J|A|C|S
ARTICLES
Published on Web 10/08/2002

The Kinetics of Thiyl Radical-Induced Reactions of Monounsaturated Fatty Acid Esters

Chryssostomos Chatgilialoglu,* Alessio Altieri, and Hanns Fischer*,†

Contribution from the ISOF, Consiglio Nazionale delle Ricerche, Via P. Gobetti 101, 40129 Bologna, Italy

Received June 24, 2002

Abstract: The time-dependent isomerizations and thiol additions of several *Z*- and *E*-monounsaturated fatty acid methyl esters catalyzed by alkanethiyl radicals during γ-radiolysis of *tert*-butyl alcohol solutions are analyzed on the basis of the radiation chemical yield of radicals and established rate data. This provides room-temperature rate constants for the reversible thiyl addition. Within experimental errors, they do not depend on the double bond position in the alkyl chains. Particularly noteworthy is the very fast β-elimination of thiyl radicals from alkyl radicals which carry a second β-substituent. It is supported by additional evidence obtained with a radical clock methodology, and the large preference of fragmentation to the *E*-isomers is attributed to different barriers for the formation of the *E*- and *Z*-transition states from the equilibrium radical structure.

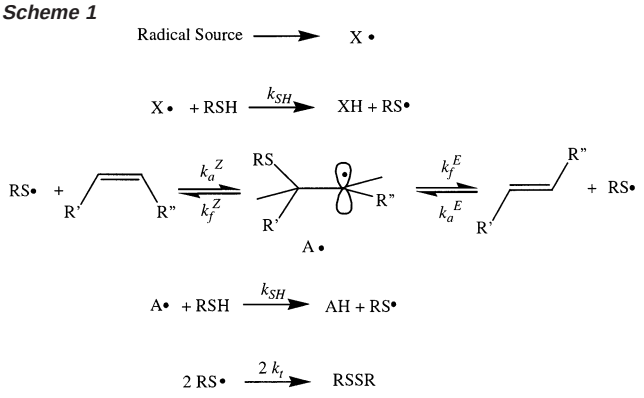
Introduction

The radical-catalyzed addition of thiols to unsaturated CC-bonds is a well-established chain reaction and has found many synthetic applications.[1,2] Thiol additions to *Z*- and *E*-alkenes are accompanied by isomerizations, and this has led to the general mechanism shown in Scheme 1 for a case including isomerization.

Sivertz et al.[3] and Walling et al.[4] found early on that the rate constants for the addition of thiyl radicals to the *Z*- and *E*-isomers differ from one another as do the fragmentation rate constants of the intermediate carbon-centered thiyl adduct radicals to the *Z*- and *E*-compounds, respectively. At small thiol concentrations, the isomerization is much faster than the thiol adduct formation. Hence, thiyl radicals efficiently catalyze the conversion of the naturally occurring *Z*-forms to the equilibrium of *Z*- and *E*-configurations of mono- or polyunsaturated fatty acid esters in which the thermodynamically more stable *E*-forms dominate.[5]

Recently, the thiyl radical-catalyzed isomerization has received additional attention because it may contribute to the *Z*- to *E*-conversion of natural unsaturated phospholipids in cell membranes and cause detrimental structural changes.[6−8] In fact, the isomerization was observed for mono- and polyunsaturated

Scheme 1



species under biomimetic conditions, that is, using organized systems such as lipid vesicles and biologically relevant thiols such as glutathione.[6] This process, which does not cause lipid degradation but a permanent modification of these membrane constituents, can ultimately influence the barrier properties and functions of biological membranes.

So far, there is little information on the rate constants of the individual reactions of Scheme 1 which could help to model

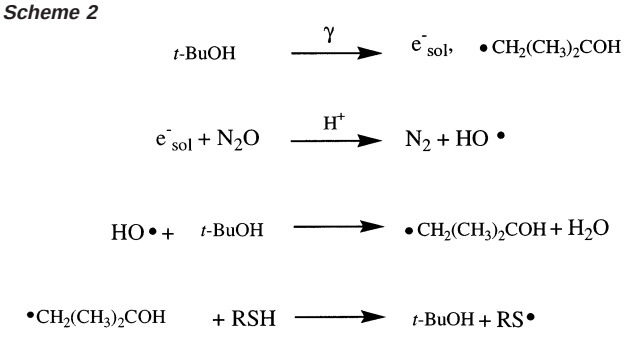
* To whom correspondence should be addressed. E-mail: chrys@area.bo.cnr.it.

† Permanent address: Physikalisch-Chemisches Institut, University of Zuerich, Winterthurer Strasse 190, 8057 Zuerich, Switzerland.

(1) Walling, C. *Free Radicals in Solution*; Wiley: New York, 1957.
(2) Chatgilialoglu, C.; Bertrand, M. P.; Ferreri, C. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; Wiley: New York, 1999. Chatgilialoglu, C.; Guerra, M. In *Supplement S: The Chemistry of Sulphur-Containing Functional Groups*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1993.
(3) Pallen, R. H.; Sivertz, C. *Can. J. Chem.* **1957**, *35*, 723. Graham, D. M.; Mieville, R. L.; Sivertz, C. *Can. J. Chem.* **1964**, *42*, 2239. Graham, D. M.; Mieville, R. L.; Pallen, R. H.; Sivertz, C. *Can. J. Chem.* **1964**, *42*, 2250.
(4) Walling, C.; Helmreich, W. *J. Am. Chem. Soc.* **1959**, *81*, 1144.
(5) Sgoutas, D. S.; Kummerow, F. A. *Lipids* **1969**, *4*, 283.
(6) Ferreri, C.; Costantino, C.; Perrotta, L.; Landi, L.; Mulazzani, Q. G.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **2001**, *123*, 4459. Chatgilialoglu, C.; Ferreri, C.; Ballestri, M.; Mulazzani, Q. G.; Landi, L. *J. Am. Chem. Soc.* **2000**, *122*, 4593. Ferreri, C.; Costantino, C.; Landi, L.; Mulazzani, Q. G.; Chatgilialoglu, C. *Chem. Commun.* **1999**, *5*, 407. Chatgilialoglu, C.; Ballestri, M.; Ferreri, C.; Vecchi, D. *J. Org. Chem.* **1995**, *60*, 3826.
(7) Adhikari, S.; Sprinz, H.; Brede, O. *Res. Chem. Intermed.* **2001**, *4*, 5, 549. Sprinz, H.; Schwinn, J.; Naumov, S.; Brede, O. *Biochim. Biophys. Acta* **2000**, *1483*, 91. Schwinn, J.; Sprinz, H.; Droessler, K.; Leistner, S.; Brede, O. *Int. J. Radiat. Biol.* **1998**, *74*, 359.
(8) (a) The effectiveness of *Z*−*E* isomerization by thiyl radicals in the presence of the most common antioxidants vitamins has also been addressed, see: Chatgilialoglu, C.; Zambonin, L.; Altieri, A.; Ferreri, C.; Mulazzani, Q. G.; Landi, L. *Free Radical Biol. Med.* **2002**, in press. (b) For $\cdot NO_2$ radical induces *Z*−*E* isomerization of arachidonic acid within cellular phospholidids, see: Jiang, H.; Kruger, N.; Lahiri, D. R.; Wang, D.; Vatèle, J.-M.; Balazy, M. *J. Biol. Chem.* **1999**, *274*, 16235.

10.1021/ja027428d CCC: $22.00

Scheme 2



biological processes. Furthermore, the literature is in part controversial. The product distribution study of Walling et al.[4] suggests quite large rate constants $k_f \gg 10^7$ s^{-1} for the β-elimination of the $CH_3S\cdot$ radical from its adducts to 2-butenes, whereas other authors[7,9] attribute at least 2 orders of magnitude smaller values to k_f for the adducts of several alkanethiyl species to mono- and polyunsaturated fatty acid esters. Moreover, in contrast to the earlier views,[1−4] equal rate constants were attributed to the different addition and fragmentation reactions.[7,9]

To obtain more kinetic information, we have extended our previous studies of thiyl radical-induced isomerizations.[6] This work presents the kinetics and the product yields of isomerization and thiol adduct formation for a variety of *Z*- and *E*-monounsaturated fatty acid esters in *tert*-butyl alcohol solution at room temperature and for a wide range of thiol concentrations. As thiol, β-mercaptoethanol was used in most cases. 1-Butanethiol was also employed and provided practically identical results. The choice of these nonbiologically relevant thiols was obligatory to quantify the corresponding adducts and the disulfides (Scheme 1). The reactions were initiated by continuous ^{60}Co γ-radiation of deoxygenated and N_2O saturated solutions. This generates thiyl radicals according to Scheme 2, and their radiation chemical yield was measured. Using this and the established rate constants for the self-termination of alkanethiyl radicals and for the hydrogen abstraction from alkanethiols, the data provide the rate constants of addition and fragmentation. The latter are rather large but are supported by the fragmentation rates of independently produced β-alkanethio substituted alkyl radicals as determined from the product yields of competing reactions.

Results and Discussion

Radiation Chemical Yield and Thiyl Radical Concentrations. The analysis of the isomerization kinetics required the knowledge of the thiyl radical concentrations during the continuous reactions. It was calculated from their radiation chemical yield. To determine this, deoxygenated and N_2O saturated 1-butanethiol solutions in *tert*-butyl alcohol were exposed to total doses of 6.5 and 18.1 kGy. Butyl disulfide RSSR was the only product found by GC analysis. It is formed by the coupling of 1-butanethiyl radicals, and blank experiments ensured its formation only in the presence of radiation. Apparently, the primary species formed by the radiolysis abstract hydrogen atoms from the thiol much faster than they terminate. The yield of the disulfide amounts to one-half of the thiyl radical yield, and two experiments with thiol consumptions of at most 13% led to $G(RS\cdot) = (0.98 \pm 0.04) \times 10^{-6}$ mol/J, which is significantly larger than $G = 0.65 \times 10^{-6}$ mol/J assumed before,[6] and we expect the same value for β-mercaptoethanol.

The thiyl radical yield provides the radical generation rate R_i from the dose rate $\dot{D}$ in Gy/min and the solvent density $\rho = 0.775$ kg/dm^3 as $R_i = \dot{D}G\rho/60$. In the studies described below, the total doses did not exceed 4 kGy and led to a maximum thiol consumption by the thiyl self-termination of 3×10^{-3} M. This is small as compared to the minimum initial thiol concentration of 4.3×10^{-2} M. Also, the thiol consumption by thiol adduct formation was much smaller than the initial thiol concentration; that is, the thiol consumption was always negligible, and R_i was constant.

From the radical generation rate R_i and the known thiyl self-termination rate constant[10] $2k_t = 3 \times 10^9$ M^{-1} s^{-1}, one obtains the steady-state concentration of thiyl radicals $[RS\cdot] = (R_i/2k_t)^{1/2}$. In the course of this work, the dose rate decreased from 23.0 to 16.8 Gy/min. Therefore, R_i decreased from about 3.0×10^{-7} to 2.2×10^{-7} M/s, and the steady-state thiyl radical concentration decreased from 10×10^{-9} to 8.6×10^{-9} M. This was taken into account in the analysis.

As mentioned above, the rate constant for the hydrogen atom transfer from the thiol to the primary alkyl radical $\cdot CH_2(CH_3)_2$-COH must be rather large. In fact, several authors[11,12] have determined rate constants very close to 1.0×10^7 M^{-1} s^{-1} for the reaction between alkanethiols and primary, secondary, and tertiary alkyl radicals and biradicals at room temperature. The Arrhenius parameters for the reaction of primary alkyl radicals with *t*-BuSH are $\log(A/M^{-1}\ s^{-1}) = 8.15(0.18)$ and $E_a = 1.86$-(0.23) kcal/mol.[12] Hence, the rate of hydrogen abstraction from the thiol by $\cdot CH_2(CH_3)_2COH$ is about $k_{SH}[RSH] = 7.5 \times 10^5$ s^{-1} for [RSH] = 0.075 M, and this is much larger than the overall termination rate $(2k_tR_i)^{1/2} \approx 30$ s^{-1}.

Isomerization Mechanism and Kinetics. The product distribution obtained by GC analysis after radiolysis of *tert*-butyl alcohol solutions of monounsaturated fatty acid esters (0.15 M) containing 0.043 to 1.3 M β-mercaptoethanol agrees very well with the mechanism of Scheme 1. The products were the isomer of the starting ester and the thiol adduct, and the yield of the latter increased with increasing thiol concentration. The disulfide formed by coupling of thiyl radicals appeared as minor product but in the concentrations expected from the thiyl radical radiation chemical yield. Traces of unidentified species amounted to at most 1% of the total products.

Figure 1 displays the development of the isomer concentrations as functions of the radiation dose during irradiations of four *Z*-compounds methyl oleate, methyl palmitoleate, methyl Z-vaccenate, and oleic acid 0.15 M in *tert*-butyl alcohol and in the presence of β-mercaptoethanol in low concentration (0.075 M). Within the experimental errors, the four compounds approach the same equilibrium of isomers within the same time; that is, the equilibrium and the rate constants are practically

(9) Schoeneich, C.; Asmus, K.-D.; Dillinger, U.; von Bruchhausen, F. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 113. Schoeneich, C.; Bonifacic, M.; Dillinger, U.; Asmus, K.-D. In *Sulfur Centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; Plenum Press: New York, 1990; p 367. Schoeneich, C.; Dillinger, U.; von Bruchhausen, F.; Asmus, K.-D. *Arch. Biochem. Biophys*. **1992**, *292*, 456.

(10) Self-termination constants of aliphatic thiyl radicals in liquid solution are close to the diffusion controlled limit, for example, *t*-BuS$\cdot$, $2k_t = (2-4) \times 10^9$ M^{-1} s^{-1} at room temperature in *n*-hexane. Ito, O.; Matsuda, M. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 427.

(11) Tronche, C.; Martinez, F. N.; Horner, J. H.; Newcomb, M.; Senn, M.; Giese, B. *Tetrahedron Lett.* **1996**, *37*, 5845. Enzinas, M. V.; Wagner, P. J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 1357.

(12) Newcomb, M.; Choi, S.-Y.; Horner, J. H. *J. Org. Chem.* **1999**, *64*, 1225.

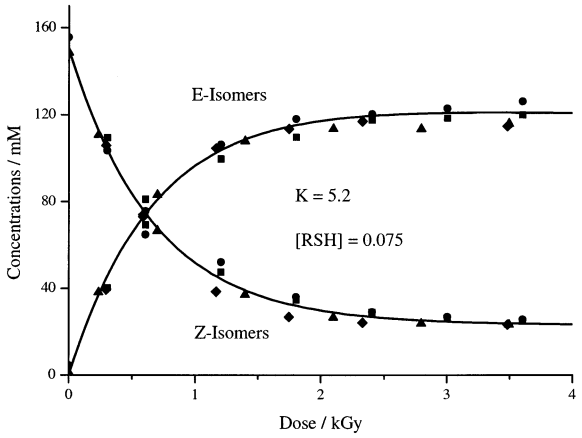


Figure 1. Isomerization of different monounsaturated fatty acid methyl esters (0.15 M) by β-mercaptoethanol derived thiyl radicals in *tert*-butyl alcohol starting from the *Z*-isomers at a low thiol concentration [RSH] = 0.075 M. ▲, oleate; ■, palmitoleate; ◆, *Z*-vaccenate; ●, oleic acid. For solid lines, see text.

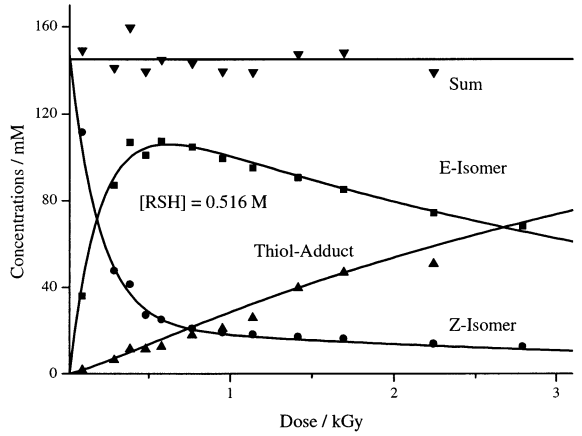


Figure 2. Isomerization of methyl oleate (0.15 M) by β-mercaptoethanol derived thiyl radicals in *tert*-butyl alcohol and formation of the thiol adduct for [RSH] = 0.516 M. For solid lines, see text.

equal. At the low thiol concentration, the total concentration of the unsaturated compounds remains approximately constant. This means that the fragmentation of the intermediate radical A• is faster than its reaction with the thiol to the adduct AH.

For larger thiol concentrations, there is loss of unsaturation. It is shown in Figure 2 for the reaction of methyl oleate in the presence of 0.516 M β-mercaptoethanol. As it was found also in many other cases, the loss of unsaturated esters is exactly balanced by the formation of the thiol adduct. Figures 3 and 4 show that the same equilibria are reached in practically equal times if one starts either from the *Z*- or from the *E*-isomers both for small and for appreciable thiol concentrations. Finally, Figure 5 compares the isomerizations of methyl oleate at two thiol concentrations as another example for the increasing loss of unsaturation at high thiol concentrations.

All of this supports the mechanism of Scheme 1, but side reactions are also at work. They give the same products but change the rates. Thus, for the rather high thiol concentrations of 0.30 and 0.93 M, blank experiments on nonirradiated samples showed some isomerization. This indicates an unknown additional thermal generation of thiyl radicals. Furthermore, several runs exhibited a small induction period such as those seen in Figures 3 and 5. It is probably due to thiyl radical removal by unknown and minor scavenging impurities. Because of their

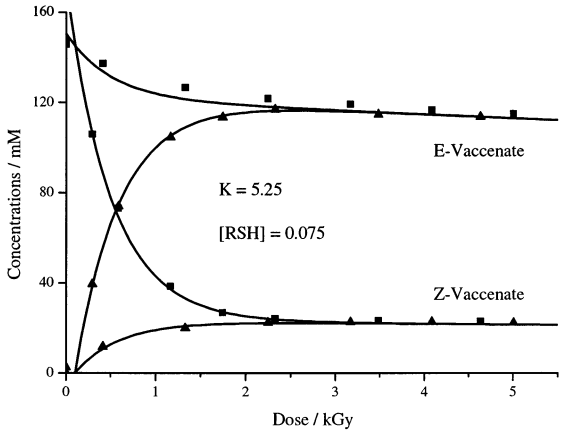


Figure 3. Isomerization of *E*- and *Z*-vaccenate (0.15 M) by β-mercaptoethanol derived thiyl radicals in *tert*-butyl alcohol at a low thiol concentration [RSH] = 0.075 M. For solid lines, see text.

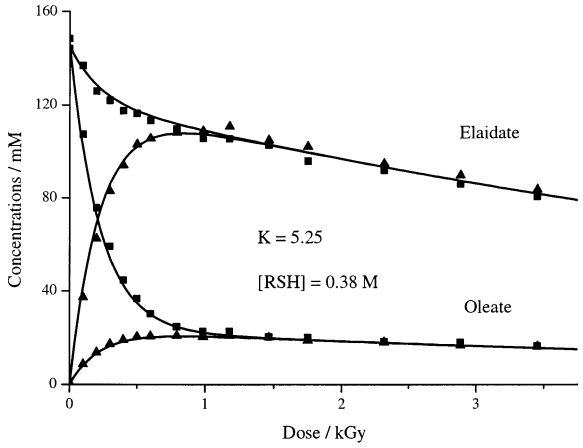


Figure 4. Isomerization of methyl oleate and methyl elaidate (0.15 M) by β-mercaptoethanol derived thiyl radicals in *tert*-butyl alcohol for [RSH] = 0.38 M. For solid lines, see text.

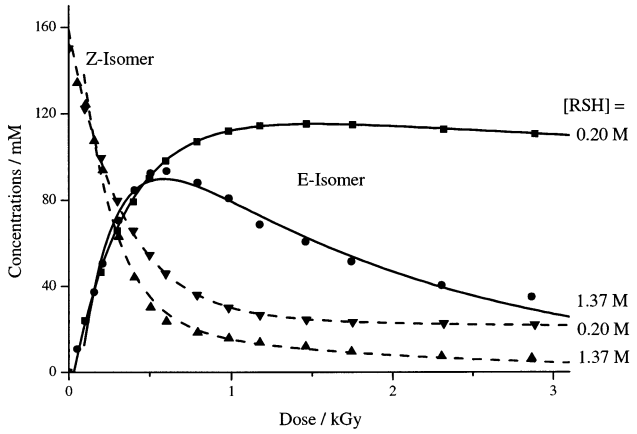


Figure 5. Isomerization of methyl oleate (0.15 M) by β-mercaptoethanol derived thiyl radicals in *tert*-butyl alcohol at thiol concentrations [RSH] = 0.2 M and [RSH] = 1.37 M. For solid lines, see text.

spurious appearance, the side reactions are ignored in the following analysis of the isomerization kinetics but lead to appreciable errors of the resulting rate constants.

According to the product distribution and supported by the large rate constant for the hydrogen abstraction, the only relevant radical termination is the bimolecular coupling of the thiyl radicals RS•. Hence, the concentrations of the primary 2-hy-

droxypropyl substituted methyl radicals X· and of the carbon-centered thiyl radical adducts A· are much smaller than the concentration of RS·.

The concentration of A· obeys

$$\frac{d[A\cdot]}{dt} = (k_a^Z[Z] + k_a^E[E])[RS\cdot] - (k_f^Z + k_f^E)[A\cdot] - k_{SH}[RSH][A\cdot] \quad (1)$$

and in the steady state, one has

$$[A\cdot] = \frac{k_a^Z[Z] + k_a^E[E]}{k_f^Z + k_f^E + k_{SH}[RSH]}[RS\cdot] \quad (2)$$

The concentrations of the unsaturated fatty acid esters obey the rate equations

$$\frac{d[Z]}{dt} = -k_a^Z[Z][RS\cdot] + k_f^Z[A\cdot] \quad (3a)$$

$$\frac{d[E]}{dt} = -k_a^E[E][RS\cdot] + k_f^E[A\cdot] \quad (3b)$$

or with eq 2

$$\frac{d[Z]}{dt} = -\frac{k_a^Z(k_f^E + k_{SH}[RSH])[RS\cdot]}{k_f^Z + k_f^E + k_{SH}[RSH]}[Z] + \frac{k_a^E k_f^Z[RS\cdot]}{k_f^Z + k_f^E + k_{SH}[RSH]}[E] = -k_{11}[Z] + k_{12}[E] \quad (4a)$$

$$\frac{d[E]}{dt} = \frac{k_a^Z k_f^E[RS\cdot]}{k_f^Z + k_f^E + k_{SH}[RSH]}[Z] - \frac{k_a^E(k_f^Z + k_{SH}[RSH])[RS\cdot]}{k_f^Z + k_f^E + k_{SH}[RSH]}[E] = k_{21}[Z] - k_{22}[E] \quad (4b)$$

The total concentration of the isomers decreases according to

$$\frac{d([Z] + [E])}{dt} = -\frac{(k_a^Z[Z] + k_a^E[E])[RS\cdot]}{k_f^Z + k_f^E + k_{SH}[RSH]}k_{SH}[RSH] \quad (4c)$$

and it is exactly matched by the thiol adduct formation. When a decay of the total unsaturation was observed, that is, for thiol concentrations larger than about 0.1 M (Figures 2 and 5), it vastly exceeded the maximum formation of the disulfide of 3 mM, and, therefore, the loss of unsaturation also equals the net loss of thiol.

Equations 4 lead to expressions for initial rate ratios and

$$\left(\frac{d[RSH]}{d[E]}\right)_{t\to 0} = -\left(\frac{d([Z] + [E])}{d[E]}\right)_{t\to 0} = -\frac{k_{SH}[RSH]}{k_f^E} \quad \text{for } [E]_0 = 0 \quad (5a)$$

$$\left(\frac{d[RSH]}{d[Z]}\right)_{t\to 0} = -\left(\frac{d([Z] + [E])}{d[Z]}\right)_{t\to 0} = -\frac{k_{SH}[RSH]}{k_f^Z} \quad \text{for } [Z]_0 = 0 \quad (5b)$$

These were used by Walling and Helmreich[4] to obtain k_{SH}/k_f for the isomerizations of *Z*- and *E*-2-butene by methyl thiyl radicals. However, because of the small induction periods, extrapolations of reaction rates to zero time were not practical here, and a different analysis was used.

For reactions starting from the pure *Z*-isomer, the integration of eqs 4a and 4b provides

$$[Z] = \left[\frac{k_{11} - \lambda_2}{\lambda_1 - \lambda_2}\exp(-\lambda_1 t) - \frac{k_{11} - \lambda_1}{\lambda_1 - \lambda_2}\exp(-\lambda_2 t)\right]\cdot[Z]_0 = [b\exp(-\lambda_1 t) + (1 - b)\exp(-\lambda_2 t)]\cdot[Z]_0 \quad (6a)$$

$$[E] = \frac{k_{21}}{\lambda_1 - \lambda_2}[\exp(-\lambda_2 t) - \exp(-\lambda_1 t)]\cdot[Z]_0 = a[\exp(-\lambda_2 t) - \exp(-\lambda_1 t)]\cdot[Z]_0 \quad (6b)$$

and for initially pure *E*-isomer

$$[Z] = \frac{k_{12}}{\lambda_1 - \lambda_2}[\exp(-\lambda_2 t) - \exp(-\lambda_1 t)]\cdot[E]_0 = c[\exp(-\lambda_2 t) - \exp(-\lambda_1 t)]\cdot[E]_0 \quad (7a)$$

$$[E] = \left[\frac{k_{22} - \lambda_2}{\lambda_1 - \lambda_2}\exp(-\lambda_1 t) - \frac{k_{22} - \lambda_1}{\lambda_1 - \lambda_2}\exp(-\lambda_2 t)\right]\cdot[E]_0 = [d\exp(-\lambda_1 t) + (1 - d)\exp(-\lambda_2 t)]\cdot[E]_0 \quad (7b)$$

where

$$\lambda_{1,2} = {}^1/_2(k_{11} + k_{22}) \pm ({}^1/_4(k_{11} - k_{22})^2 + k_{12}k_{21})^{1/2} \quad (8)$$

Hence, the time dependencies of the isomer concentrations are sums of two exponential functions. Such functions were constructed to reproduce the measured concentrations [*Z*] and [*E*] as functions of the dose, allowing for the induction when necessary. Examples are shown in Figures 1−5. This yielded the dimensionless parameters *a*−*d* and λ_1 and λ_2 per dose units. The latter were converted to reciprocal time units via 1 kGy ≡ $6 \times 10^4 \dot{D}t$ with the dose rate in Gy/min and *t* in s.

For small thiol concentrations [RSH] < 0.1 M, the total isomer concentration [*Z*] + [*E*] is practically constant (Figures 1, 3), and the fragmentation of the thiyl radical adduct occurs much faster than the hydrogen abstraction from the thiol. Hence, the hydrogen abstraction can be neglected. One then has $\lambda_2 \approx 0$, and

$$K = \frac{[E]_\infty}{[Z]_\infty} = \frac{k_a^Z}{k_f^Z}\frac{k_f^E}{k_a^E} = \frac{K^Z}{K^E} \quad (9a)$$

$$\lambda_1 = \frac{k_a^Z k_f^E + k_a^E k_f^Z}{k_f^E + k_f^Z}[S\cdot] = k_{is}[S\cdot] \quad (9b)$$

Equations 6 and 7 become monoexponential, and fits to these provide the equilibrium constant *K* and an effective isomerization rate constant $k_{is} = \lambda_1/[RS\cdot]$. The latter is the average of the addition constants weighted by the fragmentation ratios. However, *K* and k_{is} alone do not lead to the individual addition and fragmentation rate constants.

For [RSH] > 0.1 M, the hydrogen abstraction reaction competes with fragmentation. For this case, one derives from

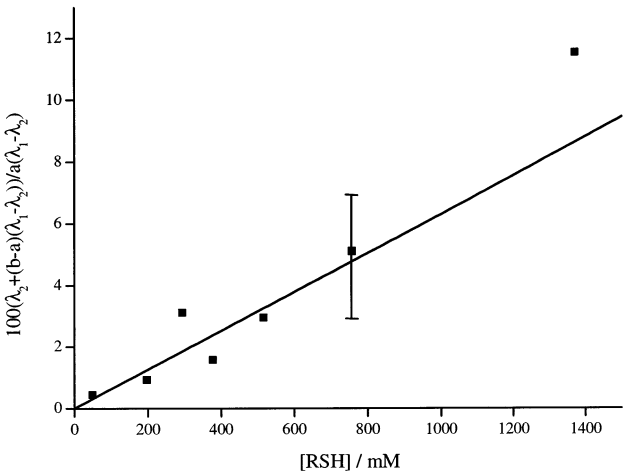


Figure 6. Competition between hydrogen abstraction and radical fragmentation during the isomerization of methyl oleate (0.15 M) versus the thiol concentration. The linear increase is expected from eq 10a.

eqs 4 and 6−8 the general relations

$$\frac{\lambda_2 + (b-a)(\lambda_1 - \lambda_2)}{a(\lambda_1 - \lambda_2)} = \frac{k_{SH}[RSH]}{k_f^E} \qquad \text{for } [E]_0 = 0 \quad (10a)$$

$$\frac{\lambda_2 + (d-c)(\lambda_1 - \lambda_2)}{c(\lambda_1 - \lambda_2)} = \frac{k_{SH}[RSH]}{k_f^Z} \qquad \text{for } [Z]_0 = 0 \quad (10b)$$

which are formally identical to eqs 5. The rate constant $k_{SH} = 1.0 \times 10^7$ M^{-1} s^{-1} for the hydrogen transfer from the thiol to the secondary thiyl adduct radical is known,[11,12] and the application of eqs 10 to the data then yields the fragmentation constants k_f^E and k_f^Z.

Furthermore, one notices from Figures 1−5 that the isomerization occurs well before the loss of unsaturation, and this held in all of the cases studied. It means $\lambda_2 \ll \lambda_1$, and, under this condition, λ_1 is still well approximated by eq 9b and can be used to obtain k_{is}. The isomer ratio at long times provides the total equilibrium constant $K = a/(1-b) = (1-d)/c$ (eq 9a), and λ_2 is given by

$$\lambda_2 = \frac{k_{SH}[RSH]}{k_f^E} \frac{K}{1+K} k_a^E[RS\cdot] \qquad \text{for } [E]_0 = 0 \qquad (11a)$$

$$\lambda_2 = \frac{k_{SH}[RSH]}{k_f^Z} \frac{1}{1+K} k_a^Z[RS\cdot] \qquad \text{for } [Z]_0 = 0 \qquad (11b)$$

In detail, for reactions starting with the *Z*-isomer ($[E]_0 = 0$), the equilibrium constant *K* was obtained from the isomer ratios at long times, and k_{is} followed from λ_1. For sufficiently large thiol concentrations, the fragmentation constant k_f^E was deduced via eq 10a (Figure 6), and the addition constant k_a^E was determined using eq 11a. The remaining parameters k_f^E and k_a^E were then extracted from *K* and k_{is}. Hence, all of the desired rate constants were obtained alone from experiments employing the *Z*-isomers as starting compounds, but the same parameters also followed from the analogous analysis of reactions starting with the *E*-isomers.

In total, isomerizations starting from *Z*-isomers methyl oleate and β-mercaptoethanol or 1-butanethiol ([RSH] = 0.049−1.37 M), palmitoleate, *Z*-vaccenate, oleic acid, and β-mercaptoethanol ([RSH] = 0.075 M) and starting from *E*-isomers methyl elaidate, *E*-vaccenate, and β-mercaptoethanol ([RSH] = 0.38 and 0.075 M, respectively) were analyzed. Essentially the same rate constants were obtained for the different starting compounds.

The reproducibilities of the equilibrium constant *K* and of the parameters *a*, *b*, *c*, and *d* were excellent, but λ_1 and λ_2 showed a considerable scatter. Especially for low and for high thiol concentrations did outliers from averages occur, but, as was mentioned above, in these cases side reactions are likely. Hence, we present in Table 1 the average rate constants obtained from a set of 10 experiments with *Z*-isomers without the apparent outliers and from three runs with *E*-isomers. The same average rate constants resulted from the two sets.

***β*-Elimination of Thiyl Radicals.** Support for the results is obtained from the fragmentation constants k_f of β-alkanethio substituted alkyl radicals measured relative to a rate constant other than the hydrogen abstraction from the thiol with a free radical clock methodology.[13] For that purpose, the reactions of Scheme 3 were used.[6]

For sufficiently high concentrations of $(TMS)_3SiH$, the radical **2** partitions between the hydrogen abstraction k_H and the β-elimination k_f. The hydrogen transfer from $(TMS)_3SiH$ to the thiyl radical is efficient. Hence, the ratio of the concentrations of the products alkane and thiol reflects the ratio of rate constants.

$$\frac{[\text{alkane}]}{[RSH]} = \frac{k_H}{k_f}[(TMS)_3SiH] \qquad (12)$$

As before,[6] the yields of alkane and thiol were determined by GC analysis, following a photochemical radical generation and using an internal standard.

The radical **2a** derived from compound **1a** carries methyl groups in the α- and β-positions and closely resembles the intermediate A· of the fatty acid ester isomerizations. From nine experiments at 203 K with silane concentrations ranging from 0.2 to 1 M, a linear dependence of the product ratio on the $(TMS)_3SiH$ concentration with negligible intercept was found. Equation 12 led to $k_H/k_f = (0.15 \pm 0.02)$ M^{-1}, where $k_f = k_f^E + k_f^Z$. In the earlier work,[6] we found $k_H/k_f = (0.37 \pm 0.09)$ M^{-1} from six data at the same temperature, but we consider this value less reliable now. At temperatures above 203 K, k_H/k_f could not be measured because the product ratio increased too little with increasing $(TMS)_3SiH$ concentration. This means that k_f increases more than k_H with increasing temperature.

The Arrhenius relation for the hydrogen abstraction from $(TMS)_3SiH$ by secondary alkyl radicals is $\log(k_H/M^{-1}\ s^{-1}) = 8.5-4.3/\Theta$, where $\Theta = 2.303RT$ in kcal/mol.[14] Hence, one has $k_H = 4700$ M^{-1} s^{-1} at 203 K and obtains $k_f = 3.1 \times 10^4$ s^{-1} for **2a** from k_H/k_f. The data given in Table 1 for room temperature are much larger, but this is in accord with a strong temperature dependence of the β-elimination.

This temperature dependence was addressed conveniently by using the same methodology with compound **1b** which lacks the α- and β-alkyl groups. Five series of experiments with silane concentrations between 0.2 and 1 M led to larger values of k_H/

(13) Newcomb, M. *Tetrahedron* **1993**, *49*, 1151. Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317.
(14) Chatgilialoglu, C.; Dickhaut, J.; Giese, B. *J. Org. Chem.* **1991**, *56*, 6399.

Table 1. Rate Constants for the Isomerization of Monounsaturated Fatty Acid Methyl Esters Catalyzed by Thiyl Radicals in *tert*-Butyl Alcohol at Room Temperature[a]

	runs	K	k_{is}/s^{-1}	k_f^E/s^{-1}	k_f^Z/s^{-1}	$k_a^E/M^{-1}s^{-1}$	$k_a^Z/M^{-1}s^{-1}$
$Z \rightarrow E$	10	5.1(0.1)	$1.4(0.3) \times 10^5$	$1.6(0.3) \times 10^8$	$2.2(0.9) \times 10^7$	$2.0(0.8) \times 10^5$	$1.5(0.3) \times 10^5$
$E \rightarrow Z$	3	5.1(0.1)	$1.1(0.4) \times 10^5$	$1.4(0.4) \times 10^8$	$2.2(0.6) \times 10^7$	$1.4(0.5) \times 10^5$	$1.1(0.4) \times 10^5$

[a] Errors in brackets represent one standard deviation.

Scheme 3

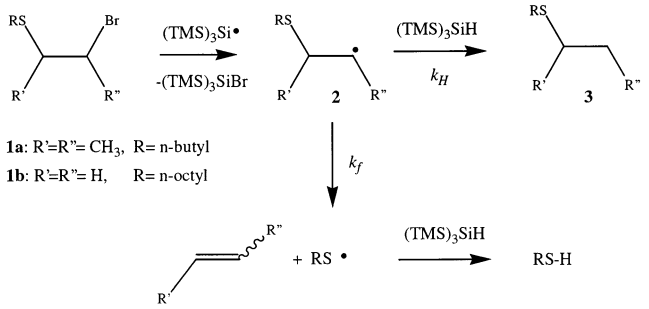


Table 2. Kinetic Data for the Reaction of Bromide **1** with $(TMS)_3SiH$ in Toluene at Various Temperatures[a]

T, K[b]	k_H/k_f, M^{-1}	intercept
253	0.82 ± 0.03	0.02 ± 0.02
273	0.48 ± 0.06	-0.02 ± 0.04
294	0.15 ± 0.01	0.02 ± 0.01
294[c]	0.18 ± 0.01	0.04 ± 0.01
313	0.08 ± 0.01	0.00 ± 0.01

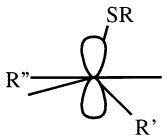
[a] Errors represent one standard deviation. Range of silane concentrations employed 0.2−1.0 M in at least five experiments. [b] Photochemical initiation. [c] Et_3B/O_2 initiation.

k_f which decrease with increasing temperature. They are given in Table 2 and provide the Arrhenius expression $\log((k_H/k_f)\ M^{-1}) = (-5.4 \pm 0.4) + (6.2 \pm 0.6)/\Theta$. Combination with $\log(k_H/M^{-1}\ s^{-1}) = (8.9 \pm 0.4) - (4.5 \pm 0.5)/\Theta$ for the hydrogen abstraction from the silane by primary alkyl radicals[14] provides $\log(k_f/s^{-1}) = (14.2 \pm 0.8) - (10.6 \pm 1.1)/\Theta$. The frequency factor is in the expected range for a unimolecular decomposition, and the rather larger activation energy supports the strong temperature dependence of the fragmentation indicated above.

For the β-elimination from the primary alkyl radical **2b**, one extrapolates $k_f = 560\ s^{-1}$ at 203 K. For the secondary alkyl radical **2a**, we found $k_f = 3.1 \times 10^4\ s^{-1}$ at the same temperature; that is, its fragmentation is 56 times faster. This is probably due to steric strain effects of the additional alkyl groups (see below). If the same rate ratio holds at room temperature, the rate constant $k_{f,298} = 2.5 \times 10^6\ s^{-1}$ for the primary species **2b** yields $k_{f,298} = 1.5 \times 10^8\ s^{-1}$ for the secondary **2a**. From the isomerization kinetics, we deduced $k_f^E + k_f^Z = (1.8 \pm 0.3) \times 10^8\ s^{-1}$ for such species at room temperature (Table 1), in excellent agreement with the extrapolated value for **2a**.

Discussion of the Isomerization Rate Data. Equilibrium Constant. $K = 5.1 \pm 0.1$ at room temperature agrees well with our earlier findings,[6] and $K = 4.3$ was reported for the equilibrium of methyl oleate and methyl elaidate in methanol by other authors.[7] For the structurally related 2-butenes, one calculates $K = 3.4$ at room temperature from the gas-phase molar enthalpy and entropy differences of the *Z*- and *E*-isomers $\Delta H = -4.4$ kJ/mol and $\Delta S = -4.3$ J/mol K.[3,15,16] Obviously, the equilibrium constant of the monounsaturated fatty acid esters is slightly larger than that of the 2-butenes presumably because of the longer alkyl chain residues. Furthermore, for dioleoyl phosphatidyl choline in *tert*-butyl alcohol, *K* decreases from 4.9 at 22 °C to 4.0 at 54 °C and 3.6 at 71 °C.[6] With $K = \exp(-\Delta G/RT)$, this provides $\Delta H = -5.4$ kJ/mol and $\Delta S = -5.5$ J/molK for the *Z*- to *E*-conversion, similar to the differences of the thermodynamic parameters of the 2-butenes.

Scheme 4



***β*-Elimination of Thiyl Radicals.** In the early classic study, Walling and Helmreich[4] related the rate of the azo-initiated isomerization of 2-butenes by methanethiyl in a 2-butene/butane mixture at 60 °C to the thiol consumption via eqs 5 and deduced $k_f^E/k_{SH} = 85$ and $k_f^Z/k_{SH} = 19.5$. With $k_{SH} = 1.0 \times 10^7\ M^{-1}\ s^{-1}$ at 25 °C and $E_a = 1.86(0.23)$ kcal/mol,[12] one derives $k_{SH} = 1.4 \times 10^7\ M^{-1}\ s^{-1}$ at 60 °C, and, hence, $k_f^E = 11.9 \times 10^8\ s^{-1}$ and $k_f^Z = 2.7 \times 10^8\ s^{-1}$. As expected, these rate constants are larger than those of the β-eliminations of the monounsaturated fatty acid esters at room temperature (Table 1) but support the latter. Further, the selectivity of the fragmentation for the 2-butenes $k_f^E/k_f^Z = 4.4$ at 60 °C[4] is smaller than $k_f^E/k_f^Z = 7.2$ observed at room temperature for the esters.[17]

$k_f^E \gg k_f^Z$ indicates that the barrier for the fragmentation to the more stable *E*-isomers is smaller than that to the less stable *Z*-alkenes. The molar enthalpy difference of the isomers of $\Delta H = h_f(E) - h_f(Z) = -(4-5)$ kJ/mol is small. It may contribute to the barrier difference, but this may also be explained by the different energies required for the formation of the transition states from the equilibrium structure of the intermediate radical A•. It carries one α- and two β-substituents, and such species adopt average conformations in which space demanding substituents avoid each other (Scheme 4). In the transition state for the β-elimination, the S-atom of the thiyl moiety eclipses the p_Z-axis of the unpaired electron orbital, and two structures are possible. The less energy demanding leads to the *E*- and the more costly one to the *Z*-configuration of the product, and consequently the formation of the *E*-isomer is preferred.

The steric effect also suggests β-alkanethio substituted alkyl radicals such as radicals A• and **2a** with a second β-substituent fragment considerably faster than species lacking this group such as **2b**, in accord with the experimental findings. α-Substituents

(15) *Landolt-Boernstein: Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik und Technik*, 6th Ed.; Schaefer, K., Lax, E., Eds.; Springer: Berlin, 1961; Vol 2, Part 4, p 263. $\Delta H = -4.4$ kJ/mol: Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Supplement 1.

(16) Graham, D. M.; Soltys, J. F. *Can. J. Chem.* **1969**, *47*, 2529. Graham, D. M.; Soltys, J. F. *Can. J. Chem.* **1969**, *47*, 2719. Graham, D. M.; Soltys, J. F. *Can. J. Chem.* **1970**, *48*, 2137.

(17) Boothe, T. E.; Greene, J. L., Jr.; Shevlin, P. B. *J. Am. Chem. Soc.* **1976**, *98*, 951 reports $k_f^E/k_f^Z = 2.3$ at 80 °C and $k_f^E/k_f^Z = 5.8$ at −67 °C for the elimination of benzenethiyl from its 2-butene adduct. $k_f^E > k_f^Z$ is also evident from the early gas phase work of Sivertz and Graham et al.,[3,16] and it is known for the fragmentation of the benzenethiyl radical from its β-methyl styrene adduct.[18]

alone do not lead to rate enhancements but seem to cause a slower fragmentation which may be due to radical stabilization. Thus, $k_{f,298} = 2.7 \times 10^5$ s^{-1} found by Wagner et al.[19] for the elimination of the 1-butanethiyl radical from a 1,4-diradical carrying an α-CH_2R and a β-CH_2SR group is smaller than $k_{f,298} = 2.5 \times 10^6$ s^{-1} for **2b** which has no α-substituent.

Addition of Thiyl Radicals to Alkenes. There are only very few literature data with which our addition rate constants can be compared. Nelson et al.[20] report an upper limit $k_a^Z < 3.4 \times 10^6$ M^{-1} s^{-1} for the gas-phase addition of the methanethiyl radical to *Z*-2-butene at room temperature. It agrees with our data, but the fast fragmentation was not taken into account. Ito et al.[18] followed the addition of arenethiyl radicals to a variety of alkenes. For the addition of benzenethiyl to monosubstituted nonconjugated monomers, the rate constants range from 1.7×10^4 (vinyl acetate) and 4.6×10^5 (acrylonitrile) to 2×10^7 M^{-1} s^{-1} (styrene) and exhibit enthalpic plus nucleophilic polar substituent effects. Alkanethiyls should add faster than the resonance stabilized benzenethiyl, and this agrees with our data. It is also noteworthy that the addition of benzenethiyl to the *E*-disubstituted alkenes is faster than to the *Z*-isomers (β-methyl styrene at room temperature $k_a^E = 6.8 \times 10^6$ M^{-1} s^{-1}, $k_a^Z = 2.0 \times 10^6$ M^{-1} s^{-1}, $k_a^E/k_a^Z = 3.4$). We find the same tendency for the alkanethiyl radical adding to the monounsaturated esters ($k_a^E/k_a^Z = 1.3$), and a faster addition to *E*- than to *Z*-isomers is also known for C-centered radicals.[21]

Finally, Griller et al.[22] determined the addition rate constant of *t*-BuS· to 1-octene at 298 K in isooctane as $k_a = 1.9 \times 10^6$ M^{-1} s^{-1} and confirmed an earlier value of Davies and Roberts.[23] Because of the absence of a substituent at the attacked site, the addition to 1-octene is expected to be faster than that to the monounsaturated fatty acid methyl esters (Table 1) as it is common for carbon-centered species.[21] Griller et al.[22] also measured $k_f/k_{SH} = 0.034$ for the 1-octene adduct. If we take $k_{SH} = 1.0 \times 10^7$ M^{-1} s^{-1} as before, the fragmentation constant becomes $k_f = 3.4 \times 10^5$ s^{-1}, similar to Wagner's value for the related elimination from a biradical[19] and smaller than the data of Table 1 because of the lacking β-substituent.

The above literature rate constants for the reactions involved in the thiyl radical-induced reactions of alkenes support the results of this work. In particular, the β-elimination of thiyl radicals from β-alkanethio substituted alkyl radicals is very fast if these carry a second β-substituent. However, recently quite different values have been suggested for the related isomerizations of polyunsaturated fatty acid residues (PUFA). Several authors[7,9] found that thiyl radicals abstract hydrogen atoms at the bisallylic positions and that this abstraction is increasingly inhibited by increasing PUFA or other alkene concentrations.[7] The latter findings were interpreted by a loss of thiyl radicals due to the formation of the adduct A·, and this was implicitly assumed to be irreversible in the observation times of about 50 μs. A competition analysis neglected the differences of the addition rate constants to the *E*- and *Z*-configurations as well as of the fragmentations, although this would lead to an unreasonable $K = 1$. It provided $k_a = 4.5 \times 10^6$ M^{-1} s^{-1} and $k_f = 3 \times 10^5$ s^{-1} for individual double bonds.[7,9] In comparison to the present results and the literature, k_a is by 1 order of magnitude larger and k_f is by nearly 3 orders smaller. Therefore, the observed inhibition of the formation of the bisallylic radical[7] may need a different explanation and experiments to clarify this point are in progress.

Experimental Section

Materials. The methyl esters of oleic, elaidic, palmitoleic, palmitelaidic, *Z*-vaccenic, *E*-vaccenic, and stearic acids, β-mercaptoethanol, 1-butanethiol, 2-hydroxyethyl disulfide, butyl disulfide, and *tert*-butyl alcohol were commercially available from Aldrich, Fluka, or Sigma and were used without further purification. 2-Bromoethylthiooctane (**1b**) was obtained by reaction of *n*-octyl thiolate with 2-bromoethanol followed by bromination,[24] whereas *threo*-2-bromo-3-butylthiobutane (**1a**) was obtained by reaction of *cis*-2-butene with in situ prepared BuSBr.[25] Ethylthiooctane (**3b**) and 2-butylthiobutane (**3a**) were obtained by reaction of bromoethane and 2-bromobutane with the corresponding thiolate, respectively.[24]

General Methods. GC analyses for the determination of the isomeric ratio of the fatty acid methyl esters were performed by using a Varian CP-3800 equipped with a flame ionization detector. As a stationary phase, a Rtx-2330 column (60 m × 0.25 mm of 10% cyanopropylphenyl and 90% biscyanopropyl polysiloxane) was used with helium as carrier gas (2 mL/min). Column heating to 156 °C for 40 min was followed by an increase of 10 °C/min up to 250 °C. The methyl esters were identified by comparison with the retention times of authentic samples.

GC analyses for the determination of the disulfide and/or methyloleate-thiol adduct yields were performed by using a Carlo Erba HRGC 5300 equipped with a flame ionization detector. As a stationary phase, a HP 5 column (30 m × 0.25 mm cross-linked 5% phenylsilicone) was used with helium as carrier gas (2 mL/min). Column heating to 50 °C for 5 min was followed by an increase of 15 °C/min up to 200 °C.

GC analyses for the kinetic experiments were performed by using a HP 5890 Series II equipped with a flame ionization detector. As a stationary phase, a HP 5 column (30 m × 0.25 mm cross-linked 5% phenylsilicone) was used with helium as carrier gas (2 mL/min). Column heating to 70 °C was followed by an increase of 15 °C/min up to 280 °C.

Continuous radiolyses were performed at room temperature (22 ± 2) °C on 100 μL samples using a ^{60}Co-Gammacell at different dose rates. The exact absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking $G(Fe^{3+}) = 1.61$ μmol J^{-1}.[26]

Preparation of Methyl Oleate/$HOCH_2CH_2SH$ Adduct. A N_2O-saturated *tert*-butyl alcohol solution (7.2 mL) containing methyl oleate (1.7 mmol) and $HOCH_2CH_2SH$ (8.16 mmol) was γ-irradiated at 22 °C (dose rate 19 Gy/min) overnight. The reaction mixture was evaporated under vacuum. Silica gel chromatography, using eluent cyclohexane with increasing amounts of acetone (up to 7.5%), provided 332 mg of pure adduct (52% yield) oil. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, $J = 7.6$ Hz, CH_3), 1.26 (m, 18H, CH_2), 1.40 (m, 4H, CH_2 γ to the sulfur atom), 1.52 (m, 4H, CH_2 β to the sulfur atom), 1.61 (m, 2H, CH_2 β to the carbonyl group), 2.30 (t, 2H, $J = 7.6$ Hz, CH_2 α to the carbonyl group), 2.58 (quintet, 1H, $J = 6.4$ Hz, H α to the sulfur atom), 2.71 (t, 2H, $J = 6$ Hz, CH_2S), 3.66 (s, 3H, OCH_3), 3.70 (t, 2H, $J = 6.4$ Hz, CH_2O).

(18) Ito, O. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; Wiley: New York, 1999. Ito, O. In *General Aspects of the Chemistry of Free Radicals*; Afassi, Z. B., Ed.; Wiley: New York, 1999. Ito, O.; Matsuda, M. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 427. Ito, O.; Matsuda, M. *J. Am. Chem. Soc.* **1979**, *101*, 1815, 5732.
(19) Wagner, P. J.; Sedon, J. H.; Lindstrom, M. J. *J. Am. Chem. Soc.* **1978**, *100*, 2579.
(20) Balla, R. J.; Weiner, B. R.; Nelson, H. H. *J. Am. Chem. Soc.* **1987**, *109*, 4808.
(21) Fischer, H.; Radom, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 1340.
(22) McPhee, D. J.; Campredon, M.; Lesage, M.; Griller, D. *J. Am. Chem. Soc.* **1989**, *111*, 7563. Griller, D.; Martinho Simoes, J. A. In *Sulfur Centered Reactive Intermediates in Chemistry and Biology*; Chatgilialoglu, C., Asmus, K.-D., Eds.; Plenum Press: New York, 1990; p 327.
(23) Davies, A. G.; Roberts, B. P. *J. Chem. Soc. B* **1972**, 1830.
(24) Salerni, O. L.; Clark, R. N.; Smart, B. E. *J. Chem. Soc. C* **1966**, 645.
(25) Trost, B. M.; Ziman, S. D. *J. Org. Chem.* **1973**, *38*, 932.
(26) Spinks, J. W. T.; Woods, R. J. *An Introduction to Radiation Chemistry*, 3rd ed.; Wiley: New York, 1990; p 100.

^{13}C NMR ($CDCl_3$): δ 14.4 (CH_3), 22.9, 25.12, 25.14, 26.8, 26.9, 27.0, 29.2, 29.3, 29.7, 29.8, 29.9, 30.0, 32.1, 34.1, 34.3, 35.1 (each CH_2), 45.9 (CH), 51.7 (CH_3), 60.8 (CH_2), 174.6 (C═O). GC/MS *m*/*z*, 329 ($M^+ - CH_2CH_2OH$), 297, 264, 243, 229, 187, 169, 155, 11, 87, 69, 55.

Isomerization of Fatty Acid Methyl Esters in *tert*-Butyl Alcohol. A N_2O-saturated *tert*-butyl alcohol solution of monounsaturated fatty acid methyl ester (0.15 M) containing thiol from 0.075 to 1.5 M was γ-irradiated at 22 °C (dose rate 17−23 Gy/min). Methyl esters of both cis and trans isomers and the thiol adduct were examined by GC analysis in comparison with the retention times of authentic samples. The saturated methyl stearate was used as the internal standard for quantitative studies.

Determination of *G*(RS·) Value. A N_2O-saturated *tert*-butyl alcohol solution of 75 mM BuSH was γ-irradiated at 22 °C (dose rate ca. 17 Gy/min). BuSSBu was the only product, and the calibration was performed by analyzing four standard solutions containing different ratios of BuSH and BuSSBu. Two experiments were carried out with a dose of 18.1 and 6.5 kGy, respectively, as determined by Fricke dosimetry with an augmentation of 2.5% to account for the different electronic densities of *tert*-butyl alcohol and water.[26] The *G*-value was calculated by assuming a solution density of 0.775 g/mL (density of pure *tert*-BuOH) and quantitative formation of disulfide. $G = (0.97 \pm 0.04)$ μmol/J is obtained.

Kinetic Experiments for the β-Elimination of the RS· Radical. Toluene containing a small amount of hexadecane as an internal GC standard was used as solvent. Bromide **1a** or **1b** (0.01 M) and $(TMS)_3SiH$ (0.2−1.0 M) were added, and the resulting solutions were degassed and photolyzed with a 1000 W high-pressure mercury lamp at the appropriate temperature for a few minutes. Alternatively, the radical initiator (Et_3B/O_2) was added via a syringe pump at 21 °C. The products of interest, that is, compounds **3a** and **3b** and RSH, were identified by comparison of their retention times with those of authentic materials.

Acknowledgment. H.F. thanks the ISOF, CNR Bologna, for the hospitality during academic visits in 2001 and 2002. We thank C. Ferreri and Q. G. Mulazzani for helpful discussions and M. Ballestri for technical assistence.

JA027428D