Autoxidation of Polyunsaturated Fatty Acids, an Expanded Mechanistic Study

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Abstract: The autoxidation of four isomeric methyl 9,12-octadecadienoates in benzene/1,4-cyclohexadiene has been investigated. Autoxidation of the isomers 9-*Z*,12-*Z* (methyl linoleate); 9-*Z*,12-*E*; 9-*E*,12-*Z*; and 9-*E*,12-*E* (methyl linoelaidate) octadecadienoates was studied. With no cyclohexadiene or with low cyclohexadiene solvent composition, the distribution of product hydroperoxides from the various isomeric precursors was equivalent or nearly so. With higher cyclohexadiene solvent composition, the product mixtures became nonequivalent and reflected the stereochemistry of the particular diene precursor. A steady-state and iterative computer kinetic analysis is reported, and a scheme for diene fatty acid autoxidation involving reversible oxygen addition to intermediate pentadienyl carbon radicals is proposed.

Toxicity by oxygen radicals has been suggested to be involved in a variety of biological events such as aging, heart disease, and cancer.[1,2] Lipid peroxidation has been proposed to play an integral role in these processes, and for this reason, peroxidation has recently attracted considerable interest.[3] By careful examination of hydroperoxide products formed in the autoxidation of polyunsaturated fatty acids, a better understanding of the mechanism of lipid peroxidation has been obtained.

The four major products formed in linoleic acid (**1**) autoxidation are conjugated diene hydroperoxides.[4] Two products have *trans,cis*-diene stereochemistry, **2** and **3**, and two have trans,trans stereochemistry, **4** and **5**. Together these products account for over 97% of the oxygen consumed in the process.

In the previously proposed kinetic scheme,[5] hydrogen atom abstraction occurs at the bis-allylic carbon of the diene system to generate the initial pentadienyl radical **6** (Scheme I). Presuming that oxygen addition to either end of the delocalized system is fast, carbon radical **6** is rapidly converted to one of two conjugated diene peroxy radicals, **7** and **8**, which are immediate precursors to hydroperoxide products **2** and **3**, respectively. We suggested that products **4** and **5** result from loss of oxygen from radicals **7** and **8** to give isomerized carbon radicals (Scheme I). The isomerized radicals ultimately lead to the trans,trans products **4** and **5**.

In order to further investigate the details of the mechanism, we have examined the autoxidation of methyl linoleate and its three geometric isomers, 9-*Z*,12-*E* (**11**), 9-*E*,12-*Z* (**12**), and 9-*E*,12-*E* (**13**) methyl octadecadienoates. We report here results obtained from oxidation studies on these isomers that lead to a new understanding of diene fatty acid autoxidation.

Results

The synthesis of dienoates **11** and **12** is outlined in Scheme II. Thionocarbonates **15** and **16** were prepared in three steps from methyl linoleate **14** and separated by column chromatography. **15** and **16** were readily converted to the respective dienes in good yield.

Dienoates **11–14** were oxidized in chlorobenzene or cooxidized with 1,4-cyclohexadiene in benzene at 30 °C. Autoxidation was carried out in air and initiated with di-*tert*-butyl peroxyoxalate.[6] The hydroperoxy diene products were reduced to the corresponding alcohols with triphenylphosphine and analyzed on high-pressure liquid chromatography. Extent of oxidation did not exceed 5% in any of the cases reported here. It has been shown previously that product distribution is invariant at this early stage of oxidation.

Scheme I

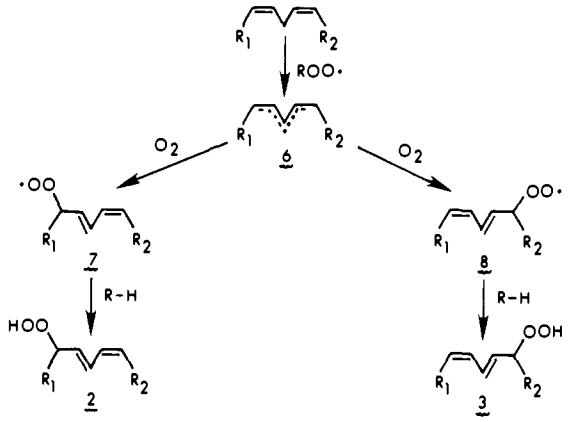

Table I. Product Distribution of Autoxidation of 0.24 M 11, 12 13, or 14 in Chlorobenzene at 30 °C[a]

substrate	13-t,c	13-t,t	9-t,c	9-t,t
11	0.10	0.41	0.05	0.44
12	0.06	0.44	0.09	0.41
13	0.06	0.45	0.05	0.44
14	0.11	0.40	0.10	0.39

[a] Standard error of analysis is ≃3%. At least duplicate runs were made for each diene with HPLC analyses of each run made in duplicate.

Table II. Product Distribution for Autoxidation of 0.24 M 11, 12, 13, or 14 in 1,4-Cyclohexadiene/Benzene at 30 °C[a]

CHD, M	substrate	13-t,c	13-t,t	9-t,c	9-t,t
0.50	11	0.35	0.23	0.05	0.37
	12	0.06	0.38	0.34	0.22
	13	0.04	0.47	0.04	0.45
	14	0.27	0.24	0.26	0.23
0.75	11	0.41	0.18	0.06	0.35
	12	0.05	0.38	0.39	0.18
	13	0.04	0.47	0.05	0.44
	14	0.32	0.19	0.30	0.19
1.50	11	0.50	0.12	0.04	0.34
	12	0.05	0.34	0.49	0.12
	13	0.05	0.46	0.05	0.44
	14	0.40	0.11	0.38	0.11
3.0	11	0.55	0.09	0.03	0.33
	12	0.06	0.32	0.54	0.08
	13	0.02	0.49	0.02	0.47
	14	0.43	0.08	0.41	0.08

[a] See footnote of Table I.

(1) "Free Radicals, Lipid Peroxidation, and Cancer"; McBrien, D., Slater, T., Eds; Academic Press: London, 1982.
(2) "Advances in PG Research"; Samuelsson, B., Ramwell, R., Paoletti, R., Eds.; Raven Press: New York, 1980; Vol. 6.
(3) "Free Radicals in Biology"; Pryor, W., Ed.; Academic Press: New York, 1980; Vol. IV.
(4) Chan, H. W-S.; Levett, G. *Lipids* **1977**, *12*, 99.
(5) Porter, N. A.; Lehman, L. S.; Weber, B. A.; Smith, K. J. *J. Am. Chem. Soc.* **1981**, *103*, 6447.
(6) Bartlett, P. D.; Benzing, E. P.; Pincock, R. E. *J. Am. Chem. Soc.* **1960**, *82*, 1762.

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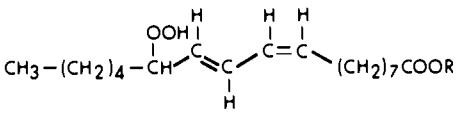
Chart I

1



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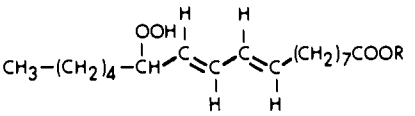

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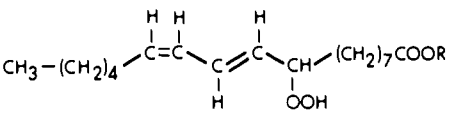

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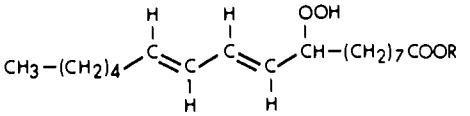

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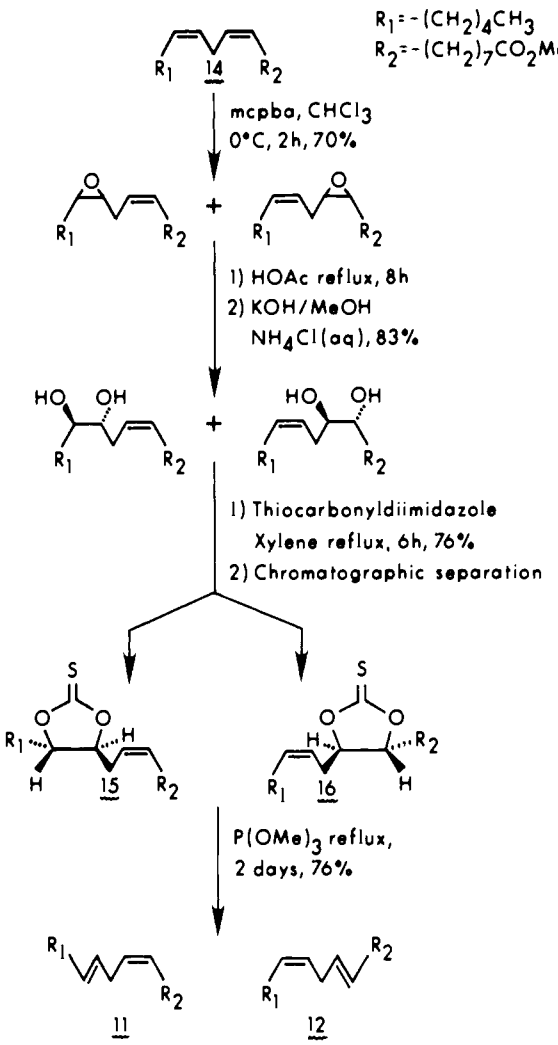
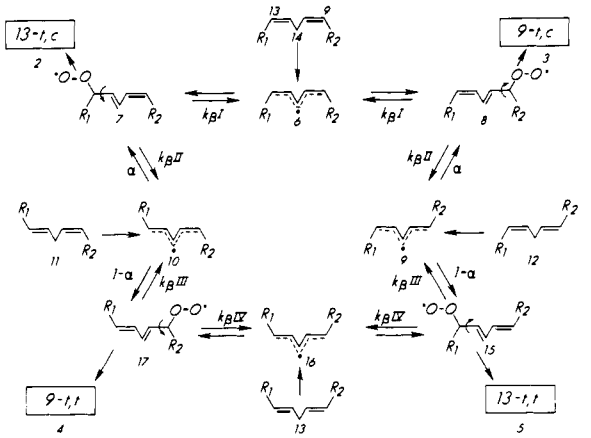
Scheme II



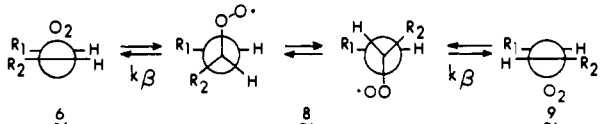
Scheme III



Product distributions for 0.24 M **11**, **12**, **13**, and **14** in chlorobenzene at 30 °C are presented in Table I. Results were shown to be independent of the solvent used with benzene and chlorobenzene giving virtually the same product distributions. In the absence of cyclohexadiene, the relative amounts of diene hydroperoxides from the various dienoates are similar, and trans,trans isomers dominate the product mixture. At progressively higher concentrations of cyclohexadiene, however, the product distribution becomes different and distinctive for each diene (Table II). In solvents of higher cyclohexadiene composition (1.5–3 M), we observe detectable amounts of conjugated diene products other than **2–4**. None of these products ever exceed 5 mol percent of the product mixture, and at low cyclohexadiene concentration they disappear from the product mixture. We believe these products to be geometric isomers of **2–4** analogous to those identified by Frankel et al., in a simpler diene autoxidation system.[7] These products probably result from generation of initial pentadienyl radicals isomeric to the radicals **6**, **9**, **10**, or **16** (Scheme III).

Discussion

Previous studies have suggested that the distribution of products in fatty acid or ester autoxidation may be understood by a reversible oxygen addition to intermediate pentadienyl radicals. This reversible addition results in isomerization of the pentadienyl radical. Consider, for example, the addition of oxygen to radical **6**. A Newman projection of **6** showing the alkyl substituents R_1 and R_2 is useful in describing this isomerization. Oxygen addition



6 8 9

to **6** occurs via an approach perpendicular to the plane of the π radical to give peroxy radical **8**, which is now free to undergo conformational equilibration. Loss of oxygen from the side of the π system opposite to that from which it entered leads to a new

(7) Frankel, E. N.; Garwood, R. F.; Vinson, J. R.; Weedon, B. C. L. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2707.

isomerized carbon radical. Similar arguments apply for isomerization of **6** to **10**.

A mechanistic scheme used for discussion of the product distribution is presented in Scheme III ($R_1 = (CH_2)_4CH_3$, $R_2 = (CH_2)_7COOCH_3$). This scheme, which is similar to the one presented earlier,[8] accounts for formation of the four major products from linoleate autoxidation. In the previous analysis, data was only available for autoxidation of linoleate **14** in benzene solution, and due to this limitation, assumptions were made for data analysis. In particular, it was necessary to assume either (a) $\alpha = 1 - \alpha$ or (b) $k_\beta^{II} = k_\beta^{III}$. Given one or the other of these assumptions, a steady-state kinetic expression could be derived that relates the product ratio **(2 + 3)/(4 + 5)** to k_p, α, and the k_β's. Assuming a common k_β (i.e., $k_\beta^{II} = k_\beta^{III}$), eq 1 could be

$$\frac{2+3}{4+5} = \frac{k_p[R\text{-}H]}{k_\beta(1-\alpha)} + \frac{\alpha}{1-\alpha} \qquad (1)$$

derived where k_p is the rate of propagation[9] of linoleate autoxidation ($k_p = 62\ M^{-1}\ s^{-1}$). By analysis of **(2 + 3)/(4 + 5)** vs. R–H, $\alpha = 0.14$ and $k_\beta = 144\ s^{-1}$ could be determined.[10] It should be reemphasized that these values are obtained with the assumption of a common k_β.

Determination of α. Autoxidation of the dienes **11–14** in the presence of 1,4-cyclohexadiene allows a more detailed kinetic analysis. Cyclohexadiene is an excellent H atom donor, and at high cyclohexadiene concentrations, kinetic product distributions are obtained. For example, autoxidation of **11–14** in 3.0 M cyclohexadiene (Table II) gives a product distribution that reflects the stereochemistry of each diene precursor. The diene **11** is converted to radicals **7** and **17** according to α and $1 - \alpha$. At high cyclohexadiene concentrations, these first-formed peroxy radicals will be trapped by H atom transfer from cyclohexadiene before competing unimolecular processes such as k_β^{I} and k_β^{II} can take place. Thus with 3.0 M cyclohexadiene, **11** provides product mole fractions of 13-t,c = 0.55 and 9-t,t = 0.33. These two products derive from the first-formed peroxy radicals **7** and **17**, and together they amount to nearly 90% of the product mixture. In a similar way, at higher cyclohexadiene concentrations **12** provides predominately **3** and **5**, **13** provides **4** and **5**, and **14** gives **2** and **3** as the major products. Each pair of products is expected from a kinetically controlled autoxidation of the corresponding precursor. Extrapolation of the data in Table II to even higher cyclohexadiene concentrations as well as autoxidations carried out in the presence of α-tocopherol, an excellent H-atom donor, suggest a product distribution from **11** at the kinetic limit of 13-t,c/9-t,t = 2.0. A similar analysis of products derived from **12** at the kinetic limit gives 9-t,c/13-t,t = 2.0. The ratio of these kinetic products gives the value α and $1 - \alpha$ directly. That is, at the kinetic limit, the only factor determining the product ratio from **11** is the partitioning of **10** via α and $1 - \alpha$. These experiments immediately expose the error of the assumption of a common k_β in our earlier analysis.[8] Assuming $k_\beta^{II} = k_\beta^{III}$, we calculated $\alpha = 0.14$ while analysis of the experiments with **11** and **12** give an $\alpha = 0.67$.

Methyl Linoleate Product Analysis. With the new value of α, a reanalysis of the linoleate autoxidation data is possible. A steady-state expression can be derived (eq 2) of products obtained

$$\frac{2+3}{4+5} = \frac{k_p[R\text{-}H]}{k_\beta^{II}(1-\alpha)} + \frac{\alpha k_\beta^{III}}{(1-\alpha)k_\beta^{II}} \qquad (2)$$

from autoxidation of **14**, the symmetric *Z,Z* precursor. It should be noted that the product ratio derived from **14** (or **13**) autoxidation is independent of k_β^{I} or k_β^{IV} while product distributions from **11** or **12** are dependent on these rate constants. Inserting

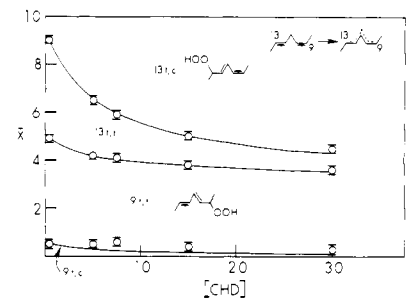


Figure 1. Mole fraction of products (**2–4**) obtained from autoxidation of **11** in benzene/cyclohexadiene vs. concentration of cyclohexadiene. Solid lines represent calculated values of products assuming values as follows: $k_\beta^{I} = 27\ s^{-1}$, $k_\beta^{II} = 430\ s^{-1}$, $k_\beta^{III} = 27\ s^{-1}$, $k_\beta^{IV} = 430\ s^{-1}$, $\alpha = 0.67$, $k_p^{CHD} = 270\ M^{-1}\ s^{-1}$.

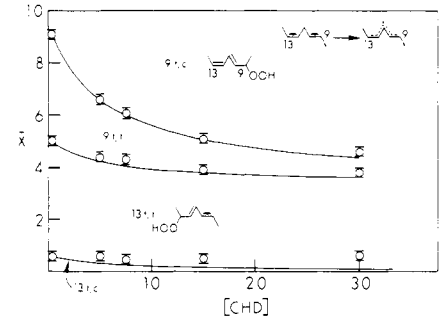


Figure 2. Mole fraction of products (**2–4**) obtained from autoxidation of **12** in benzene/cyclohexadiene vs. concentration of cyclohexadiene. Solid lines represent calculated values of products assuming values as in Figure 1.

$\alpha = 0.67$ in eq 2 allows determination of k_β^{II} and k_β^{III} from the previous product studies of linoleate.[7,11] The values obtained are $k_\beta^{II} = 430\ s^{-1}$ and $k_\beta^{III} = 27\ s^{-1}$. Equation 2 can be used to describe the ratio of products from methyl linoleate, **(2 + 3)/(4 + 5)**, vs. the $k_p[R\text{-}H]$ of any hydrogen atom donor. Thus, for cyclohexadiene donor, insertion of the known values of k_β^{II}, k_β^{III}, and α in eq 2 gives eq 3. The data obtained for methyl linoleate and

$$(2+3)/(4+5) = (k_p^{CHD}[CHD]/142) + 0.131 \qquad (3)$$

cyclohexadiene autoxidation (Table II), when fit to eq 3, give $k_p^{CHD} = 270\ M^{-1}\ s^{-1}$.

Iterative Kinetic Analysis of Products from 11–14. We have developed a straightforward iterative analysis that models the mechanism outlined in Scheme III. The approach allows product distribution to be calculated for each of the dienoate precursors **11–14** as a function of cyclohexadiene concentration and for various values of k_β^{I} and k_β^{IV}. For example, for **11** autoxidation, the program starts with 100 units of the corresponding carbon radical **10** and partitions it by α and $1 - \alpha$ to **7** and **17**. The peroxy radical **7** (100α units) is subsequently partitioned to 13-t,c (**2**), **6**, and **10** according to k_p^{CHD}, k_β^{I}, and k_β^{II}. Each radical in the cycle, as generated, is partitioned to neighboring radicals or products, and the cycle is ended when the sum of products is greater than 99.9 units. The assumptions made in the calculations are as follows: (1) **10** and **9** convert to the neighboring peroxy radicals by factors of α and $1 - \alpha$ ($\alpha = 0.67$); (b) **7** → **10** and **8** → **9** with $k_\beta^{II} = 430\ s^{-1}$; (c) **6** partitions equally to **7** and **8** while **16** partitions equally to **17** and **15**; (d) **17** → **10** and **15** → **9** with $k_\beta^{III} = 27\ s^{-1}$; (e) peroxy radicals **7**, **8**, **15**, and **17** convert to products with rate = $k_p^{CHD}[CHD] = (270\ M^{-1}\ s^{-1})[CHD]$; (f) k_β^{I} and k_β^{IV} are trial parameters. The best-fit calculated product ratios are shown in Figures 1–4. As seen in the figures the agreement between experiment and calculation is excellent for

(8) Porter, N. A.; Weber, B. A.; Weenen, H.; Khan, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 5597.
(9) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1967**, *45*, 793.
(10) The initial data was autoxidation of linoleic acid in benzene at 30 °C (five different concentrations).[8]
(11) We have extended the initial experiments and have carried out over 25 autoxidation experiments with methyl linoleate or linoleic acid in benzene or chlorobenzene. The results may all be fit on one line where (2 + 3)/(4 + 5) = 0.436[L–H] + 0.131.

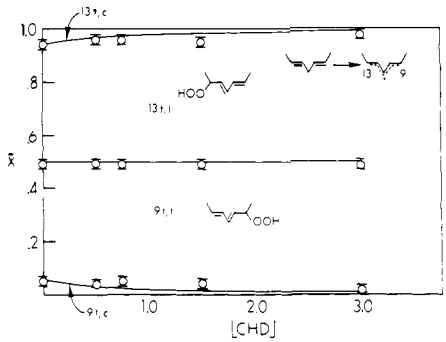


Figure 3. Mole fraction of products (**2**–**4**) obtained from autoxidation of **13** in benzene/cyclohexadiene vs. concentration of cyclohexadiene. Solid lines represent calculated values of products assuming values as in Figure 1.

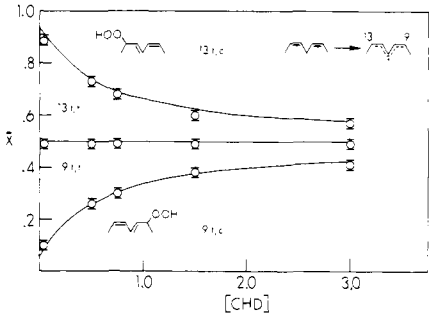
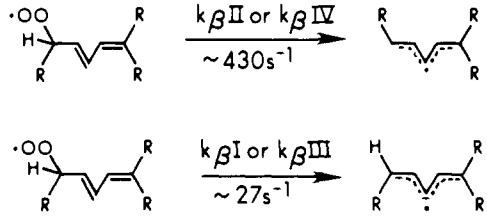


Figure 4. Mole fraction of products (**2**–**4**) obtained from autoxidation of **14** in benzene/cyclohexadiene vs. concentration of cyclohexadiene. Solid lines represent calculated values of products assuming values as in Figure 1.

each of the dienes **11**–**14** if the chosen variables are $k_\beta^{I} = 27\ s^{-1}$ and $k_\beta^{IV} = 430\ s^{-1}$. The fit of experimental data and calculation



is noticeably worse if values outside ±10% of the chosen best-fit rate constants are selected.[12]

The results of the kinetic analysis deserve some comment. In particular, we are unaware of previous studies that would have anticipated the values of α and the various k_β's determined in this analysis. The results can be generalized, however, by assuming that processes leading to and proceeding from the transoid end of a pentadienyl radical are favored relative to processes occurring at the cisoid end of the radical. As a consequence of this, β fragmentations of peroxy radicals leading to transoid centers occur with faster rates than fragmentations of peroxy radicals leading to cisoid centers.

Thus, in pathways occurring with rates of k_β^{II} and k_β^{IV}, an sp^3 center on the peroxy radical is converted to a transoid sp^2 radical center and the rate of this process is fast, 430 s^{-1}. Slower fragmentation occurs when sp^3 peroxy radical carbons are converted to cisoid sp^2 radical centers, k_β^{I} or $k_\beta^{III} = 27\ s^{-1}$.

Oxygen addition at the transoid end of the pentadienyl radical occurs more readily than at the cisoid end of the radical ($\alpha > 1 - \alpha$). The picture that then emerges is one in which a transoid terminus of a pentadienyl radical is kinetically more labile than a cisoid radical center (both toward formation from the corresponding peroxy radical and with respect to reaction with oxygen).

While the preference for formation of transoid pentadienyl radical centers from the corresponding peroxy radicals (k_β) may be understood by a simple steric argument, the propensity for oxygen to add at the transoid end of the pentadienyl radical is not so readily explained. EPR studies of pentadienyl radicals like **9** and **10** have been reported by Bascetta et al.,[13] and the results are pertinent to this work. It is of interest to note that hyperfine coupling constants for protons β to a transoid pentadienyl terminus are larger than coupling constants for protons β to a cisoid terminus. This suggests a higher spin density at transoid pentadienyl termini and, as a consequence, increased reactivity with oxygen. Our results are thus consistent with the EPR studies.

Experimental Section

High-Pressure Liquid Chromatography. A Whatman 10-μm Partisil column was used for fatty acid purification and analysis of oxidation mixtures. The solvent systems used were hexane–acetone (999:1) and hexane–acetone–isopropyl alcohol (992:4:4), respectively. Relative amounts of the hydroxy fatty acid methyl esters were corrected for their known molar absorptivities.[4]

Fatty Acid Oxidations. Methyl linoleate and methyl linoelaidate (commercial **13**) were obtained from Nu-Chek Prep. Dienoates **11** and **12** were prepared from methyl linoleate as described below. All methyl esters were HPLC purified prior to an experiment to ensure that no material was significantly oxidized.

Benzene, chlorobenzene, and 1,4-cyclohexadiene were distilled before use. The oxidations were carried out in essentially the same manner as previously described.[8] Extent of oxidation was measured via titration of peroxide products with triphenylphosphine and subsequent HPLC analysis. The entire mixture was reduced when a sufficient amount of peroxide products had been generated for convenient analysis. None of the runs exceeded 5% oxidation of the total mixture.

Autoxidations were carried out with and without 0.24 M acetic acid in solution. We had some preliminary indications that the product mixture was different with and without acid but subsequent experimentation did not support this. Identical results are obtained with and without added acid.

Preparation of Dienoates **11** and **12**. Thionocarbonates **15** and **16** were prepared from methyl linoleate by following a modification of a previous synthetic procedure.[14] Regioisomers **15** and **16** were separated on a Waters 1-in. Prep column (hexane–ethyl acetate, 92:8) and characterized using EI and CI mass spectrometry. ^{13}C, ^{1}H, and IR spectra were also obtained. Comparison of normal-phase HPLC retention times for **15** and **16** with authentic 9,10-thionocarbonate was consistent with structural assignments.[15] The thionocarbonates were subsequently converted to the respective dienoate isomers as outlined in the text.

Acknowledgment. We thank Timothy A. Porter for writing the iterative computer program used in our analysis. Financial support from NIH and NSF are also gratefully acknowledged.

Registry No. Methyl (9*Z*,12*Z*)-octadeca-9,12-dienoate, 112-63-0; methyl (9*Z*,12*E*)-octadeca-9,12-dienoate, 20221-26-5; methyl (9*E*,12*Z*)-octadeca-9,12-dienoate, 20221-27-6; methyl (9*E*,12*E*)-octadeca-9,12-dienoate, 2566-97-4.

(12) The program has also been used to calculate k_β^{II} and k_β^{III} in autoxidation of **14**. The calculated values are close to the steady-state derived values from eq 2. We also confirm with the program that product distribution from autoxidation of **13** or **14** is independent of k_β^{I} and k_β^{IV}.

(13) Bascetta, E.; Gunstone, F. D.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 603.

(14) Gunstone, F. D.; Jacobsberg, F. R. *Chem. Phys. Lipids* **1972**, *9*, 112.

(15) We thank Dr. E. D. Mihelich for the generous gift of methyl *cis*-9,10-epoxyoctadec-*cis*-12-enoate, which was ultimately converted to the corresponding thionocarbonate. Thionocarbonate **16** elutes last on normal-phase HPLC (hexane–ethyl acetate, 92:8).