CHEMISTRY OF INDIAN MEDICINAL PLANTS

A THESIS

SUBMITTED TO THE

UNIVERSITY OF POONA

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN CHEMISTRY

BY

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DIVISION OF ORGANIC CHEMISTRY : TECHNOLOGY

NATIONAL CHEMICAL LABORATORY

PUNE – 411 008

JANUARY 1999

TO MY PARENTS

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled "*Chemistry of Indian Medicinal Plants*" submitted by **Mr. Prasad Pralhad Pujar** was carried out by him at National Chemical Laboratory under my supervision for the Degree of Doctor of Philosophy in Chemistry of the University of Poona. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

Date : Jan. 12, 1999

Pune 411 008

(Dr. B. A. Nagasampagi)

Research Guide

National Chemical Laboratory

Acknowledgement

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I take this opportunity to thank the staff of spectroscopic sections, specially Mr. G. Sammuel and Mr. V.T .Sathe for recording ^{1}H and ^{13}C NMR spectra and spin decoupling studies of the compounds reported here.

I also thank, Mr. Kiran Rao, Mr. Yanjarappa, Dr. G.T. Panse, Dr. (Mrs). S.P. Joshi, Dr. A.B. Landge, Dr.(Mrs). H.V. Kamat, Dr.(Mrs).B.Sinha, Dr.(Mrs).Deodhar, Dr.(Mrs). S. Dalvoy, Mr. S.S.Pawar, Mr. S. Gurunath and Mr. K.B. Sonawane for their cooperation.

I am grateful to my parents and family members, whose constant love, support and encouragement has been the main force for whatever I am and hope I will blessed with their blessings forever.

I thank to CSIR, New Delhi, for financial assistance in the form of research fellowship during the Ph.D. program.

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

(P. P. Pujar)

Contents

General Remarks

1. The compound numbers, figure numbers, chart numbers and reference numbers, etc. given in each chapter refer to that particular chapter only. The references and figures are given at the end of each chapter.
2. All melting points are uncorrected and are recorded on Celsius scale.
3. Petroleum ether refers to the fraction boiling in the range 60-80.
4. Column chromatographic separations were carried out using the column grade (60-120 mesh) silica gel.
5. The thin layer chromatography (TLC) and preparative TLC plates were prepared by spreading an aqueous suspension of silica gel G (200-300 mesh) uniformly over glass plates using an applicator. Layer thickness: TLC plates, 0.5 mm; preparative TLC 1.2 mm. After initial drying at room temperature the plates were activated at 100 for one hour before use.
6. After development, the spot on TLC plates were visualized by exposing them to iodine vapours and/or by spraying with a mixture of H_2SO_4-HNO_3 (1:1) followed by charring in an oven. In case of preparative TLC the band of compounds (after development) were visualized by spraying a dilute solution of iodine in $CHCl_3$ to the sides (after covering the major central portion) with a glass plate.
7. Optical rotations were measured using sodium light (5893Å) as the source on a JASCO DIP – 181 digital polarimeter.
8. The UV spectrum was recorded in ethanol solution on Shimadzu UV-visible recording spectrometer UV-260.
9. The IR spectra were recorded on Perkin-Elmer 599 B, Perkin-Elmer "Infracord" 137 B model and Perkin-Elmer 1620 FT-IR spectrometers.
10. All the ^1H NMR and ^{13}C NMR spectra mentioned were recorded in $CDCl_3$ solution using tetra-methylsilane as internal reference on Bruker AC-200 MHz (50.32 MHz) FT NMR. Other ^1H NMR and ^{13}C NMR spectra were recorded on Bruker WH-90 FT and MSL-300 (75.48 MHz) spectrometers wherever mentioned. Abbreviations, viz., s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, brs = broad singlet and m = multiplet have used.
11. Mass spectra were recorded on Finnigan Mat-1020 using direct inlet system at 70 V.

Abbreviations

1.	Ac	Acetyl
2.	Ang	Angoyl
3.	iBu	isobutyl
4.	Bz	Benzoyl
5.	Cinn	Cinnamoyl
6.	Me	Methyl
7.	Mebu	2-methylbutyl
8.	Ph	Phenyl
9.	Tig	Tigloyl

ABSTRACT

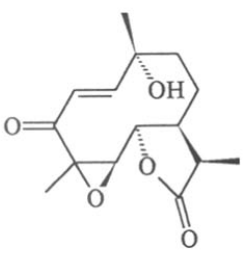
This thesis entitled "*Chemistry of Indian Medicinal Plants*" deals with the isolation of terpenes and steroids from Indian medicinal plants and their characterization by spectral methods and correlation with known compounds. It also deals with screening of the plant extracts for immunomodulatory activity. It further deals with the synthesis of perfumery intermediates using zeolite catalysts.

The thesis is divided into 4 chapters.

Chapter I : Chemical investigation of *Artemisia pallens.*

Artemisia pallens belongs to Asteraceae family. It is an essential oil yielding plant cultivated in Maharashtra and Karnataka (India). Arteether, an anti-malarial drug, has been prepared from a sesquiterpene lactone, artemisinin, a constitutent of *Artemisia annua*[1]. However, there is no report of any sesquiterpene lactone from related species *A. pallens*. With a view to explore the possible biologically active sesquiterpene lactones similar to artemisinin or its precursor, chemical investigation of *A. palllens* was carried out.

Chemical investigation of acetone extract afforded two new germacranolides **I**[2] and its epimer **II** and an ester **III** of a known monoterpene acid[3]. They were characterized by exhaustive spectral studies.



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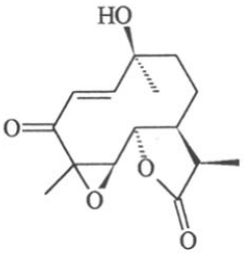

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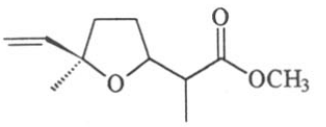
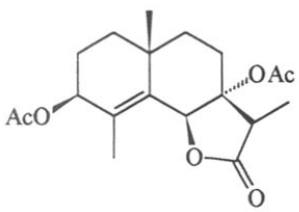


III

Chapter II : Chemical investigation of *Sphaeranthus indicus*

Sphaeranthus indicus (Asteraceae) is an Indian medicinal plant used as folk medicine. Rarely occurring 7-hydroxysesquiterpene lactones have been reported from this plant. 7-hydroxysesquiterpene lactones have been reported to have biological activities such as histamine releasing activity[4] and antimolluscicidal activity[5]. In order to isolate such compounds further chemical investigation was undertaken.

Chemical investigation of acetone extract afforded three new compounds **I, II** and **III**. The structures of these compounds were elucidated by spectral methods and correlation with known compounds.



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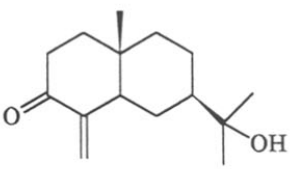

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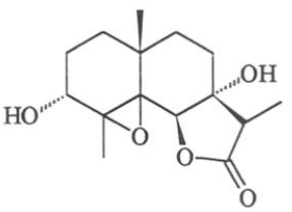


III

Chapter III : Chemical investigation of *Taxus baccata* and *Asparagus racemosus*

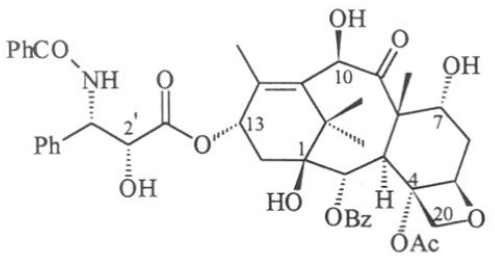
This chapter is divided into two parts.

Part I : Chemical investigation of *Taxus baccata*.

The genus *Taxus* of family Taxaceae has got much importance during last fifteen years due to occurrence of a novel anticancer compound taxol from its species[6,7]. Taxol can be prepared from its precursor 10-deacetylbaccatin III isolated from renewable sources such as leaves (needles). Different *Taxus* species have been investigated to isolate such active

compounds. *Taxus baccata* is one of them. Till now, more than 65 taxanes have been reported from *T. baccata.*

During the standardization of isolation of 10-deacetylbaccatin III, further chemical investigation of methanol extract of needles was carried out and yielded two known taxanes (i) 7-epi-10-deacetyl taxol (**I**) and (ii) brevifoliol (**II**).



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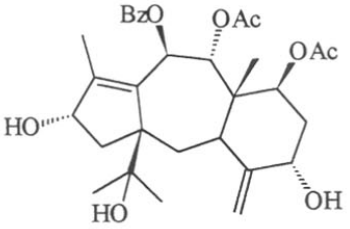


II

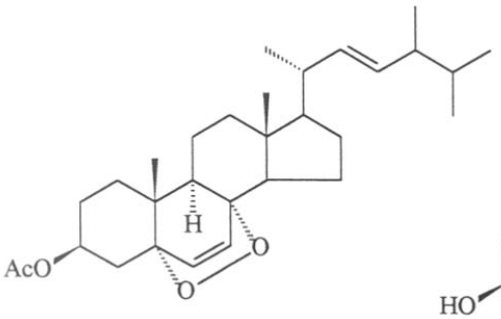
Part II : Chemical investigation of *Asparagus racemosus*

Asparagus racemosus belonging to family Liliaceae is a well-known Indian medicinal plant called Shatavari. Several therapeutic uses have been attributed in classical Ayurvedic literature.

With a view to study the immunomodulatory activity of different solvent extracts, roots were extracted with pet ether, acetone and methanol and the extracts were screened for immunomodulatory activity. For chemical investigation acetone extract was chosen.

Section I : Chemical investigation of acetone extract.

Chemical investigation of acetone extract afforded two known compounds (i) ergosterol peroxide isolated as acetate (**I**) and (ii) sarsasapogenin (**II**). Ergosterol peroxide is a biologically active compound isolated earlier only from different marine species[8]. Sarsasapogenin was reported to obtain by the hydrolysis of glycosides.



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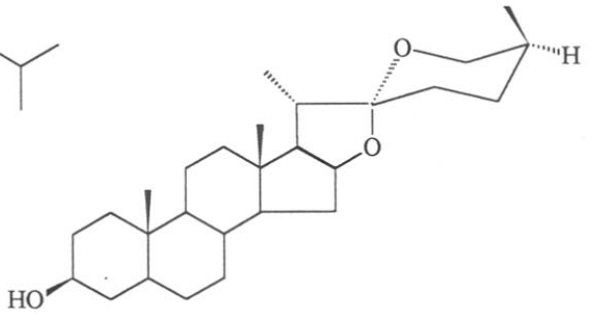


II

Section II : Immunomodulatory activity

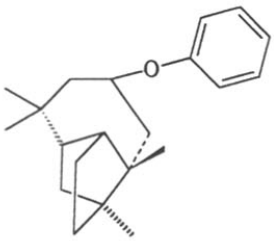
Pet ether and methanol extracts were screened for immunomodulatory activity whereas acetone extract could not be screened. Pet ether extract was found to act as immunosupressant and methanol extract was found to act as immunostimulant, based on mortality tests.

Chapter IV : Zeolite catalysed reactions of terpenes with phenol to synthesize perfumery intermediates.

Terpenyl cyclohexanol derivatives of longifolene and camphene have been studied for many years because of their perfumery uses. Intermediates of such derivatives have been prepared earlier by Friedel-Crafts alkylation using BF_3-etherate as catalyst[9]. However, use of such catalyst has many drawbacks and zeolites can overcome these drawbacks.

Considering this fact, zeolites ZSM-5, β and Y were used as catalyst for the condensation of longifolene and camphene with phenol at different temperatures. Reactions of longifolene gave products **I** and **II**. Compound **I** was earlier obtained by using BF_3-etherate as catalyst.

Reactions of camphene gave products **III** and its epimer **IV** which were also obtained by BF_3-etherate catalyzed condensation.



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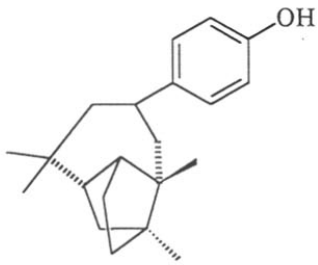

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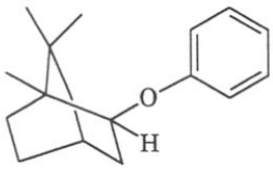
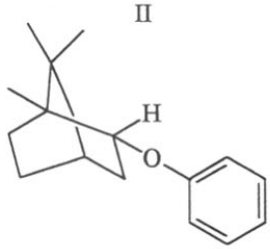


III



IV

References

1. A.M. Clark

   Pharmaceutical Research, **13**, 1133 (1996) and references cited there in.

2. S.R. Rojatkar, S.S. Pawar, P.P. Pujar, D.D. Sawaikar, S.Gurunath, V.T. Sathe and B.A. Nagasampagi

   Phytochemistry, **41**, 1105 (1996).

3. G.Appendino and P. Gariboldi

   Phytochemistry, **23**, 2545 (1984).

4. S.B. Christensen, E. Norup, U. Rasmussen and J.O. Madsen

   Phytochemistry, **23**, 1659 (1984).

5. F. Fronczek, D. Vargas and N.H. Fischer

   J. Nat. Prod., **47**, 1036 (1984).

6. M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggan and A.T. Mc Phail

   J. Am. Chem. Soc., **93**, 2325 (1971).

7. B.Das and S. Padma Rao

   Ind. J. Chem., **35B**, 883 (1996).

8. A.S.R. Anjaneyulu, K.S. Sagar and C.V.S. Prakash

   Ind. J. Chem., **35B**, 819 (1996).

9. S.G. Patnekar and S.C. Bhattacharyya

   Ind. J. Chem., **8**, 36 (1970).

CHAPTER I

CHEMICAL INVESTIGATION

OF

Artemisia pallens

Part of this work has been published in

Phytochemistry, **41**, 1105 (1996)

Introduction

The genus *Artemisia* belonging to family Asteracea, comprises of about 280 species mostly distributed in temperate regions of the world. In India, the genus is represented by about 30 species and most of these are restricted to Himalayan belt, excepting a few species growing in tropical and subtropical plains. Most of the plants belonging to this genus are bitter, characterised with stimulant, antispasmodic and antihelmenthic properties. Some of these are also prized for their essential oil for use in perfumery, food flavouring and medicine. The essential oil yielding species are represented by *A. absinthium* Linn, *A. annua* L. and *A. pallens*. Wall[1]. It is suggested by many researchers that, the sesquiterpene lactones may become useful taxonomic markers within the family Asteracae. Till now, chemical investigation of about 32 species of *Artemisia* has led to isolation of different types of sesquiterpene lactones[2].

Classification of sesquiterpene lactones

Sesquiterpene lactones are relatively stable, mostly colourless, bitter solids or gummy in nature. They are classified on the basis of their carbocyclic skeleton as germacranolides, eudesmanolides, guainolides, pseudoguainolides, eremophilanolides and xanthanolides which result from enzyme mediated cyclization. The other less common classes of sesquiterpene lactones are secoeudesmanolides, chrymoranolides, bakkenolides, secoambrosanolides, secohelenanolides etc. Germacranolides are the biogenetic precursors of all other classes of sesquiterpene lactones[3] (Chart I).

Germacrenolides posses a cyclodecadiene ring structures with double bonds[4] or their equivalent at C_1-C_{10} and C_4-C_5 positions. All naturally occurring sesquiterpene lactones have $C_7\beta$-substituents according to Hendrickson's biogenetic generalization[5]. They may be lactonized either to C_6 or to C_8, the former types being more common. The germacrenolides are further

classified into four sub-groups, (i) germacrenolide, (*trans-trans* germacrenolides) (ii) melampolides, (*cis-trans* germacrenolides) (iii) heliangolides, (*trans-cis* germacrenolides) and (iv) *cis-cis* germacrenolide depending upon the configuration of the double bonds, the germacrenolides being the largest subgroup (Chart II).

Biogenesis

The acetyl Co-enzyme A is the biogenetic origin of pyrophosphate esters of *trans- trans* farnesol, *cis-trans* farnesol or nerolidol[6]. The biogenetic theory assumes that the biosynthesis of sesquiterpenoids including sesquiterpene lactones takes place by modification and/or cyclization of the above pyrophosphate ester. The first step in biogenesis is the cyclization of pyrophosphate esters of *trans-trans* farnesol, *cis-trans* farnesol or nerolidol into a 1,10; 4,5-germacradiene cation which later undergoes various oxidative modifications to give A and B (Chart III).

The γ-lactone formation involves oxidation of isopropenyl and introduction of lactonic oxygen at C_6 or C_8 and ring closure to form either C_6 or C_8 lactones exemplied by costunolide or inunolide respectively. It represents the most elementary cyclic sesquiterpene lactone, since it retains two or three double bonds of the farnesyl pyrophosphate in the *trans-trans* configuration. For the formation of the γ-lactones two possible routes have been suggested. The first may be responsible for the occasional occurrence of the lactone.

An alternative proposal[7] involves oxidation of isopropenyl side-chain sesquiterpenes followed by introduction of oxygen at C_6 or C_8 and lactone ring closure. This mechanism appears more relevant to sesquiterpene lactones since the epoxide, alcohols, aldehyde and acids listed are widely distributed in nature (Chart III).

CHART -I

CLASSIFICATION OF SEQUITERPENE LACTONES

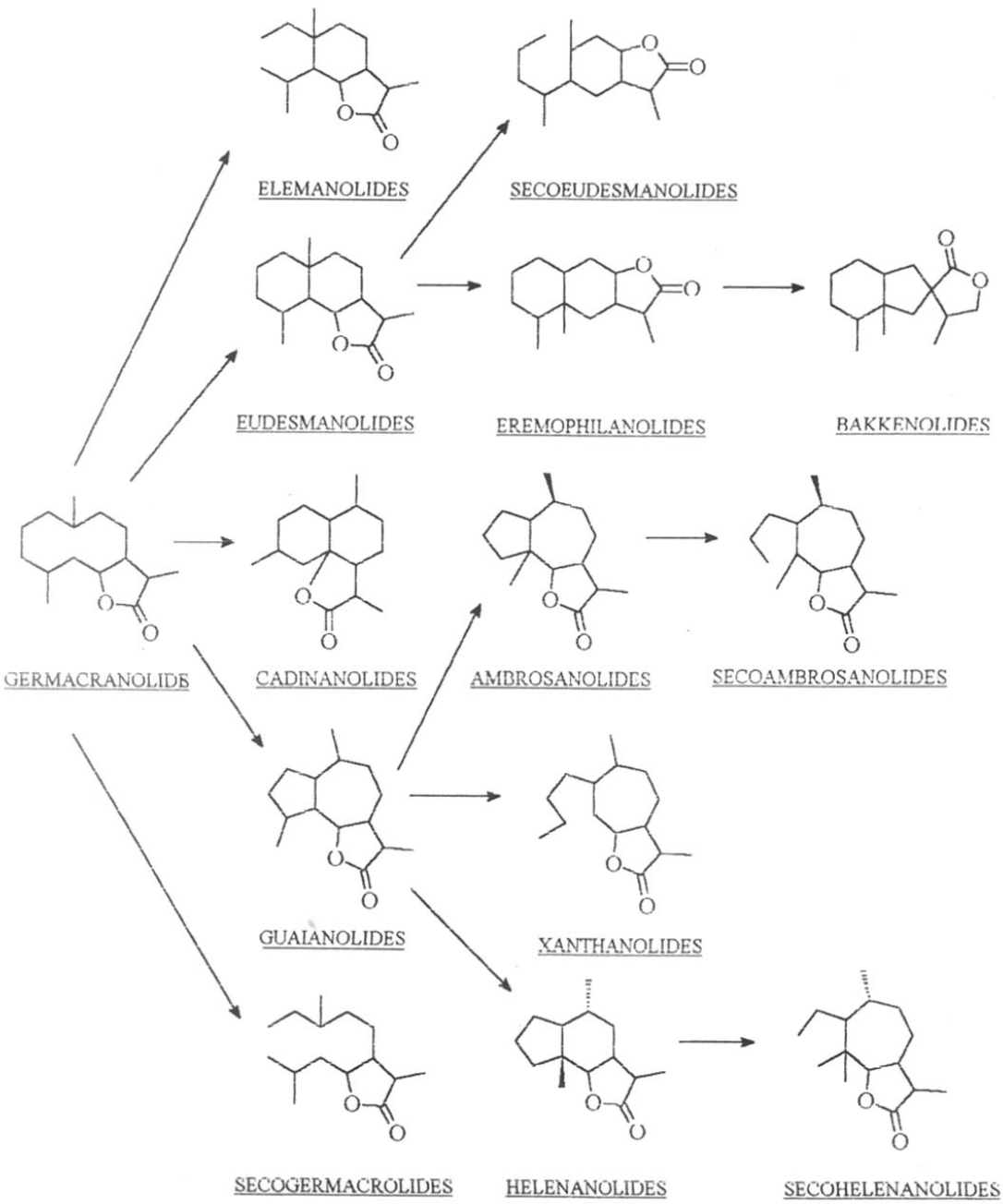
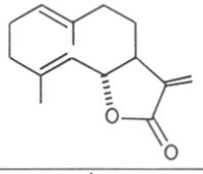
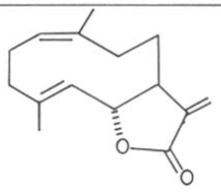
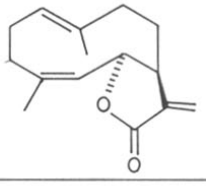
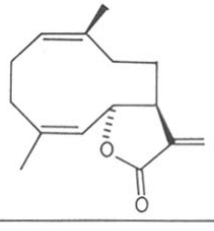
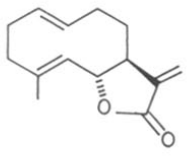


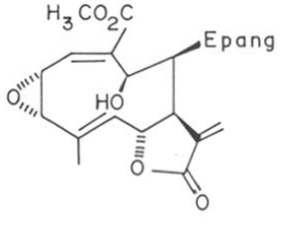
CHART-II

CLASSIFICATION OF GERMACRENOLIDES

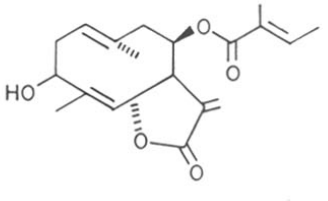
SUBGROUP	CONFIGURATION		SKELETAL STRUCTURE	EXAMPLE
	$\Delta^{1,10}$	$\Delta^{4,5}$		
1) Germacrolides	trans	trans		Costunolide
2) Melampolides	cis	trans		Melampodin – A
3) Heliangolides	trans	cis		Helangolide
4) cis-cis Germacranolide	cis	cis		Melcanthin – A



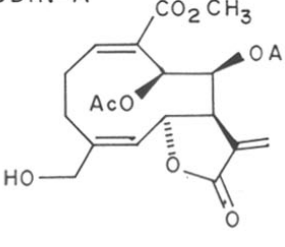
COSTUNOLIDE



MELAMPODIN-A

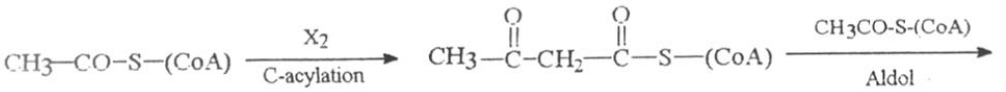


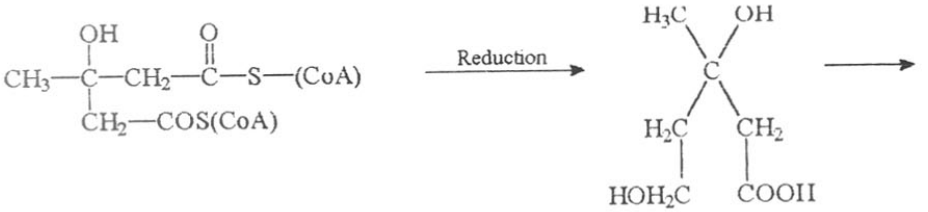
HELIANGOLIDE



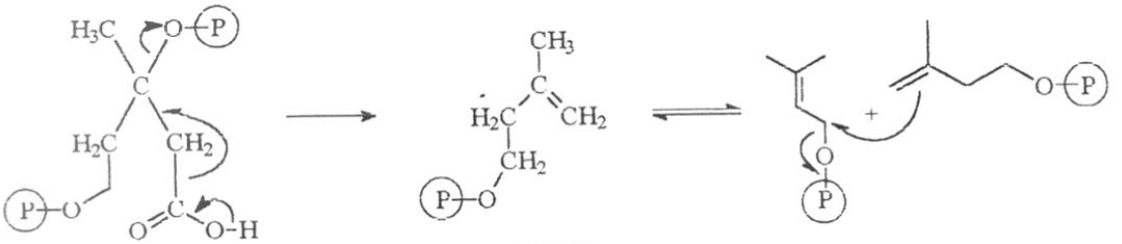
MELCANTHIN-A

CHART-III



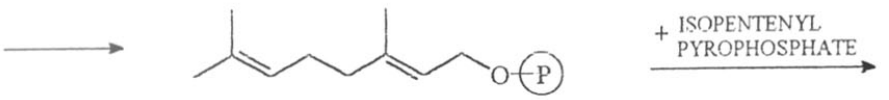


MEVALONIC ACID

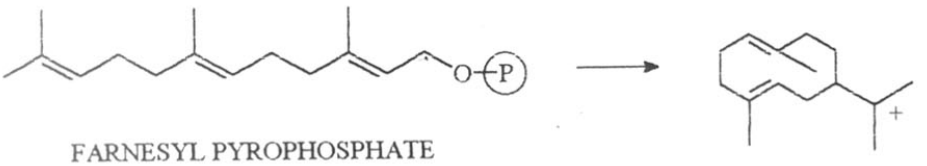


ISOPENTENYL PYROPHOSPHATE

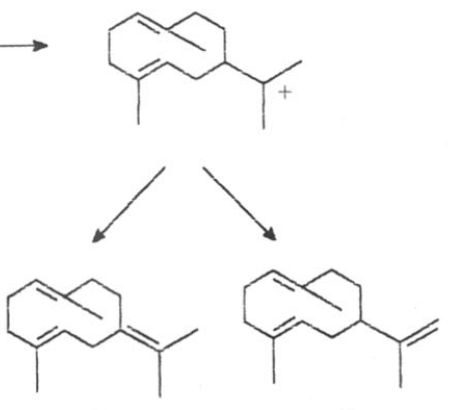
(ALLYLIC ISOMERS)



DIEMETHYL ALLYL PYROPHOSPHATE

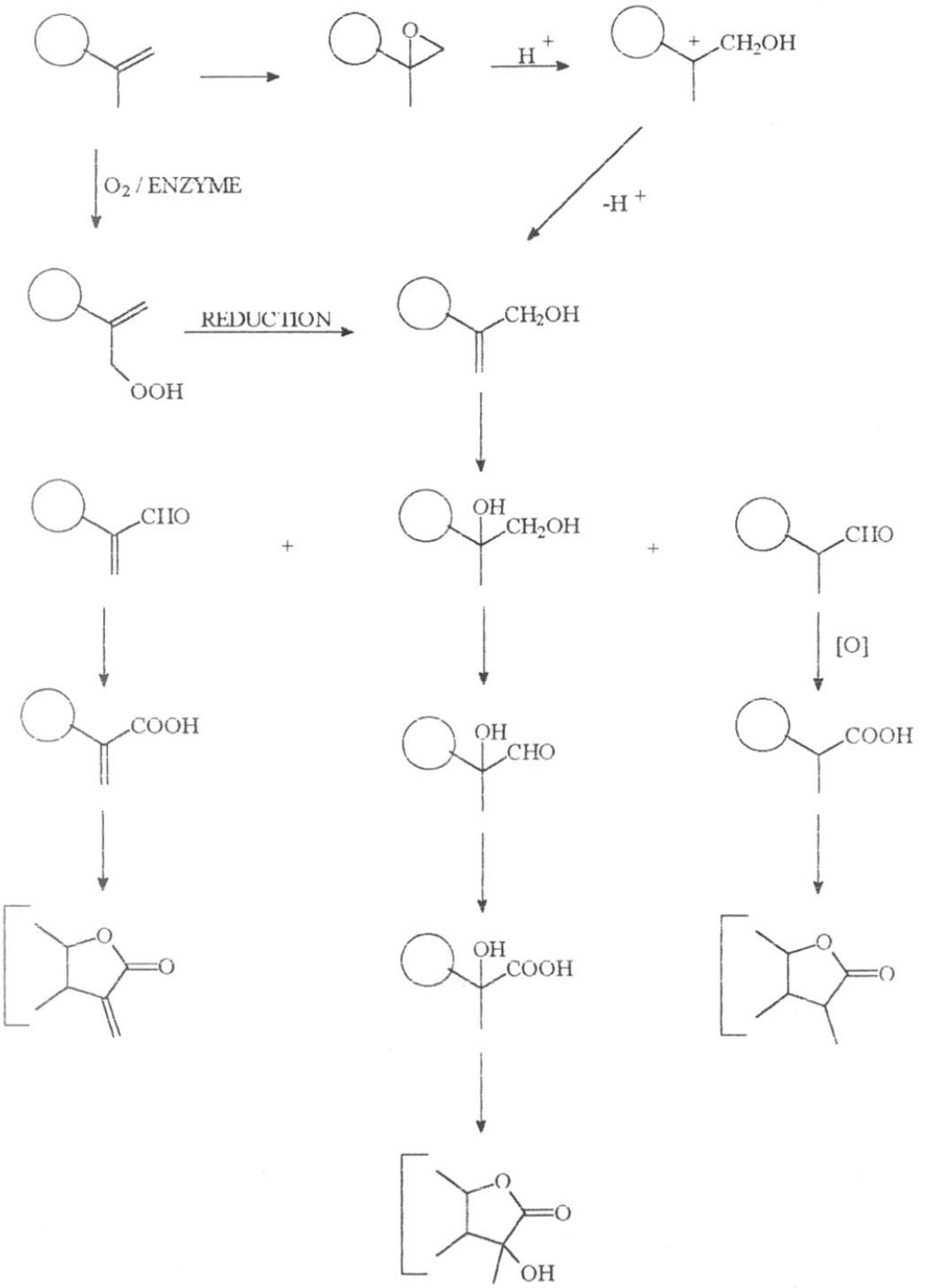


FARNESYL PYROPHOSPHATE



A B

CHART-III (CONTINUED)



Artemisia pallens

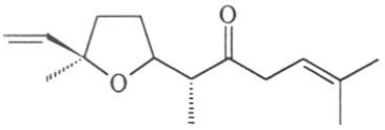
A. pallens (Davana) is a short duration winter crop remaining from November to February in field. It prefers a rich sandy loam soil with very good drainage. A few light showers in early period of growth, bright winter sunshine during reproductive phase without frost but heavy morning dew, contribute to a good crop yield. The cloudy weather or rains at the time of blooming and prior to harvest substantially affect the oil yield. Planting time is one of the important factors in obtaining optimum yield. A significantly higher oil content was recorded in November and December sown plants, while it was low in July and August sown plants[8].

A new drug arteether[9] based on the sesquiterpene lactone, artemisinin, a constituent of *A. annua* has been reported recently to be effective against malaria. A related species, *Artemisia pallens*, which is commercially cultivated in Maharashtra and Karnataka, (India) has not been studied extensively from the point of view of its utility as the source of such anti-malarial drugs. There is no report of any sesquiterpene lactones from this species. However the essential oil is reported to posses antibacterial and anti-fungal properties. The oil has mainly found its use in perfumery industry[10]. With a view to explore the possible biologically active sesquiterpene lactones similar to artemisinin or its precursors, work was undertaken for chemical investigation of the whole plant of *A. pallens.*

Previous Work

Sipma and Vander Wal isolated and characterised davanone (**1**), a sesquiterpene ketone from essential oil of *A. pallens*[11]. Nageli *et al.* isolated artemone (**2**) from the essential oil of *A. pallens, which* was also synthesised by them[12]. Thomas *et al.* reported the isolation, structure elucidation and synthesis of davana ether (**3**), an odoriferous compound present in essential oil[13].

CHART IV



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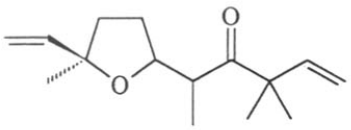

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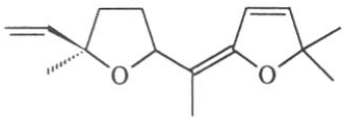

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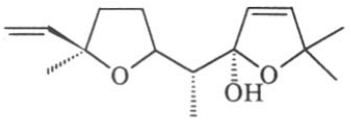

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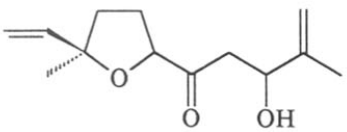

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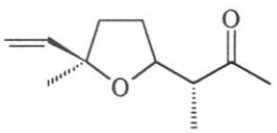

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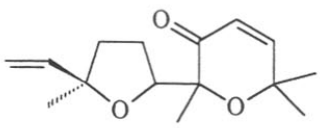
CHART IV (Continued)



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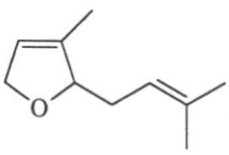

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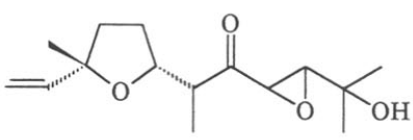


9

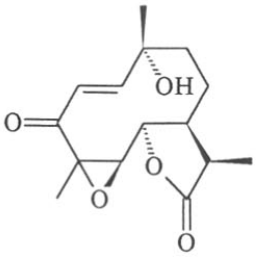
They have reported the photosensitized oxidation of davanone (**1**) to get essential oil[13]. They have reported the photosensitized oxidation of davanone (**1**) to get hemiacetal (**4**) and an allyl alcohol (**5**). They also obtained a stereoisomeric mixture of davana ether (**3**) by passing an epoxide obtained by epoxdation of davanone (**1**) with peracetic acid[14]. Thomas *et al.* reported the isolation and synthesis of nordavanone (**6**)[15], a terpenoid, present in essential oil. They have further isolated a new sesquiterpenoid (**7**)[16] and four furan type stereoisomers of Davana[17].

Lamparsky and Klimes reported some analytical results of Davana oil with respect to biogentically plausible structural features[18]. A novel biosynthesis of irregular sesquiterpene artemone (**2**) was reported by Akhila *et al.*[19] A dihydrofuranoterpenoid (**8**) has been isolated first time from the essential oil by Amitabh Chandra *et al*[20]. Catalan *et al.* reported new sesquiterpene ketones from extract of ariel parts of Davana. These compounds include mainly new 3,4-epoxy derivative of isodavanone (**9**) and cirsimaritin[21] (Chart IV). Misra *et al.* reported 34 fragrant components of Davana oil[22].

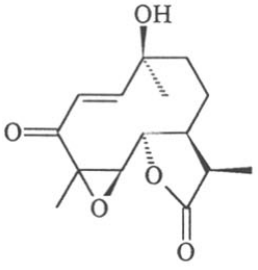
Present Work

With an intention to isolate the possible biologically active sesquiterpene lactones, we undertook the chemical investigation of this plant. The ariel parts were extracted successively with pet ether, acetone and methanol. The acetone extract, after successive chromatography over column grade silica gel and repeated preparative TLC afforded two new germacranolides (**I**) and its epimer (**II**). The methanol extract was partitioned between acidic and neutral parts. The acid part after esterification with diazomethane and further purification afforded an ester (**III**) of known monoterpene acid (Chart V).

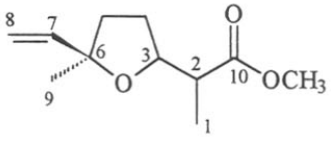
CHART V



I



II



III

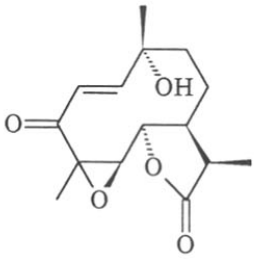
Characterisation of compound I

Compound **I** was obtained as crystalline solid, m. p. 160-162°, $[\alpha]_D^{25}$ -19° (MeOH; c, 0.14), UV λ_{max} 217 (ε_{max} 6800) (Fig 1). It showed in its mass spectrum M^+ at m/e 280 suggesting the molecular formula $C_{15}H_{20}O_5$. IR spectrum (Fig. 2) of the compound showed characteristic bands at 3480 cm^{-1}, 1785 cm^{-1} and 1685 cm^{-1} revealing the presence of hydroxy, γ-lactone (saturated) and conjugated carbonyl groups respectively. ^1H NMR spectrum (Table 1, Fig. 3) of the compound revealed secondary methyl and two tertiary methyl groups, an AB quartet and a down field proton. Its ^{13}C NMR spectrum (Table 2, Fig. 4) showed fifteen peaks indicating the number of carbon atoms in molecule to be fifteen.

All these facts showed that the compound **I** was a sesquiterpene-γ-lactone with an enone system. Presence of a hydroxyl group was further confirmed by mass spectrum, which showed a peak at m/e 262 (M-18). Attempt to acetylate the compound using pyridine and acetic anhydride, at room temperature was not successful, revealing the nature of hydroxyl group to be tertiary.

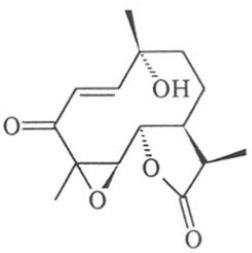
^1H NMR spectrum did not show the characteristic doublets of the exomethylene protons at C-13. Instead, it showed a doublet accounting for three protons at δ 1.23 (J = 7 Hz) for a secondary methyl group. The orientation of this methyl group was deduced from the coupling constant J = 7, 8 Hz of H-11 appearing at δ 2.35 (as doublet of quartet) as β-oriented. The doublet of doublet signal appearing at δ 4.15 (J = 10, 12 Hz) can be assigned to the lactonic proton C-6. This chemical shift and coupling constants clearly indicated that lactone is 6,7-*trans* lactone and further β-oriented H-6 is coupling with α-oriented H-5 which is appearing at δ 2.4 which is confirmed by its ^1H-^1H 2D COSY experiment (Fig. 6).

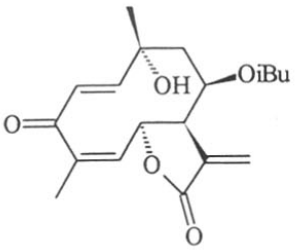
Table 1 : ^{1}H NMR spectral data of compound **I**



Proton	Chemical Shift in δ	Multiplicity	Coupling constant in Hz
H-1	6.60	d	14
H-2	5.85	d	14
H-5	2.40	d	10
H-6	4.15	dd	10, 12
H-7	1.65	m	
H-8, H-9	2.06	m	
H-11	2.35	dq	7, 8
H-13	1.23	d	7
H-14	1.50	s	
H-15	1.20	s	
OH	2.95	br	

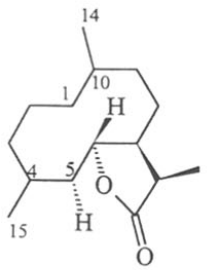
Table 2 : ^{13}C NMR spectral data of compound **I** and Tagetinin C





Carbon	Compound I		Tagetinin C	
	Chemical shift in δ	Multiplicity	Chemical shift in δ	Multiplicity
C-1	152.0	d	160.5	d
C-2	125.7	d	129.6	d
C-3	201.8	s	196.8	s
C-4	46.4	s	138.8	s
C-5	54.7	d	137.1	d
C-6	79.7	d	76.0	d
C-7	40.7	d	47.0	d
C-8	22.8	t	74.1	d
C-9	34.3	t	48.4	t
C-10	70.2	s	71.9	s
C-11	52.5	d	136.1	s
C-12	178.4	s	169.7	s
C-13	19.9	q	124.4	t
C-14	23.9	q	28.9	q
C-15	12.6	q	19.6	q

Thus the partial structure of compound **I** was deduced as follows

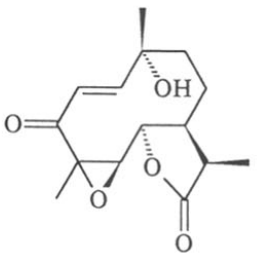


with an enone group and a *tert*- hydroxy group.

The downfield chemical shift (δ1.20 and δ1.50) of the two singlets in ^{1}H NMR spectrum revealed that two tertiary methyl groups are present on oxygenated carbon atoms. This was further confirmed in its ^{13}C NMR spectrum along with INEPT experiment (Fig. 5) where it showed two singlets at δ 46.4 and 70.2.The doublet nature of H-5 revealed C-4 to be a tetra substituted carbon atom, which was further confirmed by ^{13}C NMR spectrum and its INEPT experiment which showed a doublet at δ 54.7 and a singlet at δ 46.4 which can be assigned to oxygenated C-5 and C-4. This clearly indicated the presence of an epoxide at C-4/C-5. The other methyl group can be assigned at C-10 which is biogenetically favourable position. Since this methyl was also shown to be present on an oxygenated carbon atom, tertiary hydroxyl group can be positioned at C-10.

A signal at δ 201.8 in ^{13}C NMR and AB quartet system at δ 5.85 (d, J =14 Hz) and δ 6.60 (d, J = 14 Hz) in ^{1}H NMR, were consistent with a conjugated enone system. This system was also confirmed by absorption at λ_{max} 217 [ε_{max} 6800] in its UV spectrum. The coupling constant of AB quartet mentioned above indicated the olefinic system to be *trans*. The down field shift of the methyl at C-10 (i.e. δ 1.50) clearly indicated the position of the carbonyl at C-3 and a double bond at 1,2- position.

Based on the above spectral data, the structure of compound **I** was elucidated as 10α-hydroxy-4, 5β-epoxy-1-en-3-one-6α,7-germacranolide[23].



I

The assigned position and stereochemistry of the enone system was further supported by critical comparison of the ^{13}C NMR spectral data of compound **I** with that of a similar compound tagetinin-C (Table 2) isolated from *Tithonia diversifolia*[24]. This comparison also enables us to deduce the stereochemistry of tertiary hydroxyl group at C-10.

Compound II

Compound **II** had very close Rf value to that of compound **I** and was difficult to isolate in pure form. ^1H NMR spectrum of the mixture (Fig. 7) of **I** and **II** was similar to that of **I**, revealing that the compound **II** may have a structure similar to that of compound **I**.

However, compound **II** was separated and purified by repeated preparative TLC using different solvent systems.

Characterisation of compound II

Compound **II** obtained as viscous oil, $[\alpha]_D^{25}$ +24°, ($CHCl_3$, c, 0.38), showed in its mass spectrum M^+ at m/e 280 suggesting the molecular formula $C_{15}H_{20}O_5$. Its IR spectrum (Fig. 8)

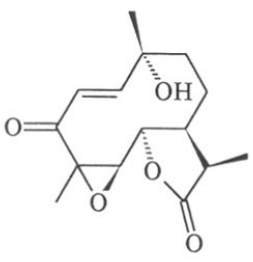
showed characteristic bands at 3681 cm^{-1}, 1775 cm^{-1} and 1670 cm^{-1} revealing the presence of hydroxyl, γ-lactone and a conjugated carbonyl system respectively. ^{1}H NMR spectrum (Table 3, Fig. 9) of compound **II** revealed one secondary and two tertiary methyl groups, an AB quartet and a down field proton at δ 4.35. Its ^{13}C NMR spectrum (Table 4, Fig. 10) along with its INEPT experiment (Fig. 11) showed 15 peaks revealing the number of carbon atoms in the compound to be 15. Thus, the compound **II** was also found to be a sesquiterpene-γ-lactone having an enone system and a hydroxyl group.

The downfield proton at δ 4.35 appearing as doublet of doublet (J = 10, 12 Hz) could be assigned to H-6, the lactonic proton. These coupling constants clearly indicated that lactone was 6,7- *trans* lactone and further β-oriented H-6 is coupling with α-oriented H-5. The secondary methyl appearing as doublet at δ 1.23 (J = 7 Hz) could be due to methyl at C-11. The tertiary methyl appearing at δ 1.32 and 1.60 revealed them to be on carbon bearing oxygen function. Thus from the molecular formula of compound **II** and its IR, ^{1}H NMR and ^{13}C NMR spectral data the structure of compound **II** was deduced as 10-hydroxy-4,5-epoxy-1-en-3-one-6,7-germacranolide, the structure similar to that of compound **I.**

Comparison of ^{1}H NMR and ^{13}C NMR spectral data of compound **I** and compound **II** (Table 3 and 4) clearly showed that the compound **II** was an epimer of compound **I**. As it can be seen, there are six chiral centres in the molecule of compound **I** and **II,** *viz.* C-4, C-5, C-6, C-7, C-10 and C-11.

Biogenetically C-7/C-11 bond is β-oriented and H-7 is α- oriented. From the coupling constsnt of the H-6 (J = 10, 12 Hz) at δ 4.35, it was clear that H-6 was β-oriented, similar to that of H-6 in compound **I**. Further it also indicated that H-5 is *trans* to H-6 and thus α- oriented as in

Table 3 : ^{1}H NMR spectral data of compound **I** and **II**



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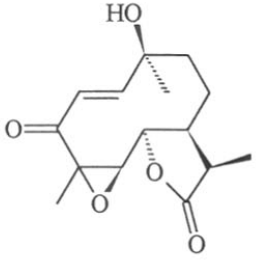


II

Proton	Chemical Shift in δ		Multiplicity	Coupling constant in Hz
	Compound I	Compound II		
H-1	6.60	6.50	d	14
H-2	5.85	5.90	d	14
H-5	2.40	2.45	d	10
H-6	**4.15**	**4.35**	**dd**	**10, 12**
H-7	1.65	1.65	m	
H-8, H-9	2.06		m	
H-11	2.35	2.30	dq	7, 8
H-13	1.23	1.22	d	7
H-14	**1.50**	**1.60**	s	
H-15	**1.20**	**1.32**	s	
OH	2.95		br	

Table 4 : ^{13}C NMR spectral data of Compounds **I** and **II**

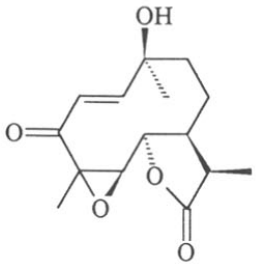
Carbon	Compound-I Chemical shift in δ (multiplicity)	Compound-II Chemical shift in δ (multiplicity)
C-1	**152.0 (d)**	**150.2 (d)**
C-2	125.7 (d)	125.1 (d)
C-3	201.8 (s)	202.8 (s)
C-4	46.4 (s)	45.9 (s)
C-5	**54.7 (d)**	**52.4 (d)**
C-6	79.7 (d)	79.1 (d)
C-7	40.7 (d)	40.7 (d)
C-8	22.8 (t)	22.7 (t)
C-9	**34.3 (t)**	**30.0 (t)**
C-10	**70.2 (s)**	**68.1 (s)**
C-11	52.5 (d)	51.2 (d)
C-12	178.4 (s)	178.7 (s)
C-13	19.9 (q)	20.5 (q)
C-14	**23.9 (q)**	**31.5 (q)**
C-15	12.6 (q)	12.3 (q)

compound **I**. From the coupling constant of H-11 at δ 2.30 (J= 7, 8 Hz) it was indicated that C-11 methyl is β-oriented as in compound **I**. Further there was only a slight change in the chemical shift of H-14 and H-15 revealing the possible change to be at C-4 or C-10.

Comparison of the ^{13}C NMR spectral data of compound **I** and **II** (Table 4) showed changes in the chemical shifts of C-1, C-5, C-9, C-10 and C-14. Though there was a slight change in chemical shift of C-5 (δ 54.7 in compound **I** and δ 52.4 in compound **II**) from the coupling constant of H-5 in ^{1}H NMR spectra of both the compounds, it was confirmed that stereochemistry at C-5 in both the compounds was same. Similarly there was no change in chemical shift of C-4 (δ 46.4 in compound **I** and δ 45.9 in compound **II**) and C-15 (δ 12.6 in compound **I** and δ 12.3 in compound **II**), it was concluded that stereochemistry at C-4 in both the compounds is same. The considerable difference in the chemical shift of C-14 (δ 23.9 in compound **I** and δ 31.5 in compound **II**) and in chemical shift of C-10 (δ 70.2 in compound **I** and δ 68.1 in compound **II**) is quite apparent. This fact clearly indicated the change in stereochemistry at C-10. i.e. C-10 methyl is α-oriented and hydroxyl group at C-10 is β-oriented. This assignment of stereochemistry was further supported by the change in chemical shift of C-9 and C-1, which are adjacent to C-10. (Table 4).

The small change in the chemical shift of C-5 in ^{13}C NMR spectrum and H-6 and H-15 in the ^{1}H NMR spectrum might be due to the conformational factors of 10-membered ring.

Based on the above spectral data, the structure of compound **II** was elucidated as 10β-hydroxy-4,5β-epoxy-1-en-3-one-6α,7-germacranolide.



II

Compound III

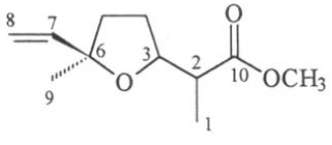
The acid part of methanol extract of *A. pallens* was esterified by diazomethane, and subjected to column chromatography and repeated preparative TLC to obtain compound **III**.

Characterisation of compound III

Compound **III** was obtained as a viscous oil, $[\alpha]_D^{25}$ + 25.1°, and analysed by mass spectrum for molecular formula $C_{11}H_{18}O_3$ (M^+ at m/e 198). IR spectrum (Fig. 12) of the compound **III** showed absorption at 1730 cm^{-1} and 1630 cm^{-1} indicating the presence of ester carbonyl group and unsaturation. ^{13}C NMR spectrum (Table 6, Fig. 15) of the compound showed 11 peaks revealing the compound to be a monoterpene ester. Its ^1H NMR spectrum (Table 5, Fig. 13) showed a singlet at δ 3.65 due to ester methyl and the presence of a secondary methyl and a tertiary methyl. ^1H NMR spectrum also revealed the presence of a monosubstituted double bond by showing three doublet of doublets accounting for one proton each in downfield region. Further it showed an oxygenated proton at δ 4.15 as multiplet.

^{13}C NMR spectrum of the compound showed the presence of two oxygenated carbon atoms and a trisubstituted double bond. ^1H-^1H 2D COSY experiment (Fig. 14) revealed the

coupling of a proton at δ 2.55 with an oxygenated proton at δ 4.15 and secondary methyl. ^{1}H-^{1}H 2D COSY experiment further showed the coupling of proton at δ 4.15 with one of the methylene which further coupled with second methylene group. From all these facts the structure of the compound under discussion was deduced as



III

The parent compound i.e. the acid has been earlier reported from *Tenacetum vulgare*[25]. The spectral data of the compound **III** was similar to the reported spectral data of acid. The acid has been earlier prepared by the oxidation of Davanone[11].

Table 5 : ^{1}H NMR spectral data of Compound **III**

Proton	Chemical shift in δ	Multiplicity	Coupling constant in Hz
H-1	1.05	d	7
H-2	2.55	m	
H-3	4.15	m	
H-7	5.90	dd	14, 10.5
H-8a	5.15	dd	14, 2.5
H-8b	4.95	dd	10.5, 2.5
H-9	1.30	s	
$COOCH_3$	3.65	s	

EXPERIMENTAL

The plant *Artemisia pallens* was collected near Jejuri (Maharashtra) during 1994 December and was shade dried. The shade dried powdered plant material (1Kg), was extracted exhaustively in a soxhlet with pet ether, acetone and methanol successively. The extracts were concentrated separately by distillation under reduced pressure to get thick viscous masses.

The acetone extract was chromatographed to isolate compounds **I** and **II**. The methanol extract was partitioned into acidic and neutral parts. The acid portion was treated with diazomethane and the crude product was purified to afford compound **III**.

Chromatography of Acetone extract

The acetone extract of *Artemisia pallens* (35 g) was chromatographed over silica gel (60-120 mesh) using pet ether, pet ether-acetone in increase percentage of acetone as eluent. The fractions showing similar composition was combined together to obtain six major fractions (A-G). (Table 7).

Rechromatography of fraction D

Fraction D (4.4 g) was rechromatographed by dry column chromatography using acetone : pet.ether mixture (20:80) as eluent with successive increase in percentage of acetone. Fraction (iii) after repeated preparative TLC afforded compound **I** in pure form and compound **II** in fairly pure form.

Table 7: Column chromatography of acetone extract (35 g, silica gel : 900 g,)

Fraction	Eluent	Total volume	Weight in g.	Composition
A	Pet.Ether	100 ml X 7	12.3	Straight chain compounds
B	Pet.Ether: Acetone 90 : 10	100 ml X 5	6.5	Straight chain compounds
C	Pet.Ether: Acetone 80 : 20	100 ml X 4	3.1	Complex mixture of unidentified compounds
D	Pet.Ether: Acetone 70 : 30	100 ml X 5	4.4	Compound **I** and Compound **II**
E	Pet.Ether: Acetone 50 : 50	100 ml X 3	2.0	Complex mixture of unidentified compounds
F	Pet.Ether: Acetone 25 : 75	100 ml X 4	3.1	Glycosides
G	Acetone	100 ml X 4	3.2	Glycosides

Compound **I**

M.P.	:	160° - 162°.
$[\alpha]_D^{25}$	:	- 19° (MeOH, c 0.14).
UV, λ_{max}^{MeOH}	:	217 nm (ε_{max} 6800). (Fig. 1)
IR ν_{max} ($CHCl_3$)	:	3480, 1785, 1685 cm^{-1}, (Fig. 2)
^1H NMR	:	Table 1, (Fig. 3)
^{13}C NMR	:	Table 2, (Fig. 4 & 5)
Mass m/e (rel. int)	:	280 [M^+] (0.5), 262 (3), 247 (11), 201(18), 173 (14), 98 (52), 69 (30), 55 (100).

Compound **II**

Compound **II** (55 mg) was obtained from rechromatography of fraction D (Table 8). It was further purified by repeated preparative TLC on silica gel to obtain compound **II** in fairly pure form as viscous oil.

$[\alpha]_D^{25}$	:	+24° ($CHCl_3$, c 0.38)
IR ν_{max} ($CHCl_3$)	:	3681, 1775, 1670 cm^{-1}, (Fig. 8)
^1H NMR	:	Table 3, (Fig. 9)
^{13}C NMR	:	Table 4, (Fig. 10 & 11)
Mass m/e (rel. int)	:	280 [M^+] (0.5), 262 (37), 247 (79), 201(37), 173 (24), 98 (49), 93 (48), 69 (15), 55 (22).

Table 8 : Rechromatography of fraction D

Fraction	Eluent	Volume	Weight in g.	Composition
I	Pet.ether : Acetone 80 : 20	50 ml X 2	1.1	Unidentified compounds
II	Pet.ether : Acetone 75 : 25	50 ml X 2	1.2	Unidentified compounds
III	Pet.ether : Acetone 70 : 30	50 ml X 4	0.7	Mixture of compounds I & II
IV	Pet.ether : Acetone 60 : 40	50 ml X 3	1.2	Unidentified compounds

Partition of methanol extract

The methanol extract (8 g) was treated with saturated sodium bicarbonate solution and mixture was extracted with $CHCl_3$. The aqueous layer was acidified with 2N HCl to pH 2 and extracted with ethyl acetate. Organic layers were washed with brine, water, dried over anhy. sodium sulphate and concentrated separately.

The acidic part (950 mg) was dissolved in cold methanol. To this a cold etheral solution of diazomethane prepared from 3 g of nitrosomethylurea was added and kept at 5° overnight. The reaction mixture after usual work up was subjected to preparative TLC in benzene : ethyl acetate (80:20) to afford compound **III**.

$[\alpha]_D^{28}$	:	+25.1° ($CHCl_3$, c, 0.14).
IR ν_{max} ($CHCl_3$)	:	1730, 1630 cm^{-1}, (Fig. 12)
^1H NMR	:	Table 5, (Fig. 13)
^{13}C NMR	:	Table 6, (Fig. 15)
Mass m/e (rel. int)	:	198 (5) $[M]^+$, 190(10) 178(35).

REFERENCE

1. A.A. Farooqui and M. Vasundhara

   Advances in Horticulture Vol. **11**, *Medicinal and Aromatic Plants*, p. 659, and references cited there in.

2. H.Z. Al-Khathaln, A.A. Mousa and H.M.G.Al-Hazimi

   J. Chem. Soc. Pak., **14**, 151 (1992).

3. T.A. Geissman in '*Recent advances in Phytochemistry*' (V. Runeckles ed.) 6, 65 Academic Press, New York. (1973).

4. E. Rodriguez, G.H.N. Tower and J.C. Mitchell

   Phytochemistry, **15**, 1573 (1976).

5. H. Yoshioka, T.J. Mabry and B.N. Timmermann

   Sesquiterpene Lactones Chemistry, NMR and Plant distribution, University of Tokyo Press, Tokyo. p. 19.

6. T.A. Geissman and M.A. Irwin

   Pure Appl. Chem. **21**, 162 (1970).

7. T.A. Geissman in '*Recent advances in Phytochemistry*' (V. Runeckles ed.) 6, 55 Academic Press, New York. (1973).

8. B.C. Gulati

   Indian Perfum. **24**, 101 (1980).

9. A.M. Clark

   Pharmaceutical Research, **13**, 1133 (1996) and references cited there in.

10. L.N. Mitra, A. Chandra and R. S. Thakur.

    Phytochemistry, **30**, 549 (1991).

11. G. Sipma and B. Vander wal.

Recl. Trav. Chim. Pays. Bas., **87**, 715 (1968).

12. P. Nageli, J.Klimes and Giuseppe

Tet. Lett, **57**, 5021 (1970).

13. A. F. Thomas and G. Pilton

Helv. Chim. Acta, **54**, 1890 (1971).

14. A. F. Thomas and R. Dubini

Helv. Chim. Acta, **57**, 2076 (1974).

15. A. F. Thomas and M. Ozainne

Helv. Chim. Acta, **57**, 2062 (1974).

16. A. F. Thomas and R. Dubini

Helv. Chim. Acta, **57**, 2081 (1974).

17. A. F. Thomas and R. Dubini

Helv. Chim. Acta, **57**, 2066 (1974).

18. D. Lamparsky and I. Klimes

Top Flavour Res. Proc. Int. Conf. 281 (1985).

19. A. Akhila, P.K. Sharma and R.S. Thakur

Tet. Lett. **27**, 5885 (1986).

20. A. Chandra, L. N. Misra and R.S. Thakur

Tet. Lett. **28**, 6377 (1987).

21. A.N. C. Cesar , M. del R. Guenca, J. Verghese, M.T. Joy, A. B. Gutbierrez and W. Herz

Phytochemistry, **29**, 2702 (1990).

22. L.N. Misra, A. Chandra and R.S. Thakur

Phytochemistry, **30**, 549 (1991).

23. S.R. Rojatkar, S.S. Pawar, P.P. Pujar, D.D. Sawaikar S. Gurunath, V.T. Sathe and B.A. Nagasampagi

Phytochemistry, **41**, 1105 (1996).

24. N.C. Baruah, R.P. Sharma K.P., Madusudanan and G.Thyagarajan

J. Org. Chem., **44**, 1831 (1979).

25. G. Appendino and P. Gariboldi

Phytochemistry, **23**, 2545 (1984).

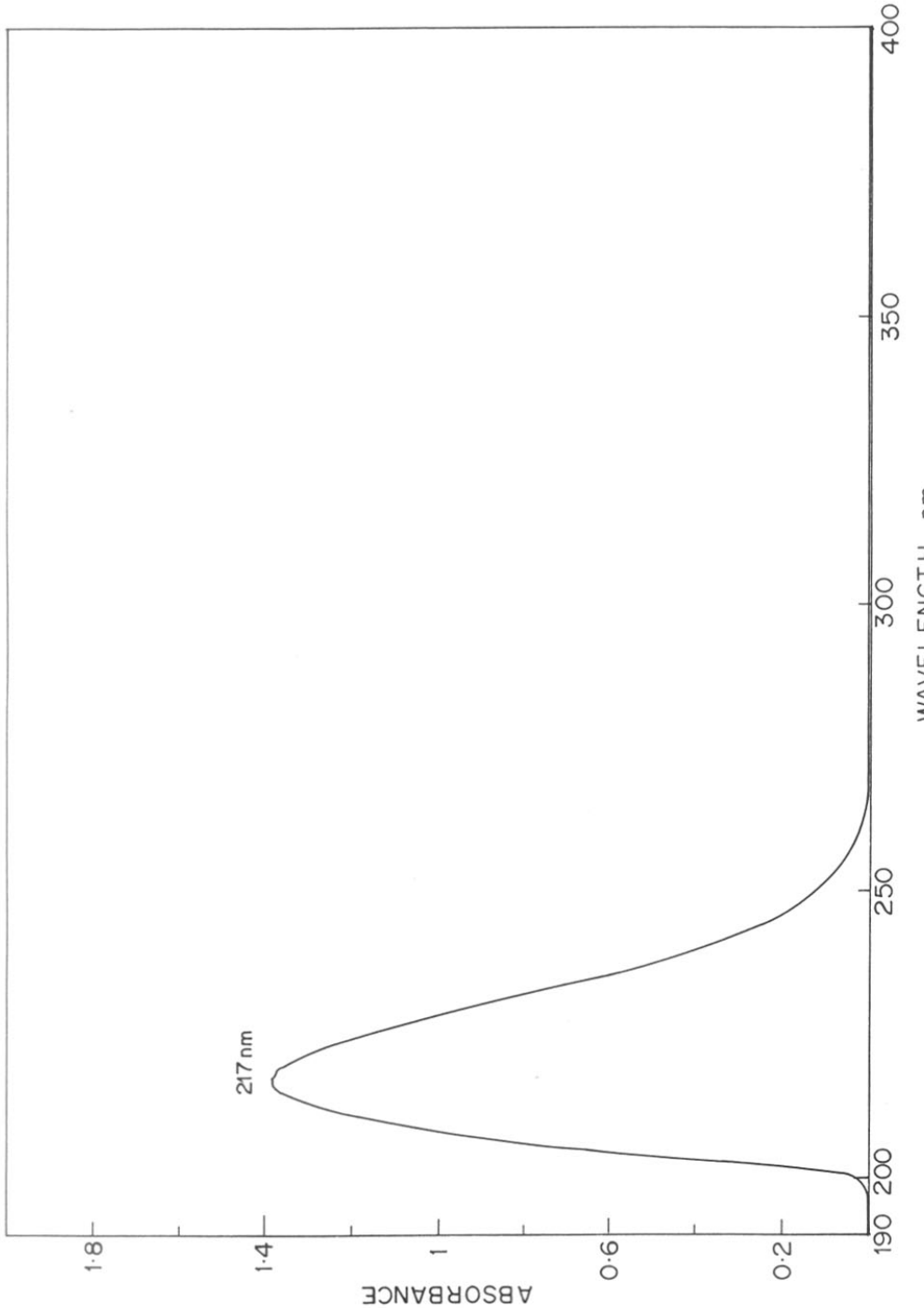


FIG 1 : UV SPECTRUM OF COMPOUND I

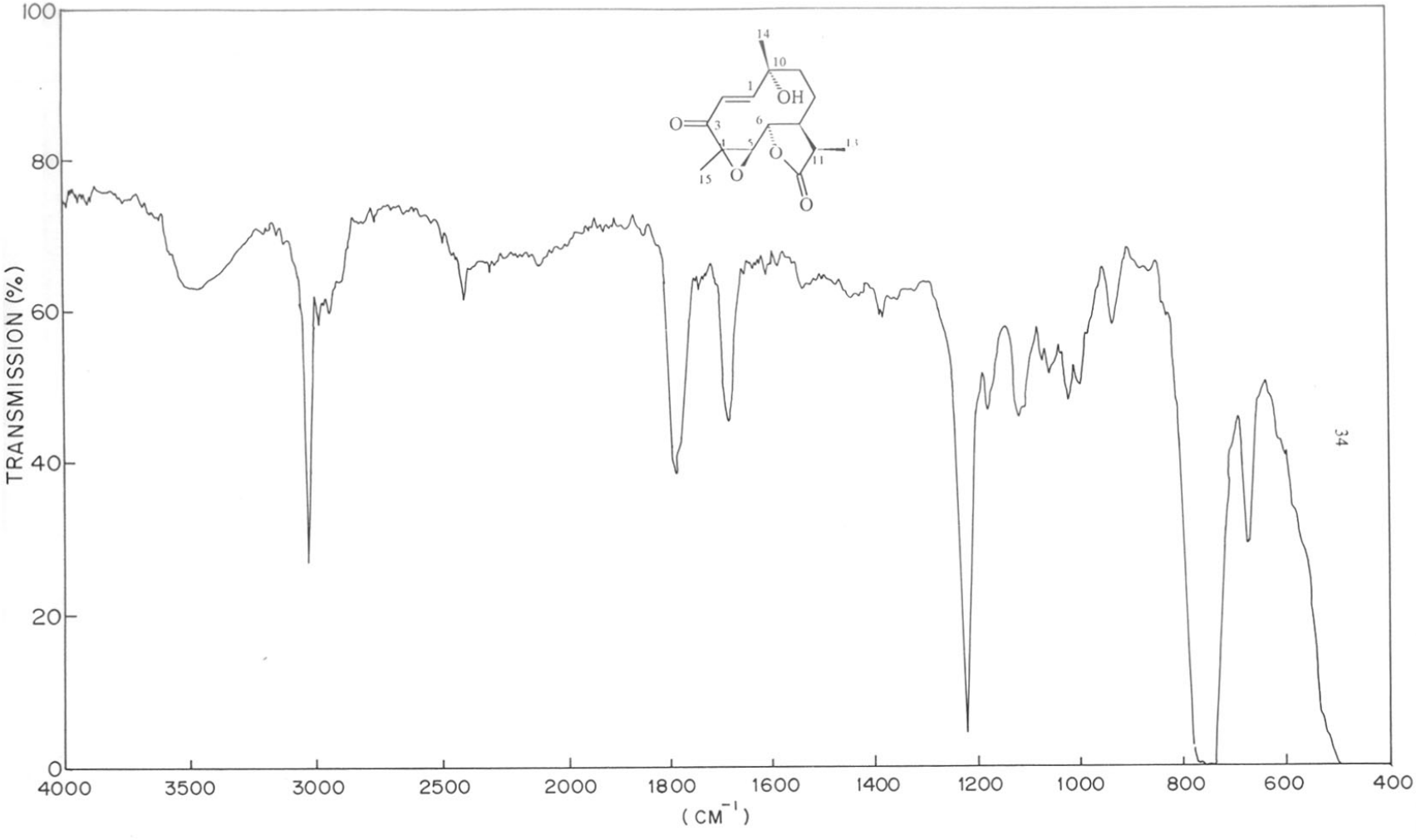


FIG 2 : IR SPECTRUM OF COMPOUND I

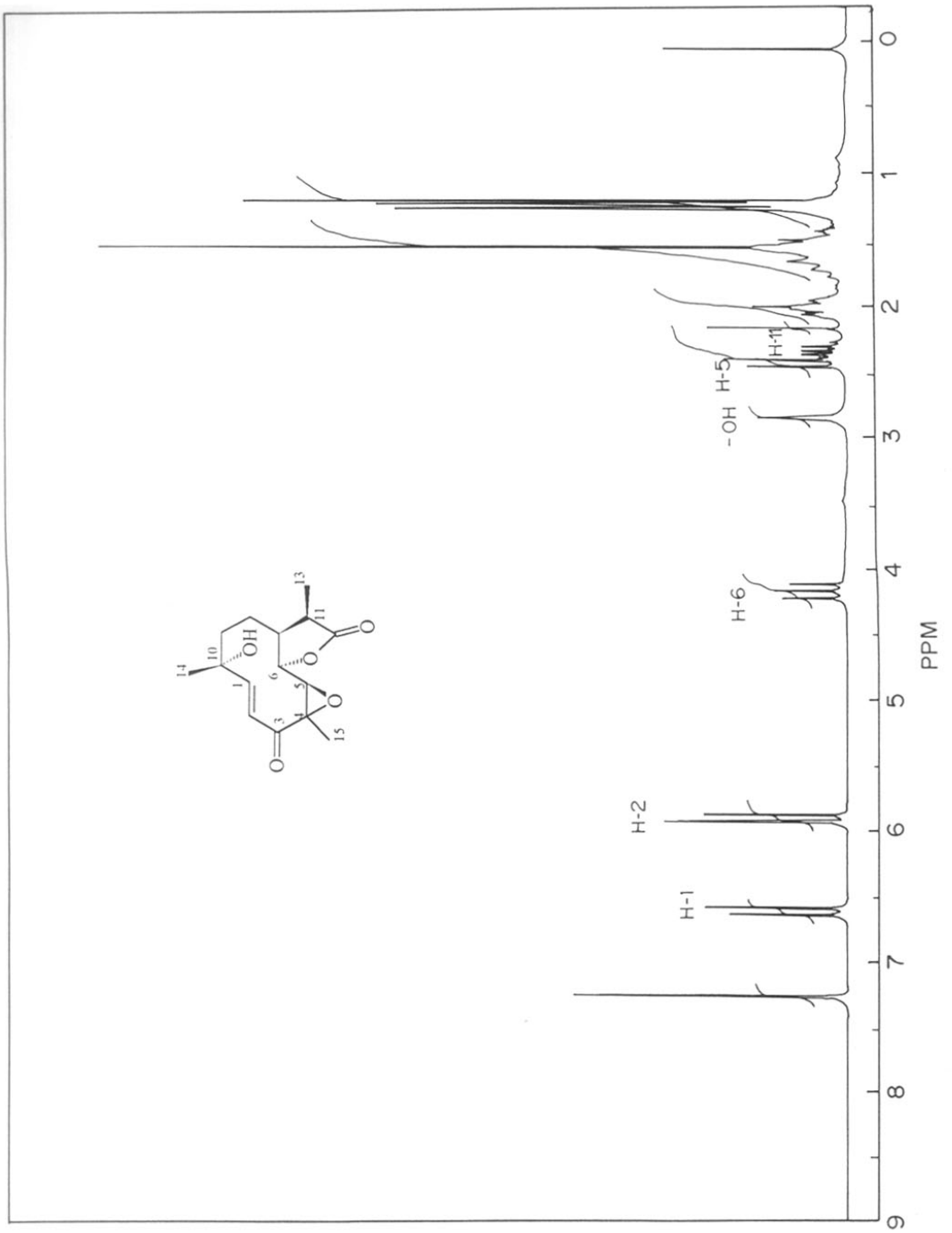


FIG 3 : ^{1}H NMR SPECTRUM OF COMPOUND I

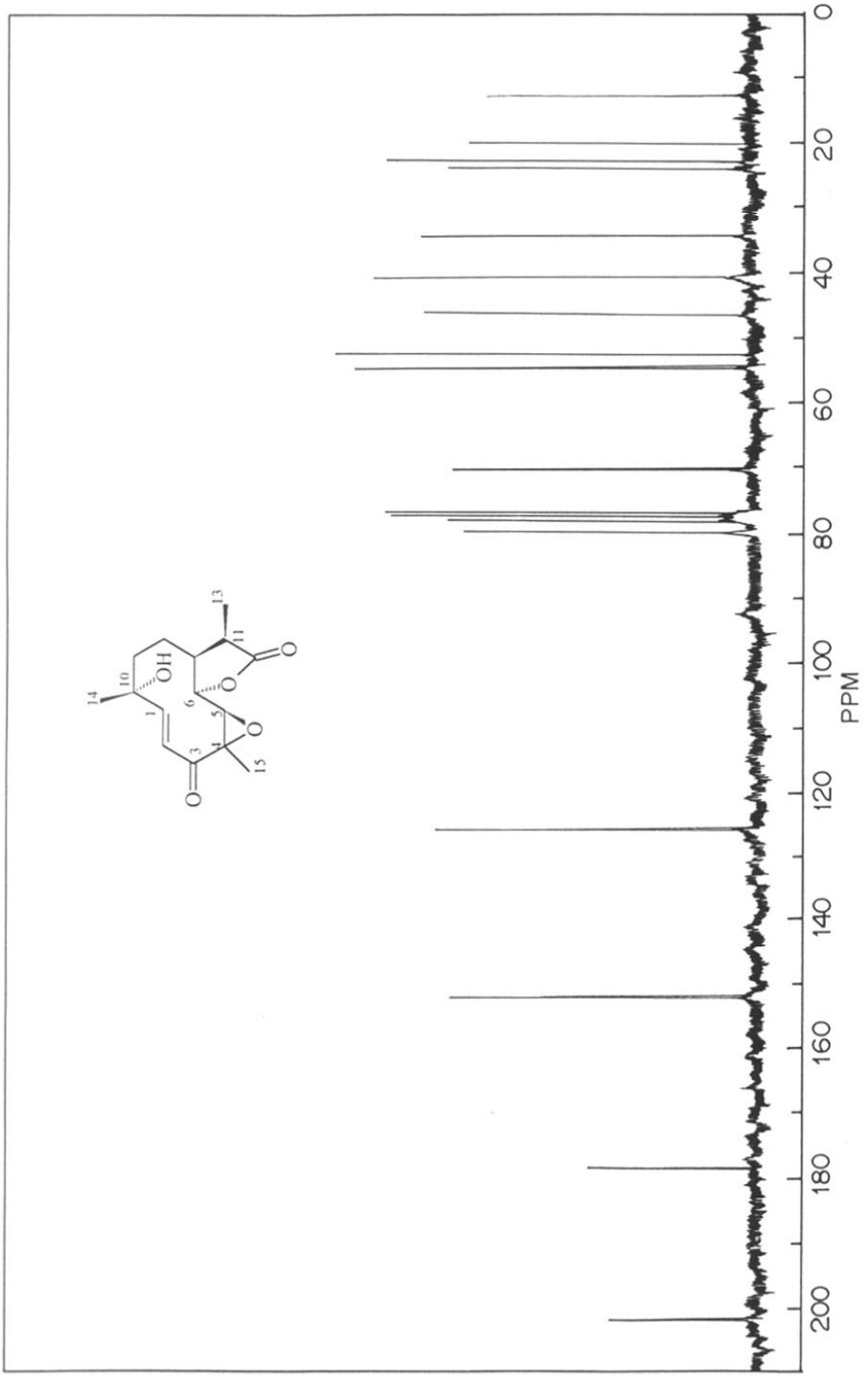


FIG 4 : ^{13}C NMR SPECTRUM OF COMPOUND I

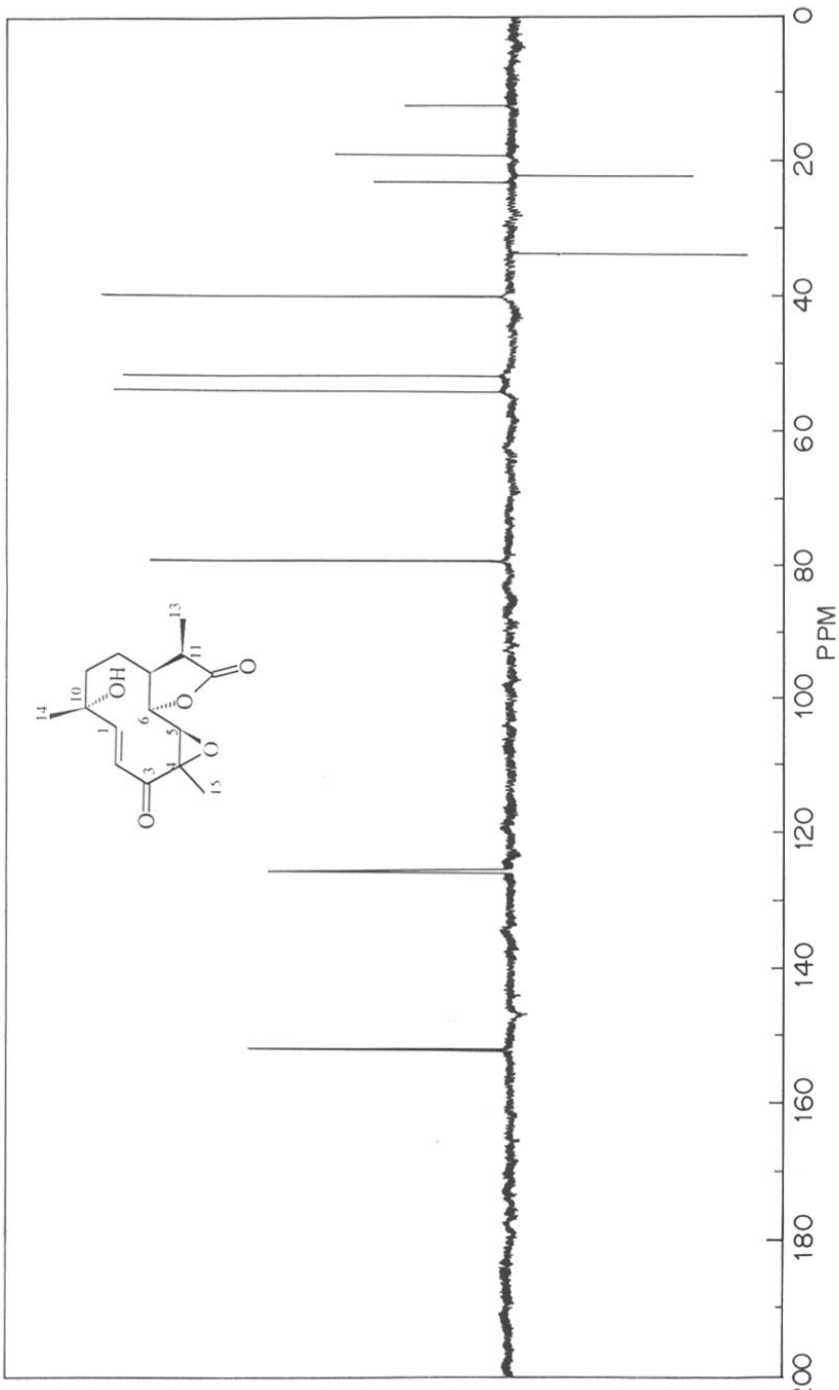


FIG 5 : ^{13}C NMR SPECTRUM (INEPT) OF COMPOUND I

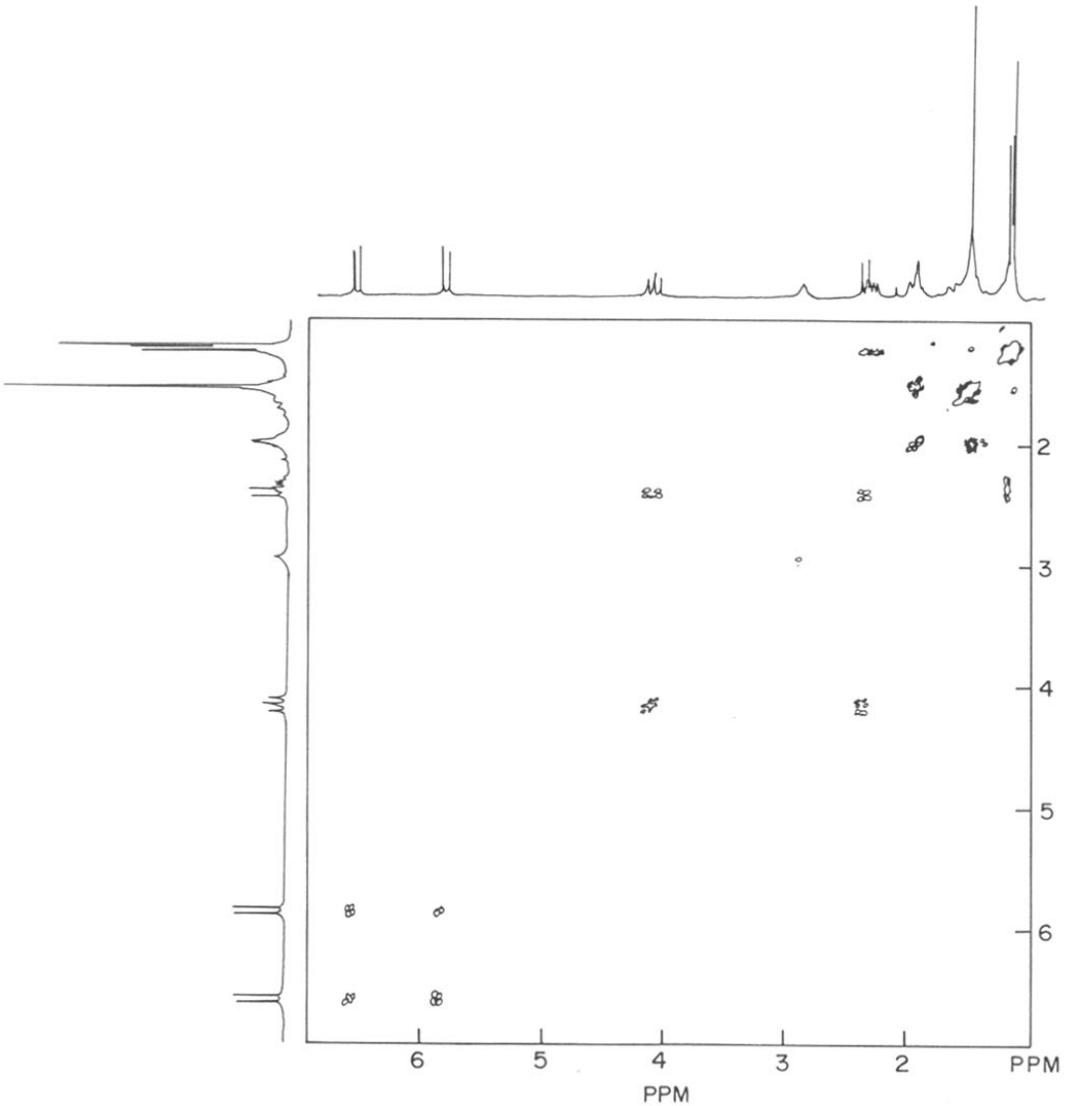


FIG 6 : $^{1}H - {}^{1}H$ 2D COSY EXPERIMENT OF COMPOUND I

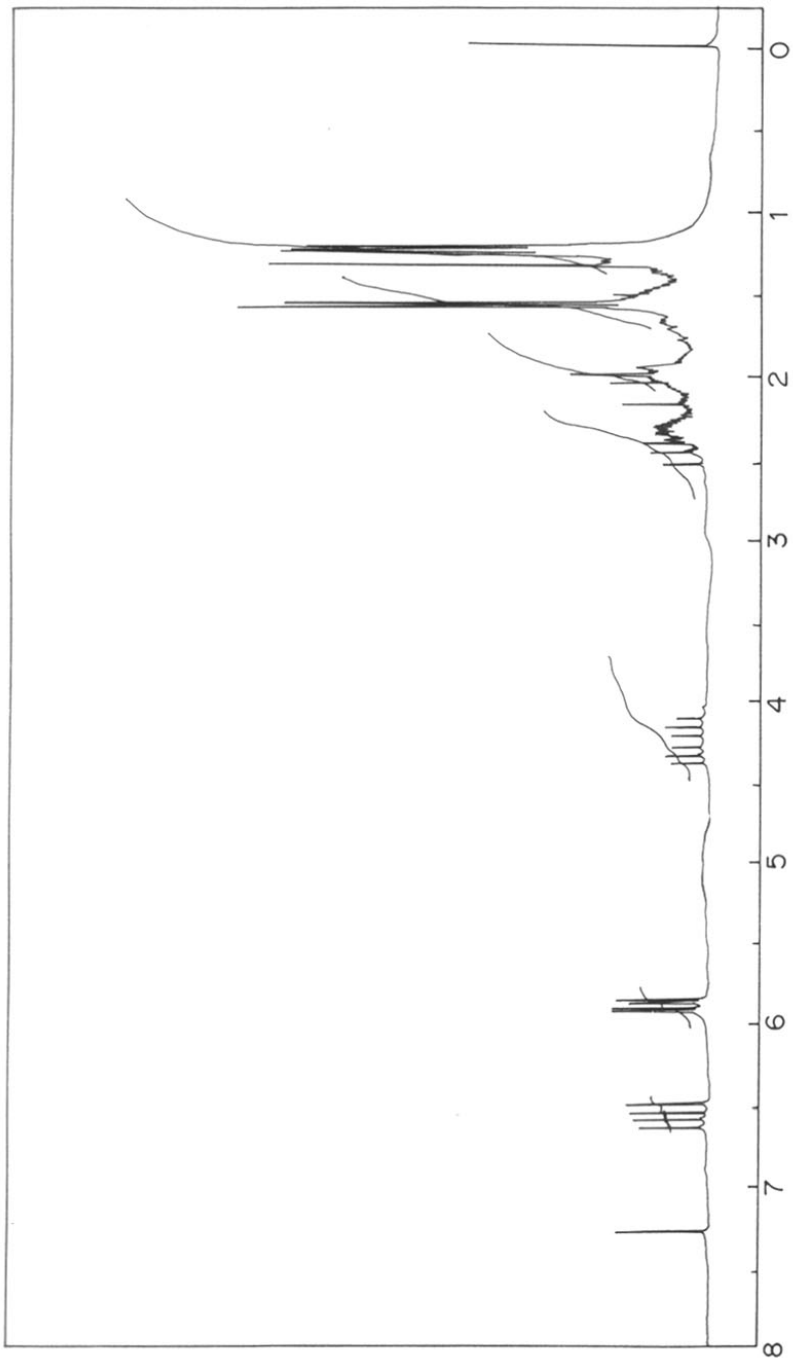


FIG 7 : ^{1}H NMR SPECTRUM OF COMPOUND I AND II

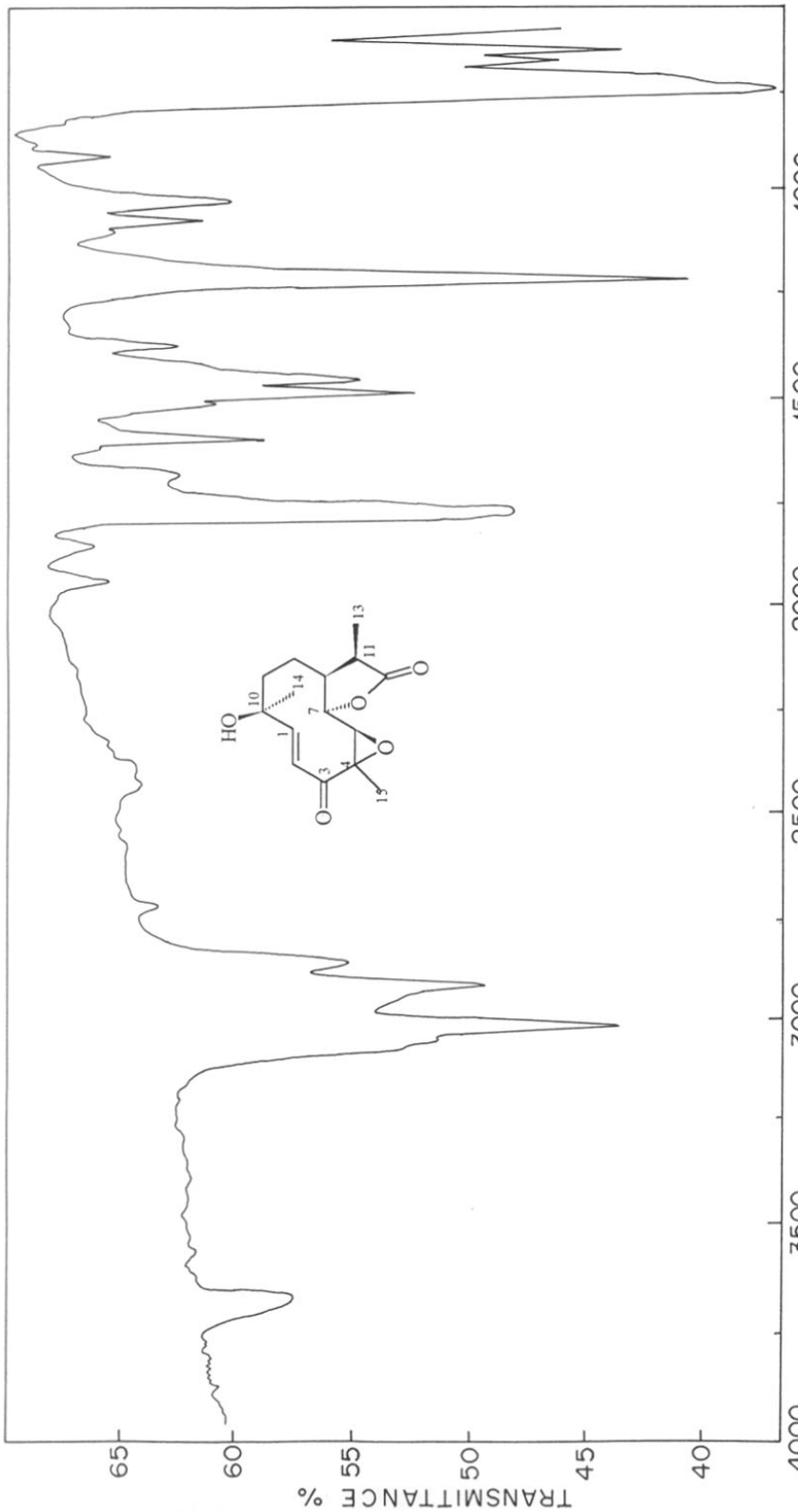


FIG 8 : FT - IR SPECTRUM OF COMPOUND II

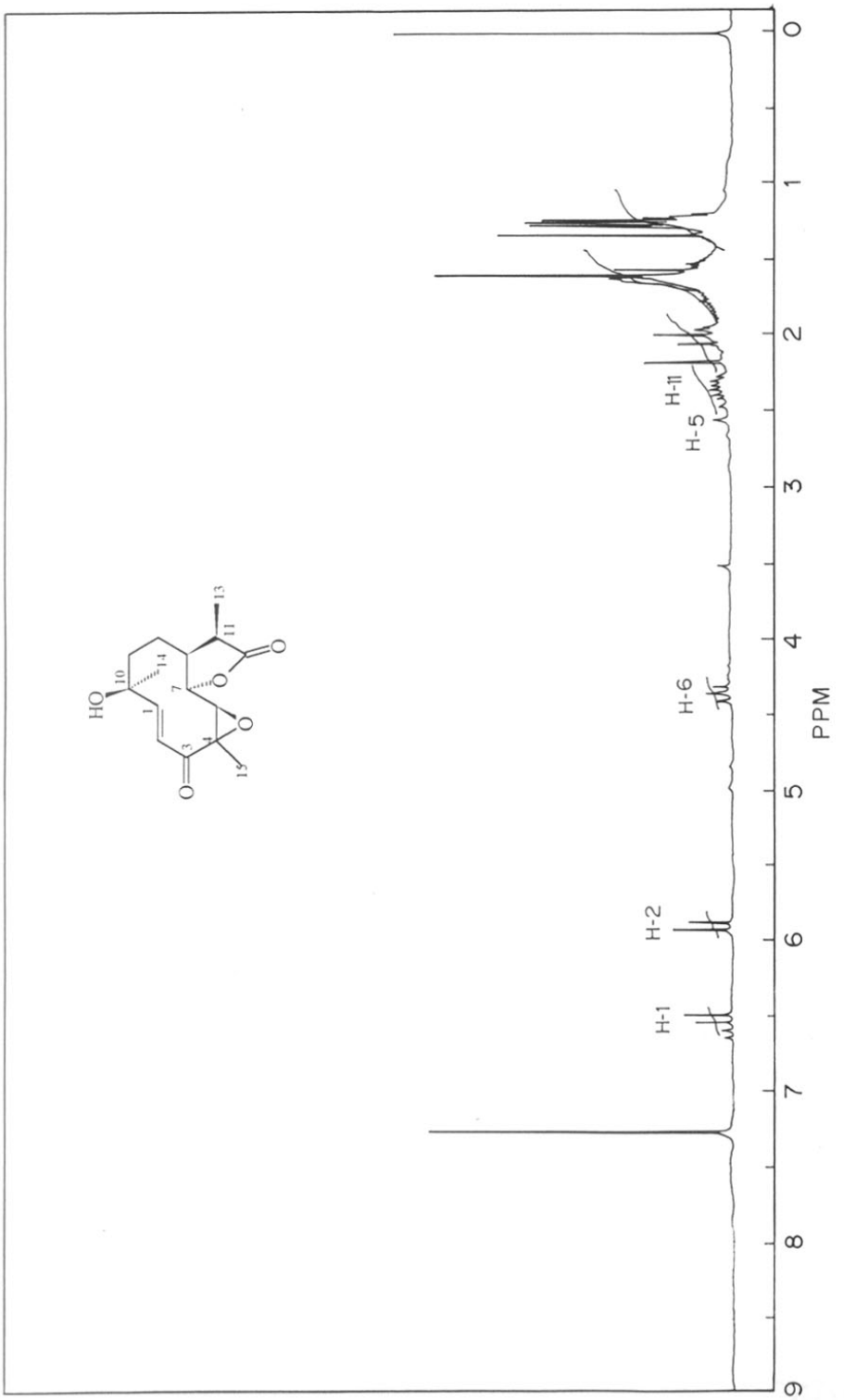


FIG 9 : ^{1}H NMR SPECTRUM OF COMPOUND II

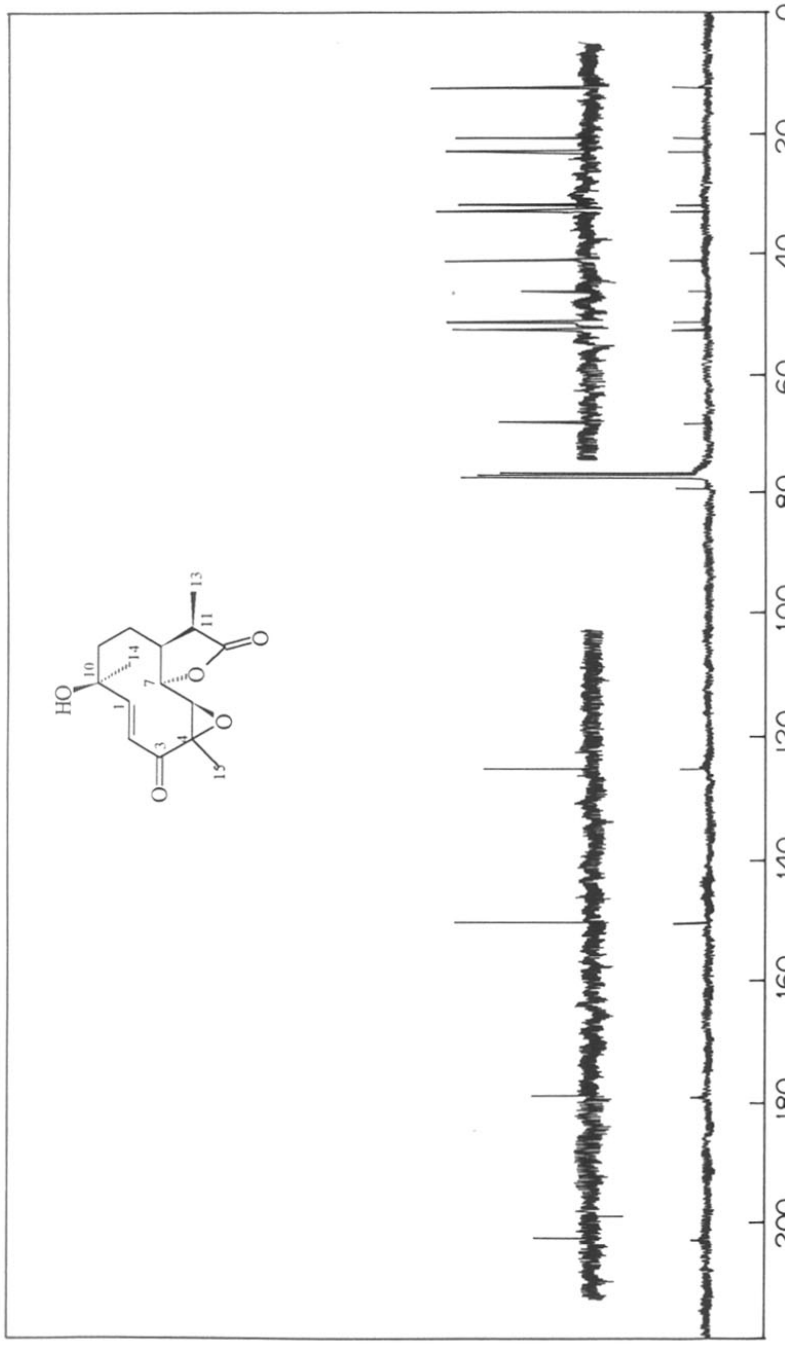


FIG 10 : ^{13}C NMR SPECTRUM OF COMPOUND II (75.48 MHz)

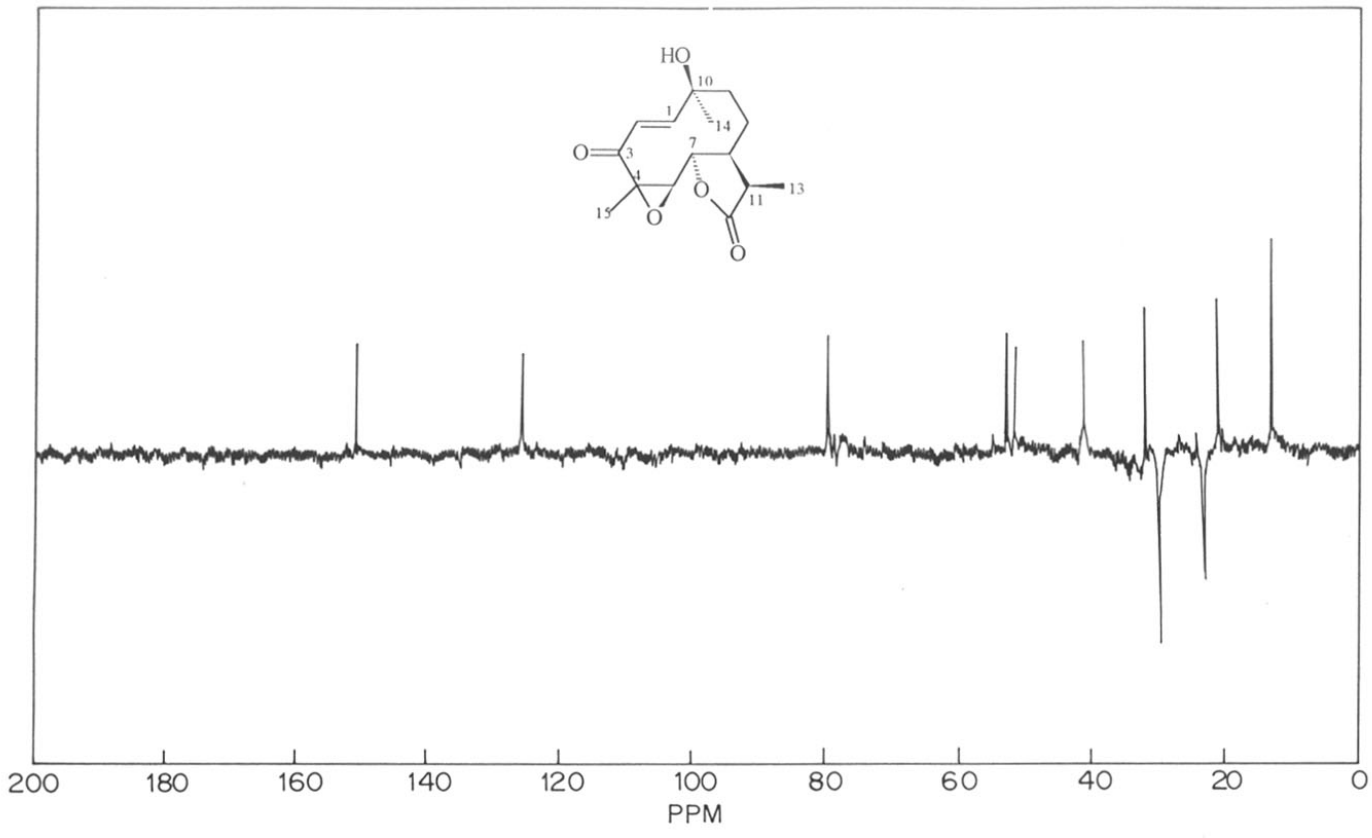


FIG 11 : ^{13}C NMR SPECTRUM (INEPT) OF COMPOUND II (75.48 MHz)

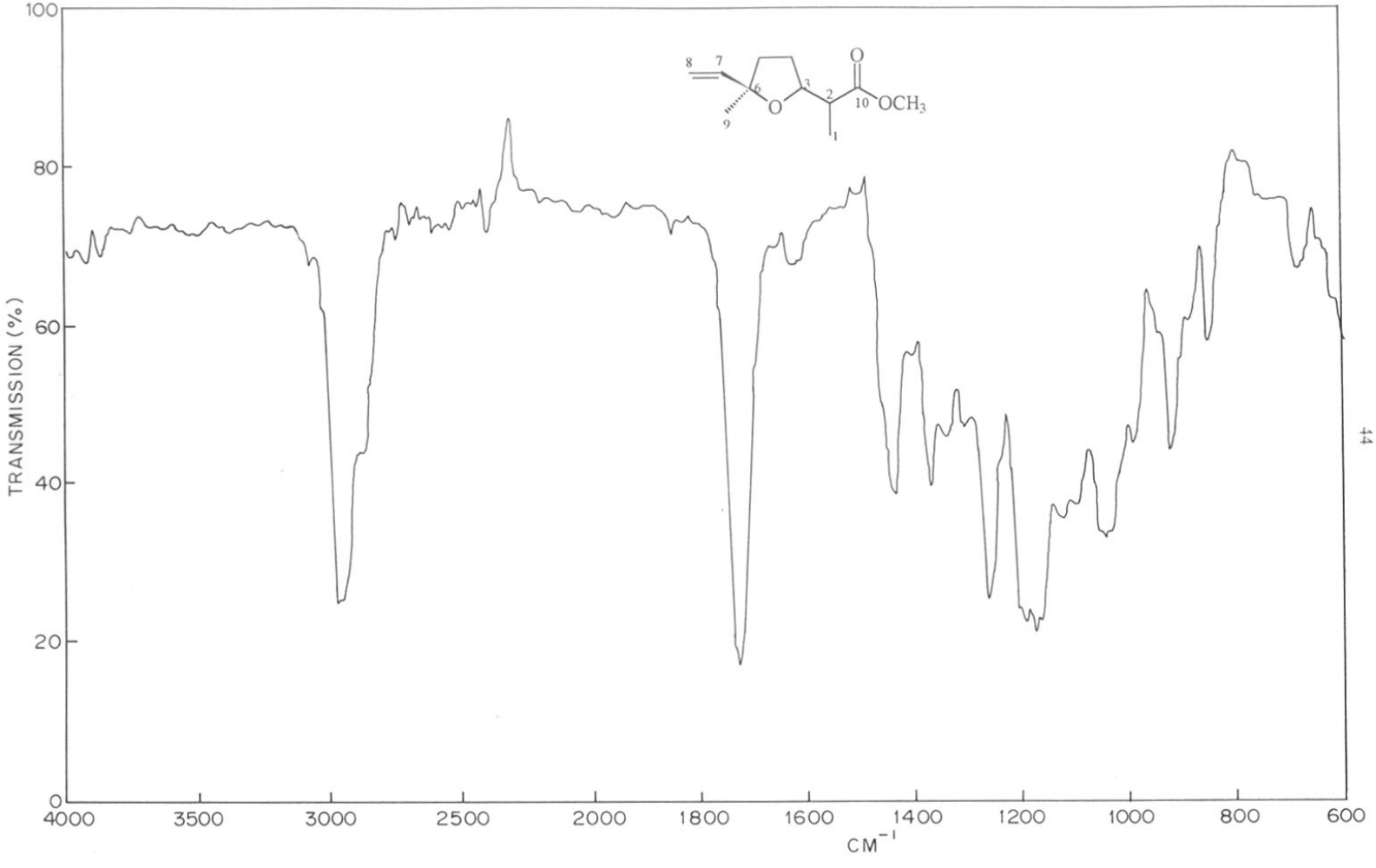


FIG 12 : IR SPECTRUM OF COMPOUND III

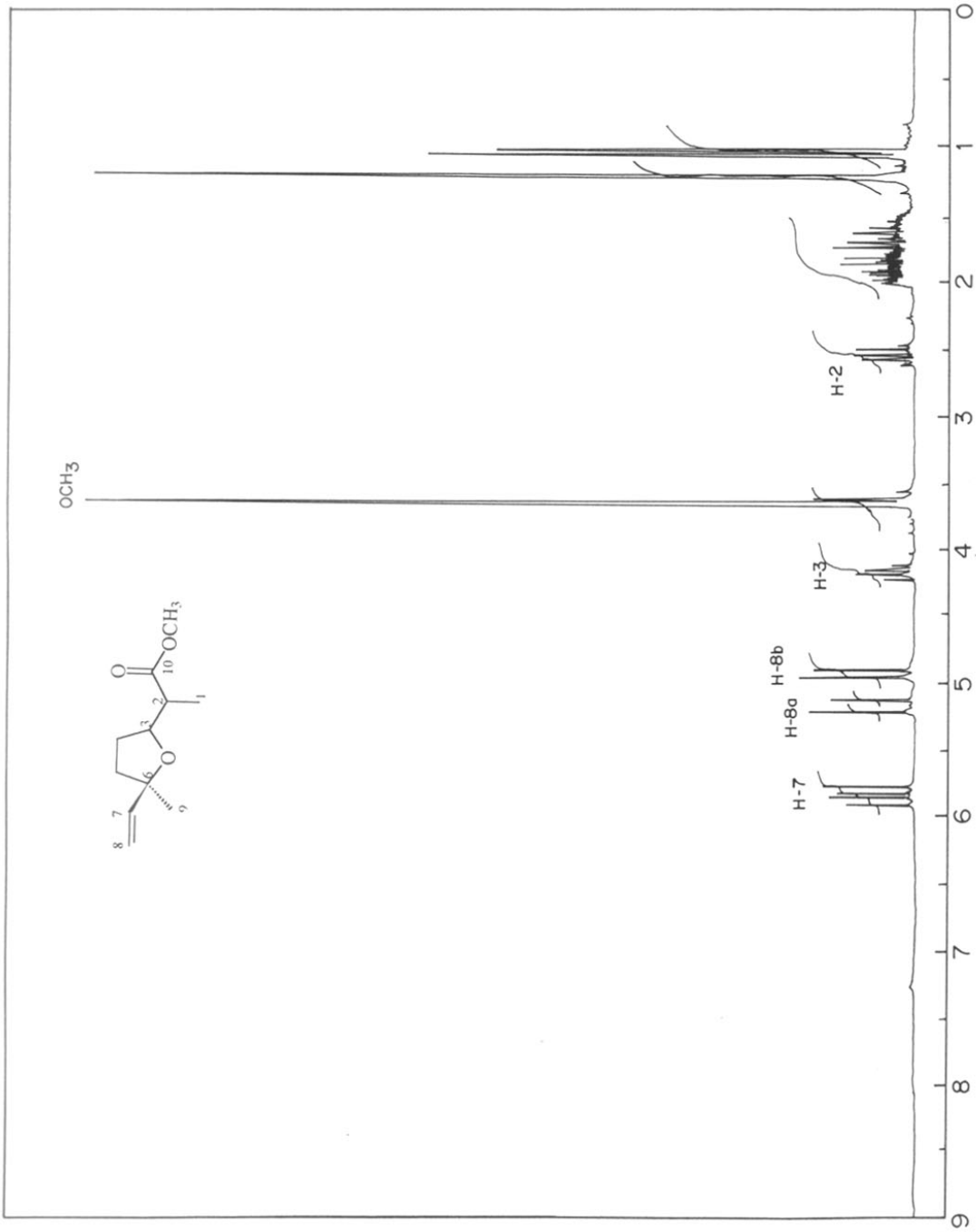


FIG 13 : ^{1}H NMR SPECTRUM OF COMPOUND III

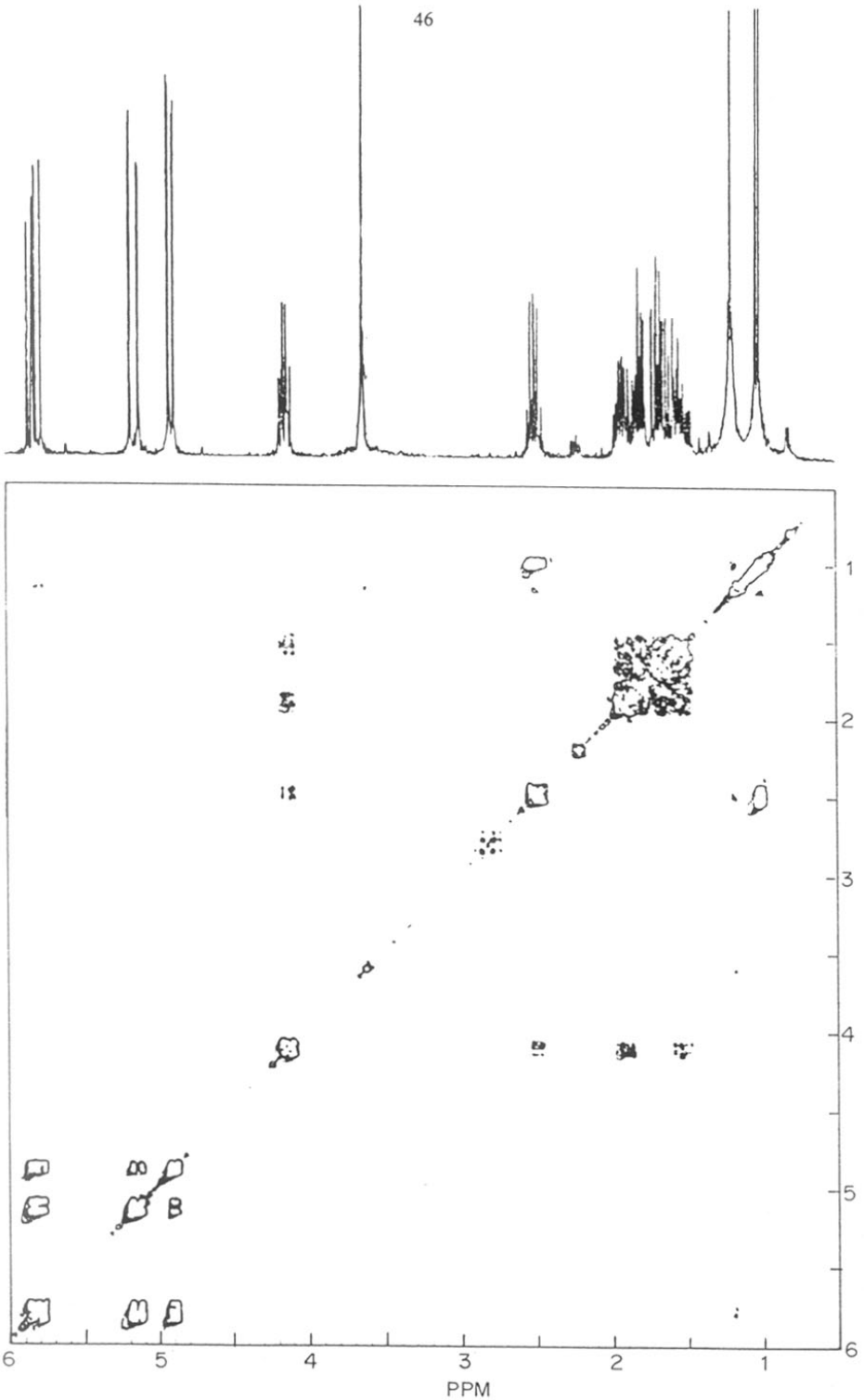


FIG 14 : $^{1}H - {}^{1}H$ 2D COSY EXPERIMENT OF COMPOUND III (300 MHz)

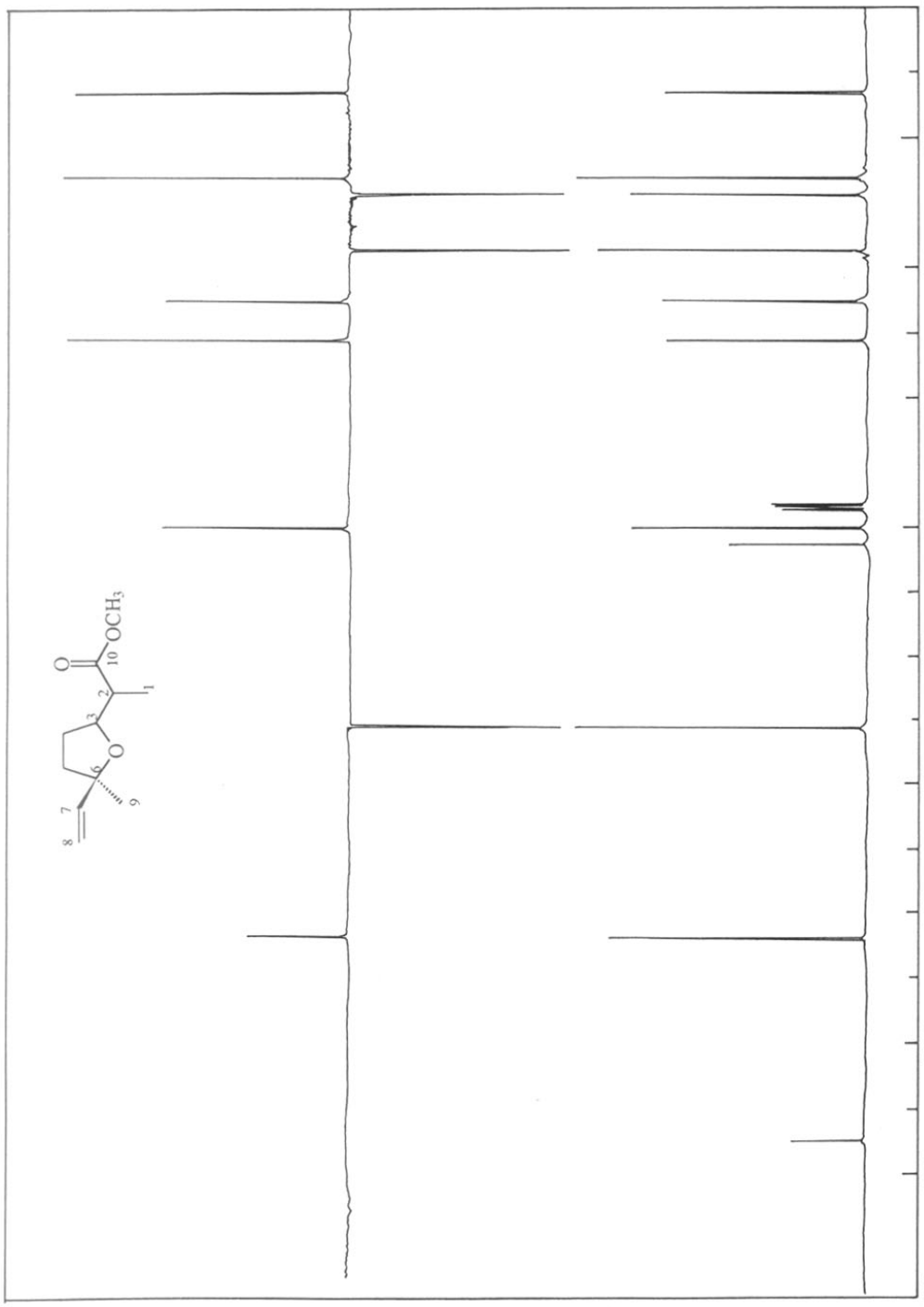


FIG 15 : ^{13}C NMR SPECTRUM AND INEPT OF COMPOUND III (75.48 MHz)

CHAPTER II

CHEMICAL INVESTIGATION

OF

Sphaeranthus indicus

Introduction

The genus *Sphaeranthus* belongs to the well-defined tribe Inulae of family Asteracea. The tribe comprises 180-200 genera with some 2100 species having cosmopolitan distribution. Inulae includes a selection of plants, which have been prized by man for their useful properties. Four of the best known, which were used medically in the past are (i) *Inula helenium* (for treating chest disease) (ii) *Pulicaria dysenterica* (in herbal remedies) (iii) *Antennaria dioeca* (for throat infections) and (iv) *Sphaeranthus indicus* (folk medicine). The genus *Sphaeranthus* is distributed in tropical Asia, Africa and Australia. In India six species of *Sphraeranthus* are reported to occur. *Sphraeranthus indicus* Linn is an aromatic herb, 30-60 cm tall, found abundantly in damp situations in the plains all over India, ascending to an altitude of 1500 metres in hills, especially as a weed in the rice fields, stem with toothed wings; leaves obovate-oblong; serrate flowers in heads are purple. The juice of the plant is used in liver disorders, the paste of the herb, made with oil is applied in chest pains, cough and bowel complaints. The bark ground and mixed with whey is said to be a useful as an application in piles. Flowers are credited with alternative, depurative and tonic properties. Leaf juice boiled with milk and sugar-candy is prescribed for cough. Anti tubercular properties have also been ascribed to the plant. The leaves of the plant are mixed with paddy and rice to prevent damage by pests during storage[1].

Previous work

The oil and hexane extract of *S. indicus* have been chemically investigated to isolate volatile compounds[2,3], fatty acids[4], alkaloids[4,5], sterols[6], glucosides of sterols[7], and some aromatic compounds[8]. The most interesting finding of the chemical investigation of this plant was the isolation of 7-hydroxyeudesmanolides, which are rare in nature. For the first time three 7-hydroxyeudesmanolides **(1, 2, 3)** were isolated from *S. indicus* by Gogte *et al*[9]. However the

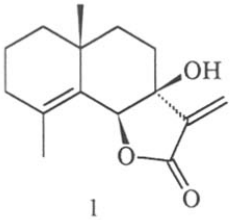
stereochemistry of the lactone ring in compound **2** was not rigorously established. Sohoni *et al.*[10] of this laboratory isolated the same 7-hydroxyeudesmanolide (**4**) and established its stereochemistry by X-ray crystallography. They also isolated β-eudesmol (**5**), 2-hydroxycostic acid (**6**) and ilicic acid (**7**). Later on Atta-ur-Rehman *et al.* isolated compound **4** and reported it to have antimicrobial activity[11]. Shekhani *et. al.*[12] have reported the isolation of three 7-hydroxyeudesmanolides (**8, 9, 10**) from this plant. A 3-O-glucoside of 7-hydroxyeudesmanolide (**11**) has been reported by Shekhani *et al.*[13] Three more 7-hydroxyeudesmanolides (**12, 13, 14**) along with cryptomeridiol (**15**) and epi-cryptomeridiol (**16**) have been reported by Rojatkar *et al.* of this laboratory[14,15] (Chart I).

7-Hydroxysesquiterpenelactones

Besides 7-hydroxyeudesmanolides isolated from *S. indicus* several other 7-hydroxysesquiterpene lactones have also been isolated from different plants.

The striking features of these lactones in the ^{1}H NMR spectra are the appearance of two singlets due to exomethylene protons (H-13a, H-13b) instead of two doublets. As expected the characteristic signals due to H-7 is absent. Another feature in ^{1}H NMR spectra is the appearance H-6 as doublet in case of 7-hydroxygermacranolides and as singlet in case of 7-hydroxyeudesmanolides and 7-hydroxyguainolides where C-5 is tetrasubstituted. In 7-hydroxysesquiterpene lactones having C-11 methyl H-11 appears as quartet instead of multiplet. The striking feature in the ^{13}C NMR spectra of 7-hydroxysesquiterpene lactones is appearance of C-7 as singlet at δ 70 to 80 instead of doublet at δ 40 to 50, indicating it to be an oxygenated carbon atom.

CHART I



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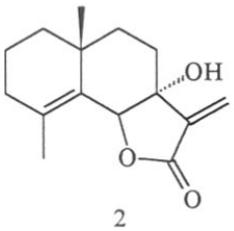

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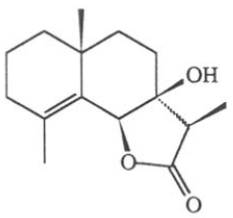

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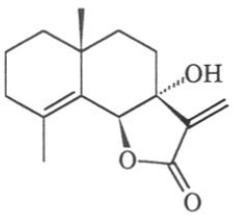

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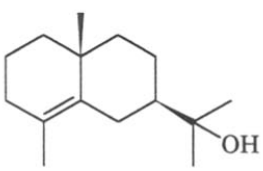

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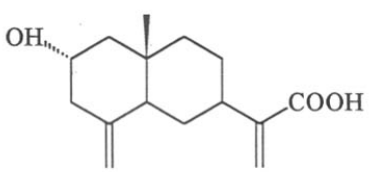

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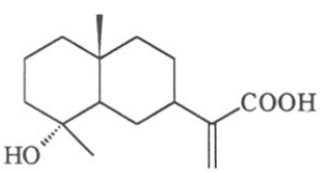


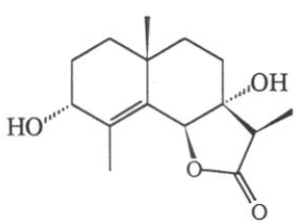
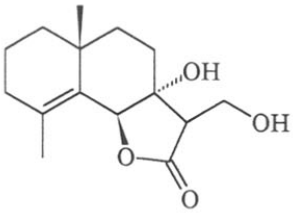


CHART I (Continued)



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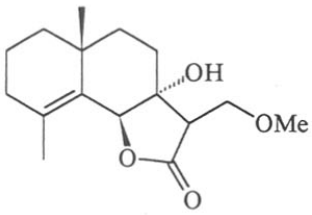

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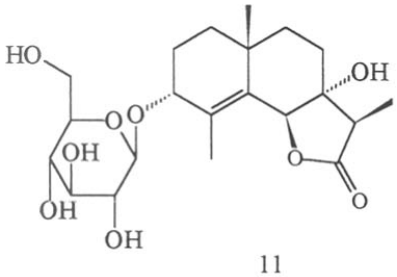

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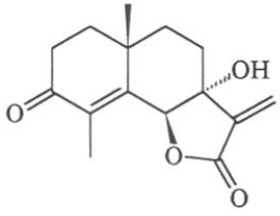

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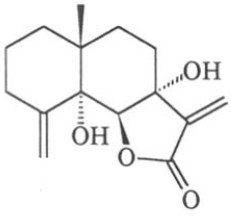

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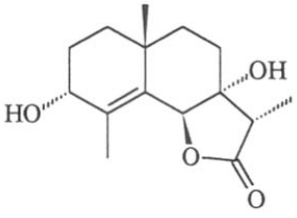

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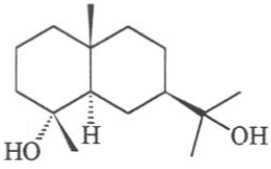

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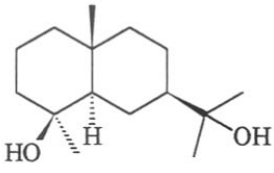


16

For the first time two 7-hydroxyguainolides (**17, 18**) have been reported from *Podochaenium emineus*[16]. 7-hydroxysesquiterpene lactones were further reported from *Montanoa* (Astereacea) and *Thapsia* (Umbelliferae) species. From the *Montanoa* species mainly 7-hydroxygermacranolides have been isolated where as 7-hydroxyguainolides have been reported from *Thapsia* species (Chart II).

From *Montanoa pteropoda* two 7-hydroxygermacranolides (**19, 20**) having *trans* (6α,7β) lactone junction have been reported[17]. From *M. artplicifolia* five more (**21, 22, 23, 24, 25**) 7-hydroxygermacranolides have been isolated[18]. The stereochemistry of lactone junction in these compounds was assigned as *cis* (6β,7β). From the ^{1}H NMR spectral data of **20** and **25** both the compounds were found to be same having *cis* lactone junction[18]. Three 7-hydroxyelmanolides (**26, 27, 28**) which were closely related to germacranolides **21, 22, 23** have also been isolated from this plant[18]. From *M. hibiscifolia* a 7-hydroxygermacranolide (**29**) has been reported[19]. Germacranolides **30, 31** have been isolated from *M. tomentosa*[20] and **32** has been reported from *M. leucantha*[21].

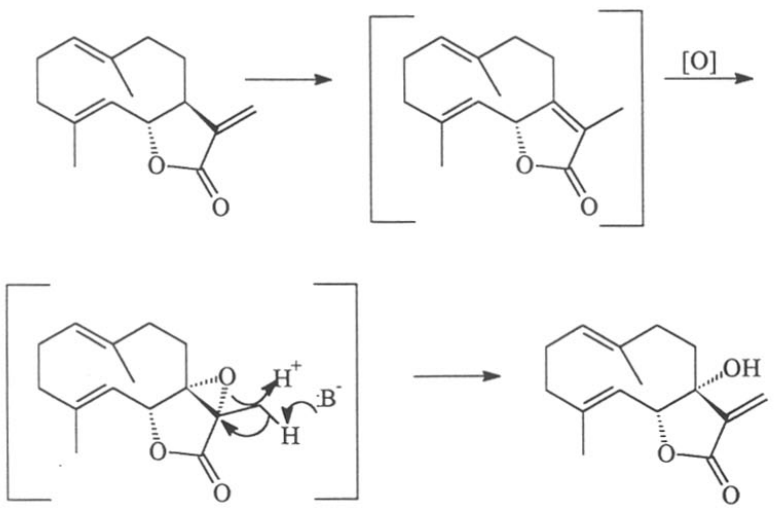
From *Thapsia* species quite a few 7-hydroxyguainolides have been reported. Guainolides (**33, 34**) have been isolated from the roots of *T. garganica* and their structures were elucidated by chemical and spectroscopic method[22] and X-ray crystallographic studies of their epoxy derivatives[23,24]. Further from the roots of *T. garganica*[25] guainolides **35, 36** have also been isolated where as guainolide **37** has been isolated from *T. villosa*[25] and **38** has been isolated from *T. maxima*[25]. The other guainolides **39-44** have also been reported from *Thapsia* species[25].

From *Decachaeta ovatofolia*[26] (Astereacae) all three types of 7- hydroxysesquiterpene lactones have been isolated. *viz.* 7-hydroxygermacranolide (**45**), a 7-hydroxyguainolide (**46**) and

two 7-hydroxyeudesmanolides (**47, 48**). A 7- hydroxyeudesmanolide (**8**) has been isolated earlier from *Grangea moderaspatana*[27] also. Two more 7-hydroxysesquiterpenelactones (**49, 50**) are reported from *Trichogonia salviaefolia*[28] and *Gongrothamnus aurantiaca*[29] respectively.

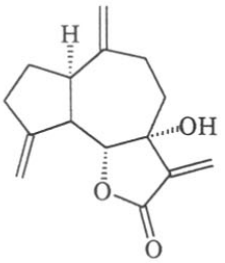
Such 7-hydroxysesquterpenlactones are reported to have high molluscicidal activity in comparison to other sesquiterpene lactones[30]. The guainolides **29, 33** have been reported to have histamine liberating activity[25].

According to T.J. Mabry *et. al.*[26] the possible biogenesis of the initial 7α-hydroxygermacranolide is as shown in scheme 1.



SCHEME 1

CHART II



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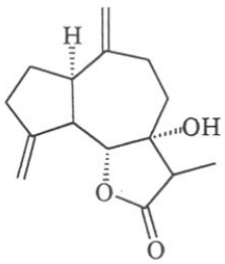

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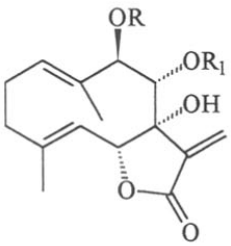
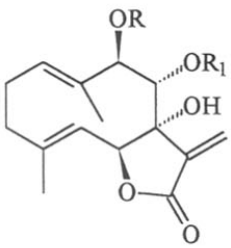
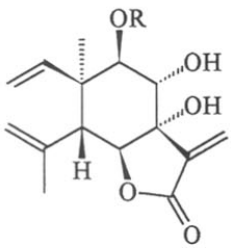


	19	20
R	Epang	Ac
R_1	Ac	Epang



	21	22	23	24	25
R	Ang	H	H	Sen	Ac
R_1	H	Ang	Sen	H	Epa



	26	27
R	Ang	Sen

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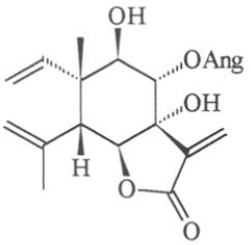

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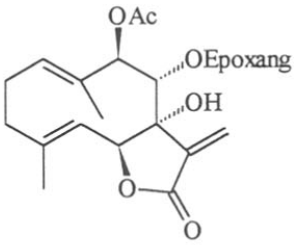

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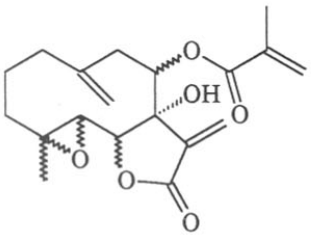

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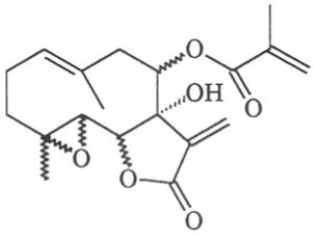

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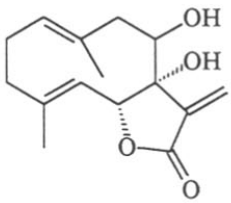

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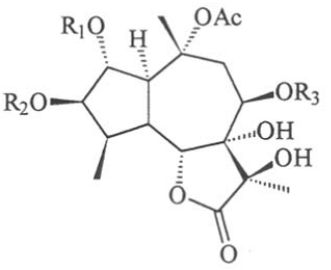
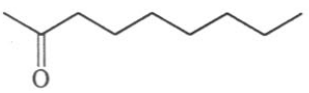
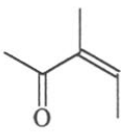
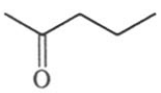
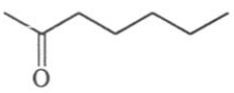
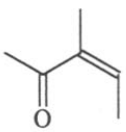
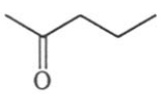
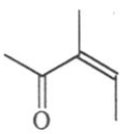
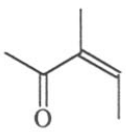
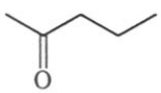
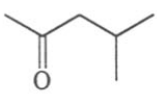
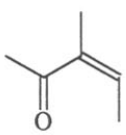
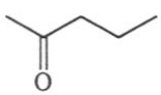
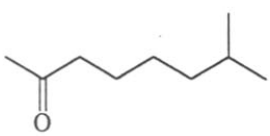
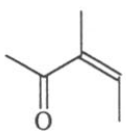
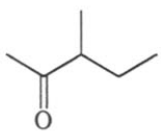
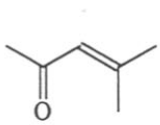
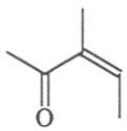


	R1	R2	R3
33			
34			
35			
36			
37			
38	$R_1R_2R_3$	2 X 	

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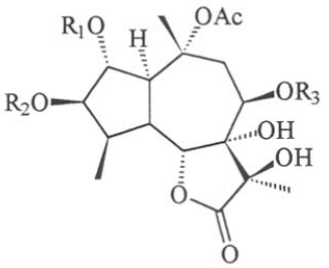
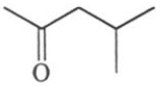
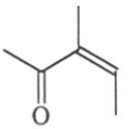
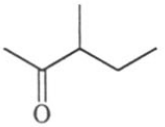
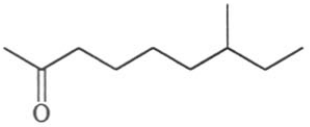
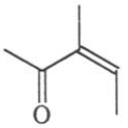
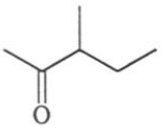
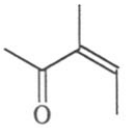
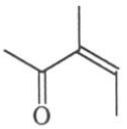
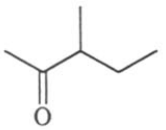
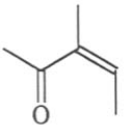
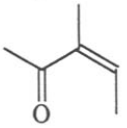
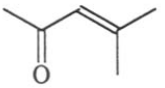
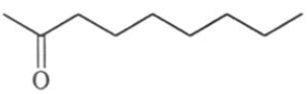
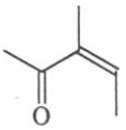
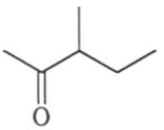
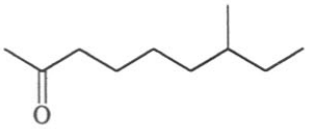
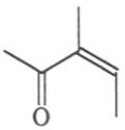
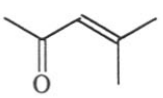


	R_1	R_2	R_3
39			
40			
41			
42			
43			
44		R_2R_3 = 	

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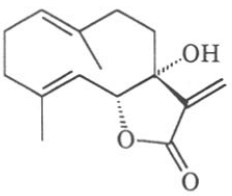

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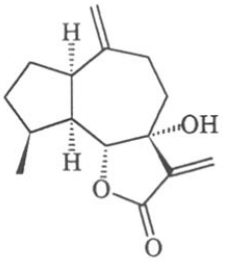

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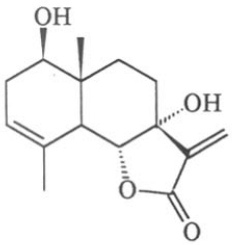

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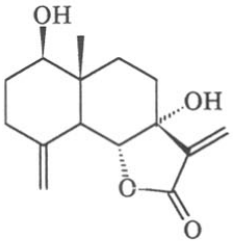

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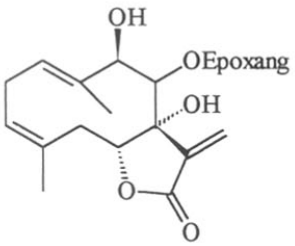

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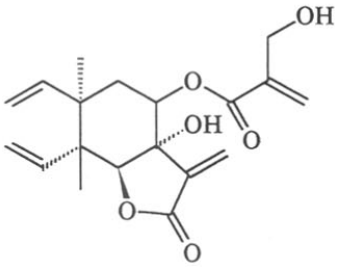


50

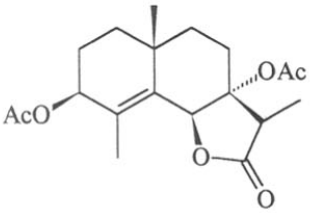
Present work

With a view to isolate biologically active compounds, chemical investigation of acetone extract of *S. indicus* was carried out. The extract was chromatographed over silica gel to afford thirteen broad fractions. Fraction 8 was acetylated at room temperature and the product was subjected to preparative TLC to yield compound **I** and a known compound **IV** isolated and characterised by earlier workers of this laboratory from the same plant[15]. Fraction 6 on further purification by column chromatography and preparative TLC afforded compound **II**, fraction 7 on further purification yielded compound **III**. All the three compounds, **I, II** and **III,** (Chart III) have been characterised by spectral analysis and correlation with known compounds and are found to be new natural products.

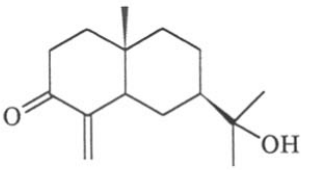
Characterisation of compound I

Compound **I**, viscous oil, showed in its mass spectrum M^+ at m/e 350 suggesting the molecular formula $C_{19}H_{26}O_6$. IR spectrum (Fig.1) of the compound showed characteristic bands at 1786 cm^{-1} (γ-lactone), 1740 cm^{-1} (ester carbonyl) and 1635 cm^{-1} (unsaturation). ^1H NMR spectrum (Fig.2, Table 1) revealed the presence of two acetate methyls at δ 2.2 and δ 2.3, an angular methyl at δ 1.1, a vinyl methyl at δ 1.75 and a secondary methyl at δ1.25 (d, J = 7 Hz). This spectral data indicated that the compound under discussion was a saturated sesquiterpene-γ-lactone having two acetate groups. The ^1H NMR spectrum further showed a quartet at δ 3.1 (J = 7 Hz) accounting for one proton which has to be H-11. This clearly indicated that C-7 is tetra substituted. From the molecular formula and presence of several 7-hydroxy eudesmanolides in this plant, it was clear that compound **I** was also an eudesmanolide having an acetoxy group at C-7 since there was no hydroxyl group in this compound as indicated by IR spectrum. ^1H NMR

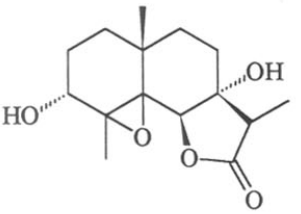
CHART III



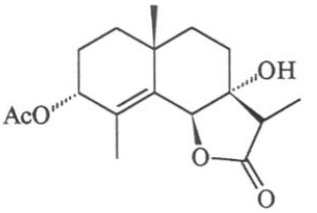
I



II



III



IV

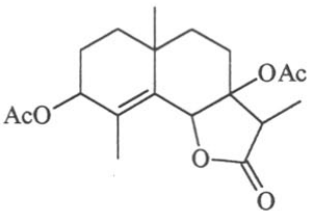
Table 1: ^{1}H NMR spectral data of compound **I** (200 MHz) and compound **IV** (90 MHz)

Chemical shift (δ), multiplicity and coupling constant (Hz)

Proton	Compound **I**			Compound **IV**		
H - 3	5.4	t	8.5	5.2	brd	5
H-6	5.1	s		4.93	s	
H-11	3.1	q	7	2.82	q	7
H-13	1.25	d	7	1.22	d	7
H-14	1.1	s		1.05	s	
H -15	1.75	s		1.82	s	
OAc	2.2	s		2.08	s	
OAc	2.3	s				

spectrum of the compound further showed a singlet accounting for one proton at δ 5.1, which clearly indicated that it was due to H-6 and hence C-5 would be tetra substituted. Thus the double bond could be placed at C-4/C-5 position, which was confirmed by the appearance of vinyl methyl at δ 1.75 in the ^{1}H NMR spectrum.

The spectrum further showed a down field triplet (J = 8.5 Hz) accounting for one proton at δ 5.4 clearly suggesting it to be due to the proton on the carbon bearing an acetoxy group. The chemical shift and multiplicity of this proton revealed it to be allylic. Thus the acetoxy group was placed at C-3. Thus the structure of the compound **I** was elucidated as 3,7-diacetoxy eudesmanolide.

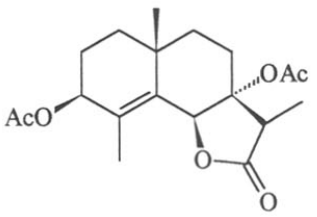


Biogenetically C-7/C-11 bond is β-oriented hence acetoxy group at C-7 would be α-oriented as in all 7-hydroxy compounds isolated till today. Further by comparing coupling constants of H-11 and H-13 of compound **I** and those of the compounds isolated from *S. indicus* configuration of C-11 methyl was deduced as β. Lactone junction in compound **I** was decided to be *cis* as assigned in compounds isolated from this plant.

As stated earlier, compound **IV** has been isolated from same fraction which has an acetoxy group at C-3 as α-oriented and fully characterised as 3α-acetoxy-7α-hydroxy-4-en-eudesmanolide (IR : Fig 3, ^{1}H NMR : Fig. 4, Table 1). Comparison of ^{1}H NMR spectra of **I** and **IV** showed striking similarities except for the chemical shift of H-3. The coupling constant and

multiplicity of H-3 in both the compounds (**I** and **IV**) were different. Hence acetoxy group at C-3 in compound **I** must be β-oriented. Further compound **I** showed two acetate groups. As compound **I** and **IV** have been isolated from the same acetylated fraction and compound **IV** has hydroxy group at C-7, the acetoxy group at C-7 must be present in the parent compound of compound **I**. Further to confirm this, acetylation of major compound **4**, having 7-hydroxy group, was carried out under same conditions. However, acetylation did not take place.

From above evidence and spectral data the structure of compound **I** was elucidated as 3β,7α-diacetoxy-4-en-6β,7-eudesmanolide and the structure of parent compound was 7α-acetoxy-3β-hydroxy-4-en-6β,7-eudesmanolide. Though there are reports of 7-hydroxy sesquiterpenoids as mentioned in prior art, this is the first report of any 7-acetoxy sesquiterpenoid.



I

Characterisation of Compound II

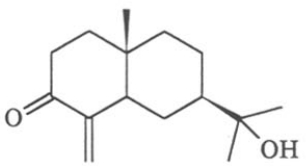
Compound **II** was obtained as a sticky mass. It showed in its mass spectrum M^+ at m/e 236 suggesting the molecular formula $C_{15}H_{24}O_2$. Its IR spectrum (Fig. 5) showed characteristic bands at 3420 cm^{-1}, 1690 cm^{-1} and 1625 cm^{-1} revealing the presence of hydroxyl group, conjugated carbonyl group and unsaturation respectively. ^1H NMR spectrum (Fig. 6, Table 2) showed three methyl singlets at δ 0.9, 1.10 and 1.22. The latter two due to their downfield shifts

Table 2: ^{1}H NMR spectral data of compound **II** (200 MHz) and β-eudesmol (90 MHz)

Proton	Chemical shift (δ)		Multiplicity
	Compound II	β-Eudesmol	
H-12	1.1	1.17	s
H-13	1.22	1.24	s
H-14	0.9	0.86	s
H-15a	5.6	4.78	s
H-15b	6.2	4.94	s

indicated that they have to be on a carbon bearing oxygen function. The ^{1}H NMR spectrum further showed two broad singlets at δ 5.6 and δ 6.2 accounting for one proton each revealing the presence of an exomethylene which was also shown by its IR spectrum (1625 cm^{-1}).

Comparison of ^{1}H NMR spectral data of compound **II** with that of β-eudesmol showed it to be similar except the chemical shift of olefinic protons. The chemical shifts of olefinic protons of compound **II** were located at down field as compared with those of β-eudesmol (Table 2) obviously due to the presence of a carbonyl group in the α-position to form a conjugated carbonyl system. Thus from the molecular formula and these spectral facts the structure of compound **II** was deduced as 3-keto-β-eudesmol.



II

Characterisation of compound III

Compound **III**, an amorphous solid (13 mg), showed in its mass spectrum molecular ion peak at m/e 282 suggesting the molecular formula $C_{15}H_{24}O_5$. Its IR spectrum (Fig.7) showed characteristic absorbance at 3420 cm^{-1} and 1780 cm^{-1} for hydroxyl group and γ-lactone respectively. ^{1}H NMR spectrum (Fig. 8, Table 3) of compound **III** showed three methyl signals at δ 0.95 (s), 1.25 (d, J = 7.5 Hz) and 1.55 (s). The down field chemical shift δ 1.55 could be due to a methyl on oxygenated carbon atom. These spectral data indicated that the compound **III** was a sesquiterpene-γ-lactone.

Table 3 : ^{1}H NMR spectral data of compound **III**

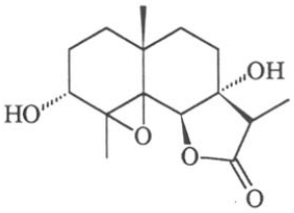
Proton	Chemical shift in δ	Multiplicity	Coupling constant in Hz
H - 3	3.3	brs	
H - 6	4.2	s	
H - 11	2.8	q	7.5
H - 13	1.25	d	7.5
H - 14	0.95	s	
H - 15	1.55	s	
3-OH	2.05	br	
7-OH	3.7	s	

^{1}H NMR spectrum did not show the characteristic two doublets of H-13a and H-13b of an α,β-unsaturated sesquiterpene-γ-lactone. However it showed the secondary methyl (H-13) at δ 1.25 and H-11 at δ 2.8 as quartet (J = 7.5 Hz) which further revealed that C-7 is tetra substituted. ^{1}H NMR spectrum showed only one down field proton at δ 4.2 as a singlet which has to be due to lactone proton at C-6, which further confirmed that C-7 as well as C-5 were tetra substituted. As there was no functional group other than hydroxyl group as revealed by IR spectrum, the C-7 had to be substituted by hydroxyl group.

Out of two methyl singlets appeared in ^{1}H NMR spectrum, the one at δ 0.95 has to be angular methyl and the other at δ 1.55 has to be on oxygenated carbon atom. Thus from the tetrasubstituted nature of C-5, it was clear that oxygenated carbon atom must be C-4 and an epoxide ring is likely to be present at C-4/C-5 position, and the angular methyl positioned at C-10. From the molecular formula and two protons exchangeable by D_2O in the ^{1}H NMR spectrum, structure to compound **III** was deduced as 7-hydroxyeudesmanolide having one more hydroxyl group.

The ^{1}H NMR spectrum showed a broad singlet at δ 3.3 accounting for one proton which has to be due to a proton on oxygenated carbon atom C-3, since it is biogenetically more favourable position which was also supported by the presence of hydroxyl group at C-3 in other 7-hydroxyeudesmanolides isolated from this plant. Stereochemistry of this hydroxyl group was assigned as α, by the comparison of multiplicity of H-3 of **III** with those of compounds **I**, **IV**, **8** and **14**.

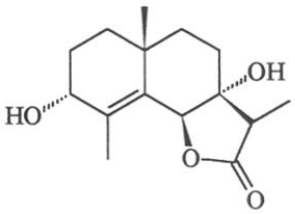
Based on the above spectral data the structure of compound **III** was elucidated as 3α,7α-dihydroxy-4,5-epoxy-6β,7-dihydroeudesmanolide.



III

Stereochemistry of lactone junction was deduced as *cis*, since the other eudesmanolide obtained from the same plant also had *cis*-lactone junction. However the stereochemistry of the epoxide ring could not be assigned.

This compound might have originated by the photo-oxidation of the parent compound of **IV** i.e. compound **V** in plant itself.



V

EXPERIMENTAL

The plant collected from Hubli, Karnataka during September 1996 was shade dried and powdered. The powder (1 kg) was extracted exhaustively with acetone to give an extract (26 g). The extract (20 g) was chromatographed over silica gel (60 –120 mesh) and thirteen broad fractions were collected with pet ether and pet-ether : acetone as eluent in increasing proportion of acetone. (Table 4).

Fraction 8 (900 mg) was dissolved in pyridine (2 ml) and acetic anhydride (2 ml) and kept at room temperature for 16 hours. The reaction mixture was poured in ice water and kept for 1 hour, acidified with dilute hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with water, dried over anhy. sodium sulphate and concentrated at reduced pressure to yield crude product mixture. The mixture was subjected to preparative TLC to afford compound **I** (12 mg) and compound **IV** (15 mg) as sticky masses.

Compound **I**

$[\alpha]_D^{25}$	:	-16.3° ($CHCl_3$, c, 0.08)
IR ($CHCl_3$, ν_{max})	:	1786, 1740, 1635 cm^{-1} (Fig. 1)
^1H NMR	:	Table 1, (Fig. 2)
Mass (rel. int)	:	m/e 350 [M^+](2), 308(8), 290(5), 248(67), 230(100), 215(30), 202(5), 192(20), 187(20), 177(20), 173(40), 95(28), 91(45), 81(14), 55(13).

Compound **IV**

IR ($CHCl_3$, ν_{max}) : 3380, 1770, 1740, 1635 cm^{-1} (Fig. 3)

^1H NMR : Table 1, (Fig. 4)

Fraction 6 (1.3 g) was subjected to rechromatography to collect five fractions (i-v). The fraction (ii) (315 mg) on preparative TLC in acetone : pet-ether (20:80) afforded compound **II** (9 mg) as sticky mass.

Compound **II**

$[\alpha]_D^{25}$: -12.2° ($CHCl_3$, c, 0.075)

IR ($CHCl_3$, ν_{max}) : 3420, 1690, 1625 cm^{-1} (Fig.5)

^1H NMR : Table 2, (Fig. 6)

Mass (rel. int) : m/e 236 $[M]^+$ (2), 218 (30), 206 (30), 190 (30)

Fraction 7 (1.2 g) was chromatographed over silica gel (100-200 mesh) using chloroform and chloroform : methanol in increasing proportion of methanol as eluents. Fraction collected in 2% methanol in chloroform was further purified on preparative TLC with acetone : pet-ether (30 :70) afforded compound **III** (13 mg).

Compound **III**

$[\alpha]_D^{25}$: - 28.3° ($CHCl_3$, c, 0.11)

IR ($CHCl_3$, ν_{max}) : 3420, 1780 cm^{-1} (Fig.7)

^1H NMR : Table 3, (Fig. 8)

Mass (rel. int) : m/e 282 $[M]^+$ (3), 264 (5), 246 (2), 236 (15)

Table 4 : Column chromatography of acetone extract (silica gel, 400 g)

Fraction No.	Eluent	Total volume collected	Weight in g	Approximate composition
1	Pet.ether	2 x 100 ml	0.7	Straight chain compounds
2	Pet.ether : Acetone 95 : 5	4 x 100 ml	2.1	Straight chain compounds
3	Pet.ether : Acetone 90 : 10	4 x 100 ml	2.0	Complex mixture of unidentified compounds
4	Pet.ether : Acetone 90 : 10	5 x 100 ml	0.9	Complex mixture of unidentified compounds
5	Pet.ether : Acetone 85 : 15	3 x 100 ml	2.1	Known major compound **4**
6	Pet.ether : Acetone 80 : 20	4 x 100 ml	1.3	Complex mixture with compound **II**
7	Pet.ether : Acetone 80 : 20	4 x 100 ml	1.2	Complex mixture with Compound **III**
8	Pet.ether : Acetone 75 : 25	2 x 100 ml	0.9	Complex mixture of parent compounds of **I & IV**
9	Pet.ether : Acetone 70 : 30	2 x 100 ml	0.7	Complex mixture of unidentified compounds
10	Pet.ether : Acetone 60 : 40	5 x 100 ml	2.2	Complex mixture of unidentified compounds
11	Pet.ether : Acetone 50 : 50	5 x 100 ml	2.3	Complex mixture of unidentified compounds
12	Pet.ether : Acetone 40 : 60	4 x 100 ml	1.1	Glycosides
13	Acetone	4 x 100 ml	1.3	Glycosides

Table 5 : Rechromatography of fraction 6

Fraction No.	Eluent	Total volume collected	Weight in g.	Approximate composition
i	Pet.ether : Acetone 85 : 15	1 X 100 ml	0.120	Known compound **5**
ii	Pet.ether : Acetone 80 : 20	2 X 100 ml	0.315	Mostly compound **II** along with impurities
iii	Pet.ether : Acetone 75 : 25	2 X 100 ml	0.215	Unidentified compounds
iv	Pet.ether : Acetone 70 : 30	2 X 100 ml	0.110	Unidentified compounds
v	Acetone	3 X 100 ml	0.225	Unidentified compounds

REFERENCES

1. *The Wealth of India, Raw materials*, Vol. X, p. 4, Publication and Information Directorate, CSIR, New Delhi, (1976).
2. K.K. Baslas

   Perfum. Essen. Oil Rec., **50**, 765 (1959).
3. N. Rao and S.K. Nigam

   Flavour India, **1**, 725 (1970).
4. K.K. Baslas

   Ind. J. Appl. Chem., **23**, 150 (1960).
5. M.I.D. Chughatai, I. Khokhar and A. Ahmad

   Sci. Int., **4**, 151 (1992).
6. R.K. Gupta, S. Chandra and V.Mahadevan

   Ind. J, Pharm. **29**, 47 (1986).
7. S.K. Singh, V.J. Tripathi and R.H. Singh

   Indian Drugs, **26**, 317(1989).
8. J.B. Harborne

   The Biology and Chemistry of the Compositae, Academic Press, London, p.603 (1977).
9. M.G. Gogte, L. Anantasubramanian, K.S. Naragund and S.C. Bhattacharya

   Ind. J. Chem. **25B**, 233 (1986).
10. J.S. Sohoni, S.R. Rojatkar, M.M. Kulkarni, N.N. Dhaneshwar, S.S. Tavale T.N. Gururow and B.A. Nagasampagi

    J. Chem. Soc., Per. Tran.- I, 157 (1988).

11. Atta-ur-Rahman, M. S. Shekhani, P. Shanaz, H. Rehman, Y. Afshani, Zia ul- Haque and D. Shaikh

J. Chem. Res. Synop., **3**, 68 (1989).

12. M.S. Shekhani, P.M. Shah, K.M. Khan and Atta-ur-Rahman

J. Nat. Prod., **54**, 882 (1991).

13. M.S. Shekhani, P. M. Shah, Y. Afshan, R. Siddiqui, P. Shanaz, K.M. Khan, K. Urooj and Atta-ur-Rahman

Phytochemistry, **29**, 2573 (1990).

14. S.R. Rojatkar and B.A. Nagasampagi

Phytochemistry, **31**, 3270 (1992).

15. S.R. Rojatkar

Ph. D. thesis, University of Poona, Pune (1995).

16. F. Bohlmann and Le Van Ngo

Phytochemistry, **16**, 1304 (1977).

17. F. Bohlmann and Le Van Ngo

Phytochemistry, **17**, 1957 (1978).

18. F. Bohlman, V. Castro and J. Jakupovic

Phytochemistry, **22**, 1223 (1983).

19. W. Herz and S.V. Govindan

J. Org. Chem., **45**, 1113 (1980).

20. F.C. Seaman, A.J. Marcolm and N.H. Fischer

Phytochemisytry, **24**, 2003 (1985).

21. Y. Oshima, S.M. wong, C. Konno, G.A. Cordell, D.P. Waller, O.D. Soejarto and H.H.S. Fong

J. Nat. Prod., **49**, 313 (1986).

22. S. B. Christensen, U. Rasmussen and C. Christophersen

Tet. Lett., **21**, 3829 (1980).

23. S. B. Christensen, I.K. Larsen, U. Rasmussen and C. Christophersen

J. Org. Chem., **47**, 649 (1982).

24. S. B. Christensen and K. Schaumburg

J. Org. Chem., **48**, 396 (1983).

25. S. B. Christensen, E. Norup, U. Rasmussen and J. O. Madsen

Phytochemistry, **23**, 1659 (1984).

26. D.H. De Juengo, M. Miski, D. A. Gage and T.J. Mabry,

Phytochemistry, **25**, 1917 (1986).

27. N. Ruangrunsi, S. Kasiworg, K. Likhitwitaywuid, G.L. Lange and C.P.Decicco

J. Nat. Prod. **52**, 130 (1989).

28. R.N. Baruah, C. Zdero, F. Bohlman, R.M. King and H. Robinson

Phytochemistry, **24**, 2641 (1985).

29. J. Jakupovic, C. Zedro, R. Boeker, U. Warning, F. Bohlman and S.B. Jones

Liebigs. Ann. Chem., 193 (1987).

30. F. Fronczek, D.Vargas and N.H. Fischer

J. Nat. Prod. **47**, 1036 (1984).

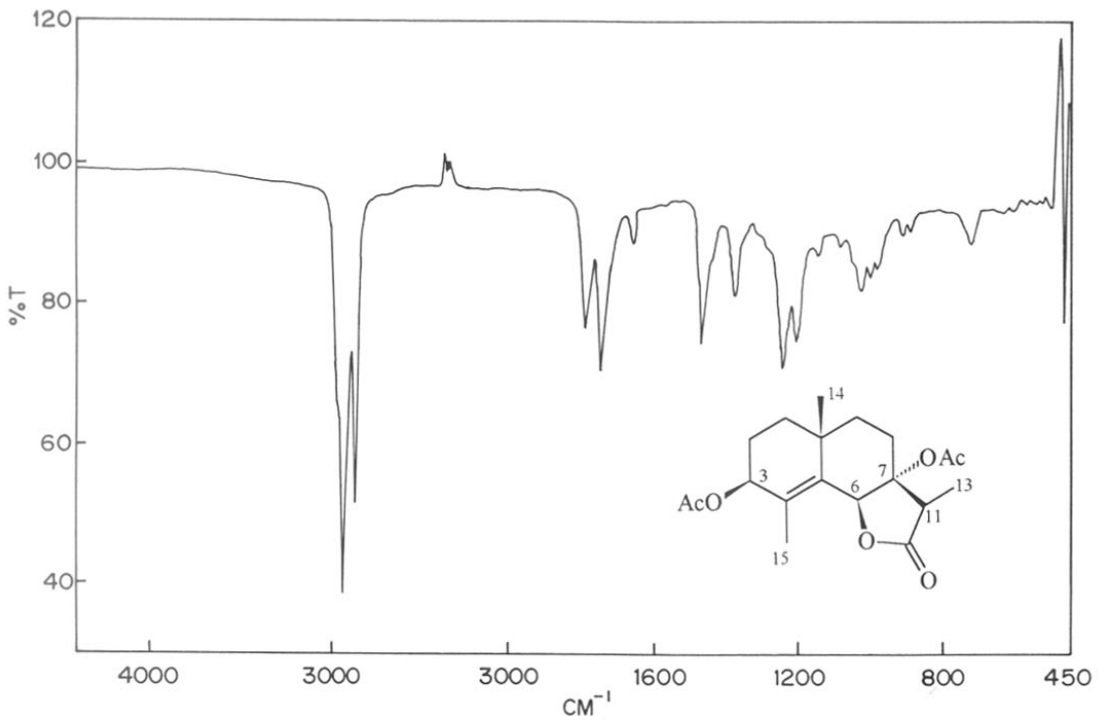


FIG 1 : FT - IR SPECTRUM OF COMPOUND I

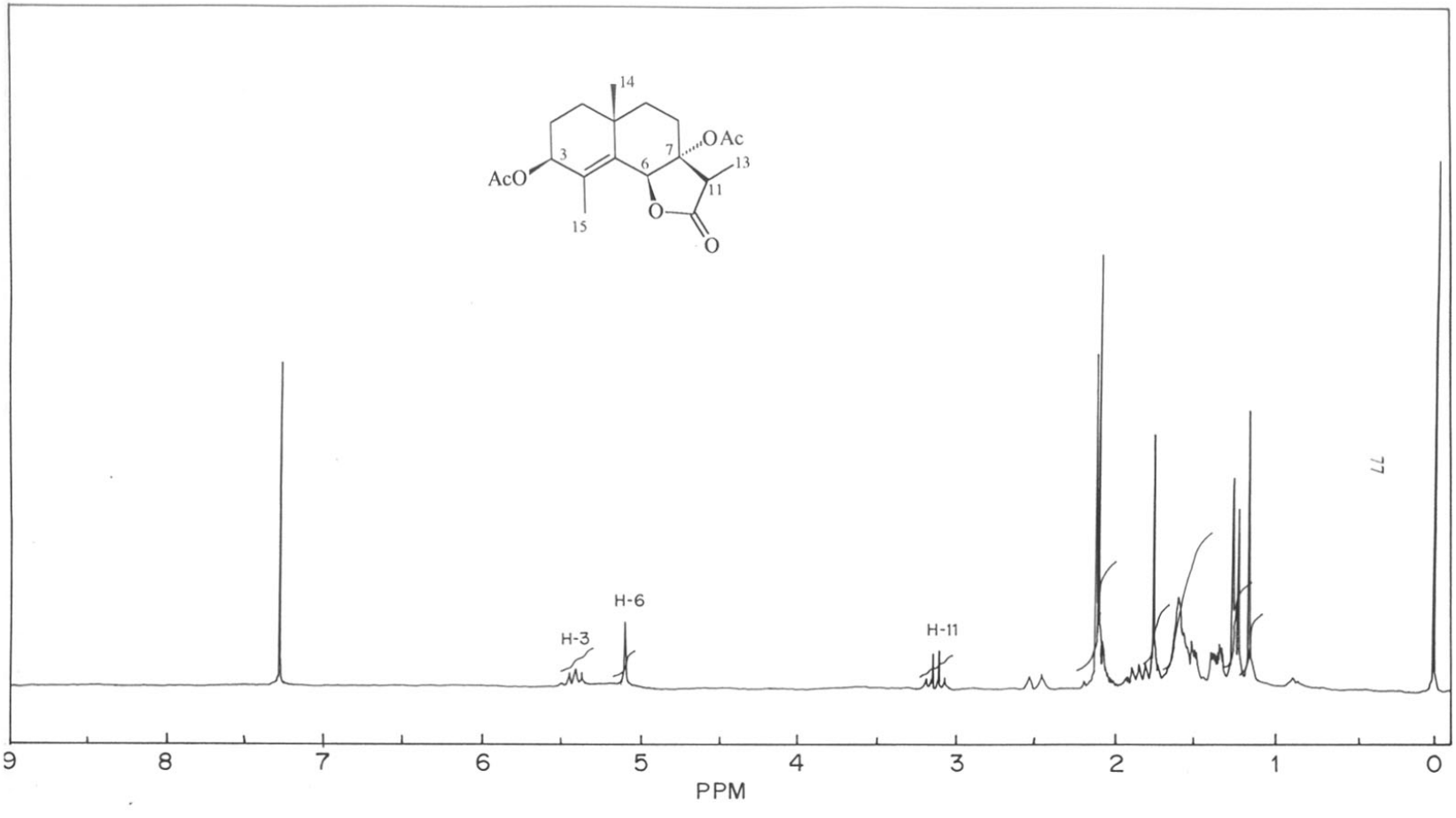


FIG 2 : ^{1}H NMR SPECTRUM OF COMPOUND I

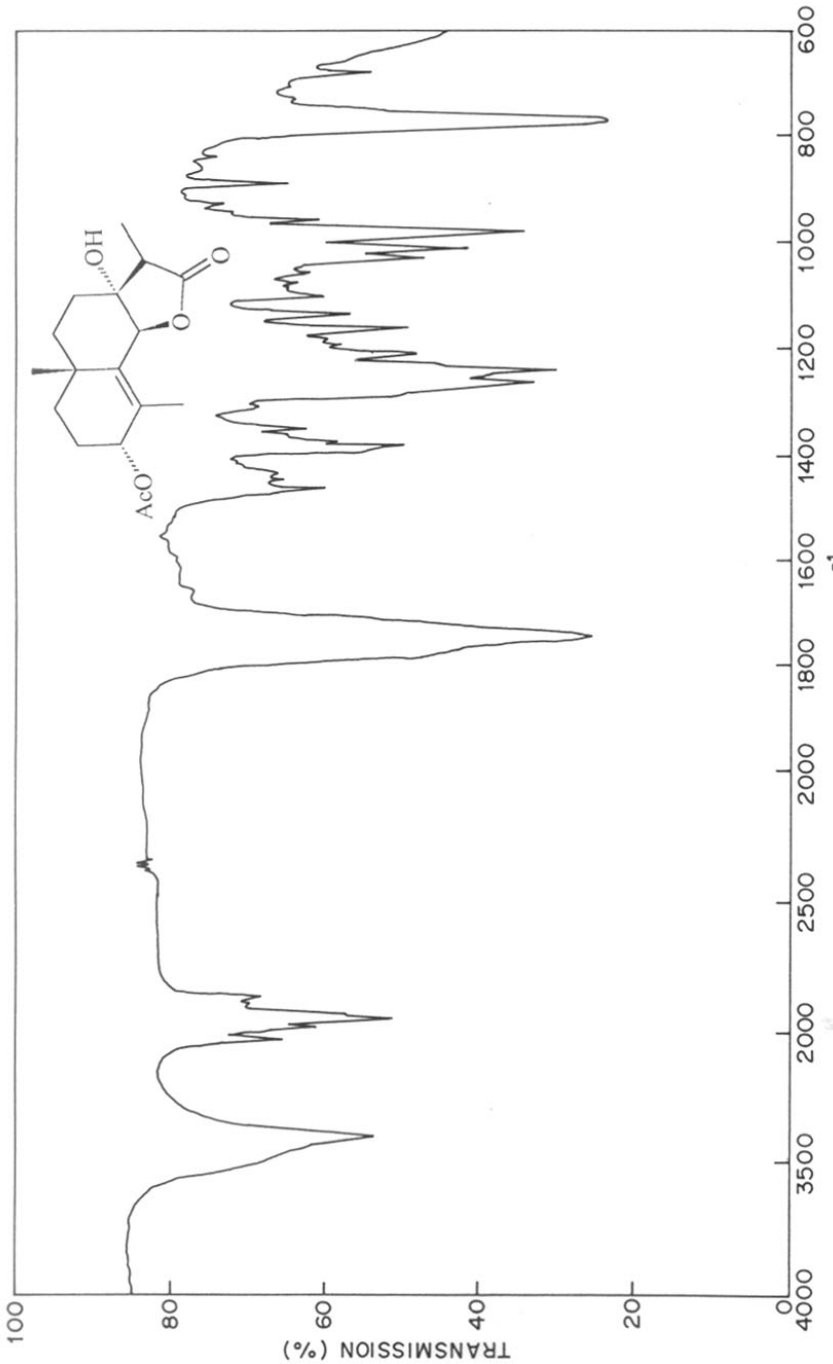


FIG 3 : IR SPECTRUM OF COMPOUND IV

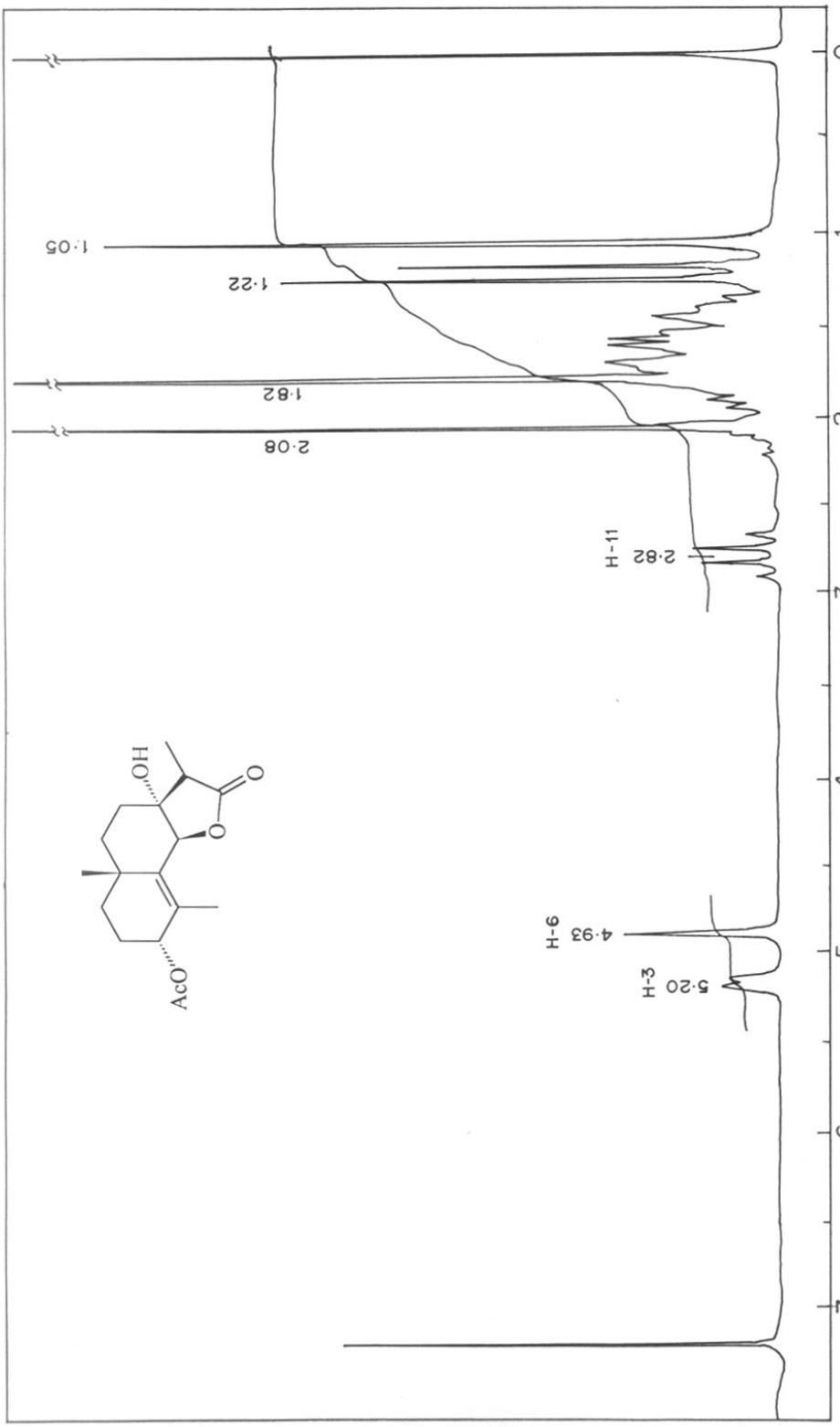


FIG 4 : ^{1}H NMR SPECTRUM OF COMPOUND IV (90 MHz)

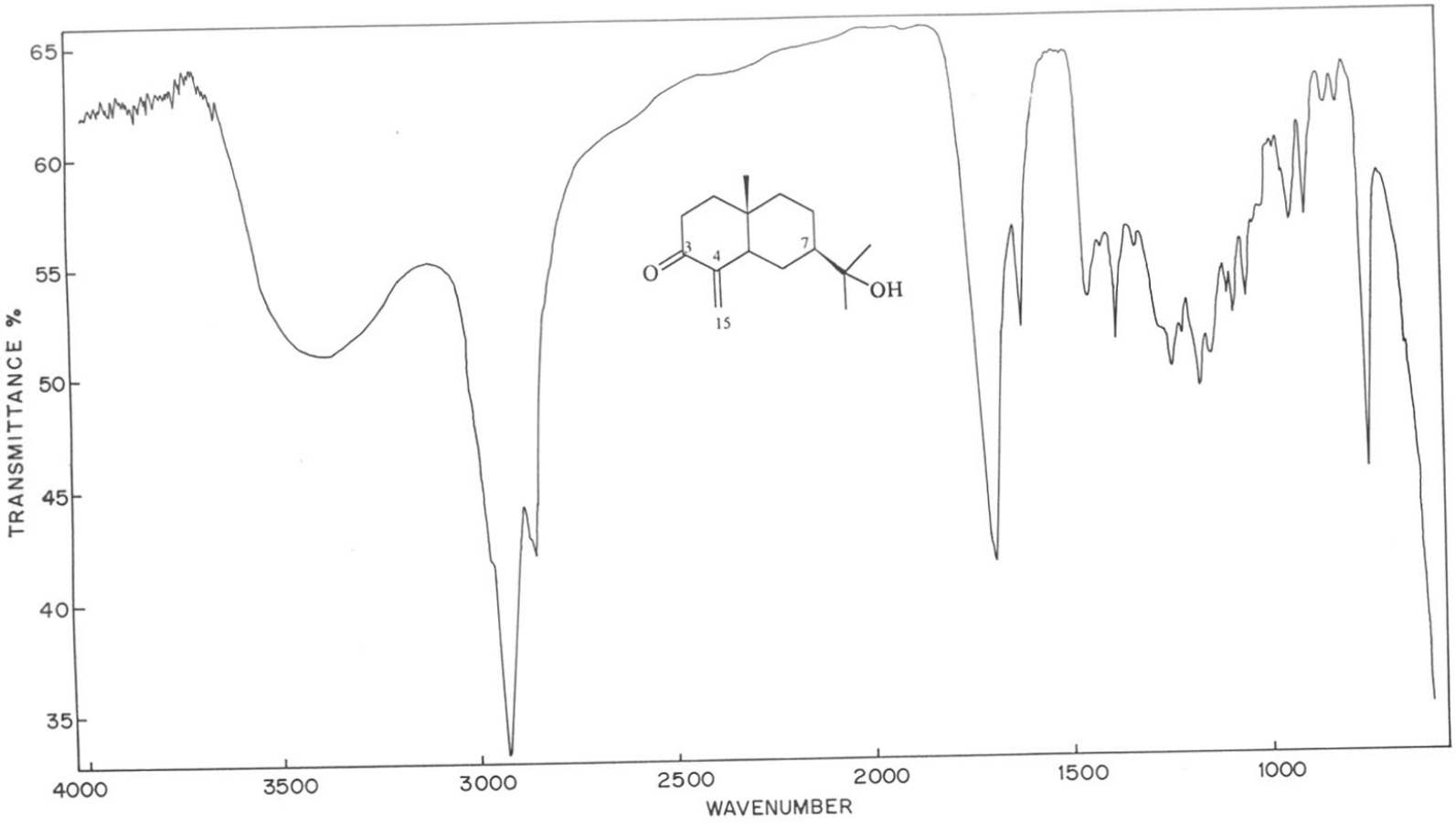


FIG 5 : FT - IR SPECTRUM OF COMPOUND II

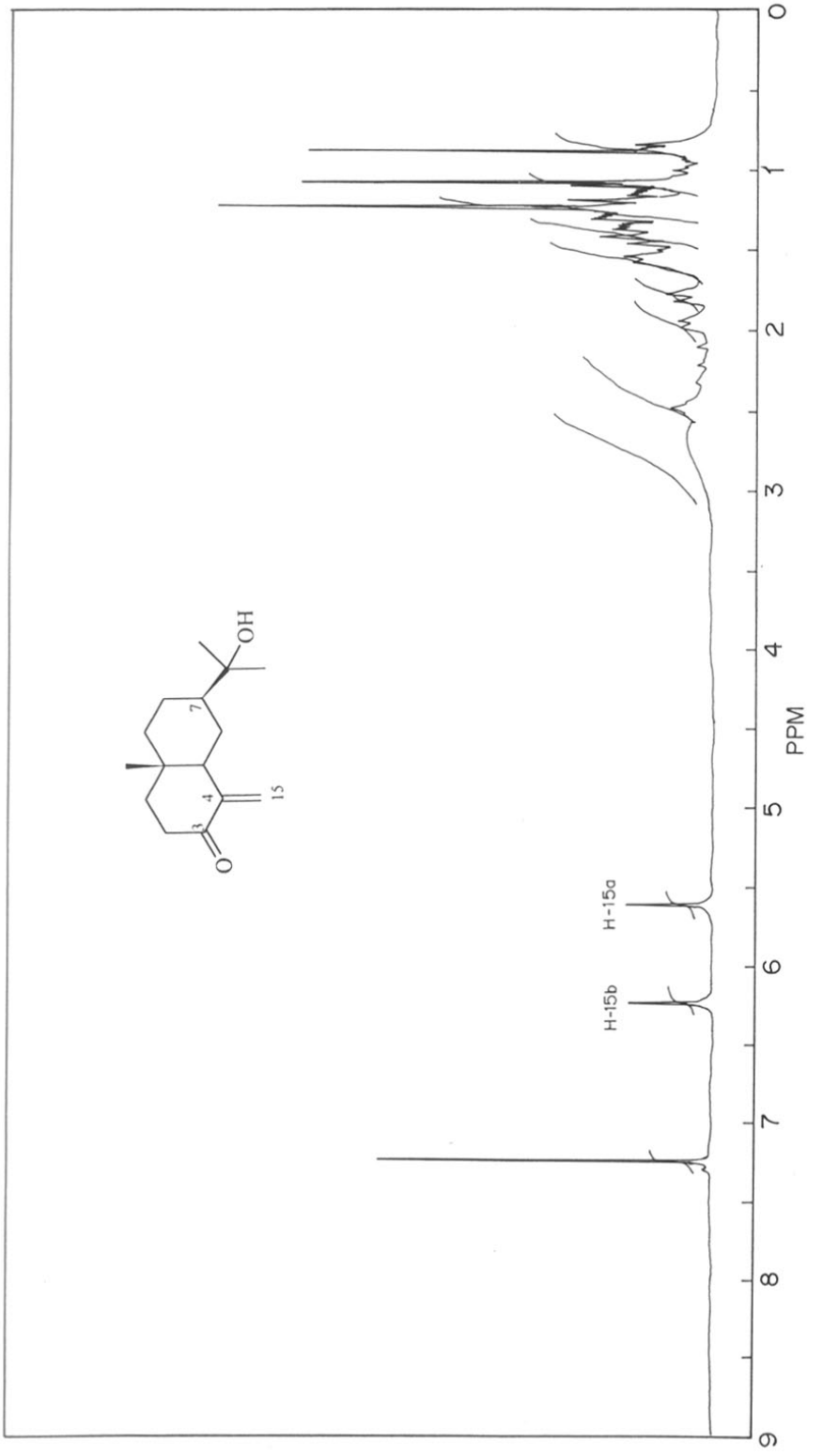


FIG 6 : ^{1}H NMR SPECTRUM OF COMPOUND II

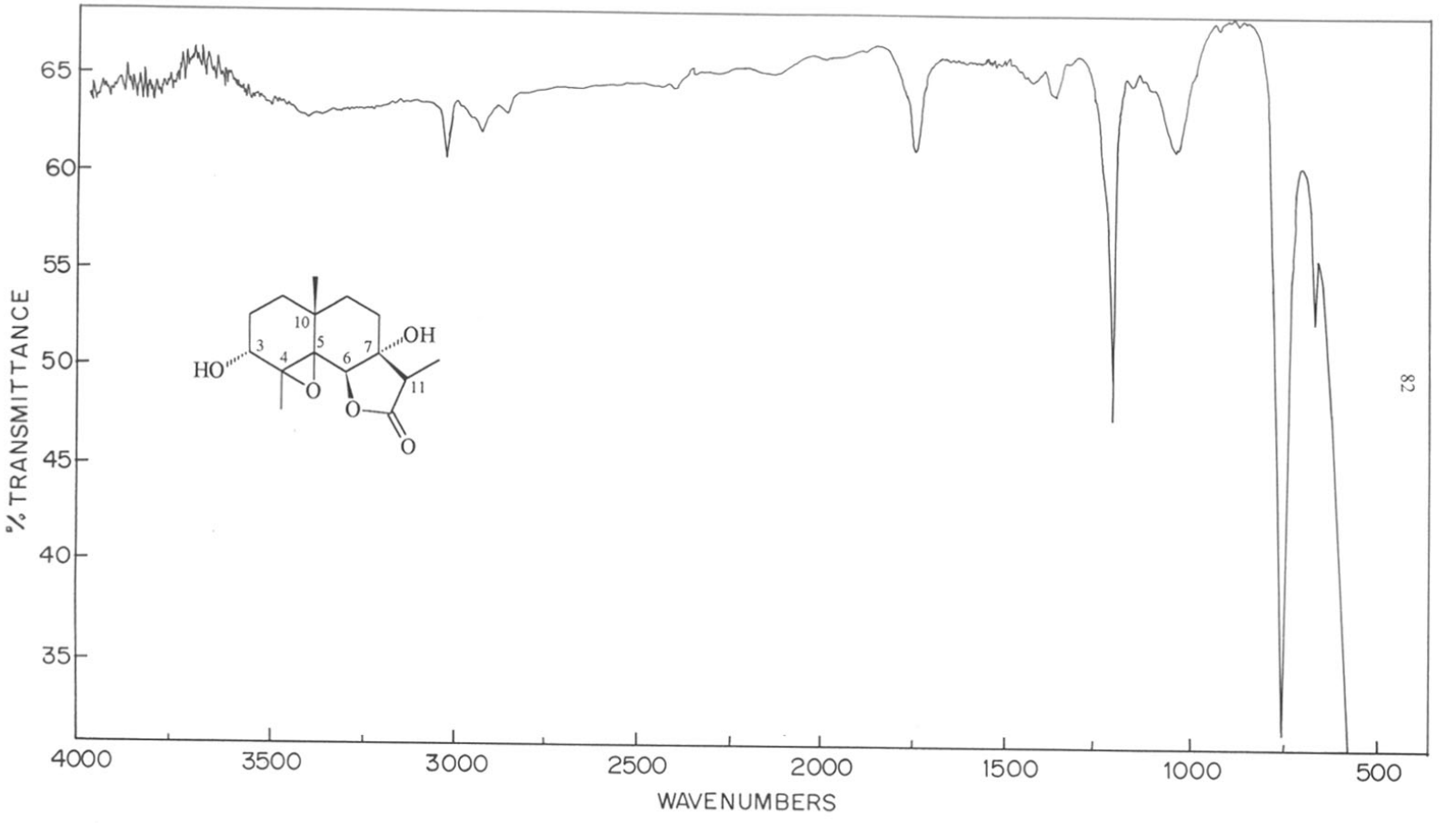


FIG 7 : FT - IR SPECTRUM OF COMPOUND III

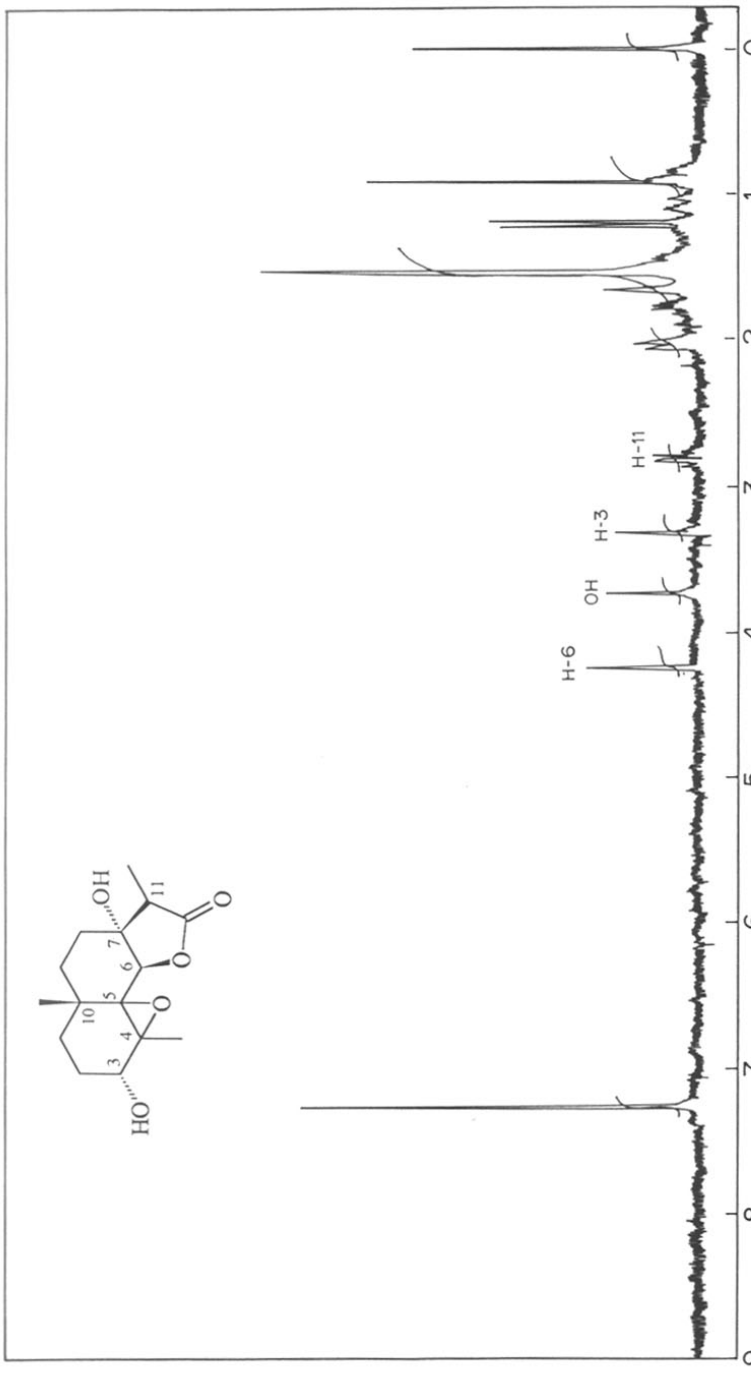


FIG 8 : ^{1}H NMR SPECTRUM OF COMPOUND III

CHAPTER III

CHEMICAL INVESTIGATION

of

Taxus baccata

and

Asparagus racemosus

PART I

CHEMICAL INVESTIGATION

of

Taxus baccata

Introduction

Taxus baccata belongs to the family Taxaceae having several species such as *T. brevifolia, T. wallichiana, T. cuspidata, T. media, T. chinensis, T. yunnanensis, T. canadensi, T. matret, T. mairet. Taxus baccata* is an evergreen tree usually 6 meters in height and 1.5-1.8 meter in girth found in the temperate Himalayas at altitudes between 1800 to 3300 meters[1]. It is known as Himalayan yew where as *Taxus baccata* growing in the temperate region of Europe is known as European yew.

A medicinal tincture made from the young shoots of *T. baccata* has long been used for the treatment of headache, giddiness, feeble and falling pulse, coldness of the extremities, diarrhoea and severe biliousness. The leaves are credited with emmenagogue and antispasmodic properties. They are employed for the treatment of hysteria, epilepsy and nervousness[1].

The genus *Taxus* has got much importance during last fifteen years due to occurrence of a novel anticancer compound taxol mainly from the barks of the *Taxus* species. Although it shows exceptionally promising antitumor property its use as a drug has been hampered due to its limited availability. Taxol produced by three whole trees is enough for the treatment of one patient[2]. This can be circumvented by semisynthesis from its precursor 10-deacetylbaccatin III,[3,4] which can be isolated from the renewable resources such as leaves (needles) and stems of *Taxus baccata*[5] and other *Taxus* species[5].

Taxol was isolated for the first time from *T. brevifolia* by Wani *et al.*[6] It was approved by Federal Drug Administration of USA as a drug in 1992 for the treatment of ovarian cancer. Taxol differs in anticancer activity from other anticancer drugs by inhibiting the cell division by a unique mechanism. Cell division takes place by duplication of chromosomes, which line up on spindles

formed by microtubles during mitosis. Taxol gums up the tubules stopping the formation of spindles, thus avoiding the cell division due to which the cancerous cell eventually dies.[2]

Chemical investigation of *T. baccata* was carried out in this laboratory during the standardisation of the process for the isolation of 10-deacetylbaccatin from needles.

Previous work

Taxol and others taxanes belong to the class of tricyclic diterpenoids. Several taxanes have been isolated from *Taxus baccata* along with other natural products such as flavonoids[7-16], lignans[14,17], sterols[18], phenolic compounds[19-21] and fatty acids[22].

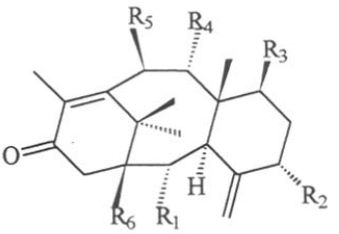
Taxanes having a typical tricyclic skeleton with C-4/C-20 double bond and carbonyl at C-13 (**1-14**) have been reported from different parts of the plant. These are listed in chart I, where as other taxanes (**15-28**) possessing C-4/C-20 double bond, but having hydroxyl or acetoxyl groups at C-13 are listed in chart II. Similarly taxanes (**29-32**) where C-20 is part of B ring also have been isolated from this plant (Chart III).

The taxanes (33-53) possessing an oxetane ring at C-4/C-5 are presently important class of taxanes owing to their excellent therapeutic potential. Such compounds reported from this plant have been listed in chart IV. Some compounds (54-56) having oxetane ring with abeotaxane type skeleton have also been reported (Chart V).

Besides these, some unusual type of taxanes have also been reported. These include, taxanes having C-12/C-15 linkage (57,58)[51], having an epoxide at C-4/C-20 (59-61)[38], a taxane with modified A ring (62[52], 63[14]) and a taxane having an unusual C-3/C-11 linkage (64)[53] (Chart VI).

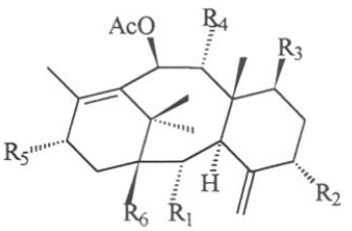
During the isolation of 10-deacetylbaccatin III, a biflavone, five taxanes (65-69)[54,55] and an aromatic compound (70)[56] have been isolated in this laboratory (Chart VII).

CHART I



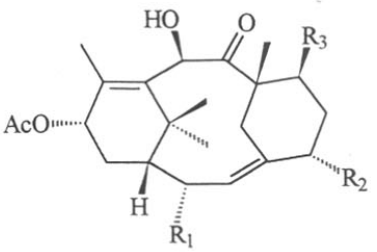
Compound	R_1	R_2	R_3	R_4	R_5	R_6	References
1	OH	$OCOCH_2CH(NMe_2)Ph$	H	OH	OAc	OH	23
2	OH	OCinn	H	OAc	OH	OH	24
3	OH	OCinn	H	OH	OAc	OH	24
4	OH	OCinn	H	OAc	OH	H	24
5	OH	OCinn	H	OH	OAc	H	24
6	OAc	$OCOCH_2CH(NMe_2)Ph$	H	OAc	OAc	H	25
7	OAc	$OCOCH_2CH(NMe_2)Ph$	H	OAc	OAc	OH	25
8	OH	OCinn	H	OAc	OAc	OH	26
9	OH	$OCOCH_2CH(NMe_2)Ph$	H	OH	OAc	H	27
10	OAc	$OCOCH_2CH(NMe_2)Ph$	H	OH	OAc	OH	27
11	OH	$OCOCH_2CH(NMe_2)Ph$	H	OAc	OH	H	27
12	OAc	$OCOCH_2CH(NMe_2)Ph$	H	OAc	OH	OH	27
13	OAc	OH	H	OH	OAc	OH	28
14	OAc	OH	OAc	OH	OAc	OH	28

CHART II



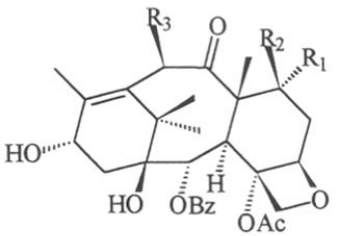
Compound	R_1	R_2	R_3	R_4	R_5	R_6	References
15	H	OH	H	OAc	OAc	H	29
16	H	OAc	OAc	OAc	OAc	H	29
17	OAc	OAc	H	OAc	OAc	OAc	29
18	OAc	OAc	OAc	OAc	OAc	H	29
19	CO_2iBu	OAc	OAc	H	H	H	29
20	CO_2iBu	OH	OAc	OAc	H	H	29
21	CO_2iBu	OAc	OAc	OAc	H	H	29
22	OH	$OCOCH_2CH[N(CH_3)_2]$-Ph	H	OH	OAc	OH	30
23	OH	$OCOCH_2CH[N(CH_3)_2]$-Ph	H	OH	OAc	H	30
24	OH	$OCOCH_2CH(NHCH_3)$-Ph	H	OH	OAc	H	30
25	OAc	OCOCH(OH)CH[N-$(CH_3)_2$]Ph	OAc	OAc	OAc	OH	28
26	H	OH	OAc	OAc	OAc	H	28
27	OH	OH	OAc	OAc	OAc	H	28
28	OAc	OH	OAc	OAc	OAc	OH	28

CHART III



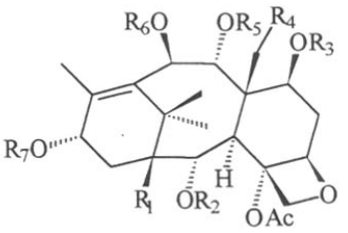
Compound	R_1	R_2	R_3	Reference
29	OAc	OH	H	26
30	OAc	OCOCH(OH)CH(N[CH_3]$_2$)Ph	OH	31
31	OAc	OCOCH(OH)CH(N[CH_3]$_2$)Ph	OAc	28
32	OH	OCOCH(OH)CH(N[CH_3]$_2$)Ph	OH	28

CHART IV



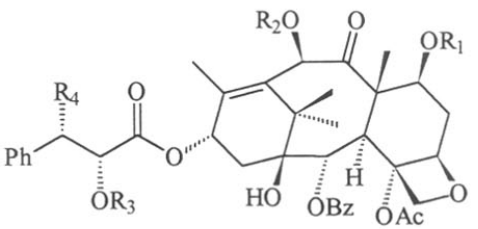
Compound	R_1	R_2	R_3	Reference
33	H	OH	OAc	32-37
34	OH	H	OAc	38,39
35	H	OH	OH	35,37,40-44,

CHART IV (Continued)



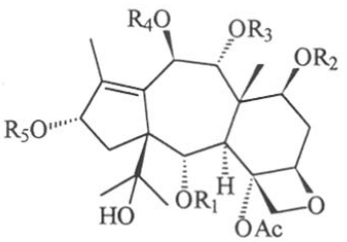
Compound	R1	R2	R3	R4	R5	R6	R7	Reference
36	OH	Ac	Ac	H	Ac	Ac	Ac	32,34
37	H	Ac	Ac	H	Ac	Ac	Ac	34
38	OH	Bz	Ac	H	Ac	Ac	Ac	34,35
39	OH	COC_5H_{11}	Ac	H	Ac	Ac	Ac	34
40	OH	Bz	H	OAc	H	H	H	45

CHART IV (Continued)



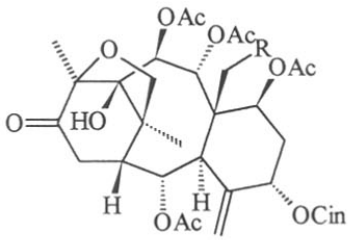
Compound	R_1	R_2	R_3	R_4	Reference
41	H	Ac	H	NHBz	5,35,36,41,46, 42,43,48,49
42	H	Ac	H	NHTig	35,36,42
43	H	H	H	NHBz	35,42
44	H	H	H	NHTig	35,42
45	β Xylose	Ac	H	NHBz	35
46	β Xylose	Ac	H	NHTig	35
47	β Xylose	Ac	H	NHC_5H_{11}	35
48	β Xylose	H	H	NHBz	35
49	β Xylose	H	H	NHTig	35
50	β Xylose	H	H	NHC_5H_{11}	35
51	H	$COCH_2$-$CH(OH)CH_3$	H	NHBz	35
52	H	$COCH_2$-$CH(OH)CH_3$	H	NHTig	35
53	β Xylose-(OAc)	Ac	Ac	NHTig	35

CHART V

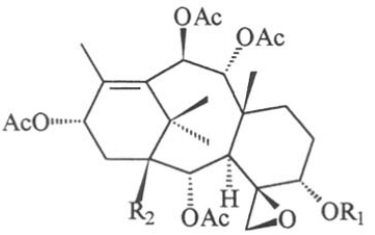


Compound	R1	R2	R3	R4	R5	Reference
54	Ac	Ac	Ac	Bz	H	14,49
55	Bz	H	H	H	Ac	50
56	Ac	Bz	Ac	H	Ac	49

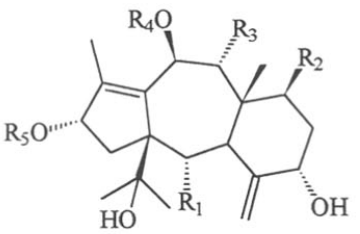
CHART VI



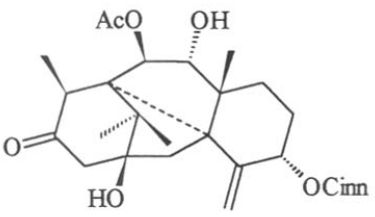
	R
57	OCOPh
58	H



	R_1	R_2
59	Ac	H
60	H	H
61	Ac	OH

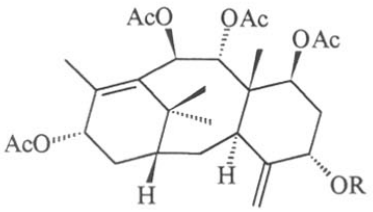


	R_1	R_2	R_3	R_4	R_5
62	OAc	H	H	Ac	Ac
63	H	OAc	OAc	Bz	H

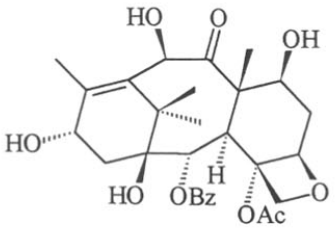


64

CHART VII



	R
65	Cinn
66	$COCH_2CH[N(CH_3)_2]Ph$



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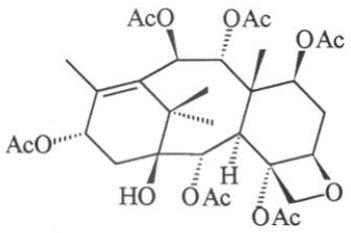

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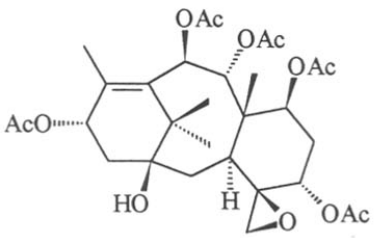

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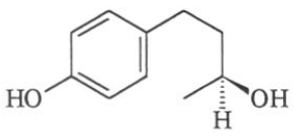


70

Present work

With an intention to isolate more bioactive compounds from the needles of *Taxus baccata*, further chemical investigation of methanol extract of needles was carried out which afforded two known taxanes, (i) 7-epi-10-deacetyl taxol (**I**) and (ii) brevifoliol (**II**). This is the first reported isolation of **I** from *T. baccata* whereas **II** has been earlier isolated from the twigs of *T. baccata*.

Characterisation of compound I

Compound **I** obtained as an amorphous solid, showed in its IR spectrum (Fig. 1) the presence of hydroxyl groups at 3380 cm^{-1}, amide and ester carbonyls at 1735-1750 cm^{-1} and absorption for aromatic protons at 1560 cm^{-1} and 1660 cm^{-1}. ^1H NMR spectrum (Fig. 2, Table 1) of the compound showed three methyls at δ1.14, 1.24 and 1.75 as singlets and a vinyl methyl at δ 1.8 as doublet (J = 1 Hz) along with an acetate methyl at δ 2.52. This methyl signal pattern indicated that compound **I** belonged to taxane type of compounds.

^1H NMR spectrum of the compound further showed a broad multiplet from δ7.7 to δ 7.35 accounting for eleven aromatic protons, a doublet of doublet at δ 8.2 (2H, J=1, 8 Hz) and another doublet of doublet at δ 7.75 (2H, J = 1, 7 Hz) indicating the presence of a benzoyl group, a benzamido group and a phenyl group. The spectrum further showed a typical NH signal characteristic of the taxol side chain at δ 7.05 (d, J = 9 Hz) along with a doublet of doublet (J = 3, 9 Hz) at δ 5.85 due to H-3' and a doublet (J = 3 Hz) at δ 4.85 due to H-2' and a broad triplet at δ 6.35 (J = 9 Hz) due to H-13 indicating the presence of taxol side chain at C-13.

It also revealed the presence of a hydroxyl group at C-1 and benzoyl group at C-2 by showing a doublet at δ 5.76 (J = 8 Hz) due to H-2 which couples with H-3 appearing at δ 3.95

Table 1 : ^{1}H NMR chemical shifts, multiplicity and coupling constants (in Hz) of baccatin, 7-epi-baccatin III, 10-deacetyltaxol, compound **I** (7-epi-10-deacetyltaxol).

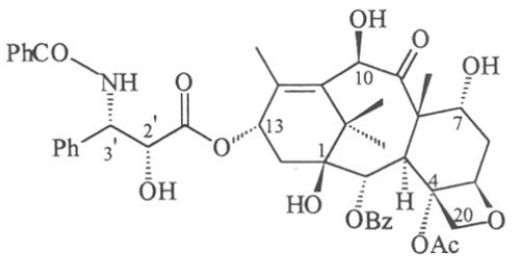
Proton	Baccatin III	7-epi-Baccatin III	10- deacetyltaxol	compound **I** (7-epi-10-deacetyltaxol)
H-2	5.58, d, J=7	5.74, d, J=6	5.67, d, J=7.5	5.76, d J=8
H-3	3.84, d, J=7	4.02, d, J=6	3.88, d, J=7.85	3.95, d, J=8
H-5	4.94, dd, J=2,8	4.93, dd, J=3,5	4.29, brd, J=9	4.95, t, J=6
H-6	2.4, m	2.1, m	1.9, m	2.4, m
H-7	4.4, m	3.68, m	4.18, m	3.75, t, J=3,12
H-10	6.28, s	6.83, s	5.18, s	5.5, s
H-13	4.82, brt, J=9	4.9, brt, J=8	6.18, brt, J=8	6.35, brt, J=9
H-14	2.4, m	2.3, m	2.4, m	2.4, m
H-16	1.08, s	1.11, s	1.19, s	1.24, s
H-17	1.08, s	1.05, s	1.10, s	1.14, s
H-18	2.01, d, J=1	1.99, d, J=1.4	1.74, brs	1.8, d, J=1
H-19	1.63, s	1.63, s	1.74, brs	1.75, s
H-20	4.11, d, J=8 4.25, d, J=8	4.38, s, (2H)	4.25 ABq	4.45, s, (2H)
OAc	2.20, s 2.24, s	2.20, s 2.35, s	2.37, s	2.52, s
H-2'			4.78, d, J=3	4.85, d, J=3

Proton	Baccatin III	7-epi-Baccatin III	10- deacetyltaxol	compound **I** (7-epi-10-deacetyltaxol)
H-3'			5.77,dd, J=3,9	5.85, dd, J=3,9
C-3'NH			7.14, d, J=9	7.05, d, J=9
C-2 OBz	7.46, m 8.05, dd, J=2,8	7.52, m 8.12, dd, J=2,8	8.12, dd, J=2,8	8.2,dd, J=1,9
C-3'NBz			7.76, dd, J=2,7	7.75, dd, J=1,7
Other Ar-H			7.43-7.47, m (11 H)	7.35-7.5, m (11 H)

(d, J = 8 Hz). The coupling constant showed clearly that these protons should be *trans* to each other i.e. H-2 as β and H-3 as α, as in other taxanes. The ^{1}H NMR spectrum further showed a singlet at δ 5.5 accounting for one proton which has to be due to H-10 indicating the presence of a carbonyl group at C-9 and hydroxyl group at C-10. The chemical shift of acetate methyl clearly revealed it to be at C-4 and an oxetane ring is present in the molecule. The proton at C-5 also appeared at δ 4.95 as broad triplet as it should be.

However, the spectrum did not show the typical doublet of doublet as AB quartet for H-20a and H-20b of oxetane ring and a multiplet at δ 4.18 for H-7 as found in 10-deacetyl taxol or 10-deacetylbaccatin. Instead it showed a broad singlet at δ 4.45 accounting for two protons and a broad triplet (J = 3, 12 Hz) at δ 3.75 accounting for one proton (H-7).

Further comparison of these spectral data with those of 10-deacetylbaccatin III, baccatin III[57], 7-epi-baccatin III (baccatin V)[38] and 10-deacetyl taxol[58] (Table 1) it became clear that compound **I** was indeed 7-epi-10-deacetyl taxol. The spectral data of compound **I** was identical to the reported spectral data of 7-epi-10-deacetyl taxol earlier isolated from *T. wallichiana*[58] and *T. chinensis*[59].



Characterisation of compound II

Compound **II** obtained as crystalline solid, m. p. 201°-203°, $[\alpha]_D^{25}$ –22.5°, showed in its IR spectrum (Fig. 3) the presence of hydroxyl group at 3360 cm^{-1}, ester carbonyls at 1730-1742 cm^{-1}, 1665 cm^{-1} and 1450-1560 cm^{-1}. ^1H NMR spectrum (Table 2, Fig. 4) revealed the presence of three tertiary methyl groups at δ 0.95, 1.05, 1.40 and a vinyl methyl at δ1.85. It also showed two acetate methyls at δ 2.1 and 2.2, a doublet of doublet at δ7.9 (2H, J = 2, 9 Hz), a multiplet at δ 7.4-7.6 accounting for three protons indicating the presence of a benzoyl group.

All these features of ^1H NMR spectrum clearly indicated that the compound was a taxane type of compound with two acetoxy and one benzoyl group. In taxol type of compounds where ring A is 6 membered, methyls at C-15 appear at δ1.15 and δ1.25 in their ^1H NMR spectrum. However in brevifoliol and similar abeotype compounds where ring A is 5 memberd, these methyls show the signals at δ1.05 and δ1.35 besides H-19 at δ 0.9 in their ^1H NMR spectra. Compound **II** under discussion showed two methyls at δ1.05 and δ1.40 besides the angular methyl at δ 0.95 indicating it to be an abeotype taxane. The ^1H NMR spectrum did not show the oxetane protons instead it showed two broad singlets at δ 4.85 and δ 5.2 revealing the presence of exomethylene at C-4. Thus from this spectral data compound **II** was found to be an abeotype taxane having two acetoxy and a benzoyl group.

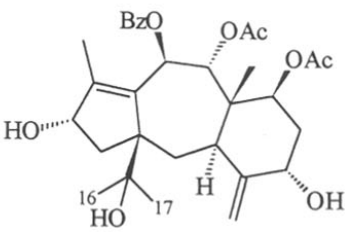
The ^1H NMR spectrum of compound further revealed the presence of an ester group at C-9 by virtue of which two doublets due to H-9 and H-10 appeared at δ 6.1 (J = 10 Hz) and δ 6.6 (J = 10 Hz) or vice versa. The other signal appearing as doublet of doublet at δ 5.55 (J = 5, 11 Hz) must be due to a proton at C-7 bearing an acetoxy group as observed in other taxanes. ^1H NMR spectrum (Fig. 5) of the acetylated product of compound showed four acetate methyls and

Table 2 : ^{1}H NMR spectral data of compound **II** (Breviofoliol)

Proton	Chemical shift in δ	Multiplicity	Coupling constant in Hz
H-2	1.47	brd	14
H-2	2.33	dq	14, 9
H-3	2.74	d	9
H-5	4.50	bs	
H-6α	1.77	m	
H-6β	1.81	m	
H-7	5.55	dd	11, 5
H-9	6.10	bd	10.5
H-10	6.60	d	10.5
H-13	4.40	bt	7
H-14α	1.28	dd	14, 7
H-14β	2.43	dd	14, 7
H-16	1.05	s	
H-17	1.40	s	
H-18	1.85	s	
H-19	0.95	s	
H-20a	5.20	bs	
H-20b	4.85	bs	
OCO$\underline{CH_3}$	2.10	s	
OCO$\underline{CH_3}$	2.20	s	
H-2',H-6'	7.90	dd	2,9
H-3',H-5'	7.41	m	
H-4'	7.53	m	

downfield shift of protons at δ 4.40 and 4.50 in the ^{1}H NMR of compound **II** to δ 5.40 and 5.50 respectively revealing the presence of two secondary hydroxyl groups which can be at C-5 and C-13 as generally observed in taxanes.

From these spectral data compound **II** was characterised as brevifoliol. The spectral data of compound **II** and its physical constants were found to be in agreement with those reported in literature[60].



EXPERIMENTAL

Taxus baccata needles were collected from Darjeeling, West Bengal, India in November 1993. These needles were shade dried and powdered .The powder (2 Kg) was extracted with methanol at room temperature. The extract was filtered and concentrated under reduced pressure to yield dark green mass (65g). The concentrated extract (35 g) was subjected to column chromatography over silica gel (60-120 mesh) using pet ether and acetone-pet ether as the elution gradient with increasing proportion of acetone to collect eight fractions (Table III). Fractions III and IV on repeated column chromatography coupled with preparative TLC yielded compound **I** and compound **II** (Table IV and V).

Isolation of compound I

Fraction (ii) (0.85 g) of chromatography **2** was purified by repeated preparative TLC using benzene : ethyl acetate (65:35) and pet ether : acetone (70:30) as eluents to obtain compound **I** as amorphous solid (13 mg).

$[\alpha]_D^{25}$	:	-33.4° ($CHCl_3$, c, 0.4)
IR_{ν}^{max} ($CHCl_3$)	:	3380, 1750-1735, 1660-1560 cm^{-1} (Fig. 1)
^1H NMR	:	Table 2, (Fig. 2)
Mass	:	m/e 811 $[M]^+$

Isolation of compound II

Fraction (iii) (1.92 g) of chromatography **3** was purified by repeated preparative TLC using benzene : ethyl acetate (60:40) and chloroform : acetonitrile (95:5) as eluents to obtain compound **II** in pure form and crystallised in pet ether-acetone to get crystalline solid (15 mg).

m.p.	:	201-203°
$[\alpha]_D^{25}$	:	-22.5° ($CHCl_3$, c 0.4)
IR_v^{max} ($CHCl_3$)	:	3360, 1742-1730, 1665, 1560-1450 cm^{-1}, (Fig. 3)
^1H NMR	:	Table 2, (Fig. 4)
Mass	:	m/e 555 $[M]^+$

Table III : Column chromatography (**1**) of total methanol extract.

Fraction	Eluent	Total volume collected	Approximate weight in g.	Composition
I	Pet Ether	250 ml x 4	7.5	Aliphatic compounds
II	Pet Ether : Acetone 10 : 90	250 ml x 4	5.6	Complex mixture
III	Pet Ether : Acetone 20 : 80	250 ml x 3	3.5	Mixture of taxanes including compound **I**
IV	Pet Ether : Acetone 30 : 70	250 ml x 5	4.9	Mixture of compound **II, 70** and 10-DAB
V	Pet Ether : Acetone 40 : 60	250 ml x 5	3.4	10-DAB and other taxanes
VI	Pet Ether : Acetone 50 : 50	250 ml x 4	2.6	Complex mixture of unidentified compounds
VII	Pet Ether : Acetone 25 : 75	250 ml x 4	2.8	Complex mixture of unidentified compounds
VIII	Acetone	500 ml x 2	3.1	

Table IV : Column chromatography (**2**) of fraction III

Fraction	Eluent	Volume	Weight in g.	Composition.
i	Pet ether : Acetone 90 : 10	100 ml x 3	0.92	Complex mixture
ii	Pet ether : Acetone 80 : 20	100 ml x 4	0.85	Compound **I** with impurities
iii	Pet ether : Acetone 80 : 20	100 ml x 4	1.16	Compound **67** and Compound **70**
iv	Pet ether : Acetone 70 : 30	100 ml x 3	0.52	Complex mixture

Table V : Column chromatography (**3**) of fraction IV

Fraction	Eluent	Volume	Weight in g.	Composition.
(i)	Pet ether : Acetone 90 : 10	100 ml x 3	0.91	Complex mixture
(ii)	Pet ether : Acetone 80 : 20	100 ml x 4	1.13	Complex mixture With traces of comp. **I**
(iii)	Pet ether : Acetone 70 : 30	100 ml x 4	1.92	10-DAB, Compound **70** and Compound **II**
(iv)	Pet ether : Acetone 60 : 40	100 ml x 4	0.83	10-DAB and compound **70**

REFERENCES

1. *The Wealth of India, Raw materials,* Vol. **X** , p. 132, Publication and Information Directorate , CSIR, New Delhi, (1976).
2. Gene Bylinsky
   Fortune, July 23, 66 (1992).
3. J.N. Denis and A.E. Greene
   J. Am. Chem. Soc., **110**, 5917 (1988).
4. C. Gennali, A.Vulpetti, M. Donghi, N. Mongelti and E. Vanotti
   Angew Chem. Int.Ed., **35**, 1723 (1996).
5. B. Das and S. Padma Rao
   Ind. J. Chem., **35B**, 883 (1996).
6. M.C. Wani, H.L. Taylor, M.E. Wall, P.Coggan and A.T. McPhail
   J. Am. Chem. Soc., **93**, 2325 (1971).
7. G.D. Modica, P.F. Rossi and A.M. Rivero
   Atti. Acad. Nazl. Linui Rend. Classe Sci. Fis Mat. Nat., **26**, 785 (1959).
8. G.D. Modica and A.M.Rivera
   J. Chromatog., **7**, 133 (1962).
9. G.D. Modica, P.R., A.M. Rivero and E. Borello
   Atti. Acad. Nazl. Linui Rend. Classe Sci. Fis Mat. Nat., **29**, 74 (1960).
10. G.D. Modica, P.F. Rossi and A.M. Rivero
    Atti. Acad. Nazl. Linui Rend. Classe Sci. Fis Mat. Nat., **32**, 87 (1962).
11. Z. Jerzmanowska and K. Samuel
    Diss. Pharm. Pharmacol., **18**, 181(1966) (Pol).

12. V.S. Parmar, A. Vardhan, K.S. Bhist, N.K. Sharma, R. Sharma, P. Taneja, O.D. Tyagi and P.M. Boll.

Ind. J. Chem., **32**, 601 (1993).

13. B. Dass, P.S. Rao, K.V.N.S.Srinivas and J.S. Yadav.

Fitoterapia, **65**, 189 (1994).

14. B. Dass, P.S. Rao, K.V.N.S. Srinivas, J.S. Yadav and R.Das.

PhytoChemistry, **38**, 715 (1995).

15. A. Ivana, P. Krsta and A. Milutin.

J. Serb. Chem. Soc., **60**, 265 (1995).

16. B. Reddy, K. Parthasarathi, and G.L David.

Ind. J. Chem., **35**, 283 (1996).

17. R.B. Mujumdar, R. Srinivasan and K. Venkatkrishnan

Ind. J. Chem., **10**, 677 (1972).

18. Fr. R. Preuss and H. Orth

Planta Med., **13**, 261 (1965) (Ger).

19. M.S.Y. Khan, I. Kumar, P. J. Siva, G.R. Nagarajan,

M.R. Parthasarthy and H.G. Krishnamurthi

Planta Med., **30**, 82 (1976).

20. B. Dass, M. Takhi, H. Kumar, M. Sampath, K.V.N.S.Srinivas and J.S. Yadav.

Phytochemistry, **33**, 697 (1993).

21. B. Dass, M. Takhi, K.V.N.S.Srinivas and J.S. Yadav.

Phytochemistry, **33**, 1489 (1993).

22. M. Hoellwarth and K. Ulrich.

Pflanzenphysiol, **91**, 325 (1979) (Ger).

23. L. Ettouti, A. Anand, C. Popuat and P. Potier

J. Nat. Prod., **54**, 1455 (1991) (Fr).

24. G. Appendino, G. Pierluiga, A. Pisetta, B. Ezio and G. Bruno.

Phytochemistry, **31**, 4253 (1992).

25. G. Appendino, G. Pierluiga, B. Ezio and G. Bruno.

J. Nat. Prod., **56**, 512 (1993).

26. G. Appendino, G. Emiru, Renata, J. Jakupovic, G. Pierluiga, B. Ezio and G. Bruno.

Phytochemistry, **36**, 407 (1994).

27. H.D. Jenniskenes, V.R. Louis, Van Rozendaal, L. M. Erik, Van Beck, A. Teris,

Wiegerinek, H.G. Peter and H.W. Scheeren.

J. Nat. Prod., **59**, 117 (1996).

28. B. Luciano, G. Appendino, E. Renata, G. Pierluiga, B. Ezio and G. Bruno.

Leibig Ann, **2**, 345 (1995).

29. D.P.Della,Casa De Marcano and T.G.Halsall

J. Chem. Soc .Chem. Comm. 1282(1969).

30. G. Appendino, H.C. Ozen, I. Fenoglio, G. Pierluiga, G.Bruono and B. Ezio.

Phytochemistry, , 1521 (1969).

31. E. Graf, A. Kirfel, G.W. Joachin, and E. Breitmaier.

Leibigs. Ann. De. Chem., 376 (1982).

32. W.R. Chan, T.G. Halsall, G.M. Hornby, A.W. Oxford, W. Sabel, K. Biamer, G. Ferguson and J.M. Robertson

J. Chem. Soc. Chem. Comm., 923 (1966).

33. Casa de Della, D.P. Marcano, T.G. Halsall and G.M. Hornby

J. Chem. Soc. Chem. Comm., 216 (1970).

34. Casa de Della, D.P. Marcano and T.G. Halsall

J. Chem. Soc. Chem. Comm., 365 (1975).

35. V. Senilh, S. Blechert, M. Colin, D. Guenard, F. Picot, P. Potier and P. Varenne.

J. Nat. Prod., **47**, 131 (1984).

36. N.C. Wheeler, K. Jech, S. Masters, W.S. Brobst, A.B. Alvarado, A.J. Hoover and K.M. Snader

J. Nat. Prod., **55**, 432 (1992).

37. E. Bombardelli, P. DeBellis, V. Foti, R. Pace, G. Zini and B. Gabetta

In abstract "Second National Cancer Institute Workshop on Taxol and Taxus" (Sept. 1992), Session III, Abstract 2.

38. Casa de Della, D.P. Marcano, T.G. Halsall, E. Castellano and O.J.R. Hodder

J. Chem. Soc. Chem. Comm., 1382 (1970).

39. E. Castellano and O.J.R. Hodder

Acta Cryst., **29B**, 2566 (1973).

40. M.W. Stasko, K.M. Witherup, T.J. Ghiorzi, T.G. McCloud, S. Look, G.M. Muschik and H.J. Issaq

J. Liq .Chromatogr., **12**, 213 (1989).

41. K.M. Witherup, S.A. Look, M.W. Stasko, T.J.Ghiorzi, G.M. Muschik and G.M. Cragg. *J. Nat P rod.*, **53**, 1249 (1990)

42. G. Chauviere, D.Guenard, F.Picot, V.Senilh and P. Poiter *C.R. Seanc Acad .Sci. Ser.*, **2**, 293,501 (1981).

43. S.K. Chattopadhyaya, V.K. Triparhi, R.S. Thakur, R.P. Sharma and S.P. Jain *Ind. J. Chem.*,**33B**, 409 (1994)

44. B. Das, S.P. Rao, K.V.N.S. Srinivasan and J.S. Jadhav. *Ind. J. Chem.*, **34B**, 672 (1995).

45. G. Appendino, H.C. Ozen, P.Gariboldi, E. Gabetta and E. Bombardelli. *Fitoterapia*, **64**(Supp-I), 47 (1993)

46 N. Videnšek, P. Lim, A. Campbell and C. Carlson *J.Nat.Prod.*, **53**,1609 (1990).

47 B.V. Sorochinski, A.I. Prokhnevski and D.M. Grodzinski *Kim.Prir. Soedin*, **6**, 702 (1990).

48 B.V. Sorochinski and A.I. Prokhnevski. *Khim-Farm Zh*, **25**, 45 (1991).

49 B. Das, S.P. Rao, K.V.N.S. Srinivasan, J.S.Jadhav and R. Das. *Phytochemistry*, **38**, 671 (1995).

50 W.M. Chen, J.Y. Zhou, P.L. Zhang and Q.C. Fang. *Chin. Chem. Lett.*, 695 (1993).

51 G. Chauviere, D. Guenard, C. Pascard, F. Picot, P. Poiter and T. Prange *J. Chem .Soc. Chem. Comm.*, 495 (1982).

52 J. Soto, M. Fuents and L. Castedo

Phytochemistry, **43**, 313 (1996).

53. G. Appendino, P.Lusso, P. Gariboldi, E. Bombardelli and B. Gabetta.

Phytochemistry, **31**, 4259 (1992).

54. S.R. Rojatkar, B. Sinha, D.D. Sawaikar, K.B. Sonavane, G.T. Panse, T. Ravindranthan and B. A. Nagasampagi.

Ind. J. Chem., **35B**, 752 (1996).

55. S.R. Rojatkar, B. Sinha, D.D. Sawaikar, K.B. Sonavane, S.S. Pawar, G.T. Panse, T. Ravindranthan and B. A. Nagasampagi.

Ind. J. Chem., **36B**, 114 (1997).

56. S.R. Rojatkar, , D.D. Sawaikar, B. Sinha, T. Ravindranathan and B. A. Nagasampagi.

Phytochemistry, **39**, 259 (1995).

57. R.W. Miller, R.G. Powell and C.R.Smith Jr.

J. Org. Chem., **48**, 1469 (1981).

58. J.L. McLaughlin, R.W. Miler, R.G. Powel and C.R. Smith Jr.

J. Nat. Prod., **44**, 312 (1981).

59. K. Fuji, K. Tanaka, Bo Li, T. Shingu, H. Sun and T. Taga

J. Nat. Prod., **56**, 1520 (1993).

60. F. Balza, S. Tachibana, H. Barios and Towers

Phytochemistry, **30**, 1613 (1991).

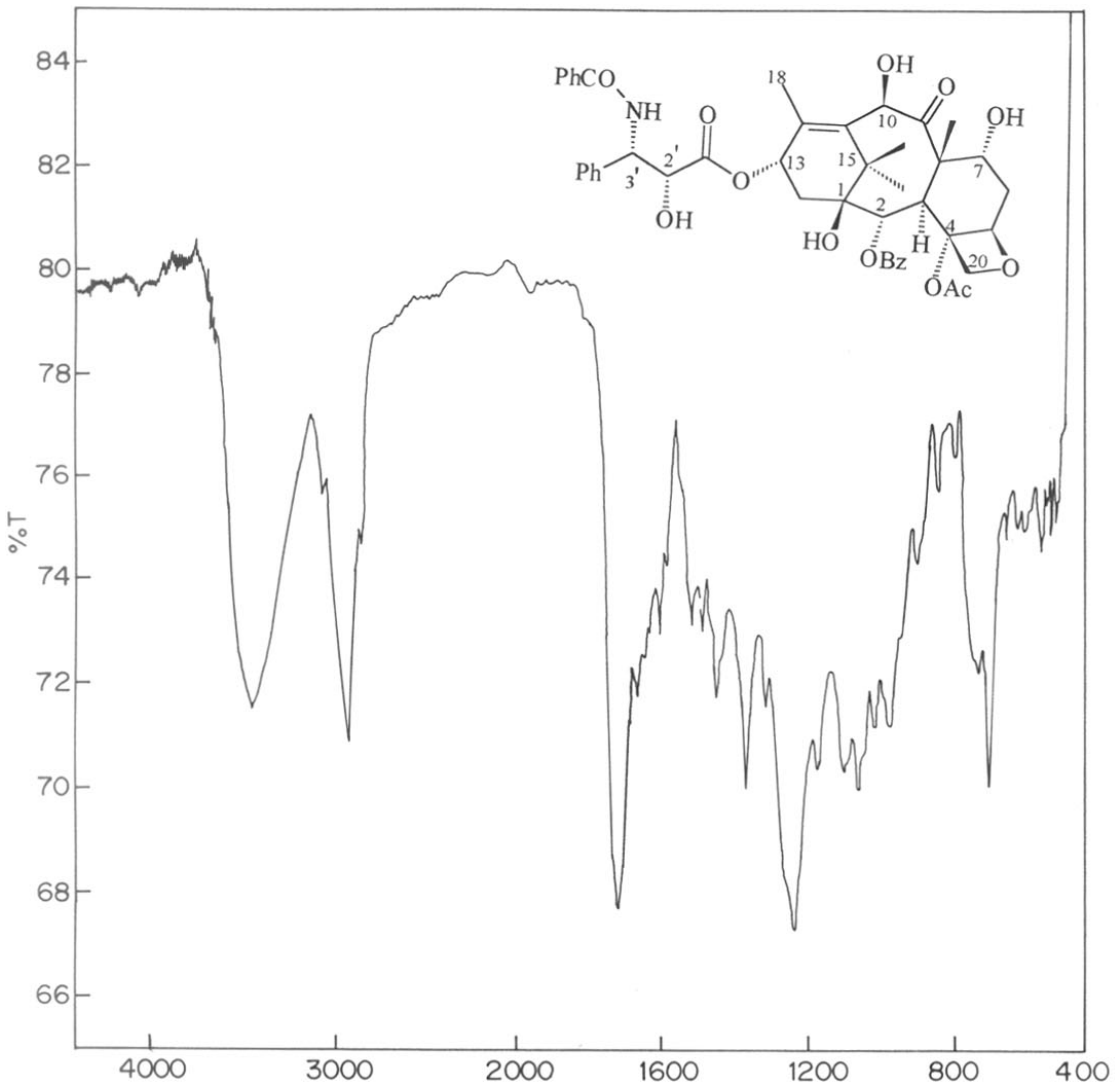


FIG 1 : FT - IR SPECTRUM OF COMPOUND I

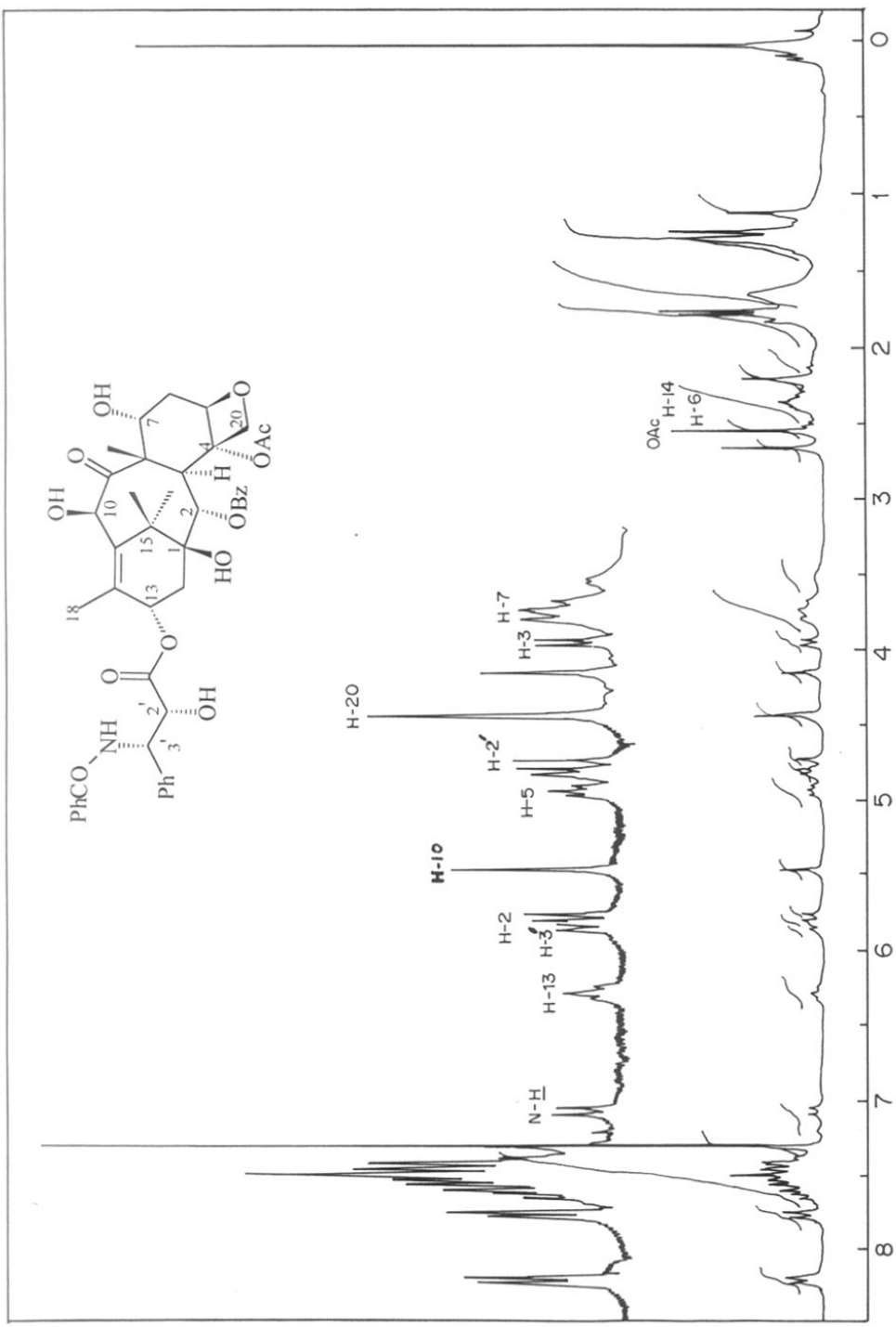


FIG 2 : ^{1}H NMR SPECTRUM OF COMPOUND I

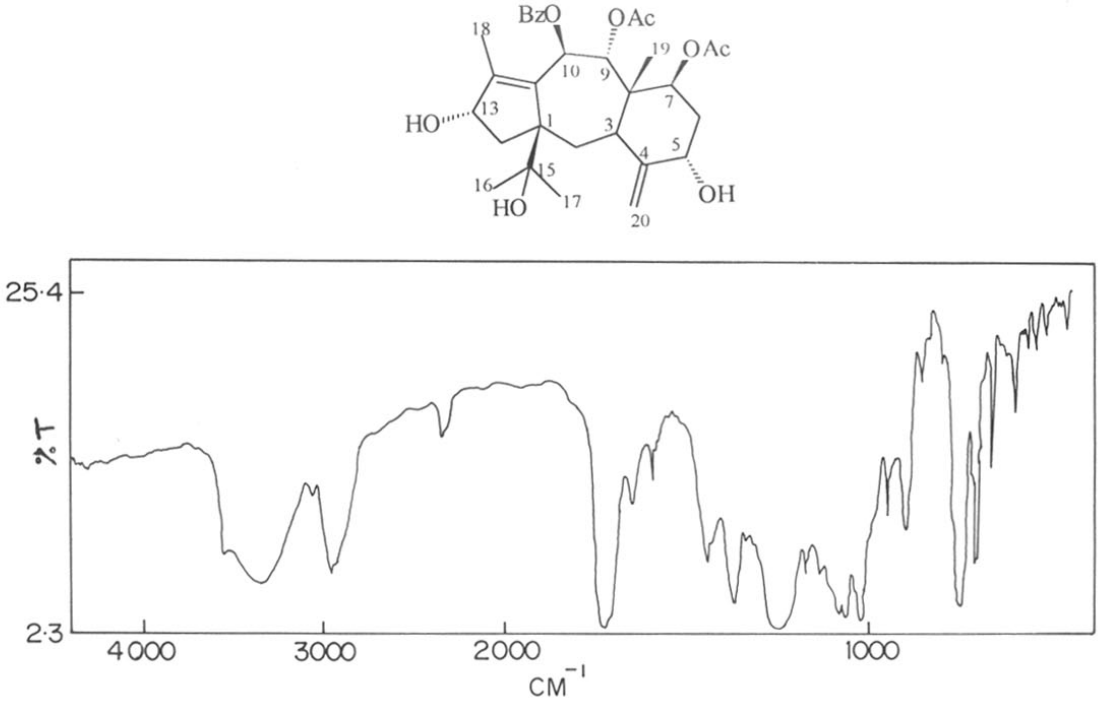


FIG 3 : FT - IR SPECTRUM OF COMPOUND II

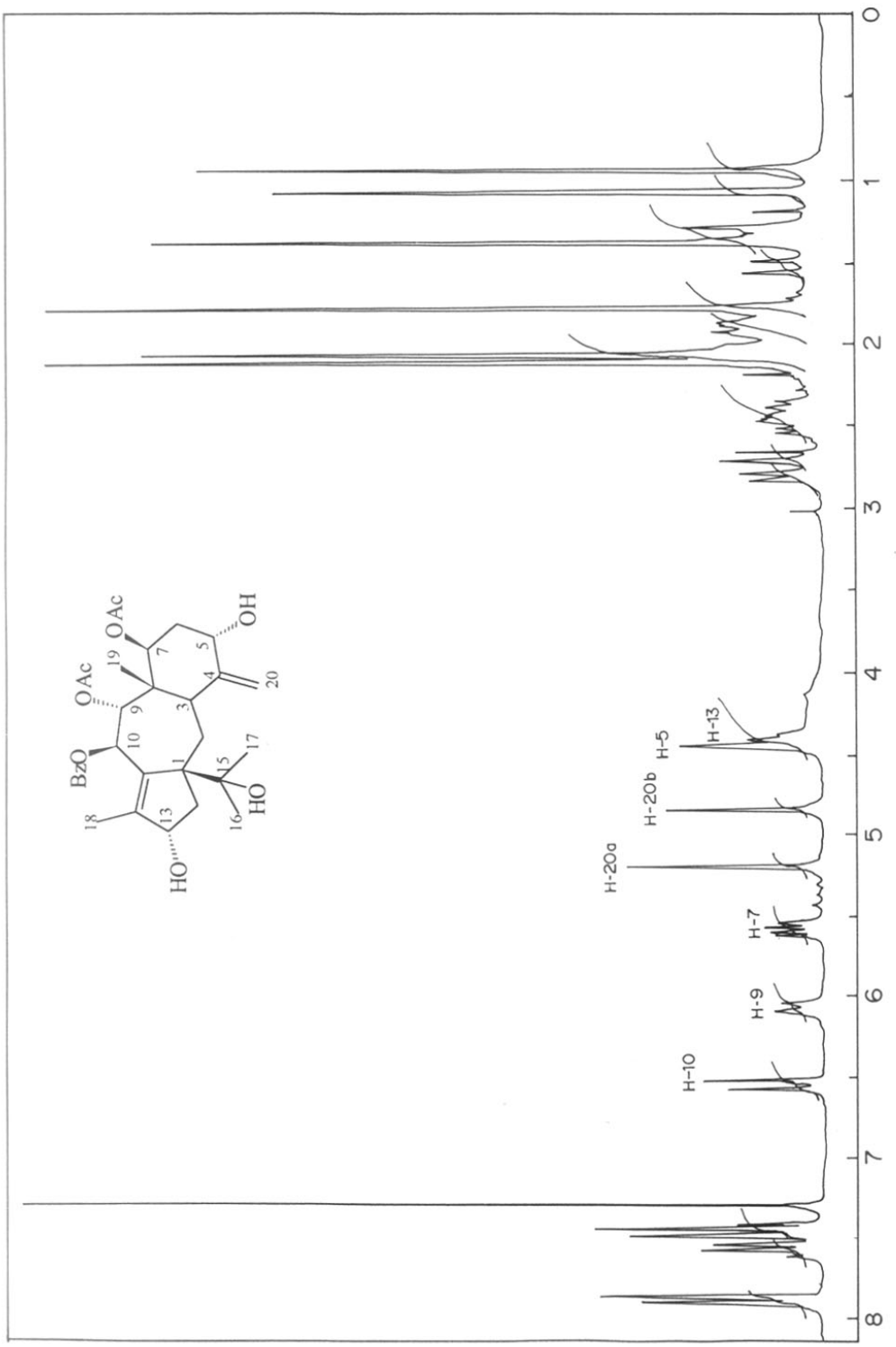


FIG 4 : ^{1}H NMR SPECTRUM OF COMPOUND II

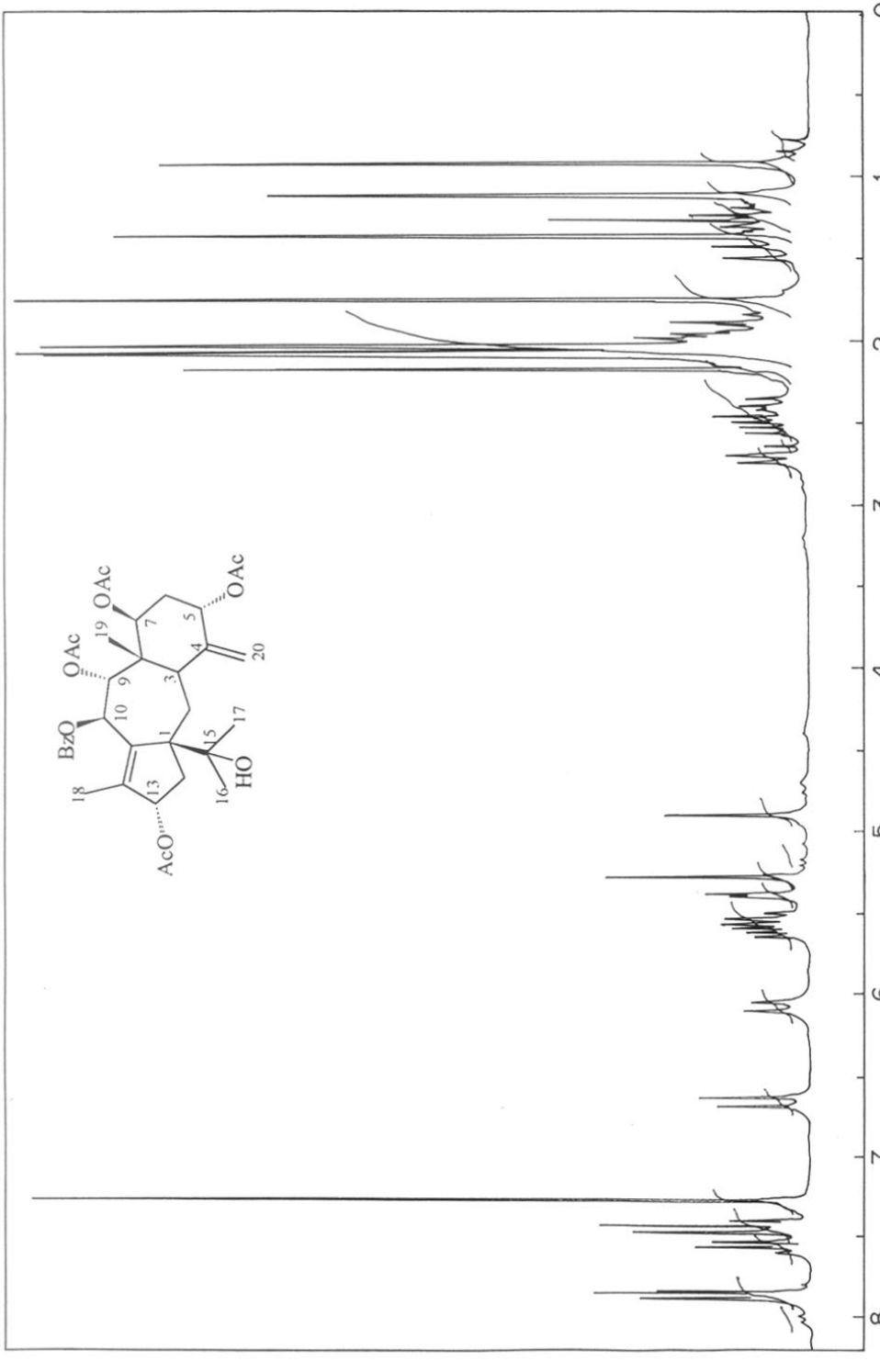


FIG 5 : ^{1}H NMR SPECTRUM OF ACETATE OF COMPOUND II

PART II

CHEMICAL INVESTIGATION

of

Asparagus racemosus

Introduction

Asparagus racemosus belongs to the family Liliaceae. The genus *Asparagus* covers well over a hundred species. *A. racemosus* is a tall spinuous, climbing, extensively branched under shrub with small white flowers which are fragrant. The roots are tuberous. This species is found in tropical and sub tropical region of India. In Ayurveda, a crude drug called 'Shatavari' comprises of dried, decorticated roots of *Asparagus racemosus* Willd. Several therapeutic attributes have been mentioned in the classical Ayurvedic literature for this drug, which has been specially recommended in cases of threatened abortion. It is reported to have antispasmodic and antioxytocin activity also. The local physicians use the root as a stimulant and restorative[1].

Previous Work

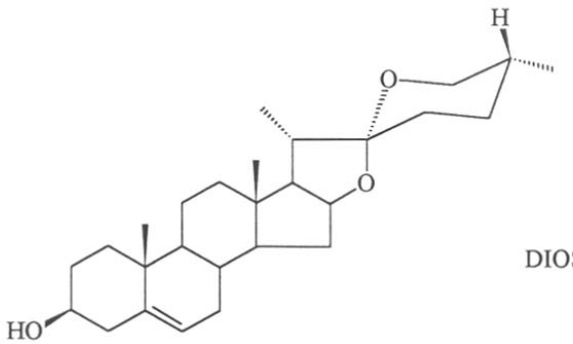
Many steroidal glycosides have been isolated from this plant. These glycosides on acid hydrolysis yielded sarsasapogenin (**1**) as major aglycone part[2]. Shatavarin-IV (**2**), a glycoside from this plant, has shown antioxytocin activity both in vivo and in vitro[3]. From its ethanol extract many mono- and di-saccharides have been isolated[4]. Diosgenin (**3**) has been reported from the leaves of *A. racemosus*[5].

The other compounds reported from this plant include alkaloids asparagamine[6] (**4**) a novel polycyclic alkaloid (Chart I) showing anticancer and uterus contracting activities[7] and 9,10-dihydroxypheanthrene[8]. Some flavonoids such as quercitin, rutin and hyperoside have also been isolated from its flowers[9].

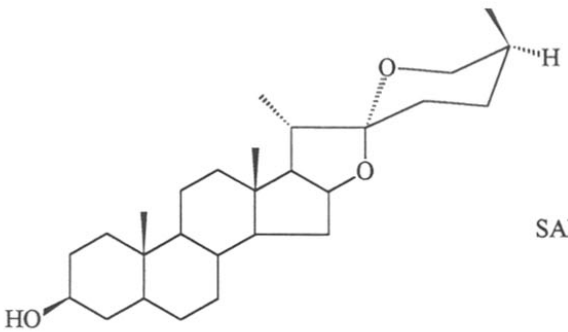
Present Work

The powdered roots of *Asparagus racemosus* collected from Karnataka, India was extracted with pet ether, acetone and methanol successively to get respective extracts. All the

CHART I

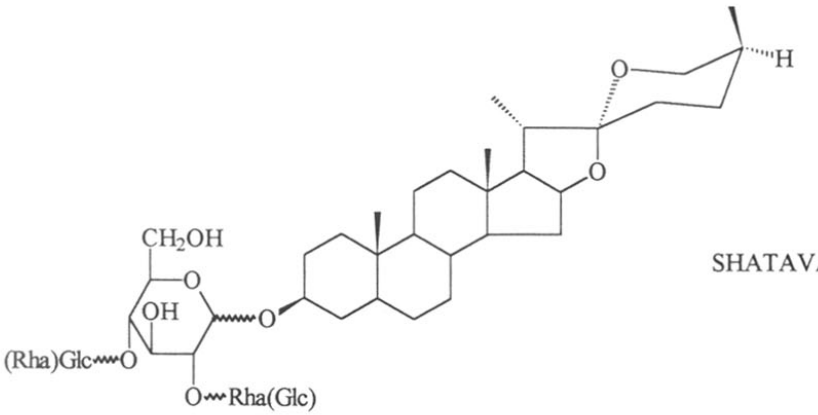


DIOSGENIN

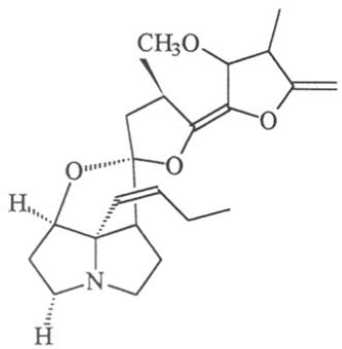


SARSASAPOGENIN

CHART I (Continued)



SHATAVARIN - IV



A NOVEL POLYCYCLIC ALKALOID

three extracts were screened for immunomodulatory activity. However pharmacological experiments could not be continued to obtain proper results with acetone extracts due to reasons beyond control. But for chemical investigation acetone extract alone was chosen (Section I).

Section-I : Chemical investigation of acetone extract

Acetone extract was chromatographed over silica gel to fractionate it into twelve broad fractions. Fraction 6 on acetylation and then further purification afforded ergosterol peroxide acetate (**I**). Ergosterol peroxide has been isolated earlier from some marine species[10-13] and it was found to be active against Walker 256 carcinomasarcoma and MCF-7 breast cells[14]. However, this is the first report of isolation of this peroxide from the terrestrial plant kingdom. Fraction 5 afforded the known compound Sarsasapogenin (**II**) which was earlier isolated by the acid hydrolysis of total extract as well as from pure glycosides. The structures of both the compounds were elucidated by extensive spectroscopic analysis and correlation.

Characterisation of compound I

Compound **I**, m.p. 193-195°, $[\alpha]_D^{25}$ -15°, showed in its mass spectrum, the molecular ion peak at m/e 470 corresponding to the possible molecular formula $C_{30}H_{46}O_4$ along with a peak at m/e 410 $[M-60]^+$. It showed in its IR spectrum (Fig.1) absorption at 1735 cm^{-1} for an ester carbonyl and 1640 cm^{-1} for unsaturation. The ^1H NMR spectrum (Table 1. Fig. 2) of the compound exhibited two angular methyls at δ 0.83, δ 0.94 and four secondary methyls at δ 0.90, 0.92, 1.02, and 1.04 along with an acetate methyl at δ 2.05. The spectrum further showed an AB quartet for two protons in the down field region at δ 6.23 and 6.52 (J = 8.5 Hz) and two doublet of doublets at δ 5.20 (J = 8, 15 Hz) and δ 5.15 (J = 8.5, 15) for one proton each along with a signal at δ 5.0 (dddd, J = 8, 8, 4, 4 Hz) accounting for one proton revealing that the proton on

carbon bearing acetoxy group is axial, i.e. α-oriented. The pattern of methyl signals clearly indicated that the compound **I** belonged to the class of steroids.

The ^{13}C NMR spectrum (Table 2, Fig. 3) of the compound showed only one signal in carbonyl carbon region at δ 170.3 which has to be due to acetate carbonyl carbon. The spectrum along with INEPT experiment (Fig. 4) further showed four vinyl carbons as doublets at δ 131.2, 132.6, 135.3 and 135.4 indicating the presence of two di-substituted double bonds, one oxygenated carbon at δ 69.8 as a doublet assignable to C-3, the most favourable position for hydroxyl or acetoxyl group in a steroid molecule, two oxygenated carbons at δ 79.6 and δ 81.9 as singlets and an acetate methyl carbon at δ 20.2 along with six methyl carbon signals. The spectrum also showed seven carbons as doublet and remaining two as singlets.

The ^{1}H-^{1}H COSY experiment (Fig. 5) showed clearly that the olefinic protons at δ 5.20 and 5.15, which coupled with each other and with the protons in methylene region. The other two olefinic protons at δ 6.23 and 6.52 were found to be coupling with one another only, revealing the olefinic bond to be between tetra substituted carbon atoms. The downfield chemical shift of these protons indicated that these tetra substituted carbon atoms must be on oxygenated carbon atoms. This indicated the presence of a peroxide group as in the case of ergosterol peroxide. Comparison of the spectral data of **I** with those of reported data of ergosterol peroxide[12] confirmed the structure of I as ergosterol peroxide-3 acetate.

Table 1 : ^{1}H NMR spectral data of compound **I**

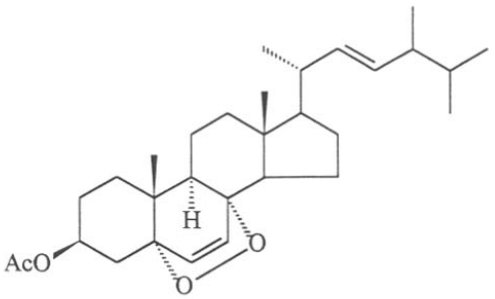
Proton	Chemical shift in δ	Multiplicity	Coupling constant in Hz
H-3	5.05	dddd	8,8,4,4
H-6	6.23	d	8.5
H-7	6.52	d	8.5
H-18	0.83	s	
H-19	0.94	s	
H-21	1.02	d	6.5
H-22*	5.20 (1H)	dd	8.5,15
H-23*	5.15 (1H)	dd	8.5,15
H-26	0.90	d	6.5
H-27	0.92	d	6.5
H-28	1.04	d	6.5
OAc	2.05	s	

* appearing like triplet

Table 2: ^{13}C NMR spectral data of compound **I**

Carbon	Chemical shift in δ	Multiplicity
C-1	28.8	t
C-2	34.6	t
C-3	69.8	d
C-4	39.9	t
C-5	79.6[b]	s
C-6	135.4[a]	d
C-7	135.3[a]	d
C-8	81.9[b]	s
C-9	33.4	d
C-10	37.3	s
C-11	20.9	t
C-12	33.5	t
C-13	44.8	s
C-14	51.9	d
C-15	26.5	t
C-16	23.6	t
C-17	56.5	d
C-18	13.1	q
C-19	17.8	q
C-20	39.6	d
C-21	21.5[c]	q
C-22	131.2[d]	d
C-23	132.6[d]	d
C-24	51.4	d
C-25	43.1	d
C-26	18.3[e]	q
C-27	19.9[e]	q
C-28	21.1[c]	q
$O\underline{C}OCH_3$	170.3	s
$OCO\underline{C}H_3$	20.2[c]	q

a-e are interchangeable



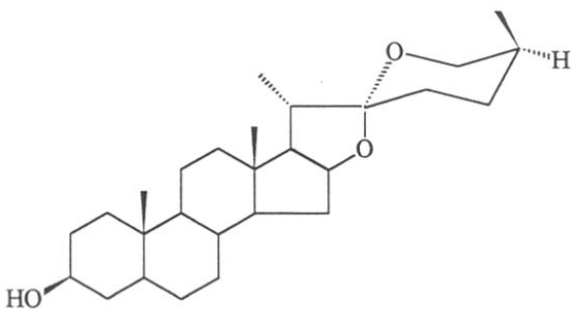
This is the first report of ergosterol peroxide from *Asparagus racemosus*.

Characterisation of compound II

Compound **II** was purified by re-crystallisation from fraction 5, m.p. 193-194°, $[\alpha]_D^{25}$ -78°. The mass spectrum of the compound showed molecular ion peak at m/e 416 suggesting the molecular formula $C_{27}H_{44}O_3$. The IR spectrum (Fig. 6) showed an absorbance for hydroxyl group at 3400 cm^{-1}. The ^1H NMR spectrum (Table 3, Fig. 7) of the compound showed two angular methyls at δ 0.75 and δ 0.95 and two secondary methyls at δ 1.03 and δ1.07 as doublets (J = 7.5 Hz). It further showed a broad multiplet for a proton on oxygenated carbon at δ 4.40 and also showed another oxygenated proton at δ 4.10 as broad singlet. Compound **II** on acetylation revealed in its ^1H NMR spectrum (Fig. 8), besides acetate methyl, a down field shift of signal at δ 4.10 to δ 5.10 indicating that this signal was due to the proton on hydroxy bearing carbon atom. The ^1H NMR spectrum of the compound **II** also showed two doublet of doublets, one at δ 3.95 (J = 8.5, 1.5 Hz) and another at δ 3.32 (J = 8.5, 1. Hz) accounting for one proton each. The chemical shift and coupling constant values of this doublet of doublet signals suggest the presence of a methylene attached to oxygen.

The ^{13}C NMR spectrum (Table 4, Fig. 9) of compound showed 25 signals out of which two at δ35.6 and δ26.8 were accountable for two carbon atoms each. The presence of 27 carbon atoms and four methyl signals in ^{1}H NMR spectrum clearly indicated that compound **II** was a steroid. The hydroxyl group present in compound was assigned at most favourable position C-3. The ^{13}C NMR spectrum along with its INEPT experiment (Fig. 10) further revealed a hemiacetal carbon atom at δ110 as singlet, a hydroxyl bearing carbon atom at δ 81.3, a methylene carbon attached to oxygen at δ 65.4 and a carbon at δ 67.3 as doublet. It further revealed four methyls, ten methylenes and three methines.

From these spectral data the structure of compound **II** was elucidated as sarsasapogenin.



The physical constants and spectral data of compound **II** were in agreement with those reported for sarsasapogenin[15].

This is the first report of isolation of Sarsasapogenin as aglycone from this plant. However it has been isolated from this plant by the hydrolysis of plant extract or purifying glycosides and hydrolysing them.

Table 3 : ^{1}H NMR spectral data of compound **II**

Proton	Chemical shift δ	Multiplicity	Coupling constant in Hz
H-3	4.10	brs	
H-16	4.40	m	
H-18	0.75	s	
H-19	0.95	s	
H-21	1.03	d	7.5
H-26α	3.32	dd	8.5, 1
H-26β	3.95	dd	8.5, 1.5
H-27	1.07	d	7.5

Table 4: ^{13}C NMR spectral data of compound **II**

Carbon	Chemical shift in δ	Multiplicity
C-1	30.0[a]	t
C-2	32.0[a]	t
C-3	67.3	d
C-4	33.8[a]	t
C-5	36.8[b]	d
C-6	21.2	t
C-7	26.1	t
C-8	35.6[b]	d
C-9	40.2[b]	d
C-10	35.6	s
C-11	26.8[a]	t
C-12	26.8[a]	t
C-13	40.9	s
C-14	27.4[b]	d
C-15	28.1[a]	t
C-16	81.3	d
C-17	56.8	d
C-18	16.3[c]	q
C-19	14.5	q
C-20	62.4	d
C-21	24.2	q
C-22	110.0	s
C-23	40.6	t
C-24	26.2[a]	t
C-25	42.4	d
C-26	65.4	t
C-27	16.7[c]	q

a-c are interchangeable

EXPERIMENTAL

Asparagus racemosus roots were collected from Karnataka during December 1995 and powdered. The powder (2 Kg.) was extracted successively with pet ether, acetone and methanol. The extracts were concentrated separately under reduced pressure to yield pet ether extract (6 g), acetone extract (5.6 g) and methanol extract (26 g).

The acetone extract (5.0 g.) was subjected to column chromatography over silica gel (60-120 mesh). The elution was started with pet-ether and continued with the mixture of acetone : pet-ether (5 : 95) and then with successive increase in the percentage of acetone. The fractions showing similar pattern on TLC were combined to obtain twelve major fractions (Table 5).

Isolation of Compound I

Fraction 6 (390 mg) was dissolved in 2 ml pyridine and 2 ml of acetic anhydride and kept overnight at room temperature. After usual working up, the crude product was subjected to preparative TLC in ethyl acetate : benzene (20:80) to isolate the compound **I** (38 mg) as white solid.

m.p.	:	193° - 195°
$[\alpha]_D^{25}$	:	-15° ($CHCl_3$, c 0..12)
IR ($CHCl_3$)	:	1735, 1640 cm^{-1} (Fig. 1)
^1H NMR	:	Table 1, (Fig. 2)
^{13}C NMR	:	Table 2, (Fig. 3, 4)
Mass (rel.int)	:	m/e 470$[M]^+$(2), 410(3), 378(10), 253(5), 197(5), 157(10), 145(8), 129(9), 107(8), 109(10), 95(22), 81(48), 50 (100).

Isolation of compound II

Fraction 5 (600 mg) was purified by preparative TLC using benzene : ethyl acetate (20 : 80) as eluent to obtain compound **II** in fairly pure form and further purified by crystallisation in pet ether-acetone to get crystalline solid (35 mg).

m.p.	:	193°-94°
$[\alpha]_D^{25}$	:	-78° ($CHCl_3$, c 0.5)
IR ($CHCl_3$)	:	3400, 1060 cm^{-1} (Fig. 6)
^1H NMR	:	Table (3) (Fig. 7)
^{13}C NMR	:	Table (4) (Fig. 9)
Mass (rel. int)	:	m/e 416 $[M]^+$(5), 398 (16), 362 (16), 178 (11), 166 (100), 120 (90), 166 (100), 107 (78), 91 (40), 55 (71).

Table 5 : Column chromatography of acetone extract

Eluent	Total volume collected	Wt. of final fraction in g	Approximate composition
Pet ether	100 ml X3	0.210	Straight chain hydrocarbons
Pet ether : Acetone 95 : 5	100 ml X 4	0.320	Straight chain hydrocarbons
Pet ether : Acetone 90 : 10	100 ml X 4	0.285	Unidentified compounds
Pet ether : Acetone 85 : 15	100 ml X 3	0.225	Unidentified compounds
Pet ether : Acetone 80 : 20	100 ml X 5	0.615	Compound **II**
Pet ether : Acetone 75 : 25	100 ml X 5	0.395	Compound **I**
Pet ether : Acetone 65 : 35	100 ml X 4	0.410	Complex mixture of unidentified compounds
Pet ether : Acetone 55 : 45	100 ml X 4	0.410	Complex mixture of unidentified compounds
Pet ether : Acetone 45 : 55	100 ml X 3	0.390	Sugars and Glycosides
Pet ether : Acetone 25 : 75	100 ml X 3	0.410	Glycosides
Acetone	100 ml X 3	0.550	Glycosides
Methanol	100 ml X 3	0.560	Glycosides

Section-II

Screening of extracts for immunomodulatory activity

Immune system

The immune system comprises of natural resistance and acquired defence mechanism. The interrelationship between natural resistance and acquired defence mechanism is illustrated in scheme 1.

The components that are involved in immune responses are Phagocytes, T- Lymphocytes, B- Lymphocytes, Null-Cells and Complement. The mechanism of action and functions of these components are explained in table 6.

Immunomodulation

Any procedure, which can alter the immune system of an organism interfering its function, is called as immunomodulation. If it results in the enhancement of the immune reaction, it is termed as immunostimulation and if it results in the decrease in host resistance it is termed as immunosuppression. The agents involved in causing these changes are called immunostimulant and immunosuppressant respectively.

Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. Hence immunostimulation and immunosuppression have their own standing and search for better agents exerting these activities is becoming an interesting field [16].

Many chemical agents available today have immunomodulatory activties. But simultaneously they have cytotoxic action and exert a variety of side effects. This has stimulated the search for natural resources showing immunological activity.

In traditional Ayurvedic medicine roots of Shatavari are claimed to have many beneficial effects against dysentery, tumour, inflammation and epilepsy. *Asparagus racemosus* was also reported to produce leucocytosis, predominant nautrophilia, increased phagocytic activity and intracellular bactericidal capacity of polymorphs[17]. It was also shown to reverse immunosuppression caused by cyclophosphamide[17].

Scheme 1 : Defence mechanism

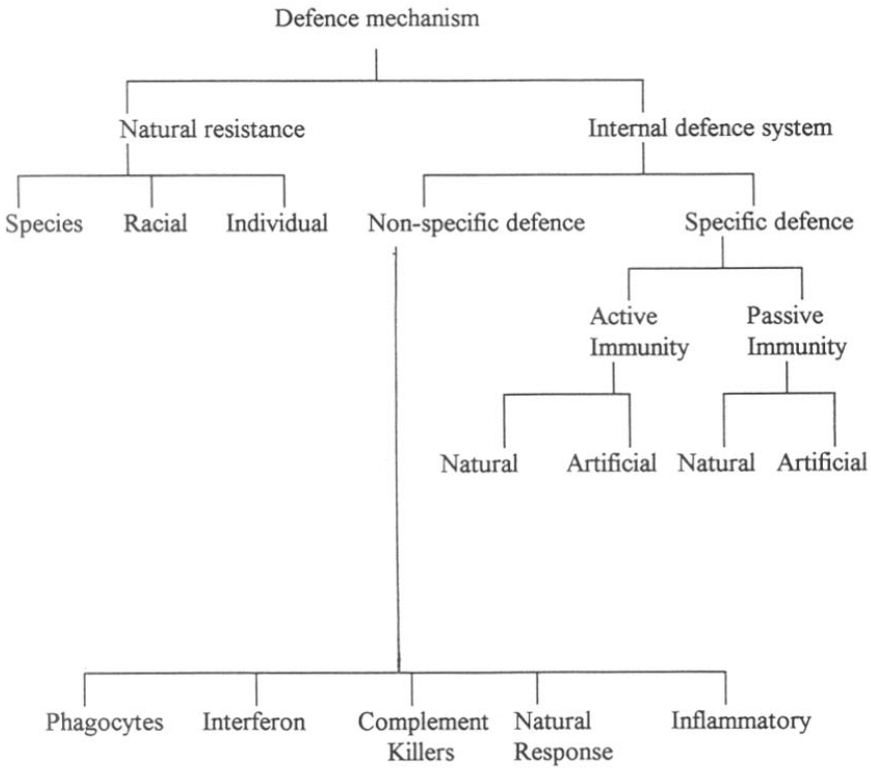


Table 6 : The Immune system

Component	Mechanism of action	Function
Phagocytes	1. Phagocytosis of the foreign particle → Intracellular destruction of foreign particle Processing of foreign particle Presentation of foreign particle to T/B cell	1. First line of defence 2. Modulates T/B cell function
	2. Phagocytosis of Ag-Ab complexes	
	3. Release of cytokines	
T-Lymphocyte	1. Contact with antigen → Blastoid transformation → Release of lymphokines	*Cellular immynity* 1. Protection against virus, fungi, intracellular bacteria
	2. Activation of macrophages	2. Immunity against cancer and transplants
	3. Modulation of B-cell function	
B-Lymphocyte	1. Contact with antigen → Blastoid transformation → Liberation of antibodies → Ag-Ab complexes → Phagocytosis by macrophages Activation of complement Activation of killer cells	*Humoral Immunity* 1. Protection against extracellular bacteria
Null - cells	1. Killer cells react with Ag-Ab complexes	1. Antibody dependent cell mediated cytotoxicity
	2. Natural killer cells	2. Non-specific killing of tumour cells, virally transformed cells
Complement	1. Activated by Ag-Ab complexex or by alternate pathway → Inflammatory response Enhanced phagocytosis Chemotaxis Immune adhrence Virus neutralisation	1. Enhancing the action of other components of the Immune system

Present work:

With view to test immunomodulatory activity of the roots, its solvent extracts were screened.

Experimental:

The screening was carried out in the Pharmacology Department of B. J. Medical College, Pune, India. Albino mice were used as experimental models for different tests. Mice were grouped in to three groups A, B, C and control group. During the experiment all the mice were allowed to acclimatise in the same environment and were given the same type of oral feeds. **Group A** was tested with **pet ether extract, Group B** with **methanol extract** and **Group C** with **acetone extract**. Since the screening experiments of the acetone extract could not be completed to obtain conclusive results only the results of pet ether and methanol extracts are reported here.

The effect of the extracts on the different components of immune system were assessed with help of three tests viz. Body weights, Leucocyte counts and Mortality of *Escherichia Coli* infected mice.Each test was performed on different sets of mice.

Body weights : Each mouse was weighed on the day prior to onset of pre-treatment. Feeding was done for 28 days. The weights were observed on 28^{th} day. Initial and final body weights were compared. Results are tabulated in table 7.

Mortality test : This test was performed on albino mice fed with extracts for 28 days .On 28^{th} day the mice were infected by intraperitoneal injection of *E. Coli*. The mortality was observed after 16 hours and 24 hours. The results are tabulated in table 8.

Table 7 : Weight of animal (Mean ± SEM)

(n = Number of mice)

	Before T/t (g)	After T/t (g)
Group A (n = 6)	48.17 ± 3.66	48.83 ± 4.88
Group B (n = 8)	46.88 ± 4.12	51.25 ± 3.81*

* $p < 0.05$ as compared to pre-treatment

(p = Index of significance)

Table 8 : Mortality on intraperitoneal injection of *E. Coli*

(n = Number of mice)

	At 16 hours		At 24 hours	
	%	Absolute	%	Absolute
Control (n = 6)	0	0	100	6
Group A (n = 6)	83.33	5	100	6
Group B (n = 8)	25.0	2	25.0	2

Table 9 : Leucocyte counts (Mean ± SEM)

(n = Number of mice)

	Control (n = 6)	Group A (n = 6)	Group B (n = 6)
Total leucocyte count (/mm^3)	7662.5 ± 1084.4	*** 4766.6 ± 855.4	**5001.6 ± 102.6
Lymphocyte % count	54.1 ± 10.0	44.6 ± 10.4	51.3 ± 3.0
Absolute count (/mm^3)	4092.3 ± 562.5	*2178.2 ± 819.2	2543.5 ± 493.2
Neutrophils % count	30.0 ± 8.0	27.5 ± 10.8	30.0 ± 4.0
Absolute count (/mm^3)	2305.5 ± 706.9	1279.5 ± 420.7	1505.2 ± 392.7
Eosinophils % count	4.0 ± 2.9	7.2 ± 5.2	2.6 ± 2.4
Absolute count (/mm^3)	311.4 ± 218.2	355.5 ± 286.8	126.2 ± 114.5
Monocytes % count	12.0 ± 6.4	*20.8 ± 7.0	16.3 ± 4.27
Absolute count (/mm^3)	956.5 ± 612.4	949.4 ± 297.6	804.8 ± 296.5

* $p < 0.05$ }
** $p < 0.0$ } as compared to control.
*** $p < 0.001$ }
(p = index of significance)

Leucocyte counts : Mice were pre-treated with extracts for 28 days. A definite volume of blood of mice was taken and diluted appropriately. The number of white cells in definite volume of the mixture was counted. Leucocytes /mm^3 was calculated by formula

$$\text{Leucocytes/mm}^3 = \frac{\text{Total no. of cells counted x 200}}{\text{No of large squares counted}}$$

Differential leucocytes counts were done by preparing a smear by spreading a small drop of blood on a clean glass slide, and staining it with hymeatoxylin and Eosin The chosen area was examined under oil immersion lens to measure the absolute and percentage counts of lymphocytes, neutrophils, eosinophils and monocytes. Results are tabulated in Table 9.

Conclusion

From the effect of the extracts on mortality of the mice following intraperitoneal injection of *E. Coli*, it may be inferred that pet ether extract showing increased mortality as compared to controls may act as a suppressant of non-specific host resistance. Where as methanol extract causing a much lower mortality may be a stimulant of non-specific host resistance. The exact arm of the immune response on which they act, needs to be investigated further.

Both the extracts decrease the total WBC count significantly ($p<0.001$,$p<0.01$) a surprising finding especially in the light of their opposite effects on non specific host resistance. A more important finding may be the significant decrease in absolute lymphocyte count as compared to control. Methanol extract decreases lymphocyte count but not significantly. This finding may explain the non-specific immunosuppressant effect of the pet ether extract. The immunostimulant effect of methanol extract however cannot be explained by the decrease in

count. One of the possible reason could be an increase in activity of lymphocytes or increased sequestration of lymphocytes in the reticulo endothelial system to be released whenever required.

REFERENCES

1. *Wealth of India, Raw Materials*, Publication and Information Directorate, CSIR, New Delhi, Vol I, p. 470 (1985)

2. S.B. Rao

   Ind. J. Pharm. Chem., **14**, 131 (1952).

3. P. Ravikumar , R. Soman , G.L. Chetty , R.C. Pandey and Sukh Dev

   Ind. J. Chem., **26B**, 1012 (1987).

4. A.B. Landage and J.L. Bose

   Ind. J. Chem., **8**, 588 (1970).

5. S.S. Subramanian and A.G.R. Nair

   Curr.Sci., **38**, 414 (1969).

6. S.T. Fukasawa, K.Y. Nakabu, R. Nijsiri and I. Murakoshi

   Chem. Pharam. Bull., **42**, 1360 (1994).

7. I. Murakoshi and J. Fuji

   Jpn.Kokai Tokyo Koho JP 07,330,774(95,330,774)

   (Cl C07 D 491/22),19 Dec.1995

   Appl.94/151,807 10 Jan. 1994, 6 pp (Japan)

   CA : 124:270547y

8. S.T. Fukasawa, I.N. Murakoshi and R. Nijsiri

   Phytochemistry , **44**, 736 (1997).

9. S.S. Subramanian and A.G.R. Nair

   Curr. Sci., **37**, 287 (1968).

10. C. Jimenez, E. Quinoa, L. Castedo and R. Riguera

J. Nat. Prod., **49**, 905 (1986).

11. L.A. Gunatilaka, A.Gopichand, Y. Schmitz and C. Dejerassi

J. Org. Chem., **46**, 3860 (1981).

12. Y. Sheikh and C. Dejerassi

Tetrahedron, **30**, 4095 (1974).

13. A.S.R Anjaneyulu, K.S. Sagar and C.V.S Prakash

Ind. J. Chem., **35B**, 819 (1996).

14. K. Kahlos, L. Kangas and R. Hiltunen

Planta Medica, **55**, 389 (1989).

15. K. Tori, S. Seo, Y. Terui, J. Nishikawa and F. Yasuda

Tet. Lett., 2407 (1981).

16. B. Patwardhan, P.S. Patki, B.A.Nagasampagi and D.Kalbag

Indian Drugs, **28**, 56 (1990) and references cited therein.

17. U.N. Thatte and S.A. Dahanukar

Phytotherapeutic Research, **3**, 43 (1989) and references cited therein.

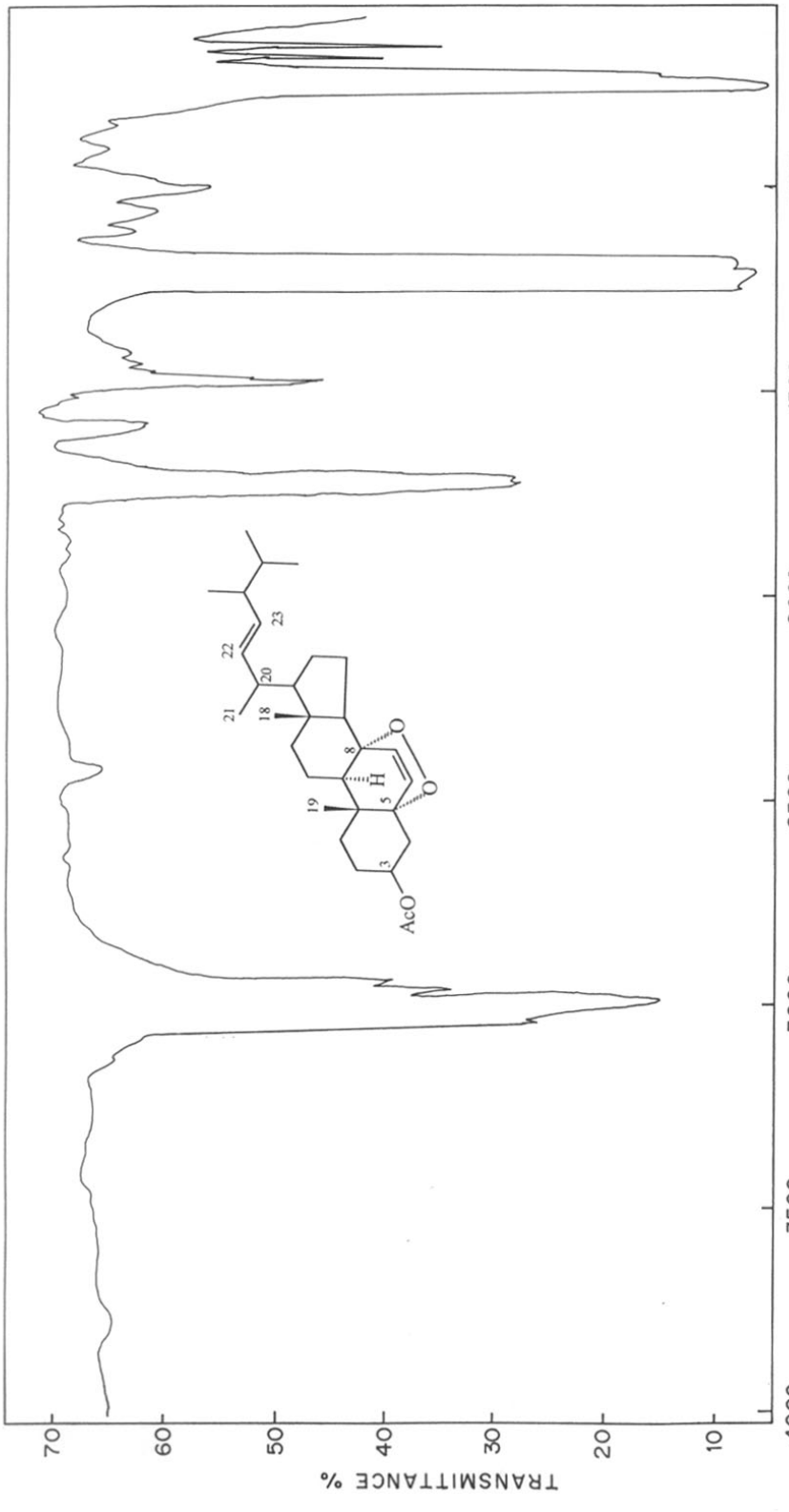


FIG 1 : FT - IR SPECTRUM OF COMPOUND I

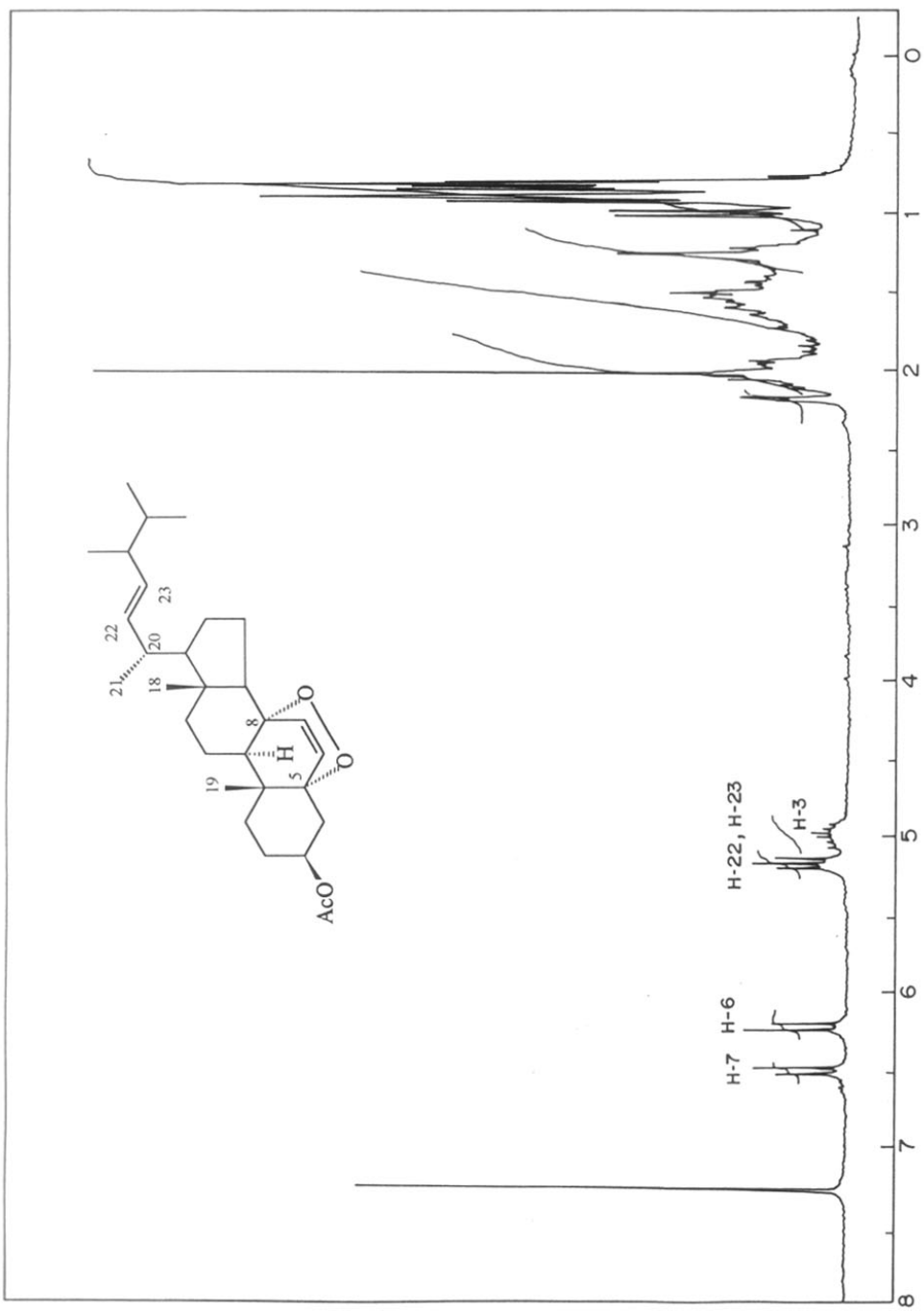


FIG 2 : ^{1}H NMR SPECTRUM OF COMPOUND I

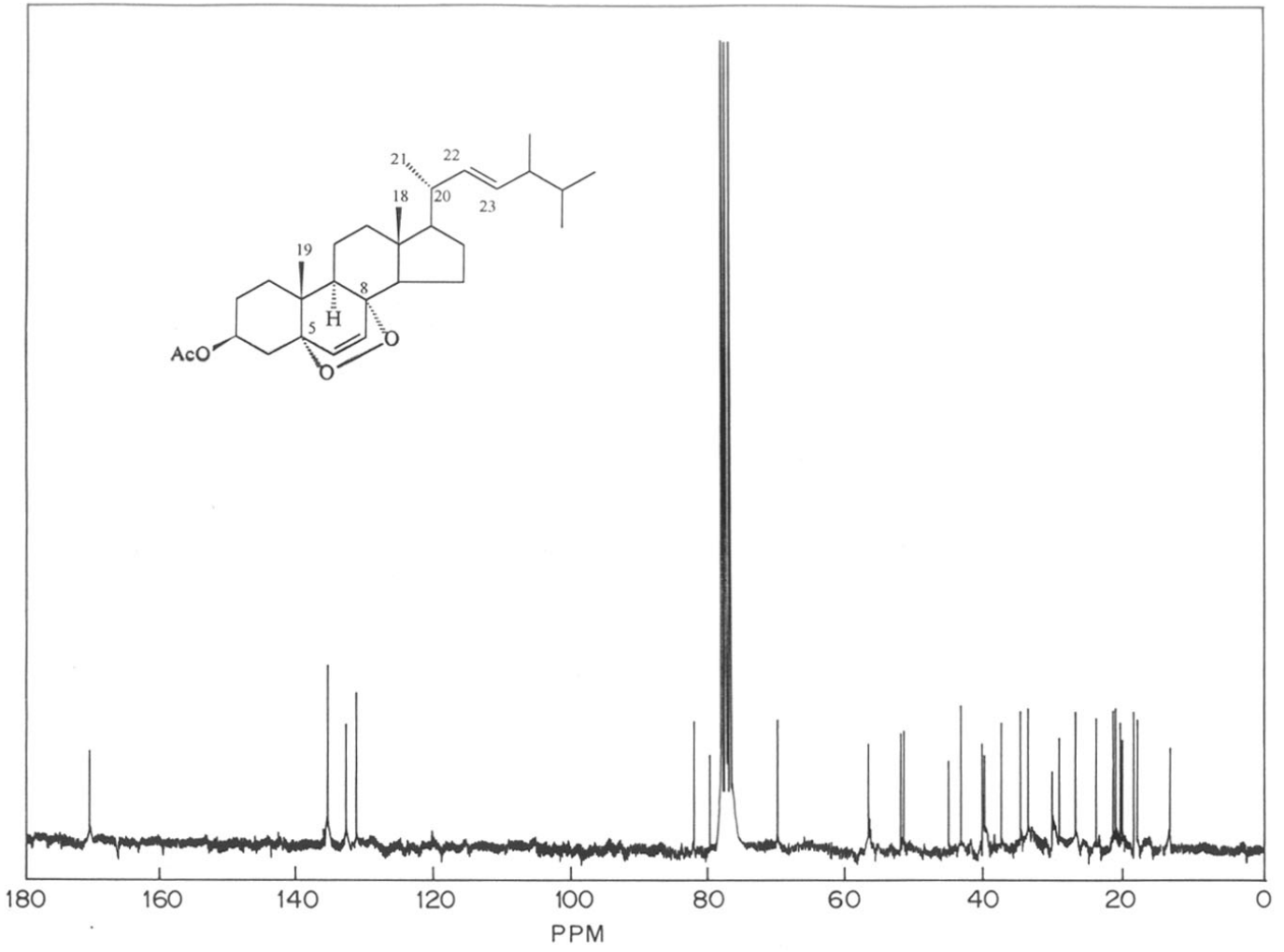


FIG 3 : ^{13}C NMR SPECTRUM OF COMPOUND I (50.32 MHz)

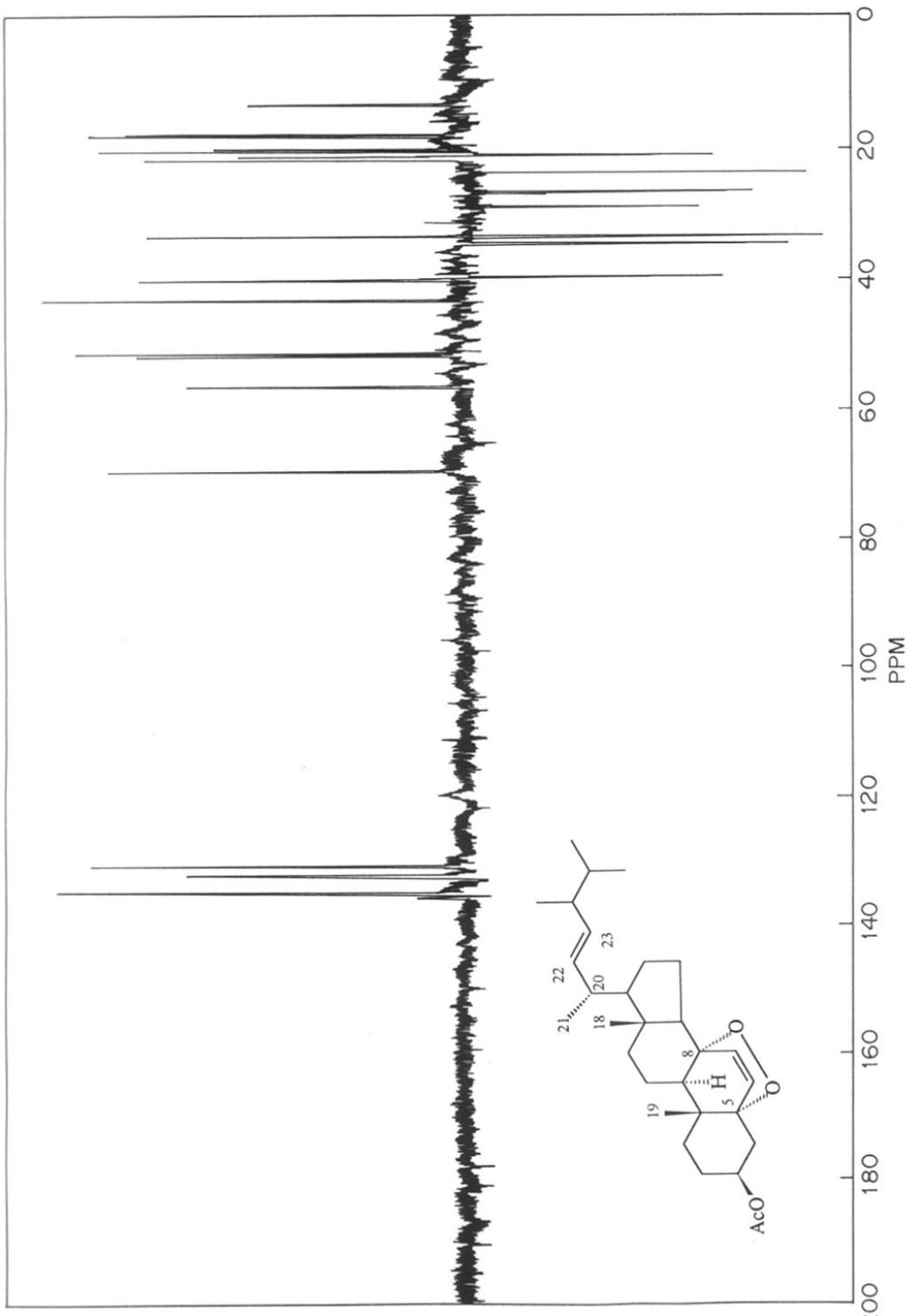


FIG 4 : ^{13}C NMR SPECTRUM (INEPT) OF COMPOUND I (50.32 MHz)

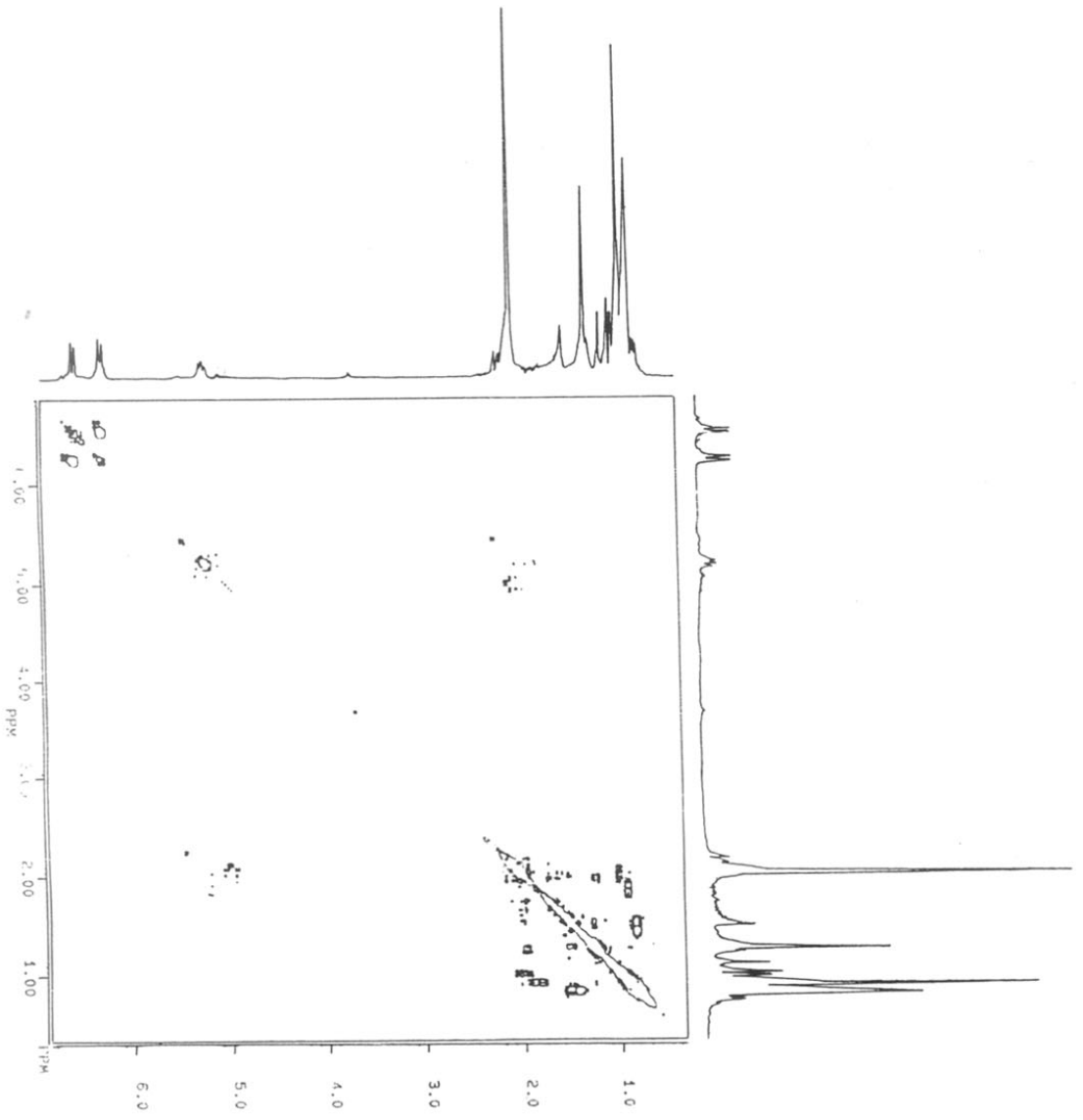


FIG 5 : $^{1}H - {}^{1}H$ 2D COSY EXPERIMENT OF COMPOUND I

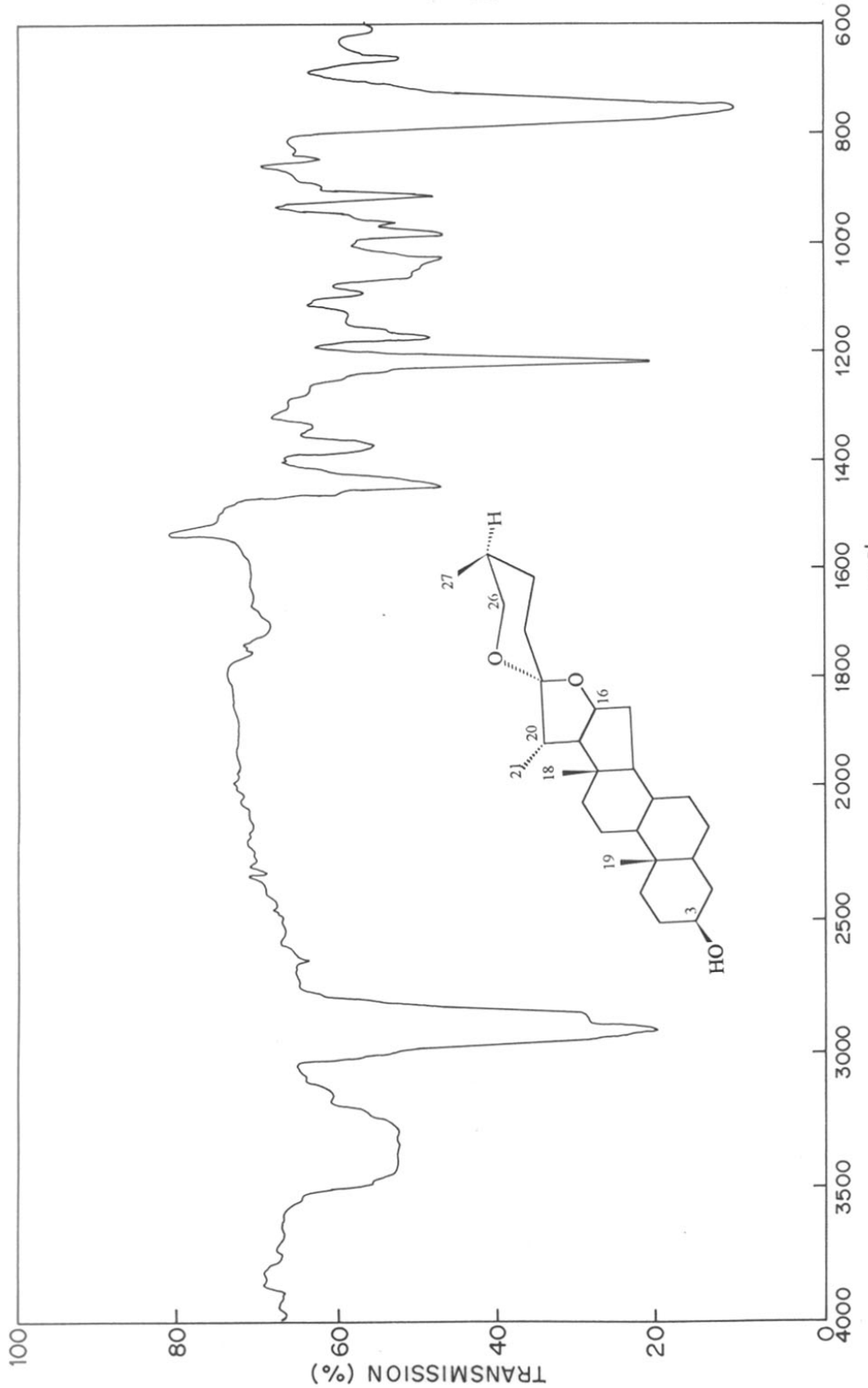


FIG 6 : IR SPECTRUM OF COMPOUND II

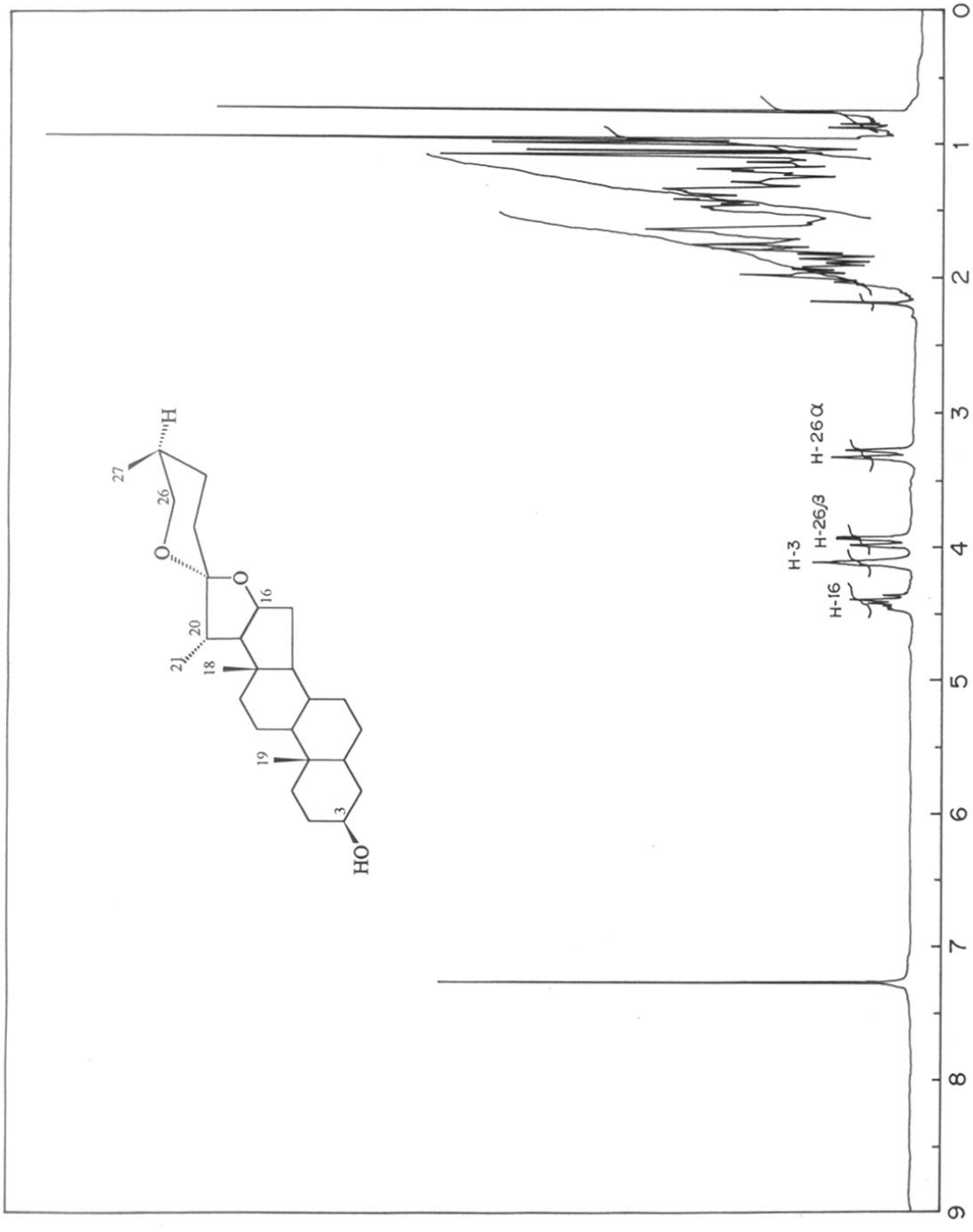


FIG 7 : ^{1}H NMR SPECTRUM OF COMPOUND II

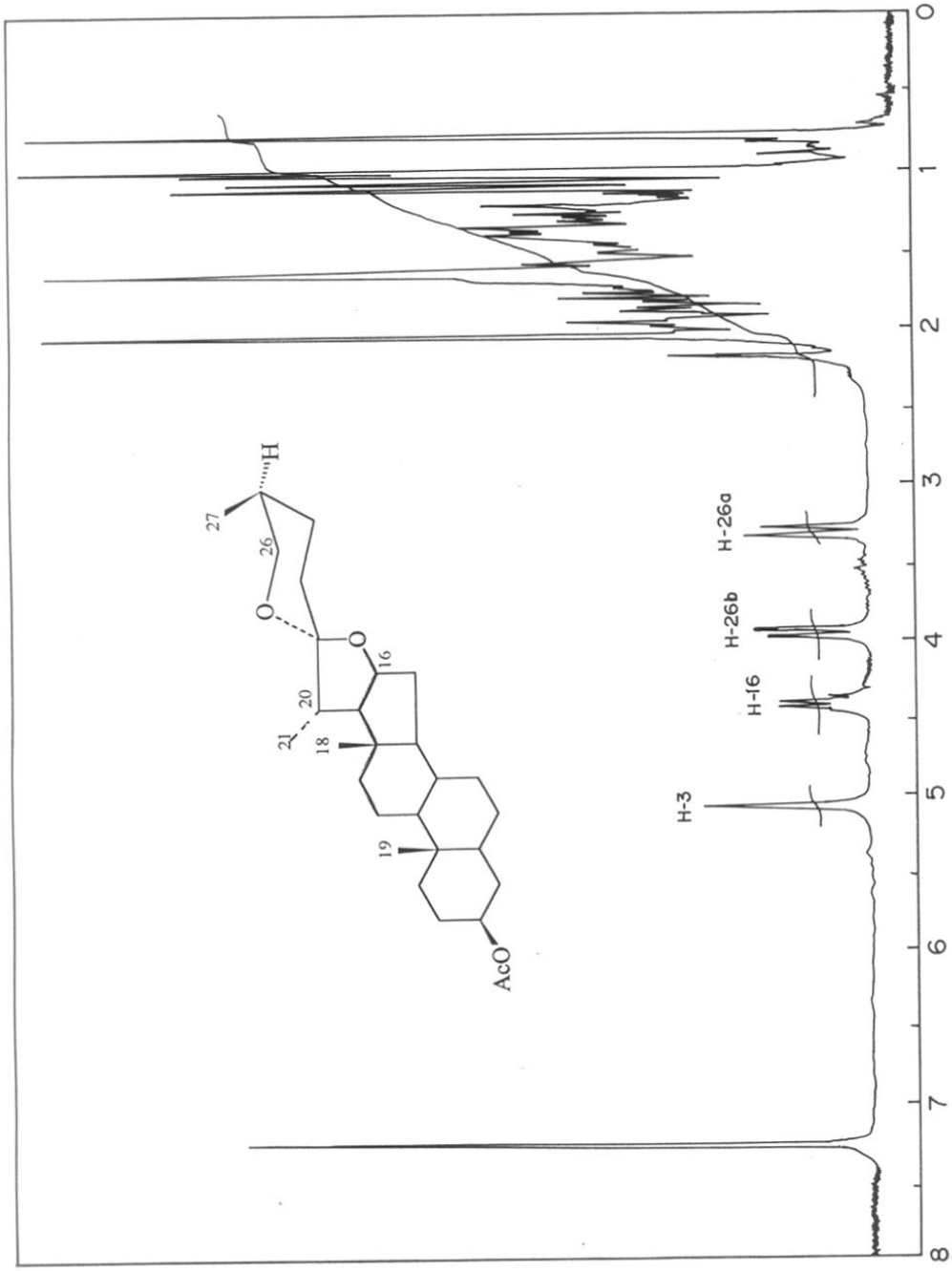


FIG 8 : ^{1}H NMR SPECTRUM OF ACETATE OF COMPOUND II

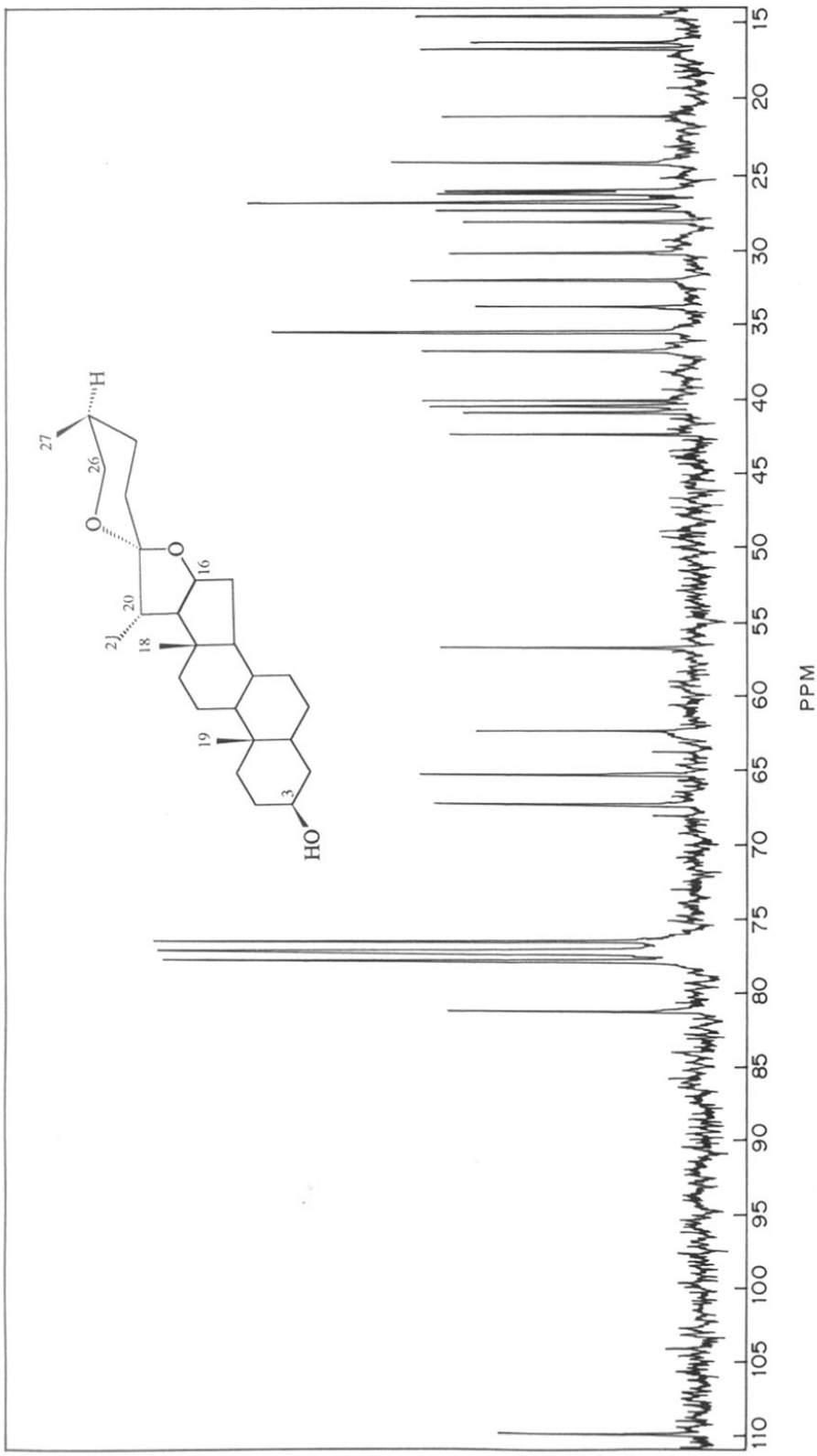


FIG 9 : ^{13}C NMR SPECTRUM OF COMPOUND II (50.32 MHz)

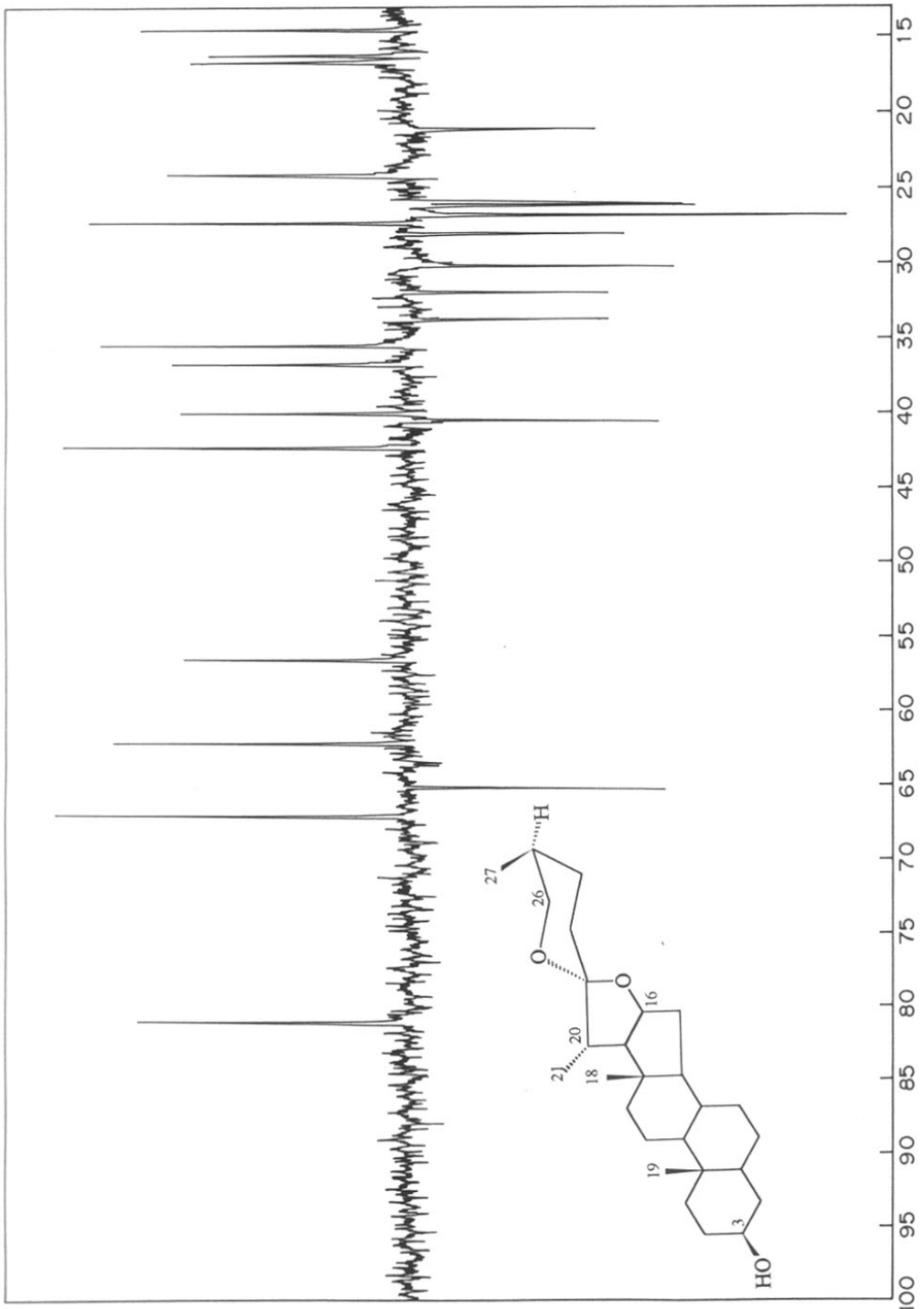


FIG 10 : ^{13}C NMR SPECTRUM (INEPT) OF COMPOUND II (50.32 MHz)

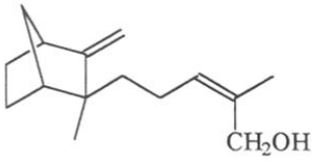
CHAPTER IV

THE ZEOLITE CATALYZED REACTIONS OF TERPENES WITH PHENOL TO SYNTHESIZE PERFUMERY INTERMEDIATES

Introduction

Natural perfumes since time immemorial are a part of human life due to their pleasant odor. However preparation of perfumes from natural resources such as plants, is quite expensive because of the low content of perfumery essential oils and difficulties and various limitations to cultivate such plants. In addition, economic feasibility also plays an important factor for processing essential oils to yield perfumes.

However, the development in the chemistry of synthetic perfumes has made it easy to synthesize the perfumes in a comparatively less expensive way using cheap raw materials like camphene and longifolene etc. For example, the famous odour of East Indian sandalwood, known for more than 4000 years, is mainly due to the presence of β-santalol (**1**), a constituent of the sandalwood oil. But the cultivation of this plant is difficult and takes long time to obtain sufficient quantity. However, a derivative of camphene 3-(2,2-exo-3-trimethyl-exo-5-norbornyl)-cyclohexanone (**2**) has similar sandalwood odour and can be synthesized by alkylation of phenol with (±) camphene (**3**) and subsequent hydrogenation[1].



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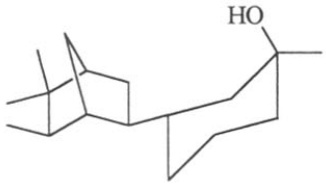

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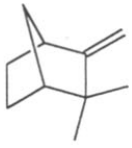


3

Such terpenyl cyclohexanols resulting from the acid-catalyzed condensation of terpenes such as camphene, α- and β-pinene and longifolene have been studied for many years. Such products are also used as antioxidants, insecticides and emulsifier etc[1].

Intermediates of terpenyl cyclohexanol derivatives of longifolene and camphene with phenol have been prepared earlier by Friedel-Crafts alkylation methods using BF_3-etherate as catalyst[2-6]. However, the use of such Lewis acid catalysts has many drawbacks. Lewis acid catalysts are corrosive to use, give poor selectivity, are difficult to separate, cannot be reused and are environmentally harmful. Many a times polymerization of hydrocarbons results in poor yield. Hence, catalysts that are safe and easy to handle, which can give high yield with good selectivity, can be easily separable from reaction mixture, can be reused and environmentally friendly are needed today. In this connection, zeolite catalysts are considered as the best candidates to act as catalysts. These heterogeneous solid catalysts fulfill these conditions and there is a growing trend to employ these catalysts in many organic reactions to commercialize various products such as perfumes, insecticides etc.

Zeolites

Zeolites are crystalline, hydrated, aluminosilicates having highly ordered rigid three-dimensional infinite framework, built up by the sharing of SiO_4 and AlO_4 tetrahedra, linked through oxygen bridges. They are represented by the general empirical unit cell formulae[7,8].

$$Mx/n\ [(AlO_2)x(SiO_2)y].zH_2O$$

Where M is a cation with the valency n. The net negative charge of the framework generated by the presence of aluminium is compensated by cation M, which is often selected from group I, II or rare earth metals or organic species. These cations are mobile and can be exchanged with

other metal ions. The unique features of zeolite catalysts are acidity, shape-selectivity and thermal stability.

Nomenclature

The structural codes for naming of natural synthetic zeolites have been assigned by International **Z**eolite **A**ssociation **S**tructure **C**ommission (IZASC) and IUPAC.[9] These codes do not depend on composition as well as distribution of various possible atoms such as Si, Al, P, Ga, Ti, etc. For example, mordanite (MOR), faujasite (FAU), sodalite (LAU), heulandite (HEW) and erionite (ERI).

Some of the synthetic zeolites are named after their inventors or institutions where they were originally synthesized. For example, ZSM for **Z**eolite **S**ociety **M**obile, VPI for **V**irginia **P**olytechnic **I**nstitute.

Classification of Zeolites

Zeolites have been classified on the basis of their morphological characteristics crystal structure, chemical composition, effective pore diameter and natural occurrence. Classification has also been done on the basis of silica/alumina ratio[10] into three type *viz.* low, intermediate and high silica/alumina zeolites. Some typical examples of the low Si/Al ratios are zeolite **A** and **X** type, which possess Si/Al ratio between 1-1.5. The intermediates, having Si/Al ratio between 2-5 are of **Y** and **L** type zeolites. And the high, having Si/Al ratio 10 to several thousand are ZSM-5, ZSM-11 type zeolites.

Barrier[10] and Sand[11] have classified zeolites into small, intermediate and large pore zeolites based on the effective diameter of pores. In the case of small pore zeolites, the diameter of the cavity is 4.1 Å, formed by eight SiO_4 tetrahedra. Medium pore zeolites have ten atom ring system with tubular diameter of 5.5 Å. These are also called as pentasil zeolites. Large pore

zeolites have cavities of 12 atom rings with diameter of 7.4 Å, these are constructed by network of SiO_4 and AlO_4 tetrahedra with oxygen bridges separating the two metal atoms.

Structure and properties

Zeolites are microporous crystalline solids. Most of them are aluminosilicates, having characteristic cage and pore structures. Each Si and Al atom is surrounded by oxygen in the crystal lattice. The central atom of the zeolite lattice can be replaced in an isomorphous manner by a large number of other tri- and tetravalent atoms. For instance B, Cr, Sb, As, Ge, Ti, and Zr can be incorporated in place of Al and Si. The isomorphous substitution in altered lattice constants, which changes catalytic properties (acidity and activity) of the zeolite. The determination of extent of incorporation can be done by MAS-NMR[12,13] spectroscopy and appropriate test reactions[14].

In general, the neutral sodium form is obtained during the synthesis of zeolites. But, by means of ion exchange with ammonium salts and subsequent calcination of ammonium form, one can obtain the proton form *i.e.* acidic form of zeolite. By ion exchange of sodium form, many other types of cation, like alkali metal ions, transition metal ions can also be introduced .The Lewis acidic sites can also be introduced in to zeolites by ion exchange with rare earth metals. The acid strength and number of acidic sites can be adjusted in a controlled manner during synthesis and/or by subsequent ion exchange.

Shape selectivity

Selectivity of the zeolites is due to shape and sizes of the pores. Only certain reactant molecules whose dimensions fall within specific limits of zeolite cavities can pass through the pores and reach the reaction sites. It is called as **reactant selectivity**. For example, in hydrocarbon cracking of n-heptane which is leniear and can easily enter in to the cavities to give

n-butane and n-heptane, where as in case of iso-heptane the entry is restricted due to reactant selectivity. **Product selectivity** is also observed where only those products are obtained in the reaction whose dimension let them diffuse out of pores of zeolite, the rest are trapped inside. An example for product selectivity can be illustrated by the alkylation of toluene with methanol in which equilibrium is reached between *o*-, *m*- and *p*-xylene being linear easily diffuse out of the pores. Restrictions imposed by the cavity dimensions on the size of the transition state of the reaction leads to **transition state selectivity**.[15] e. g. *m*-Xylene when reacted over mordenite can form 1,2,4-isomer but not 1,3,5-isomer, since the transition state for later, with alkyl group protruding downward, is too wide to be accommodated inside the pores. Obviously selectivity can operate only if the reaction occurs within zeolites pores. There are some reactions, which may take place on the outer surface of the zeolites without any selectivity.

Apart from the zeolites composed of aluminosilicates there are other families of microporous materials. One such consists of aluminium phosphate, which are known as $AlPO_4$ polymorphs (ALPO). These materials also exits in a wide range of open tetrahedral network. Structural modifications of these materials have also been carried out.

Application of zeolites in organic synthesis

The properties such as acidity, thermal stability and shape selectivity of zeolites made them to have wide applications. Zeolites can be used in two ways, (i) noncatalytic uses and (ii) catalytic uses.

Noncatalytic uses

The ability of zeolites to absorb and retain small molecules such as water, lower alcohols forms the basis for their noncatalytic use in organic synthesis of fine chemicals.[16,17] So the

zeolites can be used to dry and purify the solvents, separate the products and also can be used as a reactant disperser and slow release carrier.

Catalytic uses

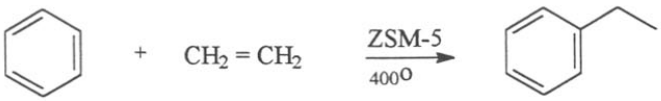
The use of zeolites as catalysts for industrial purpose began in 1960's and slowly gained importance in synthetic organic chemistry. Zeolites have several advantages over other catalysts. These are (i) they have good thermal stability, (ii) they can be reused and (iii) they are environmentally friendly. Because of these advantages they are used in for the synthesis of many intermediates and fine chemicals. Some of the important organic reactions catalyzed by zeolites are discussed below.

Alkylation

Zeolites are used for alkylation of arenes, substituted arenes, and hetero arenes.

Alkylation of arenes

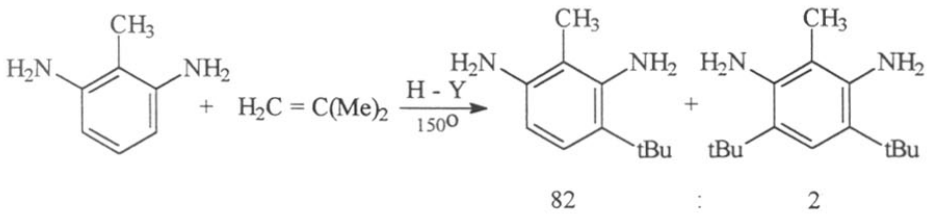
A good example of alkylation of arene is Mobil-Badger process where ethyl benzene has been prepared from ethylene and benzene over phosphorous doped ZSM-5 zeolite. Ethylene is completely converted to ethyl benzene with 99% selectivity.[18]



Similarly alkylation of benzene with either propene or isopropanol over zeolites to give industrially important cumene has also been reported[19-21].

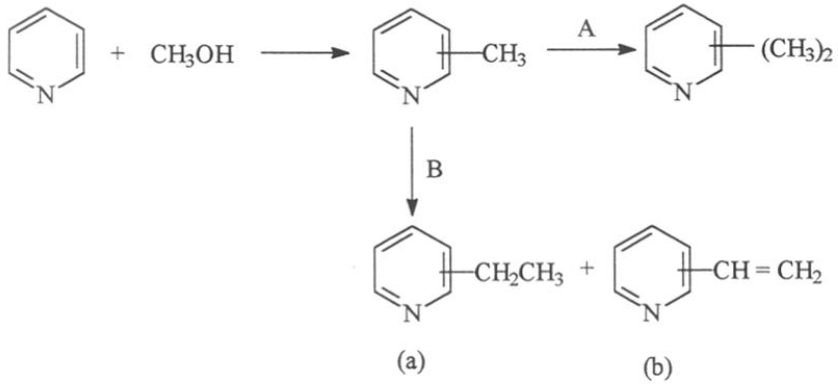
Alkylation of substituted arenes

t-Butylation of 2,6-diaminotoluene over H-Y zeolite showed shape selectivity as this process gives selectively mono-*t*-butyl compound[22,23].



Alkylation of heteroarenes

The alkylation of pyridine with methanol over faujasite[24] type zeolite has been reported. Primarily the substitution takes place at aromatic nucleus followed by secondary reaction in which the picoline formed can either undergo further ring alkylation or side chain alkylation to give compounds (a) and (b) respectively.



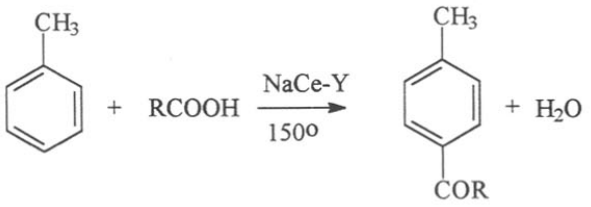
A) H-Y, Li-Y, Sr-Y

B) Na-K-Sr-Y and X

Similarly alkylation of thiophenes[25] and regioselective N-alkylation of imidazoles with alcohols over Y-type zeolite[26] have also been reported.

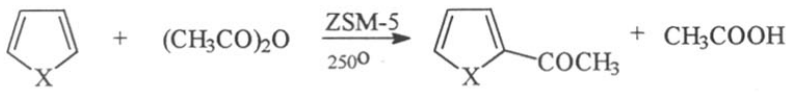
Acylation

In acylation of aromatic compounds by Friedel-Crafts reactions substantial amount of catalyst is required and the work up of the reaction involved handling of corrosive medium. To overcome this problem Chiche *et. al.*[27] reported for the first time the use of the zeolite catalyst for acylation using carboxylic acids as acylating agent. The acylaion of toulene using zeolite Na(Ce 70%) Y as a catalyst have been reported to give corresponding acylated product in good yield.



Recently, acylation of aldehydes to diacetates using β zeolite[28] has been reported. Holderich[29] for the first time reported the synthesis of 2-methyl-4-acetyl imidazole using zeolite catalyst.

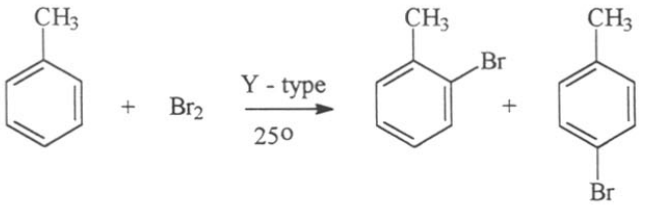
Acylation of heteroarenes have also been reported. The reaction of thiophene with acetic anhydride using boron ZSM-5 is reported to give 2-acetyl thiophene with 25 % conversion and 99 % selectivity. Similarly pyrrole and furan gave corresponding two acyl products.



X = S, O, NH

Halogenation

Halogenation using zeolite catalyst has been studied by Bekkum *et. al.*[30] and Vega *et al.*[31,32]. Bromination of toluene in presence of Y type zeolites gave high selectivity for *p*-isomer[32].



2 : 98

Similarly nitration[33] and amination reactions[34,35] using different zeolites have also been reported.

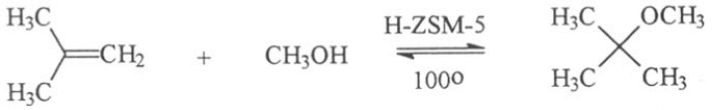
Reaction of alcohols with ammonia

Reaction of methanol and ammonia over Na-mordenite at 250° for one hour has been reported to produce methylamine along with diethyl amine and triethyl amine[36]. Recently the use of Na-mordenite[37] catalyst treated with $SiCl_4$ reported to lower the formation of trimethyl amine to 0.5 %. On the other hand the large pore H-Y zeolite under similar conditions gave 96 % trimethyl amine. The exclusive formation of dimethyl amine has also been reported by Machida *et. al*[38].

Addition Reactions

Methyl *t*-butyl ether (MTBE) has been prepared from isobutene and methanol in the presence of H-ZSM-5 zeolite at 100° with 35 % conversion and 95 % selectivity. The weekly

acidic boron zeolite affords MTBE in 86% yield[39].

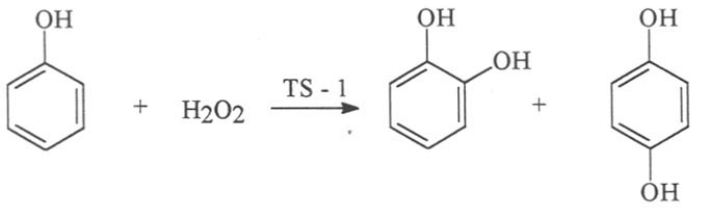


35 %

Similarly addition of ammonia to olefin[40] and addition of carboxylic acids to olefins[41] have also been reported.

Oxidation reactions

Titanium silicate (TS-1) is a widely used catalyst for oxidation of organic substrates by means of hydrogen peroxide. The titanium on TS-1 provides a high performance, highly flexible and highly stable catalytic sites. The selectivity and catalytic activity of TS-1 have provided the basis for the development of new technologies for some industrially important chemicals. A good example in which TS-1 has already formed industrial application is oxidation of phenol to hydroquinone and catechol.[42-44]

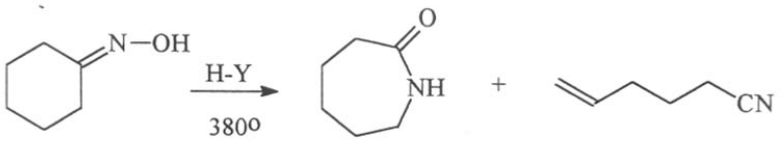


Other important applications of titanium zeolites are liquid phase conversion of cyclohexanone to its oxime in presence of ammonia and hydrogen peroxide[45] and oxidative cleavage of tosylhydrazones[46] to give corresponding carbonyl compounds.

Oxidation of various thioethers to corresponding sulfoxides and sulfones using TS-2 catalyst[47] has also been reported.

Rearrangements

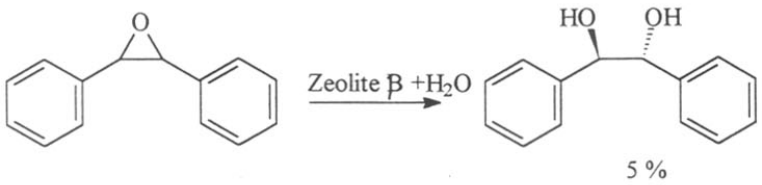
The preparation of caprolactum an important starting material for Nylon-6 involves the Beckmann rearrangement of cyclohexanone oxime. The problems associated with these reactions are the formation of huge amount of ammonium sulfate as a byproduct and handling of large amount of fuming sulfuric acid. Venuto and Landnis[48] reported the use of zeolites to achieve this rearrangement. Zeolites X, Y and H-mordenite are effective for this reaction. Cyclohexanone oxime in benzene was converted over H-Y zeolite at 380° to caprolactum in 85 % yield and 76 % selectivity in only 2 hours.



Similarly benzamine rearrangement,[49,50] Fries rearrangement[51,52] and pinacol rearrangement[53,54] using different zeolites have also been reported.

Asymmetric synthesis

The incorporation of metal ions attached to chiral ligands inside the zeolite cage offers an attractive way for achieving good asymmetric synthesis. Chiral rhodium complexes supported on zeolite have been used as catalyst for asymmetric hydrogenation of N-acyl dehydrophenyl aniline derivatives. Davis[55] has shown for the first time that zeolite especially β-zeolite with an isomorph A in slight excess can be used for asymmetric synthesis of (R,R) diol (II) starting from(I) .



I II

Although the selectivity is very low in this method, it has opened up a new area of designing chiral catalyst for asymmetric synthesis.

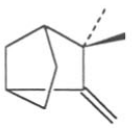
Previous work

Camphene (**3**) is a monoterpene occurring in many essential oils such as terpentine oil, cypress oil, camphor oil etc. An additional isoprene unit to camphene forms β-santalin (**4**), which on ring closure forms longifolene (**5**). Longifolene occurs in oleoresins of *Pinus longifolia* and other *Pinus* species. Thus the structures of camphene, longifolene and β-santalin are closely related.

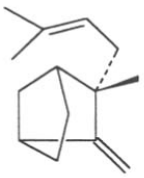
Derivatives of camphene and longifolene are useful in perfumery industries. Acetyl longifolene, ω-hydroxymethyl longifolene and isolongifolene ketones are some of the derivatives widely used in perfumery industries.[56]

The comparative study of alkylation of longifolene and camphene with phenol has been a subject of interest from many years.[57-60] It was Kitchen[2] who first showed that a mixture of camphene and phenol treated with catalytic amount of boron trifluride etherate in benzene at 0°, form isoborneol phenyl ether (**6**) (Chart I). Erman[3] has established the homogeneity as well as the structure of the above product by the study of the ^{1}H NMR spectrum and spin coupling pattern of C-2 proton. Patnekar and Bhattacharya[4] forwarded an evidence to show that the ether

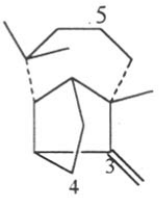
CHART I



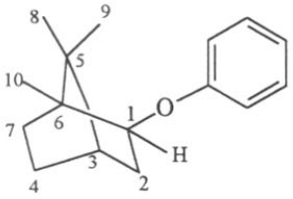
3
Camphene



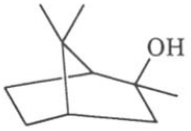
4
β-Santalin



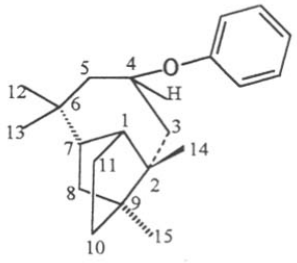
5
Longifolene



6 Isobornyl Phenyl ether

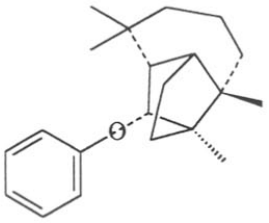


7 Isoborneol

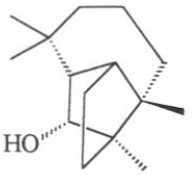


8

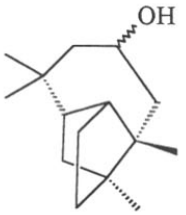
CHART I (Continued)



9

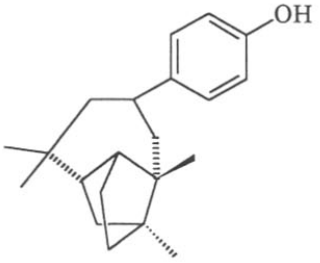


10
Longiborneol

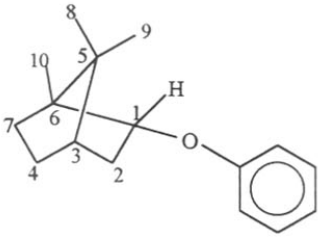


11

12
Isolongifolene



13
C-alkylated product



14

was an isoborneol derivative as it was cleaved by liquid ammonia reduction to furnish exclusively isoborneol (**7**) and cyclohexanone.

Condensation of longifolene with phenol in presence of catalytic amount of BF_3-etherate was also studied by Patnekar and Bhattacharya. At 0° two condensation products (**8**) and (**9**) were formed. The cleavage of the above mixture with liquid ammonium furnished a mixture of longiborneol (**10**) and an alcohol (**11**), the structure of which was elucidated by Nayak and Sukhdev[5] by chemical reactions and ^{1}H NMR spectrum. The reaction at 100° gave a mixture of *ortho* and *para* substituted phenols along with a hydrocarbon, isolongifolene (**12**). Similar studies were carried out by Nayak *et al*[6].

Present work

As mentioned earlier the preparation of such intermediates of terpenyl cyclohexanol derivatives using BF_3-etherte has many drawbacks and use of zeolites can overcome these drawbacks. Hence we planned a research programme to prepare the terpenyl cyclohexanol derivatives of longifolene and camphene with phenol using zeolites. The reaction of (+) longifolene and (±) camphene with phenol was studied at three different temperatures 0°, 70° and 100° using the zeolites ZSM-5, β and Y. The crystallographic and physico-chemical properties of these zeolites are given in table 1.

The reaction of longifolene with phenol using these catalysts gave mainly **8** and **13,** whereas the reaction of camphene with phenol gave mainly **6** and **14**. These products were characterized by spectral methods and by comparing the spectral data with the reported data. However, this forms preliminary part of the work and efforts are being done to optimize the conditions to obtain the maximum yield of required products, using different zeolite catalysts

Table 1 : Crystallographic and physico-chemical properties of the zeolites used

Catalyst used	Beta (β)	Y	ZSM-5
Channel structure	3-D, with interlinking channels	3-D, with interlinking channels	3-D, with two intersecting channel system
Pore opening (12 MR, A°)	5.7 x 7.5 (linear) 5.6 x 7.5 (tortuous)	7.4	5.4 x 5.6 A° (sinusoidal) 5.2 x 5.8 A°(straight)
Unit crystal Symmetry	Distorted	Cubic	Orthorhombic
Crystal size (μm)	0.5 –0.7	2.0	2.0
SiO_2/Al_2O_3	30	4.1	40
Surface area (m^2/g)	745	712	690
Sorption capacity (wt. %)			
Water	21.3	23.2	22.2
Benzene	21.9	20.3	8.8
n-Hexane	19.0	18.5	13.9
Cyclohexane	19.8	19.2	7.8

by studying the various parameters such as temperature, type of catalyst, quantity of catalyst, time period of the reaction etc.

Results and discussion

Condensation of longifolene with phenol and camphene with phenol did not take place at 0° with any of the zeolites even after 24 hours. However condensation of longifolene with phenol took place at 70° and 100° using zeolites β and Y to afford mainly compounds **8** and **13** (Table 2). But there was no reaction using ZSM-5 zeolite even at 100° for a prolonged period. Similarly condensation of camphene and phenol took place using zeolites β and Y at 70° and 100° affording mainly **6** and **14** (Table 3) whereas use of zeolite ZSM-5 did not give any product.

In both the reactions conversion of longifolene and camphene was better at 100° than that at 70° and thus the percentage yield of **8** and **13** in reactions of longifolene with phenol, and compounds **6** and **14** in condensation of camphene and phenol was more (Table 2 and 3).

However, in condensation of longifolene and phenol at both the temperatures percentage of C-alkylated product (compound **13**) was less than that of O-alkylated product (compound **8**). During the condensation of longifolene with phenol using catalytic amount of BF_3.etherate two O-alkylated products **8** and **9** were obtained[4,6] and percentage of **8** was more whereas C-alkylated product was not obtained.

In condensation of camphene with phenol percentage yield of compound **6** was more than that of compound **14** (Table 3) at both the temperatures. During the condensation of camphene with phenol using BF_3.etherate as catalyst, compound **6** was the major product along with minor quantities of *o*- and *p*-substituted phenols[3].

Table 2 : Condensation of longifolene with phenol (percentage yield based on longifolene consumed).

Catalyst	0°, 24 hrs	70°, 6 hrs.	100°, 6hrs.
ZSM-5	No reaction	No reaction	No reaction
β	No reaction	Compound 8 : 148 mg (23%) Compound 13 : 122 mg (19 %) Unidentified complex mixture : 370 mg Longifolene recovered : 1.30 g	Compound 8 : 260 mg (27%) Compound 13 : 195 mg (21 %) Unidentified complex mixture : 505 mg Longifolene recovered : 1.10 g
Y	No reaction	Compound 8 : 112 mg (19.3%) Compound 13 : 91 mg (15.6 %) Unidentified complex mixture : 377 mg Longifolene recovered : 1.46 g	Compound 8 : 133 mg (21.2%) Compound 13 : 110 mg (17.4 %) Unidentified complex mixture : 387 mg Longifolene recovered : 1.41 g

Table 3 : Condensation of camphene with phenol (percentage yield based on camphene consumed).

Catalyst	0°, 24 hrs	70°, 6 hrs.	100°, 6hrs.
ZSM-5	No reaction	No reaction	No reaction
β	No reaction	Compound 6 : 90 mg (17.6%) Compound 14 : 35 mg (19 %) Unidentified complex mixture : 385 mg camphene recovered : 850 mg	Compound 6 : 172 mg (24.5%) Compound 14 : 92 mg (13.3 %) Unidentified complex mixture : 436 mg camphene recovered : 660 mg
Y	No reaction	Compound 6 : 43 mg (11.6 %) Compound 14 : 19 mg (5.1 %) Unidentified complex mixture : 308 mg camphene recovered : 990 mg	Compound 6 : 65 mg (13.2 %) Compound 14 : 35 mg (7.1 %) Unidentified complex mixture : 150 mg camphene recovered : 870 mg

It may be possible that during zeolite catalyzed condensation also 2,6- hydride shift or Wagner-Meerwin rearrangement may be taking place as in the condensation catalyzed by BF_3-etherate.[4]

A typical experiment using the zeolite catalyst is described below.

A mixture of longifolene or camphene with phenol and catalyst was heated with stirring for 6 hours. The reaction mixture after work up was subjected to column chromatography to isolate the major products.

Identification of compound 8

Compound 8, $[\alpha]_D^{25}$ + 6.3°, showed in its mass spectrum the molecular ion peak at m/z 298 suggesting the molecular formula $C_{21}H_{30}O$. Its IR spectrum (Fig 2) showed abosrbance at 1598 cm^{-1}, 1493 cm^{-1} and 1370 cm^{-1} revealing the presence of aromatic ring. The ^1H NMR spectrum (Fig. 3, Table 4) of the compound showed the presence of four tertiary methyl groups at δ 0.85, 0.95, 0.96 and 1.0, aromatic protons at δ 6.9 (3H) and 7.3 (2H) and a multiplet at δ 4.65 (1H) assignable to a proton on an oxygenated carbon atom revealing the compound **8** to be an O-alkylated product. From these spectral data the compound was found to be longibornyl ether **8**, which was further confirmed by its ^{13}C NMR spectrum (Fig. 4 Table 5). The ^{13}C NMR spectrum along with its INEPT experiment showed four quartets, an oxygenated carbon at δ 74.5 as doublet, five triplets, two doublets and three singlets along with signals for an aromatic ring.

The compound was also obtained by the condensation of longifolene and phenol in presence of BF_3-etherate. The spectral data and physical constants were found to be same as those reported .[4]

Table 4 : ^{1}H NMR spectral data of compound **8** (300 MHz)

Proton	Chemical shift in δ	Multiplicity	No. of protons
H-4	4.65	m	1 H
H-12	0.84	s	3 H
H-13	0.94	s	3 H
H-14	1.0	s	3 H
H-15	0.96	s	3 H
H-2', H-4', H-6'	6.90	m	3 H
H-3', H-5'	7.30	m	2 H

Table 6 : ^{1}H NMR spectral data of compound **13** (300 MHz)

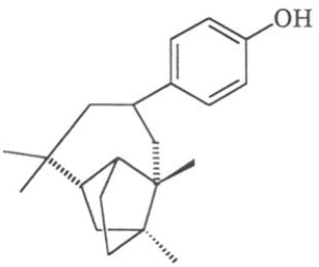
Proton	Chemical shift in δ	Multiplicity	No. of protons
H-12	0.85	s	3 H
H-13	0.96	s	3 H
H-14	0.99	s	3 H
H-15	0.98	s	3 H
H-2', H-6'	6.85	d, J = 9 Hz	2 H
H-3', H-5'	7.00	d, J = 9 Hz	2 H

Table 5 : ^{13}C NMR spectral data of compound **8** (75.48 MHz)

Carbon	Chemical shift in δ	Multiplicity
C -1	53.3	d
C –2	45.9	s
C –3	45.1	t
C –4	73.2	d
C –5	45.7	t
C –6	48.8	s
C –7	45.4	d
C –8	29.8	t
C –9	33.1	s
C –10	42.5	t
C –11	36.3	t
C –12	15.8	q
C –13	21.6	q
C –14	26.5	q
C –15	32.6	q
C –1'	158.3	s
C –2'	129.5	d
C –3'	111.0	d
C –4'	120.7	d
C –5'	111.5	d
C –6'	129.0	d

Identification of compound 13

Compound **13** $[\alpha]^{25}_{D}$ + 3.5 showed in its mass spectrum the molecular ion peak at m/z 298 suggesting the molecular formula $C_{21}H_{30}O$. Its IR spectrum (Fig. 5) showed absorbance at 3390 cm^{-1} revealing the presence of a hydroxyl group, along with absorbance at 1598 cm^{-1}, 1493 cm^{-1} and 1370 cm^{-1} revealing the presence of aromatic ring. The ^1H NMR spectrum (Fig. 6, Table 6) of the compound showed the presence of four tertiary methyl groups at δ 0.88, 0.95, 0.97 and 1.0 along with aromatic protons at δ 6.85 (d, J = 9 Hz, 2H) and 7.0 (d, J = 9 Hz, 2H) showing them to be *ortho* coupled. The absence of a multiplet due to a proton on oxygenated carbon atom clearly indicated that the compound 13 was a C-alkylated product. From these spectral data and the same methyl pattern in the ^1H NMR spectra of longibornyl ether **8** and compound **13**, the structure of compound **13** was elucidated as a para substitutated phenol derivative as below.



This is the first report of a C-alkylated product of longifolene and phenol using zeolite.

Identification of compound 6

Compound **6** showed in its mass spectrum molecular ion peak at m/z 230 indicating the molecular formula $C_{16}H_{22}O$. The IR spectrum of the compound (Fig. 8) showed absorbance at 1598 cm^{-1}, 1370 cm^{-1} revealing the presence of aromatic ring. The ^1H NMR spectrum of the

compound (Fig. 9, Table 7) showed three tertiary methyls at δ 0.94, δ 1.05 and δ 1.15 and aromatic proton signals at δ 6.9 (3H) and δ 7.3 (2H). The ^{1}H NMR spectrum also showed a triplet at δ 4.05 (J=7 Hz) indicating the presence of a proton on oxygenated carbon atom. The ^{13}C NMR spectrum (Fig. 10, Table 8) along with INEPT experiment (Fig 11) showed three quartets, three triplets, two doublets of which one was for an oxygenated carbon atom along with signals for an aromatic ring.

From these spectral data, this compound was found to be isobornyl phenyl ether. The spectral data and physical constants of compound **6** were identical to those reported[3].

Identification of compound 14

Compound showed in its mass spectrum molecular ion peak at m/z 230 suggesting the molecular formula $C_{16}H_{22}O$. Its IR spectrum (Fig. 12) showed absorbance at 1590 cm^{-1}, 1368 cm^{-1} revealing the presence of aromatic ring. The ^{1}H NMR spectrum (Fig. 13, Table 7) of the compound showed the presence of three tertiary methyl groups at δ 0.95, δ 0.96, and δ 1.0 and aromatic protons at δ 6.9 (2H) and δ 7.3 (3H) along with a doublet of doublet at δ 4.25 (J = 1,7 Hz) due to a proton on oxygenated carbon atom.

This spectral data was strikingly similar to that of compound **6** except a little change in the chemical shift and multiplicity pattern of proton on oxygenated carbon atom. In compound **6** it was at δ 4.05 (t, J = 7 Hz) where as in compound **14** it was at δ 4.25 (dd, J = 1, 7 Hz). The multiplicity pattern of this proton in both the compounds (**6** and **14**) were similar to those reported for isobornyl acetate and bornyl acetate.[61] From these spectral facts the structure of compound **14** was deduced to be bornyl phenyl ether.

Table 7 : ^{1}H NMR spectral data of compound **6** and **14**

Proton	Chemical shift in δ, multiplicity and coupling constant in Hz	
	Compound **6**	Compound **14**
H-1	4.05, t, J = 7 Hz	4.25, dd, J = 1, 7 Hz
H-8	0.95, s	0.95, s
H-9	1.05, s	0.96, s
H-10	1.15, s	1.0, s
H-2',H-4' H-6'	7.3, m	7.3, m
H-3', H-5'	6.9, m	6.9, m

Table 8 : ^{13}C NMR spectral data of compound **6**

Carbon	Chemical Shift in δ	Multiplicity
C-1	84.4	d
C-2	39.5	t
C-3	46.0	d
C-4	34.0	t
C-5	47.0	s
C-6	49.3	s
C-7	28.0	t
C-8	20.5	q
C-9	20.5	q
C-10	12.0	q
C-1'	158	s
C-2'	129.5	d
C-3'	115.3	d
C-4'	120.0	d
C-5'	115.3	d
C-6'	129.5	d

EXPERIMENTAL

Reactions of (+) longifolene with phenol :

A mixture of (+) longifolene (2.04 g, 0.01 mole), phenol (0.94 g, 0.01 mole) and freshly activated zeolite β (500 mg) was heated at 70° with stirring for 6 hours. The reaction mixture was cooled and filtered to remove the catalyst. Catalyst washed with benzene. The filtrate was treated with aqueous sodium bicarbonate solution to remove the unreacted phenol. The organic layer was washed with water, dried over anhy. sodium sulphate and concentrated to yield 1.36 g of reaction mixture which was further subjected to column chromatagrophy over silica gel to separate unreacted longifolene, longibornyl phenyl ether **8** and compound **13.**

Longibornyl phenyl ether (8):

Yield	:	148 mg
$[\alpha]_D^{25}$	:	+ 6.3° ($CHCl_3$, c 0.5)
IR (ν_{max}, $CHCl_3$)	:	1598, 1493, 1370 cm^{-1} (Fig. 2)
^1H NMR	:	Fig. 3, Table 4
^{13}C NMR	:	Fig. 4, Table 5
Mass (rel. int.)	:	m/e 298 $[M]^+$ (20), 205 (95), 149 (55), 135 (35), 121 (40), 109 (62), 95 (100), 81 (60), 69 (55), 55 (70).

Compound (13) :

$[\alpha]_D^{25}$	:	+ 3.5° ($CHCl_3$, c 0.32)
Yield	:	122 mg
IR (ν_{max}, $CHCl_3$)	:	3390, 1598, 1493, 1370 cm^{-1} (Fig. 5)
^1H NMR	:	Fig. 6, Table 6

Mass (rel. int.) : m/e 298 $[M]^+$(25), 272 (20), 198 (20), 178 (25), 161 (30) 91 (32), 79 (45), 69 (31), 55 (30), 41 (100).

Reactions at 0° and 100° were carried out using similar procedures. Further reactions using zeolites ZSM-5 and Y were carried out at 0°, 70° and 100° by similar procedures. Results are tabulated in table 2.

Reactions of (±) camphene with phenol :

A mixture of (±) camphene (1.36 g., 0.01 mole), phenol (0.94g., 0.01 mole) and freshly activated zeolite β (500 mg) was heated at 100° with stirring for 6 hours. The reaction mixture was cooled and filtered to remove the catalyst. Catalyst washed with benzene. The filtrate was treated with aqueous sodium bicarbonate solution to remove the unreacted phenol. The organic layer was washed with water, dried over anhy. sodium sulphate and concentrated to yield 1.12 g of reaction mixture which was further subjected to column chromatagrophy over silica gel to separate unreacted camphene, isobornyl phenyl ether **6** and bornylphenyl ether **14.**

Isobornyl phenyl ether (6) :

Yield : 65 mg

IR (ν_{max}, $CHCl_3$) : 1598, 1370 cm^{-1} (Fig. 8)

^1H NMR : Fig. 9, Table 7

^{13}C NMR : Fig. 10,11, Table 8

Mass (rel. int.) : m/e 230 $[M]^+$ (30), 137 (45), 95 (30), 81 (100), 67 (35), 41 (55).

Bornyl phenyl ether (14) :

Yield : 35 mg

IR (ν_{max}, $CHCl_3$) : 1590, 1368 cm^{-1} (Fig. 12)

^{1}H NMR	:	Fig. 13, Table 6
Mass (rel.int.)	:	m/e 230 $[M]^+$ (40), 137 (30), 120 (100), 95 (42), 81 (70) 67 (45), 55 (25), 41 (50).

Reactions at 0° and 70° were carried out using similar procedures. Further reactions using zeolites ZSM-5 and Y were carried out at 0°, 70° and 100° by similar procedures. Results are tabulated in table 3.

REFERENCES

1. *Fragrance Chemistry, The Science of the Sense of smell*, Ed. Ernst T. Theimer, pp. 248, 415 (1968) and references cited thee in.
2. L.J. Kitchen

   J. Am. Chem. Soc., **70**, 608 (1948).
3. W.F. Erman

   J. Am. Chem. Soc., **86**, 2887 (1964).
4. S.G. Patnekar and S.G. Bhattacharya

   Ind. J. Chem., **8**, 36 (1970).
5. U.R. Nayak and Sukh Dev

   Tetrahedron, **8**, 42 (1960).
6. U.R. Nayak, V.S. Dalavoy and V.B. Deodhar

   Ind. J. Chem., **28B**, 956 (1989).
7. D.W. Berck

   J. Chem. Edu., **41**, 678 (1964).
8. D.W. Berck

   Zeolite Molecular Seives, John Wiely and sons, New York, 1974.
9. R.M. Barrer

   Pure and Applied Chem., **51**, 1091 (1979).
10. R.M. Barrer

    Hydrothermal Chemistry of Zeolites, Acadamic Press, London, 1983.
11. L.B. Sand

    Econ. Geol., 191 (1967).

12. Z. Gabelica, J.B. Nagy, P. Bodart and G. Debras

Chem. Lett., 1059 (1984).

13. S. Hayashi, K. Suzuki, S. Shin, K. Hayamizu and O. Yamamoto

Bull. Chem. Soc. Jpn., **58**, 52 (1985).

14. J.A. Martens, M. Thielen, P.A. Jacobs and J. Witkamp

Zeolites, **4**, 98 (1984).

15. S.M. Csicsery

Zeolites, **4**, 202 (1984).

16. V.H. Bekkum and H.W. Kouwenhoven

Stud. Surf. Sci. Catal., **41**, 5 (1988).

17. V.H. Bekkum and H.W. Kouwenhoven

Rec. Trav. Chim. Pays-Bas, **108**, 283 (1989).

18. L.B. Young

US Pat. 3,962,364 (1976), CA 85 : 123537t

19. A.R. Pradhan, A.N. Kotasthane and B.S. Rao

Appl. Catal., **72**, 311 (1991).

20. A.R. Pradhan and B.S. Rao

J. Catal., **132**, 79 (1991).

21. K.S.N. Reddy, B.S. Rao and V.P. Shiralkar

Appl. Catal. A, **95**, 53 (1993).

22. W.F. Burgoyne, D.D. Dixon and J.P. Casey

Chem.Tech., **19**, 690 (1989) and references cited there in.

23. W.F. Burgoyne and D.D. Dale

Appl. Catal., **62**, 161 (1990), idem **63**, 117 (1990).

24. H. Kashiwagi, Y.Fujiki and S. Enomoto

Chem. Pharm. Bull., **30**, 404, 2575 (1982).

25. V. Solinas, K.Monaci, G. Longu and L. Forni

Acta Phy. Chem., **31**, 291 (1985).

26. O. Yoshio, I. Yuriko and Zi-hua Fu

J. Chem. Soc. Chem. Comm. 9 (1995).

27. B.Chiche, A. Finiels, C. Gauthier, J. Geneste and J. Pioch

J. Org. Chem., **51**, 2128 (1986).

28. P. Kumar, S.R. Hegde and T.P. Kumar

Tet. Lett., **36**, 601 (1995).

29. W.F. Hoeldrich, H. Lermer and M. Schwarzmann

DOS, 3, 618, 964 (1986), CA 109: 73314j.

30. Th.M.Wortel, D.Oudijn, C.J.Vleugel, D.P.Roelofsen and H.van Bekkum

J. Catal. **60**, 110 (1979).

31. F.de la Vega and Y.Sasson

Zeolites, **9**, 418 (1989).

32. F.de la Vega and Y.Sasson

J. Chem. Soc. Chem. Comm. 653 (1989).

33. Sumittomo. Chem. Co.

Jpn. Pat., 58,157,748 (1983) CA 100 : 51233u

34. M.G. Warawadekar and R.A.Rajadhykasha

Zeolites, **7**, 574 (1987).

35. H.van Bekkum ,E.M. Flanigen and J.C. Jansen

Stud. Surf. Sci. Catal., **58**, 706 (1991).

36. F.J. Wiegert

J. Catal., **103**, 20 (1987).

37. *"Acid-Base Catalysis"* eds. K. Segawa, A. Sugiyama, M. Sakaguhi, K. Sakurai, and K. Tanabe. Kodansha Ltd.Tokyo Japan (1989).

38. I. Mochida, A.Yasukute, H.Fujitsu and K.Takeshita.

J. Catal., **82**, 312 (1983).

39. M.R. Klotz

US Pat., 4,584,415 (1986), CA 105 : 45275w

40. M. Deeba, M.E. Ford and T.A. Johnson

J. Chem. Soc. Chem. Comm. 562 (1987).

41. L.B. Young

US Pat., 365,084 (1982), CA 98 : 888418

42. A. Thangaraj, R. Kumar and P. Ratnaswamy

Appl. Catal. 57 (1990).

43. T. Tatsumi, M Nakamwa, M. Neishi and H. Tominaga

J. Chem. Soc. Chem. Comm. 476 (1990).

44. D.R.C. Huybrechts, L De Bruycker and P.A. Jacobs

Nature, **345**, 240 (1990).

45. P. Roffia, G. Leofanti, A. Cesana, M. Mantegaza, M. Padovan, G. Petrini P. Touti and P. Gervasnth

Study Surf. Catal., **55**, 43 (1990).

46. P. Kumar, V.R. Hegde, B. Pande and T. Ravindranathan

J. Chem. Soc. Chem. Comm. 1553 (1993).

47. S.R. Reddy, S.J. Reddy, R. Kumar and P. Kumar

J. Chem. Soc. Chem. Comm. 84 (1992).

48. P.B. Vanuto and P.S. Ladnis

Adv. Catal., **18**, 259 (1968).

49. C.D. Chang and P.D. Perkins

Zeolites, **3**, 298 (1983).

50. H. Le Blank, L. Puppe and Wedemeyer

Ger offen., 3, 332, 687 (1985); CA, 103 : 6239b.

51. Y. Polloux, N. S. G nep, P. Magnoux and G. Perot

J. Mol. Catal., **40**, 231 (1987).

52. C.S. Cundy, R.Higgins, S.A.M. Kibby, B.M. Lowe and R. M. Paton

Tet. Lett., **30**, 2281 (1989).

53. Q. Wang and Z. Chen

Kexue Tongbao, **29**, 1130 (1984).

54. A. Molnar, S. Bucsi and H. Bartok

Stud. Surf. Sci. Catal., 203 (1982).

55. M.E. Devis

Acc. Chem. Res., **26**, 11 (1993).

56. Sukh Dev

'*Progress in the chemistry of Organic Natural Products*', 40, 49 (1981).

57. Y. Watnabe

J. Chem. Soc. Jpn., **63**, 1420 (1960).

58. A.R. Abdurasuleva and S.Achilova,

Zh. Obshch. Khimi, **32**, 707 (1962).

59. V. Kabaivanov, M. Mikhailov, M. Notov and L. Popova

Khimiya Ind., **31**, 107 (1959).

60. W.F. Erman and T.J. Flautt

J. Org. Chem., **27**, 1526 (1926).

61. T.J. Flautt and W.F Erman

J. Am. Chem. Soc., **85**, 3212 (1963).

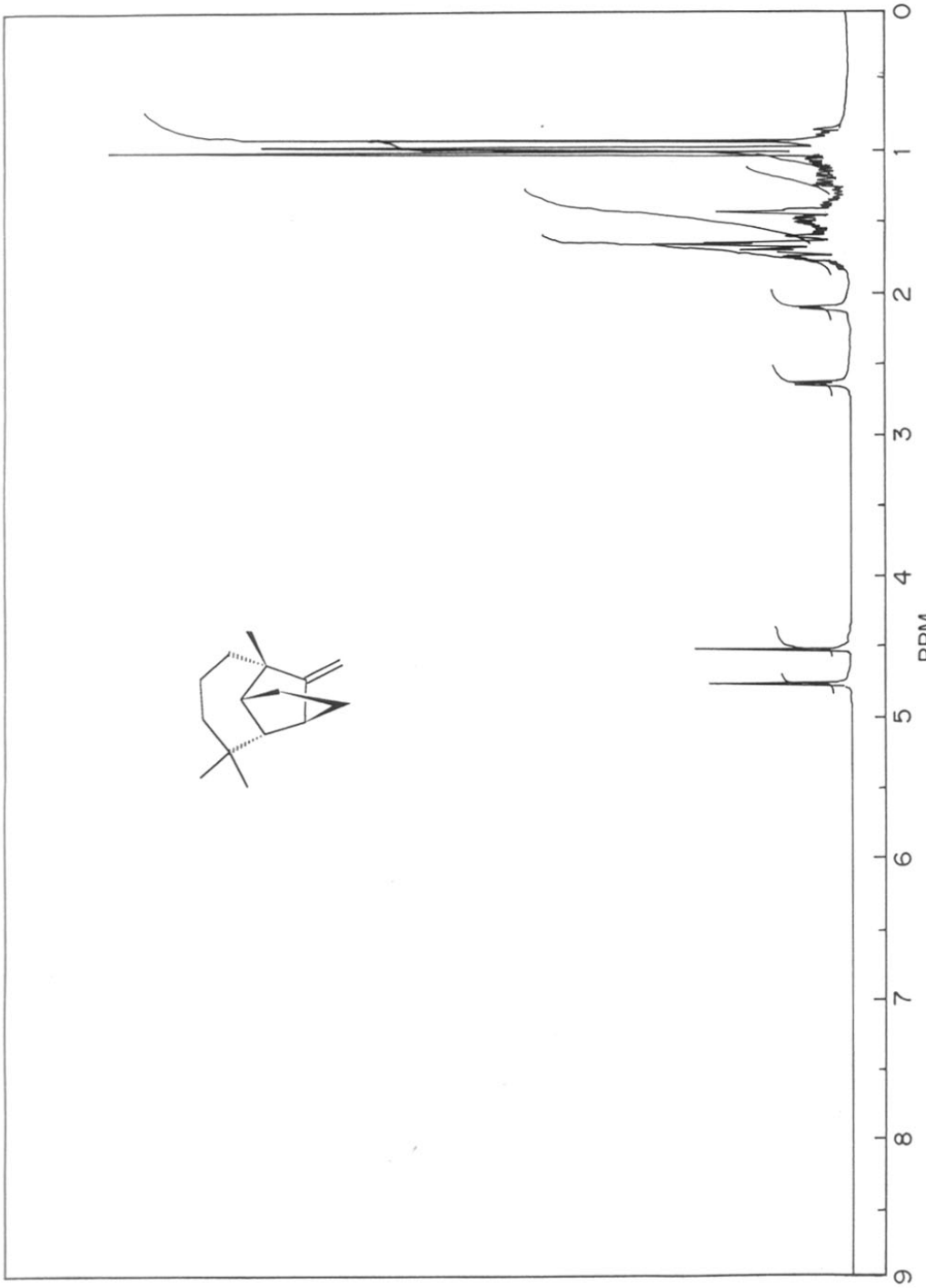


FIG 1 : ^{1}H NMR SPECTRUM OF LONGIFOLENE

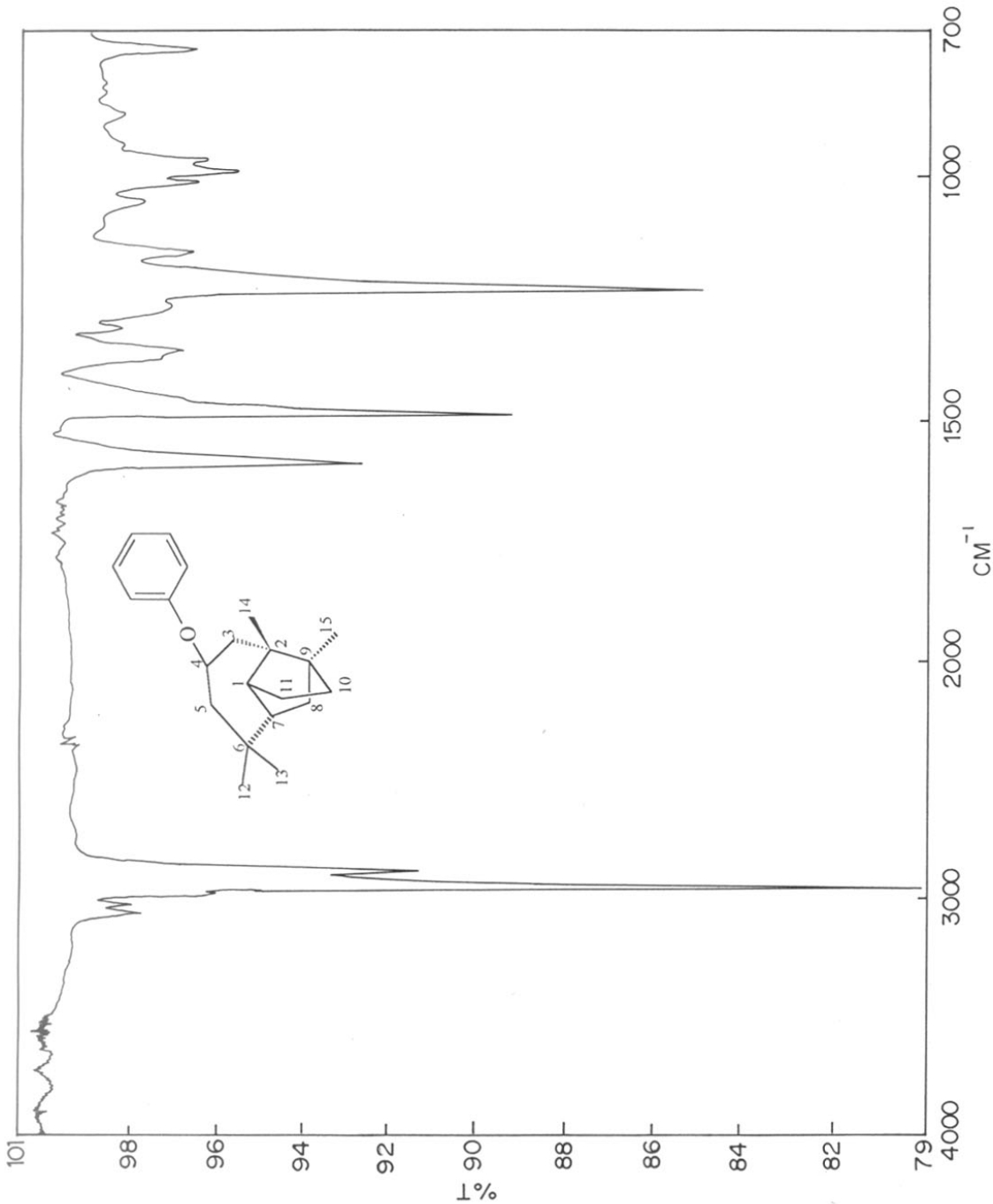


FIG 2 : FT - IR SPECTRUM OF COMPOUND 8

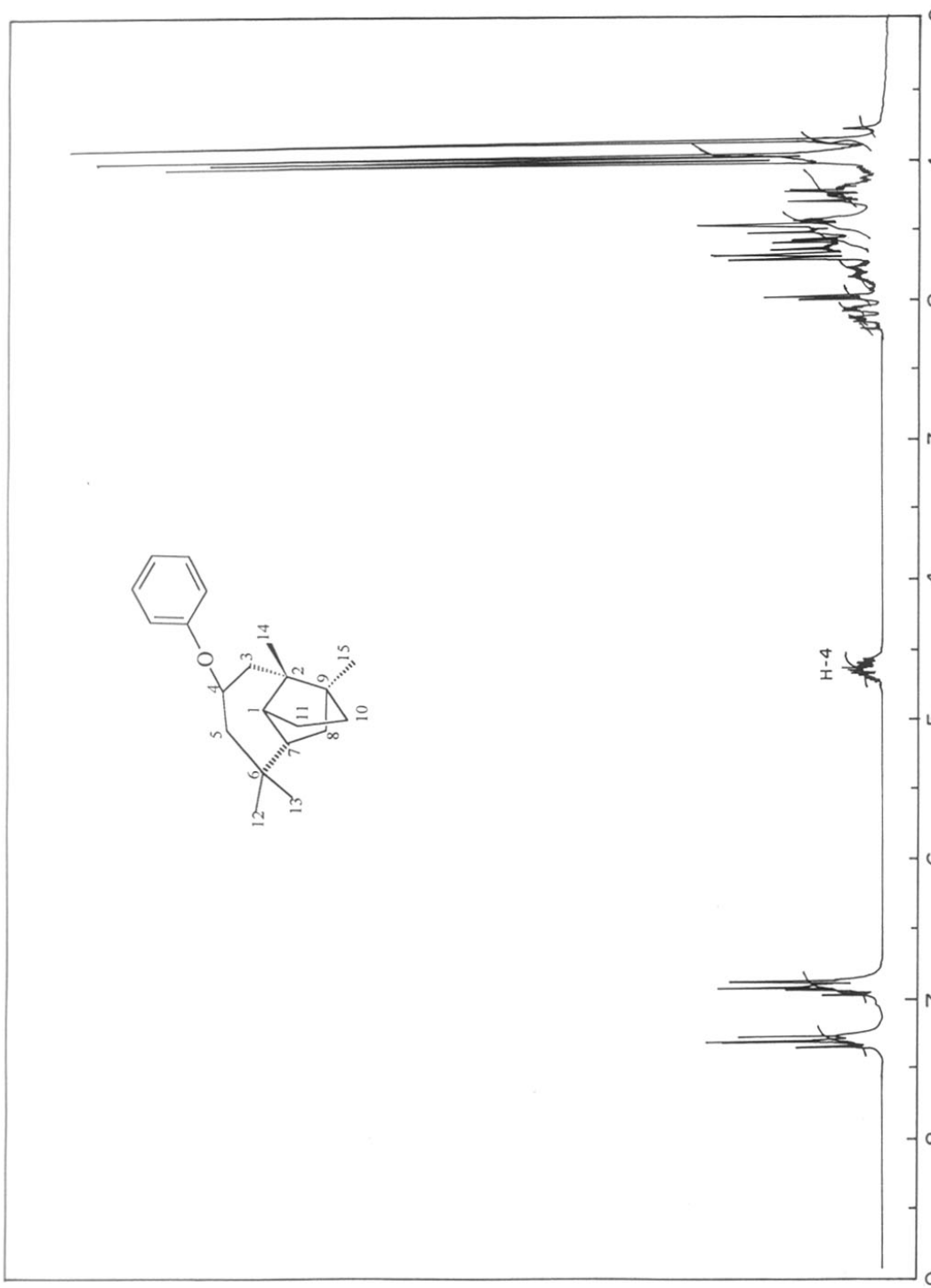


FIG 3 : ^{1}H NMR SPECTRUM OF COMPOUND 8

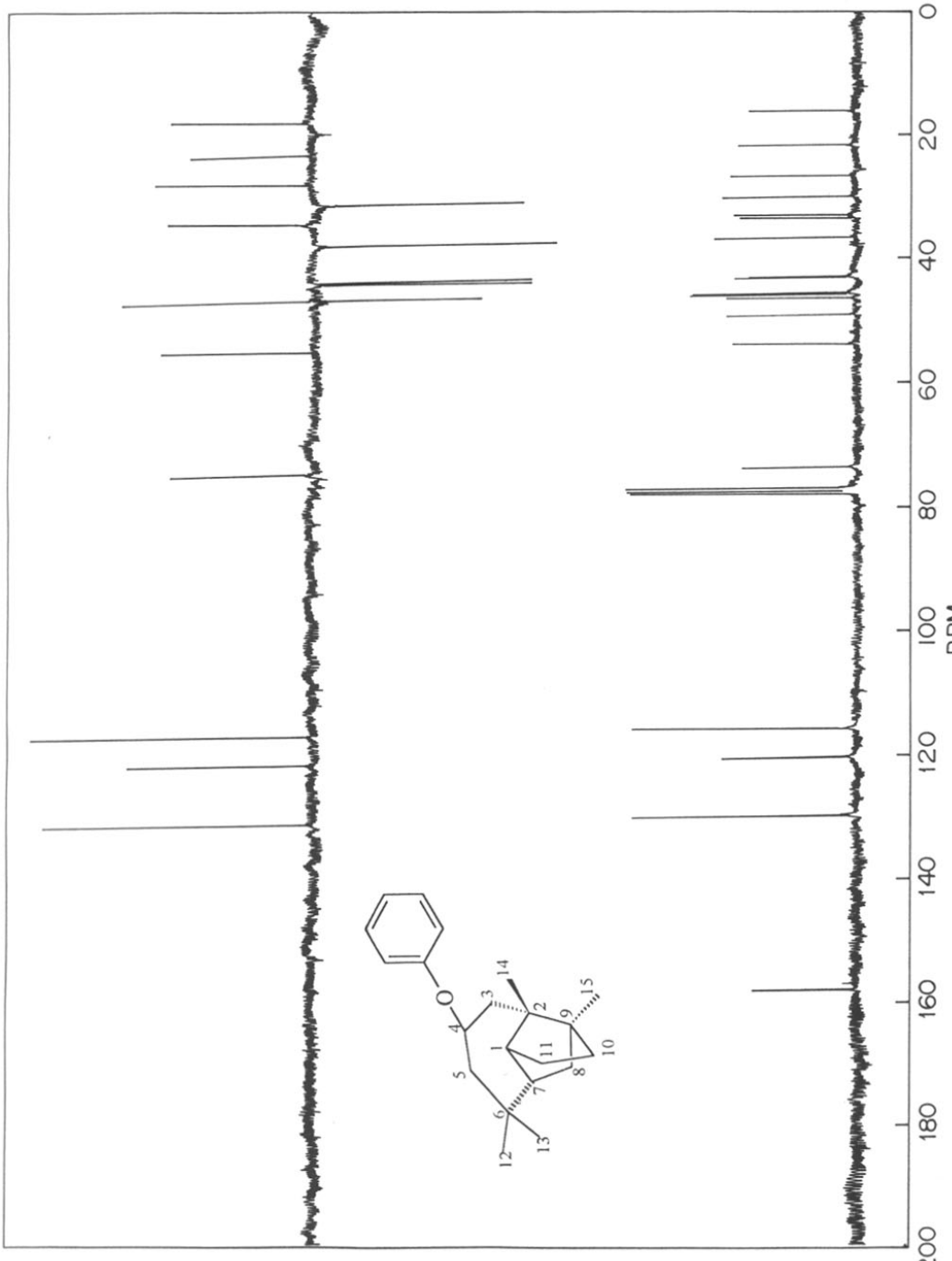


FIG 4 : ^{13}C NMR SPECTRUM AND INEPT OF COMPOUND 8 (75.48 MHz)

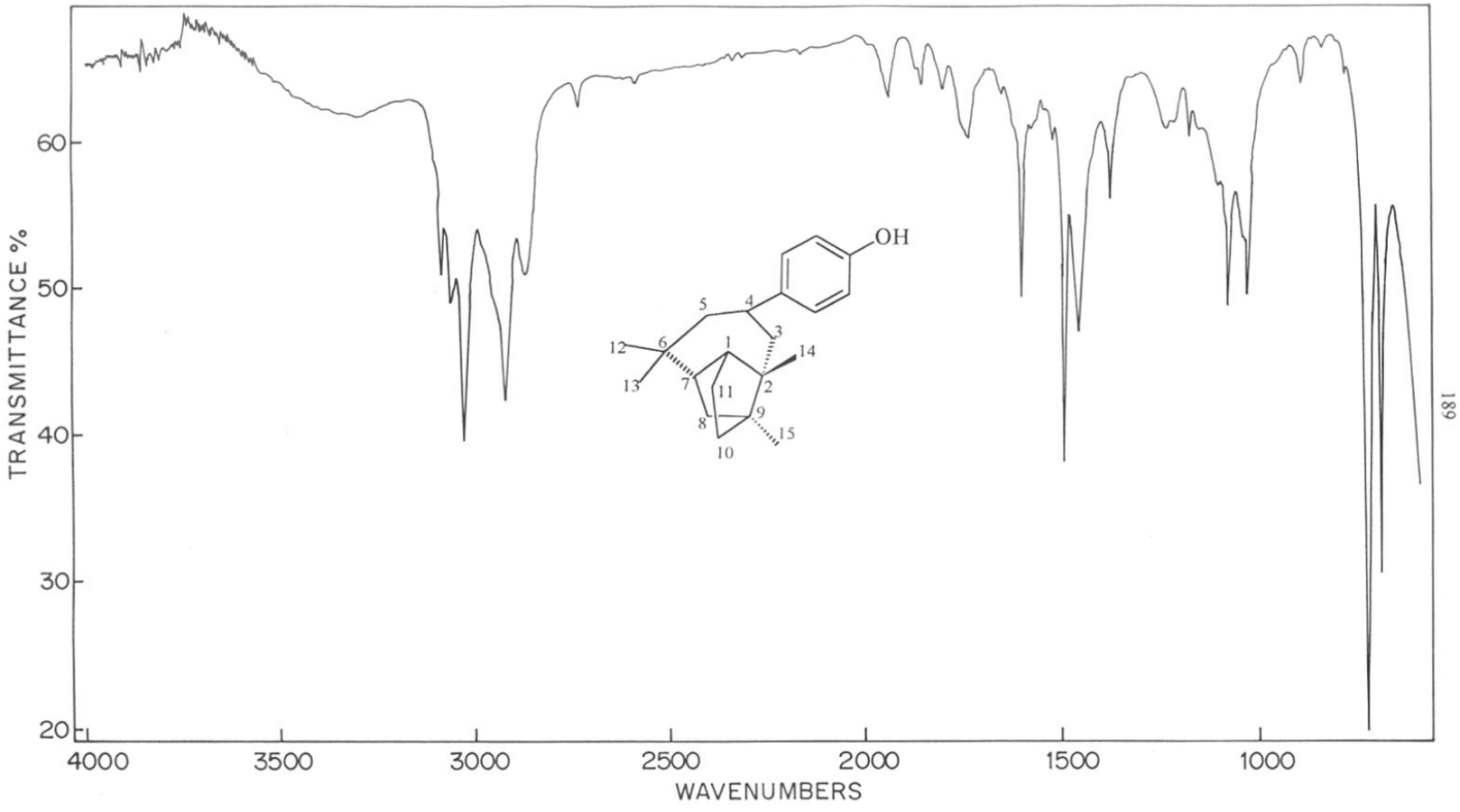


FIG 5 : FT - IR SPECTRUM OF COMPOUND 13

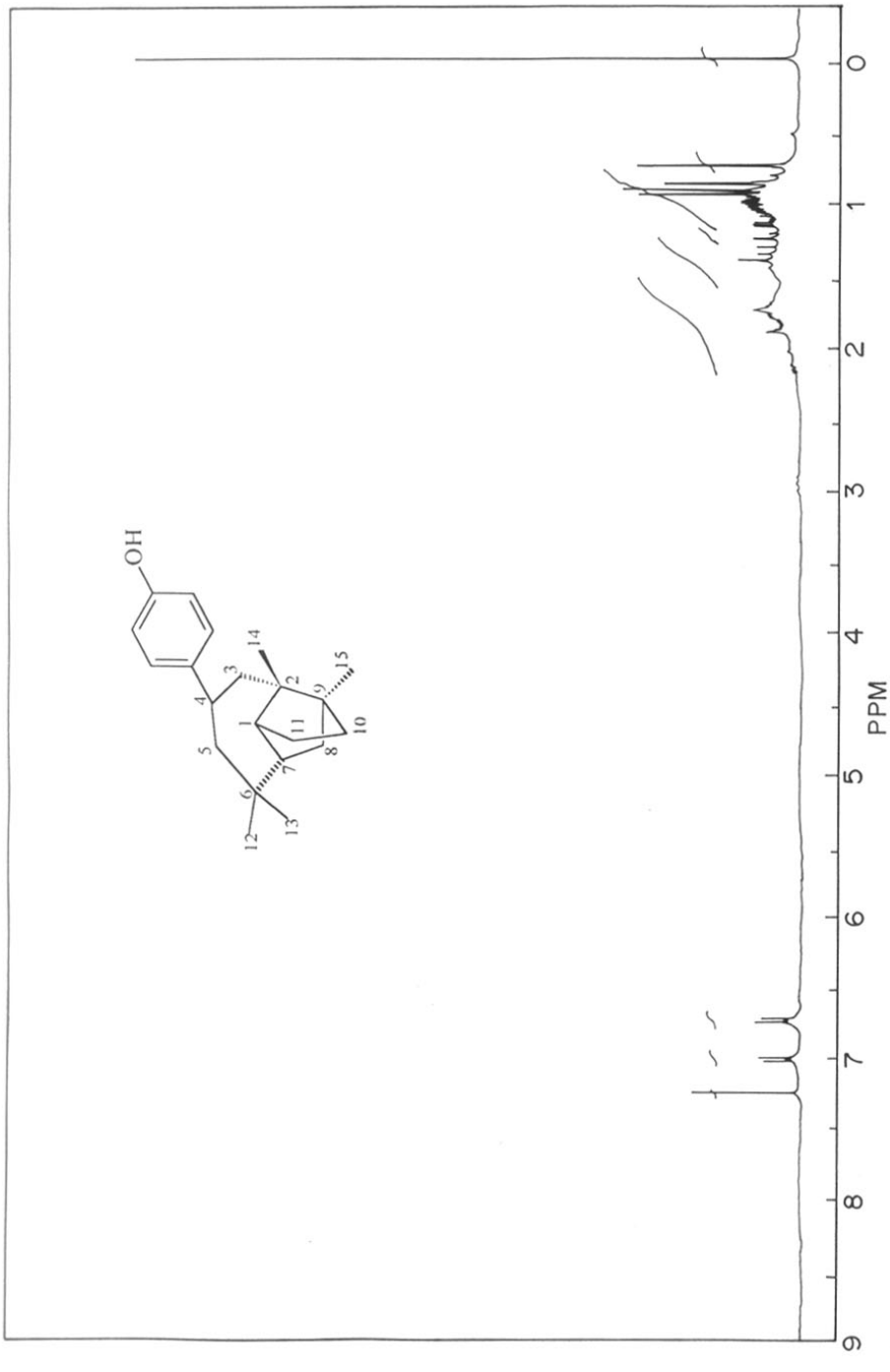


FIG 6 : ^{1}H NMR SPECTRUM OF COMPOUND 13 (300 MHz)

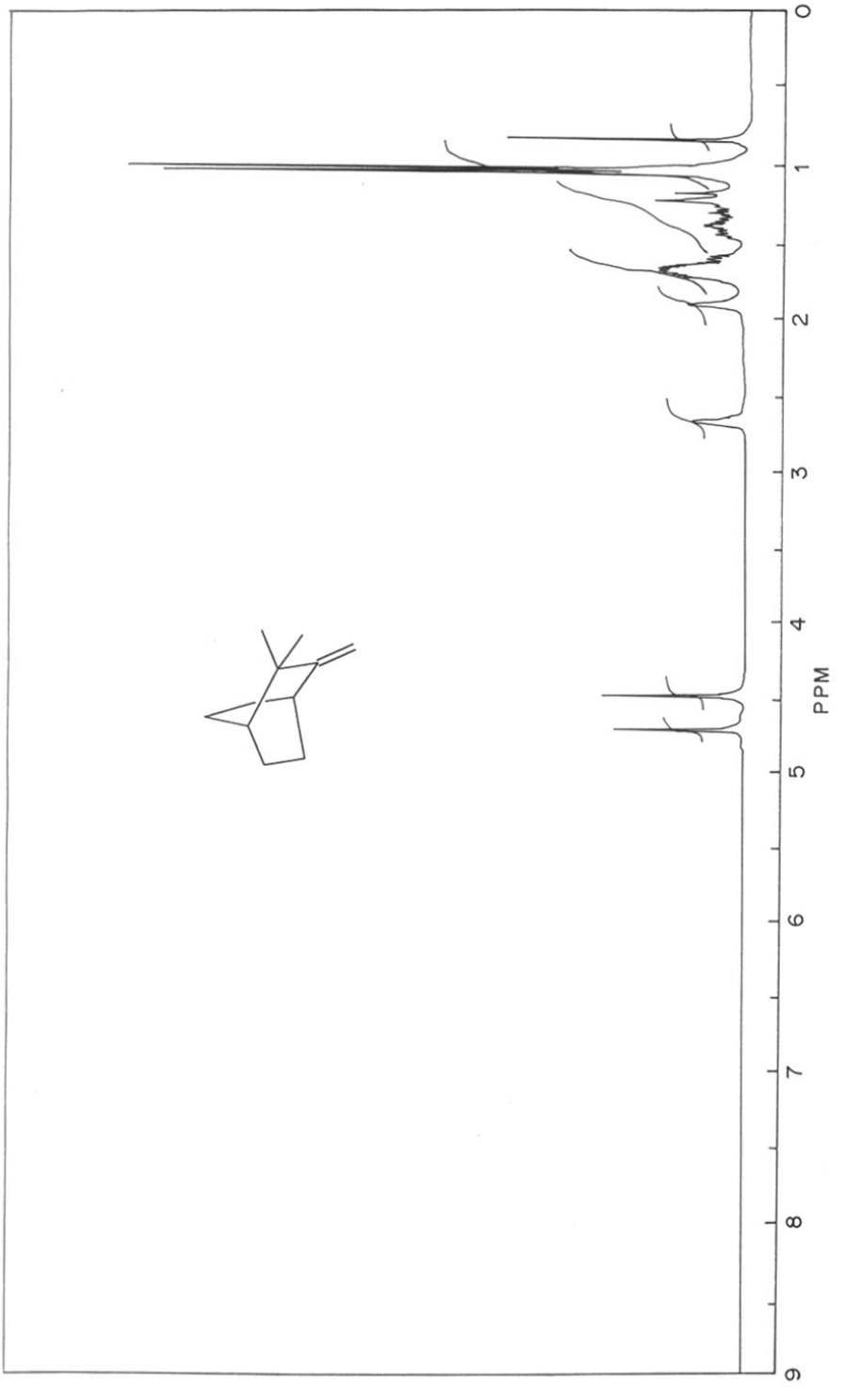


FIG 7 : ^{1}H NMR SPECTRUM OF CAMPHENE

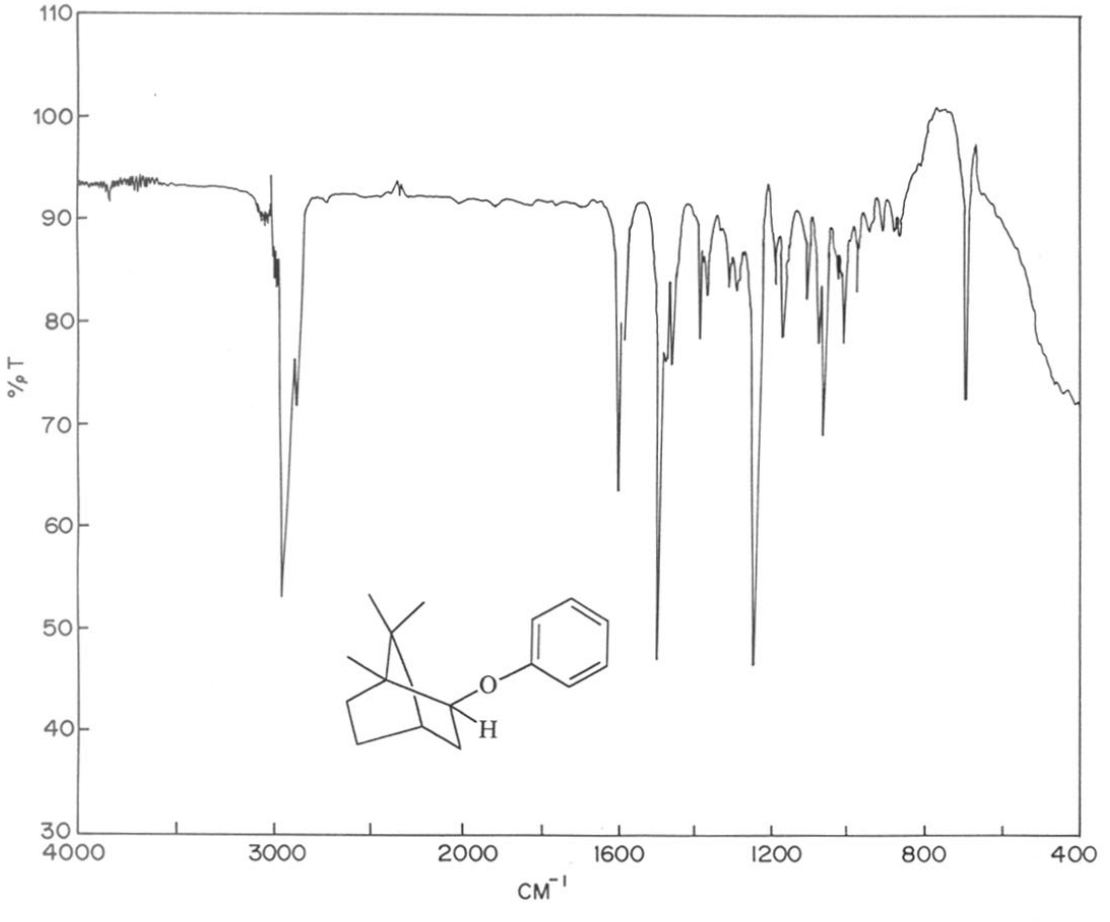


FIG 8 : FT - IR SPECTRUM OF COMPOUND 6

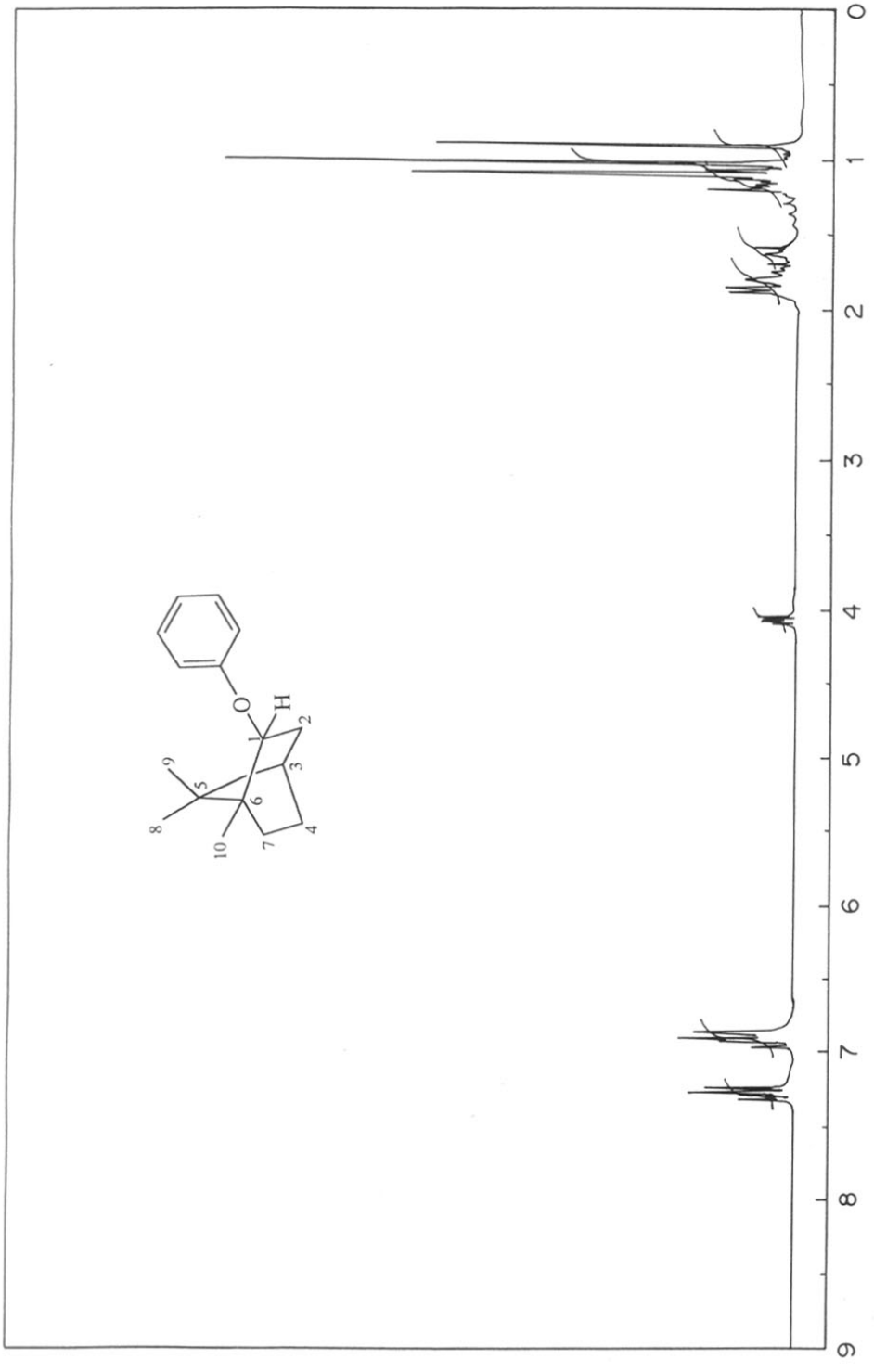


FIG 9 : ^{1}H NMR SPECTRUM OF COMPOUND 6

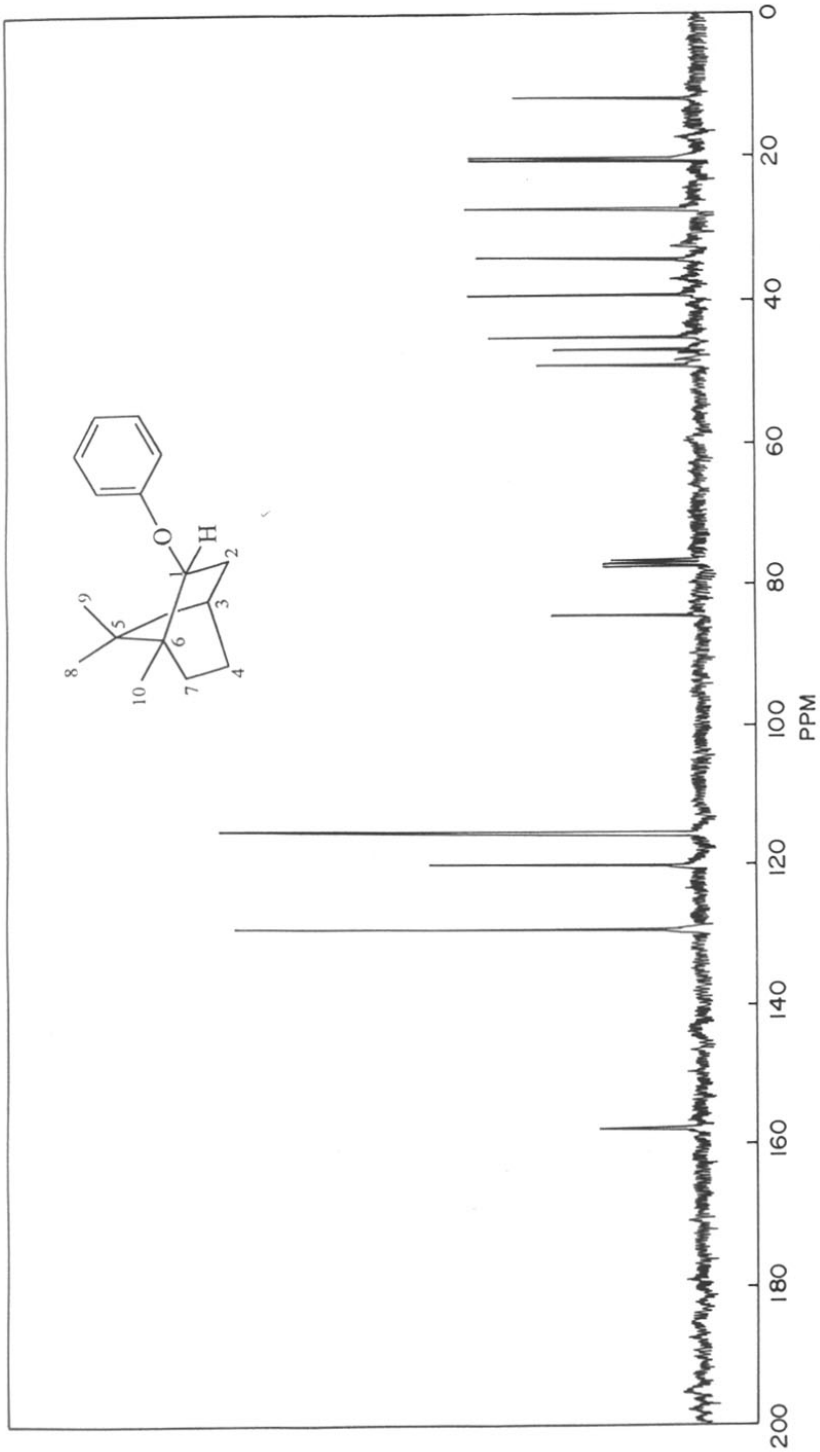


FIG 10 : ^{13}C NMR SPECTRUM OF COMPOUND 6 (50.32 MHz)

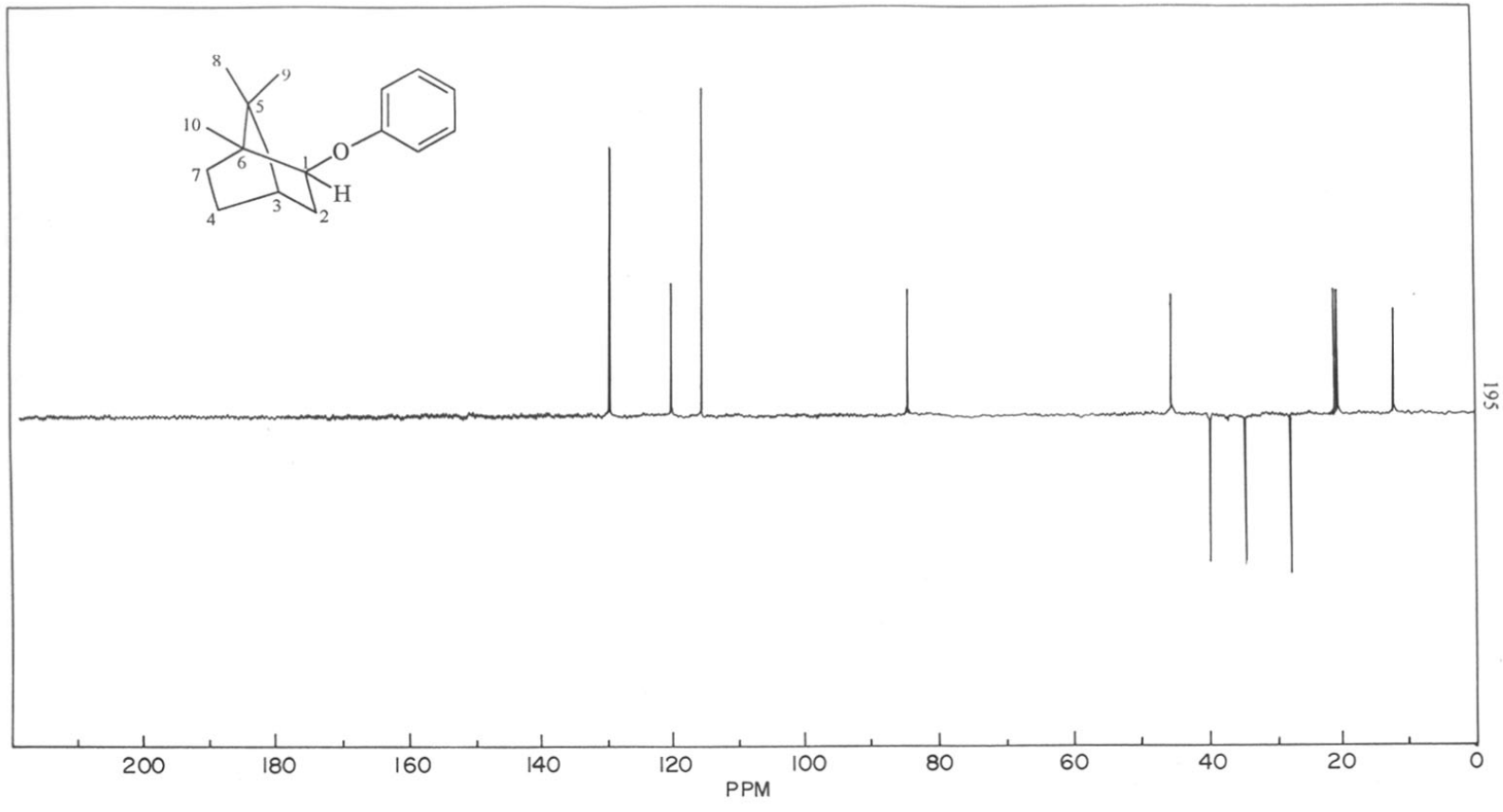


FIG 11 : ^{13}C NMR SPECTRUM (INEPT) OF COMPOUND 6 (50.32 MHz)

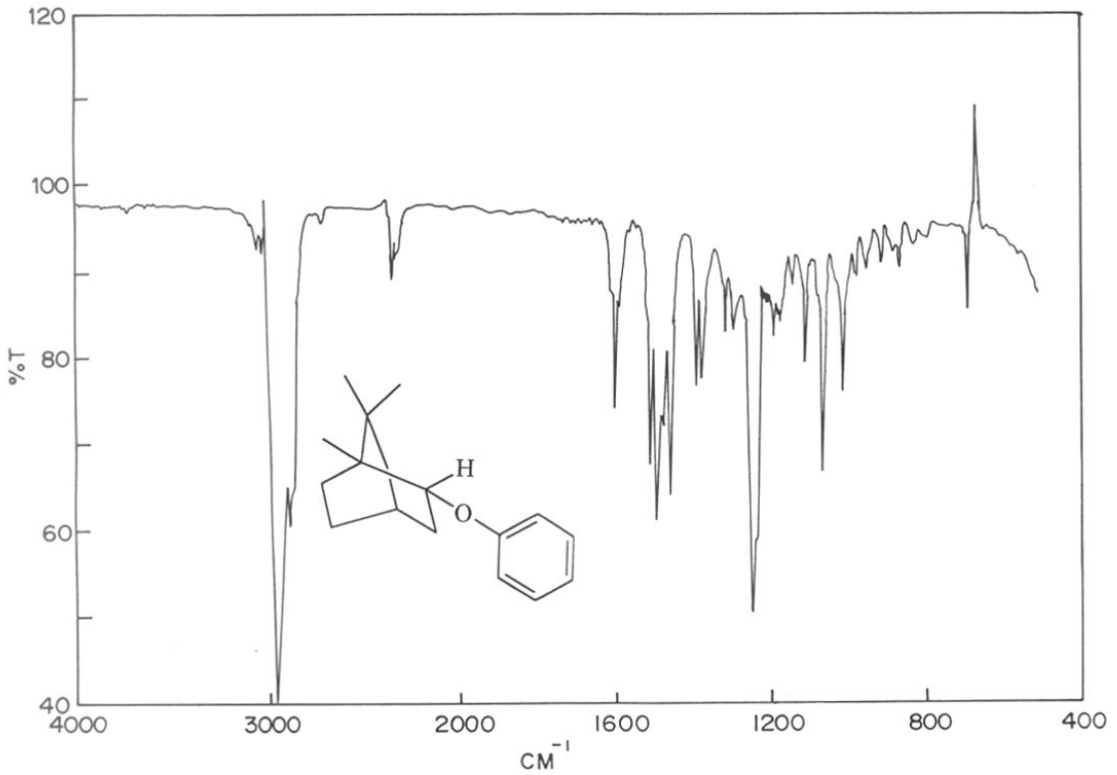


FIG 12 : FT - IR SPECTRUM OF COMPOUND 14

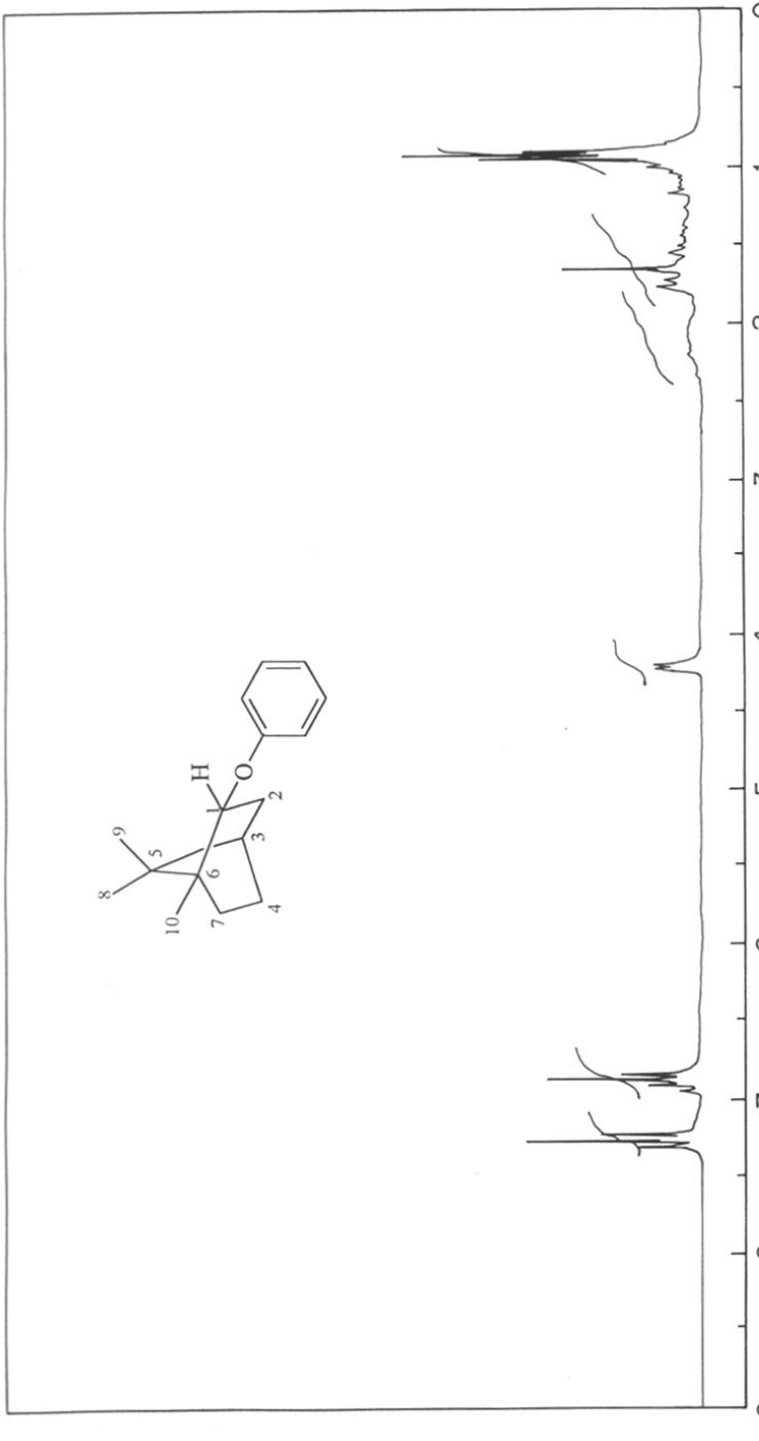


FIG 13 : ^{1}H NMR SPECTRUM OF COMPOUND 14

List of poblications

1. A new germacranolide from *Artemisia pallens*.
   S.R. Rojatkar, S.S. Pawar, **P.P. Pujar,** D.D. Sawaikar, S. Gurunath, V.T. Sathe and B.A.Nagasampgi, *Phytochemistry*, **41**, 1105 (1996).
2. Alumina catalysed derivatisation of sesquiterpene lactones.
   S.Gurunath, B.R. Patewad, D.D. Sawaikar,G.T. Panse, **P.P. Pujar**, B.A. Nagasampgi and S.R. Rojatkar, *Ind. J. Chem.* **36B**, 1047 (1997).
3. Photo-oxidation of Nimbin, a tetranortroterpenoid from *Azadirachta indica*.
   S.R. Rojatkar, B.R. Patewad, D.D. Sawaikar, G.T. Panse, **P.P. Pujar** and B.A. Nagasampgi., *Natural Product Letters*, **11**, 181 (1998).
4. Isolation of Parthenolide frpm the leaves of *Michelia champaka* (linn).
   V.C. Balurgi, S.R. Rojatkar, **P.P. Pujar**, B.K. Patwardhan and B.A. Nagasampagi *Indian Drugs*, **34**,415 (1997).
5. Guainolide from *Amberboa divericata*
   S.R. Rojatkar, **P.P. Pujar**, B.R. Patewad and B.A. Nagasampagi *Ind. J. Heter. Chem.*, **7**, 67 (1997).

Paper at Symposium

7β-Acetoxy-10-deacetyl baccatin III from the needles of *Taxus baccata*.

S.R. Rojatkar, S. Gurunath, D.D. Sawaikar, **P.P. Pujar,** G.T. Panse and B.A. Nagasampgi

7th International Symposium on Natural Products Chemistry, Dec. 28, 1997, Karachi, Pakistan. Abs. P.17

Manuscripts ready for Communication

1. A germacranolide from *Artemisia pallens*.
   P.P. Pujar, S.R. Rojatkar, and B.A.Nagasampgi.
2. New sesquiterpenoids from *Sphaeranthus indicus*
   P.P. Pujar, S.R. Rojatkar, D.D. Sawaikar, and B.A. Nagasampgi.
3. 7-Hydroxysesquiterpene lactones, a review
   P.P. Pujar, S.R. Rojatkar, D.D. Sawaikar, and B.A. Nagasampgi.
4. Biologically active steroidal constituents from *Asparagus racemosus*
   P.P. Pujar, S.R. Rojatkar, and B.A.Nagasampgi.