General introduction to macrocyclic lactones:

The macrolide structural spectrum is perhaps one of the richest in natural product chemistry. It embraces a wide variety of molecules whose number and structural type has grown enormously during the last quarter of century, and the trend is certain to continue in view of continuous development in fermentation, isolation and structural elucidation technique.

Generally, a macrocyclic lactone (A), macrolide is defined as a molecule containing a large ring (having 12 or more than 12 atoms) lactone with numerous substituents asymmetrically placed on the periphery of the ring system.

![Macrocyclic lactone structure](image)

(A)

Macrocyclic lactones are widely distributed in nature and constitute an extensive range of natural products possessing diverse biological and medicinal properties. Macrocyclic lactone macrolides are conformationally rigid molecules owing to the heavy substitution of the ring. Much of the rigidity is apparently retained in the corresponding seco acids, which in turn may persist in a conformation favouring an intramolecular reaction with proper activation. Synthetic scheme based on this seco acid approaches are new standard in macrocyclic synthesis.

The construction of macrocyclic structure is a frequent and challenging problem in synthetic organic chemistry. The advances made in the synthesis of macrocyclic natural products illustrate the difficulties and illustrate some of the ingenious ways by which this problem has been attacked and solved by synthetic chemists. In principle, macrocyclic system can be generated by cyclization of open long chain precursors or by
cleavage of internal bonds in polycyclic systems. However, in the former case, which is the most general one, the ring closure is disfavoured entropically due to the loss of entropy associated with the formation of the usually more rigid cyclic structure. Further more, polymerization due to intermolecular rather than intramolecular interactions is often a serious problem, although subject to experimental control. Despite the severe problems, however, recent interest in the chemistry of macrolide antibiotics and other biologically active macrolactones and macrolactams resulted in the discovery and development of several new synthetic methods for macrolide formation.

Apart from the problems of constructing the necessary open chain frame work, the key aspects of synthetic strategy in this field comprises the choice of protecting groups for the terminal hydroxy and carboxy functions during this construction and the development of a general high yielding intramolecular lactonization process for the resulting hydroxy acid. For work on polylactones it is additionally desirable that the hydroxy and carboxy protecting groups should be such that each may be independently removable without affecting the other, or indeed any other sensitive part of the molecule. This imposes a particularly heavy demand on the nature of the carboxy protecting group where esters functions are already present in the molecule. The constitutional structures, important biological properties and interesting conformational features of the so called macrolide antibiotics, have been the subject of elegant studies over the years. Work aimed at the assembly of the multifunctional carbon back bone of these molecules and segments, there of, constitutes a great challenge to the synthetic organic chemists. Indeed, elegant methodology has been developed as a result of this imputes and applied to the total synthesis of macrolides. The macrocyclic lactone may tansine has been the subject of intense studies on chemical and biological fronts. Several reviews have been appeared on these aspects of their chemistry\textsuperscript{1,2}.

In view of their unique structures and diverse biological activity and in continuation of our interest on the application of nitro aliphatics in the synthesis of natural products we undertook the present study and tried to develop newer routes towards the synthesis of (\(R,R\))\(-\text{(-)}\)-Pyrenophorin, Patulolide A and Patulolide B and (\(\pm\))-tridecan-12-olide.
SYNTHESIS OF (R,R)-(−)-PYRENOPHORIN

Introduction:

(R,R)-(−)-Pyrenophorin I is a naturally occurring antifungal, antibacterial 16 membered macrolide dilactone having $C_2$ symmetry isolated from the plant pathogenic fungi *pyrenophora avenae* and *stempyltium radicum*. In addition to its antifungal and antibacterial actions, it shows cytostatic properties. The $\gamma$-keto $\alpha,\beta$ unsaturated ester moiety, which is also found as structural subunits in several other biological molecule, is crucial to biological activity.

Pyrenophorin I, m.p 175° C, $[\alpha]_D -50^0$, was first isolated by Ishibashi from culture filtrates of *P. avenae*, a pathogen of oat.

Its functionalized dimeric lactone structure, biological activities and interesting $\gamma$-keto $\alpha,\beta$ unsaturated ester moiety invoked the interests of synthetic organic chemists. So far synthesis of racemic forms as well as the optically active forms and the seco acid synthesis of Pyrenophorin have been reported. Most of the reported synthetic routes to Pyrenophorin rely on synthesis of suitably functionalised hydroxy acid in racemic or chiral form followed by dimerization to dilactone.
Synthesis of optically active \((R,R)-(\text{-})\)-Pyrenophorin, A Review

Almost all synthetic approaches that have been reported are based upon the preparation of the racemic monomeric unit of hydroxy acids or chiral form followed by dimerization to dilactone. Only a few examples of chiral synthesis of the naturally occurring \(8R,16R\) isomer I have been reported. Seebach's\(^7\) group was the first to utilize an asymmetric process for the synthesis of the antipode of the natural isomer. Few years later Takano \textit{et al.}\(^7\) reported a chiral approach towards the synthesis of naturally occurring antipode of Pyrenophorin I. The only chiral center of the monomeric unit of Pyrenophorin could be obtained i) by the chiron approach, where the chirality present in a natural product is translated to the desired molecule ii) by enzymatic methods.

\((R,R)-(\text{-})\)-Pyrenophorin I has been synthesized employing all the above modes by various groups. Here we will discuss some of the recent progress made in the synthesis of this natural antipode.

**Takano's approach\(^7\):**

Takano \textit{et al.}\(^7\) (1987) synthesized the \(C_2\) symmetric 16-membered macrolide \(\text{(-)-Pyrenophorin}\) using 4-DMAP catalyzed ester exchange reaction of phosphono acetates with lactoles. Their synthetic route (scheme I) is shown below-

Ethyl-\(O\)-isopropylidene-(5)-4,5-dihydroxypentanoate (2) on reduction (LAH, THF, 25\(^\circ\)C), oxidation (PCC, CH\(_2\)Cl\(_2\), 25\(^\circ\)C) and thioacetylation [HS(CH\(_2\))\(_3\)SH, BF\(_3\).Et\(_2\)O, CH\(_2\)Cl\(_2\), 25\(^\circ\)C] gave the diol (3). Tosylation (\(p\)-TsCl, pyridine, 25\(^\circ\)C) followed by reduction (LAH, THF, 0\(^\circ\)C) yielded the alcohol (4). The alcohol (4) was then converted to lactol (5) \textit{via} the dianion (n-BuLi, THF, -30\(^\circ\)C and then DMF) according to the procedure described by Seebach\(^9\). Reaction of the lactol (5) with diisopropylmethoxycarbonyl methyl phosphonate (6) (30 mol \%, 4-DMAP, toluene, reflux, 3 days) afforded the key intermediate aldehydophosphonoacetate (7) in 75\% yield. On treatment of compound (7) with 1.1 equiv. of NaH in THF, cyclization took place at ambient temperature to give the desired diolide (8). Finally, \(\text{(-)-Pyrenophorin}\) was obtained on hydrolysis of the dithiane group of (8) (NCS-AgNO\(_3\), aq MeCN, 25\(^\circ\)C) in 57\% yield.
Scheme-I

(a) i) LAH, THF, 25°C; ii) PCC, CH2Cl2, r.t; iii) HS(CH2)3SH, BF3.OEt2, CH2Cl2, 25°C; (b) i) p-TsCl, Py, 25°C; ii) LAH, THF, 0°C; (c) n-BuLi, THF, -30°C then DMF; (d) DMAP, (1 Pro)₂-P-CH₂CO₂Me (6); (e) 1.1 equiv. of NaH in THF; (f) NCS-AgNO₃, aq MeCN, 25°C.

Kibayashi's approach⁷:

Kibayashi et al⁷ (1993) synthesized (−)-Pyrenophorin I utilizing C₂ symmetric (R,R)-diepoxide as a chiral building block. Their synthetic route (scheme-II) is shown below—
Scheme-II

a) Vitride (1 mol equiv.), THF, 0°C → r.t; b) t-BuPh₂SiCl, DMAP, CH₂Cl₂, r.t; c) PhSCH₂CO₂H, LDA (2 equiv.), THF, 0°C → r.t then CH₂N₂, Et₂O; d) NaIO₄, MeOH-H₂O, r.t; e) Py (2 equiv.), toluene, reflux, f) PDC, DMF, rt; g) CH(OEt)₃, HO(CH₂)₂OH, BF₃·OEt₂, benzene, reflux; h) 20% NaOH-MeOH, r.t then Bu₄NF, THF, reflux; i) Ph₃P, DEAD, toluene-THF (10:1), -25°C, 10 h; j) CSA, acetone.
They prepared the monoepoxide (10) by mono addition of hydride ion to the diepoxide (9) by using 1 mol equiv. of Vitride in THF with 74% yield based on 40% recovered starting material. After O-silylation (t-BuPh3SiCl, DMAP), the resulting monoepoxide (11) was further subjected to ring opening by exposure to (phenyl thio)acetic acid dianion (PhSCH2CO2H, LDA, THF) followed by diazomethane esterification, affording the α-phenyl thio hydroxy ester (12) in 48% yield from (11). The compound (12) on further oxidation with sodium metaperperiodate afforded the sulfoxide (13) which underwent pyrolysis in boiling toluene in presence of pyridine to furnish (E)-α,β-unsaturated hydroxy ester (14) (85% yield from 12). Compound (14) was converted to (16) in 67% yield via PDC oxidation and subsequent protection of the resulting ketone as the ethylene ketal. Alkaline hydrolysis of the ester followed by desilylation (Bu4NF, THF, reflux) provided the hydroxy acid (17), which was subsequently cyclodimerized to (18) according to Gerlach's procedure10 in 44% yield. Acidic removal of the carbonyl protecting group furnished (-)-Pyrenophorin I in 80% yield.

**Ohta's approach**:  

Ohta et al. (1995) synthesized (R,R)-(−)-Pyrenophorin I by a chemo-enzymatic approach, starting from commercially available 6-methyl-5-hepten-2-one. Their synthetic route (scheme-III) is shown below:

They prepared the (R)-6-methyl-5-hepten-2-ol (sulcatol) (20) by interface bioreactor mediated asymmetric reduction of the corresponding ketone (19) by a yeast, *Pichia farinosa* IAM 4682 in 51% yield with 90% ee. The subsequent carbon chain elongation via Horner-Emmons olefination of protected aldehyde (21) and cyanation afforded all of carbon skeleton in the precursor (22) with a desired β,γ-(E) double bond. The nitrile (24) was obtained by one carbon homologation using cyanide via the nucleophilic substitution of allylic halide (23), which in turn was derived from allylic alcohol (22). By the aid of a microorganism, *Rhodococcus rhodochrous* IF015564, the nitrile (24) was efficiently hydrolyzed to give the corresponding carboxylic acid (R,E)-7-hydroxy-3-octanoate (25), the key synthetic intermediate without affecting the position and configuration of the double bond. Dimeric lactone structure (26)
Scheme-III

a) Glucose-agar/ loopfuls of *P. Farinosa*; b) i) PPL, vinyl butanoate ii) K$_2$CO$_3$, H$_2$O/MeOH iii) TBDMSCl, imidazole/DMF; iv) O$_3$/CH$_2$Cl$_2$ then Me$_2$S. c) i) triphenyl phosphono acetate, LiCl, Hunig base/CH$_3$CN; ii) DIBAL-H / CH$_2$Cl$_2$; d) NBS, Ph$_3$P/ CH$_2$Cl$_2$, e) i) CuCN; ii) HF/CH$_3$CN; f) *Rhodochrous* IFO 15564, then CH$_2$N$_2$; g) *Pseudomonas Cepacia* lipase or *Candida antarctica* lipase; h) ref 7(b).

was obtained by utilizing a lipase catalyzed lactonization. While *Pseudomonas cepacia* lipase catalyzed reaction worked in an orderate efficiently, higher yield of desired dimeric lactone was obtained by the use of an immobilized form of *Candida antarctica* lipase. The lactonization was accelerated in the presence of molecular sieves 4A. *(R,R)-(-)-*
Pyrenophorin I was obtained from the dimeric lactone [Seebach's intermediate (26)] by the subsequent chemical transformation. By using this synthetic protocol they synthesized (R,R)-(−)-Pyrenophorin I in 16 steps and in 1.8% overall yield from commercially available 6-methyl-5-hept-2-one. In this synthesis they used three biocatalytic procedures: i) interface bioreactor mediated yeast reduction of aliphatic ketones for the introduction of chirality, ii) microbial hydrolysis of nitrile for the installation of β,γ-unsaturated carboxylic acid with (E)-configuration, iii) lipase catalyzed transformation for the dimeric lactonization.

Kobayashi's approach\textsuperscript{7}: 

Kobayashi \textit{et al}\textsuperscript{7} (1996) synthesized (−)-Pyrenophorin using NBS oxidation of 2-substituted furanes (29) followed by further oxidation of the enals with NaClO\textsubscript{2} to afford the hydroxy acids (31) which was then dimerized to afford the desired (−)-Pyrenophorin. Their synthetic protocol is shown below (scheme-IV).

Kobayashi \textit{et al} took (27) as a starting material, which they subsequently converted to the corresponding iodide (28) in a three step sequence with 80% yield. Compound (28) upon alkylation with furyl lithium afforded compound (29) in good yield. The NBS oxidation of the 2-substituted furane (29) yielded compound (30) in good yield, which upon further oxidation with NaClO\textsubscript{2} and subsequent ketalization furnished the intermediate (32). The dimerization of the protected acid (32) according to the literature procedure yielded the desired (−)-Pyrenophorin I.
Scheme-IV

a) TBDPSCI, imidazol, DMF, 100%; b) DIBAL, THF, -50 ~ -15°C, 3 h, 91%; c) I₂, PPh₃, C₆H₆, 88%; d) Furyl lithium, THF, r.t, 94%; e) NBS (1.2 equiv.), Pyridine (4-equiv.), THF/acetone/H₂O = 5:4:2, -20°C, 1 h, then r.t, 5 h, 64%; f) NaClO₂, 2-methyl-2-butene, t-BuOH/phosphate buffer (pH 3.6)/H₂O = 2:1:1, 83%; g) HO(CH₂)₂OH, p-TsOH (cat), C₆H₆ then LiOH, MeOH/H₂O, 70%.
SYNTHESIS OF OPTICALLY ACTIVE SECO ACID, THE PRECURSOR FOR THE SYNTHESIS OF OPTICALLY ACTIVE \((R,R)-(\cdot)-PYRENOPHORIN, A REVIEW:

So far, all synthetic approaches that have been reported towards the synthesis of optically active \((R,R)-(\cdot)-Pyrenophorin\) are based upon the preparation of the optically active monomeric unit of hydroxy acid (seco acid) followed by dimerization to dilactone. The only chiral center of the monomeric hydroxy acid could be obtained i) by the chiron approach, where the chirality present in the molecule is translated to the desired molecule ii) by asymmetric induction iii) by the enzymatic methods. We have reviewed here the development taken place during recent years towards the asymmetric synthesis of this protected seco acid derivative.

**Sih's approach**:

Sih et al\(^8\) (1989) synthesized the key penultimate intermediate \((\cdot)-7-(S)-hydroxy-4,4-(ethylene dioxy)oct-2-enoic acid\) for the synthesis of \((\cdot)-Pyrenophorin\) using a chemo-enzymatic approach. Their synthetic route (scheme-V) is shown below:

They used furfural lactone as the starting material for the synthesis of \((\cdot)-36\). The acetate \((\cdot)-34\) was obtained from \((33)\) in 80% yield by reduction with sodium borohydride followed by acetylation. The 1,4- dicarbonyl functionality was generated by Jones oxidation of the furan ring of \((\cdot)-34\). The resulting keto acid \((\cdot)-35\) was then treated with 2-mercapto benzene imidazole to catalyzed the isomerization of the \textit{cis} to the \textit{trans} isomer followed by ketalization. Hydrolysis of \((\cdot)-35\) gave the required hydroxy acid \((\cdot)-36\) in 36 % over all yield from \((\cdot)-33\). For biocatalytic resolution they prepared \((\cdot)-37\) from \((\cdot)-36a\) as the substrate. Careful condensation was given to the design of the highly functionalized substrate \((\cdot)-38\). The \textit{Pseudomonas} sp (K-10) lipase was highly enantioselective for the \textit{R} enantiomer of \((38)\) and good yield of the product was obtained. By terminating the conversion at 42%, methyl-(\cdot)-(\textit{R})-7-hydroxy-4,4-(ethylene dioxy)oct-2-enoate \((38)\) was obtained with an enantiomeric purity of > 99% ee. They Obtained the residual substrate \((-)-(S)-38, \text{methyl-}(-)-(\textit{S})-7-(chloro acetoxy)-4,4-(ethylene dioxy)-
oct-2-enoate with an ee > 99%. Completion of a formal synthesis of (-)-1 was accomplished by cleavage of the ester in (-)-(S)-38 (2 M LiOH/THF, 25°C) to afford (+)-(S)-36a, which has been chemically transformed into (I).

\[
\begin{align*}
33 & \xrightarrow{a,b} 34 \xrightarrow{c,d,e} 35 \\
37 & \xrightarrow{h} 36a : R = H \\
36 & : R = CH_3
\end{align*}
\]

\(-\)-(S)-38 \quad \text{Scheme-V} \quad \text{(-)-(R)-38}

\[
\begin{align*}
a) \text{NaBH}_4, \text{MeOH}; & \quad b) \text{Ac}_2\text{O}, \text{Py}; \quad c) \text{Jones's reagent}; \\
d) 2\text{-mercapto benzilimidazole}; & \quad e) \text{HOCH}_2\text{CH}_2\text{OH, } p\text{-TsOH}; \\
f) \text{LiOH, THF, H}_2\text{O}; & \quad g) \text{CH}_2\text{N}_2; \quad h) \text{Chloroacetyl chloride,} \\
\text{CCl}_4, \text{pyridine}; & \quad i) \text{lipase.}
\end{align*}
\]
Hoffman's approach$^8$:

Hoffman et al.$^9$ (1996) synthesized protected seco acid precursor of (R,R)-(−)-Pyrenophorin in 23% yield from a chiral β-keto ester. Their synthetic route (scheme-V) is shown below:

![Scheme-V]

**Scheme-VI**

a) i) NaH ii) n-BuLi; iii) (R)-(−)-40; iv) TBDPSCl, b) i) NaH; ii) Br$\rightarrow$CO$_2$Et; c) (NsO)$_2$, EtOAc, 0°C; d) Et$_3$N; e) 180°C, 45 min.

*tert*-Butyl acetoacetate (39) was converted to its dianion (NaH followed by n-BuLi) and reacted with (R)-(−)-40, the resulting alkoxide was quenched with *tert*-butyldiphenylsilyl chloride at 0°C to give the *tert*-butyldiphenylsilyl ether (41). Alkylation...
of (41) with ethyl bromo acetate gave keto diester (42) as a mixture of diasteromers which were converted to nosylate (43) by treating with p-nitro benzene sulfonyl peroxide (NsO)$_2$. The unsaturated ketodiester (44) from base promoted elimination of (43) was decarboxylated thermally at 180$^\circ$C to give (45).

By using this synthetic protocol they have described a eight step sequence for the enantiospecific synthesis of a protected seco acid precursor of (R,R)-(-)-Pyrenophorin in 23% yield from the chiral $\beta$-keto ester (39), which was prepared in one step from ethyl acetoacetate by a standard procedure.
RESULTS AND DISCUSSIONS
RESULTS AND DISCUSSIONS

Chiral nitro alcohols with a primary nitro group are potentially useful building blocks in organic syntheses because they can be converted into many other useful chiral products via a carbon–carbon bond formation at the position α to the nitro group. On the other hand, the easy conversion of the nitro functionality into carbonyl, amino and other functional groups has increased the synthetic potential of the nitro alkane derivatives in the synthesis of natural products. However, the use of these compounds has scarcely been reported because of the difficulty in their preparation.

Because of the unique structure, biological activities and interesting γ-keto α,β-unsaturated ester moiety, (R,R)-(−)-Pyrenophorin I have been the subject of extensive synthetic efforts, which have been culminated in several total synthesis of racemic pyrenophorin, natural pyrenophorin, and seco acid derivatives in recent years. A perusal of the literature revealed that most of the methods reported for synthesis of (−)-Pyrenophorin either require multistep process resulting in poor yields or employ costly chiral reagents. In continuation of our interest on the application of nitro aliphatics in the synthesis of natural products, we utilized readily available nitro alcohol (S)-5-nitro-2-pentanol (S)-49 as a bifunctional synthon to achieve a short essentially five steps enantioselective formal synthesis of the protected seco acid (+)-(S)-4,4-(ethylene dioxy)-7-hydroxyoct-2-enoic acid: the penultimate precursor to (R,R)-(−)-Pyrenophorin I involving a Michael addition and Nef protocol. Our synthetic scheme (scheme-VII) is shown below-

The crucial steps in our synthesis were-

i) control of the 'E' geometry of the newly formed α-enone by performing the Michael addition under the phase transfer catalytic condition (P.T.C) and

ii) Nef reaction of the nitro group in the γ-position of the adduct (51) in the presence of α-enone and ester functionalities under controlled pH using buffered titanium trichloride.

Chiral nitro alcohol (S)-5-nitro-2-pentanol (S)-49 was obtained with more than 70% yield by enantioselective reduction of the 5-nitro-2-pentanone (48) with baker's
Scheme-VII

i) Amberlyst A-21, 70%; ii) baker's yeast, glucose, 70%; iii) Ac₂O, pyridine, r.t.; 98%; iv) KF/ Bu₄NBr/ Methyl propiolate/ DMSO, 62%; v) 15% TiCl₃ solution, pH 5.3, 60%; vi) HO(CH₂)₂OH, p-TsOH, 95%; vii) KOH/MeOH, 70%.

yeast following the procedure reported by Guarna and co-workers. The spectral properties and the optical rotation value compares well with the reported ones.
5-Nitro-2-pentanone (48) was obtained in 70% yield by a Michael type coupling of nitromethane (46) and methyl vinyl ketone (47) using Amberlyst A-21 resin without any solvent.

The chiral (S)-5-nitro-2-pentanol, (S)-49 was then converted into its acetate by treating with acetic anhydride and pyridine at room temperature for over night in 98% yield. The compound (S)-50 showed sharp IR bands at 1750 and 1560 cm\(^{-1}\) indicating the presence of a carbonyl and a nitro group respectively in the molecule. In the \(^1\)HNMR spectrum, the doublet with \(J = 6.2\) Hz integrating to three protons at 1.25 ppm was assigned to the methyl group, a multiplate integrating to two protons at 1.6 ppm was assigned to be one methylene group; a multiplate integrating to two protons between 2.01-2.06 ppm was assigned to be one methylene group. A singlet integrating to three protons at 2.05 ppm was attributed to the acetate methyl group. The triplet integrating to two protons with \(J = 7.0\) Hz at 4.4 ppm indicating the protons under the \(-\text{NO}_2\) group (\(-\text{CH}_2\text{-NO}_2\) protons). The proton under acetate group showed a multiplate integrating to one proton at 4.9 ppm. The optical rotation of the compound was found to be \([\alpha]_D^{25} = +5.2\) (C 1.25, CHCl\(_3\)) \(\{\text{lit}^{7*} [\alpha]_D^{20} = +6.0, \text{CHCl}_3\}\)

Based on these data, the following structure has been assigned to this acetate derivative of (S)-4-acetoxy-1-nitro pentane (S)-50 as:

![Structure of (S)-50](image)

Our next aim was to prepare the required eight carbon atom skeleton by a Michael type coupling of (S)-4-acetoxy-1-nitro pentane (S)-50 and methyl propiolate using potassium fluoride as a base and phase transfer catalyst tetra butyl ammonium bromide under the nitrogen atmosphere. Michael adduct of the nitronate dianion generated by treating (S)-4-acetoxy-1-nitro pentane (S)-50 with variety of bases viz. Et\(_3\)N\(^{17}\), DBU\(^{18}\), DBN\(^{18}\), diisopropyl amine\(^{19}\), Amberlyst A-21 resin\(^{20}\), KF/basic alumina\(^{21}\), basic alumina(Al\(_2\)O\(_3\))\(^{22}\) led to the formation of an 'E:Z' mixtures of the newly formed double bond.
However, exclusive formation of the *trans* adduct\(^2\) (51) was achieved by treatment of (S)-4-acetoxy-1-nitro pentane (S)-50 (1 equiv.) with methyl propiolate (1 equiv.), anhydrous potassium fluoride (3 equiv.) and phase transfer catalyst tetra butyl ammonium bromide (1 equiv.) in dry DMSO (1 molar) under a nitrogen atmosphere in 62% yield as a gum. The use of tetra butyl ammonium bromide (P.T.C.) is essential for the success of the coupling process to yield exclusively *trans* adduct. Treatment of the nitronate dianion generated by treating compound (S)-50 with KF in DMSO with methyl propiolate led to the formation of an 'E,Z' mixture of the newly formed double bond.

The compound (51) showed strong IR bands at 1720 and 1550 cm\(^{-1}\) indicating the presence of a carbonyl and a nitro group respectively in the molecule. In the \(^1\)HNMR spectrum, the doublet with \(J = 6.2\) Hz integrating to three protons at 1.25 ppm indicating the presence of a methyl group. A multiplate integrating to four protons between 1.2-1.5 ppm was assigned to the two methylene group in the molecule. A singlet integrating to three protons at 2.0 ppm was attributed to the methylene group. A multiplate integrating to one proton at 3.7 ppm was assigned to the proton under nitro group (-CH-NO\(_2\)). A singlet integrating to three protons at 3.82 ppm was assigned to be presence of a methoxy group in the molecule. A multiplate integrating to one proton at 4.7 ppm indicated the presence of proton under the acetate group. A doublet with \(J = 16.0\) Hz integrating to one proton at 5.9 ppm was assigned to a vinylic proton. A double doublet with \(J = 16.0\) Hz and 2.0 Hz integrating to one proton at 7.1 ppm was indicating the presence of a *trans*-\(\alpha,\beta\)-unsaturated double bond in the molecule. From these data the structure of the Michael adduct (51) has been assigned as-

![Structure of 51](image)

The next crucial step in our approach was the conversion of nitro group in adduct (51) to a carbonyl group by Nef reaction. In this step we experienced tremendous difficulties as most of the strong acidic or basic conditions of the Nef procedures resulted
either hydrolysis of the acetate/ester functionality or reduction of the double bond or loss of materials. The different Nef procedures investigated by us for this purpose were NaOH/H₂SO₄, NaOH/HCl, basic silica gel (SiO₂-NaOMe; 0.5 equiv. NaOMe per 1 kg SiO₂), LiOMe/Na₂B₄O₇/KMnO₄, TiCl₃-H₂O/CH₃O(CH₂)₂OCH₃, TBDMS-Cl/DBU/MCPBA/CH₂Cl₂, (C₂H₅)₃N/ceric ammonium nitrate, CTMS/DBU/MCPBA, K₂CO₃/H₂O/MeOH, SnCl₂.2H₂O/THF, TiCl₃/THF (pH < 1), NaBH₄/MeOH/(NH₄)₂SO₄, KMnO₄ adsorbed on silica gel, sodium tert-butoxide/KMnO₄, Cu powder/ascorbic acid/HCl, Jacobson's procedure. However, none of these methods resulted in the desired Nef product.

We were able to successfully overcome this problem by using buffered 15% titanium trichloride and maintaining the pH at 5.3 using ammonium acetate whereupon compound (51) gave compound (S)-52 in 60% yield. To our knowledge, this is also the first report of converting the nitro group in γ-position of an α,β unsaturated system.

The compound (S)-52 showed IR bands at 1740 and 1720 cm⁻¹ respectively for carbonyl groups. The disappearance of the peak at 1550 cm⁻¹ indicated the absence of a -NO₂ group and appearance of a new peak at 1720 cm⁻¹ indicated the conversion of the nitro functionality into the corresponding carbonyl group. In the ¹H NMR spectra, the compound showed a doublet with J = 6.2 Hz integrating to three protons at 1.25 ppm indicating the presence of a methyl group. A multiplet at 1.82 ppm integrating to two protons indicated the presence of one methylene group. A singlet integrating to three protons at 2.02 ppm was attributed to the acetate methyl group. A triplet with J = 7.2 Hz integrating to three protons at 2.70 ppm was assigned to the proton adjacent to the carbonyl group. A singlet integrating to three protons at 3.82 ppm indicated the presence of a methoxy group. A multiplet integrating to one proton at 4.7 ppm was assigned to the proton under the acetate group. A doublet with J = 16.0 Hz integrating to one proton at 6.5 ppm and a double doublet with J = 16.0 Hz and 2.0 Hz integrating to one proton at 7.1 ppm indicated the presence of a trans-α-eneone system in the molecule. The optical rotation of the compound was found to be [α]₀²⁴ = +1.2 (C 1.0, CHCl₃) {lit²⁸ [α]₀²² = +1.6 (C = 0.8, CHCl₃)}.

Based on these data the structure of the compound (S)-52 has been assigned as:
The carbonyl group of the compound (S)-52 was protected as cyclic ethylene ketal by treatment with ethylene glycol and methyl orthoformate in the presence of BF₃·OEt₂ according to literature procedure, to furnished compound (S)-53 as a gum. In the IR spectrum disappearance of the peak at 1720 cm⁻¹ indicated that the carbonyl group has been protected with a dioxalane group. In the ¹H NMR spectrum, the methyl proton appeared as a doublet with J = 6.0 Hz at 1.25 ppm, the acetate group appeared as a singlet at 2.0 ppm integrating to three protons, the two methylene groups appeared as a multiplet between 1.5-1.8 ppm. The methoxy group appeared as a sharp singlet at 3.75 ppm integrating to three protons. The -O-CH₂-CH₂-O- protons appeared as a multiplet between 3.8-4.00 ppm. The proton under the acetate group appeared as a multiplet at 4.90 ppm. A doublet with J = 16.0 Hz integrating to one proton at 6.05 ppm indicated the presence of the vinylic proton. A doublet with J = 16.0 Hz integrating to one proton at 6.70 ppm indicated the presence of the trans-α-enone system in the molecule. The optical rotation of compound (S)-53 was found to be [α]D²⁵ = −4.9 (C = 1.05, CHCl₃) (lit.⁷₈ [α]D²² = −5.4 (C = 0.52, CHCl₃)

Based on these data, the structure of the compound (S)-53 has been assigned as:

After synthesizing compound (S)-53, the product was carefully saponified by alkaline hydrolysis to give hydroxy acid (S)-54 as a light yellow oil in 70% yield. In the
IR spectrum the compound (S)-54 showed a broad band at 3450 cm⁻¹ indicating the presence of a hydroxy group, and a sharp band at 1700 cm⁻¹ indicated the presence of a carbonyl group in the molecule. In the ¹H NMR spectrum, the compound showed a doublet with J = 6.0 Hz integrating to three protons at 1.20 ppm for a methyl group, a multiplet at 2.0 ppm indicated the presence of methylene group adjacent to hydroxy and methyl group. The ketal group integrating to four protons appeared as a multiplet at 3.95 ppm. A broad singlet integrating to two protons appeared at 5.20 ppm for protons adjacent to ketal group. A doublet with J = 16.0 Hz integrating to one proton at 6.05 ppm and a doublet with J = 16.0 Hz integrating to one proton at 6.80 ppm indicated the presence of the trans-α-enone system in the molecule. The optical rotation of the compound was found to be [α]²⁵ = +13.9 (C = 0.75, CHCl₃) {lit²⁸ [α]²⁵ⁿ = 14.8 (CHCl₃)}

Based on these data, the structure of the compound (S)-54 has been assigned for this hydroxy acid as-

![Structure of (S)-54](image)

**Synthesis of (R,R)-(−)-Pyrenophorin:**

Cyclodimerization of the protected seco acid (S)-54 to the protected Pyrenophorin with triphenyl phosphine/diethylazodicarboxylate (Mitsunobu method) according to Gerlach’s¹⁰ procedure and subsequent aqueous acid removal of the protecting ketal groups to (R,R)-(−)-Pyrenophorin has already been reported in the literature²⁷⁻²⁸.

![Structure of (R,R)-(−)-Pyrenophorin](image)
EXPERIMENTAL AND REFERENCES
EXPERIMENTAL

5-Nitro-2-pentanone (48):

Methyl vinyl ketone (11.5 ml, 164 mmol) was added dropwise to a stirred solution of nitromethane (10 ml, 164 mmol) and Amberlyst A-21 resin (10 g) at room temperature. The mixture was stirred for 8 hrs at room temperature. After completion of the reaction the mixture was filtered, resin was then washed with dichloromethane (3x20 ml) and solvent removed under reduced pressure (20 mm Hg). The crude material was purified by column chromatography on silica gel with Ethyl acetate: Hexane (1:3) as an eluent to afford 5-nitro-2-pentanone in 70% yield.

IR : v = 1720, 1553, 1372 cm⁻¹.

¹HNMR: δ = 2.10-2.20 (m, 2H); 2.14 (s, 3H); 2.58 (t, 2H, J = 6.8 Hz); 4.40 (t, 2H, J = 6.6 Hz) ppm.

(±)-5-nitro-2-pentanol (49):

5-Nitro-2-pentanone (10 g, 76.33 mmol) was reduced with NaBH₄ (2.85 g, 76.33 mmol) as usual and racemic 5-nitro-2-pentanol (9.4 g, 70.67 mmol) was obtained in 94% yield.

Yield = 9.4 g, 70.67 mmol, 94%.

b.p = 73°C/0.8 mm Hg.

IR : v = 3375, 1550, 1380 cm⁻¹.

¹HNMR: δ = 1.22 (d, 3H, J = 6.2 Hz); 1.45-1.57 (m, 3H); 2.03-2.22 (m, 2H); 3.81-3.90 (m, 1H); 4.42 (t, 3H, J = 8.0 Hz) ppm.

Analysis calculated for C₅H₉N₃O₃: C H N

45.10% 8.33% 10.52%

Found: 45.08% 8.48% 10.37%.
(S)-5-Nitro-2-pentanol [(S)-49]:

5-Nitro-2-pentanone (48) (133 mg) was added to a suspension of baker's yeast (10 g) and glucose (0.250 g) in 20 ml of water and the whole mixture was incubated at 30°C for 7 days. Usual workup gave a mixture of the starting material and the product, which were separated by preparative TLC with Hexane:Ethyl acetate (3:1) as solvent system to afford (S)-5-nitro-2-pentanol (S)-49 in 70% yield with 99% ee. The IR and $^1$HNMR spectra of the compound were found to be similar with that of racemic one (49).

$[\alpha]_D^{25} = +16.9$ (C = 1.70, CHCl$_3$) \{lit$^{14}$ $[\alpha]_D^{20} = +18.5$ (C = 0.92, CHCl$_3$); lit$^{15}$ $[\alpha]_D^{20} = +16.9$ (C = 1.70, CHCl$_3$); lit$^{16}$ $[\alpha]_D^{20} = +17.0$ (C = 2.0, CHCl$_3$)\}

(S)-4- Acetoxy-1-Nitro-pentane [(S)- 50]:

To a solution of (S)-5-nitro-2-pentanol (S)-49 (1.5g, 11.5 mmol) in acetic anhydride (2 ml) was added pyridine (0.5 ml) and the mixture was kept at room temperature for overnight. The reaction mixture was washed with brine and the product was extracted with chloroform (2×25 ml), dried over anhydrous Na$_2$SO$_4$ and evaporated. The crude product was purified by column chromatography on silica gel using Ethyl acetate:Hexane (1:10) as an eluent to afford (S)-5-nitro-2-pentanol acetate (S)- 50 as a pale yellow oil.

Yield = 1.9g, 11.0 mmol, 98%.

$[\alpha]_D^{25} = +5.2$ (C = 1.25, CHCl$_3$) \{lit$^7$ $[\alpha]_D^{22} = 6.0$ (CHCl$_3$)\}

IR : $\nu = 1750, 1560$ cm$^{-1}$.

$^1$HNMR : $\delta = 1.25$ (d, J = 6.2 Hz, 3H), 1.65 (m, 2H), 2.01-2.06 (m, 2H), 2.05 (s, 3H, -OAc); 4.5 (t, J = 7.0 Hz, 2H, -CH$_2$NO$_2$); 4.9 (m, 1H, -CHOAc) ppm.

Analysis calculated for C$_7$H$_{13}$NO$_4$:

<table>
<thead>
<tr>
<th></th>
<th>C</th>
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</tr>
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<tbody>
<tr>
<td>C</td>
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<td>7.48%</td>
<td>8.0%</td>
</tr>
<tr>
<td>H</td>
<td>47.25%</td>
<td>7.75%</td>
<td>7.8%</td>
</tr>
<tr>
<td>N</td>
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</table>
**Methyl-(S)-(E)-7-Acetoxy-4-nitro oct-2-enoate (51):**

(S)-4-Acetoxy-1-nitro pentane (S)-50 (1g, 5.71 mmol); KF (0.66, 11.43 mmol); tetra butyl ammonium bromide (1.84g, 5.71 mmol) and DMSO (2 ml) was stirred under nitrogen at room temperature for 30 minutes after which methyl propiolate (0.6g, 0.63 ml, 5.7 mmol) was added over 1h. The mixture was poured into water (100 ml) and extracted with EtOAc (3x25 ml) and the organic phase was dried over Na$_2$SO$_4$ (2 g). Evaporation of solvent under reduced pressure leave a yellow gum (1.5 g) which on chromatographic purification by preparative TLC (EtOAC:Hexane 1:3) gave compound (51) as an oil.

Yield : 0.91g, 3.35 mmol, 62%.

IR : $\nu = 1720, 1550$ cm$^{-1}$.

$^1$HNMR: $\delta = 1.25$ (d, $J = 6.2$ Hz, 3H, -CH$_3$); 1.2-2.5 (m, 4H, -CH$_2$-); 2.0 (s, 3H, OAc); 3.7 (m, 1H, -CHNO$_2$); 3.82 (s, 3H, -OCH$_3$); 4.7 (m, 1H, -CH-OAc); 5.9 (d, $J = 16.0$ Hz, 1H, -CH=CH-); 7.1 (dd, $J = 16.0 & 2.0$ Hz, 1H, -C=CH-) ppm.

Analysis calculated for C$_{11}$H$_{17}$NO$_5$ :

<table>
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<tbody>
<tr>
<td>C</td>
<td>50.96%</td>
<td>6.61%</td>
<td>5.40%</td>
</tr>
<tr>
<td>Found</td>
<td>51.2%</td>
<td>6.23%</td>
<td>4.98%</td>
</tr>
</tbody>
</table>

**Methyl-(S)-(E)-7-acetoxy-4-oxo oct-2-enoate [(S)- 52] :**

Ammonium acetate buffered TiCl$_3$ solution was prepared by mixing ammonium acetate (3.4g) in water (12 ml) and 15% TiCl$_3$ solution in HCl (10 ml). pH was adjusted at 5.3. To this solution methyl- (S)-(E)-7-acetoxy-4-nitro-oct-2-enoate (51) (0.34g, 1.31 mmol) in THF (5 ml) was added rapidly and the reaction mixture stirred at room temperature for 2 h. The progress of the reaction was monitored by TLC, after completion of the reaction, the reaction mixture was extracted with ethyl acetate (3x20 ml) and the organic layer was successively washed with water (20 ml), 5% aqueous sodium bicarbonate solution (20 ml) and finally with brine solution (20 ml). The organic layer was dried over anhydrous Na$_2$SO$_4$ and the solvent removed under reduced pressure.
The crude material was purified by preparative TLC (EtOAc:Hexane, 1:3) to yield pure methyl-(S)-(E)-7-acetoxy-4-oxo-2-oct-2-enoate (S)-52 as an oil.

Yield = 0.042g, 0.18 mmol, 60%.

\[\alpha\]_D^{24} = +1.2 (C = 1.0, CHCl_3) \{\text{lit} \, 7^g \[\alpha\]_D^{22} = +1.6 (C = 0.8, CHCl_3)\}.

IR : \nu = 1740, 1720 cm\(^{-1}\).

\(^1\)HNMR: \delta = 1.25 (d, J = 6.2 Hz, 3H, -CH_3); 1.89 (m, 2H); 2.02 (s, 3H, -OAc); 2.70 (t, J = 7.2 Hz, 2H); 3.82 (s, 3H, -OCH_3); 4.7 (m, 1H, -CHOAc); 6.5 (d, J = 16.0 Hz, 1H, -CH=C-); 7.1 (dd, J = 16.0 Hz \& 2.0 Hz, 1H, -C=CH-) ppm.

Analysis calculated for C₁₁H₁₈O₅: C 57.89%  H 7.07%

Found: 57.25% 7.37%.

Methyl-(S)-(E)-7-acetoxy-4,4-(ethylene dioxy)oct-2-enoate [(S)-53]:

A solution of (S)-52 (0.15g, 0.66 mmol) in dry benzene (20 ml) containing ethylene glycol (0.06 ml, 0.99 mmol) and triethylorthoformate (0.16 ml, 0.68 mmol) and two drops of BF₃·Et₂O was refluxed for 20 h. The cooled mixture was washed with saturated NaHCO₃ solution, extracted with ether, dried over anhydrous Na₂SO₄ and evaporated. The residue was purified by preparative TLC (EtOAc:PE, 1:10) to give compound (S)-53 as a colourless oil.

Yield = 0.17g, 95%

\[\alpha\]_D^{25} = -4.9 (C = 1.05, CHCl_3) \{\text{lit} \, 7^g \[\alpha\]_D^{22} = -5.4 (CHCl_3)\}.

IR : \nu = 1750, 1670 cm\(^{-1}\).

\(^1\)HNMR: \delta = 1.25 (d, J = 6.0 Hz, 3H); 1.5-1.8 (m, 4H); 2.0 (s, 3H, -OAc); 3.75 (s, 3H,-OCH₃); 3.8-4.00 (m, 4H); 4.90 (m, 1H); 6.05 (d, J = 16.0 Hz, 1H); 6.70 (d, J = 16.0 Hz, 1H) ppm.

Analysis calculated for C₁₃H₂₀O₆: C 57.34%  H 7.4%

Found: 56.95% 7.8%.
(S)-(E)-4,4-(ethylenedioxy)-7-hydroxy oct-2-enoic acid [(S)-54]:

A solution of (S)-53 (0.15g, 0.55 mmol) in methanol (20 ml) was allowed to stand at room temperature for 3.5 h with 2N KOH solution (1.74 ml). The reaction was cooled to -5°C and acidified with HCl and rapidly extracted with ethyl acetate (2×20 ml). The organic extracts were washed with brine, dried over anhydrous Na₂SO₄ and solvent removed under reduced pressure. The crude material was purified by preparative TLC (CHCl₃:acetic acid, 9.5:0.5) to afford the compound (S)-54 as a light yellow oil.

Yield = 0.09g, 78%

[α]D²⁵ = +13.9 (C = 0.75, CHCl₃) {lit 78 [α]Dn = +14.8 (CHCl₃)}

IR : ν = 3450, 1700 cm⁻¹.

¹HNMR: δ = 1.20 (d, J = 6.0 Hz); 1.5 (m, 2H); 2.0 (m, 2H); 3.95 (m, 4H); 5.20 (br s, 2H); 6.05 (d, J = 16.0 Hz, 1H); 6.80 (d, J = 16.0 Hz, 1H) ppm.

Analysis calculated for C₁₀H₁₆O₅:

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<tr>
<th></th>
<th>C</th>
<th>H</th>
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<tbody>
<tr>
<td></td>
<td>55.55%</td>
<td>7.46%</td>
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</tbody>
</table>

Found: 55.25% 7.51%.

* * * * * * * * * * * * *
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