SYNTHESIS OF SOME BIOACTIVE OXYGEN AND NITROGEN HETEROCYCLES

A THESIS
SUBMITTED TO THE
UNIVERSITY OF POONA
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
(IN CHEMISTRY)

BY
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OCTOBER 1993

CERTIFICATE

Certified that the work incorporated in the thesis entitled "**Synthesis of Some Bioactive Oxygen and Nitrogen Heterocycles**" by BEENA RAI was carried out by the candidate under my supervision. Such material as had been obtained from other sources has been duly acknowledged in the thesis.

V.H. Deshpande

(V.H. DESHPANDE)
RESEARCH GUIDE

OCTOBER, 1993

Acknowledgement

It gives me great pleasure in acknowledging my deep sense of gratitude and indebtedness to my revered guide **Dr. V.H. Deshpande**, Scientist E, Division of Organic Chemistry: Technology, National Chemical Laboratory, Pune for his inspiring guidance, keen interest and commitment to my work and helpful hints throughout my thesis work.

It is my previlege to acknowledge Dr (Mrs) R.D. Wakharkar for her valuable suggestions and discussions during the course of some part of my thesis work.

I am very thankful to Dr. T. Ravindranathan, Head, Division of Organic Chemistry : Technology for his helpful suggestions and constant encouragement.

My sincere thanks to my esteemed colleagues Mrs. Latha Sivadasan, Mrs. K. Balakrishnan, Dr. R.A. Khan and Ms. Neelam Gokhale for giving me all possible encouragement and needed help. It is my pleasure to thank Dr. (Mrs) B. Chanda, Dr. V.B. Patil, Dr. A.R. Mehendale, Dr. R.J. Lahoti, Dr. S.P. Chavan and Dr. H.B. Borate for the useful hints and help rendered by them during my tenure of research.

I wish to record my appreciation and gratefulness to all my colleagues particularly to Dr. R.A. Kasar, Dr. (Ms) P.K. Zubaidha, Ms. Chitra Govande, Ms. Tripura SubbaRao, Ms. Shubhada Dantale and Mr. K. Ethiraj from whom I received help in various ways during the investigation of work presented in this thesis.

I am very much thankful to IR, NMR, Mass and Microanalytical group of NCL. My special thanks are due to Mrs. P.P. Kulkarni for her excellent technical help in preparation of this manuscript.

I take this opportunity to acknowledge my indebtedness to my husband Mr. Kulbhushan Rai for his moral support, constant encouragement and sacrifice. I am also thankful to all other family members.

Financial assistance from CSIR, NEW DELHI in the form of research fellowship is gratefully acknowledged.

Finally, I wish to thank Director, National Chemical Laboratory, Pune for his permission to submit this research work in the form of a Ph.D. thesis.

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GENERAL REMARKS

1. All the temperatures are in °C. All the melting points and boiling points are in °C and are uncorrected.
2. ^{1}H-NMR spectra were recorded either on FT-80A or Brucker WH-90 or WH-200 FT spectrometer in $CDCl_3$ solution containing TMS as an internal standard with chemical shift (δ) expressed in ppm downfield from TMS.

   The following abbreviations are used : s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad.
3. Infra-red spectra (ν_{max} in cm^{-1}) were recorded as either thin film or nujol mull on Perkin-Elmer Infra-red 683-B spectrometer with sodium chloride optics.
4. Mass spectra were recorded on a CES-21-110B double focussing mass spectrometer operating at 70eV using direct inlet system.
5. All solvents and reagents were purified and dried by standard procedures. All evaporations were carried out under reduced pressure on Buchi rotary evaporator.
6. TLC was carried out on silica gel plates prepared by spreading the slurry (in CCl_4) and drying at room temperature.
7. Microanalyses were carried out in the microanalytical section of NCL.
8. GLS was carried out on Hewlett Packard 5890.
9. Column chromatography was performed on silica gel (60-120 mesh).
10. The list of references pertaining to a chapter/part are given at the end of that chapter/part.

ABBREVIATIONS

Ac	:	Acetyl
Ar	:	Aryl
b.p.	:	Boiling point
n-Bu	:	n-Butyl
t-Bu	:	t-Butyl
Bz	:	Benzyl
$CDCl_3$	:	Deuterated chloroform
CH_2Cl_2	:	Dichloromethane
DMAP	:	4-N,N-Dimethylaminopyridine
DMS	:	Dimethyl sulphate
DMSO	:	Dimethyl sulphoxide
EDC	:	Ethylene dichloride
h	:	Hour/s
Et	:	Ethyl
g	:	Gram/s
IR	:	Infra-red
LAH	:	Lithium aluminium hydride
LDA	:	Lithium diisopropyl amide
Me	:	Methyl
min	:	Minute/s
MOM	:	Methoxymethyl
m.p.	:	Melting point
MS	:	Mass spectrum
nm	:	Nanometer

NMR	:	Nuclear Magnetic Resonance
PCC	:	Pyridinium chloro chromate
Ph	:	Phenyl
PTSA	:	p-Toluenesulphonic acid
THF	:	Tetrahydropyran
TFA	:	Trifluoroacetic acid
TFAA	:	Trifluoroacetic anhydride
uv	:	Ultra-violet

I N D E X

The thesis entitled "Synthesis of Some Bioactive Oxygen and Nitrogen Heterocycles" in divided into four chapters.

Chapter I - Synthesis of (+) Orthosporin, Semivioxanthin, Achliso coumarin III and Saintopin.

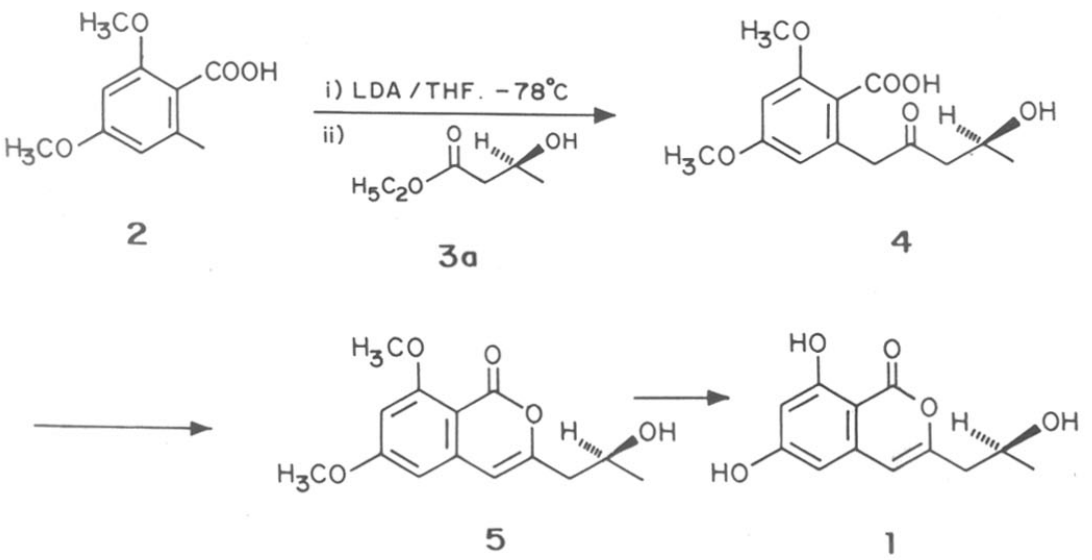
This chapter deals with the synthesis of (+) orthosporin, semivioxanthin, achlisocoumarin III and saintopin. For convenience, it has been divided into four parts:

Part 1: Stereospecific synthesis of (+) Orthosporin :

S-(+)-Orthosporin (**1**), (S)-3-(2-hydroxypropyl)-6,8-dihydroxy isocoumarin, is a phytotoxic metabolite, isolated by Ichihara et.al. from the culture filtrate of Rhynchosporium orthosporum. The structure and the absolute configuration of **1** was determined by its synthesis and resolution which involved eleven steps.

In the present work, the synthesis of (±)-orthosporin dimethyl ether was first established in two steps starting from orsellinic acid dimethyl ether (**2**) and (±)-ethyl 3-hydroxybutyrate (**3**) and then the same methodology was applied to achieve (+)-orthosporin by using (S)-ethyl 3-hydroxybutyrate (**3a**). (**Scheme 1**)

SCHEME 1

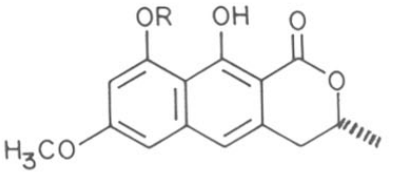


2 3a 4 5 1

The anion of **2**, generated by LDA in THF at -78°C, was treated with **3a** to obtain keto acid **4**, which on cyclization (PTSA-benzene) gave (+)-orthosporin dimethyl ether **5**. As the conversion of **5** to **1** is known, by demethylation with $AlCl_3$, this constitutes the total synthesis of (+)-orthosporin.

Part 2: Synthesis of Semivioxanthin methyl ether:

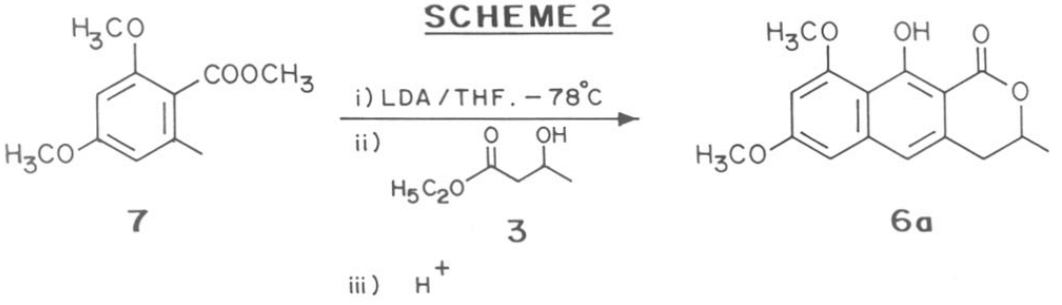
Semivioxanthin (**6**), an antifungal natural product isolated from Penicillium Cetreo-viride by Zeeck et al, was shown to be 3,4-dihydro-9,10-dihydroxy-7-methoxy-1-oxo-1H-naphtho [2,3c] pyran. Although **6** was isolated in 1979, its first racemic synthesis was reported recently in 1990 by Yamaguchi et al via a polyketide approach in eleven steps.



6 : R = H **6a** : R = CH_3

Synthesis of (±)-semivioxanthin methyl ether (**6a**) was undertaken first in order to establish the methodology, to be applied later, in the asymmetric synthesis of semivioxanthin methyl ether. A novel and elegant method has been develoepd for the synthesis of semivioxanthin methyl ether. (**Schme 2**)

SCHEME 2

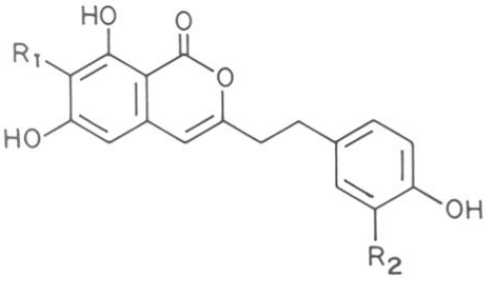


The anion of methyl orsellinate (**7**), generated with LDA in THF at -78°C, on treatment with ethyl 3-hydroxybutyrate (**3**) followed by acidic workup gave the (±)-semivioxanthin methyl ether (**6a**) in moderate yields. When the same condensation was

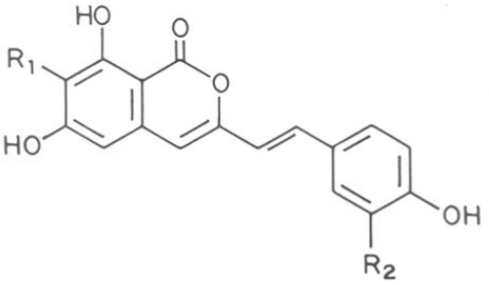
carried out using (S)-ethyl 3-hydroxybutyrate (**3a**), unnatural isomer of **6a** was obtained.

Part 3: Synthesis of Achlisocoumarin III tetramethyl ether:

A group of Japanese workers isolated three phenolic compounds (**8-10**), with a novel isocoumarin skeleton, from the underground parts of Achlys triphylla (Berberidaceae) which are being evaluated for their medicinal properties.



(I)



(II) and (III)

8 ACHLISOCOUMARIN I — R_1 = GERANYL , R_2 = H

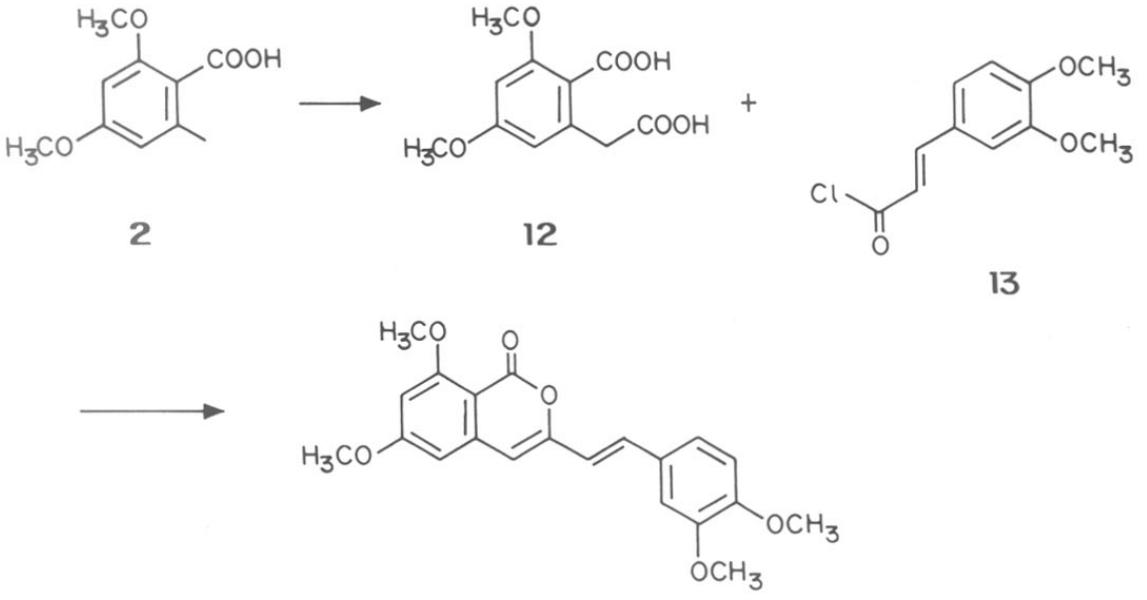
9 ACHLISOCOUMARIN II — R_1 = GERANYL R_2 = H

10 ACHLISOCOUMARIN III — R_1 = H R_2 = OH

The synthesis of achlisocoumarins have not been reported so far. In the present work, a simple method for the synthesis of achlisocoumarin III tetramethyl ether (**11**) has been developed as shown in **scheme 3**.

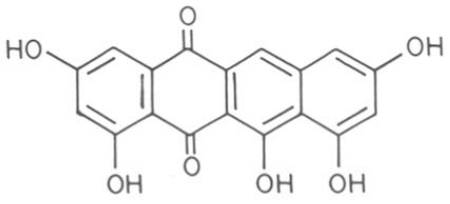
Homophthalic acid derivative **12**, prepared from **2** by known procedure, was treated with 3,4-dimethoxy cinnamoyl chloride (**13**) at 190°C to give achlisocoumarin III, tetramethyl ether **11** in good yield.

SCHEME 3



Part 4 : Synthesis of Saintopin:

Saintopin (**14**), a new antitumor antibiotic with topoisomerase II dependent DNA cleavage activity was isolated recently from the culture broth of Paecilomyces species by Japanese workers. It has got lot of potential of being developed as an antitumor drug.



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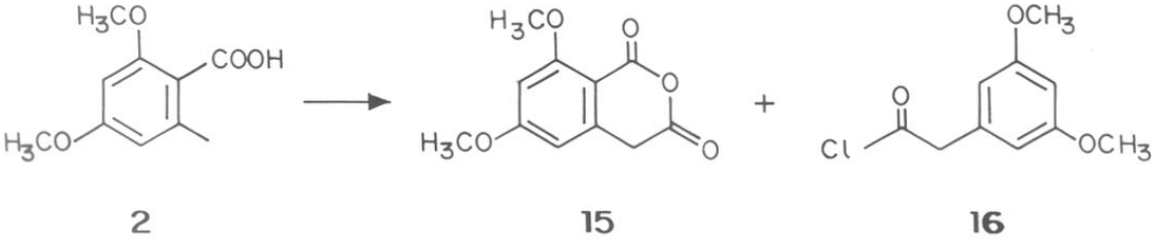
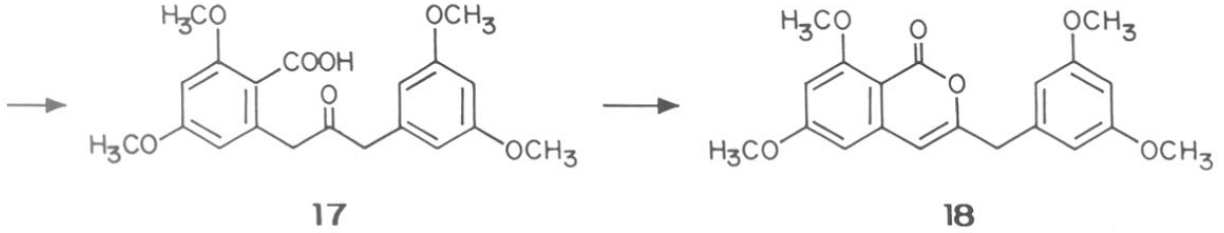
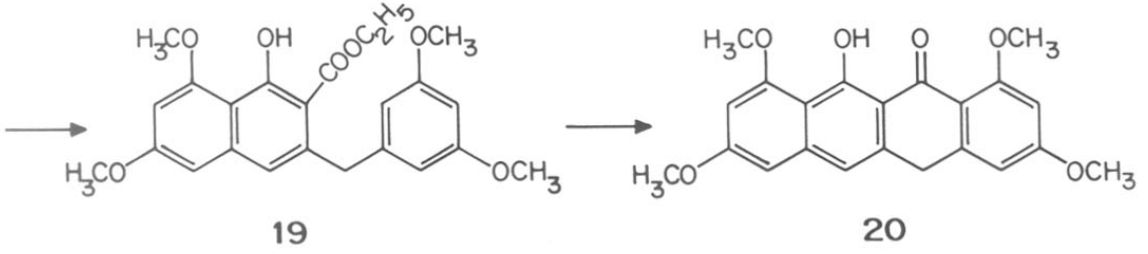
The scarcity of saintopin from natural source coupled with its interesting biological activity prompted us to attempt the total synthesis of saintopin as shown in **scheme 4.**

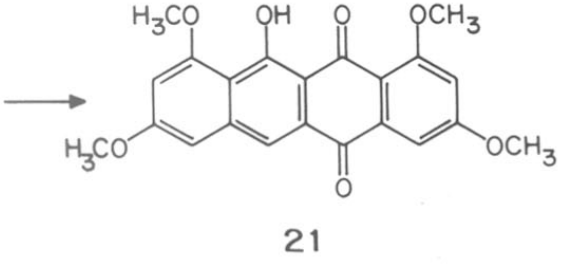
Condensation of homophthalic anhydride **15** with 3,5-dimethoxyphenyl acetyl chloride (**16**) in presence of N,N-dimethylaniline at 95°C gave the corresponding 2-carboxydibenzyl ketone **17**. It was then cyclized in 95% H_2SO_4 to give 3-(3,5-dimethoxy benzyl) 6,8-dimethoxy isocoumarin (**18**). The anion of ethyl acetate, generated by LDA in THF at -78°C was treated with **18** to give naphthalene carboxylic acid derivative **19**. It was then cyclized and oxidized to give saintopin tetramethyl ether **21**.

SCHEME 4







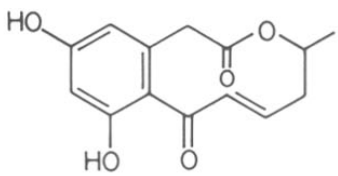


Chapter II - Stereospecific synthesis of bioactive M5032 and 12-oxocurvularin as their dimethyl ethers:

This chapter deals with the synthesis of optically active M5032 and 12-oxocurvularin as their dimethyl ethers, which has been divided into Part 1 and Part 2 respectively.

Part 1 : Stereospecific synthesis of M5032 dimethyl ether:

Bioactive substance M5032 (**22**), a new macrocyclic lactone possessing enzyme hinderance activity capacity for circular adenosyn 3',5'-monophosphoric acid phosphodiester (CAMP-PDE), is produced by microorganisms belonging to Sporomyler group.



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CAMP-PDE inhibiting property of M5032 is expected to be useful in the cure of blood vessel damage disease as curing agent, as an agent in respiratory disease cure, as a muscular pain releiving agent, as an anticancer agent etc.

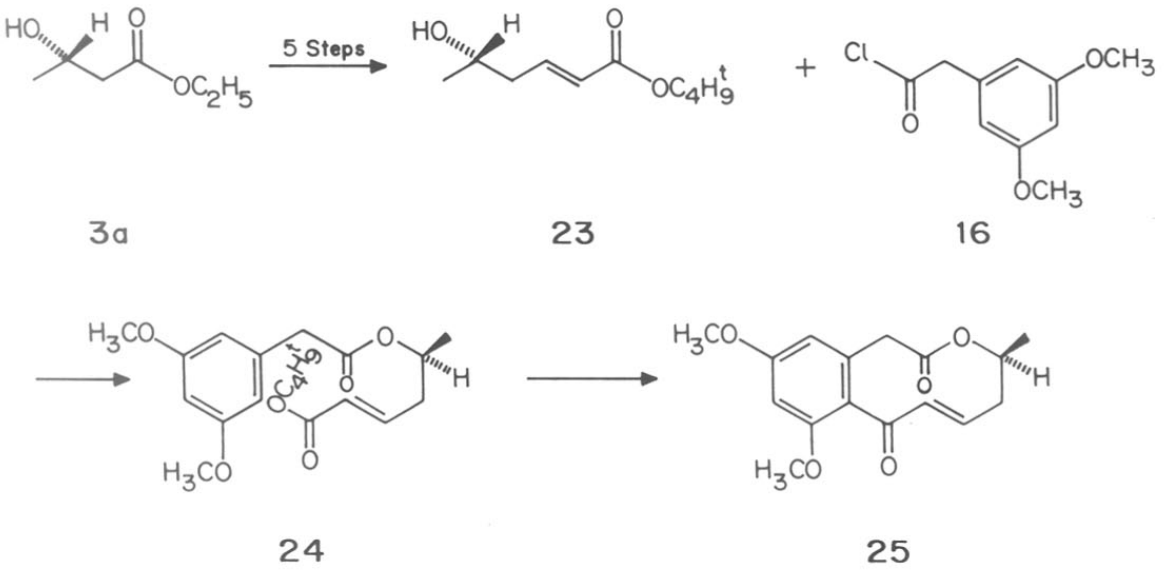
As the M5032 has got potential medicinal value and its synthesis has not been reported, the present work deals with its first stereospecific synthesis.

A reterosynthetic analysis of M5032 indicated that esterification of 3,5-dimethoxyphenylacetyl chloride (**16**) with (S)-t-butyl-5-hydroxy-2(E)-hexenoate (**23**) followed by intramolecular acylation and demethylation would give M5032 (**Scheme 5**).

The desired alcohol **23** was prepared from (s)-ethyl 3-hydroxybutyrate (**3a**) in five steps. 3,5-Dimethoxyphenylacetyl chloride (**16**) was esterified with alcohol **23** in

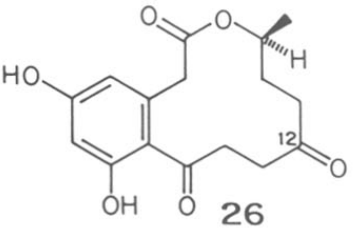
presence of pyridine and the resultant ester **24** on intramolecular acylation with a mixture of TFA-TFAA gave M5032 dimethyl ether (**25**) in moderate yield.

SCHEME-5



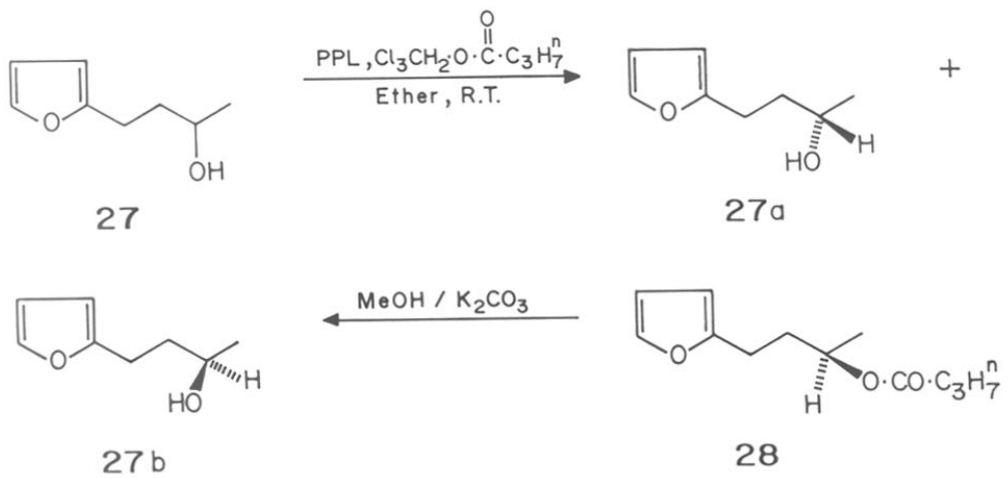
Part 2 : Stereospecific synsthesis of 12-oxocurvularin dimethyl ether:

12-Oxocurvularin (**26**) is a curvularin type metabolite isolated by Yamamura et al from the mycelium of the hybrid strain ME 0005 derived from Penicillium citreo-viride-B. It has been shown to have attractive physiological properties. It also possesses an unnatural oxygen function at C_{12} position in its structure.



The first synthesis of (±) di-O-methyl-12-oxocurvularin was reported in our laboratory and the similar strategy has been employed to obtain the natural stereoisomer of 12-oxocurvularin dimethyl ether (**31**). 4-(2-Furyl)-butan-2-ol (**27**), prepared from furfural in two steps, was enzymatically resolved by using Porcein Pancreatic lipase (PPL). (**Scheme 6**)

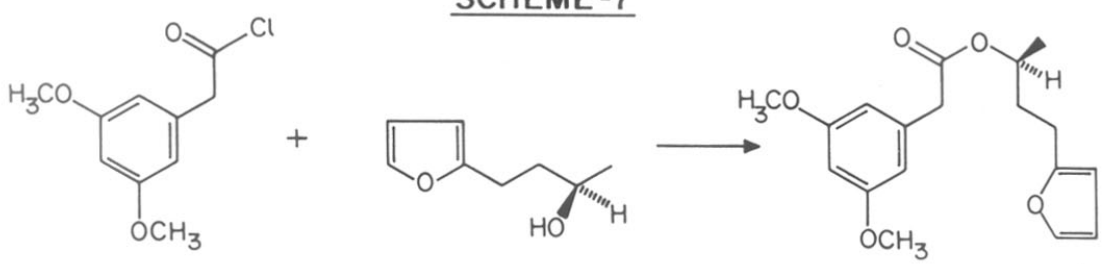
SCHEME-6



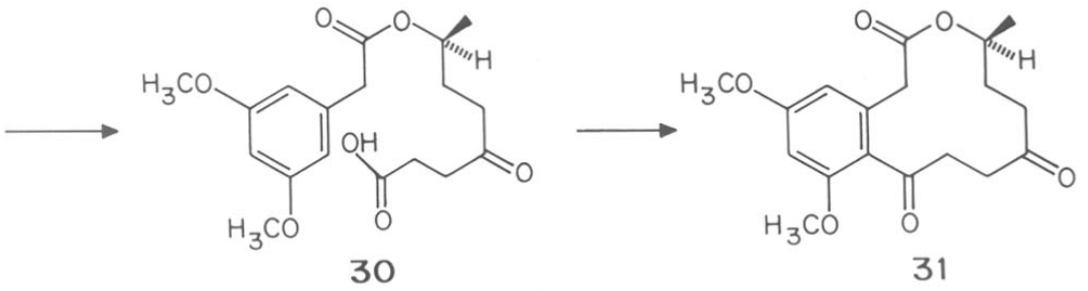
27 27a 27b 28

The desired alcohol **27b** was esterified with 3,5-dimethoxy-phenylacetyl chloride (**16**) to give **29**, which on Jones oxidation (**30**) followed by intramolecular acylation gave the optically active 12-oxocurvularin dimethyl ether (**31**) as shown in **scheme 7**.

SCHEME-7



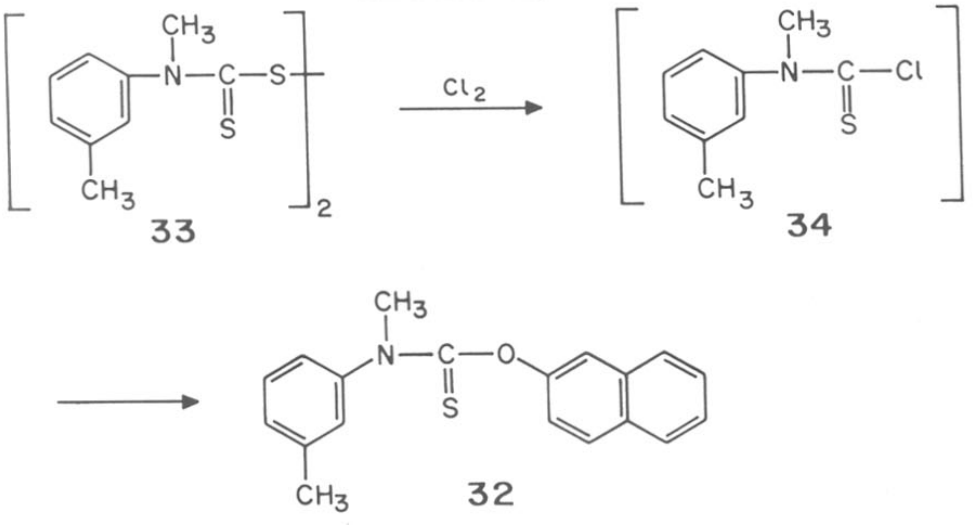
16 27b 29



Chapter III - An alternative method for Tolnaftate:

This chapter describes an alternative method for an antifungal drug tolnaftate (**32**). Tolnaftate, [2-naphthyl-N-methyl-N-(m-tolyl) thionocarbamate] a very effective antifungal drug has been known for more than a decade. It is a two step condensation product of N-methyl-m-toluidine, thiophosgene and ß-naphthol. In the present work, an alternative method which avoids the use of very toxic thiophosgene has been developed. (**Scheme 8**).

SCHEME-8



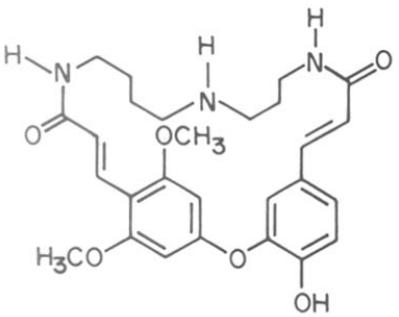
N-Methyl-m-toluidine on treatment with carbon disulphide in presence of iodine and pyridine gave dimethyl-di-(m-tolyl)-thiuram disulphide (**33**). It was then converted into N-methyl-N-(m-tolyl)-thiocarbamoyl chloride with requisite amount of chlorine absorbed in carbon tetrachloride at 5-10°C. The thiocarbamoyl chloride (**34**) was condensed with ß-naphthol in refluxing benzene in presence of powdered KOH and PTC to give tolnaftate (**32**) in good yield.

Chapter IV - Synthetic approaches towards Capparisinine and Cervinomycin:

This chapter is divided into two parts. The first part describes the synthetic approaches towards the total synthesis of capparisinine while the attempted synthesis of phenanthrene derivative, a key intermediate in the total syntheis of cervinomycin is presented in the second part.

Part 1 : Synthetic approaches towards Capparisinine:

Capparisinine (**35**) is a new spermidine alkaloid isolated from the root bark of Capparis decidua. The plant is used in oriental medicine as laxative, antidote to poison, in the treatment of cardiac troubles, toothache and inflammation. Its fairly complex macrolactam structure involves synthetic challanges to organic chemists. A regiospecific synthesis of capparisinine was undertaken in the present work.



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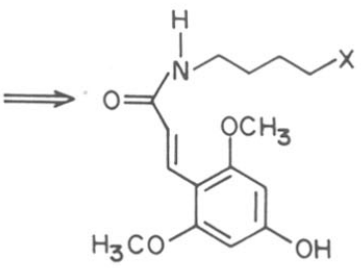
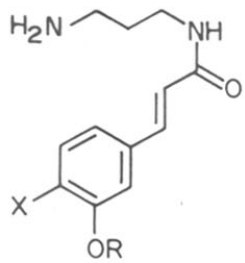
SCHEME 9



X = HALOGEN

36

+



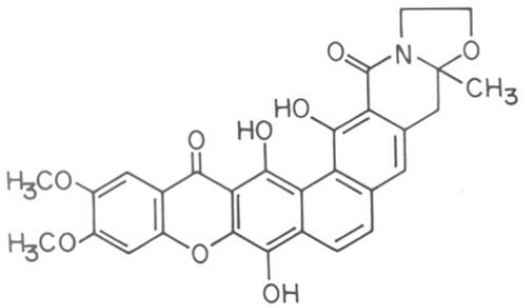
37

A reterosynthetic analysis of **35** suggested that the two fragment **36** and **37** could be coupled to give the target molecule (**Scheme 9**). The attempted synthesis of these fragments and their coupling reactions are discussed in this part.

Part 2 : Synthetic approaches towards Cervinomycin:

Cervinomycins, novel antibiotics isolated by Omura et·al·from Streptomyces cervinus sp., consists of two components A_1 (**38**) and A_2 (**39**). Triacetyl derivative of **38** has high solubility and is being developed as drug because of its low toxicity and antianaerobic activity against several bacteria.

SCHEME 10



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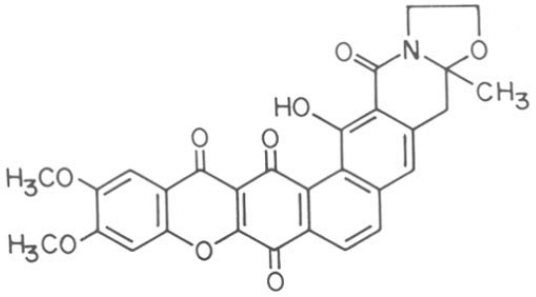

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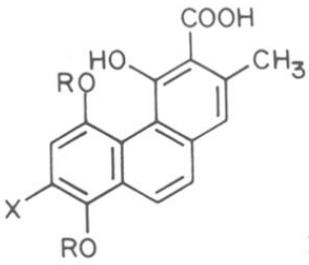


40

X = HALOGEN OR METHOXY

R = CH_3 OR H

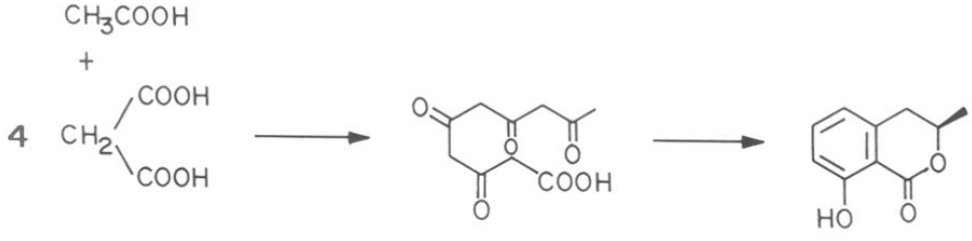
It has been considered on the basis of reterosynthetic analysis that a suitably substituted phenanthrene derivative **40** is the key intermediate which could be elaborated for the total synthesis of cervinomycin (**Scheme 10**). A number of approaches have been attempted to synthesise the key intermediate **40** which are described in this part.

CHAPTER I

SYNTHESIS OF (+) ORTHOSPORIN, SEMIVIOXANTHIN, ACHLISOCOUMARIN III AND SAINTOPIN

General Introduction

Isocoumarins, an important class of natural products have been isolated from a wide variety of microbial, plant and insect sources[1, 2]. Some isocoumarins have shown to possess an impressive array of biological activities. Biogenetically most of the natural isocoumarins are derived from acetate via the acetate-polymalonate pathway e.g. mellein (**1**) is derived from acetate and malonate.

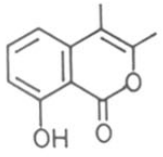


1

Biological activity

Isocoumarins display a very wide range of biological activity. Many of them exhibit antifungal activity particularly oosponolactone (**2**), cladosporin (**3**) and 6-methoxymellein (**4**). The activity probably arises as a result of competition between different fungal species, although as in other isocoumarins these antifungal agents also possess phytotoxic activity. Several 6,8-dihydroxyisocoumarins and 6,8-dihydroxy-3,4-dihydroisocoumarins are metabolites of phytopathogenic fungi e.g. sclerin (**5**) and sclerotinin A (**6**) and B (**7**) are metabolites of Sclerotinia sclerotiorum possessing plant growth regulatory activity. Hydroxydihydroisocoumarin **8** causes necrotic lesions on

the leaves of pear trees and inhibits the growth of rice seedlings.



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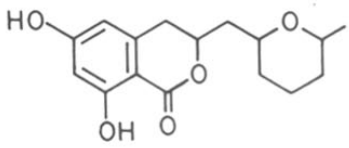

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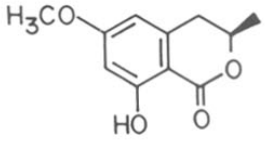

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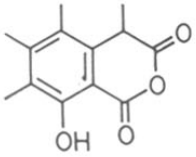

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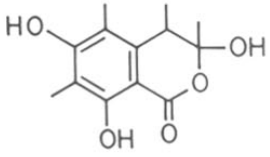

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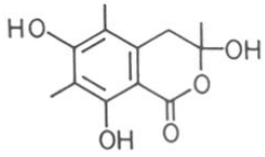

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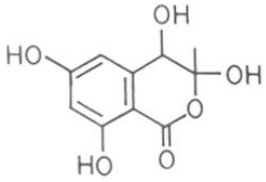

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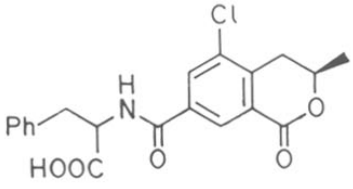

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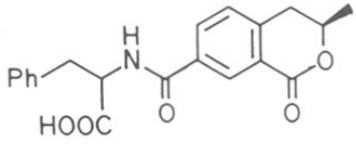

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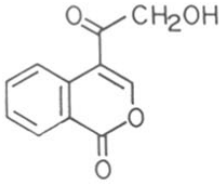

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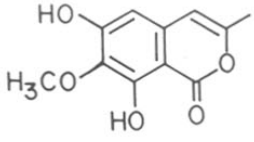

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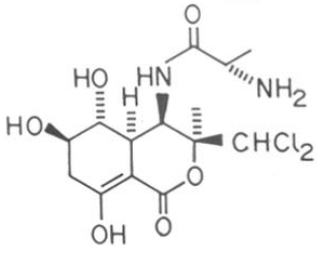


13

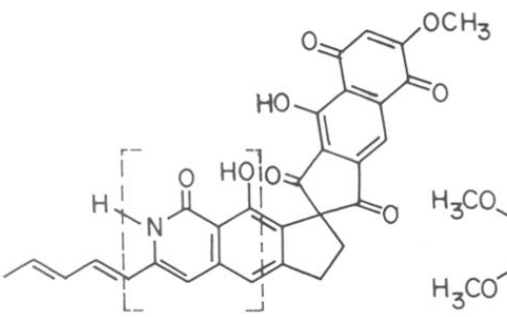
It was shown by Nakajima et al. that isocoumarins with a hydroxyl group at 8 position i.e., the position permitting chelation with carbonyl moiety are more or less antifungal.[3] Further elaboration of structure-activity relationship by the same group of workers[4] revealed that the 3,4-dihydro-isocoumarins are more active than the corresponding isocoumarins. In case of the isocoumarins bearing 8-hydroxyl group, inclusion of additional hydroxyl or methoxyl group at position 4' enhances the activity whereas reverse is true when no 8-hydroxyl group is present.

Some of these isocoumarins have been found in insects e.g. mellein (**1**) has been shown to be present in the defensive secretion of termites and Australian onerine ants, in the mandibular gland secretion of carpenter ants and also in the hair pencils of the oriental fruit moth.

Some isocoumarins are toxic to man e.g. ochratoxin A (**9**) and ochratoxin B (**10**) are nephrotoxic and hepatotoxic metabolites isolated from several Asperigillus and Penicillium species. Oosponol (**11**) inhibits dopamine ß hydroxylase and causes severe skin rashes, bronchitis and pneumonia. Reticulal (**12**) inhibits cyclic AMPase.

However, some isocoumarins are beneficial to human beings too, e.g. bactobolin A (**13**) and its analogues are antileukemic, antiviral and antibacterial. Actinobolin and baciphelacin also possess antibiotic activity. Some of them are also diuretic, antihypertensive and purgative.

Besides their attractive biological activities isocoumarins can be considered as appropriate intermediates for many complex natural products possessing isoquinolone moiety, as the isocoumarins can be easily converted to isoquinolones by treatment of aqueous ammonia.[5] Fredricamycin A[6] (**14**) and cervinomycin (**15**)[7] are the novel antibiotics possessing such isoquinolone moiety.



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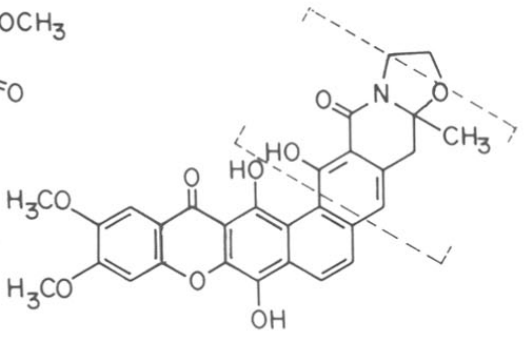


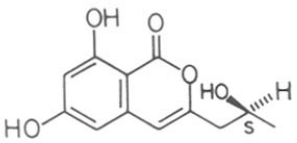
15

This chapter describes the synthesis of three naturally occurring biologically active isocoumarins namely (+) orthosporin, (-)-semivioxanthin, achlisocoumarin III and a tetracyclic anthraquinone derivative saintopin based on isocoumarin chemistry, as their methyl ethers. For convenience it has been divided into four parts.

PART 1: STEREOSPECIFIC SYNTHESIS OF (+) ORTHOSPORIN

Introduction

Orthosporin (**16**), a new isocoumarin metabolite was isolated from culture filtrate of Rhynchosporum orthosporum by Ichihara et·al[8]. Orthosporin exhibited phytotoxic activity by inhibiting the root growth of lettuce (great lake 366) by 63.2% at 250 ppm and of the host plant (Okamidori) by 50.3% at 25 ppm. Subsequently another group of scientists isolated a phytotoxin from Drechslera siccans and named it as de-O-methyldiaporthin[9] because of its structural correlation with diaporthin (**17**) isolated from Endothia parasitica[10]. However, it should be noted that orthosporin (**16**) and de-O-methyldiaporthin are structurally identical.



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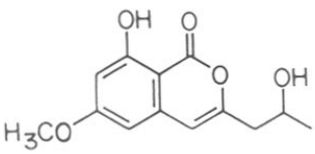


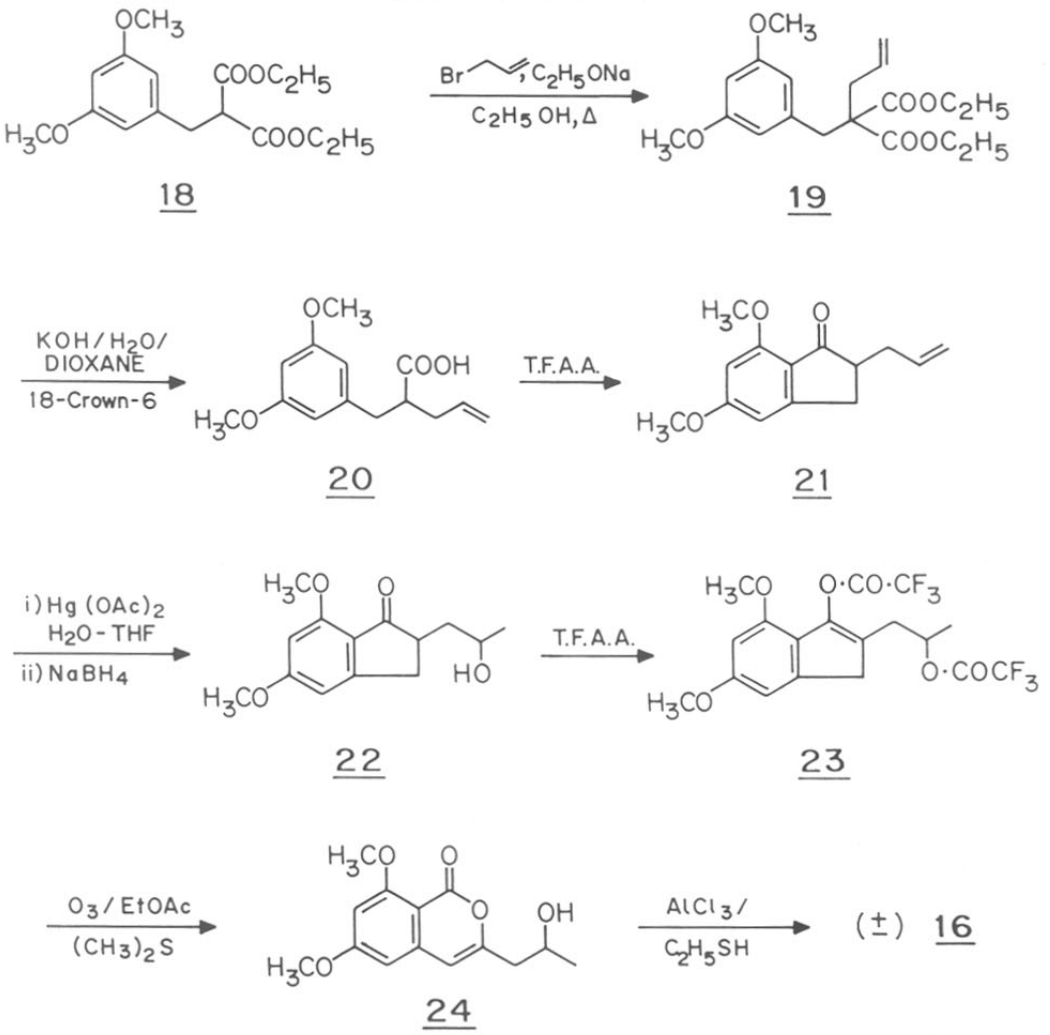
17

Structure elucidation and synthesis of (+)-orthosporin

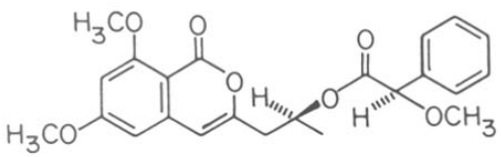
Based on the spectroscopic evidences the planar structure of (+)-orthosporin was shown to be 3-(2-hydroxypropyl)-6,8-dihydroxyisocoumarin. However its absolute configuration was established by synthesis and resolution of racemic intermediates. Thus orthosporin dimethyl ether **24** was synthesized in six steps starting from diester **18**. The alkylation of **18** with allyl bromide in presence of sodium ethoxide gave **19** which on subsequent hydrolysis and decarboxylation furnished the acid **20**. Cyclization of the acid **20** with trifluoroacetic anhydride resulted into indanone **21**. The oxymercuration and subsequent demercuration with sodium borohydride of the indanone **21**

yielded an alcohol **22**. The trifluoroacetate **23** of the alcohol **22** upon ozonolysis and decomposition of ozonide afforded (±) orthosporin dimethyl ether **24**. The removal of the protecting groups in **24** with aluminium chloride and ethanethiol furnished (±)-orthosporin (**16**) (**Scheme 1.1.1**).

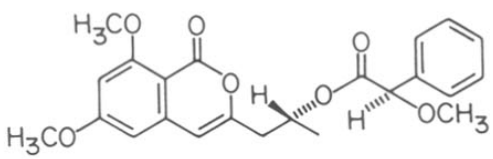
SCHEME - 1·1·1



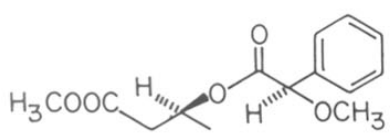
A diastereoisomeric mixture of esters **25a** and **25b**, prepared by esterification of (±) **24** with R-2-methoxy-2-phenylacetylchloride, was separated by PTLC and hydrolyzed separately to furnish both (+) and (-) isomers of **24**. The demethylation of both the isomers provided (+) and (-) orthosporin respectively. Further, to establish the absolute configuration of the natural orthosporin, **25a** was degraded and esterified to give ester **26**. The ^{1}H NMR data of **26** was in fair agreement with known (S)-**26** and hence absolute configuration of (+) orthosporin was shown to be (S).



25a



25b



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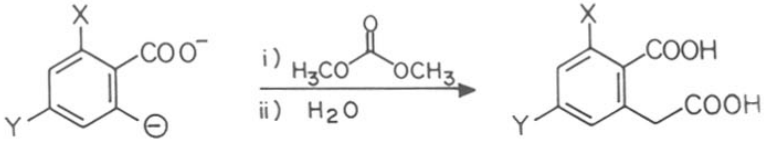
Present work

It is evident from above discussion that the only known synthesis of orthosporin involved several steps including resolution. It was felt that a simple, short and stereospecific synthesis of (+) orthosporin could be obtained and with this objective, its synthesis was undertaken and the same is reported in the present work.

It has been shown by Hauser and Rhee[11], as well as by Staunton et. al.[12] that dianions **27a** and **27b** generated from the corresponding acids by using less nucleophilic base such as lithium diisopropylamide are stable at low temperature and can be reacted with electrophiles such as dimethylcarbonate to give **29** (**Scheme 1.1.2**)

SCHEME-1·1·2



27a X=OCH_3, Y=H

27b X=Y=OCH_3

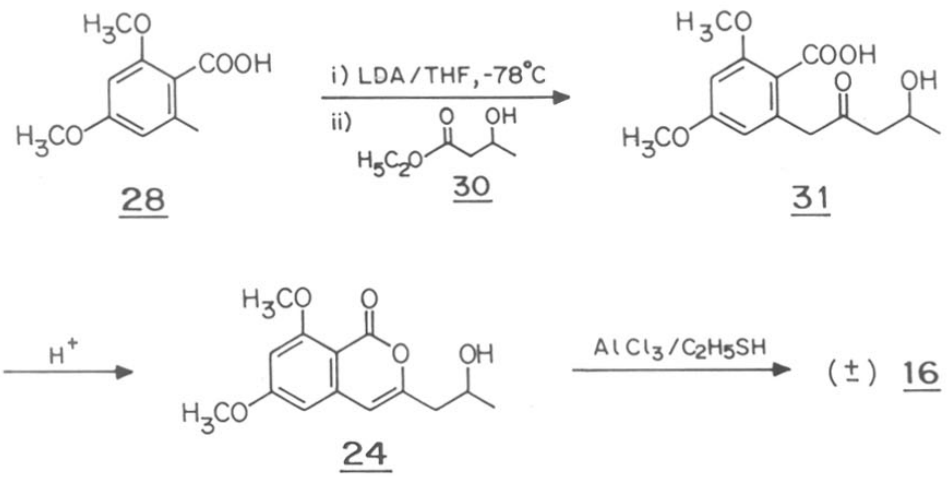
29

Based on the above observation it was considered that **28** could be the logical synthon for the synthesis of orthosporin which would displace the ethoxy group of ethyl-3-hydroxybutyrate (**30**) to furnish desired keto acid **31**. Cyclodehydration of the keto acid **31** followed by demethylation would give the target molecule (**Scheme 1.1.3**).

Thus, to test the validity of the methodology, to be applied later for stereospecific synthesis, synthesis of (±) orthosporin was first undertaken. The required orsellinic acid (**28**) was prepared from orcinol (**32**) in three steps with an overall yield of 61.5% (**Scheme 1.1.4**) Orcinol (**32**) was methylated with dimethyl sulphate and potassium carbonate in boiling acetone to give dimethyl ether **33**, which on Vilsmeier-Haack

reaction[13] furnished aldehyde **34**. Oxidation of the aldehyde **34** with aqueous potassium permanganate yielded orsellenic acid **28**, which showed identical physical and spectral properties as reported in the literature[14].

SCHEME-1·1·3



The desired electrophile ethyl-3-hydroxybutyrate (**30**) was prepared from ethyl acetoacetate by reduction with sodium borohydride in 70% yield (**Scheme 1.1.4**). The spectral and physical properties of compound **30** were consistent with those reported in the literature.[15]

In accordance with the scheme 1.1.3 next aim was to couple **28** and **30** to give keto acid **31**. The anion of **28**, generated at -78°C in THF with lithium diisopropyl amide, on treatment with ethyl-3-hydroxybutyrate (**30**) at the same temperature afforded keto acid **31** in 50% yield. The assigned structure for **31** was in good agreement with its spectral data. The IR spectrum of **31** revealed absorption bands at 3416, 1680 and 1606 cm^{-1}. The ^{1}H-NMR spectrum (**FIG. I**) showed methyl doublet at δ 1.18, a multiplet at δ 2.50 for the aliphatic methylene protons and two singlets at δ 3.81 and 3.93

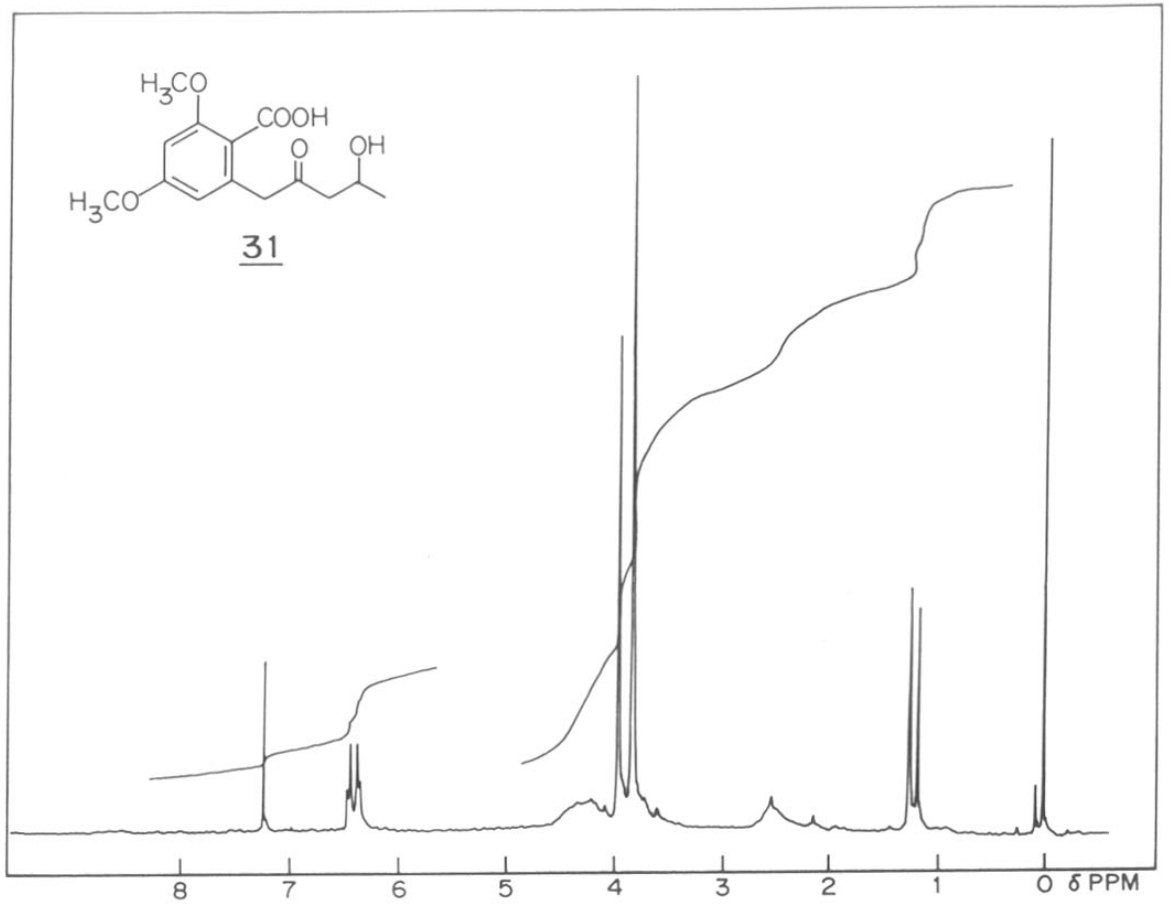
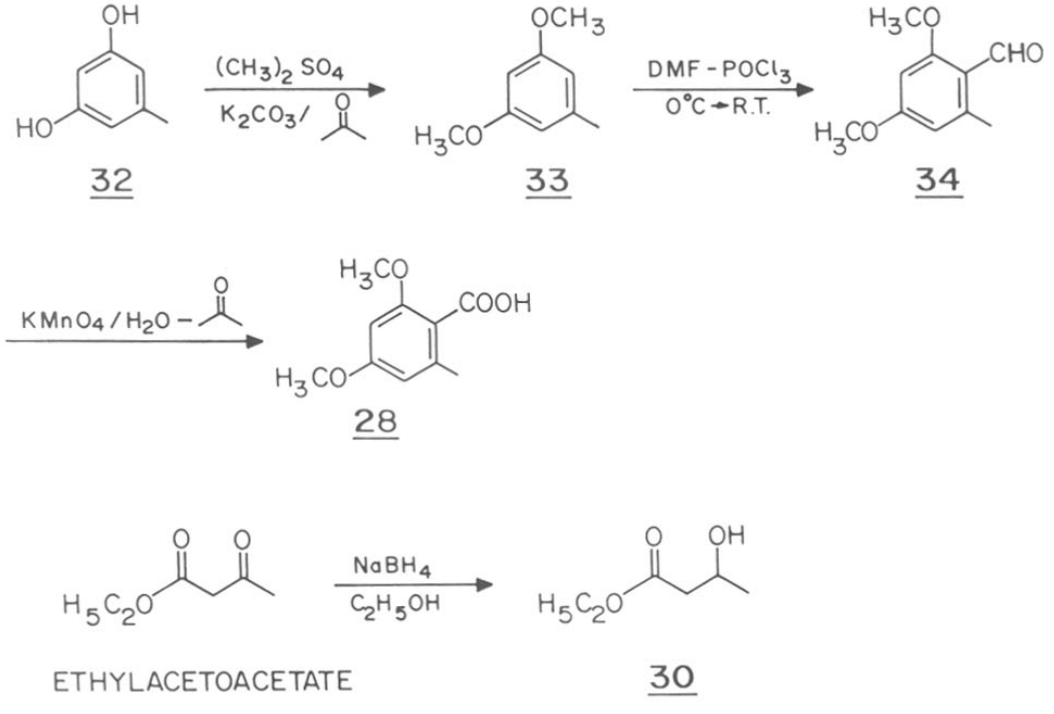


FIG. I: ^{1}H-NMR SPECTRUM OF COMPOUND (**31**) IN $CDCl_3$

integrating for benzylic methylene and two methoxyls. A multiplet between δ 4.06 - 4.56 corresponding to the single proton of -C(H)-OH was observed. Two aromatic protons appeared as two metacoupled doublets at δ 6.31 and 6.43. In the mass spectrum of **31** molecular ion peak was observed at m/e 282.

SCHEME-1·1·4



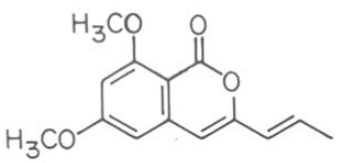
32 33 34

28

30

After obtaining the crucial intermediate **31** only task remaining was the cyclodehydration of **31** to give orthosporin di-O-methyl ether (**24**). A brisk survey of literature revealed that concentrated sulphuric acid is the common reagent used for such cyclodehydrations[16]. Hence, **31** was treated with 95% H_2SO_4 at room temperature,

however the desired product **24** could not be isolated instead another compound **35** was obtained, which was characterized on the basis of its spectral properties. The IR spectrum showed absorption peak at 1780 cm^{-1}. In the ^{1}H-NMR (**FIG.II**) methyl doublet appeared at δ 1.95 and two methoxy singlets at δ 3.92 and 3.99 were observed. The aliphatic olefinic protons appeared at δ 6.05 and 6.67 as doublet and multiplet respectively. The cyclic olefinic proton appeared as singlet at δ 6.12 and two aromatic protons appeared as metacoupled doublets at δ 6.36 and 6.45. The appearance of molecular ion peak at m/e 246, further confirmed the structure.



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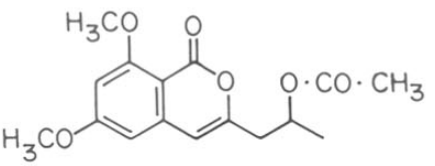

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Since the cyclodehydration of **31** with sulphuric acid posed problems besides several changes in reaction conditions, it was desirable to look for the alternative reagent. It has been reported that cyclodehydration could be achieved with perchloric acid in acetic anhydride[17]. Thus, **31** was subjected to cyclodehydration in dry ethyl acetate containing catalytic amount of perchloric acid and acetic anhydride, however **35** was obtained as the major product along with a minor product **36**, which was found to be acetate ester of orthosporin dimethyl ether (**24**). The identity of **36** was supported by its spectral properties. In the IR spectrum absorption bands at 1750 and 1680 cm^{-1} were observed. The ^{1}H-NMR (**FIG.III**) exhibited methyl doublet at δ 1.36 and two

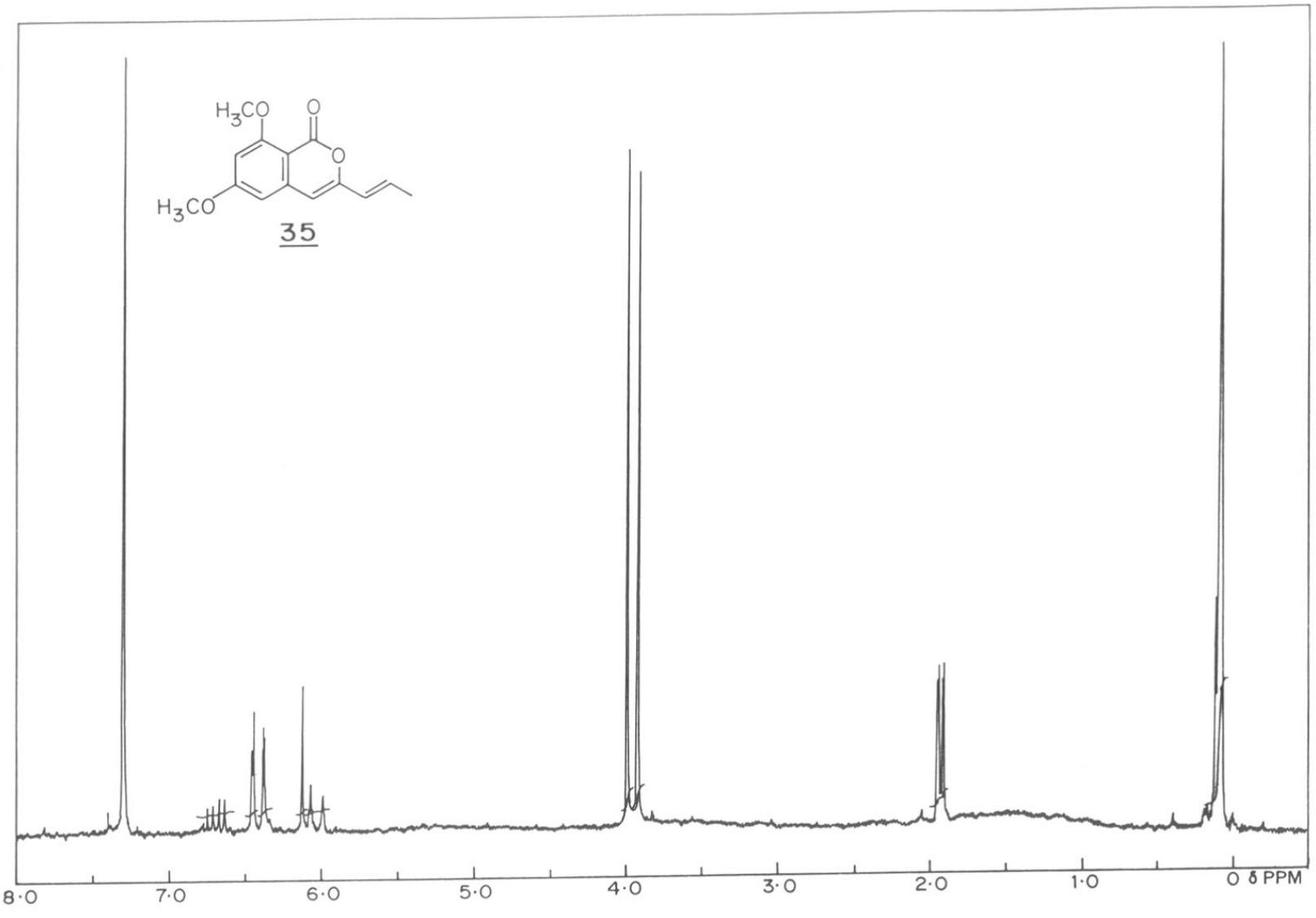


FIG. II : ^{1}H-NMR SPECTRUM OF COMPOUND (35) IN $CDCl_3$

double doublets at δ 2.68 and δ 2.81 corresponding to the two methylene protons. The acetyl protons appeared as singlet at δ 2.06. As usual two methoxy singlets were observed at δ 3.93 and 4.02. A multiplet between δ 5.22 - 5.40 clearly indicated the acetate ester formation. The singlet at δ 6.12 and two meta coupled doublets at δ 6.37 and 6.49 confirmed the isocoumarin structure. In the mass spectrum m/e at 306 was observed.

Although the cyclodehydration of the keto acid **31** with sulphuric acid had taken place, it had further dehydrated to **35**. While the reaction of **31** with perchloric acid and acetic anhydride also indicated that cyclodehydration had occured by the formation of **35** and **36**. These experiments indicated that a milder reaction condition and/or a milder reagent is desirable to achieve the selective cyclodehydration of **31**. Since p-toluenesulphonic acid is a very good dehydrating agent it seemed logical to employ the same for the conversion of **31** to **24**. Therefore, the keto acid **31** was subjected to cyclodehydration in refluxing benzene with catalytic amount of p-toluenesulphonic acid to afford **24** in 75.7% yield. The spectral (**^{1}H- NMR -FIG. IV**) and physical properties of **24** were in good agreement with those reported in literature[8].

After successful employment of the methodology for the synthesis of (±) orthosporin, attention was diverted towards its stereospecific synthesis. The desired optically active isomer of **30** was shown to be (S)-ethyl-3-hydroxybutyrate (**30a**) which can be easily obtained by the enzymatic reduction of ethyl acetoacetate using Baker's Yeast.[18]

Though the Baker's Yeast reduction of ethyl acetoacetate is a well known reaction, the yields and optical purity of the product varies according to the reaction conditions employed[19]. A number of different reaction conditions were tried to obtain **30a** with maximum optical purity and it was observed that by using 1% ethanol in fermenting glucose solution of Baker's Yeast and keeping the concentration of ethyl acetoace-

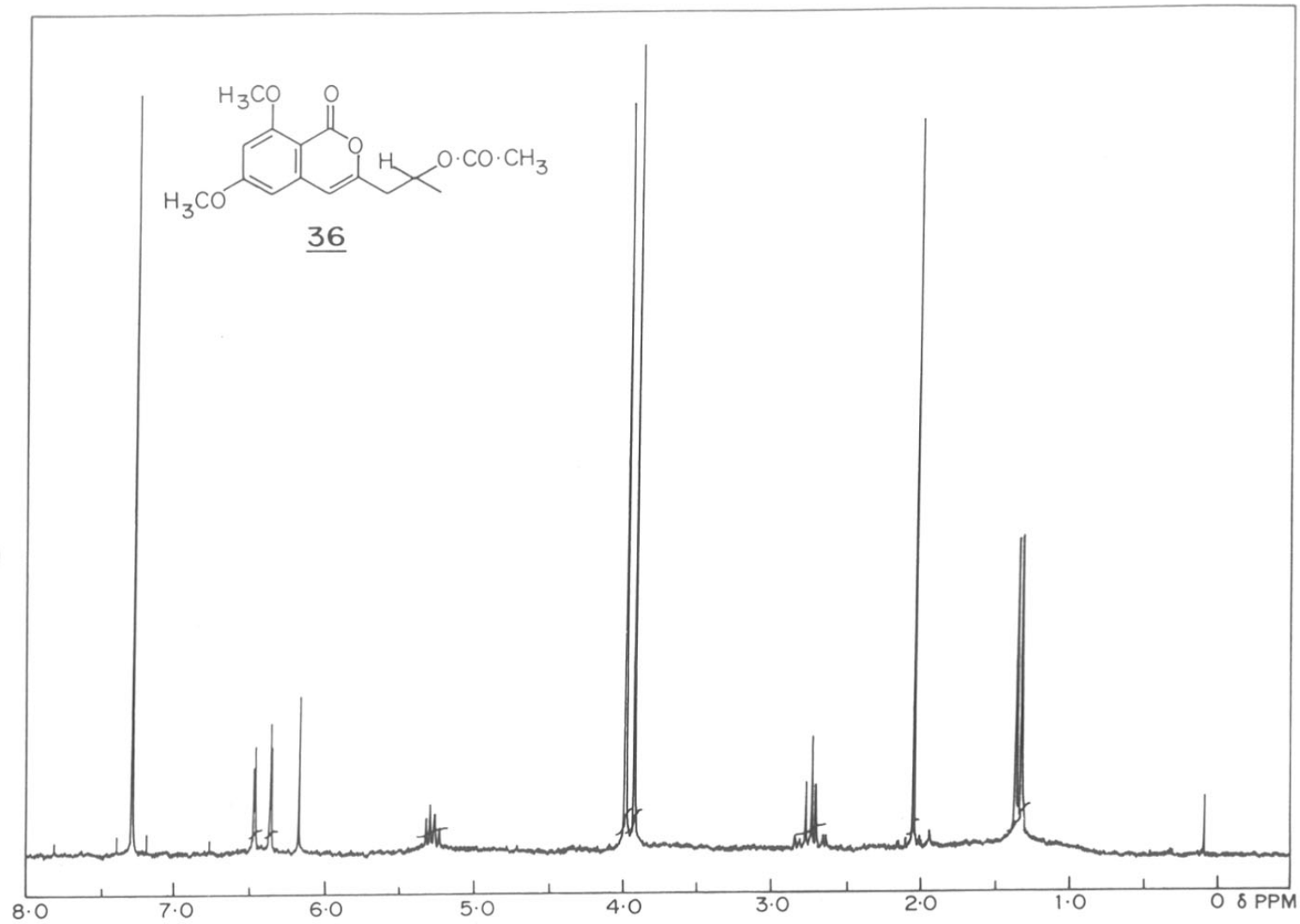


FIG. III : ^{1}H-NMR SPECTRUM OF COMPOUND (**36**) IN $CDCl_3$

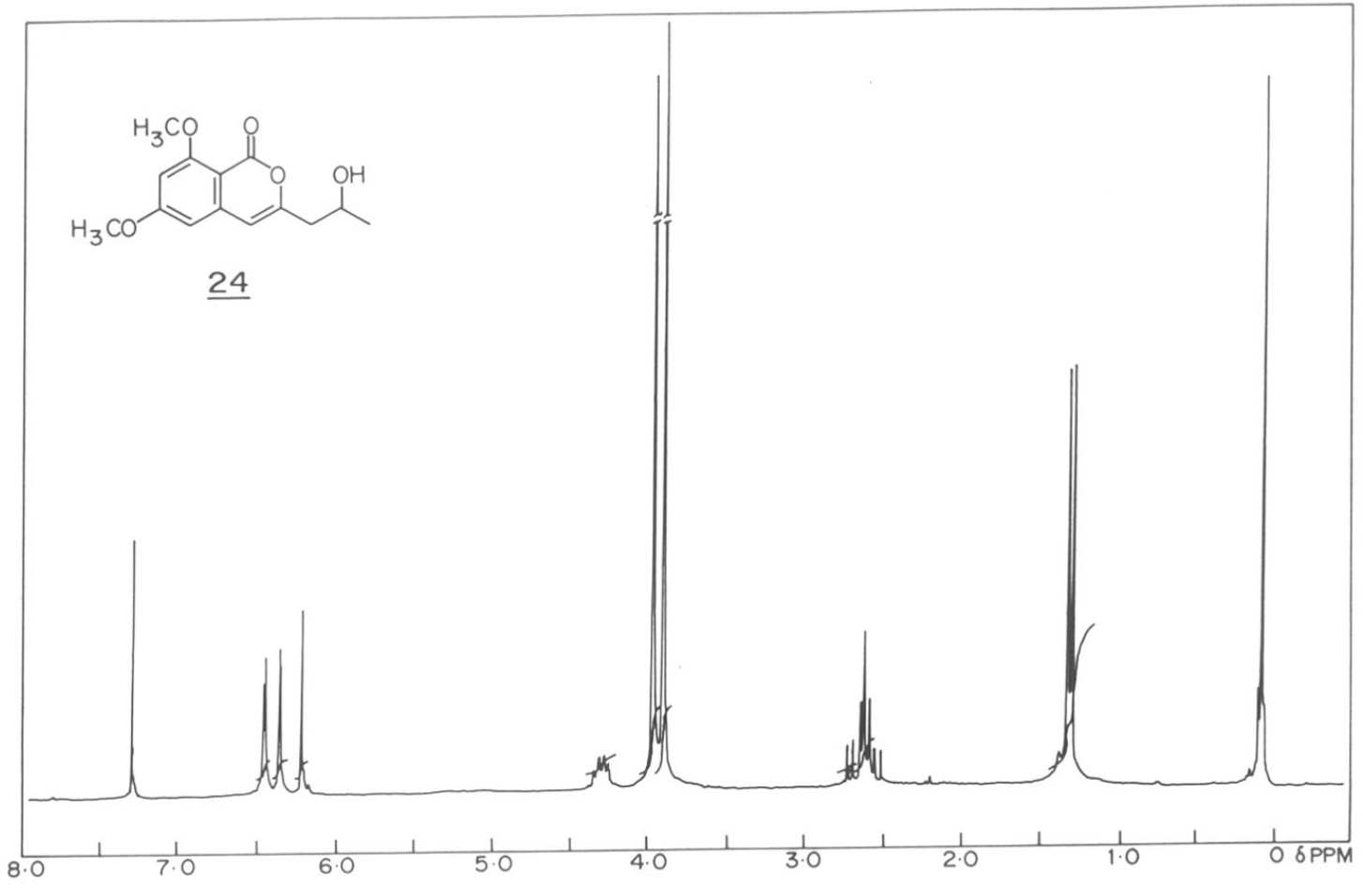
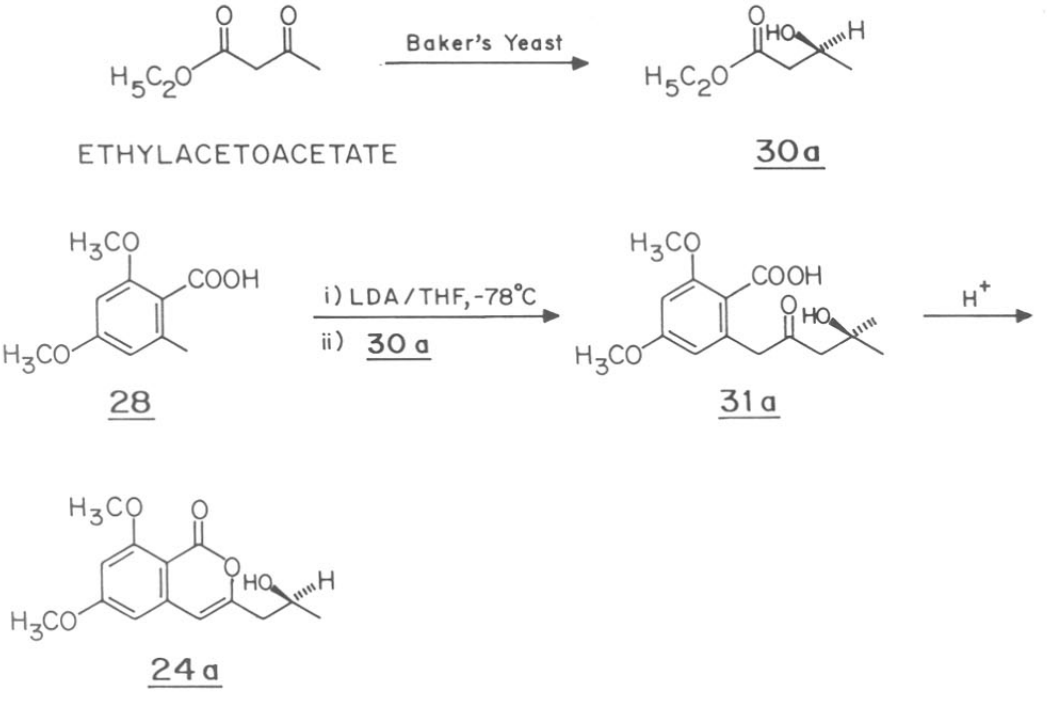


FIG. IV: ^{1}H-NMR SPECTRUM OF THE COMPOUND (24) IN $CDCl_3$

tate about 0.01 gm/ml, yielded **30a** in 95% optical purity with 60% chemical conversion (**Scheme 1.1.5**).

SCHEME-1·1·5



The chiral reactant **30a** thus obtained was condensed with anion **27b**, generated by treatment of acid **28** with LDA in THF at -78°C, to furnish optically active intermediate keto acid **31a** (53%) which exhibited optical rotation of $[\alpha]_D^{25}$ + 11° (C=2, $CHCl_3$). Cyclodehydration of **31a** with PTSA in benzene resulted into (S)-(+)-orthos-

porin di-O-methyl ether **24a** in 79.5% yield (**Scheme 1.1.5**). The synthetic (+) **24a** showed optical rotation of $[\alpha]_D^{25}$ +34.88° (C = 2.5, $CHCl_3$) (Lit.[6] - $[\alpha]_D^{25}$ + 22.4° (C = 2.7, $CHCl_3$). As the demethylation of **24a** by aluminium chloride in ethanethiol has already been reported, this constitutes a formal total synthesis of **16**.

After achieving the synthesis of (+)-orthosoprin, yet another approach was attempted for its synthesis. It was considered that condensation of methyl orsellinate and ethyl 3-hydroxybutyrate under identical conditions, followed by acidic work up would directly give orthosporin skeleton. Thus an anion of methyl orsellinate generated by LDA was treated with ethyl 3-hydroxybutyrate. However, after acidic work-up, it was found that the orthosporin methylether had not formed and the major product isolated was found to be a derivative of another natural product, semivioxanthin. Its chemistry and synthesis is described in Part 2 of this chapter.

In conclusion, a short, efficient and stereospecific synthesis of S-(+)-orthosporin has been achieved.

Experimental

3,5-Dimethoxytoluene (33) : A mixture of 3,5-dihydroxy-toluene (Orcinol) (**32**) (10 g, 80.64 mmol), dimethyl sulphate (15.28 g, 193.5 mmol) and anhydrous potassium carbonate (18.5 g, 201.6 mmol) in dry acetone (100 ml) was refluxed for 16 h. After distilling off the acetone, water (200 ml) was introduced into the residue and the reaction mixture was kept overnight to decompose excess dimethyl sulphate. The product was extracted with ethyl acetate (2 x 100 ml), the combined extracts were dried (Na_2SO_4) and evaporated to leave an oil which on distillation under reduced pressure yielded pure dimethyl ether **33** (12.08 gms, 98.6%). b.p. 92-95°C/4 mm Hg (Lit[20], b.p. 67.5-68.5°C/0.2 mm Hg).

2,4-Dimethoxy-6-methylbenzaldehyde (34) : 3,5-Dimethoxy-toluene **33** (7 g, 46 mmol) was added to the complex prepared from $POCl_3$ (14.1 g, 92 mmol) and DMF (6.7 g, 92 mmol) at 0°C. Then the mixture was warmed slowly to the room temperature and stirred for 5 h. The reaction mixture was again cooled to 0°C and decomposed with 20% aqueous sodium hydroxide solution (100 ml). A colourless solid thus separated was filtered, washed and dried. Recrystallization from pet ether (b.p. 60°-80°C) yielded the aldehyde **34** (8 gms, 96%), as a colourless crystalline solid. m.p. 182°C (Lit.[21] m.p. 182°C).

2,4-dimethoxy-6-methylbenzoic acid (28): To a stirred solution of 2,4-dimethoxy-6-methylbenzaldehyde (**34**) (5 g, 27.8 mmol) in acetone (30 ml) was added an aqueous solution of $KMnO_4$ (10 g in 150 ml of water) till the $KMnO_4$ colour persisted. The reaction mixture was stirred for 2 h at room temperature. The brown precipitate of MnO_2 formed thereafter was filtered through celite pad and the residue was washed with water. The combined filtrate was distilled under reduced pressure to remove acetone and then cooled, acidified to pH 2 with dilute HCl to give a colourless solid. The solid thus obtained was filtered, washed with cold water and air dried to afford

acid **28** as a colourless solid (3.5 gms, 65%); mp 147°C (Lit.[14], m.p. 147°C).

Ethyl-3-hydroxybutyrate (30): Ethyl acetoacetate (6.5 gms, 50 mmol) in dry ethanol (10 ml) was added to the suspension of sodium borohydride (1.9 gms, 50 mole) in dry ethanol (50 ml) at 0°C during 30 min. After the addition of ester, the stirring was continued at the same temperature for another 3 hr. Then it was diluted with methanol (150 ml) and acidified with 10% methanolic hydrogenchloride. Methylborate formed thereafter was distilled at atmospheric pressure and the suspension was cooled and neutralized with sodium hydrogen carbonate. The solid obtained was filtered and the filtrate was extracted with chloroform (2 x 100 ml). The combined extracts were dried (Na_2SO_4) and concentrated. The residue was distilled under reduced pressure to give ethyl-3-hydroxybutyrate (**30**) (4.29 gms, 65%), b.p. 90-92°C at 20 mm (lit.[15] b.p. 70-71°C/12 mm).

(S)-Ethyl-3-hydroxybutyrate (30a): Baker's Yeast (2.8 gms, Blue Bird make) was introduced in distilled water (50 ml) and then ethanol (0.58 ml, 200 mmol) and glucose (1.8 gms, 200 mmol) were added and the contents were shaken at 25-30°C for 3 hr. Then ethyl acetoacetate (0.5 gms, 77 mmol) was introduced and the shaking was continued for 48 hrs. The reaction mixture was filtered through celite pad and the filtrate was extracted with ethyl acetate (3 x 50 ml). The combined extracts were washed with brine, dried over Na_2SO_4 and concentrated. The oily residue was distilled between 65-67°C at 10 mm (lit. 70-71°C at 12 mm) to afford (S)-ethyl-3-hydroxybutyrate (**30a**) (0.3 gms, 60%) with 95% optical purity $[\alpha]_D^{25}$ = + 41.1° (chloroform, C=2); {lit[18] $[\alpha]_D^{25}$ = + 43.5° (chloroform C = 1.0}.

Condensation of 30 or 30a with orsellenic acid (28): A solution of LDA was prepared by reacting n-BuLi in hexane (1.6 M, 6.18 ml, 9.9 mmol) with dry diisopropylamine (0.99 gm, 9.9 mmol) in dry THF (10 ml) at 0°C under argon atmosphere. It was then cooled to -78°C and 2,4-dimethoxy-6-methylbenzoic acid (**28**) (0.588 gm, 3

mmol) dissolved in THF (5 ml) was added during 20 min. The stirring was continued at the same temperature for 2 more hr. and then a solution of **30** or **30a** (0.504 gms, 3.5 mmol) in THF (5 ml) was added slowly. The reaction mixture was further stirred for 2 hr. and then warmed to room temperature and stirred overnight. The reaction mixture was then poured over ice cold hydrochloric acid solution (10%) and extracted with dichloromethane (2 x 50 ml). The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. The residue was subjected to column purification on silica gel with pet ether (b.p. 60-80°C) and acetone (7:3) as eluent to yield **31** (0.42 gms, 50%) or **31a** (0.45 gms, 53%) **31a**: as a sticky solid.

Compound **31a** : $[\alpha]_D^{25} = +11°$ (2, $CHCl_3$)

IR(Nujol): 3416 (br), 1680 and 1606 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.18 (d, J = 6Hz, 3H); 2.50 (m, 2H); 3.81 (s, 5H); 3.93 (s, 3H); 4.06 - 4.56 (m, 1H); 6.31 (d, J = 2Hz, 1H); 6.43 (d, J = 2Hz, 1H).

Ms (m/e): 282 (M^+)

Analysis calc. for $C_{14}H_{18}O_6$: C: 59.56% H: 6.43%

Found: C: 59.48% H: 6.50%

Cyclization of 31 with conc. H_2SO_4: The keto acid **31** (0.282 gm, 1 mmol) was mixed with conc. H_2SO_4 (1 ml) and kept overnight at room temperature. The reaction mixture was poured over ice and extracted with ethyl acetate (2 x 10 ml). The combined organic extracts were washed with water, dried over Na_2SO_4 and concentrated. The residue thus obtained was purified on silica gel column using pet ether-acetone (9:1) as eluent to afford **35** (0.196 gms, 80%) as colourless solid, m.p. 166-167°C.

IR (Nujol): 1780 cm^{-1}

^{1}H NMR ($CDCl_3$): δ 1.95 (d, J = 6Hz, 3H); 3.92 (s, 3H); 3.99 (s, 3H); 6.05 (d, J = 15Hz, 1H); 6.12 (s, 1H); 6.36 (d, J = 2Hz, 1H); 6.45 (d, J = 2 Hz, 1H); 6.67 (m, 1H).

Ms (m/e): 246 (M^+)

Analysis calc. for $C_{14}H_{14}O_4$: C = 68.28% H = 5.72%

Found: C = 68.32% H = 5.59%

Cyclization of 31 with Ac_2O and perchloric acid: The reagent was prepared by adding 72% perchloric acid (0.05 ml, 0.575 mmol) to absolute ethyl acetate (50 ml). Then 10 ml of this solution was added to absolute ethyl acetate (30 ml) and acetic anhydride (4.8 ml, 51 mmol) and volume was made upto 50 ml with ethyl acetate. The keto acid **31** (0.200 gms, 0.70 mmol) was mixed with the reagent (20 ml) and kept at room temperature for 10-15 min. Then the reaction mixture was poured over saturated sodium hydrogen carbonate solution and the ethyl acetate layer was separated. The organic layer was washed with water, dried (Na_2SO_4) and concentrated. The residue thus obtained was purified by column to give **35** (0.120 gm, 68.9%) and **36** (0.043 gms, 19.8%) respectively.

36 (gummy material); **IR** (Nujol): 1750 and 1680 cm^{-1}.

^{1}H-NMR ($CDCl_3$) : δ1.36 (d, J = 6Hz, 3H); 2.06 (s, -COC$\underline{H}_3$); 2.68 (dd, J = 14.6 and 6.5 Hz, 1H); 2.81 (dd, J = 14.6 and 6.5 Hz, 1H); 3.93 (s, 3H); 4.02 (s, 3H); 5.22-5.40 (m, 1H); 6.12 (s, 1H); 6.37 (d, J = 2Hz, 1H); 6.49 (d, J = 2Hz, 1H)

Ms (m/e): 306 (M^+)

Analysis calc. for $C_{16}H_{18}O_6$: C = 62.73% H = 5.92%

Found: C = 62.81% H = 5.78%

Cyclodehydration of 31 or 31a with PTSA: The keto acid **31** or **31a** (0.282 gm, 1 mmol) was refluxed in dry benzene (10 ml) with catalytic amount of para-toluene sulphonic acid for 30 min. After cooling, the benzene solution was washed with sodium bicarbonate solution and water respectively and dried over Na_2SO_4. The residue obtained after removal of the solvent was purified on silica gel column (8:2 pet-ether - acetone as eluent) to give (±)di-O-methyl orthosporin (**24**) (0.20 gms, 75.7%)

or (+) di-O-methyl orthosporin (**24a**) (0.21 gms, 79.5%) as sticky solid.

24a: $[\alpha]_D^{25}$ = + 34.8° (2.5, $CHCl_3$) [lit.[6] $[\alpha]_D^{25}$ = + 22.4° (2.7, $CHCl_3$].

IR (Nujol): 3473 and 1713 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.28 (d, J = 6Hz, 3H); 2.55 (dd, J = 14.2, 7.7 Hz, 1H); 2.65 (dd, J = 14.2, 5.6 Hz, 1H); 3.90 (s, 3H); 3.96 (s, 3H); 4.25-4.40 (m, 1H); 6.22 (s, 1H); 6.35 (d, J = 2Hz, 1H); 6.45 (d, J = 2Hz, 1H).

Ms (m/e): 264 (M^+), 220 (base peak)

UV ($CHCl_3$, λ_{max}): 321.4 and 260.4 nm

Analysis calc. for $C_{14}H_{16}O_5$: C = 63.62% H = 6.10%

Found: C = 63.64% H = 6.06%

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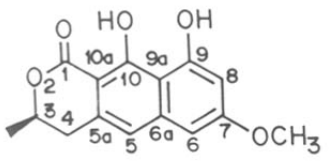
PART 2: SYNTHESIS OF SEMIVIOXANTHIN METHYL ETHER

Introduction

Zeeck et. al.[1] isolated a new antifungal antibiotic semivioxanthin (**1**) from the culture filtrate of Penicillium citreo viride along with the known compounds xanthomegnin (**2**), 3,4-dehydroxanthomegnin (**3**), viomellein (**4**) and vioxanthin (**5**). The major component xanthomegnin (**2**) was also isolated from Trichophyton strain[2,3,4] Microsporim cookii[5] and Asperigillus strain.[6] Vioxanthin (**5**) was isolated for the first time from T. Violaceum by Blank et. al.[4]. Biogenetically xanthomegnin (**2**), viomellein (**4**) and related compounds were shown to be produced from acetic acid via polyketides.[7]

The structure of semivioxanthin (**1**) was established as 3,4-dihydro-9,10-dihydroxy-7-methoxy-3-methyl-1-oxo-1H-naphto[2,3-c] pyran on the basis of the spectral evidence and the fact that it gave semixanthomegnin (**6**) when subjected to Fremy's salt oxidation. Some other antifungal natural products having similar basic skeleton as in semivionxanthin (**1**) are vioxanthin (**5**)[1], SC-28762 (**7**)[8], SC-28763 (**8**), SC-30532 (**9**)[9], semiviriditoxic acid (**10**)[10]. Dermolactone[11] (**11**) is another natural product having tetracyclic structure similar to semivioxanthin (**1**).



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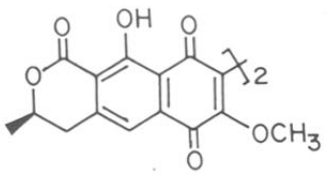

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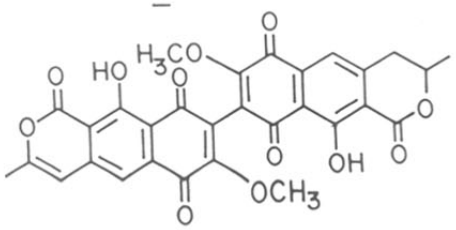

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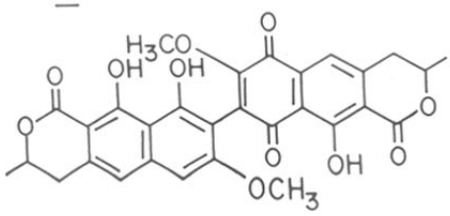

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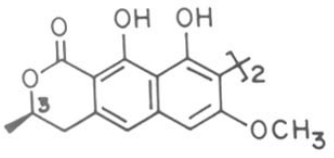

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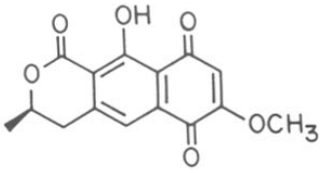

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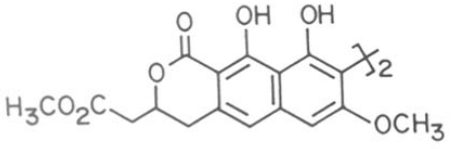

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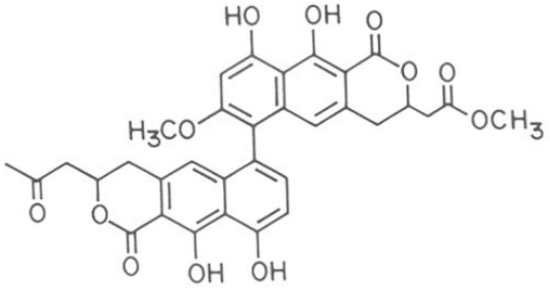

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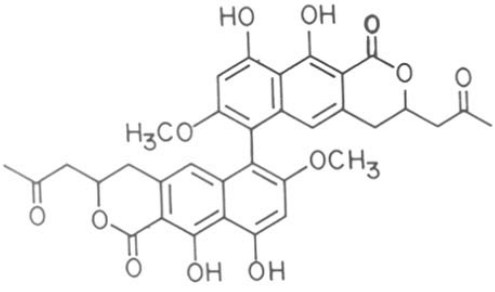

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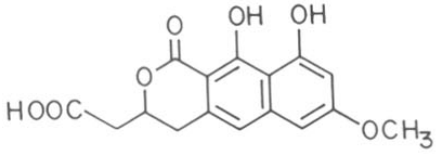

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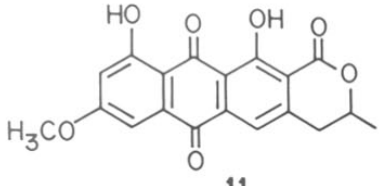

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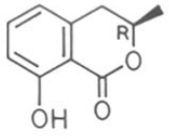


(R) – Mellein

Biological activity

Semivioxanthin (**1**), semixanthomegnin (**6**), viomellein (**4**), vioxanthin (**5**) and xanthomegnin (**2**) inhibit the growth of Gram positive and Gram negative bacteria.[1] The monomers **1** and **6** are active against E. Coli and S. aureus to considerable extent. Some of these compounds showed no influence on longivity when tested against anthropodes[12]. Xanthomegnin (**2**) has an effect on mitochondrial phosphorylation of rat-liver.[5] Xanthomegnin (**2**) and viomellein (**4**) also reduce the activity of the bean's beetle or bug (Eplilachna Varivestis MULS)[12].

Absolute stereochemistry of semivioxanthin

The absolute stereochemistry of semivioxanthin (**1**) at C_3 was assigned on the basis of the correlation of its CD curve with that of R-mellein by Zeeck et. al.[1]. The negative cotton effect at 270 nm was similar to that of R-mellein at 257 nm and hence configuration at C_3 was designated as R. However, same workers assigned S-configuration at C_3 for vioxanthin (**5**), the dimer of semivioxanthin (**1**)[1]. It should be noted that, earlier based on the degradative studies, Blank et. al.[13] confirmed R-configuration for xanthomegnin (**2**). However, no degradation product of vioxanthin (**5**) could be obtained yet the probable configuration of vioxanthin (**5**) was assigned as R, since both xanthomegnin (**2**) and vioxanthin (**5**) were isolated from the same natural source.

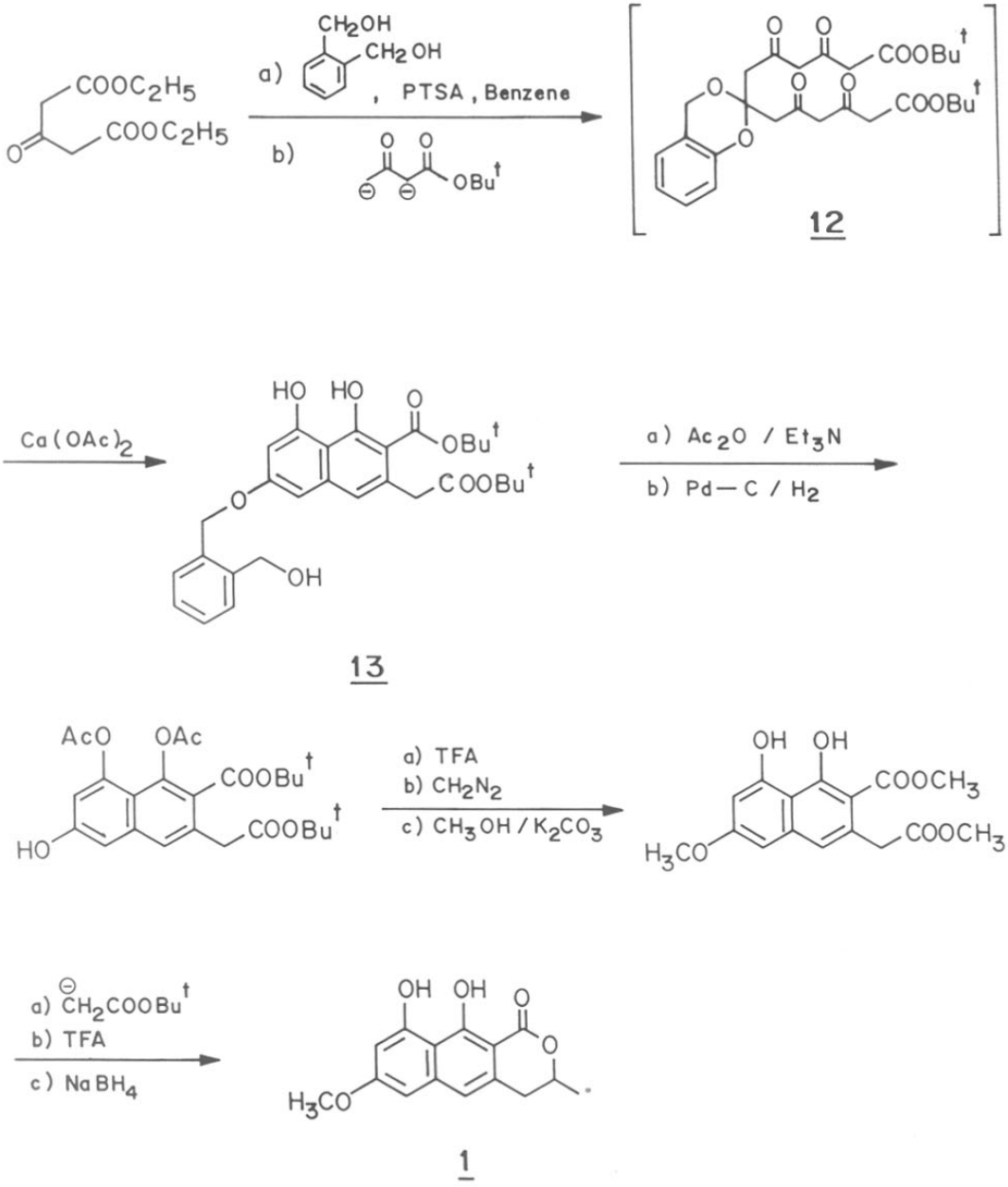
Synthesis of (±) semivioxanthin (1)

The first synthesis of semivioxanthin (**1**) was reported in 1990 by Yamaguchi et. al.[14] via a polyketide approach. The $Ca(OAc)_2$ induced intramolecular condensation of the polyketide intermediate (**12**) furnished that suitably substituted naphthalene derivative (**13**) which was further elaborated to semivioxanthin (**1**) (**Scheme 1.2.1**).

The desired polyketide intermediate (**12**) was generated by the Claisen condensation of the ß-oxo glutarate derivative with the acetoacetate dianion. The use of a novel protecting group for the ketone (o-phenylenedimethanol) allows the selective methyla-

tion of the 7-hydroxy group.

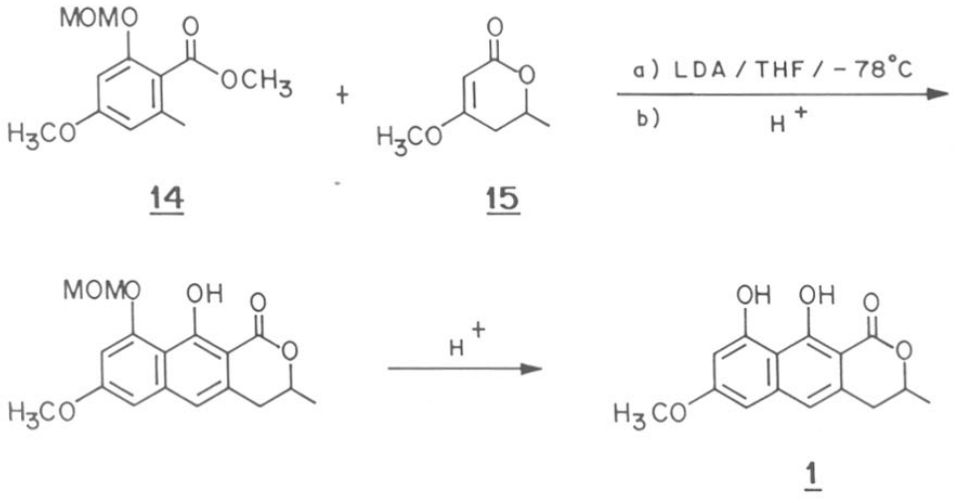
SCHEME 1·2·1



The second synthesis of semivioxanthin (**1**) has been reported recently from our group.[15] The synthesis is based on the condensation of the suitably substituted orsellinate derivative (**14**) and the pyrone (**5**) in presence of LDA to form the tricyclic lactone skeleton of semivioxanthin (**1**).

The orsellinate derivative **14** was prepared by selective demethylation of methylorsellinate dimethyl ether followed by MOM protection. The 4-methoxy-6-methyl-5,6-dihydro-2-pyrone (**15**) was obtained in quantitative yield by methylating the corresponding hydroxy compound. The condensation of **14** with the pyrone (**15**) followed by deprotection of the MOM group yielded semivioxanthin (**1**) in 82% yield (**Scheme 1.2.2**).

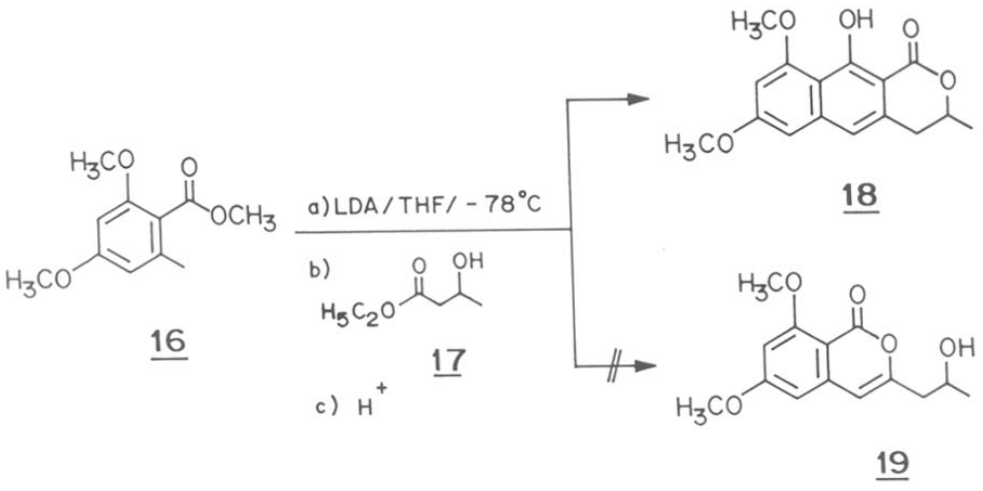
SCHEME 1·2·2



Present work

As it has been already mentioned in Part 1, that while developing the synthetic route for orthosporin, the anion of methyl orsellinate dimethyl ether (**16**), generated by LDA at -78°C, when treated with ethyl-3-hydroxybutyrate (**17**) followed by acidic work up of the reaction afforded a major product which was characterized as semiviox-anthin methylether (**18**) instead of expected orthosporin dimethylether (**19**) (**Scheme 1.2.3**).

SCHEME 1·2·3



The product so obtained was fully characterized by various spectral means. The lactone carbonyl absorption band appeared at 1665 cm^{-1} in the IR spectrum. The ^1H-NMR (**FIG.I**) spectrum exhibited a methyl doublet at δ 1.48, a doublet of doublet at δ 2.88 and a multiplet at δ 4.64 [CH_2(O)-C<u>H</u>-CH_3]. The two methoxyl singlets were seen at δ 3.84 and δ 3.93. Two aromatic protons appeared as two meta coupled doublets (J = 2 Hz) at δ 6.40 and δ 6.48 and the remaining aromatic proton showed a singlet at δ 6.77.

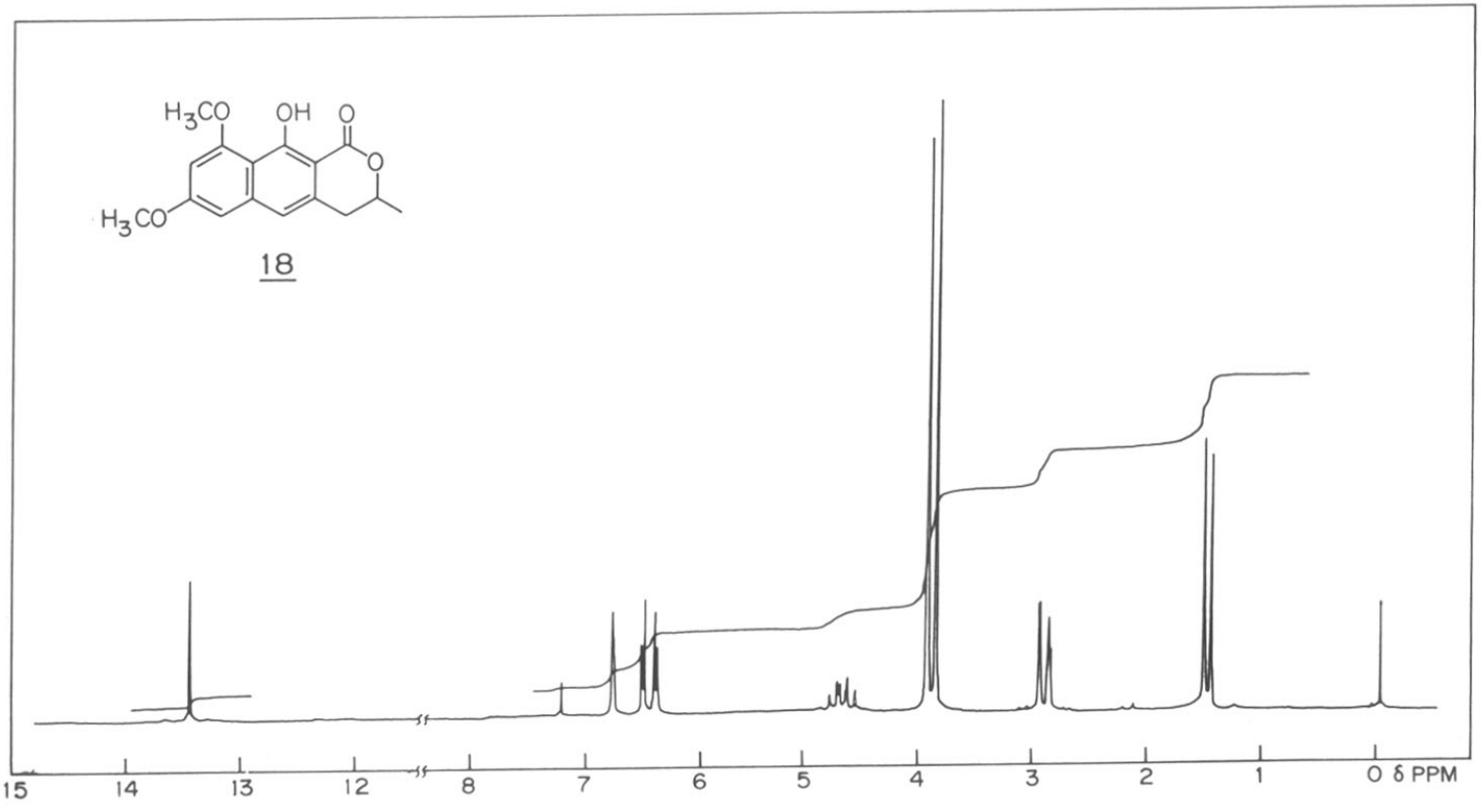


FIG. I : ^{1}H-NMR SPECTRUM OF COMPOUND (**18**) IN $CDCl_3$

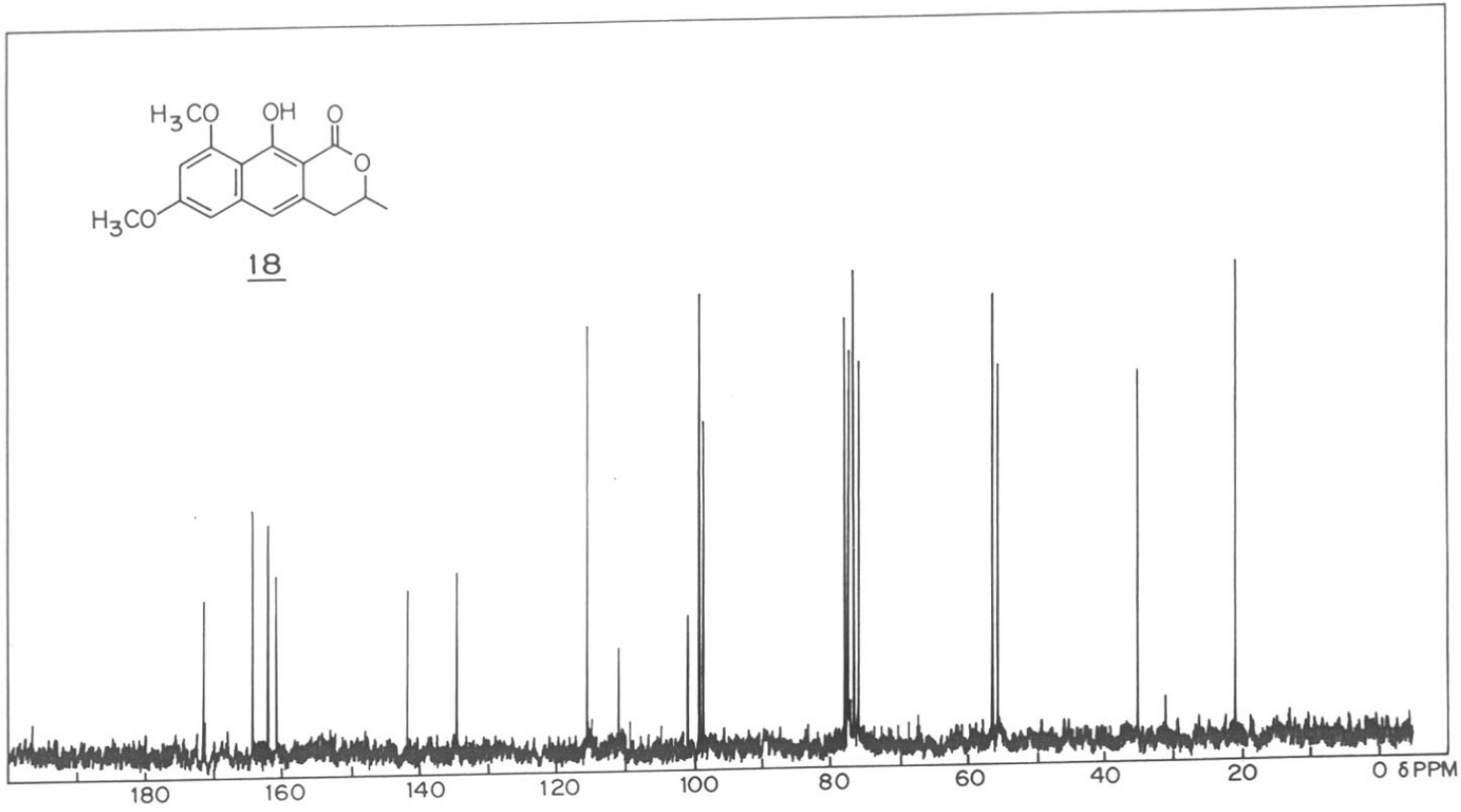
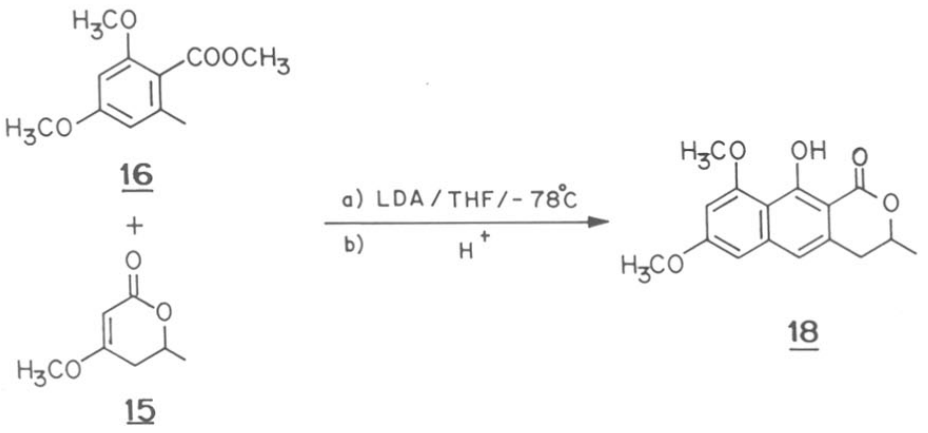


FIG. II : ^{13}C-NMR SPECTRUM OF COMPOUND (18) IN $CDCl_3$

The chelated hydroxyl proton appeared at δ 13.13 as a singlet. The presence of the molecular ion peak at m/e. 288 further extended support to the formation of **18**. The ^{13}C-NMR (**FIG.II**) spectrum finally confirmed the formation of **18**.

Further confirmation of the formation of **18** was achieved by synthesizing **18** by the condensation of the methyl orsellinate dimethyl ether (**16**) and the pyrone **15**[15] in presence of LDA. (**Scheme 1.2.4**).

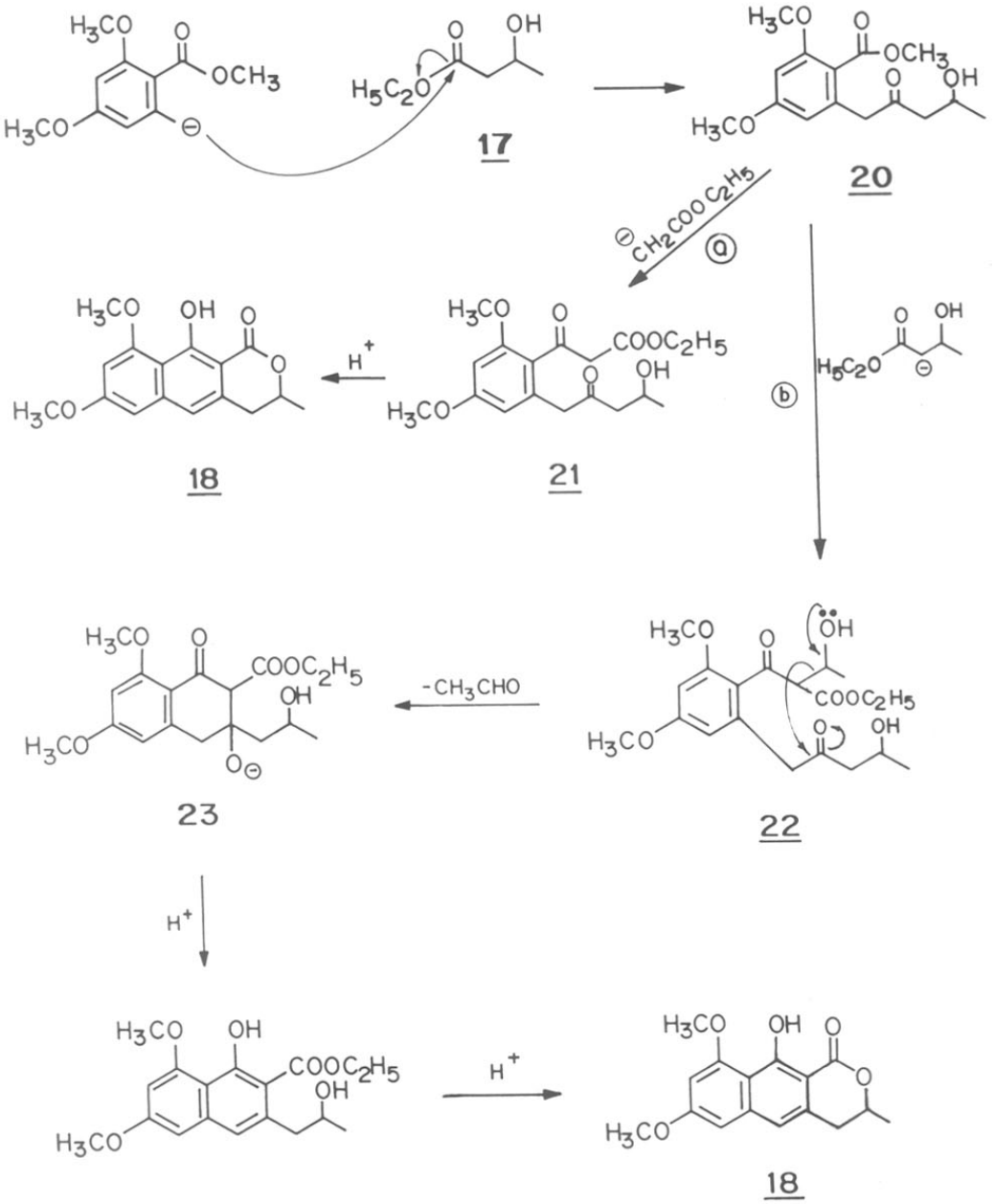
SCHEME 1·2·4



The product obtained from the above condensation was identical in all respects to the major product (**18**) obtained from the condensation of **16** and ethyl 3-hydroxybutyrate (**17**).

The formation of two different products under identical reaction conditions with the same reactant ethyl-3-hydroxybutyrate (**17**) condensing to the orsellinic acid or methyl orsellinate, indicated that probably formation of both the products can be explained by a common mechanism in which the aromatic ester group might play an

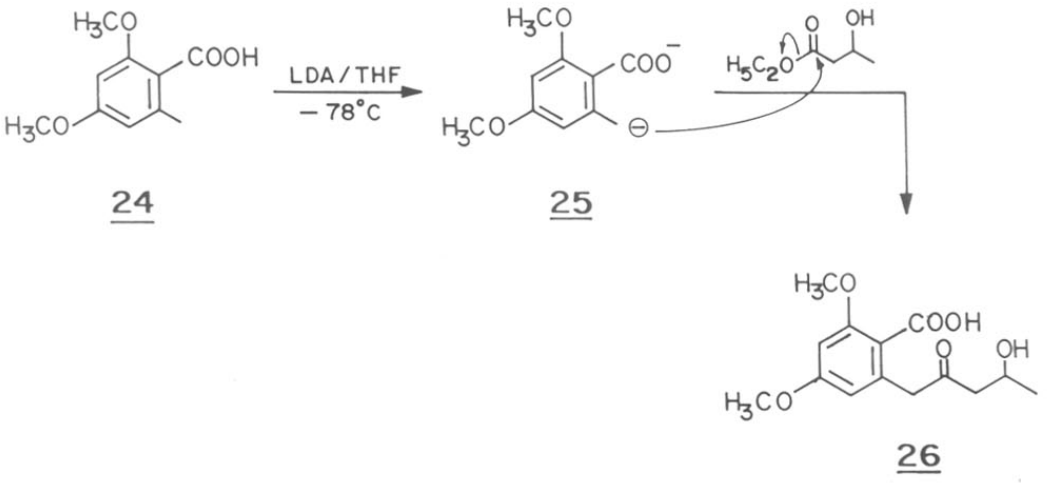
SCHEME 1·2·5



important role. This assumption envisaged a retero-aldol type of mechanism involving further condensation of the aromatic ester group to another molecule of ethyl-3-hydroxybutyrate. (**Scheme 1.2.5**).

Thus, the benzylic anion of orsellinate **16** can first be quenched with ethyl-3-hydroxybutyrate to give the keto ester **20** which can further condense with another molecule of ethyl-3-hydroxybutyrate since the anion generation is quite possible because of the acidity of its active methylene group. The anion so generated can attack the keto ester **20** in two ways: a) either it cleaves into acetaldehyde and $\overset{\ominus}{C}H_2COOEt$ anion first and then the latter attacks to give an intermediate **21** which upon cyclization would give the semivioxanthin methyl ether (**18**). b) or it can first attack to give an intermediate **22** which would rearrange intramolecularly to give the cyclic intermediate **23** which inturn would cyclize further to give semivioxanthin methyl ether (**18**).

SCHEME 1·2·6



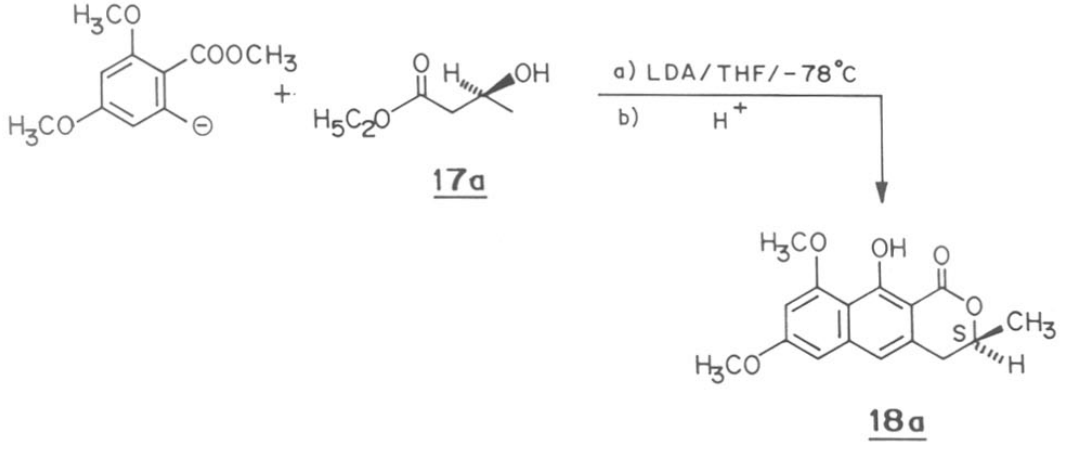
24 25 26

While in orsellinic acid **24**, the benzylic anion **25** attacks the ester carbonyl of ethyl-3-hydroxybutyrate, thereby leading to the formation of keto acid **26** (**Scheme 1.2.6**).

However, the mechanism proposed for the formation of semivioxanthin skeleton needs to be confirmed as none of the suggested intermediates could be isolated and characterized.

The synthesis of semivioxanthin methyl ether (**18**) by the condensation of methyl orsellinate with ethyl-3-hydroxybutyrate made it quite appropriate to apply the methodology for its stereospecific synthesis by using the desired optically active isomer of ethyl-3-hydroxybutyrate. So far, both the methods reported for semivioxanthin deal with its racemic synthesis, hence if the stereoinduction is possible by this new route it would generate a new methodology for stereospecific synthesis of semivioxanthin and analogs.

SCHEME 1·2·7



17a

18a

Although indirect evidence postulates R-configuration at C_3 for semivioxanthin (**1**), yet no direct proof is known till date. Therefore, the (S)-ethyl-3-hydroxybutyrate (**17a**) was employed as it was already prepared and used in the synthesis of (+) orthosporin (Part 1). The condensation of methyl orsellinate anion, generated by LDA at -78°C, with the optically active (S)-ethyl-3-hydroxybutyrate (**17a**) (Part 1) followed by acidic work up afforded the S(-)-semivioxanthin methyl ether (**18a**) (**Scheme 1.2.7**). The compound (**18a**) exhibited the optical rotation of -18° in chloroform. The physical and spectral (**^{1}H-NMR : FIG.III**) properties were identical to its racemic analogs (**18**).

The formation of (S)-(-)-semivioxanthin methyl ether (**18a**) from (S)-ethyl-3-hydroxybutyrate (**17a**) suggested that the condensation of the (R)-ethyl-3-hyroxybutyrate with methyl orsellinate would lead to the (R)-semivioxanthin methyl ether. R(-)-Ethyl-3-hydroxybutyrate is an important chiral building block used in the synthesis of natural products including macrolide, ß-lactam antibiotics and pheromones. Its preparation was reported by Mori et. al.[16] from polyhydroxybutyrate. Alternatively, it was also prepared by microbial reduction of ethyl acetoacetate with Geotrichum candidum[17]. Due to non-availability of poly-hydroxybutyrate, an attempt was made to reduce ethylacetoacetate with G. candidum. However, the product obtained in very low yield showed poor optical purity. Therefore, further work was not carried out to achieve the synthesis of natural (R)-semivioxanthin.

So, it can be concluded that a novel single step condensation methodology has been evolved for the semivioxanthin methyl ether and the same is applied for its stereospecific synthesis. The first stereospecific synthesis of the antipode of semivioxanthin methyl ether has been achieved. The methodology can be extended for building up other molecules possessing the similar basic skeleton as in semivioxanthin.

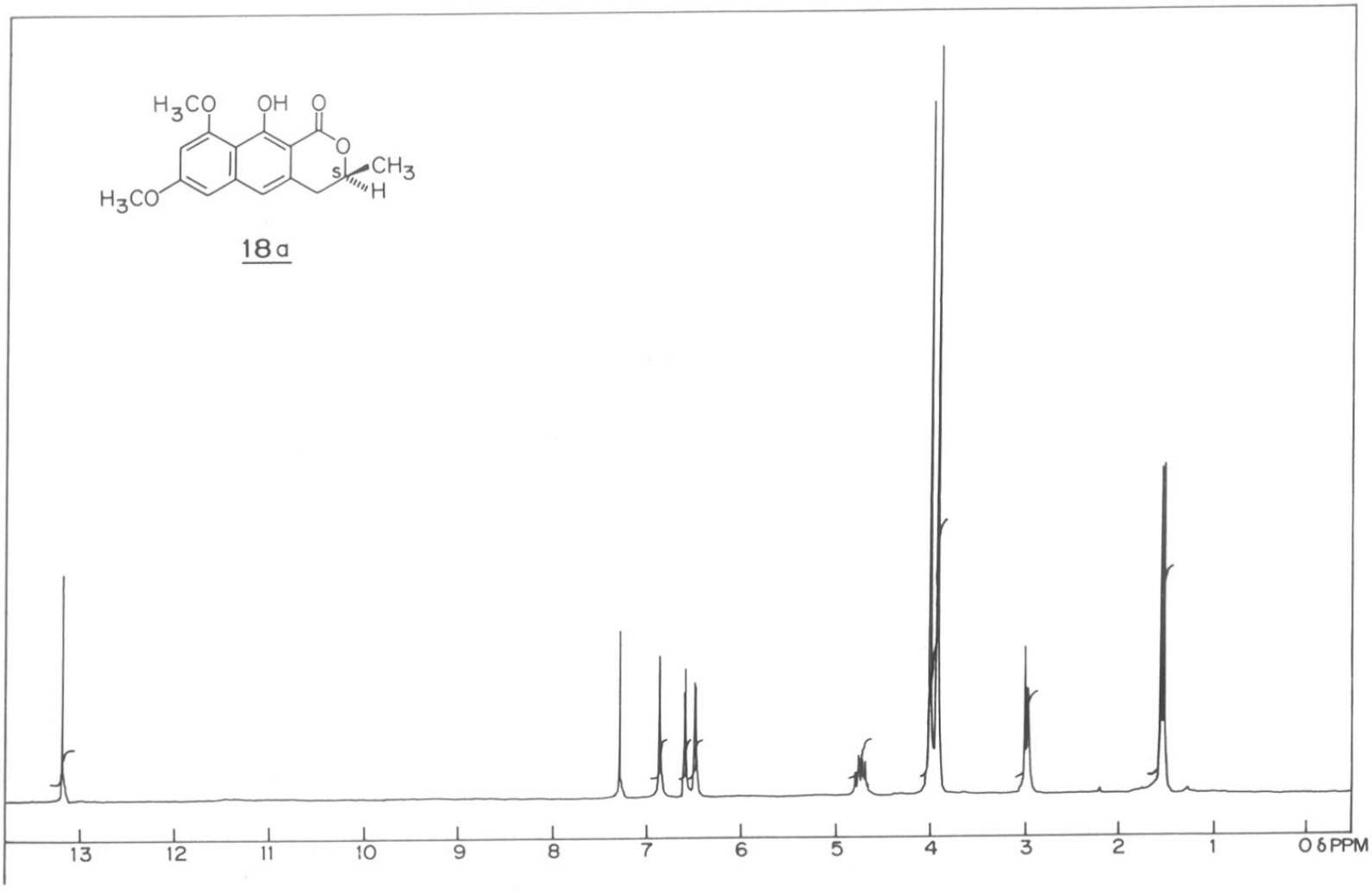


FIG. III : ^{1}H-NMR SPECTRUM OF COMPOUND (**18a**) IN $CDCl_3$

Experimental:

Methyl-2,4-dimethoxy-6-methylbenzoate (Methylorsellinate) (16): A mixture of acid **24** (3.92 g, 20 mmol), dimethyl sulphate (2.6 g, 21 mmol) and anhydrous potassium carbonate (2.9 g, 21 mmol) in dry acetone (35 ml) was refluxed on waterbath for 6 h. The acetone was distilled off completely and to the residue cold water (50 ml) was introduced. The solid separated was filtered, washed with water and dried to give ester **16** (4.1 g, 98%); m.p. 44°C (Lit.[18], m.p. 43°C).

Condensation of methyl orsellinate (16) with ethyl-3-hydroxybutyrate (17): A LDA solution was prepared at 0°C by adding n-BuLi (1M, 9.54 ml) to diisopropylamine (0.96 gm, 9.54 mmol) in THF (5 ml) under argon. The solution was cooled to -78°C and a solution of methyl orsellinate (**16**) (1.00 gm, 4.76 mmol) in THF (5 ml) was added during 10 min. The deep red solution was stirred for 15 min. at the same temperature and ethyl-3-hydroxybutyrate (0.63 gm, 4.77 mmol) in THF (3 ml) was added dropwise. The reaction mixture was stirred further for 2 h at -78°C and 1 h at room temperature. The reaction mixture was poured over ice-cold dil. HCl (20 ml), stirred and extracted with CH_2Cl_2 (2 x 20 ml). The combined organic extracts were washed with water, dried over Na_2So_4 and evaporated. The reddish yellow residue was purified on silica gel column (hexane - acetone; 8:2) to afford the semivioxanthin methyl ether (**18**) (0.468 gm, 34%) as colourless crystalline solid, m.p. 130-132°C.

IR (Nujol): 1665 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.48 (d, J = 6 Hz, 3H); 2.88 (dd, J = 6 Hz and 1 Hz, 2H); 3.84 (s, 3H); 3.93 (s, 3H); 4.64 (m, 1H); 6.40 (d, J = 2 Hz, 1H); 6.48 (d, J = 2 Hz, 1H); 6.77 (s, 1H); 13.13 (s, 1H, O<u>H</u>).

Ms (m/e): 288 (M^+) (base peak).

UV ($CHCl_3$, λ_{max}): 259, 307 and 361 nm.

^{13}C-NMR ($CDCl_3$): δ 20.927 ($C_3\underline{C}H_3$); 35.180 (C_4); 55.581 (C_9-O<u>C</u>H_3); 56.323 (C_7-

$O\underline{C}H_3$); 75.830 (C_3); 98.462 (C_{9a}); 99.141 (C_8); 100.829 (C_6); 110.832 (C_{10a}); 115.421 (C_5); 134.480 (C_{4a}); 141.632 (C_{5a}); 160.772 (C_{10}); 161.951 (C_9); 164.229 (C_7); 171.329 (C_1).

Condensation of methyl orsellinate (16) with the pyrone (15): The LDA was generated at 0°C by adding n-BuLi (1M, 2 ml) to diisopropylamine (0.20 gm, 2 mmol) in THF (2 ml) under argon atmosphere. The resultant LDA solution was cooled to -78°C and methyl orsellinate (**16**) (0.21 gm, 1 mmol) in THF (2 ml) was added and the orange-red solution was stirred for 15 min at the same temperature. The pyrone (**15**) (0.14 gm, 1 mmol) in THF (1 ml) was added at the same temperature and stirred further for 30 min. The reaction mixture was warmed to room temperature and stirred for 1/2 h. The reaction mixture was poured to an ice-cold solution of dilute HCl (10 ml) and extracted with CH_2Cl_2 (2 x 10 ml). The combined extracts were washed, dried (Na_2SO_4) and concentrated. The residue on column purification afforded semivioxanthin methyl ether (**18**) (0.20 gm, 70%); m.p. 130-132°C.

Condensation of methyl orsellinate (16) with (S)-ethyl-3-hydroxybutyrate (17a): To the LDA solution, generated by addition of n-BuLi (1M, 3.16 ml) to diisopropylamine (0.32 gm, 3.16 mmol) in THF (2 ml), cooled to -78°C was added methyl orsellinate (**16**) (0.33 gm, 1.58 mmol) in THF (2 ml) during 10 min. The reddish orange solution was stirred for 15 min and (S)-ethyl-3-hydroxybutyrate (**17a**) (0.21 gm, 1.59 mmol) in THF (1 ml) was added and stirring continued for 2 h. The reaction mixture was stirred further for 1 h at room temperature and poured into ice-cold dilute HCl (10 ml). The product was extracted in CH_2Cl_2 (2 x 5 ml), dried (Na_2SO_4) and concentrated. The residue was purified on column to give (S)-(-)-semivioxanthin methyl ether (**18a**) (0.158 gm, 35%). $[\alpha]_D^{25}$ = -18°C. (0.3, $CHCl_3$].

References:

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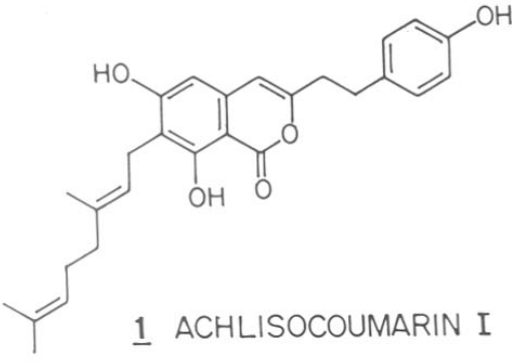
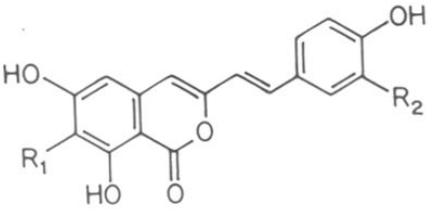
PART 3: SYNTHESIS OF ACHLISOCOUMARIN III TETRAMETHYL ETHER

Introduction

Mizuno et. al.[1] isolated three new phenolic isocoumarins from the underground parts of Achlys triphylla (Berberidaceae) while investigating the chemical constituents of the plant to determine its chemotaxonomic relationship with other members of the subtribe Epimedinae. The medicinal value of these compounds are also being evaluated.



1 ACHLISOCOUMARIN I



2 R_1 = Geranyl , R_2 = H
ACHLISOCOUMARIN II

3 R_1 = H , R_2 = OH
ACHLISOCOUMARIN III

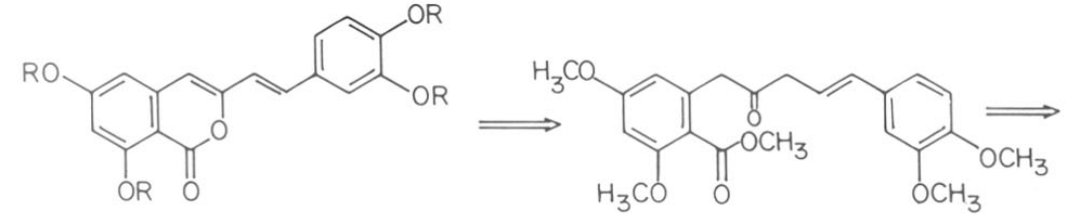
The structures of these isocoumarins established by spectroscopic means were shown to be 7-geranyl-6,8-dihydroxy-3-(4'hydroxyphenylethyl)isocoumarin (1) for achlisocoumarin I, 7-geranyl-6,8-dihydroxy-3-(4'-hydroxyphenyleth- enyl) isocoumarin (2) for achlisocoumarin II and 6,8-dihydroxy-3-(3',4'-dihydroxyphenylethenyl) isocoumarin (3) for achlisocoumarin III, respectively.

Present work

Since these new isocoumarins have not been synthesized so far besides being biologically important, the synthesis of the simplest member, achlisocoumarin III, was undertaken so as to develop a methodology which could be applied for the synthesis of other two isocoumarins too. The successful synthesis of achlisocoumarin III methyl ether has been described in this part.

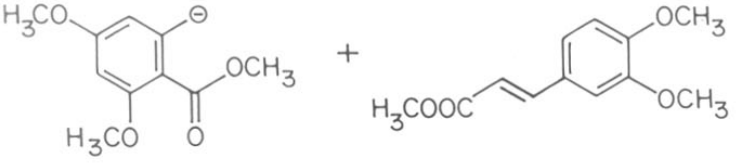
The reterosynthetic analysis (**Scheme 1.3.1**) revealed that the anion **4** generated from methyl orsellinate (**13**) (Chapter I: Part 2) could be condensed with the cinnamic acid ester (**6**) electrophillically thereby generating the keto-ester (**7**) which would cyclize to furnish the achlisocoumarin III methyl ether (**8**).

SCHEME 1·3·1



3 R = H
8 R = CH_3

7

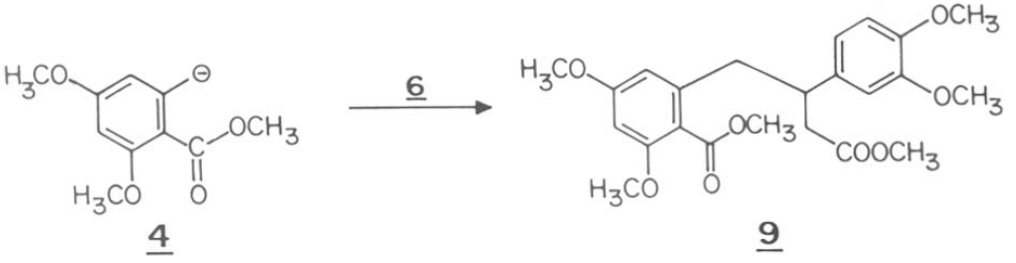


4 **6**

Although it has been reported in the literature that alkylithiums such as methyl

lithium reacts with cinnamic acid thereby giving the corresponding ketone in 65-95% yield[2], still the reactivity of the anion **4** cannot be compared to the former as the latter is a soft nucleophile. Hence, possibility of **4** undergoing 1,4-addition to theα,β-unsaturated double bond of **6** seemed equally competitive (**Scheme 1.3.2**). So there would be a possibility of getting **8** as well as Michael adduct **9** or both from the reaction mixture.

SCHEME 1·3·2

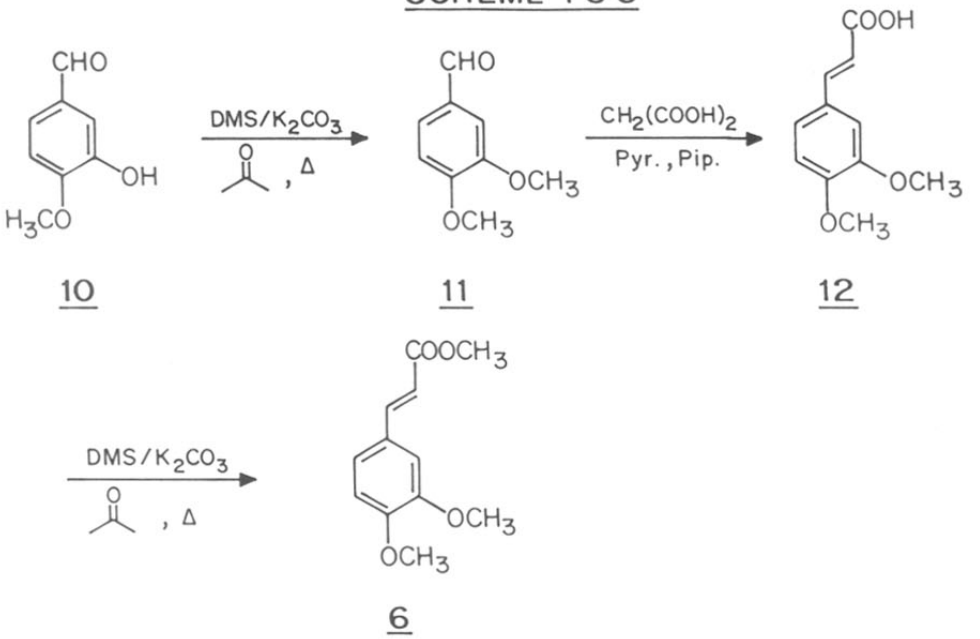


4 **9**

However, even with this pretext, the strategy still appeared promising as, if successful, it would furnish a single step methodology for making the isocoumarins analogous to achlisocoumarins.

Thus, the desired cinnamic acid ester (**6**) was prepared in three steps, with the overall yield of 67%, starting from isovanillin (**10**)(**Scheme 1.3.3**). The methylation of isovanillin (**10**) with dimethyl sulphate and potassium carbonate in boiling acetone afforded 3,4-dimethoxybenzaldehyde (Veratraldehyde **11**) in 96% yield. The Doebner condensation[3] of **11** with malonic acid in pyridine and piperidine resulted into the formation of 3,4-dimethoxycinnamic acid (**12**). The esterification of acid **12** with dimethyl sulphate and potassium carbonate in acetone gave the ester **6**. The physical and spectral properties of compound **6** were consitent with those reported in the literature[4].

SCHEME 1·3·3

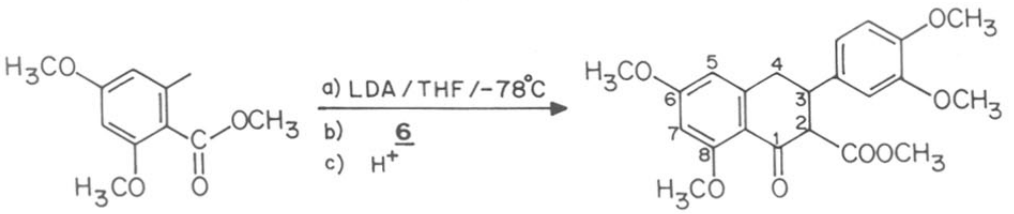


10 11 12

6

The anion **4**, generated by LDA treatment on methyl orsellinate (**13**) (Chapter I: Part 2) at -78°C, when quenched with the ester **6** at the same temperature, followed by acidic work up, furnished a new compound which was characterized on the basis of its spectral analysis as 3-(3,4-dimethoxyphenyl)-6,8-dimethoxy-1,2,3,4-tetrahydro-1-oxo-naphthalene-2-carboxylic acid methyl ester (**14**) (**Scheme 1.3.4**).

SCHEME 1·3·4

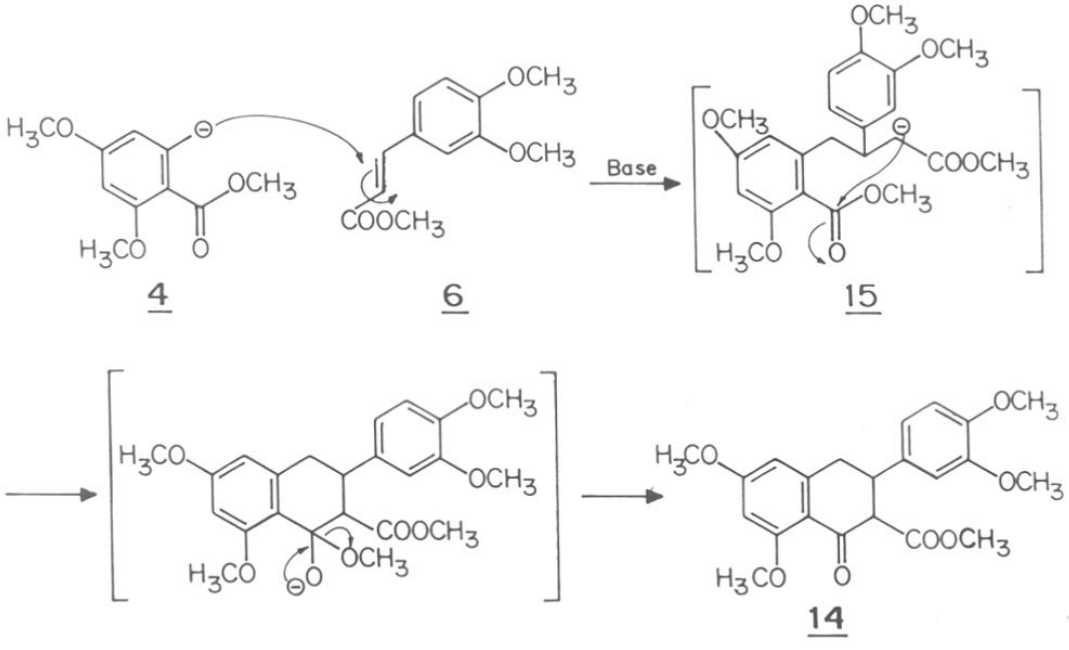


13 14

The IR spectrum showed two carbonyl absorption bands at 1720 and 1670 cm^{-1}. The ^{1}H-NMR (**FIG.I**) spectrum of compound **14** showed a benzylic methylene doublet (J = 6 Hz) at δ 3.06, a methyl ester singlet at δ 3.57 and a doublet of triplet (J = 6 Hz and 2 Hz) at δ 3.73 for single proton at C_3 position. All the twelve methoxyl protons appeared as singlet at δ 3.82. The C_2 proton appeared as a doublet (J = 2 Hz) at δ 3.88. The five aromatic protons appeared as two metacoupled doublets at δ 6.28 and 6.33 and a multiplet at δ 6.77. The appearance of molecular ion peak at m/e. 400 further supported the structure.

The formation of **14** could be visualized by a mechanism (**Scheme 1.3.5**) involving the Michael type condensation first and then the conjugated anion **15** generated thereby reacting further with the carboxylic ester to give the tetralone (**14**).

SCHEME 1·3·5



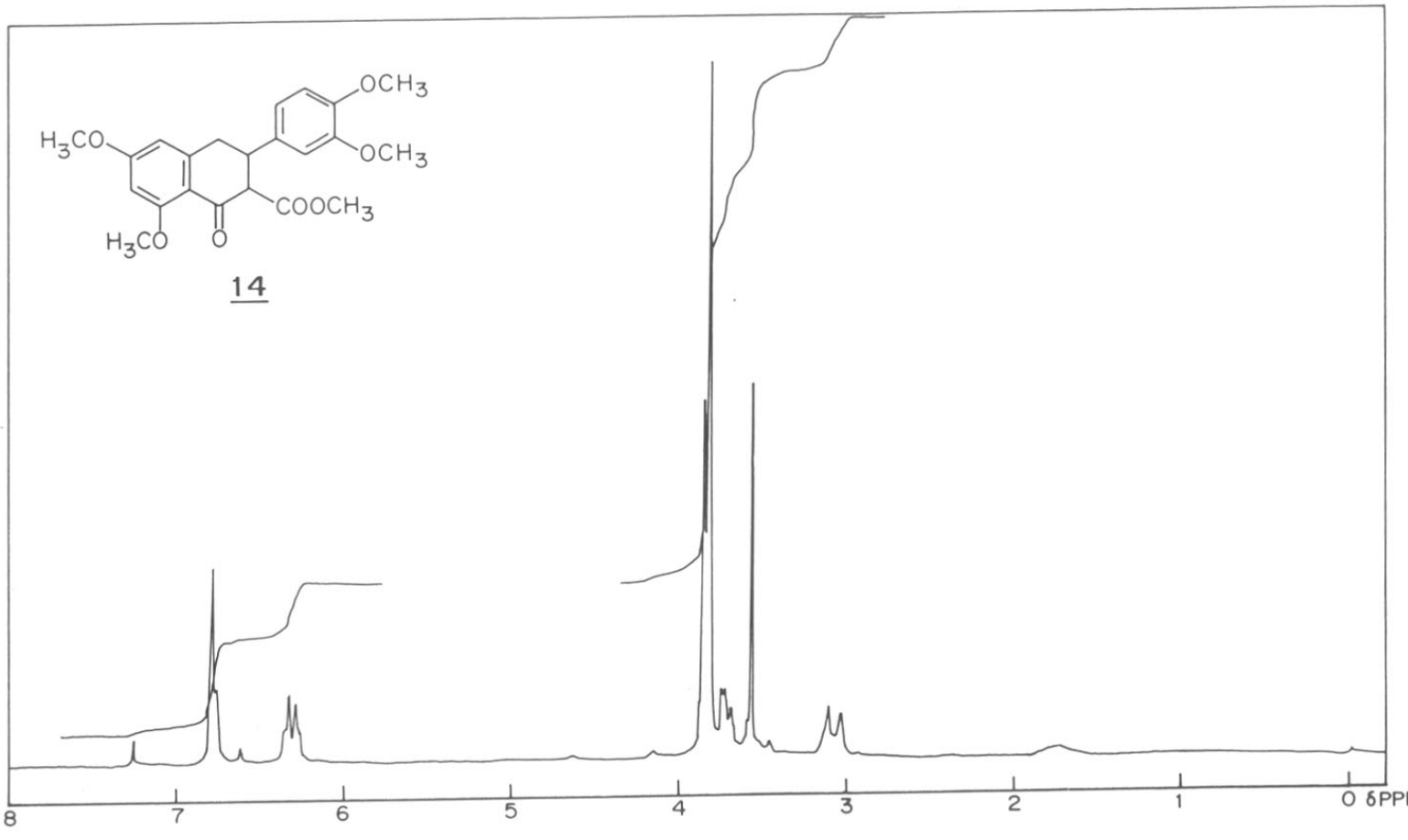
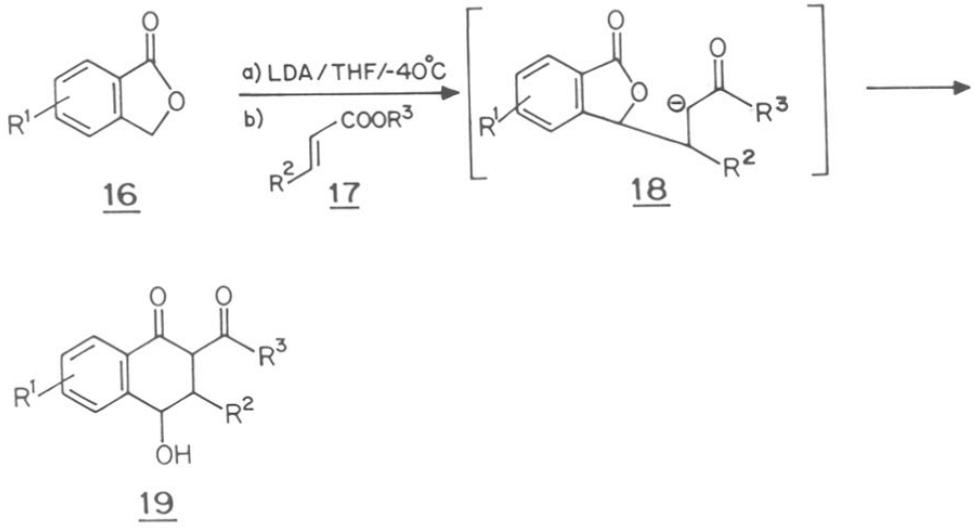


FIG. I : ^{1}H-NMR SPECTRUM OF COMPOUND (14) IN $CDCl_3$

The mechanism proposed is supported by the work of Broom and Sammes[5], who have synthesized a number of hydroxy tetralones (**19**) from phthalide (**16**) and several α,β-unsaturated compounds (**17**) (**Scheme 1.3.6**).

SCHEME 1·3·6

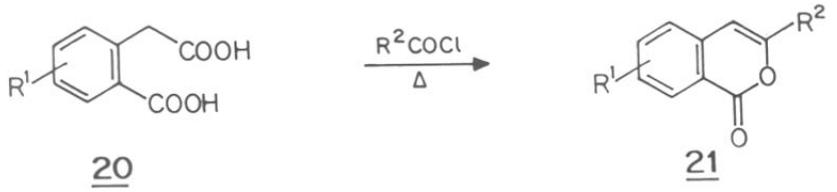


The formation of tetralone **19** was explained on the basis that the anion **18** under aprotic conditions attacks the lactone ring earlier than getting protonated itself.

Since this route involving direct condensation of **13** and **6** led to an altogether new compound **14**, the need for alternative strategy for achlisocoumarin III seemed necessary. It has been reported in the literature[6] that the homophthalic acid (**20**) when heated with acyl halides generates the isocoumarins of the type **21** (**Scheme 1.3.7**).

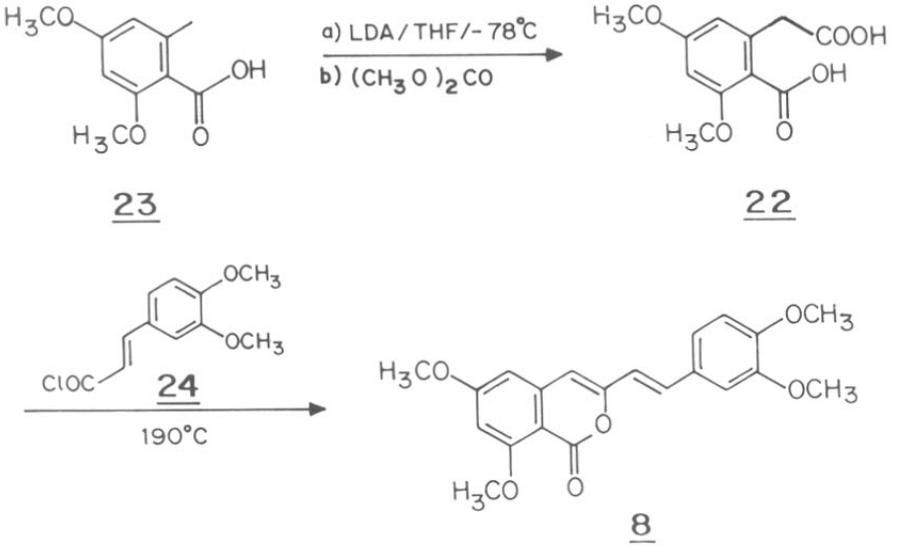
This fact lent a support to the proposed route where the desired homophthalic acid (**22**) generated from the orsellinic acid (**23**) would give the achlisocoumarin III methyl ether (**8**) when heated with 3,5-dimethoxy cinnamoyl chloride (**24**) (**Scheme 1.3.8**).

SCHEME 1·3·7



20 21

SCHEME 1·3·8



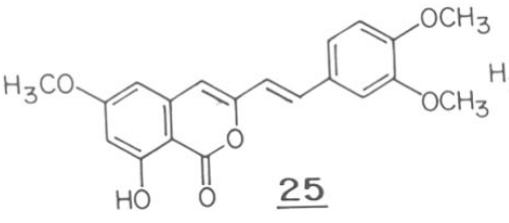
23 22 8

The desired homophthalic acid (**22**) was prepared from the orsellinic acid (**23** (Chapter I: Part 1) by the method reported by Hauser and Rhee[7]. The treatment of LDA with a solution of orsellinic acid (**23**) and dimethyl carbonate in THF at -78°C afforded the homophthalic acid (**22**) in 70% yield. The physical and spectral properties of compound **22** were in good agreement with the reported counterparts[8].

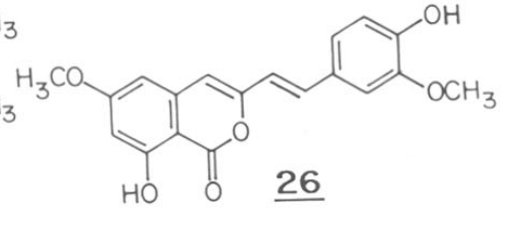
The cinnamoyl chloride (**24**) was prepared from the cinnamic acid (**12**) by refluxing it with thionyl chloride in benzene. The cinnamoyl chloride thus formed was used directly for the condensation.

The homophthalic acid **22** was heated at 190°C with the cinnamoyl chloride **24** for two hr. to generate the achlisocoumarin III tetramethyl ether (**8**) in 71% yield. The product **8** was characterized fully on the basis of its spectral analysis. The IR spectrum showed a lactone carbonyl absorption band at 1690 cm^{-1}. In the ^{1}H-NMR (**FIG. II**) three singlets appeared at δ 3.90, 4.00 and 4.05 integrating for twelve protons of the four methoxyl groups. A singlet at δ 6.30 for cyclic olefinic and two doublets (J = 16 Hz) at δ 6.55 and 7.45 for two aliphatic olefinic protons were observed. The aromatic protons were characterized by three metacoupled doublets (J = 2 Hz) at δ 6.40, 6.50 and 7.10, a doublet (J = 8 Hz) at δ 6.90 and a double doublet (J = 8 Hz, 2Hz) at δ 7.15. The appearance of the molecular ion peak at m/e 368 in the mass spectrum further confirmed the as signed structure.

The demethylation of the compound **8** with aluminium chloride in dichloromethane afforded trimethyl ether (**25**) as the major product with a trace amount of dimethyl ether (**26**).



25



26

The formation of **25** was supported by its spectral analysis. The ^{1}H-NMR (**FIG.III**) confirmed the presence of a chelated -OH group at δ11.15. There

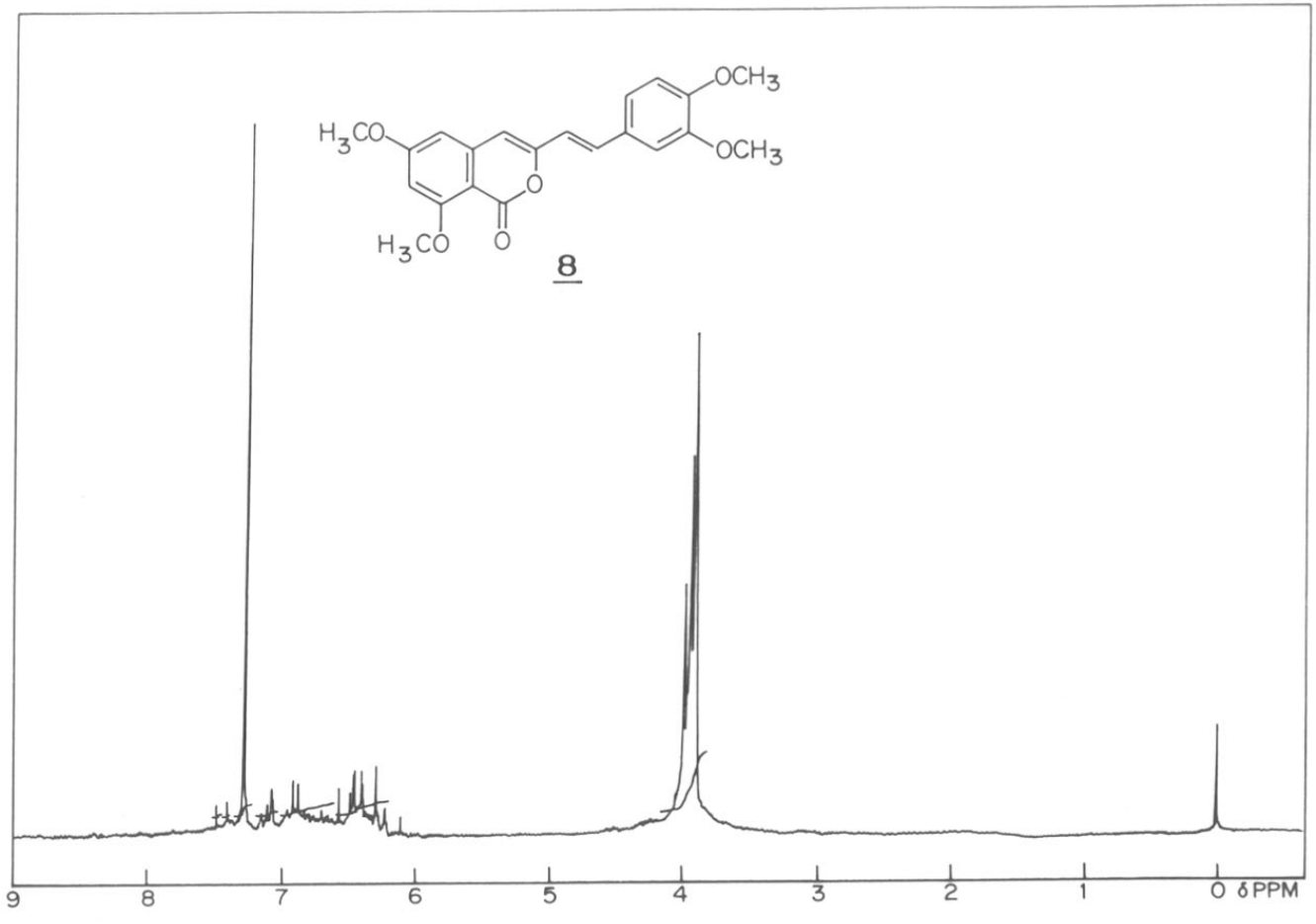


FIG. II : ^{1}H-NMR SPECTRUM OF COMPOUND (8) IN $CDCl_3$

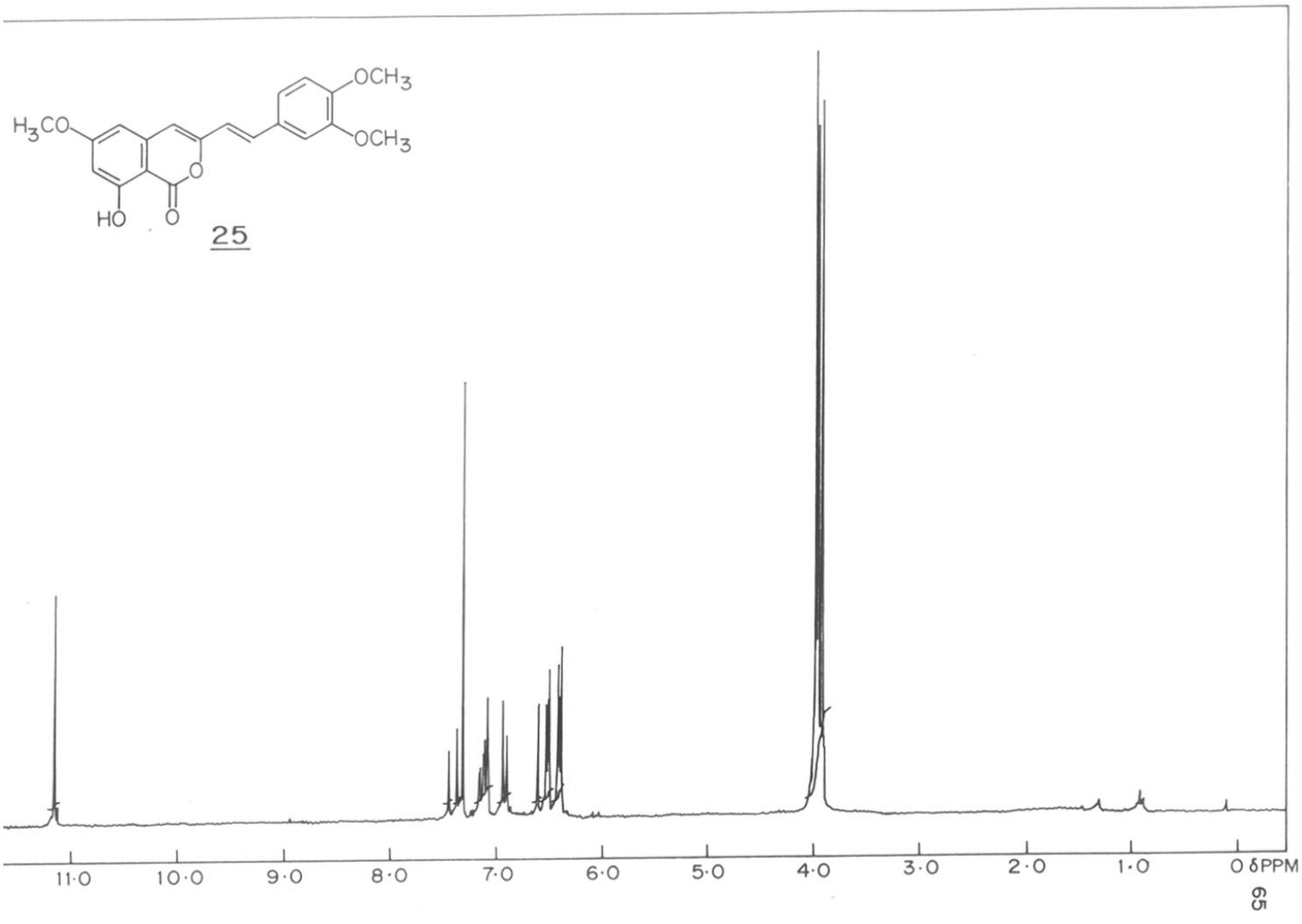


FIG. III : H-NMR SPECTRUM OF COMPOUND (25) IN $CDCl_3$

were only three methoxyl singlets at δ 3.90, 3.95 and 4.00 integrating together for nine protons. The rest of the spectrum was identical to the tetramethyl ether (**8**). The M^+ at m/e 354 was found in the mass spectrum.

However, the formation of **26** could be verified only by its mass spectrum where the M^+ at m/e 340 was present. Further characterization by ^{1}H-NMR could not be done because of the scarcity of the material available.

In conclusion, the first synthesis of achlisocoumarin III methyl ether has been achieved incorporating short and high yielding steps. The methodology thus developed can be applied for the other analogs of achlisocoumarin III. The synthesis of a new compound 3-(3,4-dimethoxyphenyl)-6,8-dimethoxy-1,2,3,4-tetrahydro-1-oxo-naphthalene carboxylic acid has also been achieved.

Experimental

3,4-Dimethoxybenzaldehyde (11): Isovanillin (**10**) (3.04 gm, 20 mmol) dimethyl sulphate (2.52 gm, 20 mmol), and potassium carbonate (2.76 gm, 20 mmol) were heated under reflux in dry acetone (50 ml) for 4 h. The acetone was distilled under reduced pressure and to the residue water (50 ml) was introduced. The solid separated was filtered, washed with water and dried in vacuum desiccator. The product was crystallized from pet.ether (40-60°C) to afford 3,4-dimethoxybenzaldehyde (**11**) as colourless crystalline solid (3.20 gm, 96%); m.p. 43°C (lit.[9] mp. 42-43°C).

3,4-Dimethoxycinnamic acid (12): A mixture of 3,4-dimethoxy benzaldehyde (**11**) (3.00 gm, 18.07 mmol), malonic acid (3.75 gm, 36.05 mmol), pyridine (10 ml) and piperidine (0.5 ml), was heated on a waterbath for 4 hr. The reaction mixture was poured over ice cold dil. HCl (50 ml). The colourless fluffy solid thus precipitated was filtered, washed and air dried. Recrystallization by acetone: hexane afforded 3,4-dimethoxycinnamic acid (**12**) (2.71 gm, 72%); mp. 180-181°C (lit.[10] mp. 180-181.5°C).

3,4-Dimethoxycinnamic acid methyl ester (6): The 3,4-di-methoxycinnamic acid (**12**) (1.04 gm, 5 mmol) was esterified with dimethyl sulphate (0.63 gm, 5 mmol) and potassium carbonate (0.76 gm, 5.5 mmol) in refluxing acetone. The residue obtained after distillation of the acetone was poured into water (20 ml). The colourless solid thus obtained was filtered, washed and dried. The product was recrystallized from hexane to give 3,4-dimethoxycinnamic acid methyl ester (**6**) (1.03 gm, 93%); m.p. 69-70°C (lit.[4] m.p., 68°C).

3-(3,4-Dimethoxyphenyl)-6,8-dimethoxy-1,2,3,4-tetrahydro-1-oxo-naphthalene-2-carboxylic acid methyl ester (14): LDA was generated by adding n-BuLi (1M, 4.8 ml) to diisopropylamine (0.48 gm, 4.8 mmol) in THF (10 ml) at 0°C under N_2 atmosphere. The methyl orsellinate (**13**) (1.00 gm, 4.76 mmol) in THF (10 ml) was added

during 10 min. to the above LDA solution at -78°C. To the resultant orange coloured solution 3,4-dimethoxycinnamic acid methyl ester (**6**) (1.05 gm, 4.72 mmol) in THF (5 ml) was added dropwise. The reaction mixture was stirred at -78°C for 2 h and further 1 hr. at room temperature. The reaction mixture was poured over dilute HCl (20 ml) and the aqueous solution was extracted with CH_2Cl_2 (2 x 25 ml). The combined organic extracts were washed, dried (Na_2SO_4) and evaporated to the yellowish-brown residue. The silica gel column purification (50:50; Hexane: Acetone) of the residue furnished compound **14** as a light yellow solid. (1.26 gm, 66%); m.p. 170°C.

IR (Nujol): 1720 and 1670 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 3.06 (d, J = 6 Hz, 2H); 3.57 (s, 3H, C-O$\underline{CH}_3$); 3.73 (dt, J = 6 Hz, 2 Hz, 1H); 3.82 (s, 12H, 4 x -O$\underline{CH}_3$); 3.88 (d, J = 2Hz, 1H); 6.28 (d, J = 2Hz, 1H); 6.33 (d, J = 2 Hz, 1H); 6.77 (m, 3H).

Ms (m/e.): 400 (M^+)

Analysis calc. for $C_{22}H_{24}O_7$: C = 65.99%; H = 6.03%

Found: C = 65.67% H = 5.98%

3,5-Dimethoxyhomophthalic acid (22): A solution of n-BuLi (1.6 M, 102 mmol) was added to diisopropylamine (10.3 gm, 102 mmol) in THF (40 ml) at 0°C and stirred for 10 min under nitrogen atmosphere. The resultant LDA solution was cooled to -78°C and a solution of orsellinic acid (**23**) (5 gms, 25.5 mmol) and dimethylcarbonate (4.59 gm, 51 mmol) in THF (50 ml) was added during 10 min. After the addition, reaction mixture was warmed to room temperature and stirred further for 4 hr. The reaction mixture was diluted with water (35 ml) and the resultant yellow solution was stirred overnight. The THF was distilled under reduced pressure and the residue was acidified with conc. HCl ($\sim$ 10 ml). The solid thus obtained was filtered, washed with water and air dried. Recrystallization of the solid with hexane: acetone mixture afforded 3,5-dimethoxyhomophthalic acid (**22**) as a colourless crystalline solid (4.3 gm, 70%); m.p.

169-170°C. (lit.[8], m.p. 170-172°C).

Achlisocoumarin III tetramethyl ether (8): The 3,4-dime-thoxycinnamic acid (**12**) (1.16 gm, 5.6 mmol) was refluxed in benzene (25 ml) with thionyl chloride (0.991 gm, 8.4 mmol) for 3 hr. The excess thionyl chloride was distilled off completely along with the benzene under reduced pressure. The crude cinnamoyl chloride (**24**) thus obtained was heated with homophthalic acid (**22**) (0.336 gm, 1.4 mmol) at 190°C for 2 hr. Methanol (10 ml) was added to the reaction mixture and refluxed for 1 hr. The residue obtained after removal of the solvent was chromatographed on silica gel to furnish achlisocoumarin III tetramethyl ether (**8**) as yellow amorphous powder (0.365 gm, 71%); m.p. 128-130°C.

IR (Nujol): 1690 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 3.90 (s, 2 x -OC$\underline{H}_3$); 4.00 (s, -OC$\underline{H}_3$); 4.05 (s, -OC$\underline{H}_3$); 6.30 (s, 1H);6.40 (d, J = 2 Hz, 1H);6.50 (d, J = 2 Hz, 1H); 6.55 (d, J = 16 Hz, 1H); 6.90 (d, J = 8 Hz, 1H); 7.10 (d, J = 2 Hz, 1H); 7.15 (dd, J = 8 Hz, 2Hz, 1H); 7.45 (d, J = 16 Hz, 1H).

Ms (m/e): 368 (M^+).

UV ($CHCl_3$ λ_{max}) 333, 293, 270 and 250 nm

Analysis calc. for $C_{21}H_{20}O_6$: C = 68.43% H = 5.47%

Found: C = 68.32% H = 5.37%

Achlisocoumarin (III) trimethyl ether (25): Achlisocoumarin (III) tetramethyl ether (**8**) (0.10 gm, 0.27 mmol) was refluxed in CH_2Cl_2 (2 ml) with aluminium chloride (0.145 gm, 1.08 mmol) for 1 hr. The reaction mixture after cooling was treated with dil. HCl (2 ml) and the organic layer was separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 2 ml). The combined organic layer was washed with water (2 x 5 ml), dried over Na_2SO_4 and concentrated. The crude residue was purified on column (Hexane-Acetone) to afford achlisocoumarin III trimethyl ether (**25**), m.p. 152-153°C,

(0.080 gm, 83%) and achlisocoumarin III dimethyl ether (**26**) (in trace amount).

25:

IR (Nujol): 1690 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 3.90 (s, -OC$\underline{H}_3$), 3.95 (s, -OC$\underline{H}_3$); 4.00 (s, -OC$\underline{H}_3$); 6.40 (s, 1H), 6.45 (d, J = 2 Hz, 1H); 6.55 (d, J = 2 Hz, 1H); 6.60 (d, J = 16 Hz, 1H); 6.95 (d, J = 8 Hz, 1H); 7.00 (d, J = 2Hz, 1H); 7.20 (dd, J = 8Hz, 2Hz, 1H); 7.40 (d, J = 16 Hz, 1H); 11.15 (s, -O$\underline{H}$).

Ms (m/e): 354 (M^+).

Analysis calc. for $C_{20}H_{18}O_6$: C = 67.77% H = 5.12%

Found: C = 67.80% H = 5.01%

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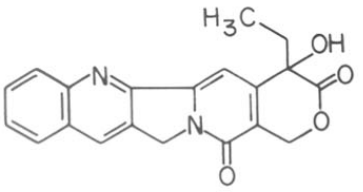
PART 4: SYNTHESIS OF SAINTOPIN

Introduction

A number of clinically important anticancer drugs have recently been shown to kill tumor cells by affecting DNA topoisomerases.[1] Topoisomerases are the essential nuclear enzymes which are important for certain functions of DNA such as replication, transcription etc. Topoisomerase targeting drugs appear to interfere with the breakage - reunion reaction of DNA topoisomerases. In the presence of these drugs a 'cleavable complex' is formed between DNA and topoisomerases which upon exposure to denaturing agents results in the induction of DNA cleavage. However, unlike the other DNA damages, these drugs induced 'cleavable complexes' are reversible and they readily dissociate when the drug is removed from the reaction. The reversibility of this novel DNA damage is probably responsible for the cell death and hence for the antitumor activity of these drugs.

The topoisomerase dependent antitumor drugs are mainly of two types i.e. 'topoisomerase I poisions' such as 'camptothecin[2] (**1**) and 'topoisomerase II poisions' e.g. genistein[3] (**2**), adriamycin [4] (**3**), amsacrine[5] (m-AMSA) (**4**), etoposide[6] (VP-16) (**5**), mitoxantrone[7] (**6**) etc.

Recently, a group of Japanese scientists isolated a new tetracyclic quinone saintopin (**7**), from the Paecilomyces sp. possessing the topoisomerase II dependent DNA cleavage (TDC-II) activity.[8] The manufacture of a novel polycyclic antibiotic UCT-1003 from Paecilomyces has also been reported by the same group of workers[9], however, it should be noted that UCT-1003 is identical to saintopin (**7**). The TDC activity of saintopin, when studied in vitro, was comparable to that of other well known topoisomerase II poisons such as m-AMSA (**4**) and VP-16 (**5**). Saintopin exhibited a weak antimicrobial activity against Gram-positive bacteria but not against Gram-negative bacteria and fungi. Saintopin also showed cytotoxicity against human tumor cell line, $HeLaS_3$ in vitro and against murine leukemia P388 in vivo. Later on Yoshinori



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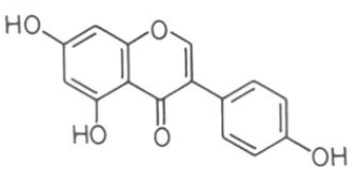

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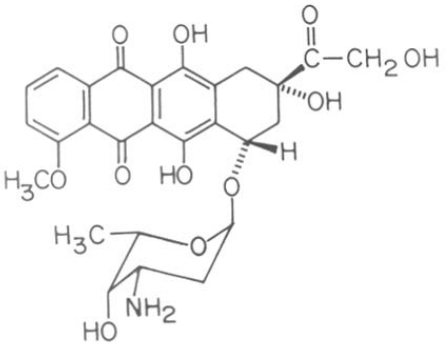

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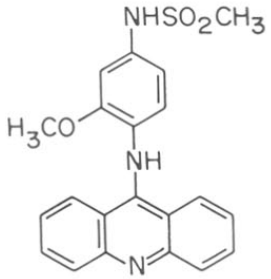

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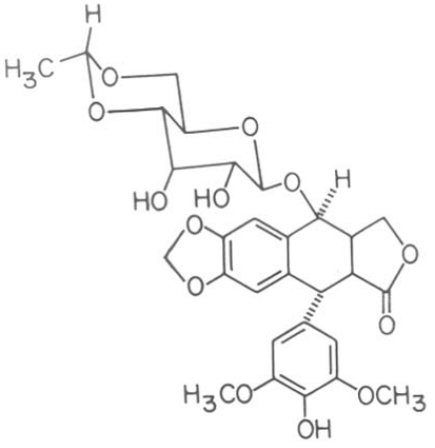

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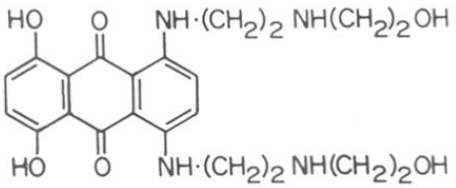

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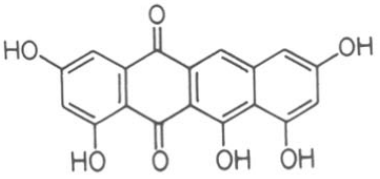


7

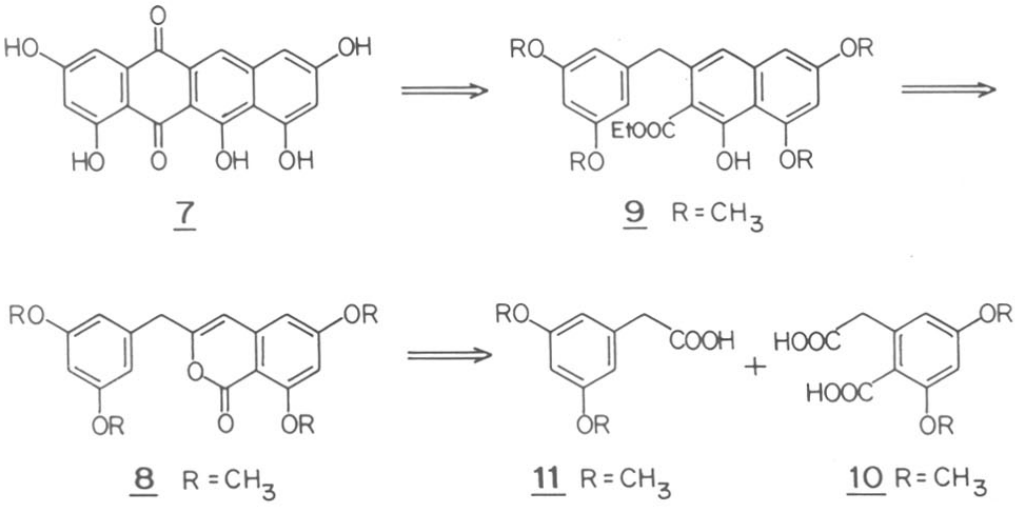
et. al.[10] further studied the biological activity of saintopin and concluded that it induced topoisomerase I mediated DNA cleavage comparable to that of camptothecin as well as topoisomerase II dependent DNA cleavage equipotent to those of well known 'topoisomerase II poisions' such as m-AMSA and VP-16. The DNA cleavage intensity pattern induced by saintopin with topoisomerase I was different from that by camptothecin. A difference in cleavage pattern was also detected between saintopin and m-AMSA or VP-16 in topoisomerase II mediated DNA cleavage. The DNA unwinding assay using T_4 DNA ligase showed that saintopin is a weak DNA intercalator like m-AMSA. Thus, saintopin represents a new class of compounds that can induce both TDC-I and TDC-II activity.

Present work

The interesting biological activity of saintopin coupled with the low yield from natural sources necessitated the need for a synthetic route to saintopin. So far, no synthesis has been reported. The structure of saintopin was assigned on the basis of spectroscopic studies as the pentahydroxy-tetracyclic quinone (**7**). The synthesis of saintopin was undertaken and the same is described in this part.

The reterosynthetic analysis of saintopin (**7**) revealed that the isocoumarin **8** could be the crucial intermediate which would condense with the anion $^{-}CH_2COOEt$ to furnish the naphthalene ester **9**, which inturn could be cyclized and oxidized thereafter to provide the saintopin frame work. The key synthon **8** however, could be derived from the homophthalic acid (**10**) and the phenylacetic acid (**11**).

RETEROSYNTHETIC ANALYSIS



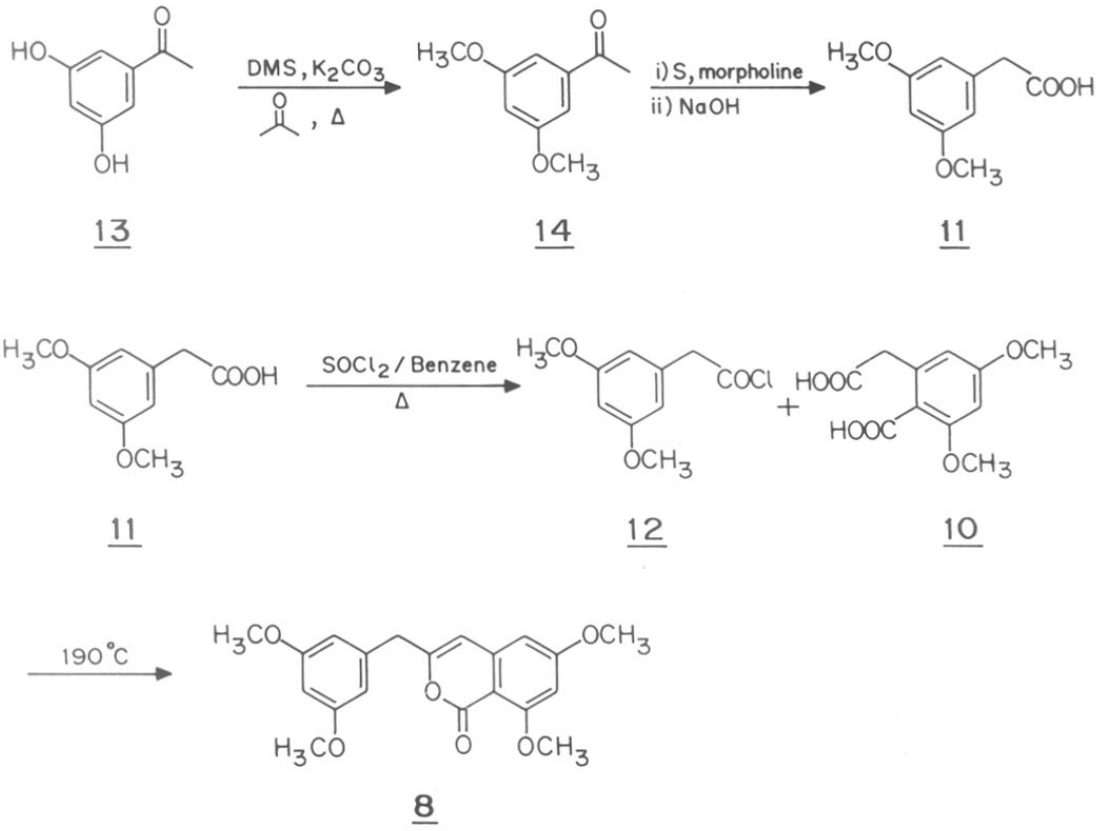
7 **9** R = CH_3 **8** R = CH_3 **11** R = CH_3 **10** R = CH_3

The synthesis of the crucial isocoumarin intermediate **8** seemed quite promising, as the strategy, applied previously for the synthesis of achlisocoumarin III methyl ether

(**Chapter 1: Part 3**), would be quite appropriate here as well. Hence, the condensation of the phenylacetyl chloride (**12**), derived from the phenylacetic acid (**11**), with homophthalic acid (**10**) at 190°C would result into the formation of desired isocoumarin **8**.

The phenylacetic acid (**11**) was prepared from 3',5'-dihydroxy-acetophenone (**13**) in two steps with an overall yield of 52.5% (**Scheme 1.4.1**).

SCHEME-1·4·1



13 14 11

11 12 10

8

Methylation of 3',5'-dihydroxyacetophenone (**13**) with dimethyl sulphate and potassium carbonate in refluxing acetone afforded 3',5'-dimethoxyacetophenone (**14**) in quantitative yield. The treatment of the acetophenone **14** with sulphur and morpholine (Willgerodt reaction) at reflux temperature followed by the hydrolysis of the morpholide formed with alcoholic sodium hydroxide at reflux temperature and subsequent acidification afforded (3,5-methoxyphenyl)acetic acid (**11**). The physical and spectral properties of the acid **11** were in good agreement with the reported values in literature.[11]

The other synthon, homophthalic acid (**10**) has been prepared and used previously in the synthesis of achlisocoumarin III methyl ether (**Chapter 1: Part 3**). Thus, the phenylacetic acid (**11**) was converted to its acyl chloride (**12**) with thionyl chloride in refluxing benzene and the resultant acyl chloride was heated at 190° with homophthalic acid (**10**) for 2 h, to furnish the 3-(3,5-dimethoxyphenyl)-6,8-dimethoxy isocoumarin (**8**) in only 10% yield along with a lot of black coloured charred material (**Scheme 1.4.1**). However, the yield of compound **8** could not be improved besides several changes in reaction conditions. The formation of isocoumarin **8** was further supported by its spectral analysis. The IR spectrum exhibited absorption peak at 1684 cm^{-1}. In the ^{1}H-NMR spectrum (**FIG. I**) three singlets at δ 3.78, 3.85 and 3.95 integrating together for twelve methoxyl protons were observed. The benzylic methylene protons appeared as singlet at δ 3.71 and the cyclic olefinic proton appeared as a singlet at δ 5.95. The aromatic protons appeared as a doublet (J = 2 Hz) at δ 6.28 and a multiplet between δ 6.36 - 6.47. The mass spectrum revealed the molecular ion peak at m/e. 356.

Since the direct condensation of phenylacyl chloride (**12**) with the homophthalic acid (**10**) led to the unambigous yield of the isocoumarin **8**, the alternative method[12], involving the condensation of homophthalic anhydride (**15**) and phenylacetyl chloride

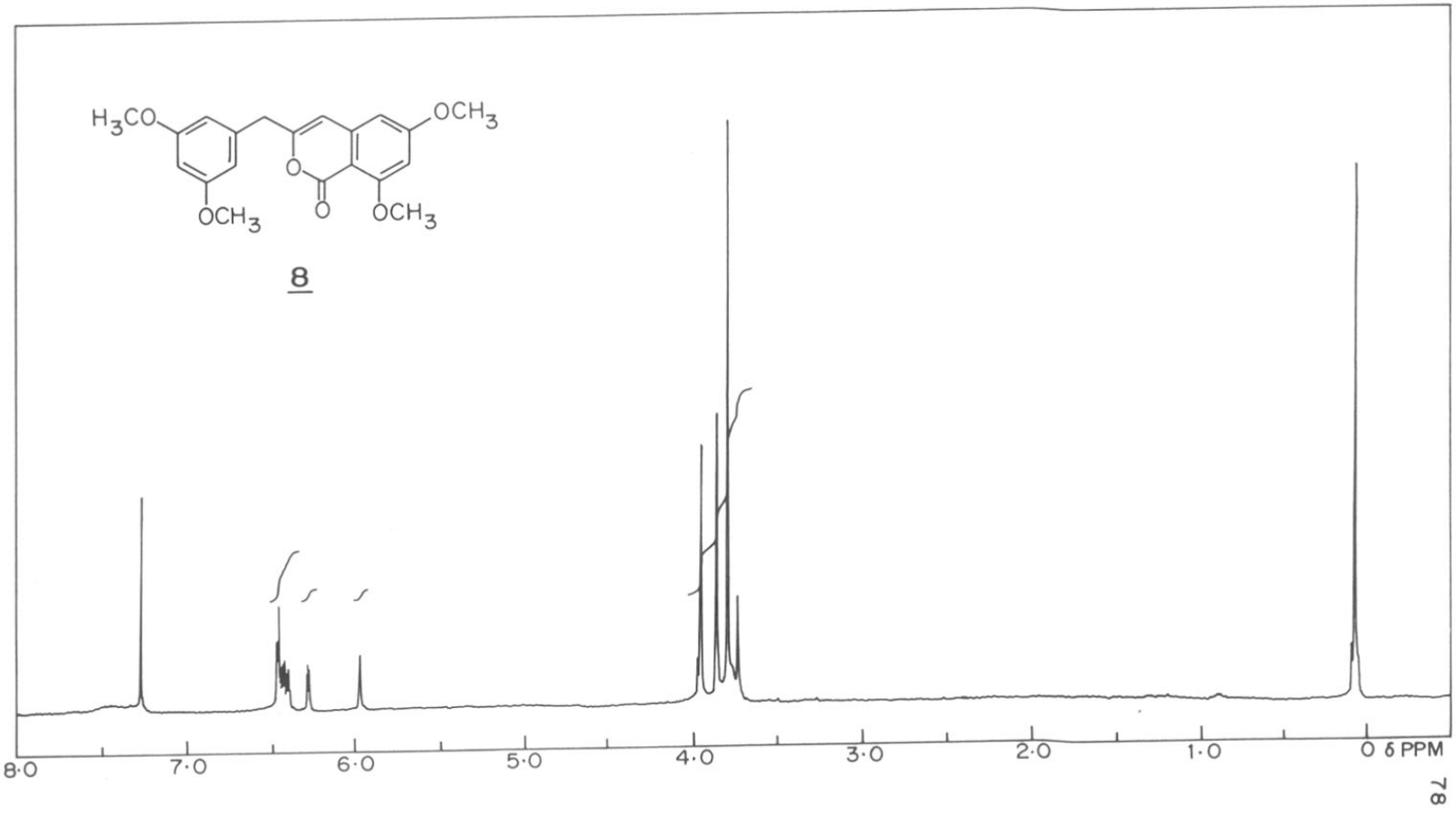
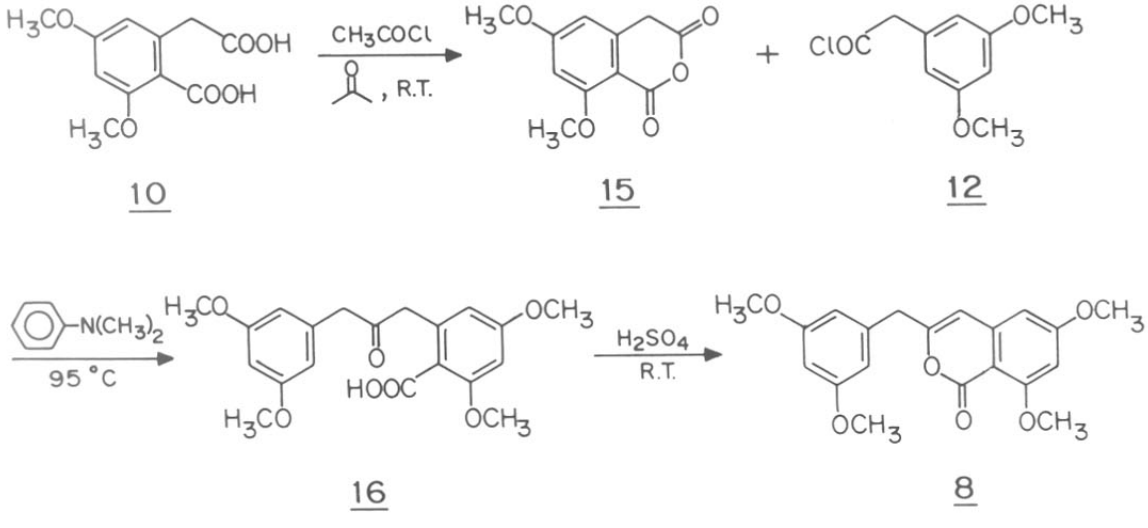


FIG. I : ^{1}H NMR SPECTRUM OF THE COMPOUND(8) IN $CDCl_3$

(**12**) to give the isocoumarin via the intermediate 2-carboxydibenzyl ketone **16** was the obvious choice of method (**Scheme 1.4.2**). Thus, the homophthalic acid (**10**) was converted to its anhydride (**15**) by treatment with acetyl chloride in acetone at room temperature. The homophthalic anhydride (**15**) when condensed with the phenylacetyl chloride (**12**) in presence of N, N-dimethylaniline at 95°C gave the corresponding 2-carboxydibenzyl ketone **16**, which on subsequent cyclodehydration in 95% sulphuric acid afforded the 3-(3',4'-dimethoxyphenyl) 6,8-dimethoxy isocoumarine (**8**) in 52% yield.

SCHEME-1·4·2



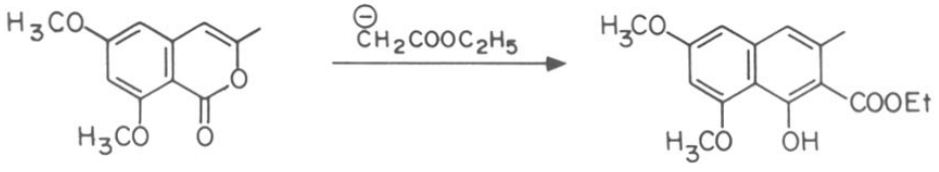
10 15 12

16 8

After obtaining the key synthon **8** the next task was to convert it to the naphthalene carboxylic acid derivative **9**. It has been reported in the literature[13], that the isocoumarin **17**, when treated with the anion of ethyl acetate generated at -78°C by LDA, furnishes a naphthalene derivative **18** (**Scheme 1.4.3**). Hence, the conversion of

8 to **9** looked refreshingly promisable with this pretext.

SCHEME - 1·4·3



17 **18** (87%)

The anion of ethyl acetate was generated at -78°C with LDA and treated with a solution of isocoumarin **8** in THF and DMSO at 0°C. The reaction after quenching with glacial acetic acid afforded the naphthalene derivative **9** in 87% yield (**Scheme 1.4.4**). The product **9** exhibited spectral properties consistent with the assigned structure. The IR spectrum revealed the absorption bands corresponding to hydroxyl and ester carbonyl group at 3380 and 1717 cm^{-1}. The ^{1}H-NMR spectrum (**FIG. II**) revealed a triplet at δ 1.27 and a quartet at δ 4.27 corresponding to the ethyl ester group. The twelve methoxyl protons appear in the form of three singlets at δ 3.82, 3.86 and 3.99. The benzylic methylene protons appeared as singlet at δ 3.71. The aromatic protons showed a multiplet between δ 6.18-6.82 and a singlet at δ 7.05. The chelated phenolic -OH appeared as singlet at δ 10.27. The presence of molecular ion peak at m/e. 426 further lent support to the assigned structure.

Further steps during the course of the synthetic scheme included cyclisation and oxidation of the naphthalene ester derivative **9** to give the saintopin methyl ether derivative. The naphthalene ester was therefore, treated with concentrated sulphuric acid for 30 minutes at room temperature and further 1 h at 60°C to furnish the tetralone **19**.

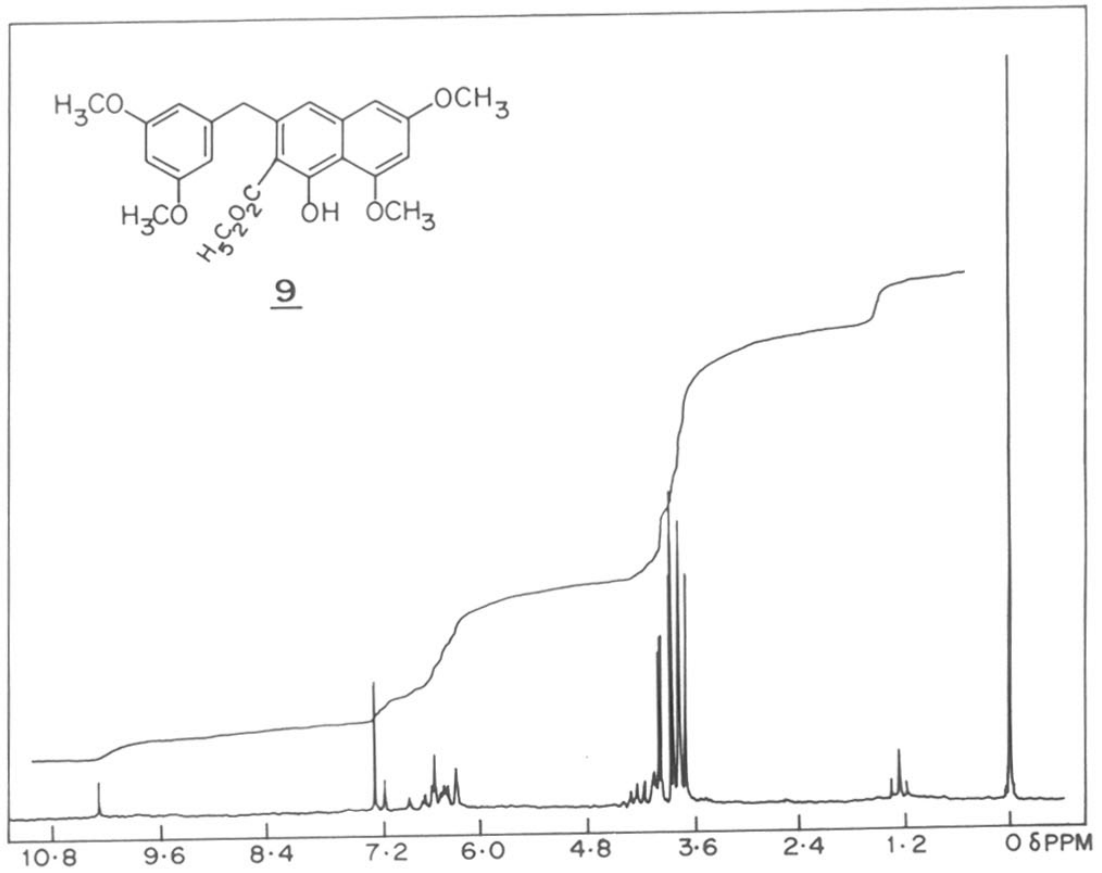
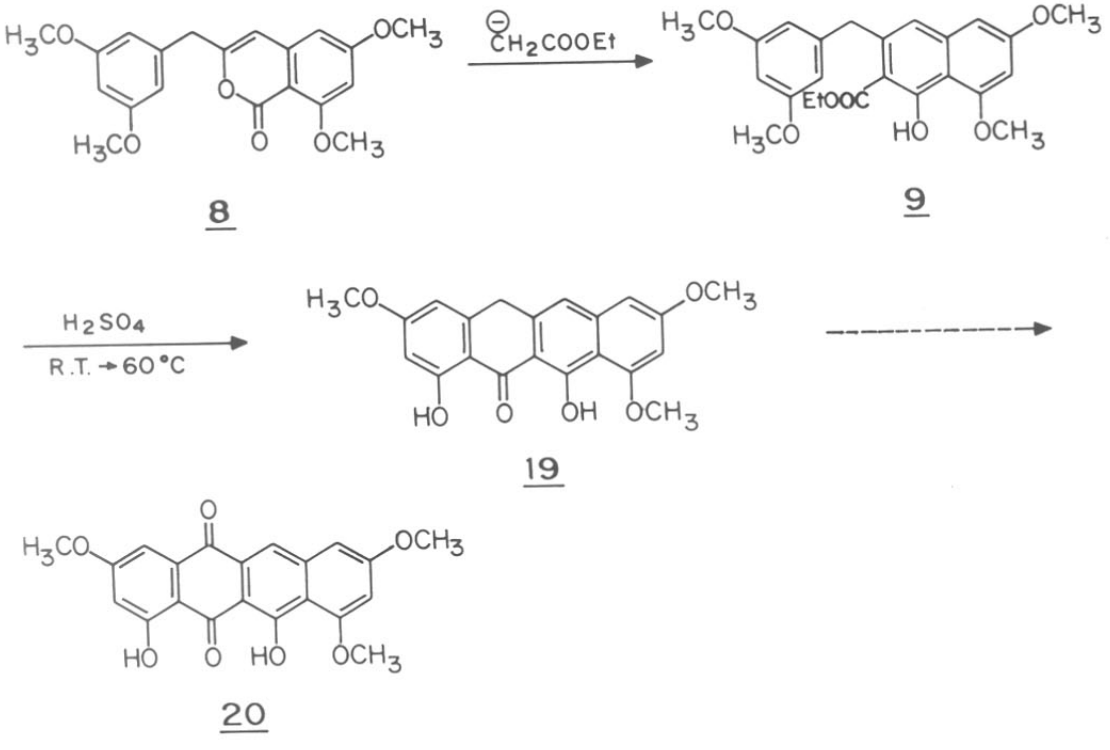


FIG. II : ^{1}H-NMR SPECTRUM OF THE COMPOUND(**9**) IN $CDCl_3$

SCHEME-1·4·4



8 9 19 20

The formation of tetralone **19** was fully confirmed by its relevant spectral data. The IR spectrum of the compound **19** showed carbonyl absorption peak at 1680 cm^{-1}. In the ^{1}H-NMR (**FIG. III**) showed two methoxy singlets at δ 3.95 and δ 4.05 and a benzylic methylene singlet at δ 3.90. The armoatic protons exhibited four meta coupled doublets (J = 2 Hz) at δ 6.35, 6.50, 6.75 and 6.85 along with a singlet at δ 7.25 each integrating separately for a single proton. The hydroxyl protons showed two singlets at δ 10.60 and 10.70. The presence of molecular ion peak at m/e 366 further supported the formation of tetralone **19**.

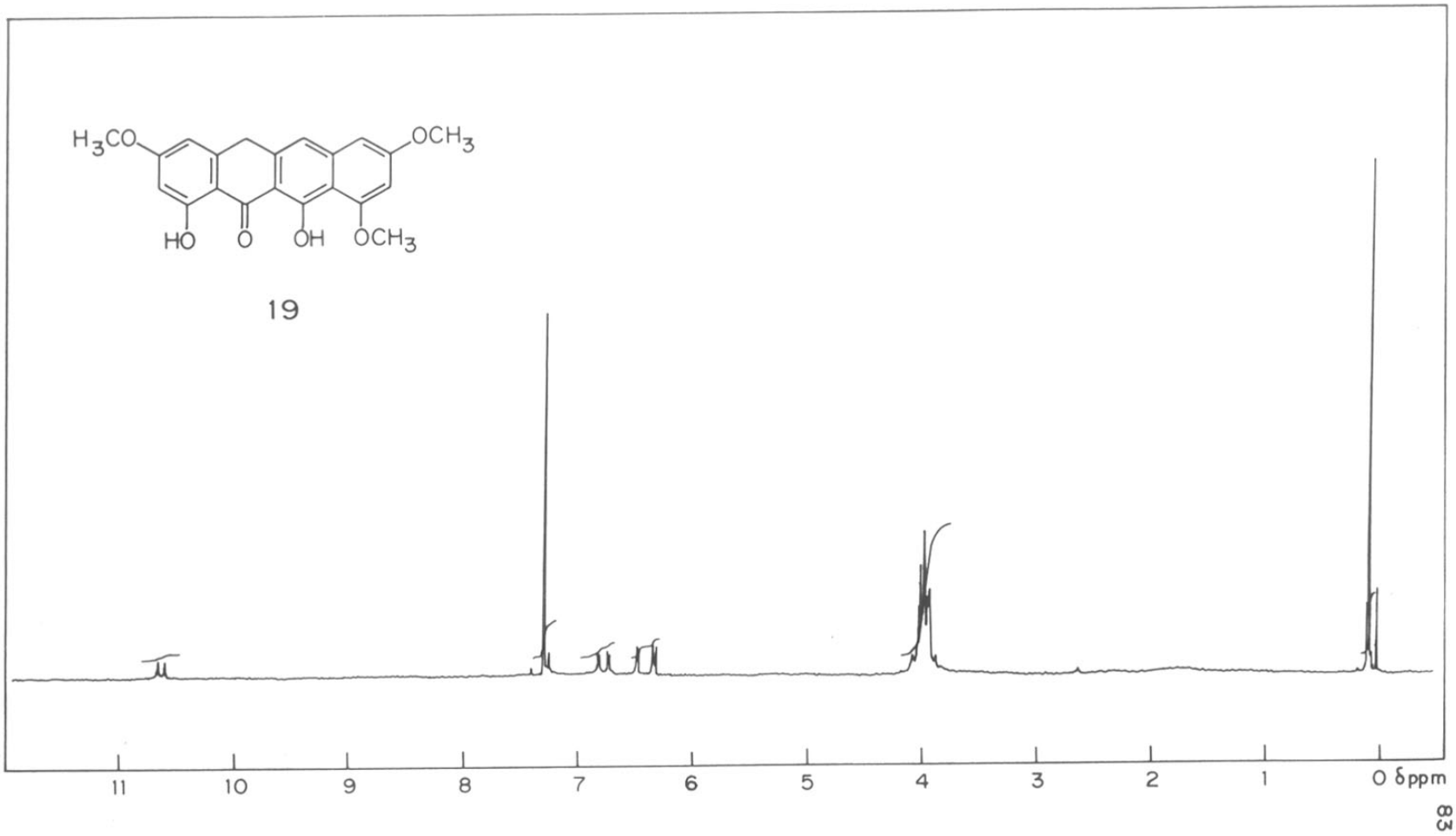


FIG. III : ^{1}H-NMR SPECTRUM OF THE COMPOUND (19) IN $CDCl_3$

The tetralone **19**, when subjected to oxidation with sodium dichromate in acetic acid, gave a mixture of products from which the desired quinone **20** could not be isolated.

In conclusion a short regiospecific synthesis of saintopin has been attempted and the desired tetracyclic framework **19** was obtained. Further conversion of **19** to saintopin is in progress.

Experimental:

3',5'-Dimethoxyacetophenone (14): A mixture of the 3',5'-dihydroxyacetophenone (**13**) (20 g, 132 mmol) and potassium carbonate (40 g, 290 mmol) in dry acetone (200 ml) was refluxed for 10 h. The acetone was distilled off and water (300 ml) was introduced. The aqueous solution was extracted with ethyl acetate (2x100 ml), washed with brine and dried (Na_2SO_4). The removal of solvent under reduced pressure furnished 3',5'-dimethoxyacetophenone (**14**) (23.0 g, 97%) as a yellow viscous oil which was used as such for the next step.

3,5-Dimethoxyphenyl)acetic acid (11): 3',5'-Dimethoxy acetophenone (**14**) (22.5 g, 125 mmol) was heated under reflux with sulphur powder (6.0 g, 188 mmol) and morpholine (13.5 g, 155 mmol). After 18 h the reaction mixture was cooled and poured into cold water (500 ml). The brown coloured thick semisolid mass thus obtained, after washing thoroughly with water, was refluxed with a 10% ethanolic sodium hydroxide solution (200 ml) for 12 h. The ethanol was distilled off under reduced pressure, the residue was diluted with water and acidified with conc. HCl. The colourless solid so obtained was filtered, washed with cold water and air dried to afford (3,5-dimethoxyphenyl)acetic acid (**11**) (13.25 g, 54%), m.p. 100-101°C (lit.[11] m.p. 100-102°C).

3-(3,5-dimethoxybenzyl)6,8-dimethoxy isocoumarin (8): (i) By the direct condensation of homophthalic acid (10) and 3',5'-dimethoxyphenylacetyl chloride (12): (3,5-Dimethoxyphenyl)acetic acid (**11**) (1.07 g, 5 mmol) was refluxed with thionyl chloride (0.644 gm, 5 mmol) for 2h. The excess of thionylchloride was distilled off along with benzene under reduced pressure. To the crude acetyl chloride **12**, homophthalic acid (0.287 g, 1.2 mmol) was added and the reaction mixture was heated at 190°C for 1.5 h. The reaction mixture was cooled and CH_2Cl_2 (20 ml) was added. The CH_2Cl_2 solution was washed with saturated sodium hydrogen carbonate solution (2

x 25 ml) and water (2 x 25 ml) and then dried over sodium sulphate. The residue obtained after concentration was purified by column chromatography (hexane-acetone, 9:1 as eluent) to give 3-(3',5'-dimethoxy-benzyl) 6,8-dimethoxy isocoumarin (**8**) (0.043 g, 10%) as a sticky solid.

IR ($CHCl_3$): 1684 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 3.71 (s, 2H); 3.78 (s, 6H); 3.85 (s, 3H); 3.95 (s, 3H); 5.95 (s, 1H); 6.28 (d, J = 2 Hz, 1H); 6.36 - 6.47 (m, 4H).

Ms (m/e): 356 (M^+).

(ii) By the indirect method via the intermediate 2-carboxydibenyl ketone (16): The homophthalic acid (**10**) (0.240 gm, 1 mmol) was dissolved in a mixture of acetyl chloride (0.3 ml) and acetone (5 ml) and stirred for 0.5 h at room temperature. The solvent was removed under vacuum to afford homophthalic anhydride (**15**) (0.215 g, 97%). A mixture of the above homophthalic anhydride **15**, (3,5-dimethoxyphenyl)acetyl chloride (**12**) [prepared from (3,5-dimethoxyphenyl) acetic acid (0.390 gm, 2 mmol) and thionyl chloride (0.236 gm, 2 mmol)] and N,N-dimethylaniline (0.5 ml) was heated at 100°C for 6 h. The reaction mixture was cooled and 25% H_2SO_4 was added with ice cooling. The aqueous solution was extracted with ethyl acetate (2 x 20 ml). The combined extracts were washed with water (2 x 25 ml) and extracted with saturated sodium hydrogen carbonate solution (2 x 25 ml). The alkaline aqueous phase was acidified with conc. HCl and extracted with ethyl acetate (2 x 20 ml). The combined organic phase was dried (Na_2SO_4) and concentrated to give a light brown residue. The residue containing the crude 2-carboxydibenzyl ketone **16** was mixed with 95% H_2SO_4 (20 ml) and left overnight. The mixture was poured carefully on ice-water and extracted with chloroform (2 x 15 ml). The chloroform extract was washed with $NaHCO_3$ solution and water and dried (Na_2SO_4). The residue obtained after concentration was purified on silica gel column using pet.ether - acetone (9:1) as eluent to give the isocoumarin **8**

(0.179 g, 52%) as a sticky solid.

3-(3,5-dimethoxybenzyl)-6,8-dimethoxy-1-hydroxy-naphthalene- 2-carboxylic acid ethyl ester (9): LDA solution was generated by adding n-butyl lithium (1 ml, 1.9 mmol) to diisopropyl amine (0.25 ml, 1.9 mmol) in THF (2 ml) at 0°C under argon atmosphere. The solution was cooled to -78°C and dry ethyl acetate (0.167 g, 1.9 mmol) was added and the reaction mixture was stirred for 0.5 h. The anion was then transferred rapidly to a solution of isocoumarin **8** (0.178 g, 0.5 mmol) in THF (1 ml) and DMSO (1 ml) at 0°C. After 0.5 h the yellow solution was quenched with acetic acid (1 ml) and stirred at room temperature for 48 h. The reaction mixture was diluted with water (10 ml) and aqueous solution was extracted with chloroform (2 x 10 ml). The combined extracts were washed with water, dried (Na_2SO_4) and concentrated. The yellow residue was purified by column chromatography (pet ether-acetone 8:2) to give the naphthoate **9** (0.186 g, 87%) as a thick oil.

IR ($CHCl_3$): 3380 and 1717 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.24 (t, 3H); 3.68 (s, 2H); 3.76 (s, 6H); 3.84 (s, 3H); 3.92 (s, 3H); 4.08 (q, 2H); 6.16-6.56 (m, 5H); 6.92 (s, 1H); 10.04 (s, 1H, O<u>H</u>).

Ms (m/e): 426 (M^+).

Cyclization of the naphthoate 9 with concentrated H_2SO_4: The naphthoate **9** (0.100 g, 0.2 mmol) was dissolved in conc. H_2SO_4 (1 ml) and stirred at room temperature for 0.5 h. The reaction mixture was warmed to 60°C and stirred for 1 h. The reaction mixture was cooled and poured into ice-water (10 ml) and extracted with ethyl acetate (2 x 10 ml). The combined extracts were washed with water (2 x 10 ml), dried (Na_2SO_4) and evaporated. The residue after column purification afforded tetralone **19** (0.086 g, 70%) as a yellow solid.

IR ($CHCl_3$): 1680 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 3.90 (s, 2H); 3.95 (s, 3H); 4.05 (s, 3H); 6.35 (d, J = 2 Hz,

1H); 6.50 (d, J = 2 Hz, 1H); 6.75 (d, J = 2 Hz, 1H); 6.85 (d, J = 2 Hz, 1H); 7.25 (s, 1H); 10.60 (s, 1H); 10.70 (s, 1H).

Ms (m/e): 366

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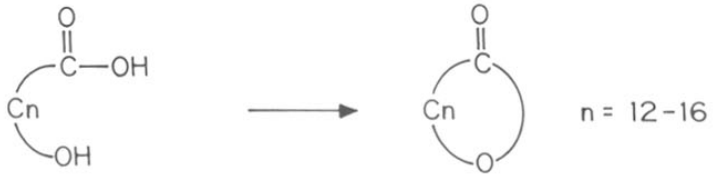
CHAPTER II

STEREOSPECIFIC SYNTHESIS OF BIOACTIVE M5032 AND 12-OXOCURVULARIN AS THEIR DIMETHYL ETHER

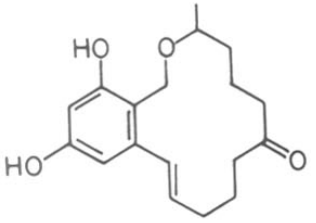
General Introduction

The macrocyclic structural spectrum is perhaps one of the richest in natural product chemistry. The origin of macrocyclic compound chemistry dates back to 1926 when the structure of civetone and muscone were elucidated.[1] However, the isolation of the first macrocyclic antibiotic picromycin[2] in 1950 boosted thereafter the isolation of several macrocyclic compounds with a wide spectrum of biological activities.

Generally, a "macrolide" is defined as a molecule containing a large ring lactone (12-16 membered) in the structure, but in somewhat broader sense it sometimes denotes the medium ring lactones (8-11 membered) as well. Even the macrocyclic lactams such as maytansinoides and rifamycins, the useful drugs for leukemia and tuberculosis respectively, are also termed as 'macrolides'[3].



There are several types of macrolides viz 12, 14 and 16 membered macrolides, polyene macrolides, ionophoric macrolides etc. The "polyoxo' macrolides constitute the 12, 14 and 16 membered macrolides. They include several biologically active compounds of either mould or bacterial origin. Some important ones worth mentioning are zearalenone (**1**)[4], curvularin (**2**)[5], vermiculine (**3a**)[6], pyrenophorin (**3b**)[7], brefeldin A (**4**)[8], patulolide A (**5**)[9], monocillin (IV) (**6**)[10], recifeiolide (**7**)[11] etc. Zearalenone (**1**), radicicol (**8**) and monocillin (IV) (**6**) are examples of "ß-resorcylic acid group of macrolide antibiotics"[10]. The polyene macrolide antibiotics constitute a group possessing strong antifungal activity. The ionophor-



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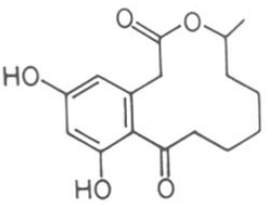

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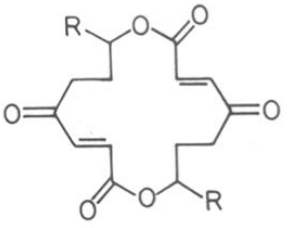
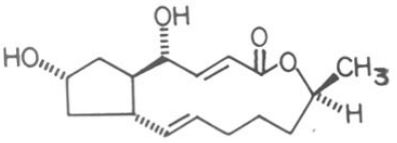


3a R=$CH_2 \cdot CO \cdot CH_3$
3b R=CH_3



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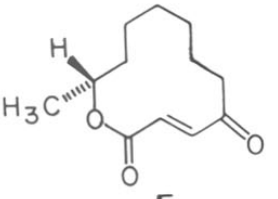

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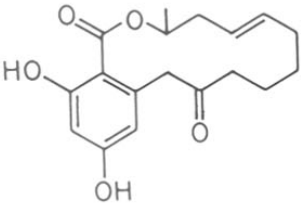

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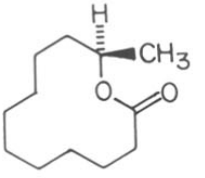

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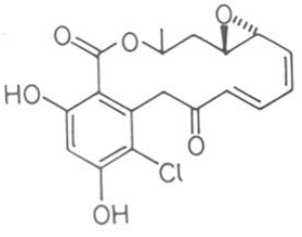

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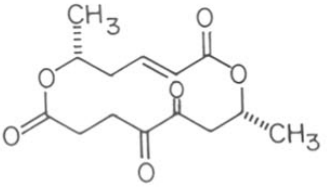

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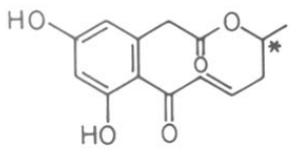
ic macrolides alternatively termed as oligonolides possess two or more lactone groups in the molecule e.g. grahamimycin A_1 (**9**)[12]. Total synthesis of several of these complex macrolides have been accomplished. The complexicity and interesting biological activity of the macrolides has made them a challenging and interesting subject for the organic chemists and consequently several reviews depicting structure, synthesis, biogenesis, biological activity etc. of macrolides have appeared.[13]

This chapter deals with the stereospecific synthesis of two biologically active macrolides, namely a new bioactive substance M5032 and 12-oxo curvularin, as their methyl ethers. For convenience it has been subdivided as part 1 and part 2.

PART 1: STEREOSPECIFIC SYNTHESIS OF M5032 DIMETHYL ETHER

Introduction

The bioactive substance M5032 (**10**) 4,5-dihydro-9 , 11-dihydroxy-4-methyl-2H-3-benzoxecin-2,8-(1H)-dione, is a new macrocyclic lactone isolated from the culture filtrate of the microorganism belonging to the Sporomylar sp. (Bacterial research number 9506)[14]. The M5032 possesses enzyme hindrance activity capacity for the circular (ring shaped) adenosyn 3',5'-monophosphoric acid phospho diester (cAMP-PDE). The AMP-PDE is one of the enzymes which regulates the concentration of the adenosyn 3',5'-monophosphoric acid (cAMP) inside the cells. The cAMP plays an important role in the metabolism of mammalian cells as a second messenger mediating the action of various hormones and is thought to be concerned with the control of many cellular functions e.g. cleavage, cultivation etc. The substance with the cAMP PDE hindrance activity raises the level of CAMP inside the cells and hence is expected to be useful as a drug for the cure of many diseases.



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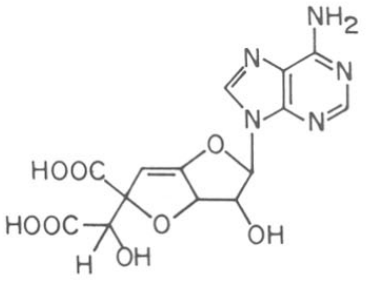

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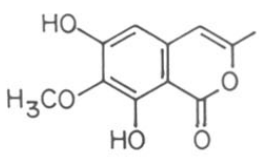

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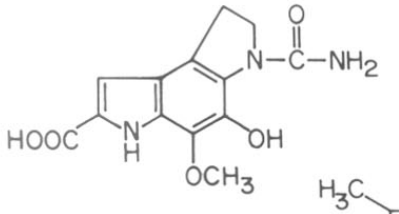

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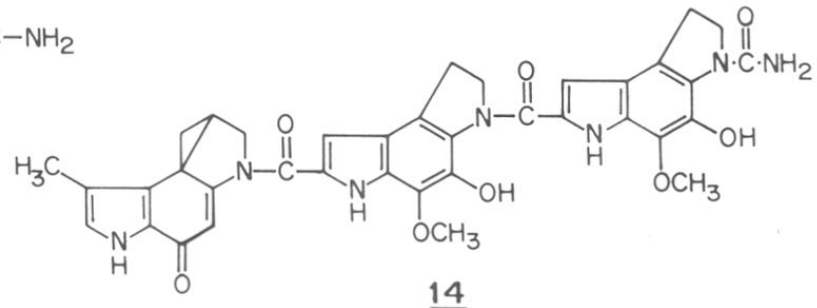

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Griseolic acid (**11**)[15], reticulol (**12**)[16], PDE-I (**13**)[17], CC 1065 (**14**)[18], terferol[19], ADP-I, II and III[20] etc. are some other natural products isolated from various microorganisms possessing the cAMP-PDE hindrance activity.

The M5032 is expected to be useful in the cure of the blood vessel damage diseases as a curing agent, as an agent in cure of respiratory diseases, as a muscular pain relieving agent, an inflammatory diseases curing agent, cancer curing agent etc. It can be dosages to humans or to the domestic animals either orally or by injection.

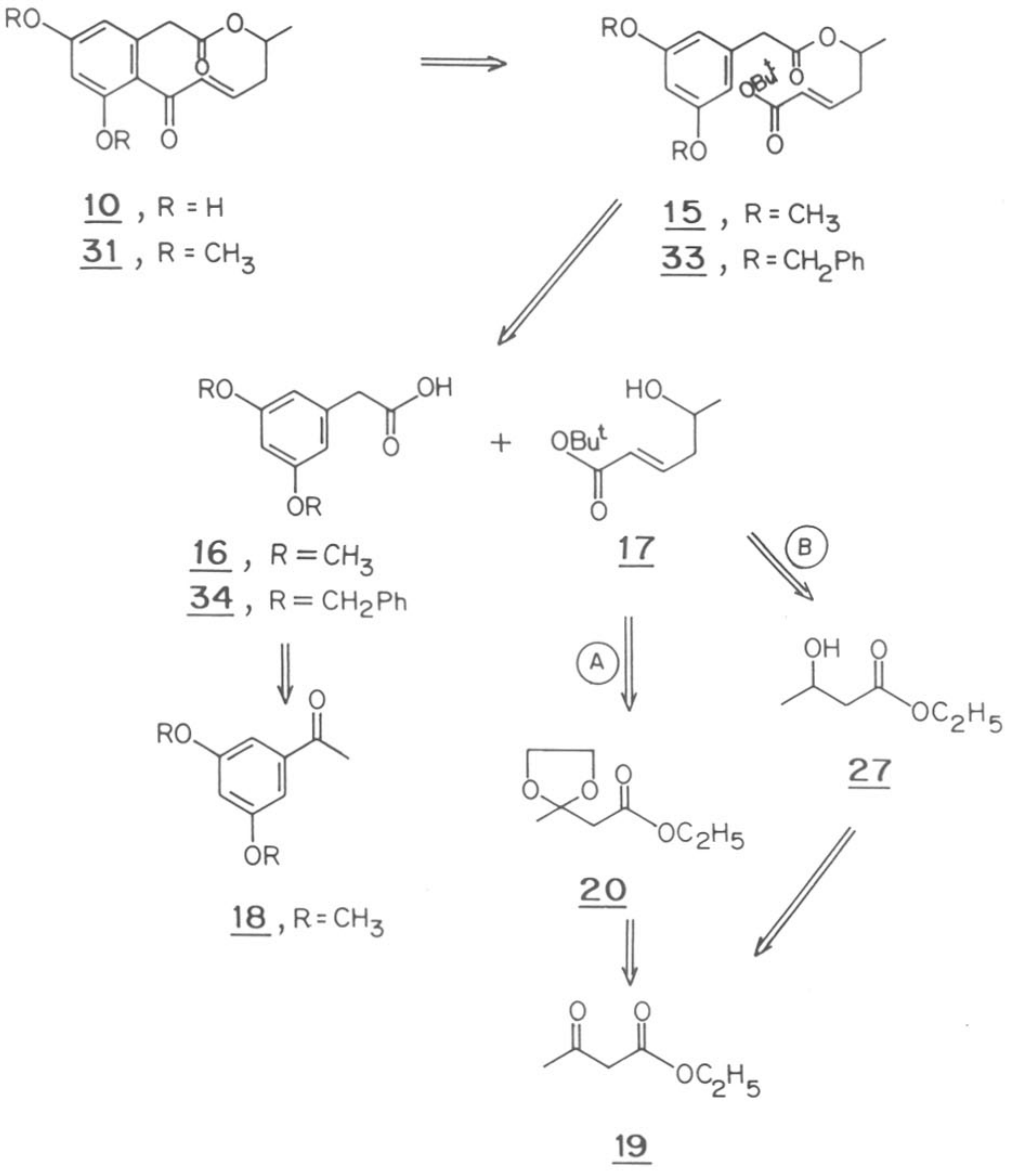
Present work

The interesting biological activity coupled with its typical chemical structure viz possessing an α-ß unsaturated double bond in the 10-membered ring with an asymmetric centre at C_4, made it quite appropriate to design a synthesis for M5032. The synthesis of M5032 has not been reported so far. Thus, the task of developing a synthetic route to M5032 was undertaken and a successful synthesis of M5032 dimethyl ether has been described in this part.

The retrosynthetic analysis (**Scheme 2.1.1**) suggested that the suitably substituted phenylacetic acid **16** and the C_6-hydroxy unit **17** would be the logical synthons which would condense to give the ester **15**. The ester **15** could be subsequently cyclized to form the desired **10** membered ring. The required phenyl acetic acid (**16**) could be obtained from 3',5'-dimethoxy acetophenone (**18**) whereas the C_6 hydroxy unit (**17**) could be derived from ethyl acetoacetate (**19**) either by route A or route B.

Desired 3,5-dimethoxyphenylacetic acid (**16**) has already been prepared and used for the synthesis of saintopin (Chap-I: Part 4). For the synthesis of the aliphatic counterpart **17**, ethyl acetoacetate (**19**) was protected as its ketal **20** by ethylene glycol in presence of PTSA. The ketal **20** was then reduced with lithium aluminium hydride in ether to afford the alcohol **21** in 91% yield. The alcohol **21** thus obtained was oxidized to aldehyde **22** with pyridinium chloro chromate (PCC) in CH_2Cl_2 in 70% yield. The Wittig reaction of the aldehyde **22** with t-butoxycarbonyl methylenetriphenylphosphorane (**23**) furnished the α-ß-unsaturated ester **24**. The deprotection of ketal of **24** by glacial acetic acid and water afforded keto-ester **25** in quantitative yield. The keto-ester **25** was subjected to carbonyl reduction with sodium borohydride in ethanol when a mixture of alco-

SCHEME 2·1·1



10 , R = H
31 , R = CH_3

15 , R = CH_3
33 , R = CH_2Ph

16 , R = CH_3
34 , R = CH_2Ph

17

18 , R = CH_3

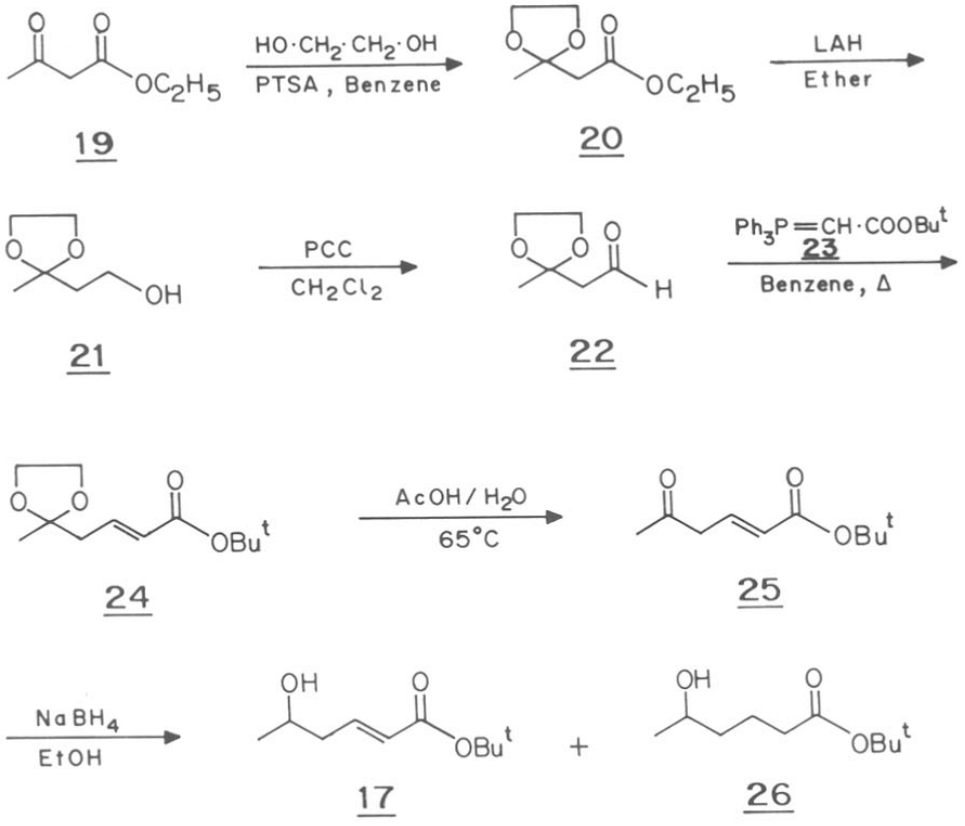
20

27

19

hols **17** and **26** were obtained (**Scheme 2.1.2**). The formation of the alcohols **17** and **26** in almost equal amounts, was determined by the ^{1}H-NMR spectrum.

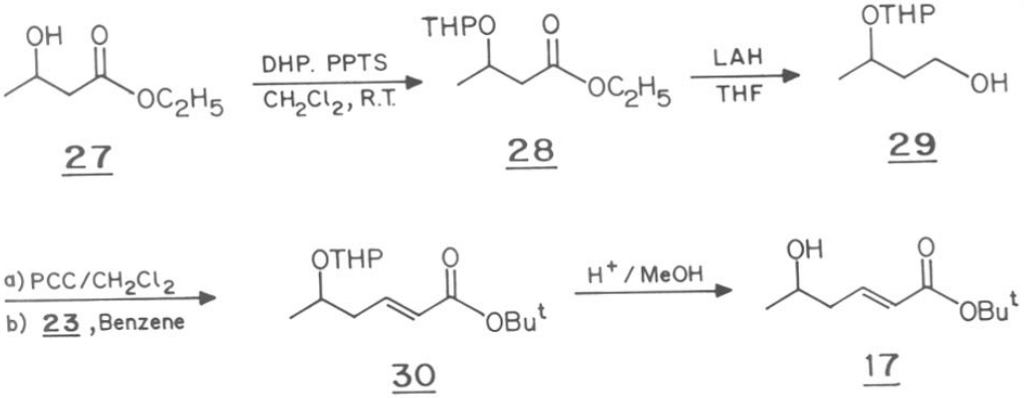
SCHEME 2·1·2



As the separation of alcohols **17** and **26** could not be achieved, route A was abandoned. The preparation of alcohol **17** was undertaken starting from ethyl 3-hydroxybutyrate by route B. Ethyl 3-hydroxybutyrate (**27**) has been prepared earlier and used in the synthesis of orthosporin (Chapter-I:Part 1) Thus, the hydroxy group of ethyl 3-hydroxybutyrate (**27**) was protected as tetrahydropyranyl ether (**28**) by treatment with dihydropyran in CH_2Cl_2 in presence of PPTS.

The THP ether **28** was then reduced with LAH to furnish alcohol **29** in 78% yield. The PCC oxidation of alcohol **29** followed by Wittig condensation with the ylide **23** gave α-ß unsaturated ester **30** in 62% yield. The tetrahydropyranyl ether of **30** was cleaved with methanol-hydrochloric acid to afford the desired six carbon unit-tert-butyl-5-hydroxy-2-(E)-hexenoate (**17**) (**Scheme 2.1.3**).

SCHEME 2·1·3



The hydroxy ester **17** was fully characterized by its spectral analysis. The IR spectrum showed absorption bands at 3420 cm^{-1} and 1720 cm^{-1} for the hydroxy and the ester carbonyl absorptions respectively. In the ^{1}H-NMR spectrum (**FIG. I**) a doublet at δ 1.23 (J = 6 Hz) for the methyl group, a singlet at δ 1.50 for the tertiary butyl group and a multiplet between δ 2.16 - 2.53 for the methylene were also seen. The multiplet between δ 3.70 - 4.13 corresponds to the -$\underset{\text{O}}{\underset{|}{\text{C}}}$H - proton. The trans olefinic protons were characterized by a doublet of triplet (J = 16 Hz, J > OHz) at 5.80 and a doublet of triplet (J = 7 Hz, J > OHz) at δ 6.83 respectively. The mass spectrum showed a peak at m/e 130 for M^+ - 56 i.e. loss of $+\underset{\text{CH}_3}{\underset{|}{\overset{\text{CH}_3}{\overset{|}{\text{C}}}}}-\text{CH}_3$ and 171 for (M^+-15).

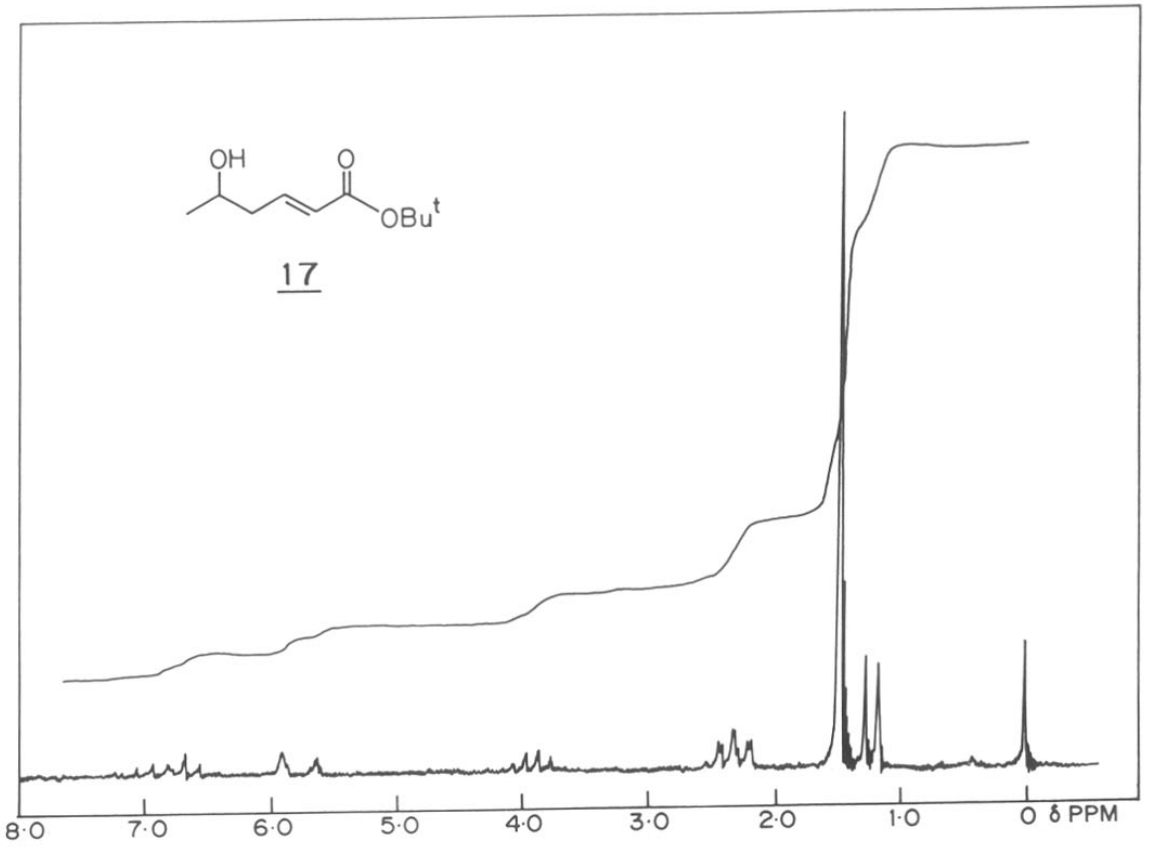
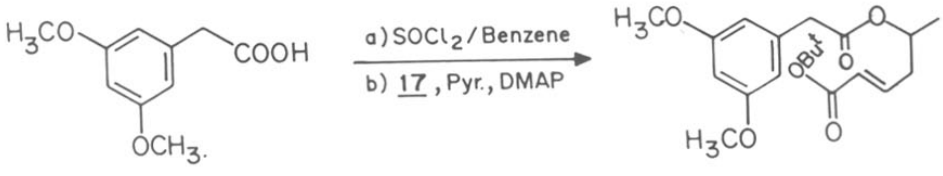


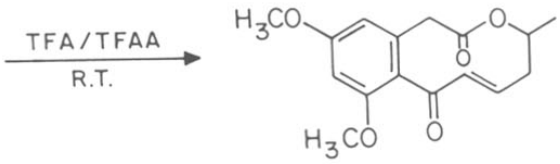
FIG. I : ^{1}H-NMR SPECTRUM OF THE COMPOUND(17) IN $CDCl_3$

Now, with both the desired synthons i.e. 3,5-dimethoxyphenylacetic acid (**16**) and t-butyl-5-hydroxy-2-hexenoate (**17**) in hand, the remaining task was to join them to form the desired 10-membered macrocyclic lactone. The 3,5-dimethoxyphenyl acetic acid (**16**) was converted into its corresponding acetylchloride by refluxing with thionyl chloride in benzene and the acetyl chloride formed was subsequently esterified with the alcohol **17** in presence of pyridine and catalytic amount of DMAP to afford ester **15** in 60% yield (**Scheme 2.1.4**).

SCHEME 2·1·4



16 15



31

The formation of **15** was supported by its spectral analysis. The IR spectrum showed two carbonyl absorption bands at 1730 and 1670 cm^{-1}. The ^{1}H-NMR (**FIG.II**) showed a methyl doublet at δ 1.18 and three singlets at δ 1.43, 3.50 and 3.75 corresponding to the tert butyl, benzylic and two methoxyl protons respectively. The methylene protons appeared as a multiplet between δ 2.12 - 2.56 and the -C<u>H</u>-proton showed a multiplet between δ 4.80 - 5.06. The olefinic pro-
|
O

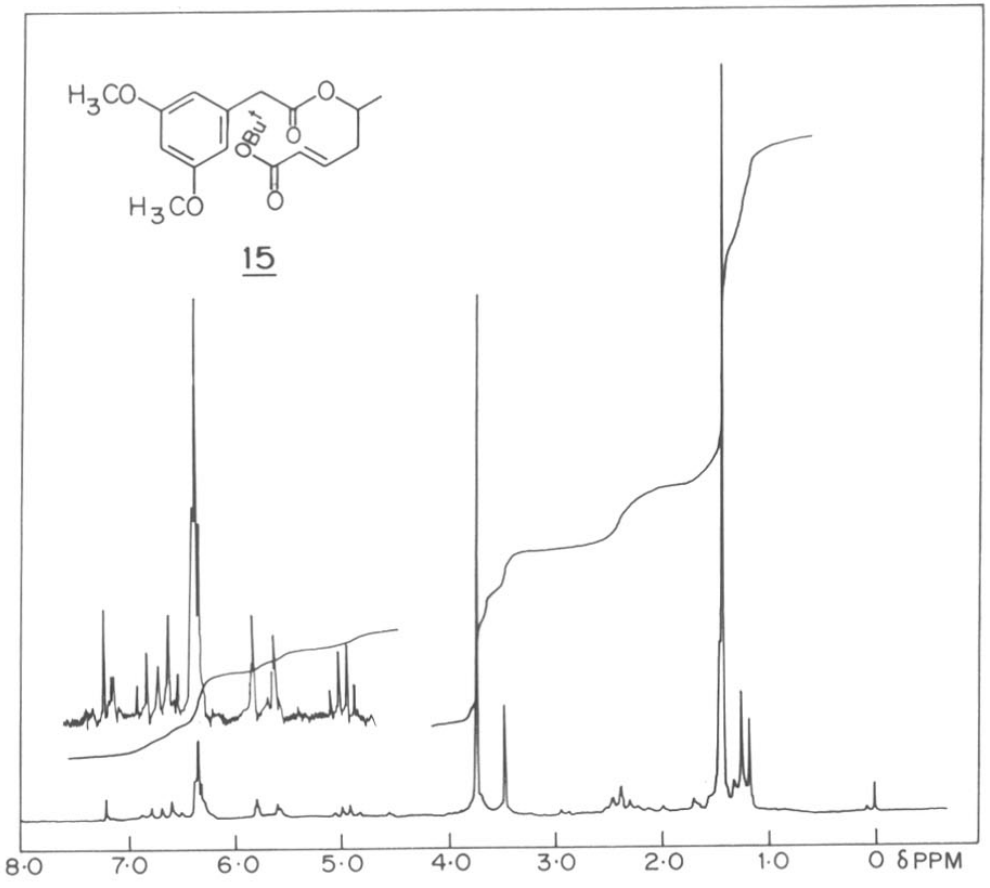


FIG. II: ^{1}H-NMR SPECTRUM OF COMPOUND(15) IN $CDCl_3$

tons appeared at δ 5.70 and 6.68 along with the three aromatic protons appearing as a broad singlet at δ 6.34. The mass spectrum revealed a peak at m/e 308 corresponding to M^+-56.

The removal of tertiary butyl group is known to take place in the presence of trifluoroacetic acid[21] and on the other hand trifluoroacetic anhydride and trifluoroacetic acid is also used for bringing out the intramolecular cycloacylation.[22]

Thus, the deprotection of the tert-butyl group in **15** and in situ cycloacylation thereby to afford dimethyl ether of M5032 (**31**), seemed refreshingly promisable in the mixture of trifluoroacetic anhydride and trifluoroacetic acid. The ester **15** was stirred with a 5:1 mixture of trifluoroacetic anhydride and trifluoroacetic acid under nitrogen at room temperature for 6 h to give, after chromatographic purification, M5032 dimethyl ether (**31**) in 19% yield (**Scheme 2.1.4**). The compound **31** showed spectral properties consistent with the assigned structure. The IR spectrum revealed two carbonyl absorption bands at 1731 and 1667 cm^{-1}. The ^{1}H-NMR (**FIG. III**) exhibited a methyl doublet (J = 6 Hz) at δ 1.20 and two multiplets at δ 2.20 and δ 3.10 each integrating for single proton. The benzylic protons appeared as two doublets (J = 16.5 Hz) at δ 3.64 and δ 4.05 respectively. Two-methoxyl singlets were present at δ 3.85 and δ 3.90. The multiplet of -C<u>H</u>- appeared as δ 5.20 and trans olefinic protons were observed as doublet of triplet at δ 5.95 (J = 7 Hz) and at δ 7.01 (J = 16 Hz, J >OHz) respectively. The two aromatic protons appeared as doublets (J = 2 Hz) at δ 6.35 and δ 6.42). The UV spectrum in $CHCl_3$ showed two absorption maxima at 273-80 and 242.00 nm. The presence of the molecular ion peak at m/e 290 in mass spectrum was observed. The ^{13}C-NMR (**FIG. IV**) of the compound (**23**) further supported the assigned structure.

The successful employment of the methodology towards the synthesis of racemic M5032 methyl ether (**31**) provided the base for its stereospecific synthe-

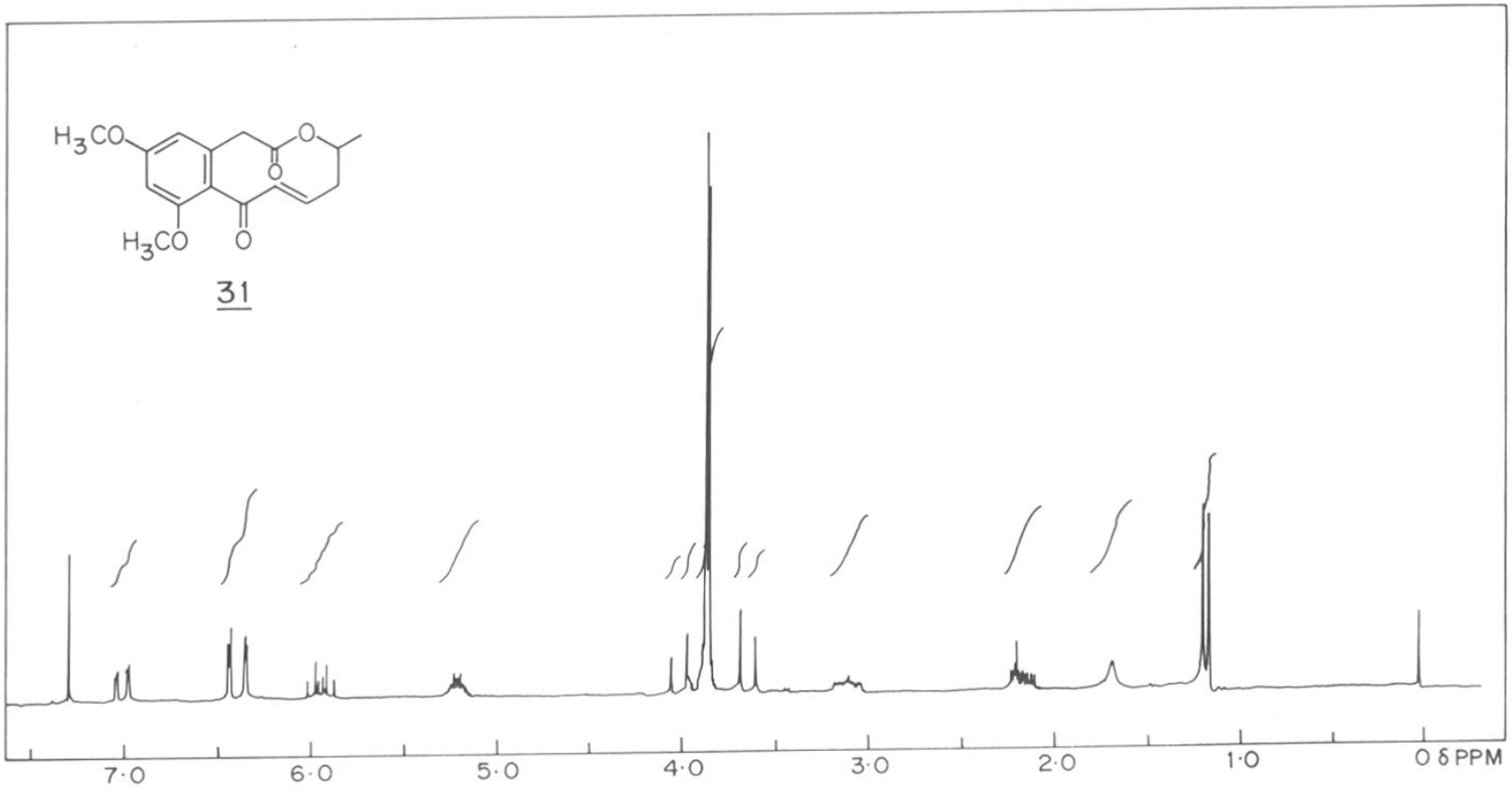


FIG. III : ^{1}H-NMR SPECTRUM OF THE COMPOUND (**31**) IN $CDCl_3$

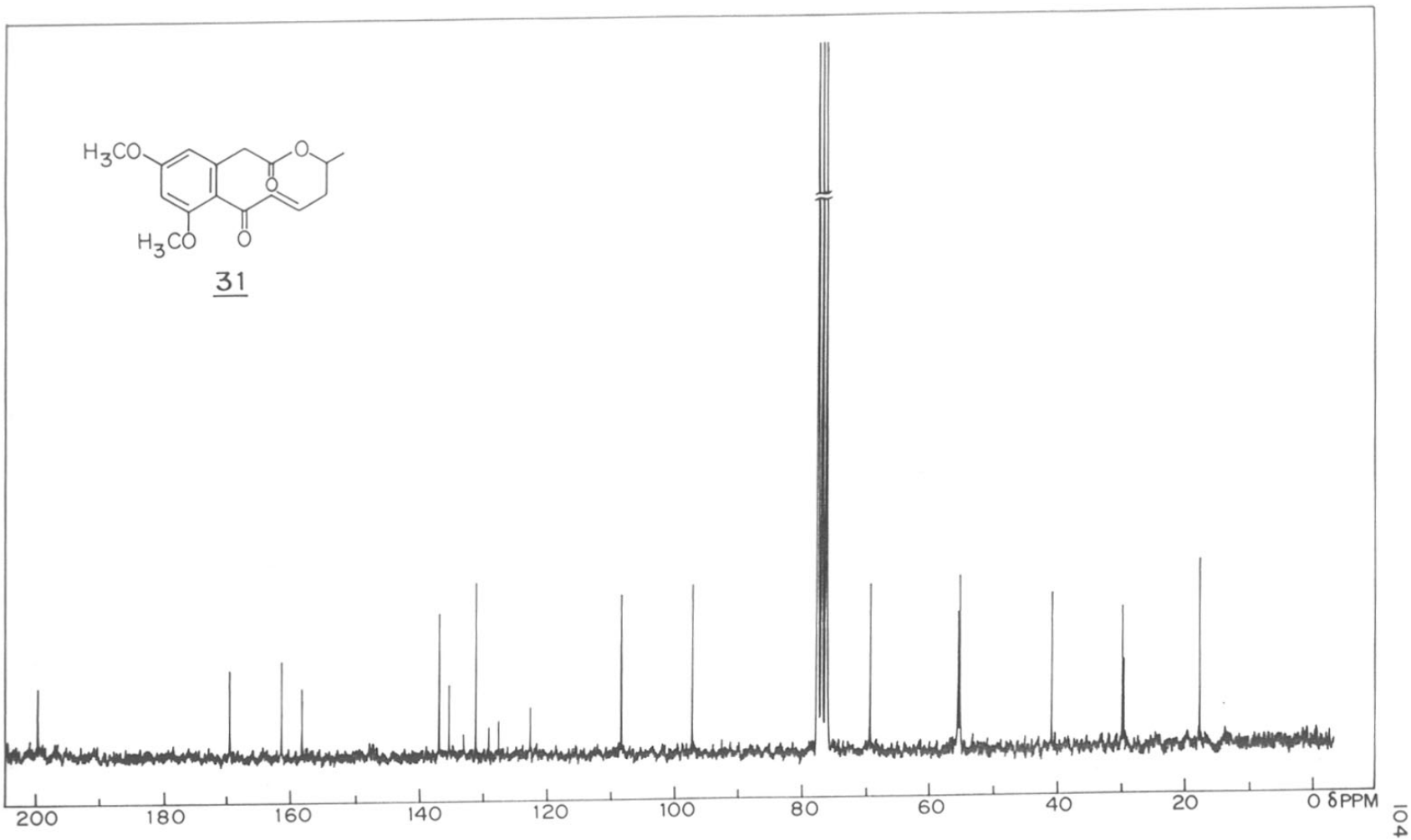
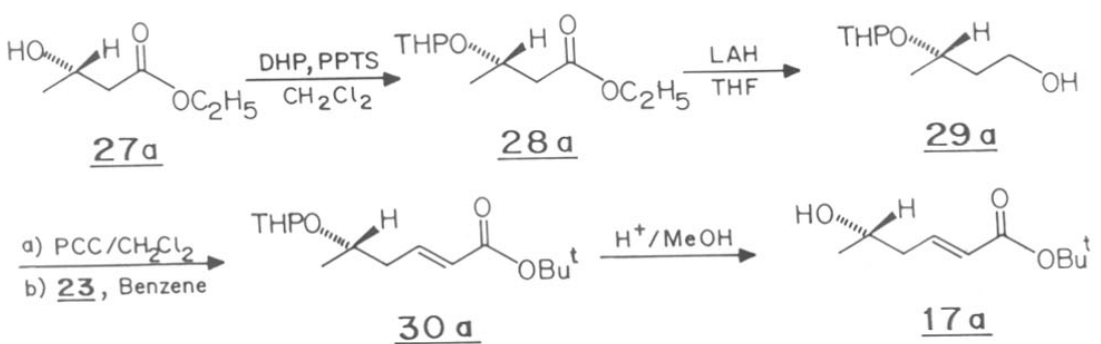


FIG. IV : ^{13}C-NMR SPECTRUM OF THE COMPOUND (31) IN $CDCl_3$

sis as the optically active counterpart (**17a**) of the alcohol **17** could be easily obtained from (S)-ethyl-3-hydroxybutyrate (**27a**).

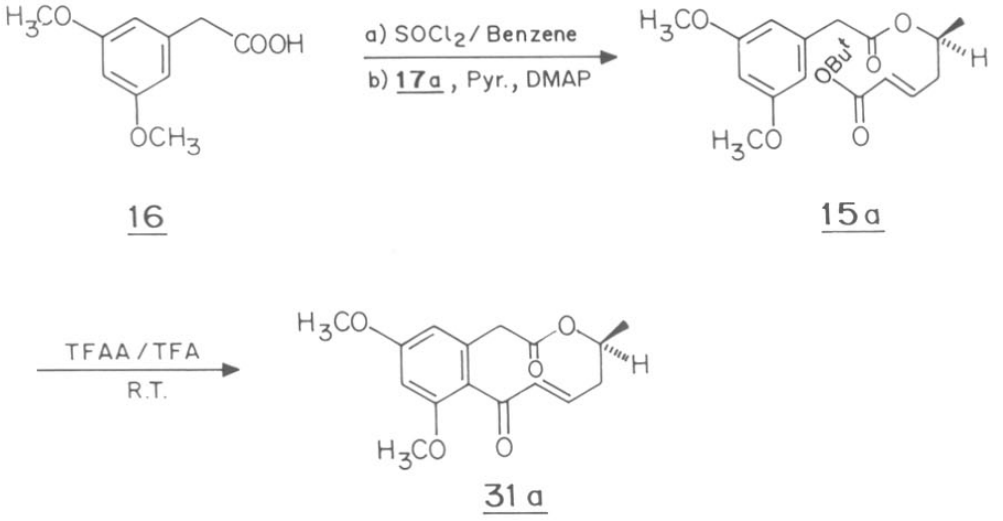
The optically active (S)-ethyl-3-hydroxy-butyrate (**27a**) has already been prepared and used in the synthesis of (+) orthosporin and (-) semivioxanthin (Chapter-I : Part 1 and Part 2). Thus, the alcohol **27a** was protected as its THP ether to give compound **28a** $[\alpha]_D^{25}$ + 15.78° (0.2, $CHCl_3$) [lit.[27] $[\alpha]_D^{25}$ + 11.4° (0.5 $CHCl_3$)]. The reduction of ester **28a** with lithium aluminium hydride in THF afforded the optically active alcohol **29a** in 72% yield $[\alpha]_D^{25}$ + 39.9° (0.2, $CHCl_3$) [lit.[27] $[\alpha]_D^{25}$ +37.3° (0.5, $CHCl_3$)]. The PCC oxidation of **29a** and subsequent Wittig reaction with the ylide **23** afforded optically active α-ß-unsaturated ester **30a**, $[\alpha]_D^{25}$ -12.02° (0.5, $CHCl_3$), [lit.[28] $[\alpha]_D^{25}$ -13° (0.5 $CHCl_3$) for methyl ester] in 60% yield. The deprotection of THP ether of **30a** with acid afforded tert-butyl-(S)-5-hydroxy-2(E) hexenoate (**17a**) in 87% yield; [$[\alpha]_D^{25}$ 13.01° (C = 0.5, $CHCl_3$)] (**Scheme 2.1.5**). The compound **17a** depicited identical physical and spectral properties as its racemic analog **17**.

SCHEME 2·1·5



The optically active (S)-alcohol **17a** was then condensed with 3,5-dimethoxyphenylacetic acid (**16**) via its acetylchloride, in the presence of pyridine and catalytic amount of DMAP. The condensed ester **15a** so obtained showed identical spectral characteristics as of the racemic ester **15** (**FIG.II**). The compound **15a** showed optical rotation $[\alpha]_D^{25}$ -12.24°(0.4, $CHCl_3$). The ester **15a** was then treated with a mixture of trifluoroacetic anhydride and trifluoroacetic acid (5:1) at room temperature to give (-) M5032 dimethyl ether (**31a**) in 18% yield (**Scheme 2.1.6**) $[\alpha]_D^{25}$ - 19.6° (0.4, $CHCl_3$) (lit.[14] $[\alpha]_D^{25}$ -18.0° for M5032].

SCHEME 2·1·6

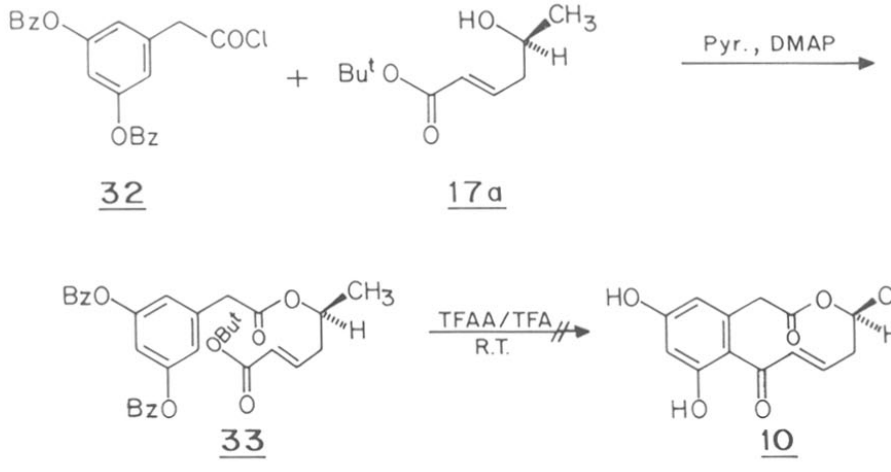


The compound **13a** exhibited identical spectral properties as of its racemic analog **31** (**FIG.III and IV**).

It should be noted here that the synthesis of (-)M5032 dimethyl ether from (S)-3-hydroxyethylbutyrate indirectly suggested (S)-absolute configuration for M5032 as both i.e the natural M5032 and the synthetic M5032 dimethyl ether exhibited negative optical rotation.

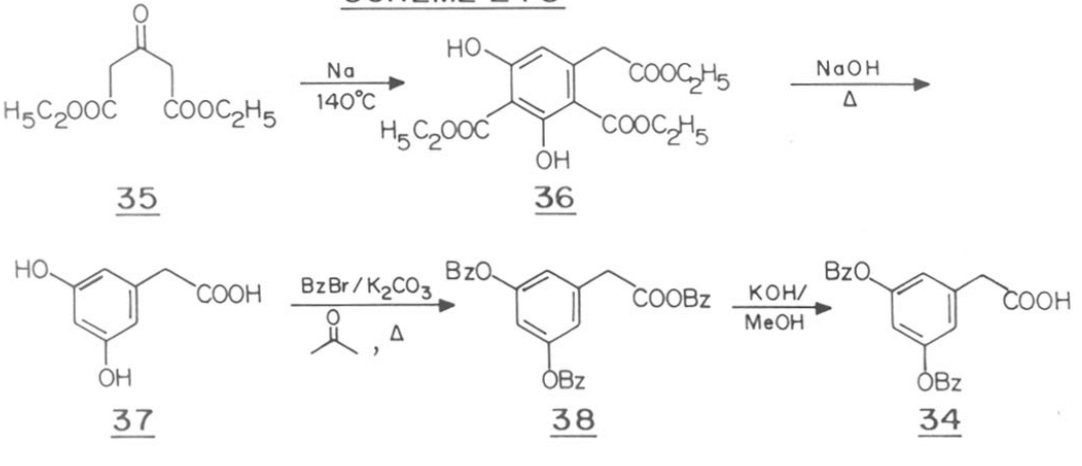
To achieve the total synthesis of M5032, it was considered in yet another approach that esterification of 3,5-dibenzyloxyphenyl acetyl chloride (**32**) with the alcohol (**17a**) followed by simultaneous intramolecular acylation and debenzylation[23] would directly give M5032 (**Scheme 2.1.7**).

SCHEME 2·1·7



The desired 3,5-dibenzyloxyphenyl acetic acid (**34**) was prepared in three steps from acetone dicarboxylic acid ethyl ester (**35**) (**Scheme 2.1.8**).

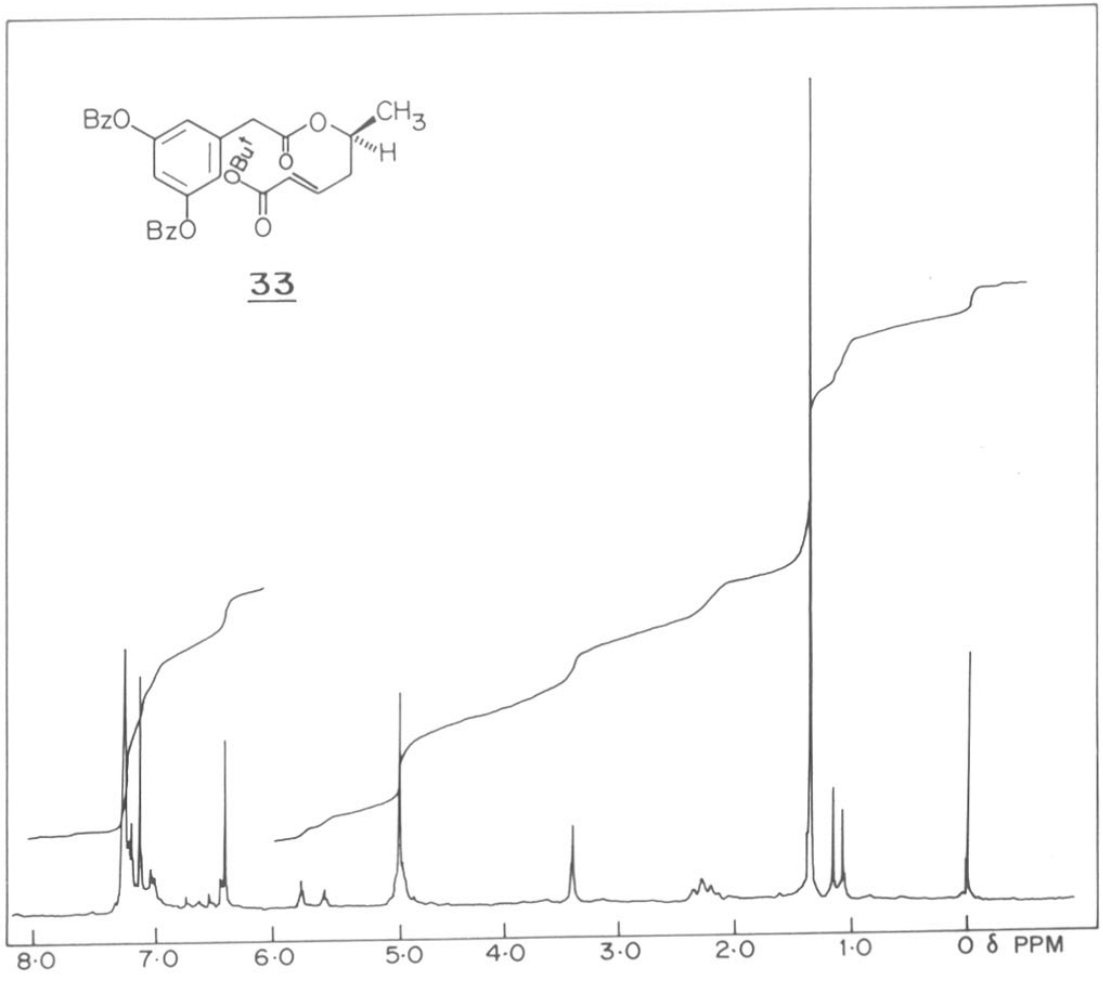
SCHEME 2·1·8



The condensation of acetone dicarboxylic acid ethyl ester (**35**) at 140°C in presence of sodium followed by hydrolysis and decarboxylation of the intermediate ester (**36**) resulted into the formation of the 3,5-dihydroxyphenyl acetic acid (**37**). The crude acid **37** was refluxed with benzyl bromide and potassium carbonate in acetone to give the 3,5-dibenzyloxyphenyl acetic acid benzyl ester (**38**). Hydrolysis of the benzyl ester **38** in methanolic potassium hydroxide afforded 3,5-dibenzyloxyphenyl acetic acid (**34**) in 60% yield.

The physical and spectral properties of the acid **34** were fully consistent to the reported data[24]. The 3,5-dibenzyloxyphenyl acetyl chloride (**32**) was prepared by refluxing the acid **34** with thionyl chloride in benzene for 2 h. The crude acetylchloride (**32**) was esterified with alcohol **17a** in benzene using pyridine and DMAP to give the ester **33** in 69% yield. The ester **33** exhibited spectral properties constitent to its structure. The IR spectrum revealed carbonyl absorption bands at 1740 and 1720 cm^{-1}. The ^{1}H-NMR (**FIG. V**) exhibited a methyl doublet at δ 1.12 and a singlet at δ1.34 integrating for nine protons of tertiarybutyl group. A methylene multiplet at δ 2.26, a benzyl methylene singlet at δ 3.38 and a broad singlet at δ 4.87 integrating for four benzylic protons of O-benzyloxy group and single proton of -C<u>H</u>-group were observed. The olefinic protons showed a doublet of triplet at δ 5.62 and a multiplet at δ 6.75 each integrating for single proton. All the three aromatic protons appeared as singlet at δ6.37 where as all the ten protons of benzyloxy group exhibited a broad singlet at δ7.21. The presence of M-57 i.e. loss of tert-butyl ion in mass spectrum at m/e. 459 further confirmed the structure. The ester **33** showed optical rotation $[\alpha]_D^{25}$ -10.35° in chloroform.

The ester **33** was subjected to intramolecular acylation and debenzylation in a mixture of trifluoroacetic anhydride and trifluoroacetic acid (5:1) at room temperature for 5 h. Usual work up of the reaction afforded a mixture of



33

FIG. V: ^{1}H-NMR SPECTRUM OF COMPOUND(33) IN $CDCl_3$

products. The ^{1}H-NMR and mass spectra of the products indicated the absence of t-butyl and benzyl groups. However, the desired product M5032 could not be isolated from the mixture.

In conclusion the first chiral synthesis of M5032 dimethyl ether has been achieved in short and simple steps. The synthesis can be utilized for the preparation of M5032 in multigram quantities.

Experimental:

Ethyl (aceto-ketal)acetate (20): A mixture of ethyl acetoacetate (**19**) (6.5 gm, 50 mmol), ethyleneglycol (25 ml) and p-toluenesulphonic acid (0.025 gm) in benzene (50 ml) was refluxed for 8 h with the azeotropic removal of water. The reaction mixture was cooled, neutralized with aq. K_2CO_3 (5 ml) and diluted with water (50 ml). The benzene layer was separated and the aqueous phase was extracted with benzene (2 x 50 ml). The combined benzene extract was dried over Na_2SO_4 and concentrated. The residue thus obtained was distilled under reduced pressure to give ethyl(aceto-ketal) acetate (**20**) as a colourless viscous liquid. (8.2 gm, 94%) b.p. 60°C/0.6 mm (Lit.[25] b.p. 88°/5 mm).

3-(Oxo-ketal)-1-butanol (21): To a stirred suspension of lithium aluminium hydride (LAH) (2.92 gm, 77 mmol) in dry diethyl ether (150 ml) was added the ethyl (aceto-ketal) acetate (**20**) (12.04 g, 77 mmol) in ether (25 ml) during 1 h at 0°C. The stirring was continued for 4 h at room temperature. The reaction mixture was again cooled to 0°C and ethyl acetate (5 ml) followed by a saturated solution of sodium sulphate (5 ml) was added slowly. The white solid thus formed was filtered and washed with ether (25 ml). The combined filtrate was dried (Na_2SO_4) and concentrated. The residue was distilled under reduced pressure to give the alcohol **21** in pure form (8.46 g, 89%) b.p. 65-67°/5 mm Hg (Lit.[25] 90-95°C/10 mm).

3-(Oxo-ketal)-1-butanal (22): To a mixture of PCC (16.08 gm, 75 mmol) and sodium acetate (1.6 gm, 20 mmol) in dry CH_2Cl_2 (50 ml) was added the alcohol (**21**) (6.6 gm, 50 mmol) with stirring at room temperature. The stirring was continued for 1 h and the reaction mixture was poured into dry ether (200 ml) and filtered through a celite pad. The filtrate on distillation of the solvent gave alde-

hyde **22** (4.9 gm, 70%) which was used as such for the next step without any further purification.

t-Butyl-5-(oxo-ketal)-2(E)-hexenoate (24): The aldehyde **22** (3.0 g, 23.07 mmol) and ylide **23** (8.67 gm, 23.07 mmol), prepared from -chloro-t-butylacetate and triphenylphosphine by known method[26], were taken in dry benzene (50 ml) and refluxed for 10 h. The benzene was distilled off under vacuum and the reddish brown residue so obtained was purified on silica gel column (hexane-Acetone 9:1) to furnish α-ß-unsaturated ester (**24**) as a thick oil. (3.5 gm, 74.4%).

IR (Neat): 1730 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.35 (s, 3H); 1.48 (s, 9H); 2.53 (bd, J = 6 Hz, 2H); 3.95 (s, 4H); 5.82 (dd, J = 16 Hz, 2 Hz, 1H); 6.84 (m, 1H).

Ms (m/e): 213 M^+ -15).

t-Butyl-5-oxo-2(E)-hexenoate (25): The ester **24** (2.4 gm, 10.5 mmol) was stirred with a mixture of glacial acetic acid (40 ml) and water (10 ml) at 65°C for 1 h. After cooling, the reaction mixture was poured carefully to an ice-cold saturated sodium bicarbonate solution (250 ml) and extracted with chloroform (2 x 50 ml). The combined organic extract was washed, dried (Na_2SO_4) and concentrated to get the keto ester (**25**) as a yellow oil. (1.92 gm, 99%).

IR (Neat): 1720 and 1670 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.43 (s, 9H); 2.23 (s, 3H); 3.12 (dd, J = 6 Hz, 2 Hz, 2H); 5.75 (dd, J = 16 Hz, 2 Hz, 1H); 6.75 (m, 1H);

Ms (m/e): 128 (M^+ 56).

Sodium borohydride reduction of the keto-ester (25): The keto ester (**25**) (1.84 gm, 10 mmol) was dissolved in ethanol (20 ml) and sodium borohydride (0.42 gm, 11 mmol) was added in portions at 0-5°C with stirring. The reaction mixture was stirred further for 0.5 h. at 0°C and 1 h at room temperature. To the reaction

mixture, 2-3 drops of conc. HCl were added, the ethanol was distilled off completely and water (50 ml) was introduced. The aqueous solution was extracted with chloroform (2x 50 ml), washed and dried over Na_2SO_4. The oily residue obtained after removal of the solvent was purified on silica gel column (8:2 Hexane: Acetone) to give an inseparable mixture of t-butyl-5-hydroxy-2-hexenoate (**17**) and t-butyl-5-hydroxyhexanoate (**26**) (1.6 gm, 86%).

Ethyl(3-tetrahydropyranyloxy)-butyrate (28) or (28a): Ethyl 3-hydroxybutyrate (**27**) or (S)-(+)-ethyl-3-hydroxybutyrate (**27a**) (1.6 gms, 12.12 mmol) and dihydropyran (1.01 gm, 12.12 mmol) were stirred in dry CH_2Cl_2 (30 ml), in presence of a catalytic amount of pyridinium p-toluenesulphonate (PPTS) for 28 h at room temperature. The reaction mixture was neutralized by a saturated sodium bicarbonate solution (5 ml) and diluted with water (50 ml). The dichloromethane layer was separated, dried Na_2SO_4 and concentrated. The residue after distillation under vacuum afforded ethyl (3-tetrahydropyranyloxy)-butyrate (**28**) (2.49 gm, 95%) or (S)-ethyl (3-tetrahydropyranoyloxy)butyrate (**28a**) (2.51 gm, 96%) b.p. 80-85°C/2 mm Hg (lit.[27], b.p. 84.5-88.5°/3 mm Hg). **28a**: $[\alpha]_D^{25}$ = + 15.78° (C = 2, $CHCl_3$) {Lit.[27] $[\alpha]_D^{25}$ = + 17.8° (1.09, $CHCl_3$)}.

3-Tetrahydropyranyloxy-1-butanol (29) or (29a) : To a stirred suspension of LAH (0.35 gm, 9.2 mmol) in dry THF (25 ml) was added ethyl (3-tetrahydropyranyloxy) butyrate (**28**) or (S)-ethyl-(3-tetrahydropyranoyloxy) butyrate (**28a**), (2.00 gm, 9.25 mmol) in THF (10 ml) at 0°C during 0.5 h. The reaction mixture was stirred for 1 h at 0°C and then for 3 h at room temperature. The reaction mixture was cooled to 0°C again and the ethyl acetate (5 ml) was added dropwise to destroy the excess LAH. Finally a saturated sodium sulphate solution was added and a solid thus formed was filtered. The filtrate was concentrated and the residue was extracted in $CHCl_3$, dried (Na_2SO_4) and the solvent was distilled off.

The residue was distilled under reduced pressure to give 3-tetrahydropyranoyloxy-1-butanol (**29**) (1.25 gm, 78%) or (S)-3-(tetrahydropyranyloxy)-1-butanol (**29a**) (1.16 gm, 72%) b.p. 90-95°C/ 1 mm Hg [Lit.[27], b.p. 76-82°C/0.35 mm Hg] **29a**: $[\alpha]_D^{25}$ = + 39.9° (C=2, $CHCl_3$), {Lit[27] $[\alpha]_D^{25}$ = + 45.2°C (1.58, $CHCl_3$)}.

t-Butyl-5-tetrahydropyranyloxy-2(E)-hexenoate (30) or (30a): The alcohol **29** or **29a** (5.00 gm, 28.7 mmol) was added to a mixture of PCC (9.29 gm, 43.1 mmol) and sodium acetate (1.2 gm, 14.35 mmol) in dry CH_2Cl_2 (50 ml) during 0.5 h and stirred for further 1 h. The reaction mixture was poured into ether (100 ml) and filtered through celite powder. The filtrate was dried (Na_2SO_4) and concentrated under vacuum. The crude aldehyde thus obtained was heated under reflux in benzene (50 ml) with the ylide **23** (8.5 gm, 23.87 mmol) for 8.5 h. The benzene was distilled off and the residue was chromatographed on silica gel column with pet ether and acetone (9:1) as eluent to afford t-butyl-5-tetrahydropyranyloxy-2(E)-hexenoate (**30**) (4.80 gm, 62%) or (S)-t-butyl-5-tetrahydropyranyloxy-2(E)-hexenoate (**30a**) (4.65 gm, 60%) as colourless thick oil.

30a: $[\alpha]_D^{25}$ = -12.02° (C=2, $CHCl_3$) {lit.[28] - 13° (0.5, $CHCl_3$ for the methyl ester).

IR (Neat): 1720 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ1.18 (d, J = 6 Hz, 3H); 1.43 (s, 9H); 1.50-1.80 (m, 6H); 2.11-2.46 (m, 2H); 3.18-4.00 (m, 3H); 4.56 (bs, 1H); 5.68 (dt, J = 16 Hz, J> OHz, 1H); 6.75 (dt, J = 7 Hz, J>OHz, 1H).

Ms (m/e): M^+ 270.

t-Butyl-5-hydroxy-2(E)-hexenoate (17) or (17a): The t-butyl- 5-tetrahydropyranyloxy-2(E)-hexenoate (**30**) or (S)-t-butyl-5-tetrahydropyranyloxy-2(E)-hexenoate (**30a**), (2.70 gm, 10mmol) was stirred in methanol (20 ml) with 2-3 drops of conc. HCl for 1 h. The methanol was distilled off and the residue was taken in chloroform (50 ml) and washed with brine. The chloroform extract was dried

(Na_2SO_4) and concentrated. Purification of the residue by column chromatography afforded t-butyl-5-hydroxy-2(E)-hexenoate (**17**), (1.58 g, 85%) or (S)-t-butyl-5-hydroxy-2(E)-hexenoate (**17a**) (1.61 gm, 87%). **17a** $[\alpha]_D^{25}$ = + 13.01° (C=2, $CHCl_3$) {lit.[29] + 10.0° ($CHCl_3$)}.

IR (Neat): 3420 and 1720 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.23 (d, J = 6 Hz, 3H); 1.50 (s, 9H); 2.16 - 2.53 m, 2H); 3.70-4.13 (m, 1H); 5.80 (dt, J = 6 Hz, J>OHz, 1H); 6.83 (dt, J = 7 Hz, 1H).

Ms (m/e): 171 (M^+-15), 130 (M^+, -56).

t-Butyl-5-(3,5-dimethoxyphenylacetoxy)-2(E)-hexenoate (15) or (15a): Thionyl chloride (1.02 gm, 8.66 mmol) was added to a stirred solution of (3,5-dimethoxyphenyl) acetic acid (**16**) (1.7 gm, 8.66 mmol) in dry benzene (20 ml) at room temperature. After refluxing for 2 h, the excess thionyl chloride was distilled off along with the benzene. The resultant acid chloride was taken in benzene (10 ml) and added to the stirred solution of the alcohol (**17**) or (**17a**) (1.5 gm, 8.06 mmol), pyridine (1 gm, 12.60 mmol) and a catalytic amount of 4-DMAP in benzene (20 ml) under nitrogen atmosphere. After stirring for 6 h, the reaction mixture was quenched with water (20 ml) and the benzene layer was separated, dried and concentrated. The reddish brown residue was then purified on column (silica gel) using 5% acetone in hexane to give the ester **15** (1.77 gm, 60%) or **15a** (1.82 gm, 62%) as thick sticky mass.

15a: $[\alpha]_D^{25}$ -12.24° (C=2, $CHCl_3$).

IR (Neat): 1730 and 1670 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.18 (d, J = 6 Hz, 3H); 1.43 (s, 9H); 2.12 - 2.56 (m, 2H); 3.50 (s, 2H); 3.75 (s, 6H); 4.80 - 5.06 (m, 1H); 5.70 (dt, J = 16 Hz and J>OHz, 1H); 6.34 (bs, 3H); 6.68 (dt, J = 7 Hz, J>OHz, 1H).

Ms (m/e): 308 (M^+ -56).

M5032 dimethylether (31) or (31a): The ester **15** or **15a** (0.364 gm, 1 mmol) was stirred with trifluoroacetic anhydride (5 ml) and trifluoroacetic acid (1 ml) at room temperature under argon atmosphere for 5 h. The reaction mixture was poured over ice-water (20 ml) and extracted with CH_2Cl_2 (2 x 10 ml). The combined extracts were washed with saturated bicarbonate solution and water. The dried (Na_2SO_4) CH_2Cl_2 solution was then concentrated and the residue was purified on column (silica gel) using pet ether-acetone (9:1) to afford M5032 dimethylether (**31**) (0.055 gm, 19%) or (**31a**) (0.060 gm, 21%) as a colourless sticky mass.

31a: $[\alpha]_D^{25}$ -19.6° (C=4, $CHCl_3$) {lit.[14] $[\alpha]_D^{25}$ -18°, of natural M5032}.

IR (Neat): 1731 and 1667 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.20 (d, J = 6 Hz, 3H); 2.20 (m, H); 3.10 (m, 1H); 3.64 (d, J = 16.5 Hz, 1H); 3.85 (s, 3H); 3.90 (s, 3H); 4.05 (d, J = 16.5 Hz, 1H); 5.20 (m, 1H); 5.95 (dt, J = 7 Hz, 1H); 6.35 (d, J = 2 Hz, 1H); 6.42 (d, J = 2 Hz, 1H); 7.01 (dt, J = 16 Hz, J>OHz, 1H).

^{13}C-NMR ($CDCl_3$): δ 17.83 (C_4); 29.88 (C_5); 40.98 (C_1); 55.31 (C_{11}); 55.59 (C_9); 69.40 (C_4); 97.18 (C_{10}); 108.32 (C_{12}); 122.62 (C_8); 131.22 (C_7); 135.31 (C_{12}); 136.83 (C_6); 158.36 (C_9); 161.49 (C_{11}); 169.61 (C_2); 199.73 (C_8).

UV (methanol, λ_{max}): 242 and 273 nm.

Ms (m/e): 290 (M^+).

3,5-Dihydroxyphenylacetic acid (37): Sodium (0.20 gm, 8.69 mmol) was dissolved in diethyl 1,3-acetonedicarboxylate (**35**) (20.0 gm, 99 mmol) and heated on an oil bath at 140° for 2 h. The thick orange mass was poured in a porcalein dish while hot and the solid obtained after cooling was washed with alcohol (2 x 20 ml) and crystallized from alcohol to give the intermediate ester **36** (8.60 gm, 51%) as colourless crystalline solid, m.p. 97°C (lit.[30] m.p. 98°C). The ester **36** thus

obtained was refluxed with 14% sodium hydroxide solution (100 ml) for 2 h. The solution was cooled, acidified with conc. H_2SO_4 and heated with animal charcoal for 20 min. and filtered. The light yellow coloured filtrate was concentrated under vacuum and the residue was extracted with ether (3 x 25 ml). The combined ethereal extracts was evaporated to give a yellowish brown oil which solidified on keeping in a vacuum desiccator to give crude 3,5-dihydroxyphenylacetic acid (**37**) (4.20 gm, 99%).

3,5-Dibenzyloxyphenylacetic acid (34): The crude 3,5-dihydroxyphenylacetic acid (**37**) (3.36 gm, 20 mmol) was refluxed with benzyl bromide (11.15 gm, 65 mmol) and potassium carbonate (8.97 gm, 65 mmol) in dry acetone (50 ml) for 6 h. The residue obtained after the distillation of the acetone was poured into water (100 ml) and extracted with ethyl acetate (2 x 50 ml). The combined extract was washed, dried and evaporated to give a thick yellow oil. The crude 3,5-dibenzyloxyphenylacetic acid benzyl ester (**38**) was refluxed with 10% methanolic potassium hydroxide solution for 2 h. The methanol was distilled off and to the residue, water (50 ml) was introduced. The aqueous solution was acidified with conc. HCl and a solid thus separated was filtered. The crude acid was crystallized with pet ether: acetone to give 3,5-dibenzyloxyphenylacetic acid (**34**) (4.17 gm, 60%) as a colourless crystalline solid. m.p. 105°C (lit.[24] m.p. 104-106°C).

IR (Nujol): 1720 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 3.56 (s, 2H); 4.98 (s, 4H); 6.52 (br.s, 3H); 7.32 (br.s, 10H).

Ms (m/e): 348 M^+.

t-Butyl-5(S)-3,5-dibenzyloxyphenylacetoxy)-2(E)-hexenoate (33): 3,5-Dibenzyloxy-phenylacetic acid (**34**) (3.50 gm, 10 mmol) was refluxed with thionyl chloride (3.00 gm, 15 mmol) in benzene (100 ml) for 2 h. The benzene and excess of thionyl chloride were distilled off completely. The crude 3,5-dibenzyloxypheny-

lacetyl chloride (**32**) was dissolved in benzene (20 ml) and added to a mixture of alcohol **17a** (1.86 gm, 10 mmol), pyridine (1.20 gm, 15 mmol) and catalytic amount of DMAP in benzene (20 ml). The reaction mixture was stirred overnight. Water (50 ml) was introduced and the benzene layer was separated. The aqueous phase was extracted with benzene (2 x 25 ml) and the combined organic phase was dried (Na_2SO_4) and concentrated. The crude residue after column purification (9:1; hexane:acetone) afforded t-butyl-5(S)-(3,5-dibenzyloxylphenylacetoxy)-2(E)-hexenoate (**33**) (3.58 gm, 69%). $[\alpha]_D^{25}$ = -10.35° (C=2, $CHCl_3$).

IR (Neat): 1740 and 1720 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.12 (d, J = 6 Hz, 3H); 1.34 (s, 9H); 2.26 (m, 2H); 3.38 (s, 2H); 4.87 (br.s, 4H and 1H); 5.62 (dt, J = 16 Hz, J >OHz, 1H); 6.37 (br.s, 3H); 6.75 (m, 1H); 7.21 (br.s, 10H).

Ms (m/e): 459 (M^+ -57).

Attempted cyclization of ester (33) in trifluoroacetic anhydride and trifluoroacetic acid (5:1): The ester **33** (0.52 gm, 1 mmol) was dissolved in trifluoroacetic anhydride (5 ml) and trifluoroacetic acid (1 ml) was added at room temperature under N_2 atmosphere. The reaction mixture was stirred for 4 h and poured over ice cold water and extracted with chloroform (2 x 15 ml), dried (Na_2SO_4) and concentrated to give a brownish red residue. However, the desired product M5032 (**10**) could not be isolated.

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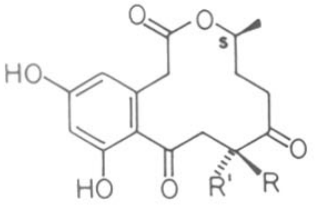
PART 2: STEREOSPECIFIC SYNTHESIS OF 12-OXOCURVULARIN DIMETHYL ETHER

Introduction

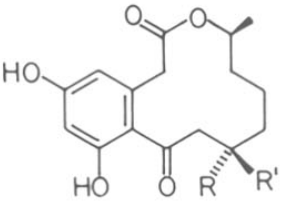
Yamamura et al[1] isolated six novel curvularin type metabolites (**1-6**) from a hybrid strain ME 0005 derived from Penicillium citreo-viride B. Though curvularin (**7**) and trans-dehydrocurvularin (**8**) were isolated way back in 1956[2] from the curvularin species, no curvularin type metabolite has been, however, isolated from the mycelium of Penicillium citreo viride-B. Thus, this is the first report of the isolation of curvularin type metabolites from Penicillium citreo viride-B. These macrolides have also been found in Penicillium gilmanii[3] and in Alternaria species.[4,5] Hyeon et al[6] isolated ß-hydroxycurvularin (**3** or **4**) and α,ß-dehydrocurvularin (**8**) as sporulation supressing factors from Alternaria tomato. Isolation of ß-hydroxycurvularin, ß-meth oxycurvularin as well as curvularin and α,ß-7dehydrocurvularin as spindle poisons from Penicillium sp. 511 have also been reported by Kobayashi et al.[7]

The new macrolides from P. citreo-viride B were identified as 12-oxocurvularin (**1**), 11-ß-hydroxy-12-oxocurvularin (**2**), 11-α- and 11-ß-hydroxycurvularin (**3**) and (**4**), cis-dehydrocurvularin (**5**) and citrofuran (**6**). The structures of these macrolides were elucidated on the basis of chemical evidence as well as by spectroscopic means. Later on further studies on P. citreo-viride B[8] led to the isolation of few more curvularin type metabolites viz 11-α-and 11-ß-methoxycurvularin (**9**) and (**10**), 11,12-dihydroxycurvularin (**11**) and 12-hydroxy-10,11-trans-dehydrocurvularin (**12**). Curvularin and curvularin type metabolites have been shown to possess S absolute configuration. The configuration of curvularin was assigned by Gerlach[9] based on the fact that the natural (-)curvularin was obtained from the known (S)-7-hydroxy octanoic acid -(2-trimethylsilyl) ether ester (**13**).



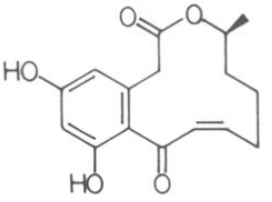
1 R = R' = H

2 R = OH, R' = H



3 R = OH, R' = H

4 R = H, R' = OH



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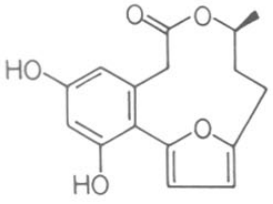

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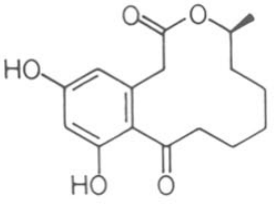

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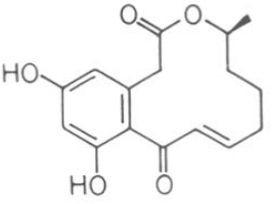

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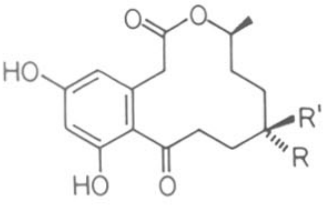
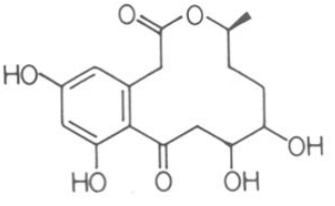


9 R = $-OCH_3$, R' = H

10 R = H, R' = $-OCH_3$



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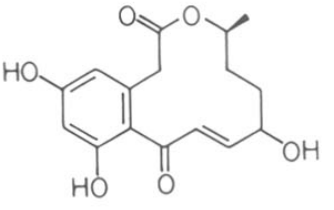
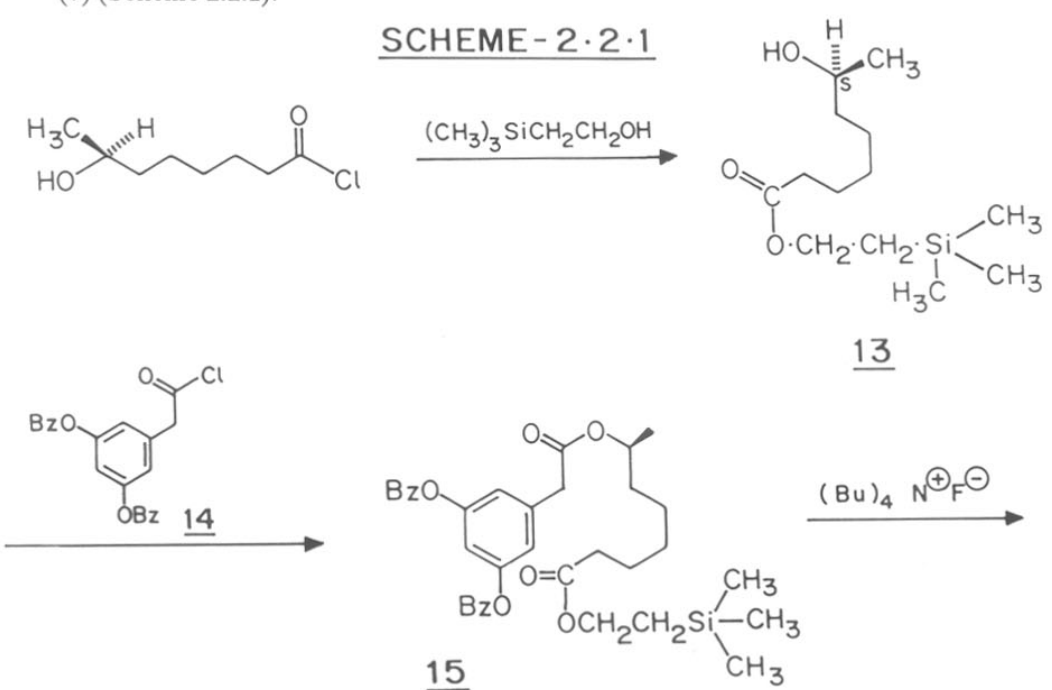


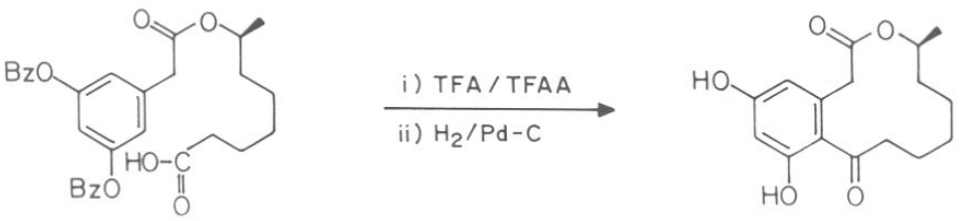
12

It has been reported by Kobayashi et·al.[7] that these curvularin type macrolides show remarkable activity against see urchin embryo cells. They induce barrel like spindles resulting in inhibition of cell proliferation. Particularly 12-oxocurvularin, citrofuran and 11-ß-hydroxy-12-oxocur vularin are more attractive not only in physiological properties but also in their structures containing an oxygen function at C_{12} position. 12-Oxocurvularin gets converted to citrofuran when treated with cesium hydroxide.

Although several syntheses of (±)curvularin are reported[10], there is only one synthesis known for natural S-(-) curvularin.[9] The synthesis involves S-(-)--valerolactone as chiral starting material. S-(-)-2-(trimethylsilyl)ethyl-7-hydroxyoctanoate (**13**) prepared from S-(-)- -valerolactone was esterified with 3,5-dibenzyloxyphenyl acetyl chloride (**14**) to form **15** having two different ester groups. The selective deprotection of 2-(trimethylsilyl) ethyl ester with tetrabutyl ammonium fluoride resulted into the formation of acid **16**. The intramolecular acylation followed by debenzylation of acid **16** gave the desired S(-)-curvularin (**7**) (**Scheme 2.2.1**).

SCHEME-2·2·1



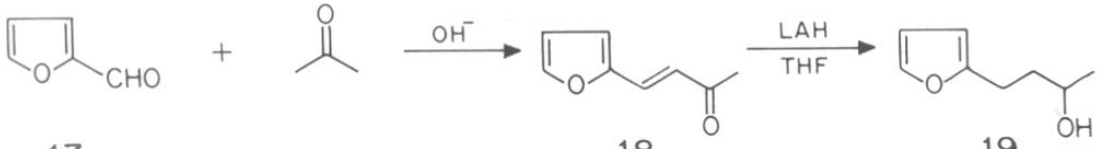


16 (-) 7

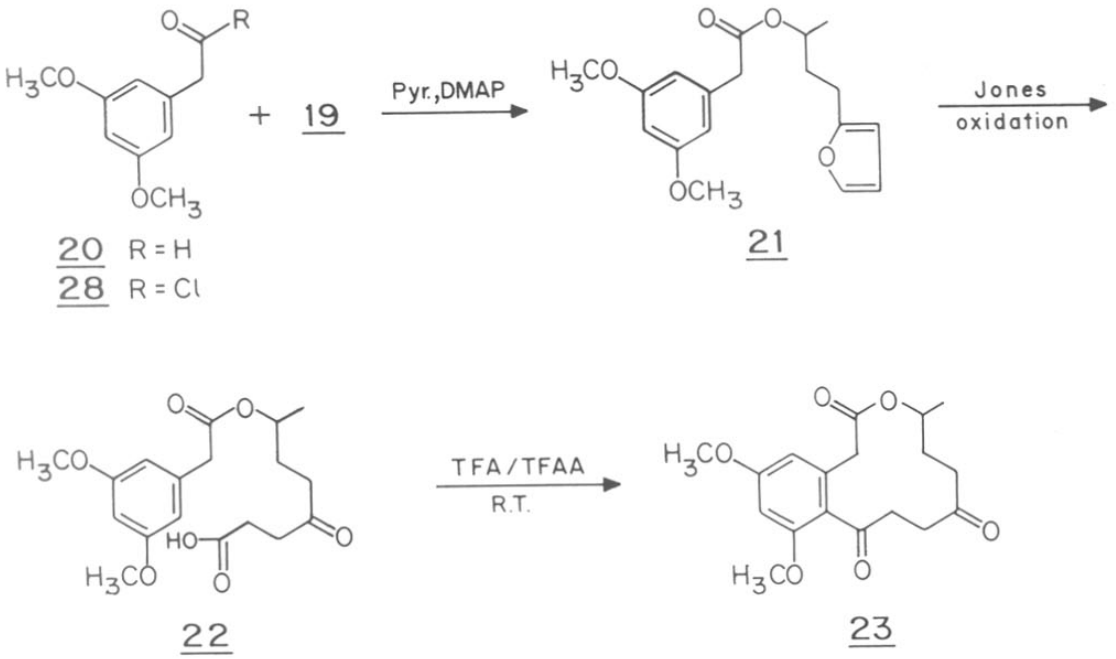
Synthesis of 12-oxocurvularin:

The first racemic synthesis of di-O-methyl-12-oxocurvularin (**23**) has been reported recently from our laboratory.[11] The synthesis incorporates the condensation of a furan alcohol 4-(2-furyl) -butan-2-ol (**19**), a two step product prepared by condensation of furfural (**17**) and acetone, followed by reduction and a properly substituted phenylacetic acid **20** to furnish as ester **21**. The oxidative cleavage of the furan ring of the ester **21** with Jones reagent furnished an intermediate keto acid **22** which underwent cycloacylation in trifluoroacetic acid-trifluoroacetic anhydride mixture to give (±) di-O-methyl-12-oxocurvularin (**23**) (**Scheme 2.2.2**).

SCHEME-2·2·2



17 18 19


H3CO
OCH3
R
O
+ 19
Pyr.,DMAP
Jones
oxidation
20 R = H
28 R = Cl
21
HO
TFA / TFAA
R.T.
22
23

Present work:

It has been discussed earlier that the racemic synthesis of 12-oxocurvularin dimethyl ether involves the furan alcohol **19** as one of the synthons. The same strategy could be applied for the stereoselective synthesis as well since the chirality introduced through the alcohol **19** could be easily transferred to the target molecule. The optically active alcohol **19a** could be obtained either by enzymatic reduction of the ketone **18** or by enzyme mediated enatioselective resolution of the racemic alcohol **19**. The latter approach however, proved to be the method of choice and based on this, the successful synthesis of (S)- (-)-di-O-methyl-12-oxocurvularin (**23a**) has been presented in this part.

Biotransformations using enzymes:

Enzymes are efficient biocatalysts with a very high degree of specificity. The immense potential of enzymes as catalysts in organic synthesis is well documented.[12] Nearly unlimited reservoir of different enzyme activities exist in nature; there is thus an enormous potential available for onward progress in the field of biotransformations. The field of application of enzymes includes all most all type of organic reactions e.g. reduction, oxidation, hydrolysis, esterification etc. The most useful aspect of biotransformations is in the asymmetric synthesis, since the conversion is highly specific in comparison to the chemical one.

A variety of enzymes are catalytically active in the non-aqueous medium as well[13] and the synthetic potential of enzymes in organic solvents has been exploited for numerous biotransformations.[14] In should be noted that most of the enzymes used as catalysts in non-aqueous medium have been hydrolases namely lipases and proteases.

Lipases are abundant, stereospecific, stable and highly versatile enzymes. The wide array of transformations possible by lipases include esterification,

transesterification, acyl exchange, lypolysis, oximolysis etc. About 20 different lipases from microbial, plant and animal sources are commercially available. Lipase catalyzed ester hydrolyses and transesterifications are the most popular reactions exploited largely for the preparation of optically active acids and alcohols.[15] The choice of organic solvent plays a crucial role in lipase catalyzed reactions. There is also a general consensus that critical amount of water present around the enzyme is absolutely necessary for the catalytic activity. The most commonly used solvents are ether, heptane, water-immiscible solvents (toluene, carbon tetrachloride), water-miscible solvents (acetone, acetonitrile, dioxane) etc. Lipases are generally stable from 25°C to 60°C with virtually no loss in catalytic activity.

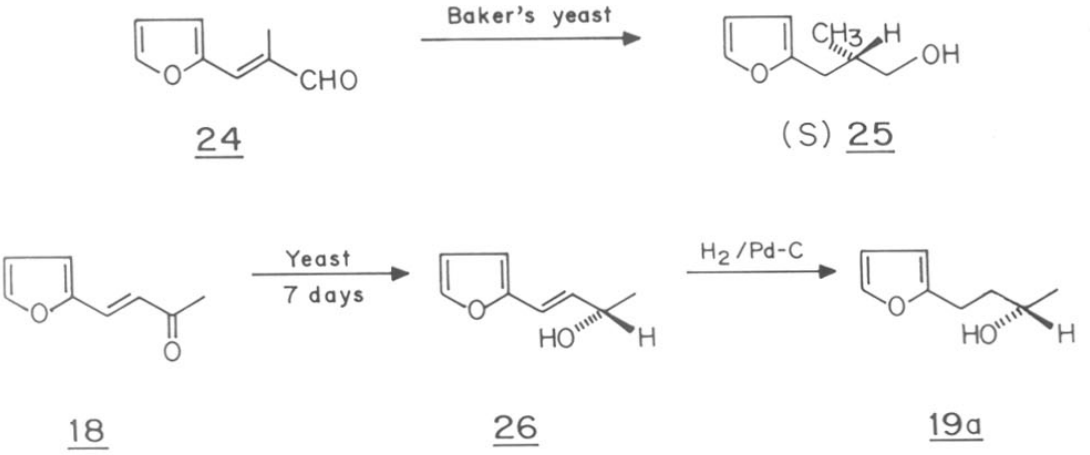
In conclusion, one might observe that there are many advantages of lipase catalyzed reactions in organic media. The simplicity and specificity of these transformations make them an obvious choice for various stereochemical conversions.

Asymmetric reduction of ketone 18 to (S)-4(2-furyl)- butan-2-ol (19a) using yeast:

Yeast mediated microbial transformations have been widely used since the early days of mankind for the production of bread, dairy products, and alcoholic beverages. However, the reduction of furfural to furfuryl alcohol[16] by means of living yeast was the first example of the microbial transformation which led to its wide range of application thereafter. Ketones with varying substituents were reduced with Baker's yeast, and the secondary alcohols obtained were mainly of S-configurations.[17]

ß-Furylacrolein (**24**) has been reduced by Baker's yeast to give (S)-alcohol **25** as main transformation product in 72% chemical yield and 100% optical purity[18] (**Scheme 2.2.3**).

SCHEME - 2·2·3



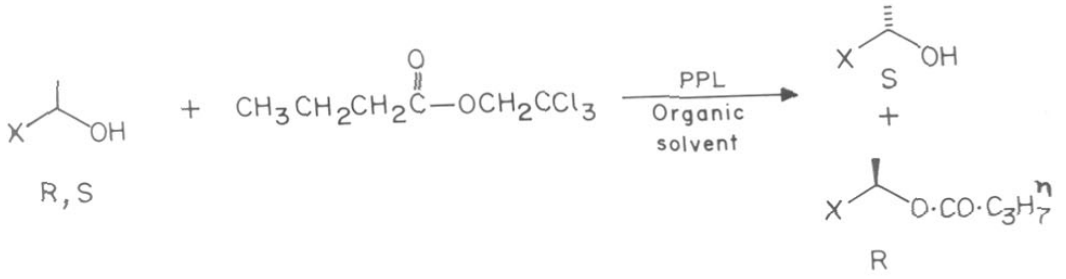
24 (S) 25

18 26 19a

Thus, the ketone **18**, prepared from furfural and acetone[11], was subjected to asymmetric reduction using various yeast species namely Saccharomyces cervisiae, Saccharomyces uvarum, Saccharomycopsis lipolytica, Saccharomyces diastaticus and Torulosis stellata. However, out of the various culture strains screened, only the culture strain NCIM-3195 of Saccharomyces cervisiae converted the ketone **18** to an allylic alcohol **26** in 14% yield after seven days. The allylic alcohol **26** thus obtained was hydrogenated using palladium on charcoal as catalyst to give the saturated alcohol **19a**. The alcohol **19a** exhibited optical rotation of $[\alpha]_D^{25}$ = -13.01° in chloroform [lit.[19] $[\alpha]_D^{25}$ = -22.6° in chloroform).]

Resolution of (±) alcohol 19 using porcine pancreatic lipase (PPL):

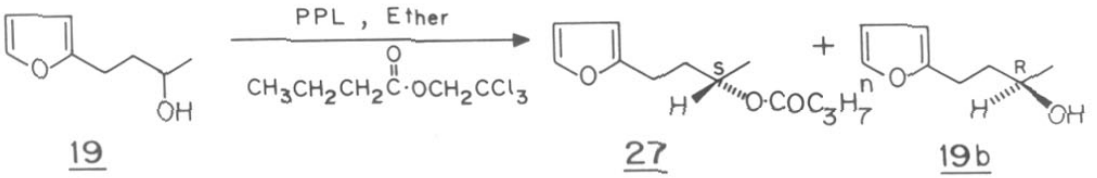
The transesterification based enzymatic resolution of racemic alcohols in the organic solvents is most preferable because of the simplicity of the process and easy recovery of the product. Several racemic secondary alcohols have been resolved in ether or heptane using PPL to give both R and S alcohols of 90-100% optical purity.[15b]

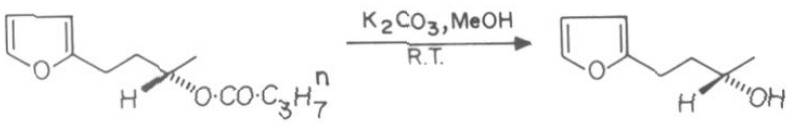


X = hexyl, decyl, phenyl etc.

Thus, the trans esterification of racemic alcohol **19**, prepared by LAH reduction of ketone **18** as reported earlier[11], when carried out with trichloroethyl-butyrate in the presence of PPL under strictly anhydrous conditions (dry ether solvent, 4A molecular sieves, nitrogen atmosphere) resulted in the formation of the optically active ester **27** and the optically active alcohol **19b** (**Scheme 2.2.4**).

SCHEME-2·2·4





In order to obtain products of high enantiomeric purity, the reaction was carried under kinetic conditions. i.e., termination of the reaction after about 45% of starting material was utilized (monitored by GLC). After 6 hrs, the reaction was discontinued, lipase was filtered through celite and ester **27** was obtained after column purification in about 30% yield and about 60% of unreacted alcohol as a mixture of **19b** and **19a** was obtained.

The IR spectrum of the compound **27** exhibited absorption band at 1740 cm^{-1} for ester carbonyl. The ^{1}H-NMR (**FIG. I**) revealed a triplet (J = 6.5 Hz) at δ 0.91 a multiplet between δ 1.40-1.75 and a triplet (J = 6.5 Hz) at δ 2.24 corresponding to butyl ester group. The rest of the aliphatic protons exhibited a doublet (J = 6.2 Hz) at δ 1.22, a multiplet between δ 1.80 -2.04, a triplet (J = 6.5 Hz) at δ 2.62 and a multiplet at δ 4.93. The furyl protons depicted three multiplets at δ 5.91, 6.20 and 7.20. The presence of molecular ion peak at m/e. 210 in mass spectrum further supported the structure. The compound exhibited optical rotation of -5.12° in chloroform [Lit.[19] $[\alpha]_D^{25}$ = -3.5° for O-acetate derivative]. The ester **27** was hydrolyzed by potassium carbonate in methanol at room temperature to give the optically active alcohol **19a**. The alcohol **19a** showed optical rotation of $[\alpha]_D^{25}$ = -18.5° in chloroform. The alcohol **19a** showed identical spectral and physical properties as reported in the literature.[19]

Thus, after obtaining the desired optically active alcohll **19a**, synthesis of (S)-di-O-methyl-12-oxo-curvularin seemed quite straightforward since it would employ the same sequence of reactions as in its racemic synthesis (**Scheme 2.2.5**). Hence, 3,5-dimethoxyphenylacetyl chloride (**28**) (Chapter 2: Part I) was esterified with alcohol **19a** in presence of pyridine and DMAP to furnish the optically active ester **21a** in 78% yield. The optically active ester **21a** exhibited optical rotation of $[\alpha]_D^{25}$ = -6.63° in chloroform. The spectral (**FIG.II, ^{1}H-NMR**) and physical proper-

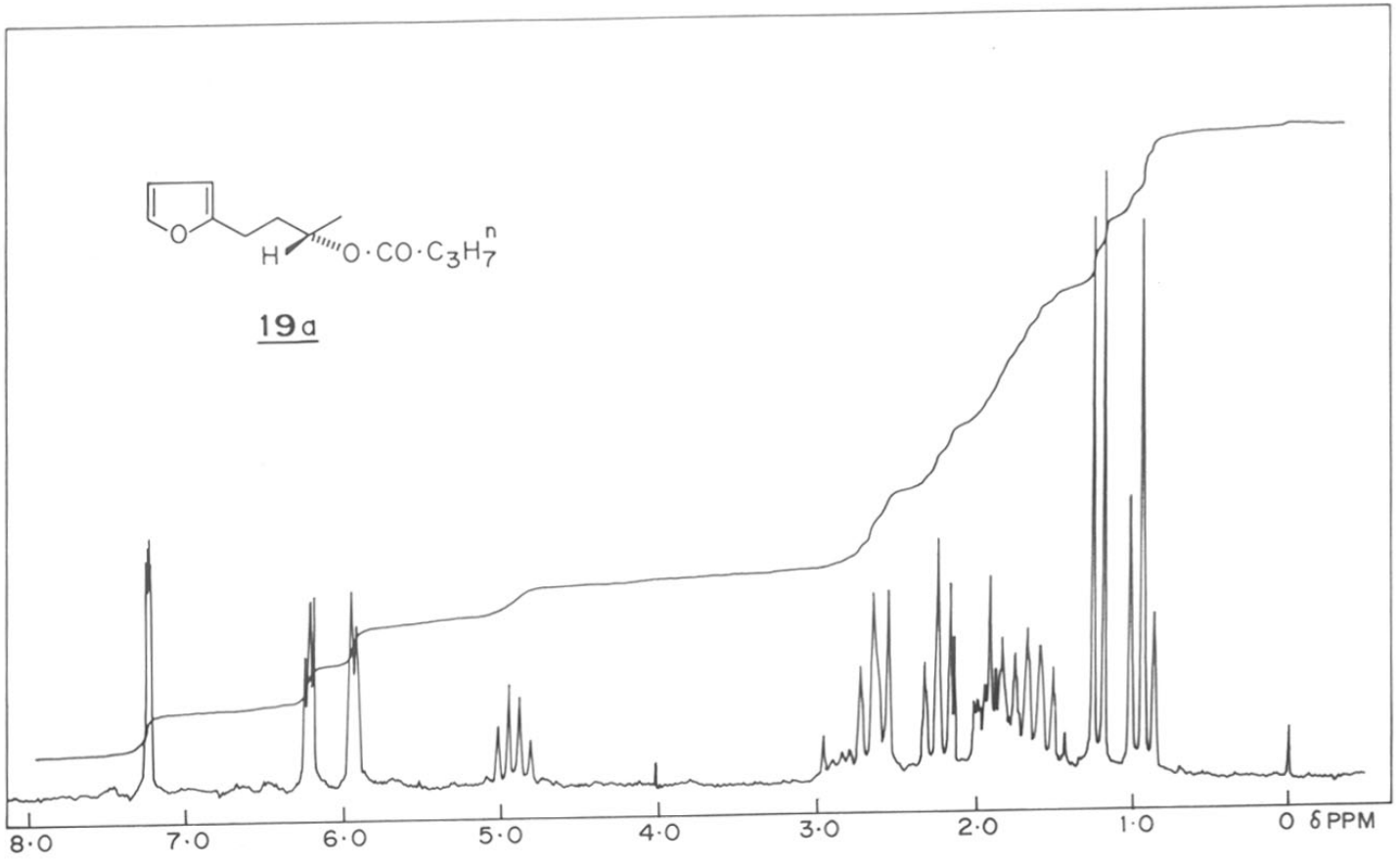


FIG. I : ^{1}H NMR SPECTRUM OF THE COMPOUND(19a) IN $CDCl_3$

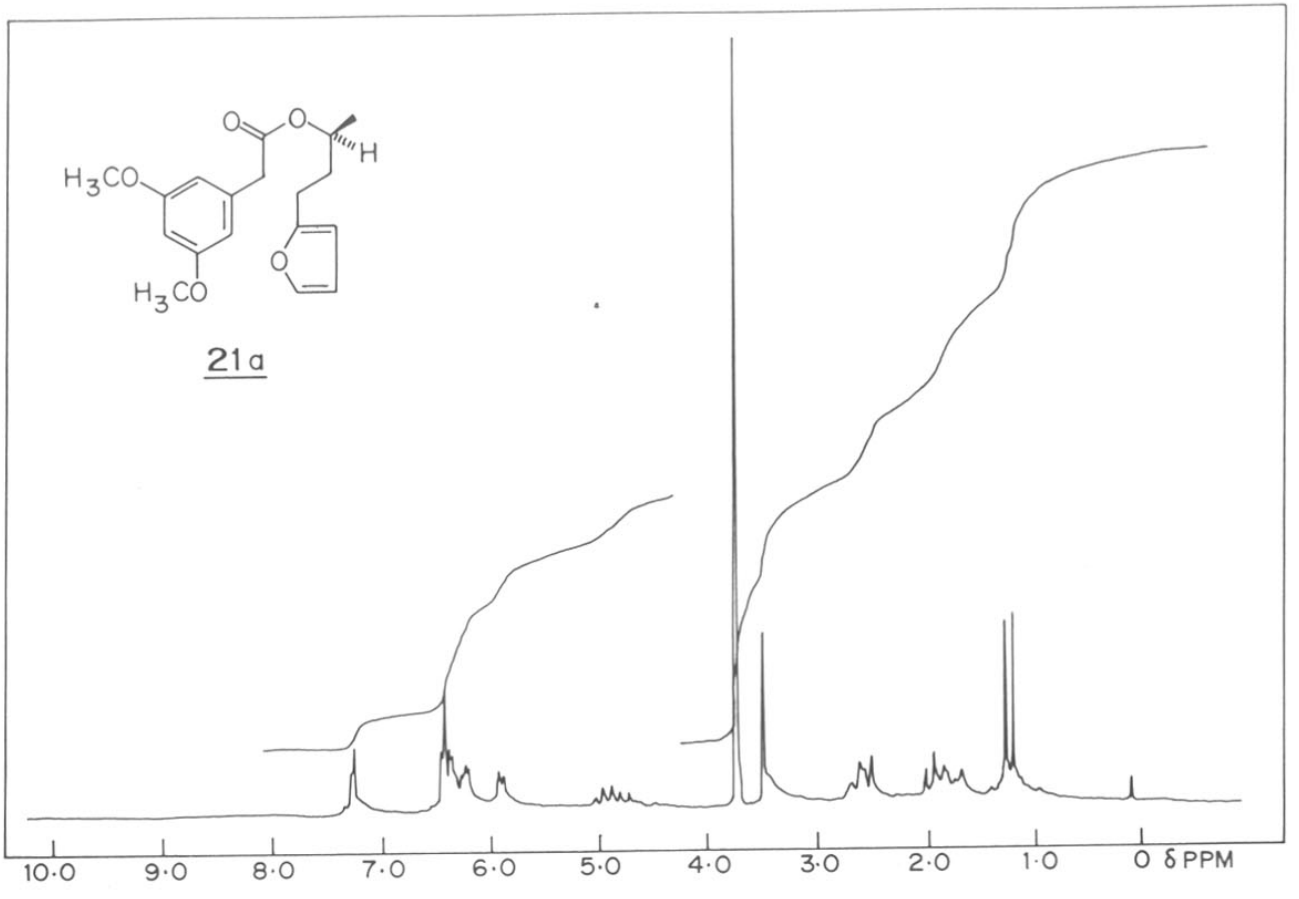
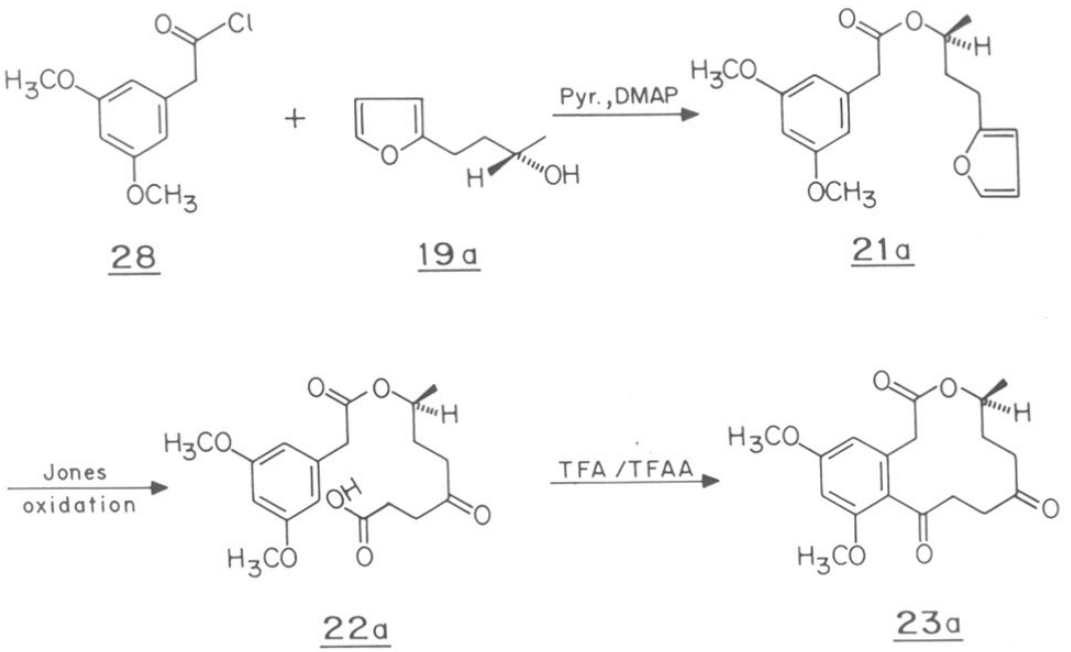


FIG. II : ^{1}H NMR SPECTRUM OF THE COMPOUND (21a) IN $CDCl_3$

ties of **21a** were in accordance of the reported data for its racemic counterpart.[11]

SCHEME-2·2·5



In accordance with the scheme, next step i.e. Jones oxidation of the ester **21a** furnished the optically active keto acid **22a** in 70% yield. Keto acid **22a** showed optical rotation of $[\alpha]_D^{25}$ = - 8.08° in chloroform. The spectral (**FIG.III, ^{1}H-NMR**) and physical properties of keto acid **22a** were in full agreement with the reported data for its racemic analog.[11]

Final cyclization of keto acid **22a** in a mixture of trifluoroacetic anhydride and trifluoroacetic acid (5:1) at room temperature resulted into the formation of S-(-)-di-O-methyl-12-oxocurvularin (**23a**) in 18% yield. Synthetic di-O-methyl-12-oxocurvularin (**23a**) exhibited optical rotation of $[\alpha]_D^{25}$ = -26.0° in ethanol which is

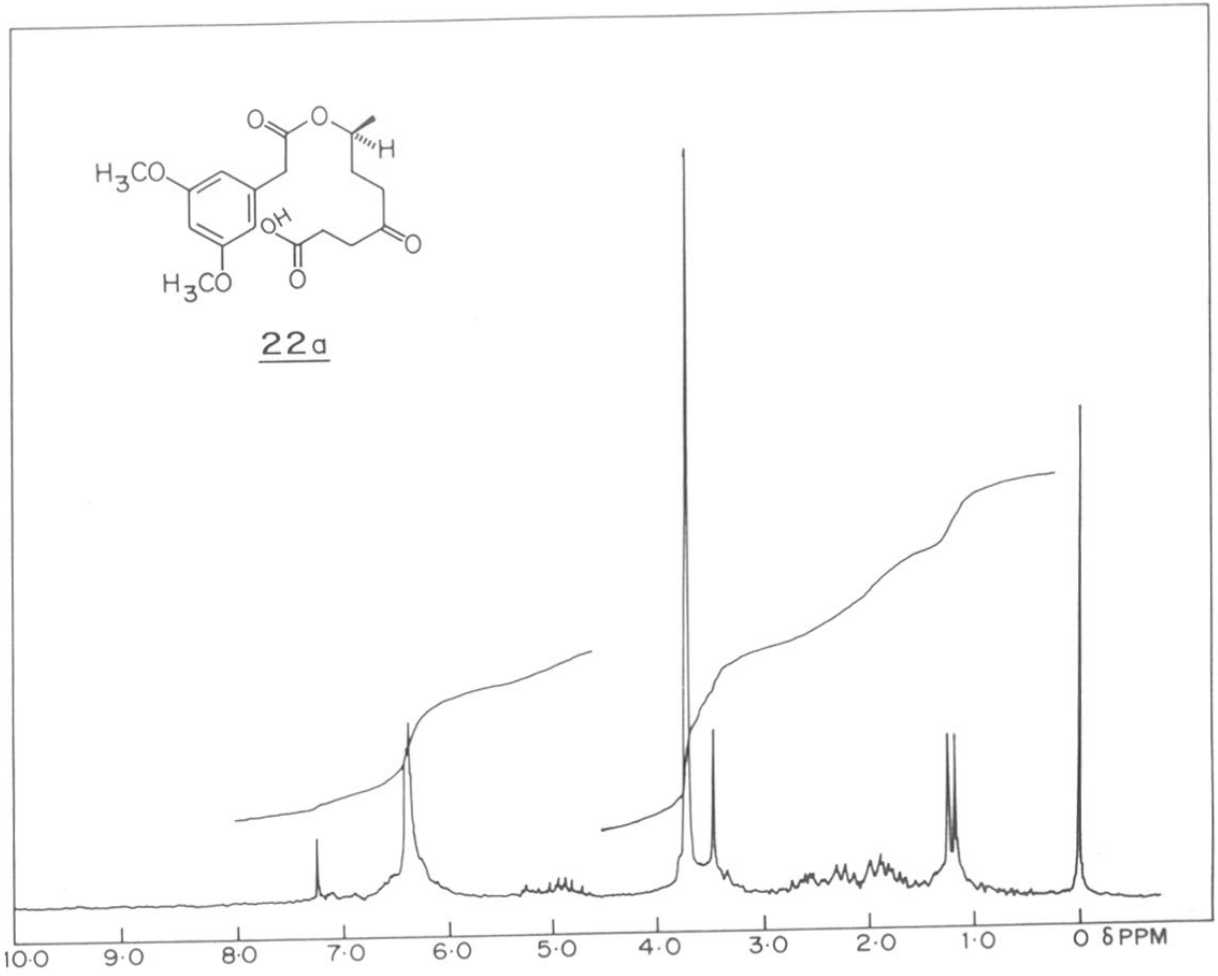
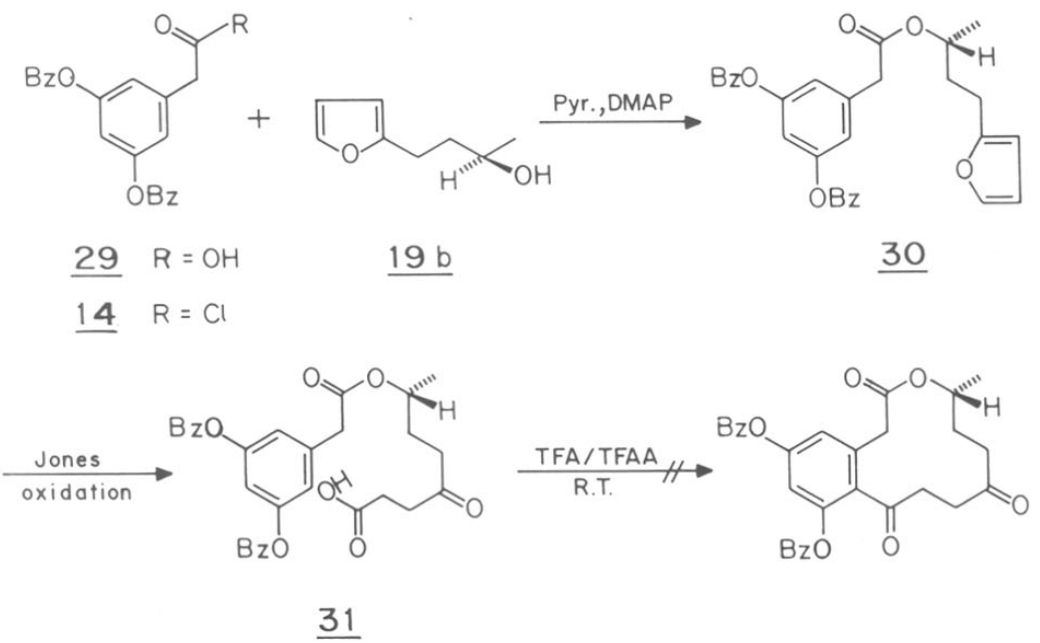


FIG. III : ^{1}H NMR SPECTRUM OF THE COMPOUND(22a) IN $CDCl_3$

comparable to the optical rotation of natural 12-oxocurvularin, $[\alpha]_D^{25}$ = -43.5° (0.47, EtOH). The (S)-(-)-di-O-methyl-12-oxocurvularin (**23a**) exhibited analogous spectral (**FIG.IV, ^{1}H-NMR**) and physical properties to the (±)-di-O-methyl-12-oxocurvularin (**23**).[11]

In order to obtain 12-oxocurvularin directly, yet another approach would be to employ 3,5-dibenzyloxyphenyl-acetyl chloride (**14**) in place of 3,5-dimethoxyphenylacetyl chloride (**28**), as during cyclization with TFA-TFAA, benzyl groups may also be cleaved to furnish 12-oxocurvularin (**Scheme 2.2.6**).

SCHEME-2·2·6



However, to test the validity of the methodology, first 3,5-dibenzyloxy-

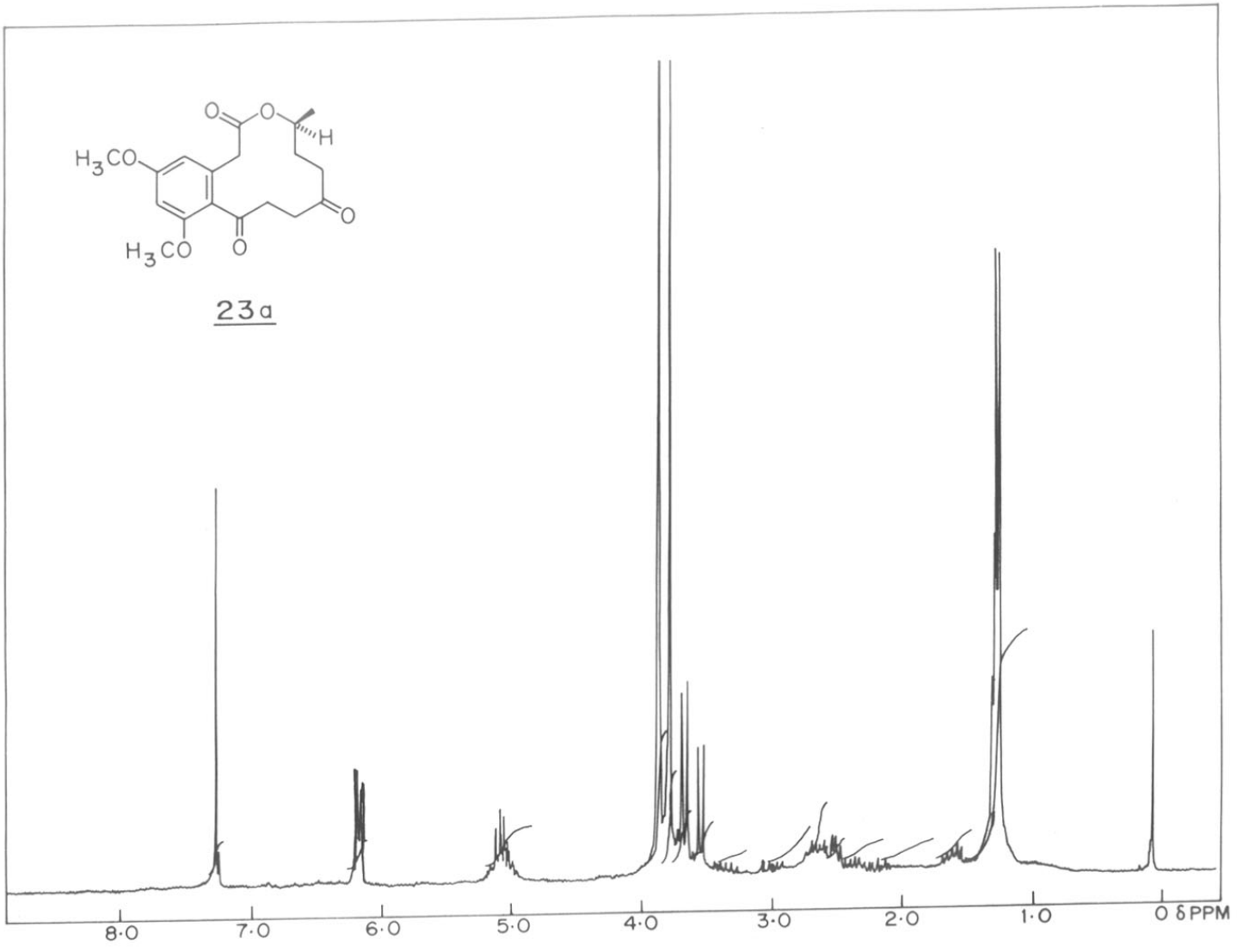


FIG. IV: ^{1}H NMR SPECTRUM OF THE COMPOUND (23a) IN $CDCl_3$

phenylacetyl chloride **14** (prepared from corresponding acid and thionyl chloride) was esterified with alcohol **19b**, the undesired isomer, to give ester **30**, which showed spectral properties consistent to its structure. The IR spectrum revealed a carbonyl absorption peak at 1730 cm^1. The ^{1}H-NMR (**FIG.V**) exhibited a methyl doublet at δ 1.20, two methylene multiplets at δ 1.84 and δ2.57. The benzylic methylene showed a singlet at δ 3.51 and four benzyloxy methylene protons appeared as singlet at δ 5.01. A multiplet between δ 4.77 - δ5.22 corresponding to C<u>H</u>- was present. The furyl protons appeared as three multiplets at δ 5.93, 6.22 and 7.11 each integrating for single proton. The aromatic protons showed a broad singlet at δ 6.55. Ten benzyloxy aryl protons exhibited a broad singlet at δ 7.35. The presence of the molecular ion peak at m/e. 470 further supported the assigned structure. The ester **30** exhibited optical rotation of +8.02° (C= 1.9, $CHCl_3$).

The ester **30** when subjected to Jones oxidation furnished the desired ketoacid **31**. The formation of the ketoacid **31** was fully supported by its spectral analysis. The IR spectrum showed absorption peaks at 3500, 1730, 1718 and 1594 cm^{-1}. The ^{1}H-NMR (**FIG.VI**) was characterized by a methyl doublet at δ1.15, two multiplets between δ 1.62-1.93 and δ 2.06-2.57 integrated together for all the eight -CH_2 protons. A singlet at δ 3.46 corresponded to benzylic methylene and the four benzyloxy methylene protons showed another singlet at δ 4.91. As usual the multiplet at δ 5.13 was present corresponding to the -C<u>H</u>- proton. The three aromatic protons showed a singlet at δ 6.42 and ten benzyloxy aryl protons depicted a broad singlet at δ 7.35. The presence of M^+ at 504 further confirmed the structure. The acid **31** showed optical rotation of + 7.020 (C = 1.2, $CHCl_3$).

The keto acid **31** was then subjected to cycloacylation in a mixture of tri-

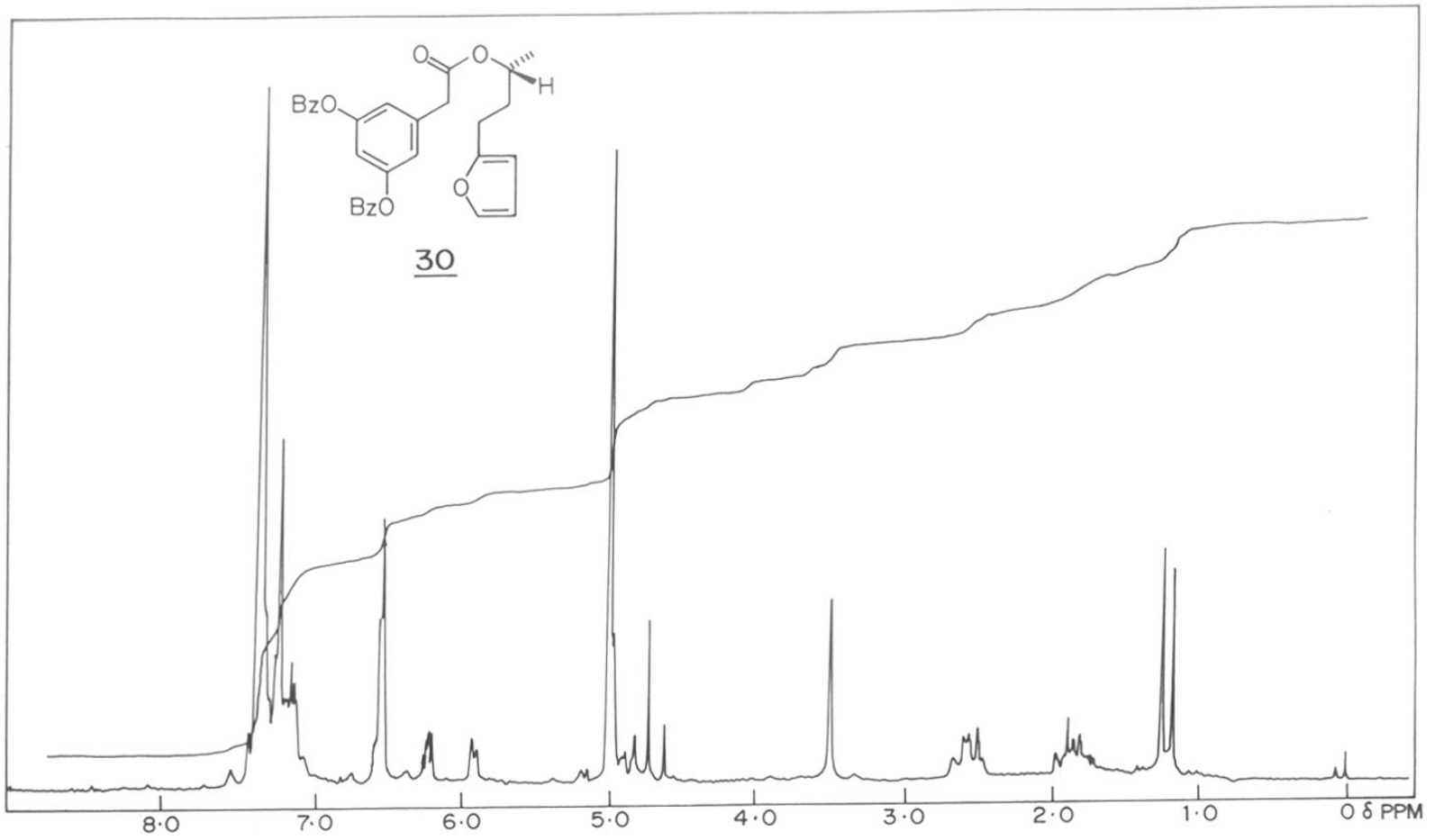


FIG. V : ^1H NMR SPECTRUM OF THE COMPOUND (30) IN $CDCl_3$

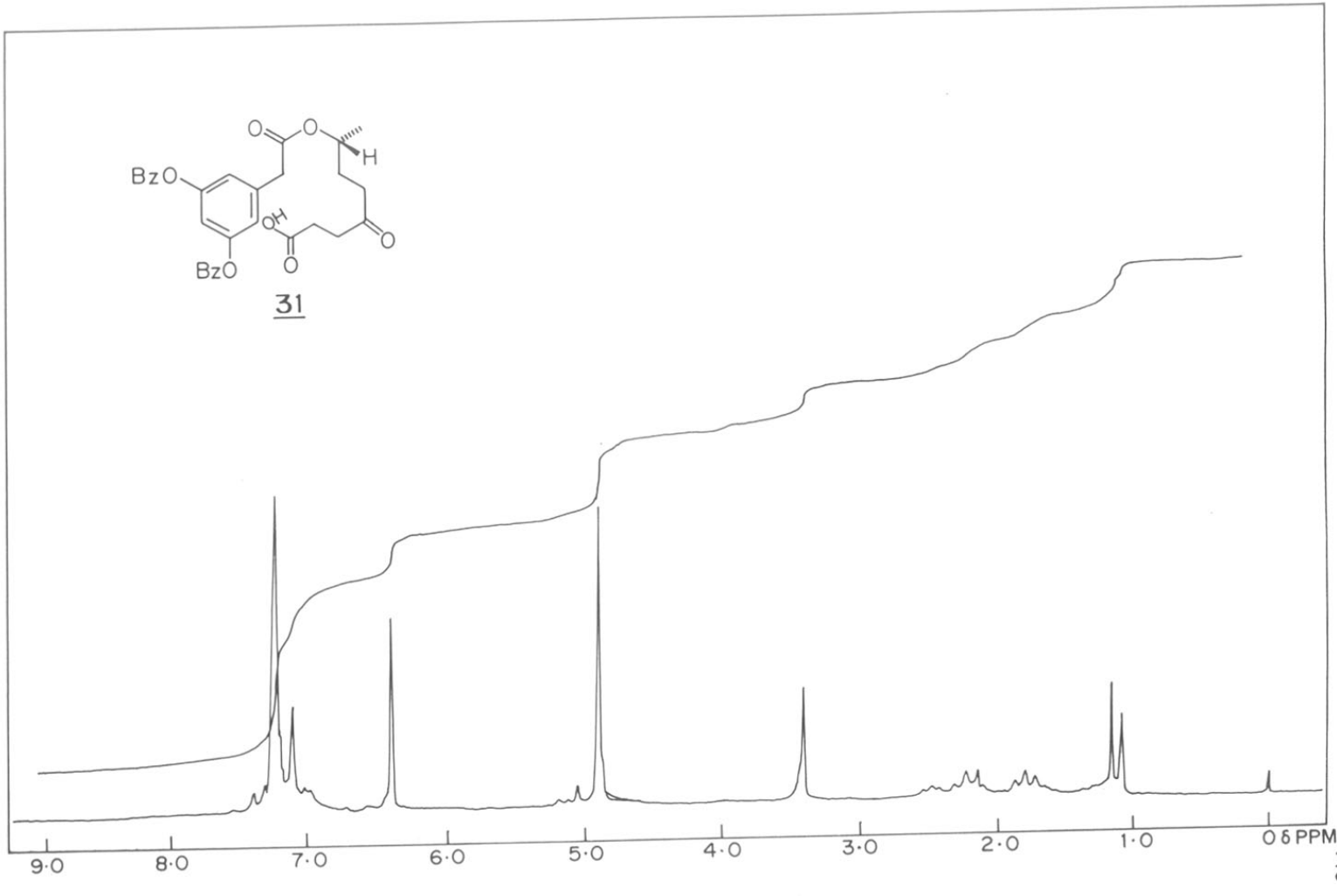


FIG. VI : ^{1}H NMR SPECTRUM OF THE COMPOUND (**31**) IN $CDCl_3$

fluoroacetic anhydride and trifluoroacetic acid (5:1) at room temperature. However, the desired (+)-curvularin could not be isolated from the reaction mixture, otherwise it would have provided the antipode of 12-oxocurvularin **32**.

It can be concluded that the PPL catalyzed transesterification of racemic alcohols can be utilized as a synthetic tool to bring in chirality in a synthetic sequence. The use of PPL for the resolution of alcohol **19** via transesterification and successful employment of the chiral alcohol **19a** so obtained in the synthesis of (-)-12-oxocurvularin dimethyl ether exemplifies the utility of the method. An interesting point to be noted is that in contradiction to the general behaviour of PPL which prefers R isomer more than the S isomer, here the S alcohol was catalysed more than R alcohol thereby leading to the formation of desired S ester (**27**). The formation of **19a** by yeast reduction which is known to give S isomer further supports the abnormal behaviour of PPL.

Experimental

4-(2-Furyl)-3-buten-2-one (18): An aqueous solution of sodium hydroxide (3.5 ml , 33%) was added to a stirred solution of furfural (**17**) (20 gm, 208 mmol) in water (150 ml) and acetone (31 ml, 530 mmol) at 0°C during 30 min. The stirring was continued for 4 h at room temperature and then it was acidified with dilute H_2SO_4 (10%). to pH 5. The organic layer was separated, dried (Na_2SO_4) and dis tilled under reduced pressure to yield 4-(2-furyl)-3-buten-2-one (**18**) (18.4 gm, 65%) as low melting solid. b.p. 86-89°C/2mm,; m.p. 36-38°C (lit.[20] b.p. 114-116°/10 mm, lit. m.p. 37-39°C).

Yeast reduction of 4-(2-fuyl)-3-buten-2-one (18): A culture of the yeast strain NCIM-3195 (Saccharomyces cervisiae) was grown on MGYP medium for 24 h. The yeast cells obtained after centrifugation were taken in phosphate buffer (pH 7) (10 ml) and the substate 4-(2-furyl)-3-buten-2-one (**18**) (1.0 gm, 7.35 mmol) was added. The contents were shaken at 30-35°C for seven days. The reaction mixture was filtered through a celite pad and the filtrate was extracted with ethyl acetate (2 x 10 ml). The combined extracts were washed with brine and dried over sodium sulphate. The residue obtained after evaporation of the solvent was purified on column (pet. ether-acetone, 9.5:0.5 as eluent) to give allylic alcohol **26** (0.15 gm, 14%) as a greenish-yellow oil. The allylic alcohol **26** so obtained was hydrogenated in ethanol (10 ml) with palladium on charcoal (0.015 gm) as catalyst at room temperature and atmospheric pressure for 8 h. The catalyst was filtered off and filtrae was evaporated to give S-(-)-4-(2-furyl)-butan-2-ol (**19a**) (0.12 gm, 80%). $[\alpha]_D^{25}$ = -12.13° (C= 1.5, $CHCl_3$) [Lit. [19] $[\alpha]_D^{25}$ = -22.6 (C= 1.5, $CHCl_3$)

(±)4-(2-Furyl)-butan-2-ol (19): The ketone **18** (5 gm, 36.38 mmol) in THF (10 ml) was added to a stirred suspension of LAH (0.7 gm, 18.38 mmol) in THF (40

ml) at 0°C under nitrogen atmosphere. The stirring was continued for additional 2 h at the same temperature. The excess LAH was destroyed with ethyl acetate (10 ml) followed by a saturated solution of sodium sulphate (20 ml). The solid obtained was filtered and washed with ethyl acetate (100 ml). The combined filtrate was dried (Na_2SO_4) and conentrated. The yellow oily residue was distilled under reduced pressure to give (±)4-(2-furyl)-butan-2-ol (**19**) (2.6 gm, 50%) as a colourless liquid, b.p. 81-84°C/25 mm (lit.[20] b.p. 71-72°C/2mm).

PPL catalyzed transesterification of (±) 4-(2-furyl)- butan-2-ol (19): A solution of 4-(2-furyl)-butan-2-ol (**19**) (0.50 gm, 3.57 mmol) and 2,2,2-trichloroethyl-butyrate (0.99 gm, 4.28 mmol) in dry ether (5 ml) was added to a stirred suspension of PPL (2.5 gm) in dry ether (5 ml) containing molecular sieves 4A (1 gm). The reaction mixture was stirred under N_2 for 6 h, the enzyme was filtered through clite powder and the filtrate was concentrated. The residue was purified by column (pet. ether-acetone, 9.5:0.5) to afford the ester **27** (0.23 gm, 30%) as a thick oil along with the unreacted alcohol **19b** (0.28 gm, 56%), **19b** $[\alpha]_D^{25}$ = +18.1° (C=1.5, $CHCl_3$) **27** = $[\alpha]_D^{25}$ = -5.12° (C=2, $ChCl_3$) [Lit.[19] $[\alpha]_D^{25}$ =-3.5°for O-acetate derivative).

IR (Neat): 1740 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 0.91 (t, J = 6.5 Hz, 3H); 1.22 (d, J = 6.2 Hz, 3H); 1.40-1.75 (m,2H); 1.80-2.04 (m, 2H); 2.24 (t, J = 6.5 Hz, 2H); 2.62 (t, J = 6.5 Hz, 2H); 4.93 (m, 1H); 5.91 (m, 1H); 6.20 (m, 1H); 7.20 (m, 1H).

Ms (m/e) : 210 (M^+)

Analysis calc. for $C_{12}H_{18}O_3$: C = 68.54; H = 8.62

Found: C = 68.51; H = 8.58

(-)4-(2-Furyl)-butan-2-ol (19a): The ester **27** (0.21 gm, 1 mmol) was stirred overnight with potassium carbonate (0.14 gm, 1 mmol) in methanol (10 ml). The

residue obtained after evaporation of methanol was diluted with water (20 ml) and extracted with chloroform (2 x 20 ml). The combined extract was dried (Na_2SO_4), concentrated and purified by column (pet-ether-acetone; 8:2) to yield S(-)-4-(2-furyl)-butan-2-ol (**19a**) (0.135 gm, 96%) as colourless oil. $[\alpha]_D^{25}$ =-18.05° (C = 1.34, $CHCl_3$) [lit[19] $[\alpha]_D^{25}$ = -22.6° (C = 1.5, $CHCl_3$).

(-)-[4-(2-Furyl)-butan-2-ol]-3,5-dimethoxyphenyl acetate (21a): 3,5-Dimethoxyphenyl acetic acid (**20**) (2.30 gm, 11.72 mmol) was refluxed with thionyl chloride (1.28 ml, 11.7 mmol) in benzene (20 ml) for 2h. The excess thionyl chloride along with the benzene was distilled off. The resultant 3,5-dimethoxyphenylacetyl chloride (**28**) was dissolved in benzene (5 ml) and added to a stirred solution of alcohol **19a** (1.5 gm, 10.71 mmol), pyridine (1.2 gm, 15.18 mmol) and catalytic amount of DMAP in benzene (20 ml) under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature and water (50 ml) was added. The benzene layer was separated and aqueous phase was extracted with benzene (2 x 20 ml). The benzene extract was dried (Na_2SO_4) and concentrated under vacuum. The residue after chromatographic purification (pet-ether - acetone 9.5:0.5) afforded (-) ester **21a** (2.54 gm, 75%) as a viscous oil. $[\alpha]_D^{25}$ = -6.63° (C =2, $CHCl_3$).

IR (neat): 1735 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.22 (d, J = 6.2 Hz, 3H); 1.88 (m, 2H); 2.57 (m, 2H);3.50 (s, 2H); 3.78 (s, 6H); 4.88 (m, 1H); 5.88 (m, 1H); 6.18 (m, 1H); 6.36 (m, 3H); 7.21 (m, 1H).

Ms (m/e): 318 (M^+).

(-)-7-(3,5-Dimethoxyphenylacetoxy)-4-oxo-octanoic acid (22a): Jones reagent (3.25 ml) was added to a stirred solution of the furan ester **21a** (2.1 gm, 6.60 mmol) in acetone (75 ml) at 0°Cduring 20 min. The reaction mixture was stirred

for 2 h. at the same temperature and the supernatant clear solution was decanted. The residue was washed with acetone (2 x 10 ml) and the combined acetone solution was concentrated. The residue was subjected to column chromatography using 30% ethyl acetate in pet ether as eluent to afford (-)-7-(3,5-dimethoxyphenylacetoxy)-4-oxo-octanoic acid (**22a**) (1.62 gm, 70%) as viscous oil. $[\alpha]_D^{25}$= -8.08° (C = 2.5, $CHCl_3$).

IR (neat): 3500 (br), 1735, 1715 and 1610 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.22 (d, J = 6.5 Hz, 3H); 1.67-1.95 (m, 2H); 2.15-2.66 (m, 4H); 2.66-2.84 (m, 2H); 3.51 (s, 2H); 3.78 (s, 6H); 4.91 (m, 1H); 6.37 (br s, 3H).

Ms (m/e): 352 (M^+)

(-)-3,5-Di-O-methyl-12-oxocurvularin (23a): To the stirred solution of acid **22a** (0.176 gm, 0.5 mmol) in trifluoroacetic anhydride (7.5 ml) was added trifluoroacetic acid (1.5 ml) at room temperature. The reaction mixture was stirred for 6h and quenched with ice water. The product was extracted with chloroform (2 x 20 ml), dried (Na_2SO_4) and concentrated. The residue was purified on column (neutral alumina) using pet ether-ethyl acetate (8:2) as eluent to give (-3,5-di-O-methyl-12-oxo-curvularin (**23a**) (0.030 g, 18%). $[\alpha]_D^{25}$ = -26.0° (C = 4.0, EtOH).

IR (neat): 1745, 1730, 1610 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.15 (d, J = 6.2 Hz, 3H); 1.62 (m, 1H); 2.05 (m, 1H); 2.25 (m, 1H); 2.62 (m, 1H); 2.70 (m, 1H); 3.02 (m, 1H); 3.45 (m, 2H); 3.55 (d, J = 15 Hz, 1H); 3.65 (d, J = 15 Hz, 1H); 3.80 (s, 3H); 3.90 (s, 3H); 4.95 (m, 1H); 6.20 (d, J = 2Hz, 1H; 6.25 (d, J = 2 Hz, 1H).

Ms (m/e): 334 (M^+).

Uv (EtOH,λ_{max}): 220, 275, 300 nm.

(+)-[4-(2-Furyl)-butan-2-ol)-3,5-dibenzyloxyphenyl acetate (30): A mixture of 3,5-dibenzyloxyphenyl acetic acid (**29**) (1.0 gm, 2.87 mmol), thionyl chloride (0.31 ml, 2.87 mmol) and benzene (20 ml) was refluxed for 2h. The excess thionyl chloride was distilled off and the crude acetyl chloride was dissolved in dry benzene (10 ml) and added to a stirred mixture of alcohol **19b** (0.37 gm, 2.61 mmol;$[\alpha]_D^{25}$ = +18.1°), pyridine (0.30 gm, 3.7 mmol) and cat. amount of DMAP in benzene (10 ml) at room temperture. The reaction mixture was stirred overnight, quenched with water (20 ml) and the benzene layer was separated. The aqueous phase was extracted with benzene (2 x 10 ml) and the combined organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by column (pet. ether-acetone 9:1) to give the ester **30** (0.85 gm, 62%) as a viscous oily mass. $[\alpha]_D^{25}$ = + 8.02° (C=1.9, $CHCl_3$).

IR (neat): 1730 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.20 (d, J = 6.5 Hz, 3H); 1.84 (m, 2H); 2.57 (m, 2H); 3.51 (s, 2H); 5.01 (s, 4H); 4.77-5.22 (m, 1H); 5.93 (m, 1H); 6.22 (m, 1H); 7.11 (m, 1H); 6.55 (bs, 3H); 7.35 (bs, 10 H).

Ms (m/e): 470 (M^+).

(+)-7-(3,5-Dibenzyloxyphenylacetoxy)-4-oxo-octanoic acid (31): To a solution of the ester **30** (0.62 gm, 1.32 mmol) in acetone (15 ml) cooled to 0°C, Jones reagent (0.65 ml) was added during 15 min. The reaction mixture was stirred for 2 h and the supernatant clear solution was decanted. The green residual mass was washed with acetone (2 x 5 ml) and the combined organic phase was concentrated under vacuum. The residue was purified by column (pet ether-ethyl acetate 7:3) to afford keto acid **31** (0.40 gm, 60%) as a sticky solid, $[\alpha]_D^{25}$ = + 7.02° (C = 1.2, $CHCl_3$).

IR (nujol): 3400 (br), 1730, 1718 and 1594 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.15 (d, J = 6 Hz, 3H); 1.62-1.93 (m, 4H); 2.06-2.57 (m, 4H); 3.46 (s, 2H); 4.91 (s, 4H); 5.13 (m, 1H); 6.42 (s, 3H); 7.35 (bs, 10 H).

Ms (m/e): 504 (M^+)

Attempted cyclization of the acid 31 with TFA-TFAA (1.5): To a solution of the keto acid **31** (0.50 gm, 1 mmol) in trifluoroacetic anhydride (5 ml) was added trifluoroacetic acid (1 ml) at room temperature under argon atmosphere. The reaction mixture was stirred for 6 h and poured over ice cold saturated sodium hydrogen carbonate solution. The aqueous solution was extracted with ethyl acetate (2 x 15 ml), combined extracts were dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography, however, the desired product could not be obtained.

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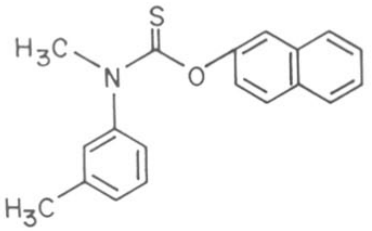
CHAPTER III

AN ALTERNATIVE METHOD FOR TOLNAFTATE

Introduction

Tolnaftate (1), [2-naphthyl-N-methyl N-(m-tolyl) thionocarbamate], is very effective antifungal drug known for more than a decade[1].



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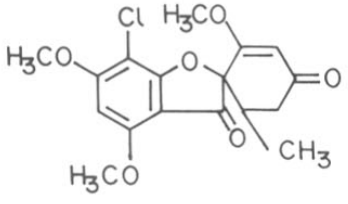

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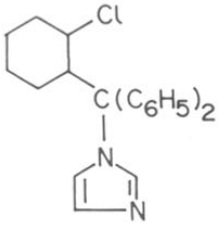

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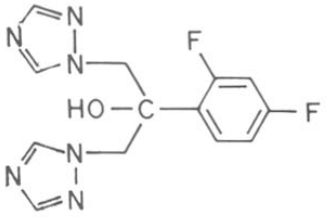

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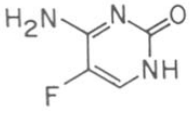

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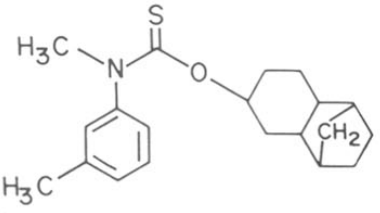

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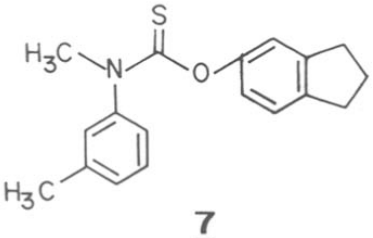


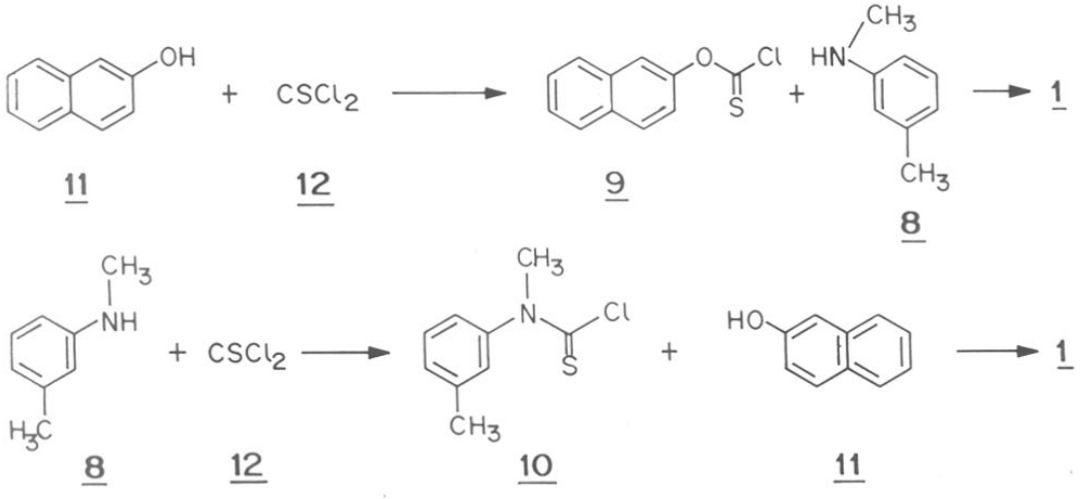
7

Tolnaftate is used for the topical treatment of ring worm and other skin infections due to Epidermophyton floccosum, Micosporum audouinii, M. canis, Trichophyton mentagrophytes and T. Verrucosun[2]. Sometimes infections due to T. rubrum, T. tonsurans and Malassezia furfur may also respond to tolnaftate[2]. However, it is inactive against Candida sp.[2]

Tolnaftate (**1**), because of being a topical agent, is not suitable for the treatment of tinea of the skin and scalp[2]. Since it is not keratolytic, the skin tolerance is very good though occasionally irritation and allergic sensation may develop.[2] It is applied in the form of cream or as a dusting powder containing 1% drug.

Some other antifungal drugs possessing similar activity to tolnaftate are griseofulvin (**2**)[3], clotrimazole (**3**)[4], fluconazole (**4**)[5], and 5-fluorocytosine (**5**)[6]. Tolciclate (**6**)[7] and tolindate (**7**)[8] are the analogs of tolnaftate possessing the antifungal activity comparable to tolnaftate.

SCHEME 3·0·1



The known methods[1] for the preparation of tolnaftate involve either the condensation of N-methyl m-toluidine (**8**) with 2-naphthylchlorothionoformate (**9**) or the condensation of thiocarbamoyl chloride (**10**) with 2-naphthol (**11**) in presence of a base in a polar solvent. The desired chlorothionoformate (**9**) and thiocarbamoyl chloride (**10**) are prepared by the condensation of thiophosgene (**12**) with 2-naphthol (**11**) and N-methyl m-toluidine (**8**), respectively (**Scheme 3.0.1.**).

Present work

The process development for drugs and drug intermediates is one of the important activities of our Division of Organic Chemistry : Technology and as a part of this programme, a laboratory scale preparation of tolnaftate was undertaken.

As discussed earlier, though the well established procedures for tolnaftate are quite simple and effective, yet the involvement of thiophosgene as one of the reactants appears as the major drawback of the methods. Toxicity and hazardous properties of thiophosgene are well known[9] and thus its handling in the process of tolnaftate manufacture would always involve some risk. Hence, there was a need for an alternative method, which could avoid the use of thiophosgene. With this view, an alternative route to tolnaftate, comprising of carbon disulphide, N-methyl m-toluidine and 2-naphthol, has been developed and the same has been described in this chapter.

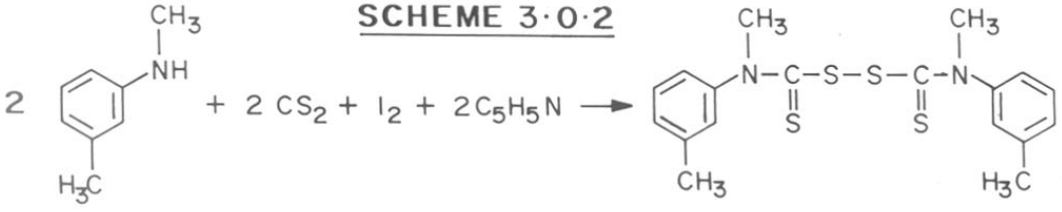
Fry and Farquhar[10] have developed a standard method for the preparation of thioureas and thiuram disulphides from primary and secondary amines, as shown below:

$$2RNH_2 + CS_2 + I_2 + 2C_5H_5N = (RNH)C + S + 2C_5H_5NHI$$

$$R_2NH\ 2CS_2 + I_2 + 2C_5H_5N = (R_2N\text{-}C\text{-}S)_2 + 2C_5H_5N.HI$$

The methodology was employed to prepare five new thiuram disulphides, including dimethyl di-(m-tolyl)-thiuram disulphide (**13**). Thus N-methyl-m-toluidine (**8**) was reacted with carbon disulphide in presence of pyridine and iodine to give the desired thiuram disulphide (**13**) in 60% yield (**Scheme 3.0.2**).

SCHEME 3·0·2



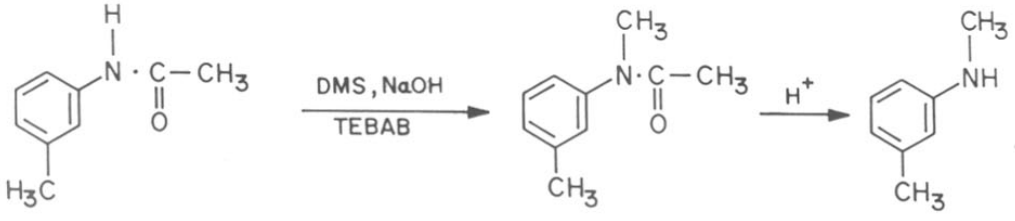
8 **13**

A general method for the conversion of a thiuram disulphide to the corresponding thiocarbamoyl chloride by treatment of chlorine has been reported by Ritter et. al.[11] These workers prepared a variety of carbamoyl chlorides from different symmetrical thiuram disulphides, as shown below:

R = C_2H_5, CH_3, $C_3H_7^i$ AND $C_4H_9^i$

A strategy based on the above two observations for tolnaftate seemed quite promisable as a combination of both the methods would lead to the preparation of desired thiocarbamoyl chloride **10** which was otherwise prepared from thiophosgene and N-methyl-m-toluidine. Methylation[12] of N-acetyl-m-toluidine (**14**) with dimethyl sulphate and sodium hydroxide in presence of triethylbutyl ammonium bromide as phase transfer catalyst gave N-methylacet-m-toluidide (**15**) which on acidic hydrolysis with 10% HCl provided N-methyl-m-toluidine (**8**) in nearly quantitative yield (**Scheme 3.0.3**). The physical and spectral properties of amine **8** were consistent to those reported in the literature.[13]

SCHEME 3·0·3

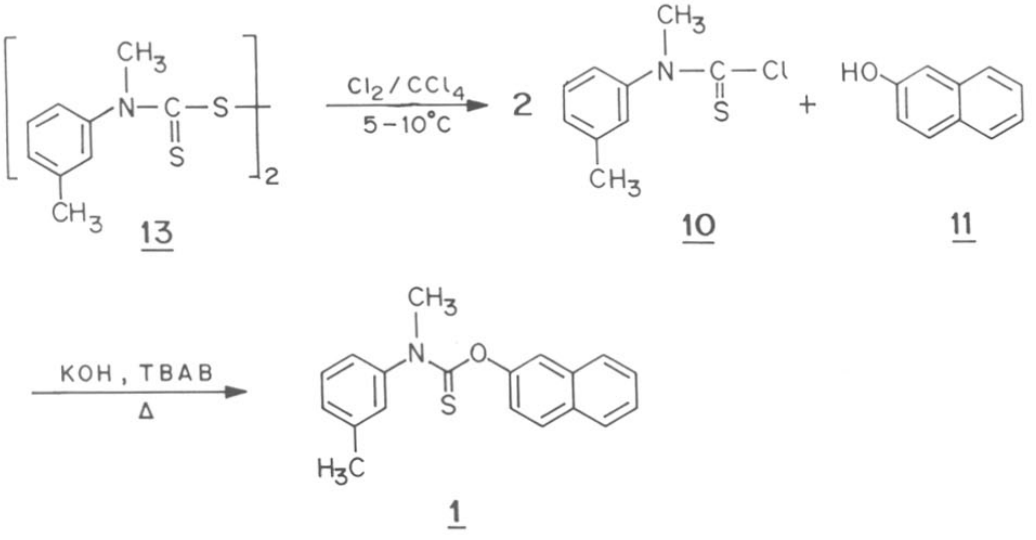


14 **15** **8**

N-methyl-m-toluidine (**8**) was then treated with carbon disulphide in presence of iodine and pyridine to afford dimethyl-di-(m-tolyl)-thiram disulphide (**13**) in 70% yield. (**Scheme 3.0.2**). The physical and spectral properties of compound **13** were in good agreement to those reported in the literature.[10]

The thiuram disulphide **13** was then treated with requisite amount of chlorine absorbed in carbon tetrachloride at 5-10°C to furnish the N-methyl-N-(m-tolyl), thiocarbamoyl chloride (**10**) in nearly quantitative yield. The crude thiocarbamoyl chloride (**10**) so obtained was condensed with ß-naphthol in benzene containing powdered potassium hydroxide and tetrabutylammonium bromide as a phase transfer catalyst, to yield tolnaftate (**1**) in 80% yield. (**Scheme 3.0.4**). The formation of tolnaftate was fully confirmed by its physical and spectral (**FIG. I**, ^{1}H-NMR) properties. The synthetic tolnaftate was found to be identical to the commercial sample.

SCHEME 3·0·4



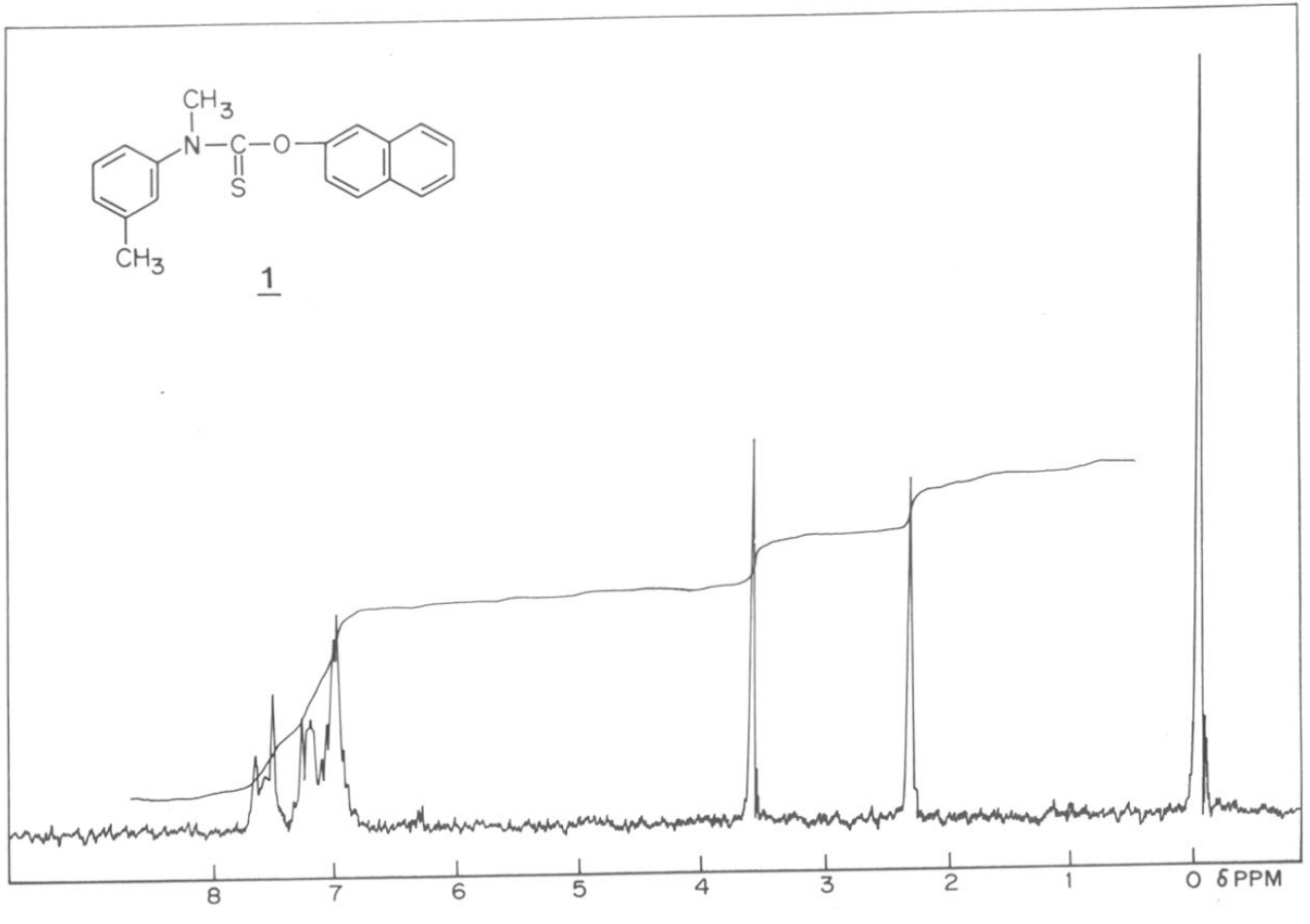


FIG. I : ^{1}H-NMR SPECTRUM OF COMPOUND (**1**) IN $CDCl_3$

It can be concluded that an alternative method for tolnaftate has been developed. The highlight of the methodology is the use of carbon disulphide and chlorine, comparatively less toxic materials in place of thiophosgene to generate the desired intermediate thiocarbamoyl chloride **10**. Thus, the involvement of less toxic materials coupled with simple and high yielding steps make the process quite appropriate to be employed industrially as well.

Experimental

N-Methylacet-m-toluidide (15): A mixture of N-acetyl-m-toluidine (**14**) (29.8 gm, 200 mmol), triethylbutyl ammonium bromide (2.38 gm, 10 mmol), benzene (300 ml) and 50% sodium hydroxide (64.0 gm, 800 mmol) was stirred at room temperature for 30 min. till a thick mass was formed. Dimethyl sulphate (27.72 gm, 220 mmol) was added slowly with stirring at 30-45° during 30 min. The reaction mixture was stirred at 80°C for 2 h and the organic phase was separated, washed with 2N HCl (100 x 2 ml), water (200 x 2 ml) and dried (Na_2SO_4). The removal of benzene under reduced pressure yielded N-methyl acet-m-toluidide (**15**) as a colourless solid (31.9 gm, 98%) m.p. 68°C [Lit.[14] m.p. 66°C].

N-methyl-m-toluidine (8): N-Methylacet-m-toluidide (**15**) (30.0 gm, 184 mmol) was refluxed in 10% HCl (200 ml) for 4 h. The reaction mixture was cooled, basified with 10% aqueous KOH to pH 12 and extracted with benzene (2 x 100 ml). The combined extracts were washed with water (2 x 100 ml), dried (Na_2SO_4) and concentrated. The brown oily residue was distilled under reduced pressure to give N-methyl m-toluidine (**8**); (21.3 gm, 96%); b.p. 90-92°C/13 mm [Lit.[13] b.p. 120-121°C].

Dimethyl di(m-tolyl)-thiuram disulphide (13): A mixture of N-methyl-m-toluidine (**8**) (24.0 gm, 196 mmol) and pyridine (32.0 gm, 405 mmol) in carbon disulphide (200 ml) was refluxed while a solution of iodine (25.2 gm, 100 mmol) in carbon disulphide (400 ml) was introduced dropwise during 1/2 h. The refluxing and stirring was continued for another 3 h. and the excess carbon disulphide was distilled and recovered. The residue was poured into cold water (500 ml) and the light yellow solid thus separated was filtered, washed with water (2 x 400 ml) and dried. The product was recrystallized from benzene and methanol to afford dimethyl di-(m-tolyl)-thiuram disulphide (**13**) (27.2 gm, 70%) as light yellow crystalline solid m.p. 170°C [Lit.[10] m.p. 170.5°C].

N-methyl-N-(m-tolyl) thiocarbamoyl chloride (10): To a stirred solution of the thiu-

ram disulphide (**13**) (24.5 gm, 62.5 mmol) in carbon tetrachloride (60 ml) was added a solution of chlorine (4.4 gm, 125 mmol) in carbon tetrachloride (25 ml) at 5-10°C. The stirring was continued for 5 h and the solvent was distilled off under reduced pressure. The residue was extracted with pet-ether (2 x 100 ml) and the combined extracts were concentrated under reduced pressure to leave the crude N-methyl-N-(m-tolyl) thiocarbamoyl chloride (**10**) (24.8 gm, 100%) as yellow oil.

2-Naphthyl-N-methyl-N-(m-tolyl) thionocarbamate (Tolnaftate) (1): The crude thiocarbamoyl chloride (**10**) (24.8 gm, 62.5 mmol) was dissolved in benzene (25 ml) and added to a mixture of powdered potassium hydroxide (13.75 gm, 250 mmol), ß-naphthol (**11**) (18.0 gm, 125 mmol) and tetrabutyl ammonium bromide (0.25 gm) in benzene (75 ml) at room temperature. The reaction mixture was refluxed for 5 h and then cooled, washed with water (2 x 100 ml), dried (Na_2SO_4) and concentrated. The brownish residue was purified on silica gel column using pet.ether - ethyl acetate (9:1) as eluent to afford 2-naphthyl-N-methyl-N-(m-tolyl)thionocarbamate (tol-naftate) (**1**) (30.75 gm, 80%) as a colourless crystalline solid; m.p. 110°C, [Lit.[15] m.p. 110.5-111.5°].

IR (Nujol): 1620, 1600, 1580 and 1460 cm^{-1}.

^{1}H-NMR ($CDCl_3$):δ 2.27 (s, 3H); 3.38 (s, 3H); 6.90-7.85 (m, 11H).

Ms (m/e): 307 (M^+).

Analysis calc. for $C_{19}H_{17}NOS$:

	C=74.23%;	H=5.58%;	N=4.56%;	S=10.43%
Found:	C=74.05%;	H=5.63%;	N=4.75%;	S=10.70%

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CHAPTER IV

SYNTHETIC APPROACHES TOWARDS CAPPARISININE AND CERVINOMYCIN

PART 1: SYNTHETIC APPROACHES TOWARDS CAPPARISININE

Introduction

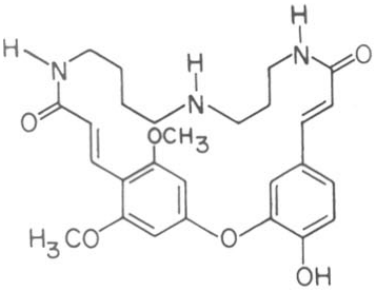
Ahmad et.al[1] isolated a new spermidine alkaloid capparisinine (**1**) while investigating the chemical constituents of the root bark of the plant Cappris decidua, a common shru of the arid plains of Pakistan. The root of the plant has been used in the oriental medicine as a laxative, antidote of poison, anthelmintic and in the treatment of cardiac troubles, boils and toothache.[2] The bark is reported to be used as cure for asthma, inflammation and gout.[3,4]

The structure of capparisinine was determined by means of spectral studies and its correlation with another spermidine alkaloid capparidisine (**2**) isolated from the root bark of Cappris decidua.[5] Capparisinine (**1**) was shown to be a positional isomer of capparidisine (**2**).

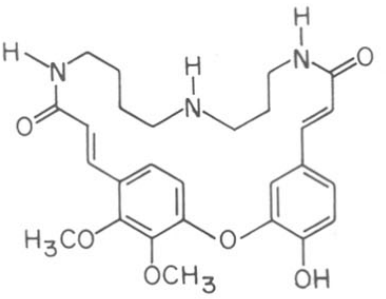
Several other spermidine alkaloids possessing related macrolactam skeleton as in capparisinine have been isolated from different plant sources Codonocarpine (**3**) was isolated from Codonocarpus australis by Doskotch et. al.[6]. Cadabicine (**4**) is another alkaloid isolated from Cadaba farinosa.[7] From the Lunaria biennis two alkaloids lunarine (**5**) and lunaridine (**6**) were isolated.[8]

All these alkaloids examplified a macrolactam skeleton consisting of a triaminespermidine, and a biaryl ether comprising of two differently substituted or unsubstituted cinnamic acid moieties. However, in lunarine and lunaridine one of the aryl ring is replaced by a cyclohexyl ring and is further joined to the other aryl ring to form a tricylic system.

Since no synthesis of capparisinine or capparidisinine has been reported so far, the total synthesis of capparisinine was undertaken and attempts made towards its regiospecific synthesis have been presented in this part.



1



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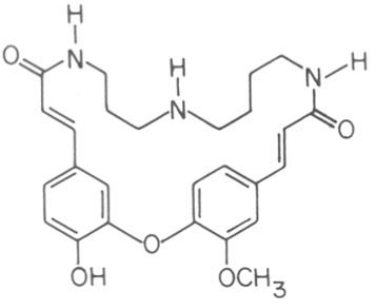

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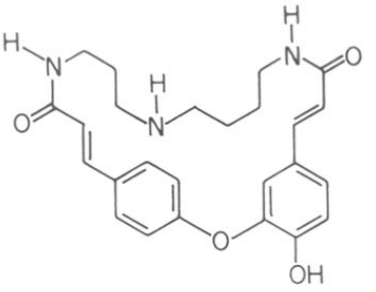

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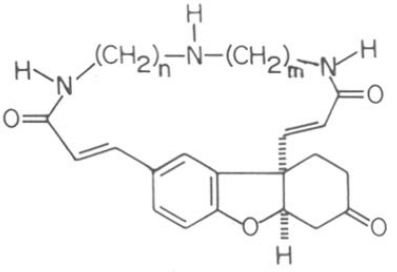
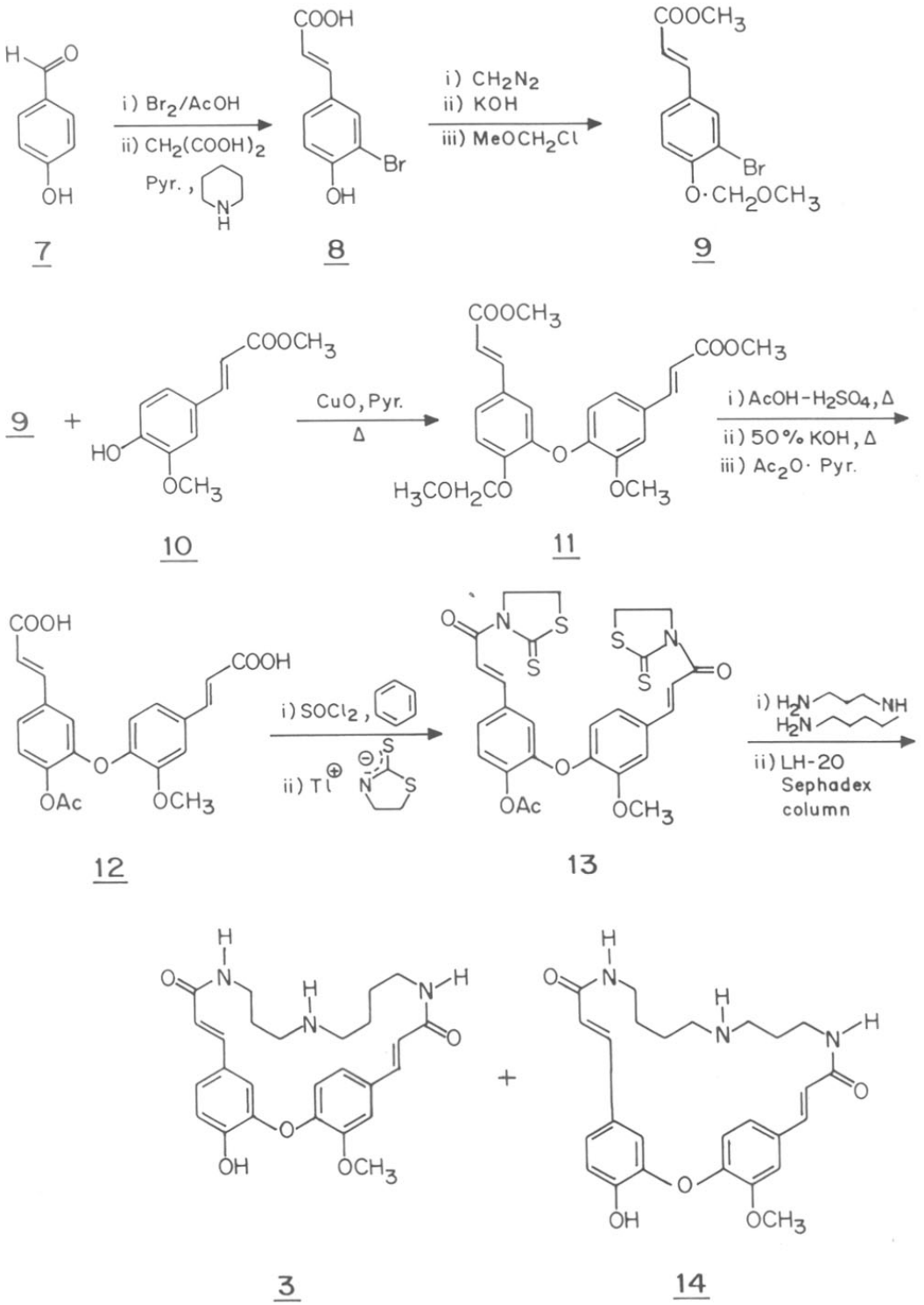


5 n = 3 , m = 4

6 n = 4 , m = 3

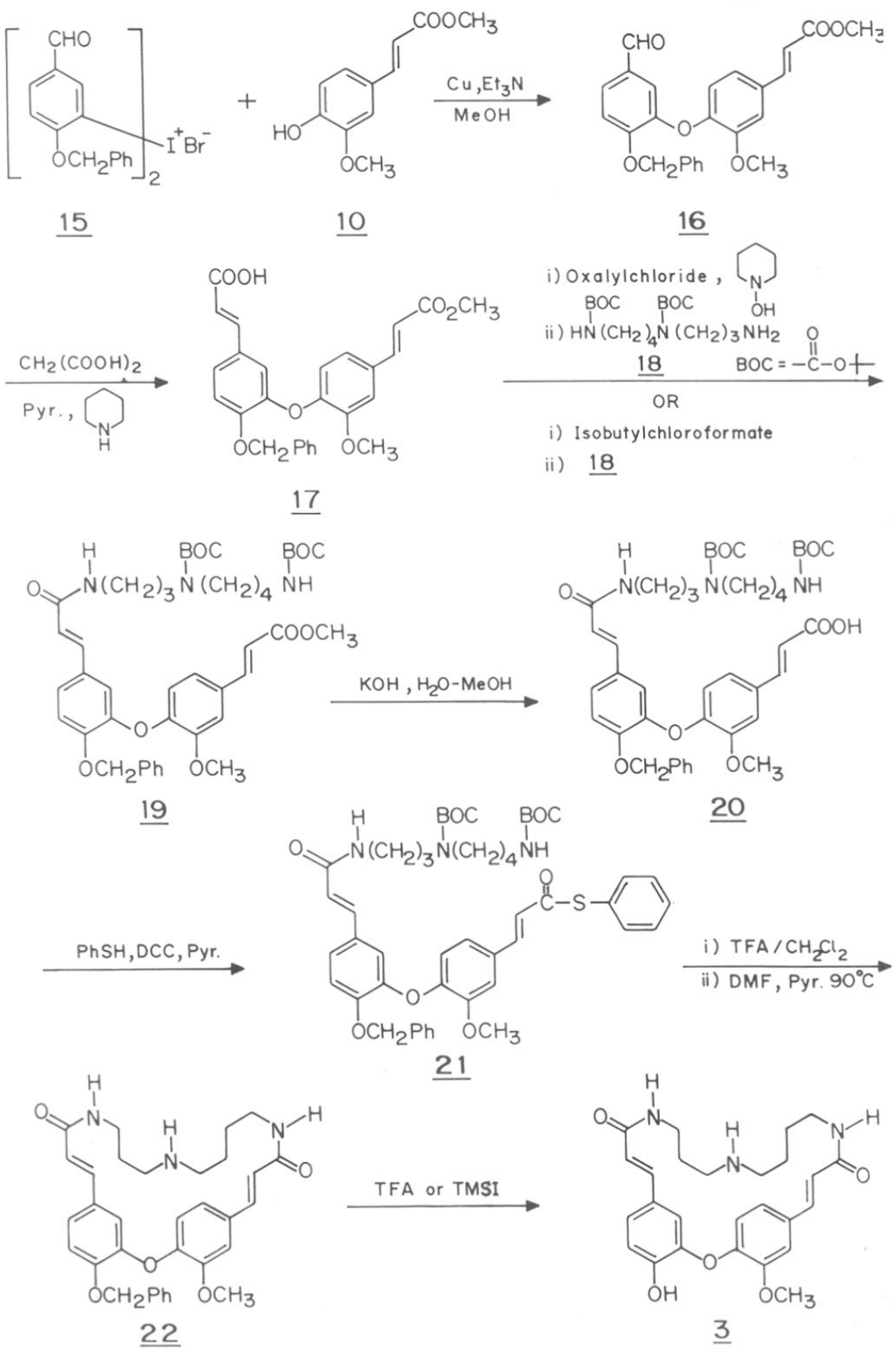
SCHEME-4·1·1


i) Br2/AcOH
ii) CH2(COOH)2
Pyr.,
i) CH2N2
ii) KOH
iii) MeOCH2Cl
CuO, Pyr.
Δ
i) AcOH-H2SO4, Δ
ii) 50% KOH, Δ
iii) Ac2O· Pyr.
i) SOCl2,
ii) Tl⊕
i) H2N NH
H2N
ii) LH-20 Sephadex column
7
8
9
10
11
12
13
3
14

However, a discussion of the total synthesis of codonocarpine (**3**) would be quite appropriate at this juncture because it has similar basic macrolactam structure as present in capparisinine (**1**) and capparidisinine (**2**). The first synthesis of codonocarpine (**3**) was reported by Fujita et. al.[9] (**Scheme 4.1.1**). The highlights of the synthesis include the biaryl ether (**11**) formation among two properly substituted cinnamic acid esters (**9** and **10**) and then aminolysis of the acid **12** with spermidine to form both codonocarpine (**3**) and regioisomer of codonocarpine (**14**). The formation of active amide **13** with thallium (I) salt of thiazolidine-2-thione facilitated the aminolysis of the desired acid **12** with spermidine. Finally codonocarpine and its isomer were separated by droplet counter-current chromatography (DCCC).

The second synthesis of codonocarpine was reported by Seitz et. al.[10] The synthesis incorporated the use of a properly protected spermidine derivative **18** to introduce regioselectivity (**Scheme 4.1.2**). Thus, the protected spermidine **18** was acylated with suitably substituted biscinnamoyl derivative **17** to give **19** either via activated ester of N-hydroxypiperidine or by mixed anhydride formed with isobutylchloroformate. Final cyclization of the acid **20** was achieved via its thioester **21** which after deprotection of BOC group in trifluoroacetic acid followed by heating in anhydrous dimethylformamide afforded o-benzylcodonocarpine (**22**). The deprotection of the benzyl group in trifluoroacetic acid or in trimethylsilyl iodide gave codonocarpine (**3**).

SCHEME - 4·1·2


CHO
OCH2Ph
I+Br-
2
15
HO
COOCH3
OCH3
10
Cu, Et3N
MeOH
16
CH2(COOH)2
Pyr.,
17
i) Oxalylchloride ,
BOC BOC
ii) HN(CH2)4N(CH2)3NH2
18
BOC = -C-O+
OR
i) Isobutylchloroformate
ii) 18
19
KOH , H2O-MeOH
20
PhSH, DCC, Pyr.
21
i) TFA / CH2Cl2
ii) DMF, Pyr. 90°C
22
TFA or TMSI
3

Present work

The fairly complex macrolactam structure of capparisinine involves synthetic challenges to organic chemists. It is evident from the earlier discussion of the synthesis of codonocarpine that the major hurdle while devising a synthesis would be the regioselective incorporation of the spermidine unit as well as the better yielding biaryl coupling of the desired cinnamoyl units. With this view, a regiospecific synthesis of capparisinine was attempted.

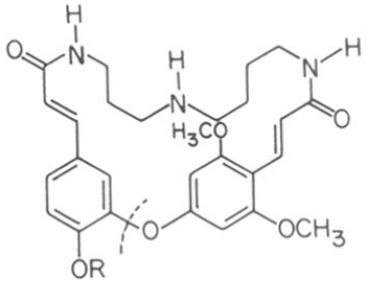
The retrosynthetic analysis of capparisinine suggested that the two fragments A and B would be the logical synthons which could be coupled to form the spermidine moiety followed by an intramolecular Ulmann's reaction to constitute the 24-membered macrocyclic ring. This strategy involving intramolecular Ulmann's reaction as the last step had not been used earlier when our work on capparisinine was in progress. However, subsequently in 1991 Boger and Yohannes reported an intramolecular, Ulmann reaction for macrocyclic ring closure in the synthesis of deoxybouvardin and RA-VII.[11] These reports supported our strategy of intramolecular Ulmann reaction. Similar methodology was successfully applied by Boger et. al. for the synthesis of a macrocyclic lactone combretastatin D-2.[12]

The desired fragment A could be derived from p-hydroxybenzaldehyde (**7**) and for the synthesis of the fragment B 1,3,5-trihydroxybenzene(phloroglucinol) (**23**) could be the proper starting material.

Synthesis of fragment A

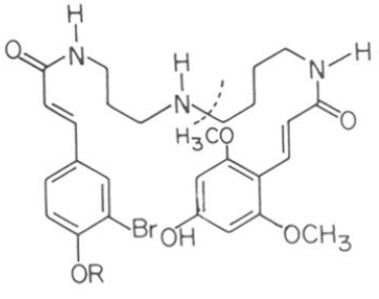
Bromination of p-hydroxybenzaldehyde (**7**) in acetic acid afforded the desired 3-bromo-4-hydroxybenzaldehyde (**24**) in 90% yield. Benzylation of **24** with benzylchloride and potassium hydroxide in refluxing ethanol furnished the benzyl ether **25** in 77% yield.

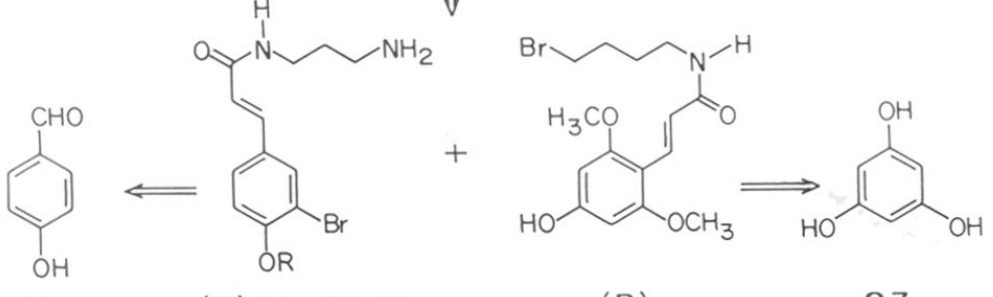
RETEROSYNTHETIC ANALYSIS



1 R = H

42 R = $-CH_2Ph$

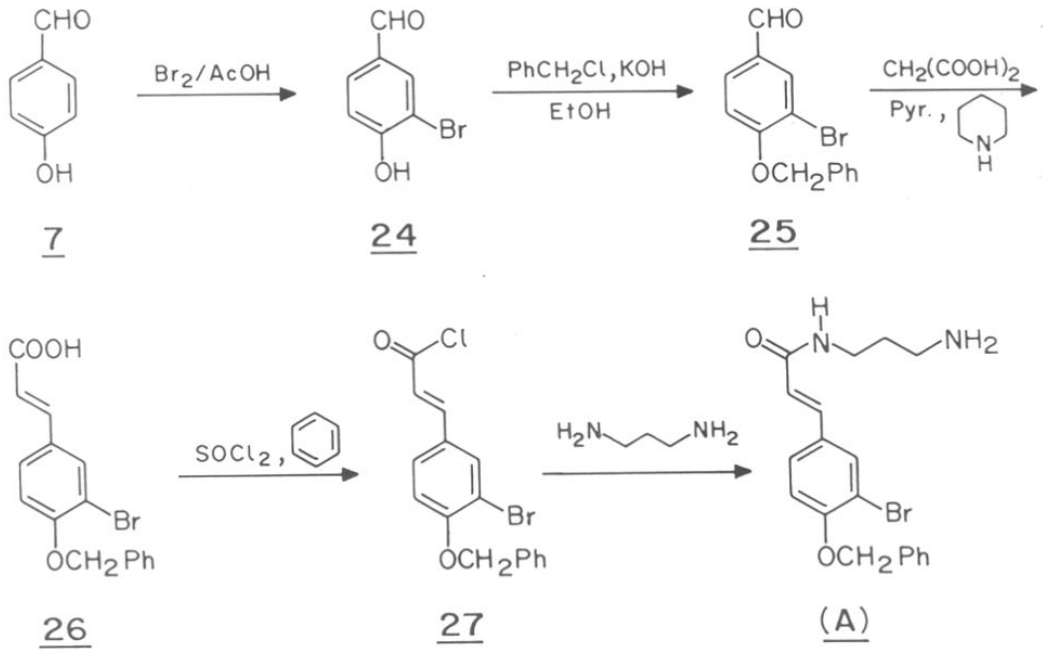




7 (A) R = $-CH_2Ph$ (B) 23

The Knoevenagel condensation[13] of the compound **25** with malonic acid in pyridine and piperidine afforded the desired cinnamic acid **26** in 79% yield (**Scheme 4.1.3**).

SCHEME - 4·1·3



The acid **26** showed physical and spectral properties consistent to those reported in the literature.[14] The acid **26** was converted to its acyl chloride with thionyl chloride in refluxing benzene and the resultant acyl chloride **27** when treated with 1,3-diaminopropane in benzene furnished the desired fragment A in 78% yield (**Scheme 4.1.3**). The spectral analysis of the fragment A further confirmed the asigned structure. In the IR spectrum absorption peaks at 1665, 3320 and 3400 cm^{-1} were observed. The ^1H-NMR (FIG.I) showed two multiplets at

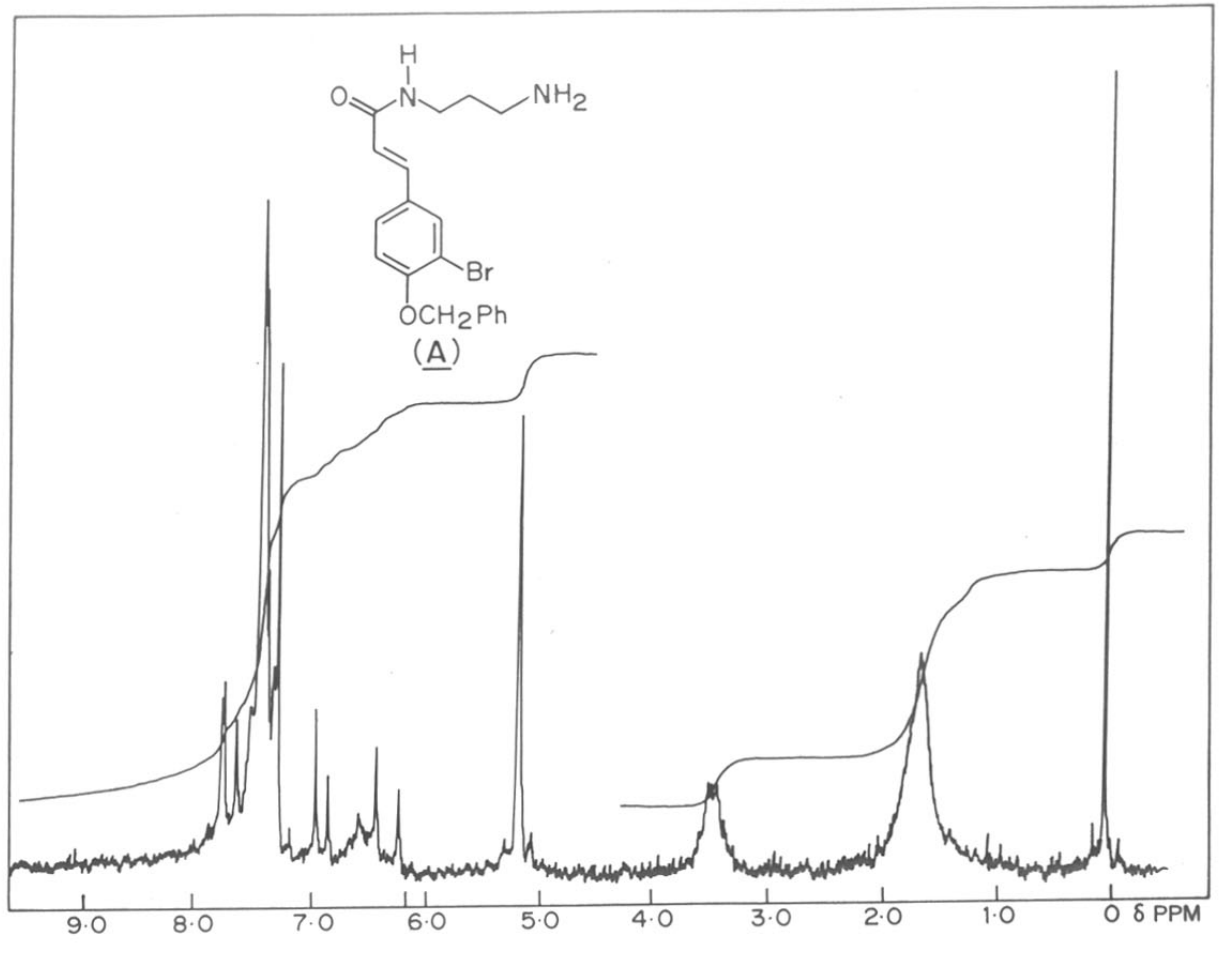


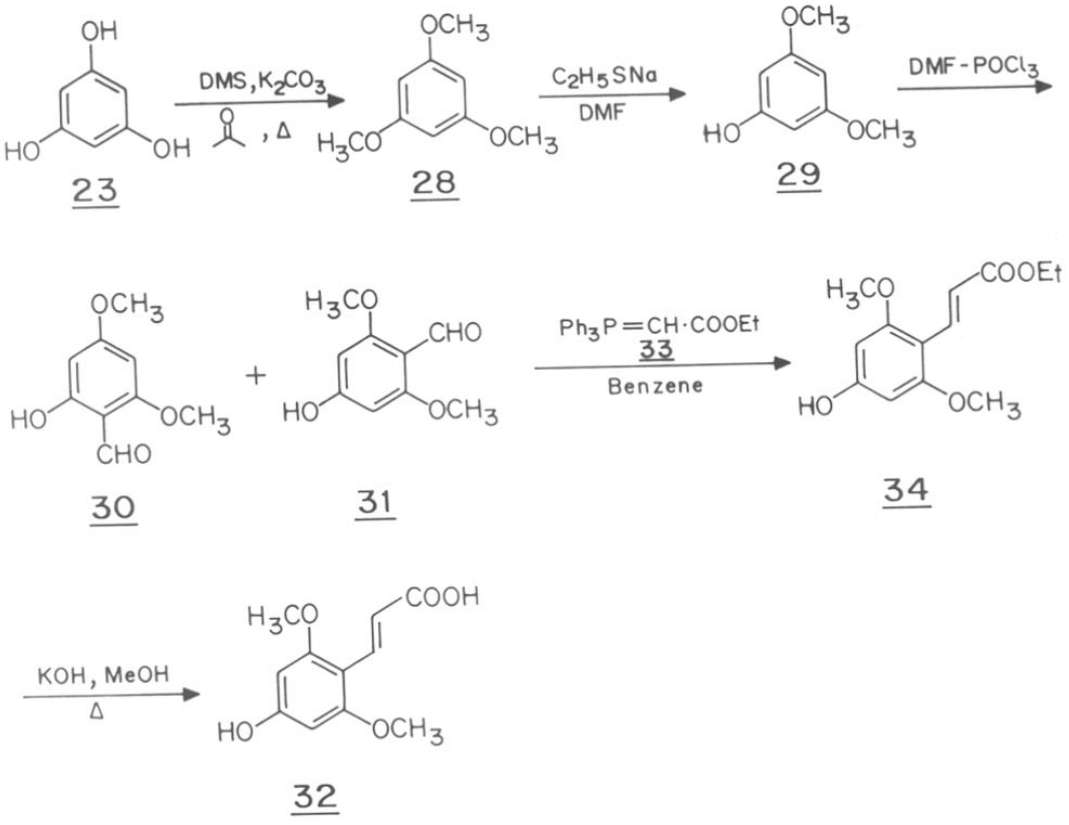
FIG. I: ^{1}H-NMR SPECTRUM OF THE FRAGMENT (A) IN $CDCl_3$

δ1.43-2.0 and δ 3.25-3.68 corresponding to three methylene groups of -N $(CH_2)_3$. NH_2 side chain. The benzylic methylene showed a singlet at δ 5.18. The trans olefinic protons appeared as doublets (J = 16 Hz) at δ 6.31 and δ 7.62. A broad singlet at δ 7.43 was observed for five protons of benzyloxy group. The aromatic protons exhibited a doublet (J = 8 Hz) at δ 6.87, a doublet of doublet at δ 7.31 and a doublet (J = 2 Hz) at δ 7.84 respectively. The presence of the molecular ion peak at m/e. 388 in the mass spectrum further confirmed the structure.

Synthesis of fragment B

After obtaining the desired fragment A, next task was to prepare the other fragment B. The presence of oxygen functionality at 2,4,6 position in the aryl ring suggested phloroglucinol (**23**) as the obvious starting material. Thus phloroglucinol (**23**) was converted to its trimethylether **28** with dimethylsulphate and potassium carbonate in refluxing acetone in almost quantitative yield. The trimethyl ether **28** on demethylation with sodium thioethoxide in dimethylformamide afforded dimethylether of phloroglucinol (**29**). The phenol **29** was subjected to Vilsmier-Haack formylation when a mixture of two aldehydes **30** and **31** was obtained. The desired aldehyde **31**, present as the major constituent, was separated from **30** by fractional crystallization. The Knoevenagel condensation of the aldehyde **31** with malonic acid in pyridine and piperdine failed to give the required cinnamic acid **32**. The aldehyde **31** also failed to give the required cinnamic acid **32** under Perkin reaction condition[15] (**Scheme-4.1.4**). Thus, the desired cinnamic acid was obtained in two steps via Wittig reaction. The aldehyde **31** was condensed with carbethoxymethylene-triphenylphosphorane (**33**) in benzene to give the ester **34** in 60% yield. The hydrolysis of the ester **34** with potassium hydroxide in methanol followed by acidification gave the requisite cinnamic acid **32** (**Scheme 4.1.4**).

SCHEME-4·1·4



The acid **32** showed spectral properties consistent to its structure. The IR spectrum revealed the carbonyl absorption peak at 1640cm^{-1} and a peak at 3280 cm^{-1} for the carboxylic hydroxy group. The ^{1}H-NMR (FIG.II) showed a singlet for six protons at δ 3.75 for two methoxyl groups. The trans olefinic protons showed two doublets (J = 16 Hz) at δ 6.46 and δ 7.84. The two aromatic protons exihibited a singlet at δ 6.06. The presence of the molecular ion peak at m/e. 224 further lent support to the asigned structure.

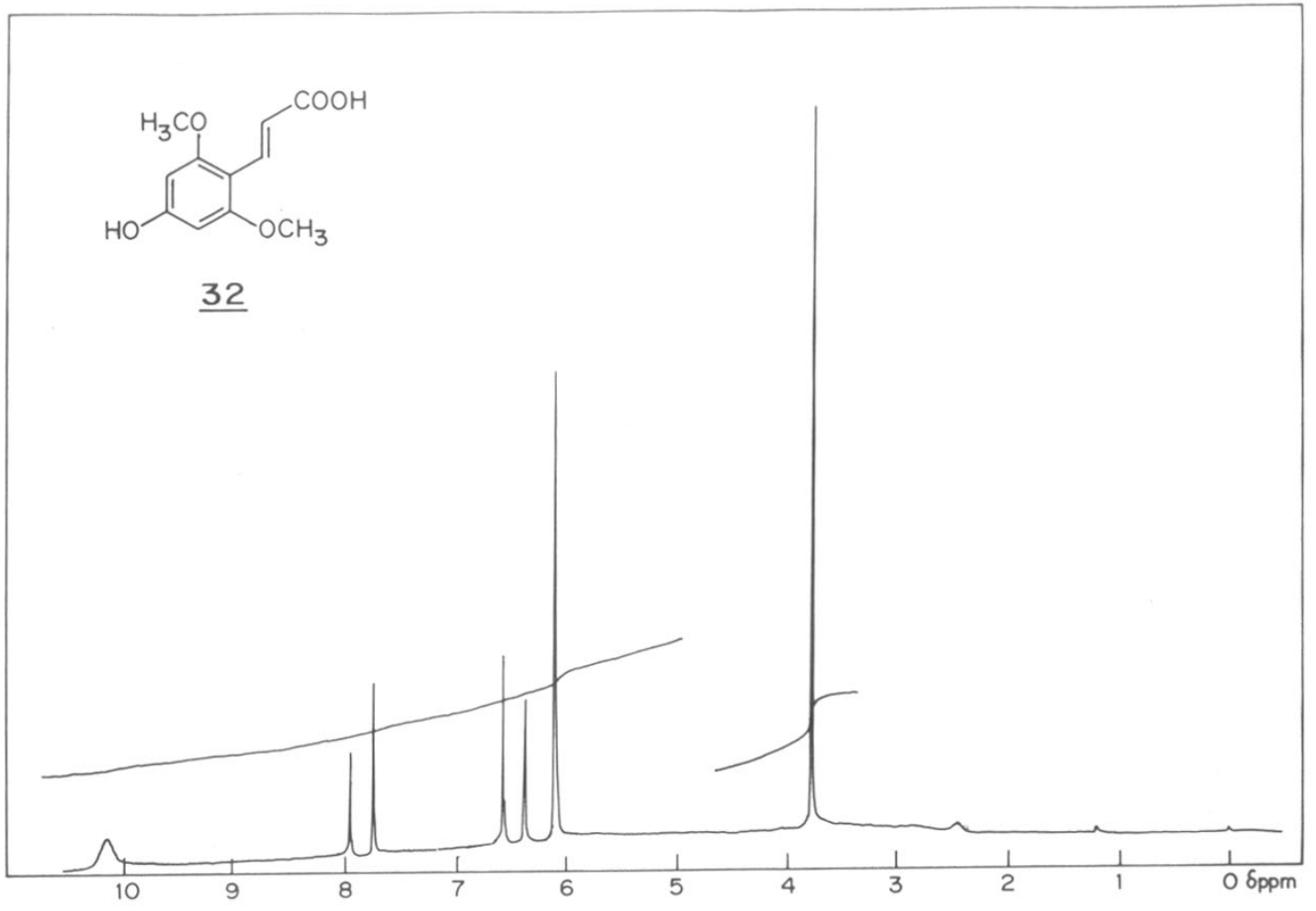
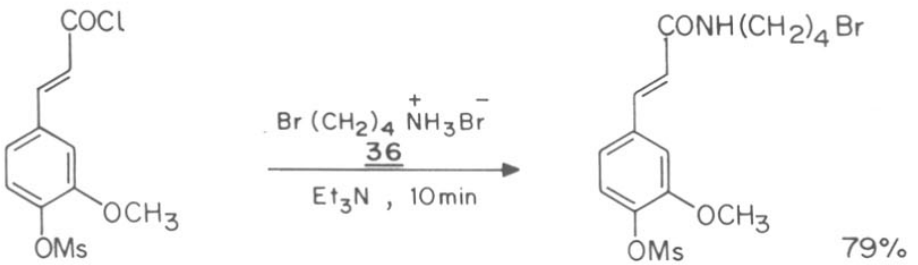


FIG. II: ^{1}H-NMR SPECTRUM OF THE COMPOUND (32) IN DMSO-d_6

After obtaining the desired cinnamic acid **32**, the only step left towards the synthesis of fragment B was to couple it with the appropriate amine. It has been reported in the literature[16] that cinnamoyl chloride **35** on condensation with the hydrobromide salt of 1-Bromo-4-amino-butane (**36**) in presence of a base like triethylamine gave the corresponding amide **37** (**Scheme 4.1.5**)

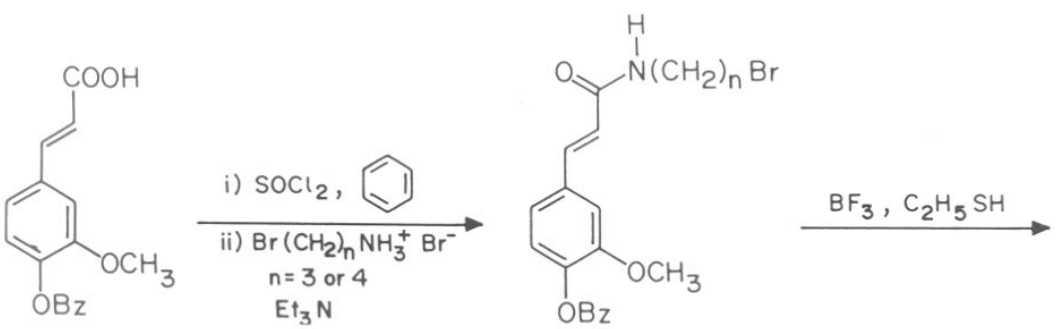
SCHEME-4·1·5

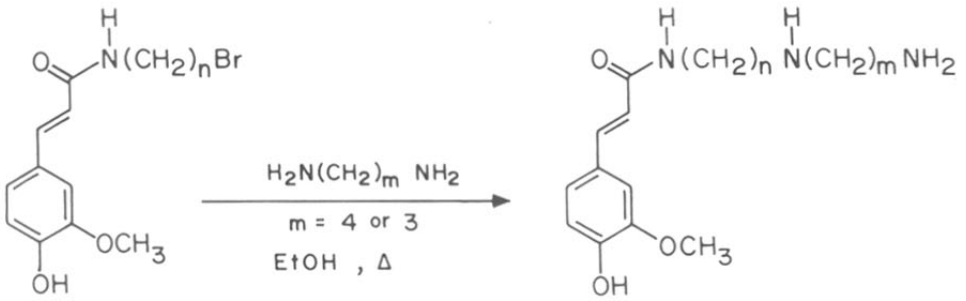


35 37

Ramiandrasoa and Milat[17] also reported similar type of amide formation and further alkylation with a primary amine to give the alkylated spermidine moiety **38**, as shown in **scheme 4.1.6.**

SCHEME-4·1·6

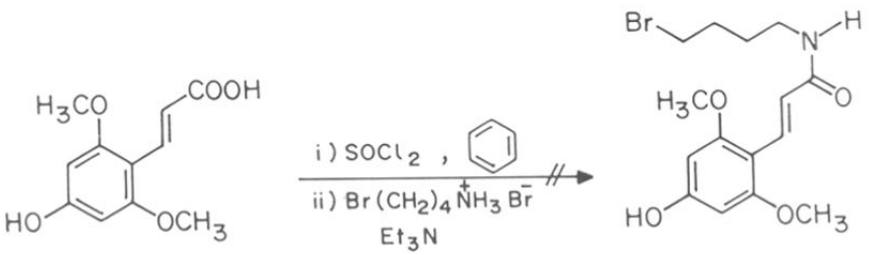




38 n=3, m=4
n=4, m=3

Based on the above observations further conversion of the acid **32** into the fragment B seemed quite promising. The acid **32** was converted to its acyl chloride by refluxing with thionyl chloride in benzene and the crude acyl chloride was condensed with hydrobromide salt **36** in presence of triethylamine. However, the desired product i.e. fragment B could not be obtained (**Scheme 4.1.7**).

SCHEME-4·1·7

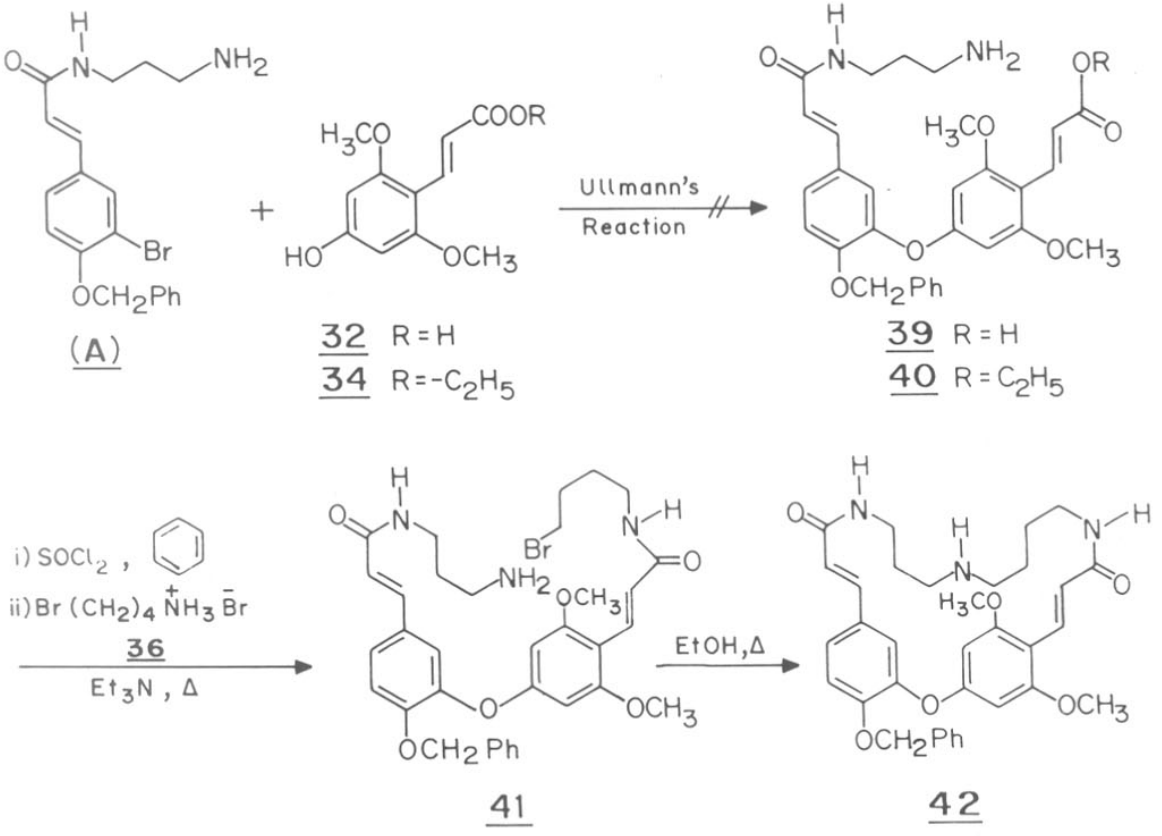


32 **(B)**

The failure of cinnamic acid **32** to provide fragment B could be attributed to the presence of a free -OH group in the acid **32**, since in the known

examples[16,17] the -OH groups are always protected. However, it was thought that protection and deprotection later on, would increase two steps which could be avoided by first bringing in the Ulmann's ether condensation among fragment A and acid **32** and then building up the desired spermidine unit, as depicted in the **scheme 4.1.8.**

SCHEME-4·1·8

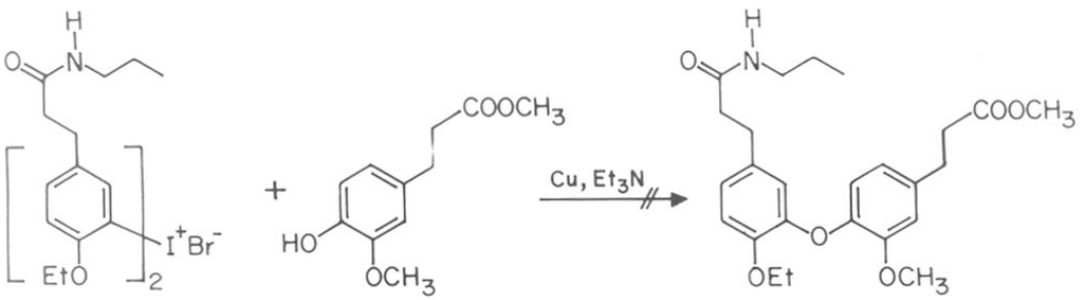


Thus, amide A and cinnamic acid **32** were subjected to Ulmann reaction in presence of potassium carbonate and cupric oxide in refluxing pyridine, but the reaction failed to furnish the desired ether **39**. The Ulmann condensation among

amide A and cinnamoyl ester **34** was also unsuccessful. Besides several changes in various parameters i.e. temperature, solvent and catalyst the desired ether **39** or **40** could not be isolated from any of the above reactions.

The point to be mentioned here is that Doskotch et·al[6] have also encountered the failure of biaryl ether formation among differently substituted phenyl derivatives, one of which had an amide side chain (**Scheme 4.1.9**).

SCHEME-4·1·9

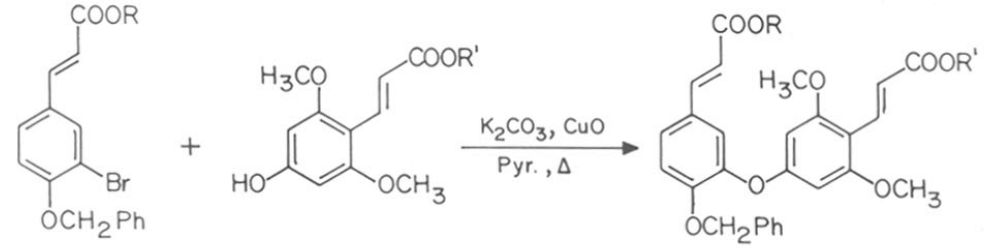


Since the planned regiospecific strategy involving an intramolecular Ulmann condensation was unsuccessful, the only option to achieve the regiospecific synthesis of capparisinine was to first bring in the aryl ether formation among two cinnamoyl moieties and later on couple them with properly protected spermidine moiety to form the macrocyclic ring as done in the synthesis of codonocarpine.[10]

Hence the two cinnamic acids **26** and **32** were refluxed in pyridine with potassium carbonate and cupric oxide for several hours, however the reaction led to no formation of the product instead the starting materials were recovered.

Similarly the two esters **43** and **34** also failed to give the bicinnamoyl derivative **45** (**Scheme 4.1.10**).

SCHEME-4·1·10



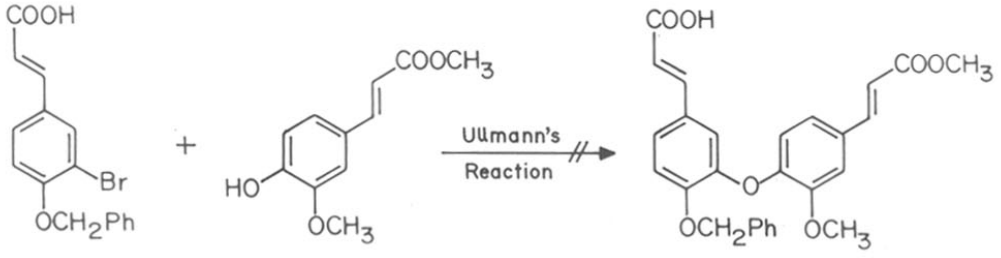
26 R = H
43 R = CH_3

32 R' = H
34 R' = C_2H_5

44 R = R' = H
45 R = CH_3, R' = C_2H_5

It should be mentioned here that during the synthesis of codonocarpine[10] Humora et. al. have reported the failure of the formation of biaryl ether **17** under Ulmann's conditions (**Scheme 4.1.11**). However, the same was prepared using the Beringer's[18] diaryliodonium salt method as shown in the **2** (**Scheme 4.1.11**).

SCHEME-4·1·11

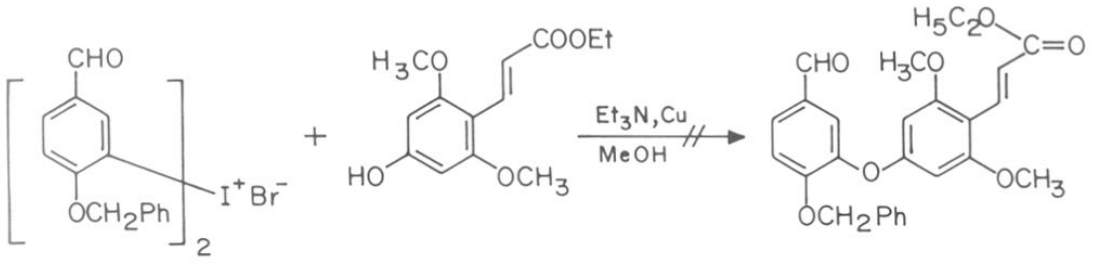


26 **10** **17**

The condensation of the ester **34** with the iodonium salt of the p-benzyloxybenzaldehyde (**15**), prepared by the known method,[6,10] failed to give the

expected biaryl ether **46**. The reaction resulted into some decomposition of the starting material leading to the formation of tar (**Scheme 4.1.12**).

SCHEME - 4·1·12



15 34 46

Finally, an activated Ulmann reaction[19] of the aldehyde **25** with the ester **34** under modified conditions i.e. using sodium hydride as base and cuprous bromide as catalyst, resulted into the formation of a biaryl ether **47** in which the aldehyde group was oxidised to the carboxylic acid group (**Scheme 4.1.13**). The low yield (~10%) of the product **47** could not be enhanced besides numorous attempts.

The formation of biaryl ether **47** was fully supported by its spectral analysis. The IR spectrum showed carbonyl absorption peaks at 1690 and 1620 cm^{-1}. The ^{1}H-NMR (**FIG. III**) showed a triplet at δ 1.31 a quartet at δ 4.25 corresponding to ethyl ester group. The six methoxyl protons showed a singlet at 3.78. The benzylic methylene exhibited a singlet at δ 5.21. The two aromatic protons of cinnamoyl moiety showed a broad singlet at δ 6.03 . The trans-olefines exhibited two doublets (J = 18 Hz) at δ 6.68 and δ 8.09. The rest of the aromatic protons appeared as a doublet (J = 8 Hz) at δ 6.93, a doublet (J = 2 Hz) at δ 8.31 and a broad singlet at δ 7.37 integrating for six protons. The presence

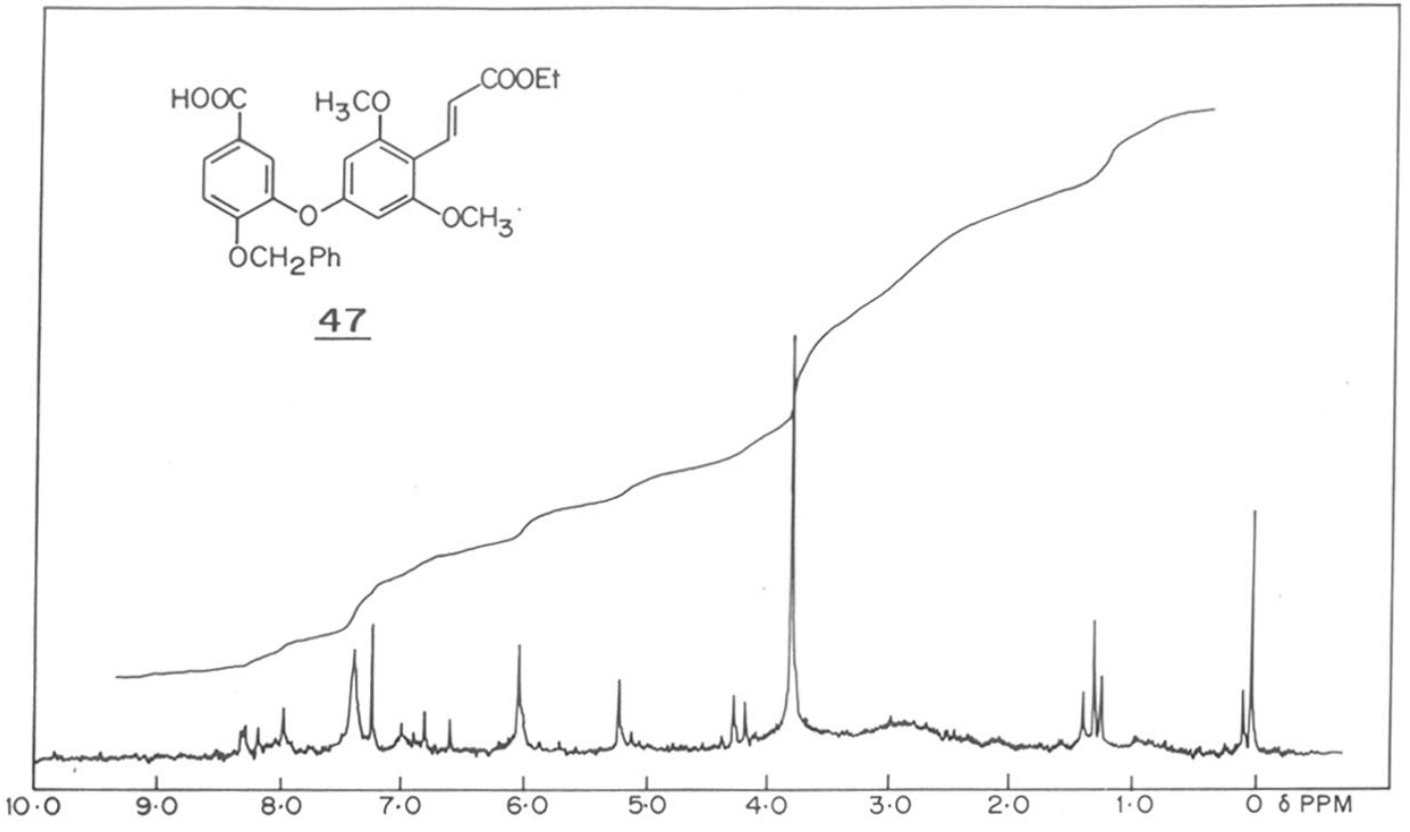
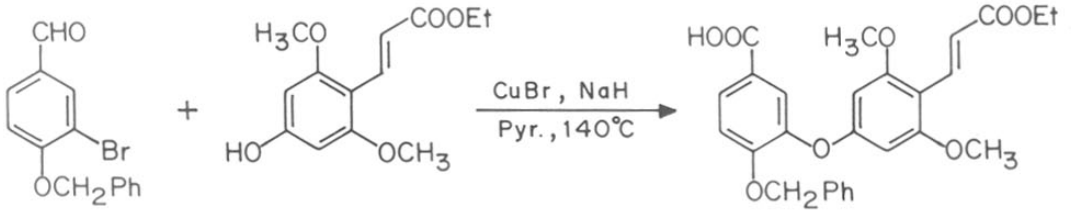


FIG. III : ^{1}H-NMR SPECTRUM OF THE COMPOUND (47) IN $CDCl_3$

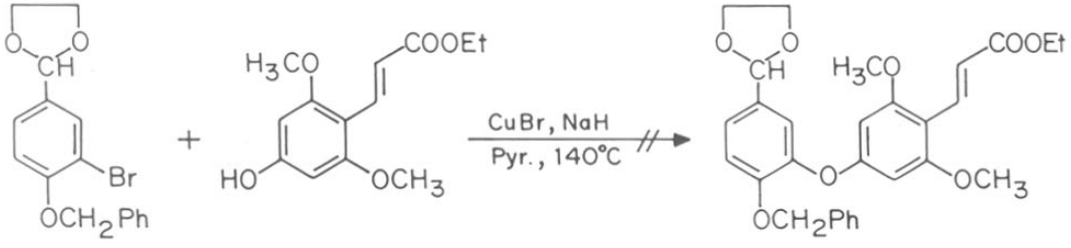
of M^+ at m/e. 478 further confirmed the carboxylic acid formation.

However, when the protected aldehyde **48** was condensed with the ester **34** in presence of sodium hydride and cuprous bromide the desired biaryl ether **49** could not be obtained (**Scheme 4.1.13**).

SCHEME-4·1·13



25 34 47



48 34 49

The formation of biaryl ether **47** in very low yield made it difficult to persuade the synthetic studies further.

In conclusion, the regiospecific synthesis of capparisinine could not be achieved even after attempting various strategies. The failure of crucial Ulmann's reaction among properly substituted cinnamic acid moieties could be explained by the facts known about the Ulmann's reaction.[20] It is reported that the presence of

an electron withdrawing group in ortho position to the halogen in the aryl halide has an activating effect whereas reverse is true for the electron donating group. Similarly, for the phenolic counterpart presence of electron donating groups reduces the activity.

Experimental

3-Bromo-4-hydroxybenzaldehyde (24): To a stirred solution of 4-hydroxybenzaldehyde (**7**) (12.2 g, 100 mmol) in acetic acid (61 ml) was added a solution of bromine (16.0 g, 100 mmol) in acetic acid (25 ml) during 1 h at room temperature. The reaction mixture was stirred for 4 h and poured into ice water (200 ml). The pinkish solid so obtained was filtered, washed with water and dried. The product was recrystallized from benzene - pet ether to give 3-bromo-4-hydroxybenz-aldehyde (**24**)(18.0 g, 90%) as a light pink coloured crystalline solid, m.p. 122-123°C [lit.[21] m.p. 125°-6°C]

3-Bromo-4-benzyloxybenzaldehyde (25): A mixture of 3-bromo- 4-hydroxybenzaldehyde (**24**) (8.04 g, 40 mmol), potassium hydroxide (2.46 g, 88 mmol) as 50% aqueous solution and benzylchloride (5.04 g, 40 mmol) in ethanol (50 ml) was refluxed for 6 h. The solvent was removed under vacuum and to the residue cold water (100 ml) was introduced. The colourless solid thus separated was filtered, washed and dried. The product was recrystallized from pet.ether to give 3-bromo-4-benzyloxybenzaldehyde (**25**) (9.01 g, 77%) as colourless shiny needles, m.p. 80°C [lit.[22] m.p. 78.80°C].

3-[3-Bromo-4-(phenylmethoxy)phenyl]2-propenoic acid or 3-bromo-4-benzyloxycinnamic acid (26): To a solution of 3-bromo-4-benzyloxybenzaldehyde (**25**) (5.0 g, 17 mmol) in pyridine (20 ml) and piperidine (~1 ml) was added malonic acid (2.0 g, 19 mmol) and the reaction mixture was heated on waterbath for 5 h. The cooled reaction mixture was poured into ice cold dil. hydrochloric acid solution (100 ml) and the solid thus separated was filtered, washed and dried. Recrystallization of the solid from ethyl acetate-benzene furnished the corresponding cinnamic acid derivative **26** (4.2 g, 79%) as a light yellow coloured solid, m.p. 206-210°C; [Lit[14], m.p. 210°C]

N-[3-bromo-4-benzyloxycinnamoyl]1,3-diaminopropane (Fragment A): 3-Bromo-4-benzyloxycinnamic acid (**26**) (4.0 g, 12 mmol) was refluxed with thionyl chloride (4.2 g, 36 mmol) in benzene (50 ml) containing few drops of DMF. After 4 h, excess of thionyl chloride was distilled off along with benzene under reduced pressure. The crude acyl chloride so obtained was dissolved in benzene (40 ml) and a solution of 1,3-diaminopropane (1.5 ml) in benzene (20 ml) was added with stirring. The reaction mixture was stirred overnight and poured into water (200 ml). The solid thus separated was filtered and stirred with sodium hydrogen carbonate solution to break the hydrochloride salt. A colourless solid thus obtained was filtered, washed with brine and dried. Recrystallization of the solid from ethyl acetate yielded fragment A (3.6 g, 78%) as a colourless solid, m.p. 187°C.

IR (Nujol): 3400, 3320 and 1665 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.43-2.0 (m, 2H); 3.25-3.68 (m, 4H); 5.18 (s, 2H); 6.31 (d, J = 16 Hz, 1H); 6.87 (d, J = 8 Hz, 1H); 7.31 (dd, J = 8 Hz, 2 Hz, 1H); 7.43 (bs, 5H); 7.62 (d, J = 16 Hz, 1H); 7.84 (d, J = 2 Hz, 1H).

Ms (m/e): 388 (M^+).

Analysis calc. for $C_{19}H_{21}BrN_2O_2$:

C: 58.61%; H: 5.43%; Br: 20.52%; N: 7.19%

Found C: 58.58%; H: 5.39%; Br: 21.01%; N: 7.05%

1,3,5-Trimethoxybenzene or phloroglucinol trimethyl ether (28): A mixture of phloroglucinol (**23**) (12.6 g, 100 mmol), potassium carbonate (50.0g, 362 mmol) and dimethylsulphate (50.4 g, 400 mmol) in acetone (200 ml) was refluxed for 3 h. The acetone was distilled off and the residue was diluted with ice cold water (200 ml). The brown crystalline solid so obtained was filtered, washed and

dried to give phloroglucinol trimethyl ether **28** (15.96 g, 95%). m.p 52°C [Lit.[23], m.p. 54-55°C].

1,5-Dimethoxy-3-hydroxybenzene or phloroglucinol dimethyl ether (29): The sodium thioethoxide reagent (7.5 eq.) was prepared by addition of a solution of ethane thiol (5.0 g, 80.6 mmol) in dry DMF (80 ml) to a stirred suspension of sodium hydride (4.0 g, a 50% oil suspension) in DMF (40 ml) under argon atmosphere. The resultant reagent was stirred for 10 min. and a solution of phloroglucinol trimethyl ether (**28**) (4.0 g, 23.8 mmol) in dry DMF (40 ml) was added. The reaction mixture was refluxed for 4 h and after cooling it was acidified with 10% aqueous HCl. The aqueous solution was extracted with ether (2 x 100 ml) and the combined ethereal extract was washed with water. The organic phase was extracted with 5% aq. NaOH solution (2 x 100 ml) and the combined alkaline extract was acidified with dil. HCl and extracted with ether (2 x 100 ml). The combined ethereal extract was washed with brine and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure furnished phloroglucinol dimethyl ether **29** (3.4 g, 94%) as a yellowish brown oil which solidified in vacuum dessiccator, m.p. 37°C [lit[23], m.p. 36-8°C].

2,6-Dimethoxy-4-hydroxybenzaldehyde (31): Phosphorous oxychloride (6.5 ml) was added to a mixture of phloroglucinol dimethyl ether **29** (10 g, 64.9 mmol) and DMF (6.2 ml) at 0°C during 30 min. The reaction mixture was stirred for 1 h and the reddish-orange viscous mass was treated with water (35 ml) and extracted with ether (2 x 50 ml). The aqueous phase was saturated with sodium acetate and left overnight. The orange solid separated was filtered and stirred with ethyl acetate (25 ml) and again filtered. The insoluble solid so obtained was recrystallized from methanol to afford 2,6-dimethoxy-4-hydroxybenzaldehyde (**31**) (5.3 g, 45%) as colourless crystals, m.p. 224°C [Lit.[24] m.p. 223-224°C]. The ethyl ace-

tate filterate was treated with pet.ether to remove amorphous impurities and the clear solution was concentrated. The crude solid was recrystallized from benzene-pet.ether to give 4,6-dimethoxy-2-hydroxybenzaldehyde (**30**) (3.3 g, 28%) m.p. 68°C, [Lit.[24], 69-70°C].

Ethyl[3-(2,6-dimethoxy-4-hydroxyphenyl)]-2-propeonate or 2,6-dimethoxy-4-hydroxy cinnamic acid ethyl ester (34): A mixture of 2,6-dimethoxy-4-hydroxybenzaldehyde (**31**) (5.0 g, 27.47 mmol), carboethoxymethylenetriphenyl phosphorane (**33**) (9.53 g, 27.5 mmol) and benzene (100 ml) was refluxed on waterbath for 10 h. The benzene was distilled off under reduced pressure and the reddish-brown residue was purified on silica gel column (benzene as eluent) to give the cinnamic acid ester **34** (5.49 g, 79%) as a colourless crystalline solid; m.p. 185°C.

IR (Nujol): 3400 (weak) and 1730 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.30 (t, J = 6.2 Hz, 3H); 3.66 (s, 6H); 4.24 (q, J = 6.2 Hz, 2H); 5.93 (s, -O<u>H</u>); 6.04 (s, 2H); 6.74 (d, J = 16 Hz, 1H); 8.10 (d, J = 16 Hz, 1H).

Ms (m/e): 252 (M^+).

3-(2,6-Dimethoxy-4-hydroxyphenyl)-2-propenoic acid or 2,6-dimethoxy-4-hydroxy cinnamic acid (32): The ester **34** (4.0 g, 15.87 mmol) was refluxed with a 10% aqueous potassium hydroxide solution in methanol (50 ml) for 10 h. The solvent was evaporated and the residue was diluted with water (50 ml) and acidified with dil. HCl to obtain a light brown solid. The solid was filtered, washed and dried. It was recrystallized from methanol to furnish 2,6-dimethoxy-4-hydroxy cinnamic acid (**32**) (3.2 g, 90%) as a light brown solid; m.p. 175°C (**d**)

IR (Nujol): 3280 and 1640 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 3.75 (s, 6H); 6.06 (s, 2H); 6.46 (d, J = 16 Hz, 1H); 7.84 (d, J = 16 Hz, 1H).

Ms (m/e): 224 (M^+)

Analysis calc. for $C_{11}H_{12}O_5$ C: 58.92%; H: 9.15%

Found: C: 58.81%; H: 9.13%.

Ulmann condensation of aldehyde 25 and ester 34 : To a stirred suspension of sodium hydride (0.08 g, as 50% oil suspension) in pyridine (5 ml) was added a solution of ester **34** (0.277 g, 1.1 mmol) in pyridine (5 ml) under N_2 atmosphere. The reaction mixture was stirred for 1 h and aldehyde **25** (0.29 g, 1 mmol) in pyridine (10 ml) was added and the reaction mixture was heated to 100°C. Then cuprous bromide (0.144 g, 1 mmol) was added and the reaction mixture was refluxed for 22 h. The pyridine was distilled under vacuum and to the residue ethyl acetate (20 ml) was added. The insoluble solid was filtered and washed with ethyl acetate. The combined ethyl acetate filterate was washed with dil HCl and brine successively and dried (Na_2SO_4). The residue obtained after concentrtion was purified on silica gel column (pet.ether-acetone 9:1) to give biaryl ether **47** as a colourless solid (0.05 g, 10%) m.p. 172-174°C (**d**).

IR (Nujol): 1690 and 1620 cm^{-1}.

^{1}H-NMR ($CDCl_3$; δ 1.31 (t, 3H); 3.78 (s, 6H); 4.25 (q, 2H); 5.21 (s, 2H); 6.03 (bs, 2H), 6.68 (d, J = 16 Hz, 1H); 6.93 (d, J = 8 Hz, 1H); 7.37 (bs, 6H); 8.09 (d, J = 16 Hz, 1H); 8.31 (d, J = 2 Hz, 1H).

Ms (m/e): 478 (M^+).

Analysis calc. for $C_{27}H_{26}O_8$: C: 67.77%; H: 5.47%

Found: C: 67.81%; H: 5.49%.

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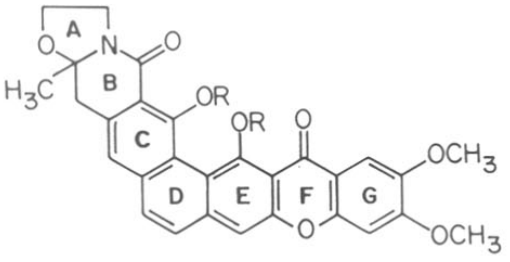
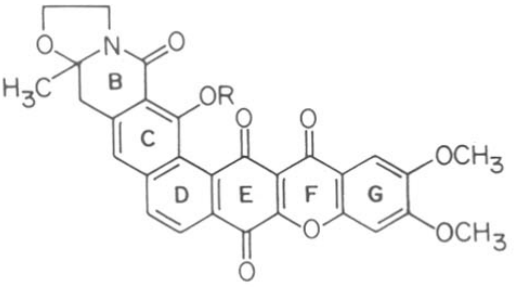
PART 2: SYNTHETIC APPROACHES TOWARDS CERVINOMYCIN

Introduction:

Cervinomycin, an anti-anaerobic and anti-micoplasmal antibiotic was isolated from Streptomyces cervinus sp. nov. by Omura et.al.[1] The antibiotic consists of two components cervinomycin A_1 (**1**) and A_2 (**2**). Owing to the high insolubility of these antibiotics in most of the solvents, various chemical modifications of cervinomycins have been carried out in order to increase the antimicrobial activity and solubility and it was found that triacetyl cervinomycin A_1 (**3**) and monoacetyl cervinomycin A_2 (**4**) have high solubility and show enhanced antianaerobic activity against Clostridium difficle, Reptococcus variabillis and Streptococcus mutans coupled with antimicoplasmal activity.[2] The triacetyl derivative **3** is being developed as a drug against anaerobes because of its potent antimicrobial activity and high solubility and low toxicity.[2]

Cervinomycins and their acetyl derivatives afforded a monocrystal in appropriate solvents. However, the X-ray crystallographic analyses were unsuccessful because of the extreme instability of the crystals when exposed to air. Hence, the structures were determined by ^{1}H-NMR spectro scopy of their methyl ethers **5** and **6**.[3] The structure was shown to be a heptacyclic skeleton comprising of a sensitive tetrahydro oxazolo-[2,3b] benz [g] isoquinolone moiety angularly fused on a novel and highly functionalized xanthone moiety.[3] Thus, cervinomycin belongs to a small group of structurally novel and biologically potent antibiotics which are recognizable through the conspicous presence of the xanthone and isoquinolone moieties within a polycyclic framework. Some other members of the group are lysolipin **I**(**7**)[4], albofungin (**8**), chloroalbofungin[5], LL D 420676 α (**9a**) and LL D42067 ß (**9b**)[6].





1 R = H, CERVINOMYCIN A_1

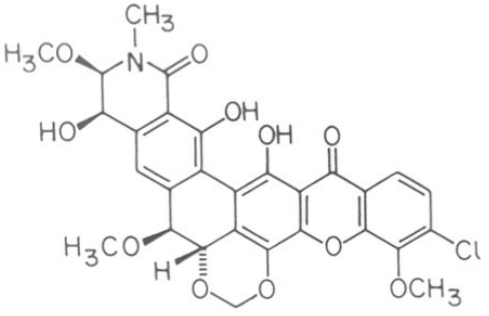
3 R = $-COCH_3$

5 R = $-CH_3$

2 R = H, CERVINOMYCIN A_2

4 R = $-COCH_3$

6 R = $-CH_3$



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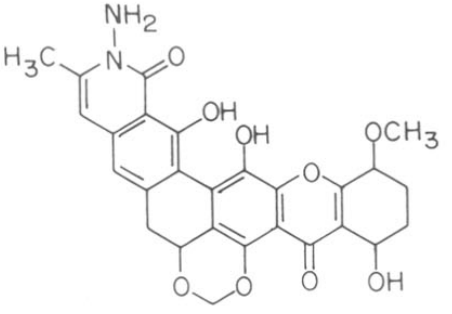
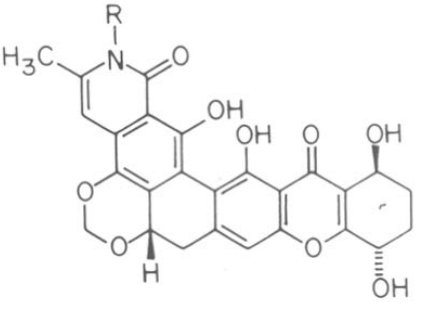


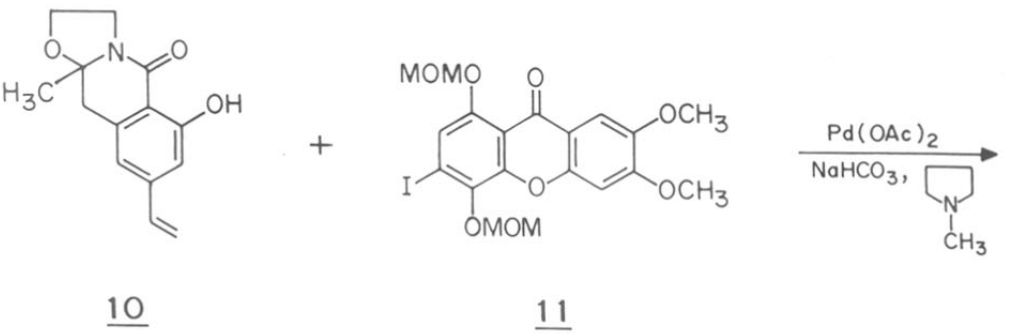
8



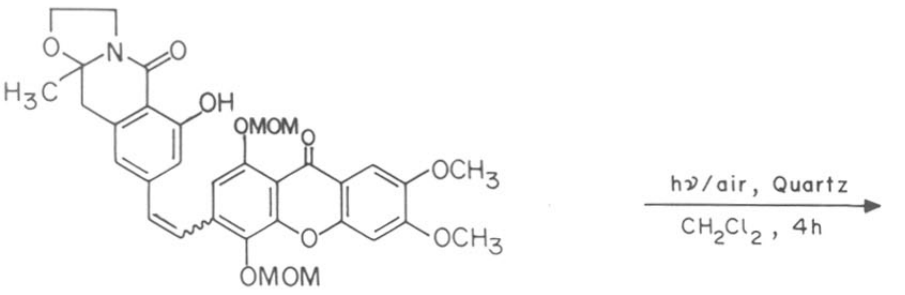
9a R = CH_3, LL-D 42067 α

9b R = H, LL-D 42067 β

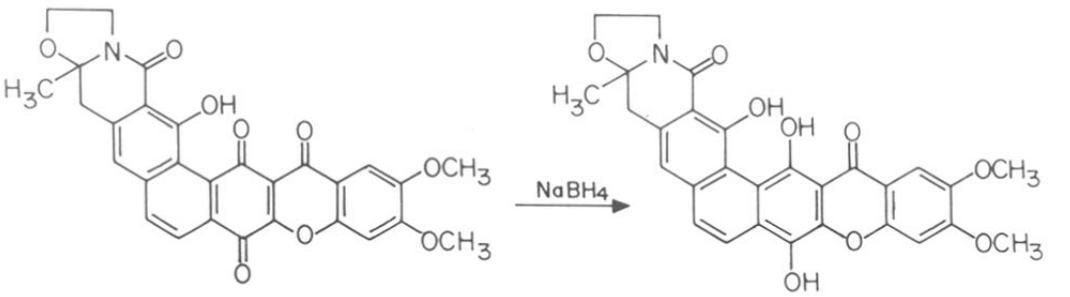
SCHEME 4·2·1



10 11



12



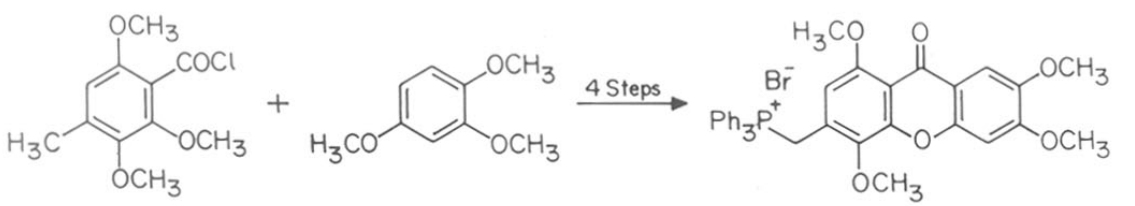
(±)2 (±)1

Ever since their isolation and structure determination cervinomycins have attracted many synthetic chemists particularly because of their skeletal novelty and potent antianaerobic activity. The first synthesis was reported by Kelly et.al.[7] in quick succession to the various model studies done by several groups.[8] Since then to date two more total syntheses have been reported.[9,10] The important aspects of these syntheses are briefly discussed here.

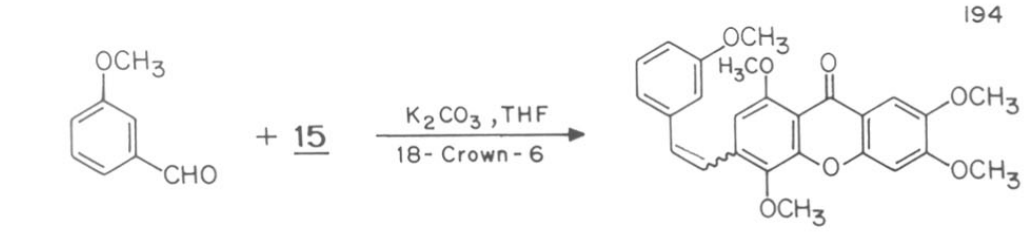
Kelly's approach[7] (**Scheme 4.2.1**) dictates a novel Pd(II) catalyzed coupling among the isoquinolone derivative to (ABC fragment) and iodoxanthone II (EFG fragment) to furnish a stillbene derivative **12**. Irradiation of **12** in dichloromethane while open to the air resulted in cyclization as well as deprotection of MOM ether followed by oxidation to provide (±) cervinomycin A_2 (**2**) in 36% yield. Further reduction of **2** with $NaBH_4$ gave (±) cervinomycin A_1 (**1**).

A model study was first executed by Mehta and Venkateswarlu[8c] for the naphthoannulation of a xanthone derivative **17** thereby constituting the ring D of the CDEFG portion of cervinomycin. Irradiation of **17** in presence of iodine furnished a mixture of two pentacyclic compounds **18** and **19** (4:1) in low yields. (**Scheme 4.2.2**).

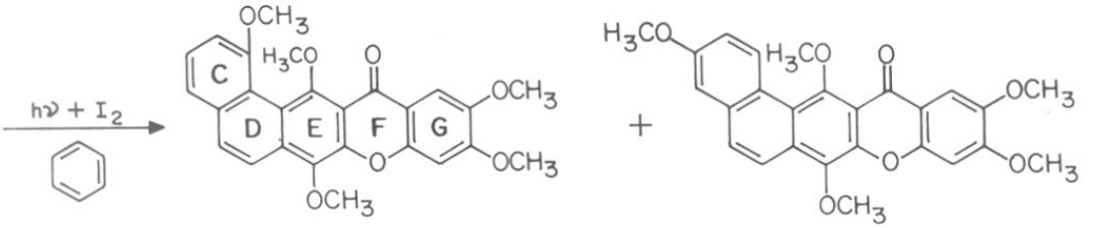
SCHEME 4·2·2



13 14 15



16 17



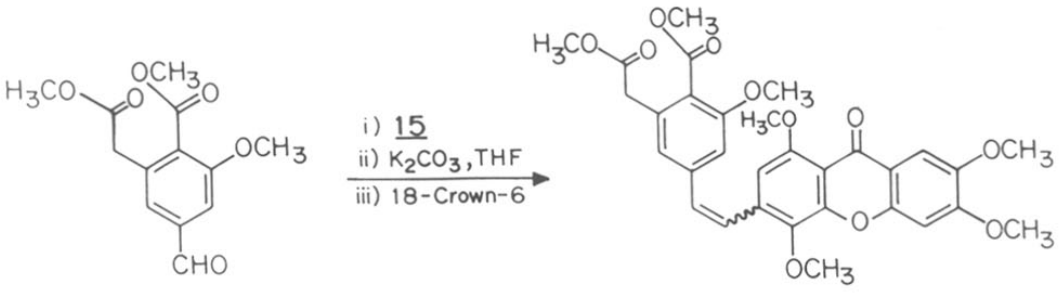
18 19

Mehta and Shah[9] further reported the synthesis of cervinomycin A_1 trimethyl ether (**5**) and cervinomycin A_2 monomethylether (**6**) in which the key central ring D was constructed through a photochemical electrocyclization strategy (**Scheme 4.2.3**). The desired stillbene derivative **22**, prepared from a Wittig condensation of the ylide **15** and aldehyde **20**, was photocyclized to give the pentacyclic compound **23**. Further elaboration of compound **23** to (±) cervinomycin A_2 monomethylether (**6**) via (±) cervinomycin A_1 trimethyl ether (**5**) was achieved in two steps.

Simultaneously Rama Rao et. al.[10] reported a regioselective synthesis of cervinomycins. A suitably functionalized naphthalene derivative **24** served as the key intermediate constituting the DE portion of cervinomycin. The acetyl functionality provided the layout for construction of the isoquinolone part while the bromine substituent allowed regiocontrolled introduction of xanthone moiety. (**Scheme 4.2.4**).

It should be noted that earlier during the course of their synthetic studies on cervinomycin Rama Rao et. al.[8d] had developed a methodology for the construction of a tetrahydro-oxazolo [3,2-b] benz[g] isoquinolone derivative **33** from the known isocoumarin **31** (**Scheme 4.2.5**) however, later on in the total synthesis

SCHEME 4·2·3



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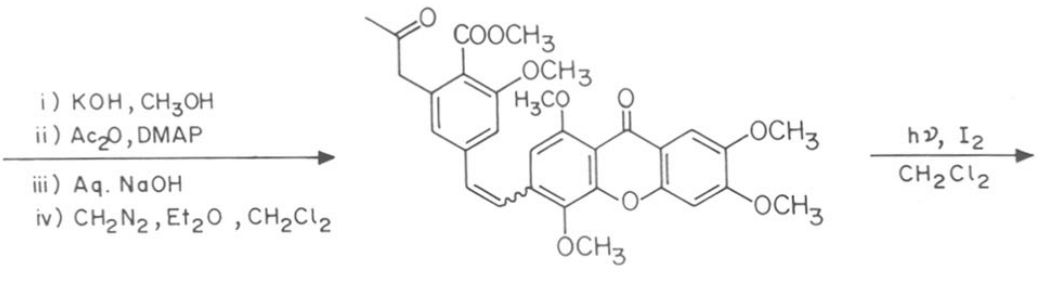

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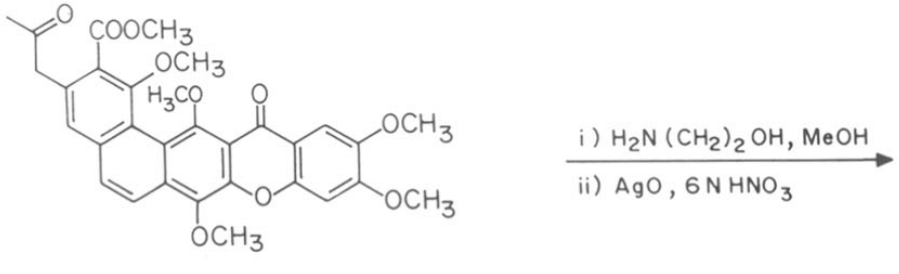

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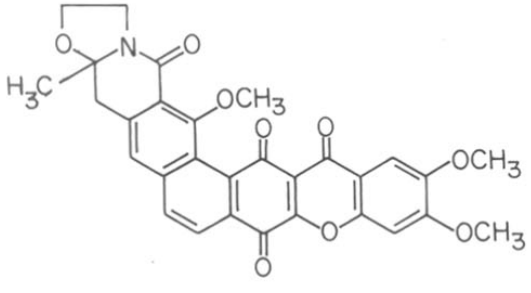
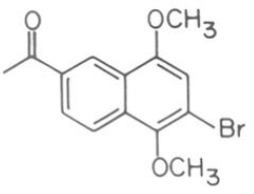


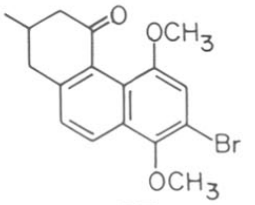
6

SCHEME 4·2·4



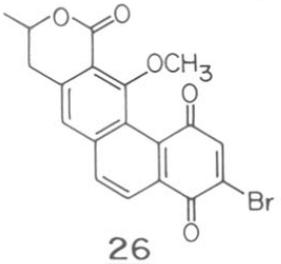
24

4 Steps



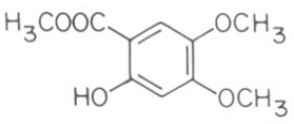
25

7 Steps



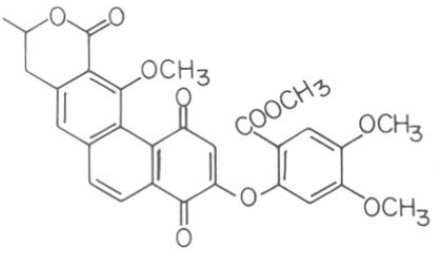
26

+



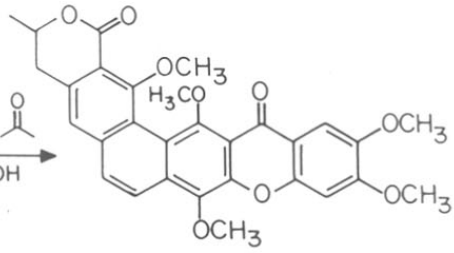
27

K_2CO_3, DMF



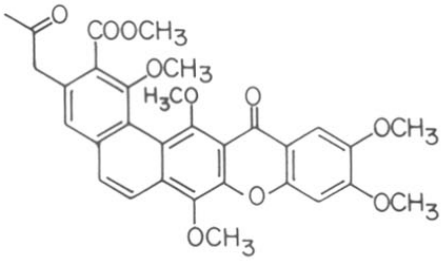
28

i) aq. $Na_2S_2O_4$
ii) DMS, K_2CO_3,
iii) aq. KOH, MeOH
iv) PPE, $CHCl_3$



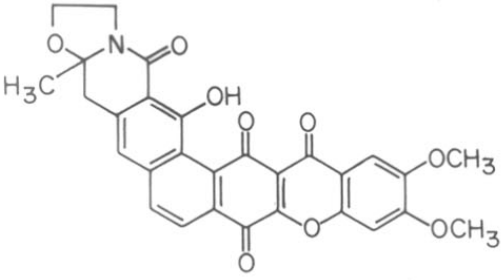
29

i) aq. KOH, EtOH
ii) CH_2N_2, Et_2O
iii) PCC, CH_2Cl_2



30

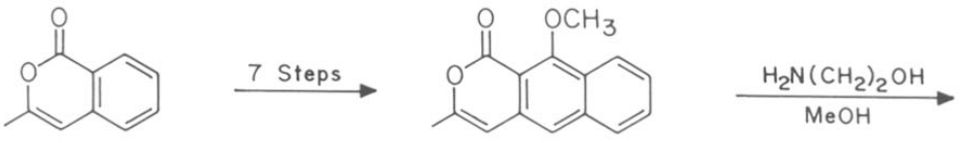
i) $H_2N(CH_2)_2OH$, K_2CO_3, MeOH
ii) CAN, CH_3CN
iii) $Et_3N:BCl_3$, CH_2Cl_2



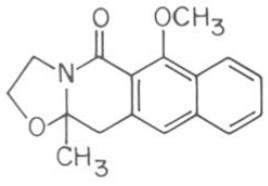
1

of cervinomycin[10] a modified methodology was employed for isoquinolone construction.

SCHEME 4·2·5



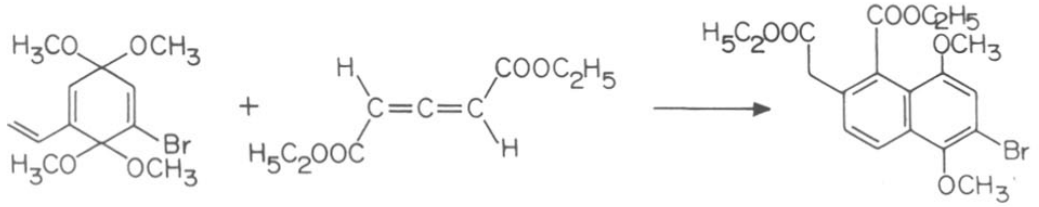
31 32



33

Parker and Ruder[8b] developed a classical approach for the suitably functionalized naphthalene derivative **36** which could be elaborated regiospecifically to cervinomycins. The desired naphthalene derivative **36** was synthesized by the Diels-Alder condensation of a vinylquinone bis-ketal **34** and allene dicarboxylate (**35**) as depicted in the **Scheme 4.2.6**.

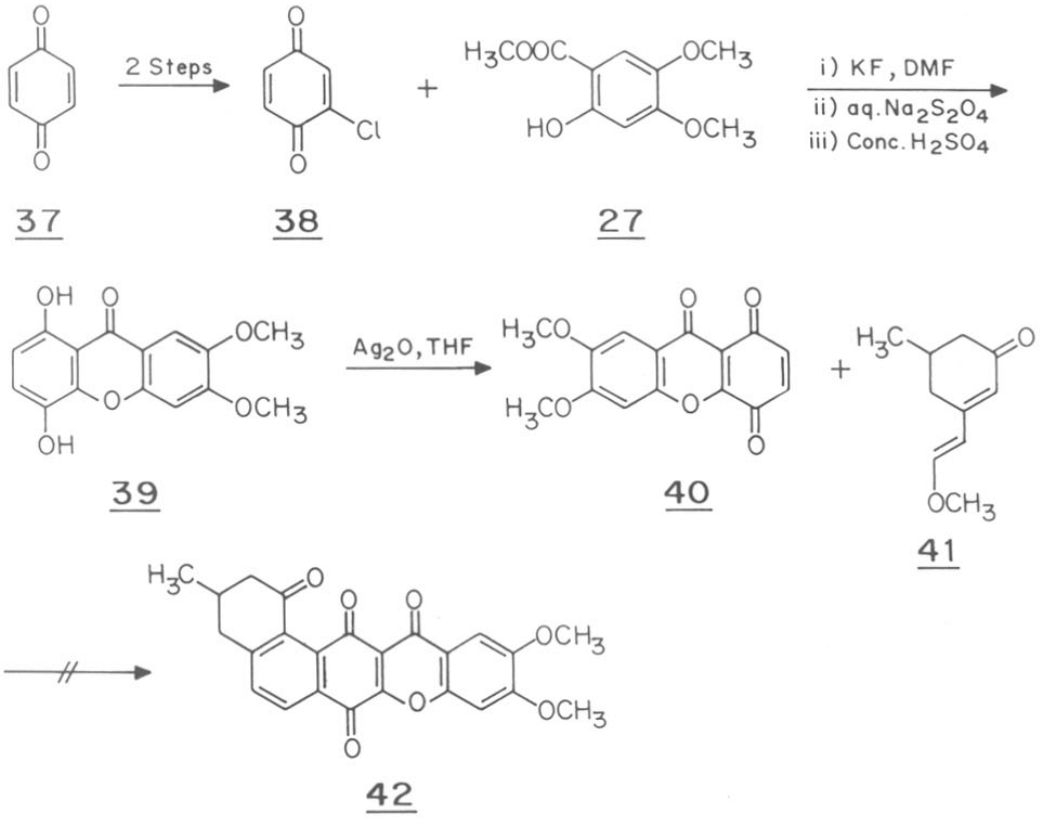
SCHEME - 4·2·6



34 35 36

Synthesis of cervinomycin was attempted in our laboratory[11] based on a Diels-Alder approach (**Scheme 4.2.7**). The xanthone derivative **40** served as the dienophile which was synthesized by condensation of 2-chloro-1,4-benzoquinone (**38**) and methyl-2-hydroxy-4,5-dimethoxybenzoate (**27**) followed by oxidation of the intermediate **39**. The diene **41** was prepared by known procedure[12]. However, the D-A reaction between the xanthone **40** and diene **41** did not give the desired pentacyclic product **42** after several attempts.

SCHEME-4·2·7



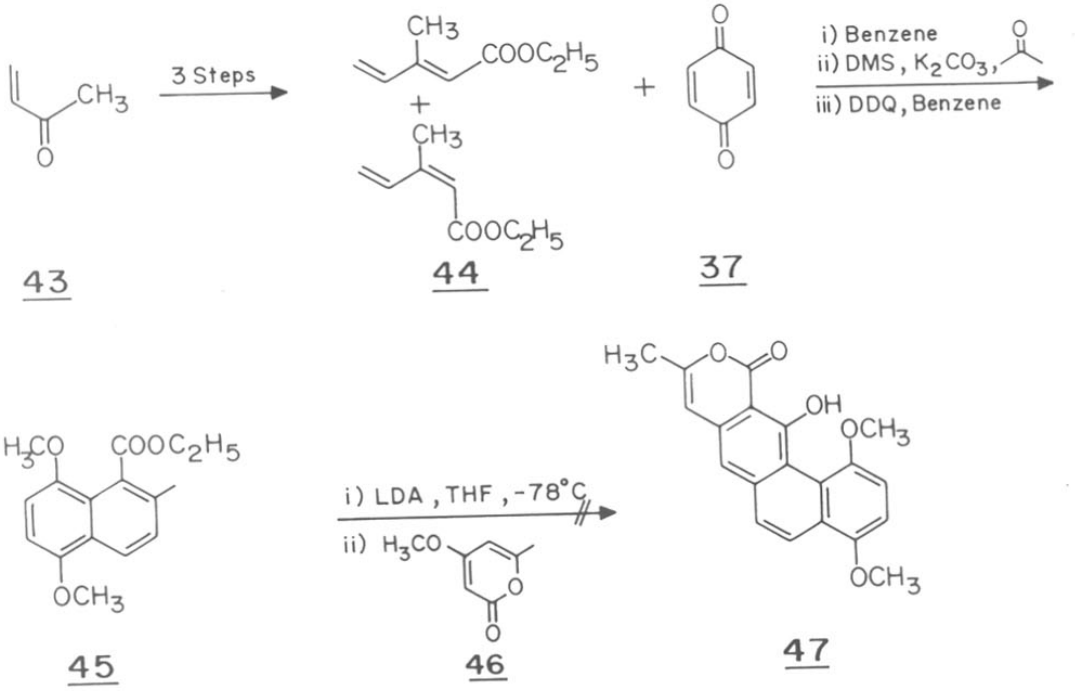
37 38 27

39 40 41

42

In another approach, the aim was to synthesize an intermediate phenanthropyrone **47** which could be elaborated to cervinomycin. Diels-Alder adduct obtained by reaction of 1,4-benzoquinone **37** and ethyl-3-methyl penta-2,4-dienoate (**44**), was methylated and aromatized to give naphthoate **45** Reaction of lithiated naphthoate **45** (LDA, THF, -78°) with triacetate lactone methyl ether (**46**) failed to give the required phenanthropyrone derivative **47**. (**Scheme 4.2.8**)

SCHEME-4·2·8



Present work:

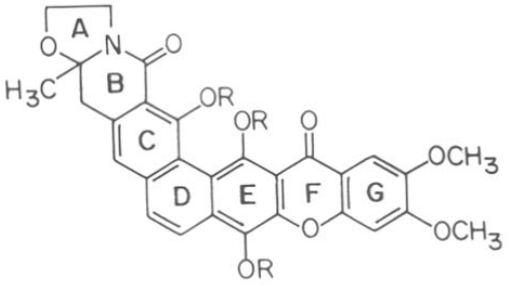
The unique structure of cervinomycin coupled with its promising biological activity made it an attractive target for many synthetic organic chemists as represented by its first synthesis in 1989 followed by two simultaneous syntheses in 1991. However, when its synthesis was initiated in early 1988 no synthesis or synthetic studies directed towards these antibiotics were reported. The present work deals with the various synthetic routes attempted for the synthesis of cervinomycin.

A reterosynthetic protocol indicated the phenanthrene derivative **48** (CDE fragment) as the logical intermediate. The presence of an ester and methyl functionality in **48** would be elaborated to the desired isoquinolone moiety (AB fragment) via isocoumarin **50** whereas the bromine or methoxyl group in **48** could be utilized to bring in the xanthone functionality (FG fragment) regiospecifically.

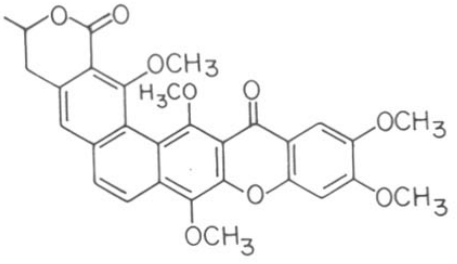
Hence, the efforts were directed towards the synthesis of the requisite intermediate **48**. A synthetic route (**Scheme 4.2.9**) starting from ß-naphthol seemed quite viable as the $-NO_2$ group present in suitabie position could be reduced and oxidized to generate the ring E and an acyl functionality at position 6 would facilitate to build the ring C.

The methylation of ß-naphthol with dimethyl sulphate and anhydrous potassium carbonate in refluxing acetone furnished methyl ether **51** in quantitative yield. Friedel-Crafts acylation of **51** with propionyl chloride in nitrobenzene followed by nitration (conc. H NO_3 in gl. AcOH) gave compound **53** in 70% yield. The α-bromination of **53** gave the monobromo derivative **54** which was alkylated with diethyl malonate in presence of sodium hydride to provide a ketodiester **55**. The compound **55** exhibited spectral properties consistent to its assigned structure. In the IR spectrum ester carbonyls appeared at 1730 and 1740

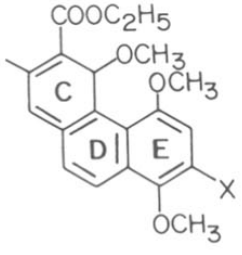
RETEROSYNTHETIC ANALYSIS



1 R = H

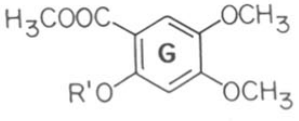


50



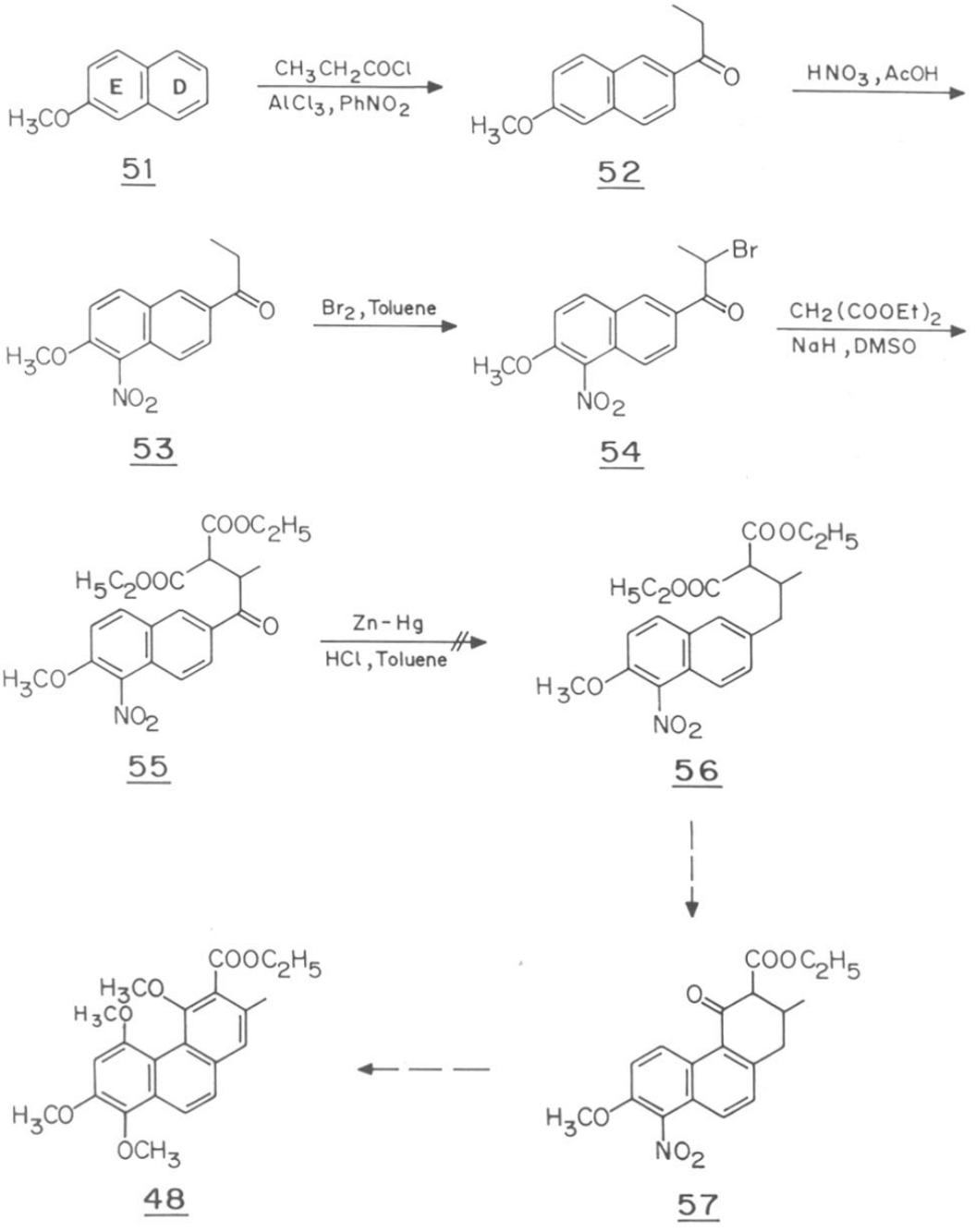
48 X = Br or $-OCH_3$

+



27 , R' = H
49 , R' = CH_3

SCHEME - 4·2·9



51 52 53 54 55 56 57 48

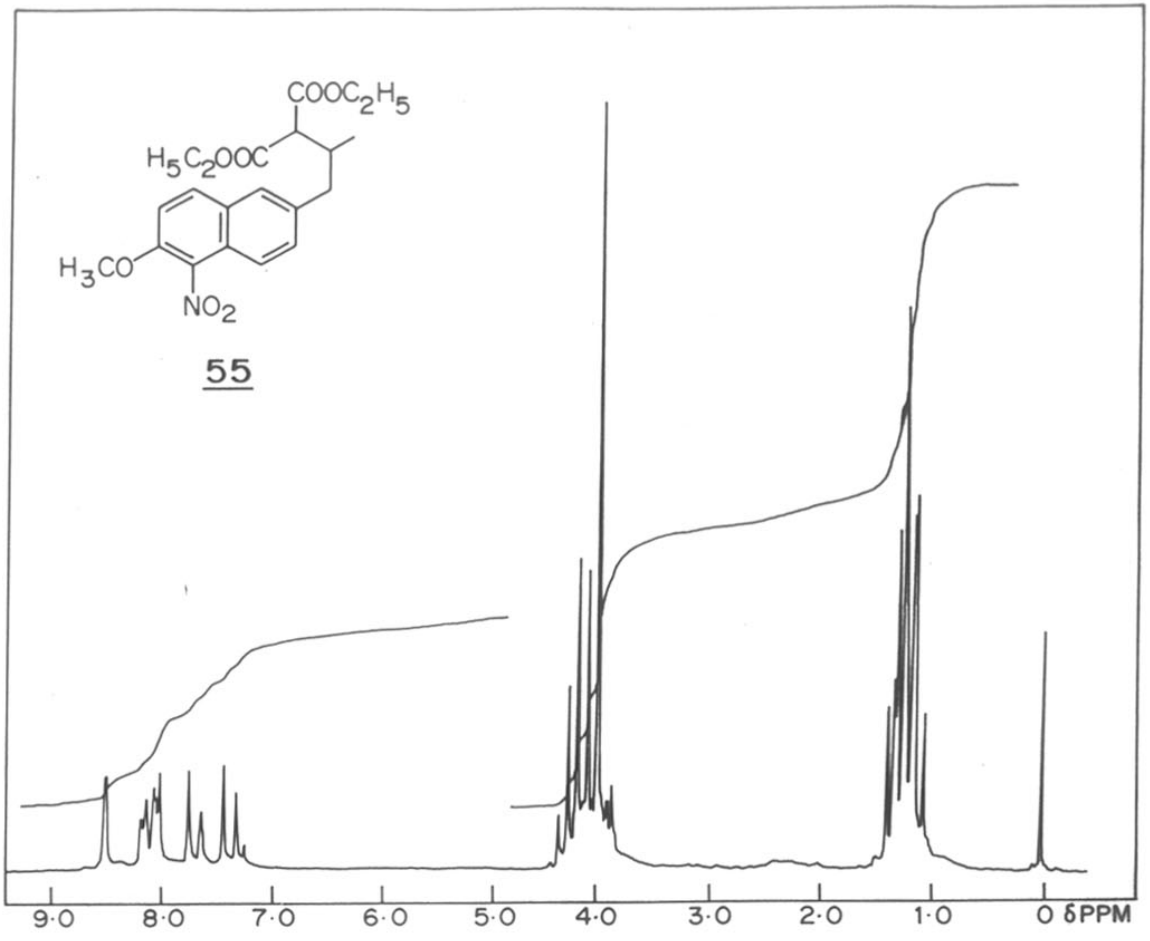


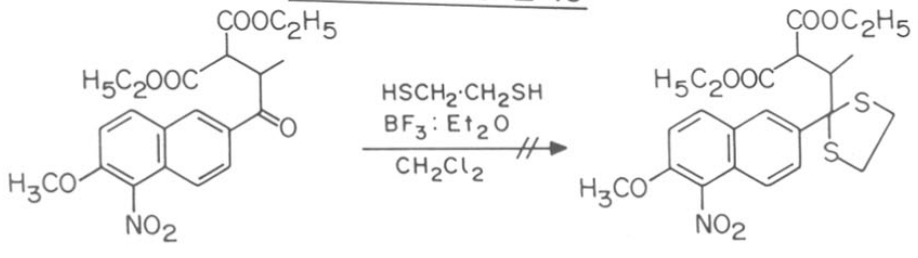
FIG. I : ^{1}H-NMR SPECTRUM OF THE COMPOUND (55) IN $CDCl_3$

cm^{-1} and ketone carbonyl showed peak at 1680 cm^{-1}. The ^1H-NMR (**FIG. I**) exhibited a multiplet between δ 1.07-1.43 integrating for nine protons of three-CH_3 groups. Another multiplet between δ 3.81-4.40 was present corresponding to six protons. The methoxyl protons showed a singlet at δ 4.06. In the aromatic region two ortho coupled doublets (J = 8 Hz) each integrating for single proton were present at δ 7.37 and δ 7.68. Two multiplets each integrating for single proton were seen at δ 8.03 and δ 8.17. A broad singlet at δ 8.51 integrating for single proton was also seen. In mass spectrum M^+ at m/e 417 was present.

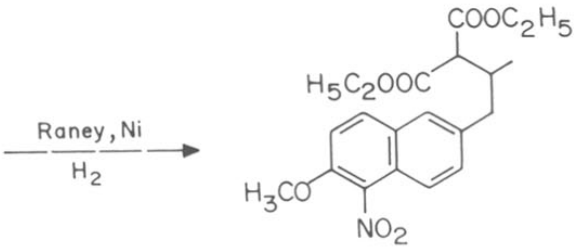
The diester **55** was then subjected to Clemmensen reduction[13] in toluene, however, the desired product **56** could not be obtained. Reduction of **55** was also unsuccessful under Wolff-Kishner reduction[14] conditions as well. Modified Clemmensen reduction[15] also failed to give the reduced product **56**. The hydrogenation of compound **55** in acetic acid under pressure (40 psi) at 65°C in presence of Pd-C as catalyst resulted into the reduction of $-NO_2$ group to $-NH_2$ group whereas the carbonyl group was found to be intact, which was indicated by the IR spectrum in which carbonyl absorption band at 1680 cm^{-1} was present and two -NH stretching bands at 3400 and 3480 cm^{-1} were seen.

Since the carbonyl reduction in the keto-ester **55** was unsuccessful, a changed methodology by incorporating a protection of carbonyl as its cyclic thio-ketal **58** followed by desulfurization with Raney-Ni seemed equally promisable (**Scheme 4.2.10**). However, the protection of **55** with 1,2-ethanedithiol in BF_3-etherate could not provide the desired thio-ketal **58**.

SCHEME - 4·2·10



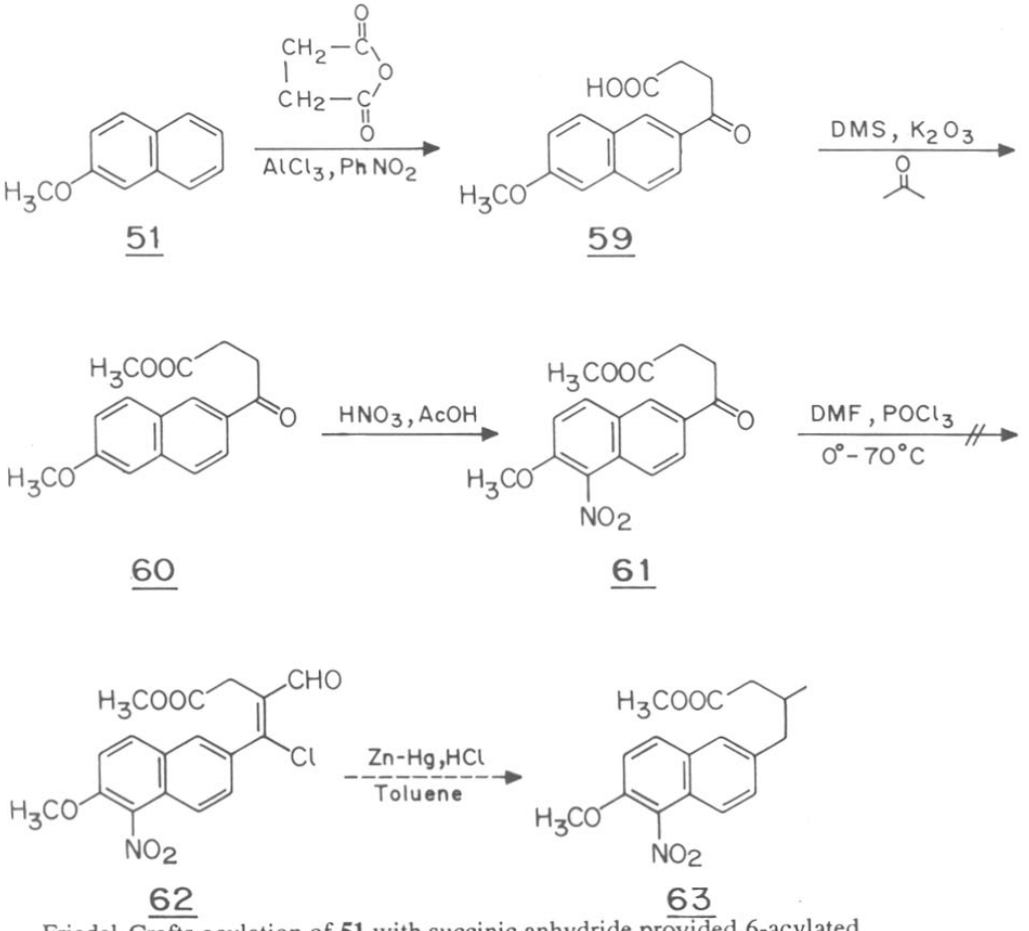
55 **58**



56

The failure in carbonyl reduction or protection of **55** may be attributed to the steric crowding due to methyl and diester groups and so a modified route (**Scheme 4.2.11**) which could overcome this problem was persuaded.

SCHEME-4·2·11



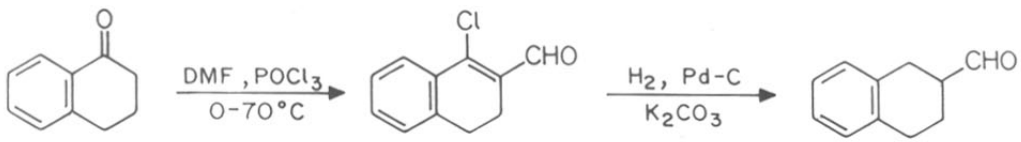
51 59 60 61 62 63

Friedel-Crafts acylation of **51** with succinic anhydride provided 6-acylated product **59**. The acid **59** was esterified with dimethyl sulphate and potassium carbonate in boiling acetone to give an ester **60** which was nitrated with fuming

HNO_3 and acetic acid to give the nitro derivative **61**. The compound **61** was fully characterized by various spectral means. The IR spectrum showed two carbonyl absorption bands at 1730 and 1680 cm^{-1}, The ^1H NMR (**FIG.II**) depicted two triplets at δ 2.78 and δ 3.38 for two $-CH_2$ groups and two singlets at δ 3.70 and δ4.05 corresponding to methyl ester and methoxyl protons respectively. The aromatic protons exhibited two doublets (J = 8 Hz) at δ 7.31 and δ 7.73, two multiplets at δ8.05 and δ8.13 and a broad singlet at δ8.43 each integrating separately for single proton. The molecular ion peak at m/e 317 was seen in the mass spectrum.

It has been reported[16] in the literature that Vilsmeier formylation of tetralone **64** gave chloroaldehyde **65** which was reduced to furnish the saturated aldehyde **66** by hydrogenation.[17]. (**Scheme 4.2.12**)

SCHEME - 4·2·12



64 **65** **66**

Analogous Vilsmeier formylation on the ketone **61** would give the chloroaldehyde **62** which inturn could be reduced under Clemmensen's conditions to give the desired product **63**. However, the ketone **61** failed to give the desired chloroaldehyde **62** besides several attempts and hence the route was abandoned.

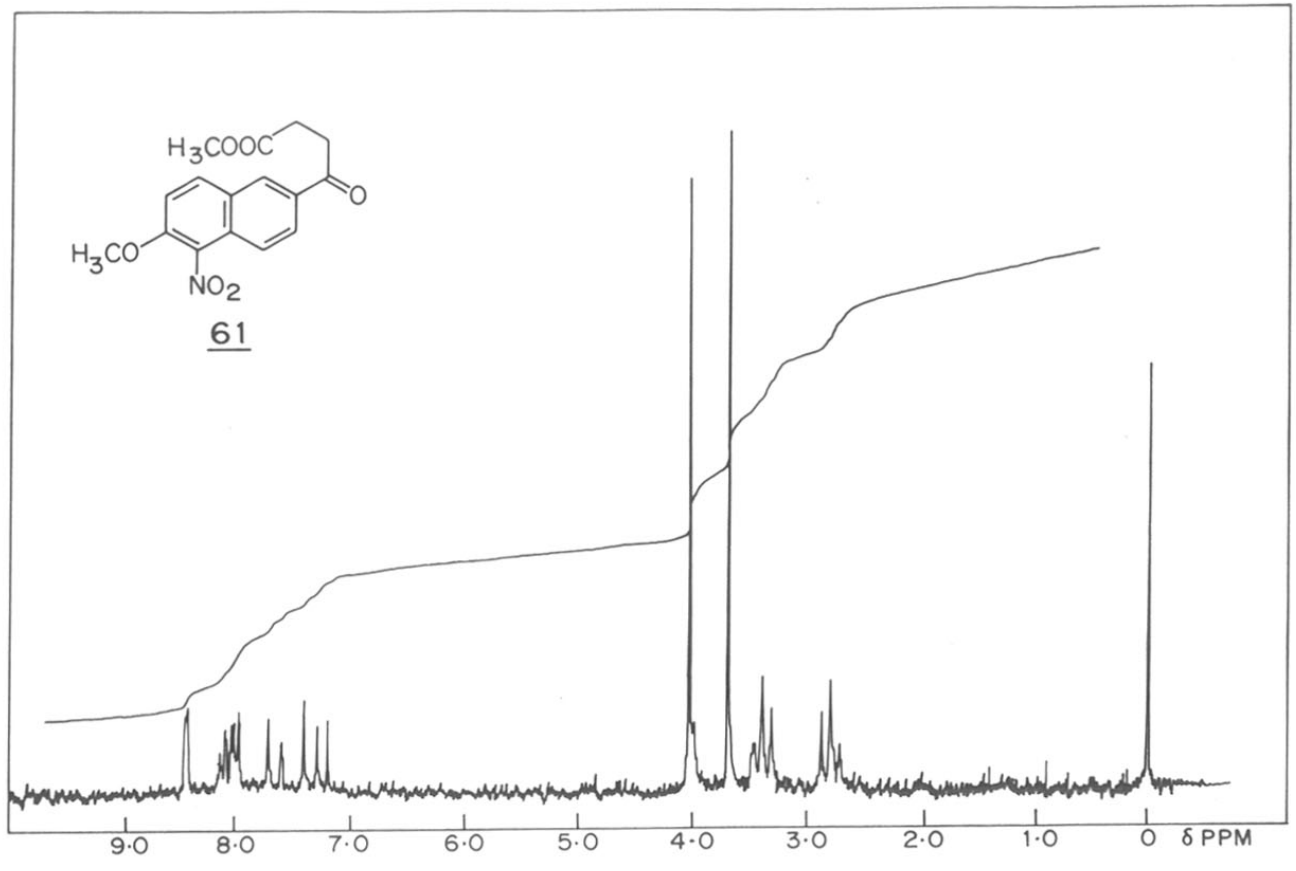
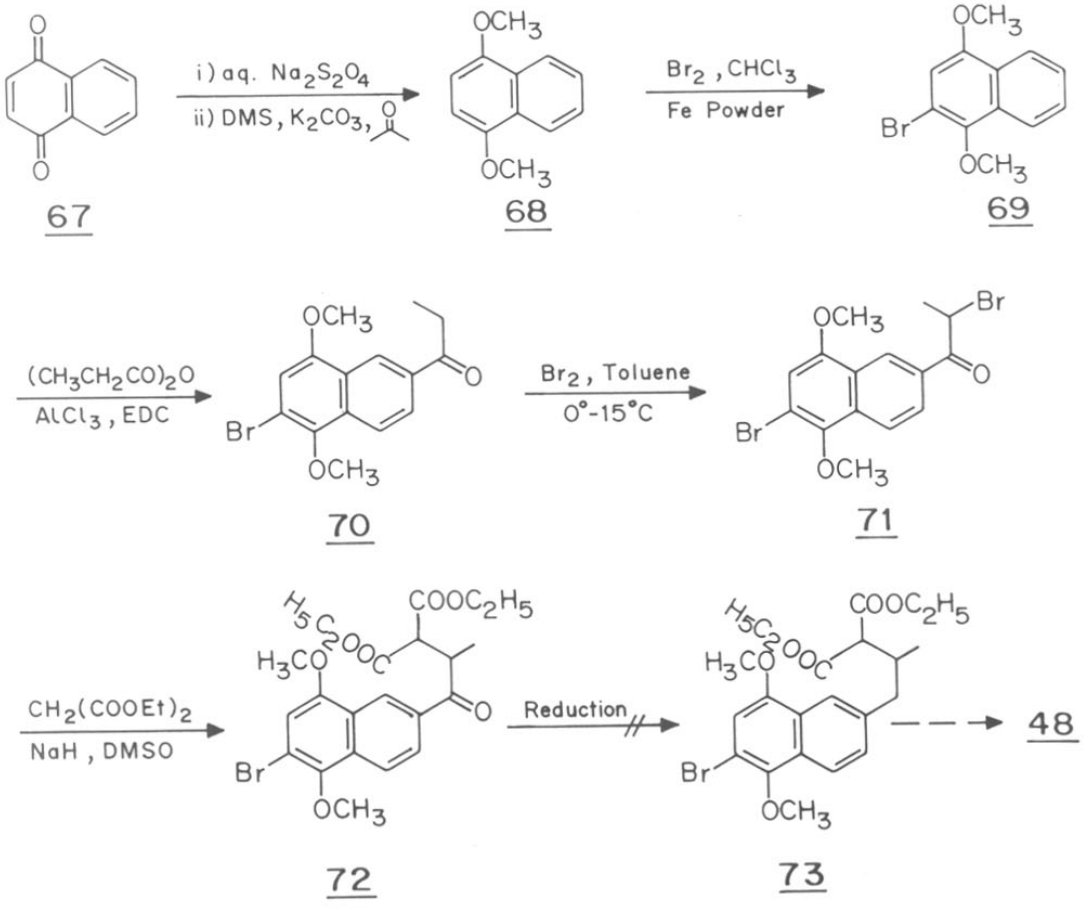


FIG. II : ^{1}H-NMR SPECTRUM OF THE COMPOUND (**61**) IN $CDCl_3$

In yet another approach towards the intermediate **48**, 1,4-dimethoxynaphthalene (**68**) was considered as the starting material of choice which could be elaborated through a synthetic sequence to **48** (**Scheme 4.2.13**).

SCHEME - 4·2·13

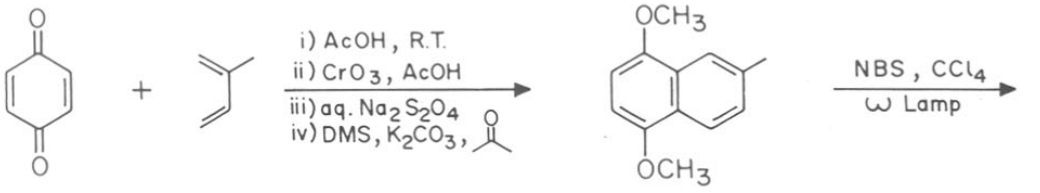


Thus, bromination of **68** with bromine in chloroform, while an inert gas was bubbled continuously, furnished 2-bromo derivative **69**. Friedel-Crafts acyla-

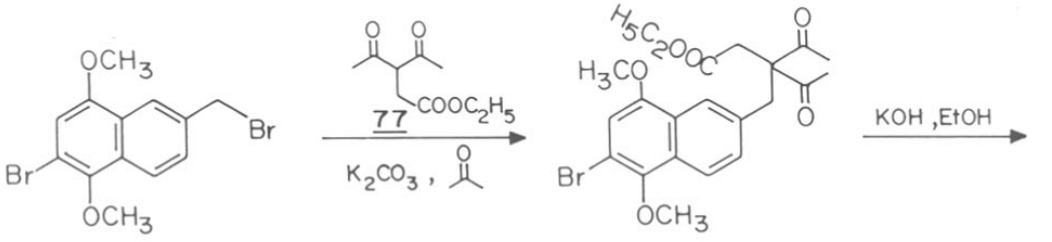
tion of **69** with propionic anhydride in ethylene dichloride gave the 6-acylated product **70**. The α-bromination of **70** gave the bromo derivative **71** which was alkylated with diethyl malonate in presence of sodium hydride to give a diester **72**. The diester **72** exihibited IR absorption bands at 1670 and 1730 cm^{-1}. The ^{1}H-NMR (**FIG.-III**) showed a doublet for -CH_3 protons at δ 1.17 and a triplet at δ1.25 for six methyl protons of the ethyl ester group. A multiplet between δ 3.81-δ4.43 corresponded to six methylene protons. The two singlets at δ 3.90 and δ4.05 were present for the six methoxyl protons. In the aromatic region a singlet at δ 6.87 for single proton, a broad singlet at δ 8.12 for two protons and a singlet at δ 8.78 for single proton were present. Further, the presence of the molecular ion peak at m/e 480 confirmed the structure. The diester **72** was then subjected to the carbonyl reduction under various known methods[13, 14, 15] but none of the reaction could give the desired product **73**.

Since, all the earlier strategies, employed for the synthesis of the intermediate **48**, posed problem with the carbonyl reduction, a new route (**Scheme 4.2.14**) which would not involve such reduction was initiated. 1,4-Dimethoxy-6-methyl naphthalene (**75**), prepared by Diels-Alder condensation of benzoquinone (**37**) and isoprene (**74**)[18], was brominated under radical conditions with NBS in carbon tetrachloride to give the dibromo derivative **76**. Alkylation of compound **76** with ethyl-(3-acetyl)-levulinate (**77**) in presence of soidum hydride in THF gave the alkylated product **78**. Hydrolysis of compound **78** with ethanolic potassium hydroxide furnished a keto acid **79**. Thio-ketalization of the keto acid **79** with 1,2-ethanedithiol in presence of BF_3-etherate afforded thio-ketal **80**. The thio-ketal **80** exhibited spectral properties consistent to its structure. The IR spectra showed carboxylic acid carbonyl absorption peak at 1660 cm^{-1}. The ^{1}H-NMR (**FIG.-IV**) exhibited a methyl singlet at δ 1.73 and a multiplet between δ 2.10 -

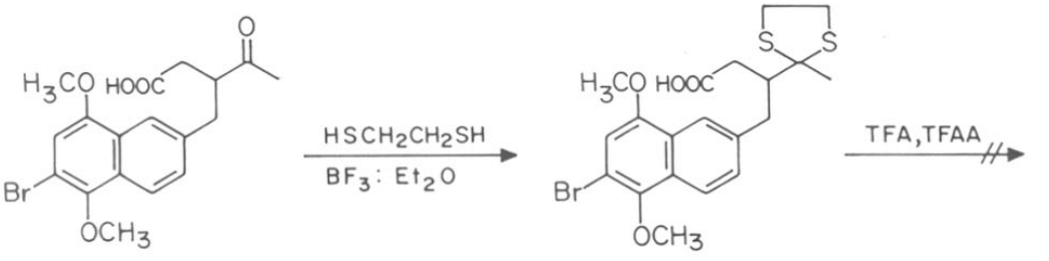
SCHEME - 4·2·14



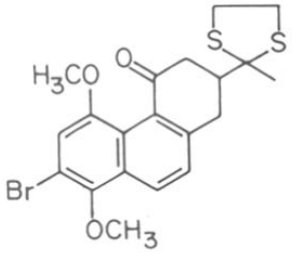
37 74 75



76 78



79 80



81

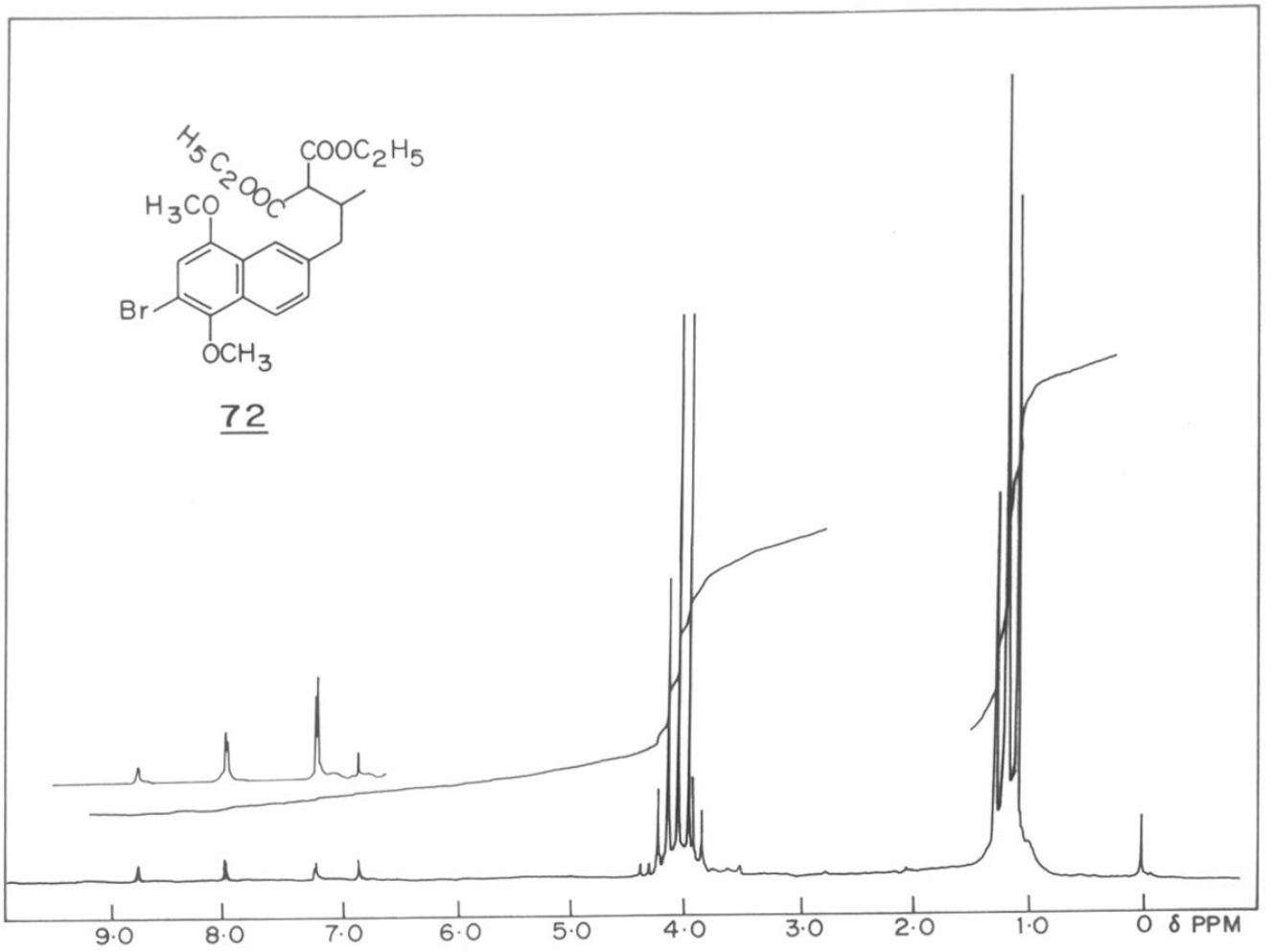


FIG. III: ^{1}H-NMR SPECTRUM OF THE COMPOUND (72) IN $CDCl_3$

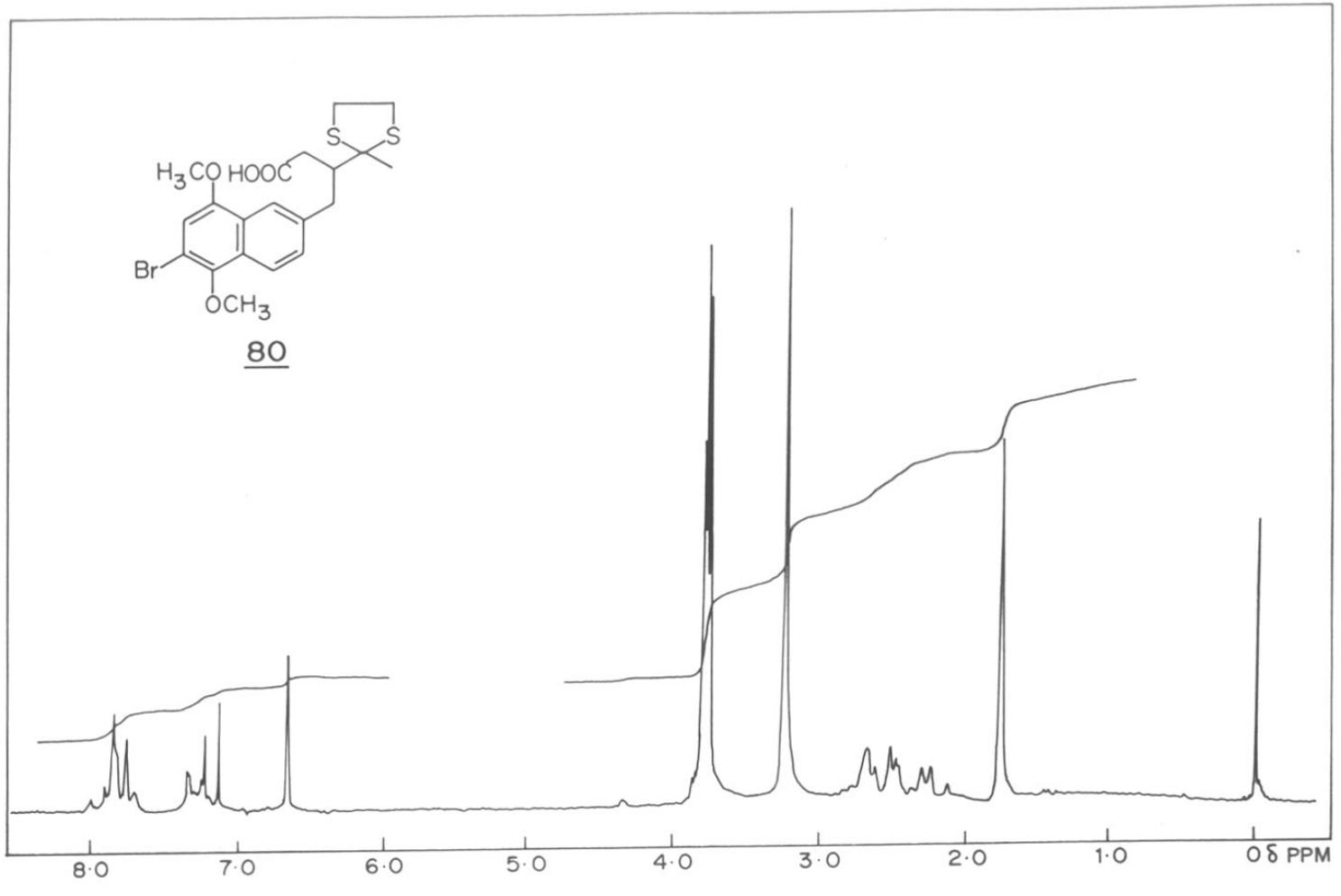


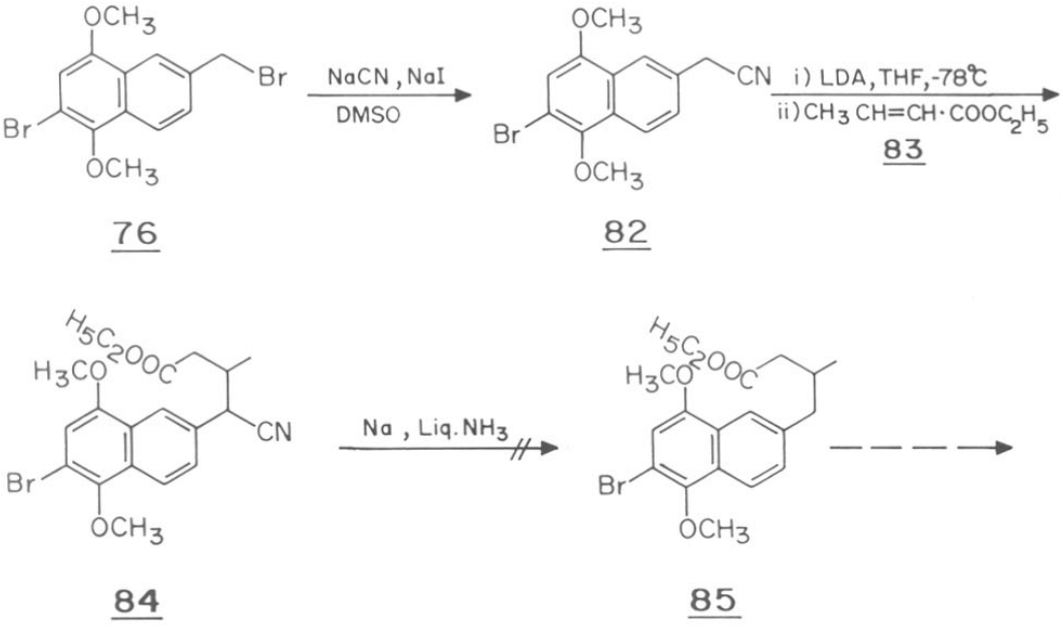
FIG. IV : ^{1}H-NMR SPECTRUM OF THE COMPOUND (80) IN $CDCl_3$

δ 2.86 integrating for five protons. The thio-ketal protons showed a singlet at δ 2.28 and two -OCH_3 groups showed singlets at δ 3.71 and δ 3.75. The aromatic protons showed a singlet at δ 6.64 and two multiplets at δ 7.24 and δ 7.82.

However, the thio-ketal **80** failed to give the cyclized product **81** when treated with trifluoroacetic anhydride and trifluoroacetic acid. The acid **80** also failed to cyclize when various other reagents[19] known to bring such cyclization were tried.

In yet another modified approach (**Scheme 4.2.15**) the dibromo derivative **76** was converted to its benzyl cyanide derivative **82** with sodium cyanide in DMSO. Aryl cyanides have been used as a nucleophilic partner in a Michael type condensation with a suitably functionalized α,ß-unsaturated ester.[20] Thus, an anion of **82**, generated by LDA at -78°C, was treated with ethyl crotonate (**83**) to give an adduct **84** in 25% yield. The poor yield of the product can be attributed to the insolubility of the lithium enolate at low temperature in THF. Hence, the reaction was carried out in presence of HMPA but the yield of the product **84** could not be improved. The product **84** was characterized by various spectral means. In the IR spectrum ester carbonyl absorption peak appeared at 1740 cm^{-1} and a nitrile asorption peak at 2260 cm^{-1}. The ^{1}H-NMR (**FIG.-V**) showed a multiplet between δ 1.10 -1.40 corresponding to six methyl protons. A doublet at δ 2.9 for two methylene protons and a multiplet between δ 3.53 - 4.35 corresponding to four protons were present. The six methoxyl protons showed a broad singlet at δ 3.92. The aromatic protons showed a singlet at δ 6.86 and two multiplets at δ 7.44 and δ 8.1. The presence of molecular ion peak at m/e. 421 further confirmed the assigned structure. The compound **84** was then subjected to Birch reduction.[21] with sodium and liquid ammonia, but the desired product **85** could not be isolated.

SCHEME-4·2·15



76 82 83

84 85

86

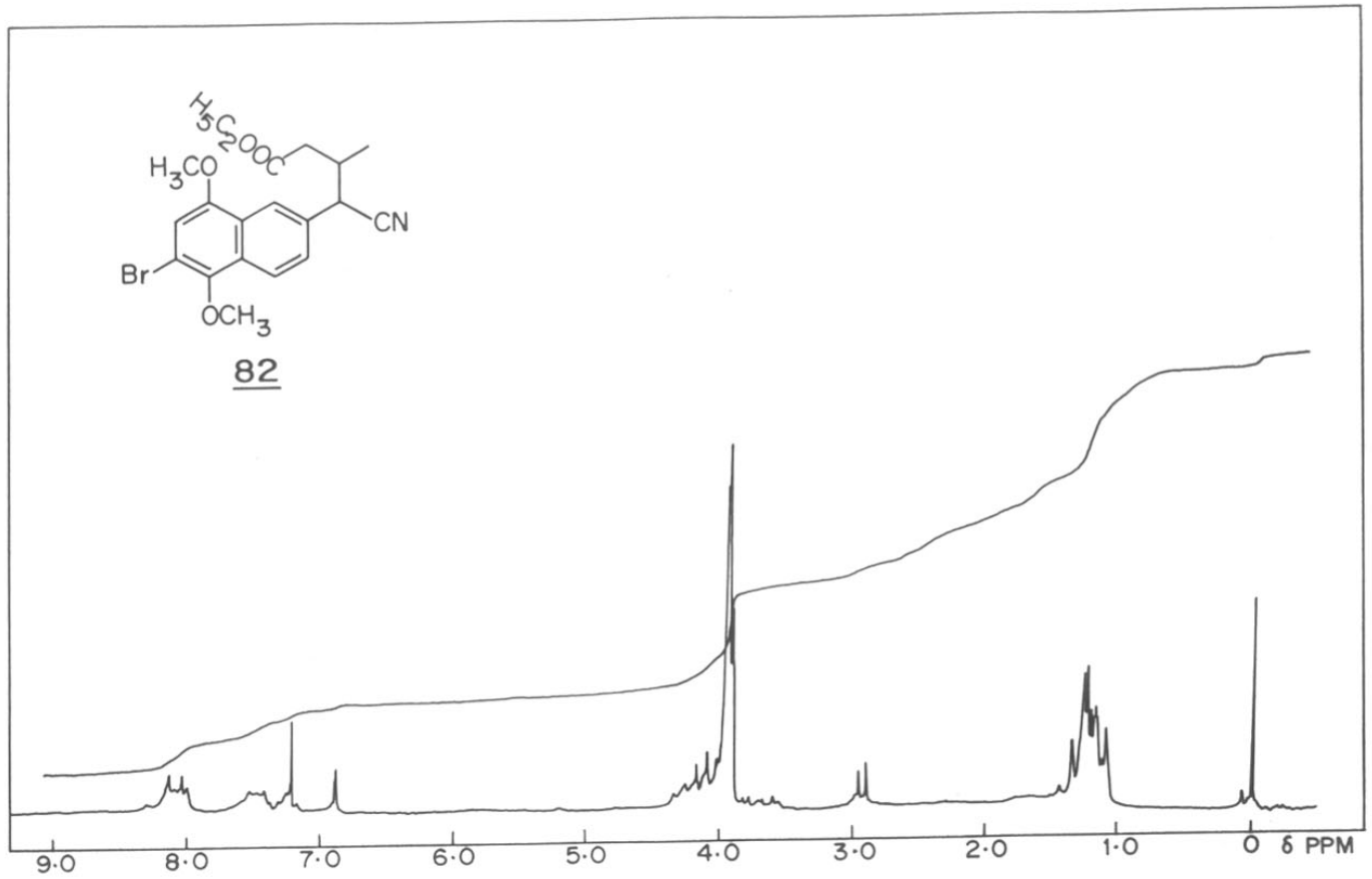


FIG. V : ^{1}H-NMR OF THE COMPOUND (82) IN $CDCl_3$

Experimental:

2-Methoxynaphthalene (ß-naphthol methyl ether)(51): A mixture of ß-naphthol (18 g, 125 mmol), potassium carbonate (25.8 g, 185 mmol) and dimethyl sulphate (15.75 g, 135 mmol) in acetone (200 ml) was heated under reflux for 4 h. The acetone was distilled off and to the residue water (200 ml) was added. The light pink solid thus obtained was filtered, washed and air dried. The product was crystallized from hexane to give 2-methoxynaphthalene (**51**) (19.5 g, 98%) as colourless crystalline solid. m.p. 72°C [Lit.[22], m.p. 73°C].

1-(6-Methoxynaphthyl)propan-1-one (52): To a stirred solution of aluminium chloride (15.35 g, 115 mmol) in nitrobenzene (65 ml) was added 2-methoxynaphthalene (**51**) (15.8 g, 100 mmol) during 30 min. The reaction mixture was cooled to 15°C and propionyl chloride (10.2 g, 110 mmol) was added slowly at the same temperature. The stirring was continued for 2 h at the same temperature and then warmed to room temperature and continued overnight. The reaction mixture was poured over a mixture of ethylene dichloride (180 ml), ice-water (200 ml) and conc. HCl (2.5 ml) and stirred for 30 min. The oily layer separated after standing overnight was removed, washed with water, 5% sodium carbonate and water successively and dried ($CaCl_2$). The solvent was removed under reduced pressure and the residue was poured into hot methanol (50 ml). The methanolic solution was heated with charcoal, filtered and cooled to 10-15°C. The light brown solid thus obtained was filtered, washed with methanol and dried. The product was crystallized from hot methanol to give compound **52** as an off-white solid. (14.9 g, 70%), m.p. 108-109°C. [Lit.[23], m.p. 109°C].

1-(6-Methoxy-5-nitro-2-naphthyl)-propan-1-one (53): To a solution of 1-(6-methoxynaphthyl)propan-1-one (**52**) (4.0 g, 18.69 mmol) in glacial acetic acid (23 ml) was added a mixture of fuming HNO_3 acid (2.9 ml, d = 1.51) and glacial

acetic acid (27 ml) at 55°C during 30 min. The reaction mixture was heated at 75°C for 10 min (till all the brown fumes disappeared). The reaction mixture was cooled and poured into ice-water (100 ml). The yellow solid so obtained was filtered, washed and air dried. Recrystallization from benzene and pet. ether furnished 1-(6-methoxy-5-nitro-2-naphthyl)propan-1-one (**53**) (3.5 g, 72%) as a yellow crystalline solid. m.p. 147-9°C.

IR (nujol): 1680 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.26 (t, 3H); 2.13 (q, 3H); 4.03 (s, 3H); 7.37 (d, J = 8 Hz, 1H); 7.71 (d, J = 8 Hz, 1h); 8.06 (m, 1H); 8.15 (m, 1H); 8.43 (bs, 1H).

Ms (m/e): 259 (M^+).

2-Bromo-1-(6-methoxy-5-nitro-2-naphthyl)propan-1-one (54):

Bromine (1.92 g, 12 mmol) was added to a solution of compound **53** (2.59 g, 10 mmol) in toluene (25 ml) and few drops of nitrobenzene at 0-10°C during 20 min. The reaction mixture was stirred at the same temperature for 2 h and then warmed to the room temperature and stirred for 4 h. The reaction mixture was poured into a 10% sodium carbonate solution (50 ml) and the organic layer was separated. The aqueous phase was extracted with toluene (2 x 20 ml) and mixed to the original organic layer. The combined organic phase was washed with water (2 x 50 ml), dried (Na_2SO_4) and concentrated. The residue was triturated with pet ether (25 ml) to give bromo compound **54** as a light yellow low melting solid, (2.8 g, 82%)

IR ($CHCl_3$): 1685 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 2.0 (d, J = 6 Hz, 3H); 4.03 (s, 3H); 5.40 (q, 1H); 7.40 (d, J = 8 Hz, 1H); 7.66 (d, J = 8 Hz, 1H); 8.01 (m, 1H); 8.16 (m, 1H); 8.50 (bs, 1H).

Ms (m/e): 337 (M^+).

Alkylation of diethyl malonate with compound 54: To a stirred suspension of sodiumhydride (0.17 g, as 50% oil suspension) in dry DMSO (5 ml) was added a solution of diethyl malonate (0.47 g, 2.95 mmol) in DMSO (5 ml) during 10 min under N_2 atmosphere. The reaction mixture was heated for 1 h at 60°C. The reaction mixture was cooled to room temperature and a solution of compound **54** (1.0 g, 2.95 mmol) in DMSO (5 ml) was added during 10 min. The reaction mixture was stirred at 60°C for 3 h, cooled, poured on ice-water (50 ml) and extracted with ether (2 x 25 ml). The combined extract was washed with water, dried (Na_2SO_4) and concentrated. The residue was triturated with pet ether (20 ml) and a solid thus obtained was filtered and dried. The product was crystallized from ethyl acetate to give the diester **55** (1.10 g, 89%) as a light brown solid. m.p. 121°C.

IR (Nujol): 1740, 1730 and 1680 cm^{-1}.

^{1}H-NMR ($CDCl_3$: δ 1.07 - 1.43 (m, 9H); 3.81-4.40 (m, 6H); 4.06 (s, 3H); 7.37 (d, J = 8 Hz, 1H); 7.68 (d, J = 8 Hz, 1H); 8.03 (m, 1H); 8.17 (m, 1H); 8.51 (bs, 1H).

Ms (m/e): 417 (M^+).

4-(6-Methoxy-2-naphthyl)4-oxo-1-butanoic acid (59): To an ice cold solution of aluminium chloride (29.5 g, 220 mmol) in nitrobenzene (75 ml) was added succinic anhydride (11 g, 110 mmol) during 30 min. After stirring for another 30 min. at the same temperature, a solution of 2-methoxynaphthalene (**51**) (15.8 g, 100 mmol) in nitrobenzene (75 ml) was added during 1 h. The stirring was continued for 6 h and the reaction mixture was poured over a mixture of conc. HCl (25 ml), ice (100 g) and ethyl acetate (300 ml). After standing overnight an oily layer thus separated was removed and washed with water (2 x 150 ml). The organic phase was extracted with saturated sodium hydrogen carbonate solution

(2 x 100 ml) and the combined aqueous phase was washed with ethyl acetate (1 x 100 ml). The aqueous phase was acidified with conc. HCl to give a cream coloured solid. The solid thus obtained was filtered, washed and air dried. The acid was recrystallized from acetone to give acid **59** (24.2 g, 93%) as a colourless crystalline solid. m.p. 146° [Lit.[24] m.p. 147-148°C].

Methyl-4-(6-methoxy-2-naphthyl)4-oxo-1-butanoate (60): A mixture of the acid **59** (23.0 g, 89.1 mmol), dimethyl sulphate (11.4 ml, 89.1 mmol) and potassium carbonate (18.45 g, 138 mmol) in acetone (250 ml) was heated under reflux for 6 h. The acetone was distilled off completely and to the residue water (200 ml) was added. The solid separated thereby was filtered, washed and dried. The product was recrystallized from hexane acetone to give the ester **60** (24.18 g, 99%) as a colourless crystalline solid; m.p. 88-89°C; [Lit[24], m.p. 108°C for ethyl ester].

Methyl-4-(6-methoxy-5-nitro-2-naphthyl)4-oxo-1-butanoate (61): The ester **60** (13.6 g, 50 mmol) was heated in glacial acetic acid (80 ml) at 55°C and to the resultant solution fuming HNO_3 (7.84 ml, 165 mmol) in acetic acid (70 ml) was added with stirring during 40 min. The reaction mixture was heated at 75°C for 2 h. The reaction mixture was cooled and poured over ice-water (200 ml). The product was filtered, washed thoroughly with water and air dried. The product was recrystallized from methanol to give the nitro derivative **61** (8.75 g, 55%) as a bright yellow crystalline solid. m.p. 190°C.

IR (Nujol): 1730 and 1680 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 2.78 (t, 2H); 3.38 (t, 2H); 3.70 (s, 3H); 4.05 (s, 3H); 7.31 (d, J = 8 Hz, 1H); 7.73 (d, J = 8 Hz, 1H); 8.05 (m, 1H); 8.13 (m, 1H); 8.43 (bs, 1H).

Ms (m/e): 317 (M^+).

1,4-Dimethoxynaphthalene (68): A solution of 1,4-naphthaquinone (**67**) (40 g, 253 mmol) in ethyl acetate (500 ml) was stirred vigorously with an aqueous solution of sodium dithionate (80 g, 459 mmol) in water (300 ml) for 15 min. The organic layer was separated, washed with brine and dried (Na_2SO_4). The solvent was distilled off under reduced pressure and the residue was dissolved in acetone (500 ml). The solution was refluxed with dimethyl sulphate (63.0 g, 500 mmol) and potassium carbonate (103.5 g, 750 mmol) for 8 h. The acetone was distilled off and residue was diluted with ice-water (500 ml). The brown solid thus separated was filtered, washed and dried. The product was recrystallized from hexane to give 1,4-dimethoxynaphthalene (**68**) (45.2 g, 96%) as a off-white crystalline solid; m.p. 86°C [Lit[25], m.p. 85-86°C].

2-Bromo-1,4-dimethoxynaphthalene (69): To a stirred solution of 1,4-dimethoxynaphthalene (**68**) (10.0 g, 53.19 mmol) in chloroform (60 ml) containing iron powder (0.150 g) was added a solution of bromine (8.5 g, 53.12 mmol) in chloroform (80 ml) at room temperature during 30 min., while a slow stream of N_2 was passed through the solution. Then the N_2 stream was stopped and the reaction mixture was stirred for 1 h. Then N_2 was bubbled vigorously to remove all the HBr from the solution and the solution was filtered and poured over water (200 ml). The organic phase was washed with 10% aqueous KOH solution and brine successively and dried (Na_2SO_4). The residue obtained after removal of the solvent was chromatographed on silica gel using hexane as eluent to afford the bromo derivative **69** (12.7 g, 90%) as a colourless crystalline solid, m.p. 56°C [Lit[26], m.p. 54-55°C].

1-(2-Bromo-1,4-dimethoxy-6-naphthyl)-propan-1-one (70): To a stirred solution of compound **69** (6.0 g, 22.38 mmol) in ethylene dichloride (150 ml) was added aluminium chloride (6.6 g, 47.82 mmol) in portions and the solution was

heated at 60°C in an oil bath. Propionic anhydride (3.0 g, 23.80 mmol) was added at the same temperature during 30 min. The reaction mixture was stirred for 4 h. at the same temperature, cooled and poured over a mixture of conc. HCl (20 ml) and ice (200 g). The organic layer thus separated was washed with water, 10% $NaHCO_3$ solution and water successively. The dried (Na_2SO_4) organic phase was concentrated under vacuum and the residue was treated with pet-ether (50 ml) to give a light yellow solid. The solid was filtered, washed with pet ether and dried to give compound **70** (4.8 g, 66%) as a light yellow solid, m.p. 95-97°C.

IR (Nujol): 1675 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.28 (t, 3H); 3.12 (q, 2H); 3.87 (s, 3H); 3.93 (s, 3H); 6.87 (s, 1H); 8.12 (bs, 2H); 8.75 (s, 1H).

Ms (m/e): 324 (M^+).

2-Bromo-1-(2-bromo-1,4-dimethoxy-6-naphthyl)propan-1-one(71): To a stirred solution of compound **70** (2.0 g, 6.17 mmol) in toluene (25 ml) and nitro-benzene (few drops) was added bromine (1.0 g, 6.25 mmol) at -10°C during 20 min. The reaction mixture was stirred at the same temperature for 1 h and then warmed to the room temperature and stirred for 2 h. The reaction mixture was poured onto 10% aqueous solution of $NaHCO_3$ (100 ml) and the organic phase was separated, washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was treated with pet ether (50 ml) to give the bromo derivative **71** (1.98 g, 80%) as a sticky solid which was used as such for the next step.

Alklyation of diethyl malonate with bromo derivative 71: To a stirred suspension of sodium hydride (0.12 g, 50% oil suspension) in DMSO (5 ml) was added diethyl malonate (0.4 g, 2.5 mmol) under nitrogen and the reaction mixture was stirred at 60°C for 1 h. The reaction mixture was cooled to room temperature and

a solution of compound **71** (1.0 g, 2.47 mmol) in DMSO (5 ml) was added and the reaction mixture was heated and stirred at 60°C for 4 h. The reaction mixture was poured over cold water (25 ml) and extracted with chloroform (2 x 25 ml). The combined extract was washed, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography to give the diester **72** (0.9 g, 75%) as a light brown solid, m.p. 108°C.

IR (Nujol): 1730 and 1670 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.17 (d, 3H); 1.25 (t, 6H); 3.81-4.43 (m, 6H); 3.98 (s, 3H); 4.05 (s, 3H); 6.87 (s, 1H); 8.12 (bs, 2H); 8.78 (s, 1H).

Ms (m/e): 480 (M^+).

1,4-Dimethoxy-6-methylnaphthalene (75): A mixture of 1,4-benzoquinone (**37**) (7 g, 60 mmol), isoprene (**74**) (4.48 g, 60 mmol) and acetic acid (20 ml) was kept standing for 3 days with occasional shaking. Water (20 ml) was introduced and the reaction mixture was refluxed for 1.5 h. Acetic acid (40 ml) was added and the reaction mixture was cooled to 75°C and a solution of CrO_3 (14.7 g) in water (15 ml) was added with stirring. The reaction mixture was stirred further for 0.5 h and diluted with water (100 ml). The greenish-yellow solid thus obtained was filtered, washed with water and air dried.

The quinone so obtained was dissolved in ethyl acetate (100 ml) and stirred with an aqueous solution of sodium dithionate (14 g in 100 ml water) for 15 min. The organic layer was separated, washed, dried (Na_2SO_4) and concentrated. The residue was dissolved in acetone (100 ml) and refluxed with dimethyl sulphate (16.2 g, 128 mmol) and potassium carbonate (18 g, 130 mmol) for 8 h. The acetone was distilled off under reduced pressure and to the residue water (100 ml) was added. The light brown solid thus obtained was filtered, washed and

dried. The product was recrystallized from pet ether to give the compound **75** (8.5 g, 65%) as a cream coloured solid. m.p. 56-57°C [Lit[27], m.p. 55-57°C].

2-Bromo-6-bromomethylene-1,4-dimethoxynaphthalene (76): A mixture of the compound **75** (5 g, 24.75 mmol), N-bromo succinimide (8.8 g, 49.43 mmol) and benzoyl peroxide (0.2 g) was refluxed in carbon tetrachloride (50 ml) in presence of a sun lamp for 2 h. The reaction mixture was cooled, filtered and concentrated under vacuum. The residue was purified on silica gel column using pet ether-acetone (9.5:0.5) as eluent to give the dibromo derivative **76** (8.61 g, 96%) as a thick oil.

IR (Neat): 1600 and 1490 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 3.84 (s, 3H); 3.93 (s, 3H); 4.60 (s, 2H); 6.84 (s, 1H); 7.51 (m, 1H); 8.01 (m, 2H).

Ms (m/e): 360 (M^+).

Ethyl-(3-acetyl)-levuinate (77): A mixture of ethyl bromo-acetate (16.6 g, 100 mmol), acetyl acetone (12.09 g, 120 mmol) and potassium carbonate (15.23 g, 120 mmol) in acetone (150 ml) was stirred for 14 h. The potassium carbonate was filtered, washed with acetone (20 ml) and the combined filtrate was concentrated under vacuum. The residue was distilled under reduced pressure to give the compound **77** (12.5 g, 60%) as a colourless oil; b.p. 100-110°C/0.5 mm [Lit[28], b.p. 165°C/55 mm].

The aklylation of compound 77 with bromo derivative 76: A mixture of the compound **77** (0.77 g, 4.13 mmol), bromo derivative **76** (1.5 g, 4.16 mmol) and potassium carbonate (0.57 g, 4.18 mmol) in acetone (20 ml) was refluxed for 8 h on a water bath. The reaction mixture was cooled, filtered and concentrated. The residue was purified by silica gel column chromatography (pet ether - acetone 8:2) to give the compound **78** (1.1 g, 51%) as a yellow oil.

IR (Neat): 1740, 1680 and 1675 cm^{-1}

^{1}H-NMR ($CDCl_3$): δ 1.26 (t, 3H); 2.17 (s, 3H); 2.26 (s, 3H), 2.88 (s, 2H); 3.86 (s, 2H); 3.93 (s, 6H); 4.20 (q, 2H); 6.84 (s, 1H); 7.20 (dd, J = 8 Hz, 2 Hz, 1H); 7.8 (d, J = 2 Hz, 1H); 8.01 (d, J = 8 Hz, 1H).

Ms (m/e): 466 (M^+).

Hydrolysis of the ester 78: The ester **78** (1.0 g, 2.1 mmol) was stirred with 10% ethanolic KOH solution (20 ml) under N_2 for 6 h. The ethanol was distilled off under reduced pressure and the residue was diluted with water (20 ml) and acidified to pH 2 with dil. HCl. The product was extracted with chloroform (2 x 50 ml) and the combined extract was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (Benzene-acetone 9:1) to give the acid **79** (0.755 g, 86%) as a sticky solid.

IR ($CHCl_3$): 1680 and 1665 cm^{-1}.

^{1}H-NMR($CDCl_3$): δ 2.13 (s, 3H); 2.35 - 3.40 (m, 5H); 3.88 (s, 6H); 6.80 (s, 1H); 7.26 (dd, J = 8 Hz, 2 Hz, 1H); 7.62 (bs, 1H); 8.01 (d, J = 8 Hz, 1H).

Ms (m/e): 394 (M^+), 376 (M^+-18).

Thio-ketalization of the acid 80: To a stirred solution of the acid **79** (0.394 g, 1 mmol) and ethanethiol (0.113 g, 1.2 mmol) in dry chloroform (10 ml) was added BF_3-etherate (0.17 g, 1.2 mmol) under N_2 atmosphere. The reaction mixture was stirred for 3 days at room temperature and quenched with water (10 ml) and methanol (2 ml). The organic layer was separated, washed and dried over Na_2SO_4. The residue obtained after concentration was chromatographed on silica gel using pet ether-acetone (8:2) as eluent to give the thio-ketal **80** (0.235 g, 50%) as a pale yellow oil.

IR (Neat): 1665 cm^{-1}.

^{1}H-NMR: δ 1.73 (s, 3H); 2.10-2.86 (m, 5H); 3.28 (s, 4H); 3.71 (s, 3H); 3.75 (s, 3H); 6.64 (s, 1H); 7.24 (m, 1H); 7.82 (m, 2H).

Ms (m/e): 470 (M^+).

2-Bromo-6-cyanomethylene-1,4-dimethoxy-6-naphthalene (82): To a solution of the dibromo derivative (**76**) (4.5 g, 12.5 mmol) in dry DMSO (20 ml) was added anhydrous sodium iodide (0.5 g) and stirred. To this stirred reaction mixture sodium cyanide (5 g) was added and the reaction mixture, was heated at 90°C for 20 h. The reaction mixture was poured over ice-water (100 ml) and extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed, dried (Na_2SO_4) and concentrated. The crude residue was purified on silica gel column using pet.ether-acetone (9:1) as eluent to give the cyano derivative **82** (2.81 g, 73%) as a yellow solid. m.p. 136°C (d).

IR ($CHCl_3$): 2260 cm^{-1} (-CN group).

^{1}H-NMR ($CDCl_3$): δ 3.88 (s, 2H); 3.93 (s, 3H); 3.97 (s, 3H); 6.86 (s, 1H); 7.44 (dd, J = 8 Hz and 2 Hz, 1H); 8.05 (d, J = 8 Hz, 1H); 8.15 (d, J = 2 Hz, 1H).

Ms (m/e): 307 (M^+).

Michael reaction of ethylcrotonate (83) with the cyano derivative 82: A LDA solution was prepared by adding n-BuLi (2.2 ml, 1.6 M) in hexane to a solution of di-isopropylamine (0.40 g, 3.5 mmol) in THF (5 ml) at -78°C under N_2 atmosphere. To the resultant LDA solution, a solution of cyano derivative **82** (0.918 g, 3 mmol) in THF (2 ml) was added and the reaction mixture was stirred at -78°C for 1 h. Ethyl crotonate (**83**) (0.342 g, 3 mmol) in THF (3 ml) was added during 15 min. and the reaction mixture was stirred for 3 h at the same temperature. Then it was warmed to room temperature and stirred for 3 more hours. Water (1 ml) was added and the reaction mixture was poured into ether (50 ml). The ethereal layer was separated, washed with 1 NHCl, water and brine successively and

dried over Na_2SO_4. The residue obtained after solvent removal was chromatographed (pet-ether-acetone 9.5:0.5) to give the compound **84** (0.326 g, 25.8%) as a thick yellow oil.

IR (Neat): 2260 and 1740 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 1.10- 1.40 (m, 6H); 2.90 (m, 2H); 3.53 - 4.35 (m, 4H); 3.92 (bs, 6H); 6.86 (s, 1H); 7.44 (m, 1H); 8.10 (m, 2H).

Ms: (m/e): 421 (M^+).

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