corollary is that as in the case of arylmethanes, LiCHA exchange of propene involves a pyramidal transition state with only partial conjugation of the reaction center with the double bond. It could well be that conjugation in the propene transition state is more developed than for toluene because less substrate reorganization is involved. In that event, the effective Brønsted slope would be greater than 0.3 and the pK difference between propene and toluene is less than 2.7. Accordingly, the pK_{CsCHA} of propene is probably best represented as 43 ± 1.

The derived ΔpK_{CsCHA} for propene and toluene of ~2 agrees well with ΔpK = 1.9–3.6 derived by Juan, Schwarz, and Breslow.[9] The latter estimate refers to an organoelectrolyte region of an electrode, but such an environment may not be too dissimilar from that in an ion pair. The close comparison of the acidities of propene and toluene in the two systems is probably more meaningful than a comparison of our propene pK_{CsCHA} with the absolute pK assigned as 47–48 in the electrochemical system, considering the uncertainties in the medium to which the latter number refers. The ion pair ΔpK for toluene and propene also compares with the gas-phase ΔpK of ≥1 estimated by Bohme et al.[4]

In kinetic exchange with LiCHA, propene is about 20–30 times more reactive than the cycloalkenes, cyclopentene through cyclooctene.[1] The primary hydrogens of propene are significantly more reactive than the secondary allylic positions of the cycloalkenes, a common result in kinetic acidities. The vinyl hydrogens of ethylene are about 1000 times less reactive than propene toward LiCHA;[31] hence, the present exchange experiments are not significantly complicated by concurrent vinyl exchange.

Acknowledgment. This research was supported in part by NIH Grant No. GM-12855, USPH, and by NSF Grant No. CHE 79-10814. The polydeuteration calculations were supported by the Academic Computer Science of Southeastern Massachusetts University.

Supplementary Material Available: A complete analysis and details of the computation (11 pages). Ordering information is given on any current masthead page.

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Unified Mechanism for Polyunsaturated Fatty Acid Autoxidation. Competition of Peroxy Radical Hydrogen Atom Abstraction, β-Scission, and Cyclization

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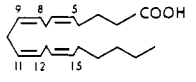
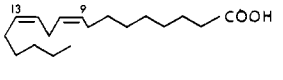
Abstract: The autoxidation of linoleic (18:2) and arachidonic (20:4) acids with several cosubstrates was investigated. Cumene, tetralin, 1,4-cyclohexadiene, and 9,10-dihydroanthracene in benzene were used as cosubstrates for the oxidation of linoleic acid. The distribution of products, trans,cis diene hydroperoxides and trans,trans diene hydroperoxides, was dependent on the ability of cosubstrates to donate hydrogen atoms to linoleate peroxy radicals. Arachidonic acid was oxidized in mixtures of benzene/1,4-cyclohexadiene with linoleic acid internal standard. Product distribution of six hydroperoxyeicosatetraenoic acids (HPETE) derived from arachidonic acid was established at different concentrations of 1,4-cyclohexadiene in the solvent mixture. A kinetic expression is derived that is useful in describing polyunsaturated fatty acid oxidation product mixtures. By the use of this kinetic derivation, the rate of cyclization of peroxy free radicals derived from arachidonic acid was determined.

Introduction

Interest in lipid oxidation[1-4] has been stimulated by discoveries that peroxide products of unsaturated fatty acid autoxidation have interesting biological properties. The enzymatic oxidation of arachidonic acid (**1**) plays a central role in a variety of biological



1, ARACHIDONIC ACID



2, LINOLEIC ACID, L-H

events such as inflammation,[5] platelet aggregation,[6] asthma,[7] and anaphylaxis.[8] Random autoxidation of polyunsaturated fatty acids and esters also appears to be an important process[9] in vivo as is evidenced by the expiration of pentane and ethane, known fatty acid oxidation products, by organisms under free-radical stress. While the enzymatic oxidation of arachidonic acid (**1**) has received considerable attention, studies of the autoxidation of this important fatty acid have been fragmentary and incomplete.[10]

Product mixtures obtained in polyunsaturated fatty acid random autoxidation, on the other hand, are complex, and the primary processes leading to products have not been firmly established. Nevertheless, some kinetic and product studies have begun to address the important mechanistic questions in free-radical lipid oxidation and the following suggestions have been made.

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(3) Chan, H. W.-S.; Levett, G.; Matthew, J. A. *Chem. Phys. Lipids* **1979**, *24*, 245.
(4) Porter, N. A.; Weber, B. A.; Weenen, H.; Khan, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 5597.
(5) Kuehl, F. A. *Science* **1980**, *210*, 978.
(6) Salmon, J. A.; Smith, D. R.; Flower, R. J.; Moncada, S.; Vane, J. R. *Biochim. Biophys. Acta* **1978**, *523*, 250.
(7) Parker, C. W. J. *Allergy Clin. Immun.* **1979**, *63*, 1.
(8) Corey, E. J.; Barton, A. E.; Clark, D. A. *J. Am. Chem. Soc.* **1980**, *102*, 4278, and references cited.
(9) Tappel, A. L.; Dillard, C. J. *Fed. Proc.* **1981**, 40, 174.
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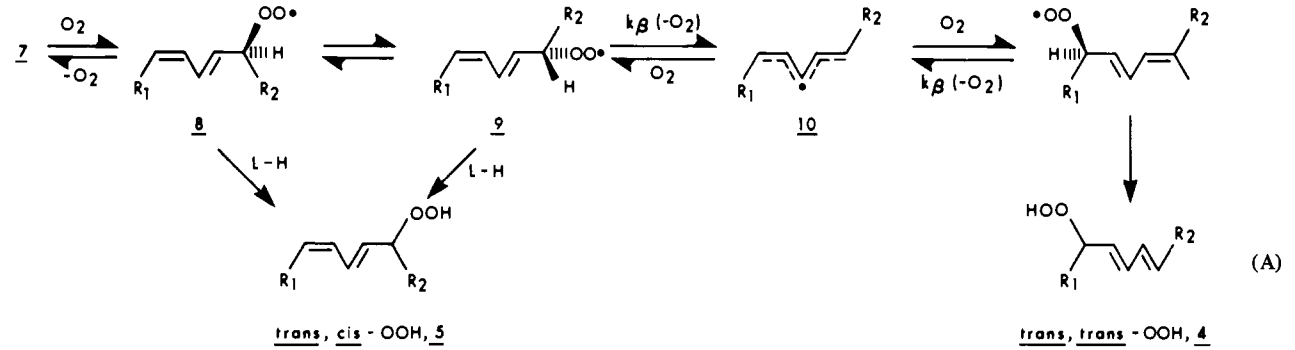
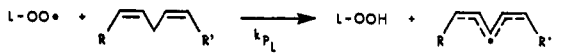
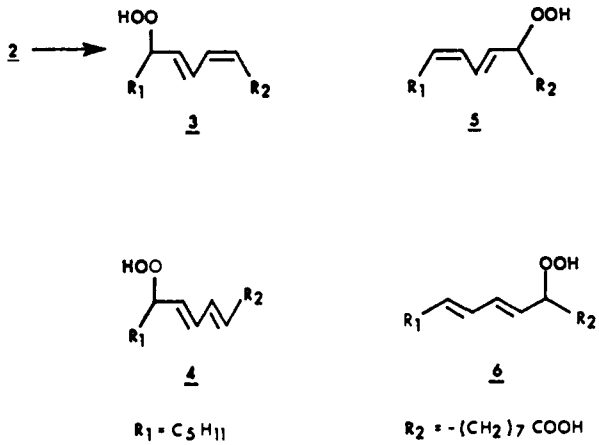


(A)

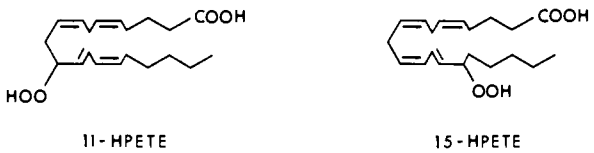
1. The abstractable hydrogens in polyunsaturated fatty acids are those attached to bis-allylic carbons. The rate of self-propagation of linoleate, k_{p_L}, has been found[11] to be 62 M^{-1} s^{-1}. The initially formed radical has the W configuration.[12]



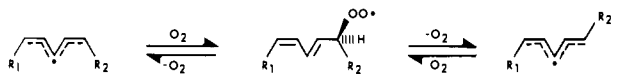
2. Primary products formed in linoleate autoxidation are trans,cis and trans,trans conjugated diene hydroperoxides.[13] At low conversion, over 97% of oxygen consumed in the autoxidation can be accounted for in these products.



3. Six major conjugated diene hydroperoxides, hydroperoxyeicosatetraenoic acids (HPETE), have been isolated and identified[10] in arachidonic acid autoxidation. These major products all have trans,cis conjugated diene stereochemistry with hydroperoxide substitution being at carbons 5, 8, 9, 11, 12, or 15. Pairs of these products derive from initial abstraction of an H atom attached to the carbon allylic to both positions of the products; e.g., 11- and 15-HPETE derive from H abstraction at carbon 13 of arachidonic acid.



4. Oxygen addition to pentadienyl radicals is reversible.



Oxygen scrambling has been established[14] in the isomerization of lipid hydroperoxides under $^{36}O_2$.

Table I. Product Distribution of Linoleic Acid–Cosubstrate Oxidation at 30 °C[a]

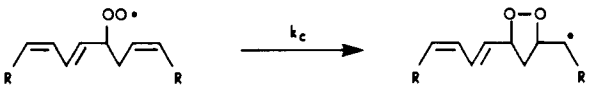
cosubstrate [M]	n	[t,c products], 3+5/ [t,t products], 4 + 6
none	1	0.24
CHD[b] [0.21]	2	1.0 (0.3)
CHD [0.50]	3	1.7 (0.1)
CHD [0.94]	2	3.0 (0.1)
CHD [2.0]	3	5.5 (0.4)
CHD [4.0]	3	12.3 (2)
CHD [9.5]	4	27.3 (2)
DHA [0.05]	1	0.44
DHA [0.10]	1	0.57
DHA [0.20]	1	0.90
cumene [neat]	1	0.27
tetralin [neat]	1	0.67

[a] Linoleic acid was 0.24 M in all experiments; inert solvent was benzene in all cases. [b] CHD = 1,4-cyclohexadiene. [c] DHA = 9,10-dihydroanthracene.

5. Reversibility of oxygen addition is the basis for understanding product distribution in linoleate oxidation.[4] Thus, it has been suggested that there are two conformations of peroxy radical, **8** and **9**, capable of undergoing β fragmentation (C–O bond undergoing fragmentation perpendicular to the diene π system). Fragmentation from conformer **8** leads to the initial W radical **7** while fragmentation of **9** leads to an isomerized radical **10** which may be converted to trans,trans products. (See eq A.)

6. Competition kinetics analysis of H-atom abstraction from linoleate by conformers **8**/**9** (leading to trans,cis products) vs. β-fragmentation leading to trans,trans products gives a value of k_β(30 °C) = 144 (±5) s^{-1} for β-scission of linoleate peroxy radicals.[4,15]

7. Radical cyclization may occur if a remote double bond is present in the peroxy radical substrate.[16-19]



8. Monocyclic peroxides, bicyclic peroxides,[20-22] and epoxy alcohols[16,22] are products from radicals formed by cyclization.

(11) Howard, J. A.; Ingold, K. U. *Can J. Chem.* **1967**, *45*, 793.
(12) The W radical has only two cisoid configurations, while the U and Z radicals would have additional cisoid orientations. See Thomas, M. J.; Pryor, W. A. *Lipids* **1980**, *15*, 544, for a discussion of this point.
(13) Chan, H. W.-S.; Levett, G. *Lipids* **1977**, *12*, 99.
(14) Chan, H. W.-S.; Levett, G.; Matthew, J. A. *J. Chem. Soc., Chem. Commun*, **1978**, 756.
(15) The value of k_β (30 °C) reported in ref 4 (111 s^{-1}) was incorrect owing to an arithmetic error. The correct value is as reported in the text.
(16) Mihelich, E. D. *J. Am. Chem. Soc.* **1980**, *102*, 7141.
(17) Porter, N. A.; Funk, M. O.; Gilmore, D. W.; Isaac, R.; Nixon, J. R. *J. Am. Chem. Soc.* **1976**, *98*, 6000.
(18) Beckwith, A. L. J.; Lawrence, T.; Serelis, A. K. *J. Chem. Soc., Chem. Commun.* **1980**, 484.
(19) Porter, N. A.; Roe, A. N.; McPhail, A. T. *J. Am. Chem. Soc.* **1980**, *102*, 7574.
(20) O'Connor, D. E.; Mihelich, E. D.; Coleman, M. C. *J. Am. Chem. Soc.* **1981**, *103*, 223.
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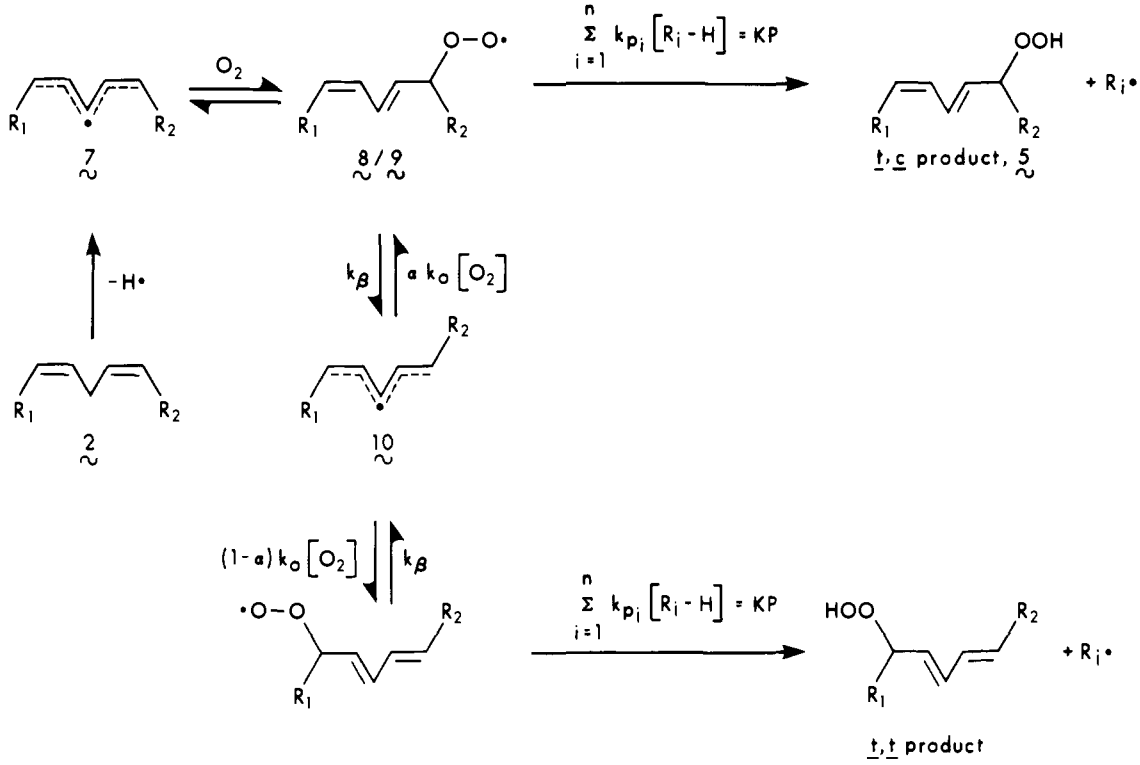
Table II. Product Distribution of Arachidonic Acid Hydroperoxides Formed in Autoxidation of Mixtures of 1,4-Cyclohexadiene and Benzene at 30 °C

	5-HPETE[b]	8-HPETE	9-HPETE	11-HPETE[c]	12-HPETE	15-HPETE
solvent	3 + 4	3 + 4	3 + 4	3 + 4	3 + 4	3 + 4
n[d]	24	12	22	24	24	24
97% CHD[a]	0.825	0.72	0.765	1.0	0.61	1.23
20% CHD	0.72	0.32	0.37	0.55	0.29	1.11
10% CHD	0.59	0.19	0.25	0.34	0.17	0.91
7% CHD	0.57	0.16	0.18	0.27	0.13	0.88
5% CHD	0.51	0.14	0.17	0.21	0.11	0.76

[a] CHD = 1,4-cyclohexadiene. [b] Ratio of t,c HPETE from arachidonic acid vs. 13-hydroperoxides from linoleic acid, t,c and t,t. [c] A shoulder on the 11-HPETE peak was subtracted from the integrated area. This shoulder was shown to be t,t 15-HPETE. [d] Total number of analyses carried out for each HPETE isomer. Standard deviations of the data are shown in Figures 3 and 4.

Scheme I



We report here on the oxidation of linoleic acid and arachidonic acids with a series of cosubstrates. The results of these studies provide a mechanistic framework for understanding product distribution in polyunsaturated fatty acid oxidation.

Results

Linoleic acid[23] (0.24 M) was cooxidized at 30 °C in mixtures of 1,4-cyclohexadiene (CHD) and 9,10-dihydroanthracene (DHA) in benzene as well as in pure tetralin and cumene. Autoxidation was initiated by di-*tert*-butyl peroxyoxalate[24] and product distribution was found to be independent of oxygen pressure between 150 and 760 nm.[4] The total oxidation of the system, fatty acid and cosubstrate, was generally less than 2%.[25] Fatty acid hydroperoxide products were reduced to the corresponding hydroxylinoleates with triphenylphosphine and analyzed by high-pressure liquid chromatography as previously described.[4] Products were detected by UV absorbance, and product distributions presented in Table I are corrected for the known absorbances of each component. The distribution of trans,cis and trans,trans products clearly depends on the overall hydrogen atom donating ability of the solution. Thus the [t,c]/[t,t] product ratio is found to be greater than 25 in 97% cyclohexadiene, an excellent hydrogen atom donating medium, while it drops to 0.27 if the cosubstrate is cumene, a relatively poor donor compared to cyclohexadiene.

Mixtures 0.1 M in arachidonic acid and 0.1 M in linoleic acid were oxidized under air or oxygen in solutions of 1,4-cyclohexadiene–benzene. Linoleic acid was added as an internal standard in the oxidation since the **9** and **13** trans,cis and trans,trans hydroperoxides of linoleic acid are the only significant products formed in bulk-phase autoxidation at low conversion. Oxidation was initiated by di-*tert*-butyl peroxyoxalate and the extent of oxidation, fatty acids and cyclohexadiene, was generally maintained at less than 2%.[25] Fatty acid hydroperoxide products were analyzed by high performance LC on a 5-μ silica column with the solvent hexane/2-propanol/acetic acid 990:10:1. A typical LC chromatogram is displayed in Figure 1. Products analyzed by LC were the 5-, 8-, 9-, 11-, 12-, and 15-HPETE's from arachidonic acid and the 13-trans,cis (**3**) and 13-trans,trans (**4**), hydroperoxides from linoleic acid, two products chosen as internal standards for the oxidation.[26] The product distribution for HPETE's formed from arachidonic acid with reference to the

(23) Linoleic acid was obtained from Nu-Chek Prep or Sigma Biochemicals. Extreme care must be taken to ensure that the fatty acid is not significantly oxidized before an experiment is begun. Significant amounts of oxidation products were detected in some freshly opened linoleic acid samples from Sigma.

(24) Bartlett, P. D.; Benzing, E. P.; Pincock, R. E. *J. Am. Chem. Soc.* **1960**, *82*, 1762.

(25) Hydroperoxide and hydrogen peroxide products derived from fatty acids and cosubstrates were titrated with PPh_3 (see Experimental Section).

(26) Measurement of HPETE's vs. linoleate hydroperoxides thus provides a common reference for extent of oxidation.

two linoleic products, **3** and **4**, is presented in Table II. We note with interest that the 5- and 15-HPETE compounds are the major products of arachidonic acid oxidation, while HPETE's substituted at internal positions (8, 9, 11, and 12) are formed to a lesser extent.

Discussion

General Mechanism for Diene Fatty Acid Autoxidation. Hydroperoxides derived from linoleic acid are suggested to be formed by a mechanism as shown in Scheme I. As noted in point 5 in the Introduction, radical **8/9** has two conformers that may lead to β-fragmentation. Fragmentation of **8** re-forms the initial carbon radical **7** while scission of **9** leads to the isomerized carbon radical **10**. The crucial competition that accounts for formation of the trans,cis and trans,trans products is the partitioning of the peroxy radical **8/9** between the β scission pathway (k_β) and the trans,cis product pathway leading to **5**. The parameters α and $(1 - \alpha)$ are inserted to represent the distribution of carbon radical **10** between the trans,cis peroxy radical **8/9** and the trans,trans peroxy radical.

It should be noted that this simplified scheme is essentially identical with the one presented earlier[4] to account for the linoleate concentration dependence of trans,cis and trans,trans hydroperoxides in linoleate oxidation. At higher concentrations of linoleate, more peroxy radical **8/9** is directed toward the trans,cis product **5** since the bimolecular pathway involving hydrogen atom transfer becomes more competitive at higher concentrations of linoleate. Scheme I has been modified here to emphasize the fact that any good hydrogen atom donor may influence the bimolecular hydrogen atom transfer pathway. The rate of formation of **5** is, in fact, directly related to the total hydrogen atom donating ability of the medium, $\sum^{n}_{i=1} k_{pi}[R_i\text{-H}]$, and for convenience we refer to this important parameter as KP. The ratio of trans,cis to trans,trans products in linoleate oxidation should thus relate to KP in a straightforward way. It is important to emphasize that KP is not a new concept in free-radical kinetics. The idea of carrying out competition kinetics by allowing the H atom availability of the medium to change has been a cornerstone of many mechanistic studies.[27] We justify the definition of this new parameter, KP, since (a) product distribution of linoleate products (trans,cis vs. trans,trans) depends directly on KP (vide supra) and (b) product studies of random oxidation in biological systems of unknown total composition will provide information about a property of that system, KP, without regard to or knowledge of the specific components that comprise that system. For example, analysis of linoleate oxidation products formed by random in vivo oxidation would provide information about KP at the site of oxidation even though the specific donors present (fatty acids, phenols such as vitamin E, thiols, etc.) are unknown.

Analysis of the mechanism shown in Scheme I by the usual steady-state kinetics leads to eq 1 where [t,c]/[t,t] is the ratio

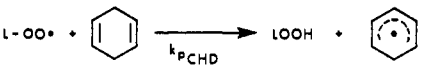
$$\frac{[t,c]}{[t,t]} = \frac{KP}{k_\beta(1-\alpha)} + \frac{\alpha}{(1-\alpha)} \qquad (1)$$

of trans,cis and trans,trans products. Insertion of $\alpha = 0.12$ (± 0.01) and $k_\beta = 144$ (± 5) s^{-1}, values previously determined for oxidation of linoleic acid in benzene at 30 °C,[4] provides a master equation, eq 2, that describes the product ratio [t,c]/t,t], in terms of the hydrogen atom donating parameter, KP. In studies where linoleic acid is the only good source of hydrogen atoms, $KP = k_{p_L}[L\text{-H}] = 62[L\text{-H}]$. For linoleic acid at 30 °C in benzene:

$$\frac{[t,c]}{[t,t]} = \frac{KP}{127} + 0.14 \qquad (2)$$

We should also point out that eq 1 does not hinge on the fact that formation of the trans,trans peroxy radical from the trans,cis peroxy radical **8/9** occurs by β-scission. Any unimolecular rearrangement connecting these two radicals in competition with bimolecular formation of the trans,cis hydroperoxide **5** would lead to eq 1. We prefer the β-scission pathway described here because of Chan's report of oxygen scrambling in linoleate hydroperoxide isomerization (Introduction, point 4). A series of 3,2 rearrangements could, however, link the trans,cis and trans,trans peroxy radical manifolds[28,29] and yield the same kinetic expression. It should also be noted that k_p for linoleate autoxidation was obtained with linoleate esters in chlorobenzene solvent, whereas our studies are carried out with the free acid in benzene. We find HPLC analysis of free acid oxidations, particularly those involving arachidonic acid, to be more straightforward than analysis of methyl ester oxidations, and for that reason we have focused on oxidations of the free acids. More limited studies of the oxidation of methyl linoleate in chlorobenzene give essentially the same product distributions as found for oxidation of the free acid in benzene, and we thus assume equivalent k_p for these similar systems.

Cooxidation of Linoleic Acid and Other Oxidizable Substrates. While the parameters (k_β and α) used to derive eq 2 come from analysis of products of the oxidation of linoleic acid in benzene alone, the equation should be applicable, as noted, to any medium of oxidation if k_β and α may be assumed to be independent of solvent (at least within the range of solvents studied here). Thus, for oxidation of linoleic acid with 1,4-cyclohexadiene (CHD) as cosubstrate, eq 2 may be applied where $KP = 62[L\text{-H}] + k_{PCHD}[CHD]$, and k_{PCHD} is the rate of hydrogen atom transfer from cyclohexadiene to the linoleate peroxy radical L–OO·. Expansion



of eq 2 for the special case of cooxidation of 0.24 M linoleic acid in 1,4-cyclohexadiene/benzene (Table I) leads to $[t,c]/[t,t] = k_{PCHD}[CHD]/127 + 0.25$. In Figure 2 is presented a plot of [t,c]/[t,t] vs. [CHD] for cooxidation of linoleic acid/cyclohexadiene in benzene. As seen in Figure 2, [t,c]/[t,t] is linear with respect to the concentration of CHD from 0.2 to 9.5 M. From the slope of the plot, we calculate $k_{PCHD} = 362$ (± 17) M^{-1} s^{-1} at 30 °C. The rate constant for lipid peroxy radical attack on cyclohexadiene has not been previously reported, but this value seems reasonable when compared to the known k_p for attack of the unhindered H–OO· radical on cyclohexadiene (1480 M^{-1} s^{-1}),[30] particularly since k_p is known to reflect peroxy radical substituent effects.

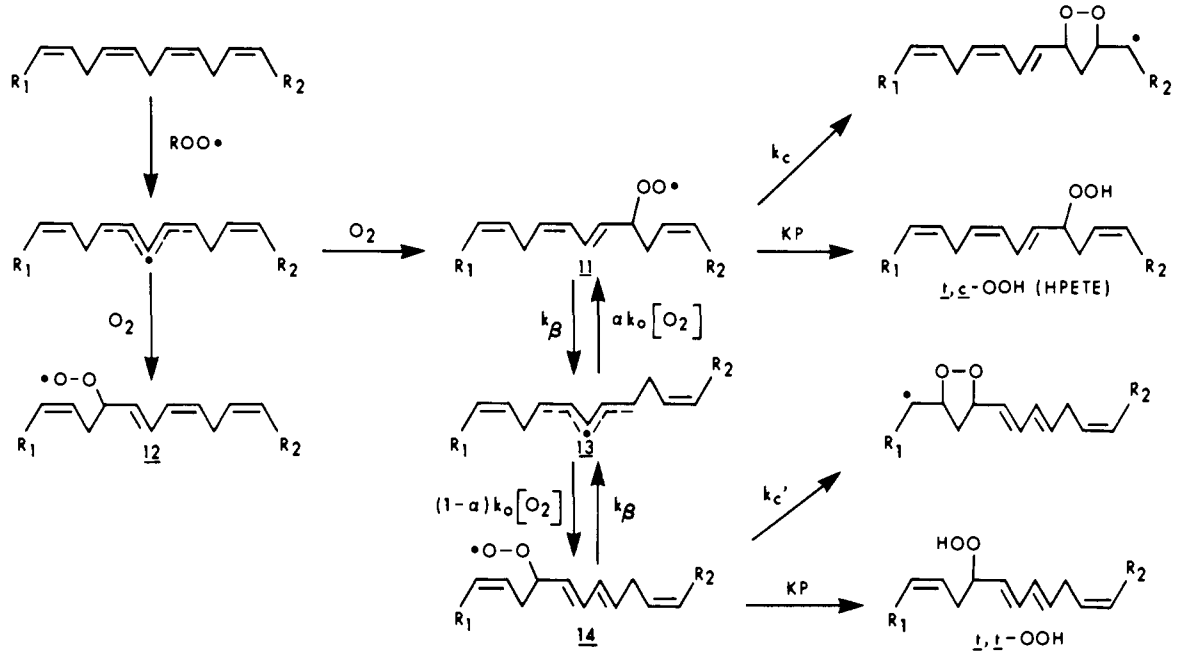
The reactivity of other substrates toward linoleate peroxy radical can be determined in a way similar to that used for cyclohexadiene. The rates of linoleate peroxy radical abstraction from cumene, tetralin, 9,10-dihydroanthracene (DHA), and cyclohexadiene as determined by the use of eq 2 are presented in Table III along with rate constants for atom abstraction from these substrates by other alkyl peroxy radicals.

The trend of rate constants as determined by the linoleate method parallels the trend seen by other kinetic methods. The only significant difference in the rate constants obtained by the different methods is for cyclohexadiene cosubstrate, and the attacking radicals (LOO· and HOO·) are dramatically different in this particular instance.

General Mechanism for Triene or Tetraene Fatty Acid Autoxidation. Autoxidation of polyunsaturated fatty acids or esters

(27) For an excellent recent review of free-radical rearrangements, see Beckwith, A. L. J.; Ingold, K. U. "Rearrangements in Ground and Excited States"; deMayo, P., Ed.; Academic Press: New York, **1980**; Vol. 1, p 161.
(28) Schenck, G. D.; Neumuller, A. O. *Justus* Liebigs *Ann. Chem.* **1958**, *618*, 202.
(29) Brill, W. F. *J. Am. Chem. Soc.* **1965**, *87*, 3286.
(30) Howard, J. A. "Advances in Free Radical Chemistry"; Williams, G. H., Ed.; Academic Press: New York, 1972; Vol. 4, p 49.

Scheme II



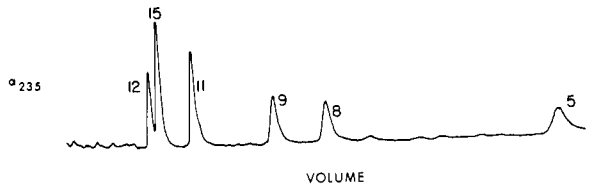


Figure 1. HPLC chromatogram of oxidation products from 0.2 M arachidonic acid in 97% cyclohexadiene/3%/benzene. HPTE isomers are indicated on the chromatogram. LC conditions are as described in the text.

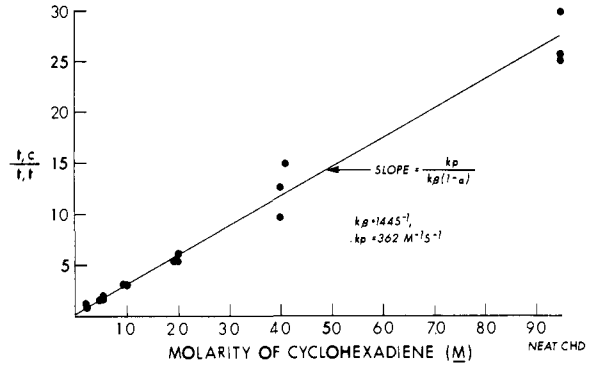


Figure 2. [t,c]/[t,t] products derived from linoleic acid oxidation in cyclohexadiene/benzene.

that contain three or more double bonds introduces an additional competitive process, peroxy radical cyclization, that must be considered in the mechanistic scheme. This modified format, shown in Scheme II, suggests that three independent pathways control product distribution in triene or tetraene fatty acid oxidation. Two of these processes, β-scission and cyclization, are unimolecular events, while the third, hydrogen atom transfer, is critically dependent on KP of the medium of oxidation. Scheme II is thus essentially identical with Scheme I with the exception that the trans,cis peroxy radical **11** may undergo cyclization with rate k_c, and the trans,trans peroxy radical **14** may cyclize with rate k_c'. Steady-state kinetics on **13** and **14** leads to $[\mathbf{14}]/[\mathbf{11}] = (1-\alpha)k_\beta/(\alpha k_\beta + \mathrm{KP} + kc')$. We should also note that we consider here only products that derive from the peroxy radical **11** and assume that a similar, but independent expression could be written for **12**.

Table III. Rate Constants for H-Atom Transfer to Linoleate Peroxy Radicals from Substrates

substrate	k_p (M^{-1} s^{-1}) L-OO· + R-H	k_p (M^{-1} s^{-1}) R'-OO· + R-H
CHD	362	1480[a]
9,10-dihydroanthracene	397	160-340[b]
tetralin	6.7	4.6-6.5[b]
cumene	0.6	0.2-0.5[b]

[a] Rate constant for HOO· + CHD. [b] Rate constant for atom abstraction from substrate by various alkyl peroxy radicals; see ref 30.

Products analyzed in the study of arachidonic acid oxidation described in this report (Table II) are the trans,cis hydroperoxide (HPETE) compounds. We thus write an expression for HPETE vs. all products derived from peroxy radical **11** as a function of constants outline in Scheme II. In eq 3, HPETE_0 is inserted for

$$\frac{\text{all products from } \mathbf{11}}{\mathrm{HPETE}} = \frac{[\mathbf{11}]\mathrm{KP} + [\mathbf{11}]k_c + [\mathbf{14}]k_c' + [\mathbf{14}]\mathrm{KP}}{[\mathbf{11}]\mathrm{KP}} \quad (3a)$$

$$\frac{\mathrm{HPETE}_0}{\mathrm{HPETE}} = 1 + \frac{k_c}{\mathrm{KP}} + \frac{(1-\alpha)k_\beta k_c'}{(\alpha k_\beta + k_c' + \mathrm{KP})\mathrm{KP}} + \frac{(1-\alpha)k_\beta}{(\alpha k_\beta + k_c' + \mathrm{KP})} \quad (3b)$$

"all products from **11**".

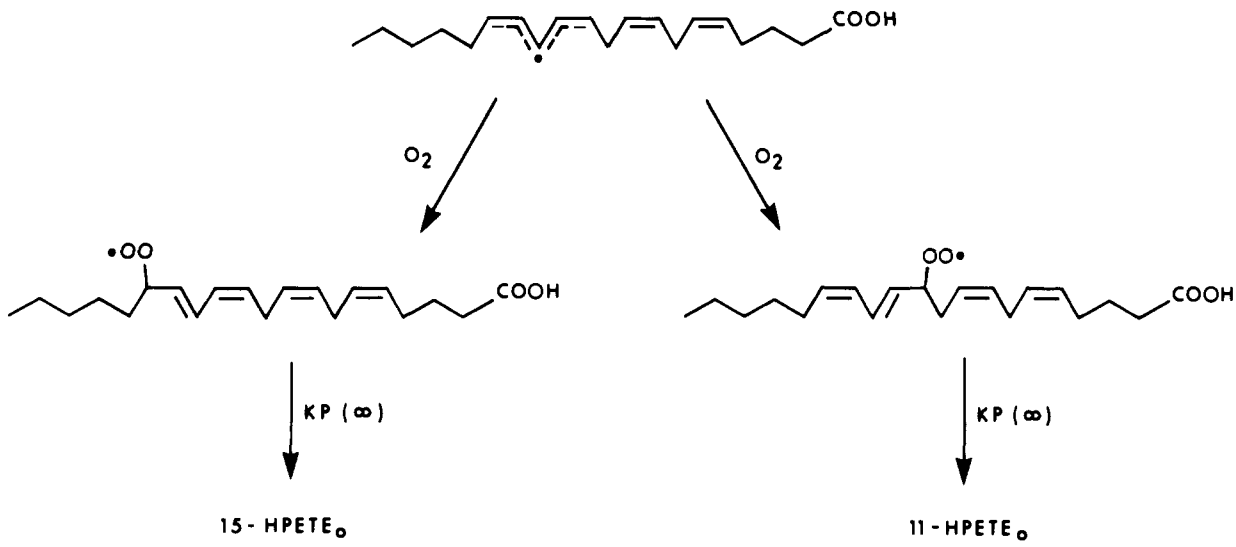
Consider the following specific cases where eq 3 might be applied:

(1) $\mathrm{KP} = \infty$, $1/\mathrm{KP} = 0$; with this boundary condition HPETE = HPETE_0.

(2) $k_c = k_c' = 0$; in this case the mechanism outlined in Scheme II reduces to Scheme I, a mechanism used to describe diene fatty acid oxidation where no radical cyclization is possible. Equation 3 yields eq 4 in this special case, and eq 4 (HPETE_0 = t,c + t,t) is equivalent to eq 1.

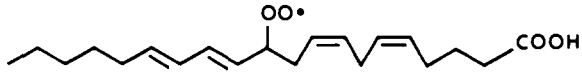
$$\frac{\mathrm{HPETE}_0}{\mathrm{HPETE}} = 1 + \frac{(1-\alpha)k_\beta}{\alpha k_\beta + \mathrm{KP}} \quad (4)$$

Scheme III



(3) $k_c = 0$, $k_c' \neq 0$; this condition is met for the trans,cis peroxy radical precursor to 15-HPETE, **15**, or the corresponding radical

15



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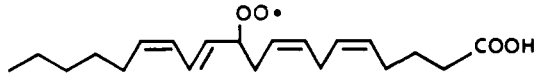
intermediate leading to 5-HPETE. In both of these radicals no remote double bond is readily available for cyclization from the trans,cis radical ($k_c = 0$) while the associated trans,trans radical, **16**, may undergo cyclization ($k_c' \neq 0$). If, additionally, $k_c' + KP >> \alpha k_\beta$ (a condition that is generally met in oxidations using cyclohexadiene cosubstrate), then eq 3 reduces to

$$\frac{HPETE_0}{HPETE} = 1 + \frac{(1-\alpha)k_\beta}{KP} \quad (5)$$

(4) $k_c' = 0$, $k_c \neq 0$; this condition is met for the trans,cis peroxy radical precursors to 11-HPETE, **17**, and 9-HPETE. The cor-



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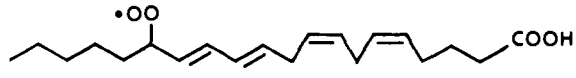

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responding trans,trans peroxy radicals, of which **18** is an example derived from **17**, do not have a cyclization pathway available. If we again assume that $KP >> \alpha k_\beta$, then eq 3 is simplified to

$$\frac{HPETE_0}{HPETE} = 1 + \frac{k_c}{KP} + \frac{(1-\alpha)k_\beta}{KP} \quad (6)$$

(5) $k_c' \neq 0$, $k_c \neq 0$; this final condition is met for peroxy radical percursors to 12-HPETE and 8-HPETE. Assuming $KP + k_c' >> \alpha k_\beta$, then eq 6 may again be used to describe this specific situation.

The general equation describing the mechanism outlined in Scheme II, eq 3, thus provides specific expressions (eq 4, 5, and 6) that may be used in considering product distributions obtained in the autoxidation of diene, triene, and tetraene fatty acids. Each particular trans,cis peroxy radical and its coupled β-scission peroxy radical (e.g., **15** and **16**) must be analyzed with regard to the potential for radical cyclization, and by such consideration the choice of an appropriate equation is made.

Cooxidation of Arachidonic Acid, Linoleic Acid, and Other Oxidizable Substrates. Equations 5 and 6, derived to describe the relationship of HPETE products and KP, suggest that 1/HPETE vs. 1/KP should be linear if $KP >> \alpha k_\beta$. In figure 3 is presented a plot of 13-t,c + 13-t,t linoleic acid hydroperoxides/HPETE vs. 1/KP. Recall that oxidations of arachidonic acid in cyclohexadiene were carried out with equimolar linoleic acid present and the comparison of arachidoniate products with the 13-linoleate hydroperoxides serves as an internal standard for oxidation. No peroxy radical cyclization occurs in linoleate oxidation, and the sum of the two 13-linoleate products should thus be invariant with KP. In addition to arachidonate HPETE products, note that a plot of 13-t,c linoleate is also shown in Figure 3 for comparison with the HPETE data.

As seen in the figure, 1/HPETE vs. 1/KP is linear for all HPETE products as well as for 13-*trans,cis*-linoleic hydroperoxide. It should be noted that the plots for 5-HPETE, 15-HPETE, and 13-*trans,cis*-linoleic hydroperoxide, all of which are described by eq 5 ($k_c = 0$), are closely parallel with low slope while plots for 12-, 8-, 9-, and 11-HPETE, described by eq 6 ($k_c \neq 0$), have significantly higher slopes. The intercepts of the plots shown in Figure 3 also deserve particular comment. At 1/KP = 0 (KP = ∞) HPETE is equal to $HPETE_0$ and the intercept thus gives 13-t,c + 13-t,t linoleate hydroperoxides/$HPETE_0$.

One might expect that HPETE products derived from the same carbon radical should have identical $HPETE_0$ values since oxygen addition at either end of the pentadienyl radical is presumed to be comparable. (See Scheme III.) Thus 11-$HPETE_0$ = 15-$HPETE_0$, 8-$HPETE_0$ = 12-$HPETE_0$, and 9-$HPETE_0$ = 5-$HPETE_0$. This expectation is realized with the 11 and 15 intercepts (open and solid circles), the 8 and 12 intercepts (open and solid triangles) and the 9 and 5 intercepts (open and solid squares). These intercepts thus relate directly to the rate of hydrogen atom abstraction from linoleate C-11 (leading to the 13-linoleate products) divided by the rate of abstraction of hydrogen atom at particular sites on arachidonic acid: C-13 abstraction giving 11-$HPETE_0$ and 15-$HPETE_0$ products, C-10 abstraction giving 12-$HPETE_0$ and 8-$HPETE_0$ products, and C-7

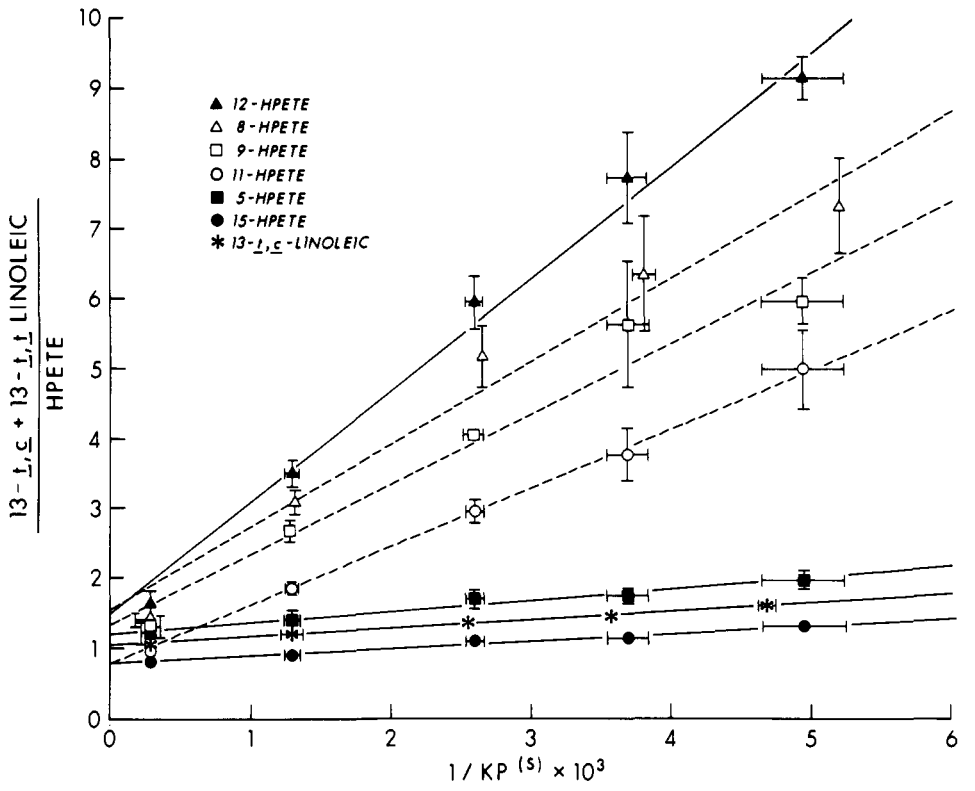

Figure 3. Product ratio of 13-linoleate hydroperoxides vs. arachidonate trans,cis hydroperoxides as a function of 1/KP.

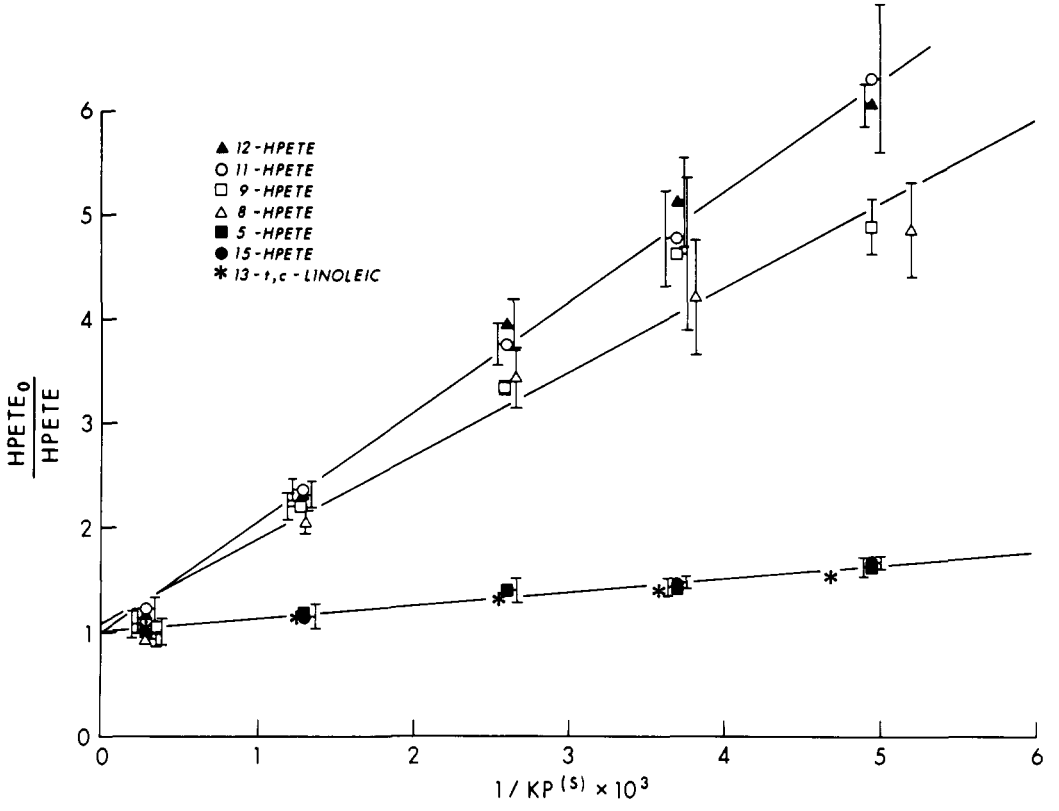

Figure 4. Product ratio of $HPETE_0/HPETE$ for arachidonate trans,cis hydroperoxides. Plotted lines represent the average slope for 12- and 11-HPETE (k_c = 931 s^{-1}); 8- and 9-HPETE (k_c = 688 s^{-1}); and 15- and 5-HPETE (k_β = 147 s^{-1}).

abstraction giving 9-$HPETE_0$ and 5-$HPETE_0$ products. From the intercepts, hydrogen atom donating abilities of arachidonic acid hydrogens at C-7, C-10, and C-13 relative to the C-11 hydrogen atom of linoleic acid in this cooxidation are found to be arachidonic C-7 = 0.86 (±0.03), C-10 = 0.70 (±0.08), C-13 = 1.33 (±0.03) vs. linoleic C-11 = 1.0 (±0.03).

By the use of the intercept values of Figure 3, a plot of $HPETE_0/HPETE$ vs. 1/KP may be made and such a plot is presented in Figure 4. Note the clear distinction between the 5 and 15 HPETE's vs. the 8, 9, 11, and 12 isomers. The 5 and 15 isomers respond to changes in KP in a manner exactly analogous to the 13-*trans,cis*-linoleic hydroperoxide. No cyclization

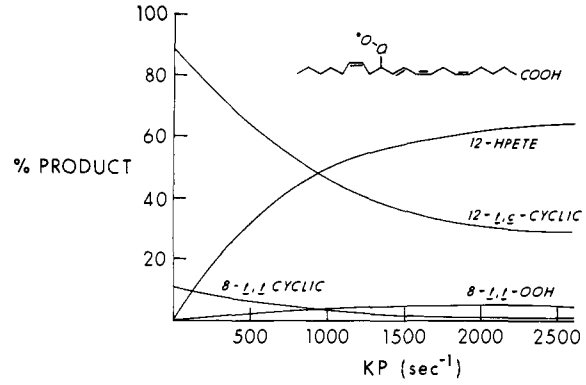

Figure 5. Products obtained from 12-HPETE peroxy radical as a function of KP. Products derived from peroxy radical cyclization include monocyclic and bicyclic peroxides as well epoxy alcohols.

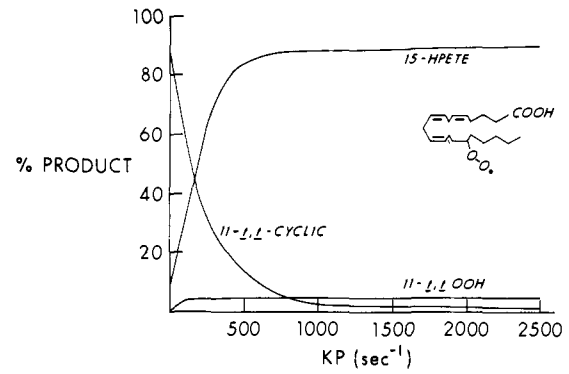

Figure 6. Products obtained from 15-HPETE peroxy radical as a function of KP.

pathway is available for peroxy radicals on the pathway leading to these products, with β-scission and H-atom abstraction being the only alternatives. The appropriate expression that describes the KP product dependence for 5-HPETE and 15-HPETE is eq 5, and rate constants calculated for β-scission of the 15 and 5 peroxy radicals by the use of this equation are: 15-HPETE, 152 ($\pm$8) s^{-1}; 5-HPETE, 145 ($\pm$16) s^{-1} (c.f. 13-linoleate, 144 ($\pm$5) s^{-1}). We conclude that the rate of β-scission for diene peroxy radicals is relatively independent of position and substitution in the fatty acid chain.

Application of eq 6 to the data presented in Figure 4 for 12-, 11-, 9-, and 8-HPETE gives values for $k_c + (1 - \alpha)k_\beta$ for each isomer. If we assume that values of α and k_β for these isomeric peroxy radicals are similar to those found for the 15- and 5-HPETE's and the 13-*trans,cis*-linoleate hydroperoxide, apparently a reasonable assumption (vide supra), then rate constants for cyclization may be calculated. These rate constants, k_c, are: 8-HPETE = 657 ($\pm$116) s^{-1}; 9-HPETE = 698 ($\pm$66) s^{-1}; 11-HPETE = 930 ($\pm$71) s^{-1}, and 12-HPETE = 936 ($\pm$132) s^{-1}. It should be noted that the rate of peroxy radical cyclization reported here, $\approx 8 \times 10^2$ s^{-1} is significantly lower than rates of corresponding carbon and alkoxy radical cyclization (10^5–10^6 s^{-1}).[27]

Product Distributions of Polyene Oxidation as a Function of KP. Equations 1, 4, 5, and 6 may be used to relate the product distribution of diene or polyene fatty acid or ester autoxidation to KP of the medium of oxidation. The importance of local KP in determining product distribution in biologically significant lipid oxidation is thus readily apparent. Autoxidation of biological lipid locally rich in good hydrogen atom donors such as vitamin E and thiols should, we suggest, lead primarily to trans,cis hydroperoxides while oxidation in mixtures with a low KP will provide trans,trans hydroperoxides from diene precursors and products derived from peroxy radical cyclization from polyene substrates. By the use of eq 1 or 4 the product distribution derived from diene fatty acids is related to KP of the medium of oxidation. At low KP, trans,trans diene hydroperoxides may dominate the product mixture. In contrast, little trans,trans products are observed in autoxidation of arachidonic acid. The lack of trans,trans products in arachidonic acid may be understood by consideration of Scheme II and eq 3. Inspection of the scheme suggests that little t,t OOH product will be formed if k_c, k_c', or KP is significantly greater than k_β. Typical rate constants for cyclization are 800 s^{-1}, while typical β scission rates are 140 s^{-1}, so β-scission will not compete effectively with cyclization under all circumstances and β fragmentation will not compete favorably with hydrogen atom abstraction at high KP.

Equation 3 provides a quantitative expression for product distribution in polyene autoxidation. The four terms of eq 3 are specifically related to disappearance of HPETE from the system by the four alternate pathways. Thus k_c/KP represents the trans,cis peroxy radical cyclization pathway, the third term reflects cyclization of the trans,trans peroxy radical and the last term indicates the fraction of HPETE product disappearing to the trans,trans hydroperoxide, with all these referred to HPETE = 1, term 1. As an example of a typical product distribution, consider products formed from the trans,cis peroxy radical as shown in Figure 5. Assuming that k_c for the 12-peroxy radical is 936 s^{-1}, k_c' for the corresponding 8-trans,trans peroxy radical is 657 s^{-1} (identical with the rate of cyclization of the 8-trans,cis peroxy radical), and k_β and α are 144 s^{-1} and 0.12, respectively, we can calculate from eq 3 the expected product distribution. At KP > 1000 s^{-1} 12-HPETE is the major product formed while products derived from cyclization become dominant at KP $\approx$ 900 s^{-1}. Cyclization products from the 8-trans,trans peroxy radical only become marginally significant ($\approx$12%) at low KP and the amount of 8-t,t hydroperoxide never exceeds 4% of the product mixture. A similar plot may be made for each HPETE product, and one other such plot is shown in Figure 6. In this plot of 15-HPETE vs. KP, the 15-trans,cis hydroperoxide dominates the product mixture at KP > 400, and products derived from cyclization of the 11-trans,trans peroxy radical only become important at KP < 200. The 11-trans,trans hydroperoxide never exceeds 8% of the product mixture. A plot similar to Figure 6 is obtained for 5-HPETE products, while Figure 5 typifies product distributions obtained from 12-, 11-, 9-, and 8-HPETE isomers.[31] Thus, in polyene oxidation trans,trans hydroperoxides make up no significant portion of the product mixture under any circumstances of oxidation according to eq 3 in support of the experimental observations.

Summary

The cooxidation of linoleic and arachidonic acids with substrates such as cyclohexadiene offers a vehicle, namely, competition kinetics, for providing quantitative information about polyene autoxidation. The rate of β-fragmentation of trans,cis linoleate peroxy radicals was earlier determined by comparison of products formed by this pathway (trans,trans hydroperoxides) compared to hydrogen atom abstraction from linoleate, a process for which the rate constant is known. We have utilized this unimolecular β-scission fragmentation pathway as a "free-radical clock"[32] for timing hydrogen atom abstraction from other donors such as cyclohexadiene. With knowledge of the rate of hydrogen atom transfer from cyclohexadiene to lipid peroxy radicals, the rate of another important process in polyene autoxidation, peroxy radical cyclization, may be evaluated. The evolution of competition kinetics described here then originates in the rate of self-propagation of linoleate. The sequence of evolution is

$$k_{\text{Plinoleate}} \rightarrow k_{\beta_{\text{linoleate}}} \rightarrow k_{\text{PCHD}} \rightarrow k_{c_{\text{arachidonate}}} + k_{\beta_{\text{arachidonate}}}$$

With knowledge of global rate constants for β-scission and cyclization, product distribution in lipid autoxidation may be addressed. In particular, product distribution is shown to depend

(31) The plots for the 12 and 8 isomers differ from those obtained for the 11 and 9 isomers in that no t,t cyclic products are formed from the 11- and 9-peroxy radical manifolds. That is, scission of the 11 and 9 radicals produces t,t 15- and 5-peroxy radicals which cannot cyclize. Only t,t hydroperoxides derive from scission, and these products decrease from a maximum of 10–12% at low KP to 0% at high KP.

(32) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317.

on KP of the medium of oxidation in a predictable way. We anticipate that products of lipid undergoing autoxidation in a more typical biological environment (membranes, lipid storage pools) will also depend on KP of that environment. Our observations may thus provide a format for further developments in the study of the autoxidation of biologically important compounds.

Experimental Section

High-Pressure Liquid Chromatography. A Whatman 5-μ Partisil analytical silica column was used for analysis of oxidation mixtures. The solvent used was hexane/2-propanol/acetic acid 990:10:1. Arachidonic oxidations could be analyzed before or after reduction of hydroperoxides to hydroxy fatty acids. Similar results were obtained by analysis of the hydroperoxy or hydroxy fatty acids. Further, analysis of hydroperoxy or hydroxy fatty acid methyl esters gave qualitatively the same results as analysis of the free acids. Relative amounts of linoleate products were corrected for their known molar absorptivity, and the arachidonate HPETE products were assumed to have identical ϵ values. This assumption was checked by oxidation of radio-labeled arachidonic acid and isolation of various HPETE products by preparative HPLC. Radioactivity and absorbance of collected fractions were measured and molar absorptivities calculated. The value of ϵ obtained in this way for various HPETE isomers (27 000) compares favorably with value obtained for synthetic HPETE's obtained by alternate routes. Comment should be made about *trans,trans*-arachidonic hydroperoxides. 8-*trans,trans*-HPETE, 15-*trans,trans*-HPETE, and 11-*trans,trans*-HPETE have been prepared by alternate routes and were analyzed by HPLC under our standard conditions. The trans,trans compounds all elute after the corresponding trans,cis isomer, and the 15-*trans,trans*-HPETE is the only compound detected in significant amount at KP > 500 s^{-1}. The 15-trans,trans compound elutes just after 11-HPETE. Significant amounts of unidentified products are observed in oxidations at low KP in the region of the chromatogram eluting after 8-HPETE and 5-HPETE. The nature of this product mixture, presumably resulting from peroxy radical cyclization, was not investigated here.

Free Fatty Acid Oxidations. Linoleic acid and arachidonic acid were obtained from Nu-Chek Prep or Sigma Biochemicals. Extreme care must be taken to ensure that the fatty acid is not significantly oxidized before an experiment is begun. Significant amounts of oxidation products were detected in some freshly opened samples from Sigma. Oxidations were carried out essentially as described earlier. Extent of oxidation was monitored by titrating hydrogen peroxide (if cyclohexadiene was cosubstrate) and fatty acid hydroperoxide formed with triphenylphosphine. Addition of a known quantity of phosphine to an aliquot of the oxidation mixture was followed by thin-layer or HPLC analysis to determine if all phosphine was oxidized to phosphine oxide. One-half percent increments of phosphine, based on starting oxidizable substrates, were generally added until some phosphine remained unoxidized.

The 9-trans,cis and 9-trans,trans hydroperoxides of linoleic acid (not analyzed in cooxidations of linoleate and arachidonate) coelute with 8-HPETE. For this reason, side-by-side oxidations were carried out with linoleic acid and arachidonic acid in cyclohexadiene/benzene and arachidonic acid alone in cyclohexadiene/benzene. The amounts of 15-, 12-, 11-, 9-, and 5-HPETE relative to the 13-linoleate could be determined from the first experiment with the 8-HPETE value compared to these other HPETE isomeric hydroperoxides being obtained by the second experiment.

Acknowledgment. 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.

Mechanism of the Photoepoxidation with and Photodecarboxylation of α-Keto Acids[1]

Yasuhiko Sawaki and Yoshiro Ogata*

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Abstract: The photooxidation of benzoylformic acid (**1a**) in benzene gave peroxybenzoic acid, hydrogen peroxide, and phenyl benzoate. The addition of α-methylstyrene to the oxidation system afforded the epoxide together with acetophenone as a C–C cleaved product, and the ester yield was significantly increased at the expense of the peracid. The photooxidation of **1a** was not sensitized by methylene blue or other sensitizers, but was efficiently accelerated by pyridine or other weakly basic solvents such as ethers. Pyridine effectively catalyzed the photoepoxidation as well as the photodecarboxylation of **1a** to benzaldehyde. The photoepoxidation gave predominantly trans epoxides, and the relative reactivities of olefins were similar to the photoepoxidation with benzoin (i.e., $PhCO_3^{\cdot}$) and quite different from the peracid epoxidation. Similar results were obtained by other α-keto acids or the corresponding esters. These facts suggest that the photoepoxidation proceeds via radical epoxidation by acylperoxy radical, affording trans epoxide predominantly. Contrary to previous reports, the photooxidation of α-keto acids via an $^1O_2$ reaction was not substantiated. The photodecarboxylation of **1a** to afford benzaldehyde was selectively catalyzed by water, and its undissociated form was about tenfold more reactive than the corresponding carboxylate ion.

Oxygen atom transfer of oxenoid intermediates are of current interest as models of monooxygenase enzymes;[2] among them are carbonyl oxides,[3] pyridine *N*-oxide,[4] and unstable cyclic peroxides.[5] In the study of the photoepoxidation of olefins with α-diketones and oxygen,[6] acylperoxy radicals have been shown to be effective epoxidizing agents,[7] based on the fact that α-diketones are not catalysts but reactants consumed and converted to peracids, etc. The same is true for the photoepoxidation with benzoins.[8] This led to our interest on the photooxidation of α-keto acids.

In relation to a model reaction of dioxygenases containing α-ketoglutarate (eq 1, S = substrate),[9] the dye-sensitized pho-

$$S + O_2 + RCOCO_2H \xrightarrow{\text{enzyme}} SO + RCO_2H + CO_2 \quad (1)$$

tooxidation of an α-keto acid (**1**) (eq 2) was recently shown to

$$RCOCO_2H \xrightarrow{O_2/h\nu/\text{dye}} RCO_2H + CO_2 \quad (2)$$

go by way of singlet oxygen.[10,11] An intermediate "trioxalone"

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