CHEMOENZYMATIC SYNTHESIS OF 4-HYDROXY ENONES

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ELİF KÖSE

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Approval of the Graduate School of the Natural and Applied Science

_____

Prof. Dr. Canan Özgen

Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science in Chemistry.

_____

Prof. Dr. Hüseyin İşçi

Head of the Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Chemistry.

_____

Prof. Dr. Ayhan S. Demir

Supervisor

Examining Committee Members

Prof. Dr. Engin U. Akkaya _____

Prof. Dr. Ayhan S. Demir _____

Assoc. Prof. Dr. Özdemir Doğan _____

Assoc. Prof. Dr. Metin Zora _____

Assist. Prof. Dr. Feray Aydoğan _____

ABSTRACT

CHEMOENZYMATIC SYNTHESIS OF 4-HYDROXY ENONES

Köse, Elif
M.S., Department of Chemistry
Supervisor: Prof. Dr. Ayhan S. Demir
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Chiral cyclic polyoxo-ketones are important structural units in many natural products, biologically active compouds, such as prostaglandins, didemnenones, sarkomycin, punaglandin, clavulone, etc.

In this work, a chemoenzymatic synthesis of both enantiomers of the α'-acetoxy-α-methyl and γ-hydroxy-α-methyl cyclic enones starting from α-methyl-β-methoxy cyclic enone is described. Manganese (III) acetate-mediated acetoxylation followed by the enzyme-mediated hydrolysis of α'-acetoxy enone provides acetoxy enones. The reduction of the hydroxy enone, obtained from hydrolysis, furnished both enantiomers of 4-hydroxy enone or γ-hydroxy enone by using $LiAlH_4$. This study is a model for the synthesis of these type compounds.

Key words: Polyoxo-ketones, γ-hydroxy enones, $Mn(OAc)_3$ mediated oxidation, Enzymatic kinetic resolution

ÖZ

4-HİDROKSİ ENONLARIN KEMOENZİMATİK SENTEZİ

Köse, Elif
Yüksek Lisans, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. Ayhan S. Demir
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Optikçe saf, halkalı polyoxo-ketonlar, prostaglandin, didemnenon, sarkomisin, punaglandin, klavulon gibi biyolojik olarak aktif olan bir çok doğal üründe bulunan önemli yapılardır.

Bu çalışmada, halkalı α-metil-β-metoksi enon'dan başlanarak halkalı α'-asetoksi-α-metil ve γ-hidroksi-α-metil enon'un her iki enantiomerlerinin kemoenzimatik sentezi gerçekleştirilmiştir. $Mn(OAc)_3$ ile α'-asetoksillemenin ardından, elde edilen α'-asetoksi enonun enzimatik hidrolizi sonucunda optikçe saf asetoksi enon ve hidroksi enon elde edilmektedir. Hidroliz sonucu elde edilen hidroksi enonun $LiAlH_4$ kullanılarak indirgenmesi sonucu 4-hidroksi enonun (ya da γ-hidroksi enon) her iki enantiomerine ulaşılmaktadır. Bu çalışma, bu türde maddelerin sentezi için model bir yöntemdir.

Anahtar kelimeler: Polyoxo-ketonlar, γ-hidroksi enonlar, $Mn(OAc)_3$ oksidasyonu, enzimatik kinetik resolüsyon.

To My Parents

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INTRODUCTION

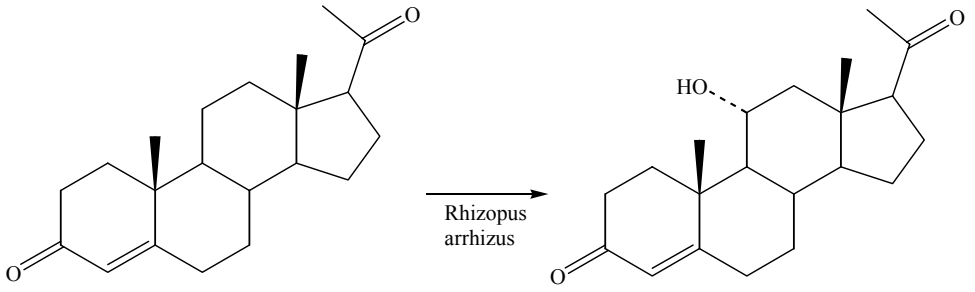
1.1. Bioconversion in Organic Chemistry

The use of biological methods to bring about chemical reactions forms a bridge between chemistry and biochemistry. Biotransformations are defined as the use of biological systems to bring about chemical changes on compounds that are not their natural substrates.

Isolated enzyme systems or intact whole organisms may be used for biotransformations. Each approach has its advantages and disadvantages. Many isolated enzyme systems are now commercially available or are relatively easy to isolate, at least in a crude form. They can be stable easy to use, often giving clean, single products. For many hydrolytic reactions, no cofactor is needed. However, for redox reactions in which a co-factor is used, the need to regenerate this can add a complication. Whole organisms do not have this disadvantage. They do tend to give more than one product, which may or may not be an advantage.

Biotransformations have a number of advantages when viewed alongside the corresponding chemical methods. Many biotransformations are not only regio- and stereospecific but are also enantiospecific allowing the production of chiral products from racemic mixtures. The conditions for biotransformations are mild and in the majority of cases do not require the protection of other functional groups. Furthermore, the features governing their regiospecifity differ from those controlling chemical specifity and indeed it is possible to obtain biotransformations at centers that are chemically unreactive (e.g. 1 transformed to 2 (scheme 1.1)). The dominant

feature in a biotransformation is the topological relationship between the substrate and the active site of the enzyme. From a commercial point of view some biotransformations can be cheaper and more direct than their chemical analogues whilst the transformations proceed under conditions that are normally regarded as 'environmentally friendly'.



1 **2**

(1)

Biological systems recognize the members of an enantiomers pair as different substances and also two enantiomers give different responses. As a result, one enantiomer may act as an efficient therapeutic drug while the other is highly toxic. The well known example is thalidomide, one enantiomer of which has sedative effect whereas the other is teratogenic. (Figure 1.1) It caused severe birth defects in children whose mothers had taken the drug in their first trimester of pregnancy. This event has shown for many pharmaceuticals that only one enantiomer contains all the desired activity, and the other is either totally inactive or toxic. Therefore, to synthesis of chiral drugs as the single appropriate enantiomer is very important issue in synthetic organic chemistry, medicinal chemistry, natural product chemistry and the pharmaceutical industry.

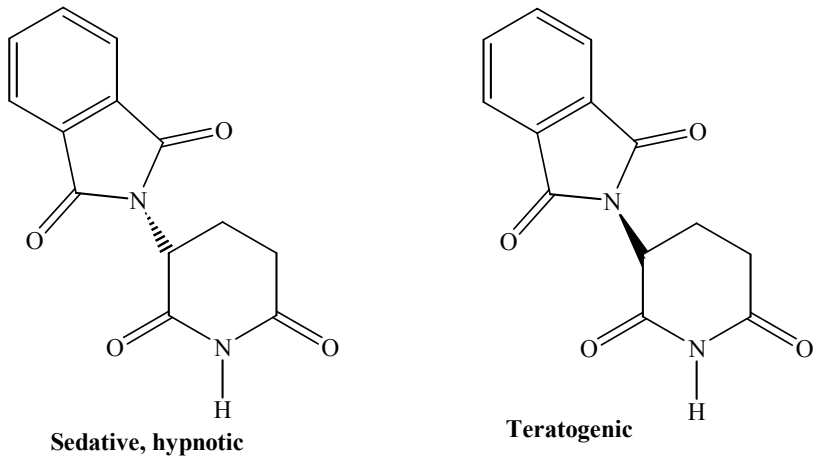


Figure 1.1

1.2. Chirality and Asymmetric Synthesis

A chiral molecule is one that is not superimposable on its mirror image; it has the property of rotating the plane of polarization of plane-polarized monochromatic light that is passed through it. This phenomenon is called optical activity.

When four different atoms or groups attached to the same carbon atom, two different arrangements, which are called as enantiomers, may exist, non-superimposable but mirror images of each other. One of them rotates the plane of polarized light to the right, and the other shows opposite rotation. If two or more groups attached to carbon are identical, the object and its mirror image become superimposable and chirality is lost.

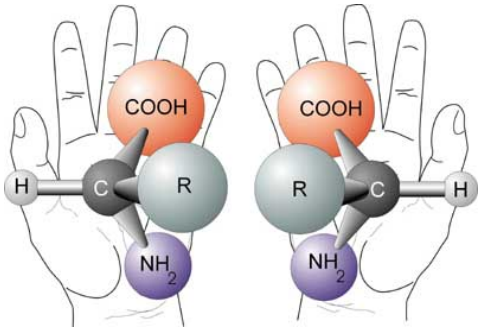


Figure 1.2

Despite the majority of optically active compounds containing at least one asymmetric carbon atom, the presence of asymmetric atoms is not an obligatory condition for the existence of optically activity. The necessary and sufficient condition for a molecule to show optically activity is that such a molecule should not be superimposable on its mirror image. To decide whether a given molecule is chiral, superimposibilty may be ascertained either by constructing models of the molecule and its mirror image or by looking for symmetry elements in the molecule. A molecule with a plane of symmetry, a centre of symmetry, or an alternating of symmetry is superimposable on its mirror image and cannot be optically active.[2]

The non-superimposibility can come about in a number of ways, and need not involve a chiral centre or even organic molecules at all. Some examples are amusing; since these do not contain asymmetric carbon atoms.[3]

Some tertiary amines might be thought to be chiral; the structure is pyramidal and if all three groups about the nitrogen are different then the mirror images appear to be non-superimposable. However, if the X, Y, Z groups are independent, then no chirality is shown. This is because the molecules flip inside out very rapidly, in the case of ammonia at a frequency of 2×10^{11} Hz. Amines are slower, but still do not permit resolution into two enantiomers. (Figure 1.3)

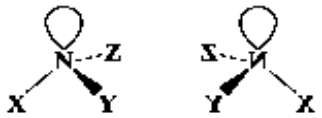
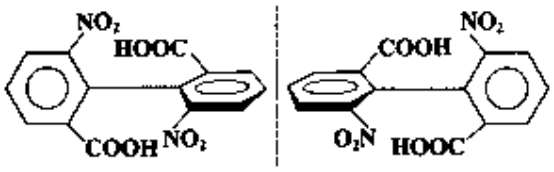
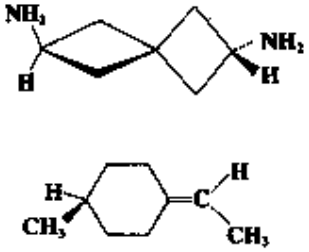


Figure 1.3 A tertiary amine and its apparently non-superimposable mirror image.

Restricted rotation about a single double bond is well-known as a potential source of geometric isomerism, though it isn't the only one. Restricted rotation can also give rise to chirality. The first example of this situation is biphenyls consisting of two benzene rings joined by a single bond. If the substituent groups are different, then the molecule will be chiral. Such enantiomers are called *atropisomers*. Restricted rotation is also shown by allenes, compounds with two double bonds side-by-side. They are chiral only if both sides are unsymmetrically substituted. Restricted rotation can also be found in *spiranes*, compounds having two rings with one carbon atom in common. This makes the rings perpendicular, and suitable substitution gives rise to chirality. (Figure 1.4)



Atropisomers



A spirane (top) and an exocylic double bond leading to chirality

Figure 1.4

To obtain enantiomericaly pure materials, there are several methods including classical optical resolution via diastereomers, chromatographic separation of enantiomers, asymmetric synthesis, chemical kinetic resolution, and enzymatic kinetic resolution.[4] In these methods there are two most popular strategies. The first one is asymmetric synthesis involving stereocontrolled formation of the new stereogenic

center. For example; as the new chiral element is formed it is done so in a non-racemic fashion. This demands that the reactive centers experience some stereo discriminating environment in the transition state. This can originate from an existing stereogenic center in the substrate or in via a chiral reagent catalyst.[5] The second approach involves resolution: this utilizes a stereoisomeric mixture and does not demand asymmetric induction in the formation of any new chiral element. Thus preparation of a single stereoisomer by resolution of a stereisomeric mixture may be achieved via a conventional separation procedure or by exploiting the difference in reactivity (kinetic resolution).

1.2.1. General Methods for Asymmetric Synthesis

The most quoted definition of an asymmetric synthesis is that of Marckwald: Asymmetric syntheses are those which produce optically active substances from symmetrically constituted compounds with the intermediate use of optically active materials, but with the avoidance of any separations. Also, an appropriate broader definition can be possible: Asymmetric synthesis is a reaction or reaction sequence that selectively creates one configuration of one or more new stereogenic elements by the action of a chiral reagent or auxiliary, acting on heterotopic faces, atoms, or groups of a substrate. The stereoselectivity is primarily influenced by the chiral catalyst, reagent, or auxiliary, despite any stereogenic elements that may be present in the substrate.[6]

There are some different approaches to the asymmetric synthesis.

Chiral Reagent: In principle this an excellent approach, since the substrate and product should require no synthetic manipulation.[7] Unfortunately, the currently available reagents for this approach often lack the generality and level of stereoselectivity, which would be required. Considerable effort and expense can be involved in the preparation of the reagent, and the stoichiometric amounts are required. If high enantiomeric excess is not produced directly, purification of the product to the desired enantiomeric purity is generally difficult since mixtures of enantiomers are isolated from the reaction.

Chiral Substrates: The best scenario is to have a chiral starting material that can then control the stereoselection of the reaction itself.[8] To achieve this, especially at the beginning of the synthetic sequence, few options are available. Nature produces chiral materials and a number of these are available in quantity. These compounds make up the 'chiral pool'. This approach is often limited to the amount of the natural product available and its price. Another consideration, sometimes overlooked, is the number of steps necessary to convert the natural product into a useful starting material for synthesis. If all of the parameters are favorable, this approach is the method of choice as it has the potential to eliminate resolutions or the necessity for an enantiospecific transformation in the synthetic design.

Chiral Auxiliaries: This approach offers significant advantages, provided that the chiral auxiliaries fulfill all the necessary conditions. The reactions are often highly predictable and reliable. The auxiliaries can be recycled. Purification to high enantiomeric excess is easy in principle, as the immediate products are diastereoisomers. Conventional purification techniques should provide diastereoisomerically pure products and removal of the chiral auxiliary produces enantiomerically pure material. However, stoichiometric quantities of the chiral auxiliaries are required, and synthetic manipulations of the starting material and product are necessary.

Self Regeneration of Stereocenters: There is a variation on the Chiron approach. A chiral center from a starting material can be transferred to another part of the molecule. This new chiral center then provides control for a stereoselective reaction, where a new center of asymmetry can be established, or the chirality at the center of the original starting material can be reestablished. Invariably, a cyclic system is involved. This approach has been used by Seebach and described as the regeneration or self-reproduction of stereogenic center.

Chiral Catalysts: Asymmetric synthesis is most efficient when chiral catalysts are used that cause one enantiomer to be selectively converted or only one enantiomer to be formed. Only catalytic amount of chiral catalyst is needed to produce large amount of chiral product. These chiral catalysts can be divided into two

groups. In the case of chemical catalysis chiral transition metal complexes[9] or organic catalyst, like cinchona alkolaids[10] are frequently used and there are many examples of very successful asymmetric reactions. The major drawback at present is that relatively few catalysts which give both enantiomers a high enantiomeric excess and accept a wide range of substrates are available, and that the products are enantiomeric mixtures so enantiomeric enrichment could be difficult.

Chiral Environments: It is possible to make the environment of a chemical reaction chiral. The majority of examples in this class utilize chiral solvents or additives. To influence the differentiation of the free energies of the diastereomeric transition states, and hence provide useful induction. These agents must be closely associated with the reaction center. In most cases, this has not been fruitful, as in the use of chiral solvents, but some reactions that use chiral ligands do provide good ee's.

In the synthesis of complex molecules, all of the above approaches may be combined. The marriage of two approaches also gives the potential for double asymmetric induction.

1.2.2. Asymmetric Synthesis Using Biotechnological Methods

Like asymmetric synthesis, kinetic resolution is most efficient when chiral catalysts are used. Kinetic resolution can be performed both using chemical catalysts and biocatalysis which encompass catalysis by bacteria, yeast, fungus, or their true components: enzymes. Enzymes, biological catalyst, are proteins that are capable of accelerating reactions under mild reaction condition by lowering the activation energy (Figure 1.5). Other advantages are the high degrees of substrate-, chemo-, regio- and stereoselectivity and high efficiency.

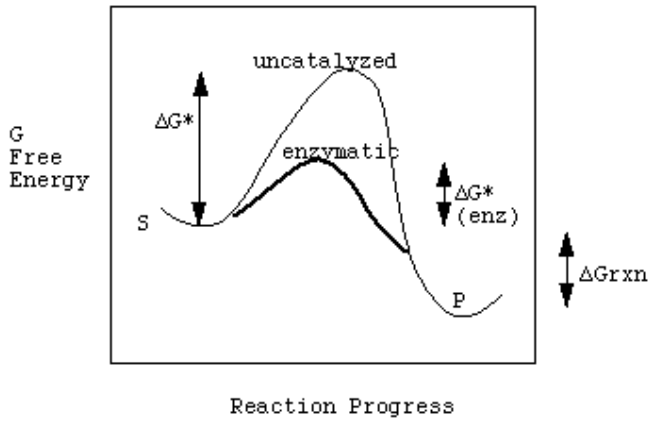


Figure 1.5

Enzymes have an active site to bind a substrate. Among the numerous theories and rationales which have been developed in order to understand relation between active site and substrate, the most illustrative models for the organic chemist are 'Lock and Key' mechanism and Induced-Fit mechanism.[11]

In 1894 E. Fisher developed the first proposal mechanism of enzymatic action. According to this mechanism, an enzyme and its substrate mechanistically interact like a lock and a key, respectively**.** (Figure 1.6 a) In spite of this assumption was quite sophisticated at that time, it assumes a completely rigid enzyme structure. Therefore, it cannot explain why many enzymes do act on large substrates, while they are inactive on smaller, similar counterparts. Furthermore, the hypothesis cannot explain why many enzymes can convert not only their natural substrates but also numerous non-natural compounds possessing different structural features.

Induced-Fit mechanism was developed by Koshland Jr. in the late 1960s. According to this, enzymes are not entirely rigid but rather represent delicate and soft structures. It assumes that upon approach of a substrate during the formation of the enzyme-substrate complex, the enzyme can change its conformation under the influence of the substrate structure so as to wrap itself around its guest. (Figure 1.6 b) This advanced model can indeed explain why in many cases several structural features on a substrate are required in addition to the reactive group. These structural features may be located at quite a distance from the actual site of the reaction

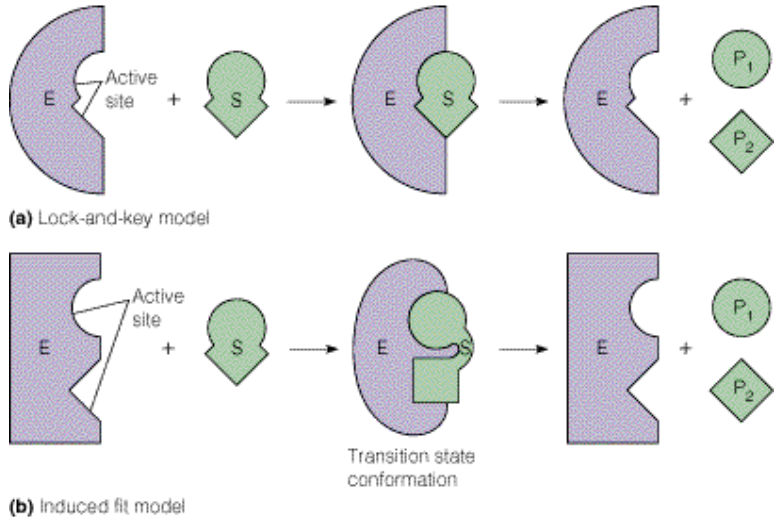


Figure 1.6

The most typical 'Induced-Fit' enzymes are lipases. They can convert an amazing variety of artificial substrates which possess structures which do not have much in common with the natural substrates-triglycerides. Lipases are among the most commonly used enzymes in organic synthesis because of their stability, availability, and acceptance of a broad range of substrates.

Diastereomers can be relatively easily separated and therefore differences in their reactivity can be explored in separate experiments.[12] With enantiomers the situation is just the opposite. Their separation is rather difficult and therefore conditions are sought under which they react at different rates, whereby ultimately their separation can be effected. When the rates are comparable, one of the enantiomers is transformed faster, and therefore an excess of the less reactive substrate gradually builds up. This excess goes through a maximum and disappears on completion of the reaction (Figure 1.7). If the reaction is interrupted before completion or if less than the necessary amount of reagent is applied, the result is a non-racemic mixture of the starting material and of a product in which an excess of the more reactive enantiomer of the substrate is incorporated. This way of partial separation of enantiomers is called kinetic resolution.

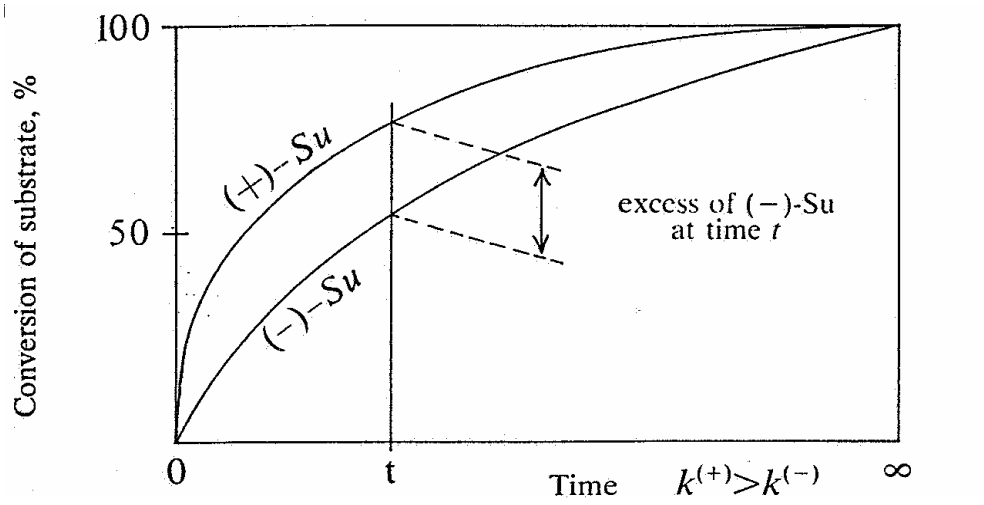


Figure 1.7 Kinetic resolution via as a function of time

Thanks to the chirality of the active site, enzyme fits one enantiomer better than the other enantiomer. Therefore, it is converted at a higher rate, so enantiomeric differentiation occurs. (Figure 1.8)

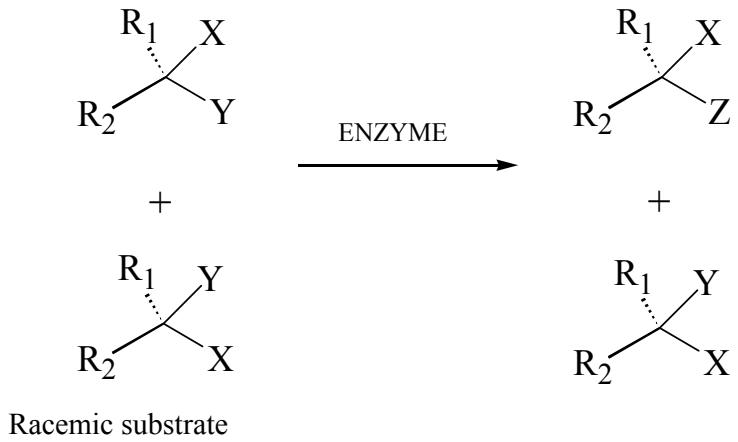


Figure 1.8

Kinetic resolutions an inherently wasteful process for producing optically active compounds and can only compete with conventional resolution when rate differences are extreme. With few exceptions this has so far only been realized with enzymes.

Enzymes can be classified according to the reactions they catalyze.[13] Hydrolytic enzymes, such as lipases are able to speed up hydrolytic reactions. This class of enzymes can be divided in to four groups with different catalytic systems. Serine proteases contain a catalytic triad with serine acting as a nucleophile. Examples of serine proteases include trypsin, chymotrypsin, pig liver esterase, and lipases. Figure 1.9 illustrates a catalytic cycle for serine proteases, which is representative for most lipases.

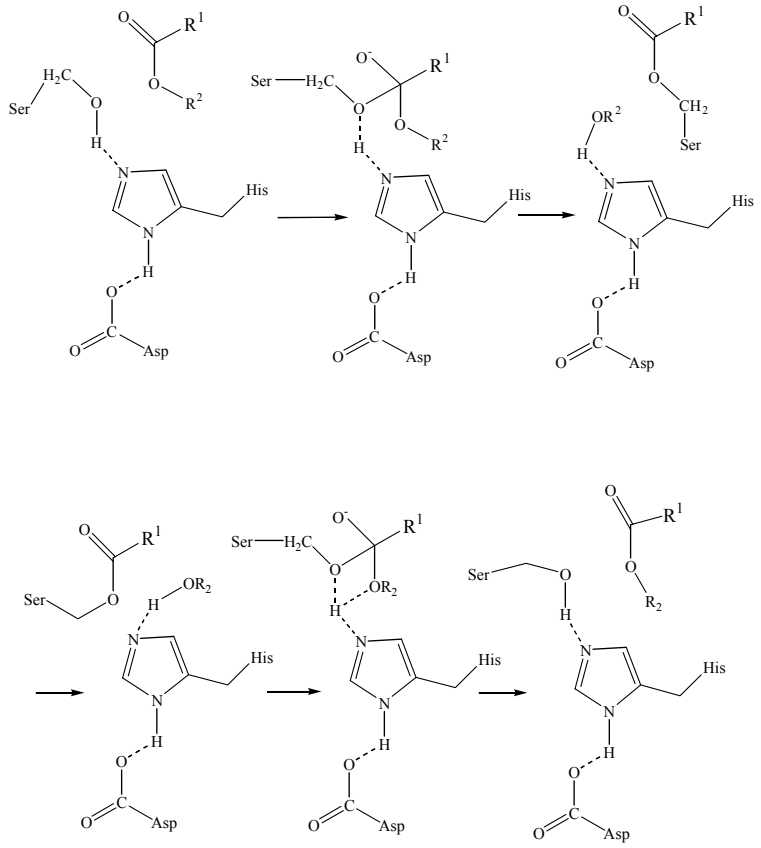


Figure 1.9 Catalytic cycles for serine proteases

In serine proteases a catalytic triad consisting of the amino acids serine, histidine and aspartic acid are responsible for the catalysis. In figure 1.9 Ser reacts as a nucleophile with a substrate molecule. Here being an ester. During substrate

binding a proton is transferred from Ser to His, making Ser more nucleophilic. The positive charge of the protonated imidazole ring is stabilized by interaction with the carboxylate group of Asp. A tetrahedral intermediate is formed in which the enzyme and substrate are covalently linked (enzyme-substrate transition state). The proton on His binds to the alkoxy group that is then eliminated as an alcohol molecule. An acyl enzyme is formed as a covalent intermediate. The highly reactive intermediate formed may react with water (R_2 = H, hydrolysis) or a second alcohol molecule (transesterification) to yield the product of the reaction, being either an acid or an ester.

1.3. α-Hydroxy Ketones

The synthesis of α-hydroxy carbonyl derivatives has been of continuous interest to organic chemists since the beginning of the century.[14] Because chiral α-hydroxy ketones are important reagents for the synthesis of complex optically active natural products and are useful stereodirecting groups.[15] There are both chemical and biotechnological methods for the synthesis of α-hydroxy ketones.

1.3.1. Chemical Synthesis

Classical methods have been supplemented in more recent years by several heavy metal-containing oxidants such as MoO_5.Py.HMPA[16] and CrO_2CI_2[17], but these type agents are potentially contaminating so chemists are trying to minimize as much as possible the use of them.

A number of studies on this subject apart from metal-containing oxidation have been reported. According to one of them, the complex, HOF.MeCN made directly by bubbling fluorine through aqueous acetonitrile, reacts quickly and efficiently with enolic forms of ketones to produce α-hydroxy ketones.[18] This complex is rapidly evolving as the best possible oxygen transfer agent, since it contains a truly electrophilic oxygen.

Other study is asymmetric oxidation of ester and amide lithium enolates to α-hydroxy carbonyl compounds using (camphorsulfonyl) oxaziridines, the configuration of whose three-membered ring determines the product stereochemistry.[19]

By use of chiral phase transfer catalysts (CPTC), catalytic enantioselective oxidation of achiral ketones with molecular oxygen was achieved to obtain α-hydroxy ketones.[20] Also; regioselective synthesis of α-hydroxy ketones starting from allylic alcohols via cyclic iodo carbonates was reported.[21]

In metal-free methods, silyl enol ethers and enol esters have been oxidized to optically active α-hydroxy ketones by the in-situ-generated dioxirane from the fructose-derived ketone. Moreover, it is well-known that vic-diols may be readily oxidized by dioxiranes to yield the corresponding α-hydroxy ketones. Using these results, it was reported that the oxidation of vic-diols to enantiomerically enriched α-hydroxy ketones was achieved by fructose-derived dioxirane.[22]

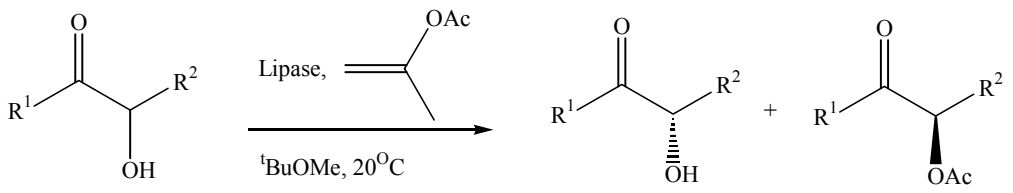
In a recent paper, a new mild method for the oxidation of a variety of olefins to α-hydroxy ketones has been described. Using the concept of a nucleophilic reoxidant, RuO_4-catalyzed ketohydroxylation of different olefins has been reported to give α-hydroxy ketones.[23]

1.3.2. Biotechnological Methods

Alternative to the chemical methods, optically active α-hydroxy ketones can be prepared enzymatically by reduction of the α-diketones with yeast as the biocatalyst.[24] However, this enzymatic method possesses the following disadvantages: further reduction of diketone to the vic-diol, formation of both regioisomeric α-hydroxy ketones and moderate chemical yields.

Esterases and lipases have a major application in the preparation of chiral molecules for synthesis. PLE (*Pig liver esterase*), PPL (*Porcine pancreatic lipase*), CCL (*Candida cylindracea lipase*), α-chymotrypsin, and PCL (*Pseudomonas*

cepacia lipase) are some commonly used enzyme systems for hydrolysis. Lipases have been widely used for the synthesis of optically active alcohols, carboxylic acids and esters via enantioselective esterification and transesterification in organic solvents. Although numerous α-hydroxy acids and esters have been resolved by lipases, reports on the kinetic resolution of structurally simple α-hydroxy ketones by these readily accessible enzymes are scarce. Recently, Gala et al. have described[25] the resolution of α-hydroxy aryl ketones (precursors of chiral azole antifungal reagents) by lipase catalyzed hydrolysis of the corresponding acetates in phosphate buffer; nevertheless, the irreversible transesterification route of this enzymatic reaction appears not to be known. Another report has been presented by Adam et al. that is the kinetic resolution of racemic α-hydroxy ketones by lipase-catalyzed irreversible transesterification with isopropenyl acetate in organic media (Scheme 2).[26]



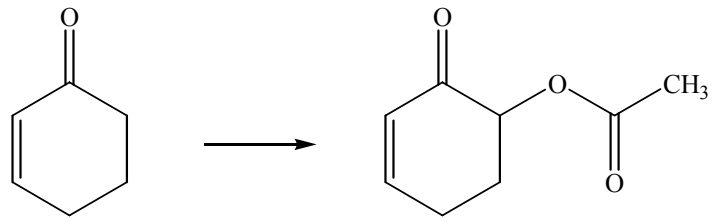
(2)

Demir and co-workers presented the enantioselective synthesis of hydroxy ketones from ketones via $Mn(OAc)_3$ mediated acetoxylation followed by enantioselective ester hydrolysis utilizing *Rhizopus oryzae.*[27] In the other study, they achieved the synthesis of α-hydroxy ketones by enzymatic kinetic resolution using a number of lipases.[28-30]

1.4. $Mn(OAc)_3$ Mediated Acetoxylation of Enones

Procedures for the selective oxidation of common functional groups occupy a central position in the synthesis of complex natural products. For the preparation of α'-acetoxyenone, methods in the literature gave unsatisfactory results.[31] To overcome this problem, Demir and his coworkers studied on the oxidation of α,β-

unsaturated enones using manganese (III) acetate.[32 - 35] They got satisfactory result for the preparation of α'-acetoxyenones.(Scheme 3)

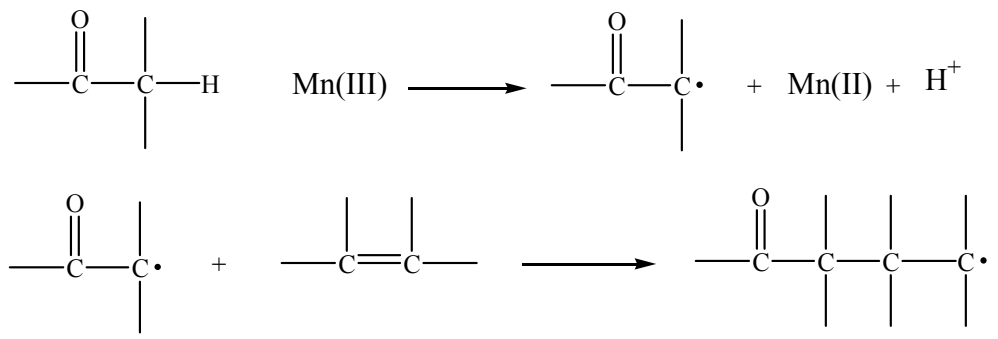


(3)

Oxidations with manganese (III) acetate can be broadly divided into two classes;

1. Direct Oxidation: Direct inner or outer-sphere one electron oxidation of the substrate; often determines the product is followed by the formation of manganese (III) complex where the subsequent oxidation of the intermediate radical occurs. Numerous examples can be found such as oxidations of alcohols, amino and thio compounds, carboxylic acids and certain aromatics.

2. Indirect Oxidation: Indirect oxidation of the substrate; takes place after the formation of an intermediate adduct free radical which is formed by the interaction of Mn(III) acetate. The result is an enolizable compound or subsequent oxidation/substitution and oxidative addition of enolizable compounds to unsaturated systems. Mn (III) acetate deals with addition reaction of compounds which have α-hydrogen atom to a carbonyl group with olefinic and aromatic unsaturated systems (Scheme 4).



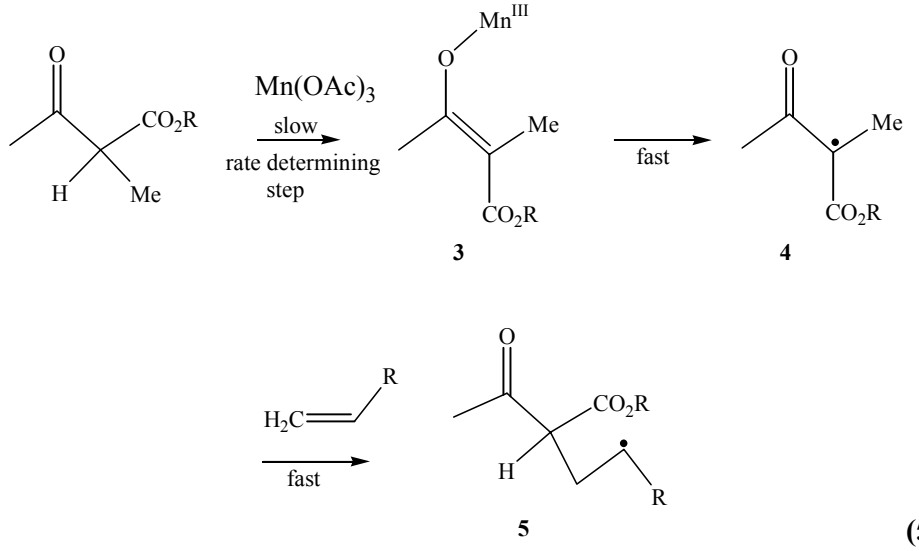
(4)

The fate of the primary radical adduct strongly depends on reaction conditions and the nature of the substrate. Manganese (III) acetate can be used as a free radical generator if substrates are less reactive to common oxidants. The one electron oxidants like Co (III), Ce (IV) and some two electron oxidants like Tl (III) and Pb (IV) also show similar properties as manganese (III) acetate.

Of all methods in the literature about α-acetoxylation, there are two most promising methods in terms of yield. One of them is lead (IV) but it is highly toxic. In general, $Mn(OAc)_3$ oxidations are characterized by higher chemical yield, higher α'-regioselectivity and milder reaction conditions, tolerating many sensitive functional groups.

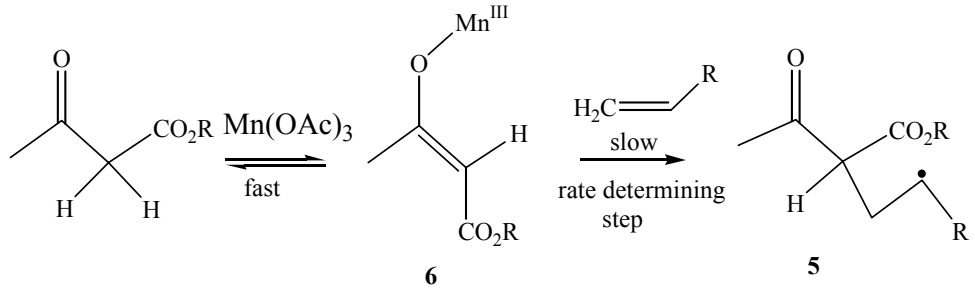
Manganese (III) acetate can be used for initiating the addition of aldehydes to olefinic unsaturated systems, the addition of ketones to olefinic unsaturated systems, the addition of haloalkanes to unsaturated systems, aromatic substitution reactions.

The mechanism of oxidation of monocarbonyl substrates with $Mn(OAc)_3.2H_2O$ has been extensively studied. Snider[36] has found a mechanism which is operative in the oxidation of α-alkyl-β-keto esters (Scheme 5)



(5)

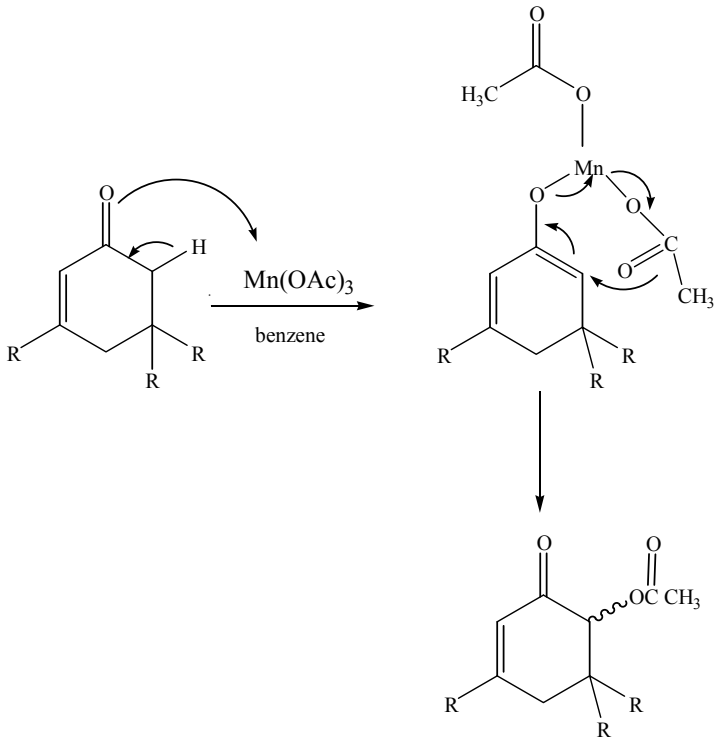
Enolization to give **3** is slow; electron transfer with loss of Mn (II) to give **4** is rapid. The rate of reaction is therefore independent of alkene concentration. This indicates that free radical **4** is involved in the Mn(III)-mediated oxidations. On the other hand, they found that the enolization of α-unsubstituted β-keto esters is fast and reversible, and electron transfer to give the radical is very slow (Scheme 6).



(6)

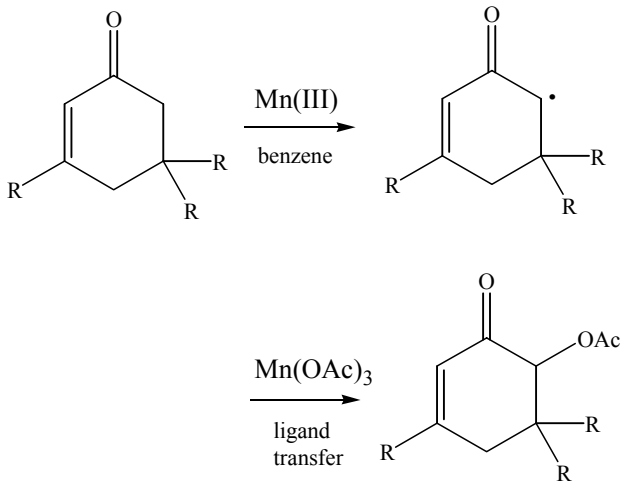
The rate determining step depends on alkene concentration and is presumably the reaction of the Mn (III) enolate **6** with the alkene to give radical **5** with loss of Mn(II). β-Keto ester radicals analogous to **4** do not appear to be intermediates in these reactions. They concluded that the nature of the reaction depends on two variables: the rate of formation of the Mn (III) enolate, which corresponds to the pKa, and the ease of oxidation of the enolate to give the free radical.

The mechanism of manganese (III) acetate oxidations in benzene remain uncertain, but it seems reasonable based on related oxidations of lead(IV) acetate. [37] The interaction of the enol or enolate of aromatic ketone with manganese(III) acetate would result in acetate transfer (Scheme 7).



(7)

Another suggested mechanism includes the formation of an α-keto radical resulting from the oxidation of an enol or enolate anion by Mn (III) (Scheme 8)



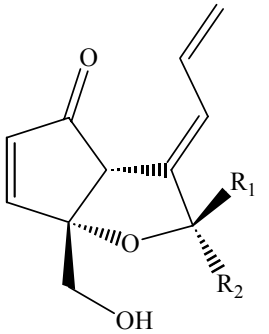
(8)

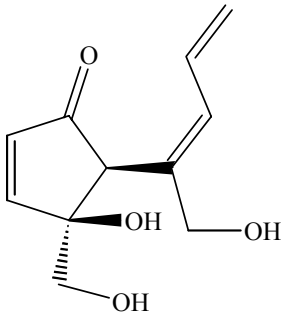
1.5. The Importance of Polyoxo Cyclopentenones

Cyclic polyoxo-ketones are valuable structures since they have many functional groups in spite of their small structures. The functional groups can be changed with any other small functional groups. Consequently, they can be used in many synthetic applications.

The synthesis of cyclic polyoxo-cyclopentenones and 4-hydroxy cyclopentenones is important subject because they are important structural units in many biologically active natural products.

The first group containing these structures is didemnenones. They display a rich abundance and variety of functionality; every carbon atom in these compounds is functionalized. In addition to their intriguing structural features, the broad-range antimicrobial and antileukemic activities displayed by the didemnenones make them ideal synthetic targets. (Figure 1.10)[38]





C

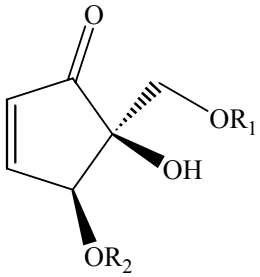
R_1 = H, R_2 = OH A

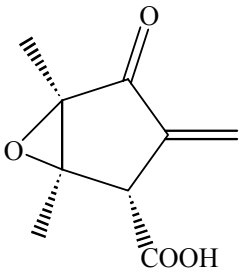
R_1 =OH,R_2 = H B

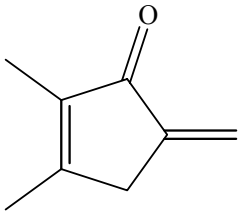
Didemnenones

Figure 1.10

Pentenomycin and methlenomycin A and B are the other group. They are antibiotics. The marine prostanoids, chlorovulone, clavulone, sarkomycin and punaglandin show antitumor activity. (Figure 1.11)







a). R_1 = R_2 = H
b). R_1=H, R_2 = Me
c). R_1 =Ac, R_2 = H

Pentenomycin Metylenomycin A B

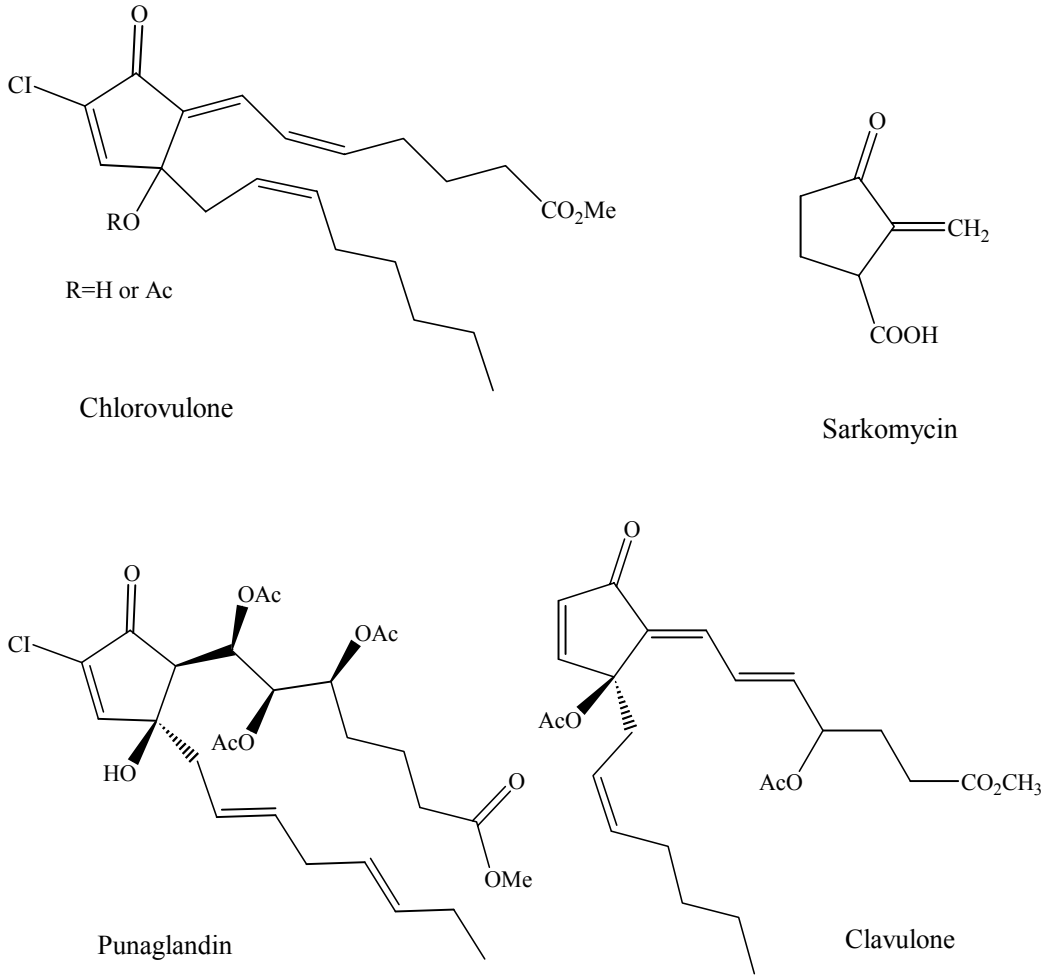


Figure 1.11

The other important type containing cyclopentenoid structure is prostaglandins and prostaglandin analogues. To date the most successful therapeutic applications of them has been in the field of obstetrics and gynaecology.[39] PGE_2, $PGF_{2\alpha}$ and synthetic analogues of them have been used successfully both for induction of labour and for the termination of unwanted pregnancy. The E-series prostaglandins have a number of actions which are potential therapeutic value. For example, their vasodilatory action could be used to treat hypertension, their bronchodilatory action to treat asthma and their gastric antisecretory action to treat ulcers. Prostacyclin, in addition to its potential value as an antithrombotic, shares the ability of E-series prostaglandins to cause vasodilatation and to inhibit gastric acid secretion. (Figure 1.12)

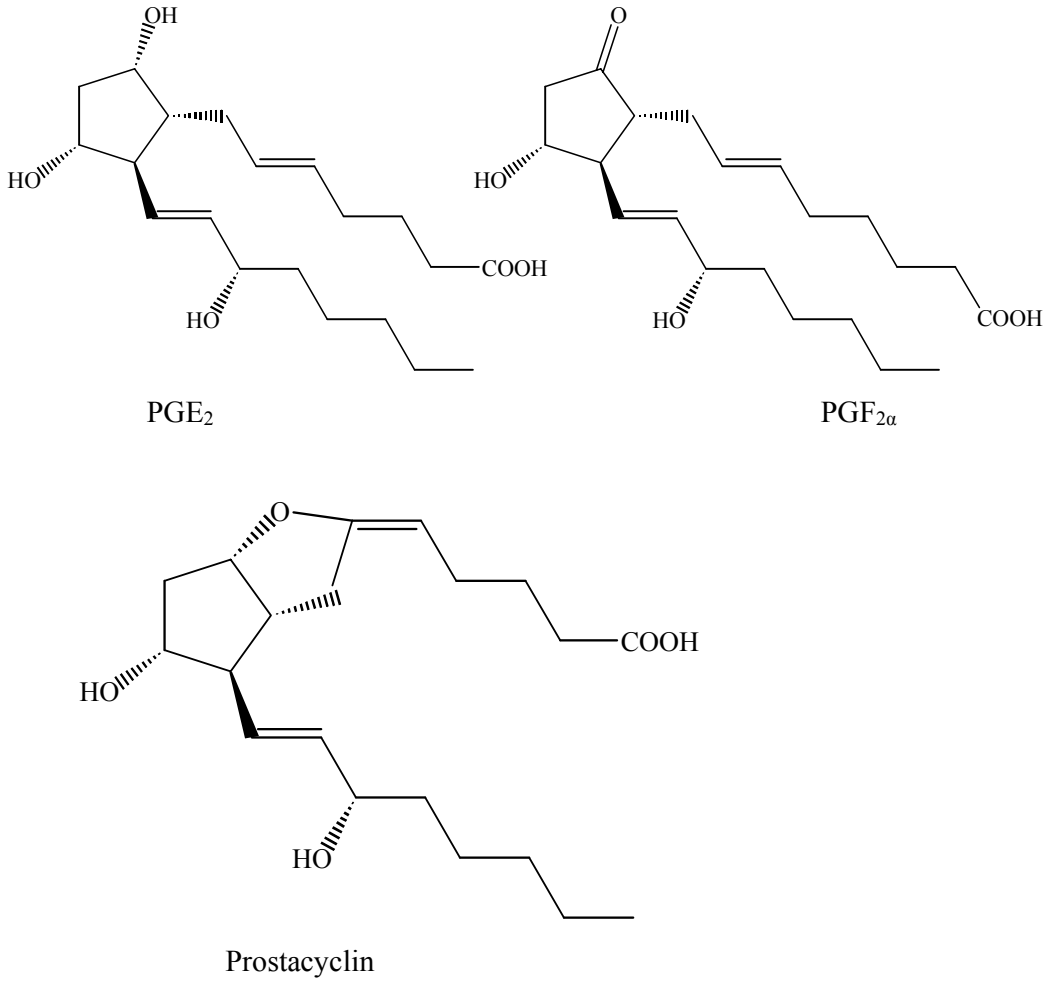
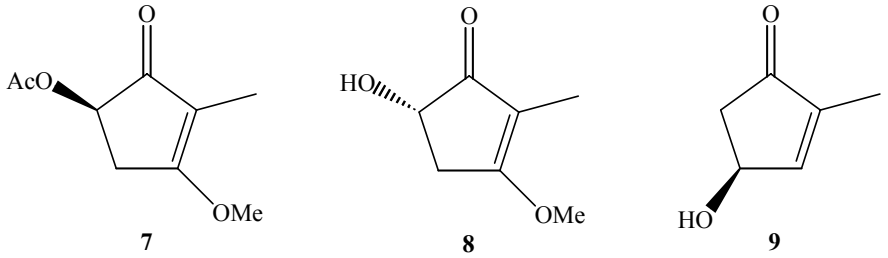


Figure 1.12

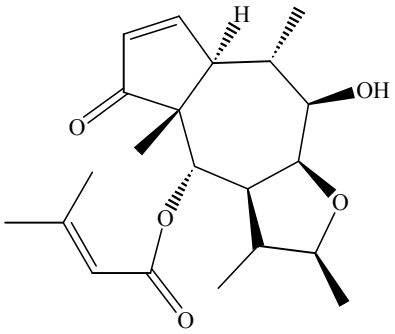
In this study, we have chosen the synthesis of 5-acetoxy and 5-hydroxy-2-methyl-2-cyclopentene-1-one **7, 8** and 2-methyl-4-hydroxy-2-cyclopentene-1-one **9** as an example study (Figure 1.13). There are several racemic preparation about the synthesis of 5-acetoxy-2-methyl-2-cyclopentene-1-one[32,34,40] **7** but few examples concerning chiral synthesis of **9**[41] have been studied.



polyoxo-ketones

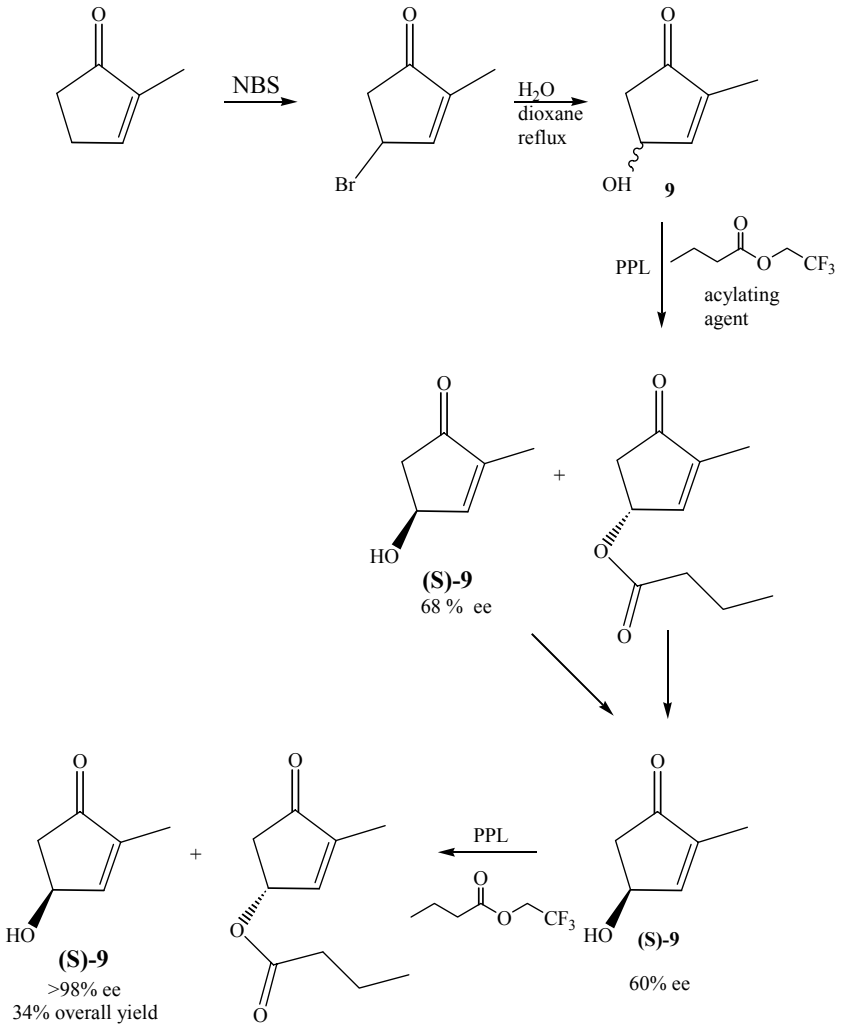
Figure 1.13

According to one report[42] outlined an 11-step ca. 6% yield synthesis of the target alcohol (S)-**9** from 2,4,6-trichlorophenol. In addition to this study, (S)-**9** is synthesized via enzymatic kinetic resolution of rac. **9** and converted to (-)-Fastigilin C (Figure 1.14), reported to exhibit cytotoxic and antineoplastic activity. A PPL mediated resolution of rac-**9** with β,β,β-trifluoroethylbutyrate in ether furnished (S)-alcohol (43%, 68% ee) together with (R)-butyrate. (R)-butyrate, isolated by column chromatography, was cleaved to the (R)-alcohol (78%, 46% ee) and the stereo center inverted via a Mitsunobu protocol, furnishing an additional quantity of the (S)-alcohol (53%, 46% ee). The combined (S)-**9** (60% ee) batches were exposed again β,β,β-trifluoroethylbutyrate and PPL in ether to provide (S)-**9** in a 52% isolated yield and 98% ee.[43] (Scheme 9)



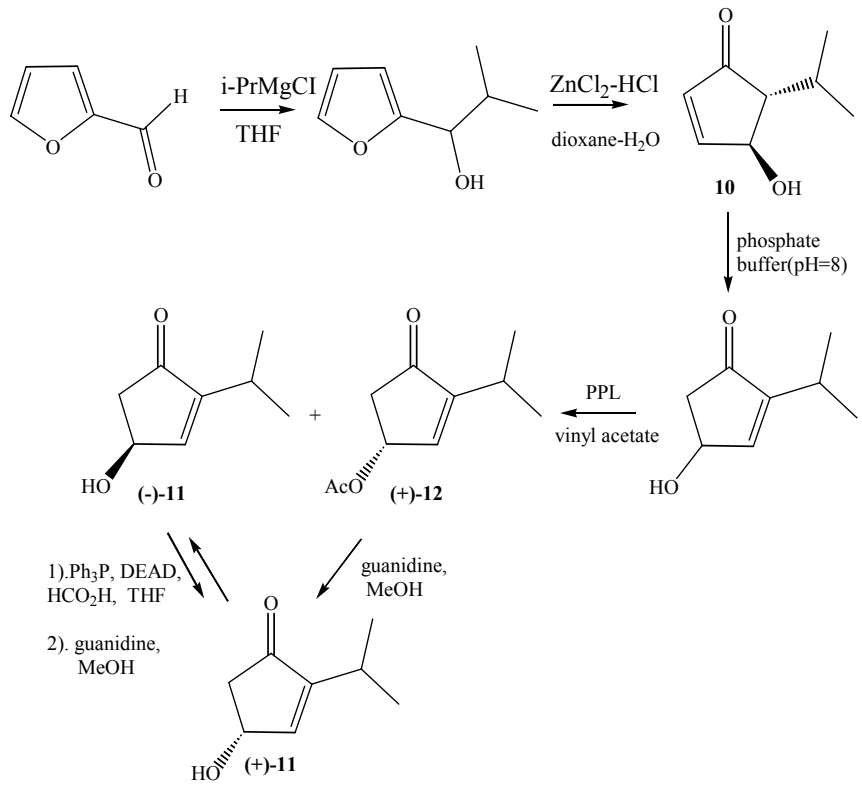
Helananolide structure

Figure 1.14 (-)-Fastigilin-C_2



(9)

In a recent study[44], chiral 4-hydroxy-2-isopropylcyclopent-2-enone has been synthesized starting from furfural by 4-steps to achieve the synthesis of prelactone B and its C-4 epimer. Kinetic resolution of racemic 4-hydroxy-2-isopropylcyclopent-2-enone rac.-**10** with PPL, using vinyl acetate as solvent and reagent, afforded (-)-(S)-**11** (45% yield, 98% ee) and acetate (+)-(R)-**12** (55% yield, 95%ee). (Scheme 10)



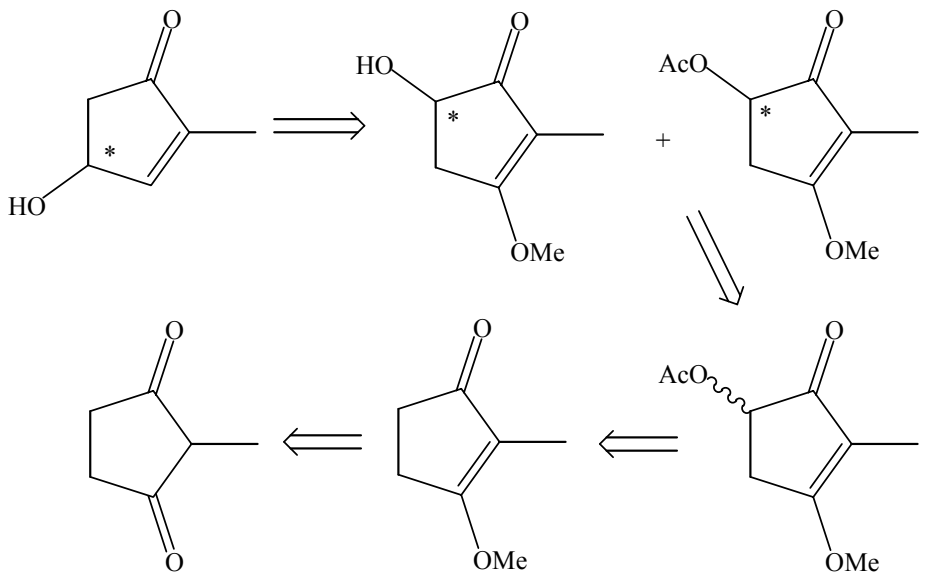
(10)

1.6. Aim of the work

The major aim of the study is to develop a simple and selective method for the synthesis of chiral 2-substituted cyclic polyoxo-ketones. For this purpose, the synthesis of 5-acetoxy and 5-hydroxy-2-methyl-2-cyclopentene-1-ones **7, 8** and 2-methyl-4-hydroxy-2-cyclopentene-1-one **9** was chosen as a model study.

As mentioned before, several racemic preparations of **7** and **9** are published, but few examples have presented the chiral synthesis of **9.** Their optical yield and chemical yield is very low. In this work, it is aimed to develop general applicable chemoenzymatic method for the enantioselective synthesis of 2-substituted cyclopentenones.

The aim of this work is shown retrosynthetically in scheme 11.



(11)

Our first approach to optically active **7, 8** and **9** is to synthesize the 2-methyl-3-methoxy-2-cyclopentene-1-one starting from 2-methyl-1,3-cyclopentenedion, which will be followed by α-oxidation of enone by using manganese(III) acetate. Then, enzymatic bioconversion of racemic 5-acetoxy-2-methyl-3-methoxy-2-cyclopentene-1-one **7** can be done by lipases and esterases. Finally, chiral hydroxy ketone can be reduced using $LiAlH_4$ to afford 4-hydroxy-enone **9**.

CHAPTER 2

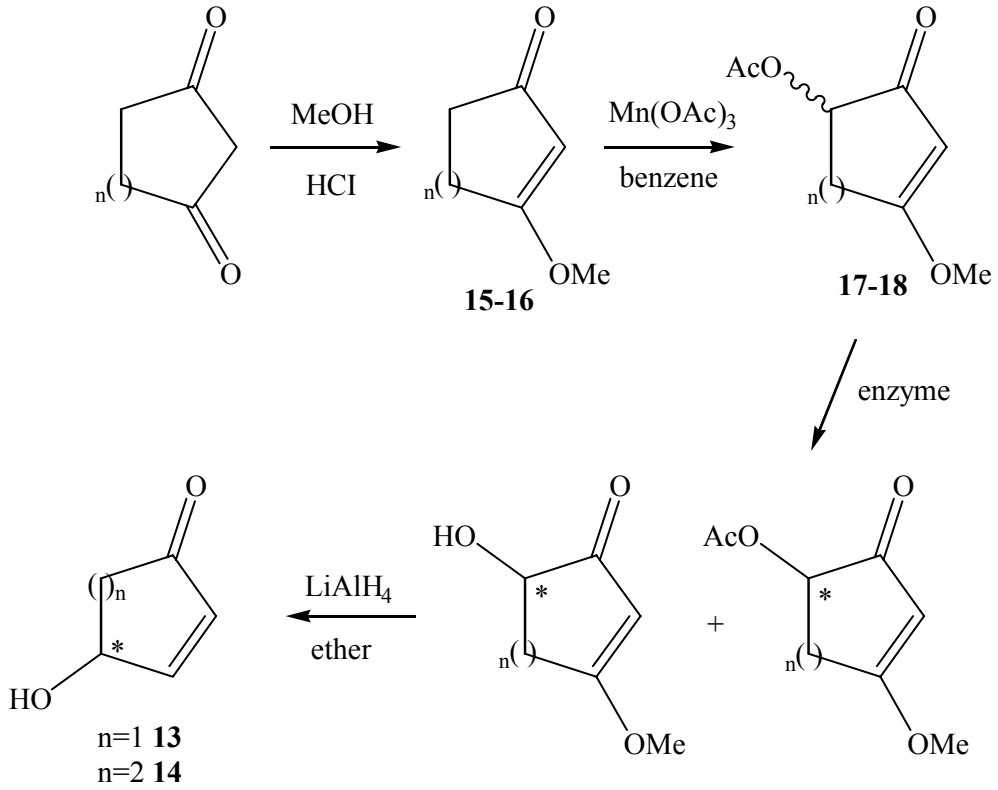
RESULTS AND DISCUSSION

2.1 Perspective of the Work

γ-hydroxy ketones **9** and polyoxo-ketones **7, 8** are versatile chiral synthones for the construction of chiral compounds due to reactive functional groups which can be transformed to other functional groups.

As mentioned before, many biologically active compounds contain these structures, such as; prostaglandins, didemnenones, sarkomycin, punaglandin, clavulone, etc.

Demir and Sesenoglu studied on the synthesis of 4-hydroxy-2-cyclopenten-1-one **13** and 4-hydroxy-2-cyclohexene-1-one **14**.[29,30] During this study, firstly 1,3-diketones were converted to the 3-methoxy-2-cyclopenten-1-one **15** and 3-methoxy-2-cyclohexen-1-one **16** and then oxidation of enones was performed with $Mn(OAc)_3$ to obtain the desired 5- and 6-acetoxy enones **17-18**. It was followed by enzymatic kinetic resolution and finally reduction. (Scheme 12)



(12)

Based on preliminary information available to us from our previous work on the biocatalyst-mediated reactions, we were able to develop a chemoenzymatic method for the synthesis of **7, 8** and **9** by using lipase and esterase type enzymes. This study is important because it provides a method with general applicability for the asymmetric synthesis of **7, 8** and **9.**

Lipase type enzymes are used extensively for the synthesis of enantiomerically pure compounds via the resolution of racemic mixture. The high stereoselectivity in organic media and their low cost make them very useful catalysts for enantioselective resolution.

To find a suitable experimental setup for the lipase and esterase catalyzed resolutions of **7, 8** and **9**, we have tried to find the reaction conditions which give the best results in the enzyme catalyzed hydrolysis. Furthermore, it was essential to find an easy and accurate way to determine both conversion of the reaction and enantiomeric excess values of the acetate (remaining substrate) and the alcohol

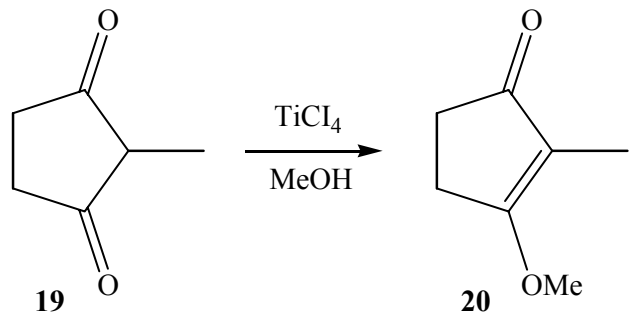
(product). For the determination of the ee of the acetate and the alcohol in the kinetic resolutions several chiral HPLC columns were used. Determination of the ee by this technique was always first performed on the racemate to optimize base-line separation. For the determination of conversion, we have decided to control the reaction with TLC. The stereoselectivity is expressed in the enantiomeric ratio or E-value, which is the ratio of reaction rates of both enantiomers of the starting material. When the difference in reaction rate between the enantiomers is large, E is high, which indicates a better enantioselectivity.

Since only very small amount of samples are needed for HPLC analysis, it was possible to perform the hydrolysis on an analytical scale. In this way the screening of a large number of enzymes becomes fast and easy.

2.2 Synthesis of Acetoxy Ketones

2.2.1 Protection of Cyclic 1,3-diketones

There are some procedures, three of which were tried, reported in the literature for the protection of 1,3-dicarbonyl compounds in enol ether form. According to the first one, 2-methyl-cyclopentane-1,3-dione **19** was dissolved in methanol and HCl gas was passed through the solution for the formation of the product **20**.[45] However, the yield of obtained product was very low (10% yield) so we tried another method. In the second one, the starting material **19,** in methanol, reacted with etheral diazomethane. Nonetheless, this method was not used owing to low yield (10%) and the bad effects on the body. As a final method, we performed the protection reaction with $TiCI_4$ according to procedure in the literature.[46] In this procedure, 1,3-diketone **19** was dissolved in methanol and then $TiCI_4$ was added under argon, which gave the highest chemical yield (90%). The reaction is monitored by TLC (Silica gel, EtOAc/Hex 3:1). After work-up procedure, the product was purified by flash column chromatography (EtOAc/Hex 3:1). The desired product, 2-methyl-3-metoxy-2-cyclopenten-1-one **20**, was obtained as colorless crystals in 90% yield. (Scheme 13)

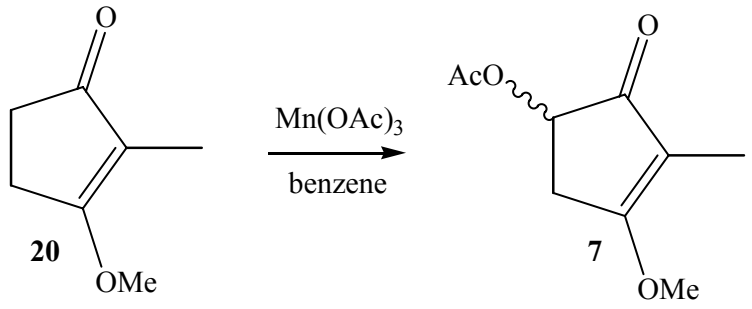


(13)

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum we observed a singlet at 3.89 ppm for -OCH_3 protons. From ^{13}C-NMR spectrum we observed a singlet at 56.5 ppm for –OCH_3 carbon.

2.2.2 $Mn(OAc)_3$ Mediated Acetoxylation of β-Alkoxy Enones

For the acetoxylation reaction, enone was allowed to react with 4 equivalent of $Mn(OAc)_3$ in benzene and refluxed under a Dean-Stark trap to give the desired acetoxy derivative **7** in racemic form. (Scheme 14)



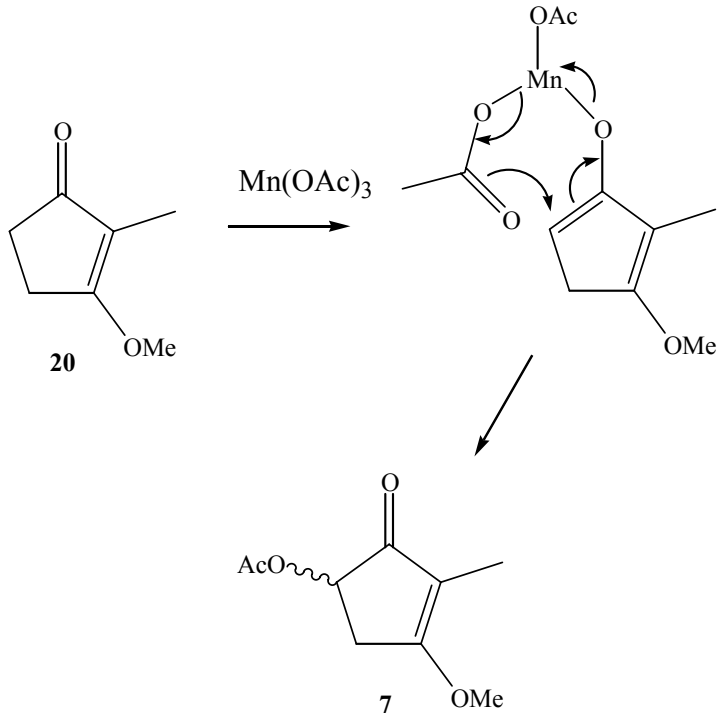
(14)

Instead of benzene, use of cyclohexane and acetonitrile as a solvent also furnished the acetoxy enone **7**, however, with undefined side products. Also, the yield of the product **7** in cyclohexane (40% yield) and acetonitrile (20% yield) was less than that in benzene (87%). Consequently, the reaction was performed in benzene.

The reaction was monitored by TLC (Silica gel, EtOAc/Hex 1:1). After the work-up and purification of the crude product by flash column chromatography (EtOAc/Hex 1:1), the desired product, 5-acetoxy-2-methyl-3-methoxy-2-cyclopenten-1-one, (±)-**7,** was obtained as yellow oil in 87% yield.

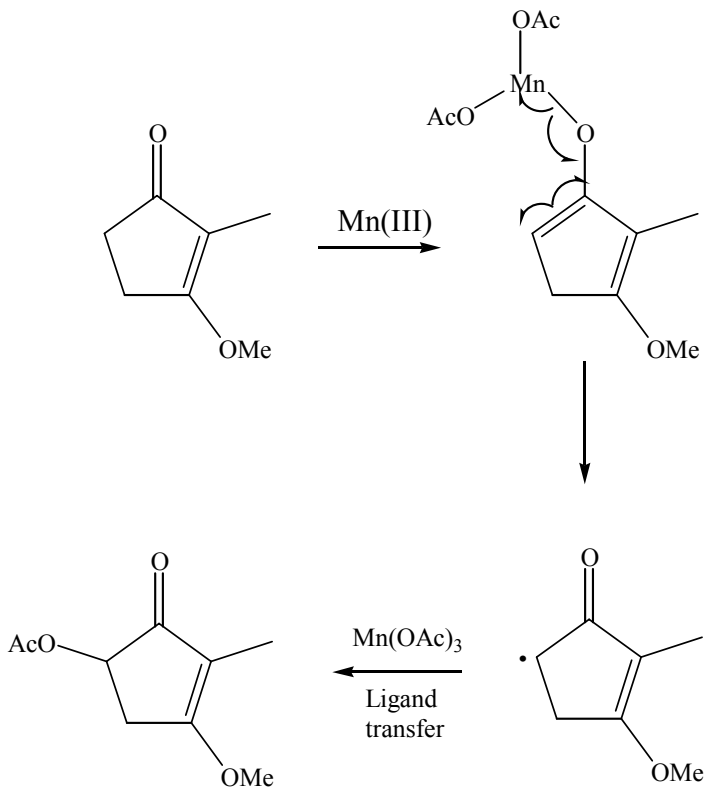
The product was identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum we observed a singlet at 2.05 ppm from $–CH_3$ group and dd at 5.03 ppm (*J* = 6.8 and 2.5 Hz) for the α-proton. From ^{13}C-NMR spectrum we observed a singlet at 19.6 ppm for the CH_3 carbon and a singlet at 169.2 ppm for the O<u>C</u>OCH_3 carbon.

As mentioned before, there are several mechanisms about α-acetoxylation with manganese (III) acetate but two of them have general acceptability. First one is based on the formation of a metal enolate followed by acetate transfer. Scheme 15 shows us the mechanism which is applied on 2-methyl-3-methoxy-2-cyclopentene-1-one **20** to obtain the desired product **7**.



(15)

According to the second one, it is possible that this reaction also proceeds via the formation of an α-oxo radical followed by ligand transfer to yield the product. (Scheme 16) Since the oxidation of carbonyl compounds with manganese (III) acetate has been reported to involve an α-oxo radical resulting from the oxidation of enol or enolate anion by Mn(III).[34]



(16)

2.3 Enzyme Mediated Hydrolysis of Acetoxy Ketones

Hydrolytic enzymes are the biocatalysts most commonly used in organic synthesis. Especially, hydrolyses of amines and esters by enzymatic methods are now routine biotransformations. A range of proteases, lipases and esterases that will accept a variety of substrates are commercially available in varying degrees of purity and homogeneity. Among them, lipases (triacylglycerolhydrolases, EC 3.1.1.3) are the most widely employed enzymes not only because they are cheap and readily available from many different sources but because they possess high enantioselectivity for a broad range of substrates and high stability in organic solvents. The enantioselectivity of lipase-catalyzed reactions in aqueous solutions, water-organic solvent mixtures, and in anhydrous organic solvents follows the classical homocompetitive equation.

At first, we did analytical screening to obtain optimum conditions for the enzymatic hydrolysis of acetoxy enone **7.** For this purpose, the reactions were performed in analytical scale. About 5 mg of acetoxy enone **7** was dissolved in minimum amount of DMSO and then 300 μL buffer was added. The reaction was controlled with TLC and when 50% conversion was observed, reaction was terminated by adding 500 μL chloroform. After the separation of organic phase from water, the ee values were determined by HPLC. (Table 1)

Table 1. Enzymatic hydrolysis of (±)-**7**-5-Acetoxy-3-methoxy-2-methyl-2-cyclopentene-1-one

			acetoxy		*hydroxy*	
enzyme	reaction time	Conversion %	ee %	yield %	ee %	Yield %
PLE	16 h	51	98	45	95	47
Amano PS	22 h	51	97	41	93	45
CCL	28 d	44	68	51	87	43
PPL	*23 d*	*53*	*28*	*54*	*32*	*37*

First, the racemic forms of acetoxy and hydroxy enones were analyzed for the determination of R_f values of enantiomers. (Figure 15) When HPLC chromatograms of racemic forms were compared with that of hydrolyzed forms, it was observed that PLE and Amano PS exhibit the high enantioselectivity for acetoxy enone, as well as hydroxy enone.

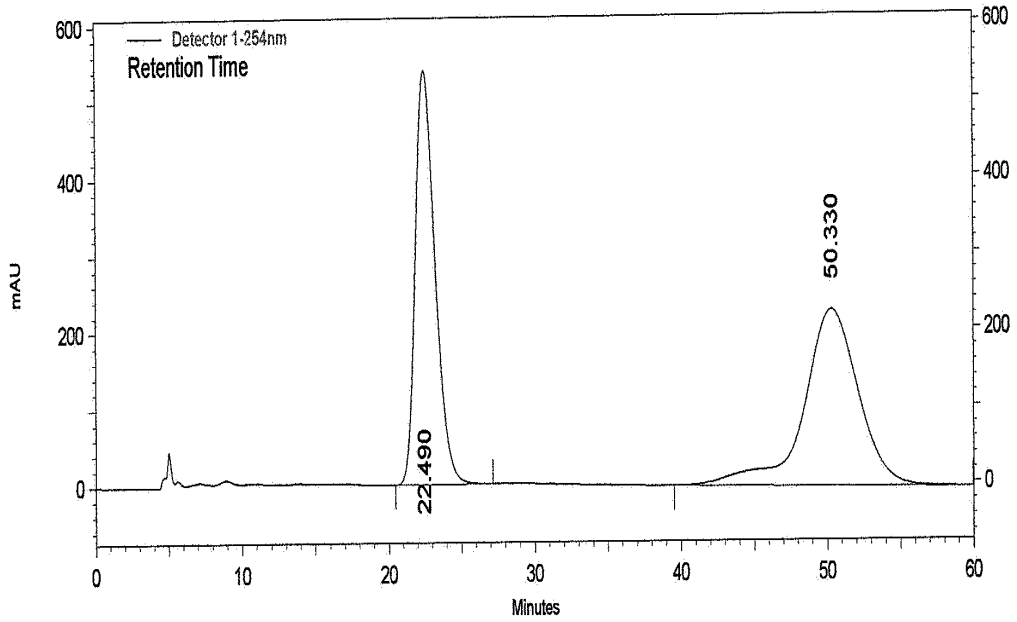


Figure 15. HPLC analysis results of rac-5-acetoxy-2-methyl-3-methoxy-2-cyclopentene-1-one

Figure 16 is HPLC spectrum of PLE catalyzed reaction in analytical scale. Amano PS and CCL also gave the same spectrums but PPL gave different spectrum. (Figure 17) It can be concluded that PLE (*Pig Liver Esterease*), Amano PS, CCL (*Candida Cylindracea Lipase*) hydrolyzed preferentially the (+)-(S)-enantiomer of racemic acetoxy enone **7** while PPL (*Porcine Pancreatic Lipase*) preferentially recognized the (-)-(R)-enantiomer of rac-**7**. After this result was recognized, for PPL different solvents, such as; toluene, xylene, THF, acetonitrile, benzene and dioxane, to increase the ee values of PPL hydrolyzed product but enzyme did not work in these solvents.

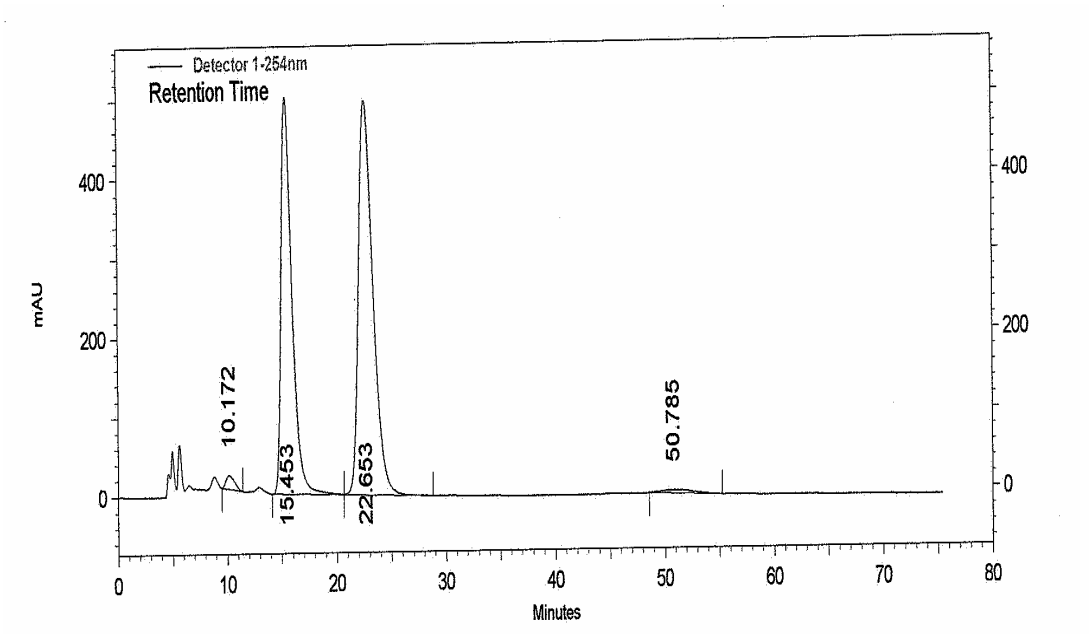


Figure 16. HPLC analysis results of PLE catalyzed hydrolysis

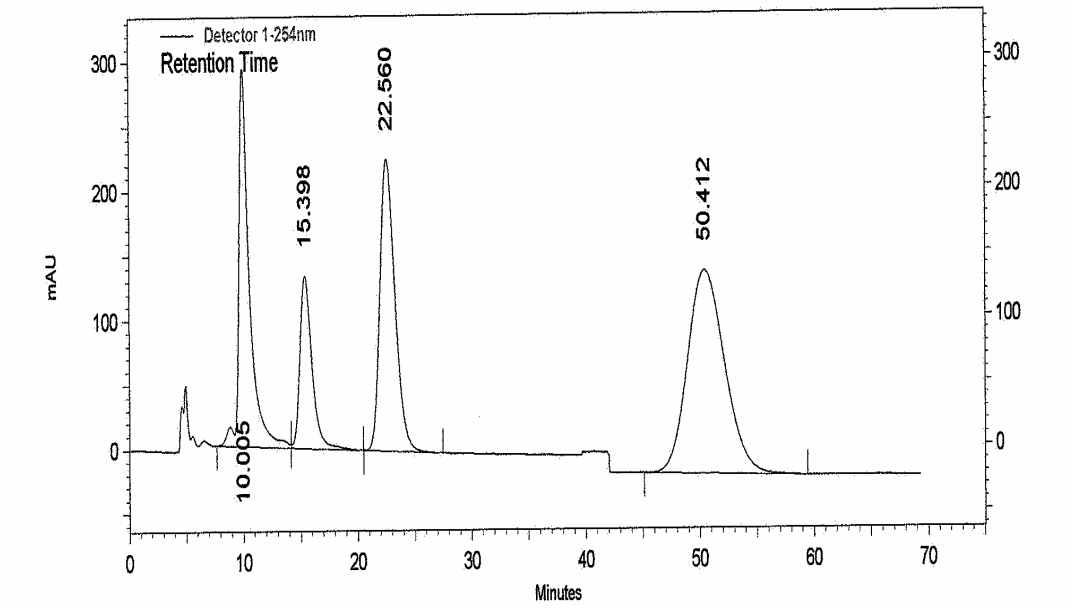
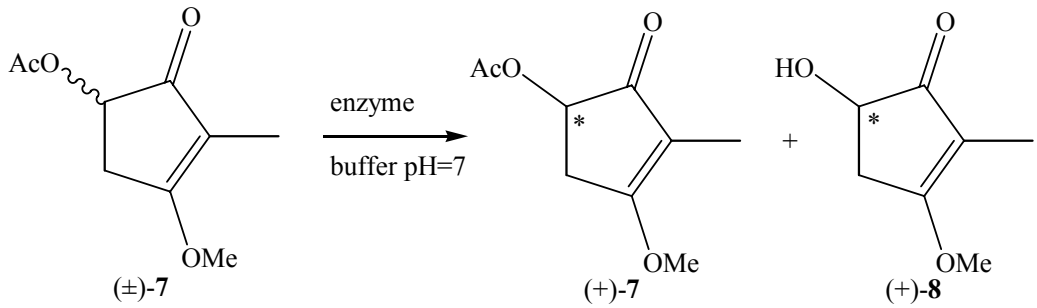


Figure 17. HPLC analysis results of PPL catalyzed hydrolysis

According to HPLC data, the enzyme which gave the highest ee value was chosen for the preparative synthesis. In a typical experiment, for enzymatic hydrolysis, the racemic acetoxy ketone, (±)**-7,** was dissolved in DMSO, then phosphate buffer (pH=7) was added and the mixture stirred at room temperature in the presence of the enzyme, PLE. The reaction was monitored by TLC and when approximately 50% conversion was attained, the crude product was separated by flash column chromatography to afford acetoxy enone **(**+)-**7** and hydroxy enone (+)-**8.** (Scheme 17)



(17)

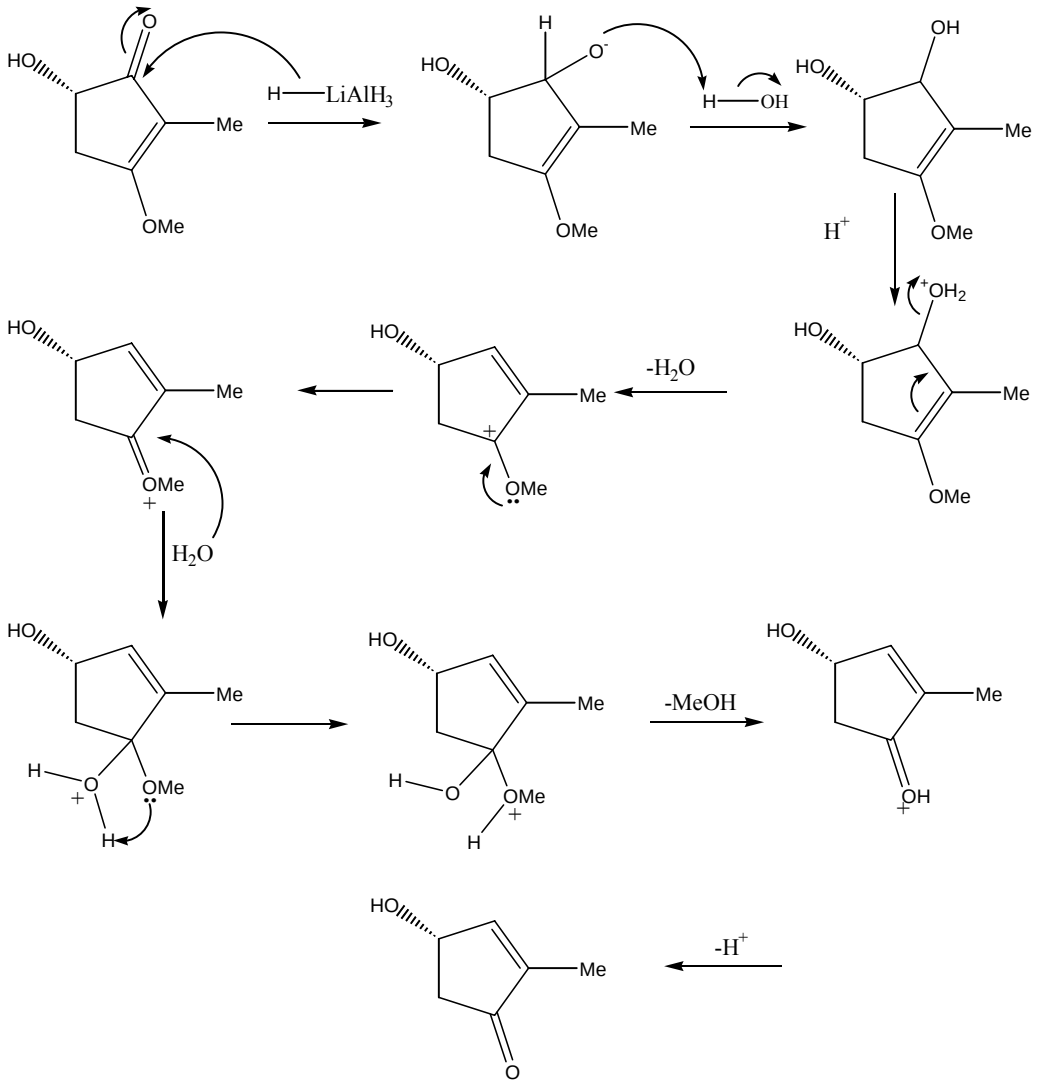
The products were identified by using NMR spectroscopy. From the ^{1}H-NMR spectrum of **7**, we observed a singlet at 2.05 ppm from $–CH_3$ group and dd at 5.03 ppm (J = 6.8 and 2.5 Hz) for the α-proton. From ^{13}C-NMR spectrum we observed a singlet at 19.6 ppm for the CH_3 carbon and a singlet at 169.2 ppm for the O<u>C</u>OCH_3 carbon. From the ^{1}H-NMR spectrum of **8,** we observed a broad singlet at 1.47 ppm for the –OH proton, and multiplet around 4.17 ppm for the α-proton. From ^{13}C-NMR spectrum we observed a singlet at 70.9 ppm for -CH-OH carbon.

Enantiomeric excess values were determined with HPLC (Chiralpak OB column, eluent: hexane/2-propanol= 75:25, flow 0.80 mL min-1 20 ° C for both **7** and **8**, using racemic compounds as references. The α values are $[\alpha]_D^{20}$ = + 32.1 (c 0.01, $CHCI_3$) for **7** and $[\alpha]_D^{20}$ = + 78.8 (c 0.1, $CHCI_3$) for **8** . Also, after reduction of acetoxy enone the other enantiomer of hydroxy enone **8** was obtained with $[\alpha]_D^{20}$ = - 64.364 (c 0.37, $CHCI_3$).

According to the published procedures racemization free conversion of acetoxy to hydroxy enone vice versa gives possibility to obtain all of the enantiomers of acetoxy and hydroxy enones.[29,30]

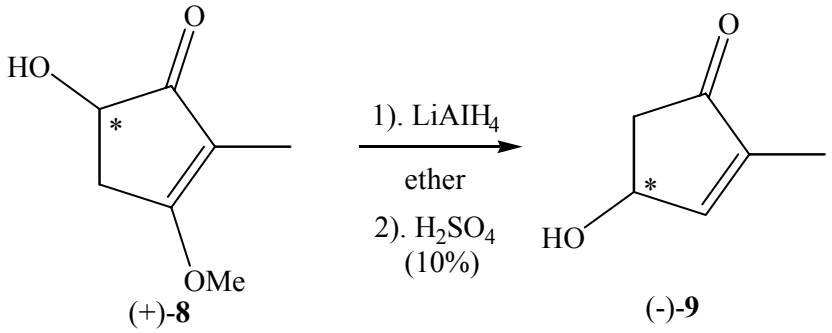
2.4 Conversion of α-Acetoxy Enones to γ-Hydroxy Enones

α'-Acetoxy enones are potential precursors for poly-hydroxy cyclopentenones. These enones are also valuable starting material for the synthesis of 4-hydroxy cyclopentenones. In general, reduction of carbonyl group into alcohol then hydrolysis of formed enol ether followed by the elimination of water should furnish 4-hydroxy enone **9**. (Scheme 18)



(18)

During this process, it is important to conserve the chirality at α' position. As shown in Scheme 19, the starting material was dissolved in ether and reacted with $LiAlH_4$. After reduction of the starting material (+)-**8**, which was checked by TLC, the mixture was hydrolyzed with 10% H_2SO_4. After work up and purification by column chromatography (EtOAc/Hex 3:1) the desired product (-)-**9** was isolated in 78% yield.



(19)

The product was identified by using NMR spectroscopy. From the ^{1}H-NMR we observed a broad singlet at 2.07 ppm for –OH proton, a multiplet at 4 .85 ppm for the –CH-OH proton and a multiplet at 7.09 ppm for the =CH proton. From ^{13}C-NMR spectrum we observed a singlet at 156.7 ppm for =CH carbon and at 68.8 ppm for –CH-OH carbon.

The same reduction reaction was carried out with $NaBH_4$ and $LiBH_4$ but the yield of product was lower than by $LiAlH_4$ reduction and also gave several side products.

The racemic 4-hydroxy enone is also synthesized starting from racemic α'-hydroxy enone **8.** When comparing of reduction of α'-hydroxy enone with reduction of α'-acetoxy enone, α'-hydroxy enone gave higher yield than acetoxy enone. The chiroptical comparison and HPLC analysis of product **9** with racemic reference compound using a chiral column showed that no racemization occurred during the reaction. For the high yield formation of **9** we suggest that either the reduction works with high selectivity or acid or base catalyzed isomerization at the α-position occurs during the elimination. Until now it has not been possible to isolate the reduction products before elimination; therefore we cannot give an exact mechanism for this step.

The absolute configuration of product (-)-**9** was assessed by the comparison of its specific rotation with data from the literature.[43] According to the measured α value which is $[\alpha]_D^{20}$ = - 33.5 (c 1.1, $CHCl_3$), the absolute configuration is determined as (S) for γ-hydroxy enone, synthesized starting from hydroxy enone (+)-**8**.

2.5 Summary of Chemoenzymatic Synthesis of α-Acetoxy and γ-Hydroxy Enones

In summary, this work describes here as the model study for the synthesis of chiral poly-oxo ketones **7, 8** and **9**. Moreover, it provides a new entry to the synthesis of 2-methyl-γ-hydroxy enone **9.**

According to the method, first of all, commercially available 2-methyl-1,3-cyclopentanedione was converted to 2-methyl-3-methoxy-2-cyclopenten-1-one. Then oxidation of the enone was done with four equivalents of manganese (III) acetate in benzene to obtain desired α'-acetoxy enone **8.** Afterwards, the enzymatic hydrolysis of acetate rac-**7** in aqueous-organic medium furnished (+)-**7** and (+)-**8** in high ee. The best results in the enzymatic hydrolysis of the rac-**7** were obtained with PLE (E: 180, >98% ee for (+)-**7** and 95% ee for (+)-**8** and with Amano PS (E: 115, 97% ee for (+)-**7** and 93% for (+)-**8** in DMSO. Finally obtained (S)-**8** alcohol was reduced to 2-methyl-γ-hydroxy enone (-)-**9** with $LiAlH_4$. The α values of (+)-**7,** (+)-**8** and (-)-**9,** which were obtained PLE catalyzed hydrolysis, were measured and then the α value of (-)-**9** was compared with literature. The absolute configuration of (-)-**9** was determined as (S). It was concluded from these results that while alcohol (+)-**8** had (S) configuration, acetate (+)-**7** had (R) configuration. Thanks to the HPLC data, it was seen that PLE, CCL and Amano PS hydrolyzed (S)-enantiomer of rac-**7,** PPL hydrolyzed preferentially (R)-enantiomer.

These results show us, same experiments can be applied to the synthesis of any other 2-substituted-polyoxo-ketones.

CHAPTER 3

EXPERIMENTAL

3.1 Materials and Methods

In this study all compounds were identified by Nuclear Magnetic Resonans Spectra (NMR) (Bruker DPX 400 MHz) by using tetramethylsilane (TMS) as an internal standard and deutereo chloroform as solvent.

Flash coloumn chromotography was done for purifying the products by using silica gel 60 (mesh size 40-63 µm)

Optical rotations were measured with an Autopol IV automatic polarimeter. Enantiomeric excesses were determined by HPLC analysis using a Thermo Quest (TSP) GC-LC-MS equipped with an appropriate column packed with an optically active material.

3.2 General Procedures

3.2.1 Synthesis of 2-methyl-3-methoxy-2-cyclopentenone (20)

Commercially available 2-0.15 mL (0.15 mmol) of a 1.0 M $TiCI_4$ solution in CH_2CI_2 was added in one portion with a syringe, at room temperature to a well stirred solution of the cyclic 2-methyl-1,3-diketone (5 mmol) in MeOH (10 mL) in the presence of molecular sieve. The reaction mixture was then stirred during a day. The reaction was monitored by TLC. After stopped, work-up was done and purified by flash column chromatography.

The product was isolated as a yellow solid after flash chromatography (3:1 EtOAc:Hexane) with 90% yield.

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4):

δ (ppm): 1.56 (s,3H)
2.36 (m, 2H)
2.56 (m, 2H)
3.89 (s, 3H)

^{13}C-NMR (100MHz, $CDCl_3$+CCl_4)

δ (ppm): 204.7, 183.8, 116.6, 56.5, 33.6, 25.0, 6.5.

3.2.2 Synthesis of 5-Acetoxy-3-methoxy-2-methyl-2-cyclopentene-1-one (7)

2.5 g, 22.3 mmol enone, $Mn(OAc)_3$ (17.2 g, 66.9 mmol) and benzene (200ml) were heated under reflux for 4 days**.** The reaction was monitored by TLC. After cooling, the reaction mixture was first filtered then washed with saturated $NaHCO_3$ solution. The solution was then dried over $MgSO_4$, concentrated and purified by flash column chromatography to yield %87 desired racemic acetoxy enone.

According to the general procedure, the product was isolated as a yellow oil after flash column chromatography (1:1 EtOAc:Hex) with 87% yield.

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 1,58 (s, 3H, CH_3)
2.05 (s, 3H, $COCH_3$)
2.43 (dd, *J* = 17.4, 1.5 Hz, 1H, CH_2)
3.14 (ddd, *J* = 17.4, 6.8, 1.5 Hz, 1H, CH_2)
3.9 (s, 3H, OCH_3)
5.03 (dd, *J* = 6.8, 2.5 Hz, 1H, CH)

^{13}C-NMR (100MHz, $CDCl_3$)

δ (ppm): 197.5, 179.9, 169.2, 114.4, 69.9, 55.7, 31.9, 19.6, 4.9.

IR ($CHCl_3$): ν = 1750, 1710, 1630 cm^{-1}.

3.2.3 Enzyme-Catalyzed Kinetic Resolution

To a stirred solution of (±)-5-acetoxy-2-methyl-3-methoxycyclo-2-pentene-1-one (165 mg, 0.9 mmol) in DMSO (2 mL) and phosphate buffer (pH 7.0, 60 mL) enzyme (PLE 200 μL) was added in one portion and the reaction mixture was stirred at rt. Conversion was monitored by TLC and HPLC up to 50%. After filtration, the filtrate was extracted with dichloramethane, dried over $MgSO_4$, concentrated and purified by flash column chromatography (1:1 EtOAc:Hex) to obtain (*R*)-5-acetoxy-3-methoxy-2-methyl-2-cyclopentene-1-one and 44-53% and (*S*)-5-hydroxy-3-methoxy-2-methyl-2-cyclopentene-1-one 37-47% yields, respectively.

3.2.3.1 Synthesis of (R)-5-Acetoxy-3-methoxy-2-methyl-2-cyclopentene-1-one (7)

The unreacted acetate (+)-**7** and product (+)-**8** were separated by flash column chromatography (3:1 EtOAc:Hex). The ee's of the acetate was determined by chiral HPLC.

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 1,58 (s, 3H, CH_3)
2.05 (s, 3H, $COCH_3$)
2.43 (dd, *J* = 17.4, 1.5 Hz, 1H, CH_2)
3.14 (ddd, *J* = 17.4, 6.8, 1.5 Hz, 1H, CH_2)
3.9 (s, 3H, OCH_3)
5.03 (dd, *J* = 6.8, 2.5 Hz, 1H, CH)

^{13}C-NMR (100MHz, $CDCl_3$)

δ (ppm): 197.5, 179.9, 169.2, 114.4, 69.9, 55.7, 31.9, 19.6, 4.9.

IR ($CHCl_3$): ν = 1750, 1710, 1630 cm^{-1}.

HPLC: Chiralpak OB column, UV detection at 254 nm, eluent: hexane/2-propanol= 75:25, flow 0.80 mL min-1 20 ° C, using racemic compounds as references; R_f: for (R)-**7**: 22 min; (S)-**7**: 50 min $[\alpha]_D^{20} = +32.1$ (c 0.01, $CHCl_3$).

3.2.3.2 Synthesis of (S)-5-Hydroxy-3-methoxy-2-methyl-2-cyclopentene-1-one (8)

The unreacted acetate (R)-**7** and product (S)-**8** were separated by flash column chromotography (3:1 EtOAc:Hex). The ee's of the hydroxy was determined by chiral HPLC.

^{1}H-NMR (400 MHz, $CDCl_3$+CCI_4)

δ (ppm): 1.47 (bs, OH)
1.58 (t, 3H, *J* = 1.9, CH_3)
2.48 (dt, *J* = 17.0, 1.9 Hz, 1H, CH_2)
2.96 (ddd, *J*=17.0, 6.5, 1.9 Hz, 1H, CH_2)
3.92 (s, 3H, OCH_3)
4.17 (d, *J* = 6.5 Hz, 1H, CH)

^{13}C-NMR (100 MHz, $CDCI_3$+CCI_4)

δ (ppm): 205.0, 182.2, 115.2, 70.9, 57.1, 34.3, 6.2

IR ($CHCI_3$): ν = 3450, 1710, 1640 cm^{-1}.

HPLC: Chiralpak OB column, UV detection at 254 nm, eluent: hexane/2-propanol= 75:25, flow 0.80 mL min-1 20 ° C, using racemic compounds as references.
R_f: for (S)-**8**: 15 min; (R)-**8**: 10 min. $[\alpha]_D^{20}$ = + 78.8 (c 0.1, $CHCI_3$) and $[\alpha]_D^{20}$ = -64.364 (c 0.0037, $CHCI_3$) after reduction of acetoxy. NMR data for **8 ;**

3.2.4 Synthesis of (*S*)-4-Hydroxy-2-methyl-2-cyclopentene-1-one (9)

To a suspension of $LiAlH_4$ (33.4 mg, 0.9 mmol) in anhydrous Et_2O (30 mL) was added acetoxy enone (S)-**8** (50 mg, 1.41 mmol) at rt over 15 min. The mixture was refluxed for 30 min., cooled to rt and quenched with water and 10% H_2SO_4. Organic phase was washed with saturated $NaHCO_3$ solution, brine and dried over $MgSO_4$. After evaporation of the solvent, flash column chromatography (3:1 EtOAc:Hex) was performed to obtain 4-hydroxy-2-methyl-2-cyclopentene-1-one.

The crude was separated by flash column chromatography (3:1EtOAc:Hex) in 78% yield. Absolute configuration of the product was determined by comparison of the [α] value with literature.[43]

$[\alpha]_D^{20}$ = - 33.5 (c 1.1, $CHCl_3$)

^{1}H-NMR (400 MHz, $CDCl_3$+CCl_4)

δ (ppm): 1.74 (s, 3H, CH_3),
2.07 (bs, 1H, OH),
2.20 (dd, *J* = 18.5, 1.8 Hz, 1H, CH_2)
2.71 (dd, *J* = 18.5, 6.07 Hz, 1H, CH_2)
4.85 (m, 1H, CHOH)
7.09 (m, 1H, =CH)

^{13}C-NMR (100 MHz, $CDCI_3$+CCI_4)

δ (ppm): 206.2, 156.7, 144.0, 68.8, 44.8, 10.3.

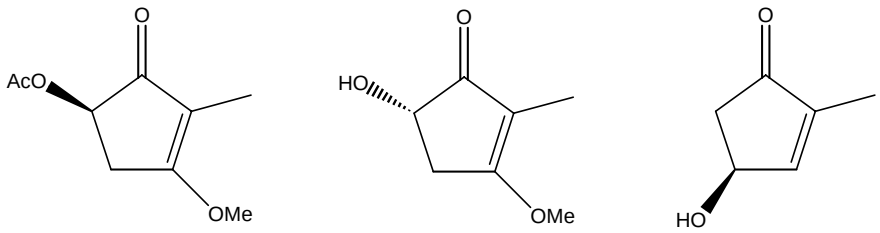
IR ($CHCI_3$): ν = 3400, 1710, 1640 cm^{-1}.

CHAPTER 4

CONCLUSION

Chiral cyclic polyoxo-ketones are important structures because they are found in the structure of many biologically active compounds such as prostaglandins, didemnenones, sarkomycin, chlorovulone etc.

The results show that manganese (III) acetate-mediated acetoxylation of enone followed by enzyme-mediated hydrolysis of the acetoxy group provides acetoxy enone **7** and hydroxy enone **8** with high enantiomeric excesses (95-98%) and in good yields. In these conversions PLE, Amano PS and CCL favor the (S)-enantiomer. On the other hand, PPL hydrolyzed (R)-enantiomer. The reduction of hydroxy enone followed by acid hydrolysis provided (S)-enantiomer of 4-hydroxy-2-methyl cyclopent-2-en-1-one **9**.



7 **8** **9**

polyoxo-ketones

This study is a model study for the synthesis of chiral hydroxy and acetoxy enonones **7** and **8**. In addition, it provides a simple new method for the synthesis of chiral cyclic 4- hydroxy enones.

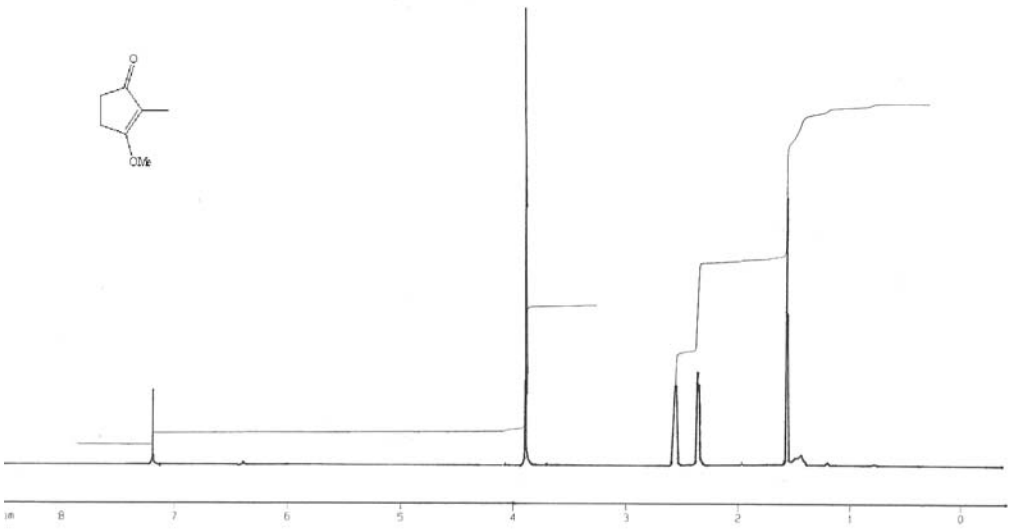


Figure 18: ^{1}H-NMR spectrum of 2-methyl-3-methoxy-2-cyclopentenone

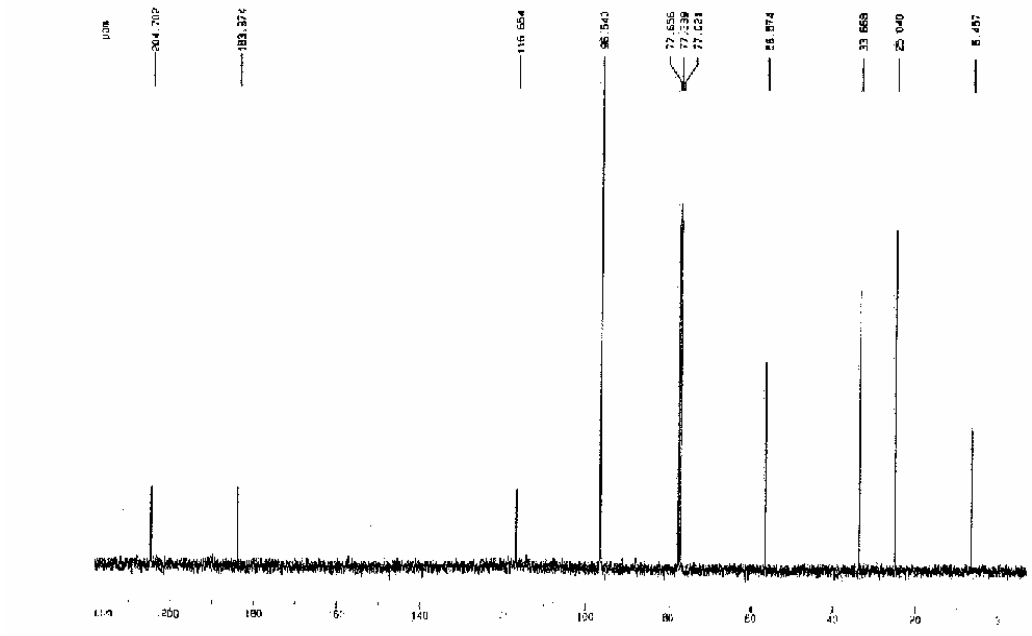


Figure 19: ^{13}C-NMR spectrum of 2-methyl-3-methoxy-2-cyclopentenone

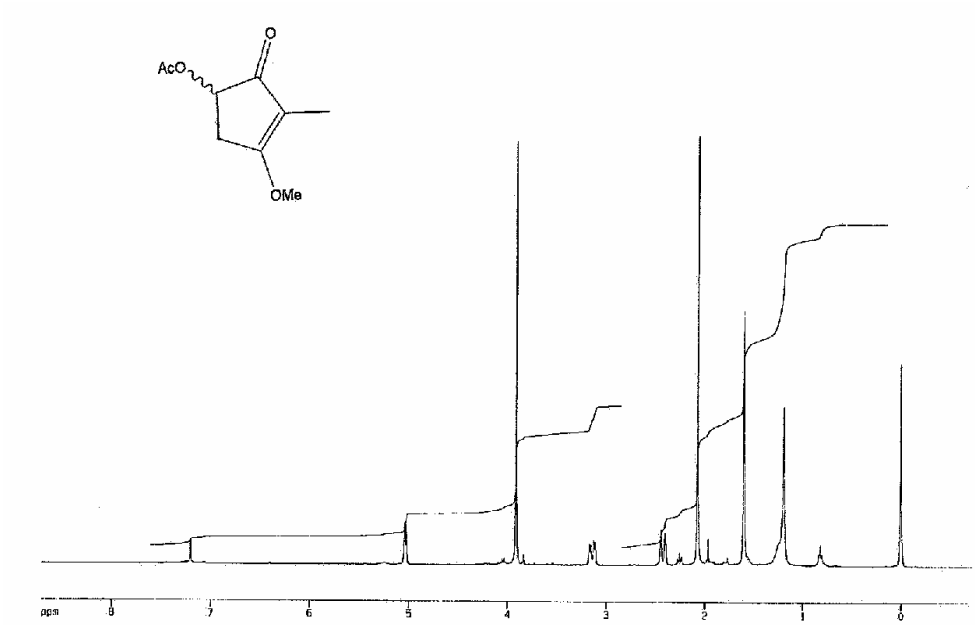


Figure 20: ^{1}H-NMR spectrum of 5-Acetoxy-3-methoxy-2-methyl-2-cyclopentene-1-one

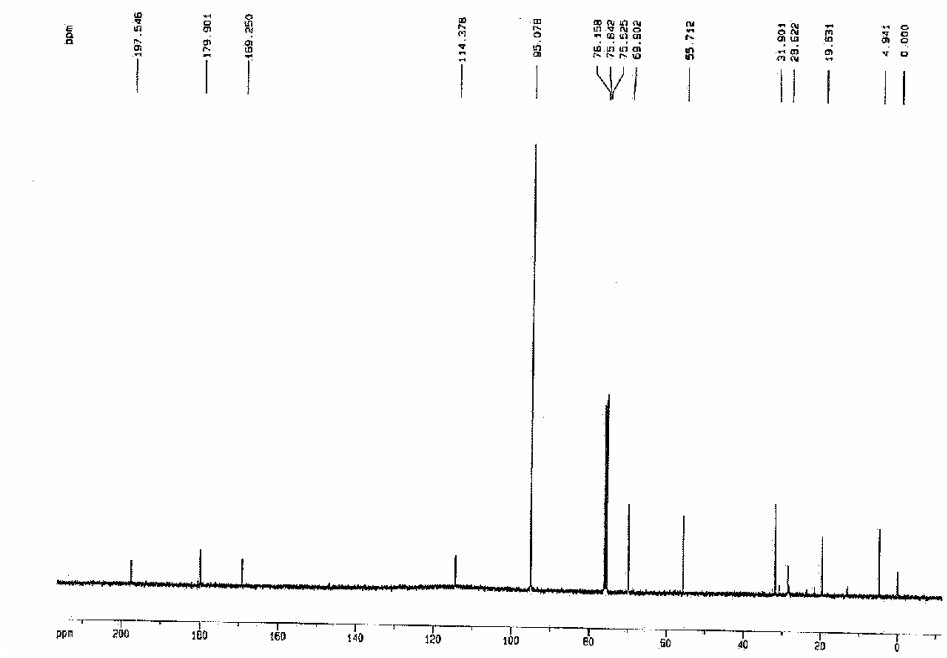


Figure 21: ^{13}C-NMR spectrum of 5-Acetoxy-3-methoxy-2-methyl-2-cyclopentene-1-one

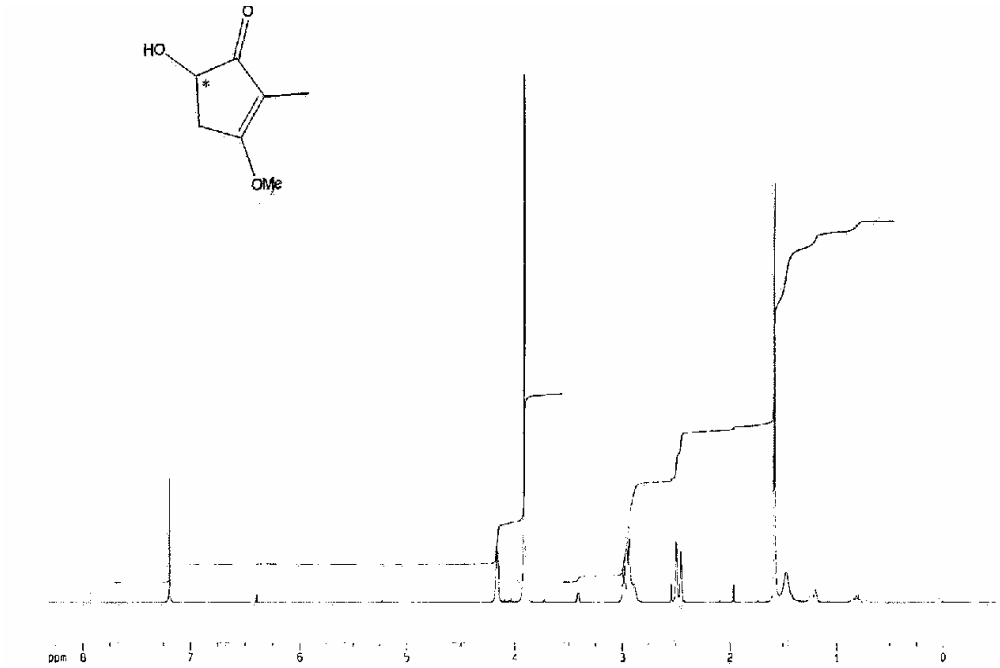


Figure 22: ^{1}H-NMR spectrum of 5-Hydroxy-3-methoxy-2-methyl-2-cyclopentene-1-one

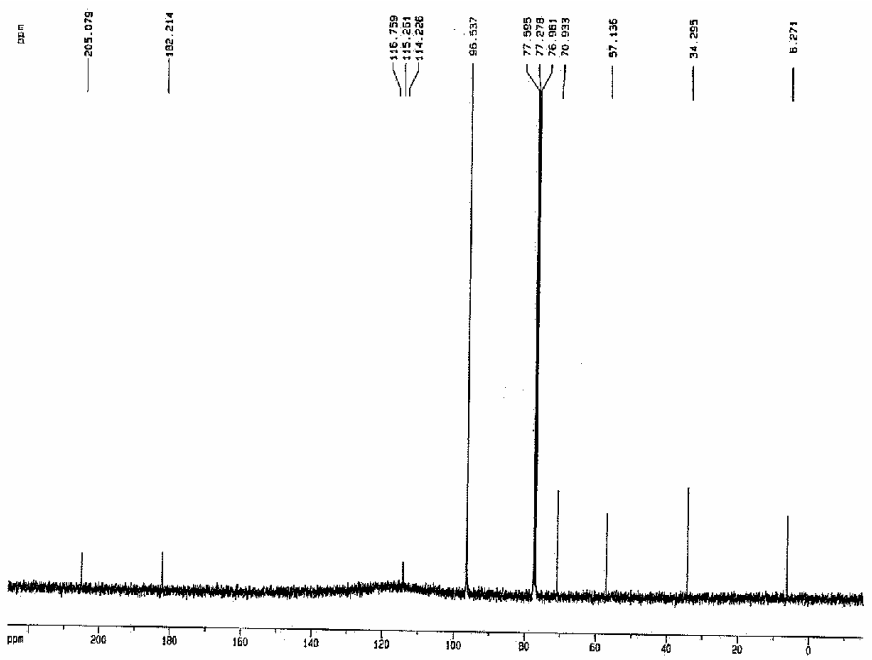


Figure 23: ^{13}C-NMR spectrum of 5-Hydroxy-3-methoxy-2-methyl-2-cyclopentene-1-one

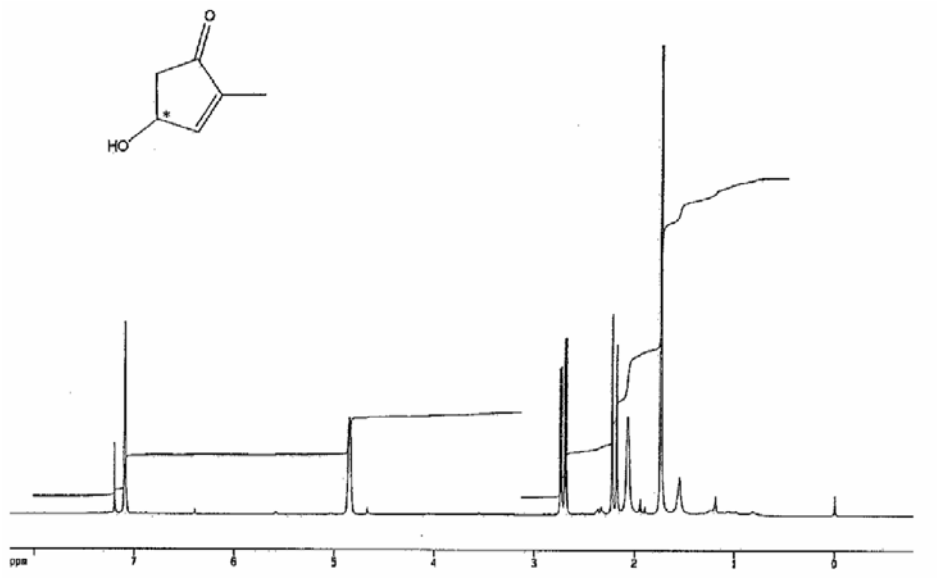


Figure 24: ^{1}H-NMR spectrum of 4-Hydroxy-2-methyl-2-cyclopentene-1-one

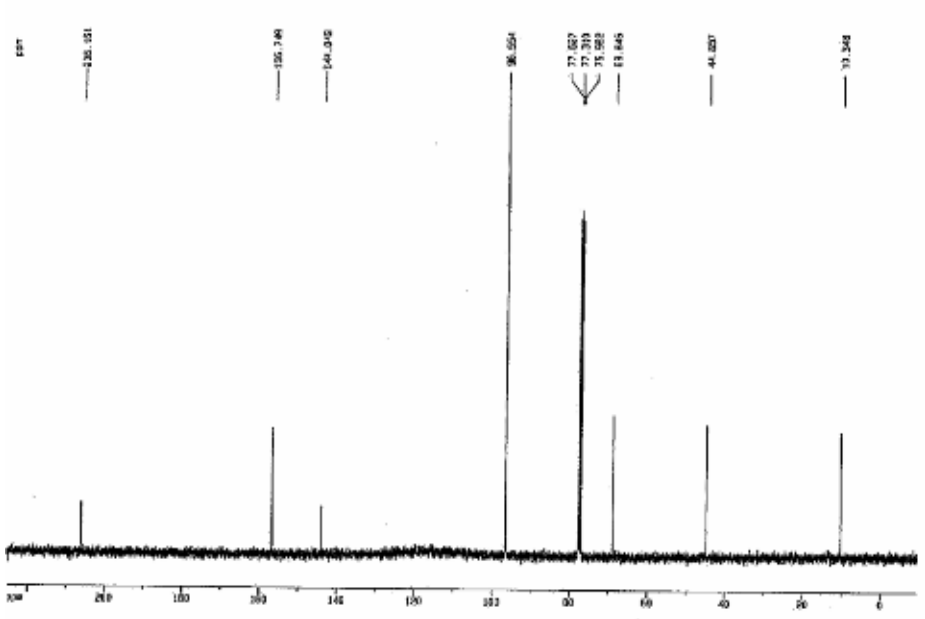


Figure 25: ^{13}C-NMR spectrumof 4-Hydroxy-2-methyl-2-cyclopentene-1-one

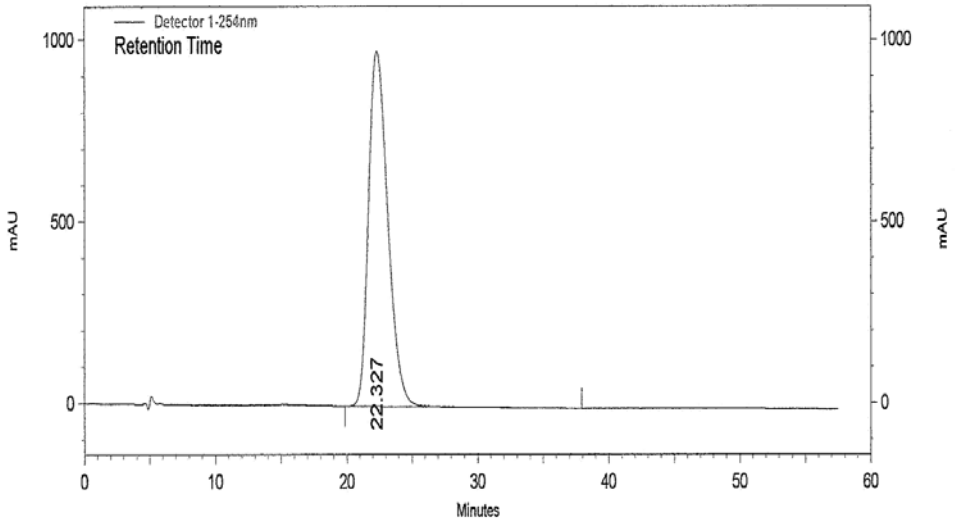


Figure 26. HPLC analysis of **7**-5-acetoxy-2-methyl-2-cyclopentene-1-one obtained from PLE catalyzed hydrolysis

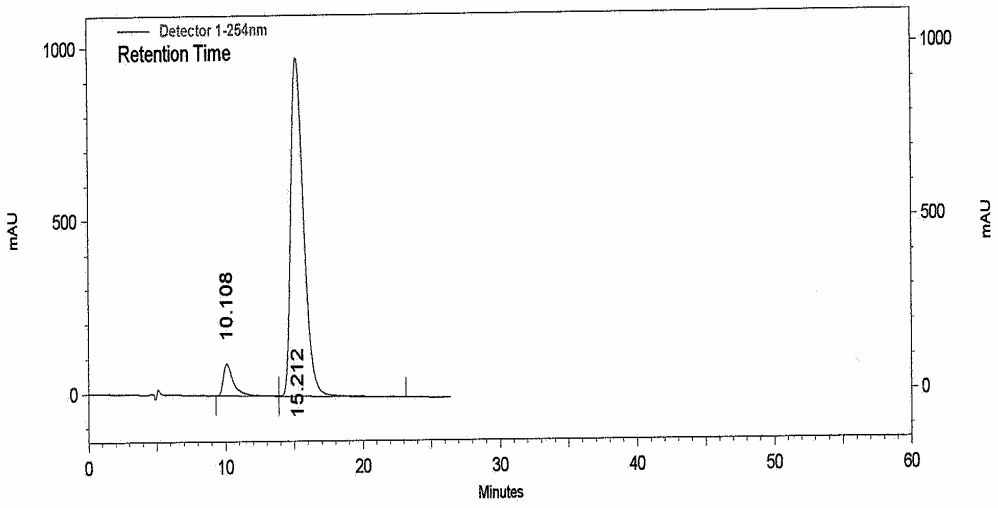


Figure 27. HPLC analysis of **8**-5-hydroxy-2-methyl-2-cyclopentene-1-one obtained from PLE catalyzed hydrolysis

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