γ-Linolenic Acid Biosynthesis: Cryptoregiochemistry of Δ⁶ Desaturation

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The valuable nutraceutical γ-linolenic acid (GLA, (6*Z*,9*Z*,12*Z*)-octadecatrienoic acid) is biosynthesized by a series of regio- and stereoselective dehydrogenation reactions that are catalyzed by a set of enzymes known as fatty acid desaturases. As part of ongoing research into the mechanism of these remarkable catalysts, we have examined the cryptoregiochemistry (site of initial oxidation) of Δ⁶ desaturation as it occurs in the protozoan *Tetrahymena thermophila*. Two complementary approaches that address this issue are described. In the first set of experiments, we measured the individual primary deuterium kinetic isotope effects associated with the C−H bond cleavages at C-6 and C-7. Competition experiments using appropriately deuterium-labeled 4-thiasubstrates revealed that a large KIE ($k_H/k_D = 7.1 \pm 0.5$) was observed for the C−H bond-breaking step at C-6, whereas the C−H bond cleavage at C-7 was insensitive to deuterium substitution ($k_H/k_D = 1.04 \pm 0.05$). These results point to C-6 as the site of initial oxidation in Δ⁶ desaturation since the first chemical step in this type of reaction is rupture of a strong, unactivated C−H bond, an energetically difficult process that typically exhibits a large KIE. This conclusion was supported by the results of our second approach, which involved locating the position of the putative diiron oxo oxidant with respect to substrate by monitoring the efficiency of oxo transfer to a series of thia fatty acid probes. Thus only a 6-thia-analogue is converted to significant amounts of the corresponding sulfoxide (9% yield). The absolute configuration of this product was determined to be *S* using (*S*)-MPAA as a chiral shift reagent. Taken together, these results point to the abstraction of the C-6 *pro S* hydrogen as the initial event in Δ⁶ desaturation as it occurs in *T. thermophila*.

Introduction

γ -Linolenic acid (GLA, (6*Z*,9*Z*,12*Z*)-octadecatrienoic acid) is a rare plant fatty acid[1] that is widely used as a dietary supplement to ameliorate the symptoms of many pathological conditions including multiple sclerosis, atopic eczema, and mastalgia.[2] The key enzymatic step in the biosynthesis of GLA in mammalian systems is the Δ⁶ desaturation of a linoleyl substrate,[3] a process that appears to be suppressed in certain disease states (Scheme 1). Our interest in this reaction forms part of an ongoing research program directed at understanding how fatty acid desaturases dehydrogenate conformationally mobile substrates with such exquisite regiochemical control. Despite recent advances in the molecular and structural biology of this class of enzymes,[4−6] the precise details of how fatty acyl substrates reside in the active site prior to oxidation remain obscure. For both soluble[7,8] and membrane-bound[9] desaturases, one wishes to locate the position of the putative diiron oxidant with respect to the substrate by determining the site of initial oxidation or "cryptoregiochemistry" of desaturation. We have developed a complementary set of substrate-based probes for this purpose on the basis of the assumption that desaturation is a stepwise process involving an initial, slow C−H cleavage step followed by rapid collapse of a short-lived radical "intermediate" or its organoiron equivalent (not shown) to give olefin (Scheme 2).[10,11] The most versatile probe for cryptoregiochemistry is our KIE method, where we determine which C−H bond cleavage is rate-determining and hence subject to a primary kinetic isotope effect. A number of desaturase systems have been examined in this manner.[13−15] The results of these analyses can in principle be corroborated by using

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(2) Horrobin, D. F. *Prog. Lipid Res.* **1992**, *31*, 163.
(3) Okayasu, T.; Nagao, M.; Ishibashi, T.; Imai, Y. *Arch. Biochem. Biophys.* **1981**, *206*, 21.
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(7) Fox, B. G.; Shanklin, J.; Ai, J.; Loehr, T.; Sanders-Loehr, J. *Biochemistry* **1994**, *33*, 12776.
(8) Lindqvist, Y.; Huang, W.; Schneider, G.; Shanklin, J. *EMBO J.* **1996**, *15*, 4081.
(9) Shanklin, J.; Whittle, E.; Fox, B. G. *Biochemistry* **1994**, *33*, 12787.
(10) We have chosen to use a generic iron oxo representation of the active oxidant, although other structures are possible. For a more thorough discussion of the diiron chemistry involved in desaturases and the related hydroxylases, see ref 5.
(11) An alternative route to the carbocationic intermediate may be insertion of OH^+ into the unactivated C−H bond as the first step, followed by rapid loss of water from a protonated alcohol intermediate.[12]
(12) Choi, S.; Eaton, P. E.; Kopp, D. A.; Lippard, S. J.; Newcomb, M.; Shen R. *J. Am. Chem. Soc.* **1999**, *121*, 12198.
(13) Buist, P. H.; Behrouzian, B. *J. Am. Chem. Soc.* **1996**, *118*, 6295.
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(15) Pinilla, A.; Camps, F.; Fabrias, G. *Biochemistry* **1999**, *38*, 15272

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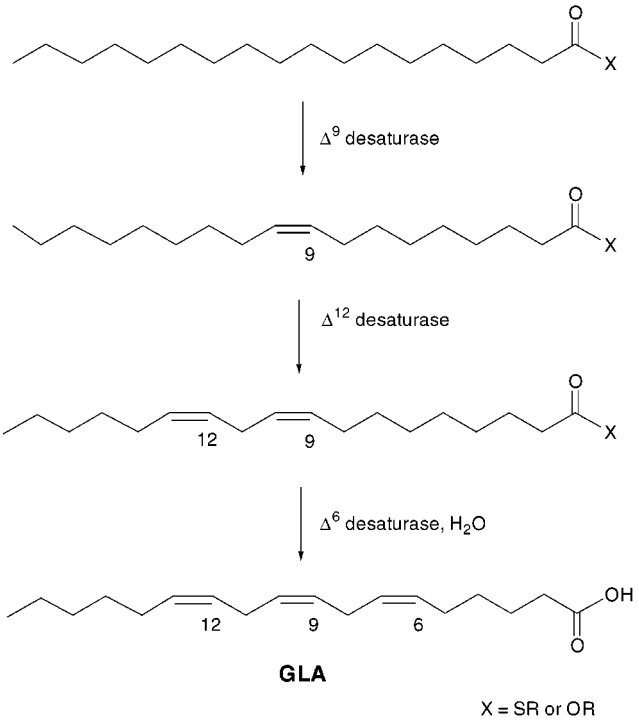
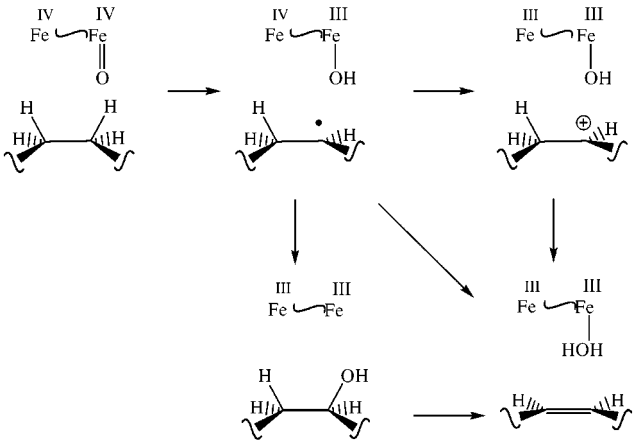
Scheme 1



Scheme 2

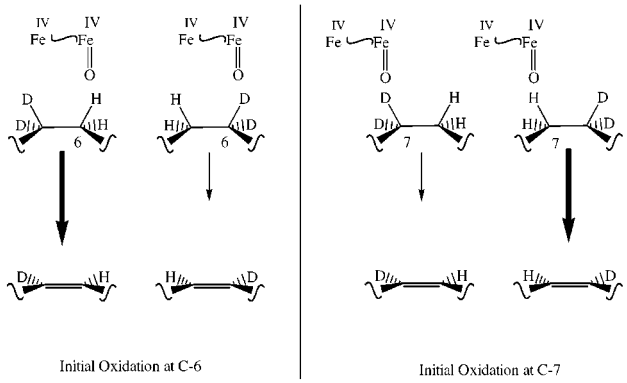


the so-called thia test in which we monitor the efficiency of oxo transfer to sulfide as a function of sulfur position in the appropriate thia substrate analogues.[16] In this paper, we show how both of these approaches can be applied to the Δ^6 desaturase system to provide an unambiguous answer to the question of whether Δ^6 desaturation is initiated at C-6 or C-7 (Schemes 3 and 4).

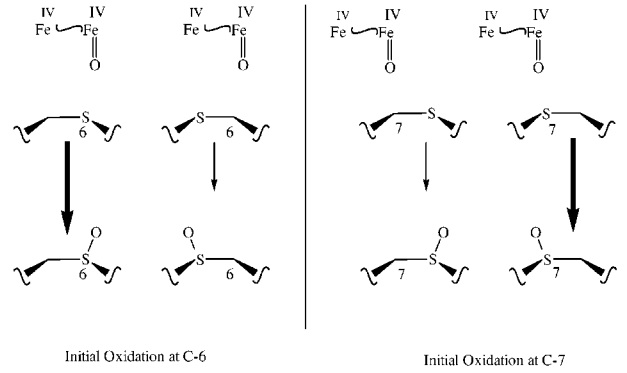
Results and Discussion

Previous studies have shown that the protozoan *Tetrahymena thermophila* is a potentially convenient microbial system for studying the cryptoregiochemistry of Δ^6 desaturation since cultures of this organism will desaturate exogenous C_{18} fatty acids derived from the growth medium at the 6, 9, and 12-positions.[17] The determination of the primary deuterium KIE on each C−H cleavage in

Scheme 3



Scheme 4



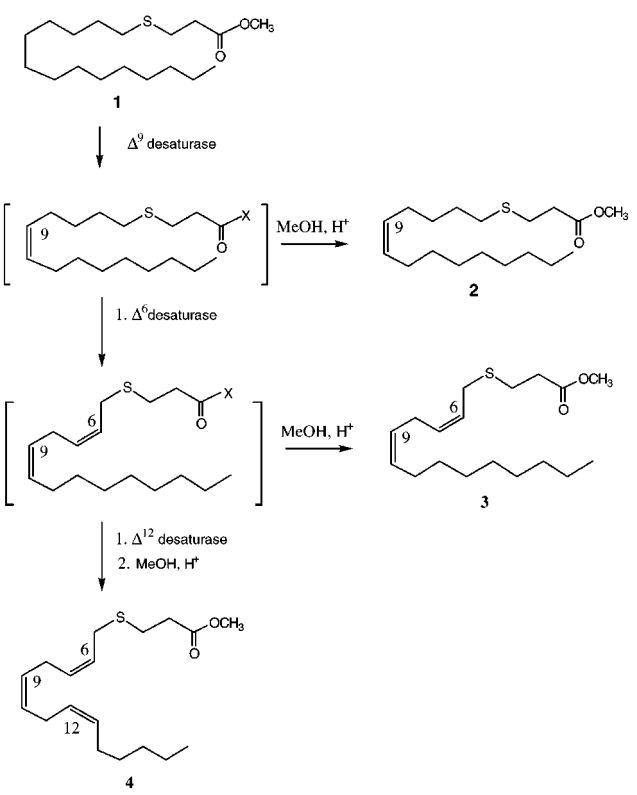
Δ^6 desaturation was carried out in a manner similar to that previously described.[13,14] Briefly, our experimental approach involves incubating an equimolar mixture of the appropriate regiospecifically dideuterated (CD_2) fatty acid analogue and the nondeuterated parent compound with the desaturase of interest. Evaluation of the d_1/d_0 ratio in the olefinic products by mass spectral examination allows one to compute the competitive primary deuterium kinetic isotope effect. We typically use deuterated substrates in which a remote methylene group is replaced by a sulfur atom in order to eliminate interference by endogenous olefinic fatty acids in the mass spectral analyses.

A preliminary experiment involving the administration of methyl 4-thiastearate **1**[18] (80 mg/L) to growing cultures of *T. thermophila* was carried out. The cell cultures were harvested after ~48 h, and the cellular fatty acids were isolated from the centrifuged cells via a standard hydrolysis/methylation sequence (see Experimental Section). Analysis of the cellular fatty acids as methyl esters by GC−MS revealed a typical fatty acid profile for this organism (Figure 1). We were gratified to see that good incorporation of 4-thia substrate can been achieved under these conditions and that the 4-thiastearoyl starting material had been transformed into the corresponding mono-, di-, and trienoic compounds **2**, **3**, and **4** as determined by mass spectral analysis. As subsequent work with deuterium-labeled substrates demonstrated (see below) these products were formed by consecutive Δ^9, Δ^6, and Δ^{12} desaturations (Scheme 5).

(16) Buist, P. H.; Marecak, D. M. *J. Am. Chem. Soc.* **1992**, *114*, 5073.
(17) Baenziger, J. E.; Smith, I. C. P.; Hill, R. J. *Chem. Phys. Lipids* **1990**, *54*, 17.

(18) The substrate in this equation is thiaoleate **2** whose deuterium content was essentially identical to that of the thiastearate **1.** Since the incubation was conducted using growing cells with a constant replenishment of thiastearate from the medium, no significant isotopic enrichment in the thiaoleate precursor was observed, as required for these types of KIE measurements[19]

Scheme 5



The two regiospecifically dideuterated methyl 4-thiastearates [6,6-$^2H_2$]-**1** and [7,7-$^2H_2$]-**1** required for the KIE study were prepared via synthetic routes similar to that previously described[13,14] in overall yields of 14% and 22%, respectively (Scheme 6). Mass spectral analysis indicated that the two deuterated substrates consisted essentially entirely of dideuterated species. A ~1:1 mixture of each deuterated material with its nondeuterated parent was administered to growing cultures of *T. thermophila* using conditions identical to that of the trial experiment. All incubations were carried out three times. The deuterium content of the olefinic fatty acid methyl esters in cellular lipid extract was assessed by GC−MS as described in the Experimental Section. As expected, the d_2/d_0 ratio of the methyl 4-thiaoleate isotopomers (**2**, S18:1) was essentially identical to that of the starting material in both incubations. Mass spectral analysis of the product of the second desaturation reaction (**3**, S18:2) revealed that in both incubations this material consisted entirely of a d_0/d_1 mixture indicating a loss of one deuterium from d_2-substrate; no dideuterated product could be detected. This indicates that Δ^6 desaturation had preceded Δ^{12} desaturation, an intriguing observation since the Δ^{12},Δ^6 pathway is thought to predominate in this organism.[17] Product kinetic isotope effects (k_H/k_D) were calculated using the ratio [%d_0 (product)/%d_1 (product)]/[%d_0 (substrate)/%d_2 (substrate)], and this analysis indicated the presence of a large primary deuterium isotope effect (7.1 ± 0.5, average of three experiments) for the carbon−hydrogen bond cleavage at C-6, whereas the C_7−H bond breaking step was shown to be essentially insensitive to deuterium substitution (KIE = 1.04 ± 0.05, average of three experiments). According to our mechanism (Scheme 2), these results demonstrate that the site of initial oxidation for Δ^6 desaturation is C-6. Our data is consistent with earlier results obtained by Baenziger et al.[17] who used Δ^9,Δ^{12} desaturase-deficient mutant *Tetrahymena* cultures to biosynthesize a series of deuterated isolinoleic ((6*Z*,9*Z*)-octadecadienoic) acids by in vivo Δ^6 desaturation of the corresponding deuterated oleates. It was observed in the latter experiments that the conversion of 6,6-d_2-oleate to 6-d_1-isolinoleate was ~7 times less efficient than that of 7,7-d_2-oleate to 7-d_1-isolinoleate, a result that is now readily interpretable in light of our findings.

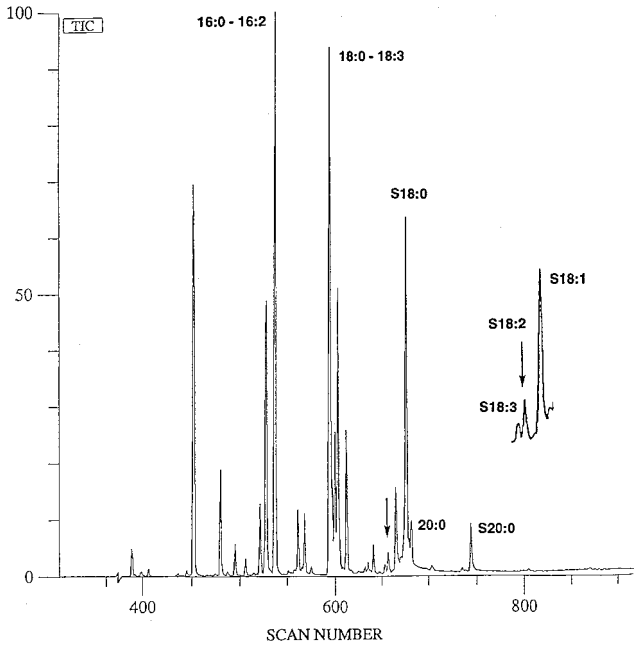
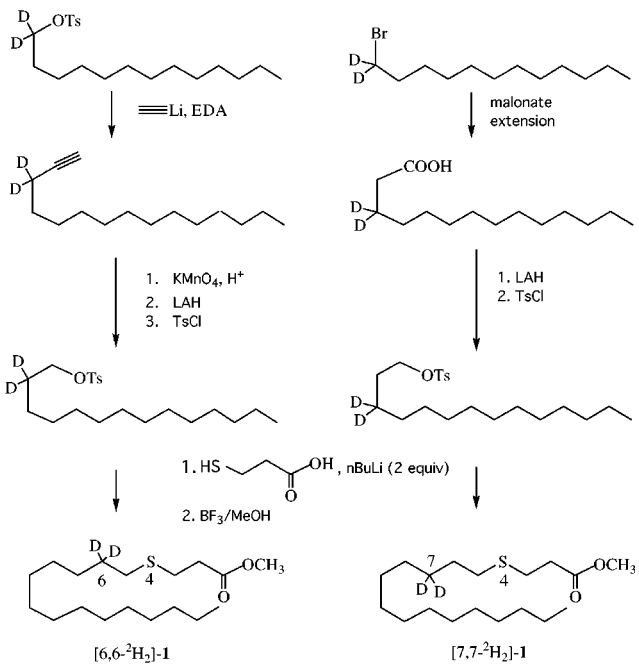


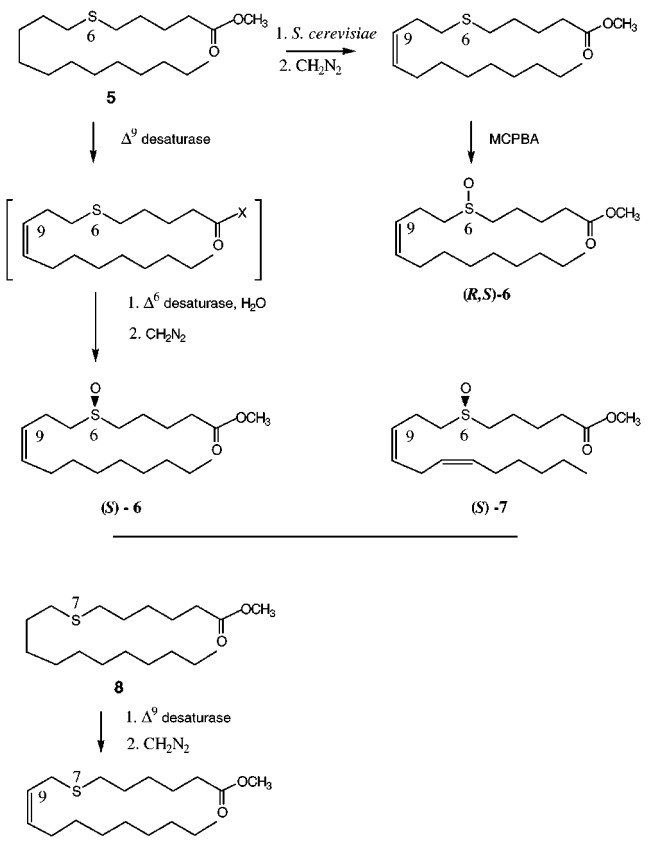
Figure 1. Cellular fatty acid profile of *T. thermophila*, grown in a nutrient media (see Experimental Section) supplemented with methyl 4-thiastearate (80 mg/L), as determined by GC−MS. S18:0 = methyl 4-thiastearate **1**; S18:1= methyl 4-thiaoleate **2**; S18:2 = methyl 4-thiaisolinoleate **3**; S18:3 = methyl 4-thia-γ-linolenate **4**.

Scheme 6



The presence of a very active Δ^6 desaturase in *T. thermophila* cultures prompted us to apply our thia test (Scheme 4) for cryptoregiochemistry. Methyl 6- and 7-thiastearate **5**, **8** (100 mg/L), as well as methyl 4-thia-

Scheme 7



stearate **1** as a control, were incubated separately with growing cultures of *Tetrahymena* for 24 h, after which the cells were centrifuged. The cellular fatty acid profile was examined in each case by GC−MS, and a search for sulfoxides in this fraction was carried out by silica gel TLC (75% EtOAc/hexanes) with the help of synthetic reference standards.[20] The fatty acids in the acidified supernatant were extracted with dichloromethane, converted to their methyl esters by treatment with diazomethane, and examined by GC−MS and silica gel TLC (75% EtOAc/hexanes). The total recovery of starting material and metabolites was typically in the 90−95% range. The results of these analyses revealed that while all of the thiastearate substrates underwent efficient initial Δ^9 desaturation to give the corresponding thiaoleates in the cell fraction, significant amounts (>1% yield) of sulfoxidized product could only be detected in the supernatant extracts of the methyl 6-thiastearate incubation. No sulfone or chain cleavage products were detected. The sulfoxide fraction (~13 mg) was isolated by silica gel chromatography and found to contain primarily methyl 6-thiaoleate *S*-oxide **6**, accompanied by ~25% of the corresponding diunsaturated material, presumably methyl 6-thialinoleate S-oxide **7** (Scheme 7). The structural assignment of **6** was based on comparison with an authentic standard of (±)-**6** using R_f (TLC), IR, ^{1}H and ^{13}C NMR, and MS data (see Experimental Section). (The reference sample of racemic **6** was synthesized by chemical oxidation (MCPBA) of methyl 6-thiaoleate, which was in turn isolated from large scale baker's-yeast-mediated Δ^9 desaturation of **5**.)

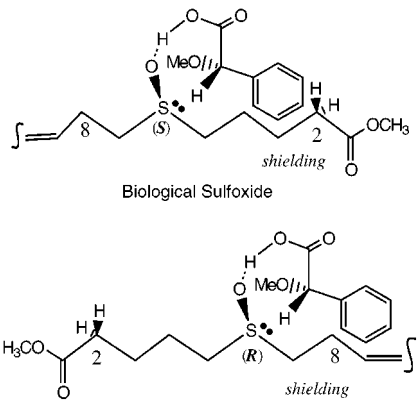


Figure 2. Complexation model showing the interaction with (*S*)-α-methoxyphenylacetic acid (MPAA) with both enantiomers of methyl 6-thiaoleate *S*-oxide **6**.

The enantiomeric purity and absolute configuration of biosynthetic methyl 6-thiaoleate *S*-oxide was assessed through the use of a chiral NMR shift reagent, (*S*)-α-methoxyphenylacetic acid, (*S*)-MPAA.[16] According to the model that we have validated,[16,21] interaction of (*S*)-**6** with this reagent should lead to an upfield shift of the ^{1}H NMR signals of the methylene group at C-2 relative to the corresponding signals of the corresponding (*R*)-sulfoxide (Figure 2). The C-2 triplet was used as a reporter group since the ^{1}H NMR signals of the other methylene groups were either too complex to analyze unambiguously or overlapped with signals from the thialinoleyl byproduct.[22] The results of the chiral NMR experiments (Figure 3) clearly show that our sample of biological 6-sulfoxide is highly enriched (>95% ee) in the "*S*" enantiomer. The correctness of this assignment was supported by our observation that in the presence of (*S*)-MPAA the ^{13}C NMR signal due to C-8[23] at 20.66 ppm experiences a *downfield* shift ($\Delta\delta$, 0.028 ppm) relative to the corresponding signal of the (*R*)-sulfoxide (data not shown). Taken together, these data suggest that the putative iron oxidant involved in Δ^6 desaturation is located near the *pro S* lone pair of the sulfur of a methyl 6-thiaoleyl substrate. This in turn implies that Δ^6 desaturation of the parent substrate is initiated by abstraction of the *pro S* hydrogen at C-6 as shown in Scheme 3 (left panel). Interestingly, a similar topological relationship between oxidant and substrate has been determined for the membrane-bound Δ,[9] Δ^{11}, and Δ^{12} desaturases;[13−16] in each case, desaturation is initiated at the carbon closest to the acyl terminus. The common cryptoregiochemical and stereochemical theme which is now emerging probably reflects a common active site architecture for this class of desaturases.

In summary, we have shown by two independent methods that Δ^6 desaturation in *T. thermophila* proceeds by initial oxidation at C-6. This represents the first direct

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(20) Buist, P. H.; Dallmann, H. G.; Rymerson, R. R.; Seigel, P. M.; Skala, P. *Tetrahedron Lett.* **1987**, *28*, 857.

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(22) The fact that the proton NMR signals for the C-2 methylene groups of **6** and **7** were coincident simplified the use of these signals in the stereochemical analysis.

(23) This ^{13}C chemical shift assignment was secured by observation of a normal (0.2 ppm) β-isotope shift on this signal by a sample of 7,7-d_2-**6** biosynthesized from 7,7-d_2-**5**, which was synthesized by methods that have been described previously.[16]

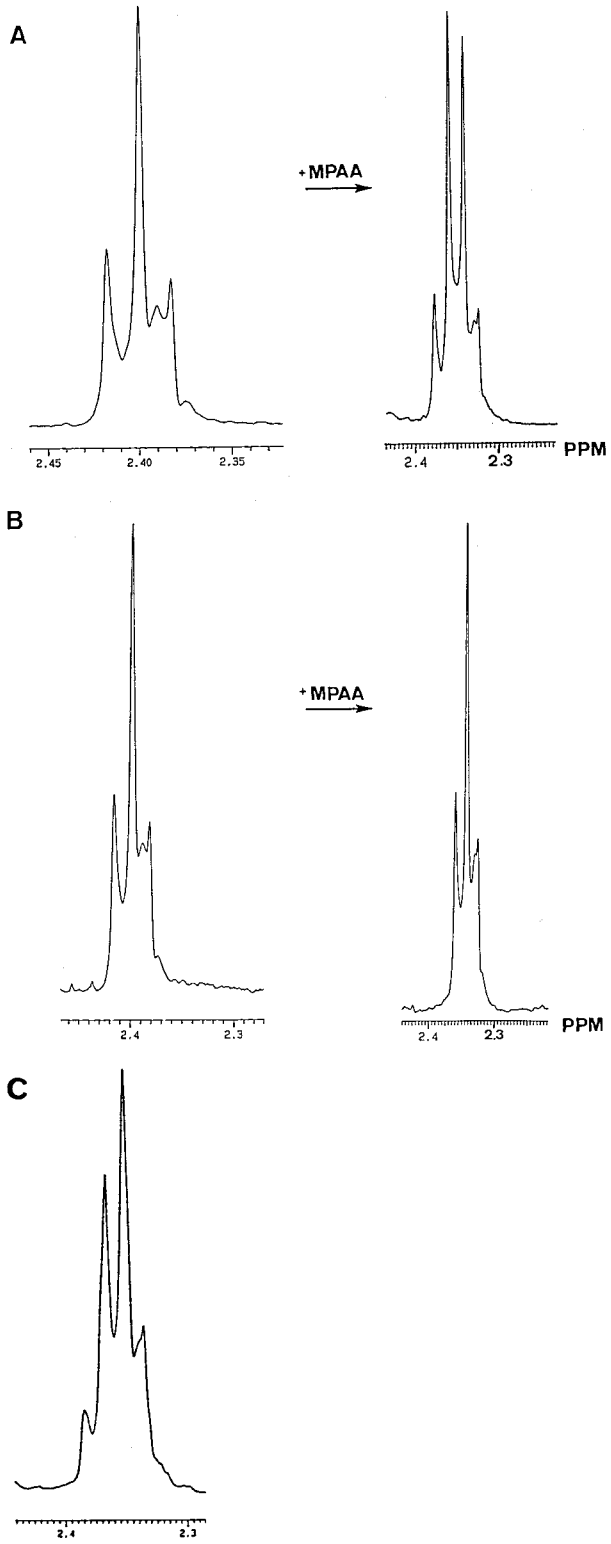

Figure 3. Effect of (*S*)-MPAA addition on ^{1}H NMR (400 MHz) due to methylene group at C-2 of (A) synthetic racemic methyl 6-thiaoleate *S*-oxide, (B) biological methyl 6-thiaoleate *S*-oxide, and (C) biological methyl 6-thiaoleate *S*-oxide spiked with the corresponding racemate.

determination of cryptoregiochemistry for a "front-end" desaturase where the introduction of a double bond occurs between a preexisting olefinic linkage and the C-1 acyl recognition point. This information can now be used in the design of selective inhibitors of this enzyme in order to assess the impact of defective Δ^6 desaturation on cellular function.

Experimental Section

General Methods. All NMR spectra were obtained using dilute $CDCl_3$ solutions at the frequency indicated. Chemical shifts are expressed in ppm and are referenced to tetramethylsilane. Mass spectra (EI, 70 eV) were obtained by GC−MS using a Kratos Concept 1H mass spectrometer either via direct probe introduction (sulfoxides) or using a HP 5980 Series 2 GC equipped with a J. & W. 30 m × 0.21 mm, DB-5 capillary column (30 m × 0.21 mm DB-5 capillary column, temperature programmed from 120 °C (held, 2 min) to 320 °C at 10 °C/min). Deuterium content was estimated using a MS scan rate of 0.5 s/decade, which resulted in 5−8 scans per GC peak; the integrated intensities of the individual ions in the pertinant ion cluster were recorded using a SUN SPARC I workstation equipped with Kratos Mach 3 software and have been corrected for natural isotopic abundances. Care was taken to include the entire GC peak in the integration to prevent errors due to fractionation of isotopic species during chromotagraphy. Isotopic ratios were determined using the following ions: *m/z* 316, M^+(methyl 4-thiastearate **1**); *m/z* 227, $(CH_3)-(CH_2)_7-CH=CH-(CH_2)_4S^+$ (methyl 4-thiaoleate **2**); *m/z* 192, $(CH_3(CH_2)_7-CH=CH-CH=CHCH=CH_2)^+$ (methyl 4-thialinoleate **3**).

Flash chromatography using silica gel (230−400 mesh) was used to purify substrates. Analytical TLC was performed using Merck glass plates precoated with silica G/UV 254. Visualization of UV-inactive materials was accomplished by using a combination of I_2 vapor followed by a water spray.

Unless otherwise stated, all reagents and starting materials were purchased from Aldrich Chemical Co. and used without purification. All air- and moisture-sensitive reactions were performed under N_2. Organic extracts were typically shaken with saturated NaCl and dried over Na_2SO_4, and solvents evaporated on a rotary evaporator. [1,1-$^2H_2$]-1-Bromododecane was prepared by treatment of the corresponding deuterated primary alcohol with HBr/H_2SO_4, and [1,1-$^2H_2$]-[(1-*p*-toluenesulfonyl)-oxy]tridecane was prepared by tosylation of the corresponding deuterated alcohol. The deuterated alcohols were in turn synthesized from the corresponding carboxylic acids by reduction using $LiAlD_4$.

Incubation experiments were carried out using a "Chx" strain derived from the standard wild type *T. thermophilia* maintained at York Unversity, Toronto by Prof. Ron Pearlman.

Synthesis of Substrates. Methyl 4- and 6-thiastearate were prepared as previously described.[20] Methyl 7-thiastearate was available from a previous study.[13] The required deuterated 4-thiafatty acids were synthesized in a similar fashion by *S*-alkylation of 3-mercaptopropanoic acid with the *p*-toluenesulfonic ester of the appropriately deuterated tetradecan-1-ol. After methylation of the carboxyl function, the crude products were purified by flash chromatography (silica gel, 4% EtOAc/hexane) to give colorless oils at room temperature. The relevant analytical data is given below:

Methyl 4-Thiastearate (1). Mp 27.5−28 °C; ^{1}H NMR (200 MHz) δ 3.71 (s, 3 H) 2.79 (t, 2 H, J = 7.4 Hz), 2.62 (t, 2 H, J = 7.4 Hz), 2.53 (t, 2 H, J = 7.3 Hz, H-5), 1.59 (m, 2 H, H-6) 1.4−1.2 (br s, 22 H) 0.89 (t, 3 H, J = 6.8 Hz); ^{13}C NMR[21] (100.61 MHz) δ 172.47, 51.75, 34.72, 32.18 (C-5), 31.91, 29.68, 29.66−29.64 (3 C), 29.59, 29.55 (C-6), 29.52 (C-9), 29.35, 29.22 (C-8), 28.87 (C-7), 26.96, 22.68, 14.10; MS *m/z* (rel intensity) 316 (M^+, 13), 285 (3), 229 (77), 120 (38), 87 (68), 69 (37), 55 (100). Anal. Calcd for $C_{18}H_3O_2S$: C, 68.30; H, 11.46. Found: C, 68.24; H, 11.42.

Methyl [6,6-$^2H_2$]-4-Thiastearate ([6,6-$^2H_2$]-1). Synthesized from [2,2-$^2H_2$]-1-*p*-toluenesulfonyloxytetradecane. The spectral data of the title compound was similar to that of **1** except for: ^{1}H NMR (400.13 MHz) δ 2.51 (s, 2 H, H-5), *1.59* (m, 2 H, H-6, absent); ^{13}C NMR (100.61 MHz) δ 31.99 (C-5, upfield β-isotope shift (0.19 ppm)), *29.55* (C-6, absent), 29.17 (C-8, upfield γ-isotope shift (0.05 ppm)), 28.67 (C-7, upfield β-isotope shift (0. 20 ppm)); MS *m/z* (rel intensity) 318 (M^+, 19), 287 (3), 231 (90), 89 (28).

Methyl [7,7-$^2H_2$]-4-Thiastearate ([7,7-$^2H_2$]-1). Synthesized from [3,3-$^2H_2$]-1-*p*-toluenesulfonyloxytetradecane. The

spectral data of the title compound was similar to that of **1** except for: ^{1}H NMR (400.13 MHz) δ 1.56 (bd. t, 2 H, J = 7.3 Hz, H-6); ^{13}C NMR (100.61 MHz) δ 32.14 (C-5, upfield γ-isotope shift (0. 04 ppm)), 29.46 (C-9, upfield γ-isotope shift (0.06 ppm)), 29.35 (C-6, upfield β-isotope shift (0.20 ppm)), 29.02 (C-8, upfield β-isotope shift (0.20 ppm)), *28.87* (C-7, absent); MS *m*/*z* (rel intensity) 318 (M^+, 42), 287 (7), 231(100), 89 (42).

Synthesis of Regiospecifically Deuterated Tetradecanol-1-ol Tosylates. [2,2-$^2H_2$]-1-*p*-Toluenesulfonyloxyundecane. The title compound was prepared from [1,1-$^2H_2$]-[(1-*p*-toluenesulfonyl)-oxy]tridecane via a standard sequence of C2-chain elongation with lithium acetylide to give [3,3-$^2H_2$]-1-pentadecyne, oxidative cleavage to give [2,2-$^2H_2$]-tetradecanoic acid, followed by $LiAlH_4$ reduction and tosylation. The overall yield for this sequence was 23%. For the known[24] unlabeled material (colorless solid): mp 34.5−35 °C, lit.[25] mp 35 °C; ^{1}H NMR (200 MHz) δ 7.77 (d, 2H, J = 8.24 Hz), 7.32 (d, 2H, J = 8.50 Hz) 4.00 (t, 2H, J = 6.6 Hz), 2.43 (s, 3H), 1.65 (m, 2H), 1.4 (br s, 20H), 0.86 (t, 3H, J = 6.64 Hz). The title compound showed similar physical and spectral characteristics.

[3,3-$^2H_1$]-1-*p*-Toluenesulfonyloxyundecane. The title compound was prepared from [1,1-$^2H_2$]-1-bromododecane via malonate chain extension to give [3,3-$^2H_2$]-tetradecanoic acid, followed by $LiAlH_4$ reduction and tosylation. The overall yield for this sequence was 38%. The physical and spectral characteristics of the title compound were similar to those of [2,2-$^2H_2$]-1-*p*-toluenesulfonyltetradecane.

Methyl 6-Thiastearate (5). Mp 24.5−25 °C; ^{1}H NMR (400 MHz) δ 3.67 (s, 3 H), 2.34 (t, 2 H, 7.4 Hz), 2.52 (t, 2 H, 7.4 Hz), 2.50 (t, 2 H, 7.4 Hz), 1.74 (m, 2 H), 1.62 (m, 2 H), 1.57 (m, 2 H), 1.37 (m, 2 H), 1.2 (br s, 16H), 0.90 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100.61 MHz) δ 173.82, 51.49, 33.60, 32.15, 31.90, 31.65, 29.67, 29. 64, 29.61, 29.58, 29.52, 29.33, 29.24, 29.07, 28.93, 24.14, 22.67, 14.09; MS *m*/*z* (rel intensity) 316 (M^+,12), 285 (6), 201 (49), 115 (100). Anal. Calcd for $C_{18}H_3O_2S$: C, 68.30; H, 11.46. Found: C, 68.92; H, 11.64.

Sulfoxide Analysis. Methyl 6-thiaoleate S-oxide **6** produced in vivo by consecutive Δ^9 desaturation and 6-sulfoxidation was compared with an authentic sample using the following criteria: R_f 0.24 (75% EtOAc/hexanes); IR ($CHCl_3$) 1737, 1673, 1014 cm^{-1}; ^{1}H NMR (400 MHz) δ 5.54 (m, 1H), 5.39 (m, 1H), 3.70 (s, 3 H), 2.62−2.79 (m, 4H), 2.53 (bd. d of t, 2H, J = 8.0, 7.2)), 2.40 (distort. t, 2 H, J = 6.8 Hz), 2.08 (bd. d of t, 2 H, J = 7.2, 6.5), 1.83 (m, 4 H), 1.28−1.40 (br s, 12H), 0.90 (t, 3H, J = 6.8 Hz); ^{13}C (100.61 MHz) δ 173.45, 133.08, 125.45, 52.41, 52.01, 51.66, 33.50, 31.87, 29. 50 (2C), 29.30, 29.29, 27.34, 24.11, 22.67, 22.23, 20.66, 14.11; MS *m*/*z* (rel intensity) 330 (M^+,1), 313 (2), 199 (41), 115 (44), 81 (61), 67 (82), 55 (97), 41 (100).

The biologically produced **6** was accompanied by methyl 6-thialinoleate *S*-oxide **7** (tentative assignment) as determined by the appearance of additional minor MS peaks: *m*/*z* 328 (M^+), 197, 164; ^{1}H NMR δ 5.3−5.5 (m, 4H), 0.904 (t, 3H); ^{13}C NMR δ 131.19, 130.93, 126.94, 125.79, 52.26, 52.04, 31.50, 27.25, 25.67, 22.57, 20.69, 14.07.

Incubation Experiments using *T. thermophila*. Cultures of *T. thermophila (Chx-2/CHx-2 (Cycl-s II)* were generously provided by Dr. Ron Pearlman (Dept. of Biology, York University, Toronto). A typical incubation experiment was carried out as follows. A starter culture (5 mL) of *T. thermophila* was grown in a rotary incubator/shaker set at 100 rpm at 30 °C for 24 h in a sterilized culture medium consisting of Difco peptone (10 g/L), glucose (2 g/L), yeast extract (1 g/L), and sequestrin (0.3 g/L). This culture was used to inoculate 200 mL of medium contained in a 1 L Erlenmeyer flask. A 20 mg portion of thia fatty acid methyl ester was added as a solution (5% w/v) in absolute ethanol to each of five culture flasks, and the innoculated cultures were incubated for 48 h. The addition of exogenous fatty acids did not affect the growth of the organism when compared with control cultures.

The cells from each incubation experiment were isolated by centrifugation and, the fatty acid fraction was isolated via a standard hydrolysis/methylation procedure. The cell pellet (ca. 10 g wet weight) was treated with refluxing 1 M 50% ethanolic KOH (100 mL) for 30 min under N_2. The cooled reaction mixture was diluted with water (50 mL), acidified to pH 2 with 3 M H_2SO_4, and extracted with dichloromethane (3 × 60 mL). This extract was heated at 70 °C for 30 min with a MeOH/H_2SO_4 (10:1 v/v) solution and extracted with CH_2Cl_2 (3 × 50 mL) to give ∼100 mg of crude fatty acid methyl esters as an oil after evaporation of solvent.

Fatty acids were isolated from the acidified supernatant (acetic acid addition to pH 3.5, blue-green, Accutint pH 0−5 wide range A indicator paper) by extraction with three 50 mL portions of dichloromethane. The crude extract was methylated with ethereal diazomethane to prevent potential racemization of sulfoxides. The sulfoxide fraction was isolated by silica gel chromatography (75% EtOAc/hexane) and quantitated.

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