J. Am. Chem. Soc. **2001,** *123,* 11827−11828

Kinetic Products of Linoleate Peroxidation: Rapid *β*-Fragmentation of Nonconjugated Peroxyls

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Received August 30, 2001

The autoxidation of polyunsaturated fatty acids and esters has been the focus of intense investigation because of its potential importance in biology.[1,2] Oxidation of neat linoleic acid or its esters, **1**, gives four major product conjugated dienes.[3,4] Two of these product hydroperoxides, **3** and **5**, are *cis,trans* dienes having hydroperoxide substitution at the 13 and 9 position of the C-18 chain. These compounds are the major products formed in linoleate oxidations in which millimolar concentrations of antioxidants, such as α-tocopherol (ArOH), are present during the reaction. Two other hydroperoxides analogous to **3** and **5** but having *trans,trans* diene geometry are formed in the absence of antioxidants.[5] Under these conditions, *β*-fragmentation of intermediate peroxyl radicals leads to the more stable *trans,trans* conjugated dienes. Recently, a nonconjugated hydroperoxide, **4**, was identified as a product formed from oxidations of methyl linoleate to which ~0.1 M α-tocopherol was added.[6] We report here that **4** is the major product formed from oxidations of methyl or cholesteryl linoleate at high α-tocopherol concentrations and that the peroxyl radical leading to **4** undergoes a rapid *β*-fragmentation, k_β in Scheme 1. This fragmentation serves as a useful radical clock for antioxidant H-atom transfer, k_{inh}.

The hydroperoxides **4** derived from methyl or cholesterol linoleate separate cleanly from the conjugated diene products by normal phase HPLC. The nonconjugated hydroperoxide from methyl linoleate has been previously characterized by spectroscopy[6] and we note that HPLC-MS utilizing silver coordination ion spray mass spectrometry (CIS-MS)[7] supports the proposed structure for the cholesteryl ester products (see Supporting Information).

Autoxidations of 0.2 M methyl linoleate initiated by 0.01 M 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile), MeOAMVN, were run in benzene at 37 °C for 4 h. Sixty-four reactions were carried out with concentrations of added α-tocopherol ranging from 0.013 to 1.76 M. To obtain clean product mixtures, the antioxidant must be freshly purified by column chromatography less than a day before use. Similar experiments were carried out with cholesteryl linoleate and the data obtained from those reactions were comparable to, although not as clean as, those from methyl linoleate. To standardize HPLC detection, the three hydroperoxides (**3**−**5**, R = Me) were isolated and several standard solutions were analyzed by both ^{1}H NMR and HPLC-UV at 207 nm.

The results of these studies are shown in Figure 1. Part A of Figure 1 shows the product ratio of **[4]/[3]** + **[5]** vs. [α-tocopherol] while the inverse of these terms is plotted in inset B. These data clearly show that the amount of nonconjugated product formed in autoxidation reaches a maximum limit at high concentrations of antioxidant, an observation that is consistent with the mechanism presented in Scheme 1. In this mechanism, oxygen partitions to the three positions of the linoleate chain as follows: 13 position = [1 − α]/2, 11 position = α, and 9 position = [1 − α]/2. Under conditions of oxidation in which millimolar concentrations of antioxidant are present, the 13- and 9-peroxyl radicals abstract hydrogen from the antioxidant, giving the hydroperoxide products **3** and **5**.

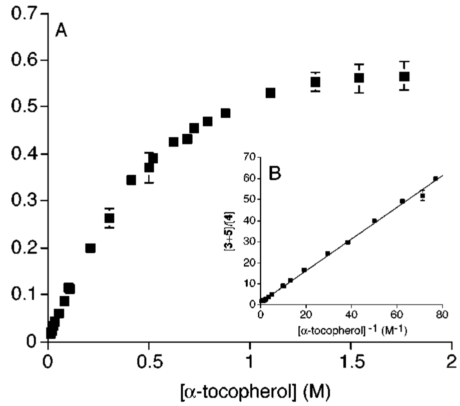
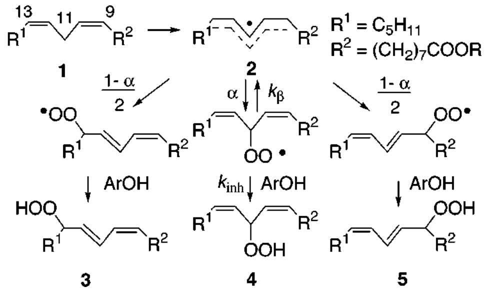


Figure 1. Methyl linoleate product composition vs antioxidant: (A) [**4**]/[**3**] + [**5**] vs [α-tocopherol] and (B) ([**3**]+[**5**])/[**4**] vs 1/[α-tocopherol].

Scheme 1. Mechanism of Linoleate Oxidation



In contrast to the conjugated diene peroxyl radicals, the 11-peroxyl radical that leads to **4** apparently undergoes a rapid *β*-fragmentation, reforming the original pentadienyl radical even in the presence of concentrations of antioxidant approaching 1 M. Competing with this fragmentation is H atom transfer to the peroxyl radical with a rate constant $k_{\text{inh}} = 3.8 \times 10^6\ \text{M}^{-1}\ \text{s}^{-1}$. As the concentration of antioxidant is increased, the yield of **4** increases until the partition limit is reached. Analysis of the mechanism in Scheme 1 leads to eq 1, which describes the product ratio plotted in Figure 1 as a function of α, k_{inh}, k_β, and [α-tocopherol]. Least-squares analysis[8] of the data shown in Figure 1 gives the best fit for an α of 0.43 ± 0.02 and a k_β of $(1.9 \pm 0.2) \times 10^6\ \text{s}^{-1}$. An α of 0.43 translates into an oxygen partition between the 13, 11, and 9 positions of pentadienyl radical **2** equal to 1:1.5:1. The nonconjugated hydroperoxide is the major product formed in autoxidations of linoleate when good H atom donors scavenge all intermediate peroxyl radicals.

$$\frac{[\mathbf{4}]}{[\mathbf{3+5}]} = \frac{k_{\text{inh}}\,[\alpha\text{-Toc}]}{k_{\text{inh}}\,[\alpha\text{-Toc}] + k_\beta} \cdot \frac{\alpha}{1-\alpha} \qquad \text{Eq. 1}$$

The kinetic product distribution for linoleate roughly parallels the distribution of spin in the intermediate pentadienyl radical.

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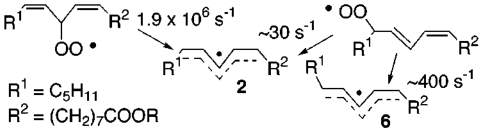
[8] Nonlinear least-squares analysis of the data in Figure 1A and linear least squares of the data in Figure 1B give essentially the same results with $R^2 = 0.99$.

10.1021/ja0169724 CCC: $20.00

Calculations of spin densities for the parent pentadienyl radical, derived from 1,4 pentadiene, and the corresponding radical derived from 2,5-heptadiene indicate that the spin density at the center position of the radical is 1 to 12% higher than that calculated for the terminal positions of the delocalized radical (see Supporting Information). Evaluation of ESR hyperfine data for the parent pentadienyl and substituted analogues indicates spin densities at the central carbon 15 to 25% higher than at the terminal carbons.[9] Oxygen does react at the site of highest spin in the intermediate pentadienyl **2**, although our data show reaction at the central carbon that exceeds expectations based upon spin density alone (50% more reaction at the center carbon than at the terminal carbons based upon the value of α determined here vs 15−25% from ESR and 1−12% from theory).[10]

The rate of β-fragmentation of the nonconjugated 11-peroxyl radical, 1.9×10^6 s^{-1}, is orders of magnitude higher than that of the corresponding conjugated 9 and 13 radicals.[5] Thus, fragmentation of the conjugated peroxyl radicals to the pentadienyl **2** was suggested to have a rate constant of ~30 s^{-1}, while fragmentation of those same radicals to the isomeric pentadienyl **6** has a rate constant of ~400 s^{-1}. The difference in rates of β-fragmentation of the 9/13-peroxyl radicals and the 11-peroxyl radical is best understood in terms of the calculated[11] C−OO$^\bullet$ bond dissociation enthalpy (BDE) of each of the two peroxyl radicals (see Supporting Information for details). The terminal peroxyl radicals (9/13) have C−OO$^\bullet$ BDEs of 9.9 kcal/mol leading to **6**,[12] whereas the bis-allylic 11 peroxyl radical has a much weaker C−OO$^\bullet$ BDE (3.8 kcal/mol). Stabilization of the 9 and 13 peroxyl radicals due to conjugation compared to the 11 peroxyl radical is one obvious source that leads to the difference in the computed BDEs.



The very rapid β-fragmentation for the nonconjugated 11 peroxyl radical provides an opportunity to use this first-order process as a standard clock for reactions of peroxyl radicals that occur in the same time domain. One example of such an application is the determination of k_{inh} for antioxidants whose inhibition rate constants are unknown. At low concentrations of antioxidant, eq 1 reduces to $[\mathbf{4}]/([\mathbf{3}] + [\mathbf{5}]) = (\alpha/[1 - \alpha])\cdot k_{\mathrm{inh}}[\mathrm{antiox}]/k_\beta$. Therefore, under these conditions, the product dependence is expected to be linear with respect to [antiox] with the slope corresponding to $(\alpha/[1 - \alpha])\cdot k_{\mathrm{inh}}/k_\beta$.

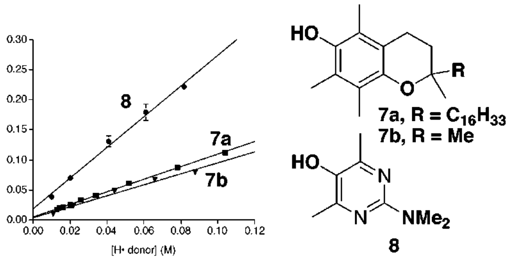


Figure 2. Methyl linoleate product composition vs antioxidant: [**4**]/[**3**] + [**5**] vs [**7a**]; [**7b**]; and [**8**].

In Figure 2 is presented the product distribution of linoleate hydroperoxides[13] vs [antioxidant] for three antioxidants at concentrations between 0.01 and 0.1 M. One of the antioxidants is α-tocopherol, **7a**, the primary standard used to establish k_β with the other two compounds, pentamethylchromanol (**7b**) and *N*,*N*-dimethyl-2-amino-4,6-dimethyl-5-pyrimidinol[14] (**8**), having been studied elsewhere. Rate constants determined in this way at 37 °C are as follows: **7b**, $k_{\mathrm{inh}} = (2.1 \pm 0.1) \times 10^6$ M^{-1} s^{-1} and **8**, $k_{\mathrm{inh}} = (6.5 \pm 0.8) \times 10^6$ M^{-1} s^{-1}.[15] It is of interest to note that these studies confirm the recent report[14] that **8**, a member of a new class of antioxidants, is a better hydrogen atom donor to peroxyl radicals than is α-tocopherol.

Other polyunsaturated fatty acids give rise to nonconjugated hydroperoxide products and the kinetic framework established here for linoleate should also apply to, for example, linolenic, arachidonic, and docosohexenoic acids and their esters. The results of studies on these fatty acid esters, including phospholipids,[16] will be reported elsewhere. While high concentrations of antioxidants are required to generate substantial quantities of nonconjugated products, other processes such as electron transfer might also provide product mixtures that contain a substantial proportion of these kinetically controlled products.

Acknowledgment. We thank Drs. Alan Brash and Martin Newcomb for helpful discussions and Jeremy Mills for help with the nonlinear least-squares analysis. N.A.P. thanks NIH (HL-17921), NIEHS (P30ES00267), and the National Science Foundation for support of this work, K.A.T. acknowledges support from T32ES07028, and D.A.P. acknowledges NSERC Canada for financial support.

Supporting Information Available: Calculated BDEs and spin densities for pentadienyl radicals, data for Figure 1, standardization of HPLC for compounds **3**−**5**, and HPLC/MS, including fragmentation for **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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[10] The autoxidation of 1,4-cyclohexadiene yields products consistent with 40% of oxygen addition to form a nonconjugated peroxyl radical, with 60% of oxygen addition giving conjugated peroxyl radicals, see: Pan, X.-M.; Schuchmann, M. N.; von Sonntag, C. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1021−1028.

[11] ROB3LYP/6-311+G(2d,2p)//UHF/6-31G(d).

[12] We calculate that the transformation of the conjugated (13/9) peroxyl radical to the pentadienyl radical **2** occurs with a BDE of 11.3 kcal/mol.

[13] Autoxidations of 0.2 M methyl linoleate were carried out in benzene at 37 °C for 4 h with 0.01 M MeOAMVN with given amounts of antioxidants.

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[15] Reported rate constants for **7b** and **8** are the following: $k_{\mathrm{inh}}^{\mathbf{7b}} = 3.8 \times 10^6$ M^{-1} s^{-1} (30 °C): Burton, G. W.; Doba, T.; Gabe, E.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053−7065. $k_{\mathrm{inh}}^{\mathbf{8}} = 8.6 \pm 0.5 \times 10^6$ M^{-1} s^{-1} (50 °C): ref 14.

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