BIOACTIVE MOLECULES : CHEMICAL AND ENZYMATIC APPROACHES TO THEIR SYNTHESIS

A THESIS
SUBMITTED TO THE
UNIVERSITY OF POONA
for the degree of
DOCTOR OF PHILOSOPHY
(IN CHEMISTRY)

by
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CERTIFICATE

Certified that the work incorporated in the thesis entitled *"Bioactive molecules : Chemical and Enzymatic Approaches to their Synthesis"* submitted by **Mrs.S.R.Gadre** was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

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DEDICATED TO MY PARENTS

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Finally, I am thankful to the Director, National Chemical Laboratory, Pune for permitting me to submit this work in the form of a thesis.

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Research Candidate

General Remarks

1. All melting points and boiling points are uncorrected and all temperatures are recorded on centigrade scale. The melting points were recorded on a Cambell Electronics - Thermonik instru ment in an open capillary.

2. The compound numbers, scheme numbers and reference numbers etc. given in each chapter refer to that particular chapter only. The references and spectra are given at the end of each chapter.

3. All solvents were distilled before use. Petroleum ether refers to the fraction boiling in the range 60-80°.

4. TLC analyses were carried out on glass plates using Silica gel : GF 254 Loba 254 (Loba Chem) and the plates were analysed by using a UV lamp or by keeping in an Iodine chamber or by spraying with $KMnO_4$ solution in acetone.

5. Optical rotations were measured at 25° on a JASCO-181-digital polarimeter using sodium light (5893A°) source at about 2% concentration.

6. The IR spectra were recorded on a Perkin-Elmer "Infracord - spectrometer using NaCl optics. ν max values are given in cm^{-1}.

7. The ^1H spectra were recorded on one of these instruments : Jeol-60, Bruker WH-90 (Spectrospin), Varian FT-80A, A.C. 200, Bruker MSL 300 using tetramethylsilane as the internal references. ^{13}C NMR spectra were recorded on A.C. 200 instruments. All chemical shifts are reported in δ units.

8. Mass spectra were recorded on a Finnigan MAT 1020 instrument.

9. Column chromatography was carried out using silica gel (60-120 mesh).

10. G.C. analysis was carried out on H.P. 5700 instrument using OV 101 column (6'x1/8").

ABBREVIATIONS

Ac_2O	Acetic anhydride
AcOH	Acetic acid
DBU	1,8-Diazabicyclo [5,4,0] undec-7-ene
DEAD	Diethylazodicarboxylate
DMF	N,N-Dimethylformamide
dppe	1,2-Bis (diphenylphosphino) ethane
GC	Gas chromatography
PPh_3	Triphenyl phosphine
PTC	Phase Transfer Catalyst
PTSA	p-Toluenesulfonic acid
TBAH	Tetrabutylammonium hydrogen sulfate
THF	Tetrahydrofuran
TEBA	Benzyl triethylammonium chloride
TDA-1	Tris (3,6-dioxaheptyl) amine

Abstract

Chapter 1 - General introduction to pyrethroids.

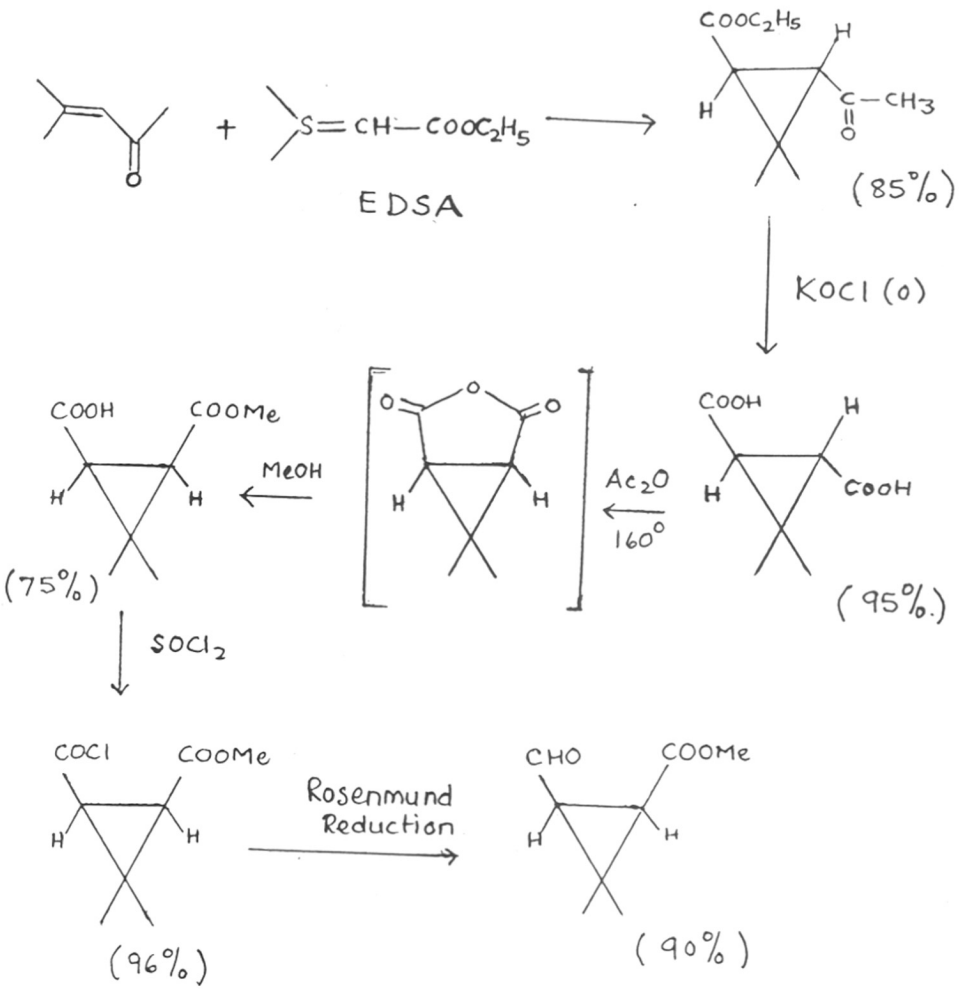
Pyrethrum obtained from the flower heads of Chrysanthemum cinerariaefolium, is a contact insecticide used since ancient times. The insecticidal activity of the pyrethrum extract is due to six active esters present in it. The two acids, (+)trans-chrysanthemic acid and (+)trans - pyrethric acid, constitute the acid component while pyrethrolone, cinerolone and jasmolone are the three alcohol components. Out of the six esters, Pyrethrin I is the most active of the natural pyrethroids.

Pyrethroid is a class of insecticides in which trans chrysanthemic acid is a common moiety. Pyrethroids, natural and synthetic, are important as insect control agents because they possess good insecticidal activity, low mammalian toxicity and rapid biodegradability. The disadvantage is that they are air and light sensitive. So while synthesizing new analogs of pyrethroids, efforts were put in to increase the photostability while keeping the required stereochemistry intact.

Different synthetic pyrethroids, their methods of preparation, structure activity correlation etc. are discussed in the first chapter.Allethrin was the first synthetic pyrethroid with strong insecticidal activity. Bioresmethrin, NRDC 132, Cypermethrin, Deltamethrin are a few more products of the same class.

Chapter 2 - An improved process for caronaldehyde ester

In this chapter an improved five step synthesis of caronaldehyde ester has been discussed. Mesityl oxide being a cheap starting material was condensed with the sulfur ylide - EDSA [Ethyl(Dimethyl sulfuranylidene)acetate] to get cyclopropanation. The yield achieved in this step was higher than the reported ones. Potassium hypochlorite oxidation yielded trans - caronic acid which was isomerised to cis - caronic acid half ester by the reported procedure. The acid chloride of the above cis analog was prepared.



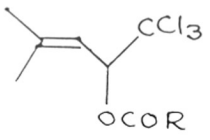
Conversion of this to the aldehyde was the most critical step. We have successfully achieved the Rosenmund reduction to get cis-caronaldehyde ester without any isomerisation to the trans - isomer. Here also the yields are excellent.

Chapter 3 - Enzymatic resolution of crucial allylic alcohol

Optically active R(-)1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene is the starting material for several potent agricultural pyrethroids such as NRDC182 and Cypermethrin. The chemical resolution of the racemic alcohol is difficult due to the low reactivity of the alcohol functionality.

In this chapter, a new enzymatic kinetic resolution of this alcohol has been discussed. This procedure leads to the required product with high chemical yields and optical purity (ee > 98%)

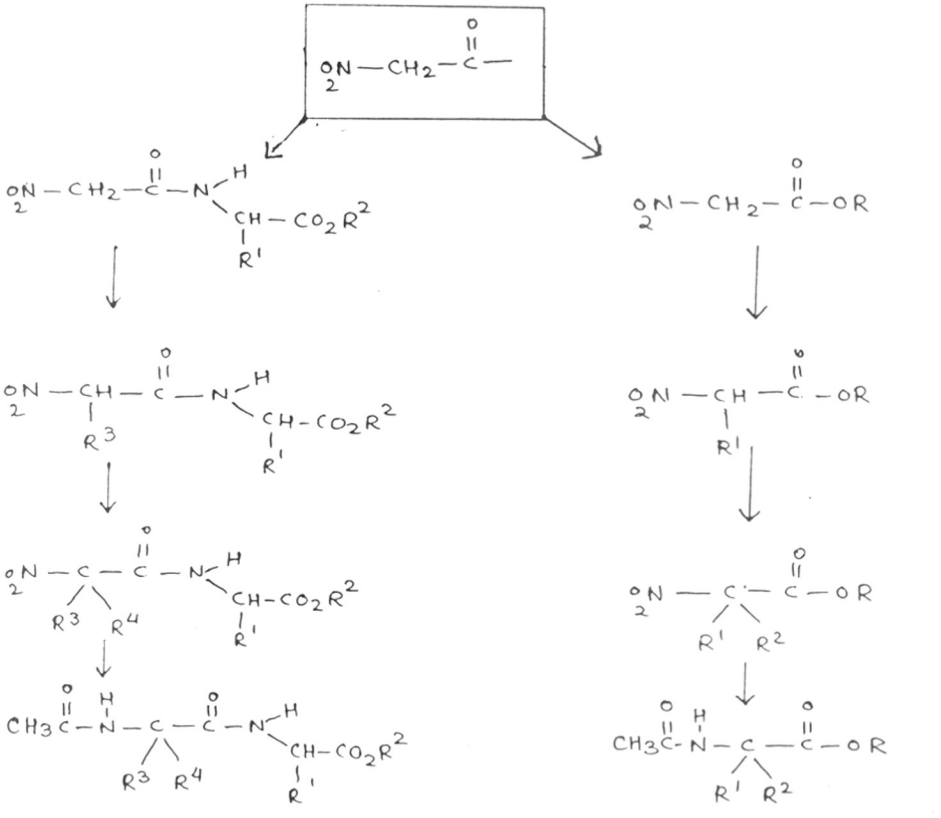
Different esters of the racemic alcohol were prepared and the esters were subjected to the enantioselective hydrolytic action of whole cell broth of Bacillus subtilis for 48 h. The acetate ester gave the best results. The alcohol, obtained after purification of the crude product, had optical rotation $(\alpha)_D$ = -12.0 with ee > 98%. The shift reagent $Eu(tfc)_3$ was used for enantiomeric excess determination.



Sr.No	R	% conversion by GC	$[\alpha]_D^{25}$	%ee
1	$-CH_3$	42	-12.0	98
2	$-C_2H_5$	40	-12.0	98
3	$-C_4H_9$	30	-11.6	96.6
4	$-C_7H_{15}$	12	-11.5	95.8
5.	C_6H_5	15	-11.9	99
6	$-C_6H_4pNO_2$	0	-	-
7	-H	100	-	-

Chapter 4 - Nitro acetyl group as a peptide synthon.

This chapter gives the background for the use of the nitroacetyl group as a peptide synthon. The use of aliphatic nitro compounds as building blocks has been on the increase recently. Our laboratory has been engaged in exploring the use of the nitroacetyl group as a peptide synthon. This group has two specific advantages. The methylene group present here is very reactive and alkylations can be done at this centre. Also the nitro group can be easily converted to other useful functional groups such as amine, oxime, keton e, hydroxylamine etc.

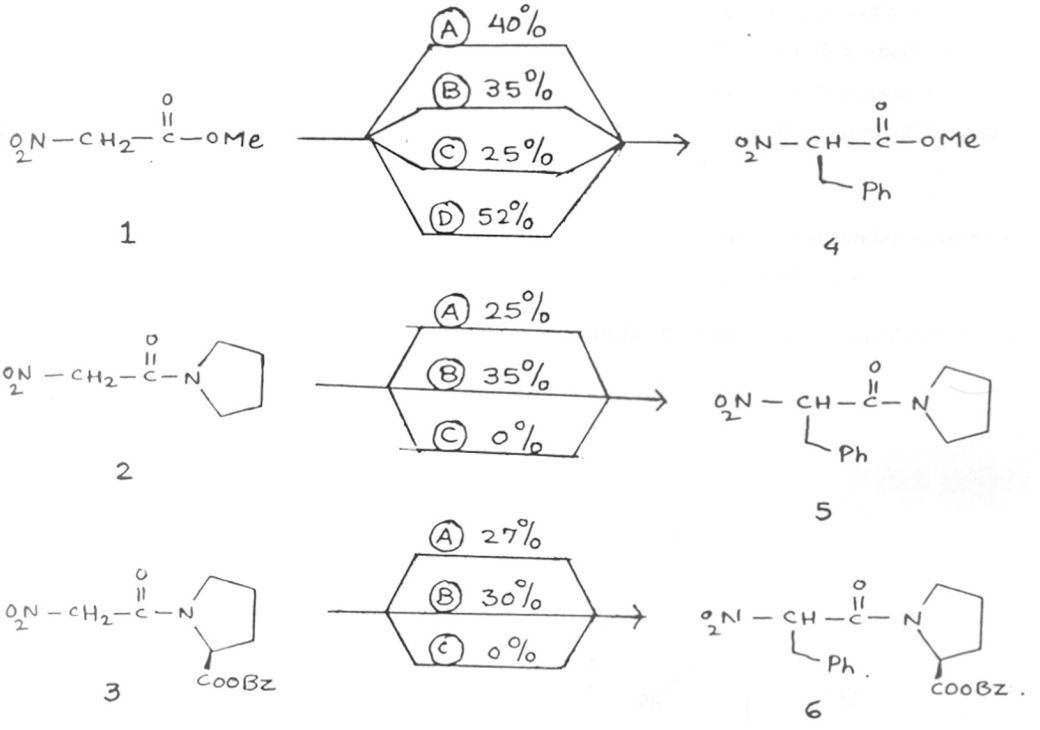


A dipeptide

N-acetyl amino acid derivative.

Chapter 5 - Alkylation of nitroacetic acid derivatives and Synthesis of phenylalanine derivatives

This chapter deals with the comparative study of four different methods of alkylation of nitroacetic acid derivatives



(A) PTC (TEBA), $PhCH_2Br$, $KHCO_3$, DMF, 60°.

(B) $PhCH_2Br$, DBU, CH_3CN, 60°.

(C) Mitsunobu, $PhCH_2OH$, PPh_3, DEAD, C_6H_6, 5-10°.

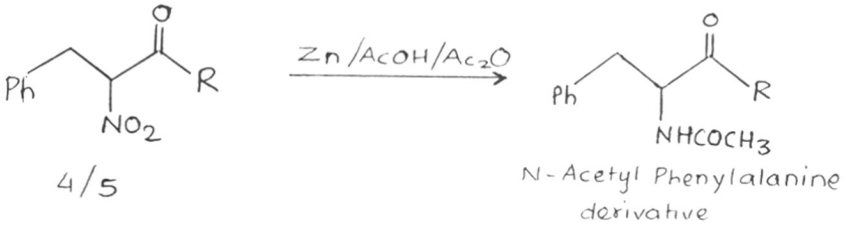
(D) i) Schiff's base of aromatic aldehyde

ii) $NaBH_4$ reduction.

In the case of methyl nitroacetate, all four procedures led to the required C-benzylated product. The best yield was obtained by the aldol condensation with benzaldehyde (in the form of its Schiff base) followed by borohydride reduction. Direct alkylation of methyl nitroacetate with benzyl bromide either under phase transfer conditions or in presence of the base DBU gave slightly lower yields. The Mitsunobu alkylation with benzyl alcohol gave the lowest yield. However, this is an extremely interesting result, since this is the first reported example of C-alkylation of a nitroalkane under Mitsunobu conditions; earlier reports (Tet.Lett., 1992, 33, 6723) have described exclusive O-alkylation under these conditions. The by-products in the reaction in our case have been identified as the oxime of glyoxalic ester and benzaldehyde; these arise by decomposition of O-benzyl nitronate.

In the case of nitroacetamides, we could not achieve any C-alkylation under Mitsunobu conditions, whereas conventional alkylation with benzyl bromide proved effective.

Reduction of the nitro group in compounds 4 and 5 with $Zn/Ac_2O/AcOH$ yielded N-acetyl phenylalanine derivatives.



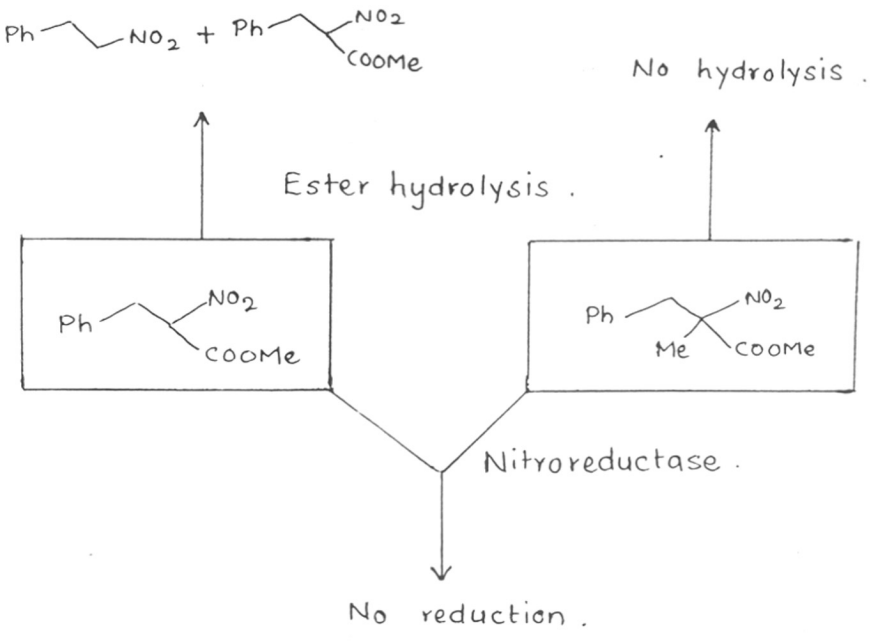
4/5

N-Acetyl Phenylalanine derivative

Chapter 6 - Attempted enzymatic transformations of some nitroacetic acid esters.

In this chapter the various attempts to obtain useful, optically active products from the mono and di alkyl methyl nitroacetates by enzymatic transformations have been discussed. 2-Nitro-3-phenylpropanoic acid methyl ester was subjected to enzymatic hydrolysis using B.subtilis. The hydrolysed acid decarboxylated to give 1-nitro-2-phenylethane. The unhydrolysed ester had no measurable optical rotation. The proton of the substrate ester is very acidic, this might have caused racemisation. To overcome this difficulty, the compound having a quaternary carbon adjacent to the nitro group was subjected to enzymatic hydrolysis. However, in this case, the substrate did not undergo hydrolysis at all.

Both the above mentioned substrates were subjected to anaerobic reduction using Clostridium sp. These attempts to reduce - NO_2 to - NH_2 were unsuccessful.



PART A

Synthesis of pyrethroid intermediates

CHAPTER 1

General introduction to pyrethroids

General Introduction to Pyrethroids

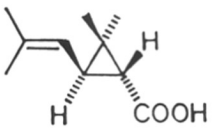
1.1 Introduction

The use of chemical insecticides to control pests has proved to be an essential part of our life. The use of such pesticides in agriculture has increased to a great extent in recent times. It is becoming increasingly evident that in addition to effectiveness in insect control, the insecticide should be non-toxic to humans and also to useful insects. The residues must dissipate fast. Also it must be favourably priced relative to its effectiveness.

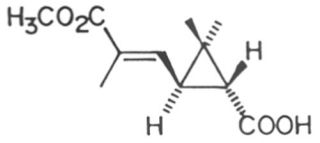
Pyrethroids are synthetic compounds which resemble natural pyrethrum in respect of structure, insecticidal activity etc. and satisfy most of the above requirements.

Since ancient times, pyrethrum[1] has been used as a contact insecticide. Pyrethrum is obtained from the flower heads of *Chrysanthemum cinerariefolium*. There are six active constituents of pyrethrum extract[2,3,4]. They are pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I and jasmolin II. These are esters[5] of two carboxylic acids (+) *trans* chrysanthemic acid and (+) *trans* pyrethric acid which are chemically (+) (1R)-*trans*- 3 (2-methylprop-1-enyl)-2,2-dimethylcyclopropane carboxylic acid and (+) (1R)-*trans*-3 (2-carbomethoxy prop-1-enyl)-2, 2-dimethyl-cyclopropane carboxylic acid respectively. The alcohol components of the esters could be any one of the three cyclopentenolones - pyrethrolone, cinerolone or jasmolone (Fig. 1).

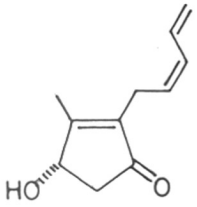
Out of these six esters, pyrethrin I and pyrethrin II together constitute 70% of the total pyrethrum extract. Of all the six esters, pyrethrin I is the most active of the natural pyrethroids.



(+) trans-chrysanthemic acid



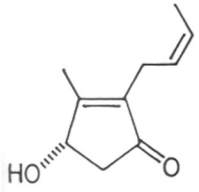
(+) trans-pyrethric acid



Pyrethrolone

Pyrethrin I

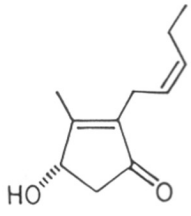
Pyrethrin II



Cinerolone

Cinerin I

Cinerin II



Jasmolone

Jasmolin I

Jasmolin II

Figure-1

1.2 Structure, Stereochemistry and Insecticidal Activity

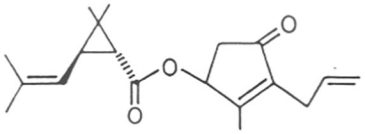
Pyrethrin possesses a unique combination of good insecticidal activity, low mammalian toxicity and high biodegradability. However, its use is limited mainly because of the high cost and photoinstability. Also after prolonged use insects develop resistance to the insecticide. Efforts were therefore directed towards the synthesis of pyrethroids bearing close resemblance to natural pyrethrins by making suitable changes in the alcohol as well as the cyclopropane - carboxylic acid moiety. The pyrethroids, thus obtained combine high and selective insecticidal activity, low mammalian toxicity, biodegradability and enhanced photostability.

Most of the pyrethroids have three chiral centres at C-1 and C-3 of the cyclopropane ring and the α-carbon of the alcohol. They can therefore exist in eight stereoisomeric forms. The stereochemistry at C-1 of cyclopropane is defined in absolute terms and for the C-3 carbon, in relative terms with C-1.

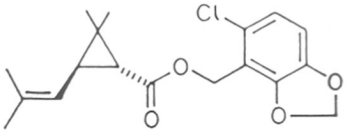
For high insecticidal activity the pyrethroid must have a precise steric relationship between an unsaturated centre in the alcohol moiety and the gem-dimethyl group or an equivalent substituent in the acid moiety[6]. This generally requires C-1 of the cyclopropane carboxylic acid to have the R configuration and the α-carbon of the alcohol part to have the S configuration.

Allethrin (**1**) was the first synthetic[7] pyrethroid with strong insecticidal activity which resulted by shortening the side chain of the alcohol part of natural pyrethrin. This indicated that the side chain in the alcohol moiety may be modified without loss of activity.

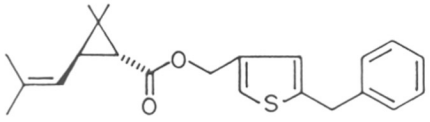
Barthrin[8](**2**) had better insecticidal activity than pyrethrin I. In this molecule the cyclopentenone ring of the alcohol was replaced by a substituted benzyl alcohol.



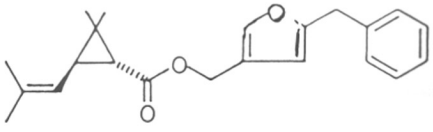
(1) Allethrin



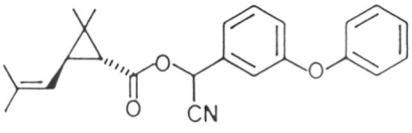
(2) Barthrin



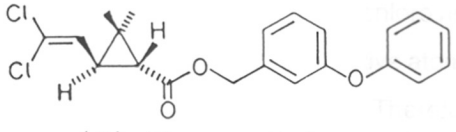
(3) Thiophenylmethyl chrysanthamate



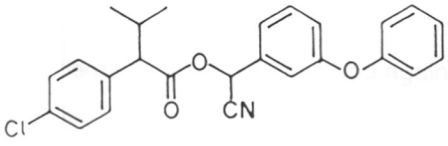
(4) Bioresmethrin



(5) Cyphenothrin



(6) Biopermethrin



(7) Fenvalerate

5-Benzyl-3-thienylmethyl chrysanthemate[9] (**3**) is one more example of this class which is superior to natural pyrethrin. Isobutenyl, *cis*-pentadienyl, allyl, furylmethyl and tetrahydropthalimidyl groups were identified as strongly photosensitive sites in pyrethroid molecules and these pyrethroids underwent rapid photodegradation[10,11]

In 5-benzyl-3-furylmethyl (1R-*trans*)-chrysanthemate i.e. Bioresmethrin[12] (**4**) the cyclopentenone ring was replaced by a benzylic system. It had high potency (LD_{50} = 0.005 μg/insect)

During the early 1970s, modifications were carried out with 3-phenoxybenzyl alcohol to develop photostable pyrethroids. An improved pyrethroid activity was observed in cyphenothrin[13] (**5**) where a cyano group was incorporated on the α carbon of 3-phenoxy benzyl alcohol.

3-Phenoxybenzyl alcohol proved to be an effective alcohol moiety in enhancing the photostability and insecticidal activity.

Trans-chrysanthemic acid is the naturally occurring one whereas *cis*- chrysanthemic acid does not occur in nature. Elliot[14] suggested that the *cis*- series of pyrethroids exhibit higher insecticidal activities.

The first photostable pyrethroid Permethrin[11] (Fig. 2) was reported after replacement of the isobutenyl side chain by a dichlorovinyl group. The dichlorovinyl side chain in which the double bond is stabilised by the two electronegative atoms, replaces the photosensitive isobutenyl unit of chrysanthemic acid. Therefore dihalovinyl pyrethroids appear extremely promising insecticides because of high activity combined with reasonable photostability and very low mammalian toxicity[11].

Biopermethrin (**6**) was as good against houseflies as bioresmethrin and still more active against mustard beetles.

A dramatic enhancement in the insecticidal activity was observed in cypermethrin (Fig.2) where a cyano group was incorporated at the α-carbon of 3-phenoxybenzyl alcohol.

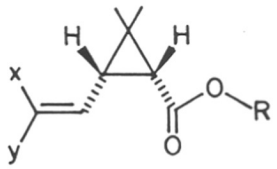
By modifying the photolabile zones, deltamethrin[15] (Fig.2) was synthesized which had high potency and increased photostability. It had (1R, 3R) *cis*- configuration in the acid moiety and α-S configuration in the alcohol part.

Deltamethrin could be used for crop protection, control of pests in stored products and in the fight against vectors of endemic diseases. Permethrin, cypermethrin and deltamethrin (Fig. 2) are in commercial use. NRDC-182[16] is a chloroanalog of deltamethrin (Fig. 2).

A second generation of pyrethroids[17] like cyhalothrin, baythroid, FMC 54800 have also come up (Fig. 2).

Most of the pyrethroids have rapid "knock-down" action on flying insects. Insecticidal action is interpreted as an ability to adopt a conformation in which all structural features essential for potency are appropriately oriented with respect to each other and to a complementary receptor.

Apart from the substituted cyclopropane carboxylic acid derivatives, some open chain compounds are also known to have appreciable insecticidal activity. Fenvalerate[18] (**7**) is one such example with activity comparable with permethrin.



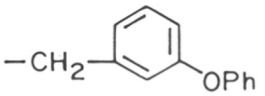
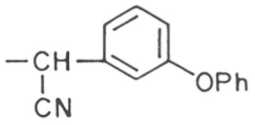
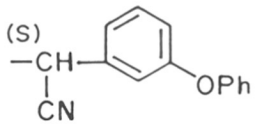
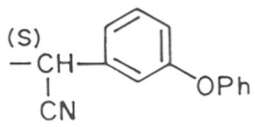
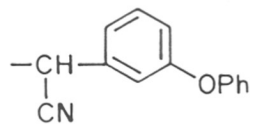
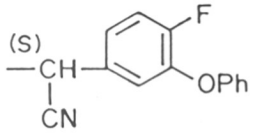
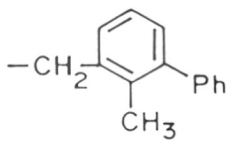
	x	y	-R
Permethrin	Cl	Cl	
Cypermethrin	Cl	Cl	
Deltamethrin	Br	Br	
NRDC-182	Cl	Cl	
Cyhalothrin	Cl	CF_3	
Baythroid	Cl	Cl	
FMC-54800	Cl	CF_3	

Figure-2

1.3 Synthesis

Due to the outstanding properties of the new pyrethroids several new synthetic routes for pyrethroids have been developed[19].

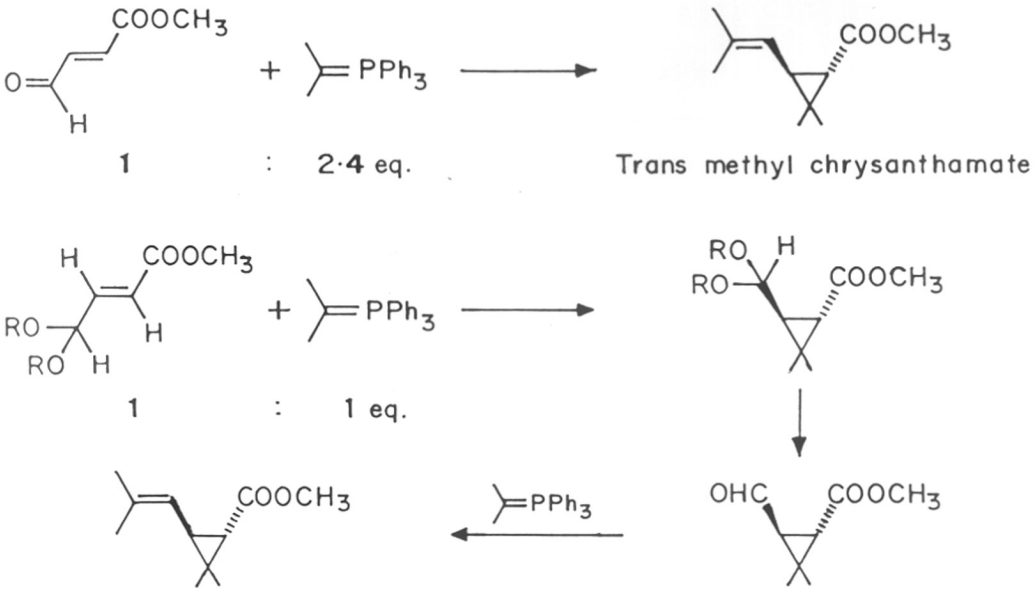
Cyclopropanation and olefin formation could be achieved in a one-pot reaction to produce *trans* methyl chrysanthemate[20] stereospecifically by simply mixing tetrahydrofuran solution of methyl *trans*-4-oxobutenoate and isopropylidene triphenyl phosphorane in the ratio 1:2.4. The ylid reacted first on the conjugated aldehyde in 1,2-mode when it was used in equivalent quantities. (Scheme I).

A Favorskii rearrangement of 2-chlorocyclobutanones leads to stereoselective pyrethroid acids. Dichlorovinylcyclopropane carboxylic acid was synthesized[21] by ring contraction of 2-chlorocyclobutanone as 80:20 *cis-trans* mixture. The *cis* compound predominantly leads to *cis* carboxylate in the rearrangement. The cyclobutanone was obtained by (2+2) cycloaddition of isobutylene with a ketene. This was then isomerised to the more stable isomer, which readily underwent a Favorskii rearrangement to predominantly *cis*-dichlorovinyl cyclopropane carboxylic acid by aqueous alkali (Scheme II).

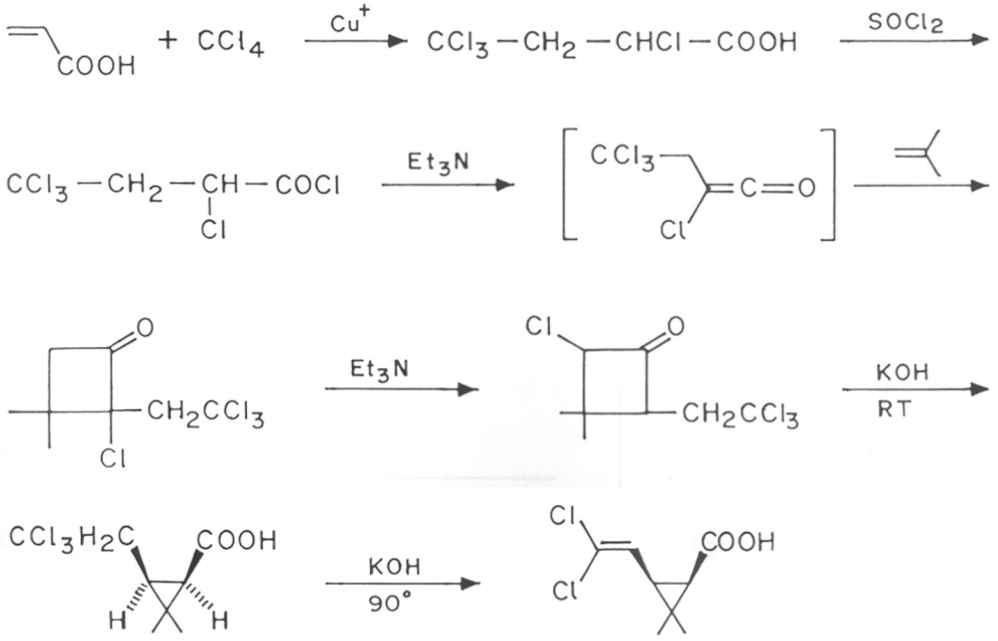
(1R)-*cis* Caronaldehyde[15] is a convenient starting material for pyrethroids. This molecule is very stable in its bicyclic form with two asymmetric carbons in the required geometry. After reacting with dibromomethylene triphenylphosphorane this gives (1R) *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (Scheme III).

It is possible to incorporate the bromine using bromoform instead of the Wittig reagent. The tribromohydroxy acid obtained lactonises under acidic treatment; subsequent metal reduction leads to the required (IR) *cis* 3 - (2,2-dibromovinyl) - 2,2 - dimethylcyclopropane carboxylic acid[15] (Scheme IV).

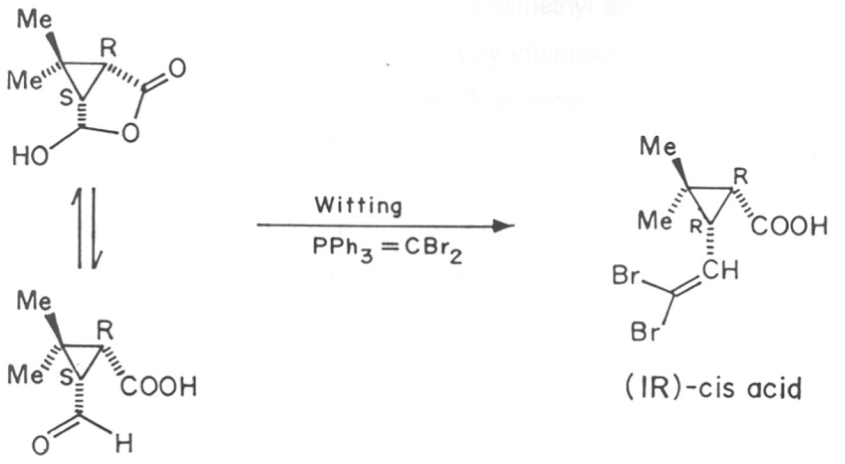
Scheme - I



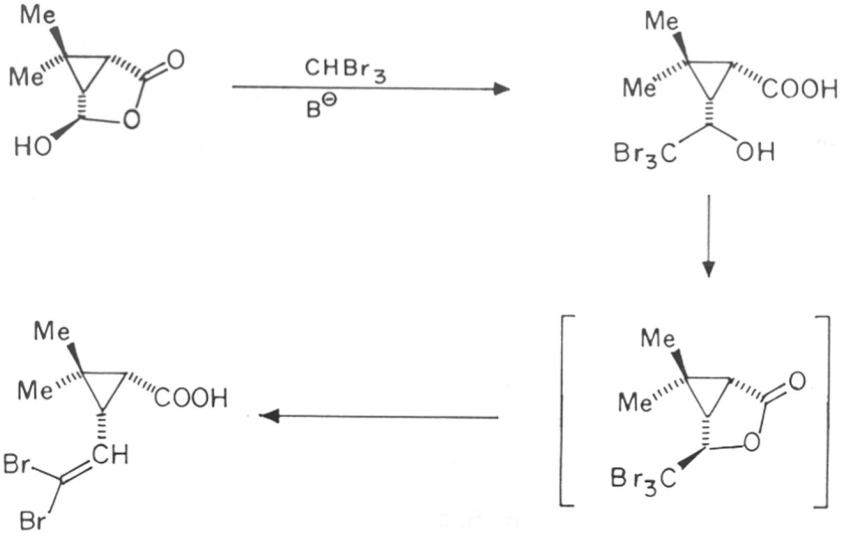
Scheme - II



Scheme - III



Scheme - IV



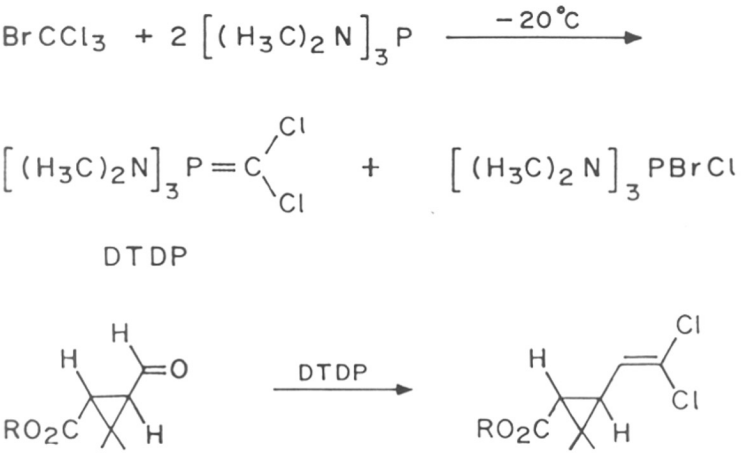
One more Wittig reagent[22] has been reported for use in the synthesis of pyrethroids. It is dichloromethylene tris (dimethyl amino)-phosphorane (DTDP) generated in dichloromethane solution by interaction of bromotrichloromethane with hexamethyl phosphorous triamide. The reagent is useful for substrates having bulky esters (e.g. t-butyl) (Scheme V).

A highly stereoselective synthesis of (1R, 3R) *cis* 3-(2,2-dichlorovinyl)2,2-dimethylcyclopropane carboxylic acid was devised by Hatch[16]. Here asymmetric reduction of 1,1,1-trichloro mesityl oxide with lithium aluminium hydride ephedrine complex produced (2R)-1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene. This was transformed to a diazoacetate via the corresponding diazoacetoacetate. Copper catalysed thermal decomposition of the diazoacetate resulted in stereospecific (2+1) cycloaddition of the carbenoid on to the olefin. The resultant bicyclic lactone was ring opened via Boord type reaction with Zn and acetic acid to afford (1R, 3R) *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid in 98% optical purity (Scheme VI).

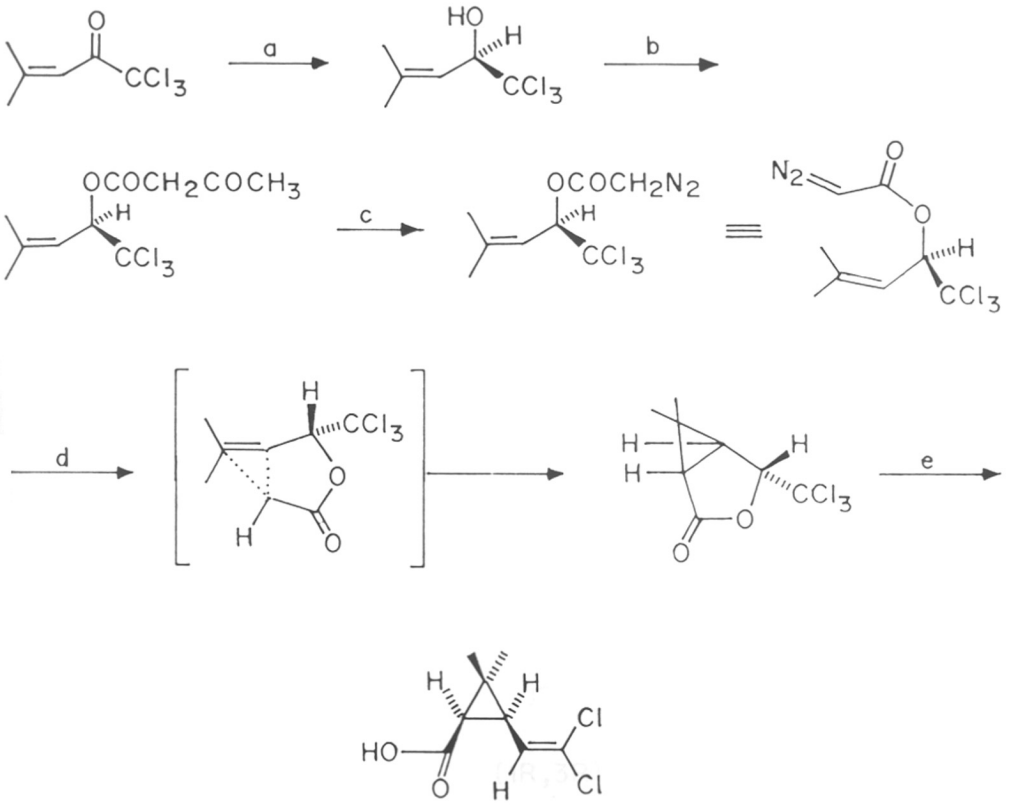
3-Formyl-2,2-dimethylcyclopropanecarboxylates were converted to more potent fluorinated pyrethroid analogs in the following manner[23]. The aldehyde was treated with trifluorotrichloroethane-zinc adduct and the product was acetylated. After reductive elimination with zinc (1R, 3S) cyhalothrins (with Z:E = 90:10) were obtained (Scheme VII)

Optically active (1R, 3R) *cis*-dihalovinylcyclopropane carboxylic acid was synthesized via intramolecular alkylation of a chiral enolate[24]. (R)-Valine was used as a chiral auxiliary for the preparation of the oxazolidone which was treated with sodium hydride followed by addition of 3,3-dimethyl-4-pentanoyl chloride. The product was reacted with $Fe(CO)_5$ in CCl_4 to afford a 3:2 isomeric mixture of addition products. The diastereomers (A) and (B) were separated by preparative HPLC. Ring closure with NaH was monitored separately for the two diastereomers. Ring closure was initiated by enolate formation. Diastereomer (B) gave major *cis*

Scheme - V

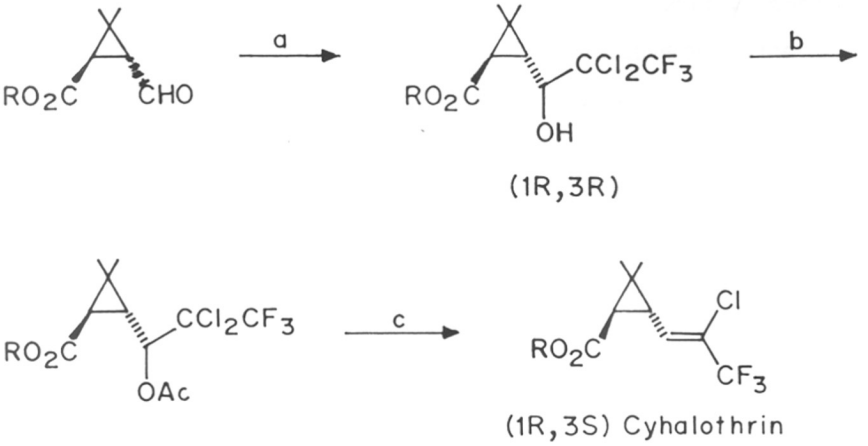


Scheme - VI



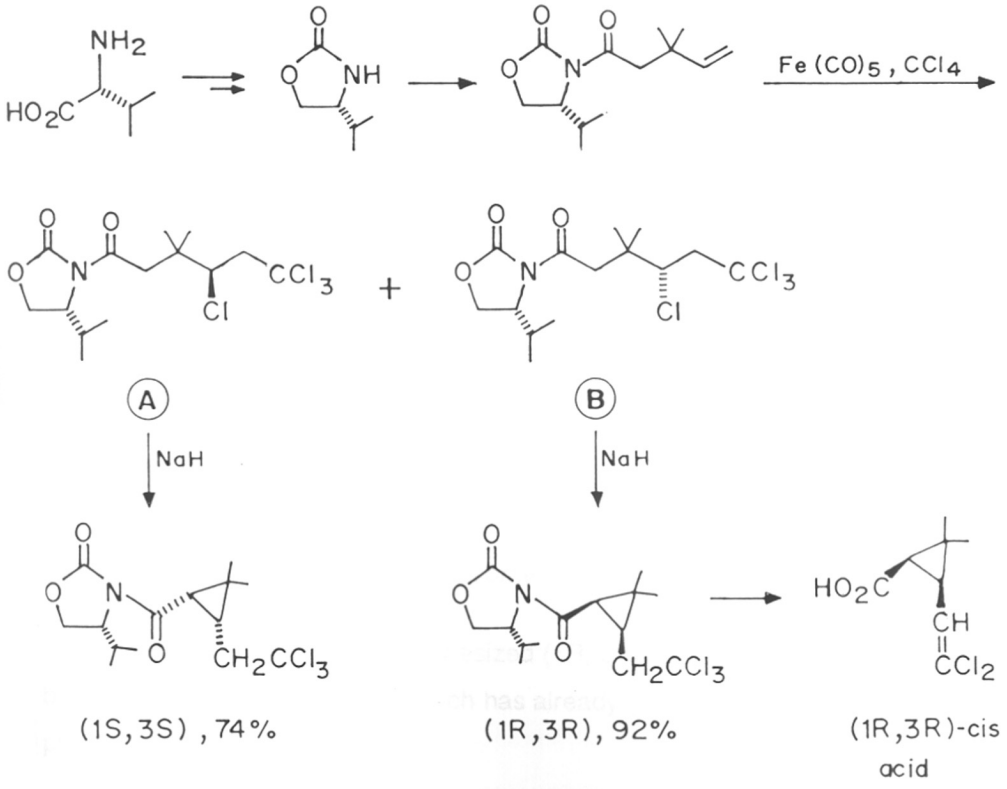
a : LAH - ephedrin ; b : diketene ; c : p-toluene sulfonyl azide or p-acetamido benzene sufonyl azide ; d : Cu (II), Δ ; e : Zn/AcOH

Scheme VII



a : CCl_3CF_3 , Zn, DMF ; b : Ac_2O, Py ; c : Zn, DMF

Scheme - VIII



stereochemistry on ring closure because of the low-energy transition state from the Z-enolate. The cyclisation is highly stereoselective due to the combined effect of facial differentiation of the enolate and steric influence of the chiral centre of the auxiliary. But the two factors oppose each other to get much less stereoselectivity in the case of diastereomer (A) (Scheme VIII).

Natural tartaric acid has been used recently by Krief[25] to synthesize methyl (1R, 3S) *cis*-hemicaronic anhydride stereospecifically. The γ-alkoxy-α,β-unsaturated esters (**13**) and (**14**) were prepared from tartaric acid.

The (E,E) stereoisomer[25] (**13**) after reacting with isopropylidene triphenyl phosphorane gave the compound with *trans* substituted cyclopropanes; this was then transformed to methyl *trans* (1R, 3R) - 2,2-dimethyl-3-formylcyclopropane carboxylate in 98% *ee*. However, similar treatment of the (Z,Z) isomer (**14**) gave only the racemic aldehyde.

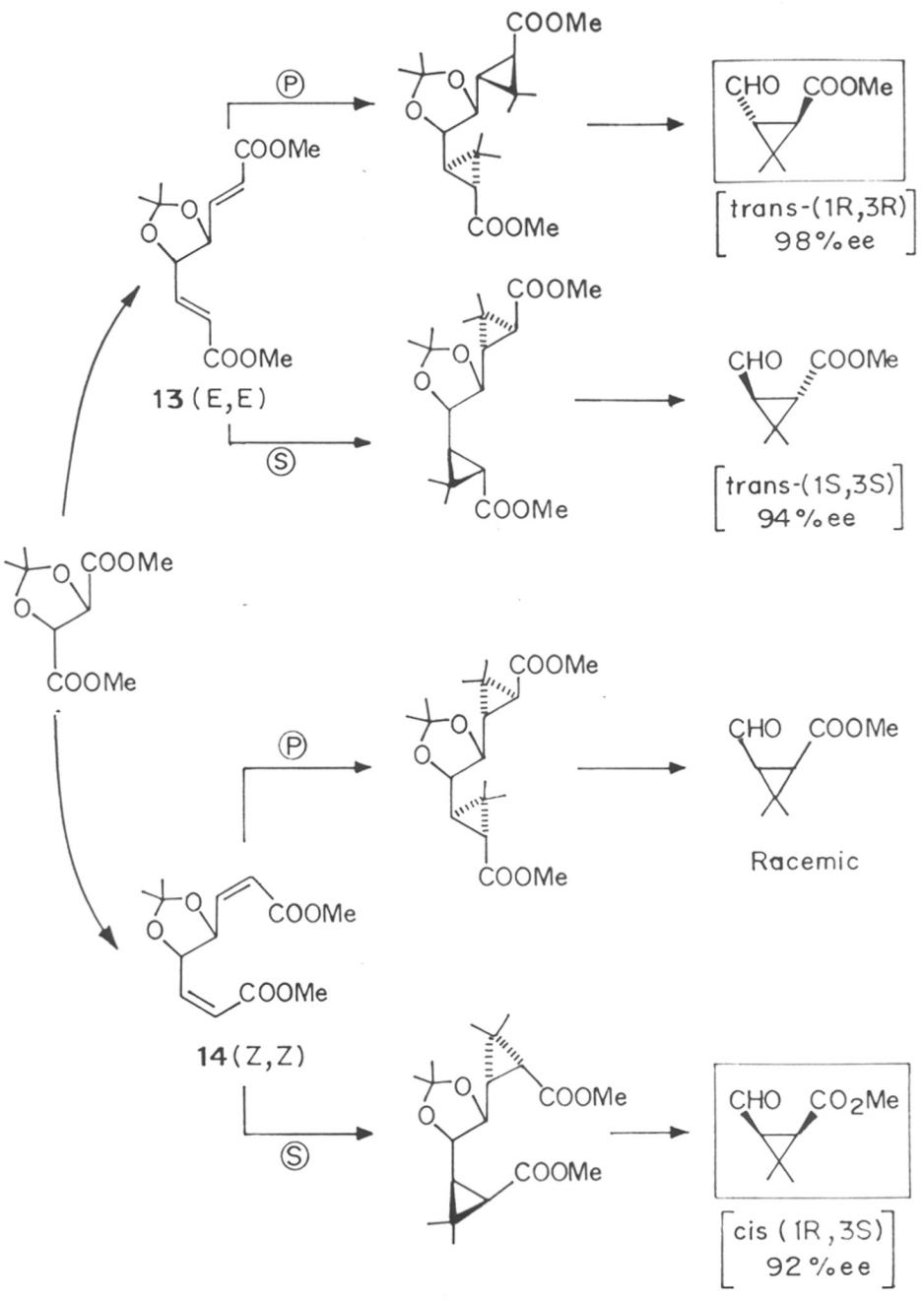
Surprisingly (Z,Z) stereoisomer (**14**) when reacted with isopropylidenediphenyl sulfurane gave *cis*-substituted cyclopropanation; this finally led to (1R, 3S)-*cis*-aldehyde in 92% *ee*. Similarly the (E,E) stereoisomer (**13**) with sulfur ylid gave (1S, 3S) *trans*-aldehyde in 94% ee.

Cyclopropanation with sulfur ylid is stereospecific while cyclopropanation with phosphorous ylid is stereoselective (Scheme IX).

Optically active natural products such as (+)3-carene (**8**) (1R, 5R) (+)- α-pinene (**9**), (2S) (+)-(**10**) and (2R) (-)-(**11**) pantolactones have been used successfully as starting materials for getting enantiomerically pure pyrethroid acids.

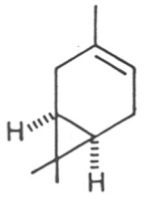
Matsui[26] for the first time synthesized (1R, 3R) (+)-*trans* chrysanthemic acid by degradation of (+) 3-carene which has already got a *cis*-fused (1R, 6S) cyclopropane ring.

Scheme - IX



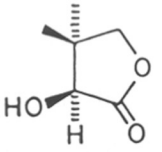
Ⓟ Isopropylidene triphenyl phosphorane

Ⓢ Isopropylidene diphenyl sulfurane

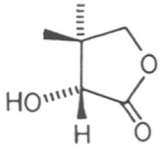


(8) : (+) 3-Carene

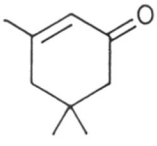
(9) : (1R,5R)(+) α-Pinene



(10) : (2S) (+) Pantolactone



(11) : (2R) (−) Pantolactone



(12) : Isophorone

Recently Mandal et al[27] reported successful conversion of (+) 3-carene to (1R)-*cis*-3-(dihalovinyl)-2,2-dimethyl-cyclopropane carboxylic acid. They also report the synthesis of (1R) *cis* (-) permethrin from (+) 3-carene.

A short synthesis[28] of (±) 3-(dihalovinyl)-2, 2 -dimethylcyclopropanecarboxylic acid, which is an important precursor for commercial permethrin and cypermethrin, from inexpensive isophorone (**12**) has also been reported.

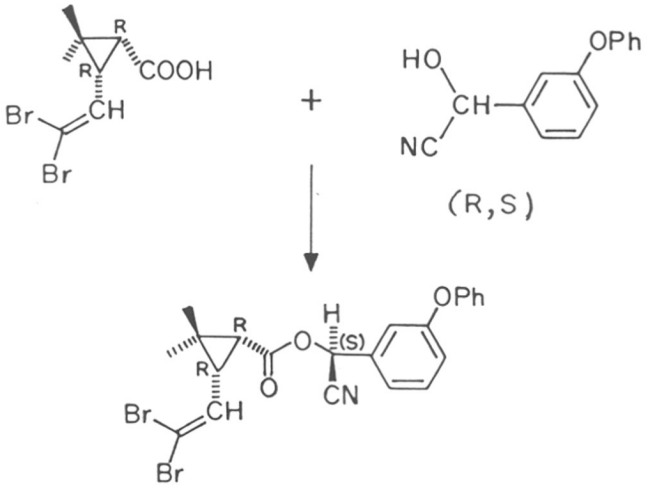
The first synthetic alcohol component for pyrethroids was allethrolone[7]. Natural pyrethrin contains α-S configuration in the alcohol part. Therefore the synthetic alcohol components were resolved to provide the pure (S)-enantiomer.

The cyanohydrin of 3-phenoxybenzaldehyde was found to be a very potent synthon for pyrethroids. The racemic aldehyde cyanohydrin was treated with the acid chloride of (1R, 3R) *cis*-3- (2,2-dibromovinyl)2,2-dimethylcyclopropane carboxylic acid. From this mixture of esters the α-S diastereomer crystallised out from hexane and the pure isomer was thus obtained[29] (Scheme X).

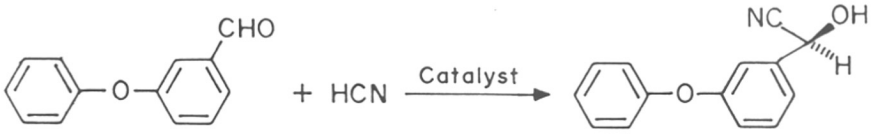
Recently[30] a cyclic dipeptide, cyclo-[(R)-phenylalanyl-(R)histidyl] has been used as catalyst for enantioselective autoinduction in asymmetric hydrocyanation of m-phenoxybenzaldehyde to get the (S)-cyanohydrin with 92% *ee*. The probable role of the cyclodipeptide is as follows: The carbonyl of the aromatic aldehyde forms a hydrogen bond with the N-H of the central ring. The imidazole gets protonated by HCN and CN^- attacks from *Si* face of the activated carbonyl group. The *Re* face is blocked by the phenyl ring of phenylalanine. (Scheme XI). However, in order to get the required S-cyanohydrin, the unnatural (R)aminoacids have to be used.

Asymmetric hydrocyanation has been achieved using a peptide titanium complex by Inoue[31]. Here a mixture of titanium ethoxide and a hindered Schiff

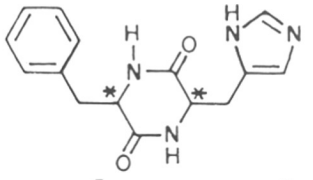
Scheme - X



Scheme - XI



(S) Cyanohydrin with 92% ee in 97% yield



Catalyst : Cyclo [(R) Phe-(R)His]

Scheme - XII

3,5 Dibromosalicylaldehyde + (S)-Valine piperidine ⟶

Hindered Schiff base $\xrightarrow{Ti(OEt)_4}$ Titanium complex (Catalyst)

PhCHO + HCN $\xrightarrow{\text{Catalyst}}$ (S)-Cyanohydrin
93 % yield, 93 % ee

(S) Amino acid (Natural) ⟶ (S)-Cyanohydrin (Required)

base formed from the amino group of (S)- Valine piperidide catalyses enantioselective hydrocyanation to provide (S)-cyanohydrin in 93% yield with 93% *ee*. The noteworthy point is that the natural (S) amino acid is used to get the required (S) cyanohydrin (Scheme XII).

Cyanohydrins have been reported to be resolved using enzymes[32]. However, the unwanted R-enantiomer is the one which is obtained by this method.

The worldwide research on pyrethroid over five decades has brought out potent insecticides non-toxic to man, biodegradable and photostable. However, there is always a pressing need to improve their performance to meet the challange in future of feeding the world and preserving the environment.

Finally, the success of the newer pyrethroids will depend upon their ability to match or surpass the performance of the older compounds or to offer a broader or different spectrum of biological activity.

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CHAPTER 2

An improved process for caronaldehyde ester

2.1 Summary

(±) *Cis*-3-formyl-2,2-dimethyl-1-cyclopropanecarboxylic acid and its esters (**1**) are the most valuable intermediates for the synthesis of pyrethroids. In this chapter we report a highly efficient synthesis of (±) *cis*-caronaldehyde methyl ester starting with cheap and easily available materials and employing operationally simple reactions.

Mesityl oxide was condensed with the sulfur ylid - ethyl (dimethylsulfuranylidene) acetate [EDSA] to achieve cyclopropanation in improved yields. The keto ester thus obtained was oxidised to *trans* caronic acid by potassium hypochlorite in high yields. The *trans* caronic acid was converted to *cis* caronic acid mono ester through caronic anhydride by a known route. The acid chloride of the mono ester was prepared using thionyl chloride. A significant step in this synthesis is the highly efficient reduction of this acid chloride to the required *cis*-caronaldehyde methyl ester by Rosenmund reduction in excellent yields without any isomerisation to the *trans* isomer.

2.2 Introduction

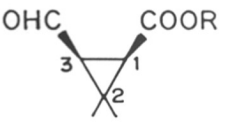
During the progress of research on synthetic pyrethroids[1] many important investigations have been carried out on the relation between structure, stereochemistry and insecticidal activity. Photoinstability and high cost were the major drawbacks of the natural pyrethrins. The isobutylene sidechain of the chrysanthemic acid was proved to be the photolabile centre in these molecules.

It was suggested by Elliott[2] that the series in which the vinyl side chain and the carboxylic acid attached to the cyclopropane ring were *cis* to each other exhibits higher insecticidal activity than the natural *trans* series. When the isobutylene sidechain was replaced by a dihalovinyl group, there was an enhancement in the photostability of the acid moiety. *Cis*-3 dihalovinyl-2,2-dimethylcyclopropane carboxylic acid (**2**) had a half life of 10 days whereas that of *trans*-chrysanthemic acid (**3**) was only 5 hours[3].

In the first phase of research chrysanthemic acid and its *cis* dihalovinyl analogues were the target molecules. *Cis* caronaldehyde (**1**) is an important intermediate for synthetic pyrethroids, since the introduction of a substituted vinyl group at the aldehyde function can lead to any desired pyrethroid, with the *cis* geometry at the cyclopropane ring. Pyrethroids in which *cis* stereochemistry is necessary for maximum insecticidal activity, include most of the commercially important products like deltamethrin (**4**) and cyhalothrin[5] (**5**) possessing acaricidal activity.

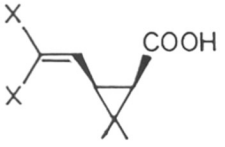
Synthesis

Many stereospecific and enantiospecific routes for the synthesis of caronaldehyde and its esters have been reported.



(±) cis-caronaldehyde

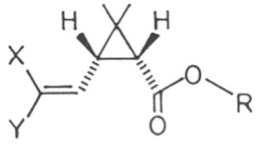
(1)

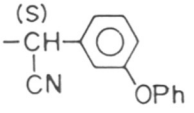
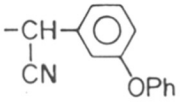


(2)

trans-chrysanthemic acid

(3)



	x	y	-R
deltamethrin (4) :	Br	Br	
cyhalothrin (5) :	Cl	CF_3	

In the beginning caronaldehyde ester[6] was produced from the appropriate chrysanthemate by ozonolysis in acetic acid followed by reductive decomposition with zinc (Scheme I)

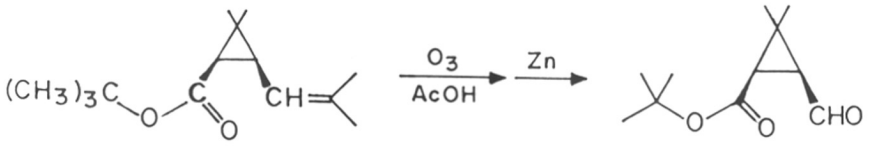
(±) *Trans*-caronaldehyde methyl ester (**6**) was synthesised for the first time stereospecifically by simply mixing tetrahydrofuran solutions of methyl *trans*-4-oxobutenoate (**7**) (as the acetal) and isopropylidenetriphenylphosphorane in equimolar quantities (Scheme II)[7].

Isopropylidenedimethylsulfurane has also been used as the cyclopropanation agent[8]. α-Butenolide (**8**) was the starting material. When this was reacted with the sulfurane, a bicyclic lactone was formed which was opened up by alkali treatment to get the alcohol. The alcohol was oxidised by Collins reagent to get (±) *cis* caronaldehyde methyl ester (**9**) (Scheme III).

When isopropylidenediphenylsulfurane was reacted with methyl 4-(tetrahydropyranyl-oxy)2-Z-butenoate (**10**) stereospecific *cis* cyclopropanation took place (Scheme III). *Trans*-caronaldehyde methyl ester (**6**) has also been produced starting from either maleic or fumaric acid[9]. Both the diesters on reacting with isopropylidenetriphenylphosphorane led to the cyclopropane derivative with *trans* stereochemistry. The *trans* caronic acid diester was then partially hydrolysed and the carboxylic acid was reduced to the alcohol by diborane. The alcohol was oxidised with chromic acid-pyridine to get *trans*-caronaldehyde methyl ester (**6**) in 66% yield (Scheme IV).

(1R,3S) *cis*-caronaldehyde ester (**11**) was obtained in 99.6% enantiomeric excess by resolution starting from prochiral caronic anhydride[3] (**12**). Caronic anhydride was converted to *cis*-caronic acid monoester and this monoester was resolved using optically active α-methyl benzyl amine to get the (1R, 3S) *cis*-enantiomer (**13**) as its ammonium salt in 25% yield. It was reduced to the alcohol and the alcohol was oxidized to (1R, 3S) *cis*-caronaldehyde dimethyl ester (**11**).

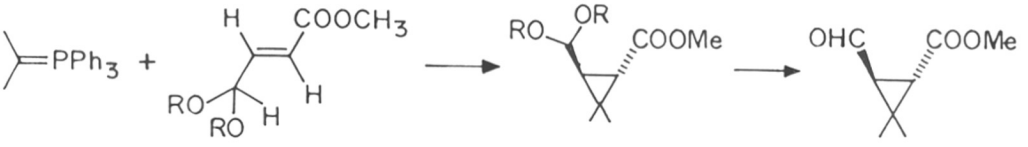
Scheme - I



(±) cis-chrysanthemate

(±) cis-caronaldehyde t-butyl ester

Scheme - II

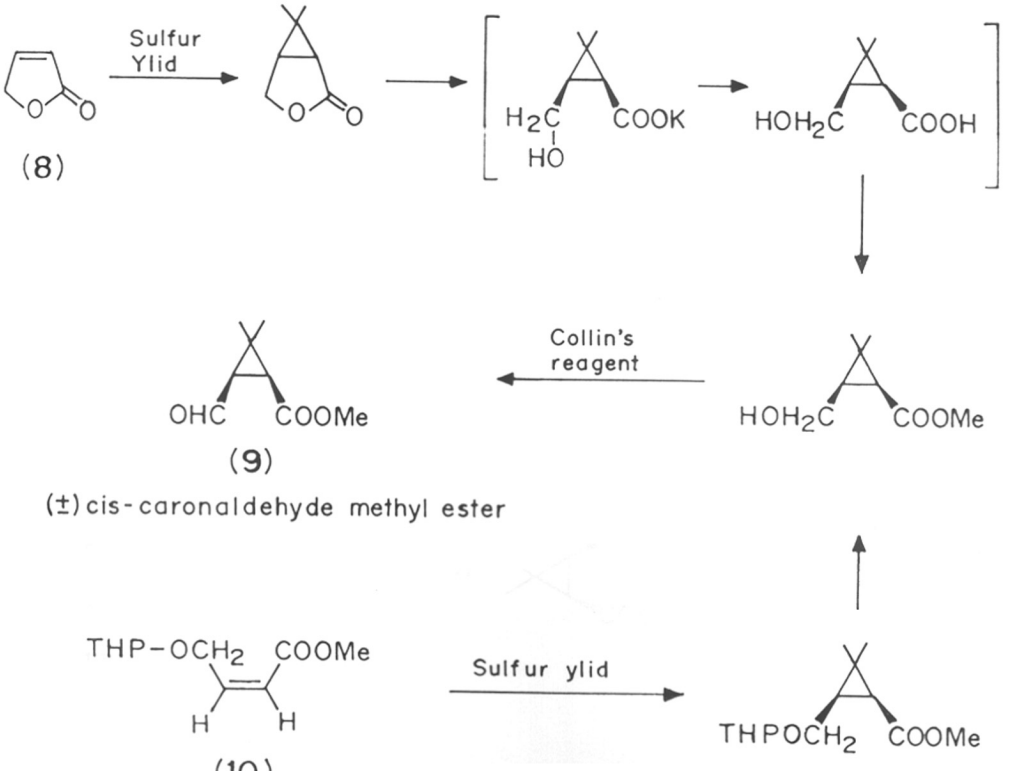


(7)

(6)

(±) trans-caronaldehyde methyl ester

Scheme - III

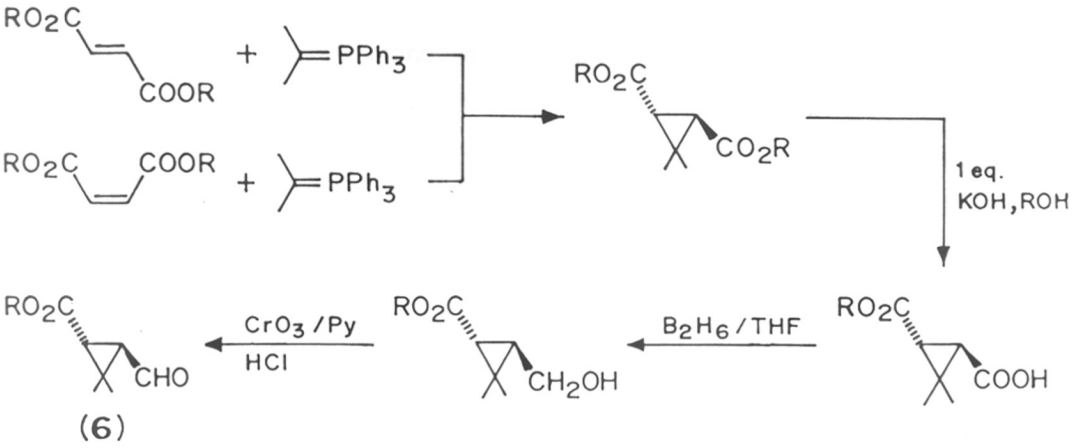


(9)

(±) cis-caronaldehyde methyl ester

(10)

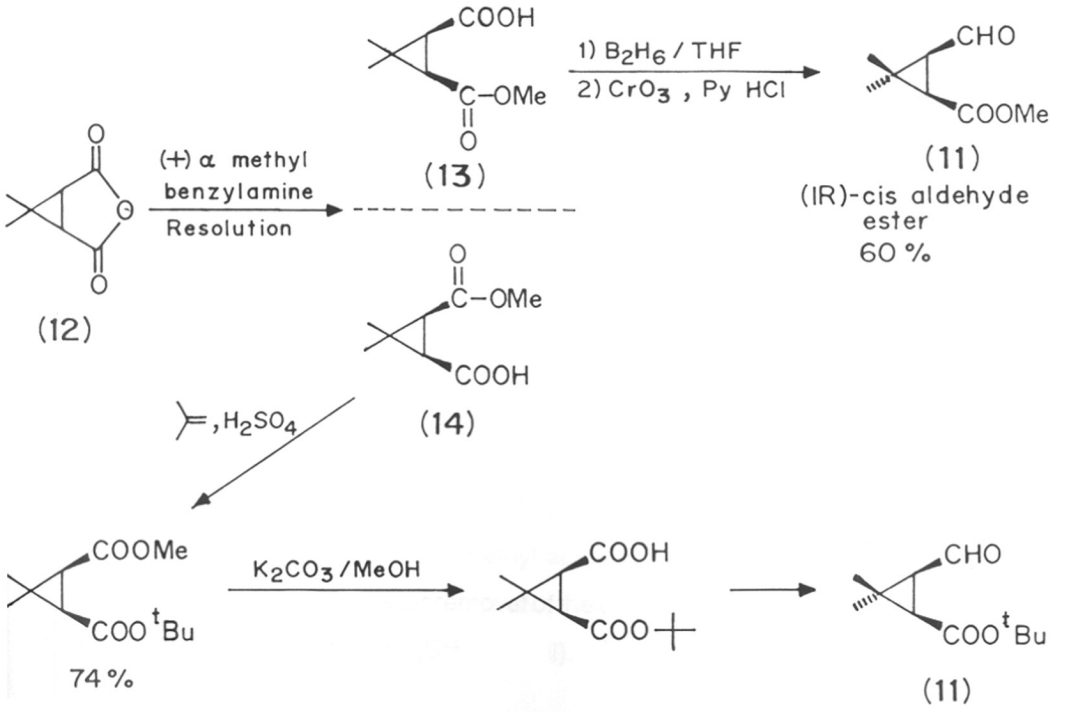
Scheme-IV



(6)

Trans-
caronaldehyde ester

Scheme-V

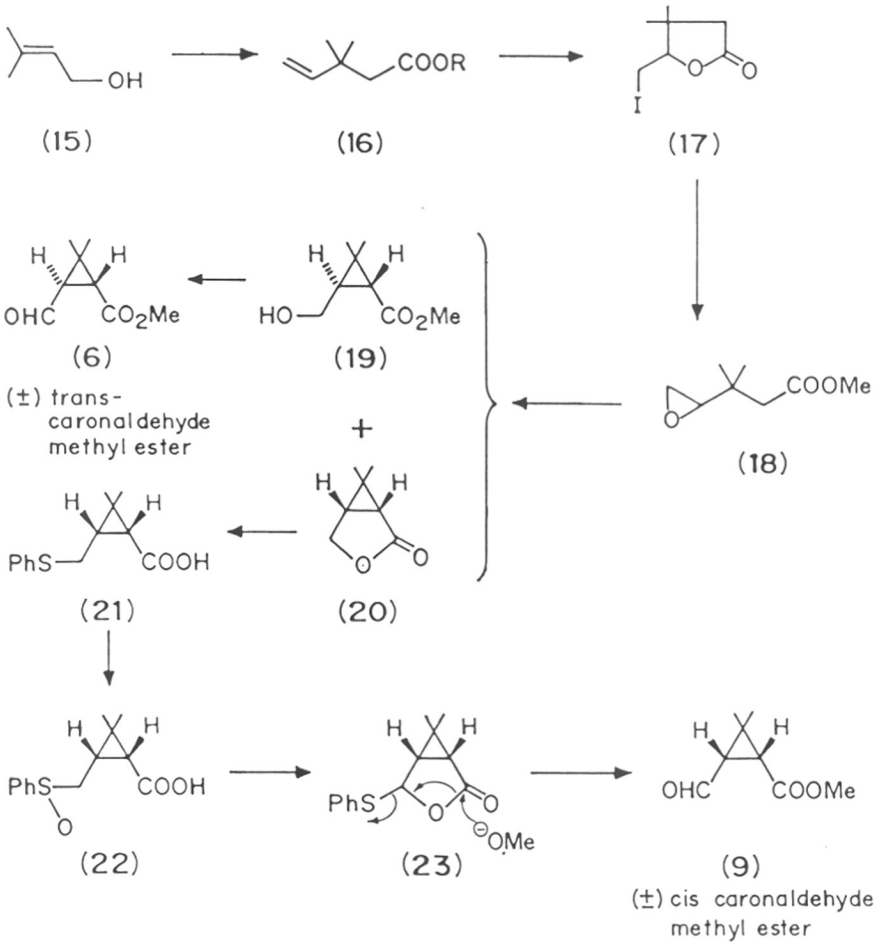


The unwanted (1S, 3R) monoester (**14**) separated by resolution was converted to the (1R,3S) *cis*-isomer as follows : The monoester was converted to the optically active mixed ester (1R, 3S)-t-butyl, methyl diester; selective hydrolysis of the methyl ester followed by the usual two step conversion of COOH to CHO yielded (1R, 3S) *cis*-caronaldehyde t-butyl ester (11) in 99.6% *ee* (Scheme V).

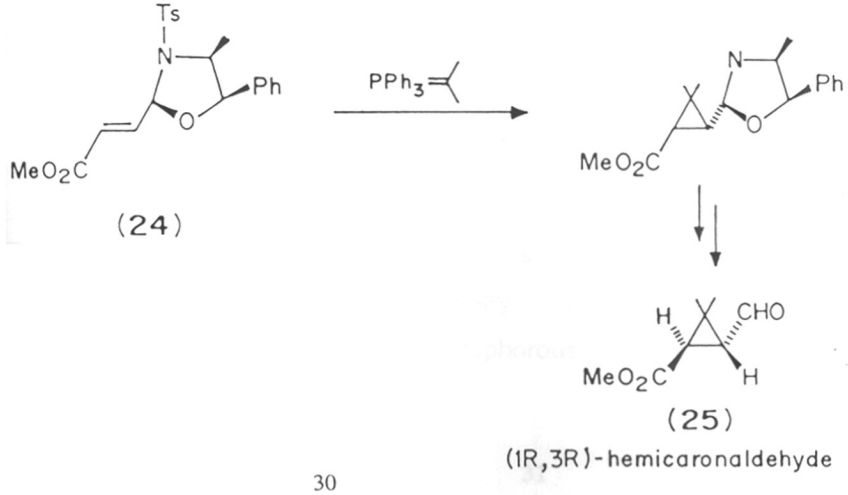
Another synthesis of (±) *trans*-and (±) *cis*-caronaldehyde derivatives starts from prenyl alcohol[10]. In this sequence, prenyl alcohol (**15**) was first converted to 3,3-dimethyl-4-pentenoic acid (**16**) which on treatment with iodine and potassium iodide in 0.5N aqueous sodium hydrogen carbonate underwent iodolactonization to yield the iodolactone (**17**). This on methanolysis in presence of potassium carbonate yielded the epoxyester (**18**). Cyclopropane ring formation via intramolecular attack on the epoxide gave methyl *trans*-3-hydroxymethyl-2,2-dimethylcyclopropanecarboxylate (**19**) and *cis*-3-hydroxymethyl-2,2-dimethylcyclopropane 1-carboxylic acid lactone (**20**) in the ratio 3:1. The *trans*-hydroxyester (**19**) was oxidised to (±) *trans*-caronaldehyde methyl ester (**6**) by pyridinium chlorochromate. The *cis*-lactone was opened up by thiophenol to get the sulfide acid (**21**) which was then oxidised to the sulfoxide (**22**). Pummerer rearrangement of this by treatment with trifluoroacetic anhydride gave the lactone hemithioacetal (**23**) which on stirring with iodine in methanol gave (±) *cis*-caronaldehyde methyl ester (**9**) (Scheme VI)

A short and highly enantioselective route for (1R, 3R) *trans*- hemicaronaldehyde methyl ester has been reported by reacting three equivalents of isopropylidenetriphenylphosphorane with the pure diastereomer of the oxazolidine (**24**) (prepared by cyclisation of N-tosyl (1R, 2S)-norephedrin and the appropriate α, β-unsaturated aldehyde ester (dimethyl acetal). Cyclopropanation took place in enantioselective manner. After removal of the chiral auxiliary (1R, 3R)-hemicaronic aldehyde (**25**) was obtained. (Scheme VII).

Scheme - VI



Scheme - VII

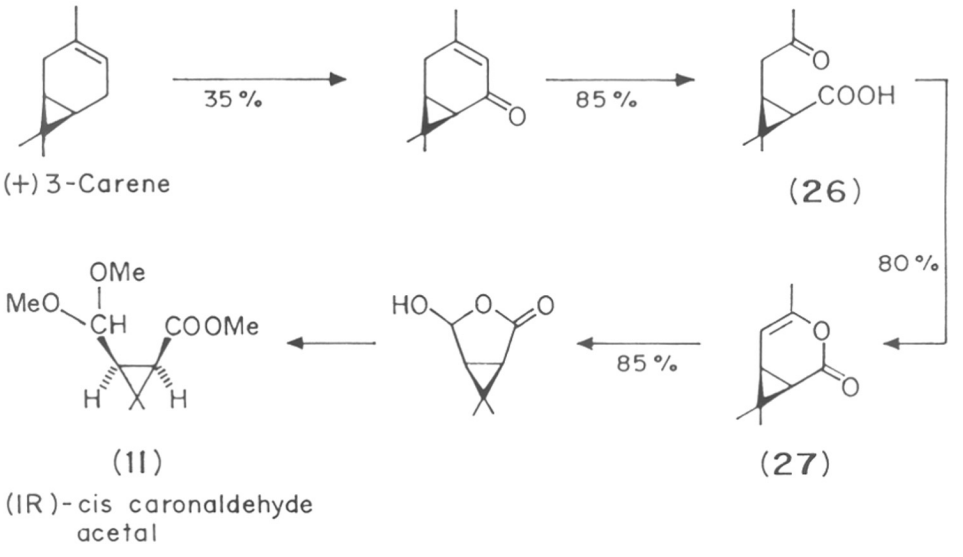


Naturally occurring (+)-3-carene has been converted to (-) *cis*-caronaldehyde hemiacetal by Sukh Dev[12]. (+) 3-Carene was first oxidised to car-3-ene-5-one by a simple transition metal catalysed air oxidation. It was further converted to the keto acid (**26**) by ozonolysis. This keto acid when exposed to acetic anhydride in presence of a macroreticular sulphonic acid resin furnished the enol lactone (**27**) which again after ozonolysis yielded (1R,3S)-*cis*-Caronaldehyde methyl ester (**11**) as the acetal (Scheme VIII).

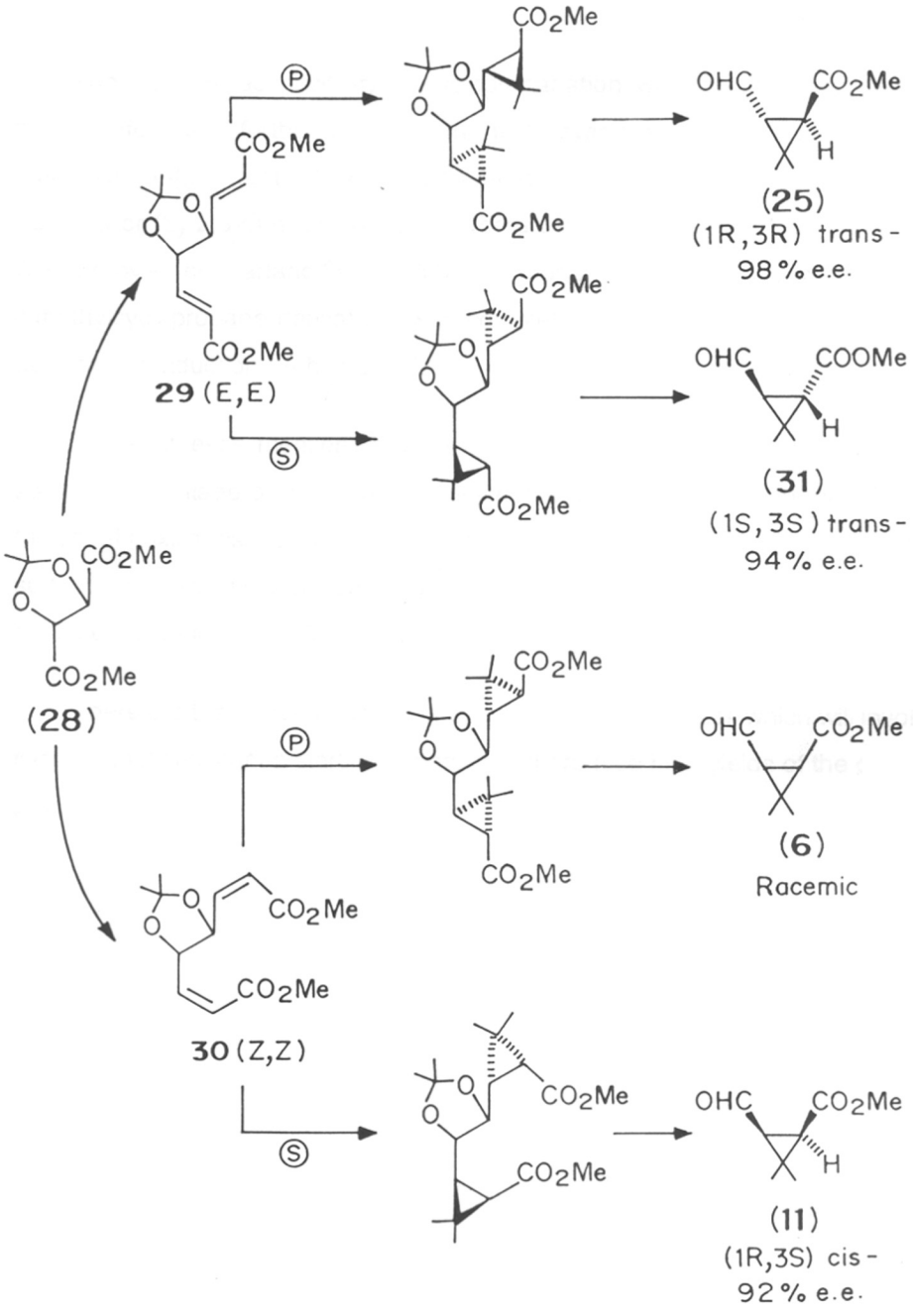
(1R, 3S)-*cis*-Caronaldehyde (**11**) and (1R, 3R) *trans*-caronaldehyde (**25**) have been synthesized enantioselectively starting from naturally occurring tartaric acid[13]. A. Krief has carried out a comparative study of phosphorous ylid and sulfur ylid as cyclopropanation agents. The substrate for the reaction was the γ-alkoxy-α,β-unsaturated ester prepared from dimethyl (2R, 3R)-O-isopropylidene tartarate (**28**). The (E,E) (**29**) and (Z,Z) (**30**) γ-alkoxy-α,β -unsaturated esters were reacted separately with the two ylids. When isopropylidenetriphenylphosphorane was reacted with the (E,E) olefin (**29**) *trans*-cyclopropanation took place which finally produced methyl *trans*-(1R, 3R)-2, 2-dimethyl -3-formylcyclopropane carboxylate (**25**) in high enantiomeric excess. The ylid when reacted with (Z,Z) olefin (**30**) finally gave the racemic aldehyde (**6**). Isopropylidenediphenylsulfurane when reacted with the (Z,Z)olefin (**30**) gave *cis* cyclopropanation which was tranformed to methyl *cis*-(1R, 3S)-3-formyl-2,2-dimethyl cyclopropanecarboxylate (**11**) in high enantiomeric excess. The same ylid when reacted with the (E,E) olefin (**29**) gave *trans* cyclopropanation which ultimately produced *trans*-caronaldehyde (**31**) in high enantiomeric excess.

With both (E,E) and (Z,Z) olefins, the phosphorous ylid achieves *trans*-cyclopropanation. But the sulfur ylid produces the cyclopropane with *cis*- stereochemistry when the substrate olefin has Z geometry and the *trans* cyclopropane when the olefin has the E geometry. The reaction with the sulfur ylid is stereospecific while that with the phosphorous ylid is stereoselective (Scheme IX).

Scheme - VIII


35 %
85 %
COOH
(+) 3-Carene
(26)
80 %
OMe
MeO
CH
COOMe
H
H
HO
O
O
85 %
(11)
(27)
(1R)-cis caronaldehyde
acetal

Scheme - IX

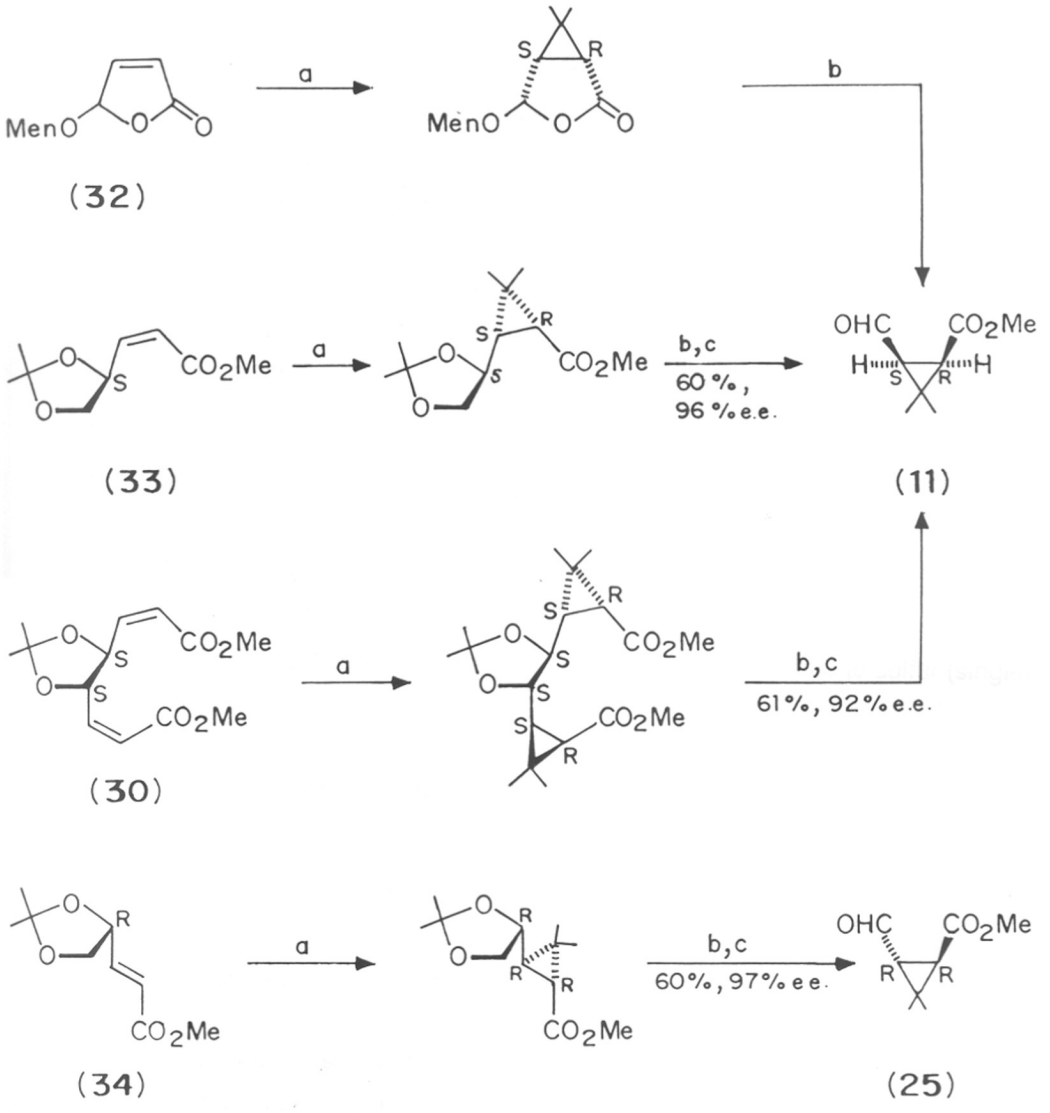

CO2Me
Ⓟ
CO2Me
CO2Me
29 (E,E)
Ⓢ
CO2Me
CO2Me
OHC
CO2Me
H
(25)
(1R,3R) trans -
98% e.e.
CO2Me
CO2Me
OHC
COOMe
H
(31)
(1S, 3S) trans -
94% e.e.
CO2Me
CO2Me
(28)
Ⓟ
CO2Me
CO2Me
OHC
CO2Me
(6)
Racemic
CO2Me
CO2Me
30 (Z,Z)
Ⓢ
CO2Me
CO2Me
OHC
CO2Me
H
(11)
(1R,3S) cis -
92% e.e.

The high stereospecificity in cyclopropanation with the isopropylidenediphenylsulfurane is further illustrated in the following examples. The sulfurane reacts with 4-[(L)-menthyl] oxy-2-buten-4-olide (**32**) and γ-alkoxy esters derived from (R) or (S)-2,3-O-isopropylidene glyceraldehyde(**33**),(**34**) or from (R,R)-2,3-O-isopropylidene tartaric acid (**30**) to produce the corresponding gem-dimethylcyclopropane derivatives with complete stereoselectivity and very high asymmetric induction (Scheme X)[14].

The syntheses mentioned above although quite ingenious do suffer from some disadvantage or the other. Sometimes the reagents, starting materials or the chiral auxiliaries are too costly. Sometimes the number of steps is too many so that the overall yield becomes too low. The experimental conditions in some cases are laborious and dangerous for scale up work.

Therefore there was a need to develop a synthetic process which will involve simple reactions, cheap starting materials and produce high yields of the desired products.

Scheme - X



Men = (L) Menthyl

a = $Me_2CLi - \overset{+}{S}Ph_2 \ \bar{B}F_4$, DMF, -78°C, 2h

b = aq. $HClO_4$ (2·5M), THF, 20°C, 3-6 h

c = $NaIO_4$, MeOH / Phosphate buffer (pH 7), 20°C, 1h

2.3 Present work and discussion

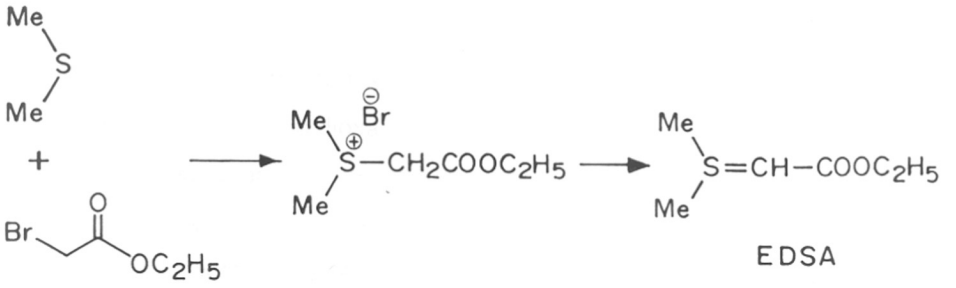
We have developed a high yield process using the cheap and easily available mesityl oxide (**35**) as the starting material. The sulfur ylid, ethyl (dimethylsulfuranylidene) acetate - [EDSA] was used as the cyclopropanating agent. The ylid was prepared as per the reported procedure[15] (Scheme XI).

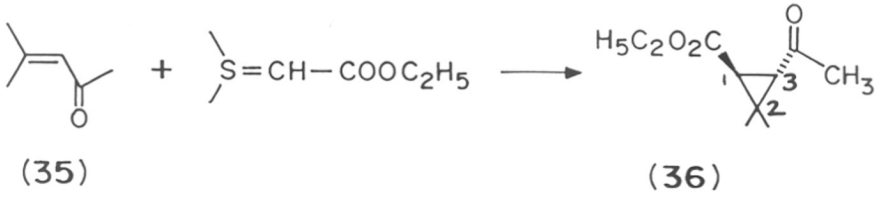
Carbethoxymethyl dimethylsulfonium bromide was prepared in 70% yield by reacting ethyl bromoacetate with dimethylsulfide in acetone at 10ºC. The salt had m.p. 79°C (Lit[15] 79°). The sulfur ylid EDSA was prepared from the salt by reacting with supersaturated potassium carbonate solution and 12.5N potassium hydroxide solution at 5°C. The reaction was very fast and took half an hour for completion. The EDSA was obtained in 85% yield. The structure of EDSA was confirmed by spectroscopic data. It exhibited I.R. absorption at 1730 and 1610 cm^{-1}; and ^1H NMR spectrum showed the presence of an olefinic proton (2.4),δ, an ethyl ester (triplet at 1.15 and quartet 3.81δ)and two methyl groups attached to sulfur (singlet at 2.81δ).

The condensation of mesityl oxide and EDSA was brought about by an improved procedure[15]. The reaction was carried out using excess of mesityl oxide at room temperature and without solvent. The reaction was completed in 4 days. The reaction mixture was fractionally distilled to get the ketoester (**36**) as a colourless liquid b.p. 63-66°/1mm in 85% yield.

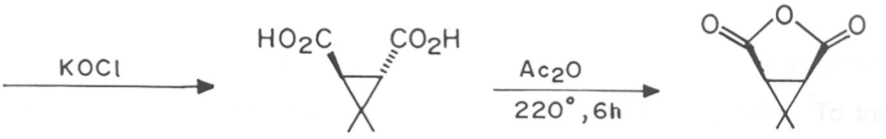
The compound showed IR absorption at 1730, 1700, 1420, 1385, 1370(cm^{-1}). The ^1H NMR spectrum of the compound showed two doublets at 2.16δ (C_1-H) and 2.4δ (C_3-H). The acetyl protons were observed at 2.2δ as a singlet. The protons of the two methyl groups attached to the ring appeared as two singlets at 1.1δ and 1.3δ

Scheme - XI

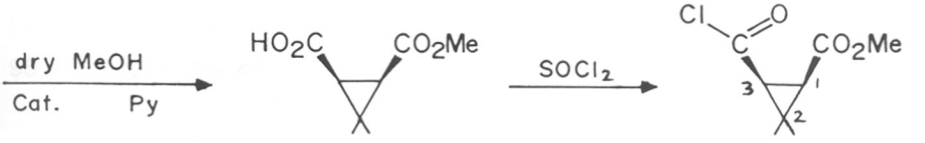




(35) (36)



(37)



(38) (39)

(1)

(±) cis - caronaldehyde methyl ester

The *trans*-ketoester (**36**) was oxidised to *trans*-caronic acid (**37**) by excess potassium hypochlorite in dioxane at room temperature. Excess of hypochlorite was neutralised after 4 hours and the *trans*-caronic acid was extracted after acidifying the reaction mixture. *Trans*-caronic acid was obtained as a white crystalline solid with m.p. 214° (Lit[16] 213°) in 95% yield.

The acid showed IR absorption at 3500-2500, 1700, 1390, 1350 cm^{-1}. The ^1H NMR spectrum (D_20) showed two singlets, one at 1.18δ for six protons of the two methyl groups on the cyclopropane ring and the second at 2.2δ corresponding to the two protons on the cyclopropane ring. In the mass spectrum, fragments at m/z 113 (100%), 95, 67 were observed.

Isomerisation of *trans*-caronic acid to *cis*-caronic acid monoester (**38**) was carried out by a known procedure[9]. *Trans*-caronic acid dissolved in acetic anhydride was heated at 220° for 6 hours in a sealed tube. The residue, after removing acetic anhydride, was vacuum distilled to get caronic anhydride. To this, dry methanol along with a catalytic amount of pyridine was added immediately. *Cis*-caronic acid monomethyl ester (**38**) was obtained as a white crystalline solid m.p. 108° in 81% yield. It had IR absorption at 3200, 1730, 1690, 1390, 1350 cm^{-1}. The ^1H NMR spectrum showed the following bands : 1.93δ (s, 2H, C_1-H and C_3-H), 3.68 (S, $COOCH_3$) and 10.6 (br, COOH). It had mass fragments at 127, 113 (100%), 95, 67 (100%),,59, 45.

The acid chloride (**39**) corresponding to the *cis*-caronic acid monoester was prepared using thionyl chloride. It was distilled at 75°/1mm to get a colourless liquid in 96% yield.

The ^1H NMR spectrum showed the following bands : 1.28, 1.4 (two singlets, $2CH_3$), 2.05 (d, C_1-H), 2.37 (d, C_3-H), 3.63 (s, $COOCH_3$)

The most important step in the synthesis was the Rosenmund reduction[17] of the acid chloride (**39**) to the required (±) *cis*-caronaldehyde methyl ester.

A mixture of the acid chloride (**39**) sodium acetate and 10% palladised carbon in dry acetone was hydrogenated at 24 psi for 40 minutes. The residue obtained after removal of the solvent from the filtrate of the reaction mixture, was distilled at reduced pressure to get *cis*-caronaldehyde methyl ester (**1**) as a colourless liquid in 88% yield. It showed IR absorption at 1730, 1700, 1380, 1320 cm^{-1}. The ^1H NMR spectrum of the product showed the following signals: 1.7 (dd, C_3-H), 2.02 (d, C_1-H), 3.66 (s, $COOCH_3$), 9.58 (d, CHO) If the geometry of the ring had been *trans*, then this aldehydic proton would have appeared as a multiplet. This confirms the *cis* geometry of the ring.

2.4 Experimental

Preparation of Carbethoxymethyl dimethylsulfonium bromide

Ethyl bromoacetate (41.75g, 0.25 mole) and dimethylsulfide (18.62g, 0.2875 mole) were mixed together in a conical flask containing acetone (75 ml) at 10°C with occasional shaking. After three hours, the two layers got separated and the flask was kept in the fridge overnight. Solid formed was filtered on a buckner funnel by applying vacuum and was washed with little quantity of acetone. The solid was dried at high vacuum. Crystalline hydroscopic solid weighed 40.0g(70% yield) with m.p.79° (dec) (lit[15]. 78-80° dec).

Preparation of Ethyl (dimethylsulfuranylidene) acetate [EDSA]

A three necked round bottom flask equipped with overhead stirrer and thermometer pocket was charged with solution of sulfonium bromide salt (31.25g) in chloroform (160 ml). The flask was cooled to 5°C. Supersaturated solution of K_2CO_3 (120 ml) and NaOH solution (12.5N, 16.4 ml) were added to the flask at once while stiring and the temperature of the reaction mixture raised upto 10-15°C. The reaction mixture was stirred vigorously at that temperature for 15 minutes. The salt separated was filtered off. The two layers of the filtrate were separated. The upper chloroform layer was dried over anhydrous K_2CO_3 for 2 hrs and solvent was removed on rotary evaporator at room temperature to get EDSA (17.25g, 85.4%). **I.R. : (Neat cm-1)**: 2990, 1730, 1610, 1410, 1320, 1140, 890, 760. **^{1}H NMR (CCl4, δ)** : 1.15 (t, J = 7Hz, 3H - -OCH_2-$\underline{CH_3}$), 2.4(s, 1H, = $\underline{CH}$), 2.81 (s, 6H,2(CH3)-S), 3.81 (q, J = 7Hz, 2H, $O\underline{CH_2}CH_3$)

Condensation of mesityl oxide with EDSA

EDSA (42.7g, 0.288 mole) was added dropwise to stirred mesityl oxide (38.7g, 0.395 mole) at room temperature. After the complete addition, the reaction mixture

was stirred for 1 hr and left at room temperature for four days. Fractional distillation of the reaction mixture gave the product (**36**) (45.67g; 85%) as colourless oil with bp 63-66°/1mm. **I.R. (Neat cm^{-1})** : 2990, 1730, 1700, 1420, 1385, 1370, 1240, 1190. **^{1}H NMR (CCl_4,δ)**: 1.1 (s,3H, CH_3-), 1.26 (t,J = 7Hz, 3H, -$OCH_2\underline{CH_3}$), 1.3 (s, 3H, $\underline{CH_3}$-), 2.16(d, J = 5Hz, 1H, $\underline{CH}$-COOEt), 2.2 (s, 3H, -COCH3), 2.4 (d, J = 5Hz, 1H, $\underline{CH}$-COCH3), 4.06 (q, J = 7Hz, 2H, -$O\underline{CH_2}CH_3$)

Preparation of KOCl

Bleaching powder (50g) was charged in a 1 lit round bottom flask. K_2CO_3 (35g) and KOH (10g) dissolved in 350 ml warm water were added to the flask. The stoppered flask was shaken for some time. The solid in the reaction mixture was filtered off on a Buchner funnel by applying vacuum. The solid was washed with water. The potassium hypochlorite solution obtained as the filtrate was adjusted to a concentration of 1.3N by iodimety.

Oxidation with KOCl : Preparation of *trans*-caronic acid (37)

KOCl (700 ml, 1.3N) was added to a stirred solution of keto-ester (**36**) (17.4g) in dioxane (17 ml). After 4 hrs, excess KOCl was destroied with sodium sulphite and the reaction mixture was acidified with 50% H_2SO_4 and extracted with solvent ether, washed with water then with NaCl brine. The organic layer was dried over anhydrous Na_2SO_4. Solvent was removed to get trans caronic acid (14.2g, 95%) as white solid m.p. 205°C. Recrystallisation from ethyl acetate- pet ether gave crystals with m.,p. 214° (Lit[16]. 213°). **I.R.(Nujol, cm^{-1})** : 3500-2500, 1700, 1430, 1390, 1350, 1120. **^{1}H NMR (D_2O,δ)** : 1.18 (s, 6H, $(CH_3)_2$), 2.2 (s, 2H, -$C\underline{H}$-COOH). **MS (m/z)** : 113 (100%) (M^+-45), 95, 67

Isomerisation of *trans*-caronic acid to *cis*-caronic acid monoester (38)

Trans-caronic acid (12g) dissolved in acetic anhydride (18 ml) was transferred to a pressure reaction tube and the tube was sealed. The sealed tube was heated in the furnace at 220° for 6 hrs. Excess acetic anhydride was distilled out from the reaction mixture and the resulting caronic anhydride was distilled at reduced pressure. Dry methanol (10 ml) and a catalytic quantity of pyridine were added immediately to the distillate and left overnight. The solvent was removed and the product was dried by applying high vacuum. *Cis*- caronic acid mono-ester (**38**) (10.5g, 81%) was obtained as a white crystalline solid with m.p. = 108°. **IR (Nujol, cm^{-1})** = 3200, 1730, 1690, 1450, 1390, 1350, 1120, 840· ^{1}H NMR ($CDCl_3$, δ) : 1.26 (s, 3H,(CH_3); 1.4 (s, 3H, (CH_3); 1.93 (s, 2H, -CH-CH-); 3.68 (s, 3H, $COOCH_3$); 10.6 (br s, 1H, -COOH). **MS (m/z)** : 127, 113 (100%), 95, 67(100%), 59, 45

Preparation of acid chloride (39)

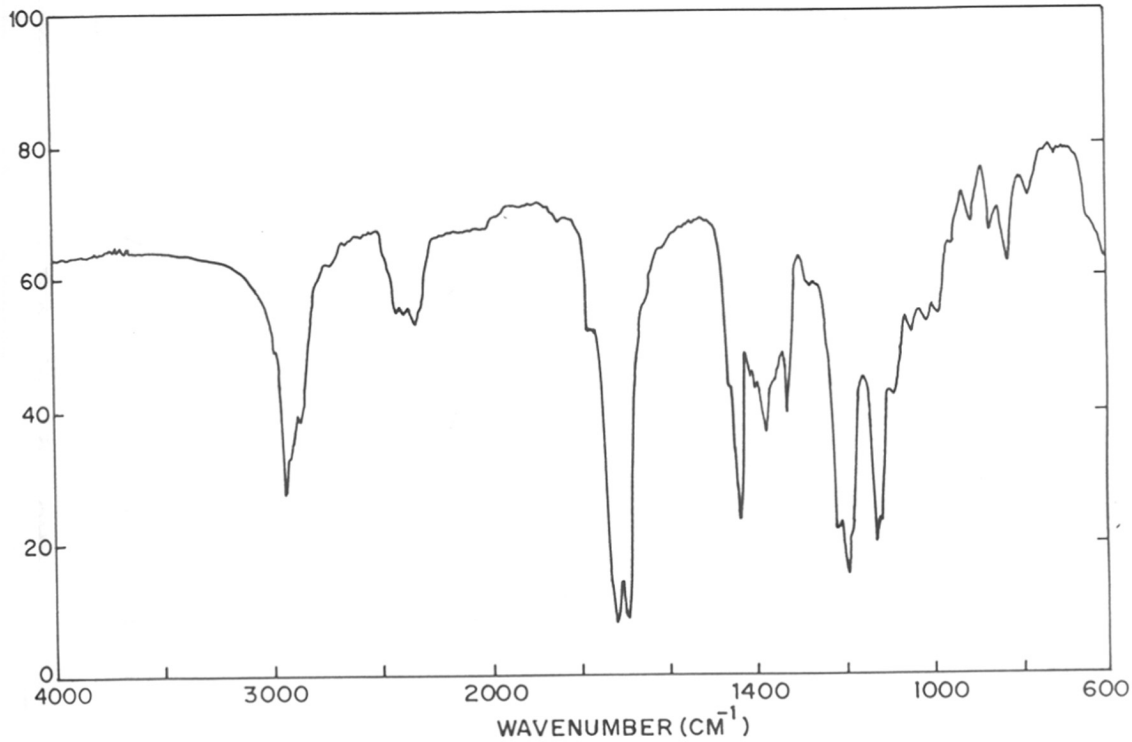
Thionyl chloride (20 ml,0.276 mole) was added to the mono ester (**38**) (20g, 0.105 mole). After thirty minutes, anhydrous benzene (25 ml) was added and was left overnight. Benzene was distilled out Additional benzene (2 x 25 ml) was added to the reaction flask and was distilled out. The residue was distilled at reduced pressure to get the acid chloride (**39**) (21.3g, 96%) as a colourless liquid b.p.75-77°/1mm· **^{1}H NMR (CCl_4,δ)** : 1.26 (s, 3H, CH_3-); 1.4 (s, 3H, CH_3), 2.05 (d, J = 9Hz, 1H, CH-COOMe), 2.37 (d,J=9Hz, 1H, CH-COCl), 3.67 (s, 3H, -OCH_3)

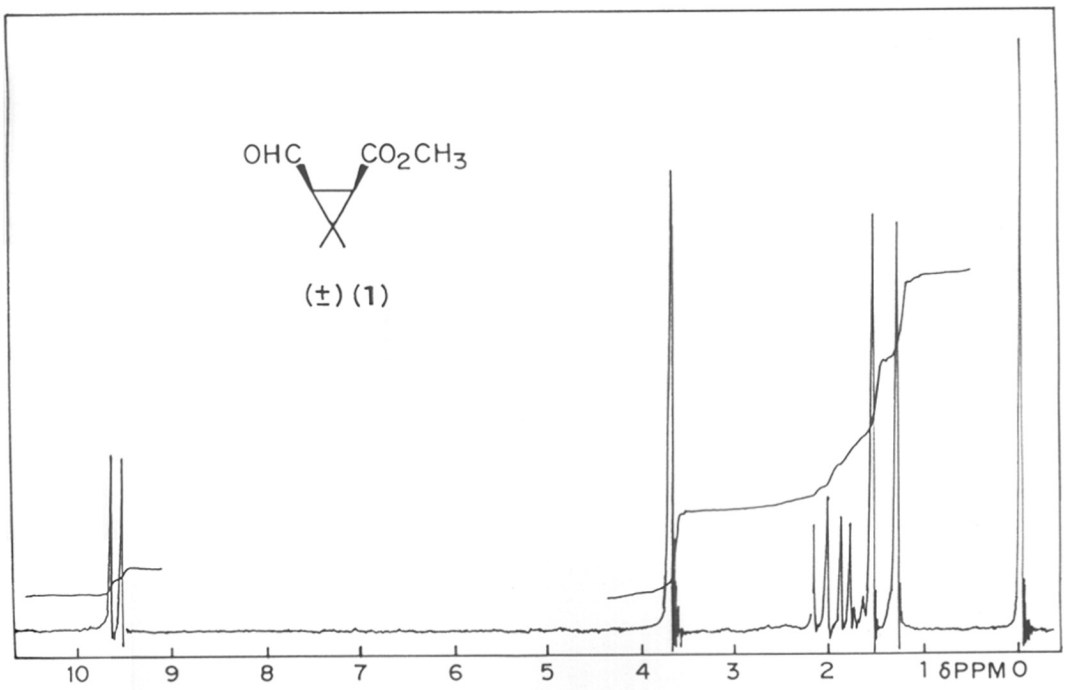
Rosenmund reduction : Preparation of *cis*-caronaldehyde methyl ester (1)

A mixture of the acid chloride (**39**) (4.536g, 0.023 mole) fused sodium acetate (5.575g, 0.068 mole), palladised carbon (10%) (900 mg) and dry acetone (225 ml) was hydrogenated at 24 psi for forty minutes. The reaction mixture was filtered and the residue was washed with acetone. Acetone was removed at room temperature and the residue was distilled by bulb to bulb distillation. The fraction

collected at 70-100° (bath temp.)/1mm was *cis*-caronaldehyde ester, obtained as a colourless liquid (3.3g, 88%). **I.R. (Neat, cm^{-1})** : 1730,1700, 1430, 1380, 1320, 1190,1130 **^1H NMR (CCl_4, δ)** : 1.25 (s, 3H, CH_3-); 1.5 (s, 3H, CH_3-); 1.7 (dd, J = 9 Hz, 6Hz, 1H, CH-CHO); 2.02 (d, J = 9Hz, 1H, CH-COOMe); 3.63 (s, 3H, $-COOCH_3$); 9.58 (d, J=6Hz, 1H, -CHO)

Spectrum No. 1





(±) (1)

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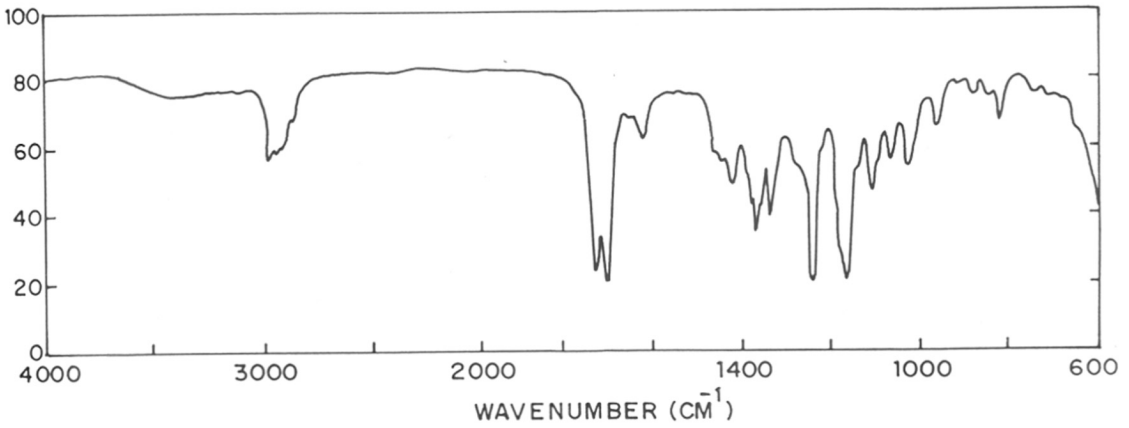

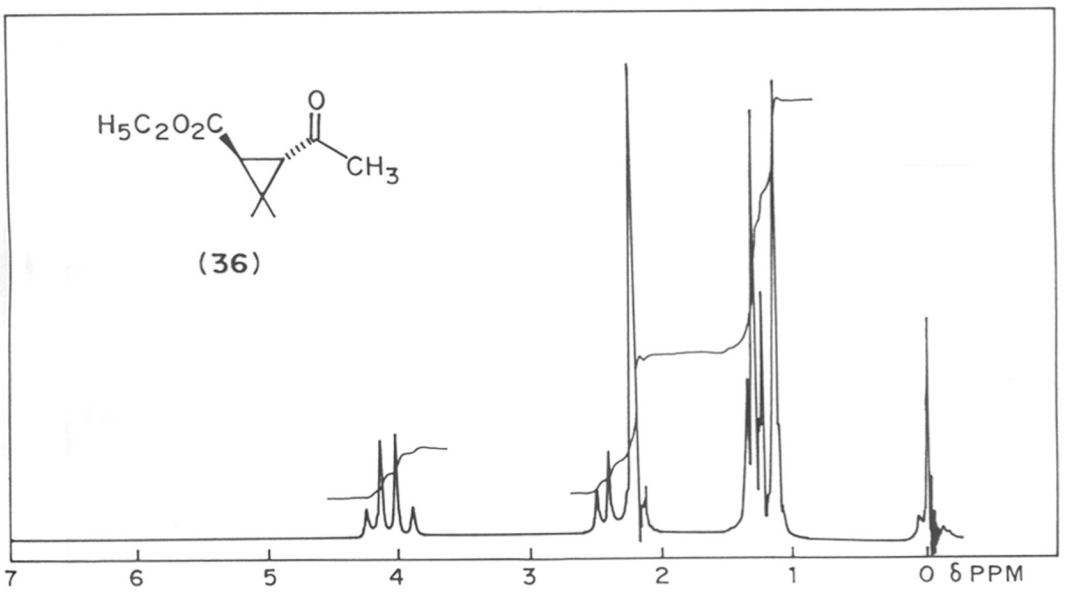

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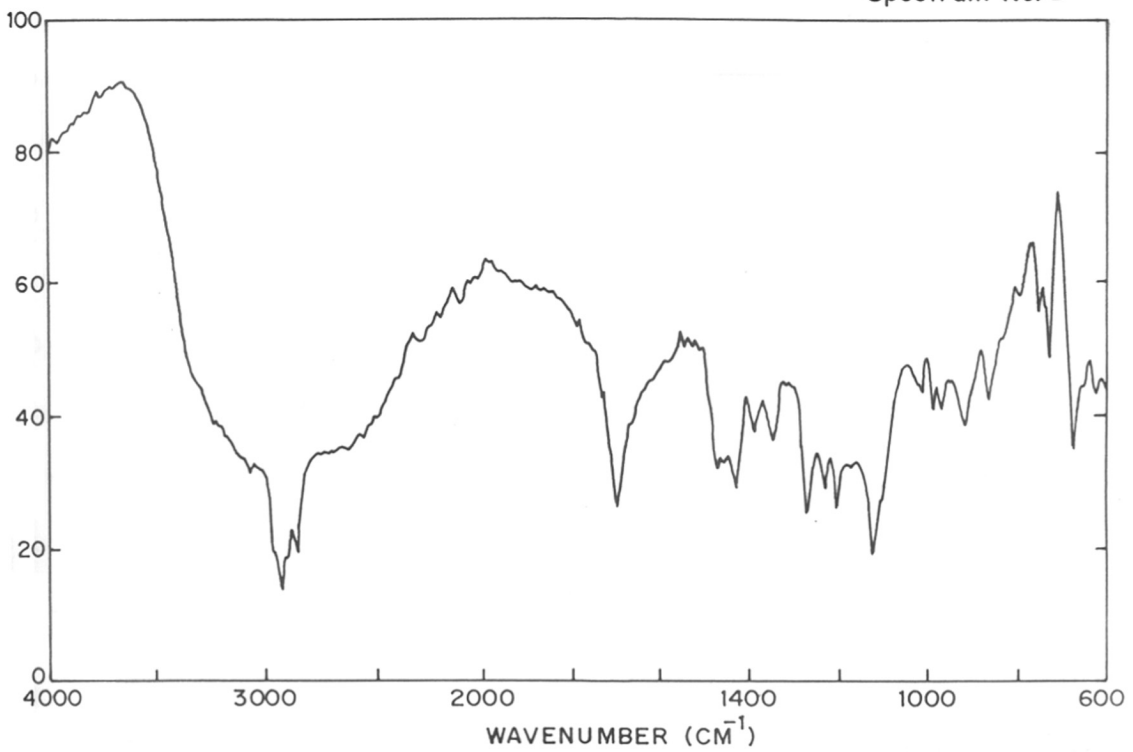

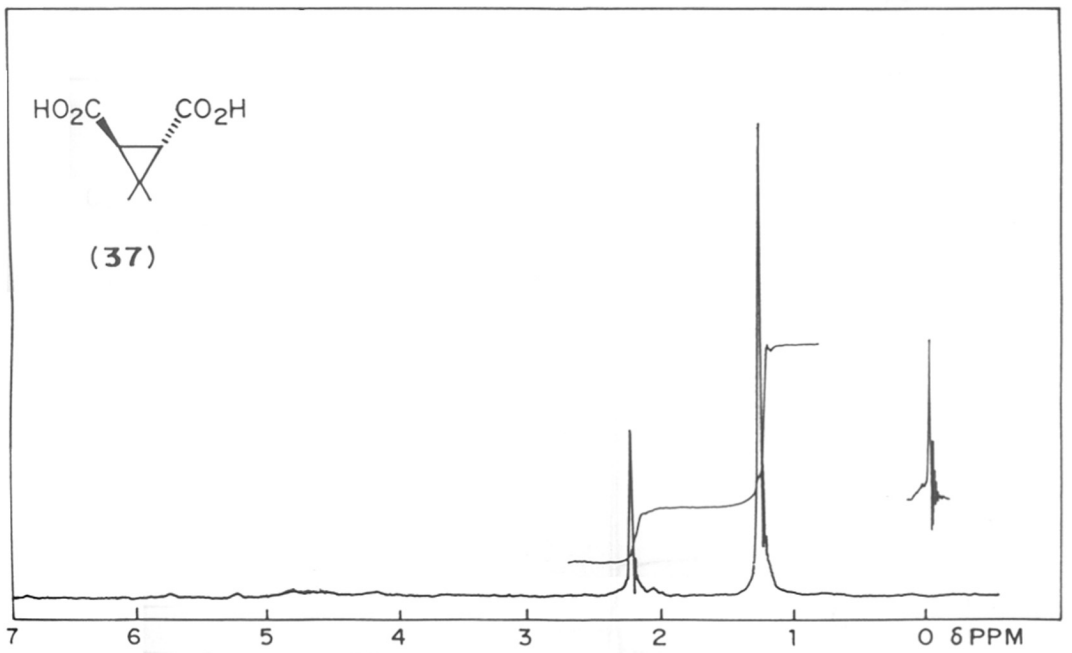

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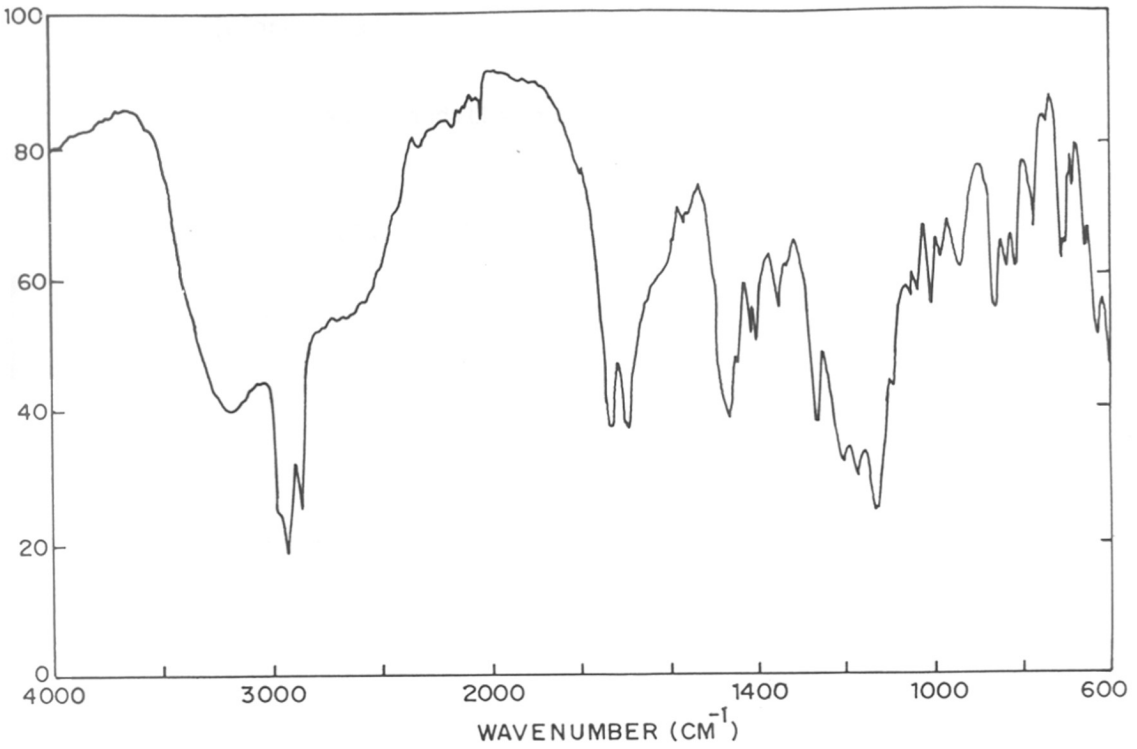

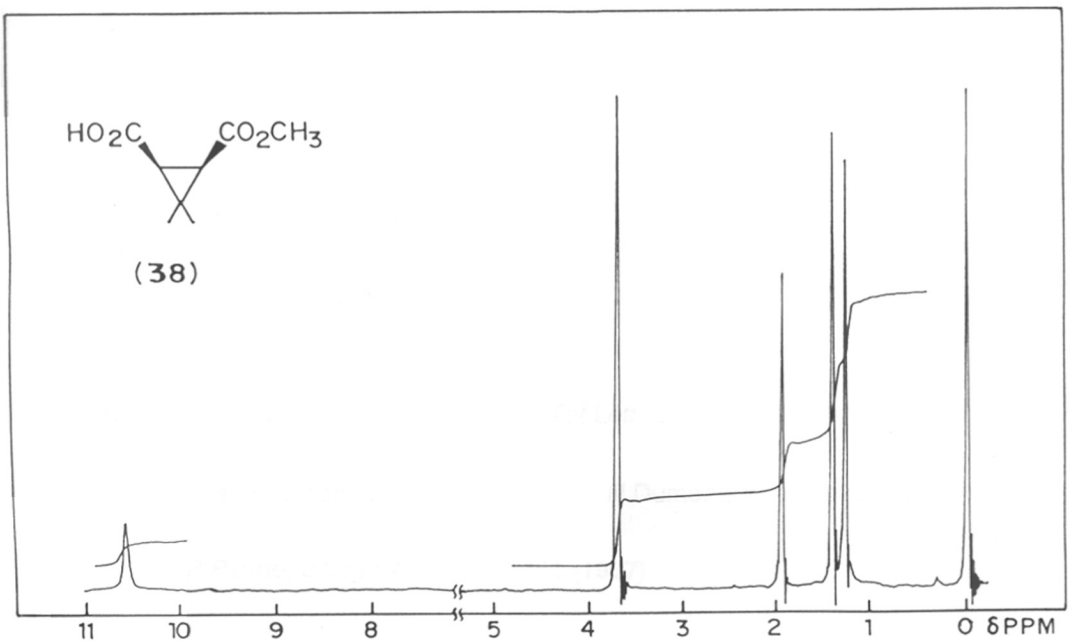

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CHAPTER 3

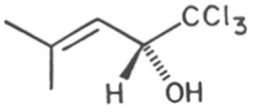
Enzymatic resolution of a crucial allylic alcohol

3.1 Summary

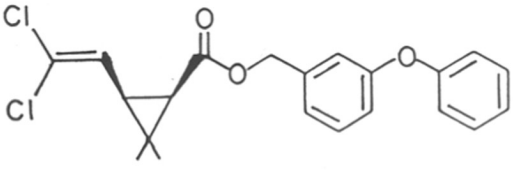
(R)(-)-1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene (**1**) is the starting material for the synthesis of the potent agricultural pyrethroids - NRDC 182[1] (**2**) and the optically active form of cypermethrin (**3**)

In order to prepare this material in its enantiomerically pure form, different esters of the racemic alcohol were prepared and subjected to enzymatic hydrolysis using whole cell broth of different micro-organisms. The best results were obtained with the acetyl derivative as the substrate and *Bacillus subtilis* to bring about the enantiospecific hydrolysis. In 48 hours, the yield of the desired alcohol was 42%. The alcohol was purified from the crude reaction product. The alcohol had $[\alpha]_D$ = -12.0 and m.p. 109°C. The enantiomeric excess of the alcohol was determined to be 98% by ^{1}H NMR studies using a chiral shift reagent on the corresponding acetyl derivative.

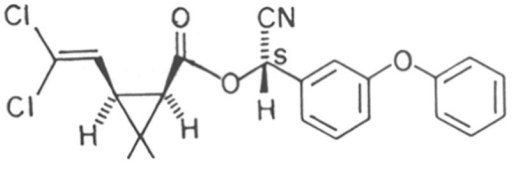
Esters of the racemic alcohol with other carboxylic acids were also subjected to the action of *B.Subtilis*. The chemical yield of the alcohol ranged from 0% to 100% while the enantiomeric excess varied from 99.1% to 0%. The formate ester was hydrolysed very fast while the p-nitrobenzoate ester did not undergo hydrolysis.



(R)(−) (1)



(2)



(3)

3.2 Introduction

In recent years enzymes have become a valuable class of catalysts in organic synthesis[2-5]. Enzymes are catalysts which achieve amazing rate enhancement for the reactions they promote. They have the ability to catalyse regiospecific and stereospecific reactions. They have a great potential for use in synthetic organic chemistry.

Most of the chemical reactions which occur in living cells are accelerated by enzymes. Enzymes are proteins with molecular weight ranging from 10^3 to 10^6. They consist of a large number of aminoacids linked covalently to form a long chain. In other words enzymes are linear polymers of aminoacids. Amide groups form the backbone of proteins; in addition, several different functional groups[6] such as hydroxyl, thiol, carbonyl and amine may be present in the side chains of the aminoacid skeleton. So these proteins can act as acidic, basic or neutral catalysts.

In comparison with other catalysts, enzymes possess certain unique properties. Enzymes have high catalytic efficiency. The rates of enzyme catalysed reaction can be faster by a factor of upto 10^{12} compared to the corresponding uncatalysed reaction[7]. Enzyme catalysis takes place under mild conditions such as atmospheric pressure, temperature around 20° to 40° and a pH near neutral. Under such mild conditions normal chemical catalysts are not expected to function effectively. Since the reaction conditions are mild, side reactions such as isomerisation, racemisation, epimerisation and rearrangement that are so troublesome in traditional methodology are not expected to pose a major problem in enzyme-catalysed reactions.

The most important property of enzymes is substrate specificity. Their catalytic activity is usually restricted to a single reaction type. Therefore it is possible to convert a single functional group in the presence of other reactive groups with

the help of enzymes. They are generally very specific about the structure and stereochemistry of the substrate. These characteristics make enzymes important catalysts for asymmetric synthesis[8].

Effect of pH

Since enzymes are proteins, pH changes will profoundly alter the ionic character of the amino group and carboxylic acid groups on the protein and will have an effect on the catalytic site and conformation of the enzyme. Each enzyme is found to function best at a specific pH; this may be different for different enzymes. This is referred to as the pH of optimum activity[9].

If the pH is made more alkaline or more acidic, the enzyme steadily loses its activity. Initially, the loss of activity is reversible but as the change in pH becomes more marked, the enzyme becomes irreversibly inactive. The optimum pH is influenced by the degree of purity of the enzyme and length of time it acts.

Effect of temperature

The rate of an enzyme catalysed reaction varies with temperature. In general, a plot of initial rate as a function of temperature first increases and then decreases, passing through a maximum which is referred to as the "optimum temperature" This type of behaviour is due to the fact that at high temperature, inactivation of enzyme takes place.

In the extreme case, if the temperature is too high, the inactivation of an enzyme is instantaneous so that the apparent rate of the enzyme catalysed reaction is zero.

Effect of enzyme concentration:

The rate of an enzyme catalysed reaction is directly proportional to enzyme concentration provided optimum substrate is present. If the quantity of the enzyme employed to catalyse the reaction is doubled then the reaction rate also doubles.

Effect of substrate concentration

Severe substrate or product inhibition may occur for enzymatic reaction with hydrophilic substrates. If substrate concentrations are too low, a certain fraction of the enzyme active sites are vacant. At high concentrations of substrate, enzymatic reactivities commonly decrease due to non-specific deactivation.

Some of the enzymes require cofactors for the manifestation of their activity. Being costly, these cofactors demand regeneration for their use in enzyme catalysed reactions on commercial scale.

The active site of an enzyme is the site on the surface of the enzyme molecule where the chemical reaction is catalysed. The active sites of enzymes are highly specific and bind the substrate in a particular way. On reaction the products are released from the enzyme molecule. The enzyme-substrate binding is like a "lock-and-key" combination. The enzyme molecule is normally bigger than the substrate and the substrate fits into the enzyme active site like a key in a specific lock. The aminoacid chain i.e. the polypeptide - folds in a specific way and results in a three dimensional enzyme molecule. A particular combination of side chains of aminoacids is present at the active site. For having an active site, the enzyme polypeptide must have a specific aminoacid sequence, it must fold in a particular way to obtain the three dimensional configuration and cofactor or metal ion, if necessary, must be available.

High temperature, ionic concentrations, drastic pH change mutations leading to change in aminoacid sequences, binding due to substrate-like antimetabolites etc. result in loss of enzyme activity due to interference in active site conformation.

A typical enzyme is likely to contain an active site per 20,000-50,000 molecular weight. This high molecular weight equivalent per active site may be balanced by a high catalytic turnover number but not always.

Most of the enzymes are water soluble and are most active in aqueous solution at pH 7-8 and at room temperature. However recently attempts have been made to use enzymes in organic solvents[10]. Enzyme catalysed dehydrations, transesterifications, aminolysis and oxidoreductions in organic solvents are now common[11]. But adjusting the pH is difficult in certain organic solvents.

More than 2000 enzymes are known[12]. Several hundreds of these are commercially available. Immobilised enzymes are also commercially available. Immobilisation facilitates easy separation of enzyme, repeated usage and regeneration of enzyme. Apart from the use of ready made enzymes, whole cells as well as whole broth (cells along with the liquid medium in which they are grown) can be used for bringing about the chemical transformations. The availability and cost of enzymes vary widely.

Chemical reactions catalysed by enzymes are essentially the same as those carried out in conventional inorganic or organic chemistry. Enzymes are classified on the basis of the type of reaction catalysed by them. They are divided into six classes[12].

1. **Oxidoreductase** : Enzymes of this class catalyse oxidation - reduction reactions involving oxygenation such as C-H -> C - OH or overall removal or addition of hydrogen atom equivalents, CH(OH) <-> C = O and CH - CH <-> C = C

2. **Transferases** : The enzymes of this class transfer groups such as acyl, sugar, amino, phosphoryl, aldehyde and ketone from one molecule to another.

3. **Hydrolases** : This group of enzymes catalyse hydrolysis of glycosides, anhydrides, esters, amides, peptides and other C-N containing functions.

4. **Lyases** : These enzymes catalyse addition usually of HX to double bonds such as C=C, C=N, C=O and the corresponding reverse processes.

5. **Isomerases** : *Cis-trans* isomerisation, double bond migration and racemisation reactions are catalysed by this group of enzymes.

6. **Ligases** : These are often called synthetase and mediate the formation of C-O, C-N, C-S, C-C and phosphate ester bonds.

Hydrolases :

This is an important class of enzymes which has wide utility in organic synthesis. They are well suited for synthetic application because they require no coenzyme and can accept a broad structural range of unnatural esters as substrates. Many of them have the ability to effect enantioselective hydrolysis of esters containing a prochiral centre.

Lipases and esterases fall under the category of hydrolases. The lipases are rapidly developing into an extremely useful class of enzymes for chiral synthesis. Lipases have the inherent advantage of low cost, high stability and high tolerance for variation in substrate structure. An additional advantage is that they do not require water soluble substrates. They operate best at water organic layer interfaces. Recently it has been proved that they retain a high degree of activity in organic systems.

Several hydrolytic enzymes have been used in stereospecific and enantiospecific organic synthesis. Some examples are given below.

When the racemic ester (**5**) was hydrolysed with Lipase amino 40, the unhydrolysed pure (s) ester (**5**) was obtained which could be used for preparation of pure (S) Propranolol[13](**4**).

The racemic ester of an epoxy alcohol (**6**) when hydrolysed with PPL (Porcine pancreatic lipase) gave alcohol (**7**) and unhydrolysed ester (**6**) in 92% *ee*[14]. This provided an alternative to the Sharpless method as a route to these useful chiral synthons.

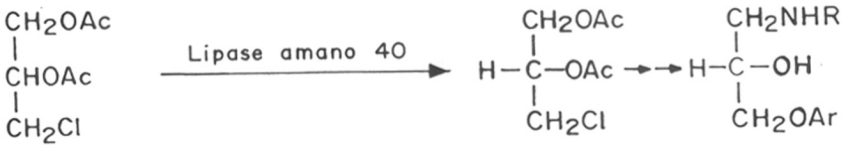
PPL was also used for the stereosepcific hydrolysis of monocyclic meso diesters (**8**) and (**10**) to get more than 95% enantiomeric excess of the monoester-monoalcohol(**9**) and (**11**)[15].

The cyanohydrin (**12**) was resolved by the hydrolysis of its acetate ester using lipase from pseudomonas[16].

Immobilised PLE (porcine liver esterase) has been used for the preparation of several chiral building blocks[17]. The diester (**13**) of cyclopentene-3,5-diol was hydrolysed using PLE to get the monoester (**14**) in 98% *e.e.*

Diastereo and enantioselective hydrolysis was observed when a (±) *cis trans* mixture of methyl chrysanthemate (**15**) was treated with PLE[18]; the (±) *cis* isomer was not hydrolysed at all while the *trans* ester underwent enantioselective hydrolysis to yield. (1R, 3R) *trans*-chrysanthemic acid (**16**) in high enantiomeric excess. Similarly (±) *cis:trans* caronic acid (**17**) diester was hydrolysed to get (1R, 3R) *trans*-caronic acid monoester (**18**) in 99% *e.e.*

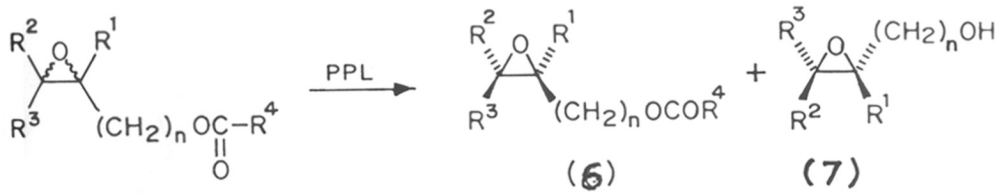
(R)-Carveol (**19**) was obtained in 95.6% *e.e* when the racemic acetate ester (**20**) was hydrolysed using *Bacillus subtilis*[19].



(±)(5)

(S)(5)
90% e.e.

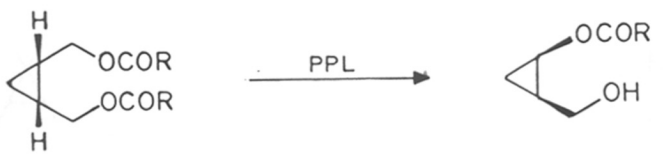
(S)(4)
R = $-CHMe_2$
Ar = -1-naphthyl



(6)

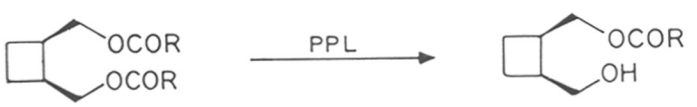
(6)
92 % e.e.

(7)



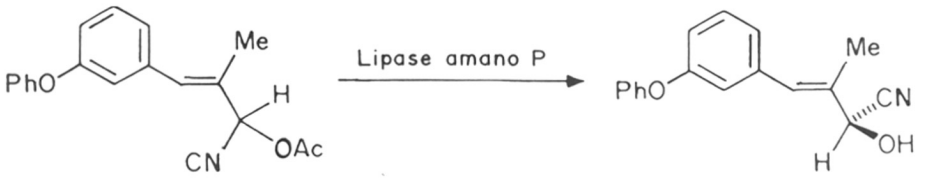
(8)

(9)
93 % e.e.

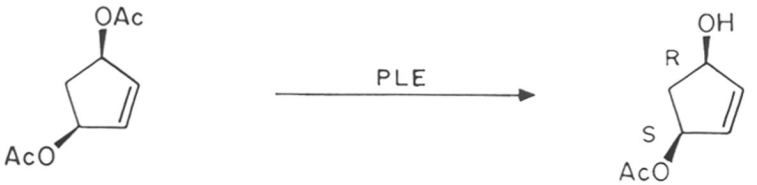


(10)

(11)
95 % e.e.

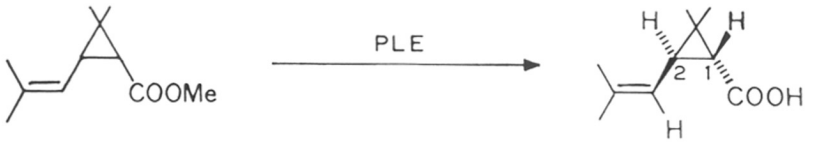


(S)(12)



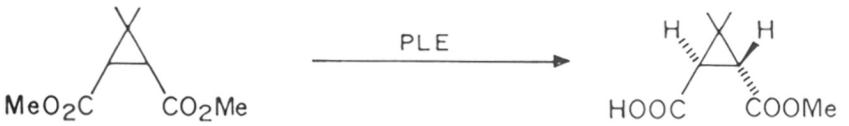
(13)

(14)
98% e.e



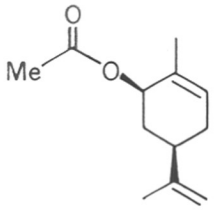
(15)
(±) (cis + trans)

(16)
(1R, 3R) high e.e



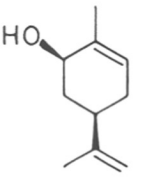
(17)
(±) (cis + trans)

(18)
(1R, 3R) 95% e.e.



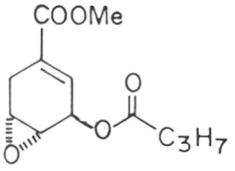
(19)

B. Subtilis



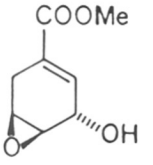
(R) (**20**)

95·6 % e.e



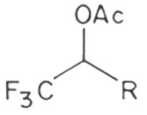
(±) (**21**)

Cholesterol esterase



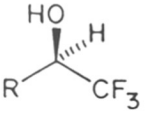
(+) (**22**)

88 % e.e.



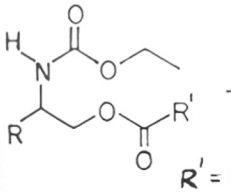
(**23**)

Lipase from C. cylindricia



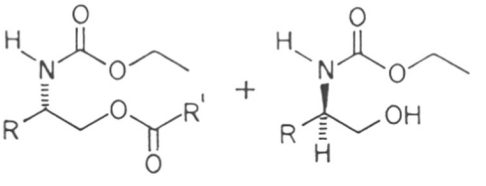
(R) alcohol (**24**)

95 % e.e.



(**25**) $R' = Me$, $R = C_2H_5$

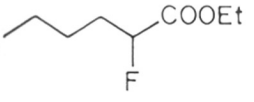
Pancreatin



(S) (**26**)

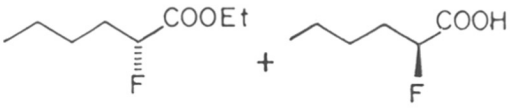
95 % e.e

$R = C_2H_5$



(**27**)

Lipase from P. fluorescens



(R) (**28**)

99·9 % e.e

The racemic butyrate ester of the epoxy alcohol (**21**) was hydrolysed with cholesterol esterase to get (+) epoxyalcohol (**22**) in 88% *e.e.*[20]

Hydrolysis of the acetates of 1-substituted 2,2,2-trifluoro ethyl alcohols (**23**) by the lipase from *Candida cylindricia* yielded the R alcohol (**24**) in 95% *e.e.*[21]

Aminoalcohols are important as chiral building blocks and as products of pharmaceutical interest. Resolution of 2-amino-1-butanols was carried out by the hydrolytic action of lipase pancreatin on the corresponding O-acyl derivative (**25**) (R^1 = Me, R = C_2H_5) in which the amino group was protected as a carbamate[22]. S-(+) 2-Amino- 1-butanol (**26**) (R = C_2H_5) was obtained in more than 95% *e.e.* as the corresponding carbamate. This is a chiral precursor in the synthesis of the antitubercular drug Ethambutol.

(R) and (S) ethyl 2-fluorohexanoates are important intermediates in the synthesis of 16-fluoroprostaglandins (antihypertensive). Lipase from *Pseudomonas fluorescens* catalyses the enantioselective hydrolysis of the racemic 2-fluorohexanoic acid ester (**27**) to leave behind the (R)-ester (**28**) in 99.9% *e.e*[23].

3.3 Present work

(R)-(-)1,1,1-Trichloro-2-hydroxy-4-methyl-3-pentene (**1**) is a synthon for the potent agricultural pyrethroids NRDC 182 (**2**) and the optically active form of cypermethrin (**3**).

Hatch[1] demonstrated the chemical resolution of the racemic alcohol (**1**). He used the optically active isocyanate (**29**) as a derivatizing agent to resolve this alcohol. Reaction of equimolar quantities of racemic alcohol (**1**) and optically active isocyanate (**29**) at 80° for 2 days yielded a diastereomeric mixture of two carbamates (**30**). The pure diastereomer α-S, 2(R)- (**30**) was separated by crystallisation in hexane. This single, pure diastereomer was cleaved using trichlorosilane-triethylamine in toluene to get (R) (-),1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene (**1**) in 60% yield with 98% optical purity (Scheme I).

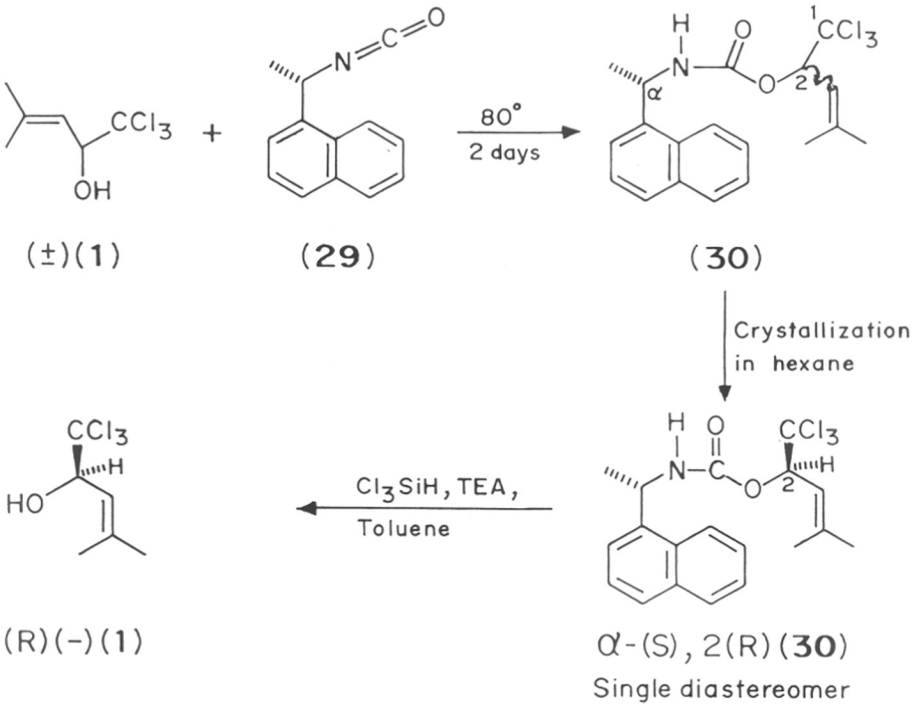
We have now achieved an enzymatic resolution of the racemic alcohol, leading to R(-)-1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene (**1**) in high chemical yield and optical purity (*e.e.* > 98%). This method avoids the tedious and expensive chemical resolution mentioned above.

Results and Discussions

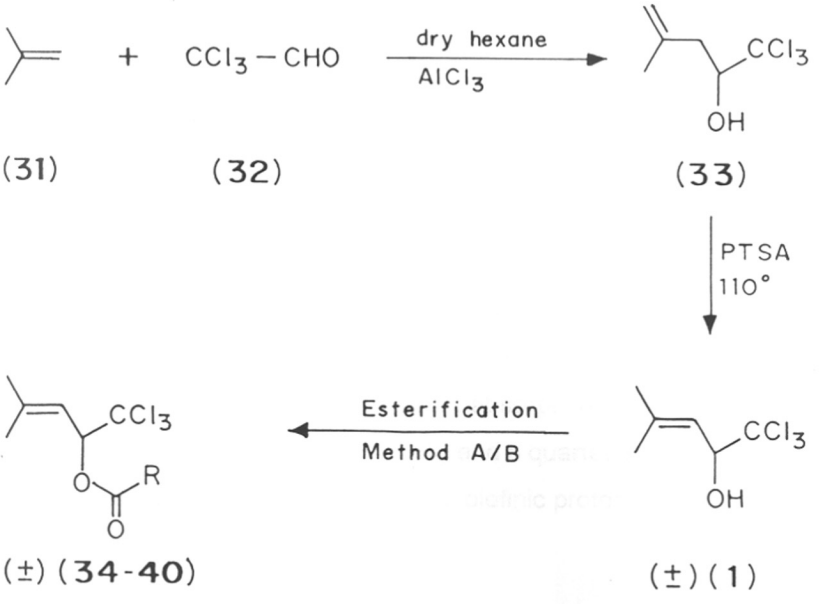
The racemic alcohol 1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene (**1**) was prepared by a modification of the reported procedure.[1]

Isobutylene (**31**) and anhydrous chloral (**32**) in dry hexane were allowed to react in presence of $AlCl_3$ to get the homoallylic alcohol (**33**) which after acid catalysed isomerisation yielded 1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene (**1**) as a white solid in 48% yield with m.p. 78-80° (Lit[1] 78-80°) (Scheme II). This allylic alcohol showed IR absorption at 3250, 1680, 1310 and 1280 cm^{-1}. The ^1H NMR spectrum showed a two singlets (very close) at 1.85δ which was assigned to the

Scheme-I



Scheme-II



protons of the two methyl groups on the olefinic bond. A broad singlet at 2.6δ was observed for the hydroxyl proton. A doublet at 4.82 δ was assigned to the proton adjacent to the CCl_3 group. The olefinic proton appeared at 5.42δ as a multiplet.

The acetate ester (**34**) of the alcohol was prepared by using acetic anhydride and pyridine as acetylating agent. The ester was distilled to get a colourless liquid bp 90°/4 mm in 92% yield. The acetate ester (**34**) showed IR absorption at 1760, 1670, 1440 and 1370 cm^{-1}. In the ^1H NMR spectrum of the compound a singlet at 1.81δ for the protons of the two methyl groups present on the olefin was observed. The protons of the CH_3CO- group appeared at 2.06δ as a singlet. The olefinic proton appeared as a multiplet at 5.2δ. The proton adjacent to -CCl_3 got shifted downfield from 4.82δ in (**1**) to 5.9δ (doublet) in (**34**) due to the esterification of the alcohol. In the mass spectrum the compound showed peaks at m/z 141, 85 (100%) and 57.

The formate ester (**40**) was prepared by esterification of the racemic alcohol using acetic-formic anhydride with a catalytic quantity of pyridine. The formate ester was distilled to get a colourless liquid, b.p. 78-80°/4mm. The compound showed I.R. absorption at 1740, 1670, 1445, 1380 cm^{-1}. In the ^1H NMR spectrum the following signals were seen: a multiplet at 1.9δ corresponding to protons of two methyl groups present on the double bond; an olefinic proton as a multiplet at 5.3δ ; a doublet corresponding to the methine proton adjacent to the -CCl_3 group at 6.06 ; and the formyl proton at 8.03 as a singlet. In the mass spectrum, fragment ions at m/z 113 (loss of -CCl_3), 85 (100%), 77 were observed.

The propionate ester (**35**) of the alcohol was prepared using propionic anhydride and pyridine. The ester was distilled, b.p. 81-84°/1mm to get a colourless liquid in 75% yield. The ester had IR absorption at 1760, 1680 cm^{-1}. In the ^1H NMR spectrum a triplet at 1.18δ (CH_3) and a quartet at 2.38δ (CH_2) were observed corresponding to the ethyl group. The olefinic proton was observed as a multiplet

at 5.2δ. The methine proton next to -CCl_3 group was seen at 5.95δ as a doublet. In the mass spectrum the propionate ester showed peaks at m/z 258 (m^+), 169, 149, 115, 85 (100%) 77, 57.

Other esters namely valerate (**36**), caprylate (**37**), benzoate (**38**) and p-nitrobenzoate (**39**) were prepared by reacting the racemic alcohol (**1**) with the corresponding acid chloride in presence of triethylamine.

The valerate ester (**36**) was distilled, bp. 98-99°/1.5mm, to get a colourless oil in 60% yield. The compound showed characteristic IR absorption at 1760, 1680cm^{-1}. In the ^1H NMR spectrum a triplet was observed at 0.95δ corresponding to the methyl protons present at the terminus of the valeric acid chain. One more triplet 2.36δ corresponding to the methylene protons adjacent to the carbonyl of the ester group was observed. The olefinic proton was seen as a multiplet at 5.23δ. The methine proton adjacent to -CCl_3 group was observed as a doublet at 5.93δ. The mass spectrum of the compound showed peaks at m/z 149, 113, 85 (100%), 77, 57.

The caprylate ester (**37**) was distilled, bp 102-104°/1.5 mm. The ester was obtained as a colourless oil in 60% yield. The ester showed characteristic IR absorption at 1760, 1680 cm^{-1}. In the ^1H NMR spectrum, a triplet was observed at 0.9δ for the methyl protons present at the terminus of the caprylic acid chain. One more triplet was observed at 2.28δ for methylene protons present adjacent to carbonyl group of the ester function. A multiplet at 1.28δ was observed for the central ten protons of the acid chain. One more multiplet at 1.83δ was assigned for two methyl groups present on the olefinic bond. The olefinic proton was observed as a multiplet at 5.2δ. The methine proton present adjacent to the -CCl_3 group was observed as a doublet at 5.9δ. In the mass spectrum of the ester peaks at m/z 185, 166, 149 (100%) 141, 131, 113 were observed.

The benzoate ester (**38**) had bp 120-23°/5mm (yield 92%). The ester showed IR absorption at 1730, 1600 cm^{-1}. In the ^1H NMR spectrum two singlets at 1.9δ and 2.0δ for the two methyl groups present on the olefin were observed. The olefinic proton was observed at 5.36δ as multiplet. The methine proton adjacent to $-CCl_3$ group occurred as a doublet at 6.15δ. A multiplet between 7.3δ - 8.2δ was observed for aromatic protons. In the mass spectrum, the compound showed fragment ion peaks at m/z 271, 189, 149, 105 (100%) 77.

The p-nitrobenzoate (**39**) was prepared in 65% yield. It is a light yellow crystalline solid with m.p. 106-107°C. The compound showed IR absorption at 1730, 1610, 1525, 1375 cm^{-1}. In the ^1H NMR spectrum, two singlets at 1.9δ and 1.96δ for two methyl groups present on the olefinic proton were observed. A multiplet at 5.4δ was observed for the olefinic proton. A doublet at 6.18δ for the methine proton adjacent to $-CCl_3$ group was observed. Deshielded aromatic protons in the p-nitrobenzoate ester were observed as a singlet at 8.2δ. The ester showed fragment ion peaks in the mass spectrum at m/z 234, 150 (100%), 104.

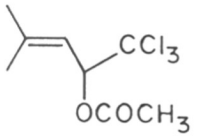
Initial experiments on the enzymatic ester hydrolysis were carried out with the acetate ester. Different organisms were tested for their efficiency in bringing about this hydrolysis. The experiments were carried out at 30°C with the substrate concentration at 0.5%. The extent of hydrolysis was in the range of 0-42% (Table 1). The best results were obtained with *Bacillus subtilis*; 42% hydrolysis took place in 48 hours at 0.5% substrate concentration.

TABLE 1

Sr.No.	Name of the organism	NCIM No	% hydrolysis of the acetate ester (34)
1	Pseudomonas aeruginosa	2053	25%
2	Saccharomycopsis lipolytica	3229	0%
3.	Candida rugose	3462	2%
4.	Pseudomonas lemonnieri	2060	30%
5.	Saccharomyces cerevisiae	3458	0%
6.	Bacillus subtilis	2010	42%

The acetate (**34**) was added to the culture broth of *B.subtilis* previously grown for 2 days at 28° in a nutrient medium (100 ml containing 1g peptone, 0.4g neat extract, 0.1g glucose and 0.1g of agar-agar). The substrate concentration was 0.5%. The flask was incubated on a rotary shaker at a speed of 240 rpm (revolutions per minute) for 48h. The contents of the flask were extracted with solvent ether. The ether layer was dried over sodium sulphate. After removal of the solvent GC analysis of the reaction product was done to find out the extent of hydrolysis. The liberated alcohol and unhydrolysed acetate were separated by column chromatography (silica gel 60-120 mesh, 10% ethyl acetate- pet. ether) to get pure alcohol (Scheme III).

Scheme - III

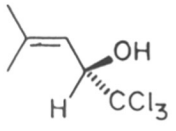


(±)(**34**)

B. subtilis
48 hrs

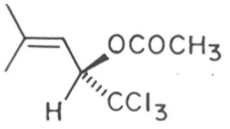
crude reaction product
after extraction with ether

Column
chromatography



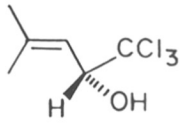
(S)(+)(**1**)

alcoholic
KOH



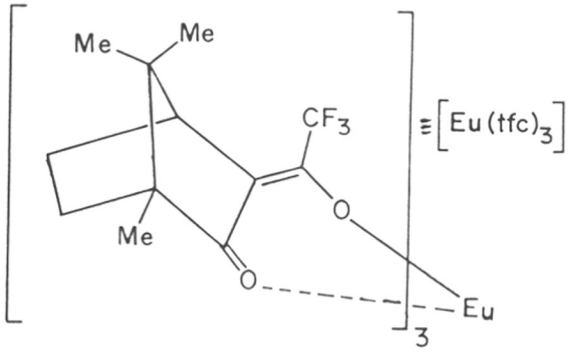
(S)(**34**)

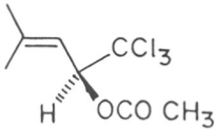
+



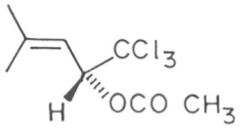
(R)(−)(**1**)

Scheme - IV

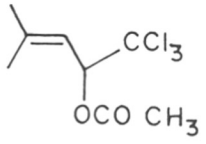




(S)(**34**)



(R)(**34**)



(±)(**34**)

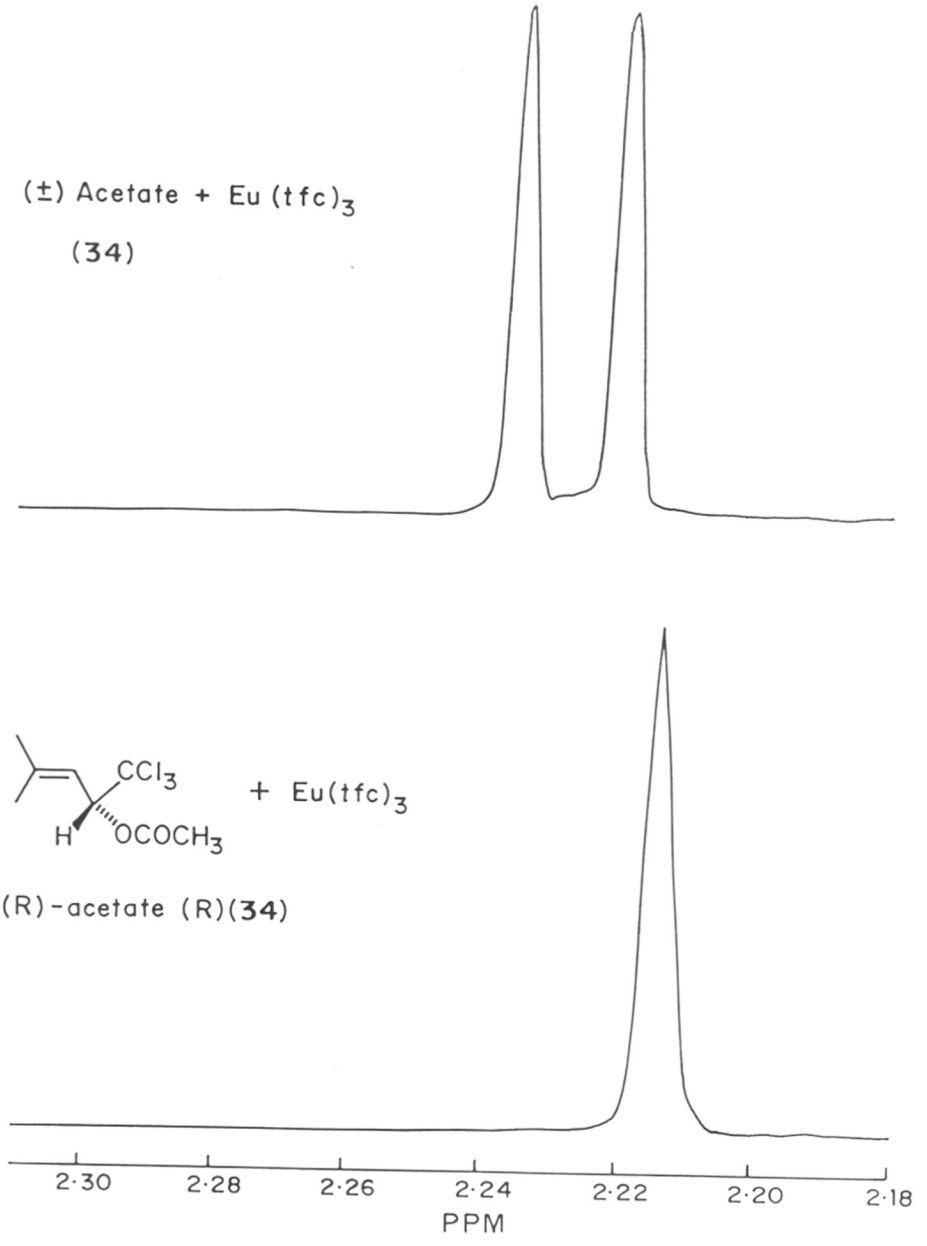
The alcohol had optical rotation $[\alpha]_D$ = -12.0[1] (Lit $[\alpha]_D$ = -12.1 for R(-) alcohol) and m.p. = 109°. The enantiomeric excess of the alcohol was determined by studying the effect of chiral shift reagent on the ^1H NMR spectrum of the acetate prepared from the alcohol. Shift reagent $Eu(tfc)_3$, Tris [3-(trifluoromethylhydroxymethylene)-(+)camphorato] europium (III) derivative] was used for this purpose (Scheme IV). The chiral shift reagent, when added to the $CDCl_3$ solution of the acetate, complexes with the compound. As a result of this complexation, a shift in the position of the signal of a particular proton/protons is observed in the ^1H NMR spectrum.

In this case the methyl protons of the acetyl group show a clear-cut shift. These protons appear as a singlet at 2.06δ in the original racemic compound. When shift reagent is added, this signal splits into two singlets in the case the of racemic acetate. The two singlets appear at 2.219δ and 2.234δ (Table 2).

TABLE 2

Chemical Shift - δ values for $COCH_3$ protons

Compound No	Before addition of shift reagent $Eu(tfc)_3$	After addition of shift reagent $Eu(tfc)_3$
(±) (34)	2.06 δ	2.219 δ2.234 δ
(R) (34)	2.06 δ	2.219 δ
(S) (34)	2.06 δ	2.249 δ



Spectrum No. I

Such an experiment was carried out for the (R)-acetate corresponding to the R(-) alcohol. Only one singlet at 2.219δ was observed (Spectrum I).

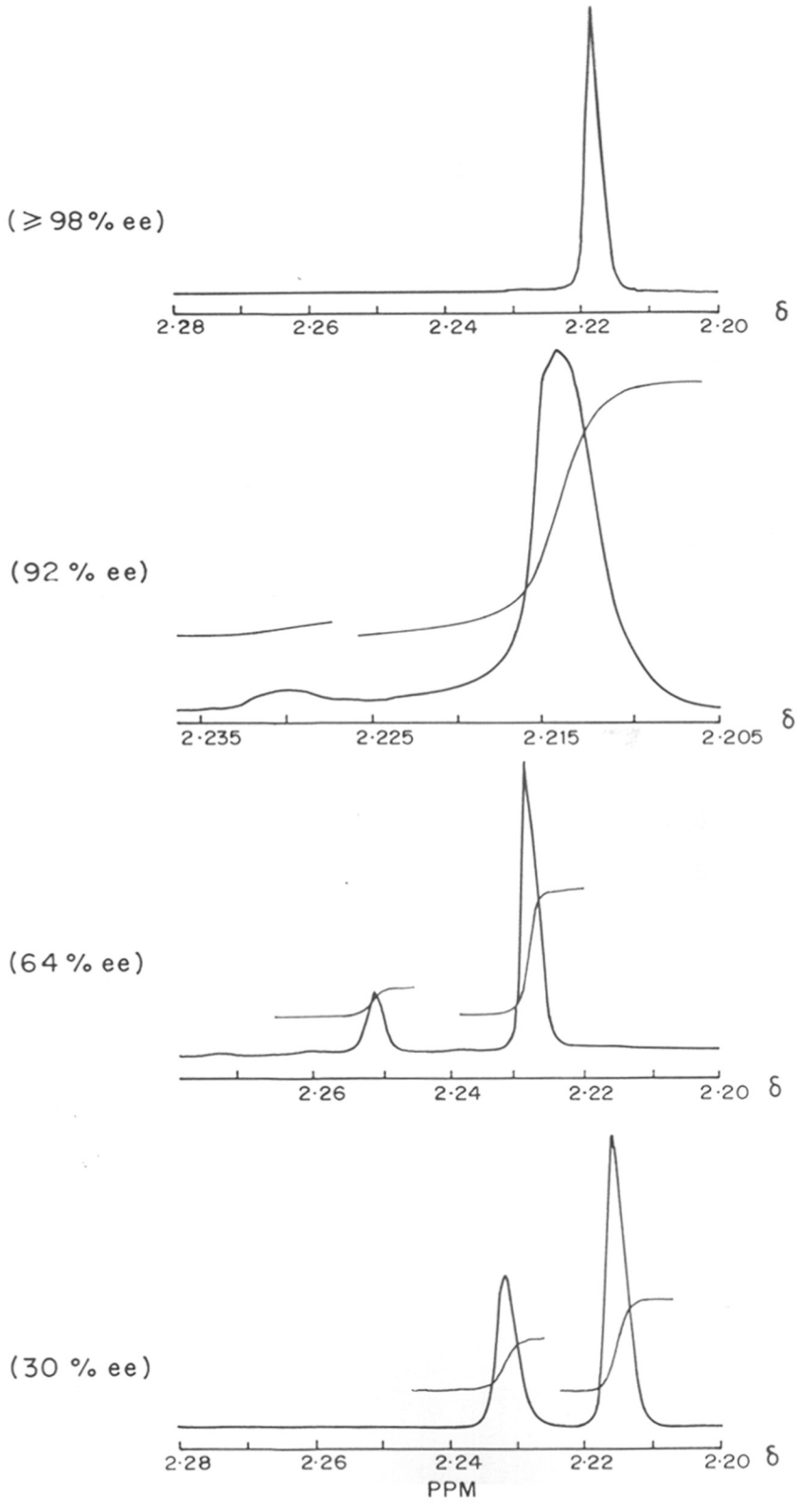
The relative area under each of these two signals (or integration) gives the proportion of the two enantiomers. For the racemic acetate the integration for the two signals is of equal value showing equal proportion of the two enantiomers resulting in the racemic compound. But for the (R)-acetate (R) (**34**) only one singlet was observed showing that it is a single pure isomer.

These ^1H NMR studies with the shift reagent were carried out on MSL 300 MHz instrument. The sensitivity of the instrument was checked by studying the effect of adding the shift reagent on the ^1H NMR spectrum of artificially prepared mixtures of pure (R) and (S) acetates. The mixture containing 96% (R) acetate and 4% (S) acetate showed two clearly separated signals for the methyl protons of the ester group on addition of 10 mg of chiral shift reagent [$Eu(tfc)_3$] to 10 mg of total acetate (Spectrum II). This shows the sensitivity of the method, and hence the validity of the calculation.

The (R)(-) alcohol was isolated in 38% yield (50% is the theoretical yield as 50:50 mixture of *R:S* is present in the starting compound) with 98% enantiomeric excess. The (R)(-) alcohol was isolated as white needles, m.p. 109° (Lit. 80°)[1].

The unhydrolysed acetate was chemically hydrolysed to get the antipodal (S)(+) alcohol (1) $[\alpha]_D$ = +11.8 (Scheme III) and m.p. 108°.

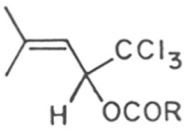
All the other esters namely formate (**40**), propionate (**35**) valerate (**36**), caprylate (**37**), benzoate (**38**) and p-nitrobenzoate (**39**) were separately subjected to enzymatic hydrolysis using *B.Subtilis* for 48h with a substrate concentration of 0.5% at 30°.



SENSITIVITY EXPERIMENT

Spectrum No. II

TABLE 3



(34-40)

Compound No	-R	Method of preparation	Conversion %GC	$[\alpha]^{25}$	%ee
(34)	$-CH_3$	A	42	-12.0	98
(35)	$-C_2H_5$	A	40	-12.0	98
(36)	$-nC_4H_9$	B	30	-11.6	96.6
(37)	$-nC_7H_{15}$	B	12	-11.5	95.8
(38)	$-C_6H_5$	B	15	-11.9	99
(39)	$-C_6H_4pNO_2$	B	0	-	-
(40)	-H	A	100	-	-

The reaction mixture in each case was extracted with solvent ether. After drying over anhydrous sodium sulphate, the solvent was removed to get the crude reaction product. The crude reaction products from each of these experiments were analysed by GC to determine the extent of hydrolysis (Table 3).

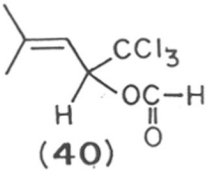
The crude reaction product was purified by column chromatography (silica gel 60-120 mesh, 10% ethylacetate in pet ether 60-80° fraction) to get the pure alcohol and unhydrolysed ester. The optical rotation of the alcohol was measured in each case. The enantiomeric excess of the alcohol was determined as before by using the chiral shift reagent on the corresponding acetate (prepared from the respective alcohols obtained after hydrolysis).

With the above mentioned conditions, the formate ester (**40**) got hydrolysed completely (100% hydrolysis) while p-nitrobenzoate ester (**39**) didnot get hydrolised at all (0% hydrolysis). The propionate (**35**), valerate (**36**), caprylate (**37**) and the benzoate esters (**38**) showed 40%, 30%, 12% and 15% hydrolysis respectively. The pure alcohol from each experiment had the same sign and value of optical rotation. The (R)(-)alcohol from these ester hydrolyses had 96-99% *ee* and $[\alpha]_D$ = -11.5 to -12.0 (Table 3).

A gradual decrease in the extent of hydrolysis was observed as the number of carbons in the ester chain increased. Thus, the formate ester showed 100% hydrolysis while the caprylate showed only 12%.

Substrate concentration and duration: these two parameters were varied in the case of the formate ester(**40**). Several experiments with higher substrate concentration and reduced duration of enzymatic hydrolysis were carried out. (Table 4). Hydrolysis to the extent of 47.1 had taken place in 4h at 1% substrate concentration [$(\alpha)_D$ = -10.1] while 33% hydrolysis took place in 6h at 2% concentation [$(\alpha)_D$ = -11.0]

TABLE 4



(40)

Sr.No	Substrate conc. (%)	Incubation time (hr.)	Conversion % GC	$[\alpha]^{25}$
1	0.5	48	100	-
2	0.5	6	65	-5.84
3	0.5	4	62	-9.3
4	0.5	2	48	-10.6
5	0.5	1.5	44	-10.2
6	0.5	1	38	-11.2
7	1	2	34	-10.4
8.	1	3	43	-10.6
9	1	4	47	-10.1
10	2	3	27	-11.0
11	2	4	29	-10.5
12	2	6	33	-11.0

In the case of the p-nitrobenzoate ester (39) the use of a miscible solvent (tetrahydrofuran) or an immiscible solvent (pet ether) (to solubilise the solid ester) in the broth failed to hydrolyse the ester.

Conclusion

The micro-organism *Bacillus subtilis* was found to be very efficient for enantioselective ester hydrolysis to get (R) (-) 1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene in high chemical yields and enantiomeric excess (>98%)

3.4 Experimental

Preparation of 1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene (±) (1)

A one litre round bottom flask, equipped with a bubbler and a pressure equalising tube, was charged with anhydrous chloral (101g) in dry pet.ether (300 ml). Isobutylene gas was generated by addition of t-butanol (150 ml) to 30% H_2SO_4 (60 ml) on a boiling hot water bath. Isobutylene gas was bubbled into the chloral solution at 0°C with stirring over a period of 2 hrs. Anhydrous $AlCl_3$ (2.4g) was then added to the reaction mixture in lots at 0°C with stirring. Stirring was continued for 4 hr. at 0°C and then at room temperature for 4 hr. Water (50 ml) was added and stirred for 0.5 hr. The two layers were separated and the organic layer was washed with water and then with brine and was dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator to yield 101g of the homoallylic alcohol.(**33**)

Isomerisation to 1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene

The homoallylic alcohol (**33**) (101g) and p-toluenesulfonic acid (0.72g) were mixed and heated at 110°C for 1.5 hrs. The reaction mixture was steam distilled to get the allylic alcohol (**1**) 48g (48% yield) m.p. = 78-80° (Lit[1]. 78-80°). **I.R. (Nujol, cm-1)** : 3250, 1680, 1310, 1280, 1130, 1060, 1000, 860, 830, 780. **NMR (CDCl3,** δ) : 1.85 (m, 6H, $(\underline{CH_3})_2$); 2.5 - 2.75 (br s, 1H, -OH); 4.82 (d, J = 9Hz, 1H, C<u>H</u>-CCl_3); 5.42 (m, 1H, <u>CH</u> =)

Esterification of the allylic alcohol (1)

Method A

Appropriate anhydride (3 equivalents) was added to allylic alcohol (**1**) Pyridine (3 equivalents) was added slowly over a period of 0.5h and was left overnight.

Little ice was added to the reaction mixture and stirred well. Some more water was then added and the reaction mixture was extracted with solvent ether. The organic layer was washed with 50% H_2SO_4 then with water to remove traces of acid. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed to get the crude product. The product was distilled at reduced pressure.

Method B

The appropriate acid chloride (1.5 eq) in CH_2Cl_2 was added to a stirred solution of the alcohol (**1**) (2.5 g,1eq.) and triethylamine (1.5 ml,1.5 eq) in CH_2Cl_2 at 0°C. The reaction mixture was stirred at 0°C for 1 h and then at room temp.for 1 h. Water was added to the reaction mixture and stirred for 15 minutes and then extracted with CH_2Cl_2. The organic layer was washed with sodium bicarbonate solution then with water and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed to get a crude product. Dry methanol was added and was stirred overnight. Methanol was removed completely and the residue was dissolved in solvent ether. The organic layer was washed with cold dil. sodium bicarbonate solution then with water. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed; and the residue was distilled at reduced pressure.

(1,1,1-trichloro-2-ol-4-methyl-3-pentene) acetate (34)

Method A for preparation - b.p. 90o/4mm; Yield = 89%

IR (Neat, cm^{-1}) : 1760, 1670, 1440, 1370, 1220, 1020, 930, 850, 800, 780 **^{1}H NMR (CCl_4,δ) :**1.81 (s, 6H, (CH3)2 =); 2.06 (s, 3H, OCOCH3); 5.2 (m, 1H, CH=); 5.9 (d, J=10 Hz, 1H, CH-CCl3). **MS (m/z) :** 113, 85 (100%), 57

Analysis Calcd. for $C_8H_{11}Cl_3O_2$; C = 39.13; H = 4.52. Found C = 39.41; H = 4.12

(1,1,1-Trichloro-2-ol-4-methyl-3-pentene) - propionate (35)

Method A for preparation: b.p. = 81-84o/1mm; Yield = 75%

I.R. (Neat, cm^{-1}) : 1760, 1680, 1480, 1390, 1290, 1180, 1100, 1050, 1020, 910, 870, 780· **^{1}H NMR (CCl_4;δ)**: 1.18 (t, J=7Hz, 3H, $\underline{CH_3}$-(CH_2); 1.88 (m, 6H, $(\underline{CH_3})_2$-); 2.38 (q, J = 7Hz, 2H, $\underline{CH_2}$ -CH3); 5.2 (m, 1H, $\underline{CH}$ =); 5.95 (d, J = 9Hz, 1H, $\underline{CH}$-CCl_3). **MS (m/z)** = 258 (M+), 169, 149, 115, 85 (100%) 77, 57

Analysis calcd. for $C_9H_{13}Cl_3O_2$ - C = 41.64, H = 5.05; Found = C = 41.83, H = 5.16

(1,1,1-Trichloro-2-ol-4-methyl-3-pentene) valerate (36)

Method B for preparation - b.p. 98-99°/1.5 mm; Yield = 60%

IR (Neat, cm^{-1}) : 1760, 1680, 1390, 1450, 1240, 1160, 1050, 940, 780, 810, 640· **^{1}H NMR (CCl_4** δ): 0.95 (t, J = 7Hz, 3H, $\underline{CH_3}$- $(CH_2)_3$,), 1.13-1.73 (m, 4H, $(\underline{CH_2})_2$; 1.85 (m, 6H, $(\underline{CH_3})_2$-); 2.36 (t, J=7Hz, 2H, CH_2-$(CH_2)_2$; 1.85 (m, 6H, $(\underline{CH_3})_2$-); 2.36 (t, J=7Hz, 2H, $\underline{CH_2}$-$(CH_2)_2$), 5.23 (m, 1H, $\underline{CH}$=), 5.93 (d, J=9Hz, 1H, $\underline{CH}$-CCl_3). **MS (m/z)** : 149, 113, 85 (100%), 77, 57. **Analysis** : Calculated C = 45.93, H = 5.96; Found = C = 45.71, H = 5.83

(1,1,1-Trichloro-2-ol-4-methyl-3-pentene) Caprylate (37)

Method B for preparation: b.p. = 102-104°/1.5 mm; Yield = 60%

IR (Neat, cm^{-1}): 1760, 1680, 1470, 1390, 1160, 1050, 950, 860, 780· **^{1}H NMR (CCl_4** δ): 0.9 (t, J = 4Hz, 3H, $(CH_2)_6$- CH_3); 1.28 (m, 10H, CH_2-$(CH_2$-$(CH_2)_5$-$CH_3)$; 1.83 (m, 6H, $(\underline{CH_3})_2$); 2.28 (t, J=7Hz, 2H, $\underline{CH_2}$-$(CH_2)_5$; 5.2 (m, 1H, $\underline{CH}$ =), 5.9 (d, J=9Hz, 1H, $\underline{CH}$-CCl_3). **MS (m/z)** : 185, 166, 149 (100%), 141, 131, 113. **Analysis** calc d. for $C_{14}H_{23}Cl_3O_2$; C = 50.99, H = 7.03; Found C = 50.69, H = 7.31

1,1,1-trichloro-2-ol-4-methyl-3-pentene - benzoate - (38)

Method B for preparation : b.p. 120-23o/5mm; Yield = 92%

I.R.(Neat, cm^{-1}) : 1730, 1600, 1450, 1310, 1250, 1100, 1090, 1020, 790, 760, 700 **^{1}H NMR (CCl_4 δ)**: 1.9 (s, 3H, ($\underline{CH_3}$)-); 2.0 (s, 3H, ($\underline{CH_3}$) -; 5.36 (m, 1H, $\underline{CH}$=); 6.15 (d, J = 9Hz, 1H, $\underline{CH}$-CCl_3); 7.3 - 8.2 (m, 5H, ArH). **MS (m/z)** = 271, 189, 149, 105 (100%), 77. **Analysis** Calcd. for $C_{13}H_{13}Cl_3O_2$ - C = 50.75, H = 4.26; Found C = 50.70, H = 4.29

1,1,1-Trichloro-2-ol-4-methyl-3-pentene-) 4-nitrobenzoate (39)

Method B for preparation : Crystalline Light Yellow Solid; m.p. = 106-107oC; Yield = 65%

I.R.(Nujol, cm^{-1}) : 1730,1610, 1525, 1375. **^{1}H NMR (CCl_4,δ)** : 1.9 (s, 3H, ($\underline{CH_3}$) -); 1.96 (s, 3H, ($\underline{CH_3}$) -; 5.4 (m, 1H, $\underline{CH}$ =); 6.18 (d, J = 9Hz, 1H, CH-CCl_3); 8.2 (s, 4H, ArH). **MS (m/z)** = 234, 150, (100%), 104. **Analysis** calcd. for $C_{13}H_{12}Cl_3NO_4$ - C = 44.27, H = 3.43; Found = C = 44.47, H = 3.72

Preparation of acetic formic anhydride.

To the solution of freshlydistilled acetyl chloride (44.5 ml) in dry solvent ether (25 ml), sodium formate (50g) was added in lots in such a way that the temperature did not exceed 27°C. the reaction mixture was stirred overnight. The inorganic salt was filtered off. Solvent ether was removed under reduced pressure below 20°C to get acetic formic anhydride (50g). IR (Neat, cm^{-1}) : 1795, 1775, (Characteristic 20 cm^{-1} difference whereas for other anhydrides the difference is 60 cm^{-1}).

(1,1,1-Trichloro-2-ol-4-methyl-3-pentene) formate (40)

Method A for preparation: (acetic formic anhydride and pyridine were used put in catalytic quantity).

IR (Neat, cm^{-1}) : 1740, 1670, 1445, 1380. **^1H NMR (CCl_4, δ) :** 1.9 (m, 6H, $(CH_3)2$); 5.3 (m, 1H, CH=); 6.06 (d, J = 9Hz, 1H, CH-CCl_3); 8.03 (s, 1H, C-CHO). **MS (m/z)** = 149, 113, 85 (100%), 77. **Analysis** calcd. for $C_7H_9Cl_3O_2$ - C=36.31, H=3.92; Found - C=36.80, H = 4.00

Ester hydrolysis using *B.Subtilis* for esters (34-39)

The micro-organism was grown for two days in 100 ml nutrient medium (100 ml containing 1g of peptone, 0.4g of meat extract, 0.1g glucose and 0.1g agar-agar)0.5 ml of the ester was added to the flask in the sterile conditions. The flask was kept on the shaker (240 rpm) for 48h. The contents of the flask were extracted with solvent ether (3 x 50 ml). The ether layer was washed with saturated sodium chloride solution and then it was dried over anhydrous sodium sulphate. After removal of the solvent, crude reaction product was examined by G.C. analysis (ov 101 column) and then the product was purified by column chromatography (silica gel 60-120 mesh, 15g, 10% ethylacetate pet-ether 60-80° fraction) to get pure (R) (-) alcohol and unhydrolysed ester.

Chemical hydrolysis of recovered (S) ester (34)

To 1g of (S) acetate (34), 2.5% NaOH solution (1.2 eq) was added. Ethanol was added to the mixture till it became homogeneous. The reaction mixture was left overnight at room temperature. The ethanol was removed at reduced pressure and the residue was diluted with water and then extracted with solvent ether, the ether layer was washed with water followed by saturated sodium chloride solution.

Then dried over anhydrous sodium sulphate. After removal of solvent ether, (S)(-) alcohol was obtained [yield = .785g (95%)] as white needles with m.p. = 108°C $[\alpha]_D$ = +11.8

(S) (+) - 1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene : (S) (+) (1)

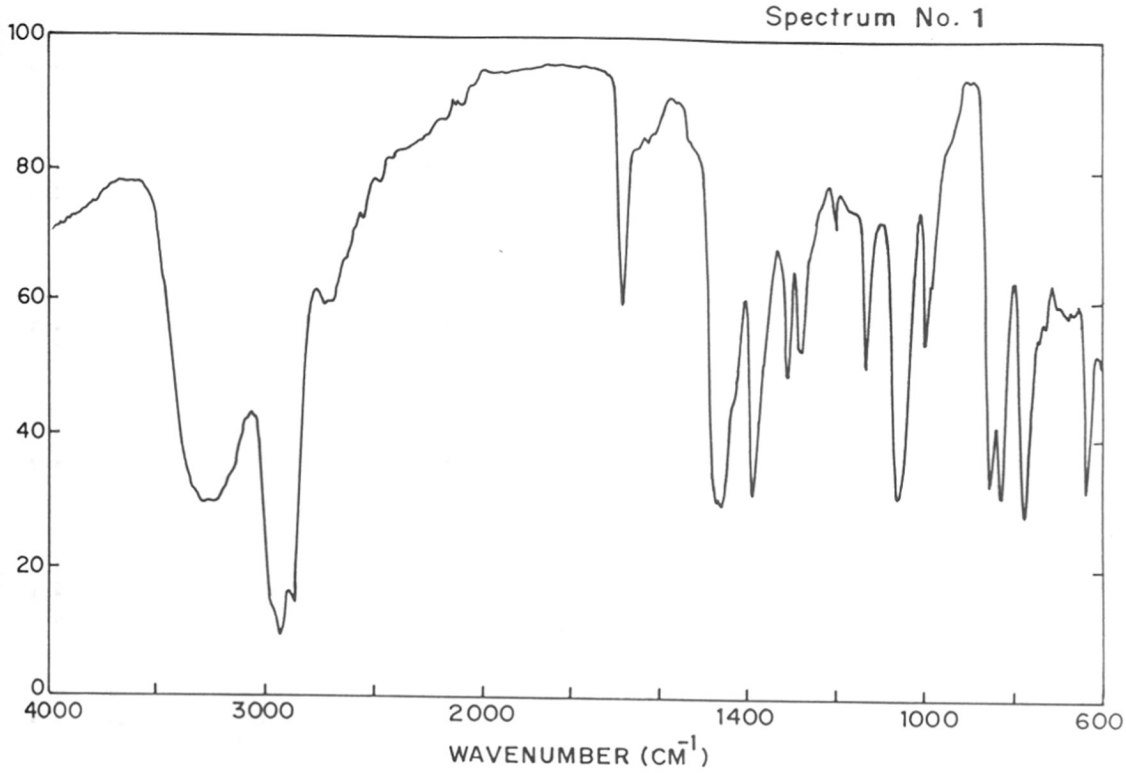
I.R. (Nujol, cm^{-1}) : 3250, 1680, 1310, 1280, 1130, 1060, 1000, 860, 830, 780

^1H NMR ($CDCl_3$, δ) : 1.85 (m, 6H, $(\underline{CH_3})_2$ -); 2.5 - 2.75 (bs, 1H, -OH); 4.82 (d, J = 9Hz, 1H, $\underline{CH}$-CCl_3); 5.42 (m, 1H, $\underline{CH}$ =)

(R) (-) 1,1,1-Trichloro-2-hydroxy-4-methyl-3-pentene : (R) (-) (1) :

m.p. 109°C; $[\alpha]_D$ = -12.0

I.R. (Nujol, cm^{-1}) : 3200, 1680, 1310, 1280, 1130, 1060, 1000, 860, 830, 780 **^1H NMR ($CDCl_3$, δ)**: 1.85 (m, 6H, $(\underline{CH_3})_2$ -; 2.5 - 2.75 (bs, 1H, -OH); 4.82 (d, J = 9Hz, 1H, $\underline{CH}$ - CCl_3); 5.42 (m, 1H, $_{CH}$=). **MS (m/z)** : 131, 117, 85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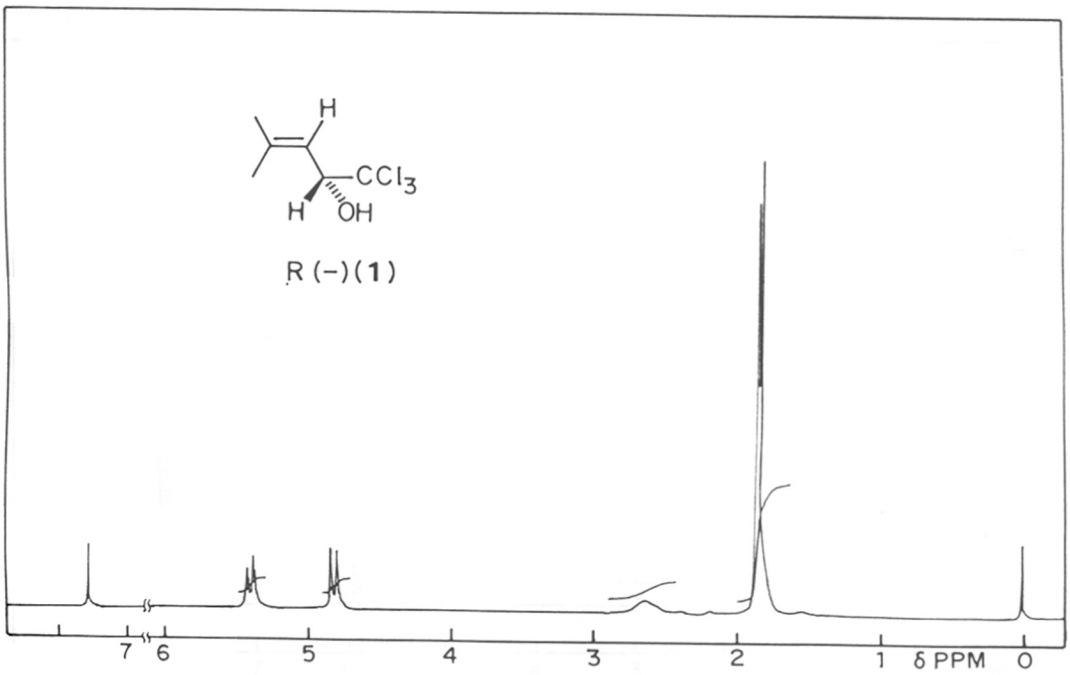
2000
1400
1000
600
WAVENUMBER (CM⁻¹)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CCl3
H
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R (−)(1)
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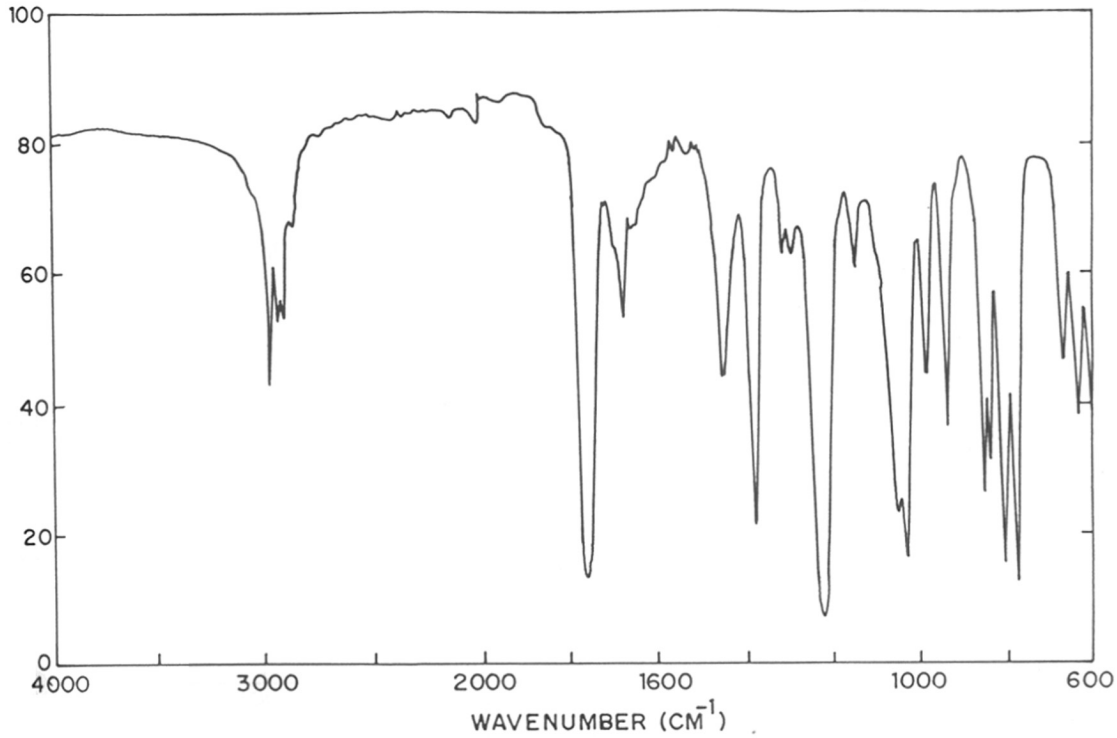

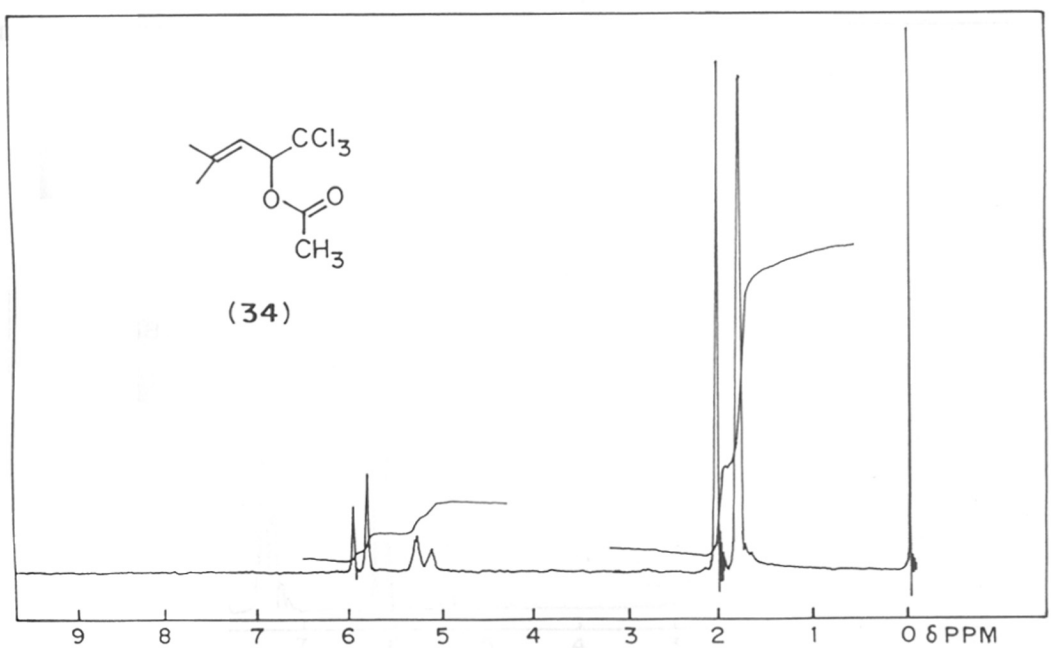

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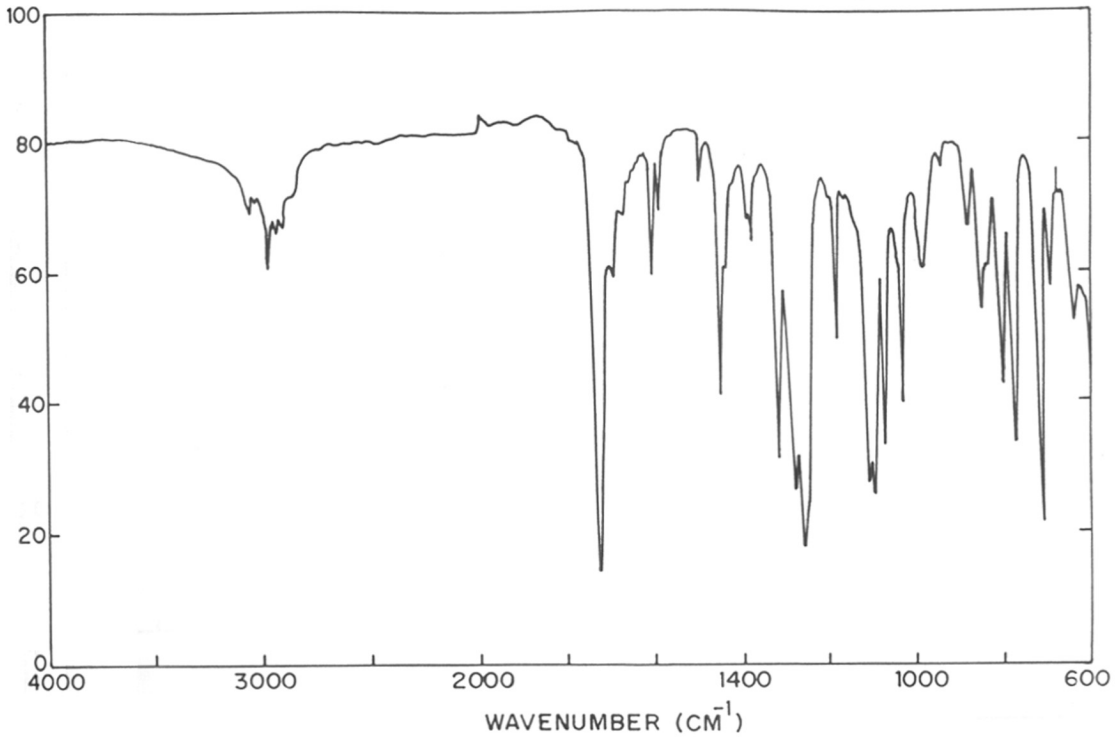

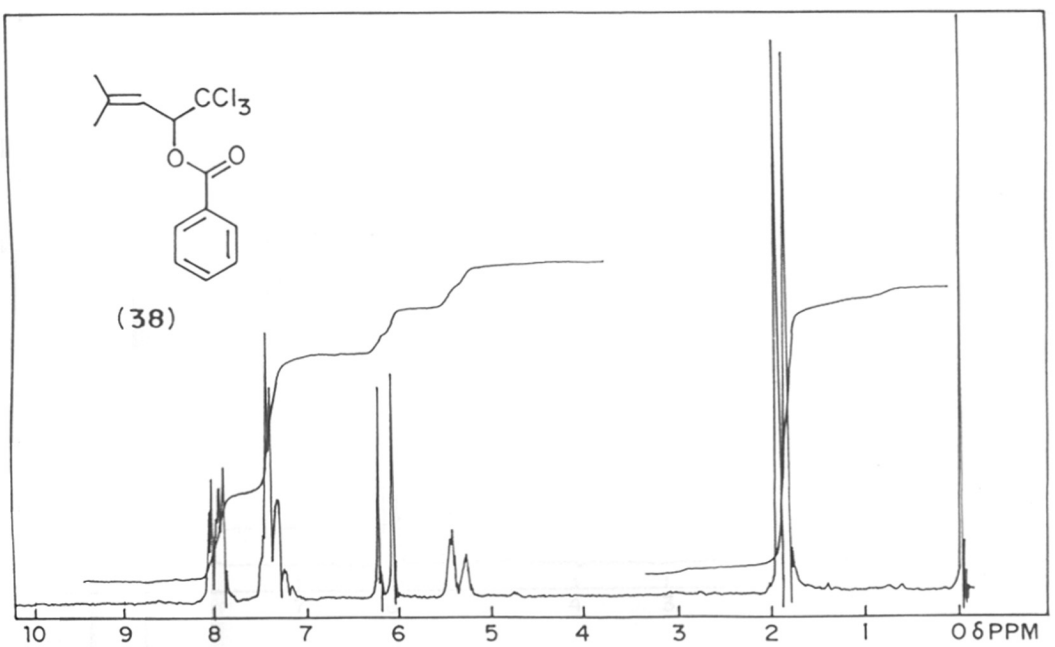

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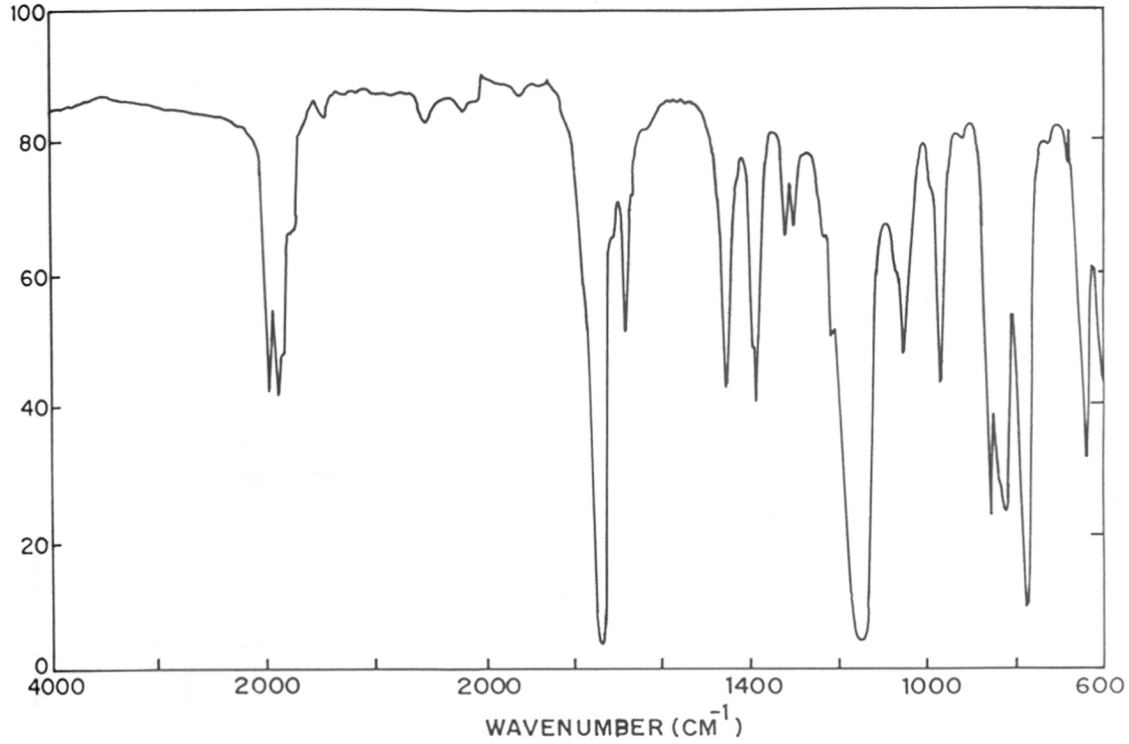

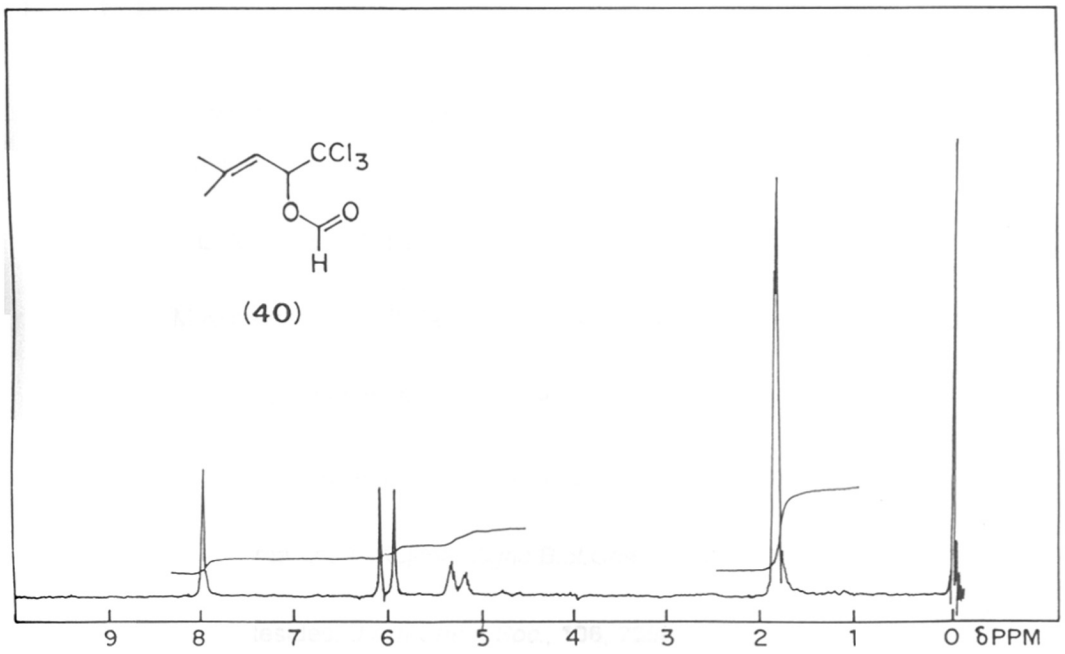

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PART B

Experiments towards asymmetric synthesis of phenylalanine derivatives

CHAPTER 4

Nitroacetyl group as a peptide synthon

Nitroacetyl group as a peptide synthon

4.1 Introduction

Aminoacids, peptides and proteins are biologically very important molecules[1]. In fact they control most of the physiological processes of the living system. Several natural and synthetic oligopeptides have been shown to possess significant biological activity. However, their use is restricted by their ease of hydrolysis both by acids and enzymes. In order to get more potent analogs, the naturally occurring peptides have been subjected to various modifications such as (i) change in the peptide chain length i.e. adding or removing some aminoacids units at specific sites (ii) replacement of individual aminoacids by other natural or unnatural ones and (iii) changes in the peptide backbone[2,3]. Such modifications are known to increase the stability of the peptide to enzymatic hydrolysis and hence one could expect an increase in the bio-activity. The understanding of the structure activity relationship of the peptide analogs would help in the construction of peptides with specific bio and chemical properties. In many instances it has been observed that most of the biological activity of a polypeptide is due to a small fragment of the peptide chain. Thus identification and synthesis of such small fragments and synthesis of their analogs have become very important in the field of drug research[4].

Polypeptides having natural aminoacid sequences can be obtained on a large scale by the standard techniques of molecular biology. But the incorporation of unnatural aminoacids at specific sites needs chemical methods where suitably protected aminoacids or peptides are coupled by the usual classical approaches to get the required oligopeptides[5,6].

A non-preteinogenic aminoacid could have one alkyl group or two alkyl groups at the α carbon. Synthesis of these aminoacids and their incorporation into synthetic oligopeptides is one of the main research objectives at present.

α-Amino isobutyric acid (Aib) is an example of this class. This amino acid is a constituent of Alamethicin and related peptides which are well known for their membrane modifying properties[7,8]

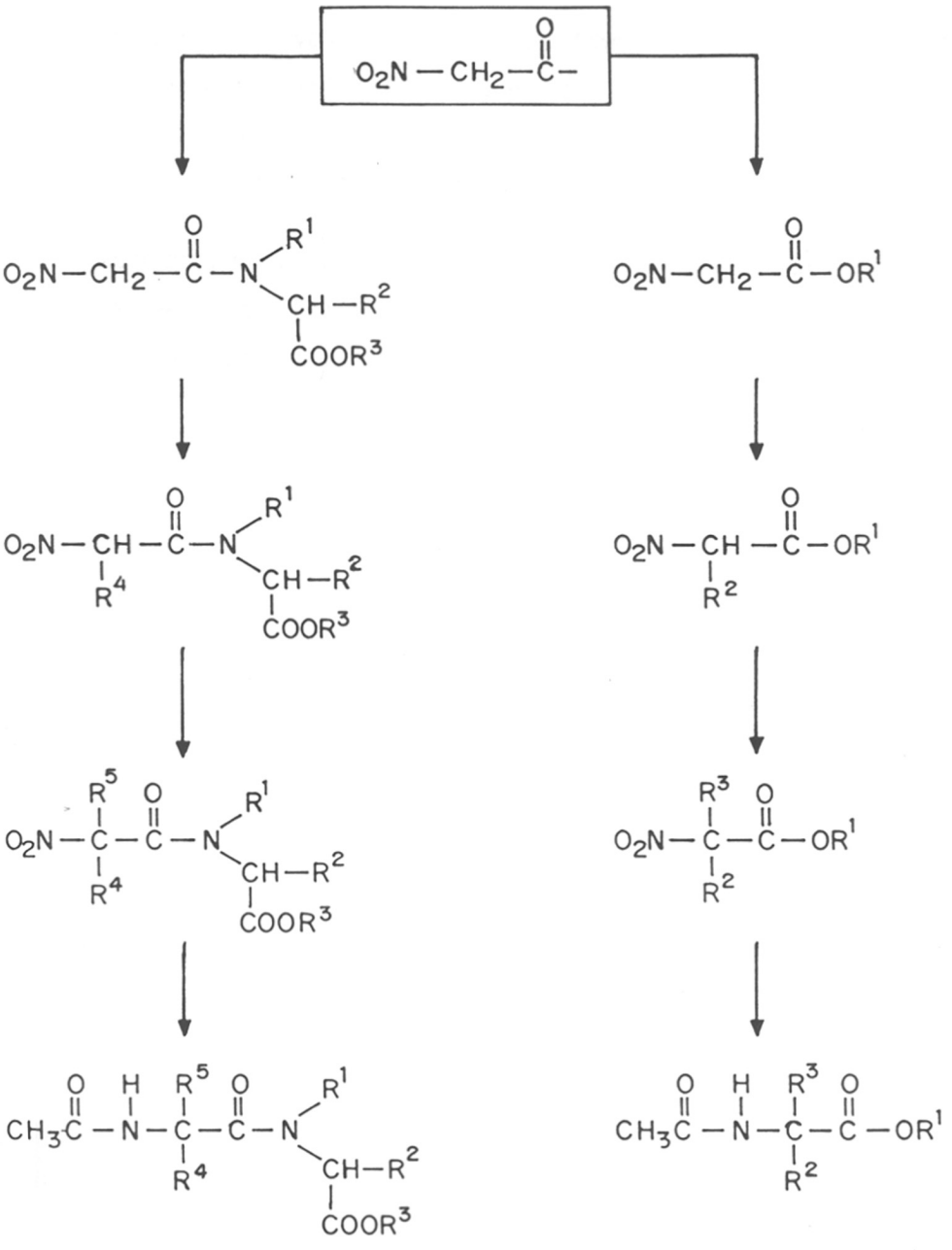
In the recent past many synthetic methods have been developed for the synthesis of such α, α-dialkyl aminoacids[9,10,11]. But the classical methods of incorporation of these aminoacids into a peptide chain have been found to be too difficult due to the steric hindrance associated with the quaternary α-carbon atom of the α, α -disubstituted glycine and hence yields of the products are generally poor[12]. If drastic reaction conditions are employed, racemization of other chiral centres in the molecule may occur[13].

In the last few years, there has been a resurgence of interest in the use of aliphatic nitro compounds as building blocks for the synthesis of various biologically important molecules.

The nitroacetyl group would be an attractive synthon for peptides especially for those involving unnatural α-aminoacids and aminoacids possessing α, α -dialkyl substituents. Such an approach would have two major advantages. First, the methylene group is flanked by a carbonyl and a nitro group, making it highly acidic; reactions with various electrophiles would therefore be feasible at this site. In fact one can expect a regiospecific reaction at this site, even in the presence of other carbonyl-activated methylene or methine groups in the substrate. Second, the nitro group is a latent primary amine and can be transformed to an amino group at the end of other functional transformations.

Thus nitroacetyl derivatives could be converted to unusual dipeptides or to α, α-disubstituted aminoacid derivatives as shown in the Scheme I.

Scheme-I



The procedure involves initial synthesis of the nitroacetamide derivative or nitroacetic ester followed by sequential mono or dialkylation at the methylene carbon atom. The last step would be reduction of the nitro group to an amine or acetyl amine. This completes the reaction sequence (Scheme I).

Such an approach to the synthesis of a dipeptide from a nitro acetamide has not been reported so far. The main reason for this is the lack of an efficient and mild synthetic method for the preparation of nitroacetamides.

4.2 Synthesis of nitroacetamides

In the first reported synthesis of nitroacetamides[14,15], the sodium salt of nitromethane was reacted with phenyl isocyanate in dry benzene to get N-nitroacetylaniline. Apart from low yields, (12.55%), the method involves the use of isocyanate which is prepared using phosgene. This is one of the major drawbacks of this method. The second method[16] involves the reaction of dimethylamine hydrochloride with ethyl nitroacetate in aqueous solution, at refluxing temperature, giving N,N-dimethylnitroacetamide in 48% yield. Due to the presence of a highly acidic proton in the nitroacetic ester, salt formation takes place on addition of dimethylamine. This is the reason why harsh reaction conditions are necesssary for amidation, leading to partial decarboxylation.

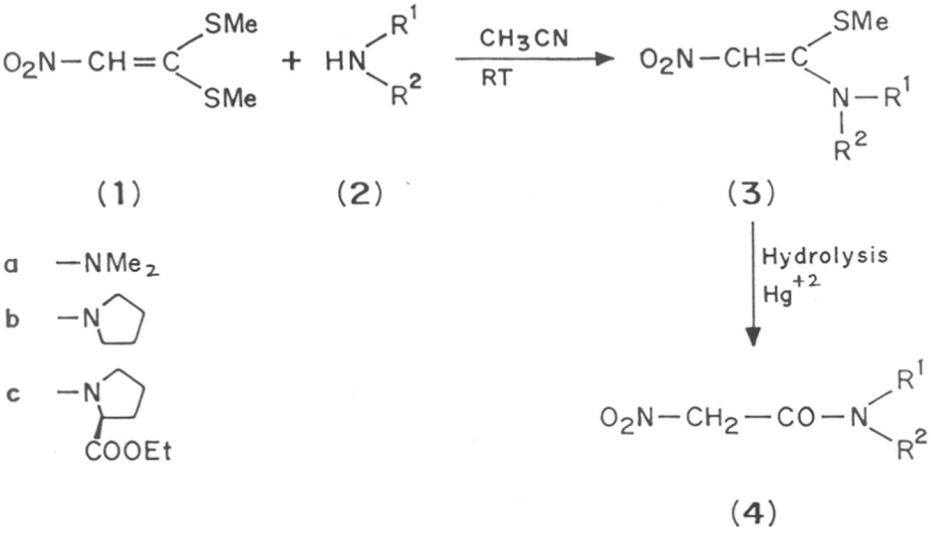
In the third method, a very strong base lithiumdiisopropylamide (LDA) is used to generate an amide enolate, then a nitro group is introduced by reaction with with isopropyl nitrate. Here the yields are good but the use of strong base (LDA) restricts its use for achiral nitroacetamides.

A mild and highly efficient general method for the synthesis of nitroacetamides has been reported by Rajappa and coworkers[18].

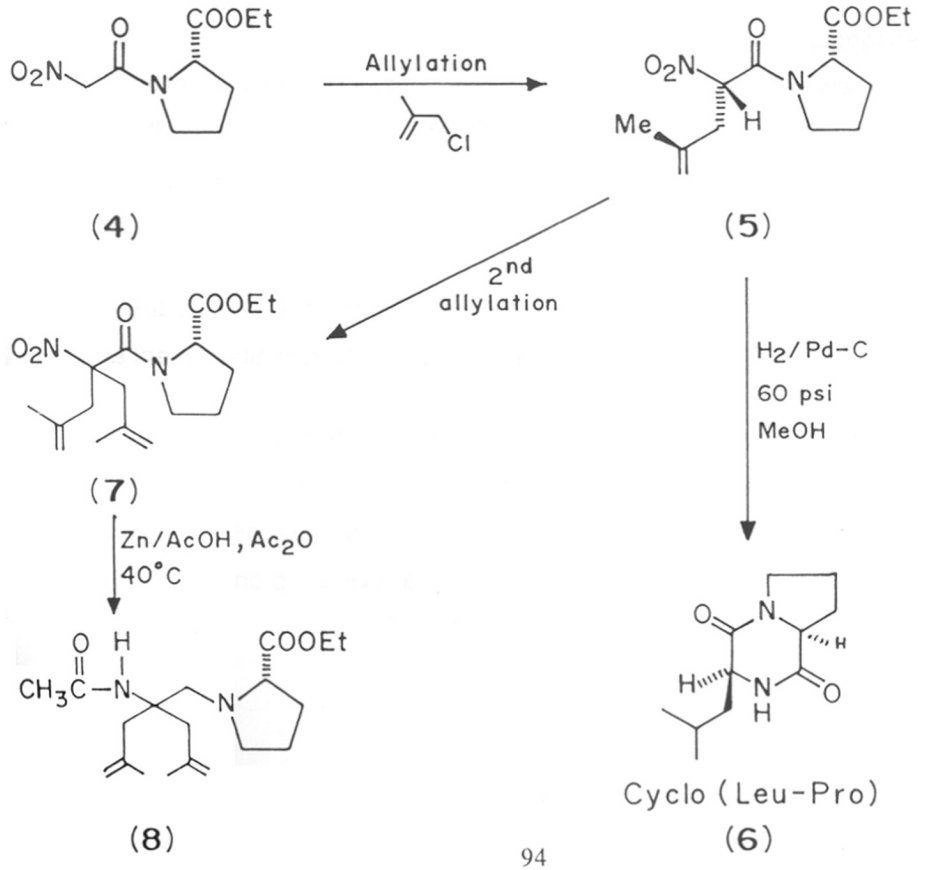
Reaction of 1,1-bismethylthio-2-nitroethene[19] (**1**) with a primary or secondary amine (**2**) at 30° in acetonitrile as solvent gave 1-methylthio-1-substituted amino -2-nitroethene (**3**) Hg^{2+} catalysed hydrolysis of this nitroenamine (**3**) gave the corresponding nitroacetamide (**4**) in high yields. The hydrolysis was carried out at 25° for 3 h in acetonitrile water mixture (3:1) (Scheme II)

This method for the synthesis of nitroacetamides has been elegantly used in the synthesis of ethyl N-(nitroacetyl) prolinate[18] (**4c**) (Scheme II)

Scheme - II



Scheme III



4.3 Nitroacetamides in peptide synthesis

These nitroacetamides were found to be good synthons for the synthesis of peptides incorporating an α,α-disubstituted glycine residue at the N-terminus. α,α-Disubstitution could be achieved by Pd(O) catalysed allylation or Michael addition.

A successful Pd(O) catalysed diastereoselective monoallylation has been demonstrated[20]. The electrophile could be allyl acetate or a substituted allyl acetate. Thus treatment of ethyl N-(nitroacetyl)-(L)-prolinate (**4a**) in degassed acetonitrile with DBU, Pd $(dba)_2$ and dppe under argon at 25° with methallyl chloride for 10h, followed by quenching at -20° with 5% aqueous HCl gave the product (**5**) in 65% yield with 25% *de* (Scheme III).

The major diastereomer was isolated and was subjected to catalytic hydrogenation with Pd-C. Under these conditions the NO_2 group was reduced to NH_2, the terminal alkene was saturated and the resultant dipeptide ester cyclised to the diketopiperazine cyclo (Leu-Pro) (**6**). The product was compared with authentic cyclo (L-Leu-L-Pro). Thus the absolute configuration of the major diastereomer of monoallyl derivative of N-nitroacetyl (L)-proline ethyl ester was established as (S.S.).

Subsequently, a second allylation under essentially the same conditions gave the α, α-disubstituted products (**7**).

These disubstituted products did not undergo reduction of nitro group on catalytic hydrogenation. However, reduction was successfully achieved using zinc dust in acetic acid - acetic anhydride mixture at 40° for 6h, the product being the acetyl amino derivative (**8**)[21].

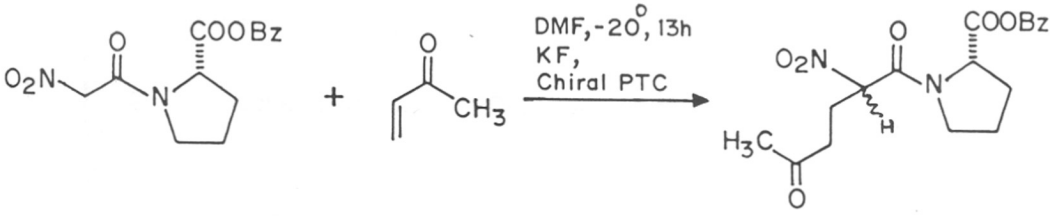
Diastereoselective alkylation was also achieved by Michael addition[22]. The diastereoselective addition of N-(Nitroacetyl) aminoacid derivatives to various Michael acceptors was successfully achieved using KF as catalyst for Michael addition. 18-Crown-6 or other chiral or achiral phase transfer catalysts have been used along with KF.

The addition of the benzyl N-(nitroacetyl)-(L)-prolinate (**9**) to methylvinyl ketone (**10**) as the Michael acceptor in DMF at -20° for 13h with KF-benzylquinidinium chloride as chiral phase transfer catalyst followed by quenching the reaction at -20° by 5% HCl gave the adduct (**11**) in excellent yields (90%) (*de* 51%) (Scheme IV)

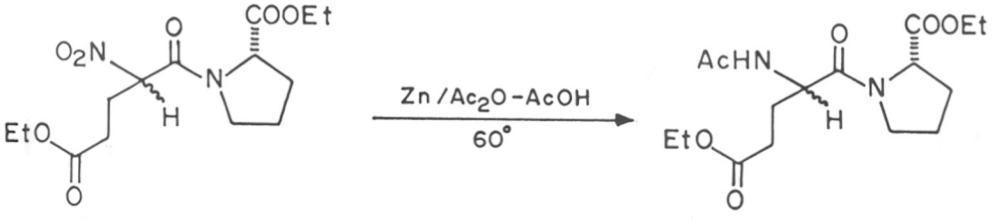
Similarly the adduct (**12**) was obtained in 89% yield with 41% *de* when ethyl acrylate was the Michael acceptor. The absolute configuration of the newly created chiral centre was established for the adduct (**12**). The nitro group of this adduct (**12**) was reduced to NHAc (**13**) and the ^{1}H and ^{13}C NMR spectra of this compound were compared with that of authentic L-Glu-L-Pro derivative. The NMR studies concluded that the major diastereomer of (**13**) had (S,S) configuration.

This leads to the conclusion that the major diastereoisomer formed in the Michael addition of N-(nitroacetyl)-L-prolinate to Michael acceptors had the (S) configuration at the newly created chiral centre.

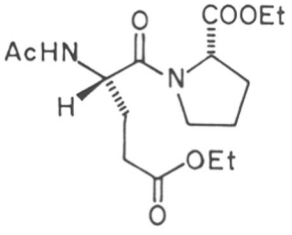
Scheme - IV



(9) (10) (11)



(12) (13)



(S,S) (13)

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CHAPTER 5

Alkylation of nitroacetic acid derivatives and synthesis of phenylalanine derivatives

5.1 Summary

α -Alkyl nitroacetic acid esters constitute a class of attractive synthons for a variety of organic molecules such as aminoacids, aminoalcohols, nitroacrylates etc. These α -alkyl nitroacetic acid esters can arise by alkylation of the C-unsubstituted nitroacetic acid derivatives.

A comparative study of four different alkylation methods was therefore carried out on three nitroacetic acid derivatives namely methyl nitroacetate (**1**), N-(2-nitroacetyl)-pyrrolidine (**2**) and benzyl N-(2-nitroacetyl)-(L)-prolinate (**3**).

The first method (Method A) is alkylation by benzyl bromide under phase transfer catalysis (PTC) with potassium bicarbonate as base. In the second method (Method B) benzyl bromide in presence of DBU is the alkylating reagent. The third method (Method C) involves alkylation under Mitsunobu conditions using diethyl azodicarboxylate (DEAD) triphenyl phosphine and benzyl alcohol as alkylating agent. In the last method (Method D) the Schiff base of an aromatic aldehyde is reacted with the nitroacetic ester to form a nitroolefin; borohydride reduction of this results in the formation of α-alkyl nitroacetic acid ester.

In the case of methyl nitroacetate (**1**) the best results were obtained by Method D. Method A & B gave 40% and 35% of the C-benzyl product (**4a**) respectively. By method C, the yield of the C-benzyl derivative was 25%, the by-product being an oxime (**5**) (25%)

For N-(2-nitroacetyl) pyrrolidine (**2**), Method A gave 25% of the C-benzyl product (**6**) while method B gave a 35% yield of this compound. No C-alkylation product was obtained by Method C.

In the case of benzyl N-(2-nitroacetyl)-(L)-prolinate (**3**) the yield of (**7**), the corresponding C-benzyl derivative was 27% by method A and 30% by method B. However, method C failed to give any C-benzylated product. The diastereomeric excess of the product obtained by methods A & B was 8% and 14% respectively.

The C-Benzylated products (**4**) and (**6**) were reduced further under mild conditions to get N-acetyl phenylalanine methyl ester (**8**) and : N-acetyl-phenylalanine pyrrolidide (**9**) respectively.

5.2 Introduction

α-Alkyl nitroacetic acid esters constitute a class of attractive synthons for a variety of organic molecules such as nitro alcohols, nitroacrylates, oxazolidines, aminoacids, aminoalcohols[1] etc.

In the literature, many methods are available for alkylation of nitroacetic acid derivatives. These methods are briefly discussed below.

1. Alkylation with alkyl halides

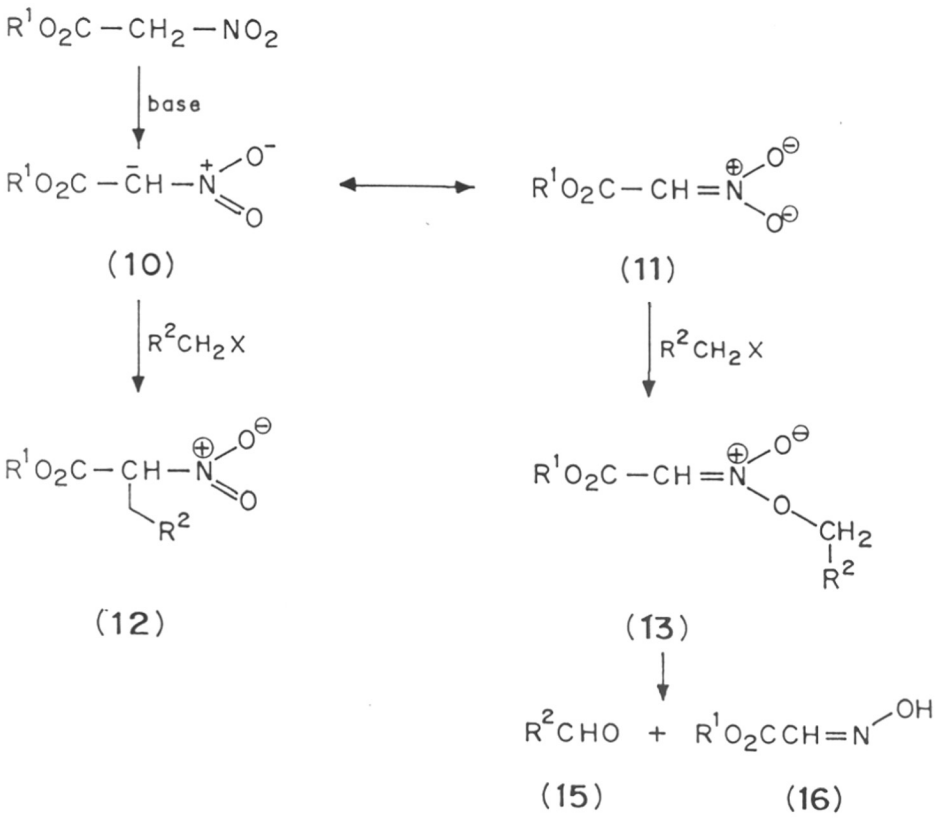
In the case of alkyl nitroacetates the carbanion generated by base is stabilised by the nitro group present; this ambident anion can be represented by either of the two structures (**10**) and (**11**). Hence alkylation can occur at either the carbon or the oxygen atom to yield products (**12**) or (**13**) respectively. (Scheme I).

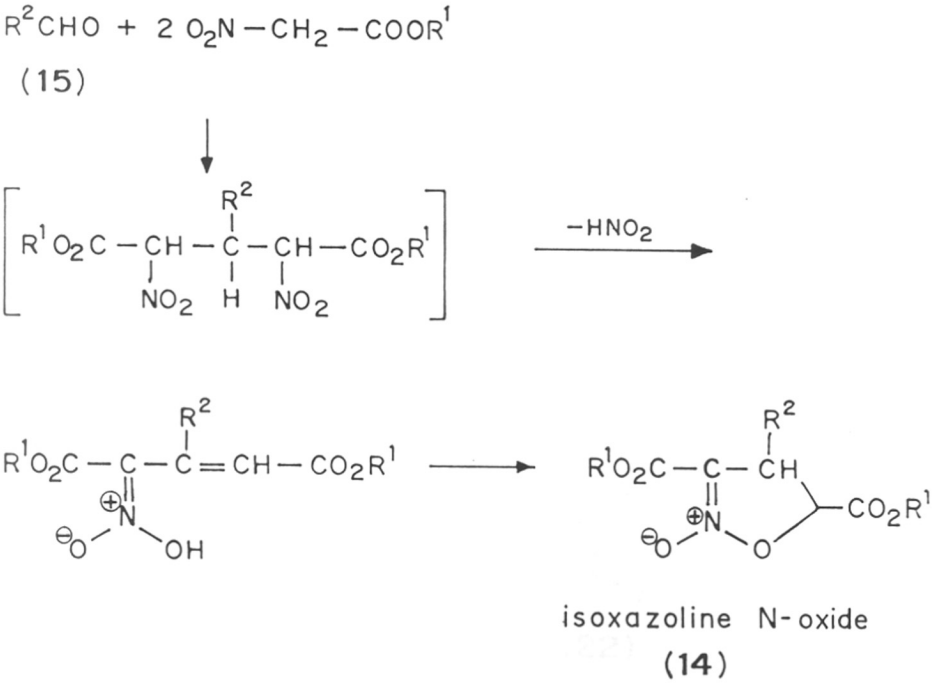
When n-alkyl iodides were used, in addition to the C-alkylated product (**12**) isoxazoline N-oxide (**14**) was isolated in 40% yield[2]. This isoxazoline N-oxide was formed as a result of the reaction of 2 moles of alkyl nitroacetate with the aldehyde (**15**) which is formed after decomposition of the initially generated O-alkylated product. An oxime (**16**) is another product of decomposition of the O-alkyl product (Scheme I). The condensation product after loss of HNO_2 cyclises to form the isoxazoline N-oxide (**14**)

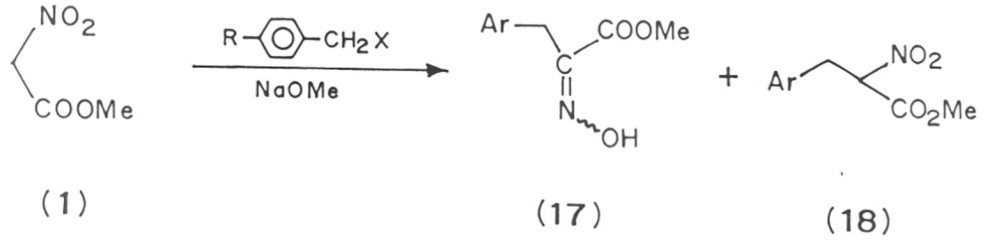
Similarly when a p-substituted benzyl halide was used in the alkylation of alkyl nitroacetate (**1**), 3-phenyl-2-hydroxyiminopropanoate (**17**) (10-17% yield) and 3-phenyl-2-nitropropanoate (**18**) (20-37%) were obtained[3].

The silver salt of ethyl nitroacetate (**19**) has been treated with methyl iodide to get ethyl 2-nitropropanoate (**20**) in 28%yield[4].

Scheme-I

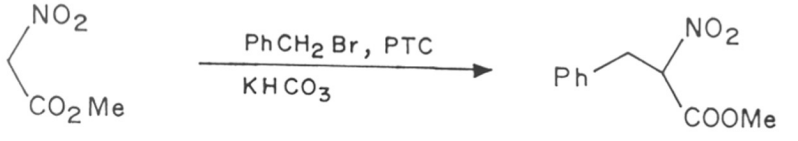

R1O2C−CH2−NO2
base
(10)
(11)
R2CH2X
R2CH2X
(12)
(13)
R2CHO + R1O2CCH=N−OH
(15)
(16)


R2CHO + 2 O2N−CH2−COOR1
(15)
−HNO2
isoxazoline N-oxide
(14)

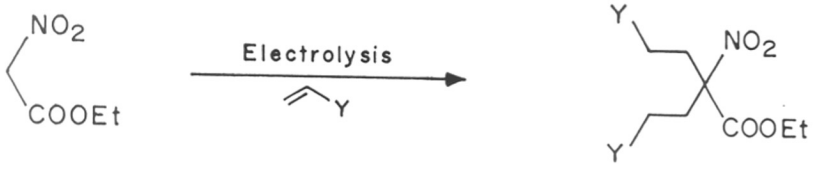


(1) (17) (18)

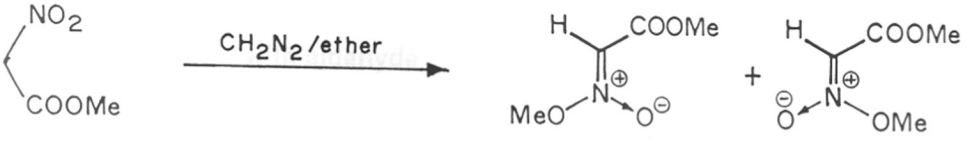
(19) (20)



(1) (4a)



(19) (21)



(1) (22) (23)

Alkylation of methyl nitroacetate (**1**) with benzyl bromide under phase transfer catalysis (PTC) yielded the C-alkylation product (**4a**) in 70% yield; TEBA was used as PTC[5].

Mono and dialkylation of ethyl nitroacetate (**19**) have been carried out electrochemically[6] in high yields (>80%) through generation of the anion followed by nucleophilic attack on an alkyl halide or by Michael addition. By the use of this methodology the symmetrical disubstituted product (**21**) was obtained in a one pot reaction.

2. Alkylation with diazomethane

Methylation of methyl nitroacetate (**1**) with diazomethane led to O-methylation i.e. methyl ester of the nitronic acid as a mixture of (E) and (Z) isomers (**22**) and (**23**) in the ratio 2:3[7].

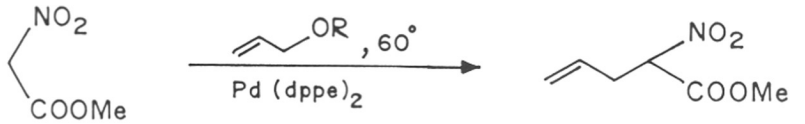
3. Transition metal catalysed alkylation

Transition metal catalysed allylation has been studied. Methyl nitroacetate (**1**) was reacted with allyl phenyl ethers in presence of Pd $(dppe)_2$[8,9] as catalyst to give C-allyl product (**24**) in good yields under mild conditions. Similarly allylation was also carried out on ethyl nitroacetate (**19**) using allylic carbonates to get the C-allylation product (**25**) in 85% yield[10].

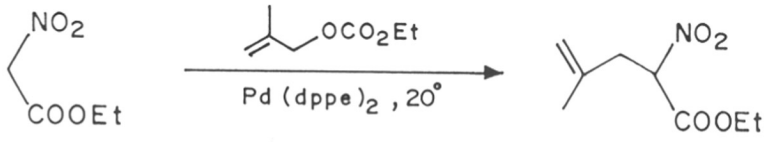
4. Reaction with alcohol

Heating of ethyl nitroacetate (**19**) with trityl alcohol in the presence of trichloroacetic acid resulted in the formation of ethyl 3,3-3-triphenyl-2-nitropropanoate (**26**) in 41% yield[11].

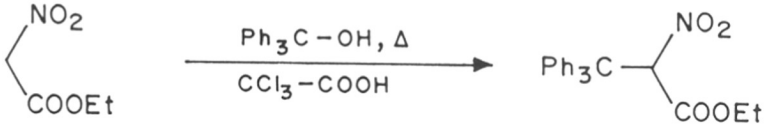
5. Reaction with aldehyde



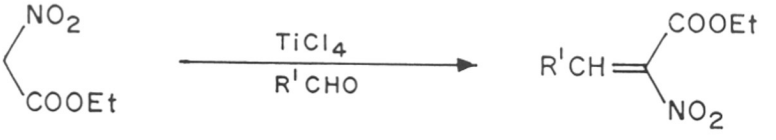
(1) (24)



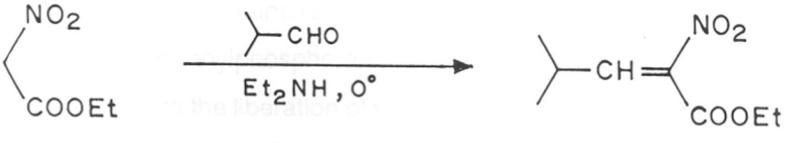
(19) (25)



(19) (26)



(19) (27)



(19) (28)

The Knoevenagel condensation of ethyl nitroacetate (**19**) with aromatic and aliphatic aldehydes in presence of titanium (IV) chloride and pyridine has given the corresponding α-nitroacrylates (**27**) in 40-45% yield[12].

When ethyl nitroacetate (**19**) was treated with isobutyraldehyde in presence of diethylamine in ligroine at 0°C followed by treatment with dil HCl, ethyl 2-nitro-4-methyl-2-pentenoate (**28**) was obtained in 54% yield[13].

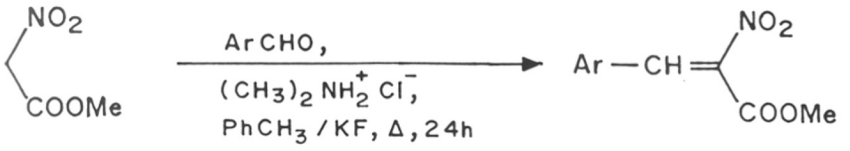
Substituted benzaldehydes have been reacted with methyl nitroacetate (**1**) in the presence of dimethylammonium chloride and catalytic amount of KF in refluxing toluene to give an E/Z mixture of α-nitrocinnamic esters (**29**)[14].

6. Reaction with Schiff bases

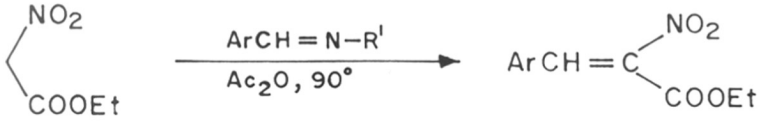
When Schiff bases of aromatic aldehydes were heated with ethyl nitroacetate (**19**) in presence of acetic anhydride, α-nitrocinnamic esters (**29**) were obtained in 11-66% yields[15].

KF catalysed cyclisation of *cis*-2,6-dihydroxy-1,4-oxathiane (**30**) with ethyl nitroacetate (**19**) was carried out at room temperature in presence of dibenzo-18-crown-6 to get isomeric products (**31**) and (**32**) in 7 and 17% yields respectively[16].

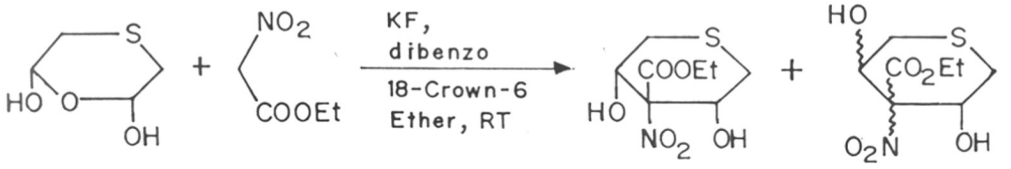
The Mitsunobu reaction is a powerful methodology for the formation of carbon-heteroatom bonds[17]. In this reaction an acidic substrate HX is alkylated by an alcohol ROH to form RX. HX could be a carboxylic acid, phenol or an active methylene compound. Diethyl azodicarboxylate (DEAD) and triphenyl phosphine (PPh_3) are the reagents. DEAD and PPh_3 react instantaneously to form the betaine (**33**). One mole of this betaine reacts with two moles of alcohol (ROH) to give one mole of dialkoxytriphenylphosphorane (**34**). This reacts further with HX to give the product RX (**35**) with the liberation of triphenylphosphine oxide. One mole of ROH is liberated in the process (Scheme II). The pKa of the carbon acid (HX) should



(1) (29)

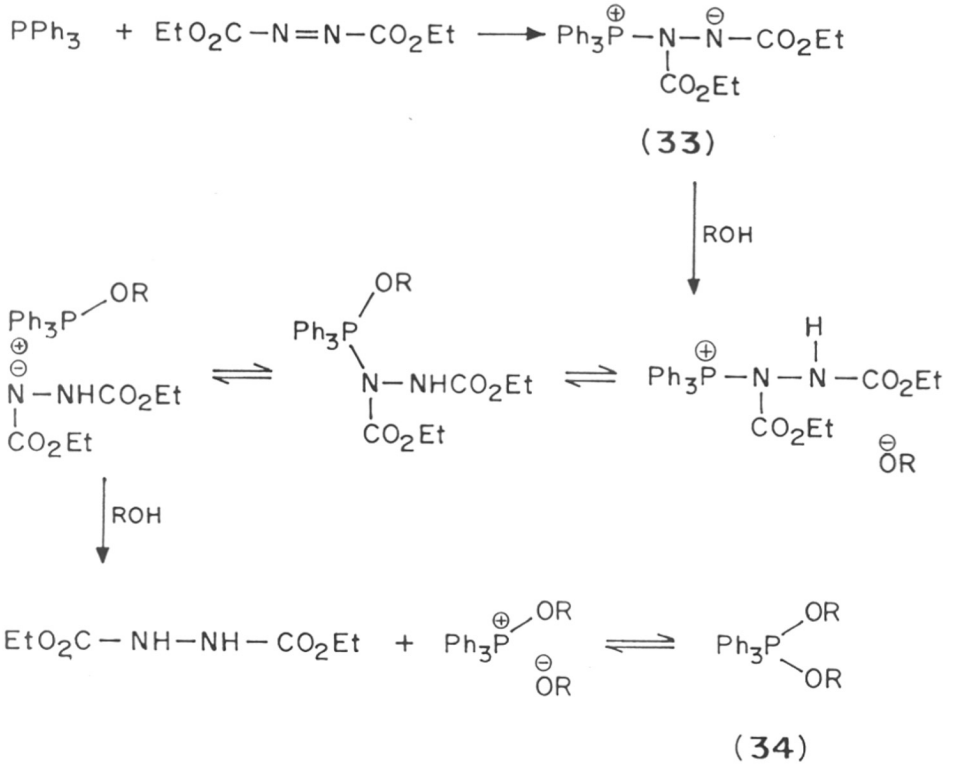


(19) (29)



(30) (19) (31) (32)

Scheme-II



(33)

(34)

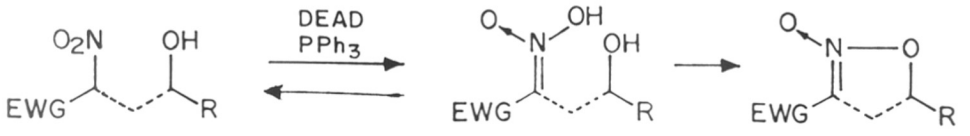
(35)

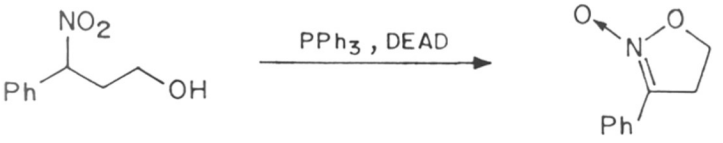
fall within the range (pKa < 14) for participation in the Mitsunobu reaction. Also in cases where there is a choice of reactive atom (i.e. O vs C), often the Mitsunobu reaction leads preferentially to the formation of carbon-hetero atom bond[17]. Its use in the formation of carbon-carbon bonds has been limited to only a few examples[18,19,20]

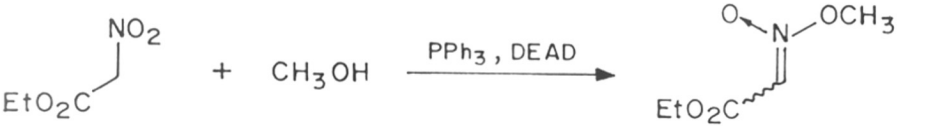
Aliphatic nitro compounds bearing electron-withdrawing or unsaturated substituents on the α-carbon experience intra or intermolecular O-alkylation by alcohols under the influence of a preformed complex of DEAD and PPh_3 affording good yields of alkyl nitronates[21] (Scheme III). C-Alkylation of the ambident anion has not been observed so far. Thus, 1-nitro-1-phenyl-3-propanol (**36**) was transformed completely to 3-phenyl-2-isoxazoline-2-oxide (**37**) by intramolecular O-alkylation under Mitsunobu conditions.

Intermolecular coupling of ethyl nitroacetate (**19**) with methanol under Mitsunobu conditions gave a mixture of *cis*- and *trans*- nitronates (**38**). This contrasts sharply with the usual preferential C-alkylation of alkyl nitroacetate anion by alkyl halides in aprotic solvents.

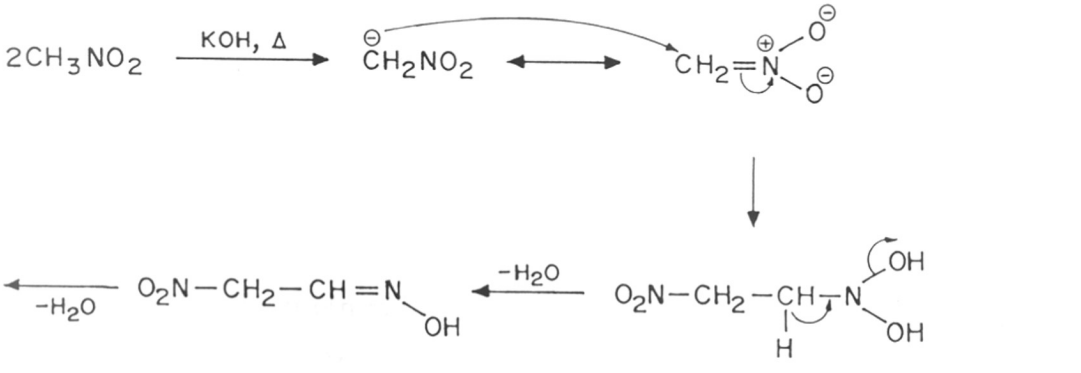
Scheme-III







Scheme-IV



5.3 Present work

Our substrates are the esters and amides of nitroacetic acid. The objective of the work was to optimize the reaction conditions for alkylation at the methylene carbon of these substrates.

Alkylation by four different methods was studied for the three compounds namely, methyl nitroacetate (**1**), N-(2-nitroacetyl) pyrrolidine (**2**) and benzyl N-(2-nitroacetyl)-(L)-prolinate (**3**). In the case of (**3**), alkylation would give a mixture of two diastereomers. It was our objective to study the stereoselectivity of this process. These three substrates were prepared by reported procedures in the literature.

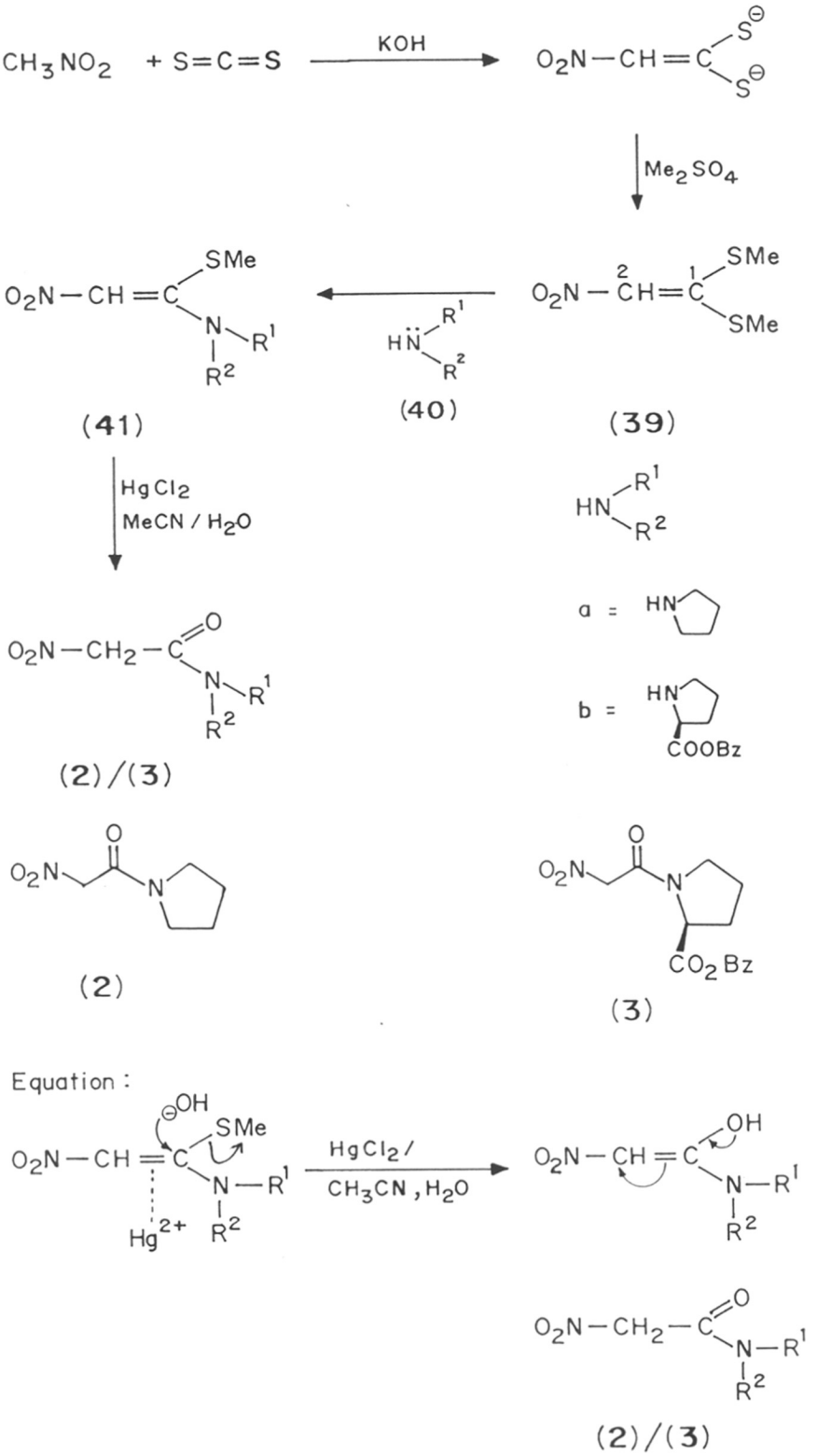
Synthesis of methyl nitroacetate (1)[22]

Nitromethane was first converted to the potassium salt of nitroacetic acid by treatment with aqueous KOH (Scheme IV). Two molecules of nitromethane are involved in the reaction. Self condensation gives rise to methazonic acid which after dehydration yields nitroacetonitrile. After hydrolysis of this at elevated temperature, loss of molecule of ammonia takes place and yields a dipotassium salt of nitroacetic acid which after acidification followed by esterification with methanol results in the formation of methyl nitroacetate (**1**). The compound had IR absorption at 1760, 1575, 1440 cm^{-1}. In the ^1H NMR spectrum, two singlets were observed, one at 3.8 δ for methyl ester protons and the second at 5.12 δ for methylene protons.

Preparation of 1,1-bismethylthio-2-nitroethene (39)

The reaction of nitromethane with carbondisulfide in ethanolic KOH gave the dithioic acid salt which was methylated using dimethyl sulphate to get 1,1-bismethylthio-2-nitroethene (**39**).(Scheme V)

Scheme - V


CH3NO2 + S=C=S
KOH
O2N—CH=C(S⊖)(S⊖)
Me2SO4
O2N—CH=C(SMe)(SMe)
HN(R1)(R2)
O2N—CH=C(SMe)—N(R1)(R2)
(41)
(40)
(39)
HgCl2
MeCN / H2O
O2N—CH2—C(=O)—N(R1)(R2)
(2)/(3)
HN(R1)(R2)
a = HN
b = HN, COOBz
O2N—CH2—C(=O)—N
(2)
O2N—CH2—C(=O)—N, CO2Bz
(3)
Equation :
⊖OH
SMe
O2N—CH=C—N—R1
Hg2+
R2
HgCl2 / CH3CN, H2O
O2N—CH=C(OH)—N—R1
O2N—CH2—C(=O)—N—R1
(2)/(3)

Preparation of N-nitroacetyl pyrrolidine (2) and benzyl N-(nitroacetyl)-(L)-prolinate (3)[23]

The secondary amine (**40**) was reacted with the 1,1-bismethylthio-2-nitroethene (**39**) to get 1-methylthio-1-substitued amino-2-nitroethene (**41**). The secondary amine attacks the electrophilic C-1 carbon of (**39**) and displaces the -SMe group as MeSH (Scheme V). In the case of primary amines like methylamine displacement of both methylthio groups can occur.

Mercuric chloride is widely used in the hydrolysis of dithioacetals and ketals to aldehydes and ketones[24]. In the hydrolysis reaction[25] using Hg^{2+}, the Hg^{2+} complexes with the olefin and makes the attack of hydroxyl group easier and the displacement of -SMe takes place. Tautomerisation of the enol to keto form yields the required product. (Equation, Scheme V).

In the present case Hg^{2+} catalysed hydrolysis of 1-methylthio-1-substituted amino-2-nitroethene (**41**) in acetonitrile-water solvent system led to N-nitroacetyl pyrrolidine (**2**) and benzyl N-(nitroacetyl) -(L)-prolinate (**3**) (Scheme V).

N-Nitroacetyl pyrrolidine (**2**) had m.p. 105-106°. The compound showed IR absorption at 1660, 1570, 1450, 1380 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 2.0δ for two β methylene group protons of the pyrrolidine ring was observed. One more multiplet at 3.5δ was observed for the two α methylene group protons adjacent to nitrogen in the pyrrolidine. A singlet at 5.2 δ was observed for the methylene protons adjacent to the nitro group.

Benzyl N-(nitroacetyl)-(L)-prolinate (**3**) had m.p. 141-142°. This compound showed IR absorption at 1750, 1680, 1570, 1460, 1380, 1180 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 2.0δ for the two (β, γ) methylene group protons of the pyrrolidine ring was observed. The δ methylene protons next to nitrogen of the pyrrolidine ring were seen as a multiplet at 3.5δ. The α methine proton of the proline group was

observed as a multiplet at 4.5δ. The methylene protons in the benzylic group were seen as a doublet of a doublet at 5.15δ. The methylene protons adjacent to the nitrogroup were observed as a doublet of doublet at 5.2 δ. The aromatic protons appeared as a multiplet at 7.4δ.

Alkylation methods:

Four different methods for the comparative study in alkylation of the above mentioned three substrates are described below.

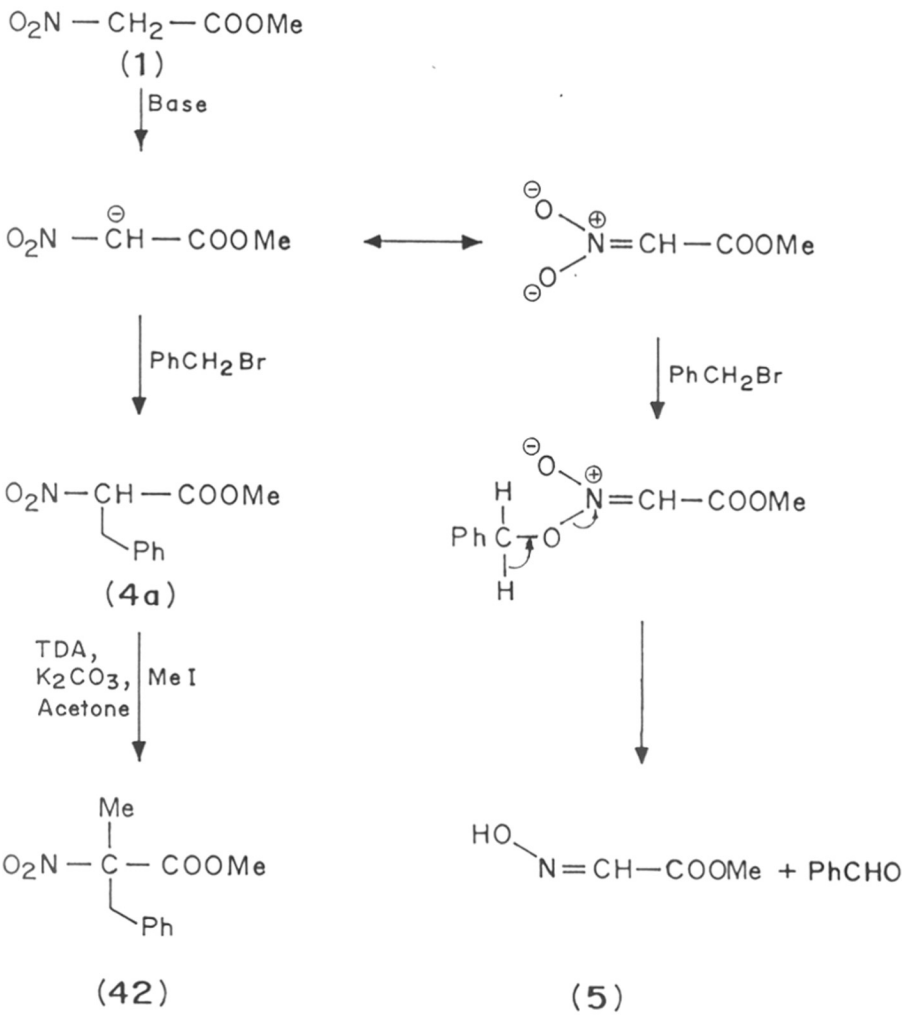
Method A : Alkylation under PTC conditions[5]

Benzyl bromide, potassium bicarbonate and benzyl triethylammonium chloride (TEBA) in DMF were used for benzylation. The carbanion is formed by abstraction of acidic proton at the methylene carbon of the substrate; it is stabilised due to the presence of the nitro group (Scheme VI). The alkylation can occur at either of the two sites of the ambident nucleophile. This would lead to either the C-benzylated product (**4**) or the O-benzylated product. The O-benzylated product is expected to decompose to benzaldehyde and an oxime (**5**).

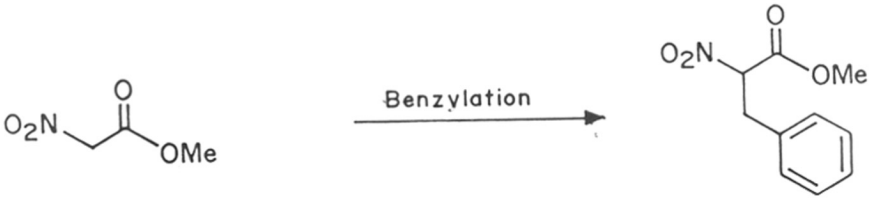
Benzylation of the three compounds (**1**), (**2**) and (**3**) was studied under the above PTC conditions (Scheme VII).

The product methyl α-nitro-β-phenylpropionate (**4a**) was isolated as a colourless liquid in 40% yield. The compound showed IR absorption at 1770, 1575, 1380 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 3.55δ for the methylene protons next to phenyl group was seen. A singlet at 3.85 δ was observed for methyl ester protons. A doublet of a doublet was observed at 5.2δ for the methine proton adjacent to the nitro group. The aromatic protons were observed at 7.3δ as a multiplet.

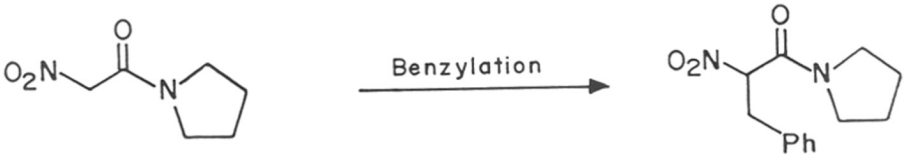
Scheme - VI


O2N — CH2 — COOMe
(1)
Base
O2N — CH — COOMe
PhCH2Br
O2N — CH — COOMe
Ph
(4a)
TDA,
K2CO3,
Acetone
MeI
Me
O2N — C — COOMe
Ph
(42)
N=CH — COOMe
PhCH2Br
H
Ph C O
H
N=CH — COOMe
HO
N=CH — COOMe + PhCHO
(5)

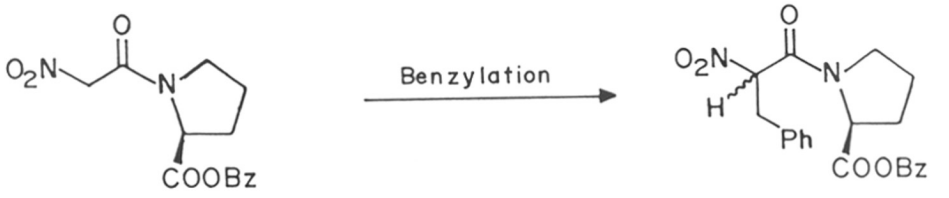
Scheme - VII



(1) (4a)



(2) (6)



(3) (7)

Method A : $PhCH_2Br$, $KHCO_3$, TEBA , DMF , 60°, 24h

B : $PhCH_2Br$, DBU, CH_3CN , 60°, 30h

C : PPh_3 , DEAD , $PhCH_2OH$, C_6H_6 , 5-10° , 24h

D : i) $ArCH=N-Ph$, Ac_2O , 40° , 4h

ii) $NaBH_4$, THF : MeOH (10:1) , R.T. , 1·5h

A second alkylation of the compound (**4a**) - the benzylated product of methyl nitroacetate was also carried out using methyl iodide, potassium bicarbonate and TDA-1 [Tris (3,6-dioxaheptyl) amine] (PTC) in acetone at room temperature to get methyl-α-benzyl-α-nitro propionate (**42**). This was obtained as a colourless liquid in 60% yield. the compound showed characteristic IR absorption at 1770, 1570, 1470, 1390, 1360 CM^{-1}. In the ^1H NMR spectrum, a singlet at 2.65δ was observed for the methyl group protons on the newly formed quaternary carbon. The two singlets at 3.45δ and 3.55 δ were seen for the benzylic methylene protons. The protons of the ester methyl group were observed at 3.8δ as a singlet. Aromatic protons were seen as a multiplet at 7.3δ

The C-benzylated product (**6**) of N-(nitroacetyl) pyrrolidine i.e. N-(2-nitro-3-phenyl) propionyl pyrrolidine was obtained in 25% yield as a light yellow solid, m.p. 65°. It showed IR absorption at 1650, (amide), 1560, 1380 (for nitro group)cm^{-1}. In the ^1H NMR spectrum a multiplet at 1.85 δ was observed for the two β methylene group protons of the pyrrolidine. One of the benzylic protons group was observed as a multiplet at 2.9δ. The second proton of this methylene group and the two α methylene group protons of the pyrrolidine appeared together as a multiplet at 3.5δ. A triplet at 5.4δ was observed for the methine proton next to the nitro group. The aromatic protons were seen at 7.3δ as a multiplet. In the mass spectrum fragment ions at m/z 202 (100%), 131, 103, 91, 77, 55 were observed.

Benzyl N-(2-benzyl-2 nitroacetyl)-(L)-prolinate (**7**) was obtained as a semi-solid in 27% yield. The compound showed characteristic IR absorption at 1730 (ester carbonyl) 1650 (amide carbonyl), 1550, 1380, (nitro group) cm^{-1}.

The ^1H NMR spectrum was complex because of the presence of diastereomers and rotamers. In the ^1H NMR spectrum, a multiplet at 2.0δ for the two methylene group protons (β, γ) proline and one more multiplet at 3.35δ for the δ methylene protons of the proline were observed. A multiplet at 3.6δ was seen for the methylene protons of the C-benzyl group. The α methine proton of the proline

was seen as a multiplet at 4.5δ; the methylene protons of the benzylic ester group were observed as a multiplet at 5.2δ. A multiplet was observed for the methine proton adjacent to the nitro group at 5.5δ . The aromatic protons appeared as a multiplet at 7.4δ. In the mass spectrum fragment ions at m/z 276 (M^+ -PhCHO) 185 (276 - $PhCH_2$) 149 (NO_2CH = CH-Ph), 136, 98, 91, 69, 55 were observed.

Estimation of diastereoselectivity by ^{13}C NMR spectroscopy

The ^{13}C NMR of the compound (**7**) was also complex. By carrying out a DEPT experiment, the signals due to the carbon attached to odd number of hydrogens could be identified from other signals. Thus the resonance signals at 129.0 - 127.3 (carbons of phenyl groups) ; 87.5, 86.7 ($\underline{C}HNO_2$); 59.4, 59.3 ($\underline{C}HCO_2Bz$)were assigned to the carbon atoms attached to an odd number of hydrogens. Similarly the resonance signals corresponding to the carbon atoms attached to an even number of hydrogens were identified and assignment was made based on their chemical shifts; 66.8 ($O\underline{C}H_2Ph$); 47.2, 47.1 ($N\underline{C}H_2$-C_5); 36.1, 35,8 ($Ph\underline{C}H_2CH$); 28.7, 28.6, 24.4 and 24.2 (C_3 and C_4 of the pyrrolidine ring).

The complexity of the ^1H and ^{13}C NMR spectra was due to the presence of *cis/trans* rotational isomers of each diastereomer. Thus four sets of peaks were seen in ^1H NMR and ^{13}C NMR spectra of this diastereomeric product (**7**). Out of the four sets, two sets were having higher intensities and were from the *trans* rotamer of the two diasteromers. The diastereomeric excess (*de*) could be estimated from the integration ratio of the corresponding proton signals and relative height of the carbon signals of the two diastereomers. The area under the curves of the carbon signals in the ^{13}C NMR spectrum do not correspond to the number of carbon atoms. This is due to the difference in the relaxation time and NOE for different carbon atoms of the molecule. However, the corresponding carbon atoms of diastereomers are expected to have nearly the same relaxation time and NOE. Hence for practical purpose, the ratio of the signal intensity of corresponding carbon

atoms in the ^{13}C spectrum of a diastereomeric mixture may be taken to indicate the ratio of diastereomers present in the solution. In fact ^{13}C NMR spectroscopy has been used earlier in many cases for the estimation of *de* [26,27].

Thus the relative intensity of the carbon signal (newly created centre O_2N-$\underline{C}H$-CH_2Ph) from the ^{13}C NMR spectrum of the product (**7**) was used to calculate *de* of the product. The *de* for (**7**) thus estimated was 8%.

Method B : Benzylation using benzylbromide and DBU

Benzyl bromide, DBU and the substrate were heated in acetonitrile at 60° for 24-30h to get the corresponding C-benzylated products.

In the case of methyl nitroacetate (**1**) the product methylα-nitro-β-phenyl propionate (**4a**) was obtained in 35% yield under these reaction conditions.

For N-(2-nitroacetyl) pyrrolidine (**2**) the product N-(2-nitro 3-phenyl) propionyl pyrrolidine (**6**) was obtained in 35% yield.

Benzyl N-(2-benzyl-2-nitroacetyl)-(L)-prolinate (**7**) was obtained in 30% yield, the starting compound being benzyl N-(2-nitroacetyl)-(L)-prolinate (**3**). The product (**7**) in this experiment had 14% *de*.

All the three products were identified by spectral data. The spectral data of these compounds were identical to those described earlier (Method A)

Method C : Benzylation under Mitsunobu conditions

DEAD and PPh_3 are the reagents. These reagents react instantaneously at 5-10° to form the betaine (**33**) (Scheme II). This reacts with 2 moles of alcohol to form dialkoxytriphenyl phosphorane (**34**). In turn this reacts with the active methylene compound to yield the product.

In the case of methyl nitroacetate (**1**) with benzyl alcohol, the product, methyl α-nitro β-phenyl propionate (**4a**) was obtained in 25% yield. Along with this product, an oxime, methyl α -hydroxyimino acetate (**5**) was obtained in 25% yield; this is a decomposition product of the O-benzylated product (same as shown in Scheme VI).

The compound methyl α-hydroxyimino acetate (**5**) had IR absorption at 3300, 1720, 1630, 1450, 1040 cm^{-1}. In the ^1H NMR spectrum, a singlet at 3.9δ for methyl ester protons was observed. The olefinic proton was observed at 7.6δ as a singlet. The hydroxyl proton on the nitrogen was observed as a broad singlet at 9.8δ. In the mass spectrum the fragment ions at (m/z) 103(M^+), 71 (100%, M^+ - MeOH), 59, were observed.

When p-chlorobenzyl alcohol was used as alkylating agent for the same substrate (**1**), the product methyl β-(4-chlorophenyl)-α-nitro propionate (**4f**) was isolated in 5% yield. This compound had IR absorpstion at 1750, 1550, 1510, 1400, 1310, 1280 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 3.45δ was observed for the methylene protons adjacent to p-chlorophenyl group. A singlet at 3.77δ was observed for the CH_3 of the methyl ester. A doublet of a doublet was observed at 5.3δ for the methine proton adjacent to the nitro group. The typical A_2B_2 pattern for a *para* substituted phenyl group was seen at 7.13 (d) and 7.31 (d).

In the case of N-(nitroacetyl) pyrrolidine (**2**) and benzyl N-(nitroacetyl)-(L)-prolinate (**3**), the compounds were unreactive towards benzylation under Mitsunobu conditions.

Method D : Benzylation by condensation with Schiff base[28] of aromatic aldehyde followed by borohydride[29] reduction.

Aromatic aldehydes as their Schiff bases (**43**) were condensed with methyl nitroacetate (**1**) in acetic anhydride at 40° to get the substituted nitrocinnamates

(**44**) (Scheme VIII). These compounds were reduced by sodium borohydride in a mixture of tetrahydrofuran and methanol at room temperature to get methyl α-nitro-β-substituted phenyl propionate (**4**).

Condensation of (**1**) with the Schiff base (**43a**) derived from benzaldehyde and aniline gave 2-nitro-3-phenyl-2-propenoic acid methyl ester (**44a**). This was obtained as a mixture of E and Z isomers in 72% yield. the (E + Z) mixture showed IR absorption at 1745, 1650, 1610, 1550, 1340 cm^{-1}. In the ^1H NMR spectrum, two sets of signals were seen for E & Z isomers. The two singlets at (3.91δ and 3.95δ) were assigned for ester methyl protons. The two singlets at (7.42δ and 7.47δ) for aromatic protons. Singlets at 7.52δ and 8.08δ were assigned for the proton on the olefinic double bond.

The pure (Z) isomer got separated from the (E+Z) mixture as a solid m.p. 58°(Lit.[28] m.p.58°). In the ^1H NMR spectrum this pure isomer exhibited the following signals : these corresponded to the upfield ones in each pair of signals of the previous (E+Z) mixture : a singlet at 3.59δ for ester methyl protons, a singlet at 7.38δ for aromatic protons and a singlet at 7.49δ for the olefinic proton.

Sodium borohydride reduction of(**44a**)in(THF - MeOH) at room temperature gave (**4a**) in 76% yield. The spectral data has already been discussed earlier.

Similarly, condensation of (**1**) with the Schiff base (**43b**) derived from 3,4,5-trimethoxybenzaldehyde and aniline gave (E+Z) 2-nitro-3-(3,4,5-trimethoxyphenyl)-2-propenoic acid methyl ester (**44b**) in 70% yield as a semisolid. The compound showed IR absorption at 1750, 1650, 1590, 1320, 1140 cm^{-1}. In the ^1H NMR spectrum a bunch of singlets at around at 3.8δ was observed for the three methoxy group protons on aromatic ring and ester methyl protons together. The olefinic proton produced singlets (E+Z) at 7.44δ and 8.0δ.

Sodium borohydride reduction of (**44b**) in (THF - MeOH) solvent at room temperature gave methyl-α-nitro-β (3,4,5-trimethoxyphenyl) propionate (**4b**) in 63% yield. The compound was obtained as a solid with m.p. 130° C (Lit[14], m.p.130°). The compound showed IR absorption at 1730, 1610, 1580, 1360, 1150 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 3.45δ for methylene protons next to aromatic ring was observed. The three methoxy protons and the methyl ester protons together were seen as a multiplet at 3.8δ. A doublet of a doublet was observed at 5.3δ for the methine proton adjacent to the nitro group. A singlet was observed at 6.4δ for aromatic protons.

Condensation of (**1**) with Schiff base (**43c**) derived from 3,4-methylene-dioxybenzaldehyde and aniline gave (E+Z) 3-(3,4-methylenedioxyphenyl) 2-nitro-2-propenoic acid methyl ester (**44c**) in 80% yield[28]. The compound showed characteristic IR absorption at 1740, 1700, 1640, 1610, 1540, 1460, 1340cm^{-1}. In the ^1H NMR spectrum two sets of signals were observed for E and Z isomers. Singlets at (3.86δ, 3.95δ) for ester methyl protons were observed. The methylene linked by ether linkage was seen as a singlet at (6.0δ and 6.05δ). Aromatic protons appeared as a multiplet at 7.0δ. The olefinic proton was seen as a singlet at (7.4δ, 7.97δ).

Sodium borohydride reduction of (**44c**) in (THF-MeOH) solvent at room temperature gave methylβ-(3,4-methylene dioxyphenyl)-α-nitro propionate (**4c**) in 67% yield. The compound was obtained as a thick liquid. The compound showed IR absorption at 1765, 1575, 1500, 1370, 1050 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 3.4δ was observed for methylene protons next to the aromatic ring. The methyl ester protons were seen as a singlet at 3.75δ. The methine proton next to the nitro group was seen as a doublet of a doublet at 5.2δ. The ether linked methylene was seen as a singlet at 5.86δ. A multiplet at 6.6δ was observed for aromatic protons.

Condensation of (**1**) with Schiff base (**43d**) derived from 4-methoxybenzaldehyde and aniline gave (E+Z) 3-(4-methoxyphenyl)-2-nitro-2-propenoic acid methyl ester[30] (**44d**) as a thick liquid in 78% yield. The compound showed IR absorption at 1720, 1650, 1540, 1300, 1180 cm^{-1}. In the ^1H NMR spectrum, two singlets at 3.84δ, 3.88δ were observed for methoxy protons. A doublet at 6.88δ was seen for two aromatic protons as part of the A_2B_2 system. The other half was merged along with the signal for the olefinic proton and was seen as a multiplet at about 7.35δ.

Sodium borohydride reduction of (**44d**) in (THF-MeOH) at room temperature gave methyl β-(4-methoxyphenyl)-α-nitro propionate (**4d**) as a thick liquid in 71% yield. The compound showed IR absorption at 1760, 1560, 1380 cm^{-1}. In the ^1H NMR spectrum a multiplet at 3.45δ was observed for the methylene protons next to the aromatic ring. Two singlets at 3.8δ and 3.85δ were observed for the two methoxy group protons (one attached to the aromatic ring, and the other as part of the ester). The methine proton adjacent to the nitro group was observed as a doublet of a doublet at 5.3δ.

Condensation of (**1**) with Schiff base (**43e**) derived from 3-methoxybenzaldehyde and aniline gave (E+Z) 3-(3-methoxyphenyl)-2-nitro-2-propenoic acid methyl ester (**44e**) as a thick liquid in 74% yield. The compound showed IR absorption at 1750, 1650, 1560, 1390 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 3.8δ was observed for methoxy protons. Aromatic protons were seen as a multiplet at 7.1δ. Two singlets at 7.5δ, 8.0δ were observed for olefinic proton.

Sodium borohydride reduction of (**44e**) in (THF-MeOH) solvent at room temperature gave methyl β-(3-methoxyphenyl)-α-nitro propionate (**4e**) as a thick liquid in 67% yield. The compound showed IR absorption at 1750, 1560, 1390. In the ^1H NMR spectrum, a multiplet at 3.55δ was seen for methylene protons next

to aromatic ring. The methoxy protons were seen as singlets at 3.8δ and 3.9δ. The methine proton was seen as a doublet of a doublet at 5.4δ. The aromatic protons were observed as a multiplet 7.0δ.

The condensation of (**1**) with Schiff base (**43f**) derived from 4-chlorobenzaldehyde and aniline gave (E+Z) 3-(4-chlorophenyl)-2-nitro-2-propenoic acid methyl ester[31] (**44f**) in 76% yield. The compound showed IR absorption at 1730, 1600, 1550, 1400, 1320 cm^{-1}. In the ^1H NMR spectrum, two singlets at 3.93δ and 3.95δ were seen for the $COOCH_3$. A multiplet at 7.3δ for aromatic protons was observed. The olefinic proton was seen as two singlets at 7.5δ and 8.05δ.

Sodium borohydride reduction of (**44f**) in (THF - MeOH) at room temperature gave methyl β-(4 chlorophenyl)-α-nitro propionate (**4f**) as a liquid 70% yield. The spectral data has already been discussed.

The reduction of nitro group in compound (**4** and **6**) to an amino or acetylamino would complete the synthesis of phenylalanine derivatives by this new route (Scheme IX). There are many methods reported in the literature for the conversion of NO_2 to NH_2 [32] The most widely used method is hydrogenation in presence of a suitable catalyst such as Pd/carbon, PtO_2 or Raney Ni[33]. The conversion of aliphatic nitro compounds to the corresponding N-acetylamino derivatives using Zn/AcOH/Ac_2O has also been reported[34].

The methyl ester (**4a**) and pyrrolidide (**6**) of 2-nitro-3-phenyl propionic acid were subjected to reductive acetylation by treatment with zinc dust in acetic anhydride and acetic acid at 60° for 6h. The resulting N-acetyl phenylalanine derivatives (**8**) and (**9**) were characterised as follows.

(±)-N-(Acetyl) phenylalanine methyl ester (**8**) was isolated in 40% yield as a liquid. The compound showed IR absorption at 3300, 1760, 1680, 1530, 1450 cm^{-1}. In the ^1H NMR spectrum a singlet at 2.05α-nitro- was observed for the

$NHCOCH_3$ group. The benzylic methylene protons were seen as a multiplet at 3.15δ. A singlet at 3.75δ was observed for ester methyl protons. Theα methine proton showed as expected, an upfield shift compared to the parent nitro compound. This proton was seen as a multiplet at 4.9δ. A broad singlet was observed for the -NH proton. The aromatic protons were observed as a multiplet at 7.3δ

(±)-N-Acetyl phenylalanine pyrrolidide (**9**) was obtained as a thick liquid. It showed IR absorption at 3300, 1660, 1480, 1440 cm^{-1}. In the ^1H NMR spectrum, a multiplet at 1.9δ was observed for the twoβ methylene group protons of the pyrrolidine. The methyl group protons of the $NHCOCH_3$ were observed at 2.25δ as a singlet. A multiplet at 3.3δ was observed for the benzylic methylene protons and the two α methylene group protons of the pyrrolidine ring. The methine proton was observed at 3.65δ as a multiplet. The proton on nitrogen was observed at 5.55δ as a broad singlet. A multiplet at 7.25δ for aromatic protons was observed.

Results

1. C-Benzylation of methyl nitroacetate (1)

Best results (52% yield) were obtained by Method D. Methods A and B gave 40% and 35% yields of the C-benzylated compounds. The Mitsunobu procedure (Method C) gave only 25% yield of the C-benzyl derivative. However, this in itself is interesting, since it has been recently reported[21] that alkylation of ethyl nitroacetate with methanol by Mitsunobu procedure gave only O-methyl nitronate and no C-methyl product.

2. C-Benzylation of N-(nitroacetyl) pyrrolidine (2)

Best results (35% yield) were obtained by Method B, By method A, 25% yield was obtained. The desired product could not be obtained by the Mitsunobu reaction (Method C). (Scheme VII, Table 1).

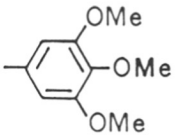
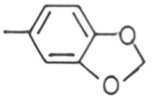
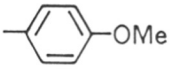
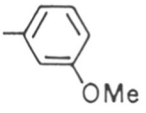
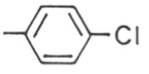
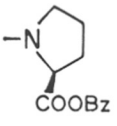
3. C-Benzylation of benzyl N-(nitroacetyl)-(L)-prolinate (3)

Practically similar results were obtained by Method A (27% yield) and Method B (30% yield). The desired product could not be obtained by the Mitsunobu reaction (Method C) (Scheme VII, Table 1). The diastereomeric excess of the product was measured by studying the ^{13}C NMR spectrum. By method A only 8% *de* was obtained while with method B, 14% *de* was achieved.

The reduction of methyl α-nitro-β-phenyl propionate (**4a**) under Zn/AcOH/Ac_2O conditions gave N-acetyl phenylalanine methyl ester (**8**) in 40% yield. Reduction of N-(2-nitro-2-benzyl acetyl) pyrrolidine (**6**) under similar conditions gave (±)-N-acetyl-phenylalanine pyrrolidide (**9**) in 40% yield.

Conclusion

Table 1

Sr. No.	Comp. No.	−R	−Ar	% yield Method			
				A	B	C	D
1	4a	−OMe	−Ph	40	35	25	52
2	4b	−OMe		—	—	—	44
3	4c	−OMe		—	—	—	54
4	4d	−OMe		—	—	—	55
5	4e	−OMe		—	—	—	50
6	4f	−OMe		—	—	5	53
7	6		−Ph	25	35	0	—
8	7		−Ph	27	30	0	—

Methyl nitroacetate (**1**) was successfully converted to N-acetyl phenylalanine methyl ester (**8**). Interestisng results were obtained when benzylation of methyl nitroacetate (**1**) was done under Mitsunobu conditions. 25% C-benzylated product was obtained in this case.

5.4 Experimental

Synthesis of methyl nitroacetate (1)

Nitromethane (61g) was added dropwise to a solution of KOH(224g) in water (112 ml) at 50°. The temperature was then raised slowly to 160° and was maintained at that temperature for an hour. Lot of frothing was observed during the reaction. The reaction mixture was cooled to room temperature and the solid, dipotassium salt precipitated was filtered. The residue was washed with methanol and dried under high vacuum for a while. The solid was suspended in methanol (465 ml) and concentrated H_2SO_4 (116g) was added slowly through a dropping funnel at -10°. The temperature was then slowly raised to 30° in 3h. and was stirred at room temperature for another 3h. The inorganic solid was filtered off and washed with methanol. The filtrate was concentrated to get a liquid. The liquid was extracted with $CHCl_3$ and the organic extract was washed well with water. Finally chloroform was removed under vacuum to get a liquid. The product was distilled under reduced pressure to get a colourless liquid(22.5 g; 50% yield) b.p. 80-82°/8mm. **IR (Neat cm-1)** : 3120, 2950, 1760, 1575, 1440, 1220. **^{1}H NMR (CDCl3,δ)** : 3.8 (S, 3H, O$\underline{CH_3}$), 5.12 (s, 2H, -$\underline{CH_2}NO_2$). **MS (m/z)** : 88, 73 (100%), 45, 42

Preparation of 1,1-bismethylthio-2-nitroethene (39)

Carbondisulfide (120 ml) was added to a solution of nitromethane (61g) and TBAH (Tetrabutylammonium hydrogen sulfate) (1.2g) in ethanol (150 ml). A solution of KOH (132 g) in ethanol (500 ml) was added slowly at 30-35°. The reaction mixture was stirred for another 1h at room temperature and then cooled in an ice bath to 15°. The dithioic acid salt was filtered and washed with ethanol (300 ml). The solid was not dried completely as the dry solid is pyrophoric. The solid was taken in cold methanol :H_2O(4:6, 1000ml) TBAH (1.2g) was added to it and dimethylsulfate (140 ml) was added slowly at 0°. The reaction mixture was

then stirred at 15-20° for 2.5h. The solid 1,1-bismethylthio-2-nitroethene was filtered and washed with cold H_2O. The solid was finally dried in air. Yield = 77g m.p. 105-106°. **IR (Nujol, cm-1)** : 1520, 1470, 1380. **^{1}H NMR ($CDCl_3$,δ)** : 2.45 (s, 6H, 2 CH_3), 6.95 (s, 1H, =C<u>H</u>).

Preparation of Benzyl L-prolinate (40b)

Thionyl chloride (14 ml,120 mmol) was added slowly to a suspension of L-proline (6.6g, 60 mmol) in benzyl alcohol (80 ml) at -10°. The reaction mixture was stirred at ice temperature for 20 min and then at room temsperature for 48 h. Benzyl alcohol was removed under high vacuum to get a gum. This gum was dissolved in ethyl acetate and was neutralised with triethylamine. The white precipitate was filtered off and the filtrate wasconcentrated to get benzyl L-prolinate (**40b**).

Synthesis of 1-methylthio-1-substituted amino-2-nitroethene (41)

A solution of the amine (**40**) (10 mmol) in dry CH_3CN (15 ml) was added slowly to a suspension of 1,1-bismethylthio-2-nitroethene (**39**) (10 mmol) and a catalytic amount of PTSA in CH_3CN (15 ml) at room temperature. A clear solution was obtained and the evolution of methanethiol was observed by its characteristic smell. The reaction was followed by TLC and the reaction mixture was stirred at room temperature for 3-24h. The solvent was removed to get a gum. The unreacted starting material (**39**) was precipitated by treating the gum with ice cold ethanol. It was filtered and the filtrate was concentrated to get a light yellow gum (**41**). The product was purified by column chromatography (silica gel, 60-120 mesh, ethyl acetate-pet. ether as a solvent).

1-Methylthio-1-pyrrolidino-2-nitroethene (41a).

Light yellow solid, yield : 80%; m.p. 70-71°. **IR (Nujol, cm^{-1})**: 3100, 1520, 1450, 1380. **^{1}H NMR ($CDCl_3$, δ)** : 2.0 (m, 4h, $2\underline{CH_2}$), 2.6 (s, 3H, $-S\underline{CH_3}$), 3.5 (m, 4H, $2N\underline{CH_2}$), 6.6 (s, 1H, $=\underline{CH}$)

Benzyl N-(1-methylthio-2-nitroethenyl) prolinate (41b)

Light yellow gum, yield 80%. **IR (Neat, cm^{-1}) :** 1760, 1580, 1530, 1470, 1340. **^{1}H NMR ($CDCl_3$, δ) :** 1.9-2.2 (m, 4H, $2\underline{CH_2}$), 2.3 (m, 3H, SCH_3), 3.35-3.75 (m, 2H, NCH_2)4.6-4.9 (m, 1H, $NC\underline{H}$), 5.15 (s, 2H, $-O\underline{CH_2}$), 6.75 (s, 1H, $=\underline{CH}$), 7.45 (s, 5H, Ph).

Mercuric chloride mediated hydrolysis of 1-methylthio-1-substitutedamino-2-nitroethene (41)

A solution of 1-methylthio-1-substituted amino-2-nitroethene (**41**)(10 mmol) in CH_3CN : H_2O (3:1, 10ml) was added slowly to a solution of $HgCl_2$ (10 mmol) in the same solvent (15 ml) and stirred at 30° for 1-24h. The clear solution obtained in the begining, turned turbid and slowly a white solid precipitated out from the reaction mixture. The reaction was followed by TLC ($CHCl_3$: MeOH(2%) 3 drops of aq. NH_3). After completion of the reaction it was filtered through a column of sodium sulfate (anhydrous) and silica gel to remove water and inorganic solid. The column was washed with CH_3CN : CH_2Cl_2. The combined filtrate was concentrated to get the product which was purified by column chromatography (silica gel, 60-120 mesh, 20% ethyl acetate in pet ether).

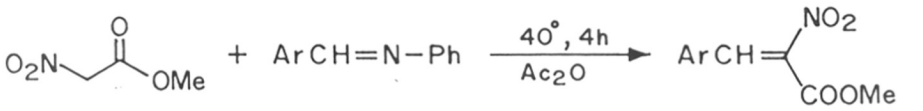
N-(2-Nitroacetyl) pyrrolidine (2)

Solid m.p. 105-106° (Yield 90%). **IR (Nujol cm^{-1})** : 1660, 1570, 1450, 1380. **^{1}H NMR ($CDCl_3$,δ)**: 1.9 - 2.2 (m, 4H, $2C\underline{H_2}$), 3.4 - 3.65 (m, 4H, $2N\underline{CH_2}$), 5.2 (s, 2H, $NO_2\underline{CH_2}$). **MS (m/z)**: 158 (M^+), 112 (M^+-NO_2), 111 (M^+-HNO_2), 83 (111-CO) 70.

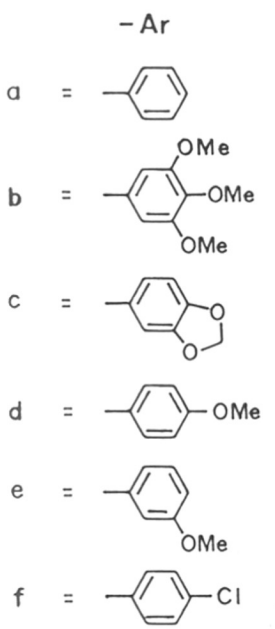
Benzyl-N-(2-nitroacetyl)-L-prolinate (3)

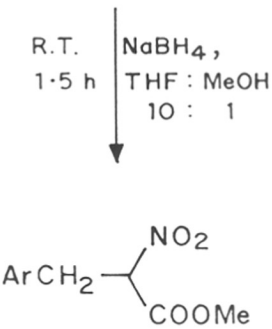
Scheme-VIII

Method-D



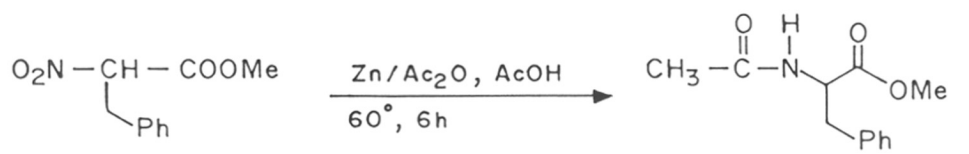
(1) (43) (44)



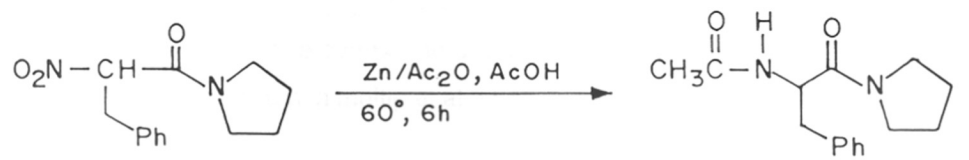


(4)

Scheme-IX



(4a) (8)



(6) (9)

Solid m.p. 141-142° (Yield : 90%). **IR (Nujol, cm^{-1})**: 1750, 1680, 1570, 1460, 1380, 1180. **^{1}H NMR ($CDCl_3$,δ)** : 1.8-2.4 (m, 4H, 2$\underline{CH_2}$), 3.4 - 3.7 (m, 2H, N$\underline{CH_2}$), 4.4-4.65 (m, 1H, NC$\underline{H}$), 5.15 (m, 2H, O$\underline{CH_2}$), 5.3 (m, 2H, $\underline{CH_2}NO_2$), 7.3 - 7.5 (m, 5H, Ar-H). **MS (m/z)** = 157 (100%) (M^+ - CO_2CH_2Ph), 111 (157-NO_2), 91, 70.

I: Method (A): Benzylation under PTC conditions : General procedure

Benzyl bromide (0.36g) was added to the stirred solution of (**1**)/(**2**) or (**3**) (2 mmol) in dimethylformamide (5 ml) containing TEBA (benzyl triethylammonium chloride) (10 mg) and anhydrous $KHCO_3$ (0.1g) at room temperature. The reaction mixture was stirred at 60° for 16-24h. DMF was removed under reduced pressure and the residue was diluted with water and acidified with 5% HCl at 0°C. It was extracted with solvent ether. The ether layer was washed with water and was dried over anhydrous sodium sulfate. The solvent was removed and the product was purified by column chromatography (Silica gel 60-120 mesh. ethyl acetate in pet ether as solvent).

Methyl α-nitro-β-phenyl propionate (4a)

Yield 40%, **IR (Neat, cm^{-1})** : 1770, 1575, 1500, 1450, 1380, 1285, 1230, 1190, 1020, 870, 660, 610 **^{1}H NMR ($CDCl_3$, δ)** : 3.52 - 3.62 (m, 2H, Ph$\underline{CH_2}$ - CH); 3.85 (s, 3H, O$\underline{CH_3}$); 5.4 (dd, J = 9Hz, 7Hz, 1H, $CH_2\underline{CH}$-NO_2); 7.20 - 7.45 (m, 5H, Ar-H). **MS (m/e)** : 161, 131 (100%), 103, 91, 77, 59. **Analysis** calcd. for $C_{10}H_{11}NO_4$; C : 57.41%, H = 5.30%; Found : C = 57.56%, H = 5.21%.

N-(2-Nitro-3-phenyl)propionyl pyrrolidine (6)

Light yellow solid. Yield 25%. m.p. : 65°, **IR (Nujol, cm^{-1})** : 1650, 1560, 1450, 1380, 1250, 1200, 1180, 1090, 980, 960, 890, 870 **^{1}H NMR ($CDCl_3$,δ)**: 1.75 - 2.0 (m, 4H, -$\underline{CH_2}$-$\underline{CH_2}$-); 2.9 - 3.0 (m, 1H, 1$\underline{CH_2}$-CH); 3.4-3.6 (m, 5H, 2N-C$\underline{H_2}$, 1C$\underline{H_2}$-CH); 5.42 (t, J = 13Hz, 1H, CH_2-C$\underline{H}$-NO_2); 7.25 - 7.4 (m, 5H, Ar-H) **^{13}C NMR** : 161.28;

134.18; 129.10; 128.73; 127.48; 87.04; 46.73; 46.42; 36.26; 25.70; 23.93. **MS (m/z)** : 202 (100%), 131, 103, 98, 91, 77, 70, 55. **Analysis** calcd. for $C_{13}H_{16}N_2O_3$: C = 62.88, H = 6.49; Found : C = 62.57, H = 6.63

Benzyl N-(2-benzyl-2-nitroacetyl)-(L)-prolinate (7)

Thick liquid. Yield 27%. *de* 8% **IR (CHCl3, cm^{-1})** : 1730, 1650, 1560, 1380 **^{1}H NMR ($CDCl_3$, δ)** : 1.7 - 2.3[m, 4H, $\underline{CH_2}$-$\underline{CH_2}$ (C_3-C_4)]; 3.3-3.7 [m, 4H, Ph$\underline{CH_2}$-C, N-$\underline{CH_2}$-(C_5)]; 4.4 - 4.6 [m, 1H, $\underline{CH}$-COOBz (C_2)]; 5.1 - 5.2 (m, 2H, O$\underline{CH_2}$Ph); 5.5 (m, 1H, -$\underline{CH}$-NO_2); 7.2 (m, 10H, Ar-$\underline{H}$) **^{13}C NMR** (minor diastereomer in parantheses) : 170.7 (170.4) ($\underline{C}$O); 161.8 (161.6) ($\underline{C}$O); 135.2, 134.3, 133.8, 129,0, 128.9, 128.8, 128.6, 128.5, 128.3, 128.1, 128, 127.9, 127.4, 127.3, (Aromatic carbons); 87.5 (86.7) ($\underline{C}$H-NO_2); (67.5), 66.8 (O$\underline{C}H_2$Ph); (59.4), 59.3 ($\underline{C}$HCOOBz (C2)); (47.2) 47.1 (N-$\underline{C}H_2$(C_5); (36.1) 35.8 (Ph$\underline{C}H_2$CH); 28.7 (28.6) and 24.4 (24.2) ($\underline{C}H_2$-$\underline{C}H_2$ C_3-C_4). **MS (m/z)** : 276 (M^+-PhCHO), 185 (276-$PhCH_2$), 149 (NO_2-CH = CH-Ph); 136 ($HCOOCH_2Ph$), 98, 91, 69, 55. **Analysis** calcd. for $C_{21}H_{22}N_2O_5$: C = 65.95, H = 5.80; Found - C=65.78, H = 5.60.

II: Method (B) :Benzylation using benzyl bromide and DBU : General procedure

Benzyl bromide (0.36g) was added dropwise to the stirred solution of (**1**)/(**2**)/(**3**) (2 mmol) and DBU (304 mg) in acetonitrile (10 ml). The reaction mixture was heated at 60° for 24-30h. The reaction mixture was cooled and acidified with 5% HCl at 0°. It was extracted with methylene chloride. The organic layer was dried over anhydrous sodium sulphate and the solvent was removed to get crude reaction product. The product was purified by column chromatography (silica gel, 60-120 mesh ethyl acetate in pet ether as solvent system).

Methyl-α-nitro-β-phenyl propionate (4a)

Yield 35%. The spectral data has already been discussed.

N-(2-nitro-3-phenyl) propionyl pyrrolidine (6)

Yield 35%. The spectral data has already been discussed.

Benzyl N-(2 benzyl-2-nitroacetyl)-(L)-prolinate (7)

Yield 30%. *de* 14%. The spectral data has already been discussed.

III: Method (C):Benzylation by Mitsunobu reaction : General procedure

To a stirred solution of PPh_3 (393 mg, 1.5 mmol) in dry benzene (25 ml), DEAD (261 mg, 1.5 mmol) was added dropwise at 5-10° in dry and inert atmosphere. The reaction mixture was stirred for 15 min at that temperature. Benzyl alcohol (1 mmol) was added to the stirred reaction mixture. The last addition of the nitroacetyl compound (1 mmol) (**1**)/(**2**)/(**3**) was done after 5 min. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed and the crude reaction product was purified by column chromatography (silica gel 60-120 mesh, ethyl acetate in pet ether as solvent system).

Methyl-α-nitro-β-phenyl propionate (4a)

Yield 25%. The spectral data has already been discussed.

Methyl-α-hydroxyimino acetate (5)

Yield 25%. **IR ($CHCl_3$, cm^{-1})** : 3300 (b), 1720, 1630, 1450, 1040. **^1H NMR ($CDCl_3$, δ)** : 3.9 (s, 3H, $COO\underline{CH_3}$); 7.6 (s, 1H, C<u>H</u>=); 9.8 (bs, 1H, N-O<u>H</u>). **MS (m/z)** : 103 (M^+), 71 (100%) (M^+-CH_3OH), 59($COOCH_3$).

Methyl-β-(4-chlorophenyl)-α-nitro propionate (4f)

Yield 5%. **IR (Neat, cm^{-1})** : 1750, 1550, 1510, 1400, 1310, 1280 **^{1}H NMR ($CDCl_3$, δ)**: 3.35-3.55 (m, 2H, Ar$\underline{CH_2}$-CH) ; 3.77 (s, 3H, COO$\underline{CH_3}$); 5.17 - 5.44 (dd, J = 9Hz, 7Hz, 1H, $CH_2\underline{CH}$-NO_2); 7.13 (d, J=8Hz, 2H, ArH); 7.31 (d, J=8Hz, 2H, ArH)

IV : Method (D): Aldol condensation with Schiff base of aromatic aldehyde followed by sodium borohydride reduction : General procedure

a) A mixture of methyl nitroacetate (**1**) (1 mmol) Schiff base of aromatic aldehyde (**43**) (1.15 eq) and 2 ml of acetic anhydride was heated at 40° for 4h. The reaction mixture was poured on stirred hot water and the upper water layer was discarded. The residue was extracted with methylene chloride and was dried over anhydrous sodium sulphate. The solvent was removed and the crude reaction product (**44**) was purified by column chromatography (silica gel, 60-120 mesh, ethyl acetate in pet. ether as solvent system).

b) Sodium borohydride reduction : $NaBH_4$ (1.5 mmol, 60 mg) was added in 4 lots to the stirred solution of nitroolefin (**44**) in tetrahydrofuran : methanol (10 : 1 ratio) (10 ml) solvent at room temperature. The reaction mixture was stireed for 1.5h. The reaction was quenched by adding water (10 ml). The solvent was removed . The reaction mixture was acidified by 5% HCl at 0° and was extracted with solvent ether. The combined ether layer was washed with water and was dried over anhydrous sodium sulphate. The solvent was removed and the product (**4**) was purified by column chromatography (silica gel 60-120 mesh ethyl acetate in pet ether as solvent).

(E+Z)-3-Phenyl-2-nitro-2-propenoic acid methyl ester (44a)

Yield 72%. **I.R. : (Neat, cm^{-1})**: 1745, 1650, 1610, 1550, 1460, 1450, 1340, 1290, 1220, 1110, 1080, 950, 780 **^{1}H NMR ($CDCl_3$, δ)** : 3.91, 3.95 (s, 3H, O$\underline{CH_3}$);

7.42, 7.47 (s, 5H, Ar-H); 7.52, 8.08 (s, 1H, CH=)

Pure (Z) isomer m.p. = 58°C. **^{1}H NMR ($CDCl_3$, δ)**; 3.89 (s, 3H, OCH_3); 7.38 (s, 5H, ArH); 7.49 (s, 1H, CH=)

(E+Z)-2-nitro-3-(3,4,5-trimethoxyphenyl)-2-propenoic acid methyl ester (44b)

Yield 70%. **IR (Neat, cm^{-1})** = 1750, 1650, 1590, 1540, 1320, 1270, 1140, 1010. **^{1}H NMR ($CDCl_3$, δ)** : 3.78 - 4.0 (m, 12H, 3-OCH_3, COOCH_3); 6.66 -7.24 (m, 2H, Ar-H); 7.44, 8.0 (s, 1H, CH=)

(E+Z) 3-(3,4-Methylendioxyphenyl)-2-nitro-2-propenoic acid methyl ester (44c)

Yield 80%. **IR (CHCl3, cm^{-1})** : 1740, 1700, 1640, 1610, 1540, 1520, 1500, 1460, 1340, 1270, 1230, 1050, 940, 680. **^{1}H NMR ($CDCl_3$, δ)**: [3.86, 3.95 (s, 3H, COOCH_3)]; [6.0, 6.05 (s, 2H, -O-CH_2-O)]; 6.71 - 7.37 (m, 3H, ArH); [7.4, 7.97 (s, 1H, CH=)]

(E+Z) 3-(4-Methoxyphenyl)-2-nitro-2-propenoic acid methyl ester (44d)

Yield 78%. **IR (Nujol, cm^{-1})** : 1720, 1650, 1600, 1540, 1520, 1450, 1300, 1270, 1220, 1180, 1080, 1030, 960, 840. **^{1}H NMR ($CDCl_3$, δ)** : 3.84 (s, 3H, OCH_3); 3.88 (s, 3H, OCH_3); 6.88 (d, J=9Hz, 2H, ArH), 7.22 - 7.48(m, 3H, 2ArH, CH=)

(E + Z) 3-(3-Methoxyphenyl)-2-nitro-2-propenoic acid methyl ester (44e)

Yield 74%. **IR (Neat, cm^{-1})** = 1750, 1740, 1650, 1610, 1590, 1560, 1550, 1450, 1390, 1340, 1250, 1200, 1060, 1000, 960, 880, 790, 700. **^{1}H NMR ($CDCl_3$, δ)** : 3.71 - 4.0 (m, 6H, OCH_3, COOCH_3); 6.84 - 7.44 (m, 4H, ArH), 7.5, 8.0 (s, 1H, CH=)

(E+Z) 3-(4-chlorophenyl)-2-nitro-2-propenoic acid methyl ester (44f)

Yield 76%. **IR (Neat, cm^{-1})** : 1730, 1600, 1550, 1510, 1450, 1400, 1320, 1280, 1220, 1110, 1090, 1030, 960, 850, 830, 810, 720. **^{1}H NMR ($CDCl_3$ δ)**: [3.93, 3.95 (s, 3H, $COOCH_3$)]; 7.2 - 7.44 (m, 4H, Ar-H); [7.5, 8.05 (s, 1H, CH=)]

Methyl-α-nitro-β-phenyl propionate (4a)

Yield 52%. The spectral data has already been discussed.

Methyl α-nitro-β-(3,4,5-trimethoxyphenyl) propionate (4b)

Solid, Yield 44% m.p. 130°C. **IR ($CHCl_3$, cm^{-1})** : 1730, 1610, 1580, 1530, 1480, 1360, 1250, 1150, 1020, 690. **^{1}H NMR (CDCl , δ)**: 3.4-3.55 (m, 2H, Ar-CH_2-CH); 3.75-3.94 (m, 12H, 3-OCH_3, $COOCH_3$); 5.22-5.48 (dd, J=9Hz, 7Hz, 1H, CH_2-CH-NO_2); 6.4 (s, 2H, ArH)

Methyl β-(3,4-methylenedioxyphenyl)α-nitro propionate (4c)

Yield 54%. **IR (Neat, cm^{-1})**: 1765, 1610, 1575, 1510, 1500, 1450, 1370, 1260, 1110, 1050, 940, 880, 820. **^{1}H NMR ($CDCl_3$, δ)**: 3.28-3.46 (m, 2H, Ar-CH_2-CH); 3.75 (s, 3H, $COOCH_3$); 5.11 - 5.33 (dd, J=9Hz, 7Hz, 1H, CH_2CH-NO_2); 5.86 (s, 2H, -OCH_2O-); 6.55-6.68 (m, 3H, ArH)

Methyl β-(4-Methoxyphenyl)-α-nitro propionate (4d)

Yield. 55%. **IR (Neat, cm^{-1})** : 1760, 1615, 1560, 1515, 1450, 1380, 1250, 1190, 1120, 1040, 870, 820. **^{1}H NMR $CDCl_3$**, δ): 3.40 - 3.55 (m, 2H, Ar-CH_2-CH); 3.8 (s, 3H, OCH3); 3.85 (s, 3H, OCH_3); 5.28 - 5.37 (dd, J=9Hz, 7Hz, 1H, CH2CH-NO_2); 6.85 (d, J=17Hz, 2H, Ar-H); 7.15 (d, J=17Hz, 2H, Ar-H)

Methyl β-(3-methoxyphenyl)α-nitro propionate (4e)

Yield 50%. **IR (Neat, cm^{-1})** : 1750, 1560, 1490, 1440, 1260, 1040, 870, 780, 690. **^{1}H NMR ($CDCl_3$, δ)** : 3.5 - 3.6 (m, 2H, Ar$\underline{CH_2}$-CH); 3.8 (s, 3H, O$\underline{CH_3}$); 3.9 (s, 3H, O$\underline{CH_3}$); 5.35 - 5.45 (dd, J = 9Hz, 7Hz, 1H, CH_2-$\underline{CH}$-NO_2); 6.75 - 7.30 (m, 4H, ArH).

Methyl β-(4-chlorophenyl)-α-nitro propionate (4f)

Yield 53%. The spectral data has already been discussed.

V Second alkylation

Methyl iodide (1 ml, excess) was added dropwise to the stirred mixture of (**4a**) (0.4g), K_2CO_3 (0.4g, 1.5 eq) and catalytic quantity of TDA-1 (PTC) in dry acetone at room temperature. The mixture was stirred for 24 h. The solvent was removed and the reaction mixture was acidified with 5% HCl at 0°. It was extracted with solvent ether. The ether layer was washed with water and was dried over anhydrous sodium sulphate. The solvent was removed and the product was distilled as bulb to bulb.

Methyl-α-benzyl-α-nitro propionate (42)

Distilled as bulb to bulb b.p. bath temp. 120-130°/1mm to get colourless liquid Yield = 60% (0.255g). **IR ($CHCl_3$, cm^{-1})** = 1770, 1570, 1470, 1390, 1360, 1280, 1220, 1130, 1040. **^{1}H NMR ($CDCl_3$, δ)**: 2.65 (s, 3H, NO_2-C-$C\underline{H_3}$); [3.45, 3.55 (s, 2H, Ph$\underline{CH_2}$)]; 3.8 (s, 3H, OCH_3); 7.0 - 7.45 (m, 5H, ArH). **Analysis** calcd. for $C_{11}H_{13}NO_4$: C = 59.18, H = 5.87; Found : C = 59.00, H = 5.57

VI Reduction of the nitro group using Zn/Ac_2O-AcOH

To a stirred solution (**4a**)/(**6**) (0.5 mmol) in acetic anhydride (2.5 ml) and acetic acid (2 ml), zinc dust (350 mg) was added in four lots and the reaction mixture was heated at 60° for 6h. The cooled reaction mixture was filtered through celite to

remove inorganic solids. The filtrate was poured on ice and was extracted with solvent ether. The ether extract was washed with cold dilute sodium bicarbonate solutin folloed by water and saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulphate and solvent was removed to get crude reaction product. The product was purified by column chromatography (silica gel, 60-120 mesh, ethyl acetate in pet.ether as solvent).

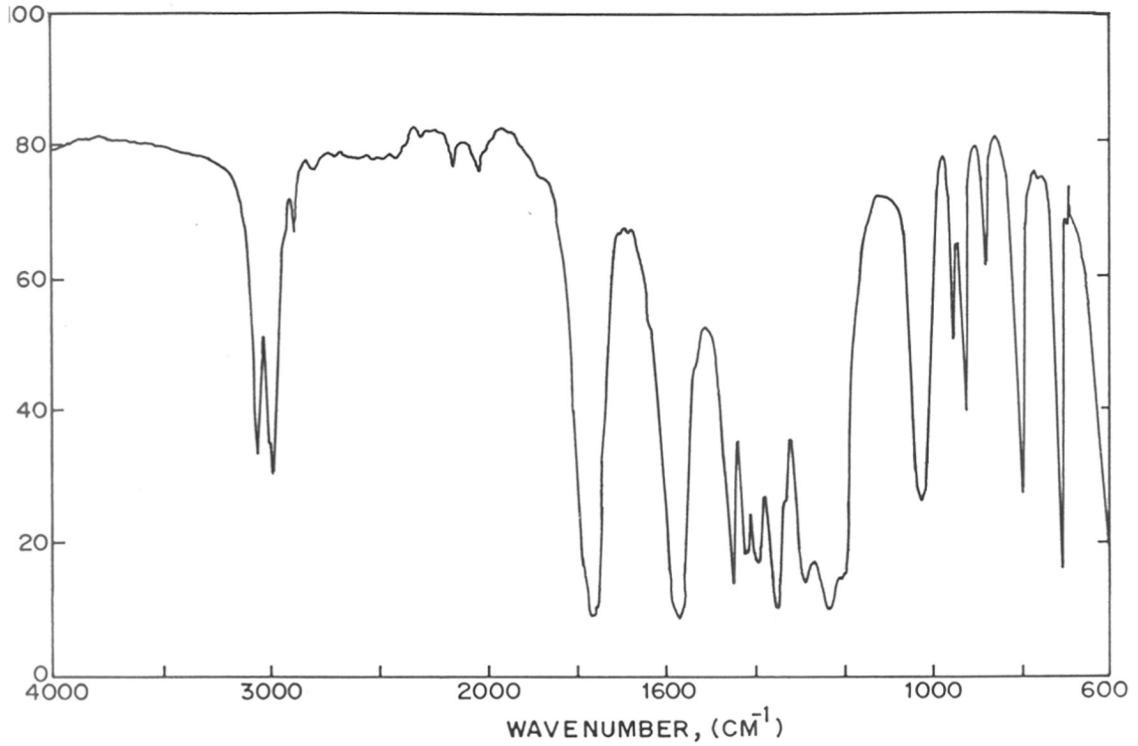
(±) N-(Acetyl) phenylalanine methyl ester (8)

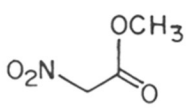
Isolated as a liquid in 40% yield. **IR ($CHCl_3$, cm^{-1})** : 3300, 3000, 1760, 1680, 1530, 1450, 1400. **^1H NMR ($CDCl_3$,δ)** : 2.05 (s, 3H, $\underline{CH_3}CONH$), 3.15 (m, 2H, $\underline{CH_2}Ph$), 3.75 (s, 3H, $COOC\underline{H_3}$), 4.9 (m, 1H, $C\underline{H}$-$NHCOCH_3$), 5.95 (bs, 1H, $N\underline{H}$), 7.1-7.5 (m, 5H, Ar-$\underline{H}$). **^{13}C NMR ($CDCl_3$**, δ):171.9, 169.5 (2$\underline{C}$=O), 135.7, 129.0, 128.4, 126.9 (Aromatic carbons), 53.03 ($\underline{C}H$ NH), 52.1 ($COO\underline{C}H_3$), 37.7 ($\underline{C}H_2Ph$), 22.9($HNCO\underline{C}H_3$). **MS (m/z)** : 221 (M^+), 163 [M^+ -58 ($NHCOCH_3$)], 131 [163 -32 (CH_3OH)],120 [163-43 ($COCH_3$)],91. **Analysis** calcd. for $C_{12}H_{15}NO_3$: C = 65.14%, H = 6.83%, Found = C = 65.37%, H = 6.42%

(±) N-Acetyl-phenylalanine pyrrolidide (9)

The pure product was isolated as thick liquid in 40% yield. **I.R. ($CHCl_3$, cm^{-1})** : 3300-3500 (b), 3000, 1660, 1480, 1400. **^1H NMR ($CDCl_3$**, δ) : 1.9 (m, 4H, $C\underline{H_2}$-$C\underline{H_2}$); 2.25 (s, 3H, $C\underline{H_3}CONH$); 3.2-3.4 (m, 6H, $PhC\underline{H_2}$, 2N-CH_2), 3.65 (m, 1H, $C\underline{H}NH$), 5.55 (bs, 1H, $N\underline{H}$), 7.25 (m, 5H, Ar-H) **^{13}C NMR ($CDCl_3$**, δ) : 168.4, 165.5 (2CO), 136.6, 129.4, 128.1, 126.6 (Aromatic carbons), 57.8 ($\underline{C}H$ NH), 46.1, 45.8 (2N$\underline{C}H_2$), 34.4 ($\underline{C}H_2Ph$), 25.6, 23.8 (-$\underline{C}H_2$-$\underline{C}H_2$-), 19.8 ($NHCO\underline{C}H_3$). **MS (m/z)** : 202 [M^+ - 58 ($NHCOCH_3$)], 185 [202 - 17 (OH)], 162 [M^+ - 98], 98, 91. **Analysis** calcd. for $C_{15}H_{20}N_2O_2$ - C = 69.20%, H = 7.74%, Found = C = 69.57%, H = 7.50%.

Spectrum No. 1





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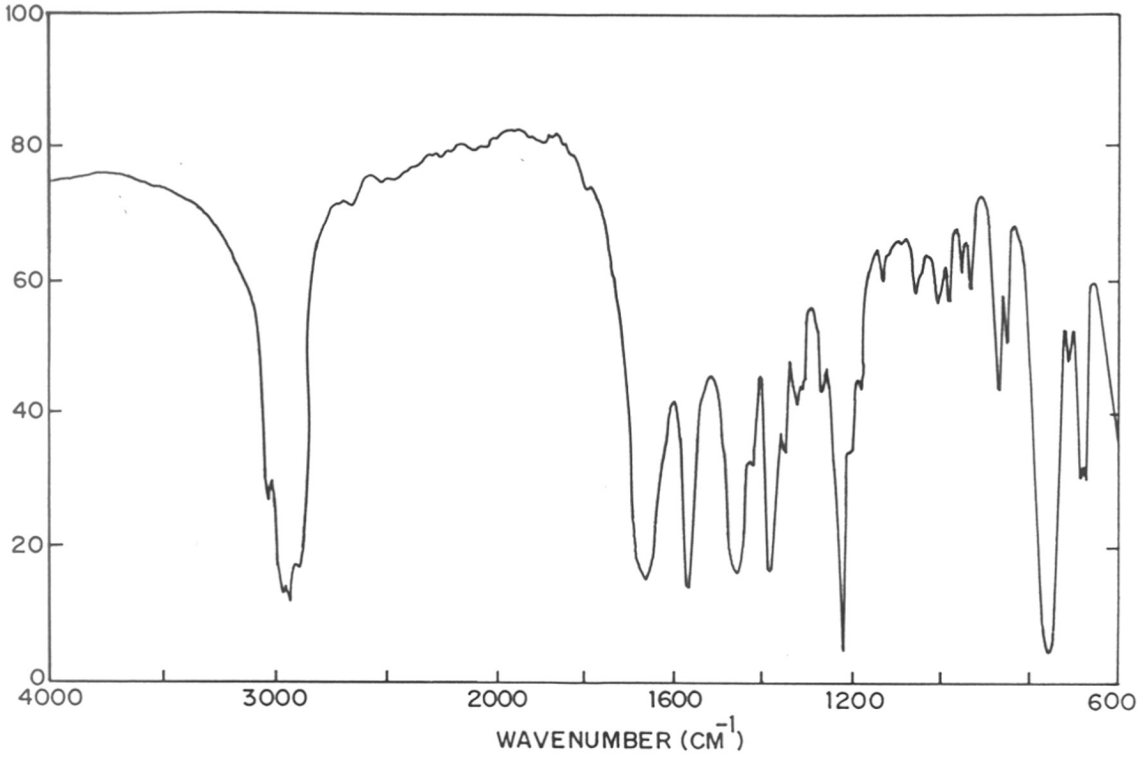

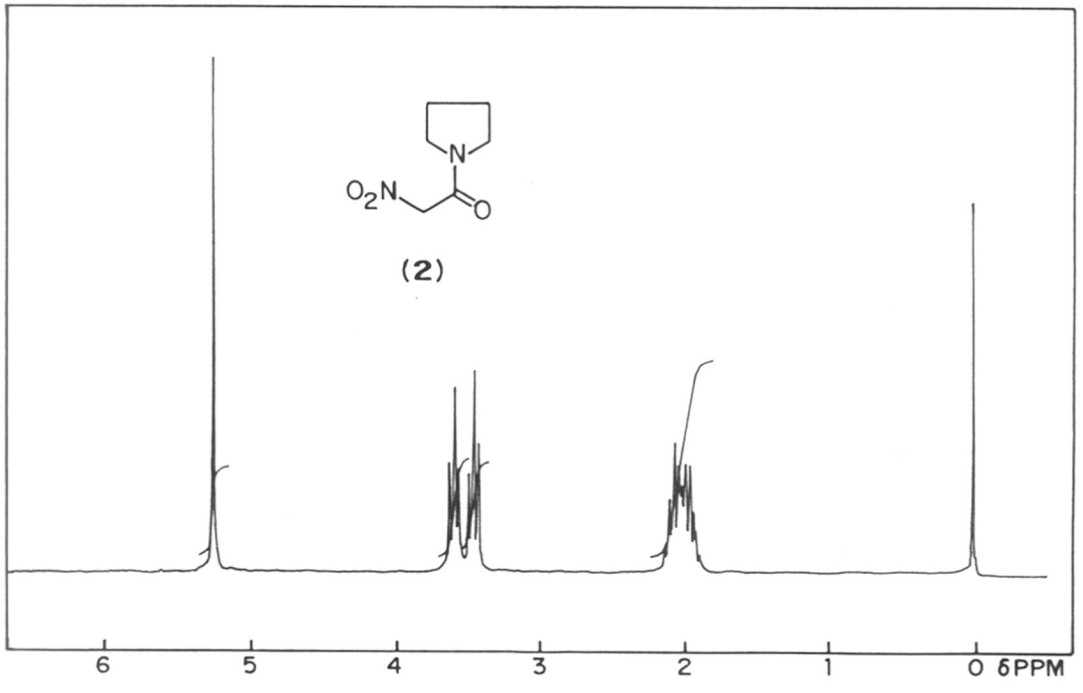

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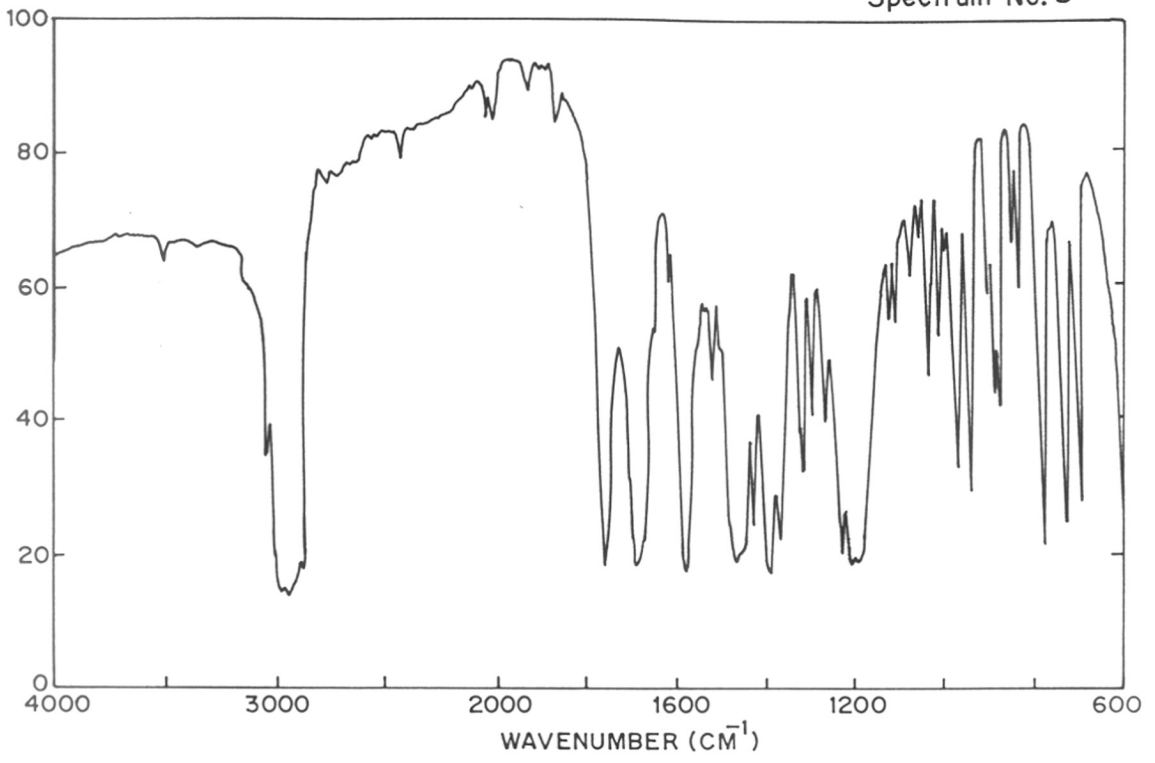

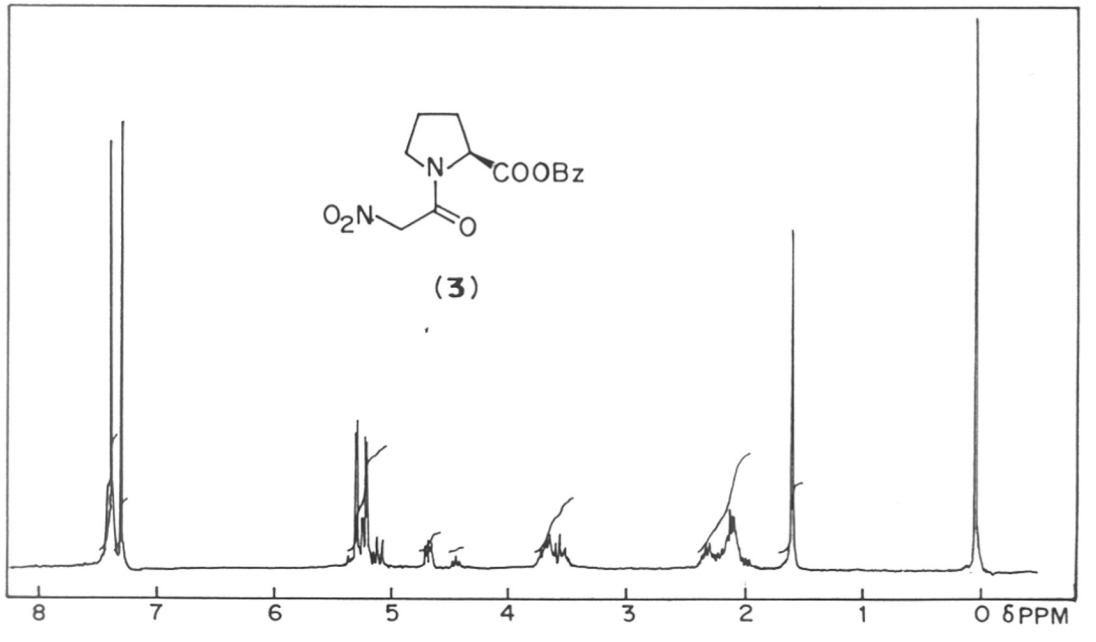

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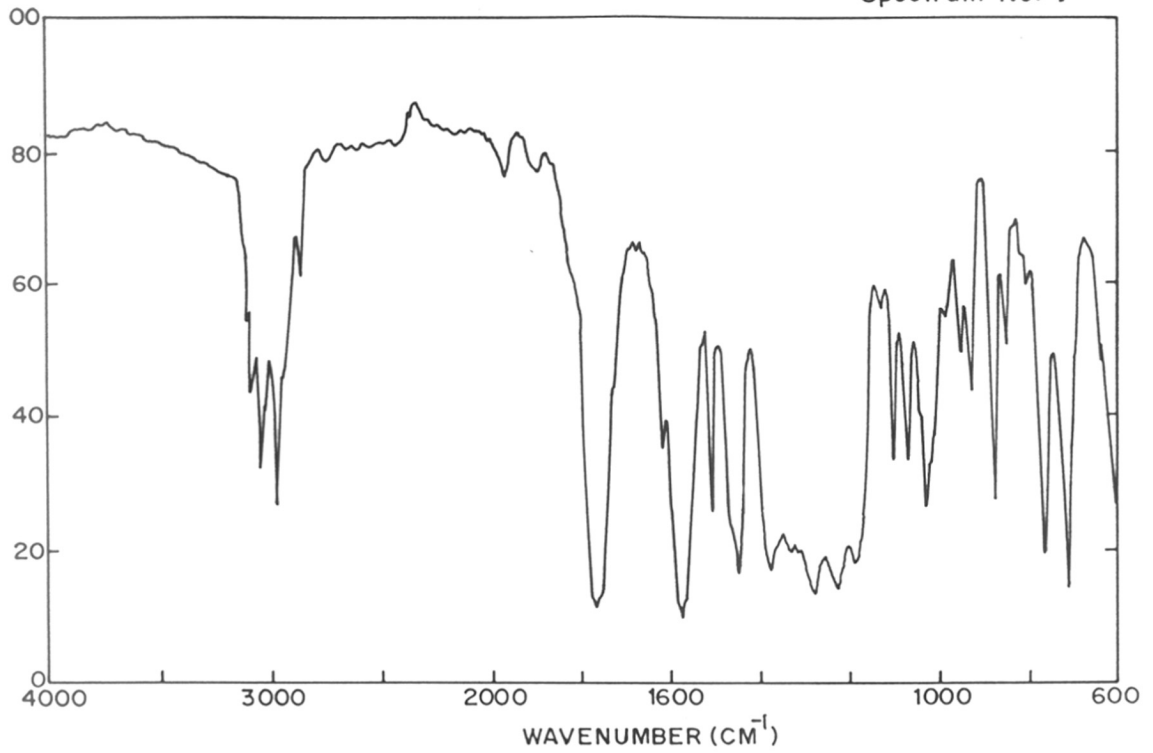
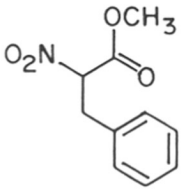
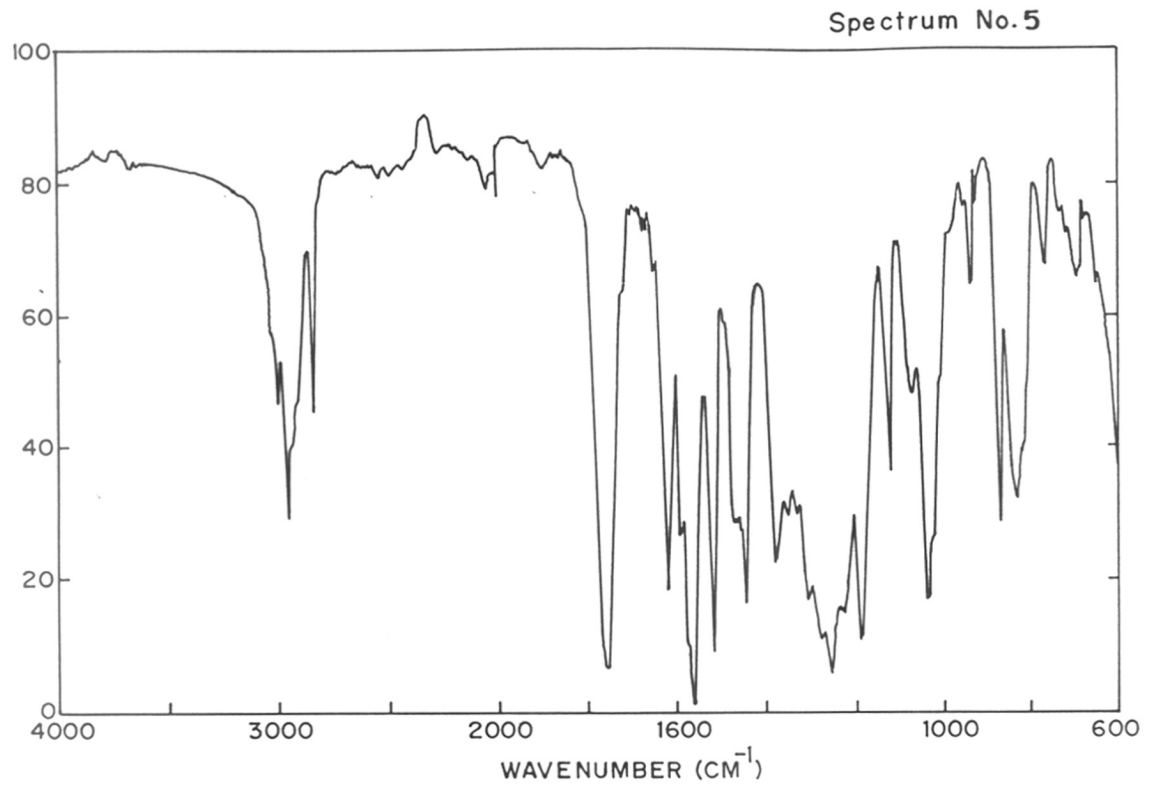
Spectrum No. 4





(4a)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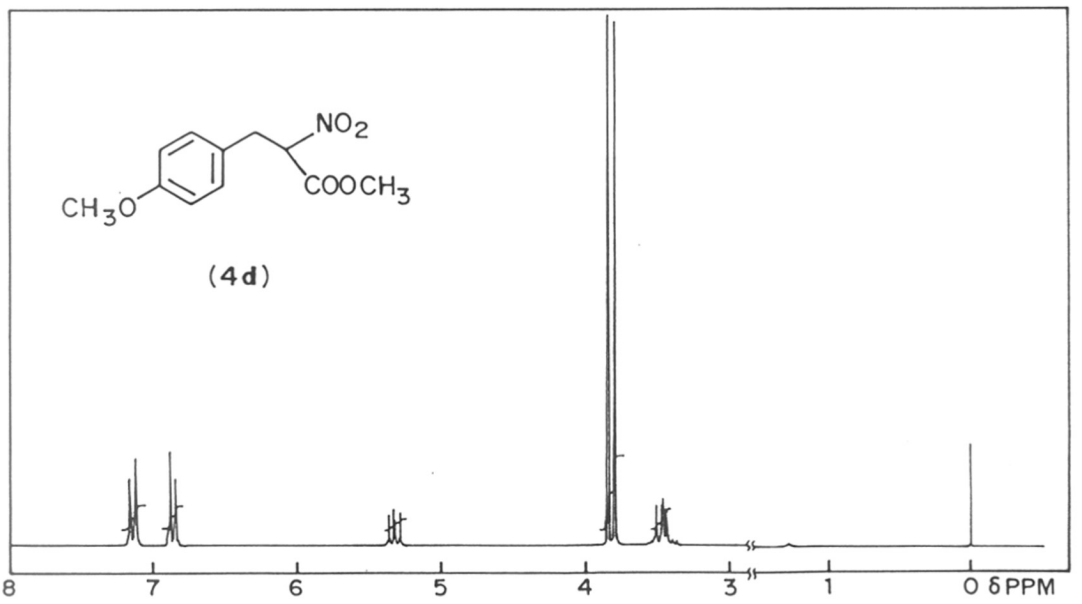
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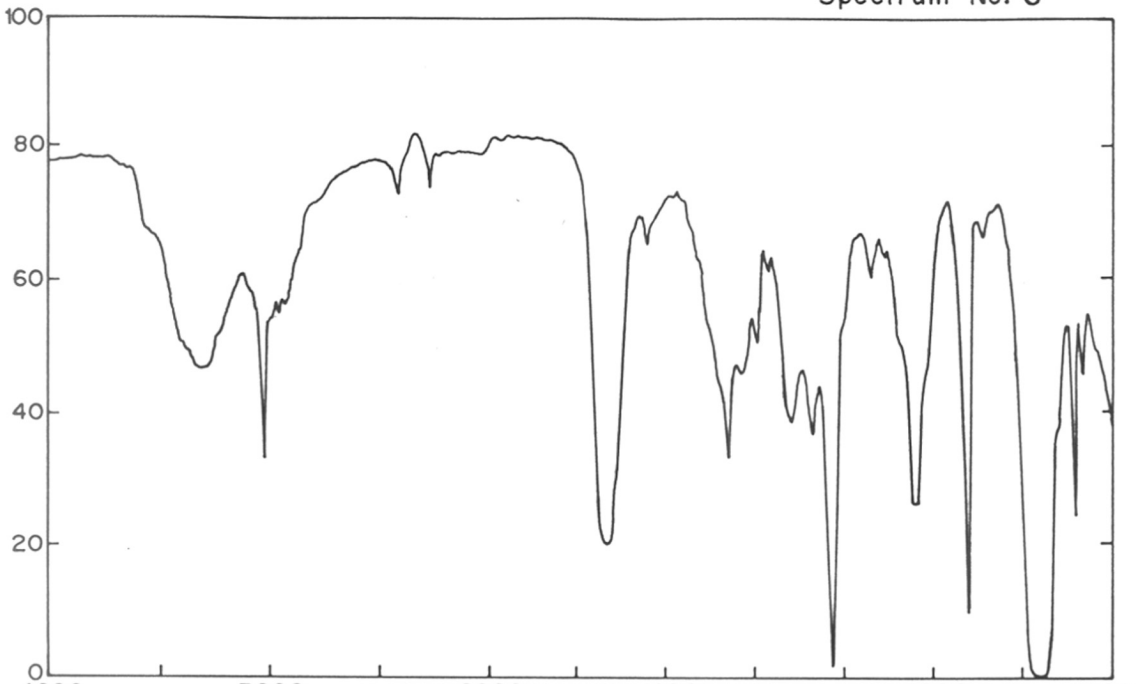

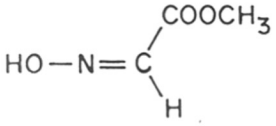

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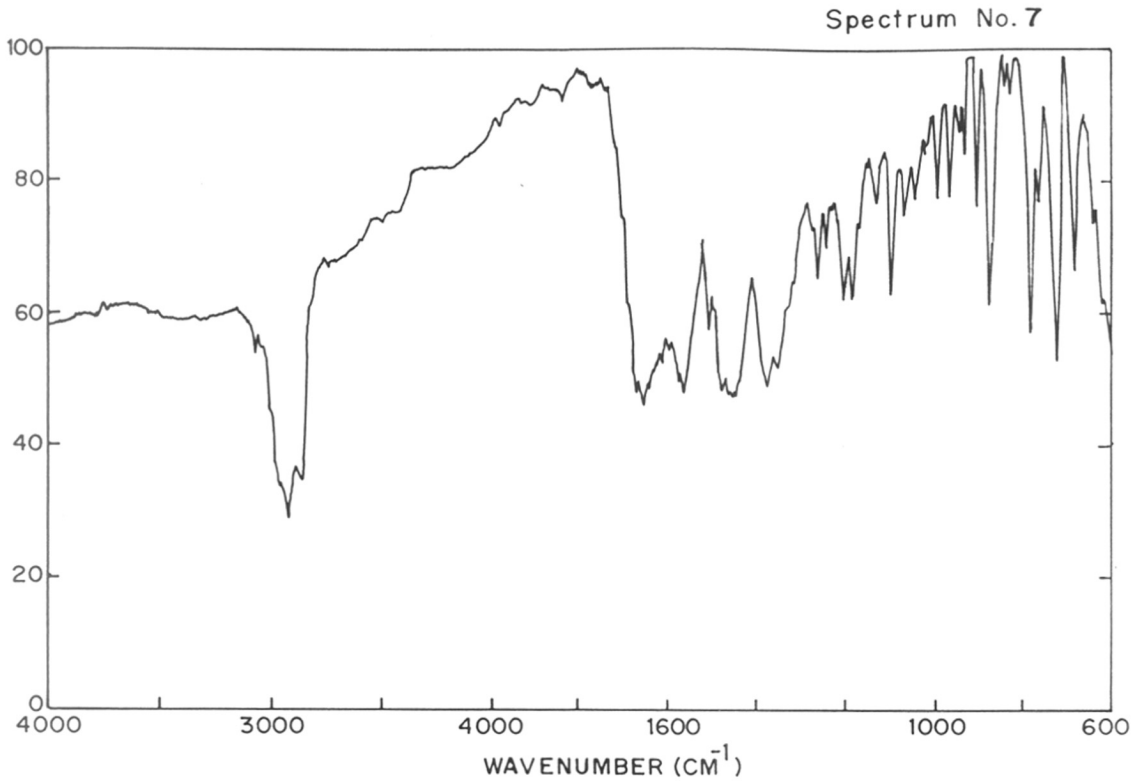
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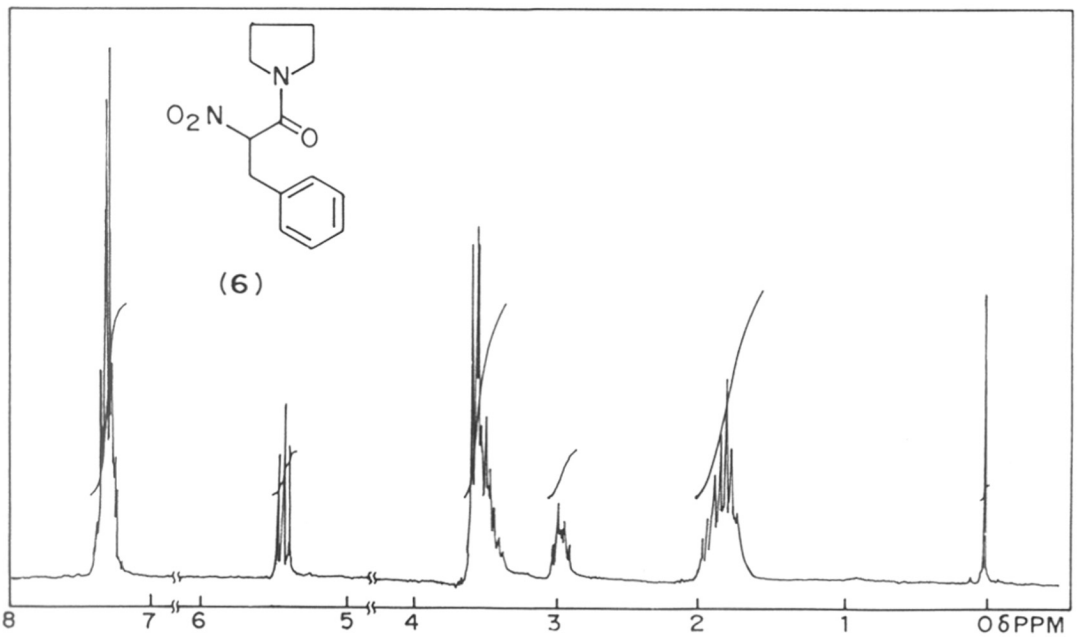
WAVENUMBER (CM⁻¹)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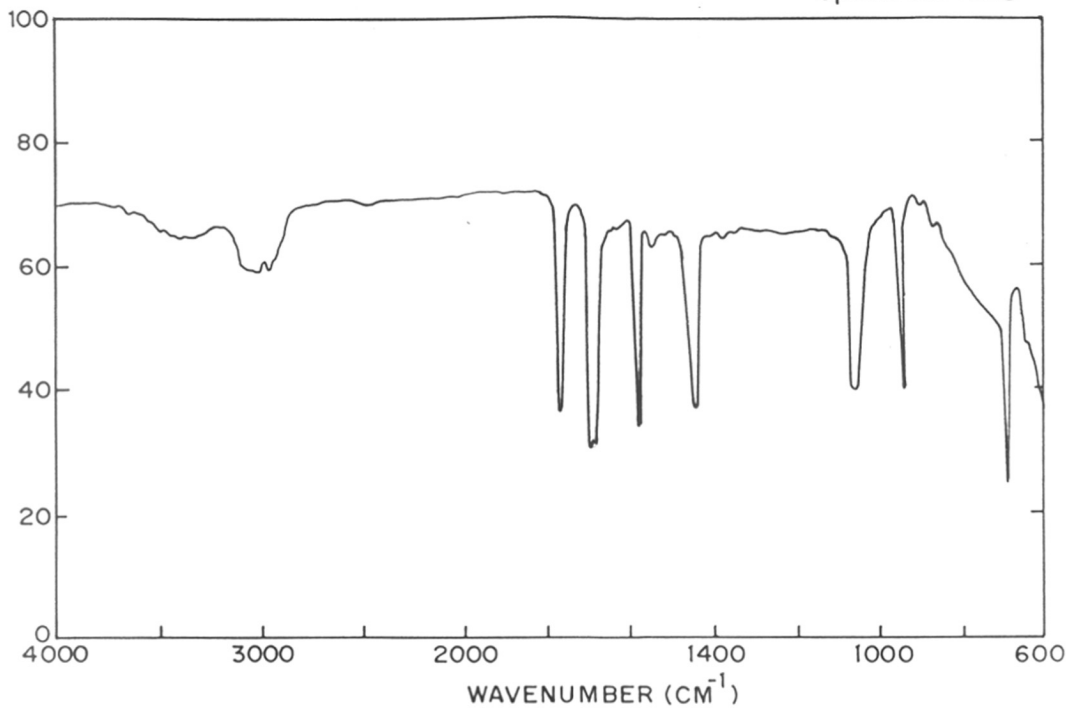

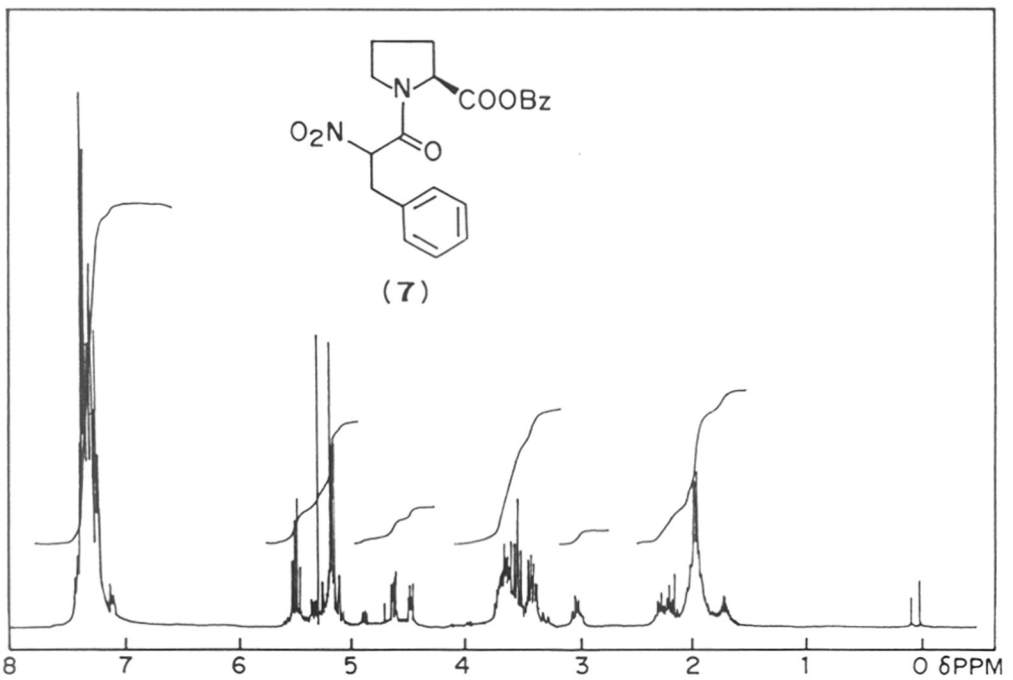

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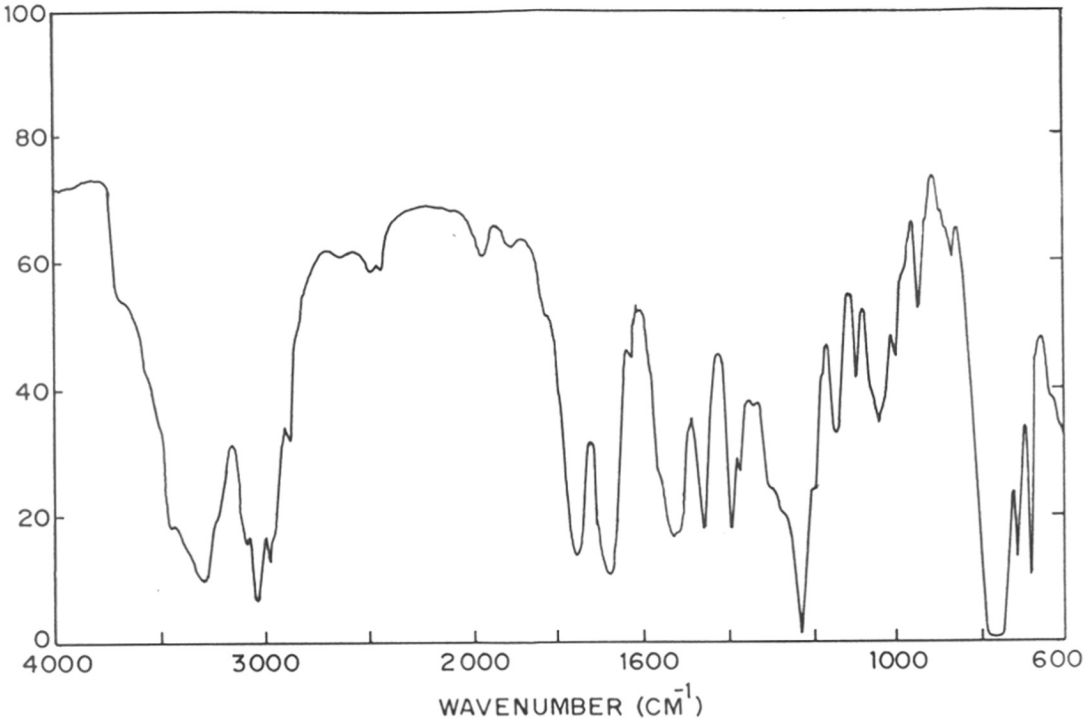

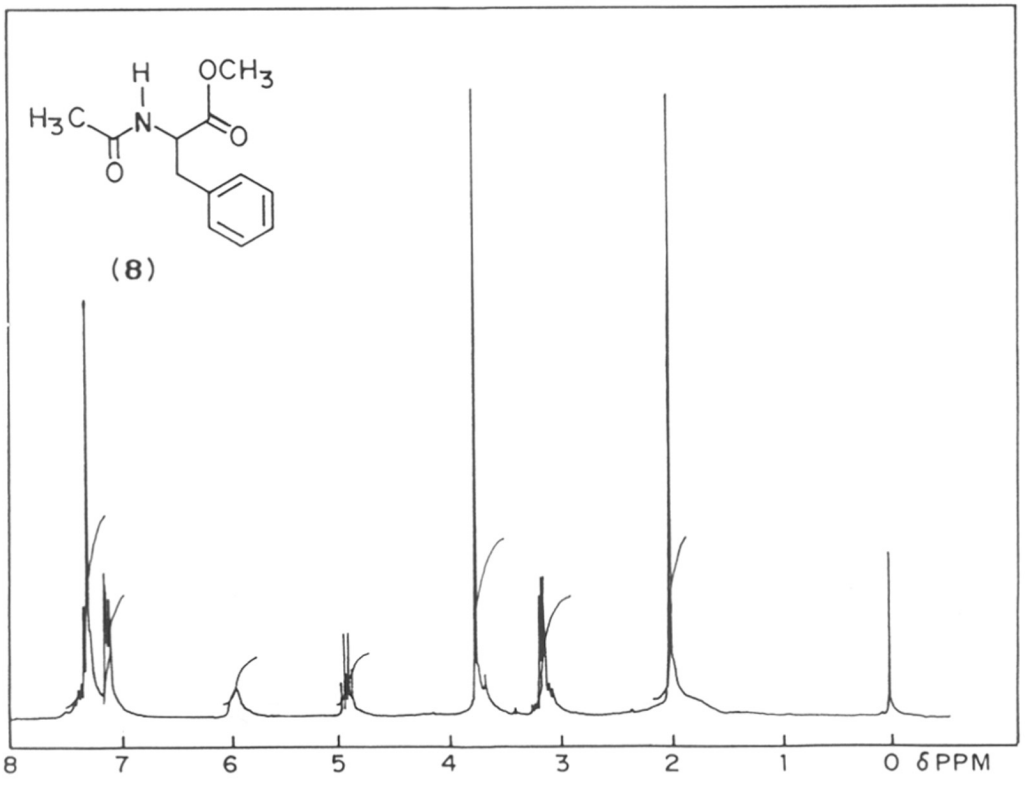

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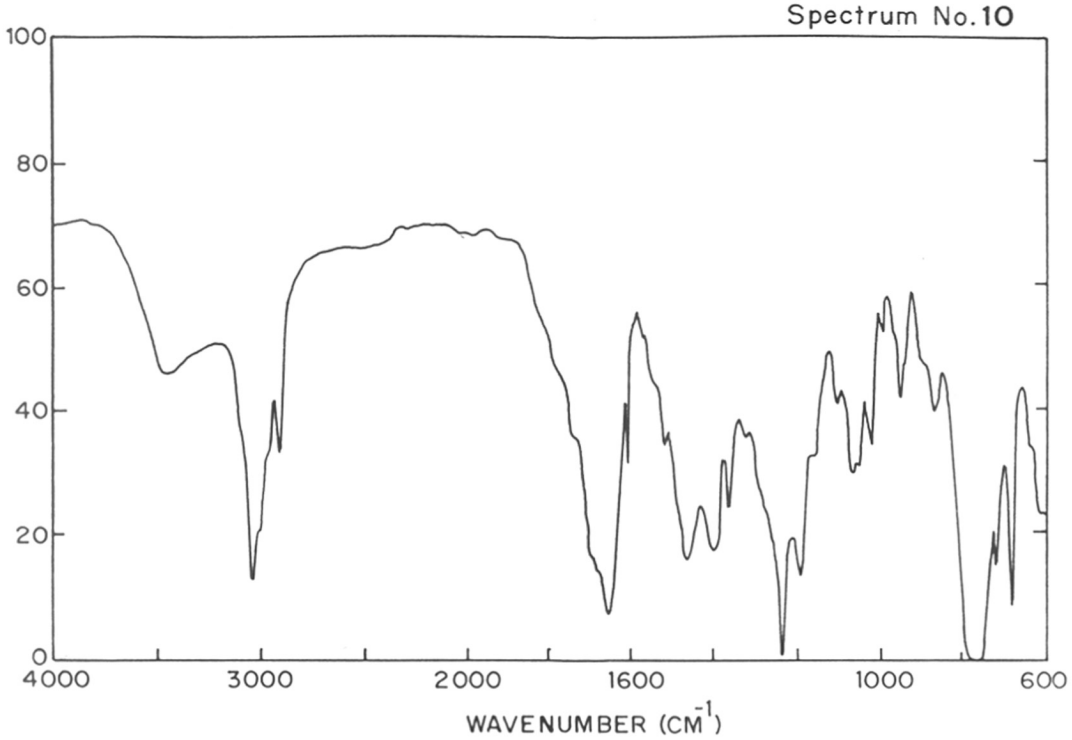

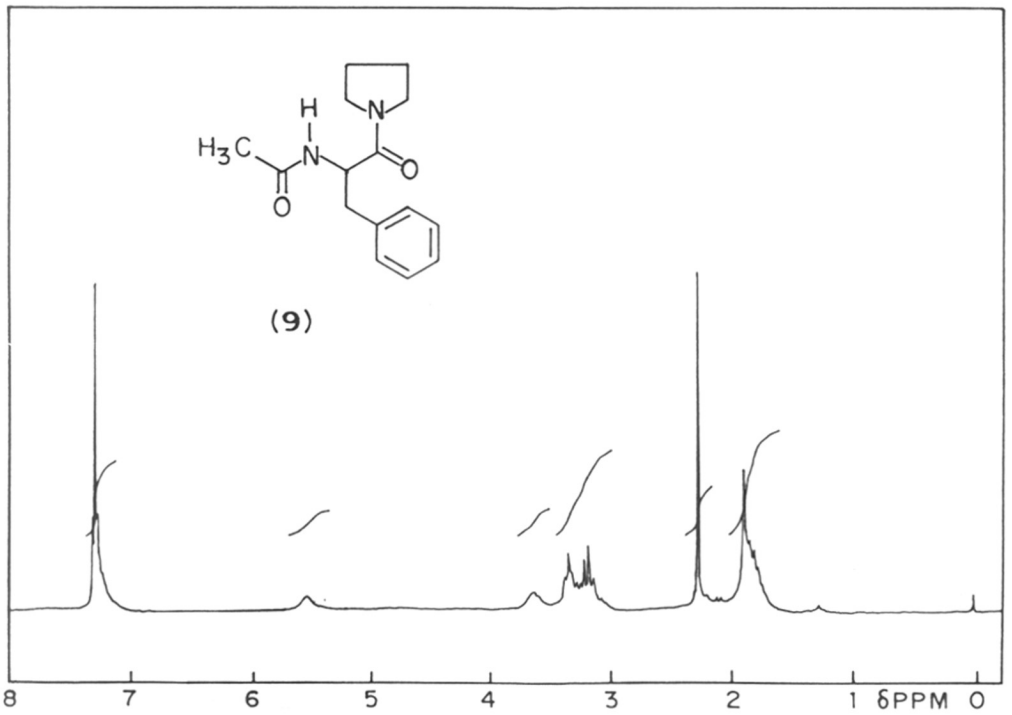

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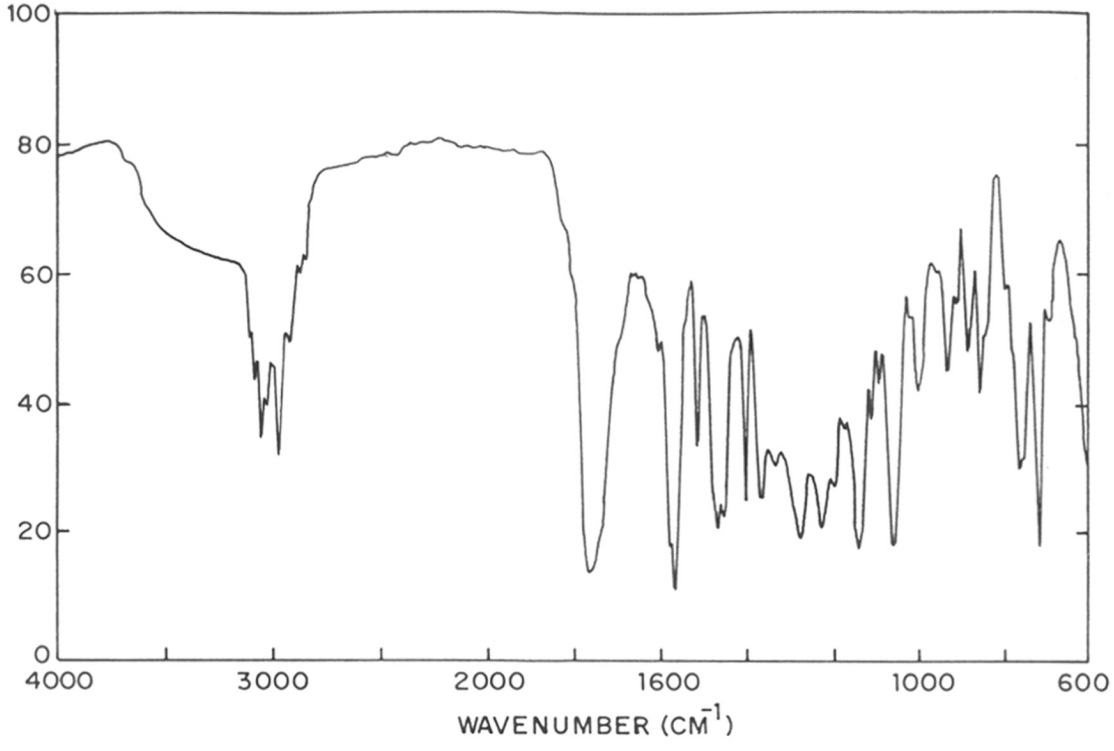

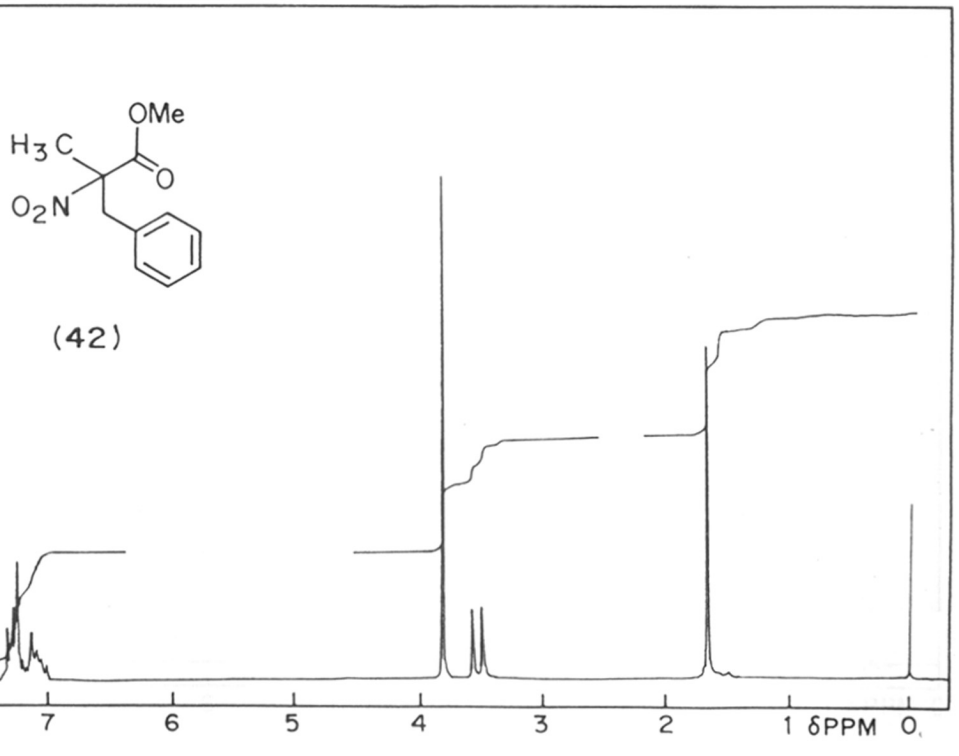

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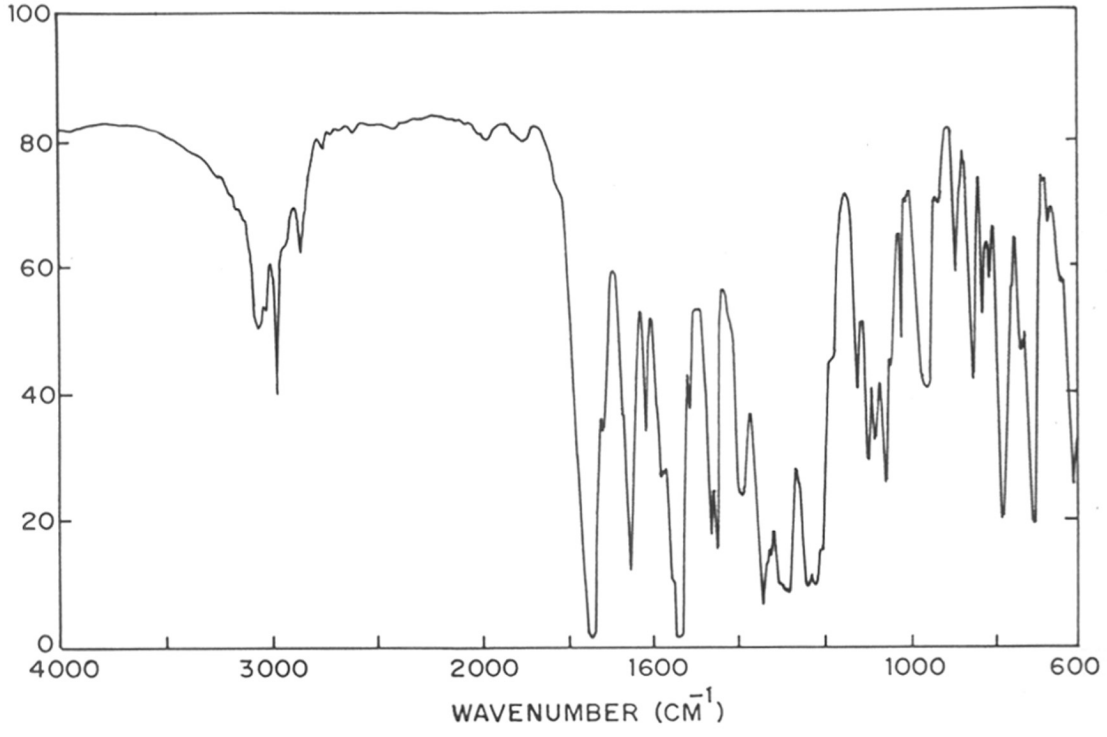

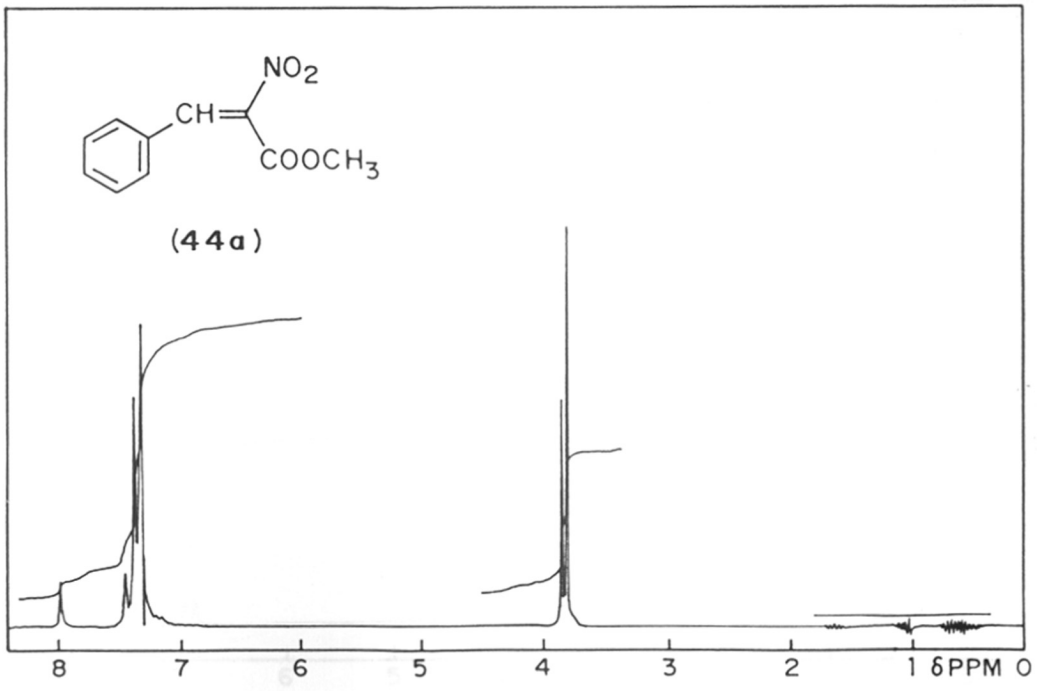

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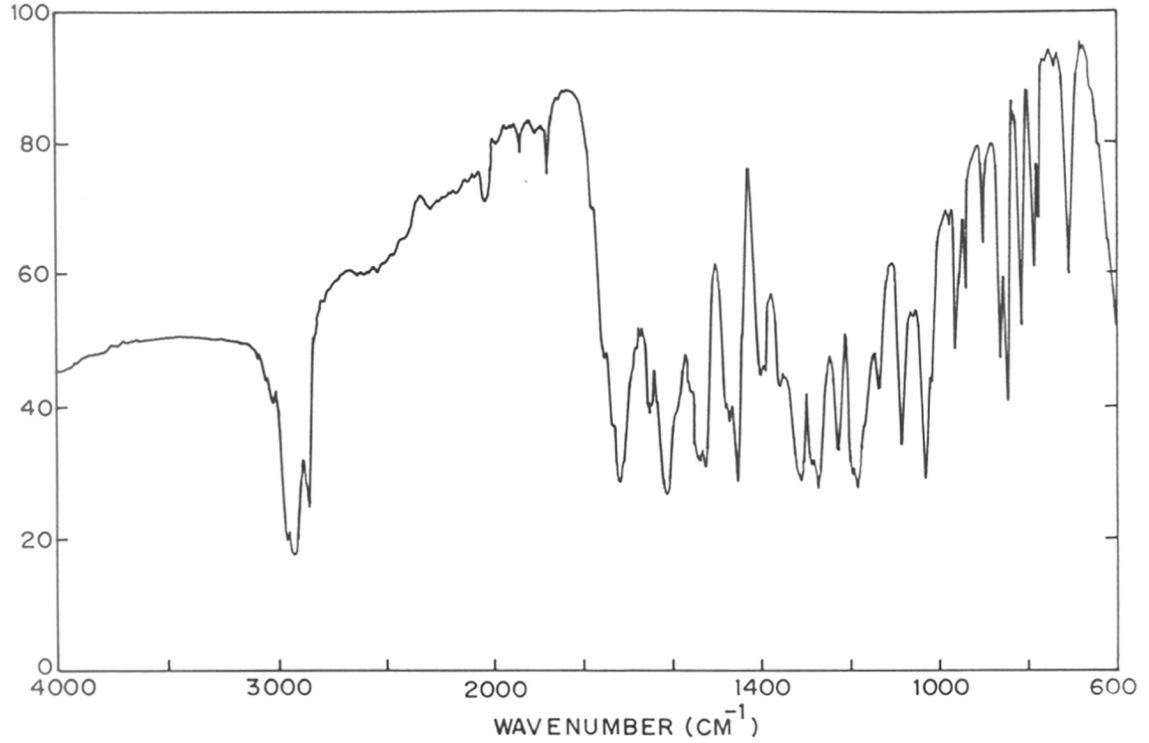

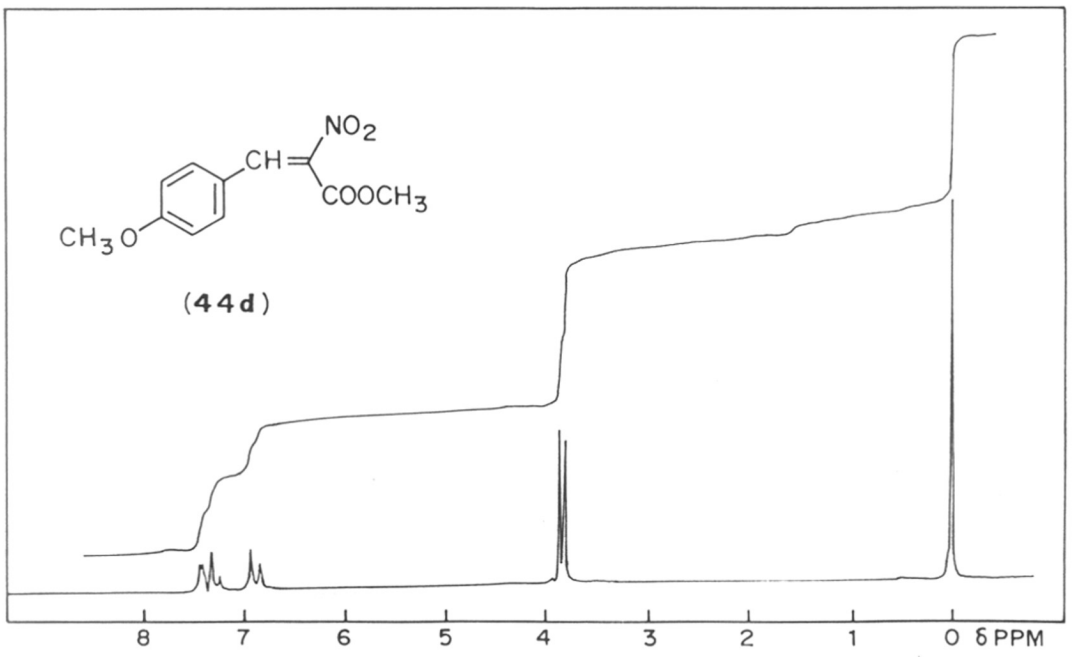

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CHAPTER 6

Attempted enzymatic transformations of some nitroacetic acid esters

6.1 Summary

α-Alkyl nitroacetic acid esters are attractive synthons for a variety of organic molecules such as aminoacids, aminoalcohols etc. The importance of optically pure organic compounds is increasing day by day.

In this chapter, we present our attempted enzymatic transformations on the following three substrates: methyl nitroacetate (**1**),racemic methyl α-nitro-β-phenyl propionate (**2**) and racemic methyl α-benzyl-α-nitro propionate (**3**). The objective was to generate enantiomerically pure products from these by the use of enzymes. The following three reactions have been attempted for this purpose:

i) The ester hydrolysis of the two compounds (**2**) and (**3**) was studied using *Bacillus subtilis*.

ii) Nitroreduction of the two esters (**2**) and (**3**) was studied using the culture *Clostridium populeti* under anaerobic conditions.

iii) The chemoenzymatic alkylation of methyl nitro acetate (**1**) was studied using bakers yeast.

In the ester hydrolysis experiment, the compound (**3**) did not undergo hydrolysis while in the case of compound (**2**), 75% hydrolysis took place in 48h with 0.5% substrate concentration. The unhydrolysed ester had no optical rotation. The reason might be that the methine proton being highly acidic racemization might have been very rapid.

In the nitroreductase experiment, compound (**3**) was recovered unchanged. With compound (**2**), no reduction of the nitrogroup took place; instead, the carboxylic ester was partially hydrolysed; however, the residual unhydrolysed ester did not exhibit any optical rotation.

In the attempted chemo-enzymatic reaction of methyl nitroacetate (**1**) with ethanol no ethylated product was obtained.

6.2 Introduction

The use of enzymes in organic synthesis, especially in ester hydrolysis, has already been discussed in Chapter 3. Some other transformations using enzymes are briefly discussed here.

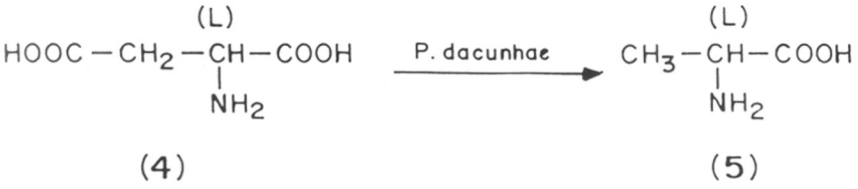
Synthesis of optically pure organic molecules has recently been a goal of many workers. The most dramatic example of the importance of chirality was the tragedy associated with thalidomide, which was sold as a racemic mixture. It was subsequently shown that our enantiomer was responsible for the desired therapeutic effect. The other enantiomer, which was originally assumed to be inert, produced fetal deformities[1]

Selective decarboxylation can be effected by pyridoxal phosphate dependent enzymes. Industrially valuable conversion of L-aspartic acid (**4**) to L-alanine (**5**) is an example of this type[2].

Hog kidney acylase-catalysed resolution of N-acylamino acids was the first major application of enzymes for resolution of racemates[3]. Acylases are stereospecific for L-enantiomers; the unhydrolysed N-acyl D-aminoacids can be recycled via chemically induced racemization. Such Acylase-mediated resolution procedures such as (±) (**6**) to (L) (**7**), are industrially important[4].

One of the most remarkable selective enzyme-catalysed reactions is the exchange of the side chains of L-aminoacids. These reactions are pyridoxal phosphate coenzyme dependent and proceed via a Schiff base intermediate.

The conversion of L-serine (**8**) and L-DOPA (**9**) illustrates the process[5,6]. Many side chain groups can be introduced in this way[7].



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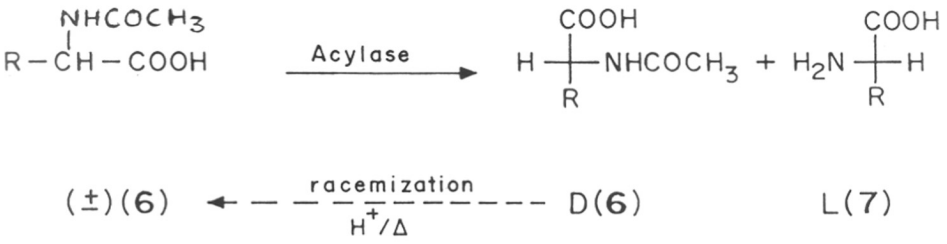
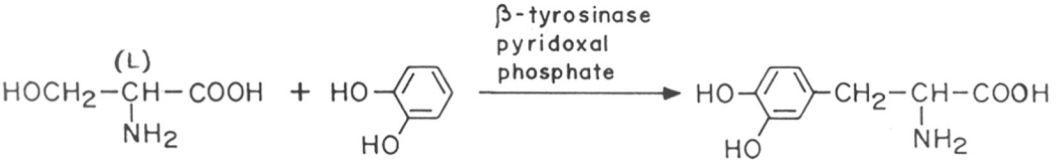
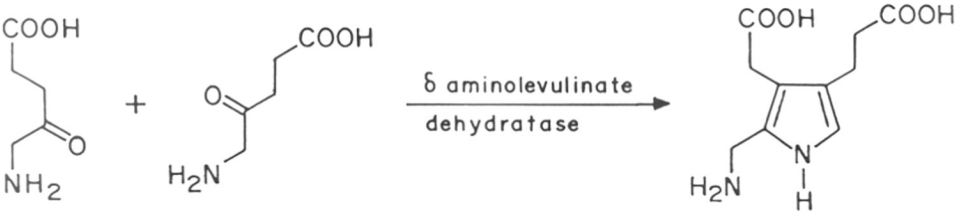




(8) (9)



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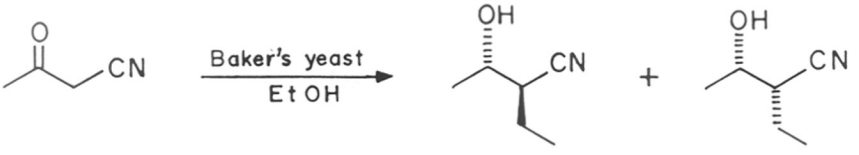


(14) (12) (13)

Another reaction that is very difficult to achieve nonenzymatically is the condensation of two molecules of δ-aminolevulinic acid (**10**) to produce porphobilinogen[8] (**11**).

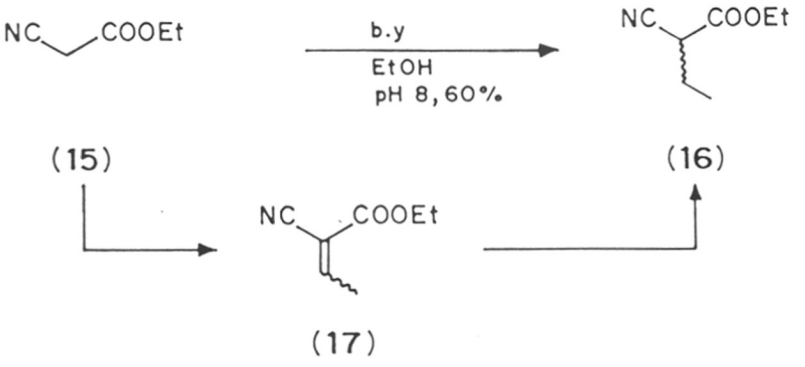
The two stereoisomeric alkylated products (**12**) and (**13**) have been obtained in high *ee* starting from cyanoacetone (**14**) in fermenting yeast[9]. This is an example of chemo-enzymatic alkylation. Active methylene compounds undergo chemical condensation in water with the aldehydes derived from yeast oxidation of the corresponding alcohols[10]. The unsaturated compound thus obtained is further reduced by yeast to produce finally the C-alkylated product.

Thus Ethyl cyanoacetate (**15**) in presence of ethanol and yeast is transformed into the ethylated product (**16**) in 60% yield at pH 8. The unsaturated condensation product (**17**) is formed initially and then gets reduced to the saturated product (**16**) (Scheme I).

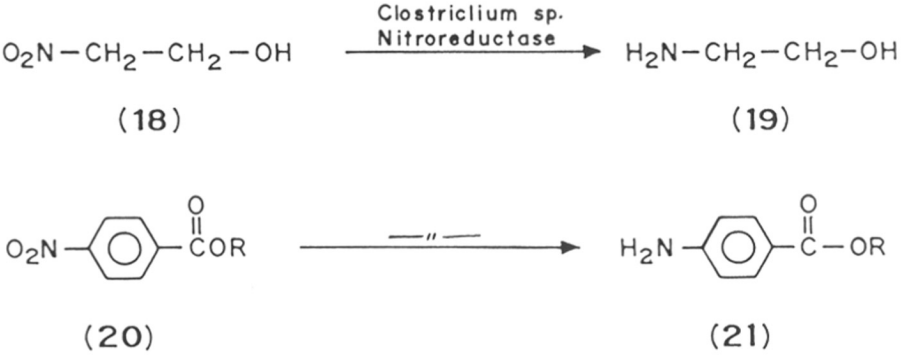
Aliphatic and aromatic nitrocompounds are reduced by variety of *Clostridium* species in the presence of hydrogen gas[11]. Thus 2-nitroethanol (**18**) is transformed to 2-aminoethanol (**19**). Similarly p-nitrobenzoate esters (**20**) are transformed to p-amino benzoate esters (**21**) (Scheme II).

α-Methyl-α-aminoacids have been employed in the pharmaceutical industry as reversible inhibitors of aminoacid decarboxylases. The increased steric bulk at the α-position of these aminoacids leads to conformational rigidity as well as resistance to hydrolysis by peptidases. These two properties have led to an increase in the importance of such compounds in structure activity relationship studies of peptide hormones. α-Methyl α-aminoacids have also been found to be constituents of peptides having antibiotic activity[12].

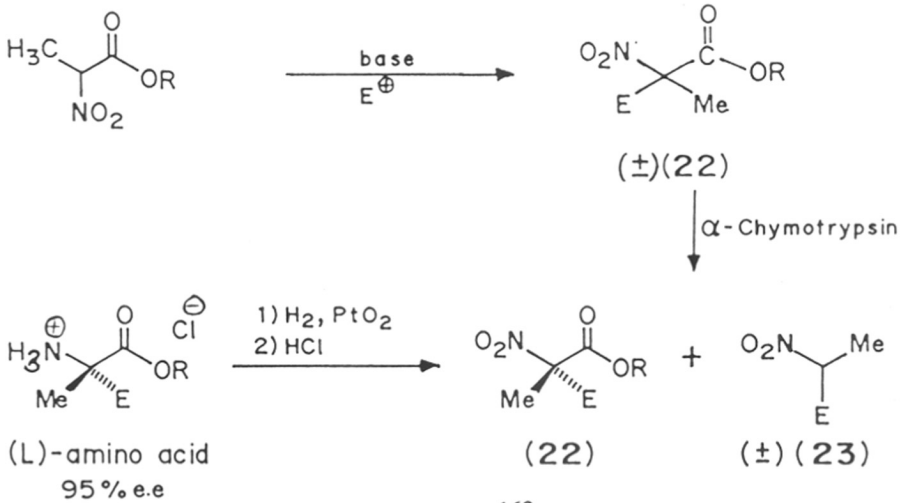
Scheme-I



Scheme-II



Scheme-III



Kinetic resolution of the 2-nitropropionic esters (**22**)using chymotrypsin has been reported[13]. These can act as precursors of α-methyl-α-aminoacid esters. The (L) aminoacid ester in is obtained 95% *ee* after reduction of the resolved α-nitropropionate (**22**). The D-isomer of the nitro compound was preferentially hydrolysed; this was followed by decarboxylation to yield (±) (**23**) (Scheme III).

6.3 Present work

Several enzymatic transformations of methyl nitroacetate (**1**) and its C-alkylated products (**2**) and (**3**) have been attempted. The preparation of these compounds (**1**), (**2**) and (**3**) has already been discussed in Chapter 5.

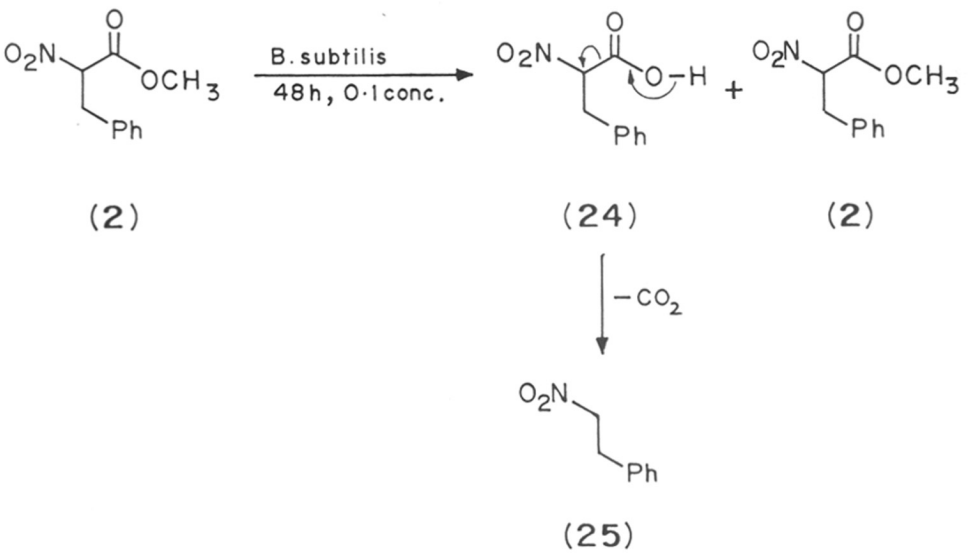
In view of our success in the resolution of 1,1,1-trichloro-2-hydroxy-4-methyl-3-pentene, by using an enzyme-mediated stereospecific hydrolysis as reported in Chapter 3, we felt that it would be worthwhile to attempt a similar enzymatic resolution of the nitroacetic acid esters (**2**) and (**3**). The organism used was *Bacillus subtillis*.

The ester hydrolysis was attempted for the two esters (**2**) and (**3**) separately with 0.1% substrate concentration for 48h. The crude reaction product in each case was purified by column chromatography.

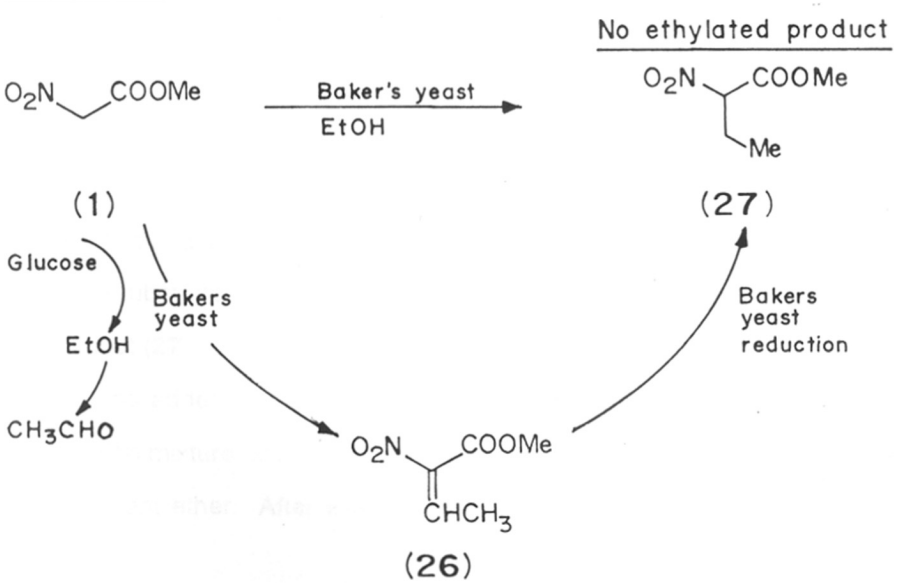
In the case of methyl α-nitro-β-phenylpropionate (**2**), two products were obtained in the ratio 3:1. These were identified as 1-nitro-2-phenylethane (**25**) and unhydrolysed ester (**2**). The product (**25**) obviously arises by decarboxylation of the hydrolysed product i.e. α-nitro β-phenyl propanoic acid (**24**). The optical rotation of the unhydrolysed ester (**2**) was measured in order to determine whether the hydrolysis was enatiospecific. This turned out to be practically zero. This might have happened because of the presence of a highly acidic methine proton which could undergo facile racemization.(Scheme IV)

To overcome this difficulty, the ester containing a quaternary carbon i.e. methyl α-benzyl-α-nitro propionate (**3**) was examined using the same enzymatic system. Unfortunately the ester did not undergo any hydrolysis.

Scheme - IV



Scheme - V



Expected enzymatic transformation

Next, the enantioselective reduction of the nitro group of (**2**) and (**3**) was attempted using *Clostridium populeti* under anaerobic conditions. The experiments were carried out by following two procedures. In the first, the compound was added to the growing culture of *C.populeti* under anaerobic conditions where the head-space was filled with oxygen free nitrogen and carbon dioxide. In the second procedure, the substrate was added to centrifuged cells of the culture *C.populeti* suspended in proper buffer. In this case the headspace gas was replaced by hydrogen and carbon dioxide.

In this case also the ester (**3**) was unreactive. The ester (**2**) showed no reduction of the nitrogroup but instead, part of it got hydrolysed to give 1-nitro-2-phenylethane (**25**). The ratio of this compound to unhydrolysed ester was 2:1. The latter showed no optical rotation.

The compound 1-nitro-2-phenylethane (**25**) was obtained as a colourless liquid with IR absorption at 1600, 1570, 1450, 1390 cm^{-1}. In the ^1H NMR spectrum, a triplet at 3.12 δ for methylene protons adjacent to phenyl group was observed. One more triplet at 4.55 δ was observed for the methylene protons adjacent to the nitro group. A broad singlet was observed at 7.1δ for aromatic protons.

Finally, a chemo-enzymatic ethylation was attempted on methyl nitroacetate (**1**). It was expected thatas the fermentation proceeds, ethanol formed would get oxidised to acetaldehyde which would condense with the active methylene group of the substrate to form the olefinic product (**26**). The further reduction to saturated product (**27**) may be brought about by the Bakers yeast. The substrate (**1**) (0.1 ml) was added to the fermenting yeast and the flask was stirred for 72h. The reaction mixture, after filtering through the celite pad, was acidified and extracted in solvent ether. After washing and drying over anhydrous sodium sulfate, the

solvent was removed to get crude reaction product. Analysis showed that the substrate had not undergone any ethylation under the reaction conditions. (Scheme V)

Conclusion

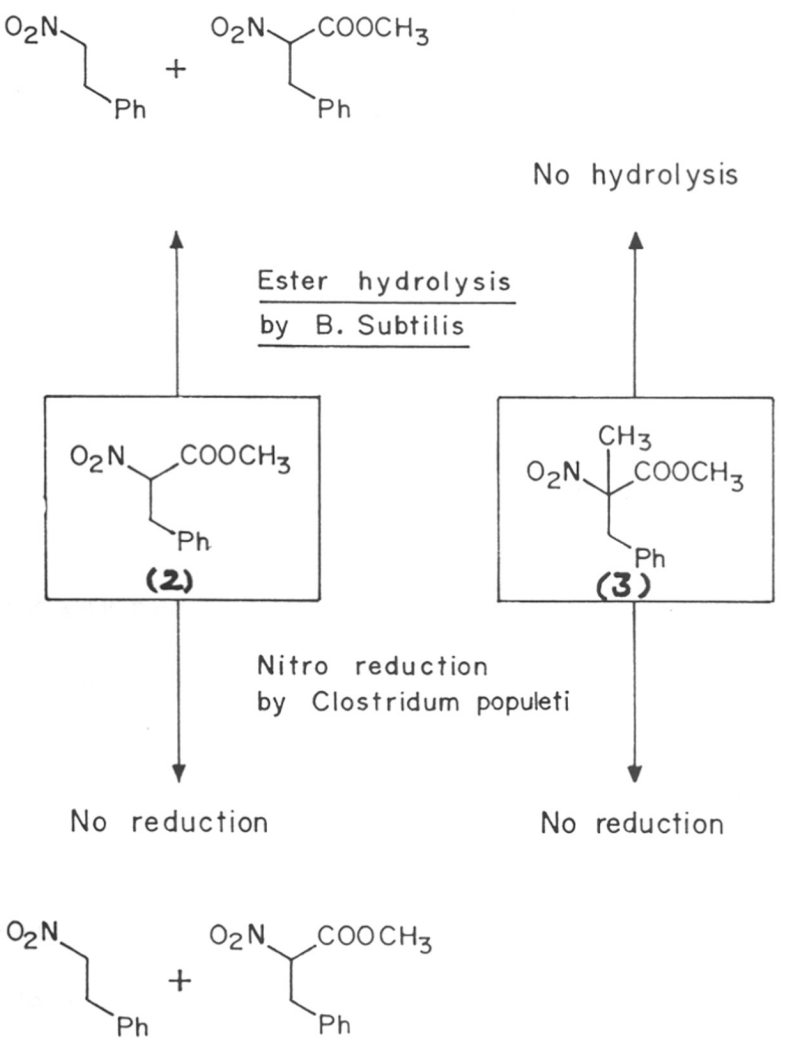
There was 75% ester hydrolysis when the ester (**2**) was subjected to enzymatic ester hydrolysis using *B.subtilis*. The unhydrolysed ester was racemic in this case.

No hydrolysis occured in case of ester (**3**). (Scheme VI)

Attempts to reduce the nitro group of (**2**) and (**3**) using *Clostridium populeti* under anaerobic conditions failed.

Chemo-enzymatic ethylation of methyl nitroacetate using Baker's yeast gave no ethylated product.

Scheme - VI


O2N
Ph
+
O2N
COOCH3
Ph
No hydrolysis
Ester hydrolysis
by B. Subtilis
O2N
COOCH3
Ph
(2)
CH3
O2N
COOCH3
Ph
(3)
Nitro reduction
by Clostridum populeti
No reduction
No reduction
O2N
Ph
+
O2N
COOCH3
Ph

6.4 Experimental

The detailed experimental procedure for the preparation of methyl nitroacetate (**1**) and its C-alkylated products namely methyl-α-nitro-β-phenyl propionate (**2**) and methyl-α-benzylα -nitro propionate (**3**) has already been discussed in chapter 5.

1. Ester hydrolysis using *Bacillus subtilis*

The substrate (**2**)/(**3**) (0.1 ml) was added to the culture broth of *B.subtilis* previously grown for 2 days at 28°C in a nutrient medium (100 ml containing 1g peptone, 0.4g of meat extract, 0.1g glucose and 0.1g of agar-agar). The flask was shaken at a speed of 240 rpm (revolutions per minute) for 48 hours. The contents of the flask were acidified by 50% HCl solution to pH4 and then it was extracted with solvent ether. The ether layer was washed with water and then dried over anhydrous sodium sulphate. After removal of the solvent, crude reaction product was obtained which was purified by column chromatography (Silica gel, 60-120 mesh, 10% ethyl acetate in pet ether as solvent for elution).

Ester hydrolysis of methyl α-nitro-β-phenyl propionate (2) by *B.subtilis*.

The crude product after enzyme hydrolysis was purified by column chromatography to get 1-nitro-2-phenyl ethane (**25**) and unhydrolysed ester (**2**) in the ratio 3:1. The optical rotation of the unhydrolysed ester was measured, it had no rotation.

1-Nitro-2-phenyl ethane (25)

I.R.(Neat, cm^{-1}) : 1600, 1570, 1450, 1390 NMR ($CDCl_3$, δ) : 3.12 (t, J=7.5 Hz, 2H, $\underline{CH_2}$-Ph), 4.55 (t, J = 7.5 Hz, 2H, $\underline{CH_2}$-NO_2) 7.1 (bs, 5H, Ar-H)

Ester hydrolysis of methyl-α-benzyl-α-nitro propionate (3) by *B.subtilis*

There was no hydrolysis by the enzyme. All the starting ester was recovered back.

2. Reduction using anaerobic culture of *Clostridium populeti*

i) Substrate (**2**)/(**3**) was added to 100 ml anaerobic culture of *Clostridium populeti* containing peptone 0.5g; yeast extract 0.5g and glucose 1g. The medium was prepared using standard anaerobic techniques in rubber stoppered serum vial. The headspace gas was mixture of oxygen free nitrogen and carbon dioxide. The vial was incubated on a rotary shaker at 200 rpm at 35°C for 48h.

ii) The culture of the *Clostridium populeti* was centrifuged and was suspended in a buffer of pH 7.2 under anaerobic conditions. Headspace gas was replaced by mixture of H_2 and CO_2 (80:20), 0.1 ml substrate (**2**)/(**3**) was added to the bottle with sterile syringe and the bottle was incubated on a rotary shaker at 200 rpm at 35° for 48h.

The contents of the bottle were acidified to pH4 and then extracted with methylene chloride. The organic layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed to get crude reaction product. The product was purified by column chromatography (silica gel, 60-120, 10% ethylacetate in pet.ether as solvent for elution).

Nitroreduction of methyl α-nitro-β-phenyl propionate (2) by *Clostridium populeti* in anaerobic conditions by method (i) and (ii)

Crude product after purification gave 1-Nitro-2-phenylethane (**25**) and methyl-αnitro-β-phenyl propionate (**2**) in the ratio (2:1). There was no reduced product at all.

Nitroreduction of methyl-α-benzyl-α-nitro propionate (3) by *Clostridium populeti* in anaerobic conditions by method (i) and (ii)

The reaction product was purified and the ester (**3**) was obtained without any reduced product. The recovery was complete.

3. Chemo-enzymatic alkylation of methylnitroacetate (1)

5 grams of freshly harvested Baker's yeast was incubated at 35° in the presence of 2.5g glucose and 0.1g. Na_2HPO_4 in 50 ml distislled water. After 1h, 0.1 ml compound (**1**) was added under stirring. Fermentation was continued for 72h. The reaction mixture was filtered through celite and was acidified with 5% HCl and extracted with solvent ether. the ether layer was washed with water and was dried over anhydrous sodium sulphate and solvent was removed to get crude reaction product.

The ^1H NMR spectrum of the crude reaction product showed no alkylated product.

6.5 References

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