gel, hexane–ethyl acetate, 4:1) gave **31** (186 mg, 34%), **32** (26 mg, 5%), **34** (124 mg, 23%), and **33** (57 mg, 10%).

4-(3′-Chloropropyl)-4-cyano-11-oxo-10-oxatricyclo[3.3.2.1]undeca-2,7-diene (13). **9b** (244 mg, 1.25 mmol) in benzene (80 mL) and furan (255 mg, 3.75 mmol) was irradiated through uranyl glass for 18 h. Removal of solvent in vacuo gave 286 mg of an oil. Flash chromatography (silica gel, hexane–ethyl acetate, 5:1) followed by preparative TLC (silica gel, hexane–ethyl acetate, 1:1) gave **13** (70 mg, >85% pure, <20% yield) and an unidentified byproduct. An analytical sample of **13** was prepared by crystallization from pentane–ethyl acetate to give a white solid (mp 108–109 °C): ^{1}H NMR ($CDCl_3$) δ 1.81–2.04 (m, 4 H), 2.61 (s, 1 H), 2.96 (m, 1 H), 3.56 (t, 2 H, J = 6 Hz), 4.80 (s, 1 H), 5.30 (s, 1 H), 5.90–6.08 (m, 2 H), 6.31 (dd, 1 H, J = 6 Hz, J = 1 Hz), 6.42 (dd, 1 H, J = 6 Hz, J = 1 Hz); IR (film) 2968, 2239, 1719 cm^{-1}; CIMS, m/z (relative intensity) 264 (M^+ + 1, 100), 237 (39), 196 (27).

Anal. Calcd for $C_{14}H_{14}ClNO_2$: C, 63.76; H, 5.35. Found: C, 63.87; H, 5.27.

Irradiation of Bicyclohexenone 11 at 366 nm. **11** (43 mg, 0.22 mmol) in benzene (7 mL) and furan (45 mg, 0.66 mmol) was irradiated through uranyl glass for 3.5 h. Removal of solvent in vacuo gave 61 mg of an oil. Flash chromatography (silica gel, hexane–ethyl acetate, 2:1) followed by preparative TLC (silica gel, hexane–ethyl acetate, 1:1) gave **13** (13 mg, 22%).

Irradiation of Bicyclohexenone 11 at >300 nm. **11** (34 mg, 0.17 mmol) in benzene (7 mL) and furan (35 mg, 0.52 mmol) was irradiated through Pyrex glass for 1.5 h. Removal of solvent in vacuo gave 38 mg of an oil. Flash chromatography (silica gel, hexane–ethyl acetate, 2:1) followed by preparative TLC (silica gel, hexane–ethyl acetate, 1:1) gave **13** (7 mg, 16%).

Acknowledgment. This work was supported by the National Institute of General Medical Science (Grant GM 26568). We thank Dr. F. S. Tham for the X-ray crystallographic structure determination.

Supplementary Material Available: Tables of crystal structure data, atomic coordinates, bond lengths, bond angles, anisotropic parameters, and hydrogen atom coordinates for **22** (8 pages). Ordering information is given on any current masthead page.

Stereochemical Analysis of Sulfoxides Obtained by Diverted Desaturation

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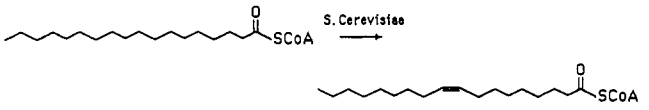
Contribution from the Department of Chemistry, Carleton University, Ottawa, Ontario, Canada K1S 5B6. *Received December 2, 1991*

Abstract: We have shown that the Δ^9-desaturase of *Saccharomyces cerevisiae* can behave as a regio- and enantioselective oxygenating agent. The stereochemistry of oxygenation matches that of hydrogen removal in the desaturation process. Thus methyl 9-thiastearate *S*-oxide (>96% ee), obtained via incubation of the corresponding sulfide with *S. cerevisiae* ATCC 12341, was shown to possess the *R* configuration. (*R*)-Methyl 10-thiastearate *S*-oxide (91% ee) was produced from the corresponding sulfide via the same catalytic system although less efficiently. (*S*)-(+)-α-Methoxyphenylacetic acid was used as a chiral NMR shift reagent to determine the optical purity and absolute configuration of these quasisymmetrical dialkyl sulfoxides. (*S*)-*S*-Benzyl-8-mercaptooctanoic acid methyl ester *S*-oxide was obtained by Δ^9-desaturase-mediated sulfoxidation in 40–50% isolated yield and with high enantioselectivity (>98% ee). The absolute configuration was established by synthesizing both optical antipodes of benzyl decyl sulfoxide from the corresponding diastereomeric menthyl phenylmethanesulfinates. The correlation was then established by polarimetry, circular dichroism, and chiral shift reagent ^{1}H NMR. *S*-Benzyl-9-mercaptononanoic acid methyl ester *S*-oxide (88% ee) was produced far less efficiently and was also shown to have the *S* configuration. In both the dialkyl and benzyl series, sulfoxidation at the 9-position is consistently more efficient than at the 10-position, which seems to indicate that dehydrogenation of stearoyl CoA is initiated at C-9.

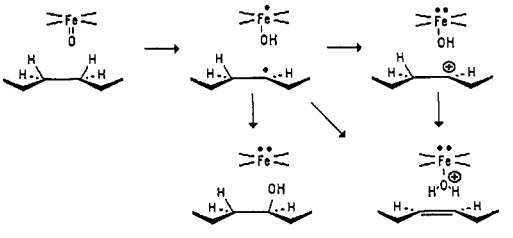
Introduction

As part of a research program directed at understanding unusual enzymatic processes, we have been interested in a "superfamily" of biological catalysts known as the desaturases. The prototypical desaturase-mediated reaction is the "syn" 9,10-dehydrogenation of stearoyl CoA, which occurs in a wide variety of biological species and plays a pivotal role in the aerobic production of unsaturated lipids.[1] This process is catalyzed by

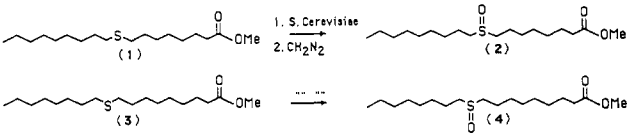


an O_2-dependent, non-heme iron protein which acquires its reducing equivalents from NAD(P)H via a NAD(P)H reductase and an intermediary electron-transfer protein such as cytochrome b_5. A topographical model, based on sequence information, has recently been constructed for the Δ^9-desaturases of rat liver and *Saccharomyces cerevisiae*.[2] However, precise information about

Scheme I



Scheme II



(1) Cook, H. In *Biochemistry of Lipids and Membranes*, Vance, D. E., Vance, J. E., Eds.; The Benjamin Cummings Publishing Co. Ltd.: Menlo Park, CA, 1985; pp 191–203.

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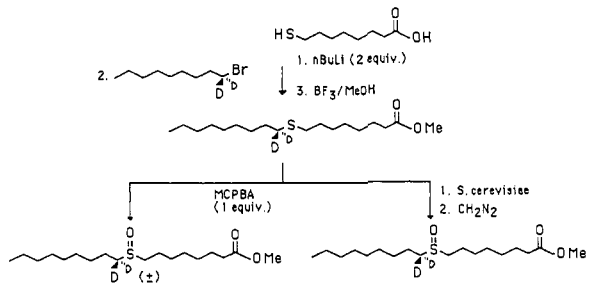
Scheme III



the ligand environment of the non-heme iron active site has been difficult to obtain.[3]

One of our fundamental objectives has been to forge a mechanistic link between desaturases and the better characterized heme (P_{450}) monooxygenases. This problem has recently been brought into sharper focus by Ortiz de Montellano,[4] who has summarized the growing number of cases where cytochrome P_{450}'s have been implicated in dehydrogenation processes. A useful mechanistic paradigm is emerging[5] which involves initial hydrogen abstraction followed by either oxygen rebound (hydroxylation) or electron transfer and deprotonation of the resultant carbocation (dehydrogenation). The possibility that dehydrogenation proceeds via sequential hydrogen atom removal cannot be excluded (see Scheme I).

This picture would suggest that desaturases might be induced to exhibit regio- and enantioselective oxygenase activity with appropriately modified substrates. We have recently carried out oxygen trapping experiments using sulfur as a methylene isostere[6] (see Scheme II). Sulfoxidation by the Δ^9-desaturase of *S. cerevisiae* is most efficient when sulfur replaces carbon 9 of stearic acid. Substantially less sulfoxide is obtained when sulfur is at the C-10 position, and sulfoxidation at the C-8 and C-11 positions is negligible. Sulfones are neither produced nor reduced to sulfoxide in our system. We decided to test the fidelity with which our thia analogues report the location of the oxidant with respect to the parent substrate by analyzing the stereochemistry of sulfoxidation. We would like to present the results of studies which show that the enantioselectivity of sulfoxidation is very high when sulfur is at the C-9 position[7] and that the stereochemistry of this process matches that of all desaturases studied to date.[8]

Results and Discussion

Determination of the Enantiomeric Purity of 2. The quasi-symmetrical nature of **2** challenged us to devise new methodology for the stereochemical analysis of dialkyl sulfoxides of this type. We reasoned that, if one of the methylene groups was labeled with deuterium, then the ^{1}H NMR resonances of the remaining methylene group could, in the presence of a suitable chiral complexing agent, be used to evaluate the enantiomeric purity of the sulfoxide.

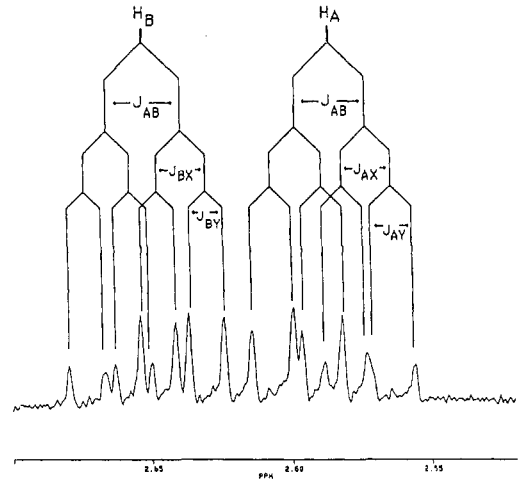


Figure 1. ^{1}H NMR (500 MHz) resonances due to the α-sulfinyl protons of racemic **2**-*10,10-d*$_2$.

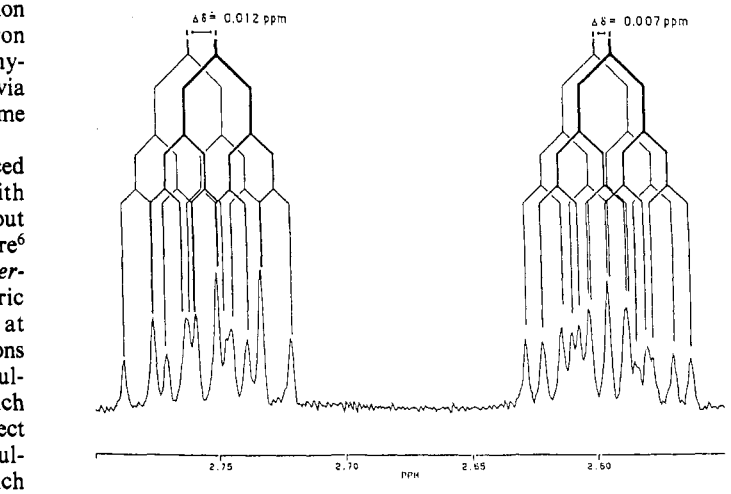
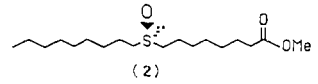


Figure 2. Effect of addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (MPAA) on ^{1}H NMR (500 MHz) resonances due to the α-sulfinyl protons of racemic **2**-*10,10-d*$_2$. Two overlapping ABXY systems of equal intensity.



(2)

Thus, substrate **1** was prepared in labeled form as shown in Scheme III. The sample of methyl 9-thiastearate-*10,10-d*$_2$ so prepared was purified by flash chromatography (silica gel, 4% EtOAc/hexane), and all spectral data obtained for this compound were in accord with our assigned structure.

Two samples of methyl 9-thiastearate-*10,10-d*$_2$ *S*-oxide were prepared from **1**-*10,10-d*$_2$ as previously reported.[6a] Racemic material was obtained by oxidation of **1**-*10,10-d*$_2$ using 1 equiv of MCPBA. Δ^9-Desaturase-produced sulfoxide (22 mg) was obtained by administering **1**-*10,10-d*$_2$ (253 mg) to growing cultures of *S. cerevisiae* ATCC 12341. The sulfoxy acid was extracted from the culture medium, methylated with diazomethane, and purified by flash chromatography (silica gel, 65% EtOAc/hexane). Mass spectral examination of the biological product indicated that no deuterium had been washed out.

^{1}H NMR analysis of racemic **2**-*10,10-d*$_2$ revealed an ABXY pattern for the methylene group at C-8 (see Figure 1). We took advantage of the known fact[9] that carboxylic acids complex strongly with sulfoxides and have been used to differentiate be-

(2) (a) Stukey, J. E.; McDonough, V. M.; Martin, C. E. *J. Biol. Chem.* **1990**, *265*, 20144. (b) Thiede, M. A.; Ozols, J.; Strittmatter, P. *J. Biol. Chem.* **1986**, *261*, 13230.

(3) Enoch, H. G.; Strittmatter, P. *Biochemistry* **1978**, *17*, 4927.

(4) Ortiz de Montellano, P. R. *TIPS Rev.* **1989**, *10*, 354.

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(6) (a) Buist, P. H.; Dallmann, H. G.; Rymerson, R. R.; Seigel, P. M.; Skala, P. *Tetrahedron Lett.* **1988**, *29*, 435. (b) Buist, P. H.; Marecak, D. *Tetrahedron Lett.* **1991**, *32*, 891. (c) For a general review of biosulfoxidations, see: Holland, H. L. *Chem. Rev.* **1988**, *88*, 473.

(7) A preliminary publication of portions of this work has appeared: (a) Buist, P. H.; Marecak, D. M. *J. Am. Chem. Soc.* **1991**, *113*, 5877. (b) Buist, P. H.; Marecak, D. M.; Partington, E. *J. Org. Chem.* **1990**, *55*, 5667.

(8) Rawlings, B. L.; Reese, P. B.; Ramer, S. E.; Vederas, J. C. *J. Am. Chem. Soc.* **1989**, *111*, 3382 and references contained therein. It has been shown that the C-10 *pro-R* hydrogen is removed by the Δ^9-desaturase of *S. cerevisiae*: McInnes, A. G.; Walter, J. A.; Wright, J. L. C. *Tetrahedron* **1983**, *39*, 3515.

(9) Nishio, M. *Chem. Pharm. Bull.* **1969**, *17*, 262.

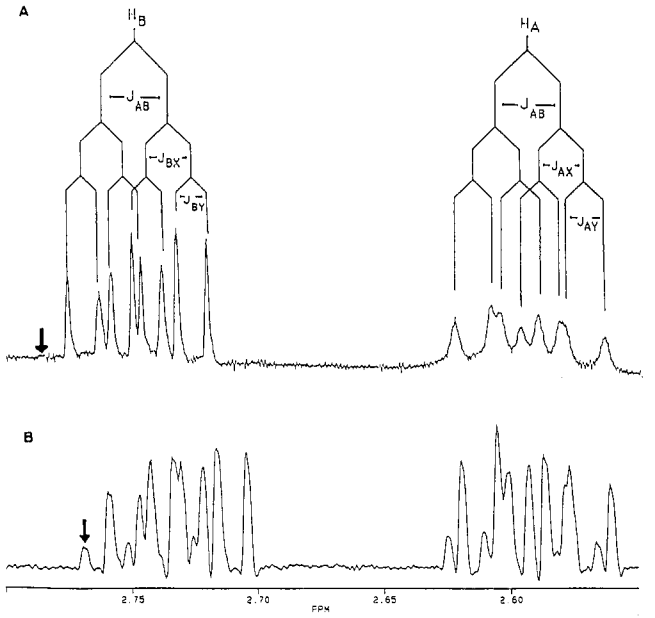
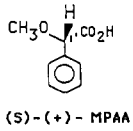


Figure 3. Effect of addition of 3 equiv of (*S*)-(+)-MPAA on ^{1}H NMR (500 MHz) resonances due to the α-sulfinyl protons of (A) biologically produced **2**-*10,10-d*$_2$ (sample dissolved in dry, DCl-free $CDCl_3$, one ABXY system (J_{AB} = 13 Hz; J_{AX} = 9 Hz; J_{AY} = 7 Hz; J_{BX} = 9 Hz J_{BY} = 6 Hz). The lower limit of % ee was estimated using the magnitude of background noise at the position indicated by arrow) and (B) biologically produced **2**-*10,10-d*$_2$ (sample dissolved in untreated, bottled $CDCl_3$).

tween adjacent diastereotopic hydrogens. We reasoned that a chiral carboxylic acid might be useful in the analysis of our system.



Thus, addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (MPAA) to a 20 mM solution of racemic **2**-*10,10-d*$_2$ in $CDCl_3$ caused the ^{1}H NMR signals of one of the diastereotopic protons at C-8 to shift downfield by 0.15 ppm, as shown in Figure 2. The racemic nature of the sample was clearly revealed by the fact that all signals in the resultant ABXY pattern were doubled. Interestingly, the observed nonequivalence ($\Delta\delta$) for the two diastereotopic protons was different; the ratio of $\Delta\delta$'s was calculated to be 0.012/0.007 = 1.7.

When the chiral shift experiment was repeated with biologically produced **2**, it was clear that the *downfield* half of each doublet had disappeared (see Figure 3a). We were thus able to estimate the enantiomeric excess of this material to be >96%. We have found that dry, DCl-free $CDCl_3$ must be used to determine % ee since significant racemization occurred when these precautions were not taken, as indicated by the appearance of the downfield signal of each doublet (see Figure 3b).

The key to the success of these chiral recognition experiments lies in the ability of the carboxylic acids to differentiate between the diastereotopic protons adjacent to the sulfinyl function to such an extent that chiral discrimination can be detected. Thus we have found that less acidic chiral shift reagents such as Eu(hfc)$_3$,[10] the Kagan amide,[11] and the Pirkle alcohol[12] do not render H_A and H_B sufficiently nonequivalent. The methyl ester of (*S*)-(+)-MPAA is also ineffective as a shift reagent because H-bonding to the sulfoxide is no longer possible.

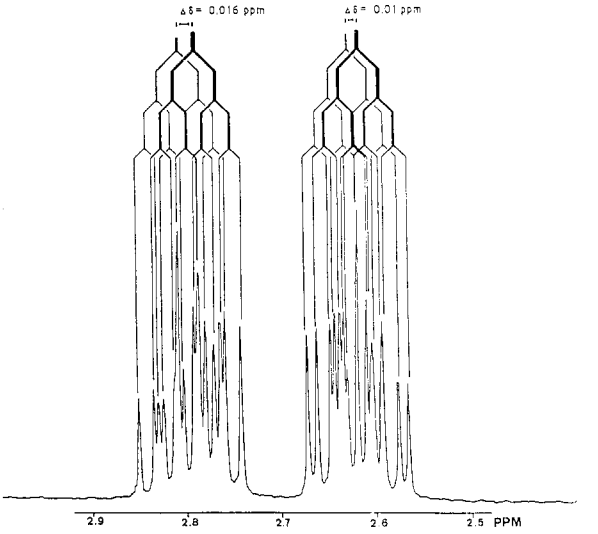
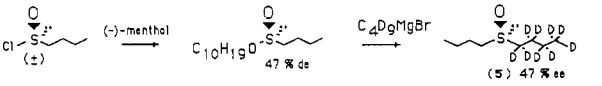


Figure 4. Effect of addition of 3 equiv of (*S*)-(+)-MPAA on ^{1}H NMR (300 MHz) resonances due to the α-sulfinyl protons of dibutyl sulfoxide. Two overlapping ABXY systems of equal intensity.

Scheme IV



Determination of the Absolute Configuration of 2. With the knowledge that our biologically produced sulfoxide was essentially optically pure, the determination of its absolute configuration became of great interest. We decided to correlate this compound with that of an optically active sulfoxide of known absolute configuration whose chirality was due to deuterium substitution. For this tactic to function, we had to first demonstrate the generality of our analytical method. This was confirmed by the addition of 3 equiv of (*S*)-(+)-MPAA to a 20 mM solution of dibutyl sulfoxide in $CDCl_3$. We were encouraged to see that the pattern of ^{1}H NMR signals and the ratio of $\Delta\delta$'s (1.6) were similar to those obtained previously (compare Figures 2 and 4).

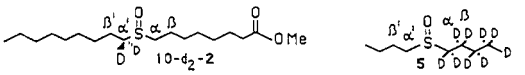
A sample of chiral, deuterated dibutyl sulfoxide suitable for use in correlation was then synthesized via the Anderson route (see Scheme IV). A diastereomeric mixture of (−)-menthyl butanesulfinates was prepared following the procedure of Mislow et al.,[13] who have shown that the major diastereomer bears the *R* configuration at sulfur and is estimated to be present in ca. 47% de. This material was treated with perdeuteriobutylmagnesium bromide to give a mixture of enantiomeric deuterated dibutyl sulfoxides in which the *R* enantiomer must be in excess. The Grignard reaction is known to proceed with clean inversion of configuration.[13]

The combination of predominantly (*R*)-dibutyl-*d*$_9$ sulfoxide with (*S*)-(+)-MPAA resulted in a set of NMR signals in which the *upfield* half of each doublet was reduced in intensity (see Figure 5).[14] It follows then, that the disposition of the labeled and unlabeled methylene groups surrounding the sulfinyl group of biologically produced **2**-*10,10-d*$_2$ is opposite to that of (*R*)-**5**-*d*$_9$. Via this analysis, the absolute configuration of the sulfoxide **2** is therefore determined to be *R*.

Confirmation of this stereochemical result was obtained using ^{13}C NMR spectroscopy as a probe. As can be seen from Table I, the ^{13}C resonances α and β to the sulfinyl function of the fatty acid sulfoxides and the dibutyl sulfoxides shift and split in similar fashion upon addition of 3 equiv of (*S*)-(+)-MPAA. To confirm the validity of our previous analysis using ^{1}H NMR, a sample of biological **2**-*10,10-d*$_2$ containing approximately 20% of (±)-**2**-

(10) Fraser, R. R.; Petit, M. A.; Saunders, J. K. *Chem. Commun.* **1971**, 1450.
(11) Deshmukh, M. N.; Duñach, E.; Juge, S.; Kagan, H. B. *Tetrahedron Lett.* **1984**, *25*, 3467.
(12) Pirkle, W. H.; Beare, S. D.; Muntz, R. L. *Tetrahedron Lett.* **1974**, 2295.
(13) Mislow, K.; Green, M.; Laur, P.; Melillo, J. T.; Simmons, T.; Ternay, A. L., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 1958.
(14) The % ee of **5** was estimated to be 47% in excellent agreement with the known de (47%) of the starting menthyl sulfinate mixture.[13]

Table I. Effect of Addition of 3 equiv of (*S*)-(+)-MPAA on the ^{13}C Shifts of Sulfoxides



	^{13}C shifts of uncomplexed sulfoxide				change in ^{13}C shifts upon complexation (ppm)			nonequivalence due to chiral recognition (ppm)		
carbon	**2**	**2**-*10,10-d*$_2$	**5**	**5**-*d*$_9$	**2**	**2**-*10,10-d*$_2$	**5**-*d*$_9$	**2**	**2**-*10,10-d*$_2$	**5**-*d*$_9$
β	22.57	22.56[a]	24.64		−0.02	−0.05		0.011	0.013	
α	52.42	52.33[a]	52.18		−0.86	−0.88		0.021	0.024	
α'	52.56		52.18	52.09[a]	−0.85		−1.07	0.024		0.036
β'	22.63	22.42[a]	24.64	24.63[a]	−0.01	−0.04	−0.03	0.013	0.013	0.015

[a] These chemical shifts are isotope-shifted upfield. The magnitude of the isotope shifts was used to assign the ^{13}C shifts of **2**.

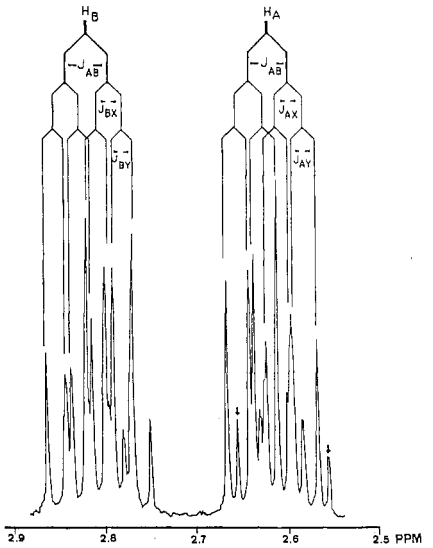


Figure 5. Effect of addition of 3 equiv of (*S*)-(+)-MPAA on ^1H NMR (300 MHz) resonances due to the α-sulfinyl protons of predominantly (*R*)-dibutyl-*d*$_9$ sulfoxide (**5**). Two overlapping ABXY systems of unequal intensity (J_{AB} = 12 Hz; J_{AX} = 8 Hz; J_{AY} = 7 Hz; J_{BX} = 8 Hz J_{BY} = 6 Hz).

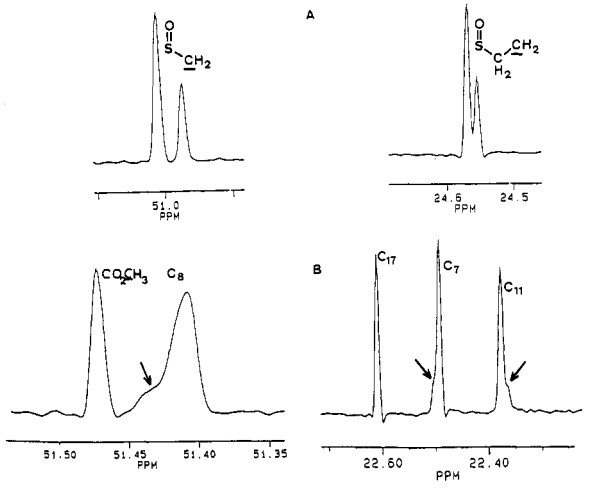


Figure 6. Comparison of the effect of (*S*)-(+)-MPAA (3 equiv on the ^{13}C NMR (125.8 MHz) resonances due to (A) the α and β carbons of predominantly (*R*)-dibutyl-*d*$_9$ sulfoxide (**5**) and (B) the corresponding carbons of a mixture of biologically produced **2**-*10,10-d*$_2$ (80%) and racemic **2**-*10,10-d*$_2$ (20%). Arrows indicate location of peak shoulders.

10,10-d$_2$ was prepared. The ^{13}C signal at C-8 possesses, in the presence of our chiral complexing agent, a *downfield* shoulder (see Figure 6b). In contrast, the corresponding signal in predominantly (*R*)-dibutyl-*d*$_9$ sulfoxide appears, under the same conditions, as a doublet in which the *upfield* component is reduced in intensity. A mirror image pattern of signals is also obtained by comparing the ^{13}C signal at C-7 of the fatty acid sulfoxide with

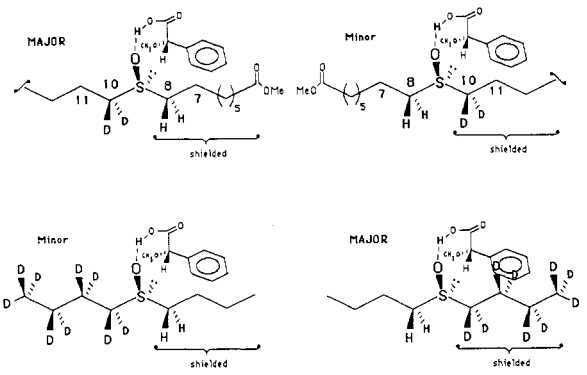


Figure 7. Complexation model showing the interaction of (*S*)-(+)-MPAA with both enantiomers of **2**-*10,10-d*$_2$ and dibutyl-*d*$_9$ sulfoxide (**5**).

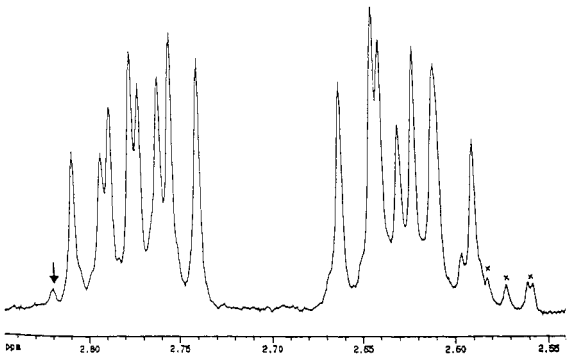


Figure 8. Effect of addition of 3 equiv of (*S*)-(+)-MPAA on ^1H NMR (400 MHz) resonances due to the α-sulfinyl protons of biologically produced **4**-*11,11-d*$_2$. The % ee was estimated using the peak indicated.

the corresponding signal in the chiral reference standard (see Figure 6a). These results are entirely consistent with our previous analysis by ^1H NMR and confirm the assignment of the *R* configuration to **2**.

The correlation between the fatty acid and dibutyl sulfoxides can be illustrated using a Pirkle-type[12] complexation model in which the hydrogens and the carbons in the vicinity of the sulfinyl function are shifted upfield when shielded by the aromatic ring (see Figure 7). A check on the validity of this picture was available in that ^{13}C NMR could be used to observe the effect of aromatic shielding at C-11. As predicted by the model, the ^{13}C signal due to C-11 of the biological sulfoxide appears at *lower* field than than the corresponding signal of its enantiomer (see Figure 6b).

Determination of the Enantiomeric Purity and Absolute Configuration of 4. With the necessary analytical methodology in hand, we were in a position to examine the stereochemistry of sulfoxidation when sulfur was at the 10-position. We synthesized both methyl 10-thiastearate (**3**) and methyl 10-thiastearate-*11,11-d*$_2$ by standard methods and administered each compound to growing cultures of *S. cerevisiae*. The product sulfoxides (**4**, **4**-*11,11-d*$_2$) were isolated in yields (4,6%) which were lower than those obtained for the corresponding 9-thiastearates (8,9%); this

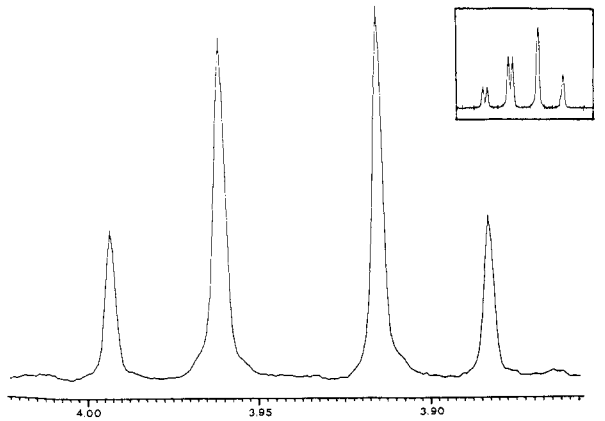
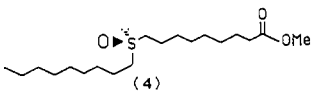


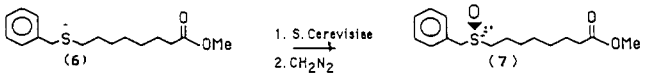
Figure 9. Effect of addition of 3 equiv of (*R*)-(−)-(3,5-dinitrobenzoyl)-α-methylbenzylamine on ^{1}H NMR (400 MHz) resonances due to the benzylic protons of biologically produced **7**. Insert: same experiment but instead using racemic **7**.

was consistent with the results of previous direct competition experiments where approximately half as much 10-sulfoxide was formed relative to 9-sulfoxide.[6a] The enantioselectivity of 10-



sulfoxidation was determined by ^{1}H NMR examination of **4**-*11,11-d*$_2$ using (*S*)-(+)-MPAA (see Figure 8). From this experiment, the % ee of **4**-*11,11-d*$_2$ was determined to be 91%; from the sense of the nonequivalence, it was clear that the absolute configuration of this sulfoxide was *R*—the same as that determined for the 9-sulfoxide. We attribute the somewhat lower % ee of the 10-sulfoxide to the presence of a small amount of sulfoxide derived by autoxidation[15]—a phenomenon which would be somewhat more important in a less efficient biological process.

Determination of the Enantiomeric Purity and Absolute Configuration of 7. We were interested in monitoring the effect that a moderate change in substrate structure would have on the stereochemistry of sulfoxidation. For this purpose, we administered the thia benzyl analogue **6** to *S. cerevisiae* and isolated the corresponding sulfoxide **7** in reproducibly high yields (43%, 54%, two separate feeding experiments)[16] compared to that obtained for the 9-thiastearate analogue (8–9% yield). The stereochemical



analysis of **7** was initially carried out by applying existing methodology. Thus the enantiomeric purity of **7** was determined by ^{1}H NMR with the assistance of the Kagan shift reagent, (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine,[11] as previously described.[8b] As can be seen from Figure 9, the optical purity of this material ([α]$_D$ −73.1 and −73.2 ($CHCl_3$, *c* 2), two separate feeding experiments) was reproducibly very high: >98% ee.[17]

We thought it would be instructive to determine whether we could predict the absolute configuration of **7** using (*S*)-(+)-MPAA. Thus, addition of (*S*)-(+)-MPAA (3 equiv) to biological **7** mixed with 30% of racemic **7** caused a splitting of both benzylic

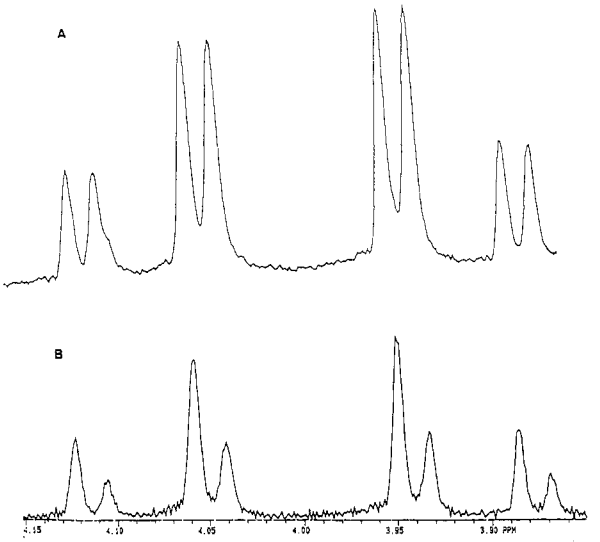


Figure 10. Effect of addition of 3 equiv of (*S*)-(+)-MPAA on ^{1}H NMR (200 MHz) resonances due to the benzylic protons of (A) racemic **7** and (B) a 2/1 mixture of biological **7** and racemic **7**.

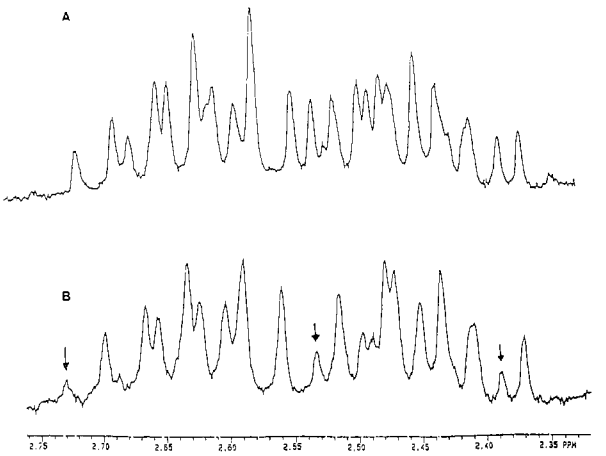


Figure 11. Effect of addition of 3 equiv of (*S*)-(+)-MPAA on ^{1}H NMR (200 MHz) resonances due to the α-sulfinyl protons at C-8 of (A) racemic **7** and (B) a 2/1 mixture of biological **7** and racemic **7**.

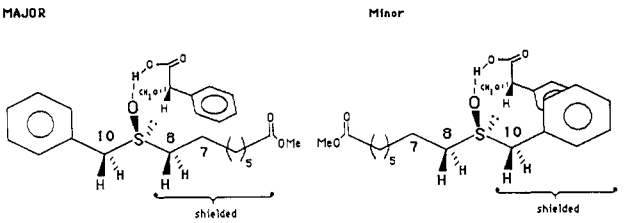


Figure 12. Predictive complexation model showing the interaction of (*S*)-(+)-MPAA with both enantiomers of **7**.

protons with an identical nonequivalence (Δδ) of 0.017 ppm (see Figure 10b). Furthermore, the α-sulfinyl protons at C-8 were also split to give a pattern very similar to that observed earlier in the analysis of **2**-*10,10-d*$_2$, although the magnitude of the nonequivalence was substantially greater: 0.03 ppm for the downfield proton and 0.017 ppm for the upfield proton, ratio = 1.8 (compare Figures 2 and 11b). Since the *upfield* signal of each doublet is reduced in intensity for the benzylic protons and the *downfield* signal of each doublet is reduced in intensity for the α-sulfinyl protons at C-8, we can predict on the basis of our complexation model (see Figure 12) that the absolute configuration of biological **7** is *S*.

This prediction was confirmed by correlation of **7** with the optical antipodes of benzyl decyl sulfoxide (**9**), which were synthesized from the appropriate menthyl sulfinate esters of known absolute configuration.[18] Thus, racemic α-toluenesulfinyl chloride,

(15) In control incubation experiments, using autoclaved yeast, trace amounts of sulfoxide are produced from the corresponding sulfide in a nonregioselective manner.[6a]

(16) The isolated yield of sulfoxide **7** has been improved substantially compared to that reported previously.[7b] We attribute this improvement to a modification of the extraction procedure (see the Experimental Section).

(17) In light of earlier experiences (see Figure 3b), we were careful to use DCl-free $CDCl_3$ in these % ee measurements. We feel that some racemization of sulfoxide probably took place during previous NMR measurements[7b] since the [α]$_D$'s of the benzyl sulfoxide **7** are within 3% of the previously reported value.[7b]

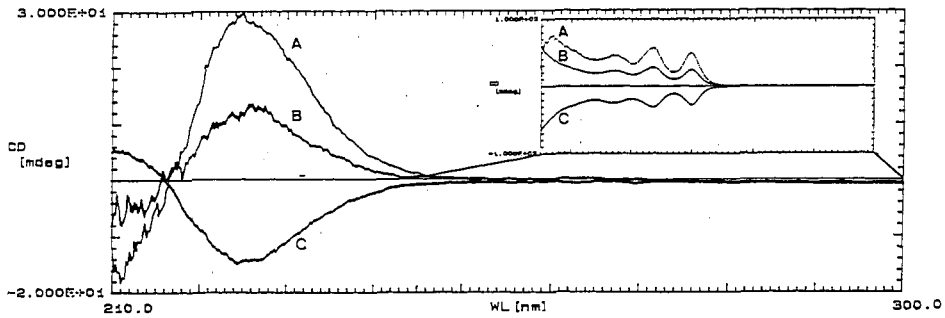


Figure 13. CD spectra of synthetic reference sulfoxides and biologically derived sulfoxide (A) **7**, (B) (*S*)-**9** and (C) (*R*)-**9**. To obtain the major CD curves centered at 225 nm, the concentrations of (*R*)-**9**, (*S*)-**9**, and **7** were 3.11×10^{-5}, 3.33×10^{-5}, and 7.9×10^{-5} M, respectively. To obtain the weaker CD curves (see insert), the concentrations were increased 100-fold.

Table II. Correlation of Chiroptical Data for (*R*)- and (*S*)-Benzyl Decyl Sulfoxide (**9**) with That of (*S*)-*S*-Benzyl-8-mercaptooctanoic Acid Methyl Ester (**7**)

compd	$[\alpha]_D$ ($CHCl_3$, *c* 2)	$[\alpha]_D$ (EtOH, *c* 2)	CD $\Delta\epsilon$ (225, 257, 263, 270 nm)[a]
(*R*)-**9**	+76.9	−4.2	−12.74, −0.26, −0.29, −0.23
(*S*)-**9**	−73.0	+3.9	+12.66, +0.22, +0.27, +0.21
7	−73.1	+5.6	+11.1, +0.19, +0.21, +0.17

[a] The $\Delta\epsilon$ values were computed from molar ellipticities $[\theta]$, which in turn were calculated from the ellipticity data shown in Figure 1 using the appropriate concentrations.

prepared by reduction[19] of α-toluenesulfonyl chloride, was reacted with (−)-menthol as described by Mislow and co-workers[18] to give a mixture of diastereomeric sulfinate esters. The major diastereomer (**8a**) formed under these conditions has previously been isolated by fractional crystallization and has been shown to have the *R* configuration at sulfur.[18,20] We were able to separate the menthyl sulfinates by flash chromatography and quantitate the diastereomeric purity by examining the benzylic resonances in the 200-MHz ^1H NMR spectra. (The minor and major components exhibited singlets at 3.987 and 4.012 ppm, respectively.) Thus, the major, more polar diastereomer (96% de) was isolated as a solid (mp 66–67 °C, lit.[20] mp 75.7–76.5 °C) and possessed an optical rotation ($[\alpha]_D$) of +99.7° (*c* 0.34, $CHCl_3$) (lit.[18] $[\alpha]_D$ +105° ($CHCl_3$)). The minor, less polar diastereomer **8b** (100% de) was isolated as an oil ($[\alpha]_D$ −217° (*c* 0.325, $CHCl_3$)).

Each menthyl sulfinate was treated with decylmagnesium bromide to give the required sulfoxides. Thus the minor sulfinate ester, bearing the *S* configuration at sulfur, yielded (*R*)-benzyl decyl sulfoxide ((*R*)-**9**), and the major ester, bearing the *R* configuration at sulfur, produced the corresponding *S* enantiomer ((*S*)-**9**). The two sulfoxide products were purified by flash chromatography (60% EtOAc/hexane) and then examined by three independent techniques: polarimetry, circular dichroism, and chiral shift reagent/^1H NMR experiments.

The polarimetric data are collected in Table II. It is clear that the specific rotation of the biological sulfoxide **7** in both $CHCl_3$ and EtOH closely matches that of synthetic reference sulfoxide ((*S*)-**9**).

To strengthen the configurational correlation, CD spectra of the sulfoxides were obtained as shown in Figure 13. (*R*)-**9** displayed a negative Cotton effect centered at 225 nm. Three weaker negative maxima could also be observed at 257, 263, and 270 nm. (*S*)-**9** yielded a CD spectrum which was the mirror image of that obtained for the *R* enantiomer. The CD data for (*S*)-**9** are consistent with published ORD spectra of some synthetic (*S*)-benzyl alkyl sulfoxides in which positive Cotton effects are observed at 225–228 nm with weak peaks in the 260–275 region.[21] It is readily apparent that the CD spectrum of our biological sulfoxide **7** matches that of (*S*)-**9** in every respect (see also Table II).

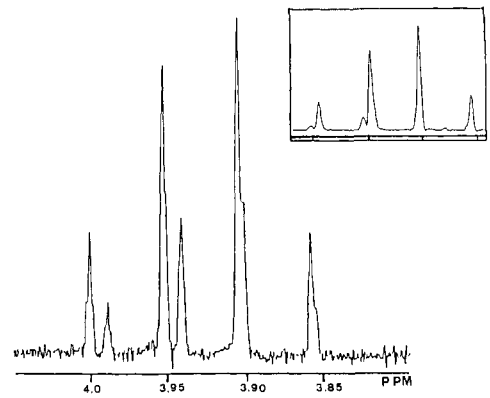
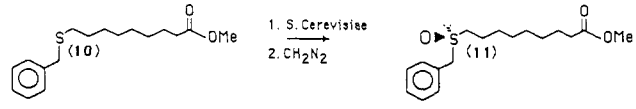


Figure 14. Effect of addition of 3 equiv of (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine on ^1H NMR (300 MHz) resonances due to the benzylic protons of a 2/1 mixture of (*R*)-**9** and (*S*)-**9**. Insert: same experiment but instead using a 70/30 mixture of biological **7** and racemic **7**.

To complete the stereochemical analysis, the two enantiomers of **9** were examined by ^1H NMR (300 MHz, in $CDCl_3$) in the presence of 3 equiv of the Kagan chiral shift reagent. As shown in Figure 14, the signals due to the low-field benzylic proton of the *R* enantiomer are shifted further downfield than those of the corresponding proton of the *S* enantiomer. The biological sulfoxide **7** mimics (*S*)-**9** in this experiment (see insert of Figure 14). These results clearly establish that the biological sulfoxide **7** bears the *S* configuration at sulfur—a result which is consistent with our complexation model and matches that obtained previously for the dialkyl analogue (**2**).

Determination of the Enantiomeric Purity and Absolute Configuration of 11. Using the methodology which we developed for the stereochemical analysis of **7**, it was a simple matter to examine the stereochemistry of the sulfoxide product obtained from **10**. Thus, a small amount (9.0 mg) of crude benzyl sulfoxide **11** was isolated from a feeding of **10** (151 mg) administered to *S. cerevisiae* in the normal way. This material was identified on the basis of MS and high-field ^1H NMR. The % ee of this material



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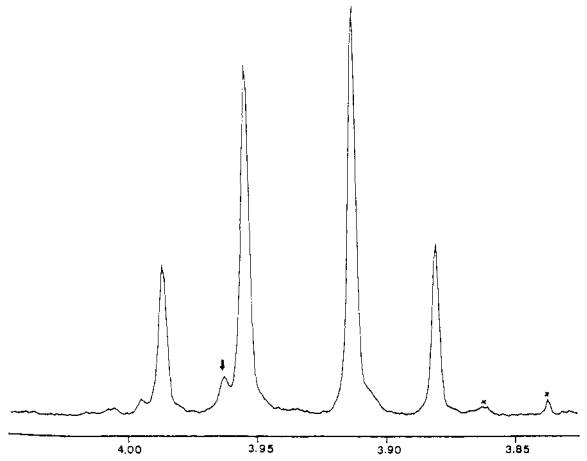
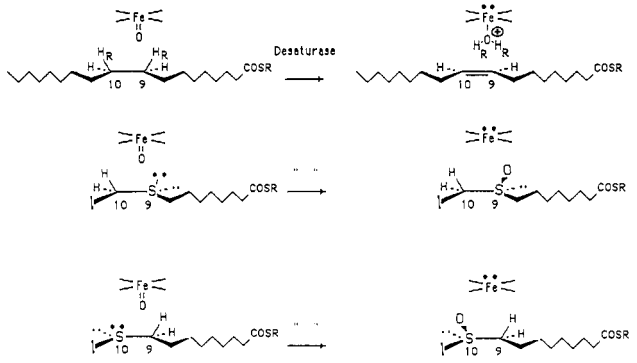


Figure 15. Effect of addition of 3 equiv of (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine on ^{1}H NMR (400 MHz) resonances due to the benzylic protons of biological **11**. The % ee was estimated using the peak indicated.

Scheme V



was determined to be 88% using the Kagan shift reagent, and the predominant enantiomer was shown to be *S* (see Figure 15). In light of the inefficiency of this particular sulfoxidation, the reduction in % ee of this material can again be attributed to the increased contribution of autoxidized material.

Conclusion

On the basis of these studies, it is clear that the stereochemistry of thia fatty acid sulfoxidation matches that of hydrogen removal in the desaturation process. Since it is known that all Δ^9-desaturases studied to date[8] remove the *pro-R* hydrogens at C-9 and C-10, it is gratifying to see that oxygenation of the 9- and 10-thia analogues proceeds with the same stereochemistry. Thus the use of sulfur as a mechanistic probe of the desaturation reaction is further validated. Furthermore, it is striking that 9-sulfoxidation is consistently more efficient than 10-sulfoxidation—a trend which is entirely consistent with the current mechanistic model for desaturation in which the iron oxidant is "asymmetrically" located between carbons 9 and 10 (see Scheme V). This picture would imply that, in addition to undergoing sulfoxidation, 10-thia analogues should be hydroxylated at C-9. A search for product(s) derived from such a process is currently underway in our laboratory.

We were also encouraged by the fact that replacing the pendant alkyl group of methyl 9-thiastearate (**1**) with a benzyl moiety had no effect on the enantioselectivity of sulfoxidation. In fact, we were somewhat surprised to find that the benzyl analogue (**6**) was processed more efficiently than the thiastearate (**1**). This information should prove extremely useful in the design of new mechanistic probes for the desaturase process.

Finally, an important spinoff from this work is the finding that (*S*)-(+)-MPAA appears to be the chiral shift reagent of choice for evaluating the enantiomeric purity of dialkyl sulfoxides and for assigning their absolute configuration; our approach should find useful applications in the field of drug and agrichemical metabolism.[22]

Materials and Methods

General Methods. All NMR spectra were obtained using dilute $CDCl_3$ solutions at the frequencies indicated. All chiral shift experiments on optically active sulfoxides were carried out using high-purity $CDCl_3$ from freshly opened vials or using bottled $CDCl_3$ which had been treated with $MgSO_4$ and Woelm basic alumina. Chemical shifts are expressed in ppm and are referenced to tetramethylsilane. To obtain accurate ^{13}C NMR deuterium isotope shifts, spectra were obtained using a mixture of the compound and its deuterated analogue in the appropriate ratio. The ^{13}C NMR chemical shifts of dibutyl sulfoxide have been incorrectly reported in the literature.[23] The correct assignments were established using routine HETCOR and COSY experiments.[24]

All polarimetric measurements were carried out at 20 °C. CD spectra were obtained on a JASCO J-600 using a cuvette path length of 1 cm.

Mass spectra were obtained by direct probe for sulfoxides or by GC/MS for substrate sulfides using a VG 7070 E mass spectrometer interfaced with a Varian 3300 GC equipped with a 30-m, megabore DB-5 column.

Flash chromatography using silica gel (230–400 mesh) was used to purify substrates and isolate sulfoxide products from the biological medium. 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. A UV lamp (254 nm) was used to locate benzyl compounds.

Unless otherwise stated, all reagents and starting materials were purchased from Aldrich Chemical Company and used without purification. Chiral materials were used without improving their optical purity. All air- and moisture-sensitive reactions were performed under N_2. Unless otherwise noted, organic extracts were shaken with saturated NaCl, dried over Na_2SO_4, and evaporated on a rotary evaporator. 1-Bromononane-*1,1-d*$_2$ and 1-bromooctane-*1,1-d*$_2$ were prepared by treatment of the corresponding deuterated primary alcohols with PBr_3. The deuterated alcohols were in turn synthesized from the corresponding carboxylic acids by reduction using $LiAlD_4$ (98 atom % D, Merck Sharpe and Dohme). Butyl-*d*$_9$ bromide (98.1 atom % D) was purchased from Merck Sharpe and Dohme. Commercially available chiral materials were used without improving enantiomeric purity.

Synthesis of Substrates. All thia fatty acids were synthesized by alkylation of the appropriate ω-thio acid with the alkyl bromide of the correct chain length.[25] After methylation of the carboxyl function, the crude products were purified by flash chromatography (silica gel, 4% EtOAc/hexane) to give colorless liquids. The relevant analytical data is given below.

A. Methyl 9-thiastearate (1): mp 19.5–21.5 °C; ^{1}H NMR (200 MHz) δ 3.66 (3 H, s, C(O)OC*H*$_3$), 2.49 (4 H, t, C*H*$_2$SC*H*$_2$), 2.30 (2 H, t, C*H*$_2$C(O)OCH$_3$), 1.60 (6 H, m, C*H*$_2$CH$_2$C(O)OCH$_3$, C*H*$_2$CH$_2$SCH$_2$C*H*$_2$), 1.29 (18 H, br s, C*H*$_2$), 0.87 (3 H, t, C*H*$_3$CH$_2$); MS (EI, 70 eV) *m/e* 316 (M^+), 285 ($(M - OCH_3)^+$), 157 ($M - CH_3(CH_2)_8S)^+$), 159 ($(CH_3(CH_2)_8S)^+$). Anal. Calcd for $C_{18}H_{36}O_2S$: C, 68.30; H, 11.46; S, 10.13. Found: C, 68.41; H, 11.88; S, 9.94.

B. Methyl 9-Thiastearate-*10,10-d*$_2$ (1-*10,10-d*$_2$). This compound was prepared using 1-bromononane-*1,1-d*$_2$ in the S-alkylation of 8-mercaptooctanoic acid. The spectral data was similar to that of **1** except the following: MS (EI, 70 eV) 318 (M^+), 287 ($(M - OCH_3)^+$), 157 ($M - CH_3(CH_2)_7CD_2S)^+$), 161 ($(CH_3(CH_2)_7CD_2S)^+$).

C. Methyl 10-thiastearate (3): mp 19–20 °C; ^{1}H NMR (200 MHz) similar to that of **1**; MS (EI, 70 eV) 316 (M^+), 285 ($(M - OCH_3)^+$), 171 ($(M - CH_3(CH_2)_7S)^+$), 145 ($(CH_3(CH_2)_7S)^+$). Anal. Calcd for $C_{18}H_{36}O_2S$: C, 68.30; H, 11.46; S, 10.13. Found: C, 68.00; H, 11.76; S, 10.04.

D. Methyl 10-Thiastearate-*11,11-d*$_2$ (3-*11,11-d*$_2$). This compound was prepared using 1-bromooctane-*1,1-d*$_2$ in the S-alkylation of 9-mercaptononanoic acid. The spectral data was similar to that of **1** except the following: MS (EI, 70 eV) 318 (M^+), 287 ($(M - OCH_3)^+$), 171 ($M - CH_3(CH_2)_6CD_2S)^+$), 147 ($(GH_3(CH_2)_6CD_2S)^+$).

E. Methyl *S*-Benzyl-8-mercaptooctanoate (6). This compound was prepared via the route used for the synthesis of **1**, except benzyl bromide was used in the S-alkylation of 8-mercaptooctanoic acid: bp 106–112 °C (10 mmHg); ^{1}H NMR (200 MHz) δ 7.35 (5 H, m, phenyl), 3.70 (2 H,

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s, $C_6H_5CH_2S$), 3.65 (3 H, s, C(O)OCH_3), 2.40 (2 H, t, SCH_2CH_2), 2.25 (2 H, t, $CH_2C(O)OCH_3$), 1.73 (2 H, m, $SCH_2CH_2CH_2$), 1.65 (2 H, m, $CH_2CH_2C(O)OCH_3$), 1.35 (6 H, br s, $(CH_2)_3$); MS (EI, 70 eV) 280 (M^+), 189 ($(M - C_7H_7)^+$), 157 ($(M - C_7H_7S)^+$), 123 ($(C_7H_7S)^+$), 91 ($(C_7H_7)^+$). Anal. Calcd for $C_{16}H_{24}O_2S$: C, 68.52; H, 8.63; S, 11.43. Found: C, 68.41; H, 8.67; S, 11.05.

F. Methyl *S*-Benzyl-9-mercaptononanoate (10). This compound was prepared via the route used for the synthesis of **3**, except benzyl bromide was used in the S-alkylation of 9-mercaptononanoic acid: bp 118–124 °C (10 mmHg); ^1H NMR (200 MHz) similar to that of **6**; MS (EI, 70 eV) 294 (M^+), 203 ($(M - C_7H_7)^+$), 171 ($(M - C_7H_7S)^+$), 123 ($(C_7H_7S)^+$), 91 ($(C_7H_7)^+$). Anal. Calcd for $C_{17}H_{26}O_2S$: C, 69.34; H, 8.90; S, 10.89. Found: C, 68.86; H, 9.07; S, 10.56.

Synthesis of Sulfoxide Products. Racemic sulfoxides were prepared by treating sulfides with 1 equiv of MCPBA at 0 °C in CH_2Cl_2 for 1 h. Contaminating sulfone and sulfide were removed by flash chromatography (75% EtOAc/hexane) to give colorless, crystalline solids. The analytical data obtained for these compounds are as follows.

A. (±)-Methyl 9-thiastearate *S*-oxide (2): mp 66.0–66.5 °C; R_f 0.15 (75% EtOAc/hexane); ^1H NMR (300 MHz) δ 3.68 (3 H, s, C(O)OCH_3), 2.65 (4 H, m, $CH_2S(O)CH_2$), 2.25 (2 H, t, $CH_2C(O)OCH_3$), 1.75 (4 H, m, $CH_2CH_2S(O)CH_2CH_2$), 1.60 (2 H, p, $CH_2CH_2C(O)OCH_3$), 1.20 (18 H, br s, $CH_2CH_2CH_2$), 0.90 (3 H, t, CH_3CH_2); MS (EI, 70 eV) 315 ($(M - OH)^+$), 301 ($(M - OCH_3)^+$), 174 ($(OS(CH_2)_7CO)^+$), 159 ($(CH_3(CH_2)_8SO)^+$), 157 ($(CH_2)_7CO_2Me)^+$); ^{13}C NMR (100.6 MHz) δ 174.15, 52.56, 52.42, 51.47, 34.0, 31.83, 29.34, 29.23, 29.21, 28.92, 28.87, 28.86, 28.71, 24.81, 22.66, 22.63, 22.57, 14.09; IR ($CHCl_3$) 1025 cm^{-1} (S=O stretch). Anal. Calcd for $C_{18}H_{36}O_3S$: C, 64.24; H, 10.78; S, 9.53. Found: C, 64.31; H, 11.09; S, 9.58.

B. (±)-Methyl 9-Thiastearate-*10,10-d*$_2$ *S*-Oxide (2). The spectral data was similar to that of **2** except for the following MS (EI, 70 eV) 317 ($(M - OH)^+$), 303 ($(M - OCH_3)^+$), 174 ($(OS(CH_2)_7CO)^+$), 161 ($(CH_3(CH_2)_7(CD_2)SO)^+$), 157 ($(CH_2)_7CO_2Me)^+$).

C. (±)-Methyl 10-thiastearate *S*-oxide (4): mp 65.5–66.0 °C; R_f 0.19 (75% EtOAc/hexane); ^1H NMR (300 MHz) similar to that of **2**; MS (EI, 70 eV) 315 ($(M - OH)^+$), 301 ($M - OCH_3)^+$), 188 ($(OS(CH_2)_8CO)^+$), 161 ($(CH_3(CH_2)_7SO)^+$), 171 ($((CH_2)_8CO_2Me)^+$); IR ($CHCl_3$) 1025 cm^{-1} (S=O stretch). Anal. Calcd for $C_{18}H_{36}O_3S$: C, 64.24; H, 10.78; S, 9.53. Found: C, 64.93; H, 11.23; S, 9.73.

D. (±)-Methyl 10-Thiastearate-*11,11-d*$_2$ *S*-Oxide (4-*11,11-d*$_2$). The spectral data was similar to that of **4** except for the following: MS (EI, 70 eV) 317 ($(M - OH)^+$), 303 ($(M - OCH_3)^+$), 188 ($(OS(CH_2)_8CO)^+$), 188 ($(OS(CH_2)_8CO)^+$), 163 ($(CH_3(CH_2)_6(CD_2)SO)^+$), 171 ($(CH_2)_8CO_2Me)^+$).

E. (±)-Methyl *S*-benzyl-8-mercaptooctanoate *S*-oxide (7): mp 73.5–74.0 °C; ^1H NMR (300 MHz) δ 7.338 (3 H, m, phenyl), 7.281 (2 H, m, phenyl), 3.959 (2 H, AB q, J_{AB} = 12.9 Hz, $C_6H_5CH_2S(O)$), 3.641 (3 H, s, C(O)OCH_3), 2.528 (2 H, t, $S(O)CH_2CH_2$), 2.267 (2 H, t, $CH_2C(O)OCH_3$), 1.728 (2 H, p, $S(O)CH_2CH_2CH_2$), 1.577 (2 H, p, $CH_2CH_2C(O)OCH_3$), 1.29 (6 H, br s, $(CH_2)_3$); MS (EI, 70 eV) 296 (M^+), 91 ($C_7H_7^+$); IR ($CHCl_3$) 1031 cm^{-1} (S=O stretch). Anal. Calcd for $C_{16}H_{24}O_3S$: C, 64.83; H, 8.16; S, 10.82. Found: C, 64.75; H, 8.44; S, 10.39.

Synthesis of Chiral Dibutyl-*d*$_9$ Sulfoxide ((*R*)-5-*d*$_9$). A mixture of diastereomeric (−)-menthyl butanesulfinates was prepared by reaction of butanesulfinyl chloride with (−)-menthol according to the method of Mislow.[13] The crude mixture (1.9 g) was purified by flash chromatography (10% EtOAc/hexane) to give 1.2 g of a colorless syrupy oil ($[\alpha]_D$ = −44.5° (*c* 2.7, acetone), lit.[20] $[\alpha]_D$ = −51° (*c* 2.58, acetone)), a 0.7-g portion of which was treated with butylmagnesium-*d*$_9$ bromide in ether to give the title compound (169 mg) as a colorless, low-melting solid in 36% isolated yield after flash chromatography: mp 20–21 °C; ^1H NMR (300 MHz) δ 2.65 (2 H, m $CH_2S(O)$), 1.73 (2 H, p, $CH_2CH_2S(O)$), 1.46 (2 H, m, CH_3CH_2), 0.94 (3 H, t, CH_3CH_2); ^{13}C NMR (125.8 MHz) δ 52.18, 24.63, 22.10, 13.69.

Synthesis of Chiral Benzyl Decyl Sulfoxides. A crude mixture (1 g) of (−)-menthyl phenylmethanesulfinates was obtained as previously described.[18,20] Each diastereomer was then isolated by flash chromatography (5% EtOAc/hexane). The properties of each compound were as follows.

A. (−)-Menthyl (+)-(*R*)-phenylmethanesulfinate (8a): major component (241.0 mg, 96% de) mp 66–67 °C (lit.[20] mp 75.7–76.5 °C); R_f 0.18 (silica gel, 10% EtOAc/hexane); $[\alpha]_D$ = +99.7° (*c* 0.34, $CHCl_3$) (lit.[18] $[\alpha]_D$ = +105° ($CHCl_3$), $[\alpha]_D$ = +108.2° (*c* 0.25, EtOH) (lit.[20] $[\alpha]_D$ = +120° (*c* 0.25, EtOH)); ^1H NMR (200 MHz) δ 7.3 (5 H, br s, phenyl), 4.008 (2 H, s, $C_6H_5CH_2S(O)$), 3.887 (1 H, t of d, CHOS(O)), 0.5–2.3 (18 H, menthyl hydrogens).

B. (−)-Menthyl (−)-(*S*)-phenylmethanesulfinate (8b): minor component (64.3 mg, 100% de) colorless oil; R_f 0.21 (silica gel, 10% EtOAc/hexane); $[\alpha]_D$ = −217° (*c* 0.325, $CHCl_3$), $[\alpha]_D$ = −208.2° (*c* 0.25, EtOH); ^1H NMR (200 MHz) δ 7.3 (5 H, br s, phenyl), 3.987 (2 H, s, $C_6H_5CH_2S(O)$), 3.896 (1 H, t of d, CHOS(O)), 0.5–2.3 (18 H, menthyl hydrogens).

C. (*S*)-Benzyl Decyl Sulfoxide ((*S*)-9). (−)-Menthyl (+)-(*R*)-phenylmethanesulfinate (140 mg, 0.5 mmol) was added to a solution of decylmagnesium bromide (0.5 mmol) in dry ether, and the resultant solution was stirred at room temperature for 6 h. The reaction was quenched with 10% ammonium chloride, and the product was extracted with CH_2Cl_2. The crude product (166 mg) was purified by flash chromatography (60% EtOAc/hexane). The title compound was obtained as a colorless solid (64.5 mg): mp 62–63 °C; (lit.[26] (racemic) mp 76–76.5 °C); $[\alpha]_D$ = −73° (*c* 2, $CHCl_3$), $[\alpha]_D$ = +3.9° (*c* 2, EtOH); ^1H NMR (300 MHz) δ 7.3 (5 H, m, phenyl), 3.96 (2 H, AB q, J_{AB} = 12.9 Hz, $C_6H_5CH_2S(O)$), 2.54 (2 H, t, $S(O)CH_2CH_2$), 1.73 (2 H, p, $S(O)CH_2CH_2CH_2$), 1.23 (br s, $(CH_2)_7CH_3$), 0.857 (3 H, t, $(CH_2)_7CH_3$).

D. (*R*)-Benzyl decyl sulfoxide ((*R*)-9) was prepared in the same manner from (−)-menthyl (−)-(*S*)-phenylmethanesulfinate (28.0 mg): $[\alpha]_D$ = +76.9° (*c* 1.9, $CHCl_3$), $[\alpha]_D$ = −4.2° (*c* 1.9, EtOH).

Biological Synthesis of Sulfoxides Using *S. cerevisiae*. A typical feeding experiment was carried out as follows. The strain of baker's yeast used in this experiment was wild type *Saccharomyces cerevisiae* ATTC #12341. Cultures were grown in YEPD medium (1.0% yeast extract, 2.0% bactopeptone, 2.0% D-glucose), at 30 °C in a rotary incubator–shaker set at 150 rpm. Four milliliters of starter culture containing 5 × 10^8 cells was used to innoculate 300 mL of sterile medium contained in a 1-L Erlenmeyer flask.

A 50-mg portion of thia fatty acid methyl ester was added as a solution (5% w/v) in absolute ethanol to each culture flask, and the innoculated cultures were then incubated for 24 h to give a final cell density of 3 × 10^{10} cells/flask. The addition of exogenous fatty acid did not affect the growth of the organism with respect to control cultures.

Culture media was collected by centrifugation (6000×g, 10 min), the supernatant was adjusted to pH 1.5 and extracted with five 80-mL portions of CH_2Cl_2, and emulsions were broken by evaporation of partially separated layers on a rotary evaporator (procedure A). Alternatively, the supernatant was brought to 5 M in NaCl, acidified to pH 0.5 with 50% H_2SO_4, and extracted with CH_2Cl_2 (5 × 70 mL), $CHCl_3$ (2 × 40 mL), and CCl_4 (2 × 40 mL) (procedure B). The combined organic layers were dried over anhydrous Na_2SO_4 (40 g, 4 h). The decanted solution was evaporated at 30–40 °C in vacuo to give a crude residue which was methylated using an ethereal diazomethane solution. TLC analysis of the extract was carried out on silica gel plates using 80% EtOAC/hexane as eluant. Purification of the sulfoxide product was performed by flash chromatography using 65% EtOAc/hexane as eluant.

Biologically produced sulfoxides were compared with the authentic synthetic standards on the basis of ^1H NMR, R_f, and MS data. The conversions were as follows: **2**-*10,10-d*$_2$ (22 mg, 8% yield) from **1** (253 mg) via extraction procedure B; **2** (14.8 mg, 9% yield) from **1** (151 mg) via extraction procedure B; **4** (14.8 mg, 4% yield) from **3** (302 mg) via extraction procedure B; **4**-*11,11-d*$_2$ (17.7 mg, 6% yield) from **3** (303 mg) via extraction procedure B; **7** (41.2 mg, 16% yield) from **6** (250 mg) via extraction procedure A; **7** (142 mg, 54% yield) from **6** (312 mg) via extraction procedure B; **7** (68 mg, 43% yield) from **6** (151 mg) via extraction procedure B; **11** (9 mg, 6% yield) from **10** (151 mg) via extraction procedure B.

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Registry No. 1, 114119-38-9; **1**-*10,10-d*$_2$, 135073-97-1; (±)-**2**, 141393-74-0; (±)-**2**-*10,10-d*$_2$, 141393-75-1; (*R*)-**2**, 135073-96-0; **3**, 114119-39-0; **3**-*11,11-d*$_2$, 141292-42-4; (±)-**4**, 141292-41-3; (±)-**4**-*11,11-d*$_2$, 141292-44-6; (*R*)-**4**, 141393-77-3; (*R*)-**5**, 135073-99-3; **6**, 130196-52-0; (±)-**7**, 130272-74-1; (*S*)-**7**, 130196-53-1; (*R*)-**8a**, 21204-21-7; (*S*)-**8b**, 141393-76-2; (*R*)-**9**, 141292-47-9; (*S*)-**9**, 141292-46-8; **10**, 141292-43-5; (*S*)-**11**, 141292-48-0; (−)-*p*-menth-3-yl (*R*)-1-butanesulfinate, 21204-20-6; (−)-*p*-menth-3-yl (*S*)-1-butanesulfinate, 81769-14-4; butyl-*d*$_9$ magnesium bromide, 141292-45-7; desaturase, 103843-28-3; stearoyl coA, 85-61-0; (*S*)-(+)-α-methoxyphenylacetic acid, 26164-26-1; (*R*)-(−)-*N*-(3,5-dinitrobenzoyl)-α-methylbenzylamine, 69632-32-2.

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