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REGIO- AND STEREOSELECTIVE OXIDATION
OF SULPHUR DERIVATIVES OF FATTY ACIDS
BY THE STEAROYL COA DESATURASE SYSTEM
OF SACCHAROMYCES CEREVISIAE.

by

Paul Skala, B.Sc.

A thesis
submitted to the Faculty of Graduate Studies
in partial fulfilment of the requirements
for the degree of
Master of Science

Department of Chemistry

Carleton University
Ottawa, Canada

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The undersigned recommended to the Faculty of Graduate Studies
acceptance of the thesis

"Regio- and Stereoselective Oxidation of Sulphur Derivatives
of Fatty Acids by the Stearoyl CoA Desaturase System
of *Saccharomyces Cerevisiae*."

submitted by Paul Skala, B.Sc.,
in partial fulfilment of the requirements for the degree of
Master of Science.

A handwritten signature in dark ink, appearing to read "W. H. Bond", is written over a horizontal dashed line.

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Chairman, Department of Chemistry
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Abstract.

Certain sulphur derivatives of fatty acids are known to be good substrates for the stearoyl CoA desaturase system. In this thesis, the fate of these compounds has been investigated. The formation of sulfoxides from several thia-fatty acids by this desaturase system has been demonstrated.

By using a number of sulphur derivatives of stearic acid as mechanistic probes we have been able to study the regioselectivity of an oxidative attack by the terminal stearoyl CoA desaturase in a whole cell system, without the need for isolation of the pure enzyme. Methyl esters of 3- to 13-thiastearic acids have been administered in competition to growing *Saccharomyces cerevisiae* cells. The composition of thiastearate S-oxide mixtures thus produced has been determined. Extensive desaturation of methyl 3- to 6- and 12- to 13-thiastearates as well as the predominant formation of methyl 9-thiastearate S-oxide in these experiments confirmed the involvement of the stearoyl CoA desaturase system in this process. It also seems to indicate that the primary attack by the active oxidant of the terminal desaturase occurs at the C9, rather than C10 position of stearic acid.

The determination of optical purity of methyl 9-thiastearate S-oxide produced by the yeast cells from methyl 9-thiastearate was attempted by chiral shift ^1H NMR.

In an exploratory experiment several S-benzyl- ω -mercapto-derivatives of fatty acids have been administered to the organism in question, resulting in a significant sulfoxide formation from methyl S-benzyl-8-mercaptooctanoate. The enantiomeric excess of this material has been determined by chiral shift ^1H NMR (33 %) and the compound has been tentatively assigned the (S) absolute configuration. The thermal and chemical instability of benzyl sulfoxides seems to be a plausible explanation for somewhat low optical purity of the above mentioned material, rather than a possible lack of absolute stereoselectivity of the oxidative attack by the enzyme in question.

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1. Introduction.

1.1. Unsaturated fatty acids.

As indispensable components of cell membranes, fatty acids are essential to cellular organisms. A remarkable variety of fatty acids exist in living organisms. The fatty acid profiles of microbial species for example define a characteristic signature of the various organisms. Several examples of typical fatty acid profiles are shown in Figure 1.¹

Figure 1: Fatty acid profiles of various organisms.

The presence of cis- unsaturated fatty acids is especially important. In the cell membranes the cis- double bonds introduce kinks in the hydrocarbon tails of the membrane fatty acids, which brings about a decrease in the gel-liquid-crystalline transition temperature.

The anaerobic pathway (Scheme 1), which appears to be restricted to bacteria is, as the name implies, a non-oxidative process.

Here the introduction of the double bond occurs at an early stage of *de novo* fatty acid synthesis, which proceeds in the usual manner, until a 10 carbon unit is reached. At this point, β -hydroxydecanoyl thioester dehydrase catalyses dehydration and double bond isomerization leading to the formation of cis-3-decenoyl ACP and trans-2-decenoyl ACP.

The trans- isomer is the normal intermediate in saturated fatty acid synthesis and undergoes reduction and further chain elongation to the saturated product. However, the cis- isomer cannot be reduced by enoyl ACP reductase but instead continues in the elongation process as an unsaturated intermediate. The principal product of anaerobic unsaturated fatty acid synthesis is vaccenic acid: $C_{18}\Delta^{11}C$.³

The aerobic pathway of unsaturated fatty acid biosynthesis is more interesting from a mechanistic point of view, since it features oxidative attack at an unactivated methylene carbon atom (Scheme 2).

Scheme 2.

The process utilizes molecular oxygen and requires NADH or NADPH as an electron source. Animal, fungal and microbial desaturases generally require CoA (coenzyme A) derivatives of fatty acids as substrates, while the desaturases of plants are specific for ACP (acyl transfer protein) derivatives of fatty acids. Desaturases requiring the CoA derivatives are associated with the microsomal fraction; the acyl ACP requiring plant desaturases are soluble and are present in the chloroplasts. The desaturases are highly regioselective in their attack on the fatty acyl chain.⁴ The production of the $\Delta 9$ double bond is the most common, although $\Delta 5$, $\Delta 6$ and other desaturases are also well known. This remarkable regioselectivity is probably due to enzyme binding of the substrate at the carboxyl end of the fatty acid, allowing the appropriate methylene groups to be properly positioned under the oxidant.

1.2. Enzymology of fatty acid desaturation.

The desaturation of fatty acids occurs by a multi-enzyme complex known collectively as the stearoyl CoA desaturating system. To date, the best characterized desaturase is that isolated from rat liver microsomes by P. Strittmatter.⁵ It consists of a flavoprotein called cytochrome b_5 reductase, the cytochrome b_5 hemoprotein and the non-heme iron stearoyl CoA desaturase (Scherer, p 3).

Scheme 3.

In the process NADH is stoichiometrically oxidized to NAD^+ by the cytochrome b_5 reductase, which then reduces the heme iron of cytochrome b_5 and this in turn effects the reduction of the non-heme iron of the stearoyl CoA desaturase. Molecular oxygen presumably binds at the non-heme iron and is reduced to H_2O while stearoyl CoA is oxidized to oleoyl CoA.

These desaturase systems are somewhat similar to three enzyme complexes for oxygen dependent hydroxylations involving cytochrome P_{450} . Like the cytochrome P_{450} systems, the stearoyl CoA desaturase system involves a hemoprotein and a non-heme iron component. However, in the stearoyl CoA desaturase system the functional positions of the hemoprotein and the non-heme iron component are reversed from those in the P_{450} systems. The cytochrome in the desaturase system does not interact directly with oxygen, presumably because it is buried deep in the interior of the protein and thus inaccessible to oxygen. Instead, the cytochrome acts as the middle component of the system, transferring electrons from a flavoprotein to the non-heme iron desaturase.

The mammalian cytochrome b_5 was isolated as an amphipathic protein (MW 16,700 daltons). It was shown to contain two domains: One is hydrophilic and contains a heme unit with an iron atom, essential for catalytic activity. The other is a single chain polypeptide of high hydrophobicity, which enables the molecule to bind to the microsomal membranes.

The cytochrome b_5 reductase is also an amphipathic protein (MW 43,000 daltons). Like cytochrome b_5 , it also contains a single chain hydrophobic tail, capable of diffusion into the lipophilic membrane material. The hydrophilic portion of this enzyme contains one FAD molecule.

The cyanide-sensitive stearoyl CoA desaturase is a single polypeptide chain of 458 residues (MW 33,000 daltons) which is hydrophobic throughout the chain. It contains one atom of tightly bound non-heme iron per molecule, which appears essential for catalysis (catalytic activity destroyed by chelators of Fe^{2+}).

Even though the amino acid sequence of this terminal desaturase component is known and genetically engineered bacteria containing a plasmid insert coding for the 358 amino acids of the desaturase can be grown to mass produce the enzyme,⁶ little is known to date about the structure of the non-heme iron oxidant of the desaturase. Chemical modification

of this enzyme has shown, that up to five tyrosyl residues are involved in the binding of iron, possibly by chelation of the phenolic oxygens.⁷ Spectroscopic studies of another non-heme iron enzyme - superoxide dismutase have also shown, that the binding of iron is five-coordinate.⁸

The presence of non-heme iron in proteins in lipid metabolism is widespread. Non-heme iron enzymes are involved in the terminal methyl oxidation of fatty acids to alcohols,⁹ epoxidation of terminal alkenes¹⁰ and hydroperoxidation of polyunsaturated fatty acids.¹¹ However, little is known about the active site of these enzymes.

1.3. Mechanism of fatty acid desaturation.

Considering the chemical similarities between the terminal desaturase and cytochrome P₄₅₀, it is tempting to infer similarities in the mechanism of action of these two enzymes as well.

In P₄₅₀-catalyzed reactions, a radical mechanism is thought to operate via a hypervalent heme iron oxo intermediate⁷³ (Scheme 4).

Scheme 4.

This reactive species is believed to be generated in a stepwise sequence, that involves substrate binding, one electron reduction, molecular oxygen binding, a second one electron reduction and the formation and repulsion of one molecule of water. Although the exact structure of the oxidant is not clear, it is currently accepted that the oxidation of the hydrocarbon is accomplished through a so-called oxygen rebound mechanism.

The active FeO^{3+} oxidant is also believed to be involved in oxidations catalyzed by non-heme iron enzymes.¹² If a sequence similar to the oxygen rebound mechanism, proposed for the action of cytochrome P₄₅₀ systems is involved in the desaturation process, radical or hydroxylated intermediates may be formed. The existence of discrete hydroxyl intermediates was ruled out by Bloch in a crude bacterial desaturase system¹³ (Scheme 5).

Scheme 5.

Attempts to convert 9- or 10-hydroxystearoyl CoA or ACP to oleoyl CoA or ACP have been unsuccessful. However, it is still possible that hydroxylated intermediates are formed during the desaturation, but are never released from the enzyme active site

before subsequent dehydration occurs.¹⁴ A purely radical pathway is also permitted on the basis of inorganic model studies.¹⁵

Even though the precise mechanism of action of the stearoyl CoA terminal desaturase is not known, the stereochemistry of the desaturation has been established. It was shown in experiments with whole bacterial (*Corynebacterium diphtheriae*),¹⁶ algae (*Chlorella vulgaris*)¹⁷ and fungal cells (*Cladosporium cladosporioides*,¹⁸ *Saccharomyces cerevisiae*,¹⁹ *Penicillium brefeldianum*²⁰) and a number of liver microsomal extracts,^{21,22} that the pro-R hydrogens from C9 and C10 are removed and the pro-S hydrogens are retained (Scheme 5). The collective evidence thus seems to suggest that all Δ^9 desaturases, irrespective of their origin, exhibit the same stereoselectivity.

This stereoselectivity suggests that part of the methylene chain may assume a thermodynamically unfavourable conformation on binding to the active site of the enzyme. Indeed, inhibition studies with the purified rat liver desaturase suggest that the methylene chain assumes an eclipsed conformation at carbon atoms 9 and 10 in the enzyme-substrate complex (for example oleyl CoA, DL cis-9,10-epoxystearoyl CoA and DL cis-9,10-methylene stearoyl CoA were strong competitive inhibitors of the desaturation, while the trans-octadecenoyl CoA being unable to assume this required conformation is only a poor inhibitor).²³

One fundamental mechanistic question that remains is whether the primary attack by the oxidant occurs at the C9 or C10 position.

1.4. Use of thia-fatty acids as mechanistic probes.

To settle this regiochemical issue and to gain more information on the nature of the desaturation process, we decided to use a number of modified substrates in which one of the methylene groups in the fatty acid chain is replaced by a sulphur atom. The thia-analogues of fatty acids have been previously used as enzyme substrates or inhibitors. Pascal and coworkers²⁴ for example found that the 10-thiastearic acid is a competitive inhibitor of dihydrostercolic acid synthesis in *Crithidia fasciculata*. The same research group has also recently found that 10-thiastearoyl CoA was an excellent substrate, but not an inhibitor for the mammalian stearoyl CoA desaturase. However, the product in this case was not characterized.

The steric requirements of a sulphur atom in thioethers are fairly close to those of a methylene group: according to Abrahams²⁵ the C-S bond length in dimethyl sulphide is 1.81 Å, and the C-S-C angle is 99.8°. ²⁶ (average sp₃ carbon - sp₃ carbon bond length is 1.54 Å ²⁷ and the C-C-C angle is close to the tetrahedral: 109° 28').

Indeed, it has already been shown that methyl 5-, 6-, 7-, 12- and 13-thiastearates are excellent substrates for the Δ 9 desaturase of *Saccharomyces cerevisiae*.²⁸ (Scheme 6).

Scheme 6.

If the sulphur atom is placed well outside the range of the oxidant, it serves merely as an inert connecting link and the resulting slight perturbation of the methylene chain is not sufficient to displace the C9 and C10 hydrogens from the active site of the enzyme. On the other hand, if the sulphur label is brought into range of the oxidant the desaturation of such a substrate cannot take place.

Indeed, it has been observed earlier in our laboratory, that a more polar compound was formed when methyl 9-thiastearate was administered to *Saccharomyces cerevisiae*. It was tentatively identified by TLC analysis as the corresponding sulfoxide.

It was the initial purpose of this thesis to fully characterize this oxidation product. Furthermore, the preference of the desaturase for primary attack at either C9 or C10

was studied by evaluating the outcome of a competitive oxidation of a suitable mixture of thia-substrate analogues. Finally, since the stearoyl CoA desaturase is a highly stereoselective enzymic system and if it is indeed involved in sulfoxide formation, then the sulfoxide thus produced is likely to be of high optical purity. This implies a potential synthetic application of the system due to current interest in this area and the lack of a suitable general method to produce chiral sulfoxides in high enantiomeric excess.

Some progress in this latter area has been made by Kagan who has recently obtained chiral sulfoxides in high enantiomeric excess from corresponding aromatic thioethers by using a modified Sharpless reagent.²⁹ In chiral sulfoxide production, however, taking advantage of the high stereoselectivity of enzymes seems to be more rewarding than chemical oxidations with chiral oxidants. For example May and coworkers³⁰ have recently used an ω -hydroxylase system to produce chiral methyl alkyl sulfoxides from thioethers. The use of both whole cell systems and purified enzymes in chiral sulfoxide production has been recently reviewed by Holland.³¹

1.5. Use of whole yeast cells as a source of stearoyl CoA activity.

In order to study the fatty acid $\Delta 9$ desaturation a convenient source of the stearoyl CoA desaturase activity - the whole cells of the yeast, *Saccharomyces cerevisiae*, NRC # 2335 was used. The stearoyl CoA desaturase system of yeast microsomes has been shown to involve cytochrome b_5 and NADH-cytochrome b_5 reductase, the properties of which are essentially similar to those of the hepatic microsomal counterparts.³² However, the terminal desaturase enzyme of the yeast system was shown to be less sensitive to cyanide than the rat liver microsomal desaturase, indicating possibly a different structure of the non-heme iron oxidant. To date, this enzyme has not been purified.

While the use of pure enzymes can yield more conclusive results, the use of the whole cell system was considered to be more convenient. In the whole cell experiment, milligram amounts of oxidation products of thia-fatty acid derivatives can be readily obtained, while similar experiments with purified enzyme typically yield only nanograms of these materials. In addition, the use of the genetically well-characterized *Saccharomyces cerevisiae* would aid in eventual isolation of the purified enzyme. In this way the results of *in vivo* and *in vitro* experiments can be directly compared. Finally, the yeasts are readily available, relatively easy to grow and quite hardy.

Despite the practical advantages, a number of assumptions have to be made in mechanistic studies using whole living cells: The outcome of a thia-substrate competition feeding experiment will be determined entirely by the regioselectivity of the desaturation, only if those processes, which are not involved in desaturation do not discriminate on the basis of sulphur position in the thia-substrate.

These processes include:

1. Transport into cells.
2. Thioester formation with CoA.
3. Excretion.
4. β -oxidation.
5. Random P₄₅₀ oxidation.
6. Biosynthetic P₄₅₀ oxidation (ergosterol pathway).

Earlier experiments in our laboratory have shown that methyl 5-, 6- and 7-thiastearates are desaturated to approximately the same extent,²⁸ which suggests that the above condition is satisfactorily met.

2. Results and Discussion.

2.1. Synthesis of thia-fatty acids.

A number of thia-fatty acid analogues, along with their oxidized derivatives were synthesized in this thesis. These compounds were required either as substrates or as authentic reference samples. In addition, a synthetic approach to a number of thiastearates bearing sulphur near the end of the chain was worked out (see Addendum).

2.1.1. Synthesis of methyl 3- to 13-thiastearates.

All methyl thiastearates used in subsequent feeding experiments were prepared by alkylating the appropriate thio-acid with a bromoalkane. Most thio-acids were prepared via alkaline decomposition of S-alkyl thiouronium salts following the original Rapoport procedure³³ (scheme 7).

Scheme 7.

Unsymmetrical dialkyl sulfides can also be prepared in a one pot reaction from the isothiuronium salts by their alkaline decomposition and alkylation of the resulting thiolates with the corresponding bromoalkanes without the need for isolation of the thiol intermediates.³⁴ However, in our case the yield of this procedure did not compare favourably with the older two step process. The 2- to 17-thiastearates have also been prepared by Lie Ken Jie³⁵ and also Pascal has synthesized several of these compounds.³⁶

Since the commercial supply of 9-bromononanoic acid as a starting material for the methyl 10-thiastearate synthesis was unreliable at the time of this experiment, it became necessary to devise an alternative route to this compound. Methyl 9-toluenesulphonyl nonanoate, easily prepared in two steps from commercially available azelaic acid monomethyl ester appeared to be a suitable substitute for the unavailable 9-bromononanoic acid (Scheme 8a). A similar procedure was reported by Pascal et al. for the synthesis of 9- and 10-thiastearic acid³⁶ (Scheme 8b).

Our approach:

Scheme 8a.

Pascal & coworkers:

Scheme 8b

However, our approach seems to be more practical than the process used by Pascal et al since the use of malodorous alkylthiols is avoided. Also the tosylation employed in our scheme seems to be a cleaner and more efficient procedure than the bromination of a hydroxyacid.

The ω -mercaptoacids were prepared from ω -bromoacids through alkaline hydrolysis of isothiuronium salts.³⁷ These isothiuronium salts were obtained by alkylation of thiourea with ω -bromo acids in absolute ethanol.³⁸ Since elevated temperatures were required for both reactions, the process was carried out under an inert (nitrogen) atmosphere in order to prevent possible oxidation of the expected mercapto-acid products to disulfides by atmospheric oxygen.

However, the work up of some of the reaction mixtures obtained did not yield the expected free thio-acid products and afforded insoluble solids instead. It was surmised, that these solids may consist of inclusion complexes (clathrates) formed from urea, a byproduct of the thiuronium salt decomposition³⁹ and the thio-fatty acids. Urea, like other primary amides, is known to have a tendency for self-association by hydrogen bonding in concentrated solutions.⁴⁰ The oligomer thus formed has a helix structure, which can incorporate a suitably shaped organic molecule in its cylindrical cavity.⁴¹ Fatty acids, as well as longer chain alkanes, are known to fit well into these cavities and form inclusion complexes (clathrates) with urea.⁴² These complexes, unlike both individual components, are insoluble in both water and organic solvents.⁴³ In the case of the mercapto acids the stability of such a complex can be further increased by hydrogen bonding between urea and the SH group (hydrogen bond donor).⁴⁴

In order to prevent this complex formation the thio-acids were emulsified with a suitable organic solvent and these emulsions were then slowly acidified under vigorous stirring. This resulted in dramatically improved yields of the longer chain (9-mercaptononanoic to 12-mercaptododecanoic) acids.

All mercaptoacids required in this project, with the exception of mercaptoacetic, 2-mercaptopropionic and partly 9-mercaptononanoic acid were prepared in this way. The mercaptoacetic and 2-mercaptopropionic acids were commercially available and 9-mercaptononanoic acid was also obtained from methyl 9-tosyl nonanoate as alluded to earlier.

The thiastearic acids were prepared from the mercaptoacids by deprotonation with *n*-butyllithium and alkylation of the resulting dianions with the corresponding

bromoalkanes. As a result of the higher nucleophilicity of the sulphur atom in thiolate compared to the oxygen of a carboxylate, the O-alkylation of the latter was not expected to compete with the S-alkylation of the former. However, a large excess of n-butyllithium can lead to the reduction of the carboxylate to a ketone or alcoholate⁴⁵ and was therefore avoided. Although aqueous KOH is also sufficiently basic to achieve complete deprotonation of the sulfhydryl group, in such case the yield of the subsequent alkylation of the dianion with a bromoalkane was found to be quite low,⁴⁶ presumably because of low solubility of the bromoalkane in the aqueous solution.

Finally, treatment with methanol in the presence of BF_3 gave the methyl thiastearates in satisfactory yields. Purification of the crude products by flash chromatography or HPLC afforded colourless solid (methyl 5- and 6-thiastearate) or liquid material in approximately 30 % yield from the ω -bromoacid (c. f. p. 71). The purities of these compounds were checked by TLC and the structures confirmed by 300 MHz ^1H NMR and direct probe MS. With the exception of methyl 3- and 4-thiastearates the methylene protons α to the sulphinyl group appeared in the NMR spectrum as overlapping triplets at 2.30 to 2.50 ppm. (c. f. p. 70). In the NMR of methyl 3-thiastearate these hydrogens appeared as a singlet at 3.15 ppm and a triplet at 2.60 ppm. The NMR spectrum of methyl 4-thiastearate exhibited a triplet at 2.75 ppm and a triplet at 2.45 ppm partly overlapping with the signal of the methylene group α - to carbonyl. The MS clearly showed the characteristic $-\text{S}(\text{CH}_2)_{y+1}\text{CH}_3$ and $\text{CH}_3\text{OCO}(\text{CH}_2)_{x+1}$ fragment ions.

2.1.2. Synthesis of thiabenzyl fatty acids.

Four thiabenzyl derivatives of fatty acids were synthesized. These were the methyl esters of S-benzyl-8-mercaptooctanoic, S-benzyl-9-mercaptanonanoic, S-benzyl-10-mercaptodecanoic and S-benzyl-11-mercaptoundecanoic acid (Scheme 9). These compounds were obtained from the corresponding mercapto acids and benzyl bromide in a process, which is analogous to that used for the synthesis of methyl 3- to 13-thiastearates.

Scheme 9.

2.1.3. Synthesis of thia-fatty acid S-oxides.

A number thia-fatty acids S-oxides were required in this work as standards. These compounds can be prepared by a selective oxidation of the corresponding thia-compounds. Even though it is not always easy to stop the reaction at the sulfoxide stage, a number of mild oxidizing agents have been successfully used to convert thioethers into sulfoxides in high yields. These include: hydrogen peroxide,⁴⁷ perbenzoic⁴⁸ and Caro's acids,⁴⁹ dinitrogen tetroxide,⁵⁰ N-bromo succinimide,⁵¹ pyridine-bromine complex⁵² or bromine in cold aqueous solution.⁵³ The same oxidants can be also used for oxidation

of thioethers to sulfones. The oxidation with *m*-chloroperbenzoic acid is recommended as a highly selective method of obtaining either sulfones or sulfoxides, compatible with many other oxidizable functions, such as α -halogen or hydroxyl⁵⁴ and thus it was the method of choice. By simply varying the reaction temperature and the amount of oxidant we were able to obtain both sulfoxides (S-oxides) and sulfones (S-dioxides) from the thia-fatty acid methyl esters in high yields (Scheme 10).

Scheme 10.

Thus S-oxides of all methyl thiastearates, methyl S-thiabenzyl-8-octanoate and S-thiabenzyl-10-decanoate were produced along with S-dioxides of several methyl thiastearates. The purity of these compounds was checked by TLC and their structures confirmed by 300 MHz ^1H NMR, direct probe MS and IR (c.f. p. 23 - 25 and 72 - 73). The signals of hydrogen atoms α to the sulfinyl group appeared as multiplets at 2.55 - 2.70 ppm in the NMR spectra of all thiastearate S-oxides with the exception of the 3- and 4-thiastearate S-oxide. The C2 hydrogens in methyl 3-thiastearate S-oxide appeared as an AB quartet at 3.35 ppm and the C3 hydrogens of methyl 4-thiastearate S-oxide as a multiplet at 2.95 ppm due to additional shielding caused by the proximity of the carbonyl group. The benzylic hydrogens in both benzyl sulfoxides appeared as AB quartets at 4.05 ppm. The MS of all thiastearates S-oxides showed characteristic $\text{---SO}(\text{CH}_2)_{y+1}\text{CH}_3$ and $\text{CH}_3\text{OCO}(\text{CH}_2)_{x+1}$ fragment ions. In the IR spectrum the methyl 9-thiastearate S-oxide exhibited a strong absorption at 1025 cm^{-1} which is consistent with literature values for the stretch of the S-O bond.⁵⁵

2.2. Feeding experiments.

2.2.1. Structure determination of polar intermediate formed from methyl 9-thiastearate.

As mentioned earlier, the confirmation of the structure of the polar metabolite formed in attempted desaturation of methyl 9-thiastearate was the first goal of this thesis.

To obtain enough of this material to allow its characterization by spectroscopic techniques, a feeding experiment was conducted on a relatively large scale, where 100 mg of methyl 9-thiastearate was administered to 600 mL of culture media containing aerobically growing *Saccharomyces cerevisiae* NRC 2335 cells. Also, blank medium was grown under the same aerobic conditions without the thia-substrate.

The methyl esters of fatty acids have been found to enter the yeast cells more rapidly than the corresponding free fatty acids, presumably as a result of being more lipophilic.⁷² Therefore the methyl esters of thia-fatty acids were used as substrates in this and all following feeding experiments, rather than the free acids.

The incubation of the above mentioned cultures for 48 hours was followed by separation of cells from the media by centrifugation.

The lipids from the supernatant media were then extracted with dichloromethane and the solvent removed. The residues thus obtained were then hydrolyzed with 1 M KOH in 50 % ethanol in order to convert all possible fatty acid esters to their free acids. Ethanol was then evaporated *in vacuo* and the basic aqueous solution was extracted with hexanes. The aqueous phase was then acidified and the fatty acids extracted with dichloromethane. Since the TLC analysis of methyl esters of these fatty acids was found to be more convenient than the analysis of the free fatty acids, these fatty acid extracts were methylated with BF_3 etherate in methanol prior to TLC analysis.

The fatty acids from the yeast cells were isolated as follows: The cells were first hydrolyzed with 1 M NaOH in 50 % ethanol to obtain the free fatty acids, then filtered and ethanol was evaporated *in vacuo*. The basic aqueous solution was then extracted with hexane and acidified. The crude fatty acids were then extracted from the acidic solution with dichloromethane and again methylated prior to TLC analysis.

A total of four extracts were obtained at this point: these were the cells and media (supernatant) extracts of both the feeding and control experiment (Figure 2).

Figure 2.

All of these extracts were analyzed by TLC (iodine vapour and water spray used for visualization) and the results compared with each other and against several standards: Since it was assumed that the earlier observed methyl 9-thiastearate metabolite may be either the corresponding sulfoxide or sulfone or a mixture of both, the purified thia-substrate and authentic samples of methyl 9-thiastearate-S-oxide and methyl 9-thiastearate-S-dioxide were used as standards to locate and tentatively identify both oxygenated compounds in the extracts.

To achieve maximum resolution, several identical plates in each analysis were developed in several solvents of different polarity: 20% ethyl acetate in hexane was used to identify the thia-substrate and the corresponding sulfone, 2% methanol / dichloromethane and ethyl acetate were used for the sulfoxide analysis of both cells and culture media extracts.

The TLC analysis showed the presence of an intense hydrophobic spot in the supernatant extract obtained from the 9-thiastearate feeding experiment. This spot had

an Rf closely corresponding to that of the synthetic methyl 9-thiastearate-S-oxide. The same spot was very weak in the corresponding cell extract and totally absent in the control cell and medium extracts (Figure 3).

Figure 3a.

Figure 3b.

No detectable amount of the methyl 9-thiastearate-S-dioxide was found in any of the extracts. An intense TLC spot corresponding to methyl 9-thiastearate was also found in the extract of the cells incubated in the presence of this compound. Only traces of this compound were found in the medium extract (Figure 3).

In this experiment and all subsequent feeding experiments performed the unreacted thia-substrates and their desaturation products were mostly found in the cell extracts, indicating their nearly complete incorporation. It appeared that those thia-substrates which undergo desaturation to thiaoleates accumulated in the membrane phospholipids or storage triglycerides just as the non thia-substrates as had previously been observed.²⁸ On the other hand, more polar metabolites formed by oxidation of the thia-substrates appeared to be excreted from the cells as a result of their unsuitability as membrane components, as these compounds were associated with the aqueous media, rather than the yeast cells.

It was decided at this point to isolate the sulfoxide from the medium extract. This was done by flash chromatography (Ethyl acetate). Since the compound obtained by this separation was judged to be impure by subsequent TLC analysis in 2% methanol / dichloromethane, another flash chromatography using 2% methanol / dichloromethane was necessary to obtain a pure product.

This procedure yielded 35 mg of a colorless crystalline material. Its TLC analysis (one spot in ethylacetate and 2% methanol / dichloromethane) and melting point range of 70.5 to 71.5°C both indicated high purity. However, the melting point appeared to be somewhat higher than that of the synthetic racemic standard (67 to 67.5°C), possibly as a result of different enantiomeric composition of the former material. The purified compound was then analyzed by 300 MHz ¹H NMR, ¹³C NMR at 75.429 MHz, IR and direct probe MS. The results were found to be identical to those obtained with the synthetic methyl 9-thiastearate-S-oxide: in 300 MHz ¹H NMR (CDCl₃) both samples exhibited a multiplet integrating for 4 hydrogens centered at 2.55 ppm. In ¹³C NMR the carbon atoms next to the sulfinyl group appeared at 52.440 and 52.308 ppm. The IR spectrum showed a distinct peak at 1025 cm⁻¹ corresponding to an SO stretch and the MS (EI, 70 eV) of both samples featured characteristic fragment ions at m/e = 157 and 159 indicating the cleavage of the sulfoxide molecule next to the sulfinyl group. Thus it was concluded, that the material isolated is indeed the methyl 9-thiastearate-S-oxide.

Direct probe MS of methyl 9-thiastearate S-oxide from culture media extract.

Figure 4.

300 MHz ^1H NMR of methyl 9-thiastearate S-oxide from culture media extract.

Figure 5.

IR of methyl 9-thiastearate S-oxide from culture media extract.

Figure 6.

Direct probe MS of synthetic methyl 9-thiastearate S-oxide.

Figure 7

300 MHz ^1H NMR of synthetic methyl 9-thiastearate S-oxide.

Figure 8.

IR of synthetic methyl 9-thiastearate S-oxide.

Figure 9.

^{13}C NMR of methyl 9-thiastearate S-oxide from culture media extract.

Figure 10.

^{13}C NMR of synthetic methyl 9-thiastearate 3-oxide.

Figure 11.

At this point, it was conceivable that some sulfoxide might be formed in the atmospheric oxidation of the thiastearate, either during the aerobic incubation, or in the work up, despite all precautions taken to prevent such oxidation. In order to check the extent of autoxidation, the following experiment was performed: methyl 9- and 10-thiastearates were administered separately to both growing yeast cells and yeast cells, which had been previously destroyed by autoclaving at 130°C for 30 min. After a 48 hour incubation, the cells were harvested and the culture media extracted, following strictly the same procedure for each. The TLC analysis of these media extracts showed, that only traces of polar material were formed during incubation of both thiastearates with the dead cells (figure 12).

Figure 12a.

Figure 12b.

Figure 12c.

On the other hand, large amounts of both sulfoxides were detected by TLC in the extracts of those culture media, where both thiastearates had been incubated with living yeast cells. Furthermore, the amount of sulfoxide formed from the 9-thiastearate seemed significantly larger, than that formed from the 10-thiastearate.

Thus it was concluded, that biological oxidation accounts for most of the sulfoxide formation, while the extent of autoxidation, presumably by atmospheric oxygen during the incubation, is quite negligible.

However, it was not quite clear at this point, whether the sulfoxide was, indeed, formed in an oxidative attack by the stearoyl CoA desaturase system, or by the action of other enzymes, such as the cytochrome P₄₅₀ dependent monooxygenases which the *Saccharomyces cerevisiae* cells are known to possess. We thus carried out further experiments to gain more information on this point as described in the next section.

2.2.2. Regioselectivity of methyl thiastearate oxidation by *Saccharomyces cerevisiae*.

As previously mentioned, the stearoyl CoA desaturase system is a highly regioselective enzymic system and indeed, earlier desaturation of several thia-analogues of stearic acid in our laboratory proved to be highly regioselective.²⁸ The sulfoxidation of a thiastearate molecule, if accomplished by the same enzyme, should also be highly regioselective. Sulfoxide formation should only occur if the sulphur atom in the thia-substrate is positioned within the reach of the active oxidant. In the case of oxidation of thiastearates by stearoyl CoA desaturase of *Saccharomyces cerevisiae* this should result in predominant formation of either 9- or 10-thiastearate-S-oxide, depending on which of these two positions is the place of primary attack by the oxidant.

The regioselectivity of the sulfoxidation was studied by feeding the yeast cells with an equimolar mixture of thiastearates bearing the sulphur at a number of different positions. In this experiment, methyl 3- to 13-thiastearates were chosen as substrates to clearly establish whether the regioselectivity of sulfoxidation matches that of the desaturation.

At this point, the problem of quantitative analysis of mixtures of methyl 3- to 13-thiastearates and their S-oxides needed to be addressed. Successful chromatographic separations of such mixtures are not likely to be achieved. Gas chromatography cannot be used due to the thermal lability of sulfoxides and several attempts at HPLC separation did not provide sufficient resolution of all isomers. Thus the analysis had to be achieved without separation using non-chromatographic techniques.

Mass spectroscopy seemed to be a useful tool. Both thioethers and sulfoxides undergo facile C-S bond cleavage under the conditions of ionization which yield characteristic fragment ions, by which each isomer can be identified. The products of McLafferty rearrangement were also observed in the MS of sulfoxides (Scheme 11).

Scheme 11.

The relative abundance of these fragment ions with respect to the most abundant ion in each spectrum (usually Mol. ion - CH_3O^- in sulfoxides) was fairly constant in the MS of each pure isomer. The relative abundance of each of these ions in the MS of a sulfoxide mixture should be directly proportional to the concentration of the corresponding compound in the mixture. Thus the composition of a sulfoxide mixture can be calculated from its MS. The accuracy of this analytical method was checked on several thiastearate and sulfoxide mixtures of known composition and the relative error of the analysis was found to be consistently under 20 % for all isomers in every analysis (Table 1 and 2).

Table 1. MS analysis of a test mixture of methyl 5- to 9-thiastearates S-oxides.

Sulphur position	3	4	5	6	7	8	9
authentic sample (%)	-	-	9.2	17.8	32.9	11.3	28.4
determined by MS (%)	-	-	11.0	19.9	31.9	11.5	25.3

Table 2. MS analysis of a test mixture of methyl 9- to 13-thiastearates S-oxides.

Sulphur position	9	10	11	12	13
authentic sample (%)	36.0	26.9	11.5	10.2	15.4
determined from MS (%)	40.4	23.8	12.4	9.5	14.3

Methyl 9- to 13-thiastearate S-oxides from media extracts

Graph 2.

Table 4. Fatty acids from media and cell extracts. Second set of feeding experiments.

exper. no.	substrates: equimolar mixture of:	f. a. extract (medium) (mg)	sulfoxide fraction (medium) (mg)	f. a. extract (cells) (mg)	non polar f. a. fraction (cells) (mg)
	3-9 thiastearates	22.5	11.5	165.5	83
	3-9 thiastearates control	5.3	1.1	205.9	106.5
	9-13 thiastearates	20.8	8.5	101.6	23
	9-13 thiastearates control	3.3	1.0	125.9	86.5

Unreacted thiastearates were recovered from the autoclaved cell extracts.

The formation of several thiaoleates from the corresponding thiastearates by the yeast cells had been previously observed in our laboratory.²⁸ In this experiment abundant ions at m/e values two units lower than those of the analytical $\text{S}(\text{CH}_2)_{17}\text{CH}_3$ ions of 3- to 9-thiastearates thiastearates were observed in the MS of the fatty acid cell extracts derived from the 5- to 9- and 3- to 9-thiastearate feeding experiments (see figure 13, next page). Also, fragment ions of m/e values two units lower than those of the $-\text{OC}(\text{CH}_2)_{n-2}-$ fragments of 12- and 13-thiastearates were observed in the MS of the fatty acid extracts of cells from the 9- to 13-thiastearate feeding experiment indicating partial desaturation of these compounds to the corresponding thiaoleates (figure 14, p. 39). No attempt was made to quantify the thiaoleate composition of these extracts. However, the presence of these compounds in the extracts is indicative of the catalytic activity of the desaturase system.

Figure 13.

Direct probe MS of thia-fatty acid methyl ester fraction from yeast cells. Methyl 9- to 13-thiastearate feeding experiment.

Figure 14.

The above mentioned MS analysis showed, that the methyl 9-thiastearate-S-oxide was predominant in all sulfoxide mixtures (figures 15 and 16 , tables 5 and 6 and graphs 1 and 2) formed by living cells and its formation exceeded that of the 10-thiastearate-S-oxide by the factor of 2 (figure 16 and table 6).

Table 5. Composition of methyl 3- to 9-thiastearate S-oxide fractions from culture media extracts.

Sulphur position	3	4	5	6	7	8	9
% in living medium extract experiment no 1	-	-	4.0	2.5	14.0	4.5	76.0
% in living medium extract experiment no 5	11.4	16.0	4.0	4.5	9.5	3.0	51.0
% in autoclaved medium extract experiment no 6	6.0	19.5	15.5	15.0	14.5	14.5	14.5

Table 6. Composition of methyl 9- to 13-thiastearate S-oxide fractions from culture media extracts.

Sulphur position	9	10	11	12	13
% in living medium extract experiment no 3	46.0	22.0	6.0	6.5	17.0
% in living medium extract experiment no 7	67.0	15.0	5.5	3.5	9.0
% in autoclaved medium extract experiment no 8	40.0	25.5	13.0	10.0	11.5

Direct probe MS of sulfoxide fraction from culture medium. Methyl 3- to 9-thiastearate feeding experiment.

Figure 15.

Direct probe MS of sulfoxide fraction from culture medium. Methyl 9- to 13-thiastearate feeding experiment.

Figure 16.

Methyl 3- to 9-thiastearate S-oxides from media extracts

Methyl 9- to 13-thiastearate S-oxides from media extracts

Graph 2.

The fact, that a small amount of sulfoxide fraction was obtained from medium extracts in experiments no. 6 and 8 may be due to autoxidation. However, the high methyl 9-thiastearate S-oxide content in the sulfoxide fraction from experiment no. 8 suggests, that a small number of yeast cells may have survived the conditions of autoclaving and subsequently caused some residual enzymic activity, even though the cultures in question did not show any growth observable by turbidity measurement of the culture media during incubation. Also, the possibility of an erroneous MS analysis due to a weak MS cannot be ruled out in this case.

The formation of methyl 10-thiastearate S-oxide may be partly due to the fact, that the thioether can be oxidized more easily than a methylene group. Assuming a substrate conformation shown in scheme 12 the sulphur atom in the 10-thiastearate may be accessible to the active oxidant in the enzyme-substrate complex. Similar oxidation at the C8 position does not occur to any significant extent because this position is inaccessible to the oxidant (Scheme 12).

Scheme 12.

It can be concluded from this experiment, that the 9- position of stearic acid appears to be the place of a primary attack by the active oxidant of the terminal stearoyl CoA desaturase of *Saccharomyces cerevisiae*. This confirms earlier indications by Bloch,

who observed an increase in the $^3\text{H}/^{14}\text{C}$ ratio of a mixture of (R)-9- ^3H and 1- ^{14}C -stearic acids recovered from partial desaturation of this substrate by *Corynebacterium diptheriae*.^{5,6} Clearly the $^3\text{H}/^{14}\text{C}$ ratio rose because the H-containing molecules were consumed in preference to the ^3H containing species. Since no such tritium enrichments of recovered unreacted substrates were observed when stearic acids monotrhitated at the 9-(S), 10-(R) and 10-(S) positions were used in similar experiments, these results seem to suggest a primary attack at the 9-(R) position of stearic acid provided, that hydrogen removal is the rate determining step.

P. Strittmatter⁵⁷ later showed by measuring the kinetic isotope effect in a desaturation of 9,10-tetradesterostearic acid by purified rat liver stearyl CoA desaturase system, that hydrogen removal is, indeed a rate determining step in this process.

Small quantities of other sulfoxide isomers found in the media extracts from experiments no 1, 3, 5 and 7 could be due to three sources:

- 1) Autoxidation. A small amount of a sulfoxide fraction was obtained in control experiment no 6. Its composition suggests a nonselective process, most likely nonenzymatic.

- 2) Oxidation by other, "detoxifying" enzymes, such as the cytochrome P₄₅₀ dependent monooxygenase systems. Some fungi are known to be rich sources of cytochrome P₄₅₀ reactivity.⁵⁸ These enzymes have also been shown to oxidize thiocethers to corresponding sulfoxides.⁵⁹ However, they tend to be neither highly regio- nor stereoselective. Thus the high predominance of the methyl 9- and 10-thiastearate-S oxide then seems to suggest that these processes occur only to a limited extent or not at all. Furthermore, the thia-substrates in our experiment should be indistinguishable from methyl stearate and therefore not likely to be dealt with as xenobiotics.

- 3) Steroid hydroxylation. It is possible that fatty acids can assume a steroidal conformation (Scheme 13) in which they may be mistaken for lanosterol or other sterols (Scheme 13) and oxidized by the enzymic systems normally involved in the conversion of lanosterol to ergosterol.

Scheme 13.

This conversion in *Saccharomyces cerevisiae* involves six general transformations.⁶⁰ (1) removal of the three methyl groups in lanosterol at C4 and C14, (2) alkylation at C24, and generation of $\Delta^{24(28)}$ -methylene, (3) isomerization of the Δ^8 double bond to Δ^7 , (4) reduction of the $\Delta^{24(28)}$ double bond generating a C24-methyl (5) introduction of a Δ^{22} double bond, (6) introduction of a Δ^5 double bond.

Since some of these transformations, namely (1), (5) and (6), are of an oxidative nature, an oxidation of a fatty acid molecule at a number of positions (Scheme 13) would presumably result if it can assume the required steroidal conformation and thus become a substrate for these enzymes. Polyunsaturated fatty acids are known to occur in yeast under certain conditions.⁶¹ Isolation of the purified enzymes would be required to settle this point.

2.2.3. Stereoselectivity of sulfoxidation by *Saccharomyces cerevisiae*.

As mentioned earlier, the oxidative attack by the stearyl CoA desaturase is highly stereoselective. Thus the sulfoxide produced from a thioether by this enzyme is expected to be one enantiomer in high optical purity and the synthesis of a number of synthetically useful intermediates can be envisioned.³¹

Therefore the determination of optical purity of sulfoxides produced by *Saccharomyces cerevisiae* was considered important at this point. As mentioned earlier, the methyl 9-thiastearate S-oxide produced by the yeast cells had a slightly higher melting point, than a synthetic racemic standard, which might indicate a different enantiomeric composition. Also, the former material exhibited a negative optical rotation of $\alpha_D = 0.06^\circ$ (2 % sol. in CHCl_3 , 25°C , 10 cm cell, 589 nm).

An attempt was made to determine the optical purity of this material by chiral shift ^1H NMR.

Tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato], europium(III) derivative (Aldrich) served as chiral shift reagent and authentic racemic methyl 9-thiastearate-S-oxide was used to test the method and examine the magnitude of induced nonequivalence of the sulfoxide α -hydrogens in both diastereomeric complexes formed between (R)- and (S)- methyl 9-thiastearate-S-oxide and the chiral shift reagent. However, addition of up to 2 mol of this reagent per 1 mole of the methyl 9-thiastearate-S-oxide failed to produce any peak splitting of the α -hydrogen signals of the sulfoxide observable by 300 MHz ^1H NMR. On the other hand a significant peak enlargement caused by the paramagnetic europium was observed, which might have obscured a small extent of the desired peak splitting. Also, the NMR spectrum of the sulfoxide in question is further complicated by spin coupling of the α - and β -hydrogens in both alkyl chains. The chemical shifts of these α -hydrogens on either side of the sulfinyl function are not sufficiently different to allow the use of spin-spin decoupling techniques or even a complete separation of their signals in a ^1H NMR spectrum. Furthermore, the magnitude of an induced nonequivalence of chemical shifts of α -hydrogens in both enantiomers of methyl 9-thiastearate S-oxide is expected to be relatively small because of the pseudo-symmetry of the molecule. This pseudo-symmetry can be eliminated and some of the above mentioned problems avoided, if a more dissymmetric substrate can be oxidized by the enzymic system in question and

the optical purity of the resulting sulfoxide determined instead

We therefore decided to carry out an exploratory experiment with a number of S-benzylthia-fatty acids to determine whether these compounds can be sulfoxidized by the enzymic system in question.

It is not known whether the fatty acyl chains in the enzyme substrate complex are fully extended. It is thus conceivable that fatty acids bearing certain substituents at the end of the fatty acyl chain may be recognized as substrates by the stearoyl CoA desaturase (Scheme 14).

Scheme 14.

Thus methyl S-benzyl-8-mercaptooctanoate, S-benzyl-9-mercaptononanoate, S-benzyl-10-mercaptodecanoate and S-benzyl-10-mercaptoundecanoate were prepared and 100 mg of each of these compounds was administered to a separate batch of 600 mL yeast culture.

In this experiment the extent of non-enzymic oxidation was again checked in a series of control runs, where each substrate was administered to a batch of previously autoclaved cells, and then processed following strictly the same procedure as that used with living cells.

It was found that a significant amount of sulfoxide was formed only from methyl S-benzyl-8-mercaptooctanoate (see table 7). 23 mg of this compound was isolated from the culture medium extract, while the cell extract contained the residual thia-substrate.

culture medium extract, while the cell extract contained the residual thia-substrate.

Table 7. Fatty acids from media and cell extracts. First set of feeding experiments with thiabenzyl fatty acids.

exper. no.	substrate: methyl S-benzyl-:	f. a. extract (medium) (mg)	sulfoxide fraction (medium) (mg)	f. a. extract (cells) (mg)	non polar f. a. fraction (cells) (mg)
9	8-mercapto- octanoate	56	23	45	26
10	8-mercapto- octanoate, control	8.5	<1	65	30.5
11	9-mercapto- nonanoate	8.5	<1	57.5	25
12	9-mercapto- nonanoate, control	10.5	<1	52	23.5

Table 8. Fatty acids from media and cell extracts. Second set of feeding experiments with thiabenzyl fatty acids.

exper. no.	substrate: methyl S-benzyl-:	f. a. extract (medium) (mg)	sulfoxide fraction (medium) (mg)	f. a. extract (cells) (mg)	non polar f. a. fraction (cells) (mg)
13	10-mercapto- decanoate	9.5	<1	59	29.5
14	10-mercapto- decanoate, control	6	<1	57.5	32
15	11-mercapto- undecanoate	7.5	<1	52	28
16	11-mercapto- undecanoate, control	5	<1	72.5	36.5

The methyl S-benzyl-8-mercapto octanoate S-oxide was characterized by 300 MHz ^1H NMR (Figure 17, p. 50), direct probe MS (Figure 18, p. 51), and its melting point range. The NMR spectrum exhibited a characteristic AB quartet of the benzylic α -hydrogens at 4.10 ppm. The mass spectrum showed a distinct molecular ion peak at $m/e = 296$ and a peak at $m/e = 265$ corresponding to the loss of a methoxy group from the molecular ion. The PhCH_2 - fragment is found at m/e 91 as the base peak of the spectrum. The melting point range of $75.5 - 76.5^\circ\text{C}$ was slightly higher than that of a synthetic racemic standard ($70.5 - 71.5$), again indicating possibly a different enantiomeric composition of this material. Also, this compound exhibited a negative optical rotation $\alpha_D^{25} = 1.04^\circ$ (2% sol. in CHCl_3 , 10 cm cell, 589 nm).

The other three thia-substrates yielded only trace amounts of sulfoxides in the culture media extracts and mostly unreacted thia-compounds were recovered from the cells extracts following 48 hour incubation with the living yeast cells. Further, these traces of sulfoxides were also detected by TLC (ethyl acetate) in the culture media extracts of previously autoclaved cells and therefore were attributed to autoxidation. Since no measurable amount of any of these sulfoxides could be recovered from flash chromatography of any of the extracts, these compounds could not be further investigated.

Unlike in previous experiments with methyl thiastearates, the desaturation of the thia-substrates with the sulphur atom outside of the range of the active oxidant (methyl S-benzyl-11-undecanoate in this series) was not observed with any of the thiabenzyl fatty acids. This may be partly due to different chain lengths of these compounds. Depending on the conformation of the substrate fatty acyl chain in the enzyme-substrate complex the methyl S-benzyl-11-undecanoate may have an effective chain length of 17 or 19 carbon atoms and its rate of incorporation and desaturation may be similar to that of heptadecanoate or nonadecanoate. The nonadecanoate has been shown to be poorly incorporated into the yeast cells.

Direct probe MS of methyl S-benzyl S-mercaptooctanoate S-oxide from culture media extract.

Figure 17.

300 MHz ^1H NMR of methyl S-benzyl-8-mercaploctanoate S-oxide from culture media extract.

Direct probe MS of synthetic methyl S-benzyl-8-mercaptooctanoate S-oxide.

Figure 19.

300 MHz ^1H NMR of synthetic methyl S-benzyl-8-mercaptooctanoate S-oxide.

The above evidence suggests that the sulfoxide obtained from methyl S-benzyl-8-mercaptooctanoate was most likely produced by the same stearoyl CoA desaturase system of the yeast cells, that had been involved in the formation of the methyl 9-thiastearate S-oxide which had been observed previously.

The optical purity of this material was then determined. In this case the benzyl group offers a distinct advantage, since the two benzylic α -hydrogens are not spin coupled to any other hydrogens. The long range coupling of benzylic hydrogens to ortho or para protons of the aromatic ring⁶² ($J_{AB} < 1$ Hz), which can possibly somewhat complicate the spectrum, was not observed in a 300 MHz ^1H NMR spectrum of methyl S-benzyl-8-mercaptooctanoate S-oxide (Figure 19). However, the dissymmetric sulfinyl function will still cause the two α -hydrogens to be diastereotopic and therefore magnetically nonequivalent. As a result the benzylic protons in the sulfoxide in question indeed appear as an AB quartet, rather than a singlet in the ^1H NMR spectrum. Also, unlike in dialkyl sulfoxides, the chemical shift of benzylic hydrogens is sufficiently different from that of the α -methylene group on the other side of the sulphur atom, so that their NMR signals do not overlap. Thus any further splitting of the relatively simple ^1H NMR signals of the benzylic hydrogens due to induced nonequivalence of their chemical shifts in both diastereomeric complexes with the chiral shift reagent was easier to observe than similar splitting of complex overlapping multiplets in dialkyl sulfoxides.

Also, the problem of peak broadening, earlier encountered with the lanthanide based shift reagent had to be addressed. This can be overcome by use of certain chiral hydrogen bonding solvents.⁶³ A new class of these reagents was recently developed by Kagan⁶⁴ and successfully applied to a number of methyl alkyl and aryl sulfoxides.⁶⁵ One of these reagents, (R)-(-)-N-(3,5-Dinitrobenzoyl)- α -methylbenzylamine, which is particularly recommended by Kagan for chiral shift ^1H NMR analysis of sulfoxide enantiomers has recently become commercially available.

Indeed, the addition of this reagent to a sample of racemic methyl S-benzyl-8-mercaptooctanoate S-oxide (approx 8 moles per 1 mole of sample) resulted in separation of one of the benzylic α -hydrogen signals in the 300 MHz ^1H NMR of this compound into two signals of equal intensity, without any significant peak enlargement (figure 21).

moles of (R)-DBMBA
per mole of substrate

Figure 21.

The addition of the above mentioned shift reagent to a sample of methyl S-benzyl-8-mercaptooctanoate S-oxide produced by the yeast cells resulted in the splitting of the NMR signal of one of the α -hydrogens into two new signals of unequal intensities with the downfield signal being more intense than its upfield counterpart (figure 22).

Figure 22

The enantiomeric excess of the sulfoxide material in question was thus calculated from the relative intensities of these two peaks as $ee = (I_A - I_B) / (I_A + I_B) = 33\%$ (I_A, I_B , where $I_A > I_B$ are the respective peak areas of both α hydrogen signals in the NMR spectrum).

The fact that the sulfoxide material obtained in this experiment was not 100 % enantiomerically pure may be due to two reasons:

1) the sulfoxidation of the S-benzylthio-substrate by the enzyme in question may not be absolutely stereoselective.

2) sulfoxide produced may undergo partial racemization during the work-up part of the experiment. This can occur by several different mechanisms

Thermal racemization⁶⁶ of alkyl and aryl sulfoxides is known to occur at temperatures above 190°C.⁶⁷ However, benzyl sulfoxides can undergo facile homolytic cleavage of the S-Bz bond with subsequent recombination leading to racemization. Thus temperatures significantly lower than those observed for alkyl and aryl sulfoxides may be sufficient to cause racemization of benzyl sulfoxides. Slow racemization of these compounds on storage was observed by Kagan

An oxygen exchange on the sulfinyl group is also accompanied by racemization. This reaction can be catalyzed by strong acids, chloride ions or Lewis acids.

Since the methyl S-benzyl-8-mercaptooctanoate S-oxide produced by the yeast cells was subjected to both elevated temperature and the action of BF_3 etherate, it is conceivable that such treatment may have caused partial racemization of the sulfoxide material.

However, time constraints did not allow additional experiments to correlate these factors to the optical purity of the sulfoxides produced

In addition, the absolute configuration of the predominant enantiomer in this mixture was tentatively assigned:

By analogy with the better known and more extensively studied Pirle reagent⁶⁸ the Kagan shift reagent is expected to form a hydrogen bond between the primary amide of the shift reagent and the sulfinyl group. The resulting adduct could be further stabilized by an interaction between the methine hydrogen of the shift reagent and the electron pair on sulphur (Scheme 15).

Scheme 15.

In these complexes one of the α -hydrogens in the complex formed from the (S)-enantiomer of methyl S-benzyl-8-mercaptooctanoate S-oxide and the shift reagent will be positioned directly above the plane of one of the aromatic rings of the shift reagent (Scheme 15)

Inside this ring a current situated approximately 0.7 Å above and below the plane of the ring⁶⁹ generates an induced magnetic field, which opposes the applied field. This will result in a shielding effect by the induced magnetic field inside the aromatic ring upon the above mentioned hydrogen. Consequently, the signal of this hydrogen atom will appear at a higher field in the NMR spectrum.

On the other hand, in a similar complex formed from the (R)- isomer of methyl S-benzyl-8-mercaptooctanoate S-oxide and the same shift reagent the α -hydrogen in question will be situated far from the above mentioned aromatic ring and therefore its chemical shift will not be as greatly affected. Thus its NMR signal should appear downfield relative to that of the (S)- isomer.

We can therefore conclude, that the absolute configuration of the predominant enantiomer of methyl S-benzyl-8-mercaptooctanoate S-oxide formed by the yeast cells is (S). This in turn implies that the predominant enantiomer of methyl 9-thiastearate S-oxide produced by the same desaturase system should have the (R)- configuration (Scheme 16). It is also consistent with the known enantioselectivity of stearoyl CoA desaturation by the desaturase system in question (Scheme 16).

Scheme 16.

However, other methods, such as X-ray analysis will have to be used in order to confirm these assignments and to clearly establish the absolute stereochemistry of the sulfoxides produced by *Saccharomyces cerevisiae*.

2.3. Conclusions.

In this thesis the use of sulphur analogues of fatty acids as mechanistic probes for the stearoyl CoA desaturase system of *Saccharomyces cerevisiae* enabled us to study some mechanistic aspects of the fatty acid desaturation without the need for purification of the desaturase system in question. The formation of sulfoxides from sulfides by this system was established. To our knowledge, this is the first known example of a conversion of a sulfide to a sulfoxide by a dehydrogenase enzyme. The results obtained in the competitive feeding experiments indicate that the primary attack by the active oxidant occurs at C9 rather than C10 of the stearoyl CoA. The sulfoxide produced from methyl S-benzyl-8-mercaptooctanoate by this desaturase system was tentatively assigned the (S)- configuration, suggesting that (R)-9-thiastearate S-oxide may have been produced from 9-thiastearate. To gain even more insight into the mechanism of these processes and to clearly confirm the involvement of stearoyl CoA desaturase in the above transformations similar experiments will soon be performed with yeast microsomal extracts containing the enzymic system in question. These experiments will use stearoyl CoA as a substrate, rather than methyl stearate to clearly distinguish between the action of this desaturase system and other oxidative enzymes, such as the P₄₅₀ dependent oxygenases. However, in order to definitely settle these and many other questions the desaturase system will eventually have to be purified.

As mentioned earlier,³² chiral sulfoxides are currently considered to be potentially useful synthetic intermediates. Since most chemical methods of chiral sulfoxide production proved unsatisfactory in terms of chemical yields and / or enantiomeric purity for most sulfoxides³² a search for more stereoselective enzymic processes is currently underway. The enzymes most frequently used, however, are of the cytochrome P₄₅₀ dependent monooxygenase variety, which lack the absolute stereospecificity of most other enzymes. By using an absolutely stereospecific enzyme to carry out the reaction, it should be possible to produce chiral sulfoxides from thioethers in unprecedented optical purity.

The oxidation of thioethers disguised as 9-thia fatty acids to corresponding sulfoxides by stearoyl CoA desaturase system of yeast whole cells appears to be a promising biosynthetic method for chiral sulfoxides in high optical purity. The chemo-, regio-, and stereoselectivity of this process have been successfully demonstrated in this thesis.

The high extent of sulfoxide production from the methyl S-benzyl-8-mercapto-octanoate also seems to indicate, that the process is applicable to a reasonably broad range of substrates. Furthermore, the excretion of sulfoxides formed by *Saccharomyces cerevisiae* from the growing cells can be successfully utilized to simplify the product separation.

However, the anticipated 100 % stereoselectivity of this method is yet to be demonstrated and further refinements in both the yeast growing and the product separation can be made to improve the yields and attain the full stereochemical potential of the process.

3. Experimental

3.1. General

The low field ^1H NMR spectra in this work (60 MHz) were recorded on a Varian EM 360 A instrument. The high field ^1H NMR (300 MHz) and the ^{13}C NMR (75.429 MHz) were recorded on a Varian XL 300 spectrometer. All chemical shifts are reported with reference to tetramethylsilane and are quoted in δ values. The mass spectra were obtained on a VG7070 E mass spectrometer, at 70 eV using a direct introduction probe at 20 to 150°C and 10^{-7} mm pressure or a GC inlet system for GC-MS (Varian 3300, megabore DB-5 column 30 m long, temperature 150°C for 2 minutes, then increased by 10°C/min, carrier gas He). The MS and GC-MS data were collected using a DEC PD P8 system.

Optical rotations were measured on a Perkin - Elmer 141 polarimeter using a 1 mL sample cell 10 cm long and a sodium lamp as a source of 589 nm radiation at 25°C.

Several chromatographic techniques were used in this work:

Thin layer chromatography, was performed using glass plates pre-coated with a 0.25 mm layer of silicagel 60 as stationary phase. The fatty acids on developed plates were visualized by iodine vapour treatment followed by water spray and slow drying of the plates. The spots of thiabenzyl derivatives of fatty acids were observed under UV light.

Most synthetic fatty acids and some intermediates were purified by flash chromatography. A glass column (10-30 cm long, 3 cm in diameter) was filled with silicagel, (0.04-0.063 mm particle size 230-400 mesh ASTM, Merck). Pressures up to 10 psi were applied. The separation was monitored by spotting 5 μL of each fraction of eluate on a TLC plate (silicagel 60). The fatty acids were then visualized in the same manner as those on developed TLC plates.

The purifications of several thiastearates S-oxides were performed by HPLC on a Waters 500 model liquid chromatograph with Waters 410 differential refractometer as detector using a Novapak C18 column and ethylacetate or 30% ethylacetate / hexane as mobile phase at 2 mL/min unless stated otherwise.

All solvents used in synthetic experiments and extractions of culture media and cells were freshly distilled. All extractions were carried out using a total volume of solvent approximately equal to the volume of extracted solution unless stated otherwise. All extractions were repeated three times.

The dry solvents used in the synthetic part of this thesis were obtained as follows:

- pyridine: distilled from and stored over anhydrous KOH under dry nitrogen atmosphere

- DMSO, THF, HMPA: distilled from CaH_2 at atmospheric pressure, (HMPA at reduced pressure) and stored over molecular sieves under dry nitrogen.
- Absolute ethanol: used as obtained from manufacturer (Consolidated Alcohols Ltd., Toronto).
- Absolute methanol: used as obtained from manufacturer (Anachemia).

All organic solutions obtained upon workup were dried by washing with 30 mL of saturated sodium chloride followed by stirring for 10 minutes with anhydrous sodium sulphate. A rotary evaporator (Buchi, Rotovapor R) was used to remove solvents *in vacuo*.

The origins and grades of reagents used in the synthetic part of this project are listed below:

Reagent	Manufacturer	Grade	Reagent	Manufacturer	Grade
1-bromohexane	A	99+%	Azelaic acid mono methyl ester	A	96%
1-bromononane	A	98%	Boron trifluoride etherate	A	
1-bromodecane	A	98%	n-Butyl lithium (1.6 M in hexane)	A	
1-bromododecane	A	98%	m-Chloro peroxybenzoic acid	L	55%
All remaining 1-bromoalkanes	A	99%		A	85%
Mercaptoacetic acid	A	99+%	3,4 dihydropyran	A	97%
Mercaptopropionic acid	A	99+%	1,5-pentanediol	A	97%
ω -bromo butyric acid	A	99%	1,6-hexanediol	A	99%
ω -bromo decanoic acid	ICN		1,8-octanediol	/	93%
ω -bromo dodecanoic acid	ICN, A	99%	1,12-dodecanediol	A	97%
ω -bromo heptanoic acid	ICN		Diborane, (1 M in THF)	A	
ω -bromo hexanoic acid	A	99%	Lithium acetylide ethylene	A	90%
ω -bromo nonanoic acid	ICN		diamine complex		
ω -bromo octanoic acid	A	98%	p-Toluenesulfonyl chloride	A	98%
ω -bromo undecanoic acid	A	99%	Thiourea	A	99%
ω -bromo valeric acid	A	97%	Chromium trioxide	A	99+%
Hydrobromic acid	B	48%	Palladium black	NBC	

A	Aldrich Chemical Comp. Ltd, Milwaukee, Wis.
ICN	ICN Biomedicals Inc., Plainview, NY.
B	J. T. Baker Chemical Comp. Phillipsburg, NJ.
L	Lancaster Synthesis Ltd., Wyndham NH.
NBC	Nutritional Biochemical Corp., Cleveland, OH.

The microorganism used in this work was *Saccharomyces cerevisiae* NRC 2335, which was obtained from the National Research Council, Ottawa, Canada. It was stored at 4°C and grown at 30°C in a medium containing Bacto yeast extract (Difco) and Bacto peptone (Difco) in a New Brunswick incubator / shaker. The cells were harvested either by centrifugation at 2500 rpm, or by microfiltration, using a Millipore Minitan ultrafiltration unit equipped with four Minitan 4/PK membrane plates. All glassware and culture media to be used in these experiments were sterilized by autoclaving at 130°C for 30 minutes.

3.2. Synthesis

3.2.1. Methyl 3- to 13-thiastearates.

3.2.1.1. ω -Mercaptoalkanoic acids from ω -bromoalkanoic acids.

The ω -bromoacids were dissolved in absolute ethanol to make a 10% solution and refluxed (under dry nitrogen) for 2.5 h with a slight excess of thiourea (1.2 mole per 1 mole of bromoacid) and then immediately hydrolyzed with a large excess of 2 N aqueous NaOH (50 - 100 mL), which resulted in heavy precipitation. The basic solution was then refluxed under dry nitrogen for another 3 h (the precipitate re-dissolved upon heating) and subsequently diluted with approximately 100 mL of water and extracted with ether to remove excess thiourea.

Each aqueous layer thus obtained was mixed with an approximately equal volume of dichloromethane / n-hexane (1:1) and stirred vigorously until an emulsion was formed. The emulsion was then acidified to pH = 2, under continued vigorous stirring. The emulsion typically coagulated upon acidification, and the reaction product entered the organic layer, which was then separated, combined with two additional dichloromethane extracts of the acidified

aqueous solution, dried over anhydrous sodium sulphate and evaporated in vacuo to yield a colourless liquid ($x = 2, 3$), semisolid ($x = 4$) or solid ($x = 5$ to 10) residue.

The identity of each product was confirmed by 60 MHz ^1H NMR:

δ 11.00 (s, 1 H):	-COOH
2.10 (t, 2 H):	-CH ₂ CH ₂ COOH
2.30 (t, 2 H):	-CH ₂ CH ₂ SH
1.20 (b, 2[x-1] +1 H):	-CH ₂ CH ₂ CH ₂ - and -CH ₂ SH

The absence of a triplet of -CH₂CH₂Br at 3.45 ppm, 2H was noted.

The scales and yields of this preparation for 4-mercaptoputyric to 12-mercaptododecanoic acid are summarized below:

Starting material	Scale		Product	Yield	
	g	mmol		g	%
4-bromobutyric acid	1.25	7.5	4-mercaptoputyric acid	0.62	61
5-bromovaleric acid	1.35	7.4	5 mercaptovaleric acid	0.4	41.5
6-bromohexanoic acid	1.46	7.5	6-mercaptophexanoic acid	0.92	73
7-bromoheptanoic acid	1.56	7.5	7-mercaptopheptanoic acid	1.10	90
8-bromooctanoic acid	1.52	6.7	8 mercaptooctanoic acid	0.86	72
9-bromononanoic acid	1.62	6.7	9-mercaptanonanoic acid	1.04	80
10-bromodecanoic acid	1.88	7.5	10-mercaptodecanoic acid	1.55	94
11-bromoundecanoic acid	2.00	7.5	11-mercaptoundecanoic acid	1.49	90
12-bromododecanoic acid	2.09	7.5	12-mercaptododecanoic acid	1.55	88

The crude product was deemed sufficiently pure to be used in the following step without further purification.

3.2.1.2. Thiastearic acids from ω -mercaptoacids.

An oven dried three necked round bottomed flask was charged with 3:1 THF / HMPA under dry nitrogen. The ω -mercaptoacid was added to make a 10 % solution and cooled to 0°C (ice bath).

2 equivalents of a 1.6 M solution of *n*-butyllithium in hexanes was added slowly via syringe while stirring and the solution was stirred for approximately 30 minutes at 0°C. By this time a reddish brown precipitate appeared.

The appropriate 1-bromoalkane was then added dropwise. Several minutes after the addition the precipitate mostly dissolved to give a reddish colored, but almost clear solution.

The solution was stirred overnight at room temperature and then quenched with 50 mL of water. The resulting basic aqueous layer was then washed with hexanes and acidified to pH = 2. The acidic aqueous solution was then extracted with dichloromethane or ether, dried over anhydrous sodium sulphate and evaporated to afford an almost colourless solid residue.

All products were analyzed by 60 MHz ^1H NMR and found to be sufficiently pure to proceed to the next step without further purification (see below).

60 MHz ^1H NMR of products:

Sulphur position	Signal a	b	c	d	e	f	g
3	11.00 (s, 1 H)	abs.	abs.	3.15 (s, 2 H)	2.60 (t, 2 H)	1.30 (b, 26 H)	0.90 (t, 3 H)
4	11.00 (s, 1 H)	2.40 (m, 4 H)	abs.	2.60 (t, 2 H)	2.40 (m, 4 H)	1.30 (b, 24 H)	0.90 (t, 3 H)
5	10.50 (s, 1 H)	2.30 (m, 4 H)	1.80 (m, 2 H)	2.40 (t, 2 H)	2.30 (m, 4 H)	1.30 (b, 22 H)	0.90 (t, 3 H)
6 to 13	11.50 (s, 1 H)	2.35 (m, 6 H)	abs.	2.35 (m, 6 H)	2.35 (m, 6 H)	1.30 (b, 24 H)	0.90 (t, 3 H)

abs.: Signal not present

The scales and yields of this process for 3- to 13-thiastearic acids are summarized below:

Starting material	Scale		n-Bromoalkane added	Amount ml	Yield of product	
	g	mmol			g	%
mercaptoacetic acid	2.00	21.0	n-bromopentadecane	7	2.50	36
3-mercaptopropionic acid	2.20	21.0	n-bromotetradecane	6.5	3.10	47
4-mercaptobutyric acid	0.62	6.0	n-bromotridecane	8	0.93	53
5-mercaptovaleric acid	0.40	3.3	n-bromododecane	4	0.65	65
6-mercaptohexanoic acid	0.91	6.7	n-bromoundecane	8	1.32	64
7-mercaptoheptanoic	1.10	7.5	n-bromodecane	10	1.40	62
8-mercaptooctanoic acid	1.20	7.5	n-bromononane	10	1.32	71
9-mercaptanonanoic acid	1.04	6.0	n-bromooctane	8	1.45	81
10-mercaptodecanoic acid	1.29	6.7	n-bromoheptane	7.5	1.40	68
11-mercaptoundecanoic acid	1.00	4.5	n bromohexane	6	0.73	49
12-mercaptododecanoic acid	1.15	6.7	n-bromopentane	7.5	1.12	70

3.2.1.3. Methyl 3- to 13-thiastearates from 3- to 13-thiastearic acids.

Each crude thiastearic acid was dissolved in approximately 20 mL of 12.9 % methanolic solution of BF_3 / etherate and refluxed under nitrogen for 1 h. The mixture was then cooled, the BF_3 hydrolyzed with approximately 30 mL of distilled water and the solution was extracted with hexanes. The organic layer was then dried in the usual manner and the solvent removed *in vacuo*. The colourless residue obtained was a low-melting crystalline solid (3- to 6-thiastearates) or a liquid (7- to 13-thiastearates) at room temperature.

The crude thiastearates were purified by flash chromatography.

Solvent: 4% Ethylacetate/hexanes, Retention volume = 4 to 6 column volumes.

The purified thiastearates were analyzed by 300 MHz ^1H NMR and a direct probe MS (see below).

MS of methyl 3- to 13-thiastearates:

Sulphur position	Molecular ion $m/e = 316$ Abundance:	Fragment ions: m/e ; abundance		
		A	B	C
3	9.8	285 ; 5.7	73 ; 45.2	243 ; 66.9
4	14.5	285 ; 7.1	87 ; 66.8	229 ; 76.7
5	21.2	285 ; 7.8	101 ; 68.3	215 ; 100.0
6	11.9	285 ; 6.0	115 ; 100.0	201 ; 48.9
7	18.6	285 ; 9.9	129 ; 100.0	187 ; 68.0
8	21.8	285 ; 9.1	143 ; 98.5	173 ; 94.7
9	25.7	285 ; 12.2	157 ; 92.3	159 ; 100.0
10	24.6	285 ; 14.1	171 ; 80.3	145 ; 82.7
11	22.5	285 ; 9.6	185 ; 81.1	131 ; 62.5
12	14.0	285 ; 6.6	199 ; 87.8	117 ; 44.2
13	21.8	285 ; 9.9	213 ; 60.9	103 ; 48.3

The methyl 3- to 13-thiastearates exhibited the following 300 MHz ^1H NMR spectra:

Sulphur position	Signal								
	a	b	c	d	e	f	g	h	i
3	3.65 (s, 3)	abs.	abs.	3.15 (s, 2)	2.60 (t, 2)	abs.	1.75 (m, 2)	1.30 (b, 24)	0.90 (t, 3)
4	3.68 (s, 3)	2.45 (m, 4)	abs.	2.75 (t, 2)	2.35 (m, 4)	abs.	1.75 (m, 2)	1.30 (b, 22)	0.90 (t, 3)
5	3.65 (s, 3)	2.35 (m, 4)	abs.	2.45 (m, 2)	2.35 (m, 4)	1.80 (m, 4)	1.80 (m, 4)	1.30 (b, 20)	0.90 (t, 3)
6	3.68 (s, 3)	2.20 (t, 2)	1.65 (m, 6)	2.35 (m, 4)	2.35 (m, 4)	1.65 (m, 6)	1.65 (m, 6)	1.30 (b, 18)	0.90 (t, 3)
7 to 13	3.65 (s, 3)	2.25 (t, 2)	1.60 (m, 6)	2.38 (m, 4)	2.38 (m, 4)	1.60 (m, 6)	1.60 (m, 6)	1.25 (b, 18)	0.90 (t, 3)

The yields of crude and purified methyl thiastearates and their melting points are shown below:

Starting material (sulphur position in thiastearic acid)	Scale		Product yield (purified):	
	g	mmol	g	%
3	2.50	8.28	2.06	78.5
4	3.10	10.26	2.13	63.5
5	0.90	2.98	0.73	77
6	0.65	2.15	0.31	45.5
7	1.32	4.37	0.83	60
8	1.40	4.64	0.87	59
9	1.32	4.37	0.76	67.5
10	1.45	3.97	0.74	59
11	1.40	4.64	0.90	67
12	0.73	2.42	0.25	32.5
13	1.12	3.71	0.86	73.5

3.2.2. Methyl 3- to 13-thiastearate S-oxides from methyl 3- to 13-thiastearates.

Each methyl thiastearate was dissolved in 2 mL (9-thiastearate in 5 mL) of dichloromethane and cooled to 0°C. 10% MCPBA solution in dichloromethane pre-cooled to 0°C was added slowly in stoichiometric amounts (1 equivalent). The solution was then stirred in an ice bath for 2 h and extracted with 1 M aqueous sodium bicarbonate, dried in the usual manner and the solvent

removed *in vacuo*, affording a colorless solid residue, which was subsequently purified by HPLC (5-, 10- and 13-thiastearates) or flash chromatography.

HPLC: column: Novapak C18, mobile phase: 30% ethylacetate / dichloromethane at 2 mL/min.

Flash: solvent: 50% ethylacetate / hexane → ethylacetate.

The purified methyl 3- to 13-thiastearates S-oxides were analyzed by 300 MHz ^1H NMR:

Sulphur position	Signal a	b	c	d	e	f	g	h	i
3	3.68 (s, 3)	abs.	abs.	3.50 (ABq, 2)	2.80 (t, 2)	abs.	1.75 (m, 2)	1.20 (b, 24)	0.90 (t, 3)
4	3.65 (s, 3)	2.65 (t, 2)	abs.	2.95 (t, 2)	2.75 (t, 2)	abs.	1.75 (m, 2)	1.20 (b, 22)	0.90 (t, 3)
5	3.68 (s, 3)	2.50 (t, 2)	abs.	2.78 (t, 2)	2.65 (t, 2)	2.10 (m, 2)	1.75 (m, 2)	1.20 (b, 20)	0.90 (t, 3)
6	3.68 (s, 3)	2.40 (t, 2)	1.75 (m, 6)	2.65 (m, 4)	2.65 (m, 4)	1.75 (m, 6)	1.75 (m, 6)	1.20 (b, 18)	0.90 (t, 3)
7	3.65 (s, 3)	2.33 (t, 2)	1.70 (m, 6)	2.65 (m, 4)	2.65 (m, 4)	1.70 (m, 6)	1.70 (m, 6)	1.20 (b, 18)	0.90 (t, 3)
8 to 13	3.68 (s, 3)	2.25 (t, 2)	1.60 (m, 2)	2.65 (m, 4)	2.65 (m, 4)	1.75 (m, 6)	1.75 (m, 6)	1.20 (b, 18)	0.90 (t, 3)

The scales and yields of methyl 3- to 13-thiastearate S-oxide synthesis are summarized below:

Starting material (sulphur position in thiastearate)	Scale		Product yield (purified)	
	mg	mmol	mg	%
3	100	32	63.3	60.5
4	50	16	33.8	64.5
5	60	19	38.9	61.5
6	50	16	32.5	62
7	50	16	28.6	54.5
8	50	16	35.1	67
9	100	32	63.2	60
10	100	32	36.8	35.
11	60	19	39.3	62.5
12	60	19	47.0	74.5
13	50	16	28.1	53.5

MS of methyl 3- to 13-thiastearates S-oxides:

Sulphur position	Mol. ion - OH m/e = 315	Fragment ions: m/e : abundance			
		A m/e = 301	B	C	D
	Abundance				
3	45.8	3.3	90 ; 20.5	243 ; 5.9	73 ; 1.0
4	23.9	6.5	104 ; 56.8	229 ; 100.0	87 ; 10.1
5	32.4	8.8	118 ; 48.5	215 ; 100.0	101 ; 100.0
6	38.8	10.6	132 ; 48.6	201 ; 9.2	115 ; 100.0
7	80.8	13.4	146 ; 62.6	187 ; 16.6	129 ; 35.9
8	100.0	13.6	160 ; 56.3	173 ; 17.5	143 ; 14.5
9	94.0	12.4	174 ; 61.0	159 ; 46.8	157 ; 15.9
10	100.0	12.7	188 ; 53.8	145 ; 16.8	171 ; 10.0
11	100.0	12.8	202 ; 45.5	131 ; 19.5	185 ; 8.8
12	81.1	10.3	116 ; 38.2	117 ; 18.7	199 ; 7.5
13	100.0	14.5	230 ; 37.3	103 ; 21.7	213 ; 15.5

3.2.3. Methyl 5-, 3- and 13-thiastearates S-dioxides from 5-, 9- and 13-thiastearates.

Each methyl thiastearate was dissolved in dichloromethane and a solution of MCPBA was added with stirring (2 equivalents). The solution was stirred for 30 minutes at room temperature and then refluxed for another 30 minutes and subsequently extracted with 1 M aqueous sodium bicarbonate, dried and evaporated, affording a solid colorless residue. The residue was then purified by HPLC using 30% ethylacetate / hexane as mobile phase at 2 ml/min.

The purified products were analyzed by 300 MHz ^1H NMR and direct probe MS (below):

300 MHz ^1H NMR:

Sulphur position	Signal:								
	a	b	c	d	e	f	g	h	i
5	3.68 (s, 3)	2.30 (t, 2)	1.62 (m, 2)	3.00 (m, 4)	3.00 (m, 4)	1.90 (m, 4)	1.90 (m, 4)	1.30 (b, 20)	0.85 (t, 3)
9, 13	3.68 (s, 3)	2.30 (t, 2)	1.60 (m, 2)	2.94 (t, 4)	2.94 (t, 4)	1.84 (t, 4)	1.84 (t, 4)	1.25 (b, 18)	0.85 (t, 3)

Direct probe MS:

Sulphur position	Mol ion $m/e = 348$	Fragment ion: m/e ; abundance		
		A $m/e = 317$	B	C
5	0.1	14.2	101 ; 100.0	70 ; 23.5
9	1.2	14.5	157 ; 70.9	126 ; 9.1
13	1.1	14.0	213 ; 65.5	182 ; 10.5

The scales and yields of the methyl 5-, 9- and 13-thiastearate S-dioxide are summarized below:

Starting material (Sulphur position in thiastearate)	Scale		Product yield (purified)	
	mg	mmol	mg	%
5	50	0.173	36.9	67
9	186.5	0.590	111.3	54.5
13	50	0.173	41.1	74.5

3.2.4. Alternative synthetic route to 9-mercapto nonanoic acid.

3.2.4.1. Methyl 9-hydroxy nonanoate from azelaic acid monomethyl ester

Azelaic acid monomethyl ester (4.5 g) was dissolved in 15 mL of dry THF under dry nitrogen and cooled to 0 °C. 25 mL of a 1 M solution of BH_3 in THF was then added over a 20 min period via syringe, while the mixture was stirred in an ice bath. The solution was subsequently brought to room temperature, stirred for an additional 2 h, and hydrolyzed with 10 mL of distilled water (ice bath). The THF was then evaporated *in vacuo*, the remaining solution was diluted with 50 mL of water and extracted with ether. The ether extract was dried and solvent was removed.

3.6 g of a colorless residue was obtained and analyzed by 60 MHz 1H NMR.

- δ 4 - 5 (s, 1 H): $-CH_2OH$
- 3.70 (s, 3 H): $-OCH_3$
- 3.60 (t, 2 H): $-CH_2-CH_2OH$
- 2.30 (t, 2 H): $-CH_2COOH$
- 1.30 (b, 12 H): $-CH_2-CH_2-CH_2-$

The material was judged to be sufficiently pure for use in the next synthetic step.

3.2.4.2. Methyl 9-p-toluenesulphonyl nonanoate.

1 g of methyl 9 hydroxy nonanoate was dissolved in 10 mL of dry pyridine and cooled to 0°C (ice bath) under a dry nitrogen atmosphere.

p Toluenesulphonyl chloride (4 g) was slowly added and the solution stirred for 1 h in ice bath. Once the ice had melted the solution was stirred overnight at room temperature, then neutralized by pouring over a 20% H_2SO_4 / ice mixture and extracted with ether. The ethereal extract was dried and evaporated. A yellowish semi-solid residue was obtained, and subsequently purified by flash chromatography using 50% dichloromethane / hexane as mobile phase.

1.2 g of a colorless liquid was obtained and analyzed by 60 MHz ^1H NMR:

- 7.85 (d, 2 H) Ar-H
- 7.45 (d, 2 H) Ar-H
- 4.10 (t, 2 H) $-\text{CH}_2-\text{CH}_2-\text{OTs}$
- 3.70 (s, 3 H): OCH_3
- 2.50 (s, 3 H): $-\text{CH}_3-\text{Ph}$
- 2.30 (t, 2 H): $-\text{CH}_2-\text{CH}_2-\text{COOMe}$
- 1.30 (b, 12 H): $\text{CH}_2-\text{CH}_2-\text{CH}_2-$

At this point the material was judged sufficiently pure for use in the next synthetic step.

3.2.4.3. 9-mercapto nonanoic acid from methyl 9-p-toluenesulphonyl nonanoate.

The procedure described in section 3.2.1.1. yielded 0.4 g of a colorless low melting solid from 1.2 g of methyl 9-(p-toluenesulphonyl) nonanoate.

The product was analyzed by 60 MHz ^1H NMR and found sufficiently pure to proceed to the next step without further purification:

- δ 10.80 (s, 1 H): $-\text{COOH}$;
- 2.30 (m, 4 H): $-\text{CH}_2-\text{CH}_2-\text{SH}$ and $\text{CH}_2-\text{CH}_2-\text{COOH}$;
- 1.30 (b, 13 H): $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$ and CH_2SH .

The material was then used in the synthesis of 10-thiastearic acid and its methyl ester as described in sections 3.2.1.2. and 3.2.1.3 of this thesis.

3.2.4.3. 9-mercapto nonanoic acid from methyl 9-p-toluenesulphonyl nonanoate.

The procedure described in section 3.2.1.1. yielded 0.4 g of a colorless low melting solid from 1.2 g of methyl 9-(p-toluenesulphonyl) nonanoate.

The product was analyzed by 60 MHz ^1H NMR and found sufficiently pure to proceed to the next step without further purification:

δ 10.80 (s, 1 H): $-\text{COOH}$;

2.30 (m, 4 H): $-\text{CH}_2-\text{CH}_2-\text{SH}$ and $\text{CH}_2-\text{CH}_2-\text{COOH}$;

1.30 (b, 13 H): $-\text{CH}_2-\text{CH}_2-\text{CH}_2$ and CH_2SH .

The material was then used in the synthesis of 10-thiastearic acid and its methyl ester as described in sections 3.2.1.2. and 3.2.1.3. of this thesis.

**3.2.5. Methyl S-benzyl-8-mercaptooctanoate,
S-benzyl-9-mercaptanonanoate,
S-benzyl-10-mercaptodecanoate and
S-benzyl-11-mercaptoundecanoate.**

3.2.5.1. S-benzyl ω -mercaptoalkanoic acids from ω -mercaptoalkanoic acids.

The mercapto acids (8-mercaptooctanoic, 9-mercaptanonanoic, 10-mercaptodecanoic, 11-mercaptoundecanoic) prepared previously in this work (c.f. p. 66 - 67) were dissolved under dry nitrogen in 2 : 1 dry THF / HMPA to make a 10% solution and cooled to 0°C.

A 1.6 M solution of butyllithium in hexanes was added dropwise and the solution was stirred for 30 minutes at 0°C. Benzyl bromide was then added dropwise and the solution was stirred overnight and worked up as reported previously (c.f. p. 67). The workup afforded colourless solid residues, which were analyzed by 60 MHz ^1H NMR:

δ : 11.00 (s, 1 H):	-COOH
7.30 (b, 5 H):	Ar-H
3.70 (s, 2 H):	PhCH ₂ S-
2.35 (m, 4 H):	-CH ₂ CH ₂ S- and -CH ₂ CH ₂ COOH
1.35 (b, 2[n - 2] H)	-CH ₂ CH ₂ CH ₂ -

The scales and yields of this preparation for all four products are summarized below.

Starting material	Scale		Product yield	
	g	mmol	g	%
8-mercaptocctanoic acid	1.00	5.68	1.32	87.5
9-mercaptanonanoic acid	1.08	5.68	0.66	41.5
10-mercaptodecanoic acid	1.16	5.68	1.31	78.5
11-mercaptoundecanoic acid	1.24	5.68	0.54	31

3.2.5.2. Methylation of S-benzyl mercaptoalkanoic acids.

The four S-benzyl mercaptoalkanoic acids were methylated following the procedure described for thiastearic acids (c.f. p. 70). The corresponding methyl esters were obtained as nearly colourless liquids and purified by flash chromatography using the same analytical conditions reported earlier for purification of thiastearates. The colourless liquids obtained were analyzed by direct probe MS and 300 MHz ^1H NMR (below) and judged to be satisfactorily pure to be used in subsequent feeding experiments.

Direct probe MS of S-benzyl mercaptoalkanoic acid methyl esters obtained:

Homologue: Mol. ion		Fragment ions: m/e ; abundance			
z =	m/e ; abundance	A	B	C	D
7	280 ; 6.9	249 ; 3.8	91 ; 100.0	189 ; 3.2	123 ; 28.7
8	294 ; 6.4	263 ; 3.0	91 ; 100.0	203 ; 5.2	123 ; 24.5
9	308 ; 11.5	277 ; 5.1	91 ; 100.0	217 ; 9.6	123 ; 52.9
10	322 ; 7.1	291 ; 2.8	91 ; 100.0	211 ; 1.0	123 ; 34.3

The S-benzyl mercaptoalkanoic acid methyl esters exhibited the following 300 MHz ^1H NMR:

δ : 3.65 (s, 3 H):	$-\text{COOCH}_3$
7.35 (m, 5 H):	Ar-H
3.70 (s, 2 H):	$\text{PhCH}_2\text{S}-$
2.40 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{S}-$
2.25 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{COOH}$
1.73 (m, 2 H):	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{S}-$
1.65 (m, 2 H):	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$
1.35 (m, 2z H):	$-\text{CH}_2\text{CH}_2\text{CH}_2-$

The scales and yields of this process for all products are summarized below:

Starting material	Scale		Product yield (purified)	
	g	mmol	g	%
S-benzyl-				
8-mercaptooctanoic acid	1.32	7.51	1.02	67.5
9-mercaptononanoic acid	0.66	3.54	0.42	26.5
10-mercaptodecanoic acid	1.31	6.40	1.10	66
11-mercaptoundecanoic acid	0.54	2.57	0.39	22.5

3.2.6. Methyl S-benzyl-8-mercaptooctanoate S-oxide and methyl S-benzyl-10-mercaptodecanoate S-oxide.

The procedure reported earlier for the synthesis of methyl thiastearates S-oxides gave both products in satisfactory yields from methyl S-benzyl-8-mercaptooctanoate and S-benzyl-10-mercaptodecanoate. Both were purified by flash chromatography using the procedure described for thiastearate S-oxides (c. f. p. 72) and judged sufficiently pure by TLC (silica gel 60, ethyl acetate) to be used as chromatographic standards.

Their structures were confirmed by 300 MHz ^1H NMR and direct probe MS:

300 MHz ^1H NMR:

δ : 7.35 (m, 5 H):	Ar-H
4.05 (AB q, 2 H):	$\text{PhCH}_2\text{SO}-$
3.68 (s, 3 H):	$-\text{COOCH}_3$
2.60 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{SO}-$
2.30 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{COOH}$
1.75 (m, 2 H):	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}-$
1.62 (m, 2 H):	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{COOH}$
1.35 (m, 2 z H):	$-\text{CH}_2\text{CH}_2\text{CH}_2-$

Direct probe MS:

Homologue:	Fragment ions: m/e ; abundance				
z =	A	B	C	D	E
7	265 ; 13.5	279 ; 4.5	91 ; 100.0	205 ; 2.8	139 ; 35.6
9	293 ; 9.5	307 ; 3.7	91 ; 100.0	233 ; 4.9	139 ; 41.1

The scales and yields of this preparation are summarized below:

Starting material	Scale		Product yield (purified)	
	mg	mmol	mg	%
methyl S-benzyl-8-mercaptooctanoate	50	0.179	35.6	67
10-mercaptodecanoate	50	0.162	34	64.5

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3.3. Biological experiments.

3.3.1. General procedure.

All feeding experiments described in this thesis were carried out following the same general procedure outlined below. The only variables were:

- the substrate administered to the yeast cells
- the scale of the experiment.

All water used in the feeding experiments was distilled and deionized, obtained from Millipore Milli Q Water System CD0F01205 equipped with a Super C Carbon Cartridge, two Ion Ex Cartridges, an Organex Q Cartridge and a Milli Pak Filter.

The yeast, *Sacharomyces cerevisiae*, strain NRC 2335 was freshly grown on slants prior to each feeding experiment. The slant medium was prepared as follows: 10 g of bacto peptone, 5 g of Bacto yeast extract, 15 g of Bacto agar were dissolved in 400 mL of distilled deionized water. In a separate flask 10 g of glucose was dissolved in 100 mL of distilled deionized water and both solutions were autoclaved separately, cooled and combined. Approximately 5 mL aliquots of this solution were then pipetted into each of the culture tubes, which were also previously autoclaved. All transfers were carried out in sterile conditions.

The agar was allowed to solidify while the tubes were on a slight slant and the set slants were then stored at 4°C until needed.

To obtain a batch of freshly grown cells for use in a feeding experiment the required number of slants were streaked with dormant *Saccharomyces cerevisiae* cells (stored at 4°C) and incubated at 30°C for 24 h in a New Brunswick incubator / shaker, until a distinct growth pattern appeared.

3.3.2. Feeding experiments.

The culture medium was prepared as follows: 7 g of Bacto peptone and 3.5 g of Bacto yeast extract was dissolved in 300 mL of distilled deionized water. 14 g of glucose were dissolved in 100 mL of distilled deionized water in a separate flask and both solutions autoclaved separately, cooled and combined. All transfers were carried out in a sterile atmosphere. The cells of one freshly grown slant were suspended by shaking with 2 mL of sterilized distilled water and added to the culture medium. 40 mg of substrate dissolved in 2 mL of absolute ethanol were then added

to the medium and the culture was incubated for 48 h in a New Brunswick incubator / shaker at 1500 rpm and 30°C.

The cells were then separated from the media by centrifugation (2500 rpm) or ultrafiltration (Millipore Minitan, 4 Minitan 4/PK membrane plates, up to 30 psi backpressure at approx. 100 mL/min) and washed twice with 100 mL of distilled water. This procedure typically yielded approximately 90 mg of wet cells per 1 mL of culture media with little variation from feeding experiments and approximately 8 mg/mL of media from control experiments. The cells were stored at -20°C until needed.

In control experiments, the cells used to inoculate the culture media were previously destroyed by autoclaving at 130°C for 30 minutes.

3.3.3. Extraction and methylation of fatty acids from yeast cells.

The harvested yeast cells were refluxed under nitrogen atmosphere with 100 mL of 1 M KOH in 50 % ethanol for about 2 h and the ethanol was removed *in vacuo*. The resulting solution was washed with ether, acidified to a pH of 2 and extracted with dichloromethane. The dichloromethane extract was then washed with saturated sodium chloride and dried over anhydrous sodium sulphate and the solvent was removed. The mixture of fatty acids thus obtained was then methylated by refluxing for about 1 h with approximately 30 mL of 12.9 % BF_3 etherate solution in methanol. The solution was cooled, diluted with 50 mL of distilled water and then extracted with ether or dichloromethane. 10 mL of saturated sodium chloride was added to speed up the separation and to make the dichloromethane or ether less soluble in the methanol / water phase. The organic extract was washed with saturated sodium chloride and dried over anhydrous sodium sulphate and the solvent was removed *in vacuo*.

The resulting mixture of fatty acid methyl esters was then analyzed by TLC on silicagel 60 plates (20 % ethylacetate/hexane for methyl esters of thia fatty acids, ethylacetate for the thia-fatty acid S-oxides) with the aid of synthetic methyl 9-thiastearate and 9-thiastearate S-oxide standards. The fractions containing the methyl esters of thia-fatty acids or their S-oxides were separated from these extracts by flash chromatography: (silicagel, 4 % ethylacetate/hexanes for the thia-fatty acid fraction, followed by second elution with ethylacetate for the S-oxide fraction).

3.3.4. Extraction and methylation of fatty acids from culture media.

Each culture medium was acidified to a pH of 2 and extracted with 3 x 75 ml of dichloromethane. The dichloromethane extract was then evaporated and the residue refluxed for 2 h with 1 M NaOH under nitrogen atmosphere and the ethanol was then evaporated *in vacuo*. The aqueous solution was again acidified to a pH of 2, extracted with dichloromethane and dried in the usual manner. The crude residue was then methylated, worked up and analyzed by TLC as previously described. The S-oxides of thia-fatty acids were then separated and purified by repeated flash chromatography. Mobile phase: 1) ethyl acetate, 2) 2 % methanol/dichloromethane.

4. Addendum.

A synthetic approach to methyl 14- to 17-thiastearates.

To expand the scope of the regioselectivity studies of thiastearate oxidation by *Saccharomyces cerevisiae* the methyl 14- to 17-thiastearates were to be administered to the cells in competition with the remaining isomers. Time constraints did not permit the completion of the synthesis of these compounds, however, a general approach to methyl thiastearates bearing the sulfur atom near the end of the alkyl chain was worked out.

Since suitably difunctionalized hydrocarbons of proper chain length for the synthesis of 14- to 17-thiastearates (i.e. tridecane to hexadecane) were not commercially available, it became necessary to develop a general method for their synthesis. A strategy based on scheme 17 was proposed (next page).

Even though many other reactions were also considered, the alkylation of a terminal alkyne was chosen to assemble the difunctionalized hydrocarbon skeleton. This method, once the optimum conditions are found, can be easily adapted for the alkylation of terminal alkynes with terminal epoxides to yield β -hydroxyalkynes, which can be resolved and used as intermediates for the synthesis of chiral fluoroderivatives of fatty acids.

Also, this synthesis is a convergent one, where the key intermediates, the THP ethers of α -bromo alcohols and the alkynoacids can be combined as needed so as to enable the synthesis of a broad range of products from a relatively limited number of intermediates, thus in effect reducing the number of steps necessary for each of these syntheses.

Scheme 17.

4.1. Monobromination of 1, ω -alkanediols.

The ω -bromoalcohols of general formula $\text{Br}(\text{CH}_2)_n\text{OH}$, needed for the synthesis of the key bromoalcohol THP ether intermediates were obtained in a slow bromination of the corresponding diols, while extracting the monobrominated product with a suitable solvent in a continuous extraction apparatus.⁷⁰

The diol and a 3:1 v/v mixture of 48% aq. HBr / concentrated H_2SO_4 (1 mole of HBr per 1 mole of diol) was placed in a continuous extraction apparatus.

The reaction mixture in the extraction apparatus was then heated to approximately 80°C and extracted with refluxing heptane for 24 h ($n = 5$ for 48 h). The apparatus was then cooled, the heptane solution stirred with anhydrous sodium sulphate and sodium bicarbonate for 1 h and the solvent was removed *in vacuo*. A yellowish dense liquid ($n = 5, 6$), semisolid ($n = 8$), or solid ($n = 12$) residues were obtained and analyzed by 60 MHz ^1H NMR in CDCl_3 :

- δ : 3.65 (t, 2H): $-\text{CH}_2\text{CH}_2\text{OH}$
 3.45 (t, 2H): $-\text{CH}_2\text{CH}_2\text{Br}$:
 2 - 3 (concentration dependent) (s, 1H): $-\text{CH}_2\text{OH}$
 1.30 (b, $2[n - 2]$ H): $-\text{CH}_2\text{CH}_2\text{CH}_2$

The scales and yields of this reaction for each homologue are shown below:

Starting material	Scale		Product	Yield	
	g	mol		g	%
1,5-pentanediol	80	0.77	5-bromo-1-pentanol	57.8	45
1,6-hexanediol	23.6	0.2	6-bromo-1-hexanol	27.7	76.5
1,8-octanediol	29.2	0.2	8-bromo-1-octanol	32.8	78
1,12-dodecanediol	40.4	0.2	12-bromo-1-dodecanol	37.9	71.5

4.2. Formation of tetrahydropyranyl ethers of bromoalcohols

In order to prevent undesirable side reactions and to optimize the yields in the subsequent alkylation of alkynoacids or lithium acetylide with the bromoalcohols, the protection of the hydroxyl groups of these alcohols against deprotonation seemed to be a sensible precaution. The tetrahydropyranyl group provides the desired stability to strong bases.

The THP ethers were formed through the acid catalyzed alkylation of the corresponding alcohols with dihydropyran in good yields.

Dihydropyran containing catalytic amount of HCl (0.2 g) was slowly added to the bromoalcohol, while stirred at 20°C. Stirring was continued at room temperature for 1 hour, then anhydrous sodium sulphate and sodium bicarbonate were added and the solution stirred for an additional 2 h and subsequently filtered and the excess dihydropyran was evaporated *in vacuo*, yielding an almost colorless liquid residue.

All products were analyzed by 60 MHz ^1H NMR:

The scales and yields of this preparation for the THP ethers of 5-bromo-1-pentanol, 6-bromo-1-hexanol, 8-bromo-1-octanol and 12-bromo-1-dodecanol are summarized below:

Starting material	Scale		Product	Yield	
	g	mol		g	%
5-bromo-1-pentanol	57	0.34	5-bromo-1-pentanol THP ether	68	79
6-bromo-1-hexanol	11.5	0.075	6-bromo-1-hexanol THP ether	12.1	72
8-bromo-1-octanol	16	0.075	8-bromo-1-octanol THP ether	15.7	68.5
12-bromo-1-dodecanol	40.2	0.14	12-bromo-1-dodecanol THP ether	46	89.5

4.3. Alkynylation of THP ethers of ω -bromoalkane-1-ols.

The THP ethers of ω -bromoalcohols thus prepared were alkynylated with lithium acetylide to obtain the THP ethers of the corresponding alkynols. Ethylene diamine complex of lithium acetylide was used in this experiment, rather than pure lithium acetylide.

The complex was dissolved in dry DMSO (distilled from CaH_2) under dry nitrogen to make a 2 M solution. This solution was then cooled in ice bath until it began to freeze (at about 8°C). The bromoalcohol was then added slowly via syringe (0.9 mole / 1 mole of complex) with stirring. The solution was kept below 10°C throughout the addition by external cooling, then stirred for additional 2 h at room temperature and subsequently cooled again to near freezing. At this point the excess lithium acetylide was destroyed by dropwise addition of 100 mL of water, while stirring the solution vigorously in an ice bath. Hexane extraction of the resulting mixture followed by routine drying and solvent removal afforded dense yellow oils in acceptable yields and purity (see below).

Starting material	Scale		Product	Yield	
	g	mmol		g	%
1-Tetrahydropyranoxy-:			1-Tetrahydropyranoxy-:		
-5-bromopentane	60	240	-6-heptyne	33.6	71
-6-bromohexane	12	45.5	-7-octyne	7.9	82
-8-bromooctane	10	34	-9-decyne	5.6	68.5

The structures of all products were confirmed by 60 MHz ^1H NMR:

δ : 5 and 4.60 (b, b, $\Sigma=1$ H):	and
3.65 (m, 4 H):	$-\text{CH}_2\text{CH}_2\text{O}-$
2.10 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{C}\equiv\text{CH}$
1.90 (t, 1 H):	$-\text{CH}_2\text{C}\equiv\text{CH}$
1.65 (b, 6 H):	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{O}-$
1.40 (b, 2[n - 3] H):	$-\text{CH}_2\text{CH}_2\text{CH}_2-$

4.4. Deprotection of THP ethers of (n-1)-alkyne-1-ols.

Each tetrahydropyranoxyalkyne was deprotected to a free alcohol by treatment with a large excess of methanol and catalytic amount of p-toluenesulphonic acid. The methoxypyran formed in the trans- acetal formation was removed by evaporation.

The THP ethers were dissolved in 50 mL (large excess) of methanol and a catalytic amount of p-toluenesulphonic acid (0.2 g) was added. The solution was stirred for 90 min at room temperature and then refluxed for 15 minutes. 5 g of sodium bicarbonate and 10 g of anhydrous sodium sulphate was added and stirred for 1 hour and the solids were filtered off. Solvent evaporation afforded almost colourless solids. Heating the solutions *in vacuo* to about 80°C for more than 30 min was typically required to remove 2-methoxytetrahydropyran along with the solvent.

The products were judged by subsequent 60 MHz ^1H NMR analysis to be sufficiently pure to proceed to the next step without further purification:

δ : 3.60 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{OH};$
2 - 4, concentration dependent, (s, 1 H):	$-\text{CH}_2\text{OH}$
2.15 (t, 2 H):	$-\text{CH}\equiv\text{C}-\text{CH}_2-\text{CH}_2$
1.95 (t, 1 H):	$\text{CH}\equiv\text{C}-\text{CH}_2-$
1.35 (b, 2(n-2) H):	$-\text{CH}_2-\text{CH}_2-\text{CH}_2-$

The scales and yields of this preparation are summarized below:

Starting material	Scale		Product	Yield	
	g	mmol		g	%
1-Tetrahydropyranoxy-:					
-6-heptyne	20		6-heptyne-1-ol	7.1	68.5
-7-octyne	7.9		7-octyne-1-ol	3.3	74
-9-decyne	5		9-decyne-1-ol	2.2	71

4.5. Oxidation of (n-1)-alkyne-1-ols to terminal alkynoic acids.

Chromium trioxide in the presence of many oxygen containing solvents, such as diethylether and acetone is known to be a mild and selective oxidizing agent. Numerous precedents are known of the conversion of unsaturated alcohols to aldehydes and ketones⁷¹ with this reagent. It was therefore used to oxidize alkyno alcohols to the corresponding alkyno acids.

Chromium trioxide was dissolved in minimum amount of water and 25% sulphuric acid was added and the solution cooled in ice bath. The alkynols were dissolved in acetone and cooled in an ice-salt bath to below 0°C. The pre-cooled chromic acid solution was then added very slowly via Pasteur pipette, (1.4 mole of CrO₃ per 1 mole of alcohol) with vigorous stirring in the ice-salt bath, keeping the temperature below 10°C throughout the oxidation. 10 g of solid sodium bisulphite was then added and stirring resumed at room temperature for 30 min. The solution was then extracted with hexanes and the hexanes fraction extracted again with 1 M NaOH. The alkaline aqueous extract was then acidified to a pH of 2 with HCl and extracted with dichloromethane. The dichloromethane solution was dried and a colorless solid residue was obtained upon solvent evaporation *in vacuo*.

The scales and yields of this preparation for 6-heptynoic, 7-octynoic and 9-decynoic acid are shown below:

Starting material	Scale		Product	Yield	
	g	mol		g	%
6-heptyne-1-ol	7	0.069	6-heptynoic acid	4.1	52
7-octyne 1-ol	3.3	0.028	7-octynoic acid	2.1	54
9 decyne-1-ol	5.6	0.038	9-decynoic acid	4.3	68

60 MHz ¹H NMR of products:

a. 11 ppm, concentration dependent (s, 1 H): -COOH;

2.30 (m, 4 H): -CH₂CH₂COOH

2.10 (t, 2 H): -CH₂-CH₂-C≡CH

1.90 (t, 1 H): -CH₂C≡CH

1.35 (b, 2[n - 3] H): -CH₂-CH₂-CH₂.

4.6. Alkylation of (n-1)-alkynoic acids with THP ethers of n-bromoalkane-1-ols.

As mentioned above, the yield of each ω -bromo alcohol THP ether was split into several parts, one of which was later deprotected and oxidized to the corresponding acid. The remaining material could then be used to alkylate one or more of the alkyno acids thus produced to obtain hydroxyacids of varying chain lengths.

Thus the dianions, produced by treatment of 6-heptynoic and 7-octynoic acids with butyllithium were both alkylated with 8-bromo-1-octanol THP ether to obtain 15-hydroxy pentadecanoic acid and 16-hydroxy hexadecanoic acid by subsequent deprotection and hydrogenation of the alkylation products of general formula $\text{THPOCH}_2(\text{CH}_2)_n\text{C}=\text{C}(\text{CH}_2)_m\text{COOH}$.

6-heptynoic acid (4 g) and 7-octynoic acid (3.1 g) were dried by dissolving in dry THF and re-evaporating the solvent *in vacuo* and then dissolved in dry hexamethylphosphorus amide and cooled to about 10°C in an ice bath. A 1.6 M solution of n-butyllithium in hexane was then added slowly via syringe (2.2 mol / 1 mol of acid), with vigorous stirring. The stirring and cooling was continued for approximately 30 minutes after the addition of n-butyllithium was complete.

The THP ether of the bromoalcohol, also previously dried by dissolving in dry THF and re-evaporating the solvent, was then added dropwise via syringe (1 mL/g of acid) and the solution was stirred overnight at room temperature.

30 mL of 1 M HCl was then slowly added and the solution was refluxed for 30 minutes and extracted with hexanes (1 x 40 mL) and ether (2 x 40 mL). Both hexanes and ether fractions were extracted with 1 M NaOH, which resulted in a separation of a dense yellow oil, insoluble in both the aqueous and organic (ether or hexane) phase, which eventually solidified, when separated from both aqueous and organic solution.

A 60 MHz ^1H NMR of this material taken in CDCl_3 , in which it was partly soluble, proved it to be mainly the sodium salt of the expected hydroxyacid product. The solid was then dissolved in 1 M solution of H_2SO_4 in THF. The addition of water resulted in heavy precipitation. The precipitate thus formed was collected, washed with distilled water and dried.

The hexane and ether extracts of the original reaction mixture (after reflux with HCl) were not combined, but, instead, treated separately as above. Two solid residues were obtained in each preparation: the one obtained from the ether extract contained mostly the free hydroxy acid, while the residue derived from the hexanes extract consisted mainly of the THP ether of this

compound.

60 MHz ^1H NMR of THP ethers of 15-hydroxy-6-pentadecynoic and 16-hydroxy-7-hexadecynoic acid:

δ : + 11 (s, 1 H):	-COOH
4.55 (b, 1 H):	-OCH(CH ₂)O-
3.65 (m, 4 H):	-CH ₂ CH ₂ O-
2.30 (t, 2 H):	-CH ₂ CH ₂ COOCH ₃ overlapping with
2.10 (t, 4 H):	-C $\equiv$ CCH ₂ CH ₂ -
1.60 (b, 6 H):	-CH ₂ CH ₂ CH ₂ O-
1.30 (b, 2[m + n - 2] H):	-CH ₂ CH ₂ CH ₂ -

When both hexanes and ether residues obtained from both preparations were combined 2.1 g of 15-hydroxy-6-pentadecynoic acid and its THP ether were obtained along with 1.1 g of 16-hydroxy-7-hexadecynoic acid and its THP ether.

4.7. Deprotection of ω -hydroxyacid THP ethers and methylation of hydroxy acids.

Since the deprotection of terminal hydroxyl groups in the products of the above reaction was not complete despite 30 min reflux with dilute HCl, it was necessary to complete the deprotection before the tosylation and thiolation is attempted. To prevent polyesterification of the hydroxyacid the THP ether was first treated with a large excess of 6% solution of BF_3 etherate in methanol. When the methylation was judged complete, catalytic amount of p-toluenesulphonic acid was added and the deprotection was allowed to take place.

The two free hydroxyacid / hydroxyacid THP ether mixtures obtained in the previous step were dissolved in 30 mL of 6% BF_3 etherate in methanol and stirred for 1 hour. p-Toluenesulphonic acid (0.2 g) was then added and stirring was continued for another 2 h. The solution was then diluted with 30 mL of water and extracted with dichloromethane. The extract was dried and solvent evaporated *in vacuo* (80°C for 40 min to remove all 2-methoxy-tetrahydropyran).

Two colourless low melting solid residues were obtained:

methyl-15-hydroxy-6-pentadecynoate: 1.2 g.

methyl-16-hydroxy-7-hexadecynoate: 0.9 g.

Both compounds were analyzed by TLC and 60 MHz ^1H NMR and found to be sufficiently pure to proceed to the next step without further purification.

60 MHz ^1H NMR:

δ : 3.70 (s, 3 H):	$\text{CH}_3\text{O}-$
3.65 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{O}-$
+ 3 (s, 1 H):	$-\text{CH}_2\text{OH}$
2.35 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{COOCH}_3$
2.05 (t, 4 H):	$-\text{C}\equiv\text{CCH}_2\text{CH}_2-$
1.30 (b, $2[m + n - 2]$ H):	$-\text{CH}_2\text{CH}_2\text{CH}_2-$

4.8. Hydrogenation of alkyne carboxylic acid methyl esters.

The substrates ($m + n = 15$: 1.2 g; $m + n = 16$: 0.9 g) were dissolved in 10 mL of ethylacetate. 0.1 g of palladium black was added to each solution and stirred in a hydrogenation apparatus until no further intake of hydrogen was observed, then filtered and the solvent evaporated. Two low melting solid residues were obtained:

methyl 15-hydroxypentadecanoate: 1.1 g and methyl 16-hydroxyhexadecanoate: 0.85 g.

Both products were analyzed by 60 MHz ^1H NMR:

δ : 3.70 (s, 3 H):	$\text{CH}_3\text{O}-$
3.65 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{O}-$
+ 3 (s, 1 H):	$-\text{CH}_2\text{OH}$
2.30 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{COOCH}_3$
1.30 (b, $2[m + n + 2]$ H):	$-\text{CH}_2\text{CH}_2\text{CH}_2-$

4.9. Tosylation of hydroxyacid methyl esters.

The methyl 15-hydroxypentadecanoate and 16-hydroxyhexadecanoate were tosylated using the same procedure described for the tosylation of 9-hydroxynonanoic acid (c.f. p. 78). The crude residue obtained was purified by flash chromatography in the same manner as the methyl 9-toluenesulfonylnonanoate (c.f. p. 78). 1.05 g of purified methyl 15-(p-toluenesulfonyl)-pentadecanoate and 0.65 g of purified methyl 16-(p-toluenesulfonyl)hexadecanoate was obtained.

Both products were analyzed by 60 MHz ^1H NMR:

δ : 7.90 (d, 2 H):	Ar-H
7.40 (d, 2 H):	Ar-H
4.10 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{OTs}$
3.70 (t, 3 H):	$\text{CH}_3\text{O}-$
2.50 (s, 3 H):	$\text{CH}_3\text{-Ph}$
2.30 (t, 2 H):	$-\text{CH}_2\text{CH}_2\text{COO}-$
1.30 (b, 2[m + n + 2] H):	$-\text{CH}_2\text{CH}_2\text{CH}_2-$

5. References.

- 1) a) Lechevalier M. P.: "Lipids in Bacterial Taxonomy - A Taxonomists View" *CRC Critical Reviews in Micro.*, **5**, 109-210 (1977)
 b) Marr A. G., Ingraham J. L., *J. Bacteriol.*, **84**, 1260 (1962)
 c) Moss C. W., Shinoda T., Samuels J. W.: "Determination of Cellular Fatty Acid Composition of Various Yeasts by Gas-Liquid Chromatography" *J. Clin. Micro.*, **16**, 1073 (1982)
- 2) Bloch K., Baronowsky P., Goldfine H., Lennarz W. L., Light R., Norris A. T., *Lipid Metabolism*, **20**, 921 (1961) and references cited therein.
- 3) Bloch K., *Acc. Chem. Res.*, **2** (7), 193 (1969)
- 4) James A. T., Gurr M. I.: "Lipid biochemistry. An Introduction" Chapman and Hall, London, 1980, 3-rd edition.
- 5) a) Strittmatter P., Spatz L., *Proc. Nat. Acad. Sci. USA*, **8** (5), 1024 (1971)
 b) Strittmatter P., *J. Biol. Chem.*, **246** (4), 1017 (1971)
 c) Strittmatter P. Spatz L., *J. Biol. Chem.*, **248** (30), 793 (1973)
 and references contained therein.
- 6) a) Strittmatter P., Thiede M. A., Ozols J., *J. Biol. Chem.*, **261** (28) 13230 (1987)
 b) Strittmatter P., Thiede M. A., *J. Biol. Chem.*, **260** (27), 14459 (1985)
 c) Strittmatter P., Thiede M. A., Hackett C. S., Ozols J., *J. Biol. Chem.*, **263** (5), 2532 (1988)
- 7) Strittmatter P., Enoch G. H., *Biochemistry*, **17** (23), 4927 (1978)
- 8) Whittaker J. W., Solomon E. I., *J. Am. Chem. Soc.*, **108**, 835 (1986)
- 9) Katapodis A. G., Wimalasena K., Lee J., May S. W., *J. Am. Chem. Soc.*, **106**, 1928 (1984)
- 10) Ohta H., Tetsukawa H., *J. C. S. Chem. Comm.*, 849 (1978)
- 11) Funk M. O., Alteneder A. W., *Biochem. Biophys. Res. Comm.*, **114**, 937 (1983)
- 12) see ref 9.
- 13) Bloch K., Lennarz W. L., Light R. J., *J. Biol. Chem.*, **237**, 1793 (1962)
- 14) see ref 3.
- 15) Kochi J. K., Bemis A., Jenkins C. L., *J. Am. Chem. Soc.*, **90**, 4616 (1968)
- 16) Bloch K., Scheuerbrandt G., *J. Biol. Chem.*, **237**, 2064 (1962)
- 17) Morris M. J., Harris R. U., Kelly W., James A. T., *Biochem. Biophys Res. Comm.*, **28**, 904 (1967)

- 18) Rawlings B. J., Reese P. B., Ramer S. E., Vederas J. C., *J. Am. Chem. Soc.*, **111**, 3382 (1989)
- 19) McInnes A. G., Walter J. A., Wright L. C., *Tetrahedron*, **39** (21) 3515 (1983)
- 20) McInnes A. G., Walter J. A., *Tetrahedron*, **39** (21), 3507 (1983)
- 21) in hen liver extracts: Johnson A. R., Gurr M. I., *Lipids*, **6**, 78 (1971)
- 22) in pig liver extracts: Biellmann J. F., Hirth C. G., *FEBS Lett.*, **8**, 55 (1970)
- 23) Strittmatter P., Spatz L., Corcoran D., Rogers M. J., Setlow B., Redline R., *Proc. Nat. Acad. Sci. USA*, **71** (11), 4565 (1974)
- 24) a) Pascal R. A. Jr., Rahman M. D., Ziering D. L., Mannarelli S. J., Swartz K. L., Huang D. S., *J. Med. Chem.*, **31**, 1656, (1988)
 b) Pascal R. A. Jr., Mannarelli S. J., Ziering D.L., *J. Biol Chem*, 12441 (1988)
- 25) Abrahams A., *Q. Rev., Chem. Soc.* **10**, 407-436 (1956)
- 26) a) P. H. Laur in "Sulphur in Organic and Inorganic Chemistry", ed. Senning; Deker, New York, 1972, vol 3. p. 91.
 b) Ollis W. D., Barton D.: *Comprehensive Org. Chemistry*, vol. 3, p. 34.
- 27) Mc Murry J.: *Fundamentals of Organic Chemistry*, Brooks, Cole 1986, p. 15.
- 28) Buist P. H., Dallmann H. G., Rymerson R. T., Seigel P. M., *Tetrahedron Letters*, **28** (8) 857 (1987)
- 29) Desmukh M. N., Dunach E., Kagan H. B., Pitchen P., *J. Am. Chem. Soc.*, **106**, 8188 (1984)
- 30) Katapodis A. G., Smith H. A., May S. W., *J. Am. Chem. Soc.*, **110**, 897 (1988)
- 31) Holland H. L., *Chem reviews*, **88** (3) 473-485 (1988)
- 32) Tamura Y., Yoshida Y., Sato R., Kumaoka H., *Archieves of Biochemistry and Biophysics*, **175**, 284 (1976)
- 33) Rapoport L., Smith A., Newman M.S., *J. Am. Chem. Soc.*, **69**, 693 (1947).
- 34) Luzzio F. A., *Synth. Commun.* **14** (3), 209 (1984)
- 35) M. S. F. Lie Ken Jie, University of Hong Kong, Hong Kong; personal communication.
- 36) Pascal R. A. Jr., Ziering D. L., *J. Lip. Res.*, **27**, 221 (1986)
- 37) Urquhart G. G., Gates J. W. Jr., Connor R., *J. Org. Synth. Coll. Vol.* **3**, 363 (1955)
- 38) Lofod H., *J. Org. Synth., Coll. Vol.* **4** (1963)
- 39) Speziale J., *J. Org. Synth., Coll. Vol.* **4**, 401 (1963)
- 40) a) Saito H., Tanaka Y., Nukada K., *J. Am. Chem. Soc.*, **93**, 1077 (1971)
 b) McLachlan R. D., Nyquist R. A., *Spectrochim. Acta*, **20**, 1397 (1964)
 c) Ollis W. D., Barton D. ed., "Comprehensive Organic Chemistry", Pergamon Press,

- 1-st ed., 1979, vol 2, p. 997 and references contained therein.
- 41) a) Schiessler R. W., Flitter D., *J. Am. Chem. Soc.*, **74**, 1720 (1952)
 b) Fieser L. F., *J. Chem. Ed.*, **42**, 408 (1965)
 - 42) Knight H. B., Wittnauer L. P., Coleman J. E., Noble W. R. Jr., Swern D., *Anal Chem.*, **24**, 1331 (1952)
 - 43) a) Fieser L. F., Fieser M, Reagents for Organic Synthesis, vol 1. J. Willey & Sons, 1967, p.1262
 b) Schlenk H., Hollmann R. T., *J. Am. Chem. Soc.*, **72**, 5001 (1950)
 c) Linstead R. P., Whalley M., *J. Chem. Soc.*, 2987 (1950)
 - 44) Homer R. B., Johnson C. D. in "The Chemistry of Amides", J. Zabicky ed., Interscience, London, 1970, chap. 2.
 - 45) a) Levine R., Karten M. J., *J. Org. Chem.*, **41**, 1176 (1976)
 b) Wakefield B. J.: The Chemistry of Organic Lithium Compounds, Pergamon Press, Oxford, 1974
 - 46) Buist P. H., Dallmann H., Seigel P. M., unpublished results
 - 47) Oae S., Organic Chemistry of Sulphur, Plenum Press, London, 1977, p. 385 and references cited therein.
 - 48) Curci R., Edwards J. D., Modena G.: Hydrogen-Bonded Solvent Systems. Proc. Symp. (1968), 303: *Chem Abstr.* **71**, 489904r (1969)
 - 49) Gragerov I. P., Levit A. F., *Zh. Obshch. Khim.*, **33**, 544 (1963) *Chem. Abstr.*, **59**, 1506h (1963)
 - 50) Addison C. C., Sheldon J. C., *J. Am. Chem. Soc.*, 2705 (1956)
 - 51) Harville R., Reed S. F. Jr., *J. Org. Chem.*, **33**, 3976 (1968)
 - 52) Oae S., Ohnishi Y., Kozuka S., Tagaki W., *Bull. Chem. Soc. Jap.*, **39**, 364 (1966)
 - 53) Mioti U., Modena G., Sedeia L., *J. Chem. Soc.*, **88**, 3874, 5486 (1966)
 - 54) Ollis W. D., Barton D.: Comprehensive Org. Chemistry, Pergamon Press, 1977, vol. 3, p. 125
 - 55) Lambert J. B., Shurwell H. F., Verbit L., Cooks R. G., Stout G. H.: Organic Structural Analysis; Macmillan, London, 1976, p. 283.
 - 56) a) Bloch K., Schroepfner G. J., *J. Biol. Chem.*, **240**, 54 (1965)
 b) see also ref. 3.
 - 57) Strittmatter P., Enoch H., Catala A., *J. Biol. Chem.*, **251**, 5095 (1976)
 - 58) Rosazza J.P., Smith R. V., *Adv. Appl. Microbiol.*, **25**, 169 (1979)

- 59) a) Fukushima D., Kim Y. H., Iyanagi T., Oae S., *J. Biochem.*, **83**, 1019, (1978)
b) Waxman D. J., Light D. R., Walsh C., *Biochem.*, **21**, 2499 (1982)
- 60) a) Mulheim L. J., Ramm P. J., *Chem Soc. Rev.*, **27**, 259 (1972)
b) Fryberg M., Oehlschlager A. C., Unrau A. M., *J. Am. Chem. Soc.*, **95** (17) 5747 (1973)
- 61) Buist P. H.; personal communication.
- 62) Kishi M., Tori K., Komeno T., Shingu T., *Tetrahedron Letters*, 3525 (1971)
- 63) Pirkle W. H., Beare S. D., Muntz R. L., *Tetrahedron Letters*, 2295 (1974)
- 64) Kagan H. B., Desmukh M., Dunach E., Juge S., *Tetrahedron Letters*, 3467 (1984)
- 65) Kagan H. B., Pitchen P., Dunach E., Deshmukh M. N., *J. Am. Chem. Soc.* **106**, 9188 (1984)
- 66) a) Oae S.: *Organic Chemistry of Sulphur*; Plenum Press, London, 1977, p. 397
b) Mislow K., Miller E. G., Rayner D. R., Thomas H. T., *J. Am. Chem. Soc.*, **90**, 4861, (1968)
- 67) a) Andersen K. K., *J. Org. Chem.*, **29**, 1953 (1964)
b) Mislow K., Rayner D. R., Miller E. G., Bickart P. B., *J. Am. Chem. Soc.*, **88**, 3138 (1966)
- 68) Pirkle W. H., Hoover D. J., *Top. Stereochem.*, **13**, 263 - 331 (1982)
- 69) a) Rottendorf H., Sternhell S., *Tetrahedron Letters*, 1289 (1963)
b) Rottendorf H., Sternhell S., *Aust. J. Chem.*, **17**, 1315 (1964)
- 70) Hendry L. B., Korzeniowski D. M., Hindenlang D. M., Kosarych Z., Mumma R. O., Jugovich J., *J. Chem. Ecol.* **1**, 317 (1975)
- 71) a) Augustine R. L.: *Oxidation, Techniques and Applications in Organic Synthesis*. Marcel Dekker Inc., New York 1969, vol 1, p. 61
b) Djerassi C., Engle R. R., Bowers A., *J. Org. Chem.*, **21**, 1547 (1956)
- 72) Buist P. H.; personal communication.
- 73) McMurry T. J. and Groves J. T. in *Cytochrome P₄₅₀ - Structure, Mechanism and Biochemistry*, ed. P. R. Ortiz de Montellano, Plenum Press, N.Y., 1986, p. 1.

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