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Substituent Effects on Regioselectivity in the Autoxidation of Nonconjugated Dienes

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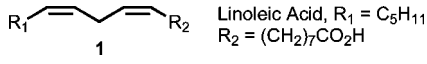
Abstract: Free radical oxidation of several 1,4-dienes was carried out in the presence of variable concentrations of α-tocopherol to investigate the effect of diene structure on product distribution. Oxidations carried out at low tocopherol concentration gave only C-1 and C-5 conjugated diene hydroperoxides, while higher concentrations of the antioxidant resulted in formation of substantial amounts of the nonconjugated C-3 diene hydroperoxide. Increasing size of the substituents at C-1 and C-5 of the diene favors kinetic products arising from oxygen addition at the nonconjugated position, C-3, of the pentadienyl radical intermediate. Substituents at C-1 or C-5 of the pentadienyl radical also have a significant effect on the regioselectivity of the conjugated diene hydroperoxides formed, larger substituents directing oxygen addition to the pentadienyl radical at the site of least steric hindrance. This trend is also observed in oxidations of ω-3 and ω-6 linolenate fatty acid esters. Groups at C-1 and C-5 of the diene can influence product distribution based upon (a) steric demand in the oxygen-radical reaction and (b) the influence of substituents on the rearrangement of the C-3 peroxyl radical to give conjugated diene products.

Introduction

Oxidative stress, the generation of reactive oxygen species *in vivo*, leads to the free radical-mediated reaction of polyunsaturated fatty acids (PUFA) with oxygen, a process that gives rise to a host of peroxidic products.[1] Oxidative stress has been associated with a number of disease states such as atherosclerotic cardiovascular disease,[2] neurodegenerative disorders,[3] and cancer.[4] Due to the importance of these and other pathologies that are linked to oxidative stress, this process along with the resultant lipid-oxygen free radical chemistry has been the subject of extensive investigation.

The reactive substructure of PUFA is the homoconjugated *cis*,*cis*-1,4-diene unit common to all diene or polyene fatty acids and esters. Linoleic acid, for example, is an 18-carbon diene lipid that undergoes free radical oxidation readily and gives conjugated diene hydroperoxide products. We have previously



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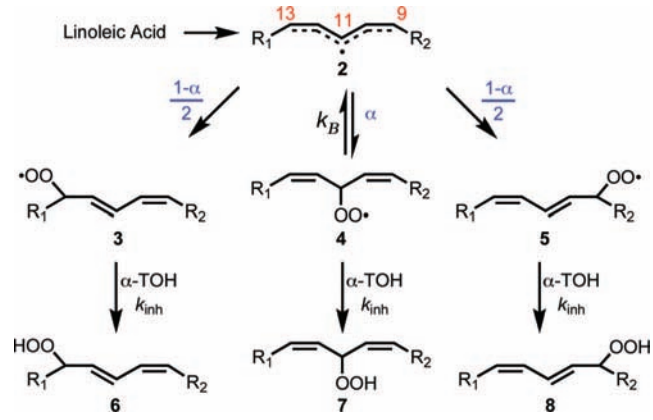
explored the oxidative free radical chemistry of linoleic acid and its geometric isomers and have noted that the distribution of products can be affected by co-oxidation of the lipid in the presence of phenolic antioxidants.[5] When peroxidation was carried out in the presence high concentrations of the good phenolic antioxidant α-tocopherol (Scheme 1), the major products formed were the nonconjugated hydroperoxide (**7**) and the conjugated diene hydroperoxides having *trans*,*cis* double bond geometry (**6** and **8**). The yield of nonconjugated product **7** depended directly on α-tocopherol, this product being negligible at α-tocopherol ∼0.05 M but comprising as much as 40% of the mixture at [α-tocopherol] = 1.8 M.

Scheme 1. General Mechanism of Kinetically Controlled Linoleate Oxidation

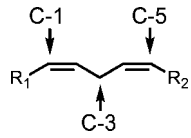


Product and kinetic studies of oxidations carried out with the linoleic acid stereoisomeric *trans*,*cis*- and *trans*,*trans*-diene fatty acids (i.e., *trans*-fatty acids) were also undertaken since there is evidence that *trans*-fatty acids may have a deleterious effect

(1) (a) Porter, N. A. *Acc. Chem. Res.* **1986**, *19*, 262–268. (b) Porter, N. A.; Caldwell, S. E.; Mills, K. A. *Lipids* **1995**, *30*, 277–290.
(2) (a) Steinburg, D.; Parhsarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. *N. Engl. J. Med* **1989**, *320*, 915–924. (b) Chisholm, G. M.; Steinburg, D. *Free Radical Biol. Med.* **2000**, *28*, 1815–1826.
(3) Montine, K. S.; Quinn, J. F.; Zhang, J.; Fessel, J. P.; Roberts, L. J.; Morrow, J. D.; Montine, T. J. *Chem. Phys. Lipids* **2004**, *128*, 117–124, 2004.
(4) (a) Marnett, L. J. *Carcinogenesis* **2000**, *21*, 361–370. (b) Sevanian, A.; Ursini, F. *Free Radical Biol. Med.* **2000**, *29*, 306–311. (c) Spiteller, G.; Spiteller, D.; Jira, W.; Kiessling, U.; Dudda, A.; Weisser, M.; Hecht, S.; Schwarz, C. *Peroxide Chem.* **2000**, 179–208.
(5) Tallman, K. A.; Roschek, B., Jr.; Porter, N. A. *J. Am. Chem. Soc.* **2004**, *126*, 9240.

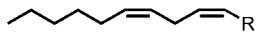
on human health.[6] These studies, along with calculations providing relative energetics and spin distributions for stereoisomeric radical intermediates,[7] led us to the conclusion that the nonconjugated diene peroxyl radical **4** undergoes rearrangement to the conjugated diene peroxyls **3** and **5** with a rate that competes with H-atom abstraction from tocopherol. The nonconjugated peroxyl is trapped at high [α-tocopherol], but rearrangement to the thermodynamically more stable conjugated peroxyls occurs at lower concentrations of the antioxidant. The mechanism of the rearrangement of allyl peroxyl radicals like **4** has been in question for some time. Both an associative mechanism involving direct transfer of oxygen across the allyl fragment and a dissociative mechanism that returns **4** to **2** by β-fragmentation of the peroxyl radical as shown in Scheme 1 have been proposed.

We report here a study of the autoxidation of several model nonconjugated dienes that was undertaken to provide more information about the factors that control product distribution in diene autoxidation. We find that the distribution of hydroperoxides formed from these model dienes depends dramatically on the size of substituents at C-1 and C-5 of the diene and we rationalize the distribution of these products based on simple steric effects of the substituents on the transition state for reaction of intermediate pentadienyl radicals with oxygen.

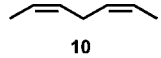


Results

A series of *cis*,*cis*-nonconjugated 1,4-dienes (**9a**−**f**) were synthesized with each having a pentyl side chain at the C-1 terminus. The substituents at C-5 were alkyl groups of different length and steric size (R = Me, Et, Pr, pentyl, *i*-Pr, *t*-Bu). It was previously shown that *cis,cis*-6,9-pentadecadiene (**9d**) behaves identically to methyl linoleate in a study of free radical oxidation and it therefore serves as a good model for the lipid.[5] We also chose to study *cis,cis*-2,5-heptadiene (**10**) since this compound is commonly used as a model for methyl linoleate in both experiment and computation.[7] In addition, methyl α-linolenate (**11**) and methyl γ-linolenate (**12**) were studied as representative ω-3 and ω-6 fatty acids.



9a R = Me **d** R = pentyl
b R = Et **e** R = *i*Pr
c R = Pr **f** R = *t*Bu



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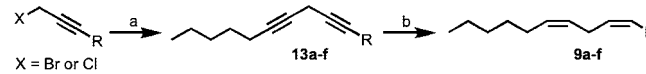
Synthesis of Nonconjugated Dienes. Methyl α-linolenate (**11**) and methyl γ-linolenate (**12**) are commercially available, whereas 2,5-heptadiene (**10**) was synthesized as previously described.[8] The model dienes **9a**−**f** were synthesized in a straightforward manner following a common strategy (Scheme 2). The diynes (**13a**−**f**) were synthesized by a copper-promoted coupling of a 1-alkyne with the requisite propargyl halide.[9,10] Lindlar hydrogenation using the more reactive $Pd/BaSO_4$ catalyst produced the desired nonconjugated dienes having the *cis*,*cis* configuration. In the case of the *t*-Bu-substituted diene (**9f**), $Pd/BaSO_4$ was only effective in the reduction of one of the double bonds, presumably due to steric effects. In that case, the more reactive Ni P-2 was used as the hydrogenation catalyst.[10] This approach produced the requisite nonconjugated dienes in good to moderate yields and with a purity required for these studies.

Scheme 2. Synthetic Approach to Nonconjugated Dienes[a]



[a] Reagents: a) 1-alkyne, CuI, K_2CO_3, NaI; b) $Pd/BaSO_4$, quinoline, H_2, EtOAc or Ni P-2.

Oxidation of Model Dienes. Oxidations of the model dienes (0.2 M) were carried out in benzene in the presence of varying concentrations of α-tocopherol (0.05−1.8 M). The reactions were initiated by 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile)(MeOAMVN) at 37 °C for 4 h. The relatively short reaction times allowed for low conversion of diene and consumption of α-tocopherol, ensuring pseudo-first-order conditions. The diene hydroperoxides from **9a**−**f** were reduced to the corresponding alcohols with PPh_3 and subsequently analyzed by HPLC or GC. The HPLC-UV profile for the oxidation products was as described in previous reports.[5,11] Under these oxidation conditions, only the nonconjugated and *trans*,*cis*-conjugated diene products were observed as significant oxidation products, as shown in Scheme 3. Larger-scale oxidations of **9** were carried out in reactions initiated with MeOAMVN in the presence of *N*-methylbenzhydroxamic acid (NMBHA), conditions under which only the conjugated hydroperoxides are formed in high yields.[12] For purposes of discussion, we define the products as **15p** and **16R**, indicating that the alcohol is proximal to the pentyl group for **15** and proximal to the R group for **16**.

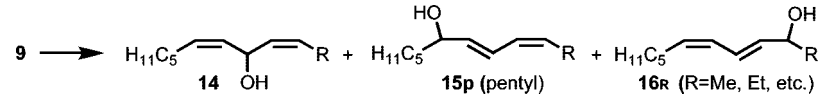
The dependence of product ratio on the concentration of α-tocopherol is consistent with previous reports (Figure 1).[5,11b,c] The data clearly show that for all compounds studied, the amount of nonconjugated alcohol (**14**) increases at the expense of the conjugated products (**15** + **16**) until a saturation limit is reached at α-tocopherol concentrations greater than approximately 1 M.

From Figure 1, it can be seen that the diene substitution significantly influences the partitioning of O_2 across the pentadienyl radical. The amount of nonconjugated product (**14**) arising from the oxidation of 2,5-heptadiene (**10**) is the lowest for all of the compounds studied, while dienes having bulky R-groups (*t*-Bu, *i*-Pr) generally give rise to more of this product, the notable exception to this trend being R = *i*-Pr vs R = *t*-Bu. The partitioning of O_2 to the center position of the pentadienyl radical is clearly affected by the size of the R substituents on the ends of the radical.

The effect of substituents on the product distribution is presented in an alternative graphical form in Figure 2 and in

(6) (a) Sargis, R. M.; Subbaiah, P. V. *Biochemistry* **2003**, *42*, 11533. (b) Katan, M. B.; Zock, P. L.; Mensink, R. P. *Annu. Rev. Nutr.* **1995**, *15*, 473. (c) Mensink, R. P.; Katan, M. B. *New Engl. J. Med.* **1990**, *323*, 439.
(7) Pratt, D. A.; Mills, J. H.; Porter, N. A. *J. Am. Chem. Soc.* **2003**, *125*, 5801.
(8) Frankel, E. N.; Garwood, R. F.; Vinson, J. R.; Weedon, B. C. L. *J Chem. Soc., Perkin Trans. 1* **1982**, 2707.
(9) Ivanov, I. V.; Groza, N. V.; Romanov, S. G.; Kühn, H.; Myagkova, G. I. *Synthesis* **2000**, 691.
(10) (a) Dasse, O.; Mahadevan, A.; Han, L.; Martin, B. R.; Di Marzo, V.; Razdan, R. K. *Tetrahedron* **2000**, *56*, 9195. (b) Jeffery, T.; Gueugnot, S.; Linstrumelle, G. *Tetrahedron Lett.* **1992**, *33*, 5757.
(11) (a) Brash, A. R. *Lipids* **2000**, *35*, 947. (b) Tallman, K. A.; Pratt, D. A.; Porter, N. A. *J. Am. Chem. Soc.* **2001**, *123*, 11827. (c) Roschek, B., Jr.; Tallman, K. A.; Rector, C. L.; Gillmore, J. G.; Pratt, D. A.; Punta, C.; Porter, N. A. *J. Org. Chem.* **2006**, *71*, 3527.
(12) Punta, C.; Rector, C. L.; Porter, N. A. *Chem. Res. Toxicol.* **2005**, *18*, 349.

Scheme 3. Oxidation Products of Nonconjugated Dienes **9a**−**f**[a]



[a] Product hydroperoxides are reduced to alcohols with triphenylphosphine.

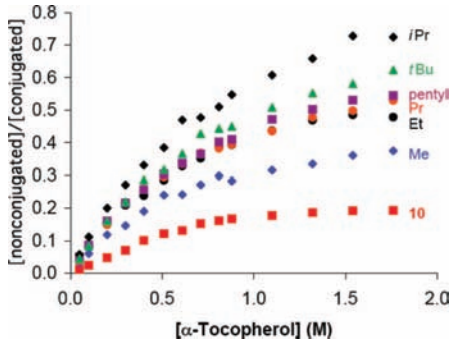


Figure 1. Oxidation profile of dienes **9a**−**f** and **10**, ratio of nonconjugated products vs α-tocopherol. Oxidations were carried out in benzene with diene (0.1 M), MeOAMVN (0.01 M) and α-tocopherol (0.05−1.8 M) at 37 °C for 4 h. The oxidations were stopped by the addition of BHT and PPh_3.

Table 1. Product Distribution for Oxidation of **10** and **9a**−**f** in the Presence of 1.8 M α-Tocopherol[a]

substrate	15p	nonconjugated, 14	16R
10 (heptadiene)	0.38	0.24	0.38
9a (Me)	0.25	0.34	0.41
9b (Et)	0.25	0.41	0.34
9c (Pr)	0.28	0.44	0.28
9d (pentyl)	0.28	0.44	0.28
9e (*i*-Pr)	0.30	0.53	0.17
9f (*t*-Bu)	0.45	0.49	0.06

[a] Oxidation conditions are as described in Figure 2.

tabular form in Table 1. In the case of **9a**, the major product formed at all concentrations of α-tocopherol is the conjugated alcohol substituted proximal to the small methyl group, **16R** (Figure 2A). However, as the steric size of the substituent is increased in the series, the pentyl-proximal conjugated alcohol, **15p**, becomes the major product (Figure 2B). The results of experiments with the Pr and pentyl-substituted dienes (**9c** and **d**) show that with R = Pr the diene is effectively symmetrical in terms of the substituent's effect on product distribution.

The ratio of **15p**:**16R** is relatively insensitive to α-tocopherol concentration, the apparent effect of antioxidant being only to cause an increase in the nonconjugated product relative to both conjugated dienes. Table 2 presents the product distribution for oxidation of the unsymmetrical dienes **9a**−**c**, **9e** and **9f** at

Table 2. Product Ratio of Pentyl-Proximal (**15p**) and R-Proximal (**16R**) Dienes Formed at 37 °C in the Presence of ∼0.05 M α-Tocopherol.[a]

substrate **9**	15p	16R
9a (Me)	0.38	0.62
9b (Et)[b]	0.42	0.58
9c (Pr)	0.5	0.5
9d (pentyl)	0.5	0.5
9e (*i*-Pr)	0.65	0.35
9f (*t*-Bu)	0.88	0.12

[a] Ratios are based on GC analysis of the product mixture. [b] Conjugated products are not separable on GC, so the ratio was based on HPLC analysis.

α-tocopherol ∼0.05 M, conditions under which less than a few percent of the nonconjugated product is formed.

Oxidation of Linolenates. Comparison of the oxidation of the ω-3 lipid methyl α-linolenate (**11**), and its ω-6 isomer methyl γ-linolenate (**12**) offers an opportunity to examine substituent effects in two natural isomeric triene lipids. The Δ_{15-16} double bond of α-linolenate has a terminal ethyl substituent, while the functionally analogous double bond in γ-linolenate at Δ_{12-13} has a pentyl substituent group. A small but detectable product-directing substituent effect is anticipated based upon the studies with the model dienes **9b**−**d**.

The methyl esters of α-linolenate and γ-linolenate were oxidized in the presence of low concentrations of α-tocopherol (0.018 M) or with NMBHA (0.3 M) in acetonitrile at 37 °C. Aliquots were removed at 30-min intervals, and after reduction with PPh_3, the alcohols from the oxidations were analyzed by HPLC. The only products observed under these oxidation conditions were the conjugated alcohols having *trans*,*cis*-conjugated double bond geometry (Scheme 4).[13] Oxidation of polyunsaturated lipids at these concentrations of NMBHA and α-tocopherol eliminates the formation of nonconjugated diene, *trans*,*trans*-conjugated diene as well as complex mixtures of polycyclic peroxides.

The results of these studies are summarized graphically in Scheme 5. For oxidation of methyl α-linolenate, abstraction of H_{14} gives rise to the C-16 and C-12 products, indicated graphically in red in Scheme 5 while the C-9 and C-13 products, formed after abstraction at H_{11}, are indicated in blue. Products

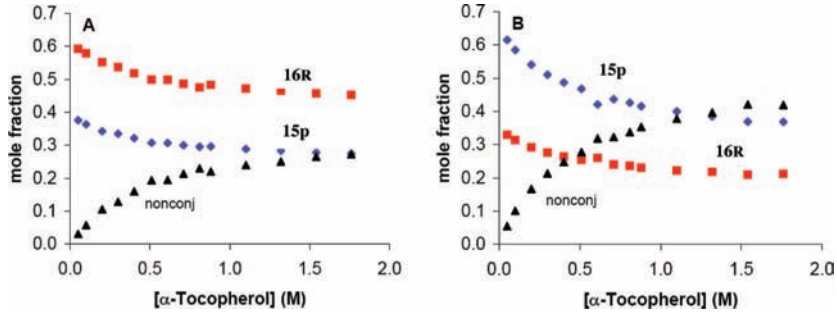
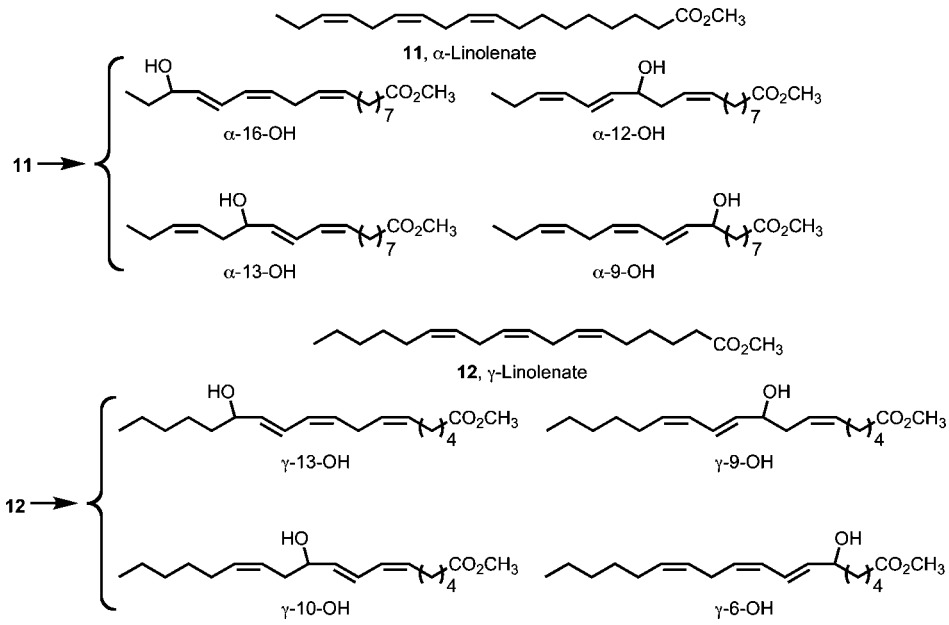
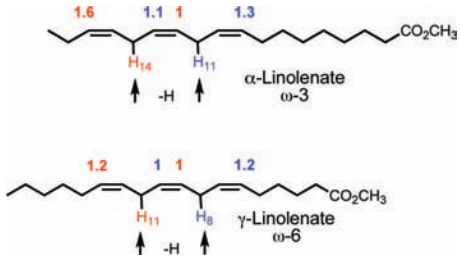


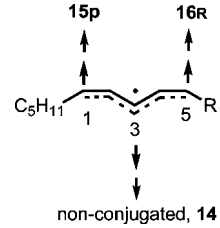
Figure 2. Mole fractions of oxidation products vs [α-tocopherol]. (A) oxidation of **9a** (R = Me); (B) oxidation of **9e** (R = *i*-Pr),▲ nonconjugated alcohol, ♦ **15p**, ■ **16R**. Oxidations were carried out in benzene with **9a** or **9e** (0.1 M), MeOAMVN (0.01 M), and α-tocopherol (0.05−1.8 M) at 37 °C for 4 h. The oxidations were stopped by the addition of BHT and the product mixture reduced with PPh_3.

Scheme 4. Oxidation Products of Methyl Linolenates **11** and **12**



Scheme 5. Relative Product Ratios from Autoxidation of Linolenates





formed in the oxidation of the γ-6 ester, methyl γ-linolenate are shown in the scheme with a similar shorthand.

A symmetrical product pattern is observed in the oxidation of the ω-6 ester, oxygen addition at carbons toward the center of the 18-carbon chain at C-9 and C-10 being somewhat disfavored compared to addition at carbons nearer the ends of fatty ester at C-13 and C-6. On the other hand, the product pattern that results from oxidation of the ω-3 ester is skewed toward the C-16 product. Oxygen addition at the ethyl-substituted end of the intermediate pentadienyl radical formed by abstraction of H_{14} leads preferentially to C-16 instead of C-12.

Discussion

Steric Effects and Diene Oxidation Products. The observations reported for oxidation of dienes **9a**−**f** and **10** are consistent with the proposal that O_2 is partitioned among the three positions of the pentadienyl radical as shown in Scheme 1. At low concentrations of α-tocopherol, H-atom transfer to the nonconjugated peroxyl radical is not competitive with rearrangement of that radical. As the concentration of α-tocopherol is increased, H-atom transfer dominates rearrangement, and a kinetic product limit is reached that reflects the O_2 partition to the three positions of the pentadienyl radical. This is clearly demonstrated in Figure 1 for the oxidation of **10** and **9a**−**f**. For each of these dienes, the amount of nonconjugated product formed is dependent on the concentration of α-tocopherol present during oxidation.

The kinetic product distribution for oxidation of **10** and **9a**−**f** can be understood based upon a simple model in which the steric bulk of substituents at C-1 and C-5 of intermediate pentadienyl radicals suppresses the addition of oxygen at those positions. If one assumes that the substituents at C-1 and C-5 do not affect the rate of addition of oxygen to C-3, then the product ratio of C-3 derived product to products formed by addition of oxygen at C-1 + C-5 should be reflected in the sum of the Taft steric parameters[14] for groups at C-1 and C-5. For the series **9**, R_1 is pentyl and R varies in size from methyl to *tert*-butyl. For this series, addition of oxygen at C-3 gives rise to the nonconjugated product **14**, addition to C-1 gives **15p** and addition to C-5 gives **16R**. Equation 1 describes this relationship in terms of the Taft E_s parameters.

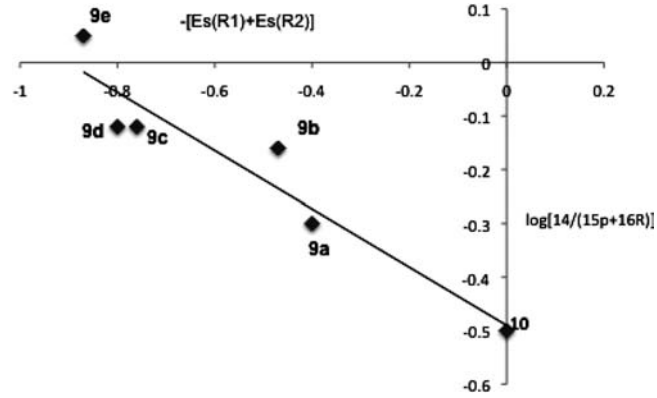


Figure 3. Dependence of oxygen addition at C-3 vs C-1 + C-5 on the basis of steric parameters for substituents at C-1 and C-5 of intermediate pentadienyl radicals. Conditions of oxidation, benzene at 37 °C in the presence of 1.8 M α-tocopherol.

$$\log\left(\frac{[\mathbf{14}]}{[\mathbf{15p}]+[\mathbf{16R}]}\right) = \delta(E_s^{R1} + E_s^{R2}) \quad (1)$$

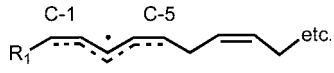
$$\log\left(\frac{[\mathbf{16R}]}{[\mathbf{15p}]}\right) = \delta(E_s^{R} - E_s^{P}) \quad (2)$$

In Figure 3 is presented a plot of the sum of the Taft steric parameters, E_s, for substituents R_1 and R_2 in dienes $R_1CH{=}CH\text{-}CH_2{-}CH{=}CHR_2$ (**10** and **9a**−**e**) vs log[**14**]/([**15p**]+[**16R**]) for data taken from Table 1. There is a modest correlation (R^2 = 0.91) of product ratio vs steric size of substituents, supporting the notion that the amount of nonconjugated product formed is controlled by retardation of oxygen addition at the sterically congested termini of the pentadienyl radical.

One notable exception to this correlation is the diene **9f**, in which R = *t*-Bu. Much less of the nonconjugated product **14** is formed in the oxidation of **9f** than is anticipated by the correlation in Figure 3. We suggest that the sterically encumbered *tert*-butyl substituent group suppresses oxygen addition not only at the C-5 group but also at C-3. The *t*-Bu is *cis*-substituted on the pentadienyl radical and cis-substituted alkenes bearing *t*-Bu groups are known to be highly sterically congested with substantial associated strain energy.[15]

The Taft E_s equation can be used more successfully to evaluate factors influencing the addition of oxygen at the terminal positions of the pentadienyl radical. The analysis in this case leads to eq 2 and the data for **15p** and **16R** products reported from **9a**−**f** in Table 1 are plotted in Figure 4 according to this equation. The least-squares for this plot of $y = mx + b$ gives a best fit for $m = -0.07$ and $b = -0.09$ with $R^2 = 0.98$. A similar analysis of the **16R**: **15p** product ratio obtained in oxidations in the presence of 0.05 M α-tocopherol (Table 2) gives a similarly good correlation with $m = -0.07$ and $b = -0.10$ with $R^2 = 0.97$. The correlation is essentially the same in oxidations carried out in the presence of 1.8 M α-tocopherol, the kinetic limit, and those carried out at 0.05 M α-tocopherol, conditions under which no nonconjugated product is formed.

The linolenate product distributions shown in Scheme 5 can also be understood based upon substituent steric effects. The substituents that control addition of oxygen at C-1 and C-5 of the intermediate radical formed in the oxidation of methyl α-linolenate and the corresponding γ-linolenate are R_1 = ethyl or pentyl and R_2 = $CH_2CH{=}CH\text{-}CH_2$-. Based upon the fact



R_1= pentyl for γ-linolenate
R_1= Et for α-linolenate

that C-1 addition in γ-linolenate (R_1 = pentyl) is favored by a factor 1.2:1 over addition at C-5, the -$CH_2CH{=}CH\text{-}CH_2$- substituent must have a larger E_s than pentyl (E_s = 0.40).[14a] For α-linolenate, where R_1 = ethyl, the C-1:C-5 addition ratio

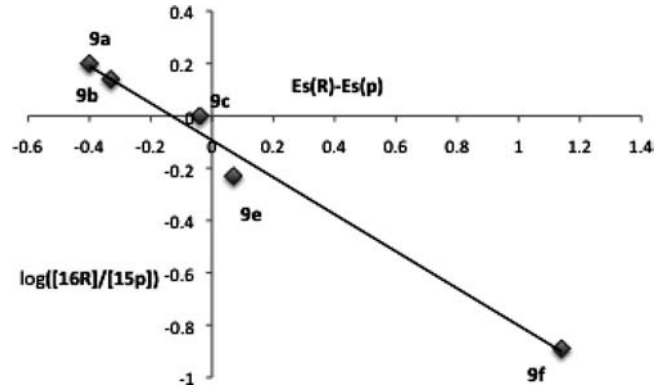
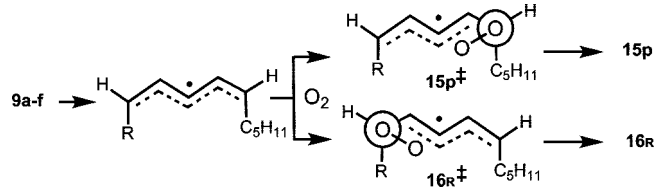


Figure 4. Dependence of oxygen addition at C-5 vs C-1 on the basis of steric parameters for substituents at C-1 and C-5 of intermediate pentadienyl radical. Conditions of oxidation, benzene at 37 °C in the presence of 1.8 M α-tocopherol.

of 1.6:1 reflects the even smaller Taft substituent parameter for ethyl (E_s = 0.07) as compared to that for pentyl.[14a]

Preferred Transition-State Geometry for Pentadienyl Radical-Oxygen Addition. Both the model diene experiments and oxidations of linolenates demonstrate that a steric substituent effect exists that influences the site of oxygen addition to intermediate radicals. The nature of these substituents' influence on the spin distribution in the pentadienyls is not known, but the link between spin and reactivity with oxygen is in and of itself questionable.[16] This is demonstrated in studies reported earlier on the oxidation of a geometric isomer of **9d**, see Figure 5. While the radical derived from the *cis*,*trans*-isomer has more unpaired spin at the *cisoid* end of the radical, oxygen addition at the *transoid* end is favored by almost 2 to 1. These studies with *cis*,*trans*-**9d** also make clear that oxygen addition is dependent on the geometry of substitution on the pentadienyl, a *cis*-alkyl substituent at a radical terminus suppresses addition relative to a *trans* substituent.

A picture that emerges from these studies is a transition state for oxygen addition to C-1 or C-5 of substituted pentadienyl radicals that minimizes steric interactions in transition states for addition. The transition state leading to **15p** has a pentyl O−O *gauche* interaction;the transition state leading to **16R** has a *gauche* interaction with the R group. Larger R groups suppress the formation of the **16R** product because of this steric interaction.



This transition-state picture also provides an understanding for the oxidation of *E*,*Z*-**9d**, a substrate that gives both *cis*,*trans*- and *trans*,*trans*-products. Addition of oxygen via the *anti* transition state leads preferentially to the *cis*,*trans*-product, as is observed experimentally. This transition state minimizes steric interactions between the substituent group, pentyl in this case

(13) The products of γ-linolenate have been previously identified as described in ref 12. α-Linolenate oxidation products have also been described. Chan, H. W.-S.; Levett, G. *Lipids* **1977**, *12*, 837.

(14) (a) MacPhee, J. A.; Panaye, A.; Dubois, J.-E. *Tetrahedron Lett.* **1978**, *19*, 3293. (b) Shorter, J. *Advances in Linear Free Energy Relationships*; Chapman, N. B.; Shorter, J., Eds.; Plenum Press: London, 1972; Chapter 2. (c) Anslyn, E.; Dougherty, D. A. *Modern Physical Organic Chemistry*; University Science Books: Mill Valley, CA, 2006; p 104.

(15) (a) Tidwell, T. *Tetrahedron* **1978**, *34*, 1855. (b) Ermer, O.; Lifson, S. *Tetrahedron* **1974**, *30*, 2425.

(16) (a) Bascetta, E.; Gunstone, F. D.; Scrimgeour, C. M.; Walton, J. C. *J. Chem. Soc., Chem. Commun.* **1982**, 110. (b) Bascetta, E.; Gunstone, F. D.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 603.

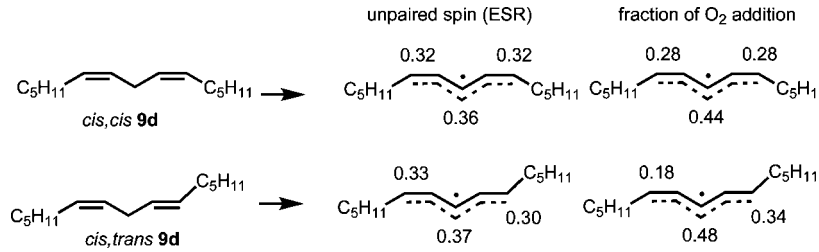
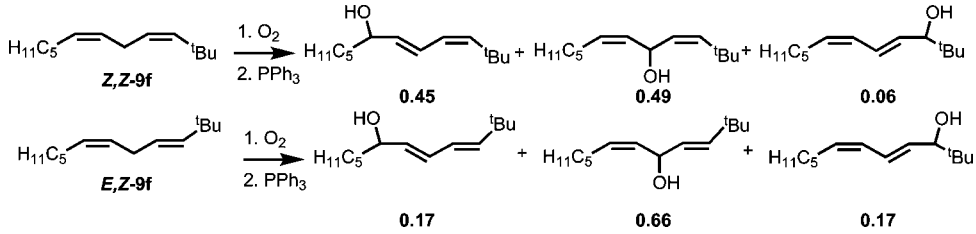
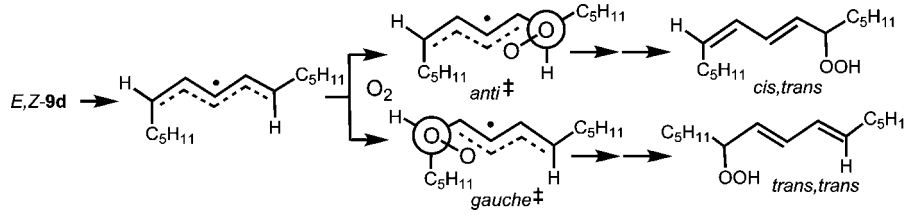


Figure 5. Unpaired spin in isomeric pentadienyl radicals derived from *cis,cis*-**9d** and its *cis,trans*-isomer and mole fraction of products formed upon addition of oxygen to these radicals at C-1, C-3 and C-5. Data taken from ref 5.

Scheme 6. Product Distribution (Mole Fraction) for Oxidation of **9f** and an *E,Z* Geometric Isomer[a]



[a] Conditions of oxidation, benzene at 37°C in the presence of 1.8 M α-tocopherol.



and the approaching oxygen. In contrast, addition to the *cisoid* end of the radical must proceed by a *gauche* transition state.

As a test of this transition-state model, we prepared and studied the oxidation of a *trans* geometric isomer of **9f**, *E*,*Z*-2,2-dimethyl-3,6-dodecadiene. The results of those studies are presented in Scheme 6. While the *Z*,*Z* isomer leads to the compound that results from oxygen addition at the site bearing the *t*-Bu group with a product mole fraction of 0.06, the *E*,*Z* isomer gives nearly 3 times as much of the equivalent product. Note also that the nonconjugated product from the *E*,*Z* isomer is 66% of the product mix compared to a value of 49% for the equivalent nonconjugated *Z*,*Z* product. Both of these observations are consistent with the transition-state model proposed. The *t*-Bu group occupies an *anti* orientation in the transition state for oxygen addition for the *E*,*Z* isomer, minimizing steric interactions compared to the *gauche t*-Bu for the *Z*,*Z* diene. The *E*,*Z* diene gives rise to a pentadienyl radical that also minimizes allylic interactions that arise in the transition state that leads to the nonconjugated product. Thus, both the nonconjugated compound and the product resulting from oxygen addition proximal to the *t*-Bu group are formed in greater amount for the *E*,*Z* isomer than was observed for the *Z*,*Z* compound.

Rearrangement of Nonconjugated Peroxyl Radicals. In oxidations carried out at low concentrations of α-tocopherol, no nonconjugated product (**14** in Scheme 3) is formed since the peroxyl radical leading to this compound undergoes rearrangement to more stable conjugated diene peroxyls. Oxygen labeling and stereochemical studies of rearrangements of simple allyl peroxyl radicals suggest an associative mechanism for this transformation,[17,18] and we have previously proposed an envelope-like transition state to explain the experimental observations.[5] A radical-dioxygen triplet complex has also been speculated to be an intermediate in this chemistry.[19] The mechanism for the rearrangement of the nonconjugated dienyl peroxyl radicals that are intermediates in this study is an open question, but we have generally suggested that the rearrangement involves a peroxyl radical β-fragmentation:oxygen readdition as shown in Scheme 1.[20]

It is notable that the results obtained in the oxidation reactions of the unsymmetrical dienes **9a**−**f** show that the ratio of **15p**:**16R** is independent of α-tocopherol concentration. One concludes from this that the steric factors that influence the addition of oxygen to the pentadienyl radical under kinetically controlled conditions have a similar influence on the course of the rearrangement of the nonconjugated peroxyl radical to **15p** and **16R**. A dissociative rearrangement mechanism in which the nonconjugated peroxyl radical undergoes fragmentation as shown in Scheme 1 is consistent with the results. In this case the steric factors that govern oxygen addition to the pentadienyl

(17) (a) Mills, K. A.; Caldwell, S. E.; Dubay, G. R.; Porter, N. A. *J. Am. Chem. Soc.* **1992**, *114*, 9689. (b) Porter, N. A.; Mills, K. A.; Carter, R. L. *J. Am. Chem. Soc.* **1994**, *116*, 6690. (c) Porter, N. A.; Mills, K. A.; Caldwell, S. E.; Dubay, G. R. *J. Am. Chem. Soc.* **1994**, *116*, 6697. (d) Lowe, J. R.; Porter, N. A. *J. Am. Chem. Soc.* **1997**, *119*, 11534.

(18) Previous studies have ruled out the intermediacy of the cyclic 1,2-dioxolanyl-4-yl radical. (a) Brill, W. F. *J. Chem. Soc., Perkin Trans. 2* **1984**, 621. (b) Porter, N.; Zuraw, P. *J. Chem. Soc., Chem. Commun.* **1985**, 1472. (c) Porter, N. A.; Wujek, J. S. *J. Org. Chem.* **1987**, *52*, 5085. (d) Boyd, S. L.; Boyd, R. J.; Shi, Z.; Barclay, R. C.; Porter, N. A. *J. Am. Chem. Soc.* **1993**, *115*, 687.

(19) Olivella, S.; Solé, A. *J. Am. Chem. Soc.* **2003**, *125*, 10641.

(20) (a) Porter, N. A.; Lehman, L. S.; Weber, B. A.; Smith, K. J. *J. Am. Chem. Soc.* **1981**, *103*, 6447. (b) Porter, N. A.; Wujek, D. G. *J. Am. Chem. Soc.* **1984**, *106*, 2626.

radical under kinetic conditions also control the rearrangement since the **15p** *and* **16R** *selectivity is controlled in the same step for both processes: addition of oxygen to the intermediate pentadienyl radical*.

An alternative proposal that seems less attractive is an associative rearrangement of nonconjugated to conjugated peroxyls through two distinct isomeric envelope-like transition complexes in which the size of the pentyl and R groups is important.[5,19] Indeed, the transition state for this rearrangement would look sufficiently enough like the proposed transition state for oxygen addition to C-1 and C-5 of an intermediate pentadienyl radical to suggest that substituents would affect both processes to a comparable extent, see Figure S2 in Supporting Information.

Conclusion

The autoxidation of a variety of nonconjugated dienes demonstrates that the substitution of the diene plays a significant role in the distribution of products. Formation of the nonconjugated peroxyl radical and its subsequent rearrangement depends on the substitution of the alkene precursor and consequently the pentadienyl radical intermediate. Significant unpaired electron spin density is present at the central carbon of pentadienyls and the bis-allylic hydroperoxide product that arises from addition at this position is a significant kinetic product for each of the systems studied. Steric effects of substituents on intermediate pentadienyl radicals play an important role in controlling the product distribution for the overall conversion. These experiments also help to explain the preference for oxygen addition at the ω-3 site of those lipids relative to addition at the ω-6 site of those fatty acids and esters. The ω-3 carbon bears a smaller ethyl substituent than the pentyl group attached to the ω-6 carbon of fatty acids in that class.

Experimental Section

Methods and Materials. ^{1}H and ^{13}C NMR spectra were collected on a 300 or 400 MHz NMR. Purification by column chromatography was carried out on silica gel, and TLC plates were visualized by UV and stained with phosphomolybdic acid. Polyunsaturated fatty acid methyl esters were purchased from NuChek Prep (Elysian, MN, USA) and chromatographed on silica (10% EtOAc/hexanes) prior to use. All nonconjugated dienes were also chromatographed on silica (hexanes) immediately before use to remove any oxidation products. 2,2′-Azobis(4-methoxy-2,4 dimethylvaleronitrile) (MeOAMVN) was purchased from Wako Chemicals USA, Inc. (Richmond, VA, USA) and dried under high vacuum for 2 h. α-Tocopherol was purified by flash chromatography (10% EtOAc/hexanes) and protected from light. Benzene used in autoxidations was passed through a column of neutral alumina. The syntheses of 6,9-pentadecadiene[5] and 2,5-heptadiene[8] were previously reported. NMBHA was synthesized by literature procedures.[12,21]

Autoxidation of Model Dienes. Stock solutions of the dienes (1.5−1.7 M), MeOAMVN (0.1 M), and α-tocopherol (1.0 M) were prepared in benzene. Samples were prepared in 1.0 mL autosampler vials with a total reaction volume of 100 μL. It is important to add the solutions in the following order to avoid premature oxidation: α-tocopherol (0.05−1.8 M), diene (0.10 M), MeOAMVN (0.01 M) and diluted to 100 μL with benzene. The sealed samples were then incubated at 37 °C for 4 h.

After 4 h, the oxidations were stopped by the addition of BHT (50 μL of a solution in hexanes) and reduced with PPh_3 (50 μL of a solution/hexanes). BHT does not propagate the radical chain, whereas the tocopheryl radical does. The samples were analyzed by normal-phase high performance liquid chromatography (HPLC) or gas chromatography (GC). For HPLC analysis, the samples were diluted to 1.0 mL with hexanes and analyzed using 0.5% *i*-PrOH/hexanes (1 mL/min, detection at 207 nm). The samples were also analyzed by GC (100−180 °C at 5 °C/min, 180−280 °C at 20 °C/min, 10 min). For the data presented in Figures 1 and 2, GC analysis was used, with the exception of diene **9b**. The conjugated products were inseparable on GC, so the product mixture was analyzed by HPLC.

NMBHA Oxidation of Dienes. The nonconjugated products formed upon autoxidation of the dienes were identified based on HPLC-UV analysis and compared to previous results.[5,11] The nonconjugated alcohol absorbs light at 210 nm, whereas the conjugated products absorb at 234 nm. In order to identify the regioisomeric conjugated alcohols, large-scale oxidations with NMBHA (0.3 M) were carried out with dienes (0.2 M) and MeOAMVN (0.02 M) in CH_3CN at 37 °C for 18 h. These oxidations were stopped by the addition of BHT,[22] and the resulting hydroperoxides were reduced to their corresponding alcohols by addition of excess PPh_3. The products were separated by NP HPLC as described above, and they were analyzed by NMR. These samples were subsequently used as authentic standards for GC analysis.

Autoxidation of Linolenates. The oxidations of methyl α-linolenate and methyl γ-linolenate were performed with 0.2 M linolenate, 0.3 M NMBHA or 0.018 M α-tocopherol, and 0.02 M MeOAMVN. The reactions were carried out in CH_3CN at 37 °C, removing aliquots every 30 min. After stopping the reactions with BHT, the solvent was removed, and the resulting hydroperoxides were reduced to their corresponding alcohols by addition of excess PPh_3. NP-HPLC was performed using a Beckman Ultrasphere 5-μm silica column (4.6 mm × 25 cm) with 0.5% *i*-PrOH/hexane at 1 mL/min monitoring at 234 nm. The oxidation products for methyl γ-linolenate[12] and methyl α-linolenate[23] have been previously described.

Acknowledgment. This project was supported by funding from the National Science Foundation and the NIH ES013125. C.L.R. also acknowledges support from the Center in Molecular Toxicology, Vanderbilt University. Helpful discussions with Professor Derek Pratt of Queen's University are gratefully acknowledged.

Supporting Information Available: Experimental procedures for the synthesis of all compounds, NMBHA oxidations of dienes (Table S1), α-tocopherol-mediated oxidations of methyl α-linolenate (Figure S1), and transition-state models for an associative rearrangement (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Coates, R. M.; Firsan, S. J. *J. Org. Chem.* **1986**, *51*, 5198.

(22) BHT radicals do not propagate the oxidative chain, whereas tocopheryl radicals may do so. Bowry, V. W.; Stocker, R. *J. Am. Chem. Soc.* **1993**, *115*, 6029.

(23) Chan, H. W.-S.; Levett, G. *Lipids* **1977**, *12*, 837.