

Biotransformation of Aromatic Hydrocarbons
and
Organic Sulphides
by
Fungi

by

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Abstract

Toluene is converted to benzyl alcohol by the fungi Mortierella isabellina and Helminthosporium species; in the latter case, the product is further metabolized. Toluene- α - d_1 , toluene- α, α - d_2 , and toluene- α, α, α - d_3 have been used with Mortierella isabellina in a series of experiments to determine both primary and secondary deuterium kinetic isotope effects for the enzymic benzylic hydroxylation reaction. The values obtained, intermolecular primary $k_H/k_D =$ intramolecular primary $k_H/k_D = 1.02 \pm 0.05$, and secondary $k_H/k_D = 1.37 \pm 0.05$, suggest a mechanism for the reaction involving benzylic proton removal from a radical intermediate in a non-symmetrical transition state.

^2H NMR (30.7 MHz) studies using ethylbenzene-1,1- d_2 , 3'-fluoroethylbenzene-1,1- d_2 , 4'-fluoroethylbenzene-1,1- d_2 , and toluene- d_8 as substrates with Mortierella isabellina suggest, based on the observable differences in rates of conversion between the substrates, that the hydroxylation of hydrocarbons at the benzylic position proceeds via a one electron abstraction from the aromatic ring, giving a radical cation.

A series of 1,3-oxathiolanes (eight) were incubated with Mortierella isabellina, Helminthosporium, Rhizopus arrhizus, and Aspergillus niger. Sulphoxides were obtained from Mortierella isabellina and Rhizopus arrhizus using the substrates 2-phenyl-, 2-methyl-2-phenyl-, and 2-phenyl-2-tert.

butyl-1,3-oxathiolane. The relative stereochemistry of 2-methyl-2-phenyl-1,3-oxathiolan-1-oxide was assigned based on ^1H decoupling, n.o.e, and ^1H NMR experiments. The ^1H NMR (200 MHz) of the methylene protons of 2-methyl-2-phenyl-1,3-oxathiolan-1-oxide was used as a diagnostic standard in assigning the relative stereochemistry of 2-phenyl-1,3-oxathiolan-1-oxide and 2-phenyl-2-tert-butyl-1,3-oxathiolan-1-oxide. The sulphoxides obtained were consistent with an oxidation occurring from the opposite side of the molecule to the phenyl substituent.

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To my parents and Juanita
thank you for
all the
love, never ending support,
and
encouragement
throughout the years.

Introduction

Cytochrome P-450 dependent hydroxylase enzymes

The oxygenases are a general class of enzymes capable of introducing molecular oxygen into organic compounds. Those enzymes that incorporate both atoms of molecular dioxygen into a compound are specifically referred to as dioxygenases, whereas monooxygenases are classified as enzymes that introduce one atom of molecular dioxygen with reduction of the second atom to water¹ (equation one).



Cytochrome P-450 is one of a number of cofactors that belong to this latter class of enzymes, the monooxygenases.

It has been nearly thirty years since Williams first detected a strong absorption at 450 nm when he bubbled carbon monoxide through a dithionite-reduced microsomal suspension². In light of this discovery, a co-worker Klingenberg³ attempted to determine the nature of this newly discovered microsomal pigment. However, it was not until 1962 that Omura and Sato proposed the name P-450 indicative of a pigment with an absorption at 450 nm⁴.

Although the enzyme was first described in mammalian liver, later investigations have detected its presence in the kidney, small intestines, lungs, skin, as well as other mammalian tissues⁵. However, cytochrome P-450 is not totally restricted to mammalian systems. It has been

reported in invertebrates⁶ such as the housefly and Drosophila . In higher plants, the cytochrome has been detected during studies of the pathways involved in specialized metabolisms such as the biosynthesis of phytochromes, lignins, and alkaloids. The cytochrome is known to be active in bacterial systems of Pseudomonas putida grown on camphor as a carbon source⁷, and in the yeast Saccharomyces cerevisiae⁸.

In the mammalian system, the cytochrome is involved in the steroid 21-hydroxylation⁹: the biosynthesis of cholesterol and ergosterol from acetate and mevalonate, respectively¹⁰. Furthermore, it has been established that a number of lipophilic xenobiotics including drugs, insecticides, carcinogens, food additives, and environmental pollutants are oxidatively converted to more polar compounds by cytochrome P-450¹¹. As a result of this oxidative conversion the xenobiotics can lose their toxic characteristics. Thus, it is thought that cytochrome P-450's are a device elaborated by organisms to effect detoxification.

Sometimes xenobiotics are converted by the cytochrome into potentially more toxic metabolites. For example, the route of detoxification for a halogenated benzene would be to convert this relatively non-polar substrate into a halogenated phenol that is polar and hence more readily excreted. This halogenated benzene is converted to a phenol via an arene oxide, a molecule that is known to bond

covalently to cellular macromolecules and cause cellular damage^{12,13}. Moreover, polycyclic hydrocarbons are converted to proximate and true carcinogens via the same pathway. The ability of cytochrome P-450 to metabolize compounds and turn them into less toxic counterparts, most of the time, is one very important reason why cytochrome P-450's are being studied so actively in the field of metabolism.

Cytochrome P-450 dependent monooxygenases can be divided into two distinct parts: a cofactor protoheme prosthetic group and a polypeptide (apoenzyme). The function of the polypeptide portion of the enzyme is to bind the substrate. It is from here, the polypeptide, that the substrate regio- and stereo-specificity of reaction is controlled¹⁴. Variations in the polypeptide portion of the enzyme gives rise to a number of different types of cytochrome P-450¹⁵. This would explain why cytochrome P-450 is capable of catalyzing various reactions ranging from oxidative degradation of aliphatic and aromatic hydrocarbons to drug detoxification.

The role of the cofactor is to bind molecular oxygen, activate, and deliver the oxygen to the substrate. The cofactor is a quadridentate chelate complex between iron and a protoporphyrin (figure one). The equatorial ligand positions about the iron centre are occupied by the four nitrogens of the protoporphyrin complex. Of the two axial positions about the iron centre, one is known to be occupied

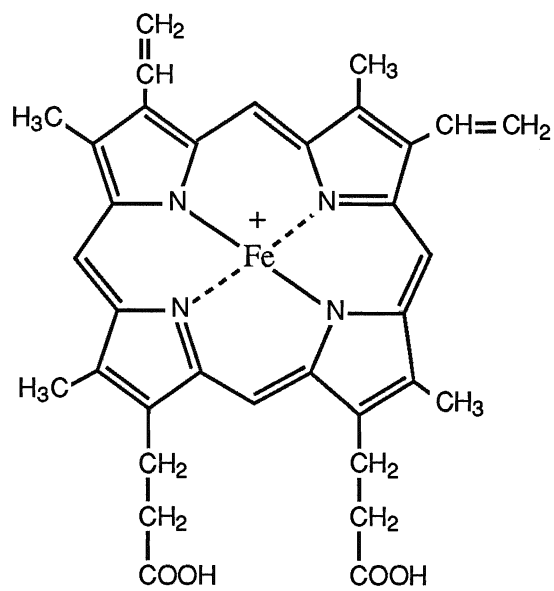


Figure One: Cytochrome P-450

by a cysteine residue of the apoenzyme¹⁶. However, the nature of the ligand in the other axial position has not been fully determined. It has been speculated, based on comparative epr and electron spin studies¹⁷, that this second axial position could be occupied by the imidazole nitrogen of a histidine residue. It is this unidentified portion that is displaced by oxygen during the catalytic cycle.

The catalytic cycle of cytochrome P-450 (figure two) was largely deduced from studies pertaining to the camphor hydroxylase of P. putida¹⁸. The cycle begins with ferric iron (Fe(III)) in low spin configuration¹⁹. Binding of the substrate(s) to the enzyme, the next step, causes the ferric iron (Fe(III)) to move from a low spin to a high spin electron configuration²⁰.

It is this change in electron configuration that results in the displacement of the unidentified ligand. Since the binding of the substrate seems to be directly responsible for the transition in spin states, as well as a change in the redox potential²¹, it appears that the binding site of the apoenzyme must be in close proximity to the cofactor. Next the ferric iron (Fe(III)) is reduced by one electron to the ferrous iron (Fe(II)).

Oxidation of Krebs cycle intermediates give rise to the electron that is necessary for the reduction²². This electron is delivered to the substrate-bound ferric enzyme through a complex electron transport system (figure three).

FIGURE TWO: The Catalytic Cycle of Cytochrome P-450

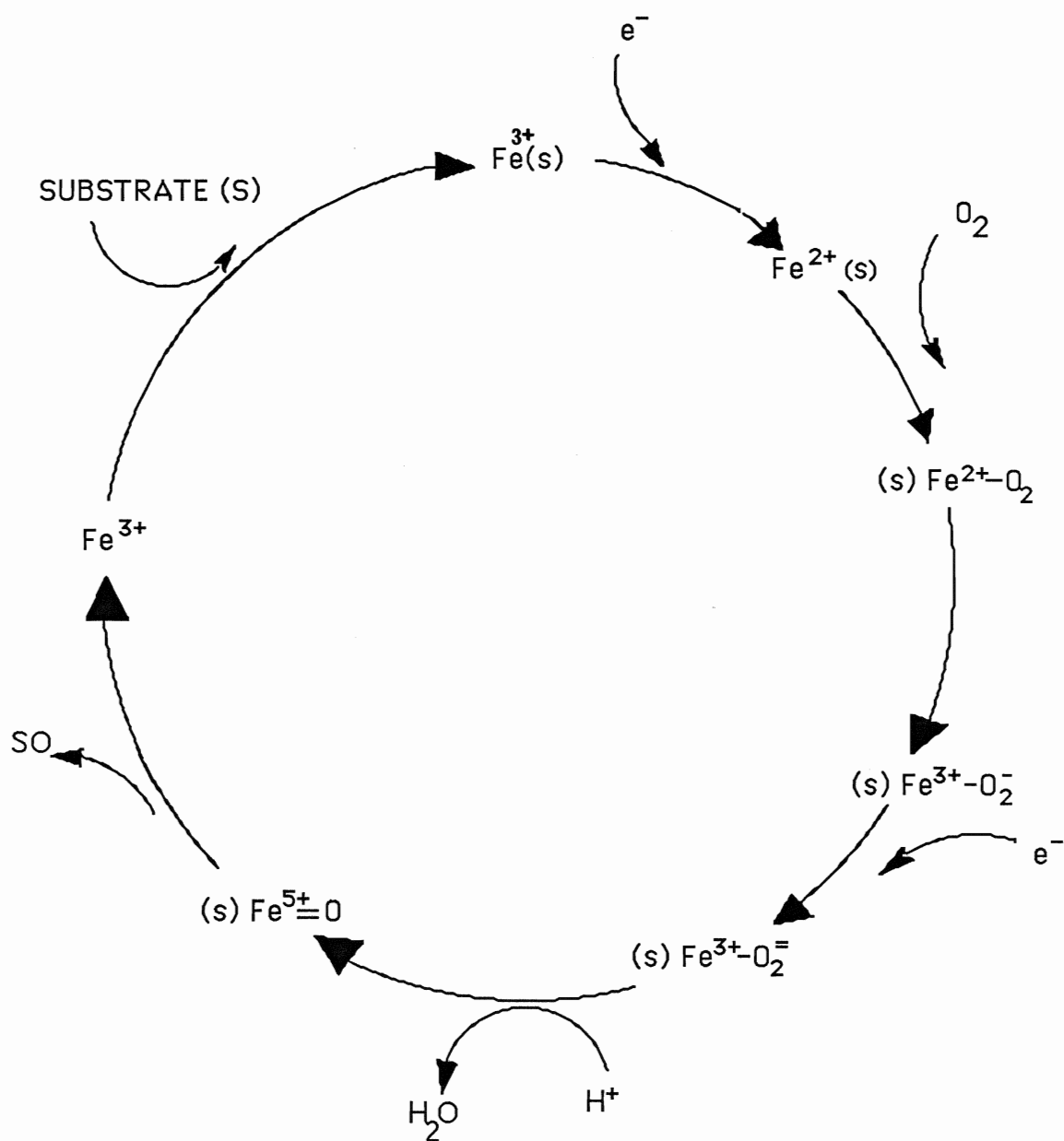
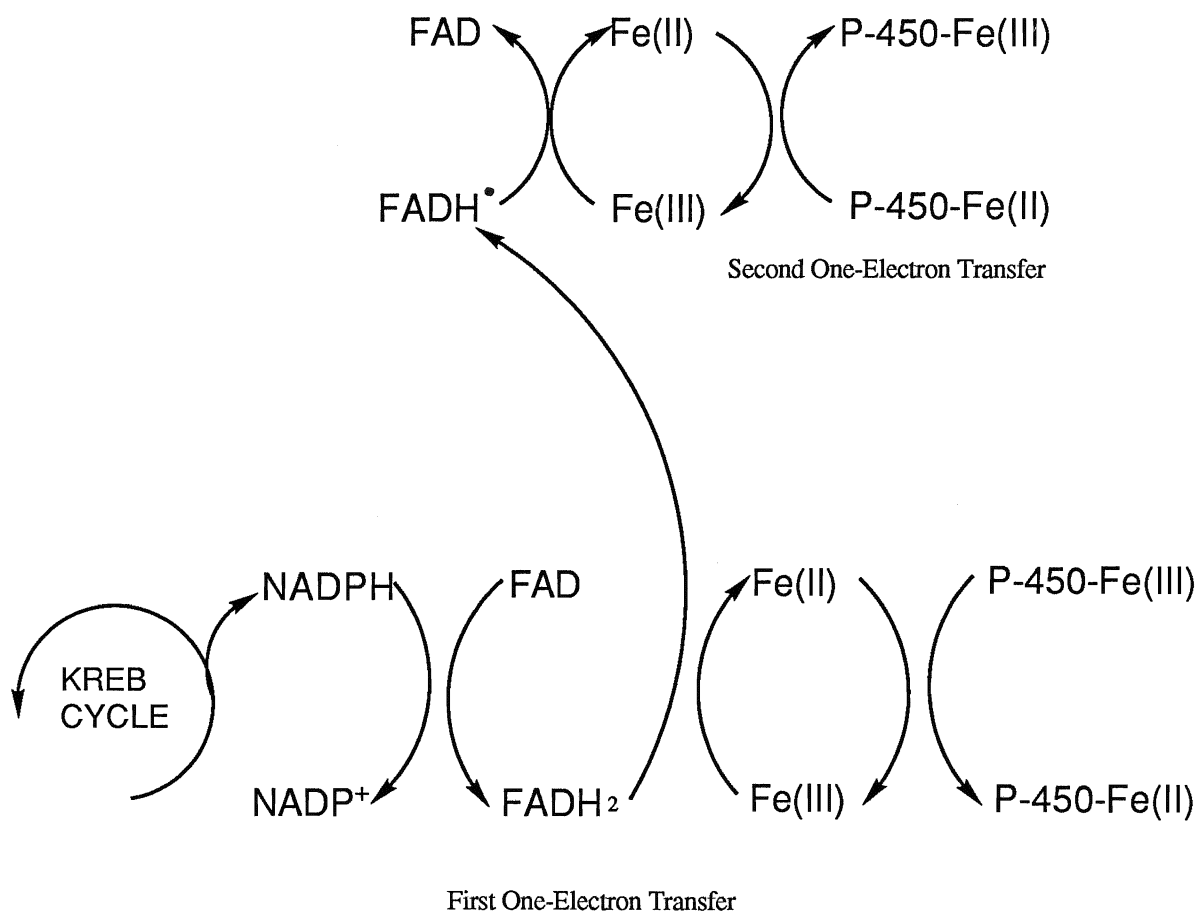
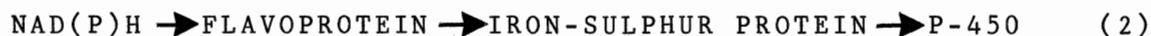


FIGURE THREE: Reduction of Cytochrome P-450



The Krebs cycle provides the first cofactor, NADPH. However, NADPH is not capable of reducing cytochrome P-450 since NADPH can only carry out its reduction with the loss of a hydride representing a direct two electron reduction. As previously mentioned, a one electron reduction is required. In order to accomplish this one electron reduction, NADPH transfers its two electrons to a flavin cofactor, FAD, to give rise to FADH_2 (figure four). Although FAD has accepted two electrons, unlike NADPH it is capable of transferring one electron at a time. The transfer of this one electron from FADH_2 to a ferredoxin, leaves the flavin cofactor in the form of FADH^\cdot which is utilized in the later second electron transfer.

The iron-sulphur centres of the ferredoxin proceed to reduce ferric iron (Fe(III)) to ferrous iron (Fe(II)) (equation two).



In higher organisms, such as animals cytochrome b_2 rather than ferredoxins are part of the electron shuttle system²³ (equation three).

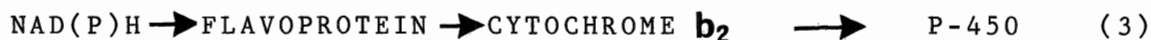
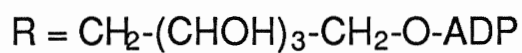
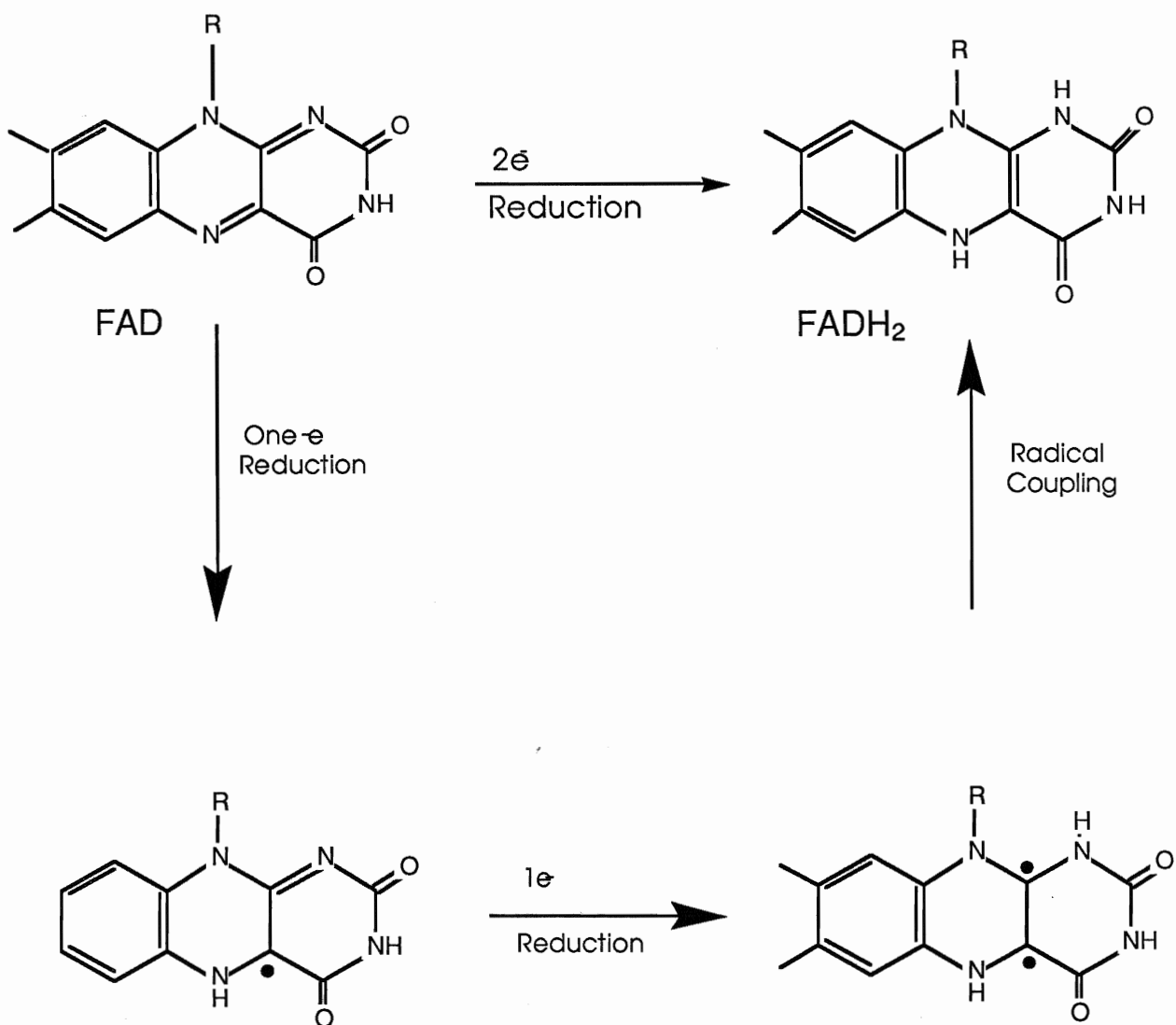


FIGURE FOUR: Electron Transfer between NADPH & FAD

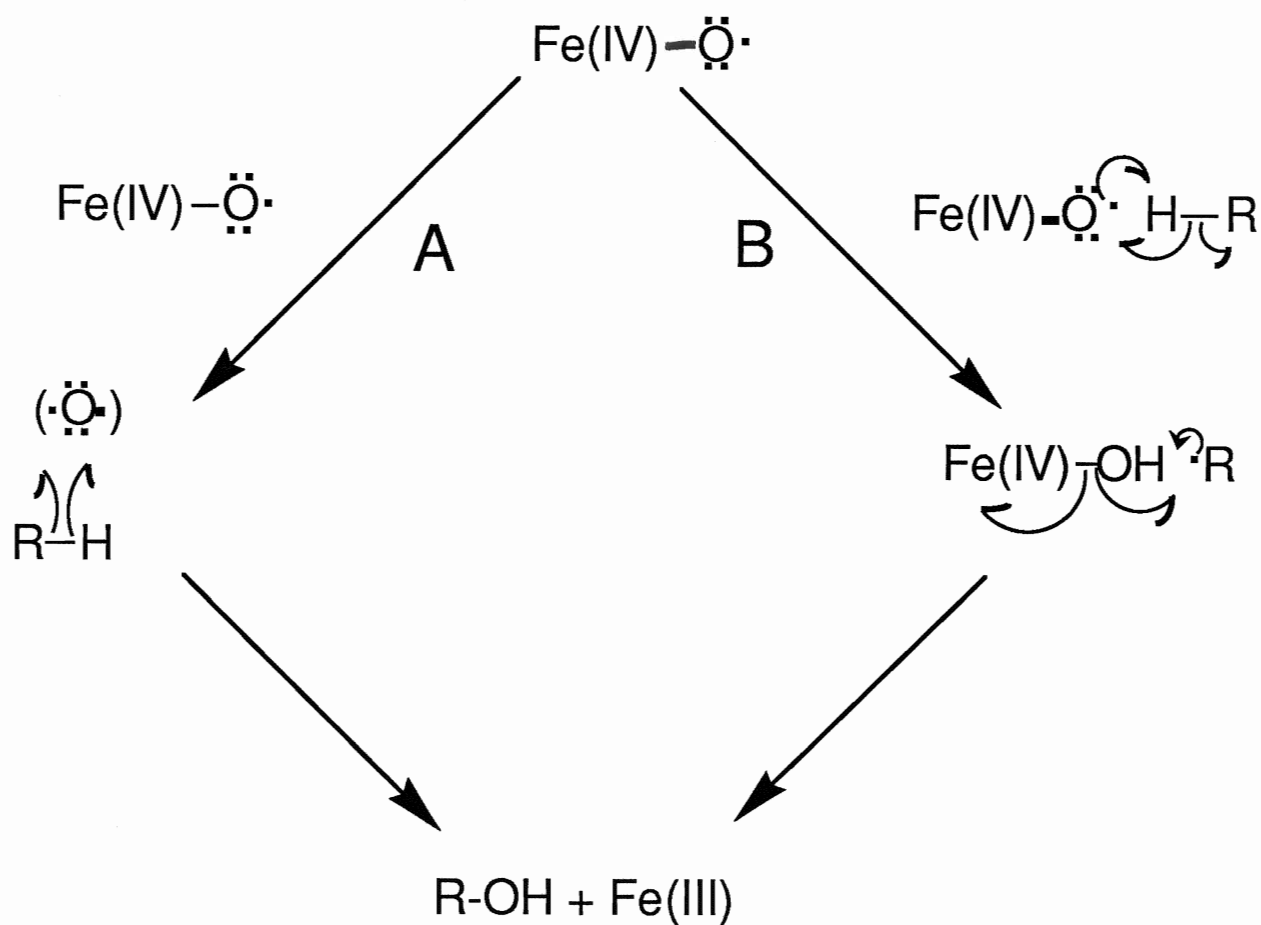
In this reduced state the cytochrome substrate complex can readily bind with oxygen to yield the six coordinate ferrous oxy-complex, $(S) Fe^{+2}-O_2$. The presence of such an oxygenated intermediate in the catalytic cycle has been substantiated by researchers²⁴. The ferrous iron (Fe(II)) then transfers an electron to the ferric-oxy complex, $(S) Fe^{+3}-O_2^-$. A second one electron reduction from the remaining flavin cofactor, $FADH^{\cdot}$, generates the ferric-peroxo complex, $(S) Fe^{+3}-O=2$. Finally, the loss of water associated with a heterolytic cleavage of the peroxide bond generates the ferryl oxygen formally²⁵ $(S) Fe^{+5}=O$. This species is quite reactive in order to hydroxylate unreactive carbon bonds. The final step of the catalytic cycle requires the introduction of this activated oxygen species into the bound substrate.

Two possible pathways for the introduction of the activated oxygen into an unreactive C-H bond have been proposed (figure five).

- A) direct insertion (path A) or
- B) hydrogen abstraction=recombination (path B).

Until very recently, the direct insertion pathway (pathway A) had been the favoured of the two mechanisms. Evidence favouring such a mechanism has arisen from comparative studies of known chemical reactions with those reactions catalyzed by the cytochrome.

FIGURE FIVE: Possible Routes for Hydroxylation at Saturated Carbon

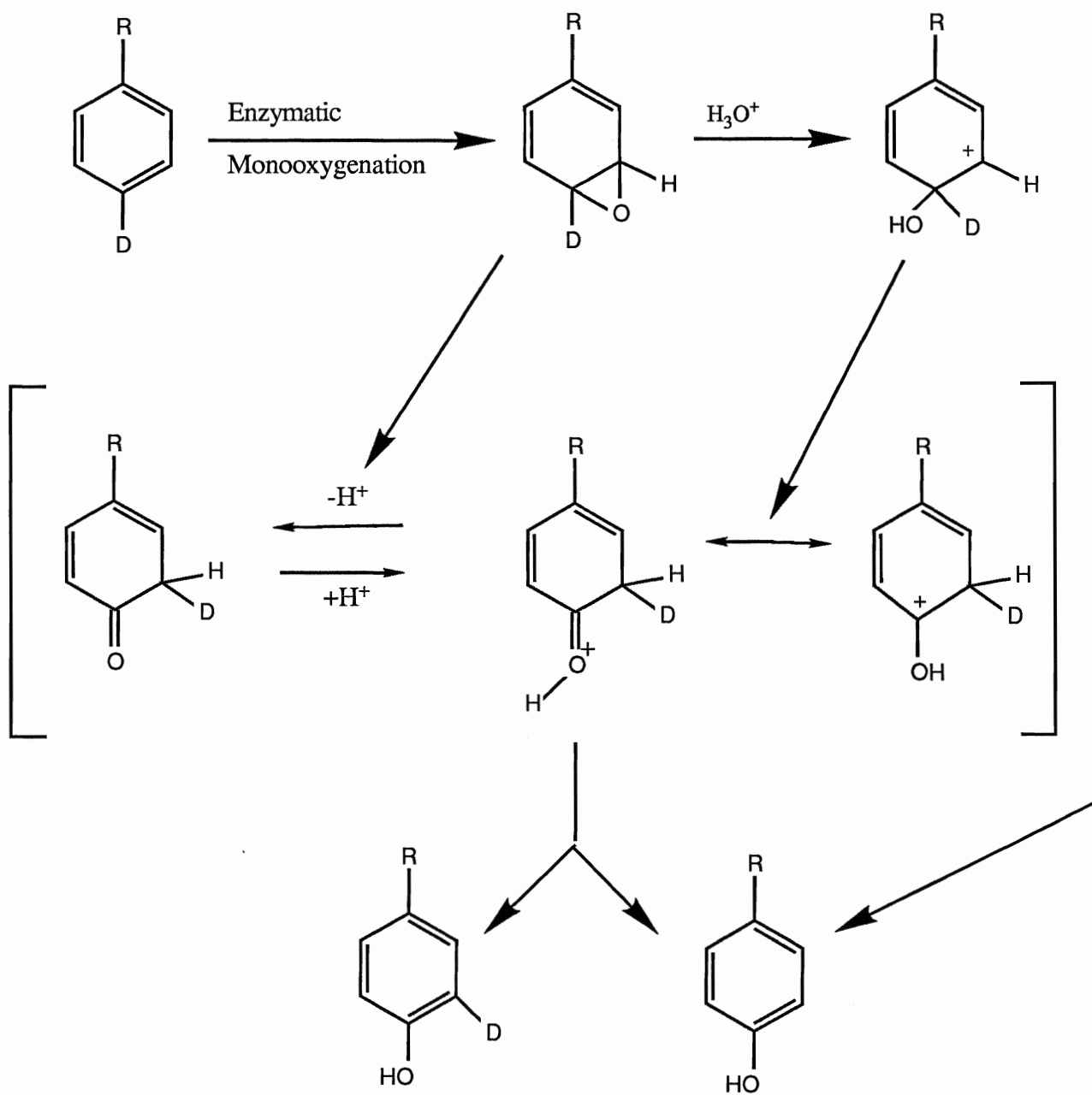


The six-electron oxene species, observed in path A, is isoelectronic with carbene and nitrene. Insertion and addition reactions by carbene and nitrene are well established. Thus, the oxene species involved in the enzymic hydroxylation according to the above analogy should react via a similar insertion mechanism²⁶.

Another chemical analogy in support of the insertion mechanism is based on the observation that hydroxylation and epoxidation reactions for the enzyme are catalyzed by the same cytochrome²⁷. This observation suggests that the mechanism for enzymic epoxidation and hydroxylation may be similar. Since the epoxidation reaction of peroxyacid with an alkene has been demonstrated to proceed via a concerted insertion mechanism²⁸, then this analogy could be extended to the mechanism involved in the epoxidation as well as the hydroxylation reactions catalyzed by cytochrome P-450.

The isolation of 1,2 naphthalene oxide, an intermediate from the incubation of naphthalene, with rat liver microsomes by Jerina et al.²⁹ indicates that an oxene addition to a carbon-carbon double bond had occurred assuming that this reaction is analogous to that of a carbene addition.

Studies pertaining to the hydroxylation of aromatic compounds by cytochrome P-450 have been shown to proceed via an NIH shift³⁰. In an NIH shift the substituent on the para position migrates to the meta position during the course of the hydroxylation (figure six). Since

FIGURE SIX:NIH Shift Mechanism for Hydroxylation
of an Aromatic Compound by Cytochrome P-450

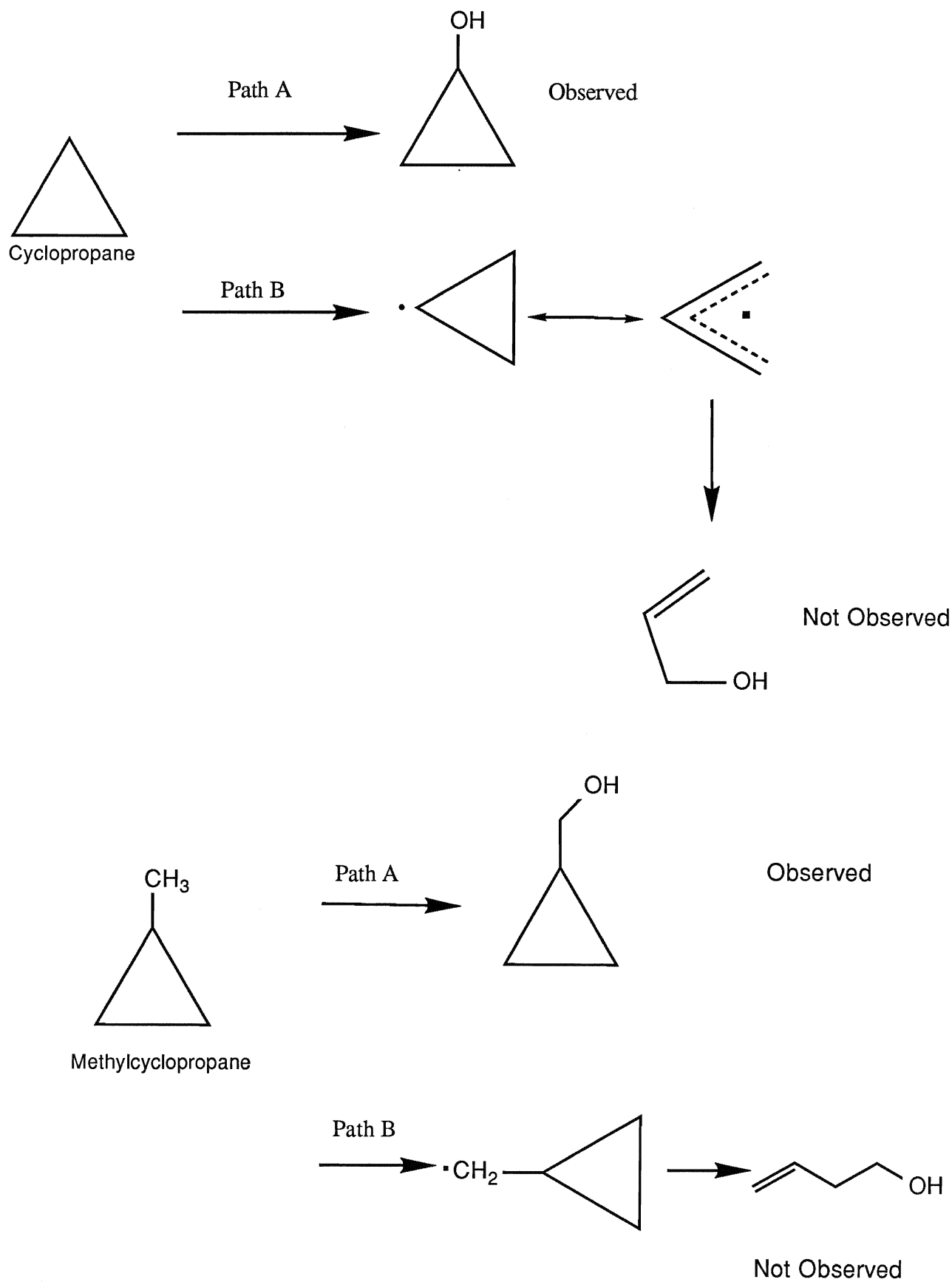
rearrangements similar to the migration described above have been observed in non-enzymatic isomerization of arene oxides, it has been proposed that an intermediate arene 3,4 oxide could be involved in such a hydroxylation³¹. It is also possible that an intermediate radical could rearrange to give an NIH shift, but there is no direct evidence supporting such a radical rearrangement.

Furthermore, hydroxylation reactions of steroids catalyzed by cytochrome P-450 are known to proceed with retention of configuration at the carbon involved in the hydroxylation³². According to the proponents of an insertion mechanism, if the hydroxylation reaction was to proceed through a radical-abstraction recombination mechanism, some degree of epimerization and/or rearrangement may have been observed.

Epimerization and rearrangement products were not observed in cytochrome P-450 oxidation of cyclopropane and methylcyclopropane³⁰. The only products observed were cyclopropanol and cyclopropylmethanol, respectively. If a radical intermediate had existed, then allyl alcohol as well as but-3-en-1-ol should have been observed along with cyclopropanol and cyclopropylmethanol (figure seven).

Intermolecular isotope effects obtained for steroid hydroxylation at C-6 β , C-7 α , and C-11 α and also for non-steroid substrates are low ($k_H/k_D < 2$) and tend to lend support to a direct insertion mechanism by analogy with kinetic isotope effect values observed for carbene

FIGURE SEVEN: Oxidation of Cyclopropane and Methylcyclopropane by P-450



insertions^{33,34}.

However, evidence obtained from recent studies favours a hydroxylation mechanism that proceeds via a carbon centred free radical. The earliest of these studies, an investigation into the metabolism of the insecticide dieldrin in mammalian systems, gave a bridged metabolite as one of a number of metabolic products³⁵. The formation of a bridged metabolite was explained by a mechanism that required a transannular reaction of a radical intermediate (figure eight).

Recent studies using the fungus Mortierella isabellina NRRL 1757 with ethylbenzene and its deuterated analogue ethylbenzene-1,1-d₂ as substrates, afforded kinetic isotope values which favoured a radical abstraction mechanism³⁶ (figure nine). Holland et al. observed an intermolecular value of 1.5 ± 0.1 which is typical of intermolecular studies. Since the intermolecular isotope effect is greater than one, the results tend to support a radical-recombination mechanism. However, since this value is not much greater than one and the certainty of the rate-determining step was questionable, the results are inconclusive. The intramolecular investigation, however, gave a large kinetic isotope effect of 4.6 ± 0.2 . This latter isotope effect of 4.6, according to the authors, is the true isotope effect and lends additional evidence towards a radical-recombination mechanism.

Further evidence supporting a radical-recombination

FIGURE EIGHT: Mechanism for the P-450 Mediated Oxidation of Dieldrin

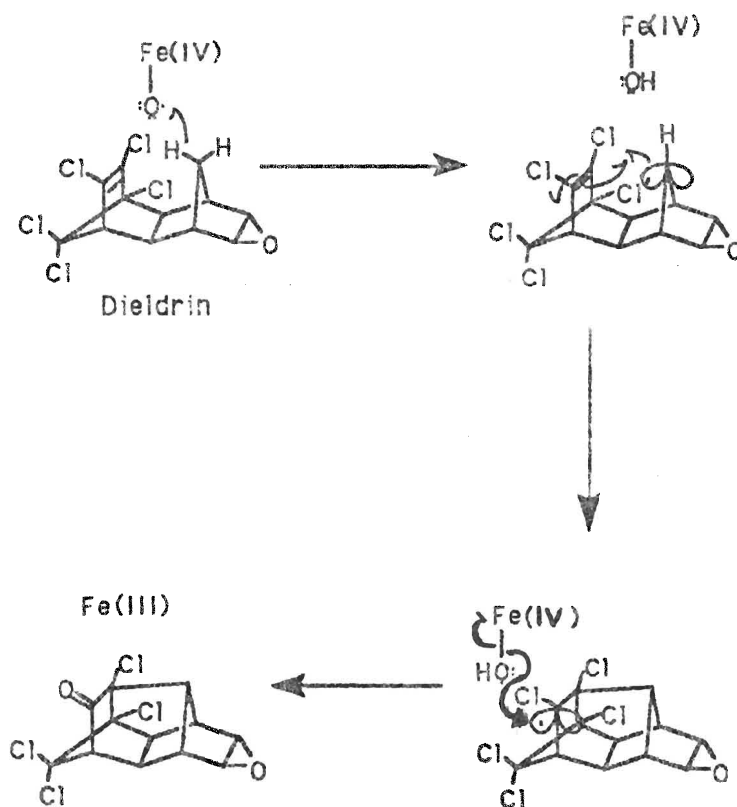
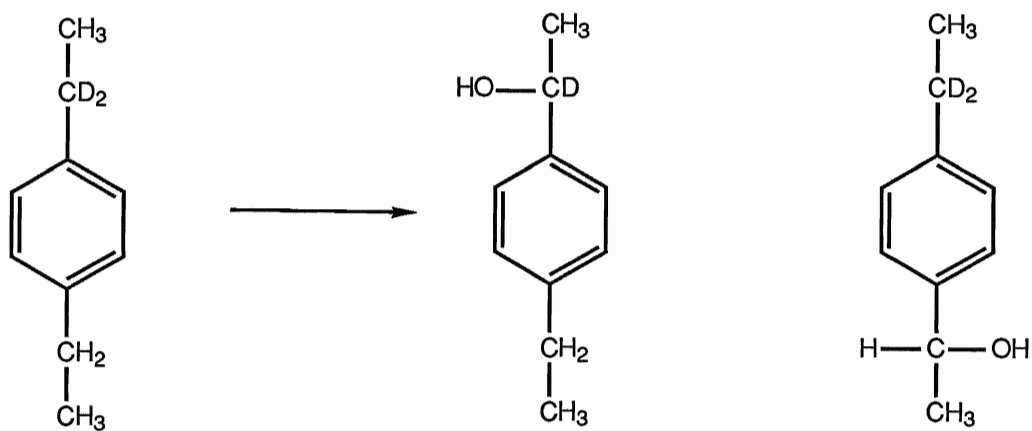
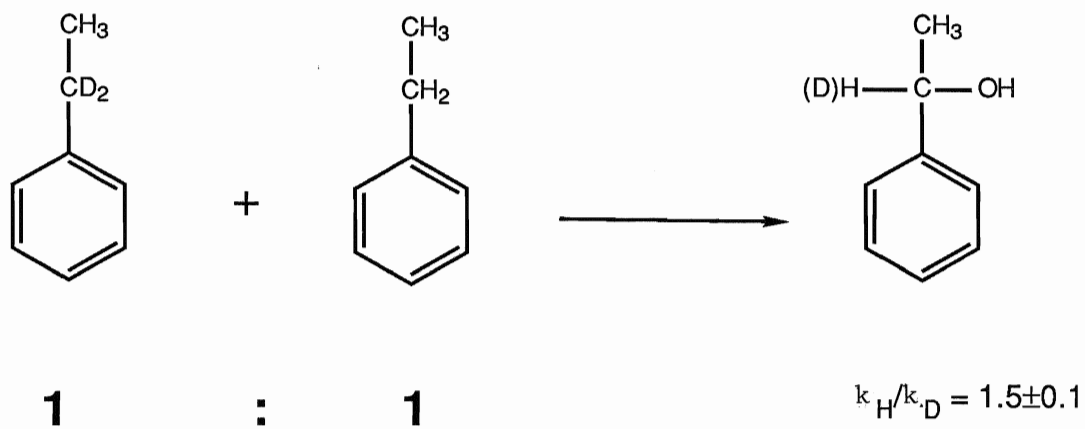


FIGURE NINE: The Hydroxylation of ethylbenzene with *Mortierella isabellina* NRRL 1757

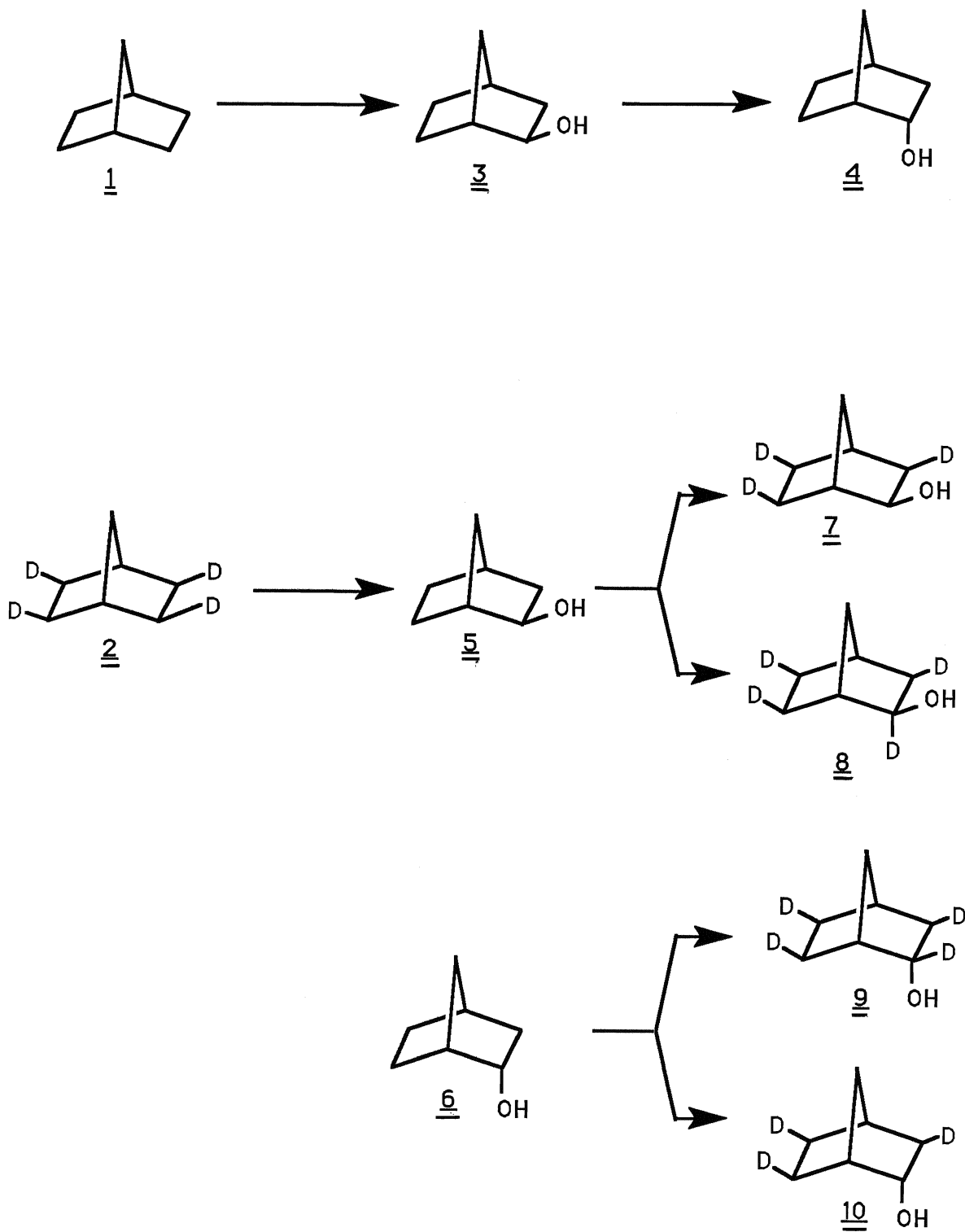


$$k_{\text{H}}/k_{\text{D}} = 4.6 \pm 0.2$$

mechanism arises from the study of the hydroxylation of norbornane (1), and the corresponding deuterated norbornane, exo,exo,exo,exo-2,3,5,6-tetradeutero-norbornane (2), by Groves and McClusky³⁷ (figure ten). The hydroxylation of norbornane (1) by reconstituted liver microsomes yielded exo- and endo-2-norborneol (3 and 4 , respectively) as products in the ratio of 3.4:1. Hydroxylation of the substrate, 2 , also afforded exo- and endo-2-norborneol(5 and 6 , respectively). However, the ratio of exo to endo products was 0.76:1. The difference in stereoisomer ratio with deuterium substitution contrary to other investigations is best interpreted as a result of a significant kinetic isotope effect as well as some degree of intrinsic stereospecificity. Analysis of the deuterium content of the exo- and endo-2-norborneol products (5 and 6) from 2 by mass spectrometry indicated that the deuterium content of the exo alcohol (5) was 75% d₃ (7) and 25% d₄ (8), and the deuterium content of the endo alcohol (6) was 91% d₄ (9) and 9% d₃ (10). This result indicates that a certain degree of epimerization at the hydroxylated carbon was observed. In order for epimerization to occur, a free valence or carbonium ion would have had to exist at the carbon undergoing hydroxylation. The d₄ exo alcohol (8) resulted from an 18% endo→exo inversion, whereas the d₃ endo alcohol (10) had arisen from an 14% exo→endo crossover.

Correcting for stereochemical crossover, the isotope

FIGURE TEN: P-450 Catalyzed Hydroxylation of **Norbornane**



effect was determined to be 11.5 ± 1 . This value contradicts previously reported kinetic isotope values ($k_H/k_D < 2$). However, it is consistent with reported intramolecular isotope effects for similar microsomal systems. A large observable isotope effect and a noticeable degree of epimerization provides convincing evidence for a radical-abstraction recombination mechanism.

Inter- and intra-molecular isotope effects determined for the same substrate indicate that a noticeable difference exists between the two³⁸. The intramolecular effects were much larger than the reported intermolecular values. The observed intramolecular effects ($k_H/k_D > 5$) are consistent with a radical-abstraction mechanism but not with a direct insertion mechanism.

Theoretical calculations for the reaction of singlet carbene³⁹ and oxene⁴⁰ with a carbon-hydrogen bond indicate that the reaction is most likely to proceed via an attachment-rearrangement mechanism as opposed to a direct insertion mechanism. Similar calculations for triplet oxene also favour a radical abstraction mechanism. Thus, the formation of an oxene intermediate does not necessitate a direct insertion mechanism⁴⁰. However, since no information on the nature of the enzymic intermediate is currently available, the oxene intermediate could be consistent with either mechanism.

Holland et al. observed that the enzymic epoxidation of

the $\Delta^{4,6}$ -dien-3-ones by the fungus R. arrhizus ATCC 11145 proceeded with β stereochemistry⁴¹. However, the peracid oxidation of $\Delta^{4,6}$ -en-3-ones, which is an established concerted epoxidation, afforded products with α stereochemistry⁴². The observed epoxidation of $\Delta^{4,6}$ -dien-3-ones by R. arrhizus in the absence of any effects due to substrate binding cannot be attributed simply to enzyme specificity. Based on these results a stepwise oxidation (figure eleven) that is consistent with known features of the enzyme (figure five and twelve) and known steric and stereoelectronic properties was proposed. Their conclusions lend additional evidence towards a radical mechanism.

A mechanism involving a carbon centred free radical was proposed for the P-450 system of camphor dependent P. putida (P-450_{cam})⁴³. The study demonstrated that the hydroxylation of 5-endo and 5-exo-monodeuterated camphor (figure thirteen) gave a single product, 5-exo-hydroxycamphor. The hydroxylation of the monodeuterated camphors not only proceeded with a small loss of stereochemistry at carbon-5, but also with a large intramolecular isotope effect.

FIGURE ELEVEN: Mechanism of 6,7 β Epoxidation of Δ -3-ketosteroids by *R. arrhizus*

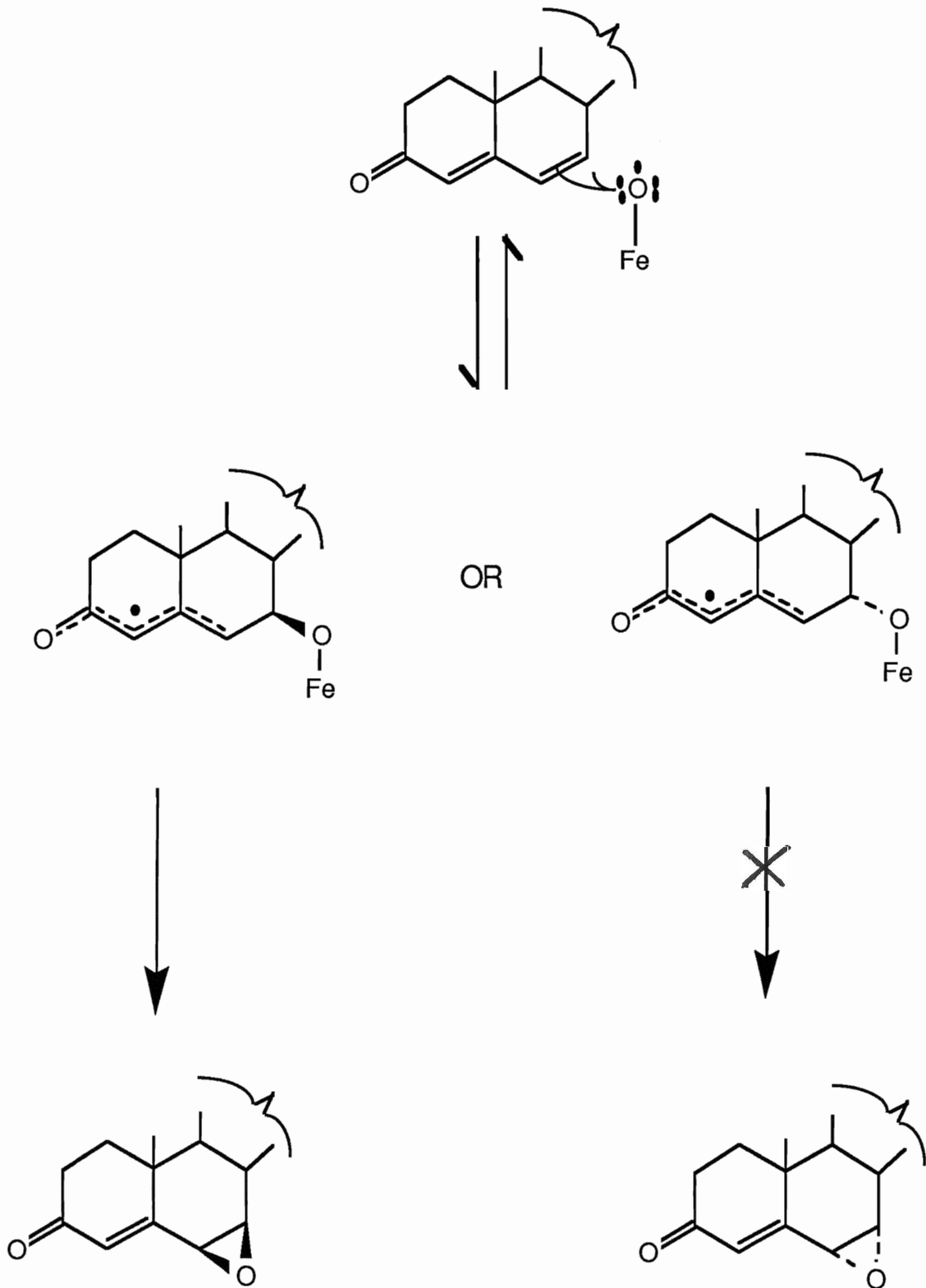


FIGURE TWELVE: Possible Routes for Epoxidation of Olefins by Cytochrome P-450 Dependent Monooxygenases

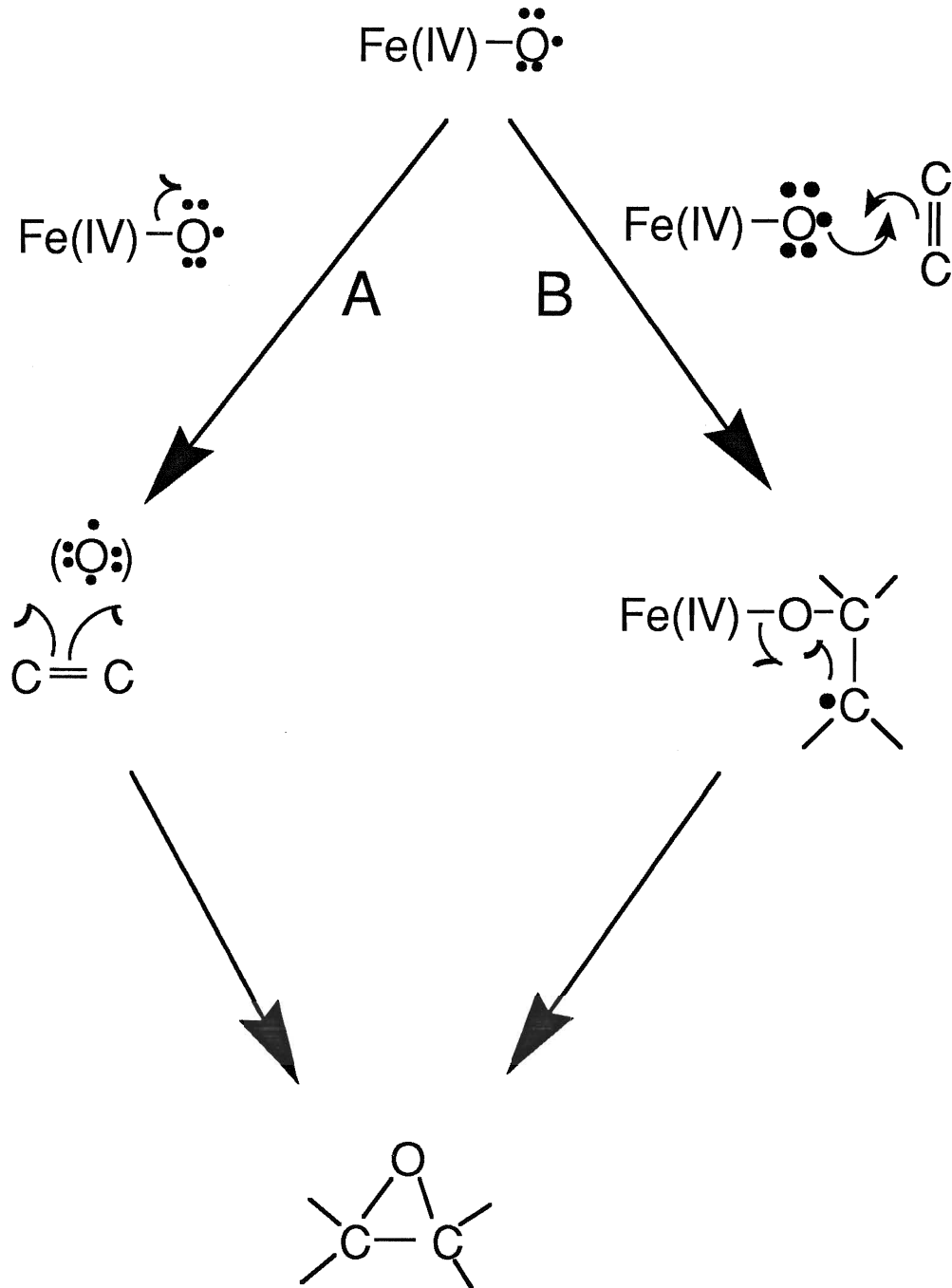
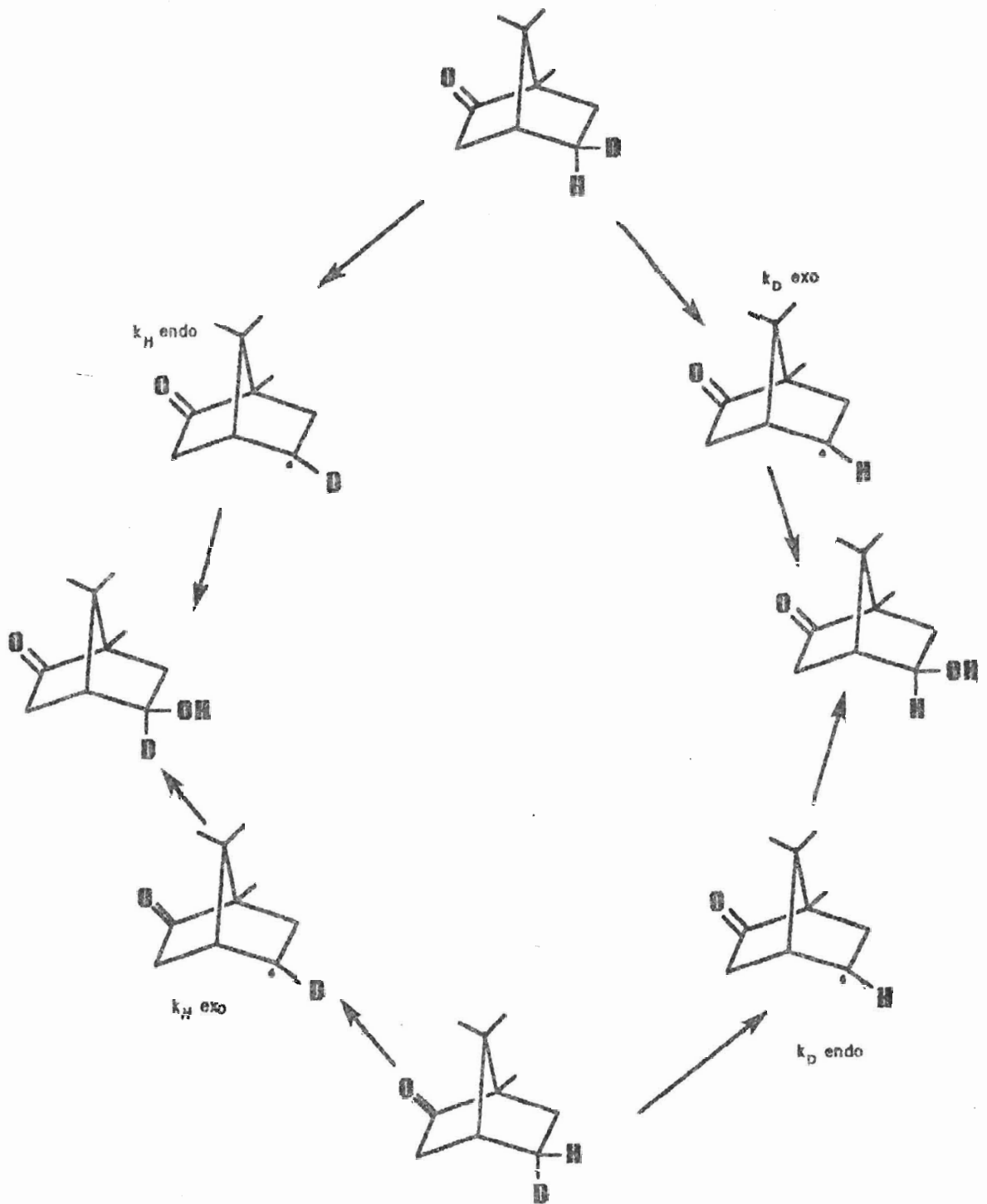


FIGURE THIRTEEN: Hydroxylation of Camphor

Cytochrome P-450 dependent sulphur enzymic oxidations

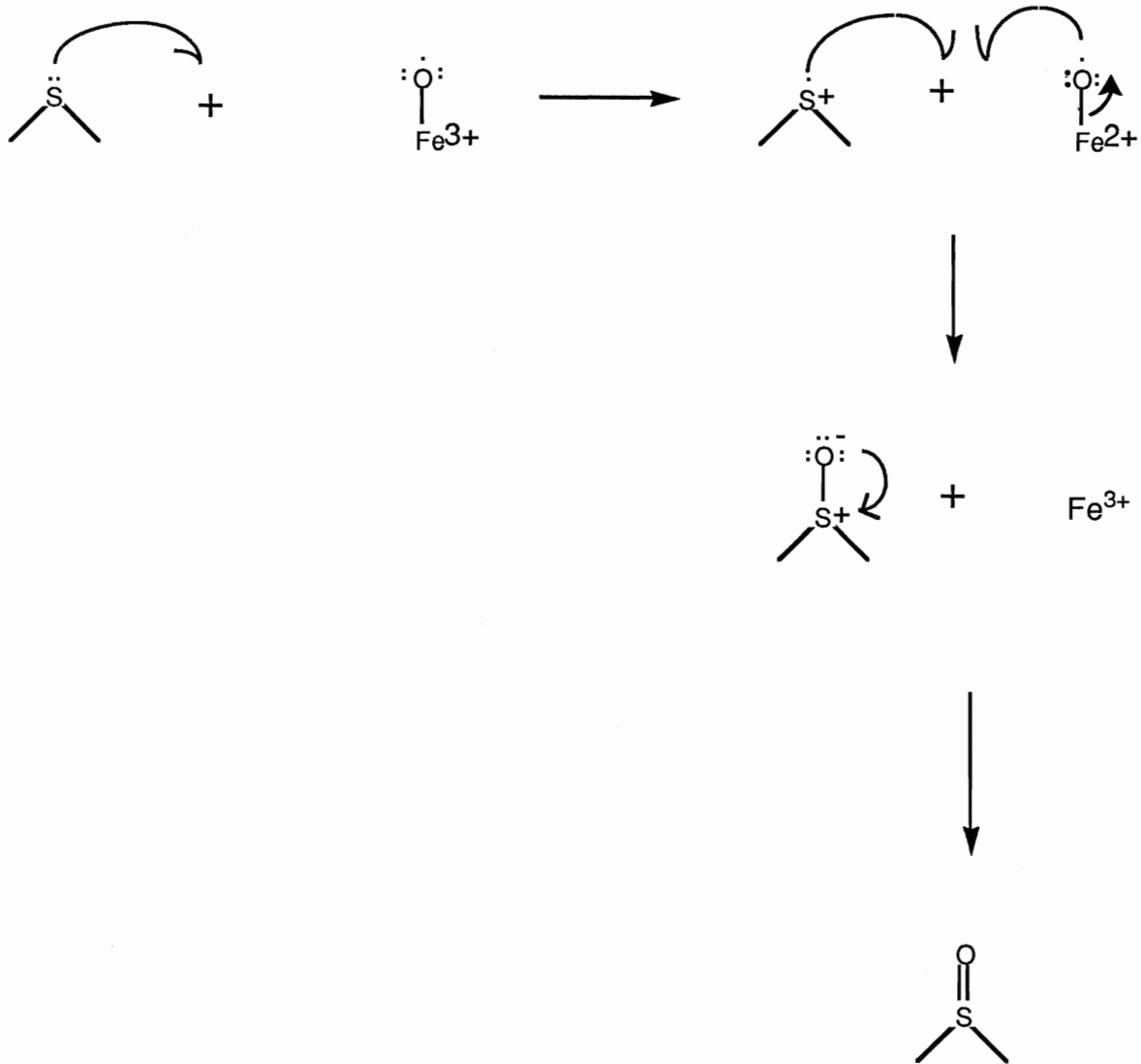
Recent studies involving cytochrome P-450 dependent mammalian and microbial sources have shown that these systems are capable of oxidizing sulphur. Sulphur enzymic oxidations involve the oxidation of a sulphide to the corresponding sulphoxide^{44,45,46}. Further oxidation of the sulphoxide to a sulphone has also been reported^{44,47}.

Kinetic investigations pertaining to sulphoxidation of phenyl methyl sulphides using rat liver microsomes and studies involving the chemical activities of sulphur radicals have resulted in the proposal of a mechanism for the enzymic oxidation. The rate-determining step in this mechanism involves an electron transfer from a divalent sulphide to the oxidized species to afford the sulphoxide (figure fourteen). The enzymic oxidation of substituted sulphoxides to sulphones is consistent with an oxidation that originates with a one electron transfer from sulphur to the iron-oxygen intermediate to give an activated oxenoid species that further reacts with the sulphoxide to yield the appropriate sulphone.

Abushanab et al have reported that the oxidation of methyl p-tolyl sulphide by Helminthosporium species NRRL 4671 gave a sulphoxide⁴⁸. The isolated sulphoxide was the S(-) enantiomer of 1-methyl-4-(methylsulphinyl)-benzene. More importantly, the S(-) enantiomer was obtained in a 50% yield with a reported optical purity of 100%. Further study with

FIGURE FOURTEEN:

Proposed mechanism for sulphoxidation by Cytochrome P-450.



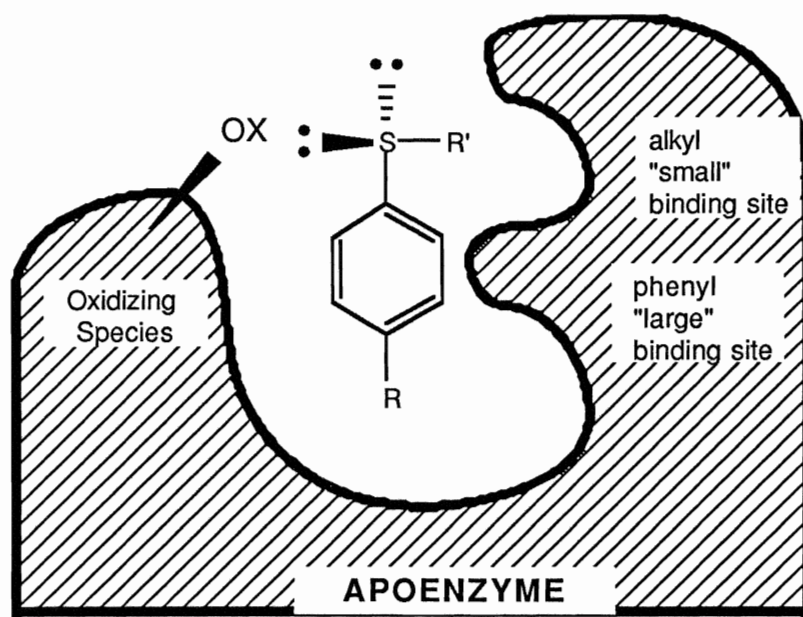
the same substrate, methyl p-tolyl sulphide, using the fungus Mortierella isabellina NRRL 1757 afforded R(+) 1-methyl-4-(methylsulphinyl)-benzene in 60% yield and with an observed optical purity of 100%.

In order for these oxidations to occur stereospecifically, the enzyme must be capable of distinguishing between the enantiotopic electron pairs of the pro-chiral sulphides. Such an enantiotopic selection could occur if the enzyme provides two different binding sites: one binding site for a large group and another for a small group. Moreover, the binding of the substrate to these two different sites should be stereospecific or non-reversible (figure fifteen). This model (figure fifteen) would then lend to asymmetric oxidation, if the oxidative functional species was fixed within the enzyme.

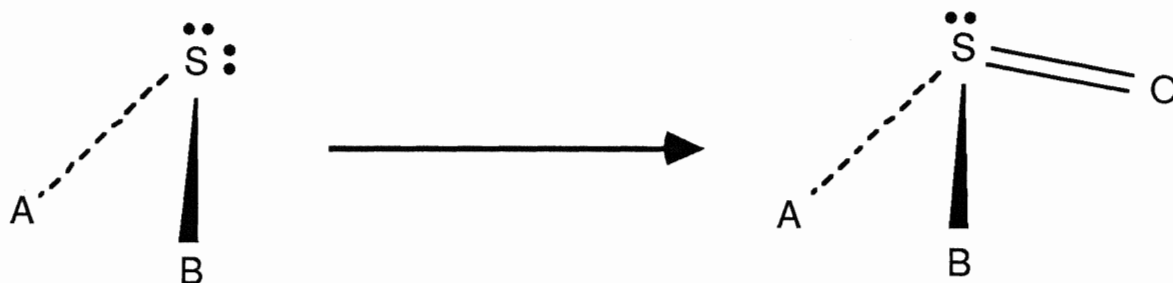
Auret et al. have investigated the stereoselectivity involved in the oxidation of sulphides to sulphoxides⁴⁴. In their studies they used the fungal species Aspergillus niger ATCC 18500. They observed that the stereoselectivity of the oxidation was depended on the structure of the sulphide. The observed optical purities of the sulphoxides indicated that the sulphides must orientate themselves in a stereospecific manner within the enzyme (figure sixteen). For the compounds studied, a p-tolyl group preferred position A, while a tert. butyl group preferred position B. The presence of both these groups, tert. butyl and p-tolyl, on the same substrate produced the corresponding sulphoxide with a high degree of

FIGURE FIFTEEN:

Model for the binding of alkyl phenyl sulphides to cytochrome P-450.

**FIGURE SIXTEEN:**

Preferred Direction of Oxidation of Thioethers in the presence of *Aspergillus niger*.



stereoselectivity. A definite trend in the stereoselectivity of the reaction was observed in the oxidation of alkyl p-tolyl sulphides.

Reverse binding experiments have been performed in an attempt to elucidate the exact nature of these two binding sites. Since the oxidation of methyl phenyl sulphide by Mortierella isabellina NRRL 1757 has been well documented⁴⁸, then a study involving variations in the alkyl substituents could prove to be quite informative. If there are indeed two distinctive binding sites, then alterations in either substituents could result in possible reverse binding. For example, increasing the size of the large group on the substrate should result in the preferential binding of the large group in the large binding site, therefore reducing the potential for reverse binding.

A more interesting approach which was undertaken by Holland et al. involved alkyl groups that were the same size or larger than the phenyl substituent⁴⁹. Comparing the optical purity of the resulting sulphoxides, it was observed that they were consistent and in the same direction as the methyl phenyl sulphoxide example. From these results the authors concluded that no reverse binding occurred. There is the suggestion, however, that one of the binding sites maybe independent of steric factors, but dependent on the phenyl substituent: one site binding the aromatic substituent while the other site binds the non-aromatic or alkyl substituent (figure fifteen).

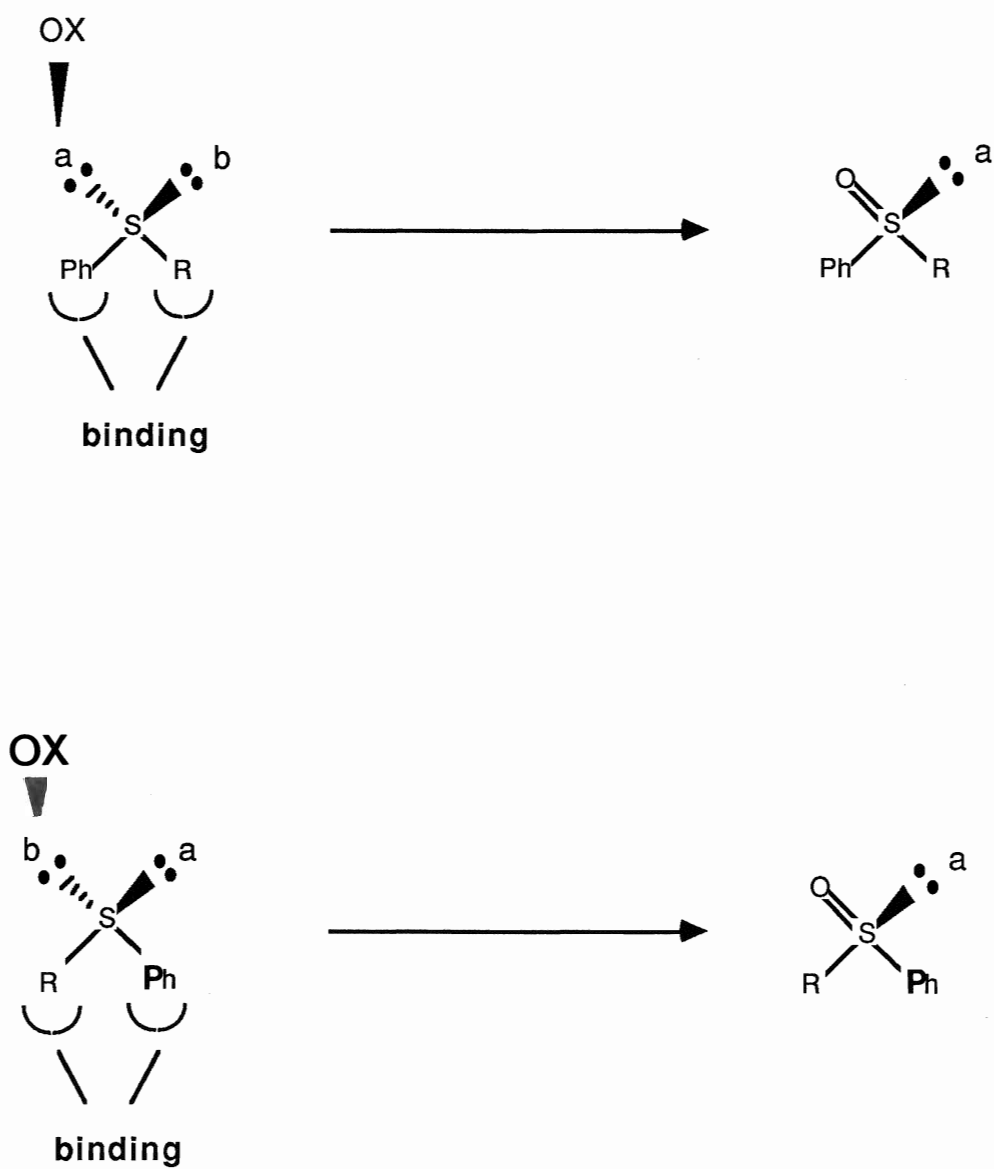
Another possibility may be that binding of the substrate may not be entirely rigid (figure seventeen). Evidence from sulphoxidation studies with liver microsomes using methyl, phenyl, and tert. butyl phenyl sulphides gave results which are consistent with a mechanism supportive of non-specific binding⁵⁰.

The results obtained from the sulphur oxidation of methyl p-tolyl sulphide using Helminthosporium species NRRL 4671 and Mortierella isabellina NRRL 1757, described previously⁴⁸, were favourable as each gave products that were reported to be either R(+) or S(-) and optically pure. From this point of view the nature of binding sites and the position of the oxidative species can be speculated with a certain degree of confidence.

However, as observed in most other cases the results have not yielded enantiomerically pure products. Rather, oxidation of sulphides to sulphoxides generally produces a mixture of both enantiomers. These results complicate the exact nature of the enzymic sulphur oxidation and the original model, figure fifteen, requires modification. Most of these modifications are concerned with the nature of binding with respect to the enzyme.

Another model not discussed is concerned with the oxidative species. In this model the substrate binds stereospecifically with no possibility to rotate or loosen, but the actual position of the oxidizing species may be flexible. Thus, the general non-stereospecific oxidations

FIGURE SEVENTEEN: Proposed non-specific binding of alkyl phenyl sulphides



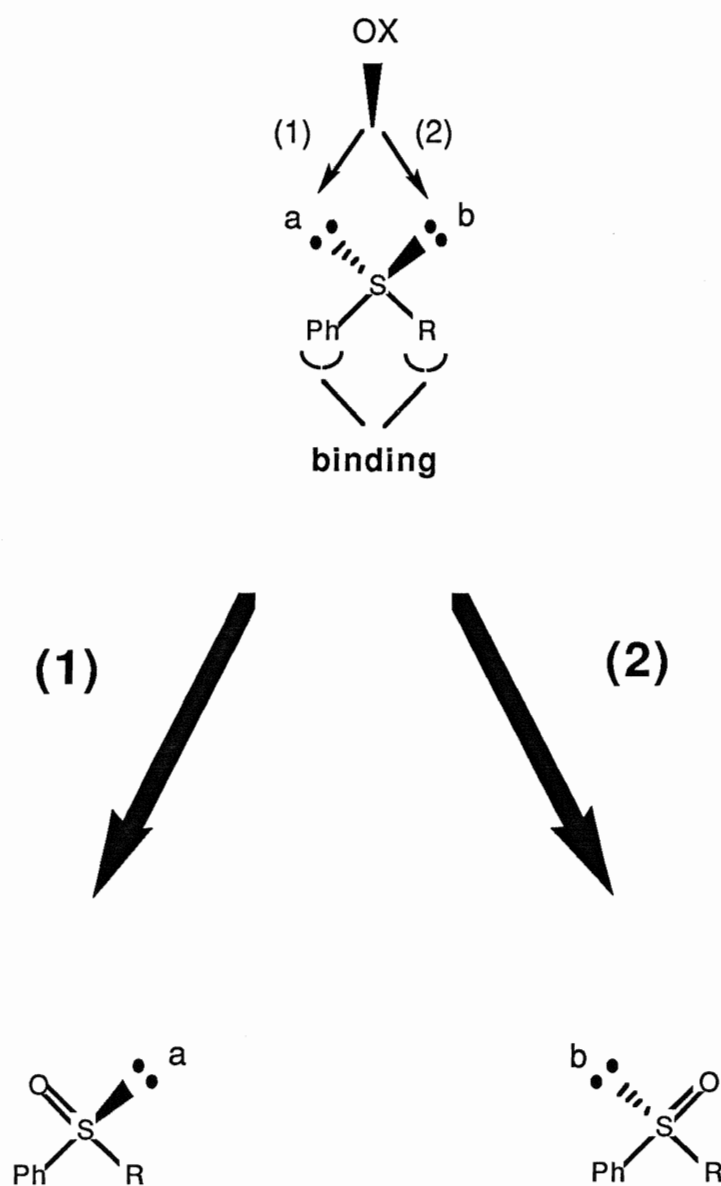
observed may not be due to binding but instead to the position of the oxidizing species. Such a model (figure eighteen) could explain the varying yields in the optical purity as well as allowing inversion to occur.

However, it is difficult to determine the relative position of the oxidizing species. Although the sulphide has two pro-chiral pairs of electrons, these electrons are chemically indistinguishable. Hence, it is impossible to know which of the lone pairs has reacted to yield the sulphoxide. One cannot determine whether the optical purity is due to inversion of the radical cation intermediate, or results from a variable oxidative intermediate.

Investigations involving the microbial metabolism of 1,3-dithiane using the fungi Aspergillus foetidus NRRL 377, Mortierella isabellina NRRL 1757, and Helminthosporium NRRL 4671, all afforded the monosulphoxide⁵¹ (figure nineteen). The observed optical purities of the corresponding sulphoxide were low in comparison with results obtained from fungal transformation of alkyl aryl sulphides. Aspergillus foetidus afforded a monosulphoxide with a preferred R(+) enantiomer, Helminthosporium a S(-) enantiomer, while a racemic monosulphoxide was obtained from Mortierella isabellina. Biotransformation studies using the racemic monosulphoxide 1,3-dithian-1-oxide as a substrate gave monosulphoxides with an increased optical purity. Helminthosporium and Aspergillus foetidus both yielded 1,3-dithian-1-oxide with higher optical

FIGURE EIGHTEEN:

Proposed specific binding of alkyl phenyl sulphides with flexible oxidative species.

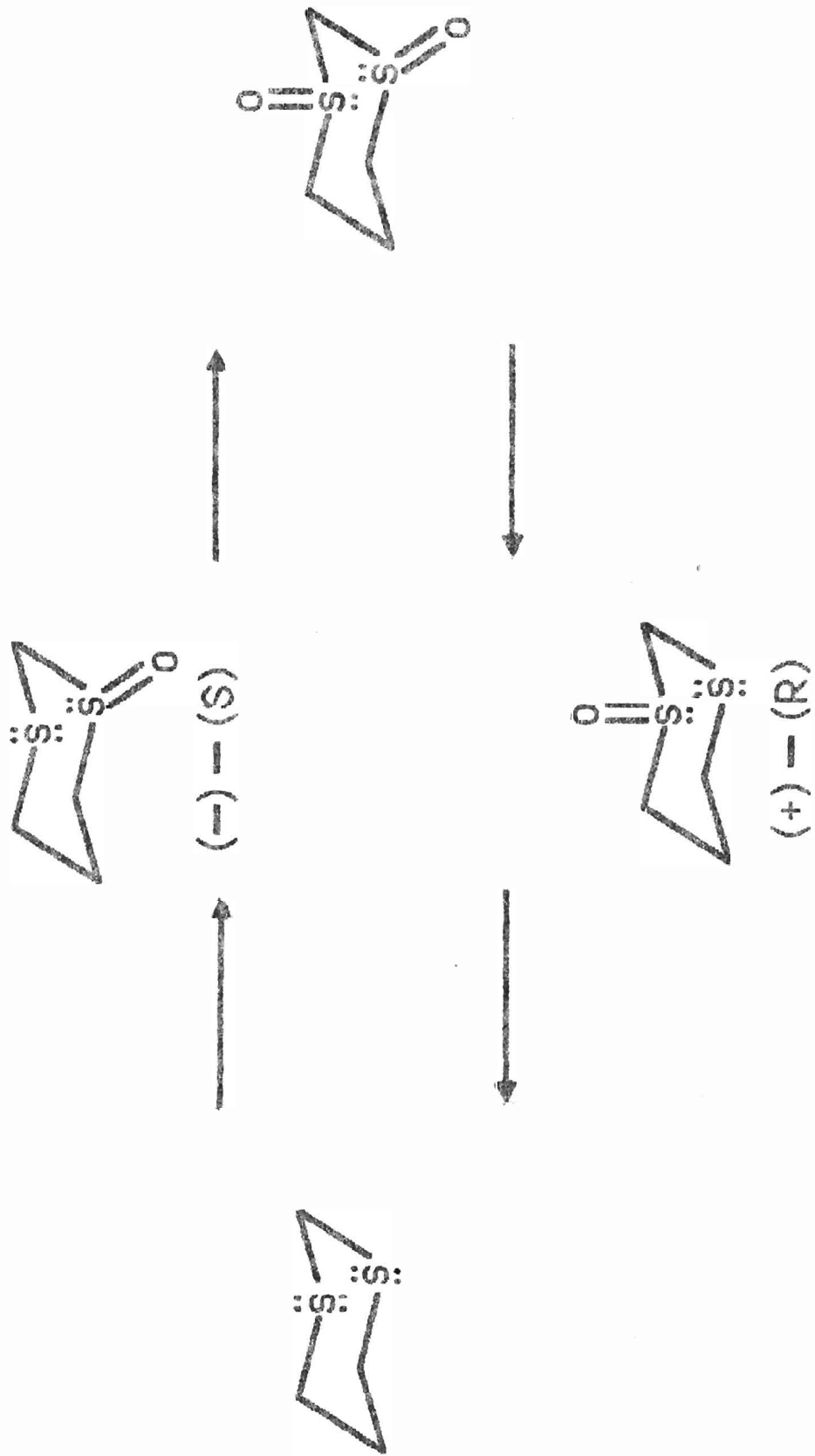


activity and unchanged in their preferred enantiomers, S(-) and S(+), respectively. Mortierella isabellina gave a monosulphoxide with S(-) enantiomer. These results indicate that asymmetric destruction of one enantiomer had occurred. Since the only recoverable product from the incubation of racemic 1,3-dithian-1-oxide was optically enriched sulphoxides, the authors proposed that the enantioselectivity may have arisen from a selective oxidation of one of the enantiomers to a bis-sulphoxide. Attempted incubation of a bis-sulphoxide with Helminthosporium and Aspergillus foetidus gave no recoverable products or starting material, suggesting that the bis-sulphoxide was most likely rapidly hydrolyzed and metabolized to water soluble products. Similar incubations using Mortierella isabellina with the bis-sulphoxide afforded 1,3-dithiane.

The latter part of Auret's investigation is unusual because a single enzymic deoxygenation is relatively uncommon. However, the enzymic removal of two oxygens from a single substrate appears to be without precedent. These enzymic deoxygenation reactions, especially the latter example, may be a common transformation, but no evidence for it yet exist in other systems.

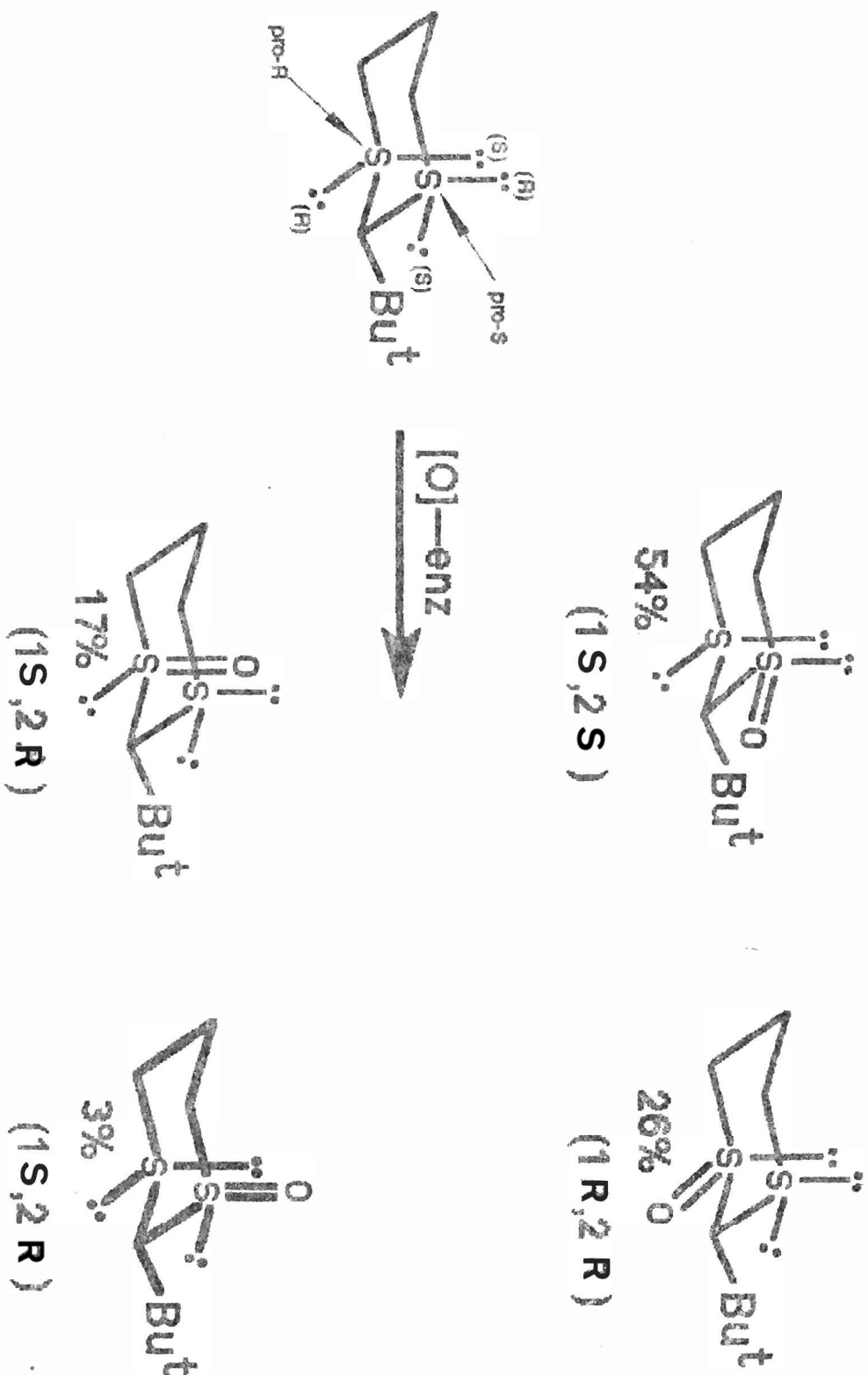
Recently, Auret et al. have demonstrated that the fungal metabolism of 2,2-disubstituted-1,3-dithianes to the corresponding monosulphoxide results in a stereodifferentiation between prochiral, diastereotopic lone pairs on sulphur and between prochiral, enantiotopic thioalkyl

Figure Nineteen: Microbial Metabolism of 1,3- dithiane



substituents on carbon⁵². The fungal species Mortierella isabellina NRRL 1757, Helminthosporium NRRL 4671, and Aspergillus foetidus NRRL 337, all afforded 2,2-dimethyl-1,3-dithian -1-oxide with a stereopreference for the (-) enantiomer. Incubation of the racemic monosulphoxide with all fungi gave no enrichment of the optical purity as previously observed. Thus, the optical purity of the sulphoxide is due to a single asymmetric oxidation. Fungal metabolism of 2-alkyl-1,3-dithianes (methyl, butyl, and methyl butyl) all yielded their respective monosulphoxide with a preferential trans sulphoxide (90%). Microbial deoxygenation of a racemic sulphoxide was observed with fungi Aspergillus foetidus and Mortierella isabellina. However, enzymic deoxygenation was not observed with Helminthosporium.

Thus, with the fungus Helminthosporium the overall biotransformation occurred with a 68% preference for the pro-S versus a 32% preference for the pro-R lone pair during equatorial oxidation of the 1,3-dithian. Axial oxidation occurred with a 86% selectivity for the pro-R lone pair compared to a 14% selectivity for the pro-s thioalkyl (figure twenty). The overall result is an observed stereoselectivity for the pro-S thioalkyl of 57%.

**FIGURE TWENTY:**

Microbial Transformation of 2-(tert-butyl)-1,3-dithiane

Origin of the kinetic isotope effect

Since isotope substitution causes the least possible change in the reactants, the transition state species, and the potential energy surface of the reaction⁵³, isotopic substitution is one of the most effective methods available for elucidating reaction mechanisms or transition state species structure⁵⁴.

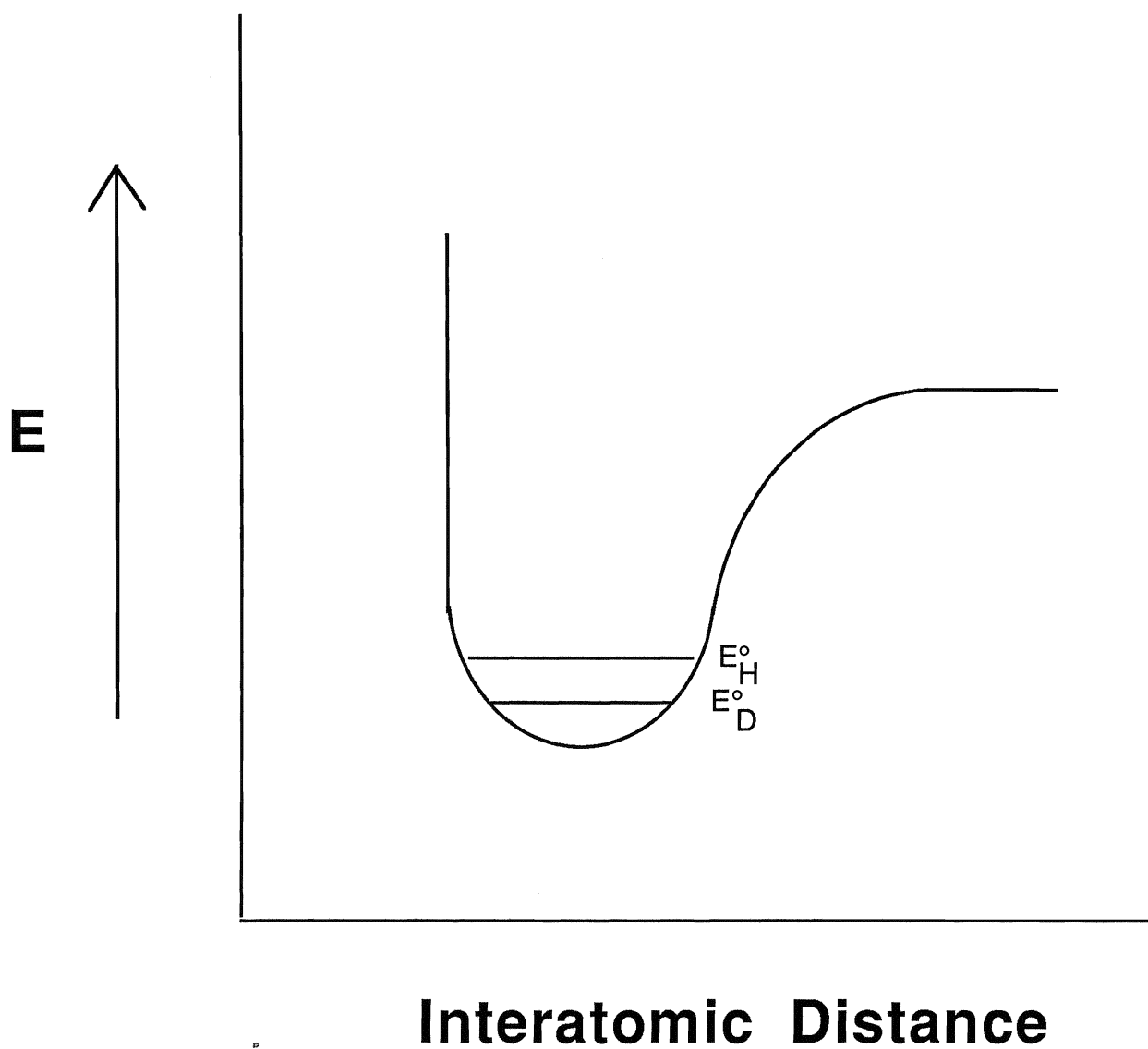
Isotope substitution either at equivalent positions in a single molecule (intramolecular) or at identical positions in two molecules of the same substrate (intermolecular) does result in a small measurable difference in the rate of abstraction of one isotope compared to the rate of abstraction for the other isotope. It is not common to observe isotope effects that involve heavier atoms such as carbon-12 and -13. The most commonly used isotopic substitution is that of deuterium (D) for hydrogen (H).

Substitution of deuterium for hydrogen results in a carbon-deuterium bond (C-D) which is less reactive than the corresponding carbon-hydrogen bond (C-H) (figure twenty-one). This reduced reactivity is a result of⁵⁵:

- A) differences in free energy resulting from differences in zero point energies for the two bonds.
- B) effects due to the difference in mass on the velocity of passage over the potential energy barriers.

FIGURE TWENTY-ONE:

Morse Curve showing the relative zero point energies for a bond to hydrogen and a corresponding bond to deuterium.



- C) the possibility for non-classical penetration of the energy barriers (tunneling).

The energy levels for a diatomic molecule are governed by the following quantized equation⁵⁶ (equation four):

$$E = (n + 0.5) h\nu \quad n = 0, 1, 2, \dots \quad (4)$$

Where ν is the oscillation frequency of the molecule, h is Planck's constant, and n represents the energy level. The lowest value of energy permitted by the above equation occurs when n is equivalent to zero. This is commonly referred to as the zero point energy (equation five).

$$E^0 = 0.5 h\nu \quad (5)$$

The oscillation frequency, ν , is inversely proportional to the square root of the reduced mass (equation six) of the oscillation:

$$u = m_1 m_2 / (m_1 + m_2) \quad (6)$$

where m_1 and m_2 represent the masses of the appropriate atoms involved in the oscillator. It is the change in the reduced mass (u) resulting from isotopic substitution that gives rise to the differences in E^0 observed for C-D and C-H bond. The differences are a result of the effect of the mass change on

the stretching frequencies, since the zero point energy is an inverse function of the mass of the isotope at absolute zero.

Thus,

$$E_H^0/E_D^0 = (u_D/u_H)^{0.5} = 2 \quad (7)$$

Using the standard relationships $E = h\nu$ and $\nu = \lambda c$, the zero point energy differences for C-H and C-D can be determined given that ν_{C-H} is equal to 3000 cm^{-1} and ν_{C-D} is equal to 2200 cm^{-1} . Thus, the difference in zero point energies is 400 cm^{-1} ; when converted to kcal/mole, this value is approximately equal to 1.15 kcal/mole. This calculation demonstrates that more energy is required to break a C-D bond than is necessary to break a C-H bond. The difference in E^0 of the reactants is due to differences in the height of the potential energy barrier for the reaction. Therefore, there is a higher energy of activation for the deuterated compound relative to the hydrogen compound.

The magnitude of the rate difference which may be observed can be calculated from the Arrhenius equations:

$$k_H = A_H e^{(-E_H^a/RT)} \quad (8)$$

and

$$k_D = A_D e^{(-E_D^a/RT)} \quad (9)$$

where A is the pre-exponential factor and E° is the energy of activation. Thus,

$$k_H/k_D = (A_H e^{(-E_H^a/RT)}) / (A_D e^{(-E_D^a/RT)}) \quad (10)$$

Assuming the ratio of the pre-exponential factors A_H/A_D is equal to one, then equation ten simplifies to:

$$k_H/k_D = (e^{(-E_H^a/RT)}) / (e^{(-E_D^a/RT)}) \quad (11)$$

further simplification gives:

$$k_H/k_D = e^{(E_D^a - E_H^a)/RT} \quad (12)$$

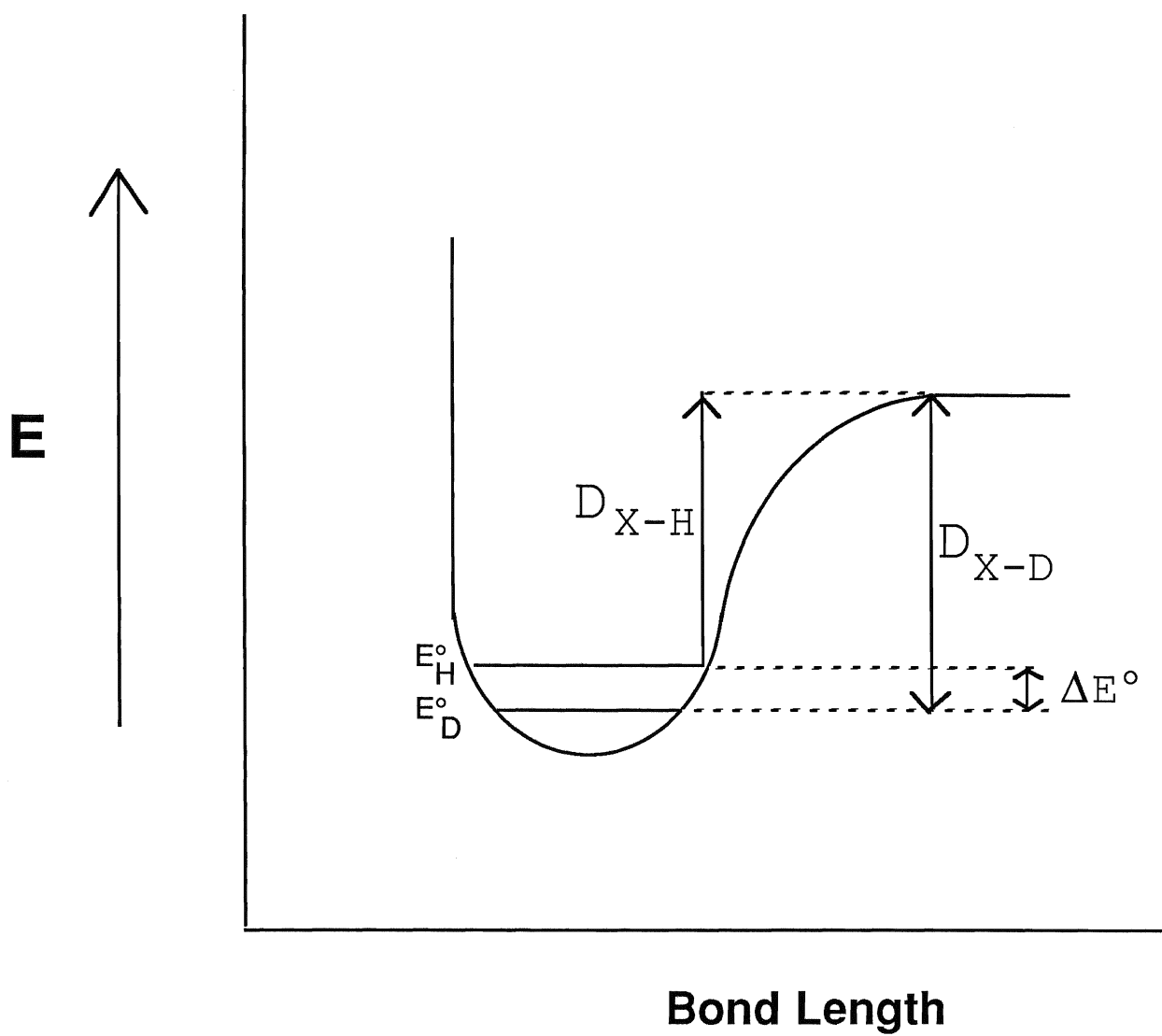
where the $E_D^a - E_H^a$ factor is E° calculated earlier to be equal to approximately 1.15 kcal/mole for deuterium substitution. Thus, at 300°K;

$$k_H/k_D = e^{1150/(1.98)(300)} = e^{1.97} = 7 \quad (13)$$

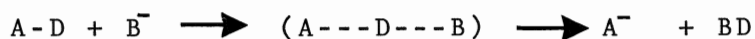
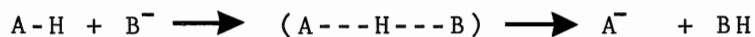
This is the maximum calculated isotope effect based on zero point energy difference and corresponds to the complete cleavage of the hydrogen or deuterium bond in the transition state (figure twenty-two).

Kinetic isotope effects are divided into two types: intramolecular and intermolecular. In the intermolecular isotope effect, hydrogen and deuterium occupy identical

FIGURE TWENTY-TWO: Role of the zero-point energy difference in bond breaking



positions in two separate but structurally similar molecules. A proton transfer reaction that occurs can be interpreted as follows:



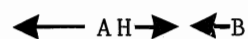
Thus, the intermolecular isotope effect is a comparison of the rates of the two different reactions occurring at the same time. The transition state that occurs in the course of the reaction is assumed to be linear and independent of bending vibrations.

A typical linear molecule would have two stretching frequencies as indicated below⁵⁷.



symmetric

vibration



asymmetric

vibration

In the case of asymmetric vibrations the movement of the atoms occurs along the reaction coordinate. This is not a true vibration but rather translational motion along the reaction coordinate. The symmetric vibrations represent the stretching frequency that occurs at right angles to the reaction coordinates. Since this is a symmetrical motion, A and B move in opposite directions and the H atom remains

stationary⁵⁸. This motion assumes that the force constants for the A-H and B-H bonds are equal. Thus, the vibration frequency is independent of whether hydrogen or deuterium is present in the transition state species. Therefore, the isotope effect that is observed results from the differences in the zero point energies of the reactants (figure twenty-three).

However, sometimes the observed isotope effect is much less than the maximum predicted value. One reason for this difference is because the force constants for the A-H and B-H bonds are not equal. As a result, the transition state species is no longer perfectly symmetrical as originally assumed and the stationary H atom begins to move. The result of this imperfect situation is a small dependence of the vibration frequency on the mass of the H atom. Therefore, the isotopic substitution would affect the energy of the transition state species in a manner similar to the effect observed in the reactants. The resulting change decreases the kinetic isotope effect and sometimes the effect disappears entirely (figure twenty-four).

Low intermolecular isotope effects which have been observed in investigations involving enzymic systems are not thought to be the result of differing energies in the transition state. Instead, these low values have been observed because in all these investigations the methodology of the studies involved a comparison between the measured overall velocities of the reactions of deuterated and

FIGURE TWENTY-THREE:

Origin of the Intermolecular Kinetic Isotope Effect in a symmetrical transition state.

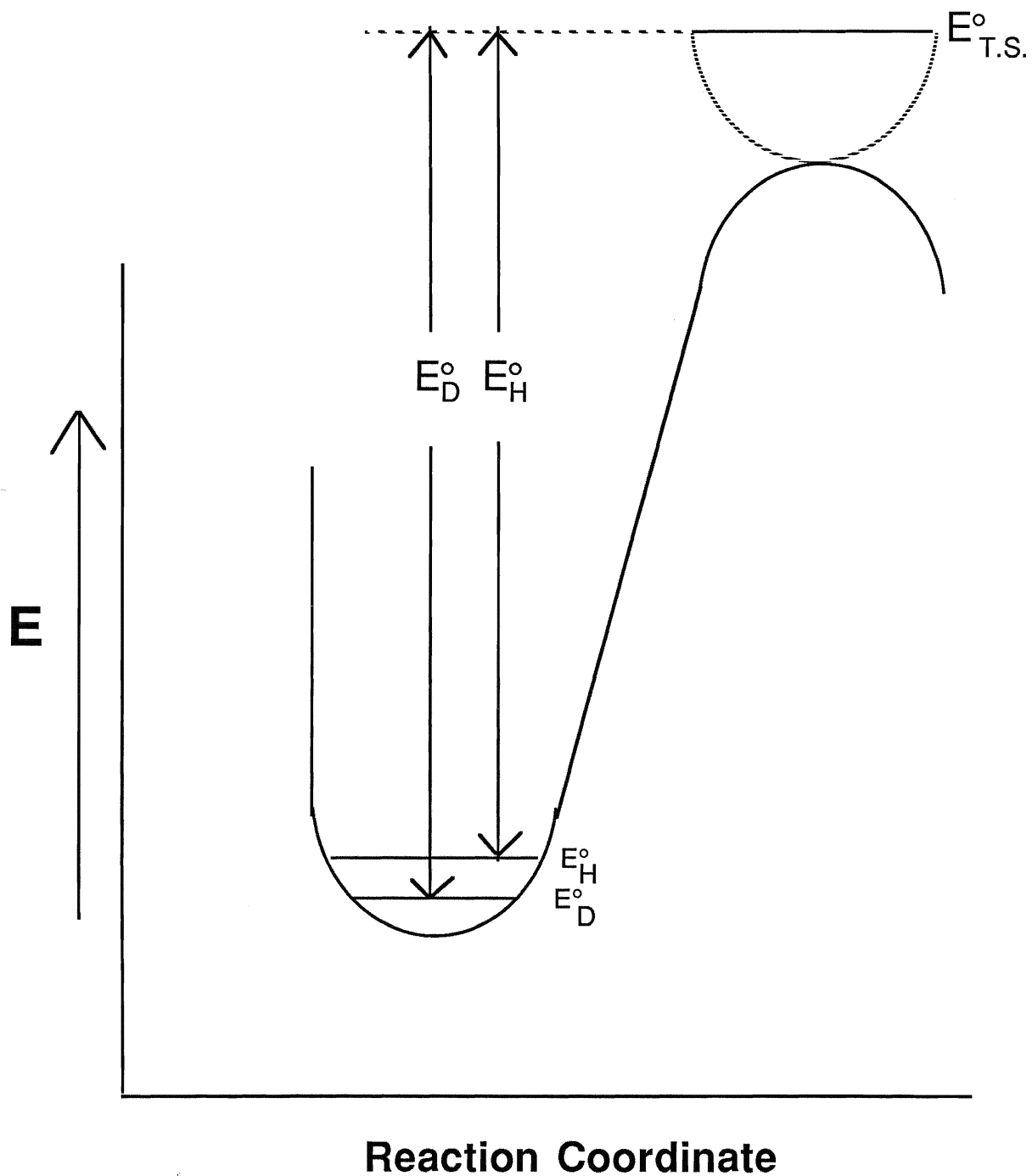
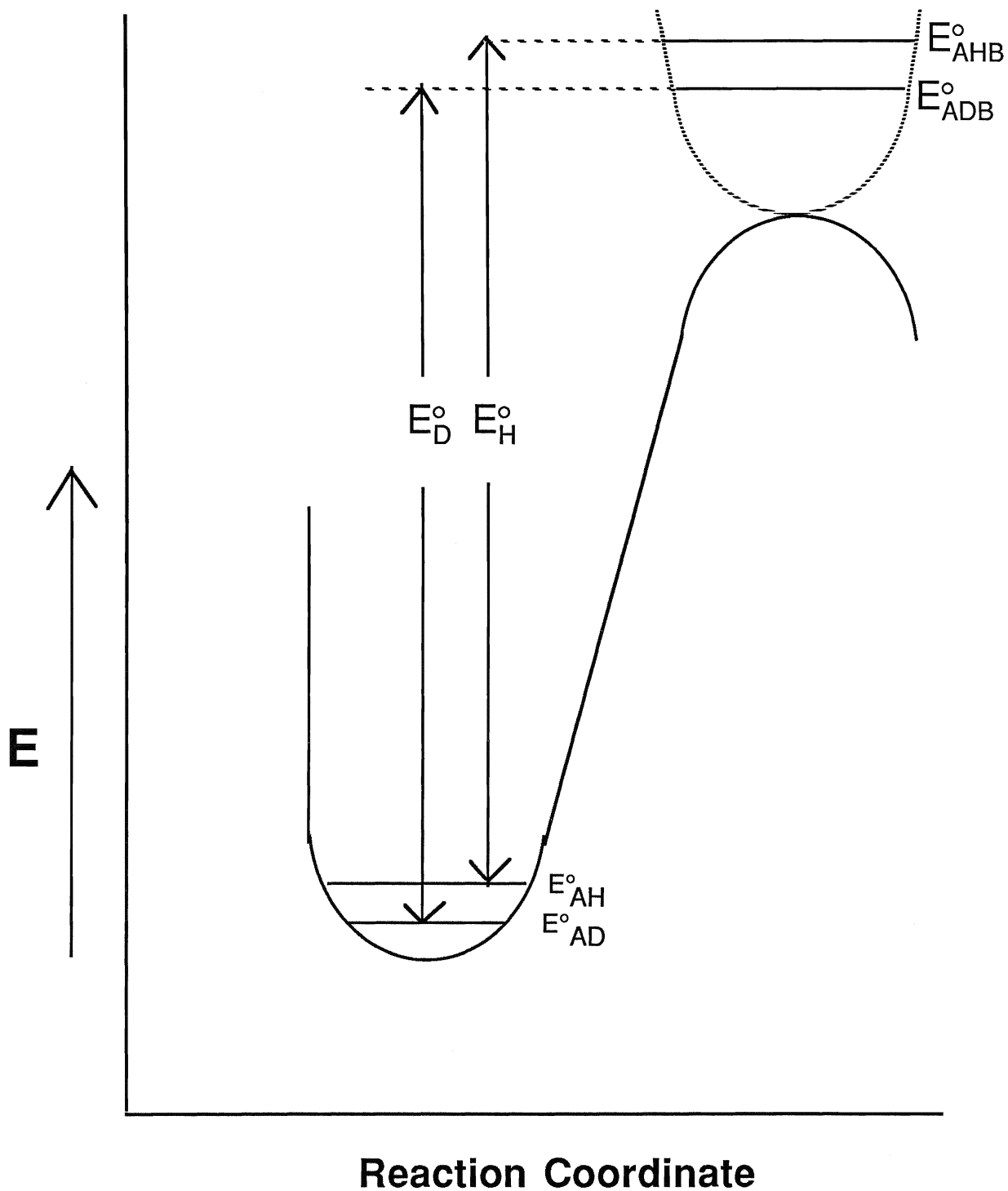


FIGURE TWENTY-FOUR:

Origin of a less than maximum Intermolecular Kinetic Isotope Effect in a nonsymmetrical transition state species.



non-deuterated substrates⁵⁹. The velocities of such reactions, however, are complex functions of many individual rates which make up the total reaction.

Thus, the actual validity of the intermolecular isotope effect is questionable since it appears that the true isotope effect could be hidden or absorbed in one or many of the individual rates or steps⁵⁹. It is also possible that the actual step that is measured is not the rate-determining step or close to the rate-determining step but the final release of product.

However, if hydrogen and deuterium atoms are in equivalent positions in the same substrate an "intramolecular" isotope effect is observed. In this case, the isotope effect does not arise from the comparison of reaction velocities between deuterated and non-deuterated substrates. Instead, the effect is a result of deuterium substitution on the rate of the individual step involving covalent modification of the substrate⁵⁹. Thus, the intramolecular isotope effect is interpreted as the true observable isotope effect. Investigations that involve intramolecular studies generally yield large values. It is considered that in enzymic studies the intramolecular isotope effect provides more relevant and accurate mechanistic data.

Secondary Isotope Effects

The effects of isotopic substitution on reaction rates arise from changes in vibrational frequencies associated with the process of activating a molecule from its ground state (zero-point vibrational energy) to a transition state (figure twenty-two). Primary isotope effects are observed when the bond to the isotopic atom is broken in the rate-determining step. Secondary isotope effects on the rate of a reaction may be observed when the isotopic atom is in a position (usually near to the reacting centre) from which transfer of the atom does not take place, but changes in vibrational frequency do occur. These secondary isotope effects can give valuable information about the geometry of the transition state^{60,61}.

Secondary isotope effects are usually expressed as either an α or β effect depending on the position of the deuterium with respect to the reacting centre. A β secondary isotope effect occurs when deuterium has been substituted for hydrogen β to the position of bond breaking. These effects are usually small (close to unity) but can range as high as 1.5⁶².

There is some evidence that suggests that β secondary isotope effects are a result of steric interaction^{63,64} (a CD_3 group has a smaller steric requirement than a CH_3). It has also been proposed that since CD_3 is a better electron donor than CH_3 , a field effect could explain the effect⁶⁵. However, it is widely considered that β secondary isotope effects arise solely from hyperconjugation⁶⁶ which lowers the bending

frequencies of the weakened C-H bond at the reacting centre.

α secondary isotope effects result when deuterium has been replaced for hydrogen at the reacting carbon centre. These effects range from 0.87 to 1.26⁶⁷ and appear to correlate with carbocation character. It has been demonstrated that nucleophilic reactions that do not proceed via a carbocation intermediate have α secondary isotope effects less than or near to one⁶⁸, whereas those that involve a carbocation intermediate have a high α secondary isotope effect (1.0 to 1.4)⁶⁹. The accepted explanation for secondary isotope effects is that one of the bending C-H vibrations (out-of-plane bending) in the transition state is affected by substitution of deuterium for hydrogen⁷⁰.

The Present Problem

This thesis attempts to investigate two different aspects of enzymic chemistry. The first investigation attempts to elucidate the nature of the enzymic hydroxylation catalyzed by the enzyme. Until very recently the literature tended to support a direct insertion mechanism. It now appears, in light of new information, that a radical recombination mechanism may be the correct mechanism.

In the present study a series of specifically deuterated toluenes will be prepared and used to determine primary and secondary isotope effects for the enzymic hydroxylation reaction. Furthermore, a number of benzylic deuterated ethylbenzenes will be prepared and the hydroxylation will be monitored by ^2H NMR (30.7 MHz). It is hoped that this investigation will lend support to either a direct insertion or a radical-abstraction recombination mechanism.

The second investigation attempts to utilize the ability of fungal enzymes which can perform the asymmetric oxidation of organic sulphides to sulphoxides in high chemical and stereochemical yields^{44,45,46}. It has been demonstrated that fungi are capable of enantio- and diastereoselection in the oxidation of 1,3-dithanes⁵². The current proposal is to investigate the use of fungal enzymes in the asymmetric oxidation of 1,3-oxathiolanes with the intention of generating chiral alcohols of known stereochemistry.

Experimental

I) Apparatus, Materials, and Methods

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded with an Analect FX-6260 FTIR spectrophotometer interfaced with an Analect MAP-66 data system. ^1H NMR spectra were obtained from a Bruker WP-80 CW NMR or a Bruker AC-200 FT NMR using deuteriochloroform or NMR reagent grade carbon tetrachloride as the solvent and TMS as the internal standard. 50.3 MHz ^{13}C NMR spectra were recorded with a Bruker AC-200 FT NMR using deuteriochloroform as the solvent and as the internal reference. 30.7 MHz ^2H NMR spectra were recorded with a Bruker AC-200 FT NMR using deuterium depleted water as the solvent. ^2H NMR spectra were obtained unlocked with deuteriochloroform as an external standard. The fast atom bombardment (FAB) spectra were obtained from a KRATOS MS 30 with a saddle field gun (Ion Tech. Ltd.) operating at an energy of 7 Kev using a glycerol matrix for the the sample. Electron Impact (EI) mass spectra were recorded on a KRATOS/AEI MS 30 with a DS55 data system at 70 ev. The isotope composition of the various samples were obtained by repeated accumulation over the appropriate mass range and suitable corrections were applied for the presence of the natural isotope abundancies. These values were accurate to \pm 2%. The optical rotation measurements were obtained either at McMaster University using a Perkin-Elmer 241 MC

Polarimeter at ambient temperature and 598 nm with a 10 cm cell ,in ethanol, or with a Rudolph Research AUTOPOL III automatic polarimeter under the same operating conditions.

Computer simulated ^1H NMRs were generated on the Bruker WP-60 FT NMR using an Iteration of Calculated NMR spectrum using Least Squares Criteria (ITRCAL) or a Nuclear Magnetic Resonance Spectrum Calculation (NMRCAL) program.

Column chromatography was performed using silica gel, flash chromatography with Merck Kieselgel 60 (230-400 mesh), and thin layer chromatography on Merck thin layer silica gel 60F 245 (0.2mm).

The pH of solutions were measured with a Fisher Accumet pH meter model 800.

Mortierella isabellina NRRL 1757, Aspergillus niger ATCC 9142, and Rhizopus arrhizus ATCC 11145 were maintained refrigerated on 4% malt agar slopes. Helminthosporium species NRRL 4671 was maintained on V-8 juice agar slopes. The incubation flasks were kept on a New Brunswick shaker at 26° C.

II) Preparation of Substrates

A) Preparation of toluenes and ethylbenzenes

Toluene- α -d₁ (2)

Distilled benzyl chloride (1) (8.0g, 0.063 moles), was transferred to a 250 mL round bottom flask which had previously been sealed with a rubber septum and filled with nitrogen. Anhydrous ether (25 mL) was then added to the aforementioned flask and the resulting mixture was stirred. This was followed by the slow addition of 80 mL of 1.0 M lithium triethylborodeuteride solution (super deuteride) under anhydrous transfer conditions.

The above solution was allowed to stir for 16 hours. An additional 20 mL of super deuteride solution was slowly added and stirring was continued for two more hours. The reaction mixture was then cooled in an ice/acetone bath and the sealed round bottom flask was opened to atmosphere.

Water was added dropwise to the resulting mixture until the excess super deuteride had been destroyed. This was followed by the addition of 60 mL of a 3 N sodium hydroxide solution. Hydrogen peroxide solution (60 mL of a 30% solution) was then added carefully to the reaction mixture.

The mixture was extracted with low boiling pentane (4 * 50 mL). The pentane extracts were washed with a 50% sulphuric acid solution (4 * 100 mL), water (2 * 75 mL), a saturated sodium bicarbonate solution (1 * 75 mL), water (1 * 75 mL), and dried over anhydrous sodium sulphate. The pentane solution was passed through silica gel in a buchner funnel and removed using the rotary evaporator at room temperature. 2 was obtained in a 27% yield (1.6 g).

The ^1H NMR (80 MHz) spectrum showed signals at δ : 2.3 (2H,s), 7.2 (5H,s). The Electron impact (EI) mass spectrum m/e (%): 94 (5.6), 93 (78.1, M^+), 92 (100.0), 91 (20.7), 90 (3.8), 89 (2.1), 88 (0.8), 87 (0.6). Deuterium content of 2 as calculated from mass spectrum was in excess of 99% d_1 species.

Benzyl alcohol- α - d_1 (3)

Distilled benzaldehyde (4) (10.0 g, 0.094 moles) was added to 100 mL of anhydrous ether. To the mixture of 4 and anhydrous ether was slowly added 1.0 g (0.024 moles) of lithium aluminum deuteride. The resulting solution was gently refluxed with stirring for 24 hours. The condenser was fitted with a drying tube to ensure anhydrous reaction conditions.

After 24 hours 1.0 mL of water, 1.0 mL of a 10% sodium

hydroxide solution, and 3 mL of water were added sequentially to the reaction mixture. The reaction mixture was filtered and the ether solution was washed with a 10% sodium bicarbonate solution (2 * 25 mL), water (2 * 10 mL), and dried over anhydrous sodium sulphate. The ether was removed using the rotary evaporator to give 7.47 g (73% yield) of 3. The ^1H NMR (80 MHz) spectrum showed signals at δ : 2.9 (1H,s), 4.5 (1H,s), 7.19 (5H,s).

Benzyl chloride- α -d₁ (5)

The synthesis of 5 was accomplished by the adapted method of Pereira⁷¹. Thionyl chloride (15.0 g, 0.13 moles) was added dropwise over a one hour period with stirring to 7.47 g (0.059 moles) of 5. After addition was completed, stirring was continued for two hours. The reaction mixture was poured onto 100 mL of water and then extracted with ether (3 * 25 mL). The ether extracts were dried over anhydrous sodium sulphate. The ether was removed using the rotary evaporator. 5 was isolated in a 31% yield (5.2 g). The ^1H NMR (200 MHz) spectrum showed signals at δ : 4.3 (1H,s), 7.3 (5H,s).

Toluene- α,α -d₂ (6)

6 was synthesized by following the procedure described for the preparation of 2. From 5.2 g (0.04 moles) of 5 and 100 mL of a 1.0 M super deuteride solution was isolated 2.1 g (54% yield) of 6. The ¹H NMR (200 MHz) spectrum showed signals at δ : 2.3 (1H,s), 6.9-7.1 (5H,m). The EI mass spectrum m/e (%): 95(8.0), 94(85.4,M⁺), 93(100.0), 92(37.5), 91(6.2), 90(3.1), 89(0.7), 88(0.6), 86(2.8). The deuterium content of 6 as calculated from the mass spectrum was in excess of 99% d₂ species.

1-(3'-fluorophenyl)-ethanol (7)

3-fluoroacetophenone (8) (10.0 g, 0.073 moles) was reduced as outlined in the preparation of 3 except that 1.0 g (0.026 moles) of lithium aluminum hydride was substituted for 1.0 g (0.024 moles) of lithium aluminum deuteride. Moreover, the resulting reaction mixture was allowed to gently reflux for 72 hours instead of the prescribed 24 hours. A yield of 80% (8.2 g) was obtained for 7. The ¹H NMR (80 MHz) spectrum showed signals at δ : 1.4 (3H,d), 2.9 (1H,s), 4.75 (1H,q), 7.0-7.3 (4H,m).

1-chloro-(3'-fluorophenyl)-ethane (9)

Synthesis of 9 was completed as outlined in the

preparation of 5 . From 8.2 g (0.059 moles) of 7 and 15.0 g (0.13 moles) of thionyl chloride was isolated 7.82 g (83% yield) of 9 . The ^1H NMR (80 MHz) spectrum showed signals at δ : 1.8 (3H,d), 5.0 (1H,q), 6.6-7.3 (4H,m).

3-fluoroethylbenzene (10)

Reduction of 7.82 g (0.05 moles) of 9 with super hydride instead of super deuteride by the methods prescribed under preparation of 2 afforded 1.8 g (29% yield) of 10 . The ^1H NMR (80MHz) spectrum showed signals at δ : 1.3 (3H,t), 2.5 (2H,q), 6.9-7.2 (4H,m). The EI mass spectrum m/e (%) : 125(3.2), 124(30.0, M^+), 123(3.7), 122(1.6), 120(0.8), 110(9.7), 109(100).

1-(3'-fluorophenyl)-ethanol-1-d₁ (11)

Synthesis of 11 was completed as outlined in the preparation of 3 except that the reaction mixture was refluxed for 72 hours instead of 24 hours. From 10.0 g (0.073 moles) of 8 was isolated 7.43 g (72% yield) of 11 . The ^1H NMR (200 MHz) spectrum showed signals at δ : 1.4 (3H,s), 6.9-7.3 (4H,m).

1-chloro-(3'-fluorophenyl)-ethane-1-d₁ (12)

Addition of 15.0 g (0.13 moles) of thionyl chloride to 7.43 (0.053 moles) of 11 as described under the preparation of 5 , afforded 7.7 g (91% yield) of 12 . The ¹H NMR (200 MHz) spectrum showed signals at δ : 1.75 (3H,s), 6.8-7.2 (4H,m).

3'-fluoroethylbenzene-1,1-d₂ (13)

Reduction of 7.7 g (0.048 moles) of 12 with 100 mL of a 1.0 M super deuteride as described in the preparation of 2 gave 2.0 g (33% yield) of 13 . The ¹H NMR (200 MHz) spectrum showed signals at δ : 1.7 (3H,s), 6.9-7.2 (4H,m). The EI mass spectrum m/e (%) : 127(2.9), 126(31.9, M⁺), 125(3.0), 123(1.4), 122(1.0), 121(0.6), 112(7.9), 111(100). The deuterium content of 13 as calculated from the mass spectrum was in excess of 99% d₂ species.

1-(4'-fluorophenyl)-ethanol-1-d₁ (15)

Standard reaction conditions and extraction procedures as previously outlined under preparation of 11 gave 7.8 g (76% yield) of 15 from 10.0 g (0.072 moles) of 14 . The ¹H NMR (200 MHz) spectrum showed signals at δ : 1.4 (3H,s), 7.3

(4H,AB).

1-chloro-(4¹-fluorophenyl)-ethane-1-d₁ (16)

Preparation of 16 was achieved by following the identical procedure used in the synthesis of 9 . From 7.8 g (0.055 moles) of 15 was isolated 7.6 g (86% yield) of 16 . The ¹H NMR (200 MHz) spectrum showed signals at δ : 1.8 (3H,s), 7.0 (4H,AB).

4¹-fluoroethylbenzene-1,1-d₂ (17)

17 was prepared by reacting 7.6 g (0.048 moles) of previously prepared 16 with 100 mL of a super deuteride solution as outlined in the preparation of 2 to give 1.6 g (26% yield) of 17 . The ¹H NMR (200 MHz) showed signals at δ : 1.3 (3H,s), 7.0 (4H,AB). EI mass spectrum m/e (%) : 125(2.3), 124(27.3,M⁺), 123(2.9), 122(1.8), 121(1.6), 120(0.8), 110(7.8), 109(100). The deuterium content of 17 as calculated from the mass spectrum was in excess of 99% d₂ species.

4-fluorotoluene (18)

4-fluorobromobenzene (19) (1.0 g, 0.058 moles) was transferred to a 250 mL round bottom flask which had previously been dried, sealed with a rubber septum, and filled with nitrogen. Anhydrous ether (25 mL) was then added to the flask and the resulting mixture was cooled in a dry ice/acetone bath and stirred. To this mixture was slowly added 50 mL of a 1.6 M tert. butyl lithium solution under anhydrous transfer conditions.

The reaction mixture was allowed to stir for one hour after which 3.0 g (0.021 moles) of methyl iodide was added. The mixture was stirred for an additional two hours.

After two hours the sealed round bottom flask was opened to atmosphere and a saturated solution of ammonium chloride was added dropwise until the excess tert. butyl lithium had been destroyed.

The mixture was extracted with ether (4 * 25 mL). The ether extracts were washed with water (2 * 15 mL), a saturated sodium bicarbonate solution (2 * 25 mL), water (2 * 15 mL), and dried over anhydrous sodium sulphate. 18 was isolated in an 18% yield (0.12 g). The ^1H NMR (80 MHz) showed signals at δ : 2.4 (3H,s), 6.9-7.4 (4H,m).

4-fluorotoluene- α, α, α -d₃ (20)

Synthesis of 20 was completed as outlined in the preparation of 18 . From 1.0 g (0.006 moles) of 19 and 2.0 g

(0.013 moles) of methyl iodide- d_3 was isolated 0.12 g (17% yield) of 20 . The 1H NMR (80 MHz) spectrum showed signals at δ : 7.0-7.4 (m). The EI mass spectrum proved to be inconclusive due to the presence of impurities.

3-fluorotoluene- $\alpha, \alpha, \alpha-d_3$ (21)

Preparation of 21 was achieved by following the identical procedure used in the synthesis of 20 . From 1.0 g (0.006 moles) of 3-fluorobromobenzene (22) was isolated 0.16 g (23% yield) of 21 . The 1H NMR (80 MHz) spectrum showed signals at δ : 7.1-7.5 (m). The EI mass spectrum for 21 also proved to be inconclusive due to the presence of impurities.

B) Preparation 1,3-oxathiolanes

2,2-dimethyl-1-phenyl-1-propanol (25)

The title compound (25) was prepared by reacting 10.0 g (0.094 moles) of distilled benzaldehyde (4) in 60 mL of anhydrous ether with 55 mL of a 1.6 M tert. butyl lithium solution as described in the preparation of 4-fluorotoluene (18). A yield of 90% (10.4 g) was obtained for 25 . The ^1H NMR (200 MHz) showed signals at δ : 0.9 (9H,s), 1.9 (1H,s), 4.4 (1H,s), 7.4 (5H,s). EI mass spectrum m/e (%) : 164(2.4, M^+), 163(10.4), 162(1.6), 161(6.3), 147(8.1), 108(9.9), 107(47.0), 106(6.0).

2,2-dimethylpropiophenone (26)

Oxidation of 10.4 g (0.063 moles) of 25 with Jones' reagent⁷² gave the ketone 26 . Jones' reagent was added dropwise to a mixture of 25 in 50 mL of acetone while cooling in an ice/acetone bath. The reagent was added until the solution became brown in colour. At this point the reaction mixture was allowed to stir for two hours. Isopropyl alcohol was then added to the reaction mixture until the solution turned green. 150 mL of water was added to the mixture.

The mixture was extracted with ether (4 * 50 mL). The ether extracts were washed with water (2 * 25 mL) and dried over anhydrous sodium sulphate. The ether was removed using a rotary evaporator. An 80% yield (8.2 g) was obtained for 26. ^1H NMR (200 MHz) spectrum showed signals at δ : 1.5 (9H,s), 7.2 (5H,AB).

2-phenyl-2-tert. butyl-1,3-oxathiolane (27)

27 was synthesized as outlined by C. Djerassi and M. Gorman⁷³ using 10.0 g (0.062 moles) of 26 obtained either commercially (Aldrich) or synthesized as described in the preparation of 25 and 26, 5.0 g (0.064 moles) of 2-mercaptoethanol (28), 100 mL of anhydrous benzene, and 1.0 g (0.0057 moles) of p-toluene sulphonic acid monohydrate (p-tsa) was refluxed in a round bottom flask fitted with a Dean-Stark water separator until no more water appeared in the separator (approximately three hours).

The benzene solution was cooled, washed with a saturated sodium bicarbonate solution (2 * 25 mL), water, (2 * 25 mL), dried over anhydrous sodium sulphate, and evaporated using a rotary evaporator to yield 13.0 g (88% yield) of 27 (m.p. 63.0-67.0°C). The ^1H NMR (80 MHz) showed signals at δ : 1.1 (9H,s), 2.7-3.0 (2H,m), 3.5-3.9 (1H,m), 4.3-4.5 (1H,m), 7.0-7.5 (5H,m). The EI mass spectrum m/e (%) : 222(0.1,M⁺), 208(0.1), 162(1.4), 147(1.0), 121(1.2), 119(2.1), 115(0.9),

107(2.1), 106(9.2), 105(100.0)

The synthesis of 27 as outlined above has been adopted as the general procedure for synthesis in the series of 2-substituted-1,3-oxathiolanes which are described below.

2-methyl-2-phenyl-1,3-oxathiolane (29)

A yield of 85% (21.5 g) for 29 was obtained from 17.0 g (0.14 moles) of acetophenone (30), 12.0 g (0.15 moles) of 28 , and 1.0 g (0.0057 moles) of p-tsa. The ^1H NMR (80 MHz) showed signals at δ : 1.9 (3H,s), 2.4-3.1 (2H,m), 3.8-4.5 (2H,m), 7.1-7.6 (5H,m). The EI mass spectrum m/e (%) : 180(10.0, M^+), 179(1.0), 165(1.9), 120(2.7), 106(1.3), 105(9.5), 91(0.4), 78(100.0).

2,2-diphenyl-1,3-oxathiolane (31)

Diphenyl ketone (32) 10.0 g (0.055 moles), 5.0 g (0.06 moles) of 28 , and 0.8 g (0.0047 moles) of p-tsa gave 11.2 g (83.5% yield) of 31 (m.p. 42-44°C). The ^1H NMR (80 MHz) showed signals at δ : 3.1 (2H,t), 4.2 (2H,t), 7.0-7.6 (10H,m). The EI mass spectrum m/e (%) : 243(5.9, M^+), 242(3.2), 241(5.8), 197(2.8), 195(2.3), 183(42.5), 182(13.9),

167(11.9).

2-phenyl-1,3-oxathiolane (33)

The title compound (33) was obtained in an 84% yield (6.6 g) from 5.0 g (0.047 moles) of 4 , 4.2 g (0.053 moles) of 28 , and 0.5 g (0.003 moles) of p-tsa. The ^1H NMR (80 MHz) showed signals at δ : 3.1-3.3 (2H,m), 3.8-4.0 (1H,m), 4.4-4.6 (1H,M), 6.0 (1H,s), 7.0-7.4 (5H,m). The EI mass spectrum m/e (%) : 166(14.4), 165(1.1), 133(3.7), 121(3.3), 107(8.8), 106(76.9), 77(92.0).

2-ethyl-2-methyl-1,3-oxathiolane (34)

Ethyl methyl ketone (35) 8.0 g (0.11 moles), 9.0 g (0.12 moles) of 28 , and 0.8 g (0.0047 moles) of p-tsa yielded 13.2 g (90% yield) of 34 . The ^1H NMR (80 MHz) showed signals at δ : 1.6 (3H,s), 1.8 (2H,q), 3.0 (2H,t), 4.2(2H,t). The EI mass spectrum m/e (%) : 132(11.3, M^+), 117(3.0), 103(53.3), 78(32.5), 60(100.0), 57(18.4), 43(85.7).

2-methyl-2-n-propyl-1,3-oxathiolane (36)

A yield of 91% (16.4 g) for 36 was isolated from 10.0 g

(0.12 moles) of n-propyl methyl ketone (37), 9.4 g (0.12 moles) of 28 , and 1.0 g (0.0057 moles) of p-tsa. The ^1H NMR (80 MHz) of 36 showed signals at δ : 0.3 (2H,t), 1.1-1.3 (3H,m), 1.5 (3H,s), 1.6-1.8 (2H,m), 3.0 (2H,t), 4.2 (2H,t). The EI mass spectrum m/e (%) : 146(1.6, M^+), 132(6.1), 103(40.2), 78(66.9), 60(81.9), 43(100.0).

2-cyclohexyl-2-methyl-1,3-oxathiolane (38)

From 5.0 g (0.04 moles) of cyclohexyl methyl ketone (39), 3.1 g (0.04 moles) of 28 , and 0.5 g (0.003 moles) of p-tsa was isolated 7.0 g (92% yield) of 38 . The ^1H NMR showed signals at δ : 1.0-2.0 (11H,m), 2.2 (3H,s), 2.9-3.1 (2H,m), 3.9-4.2 (2H,m). The EI mass spectrum m/e (%) : 126(5.7), 103(43.1), 78(100.0), 60(39.3), 59(21.0), 57(27.0), 43(62.7).

2-methyl-2-tert. butyl-1,3-oxathiolane (40)

From 15.0 g (0.15 moles) of methyl tert. butyl ketone (41), 12.0 g (0.16 moles) of 28 , and 0.8 g (0.0047 moles) of p-tsa was obtained 21.0 g (86% yield) of 40 . The ^1H NMR (80 MHz) showed signals at δ : 1.0 (9H,s), 1.5 (3H,s), 2.7-3.0 (2H,m), 3.7-4.3 (2H,m). The EI mass spectrum m/e (%) : 160(2.2, M^+), 146(1.8), 103(95.1), 78(65.7), 57(98.4).

C) Preparation of Miscellaneous Substrates

1-phenylethyl 2'-hydroxyethyl sulphide (42)

2-mercaptoethanol (28) (5.0 g, 0.069 moles) was added slowly to a mixture of 2.5 g (0.10 moles) of sodium hydride in 25 mL of anhydrous benzene. The resulting solution was gently refluxed with stirring for 24 hours. The condenser was fitted with a drying tube to ensure anhydrous reaction conditions. After 24 hours of refluxing 8.9 g (0.064 moles) of 1-chloro phenyl ethane (43) was added to the sodium salt and the resulting mixture was allowed to reflux under anhydrous conditions for an additional 24 hours.

The reaction mixture was poured onto 50 mL of water and then extracted with ether (3 * 25 mL). The ether extracts were dried over anhydrous sodium sulphate and evaporated using the rotary evaporator to yield 5.2 g of crude product (42). Purification by flash chromatography using 1:4 ethylacetate/hexane solution afforded 2.8 g (23.5% yield) of pure 42 . The ¹H NMR (80 MHz) showed signals at δ : 1.5 (3H,d), 2.0 (1H,s), 2.5 (2H,t), 3.5 (2H,t), 4.0 (1H,q). The EI mass spectrum m/e (%) : 182(1.2,M⁺), 181(1.7), 165(0.7), 137(0.4), 122(0.8), 105(53.0), 104(100.0), 78(38.6). The IR confirmed the presence of a OH absorption at 3,300 cm⁻¹ and the absence of a S-H peak (2,645 cm⁻¹).

III) Incubation with Fungi

A) Incubation with Mortierella isabellina NRRL 1757

The growth medium used in the cultivation of the micro-organism Mortierella isabellina consisted of a mixture of glucose (40 g), soy grits (5 g), yeast extracts (5 g), sodium chloride (5 g), and dibasic potassium phosphate (5 g) per Litre of distilled water. The growth medium (150 mL) was transferred to one Litre flasks, stoppered with sponge plugs and sterilized in the autoclave at 15 psi and 121°C for fifteen minutes. Upon removal from the autoclave, the flasks were allowed to cool to room temperature. The fungus was transferred from the slopes into the growth medium under sterile conditions. One slope was usually enough to incubate approximately eight to eleven flasks. The flasks were set on a rotary shaker (180 rpm) for three days at 26°C.

After three days, the flasks were filtered and the isolated mycelial growth was washed a number of times with distilled water. The resulting mycelia were divided into equal parts and resuspended in distilled water (150 mL/flasks). The substrate was dissolved in 95% ethanol in the ratio of 1.0 g of substrate to 10 mL of solvent. To each flask was added approximately one mL of substrate solution. If volatile substrates were used, then the flasks were stoppered with rubber stoppers, otherwise sponge stoppers

were used and the incubations were carried out for 72 hours. After the incubation the medium was separated by filtration.

The medium layer was continuously extracted over a 72 hour period with dichloromethane, dried over anhydrous sodium sulphate, and evaporated using a rotary evaporator. The medium residue was weighed and chromatographed either by column using benzene-10% ether gradient or flash using mixtures of hexane and ethyl acetate as eluants. The fractions obtained were analysed by TLC and based on these results the appropriate fractions were chosen for ^1H NMR, ^{13}C NMR, IR, optical rotation, and mass spectrometric analysis.

The filtered fungus was blended twice in a Waring Blender with dichloromethane (200 mL) and the filtrate was dried over anhydrous sodium sulphate using the rotary evaporator. The residue was weighed and analysed by ^1H NMR.

B) Incubation with Aspergillus niger ATCC 9142

Aspergillus niger ATCC 337 was grown in a Czapek Dox broth medium (35 g/Litre). The growth medium (150 mL) was transferred to one Litre flasks. Standard sterilization, filtration, incubation, extraction, and analysis procedures as previously outlined under Incubation with Mortierella isabellina NRRL 1757 were followed.

C) Incubation with Helminthosporium NRRL 4671

The same procedures was followed for this fungus as those used for Aspergillus niger ATCC 9142 and Mortierella isabellina NRRL 1757. However, the growth medium used for Helminthosporium NRRL 4671 consisted of V-8 tomato juice (200 mL) and calcium carbonate (3 g) per Litre of distilled water, adjusted to a pH of 7.2.

D) Incubation with Rhizopus arrhizus ATCC 11145

The procedure was similar to those previously listed with two alterations: firstly, the growth medium consisted of a mixture of glucose (40 g), peptone (20 g), and corn steep liquor (5 mL) per Litre of distilled water. Secondly, prior to incubation with the substrate, the mycelia were blended in a Waring Blender for approximately two, one second periods and filtered.

IV) Incubation of toluenes and ethylbenzenes

A) Incubation of toluenes and ethylbenzenes

with Mortierella isabellina NRRL 1757

Toluene (24)

Incubation of commercially purchased 24 (1.0 g) gave extracts from the medium (0.20 g) and mycelium (0.18 g). Fungal material was the only product detected in the mycelium extract while the chromatographed medium extract gave benzyl alcohol (48) (70.0 mg). The ^1H NMR (80 MHz) of 48 included signals at δ : 1.5 (1H,s), 4.5 (2H,s), 7.2 (5H,s).

4-fluorotoluene (18)

Incubation of commercial 18 (1.0 g) yielded extracts from the medium (0.23 g) and mycelium (0.12 g). The mycelium extract contained the typical fungal material while the chromatographed medium extract gave 4-fluorobenzyl alcohol (46) (50.0 mg). The ^1H NMR (80 MHz) of 46 showed signals at δ : 2.4 (1H,s), 4.7 (2H,s), 7.3 (4H,AB).

3-fluorotoluene (60)

Incubation of commercially available 3-fluorotoluene (60) (1.0 g) gave extracts from the medium (0.22 g) and mycelium (1.5 g). The chromatographed medium extract yielded 3-fluorobenzyl alcohol (47) (65.0 mg) while the mycelium extract contained only fungal material. The ^1H NMR (80 MHz) of 47 showed signals at δ : 1.6 (1H,s), 4.7 (2H,s), 7.2 (4H,m).

Toluene (24) and toluene- α, α, α -d₃ (23)

Incubation of a mixture of 24 (0.56 g) and 23 (0.44 g) gave extracts from the medium (0.11 g) and mycelium (0.2 g). The mycelium contained the typical fungal product. 50.0 mg of benzyl alcohol (48) and benzyl alcohol- α, α -d₂ (49) was isolated from the chromatographed medium extract. The ^1H NMR (80 MHz) spectrum of the above included signals at δ : 4.7 (7.7 sq,s), 7.2 (27.0 sq,s).

Toluene- α -d₁ (2)

Incubation of 1 (1.0 g) yielded extracts from the medium

(0.19 g) and mycelium (0.28 g). Fungal material was the only product detected in the mycelium residue. The chromatographed medium extract gave 48 and benzyl alcohol- α -d₁ (3) (40.0 mg). The ¹H NMR (80 MHz) of 48 and 3 showed signals at δ : 4.7 (9.3 sq,s), 7.3 (33 sq, s).

Toluene- α, α -d₂ (6)

6 (1.0 g) afforded extracts from the medium (0.17 g) and mycelium (0.24 g). The chromatographed medium gave 50.0 mg of benzyl alcohol- α, α -d₂ (49) and benzyl alcohol- α -d₁ (3) while the mycelium indicated the presence of fungal material only. The ¹H NMR (200 MHz) of 3 and 49 showed signals at δ : 4.6 (4.02 sq,s), 7.3 (29.3 sq, s).

Toluene- α, α, α -d₃ (23)

23 (1.0 g) yielded extracts from the medium (0.33 g) and the mycelium (0.21 g). Typical fungal results were obtained from the mycelium while the crude medium extract gave ¹H NMR spectral data that was consistent with 49. The ¹H NMR (200 MHz) of the crude medium showed signals at δ : 7.2 (s).

Benzyl alcohol (48)

48 (0.5 g) gave 0.38 g of medium and 0.21 g of mycelium extracts. The ^1H NMR (200 MHz) of the crude medium showed signals at δ : 2.4 (1H,s), 4.5 (2H,s), 7.2 (5H,s). The mycelium gave fungal material only.

Incubation of 3-fluoroethylbenzene (10)

Incubation of commercial 10 (1.0 g) yielded extracts from the medium (0.20 g) and the mycelium (0.15 g). The mycelium extract contained typical fungal material while the chromatographed medium gave 1-(3'-fluorophenyl)ethanol (7) (110.0 mg). The ^1H NMR (80 MHz) of 7 showed signals at δ : 1.6 (2H,d), 4.9 (1H,q), and 7.0-7.6 (5H,m). $[\alpha]_{\text{D}}^{20} = +2.16^\circ$. 7 had an EE of 18% as determined from ^1H NMR of the benzylic proton in the presence of tris(3-heptafluoropropyl-hydroxymethylene-(+)-camphorato)-europium(III). Based on previous results 7 was assigned an R configuration.³⁶

Cross-Induction incubations with 24

Induction of the enzyme was carried out by the addition of a solution of ethylbenzene (30.0 mg in 7.5 mL of ethanol; 2 mg/0.5 mL) to the initial growth medium (1.5 Litres). After

the normal growth period (three days) the fungus was washed and resuspended in distilled water containing cycloheximide (0.1 mg/1 mL). Subsequent incubation with toluene (24) (100.0 mg/1 mL of ethanol) afforded, after normal incubation and extraction, 25.0 mg of benzyl alcohol (48). The ^1H NMR (200 MHz) spectra showed signals at δ : 4.6 (2H,s), 7.7 (5H,s).

Incubation of 24 (1.0 g) under non-induction conditions (absence of ethylbenzene), but in conditions where de novo protein synthesis was stopped with cycloheximide (0.1 mg/mL) during the incubation, gave no benzyl alcohol.

B) Incubation of toluenes with Helminthosporium NRRL 4671

Toluene (24)

Incubation of commercially acquired 24 (1.0 g) yielded extracts from the medium (0.09 g) and mycelium (0.12 g). ^1H NMR (200 MHz) run on both mycelium and medium residues showed the absence of benzyl alcohol (48); only fungal material was detected.

Benzyl alcohol (48)

Benzyl alcohol (48) (1.0 g) allowed for the crude recovery of 0.23 g of 48 from the medium. The mycelium extract contained the typical fungal material. The ¹H NMR (200 MHz) of the crude medium extract showed signals excluding fungal peaks at δ : 4.5 (2H,s), 7.3 (5H,s).

TABLE ONE

Incubation of toluenes with Mortierella isabellina (M.i.)
and Heliminthosporium species (H.s.)

Substrate	fungus	product	isolated yield (%)	benzylic H content
<u>24</u>	M.i.	<u>48</u>	7-10	-
<u>24</u>	H.s.	<u>48</u>	0-3	-
<u>48</u>	M.i.	<u>48</u> ⁺	76	-
<u>48</u>	H.s.	<u>48</u> ⁺	7	-
<u>2</u>	M.i.	<u>48</u> + <u>3</u>	4	1.40
<u>6</u>	M.i.	<u>49</u> + <u>3</u>	5	0.73
<u>23</u>	M.i.	<u>49</u>	10	-
<u>24</u> + <u>23</u> (56:44)	M.i.	<u>48</u> + <u>49</u>	5	1.42
<u>24</u> + cycloheximide	M.i.	-	-	-
<u>24</u> + cycloheximide*	M.i.	<u>2</u>	2.5	-

⁺ recovered

* enzymes induced by ethylbenzene

V) Incubation of 1,3-oxathiolanes

Incubation of 1,3-oxathiolanes

A series of eight 2-substituted-1,3-oxathiolanes were incubated with four types of fungi, Rhizopus arrhizus ATCC 11145, Mortierella isabellina NRRL 1757, Helminthosporium NRRL 4671, and Aspergillus niger ATCC 9142. Table two summarizes the results of these incubations. In order to simplify matters, results will only be given for those incubations which gave product(s). Furthermore, unless otherwise mentioned the mycelium residue contained only fungal product.

A) Incubation of 2-substituted-1,3-oxathiolanes with
Mortierella isabellina NRRL 17572-phenyl-1,3-oxathiolane (33)

Incubation of 33 (1.0 g) gave extracts from the medium (0.09 g) and from the mycelium (0.12 g). The chromatographed medium extract yielded 2-phenyl-1,3-oxathiolan-1-oxide (50)

(40.0 mg). The ^1H NMR (200 MHz) of 50 showed signals at δ : 2.5-2.9 (2H,m), 4.4-4.8 (2H,m), 5.4 (1H,s), 7.4 (5H,s). Expansion of the ^1H NMR (200 MHz) between 2.0 and 5.5 ppm gave the following signals at δ : 2.66, 2.71, 2.72, 2.74, 2.76, 2.77, 2.80, 2.81, 2.83, 2.87 (1H,m) (1); 3.0, 3.06, 3.10, 3.12, 3.15, 3.16, 3.18, 3.19 (1H,m) (2); 4.45, 4.47, 4.50, 4.51, 4.52, 4.53, 4.55, 4.57 (1H,m) (3); 4.71, 4.75, 4.76, 4.79 (1H,m) (4) (see figure thirty-nine for relative stereochemistry, page 138). The ^{13}C NMR (50.3 MHz) showed signals at δ : 52.66, 69.84, 11.65, 125.90, 128.90, 129.38, 133.2. The EI mass spectrum m/e (%) : 182(16.0, M^+), 154(26.6), 126(11.8), 122(3.5), 121(3.2), 107(6.5), 105(100.0), 77(65.0). $[\alpha]_{\text{D}}^{20} = +0.008^\circ$ (ethanol).

2-methyl-2-phenyl-1,3-oxathiolane (29)

Incubation of the title compound (1.0 g) yielded extracts from the medium (0.26 g) and mycelium (0.20 g). The chromatographed medium extract gave 29 (10.0 mg), acetophenone (30) (20.0 mg), phenyl ethanol (52) (20 mg), and 2-methyl-2-phenyl-1,3-oxathiolan-1-oxide (51) (50 mg, m.p. 68-75°C). The ^1H NMR (80 MHz) of 29 , 30 , and 52 were similar to standard and previously obtained ^1H NMRs.

The ^1H NMR (200 MHz) of 51 gave signals at δ : 1.7 (3H,s), 2.7-2.8 (1H,m), 2.9-3.0 (1H,m), 4.3-4.5 (1H,m), 4.6-4.8 (1H,m), 7.3 (5H,s). Expansion of the above ^1H NMR

between 2.0 and 5.5 ppm gave the following signals at δ :
 2.73, 2.76, 2.77, 2.79, 2.81, 2.83, 2.84, 2.88 (1H,m) (1);
 2.93, 2.95, 2.96, 2.98, 2.99, 3.01, 3.03, 3.05 (1H,m) (2);
 4.34, 4.36, 4.38, 4.39, 4.40, 4.41, 4.43 (1H,m) (3); 4.63,
 4.66, 4.68, 4.71, 4.72, 4.75 (1H,m) (4) (see figure
 thirty-seven for relative stereochemistry, page 135). The
 following coupling constants (in Hz) were measured; $J_{1,2 \text{ gem}} =$
 14.7, $J_{1,4 \text{ trans}} = 6.6$, $J_{1,3 \text{ cis}} = 6.4$, $J_{2,4 \text{ cis}} = 7.5$, $J_{2,3 \text{ trans}} =$
 5.3, $J_{3,4 \text{ gem}} = 10.5$. A n.o.e of 50% was observed for
 proton 2 and 10% for 3 upon irradiation at 1.7 ppm. The ^{13}C
 NMR (50.3 MHz) showed signals at δ : 22.17, 51.13, 68.11,
 106.72, 125.22, 128.50, 128.88, 139.03. The FAB mass spectrum
 m/e (%) : 197(13.3, M+1), 196(19.3), 126(5.9), 121(34.3),
 105(20.8), 103(6.8), 97(13.9), 77(71.1). The IR spectrum
 showed a typical S=O absorption between 1400-1500 cm^{-1} . $[\alpha]_D^{20} = + 0.08^\circ$ (ethanol). 51 had a melting point of
 76.5-79.0°C.

29 at pH 7.1

Incubation of 29 (1.0 g) in a buffered solution at pH 7.1
 for the normal incubation period (3 days) afforded extracts
 from the medium (0.21 g) and mycelium (0.16 g). The medium
 extract yielded acetophenone (30) (30.0 mg) as determined by
 ^1H NMR (80 MHz).

29 with Dead Mortierella isabellina NRRL 1757

29 (1.0 g) was incubated with Mortierella isabellina as previously described under Incubation with Mortierella isabellina NRRL 1757. However, prior to addition of the substrate, the fungus was autoclaved for 20 minutes in order insure the denaturation of all the enzymes. After a normal incubation and extraction period, extracts were obtained from the medium (0.86 g) and mycelium (0.26 g). The medium residue gave a clean ^1H NMR (80 MHz) spectrum of the title compound δ : 1.9 (3H,s), 2.4-3.3 (2H,m), 3.8-4.5 (2H,m), 7.1-7.5 (5H,m).

2,2-diphenyl-1,3-oxathiolane (31)

2,2-diphenyl-1,3-oxathiolane (1.0 g) gave extracts from the medium (0.22 g) and mycelium (0.15 g). The crude medium residue yielded diphenyl methanol (54). The ^1H NMR (80 MHz) for the crude product gave signals at δ : 2.2 (1H,s), 5.9 (1H,s), 7.2 (10H,s).

2-methyl-2-tert. butyl-1,3-oxathiolane (40)

40 (1.1 g) gave extracts from the medium (0.25 g) and mycelium (0.16 g). The chromatographed medium extract gave 40

(0.015 g), tert. butyl methyl ketone (41) (0.01 g), 2,2-dimethyl-3-butanol (55) (0.02 g), and 28 (0.01 g). The ^1H NMR of the above four compounds were identical to the standard and previously obtained ^1H NMRs. $[\alpha]_D^{20} = + 0.5^\circ$ (ethanol).

Tert. butyl methyl ketone (41)

Tert. butyl methyl ketone (41) (2.0 g) gave extracts from the medium (1.52 g) and mycelium (0.26 g). The crude extract yielded 2,2-dimethyl-3-butanol (55). The ^1H NMR of 55 gave signals at δ : 0.9 (9H,s), 1.2 (3H,d), 2.3 (1H,s), 4.5 (1H,q). $[\alpha]_D^{20} = + 0.6^\circ$ (ethanol).

40 with Dead Mortierella isabellina NRRL 1757

Incubation of 40 (0.5 g) as outlined under the Incubation of 29 with Dead Mortierella isabellina NRRL 1757 gave extracts from the medium (0.16 g) and the mycelium (0.10 g). The crude medium extract yielded starting material (40) as determined by ^1H NMR.

2-phenyl-2-tert. butyl-1,3-oxathiolane (27)

Incubation of 27 (1.0 g) gave extracts from the medium (0.22 g) and mycelium (0.16 g). The chromatographed medium extract afforded 26 (10.0 mg), 25 (30.0 mg), and dl-threo-2-phenyl-2-tert. butyl-1,3-oxathiolan-1-oxide (53) (30.0 mg). The ^1H NMR (200 MHz) obtained for 25 and 26 were the same as those obtained in the preparation of 25 and 26, respectively.

The ^1H NMR (200 MHz) of 53 including the expansion between 2.0 and 5.5 ppm gave signals at δ : 1.2 (9H,s); 2.44, 2.48, 2.51, 2.53, 2.55, 2.60 (1H,m) (1); 2.82, 2.84, 2.85, 2.87, 2.89, 2.90, 2.92, 2.94 (1H,m) (2); 4.18, 4.19, 4.22, 4.24, 4.27, 4.29 (1H,m) (3); 4.76, 4.78, 4.82, 4.86 (1H,m) (4); 7.4 (5H,s) (see figure forty-one for relative stereochemistry, page 142). There was no observable n.o.e on protons 1, 2, 3, and 4 when the tert. butyl group was irradiated. The following coupling constants (in Hz) were measured; $J_{1,2 \text{ gem}} = 13.6$, $J_{1,3 \text{ trans}} = 7.3$, $J_{1,4 \text{ cis}} = 6.4$, $J_{3,4 \text{ gem}} = 8.9$, $J_{4,2 \text{ trans}} = 6.9$, $J_{2,3 \text{ cis}} = 5.2$. The ^{13}C NMR (50.3 MHz) showed signals at δ : 26.96, 39.43, 50.74, 67.68, 112.90, 127.0, 128.2, 128.4, 136.10. The IR indicated the presence of a S=O absorption between 1400-1500 cm^{-1} . Melting point of 53 was 63-67°C. $[\alpha]_{\text{D}}^{20} = -13.3^\circ$ (ethanol). 53 had an EE of 4% as determined from the tert. butyl signal using a europium shift reagent.

41 with dead Mortierella isabellina

Tert. butyl methyl ketone (41) (0.5 g) gave extracts from the medium (0.23 g) and the mycelium (0.09 g). The mycelium afforded fungal material while the ¹H NMR (80 MHz) of the crude medium was identical to 41 .

B) Incubation of 2-substituted-1,3-oxathiolanes
with Rhizopus arrhizus ATCC 11145

2-phenyl-2-tert. butyl-1,3-oxathiolane (27)

Incubation of 27 (1.0 g) yielded medium (0.22 g) and mycelium (0.16 g) extracts. The chromatographed medium afforded 2,2-dimethylpropiophenone (26) (10.0 mg), 2,2-dimethyl-1-phenyl-1-propanol (25) (30.0 mg), and 2-phenyl-2-tert. butyl-1,3-oxathiolan-1-oxide (53) (30.0 mg, M.p. 63-67°C). ¹H NMR (80 MHz) of 25 and 26 are consistent with previous ¹H NMRs that have been obtained.

The ¹H NMR (200 MHz) of 53 including the expanded region between 2.0 and 5.5 ppm gave signals at δ : 1.2 (9H,s); 2.44, 2.48, 2.51, 2.53, 2.55, 2.60 (1H,m) (1); 2.82, 2.84, 2.85, 2.87, 2.89, 2.90, 2.92, 2.94 (1H,m) (2); 4.18, 4.19, 4.22, 4.27, 4.29 (1H,m) (3); 4.76, 4.78, 4.82, 4.86 (1H,m) (4), 7.4 (5H,s) (see figure forty-one for relative stereochemistry, page 42). The coupling constants measured, within experimental error, are the same as those previously observed for 53 (Incubation of 27 with Mortierella isabellina NRRL 1757). The ¹³C NMR (50.3 MHz) showed signals at δ : 26.89, 39.44, 50.75, 67.69, 112.95, 126.91, 128.18, 128.41, 136.09. FAB mass spectrum m/e (%) : 239(49.7,M+1), 196(5.9), 182(3.6),

164(92.6), 162(4.6), 146(24.0), 119(31.0), 105(66.7). IR absorption confirmed the presence of a S=O stretch, 1400-1500 cm^{-1} . Melting point of 53 was 62-67°. $[\alpha]_D^{20} = + 0.5^\circ$ (ethanol).

2,2-diphenyl-1,3-oxathiolane (31)

31 (1.0 g) afforded a medium extract (0.22 g) which yielded upon chromatography diphenyl methanol (54) (0.05 g). The ^1H NMR (80 MHz) of 54 showed signals at δ : 5.9 (1H,s), 7.4 (10H,s).

TABLE TWO

Incubation of 1,3-oxathiolanes with Rhizopus arrhizus
(R.a.), Mortierella isabellina (M.i.),
Helminthosporium (H.s.), and Aspergillus niger (A.n)

Substrate	R.a.	M.i.	H.s.	A.n.
<u>33</u>	*	<u>50</u>	*	*
<u>29</u>	-	<u>51</u> , <u>52</u> , <u>29</u> , <u>30</u>	-	-
<u>29</u> (ph = 7.1)	*	-	*	*
<u>29</u> (dead)	*	<u>29</u>	*	*
<u>27</u>	<u>53</u> , <u>26</u> , <u>25</u>	<u>53</u>	-	-
<u>31</u>	<u>54</u>	<u>54</u>	-	-
<u>35</u>	-	-	-	-
<u>36</u>	-	-	-	-
<u>38</u>	-	-	-	-
<u>40</u>	-	<u>40</u> , <u>41</u> , <u>55</u>	-	-
<u>40</u> (dead)	*	<u>40</u>	*	*
<u>41</u>	*	<u>51</u>	*	*
<u>41</u> (dead)	*	<u>41</u>	*	*

* incubation not performed.

- incubation performed but no results were obtained.

VI) Incubation of toluenes and ethylbenzenes

with Mortierella isabellina NRRL 1757 using ^2H NMR

A portion of mycelia from the regular growth of Mortierella isabellina as previously described (see Incubation with Fungi) was dried between pieces of filter paper, washed with deuterium depleted water (3 * 2 mL), and resuspended in 2 mL of deuterium depleted water in a 10 mm NMR tube. To the NMR tube containing the fungal species was delivered 20 uL of a 100 mg of substrate per mL of ethanol solution or, 2 mg of substrate. The NMR tube was stoppered and sealed. The ^2H NMR (30.7 MHz) was taken at approximately 24 hour intervals. During the intervals in which the ^2H NMR (30.7 MHz) spectra were not being acquired, the NMR tubes and their contents were placed on a rotary shaker.

$4'$ -fluoroethylbenzene-1,1- d_2 (17),
 $3'$ -fluoroethylbenzene-1,1- d_2 (13), ethylbenzene-1,1- d_2 (45), toluene- d_8 (61), and a blank were studied by this method. The blank was incubated with 20 uL of ethanol (substrate excluded) and the ^2H NMR (30.7 MHz) obtained over the next five days indicated no noticeable change. The results obtained for 17 , 13 , 45 , and 61 are given in tables three and four.

TABLE THREE

Deuterium content of substrate and product as determined
by ^2H NMR

Day	<u>17</u>	<u>15</u>	<u>13</u>	<u>11</u>	<u>45</u>	<u>58</u>	<u>62</u>	<u>61</u>
1	.05	2.0	.08	2.0	.06	2.0	.128	3.0
2	.17	2.03	.10	2.0	.264	2.04	.382	2.53
3	.26	2.0	.34	2.0	.55	2.0	.653	2.25
4	.50	2.0	.50	2.0	.84	2.0	.85	2.25
5	.49	2.0	.50	2.0	1.11	2.02	1.62	2.75

TABLE FOUR

Percent conversion of deuterated substrate by
Mortierella isabellina determined by ^2H NMR

Day	<u>17</u>	<u>15</u>	<u>13</u>	<u>11</u>	<u>45</u>	<u>58</u>	<u>62</u>	<u>61</u>
1	-		-		-		-	
2	7.5		5.0		11.5		8.5	
3	12.0		14.0		21.5		17	
4	20.0		20.0		30.0		24	
5	19.7		20.0		35.5		34	

errors from NMR suggest conversion $\pm 2\%$

VII) Incubation of toluene-1-¹³C (59)

with Mortierella isabellina NRRL 1757 using ¹³C NMR

Using the procedure outlined above, 2 mg of commercially purchased 59 (20 uL of a 100 mg per mL of ethanol solution) was incubated and monitored by ¹³C NMR (50.3 MHz) over a five day period. In order to facilitate integration of results, the FID spectrum was acquired using a Inverse Gated Decoupling (INVGATE.AU) routine. ¹³C NMR (50.3 MHz) spectra obtained over the five day interval were identical to the initial spectrum of 59 and ethanol obtained on day one.

VIII) Incubation of Miscellaneous Substrates with Mortierella isabellina NRRL 1757

1-phenyl-2'-hydroxyethyl sulphide (42)

Incubation of the above title substrate (1.0 g) yielded extracts from the medium (0.52 g) and the mycelium (0.21 g). The chromatographed medium residue afforded 56 (58.0 mg). The ^1H NMR (80 MHz) of 56 gave signals at δ : 1.1 (2H,t), 1.6 (3H,d), 2.3 (2H,t), 3.1 (1H,s), 3.6 (1H,q), 7.2 (5H,s). The FAB mass spectrum m/e (%) : 199(30.5,M+1), 165(0.8), 154(3.6), 137(2.5), 136(3.6), 121(2.2), 120(0.9), 105(100.0). S=0 stretch was evident in the IR spectrum, 1400-1500 cm^{-1} . $[\alpha]_D^{20} = -0.85^\circ$ (ethanol). M.p. 77-79.7°C.

Incubation of 4-methyl anisole (44)

44 (1.0 g) gave 0.36 g of medium residue. The ^1H NMR (80 MHz) of the crude medium residue gave signals at δ : 2.3 (19sq,s), 3.7(23sq,s), 4.6(6sq,s), 6.8-7.5 (42sq,m). Based on the ^1H NMR data the crude residue was a mixture of 44 and 57 , in a 66 to 33% ratio, respectively.

IX) Attempted Chemical Synthesis of 1,3-oxathiolan-1-oxides

Oxidation of 29 with Sodium meta-periodate

The title compound (1.68 g, 0.009 moles) was dissolved in approximately 50 mL of methanol and stirred. To this solution was added dropwise over a 10 minute interval a solution of 2.0 g (0.009 moles) of sodium meta-periodate in 10 mL of water. The resulting mixture was allowed to stir for 24 hours.

After 24 hours, the reaction mixture was extracted with dichloromethane (3 * 40 mL). The dichloromethane extracts were washed with water (1 * 25 mL), sodium bicarbonate solution (1 * 25 mL), water (1 * 25 mL), and dried over anhydrous sodium sulphate. The dichloromethane was removed using the rotary evaporator. The ^1H NMR (80 MHz) spectrum of the crude extract (1.2 g) gave signals that were consistent with 30 .

This procedure was repeated using 1.0 g (0.006 moles) of 29 and 1.0 g (0.006 moles) of sodium meta-periodate for reaction periods of 1, 2, 4, and 8 hours. In each case, the only recoverable product was 30

Oxidation of 29 with meta-chloroperoxybenzoic acid

29 (1.9 g, 0.009 moles) was dissolved in 50 mL of

dichloromethane and refrigerated at 4°C. Similarly, 2.1 g (0.01 moles) of meta-chloroperoxybenzoic acid was dissolved in 50 mL of dichloromethane and refrigerated at 4°C. The meta-chloroperoxybenzoic acid solution was slowly added to 29 and the resulting reaction mixture was allowed to stir while refrigerated for 24 hours.

After 24 hours, the reaction mixture was washed with water (1 * 25 mL), sodium bicarbonate (3 * 20 mL), water (1 * 25 mL), and dried over sodium sulphate. The ¹H NMR (80 MHz) spectrum of the crude extract (1.5 g) gave signals that were identical to those of 30.

Similar reactions worked up after 1, 2, 4, and eight hours afforded, in each example, 30 as the only recoverable product.

Oxidation of 27 with meta-chloroperoxybenzoic acid

27 (0.25 g, 0.001 moles) was dissolved in 500 mL of dichloromethane and stirred. Meta-chloroperoxybenzoic acid (0.20 g, 0.01 moles) in 50 mL of dichloromethane was added to the above solution and allowed to stir for two hours. Similar work up as outlined in the oxidation of 29 with meta-chloroperoxybenzoic acid yielded only 26 (0.16 g) as indicated by the ¹H NMR (80 MHz) spectrum.

X) Decomposition study of

dl-threo-2-methyl-2-phenyl-1,3-oxathiolan-1-oxide (51)

A sample of 51 suspended in a sealed NMR tube containing acetonitrile- d_3 as solvent (no internal standard) gave a 1H NMR (200 MHz) spectrum of the decomposition product(s) after three days. The decomposition product(s) showed signals at δ : 1.9 (3H,s), 2.1-2.2 (1H,m), 2.25 (1H, broad s), 2.6-2.7 (1H,m), 3.05-3.18 (1H,m), 3.16-3.19 (1H,m), 6.8-7.0 (3H,m), 7.2-7.4 (2H,m). The signals at δ 1.9, 6.8-7.0, and 7.2-7.4 correspond to acetophenone (30). The nature of the signals at δ 2.1-2.2, 2.25, 2.6-2.7, and 3.05-3.18 must arise from the other decomposition product(s).

The ^{13}C NMR (50.3 MHz), due to the intense signal from the solvent (acetonitrile- d_3), gave no additional useful information.

Discussion

Preparation of Labelled Substrates

In this investigation, hydroxylation of a series of toluenes and ethylbenzenes deuterated in the benzylic position were studied because P-450 catalyzed hydroxylations have been shown to occur at the benzylic position⁷⁴.

The synthetic strategy employed in introducing deuterium at the benzylic position in these substrates required the reduction of the carbonyl function or the corresponding chloride with lithium aluminum deuteride and lithium triethyl borodeuteride (super deuteride), respectively.

Preparation of 3-fluoroethylbenzene-1,1-d₂ and 4-fluoroethylbenzene-1,1-d₂ (13 and 17, respectively) was achieved in a three step synthesis (figure twenty-five). The first step required the reduction of the fluoro substituted acetophenones, 8 and 14, with lithium aluminum deuteride to give the corresponding fluoro substituted deuterated fluorophenylethanol 11 and 15 in high yield. The second step, the addition of thionyl chloride to 11 and 15 gave, in very good yield, the chlorides 12 and 16. The third and final step in the synthesis required the reduction of the aforementioned chlorides with the reducing agent super deuteride. Contrary to the previous two steps, 13 and 17 were obtained in very low yields. The low yields afforded have been attributed to difficulties associated in separating the products, 13 and 17, from the solvent (pentane).

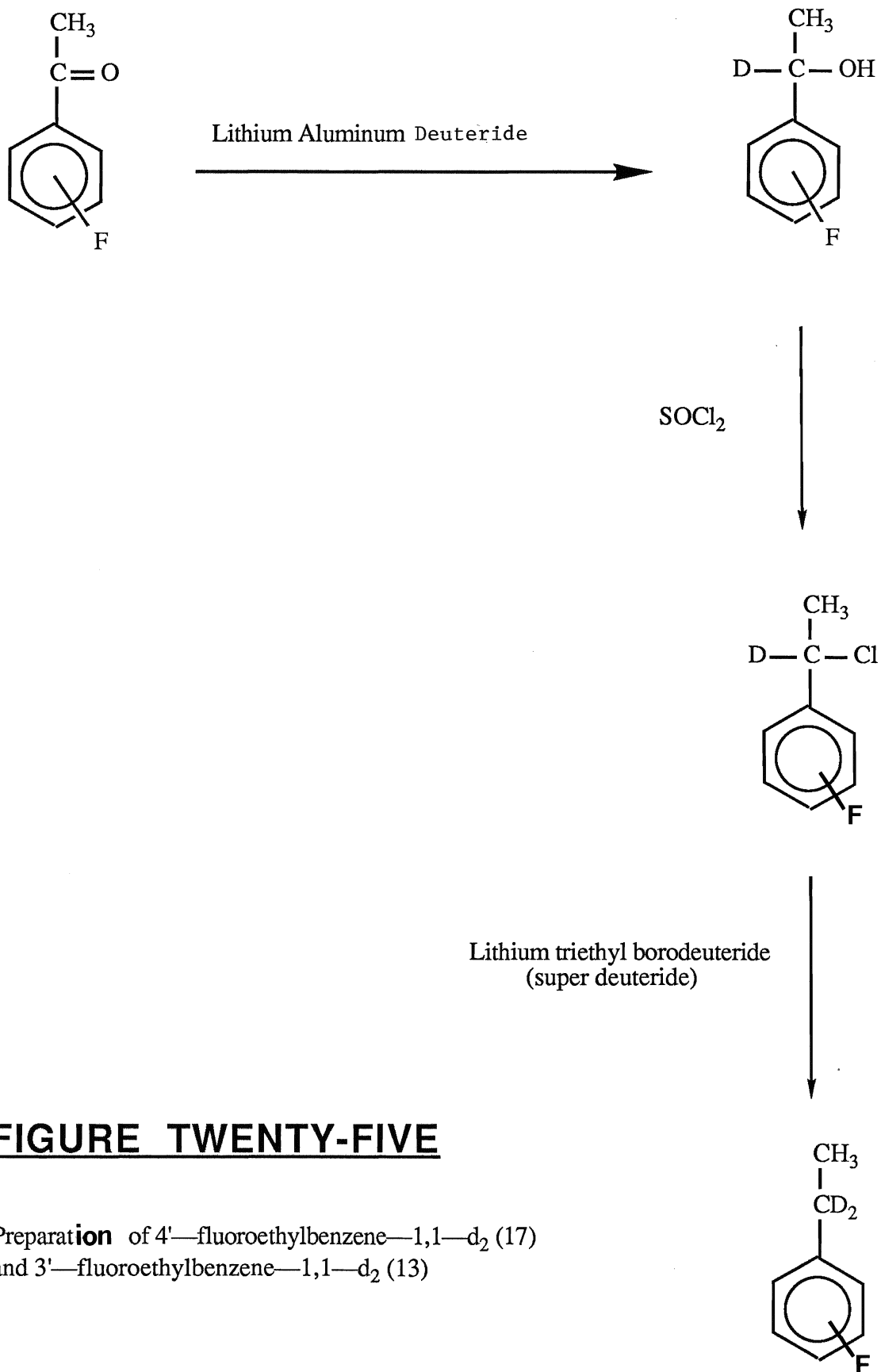


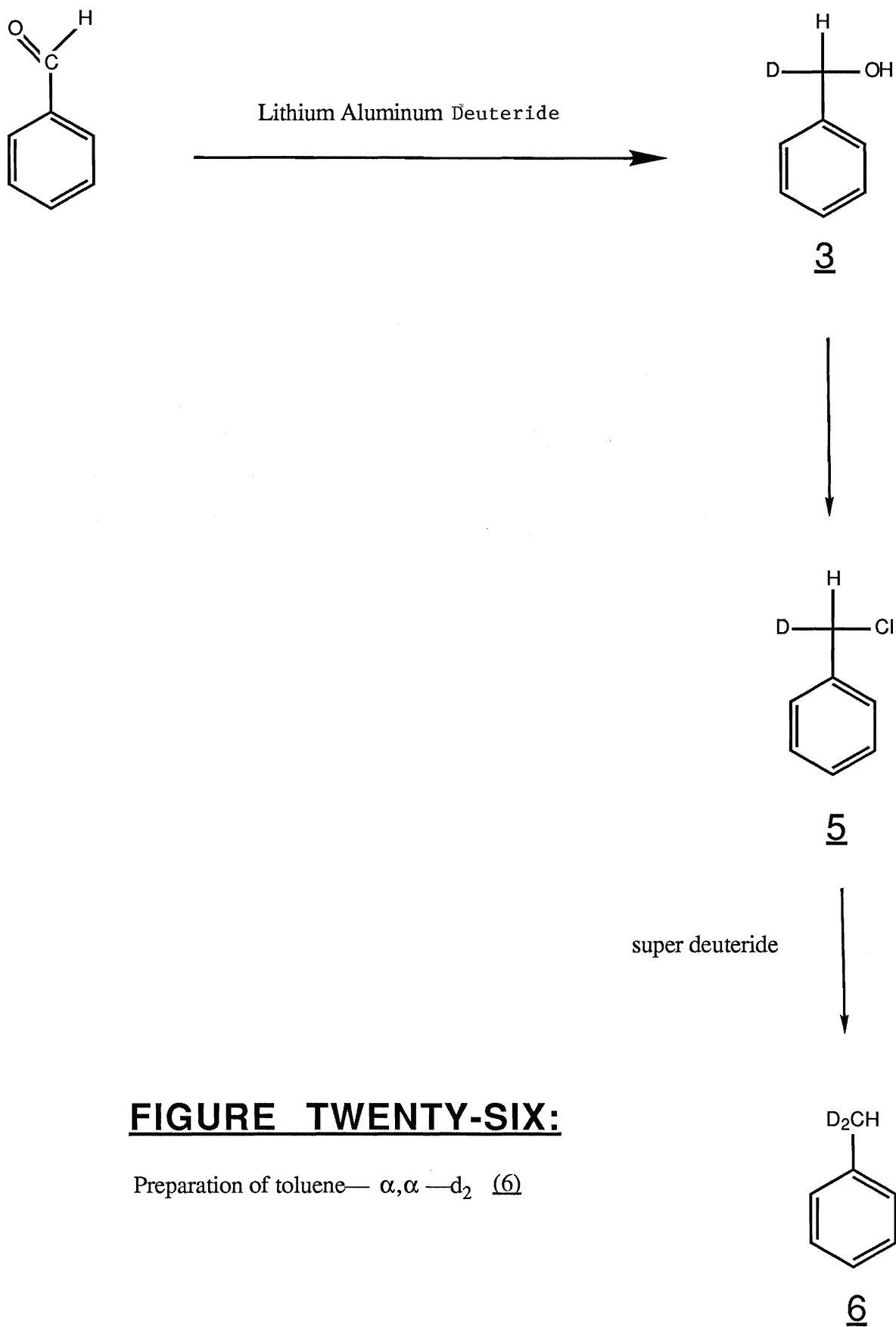
FIGURE TWENTY-FIVE

Preparation of 4'-fluoroethylbenzene-1,1-d₂ (17)
and 3'-fluoroethylbenzene-1,1-d₂ (13)

Toluene- α, α -d₂ was prepared in a similar three step synthesis (figure twenty-six). The first step was the reduction of benzaldehyde (4) with lithium aluminum deuteride to the corresponding alcohol, benzyl alcohol-1-d₁ (3), in good yield. Addition of thionyl chloride (the second step) yielded benzyl chloride-1-d₁ (5) in high yield. The final step in the synthesis, reduction of 5 with super deuteride afforded toluene- α, α -d₂ in low yield. Similar difficulties associated with the final step in the synthesis of 13 and 17 were responsible for the low yield of 6 .

Toluene- α -d₁ (2) was obtained in a one step reduction utilizing commercially available benzyl chloride (1) and super deuteride (figure twenty-seven). A low yield for reasons previously discussed was observed.

The syntheses of 4- and 3-fluorotoluene- α, α, α -d₃ (20 and 21) was accomplished in one step (figure twenty-eight). Treatment of the precursor fluoro substituted bromobenzenes (19 and 22) with a strong base, tert. butyl lithium, generated in situ fluorophenyl lithium. Addition of deuterated methyl iodide (CD₃I) gave 20 and 21 in low yield. Although this method proved successful, the presence of a low boiling hydrocarbon(s) in very low concentrations proved to be detrimental to the fungus.

**FIGURE TWENTY-SIX:**

Preparation of toluene— α,α —d₂ (6)

Reversibility Study

The mammalian metabolism of toluene (24), for which the fungal hydroxylation serves as a model, produces benzoic acid as the ultimate metabolite⁷⁵. However, prior to the formation of benzoic acid, 24 is initially converted to benzyl alcohol (48)⁷⁶.

We have shown that the fungal species Mortierella isabellina and Helminthosporium are both capable of biotransforming toluene (24) to benzyl alcohol (48). However, Helminthosporium appears capable of further metabolizing the primary metabolite, 48 , as evident by the low recoveries obtained in the control experiments using 24 and 48 as substrates for the fungal biotransformation.

Similar control experiments utilizing 48 as a substrate with Mortierella isabellina gave 48 in high yield. This result suggests that Mortierella isabellina , unlike Helminthosporium is not capable of further metabolizing 48 . This does not exclude the possibility that 48 , in the case of Mortierella isabellina , is further oxidized to a carbonyl and then subsequently reduced by the fungus to 48 .

Although it may appear that Mortierella isabellina is not capable of further converting 48 , the above control experiment does not exclude the possibility that 48 with Mortierella isabellina is oxidized to the benzaldehyde (4)

and subsequently is reduced by the fungus to 48 . As a result it may appear that 48 is not further metabolized by Mortierella isabellina .

It has been demonstrated in a previous study that Mortierella isabellina is capable of reducing ketones to alcohols⁷⁷. Incubation of acetophenone (30) and 4-ethylacetophenone with Mortierella isabellina gave phenylethanol (52) and 4-ethylphenylethanol, respectively.

The possible oxidation of 48 to the corresponding benzaldehyde (4) by NAD^+ followed by reduction to 48 by NADPH would result in the loss of deuterium label (figure twenty-nine)⁷⁸. Such a loss of deuterium label would invalidate any isotope effects whether they be inter- or intramolecular.

The results obtained from the incubation of commercially purchased toluene- α, α, α - d_3 (23) with Mortierella isabellina indicated that the reversible reaction proposed in figure twenty-nine was not occurring evident by the absence of any unlabelled benzyl alcohol (48) in the crude medium extract. The only compound detected was benzyl alcohol- α, α - d_2 (49).

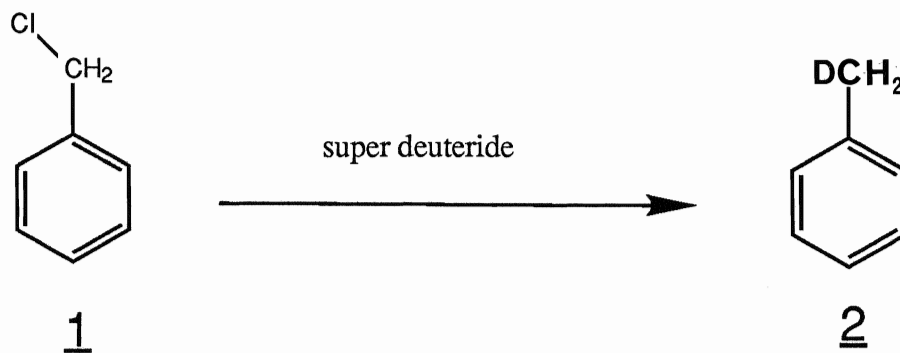


FIGURE TWENTY-SEVEN:

Preparation of toluene— α — d_1 (2)

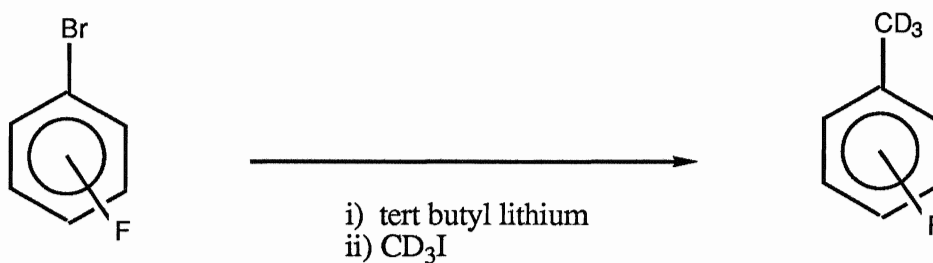


FIGURE TWENTY-EIGHT:

Preparation of 4—fluorotoluene— α,α,α — d_3 (20)

and 3—fluorotoluene— α,α,α — d_3 (21)

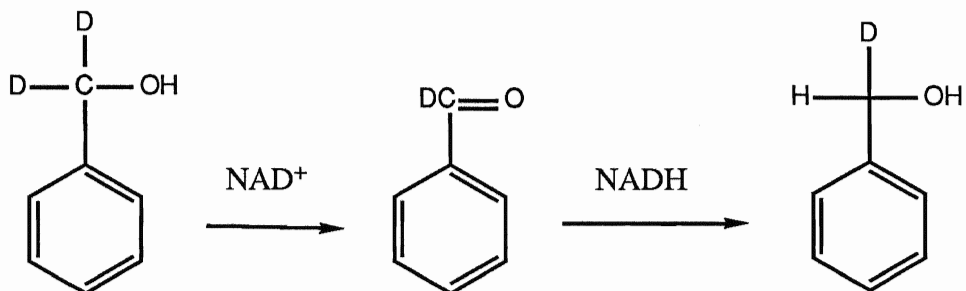


FIGURE TWENTY-NINE:

Reversibility Study

Inter- and Intramolecular Isotope Effects

Hanzlik and co-workers utilizing hydroxylase enzymes found in rat-liver microsomes have investigated the mammalian metabolism of toluene (24)⁷⁶. Their study yielded inconsistent primary (p) and secondary (s) isotope effects for substrates 2 and 6. According to their data analysis (outlined in Table six) the ratio of r_1/r_2 should theoretically be equal to four, however, they obtained from their data a value of 2.13 for r_1/r_2 . This number of 2.13 reflects a variant p/s ratio of 3.01 and 5.64 for the hydroxylations of 2 and 6, respectively.

The results that we have obtained from the incubations of 2, 6, and 23 : 24 with Mortierella isabellina given in Tables one, five, and six are internally consistent. The hydroxylation of 2 and 6 both gave deuterium isotope effect ratio (p/s) of 0.75 and an r_1/r_2 equal to 4 (as required by the method of analysis). It should be noted that the calculation of kinetic isotope effects directly from products is valid at only low degrees of conversion⁷⁹, the situation which exists here.

The results obtained from our study do not allow for the unequivocal deconvolution of p (intramolecular primary isotope effect), p^* (intermolecular primary isotope effect), and s (secondary isotope effect) without at least assuming a limiting value for one of these terms. If the assumption is made that

the minimum value for the intramolecular primary kinetic effect (k_H/k_D) is one, then the secondary effect is 1.33 ± 0.06 . Furthermore, assuming that p and p^* are similar gives $p = p^* = 1.02 \pm 0.05$ and $s = 1.37 \pm 0.06$. These values are all internally consistent with the ratios proposed in Table six. Although inter- and intramolecular primary kinetic isotope effects for cytochrome P-450 catalyzed hydroxylations are usually different^{38,59} precedent does exist for intrinsic, intramolecular, primary isotope effects which are very low⁸⁰ and identical to intermolecular effect⁸¹.

In an attempt to demonstrate the method of analysis used to arrive at these isotopic values (p , p^* , s), the incubation of toluene- α -d₁ (2) with Mortierella isabellina will be used as an example. The incubation of toluene- α -d₁ (1.0 g) gave after purification by column, 40.0 mg of product. The product from this incubation is a mixture of benzyl alcohols: benzyl alcohol (48) and benzyl alcohol- α -d₁ (3). The ¹H NMR (80 MHz) of the product mixture (48 and 3) gave signals at 4.7 (9.3 sq, s) and 7.3 (33 sq, s). The signal at 7.3 represents the total number of aromatic protons which in this case is five, whereas the signal at δ 4.7 represents the total number of benzylic protons resulting from 48 and 3 .

The number of benzylic protons are presently unknown but can be determined if one assumes the following: Since 33 squares represents five protons then one proton is equivalent to 6.6 squares ($33/5 = 6.6$). According to this relationship, one proton is equal to 6.6 squares, the number of benzylic

protons (or the total benzylic content of the product) is 9.3/6.6 or 1.40 protons (see Table one).

The benzylic content is then interpreted as a % composition of 48 and 3 which is 40% and 60%, respectively (see table six). The loss of hydrogen versus the loss of deuterium or the product ratio is then represented as a ratio of 3 versus 48 (since 3 arises from the loss of hydrogen and 48 from loss of deuterium).

The product ratio of 1.5 is composed of primary and/or secondary isotope effects. In this case the loss of hydrogen which results in 3 is subjected to a double primary isotope effect (since there is an equal probability that either proton may have been abstracted). Similarly, 48 which arises from the loss of deuterium is subjected to one inverse secondary effect. As a result, the product ratio (loss of hydrogen (H):loss of deuterium (D)) is given by $2p/s$.

The approach outlined above for the product obtained from the incubation of toluene- α -d₁ (2) with Mortierella isabellina was repeated for toluene- α , α -d₂ (6) and toluene:toluene- α , α , α -d₃ (24 + 23). These results were then used as outlined in Table six to give final values for p, p*, and s.

The inter- and intramolecular isotope effects (p* and p, respectively) observed in this investigation, within experimental error, can be reported as unity. As previously discussed in the introduction, intermolecular isotope effects

TABLE FIVE

Kinetic isotope effect for toluene hydroxylation by

Mortierella isabellina

Substrate	product (%) [*]	ratio	
		H loss/D loss	composed of ⁺
<u>2</u>	<u>48</u> (40) + <u>3</u> (60)	1.5 ± 0.05	2p/s
<u>6</u>	<u>49</u> (27) + <u>3</u> (73)	0.37 ± 0.015	p/2s
<u>24</u> + <u>23</u> (56:44)	<u>48</u> (71) + <u>49</u> (29) <u>48</u> (66.5) + <u>49</u> (34.5) ⁻	1.92 ± 0.0.06	p*s ²

⁻ corrected for 1:1 substrate ratio⁺ p = intramolecular primary k_H/k_Ds = secondary k_H/k_Dp^{*} = intermolecular primary k_H/k_D^{*} ± 2%

TABLE SIX

Isotope effect ratios for hydroxylation of 2 , 6 , and 24

<u>2</u>	PhCH ₂ D	PhCH ₂ OH	+	PhCHDOH
	product composition	=	x%	+ (100-x)%
	product H ₁ content	=	2x	+ (100-x)
		=	(100+x)	= 1.40*
		x =	40%	
	product ratio ⁺	=	(100-x)/x = 2p/s = 1.50 ⁻	= r ₁
<hr/>				
<u>6</u>	PhCD ₂ H	PhCHDOH	+	PhCD ₂ OH
	product composition	=	y%	+ (100-y)%
	product H ₁ content	=	y	= 73%*
	product ratio ⁺	=	(100-y)/y = p/2s = 0.37 ⁻	= r ₂
<hr/>				
<u>24</u>	PhCD ₃ + PhCH ₃	PhCD ₂ OH	+	PhCH ₂ OH
	product composition	=	z%	+ (100-z)%
<u>23</u>	product H ₁ content	=	2(100-z)	= 1.42*
		z =	29%	
	product ratio ⁺	=	(100-z)/z	= 2.45*
	corrected product ratio	=	(2.45*44)/56 = 1.92 = p*s ²	-

* see Table TWO

⁻ see Table FIVE⁺ loss of H:loss of D.

observed in biological systems are frequently lower than those observed in chemical reactions⁸². These observed lower values are attributed to a kinetically complex reaction in which the removal of hydrogen or deuterium is only partially rate determining. The rate-determining step in the cytochrome P-450 cycle (figure two) has been reported as the addition of the second electron⁸³ and the decomposition of the P-450 substrate-oxygen complex to product⁸⁴.

A value greater than unity for the intermolecular isotope effect would suggest that the hydroxylation step is rate or partially rate-determining. If p^* had been greater than unity the results could have lent support to a radical abstraction-recombination mechanism. However, since this value is unity and the certainty of the rate-determining step is questionable, the intermolecular isotope effect is inconclusive.

The intramolecular isotope effect (p), in view of complicating factors discussed in the introduction, is potentially more useful than the intermolecular effect in providing data for mechanistic interpretation^{37,85}. A large intramolecular isotope effect would have been consistent with a radical abstraction-recombination mechanism. This does not, however, mean that our intramolecular isotope of unity is not consistent with the aforementioned mechanism.

The maximum observable isotope effect occurs when hydrogen or deuterium are shared equally between the abstracting species and the carbon to which it is originally

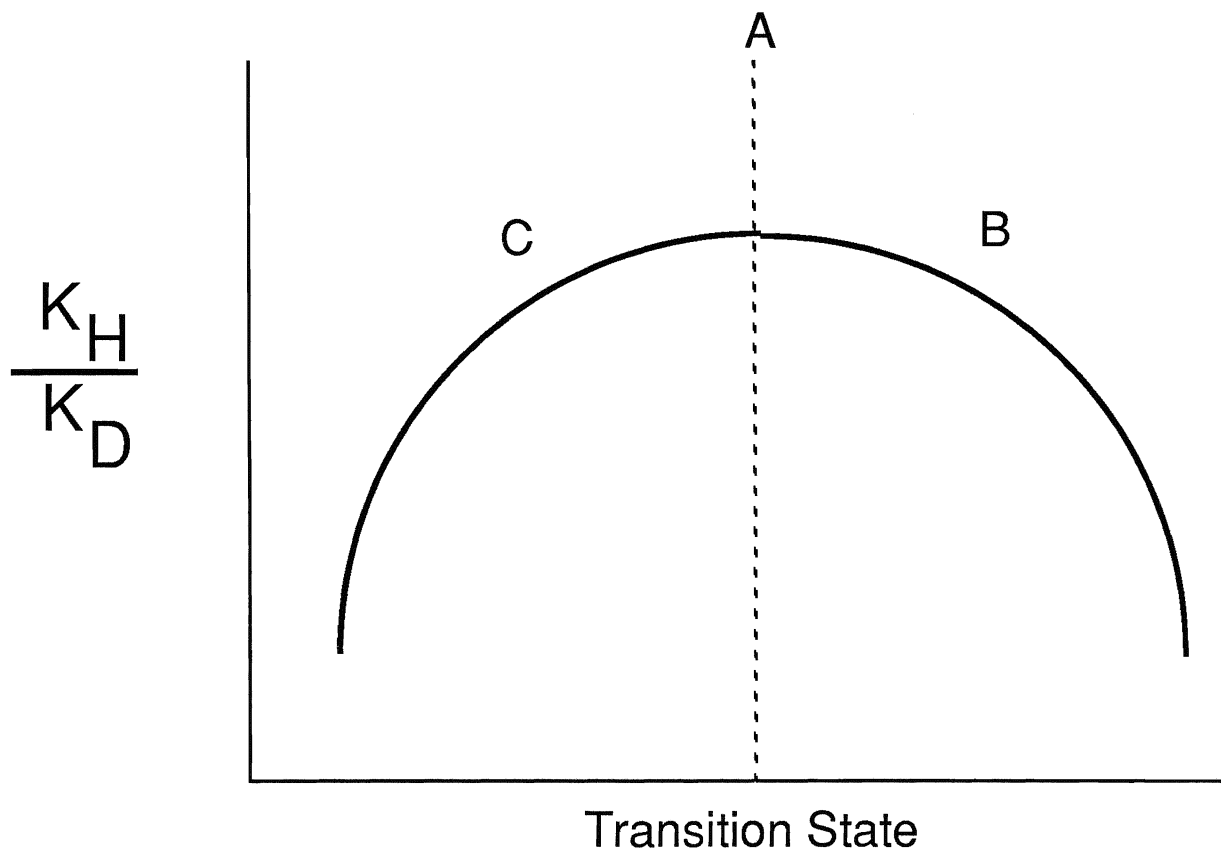
attached in the transition state. This can be described as a symmetrical transition state. However, benzylic hydrogens, and therefore benzylic deuteriums, are easy to abstract⁸⁶. Thus, the transition state is unlikely to be one that is symmetrical, but rather one that may have product character. This can result in an unsymmetrical transition state in which the abstracting bond is stronger than the original C-H and C-D bond. Therefore, the transition state is no longer situated at the maximum position on the curve but is shifted to the right-hand side of the curve (figure thirty). The end result is a lower observable isotope effect.

Although an intramolecular isotope effect of one may not provide convincing evidence in favour of a radical abstraction-recombination mechanism, a large secondary isotope effect (s) of 1.37 is supportive of a intermediate which has considerable sp^2 character.

Thus, the values of p , p^* , and s discussed above are consistent with a route of hydroxylation for toluene (24) by a cytochrome P-450 dependent hydroxylase enzyme which proceeds via a radical abstraction-recombination mechanism.

In the absence of purified enzyme preparation it cannot be stated definitely that the same enzyme that metabolizes ethylbenzenes is responsible for the hydroxylation of 24 . However, the results obtained from induction and induction/inhibition experiments suggest that ethylbenzene hydroxylase is capable of accepting toluenes as a substrate.

FIGURE THIRTY: Varying intramolecular isotope effect with varying initial transition state.



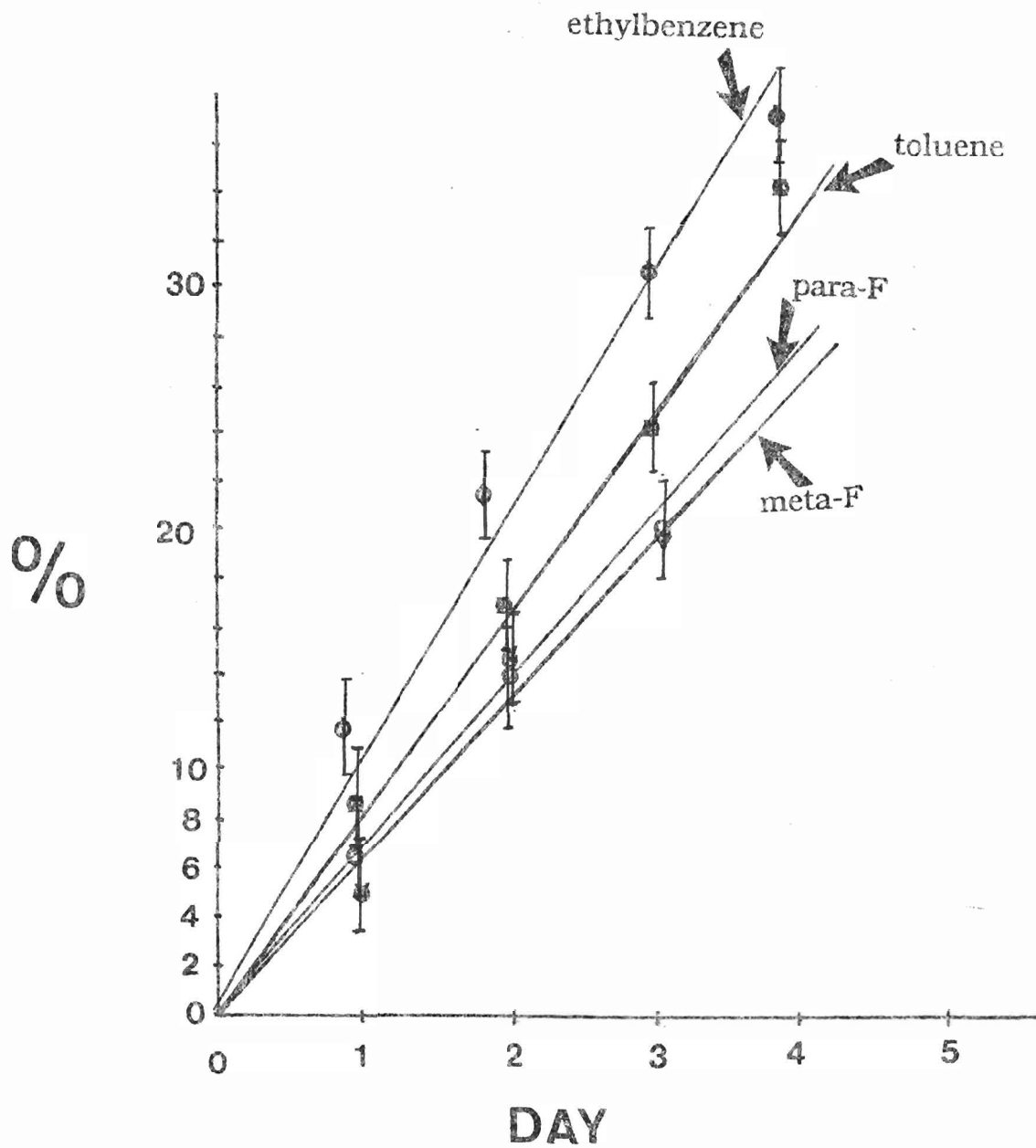
- A = maximum intramolecular isotope effect
- B = lower intramolecular isotope effect due to unsymmetrical transition state with more product character.
- C = lower intramolecular isotope effect due to unsymmetrical transition state with more starting material character.

²H NMR Study

The hydroxylation reaction catalyzed by cytochrome P-450 was examined by following the conversion of substrate to product by ²H NMR (30.7 MHz). The results obtained from this study using 4'-fluoroethylbenzene-1,1-d₂ (17), 3'-fluoroethylbenzene-1,1-d₂ (13), ethylbenzene-1,1-d₂ (45), and toluene-d₈ (61) as substrate are summarized as a graph in figure thirty-one, and in table seven.

Figure thirty-one demonstrates that the conversion of substrate to product over five days is apparently linear, reaching a maximum on the fifth day. Metabolism ceased after the fifth day and thus conversions are not reported. This discontinuation in the metabolism has been attributed to the death of the fungus. Since the fungus is incubated in the absence of any growth medium, it is only a matter of time before it dies.

The metabolism is most rapid for ethylbenzene-1,1-d₂ (45) which demonstrates the greatest overall conversion and least rapid for the fluoro substituted ethylbenzenes 13 and 17 . The differences in the rates of conversion can be attributed to the effect of fluoro substitution ($\sigma_m = 0.34$ and $\sigma_p = 0.06$ for fluorine)⁸⁹. If the hydroxylation proceeds via an initial abstraction of a benzylic proton or a proton radical as originally assumed, then the decrease in rate of conversion observed for 3'-fluoroethylbenzene-1,1-d₂ (13) with respect

**FIGURE THIRTY-ONE**

% conversion versus days for
substrates 17, 13, 45, and 61

TABLE SEVEN

Percent hydroxylation of deuterated substrates by
Mortierella isabellina determined by ^2H NMR

Substrate	Product	% Conversion after ⁺			
		1 day	2 days	3 days	4 days
<u>61</u>	<u>62</u>	8.5	17	24	34
<u>45</u>	<u>58</u>	11.5	21.5	30	35.5
<u>13</u>	<u>11</u>	5	14	20	20
<u>17</u>	<u>15</u>	7.5	12	20	20

⁺ ± 2%

to 45 may be influenced by the meta (electron withdrawing) influence of fluorine on the benzylic position. A fluoro substituent in the meta position would make the benzylic position less susceptible to hydroxylation. As a result, a lesser degree of conversion should be observed.

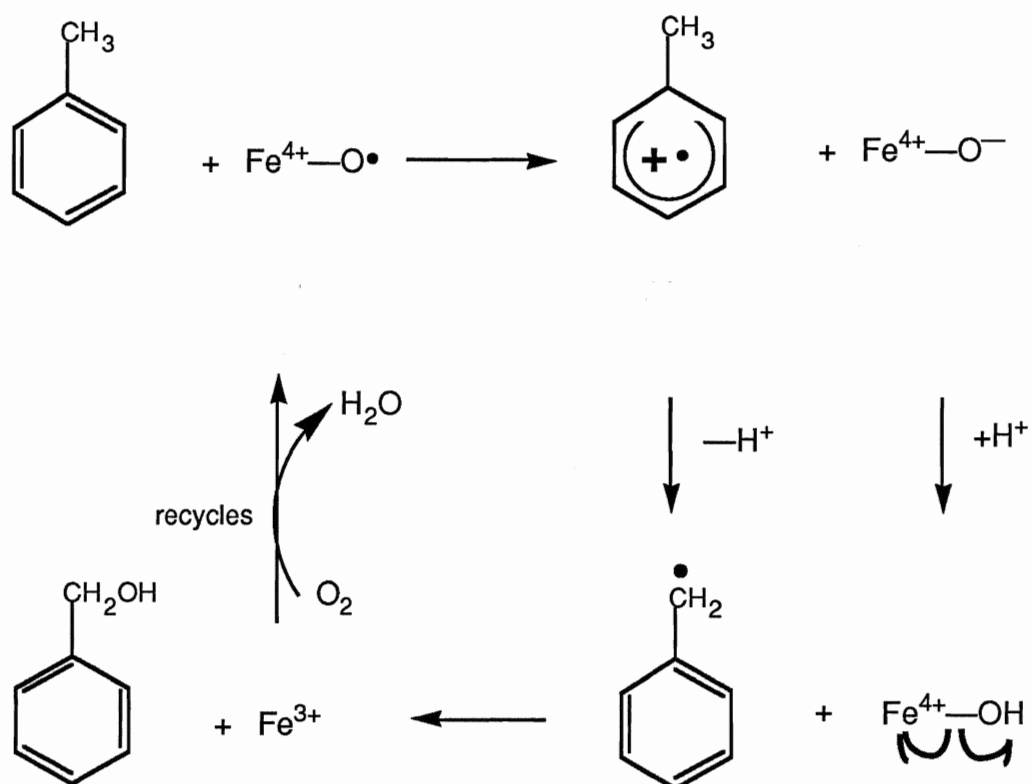
Substrate 17, which is para substituted, also demonstrates a considerable difference in the rate of conversion with respect to 45. The rate of conversion for 17 is almost identical to that of 13. Unlike 13, the fluoro substituent of 17 has little or no effect on the benzylic position.

If the first step in the hydroxylation is the removal of an electron from the aromatic ring by the enzyme's active site species (figure thirty-two), then a fluoro substituent at either the meta or para position could have similar effects on the rate of conversion. The hydroxylation proposed in figure thirty-two is analogous to that which has been proposed for the benzylic hydroxylation of ethylbenzene by the same fungus³³. The large positive secondary deuterium isotope effect for toluene hydroxylation is also consistent with the route outlined in figure thirty-two. Similar large secondary isotope values have been reported for radical cation deprotonation⁸⁷.

The conversion of toluene-d₈ (61) to benzyl alcohol-d₇ (62) occurs at a rate which is in between the metabolism of ethylbenzene-1,1-d₂ (45) and the fluoro substituted

FIGURE THIRTY—TWO:

Proposed route for Hydroxylation of toluene by fungi



ethylbenzenes (13 and 17). The differences in the rates can be attributed to the effects of the different substituents. The presence of fluoro substituents has been shown to reduce the rate of metabolism. Since toluene- d_8 (61) is not subjected to such substitution, one would predict that it should be metabolized at a rate which is greater than 13 and 17 . The observed increase in the metabolism between 45 and 61 where the metabolism of 45 is more prominent may be due to the inductive effects that the methyl substituent has on the benzylic position of 45 . This inductive effect may make the benzylic position of 45 more attractive towards benzylic deprotonation. It is also possible that the differences in the rates between 61 and 45 , 17 , and 13 may be due to substrate specificity.

However, the initial intent of the toluene- d_8 (62) study by ^2H NMR (30.7 MHz) was to compare the metabolism of 62 with that of 4-fluorotoluene- α, α, α - d_3 (20) and 3-fluorotoluene- α, α, α - d_3 (21) since we have demonstrated that substrates 20 and 21 can be metabolized by Mortierella isabellina . If the first step in the benzylic hydroxylation is the removal of an electron from the aromatic ring, then the hydroxylation rates of 20 and 21 should be approximately the same. Furthermore, the rate of hydroxylation of 20 and 21 should be less than that of 62 . In other words, the results should be similar to those observed in the ethylbenzene study.

Unfortunately, the presence of low boiling hydrocarbon(s) in very low concentrations in 20 and 21 proved to be

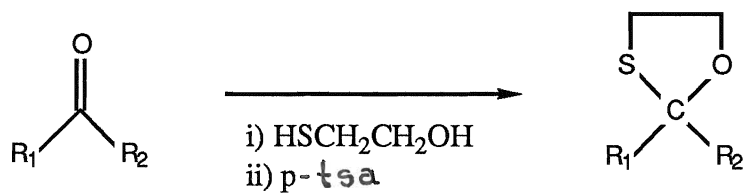
detrimental to the fungus. In an attempt to rectify this situation an alternative approach to the synthesis of 20 and 21 is presently underway. This new method should avoid the contamination by low boiling hydrocarbon and allow for the metabolism of 20 and 21 by Mortierella isabellina to be followed by ^2H NMR (30.7 MHz).

Preparation of 1,3-oxathiolanes

A series of 1,3-oxathiolanes (eight) were obtained in high yield by the condensation of the appropriate ketone with 2-mercaptoethanol (28) and a catalytic amount of p-tsa (figure thirty-three). Although enzymic oxidation of oxathiolane systems by fungi have not been reported, analogous fungal studies involving dithiane ring systems have yielded sulphoxides and sulphones. Thus, 1,3-oxathiolanes have the potential of yielding new and novel sulphoxides and/or sulphones.

The synthesis of 2-phenyl-2-tert. butyl-1,3-oxathiolane (27) was accomplished by the condensation of 2,2-dimethylpropiophenone (26) (figure thirty-three), and 26 was prepared in a two step synthesis (figure thirty-four). The first step in the preparation of 26 was the addition of tert. butyl lithium to benzaldehyde (4) which gave in high yield 2,2-dimethyl-1-phenyl-propanol (25). In the final step, 25 was oxidized (by Jones' reagent) to the ketone, 26 , in high yield.

1-phenylethyl-2'-hydroxyethyl sulphide (42), a miscellaneous substrate, was prepared in a one step synthesis (figure thirty-five). To the sodium salt of 2-mercaptoethanol (28), which was generated in situ using equal molar amounts of sodium hydride and 28 , was added 1-chloro phenyl ethane (43) to give, after purification, 42 in low yield.



$\text{R}_1 = \text{C}_6\text{H}_5$	$\text{R}_2 = \text{H}$	<u>(4)</u>	<u>50</u>
$\text{R}_1 = \text{C}_6\text{H}_5$	$\text{R}_2 = \text{CH}_3$	<u>(30)</u>	<u>51</u>
$\text{R}_1 = \text{C}_6\text{H}_5$	$\text{R}_2 = \text{CH}_3$	<u>(26)</u>	<u>53</u>
$\text{R}_1 = \text{R}_2 = \text{C}_6\text{H}_5$		<u>32</u>	<u>31</u>
$\text{R}_1 = \text{CH}_3\text{CH}_2$	$\text{R}_2 = \text{CH}_3$	<u>35</u>	<u>34</u>
$\text{R}_1 = \text{CH}_3\text{CH}_2\text{CH}_2$	$\text{R}_2 = \text{CH}_3$	<u>37</u>	<u>36</u>
$\text{R}_1 = \text{cyclohexyl}$	$\text{R}_2 = \text{CH}_3$	<u>39</u>	<u>38</u>
$\text{R}_1 = (\text{CH}_3)_3\text{C}$	$\text{R}_2 = \text{CH}_3$	<u>41</u>	<u>40</u>

FIGURE THIRTY-THREE:

Preparation of 1,3—oxathiolane series

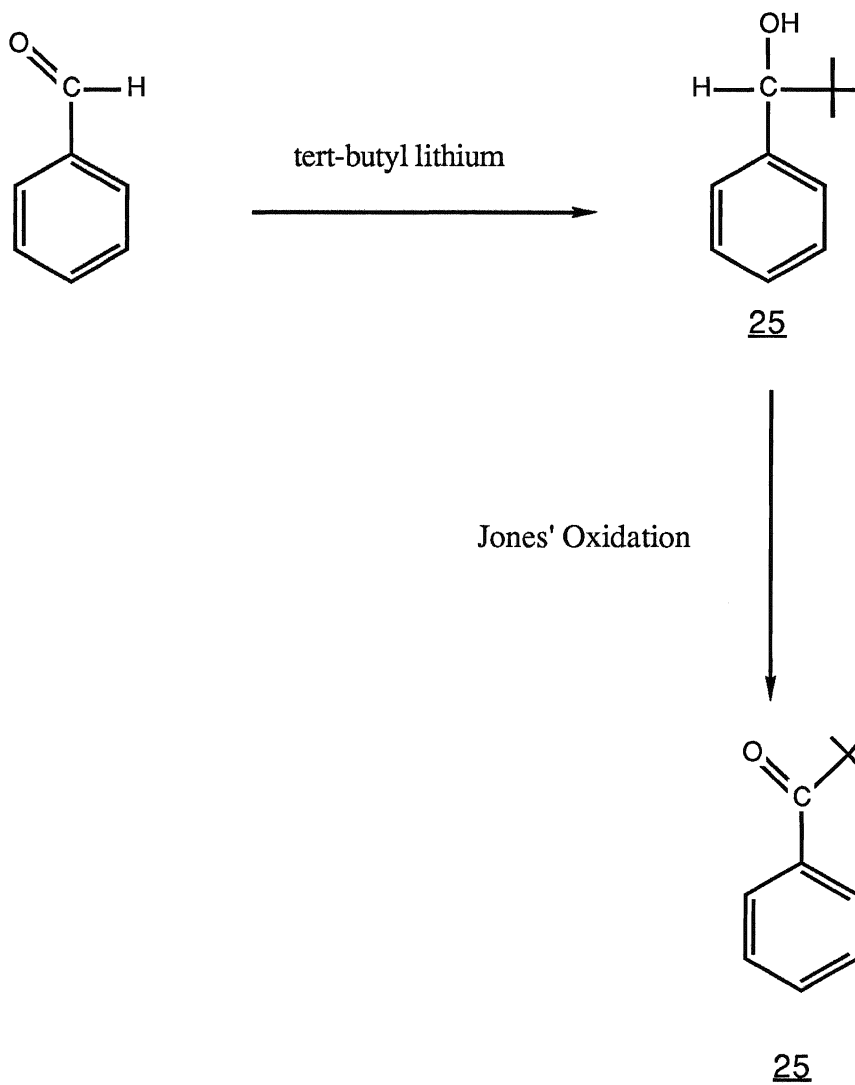


FIGURE THIRTY-FOUR:

Preparation of
2,2—dimethyl propiophenone (26)

Although the substrate 42 is not a 1,3-oxathiolane it does resemble the system in an open chain configuration. Furthermore, since 42 was used in the 1,3-oxathiolane study it would simplify matters if it was generalized under this and other oxathiolane sections in the discussion.

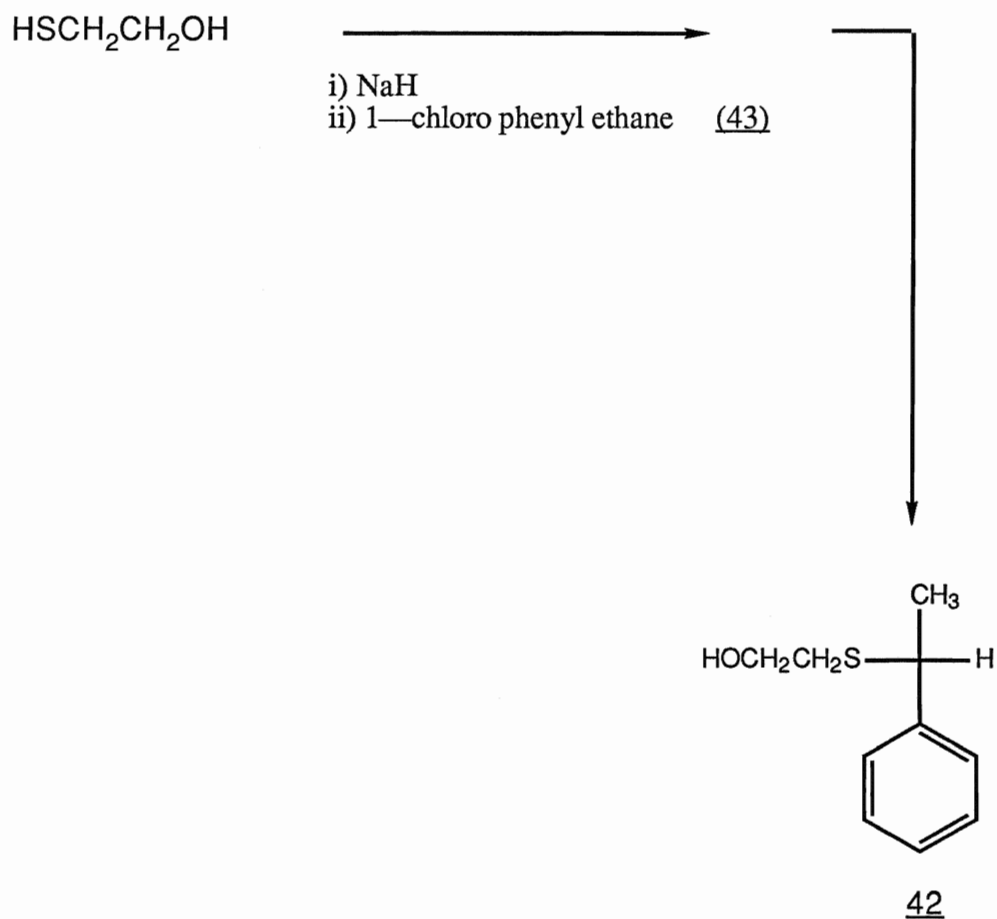


FIGURE THIRTY-FIVE:

Preparation of 1—phenylethyl-2¹—hydroxyethyl sulphide (42)

Incubation of 1,3-oxathiolanes

The products obtained from the incubation of the substrates with the fungi are given in Table two (in the experimental section, page 92). In attempts to simplify the discussion, the substrates have been divided into two groups. Those that have an aromatic substituent at carbon-2 are listed in Table nine, and substrates with no aromatic moiety at carbon-2 can be found in Table eight.

Incubation of substrates 35 , 36 , and 38 (Table eight) gave no recoverable product(s) or starting material. Substrate 40 gave products 41 and 55 with Mortierella isabellina .

In order to determine whether the products obtained from the incubation of 40 with Mortierella isabellina were of enzymic nature the incubation was performed under normal conditions using fungus that had been destroyed by autoclaving. Death of the fungus insures that the enzymic activity of the fungus ceases. Incubation of 40 with dead Mortierella isabellina yielded starting material (40) as the only recoverable product. Thus, these two cross incubations, one under live and an other under dead conditions, suggests that substrate 40 was subjected to a biotransformation since products 41 and 55 were only obtained under live conditions. Furthermore, isolation of 40 from dead Mortierella isabellina indicates that the 1,3-oxathiolane

TABLE EIGHT

Incubation of 1,3-oxathiolanes with Rhizopus arrhizus
(R.a.), Mortierella isabellina (M.i.),
Helminthosporium (H.s), and Aspergillus niger (A.n.)

Substrate	R.a.	M.i.	H.s.	A.n.
<u>35</u>	-	-	-	-
<u>36</u>	-	-	-	-
<u>38</u>	-	-	-	-
<u>40</u>	-	<u>40</u> , <u>41</u> , <u>55</u>	-	-
<u>40</u> (dead)	*	<u>40</u>	*	*
<u>41</u>	*	<u>51</u>	*	*
<u>41</u> (dead)	*	<u>41</u>	*	*

* incubation not performed.

- incubation performed but no results were obtained.

ring system (in this case) is stable and does not degenerate while suspended in the medium to give 41 .

The isolation of 41 may arise if 40 was transformed to a sulphoxide (or sulphone) which subsequently gave 41 . 41 could then be reduced by the enzyme to the corresponding alcohol 55 . This latter possibility, reductase activity, which is not uncommon with fungi, was investigated⁸⁸ . Incubation of 41 with Mortierella isabellina afforded the racemic alcohol 55 whereas incubation of 41 with dead Mortierella isabellina gave 41 only. These results tend to confirm the reductase activity of the fungus.

The results obtained solely from incubations of 40 and 41 with Mortierella isabellina suggests a complex biotransformation (figure thirty-six).

The evidence obtained from the incubation of non-aromatic moties is inconclusive since it is based on one result. This one result has allowed for the speculative transformation of 40 (figure thirty-six) which requires a non-isolable sulphur oxidized product. It is not known whether this intermediate could yield a ketone. Without any other evidence, the only conclusions that can be stated are that in one instance incubation of substrate 40 with Mortierella isabellina gave 41 which was reduced by the fungus to give 55 .

Incubation of substrates with aromatic characteristics at carbon-2, unlike the latter series of 1,3-oxathiolanes, proved to be more informative (table nine). Since no products or

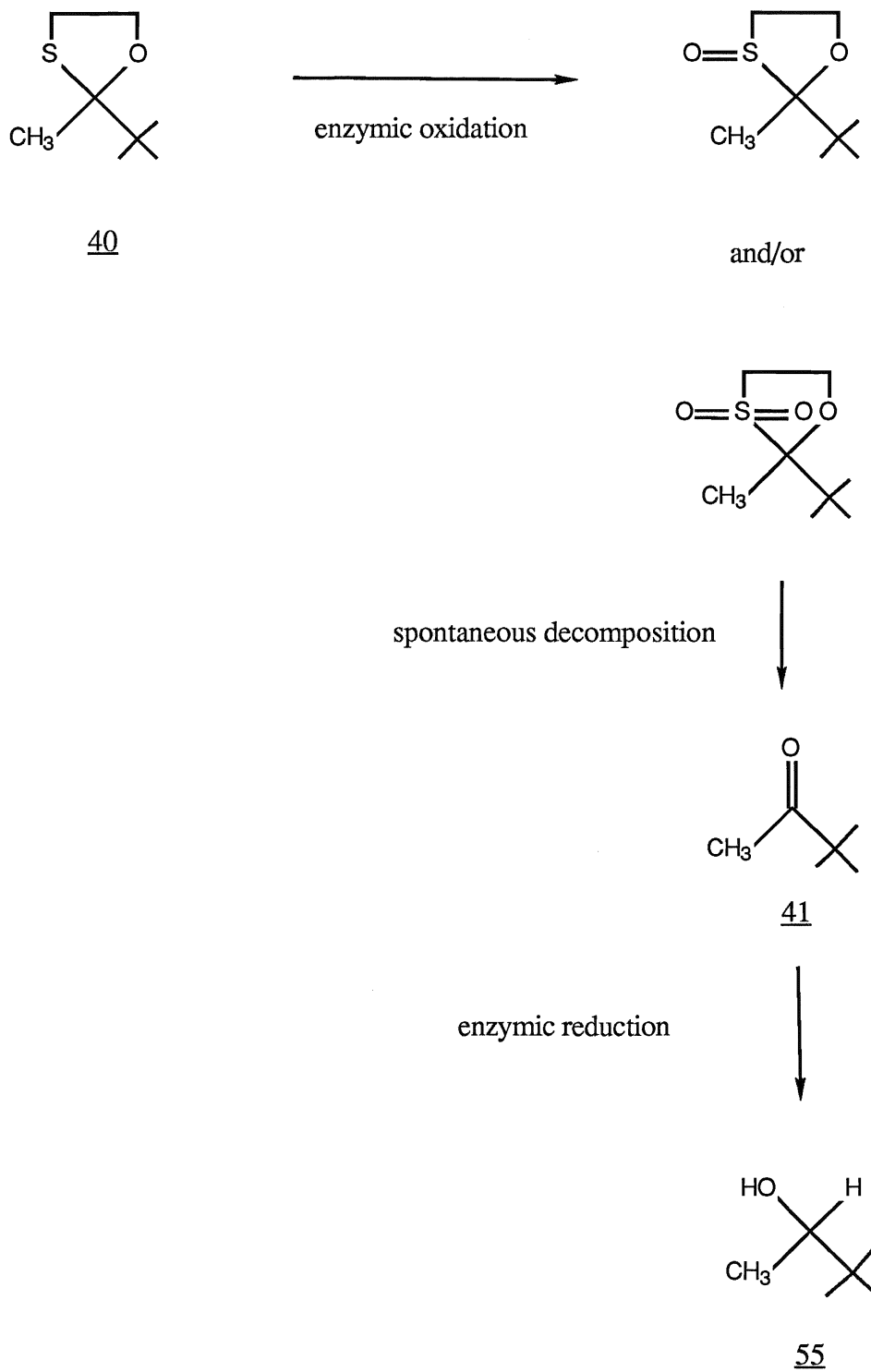


FIGURE THIRTY-SIX: Possible complex biotransformation of 40

TABLE NINE

Incubation of 1,3-oxathiolanes with Rhizopus arrhizus (R.a.), Mortierella isabellina (M.i.), Helminthosporium (H.s.), and Aspergillus niger (A.n)

Substrate	R.a.	M.i.	H.s.	A.n.
<u>33</u>	*	<u>50</u>	*	*
<u>29</u>	-	<u>51</u> , <u>52</u> , <u>29</u> , <u>30</u>	-	-
<u>29</u> (dead)	*	<u>29</u>	*	*
<u>29</u> (ph = 7.1)	*	-	*	*
<u>27</u>	<u>53</u> , <u>26</u> , <u>25</u>	<u>53</u>	-	-
<u>31</u>	<u>54</u>	<u>54</u>	-	-

* incubation not performed.

- incubation performed but no results were obtained.

starting material were isolated with any of the four substrates 33 , 29 , 27 , and 31 using the fungi Helminthosporium and Aspergillus niger , the discussion will be limited to the results obtained from Rhizopus arrhizus and Mortierella isabellina .

The first substrate in the series, 2-phenyl-1,3-oxathiolane (33) gave the sulphoxide dl-threo-2-phenyl-1,3-oxathiolan-1-oxide (50) as the only product with Mortierella isabellina . This product, however, upon isolation, was not stable and rapidly decomposed to give benzaldehyde (4). Attempts to preserve 50 under nitrogen atmosphere at - 70.0°C eventually yielded 4 as the only recoverable product. Fortunately, the product (50) was stable long enough to obtain spectroscopic information. Since 50 was racemic and unstable, further incubations were not pursued.

2-methyl-2-phenyl-1,3-oxathiolane (29), the second substrate in the series, gave 29 , acetophenone (30), phenyl ethanol (52), and dl-threo-2-methyl-2-phenyl-1,3-oxathiolan-1-oxide (51) as products with Mortierella isabellina , whereas Rhizopus arrhizus gave no recoverable product(s) or starting material. The sulphoxide 51 decomposed to acetophenone (30) but at an observable slower rate. Incubation of 29 with dead Mortierella isabellina , in an attempt to elucidate the nature of the products 30 , 52 , and 51 , afforded starting material (29) as the only product.

Incubation of 29 with Mortierella isabellina suggests that the 1,3-oxathiolane ring system is stable under non-enzymic conditions. The absence of 30 and 52 with dead Mortierella isabellina suggests that the products, 30 and 52, may be associated with 51. This is not unlikely since it has been demonstrated in two cases that the sulphoxide 50 and 51 upon isolation are unstable and generate 4 and 30, respectively.

Incubations of 29 with Mortierella isabellina in a medium buffered to pH of 7.1 gave 30 as the only isolable product. It was proposed that if 51 was acid sensitive then buffering the medium would enhance the yield of 52 and eliminate 30. Unfortunately, the reverse occurred.

The relative stereochemistry of 51 (figure thirty-seven) was elucidated using n.O.e (nuclear Overhauser effect), 2-D COSY, ^1H decoupling, and ^1H NMR experiments. The chemical shifts of the methylene protons in the 1,3-oxathiolan-1-oxide ring system were assigned in accordance to their adjacent functional groups. The proton from methylene group that was furthest downfield was assigned as the cis proton to the sulphoxide oxygen (proton two). n.O.e (nuclear Overhauser effect) experiment showed a 45% increase in the signal for proton two and a 10% increase for proton three on irradiation at 1.7 ppm (the C-2 methyl group). Based on these results the relative configuration of 51 was assigned the structure give in figure thirty-seven. The optical rotation of 51 indicates that the product, 51, was racemic. The coupling constants

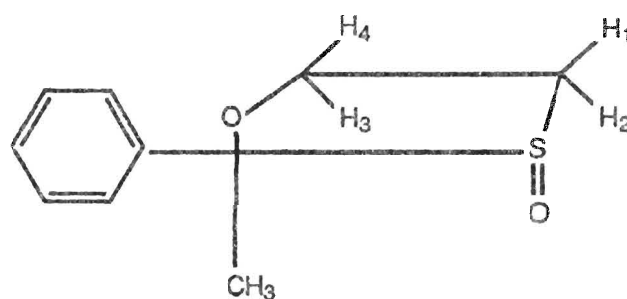


FIGURE THIRTY-SEVEN:

Relative stereochemistry of dl-threo-2-methyl-2-phenyl-1,3-oxathiolan-1-oxide (51)

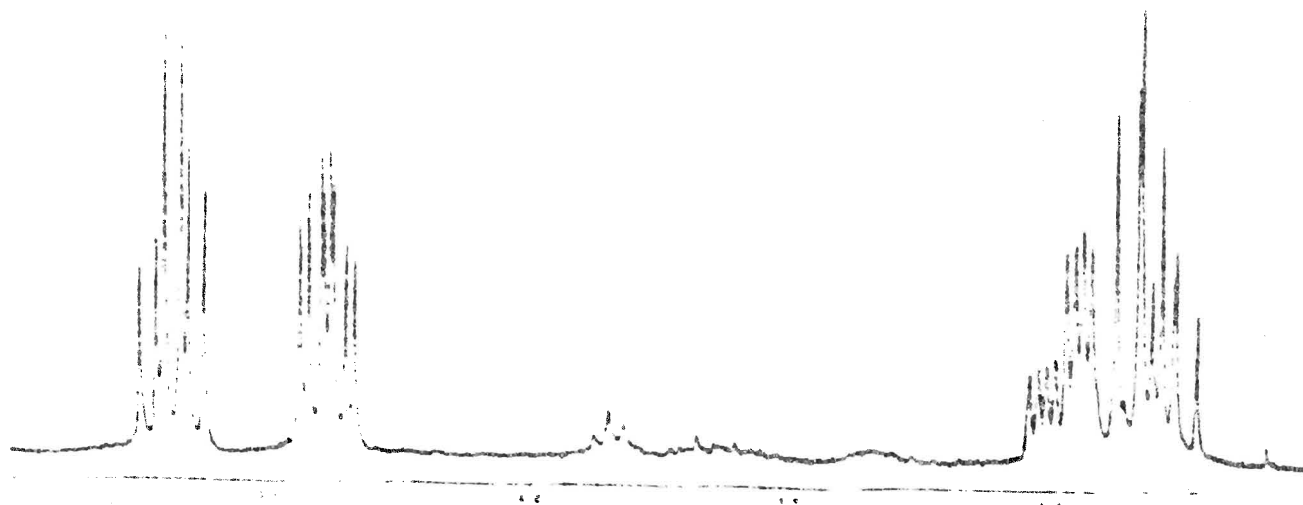
determined for 51 are consistent with the structure given in figure thirty-seven.

Attempted computer simulation of the methylene proton pattern using an Intercal and NMRcal program with coupling constants determined by decoupling experiments gave very similar patterns (figure thirty-eight). The pattern could not be exactly duplicated due to long range coupling between the methyl group and protons one and two. This long range coupling which was less than 0.5 Hz was observed in the 2-D COSY spectra.

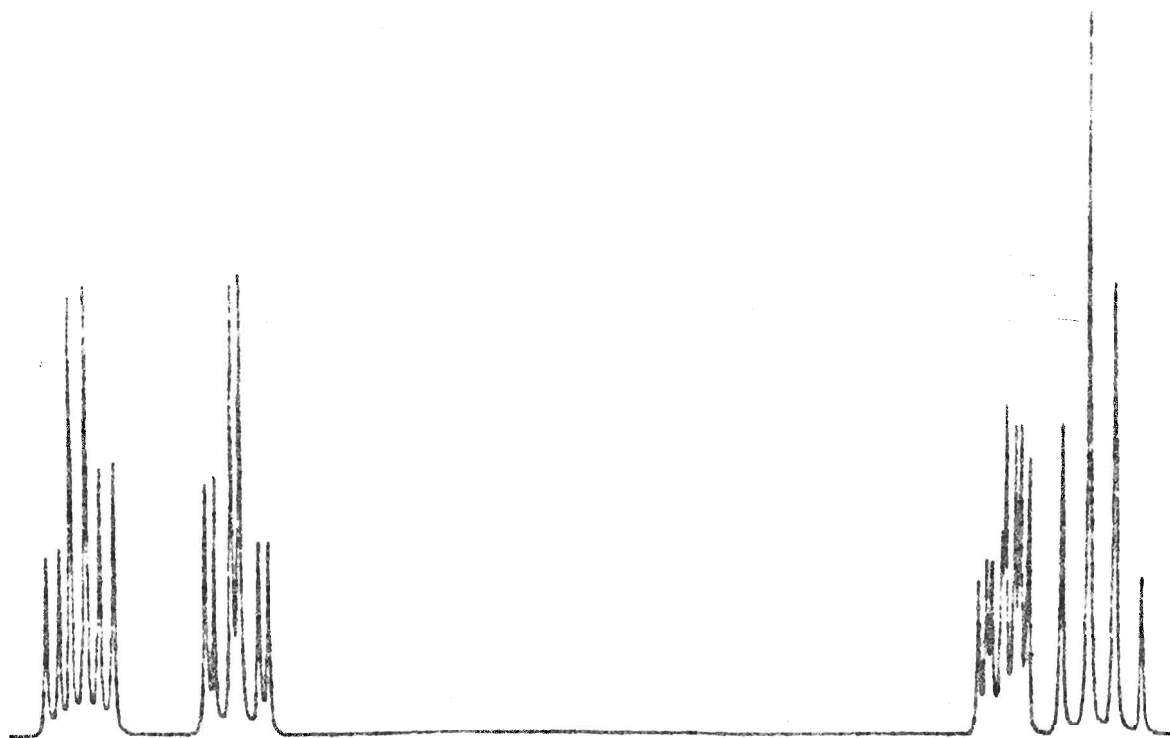
The methylene splitting pattern observed for 51 is characteristic of a 1,3-oxathiolan-1-oxide having a structure similar to that given in figure thirty-seven. If the oxidation had been reversed, then the splitting pattern within each pair of methylene protons should have been reversed or at least different. Thus, 51 provides a diagnostic standard for the determination of the relative configuration of 1,3-oxathiolan-1-oxide.

The relative stereochemistry of dl-threo-2-phenyl-1,3-oxathiolan-1-oxide (50) was determined by comparing the methylene ¹H NMR pattern of 50 with 51 . Based on this comparison 50 was assigned the structure given in figure thirty-nine.

51 was assigned the threo configuration based on the configurational assignments shown in figure forty. This assignment has been used for all other



Bruker AC 200 ^1H NMR (200 MHz)



Simulated ^1H NMR (200 MHz)

FIGURE THIRTY-EIGHT : Comparison of ^1H NMR (200 MHz) to computer simulated ^1H NMR (200 MHz)

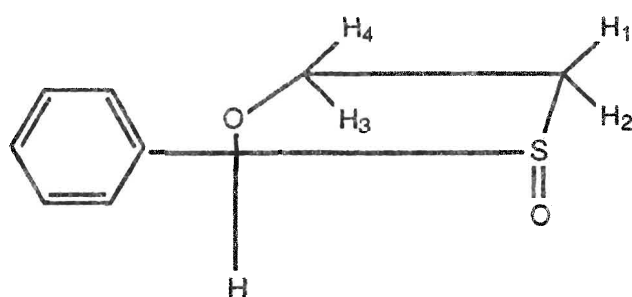
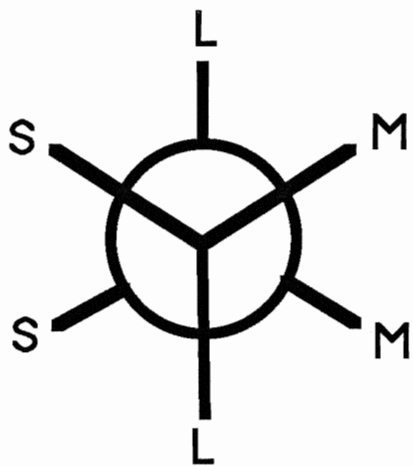
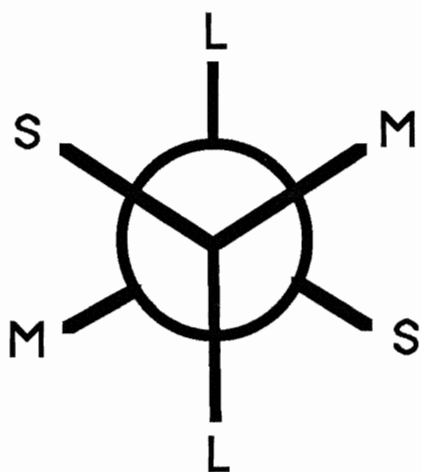


FIGURE THIRTY-NINE:

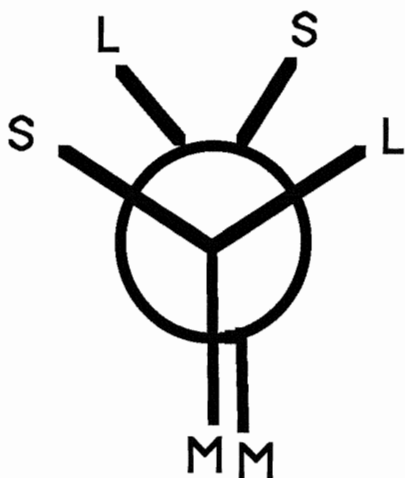
Relative stereochemistry of dl-threo-2-phenyl-1,3-oxathiolan-1-oxide (50)



erythro



threo



E1-threo configuration

FIGURE FORTY:

Definitions of erythro and threo configurations.

1,3-oxathiolan-1-oxide obtained from the incubations.

Incubation of 2-phenyl-2-tert. butyl-1,3-oxathiolane (27) with Mortierella isabellina gave the dl-threo-2-phenyl-tert. butyl-1,3-oxathiolan-1-oxide (53), 2,2-dimethyl-1-phenyl-1-propanol (25) as products. Incubation of 27 with Rhizopus arrhizus also gave 53 , 26 , and 25 .

The sulphoxide, 53 , obtained from both fungi had identical spectroscopic properties. 53 was also unstable and decomposed over a number of days to yield 26 . The methylene splitting patterns were identical to the splittings observed with 51 . 53 was assigned the relative configuration given in figure forty-one based on the comparison of the methylene splitting pattern of 53 to that observed for 51 . The only difference between the two sulphoxides was that the sulphoxide obtained from Mortierella isabellina had an optical rotation ($[\alpha]_D^{20}$) of -13.3° , while 53 obtained from Rhizopus arrhizus was racemic. Using a europium shift reagent, it was calculated that 53 obtained from Mortierella isabellina had an EE of 4%.

The final substrate in the series, 2,2-diphenyl-1,3-oxathiolane (31), gave with Rhizopus arrhizus and Mortierella isabellina diphenyl methanol (54) as the only isolable product.

Incubation of 1-phenylethyl-2'-hydroxyethyl sulphide (42) with Mortierella isabellina gave the sulphoxide, 56 , which was racemic. This substrate was used in order to observe the effect that an open chain analogue of a 1,3-oxathiolane would

have on the product. Since 42 did not give a chiral sulphoxide, the results proved to be inconclusive. It is interesting to note that a polar substrate like 42 was metabolized by the fungus.

Incubation of 33 , 29 , 27 , and 31 with Rhizopus arrhizus and Mortierella isabellina in all but one case gave products. The sulphoxides that were obtained (50 , 51 , and 53) were all the result of an enzymic oxidation of the sulphide to the sulphoxide. The presence of alcohols 54 , 25 , and 52 most likely resulted from the enzymic reduction of the corresponding ketone. However, the nature of the ketone is speculative since no information is available on the stabilities of the sulphoxide in the medium. It has been observed that the sulphoxides after isolation yielded the corresponding ketones. Attempts to isolate or determine the other product(s) of the decomposition by ^1H NMR has yielded results which have not yet been explained. These ^1H NMR (200 MHz) studies have shown that the sulphoxide 51 decomposes to acetophenone (30) over a period of days. However, the nature of the other product(s) of decomposition have yet to be determined. It is not unlikely that the sulphoxides in the fungal medium slowly decompose to yield the ketone and some other unknown product(s).

Speculatively, it is possible that the sulphide is oxidized to the sulphoxide which then decomposes to give the ketone. The ketone is subsequently reduced by the enzyme to the alcohol (figure forty-two).

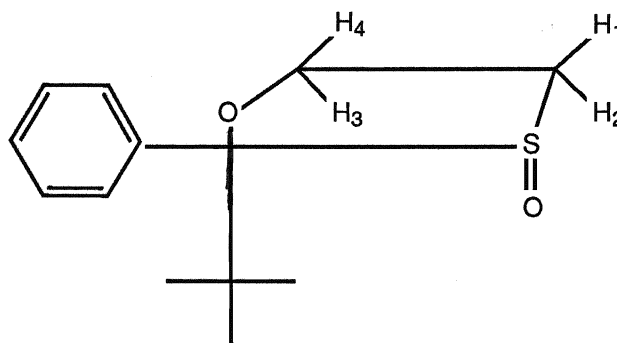


FIGURE FORTY-ONE:

Structure of dl-threo-2-phenyl-2-tert-butyl-1,3-oxathiolan-1-oxide (53)

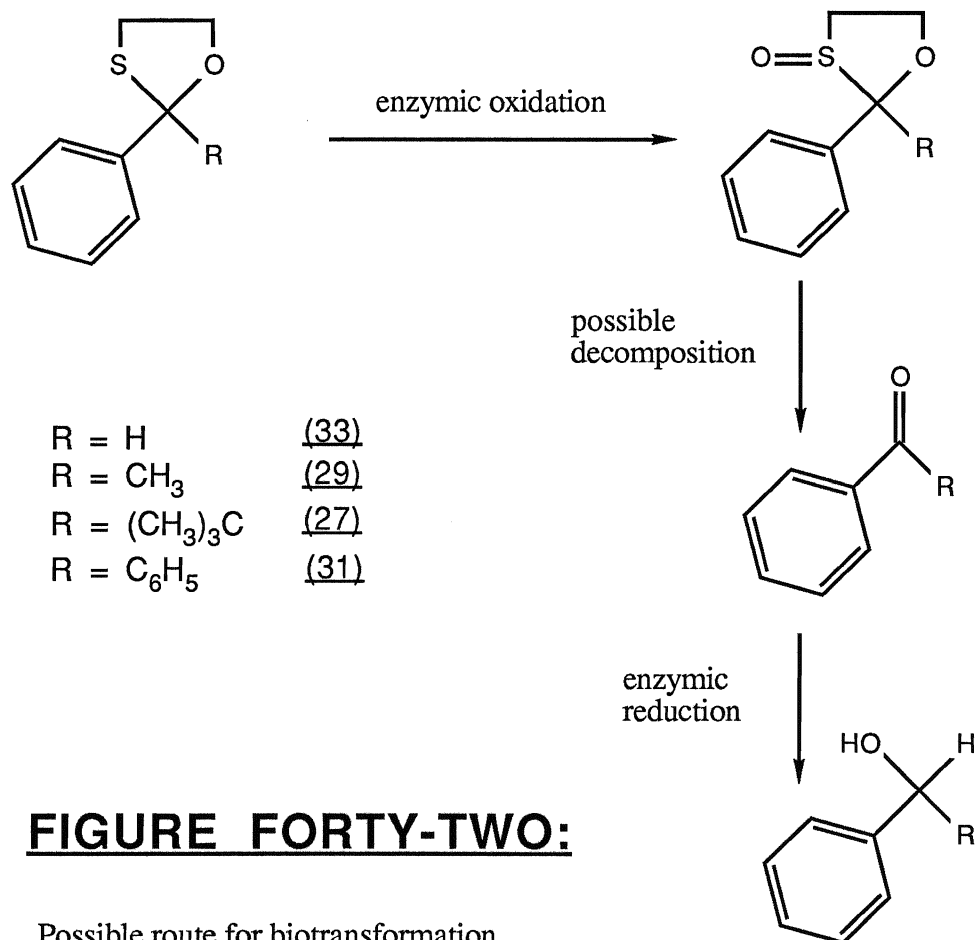


FIGURE FORTY-TWO:

Possible route for biotransformation pathway of 1,3-oxathiolanes

The sulphoxides obtained from substrates 33 and 29 are consistent with an oxidation occurring from the least hindered side of the molecule. While, the sulphoxide obtained from 27 is not. However, all three sulphoxides are consistent with an oxidation occurring from the opposite side of the molecule to that occupied by the phenyl substituent. These results could be explained if one of the enzyme's binding sites was specific for groups that had aromatic character and if the oxidizing species was fixed within the enzyme (figure fifteen, page 30). Thus, the substrate could bind randomly (with aromatic dependence) but oxidized by a single site (pseudoaxially) to yield the sulphoxides (figure forty-three). If the oxidizing species is below the plane of the molecule then the sulphoxides A and B are produced, whereas if the oxidizing species is above, C and D are obtained. Sulphoxides C and D are consistent with sulphoxides 50 , 51 , and 53 .

The racemic nature of the sulphoxides ruled out the initial intent of this project which was to generate chiral sulphoxides with the eventual purpose of obtaining chiral alcohols (figure forty-four). However, the instability of the sulphoxide, even if it had been chiral, would have more than likely terminated the project, since it is doubtful that the sulphoxide would have survived any further chemical reactions.

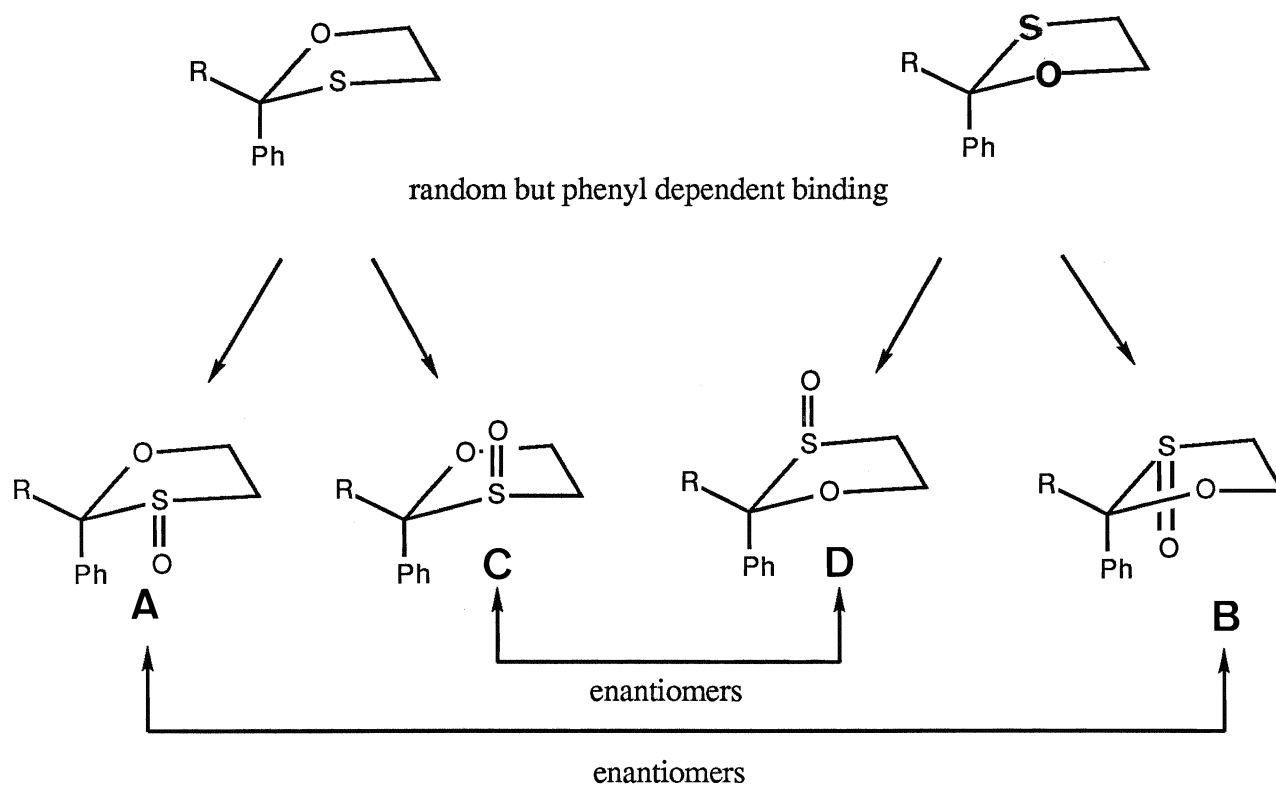
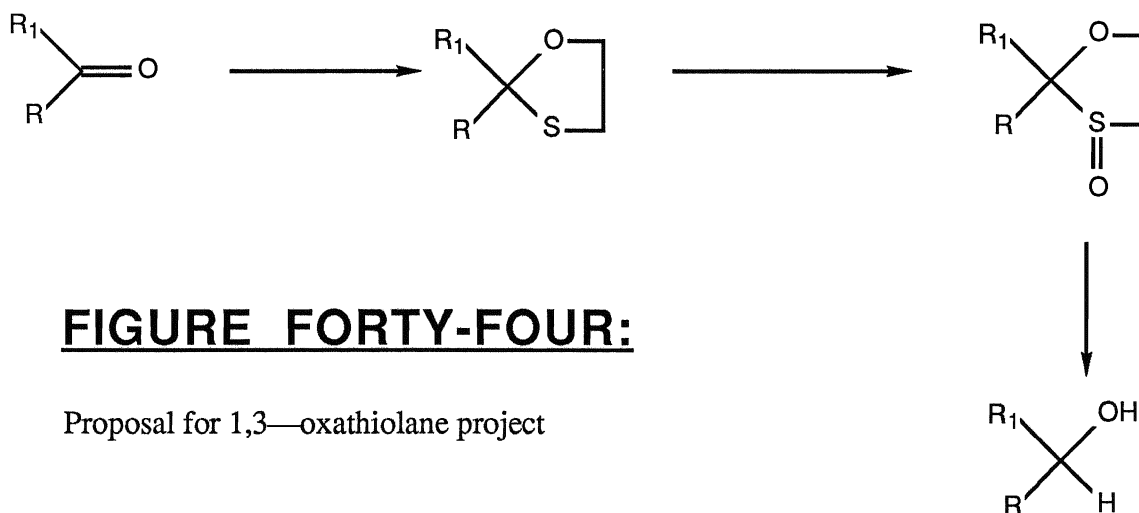


FIGURE FORTY-THREE:

Possible explanation of enzymic sulfoxidation of 1,3—oxathiolanes

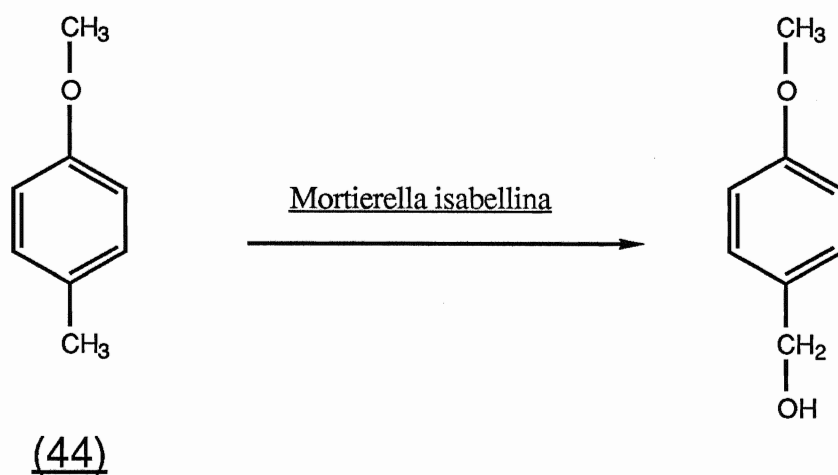


Incubation of 4-methoxytoluene (44)

Since we have demonstrated that Mortierella isabellina is capable of selectively metabolizing the methyl substituent of 44 (figure forty-five), an intra- and intermolecular isotope study similar to that of toluene (24) has been proposed. This study which is being conducted in conjunction with Hanzlik and co-workers in Kansas requires the incubation of the deuterated analogues of 44 ; 4-methoxytoluene- α -d₁, - α , α -d₂, and - α, α, α -d₃. These deuterated substrates are presently being synthesized by Hanzlik and co-workers. However, due to the time frame involved, this project will be completed at a later date by someone else.

FIGURE FORTY—FIVE:

Incubation of 4—methoxy—toluene (44) with Mortierella isabellina



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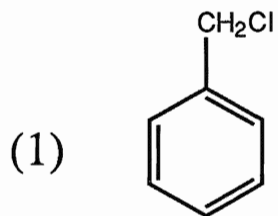
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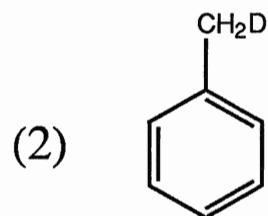
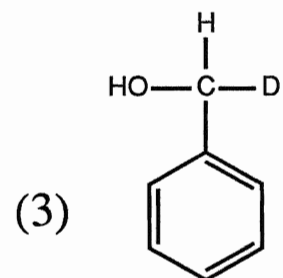
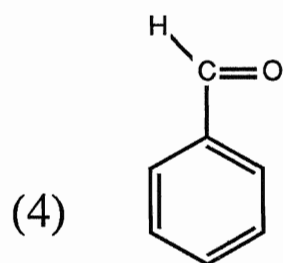
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Appendix I

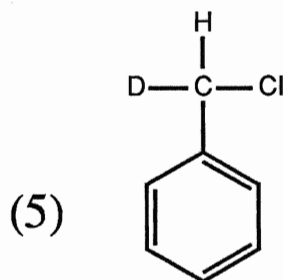
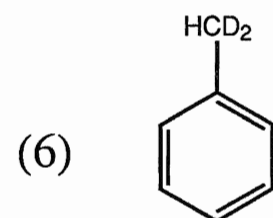
Structures

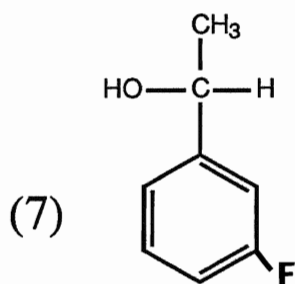
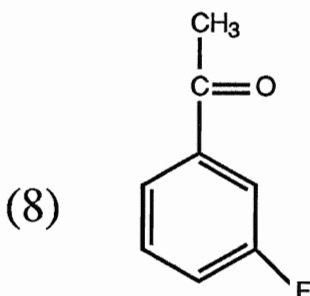


Benzyl Chloride

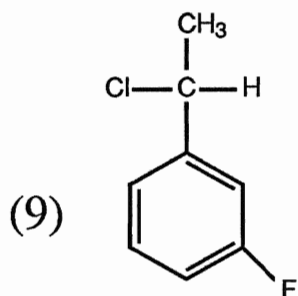
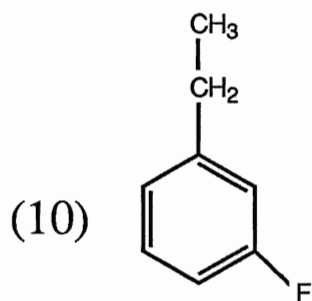
Toluene— α — d_1 Benzyl alcohol— α — d_1 

Benzaldehyde

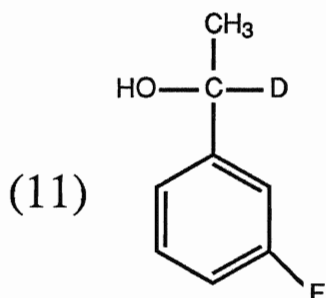
Benzyl chloride— α — d_1 Toluene— α, α — d_2

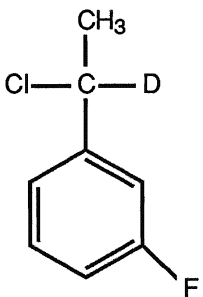
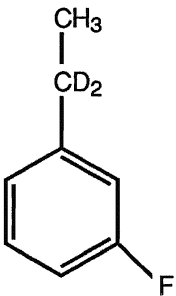
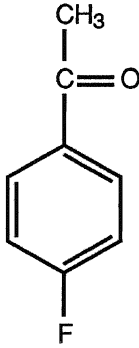
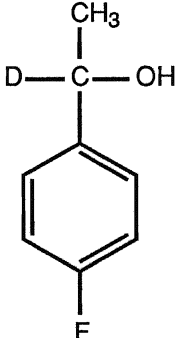
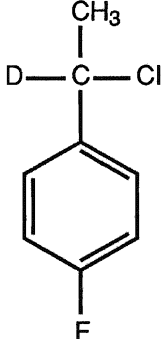
1—(3'—fluorophenyl)—
ethanol

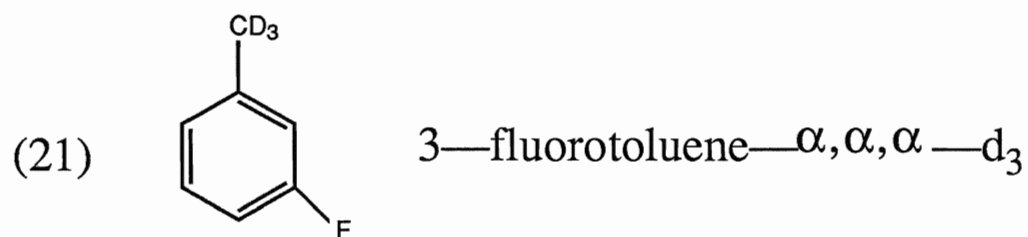
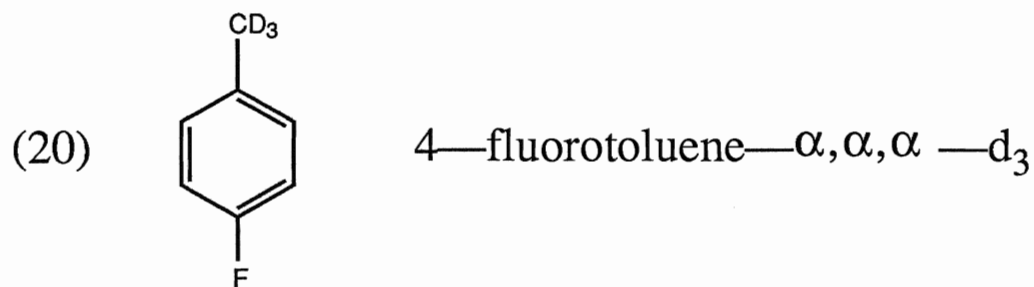
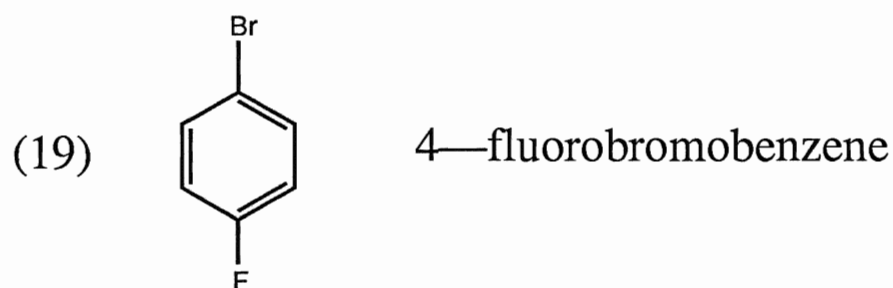
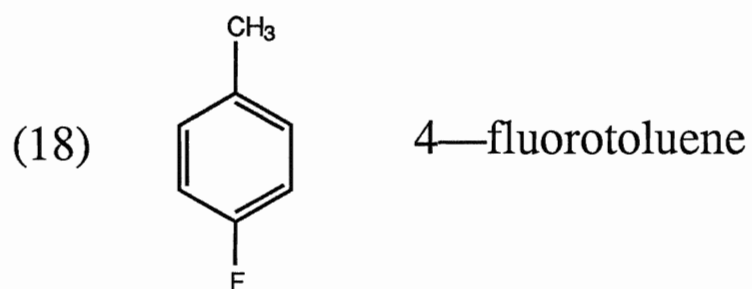
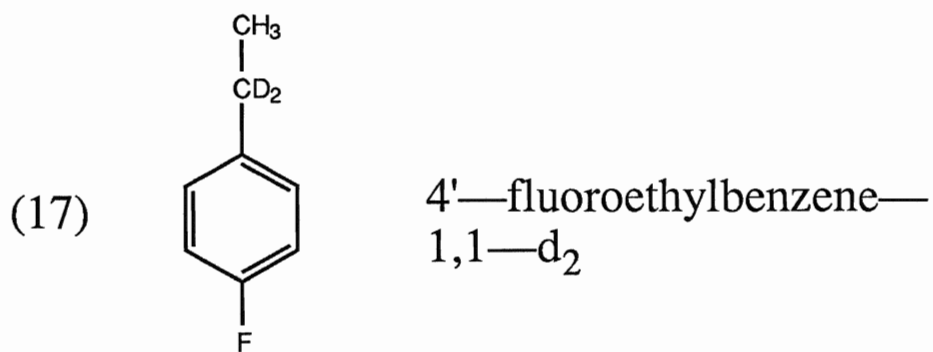
3—fluoroacetophenone

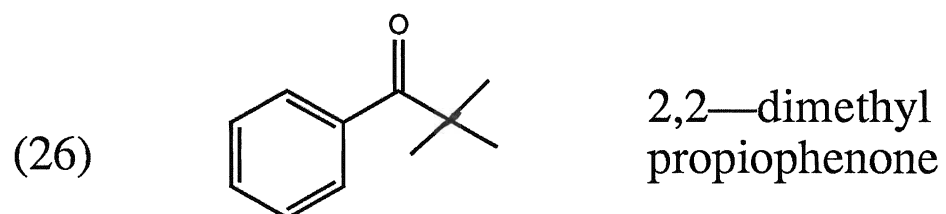
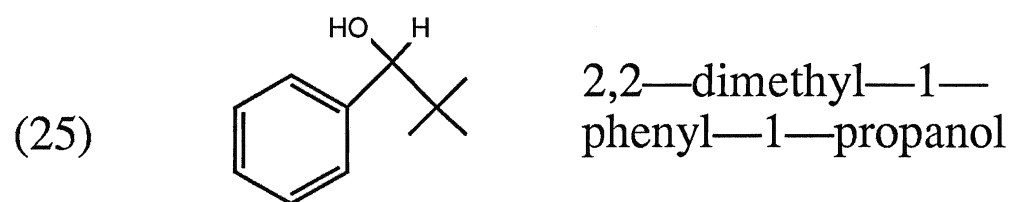
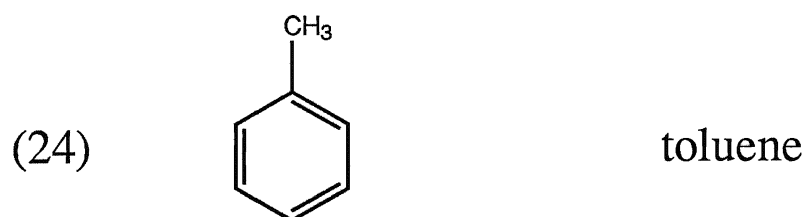
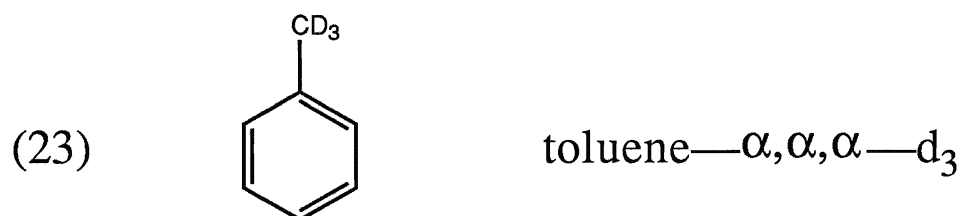
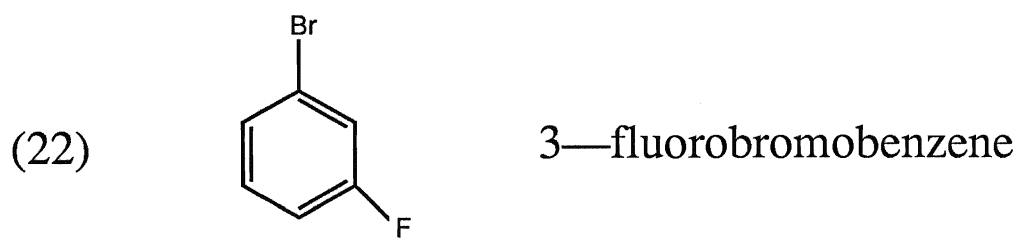
1—chloro—(3'—
fluorophenyl)—ethane

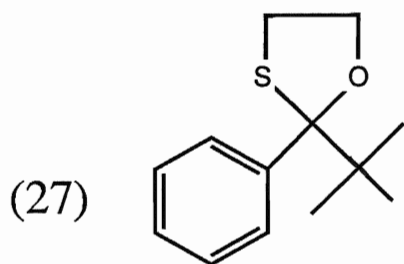
3—fluoroethylbenzene

1—(3'—fluorophenyl)—
ethanol—1—d₁

- (12)  1—chloro—(3'—
fluorophenyl)—ethane—1—d₁
- (13)  3'—fluoroethylbenzene—1,1—d₂
- (14)  4—fluoroacetophenone
- (15)  1—(4'—fluorophenyl)—
ethanol—1—d₁
- (16)  1—chloro—(4'—fluorophenyl)—
ethane—1—d₁



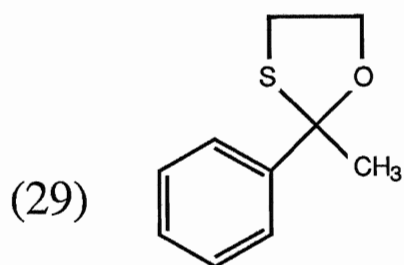




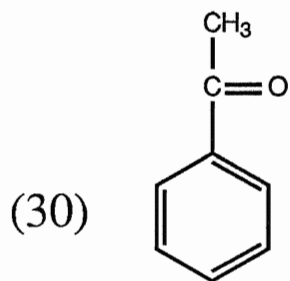
2—phenyl—2—
tert-butyl—1,3—
oxathiolane



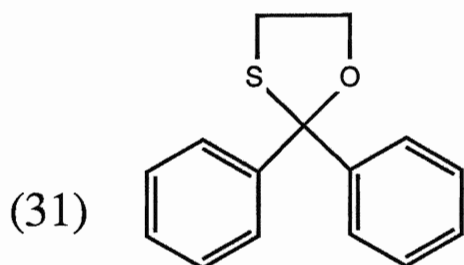
2—mercaptoethanol



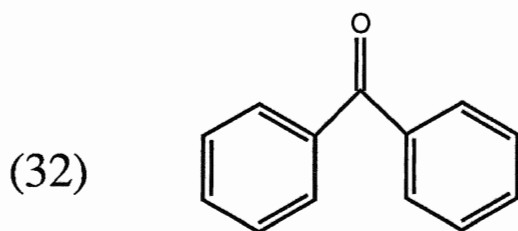
2—methyl—2—phenyl
—1,3—oxathiolane



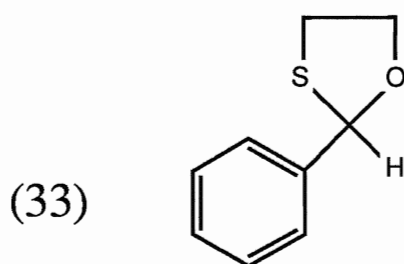
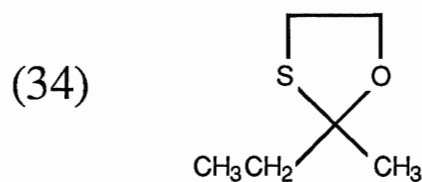
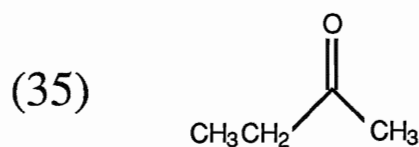
acetophenone



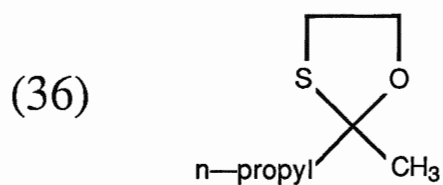
2,2—diphenyl—1,3—
oxathiolane

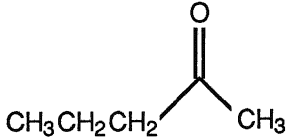
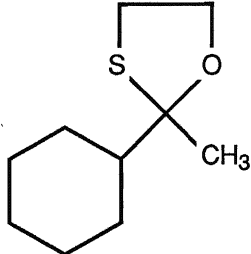
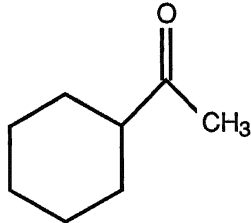
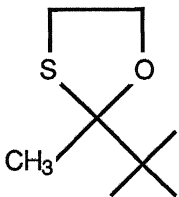
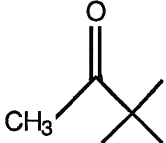


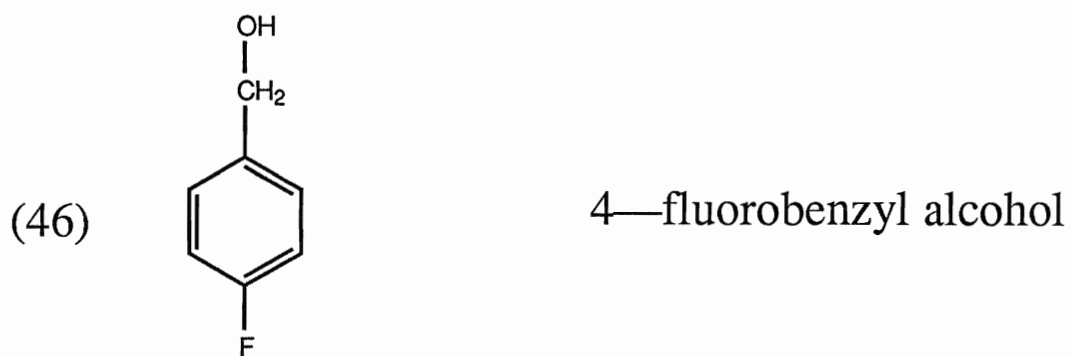
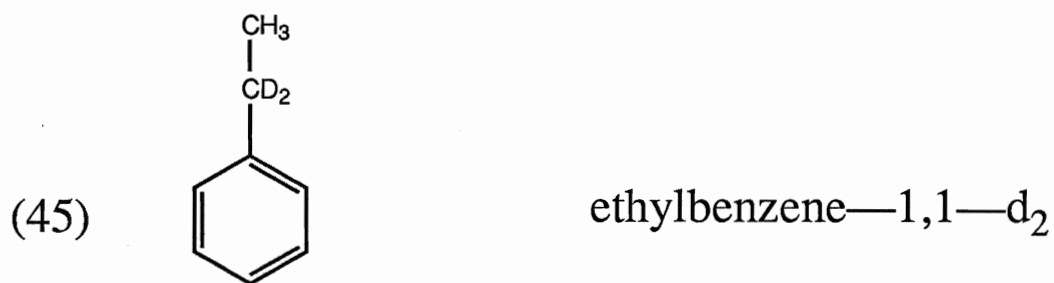
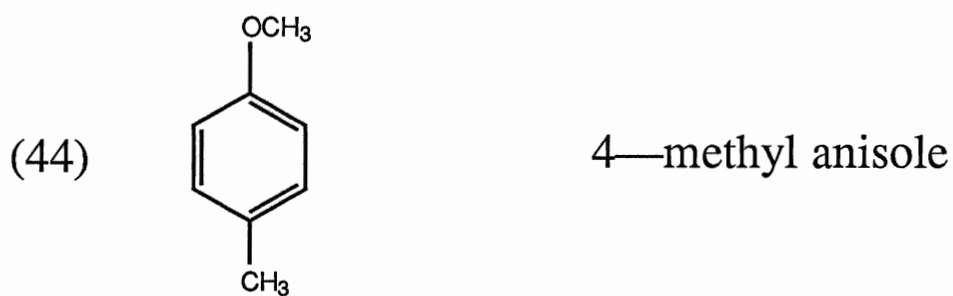
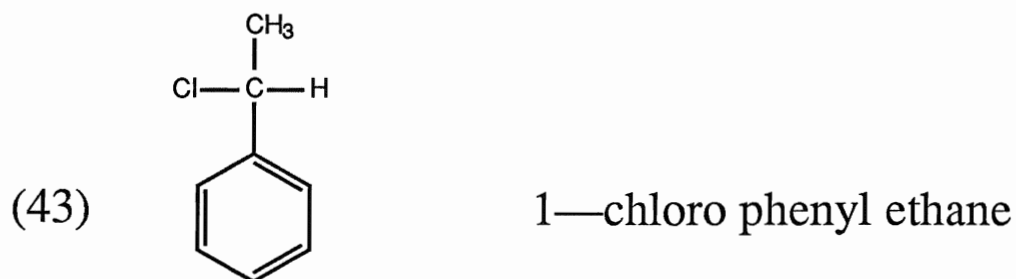
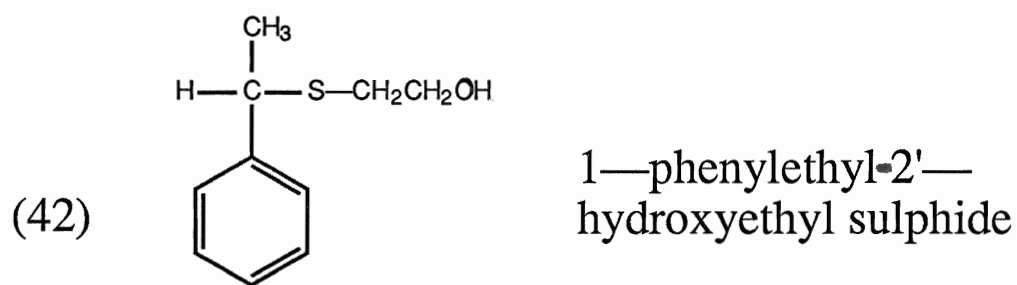
diphenyl ketone

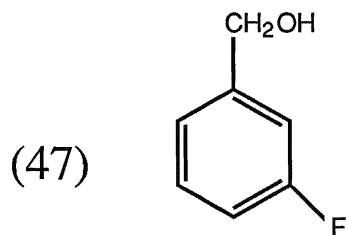
2—phenyl—1,3—
oxathiolane2—ethyl—2—methyl
—1,3—oxathiolane

ethyl methyl ketone

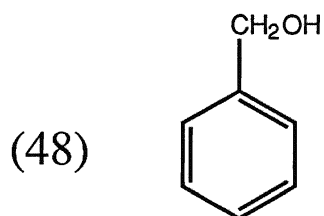
2—methyl-2-n-propyl
—1,3—oxathiolane

- (37)  methyl n-propyl ketone
- (38)  2—cyclohexyl—2—
methyl—1,3—oxathiolane
- (39)  cyclohexyl methyl ketone
- (40)  2—methyl-2-tert-butyl—
1,3—oxathiolane
- (41)  methyl tert-butyl ketone

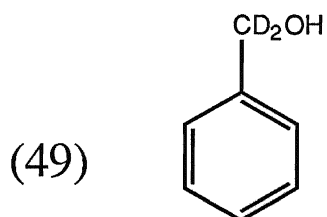
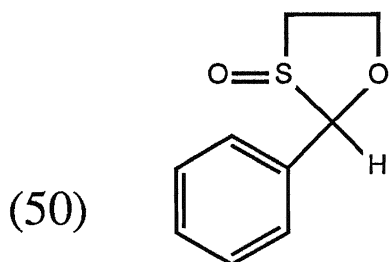
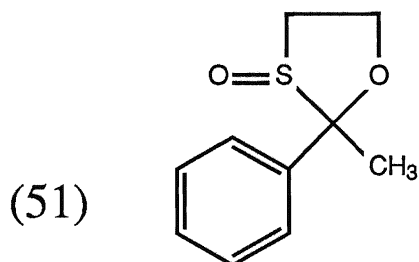


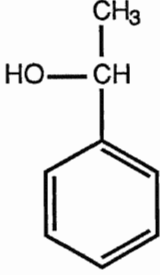
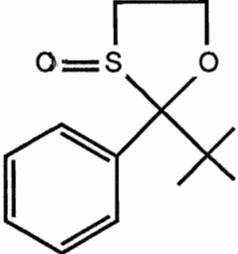
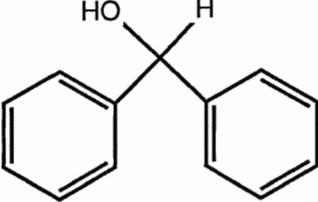
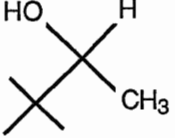
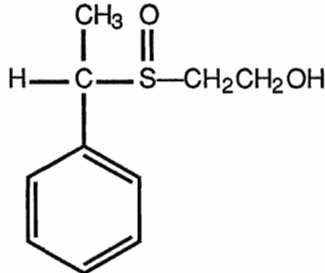


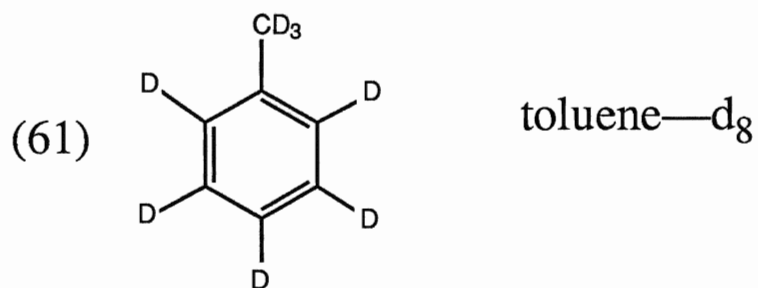
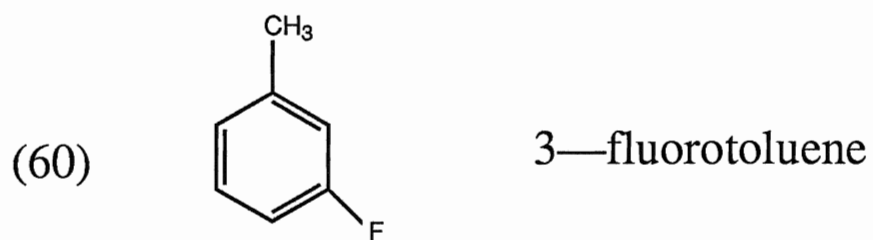
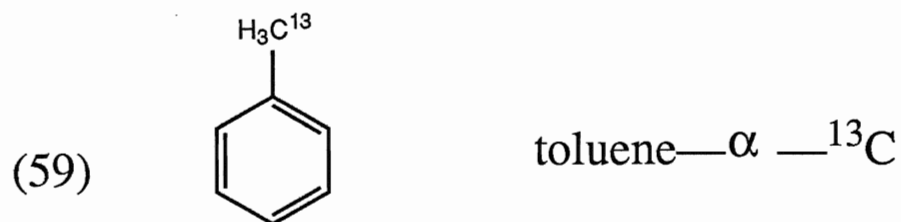
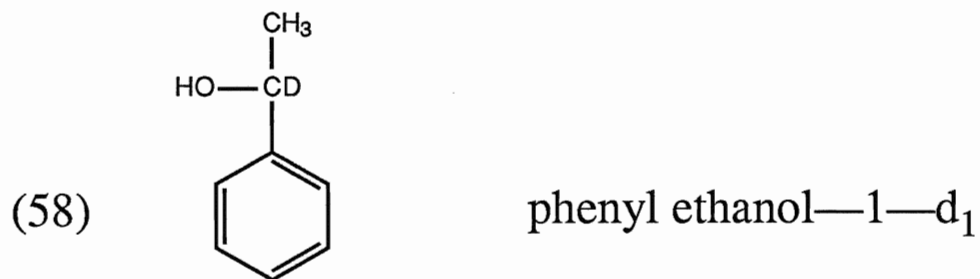
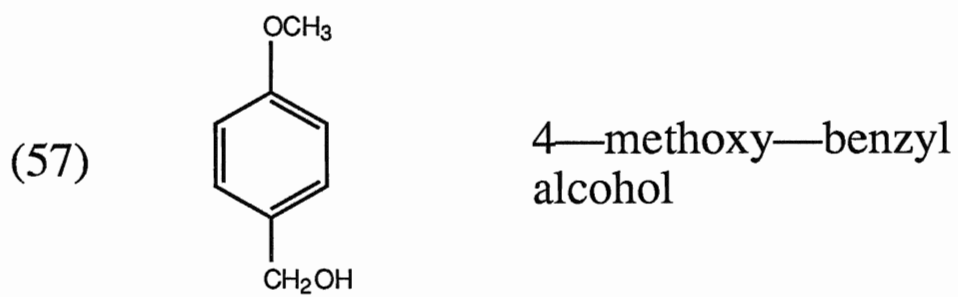
3—fluorobenzyl alcohol

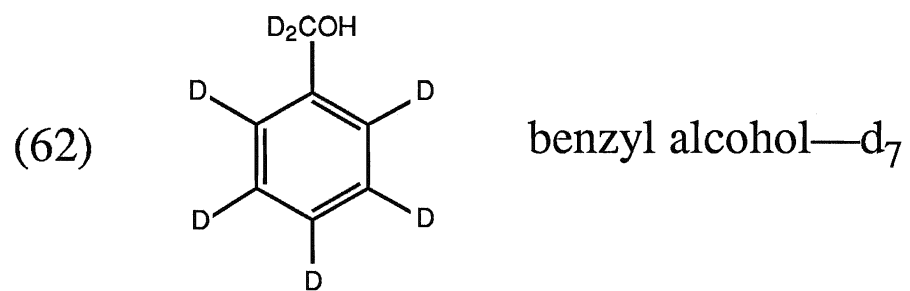


benzyl alcohol

benzyl alcohol— α, α — d_2 dl—threo—2—phenyl—
1,3—oxathiolan —1—oxidedl—threo—2—methyl—
2—phenyl—1,3—
oxathiolan —1—oxide

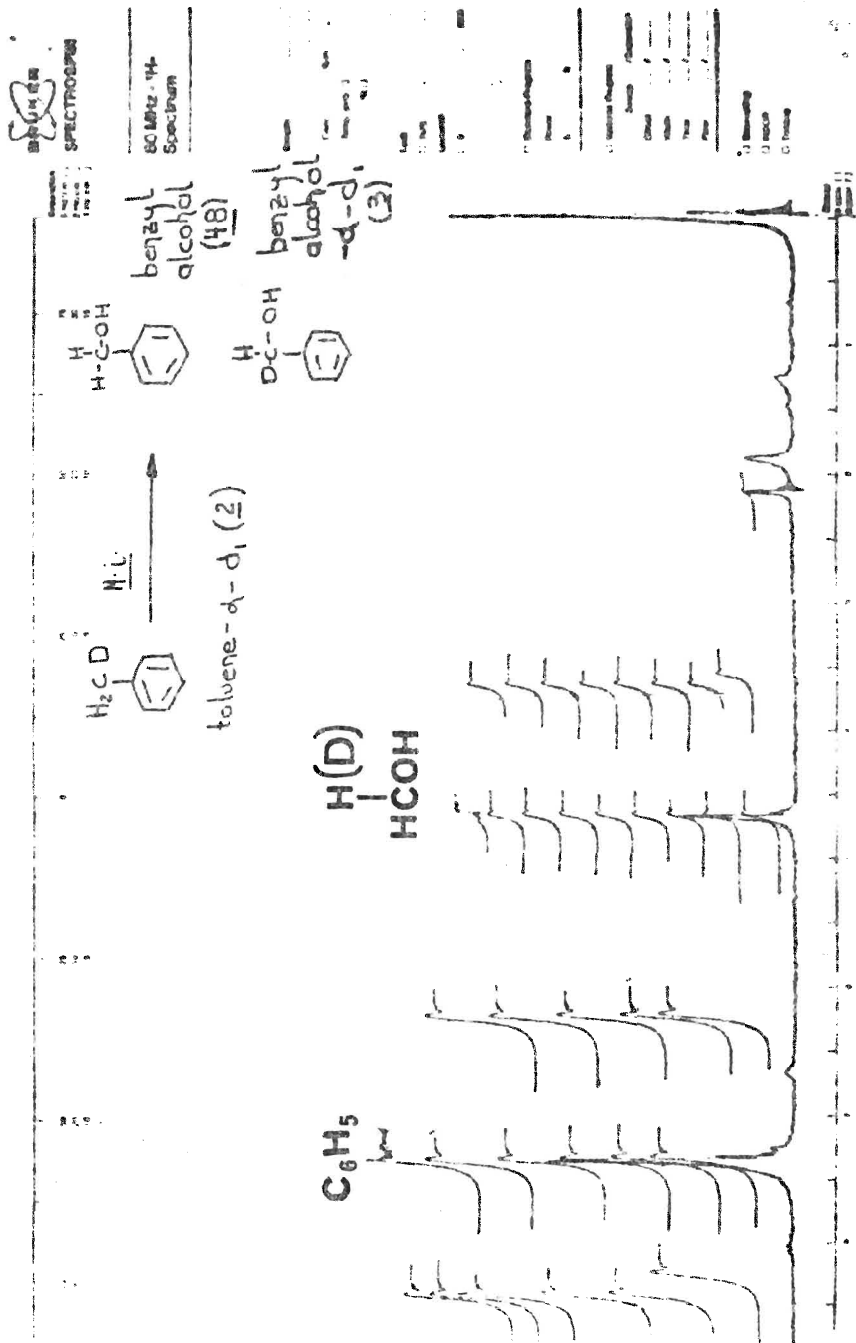
- (52)  **1-phenyl ethanol**
- (53)  **dl-threo-2-phenyl-2-tert-butyl-1,3-oxathiolan-1-oxide**
- (54)  **diphenyl methanol**
- (55)  **2,2-dimethyl-3-butanol**
- (56)  **1-phenylethyl-2'-hydroxyethyl sulphoxide**





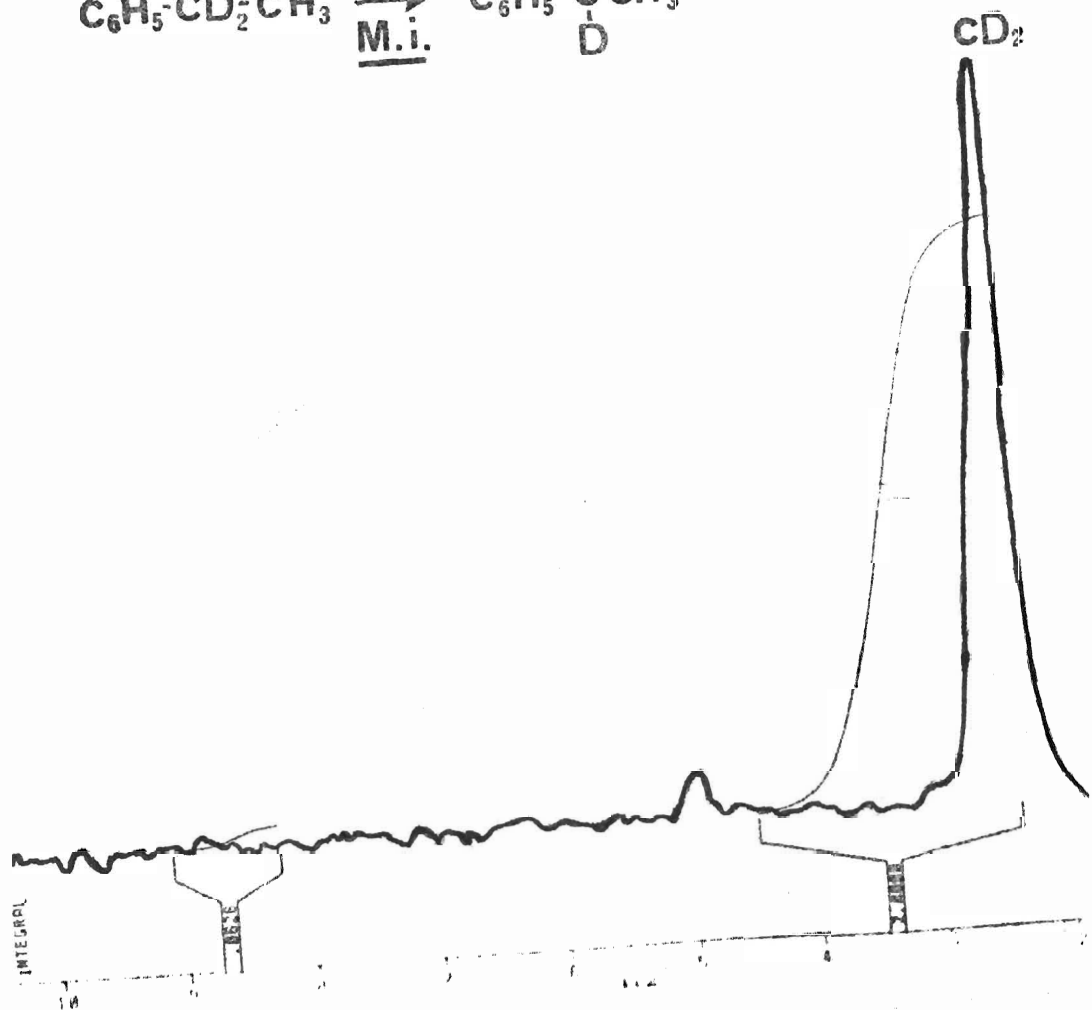
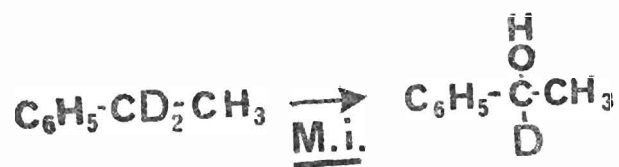
Appendix II

^1H NMR of benzyl alcohol (48) and
benzyl alcohol- α - d_1 (3). Products
from incubation of toluene- α - d_1 with
Mortierella isabellina .

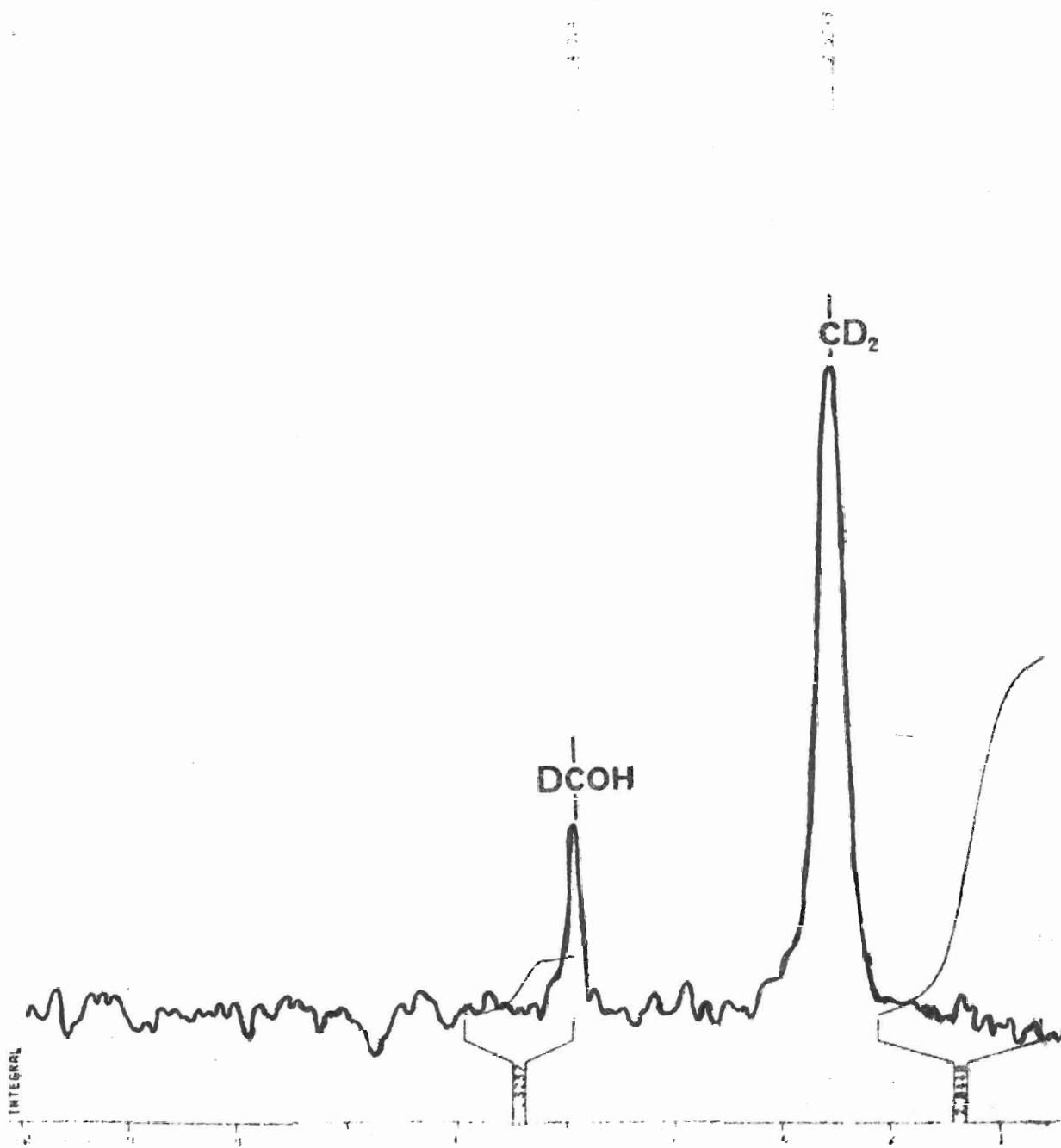


Appendix III

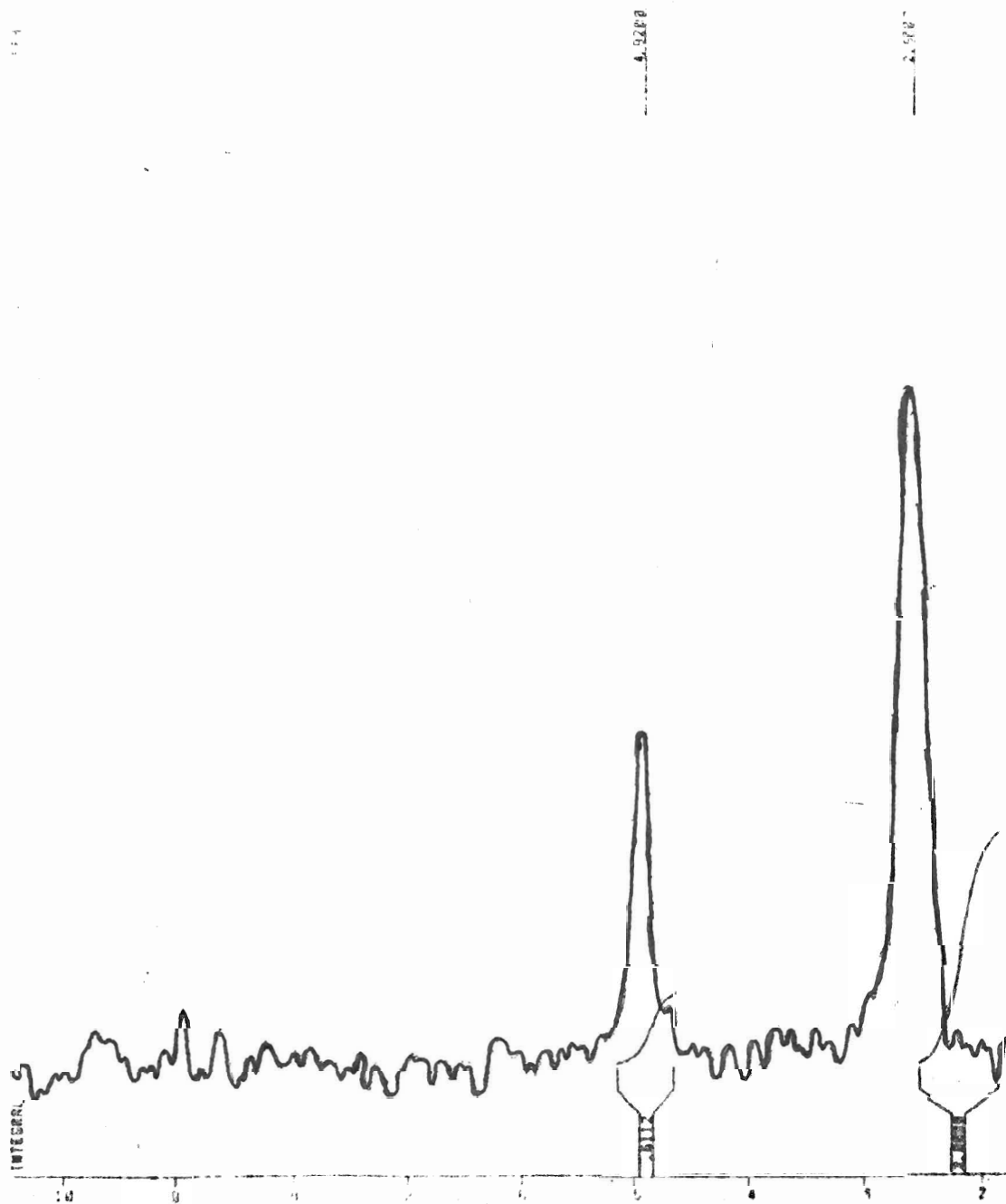
^2H NMR of ethylbenzene-1,1-d₂ (45)
with Mortierella isabellina over a five
day interval.

ETHYLBENZENE-CD₂ DAY 1

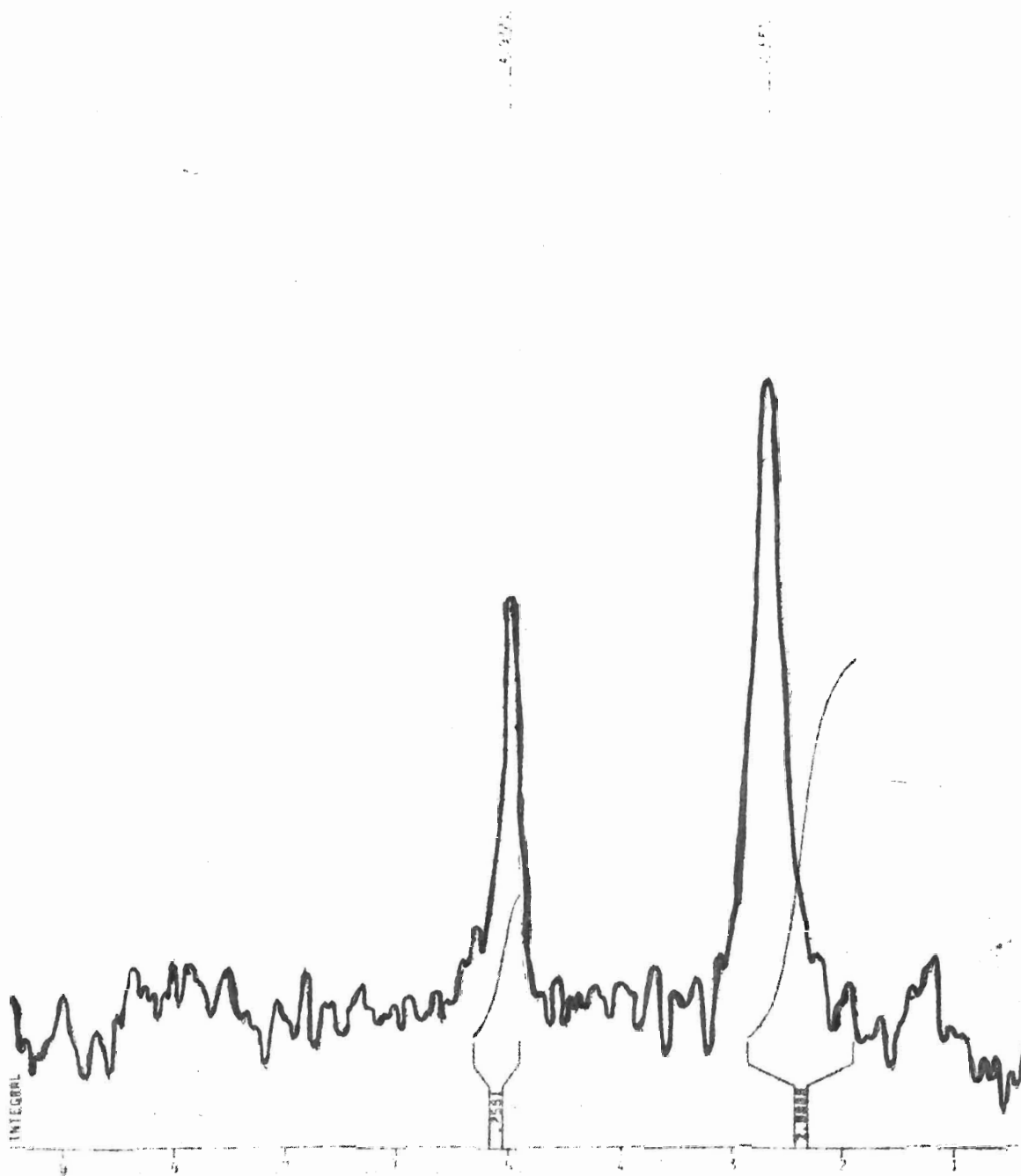
ETHYLBENZENE-D2 DAY 2



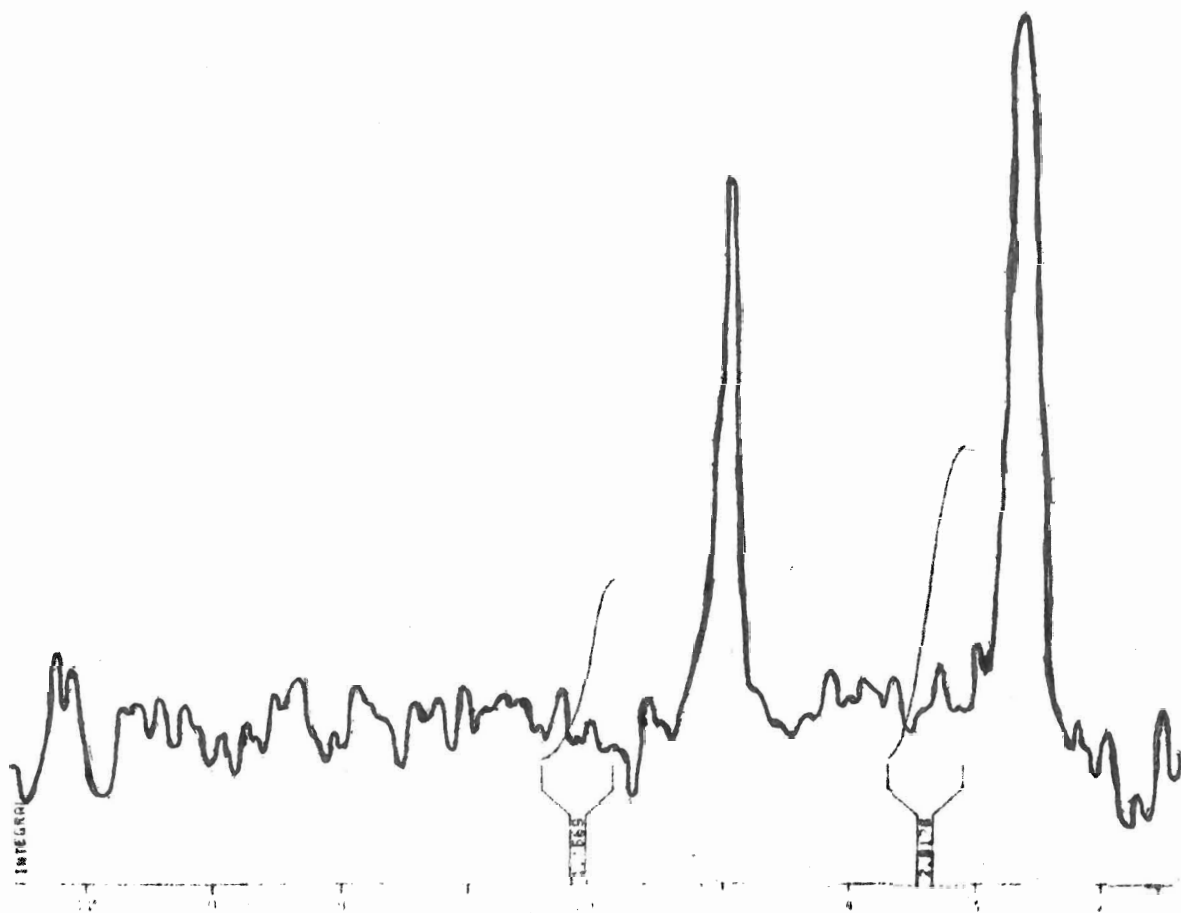
ETHYLENE-02 DAY3



ETHYLENZENF-D2 DAY4



ETHYLBENZENE-02 DAYS



Goodbye

and

Thanks

for

the

fish