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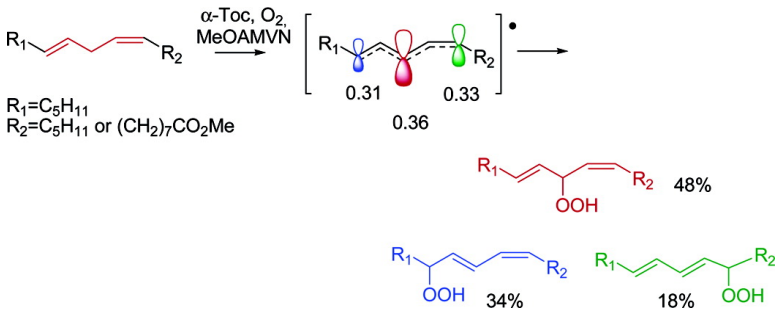

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Factors Influencing the Autoxidation of Fatty Acids: Effect of Olefin Geometry of the Nonconjugated Diene

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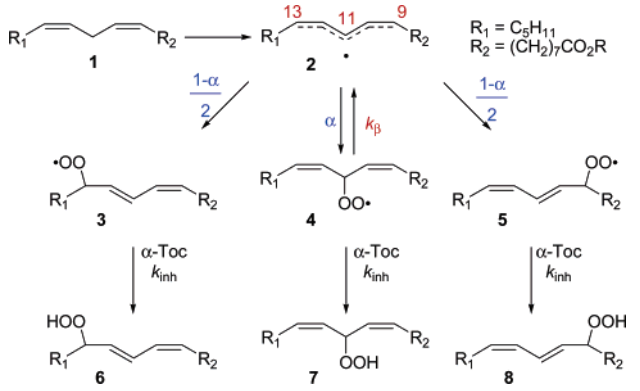
Abstract: Autoxidations of cis,cis, cis,trans, and trans,trans nonconjugated octadecadienoates and pentadecadienes were carried out in the presence of α-tocopherol to investigate the effect of olefin geometry on this oxidation process and provide insight into the factors that influence the autoxidation of fatty acids. We have found that as the trans character of the diene increases, the amount of O_2 trapping at the central (bis-allylic) position of the pentadienyl radical also increases. In addition, the rate constant for β-fragmentation ($k_\beta \approx 10^6\ s^{-1}$) of the bis-allylic peroxyl radical decreased on going from the cis,cis to the trans,trans diene. We have also found that for the cis,trans nonconjugated dienes, there is a preference for trapping of the pentadienyl radical by O_2 at the *transoid* end, generating the cis,trans conjugated hydroperoxide as the major product.

Introduction

The autoxidation of polyunsaturated fatty acids (PUFAs) and the corresponding esters has been the focus of intense investigation due to its potential importance in biology.[1] When PUFAs and their esters are exposed to oxidative stress, the primary products are hydroperoxides.[2] This oxidation process is a free radical chain reaction, which targets the nonconjugated diene moieties of PUFAs (**1**). Oxidation is initiated by abstraction of the bis-allylic hydrogen atom generating a pentadienyl radical (**2**), which can undergo trapping at either terminus or at the central (bis-allylic) position. For example, the pentadienyl radical derived from methyl linoleate reacts with O_2 at the 13, 11, or 9 positions (Scheme 1). A hydrogen atom donor subsequently traps the peroxyl radical (**3**−**5**) generating a hydroperoxide (**6**−**8**). The distribution of hydroperoxides formed is highly dependent on the efficiency and concentration of the hydrogen atom donor present. For example, when oxidations of linoleates are carried out in the presence of low concentrations of α-tocopherol (α-Toc, <10 mM), trans,cis conjugated hydroperoxides (**6**, **8**) are the major products formed. However, in the absence of antioxidant, β-fragmentation of the intermediate peroxyl radicals leads to products having the more stable trans,trans diene geometry.[3]

Scheme 1. General Mechanism of Kinetically Controlled Linoleate Oxidation



The formation of conjugated hydroperoxides, which result from trapping of the pentadienyl radical at the terminal positions, has been well documented. Although the formation of bis-allylic hydroperoxides of cyclic dienes has been reported, products arising from O_2 trapping at the bis-allylic position of PUFAs have eluded researchers until recently.[4] When methyl linoleate was oxidized in the presence of a high concentration of α-tocopherol (∼0.1 M), the nonconjugated hydroperoxide (**7**) was observed.[4b] We have previously reported that the bis-allylic hydroperoxide is the major product formed when oxidations of methyl linoleate were carried out in the presence of high concentrations of α-tocopherol (0.05−1.8 M).[5] The mechanism shown in Scheme 1 was used as a basis for analysis of product distribution in these oxidations. The high concentrations of antioxidant were necessary in order to observe the bis-allylic

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(3) Porter, N. A.; Wujek, D. G. *J. Am. Chem. Soc*. **1984**, *106*, 2626. A value of 430 s^{-1} was reported in this publication, but we are currently re-evaluating rate constants for fragmentation of the conjugated peroxyl radicals for use as slow free radical clocks. These studies suggest a rate constant for this fragmentation of 620 s^{-1}. Gillmore, J. G.; Porter, N. A. To be published.

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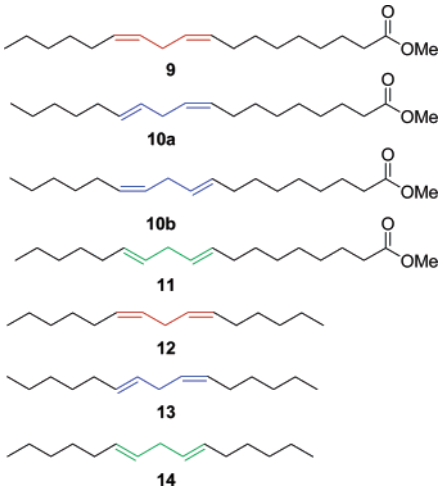
product, since the peroxyl radical leading to the hydroperoxide undergoes a very rapid β-fragmentation (10^6 s^{-1}). In addition, we have shown that this fragmentation serves as a useful radical clock for antioxidant H-atom transfer to peroxyl radicals with bimolecular rate constants of ca. 10^6 M^{-1} s^{-1}.

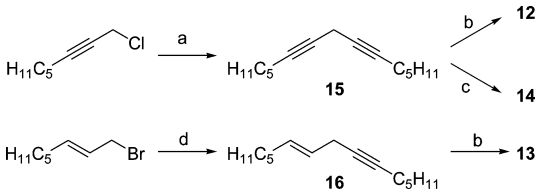
Herein we report more detailed studies on the formation of bis-allylic peroxyl radicals and their subsequent β-fragmentation in the oxidation of octadecadienoate and model diene systems. In addition to studies involving methyl linoleate previously reported, we have investigated the effect of olefin geometry on the formation and reactivity of bis-allylic peroxyl radicals. Although little is known about their reactivity, there is evidence that geometric isomers of methyl linoleate (i.e., trans fatty acids) can play a significant role in biological systems.[6]

Results and Discussion

The effect of olefin geometry on the formation of the bis-allylic hydroperoxides was studied using methyl octadecadienoates having cis,cis (linoleate), cis,trans, and trans,trans (linoelaidate) geometry (**9**−**11**). Studies were also carried out using the analogous model diene compounds (**12**−**14**). Oxidations of the model dienes enabled us to probe the generality of the proposed mechanism (Scheme 1) by focusing only on the reactivity of the nonconjugated diene moiety without potential interference from any other functional group in the compound. Although the ester present in the linoleate compounds would appear to be too far removed from the reactive site to play any role, oxidation of the model dienes addresses this issue. The oxidation profiles for these three model compounds were identical to the octadecadienoates, suggesting that the ester indeed has no influence at the site of oxidation. In addition to probing the generality of the oxidation mechanism, the use of the octadecadienoates and model dienes offers the opportunity to use complimentary methods of product analysis. The fatty ester hydroperoxides were analyzed by HPLC, whereas the diene oxidation products were analyzed by GC. These two methods allowed us to verify the product distribution from each substrate. Finally, the model dienes offer the advantage that the oxidation products could be confirmed through straightforward independent synthesis.



Scheme 2. Synthesis of Model Dienes[a]



[a] Reagents: (a) 1-heptyne, CuI, NaI, K_2CO_3, DMF; (b) Pd/$BaSO_4$, H_2, EtOAc; (c) NH_3/ether, Li^0, $(NH_4)_2SO_4$; (d) 1-heptyne, *n*-BuLi, DMPU, THF, −78 °C.

Synthesis of Octadecadienoates and Model Dienes. Methyl linoleate (**9**) and linoelaidate (**11**) are commercially available, whereas the cis,trans octadecadienoates (**10a**, **10b**) were synthesized as previously reported. Although none of the model dienes are commercially available, they could be synthesized in a straightforward manner (Scheme 2). The cis,cis (**12**) and trans, trans (**14**) dienes were synthesized from a common intermediate diyne (**15**). The diyne (**15**) was synthesized by a copper-promoted coupling of 1-heptyne with 1-chloro-2-octyne.[7] Lindlar hydrogenation using the more reactive Pd/$BaSO_4$ catalyst or a buffered Birch reduction[8] of **15** yielded **12** or **14**, respectively. The unsymmetrical diene (**13**) was synthesized by addition of 1-heptynyllithium to *trans*-1-bromo-2-octene,[9] followed by the same Lindlar hydrogenation. The synthesis of **16** via the copper coupling has been reported,[10] but we found that this procedure resulted in a mixture of regioisomers. Carrying out the coupling of 1-heptynyllithium with the allylic bromide in the presence of DMPU gave exclusively the desired regioisomer. All model dienes were purified by column chromatography on silver-nitrate-impregnated silica gel (SNIS) to ensure high isomeric purity.[11]

Oxidation Products of Octadecadienoates and Model Dienes. Oxidations of the model dienes and octadecadienoates (0.2 M) were carried out in benzene in the presence of varying concentrations of α-tocopherol (0.05−1.8 M) and were initiated by 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeOAMVN). The reaction vials were incubated at 37 °C for 4 h. The relatively short reaction times allowed for low conversion so that the α-tocopherol was not consumed to a significant extent during any of the oxidations, ensuring pseudo-first-order conditions. The octadecadienoate hydroperoxides were analyzed directly by normal phase HPLC. The diene hydroperoxides from **12**−**14** were reduced to the corresponding alcohols with PPh_3 and subsequently analyzed by GC. Under these oxidation conditions, only the kinetic products were observed, as depicted in Scheme 3.

The nonconjugated hydroperoxide formed in each oxidation contained the same olefin geometry as the parent substrate. The cis,cis and trans,trans substrates generated only the cis,trans and trans,trans conjugated hydroperoxides, respectively, while a mixture of the two conjugated hydroperoxides were generated upon oxidation of the cis,trans substrates. The products of the oxidations were identified by several methods. In the case of

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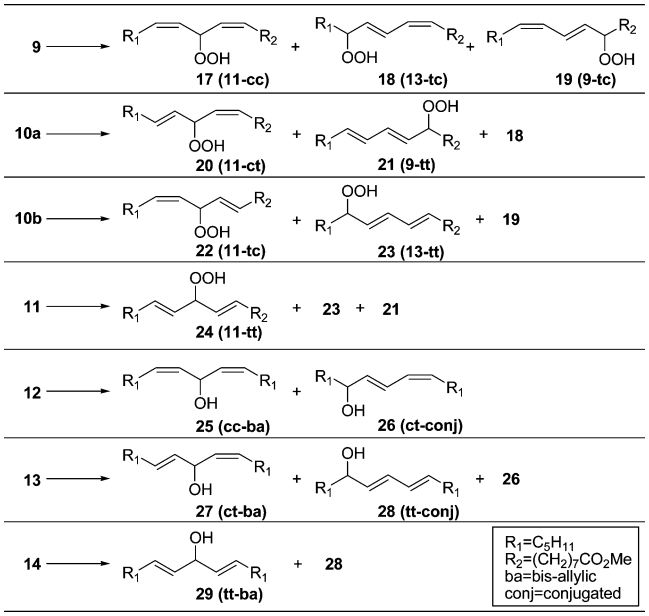
(8) Brandsma, L.; Nieuwenhuizen, W. F.; Zwikker, J. W.; Mäeorg, U. *Eur. J. Org. Chem.* **1999**, 775.

(9) Chun, J.; Li, G.; Byun, H.-S.; Bittman, R. *J. Org. Chem.* **2002**, *67*, 2600.

(10) Capon, R. J.; Barrow, R. A. *J. Org. Chem.* **1998**, *63*, 75.

(11) Williams, C. M.; Mander, L. N. *Tetrahedron* **2001**, *57*, 425.

Scheme 3. Oxidation Products of Octadecadienoates (**9**−**11**) and Dienes (**12**−**14**)



the octadecadienoates (**9**−**11**), the product mixtures were analyzed by HPLC−MS utilizing silver coordination ion spray mass spectrometry (Ag-CIS-MS).[12] This method has proven to be very useful in identifying intact hydroperoxides, as well as distinguishing the regioisomers present in the oxidation mixture.[13] Coordination of silver to the hydroperoxides provides a mild method of generating a positively charged species that is detected by MS (Figure 1).

In addition, the silver promotes Hock fragmentation[14] of the hydroperoxides in the MS to generate two aldehydes (Scheme 4). For example, the 13- and 9-hydroperoxides (**18**, **19**) fragment to generate aldehydes having a mass of 333 and 293, respectively. The 11-hydroperoxide (**17**) undergoes fragmentation on either side of the hydroperoxide generating a mixture of two aldehydes (*m*/*z* 319 and 307). The characteristic fragments that arise from each hydroperoxide are diagnostic and allow the assignment of each regioisomer present in the oxidation mixture.[15] It should be noted that only the aldehyde fragment containing the ester functionality (shown in Scheme 4) was detected by MS. In addition to HPLC-MS analysis, the hydroperoxides were isolated and analyzed by ^{1}H NMR to confirm the double-bond geometry.

The products formed from oxidation of the model dienes **12**−**14** were identified and verified by independent synthesis of the alcohols. The cis,cis bis-allylic alcohol (**25**) was synthesized by addition of 1-heptynyllithium to ethyl formate, followed by

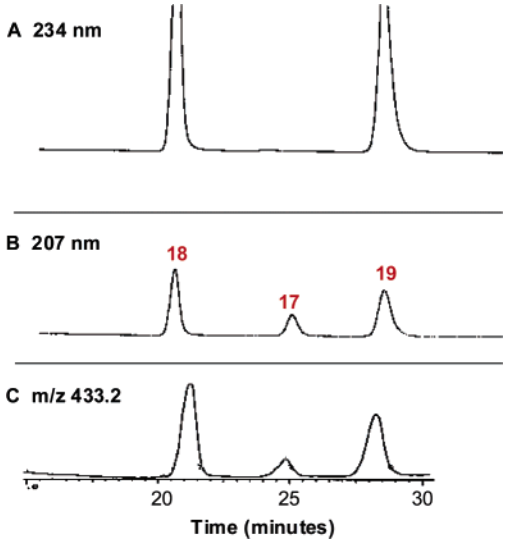
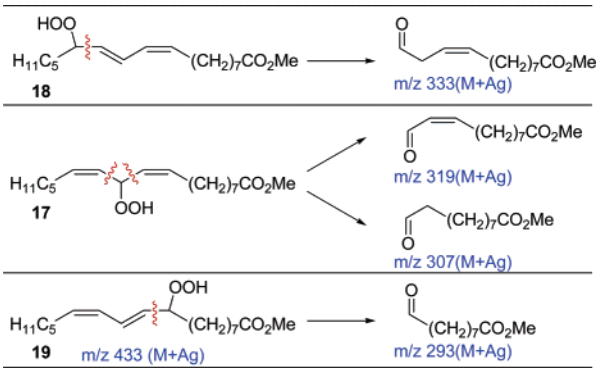
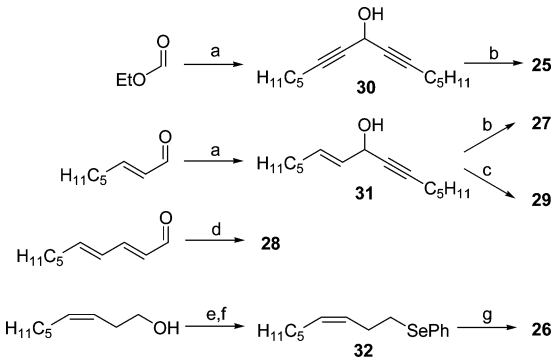


Figure 1. HPLC chromatograms of methyl linoleate oxidation mixture. (A) HPLC−UV detection at 234 nm, (B) HPLC−UV detection at 207 nm, (C) HPLC−MS (Ag CIS), intact hydroperoxides detected at 433.2 (M + Ag). HPLC-MS analyses were carried out using a Beckman Ultrasphere narrowbore (0.20 × 25 cm^2) silica column with 0.5% ^{i}PrOH/hexanes (0.15 mL/min) and postcolumn addition of $AgBF_4$/^{i}PrOH (0.3 mM, 0.075 mL/min) as described in ref 13a.

Scheme 4. Hock Fragments of Linoleate Hydroperoxide Silver Adducts



Scheme 5. Synthesis of Diene Alcohols[a]



[a] Reagents: (a) 1-heptyne, *n*-BuLi, THF, −78 °C; (b) Pd/$CaCO_3$/Pb, H_2, EtOAc; (c) $LiAlH_4$, THF, reflux; (d) $H_{11}C_5MgBr$, THF; (e) MsCl, pyr; (f) $(PhSe)_2$, $LiAlH_4$, THF; (g) *m*-CPBA, THF, −78 °C; LDA, hexanal, −78 °C; *i*-Pr_2NH, reflux.

Lindlar hydrogenation with the mild Pd/$CaCO_3$/Pb catalyst (Scheme 5).[16] Addition of 1-heptyne to *trans*-2-octenal yielded **31**. This intermediate was converted to the cis,trans (**27**) and

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(14) Hock fragmentation is commonly observed in solution for lipid hydroperoxides and is promoted by protic or Lewis acids. (a) Hock, H. *Angew. Chem.* **1936**, *49*, 595. (b) Frimer, A. A. *Chem. Rev.* **1979**, *79*, 359. (c) Tanigawa, S.; Kajiware, T.; Hatanaka, A. *Phytochemistry* **1984**, *23*, 2439. (d) Gardner, H. W.; Planter, R. D. *Lipids* **1984**, *119*, 294.

(15) Regardless of the olefin geometry in the hydroperoxide, the Hock fragmentation of each regioisomer was virtually identical. Olefin geometry was confirmed by ^{1}H NMR analysis and comparison to published spectra for similar compounds (cholesteryl linoleate). Kenar, J. A.; Havrilla, C. M.; Porter, N. A.; Guyton, J. R.; Brown, S. A.; Klemp, K. F.; Selinger, E. *Chem. Res. Toxicol.* **1996**, *9*, 737.

(16) Guo, C.; Lu, X. *Synlett* **1992**, 405.

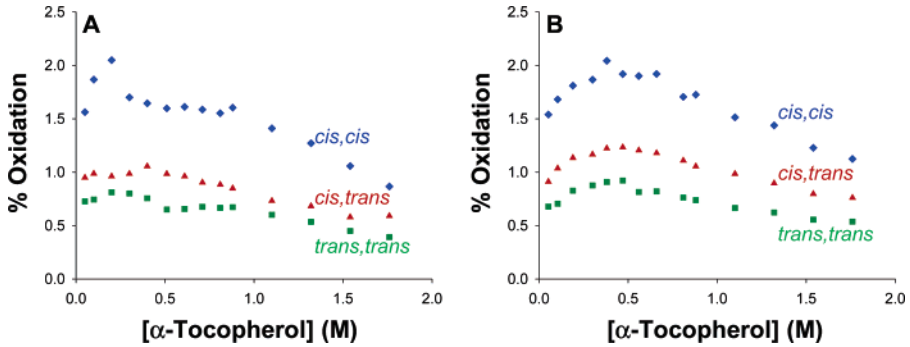

Figure 2. % Oxidation of octadecadienoates and dienes in the presence of α-tocopherol. (A) Octadecadienoates (blue diamonds) **9**, (red triangles) **10a**, (green squares) **11**. (B) Dienes (blue diamonds) **12**, (red triangles) **13**, (blue squares) **14**. Oxidations were carried out in benzene with octadecadienoate or 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 the diene hydroperoxides reduced with PPh_3. The octadecadienoate and diene products were analyzed by HPLC and GC, respectively, as described in the Experimental Section.

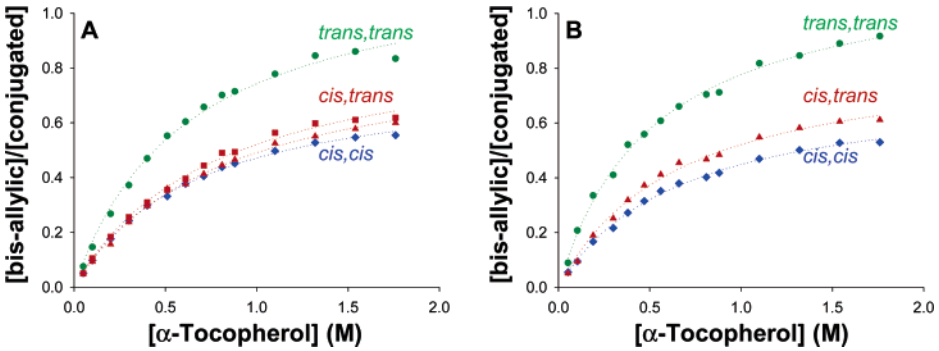

Figure 3. Oxidation profile of octadecadienoates and dienes, ratio of bis-allylic/conjugated products versus [α-tocopherol]: (A) Octadecadienoates (blue diamonds) **9**, (red squares) **10a**, (red triangles) **10b**, (green circles) **11**. (B) Dienes (blue diamonds) **12**, (red triangles) **13**, (green circles) **14**. Oxidations were carried out in benzene with octadecadienoate or 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 the diene hydroperoxides reduced with PPh_3. The octadecadienoate and diene products were analyzed by HPLC and GC, respectively, as described in the Experimental Section.

trans,trans (**29**) alcohols by Lindlar hydrogenation and $LiAlH_4$ reduction, respectively. The trans,trans conjugated alcohol (**28**) was easily prepared in one step by addition of pentylmagnesium bromide to *trans,trans*-2,4-decadienal. Synthesis of the cis,trans conjugated alcohol was achieved through an interesting cascade of reactions.[17] First, *cis*-3-nonenol was converted to the selenide (**32**) by displacement of the mesylate. Oxidation of the selenide to the selenoxide with *m*-CPBA increases the acidity of the α-protons. Hence, deprotonation followed by addition of hexanal generates the *β*-phenylseleno alcohol, which undergoes a selenoxide elimination to yield the desired compound.

Extent of Oxidation. Oxidations of the octadecadienoates and model dienes were carried out side-by-side, and the amount of oxidation products were determined by HPLC and GC, respectively. GC detection was standardized using the authentic synthetic alcohols (**25**−**29**). To standardize HPLC detection, the hydroperoxides were first isolated by semipreparative HPLC. The concentration of each solution was determined by ^{1}H NMR relative to an internal standard and subsequently standardized by HPLC−UV at 207 nm.

The oxidation profile somewhat demonstrates the delicate balance between the prooxidant and antioxidant properties of α-tocopherol (Figure 2).[18] This prooxidant behavior is more clearly observed in oxidations of the dienes (Figure 2B). As the concentration of α-tocopherol increases, the amount of oxidation also increases, indicative of the ability of the α-tocopheroxyl radical to mediate oxidation. However, at the highest concentrations, the amount of oxidation decreased. At higher concentrations of antioxidant, peroxyl radicals are more effectively scavenged, interrupting chain propagation. It is also clear from the plot that olefin geometry influences the extent of oxidation. The cis,cis dienes (**9**, **12**) undergo at least twice as much oxidation as the trans,trans dienes (**11**, **14**), with the cis,trans dienes (**10a**, **10b**, **13**) falling between these two. This is consistent with the BDEs that have been calculated for these types of compounds. The bis-allylic C−H BDEs for cis,cis, cis,trans, and trans,trans 2,5-heptadiene have been calculated as 72.7, 73.1, and 73.5 kcal/mol, respectively.[19] Since the trans,trans diene has the strongest C−H bond, it is less prone to oxidation than the other compounds. This is also consistent with reports that trans fatty acids undergo less oxidation than their cis counterparts in LDL oxidations.[6a]

Oxygen Partitioning and Peroxyl Radical Fragmentation. The plot of the ratio of bis-allylic to conjugated products versus the concentration of α-tocopherol is shown in Figure 3. The profile for the product distribution is essentially the same for the octadecadienoates and dienes. This indicates that the ester functionality does not influence the site of oxidation. In addition, regardless of the substrate or the method of analysis, the oxidation profiles are nearly identical.

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(18) (a) Upston, J. M.; Terentis, A. C.; Stocker, R. *FASEB* **1999**, *13*, 977. (b) Bowry, V. W.; Ingold, K. U. *Acc. Chem. Res.* **1999**, *32*, 27. (c) Culbertson, S. M.; Vinqvist, M. R.; Barclay, L. R. C.; Porter, N. A. *J. Am. Chem. Soc.* **2001**, *123*, 8951.
(19) Pratt, D. A.; Mills, J. H.; Porter, N. A. *J. Am. Chem. Soc.* **2003**, *125*, 5801.

Table 1. Values for O_2 Partitioning to the Bis-Allylic Position (α) and the Rate Constant for Subsequent β-Fragmentation (k_β) of the Bis-Allylic Peroxyl Radical

substrate	α	k_β (10^6 s^{-1})
9	0.44 (±0.01)	2.32 (±0.09)
10a	0.48 (±0.01)	2.74 (±0.18)
10b	0.47 (±0.01)	2.84 (±0.14)
11	0.55 (±0.02)	2.18 (±0.17)
12	0.43 (±0.01)	2.36 (±0.10)
13	0.48 (±0.01)	2.69 (±0.11)
14	0.55 (±0.01)	2.02 (±0.09)

The data clearly shows that, for all compounds studied, the amount of bis-allylic hydroperoxide increases with increasing α-tocopherol concentration at the expense of the conjugated products until a maximum limit is reached. This observation is consistent with the proposed mechanism for linoleate shown in Scheme 1.[5] In this mechanism, O_2 is partitioned among the three positions of the pentadienyl radical as follows: terminal = $1 - \alpha/2$, bis-allylic = α, and terminal = $1 - \alpha/2$. The peroxyl radicals subsequently abstract a hydrogen atom from α-tocopherol to generate the hydroperoxides. Under the oxidation conditions used in these studies, the rate constant for β-fragmentation of the conjugated peroxyl radical is slow (620 s^{-1}) relative to trapping by α-tocopherol and formation of the thermodynamically favored trans,trans conjugated hydroperoxides is not observed.

In contrast to the conjugated peroxyl radicals, the nonconjugated peroxyl radical undergoes a rapid β-fragmentation (k_β) to regenerate the pentadienyl radical (see Scheme 1). Competing with this β-fragmentation is hydrogen atom transfer (k_{inh}) to the peroxyl radical by α-tocopherol with a rate constant of 3.5×10^6 M^{-1} s^{-1}.[20] As the concentration of α-tocopherol increases, β-fragmentation becomes negligible and a limit is reached. At this limit, the product distribution reflects the O_2 partition to the three positions of the pentadienyl radical.

From Figure 3 it can be seen that olefin geometry influences the partitioning of O_2 across the pentadienyl radical. The fraction of bis-allylic products arising from the oxidation of trans,trans compounds reaches a higher limit than the other compounds. This indicates that as the trans character of the pentadienyl radical increases, so does O_2 addition at the bis-allylic position. Analysis of the mechanism leads to a kinetic expression (eq 1), which describes the product ratio as a function of α, k_β, k_{inh}, and [α-tocopherol].

$$\frac{[\text{bis-allylic}]}{[\text{conjugated}]} = \left(\frac{\alpha}{1-\alpha}\right)\frac{k_{inh}[\alpha\text{-Toc}]}{k_{inh}[\alpha\text{-Toc}] + k_\beta} \qquad (1)$$

Nonlinear least-squares analysis of the data using eq 1 allowed us to determine values for α and k_β for the various substrates (Table 1). The data clearly shows that olefin geometry has an effect on the O_2 partitioning (α) and on fragmentation (k_β) of the bis-allylic peroxyl radical (Table 1).

As the trans character of the pentadienyl radical increases, the addition of O_2 at the bis-allylic position (α) increases, consistent with ESR and theoretical predictions. We note that ESR[21] and theory[19] (Table 2) indicate that the pentadienyl radical formed from the trans,trans diene precursor has higher unpaired spin (38% from the ESR data) at the bis-allylic carbon than does the radical formed from the cis,cis diene (36%). For this pair of reactants, unpaired electron spin density on the intermediate pentadienyl radical parallels trapping by O_2 at the bis-allylic position and a higher value for α (0.55) is observed for the trans,trans diene oxidations compared to the cis,cis precursor (0.43). Likewise, ESR experiments suggest that the pentadienyl from the trans,cis precursors has 37% unpaired spin at the bis-allylic position and the α values obtained (0.47) for these substrates (**10a**, **10b**, and **13**) are consistent with unpaired spin density being important in controlling the kinetic product distribution. The experimentally determined values for α are somewhat higher than the spin density ratios that have been calculated for model compounds and that are observed by ESR, although experiment and theory show the same trends. The calculations were carried out using 2,5-heptadiene as the model for octadecadienoates, and experiments to be published indicate that there is a significant substituent effect on the partitioning of oxygen to pentadienyl radicals.[22,23]

There is also a rather small influence of olefin geometry on the propensity for β-fragmentation of nonconjugated peroxyl radicals. The BDEs for the bis-allylic 4-peroxyl(s) derived from cis,cis, cis,trans, and trans,trans 2,5-heptadiene have been calculated to be 7.4, 7.9, and 8.4 kcal/mol, respectively.[19] Since the trans,trans peroxyl radical has a stronger C−OO• bond than the analogous cis,cis substrate, k_β is expected to be slower for the trans,trans peroxyl. However, the cis,trans substrates do not follow this trend. In fact these compounds have the highest observed k_β of the substrates studied.

Terminal Trapping of Pentadienyl Radical. The results for oxidation of the octadecadienoates with both alkenes having the same geometry (cis,cis or trans,trans) showed that O_2 trapping at the terminal positions of the pentadienyl radicals (C9 and C13) was identical (Figure 4A, data for **11** not shown). The yield of the 13- and 9-conjugated hydroperoxide was always equal for oxidations of **9** and **11**. This is not surprising, since one expects the unpaired electron spin density to be identical at the terminal pentadienyl positions for these substrates. Unpaired spin density for the radicals derived from cis,trans dienes is not equal at the pentadienyl terminal positions, as indicated by theory and ESR spectroscopy.[19,21] Indeed, spin appears to be slightly greater at the *cisoid* end of the pentadienyl compared to the *transoid* position for these unsymmetrical radicals (see Table 2). If spin density controls the site of initial addition of oxygen to delocalized radicals, the major conjugated product would be expected to be the trans,trans conjugated diene hydroperoxide, as shown in Scheme 6.

A previously reported study of the oxidations of **10a** and **10b** in the absence of phenolic antioxidants showed, however, that twice as much trans,cis conjugated diene product was formed

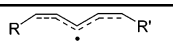
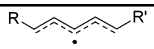
(20) The rate constant for α-tocopherol at 37 °C was derived from an Arrhenius plot of known rate constants: 30 °C (3.2×10^6 M^{-1} s^{-1}) (a) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc*. **1985**, *107*, 7053. (b) Burton, G. W.; Hughes, L.; Ingold, K. U. *J. Am. Chem. Soc*. **1983**, *105*, 5950. 50 °C (4.1×10^6 M^{-1} s^{-1}) (c) Pratt, D. A.; DiLabio, G. A.; Brigati, G.; Pedulli, G. F.; Valgimigli, L. *J. Am. Chem. Soc*. **2001**, *123*, 4625.

(21) (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.

(22) In comparing 6,9-pentadecadiene, 2,5-undecadiene, and 2,5-heptadiene, we found that O_2 trapping at the bis-allylic position (α) decreases in this order.

(23) The stability of peroxyl radicals has been shown to be influenced by substituent effects due to hyperconjugation. (a) Pratt, D. A.; Porter, N. A. *Org. Lett*. **2003**, *5*, 387. (b) Kranenburg, M.; Ciriano, M. V.; Cherkasov, A.; Mulder, P. *J. Phys. Chem. A* **2000**, *104*, 915.

Table 2. Unpaired Spin as Determined by ESR and Theory on Isomeric Pentadienyl Radicals

			
ESR[a]	0.32 : 0.36 : 0.32	0.30 : 0.37 : 0.33	0.31 : 0.38 : 0.31
Theory[b]	0.326 : 0.353 : 0.326	0.31 : 0.36 : 0.33	0.313 : 0.365 : 0.313
O_2 Trapping[c]	0.28 : 0.44 : 0.28	0.34 : 0.48 : 0.18	0.225 : 0.55 : 0.225

[a] R and R′= methyl; see ref 21b. [b] R and R′= methyl; see ref 19. The values given here were the result of UB3LYP/6-311+G(2d,2p). [c] This work, product distributions at the kinetic limit. Average distribution from diene substrates having identical diene geometry: **9** and **12**; **10a**, **10b**, and **13**; and **11** and **14**.

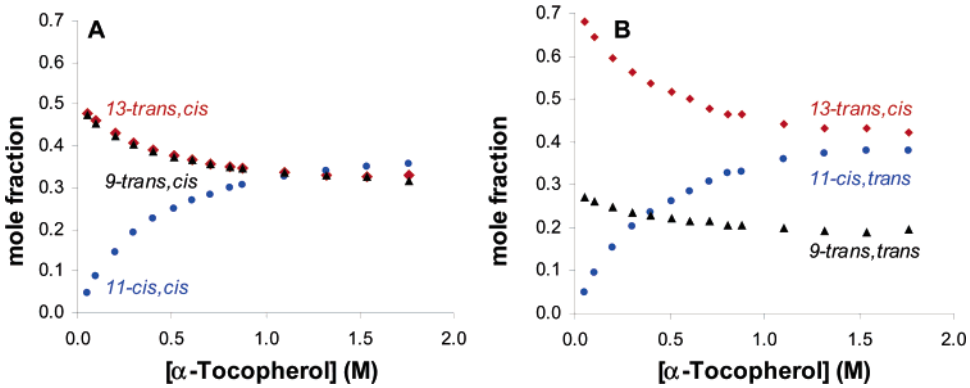
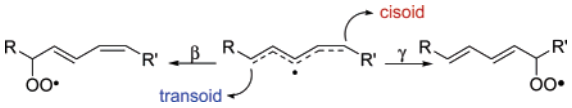


Figure 4. Mole fractions of oxidation products. (A) Oxidation of **9**, (red diamonds) 13-cis,trans conjugated, (black triangles) 9-cis,trans conjugated, (blue circles) 11-cis,cis. (B) Oxidation of **10a**, (red diamonds) 13-cis,trans conjugated, (black triangles) 9-trans,trans conjugated, (blue circles) 11-cis,trans. Oxidations were carried out in benzene with **9** or **10a** (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. The products were analyzed by HPLC as described in the Experimental Section.

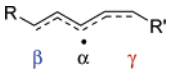
Scheme 6. Terminal Trapping of Pentadienyl Radical



as compared to the trans,trans product. Our data for O_2 trapping (Table 2) also indicate that the trans,cis conjugated diene product is formed in preference to the trans,trans compound at the kinetic limit. Theory and ESR experiments cannot account for the observed distribution of products based upon unpaired spin for these "unsymmetrical" systems. Because of this discrepancy, we analyzed the distribution of products formed from **10a**, **10b,** and **13** as a function of antioxidant concentration by a more complex kinetic model. In Figure 4 the distribution of the three products formed from oxidation of an isomerically symmetrical diene **9** (cis,cis) and one of the isomerically unsymmetrical dienes, **10a**, is presented. The analysis of products from the other two unsymmetrical dienes, **10b** and **13**, gives results that are essentially identical to those of **10a**.

The data from Figure 4B are analyzed under the assumption that formation of each conjugated hydroperoxide is a separate pathway leading to eqs 2 and 3. These equations are essentially the same as eq 1, but now it is *not* assumed that O_2 is partitioned equally at the terminal positions of the pentadienyl radical. The fraction of trapping at the bis-allylic position is still denoted as α, whereas trapping at the *transoid* and *cisoid* ends of the pentadienyl radical are denoted as β and γ, respectively (Scheme 6). Instead of collectively referring to terminal trapping as $1 - \alpha$, the two termini are treated separately (i.e., $\beta + \gamma = 1 - \alpha$). Analysis of the data using these equations, in conjunction with eq 1, gives the ratio of O_2 trapping at the three positions of the pentadienyl radical (Table 3). The data presented in Table 3 show the results of this analysis for **10a**, **10b**, and **13**.

Table 3. Kinetically Controlled O_2 Trapping of Unsymmetrical Pentadienyl Radicals



substrate	β[a]	α	γ[b]
10a	0.35	0.48	0.16
10b	0.36	0.47	0.17
13	0.32	0.48	0.21

[a] Refers to trapping at *transoid* end of pentadienyl radical to generate cis,trans conjugated product. [b] Refers to trapping at *cisoid* end to generate the trans,trans conjugated product.

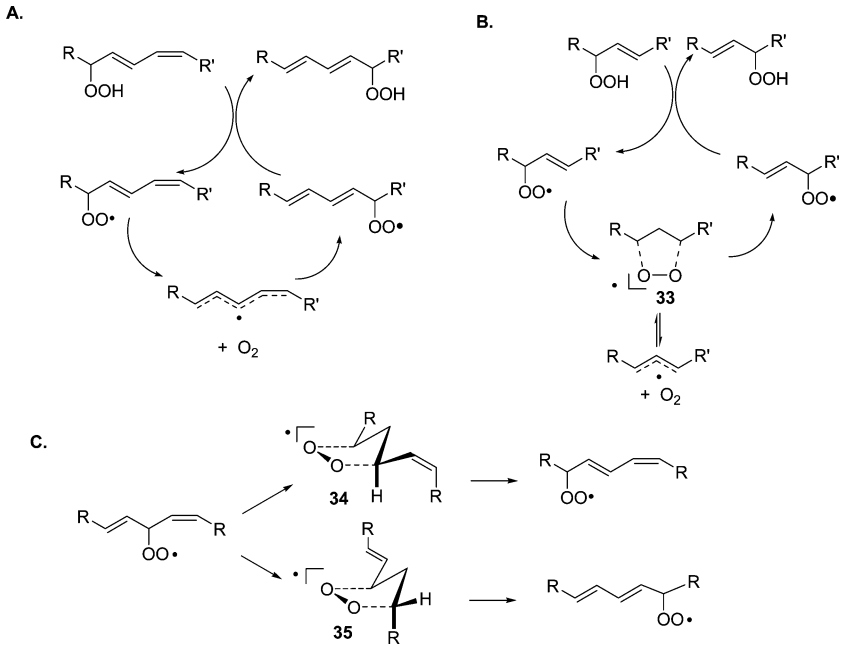
$$\text{Transoid:}\quad \frac{[\text{bis-allylic}]}{[\text{conjugated}]} = \left(\frac{\alpha}{\beta}\right)\frac{k_{\text{inh}}[\alpha\text{-Toc}]}{k_{\text{inh}}[\alpha\text{-Toc}] + k_\beta} \qquad (2)$$

$$\text{Cisoid:}\quad \frac{[\text{bis-allylic}]}{[\text{conjugated}]} = \left(\frac{\alpha}{\gamma}\right)\frac{k_{\text{inh}}[\alpha\text{-Toc}]}{k_{\text{inh}}[\alpha\text{-Toc}] + k_\beta} \qquad (3)$$

The best-fit parameters for the analysis shown in Figure 4B for **10a** and similar analyses for **10b** and **13** indicate that β, representing oxygen addition at the *transoid* end of a pentadienyl, is almost twice the value of γ, oxygen addition at the *cisoid* end of the radical. This analysis is consistent with the earlier experimental results and substantiates the conclusion that the unpaired spin density apparently does not control the partitioning of oxygen in this geometrically unsymmetrical pentadienyl radical.

Dioxygen-Radical Complexes as Intermediates in Chain Oxidation. The results for oxidation of the octadecadienoates have been analyzed here assuming that a simple β-fragmentation of peroxyl radicals is the critical process that interconverts the bis-allylic and conjugated peroxyl radicals. There is, however, a rich experimental history for peroxyl rearrangements that

Scheme 7. Mechanisms for Peroxyl Rearrangements



should not be ignored when considering product distributions in autoxidations of unsaturated substrates.[24] The use of isotopically labeled hydroperoxides or molecular oxygen in studies of hydroperoxide rearrangements has led to the conclusion that the rearrangement of conjugated diene hydroperoxides proceeds via a β-fragmentation (see Scheme 7A).[2a,25] The rearrangement of simple allylic hydroperoxides, on the other hand, is more complex. A recent computational study has led to the proposal of an allyl-triplet dioxygen complex **33** (**CX**), as an intermediate in the rearrangement; see Scheme 7B.[26] Oxygen labeling and stereochemical studies of such rearrangements are consistent with this proposal.[27,28] Calculations suggest that oxygen and an allyl radical are held in the complex **33** by dispersion forces and that "The observed high degree of stereoselectivity of the rearrangement results from the formation of the **CX** complex, which prevents the diffusion of the allyl radical and triplet dioxygen partners and maintains the stereocontrol along the fragmentation-recombination processes."[26]

We speculate that such radical-dioxygen triplet complexes may intervene in the chemistry of the bis-allylic peroxyl radicals that are intermediates in this study. The bis-allylic peroxyl radical derived from oxidation of an unsymmetrical diene such as **13** could rearrange to the conjugated peroxyls through two distinct isomeric radical-dioxygen complexes, **34** and **35**. The complex **34** derives from rearrangement across the trans alkene, while **35** is formed from rearrangement across the cis double bond. The calculated low-energy conformation of **33** is a typical five-membered ring envelope structure, and based upon that structure, one anticipates that the formation of **34** would be favored over that of **35**.[29] Substitution in **34** is diequatorial on the ring, while **35** must have one axial substituent. Thus, product distribution in the oxidation may be biased by the preferential formation of **34** over **35**, the consequence of which is the formation of the trans,cis hydroperoxide. Experiments on rearrangement of the bis-allylic hydroperoxides are currently underway in our laboratory to support or deny the intermediacy of radical-dioxygen complexes in these rearrangements. We believe such species may well be intermediates in the first addition of oxygen to unsaturated carbon radicals in chain oxidation.

Conclusion

The autoxidations of cis,cis, cis,trans, and trans,trans nonconjugated dienes and octadecadienoates give rise to three kinetically controlled hydroperoxides. Formation of the bis-allylic peroxyl radical and its subsequent fragmentation depend on the geometry 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 the major kinetic product for each of the systems studied. Unpaired spin density is not the only factor that determines the position of oxygen addition to a delocalized radical, however, and we speculate that radical-triplet dioxygen complexes may be intermediates in the formation and rearrangement of delocalized radicals. Rearrangement of the bis-

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(29) It has been shown that for allylperoxyl rearrangements, the trans allyl radical-dioxygen reaction is faster than the cis allyl radical-dioxygen reaction (see ref 27c).

allylic peroxyls to conjugated peroxyls occurs with a rate constant between 2.2 and 2.8 $\times$ 10^6 s^{-1}. Regardless of the mechanism by which this rearrangement takes place, this rearrangement can be used as a peroxyl radical clock.[5] Although the previous studies were carried out using methyl linoleate in the clock reactions, any of the substrates discussed here could be used depending on the preference for HPLC or GC analysis. This offers a very straightforward method for determining the rate constant of H-atom transfer to a peroxyl in the kinetic range of 10^5 to 10^6 M^{-1} s^{-1}.[5,30] Although the formation of bis-allylic hydroperoxides only can be observed at concentrations of α-tocopherol that are not normally encountered in vivo, the formation of these products may become important in confined systems, such as an enzyme active site[4b] or a lipid particle having excellent H-atom donors present.[20c,30,31]

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were collected on a 300 or 400 MHz NMR, using Bruker software. HPLC analyses were carried out with a Waters 600 liquid chromatograph interfaced to a Waters 996 PDA detector. Linoleate hydroperoxides were separated on a Beckman Ultrasphere silica column (0.46 $\times$ 25 cm^2) using 0.5% ^{i}PrOH/hexanes at 1.0 mL/min and detected at 207 nm. GC analyses were carried out with a Hewlett-Packard 6890 gas chromotagraph equipped with a DB-5 (30 m $\times$ 0.32 mm $\times$ 0.25 mm) fused silica column from J&W Scientific. The diene alcohols were separated using a temperature program of 100−180 °C @ 5 °C/min, 180−280 °C @ 20 °C/min (10 min). HPLC−MS analyses were carried out as previously described[13a] using a Beckman Ultrasphere narrowbore silica column (0.20 $\times$ 25 cm^2) with 0.5% ^{i}PrOH/hexanes (0.15 mL/min) and postcolumn addition of $AgBF_4$/^{i}PrOH (0.3 mM, 0.075 mL/min).

Methyl linoleate and linoelaidate were purchased from Nu-Chek Prep and chromatographed on silica (10% EtOAc/hexanes) prior to use. All other compounds were synthesized (see Supporting Information) and freshly chromatographed on silica (**10a** and **10b** with 10% EtOAc/hexanes, **12**−**14** with hexanes) prior to use to remove any oxidation products. The initiator, 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeOAMVN), was obtained from Wako and dried under high vacuum for 2 h. α-Tocopherol was purchased from Aldrich and purified by flash chromatography (10% EtOAc/hexanes on silica), protected from light and oxygen. It is crucial that the α-tocopherol be purified prior to use and dried overnight under high vacuum. Benzene was passed through a column of alumina.

General Procedure for Oxidations. Stock solutions of each octadecadienoate or diene (1.5−1.7 M), MeOAMVN (0.1 M), and α-tocopherol (1.0 M) were made up in benzene. For samples containing high concentrations, neat α-tocopherol (2.2 M) was used. Samples were made up in autosampler vials with a total reaction volume of 100 μL. It was important to add the solutions in the following order to avoid premature oxidation: α-tocopherol (0.05−1.8 M), octadecadienoate or diene (0.15−0.17 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 oxidation was stopped by the addition of BHT (50 μL of 1.0 M solution in hexanes), followed by the addition of the internal standard (for octadecadienoates, 5 mM benzyl alcohol; for dienes, 5 mM tetradecane). The octadecadienoate samples were diluted to 1.0 mL with hexanes and analyzed by HPLC as their hydroperoxides. The diene samples were reduced with PPh_3 (50 μL of 1.0 M solution/hexanes) and analyzed by GC.

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Supporting Information Available: Experimental procedures for the synthesis of all compounds, HPLC and GC standardization of octadecadienoates and dienes, and plots of mole fraction of oxidation products of **10b**, **11**, and the dienes (**12**−**14**). This material is available free of charge via the Internet at http://pubs.acs.org.

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