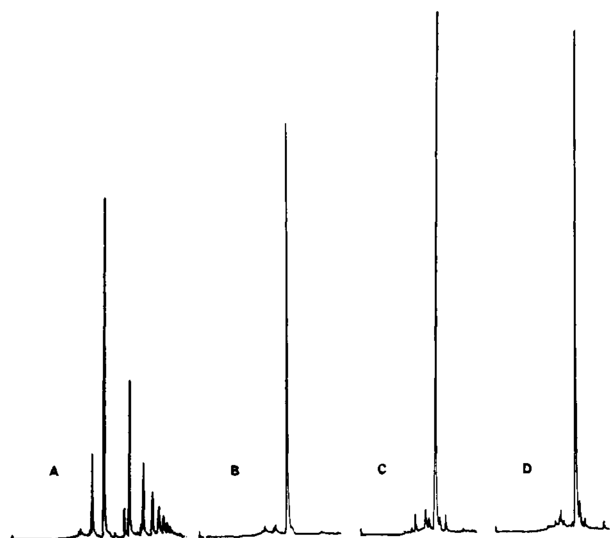

Figure 1. HPLC of crude products from syntheses with unprotected bases: A and D show products from preparation of d(AT) and d(GT), respectively, using a standard reaction cycle; B and C show products from preparation of d(AT) and d(CT) using a $C_5H_5N{\cdot}HCl/C_6H_5NH_2$ transfer step. A C18 ODS column (4.6 × 200 mm) was used, with 0.03 M $Et_3N{\cdot}HOAc$ (pH 7.0) and a CH_3CN gradient increasing from 0% at 1%/min; flow rate 1 mL/min. Elution times for major peaks in A–D are 15.2, 15.2, 13.0, and 13.9 min, respectively.

concentrated NH_4OH, the reagent used in standard protocols. As a preliminary example, dimers dC(OMe)T, dA(OMe)T, and dG(OMe)T were readily obtained by utilizing an oxalyl-CPG support, a transfer step (pyridine hydrochloride/aniline), and cleavage with 5% NH_4OH in MeOH (3 min).[15]

Acknowledgment. This work was supported by Grant 5R37 GM10265 from the National Institute of General Medical Sciences.

(15) For dA(OMe)T, dC(OMe)T, and dG(OMe)T, respectively: HPLC elution time (conditions in Figure 1) 23.0 and 23.3 min (stereoisomers), 18.5 min, 21.4, and 21.7 min; M + H$^+$ (FAB MS) 570, 546, 586; λ_{max} 262, 268, 256 nm. These compounds were further characterized by conversion to the dinucleoside phosphates by treatment with NH_4OH.

Stereochemical Analysis of a Quasisymmetrical Dialkyl Sulfoxide Obtained by a Diverted Biodehydrogenation Reaction[†]

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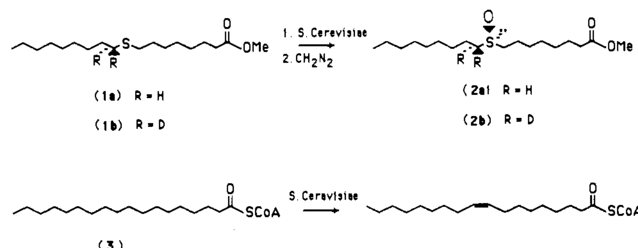
Received December 27, 1990

In a previous communication, we have reported that methyl 9-thiastearate (**1a**) is converted to the corresponding sulfoxy acid by cultures of bakers' yeast.[1] It appears that this sulfoxide is the product of a diverted fatty acid desaturase reaction which normally introduces a cis double bond into the hydrocarbon chain of stearoyl-CoA (**3**).[2] We now report that the quasisymmetrical sulfoxide **2a** is produced with very high enantioselectivity and has the *R* configuration.

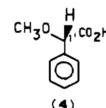
The first step in our stereochemical analysis was to label the C-10 position of the sulfide (**1a**) with deuterium. Thus the dianion of 8-mercaptooctanoic acid was S-alkylated with the tosylate of nonan-1-ol-*1,1*-d_2[3] in the manner previously described.[4] The



sample of 9-thiastearic-*10,10*-d_2 acid so prepared was methylated by using BF_3/MeOH and the resultant ester (**1b**) purified by flash chromatography (silica gel, 4% EtOAc/hexane).

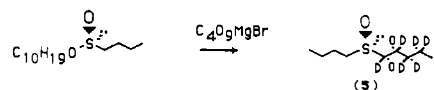
A sample of methyl 9-thiastearate-*10,10*-d_2 *S*-oxide (**2b**, 22 mg) was obtained essentially as previously reported[1] by administering **1b** (253 mg) to growing cultures of *Saccharomyces cerevisiae* ATCC 12341.

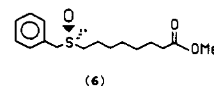
The optical purity of our biologically produced sulfoxide was assessed by taking advantage of the known ability of carboxylic acids such as trifluoroacetic and acetic acids to shift the ^1H NMR signals of diastereotopic protons adjacent to the sulfinyl group.[5] We reasoned that a chiral carboxylic acid might discriminate between the protons at C-8 of (*R*)- or (*S*)-**2b**. Thus addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (**4**) to a 20 mM solution of racemic **2b**[6] in $CDCl_3$ caused the ^1H NMR signals of one of the diastereotopic protons at C-8 to shift downfield by 0.15 ppm as shown in Figure 1A. That chiral discrimination had



occurred was apparent from the fact that all signals in the resultant ABXY pattern were doubled. When the chiral shift experiment was repeated with biologically produced **2b**, it became clear that the *downfield* half of each doublet had disappeared. (See Figure 1B.) We were thus able to estimate the enantiomeric excess of this material to be >96%.[7]

Our method for assessing optical purity is sufficiently general to allow us to correlate the absolute configuration of **2b** with that of a simpler chiral dialkyl sulfoxide. We thus synthesized a mixture of enantiomeric deuterated dibutyl sulfoxides in which the *R* enantiomer **5** was in excess. This material was prepared





via Grignard attack of (perdeuteriobutyl)magnesium bromide on a diastereomeric mixture of (−)-menthyl 1-butanesulfinates where the major diastereomer is known to bear the *R* configuration at sulfur.[8] The Grignard reaction is known to proceed with inversion of configuration.[8] Combination of the mixture of enantiomeric deuterated dibutyl sulfoxides with our chiral shift reagent (**4**) resulted in a set of NMR signals similar to that obtained for the

† This paper is dedicated to Karl Diedrich.

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

(6) Racemic **2b** was prepared by oxidation of **1b** with MCPBA as previously described.[1]

(7) We have found that DCl-free $CDCl_3$ must be used to determine % ee. Significant racemization occurs when these precautions are not taken as indicated by appearance of the low field set of signals. Interestingly, we observe substantial broadening of the upfield sulfinyl multiplet when very dry solvent is used.

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J. Am. Chem. Soc. **1991**, *113*, 5878–5880

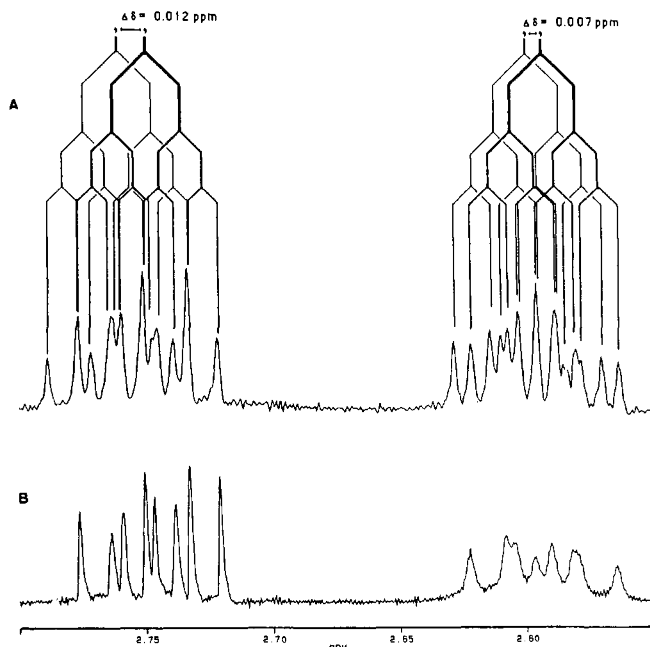

Figure 1. Effects of the addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (**4**) on ^{1}H NMR (500 MHz) resonances due to (A) the α-sulfinyl protons of racemic **2b** and (B) the α-sulfinyl protons of biologically produced **2b**.

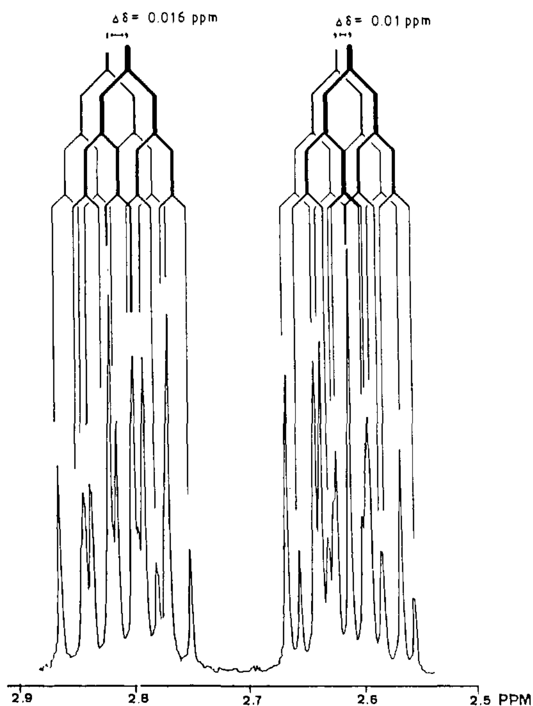

Figure 2. Effects of the addition of 3 equiv of (*S*)-(+)-α-methoxyphenylacetic acid (**4**) on ^{1}H NMR (300 MHz) resonances due to the α-sulfinyl protons of predominantly (*R*)-butyl butyl-d_9 sulfoxide (**5**).

fatty acid sulfoxide; however, in this case, the *upfield* half of each doublet was reduced in intensity.[9] (See Figure 2.) It clearly follows that the disposition of the labeled and unlabeled methylene groups surrounding the sulfinyl group of biologically produced **2b** is opposite to that of **5**. The absolute configuration of the parent sulfoxide **2a** is therefore *R*, a stereochemical result that is consistent with that obtained for a benzyl analogue (**6**).[10]

In conclusion, we have demonstrated that biosulfoxidation of a quasisymmetrical thia fatty acid analogue is a highly enantioselective process.

Acknowledgment. We thank the NSERC for a postgraduate scholarship to D.M. and Carleton University for financial support of this work. The assistance of Dr. Barbara Blackwell, John Nikiforuk (Agriculture Canada), and Professor G. W. Buchanan (Carleton University) is greatly appreciated.

(9) The ee of **5** was estimated to be 47%, in excellent agreement with the known de (47%) of the starting menthyl sulfinate mixture.[8]

(10) Buist, P. H.; Marecak, D. M.; Partington, E. *J. Org. Chem.* **1990**, *55*, 5667. The absolute configuration of **6** has recently been confirmed by synthesis (unpublished results).

Pseudomonas oleovorans Monooxygenase Catalyzed Asymmetric Epoxidation of Allyl Alcohol Derivatives and Hydroxylation of a Hypersensitive Radical Probe with the Radical Ring Opening Rate Exceeding the Oxygen Rebound Rate

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The non-heme monooxygenases are NAD(P)H/O_2-dependent metalloenzymes which often contain iron(s) in the active site for catalysis.[1] These enzymes catalyze the incorporation of molecular oxygen into unactivated organic molecules in a selective manner.[2] It has been demonstrated, with support from model studies,[3] that the putative "iron–oxo" species[4] generated from the reduced enzyme and molecular oxygen are like that of the heme-containing monooxygenases[5] and capable of hydroxylation of alkanes, epoxidation of olefins, oxidation of heteroatoms such as S or N, and O-demethylation of methyl ethers. *Pseudomonas oleovorans*

[†] Scripps Research Institute.
[‡] Texas A&M University.

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(4) (a) For a detailed discussion, see: Ortiz de Montellano, P. R. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 217–271. Also see: (b) Mcurry, T. J.; Groves, J. T.; In Cytochrome P-450: *Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum: New York, 1986; pp 3–5. (c) Groves, J. T.; McClusky, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 859.

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