STUDIES TOWARDS THE SYNTHESIS OF NATURAL PRODUCTS USING CHEMOENZYMATIC TECHNIQUES

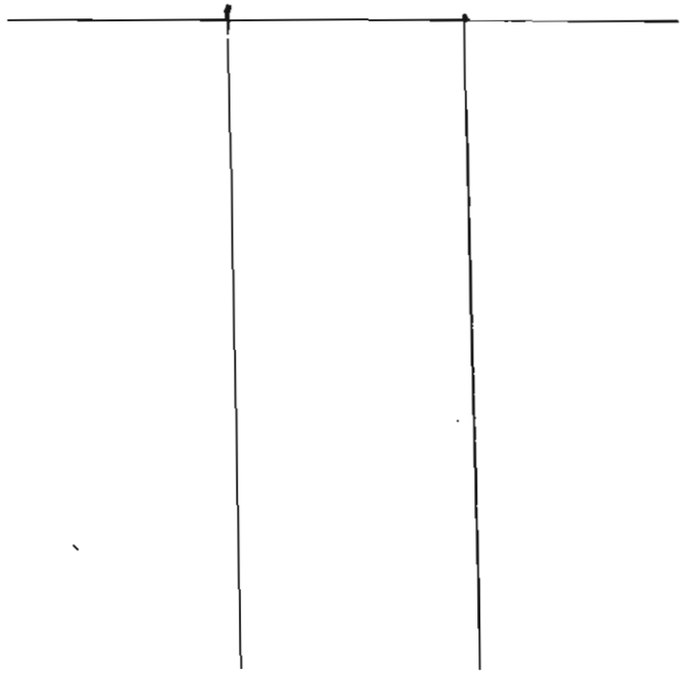
THESIS SUBMITTED TO THE UNIVERSITY OF KERALA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY UNDER THE FACULTY OF SCIENCE

BY

A. T. ANILKUMAR

ORGANIC CHEMISTRY DIVISION
REGIONAL RESEARCH LABORATORY (CSIR)
TRIVANDRUM-695 019, KERALA, INDIA

MAY, 1995



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STATEMENT

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me at the Organic Chemistry Division of the Regional Research Laboratory, Trivandrum, under the supervision of ***Dr. MANGALAM S. NAIR*** *and the same has not been submitted elsewhere for a degree.*

In keeping with the general practice of reporting scientific observations, due acknowledgement has been made wherever the work described is based on the findings of other investigators.

A. T. ANILKUMAR

वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद्
Council of Scientific & Industrial Research
क्षेत्रीय अनुसंधान प्रयोगशाला, तिरुवनन्तपुरम 695 019
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CERTIFICATE

Certified that the work described in this thesis entitled ***STUDIES TOWARDS THE SYNTHESIS OF NATURAL PRODUCTS USING CHEMOENZYMATIC TECHNIQUES*** *has been carried out by* ***Mr. A. T. ANILKUMAR*** *under my supervision and the same has not been submitted elsewhere for a degree.*

Mangalam S. Nair

MANGALAM S. NAIR
THESIS SUPERVISOR

ACKNOWLEDGEMENT

With great pleasure I place on record my deep sense of gratitude to my research supervisor, ***Dr. Mangalam S. Nair*** *for suggesting the research problem and encouraging me for the successful completion of this work.*

I would like to express my gratitude to Dr. A. D. Damodaran, Director, R. R. L, Trivandrum for permitting me to utilize all the facilities during my stay at R.R.L, Trivandrum.

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Financial assistance from CSIR, New Delhi and DST, Govt. of India is gratefully acknowledged.

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A. T. ANILKUMAR.

Trivandrum
May 1995

CONTENTS

Abbreviations

CCL	Candida cylindracea lipase
PFL	Pseudomonas fluorescens lipase
PPL	Porcine pancreatic lipase
WGL	Wheat germ lipase
DME	1, 2- Dimethoxyethane
DBU	1,8- Diazabicyclo[5.4.0]undec-7-ene
DMSO	Dimethylsulfoxide
$Eu(hfc)_3$	Tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato], europium(III) derivative
PTS	*p*-Toluenesulfonic acid
py	Pyridine
s	singlet
d	doublet
m	multiplet
br	broad

PREFACE

The asymmetric synthesis of biologically active compounds, especially, complex natural products is a challenging and rewarding task. Todate, several methodologies involving organometallic reagents, phase transfer catalysts, chiral auxilliaries, chiral reducing agents, biocatalysts *etc.* have been developed to meet this challenge. Among these, the use of biocatalysts, especially, enzymes has attracted much attention due to the manifold advantages offered by them in terms of efficiency, selectivity and specificity.

In this thesis titled "**STUDIES TOWARDS THE SYNTHESIS OF NATURAL PRODUCTS USING CHEMOENZYMATIC TECHNIQUES**", the results of an investigation of the use of lipases for the enantioselective synthesis of various chiral intermediates enroute to the biologically active natural products viz., *Polygodial*, *Warburganal*, *Albicanol*, *Albicanyl acetate*, *Coronarin E* and *Zonarol* (structures **59 - 64** in the text) are presented. The thesis is divided into four chapters. Relevent references are given at the end of each chapter.

Chapter I is divided into four sections. Section 1.1 gives a general introduction. Section 1.2 constitutes the literature survey and is divided into two parts. Part I contains *selected examples from the recent literature* highlighting the use of enzymes for the enantioselective synthesis of pharmaceutical intermediates and natural products. In Part II, a study of the existing synthetic methodologies for the enantioselective synthesis of the

natural products of our interest has been made. The objectives of the present investigation are specified in section 1.3.

In Chapter II, the results of the initial attempts at obtaining chiral intermediates through the use of Baker's yeast is discussed.

The efficiency of various commercially available lipases for catalysing regio- and enantioselective transesterification of a bicyclic substrate containing a 1,3- diol system has been investigated in detail and the results are given in Chapter III. In addition, the methodology for converting products obtained from such biotransformations to advanced intermediates enroute to the natural products **59 - 66** have also been delineated.

Using the lipase catalysed transesterification method on a carefully chosen bicyclic substrate, several intermediates enroute to **59 - 66** have been synthesised. The details of these as well as the total synthesis of the natural products (+)-Albicanol and (+)- Albicanyl acetate are given in Chapter IV.

CHAPTER I

1.1 GENERAL INTRODUCTION

Over the past two decades, there has been a tremendous surge of interest in enantioselective synthesis, catalysed and necessitated by the deeper understanding of the intricate relationship between chirality and biological activity. This has led to the development of various synthetic methodologies involving chiral-catalysts, reagents and auxilliaries for asymmetric induction. Interestingly, the vast array of structurally complex natural products found in nature have been crafted with the aid of enzymes. For almost every type of reaction, there exists an enzymatic counterpart. Such reactions occur with a high degree of selectivity under relatively mild conditions (*i.e.* at ambient temperature and nearly neutral pH) and are usually free from associated environmental hazards (ie. byproducts, solvents etc). This realisation has attracted a large number of synthetic organic chemists to the idea of using biocatalysts for asymmetric synthesis. The biocatalysts usually used are whole cells (microorganisms), purified enzymes and partially purified enzymes.

In particular, the development of enzymatic techniques for the synthesis of key chiral intermediates enroute to pharmaceuticals and biologically active natural products is an area of high contemporary interest.

The present work is focussed on the asymmetric synthesis of an extremely interesting group of natural products (containing a bicyclo[4.4.0] ring system) through chiral induction using enzymes. Appropriately, the thesis is titled 'Studies towards the synthesis of natural products using chemoenzymatic techniques'.

Since the aim was to utilize enzymatic transformations for enantioselective synthesis of a specific group of natural products, it was necessary to be familiar with their chemistry as well as with existing biotransformation techniques. Accordingly, the literature survey presented here encompasses both aspects.

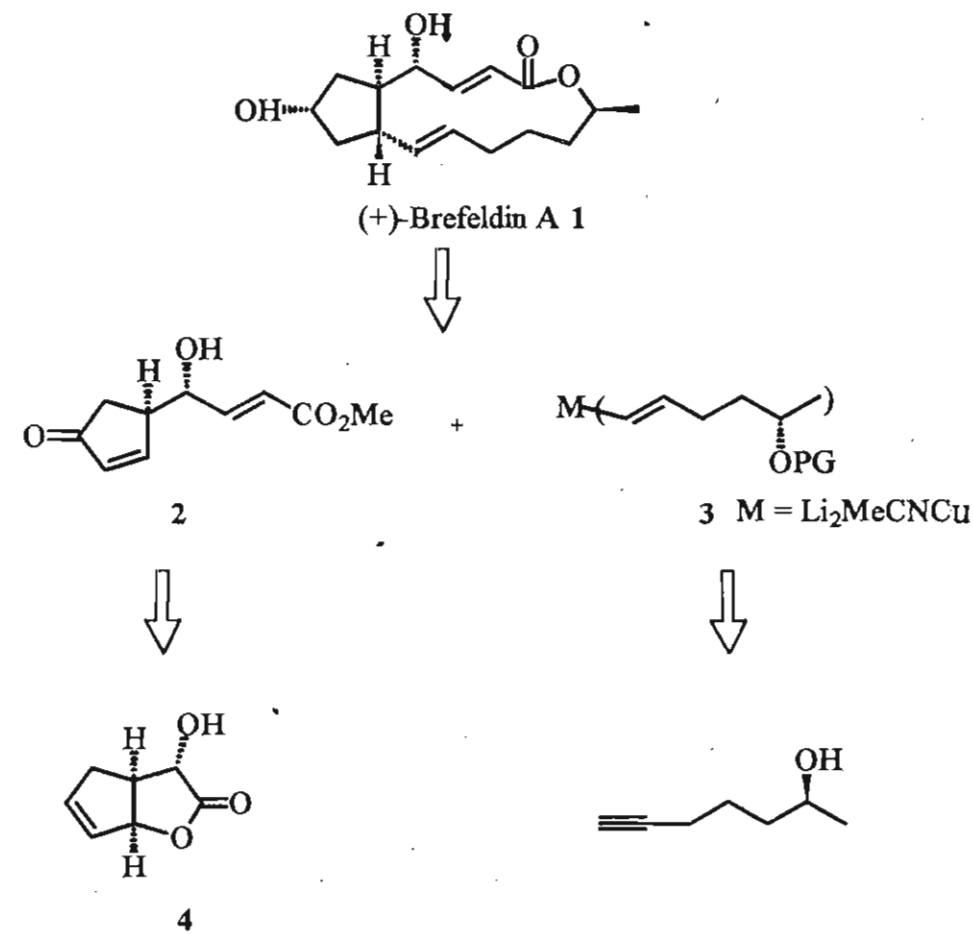
1.2 LITERATURE SURVEY

1.2.1 PART I: Selected examples from recent literature on the use of lipases for the syntheses of natural products and pharmaceutical intermediates

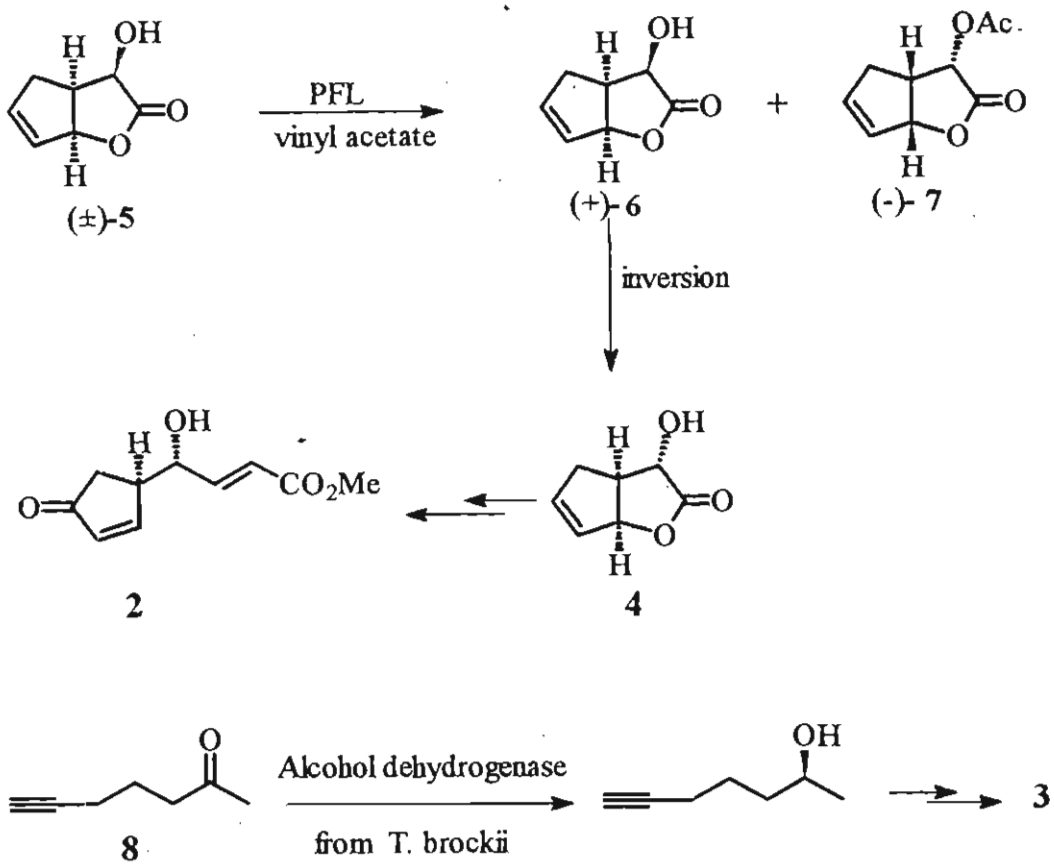
The stereoselective elaboration of chiral centres has become a central issue in pharmaceutical industry and in the synthesis of natural products and their analogs. In this part, a selected number of papers dealing with the

efficient use of biocatalysts for the construction of key chiral intermediates in the synthesis of natural products and pharmaceuticals of high contemporary interest is summarised. These are examples that have not been included in any reviews and only the enzymatic transformation step is highlighted. However, in order to project the overall view about the synthesis, a few advanced intermediates obtained through further chemical transformations enroute to the final products are also depicted using retrosynthetic arrows. For the enzymatic transformation, in most cases, the biocatalysts used are hydrolases. The principal reason for this is their ease of application and their ability to function without coenzymes. Further, the utility of these hydrolases(*viz.*, lipases and esterases) have been magnified several fold following the Klibanov[1] discovery that they can function well in many organic solvents and has therefore, attracted a large number of organic chemists into this area.

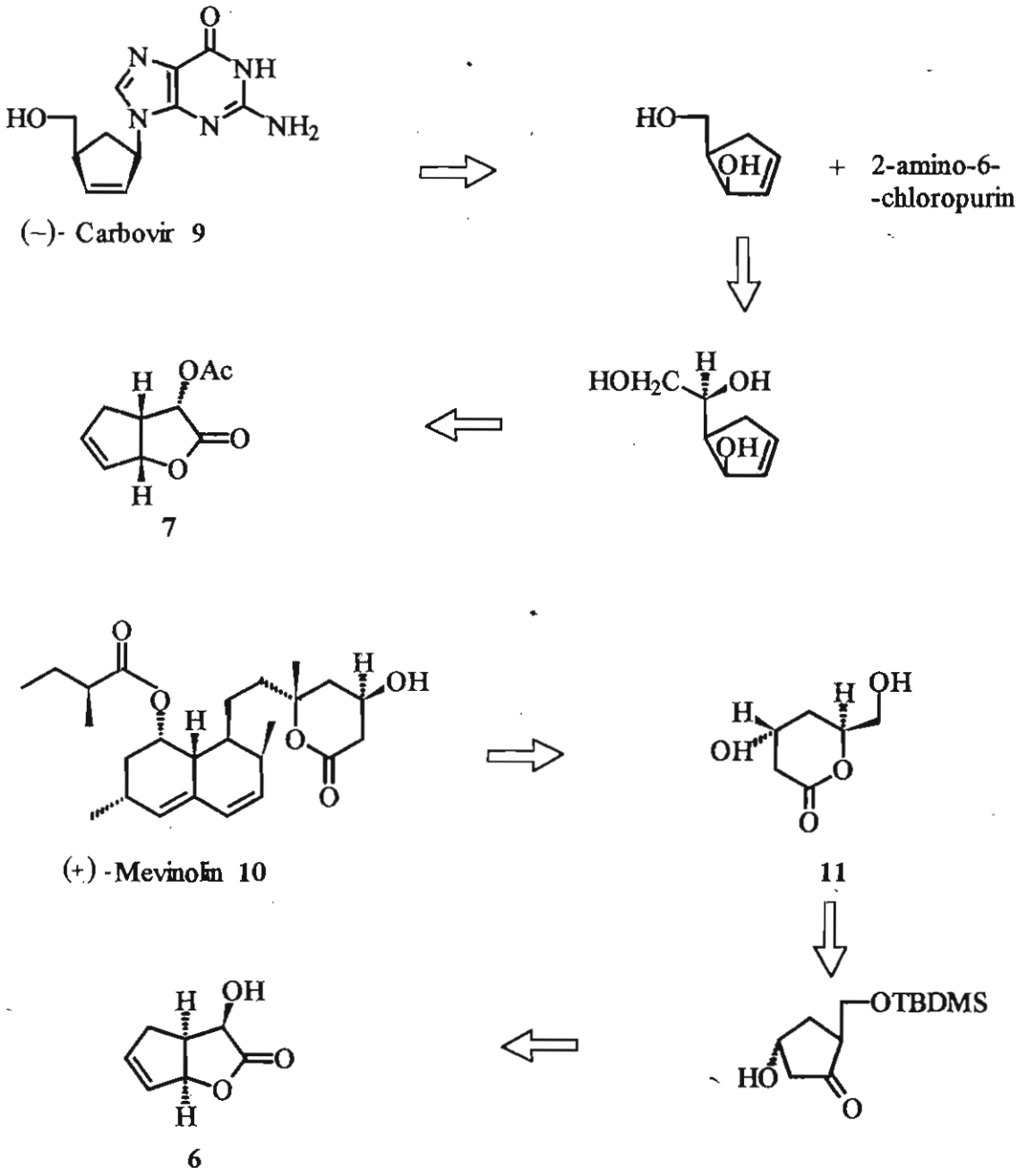
A diverse number of enantiomerically pure compounds have been synthesised by Roberts' group at Exeter through the judicial and strategic use of enzymes. Brefeldin A **1**, first isolated from *Pencillium decumbens* in 1958 and subsequently found as a secondary metabolite in other cultures is known to possess antibiotic, antiviral, cytotoxic and antimitotic effects. Its synthesis has been pursued actively around the world. The synthesis of **1** has been achieved by Roberts' team[2] through the coupling of two chiral units **2** and **3** as shown in the retrosynthetic analysis.



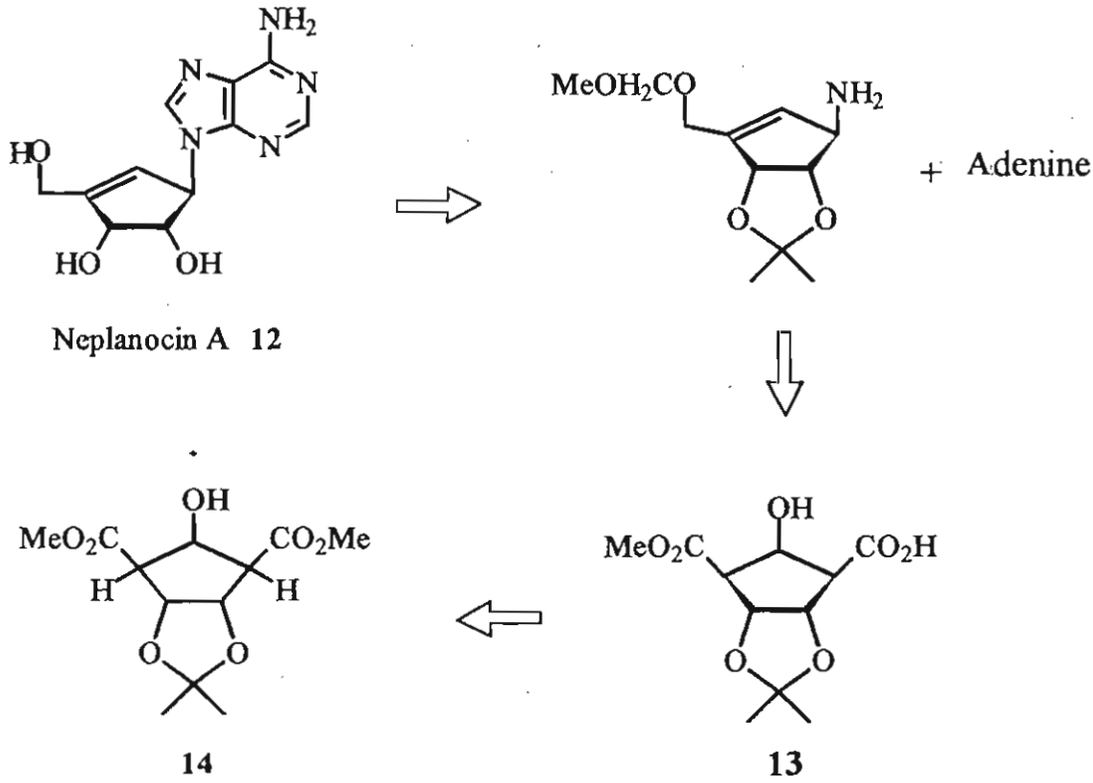
Enantiomerically pure **2** has been obtained through the steps including (i) PFL catalysed resolution of the racemic hydroxy lactone **5**, resulting in the alcohol **6** and acetate **7**, (ii) inversion at the hydroxy centre of **6** to give **4** and (iii) further chemical reactions leading to **2** retaining the two required stereocentres. The chiral centre in the lower side chain was generated through enzymatic reduction of hept-6-yne-2-one **8** using alcohol dehydrogenase from *Thermoanaerobium brockii*. This process was found to be superior to the enzymatic resolution of (±) - heptyn -2- ol.



The successful resolution of **5** recorded above has also been instrumental in the synthesis of the anti HIV agent (-)carbovir[3] **9** and the chiral intermediate **11** enroute to the hypocholestemic agent mevinolin[4] **10**. Interestingly, when PFL catalysed acylation of **5** was stopped at 40% conversion, **7** was obtained in >95% ee whereas after 60% conversion, alcohol **6** could be isolated in >95% ee.



The chemoenzymatic approach has been successfully used by the same group for the synthesis of carbocyclic nucleosides neplanocin A[5] **12** and the carbon analogue of oxetanocin A[6] **15**.

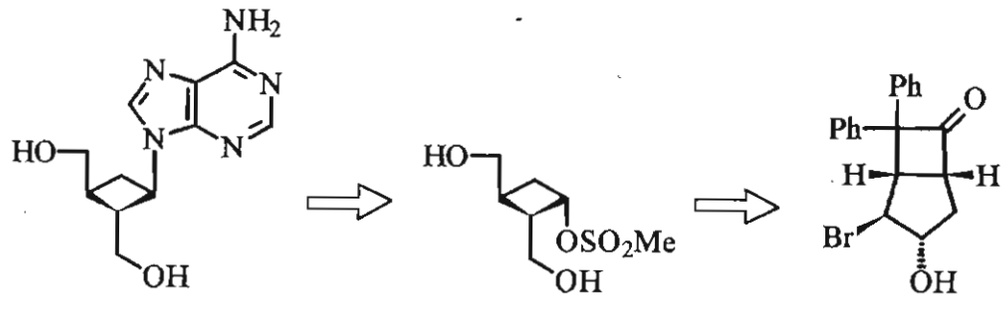


Neplanocin A 12

14

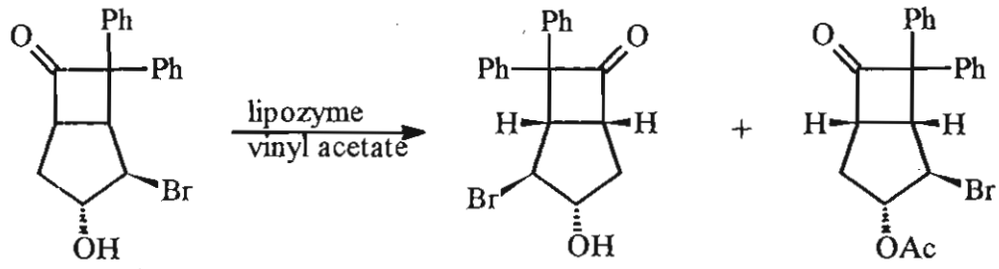
13

The chiral intermediate **13** required for elaboration to **12** was obtained through PLE catalysed enantiospecific hydrolysis of the meso ester **14**, which was first reported by Zemlicka in 1988[7]. The enzymatic resolution of the bromohydrin **16** using lipozyme in vinyl acetate was the pivotal step in the approach to **15,** a carbocylic analogue of oxetanocin A .



15

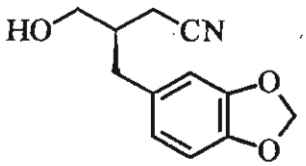
(+)-16



(±) (+)-16

Since these carbocyclic nucleosides are not susceptible to degradation in vivo by nucleosidases and phosphorylases, they have attracted much attention in the study of HIV virus.

Another remarkable example of the use of enzymes is Itoh's synthesis[8] of the three types of naturally occuring antitumor lignans (-)-hinokinin **17**, (-)-isodeoxypodophyllotoxin **18** and (+)-isostegane **19** from the optically active intermediate **20**. This compound in turn, has been obtained from the chiral hydroxynitrile **21**. As a means of access to **21**, the kinetic resolution of the diacetate **22a** using PLE, PPL, or lipases from *Aspergillus niger*, *Candida Sp.*, *Rhizopus Sp.* etc proved to be unsatisfactory. However, asymmetrisation of the prochiral diol **22b** was found to occur leading to the monoacetate **23** with lipase PS. Further chemical transformations yielded the hydroxynitrile **21** which was elaborated to the natural product through the intermediacy of **20**.



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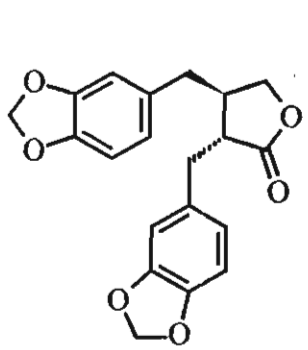
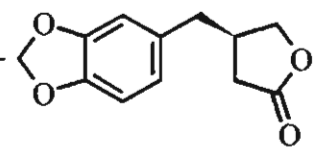


(–)-Hinokinin **17**



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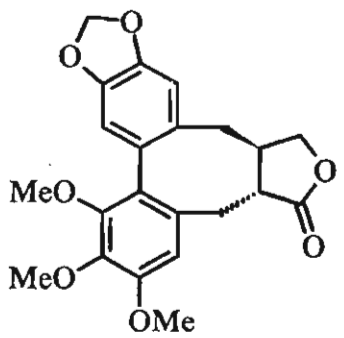
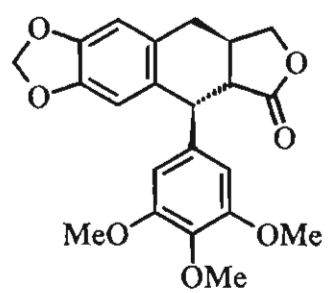
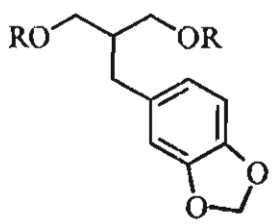


(+) - Isostegane **19**

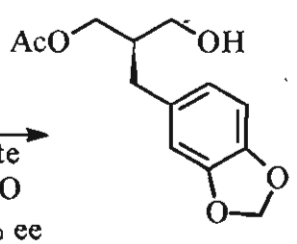


(–) - Isodeoxypodophyllotoxin **18**

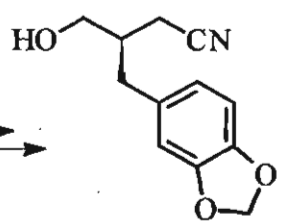


22 a. R = OAc
b. R = H

Lipase PS
vinyl acetate
i- Pr_2O-H_2O
97%, >98% ee



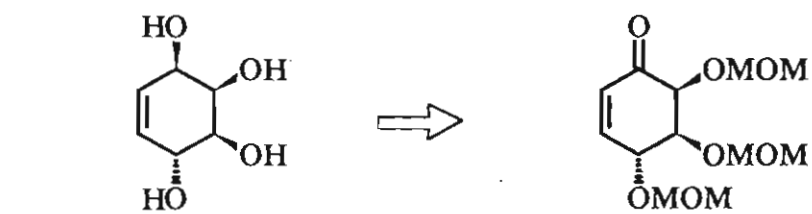
23



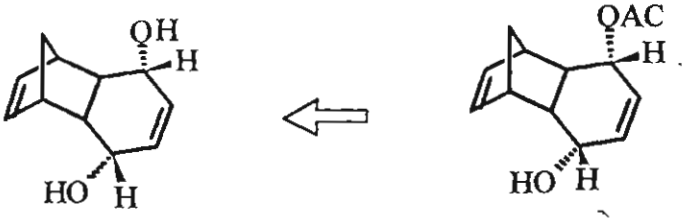
21

The glucosidase inhibitor (-) Conduritol C **24** and the fungal secondary metabolite Eutypoxide B **27** are formidable synthetic targets having four and

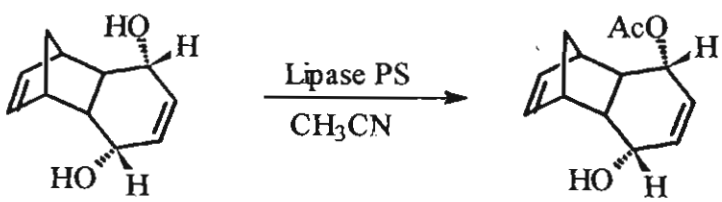
five stereocentres on the cyclohexyl ring each, respectively. The total synthesis of both have been achieved by Takano *et al.* from the key chiral compound **26**. After introducing asymmetry through lipase PS catalysed acetylation of the meso diol **25** and building up the additional two hydroxyl functionalities in a stereospecific manner using OsO_4 as catalyst, the cyclohexenyl ring was unravelled elegantly through a retro Diels-Alder reaction. The key intermediates are depicted in the retrosynthetic scheme shown below.[9]



(-)-Conduritol C **24**

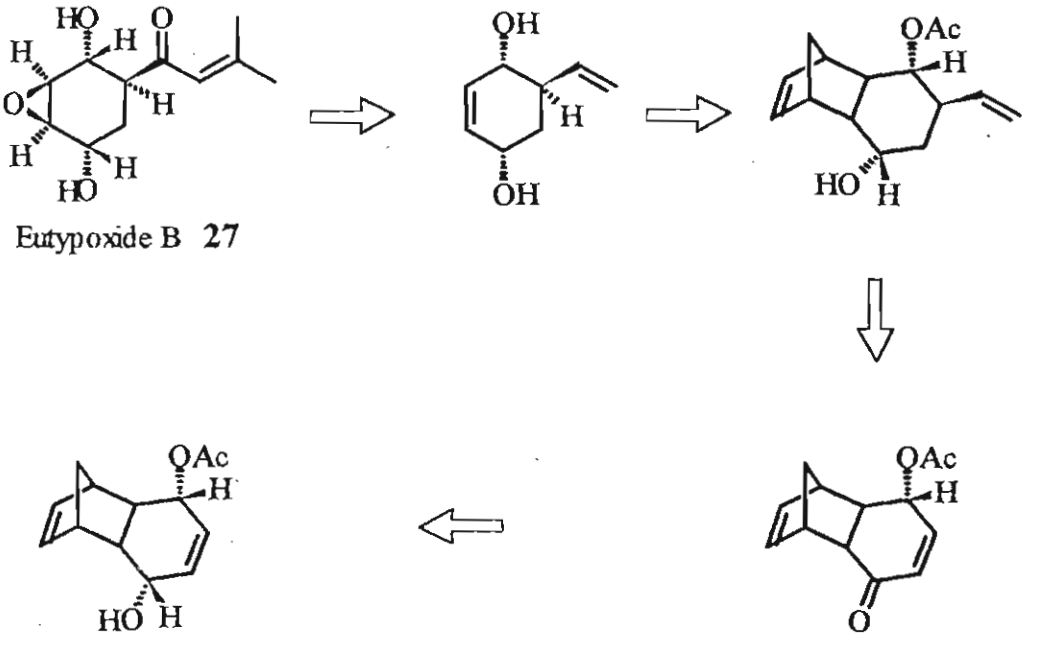


25 (+)-**26**



25 (+)-**26**

For the synthesis of Eutypoxide B **27**, the same enantiomerically pure compound **26** was subjected to a series of chemical modifications yielding the various intermediates shown in the retrosynthetic scheme.[10]



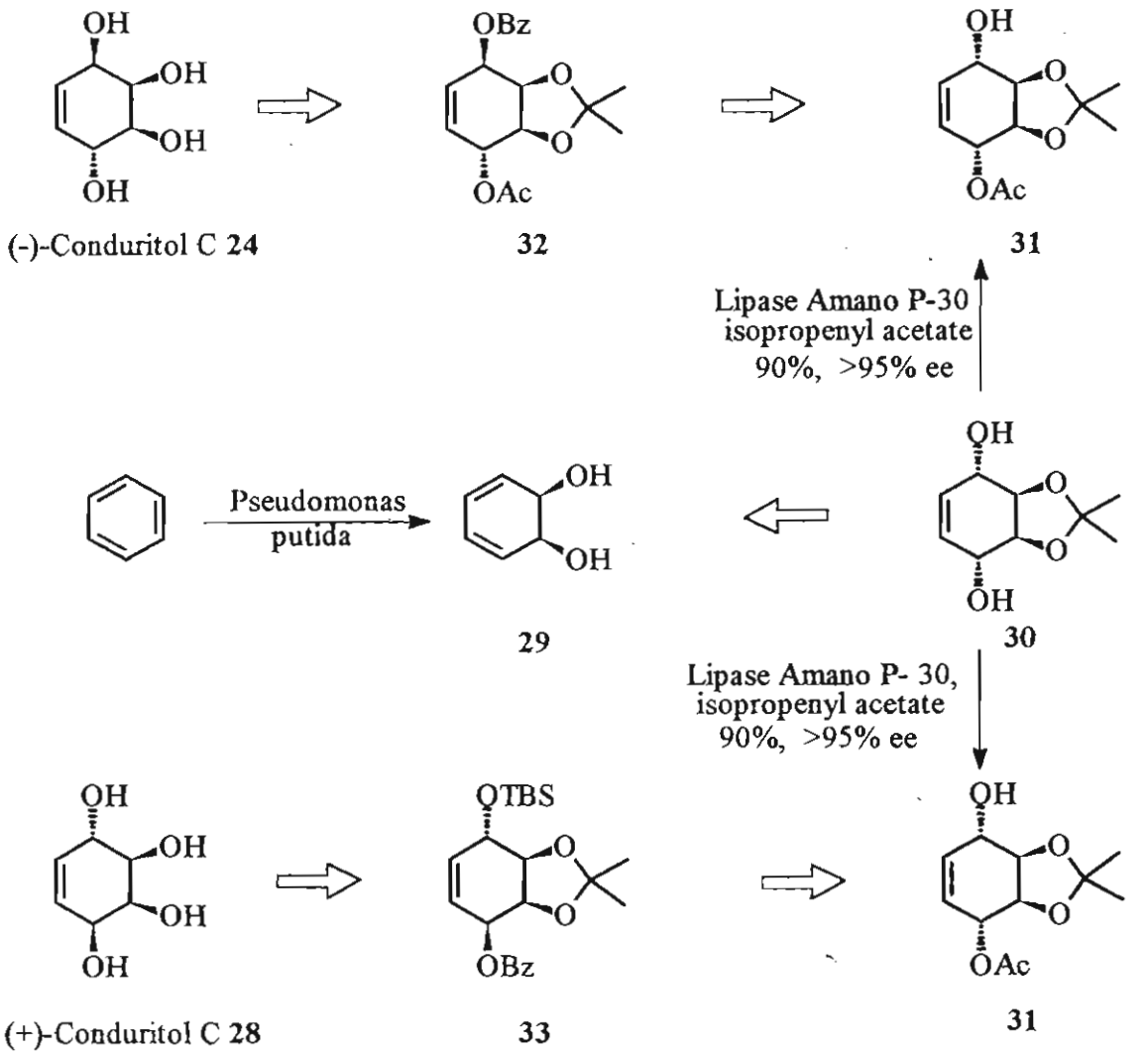
Eutypoxide B **27**

26

Takano has also been among the first to recognise and demonstrate the potential of enzymatic transformations of meso diols in the synthesis of enantiomerically pure compounds.

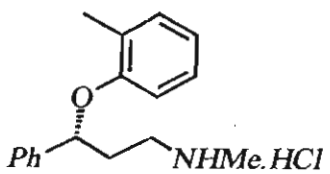
Both the enantiomers of Condüritol C *viz.*, **24** and **28** have been synthesised by Johnson *et al.* using two biotransformations as key steps.[11] The first step involved a microbial oxidation of benzene to cyclohexa-3,5-diene-1,2-diol **29** with a mutant of *Pseudomonas putida*. This was followed by chemical transformations to give **30**. This meso diol on treatment with Lipase PS-Amano in isopropenyl acetate provided the monoacetate **31** in excellent yield and optical purity. Selective use of Mitsunobu reaction in

tandem with protection-deprotection sequences led to the intermedates **32** and **33** enroute to (-)-**24** and (+)-**28** as depicted in the retrosynthetic scheme.

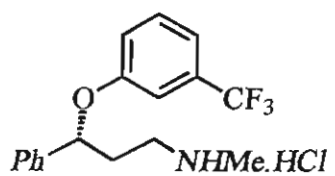


Tomoxetine **34** and Fluoxetin **35** are currently used antidepressants marketed in racemic form. The *S*-enantiomer of **34** is twice as active as *R*-enantiomer while *S*-**35** is nine times as active as *R*-**35**. Since, through the use of lower dosages of highly active *S*-enantiomers, the side effects can be minimised, their asymmetric syntheses have been pursued by many groups. The progenitor for *R* and *S* forms of **34** and **35** *viz.*, *R*-and *S*-3-chloro-1-

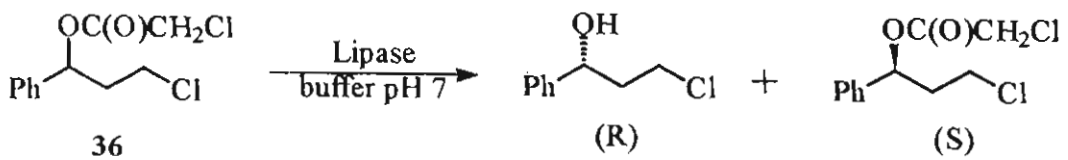
phenyl-1-propanol has been obtained by Schneider[12] through the hydrolysis of the corresponding chloroacetate **36** with lipase from *Pseudomonas sp* (SAM-2).



Tomoxetin **34**

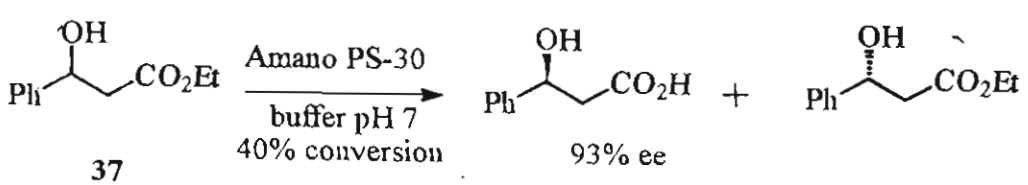


Fluoxetin **35**



36 (R) (S)

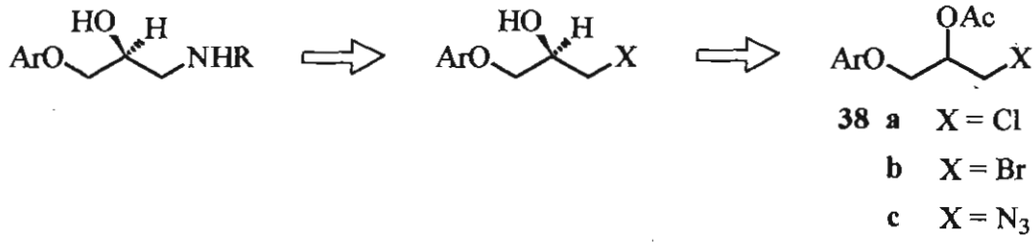
For the same purpose , the *R* and *S* forms of 3-hydroxy-3-phenyl-propanoic acid has been synthesised by Boaz[13] through Amano Lipase PS-30 catalysed hydrolysis of ethyl-3-hydroxy-3-phenyl proponoate **37** as shown below.



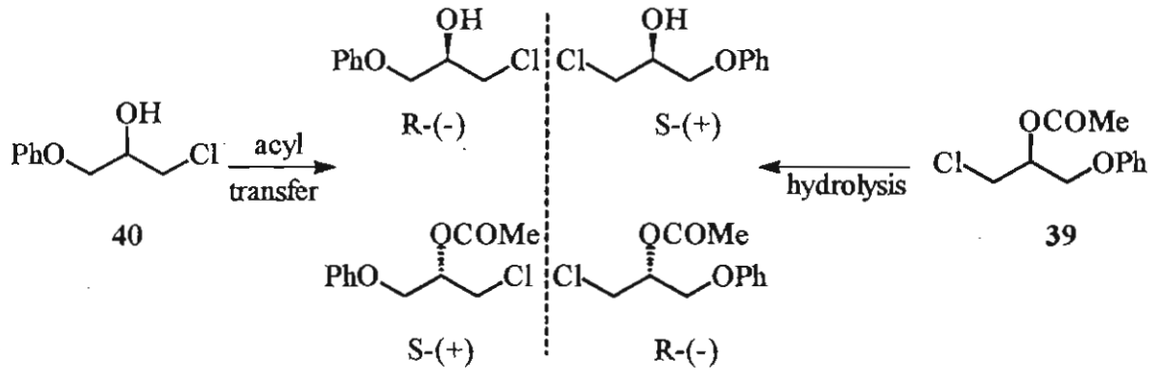
37

Schneider has also studied in depth, the use of biocatalysts for the synthesis of β-adrenergic blockers. His important findings are that (i) activated esters such as α-chloro, α–bromo or α-azido derivatives **38a - 38c** hydrolyse faster and selectively in the presence of lipase from *Pseudomonas species*[14] and (ii) by using it in the 'hydrolysis mode' in

aqueous solution or 'acyl transfer' mode in acylating agents, products of complementary stereochemistry can be obtained[15] as shown for the examples **39** and **40**.



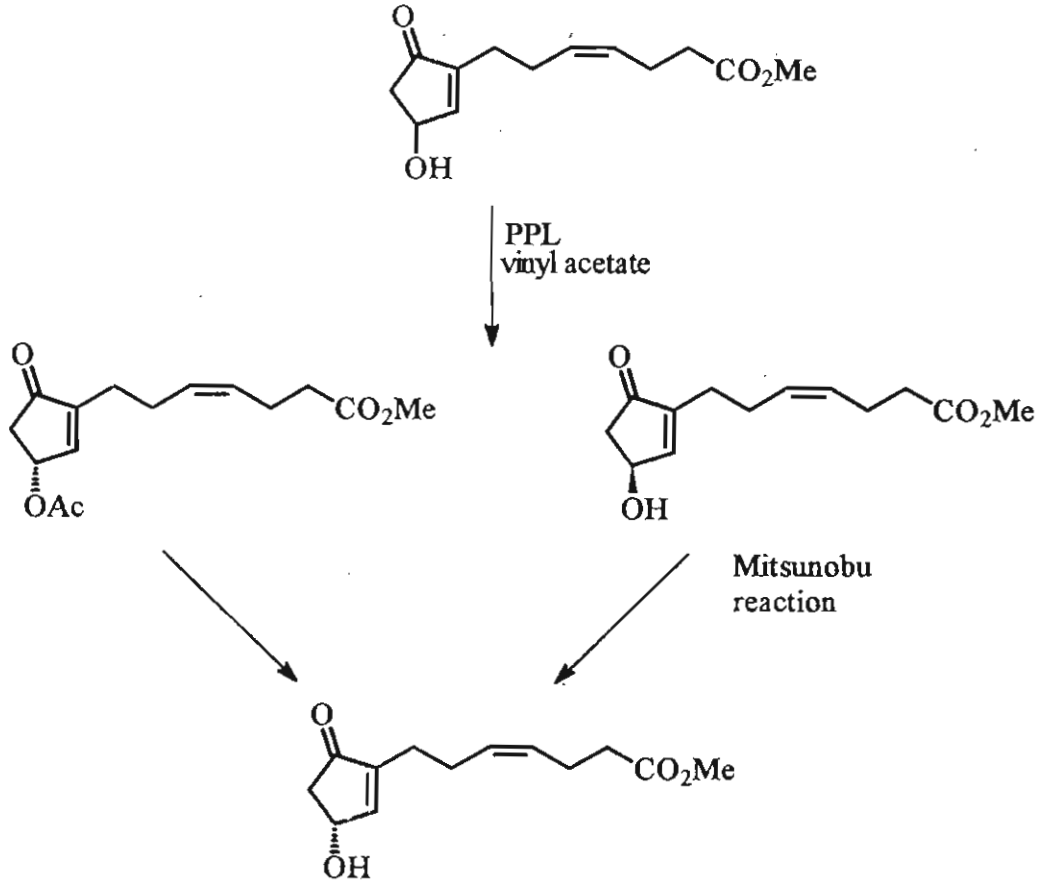
38 **a** X = Cl
b X = Br
c $X = N_3$



An important group of compounds where chirality plays a crucial role is pheromones. The use of biocatalysts for their synthesis has been widely favoured and has been covered in Mori's extensive review on the subject.[16]

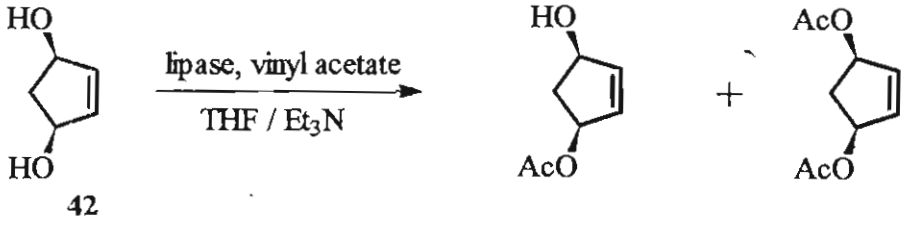
Several suitably functionalised cyclopentanols as potential prostaglandin intermediates have been synthesised through enzymatic mediation. The most noteworthy examples are (i) the synthesis of **41** by Wong[17] (ii) the resolution of **42** and **43** by Theil[18] and also by Johnson[19] and (iii) the large scale preparation of **44** by Mori.[20]

Wong intermediates

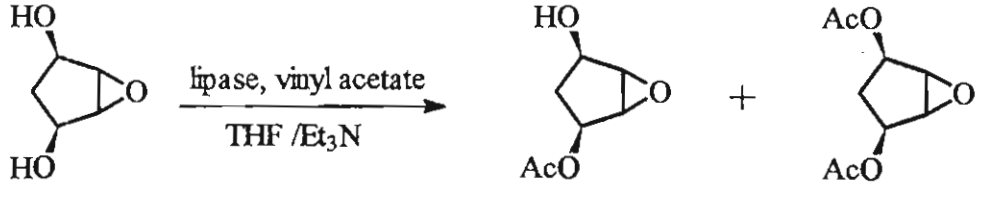


41

Theil intermediates

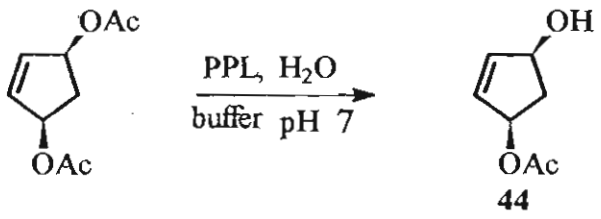


42



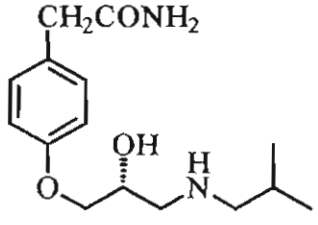
43

Mori intermediate

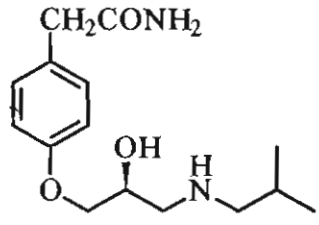


44

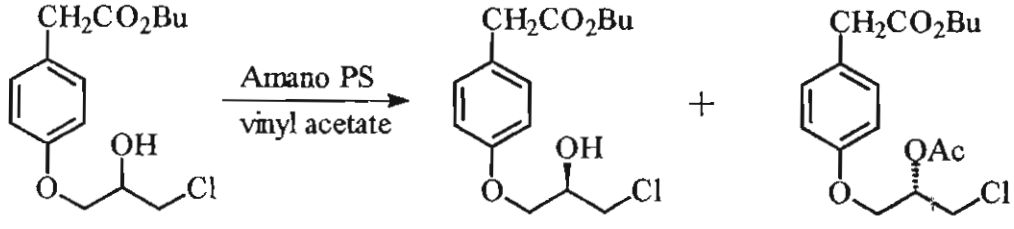
Atenolol, sold in recemic form is among the best selling drugs in the world today for hypertension and angina. However, its *R* and *S* enantiomers *viz.*,**45** and **46** posses further interesting pharmaceutical profile when used as enantiomerically pure compounds and have been synthesised by Bevinakatty[21] with the help of enzymes. The key step in their synthesis is the transesterification of **47** with lipase PS from Amano. Stopping the reaction after 50% conversion provided *R*-(-)-**47** and *S*-(+)-**48** with high optical purity. In an alternative approach (±)-**48** was subjected to deacylation using 1-butanol in the presence of Amano PS lipase. This showed excellent selectivity toward the *S* isomer giving complimentary products *S*-(+)-**47** and *R*-(-)-**48**.



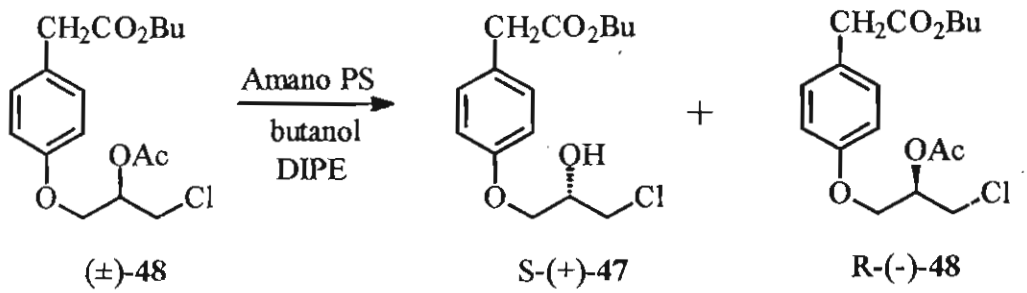
(R)- Atenolol-**45**



(S)-Atenolol-**46**

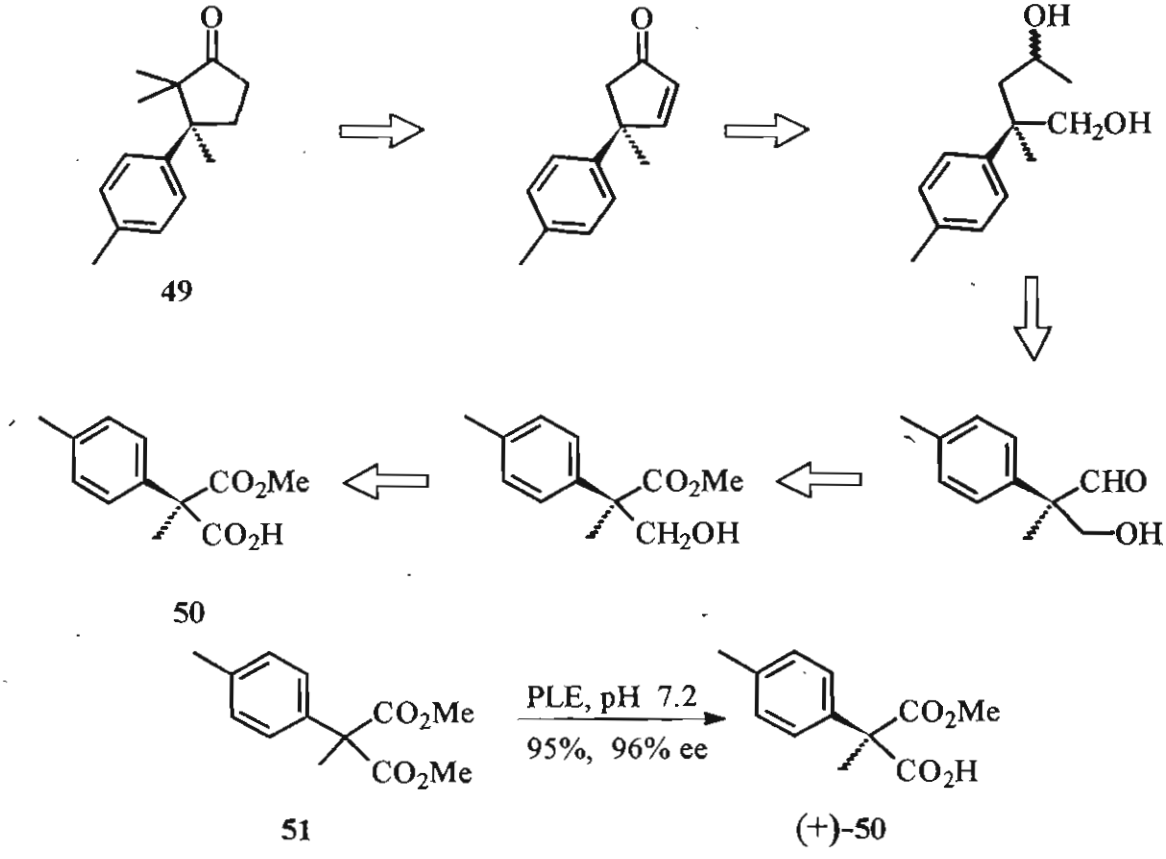


(±)- **47** R- (-)-**47** S-(+)-**48**



(±)-48 S-(+)-47 R-(-)-48

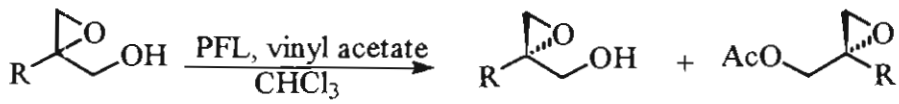
Chiral synthon **50** enroute to the naturally occurring sesquiterpene (+)-α-cuparenone **49** has been synthesised by Fadel *et al.*[22] through a key step involving the enzymatic generation of a chiral quarternary centre. A few intermediates encountered in their synthesis is depicted here.



49 **50** **51** (+)-**50**

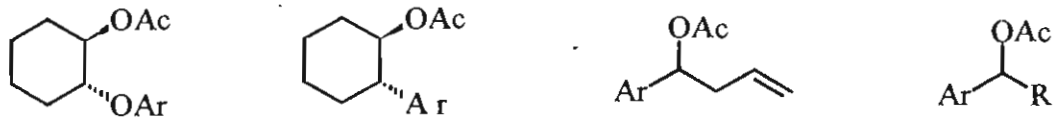
The construction of the asymmetric quarternary carbon was carried out by the enantioselective enzymatic hydrolysis of the prochiral dimethyl malonate **51** leading to (+)-**50** in 95% yield and 96% ee.

Synthetically useful building blocks bearing chiral quarternary centres have also been obtained by Santaniello *et al.*[23] using enzymatic resolution of 2-substituted oxirane methanols like **52** depicted below.



(±)-**52** (-) (+)

Basavaiah *et al.*[24] have shown that crude enzyme preparations like pig liver acetone powder, chicken liver acetone powder, bovine liver acetone powder and goat liver acetone powder can be used effectively for resolving and thereby obtaining various synthetically important intermediates having the general structures **53, 54, 55** and **56**. These crude enzymes have been prepared by the method developed by Ohno *et al.*[25]



53 **54** **55** **56**

Eventhough it does not fall into the theme of this chapter the large amount of work carried out by Crout *et al.*[26] using protease and glycosidase

to catalyse the synthesis of peptides, glycosides, oligosaccharides etc. merits mention here.

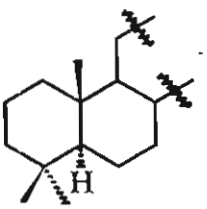
Similarly, mention must be made of the extensive investigations carried out by Goter *et al.*[27] on enzymatic aminolysis and transamidation reactions using lipases.

Two extremely useful reviews[28,29] and several books[30,31] that have been published during the last few years have helped us to appreciate the wide scope of selective biocatalysis.

1.2.2 PART II: Some biologically active natural products containing the bicyclo[4.4.0] ring system and the strategies developed for their asymmetric synthesis

Structural diversity combined with biological activity has continued to keep terpenoid synthesis an area of constant interest. It remains the fertile testing ground for new synthetic methodologies and strategies, as well as the armoury of the more utilitarian pharmaceutical industry. The spectacular advances made in isolation methods and spectroscopic techniques during the last few decades have helped to unravel larger number of structurally intriguing compounds from nature's mysterious hidden places.

A common feature found in many terpenoids, considered to be arising *through electrophile initiated cyclization of farnesyl pyrophosphate and squalene epoxide, is the bicyclo[4.4.0] ring system* **57** *with three pendant* methyl groups as shown below. This core is found in hopanes, sclaranes,



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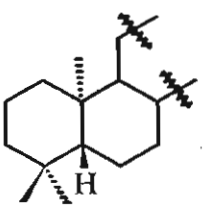
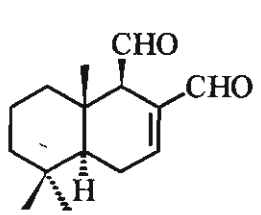


58

labdanes, drimanes etc. Among these, the highly oxygenated drimane compounds **Polygodial 59** and **Warburganal 60** have attracted considerable attention due to their antifeedant activity and potential for use in

agriculture.[32] Few other natural products containing the same ring system, viz., **Albicanol 61**, **Albicanyl acetate 62** and **Coronarin E 63** also possess interesting biological activity. Their structures are shown in Figure 1.

Figure 1



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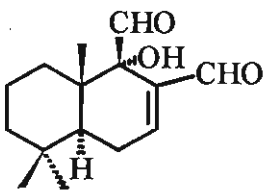

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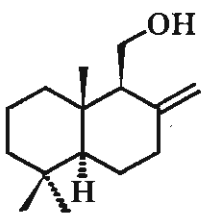

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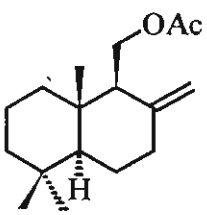

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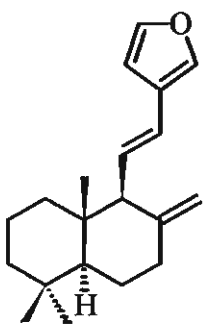

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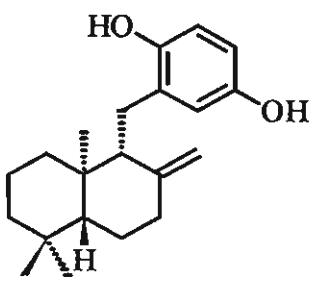

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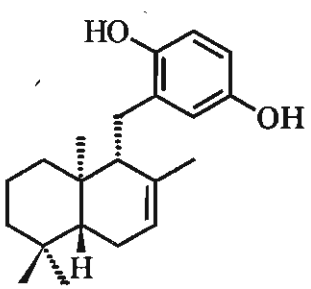

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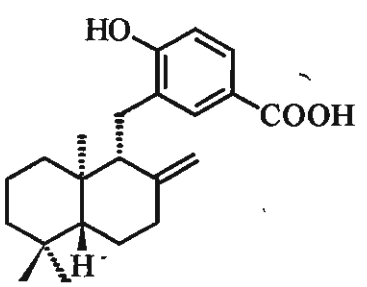


66

The optical antipode of **57** viz., the structural core **58**, although rare, can be found in the marine natural products **Zonarol 64**, **Isozonarol 65** and **Zonaroic acid 66** (Figure 1). A few *ent*-labdanes have also been isolated recently,[33] which bear similar ring system.

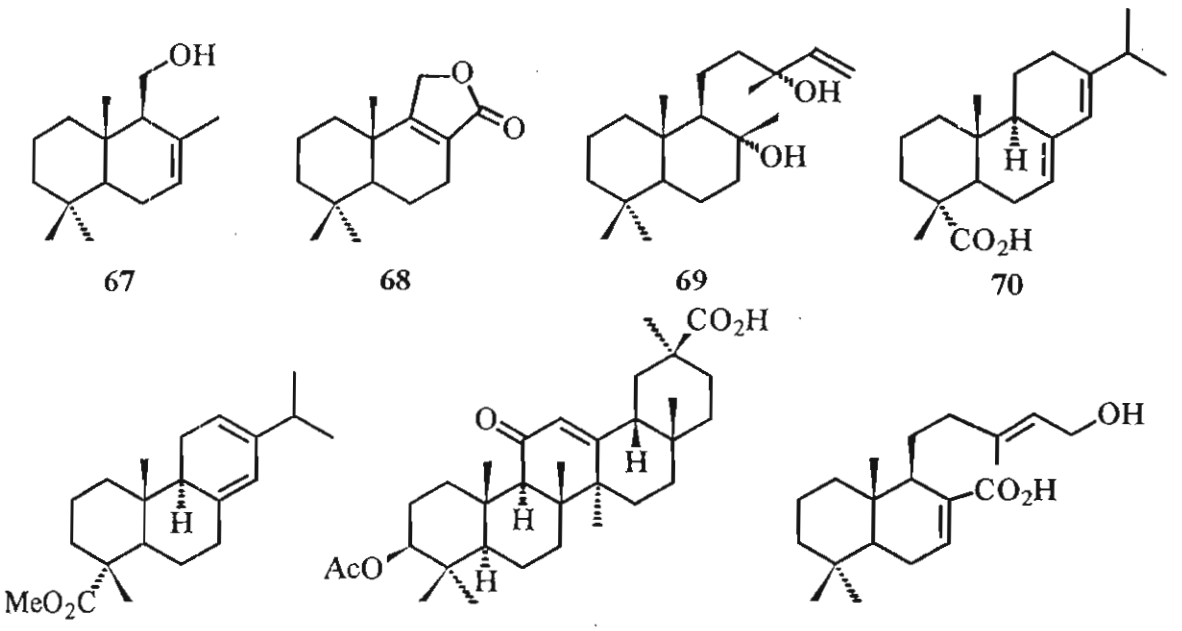
The prevalence of the structural unit **57** and **58** in terpenoids, as well as the interesting biologcal profile of compounds **59-66** prompted us to investigate suitable methods for obtaining well functionalised chiral intermediates that contain these basic units. Prior to embarking upon this task, a literature survey of synthetic approaches to enantiomerically pure **59-66** were carried out.

To our suprise, the literature survey revealed that only few attempts had been made previously for synthesizing compounds **59-66** in an optically pure form from simple starting materials. Most of the synthetic efforts towards these compounds relied on the degradation of other optically pure natural products which already contained the structural unit **57**. To put the problem in the correct perspective, a few salient aspects of the chemistry of compounds **59-66** are given here.

Polygodial

(-)-Polygodial **59** was first isolated from *Polygonum hydropiper*[34] and later from the African plant *Warburgia stuhlmanii.*[35] It has been found to possess antifeedant activity against several African crop pests, especially *Spodoptera littoralis* and *Spodoptera exempta.*[35] Several synthesis of racemic **59** have been reported and are well summarised in de Groot's review on Drimane sesquiterpenoids in *'Natural Products Reports'.*[36] It has been synthesized in optically pure form, starting from the natural products[36] (-)-drimenol **67**, (+)-confertifolin **68**, (-)-sclareol[37] **69**, (-)-abietic acid **70**, levopimaric acid **71**, glycerrhetinic acid **72** and zamoranic acid[38] **73**, all depicted in Figure 2. The only total synthesis of (-)-**59** that

Figure 2

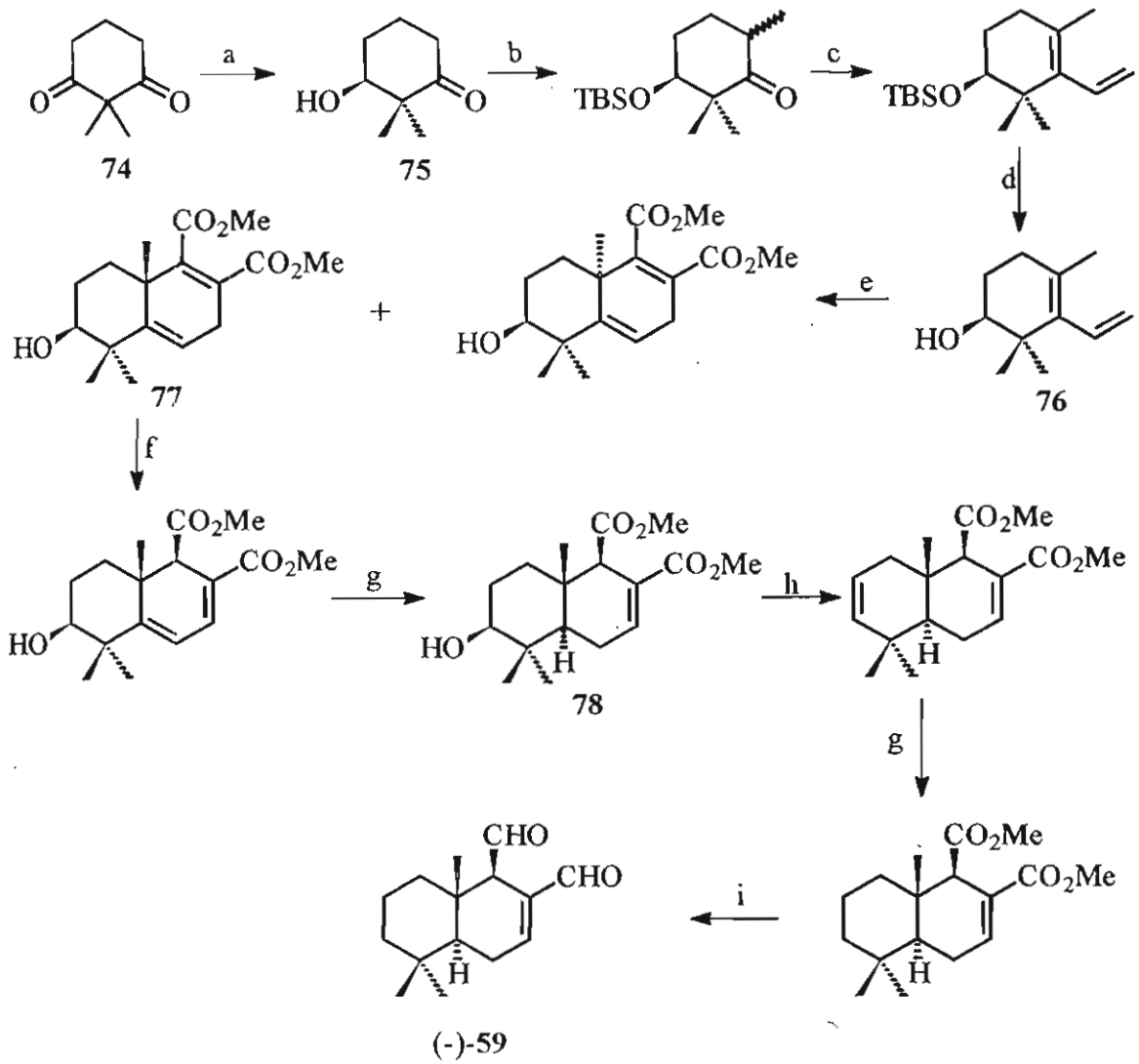


67 68 69 70

71 72 73

does not utilize a chiral natural product as starting material has been reported by Mori.[39] In this approach, shown in Scheme I, the diketone **74** was initially converted to the optically active intermediate (*S*)-ketol **75** through enzymatic reduction using bakers yeast and further transformed to the diene **76**. Following the widely used Diels-Alder approach for the construction of functionalised decalin systems, the diester **77** was synthesized through the addition of dimethyl acetylenedicarboxylate to **76**. Delocalization of the double bond followed by hydrogenation resulted in **78** which was converted to (-)-**59** through routine transformations as depicted here.

Scheme I



Reagents : a. Bakers yeast; b. (i) t-BuMe_2SiCl, imidazole, DMF; (ii) LDA, MeI; c. (i) sodium acetylide, ammonia; (ii) $CuSO_4$, xylene; (iii) H_2, Pd on $CaCO_3$, quinoline; d. HF, CH_3CN; e. DMAD; f. DBU, THF; g. H_2, Pd on C; h. CF_3SO_2Cl, DMAP, CH_2Cl_2; i. (i) $LiAlH_4$; (ii) DMSO, NEt_3, $(COCl)_2$

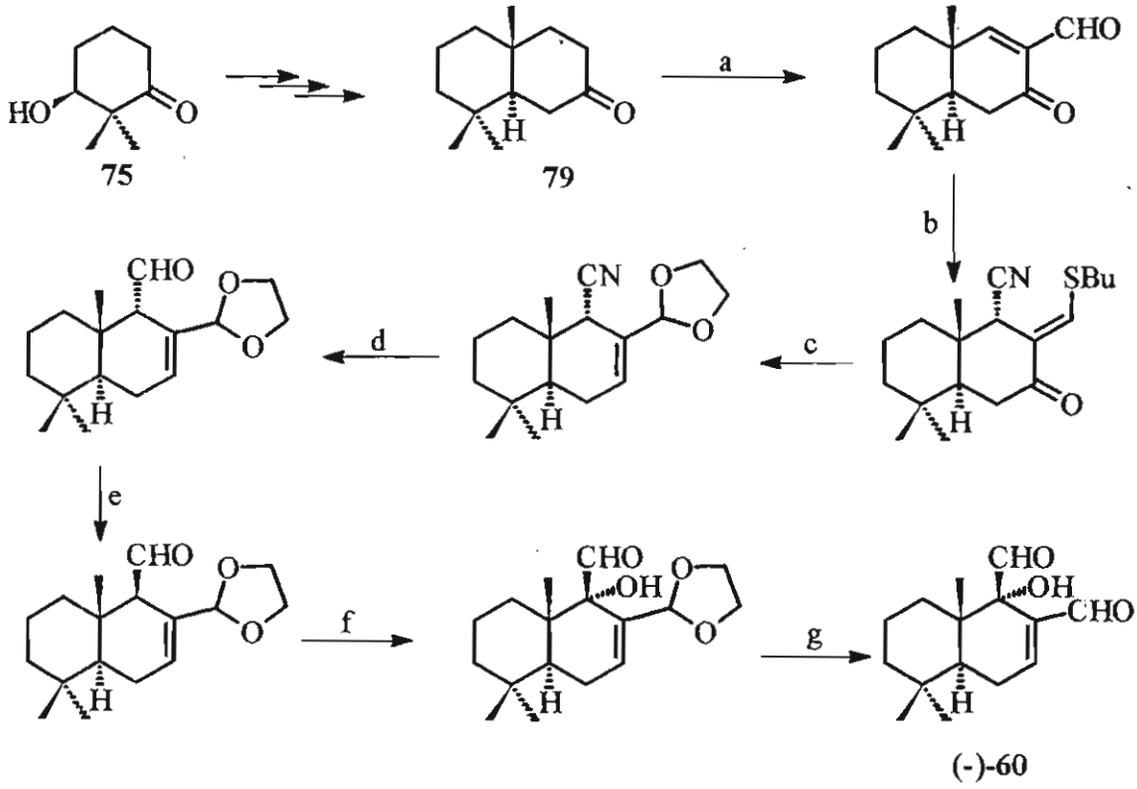
Warburganal

(-)-Warburganal **60** has been isolated from the bark of East African medicinal trees *Warburgia ugandensis* and *Warburgia stuhlmanii*.[35,40]

Following detailed studies carried out by Kubo, Nakanishi *et al.*, it has been found to possess three distinctly different yet, useful types of biological activities.[40b] They are (i) irreversible and species specific antifeedant activity on the African army worm, *Spodoptera exempta*, (ii) cytotoxic and molluscicidal activity against the schistosome transmitting snail, *Biomphalaria glabaratus* and (iii) broad spectrum antimicrobial activity. Warburganal is a further oxidised form of polygodial itself and like in the case of **59,** it has been synthesised[36,38] starting from (-)-drimenol **67,** (-)-confertifolin **68**, abietic acid **70,** levopimaric acid 71, glycerrhetinic acid **72** and zamoranic acid **73 .**

Once again Mori *et al.*[41] have carried out a total synthesis of (-)-**60** starting from the chiral intermediate **75** (Scheme II). Following further transformations that included, deoxygenation, alkylation, ring formation *etc.* the chiral intermediate **79** was obtained. Starting from here, Mori *et al.* have followed the same path to synthesise (-)-**60** , which has been worked out on (±)-**60** by de Groot *et al.*[42] The key transformations included (i) introduction of a formyl group at C-2 (ii) introduction of a cyano group at C-1 *via* conjugate addition (iii) base catalysed epimerisation at C-1 (iv) introduction of 1α hydroxyl group and (v) hydrolysis.

Scheme II



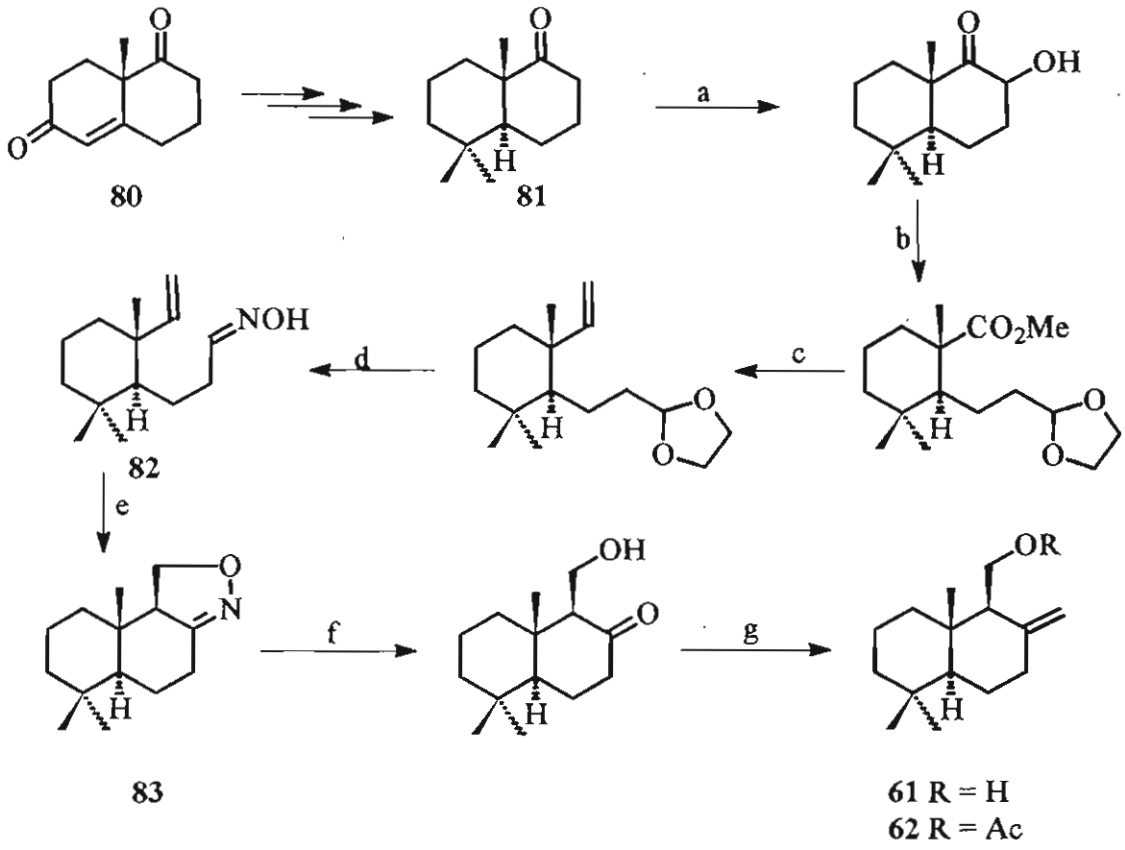
Reagents : a. (i) NaH, HCO_2Et; (ii) PhSeCl, py., H_2O_2; b. (i) KCN; (ii) n-BuSH, p-TsOH; c. (i) $NaBH_4$, H_2O, H^+, $HgCl_2$; (ii) $HO(CH_2)_2OH$, p-TsOH; d. DIBAH; e. KO-t-Bu; f. LDA, MoO_5. HMPA. py.; g. H_2O, H^+

Albicanol and Albicanyl acetate

(+)-Albicanol **61** was first isolated from the liverwort *Diplophyllum albicans*[43] and later found to occur along with albicanyl acetate **62** in the dorid nudibranch *Cadlina luteomarginata*[44] also. (+)-Albicanyl acetate **62** has been reported to possess fish antifeedant activity. The first synthesis of racemic **62** was carried out by Armstrong *et al.*[45] utilizing electrophilic cyclization of olefinic allylsilane as the key step.

Recently, (+)-**62** has been synthesized starting from the natural product (-)-sclareol **69** by Barrero *et al.*[37] Apart from this, the only other synthesis of (+)-**61** and (+)-**62** has been reported by Fukumoto *et al.*[46] starting with the readily available (+) Wieland-Miescher ketone **80.** Using standard procedures **80** was first converted to the ketone **81** and further to the olefinic oxime **82** as shown in Scheme III. Treatment of **82** with sodium hypochlorite generated the nitrile oxide *in situ* which underwent a highly diastereoselective intramolecular (3+2) dipolar cycloaddition leading to the isoxazoline **83**. Reductive hydrolysis of **83** followed by methylenation resulted in (+)-**61**. Facile acetylation of **61** using acetic anhydride/pyridine system produced (+)-**62**.

Scheme III



Reagents : a.(i) Py^+. HBr_3^-; (ii) NaOH; b. (i) LTA, MeOH; (ii) $HO(CH_2)_2OH$
c.(i) $LiAlH_4$; (ii) $(COCl)_2$, DMSO, Et_3N; (iii) Ph_3PCH_2; d.(i) H_3O^+;
(ii) NH_2OH. HCl, NaOAc; e. 7% aqueous NaOCl; f. H_2, Raney Ni,
$B(OMe)_3$; g. Zn- CH_2Br_2-$TiCl_4$; (ii) Ac_2O, py.

Coronarin E

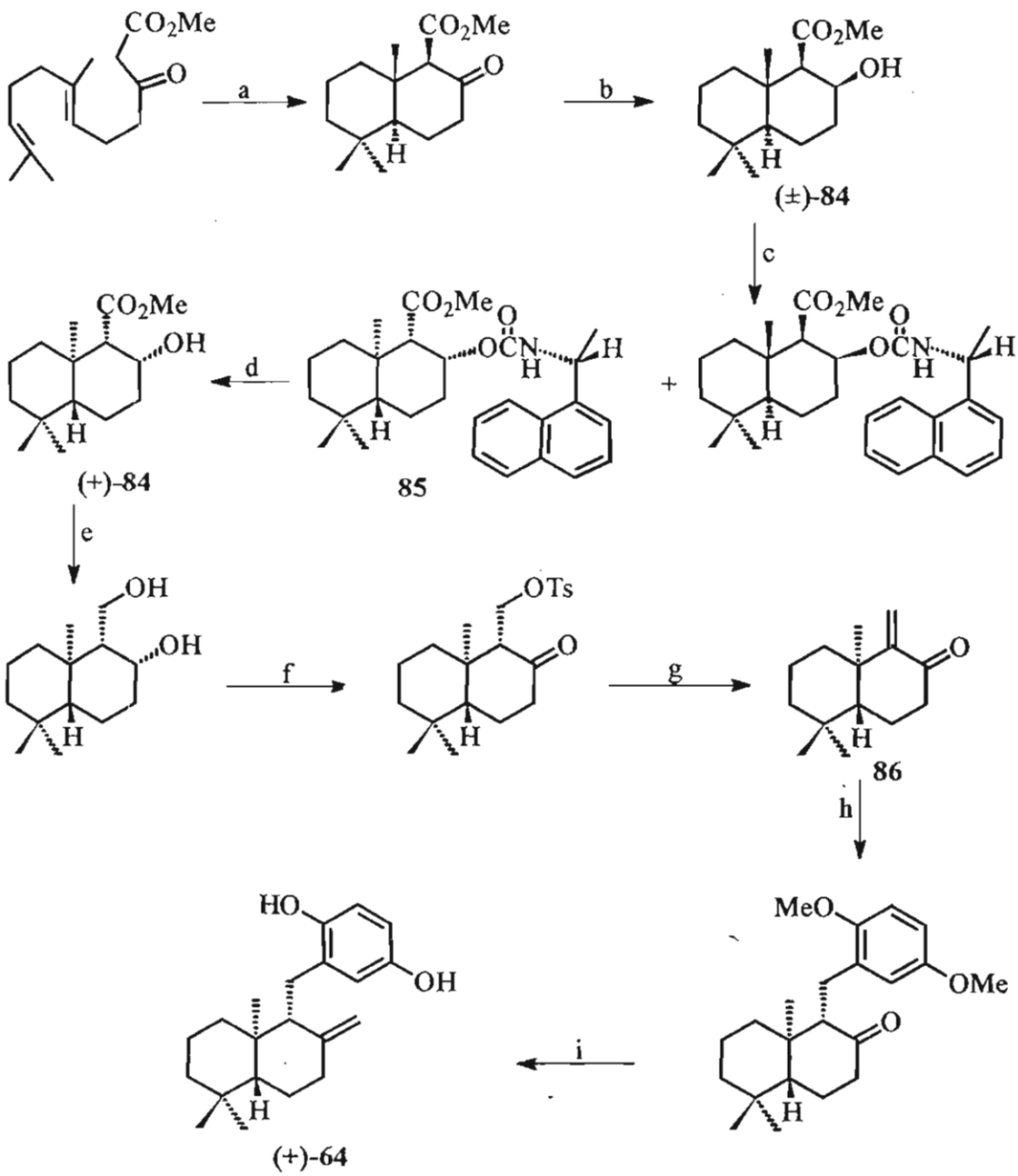
Coronarin E **63** (Figure I) was isolated from the Brazilian medicinal plant *Hedychium coronarium* by Itokawa *et al.*[47] To date, no synthesis of this compound has been published.

Zonarol

Zonarol **64** was isolated from the brown seaweed *Dictyopteris undulata* collected from the Pacific ocean by Fenical *et al.*[48] Interestingly, *D. undulata* collected from the Gulf of California by the same group contained only isozonarol **65**. It displays fungitoxic activity against certain species of Phytophthora, Rhizoctonia and Sclerotinia fungi. A synthesis of racemic zonarol **64** and isozonarol **65** has been reported by Welch *et al.* in 1978.[49]

Configuration of natural zonarol **64** was established by Mori *et al.*[50] through their synthesis of (+)-**64** as depicted in Scheme IV. The key intermediate in their approach was the optically active hydroxy ester **84** obtained through the resolution of (±)-**84** *via.* its naphthylethyl carbamate derivative **85**. After obtaining optically pure **84,** it was converted to the versatile synthon **86** *viz.*, 1-methylene-5,5,8a-trimethyl-2-oxodecahydro-naphthalene. The enone **86** readily underwent 1,4 addition with Grignard reagent prepared from 1-bromo-2,5-dimethoxybenzene. Further manipulations necessary to attain (+)-**64** included a Wittig methylenation and hydrolysis of methoxy substituents.

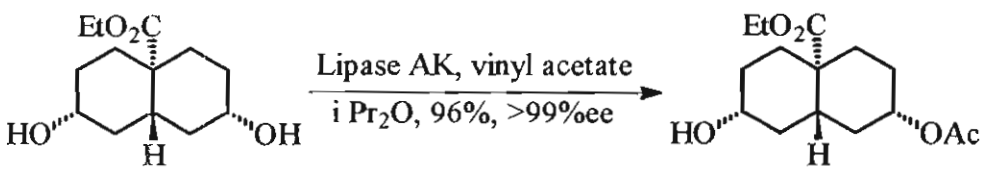
Scheme IV



Reagents : a. $SnCl_4$, CH_2Cl_2; b. $NaBH_4$, MeOH; c. (R)-1-(1-naphthyl)ethyl--isocynate; d. $HSiCl_3$, Et_3N, benzene; e. $LiAlH_4$; f. (i) p-TsCl, py.; (ii) Jone's reagent; g. DBU; h. Mg, 1-bromo-2,5-dimethoxybenzene, CuI; Ac_2O, KOH; i. (i)$Ph_3P{=}CH_2$;(ii) n-BuSLi, HMPA.

No efforts have been made towards the synthesis of optically pure isozonarol **65** and zonaroic acid **66.**

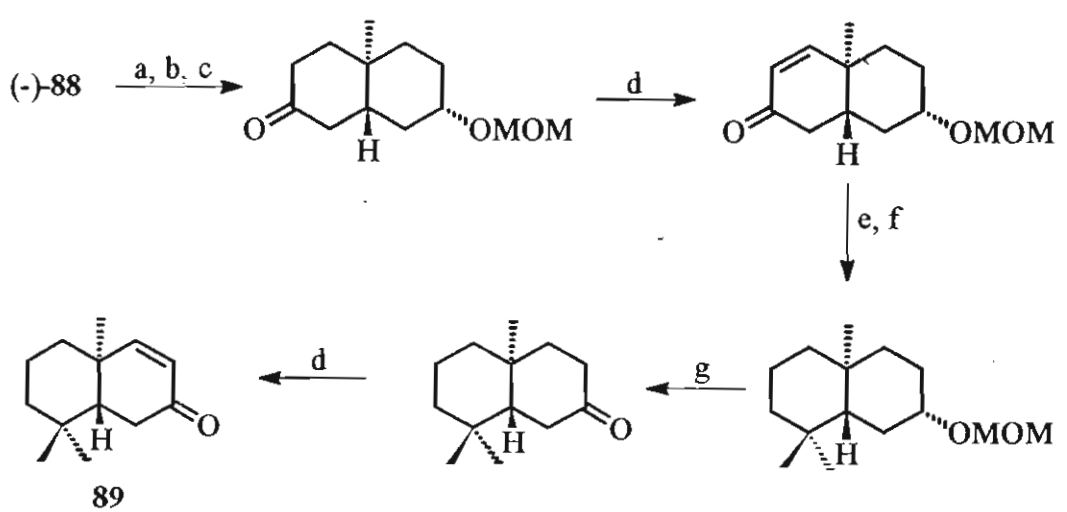
Recently, Momose *et al.*[51] have synthesised the chiral bicyclic enone **89**, an intermediate enroute to Polygodial **59** and Warburganal **60**. Eventhough the approach is lengthy, the enzymatic step is highly efficient. In their approach chiral bicyclo[4.4.0]decane system (**88**) was synthesised



87 **(-)-88**

through enantioselective transesterification of the mesodiol **87**. The key intermediates in their synthesis are depicted in Scheme V.

Scheme V



89

Reagents: a. (i) MOMCl, Hunig base; (ii) K_2CO_3; (iii) TBSCl, Et_3N, DMAP;

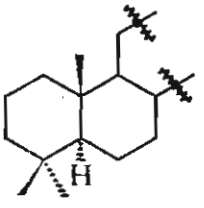
b. (i) $LiAlH_4$; (ii) I_2, Ph_3P, imidazole; (iii) Zn, AcOH; c. (i) TBAF; (ii) PCC; d. LDA, TMSCl then $Pd(OAc)_2$; e. (i) LDA, MeI; (ii) LDA, MeI; (iii) H_2, 5% Rh/C; f. (i) $TsNH.NH_2$, $BF_3.Et_2O$; (ii) MeLi; (iii) H_2, 5% Pd/C g. (i) HCl, MeOH, (ii) PCC.

It is thus clear that the use of enzymes for the enantioselective synthesis of biologically active natural products has begun to attract the attention of chemists around the world.

1.3 OBJECTIVE OF THE PRESENT INVESTIGATION

Part I of the foregoing literature survey makes it clear that while myriad examples exist in literature dealing with the use of hydrolases for the synthesis of pharmaceutical intermediates, they have not been deployed extensively for the synthesis of terpenoid natural products. In Part II of the same chapter, we have surveyed the existing approaches to the asymmetric synthesis of the biologically active natural products Polygodial **59,** Warburganal **60,** Albicanyl acetate **62,** Coronarin E **63** and Zonarol **64.** Because of the current interest in the synthesis of **59-64** and our fascination for biotransformations, the idea of exploiting the wide scope and applicability of the latter for the asymmetric synthesis of such natural products appealed to us. The first programme we set out to do was seek points of convergence in a retrosynthetic manner. Thus a quick glance at structures **59-64** revealed their

most striking feature *viz.*, the commonality of the bicyclo[4.4.0] ring system with three pendant methyl groups either as in core **57** or **58.** Identifying this



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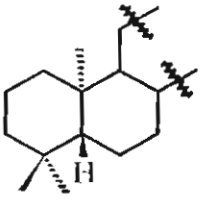

58

structure rapidly helped in crystallising our ideas and our aim, and efforts therefore, focussed on developing methods for the synthesis of suitably functionalised optically active intermediates enroute to **59-64** contaning the units **57** or **58** using biocatalysts in key reactions. The route we followed is discussed in the rest of the thesis.

1.4 REFERENCES

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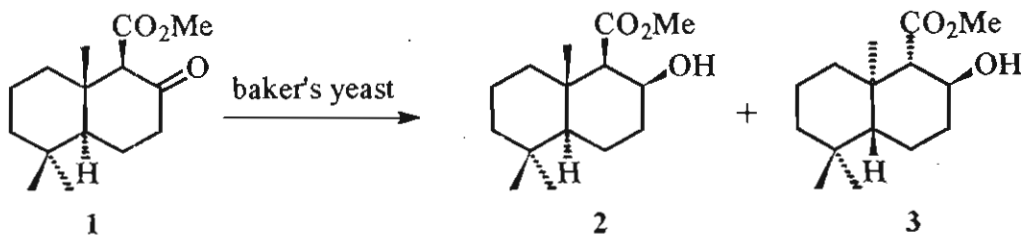
CHAPTER II

INITIAL STUDIES USING BAKER'S YEAST FOR CHIRAL REDUCTION

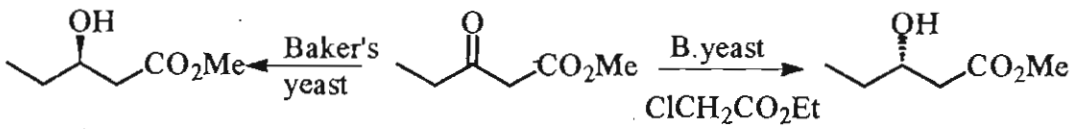
2.1 INTRODUCTION

As already mentioned in the last chapter our aim was to synthesise suitably functionalised chiral intermediates containing the bicyclo[4.4.0] ring system with the help of enzymatic techniques. As any other novice to the field of biotransformations is wont to do, we too at first, studied the literature on the use of baker's yeast.[1] Baker's yeast, *Saccharomyces cerevisiae* has been used widely for the chiral reduction of carbonyl groups and the reduction of β-ketoesters to chiral β-hydroxy esters proceed in general with good enantioselectivity. Familiarity with the literature on drimane natural products, prompted us to consider the β-ketoester **1** containing the required bicyclo[4.4.0] ring system as a possible substrate for baker's yeast mediated reduction. Since, in general, such reductions lead to products with *S*-configuration at the newly generated hydroxyl centre, **1** was expected to lead to diastereomers **2** and **3**, which should be readily separable.



1 2 3

The reduction of the sterically crowded β-ketoester **1** with various strains of yeasts like *Saccharomyces bailii* KI 0116, *S. bailii* IFO 0468, *S. bailii* IFO 0488, *S. bailii* IFO 1091, *S. bailii* IFO 1611, *S. bailii* IFO 1801, and *S. cerevisiae* had not yielded satisfactory results in Mori's hands.[2] However, we were buoyed up by several newer findings on the use of baker's yeast. For example, Nakamura has shown that the introduction of certain additives like ethyl chloroacetate and methyl vinyl ketone to the reaction medium can alter the enantioselection process considerably.[3] The yeast reduction of β-ketoesters is undertaken by a complex of dehydrogenases that afford L or D - hydroxyl esters. Additives such as ethyl chloroacetate and methyl vinyl ketone selectively deactivate certain dehydrogenases thus amplifying the enantioselectivity of others and leading to products of higher optical purity. The example shown below is demonstrative.

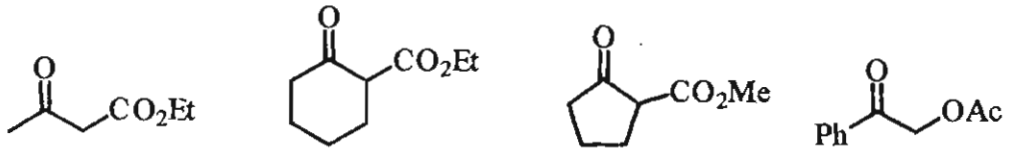


D-Hydroxy ester
12% ee

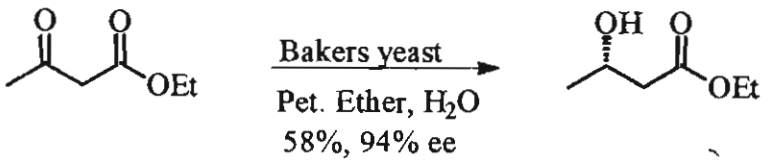
L- Hydroxyester
69% ee

Usual bioreduction using baker's yeast is carried out in aqueous glucose or sucrose. The actual reducing agent, NADPH is regenerated from $NADP^+$ through hexose monophosphate pathway for glucose oxidation in yeast cells.

Kometani *et al.*[4] have shown that NADPH could be regenerated through the oxidative pathway of ethanol in the presence of oxygen. Thus large quantities of aqueous glucose/sucrose can be replaced with a smaller amount of EtOH in water, thus making the reactions easier to handle. They have successfully carried out the baker's yeast mediated asymmetric reduction of various β-ketoesters and ketones shown below by this method.



A major problem faced with β-ketoester reductions with baker's yeast is the insolubility of the substrate in aqueous medium. In an interesting report, Smallridge *et al.*[5] have addressed this problem. They have carried out the conversion of ethyl acetoacetate to *S*-ethyl-3-hydroxybutyrate by

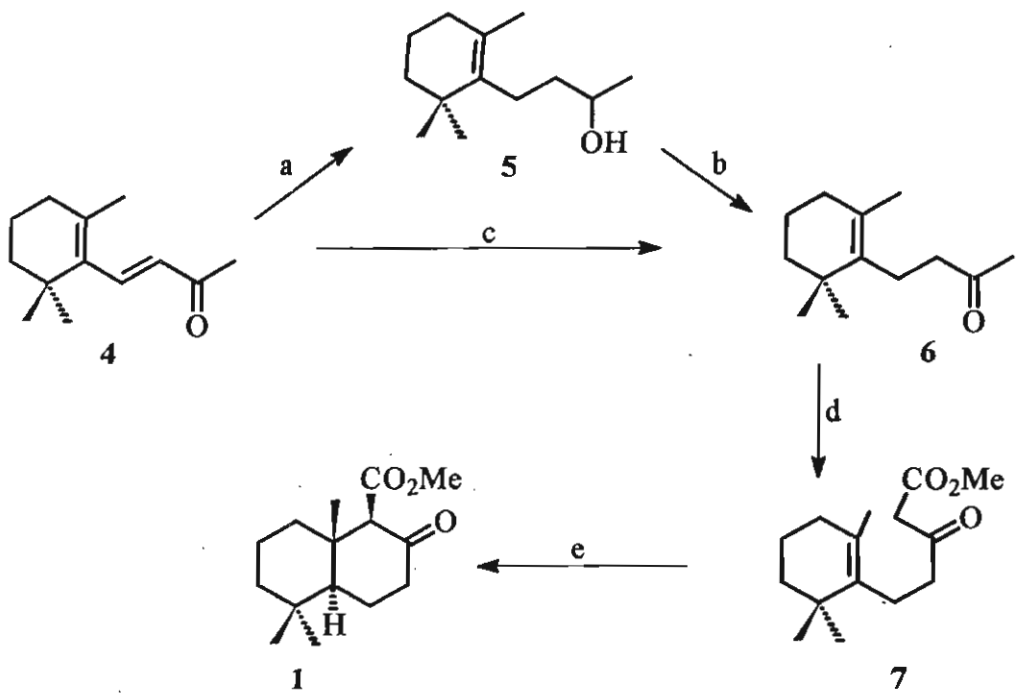


the use of freeze dried baker's yeast in petroleum ether containing a trace amount of water.

2.2 RESULTS AND DISCUSSION

In order to try these new methods, we synthesised **1** from readily available β-ionone **4**. Initially, due to our laboratory limitations, **4** could be converted to dihydro β-ionone **6** in a two step manner only, *i.e.*, through 1,4-reduction using sodium in ethanol followed by pyridinium chlorochromate

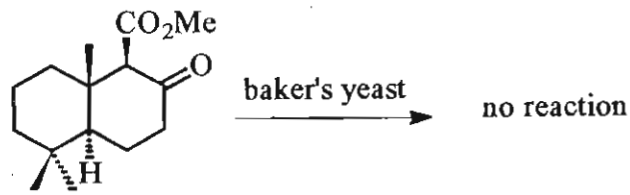
oxidation. Later, we utilized the Pd/C-ammonium formate in MeOH mileu with **4** for obtaining this in one step.[6] **6** was converted to the monocyclic β-ketoester **7** by condensation with dimethyl carbonate and sodium hydride. The required β-ketoester **1** was then obtained through stannic chloride mediated cyclisation of **6** as reported earlier.[7]



Reagents and conditions: a. Na, EtOH, 10°C-rt, 65%; b. PCC, CH_2Cl_2, 93% c. HCO_2NH_4, Pd on C, MeOH, rt, 52%; d. NaH, $(MeO)_2CO$, THF, reflux, 66%; e. $SnCl_4$, CH_2Cl_2, 5°C-20°C, 66%

First, the β-ketoester **1** was dissolved in a small amount of ethanol and fed to fermenting baker's yeast *viz.*, *Saccharomyces cerevisiae* in the presence of aqueous sucrose. Active culture was maintained by the addition of further yeast and aqueous sucrose for a period of 48 h. The total reaction

mixture was then centrifuged and the aqueous layer extracted with methylene chloride. Unfortunately, **1** was found unchanged.



1

Secondly, the Kometani modification, *i.e.*, subjecting the β-ketoester **1** to *S. cerevisiae* in water containing EtOH as the energy source was tried. Here again, **1** resisted reduction.

Equally disappointing was the effect of *S. cerevisiae* on **1** in petroleum ether solvent. It was then clear that *S. cerevisiae* would not reduce **1** even under the new conditions and that alternate methods would have to be sought for introducing chirality in this system.

2.3 EXPERIMENTAL

All melting points are uncorrected and were determined on a Buchi-530 melting point apparatus. IR spectra were recorded on a Perkin-Elmer Model 882 infrared spectrophotometer. ^{1}H NMR spectra were recorded on Hitachi-60, JEOL EX-90 and Bruker-200 NMR spectrometers and ^{13}C on JEOL EX 90 spectrometer using tetramethylsilane as internal standard. The mass spectra were recorded on a Hewlett Packard 5890 Series II GC connected to a 5890 mass selective detector. Elemental analysis were obtained using Perkin-Elmer model 240C-CHN analyser. All dry reactions

were conducted under atmosphere of argon or nitrogen. Reagents were transferred using standard syringe - septa techniques. All the solvents were distilled before use. THF and Et_2O were dried by distillation over sodium wire followed by sodium benzophenone ketyl. Dichloromethane was distilled from P_2O_5. Column Chromatography was done using 100 - 200 mesh silica gel.

Two types of Baker's yeast were utilised. One was obtained as dry granules from the market where as the other was obtained from M/s. Asian Bakers, Kerala, as wet cake. Experiments with Baker's yeast were carried out in triplicate.

Dihydro β- ionone (6)

(a) To a solution of β-ionone **4** (16 g, 83.3 mmol) in absolute ethanol (400 mL) at 10°C was added metallic sodium (12 g, 520 mmol) as small pieces, maintaining the same temperature throughout. The reaction mixture was stirred at 10°C for 3 h and further at rt till all the sodium had reacted. When the reaction was complete the mixture was cooled in an ice bath and diluted with water (100 mL). Ethanol was removed under reduced pressure and product extracted with CH_2Cl_2 (4 x 75 mL). The combined CH_2Cl_2 extracts were washed with aqueous 5% HCl, saturated aqueous $NaHCO_3$ solution and brine and dried over Na_2SO_4. After concentration crude product was purified on a silica gel column using 5% EtOAc in petroleum ether as eluent which furnished alcohol **5** (10 g, 65%).

IR (film)ν_{max} : 3424 (-OH), 2958, 1680 (>C=C<) , 1470, 1360 cm^{-1}.

^1H NMR (60 MHz, CCl_4) : δ 1.0 (s, 6 H), 1.1 (d, 3H, J = 6 Hz, $C\underline{H}_3$-HC-O), 1.55 (s, 3 H, >C=C-$C\underline{H}_3$), 1.15-2.3 (10 H), 2.65 (br s, 1 H, -O$\underline{H}$), 3.6 (m, 1 H, C-$\underline{H}$C-OH).

(b) To a well stirred suspension of pyridinium chlorochromate (12.2 g, 56.6 mmol) and 4Å molecular sieves (8 g) in dry CH_2Cl_2 (100 mL), was added dropwise, a solution of the hydroxy compound **5** (10 g, 51 mmol) in dry CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 2 h, diluted with ether (100 mL) and filtered through a silica gel column. Removal of the solvent furnished dihydro β-ionone **6** (9.2 g, 93%) which was used as such for the next reaction.

IR (film)ν_{max} : 2957, 2875, 1720 (>C=O), 1674(>C=C<), 1476 cm^{-1}.

^1H NMR (60 MHz, CCl_4) : δ 0.95 (s, 6 H), 1-2.4 (m, 10 H), 1.5 (s, 3 H, C=C-$C\underline{H}_3$), 2.0 (s, 3 H, $C\underline{H}_3$CO).

Alternate procedure for the synthesis of Dihydro β -ionone (6)

To a solution of β-ionone **4** (4.13 g, 22.45 mmol) in dry MeOH (40 ml) were added ammonium formate (8.49 g, 134.68 mmol) and 10% Pd-C (200 mg) and vigorously stirred at room temperature for 2 h. The catalyst was filtered through a short plug of silica gel. The reaction mixture was

diluted with water and extracted with CH_2Cl_2 (3 x 50 ml). The combined extracts were washed with water and brine and dried over Na_2SO_4. Solvent was removed and the residue purified by chromatography over silica gel using 1% EtOAc in petroleum ether as eluent, to furnish **6** (2.2 g, 52%).

Methyl 3-oxo-5-(2',6',6'-trimethylcyclohex-1'-enyl) pentanoate (7)

To a well stirred suspension of sodium hydride (50% suspension in mineral oil, 3.96 g, 82.5 mmol, washed twice with hexane) in dry THF (70 mL) under N_2, was added dimethyl carbonate (7.4 g, 7 mL, 82.5 mmol) and refluxed for 1 h. After cooling the mixture, a solution of dihydroionone **6** (8 g, 41.2 mmol) in dry THF (15 mL) was added and refluxed further for 2 h. The reaction mixture was then cooled to rt, poured into ice-water containing acetic acid (10 mL) and extracted with ether (3 x 50 mL). Combined ether extracts were washed with saturated aqueous $NaHCO_3$ solution, water and brine and dried over Na_2SO_4. Removal of the solvent and purification of the residue on silica gel column using 2% EtOAc in petroleum ether as eluent furnished ketoester **7** (6.85 g, 66%) as an oil.

IR (film)ν_{max} : 2936, 2872, 1758 (-COOMe), 1725 (>C=O), 1660(>C=C<), 1634, 1441 cm^{-1}.

^1H NMR (60 MHz, CCl_4) : δ 1.0 (s, 6 H), 1.6 (s, 3 H, C=C-C$\underline{H}_3$), 1.1-2.75 (10 H), 3.3 (s, 2 H, (O)C-C$\underline{H}_2$-C(O)-), 3.65 (s, 3 H, -OC$\underline{H}_3$).

Methyl 5,5,8a-trimethyl-2-oxodecahydronaphthalene-1-carboxylate (1)

To a solution of ketoester 7 (6.8 g, 27 mmol) in CH_2Cl_2 (75 mL) maintained at 5°C, was added $SnCl_4$ (8.5 g, 3.8 mL, 32.6 mmol) dropwise and stirred at 5°C for 30 mts and further at 20°C for 8 h. The reaction was quenched with cold water, CH_2Cl_2 layer was separated and aqueous layer extracted further with CH_2Cl_2 (3 x 25 mL). The combined organic extracts were washed with water, brine and dried over Na_2SO_4. After removing the solvent the crude product was chromatographed on a silica gel column using 5% EtOAc in petroleum ether as eluent which yielded β-ketoester **1** (4.48, 66%), mp. 86-88°C (hexane) (lit[2] 85 - 87 °C).

IR (KBr)ν_{max} : 2951,2856,1750($COOCH_3$),1710 (>C=O), 1468, 1351, 1168, 1042 cm^{-1}.

^1H NMR (60 MHz, CCl_4) : δ 0.9 (s, 3 H), 0.95 (s, 3 H), 1.1 (s, 3 H), 1.15-2.5 (11 H), 3.0 (s, 1H, O-C(O)-C<u>H</u>-C(O)-), 3.5 (s, 3 H, -OC<u>H</u>$_3$).

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 14.6, 18.5, 21.8, 23.0, 33.5, 39.0, 41.4, 42.0, 51.4, 53.2, 69.9, 168.8, 205.5.

Attempted Baker's yeast reduction of β-ketoester 1

Trial 1

A mixture of sucrose (3 g) and baker's yeast (2 g) in water (20 mL) was stirred at ~30°C for 30 mts. To this mixture was added a solution of β-ketoester **1** (102 mg, 0.4 mmol) in ethanol (0.2 mL) and the fermenting suspension was stirred for 24 h. A second lot of baker's yeast (2 g) and sugar solution (3 g in 20 mL water) was then added and stirred for another 24 h at room temperature. The mixture was then centrifuged, aqueous layer was saturated with NaCl and extracted with CH_2Cl_2 (6 x 15 mL). Combined extracts were dried over Na_2SO_4 and concentrated. Starting material was recovered unchanged.

Trial 2

A solution of β-ketoester **1** (102 mg, 0.4 mmol) in ethanol (0.2 mL) was added to a suspension of baker's yeast (2 g) in water (30 mL) containing ethanol (0.2 mL). The mixture was stirred at ~30°C for 48 h and worked up as before. Starting material was recovered unchanged.

Trial 3

β-Ketoester **1** (102 mg, 0.4 mmol) was added to a suspension of pulverised baker's yeast (1 g) in petroleum ether (50 mL) containing water (0.8 mL). The mixture was stirred at room temperature for 24 h and filtered. Filtrate was concentrated. Only starting material was obtained.

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CHAPTER III

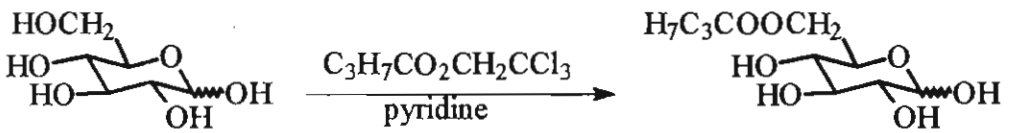
LIPASE CATALYSED TRANSESTERIFICATION STUDIES ON 1-HYDROXYMETHYL-2-HYDROXY-5,5,8a-TRIMETHYLDECAHYDRONAPHTHALENE

3.1 INTRODUCTION

Following the difficulty we faced in obtaining chiral intermediates of our interest with the help of Baker's yeast, attention was turned to the use of commercially available lipases. By this time, the synthetic potential of enzymes in organic solvents was already well recognised and a number of publications on this topic had appeared.[1] The majority of these publications dealt with enantioselective lipase catalysed esterification and transesterification. Among these were two seminal findings that opened up new horizons in the use of such biocatalysts. The first was the finding by Maillard[2] in 1987 and by Wong[3] in 1988 that, through the use of enol esters as irreversible acylating agents, the two often encountered problems in enzyme catalysed reactions *viz.* slow reaction rate as well as consequent loss of selectivity, due to the reversible nature of such reactions, could be overcome.[4]

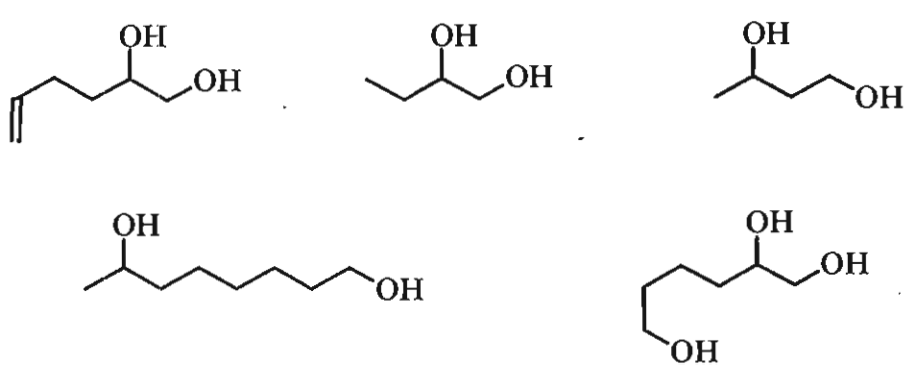
The second valuable finding was that regio- and sometimes, even enantioselectivity could be obtained in enzyme catalysed transesterification of 1,2 and 1,3-diols containing both primary and secondary hydroxyl sites. This potential was recognised early by Klibanov *et al.* who demonstrated the use

of PPL for regioselective monoacylation of various glycols in ethyl carboxylates (used both as solvent and acylating agent).[5] The same group has also successfully carried out regioselective acylation of the primary hydroxyl group of carbohydrates as shown below.[6]

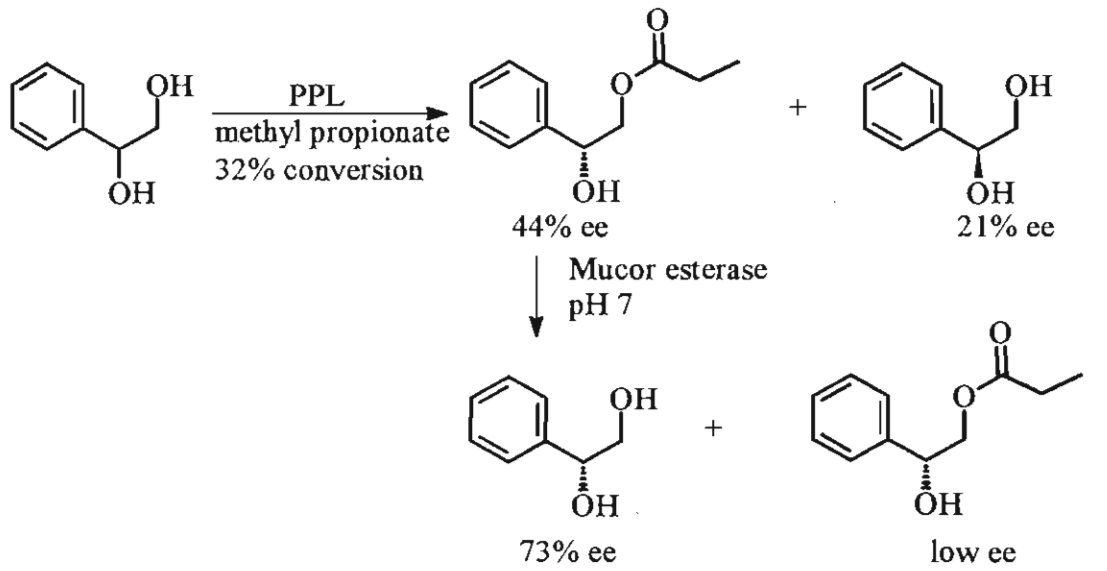


Interestingly, these reactions have been carried out by PPL in pyridine or dimethyl formamide since the unprotected sugars were soluble only in few such hydrophilic solvents. This type of regioselectivity has been further established in numerous furanose and pyranose derivatives by Wong *et al.*[7]

Oehlschlager has shown that by using PPL with acetic or butyric anhydride as acylating agent, high yields of primary acylated products can be obtained.[8] A systematic investigation of this method using various 1,n-diols and triols shown below revealed the method to be general.

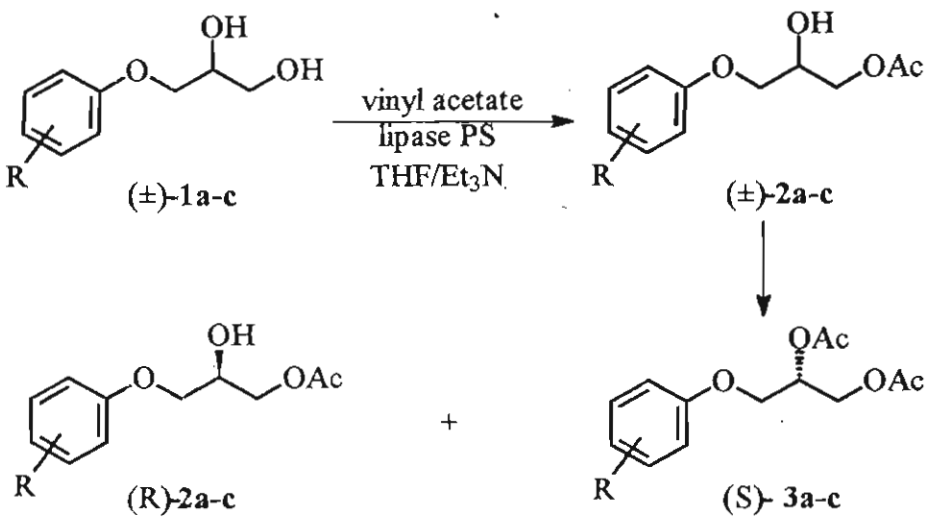


Of particular interest to us was the Zwanenburg[9] paper on the tandem use of enzymes. Depicted below is their sequence of reactions. Here, the



diol on treatment with PPL in methyl propionate proceeded to give the monoacetate through regioselective acylation at the primary site. While this resulted only in moderate enantioselectivity, hydrolysis of this product with a second enzyme *viz*. Mucor esterase, gave the product of high optical purity. Thus, in a two step procedure high enantioselectivity was achieved.

Equally interesting was the Thiel disclosure that kinetic resolution of acyclic 1,2-diols like the pharmaceuticals Mephenesin, Guaifenesin and Chlorphenesin could be carried out using sequential lipase catalysed transesterifications.[10] As depicted here, transformations were carried out

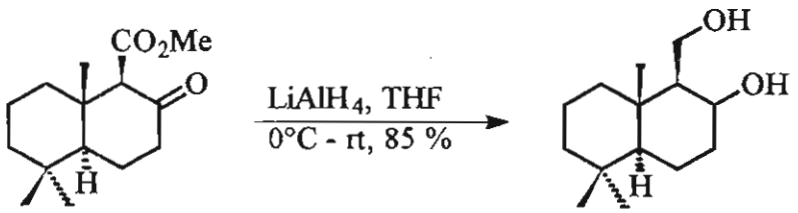


1a R = 2-Me, Mephenesin
1b R = 4-Cl ,Chlorphenesin
1c R = 2-OMe, Guaiphenesin

on(±)-**1a**-c with vinyl acetate in the presence of lipase PS in tetrahydrofuran-triethyl amine until ~50% of the fastly formed primary monoacetates (±)-2a-c were converted enantioselectively into the diacetates **3a-c**. The *S*-enantiomers of the primary monoacetates (±)-**2a-c** were converted at a higher rate into the *S*-diacetates **3a-c**. The corresponding *R*-enantiomers are slow reacting and resist further diacetylation to a greater extent. This procedure is especially attractive since only one enzyme is employed.

3.2 RESULTS AND DISCUSSION

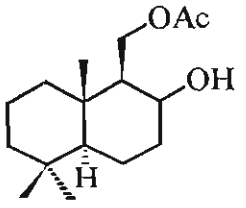
Having gained some insight into such enzyme catalysed site-selective modification of polyfunctional organic compounds, we felt that our next attempt should be aimed at lipase catalysed enantioselective acylation of a diol containing the required bicyclo[4.4.0] ring system. Such a system **5** was obtained without much ado through the reduction of the β-ketoester **4** with lithium aluminium hydride as reported earlier.[11]



4 5

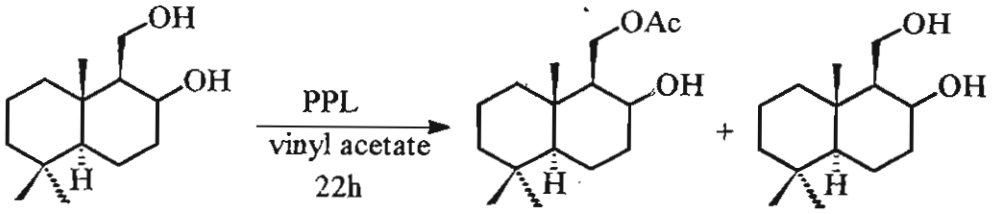
Due to the ready availability and affordable price of PPL, we carried out our initial experiments for selective biotransformation with this enzyme. The use of PPL with alkyl carboxylates such as methyl propionate, ethyl acetate, chloroethyl acetate *etc.* as effective acyl transfer agents for achieving regioselective acylation has been established.[9] The diol **5** was treated likewise. However, even after stirring with PPL for several days in ethyl acetate, methyl propionate or chloroethyl acetate, the substrate **5** was unchanged. Thus, it was clear that more reactive acylating agents were necessary. The diol **5** was then treated with PPL and vinyl acetate in various solvents such as ethyl acetate, tetrahydrofuran, acetonitrile and ether. Again, no discernable amount of acetylated material was obtained.

In an effort to force the reaction to go forward, **5** was treated with PPL in a larger quantity of vinyl acetate (as acylating agent as well as solvent). On observing the reaction, almost 50% conversion was found to occur after 22 h. The lipase was removed by filtration and the product rapidly purified on a silica gel column. The spectral data of the product clearly indicated that monoacetylation had taken place at the primary hydroxyl site to give **6.**



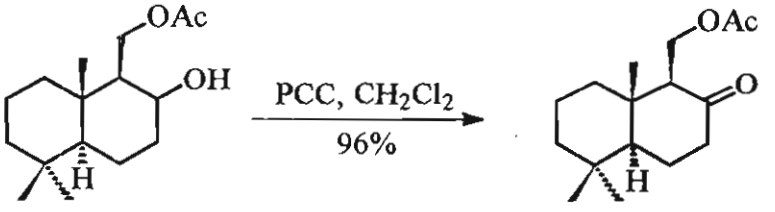
(±)-6

^{1}H NMR and ^{13}C NMR of **6**



(±)-**5** **6** **5**

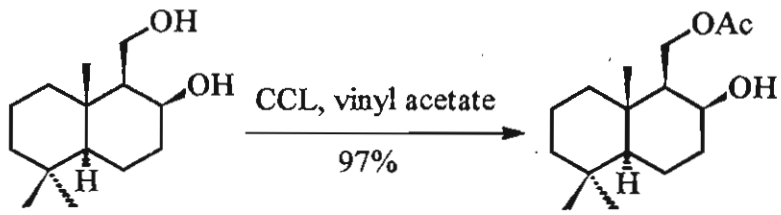
To probe the enantioselectivity of the reaction using ^1H NMR it was necessary to convert **6** to a product that would complex with a chiral nmr shift reagent. For this purpose, **6** was oxidised with pyridinium



(±)-**6** (±)-**7**

chlorochromate to the keto acetate **7** and was fully characterised using IR, ^1H NMR and ^{13}C NMR spectra. Additional nmr spectra were recorded with different concentrations of $Eu(hfc)_3$ as the chiral shift reagent. However, **7** was found to contain a near equal mixture of enantiomers.

When PPL failed to demonstrate enantioselectivity, the diol **5** was treated with CCL(Aldrich) in vinyl acetate. To our surprise, complete acylation occured rapidly in 3 h to give 97% of the monoacetate **6**. This CCL from Aldrich Chemical Co. had an activity of 700 - 1500 U/mg.



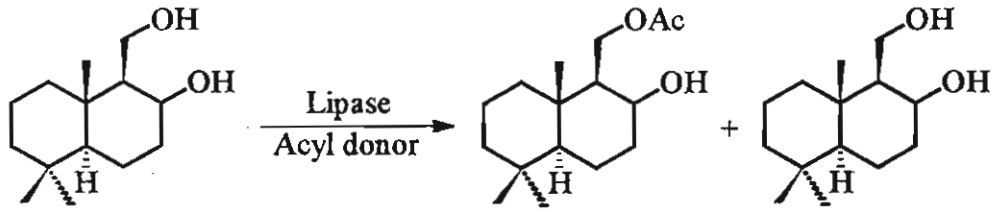
(±)-5 (±)-6

In order to check whether CCL(Aldrich) would catalyse the enantiospecific acylation, the reaction was slowed down by limiting the volume of vinyl acetate. Thus **5** was treated with CCL in ethyl acetate containing 4% of vinyl acetate which resulted in 47% conversion to the monoacetate **6** after 18 h.

Once again, this product was oxidised with PCC and the ^{1}H NMR of the keto acetate **7** thus obtained examined after complexation with Eu(hfc)$_3$. No *enantioselectivity* was observed. However, on acylation catalysed by CCL(Fluka) in isopropenyl acetate ~ 40% conversion occured after 4 days with the product showing a meagre 10% enantiomeric excess. This CCL from Fluka Chemical Co. had an activity of 24.2 U/mg.

In addition to these, the lipase catalysed acylation of the diol **5** was examined with different lipases and under a variety of solvent systems. Details of these are summarised in Table I.

Table 1



5 6 5

Entry[a]	Lipase	Acyl donor/ Solvent	Time h	Ester 6 % y[b] (% ee)[c]	Diol 5 %y
1	PPL	Methyl propionate	73	no reaction	*
2	PPL	Ethyl acetate	138	no reaction	*
3	PPL	Chloro ethyl acetate	72	no reaction	*
4	PPL	Vinyl acetate	22	38 (4)	50
5	PPL	Vinyl acetate/ t-BuOH	72	no reaction	*
6	PPL	Vinyl acetate/ THF	168	no reaction	*
7	PPL	Vinyl acetate/ CH_3CN	244	no reaction	*
8	PPL	Vinyl acetate/ THF. Et_3N	72	no reaction	*
9	CCL (Aldrich)	vinyl acetate	3	97 (0)	

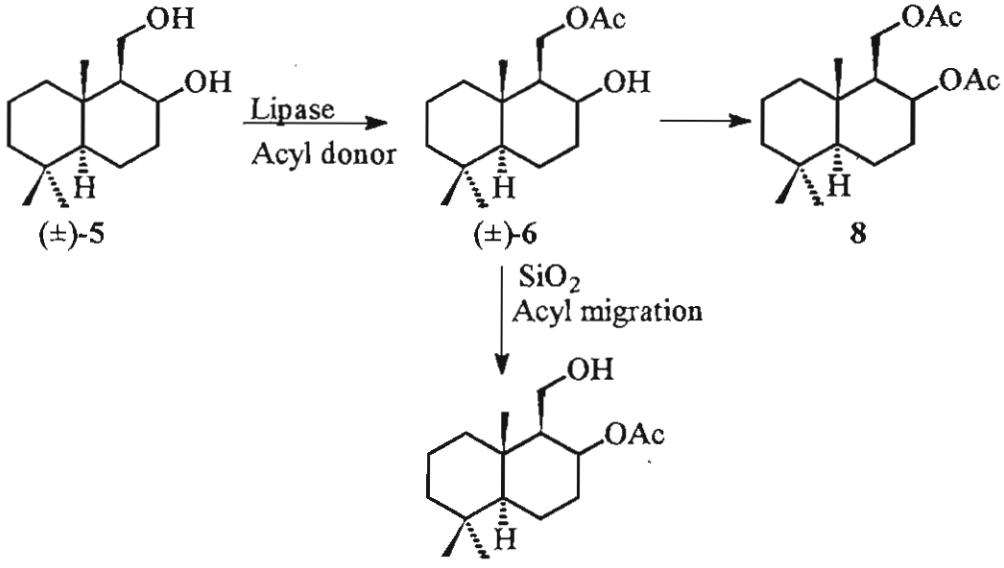
Table I (contd)

10	CCL (Aldrich)	Ethyl acetate	48	no reaction	*
11	CCL (Aldrich)	Methyl propionate	48	no reaction	*
12	CCL Aldrich)	Vinyl acetate/ Ethyl acetate	18	47 (5%)	34
13	CCL (Aldrich)	Vinyl acetate/ THF-Et_3N	48	no reaction	*
14	CCL	Vinyl acetate/ Et_2O - THF	20	40 (5)	50
15	WGL	Vinyl acetate	48	no reaction	
16	SAM 2	Vinyl acetate	336	34 (0)	50
17	CCL (Fluka)	Isopropenyl-acetate	96	40 (10)	58

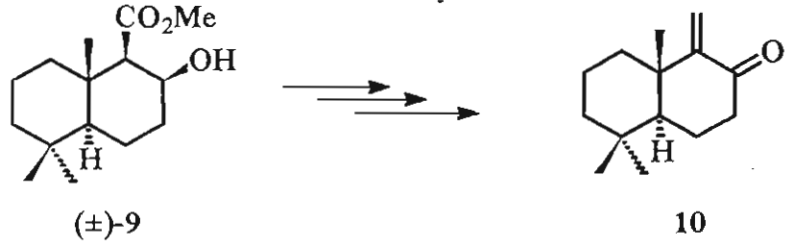
*Almost complete recovery of starting diol. [a]All the reactions were carried out in triplicate. [b] Isolated yield after column chromatography. [c] Determined by ^{1}HNMR in the presence of $Eu(hfc)_3$.PPL , Type II with activity 110-220 U/mg and CCL, Type VIII with activity 700 - 1500 U/mg were purchased from Aldrich. CCL (Fluka) with activity 24.2 U/mg and SAM 2 (Pseudomonas fluorescens lipase) with activity 31.5 U/mg were purchased from Fluka. WGL (Wheat germ lipase) with activity 9.5 U/mg was purchased from Sigma. y-Yield

In any of the reactions mentioned above, the formation of diacetate **8** was not observed. However, the monoacetate **6** was found to undergo 1,3-transacylation on keeping at room temperature for long periods or a few hours on a silica gel column. Therefore, our efforts to follow the Thiel procedure[10]

of carrying out sequential acylation of the first formed monoacetate **6** to give diacetate **8** perhaps with higher optical activity, had to be abandoned.

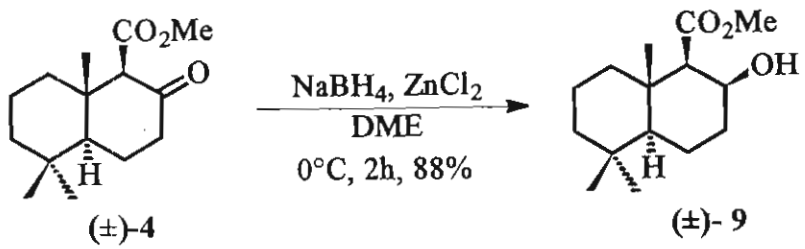


In general, lipase catalysed acylation of secondary hydroxyl groups are found to be more enantioselective than primary, since the enzyme binding takes place at the site nearer to the centre of chiral induction. The lipase catalysed acylation of the β –hydroxy ester **9** therefore, appeared to be worth examining , especially, as its chemical resolution has been used by Mori *et al.* for the synthesis of the enone **10** in optically pure form.[11]



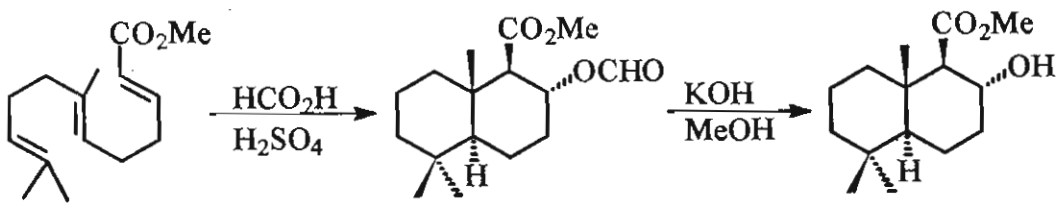
For this purpose, (±)-**9** has been synthesised by the same group through the reduction of **4** with $NaBH_4$ in MeOH at -60 to -40°C. We found that $Zn(BH_4)_2$ prepared *in situ* readily reduced **4** at 0°C in DME within 2h to

yield 88% of the 2β -hydroxy compound **9**. The structure of (±)-**9** was confirmed based on its spectral data comparison with that reported earlier.[11a]



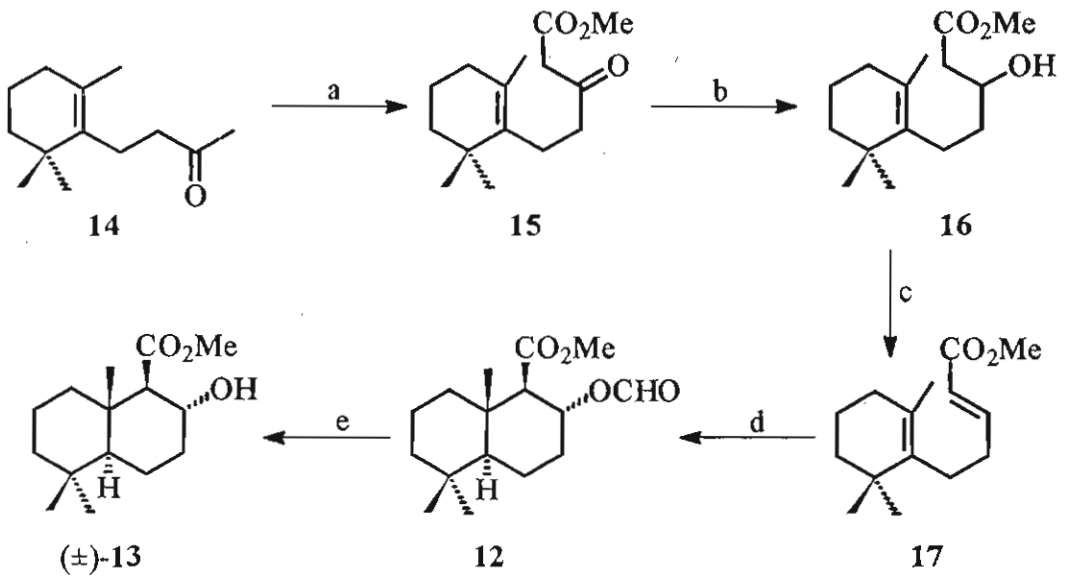
(±)-**4** (±)- **9**

Simultaneously, the synthesis of the 2α-hydroxy compound **13** was also undertaken. (±)-**13** has been synthesised earlier by Liapis *et al.*[12] from **11** through the biomimetic type cyclisation method developed by Eschenmoser *et al.*[13] as shown below.



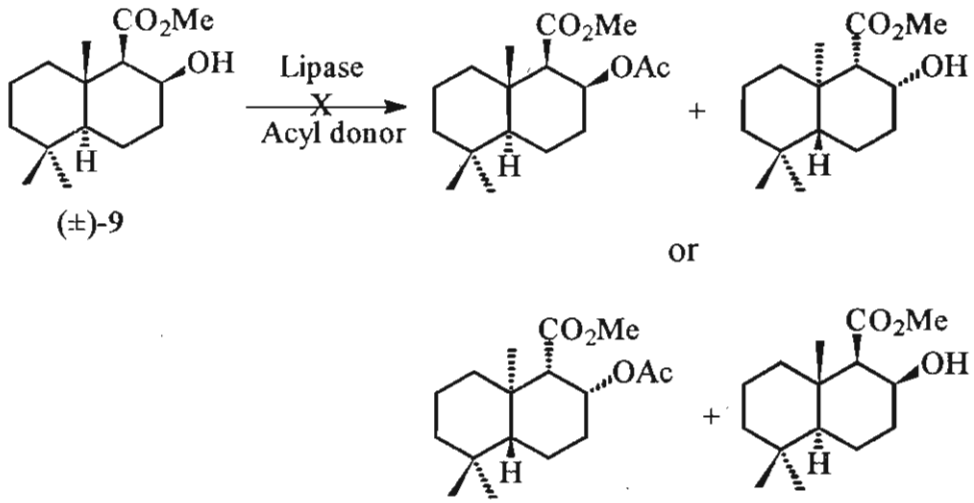
11 (±)-**12** (±)-**13**

We utilized dihydro-β-ionone **14** since it was readily available and converted it to the monocyclic olefinic ester **17** through intermediacy of compound **15** and **16** as shown below. Once again the cyclisation method developed by Eschenmoser[13] readily yielded **13** through the intermediate formate ester **12** as shown below.



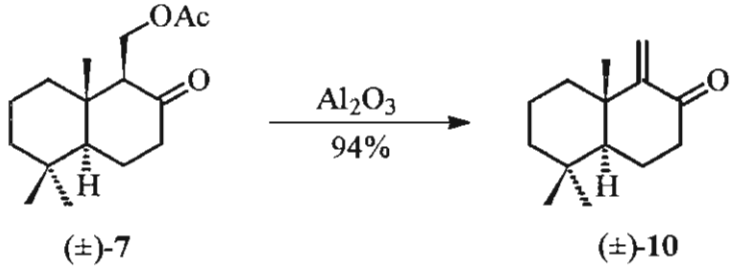
Reagents and conditions: a. NaH, $(MeO)_2CO$, THF, reflux, 66%; b. $NaBH_4$, $ZnCl_2$, DME, 72% ;c. (i) MsCl, Hunig base, . CH_2Cl_2, 0 -30 °C, 79%; (ii) DBU, Benzene, 60°C, 70%; d. HCO_2H , H_2SO_4, 0 -30°C 53%; e. NaOH, MeOH, 78%

Having obtained hydroxy esters **9** and **13**, enzyme catalysed transesterifications were attempted. Unfortunately the following conditions, *viz.*, (i) hydroxy alcohol **9**, CCL and vinyl acetate; stirred at 30°C for 4 days (ii) **9** , PPL and vinyl acetate; stirred at 30°C for 5 days (iii) **9** , PFL and vinyl acetate; stirred at 30°C for 5 days (iii) **13,** CCL and vinyl acetate; stirred at 30°C for 5 days, all failed to yield any acetylated product.



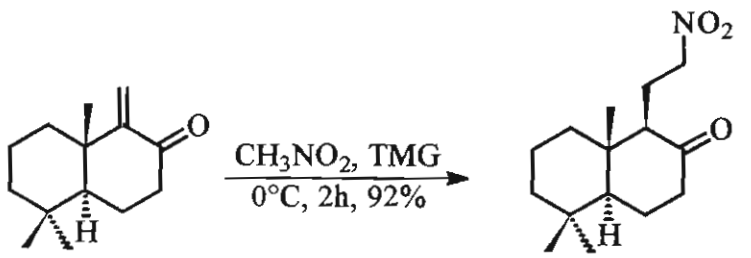
Thus it was clear that using the enzymes available with us, substrate **5** alone would undergo acylation, and that too only with regioselectivity, but not enantioselectivity. Still this result *per se* was quite useful within our overall objective of constructing versatile intermediates enroute to the natural products of our interest (see Fig. I , Chapter I) , albeit without optical purity.

An intermediate of great versatality that has been utilized for the synthesis of *Zonarol*[11a] (Fig. I, Chapter I), *Ambrox*® [14] *etc.* is the enone **10.** Interestingly, we observed that the ketoacetate (±)-**7** on adsorption on a neutral alumina column for 2h followed by elution with 20% ethyl acetate in petroleum ether would readily furnish the enone (±)-**10** in 94% yield. Its structure was confirmmed based on IR, ^{1}H NMR and ^{13}C NMR spectral data comparison with that reported in literature.[11]



(±)-7 (±)-10

Next an intermediate **18** with wider applicability, especially suitable for elaboration to *Coronarin E* (See Fig. I, Chapter I) was synthesised through the addition of nitromethane to the enone **10.** The reaction, carried out under argon and in the presence of tetramethyl guanidine at 0°C readily resulted in addition from the less hindered face, thereby yielding 92% of (±) **18** where nitromethyl and the angular methyl groups are *cis* to each other.



(±)-**10** (±)-**18**

The IR spectrum of **18** contained absorption at 1716 cm^{-1} due to carbonyl and at 1554 cm^{-1} and 1378 cm^{-1} due to the $-CH_2-NO_2$ group. The ^1H NMR spectrum exhibited a characteristic multiplet centered at δ 4.3 due to the $-C\underline{H}_2-$ proton adjacent to the nitro group. Its ^{13}C NMR exhibited 15 signals including the signals at δ 210.7(C=O) and at δ 74.7 due to $C\underline{H}_2NO_2$. The mass spectrum clearly showed m/z 267 for the molecular ion peak thus confirming the structure **18.**

3.3 EXPERIMENTAL

For a general section see experimental part of Chapter II

PPL , Type II with activity 110-220 U/mg and CCL, Type VIII with activity 700 - 1500 U/mg were purchased from Aldrich. CCL (Fluka) with activity 24.2 U/mg and SAM 2 (Pseudomonas fluorescens lipase) with activity 31.5 U/mg were purchased from Fluka. WGL (Wheat germ lipase) with activity 9.5 U/mg was purchased from Sigma. Lipase AY "Amano" 30 (from Candida rugosa) with activity 30 U/mg and Lipase PS "Amano" (from Pseudomonas cepacia) with activity 30 U/mg were obtained from Amano Pharmaceutical Co., Japan.

Optical rotations were recorded on JASCO DIP-370 digital polarimeter in $CHCl_3$, where concentrations are expressed in g / 100 mL.

1-Hydroxymethyl-2-hydroxy-5,5,8a-trimethyldecahydronaphthalene-(5)

To a well stirred suspension of $LiAlH_4$ (360 mg, 9.52 mmol) in dry THF (60 mL) cooled to 0°C, was added dropwise a solution of ketoester **4** (1.2 g, 4.76 mmol) in dry THF (10 mL). After the addition was over, the mixture was stirred at room temperature for 4 h and excess $LiAlH_4$ was then destroyed by careful addition of 2mL of water-THF mixture (10 : 90) at 0°C. The precipitate formed was filtered off and washed with THF. Combined filtrate was dried over anhydrous Na_2SO_4 and concentrated. The product readily recrystallized from ethylacetate and pure diol **5** (0.91 g, 85%) was obtained. mp.134-136°C (EtOAc), (lit.[11a] 132-134° C)

IR (KBr) ν_{max} : 3300 (-OH), 2926, 1034 cm^{-1}

^1H NMR (90 MHz, $CDCl_3$) : δ 0.88 (s, 6 H), 1.1 (s, 3 H), 1.1-2.2 (14 H), 3.85-4.3 (m, 3 H, -C<u>H</u>$_2$-O, -C<u>H</u>-O).

^{13}C (22.4 MHz, DMSO. d_6) : δ 17.6, 17.9, 19.0, 22.7, 34.0, 34.7, 36.0, 37.7, 40.4, 42.8, 56.5, 57.3, 58.6, 65.7.

PPL catalysed Transesterification of Diol (±)-5

1-[(Acetyloxy)methyl]-2-hydroxy-5,5,8a-trimethyldecahydro-naphthalene- (6)

To a solution of the diol (±)-**5** (500 mg, 2.2 mmol) in vinyl acetate (75 mL) was added PPL (1.25 g, Aldrich) and stirred at 28°C for 22 h. Lipase was then removed by filtration through a small layer of silica gel. Solvent was removed under reduced pressure and the residue purified by rapid chromatography on a silica gel column using 15% EtOAc in petroleum ether as eluent to furnish acetate **6** (190 mg, 32%) which was crystallised from CH_2Cl_2. mp. 70 -71 °C

IR (KBr)ν_{max} : 3553 (-OH), 2925, 1745 (>C=O), 1462,1267 cm^{-1}

^1H NMR (90 MHz, $CDCl_3$) : δ 0.85 (s, 3 H), 0.88 (s, 3 H), 1.04 (s, 3 H), 1.05-1.95 (12 H), 2.05(s, 3H,OC(O)-C<u>H</u>$_3$), 2.36 (br s, 1 H, -OH), 3.85-4.55 (m, 3 H, C<u>H</u>$_2$-OAc, -C<u>H</u>-O).

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 16.4, 16.9, 18.2, 21.0, 21.7, 33.2, 33.6, 34.6, 37.0, 39.5, 41.8, 53.0, 55.7, 62.0, 66.3, 171.8.

GC-MS m/z (relative intensity) : 208 (M^+-60, 15), 193 (25), 190 (20), 178 (27), 174 (25), 163 (30), 149 (30), 136 (40), 124 (68), 123 (58), 109 (100), 95 (72).

Further elution of the column gave unreacted diol **5** (250 mg, 50%).

CCL catalysed transesterification of Diol - (±)-5

A mixture of diol **5** (100 mg, 0.44 mmol) and CCL (Aldrich, 200 mg) in vinyl acetate (15 mL) was stirred at 30°C for 3 h and worked up as before. A rapid chromatography on silica gel using 15% EtOAc in petroleum ether as eluent furnished acetate **6** (115 mg, 97%).

Conditions for other transesterification studies are given in Table I

1-[(Acetyloxy)methyl]-5,5,8a-trimethyl-2-oxodecahydronaphthalene (7)

To a stirred suspension of PCC (195 mg, 0.9mmol) and 4Å molecular sieves powder (200mg) in dry CH_2Cl_2 (10mL) was added a solution of the hydroxy acetate **6** (200mg, 0.74mmol) in dry CH_2Cl_2 (5mL). After stirring for 30 minutes at rt , the mixture was diluted with Et_2O and filtered through a short column of silica gel. Removal of the solvent furnished pure keto acetate 7 (191mg, 96%).

IR (KBr)ν_{max} : 2979, 2931, 1744 (OCO-CH_3), 1723 (>C=O), 1464, 1374, 1249, 1045 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$) : δ 0.77 (s, 3 H), 0.86 (s, 3 H), 0.98 (s, 3 H), 1.2-1.8 (9 H), 2.0 (s, 3 H, -C(O)-C<u>H</u>$_3$), 2.05-2.6 (m, 3 H, -C(O)-C<u>H</u>-C, C(O)-C<u>H</u>$_2$-C), 4.2 (m, 2 H, C<u>H</u>$_2$-O-)

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 15.1, 18.6, 20.7, 21.4, 23.5, 33.3, 33.4, 38.9, 41.5, 41.7, 41.8, 53.6, 58.6, 62.1, 170.7, 209.1

Analysis
Calcd. for $C_{16}H_{26}O_3$: C, 72.14; H, 9.84

Found : C, 72.38; H, 9.85

GC-MS m/z (relative intensity) : 266 (M^+, 5), 206 (20), 191 (26), 178 (24), 173 (24), 163 (24), 136 (60), 128 (52), 122 (51), 95 (80), 85 (70), 55 (100)

Determination of the enantiomeric excess of (7)

A mixture of ketoacetate **7** (5mg) obtained from each of the reaction and (+)-Eu(hfc)$_3$ (21mg, 0.93mol equiv) was dissolved in $CDCl_3$(0.5 - 0.6 mL). After 5 - 8 minutes a ^1H NMR spectra were taken. The singlet at δ 2.0 due

to $C\underline{H}_3CO$ was shifted and split into a doublet showing a major peak at δ 3.62 and a slightly minor one at δ 3.76. The samples were thus found to be near racemic mixture.

(1β, 2β, 4aα, 8aβ) Methyl (±)- 2-hydroxy-5, 5, 8a-trimethyldecahydro-naphthalene-1-carboxylate (9)

To a stirred suspension of $ZnCl_2$ (974 mg, 7.16 mmol) in DME (10 mL) cooled to 0°C, was added $NaBH_4$ (543 mg, 14.3 mmol) and the mixture stirred for 40 minutes at 0°C. A solution of ketoester **4** (600 mg, 2.38 mmol) in DME (6 mL) was then added to the resultant $Zn(BH_4)_2$ reagent formed. The reaction mixture was further stirred at 0°C for 2 h and quenched by the addition of ice-water (1 mL). After diluting the mixture with ether (40 mL), the solid residue was filtered off and washed with ether. Combined organic phases were washed with water and brine and dried over anhydrous Na_2SO_4. Removal of solvent and purification of the crude product on a silica gel column using 5% EtOAc in petroleum ether as eluent furnished the alcohol 9 (532 mg, 88%). mp. 55- 56°C (MeOH/H_2O) (lit.[11a] 53-54°C)

IR (KBr)ν_{max} : 3501, 2959, 2953, 1706, 1440, cm^{-1}.

^1H NMR (60 MHz, CCl_4) : δ 0.86 (s, 6 H), 1.00-1.98 (11 H), 1.16 (s, 3 H), 2.09 (d, 1 H, J = 2 Hz, -$\underline{H}$C-CO_2Me), 3.53(br s, 1 H, -OH), 3.63(s, 3H, -OCH_3), 3.98 (m, 1 H, -$\underline{H}$C-O).

GC-MS m/z (relative intensity) : 254 (M^+, 7), 237 (16), 221 (40), 177 (15), 161 (33), 147 (27), 137(72), 123 (86), 116 (100), 109 (60).

Methyl 5- (2', 2', 6'-trimethylcyclohex-1'-enyl)-2-pentenoate (17)

(a) To a well stirred suspension of $ZnCl_2$ (160 mg, 1.18 mmol) in DME (5 mL) cooled to 0°C, was added $NaBH_4$ (90 mg, 2.36 mmol) and the mixture stirred at 0°C for 30 mts. A solution of the ketoester **15** (100 mg, 0.4 mmol) in DME (2 mL) was then added to the resultant $Zn(BH_4)_2$ reagent formed. The reaction mixture was further stirred at 0°C for 2 h and quenched by the addition of a few drops of cold water. Following dilution of the mixture with ether (20 mL), the solid residue was filtered off and washed with ether. Combined filtrate was washed with water and brine and dried over anhydrous Na_2SO_4. Solvent was removed and crude product chromatographed on silica gel column using 10% EtOAc in petroleum ether as eluent to give hydroxyester **16** (73 mg, 72%)

IR (film)ν_{max} : 3455 (-OH), 2933, 2872, 1740 (-COO), 1442, 1081 cm^{-1}.

^1H NMR (60 MHz, CCl_4) : δ1.05 (s, 6 H), 1.6 (s, 3 H), 1.1-2.4 (10 H), 2.3 (d, 2 H, J = 6 Hz, -OOC-C<u>H</u>$_2$-CO), 2.85 (br s, 1 H, -O<u>H</u>), 3.6 (s, 3 H, -OC<u>H</u>$_3$), 3.86 (m, 1 H, -C<u>H</u>-O)

(b) To a solution of the hydroxy ester **16** (220 mg, 0.87 mmol) in dry CH_2Cl_2 (10 mL) cooled to 0°C was added N-Ethyl diisopropylamine (Hunig base, 150 mg, 0.2 mL, 1.16 mmol) and mesyl chloride (296 mg, 0.2 mL, 2.58 mmol). The reaction mixture was stirred at 30°C for 18 h and then washed with water and brine and dried over anhydrous Na_2SO_4. Solvent was removed and residue chromatographed on a silica gel column using 10% EtOAc in petroleum ether as eluent to furnish mesylate (230 mg, 79%).

IR (film)ν_{max} : 2932, 2871, 1743, 1442, , 1261, 1175cm^{-1}

^{1}H NMR (60 MHz, CCl_4) : δ 0.98 (s, 6 H), 1.6 (s, 3 H, C=C-C<u>H</u>$_3$), 1.1-2.1(10H), 2.45- 2.7(m,2H, (O)CC<u>H</u>$_2$C(O), 2.9 (s, 3H, O_2SC<u>H</u>$_3$), 3.55 (s, 3 H, -OC<u>H</u>$_3$), 4.75 (m, 1 H, <u>H</u>C-OMs).

(c)To a solution of the above mesylate (200 mg, 0.60 mmol) in dry benzene (12 mL) was added DBU (0.45 mL, 3 mmol) and stirred at 60°C for 2 h. Solvent was removed under reduced pressure and residue chromatographed on a silica gel column using 4% EtOAc in petroleum ether as eluent to furnish unsaturated ester **17** (100 mg, 70%).

^{1}H NMR (90 MHz, $CDCl_3$) : δ 1.0 (s, 6 H), 1.58 (s, 3 H, C=C-C<u>H</u>$_3$), 1.1-2.4 (10 H), 3.72 (s, 3 H,-OC<u>H</u>$_3$), 5.75(d, 1 H, O_2C-C<u>H</u>=C), 5.92 (d, 1 H, CH=C-CO_2).

Formate of α-Hydroxy ester (12)

To a mixture of concentrated H_2SO_4 (0.2 mL) and 98% formic acid (2.1 mL), cooled in an ice bath, was added diene-ester **17** (100 mg, 0.42 mmol). After the addition was completed, ice-bath was removed and the reaction mixture was stirred at rt for 2 h, then poured into ice water (5 mL). Product was extracted with ether (4 x 5 mL). Combined ether extracts were washed with water, aqueous $NaHCO_3$ and brine and dried over anhydrous Na_2SO_4. Solvent was removed and crude product purified by crystallisation from hexane to give formate **12** (63 mg, 53%). mp. 109-110°C (lit. [12] 106-109°C)

^{1}H NMR (60 MHz, CCl_4) : δ 0.85 (s, 3 H), 0.9 (s, 3 H), 1.0 (s, 3 H), 1.0-2.5(12 H), 3.55 (s, 3 H), 4.7-5.4 (m, 1 H), 7.75 (s, 1 H)

(1β ,2α, 4aα, 8aβ,) Methyl (±)-2-hydroxy-5,5,8a-trimethyldecahydro-naphthalene-1-carboxylate (13)

To a solution of formate **12** (100 mg, 0.35 mmol) in MeOH (4 mL) was added 20% aqueous NaOH solution (0.3 mL) and stirred at room temperature for 8 h. Mixture was diluted with water (8 mL), acidified with AcOH (0.15 mL) and extracted with ether (4 x 10 mL). Combined ether extracts were washed with water, aqueous sodium bicarbonate and brine and dried over anhydrous Na_2SO_4. Solvent was removed and residue was crystallised from hexane to furnish alcohol **11** (70 mg, 78%). mp. 94 - 96°C (lit. [12] 90 - 92 °C)

IR (KBr)ν_{max} : 3532, 2958, 2853, 1717, 1467, 1439, 1047, 1035 cm-1.

^{1}H NMR (60 MHz, CCl_4) : δ 0.82 (s, 3 H), 0.88 (s, 3 H), 0.92 (s, 3 H),1.0-2.1 (11 H), 1.92 (d, 1 H, J = 10 Hz,O_2C-C<u>H</u>-C-), 2.15 (br s, 1 H, -O<u>H</u>), 3.58 (s, 3 H, -OC<u>H_3</u>), 3.88 (m, 1 H. <u>H</u>C-O).

GC-MS m/z (relative intensity) : 254 (M^+, 5), 236 (11), 222 (23), 221 (35), 180 (10), 161 (35), 147 (30), 137 (90), 123 (70), 109 (68), 95 (93), 81 (100).

1-Methylene-2-oxo-5,5,8a-trimethyldecahydronaphthalene (10)

The keto acetate 7 (190 mg, 0.71 mmol) was adsorbed on a short column of neutral alumina (Brockmann grade 1, 10 g). After 1 h the column was eluted with 10% EtOAc in petroleum ether which furnished pure enone **10** (138g, 94%) as an oil.

IR (film)ν_{max} : 2928, 2900, 1690 (>C=O), 1600 (>C=C<), 1435, 1240 cm^{-1}

^{1}H NMR (90 MHz, $CDCl_3$) : δ 0.9 (s, 3 H), 0.95 (s, 3 H), 1.0 (s, 3 H), 1.05-2.8 (11 H), 5.0 (d, 1 H, J = 2 Hz, C=C<u>H</u>-), 5.55 (d, 1 H, J = 2 Hz, C=C<u>H</u>-).

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 18.7, 20.6, 21.2, 21.9, 33.1, 33.7, 37.4, 40.5, 40.7,41.8, 50.3, 113.4, 159.0, 203.9.

GC-MS m/z (relative intensity) : 207 $(M + 1)^+$, 7), 206 (M^+, 36), 191 (42), 178(58), 163 (82), 149 (48), 135 (72), 122 (100), 121(58), 109 (100), 95 (67), 79(80)

1-(2'-Nitroethyl)-5,5,8a-trimethyl-2-oxodacahydronaphthalene (18)

A solution of enone (±)-**10** (80 mg, 0.388 mmol) in nitromethane (2 mL) under argon was cooled to 0°C, and 1,1,3,3-tetramethylguanidine (0.04 mL, 0.319 mmol) was added to it. The reaction mixture was stirred at 0°C for 2 h. When the reaction was complete as shown by tlc, it was diluted with ether (10 mL). The organic layer was washed with aqueous 5% HCl, water, saturated $NaHCO_3$ solution, brine and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure and the residue was chromatographed over silica gel using 10 % EtOAc in petroleum ether as eluent to furnish nitrocompound **18** (95 mg, 92 %).

IR (KBr)ν_{max} : 2934, 2856, 1716 (>C=O), 1554 (asym-NO_2), 1444, 1378 (sym-NO_2), 1187, 1079 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$) : δ 0.76 (s, 3 H), 0.87 (s, 3 H), 0.98 (s, 3 H), 1.1-1.8 (9 H), 2-2.55 (m, 5 H, HC-C(O),

$C\underline{H}_2$-C(O), $C\underline{H}_2$-C-NO_2), 4.25-4.53 (m, 2H, -$C\underline{H}_2$-NO_2).

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 14.4, 18.6, 20.3, 21.4, 23.4, 33.2, 33.4, 38.8, 41.5, 41.9, 42.0, 53.6, 60.0, 74.7, 210.7.

Analysis

Calcd. for $C_{15}H_{25}O_3N$: C, 67.39; H, 9.43; N, 5.24.

Found : C, 67.45; H, 9.45; N, 5.28.

GC-MS m/z (relative intensity) : 267 (M^+, 5), 252 (5), 234 (5), 220 (19), 205 (13), 163 (22), 137 (100), 123 (58), 109 (38), 95 (63), 81 (60).

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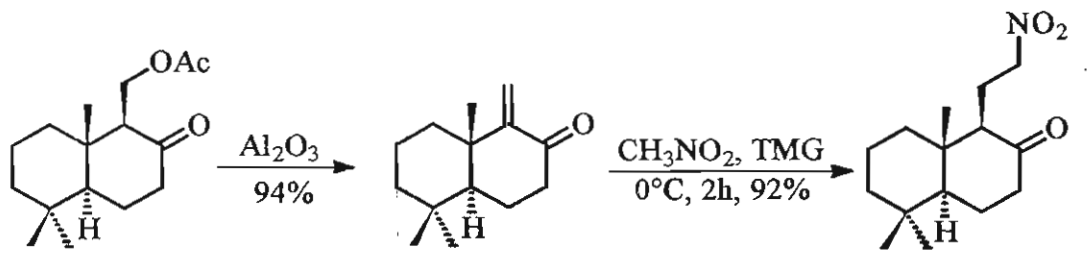
CHAPTER IV

SYNTHESIS OF CHIRAL INTERMEDIATES THROUGH LIPASE CATALYSED ENANTIOSELECTIVE ACYLATION OF 1-HYDROXYMETHYL-5,5,8a TRIMETHYL- 2-OXODECAHYDRONAPHTHALENE AND THE TOTAL SYNTHESIS OF (+)- ALBICANOL AND (+)-ALBICANYL ACETATE

4.1 INTRODUCTION

From the studies conducted so far, it became clear that the versatile intermediates **2** and **3** could be accessed rapidly from the ketoacetate **1** as shown below. The objective of obtaining these intermediates in chiral form

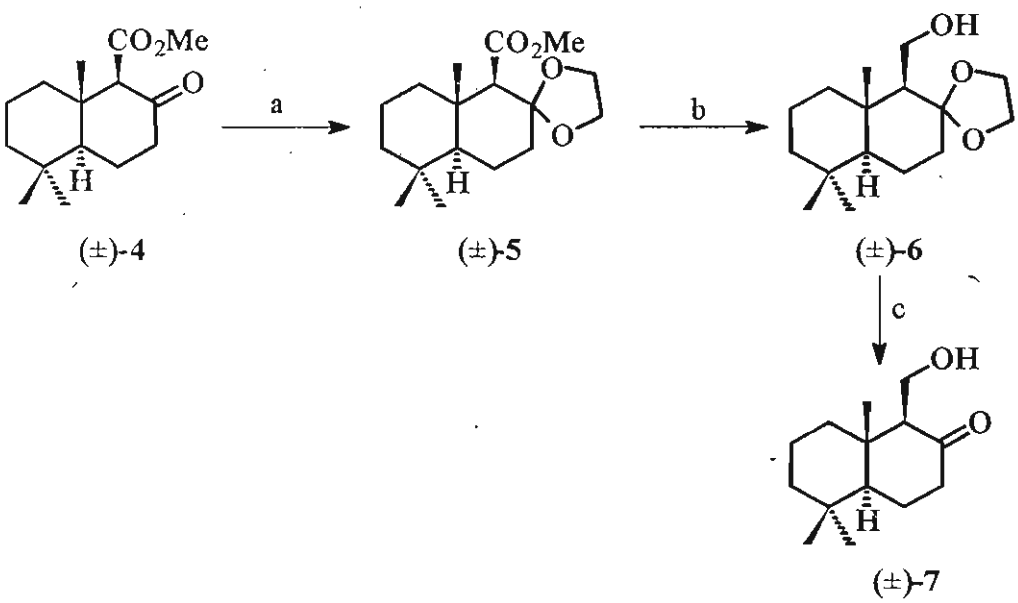


(±)-1 (±)-2 (±)-3

thus rested on the synthesis of optically pure ketoacetate **1**. The most logical approach under the circumstances, *viz*., the enzymatic enantioselective synthesis of **1** was therefore attempted.

4.2 RESULTS AND DISCUSSION

Once again, the readily available bicyclic β-ketoester **4** served as a suitable precursor. The keto group was protected as ethylene ketal leading to **5** and later reduced with $LiAlH_4$ at 0°C - rt in ether, thus providing **6** in 82 % yield. Structure of each of these compounds were established from their spectral data. Subsequent deketalisation of **6** in wet acetone using *p*-toluene sulfonic acid as catalyst furnished the ketoalcohol **7** in 99 % yield. Its IR spectrum showed absorptions at 3567 cm^{-1} and 1709cm^{-1}



Reagents and conditions: a. Ethylene glycol, PTS, Benzene, reflux, 96%; b. $LiAlH_4$, Et_2O, 0°- rt, 86%; c. PTS, Acetone (wet) rt, 99%.

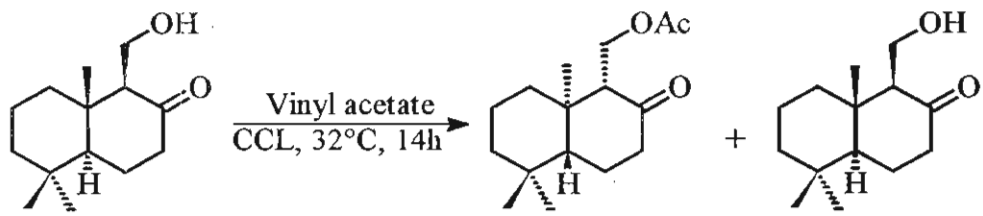
confirming the presence of hydroxyl and carbonyl groups respectively. In the ^1H NMR spectrum, the signal at δ 3.8 was indicative of the two protons of -$C\underline{H}_2$-OH functionality. The ^{13}C NMR spectrum containing 13 signals including those at δ 214.4 due to the carbonyl group and at δ 65.3 due to the carbon of -CH_2-OH group further confirmed the structure.

Having acquired adequate amount of the racemic ketoalcohol **7**, its propensity for undergoing enzymatic resolution under different conditions were examined. The results of various attempts at catalysing the transesterification of **7** with PPL in vinyl acetate was disappointing. However, our next attempts with CCL in vinyl acetate paid rich dividends.

When the ketoalcohol 7 was treated with CCL (Aldrich) which had high activity (700 - 1500 U/mg) in vinyl acetate at 28°C, transacylation proceeded rapidly and produced 56 % of the acetate **8** in 45 minutes. However, ^1H NMR studies using $Eu(hfc)_3$ as chiral shift reagent indicated that no enantiodifferentiation had occurred. Factors such as growth conditions of the source, mode of purification *etc.* have great impact on the activity and enantiodifferentiating capability of enzymes. An indication of this was visible in the studies carried out on the diol (Chapter III). While CCL (Aldrich) rapidly catalysed the formation of acylated product without enantioselectivity, CCL (Fluka) with activity 24.2 U/mg reacted much more slowly but indicated the propensity for enantiodifferentiation (see Table I in Chapter III). Therefore, we studied the effect of CCL (Fluka) on substrate **7**.

As envisaged the addition of CCL(Fluka) to **7** in vinyl acetate followed by stirring at 32°C for 14 h resulted in 36.6% conversion to the

(+)-acetate **8** with 70% ee, while 62% of the enriched (-)-ketoalcohol **7** of 40% ee was recovered. The absolute stereochemistry has been depicted tentatively based on the rotation of the keto alcohol reported in literature.[1]



(±)-7 (+)-**8** (-)-7

36.6% yield 62 % yield
70 % ee 40 % ee

Small differences in the rates of conversion and enantioselectivity were observed on repeating the reactions. This was attributed to the moisture content and temperature differences as the experiments were not carried out in a thermostated bath. For example, in a second attempt at transesterification catalysed by CCL in vinyl acetate at 30°C, an yield of only 31 % for (+)-**8** with 65% ee was realised after 19 h. Assuming that some of the enzyme was losing its activity after several hours in the reaction medium, an effort was made to improve the yield and ee of this transformation through the addition of CCL in several smaller lots. The most encouraging result was obtained on treatment of (±)-**7** with CCL (added in two equal lots with an intermission of 12 h) in vinyl acetate at 30°C for 24 h. Here, 47% conversion to the ketoacetate (+)-**8** of 68% ee was observed. Single crystallisation of the above acetate using pentane as solvent readily yielded material of 92% ee, while a second crystallisation delivered (+)-**8** of ~100% ee. The enantiomeric excess at each stage was ascertained by ^{1}H NMR using Eu(hfc)$_3$ as shift reagent (see Fig. 3-5).

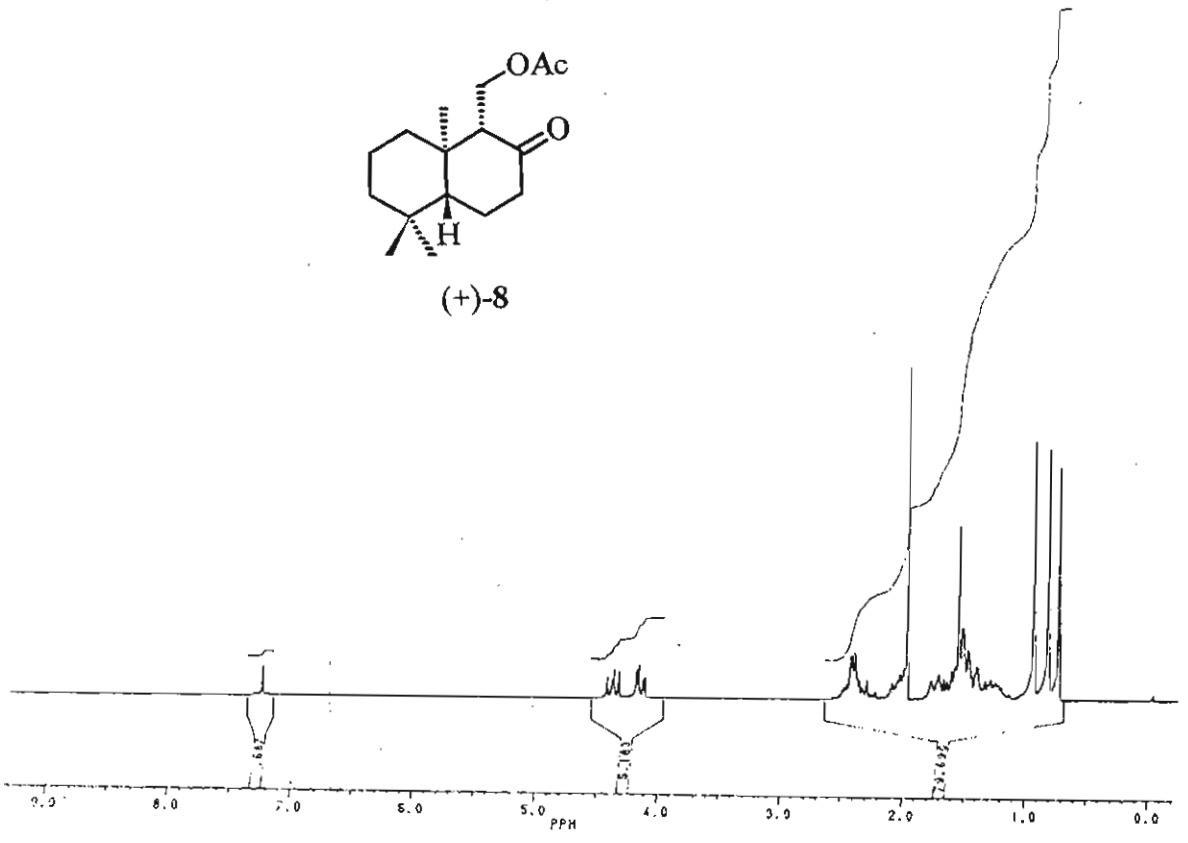


Fig 1 ^{1}H NMR of (+)-**8**

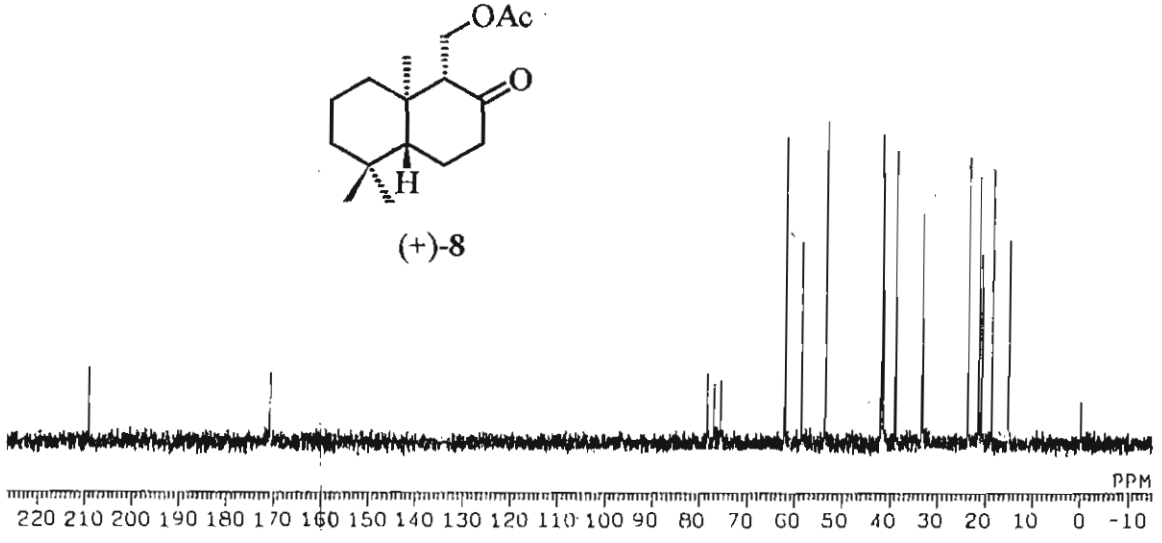
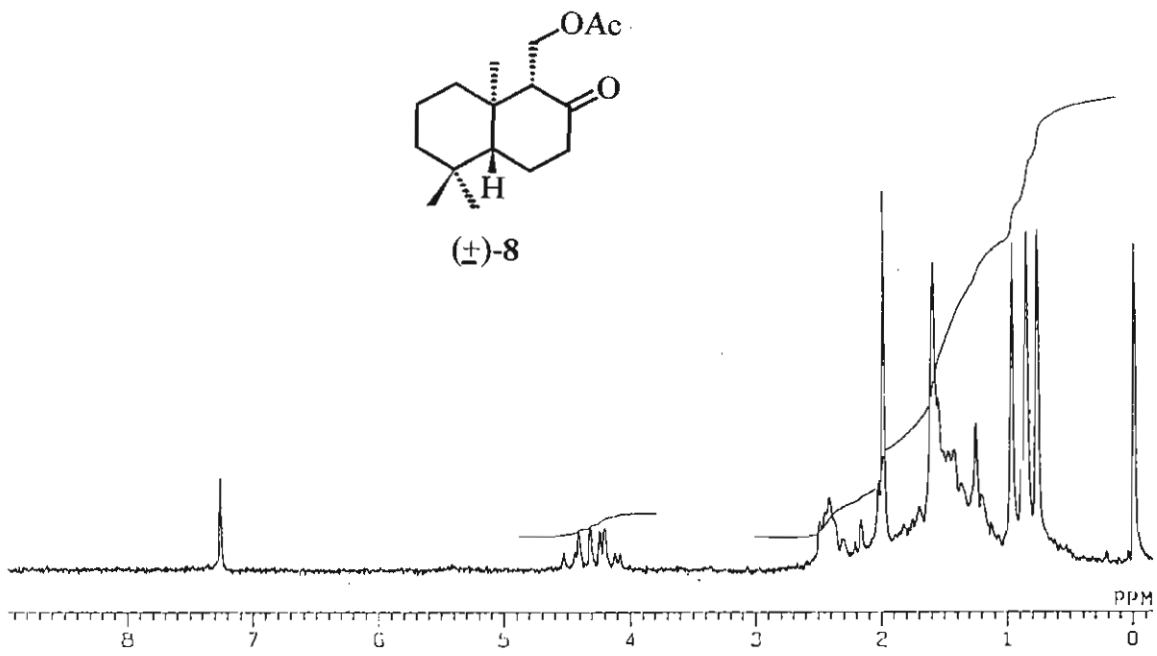


Fig 2 ^{13}C NMR of **8**



^{1}H NMR of (±)- **8**

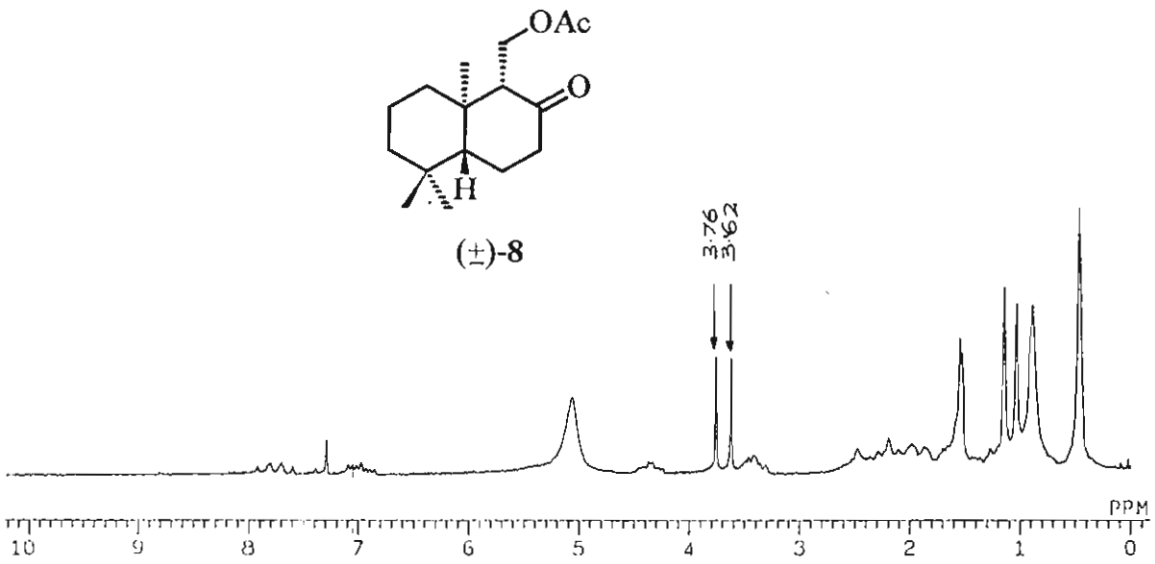


Fig 3 ^{1}H NMR of **(±)-8** (5 mg) in presence of $Eu(hfc)_3$(21 mg)

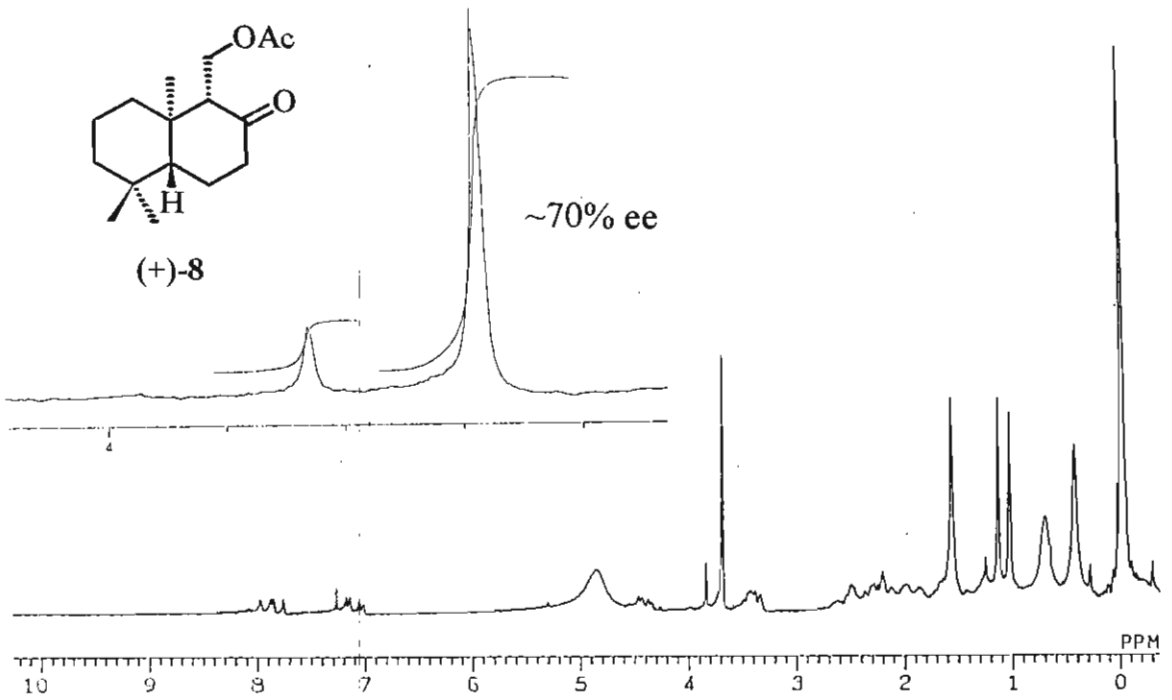


Fig 4 ^{1}H NMR of **(+)-8** (5 mg) in presence of Eu(hfc)$_3$(21 mg)

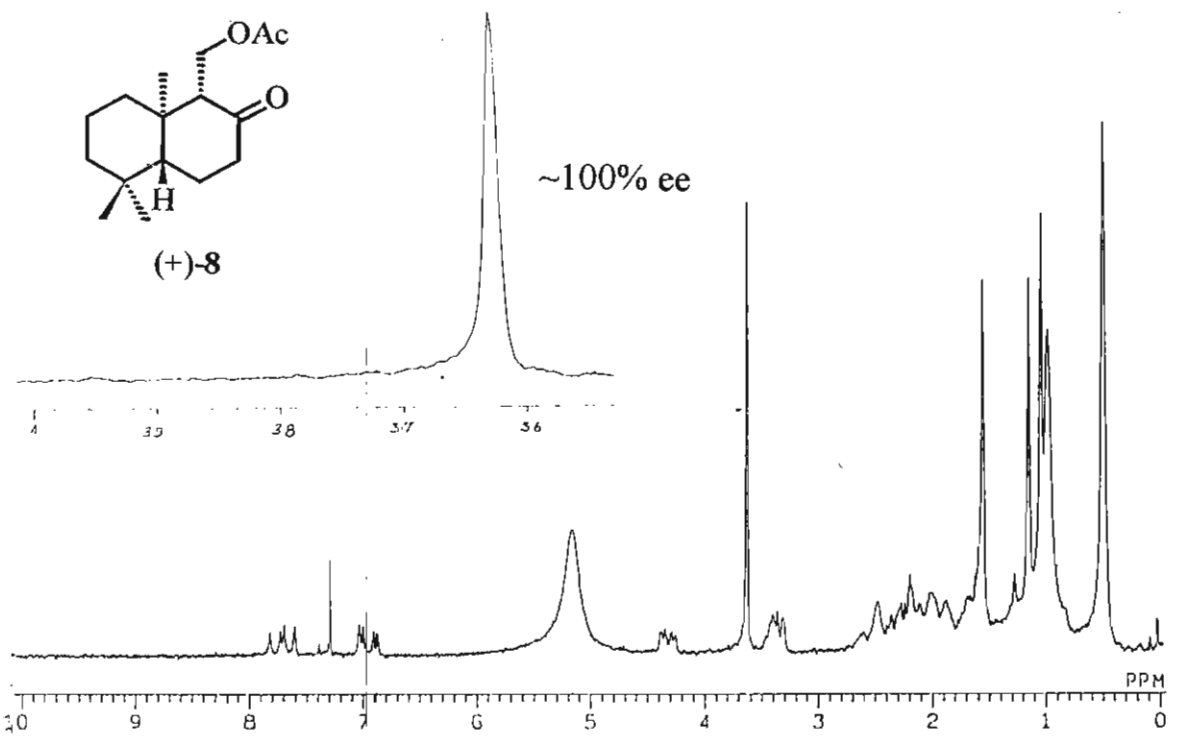
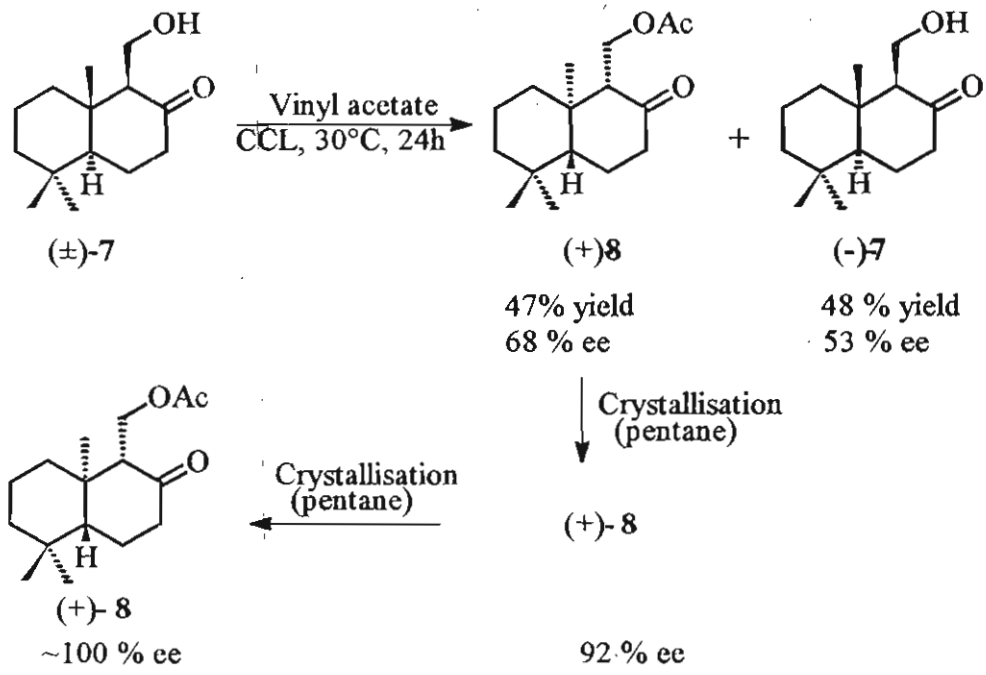
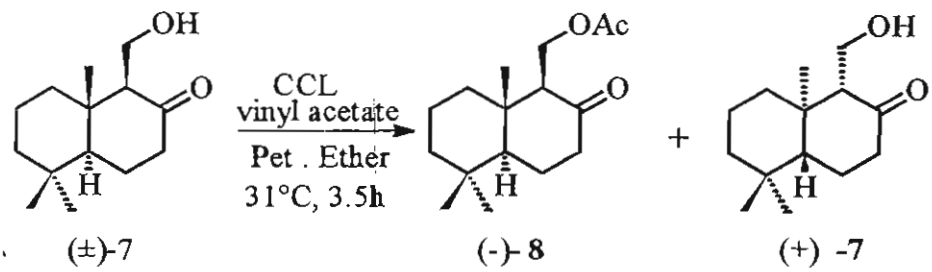


Fig 5 ^{1}H NMR of **(+)-8** (5 mg) in presence of Eu(hfc)$_3$(21 mg)



The structure of the ketoacetate **8** was firmly established from its spectral data including IR, ^{1}H NMR (Fig 1) and ^{13}C NMR (Fig 2).

Subsequently, the effect of different solvents on the resolution process was diligently examined. Most interestingly, a reversal of enantioselectivity was observed when (±)-7 was treated with CCL in petroleum ether solvent containing a limited quantity of the acylating agent, vinyl acetate for 3.5 h at 31°C. This reaction resulted in 31% conversion to the (-)-ketoacetate **8** of 18% ee.

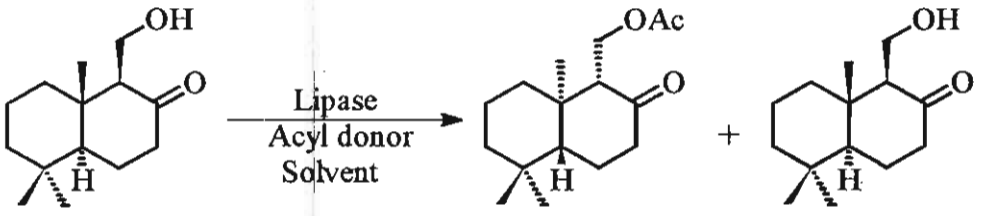


Reaction of (±)-7 with CCL in diisopropyl ether (DIPE) containing vinyl acetate at 28°C for 76 h furnished 55.8 % of the acetate **8** and 43 % of the keto alcohol **7.** However, no enantioselectivity had occurred.

The use of lipase AY (Amano) in isopropenyl acetate failed to deliver transesterification products even after 9 days of stirring. The treatment of (±)-7 with CCL in benzene containing isopropenyl acetate for 76 h at 28°C produced 47% of the keto acetate **8.** However the product **8** showed only 10% ee.

For a quick review, the results of the various reactions carried out on (±)- 7 are given in Table 1. Many of these reactions have been carried out several times to confirm the results.

Table I



(±)-7 8 7

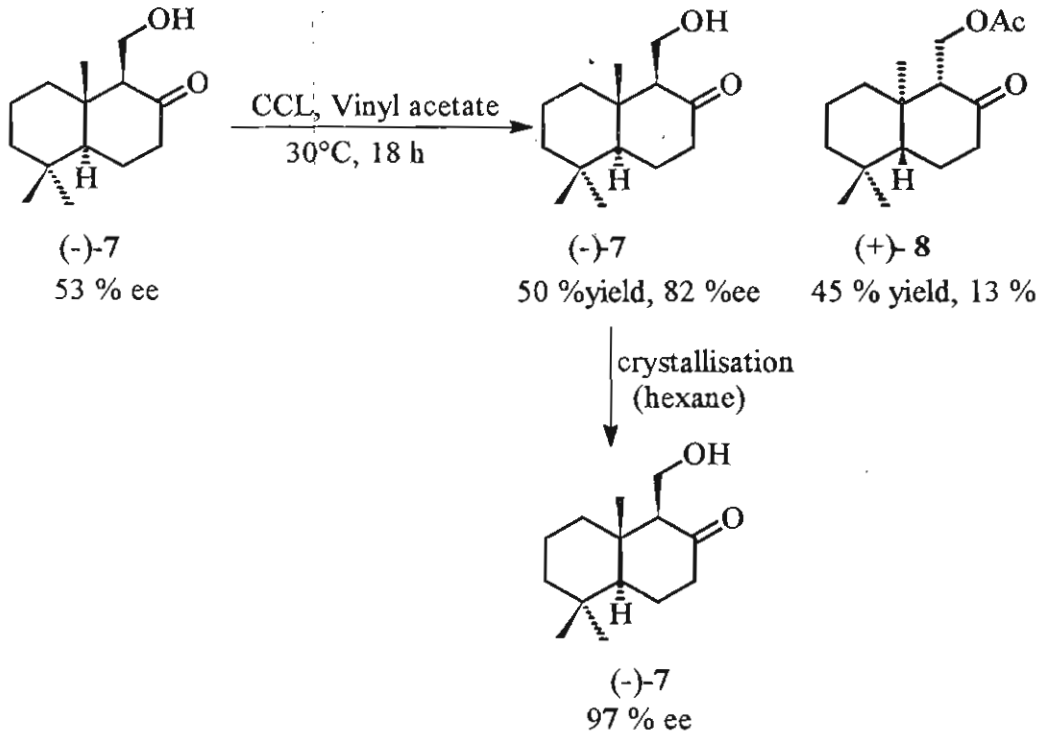
Entry	Lipase	Acyl donor / Solvent	Time h	Temp °C	Ester 8 % y[a] (% ee)[b]	Alcohol 7 %y[a] (%ee)[c]
1	PPL	Vinyl acetate	70	28-32	no reaction	d
2	CCL (Aldrich)	Vinyl acetate	0.75	28	56 (0)	38 *
3	CCL (Fluka)	Vinyl acetate	14	32	36.6 (68)	62.7 (40)
4	CCL (Fluka)	Vinyl acetate	21	28	33.6 (70)	64 *
5	CCL (Fluka)	Vinyl acetate	19	28	31 (65)	60 *
6	CCL (Fluka)	Vinyl acetate	21	28	41.6 (60)	47 *
7	CCL (Fluka)	Vinyl acetate	15	30	43.5 (72)	54 *
8	CCL (Fluka)	Vinyl acetate	24	30	47 (68)	48 (53)
9	CCL (Fluka)	Vinyl acetate/ DIPE	76	28	55.8 (4)	43 *
10	CCL (Fluka)	Isopropenyl acetate/Benzene	53	30	47 (10)	51 *

Table I (contd)

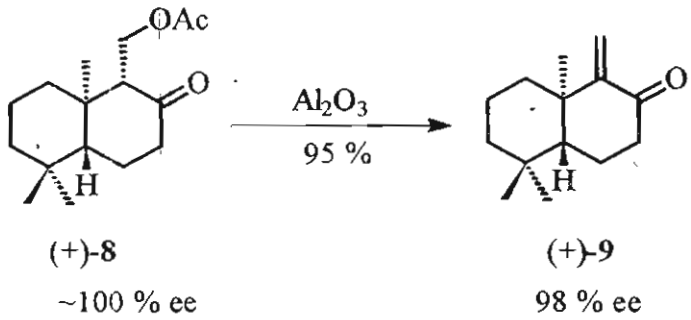
11	CCL (Fluka)	Vinyl acetate/ Pet. Ether	3.5	31	31 (18)	64 *
12	Lipase AY Amano 30	Isopropenyl acetate	216	28-31	no reaction	d
13	LipasePS Amano	Vinyl acetate	288	28-31	20.5 (10)	62 *

* ee not determined. [a]isolated yield after column chromatography [b]determined by ^{1}HNMR in the presence of Eu(hfc)$_3$. [c]determined by comparison of the optical rotation with literature value. [d]almost complete recovery of alcohol **7.** PPL , Type II with activity 110-220 U/mg and CCL, Type VIII with activity 700 - 1500 U/mg were purchased from Aldrich. CCL (Fluka) with activity 24.2 U/mg was purchased from Fluka. Lipase AY "Amano" 30 (from Candida rugosa) with activity 30 U/mg and Lipase PS "Amano" (from Pseudomonas cepacia) with activity 30 U/mg were obtained from Amano Pharmaceutical Co., Japan y-yield

For obtaining optically pure ketoalcohol **7,** a sequential enzymatic acylation method was used. The keto alcohol (-)-**7** obtained after the removal of (+)-**8** from the best yielding CCL catalysed transesterification reported earlier only had an ee of 53%. Taking advantage of the higher acylation selectivity shown by CCL for the (+)-enantiomer, a second transesterification was carried out on the above material. This resulted in keto acetate **8** of poor ee, but provided unreacted ketoalcohol (-)-**7** in 50 % yield and 82 % ee. On crystallisation from hexane, ketoalcohol **(-)-7** of 97 % ee was obtained. Once again the ee was determined on comparison of the optical rotation with the reported values.[1] At this stage, the absolute stereochemistry of **8** was also confirmed.



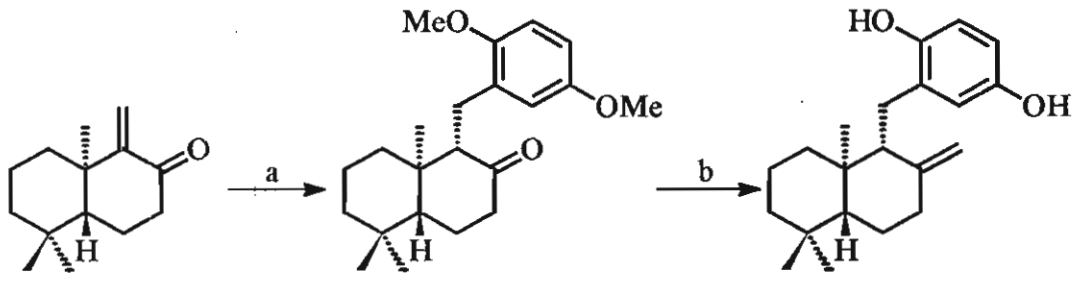
The availability of (+)-ketoacetate **8** with high optical purity made possible further advancement towards the synthesis of natural products of our interest. The first priority was given to the synthesis of enone **9**. Accordingly, the elimination was carried out using the method developed earlier, whereby **8** was adsorbed on neutral alumina column and eluted with 10 % ethylacetate in petroleum ether after a period of 1h. This process readily furnished enone (+)-**9** in 95 % yield with an ee of 98 %. The enantiomeric excess was



(+)-**8**
~100 % ee

(+)-**9**
98 % ee

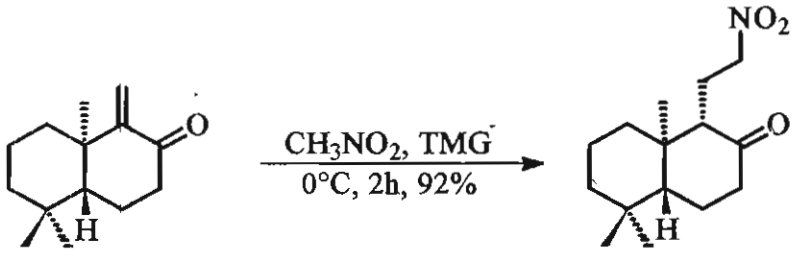
calculated from the comparison of its optical rotation with the value reported earlier.[2,3] The conversion of (+)-**9** to (+)-*Zonarol* in 3 steps involving (i) cuprate mediated 1,4-addition (ii) methylenation and deprotection has been reported earlier by Mori.[2]



(+)- 9 **(+) Zonarol**

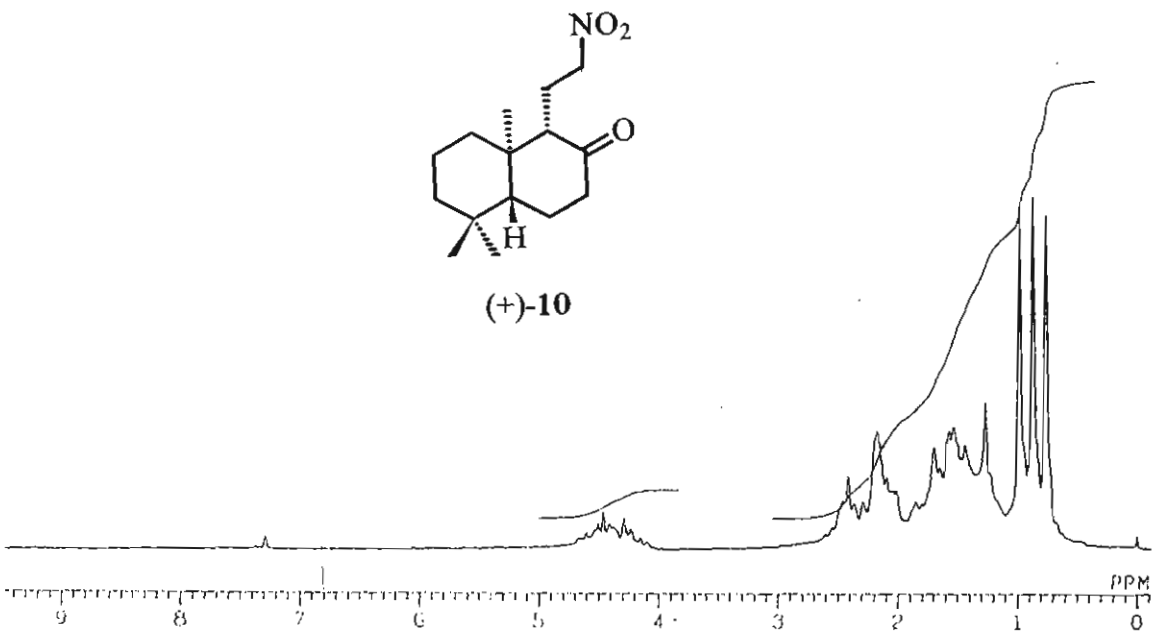
Reagents and conditions : a. Mg, 1-bromo-2,5-dimethoxybenzene, CuI; Ac_2O, KOH; b(i) $Ph_3P{=}CH_2$; (ii) n-BuSLi, HMPA.

To extend the utility of this enone further, a one carbon annulation was carried out as before through the addition of nitromethane to optically pure (+)-**9** at 0°C in the presence of tetramethyl guanidine. This readily gave **10** in 92 % yield as a single diastereoisomer as evidenced from the high resolution ^1H NMR (Fig.6). The optical rotation was recorded as $[\alpha]^{24}_D$ + 19. 7°(*c* 1.45, $CHCl_3$)



(+)-**9** (+)-**10**

The synthesis of the (-) bicyclic enone **9** was achieved in two steps including acetylation of (-)- 7 and subsequent elimination.



(+)-**10**

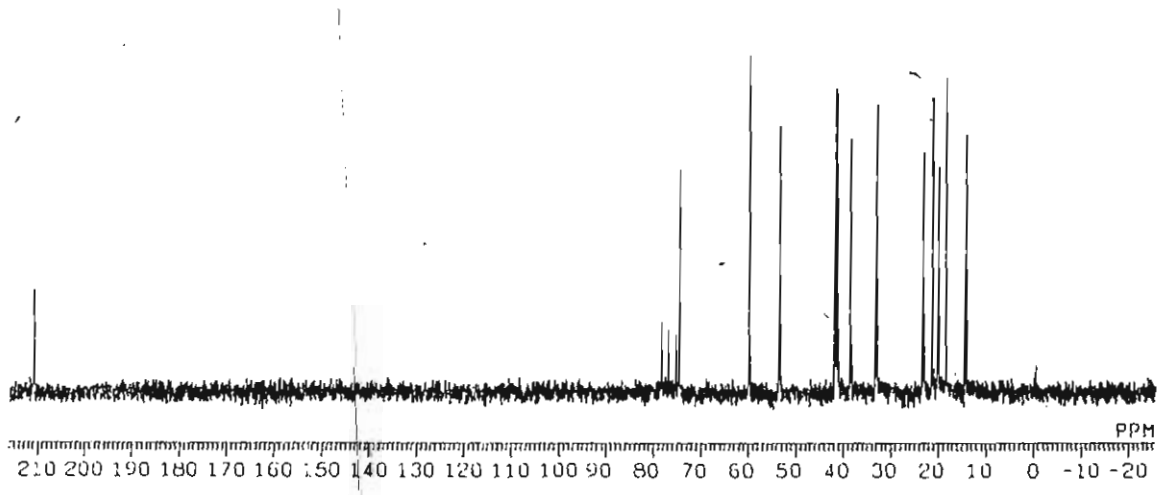
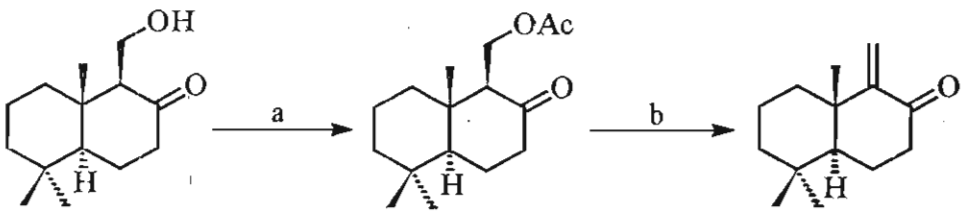


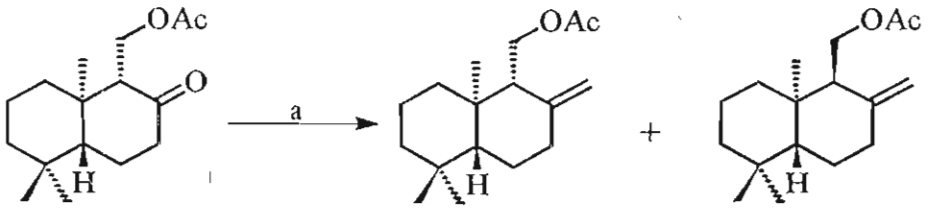
Fig 6 ^{1}H NMR and ^{13}C NMR of **10**



(-)-7 (-)-8 (-)-9

Reagents and conditions : a. Ac_2O, py, 0°C, 91% ; b. Al_2O_3, 94% .

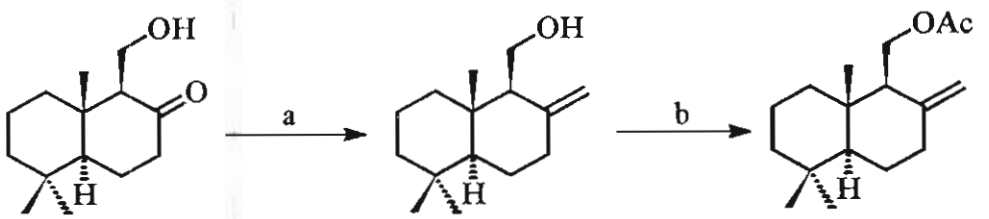
Having synthesised these versatile chiral intermediates, attention was turned to the synthesis of unnatural (-) Albicanyl acetate **11**, since the closest precursor in hand was the (+)-ketoacetate **8**. It has been reported earlier by Fukumoto *et al.*[1] that methylenation of such system with Wittig or Peterson olefination methods do not give satisfactory results. Therefore, the Nozaki[4a] and Lombardo[4b] procedure of methylenation using $TiCl_4$- Zn- CH_2Br_2 reagent system was tried on (+)-**8** . Out of the several attempts that were carried out, the methylenation product could be obtained only twice and in 28 % yield. To our dismay, the product was found to be a diastereomeric mixture contaning products **11** and **12** in 3 : 1 ratio when the nmr was studied with $Eu(hfc)_3$ chiral shift reagent. This might have been caused by some equilibration during the prolonged time in the presence of $TiCl_4$. (-)-Albicanyl acetate could not be separated from the mixture using column chromatography.



(+)- 8 (-)-11 12

Reagents and conditions : a. CH_2Br_2- Zn-$TiCl_4$, THF, CH_2Cl_2, 0°C-rt, 28 %

While the work was in progress, Takai *et al.*[5] reported that addition of $PbCl_2$ or PbI_2 to the methylenation reagent system CH_2Br_2 (CH_2I_2)-$TiCl_4$-Zn resulted in providing more reproducible results. After obtaining this information, we made the methylenation reagent by stirring CH_2I_2, $TiCl_4$, Zn and PbI_2 (catalytic amount) in dry THF at 0 °C for 1h and added the ketoalcohol (-)-7 to it. Stirring at rt for 12h, work up and purification of the compound resulted in providing (+) Albicanol in 30 % yield. It was converted to (+)- Albicanyl acetate by treatment with acetic anhydride in



(-)-7 (+)-**Albicanol** (+) **Albicanyl acetate**

Reagents and conditions : a. CH_2Br_2- Zn-(PbI_2)-$TiCl_4$, THF, CH_2Cl_2, 0°C-rt, 30 %, b. Ac_2O, py. 0°C, 90 %

pyridine. The spectral data of (+)-**11** including IR, ^1H NMR, and the optical rotation were in accordance with that reported by Fukumoto *et al.*[1] While this modified methylenation procedure was reproducible, the yield could not be improved further.

In summary, we have developed the methodology for obtaining several versatile chiral intermediates that have wide utility in natural product synthesis through the use of simple starting materials and commercially available lipases. We have also been able to synthesise the natural product

(+)- Albicanol and (+)- Albicanyl acetate in an optically pure form, albiet in low yield.

4.4 EXPERIMENTAL

For general section see experimental part in Chapter II and Chapter III

Methyl 2,2-ethylenedioxy -5,5,8a- trimethyldecahydronaphthalene -1-carboxylate -(5)

A mixture of the ketoester **4** (4.3 g, 17.06 mmol) and ethylene glycol (5 mL) in dry benzene (45 mL) containing catalytic amount of *p*-toluene sulfonic acid monohydrate (100 mg) was refluxed and the water removed using Dean-Stark condenser for 4 h. The reaction mixture was cooled to rt, diluted with ether (45 mL), washed with aqueous $NaHCO_3$ solution, water and brine and finally dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue on crystallization from hexane furnished ketal ester **5** (4.9 g, 97%) mp. 118-119°C (lit [6] 103 -104 °C).

IR (KBr)ν_{max} : 2962, 2930, 1740 (-$COOCH_3$), 1440, 1375, 1196, 1074 cm^{-1}.

^1H NMR (60 MHz, CCl_4) : δ 0.85 (s, 6H), 1.10 (s, 3H), 1.1-1.9 (11 H) 2.3 (s, 1 H, C<u>H</u>-COOMe), 3.5 (s, 3 H , O<u>CH</u>$_3$), 3.5-4.00 (m, 4 H, O<u>CH</u>$_2$-<u>CH</u>$_2$O).

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 14.7, 18.4, 20.1, 21.6, 33.2, 33.6, 37.7, 39.1, 39.8, 42.0, 50.9, 54.9, 62.8, 64.0, 65.6, 109.2, 171.2.

Analysis

Calcd. for $C_{17}H_{28}O_4$: C, 68.89; H, 9.52

Found : C, 68.95; H, 9.55

GC-MS m/z (relative intensity) : 296 (M^+, 4), 113 (4), 100 (7), 99 (100), 86 (15), 69 (6), 67 (5), 55 (12).

2,2-Ethylenedioxy-1-hydroxymethyl-5,5,8a-trimethyldecahydro-naphthalene-(6)

To a stirred suspension of $LiAlH_4$ (1.25 g, 32.9 mmol) in dry ether (100 mL) under argon cooled to 0°C, was added a solution of ketal ester **5** (5 g, 16.9 mmol) in dry ether (30 mL). The reaction mixture was stirred at rt for 12 h and excess $LiAlH_4$ was destroyed at 0°C by the careful addition of ether-water (90:10) mixture. The precipitate formed was filtered and washed with ether. Combined filtrate was dried over anhydrous Na_2SO_4 and concentrated. Residue was chromatographed on a silica gel column using 10 % EtOAc in petroleum ether as eluent and yielded ketal alcohol **6** (3.9 g, 86%) mp.106- 107°C (hexane) (lit[6] 92 - 93° C).

IR (KBr)ν_{max} : 3541 (-OH), 2967, 2673, 1472, 1422, 1366, 1147, 1077, 904 cm^{-1}.

^{1}H NMR (90 MHz, $CDCl_3$) : δ 0.8 (s, 3 H), 0.84 (s, 3 H), 0.86 (s, 3 H), 0.9-2.0 (12 H), 2.92 (m, 1 H, -OH), 3.4-4.1(m, 6 H, -C<u>H</u>$_2$-O, -O-(C<u>H</u>$_2$)$_2$-O-)

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 15.5, 18.5, 19.6, 21.6, 33.2, 33.6, 35.5, 38.3, 39.4, 41.8, 55.0, 58.8, 62.6, 64.9, 112.3

Analysis

Calcd. for $C_{16}H_{28}O_3$: C, 71.6; H, 10.52

Found : C, 71.9; H, 10.53

GC-MS m/z (relative intensity) : 268 (M^+, 6), 139 (4), 100 (12), 99 (100), 86 (14), 81 (6), 79 (6), 67 (8), 55 (18).

1-Hydroxymethyl-5,5,8a-trimethyl-2-oxodecahydronaphthalene- (7)

To a solution of ketal **6** (2.1 g, 7.8 mmol) in wet acetone (20 mL) was added *p*-toluene sulfonic acid monohydrate (200 mg, 1.05 mmol) and stirred at rt for 3 h. The reaction mixture was then neutralized with aqueous $NaHCO_3$ solution, diluted with water (40 mL) and extracted with CH_2Cl_2 (3 x 25 mL). Combined organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4. Solvent was removed and residue chromatographed over silica gel, with 10% EtOAc in petroleum ether as

eluent to furnish keto alcohol 7 (1.74 g, 99 %) mp. 67-68°C (hexane) (lit[1] 69.5 -70.5°C) .

IR (KBr)ν_{max} : 3567 (-OH), 2932, 1709 (>C=O), 1464, 1391, 1369, 1041 cm^{-1}

^1H NMR (90 MHz, $CDCl_3$) : δ 0.8 (s, 3 H), 0.86 (s, 3 H), 0.96 (s, 3 H), 1.05-2.55 (13 H), 3.8 (m, 2 H, -$C\underline{H}_2$-O).

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 15.7, 18.7, 21.7, 23.2, 33.4, 39.0, 41.0, 41.6, 41.9, 53.4, 57.5, 65.3, 214.4.

Analysis:

Calcd. for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78

Found : C, 75.02; H, 10.80

GC-MS m/z (relative intensity) : 224 (M^+, 8), 206 (5), 191 (8), 179 (7), 138 (15), 123 (20), 109 (21), 99 (50), 86 (100), 81 (45), 69 (48), 55 (55)

(+)-1-(Acetyloxy)methyl-5,5,8a-trimethyl -2-oxodecahydronaphthalene (8)

To a solution of (±)-7 (520 mg, 2.32 mmol) in vinyl acetate (45 mL) was added Candida cylindrcea lipase (CCL, 24 U/mg, 490 mg) and stirred at 30°C for 12h following which an additional amount of lipase (490 mg) was added. The reaction mixture was stirred for a total period of 24 h. Lipase was

then filtered off and washed with ethyl acetate. Combined filtrate was concentrated under reduced pressure and the residue chromatographed over silica gel using 10% EtOAc in petroleum ether as eluent to furnish (+)-(1*R*, 4a*R*, 8a*R*)-1-[(Acetyloxy)methyl]-5,5,8a-trimethyl-2-oxodecahydro-naphthalene **8** (290 mg, 47%, 68% ee). and (-)-ketoalcohol **11** (250 mg, 48%, 53% ee). Crystallisation of optically enriched acetate **8** (290 mg, 68% ee) from pentane furnished (+) -8 (144 mg) with >92% ee. Recrystallisation of >92% ee sample (140 mg) finally furnished (+)-8(100 mg, >99% ee). mp. 57- 58.2°C

$[\alpha]^{24}_D$: +35.06°(*c* 0.96 , $CHCl_3$)

IR (KBr)ν_{max} : 2979, 2931, 1744 ($OCO-CH_3$), 1723 (>C=O), 1464, 1374, 1249, 1045 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$) : δ 0.77 (s, 3 H), 0.86 (s, 3 H), 0.98 (s, 3 H), 1.2-1.8 (9 H), 2.0 (s, 3 H, -C(O)-C<u>H</u>$_3$), 2.05-2.6 (m, 3 H, -C(O)-C<u>H</u>-C, C(O)-C<u>H</u>$_2$-C), 4.2 (m, 2 H, C<u>H</u>$_2$-O-)

^{13}C NMR (22.4 MHz, $CDCl_3$) : δ 15.1, 18.6, 20.7, 21.4, 23.5, 33.3, 33.4, 38.9, 41.5, 41.7, 41.8, 53.6, 58.6, 62.1, 170.7, 209.1

Analysis

Calcd. for $C_{16}H_{26}O_3$: C, 72.14; H, 9.84

Found : C, 72.38; H, 9.85

GC-MS m/z (relative intensity) : 266 (M^+, 5), 206 (20), 191 (26), 178 (24), 173 (24), 163 (24), 136 (60), 128 (52), 122 (51), 95 (80), 85 (70), 55 (100).

(-)-(1*S*, 4a*S*, 8a*S*)-1-Hydroxymethyl-5,5,8a-trimethyl-2-oxodecahydro-naphthalene- (7)

The ketoalcohol (-)-7 (200 mg) of 53% ee was resubmitted to transesterification under the same conditions using CCL (300 mg) and vinyl acetate (20 mL). Reaction was stopped after 18h and the crude mixture was chromatographed over silica gel to furnish ketoacetate **8** (90 mg, <13% ee) and further enriched (-)-7 (100 mg, 50%, 82 %ee). Crystallisation of this alcohol from hexane furnished optically pure (-) -7 (50 mg) of 97% ee, $[\alpha]^{25}_D$ -37.6° (*c* 0.46, $CHCl_3$) {lit[1] $[\alpha]_{26D}$ -38.9° (*c* 1.24 , $CHCl_3$) lit[3] $[\alpha]_D$ -38.3° (*c* 1.0).

Different lipase catalysed transesterification of (±)- 7

(1) A mixture of ketoalcohol (±)-7 (58 mg, 0.26 mmol) vinyl acetate (0.5 mL) and CCL (116 mg) in diisopropyl ether (10 mL) was stirred at 28°C for 76 h and worked up. Residue was chromatographed over silica gel to furnish keto acetate(+)-**8** (38 mg, 55%, <4% ee) and ketoalcohol 7 (25 mg, 43%).

(2) A mixture of ketoalcohol (58 mg, 0.26 mmol) and lipase Amano PS (88 mg) in vinyl acetate (8 mL) was stirred at 28°C for 12 d and worked up. The residue was chromatographed over silica gel to furnish keto acetate (+)-8 (14 mg, 20%,10% ee) and ketoalcohol 7 (36 mg, 62%).

(3) A mixture of ketoalcohol (48 mg, 0.21 mmol) and CCL (Aldrich, 700- 1500 U/mg, 95 mg) in vinyl acetate (7 mL) was stirred at 28°C for 45 minutes and worked up as before. The residue was chromatographed over silica gel using 8% EtOAc in petroleum ether as eluent to furnish keto acetate **8** (32 mg, 56%, racemic) and unreacted alcohol 7 (18 mg, 30%).

(4) To a solution of ketoalcohol 7 (56 mg, 0.25 mmol) and isopropenyl acetate (0.5 mL) in benzene (10 mL) was added CCL (Fluka, 24 U/mg, 84 mg) and stirred at 28°C for 53 h. Lipase was filtered, washed with 5 ml of benzene and the solvent removed under reduced pressure. The residue was chromatographed over silica gel using 8% EtOAc in petroleum ether and yielded ketoacetate **(+)**-8 (31 mg, 47%, 10% ee) and unreacted alcohol 7 (29 mg, 51%).

(5) A mixture of ketoalcohol (603 mg, 2.69 mmol) and CCL (Fluka, 24.2 U/mg , 900 mg) in vinyl acetate (50 mL) was stirred at 30°C for 15 h and worked up as before to furnish keto acetate (+)-**8** (310 mg, 43%, 70% ee) and ketoalcohol 7 (325 mg, 54%).

(6) A mixture of ketoalcohol (100 mg, 0.45 mmol), vinyl acetate (0.2 mL) and CCL (Fluka, 150 mg) in distilled petroleum ether (60-80°C) was stirred at 31°C for 3.5 h and worked up as usual. Residue on

chromatography (SiO_2) provided ketoacetate (-)-**8** (37 mg, 31%, 18% ee) and unreacted ketoalcohol 7 (70 mg, 70%).

Determination of the enantiomeric excess of 8

A mixture of enantiomerically enriched ketoacetate (+)-**8** (5mg) and (+)-Eu(hfc)$_3$ (21mg, 0.93mol equiv) was dissolved in $CDCl_3$ (0.5 - 0.6 mL). After 5 - 8 minutes a ^1H NMR was taken. The singlet at δ 2.0 due to $C\underline{H}_3CO$ was shifted and split into a doublet showing a major peak at δ 3.62 and a minor one at δ 3.76. The ee of the sample was then determined from the ratio of their integration.

(4a*R*,8a*R*)-(+)1-Methylene-5,5,8a-trimethyl-2-oxodecahydronaphthalene (9)

The ketoacetate (+)-8 (100 mg,0.38 mmol) of ~100% ee was adsorbed on a short column of neutral alumina (Brockmann grade 1, 5g) and on elution with 10% ethyl acetate in petroleum ether after 1h readily furnished optically pure enone (+)-**9** (73 mg, 95%, 98% ee). mp.52-53.5° C (pentane) (lit[2] 55 - 56° C) $[\alpha]^{25}_D$ +73.6° (*c* 1.00, $CHCl_3$) {lit[2], $[\alpha]^{23}_D$ +71.9° (*c* 0.695 ,$CHCl_3$), lit[3], $[\alpha]^{23}_D$ -75° (*c* 1.0 , $CHCl_3$, for (-) -**9**)

(4a*S*,8a*S*)-(-)-1-Methylene-5,5,8a-trimethyl-2-oxodecahydro-naphthalene -(9)

(a) To a solution of the ketoalcohol (-)-7 (50mg, 0.22mmol, 97 % ee) in pyridine (1mL) cooled to 0°C was added acetic anhydride (0.1 mL,

1.06mmol) and stirred at 0° C for 3h. The mixture was then diluted with CH_2Cl_2 (10mL) and pyridine washed off with saturated aqueous $CuSO_4$. The organic layer was further washed with water, brine and dried over Na_2SO_4. Solvent was removed and the residue chromatographed over silica gel using 10 % EtOAc in petroleum ether as eluent to furnish (-)-**8** (53mg, 90 %).

(b) The keto acetate (-)-8 (50 mg, 0.187 mmol) of 97 % ee was adsorbed on a short column of neutral alumina (Brockmann grade 1, 5g) and on elution with 10 % ethyl acetate in petroleum ether after 1h readily furnished optically pure enone (-)-**9** (36 mg, 93%, 95 % ee). mp 52.5 - 53.5° C(pentane) (lit[3] 52 - 53° C) $[\alpha]^{24}_D$ -71.6 (*c* 0.5, $CHCl_3$) lit[3] $[\alpha]^{23}_D$ - 75° (*c* 1.0 , $CHCl_3$)

1-(2'-Nitroethyl)-5,5,8a-trimethyl-2-oxodacahydronaphthalene -(10)

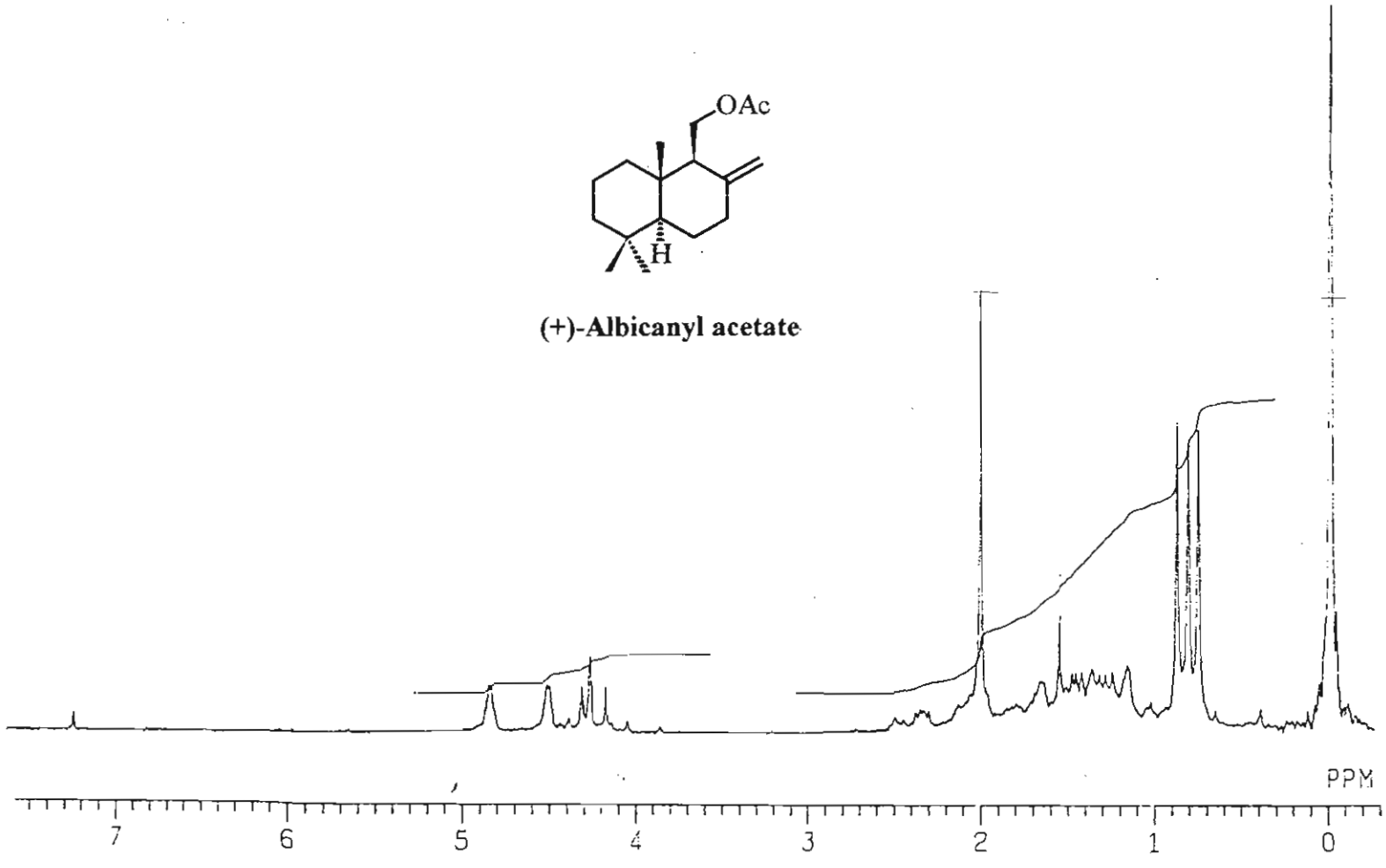
A solution of enone(+)-**9** (70 mg, 0.34 mmol) in nitromethane (2 mL) under argon was cooled to 0°C, and 1,1,3,3-tetramethylguanidine (0.04 mL, 0.319 mmol) was added to it. The reaction mixture was stirred at 0°C for 2 h. When the reaction was complete as shown by tlc, it was diluted with ether (10 mL), washed with aqueous 5% HCl, water, saturated $NaHCO_3$ solution, brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue was chromatographed over silica gel using 10% EtOAC in petroleum ether as eluent to furnish nitrocompound (+)-**10** (82 mg, 90%).
$[\alpha]^{24}_D$ +19.7°(*c* 1.45, $CHCl_3$)

(-)-Albicanyl acetate -(11)

To a well stirred mixture of Zn (244 mg, 3.73 mmol) and dibromomethane (0.09 mL, 215 mg, 1.24 mmol) in dry THF (4 mL) under argon cooled to -5° C, was added $TiCl_4$ (0.1 mL, 173 mg,0.91mmol) dropwise and stirred for 5h at 5 - 0° C. A solution of ketoacetate(+)-**8** (43 mg, 0.16 mmol, ~100 % ee) in dry CH_2Cl_2 (1mL) was added to the methylenation reagent thus prepared and stirred at 0° C for 4h and 28 °C for 20h. The mixture was diluted with hexane (8mL) and a saturated aqueous $NaHCO_3$ solution was added. Hexane layer was separated and aqueous layer extracted with hexane (3 x 5 mL). Combined extracts were washed with water, brine and dried over $Na_2SO_4/NaHCO_3$ mixture. Solvent was removed under reduced pressure and residue chromatographed over silica gel using 4% EtOAc in petroleum ether as eluent to yield (-)- Albicanyl acetate (**11**) (12 mg, 28 %) as a mixture of diastereomers.

(+)- Albicanyl acetate

(a)Methylenation reagent was prepared by stirring a mixture of Zn (244 mg, 3.73 mmol), catalytic amount of PbI_2 (8 mg), diidomethane (0.09mL, 215 mg, 1.24 mmol) and $TiCl_4$ (0.1mL, 0.91mmol) in dry THF (4mL) at 0° C for 1h. A solution of (-)-keto alcohol 7 (38 mg, 0.17 mmol, ~97 % ee) was added to the reagent and stirred at 0 -28 °C for 12h. Reaction mixture was worked up as before and residue purified over silica gel using 6 % EtOAc in petroleum ether as eluent to furnish (+)- Albicanol (11.5 mg, 30 %)



(+)-Albicanyl acetate

^{1}H NMR of Albicanyl acetate

(b) **Acetylation of (+)-Albicanol** : Acetic anhydride (21 mg, 0.2 mmol) was added to a solution of the (+)- Albicanol (11.5 mg, 0.05 mmol) in pyridine at 0°C and stirred at rt for 6h and worked up. The residue was chromatographed over silica gel using 4 % EtOAc in petroleum ether as eluent to afford (+)-Albicanyl acetate (12 mg, 88 %) $[\alpha]^{24}_D$ + 21.6° (*c* 0.46 $CHCl_3$) lit[1] $[\alpha]^{26}_D$ + 21.9° (*c* 0.37, $CHCl_3$)

IR (film)ν_{max} : 3091, 2931, 2863, 1746 (>C=O), 1651(>C=C<), 1465, 1032cm^{-1}

^1H NMR(90 MHz, $CDCl_3$) : δ 0.75 (s, 3 H), 0.8 (s, 3 H), 0.86 (s, 3 H) 1.0 - 2.3 (12 H), 2.0 (s, 3 H), 4.1 - 4.4 (m, 2 H), 4.5 (br s, 1 H), 4.85 (br s, 1 H)

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List of Publications

1. Lipase Catalysed Regioselective Acylation: A Facile Method for the Synthesis of Commercially Important Ambrox® Intermediate.
   M. S. Nair and A.T. Anilkumar, *Biotechnology Letters* **1994**, *16*, 161.

2. Facile Synthesis of the Versatile Synthon 1-Methylene-5,5,8a-trimethyl-2-oxodecahydronaphthalene.
   M. S. Nair and A. T. Anilkumar, *Synth. Commun.*, **1994**, *24*, 1085.

3. Versatile Chiral Intermediates For Terpenoid Synthesis Using Lipase Catalysed Acylation: Synthesis of (+)-1[(Acetyloxy)-methyl]-5,5,8a-trimethyl-2-oxodecahydronaphthalene, (-)-1-Hydroxymethyl-5,5,8a-trimethyl-2-oxodecahydronaphthalene and (+)-1-Methylene-5,5,8a-trimethyl-2-oxodecahydronaphthalene.
   M. S. Nair and A. T. Anilkumar (communicated).

4. Chiral Intermediates Through Lipase Catalysed Transesterification: Facile Synthesis of (-)-Albicanyl acetate.
   M. S. Nair and A. T. Anilkumar, Presented at the 10th International Conference on Organic Synthesis held at Bangalore, India, December-1994.