 50 mM KCl solution was added, and the mixture was gently agitated at 37 °C. The emulsion was then vigorously shaken on a vortex mixer until it was homogeneous and milky white. It was only possible to obtain homogeneous emulsions of **9** and **10** (at 37 °C) if the ratio of **9**:**10** was greater than or equal to 1:5.

The oxidation was carried out in a 10-mL two-neck flask with a condenser (cooling fluid temperature approximately 0 °C) connected to a Claisen adapter. Dried air was pulled through the Claisen adapter to supply a constant O_2 concentration. The emulsion was stirred magnetically and kept at 37 ± 1 °C with an oil bath and thermoregulator. All emulsion oxidations were stopped when diene conjugation was 10 ± 1%, which corresponded to a reaction time of 16–22 h. The water was removed under vacuum, the oxidized lipid was reduced with PPh_3 in wet ether and hydrolyzed with snake venom,[28] and the resulting fatty acid alcohols were analyzed on LC.

Acknowledgments. This research was supported by grants from NIH (HL 22219) and the National Science Foundation. N.A.P. acknowledges receipt of an NIH RCDA, 1977–1982. We thank Dr. B. Zwanenburg of the University of Nijmegen for his generous assistance.

(22) Wills, E. D. *Int. J. Radiat. Biol.* **1970**, *17*, 217.
(23) Recknagel, R. O. *Pharm. Rev.* **1967**, *19*, 145.
(24) Weddle, C. C.; Hambeck, K. K.; McKay, P. B. *J. Biol. Chem.* **1976**, *251*, 2973.
(25) Kellog, E. W. III; Fridovich, I. *J. Biol. Chem.* **1975**, *250*, 8812.
(26) Lynch, R. E.; Fridovich, I. *J. Biol. Chem.* **1978**, *253*, 1838.
(27) Porter, N. A.; Logan, J.; Kontoyiannidou, V. *J. Org. Chem.* **1979**, *44*, 3177.
(28) Porter, N. A.; Wolf, R. A.; Nixon, J. R. *Lipids* **1979**, *14*, 20.
(29) De Gier, J.; Mandersloot, J. G.; Van Deenen, L. L. M. *Biochim. Biophys. Acta* **1968**, *150*, 666.