

STUDIES IN SESQUITERPENE LACTONES

A THESIS
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FOR THE DEGREE OF
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IN CHEMISTRY



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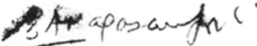
1982

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*Dedicated To
My Parents*

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Certified that the work incorporated in the thesis "Studies in sesquiterpene lactones" submitted by Shri J.S. Sohoni was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis.


(Dr. B.A. Nagasampagi)
SUPERVISOR

A C K N O W L E D G E M E N T

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(J.S. Schoni)

C O N T E N T S

		Page No.
GENERAL REMARKS	..	1-3
<u>CHAPTER I</u>		
sesquiterpene lactones of Compositae	..	4-71
Introduction	..	4
Classification	..	5
biogenesis	..	9
Isolation	..	13
Chemical transformations	..	16
Physical methods for structure elucidation	..	34
Stereochemistry and position of attachment of lactone ring	..	43
biological activity	..	49
References	..	58
<u>CHAPTER II</u>		
Chemical investigation of <u>Cyathocline purpurea</u>		72-137
Introduction	..	72
Present work	..	73
Experimental	..	95
References	..	108
Figures	..	110
<u>CHAPTER III</u>		
Chemical investigation of <u>Cyathocline lutea</u>		138-184
Introduction	..	138
Present work	..	138

Experimental	..	155
References	..	164
Figures	..	165

CHAPTER IV

Chemical investigation of <u>Sphaeranthus Indicus</u>	..	185-231
Introduction	..	185
Present work	..	188
experimental	..	201
References	..	210
Figures	..	211

CHAPTER V

Structure activity relationship of insect antifeedants	..	23P-286
Introduction	..	232
Present Work	..	246
Experimental	..	255
References	..	263
Figures	..	267

ABSTRACT	..	287-292
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GENERAL REMARKS

1. The figure (spectra) numbers, chart numbers, scheme numbers and reference number etc. given in each chapter refer to that particular chapter only. The references and figures are given at the end of each chapter.
2. The temperatures are given in centigrade scale.
3. All the solvents were distilled before use. Petroleum-ether refers to the fraction boiling in the range 60-80°.
4. Unless otherwise mentioned all the column chromatographic separations were carried out using the TLC grade silicagel using the dry column technique. The silica gel used had a particle size range of 200-300 mesh. The silica-gel was activated at 300° for five hours before using for chromatography.
5. The thin layer chromatographic (TLC) and preparative TLC plates were prepared by spreading an aqueous suspension of silica-gel (200-300 mesh, containing 13% CaSO_4 as binder) uniformly over glass plates using an applicator.
Layer thickness: TLC plates: 0.2 mm; preparative TLC: 1-1.5 mm.
After initial drying at room temp. the plates were activated at 100° for one hour before use.
6. Unless otherwise mentioned, all TLC and preparative TLC were run using mixtures of acetone and pet.ether as solvent. The percentage composition is given in parenthesis. (For example, eluent: 40:60 means eluent:acetone-pet.ether 40:60).

7. After development, the spots on TLC plates were visualized by exposing them to iodine vapours and/or by spraying with a mixture of $\text{H}_2\text{SO}_4\text{-HNO}_3$ (1:1) followed by charring in an oven. In case of preparative TLC plates the bands of compounds (after developing) were visualised by spraying a dilute solution of iodine in CHCl_3 to the sides (after covering the major central portion with a glass plate).
8. All the melting points ~~points~~ reported are uncorrected.
9. Optical rotations were measured in CHCl_3 solution using sodium light (5893) as the source on a Jasco Dip 181 digital polarimeter.
10. The UV spectra were recorded in ethanol solution on a Carl Zeiss 'Specord' UV-visible spectrophotometer.
11. Unless otherwise stated, the IR spectra of compounds were recorded in nujol mull with sodium chloride optices. The IR spectra were recorded on Perkin-Elmer 599B and Perkin-Elmer "Infracord" 137B model.
12. Mass spectra were recorded on a CEC-2-110B double focussing spectrometer using direct inlet system at 70 eV.
13. All the ^1H NMR and ^{13}C -NMR spectra were recorded in CDCl_3 solution (10%) using TMS as internal standard. ^1H NMR chemical shifts are given in δ -scale. Most of the ^1H NMR spectra were recorded at 90 MHz on a Bruker WH-90 (spectrospin) spectrometer. 60 MHz ^1H -NMR spectra were recorded on a Varian T-60 spectrometer.

14. The ^{13}C -NMR spectra were recorded at 22.63 MHz on a Bruker WH-90 spectrometer. All ^{13}C -NMR spectra were recorded twice as (1) proton noise decoupled or single line spectrum and (2) single frequency offresonance decoupled (SFOD) spectrum.

CHAPTER I

SESQUITERPENE LACTONES OF COMPOSITAE

INTRODUCTION

Sesquiterpene lactones are a class of naturally occurring plant terpenoids formed via head to tail condensation of three isoprene units and subsequent cyclization and oxidative transformations to produce a cis or transfused lactone ring. Sesquiterpene lactones are common constituents of most genera of compositae with the exception of the evolutionary advanced tribe the tagatae¹. They are also reported to occur sporadically in the genera of Umbelliferae, Magnoliaceae, Louraceae, Winteraceae, Illiciaceae, Aristolochiaceae, Menispermaceae, Cortiariaceae, Acanthaceae².

Reports dealing with the isolation and structure elucidation of these compounds have increased dramatically in last two decades. Two reasons can be given for the strongly increasing interest in this group of natural products. First: sesquiterpene lactones have been successfully used as markers in the biochemical systematic (chemotaxonomy) studies mainly in compositae³⁻⁸. Second: more recently a number of compounds received considerable attention due to their various biological activities, which resulted in an additional increase in the biological activity related publications⁹⁻¹⁴.

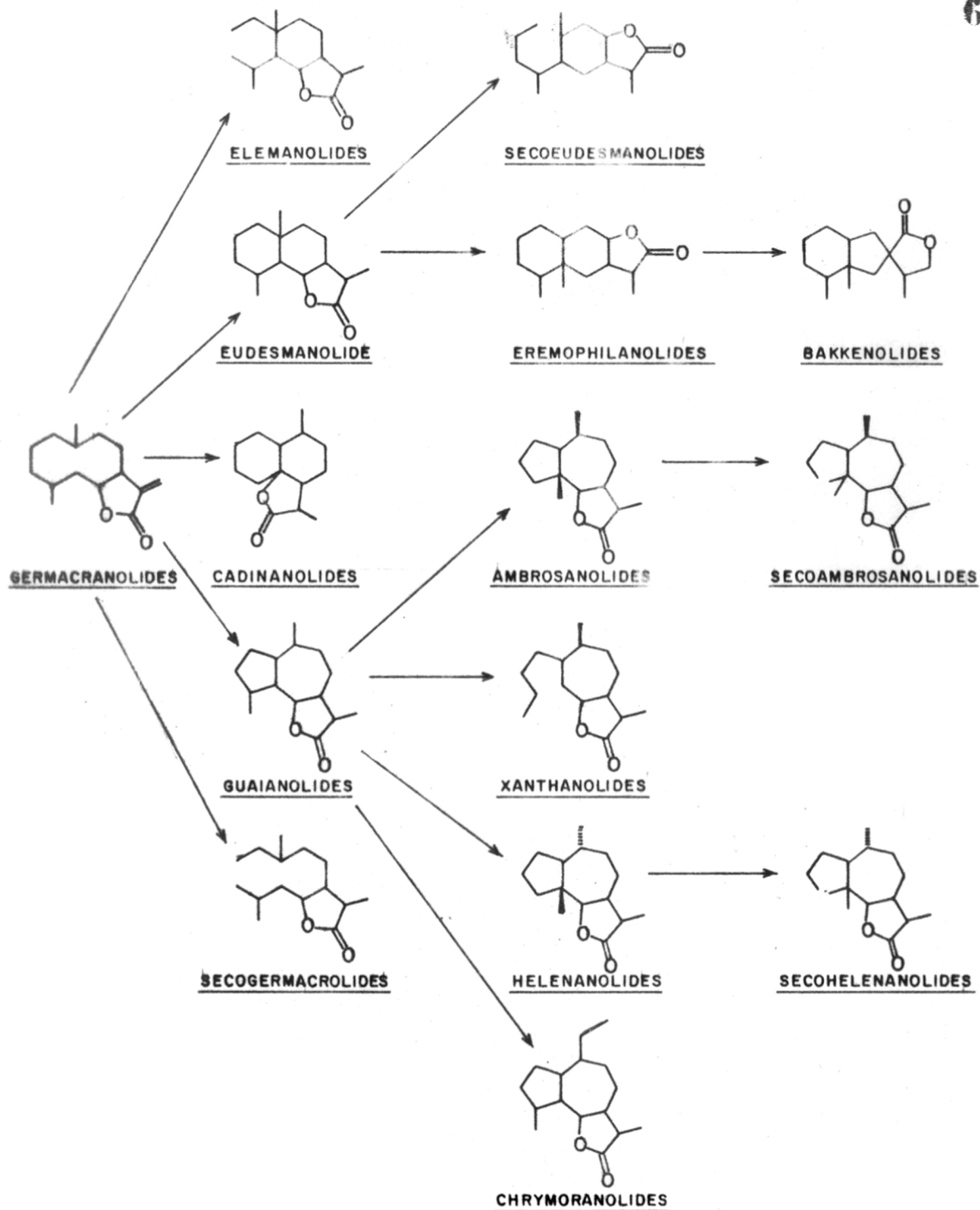
In addition to above said reasons, the recent advances in the isolation techniques such as adsorption chromatography,

gas liquid chromatography and high performance liquid chromatography and the developments in the physical methods for structure elucidation such as IR, NMR, ^{13}C NMR, Mass spectroscopy, X-ray diffraction and CD also have a marked effect on the increase in the number of publications related to isolation and structure elucidation of these compounds.

✓ CLASSIFICATION

Sesquiterpene lactones are colourless, bitter, relatively stable, lypophyllic constituents which are biogenetically derived from trans-trans farnesyl pyrophosphate¹⁵ following an initial cyclization and subsequent oxidative modifications¹⁶. The major types of lactones resulting from such enzyme mediated cyclizations are classified on the basis of their carbocyclic skeleton as germacranolides, eudesmanolides, guaianolides, pseudo-guaianolides, eremophilanolides and xanthanolides. The less common classes of sesquiterpene lactones are secoeudesmanolides, chrymoranolides, bakkenolides, secoambrosanolides and secohelenanolides. Germacranolides are considered to be the biogenetic precursors for all other classes of sesquiterpene lactones¹⁵.) The various classes of sesquiterpene lactones and their biogenetic relationship are represented in Chart I¹⁷.

The germacranolides are characterized by a cyclo-decadiene skeleton with double bonds in C-1, 10 and

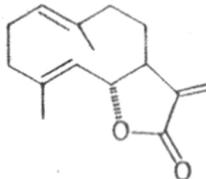
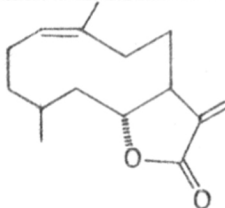
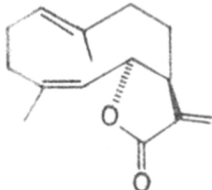
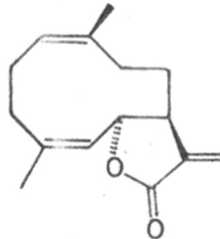


CLASSIFICATION OF SESQUITERPENE LACTONES

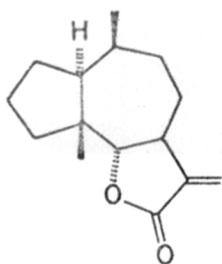
C-4,5 positions. Depending upon the configuration of the double bonds¹⁸ the germacranolides are reclassified into four subgroups^{19,20}, namely (i) germacrolides (ii) melampolides (iii) heliangolides and (iv) cis-cis germacranolides. Among the four germacranolide subgroups the germacrolides represent the largest sub-group although an increasing number of melampolides²¹ and heliangolides²² have been isolated. The cis-cis germacranolides represents the smallest sub-group, members of which have been isolated only recently²³⁻²⁵. The Chart IIA shows the basic configurational types.

The eudesmanolides are based on the eudesmane (selinane) skeleton with double bonds in 3,4, 4,5 or 4,15 positions whereas the guaianolides and pseudoguaianolides are based upon the 5,7 ring skeleton. The guaianolides contain methyl groups at C-4 and C-10 positions, whereas in pseudoguaianolides the C-4 methyl group has migrated to C-5 ring **junction**. The pseudoguaianolides are further classified into two subgroups i.e. (i) ambrosanolides and (ii) helenanolides (Chart IIB) both of which possess a trans-fusion of the 5,7 ring system. The ambrosanolide skeleton typically contains a C-10 β -methyl group whereas in helenanolides the C-10 methyl group is α .

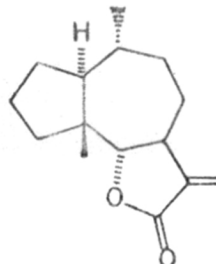
[A] CLASSIFICATION OF GERMACRANOLIDES

SUBGROUP	CONFIGURATION		SKELETAL STRUCTURE	EXAMPLE WITH REFERENCE
	$\Delta^{1,10}$	$\Delta^{4,5}$		
1) Germacrolides	<u>trans</u>	<u>trans</u>		Costunolide
2) Melampolides	<u>cis</u>	<u>trans</u>		Melampodin - A
3) Heliangolides	<u>trans</u>	<u>cis</u>		Heliangin
4) cis cis Germa- cranolide	<u>cis</u>	<u>cis</u>		Melcanthin - A

[B] CLASSIFICATION OF PSEUDOGUAIANOLIDES



Ambrosanolide



Helenanolide

BIOGENESIS

Biogenetic theory assumes that the biosynthesis of sesquiterpenoids involves modifications and/or cyclization of pyrophosphate esters of trans-trans farnesol, cis-trans farnesol or nerolidol (1)²⁶ which in turn has its biogenetic origin from the acetyl co-enzyme A, as depicted in Chart III, Scheme I. The initial step in the biogenesis is the cyclization of (1) into a 1,(10),4,5-germacradiene cation (2) which later undergoes various oxidative modifications and recyclizations to give various types of sesquiterpene lactones (Chart I).

Formation of the γ -lactone, typical of Compositae involves (Chart III) oxidation of side-chain and introduction of lactonic oxygen at C-6 or C-8 and eventual ring closure to form either a costunolide (3) or inunolide (4) respectively which represents the most elementary cyclic sesquiterpene lactone, since it retains two of the three double bonds of the farnesyl pyrophosphate in the trans-trans configuration. The details of this process are not known although two possible biogenetic schemes have been suggested. The first of these²⁷ may well be responsible for the occasional occurrence of the lactones of type A (Chart III, Scheme II) in those plant groups which normally elaborate furano-sesquiterpenoids (Lauraceae, Senecionae) although its relevance to the formation of type B lactones so prevalent in Compositae is less clear (Chart III, Scheme II). An

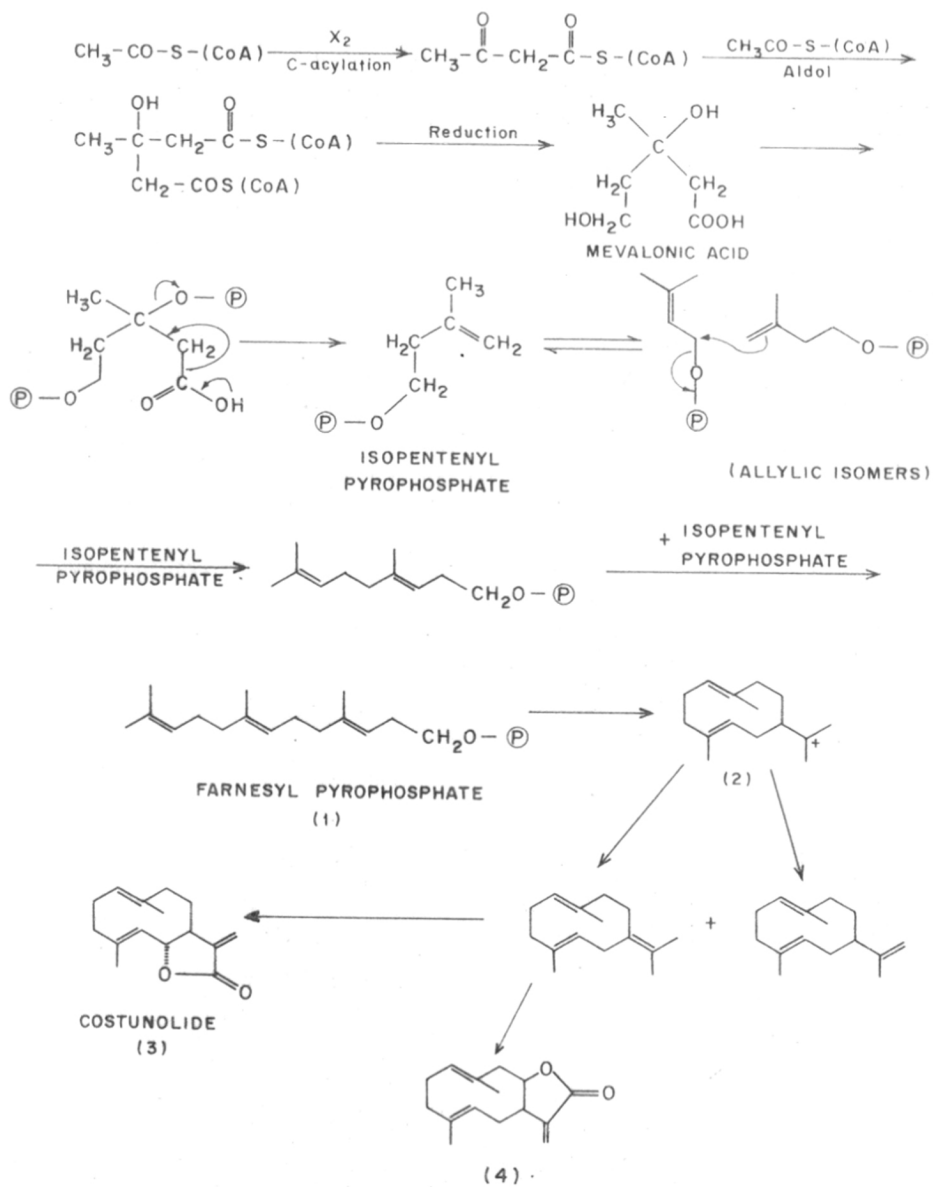
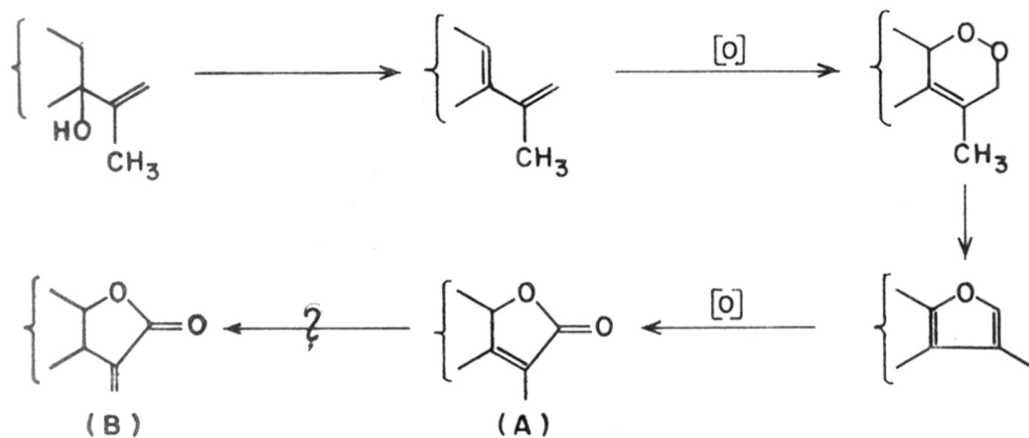
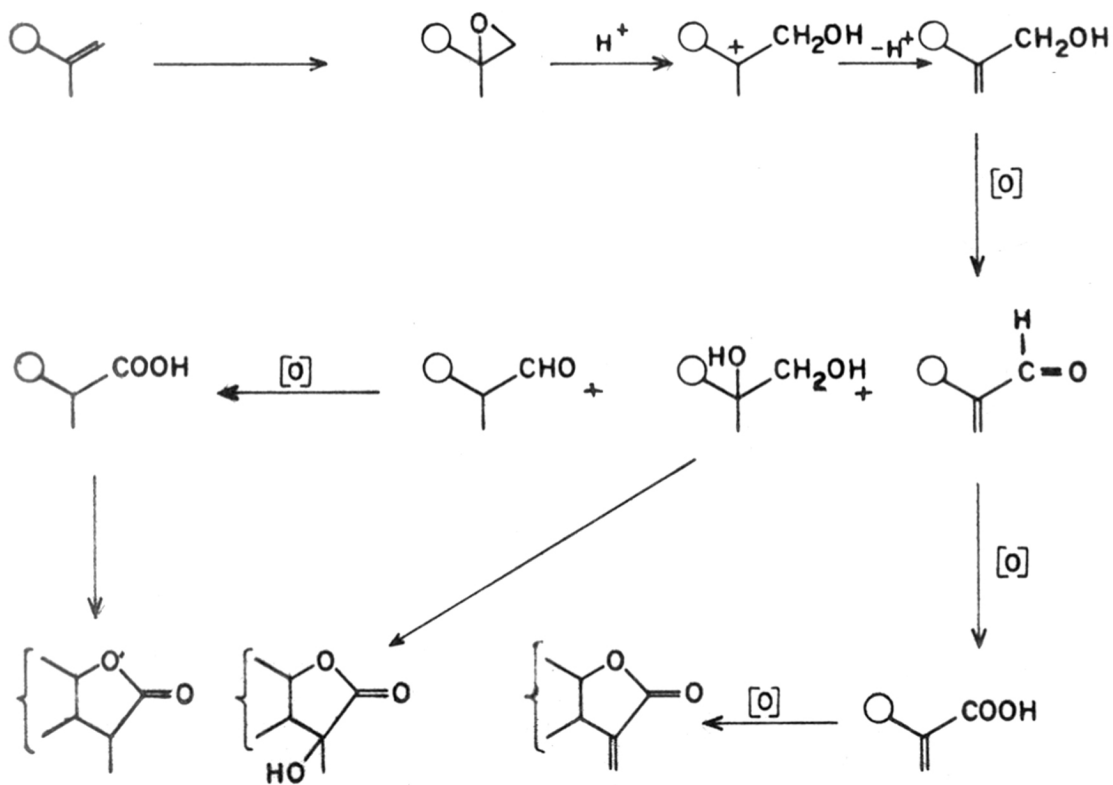


CHART III (SCHEME I)

CHART - III



SCHEME II



SCHEME III

alternative proposal²⁸ (Chart III, Scheme III) involves oxidation of isopropenyl side-chain of sesquiterpenes followed by introduction of oxygen at C-6 or C-8 presumably after cyclization of germacrodien and lactone ring closure. This mechanism appears more attractive as a general route to sesquiterpene lactones since the epoxides, alcohols, aldehydes and acids listed in the scheme, sometimes already oxidized at requisite ring position are widely distributed in nature and since the postulated intermediates occasionally accompany the lactone end products.

It is not yet clear whether the four subgroups of germacranolides (Chart IIa) are biosynthesised directly from the double bond isomers of farnesyl pyrophosphate or the double bond isomerization of the germacrolide skeleton takes place at a later stage. However, the co-occurrence of more than one skeletal type of germacranolides in some species^{22,24} and the fact that almost all the melampolides isolated so far have their C-14 oxidized, indicated a probable interconversion from one skeletal type to another during or after oxidation of C-14 and/or C-15.

Among the other major classes of sesquiterpene lactones, eudesmanolides and guaianolides are formed by cyclization of germacranolides or their precursors while further modification by methyl shift in guaianolides lead to pseudoguaianolides. Eudesmanolides (7) appear to have their origin through an acid catalyzed or enzymatic

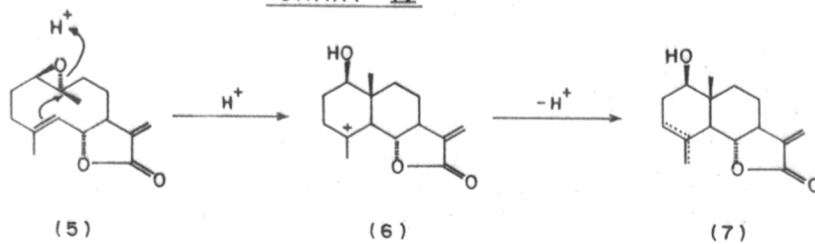
Markownikow oriented cyclization of 1,10 epoxygermacranolide (5)^{29,30} (Chart IV, Scheme I). A similar cyclization of a chair-folded conformer of 4,5-epoxygermacrolide precursor (8) followed by addition of water to the resulting guaianolide cation (9) is the probable biosynthetic pathway for the more common cis fused guaianolides of type (10)³¹ (Chart IV, Scheme II). On the other hand, the precursor (9) can undergo a double hydride and methyl shift to give an ambrosanolide precursor which has a transfused 5,7-ring system with a β -oriented methyl group at C-10. The latter intermediate then preferentially lactonizes with a 6- β -hydroxyl group to give a 6,7 cis-lactone (11)³¹ (Chart IV, Scheme II). The less common trans (i.e. H-1 β , H-5 α) guaianolides (14) appear to be originated from an acid catalyzed cyclization of melampolide 4,5 epoxide (12) through the cation (13). The indicated shifts in the cation (13) can lead to formation of helenanolides (15)³² which have a transfused 5-7 ring system with α -oriented methyl group at C-10.

Other minor classes of sesquiterpene lactones probably arise from further modification (such as oxidation, ring fission etc.) of the major types or of their precursors.

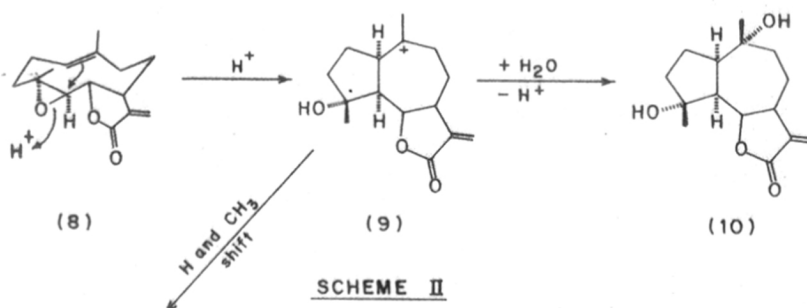
ISOLATION

The shade dried plant material is coarsely powdered before extraction. Chloroform is the most commonly used solvent for extraction of sesquiterpene lactones from plant

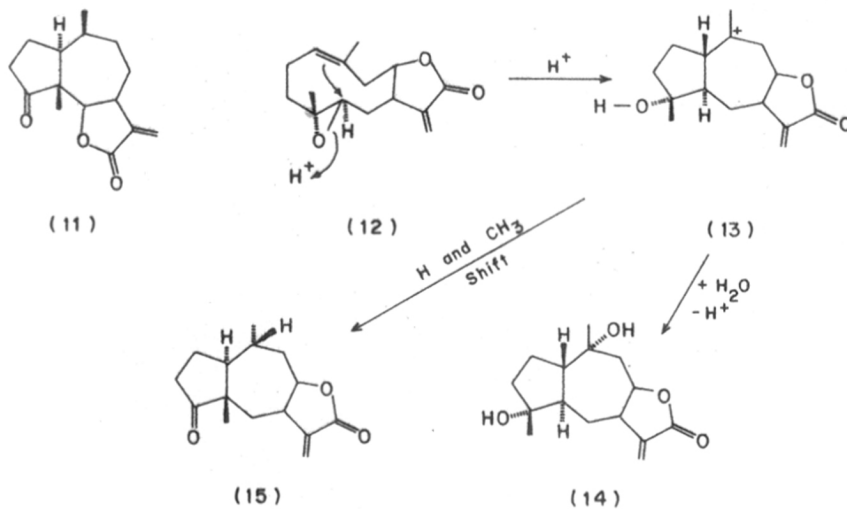
CHART IV



SCHEME I



SCHEME II



SCHEME III

material. However, other solvents such as methanol, ethanol, acetone, dichloromethane, ether, ether:petrol, ethylacetate, benzene etc. are also used for extraction. Maceration, percolation and Soxhlet extraction are the most commonly employed techniques for extraction. The residue obtained after removing the solvent from extract is usually subjected to column chromatographic separation. Silica gel (60-120 mesh size, or 200 mesh) is most commonly used as adsorbant, however alumina is also used occasionally. A wide variety of solvents (ranging in polarity from pet.ether to methanol) in various proportions have been used in the column chromatographic separation of sesquiterpene lactones. The choice of the solvent system depends mainly on the nature and polarity of the compound to be separated. The residue, before subjecting to the chromatographic separation, can be treated with 4% lead acetate solution so as to remove unwanted material and then subjected to the chromatographic separation³³. The progress of the chromatographic separation is monitored by thin-layer chromatography (TLC). The sesquiterpene lactones are detected by placing the plate in a chamber containing crystals of iodine or by spraying the plate with reagents such as 2-3% aq. potassium permanganate solution, concentrated sulfuric acid (followed by heating) ethanol-hydrochloric acid (heating). Recently Picman *et al.*³⁴ have suggested a spray reagent consisting of 5% vanillin or p-dimethyl aminobenzaldehyde, 5% concentrated sulfuric

acid and traces of acetic acid in ethanol (95%). This reagent is said to be very sensitive and gives characteristic colours with different sesquiterpene lactones.

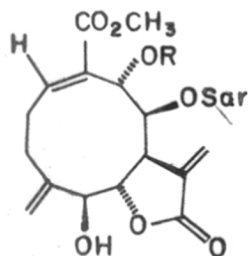
More recently high performance liquid chromatography (HPLC) has received considerable attention as a method of choice for the separation of complex mixtures of sesquiterpene lactones possessing the same carbocyclic skeleton but differing in nature of the side chain. For example rependins A and B (16) (Chart V) isolated as a mixture from Tetragonotheca repens have been separated by HPLC on a Bondapak C-18 column using CH_3CN -SM phosphate buffer as the eluent³⁵. Similarly melnerins A and B and their 9-acetoxy derivatives (17) (Chart V) isolated from Melampodium leucanthum³⁶ and melcanthins D-G (18) (Chart V) isolated from M. cinereum³⁷ have been separated by Bondapak C-18 reverse phase column using methanol-water (1:1) as the eluent.

CHEMICAL TRANSFORMATIONS

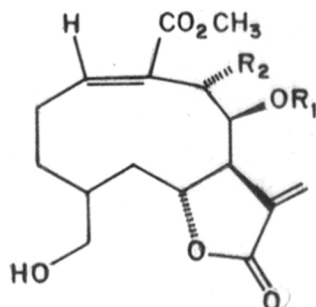
(A) Germacranolides: Hydrolysis and Relactonization:

In the course of structure elucidation alkaline hydrolysis of sesquiterpene lactones is frequently desired. A distinct difference in the ease of hydrolysis of various ester side-chains attached to germacranolide was described by Herz and Sharma³⁸. The authors demonstrated that a solution of K_2CO_3 in methanol water at room temperature hydrolyzes 3-hydroxy, 2-methyl, but-2-enoate in 20 to 30

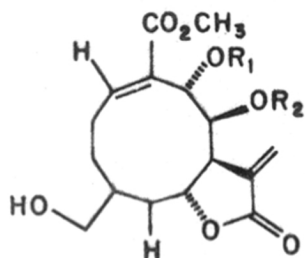
CHART V



(16)

Repandin A $R = i\text{-Bu}$ Repandin B $R = \text{Me-Bu}$ 

(17)

Melnerin A $R_1 = i\text{-Bu}, R_2 = \text{H}$ Melnerin B $R_1 = \text{Me-Bu}, R_2 = \text{H}$ 9 α -Acetoxy Melnerin A $R_1 = i\text{-Bu}, R_2 = \text{OAc}$ 9 α -Acetoxy Melnerin B $R_1 = \text{Me-Bu}, R_2 = \text{OAc}$ 

(18)

Melcanthin D $R_1 = \text{Ac}, R_2 = \text{Meacr}$ Melcanthin E $R_1 = \text{Ac}, R_2 = i\text{-Bu}$ Melcanthin F $R_1 = \text{Ac}, R_2 = \text{Me-Bu}$ Melcanthin G $R_1 = \text{Me-Bu}, R_2 = \text{Ac}$ 547.913(043)
50H

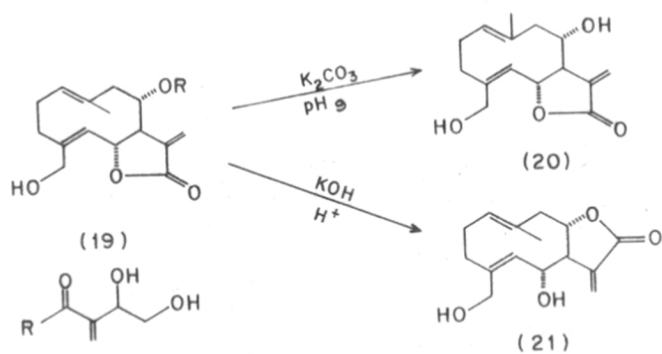
minutes whereas the isobutrate, α -methylene n-butate, tiglate and angelate give no reaction under same conditions.

Mild hydrolysis with K_2CO_3 of cinicin (19) provides solanitanolide (20) without opening the γ -lactone ring³⁹. However treatment under stronger alkaline conditions followed by acidification resulted in formation of C₇₋₈ lactonized germacranolide artemisiifolin (21) (Chart VI, Scheme I) similar relactonization of 7-6 lactonized germacranolides to C₇₋₈ lactonized germacranolides have been reported for chichuahuin which can be converted to chamissonin³⁹, the dilactones elephantopin and elephantin to C-8 lactonic elephantol⁴⁰. The data presented above led to a general relactonization rule for germacranolides containing both C-6 and C-8 oxygen functions. The rule states that upon strong alkali treatment followed by acidification, this type of germacranolides always relactonize to C-8³⁹.

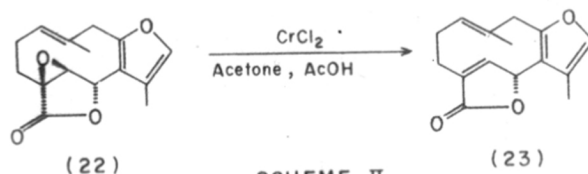
Reduction and Oxidation:

Generally catalytic hydrogenation of sesquiterpene lactones with Pt/C as catalyst as well as chemical reduction with sodiumborohydride in methanol proceeds with ease to give the corresponding 11,13-dihydroderivative (saturation of exocyclic methylene). The epoxides are reduced to alkenes by using either Zn-Cu couple^{41,42} or $CrCl_2$ ⁴³ as reducing agents. Linderane (22) was correlated with linderolactone (23) by reduction of linderane (22) with $CrCl_2$ in 50% yield⁴³ (Chart VI, Scheme II).

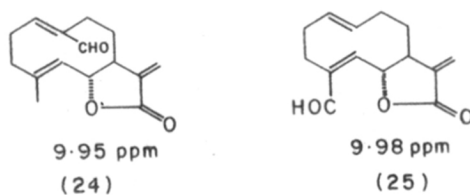
CHART VI



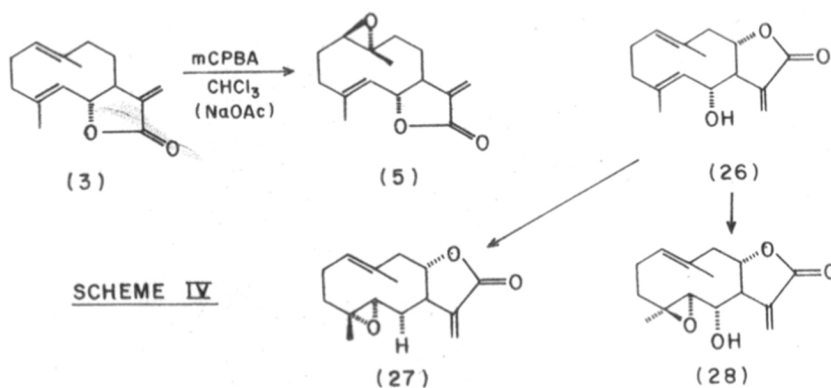
SCHEME I



SCHEME II



SCHEME III



SCHEME IV

Oxidation reactions have been frequently applied in structural elucidation of various types of germacranolides. MnO_2 oxidations generally transform primary allylic alcohols to α,β -unsaturated aldehydes. This reaction can be used for making configurational assignments to double bonds in cyclo-terpene skeleton, and for siting OH groups at C-14 or C-15. Herz and Sharma⁴⁴ have pointed out that the NMR chemical shift of aldehyde protons in α,β -unsaturated medium ring aldehydes depend upon the configuration of carbon carbon double bond. Compounds with trans double bonds generally exhibit NMR chemical shift for aldehyde protons at 10 ppm and above whereas for cis configuration the NMR absorptions are near 9.5 ppm. Aldehyde shift of germacranolide derivatives obtained from 14 hydroxy costunolide (24)⁴⁵ and from 8 desoxysalonitanolide (25)⁴⁶ are in good agreement with the predicted values (Chart VI, Scheme III).

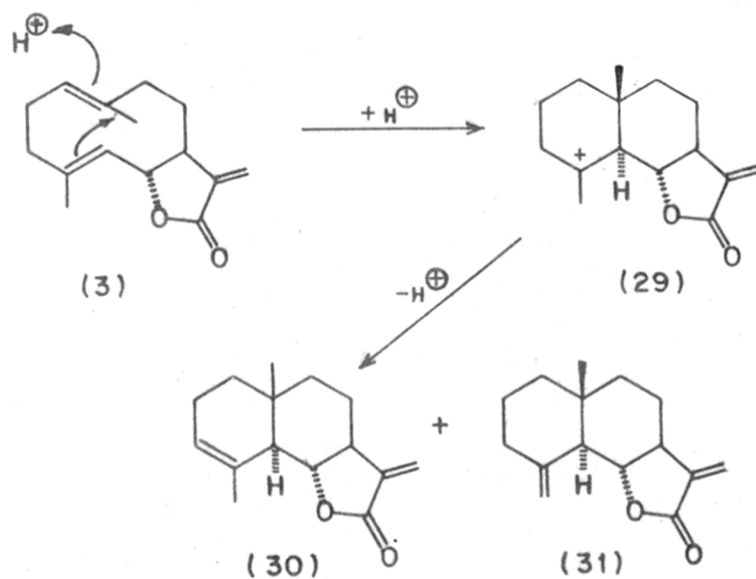
When 7,6-lactonic germacranolides are oxidized with per acids the 1,10 epoxides are formed preferentially. For instance costunolide (3) is converted to acid labile 1,10 epoxide (5) with *m*-chloroperbenzoic acid in chloroform in presence of sodium acetate as buffer⁴⁷. Without buffer the epoxide cyclizes under the reaction conditions forming mixture of eudesmanolides⁴⁸. Epoxidation of 7,8-lactonized desacetylauranobiolide (26) which exists in equilibrating mixture of conformers⁴⁹ results in mixture of two 4,5 epoxide derivatives (27), (28)⁵⁰. The preferential 4,5 epoxidation of

desacetyllaurenobicolide might be due to anchimeric assistance of the C-6 hydroxyl group in parent compound (Chart VI, Scheme IV).

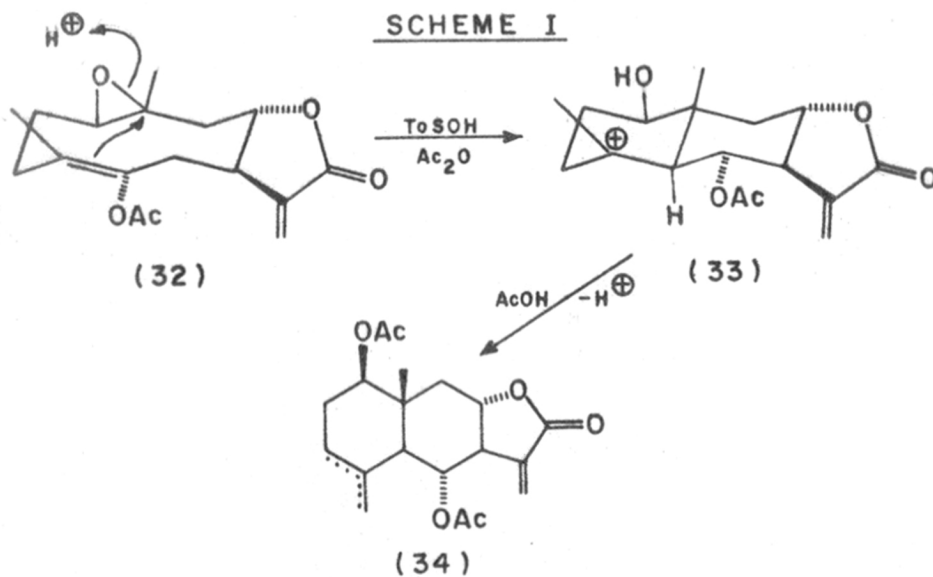
Cyclization reactions:

Lewis acid catalyzed cyclization reactions of cyclo-decaliene system or 1,10 and 4,5 epoxide derivatives play an important role in the structure elucidation of many sesquiterpene lactones of germacranolide class⁵¹. In general cyclization of germacradiene provides eudesmanolides. Thus costunolide (3) when treated with a cation exchange resin^{52,53} or $\text{HClO}_4/\text{AcOH}$ ⁵⁴ undergoes an acid initiated cyclization via cation (29) to give eudesmanolides (30, 31) (Chart VII, Scheme I). Analogous reactions with various lewis acid catalyst are known for arctiopicrin⁵⁵, chamissonin⁵⁶, novanin⁵⁷, tulipinolide and epitulipinolide⁵⁸, laserolide⁵⁹, lauranobicolide⁶⁰ and other germacranolides.

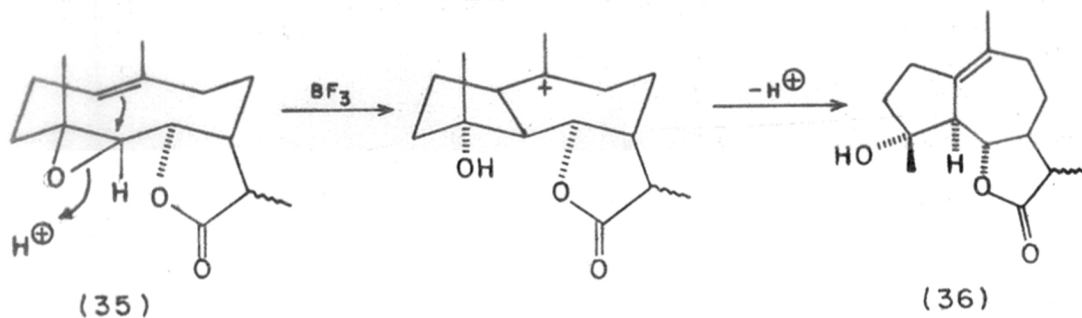
The conversion of double bond into an epoxide function either at 1,10 or 4,5 positions of a germacranolide increases the reactivity, the regiospecificity and the stereospecificity of cyclization reactions⁶¹. Pyrethrasin a 1,10 epoxide (32) cyclizes by an acid catalyzed Markovnikov type attack of C-5 at C-10 to form a cation (33) (Chart VII, Scheme II) which is stabilized by the uptake of acetic acid to give the eudesmanolide (34)⁶²⁻⁶⁴. In contrast BF_3 catalyzed reaction of 4,5 epoxide dihydroparthenolide (35) proceeds in a Markovnikov type attack of C_1 at C_5 to form a



SCHEME I



SCHEME II



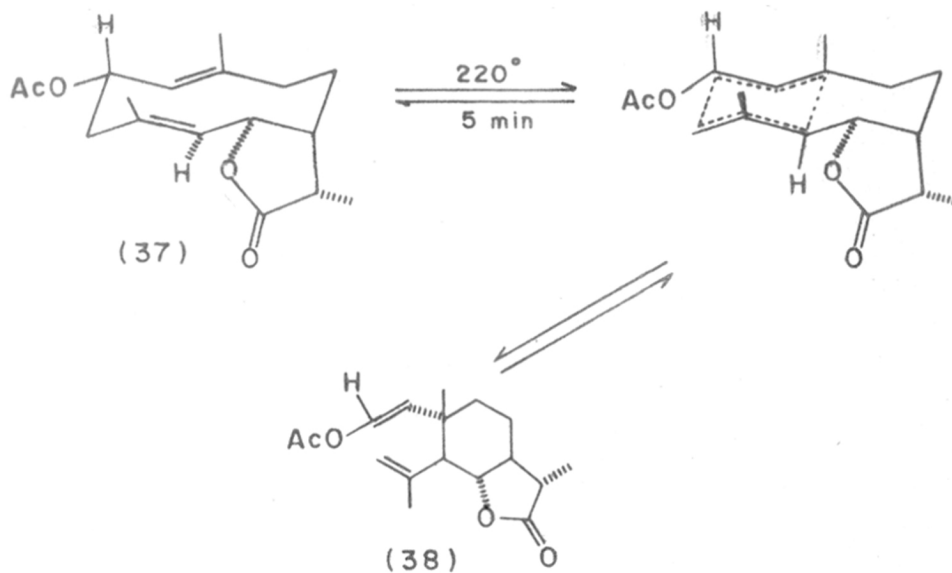
SCHEME III

guaianolide (36)⁶⁵ (Chart VII, Scheme III).

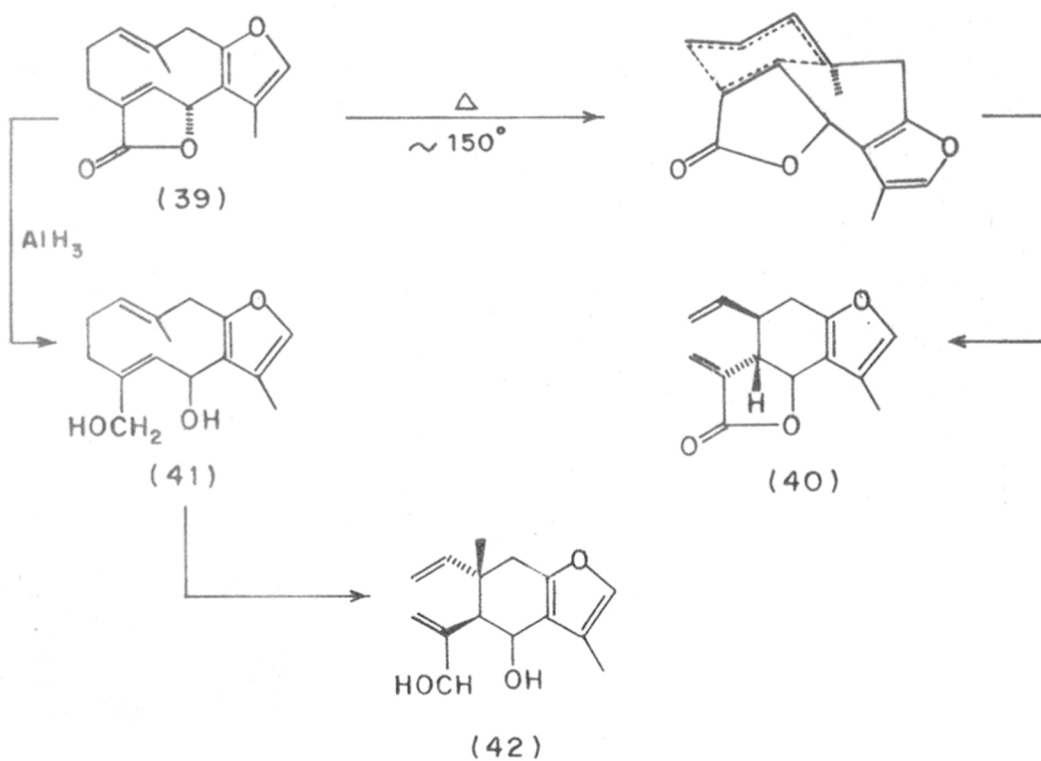
Cope Rearrangement:

Cope rearrangement of germacranolides have been reviewed by Takeda^{66,67}. Thermal rearrangements of cyclodeca 1,5-dienic sesquiterpene lactones proceed in a highly stereospecific manner through a chair like transition state resulting in a divinylcyclohexane skeleton. Thus thermolysis of dihyrotamaulipin A acetate (37) at 220°C for five minutes gives a 2:3 equilibrium mixture of starting material and divinylcyclohexane derivative (38)⁶⁸ (Chart VIII, Scheme I).

Linderalactone (39) on thermolysis gives an elemadiene compound with an α -methyl group at C-10 angular position (40)⁶⁹ (Chart VIII, Scheme II). This change in the stereochemistry of cope rearrangement product was ascribed to the presence of 4,6-lactone ring which prohibits conversion into a conformation analogous to (37) (dihyrotamaulipin A acetate) by rotation of double bond. When the lactone ring of linderalactone is reductively opened the resulting diol (41) can adopt a transition state similar to (37) to provide the elemadiene⁶⁹ which has the same stereochemistry at C-5 and C-10 (42) as in (38) (Chart VIII, Scheme II).



SCHEME I



SCHEME II

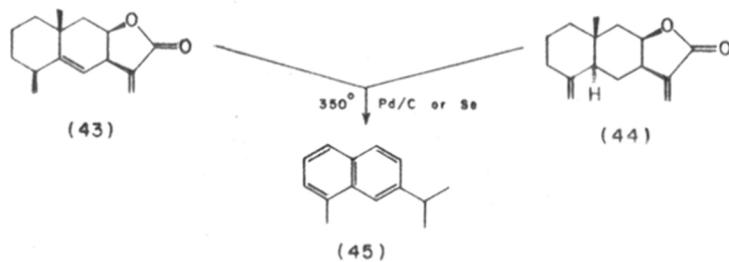
(B) Eudesmanolides: Dehydration, Hydrogenation and oxidation:

Pyrolysis of alantolactone (43) and isoalantolactone (44) at 350° in presence of Pd/C or selenium results in the naphthalene derivative eudalene (45) with loss of C-10 methyl group⁷⁰ (Chart IX, Scheme I).

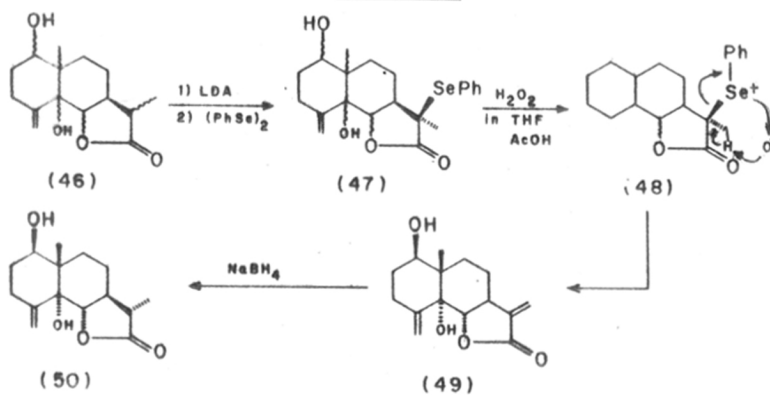
More recently Grieco⁷¹ described a novel and useful dehydrogenation process involving the conversion of α -methyl γ -lactones into α -methylene γ -lactones. Gonzalez *et al.*⁷² applied this method to determine the stereochemistry of artemin (46). Artemin after exposure to diisopropyl amide (IDA) and subsequent treatment of enolate with diphenyldiselenide gave the phenyl selenide (47), the selenide was then oxidized with H_2O_2 to (48) which underwent spontaneous elimination to give tanacetin (49) a compound of established stereochemistry⁷³. Reduction of compound (49) with sodium borohydride resulted in artemin (50) which established its entire stereochemistry as (50) since all sodiumborohydride reductions in this series provide a C-11 α -methyl epimer⁷⁴ (Chart IX, Scheme II).

Examples demonstrating catalytic hydrogenation of eudesmanolide are the conversion of reynosin (51) to C-4 epimeric mixtures of tetrahydroderivatives (53) and (54) with Pd/C and the formation of (54) and isodihydroderivative (52) upon reaction with PtO_2 ⁷⁵ (Chart IX, Scheme III). A number of other conversions involving hydrogenations and oxidations with various reagents are known⁷⁶.

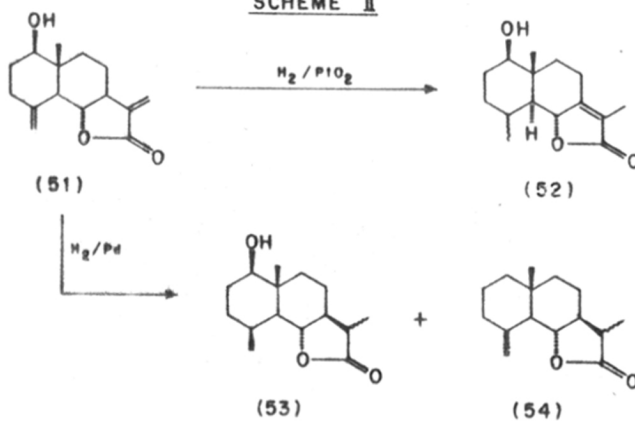
CHART IX



SCHEME I



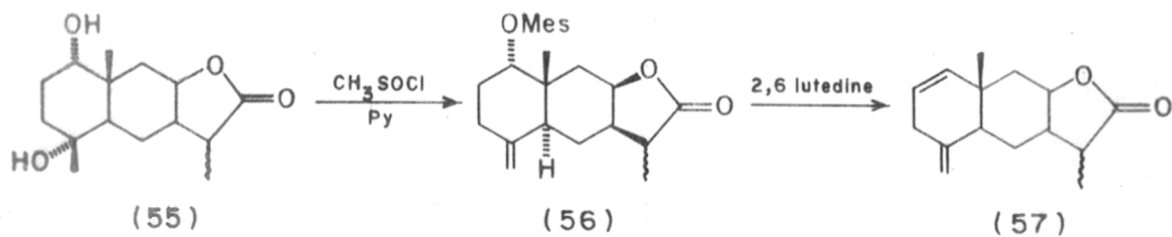
SCHEME II



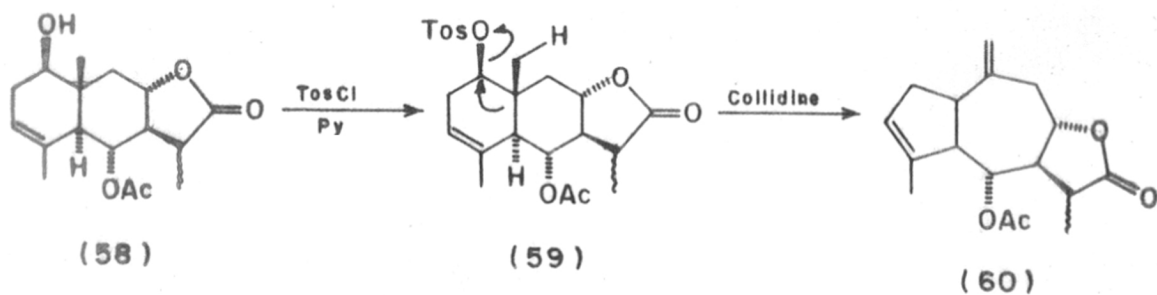
SCHEME III

Eudesmanolides have been frequently used as starting material for chemical and photochemical rearrangement processes which lead to other skeletal types of sesquiterpene lactones. Dihydromicrocephalin (55) on treatment with $\text{CH}_3\text{SO}_2\text{Cl/Py}$ forms a mesylate (56) which then undergoes the antielimination with 2,4 lutidine to give the product⁷⁷ (57) (Chart X, Scheme I). In contrast the dihydrocyclopyrethrosin (58) takes the different course. The compound (58) upon tosylation forms a C-1 β -tosylate (59) which in contrast to previous example has no proton at C-2 which is antiperiplanar to tosylate leaving group to permit antielimination. Instead, the stereochemistry is appropriate for elimination rearrangement reaction which occurs upon treatment of (59) with collidine to give a guaianolide (60)⁶² (Chart X, Scheme II). Hendrikson and coworkers⁷⁸ have prepared a pseudoguaianolide (63) starting from α -santonin 4,5 epoxide (61) which forms a cis decalin type bromolactone (62) through a series of steps. Stereospecific rearrangement of (62) resulted in formation of pseudoguaianolide (63) (Chart X, Scheme III).

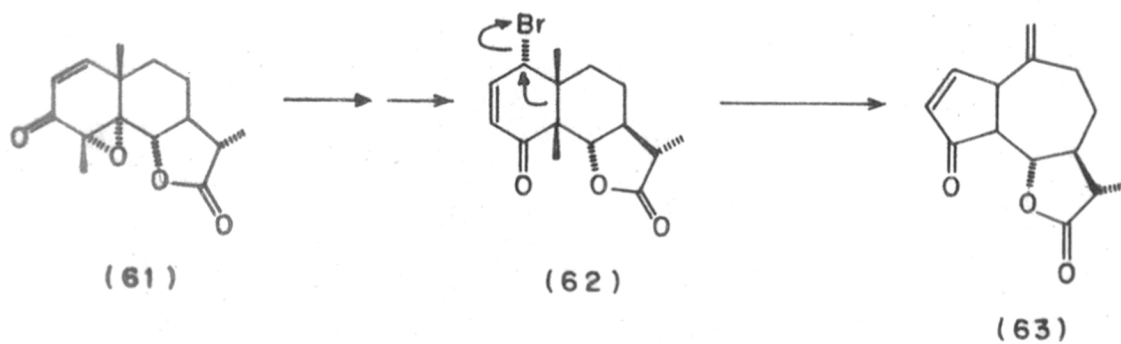
The base promoted conversion of α -santonin into santonic acid elucidated by woodward and co-workers⁷⁹, the acid catalyzed rearrangement of santonin yielding desmotroposantonin⁸⁰ and the photochemical transformations of santonin to lumisantonin, photosantonic acid and isophotosantonic lactone^{81,82} are additional important examples



SCHEME I



SCHEME II



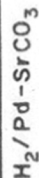
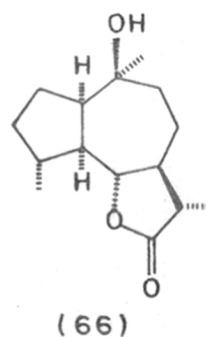
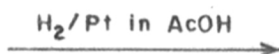
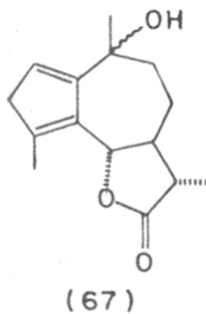
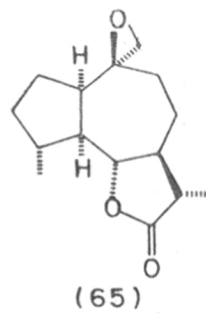
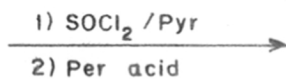
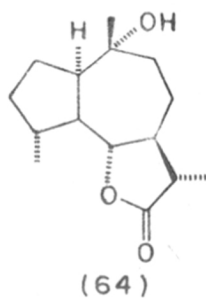
SCHEME III

of chemical and photochemical transformations of eudesmanolides.

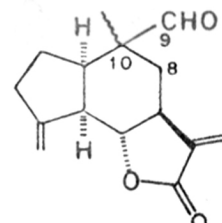
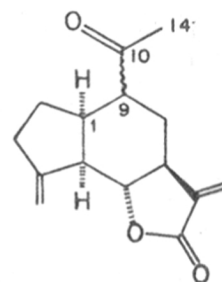
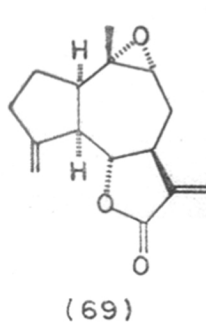
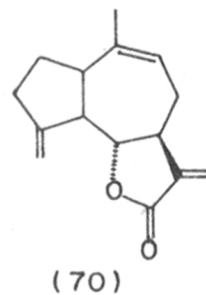
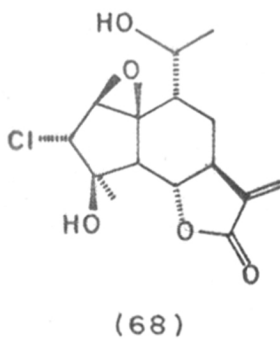
(C) Guaianolides:

Correlations by simple chemical modifications have been frequently used in structure elucidation and making stereochemical assignments in guaianolide series. The stereochemistry at C-10 of artabsin (67) was determined by a sequence of reactions which are outlined in Chart XI, Scheme I. Isophoto α -santonin lactone (64) a compound of known stereochemistry was converted to spiroepoxide (65) by SOCl_2/Py mediated elimination reaction followed by peracid oxidation. Subsequent catalytic hydrogenation over Pd/SrCO_3 gave the tetrahydroartabsin (66) with a C-10 β -OH, a compound also obtained by saturation of two double bonds of artabsin (67) with H_2/Pt in acetic acid⁸³, thus establishing the stereochemistry at C-10 of artabsin.

The only naturally occurring guaianolide in which the seven membered ring has undergone biogenetic ring contraction is chlorochrymorin (68). The biogenesis of which most likely involves the C-10 to C-9 alkyl shift of C-1⁸⁴. As outlined in Chart XI, Scheme II Rabi and coworkers^{85,86} studied the lewis acid catalyzed rearrangement of 9,10 α -epoxide (69) which was obtained from eremanthin (70) by peracid oxidation. Treatment of epoxide with one equivalent of $\text{BF}_3\text{Et}_2\text{O}$ did not give (71) an analogue of chlorochrymorin but resulted in an aldehyde (72) derived from shift of C-8 to C-10.



SCHEME I

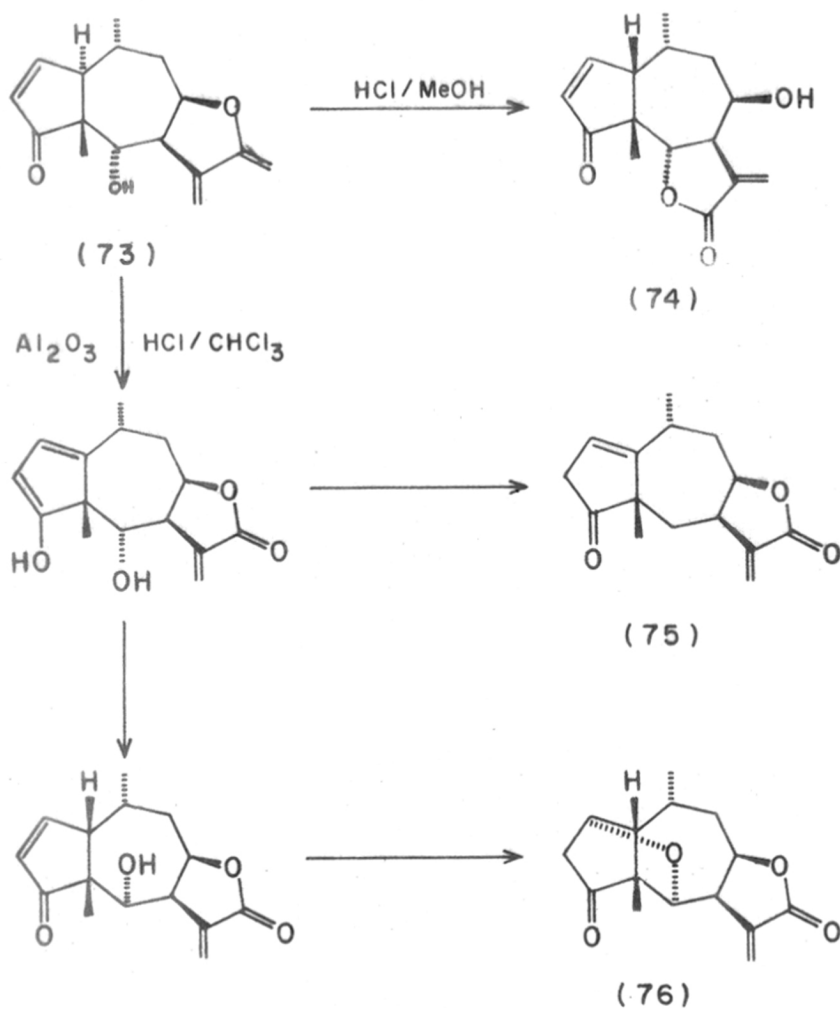


SCHEME II

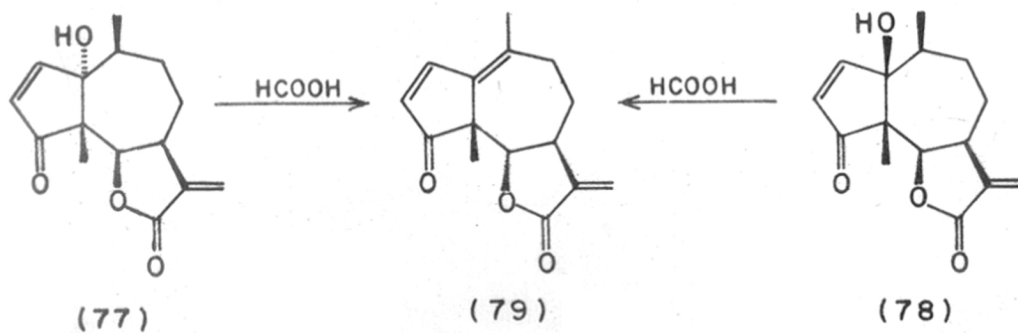
Chemical transformations as well as NMR studies of a number of 10-hydroxyguaianolides were reported by Vokac and Samek⁸⁷.

(D) Pseudoguaianolides:

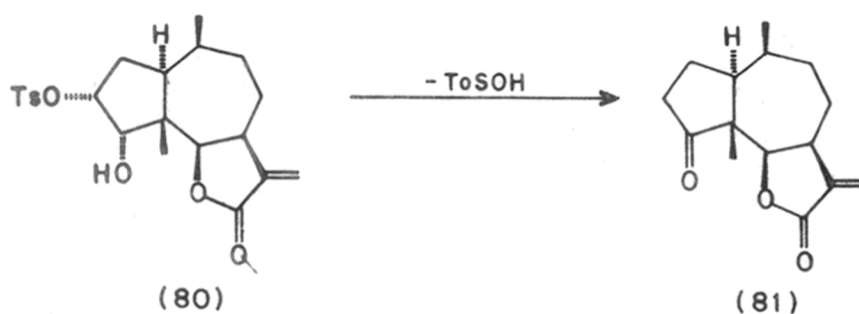
A number of elimination reactions are used as simple laboratory modifications in structure elucidation of a number of closely related ambrosanolides and helenanolides. Treatment of helenalin (73) or ambrosin with mineral acids resulted in epimerization of C-1 position⁸⁸⁻⁸⁹, helenalin upon treatment with HCl in methanol gave epiallohelenalin (74) which is the product of C-1 epimerization and relactonization to a 7-6 trans lactone. Acid treatment of helenalin under different conditions afforded different products, mexicanin A (75)⁸⁸ and the ether (76)⁹⁰ a compound whose structure was established by X-ray crystallography⁹¹ (Chart XII, Scheme I). Acid mediated elimination of the epimeric angular C-1 hydroxyls in parthenin (77) and hymenin (78) provide the same doubly conjugated ketone (79) thus allowing correlation between hymenin and known parthenin⁹² (Chart XII, Scheme II). Treatment of the tosyl derivative of ambrosiol (80) with dilute sulfuric acid resulted in lamsin (81) a compound with known stereochemistry, thus establishing five chiral centers in ambrosiol⁹³ (Chart XIII, Scheme I). The β -hydroxyl group of bipinatin (82) was eliminated under mild acetylation conditions to provide ambrosin (83)⁹⁴ (Chart XIII, Scheme II).



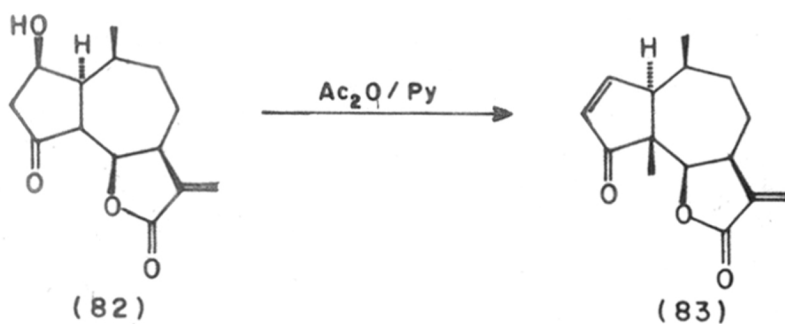
SCHEME I



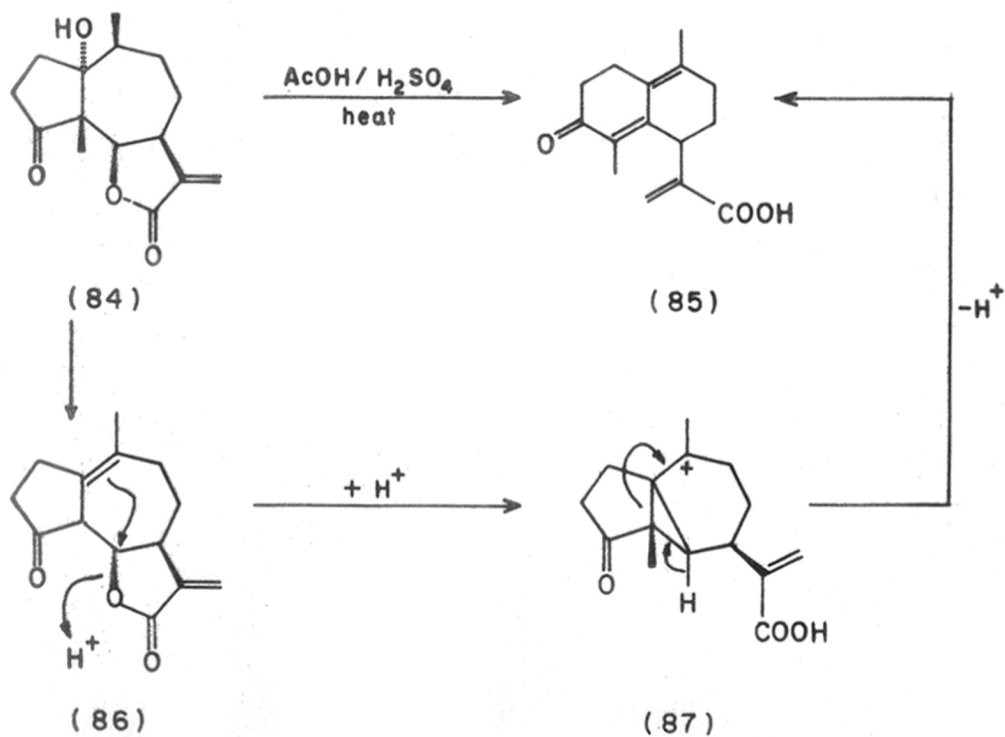
SCHEME II



SCHEME I



SCHEME II



SCHEME III

Coronopilin (84) when heated with H_2SO_4 in acetic acid gives coronopilic acid (85)^{95,96}. Since this reaction cannot be initiated by $SOCl_2$ in pyridine, a wagner-meerwin type rearrangement by direct migration of C-6 to C-1 can be excluded. Instead an elimination substitution reaction is more likely involved in formation of coronopilic acid (85) as indicated in chart XIII, scheme III. Dehydration of coronopilin provides the alkene (86) which attacks C-6 after or under simultaneous lactone ring opening to form the cyclopropane ring (87). Stabilization of the cation (87) by loss of proton from C-6 and cleavage ^{of} C-1-5 bond results in coronopilic acid (85).

PHYSICAL METHODS FOR STRUCTURE ELUCIDATION

The introduction of new physical methods such as UV, IR, NMR, Mass spectroscopy, X-ray crystallography and C.D. has revolutionized the subject of natural products chemistry and opened up a new era, not only by speeding up the work, but also by making it possible to deal with smaller quantities of material and by providing detailed informations of kind not previously obtainable, for example certain aspects of stereochemistry.

The variety and scope of these physical methods are vast and each has its own particular sphere of application. Although in some respects the information provided by one method simply confirm that obtained by another, it is usually desirable to have all available, since each has

certain specific advantages.

U.V. Spectroscopy :

The U.V. spectra of sesquiterpene lactones offer very little structural information, as almost all sesquiterpene lactones exhibit a strong end absorption or maxima in the 210-220 nm region. However, compounds having additional conjugated system show characteristic absorptions at higher wave lengths.

I.R. Spectroscopy:

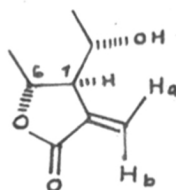
IR Spectroscopy helps in identification of α,β -unsaturated γ -lactone, presence of which is a characteristic feature of most of the sesquiterpene lactones. α,β -Unsaturated γ -lactone show a strong carbonyl absorption band around 1750 to 1780 cm^{-1} . In addition to this IR spectroscopy also helps in identification of the presence or absence of the functional groups, such as hydroxyl groups, esters, cyclopentenone or cyclohexenone systems, unsaturation etc.

N.M.R. Spectroscopy:

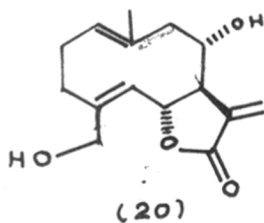
a) Proton spectroscopy: The proton magnetic resonance spectroscopy is the most important and most commonly used technique in structure elucidation of sesquiterpene lactones. In general considerable structural information is obtained from proton spectra together with the application of double resonance experiments. In addition NMR spectra of acetates

and other derivatives of hydroxyl containing compounds strongly assist spectral interpretations. Trichloroacetyl isocyanate (TAI) has been introduced for in situ reactions with alcohols for NMR studies⁹⁷ and has been recently applied to sesquiterpene lactones⁹⁸⁻¹⁰⁰.

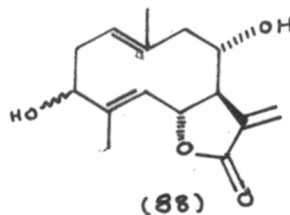
The typical feature in the NMR spectra of sesquiterpene lactones containing the α -methylene γ -lactone group is the appearance of two doublets ($J = 1-4$ Hz) generally one below and other above 6 ppm, which are due to two C-13 methylene protons, the low-field absorption being due to the proton oriented towards the lactone carbonyl group. This coupling is due to the allylic interactions between H-7 and the C-13 methylene protons¹⁰¹⁻¹⁰². When an α -hydroxyl group is present at C-8 in a C₇₋₆ lactonized compound a geminal ($J = 0.7 - 2.0$ Hz) between the two C-13 methylene protons together with a paramagnetic shift of H_{13a} (partial structure (a) Chart XIV, Scheme I) below 6 ppm is observed¹⁰³⁻¹⁰⁴. For example salonitenolide (20) and tetriline A (88) (Chart XIV, Scheme I) exhibit doublets of doublets between 6 and 6.5 ppm. It has been proposed that the geminal coupling and the concurrent paramagnetic shift for H_{13a} mainly results from the Van-der-waals proximity effect^{105,106} of the C-8 α -hydroxyl group upon the bonding orbital of H_{13a}. The paramagnetic shift and the geminal coupling data can be used for the following stereochemical assignment¹⁰⁷.



(a)

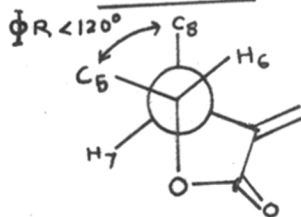
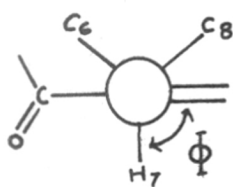


(20)

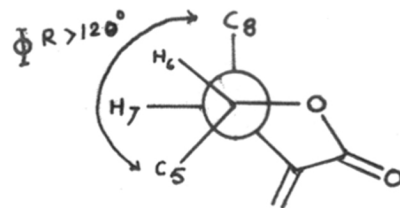


(88)

SCHEME I

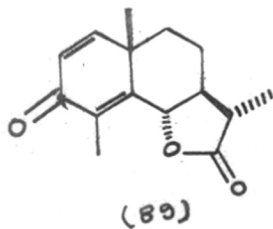


(B)

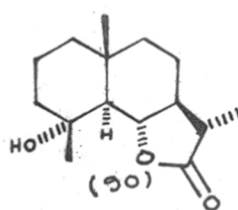


(C)

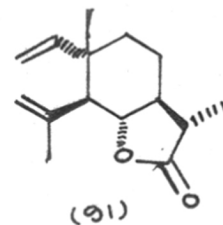
SCHEME II



(89)



(90)



(91)

SCHEME III

The stereochemistry for a C-8 hydroxyl group in C-6 γ -lactones which belong to either the eudesmanolide or the elemanolide class can be assigned. For example a positive shift (in the range of 0.4 - 0.7, average 0.5 ppm relative to chemical shift of H_{13a} in a compound without a C-8 hydroxyl group) with concurrent geminal coupling for H_{13a} denotes an α -orientation for the C-8 hydroxyl group and negative result determines a β -orientation. The rule also appears to be applicable to the C_6 γ -lactones of germacranolide and guaianolide classes.

Based on the size of the allylic coupling between the C-13 exocyclic methylene protons and H-7 Samek¹⁰¹ has proposed a rule for determining the stereochemistry of α -methylene γ -lactones which states that trans lactones have larger allylic couplings than cis lactones, the allylic couplings in trans lactones being equal to or greater than 3 Hz whereas the allylic couplings for cis lactones are ≤ 3 Hz.

$$J_{trans} \geq 3 \text{ Hz} \geq J_{cis}$$

The rule is based upon the correlation between the allylic dihedral angle and the magnitude of allylic couplings¹⁰², certain characteristic J ranges for J_{13a-7} and J_{13b-7} can be expected with maximal couplings when $\Phi = 90^\circ$ (Chart XIV, Scheme II, Fig. A). The above rule is applicable to a number of sesquiterpene lactones including most of the 7,6-germacrolides, melampolides and eudesmanolides^{104,108,109}. Herz and Whalberg¹¹⁰ pointed out

that heliangolides constitute an exception to Samek's rule and show $J_{7,13}$ values between 1.5 and 2.5 Hz¹¹¹⁻¹¹³. The differences can be explained as follows:

Based on X-Ray results, 7,6 trans lactonized germacrolides and melampolides^{114,115} and their 4,5 epoxide derivatives preferentially adopt a conformation with $\Phi R < 120^\circ$ (Chart XIV, Scheme II, Fig. B) and H_6 and H_7 in an antiperiplanar relationship with $J_{6-7} \approx 10$ Hz which is commonly observed for germacrolides and melampolides¹¹⁶. In contrast heliangolides adopt conformations¹¹⁷ with $R > 120^\circ$ (Chart XIV, Scheme II, Fig. C) narrowing the angle between H_6 and H_7 to nearly 90° thus giving J_{6-7} values < 3 Hz. In all cases the J values J_{6-7} , $J_{7,13}$ and ΦR are strongly interrelated and 7,6 trans lactonized germacrolides with $\Phi R < 120$ should generally give values $J_{7-6} > 10$ Hz and $J_{7,13} > 3$ Hz (germacrolides and melampolides) and lactones with $OR \geq 120$ would result in $J_{6,7} < 3$ and $J_{7,13} < 3$ Hz (heliangolides)¹¹¹.

11,13-Dihydro-eudesmanolides and elemanolides often give rise to a broadened triplet for the C-6 lactonic proton e.g. α -Santonin (89), celartin (90) and saussurea lactone (91) etc. (Chart XIV, Scheme III). This phenomenon appears to result from long range coupling between the β -C-6 and β -C-11 protons, since none of the NMR spectra for the 11,13 unsaturated analogs exhibit the broadening¹¹⁸.

(B) ^{13}C NMR Spectroscopy:

Although the potential utility of ^{13}C NMR was recognized very early, essentially no application of ^{13}C NMR appeared in the literature prior to 1966 and 95% of the existing data are less than 10 years old. The initial slow growth had its cause in inadequate instrumentation, insufficient sensitivity being the main obstacle. With the advent and commercial availability of broad band excitation and fourier transform methods, the situation changed drastically, giving natural abundance ^{13}C NMR and its numerous chemical applications a tremendous impetus. Several introductory review articles have appeared recently to demonstrate the usefulness of ^{13}C NMR for structural analysis of natural products¹¹⁵⁻¹²¹.

In general, information on the various types of the carbon present in sesquiterpene lactones is obtained by proton noise decoupling experiments (PND) in which non-equivalent carbons resonate as separate single lines which yield chemical shift information. With the aid of single frequency off resonance decoupling technique (SFOD), residual carbon hydrogen splitting allows determination of the number of hydrogens at each carbon. Selectively decoupled spectra permit assignment of non-proton bearing carbons¹²². F.W. Wehrli and T. Nishida¹²³ have reviewed the use of ^{13}C NMR spectroscopy in natural products chemistry and have given the ^{13}C spectral data for all the major classes of sesquiterpene lactones.

Mass Spectroscopy:

Due to the great variation in the skeletal substitution patterns within the sesquiterpene lactones, the mass spectral fragmentation vary considerably and do not allow the presentation of an unified picture. The use of the mass spectra of sesquiterpene lactones is commonly restricted to the finding of the parent peak and/or detection of certain side chains attached to the carbocyclic skeleton. Frequently the parent peak is missing in the compounds containing hydroxyl and/or ester function due to loss of water and/or the ester side-chain by McLafferty rearrangements ($M-RCOOH$)¹²⁴. In such cases the acylium ion of the ester group is usually observed as an intense peak and very often represents the base peak^{125,126}.

C.D. Spectroscopy:

Application of circular dichroism (C.D.) has been of great value in determination of the stereochemistry of many sesquiterpene lactones. A large number of sesquiterpene lactones contain α -methylene- γ -lactone groups which are optically active. Geissman and co-workers^{127,128} analyzed the cotton effect in the range of 245-250 nm due to $n \rightarrow \pi^*$ transitions of this chromophore in a number of known sesquiterpene lactones and suggested a correlation between the sign of cotton effect and stereochemistry of lactone ring. Their rule relating the sign of the CE and position (6 or 8)

and stereochemistry of lactone ring fusion is summarized in Table I.

A number of germacranolides exhibit two strong bands of opposite sign at two low wave lengths. A positive CE is usually observed near 220 nm and a negative band at 200 nm or below. These bands are highly characteristic of homoconjugation¹²⁹ between the crossed 1,10- and 4,5-trans double bonds and indicate the chirality of the diene system. The bands have been ascribed to the exciton splitting caused by chiral overlap of homoconjugated double bonds in the cyclo-decaliene system¹³⁰.

Table I

The Stocklin-Waldell-Geissman Rule

Predictions of the type of lactonizations from the sign of CE of $N \rightarrow \pi^*$ transitions of sesquiterpene lactones.

Lactone position	Lactone ring fusion	
	cis	trans
C-6	+	-
C-8	-	+

X-Ray diffraction:

In recent years an increasing number of sesquiterpene lactones have been analyzed by X-Ray diffraction methods^{131,132}. The germacranolides have attracted special attention due to their conformational and configurational variations.

STEREOCHEMISTRY AND POSITION OF ATTACHMENT OF

LACTONE RING:

By the combined use of the ^1H NMR spectroscopy and the CD spectroscopy one can assign the stereochemistry and the position of attachment (i.e. whether a 6,12 olide or a 8,12 olide) of the lactone ring. Based on the size of allylic coupling between C-13 exomethylene protons and H-7 the stereochemistry of the α -methylene γ -lactone could be determined. The trans lactones always have larger coupling than the cis lactones. The allylic couplings in the trans lactones being equal to or greater than 3 Hz whereas allylic couplings in the cis lactones is equal to or less than 3 Hz (SAMEK's Rule)¹⁰¹. Further more the compounds having trans lactone ring generally show larger coupling ($J = 10$ Hz) between the lactonic proton and the H-7, whereas in the compounds having cis lactone the coupling between the lactonic proton and the H-7 is always small ($J = 3$ Hz).

W. Herz et al.^{101a} has pointed out that in eulesmanolides guaianolides and pseudoguaianolides, a small couplings (on the order of 1-1.5 Hz) between H-13 and H-7 protons were characteristic of C-8 cis lactonized eulesmanolides and that somewhat larger couplings 2-3 Hz generally 2.5 - 3 Hz) between H-13 and H-7 were characteristic of guaianolides, pseudoguaianolides and C-6 trans lactonized eulesmanolides.

Once the stereochemistry of the lactone ring is known then by application of circular dichroism it is possible to identify the position of attachment of the lactone ring. A large number of sesquiterpene lactones contain α -methylene γ -lactone group, which shows a cotton effect in the range of 245-250 nm due to $n \rightarrow \pi^*$ transitions of this chromophore. Based on stereochemistry of lactone ring and sign of cotton-effect the position of attachment of lactone ring could be determined. (The Stocklin-Waddell-Geissman rule)^{127,128}. Since the lactones cis(trans) fused at C-6 are pseudoenantiomeric to those cis(trans) fused at C-8, one expects opposite CE signs for the lactones cis(trans)fused at C-6 and C-8 respectively. This expectation assumes that the asymmetry of the chromophore or asymmetry immediately surrounding the chromophore is responsible for the sign of the CE. Thus a cis C-6 fused lactone shows +ive CE, whereas the cis C-8 fused lactone shows the -ive CE. Similarly the trans C-6 lactone shows -ive CE and the trans C-8 fused lactone show +ive CE. Structures of some of the sesquiterpene lactones demonstrating the usefulness are given in Chart (XIVa), whereas Table II gives the CD of the CD of the $N \rightarrow \pi^*$ transition of α -methylene γ -lactones chromophore of sesquiterpene lactones.

Out of the twenty two sesquiterpene lactones listed in the table only five showed CE sign opposite that predicted by Stocklin-Waddell-Geissman rule, all other compounds showed

Table CD of the $n \rightarrow \pi^*$ transition of the α -methylene γ -lactone chromophore

Compound	lactone	CE Predicted	CE Found λ_{max}	(O) ^a
<u>Eudesmanolides:</u>				
Douglanine	Trans C-6	-	250	-2780
Cyclized chamissonin diacetate	Trans C-5	+	255	+1280
Artecalin	Trans C-6	-	257	-1210
Arbusculin A	Trans C-6	-	253	-3620
Pinnatifidin	Cis C-8	-	269	-1210 (EtOH)
<u>Guaianolides:</u>				
Cumambrin B	Trans C-6	-	260	-2920
Canin	Trans C-6	-	250	-2870
Pleniradin acetate	Trans C-8	+	253	+5600
Rupin A	Trans C-6	-	250	-2660
<u>Ambrosanolides:</u>				
Apoludin	Cis C-6	+	255	-1080
Ambrosiol	Cis C-6	+	255	-1870
Ambrosiol diacetate	Cis C-6	+	246	-2370
Neoambrosin	Cis C-6	+	255	+ 320
Damsin	Cis C-6	+	250	+ 496
Coronopilin	Cis C-6	+	246	+1785
<u>Xanthanolides</u>				
Xanthumin	Cis C-8	-	256	-4730
Xanthinin	Trans C-8	+	255	+3280
<u>Germacranolides</u>				
Baileyin	Trans C-8	+	255	+1550
Costunolide	Trans C-6	-	261	-5460
Parthenolide	Trans C-6	-	250	-1400
Chamissonin diacetate	Trans C-8	+	248	-3630
Heliangenol	Trans C-6	-	249	+3910

the same CE sign as predicted for the compound. Out of the five compounds, showing CE signs opposite to that of predicted, three compounds apolulin (92) ambrosiol (93) and ambrosiol diacetate (94) (Chart XIVa) all contain an oxygen function near the ring oxygen of a C-6 cis fused lactone. It is possible that this oxygen function is responsible for this anomaly. A similar inverted CE has been found in 17-oxo-steroids containing a 16 β -acetoxy group.

A second group of deviations contain two compounds chamissanin diacetate (95) and heliangelol (96) of germacranolide type. The conformational flexibility of the $\Delta^{10(1)}, \Delta^4$ -cyclodecadiene ring may be responsible for these exceptions. More recently it has been shown that the heliangolides and some melampolides with 7-6 trans lactone ring fusion constitute the major classes of compounds which disobey the Stocklin-Wadfell-Geissman rule.

An explanation of these observations can be found in Beecham's suggestion¹⁷³ that the sign of the cotton effect of the α -methylene γ -lactone chromophore is determined by its chirality. The conformations of the various types of germacranolides depend mainly on the cis or trans nature of the 1(10) and 4,5 double bonds¹⁷⁴. Since the exocyclic $C_{13}=C_{11}-C_{12}=O$ torsion angle depends on the endocyclic $C_{11}-C_7-C_8-O$ torsion angle (or $C_{11}-C_7-C_8-O$) a change in cyclo-decadiene conformation can result in change in chirality of the $C=C-C=O$ chromophore and there for in the sign of CE¹⁷⁵.

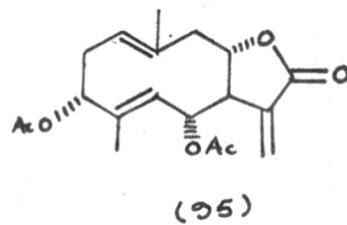
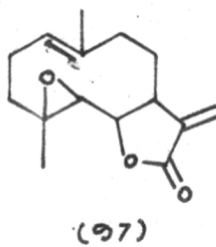
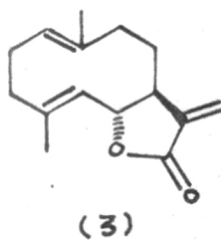
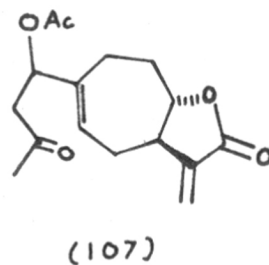
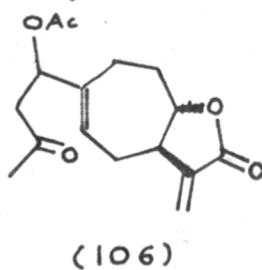
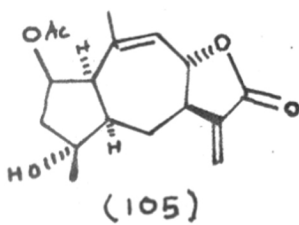
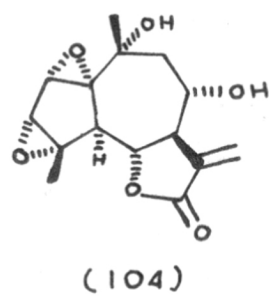
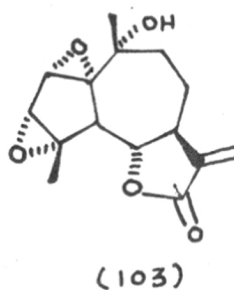
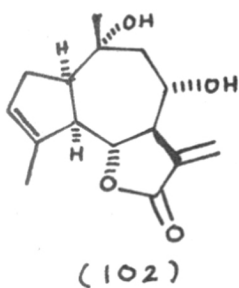
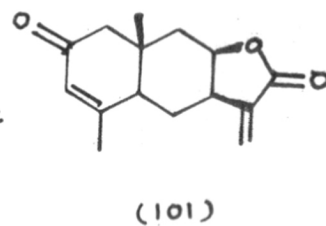
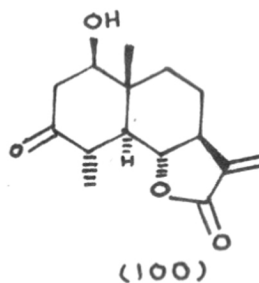
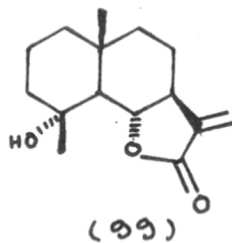
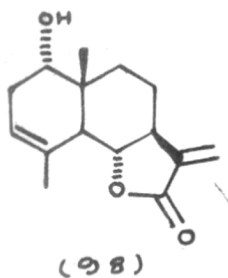
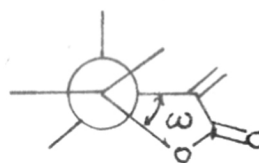
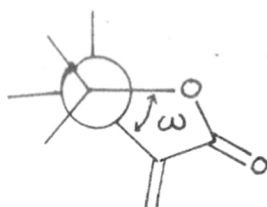
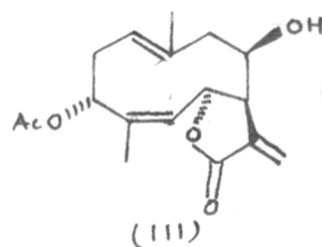
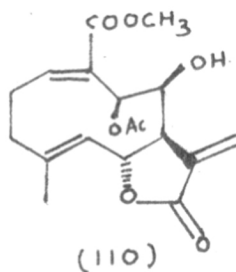
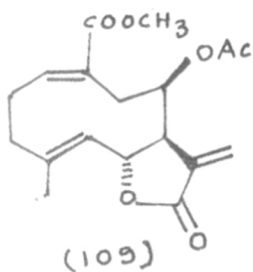
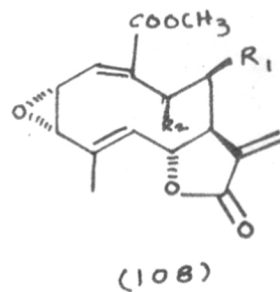
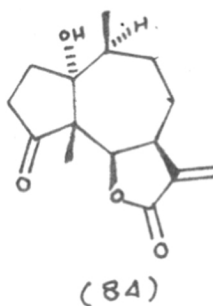
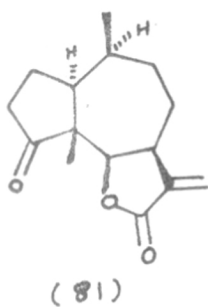
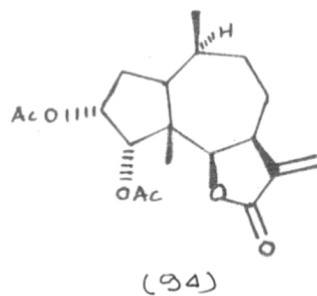
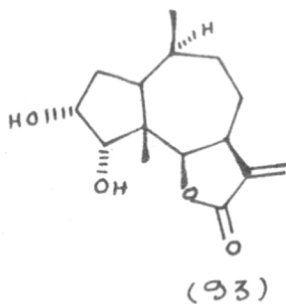
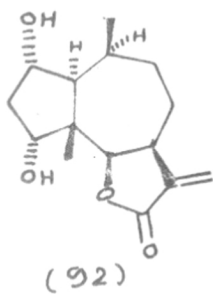
CHART XIV_(b)

CHART XIV(b)



Hence the absolute configuration of sesquiterpene lactone can be determined if the CE and torsion angles of lactone chromophore are known. McPhail and Onan¹⁷⁶ compared the X-Ray derived C=C-C=O torsion angles of the germacrolide eupatolide (IIIa) with torsion angles of the heliangolide eupaeformonin (III) and found that they were of opposite sign thus leading to opposite Cotton effect in accordance to Beecham's rule (Chart XIVb).

Generally heliangolides show CE's of the same sign (positive) as that of eupaeformonin¹⁷⁷⁻¹⁷⁹ indicating that the conformations around the lactone portion of the medium ring are similar.

C.D. Data within the melampolide series are inconsistent. A number of compounds related to melampodin A (108) exhibit relatively strong positive CE's near 245 nm as exemplified by melampodin A¹⁸⁰⁻¹⁸². Other melampolides such as uvedalin (109) and polydalin (110)¹⁸³ exhibit negative CE values but reverse their CE sign upon epoxidation of 4,5 double bond¹⁸².

BIOLOGICAL ACTIVITY

(A) Antitumor activity:

The availability of modern, refined methods for testing anticarcinogenic agents has encouraged the systematic search for cancerostatic agents among natural products. Many of the sesquiterpenes in compositae family, chiefly sesquiterpene lactones from germacranolides, guaianolide, pseudoguaianolide and elemanolide class show antitumor activity. The structure of the cytotoxic sesquiterpene lactones show considerable

variation in carbon skeleton and contain a variety of combinations of functional groups. However, it has been possible to demonstrate that cytotoxicity is primarily dependent upon the presence of a free conjugated α -methylene γ -lactone grouping. Modification of the α -methylene γ -lactone results in diminution in cytotoxicity or leads to derivatives which are essentially inactive^{133,134}. According to Kupchan et al.¹³³ for the in vivo tumor inhibiting activity, presence of a second α,β -unsaturated carbonyl function in sesquiterpene lactone skeleton is essential.

It is believed that the mechanism by which these compounds exert their biological activity, depend on an interaction of α -methylene γ -lactones and other conjugated systems with biologically important sulfhydryl group of enzymes, as the double bond of the lactone ring reacts extremely easily with the sulfhydryl group of cystin at pH 7.4¹³⁵⁻¹³⁷. The tested sesquiterpene lactones were active against different tumors e.g. in vitro against human carcinoma of nasopharynx (K.B.Cells) He La cells (carcinoma cervicis uteri), normal rabbit kidney cells R.K.B., Ehrlich ascites carcinoma cells and in vivo against walker carcinoma-256 in the rat, P-388 lymphocytic leukemia in the mouse or sarcoma Sa-180, Ehrlich ascites carcinoma and exulative leukemia L1210 in the mouse^{134,138,139}.

In recent study of bakkenolides from Petasites albus P.fragrans and P.hybridus¹⁴⁰ it was noted that bakkenolide A

(120) (Chart XV) a β -methylene α -lactone (which does not have an $O=C-C=CH_2$ system) gave results against cells derived from human carcinoma (HEP-2) similar to that reported for other sesquiterpene lactones. This recent finding suggests that other structural parameters must be taken into consideration when evaluating the cytotoxic potential of sesquiterpene lactones. Chart XV shows some of the sesquiterpene lactones which are reported to be cytotoxic.

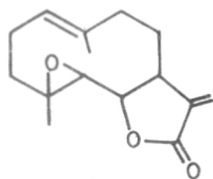
(B) Microbial growth inhibitor: (Antibiotics)

Some sesquiterpene lactones have been shown to possess antibacterial, -fungal or helminthic properties¹⁴¹. The germacranolides mikanolide (121) and dihydromikanolide (Chart XVI) from Mikania monagasensis inhibit the growth in culture of a bacterium, staphylococcus and also of a yeast Candida albicans¹⁴². Helenalin (71) a pseudoguaianolide common in species of helenium, was shown to exhibit activity against the human pathogenic fungi, Trichophyton mentagrophytes, T. acuminatum and epidermophyton sp.¹⁴³. Parthenin (77) the major lactone from Parthenium hysterophorus was reported to inhibit sporangial germination and zoospore mobility in Sclerospora graminicola¹⁴⁴.

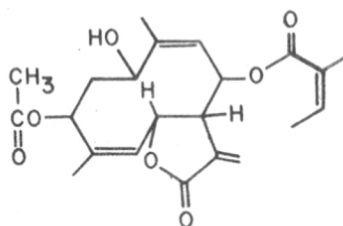
(C) Allergic contact dermatitis in man:

Sesquiterpene lactones form a major class of allergens causing allergic contact dermatitis in human^{145,147}. Over 68 sesquiterpenes were used in patchtests to determine the allergenic potential and the presence of an α -methylene group

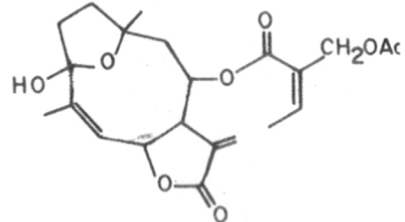
CHART XV



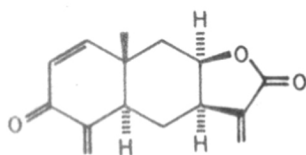
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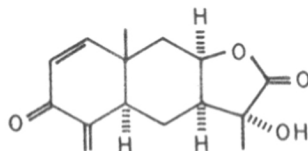
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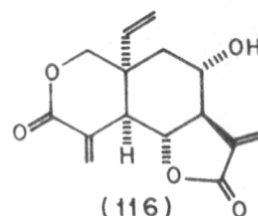
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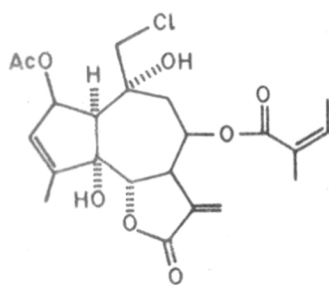
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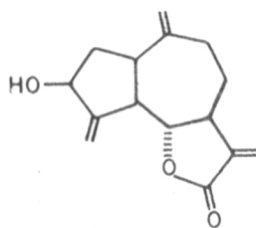
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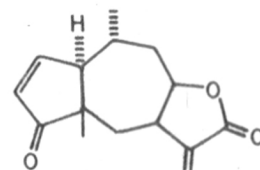
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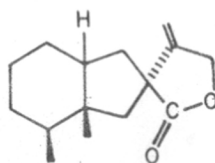
(117)



(118)

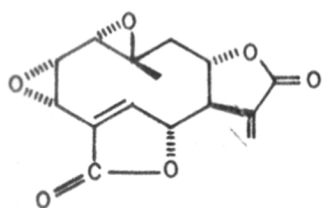


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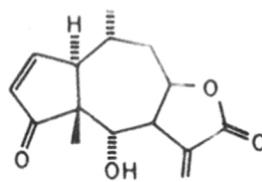


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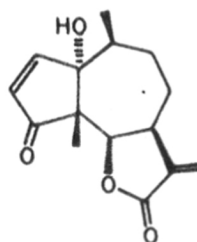
CHART XVI



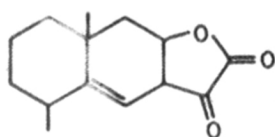
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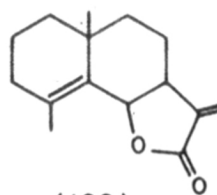
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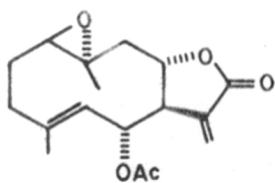
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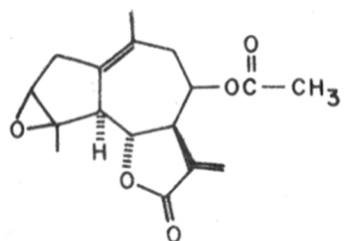
(43)



(122)



(32)



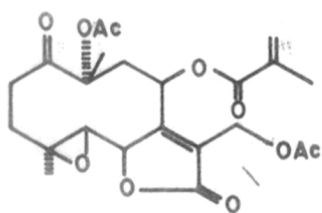
(123)

exocyclic to γ -lactone was shown to be the principal immunochemical requisit for the production of dermatitis¹⁴⁸⁻¹⁵⁰. Parthenium hysterophorus an extraordinary aggressive weed was accidentally introduced into India from America in 1956 and in certain cities of India e.g. Poona allergic contact dermatitis from P.hysterophorus has become an important dermatological and public health problem. Parthenin (77) a pseudoguaianolide is the major allergenic lactone in P.hysterophorus Frullania, many varieties of chrysanthemum, sassurea lappa and Laurus nobilis are the other important plants responsible for dermatitis and all of them are known to have sesquiterpene lactones^{149,151-57}. Chart XVI shows some of the sesquiterpene lactones responsible for dermatitis.

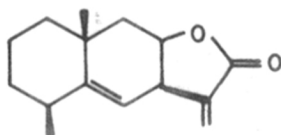
All the known sesquiterpene lactones responsible for dermatitis contain exocyclic α -methylene γ -lactone function which may conjugate with sulfhydryl groups of proteins in cells by a michel type addition to form complete antigens capable of producing cell mediated contact allergic reaction¹⁵⁸.

(D) Insect feeding deterrents :

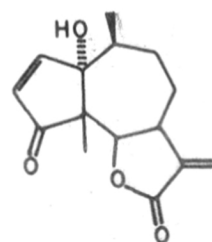
Studies on Vernonia sp. provided an experimental evidence that sesquiterpene lactones act as insect feeding deterrents. Larval feeding experiments were conducted on Spodoptera eridania, S. frugiperia, Diacrision virginia, Trichoplusia ni and S. ornithogalli to determine whether the feeding preference of the six species was related to the presence of glaucolide A (124) (Chart XVII, Scheme I) the major lactone in numerous species



(124)

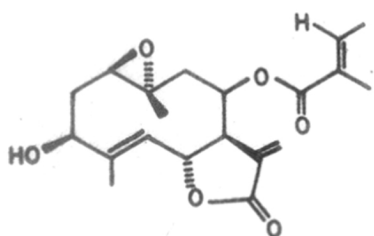


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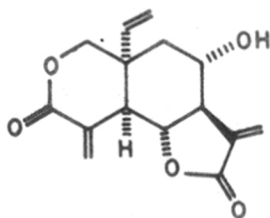


(77)

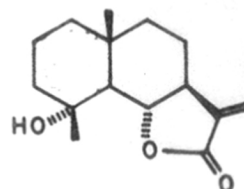
SCHEME I



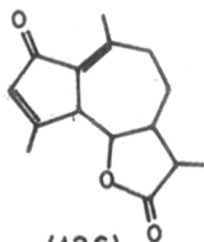
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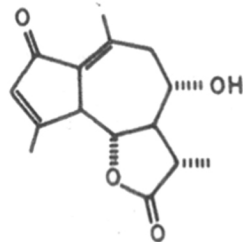
(116)



(99)

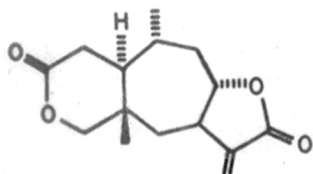


(126)

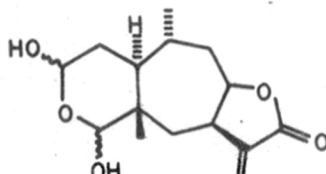


(127)

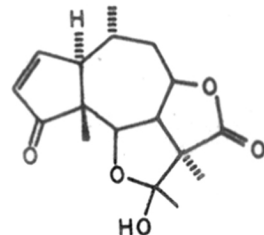
SCHEME II



(128)



(129)



(130)

SCHEME III

of Vernonia¹⁵⁹. Supplementing the agar medium with glaucolide-A resulted in greatly reduced larval feeding; feeding was inversely proportional to the concentration of glaucolide-A in the medium. Further more these insects selected Vernonia flauidifolia medium (which does not contain glaucolide-A) in preference to the same diet containing the lactone¹⁵⁹. In addition to glaucolide-A, parthenin (77) a pseudoguaianolide and alantolactone (43), (Chart XVII, Scheme I) are also reported to show antifeedant activity¹⁶⁰⁻⁶¹.

(E) Plant growth inhibitors; (Phytotoxins)

A variety of sesquiterpene lactones of different skeletal types have been reported to show plant growth regulatory activity¹⁶². Heliangin (125) the major germacranolide of Helianthus tuberosus, inhibits the elongation of Avena coleoptil sections but promotes adventitious root formation of Phaseolus cutting¹⁶³. Promotion of adventitious root formation was reduced by the addition of cystine or by hydrogenation of exocyclic methylene group. Vernolepin (116) an antineoplastic agent from Vernonia hymenolepis inhibits growth of wheat coleoptile sections¹⁶⁴. Similar results were noted for parthenin (77) on Phaseolus vulgaris¹⁶⁵ and the crop plant Eleusine coracana¹⁶⁶. The sesquiterpene lactones arbusculin-A (99) achillin (126) deacetoxymatricarin (127) viscidulin-B and C obtained from Artemisia tridentata vareyana and other species

of *Artemisia* were shown to inhibit lateral root growth but stimulate respiration in *Cucumis sativum*¹⁶⁷. Alantolactone (43) was shown to be a potent inhibitor of seed germination and of seedling growth. It was postulated that the lactone inhibits the enzymes associated with the degradation of starch (amylases) and of protein (proteases) but there is no evidence to support this¹⁶⁸. Chart XVII, Scheme II gives structures of the active compounds.

(F) Vertebrate poisoning:

Live stock poisoning from foraging on bitter tasting plants of compositae is well documented in agricultural literature^{169,170}. *Hymenoxys odorata* is an important livestock toxicant that affects sheep and goats. Chemical studies on *hymenoxys odorata* have shown that hymenoxin (129) (Chart XVII, Scheme III) the major sesquiterpene lactone in populations of *H.odorata* is the toxin involved in the death of sheep¹⁷¹. Similar poisoning (vomiting disease in sheep) has been noted among sheep grazing on South African species of *Geigeria* which contain the compound vermeerin (128)¹⁷². (Chart XVII, Scheme III). Besides their toxicity to sheep and goats, plants containing lactones when eaten by dairy cattle, impart a bitter taste to their milk which is thus rendered unpalatable¹⁶⁹. The lactone tenulin (130) (Chart XVII, Scheme III) a constituent of *Helenium amarum* imparted a bitter taste to milk after oral administration to lactating cow¹⁷⁰.

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CHAPTER II

CHEMICAL INVESTIGATION OF CYATHOCLINE PURPUREA

I N T R O D U C T I O N

The genus Cyathocline Cass. belongs to the tribe Astereae of family Compositae. It is an annual herb, four species of which are known to occur in Southern Asia and Ethiopia. The total number of Astereae today is 135 genera with about 2500 species. The Astereae can be characterized by the styles of bisexual disc florets which have distinct, linear to triangular sterile appendages. The main centres of distribution of this tribe are both Americas and Southern Africa¹, while the other centres of distribution being Asia, Australia and New Zealand.

Not more than 10% of the approximately 2500 species of the tribe Astereae have been investigated chemically. Most of the work done so far has been confined largely to the root constituents². Polyacetylenes, polyenes and related substances are characteristic constituents of roots. In certain groups the coumarin are very characteristic². Less widely distributed are the diterpenes of labiane and clerodane type³⁻⁶. Epigeal parts have been studied only sporadically. As a result, the information on distribution of flavanoids and other low molecular weight secondary metabolites is very spotty⁷⁻⁹. However rarity or absence of sesquiterpene lactones and alkaloids appear to distinguish Astereae from several other tribes of Compositae. A stereo-isomer of coumambirin B, isolated from Haplopappus rigidifolius,

is the only sesquiterpene lactone so far found in Astereae¹⁰.

So far only one species of the genus Cyathocline has been investigated chemically i.e. O.N. Devangan et al.¹¹ isolated a monoterpene alcohol lyratol (Ia) from Cyathocline lyrata.

PRESENT WORK

Cyathocline purpurea is a slender, delicate, erect herb, 1-2 ft. in height. The stem is branched, grooved more or less pubescent. Leaves sessile, 1-5 inch long, pinnatifid with toothed segments. Leaves also are more or less pubescent. Flowers (both ray and disc floret) rose purple, in heads 1/6 inch in diameter, arranged in small rounded corymbose panicles.

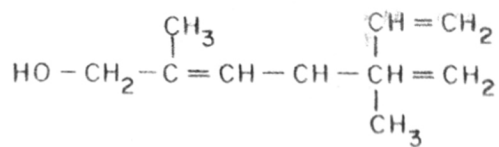
The chromatographic separation of the acetone extract of Cyathocline purpurea resulted in the isolation of four new sesquiterpene lactones, two guaianolides (I) and (II) (Chart I) closely related to each other and two closely related eudesmanolides (IV) and (V) (Chart II). In addition to these compounds one more sesquiterpene lactone of known structure ivangustin (III) was present as a minor constituent in the extract. Compound (I) was present as major sesquiterpene lactone and was obtained in 0.22% yield (based on dry weight of plant), whereas compound (IV) was

obtained in 0.062% yield (based on dry weight of plant). The other sesquiterpene lactones were present as minor constituents. Structures of all these compounds and their transformation products could be elucidated by extensive spectral studies of compounds and their transformation products. The structure elucidation of these compounds is described below.

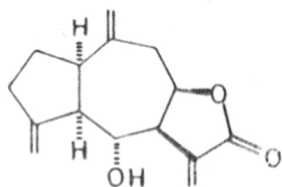
Compound (I):

Compound (I) m.p. 115-16° (α_D^{32} - 111.8° (CHCl₃C = 0.5) analyzed for C₁₅H₁₈O₃ which was supported by its mass spectrum which exhibited M^+ peak at m/e 246.

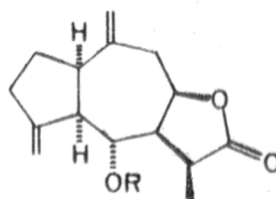
IR spectrum of (I) (Fig. 1) suggested the presence of a hydroxyl group (band at 3280 cm⁻¹) which was further confirmed by appearance of a D₂O exchangeable signal at 2.46 ppm in the ¹H NMR spectrum of (I) (Table I, Fig. 2), a M-H₂O peak in its mass spectrum and its conversion to monoacetate (VI) on treatment with acetic anhydride in pyridine. The ¹H NMR spectrum of (I) also showed a double doublet at 3.68 ppm (one proton) which suggested the secondary nature of hydroxyl group. This was further supported by the ¹H NMR spectrum of monoacetate (Table I, Fig. 4) in which the signal assignable to proton on carbon carrying hydroxyl group shifted to 5.16 ppm from its position at 3.68 ppm in the ¹H NMR spectrum of acetate (VI). The SFOD ¹³C NMR of the acetate (VI) (Table II, Fig. 5) showed signals at δ 76.29 (d)



I a

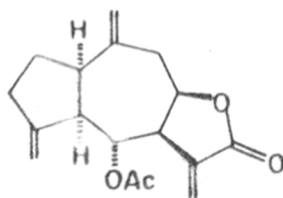


I

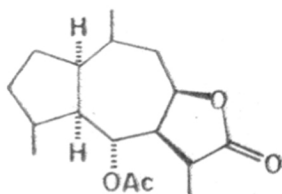


II R = H

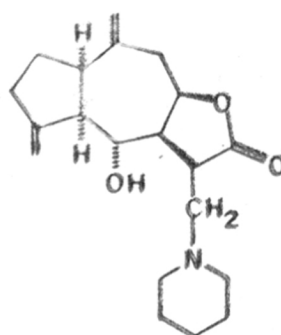
II a R = Ac



VI



VII



VIII

Table (I) ^1H NMR spectral data of compounds (I), (IIa) and derivatives of compound (I)

I (270 MHz)	IIa	(270 MHz) (VI)	VII	VIII
1-H 2.46 dd br		2.45 m		2.45 m
2-H 1.68-1.88 m		1.68 - 1.88 m	1.7 - 1.9 m	1.68 - 1.88 m
3-H 2.31 m		2.3 m		2.3 m
4-H -	2.57	-	2.4 m	-
5-H 2.31 m		2.3 m		2.3 m
6-H 3.68 du	4.51 m	5.16 dd	4.7 m	3.53 m
7-H 3.07 dddd	2.9 m	2.93 dddd	2.4 m	3.03 m
8-H 4.44 ddd	4.51 m	4.43 ddd	4.42 m	4.48 ddd
9-H 2.75 du		2.5 d and 3.13 d(m)	1.8 m	2.55 dd and 3.15
10-H -	-	-	2.4 m	-
11-H 2.57		-	2.4 m	-
12-H 6.3 dd	1.13 d	6.08	1.16 d	2.5 m
13'-H		5.43		
14-H 4.97 d	4.8 s(br)	4.95 br.s.	1.06 d	4.88 s(br)
15-H 5.08 s(br)	4.71 and	4.73 and	1.03 d	5.03 s(br) and 5.46 s(br)
OAc -		2.05 s	2.06	1.5 - 2.41 (CH ₂ of pipridine ring)

J(H₂): (I) 1,2 = 8,1; 1,5 = 8; 2,3 = 7,10; 5,6 = 10; 6,7 = 10; 7,8 = 9; 7,13 = 3.3;

7,13 = 3.3; 7,13' = 3; 8,9 = 11,4; 9,14 = 1.5;

(IIa) 6,7 = 10; 7,8 = 9; 11,13 = 6;

(VI) 1,2 = 8,1; 1,5 = 8; 2,3 = 7, 10,5; 5,6 = 10; 6,7 = 10; 7,8 = 9;

7,13 = 3.4; 7,13' = 3; 8,9 = 11,4; 9,14 = 1.5;

(VII) 6,7 = 10; 7,8 = 9; 11,13 = 10,14 = 4,15 = 6;

(VIII) 1,2 = 8,1; 1,5 = 8; 5,6 = 10; 6,7 = 10; 7,8 = 9; 8,9 = 11,4; 9,14 = 1.5

Table II) ^{13}C NMR spectral data of compound (VI)
(in CDCl_3 , at 22.63 MHz , TMS as internal
standard. Chemical shift in $\delta\text{c ppm}$)

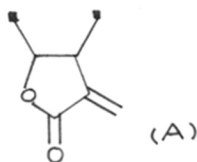
Carbon No.	Chemical shift in $\delta\text{c ppm}$	Carbon No.	Chemical shift in $\delta\text{c ppm}$
C-1	46.01 (d)	C-10	137.06 (s) ⁺
C-2	30.15 (t)	C-11	150.97 (s)
C-3	34.05 (t)	C-12	170.27 (s)
C-4	144.14 (s) ⁺	C-13	125.17 (t)
C-5	51.79 (d)	C-14	111.71 (t) [*]
C-6	76.29 (d) ⁺⁺	C-15	112.83 (t) [*]
C-7	54.65 (d)	Acetate carbonyl	169.49 (s)
C-8	75.12 (d) ⁺⁺	Acetate methyl	21.51 (q)
C-9	39.70 (t)		

Note: (1) s = singlet; d = doublet; t = triplet;
q = quartet. Signal multiplicity in single
frequency off resonance decoupled
spectrum (SFOD)

(2) Chemical shifts with similar marks
(i.e., ++ * etc.) are interchangeable

ppm which was in good agreement with the above statement. The acetate (VI) in its ^1H NMR spectrum showed one acetate methyl singlet at 2.06 ppm and its IR spectrum (Fig. 3) showed bands at 1720 cm^{-1} and 1240 cm^{-1} for the acetate group, but it did not show any hydroxyl absorption band which showed that (I) contained only one hydroxyl group.

IR Spectrum of (I) exhibited a carbonyl absorption band at 1735 cm^{-1} and exomethylene absorption bands at 1630 and 835 cm^{-1} which suggested the presence of an α,β -unsaturated γ -lactone moiety in the compound as represented in partial structure (A). This was further supported by the appearance of a two proton quartet at 6.3 ppm in the ^1H NMR spectrum of (I) (Fig. 2) and signals at δ 170.27(s), 125.17(t) and 150.97(s) ppm in the SFOD ^{13}C NMR spectrum of the acetate (VI) (Fig. 5) as well as by conversion of (I) into piperidine adduct on treatment with piperidine (VIII). The ^1H NMR spectrum of piperidine adduct (VIII) (Fig. 9) did not show quartet at 6.3 ppm observed in ^1H NMR spectrum of (I).

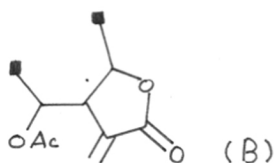


The lactone function and the hydroxyl group accounted for all the three oxygen atoms in the molecular formula $\text{C}_{15}\text{H}_{18}\text{O}_3$ of (I).

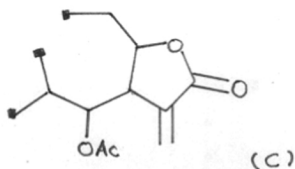
Appearance of doublet at 4.97 ppm and a broad singlet at 5.08 ppm (4 protons) in the ^1H NMR spectrum of (I) suggested the presence of two exocyclic methylene groups (in addition to lactone exomethylene). The molecular formula $\text{C}_{15}\text{H}_{18}\text{O}_3$ and the presence of three double bonds (one lactone exomethylene and two exocyclic methylenes) suggested a bicyclic skeleton for the compound (I).

Critical comparison of the ^1H NMR spectral data (Table I) of (I) with those of numerous known compounds suggested that it belonged to guaianolide class as the signals in the ^1H NMR spectrum of (I) were similar to those of chrysostomalide (Ib)¹². The position of attachment of various groups in (I) could be deduced from careful decoupling studies of its acetate (VI), the ^1H NMR spectrum of which showed well resolved signals. The proton on carbon-7 (i.e. H-7) could be located as a four-fold doublet centered at 2.93 ppm as the irradiation of this signal collapsed the two doublets at 6.08 ppm and 5.43 ppm into singlets. The two doublets at 6.08 ppm and 5.43 ppm are due to the lactone exomethylene protons and the multiplicity of these signals is known to be due to the allylic coupling of these protons with H-7 proton¹³. The same irradiation (i.e. at 2.93 ppm) also collapsed a four-fold doublet at 4.43 ppm into double doublet and the double doublet at 5.16 ppm into a doublet. The former signal (i.e. at 4.43 ppm) could be assigned to lactonic proton, whereas

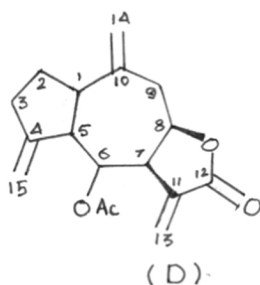
the latter signal (i.e. at 5.16 ppm) could be assigned to proton on carbon carrying the acetate group. The above decoupling studies suggested that these protons are on the carbon atoms vicinal to C-7 i.e. they are on the carbon atoms 6 & 8. Thus the partial structure (A) for compound (I) can now be extended to (B).



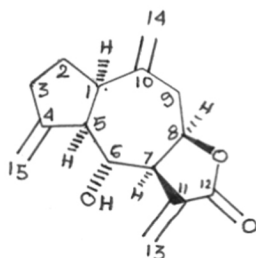
Based on the observed couplings of above two signals it could be proposed that the proton on carbon carrying lactone oxygen is at C-8 and the acetate group is present at C-6 i.e. the lactone ring is C-8 fused. This was further confirmed by decoupling studies. Irradiation of signal at 4.43 affected the multiplicity of two proton double doublet at 2.5 and 3.13 ppm and the same decoupling also affected the multiplicity of a signal at 2.93 ppm (i.e. H-7). Irradiation of double doublet at 5.16 affected the multiplicity of signal at 2.3 ppm (one proton) as well as of the multiplet at 2.93 ppm (i.e. H-7). Based on the above discussion the partial structure for (I) can now be further extended to C.



The two exocyclic methylene groups in the compounds have to be present at C-4 and C-10 positions on biogenetic considerations. Thus by summing up all the above data i.e. guaianolide skeleton of the compound, partial structure (C) and two exocyclic methylene groups at C-4 and C-10, the following gross structure (D) could be written for compound (I) and its acetate.



The stereochemistry of compound (I) at various positions was proposed on the basis of coupling constants in its NMR spectrum. The hydrogen atom at C-7 has to be α -oriented in accordance with Hendrickson's¹⁴ biogenetic generalization that all sesquiterpenes have $C_7\beta$ -substituents. The coupling J_{7-8} and J_{8-9} (and 9 Hz) suggested the presence of a 8, 12 cis-lactone ring system. The negative sign of the cotton effect supported this assumption. A large value of J_{6-7} (10 Hz) indicated that H-6 is β -oriented and hence the hydroxyl group is α -oriented. The 1,5 ring fusion has to be cis based on biogenetic considerations. Thus the structure and stereochemistry of the major sesquiterpene lactone from Cyathocline purpurea can be depicted as in (I).

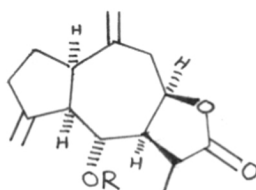


Compound (II):

Due to very close R_f values of (I) and (II) and the paucity of the compound, (II) could not be purified by the usual chromatographic techniques. However, it was obtained in fairly pure form as its acetate (IIb) (Chart I).

The acetate (IIb) m.p. 162° (α_D^{30} - 117 (CHCl_3 C: 0.2) analyzed for $\text{C}_{17}\text{H}_{22}\text{O}_4$ (M^+ at m/e 290). A carbonyl absorption band at 1780 cm^{-1} in the IR spectrum of (IIb) (Fig. 10) suggested the presence of a γ -lactone moiety in the compound. IR spectrum of (IIb) also showed bands at 1720 cm^{-1} and 1240 cm^{-1} , which suggested the presence of acetate group in (IIb). This was further supported by the appearance of a three proton singlet at 2.02 ppm (acetate methyl) in the ^1H NMR spectrum of (IIb) (Fig. 11). The ^1H NMR spectrum of (IIb) also showed a one proton multiplet at 4.5 ppm which suggested the secondary nature for acetate group. The two oxygen atoms in the lactone ring and two in the acetate group accounted for all the four oxygen atoms in the molecular formula ($\text{C}_{17}\text{H}_{22}\text{O}_4$) of the compound.

A close comparison of the ^1H NMR spectral data of (IIb) with that of the acetate (VI) (acetate of compound I) (Table I gives spectral data of both the compound) suggested that compound (II) is a dihydroderivative of compound (I) and contained a saturated γ -lactone function instead of an α -methylene γ -lactone function. The only difference in the ^1H NMR spectrum of (IIb) and (VI) was that the two one-proton doublets at 6.08 ppm and 5.43 ppm for the exomethylene protons of the α -methylene γ -lactone in ^1H NMR spectrum of (VI) were absent in ^1H NMR spectrum of (IIb). Instead it showed a three proton doublet at 1.19 ppm and a one proton double quartet at 2.57 ppm for a secondary methyl group. Thus based on above discussion the structure and stereochemistry for the compound and its acetate could be depicted as in (II).



(II) $\text{R} = \text{H}$

$\text{R} = \text{Ac}$

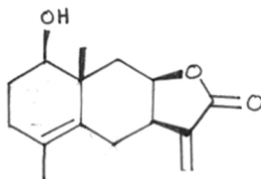
The structure and stereochemistry proposed for (II) on the basis of above observations is tentative since further structural studies were not possible due to the paucity of the compound. However, in view of its

co-occurrence with (I) and its spectral similarities, (II) is the most probable structure for this minor constituent from Cyathocline purpurea.

Compound III:

Attempts to isolate (III) as such by chromatography were unsuccessful because of the presence of overlapping compounds and the paucity of the compound. However, purification of the compound (III) could be achieved by converting (III) into its piperidine adduct followed by regeneration of compound (III) by the method of Jain et al.¹⁵

The compound (III) m.p. 121-123° analysed for $C_{15}H_{20}O_3$ (M^+ at m/e 248). The spectral data and the melting point revealed that (III) is a known sesquiterpene lactone ivangustin, earlier isolated from Ix angustifolia¹⁶.



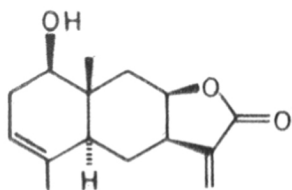
(III)

Compound IV:

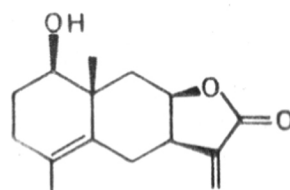
Because of the very close R_f values of (III), (IV) and (V), compound (IV) could not be obtained ⁱⁿ pure form by the usual chromatographic techniques. However (IV) could be obtained in pure crystalline form via its piperidine adduct.

Compound (IV) m.p. 141-142°C, $(\alpha)_D^{30} + 123.4^\circ$
 (CHCl₃ $c = 0.26$) analyzed for C₁₅H₂₀O₃ (M^+ at m/e 248)
 IR spectrum of compound (IV) (Fig. 14) showed a band at
 3620 cm⁻¹ which suggested the presence of a hydroxyl group
 in the compound which was further supported by the
 appearance of a D₂O exchangeable signal in the ¹H NMR spectrum
 of (IV) (Fig. 15, Table III) and M-H₂O peak in its mass
 spectrum. On acetylation at room temperature (IV) gave a
 monoacetate (X). The IR spectrum (Fig. 17) of which
 showed acetate carbonyl band at 1740 cm⁻¹ as well as a band
 at 1240 cm⁻¹, but it did not show any hydroxyl absorption
 band, which suggested the presence of only one hydroxyl group
 in the compound. That the hydroxyl group is secondary was
 proposed by appearance of a one proton double doublet at
 3.46 ppm in the ¹H NMR spectrum of (IV) which shifted to
 4.76 in the ¹H NMR spectrum of acetate (X) (Table III,
 Fig. 18) and also by the appearance of a doublet at δ 76.68
 ppm in the SFOD ¹³C NMR spectrum of (IV). (Table IV, Fig. 16).

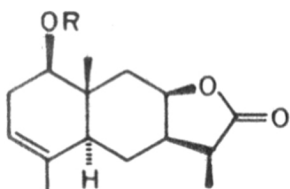
The carbonyl absorption band at 1760 cm⁻¹ and a band at
 1650 cm⁻¹ for unsaturation suggested the presence of an
 α -methylene γ -lactone moiety in the compound (partial
 structure A) which was further supported by appearance of
 two characteristic one-proton doublets at 5.56 ppm and
 6.11 ppm ($J = 1.5$ Hz) in the ¹H NMR spectrum of (IV). This
 was confirmed by its conversion into its piperidine



IV



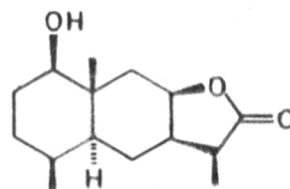
III



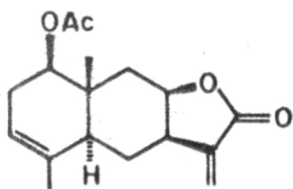
V R = OH

Va R = Ac

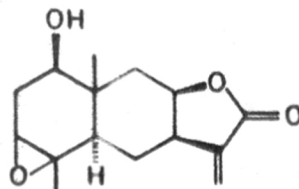
XII R = Ac



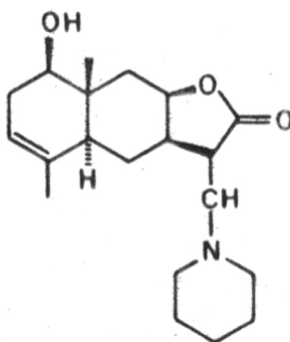
IX



X



XI



XIII

Table III ^1H NMR Spectral Data of Compound (IV) and its derivatives (in CDCl_3 , TMS as internal standard, chemical shifts in δ scale) (values in parenthesis are coupling constants in Hz)

	(IV) (270 MHz)	(IA) (C_6D_6) (270 MHz)	(A)	(AII)	(XI)	(XIII)
1-H	3.46 dd	2.91 dd	4.76 dd	4.66 dd	3.35 dd	3.46 dd
2-H	1.95-2.30 m	1.0 m	1.95-2.21 m		2.30 m	1.95 m and 2.30 m
3-H	5.31 s(br)		5.31 s(br)	5.26 s(br)	3.00 dd	5.26 s(br)
4-H	-	1.38 m	-	-	-	-
5-H	1.95 m					1.95 m
6-H	1.33 - 1.95 m	0.82 - 1.21 dd				1.21 and 1.95 m
7-H	2.99 dd	1.57 dd	2.98 dd	2.53 m	2.37 dd	2.91 dd
8-H	4.55 dd	3.87 dd	4.56 dd	4.44 dd	4.48 dd	4.48 dd
9-H	1.45 and 2.60	0.92 dd				1.45 dd
		2.43 dd				2.59 dd
11-H	-	2.17 dd		2.68 dd		
13-H	6.11 d	1.06 d	6.11 d	1.18 d	6.01 d	2.5 m
13'-H	5.57 d		5.58 d		5.60 d	
14-H	0.86 s	0.97 s	0.98 s	0.93 s	0.91 s	0.82 s
15-H	1.62 d	0.70 d	1.63 d	1.65 d	1.24 s	1.63 d
OAc	-	-	2.08 s	2.03 s	-	2.4 - 2.6 m
						(CH_2 's of piperidine ring)

$^J\text{H}_2$ (IV): 1,2 = 6,10; 1,OH = 6; 2,2' = 17; 5,6 = 12; 6,7 = 6,12; 7,8 = 5; 7,13 = 1; 8,9 = 1.5; 9,9' = 16;
 (IX): 1,2 = 5,10; 4,15 = 7; 5,6 = 6,12; 6,7 = 2,12; 7,8 = 5; 7,11 = 7; 8,9 = 4.52, 2;
 (X): 5,6 = 12; 6,7 = 6,12; 7,8 = 5; 7,13 = 1.5; 7,13' = 1; 8,9 = 1.5; 1,2 = 5,10;
 (XII): 1,2 = 6,10; 5,6 = 12; 6,7 = 2,12; 7,8 = 5; 7, 11 = 7; 11,13 = 6.5;
 (XI): 1,2 = 6,10; 5,6 = 12; 6,7 = 6,12; 6,7 = 6,12; 6,7 = 6,12; 7,8 = 5; 7,13 = 1.5, 7,13' = 1
 (XII): 1,2 = 5,10; 1,OH = 6; 5,6 = 12; 6,7 = 6,12; 7,8 = 5; 8,9 = 1.5

Table IV ^{13}C NMR Spectral data of compound (IV)
(in CDCl_3 , at 22.63 MHz, TMS as internal
standard, chemical shifts in $\delta\text{c ppm}$)

Carbon No.	Chemical shift in ppm	Carbon No.	Chemical shift in ppm
C-1	76.68(1)*	C-9	40.81 (t) ⁺
C-2	36.91(t) ⁺	C-10	32.23(s)
C-3	120.49(d)	C-11	142.26 (s)
C-4	133.61 (s)	C-12	170.6 (s)
C-5	36.39 (d)	C-13	120.94 (t)
C-6	27.03 (t)	C-14	11.04 (q)
C-7	44.19 (d)	C-15	20.79 (q)
C-8	76.29 (d)*		

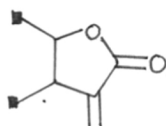
Note: (1) s = singlet, d = doublet, t = triplet,

q = quartet.

Signal multiplicity in single frequency off
resonance decoupled (SFOD) spectrum.

(2) Chemical shifts with similar marks (i.e. *,+etc.
are possibly interchangeable.

adduct (XIII) (Chart II), the ^1H NMR spectrum (Fig. 6, Table III) of which did not show the signals due to lactone exomethylene at 5.56 and 6.11 ppm. The SFOD ^{13}C NMR spectrum of (IV) (Table IV, Fig. 16) which showed signals at δ 170.64(s), 142.26(s) and 120.94(t) also confirmed the above statement.



(A)

The lactone moiety and one hydroxyl group accounted for all three oxygen atoms in the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_3$ of (IV).

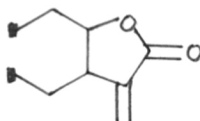
The presence of a double bond in addition to the lactone exomethylene was suggested by appearance of a three proton doublet at 1.62 ppm ($J = 1 \text{ Hz}$) (methyl on double bond) in the ^1H NMR spectrum of (IV). The ^1H NMR spectrum of (IV) also showed a one proton broad singlet at 5.31 ppm, which suggested the trisubstituted nature for the double bond. This was further confirmed by the ^1H NMR spectrum of epoxide (XI) (Table III, Fig. 22) in which the signal due to methyl on double bond at 1.62 ppm in the ^1H NMR spectrum of (IV) shifted to 1.24 ppm and the signal due to vinyl proton at 5.31 ppm had shifted to 3.00 ppm as the epoxide proton. In addition to this ^1H NMR spectrum of (IV)

exhibited the presence of an angular methyl group as a singlet at 0.86 ppm. Appearance of signals at δ 133.61(s), 120.49(d), 20.79(q) and 11.04(q) in the SFOD ^{13}C NMR spectrum of (IV) (Table IV, Fig. 16) supported the above statement.

The molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_3$ and the presence of two double bonds (one lactone-exomethylene and one trisubstituted double bond), one angular methyl group and one methyl on the double bond suggested a bicyclic skeleton for compound (IV).

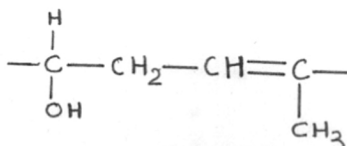
Critical comparison of the ^1H NMR spectral data of (IV) with those of known compounds suggested that it belonged to an eudesmanolide class as the signals in the ^1H NMR spectrum of (IV) were very much similar to those of alantolactone (XIV) and ivangustin (III)^{16,17}. The position of attachment of various groups in (IV) could be deduced from the extensive decoupling studies of (IV). The proton on carbon-7 (i.e. H-7) could be located as a three-fold doublet at 2.99 ppm as the irradiation of this signal collapsed the two doublets at 6.11 ppm and 5.57 ppm into singlets (13 and 13' protons of lactone exomethylene). The same irradiation also collapsed the three-fold doublet at 4.55 ppm into double doublet and also affected a two proton multiplet at 1.33 - 1.95 ppm. This suggested that these two signals are due to the protons on the carbon atoms vicinal to C-7 (i.e. they are on carbon atoms 6 and 8). The former signal could be assigned to a proton on carbon atom carrying lactone oxygen whereas the latter

signal is due to a $-\text{CH}_2-$ grouping. The observed chemical shift and the multiplicity (4.55 ppm) for the proton on carbon carrying lactone suggested a C-8 fused lactone ring in the compound. The decoupling studies also were in good agreement with the above statement, which showed that the irradiation of the three-fold doublet at 4.55 ppm affected the multiplicity of three-fold doublet at 2.99 ppm (H-7) as well as affected the multiplicity of two proton doublet at 1.45 and 2.60.. Based on above discussion the partial structure (A) now can be extended to (B) for compound (IV).



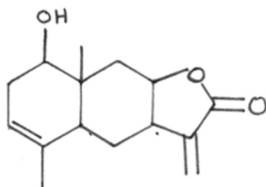
(B)

Irradiation of 'broad singlet at 5.31 ppm collapsed the doublet ($J = 1 \text{ Hz}$) at 1.62 ppm into singlet and also affected the multiplicity of the signal at 1.95 - 2.30 ppm, the same multiplet was also affected by irradiation of the signal at 3.46 ppm (proton on carbon carrying hydroxyl group), which suggested the presence of partial structure (C) in compound (IV).



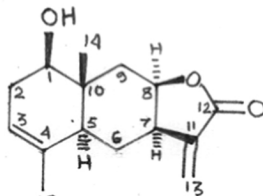
(C)

Based on biogenetic considerations, the double bond in eudesmanolides is normally present either at C_{3,4}, C_{4,5} or at C_{4,15} positions. As the double bond in (IV) is trisubstituted it has to be present at C_{3,4} position and thus the hydroxyl group should be present at C-1. Thus based on above discussion following gross structure (D) could be written for (IV).



(D)

The ¹H NMR spectral data of the compound revealed that the compound (IV) is a double bond isomer of ivangustin (III). Instead of a tetrasubstituted double bond in ivangustin at C₄₋₅ position, compound (IV) contained a trisubstituted double bond at C_{3,4} position, other structural parameters and stereochemistry at various centres in both compounds being same, hence the name isoivangustin was coined for compound (IV). Thus the structure and stereochemistry for isoivangustin could be depicted as in (IV)



(IV)

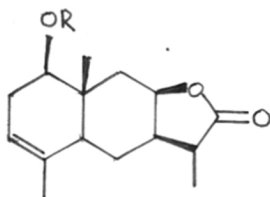
Compound V):

Attempts to isolate compound (V) in pure form by chromatography were unsuccessful because of the very close R_f values of compounds (III), (IV) and (V). However compound (V) was isolated in fairly pure form as its acetate (Va).

The acetate (Va) m.p. 116.0° (α)_D³⁰ + 80.00 (CHCl₃ C = .003 g/ml) analyzed for C₁₇H₂₄O₄ (M⁺ at m/e 292). The IR spectrum of (Va) (Fig. 27) showed the acetate carbonyl band at 1720 cm^{-1} and a band at 1240 cm^{-1} but it did not show any hydroxyl absorption band. The presence of the acetate group in (Va) and its secondary nature ~~on~~ was suggested by the appearance of a three proton singlet at 2.00 ppm (acetate methyl group) and a one proton double doublet at 4.7 ppm assignable to the proton on the carbon atom carrying acetate group in its ¹H NMR spectrum (Fig. 28).

Band at 1760 cm^{-1} in the IR spectrum of (Va) suggested the presence of a γ -lactone function in the compound. The γ -lactone and the acetate group accounted for all the four oxygen atoms in the molecular formula C₁₇H₂₂O₄ of compound (Va). A close comparison of the ¹H NMR spectrum of (Va) with that of acetate (X) (Table III) , revealed that the only difference in the ¹H NMR spectrum of two compounds was that the two one proton doublets (J = 1.5 Hz) at 6.11 and 5.58 ppm observed in ¹H NMR spectrum of (X) were not observed in

^1H NMR spectrum of (Va). Instead the ^1H NMR spectrum of (Va) showed a three proton doublet ($J = 8 \text{ Hz}$) at 1.19 ppm and a double quartet at 2.6 ppm. assignable to a secondary methyl group. This suggested that the lactone ring in (Va) is saturated and thus it is a 11,13-dihydroderivative of acetate (X). By combining all the above data, the following structure could be written for compound (V) and its acetate.



(V) $R = \text{H}$

(Va) $R = \text{Ac}$

A direct correlation between compound (IV) and (V) was achieved by converting the acetate of isocivangustin into (Va) by selective hydrogenation of exomethylene double bond.

EXPERIMENTAL

Plant material:

The plant Cyathocline purpurea was collected from Lonavala (Maharashtra) in December 1979 and was shade dried.

Extraction:

The shade dried and coarsely powdered plant material (8 kg) was extracted with acetone in a soxhlet apparatus on water-bath. The extraction was continued till the solvent ^{sample} removal from the test (100 ml) left no or negligible residue. The solvent was then recovered from extract by distillation. the last traces of the solvent were removed under vacuum to obtain a dark coloured gummy residue (300 g).

Chromatographic separation:

The acetone extract of Cyathocline purpurea (200 g) was then loaded on the column (115 cms long, and 9.5 cm in diameter) for separation of its constituents. The column was packed with silica gel (200 mesh, 3.5 kg). The elution was started with acetone: pet. ether mixture (20:80) and continued with successive increase in the percentage of acetone. Fractions of 150 ml size were collected. All fractions collected were evaporated to dryness and examined on TLC for their composition. The fractions showing similar chromatographic pattern were pooled together to obtain nine major fraction (A-I). The details of the chromatographic separation are summarized in Chart (III) and Table (V).

Table V Column chromatographic separation of acetone extract of Cyathocline purpurea

Fraction No.	Eluent	Total volume collected	Net weight of fraction	Approximate composition
(A)	Acetone (20) + Pet.ether (80)	150 ml x 3	25 g	straight chain hydrocarbon
(B)	Acetone (20) + Pet.ether (80)	150 ml x 4	22 g	Complex mixture of unidentified compounds
(C)	Acetone (20) + Pet.ether (80)	150 ml x 4	24 g	Mixture of unidentified compounds + sterol
(D)	Acetone (20) + Pet.ether (80)	150 ml x 5	23 g	sterol + unidentified compound
(E)	Acetone (30) + Pet.ether (70)	150 ml x 3	10 g	sterol + compounds (I) and (II)
(F)	Acetone (30) + Pet.ether (70)	150 ml x 7	35 g	Mixture of compounds (I), (II), (III), (IV) and (V)
(G)	Acetone (40) + Pet.ether (60)	150 ml x 4	4.8 g	Mixture of compounds (III), (IV) and (V)
(H)	Acetone (50) + Pet.ether (50)	150 ml x 4	5 g	Mixture of compounds (IV) (V)
(I)	Acetone + MeOH	150 ml x 10	45 g	Plant acids and sugars

Rechromatography of Fraction F:

Thin-layer-chromatographic examination of fraction (F) revealed that it was a mixture of minimum five compounds having very close R_f values (eluent:30:70). The fraction was rechromatographed on column (silica gel 1 kg). Elution was started with acetone:pet. ether mixture (20:80) with successive increase in proportion of acetone. Fractions having similar composition were combined to obtain seven major fractions (Chart III, Table IV).

Isolation of compound (I):

Fraction (III) from rechromatography of fraction (F) gave compound (I) as a greenish solid (16 g). The compound (I) was further purified by its crystallization from benzene to get colourless crystalline solid (15.5 g) m.p.115-116°C.

Analysis: Found C, 72.93%; H, 7.41%

$C_{15}H_{18}O_3$ required C, 73.15; H, 7.37%.

$(\alpha)_D^{32} = -111.8^\circ$ ($CHCl_3$ $c = .5$)

U.V. : end absorption.

IR (Fig.1) $\gamma_{\max}^{nujol} \text{ cm}^{-1}$ 3280 (OH), 1745 (lactone carbonyl)
1630 and 805 (unsaturation)

Mass: (m/e, rel intensity).

Isolation of compound (II):

Compound (II) was obtained as a mixture along with compound (I), as both the compounds had very close R_f values. Further purification of compound (II) by chromatographic

Table VI Rechromatography of Fraction F

Fraction No.	Eluent	Total volume collected	Net weight of fraction	Approximate composition
(I)	Acetone (20) + Pet.ether (80)	50 ml x 2	5 g	Straight chain hydrocarbons
(II)	Acetone (20) + Pet.ether (80)	50 ml x 4	3 g	unidentified compounds sterols
(III)	Acetone (25) + Pet.ether (75)	50 ml x 4	16 g	Compound (I)
(IV)	Acetone (25) + Pet.ether (75)	50 ml x 6	2 g	Compounds (I) and (II)
(V)	Acetone (30) + Pet.ether (70)	50 ml x 5	2.3 g	Compounds (III), (IV) and (V)
(VI)	Acetone (30) + Pet.ether (70)	50 ml x 4	5 g	Compound (IV) and (V)
(VII)	Acetone	50 ml x 6	2 g	Plant acids

techniques was not possible. However, compound (II) was obtained in fairly pure form as its acetate (IIa). For acetylation, a mixture of compound (I) and (II) (200 mg) was dissolved in pyridine (2 ml) and to it acetic anhydride (2 ml) was added dropwise at room temperature. The mixture was allowed to stand at room temperature for 12 hours. The usual work up of the mixture gave a mixture of two acetates (210 mg). The two acetates were separated by preparative TLC (eluent: 30:70) to get the acetate of compound (II) as colourless crystalline solid m.p. 162-63°.

$$(\alpha)_D^{30} = -117^\circ \text{ (CHCl}_3 \text{ } c = .2)$$

IR: (Fig. 10) $\nu_{\text{max}}^{\text{nujol}} \text{ cm}^{-1}$ 1780 (γ -lactone) 1740, 1240 (acetate)
1650 (unsaturation).

Isolation of compound (III):

Fraction (V) from the rechromatography of fraction (F) was a mixture of three compounds having very close R_f values (TLC eluent: 30:70). The fraction (V) (2.5 g) was again subjected to column chromatographic separation (silica gel, 200 mesh, 100 g). Acetone-pet.ether mixture (20:80) was used as eluent, with successive increase in percentage of acetone. Fractions showing similar composition were mixed together (TLC eluent 30:70) to obtain five major fractions (Table VII). Compound (III) was obtained as a gummy solid from fraction C (Table (VII) (.8 g). The compound (III) was further purified via its piperidine adduct. The compound (III) was dissolved in ethanol (10 ml) and to it piperidine (15 ml) was added

Table VII Column chromatography of Fraction (V)
from rechromatography of fraction (F)

Fraction No.	Eluent	Total volume collected	Net weight of fraction	Approximate composition
(a)	Acetone (20) + Pet.ether	25 ml x 4	.3 g	unidentified compounds
(b)	Acetone (20) + Pet.ether (80)	25 ml x 4	.3 g	compound (III) + unidentified compound
(c)	Acetone (25) + Pet.ether (75)	25 ml x 6	.8 g	compound (III)
(d)	Acetone (30) + Pet.ether (70)	25 ml x 3	.2 g	compound III, IV and (V)
(e)	Acetone (30) + Pet.ether (70)	25 ml x 4	.6 g	compound (IV) and (V)

dropwise at room temperature. The mixture was allowed to stand at room temperature for 12 hours. The usual work up of the reaction mixture gave the crude piperidine adduct (0.6 g). The crude adduct was purified by its preparative layer chromatography (eluent: 40:60) to obtain pure piperidine adduct as viscous oil (0.54 g).

IR: $\nu_{\text{max}}^{\text{nujol}}$ cm^{-1} 3260 (OH) 1745 (Y-lactone) 1640 (unsaturation)
 ^1H NMR: (CCl_4 soln.) 1.1 (s, 3H H-14) 1.66 (s, 3H, H-15) 3.33 (1Hm H-1) 4.41 (1H, m, H-8) 1.60-2.5 ($-\text{CH}_2-$) of piperidine ring.

The compound (III) was regenerated from its piperidine adduct as described below. To the piperidine adduct (400 mg) in a round-bottom flask, methyl iodide (6 ml) was added and solution was stirred at room temperature for 1/2 hour. Sodium bicarbonate solution (5% aqueous, 50 ml) was then added to the above solution and it was stirred for additional 24 hours. Reaction mixture was then diluted with water and extracted with chloroform (20 ml x 3), the chloroform extract was washed with water (20 ml x 3) dried over anhydrous sodium sulphate and solvent removed to get the crude compound (III) (300 mg) which was then crystallised from acetone:pet.ether mixture to get colourless crystalline solid, m.p. 121-123° (200 mg).

IR: (Fig. 12) $\nu_{\text{max}}^{\text{nujol}}$ cm^{-1} 3300 (OH) 1750 (Y-lactone) 1650 (unsaturation).

Mass: m/e (relintensity) 248 (M^+ , 68) 230 ($\text{M}-\text{H}_2\text{O}$, 21) 206 ($\text{M}-\text{CO}_2$, 20), 81 (100)

Isolation of compound (IV):

Fraction (VI) from the rechromatography of fraction (F) (Chart III) gave compound (IV) as a mixture along with compound (V) (TLC, eluent:35:65). Attempts to purify the compound (IV) by chromatography were unsuccessful due to the very close R_f values of compounds (IV) and (V). However, compound (IV) was obtained in pure crystalline form via its piperidine adduct. The mixture of (IV) and (V) (.8 g) was dissolved in ethanol (25 ml) and piperidine (1 ml) was added to it at room temperature dropwise and the mixture was allowed to stand at room temperature for 12 hours. The mixture was then worked up to get piperidine adduct (950 mg). The crude piperidine adduct (XIII) was crystallized from benzene pet.ether mixture to get colourless crystalline solid (.75 g) m.p.160-61°.

IR: (Fig. 25) $\gamma_{\text{max}}^{\text{nujol}} \text{ cm}^{-1}$ 3620 (OH) 1770 (γ -lactone)
1650 (unsaturation).

Mass: m/e (rel.intensity) 333 (M^+ , 100), 248 ($M-85$, 48)
220 (248-OH 12.5) 215 (248 - H_2O - CH_3 , 10).

Compound (IV) was obtained from its piperidine adduct as described for compound (III). The compound (IV) (0.5 g) was crystallized from acetone pet.ether mixture to get colourless crystalline solid, (.4 g), m.p. 141-142°.

$(\alpha)_D^{30} = +125.4$ ($CHCl_3$ $c = 0.26$)

UV: and absorption.

IR (Fig. 14) $\gamma_{\text{max}}^{\text{nujol}}$ cm^{-1} 3620 (OH) 1770 (Y-lactone) 1650 (unsaturation).

Mass: m/e (rel. intensity) 248 (M^+ , 68), 230 ($\text{M}-\text{H}_2\text{O}$, 21) 206 ($\text{M}-\text{CO}_2$, 20), 81 (100).

Isolation of compound (V):

Compound (V) was isolated from the mother liquor of piperidine adduct (XIII) (please see isolation of the compound (IV) by its preparative TLC (eluent: 35:65). Compound (V) (150 mg) thus obtained still contained some overlapping impurity. The attempts to purify (V) by chromatography (column, PLC) were unsuccessful due to overlapping impurity and paucity of compound. However (V) could be isolated in fairly pure form as its acetate (Va). For acetylation, the compound (V) (150 mg) was dissolved in pyridine (1.5 ml) and to it acetic anhydride was added dropwise at room temperature. The mixture was allowed to stand at room temperature for 12 hours. The usual work-up of the reaction mixture gave the crude acetate (160 mg). The resulting acetate (Va) (160 mg) was purified by its preparative TLC (eluent: 30:70) to get pure acetate (Va) (130 mg) as a colourless crystalline solid, m.p. 116° .

$(\alpha)_D^{30} + 80.00^\circ$ (CHCl_3 $c = .3$).

IR: (Fig. 27) $\gamma_{\text{max}}^{\text{nujol}}$ cm^{-1} 1760 (Y-lactone) 1720 and 1240 (acetate).

Chemical transformation products of compound (I):

a) Mono acetate (VI): Compound (I) (100 mg) was dissolved in pyridine (1 ml) and to it acetic anhydride (1 ml) was added at room temperature and mixture was allowed to stand at room temperature for 12 hours. The usual work up of the product gave crude mono-acetate (110 mg) as crystalline solid, which was recrystallized from acetone to get colourless crystalline solid (95 mg), m.p. 128°.

(α)_D³⁰ -128.0 (CHCl₃ C = 0.17)

IR: (Fig. 3) γ $\begin{matrix} \text{nujol} \\ \text{max} \end{matrix}$ cm⁻¹ 1765 (γ-lactone), 1740, 1240 (acetate), 1650, 885 (unsaturation)

b) Hexahyromonoacetate (VII): The monoacetate (VI) (50 mg) was catalytically hydrogenated at atmospheric pressure/room temperature using palladium/carbon 10% as catalyst, to obtain hexahyromonoacetate (VII) as a thick liquid.

IR: (Fig. 6) γ $\begin{matrix} \text{nujol} \\ \text{max} \end{matrix}$ cm⁻¹ 1780 (γ-lactone) 1750 and 1240 (acetate).

Transformation products of compound (IV):

Tetrahydro compound (IX): Compound (IV) (100 mg) dissolved in ethanol was stirred in atmosphere of hydrogen at atmospheric pressure and room temperature in presence of Pd/C 10% as catalyst, till there was no absorption of hydrogen. The usual work up of reaction mixture afforded tetrahydro-compound (IX) as colourless crystalline solid m.p. 151-152°.

IR: (Fig. 19) γ $\begin{matrix} \text{nujol} \\ \text{max} \end{matrix}$ cm⁻¹ 3620 (OH) 1775 (γ-lactone).

Mass: m/e (rel.intensity) 252 (M^+ , 100) 234 ($M-H_2O$, 10)

219 (234-Me, 8) 208 ($M-CO_2$, 18) 193 (208-Me, 83).

Monoacetate (X): Compound (IV) (100 mg) was dissolved in pyridine (1 ml) and to it acetic anhydride (1 ml) was added at room temperature. The reaction mixture was allowed to stand at room temperature for 12 hours and it was then worked up as usual, to get crude monoacetate (X) (110 mg) which was crystallized from ethanol to get colourless crystalline solid (95 mg) m.p.166.6°.

$(\alpha)_D^{30} = +158.66$ ($CHCl_3$ $C = 0.15$)

IR: (Fig. 17) $\nu_{\max}^{nujol}$ cm^{-1} : 1750 (γ -lactone) 1725 and 1240 (acetate) 1650 and 880 (unsaturation)

Dihyromonoacetate (XII): Monoacetate (X) (50 mg) was selectively hydrogenated, by stirring ethanol solution of monoacetate in hydrogen atmosphere at room temperature/pressure in presence of palladium hydroxide on calcium carbonate as catalyst. The usual work up of the product gave (XII) as colourless crystalline solid (46 mg) m.p.116.8°.

$(\alpha)_D^{30} = +80^\circ$ ($CHCl_3$ $C = 0.3$)

IR: (Fig. 23) $\nu_{\max}^{nujol}$ cm^{-1} 1750 (γ -lactone) 1725 and 1240 (acetate) 1650 (unsaturation).

Epoxide (XI): The compound (IV) (80 mg) was dissolved in minimum amount of chloroform and solution was cooled to 0° in an ice bath. To the above cold solution, m-chloroperbenzoic acid (240 mg) dissolved in minimum amount of chloroform was

added slowly. The mixture was allowed to stand at 0° for 48 hours with occasional shaking. The reaction mixture was then washed with water till it was free from acid (pH 7), dried over anhydrous sodium sulphate and solvent removed to get the product (XI) as colourless crystalline solid m.p. 148-50°.

IR : (Fig. 21) ν_{max} ^{nujol} cm⁻¹ 3600 (OH) 1760 (γ -lactone)
1650 and 875 (unsaturation)

Piperidine adduct (XIII)

For preparation of piperidine adduct and its spectral data please see isolation of compound (IV).

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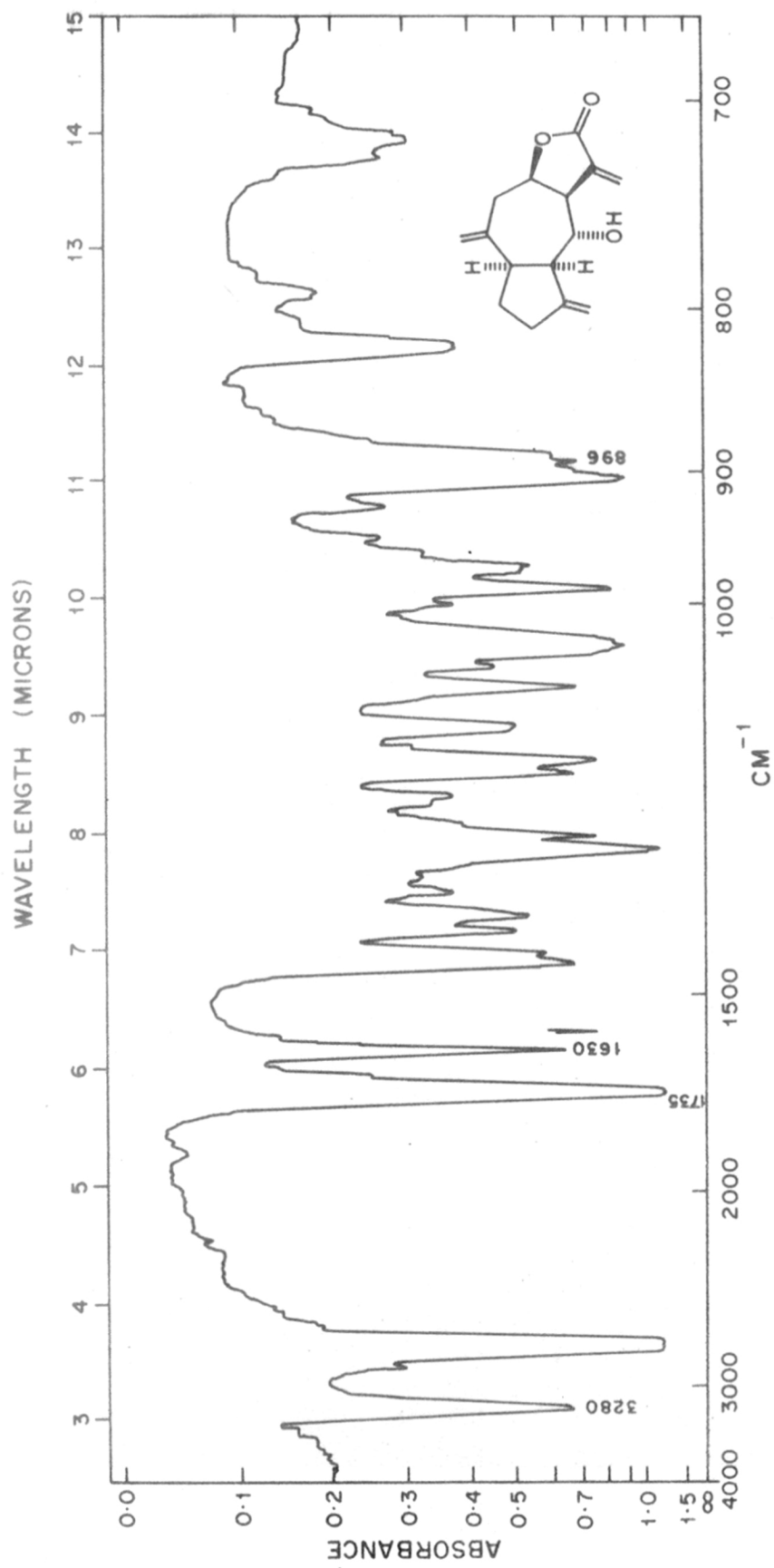


FIG. 1 : IR SPECTRUM OF COMPOUND (I) .

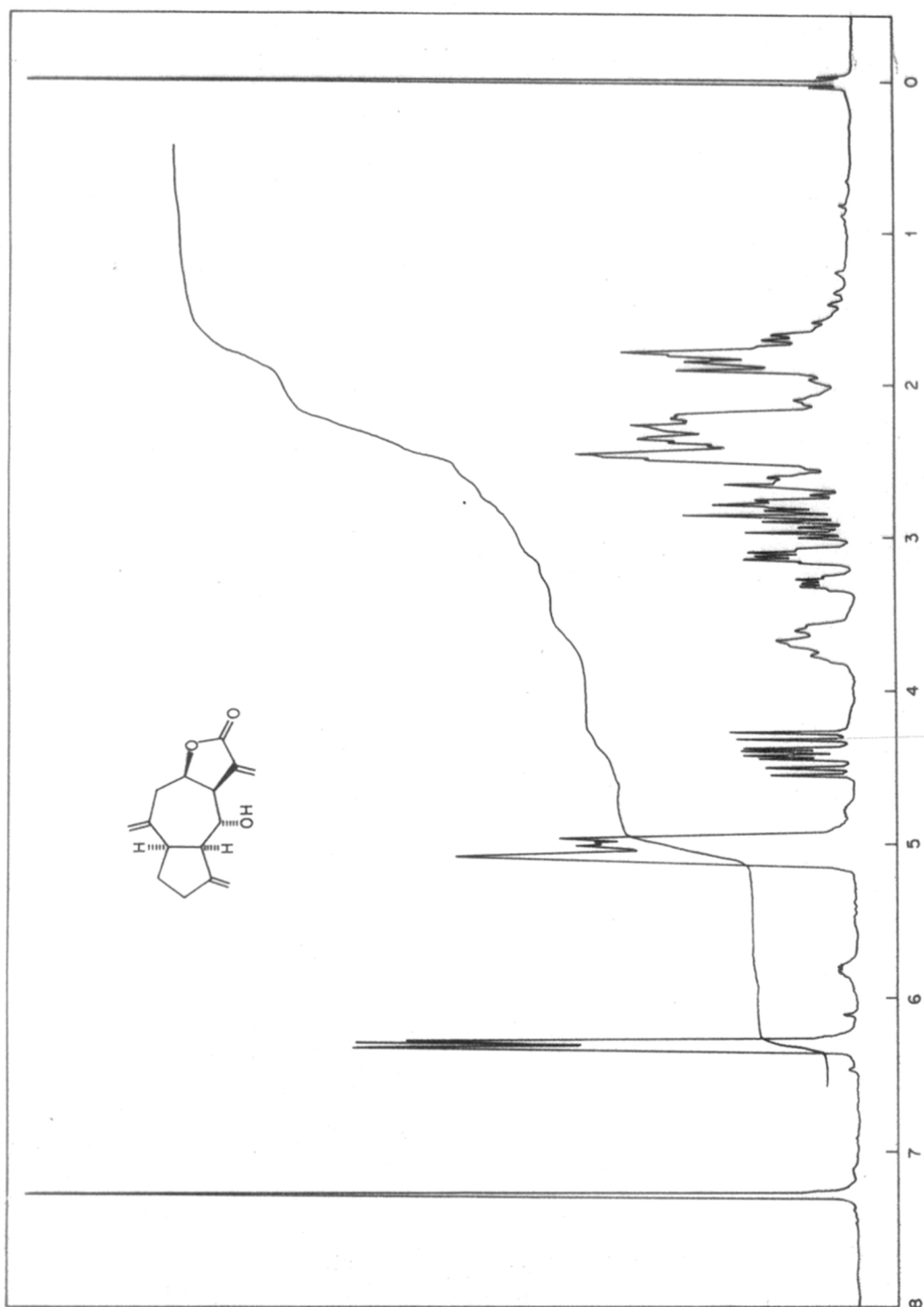


FIG. 2 : ^1H NMR SPECTRUM OF COMPOUND (I) (90 MHz).

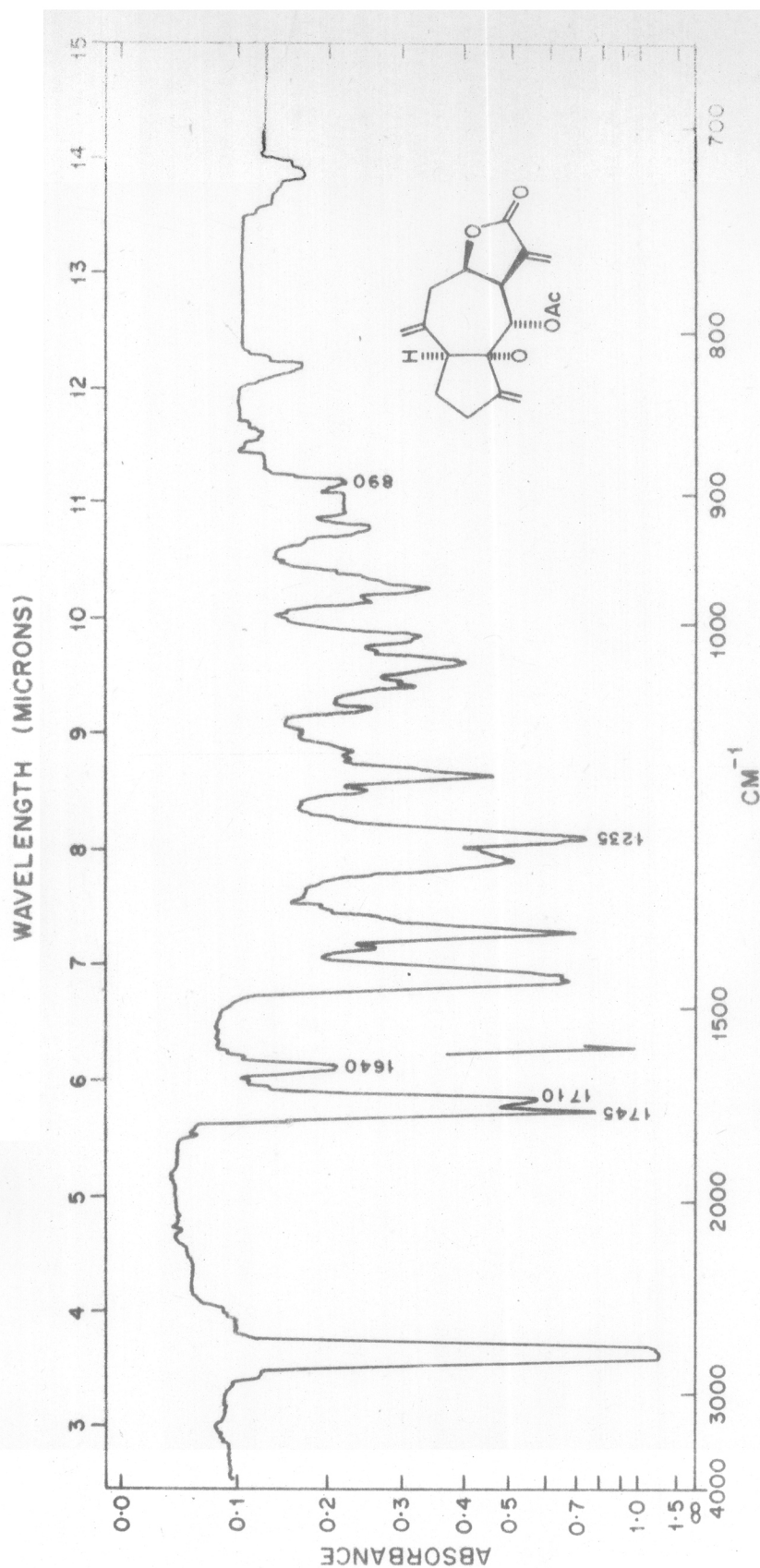


FIG. 3 : IR SPECTRUM OF ACETATE (VI).

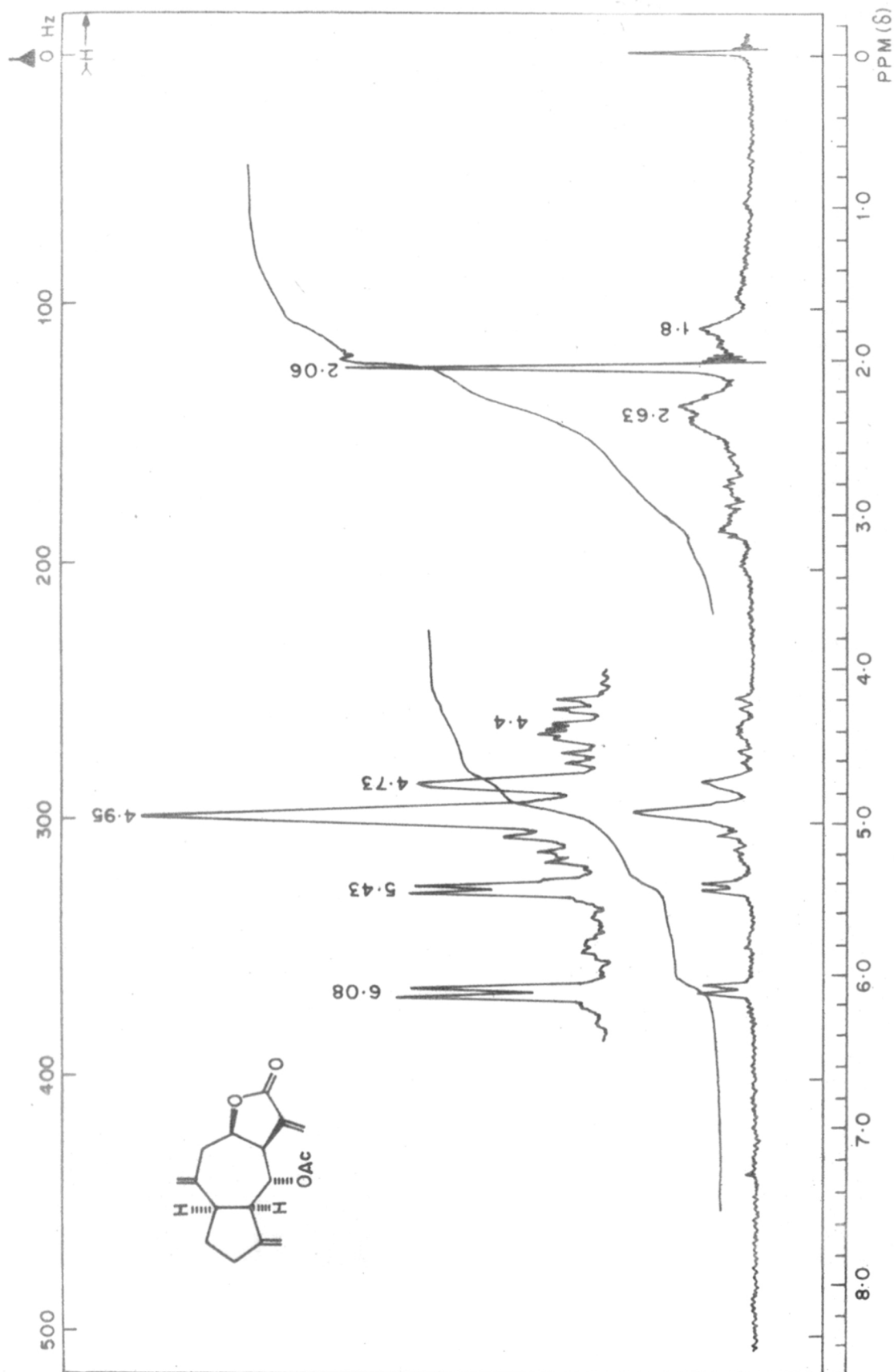
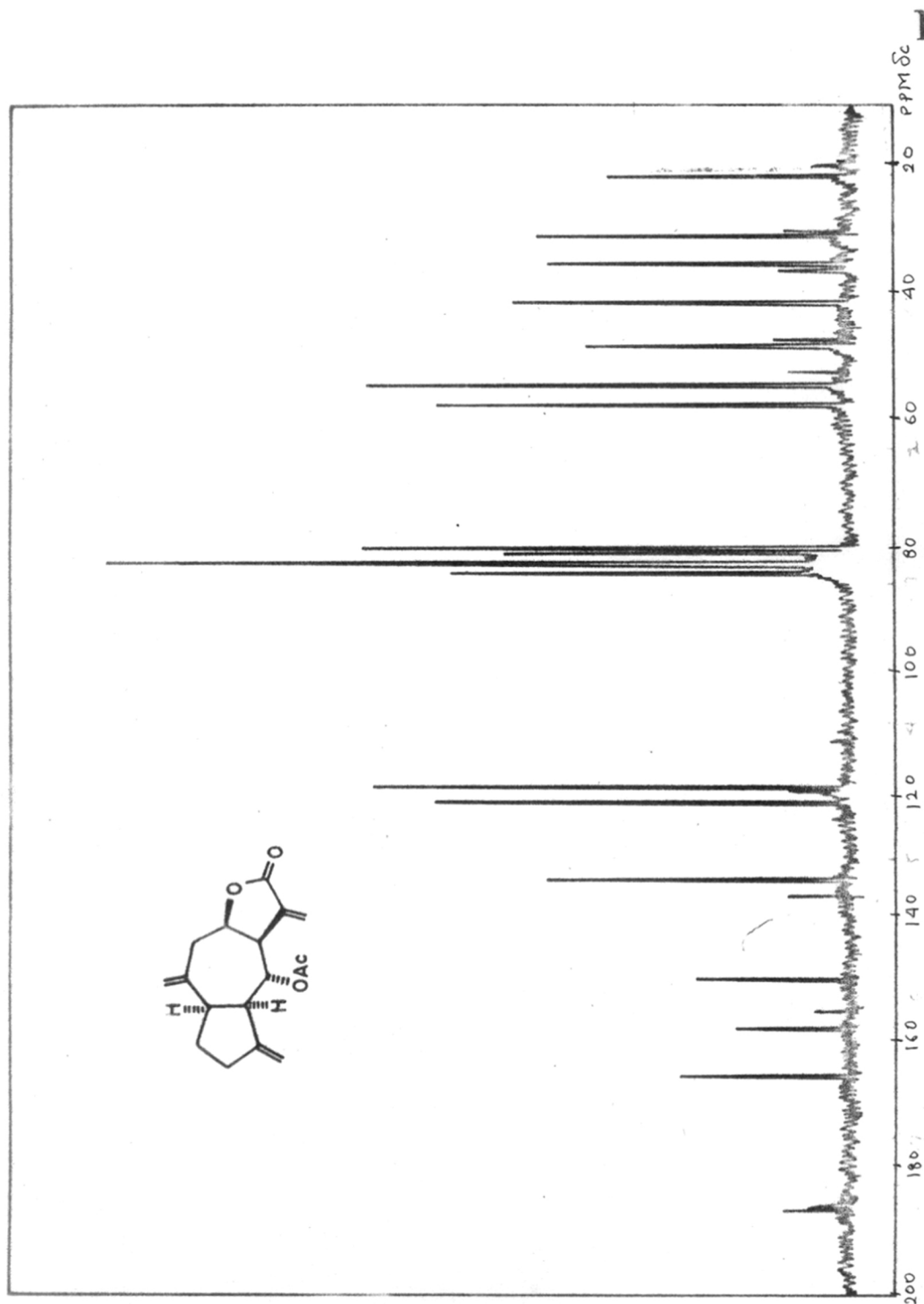


FIG. 4 : ^1H NMR SPECTRUM OF ACETATE (VI).



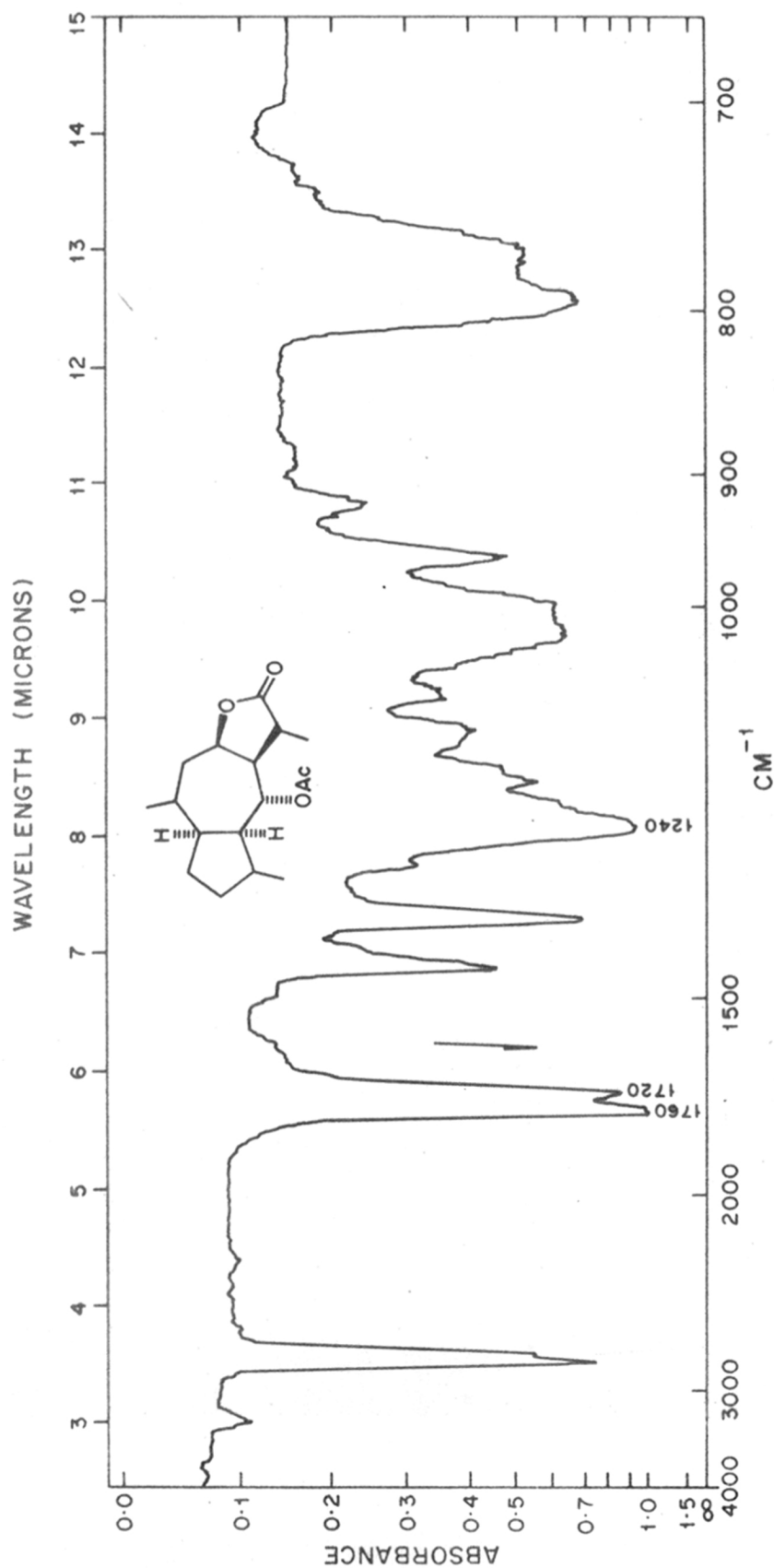


FIG. 6 : IR SPECTRUM OF HEXAHYDROMONOACETATE .

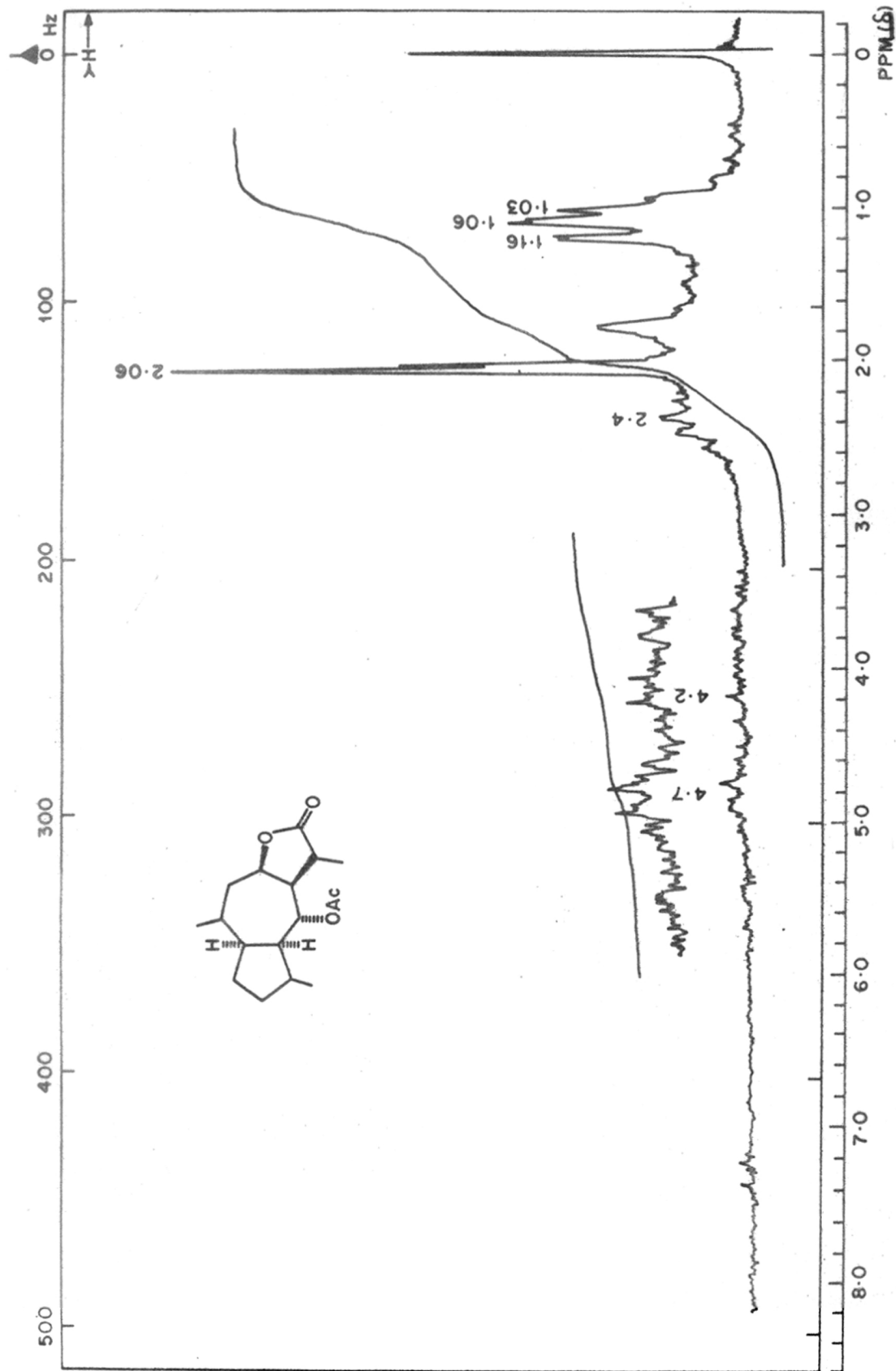


FIG. 7 : HNMR SPECTRUM OF HEXAHYDROMONOACETATE (60 MHz)

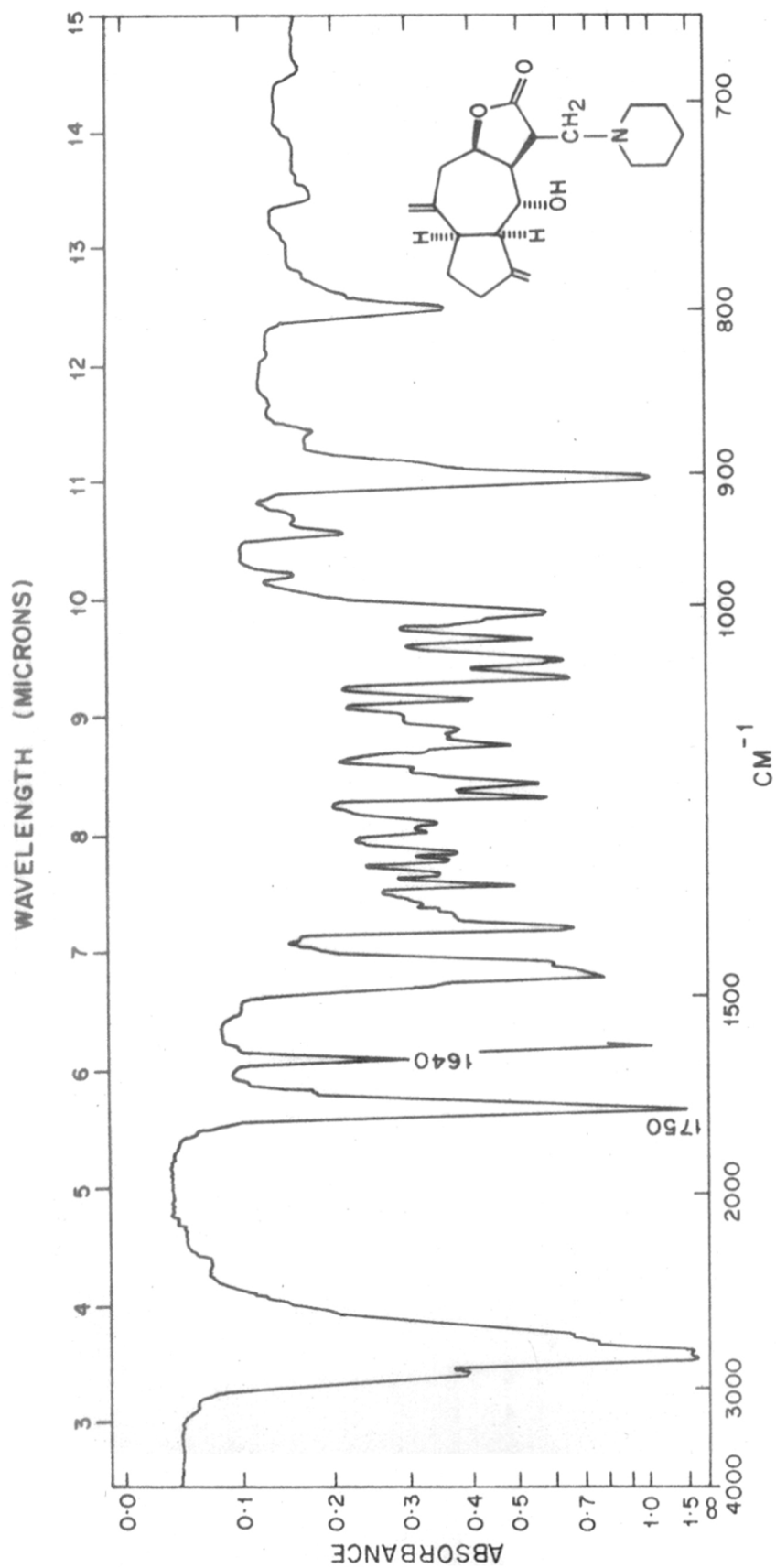
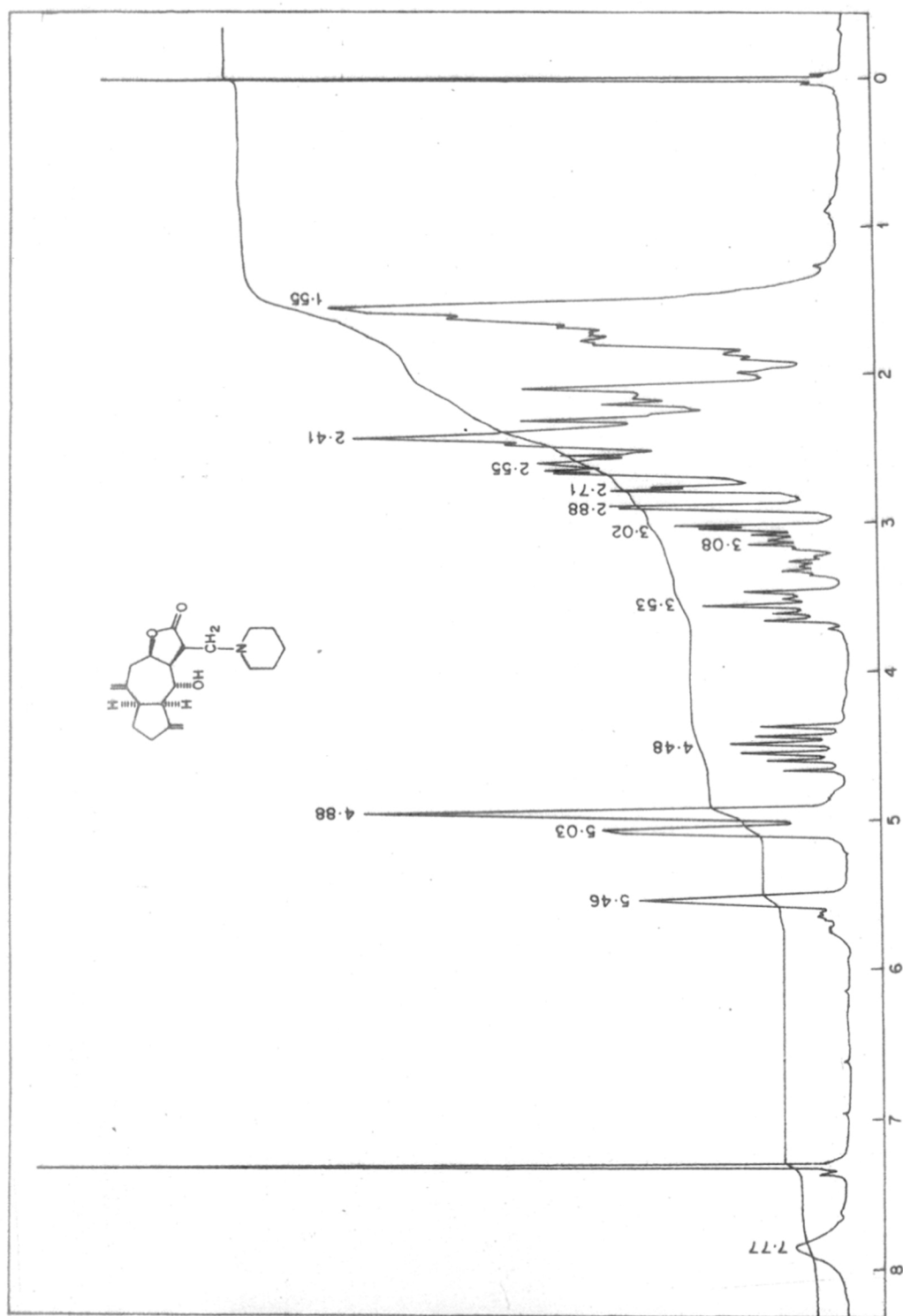


FIG. 8 : IR SPECTRUM OF PIPERIDINE ODDUCT (VIII).

FIG. 9 : ^1H NMR SPECTRUM OF PIPERIDINE ADDUCT (VIII) (90 MHz).

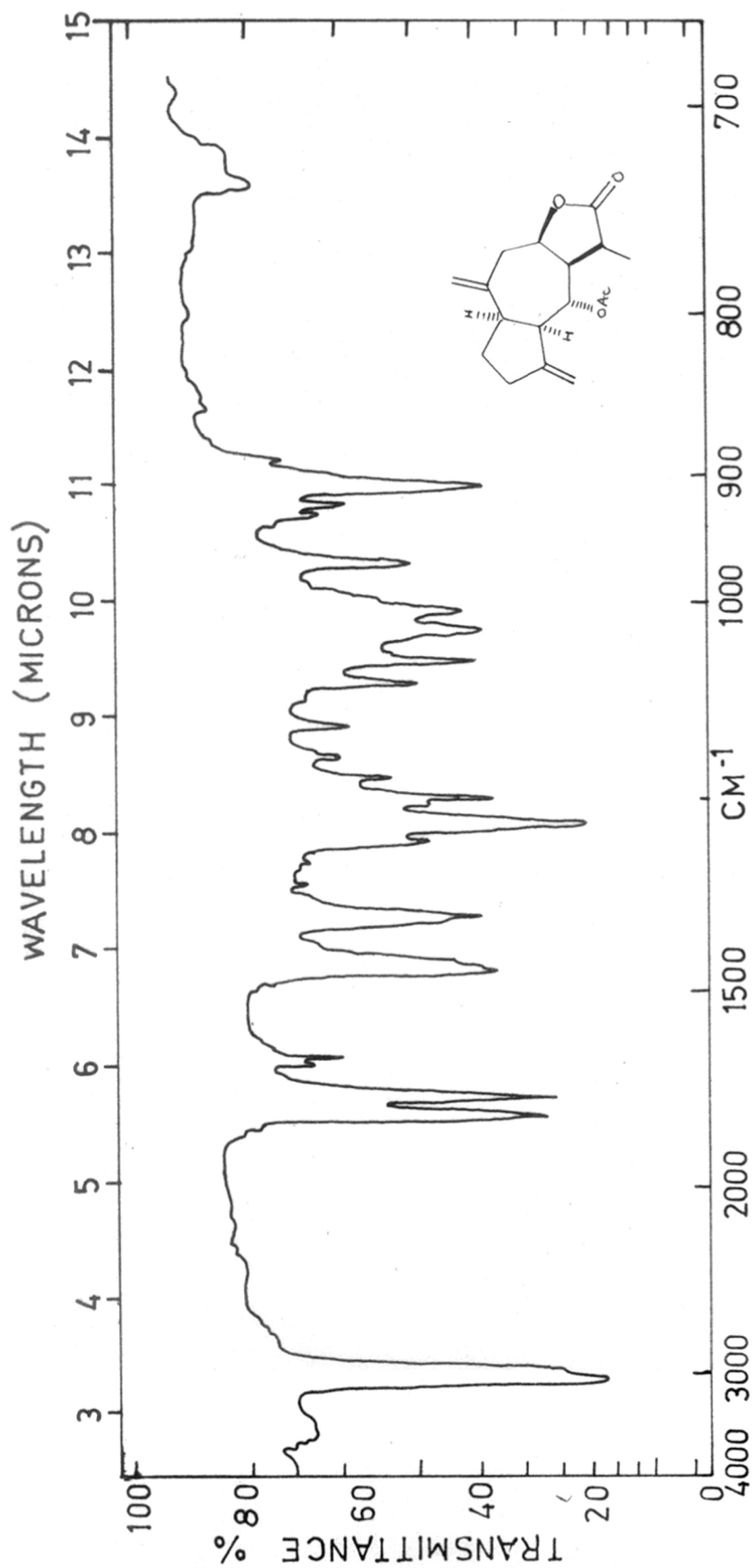


FIG 10: I R SPECTRUM OF MONOACETATE (IIa)

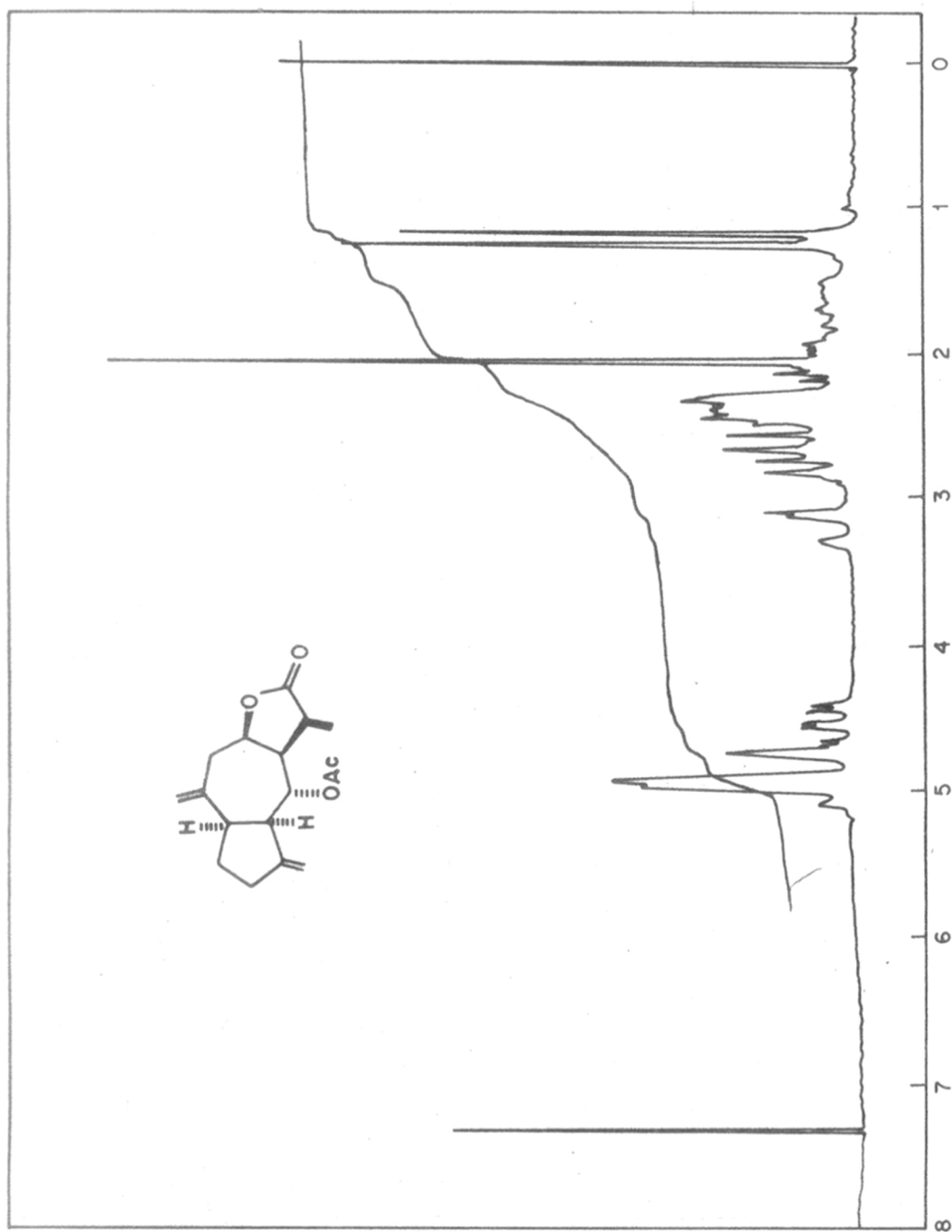


FIG. 11 : ^1H NMR SPECTRUM OF SPECTRUM OF ACETATE OF COMPOUND (II) (90MHz).

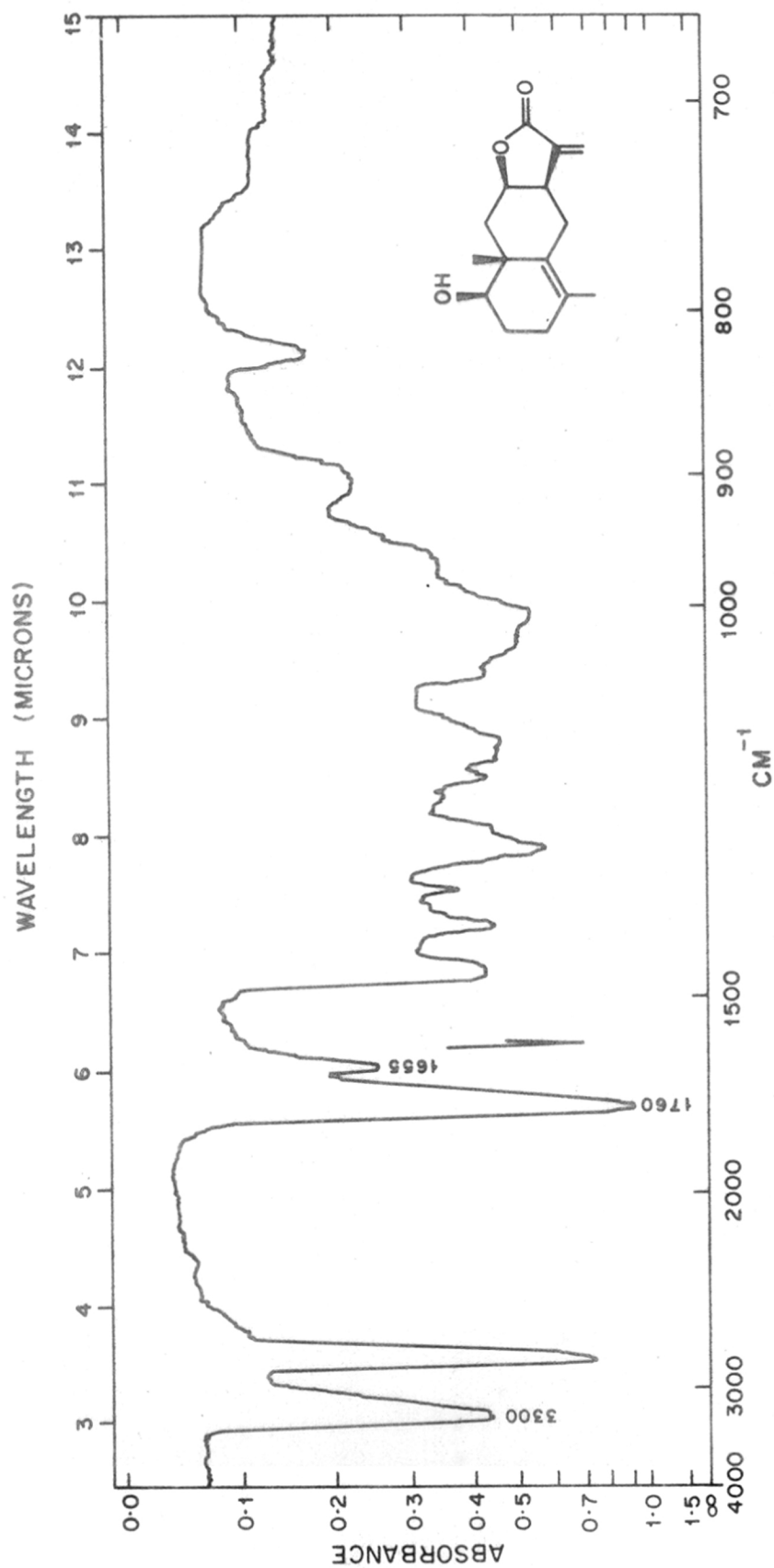


FIG12: I R SPECTRUM OF COMPOUND (III)

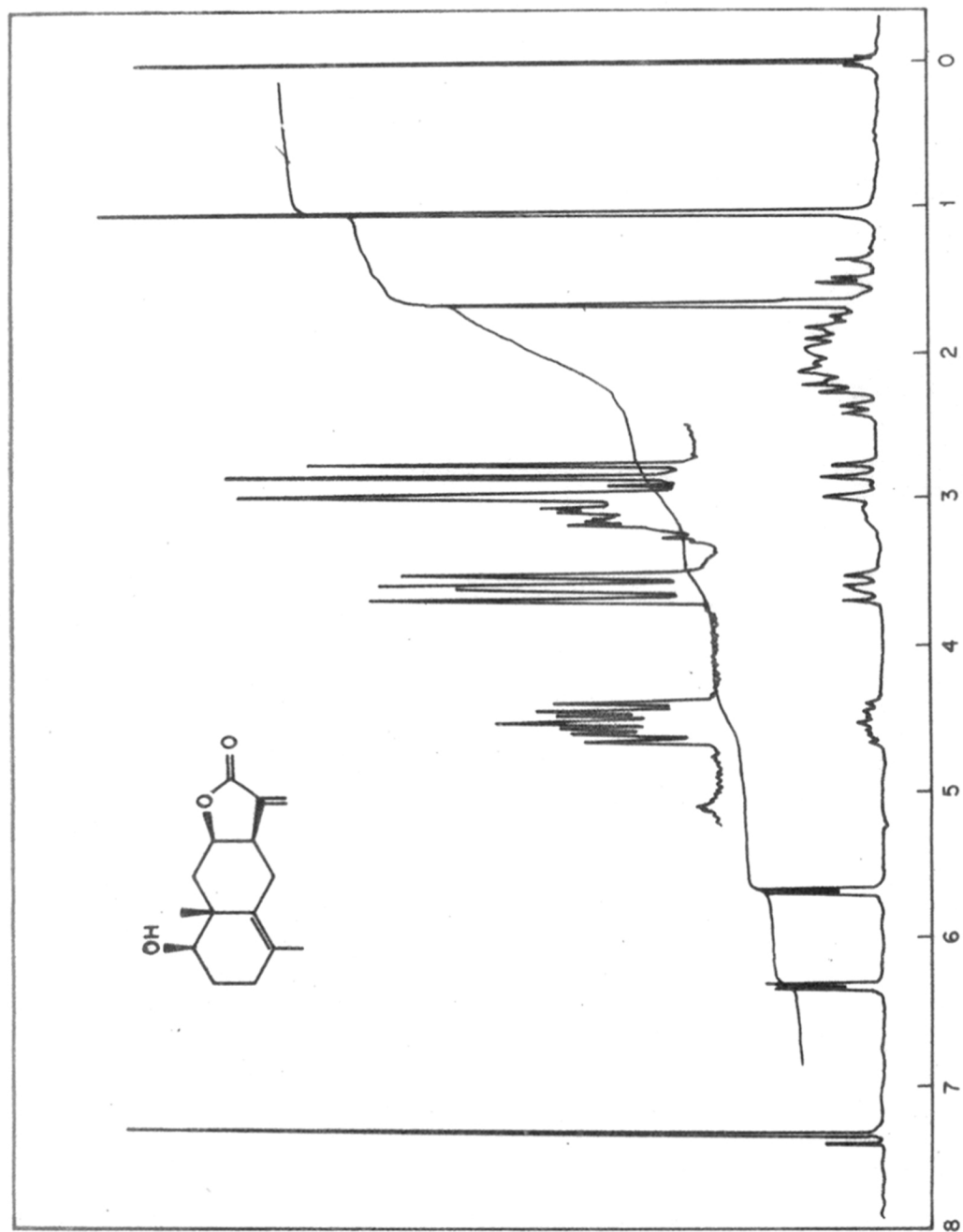
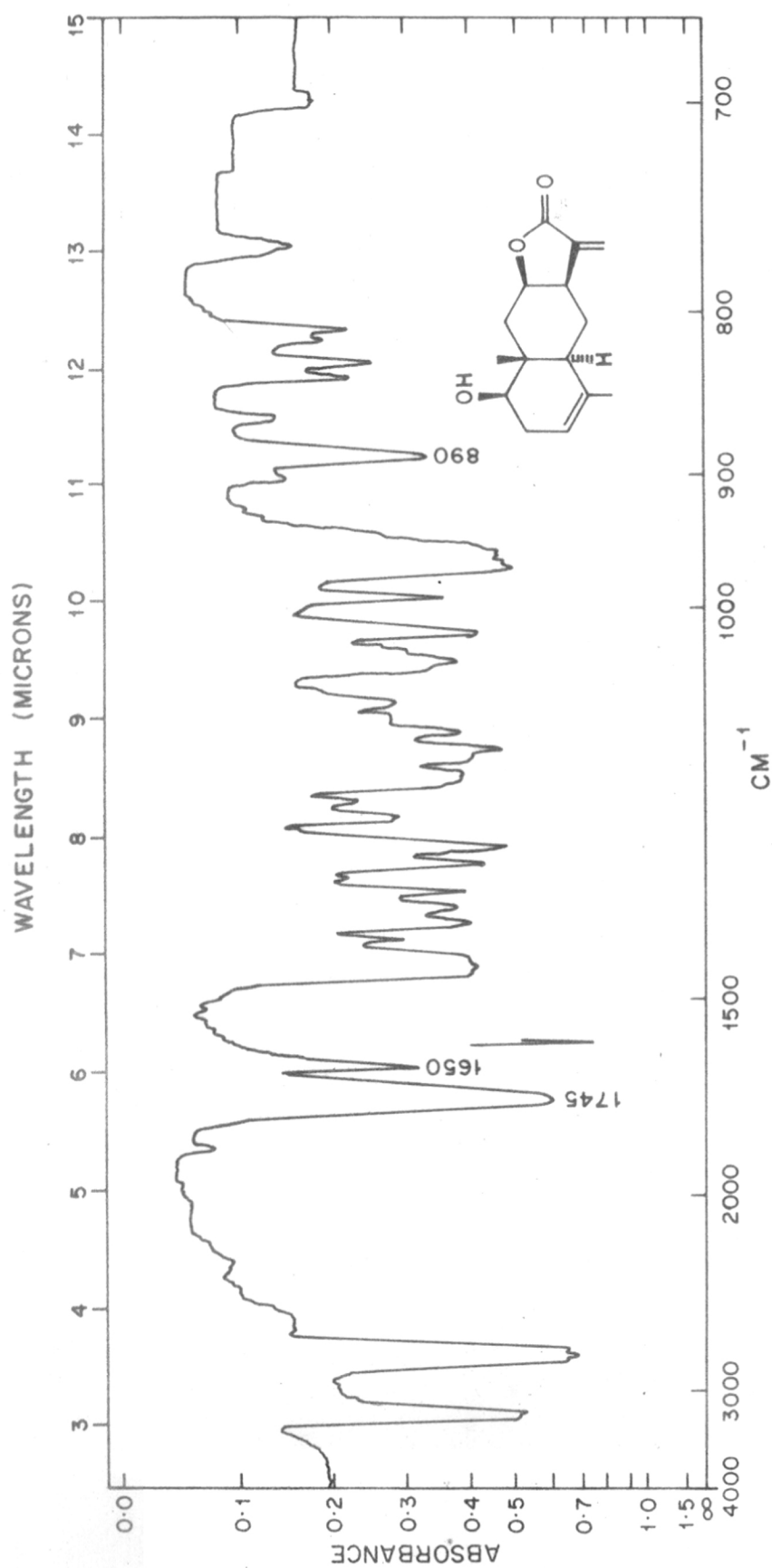
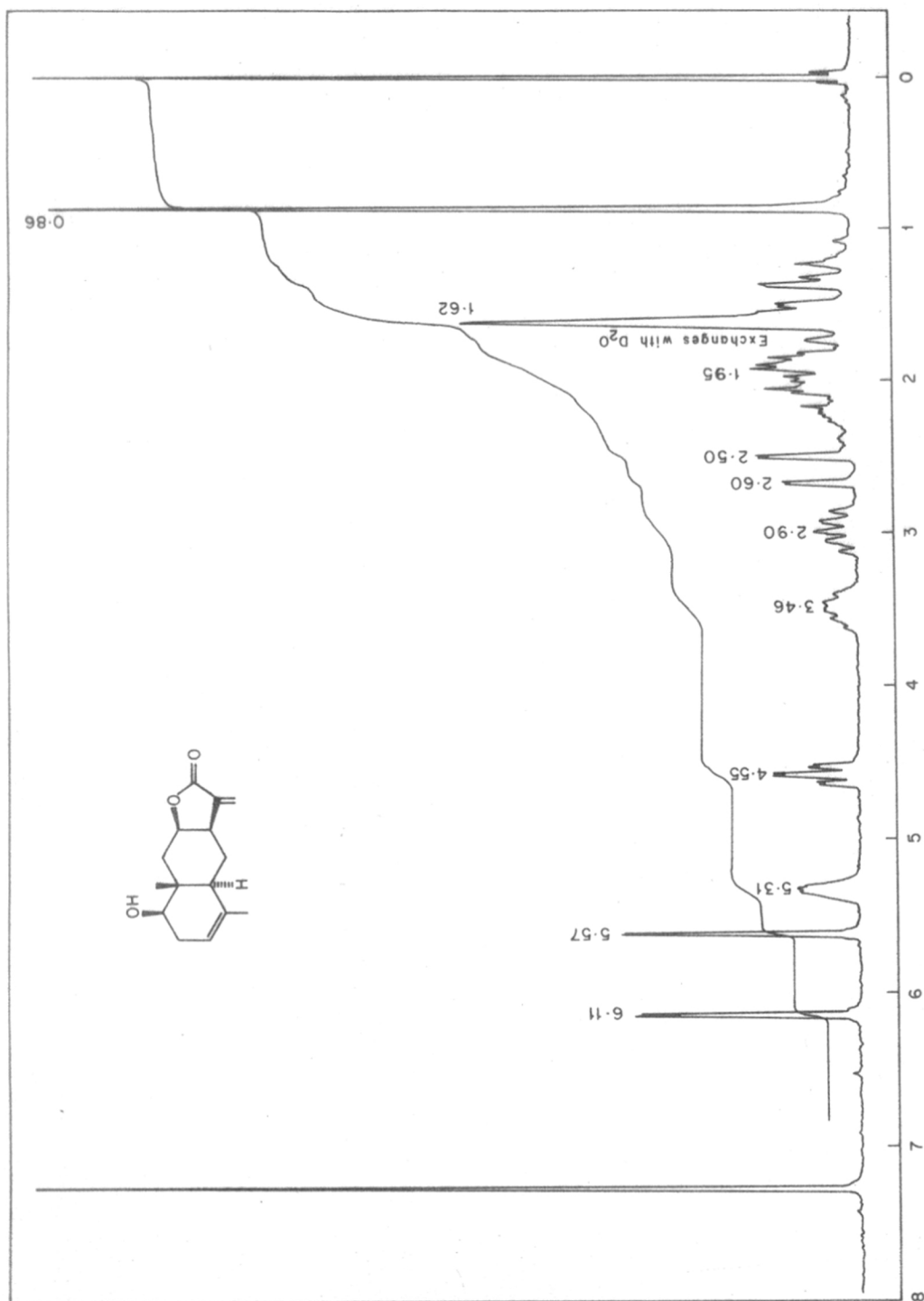
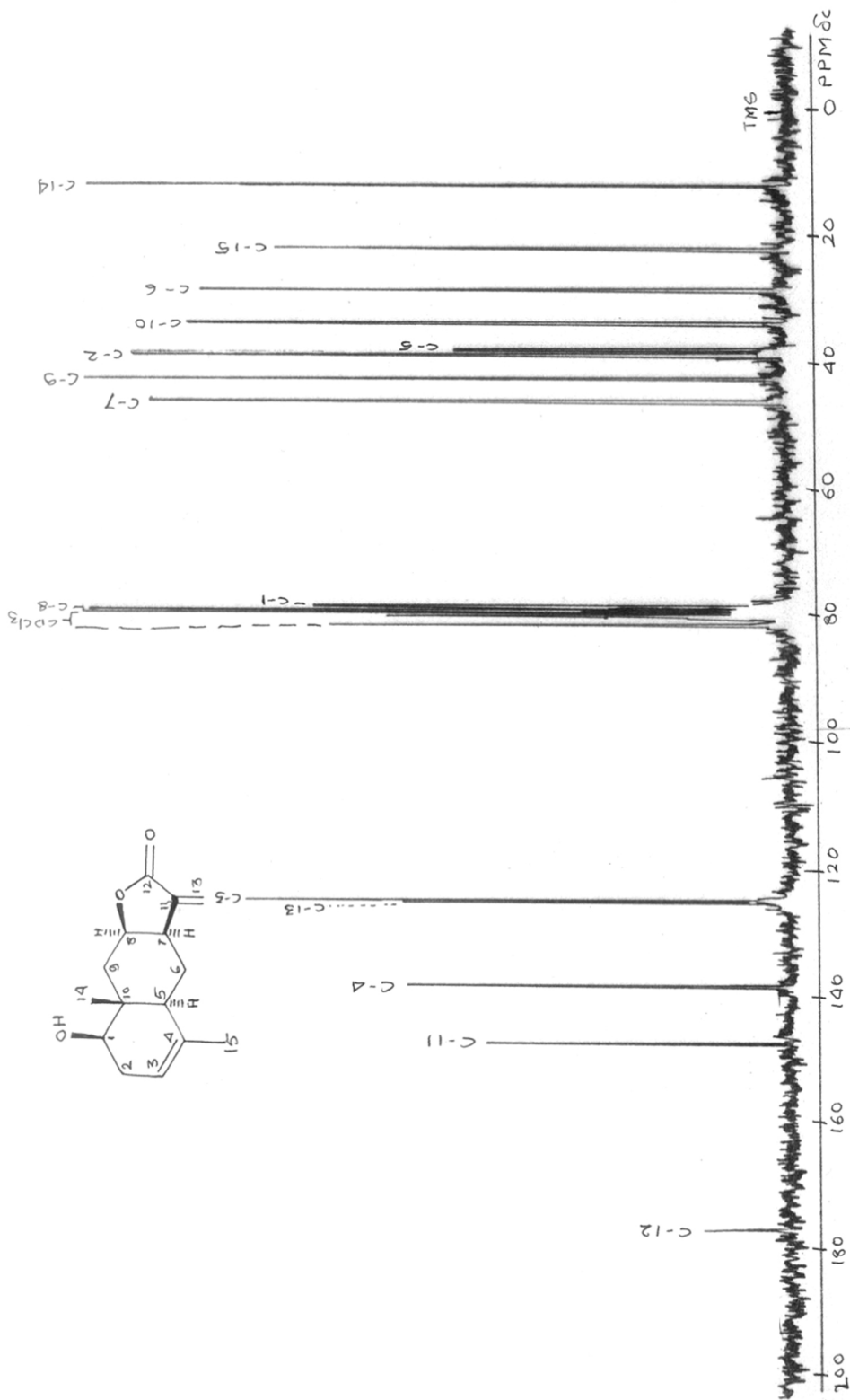
FIG. 13 : ^1H NMR SPECTRUM OF COMPOUND. (III) (90 MHz)

FIG. 14 : IR SPECTRUM OF COMPOUND (IV).



FIG. 15 : ^1H NMR SPECTRUM OF COMPOUND (IV) (90 MHz).



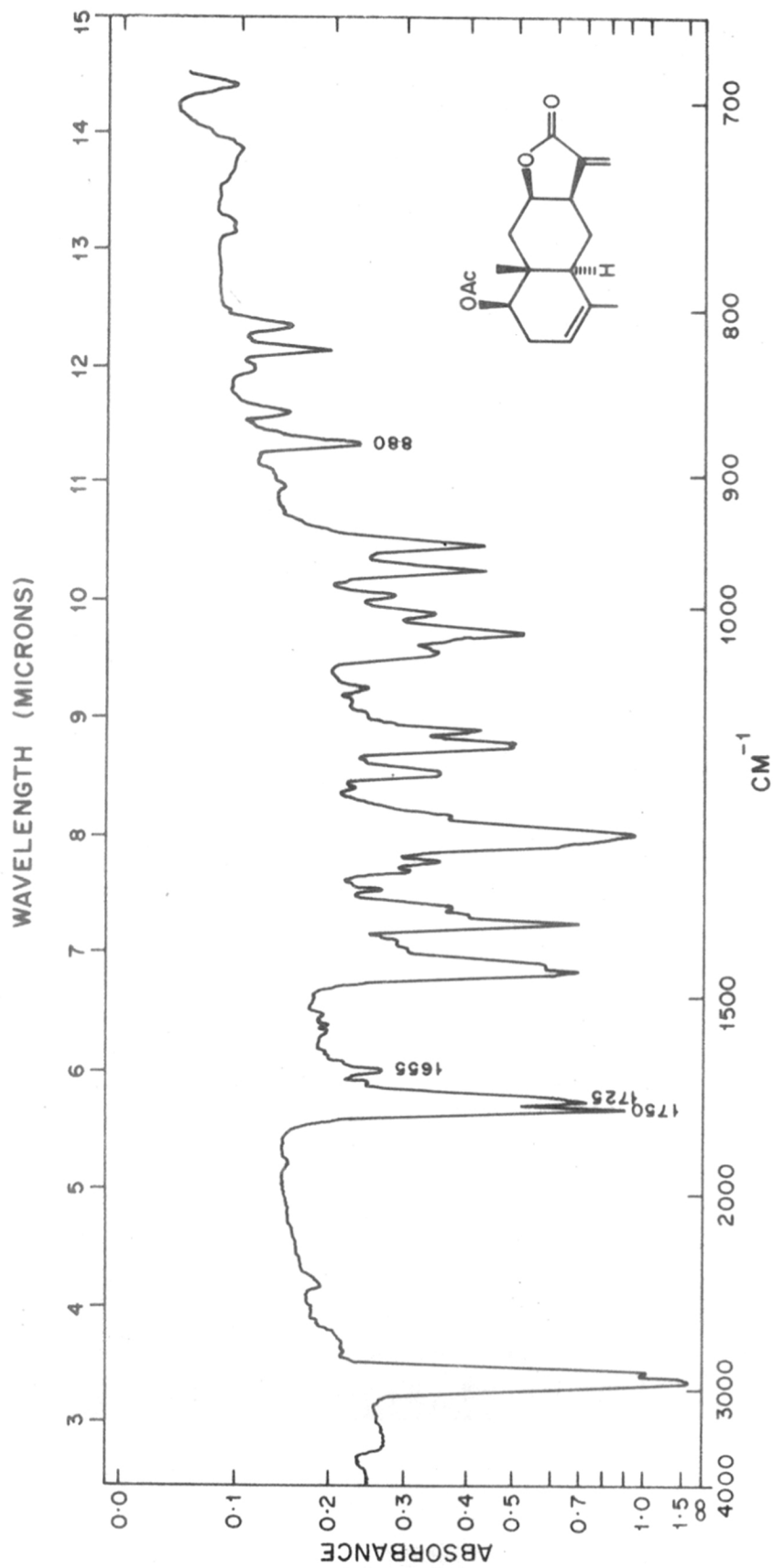
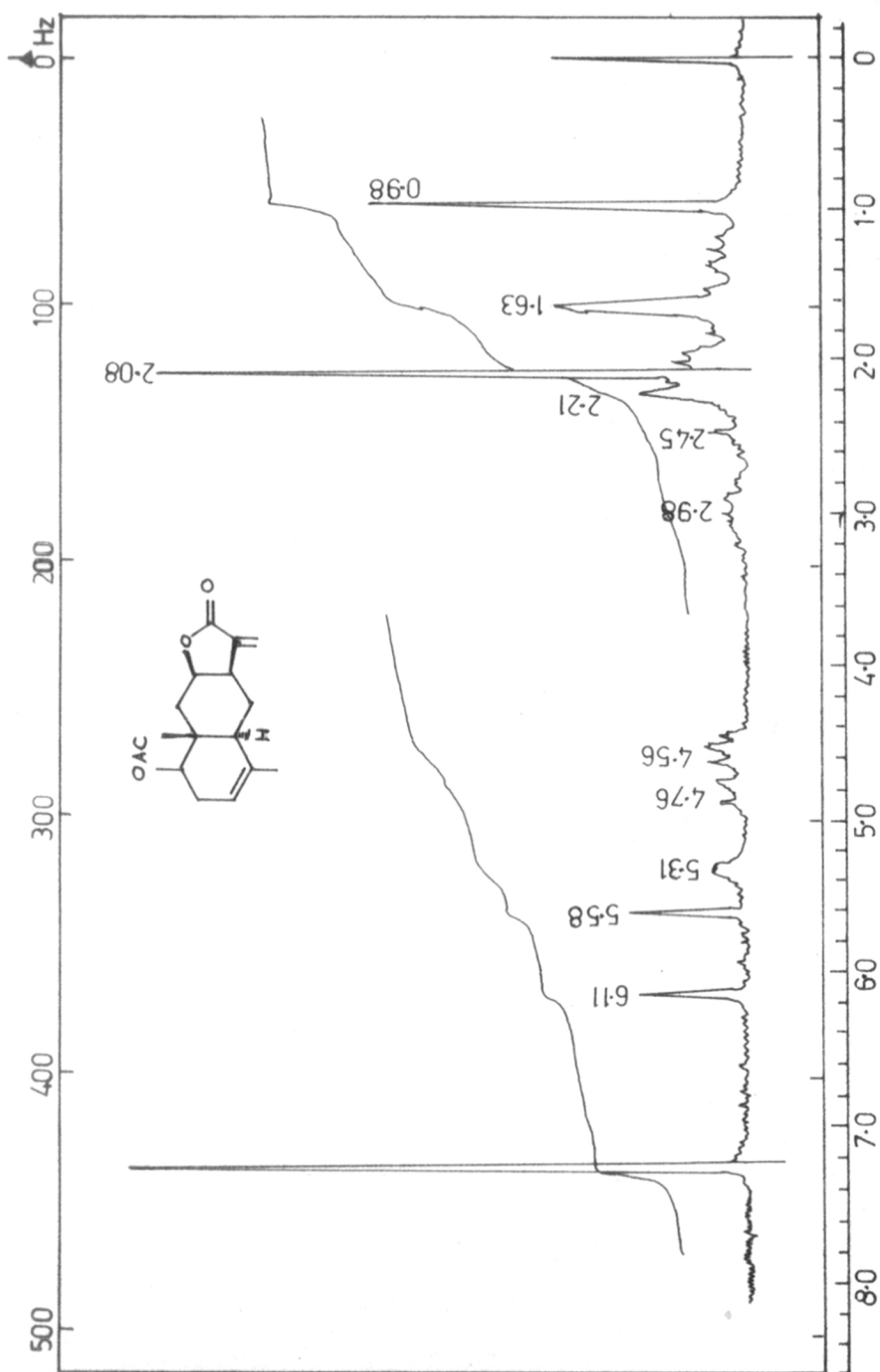


FIG. 17 : IR SPECTRUM OF ACETATE (X).

FIG 18: ^1H NMR SPECTRUM OF ACETATE

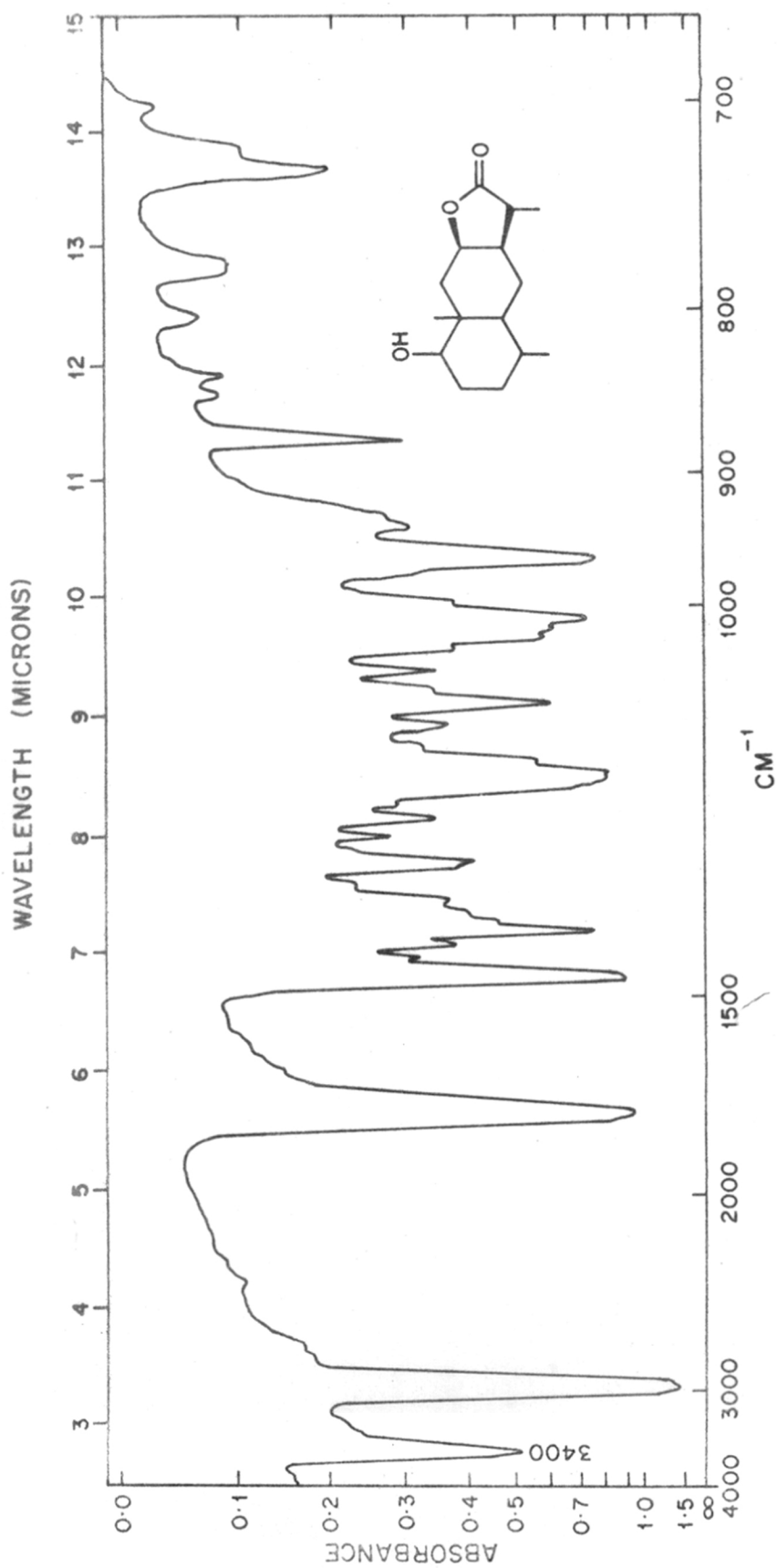
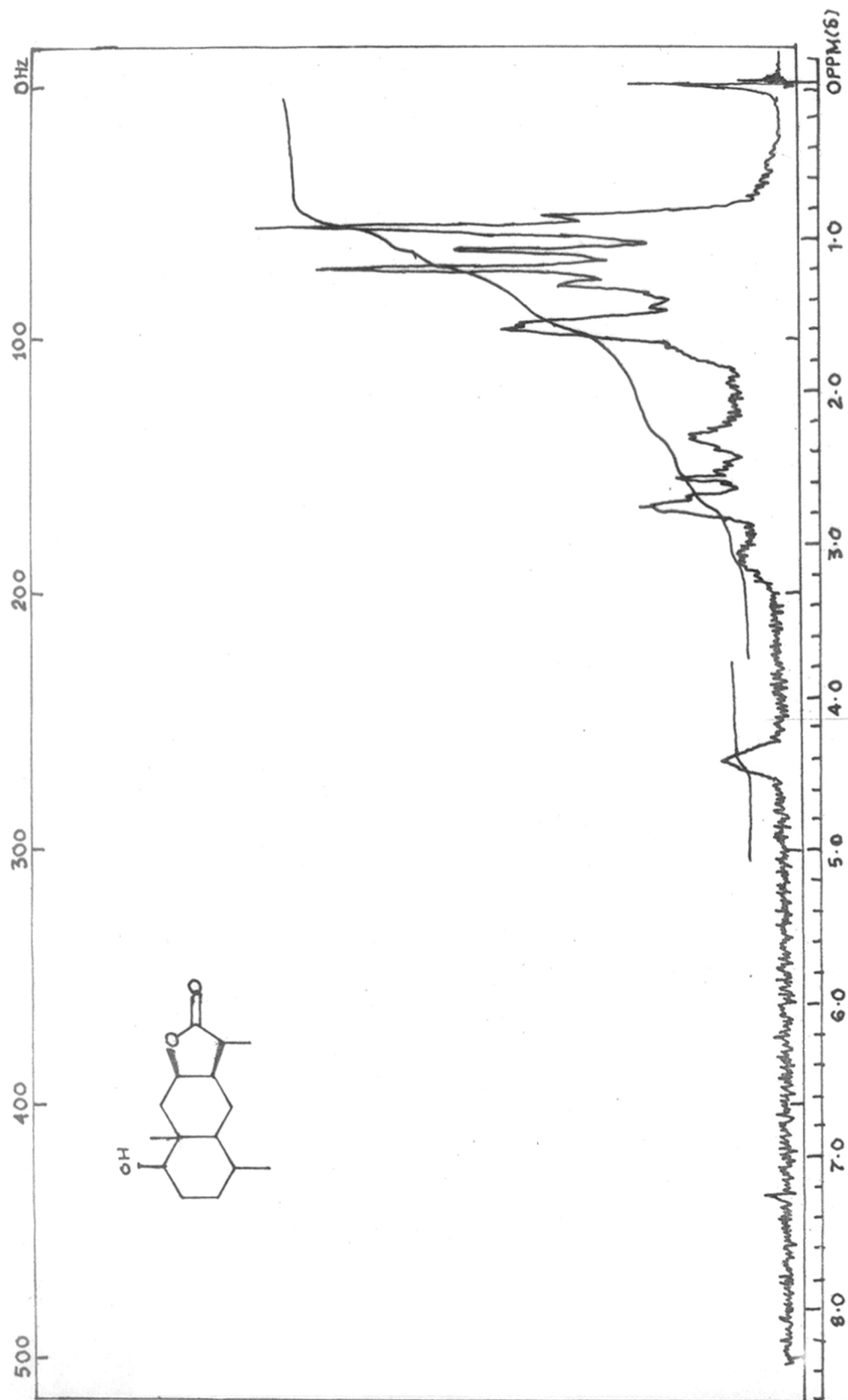


FIG.19: IR SPECTRUM OF TETRAHYDRODERIVATIVE.

FIG 20: ^1H NMR SPECTRUM OF TETRAHYDRO DERIVATIVE(IX) (60MHz)

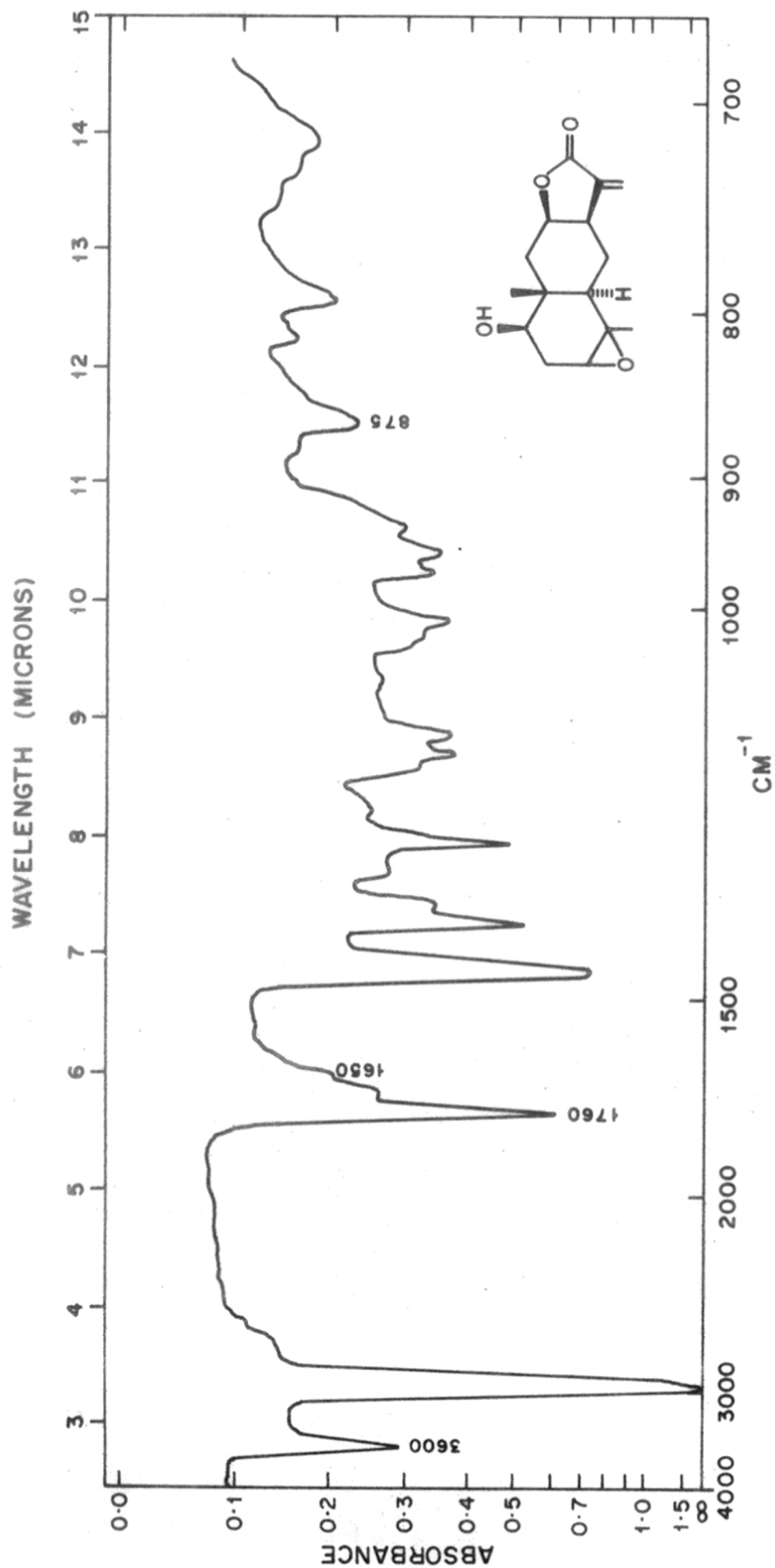
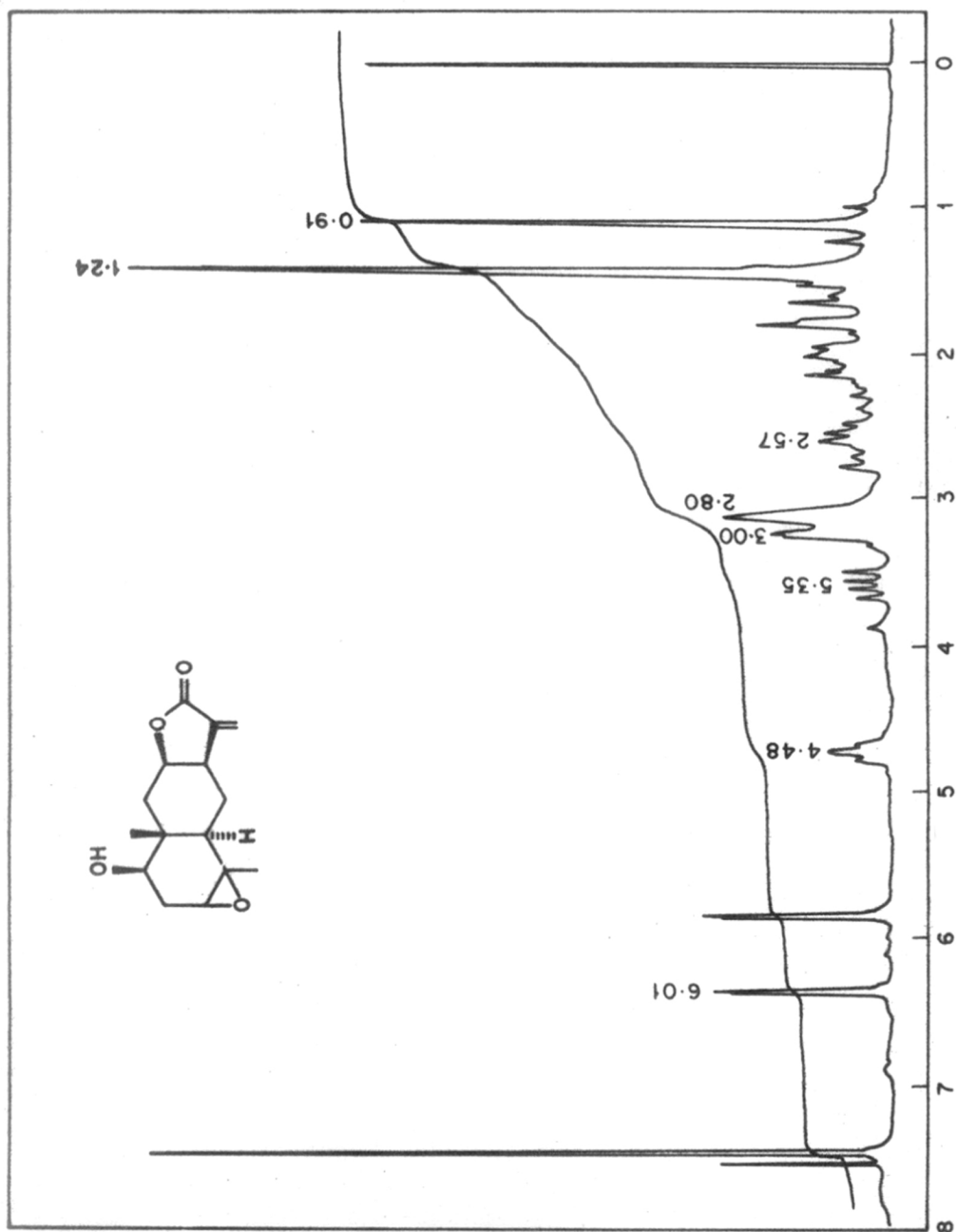


FIG. 21 : IR SPECTRUM OF EPOXIDE (XI)

FIG. 22: ^1H NMR SPECTRUM OF EPOXIDE (XI) (90MHz).

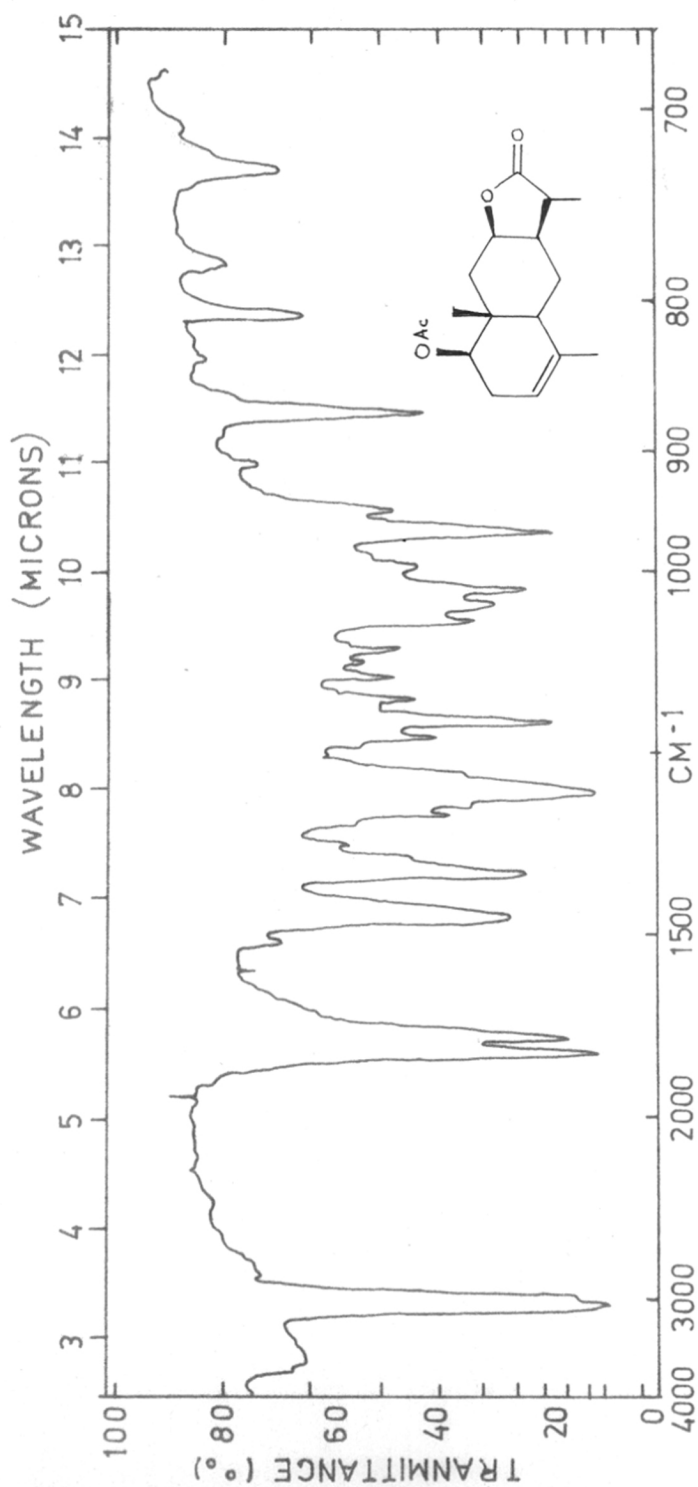


FIG 23:1 R SPECTRUM OF MONOACETATE (Va)

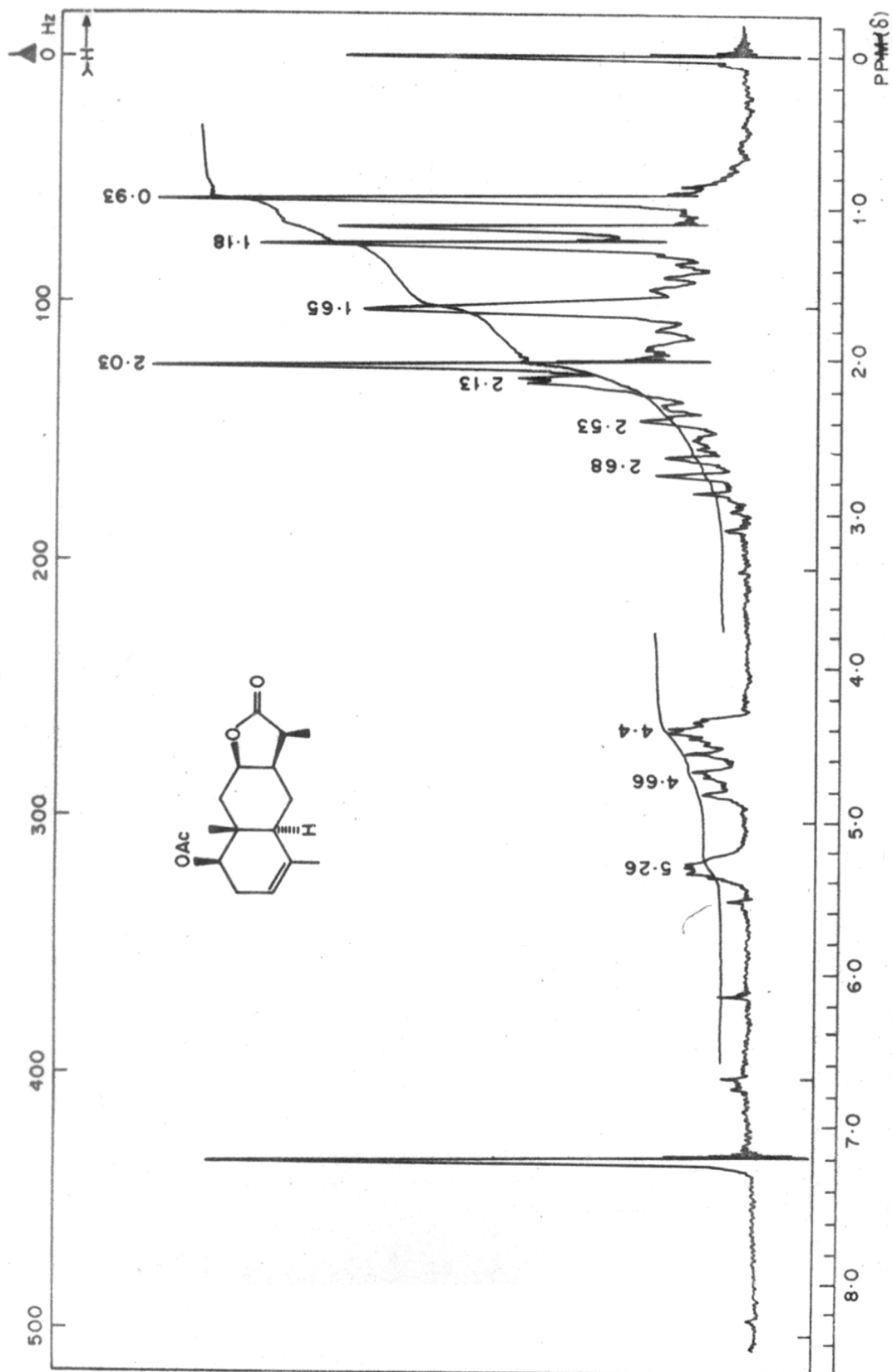


FIG. 24 : ^1H NMR SPECTRUM OF DIHYDROMONOACETATE (60 MHz).

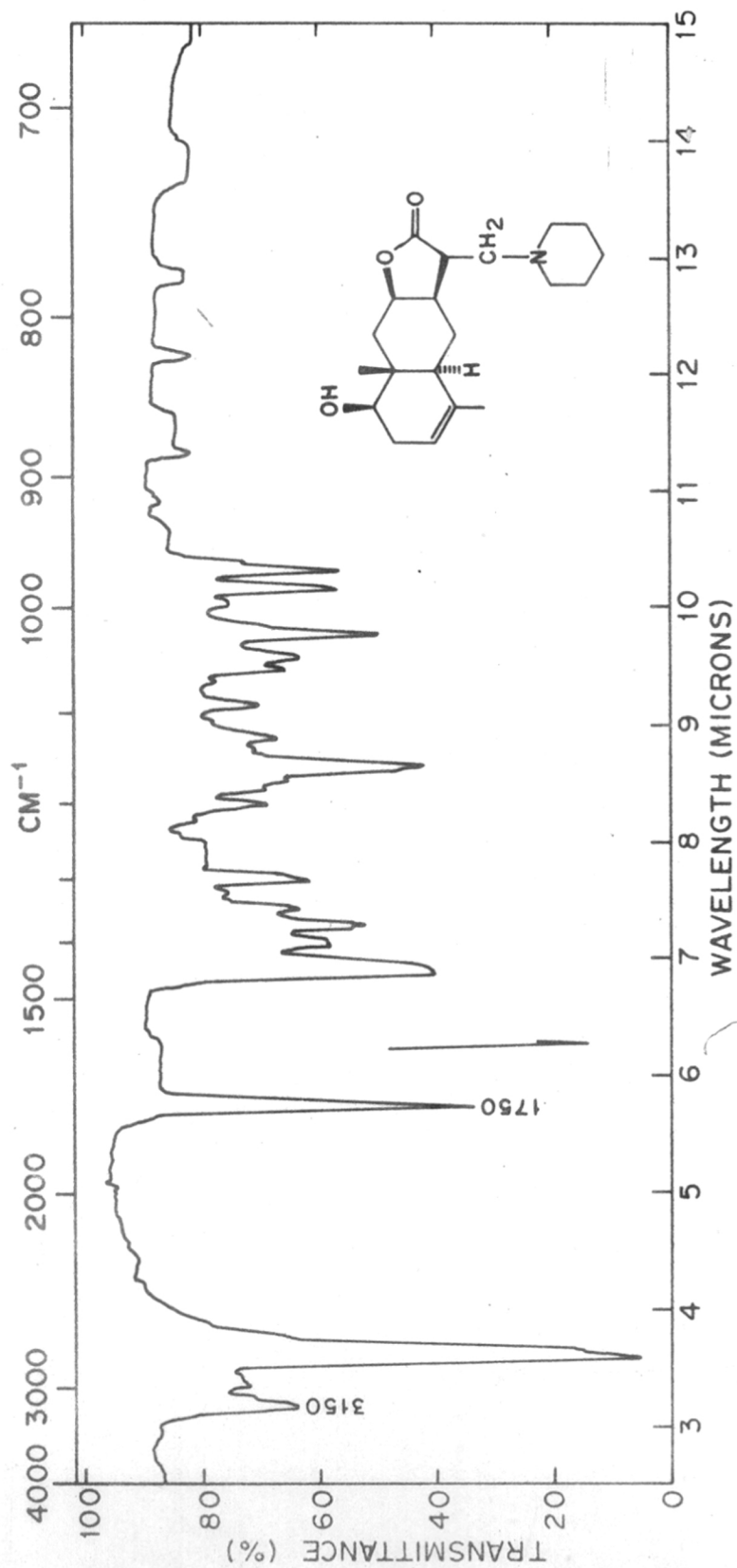
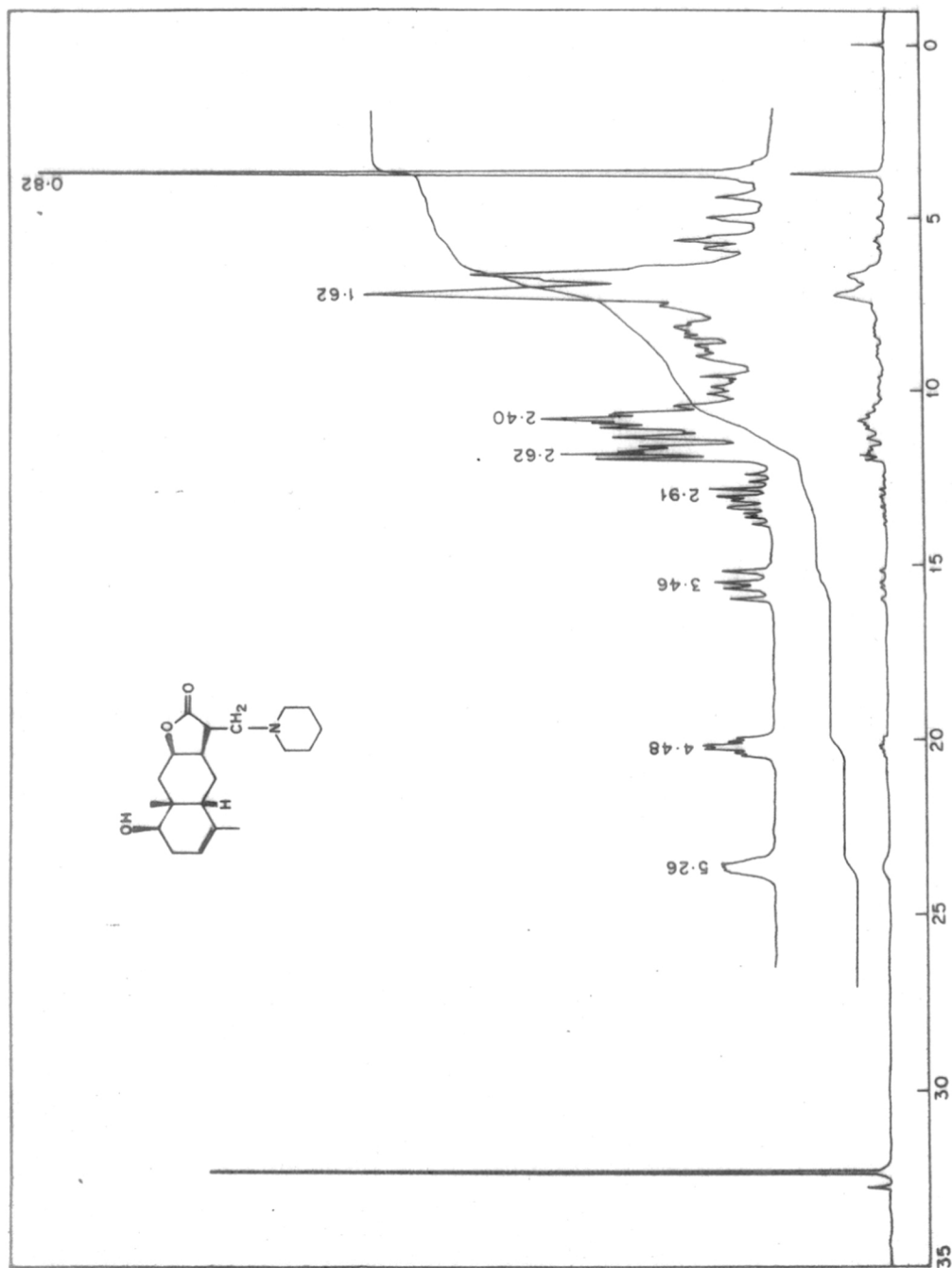


FIG. 25 : IR SPECTRUM OF PIPERIDINE ADDUCT (XIII).



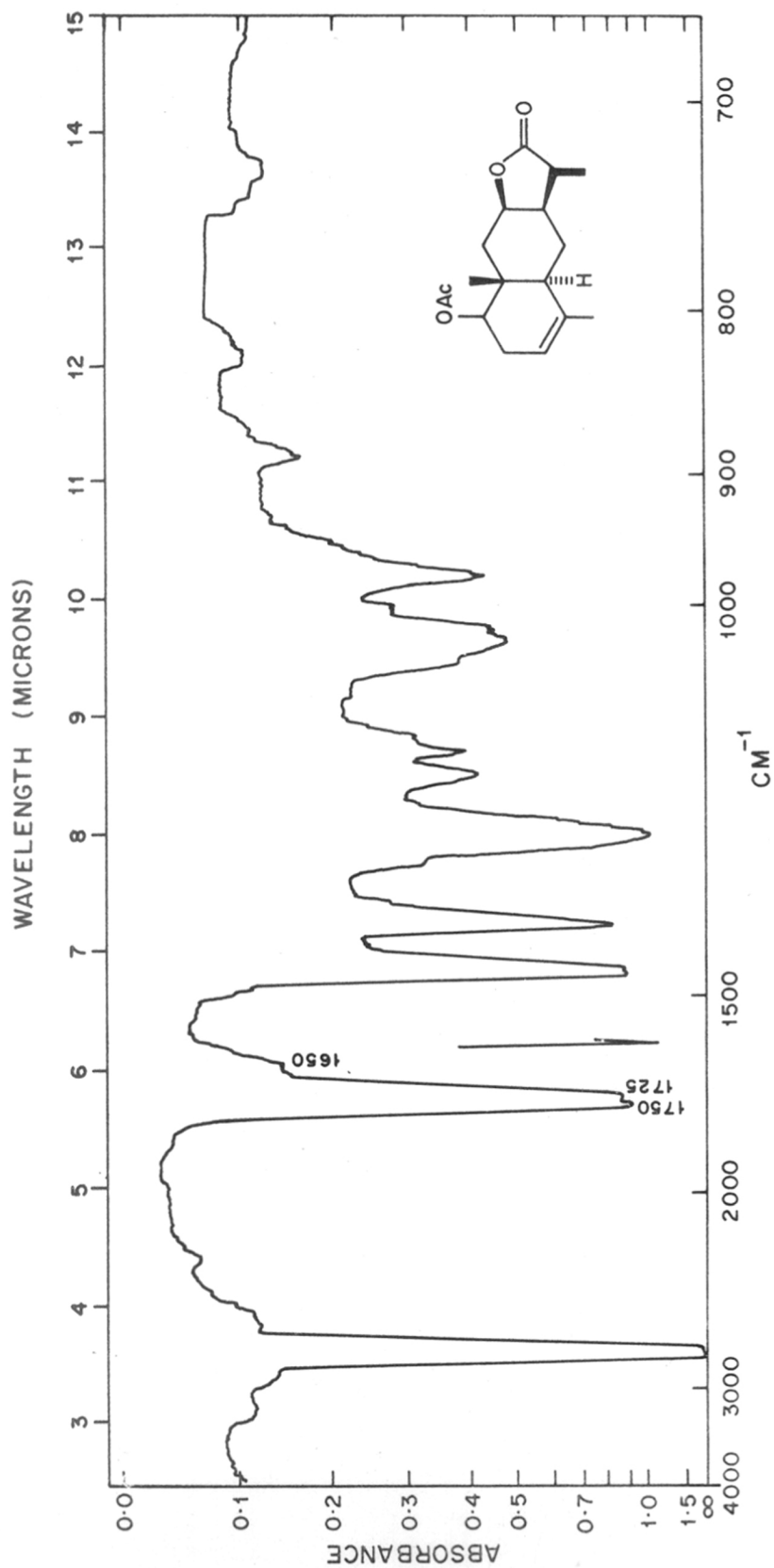


FIG. 27 : IR SPECTRUM OF ACETATE OF COMPOUND (V).

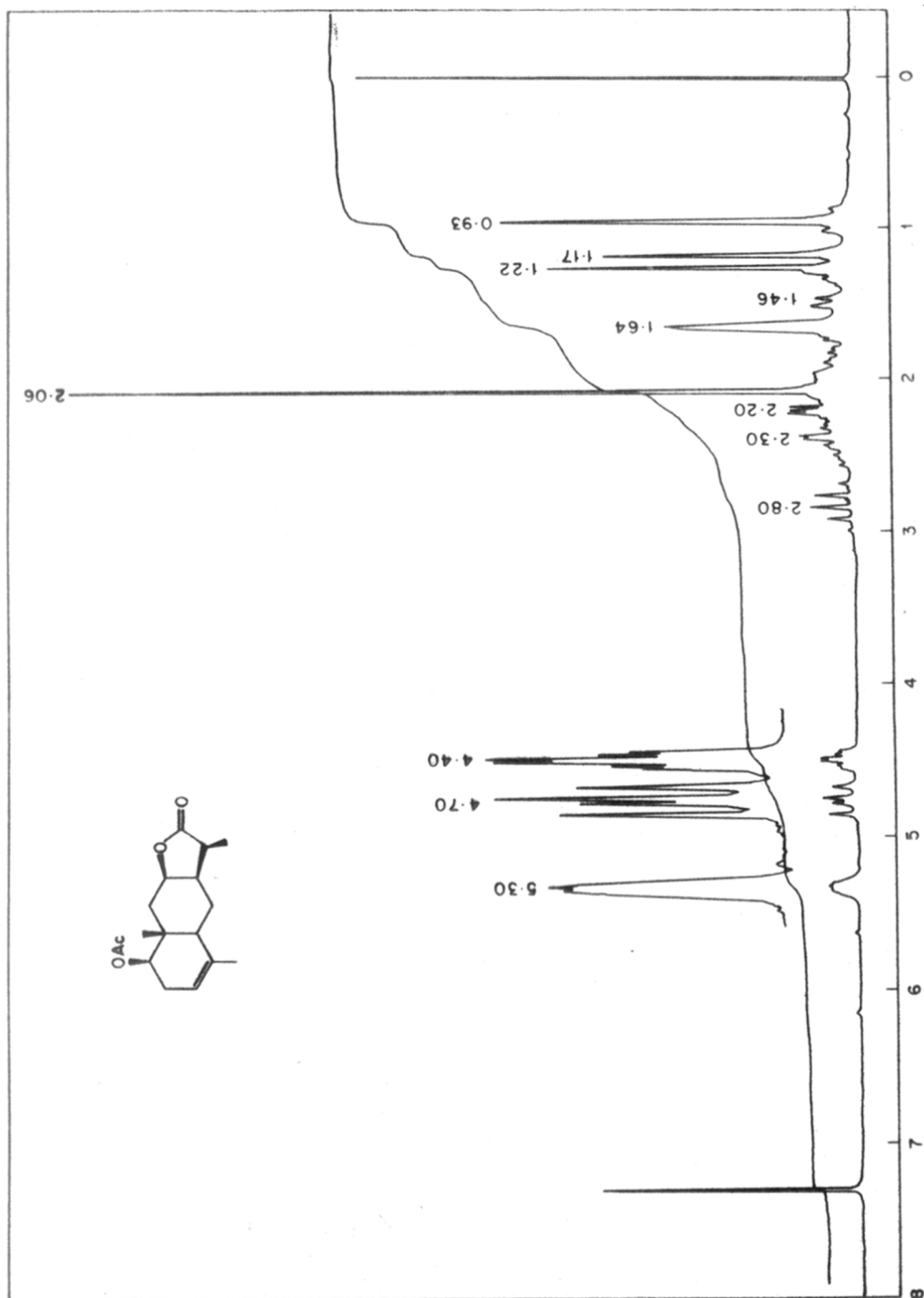


FIG. 28 : ^1H NMR SPECTRUM OF ACETATE
OF COMPOUND (V) (90 MHz)

CHAPTER III

CHEMICAL INVESTIGATION OF CYATHOCLINE

LUTEA

I N T R O D U C T I O N

Cyathocline lutea Cass, belongs to the tribe Astereae of family Compositae. It is a small herb 4-9 inches in height with a scape like stem which is very slender. Leaves are sessile, glandular, bipinnatifid, mostly radical and are forming a stellately spreading rosette. Heads are 1/8 - 1/6 inch in diameter few and arranged in a branched corymbose panicles. Flowers are bright yellow in colour¹.

The species Cyathocline lutea has not been investigated chemically. As mentioned in previous chapter so far only one species, Cyathocline lyrata of the genus Cyathocline has been investigated chemically².

P R E S E N T W O R K

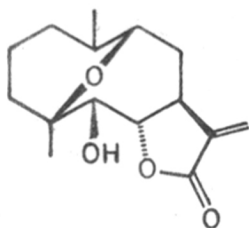
The chromatographic separation of the constituents of acetone extract of Cyathocline lutea resulted in isolation of two new sesquiterpene lactones (I) and (II) belonging to the class germacranolides. The compound (I) was a major sesquiterpene lactone obtained in 0.3% yield (based on dry weight of plant). Whereas compound (II) was obtained in 0.175% yield (based on dry weight of plant). The structure elucidation of the compounds isolated is described below.

Compound (I):

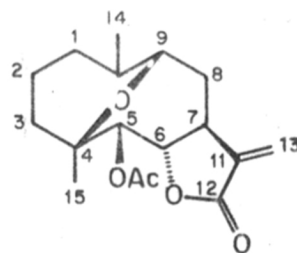
Compound (I) m.p. 136-8° (α)_D³⁰ + 53.33 (CHCl₃ C = .6) have molecular formula C₁₅H₂₂O₄ as indicated by its elemental analysis and also by the mass spectrum which showed M⁺ at m/e 266.

Presence of an hydroxyl group in the compound (I) was suggested by hydroxyl absorption band at 3300 cm^{-1} in the IR spectrum (Fig. 1) of (I), which was further confirmed by the appearance of D_2O exchangeable signal in its ^1H NMR spectrum (Table I, Fig. 2), $\text{M}-\text{H}_2\text{O}$ peak in its mass spectrum and its conversion to monoacetate (IV) (Chart I). The compound (IV) showed the acetate methyl signal in its ^1H NMR spectrum (Table I, Fig. 7) and showed bands at 1720 and 1240 cm^{-1} in its IR spectrum (Fig. 6) which did not show any hydroxyl absorption band. This indicated that compound (I) contained only one hydroxyl group. The secondary nature of the hydroxyl group was indicated by the appearance of one proton signal at 4.12 ppm in the ^1H -NMR spectrum of (I) which shifted to 5.36 ppm in the ^1H NMR spectrum of acetate (IV), as well as by appearance of a doublet at δ 74.47 ppm in the SFOD ^{13}C -NMR spectrum of (I) (Table II, Fig. 3). This was confirmed by its conversion to oxide (V) on treatment with Jones reagent.

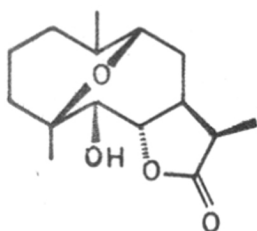
IR spectrum of (I) exhibited a carbonyl absorption band at 1760 cm^{-1} and an exomethylene absorption at 1660 cm^{-1} , which suggested the presence of an α,β -unsaturated lactone moiety in the compound (partial structure A). Appearance of two doublets ($J = 3\text{ Hz}$) at 5.41 and 6.13 ppm in the ^1H NMR spectrum of (I) as well as the signals at δ 169.42 (s) , 140.31 (s) and 118.99 (t) in the SFOD ^{13}C -NMR spectrum confirmed the presence of α -methylene γ -lactoneⁱⁿ (I). Conversion of (I)



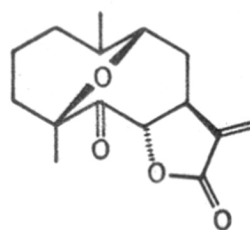
I



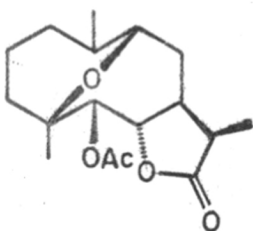
IV



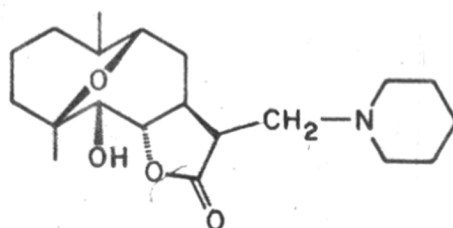
III



V



VI



VII

CHART - I

Table I ¹H NMR spectral data of compound (I) and its derivatives

	(I) ⁺	(III) ⁺⁺ (benzene)	(IV) ⁺⁺	(V) ⁺⁺	(VI) ⁺⁺	(VII) ⁺⁺
1-H	*	*	*	*	*	*
2-H	*	*	*	*	*	*
3-H	*	*	*	*	*	*
4-H	-	-	-	-	-	-
5-H	4.12 (d)	3.76 (d)	5.36 (d)	-	5.4 (d)	4.1 (d)
6-H	4.12 (t)	3.76 (t)	4.03 (t)	5.3 (d)	4.13 (t)	4.1 (t)
7-H	2.85	1.98 (m)	2.00 (m)	2.3 (m)	2.2 (m)	-
8-H	3.7* (dddd)	3.3* (dddd)	3.63 (dddd)	3.8 (m)	3.6* (dddd)	3.6 (dddd)
9-H	*	*	*	*	*	*
10-H	-	-	-	-	-	-
11-H	6.13 (d)	0.92 (d)	5.96 (d)	6.16 (d)	1.2 (d)	1.53 (m)
13-H	5.41 (d)	1.23 (s)	5.26 (d)	5.46 (d)	-	1.53 (m)
13-H	1.2 (s)	0.58 (d)	1.16 (s)	1.33 (s)	1.2 (s)	1.2 (s)
14-H	0.88 (d)	-	0.91 (d)	0.86 (d)	1.	0.86 (d)
OAc	-	-	2.06 (s)	-	2.1 (s)	-

Note: *Spectrum recorded at 90 MHz ⁺⁺Spectrum recorded at 60 MHz *signals unresolved

J (Hz): I): 5,6 = 10; 6,7 = 10; 8,8 = 3.5,6; 7,13 = 3.2; 7,13' = 3; 10,14 = 6.5;

III): 5,6 = 10; 6,7 = 9.5; 8,9 = 3.5,6; 10,14 = 6.5; 11,13 = 6

IV): 5,6 = 10; 6,7 = 10; 8,9 = 3.5,6; 7,13 = 3; 7,13' = 3; 10,14 = 6;

V): 6,7 = 10; 7,13 = 3; 7,13' = 3; 10,14 = 6;

VI): 5,6 = 10; 6,7 = 9.5; 8,9 = 3.5,6; 11,13 = 6.5; 10,14 = 6;

VII): 5,6 = 10; 6,7 = 9.5; 8,9 = 3.5,6; 11,14 = 6.5;

Table II ^{13}C NMR spectral data of compound (I)
 (CDCl_3 , TMS as internal standard, values in
 δ ppm)

Carbon No.	ppm	Carbon No.	ppm
C-1	35.13 (t) ⁺	C-9	82.66 (d) ⁺⁺⁺
C-2	30.93 (t) ⁺	C-10	26.06 (d)
C-3	38.47 (t) ⁺⁺	C-11	140.31 (s)
C-4	80.54 (s)	C-12	169.42 (s)
C-5	74.47 (d)	C-13	118.99 (t)
C-6	82.47 (d) ⁺⁺⁺	C-14	21.77 (q)
C-7	42.30 (d)	C-15	25.21 (q)
C-8	39.13 (t) ⁺⁺		

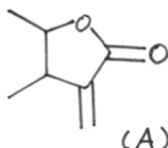
Note: 1. Spectrum recorded at 22.63 MHz

2. s = singlet, d = doublet, t = triplet,
 q = quartet

Signal multiplicity in single frequency off
 resonance decoupled (SFOD) spectrum

3. Chemical shifts with similar marks
 (i.e., +, ++, +++ etc.) are possibly interchangeable.

into its piperidine adduct (VII) (Chart I) supported the above statement.



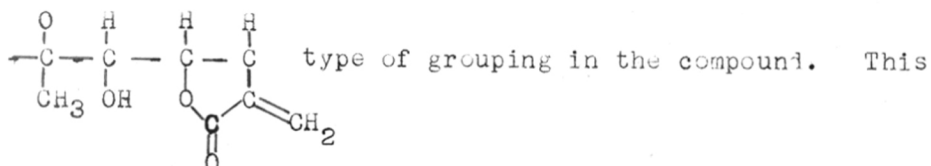
Two oxygen atoms in the lactone ring and one oxygen in the form of hydroxyl group accounted for the three out of the four oxygen atoms present in the molecule (molecular formula $C_{15}H_{22}O_4$). As the IR spectrum of the acetate (IV) did not show any hydroxyl absorption band, the remaining oxygen atom has to be present either as an epoxide or an ether bridge. The observed one proton multiplet centred at 3.71 ppm in the 1H NMR spectrum of (I) suggested that the remaining oxygen atom is present in the form of an ether bridge and not as an epoxide, as almost all the sesquiterpene lactones of known structures having epoxide rings show signals for the epoxide proton in the region 2.7 - 3.00 ppm³. This assumption was further supported by the fact that all the known methods for opening the epoxide ring e.g. treatment with (1) formic acid (2) lithium ethylamine (3) mineral acids, (4) BF_3 etherate and (5) with lithium liquid ammonia failed to open the oxirane ring. in (I). In contrast, the observed chemical shift (3.71 ppm) was in good agreement with the chemical shift expected for protons on carbon carrying ether bridge. The SFOD ^{13}C -NMR spectrum of (I) showed a doublet at δ 82.66 and a singlet at δ 80.54 ppm thus supporting

the presence of an ether bridge in the compound.

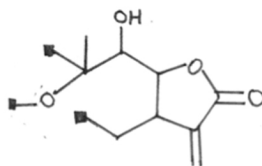
The molecular formula $C_{15}H_{22}O_4$ (M^+ at m/e 266) and appearance of one secondary methyl and one methyl on ether bridge suggested a germacranolide skeleton for the compound (I). The position of attachment of various functional groups could be deduced from the detailed spin decoupling studies. The decoupling experiments were carried out mostly with compound (I), but as the signals due to the proton on the carbon atom carrying hydroxyl group and that of the lactonic proton were overlapping, to deduce position of attachment of hydroxyl group, spindecoupling experiments were carried out with the mono-acetate (IV), the 1H -NMR spectrum of which showed very well resolved signals.

The signal of H-7 was located as a multiplet centered at 2.85 ppm, as irradiation of this signal caused collapsing of the two lactone exomethylene doublet at 5.41 and 6.13 ppm into singlets. The multiplicity of these exomethylene signals is known to be due to the allylic coupling with - H-7 proton⁴. The same irradiation also converted the double doublet at 4.12 into a doublet and also converted multiplets at 2.05 and 2.22 ppm into broad singlets. This indicated that these signals are due to the protons on carbon atoms vicinal to C-7 (i.e. they are on C-6 and C-8). In the 1H NMR spectrum of oxide (Table I) the double doublet observed at 4.12 ppm in 1H NMR spectrum

of (I), shifted down-field and appeared as a doublet at 5.3 ppm ($J = 10 \text{ Hz}$). Further more the ^1H NMR spectrum of oxide (V) also showed that the signal for methyl on ether bridge shifted to 1.33 ppm from its position at 1.2 ppm in ^1H NMR spectrum of (I). This indicated the presence of

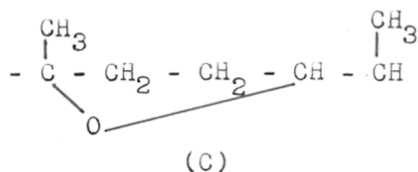


was further supported by the careful spin-decoupling studies with the acetate (IV). Irradiation of signal at 4.03 collapsed the doublet at 5.36 ($J = 10 \text{ Hz}$) into a broad singlet and also affected the multiplicity of signal centered at 2.90 (i.e. H-7). Irradiation of signal at 5.36 (signal due to proton on carbon carrying acetate group) collapsed the double doublet at 4.03 ppm into doublet. No other signal was affected by this irradiation. Based on above discussion the partial structure (A) could be extended further to partial structure (B).

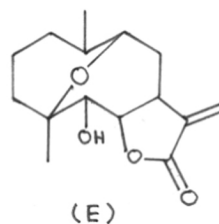
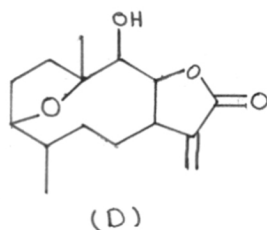


(B)

Irradiation of the signal at 3.71 affected the multiplicity of a two proton multiplet centered at 1.66 which collapsed into a doublet. Same irradiation also collapsed the one proton multiplet at 1.77 into a broad triplet which suggested the presence of a $-\text{CH}_2-\text{CH}-\text{CH}$ type of grouping in the compound. Irradiation of signal at 1.77 collapsed the three proton doublet at 0.88 into singlet and two proton multiplet at 1.66 into broad doublet. In addition to this same irradiation also affected the multiplicity of a one proton-multiplet at 3.71 ppm. Based on above discussion partial structure (C) could be written for the other part of compound (I).

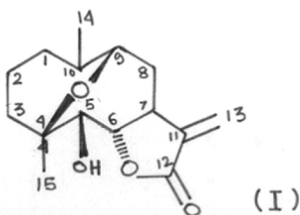


By combining the two partial structures (B) and (C), two probable structures (D) and (E) could be written for the compound (I). Proof for the correct gross structure in favour of E was obtained as follows:



The position of attachment of lactone ring in compound (I) could be deduced by the use of its CD spectrum based on the sign of the cotton effect and the stereochemistry of lactone ring, the position of attachment of lactone ring could be determined from the stocklin-Waddell-Giessman rule⁵ (please see Chapter I page No.). Compound (I) showed a -ive cotton-effect and the lactone ring in transfused as indicated by the large coupling ($J = 10 \text{ Hz}$) between the lactonic proton and H-7. Thus according to the Stocklin-Waddell-Giessman rule compound (I) should be a trans 6,12 olide and hence (E) represents the correct structure of compound (I).

The stereochemistry of compound (I) at various positions was proposed on the basis of coupling constants in its ¹H-NMR spectrum. The hydrogen atom at C-7 (i.e. H-7) had to be α -oriented as is known for all naturally occurring sesquiterpene lactones from higher plants. A large coupling constant (10 Hz) between H-7 and H-6 required that the latter is β -oriented and thus the lactone ring is transfused to C-6. Similarly the large value of J_{5-6} (10 Hz) indicated that H-5 is α -oriented and thus the hydroxyl is β -oriented. Thus the structure and stereochemistry of the major sesquiterpene lactone from Cyathocline lutea can be depicted as in (I).

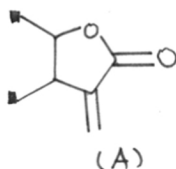


Compound (II):

The compound (II) a thick oil, $(\alpha)_D^{30} + 75.66$ (CHCl_3 $c = 0.7$) analyzed for $\text{C}_{15}\text{H}_{22}\text{O}_3$ (M^+ at m/e 250). The presence of an hydroxyl group in the compound was suggested by appearance of a hydroxyl absorption band at 3300 cm^{-1} in the IR spectrum of compound (II) (Fig. 14) and was confirmed by appearance of $\text{M}-\text{H}_2\text{O}$ peak in its mass spectrum. On acetylation at room temperature compound (II) gave a monoacetate (IX) (Chart II). The latter compound in its ^1H -NMR spectrum (Fig. 19, Table III) showed one acetate methyl signal. The IR spectrum of the acetate (IX) (Fig. 18) showed carbonyl absorption band at 1720 cm^{-1} and a band at 1240 cm^{-1} but did not show any hydroxyl absorption band which indicated that compound (II) contained only one hydroxyl group. Appearance of a one proton double doublet at 3.56 ppm in the ^1H -NMR spectrum of compound (II) (Table III, Fig. 15) and its down-field shift to 4.71 ppm in the ^1H NMR spectrum of acetate (IX) and compound's easy oxidation on treatment with Jones reagent indicated that the hydroxyl group is secondary.

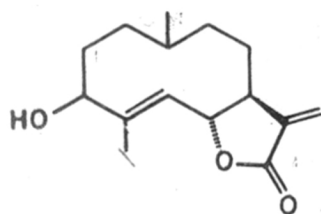
The carbonyl absorption band at 1745 cm^{-1} and a band at 1650 cm^{-1} for unsaturation in the IR spectrum of (II) (Fig. 14) suggested the presence of an α,β -unsaturated lactone function (partial structure A) in the compound. It was further confirmed by appearance of two characteristic doublets ($J = 3\text{ Hz}$) at 5.5 and 6.16 ppm in the ^1H -NMR spectrum of (II) and its easy reaction with piperidine to

from a piperidine adduct (XI) (Chart II).

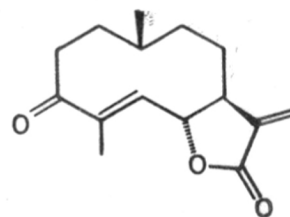


Two oxygen atoms in the lactone ring and one oxygen atom in form of hydroxyl group accounted for all the three oxygen atoms in the molecular formula of compound (II). The ^1H NMR spectrum of compound (II) (Table III, Fig. 15) showed a broad doublet at 5.17 ppm (one proton) and a doublet ($J = 1 \text{ Hz}$) at 1.76 ppm (three protons, methyl on double bond) suggested the presence of a trisubstituted double bond in the compound. The signal at 5.17 was not observed in the ^1H NMR spectrum of the hydrogenated product (X) (Table III, Fig. 21) as well as the signal at 1.76 ppm in ^1H NMR spectrum of (II) had shifted to 0.92 ppm (1, $J = 6 \text{ Hz}$) in the ^1H NMR spectrum of (X) which supported the above statement. The ^1H NMR spectrum of (II) also showed the presence of one secondary methyl group at 1.03 ppm ($J = 8 \text{ Hz}$). The molecular formula and appearance of one secondary methyl and one methyl on double bond suggested a germacranolide skeleton for (II).

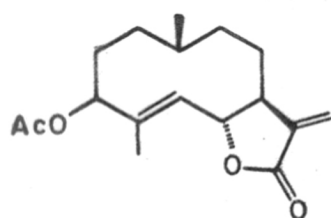
The position of attachment of various functional groups could be deduced from the extensive decoupling studies carried out with the monoacetate (IX), as the ^1H NMR spectrum of monoacetate showed well resolved signals. The signals for



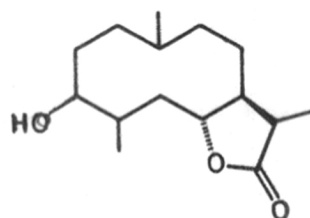
II



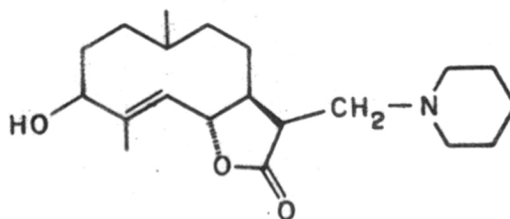
VIII



IX



X



XI

CHART II

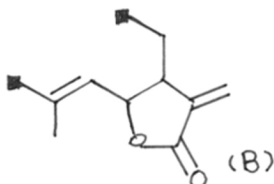
Table III ^1H NMR spectral data of compound II and its derivatives (in CDCl_3 , TMS as internal standard, chemical shifts in δ scale) (values in parenthesis are coupling constants in Hz)

	(II)	(VIII)	(IX)	(X)
H-1	*	*	*	*
H-2	*	3.17 m	*	*
H-3	3.56(dd) (10, 3 Hz)	-	4.71(dd) (10, 3)	4.02 (m)
H-4	-	-	-	*
H-5	5.13(d) (10 Hz)	5.30(d) (10 Hz)	5.17(d) (10 Hz)	*
H-6	4.53(t) (10 Hz)	4.57(t) (10 Hz)	4.55(t) (10 Hz)	4.02 (m)
H-7	2.26 (m)	2.28(m)	2.28(m)	2.3(m)
H-8)	*	*	*	*
H-9	*	*	*	*
H-10	*	*	*	*
H-11	-	-	-	*
H-13	6.46(d) (3 Hz)	6.22(d) (3 Hz)	6.22(d) $\emptyset$ (3 Hz) $\emptyset$	1.11(d) (6 Hz)
H-13'	5.5(d) (3 Hz)	5.44(d) (3 Hz)	5.57(d) $\emptyset$ (3 Hz) $\emptyset$	
H-14	1.03(d) (6 Hz)	1.03(d) (6 Hz)	0.91 (d) (6Hz)	0.91(d) (7 Hz)
H-15	1.76(d) (1 Hz)	1.62(d) (1 Hz)	1.82 (d) (1 Hz)	0.93(d) (6 Hz)
OAc	-	-	2.04(s)	

H-7 was located as a multiplet at 2.28 ppm, as irradiation of this signal caused the collapsing of the two lactone exomethylene doublets ($J = 3$ Hz) at 5.57 and 6.22 ppm in the singlets. The same irradiation also converted the double doublet centered at 4.55 ppm into a doublet and also collapsed a multiplet at 1.66 ppm into broad doublet. This indicated that these signals are due to the protons on carbon atoms vicinal to C-7 (i.e. they are on C-6 and C-8). The double doublet at 4.55 ppm could be assigned to a lactonic proton whereas the multiplet at 1.66 ppm could be assigned to a $-\text{CH}_2-$ grouping. Irradiation of double doublet at 4.55 ppm affected the multiplicity of signal at 2.28 ppm (i.e. H-7) as well as collapsed the broad doublet at 5.17 ppm into broad singlet. The broad doublet at 5.17 ppm could be assigned to vinyl proton. Irradiation of the signal at 5.17 ppm collapsed the double doublet at 4.55 ppm into doublet. The same irradiation also collapsed the doublet ($J = 1$ Hz) at 1.82 ppm (methyl on double bond) into singlet. From the above discussion it could be concluded that the lactonic proton and the vinyl proton are on the vicinal carbon atoms.

Based on biogenesis of the germacranolides the double bonds in the germacranolides are generally present at C_{1,10} and C_{4,5} positions. As the compound (II) contains only one double bond which is vicinal to lactonic proton, the double bond must be present at C_{4,5} position with a lactone ring fusion at C-6. Based on above data the partial structure

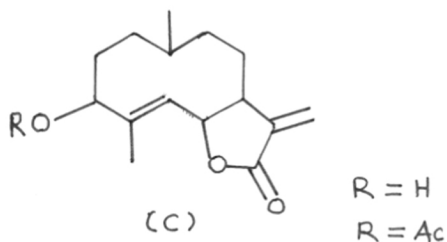
(a) now could be extended to (b).



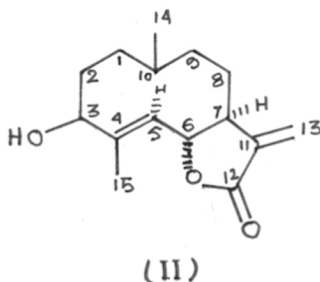
The IR spectrum of the oxide (VIII) (Fig. 16) showed a carbonyl absorption band at 1690 cm^{-1} which suggested the presence of an α,β -unsaturated ketone (in addition to α -methylene γ -lactone) in compound (VIII). This was further supported by the $^1\text{H-NMR}$ spectrum of oxide (VIII) (Table III, Fig. 17) in which the signal due to vinyl proton had shifted to 5.30 ppm from its position at 5.13 ppm in the $^1\text{H-NMR}$ spectrum of (II). The UV spectrum of the oxide (VIII) and (II) was in good agreement with above statement. Thus based on above discussion it could be concluded that the hydroxyl group in compound (II) is present at C-3. The above statement was further supported by the decoupling studies carried out with the acetate (IX). Irradiation of the doublet ($J = 1.0\text{ Hz}$) at 1.82 ppm affected the multiplicity of the four-fold doublet centered at 4.71 ppm. The signal at 4.71 ppm could be assigned to proton on carbon atom carrying acetate group. Same irradiation also converted the broad doublet at 5.17 (vinyl proton) into sharp doublet.

By combining all the above data i.e. presence of partial structure (B), hydroxyl group at C-3 position and presence of one methyl on the double bond and one secondary methyl group, following gross structure (C) could be written

for compound (II) and its acetate (IX).



The stereochemistry at various position of (II) was proposed on the basis of coupling constants in its $^1\text{H-NMR}$ spectrum. The hydrogen atom at C-7 (i.e. H-7) has to be α -oriented as is known for all naturally occurring sesquiterpene lactones from higher plants. A large coupling constant (10 Hz) between H-7 and H-6 required that the latter is β -oriented and thus the lactone ring is transfused to C-6. Similarly the large values of J_{5-6} (10 Hz) indicated that H-5 is α oriented. Thus the stereochemistry and structure of compound (II) from Cyathocline lutea can be depicted as in (II).



EXPERIMENTAL

Plant Material:

The plant Cyathocline lutea was collected from Lonavala (Maharashtra, India) during December 1979 and was shade dried.

Extraction:

The dried and coarsely powdered plant material (4 kg) was extracted with acetone (40 lit) in a soxhlet apparatus. The progress of the extraction was monitored by taking out the test sample of extract (100 ml) and evaporating it to dryness. When the residue left after evaporation of solvent from test sample weighed negligible the extraction was stopped. The solvent was recovered from the extract by distillation. Last traces of the solvent were removed under vacuum to obtain a dark gummy residue (200 g).

Chromatographic separation:

The acetone extract of Cyathocline lutea (100 g) was loaded on a column (size 100 cms long and 6 cm in diameter). Packed with silica gel (200 mesh, 3 kg). The elution was started with acetone-pet.ether mixture (20:80) with successive increase in the percentage of acetone. Fractions of 150 ml size were collected. The fractions showing similar composition on TLC plates (eluent: 30:70) were mixed together to get eight major fractions (A-H). The details of the chromatographic separation are summarized in Chart (III) and Table (IV).

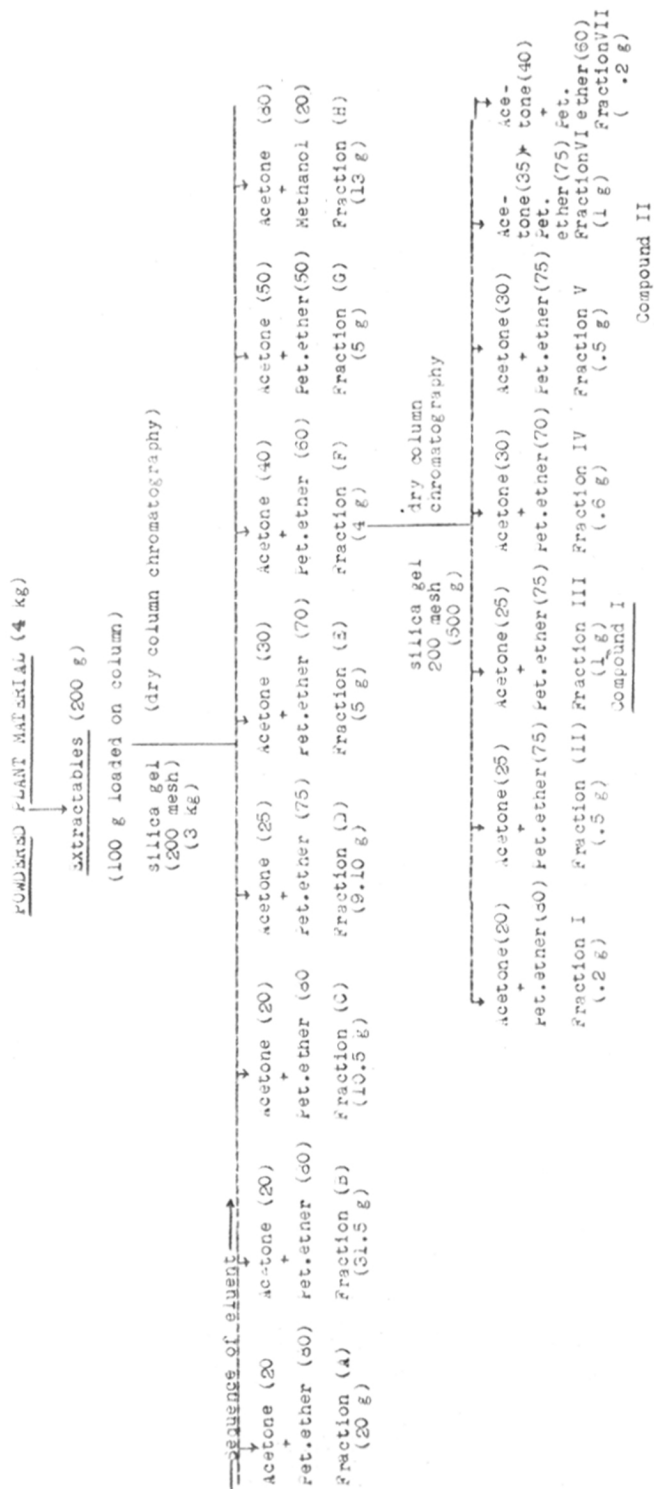


Chart III Chromatographic separation of constituents of acetone extract of Cyatococcine lutea

Table IV Chromatographic separation of acetone
extract of Cyathocline lutea

Fraction No.	Eluent	Volume collected	Total weight (in gms)	Approximate composition
(A)	Acetone (20) + Pet.ether (80)	150 ml x 3	20 g	straight chain hydrocarbons
(B)	Acetone (20) + Pet.ether (80)	150 ml x 8	31.5 g	straight chain hydrocarbon and other unidenti- fied compounds
(C)	Acetone (20) + Pet.ether (80)	150 ml x 4	10.5 g	sterol + uniden- tified compounds
(D)	Acetone (25) + Pet.ether (75)	150 ml x 6	9.0 g	sterol + compound (I)
(E)	Acetone (30) + Pet.ether (70)	150 ml x 2	5.3 g	impure compound (I)
(F)	Acetone (40) + Pet.ether (60)	150 ml x 12	4 g	compound I + compound II + unidentified compound
(G)	Acetone (50) + Pet.ether (50)	150 ml x 10	5.0 g	impure compound (II)
(H)	Acetone (80) + Methanol (20)	150 ml x 13	13 g	plant acids and sugar

Rechromatography of fraction F:

Fraction F on TLC examination showed that it was a mixture of atleast three compounds, compound (I), compound (II) and some unidentified compound having very close R_f value. To isolate compound (I) and (II), fraction (F) (4 gms) was rechromatographed on silica gel (200 mesh, 200 g) using acetone:pet.ether mixture (20:80) as eluent with successive increase in percentage of acetone. Seven major fractions were obtained as shown in Table V. Compound (I) was obtained in pure crystalline form from fraction (III) (1g) whereas fraction (VI) afforded compound (II).

Compound I:

The compound(I) was obtained as a crystalline solid from fraction (III) (Table V). The solid obtained was recrystallized from acetone:pet.ether mixture (70:30) to get colourless crystalline solid m.p.136-8°.

$(\alpha)_D^{30}$: + 53.33 (CHCl₃ C = 0.6)

UV: end absorption CD: (MeCN) ϵ 258 - 0.92.

IR (Fig. 1) $\gamma_{\text{max}}^{\text{nujol}}$ cm⁻¹ 3300 (OH), 1760 (γ -lactone) 1660 (unsaturation).

Mass: m/e (rel.int.) 266.152 (M⁺, 7) (C₁₅H₂₂O₄) 248 (M-H₂O, 7) 230 (248-H₂O, 7) 55 (100)

Compound (II):

Compound (II) (1 g) obtained from fraction (VI), (Table V) was further purified by its preparative TLC on

Table V Chromatographic separation of
Fraction F

Fraction No.	Eluent	Volume collected	Total weight (in g)	Approximate composition
(I)	Acetone (20) + Pet.ether (80)	50 ml x 2	0.2 g	straight chain hydrocarbon
(II)	Acetone (25) + Pet.ether (75)	50 ml x 2	0.5 g	straight chain compound
(III)	Acetone (25) + Pet.ether (75)	50 ml x 5	1.0 g	compound (I)
(IV)	Acetone (30) + Pet.ether (70)	50 ml x 5	0.6 g	compound (I) + more polar impurity
(V)	Acetone (30) + Pet.ether (70)	50 ml x 4	0.5 g	impure compound II
(VI)	Acetone (35) + Pet.ether (65)	50 ml x 5	1.0 g	compound II
(VII)	Acetone (40) + Pet.ether (60)	50 ml x 3	.2 g	mixture of unidentified compound

silicagel (eluent: 35:65) to get pure compound (II) (700 mg) as a colourless viscous oil.

$(\alpha)_D^{30} + 75.66$ (CHCl_3 $C = 0.7$).

UV: end absorption.

IR: (Fig. 14) $\gamma_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} 3300 (OH) 1745 (γ -lactone) 1650 and 870 (unsaturation).

Mass: m/e (rel. int.) 250 (M^+ , 15) ($\text{C}_{15}\text{H}_{22}\text{O}_3$) 232 ($M-\text{H}_2\text{O}$, 7).

Chemical transformations of compound(I):

Monoacetate (IV): The compound (I) (150 mg) was dissolved in pyridine (1.5 ml) and acetic anhydride was then added slowly at room temperature. The mixture was then allowed to stand at room temperature for 12 hours and then it was worked up as usual to get the crude monoacetate (158 mg). The crude acetate was further purified by its preparative TLC (eluent: 30:70) to obtain pure mono acetate as colourless oil.

$(\alpha)_D^{30} + 26^0$ (CHCl_3 $C = 0.6$)

IR: (Fig. 6) $\gamma_{\text{max}}^{\text{nujol}}$ cm^{-1} 1780 (γ -lactone) 1725, 1240 (acetate).

Oxide (V): To the acetone (dry, 5 ml) solution of compound (I) (200 mg) Jones reagent (1 ml) was added slowly under stirring at room temperature. The mixture was stirred at room temperature for an additional hour. The usual work up of the reaction mixture gave the oxide (V) (180 mg) as crystalline solid m.p. 195-7 0 .

$(\alpha)_D^{30} + 2^0$ (CHCl_3 $C = .1$)

IR: (Fig. 8) $\gamma_{\text{max.}}^{\text{nujol}}$ cm^{-1} 1780 (γ -lactone) 1740 (ketone)
1660 and 885 (unsaturation).

Dihydro derivative (III): Compound (I) (200 mg) was hydrogenated in ethanol solution at room temperature/pressure using 10% Pd/C as catalyst. The usual work-up of the reaction mixture gave crude dihydro compound (205 mg). The crude dihydro compound was then crystalized from benzene:pet. ether (1:1) mixture to obtain dihydro derivative as a colourless crystalline solid, m.p. $65-8^{\circ}$.

IR: (Fig. 4) $\gamma_{\text{Max}}^{\text{nujol}}$ cm^{-1} 3450 (OH) 1780 (γ -lactone).

Dihydromonoacetate (VI): The dihydro derivative of compound (I) (50 mg) was dissolved in pyridine (0.5 ml) and to it acetic anhydride (0.5 ml) was added at room temperature. The mixture was allowed to stand at room temperature for 12 hours and worked up as usual to get a dihydromonoacetate (45 mg).

IR: (Fig. 10) $\gamma_{\text{max}}^{\text{nujol}}$ cm^{-1} 1780 (γ -lactone) 1725
1240 (acetate).

Treatment of compound (I) with lithium ethylamine:

To a stirred solution of compound (I) (100 mg) in ethylamine (5 ml) freshly cut lithium was added (0.1 g) at 10°C . The mixture became deep blue (20 minutes). The mixture was stirred for additional fifteen minutes and ethylamine was then allowed to evaporate. The usual work up of the reaction mixture gave the product (20 mg). The spectral data of the compound showed that the required oxiran ring opened product

was not obtained.

Treatment of compound (I) with BF_3 etherate:

To a stirred solution of (I) (100 mg) in benzene (dry) (25 ml) BF_3 etherate (.5 ml) was added at room temperature and mixture was stirred for one hour at room temperature. The reaction mixture was worked up as usual. TLC examination of the product showed that compound (I) did not react with BF_3 etherate.

Treatment of compound (I) with formic acid:

A mixture of compound (100 mg) and formic acid (1 ml) was refluxed on water-bath for two hours. The reaction mixture was then worked up as usual to get the product.

The required oxirane ring opened product was not obtained as revealed by the ^1H NMR spectrum of the product.

Treatment of compound (I) with lithium liquid ammonia:

To a stirred liquid ammonia (50 ml) in three necked round bottom flask fitted with a dropping funnel, freshly cut lithium (250 mg) was added slowly (35 minutes). To the above stirred lithium liquid ammonia solution compound (I) (100 mg) dissolved in dry THF was added slowly and mixture was stirred for additional 1/2 hour. Ammonium chloride (0.5 g) was then added to reaction mixture and ammonia was allowed to evaporate. The reaction mixture was diluted with water and extracted with ether (3 x 20 ml). The ether extract was washed with water, dried over anhydrous sodium sulphate and

solvent removed to get the product.

$^1\text{H-NMR}$ spectrum of the product showed that the oxiran ring was intact.

Chemical transformations of compound (I):

Oxide (VIII): To an acetone solution (dry, 10 ml) of compound (II) (80 mg) Jones reagent (0.03 ml) was added slowly under stirring, at room temperature. The stirring was continued for additional 1/2 hour and then the reaction mixture was worked up as usual, to get crude oxide which was then purified by its preparative TLC (eluent: 30:70) to get pure oxide as colourless crystalline solid m.p.

$(\alpha)_D^{30} + 90^\circ$ (CHCl_3 $c = 0.1$)

IR: (Fig. 16) $\gamma_{\text{max}}^{\text{nujol}}$ cm^{-1} 1750 (γ -lactone), 1690 (ketone)
1650 (unsaturation).

Mass: m/e (rel.inte.) 248 (M^+ , 20) 232 (20) 181 (12), 151 (75)
and 55 (100).

Monoacetate (IX): To a pyridine (2 ml) solution of compound (II) (80 mg) acetic anhydride (0.8 ml) was added at room temperature. Mixture was allowed to stand at room temperature for 12 hours and was then worked up as usual to get crude monoacetate (65 mg). The acetate was crystallized from ethanol to get colourless crystalline solid m.p. 129-31 $^\circ$.

IR: (Fig. 18) $\gamma_{\text{max}}^{\text{nujol}}$ cm^{-1} 1750 (γ -lactone), 1720 and
1240 (acetate) 1650 (unsaturation).

R E F E R E N C E S

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FIG. 1 : IR SPECTRUM OF COMPOUND. (I)

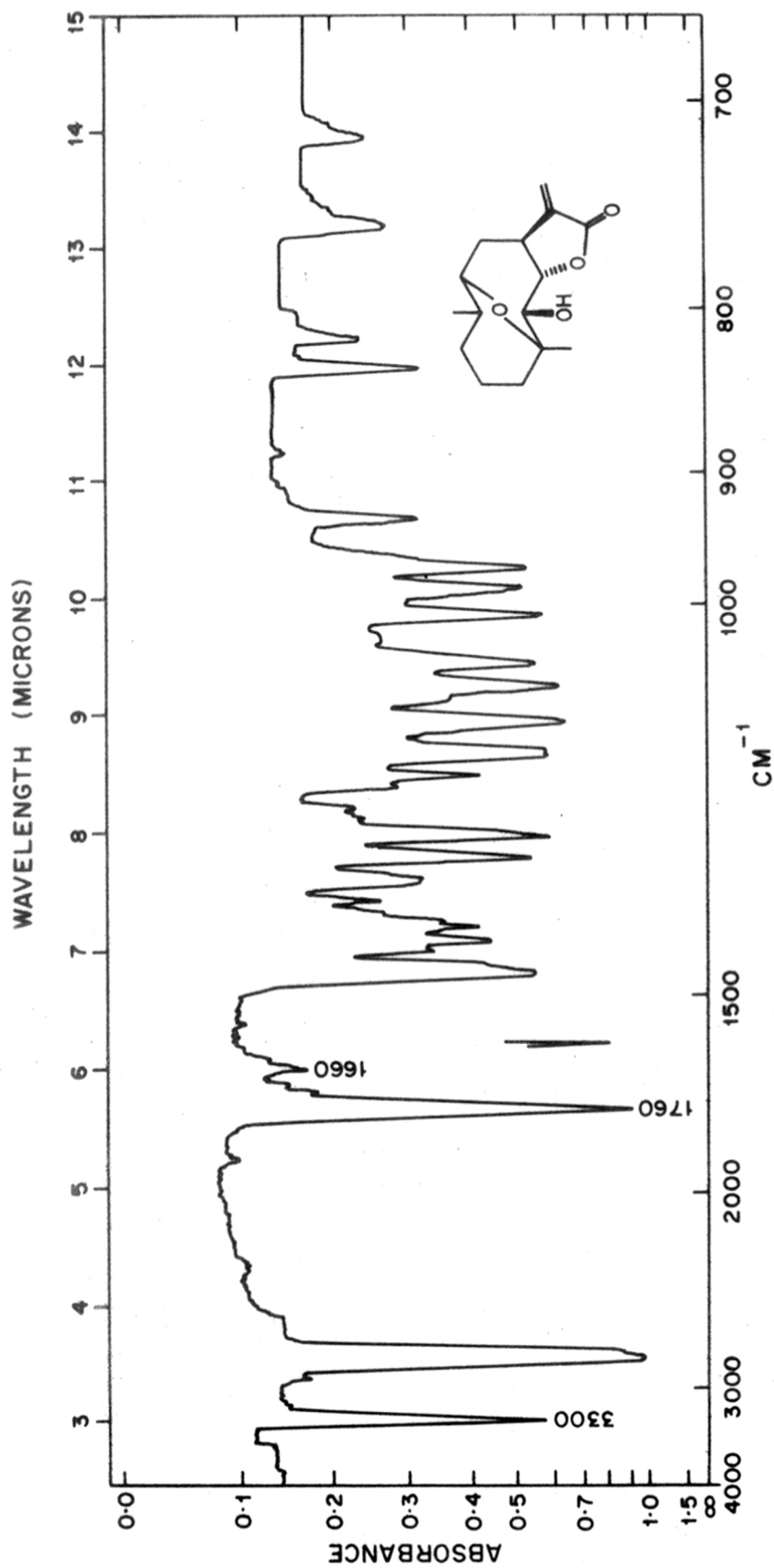
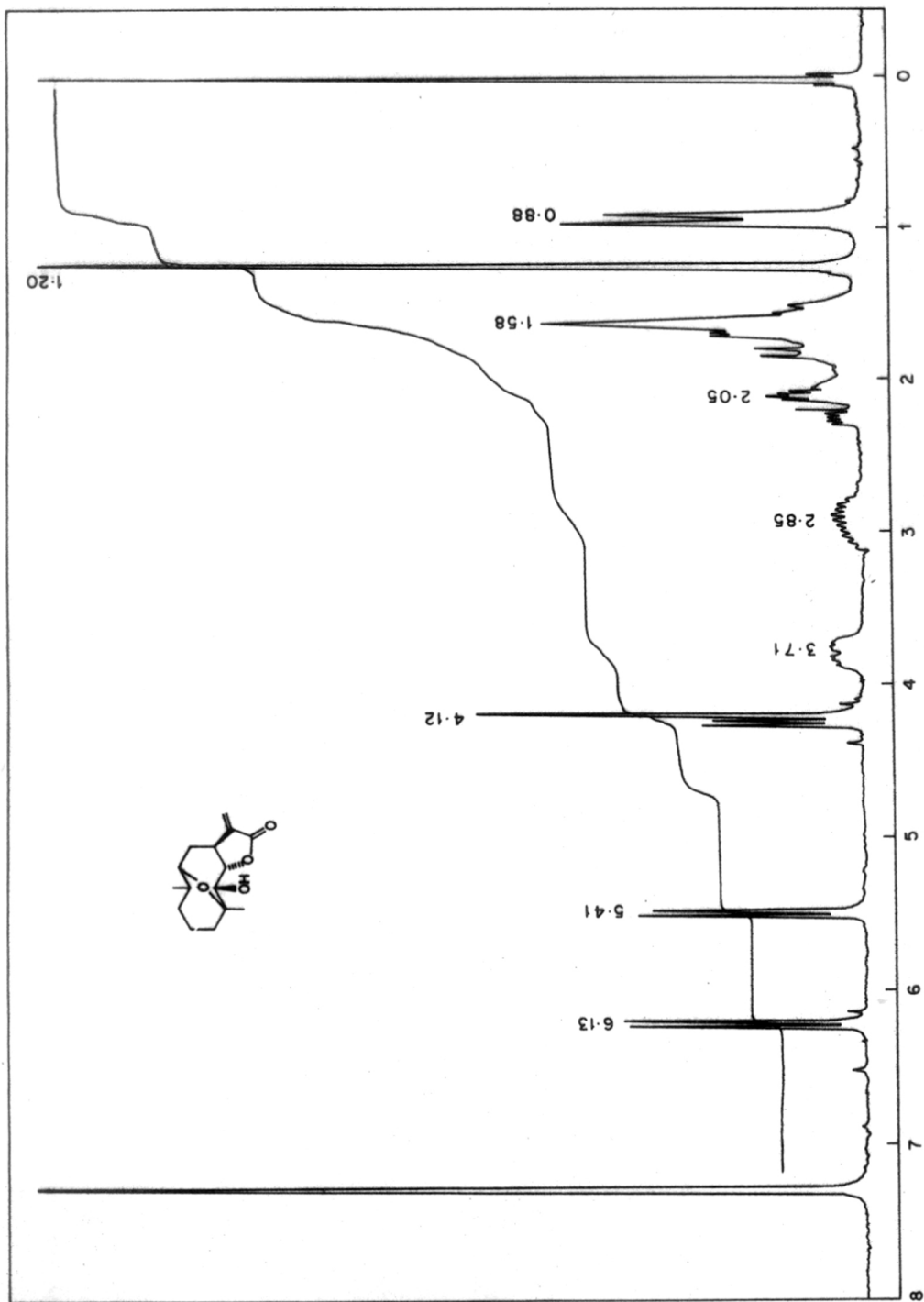
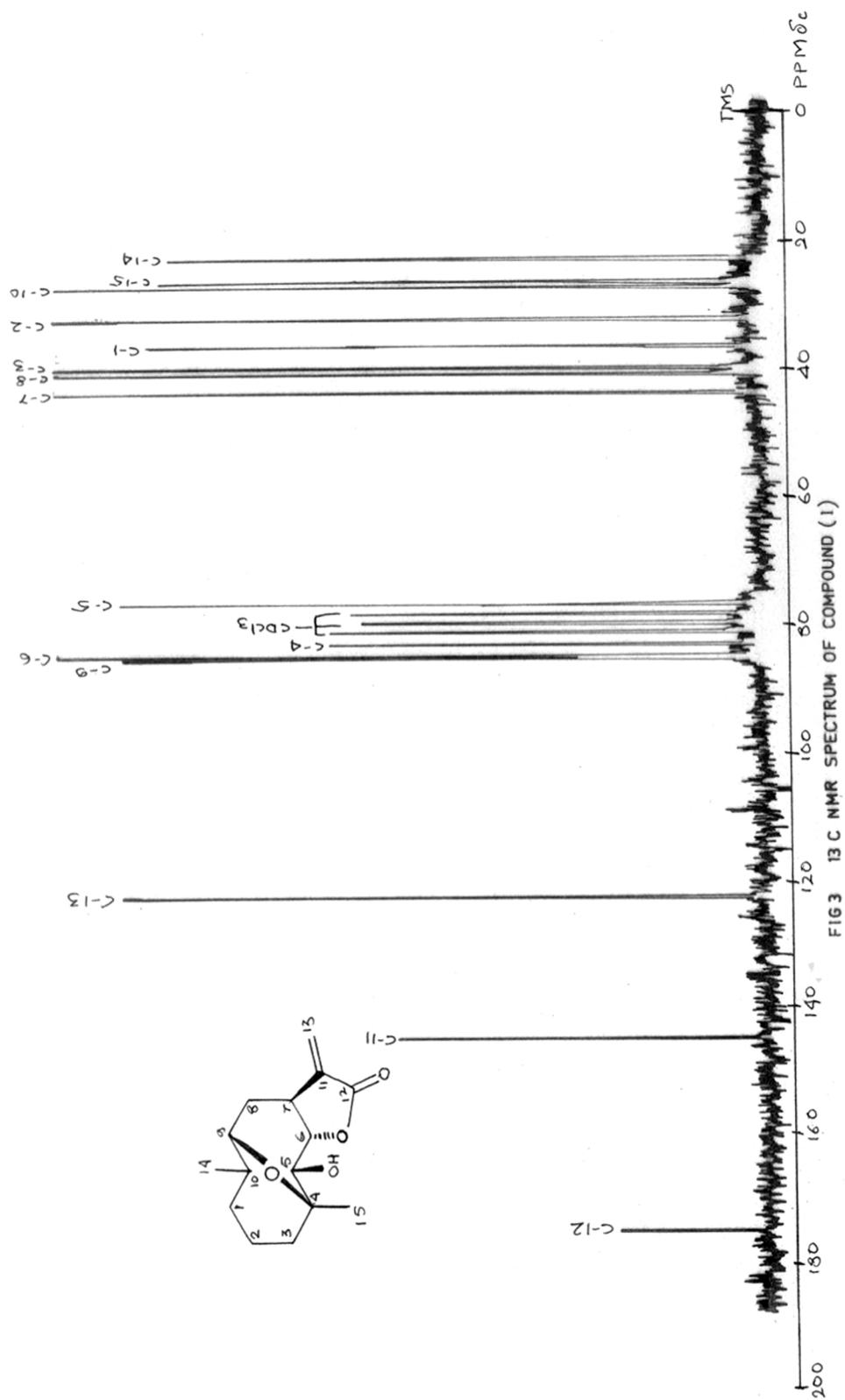


FIG. 2 : ¹H NMR SPECTRUM OF COMPOUND (I) (90 MHz).



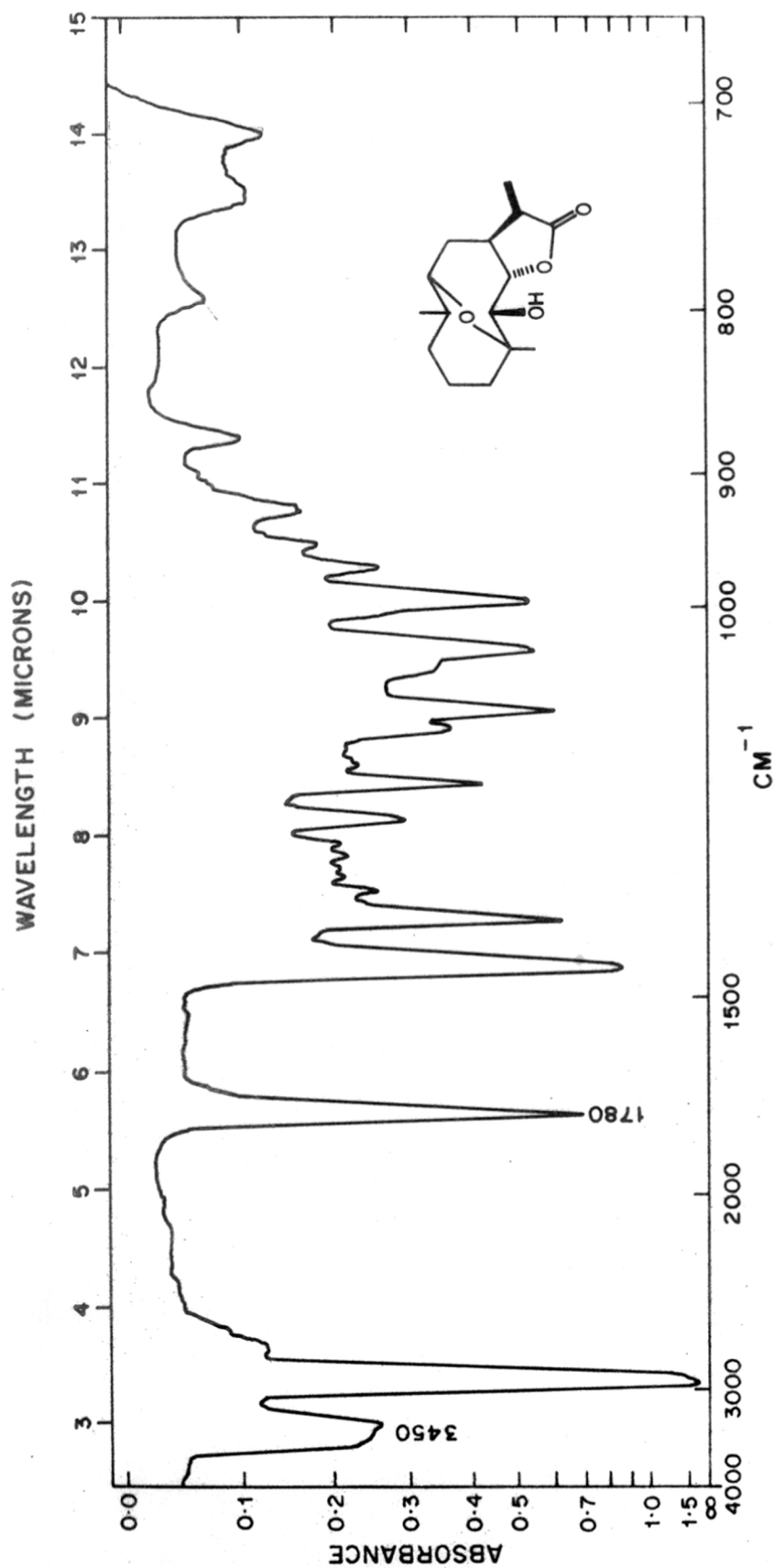
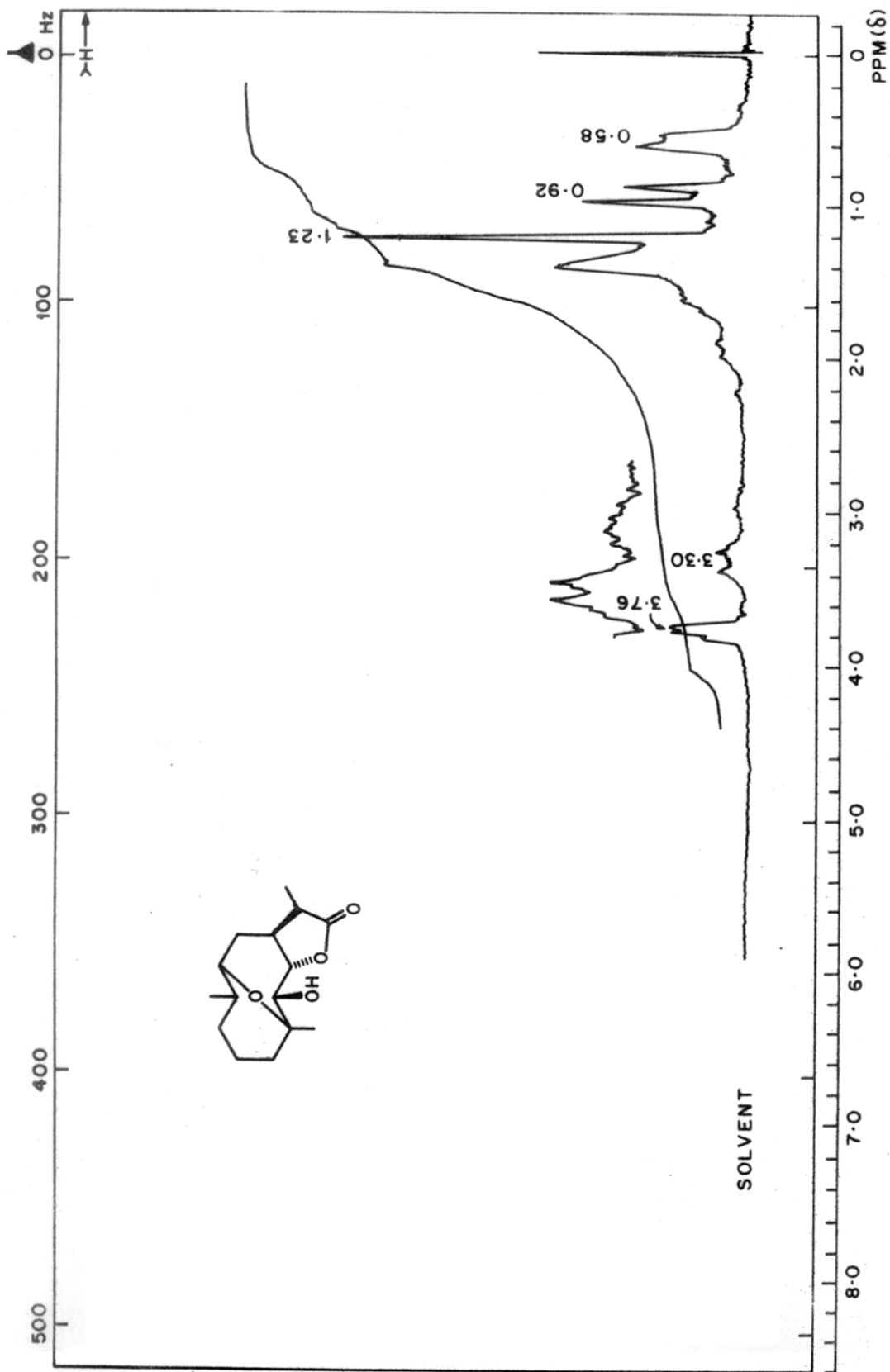


FIG. 4 : IR SPECTRUM OF DIHYDRODERIVATIVE. (III)

FIG. 5 : ^1H NMR SPECTRUM OF DIHYDRODERIVATIVE.(III) (60MHz)

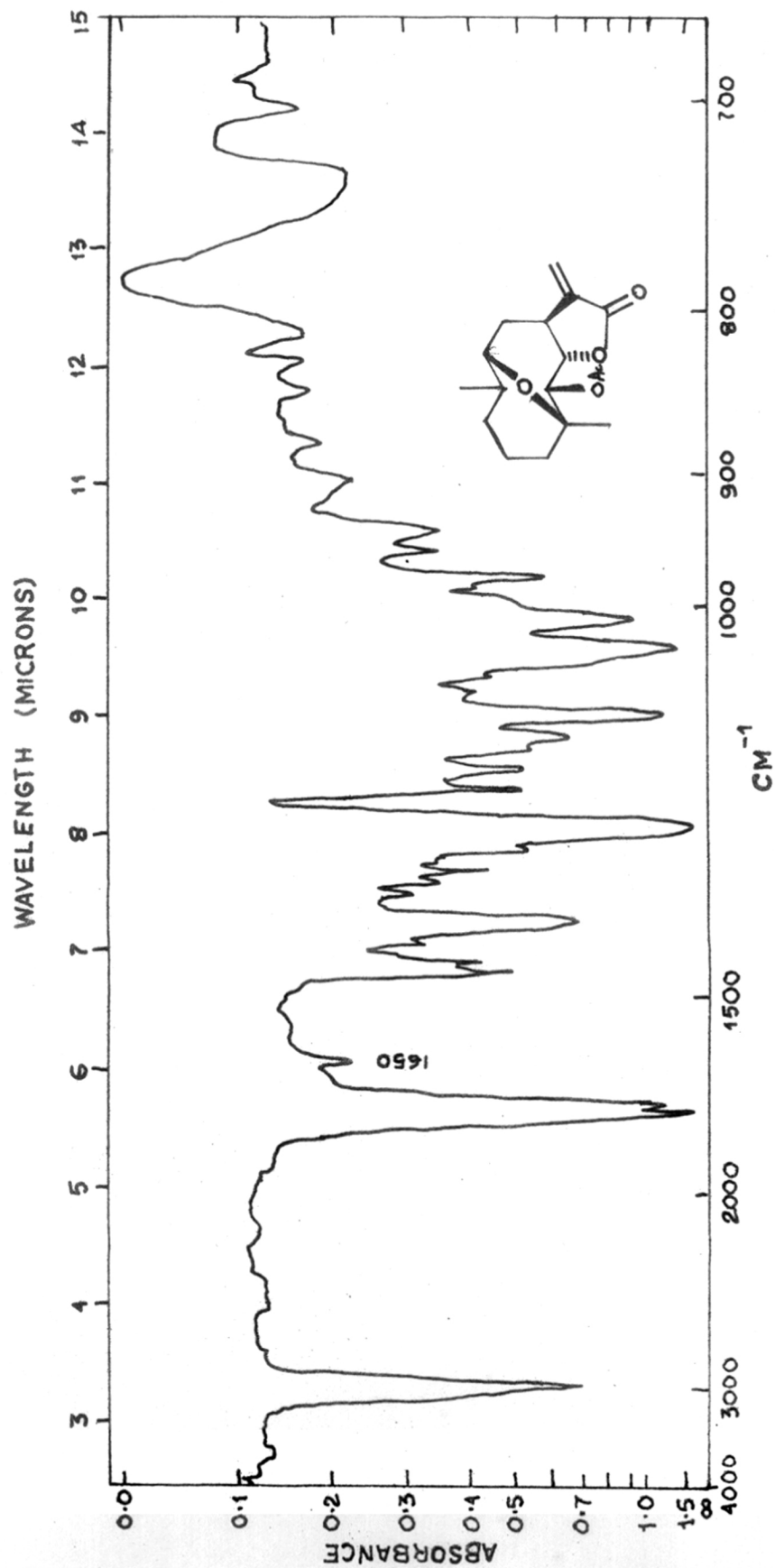


FIG 6. : IR SPECTRUM OF ACETATE.

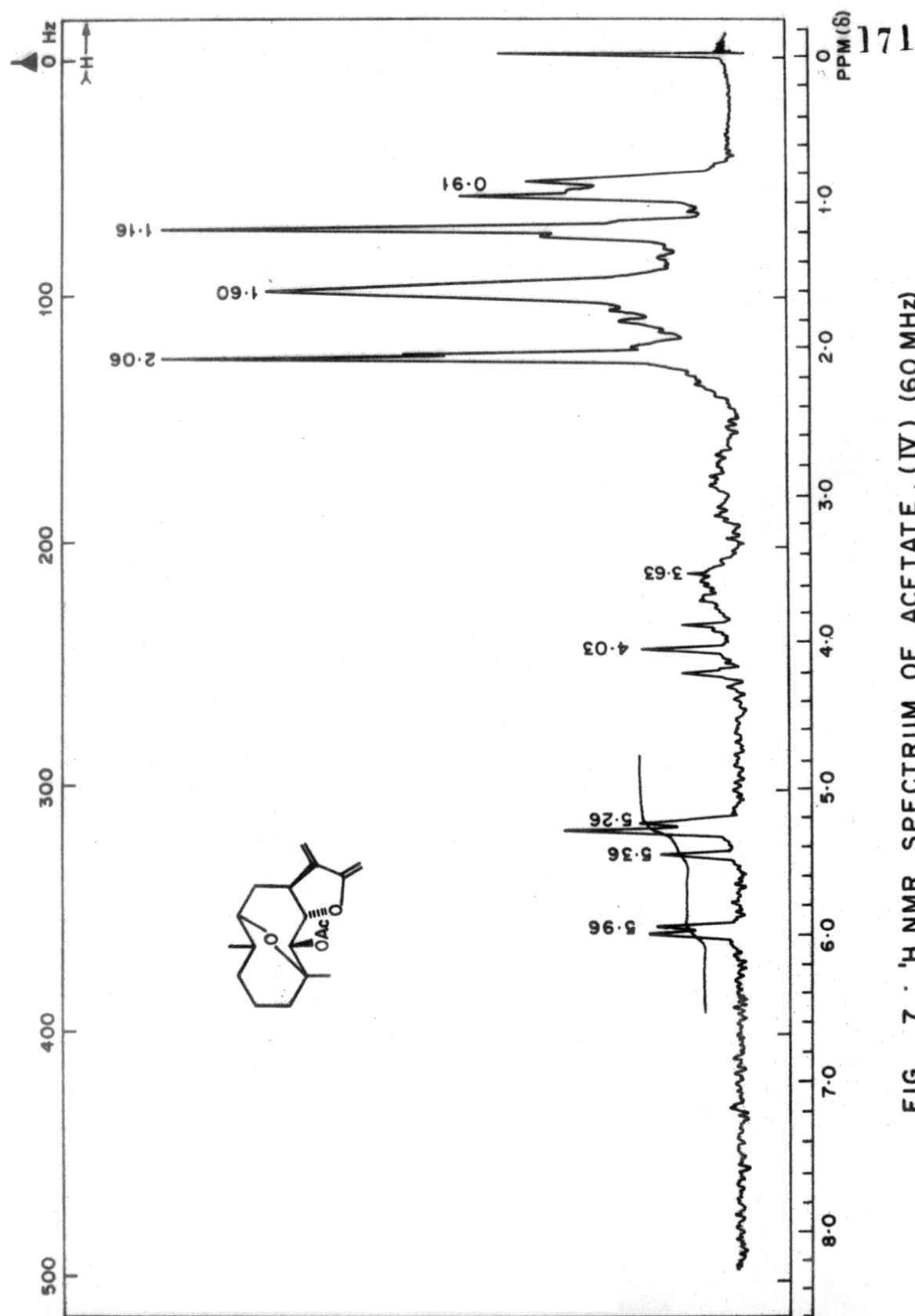


FIG. 7 : ${}^1\text{H}$ NMR SPECTRUM OF ACETATE . (IV) (60 MHz)

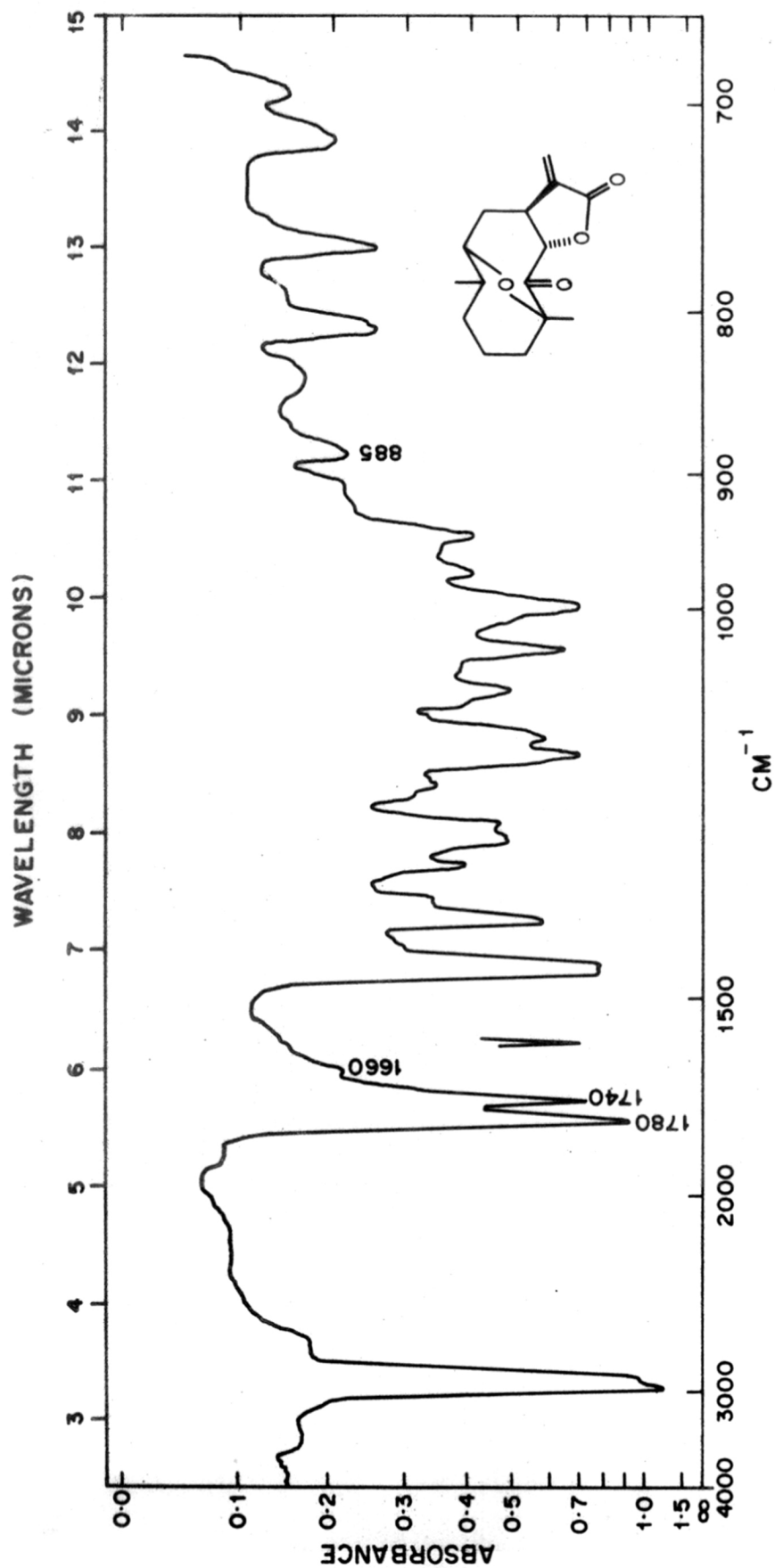
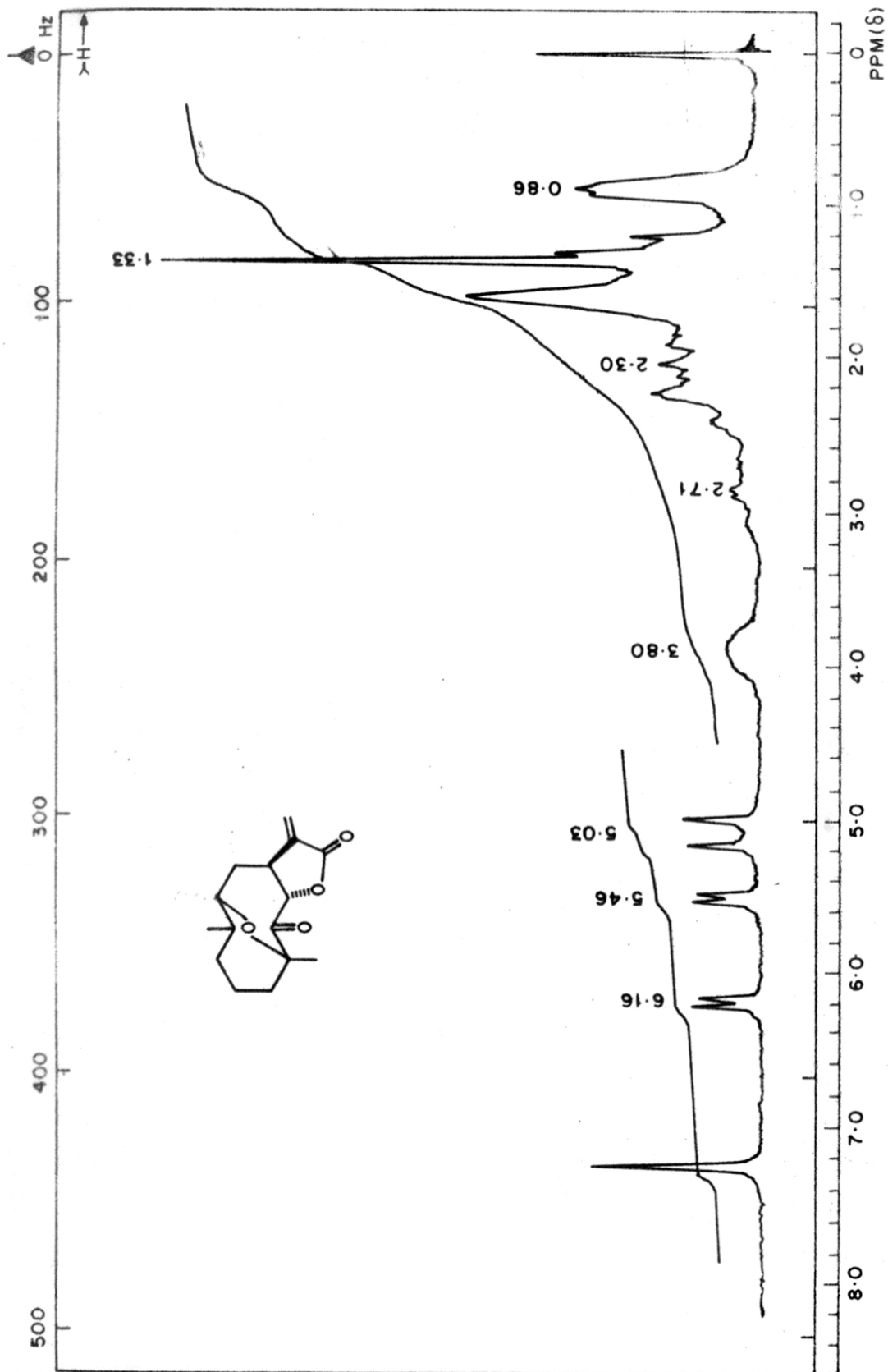


FIG. 8 : IR SPECTRUM OF OXIDE . (V)

FIG. 9 : ^1H NMR SPECTRUM OF OXIDE . (V) (60 MHz)

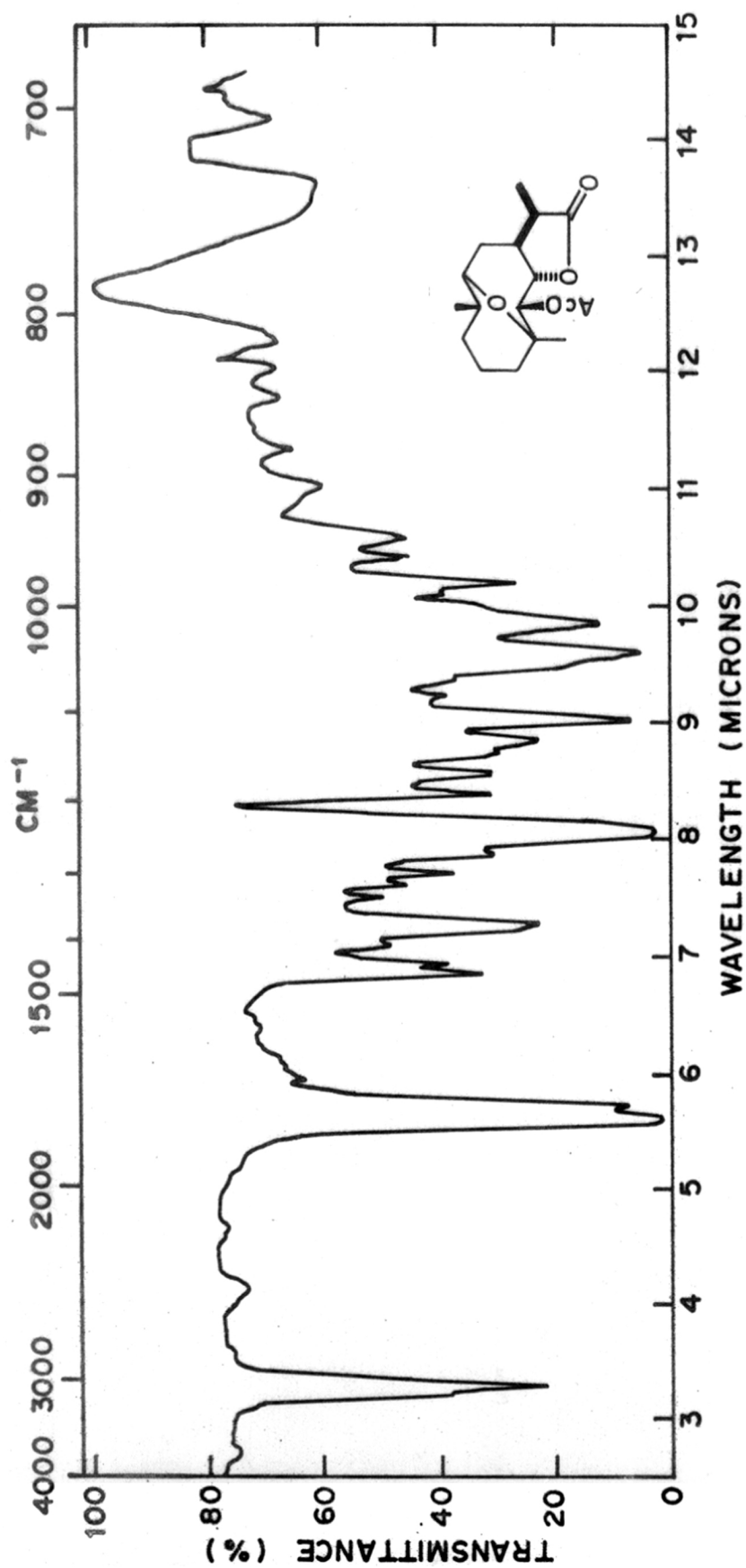


FIG. 10. IR SPECTRUM OF ACETATE (VI)

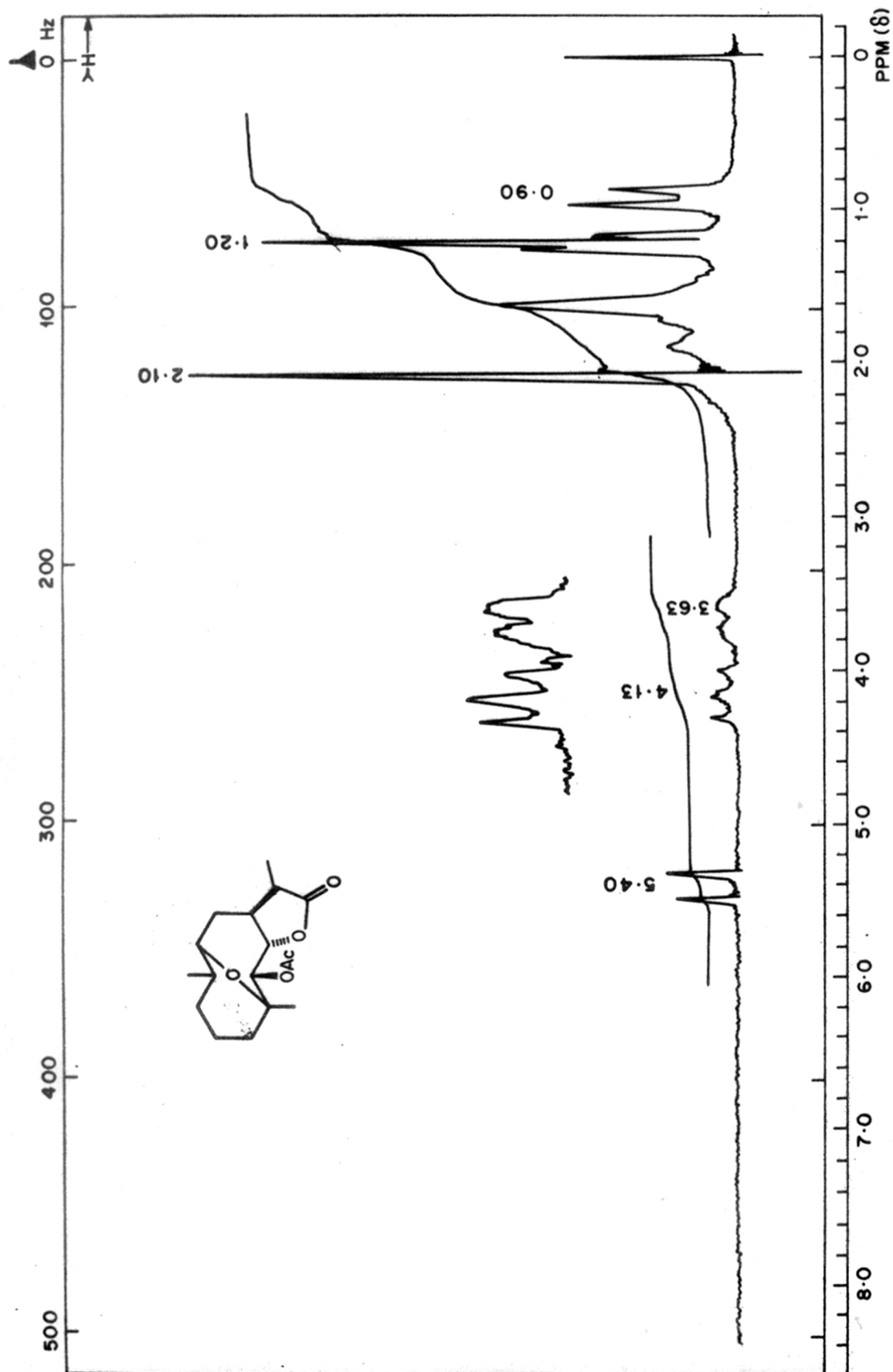
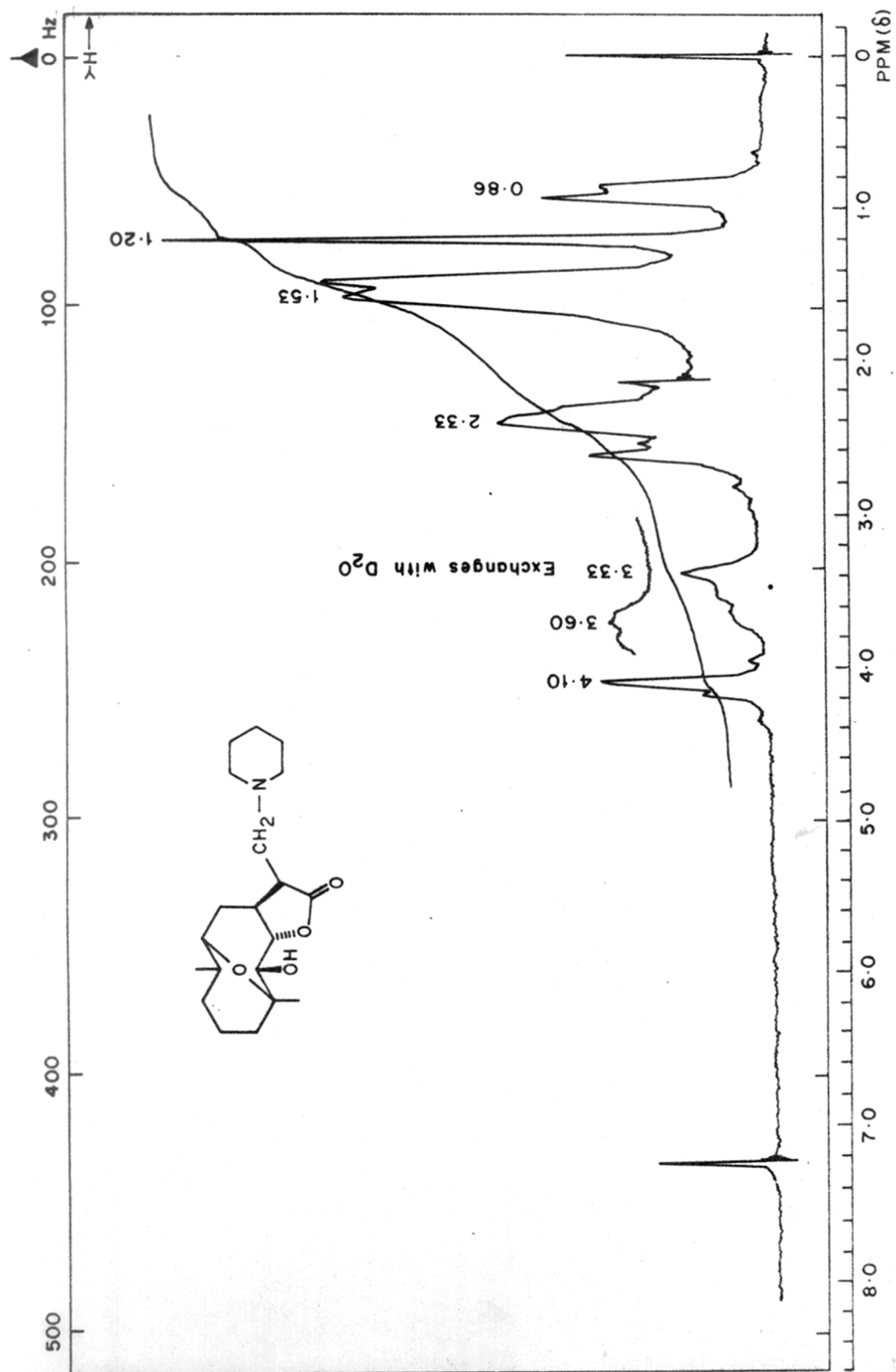
FIG. 11 : ^1H NMR SPECTRUM OF ACETATE OF DIHYDRODERIVATIVE.(VI) (60 MHz)

FIG. 13 : ^1H NMR SPECTRUM OF PIPRIDIN ADDUCT. (VII) (60 MHz)

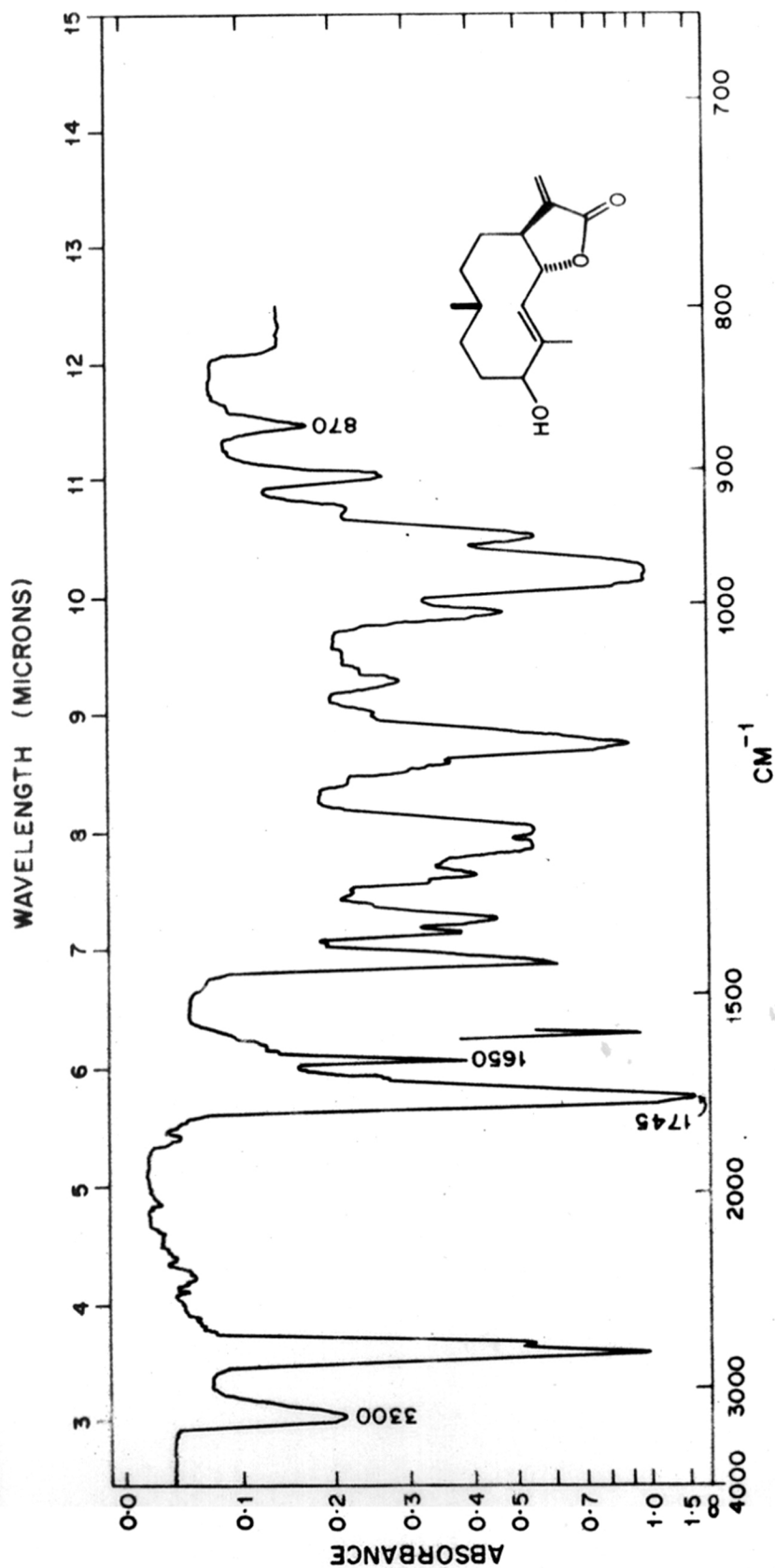
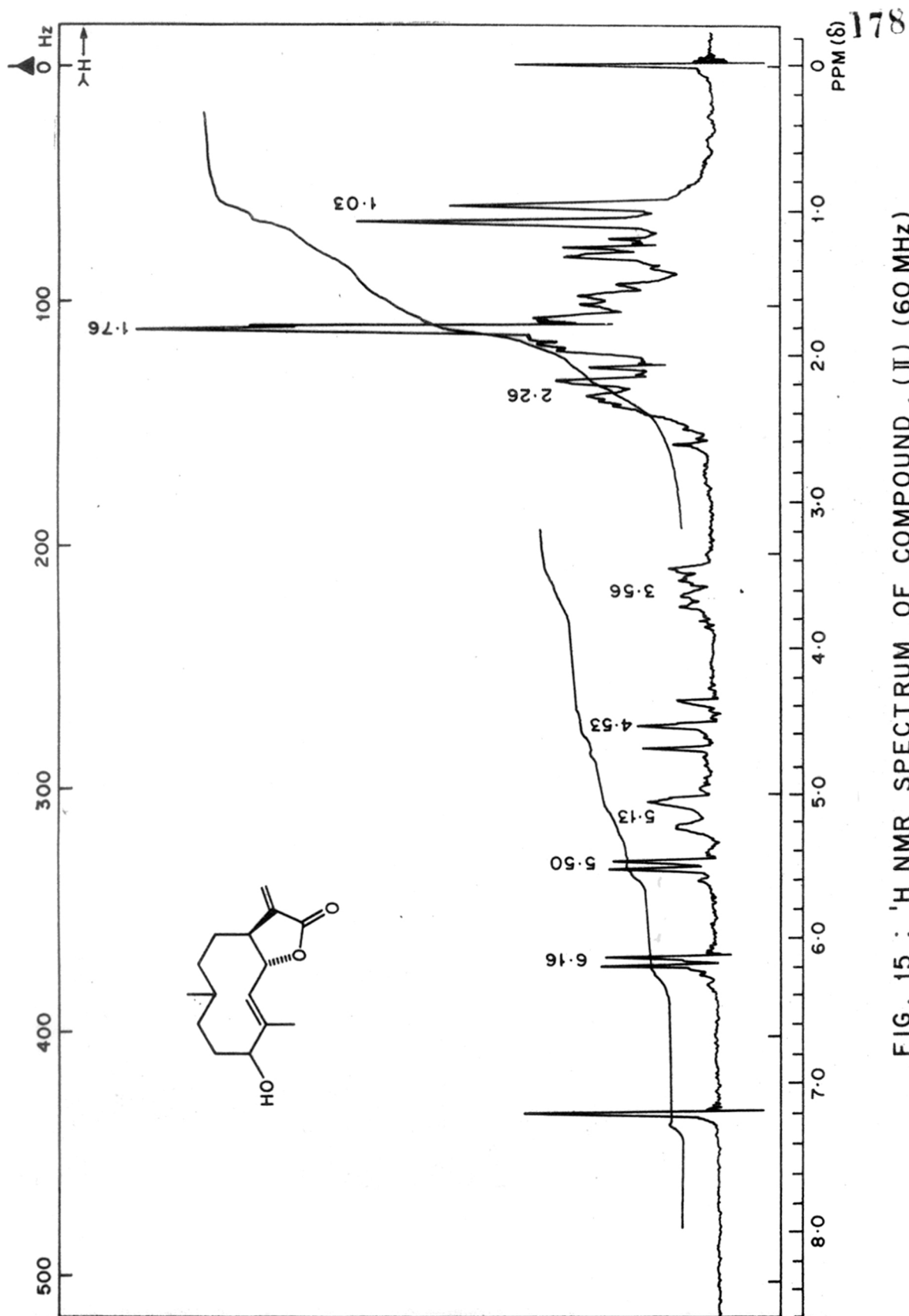


FIG. 14 : IR SPECTRUM OF COMPOUND . (II)



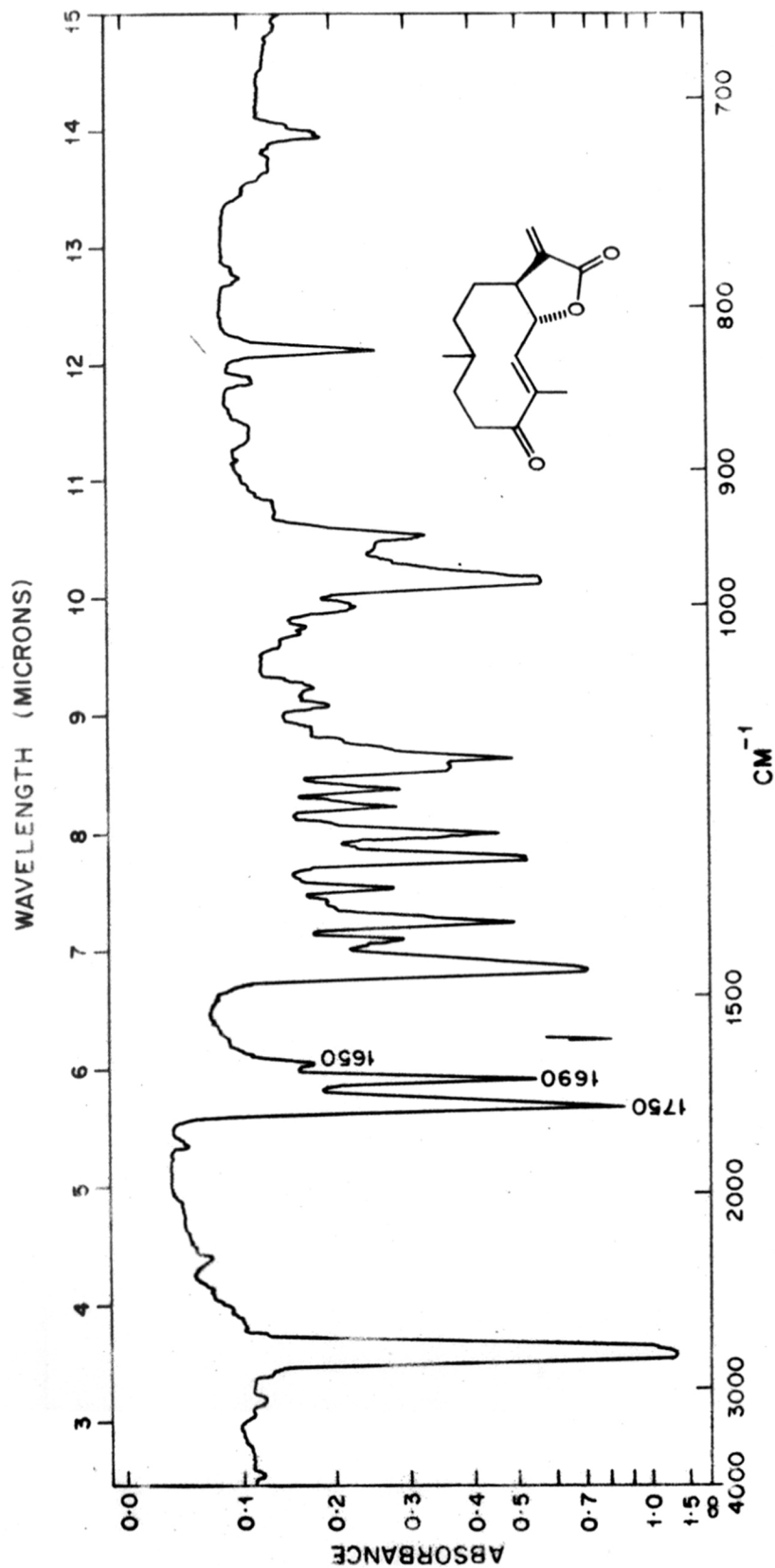
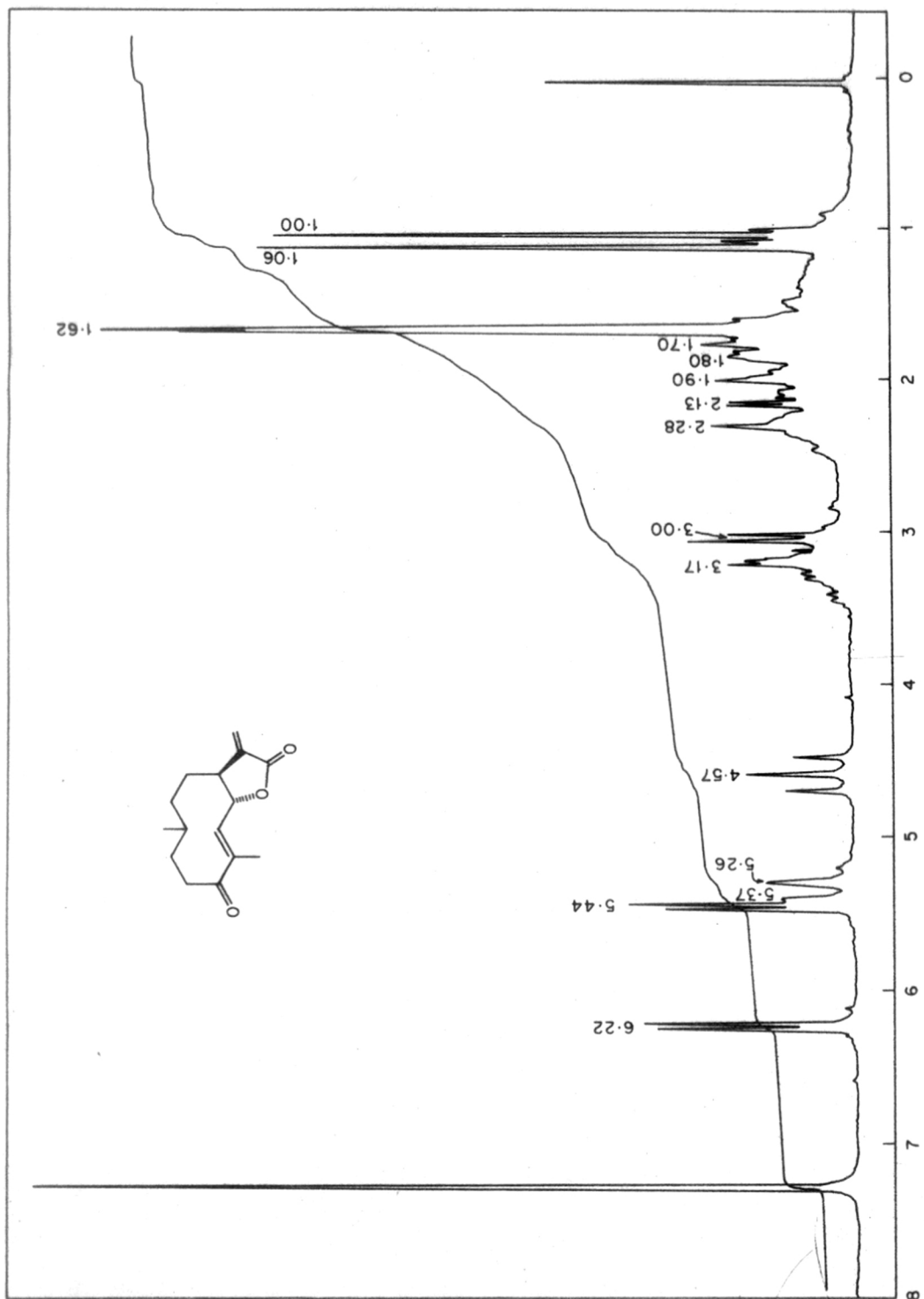


FIG. 16 : IR SPECTRUM OF OXIDE . (VIII)

FIG. 17 : ^1H NMR SPECTRUM OF OXIDE. (VIII) (90 MHz)

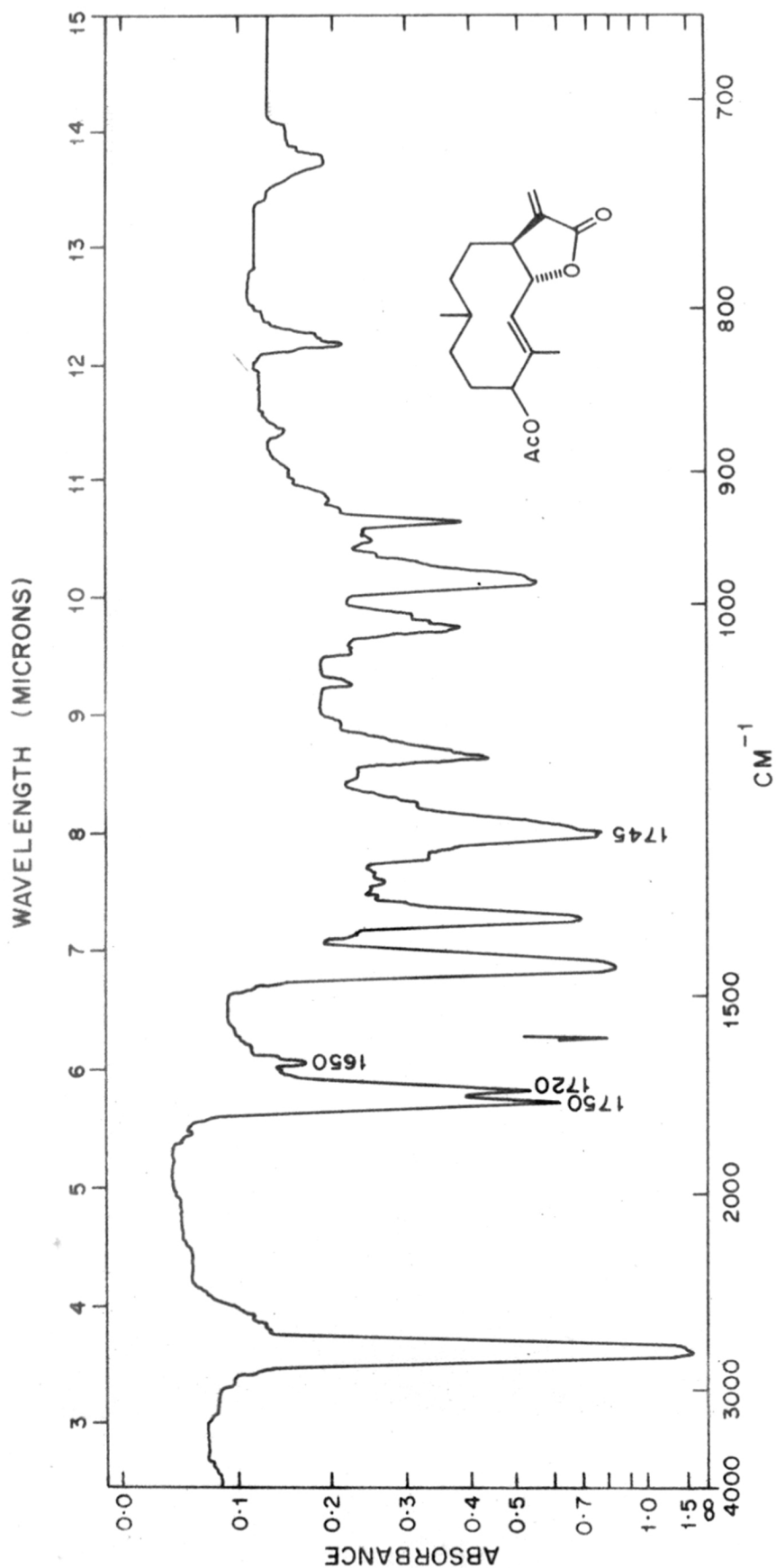
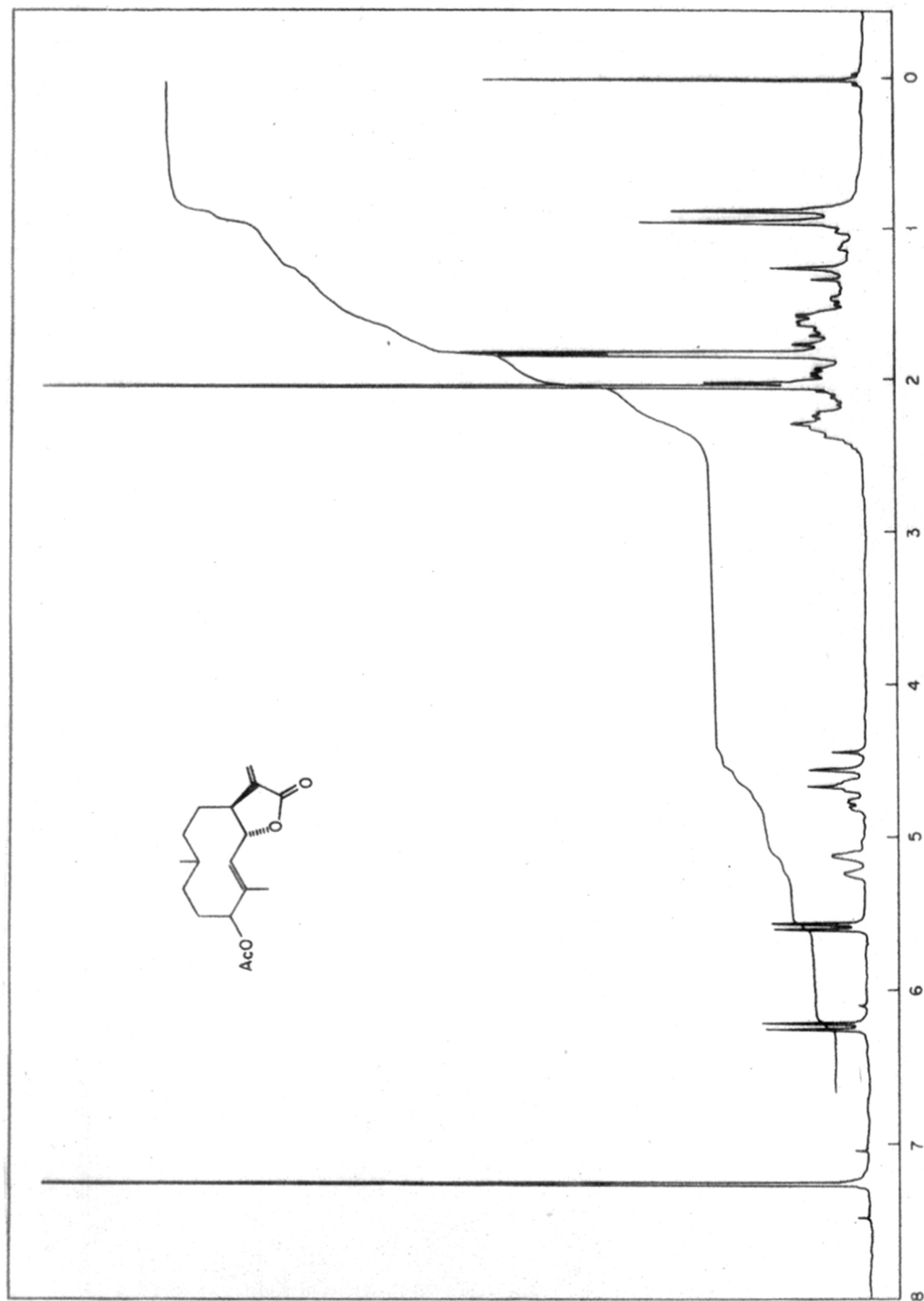


FIG. 18 : IR SPECTRUM OF ACETATE. (IX)

FIG. 19 : ^1H NMR SPECTRUM OF ACETATE. (IX) (90 MHz)

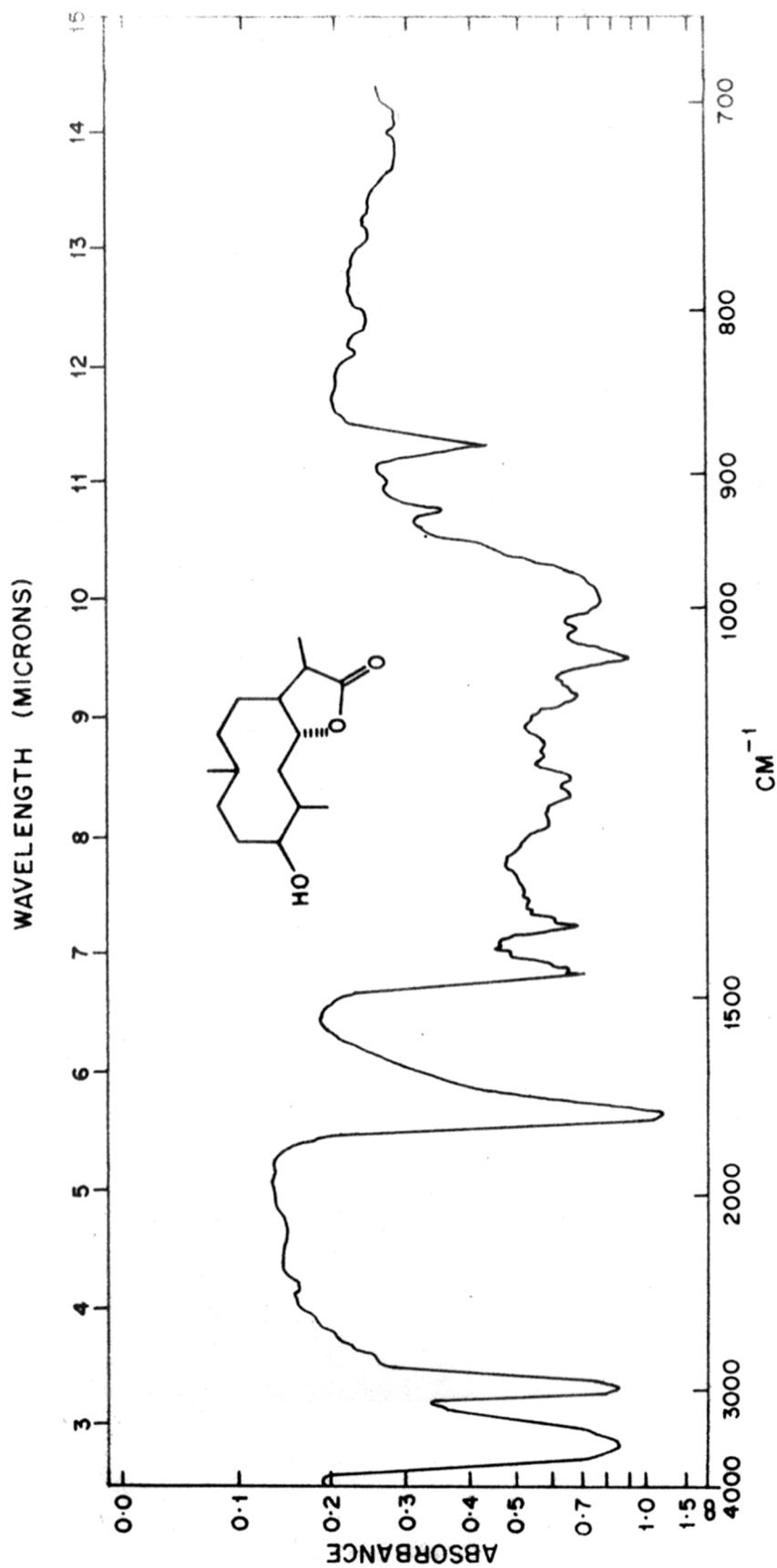


FIG.20.: IR SPECTRUM OF TETRAHYDRODERIVATIVE.

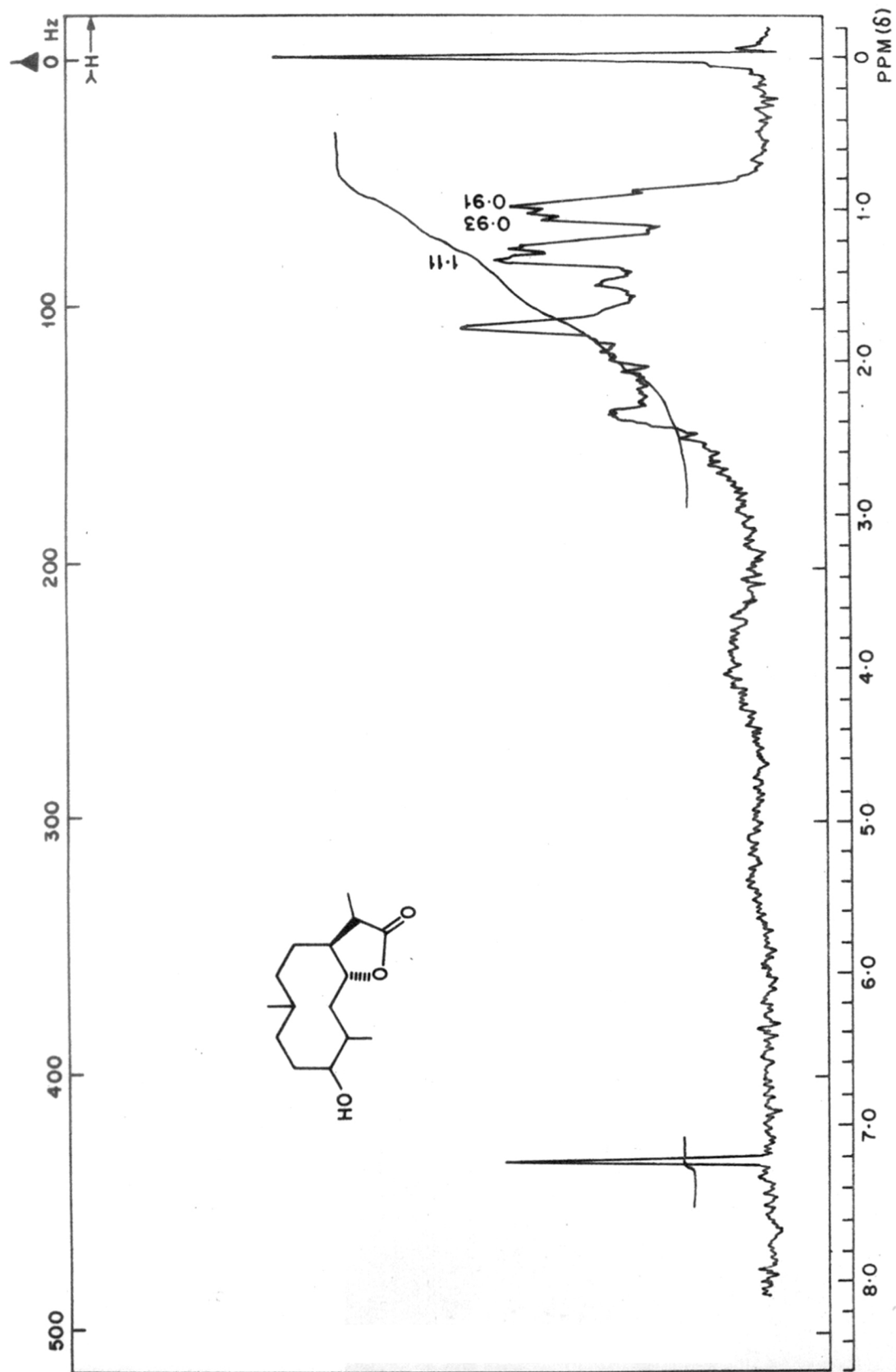


FIG. 21: ^1H NMR SPECTRUM OF TETRAHYDRODERIVATIVE.

CHAPTER IV

CHEMICAL INVESTIGATION OF SPHAERANTHUS INDICUS

I N T R O D U C T I O N

The genus Sphaeranthus belongs to the well defined tribe Inulae of family Compositae. The main character of the tribe is the combination of usually caudiculate ~~or~~ sagittate anthers, the predominance of heterogamy, yellow flowers and setose and plumose pappus elements. The tribe comprises 180-200 genera with some 2100 species having cosmopolitan distribution. Plants are specially abundant in South Africa and Australia¹.

The Inulae include a selection of plants which have been prized by man for their useful properties. Four of the best known, which were used medicinally in past are (i) Inula helenium (for treating chest diseases) (ii) Pulicaria dysenterica (in herbal remedies), (iii) Atennaria dioica (for throat infections) and (iv) Sphaeranthus indicus (folk medicine). The leaves of Inula and Pulicaria species have also been employed as insecticidal sources¹. In spite of the practical interest in plants of Inulae, the chemistry of the tribe has not been extensively explored. The only representative survey seems to be of the polyacetylenes in roots and leaves by Bohlmann et al.². The sesquiterpene lactones which have been so thoroughly explored in other Compositae tribes have only been identified in relatively few members. Fragmentary information on range of other constituents is also available³.

The genus Sphaeranthus is distributed in tropical Asia, Africa, and Australia. Six species of Sphaeranthus are reported to occur in India. Sphaeranthus 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 1,500 meters in the hills, especially as a weed in the rice fields, stem with toothed wings; leaves obovate, oblong, serrate; flowers in heads and are purple⁴.

All parts of plants find medicinal uses. The juice of the plant is styptic and said to be useful in liver and gastric 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 application in piles. Flowers are credited with alterative, depurative and tonic properties. Leaf juice is boiled with milk and sugar-candy is prescribed for cough. Antitubercular properties have also been ascribed to the plant. The leaves of the plant are mixed with paddy and rice to prevent damage by insect pests during storage⁵.

K.K. Baslas⁶ investigated the plant chemically for the first time in 1959 and reported that steam distillation of the fresh flowering herb yields a red viscous essential oil which is highly soluble in water. A sample of the oil (yield 0.02% d_{40}^{30} 0.9935; n_D^{30} 1.5055; $(\alpha)_D^{30} \pm 0^\circ$, acid value 5.8 and ester value 75.8) from Tambaram (Tamilnadu) obtained by steam distillation of plant showed following constituents:

cadinene 15.3; α -ionone 12.6; β -caryophyllene 7.4; p-methoxycinnamaldehyde 7.4; eugenol 7.0; α -phellanderene 7.0; ocimene 6.1; citral 5.4; α -terpinene 2.2; and an unidentified sesquiterpene (b.p. 127-128°/10 mm).

The constants and constituents of the sample of oil obtained from varanasi were slightly different, as reported by the same author⁵ (yield 0.01% (α)_D²⁰ $\pm$ 3.5, ester value 42.6). This sample of oil contained methyl eavicol, α -ionone, δ cadinene and p-methoxy cinnamaldehyde as major constituents and ocimene, α -terpinene, citral, geraniol, geranyl acetate, β -ionone, a new sesquiterpene alcohol sphaeranthol (C₁₅H₂₆O b.p.179-80°/10 mm), two new sesquiterpenes (sphaeranthene b.p.140-142°/10 mm and indicusene, b.p.148-150°/10 mm) and a phenolic ketone as the minor constituents⁵. Besides the essential oil, the herb yielded 3% of fatty oil, yellowish green in colour. A bitter alkaloid, sphaerenthine (C₁₅H₁₉NO₅ m.p.166-8° cecomp) has been reported to occur in the plant⁷. However structures for the new compounds (i.e. sphaeranthol, sphaeranthene, indicusene and sphaerenthine) were not reported.

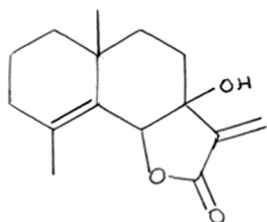
PRESENT WORK

In view of the incomplete work on the chemical investigation on this plant and reported insect control activity, we undertook the further chemical investigation. As a result of our chemical investigation of the acetone extract of dried plant material three sesquiterpenes (I) (II) and (III) were isolated (Chart I and II), however we could not isolate the compounds reported by earlier workers⁵⁻⁶. The compound (I), a sesquiterpene lactone belonging to eudesmanolide class was present as a major constituent (yield 0.26% based on dry weight of plant) and the compounds (II) and (III) were present as minor constituents. In addition to compounds (II) and (III), the acetone extract also contained two minor compounds one of which was identified as β -eudesmol a known sesquiterpene alcohol and the other compound was an acetylenic compound as revealed by its IR and NMR spectrum (Fig.). Due to paucity of the acetylenic compound and its instability structure elucidation of acetylenic compound was not possible. The structure elucidation of compounds (I), (II) and (III) is described below.

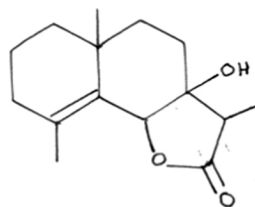
Compound (I): Compound (I) a thick oil, $(\alpha)_D^{30} -56.81$ (CHCl_3 $C = 0.4$) analysed for $\text{C}_{15}\text{H}_{20}\text{O}_3$ (M^+ at m/e 248).

The presence of a hydroxyl group in (I) was indicated by appearance of a band at 3250 cm^{-1} in the IR spectrum (Fig. 1) of (I). This was further confirmed by appearance of a D_2O exchangeable signal in the ^1H NMR spectrum (Fig. 2) of (I) and also by appearance of $\text{M}-\text{H}_2\text{O}$ peak in its mass spectrum. That the hydroxyl group is tertiary was proposed on the basis of the absence of a signal which could be assigned to a proton on carbon atom carrying hydroxyl group in the ^1H NMR spectrum of (I) (Fig. 2)(Table I). This was further supported by the appearance of a signal at δ 76.10(s) in the SFOD ^{13}C -NMR spectrum of (I) (Fig. 3) (Table II) and also by the fact that the compound did not react with Jones reagent and did not form an acetate even under forcing conditions.

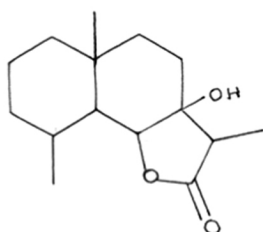
A carbonyl absorption band at 1750 cm^{-1} and a vinyl absorption band at 1650 cm^{-1} suggested the presence of an α,β -unsaturated γ -lactone moiety in the compound, which was further supported by the appearance of two one protons singlets at 5.75 ppm and 6.31 ppm in the ^1H -NMR spectrum of (I) (Fig. 2) (Table I) and appearance of signals at δ 169.75(s), 127.31(s) and 121.27(t) in the SFOD ^{13}C NMR spectrum (Fig. 3) (Table II) of (I). Compounds conversion into piperidine adduct (VII) (Chart I) on treatment with piperidine and formation of pyrozone derivative (VIII) (Chart I) on treatment with diazomethane, also supported the above statement. Two oxygen atoms in the



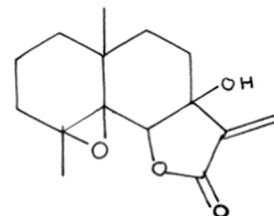
(I)



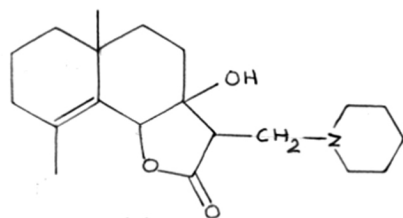
(IV)



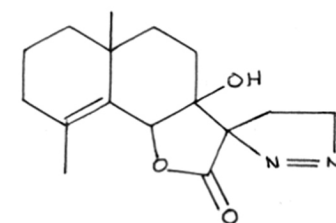
(V)



(VI)



(VII)



(VIII)

CHART(I)

Table I
¹H NMR spectral data of compound (I) and derivatives
 (in CDCl₃, TMS as internal standard, chemical shifts in
 δ scale) (values in parenthesis are coupling constants)

	(I) ⁺⁺	(IV) ⁺⁺	(V) ⁺	(VI) ⁺⁺	(VII) ⁺	(VIII) ⁺
6-H	5.00(s)	4.95(d) (J = 1Hz)	*	3.94(s)	5.03(s)	5.83(s)
7-H	-	-	-	-	-	-
8-H	*	*	*	*	*	*
9-H	*	*	*	*	*	*
10-H	-	-	-	-	-	-
11-H	-	*	*	-	-	-
13-H	3.31(s)†	1.17(d) (J = 6 Hz)†	1.13(d) (J = 6Hz)	6.28(s)†	3.1(d) (J = 10 Hz)†	2.1 (m)
13-H	5.75(s)†	†	†	5.88(s)†	†	†
14-H	1.04(s)	1.04(s)	0.88(s)	1.1(s)	1.06(s)	1.06(s)
15-H	1.73(s)	1.8(s)	0.84(d)	1.36(s)	1.8(s)	1.83(s)
16-H				1.63 and 2.05 CH ₂ 's of piperidine ring		4.51(dd) (J=10 Hz)

Note: † Spectrum recorded at 60 MHz. †† Spectrum recorded at 90 MHz

* Signals unresolved.

Table (II) ^{13}C NMR Spectral data for compound (I)
(in CDCl_3 at 22.63 MHz , TMS as internal
standard values in δ scale)

Carbon No.	Chemical shift	Carbon No.	Chemical shift
C-1	32.885 (t) ⁺	C-9	26.32(t) ⁺
C-2	31.780(t) ⁺	C-10	33.27(s)
C-3	38.99(t)*	C-11	127.31(s)
C-4	145.25(s)	C-12	169.75(s)
C-5	140.50(s)	C-13	121.27(t)
C-6	81.75(d)	C-14	18.39(q)
C-7	76.10(s)	C-15	19.49(q)
C-8	35.09(t)*		

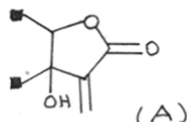
Note: (1) s = singlet, d = doublet, t = triplet, q = quartet
signal multiplicity in single frequency off
resonance decoupled (SFOD) spectrum.

(2) Chemical shifts with similar marks (i.e. *, + etc.)
are possibly interchangeable.

lactone ring and one in hydroxyl group accounted for all the three oxygen atoms in the molecular formula ($C_{15}H_{20}O_3$) of the compound.

A striking feature in the 1H NMR spectrum of (I) (Fig. 2) is that the signals due to two exomethylene protons of γ -lactone (i.e. H-13 and H-13') appeared as two singlets at 5.75 ppm and 6.31 ppm and did not show any allylic coupling with H-7 as is observed for almost all the α -methylene γ -lactones of known structures having both hydrogen at C-7 and α -methylene γ -lactone protons in their usual positions (i.e. H-13 and H-13')⁸. Further more the signal due to H-7 proton could not be located in 1H NMR spectrum of (I) and the signal for lactonic proton appeared as a singlet at 5.00 ppm. This indicated that carbon carrying lactone oxygen was attached to the quaternary carbons on both the sides. Based on these observations it was proposed that the hydroxy group must be present at C-7. This proposal was further supported by the SFOD ^{13}C NMR spectrum (Fig. 3) (Table II) of compound (I), which showed a singlet at δ 76.10(s), for C-7, and also by the failure of compound (I) to form acetate even under forcing conditions and failure of compound (I) to give a dehydration product with various dehydrating agents e.g. formic acid, thionyl chloride pyridine (reflux), p-toluene sulphonic acid benzene (reflux) etc. In all the above reactions compound decomposed. Thus based on the above discussion following

partial structure (A) could be written for compound (I).

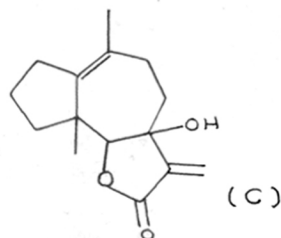
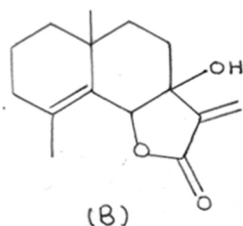


Band at 1650 cm^{-1} in the IR spectrum of (I) and signal at 1.73 ppm (3 protons, methyl on double bond) in the ^1H NMR spectrum of the compound suggested the presence of one more double bond (in addition to lactone exomethylene) in the compound. The ^1H -NMR spectrum also showed a singlet for an angular methyl group at 1.04 ppm. The tetrasubstituted nature of the double bond was suggested on the basis of appearance of signals at δ 145.25 ppm (s) and 140.50 ppm (s) in the SFOD ^{13}C NMR spectrum of compound (I) (Fig. 3). The presence of double bond (in addition to lactone exomethylene) was also confirmed by conversion of (I) into dihydro (IV) (Chart I) and tetrahydroderivative (V) (Chart I) on catalytic hydrogenation using palladium hydroxide on calcium carbonate and Pd/C 10% as catalysts respectively, as well as by compounds conversion to epoxide (VI) (Chart I) on treatment with *m*-chloroperbenzoic acid. ^1H NMR spectrum of epoxide (VI) (Fig. 9) showed a signal at 1.36 (methyl on epoxide ring) but did not show any signal assignable to proton on epoxide ring.

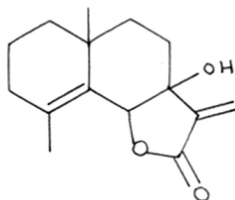
That the signal at 5.00 ppm in the ^1H NMR spectrum of (I) is due to lactonic proton was supported by the

^1H NMR spectrum of dihydroderivative (IV) (Fig. 5) in which the same signal (at 4.95 ppm) showed a little broadening ($J = 0.8 \text{ Hz}$). This broadening could be attributed to the allylic interactions between the C-6 and H-11 protons⁹.

By combining all the above data i.e. (i) partial structure (A), (ii) presence of tetrasubstituted double bond (iii) one methyl on double bond and one angular methyl group, two possible structures (B) and (C) could emerge for compound (I).

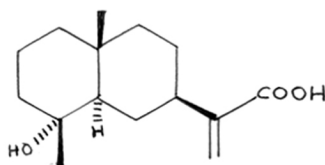


Proof in favour of structure (B) came from the ^1H NMR spectrum of epoxide (VI) (Fig. 9) in which the signal assignable to lactonic proton had shifted to 3.94 ppm(s) from its position at 5.00 ppm(s) in the ^1H -NMR spectrum (I). This suggested that the carbon atoms carrying the epoxide ring (and hence the double bond in compound I) and the carbon atom carrying lactonic oxygen are on the adjacent carbon atoms, thus the structure of the major compound from Sphaeranthus indicus could be depicted as in (I).



(I)

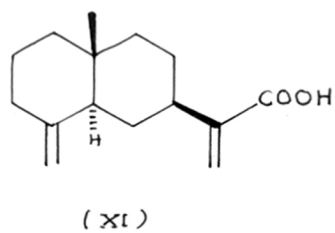
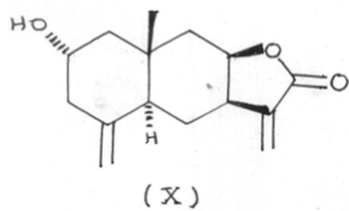
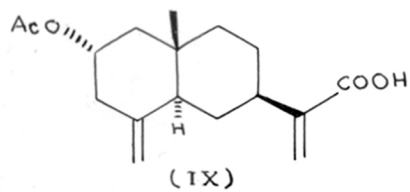
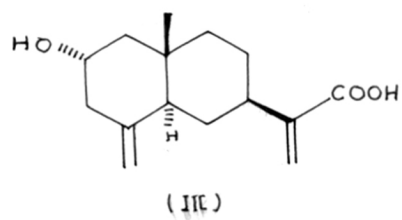
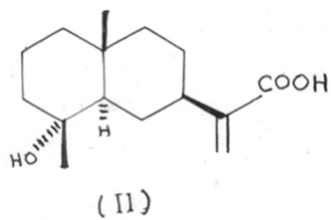
Compound (II): Compound (II) a colourless crystalline solid m.p. 177-79° (α)_D³⁰ - 32.8° (CHCl₃ C = 0.5) analyzed for C₁₅H₂₄O₃ (mass spectrum M⁺ at m/e 252). The spectral data (Figs. 13, 14 and Table III) and the melting point revealed that it is a known compound ilicic acid (II) earlier isolated by W. Herz et al.¹⁰ from Ambrosia ilicifolia.



(II)

The ¹³C NMR spectrum of the compound which has ^{not} been reported earlier (II) (Table IV, Fig. 15) also confirmed the structure of ilicic acid.

Compound (III): Compound (III) a colourless crystalline solid m.p. 187-189°, (α)_D³⁰ (CHCl₃ C = oil). Analysed for C₁₅H₂₂O₃. IR spectrum of compound (III) (Fig. 16) showed a band at 3500 cm⁻¹ which suggested the presence of a hydroxyl group in the compound, which was further supported by the appearance of a D₂O exchangeable signal in the ¹H NMR spectrum (Table III, Fig. 17) of (III). The ¹H NMR



CHART(II)

Table (III) ^1H NMR spectral data of compound (II), (III), (XI) and derivatives of compound (III) (values in parenthesis are coupling constants)

	(II) (90 MHz)	(III) ⁺ (100 MHz)	(IX) (90 MHz)	(XI)
H-2	*	3.98(dddd J=	5.02(dddd, J =	*
H-3	*	2.75 (m)	2.68 (m)	*
H-7	2.48 (m)	2.70 (m)	2.66 (m)	*
H-13	5.55 (s)	5.57 (s)	5.73 (s)	5.73(s)
H-13'	6.22 (s)	6.30 (s)	6.37 (s)	6.40(s)
H-14	0.94 (s)	0.80 (s)	0.80 (s)	0.77 (s)
H-15		4.52 (s)	4.57 (s)	4.46 (s)
H-15'	1.11 (s)	4.80 (s)	4.88 (s)	4.76
Others	6.06(OH)		2.04(s, OAc)	-

Note: *Signals not resolved

⁺Spectrum in pyridine D₅

Table (IV) ^{13}C NMR spectral data of compound (II)
(in CDCl_3 at 22.63 MHz, TMS as internal standard
chemical shifts in $\delta\text{c ppm}$ scale)

Carbon No.	Chemical shift	Carbon No.	Chemical shift
C-1	27.49(t) ⁺	C-9	26.90 ⁺ (t)
C-2	20.34(t)	C-10	34.88(s)
C-3	41.26(t)	C-11	145.77(s)
C-4	72.78(s)	C-12	171.83(s)
C-5	40.42(d)	C-13	124.39(t)
C-6	44.77(t)*	C-14	18.91(q)
C-7	55.24(d)	C-15	22.68(q)
C-8	43.60(t)*		

Note: 1) s = singlet, d = doublet, t = triplet,

q = quartet signal multiplicity in single

frequency off resonance decoupled (SFOD) spectrum.

2) Chemical shifts with similar marks (i.e. *, +)
are possibly interchangeable.

spectrum of (III) also showed a fourfold doublet centered at 3.98 ppm (one proton) suggesting that the hydroxyl group is secondary in nature. The secondary nature of hydroxyl group was supported by conversion of (III) into its mono acetate (IX) on treatment of (III) with acetic anhydride and pyridine. The ^1H NMR spectrum of acetate showed one acetate methyl singlet at 2.04 ppm (Fig. 19, Table III) and also showed a four-fold doublet at 5.02 ppm which had appeared at 3.98 ppm in the ^1H NMR spectrum of (III). The IR spectrum of acetate (IX) (Fig. 18) showed bands at 1750 and 1240 cm^{-1} for acetate group but did not show any absorption due to hydroxyl group which suggested the presence of only one hydroxyl group in compound (III).

IR spectrum of (III) (Fig. 16) showed a band at 2700 cm^{-1} which suggested the presence of a carboxylic acid group in the compound. Thus two oxygen atoms in the acid group and one in form of hydroxyl group accounted for all the three oxygen atoms in the molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_3$ of compound (III).

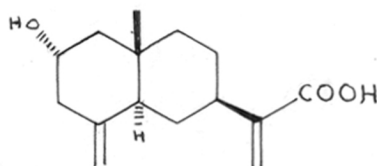
The appearance of a band at 1630 cm^{-1} in the IR spectrum of compound (III) suggested the presence of double bond in compound. This was supported by the ^1H NMR spectrum of compound (III) which suggested the presence of two exocyclic methylene groups in the compound. The ^1H NMR spectrum (Fig. 17, Table III) showed two one proton broad singlets at 5.57 and 6.30 ppm and two one proton broad

singlets at 4.52 and 4.80 ppm which suggested that one of the exomethylene groups is in conjugation with a carbonyl group (acid-carbonyl). The two one proton singlets which are down-field than the other two (i.e. at 5.57 and 6.30 ppm) are due to the exomethylene groups which are in conjugation with carbonyl group. The ^1H NMR spectrum of compound (III) (Fig. 17, Table III) also showed a singlet at 0.80 ppm for angular methyl group.

Critical comparison of the ^1H NMR spectrum of compound (III) (Table III) with those of the number of known compounds showed that the signals in the ^1H NMR spectrum of compound (III) were similar to those of costic acid (XI)¹⁰ (Table III) which suggested the eudesman skeleton for compound (III). In fact the only difference in the ^1H NMR spectrum of (III) (Table III) and that of costic acid (XI) (Chart II, Table III) is that the former showed a one proton four-fold doublet centered at 3.98 ppm (assignable to proton on carbon atom carrying hydroxyl group) which was not observed in the ^1H NMR spectrum of costic acid. Thus the compound (III) is a hydroxy costic acid.

That the hydroxyl group is present at 2 position of costic acid was proposed on the basis of the observed chemical shift and multiplicity of the signal due to proton on carbon atom carrying hydroxyl group, which was very much similar to that of Ivalin (X)¹¹ (Chart II). This was further confirmed by the decoupling studies on the

compound (III). Irradiation of the four-fold doublet centered at 3.98 ppm (assignable to proton on carbon atom carrying hydroxyl group) affected the multiplicity of a two proton multiplet centered at 2.75 ppm as well as affected the multiplicity of a two proton signal at 2.15 ppm which suggested the presence of a $\text{CH}_2\text{-CH-CH}_2$ type of grouping in the molecule. Irradiation of the broad singlet at 4.51 ppm (15-H) sharpened the multiplet centered at 2.75 ppm thus indicating that the signal due to 15-H and that due to proton on carbon atom carrying hydroxyl group are coupled to the same $\text{-CH}_2\text{-}$ group (allylic $\text{-CH}_2\text{-}$). Thus based on above data, structure and stereochemistry for minor constituent from acetone extract of Sphaeranthus indicus is tentatively assigned as in (III).



(III)

EXPERIMENTAL

Plant material: The plant Sphaeranthus indicus was collected near Lonawala (Maharashtra, India) during December 1979 and was shade dried.

Extraction: The shade dried and coarsely powdered plant material (3 kg) was extracted with acetone (40 lit) in a soxhlet extractor on water-bath. The progress of the extraction was monitored by taking out the test sample (100 ml) from the extract and evaporating it to dryness. The extraction was stopped when the residue left after evaporating the solvent from the test sample weighed negligible. The solvent was recovered from the extract by distillation on water-bath, the last traces of the solvent were removed under vacuum to get a dark coloured residue (150 g).

Chromatographic separation: The acetone extract of Sphaeranthus indicus (100 g) was adsorbed on minimum amount of silica gel (200 mesh, 250 g) and then was loaded on column (115 cm long and 9.5 cm in diameter) for separation of its constituents. The column was packed with silica-gel (200 mesh, 3 kg). The elution was started with acetone; pet.ether mixture (20:80) and was continued with successive increase in the % of acetone. Fractions of 150 ml size were collected. The progress of the column chromatographic separation was monitored by thin layer chromatography of the fraction. Fractions showing similar composition were mixed

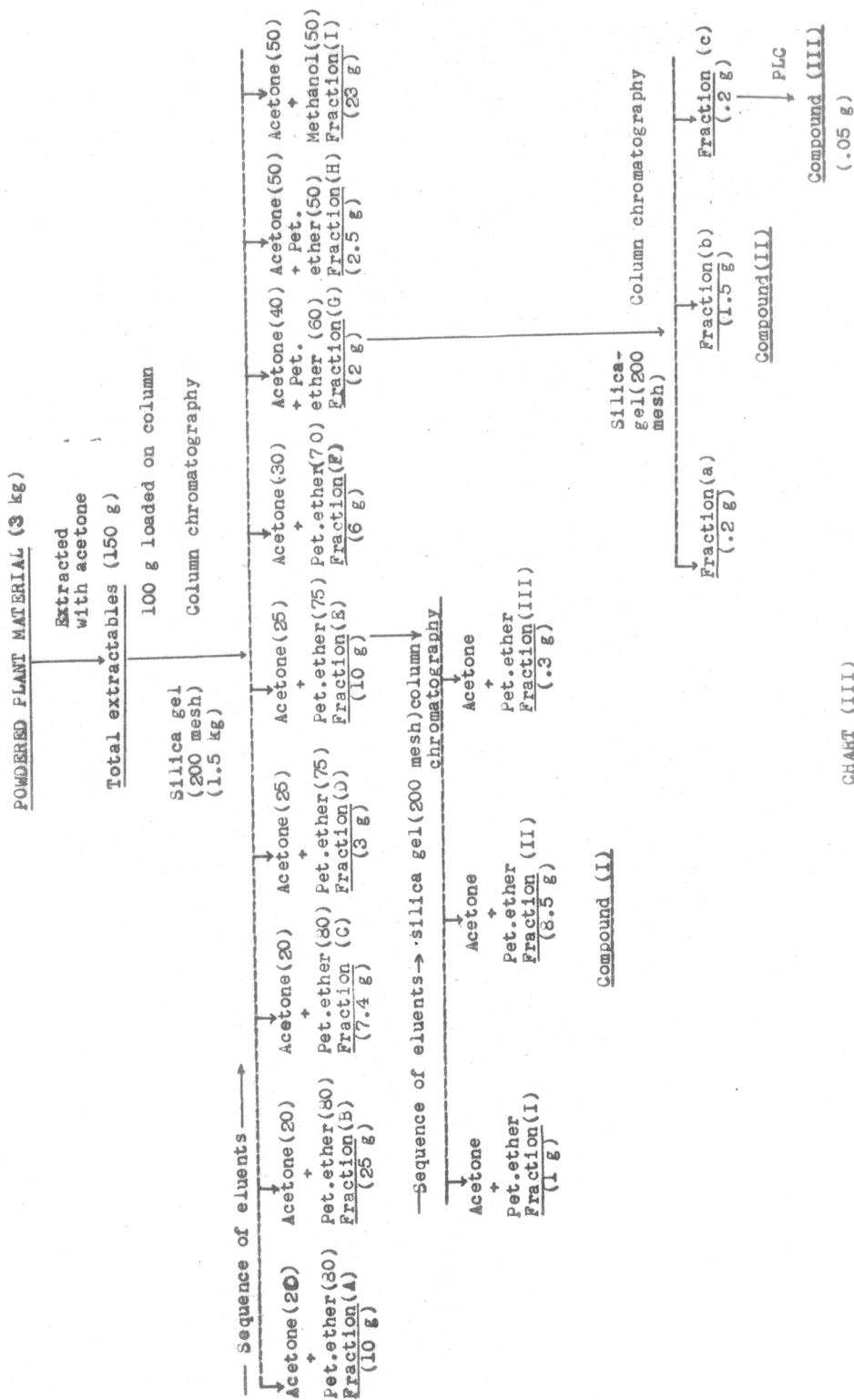


CHART (III)

Table (V) Chromatographic separation of constituents of acetone extract of Sphaeranthus indicus

Fraction No.	Eluent	Total volume collected	Net weight of fraction	Approximate composition
(A)	Acetone (20) Pet.ether(80)	4 x 150 ml	10 g	straight chain hydrocarbons
(B)	Acetone (20) Pet.ether (80)	6 x 150 ml	25 g	Mixture of unidentified compounds
(C)	Acetone (20) Pet.ether(80)	5 x 150 ml	7.4 g	mixture of unidentified compounds and sterol
(D)	Acetone (25) Pet.ether (75)	4 x 150 ml	3 g	mixture of sterol compound (I)
(E)	Acetone (25) Pet.ether (75)	8 x 150 ml	10 g	impure compound (I)
(F)	Acetone (30) Pet.ether (70)	4 x 150 ml	6 g	mixture of unidentified compounds
(G)	Acetone (40) Pet.ether (60)	4 x 150 ml	2 g	compound (II) (impure)
(H)	Acetone (50) Pet.ether (50)	4 x 150 ml	2.5 g	compound II + unidentified compound (III)
(I)	Acetone (50) Methanol (50)	10 x 150 ml	23 g	plant acids and sugars

together to obtain nine major fractions (A-I). The details of the chromatographic separation are summarized in Chart (III) (Table V).

Rechromatography of fraction (E): Fraction (E) (10 g) contained compound (I) as major compound along with some impurity (TLC:eluent 30:70) to isolate compound (I) in pure form the fraction (E) was rechromatographed by dry column chromatography using acetone:pet.ether mixture (20:80) as eluent with successive increase in % of acetone. Fraction (II) from the chromatography afforded compound (I) in fairly pure form. The details of the chromatography are summarized in Chart(III), Table (VI).

Table (VI) Rechromatography of fraction (E)

Fraction No.	Eluent	Total volume collected	Net weight of fraction	Approximate composition
(I)	Acetone (20) + pet.ether(80)	2 x 50 ml	1 g	unidentified compounds
(II)	Acetone (25) + pet.ether(75)	4 x 50 ml	8.5 g	compound (I)
(III)	Acetone (30) + Pet.ether(70)	3 x 50 ml	0.3 g	mixture of unidentified compound

Rechromatography of fraction (G): Fraction (G) (2 g) was a mixture of two compounds (II) and (III) along with some other impurity. (TLC, eluent 40:60). To isolate compound (II) and (III) in pure form fraction (G) was chromatographed on silica-gel (200 mesh activated, 200 g). Elution was started with acetone:pet.ether mixture (30:70) with successive increase in the % of acetone. Fractions of 50 ml size were collected. Progress of the chromatographic separation was monitored by TLC (eluent: 40:60) and fractions showing similar composition were mixed together to get three major fraction (a-c). Fraction (b) gave compound (II) as crystalline solid where as compound (III) was obtained in fairly pure form from fraction (c). The details of the chromatographic separation are summarized in Chart (III) and Table (VII).

Table (VII) Rechromatography of Fraction (G)

Fraction No.	Eluent	Total volume collected	Net weight of fraction	Approximate composition
(a)	Acetone(30) + Pet.ether	0 x 50 ml	0.2 g	mixture of unidentified compounds
(b)	Acetone (40) + Pet.ether (60)	3 x 50 ml	1.5 g	compound (II)
(c)	Acetone (50) + Pet.ether (50)	4 x 50 ml	0.3 g	compound (III) + unidentified compound

Compound (I): Compound (I) (8.5 g) was obtained in fairly pure form from rechromatography of fraction (E) (Table VI). Compound (I) was further purified by its preparative TLC on silica gel (eluent = 30:70) to obtain compound (I) as yellowish viscous oil.

$$(\alpha)_D^{30} - 56.81 \text{ (CHCl}_3 \text{ } c = 0.43)$$

UV: $\lambda_{\text{max}}^{\text{EtOH}}$ and absorption.

IR: (Fig. 2) $\gamma_{\text{max}}^{\text{nujol}} \text{ cm}^{-1}$ 3250 (OH) 1750 (γ -lactone)
1650 (unsaturation)

Mass: m/e (rel.int.) 248 (M^+ , 20), 233 ($M-\text{CH}_3$, 18)

215 ($233-\text{H}_2\text{O}$, 43) 204 ($M-\text{CO}_2$, 13) 117 (100)

Compound (II): Compound (II) (1.5 g) was obtained as a crystalline solid from fraction (b) from rechromatography of fraction (G). It was recrystallized from ethanol water mixture to get compound (II) as colourless crystalline solid (1.2 g).

m.p. 177-9°

$$(\alpha)_D^{30} - 32.8 \text{ (CHCl}_3 \text{ } c = 0.5).$$

IR: (Fig. 13) $\gamma_{\text{max}}^{\text{nujol}} \text{ cm}^{-1}$ 3300 (OH) 2700, 1710 (acid carbonyl) and 1650.

Mass: m/e (rel.int.) 252 (M^+ , 9) 234 ($M-\text{H}_2\text{O}$, 100)

219 ($234-\text{CH}_3$, 85), 205 (50)

Compound (III): Compound (III) (.3 g) obtained in impure form from fraction (c) from rechromatography of fraction (G) was further purified by its preparative layer chromatography on silica-gel (eluent 40:60) to obtain compound (III) (.06 g)

as colourless crystalline solid. Compound (III) was crystallized from ethanol water mixture to obtain compound (III) as colourless crystalline solid. m.p. 187-89°.

IR: (Fig. 16) $\gamma_{\text{max}}^{\text{nujol}}$ cm⁻¹ 3350, 2700, 1690, 1630 and 890.

Chemical transformations of compound (I)

(i) Dihydroderivative (IV): Compound (I) (100 mg) was hydrogenated at room temp. and atmospheric pressure using paladium hydroxide on calcium carbonate as catalyst to obtain dihydroderivative as colourless crystalline solid (95 mg) m.p. 143-5°.

IR: (Fig. 4) $\gamma_{\text{max}}^{\text{nujol}}$ cm⁻¹ 3300 (OH) 1760 (γ-lactone) 1650 (unsaturation)

Mass: m/e (rel.int.) 250 (M⁺, 10) 235 (M-CH₃, 50)

217 (235-H₂O, 25) 189 (20) 149(50) 104(50) 44(100).

(ii) Tetrahydroderivative (V): Compound (I) (100 mg) was hydrogenated at atmospheric pressure (room temperature using Pd/C 10% as catalyst to obtain tetrahydroderivative as colourless viscous oil.

IR: Fig. 6) $\gamma_{\text{max}}^{\text{nujol}}$ cm⁻¹ 3500 (OH), 1765 (γ-lactone).

Epoxide (VI): Compound (I) (80 mg) was dissolved in minimum amount of chloroform and was cooled to 0°C. To the above cold solution ~~in~~-chloroperbenzoic acid (120 mg) dissolved in minimum amount of chloroform was added slowly. The mixture was then allowed to stand at 0° for 48 hours with

occasional shaking. It was then worked up as usual to get the epoxide (85 mg) as crystalline solid m.p.

IR: (Fig. 8) $\nu_{\text{max}}^{\text{nujol}}$ cm^{-1} 3300 (OH) 1750 (γ -lactone).

Mass: m/e (rel. intensity) 264 (M^+ , 5) 246 ($M-H_2O$, 5)

231 ($246-CH_3$, 5) 221 (100)

Piperidine adduct (VII): To the ethanol solution of compound (200 mg) piperidine (2.5 ml) was added slowly at room temperature, the mixture was allowed to stand at room temperature for 12 hours and then excess of piperidine was removed under vacuum to obtain the piperidine adduct (250 mg) as viscous oil.

Pyrazoline derivative (VIII): Compound (I) (100 mg) was dissolved in minimum amount of dry methanol and to it ether solution of diazomethane (prepared from 200 mg of nitroso-methyl urea) was added at 0-5°C. The mixture was allowed to stand at room temperature for 12 hours. The solvent was then removed to obtain pyrazoline derivative (VIII) as a yellowish crystalline solid m.p. 145.7° (with decomposition).

IR: Fig. (11) $\nu_{\text{max}}^{\text{nujol}}$ cm^{-1} 3540 (OH) 1750 (γ -lactone)

Chemical transformations of compound (III):

Monoacetate (IX): Compound (III) (50 mg) was dissolved in pyridine (0.5 ml) and to the above solution acetic anhydride (0.5 ml) was added slowly at room temperature. The reaction mixture was allowed to stand at room temperature for 12 hours and was then worked up as usual to obtain the

acetate (IX) as a colourless crystalline solid (yield 52 mg)

m.p. 245-6°.

$(\alpha)_D^{30}$

IR: (Fig. 18) $\gamma_{\text{max}}^{\text{nujol}}$ cm⁻¹ 1750 (acetate) 1700 (acid
carbonyl) 1650 (unsaturation) and 1240 (acetate)

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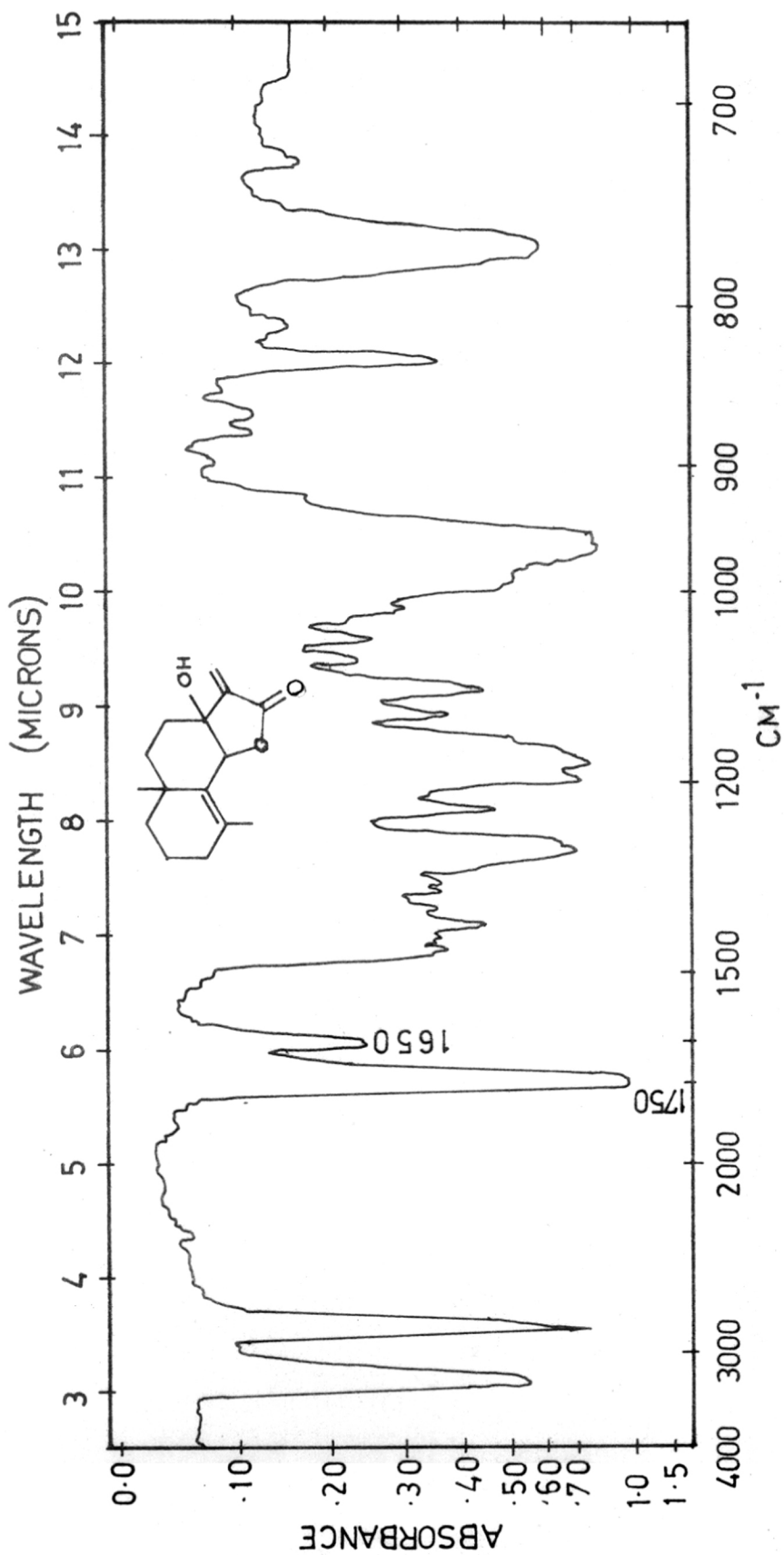
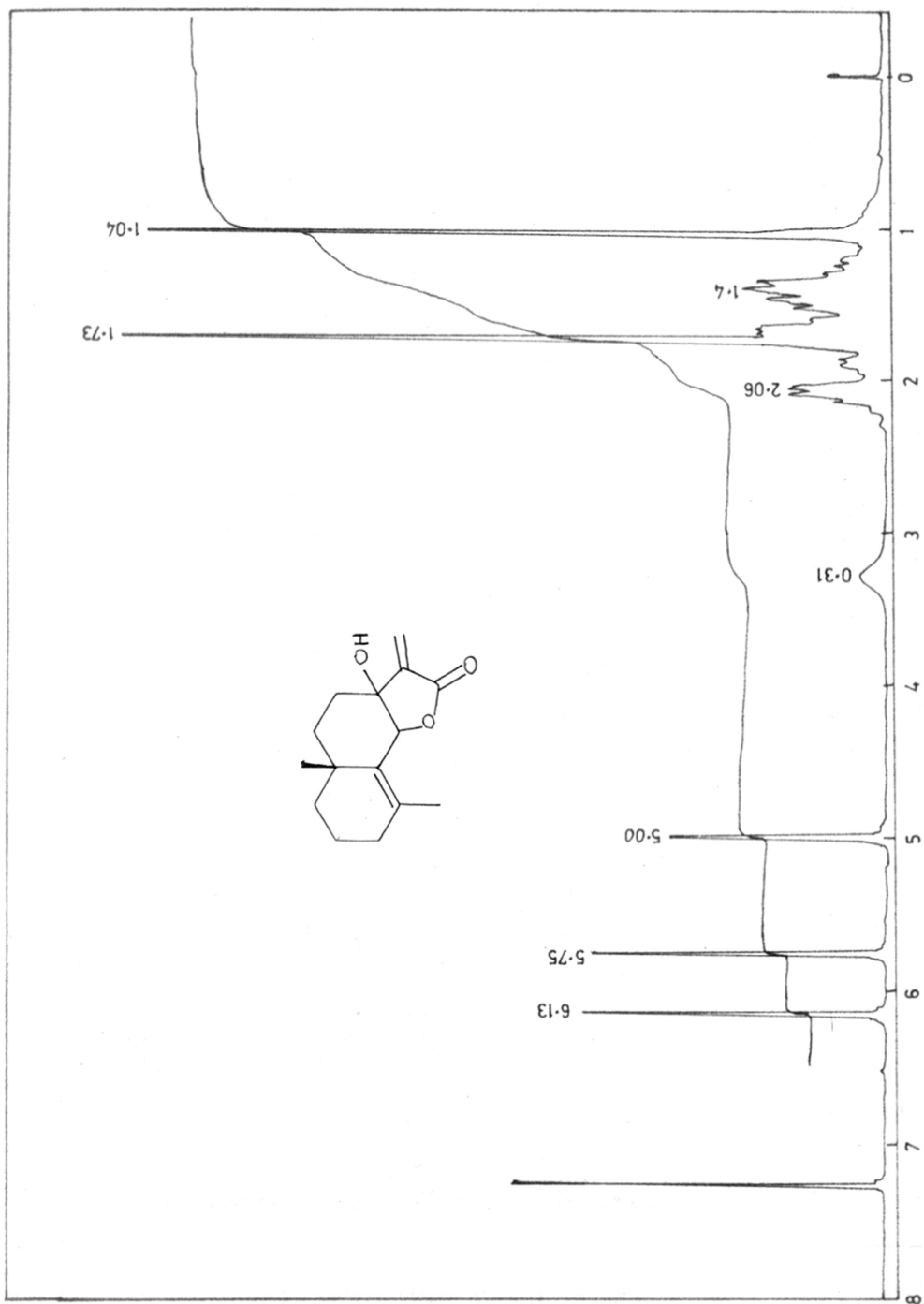
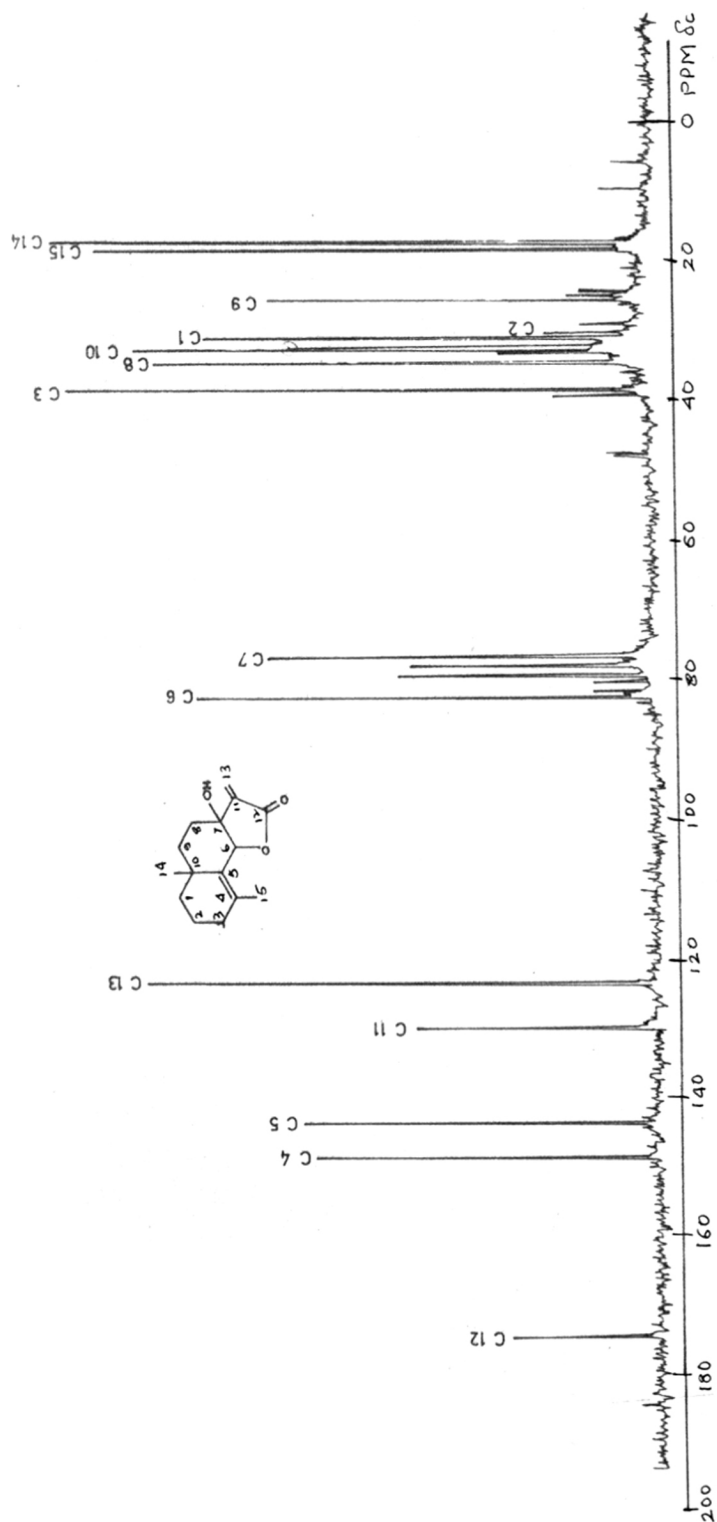


FIG.1: I R SPECTRUM OF COMPOUND (I)

FIG.2 ^1H NMR SPECTRUM OF COMPOUND (I) (90 MHz)

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

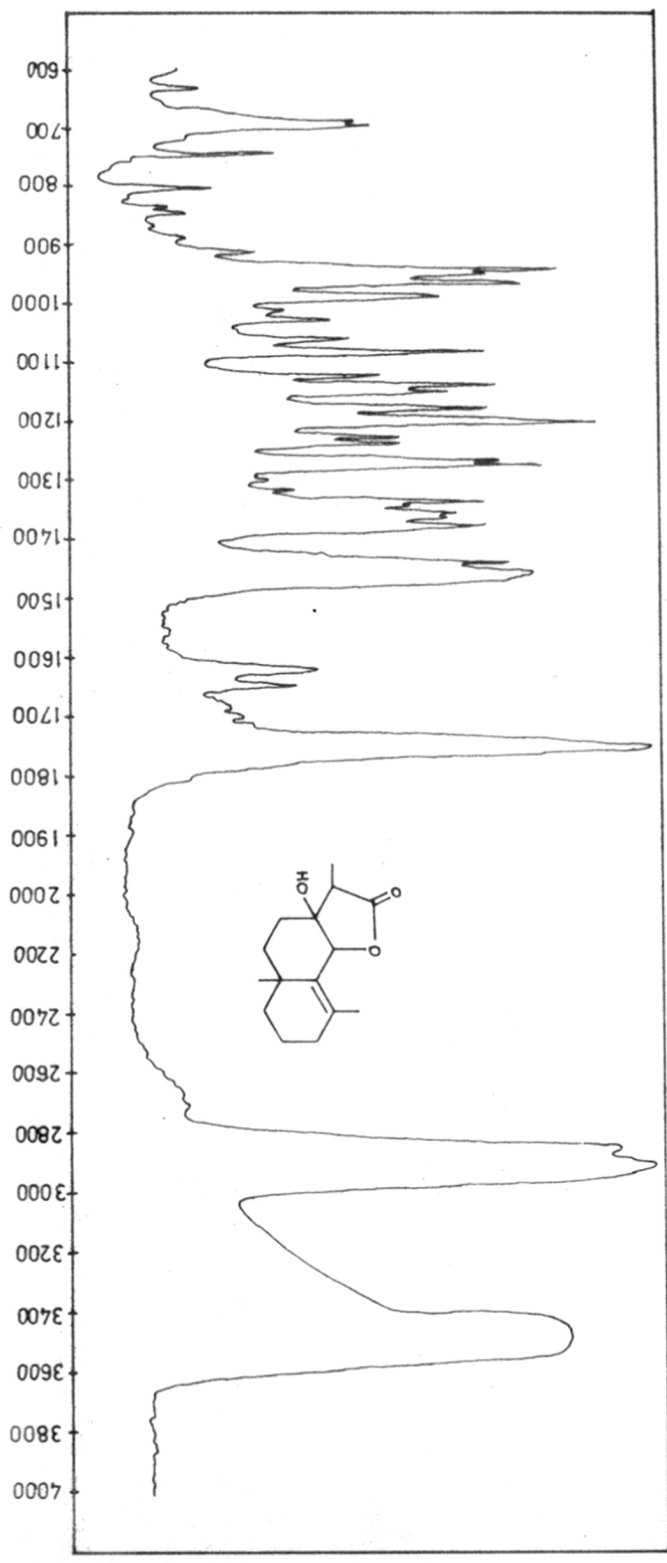
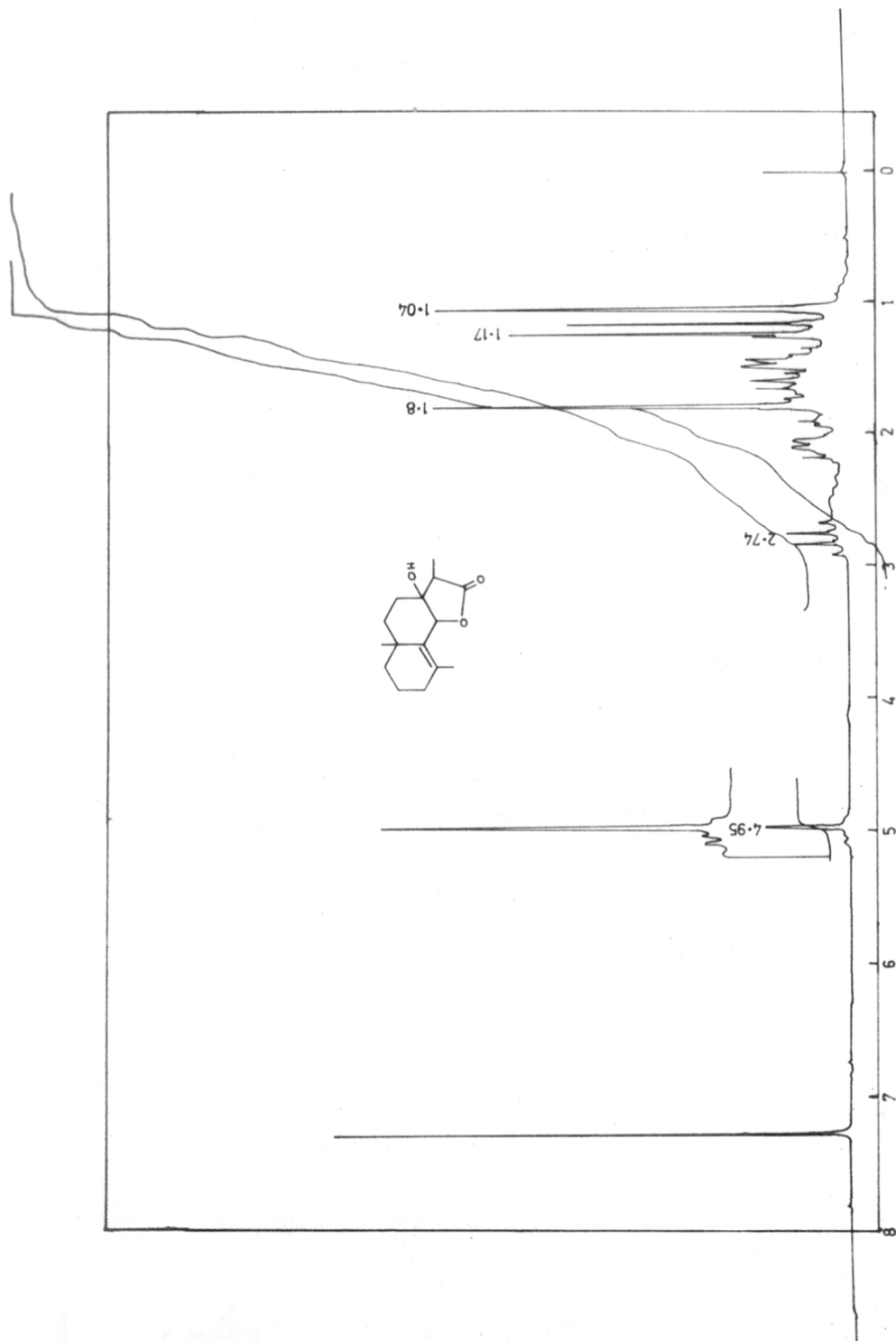


FIG 4: I R SPECTRUM OF DIHYDRODERIVATIVE (IV)

FIG 5: ^1H NMR SPECTRUM OF DIHYDRODERIVATIVE (IV) 90 MHz

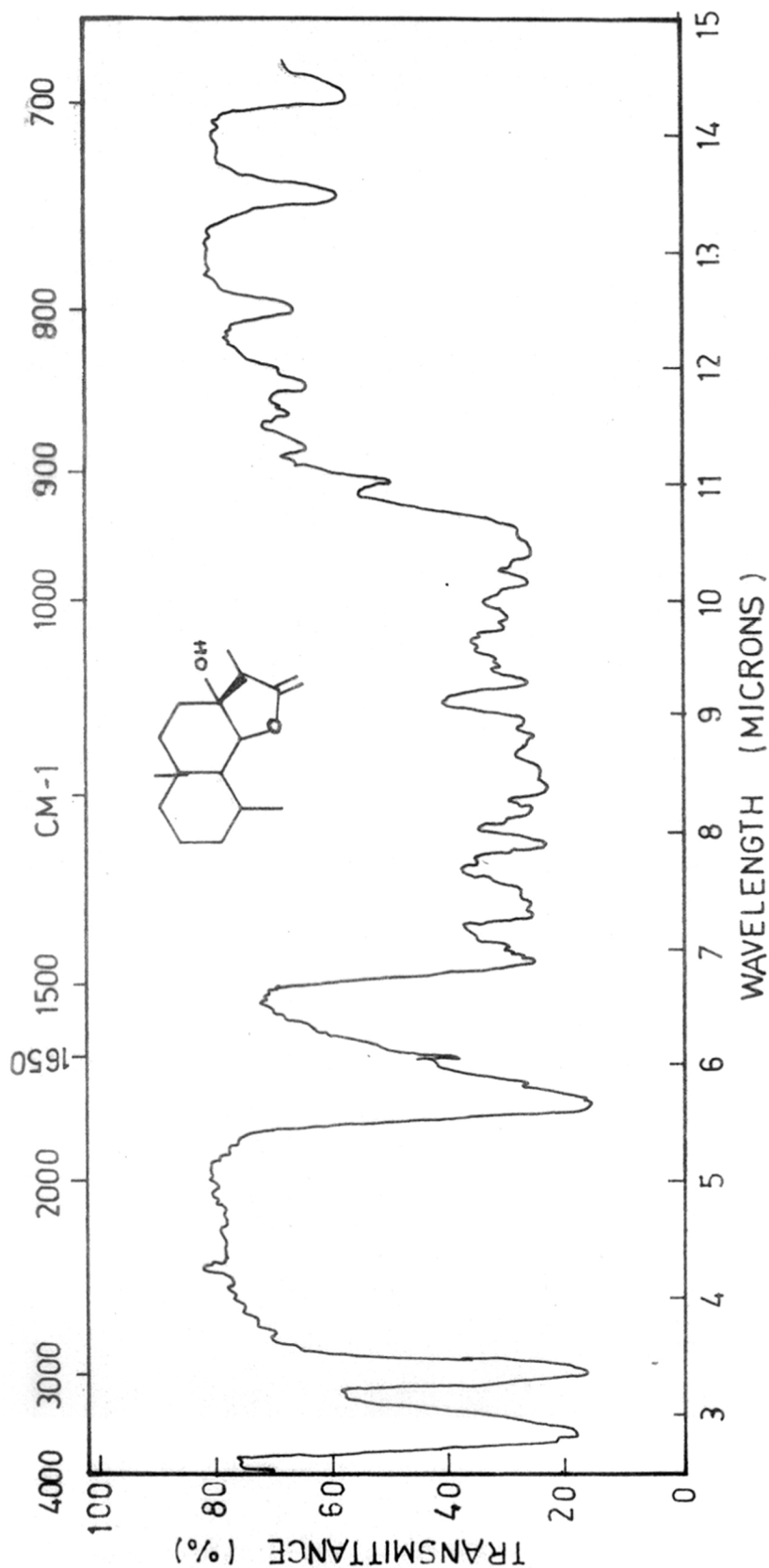


FIG. 6: I'R SPECTRUM OF TETRAHYDRO DERIVATIVE

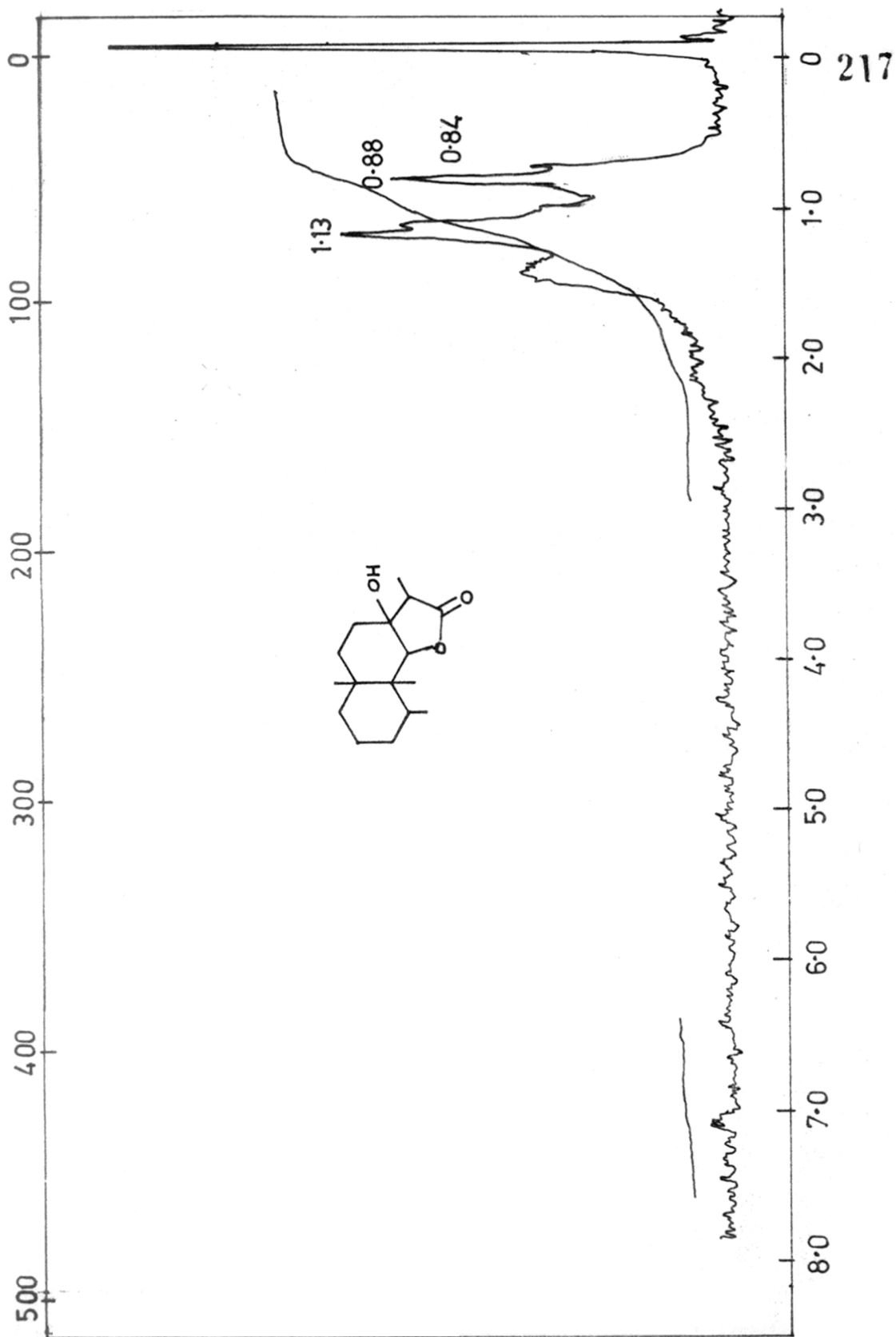


FIG.7: ¹H NMR SPECTRUM OF TETRAHYDRODERIVATIVE

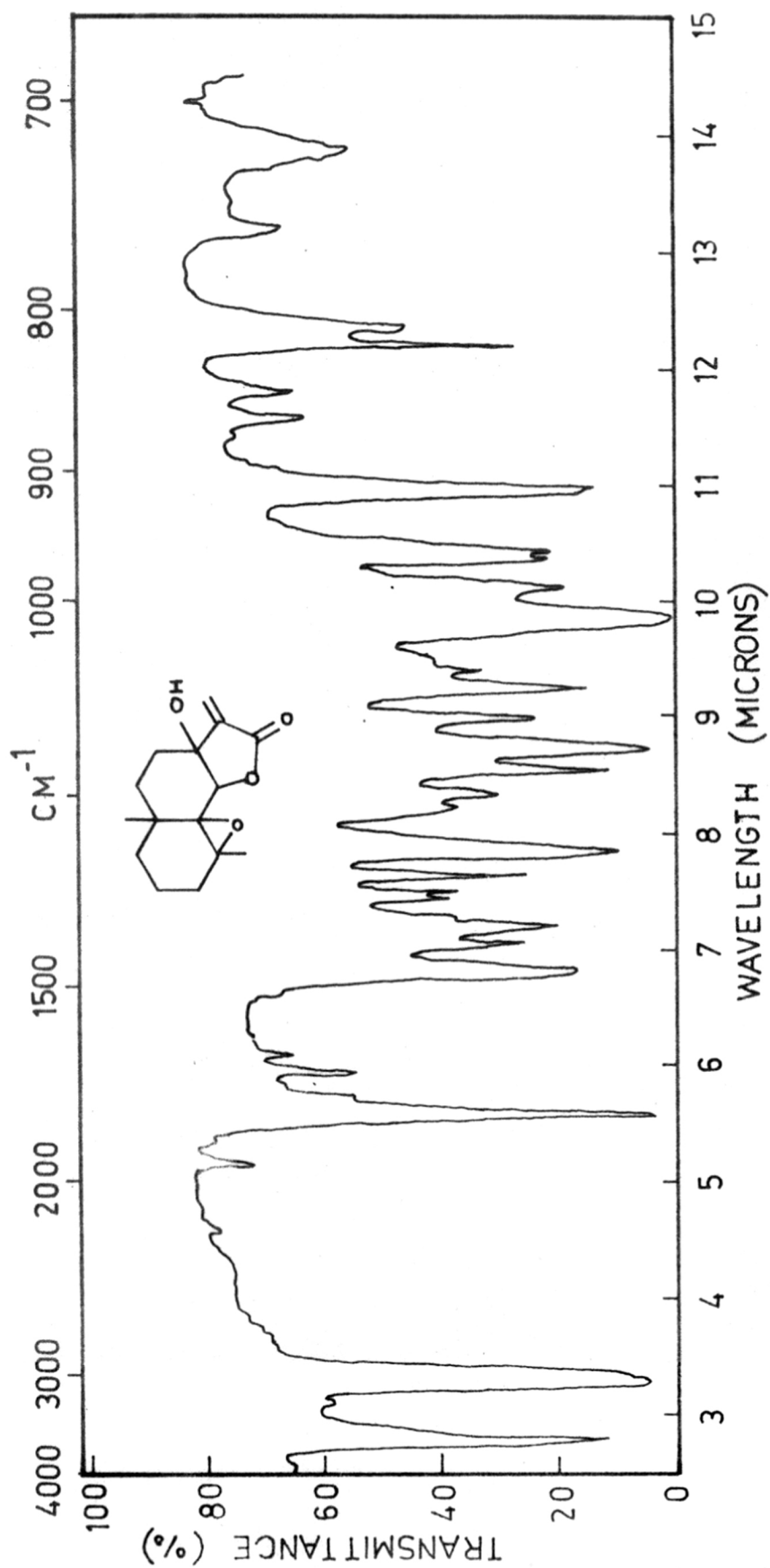
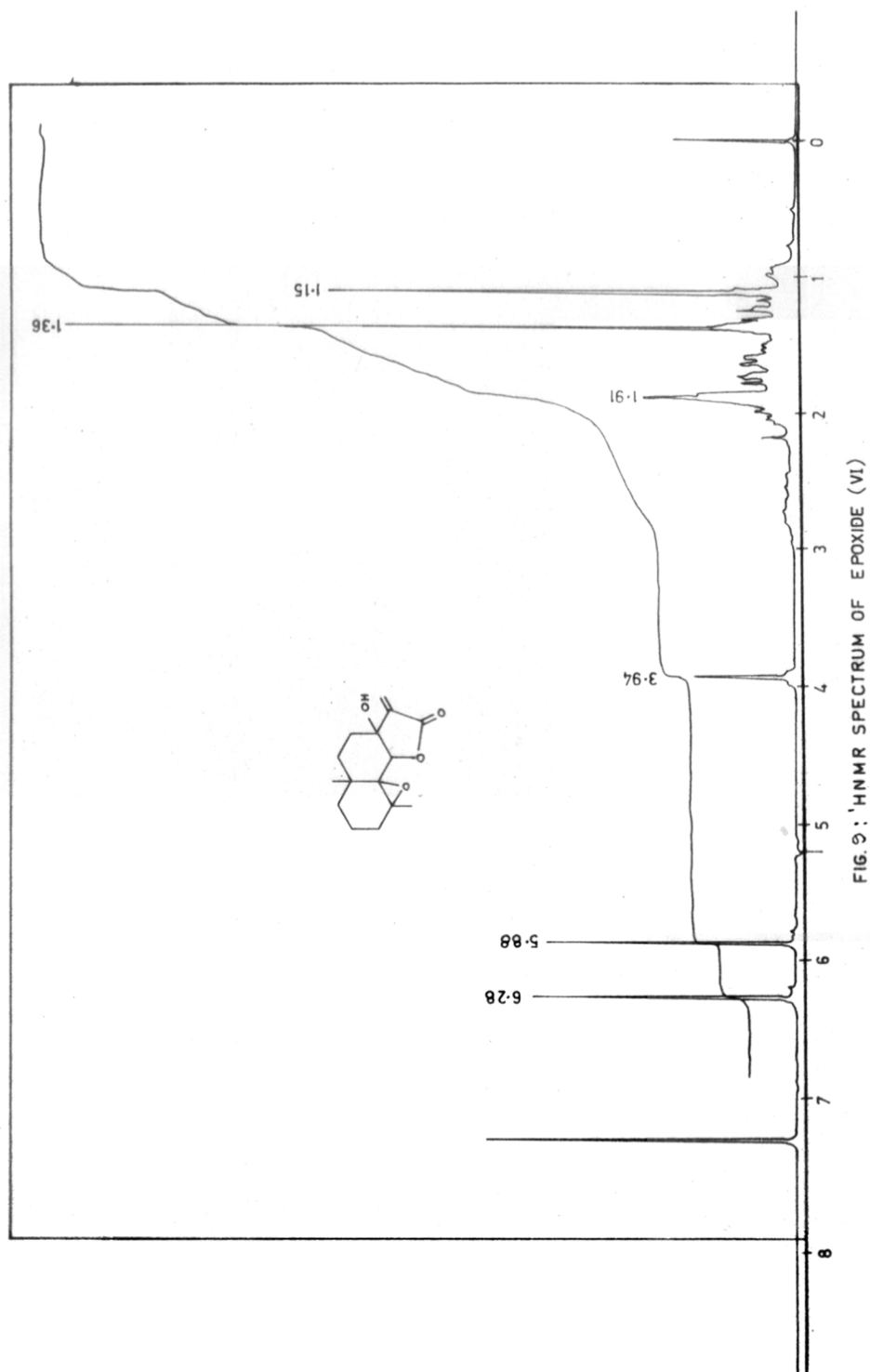
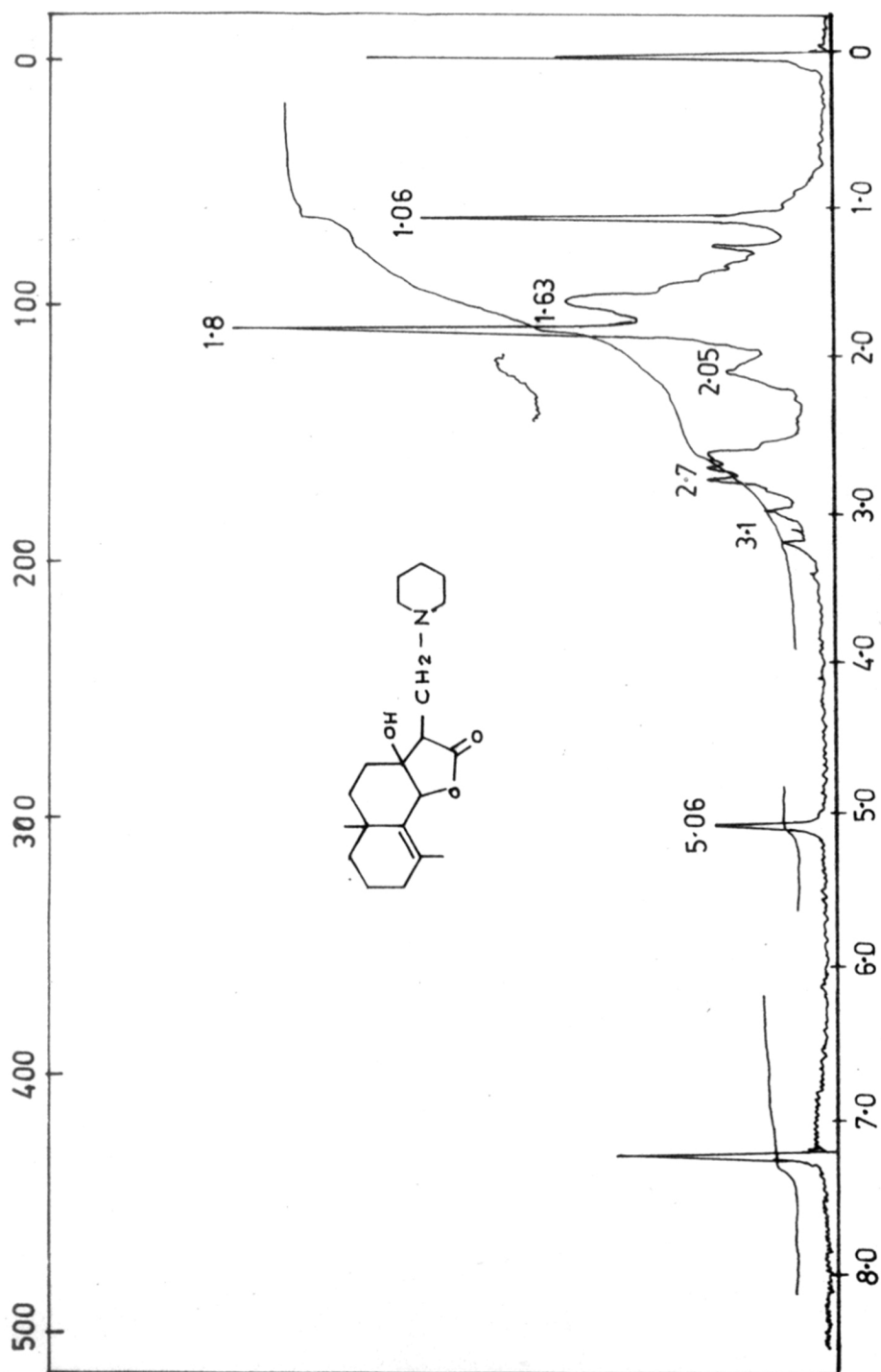


FIG. 8: I R SPECTRUM OF EPOXIDE



FIG. 10 ^1H NMR SPECTRUM OF PIPERIDINE ADDUCT

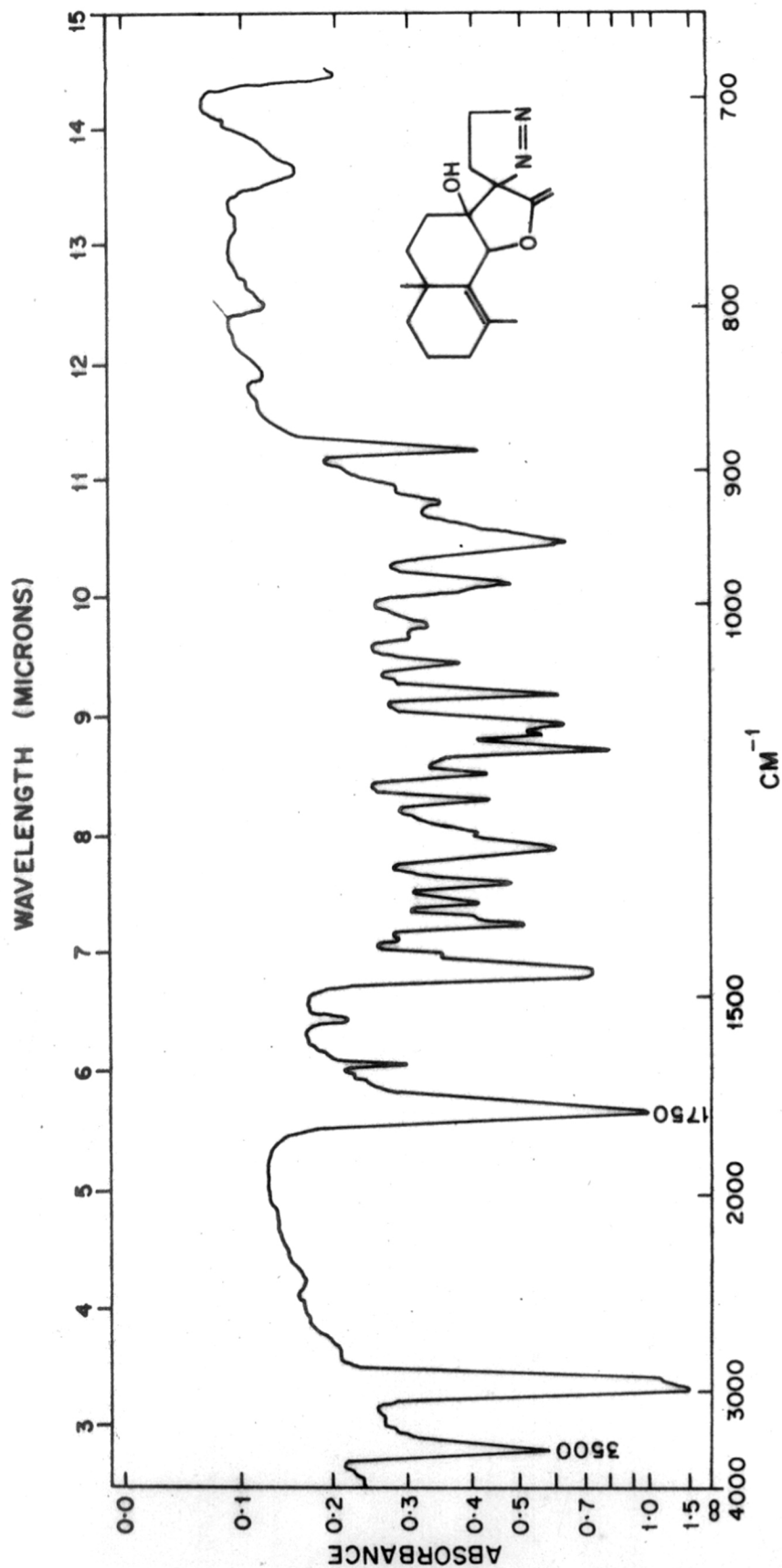
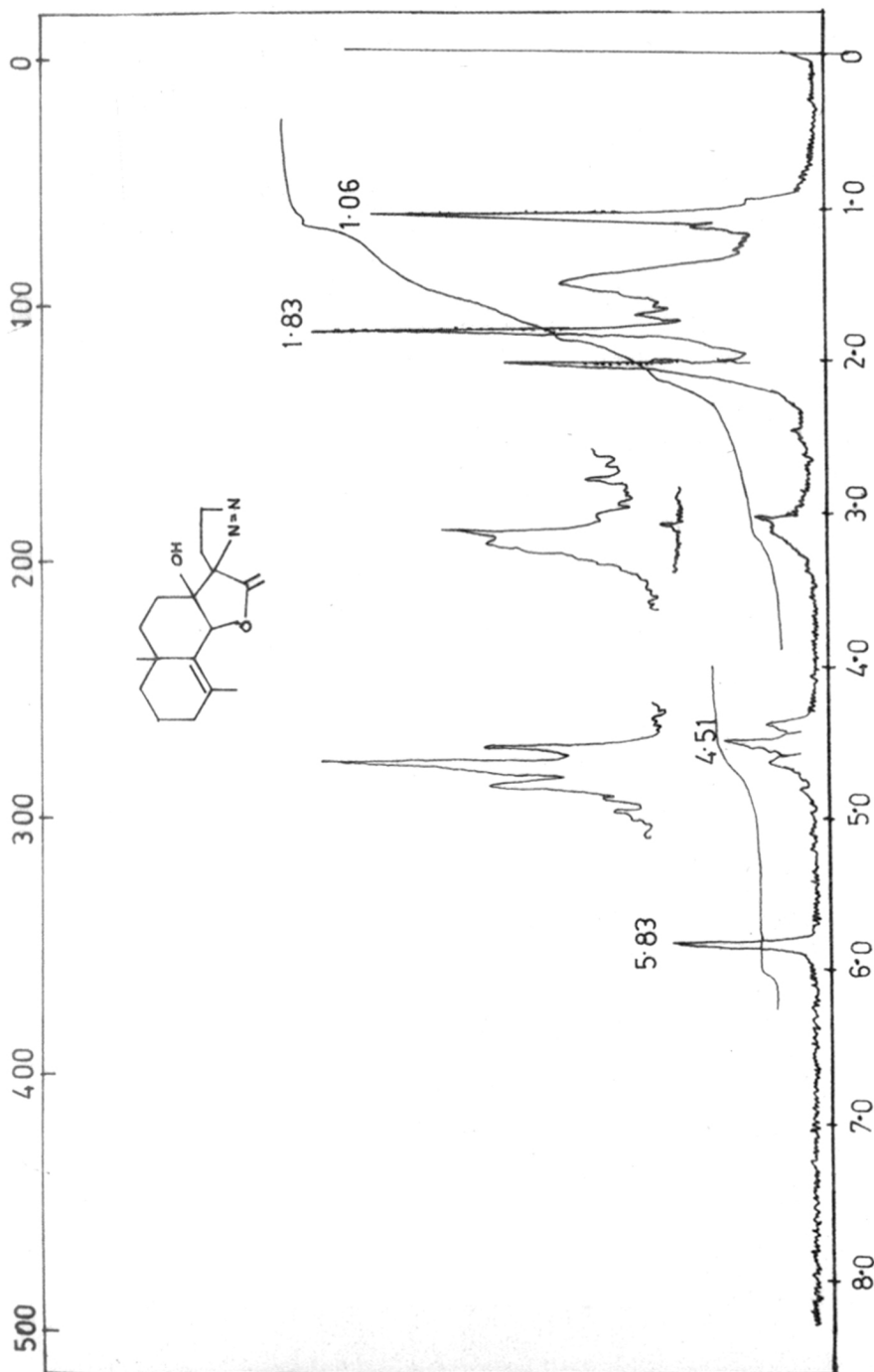


FIG. 11 : IR SPECTRUM OF PYROZOLINE DERIVATIVE (VIII).

FIG. 12: ^1H NMR SPECTRUM OF PYROLOZOLIN DERIVATIVE

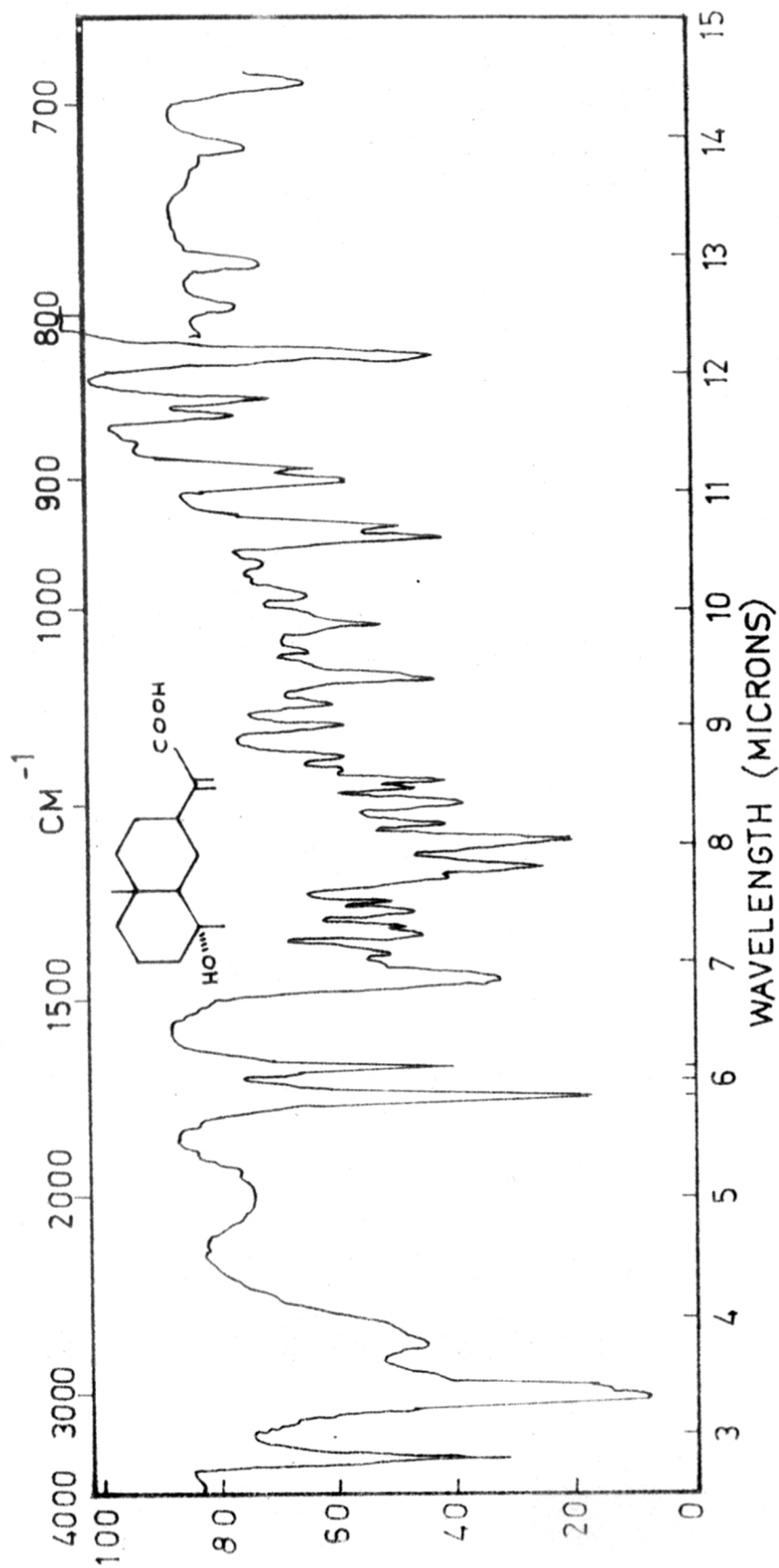
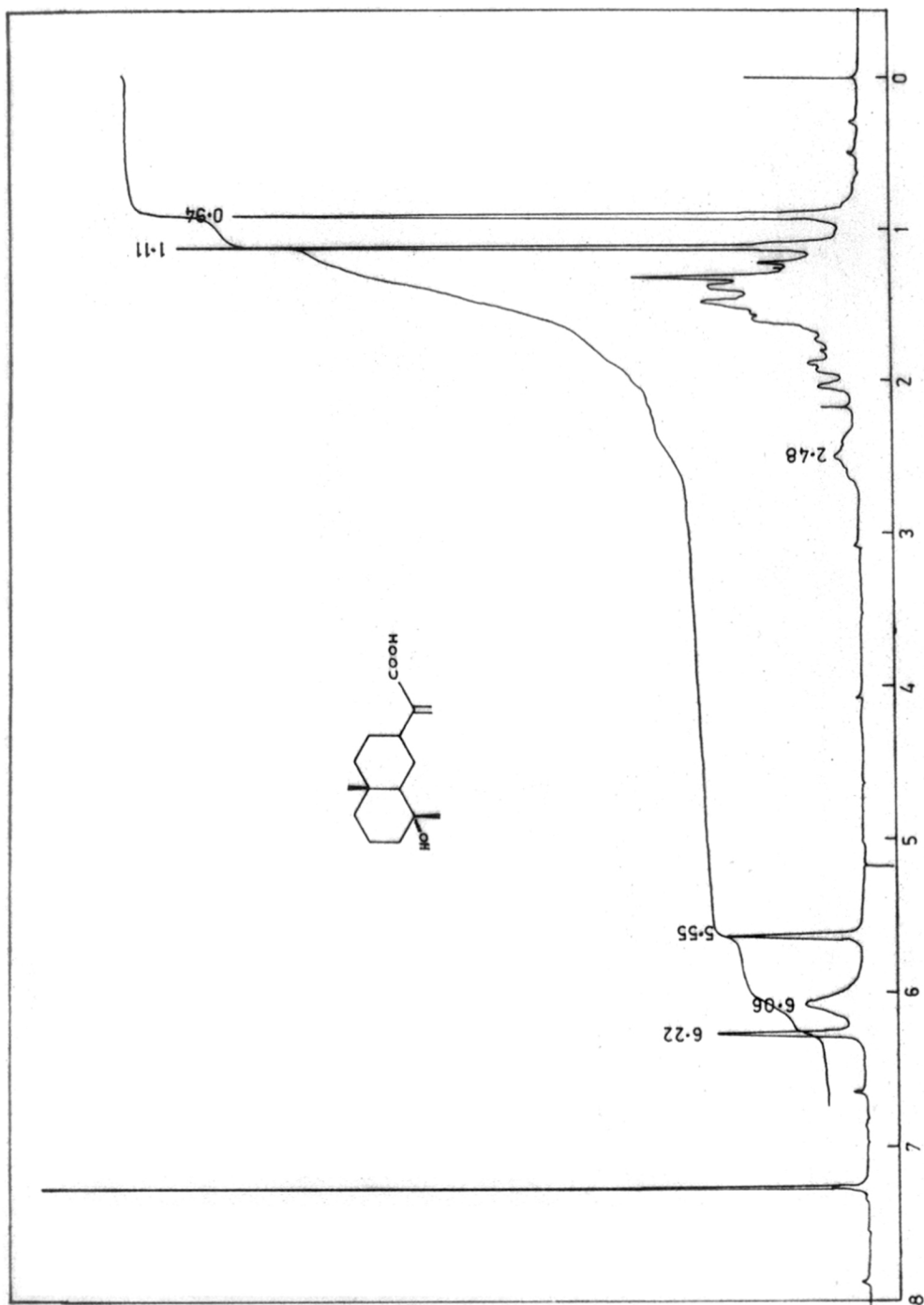
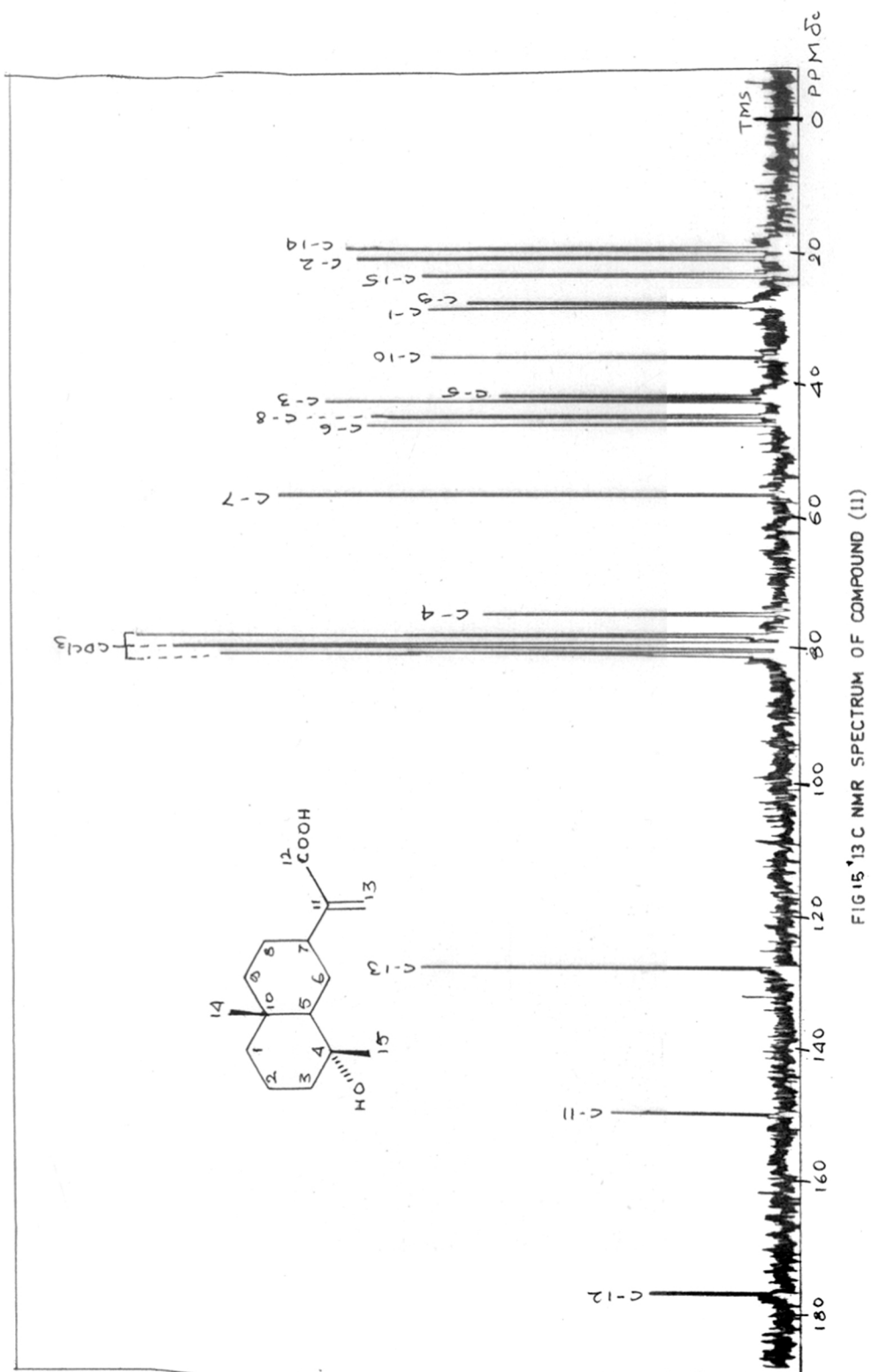


FIG.13: I R SPECTRUM OF COMPOUND (II)

FIG. 14 ^1H NMR SPECTRUM OF COMPOUND (II)



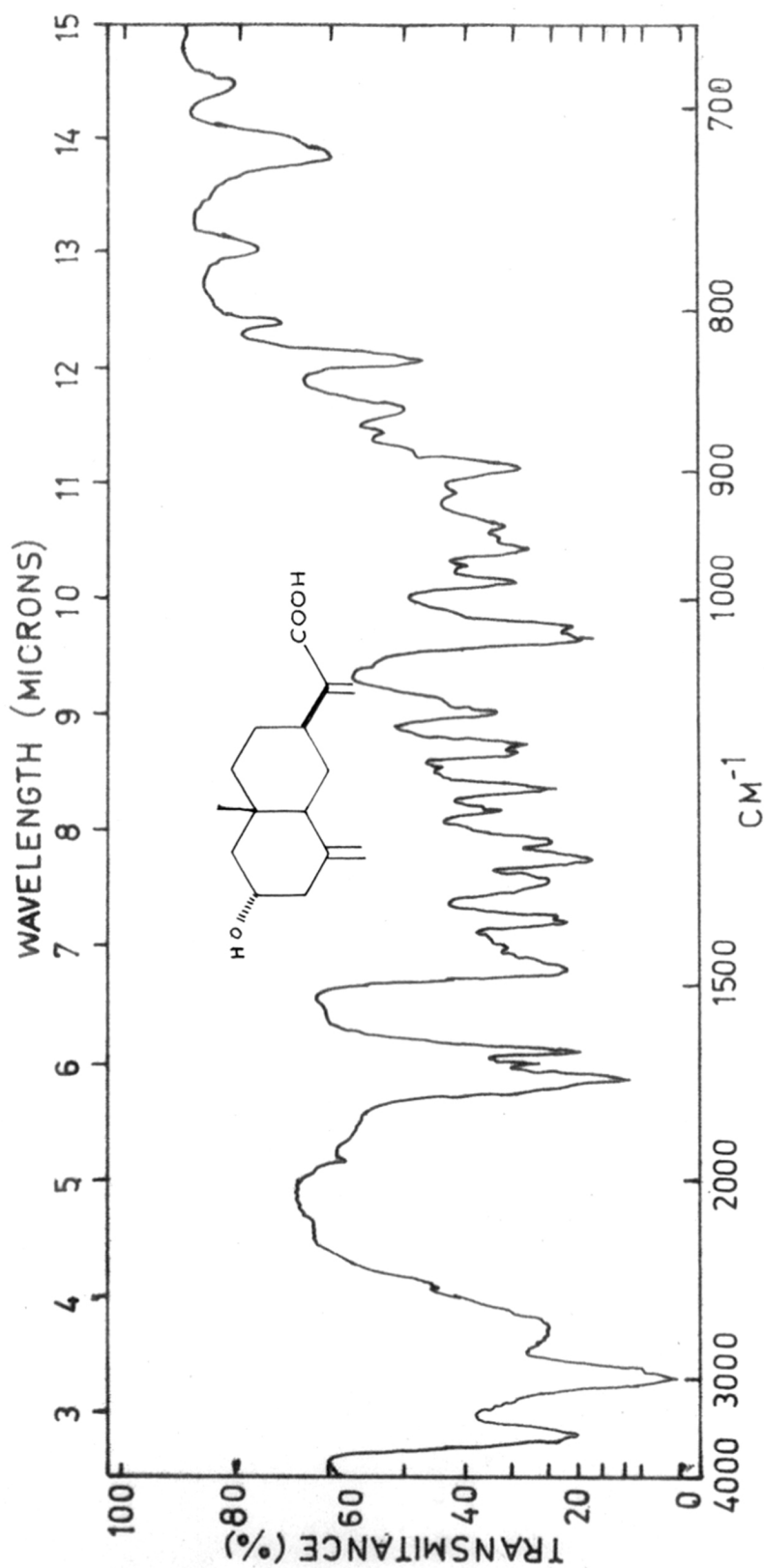
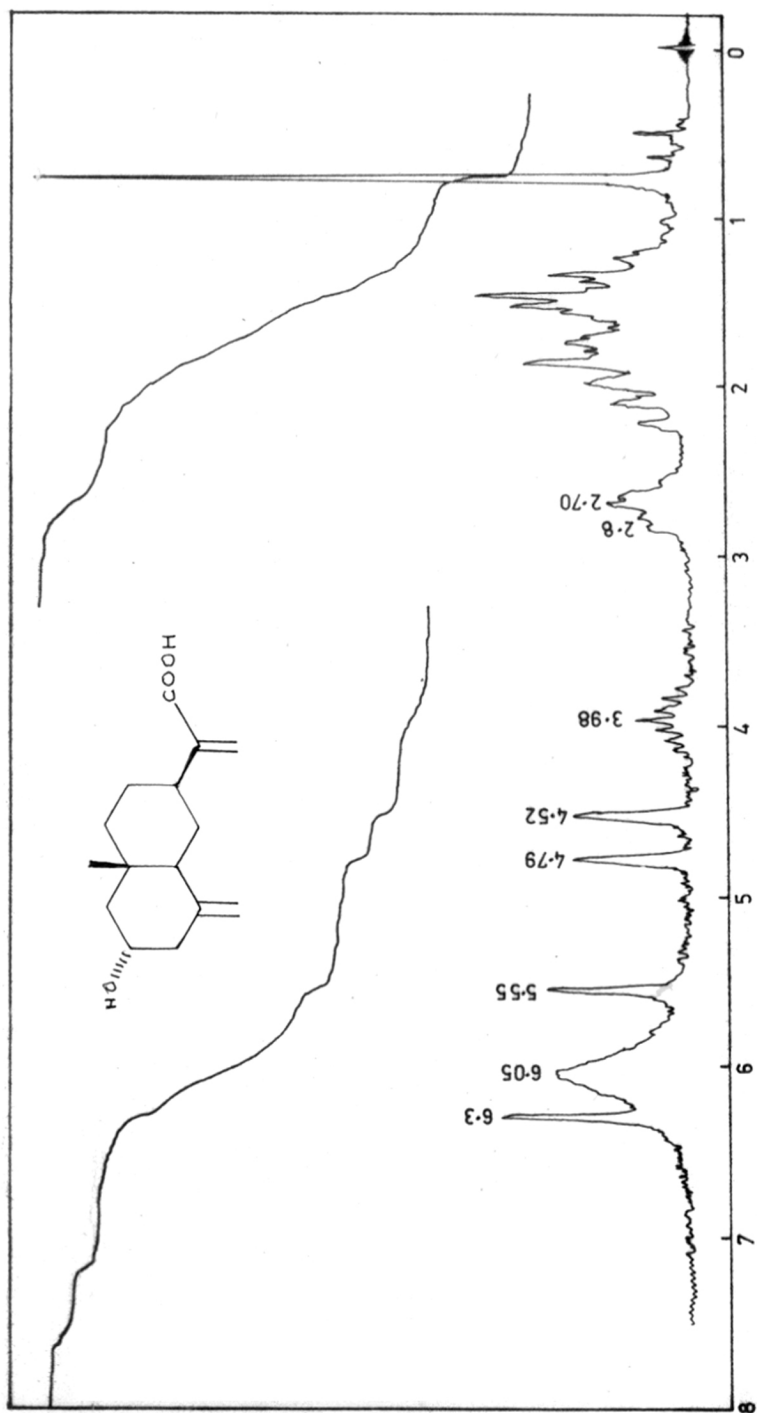


FIG 16: I R SPECTRUM OF COMPOUND (III)

FIG 17: ^1H NMR SPECTRUM OF COMPOUND (III)

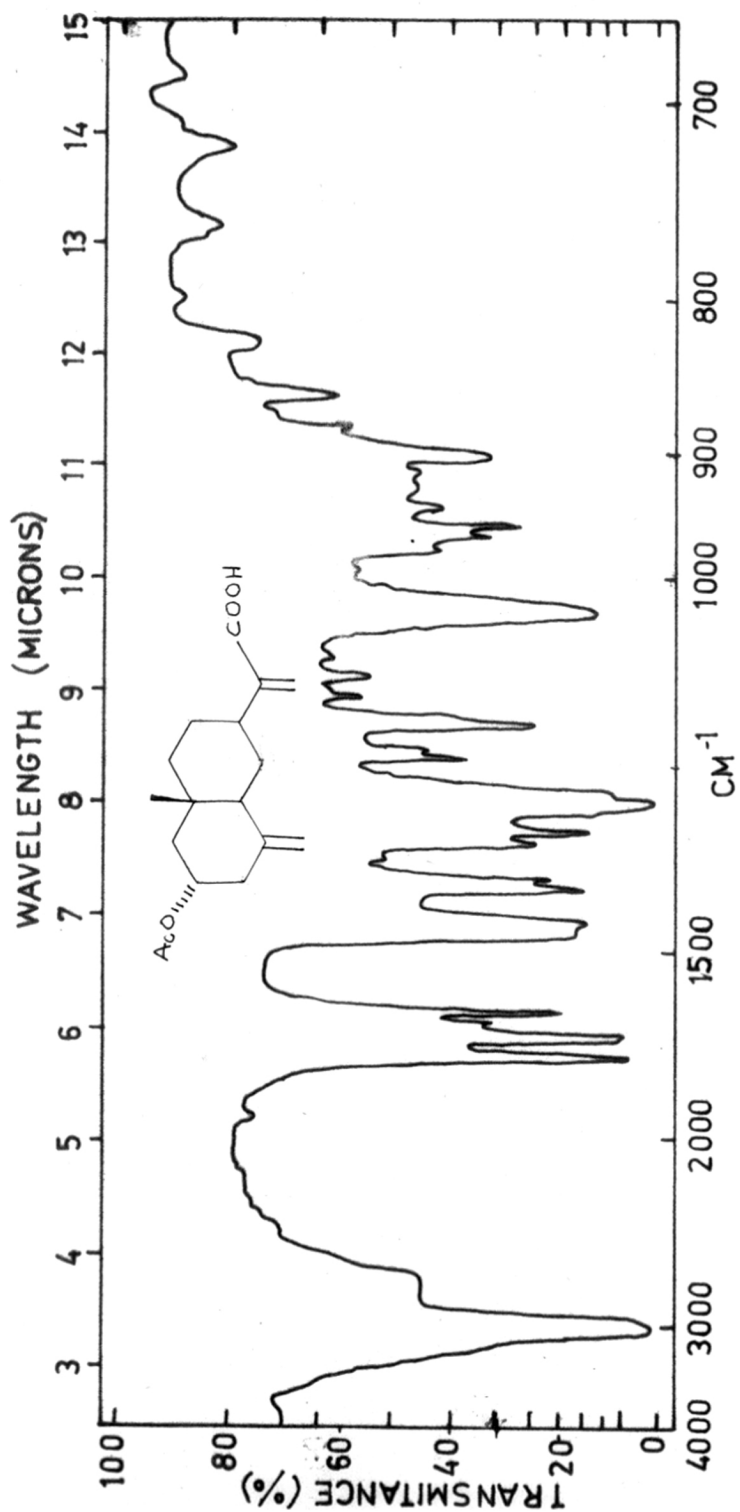


FIG 18: I R SPECTRUM OF ACETATE (VIII)

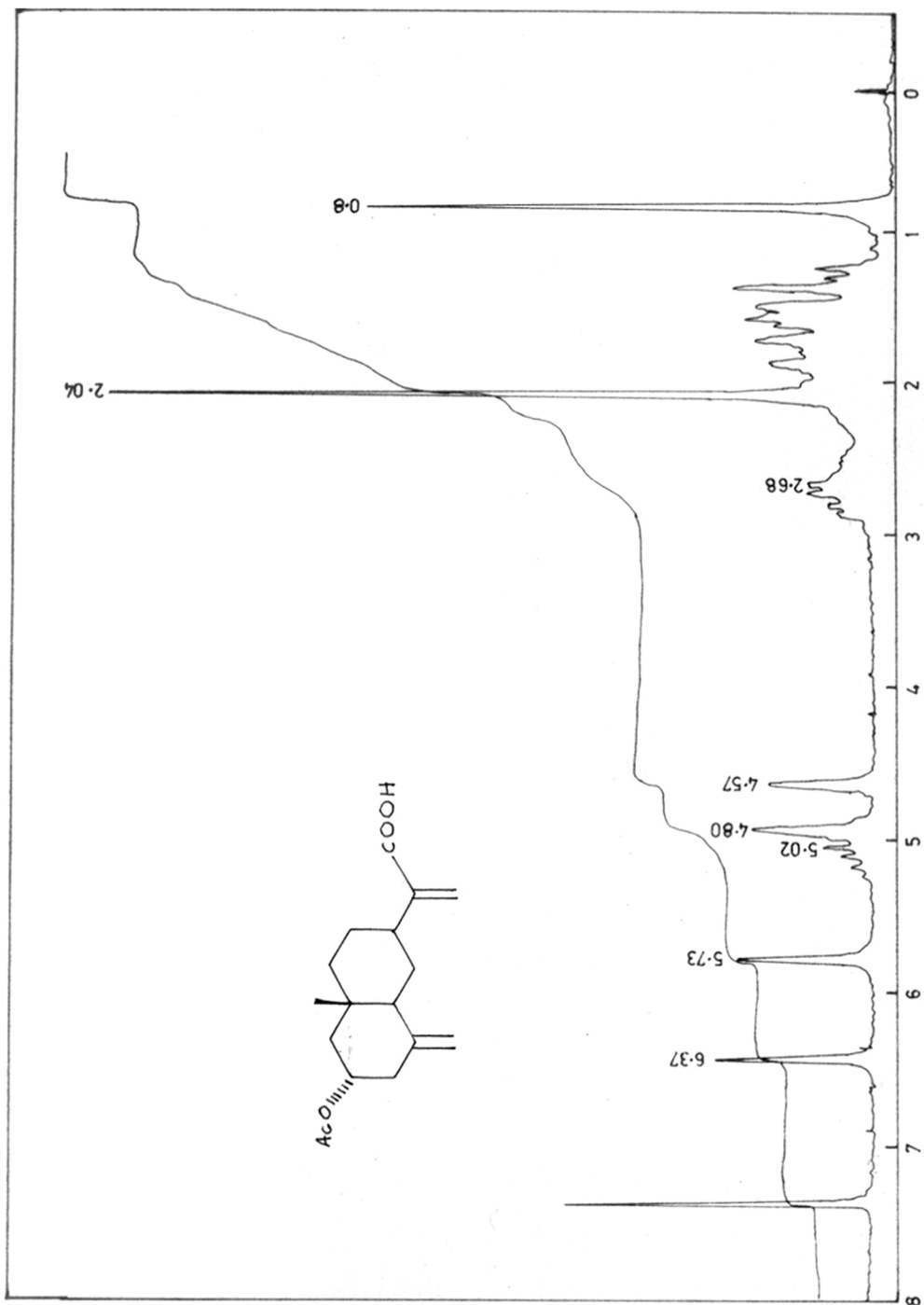
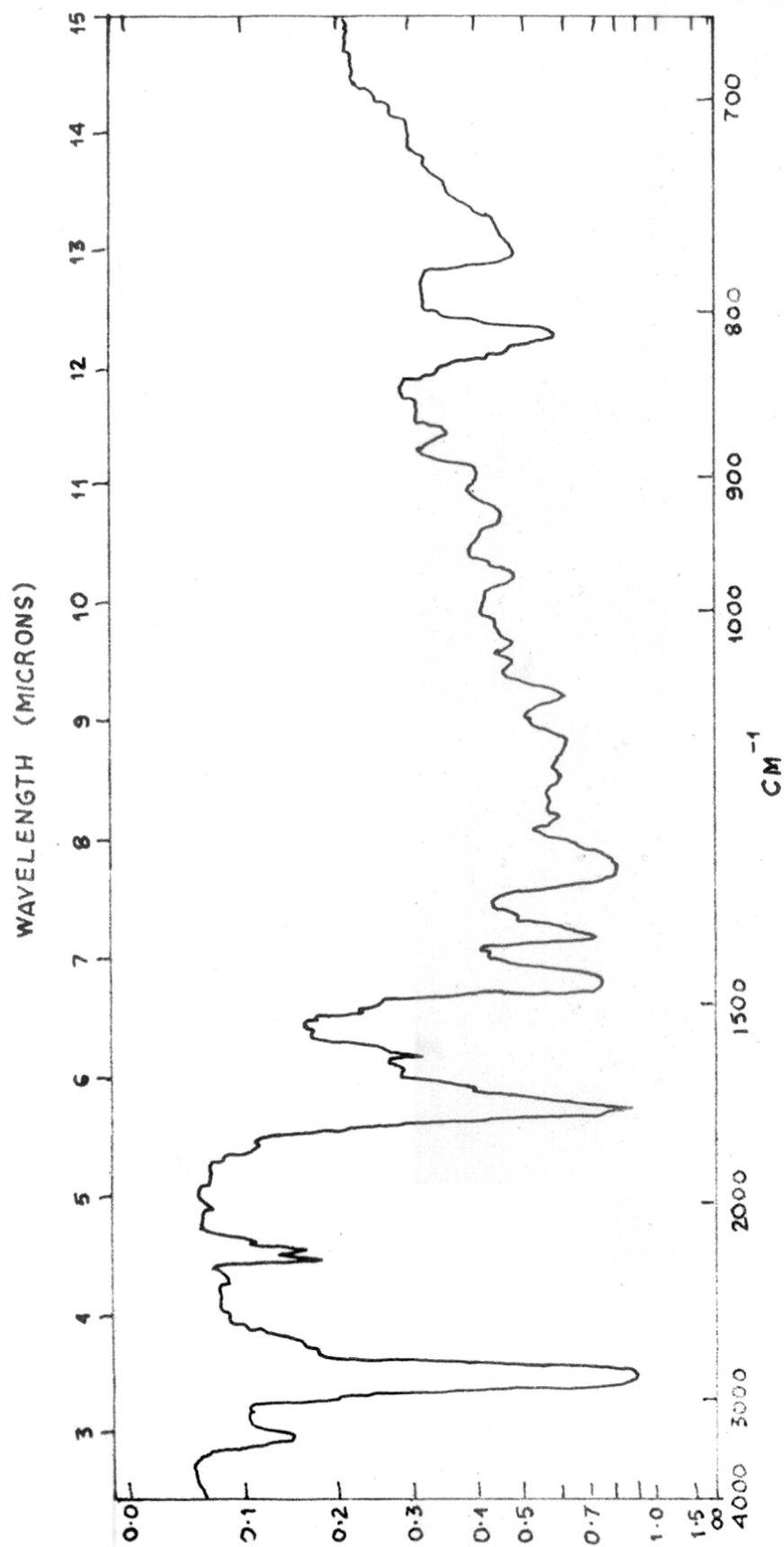
FIG. 12. ^1H NMR SPECTRUM OF MONOACETATE (VIII) (90 MHz)

FIG 20 : IR SPECTRUM OF ACETYLINIC COMPOUND



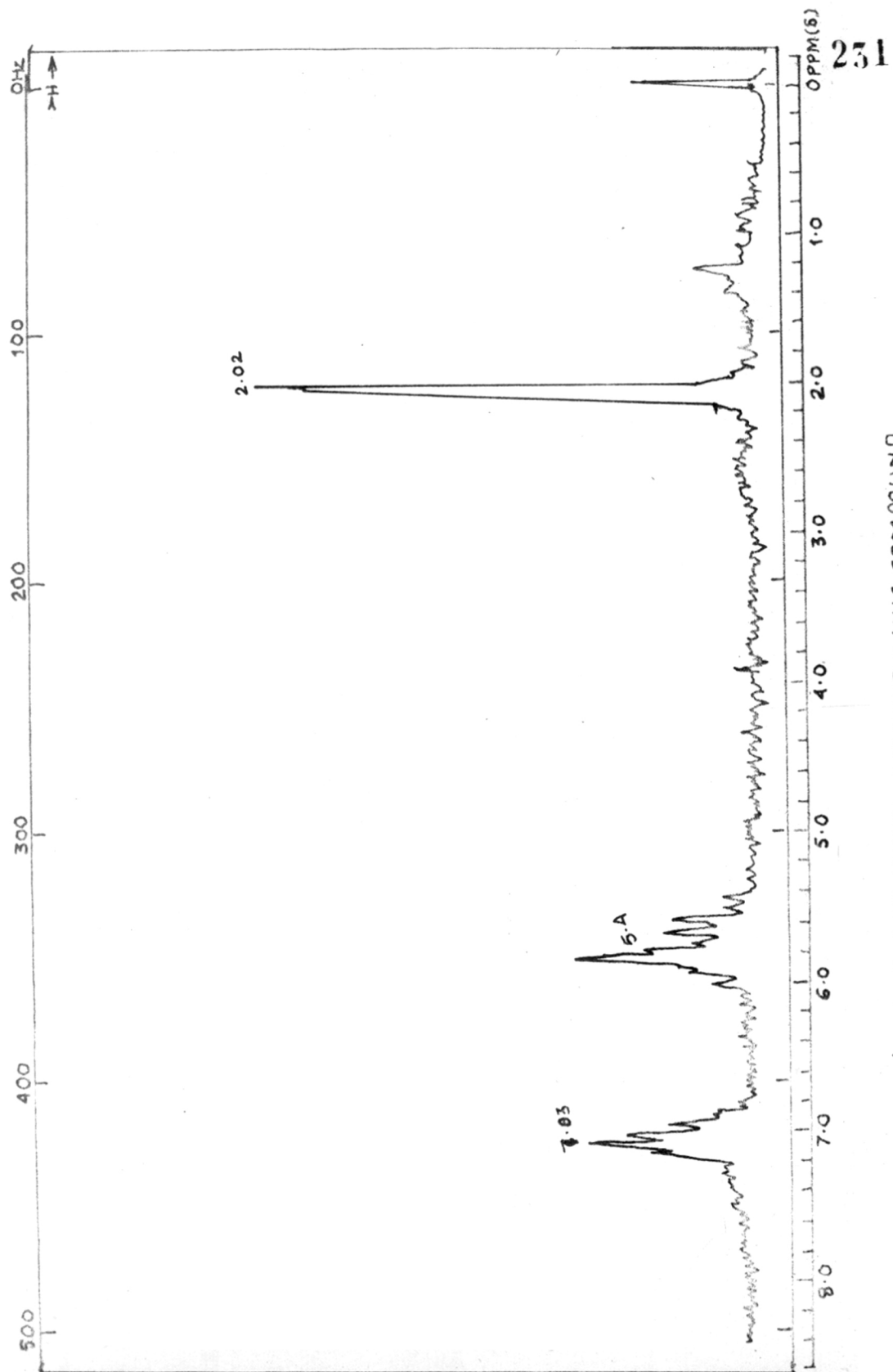


FIG. 21: ^1H NMR SPECTRUM OF ACETYLINIC COMPOUND

CHAPTER V

STRUCTURE ACTIVITY RELATIONSHIP OF
INSECT ANTIFEEDANT AGENTS

I N T R O D U C T I O N

GENERAL:

Pesticide chemicals have played a significant role in increasing agricultural production and productivity. However the continuous use of insecticides has not only caused leath in man through poisoning but also caused resistance in pest population and destroyed parasites, predators and pollinators. In these circumstances research needs are aimed at establishing alternative means of insect control i.e. non-insecticidal approaches. One of the wisest approaches to alternative insect control would come from the use of the knowledge on natural products able to influence insect chemosensory behavior as attractants, repellants, stimulants, antifeedants and arrestants.

Studies of natural phenomenon of antifeeding (i.e. no consumption of plant material by insect) could reveal the presence of new antifeeding substances in plants and provide correlations between chemical structure and antifeeding activities. Antifeeding could be of great value in protecting crops from noxious insects as alternative insect control means to insecticides.

The feeding behaviour of the insect can be divided into four steps: (1) host plant recognition and orientation, (2) initiation of feeding (3) maintenance of feeding and (4) cessation of feeding. Antifeedant is concerned with steps (2) and (3). The term antifeedant is defined as

a chemical that inhibits feeding but does not kill the insect directly, the insect remaining near the treated leaves and dying through starvation. The term relative antifeedant is used for compounds retarding the feeding of larvae only for defined time, whereas the term absolute antifeedant is used for the compound whose activity is not limited to a short span of time, instead the larvae eventually starve to death. Gustatory repellants, feeding deterrents and rejectants are synonymous with antifeedants.

BIOASSAY OF ANTIFEEDANTS:

a) Electro physiology: The most revealing bioassay for the phagostimulants and deterrents is that of electrophysiology with the eight sensilla at the tip of the maxillary palp which correspond to test buds¹. A microelectrode is inserted into the maxillary palp and another into the one of the sensillum. The tip of the sensillum is brought into contact with the filter paper impregnated with the test solution and the scoring, which is recorded as impulses per second, is done with an oscilloscope¹.

b) Leaf disk method: the leaf disk method is the most conventional method of bioassay of antifeedants². The method is shown in Fig. 1. Leaf disks made with a 20 mm cork, borer are immersed in test acetone solution for two seconds. Immersion for longer time should be avoided because the phagostimulants are extracted out. These leaf disks are placed in a petridish with control leaf disks and the test

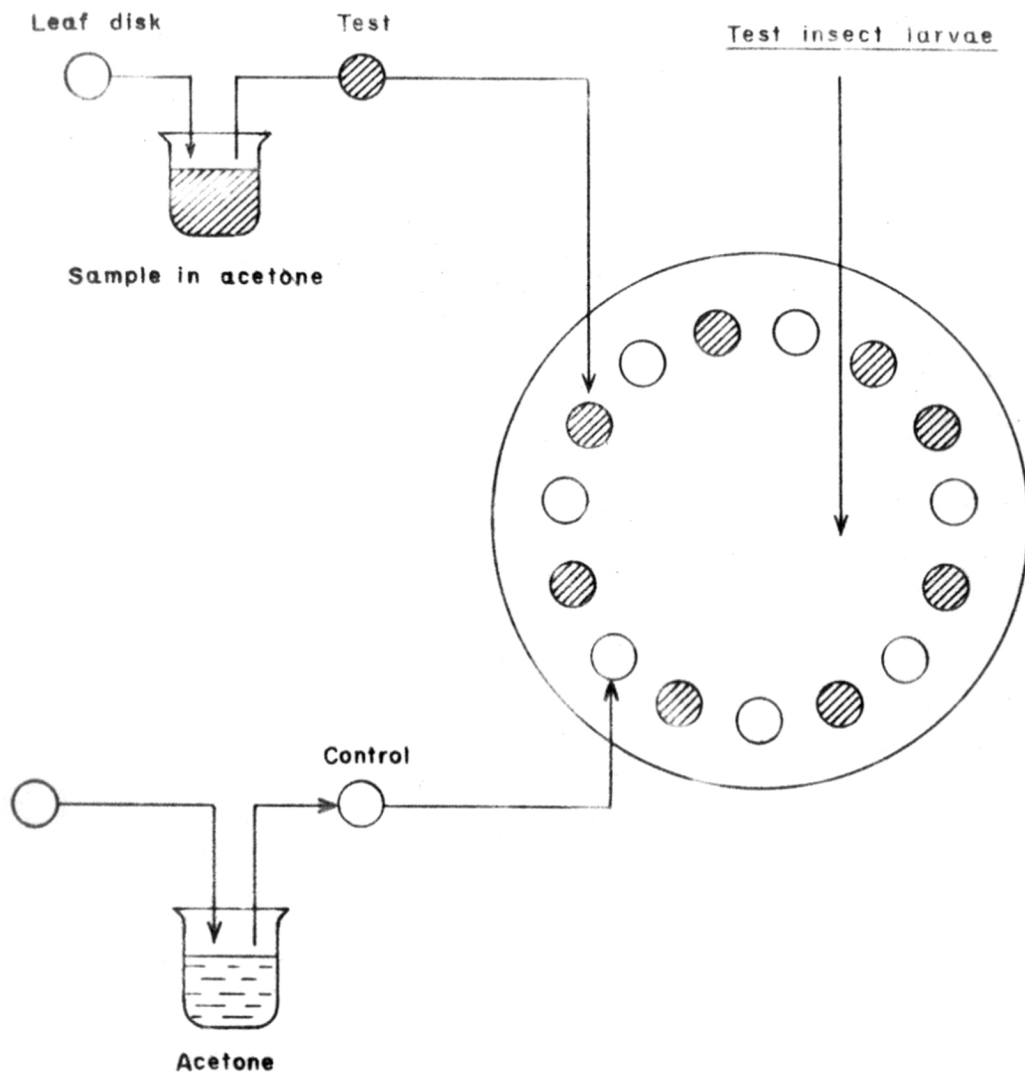


FIG. 1 THE LEAF DISK BIOASSAY A CHOICE METHOD

insect larvae. This constitutes a "choice" test, whereas the tests performed by putting the treated leaf disks and the control in separate petri dishes constitute the "no choice" test. The scoring is carried out over a period of upto 2 days depending on the antifeedant potency.

Alternatively the sample solution can be applied directly onto several leaves of plants in a pot and scored against control leaves.

ANTIFEEDANT CHEMICALS:

The antifeedant chemicals show a wide range of structural diversity. A large number of compounds belonging to different classes such as terpenoids, coumarins, flavonoids, quinones, lignans, phenyl propanoids, steroids and metal complexes are shown to possess the insect antifeedant activity. A number of review articles on various aspects of insect antifeedants have appeared in last decade or so³⁻¹². It is beyond the scope of this small review to accommodate all the classes of insect antifeedants, hence we will restrict ourselves to description of the (1) sesquiterpenic, (2) diterpenic and (3) aromatic insect antifeedants and the change in the activity in relation to the structure.

1) Sesquiterpenes:

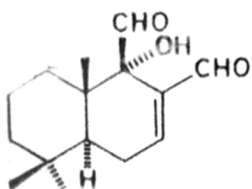
Not many sesquiterpenoids are known to exhibit the antifeedant activity. Some of the sesquiterpenoids which show antifeedant activity are discussed below.

Polygodial (1) ugandensifial (2) and warburginal

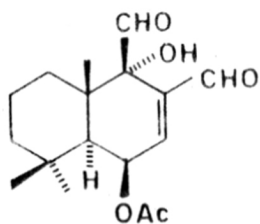
(3) Chart I) isolated from the bark of *Warburgia stuhlmannii* and *W. ugandensis*¹³ showed antifeedant activity against *Spodoptera exempta*. Out of the three compounds isolated, warburginal (3) is by far the most active antifeedant. Some structure activity relationship studies carried out with polygodial (1) revealed that the activity was lost when one or both aldehyde groups were reduced to hydroxy groups or oxidized to carboxylic groups. Epimerization of the equatorial 9 β -CHO to more stable axial 9 α -CHO also resulted in loss of activity¹³.

Shiromolol diacetate (4) shiromolol monoacetate (5) and shiromool (6) (Chart I) three sesquiterpene alcohols belonging to germacrane class, isolated from *Parabenzoin trilobum* Nakai are reported to show feeding inhibitory activity against *Spodoptera littoralis*¹⁴. Other examples of sesquiterpenoid antifeedants are the antifeedant activity of a secoaromadendrane type sesquiterpene (7) (Chart I) isolated from *Plagiochilla* sp.¹⁵ against *Spodoptera exempta* and the antifeedant activity of nerolidol a sesquiterpene alcohol isolated from *Melaleuca leucadendron* against gypsy moth larvae¹⁶.

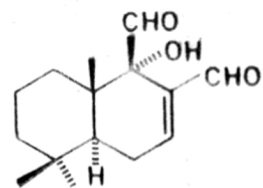
The feeding deterrent activity of glaucolide A (8) (Chart I) a sesquiterpene lactone isolated from *Vernonia gigantea* and *V. glauca* against the larvae of *Spodoptera eridania*, *S. frigiperia* and *S. orhithogalli*¹⁷ is perhaps the first report on the feeding deterrent activity of sesqui-



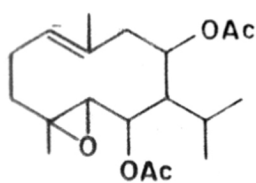
(1)



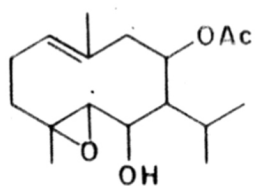
(2)



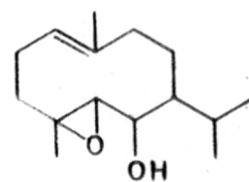
(3)



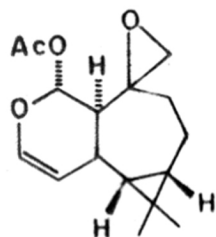
(4)



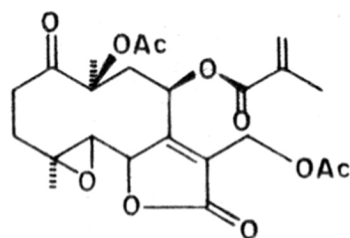
(5)



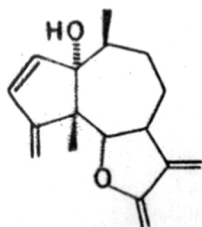
(6)



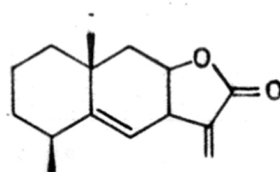
(7)



(8)



(9)



(10)

CHART (I)

terpene lactones. In addition to glaucolide-A (8) parthenin (9) a pseudoguaianolide and alantolactone (10) (Chart I) are also reported to show antifeedant activity^{18,19}

2) Diterpenoids:

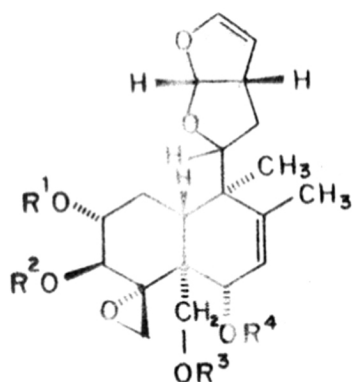
A number of diterpenoids having clerodane skeleton are reported to possess insect antifeeding activity. Clerodendrins A and B (12) and (13) isolated from clerodendron tricotomum thunberg^{20,21}, clerodin (13)²² caryoptin (14), dihydroclerodin (17), dihydrocaryoptin (18), clerodin hemiacetal (19), caryoptin hemiacetal (20), caryoptinol (15) and dihydrocaryoptinol (21) isolated from Caryopteris divaricata Maxim^{23,24} and 3-epicaryoptin (16)²⁵ isolated from Clerodendron clamitosum all showed a strong antifeedant activity against Spodoptera litura. Chart II gives structures of all the compounds (11-21) and the Table 1 gives activities of all compounds.

Table 1 antifeedant activity of compounds 11-21

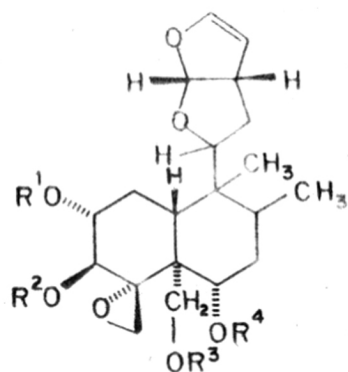
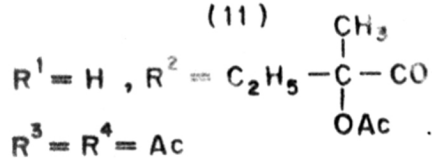
Compound	ppm ^a	Compound	ppm ^a
Clerodendrin A	300	Dihydroclerodin I	50
Clerodendrin B	200	Clerodinhemiacetal	50
Caryoptin	200	Caryoptinol	200
Dihydrocaryoptin	80	Dihydrocaryoptinol	100
Caryoptin hemiacetal	200	3 Epicaryoptin	200
Clerodin	50	3 Epidihydrocaryoptin ^b	100

a: The concentrations of the sample solution showing 100% antifeeding activity within 2 hr.

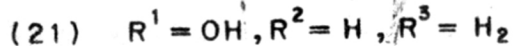
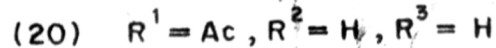
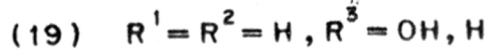
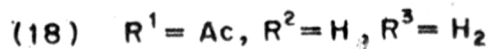
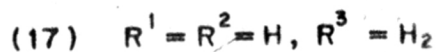
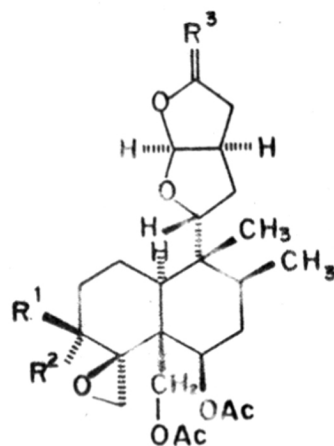
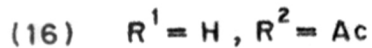
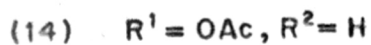
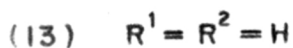
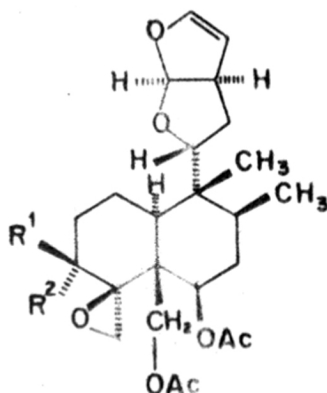
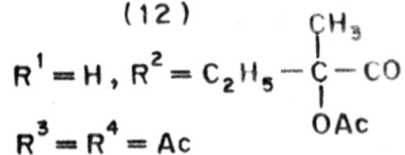
b: Catalytic reduction product of (21)



(11)



(12)



The antifeeding activities of a series of clerodin derivatives are much stronger than (Table 1) those of the caryoptin derivatives. The difference in the activities may be attributed to the presence of 3 β -acetoxy group in ring A. It could be concluded that the functional groups in the ring A contribute greatly to antifeeding activity of these diterpenes.

3) Aromatics: (a) Furocoumarins:

The furocoumarins constitute one of the major classes of insect antifeedants isolated from plants. Several furocoumarins isolated from Orixa japonica showed potent antifeedant activity against Spodoptera litura²⁶. The phloroglucinol type furocoumarins which bear 1,3,5-trihydroxybenzene moiety in molecules e.g. isopimpinellin (22), bergapten (23), isobergapten (26) and pimpinallin (27) showed antifeedant activity against Spodoptera litura with low threshold concentrations over the range of 5 - 100 ppm, but byakangelicol (24) and byakangelicin (25) showed no activity although they also contained the same 1,3,5 trihydroxybenzene moiety in the molecule. The absence of the antifeedant activity in (24) and (25) could be attributed to the presence of bulky substituents on phloroglucinol type molecule as the other structural features of these compounds are same as in (22), (23) etc. The pyrogallol type furocoumarins (1,2,3 trihydroxy benzene type) e.g. Xanthotoxin (28) and resorcinol type furocoumarins (1,3 dihydroxy benzene type) e.g. angelicin (29), psoralen (30) and pauciflorin (31) exhibited

antifeedant activity to Spodoptera litura only at higher concentrations over the range of 500 - 1000 ppm (see chart III for structures of all compounds).

It has been reported that many examples of insect antifeeding substances carry furan or saturated furan ring for e.g. clerodienirin A and B (11), (12) clerodin (13), caryoptin (14), piperenone (54), meliantriol (53) azadirachtin (55) and pinguison (56), (Chart IIIb) besides furocoumarins, furoquinolins and khelin²⁷⁻²⁹. It shows that possibly the presence of furan group is necessary for the insect antifeedant activity in such type of compounds. K. Munakata et al.²⁶ examined the antifeedant activity of simple coumarins e.g. 5-methoxy 7-hydroxy coumarin (34), 5-methoxy 7-acetoxy coumarin (35), umbelliferon (36) and coumarin (37) (Chart III) which did not contain fused furan ring and found that none of the compounds showed remarkable activity. This showed that the removal of the fused furan moiety in furocoumarins is possibly required for having the insect antifeedant activity. These results were in good agreement with the above statement.

The activity of the dihydroisopimpinellin (32)(Chart III) is almost same as that of isopimpinellin (22) and that of dihydroxanthotoxin (33) (Chart III) is about two times as large as that of xanthotoxin (23). Thus saturation of the fused furan segment in furocoumarin molecules does not reduce the antifeedant activity in S.litura. These results are in good agreement with antifeeding activity of kokusagine

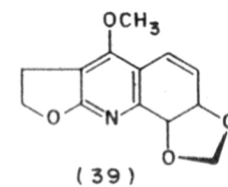
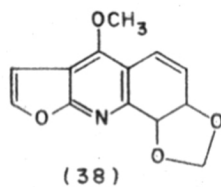
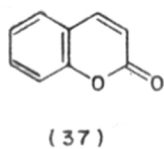
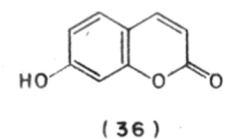
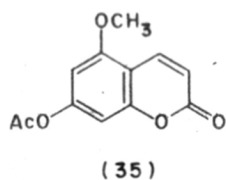
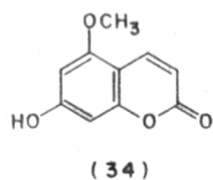
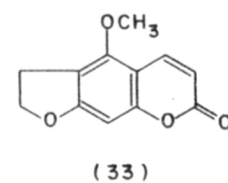
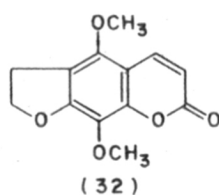
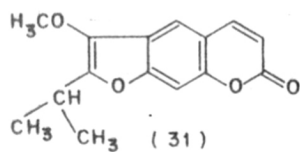
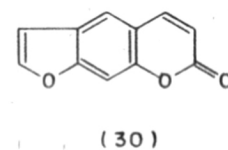
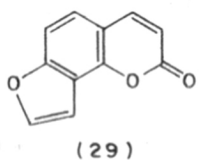
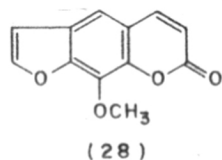
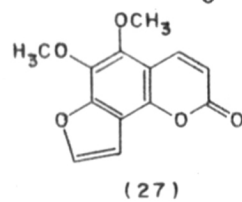
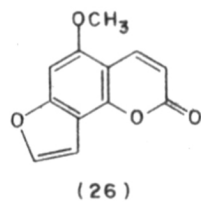
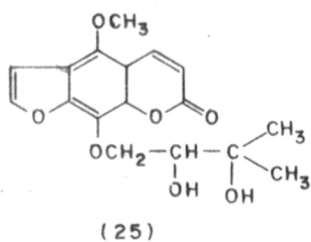
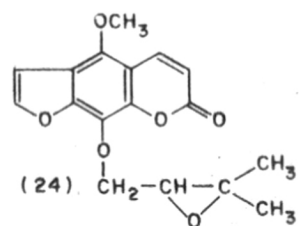
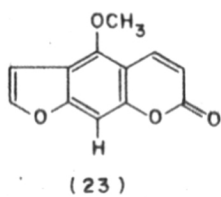
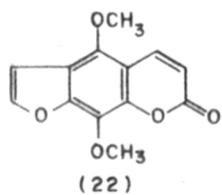
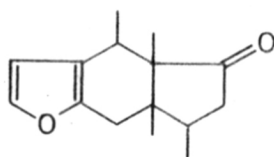
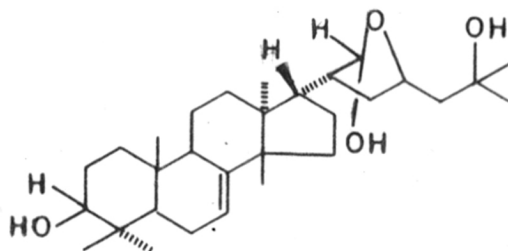


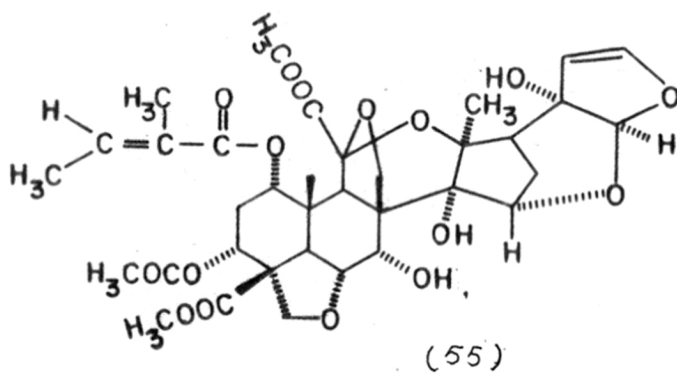
CHART (III)



(56)



(53)



(55)

CHART III

(38) and dihydrokokusagine (39) (Chart III)²⁶.

(b) Phenyl propanoids:

Phenyl propanoids are a class of aromatic natural products having a $\text{CH}_2\text{-CH=CH}_2$ side chain on aromatic ring. A number of compounds belonging to this class are reported to be having insect antifeeding activity. A. Isogai et al.³⁰ isolated a number of phenyl propanoids (40-52) (Chart IV) from nutmeg. All the compounds were tested for insect antifeedant activity against the silkworm larvae Bombyx mori L.

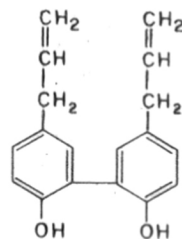
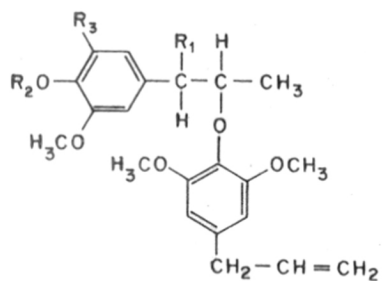
All the tested compounds showed antifeedant activity with the sole exception of dehydrodiisoeugenol (52) which did not show any activity even at concentrations as high as 500 ppm. The compound (50) showed most striking activity causing death in all fourth instar larvae of silkworm Bombex mori L. in 3 days due to starvation at a dosage of 300 ppm in diet, whereas compound (43) revealed antifeeding effect on larvae at 50 ppm³⁰.

Isoasaron (53) and piperenone (54) (Chart IV), two phenyl propanoids isolated from Piper futokazura showed 90 to 100% antifeedant activity against Spodoptera litura at concentrations of 0.5% and 0.005% respectively^{31,32}.

All active compounds (40-53) contained a $\text{CH}_2\text{-CH=CH}_2$ grouping in the molecule which suggests that the presence of a $\text{CH}_2\text{-CH=CH}_2$ side-chain in phenyl propanoids is essential for the activity of these compounds.

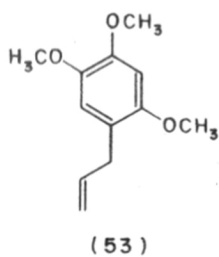
(c) Other aromatics:

In addition to furocoumarins and phenyl propanoids, a number of other classes of aromatic compounds such as quinones and lignans³³, flavonoids and isoflavonoids and benzoic acid derivatives also show insect antifeedant activity^{34,35}.

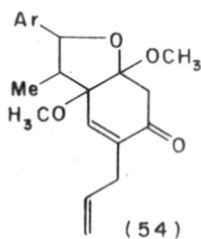
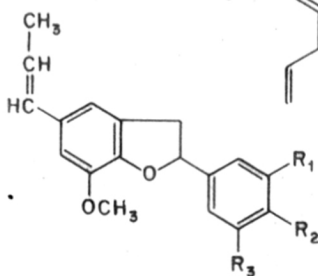


(45)

Compound	R ₁	R ₂	R ₃
(40)	H	CH ₃	OCH ₃
(41)	OAc	CH ₃	H
(42)	OH	CH ₃	H
(43)	OH	CH ₃	OCH ₃
(44)	OH	H	H

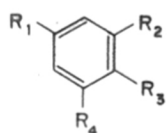


(53)



(54)

Compound	R ₁	R ₂	R ₃
(50)	O-CH ₂ -O	H	
(51)	O-CH ₂ -O	OCH ₃	
(52)	OCH ₃	OH	H



Compound	R ₁	R ₂	R ₃	R ₄
(46)	CH ₂ -CH=CH ₂	O-CH ₂ -O	OCH ₃	
(47)	CH ₂ -CH=CH ₂	OCH ₃	OCH ₃	H
(48)	CH ₂ -CH=CH ₂	OCH ₃	OCH ₃	H
(49)	CH ₂ -CH=CH ₂	OCH ₃	OH	OCH ₃

CHART IV

PRESENT WORK

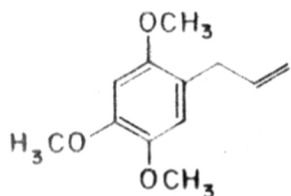
In our studies directed towards the isolation of new insect control agents such as (i) antifeedants, (ii) repellants, (iii) juvenile hormones (iv) antijuvenile hormones, (v) ovicidals, (vi) oviposition deterrents (vii) antigonadials and (viii) insecticides, we screened a large number of plants belonging to family Compositae. The present work deals with our investigations on isolation and biological evaluations of constituents of Caesulia axillaris Roxb and some structure activity relationship studies carried out with γ -asarone and some substituted cinnamic acids and their cyclopropane analogues.

The plant Caesulia axillaris Roxb is of botanical interest since it represents the only known species of the genus Caesulia^{36a} (i.e. mono-typic genus). It belongs to tribe Inulae of family Compositae and is a succulent, prostrate or suberect herb 6-18 in high. Stem stout, more or less branched, steriate and glabrous. Leaves sessile, lanceolate, acute, distinctly serrulate, narrowed at the base and glabrous. Heads axillary, sessile, flowers pale-blue or white. Involucre large and membranous^{36b}.

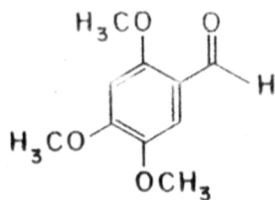
The acetone extract of the plant showed a strong antifeedant activity against the larvae of Achea janata and showed good JH activity against Dysdercus koengii. To isolate the active components the acetone extract was subjected to column chromatographic separation and six

fractions, 1-6 were collected (Chart VI) and bio-assayed, when fractions 2 and 3 only showed the antifeedant activity. Rechromatography of above two fractions afforded the active compound: (I) (Chart V) as a yellow oil, b.p. 145-7°/2 mm (bath temp.). The IR and NMR spectra (Fig. 1 and 2) of the active compound (I) revealed that it belonged to a phenyl propanoid class of natural products and was identified as γ -asarone, a known compound earlier isolated by Devangan *et al.*³⁷ from the same source. K. Matsui *et al.*^{31,32} isolated the same compound γ -asarone from Piper futokazura and reported that it showed antifeedant activity against the larvae of Spodoptera litura.

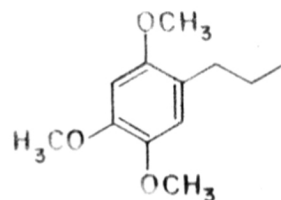
In addition to the testing of γ -asarone for antifeedant activity against the larvae of Achea janata and Musca domestica, we also tested it against Spodoptera litura to confirm the report of earlier workers^{31,32}. It showed 60-70% antifeedant activity against the larvae of Achea janata (Table III) and no activity against the Musca domestica adults and this is the first report of these data. That it showed 100% activity at 0.5% concentration as reported earlier^{31,32} against the larvae of Spodoptera litura was confirmed by us.



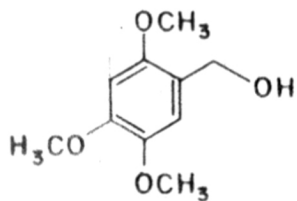
(I)



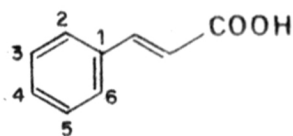
(II)



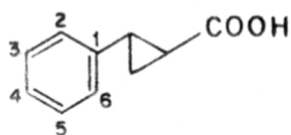
(III)



(IV)



	2	3	4	5	6
(V)	OCH ₃	H	OCH ₃	OCH ₃	H
(VI)	H	OCH ₃	OCH ₃	H	H
(VII)	H	O-CH ₂ -O		H	H
(VIII)	OCH ₃	H	OCH ₃	H	H
(IX)	H	H	H	H	Cl
(X)	H	H	H	H	NO ₂



	2	3	4	5	6
(XI)	OCH ₃	H	OCH ₃	OCH ₃	H
(XII)	H	OCH ₃	OCH ₃	H	H
(XIII)	H	O-CH ₂ -O		H	H

Table III Antifeedant activity of γ -asarone
against Achea janata larvae

Compound	% dose	mean % area eaten after 24 hours
γ -Asarone	1.0	35.07
Control	1.0	92.49

Fourth fraction of the column chromatography of acetone extract of Caesulia axillaris showed a very good JH activity against Dysodercus koengii. Hence it was again fractionated and bioassayed to pinpoint the JH active component. Due to paucity of the compound, its structure elucidation was not possible, however from the ^1H NMR spectrum (Fig.) of compound, it is suspected that the JH active compound is most probably a straight-chain unsaturated compound containing a carbonyl group.

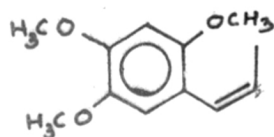
γ -Asarone is the major constituent, of the acetone extract of Caesulia axillaris and was isolated in 0.41% yield (based on dry weight of plant). In addition of γ -asarone, the extract also furnished a crystalline compound (II) m.p.152-4° as a minor constituent. The IR and NMR spectra (Fig. 3 and 4) of the compound (II) suggested that it is a 2,4,5 trimethoxy benzaldehyde and was confirmed by comparing the data with an authentic

sample. Compound (II) could be an auto-oxidation product of γ -asarone as the same aldehyde is obtained by oxidation of γ -asarone. Thus compound (II) may be an artifact.

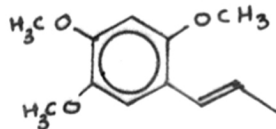
A large number of phenyl propanoids having $-\text{CH}_2\text{-CH}=\text{CH}_2$ side-chain are reported to show insect anti-feedant activity³⁰⁻³². It is assumed that the presence of a $-\text{CH}_2\text{-CH}=\text{CH}_2$ side-chain in such type of compounds is possibly required for the insect antifeedant activity. The structure activity relationship studies carried out with γ -asarone showed that the presence of a $-\text{CH}_2\text{-CH}=\text{CH}_2$ side-chain is required atleast in the case of γ -asarone as none of the compounds (II-V, Chart V) in which side-chain of γ -asarone was modified either to an aldehyde (II), saturated to get compound (III), converted to alcohol (IV) or converted to an acid (V) (Chart V) show any appreciable activity against Spodoptera litura or Musca domestica.

It is interesting to note that the α , and β -isomers of γ -asarone (54,55) (Chart Va) isolated from Acorus calamus by B.P. Saxena et al.³⁸ showed antigonadial activity suggesting that the cis conformation of the double bond in the side-chain is responsible for the antigonadial activity. While the trans conformation of the double bond in the side-chain of asarone is responsible for the antifeedant activity. In asarones and some other bioactive compound such as precocenes, the presence and the position of o-methyl groups on the aromatic ring also play an important role in

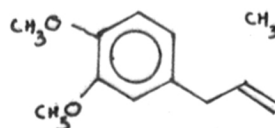
biological activity of the compounds^{38,39} e.g. B.P. Saxena et al.³⁸ after testing various compounds (54, 57, Chart Va) showed that *o*-methyl group at position 2 in the ring is important for resorption of the oocyte which is further supported by the suppression in ovary due to *o*-methyl group at 5 position (as in eugenol (56) and methyl isoeugenol (57)). The suppression in ovary due to *o*-methyl group at 5 position on ring seems true of the precocene II (58) also, because removal of the *o*-methyl group from 5 position in that molecule reduces the activity. (Precocene I (59) of Bowers, Chart Va)³⁹. Cinnamic acid is reported to show insecticidal activity against screw worms at 0.67% concentrations⁴⁰. Thus keeping in view the activity of cinnamic acid and the role played by the *o*-methyl groups on aromatic ring and their position of attachment in various compounds biological activity, it was thought imperative to prepare cinnamic acids with different substitution pattern of *o*-methyl and other functional groups on the aromatic ring (V-X, Chart V) and to study the structure activity relationship of these compounds, as reported on the biological activity of such type of compounds are too spotty⁴¹⁻⁴³. The cinnamic acids (V-X) were prepared by the known procedure and the structures of all the compounds were established by the comparison of the NMR spectra of compounds (Figs. 9-15) with the reported NMR spectra of these compounds^{44,45}. 3,4-Dimethoxy cinnamic acid (VI) is also reported to occur in nature⁴⁶.



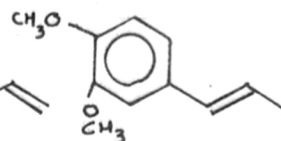
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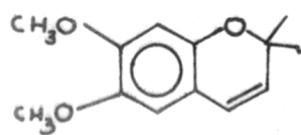
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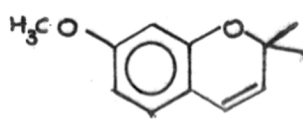
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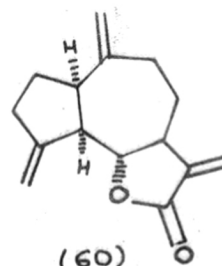
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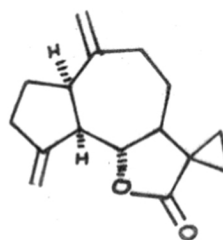
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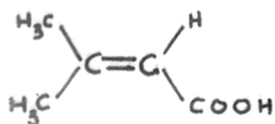
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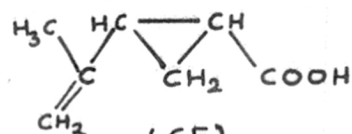
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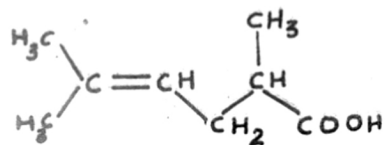
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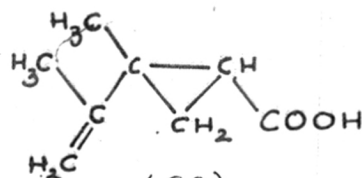
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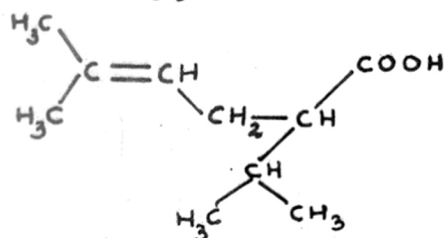
(65)



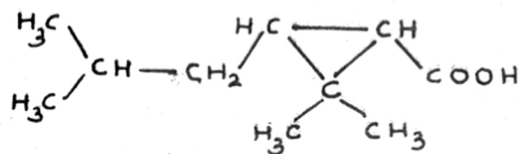
(63)



(66)



(64)



(67)

CHART (Va)

The above cinnamic acids (V-X) were tested for the antifeedant activity against the larvae of Spodoptera litura and Musca domestica adults. None of the compounds (V-X) however showed any appreciable antifeedant activity against Spodoptera litura or Musca domestica.

A number of cyclopropane compounds are known for their biological activity^{47,48} and it has been shown that when the double bond in the active compounds was replaced by a cyclopropane ring the activity of the compounds enhanced⁴⁸⁻⁵⁰ e.g. P.S. Kalsi⁴⁹ based on his structure activity relationship studies on plant growth regulating activities of sesquiterpene lactones (60, 61) suggested that the presence of α -methylene γ -lactone moiety is essential for the activity. When the exomethylene of α -methylene γ -lactone was replaced by a cyclopropane ring the compound showed even more activity than the parent compound. Similarly the structure activity relationship studies in natural and synthetic pyrethroids revealed that the carboxylic acid moiety in the pyrethroids must contain a cyclopropane ring so as to show toxicity when esterified (65-67) (Chart Va), as the esters of other acids which are not having cyclopropane ring (62-64, Chart Va) did not show toxicity or showed very little toxicity⁵⁰. Hence we replaced the double bonds in acid side-chains of substituted cinnamic acids by cyclopropane ring employing the known procedure⁴⁴ to obtain the cyclopropane analogues of substituted cinnamic acids (XI-XIII) (Chart V). The

structures of these compounds were deduced from the NMR spectra of methyl esters of these compounds (Figs. 16-18). These compounds also were tested for the antifeedant activity against larvae of Spodoptera litura and Musca domestica adults. However, none of the compounds showed antifeedant activity against both the insects.

However, an interesting observation was in store for us when we subjected the substituted cinnamic acids (V - X) and their cyclopropane analogues (XI - XIII) for other insect control tests. The compound (VI) i.e. 3,4-dimethoxy cinnamic acid and its cyclopropane analogue (XII) exhibited good growth disruption in the adults of Dysdercus koengii. Here again, as expected the activity was more pronounced in the cyclopropane analogue (XII) as compared with the corresponding unsaturated analogue (VI). The other compounds however, did not show any such activity.

E X P E R I M E N T A L

Isolation of Y-asaron (I)

- a) Plant material: The plant *Caesulia axillaris* was collected from Pune (Maharashtra, India) and was shade dried.
- b) Extraction: The dried and coarsly powdered plant material (6 kg) was extracted in soxhlet extractor. Acetone (40 lit) was used as solvent for extraction. The progress of the extraction was monitored by taking out the test sample (100 ml) of the extract, evaporating it to dryness and weighing the residue. When the residue left after evaporation of the solvent from test sample weighed negligible the extraction was stopped. The solvent was then recovered from the extract by distillation on waterbath. The last traces of solvent were removed by applying vacuum to obtain a dark coloured gummy residue (200 g).
- c) Chromatographic separation: The acetone extract of *Caesulia axillaris* (100 g) was subjected to column chromatographic separation. The column was packed with silica gel (column grade, 2.5 kg). The column was eluted successively with pet.ether, benzene and benzene ethylacetate mixture (with successive increase in % of ethylacetate). Fractions of 100 ml size were collected. The progress of the chromatographic separation was monitored by TLC of the fractions. Fractions showing similar composition were mixed together to obtain six major fractions (A-F). The

details of the chromatographic separation are outlined in Chart (VI) and Table (IV).

Y-Asaron was obtained as a pure liquid from the second fraction from rechromatography of fraction (B) (Chart VI). Y-Asaron (25 g) thus obtained was further purified by its distillation under vacuum to get yellowish viscous oil.

b.p.: 145-7°C (bath temp.) at 2 mm.

U.V.: $\lambda_{\text{max}}^{\text{EtOH}}$ 210 nm (ϵ 25626), 234 nm (ϵ 11986) and
(Fig 1) 292 nm (ϵ 6406).

IR: $\lambda_{\text{max}}^{\text{liq.film}}$ cm^{-1} 1620, 1600, 1530, 1500.

Mass: (m/e) M^+ at 208.

Isolation of 2,4,5 trimethoxy benzaldehyde (II)

2,4,5-Trimethoxybenzaldehyde was isolated from fraction (D) (Chart VI) by its rechromatography on silica gel (10 g) using benzene + ethylacetate mixture as solvent for elution with successive increase in the % of ethylacetate. Fractions of 50 ml size were collected. Fractions showing similar composition on TLC (eluent 25:75) were mixed together to get three major fractions (I-III) 2,4,5-trimethoxy benzaldehyde was obtained from fraction (II) as a greenish yellow solid (200 mg). The solid thus obtained was crystallized from ethanol to obtain yellowish crystalline solid (150 mg).

m.p. 152-4°C

IR: (Fig. 3) $\lambda_{\text{max}}^{\text{nujol}}$ cm^{-1} 1730, 1650, 1600, 1500.

POWDERED PLANT MATERIALS

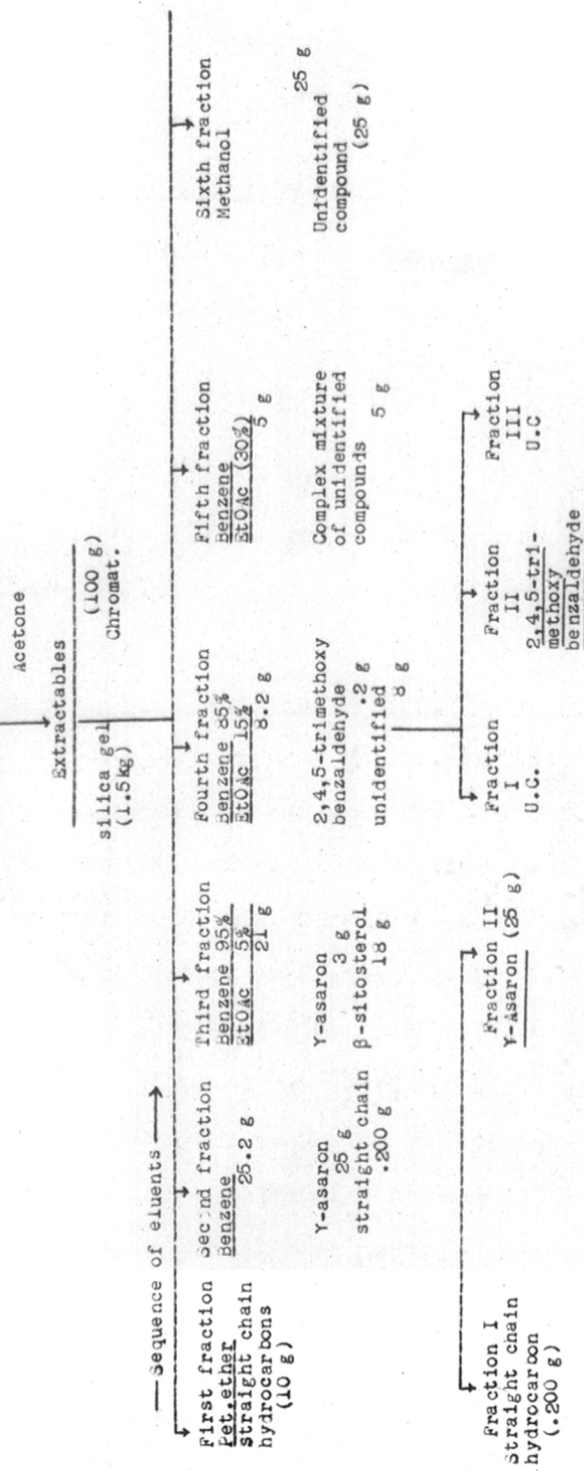


CHART II Chromatographic separation of constituents of acetone
extract of *Caesulia axillaris*

Dihydro Y-asaron (III)

Y-Asaron (100 mg) was hydrogenated catalytically using Pd/C (10%) as catalyst in ethanol at atmospheric pressure and room temperature to get the dihydroderivative (98 mg) as a colourless oil.

b.p. 130° at 0.5 mm (bath temp.)

IR: (Fig. 5) $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} 1600, 1510, 1500.

Mass: (m/e, rel.int.) 210 (M^+ , 80) 195 (M-15, 100)
121 (60)

2,4,5 Trimethoxy benzaldehyde from Y-asaron

A mixture of Y-asaron (2 g), sodium dichromate (6 g) and acetic acid (60 ml) in benzene (50 ml) was refluxed on waterbath for six hours. The mixture was then diluted with water and extracted with benene (4 x 25 ml). The combined benzene extract was successively washed with water (3 x 20 ml), 10% aq. sodiumbicarbonate (3 x 20 ml), water (3 x 20 ml) and finally ^{with} brine. It was then dried over anhydrous sodium sulphate and solvent removed to get the product, (yield 1.8 g). TLC examination of the product showed it to be a mixture of three compound, hence it was purified by its column chromatographic separation on silica gel. The 2,4,5-trimethoxybenzaldehyde was obtained as yellowish crystalline solid from first fraction (0.6 g). It was recrystallized from ethanol m.p. $152-3^{\circ}$.

IR: (Fig. 3) $\nu_{\text{max}}^{\text{nujol}}$ cm^{-1} 1730, 1650, 1600, 1500.

2,4,5-Trimethoxy benzyl alcohol (IV)

A mixture of compound (II) (2,4,5 trimethoxybenzaldehyde) (100 mg), 2-propanol (200 mg), carbontetrachloride (6 ml) and alumina (neutral, Grade I, 2 g) was stirred for two hours at room temperature. The mixture was then filtered, alumina was washed with methanol twice (20 ml) and solvent removed to get the product. The crude product was purified by its preparative TLC (eluent: 25:75) to get pure trimethoxy benzylalcohol (IV) (60 mg) as a colourless crystalline solid, m.p.64-65.

IR: (Fig. 7) $\nu_{\text{max}}^{\text{nujol}}$ cm^{-1} 3200 (OH) 1600 and 1520 (aromatic).

General procedure for preparation of substituted cinnamic acids (V-XI)

The condensation of malonic acid with appropriately substituted benzaldehyde in presence of pyridine and piperidine was used as a general procedure for preparation of substituted cinnamic acids (V-XI)⁴⁴. The process is described below for preparation of 2,4,5 trimethoxy cinnamic acid.

2,4,5 Trimethoxy cinnamic acid (V)

A mixture of 2,4,5 trimethoxy benzaldehyde (0.5 g) malonic acid (0.6 g), pyridine (1.2 ml) and piperidine (0.03 ml) was refluxed for three hours. The reaction mixture was then cooled and poured on ice water mixture (30 ml) containing 1.5 ml conc. hydrochloric acid. The

resulting ppt. was filtered and dried to get the crude acid (V) (600 mg). The crude acid was then crystallized from ethanol to get a yellow crystalline solid m.p.

General procedure for preparation of cyclopropane analogues of substituted cinnamic acids (XI-XIII)

Preparation of compound (XI)

a) Esterification: The 2,4,5 trimethoxy cinnamic acid (V) (60 mg) was esterified by using ether solution of diazomethane, to obtain the methyl ester of 2,4,5 trimethoxy cinnamic acid (V) (65 mg).

b) Cyclopropanation of the methyl ester: The mixture of methyl ester (50 mg) and excess of diazomethane (ether solution) was stirred at 0-2°C in presence of palladium acetate (2 mg) as catalyst for twelve hours. The mixture was then filtered and solvent removed to get the methyl ester of cyclopropane acid (XI) (55 mg) as a viscous oil.

c) Hydrolysis of the methyl ester of cyclopropane acid (XI)

A mixture of methyl ester (50 mg), potassiumhydroxide (0.01 g), methanol (5 ml) and water (1 ml) was refluxed on water bath for two hours and was then poured on 20% aqueous solution of hydrochloric acid (10 ml). The resulting solid was filtered and washed thoroughly with water dried and weighed (yield: 39 mg).

The compounds (XII) and (XIII) were prepared similarly employing the above procedure. Table II gives the yields and the m.p. of the compounds (V-XIII).

Table II

Compound	molecular formula	% yield	m.p./b.p.
(V)	$C_{12}H_{14}O_5$	98%	172 - 5°
(VI)	$C_{11}H_{12}O_4$	96.4%	192 - 3°
(VII)	$C_{10}H_8O_4$	97%	238 - 40°
(VIII)	$C_{11}H_{12}O_4$	98%	200 - 201°
(IX)	$C_9H_7O_2Cl$	93%	160 - 64°
(X)	$C_9H_7NO_4$	93.6%	156 - 7°
(XI)	$C_{13}H_{16}O_5$	83.3%	140 - 45° (decomp)
(XII)	$C_{12}H_{14}O_4$	80.4%	135 - 40°(decomp)
(XIII)	$C_{11}H_{10}O_4$	81.2%	125 - 30°(decomp)

Testing procedure for antifeedant activity

a) Against Larvae of Spodoptera litura: A 1% solution of test sample was prepared by dissolving appropriate amount of test sample in acetone. A leaf of measured area was then dip in the above solution for two seconds and allowed to dry. Another leaf of measured area was dip in acetone for two seconds and dried (control). Both the leaves were kept in separate petri dishes and the 4th instar larvae of Spodoptera litura were allowed to feed in both petridishes. The area eaten up after 24 hours was measured. The difference in the mean % area of the treated leaf and the control leaf eaten after 24 hours gives the % antifeedant activity of the compound.

The antifeedant activity of γ -asaron is given in Table III. Whereas the compounds (II-XIII) all showed 80-90% feeding.

b) Against Musca domestica adults

10 mg of the test sample was dissolved in 1 ml acetone and 0.8 ml of this solution was added to 3.2 ml of the stock solution (the stock solution was prepared from 0.1% triton x 100 + 0.05 molar sucrose solution + 0.01 g light green dye) the above solution was then put in a small petridish and soaked on cotton. The Musca domestica adults (0-24 hr old, fed on water No. of adults/Replicate = 10) were allowed to feed on the above test sample. The observations were

taken after 24 hours for the presence or absence of dye.

None of the compounds I-XIII tested showed antifeedant activity as about 85-90% of the adults in all the experiments showed +ive test for the presence of dye.

c) Against Larvae of Achea janata:

For insect antifeedant activity testing against Achea janata same procedure, as employed against Spodoptera litura was used.

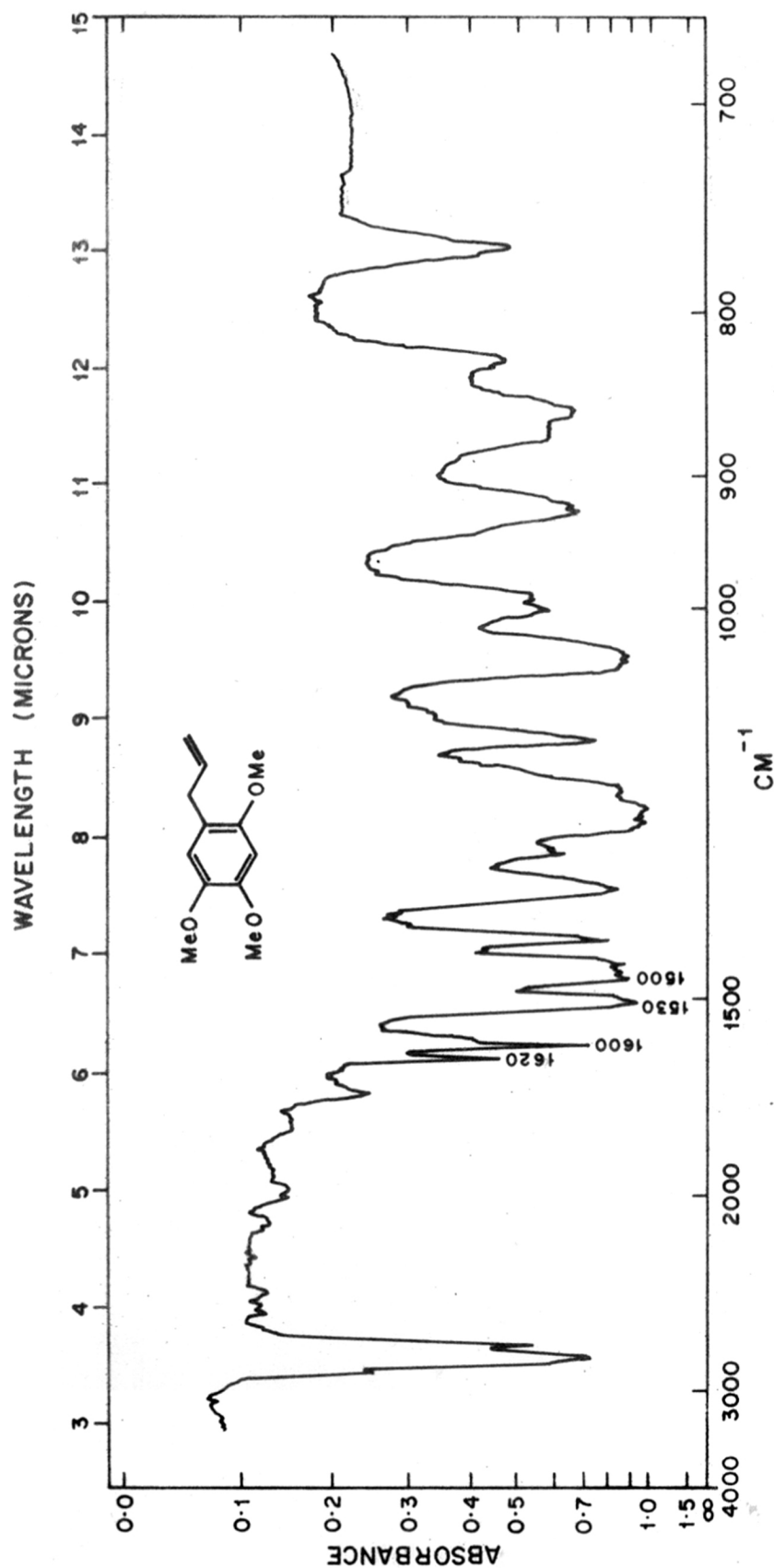
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FIG. 1 : IR SPECTRUM OF γ -ASARON. (I)

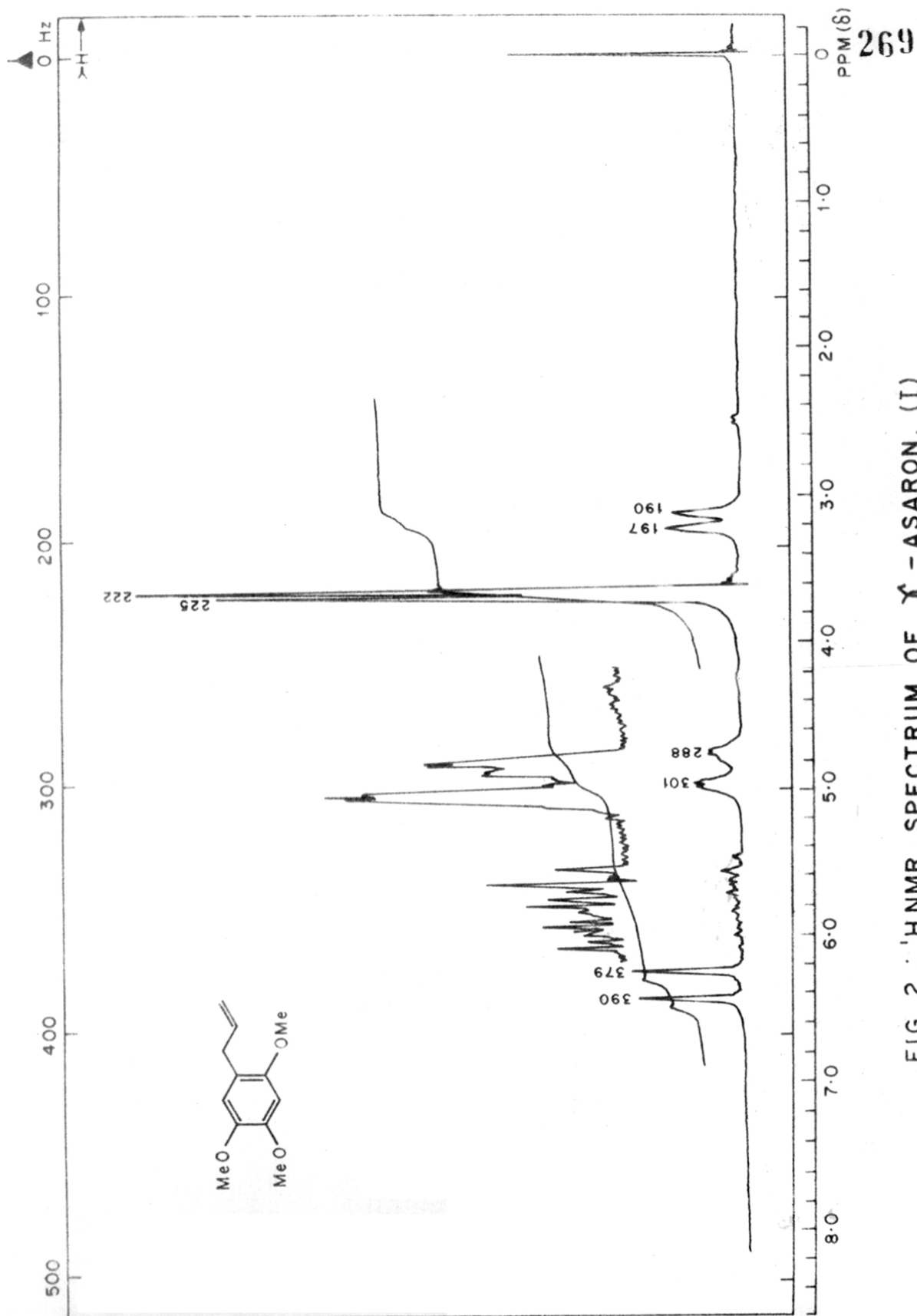


FIG. 2 : ¹H NMR SPECTRUM OF γ - ASARON. (I)

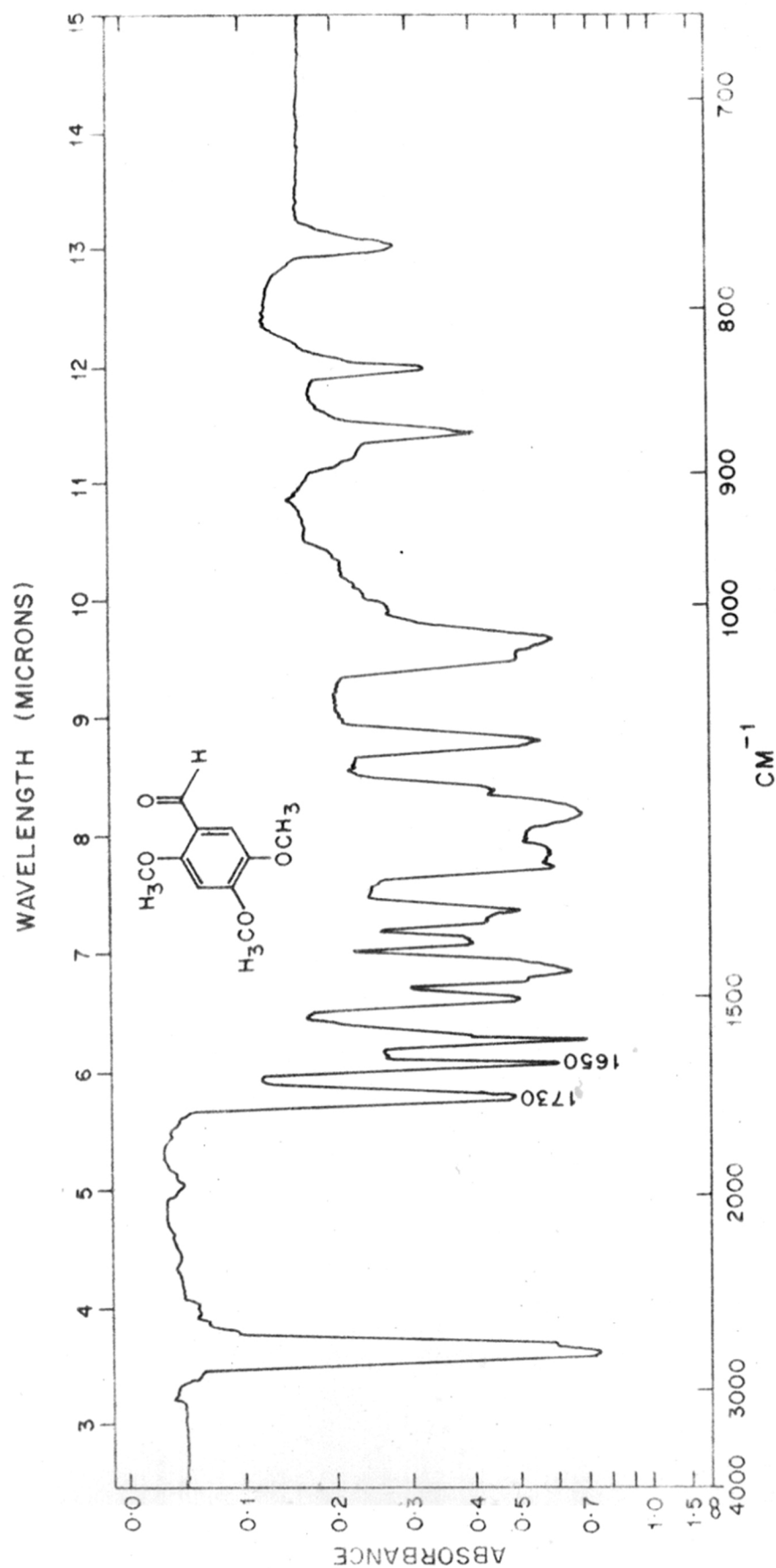
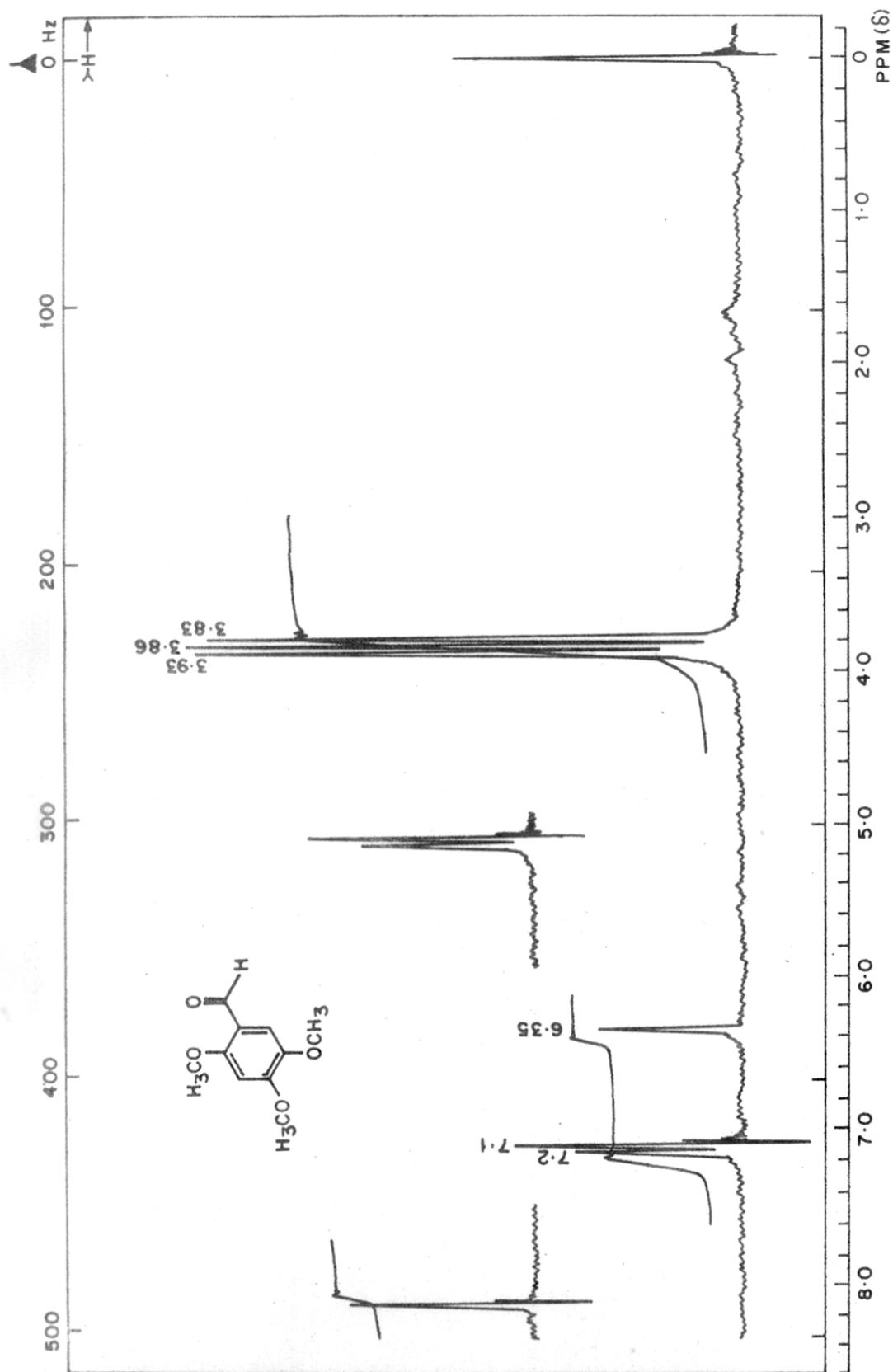


FIG. 3 : IR SPECTRUM OF COMPOUND. (II)

FIG. 4 : ^1H NMR SPECTRUM OF COMPOUND. (II)

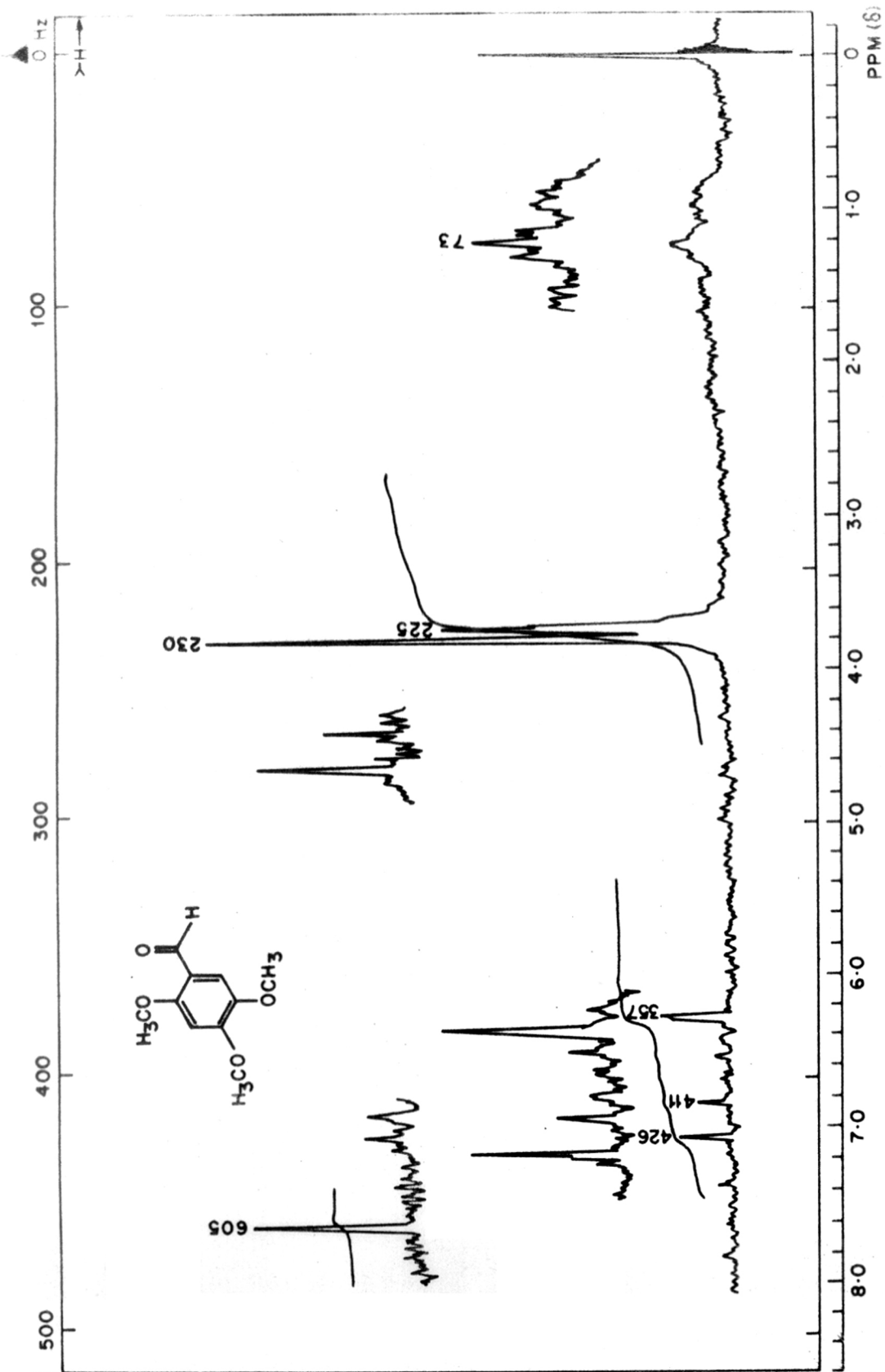


FIG. 4 : ^1H NMR SPECTRUM OF COMPOUND (I)

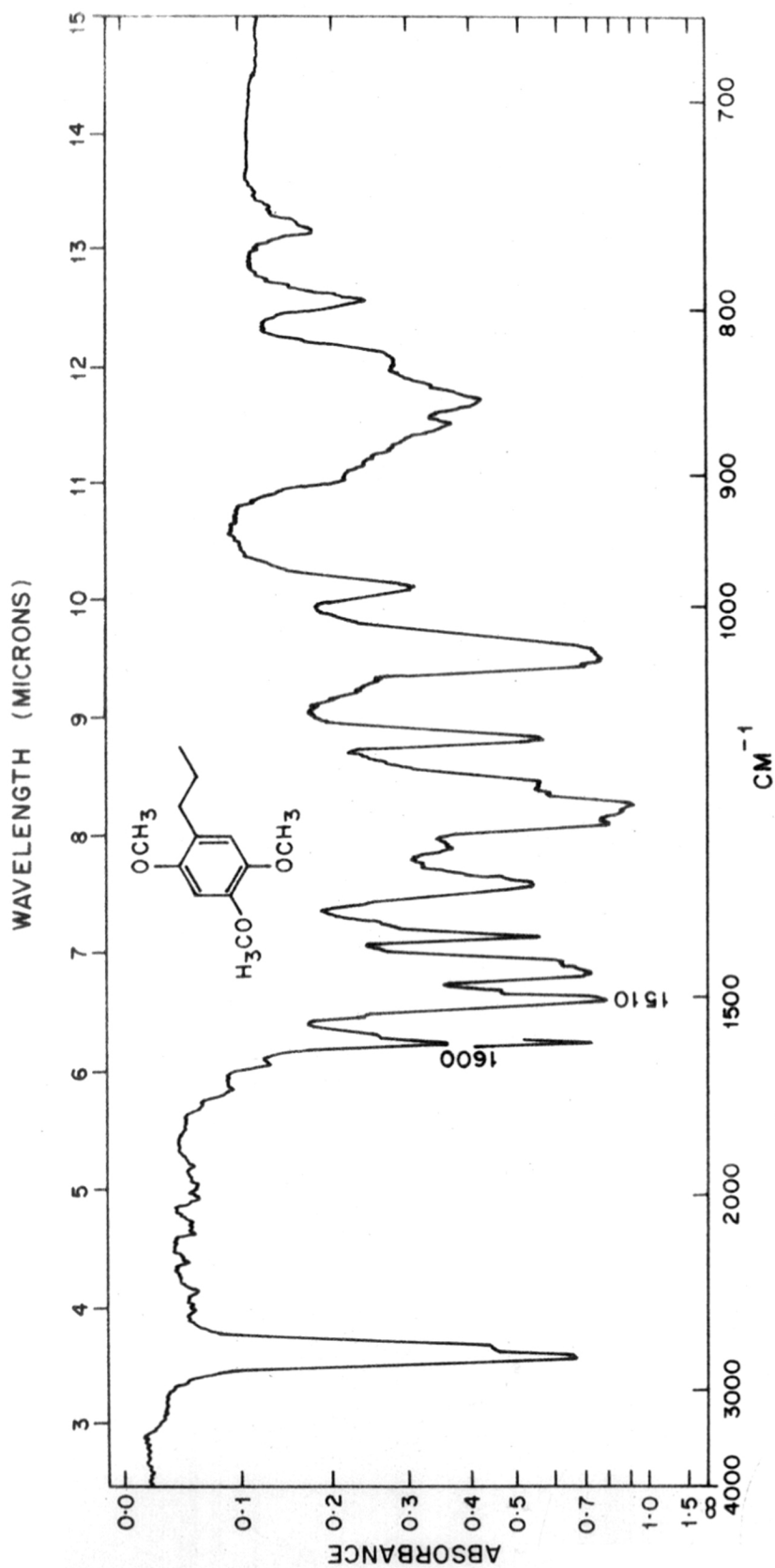
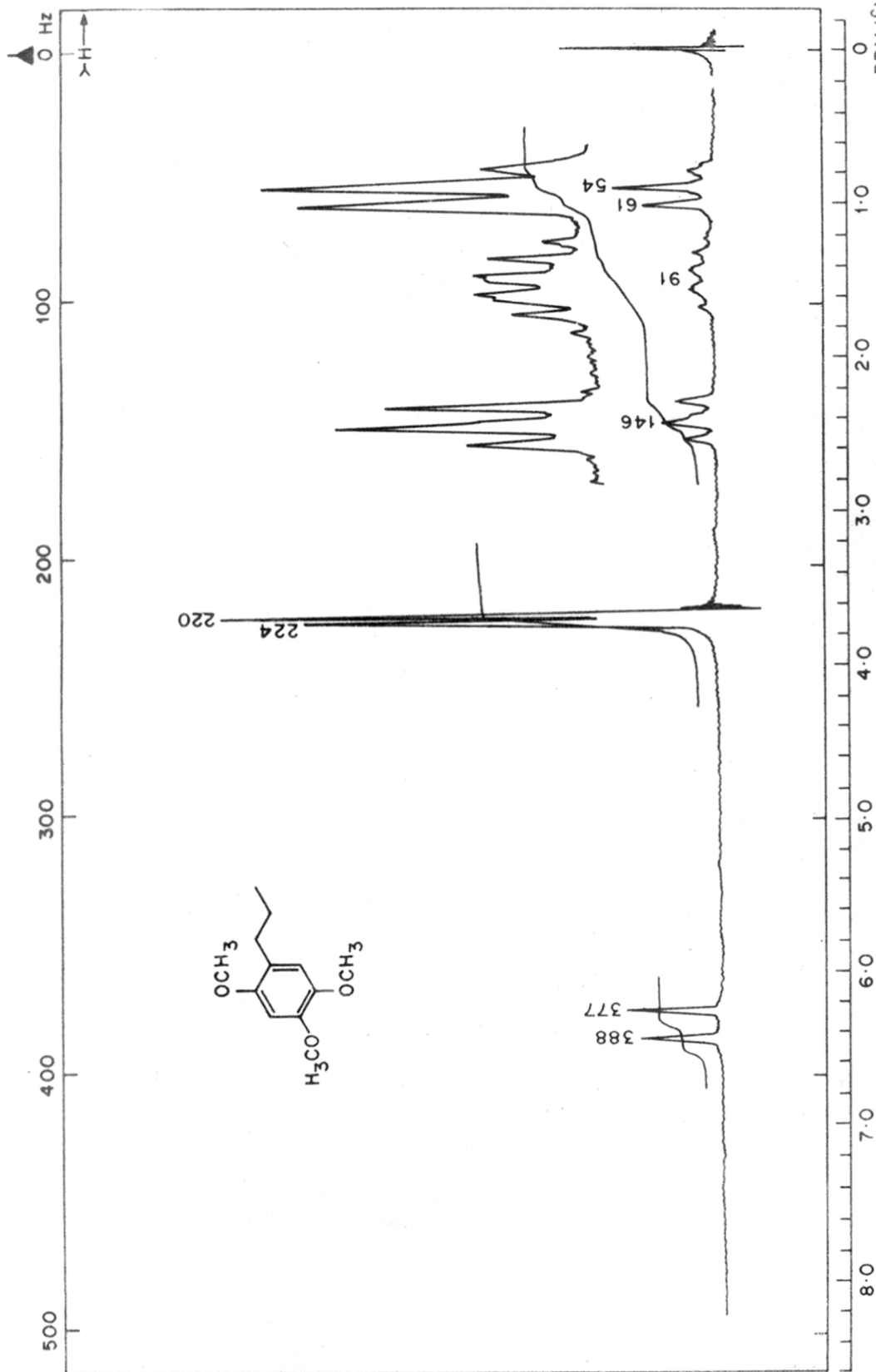


FIG. 5 : IR SPECTRUM OF COMPOUND . (III)

FIG. 6 : ^1H NMR SPECTRUM OF COMPOUND. (III)

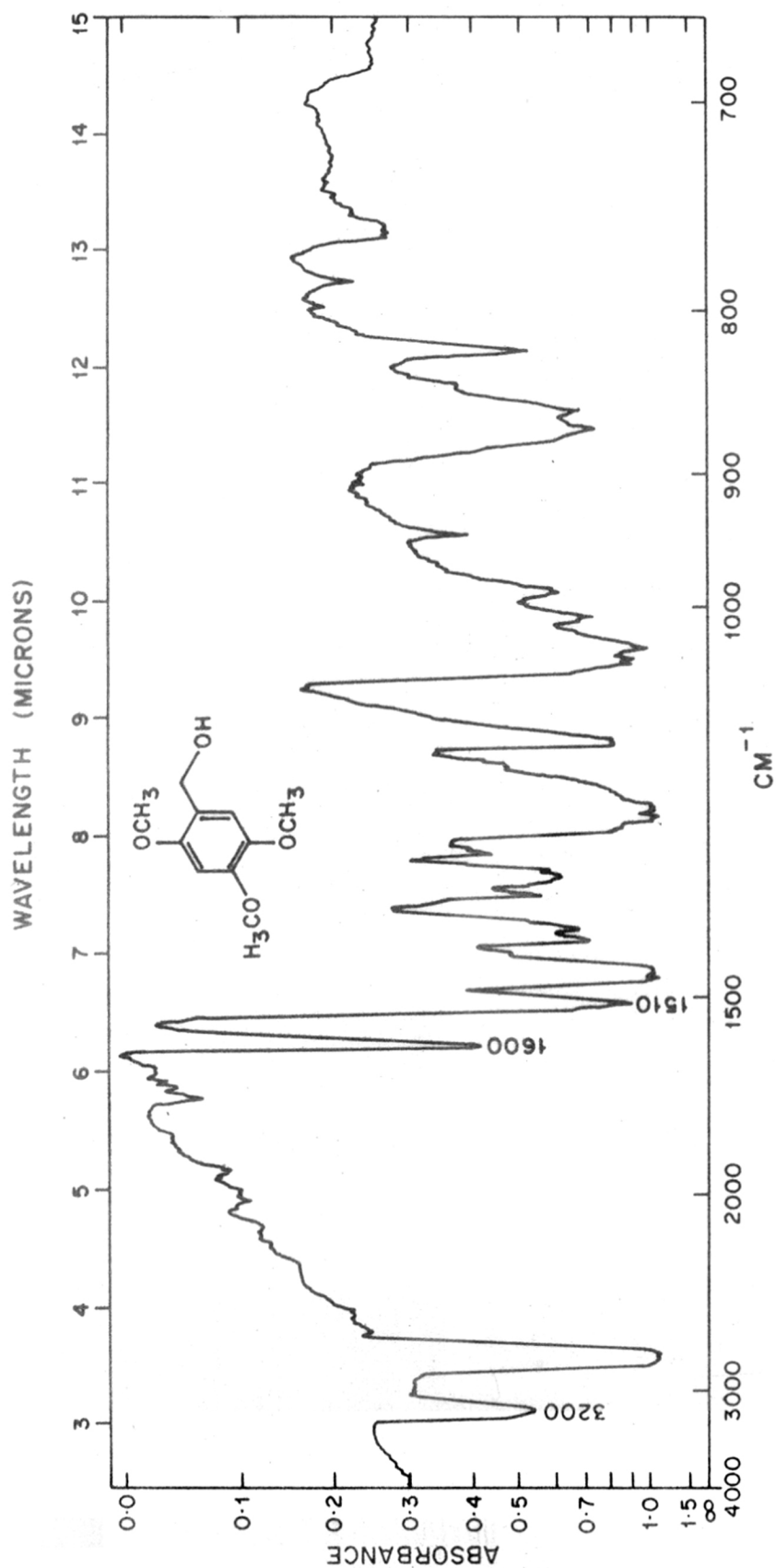
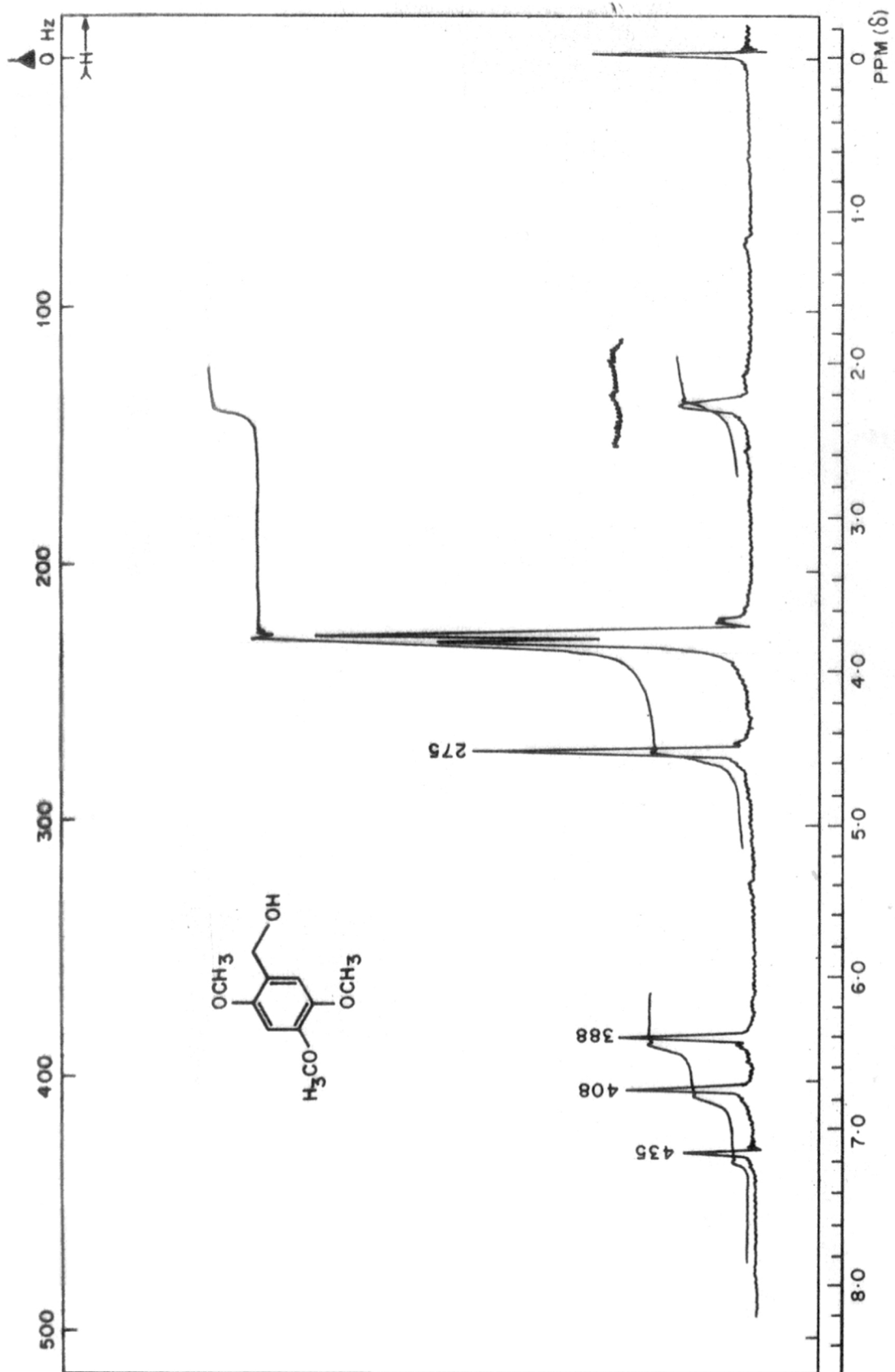


FIG. 7 : IR SPECTRUM OF COMPOUND. (IV)

FIG. 8 : ^1H NMR SPECTRUM OF COMPOUND. (IV)

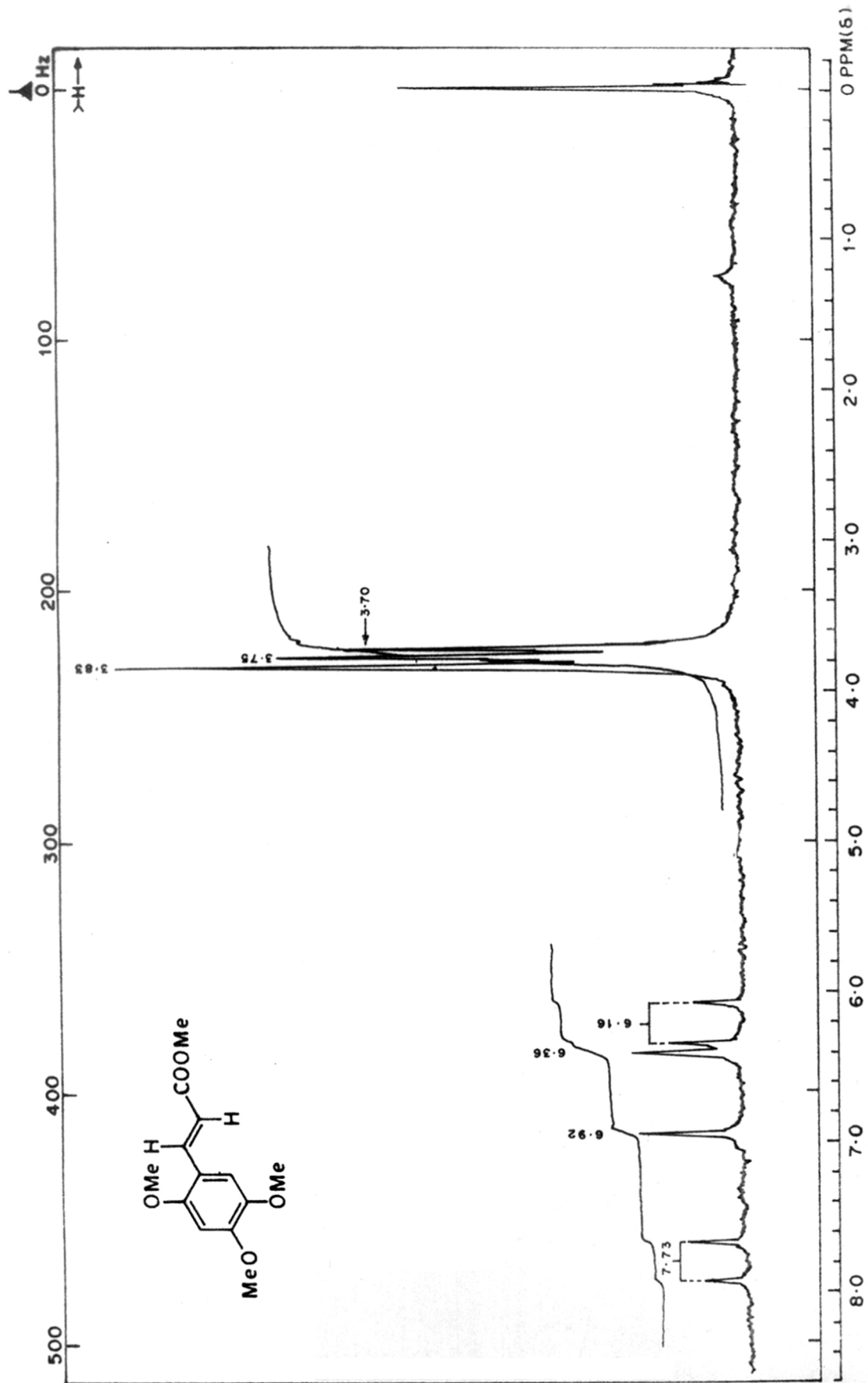
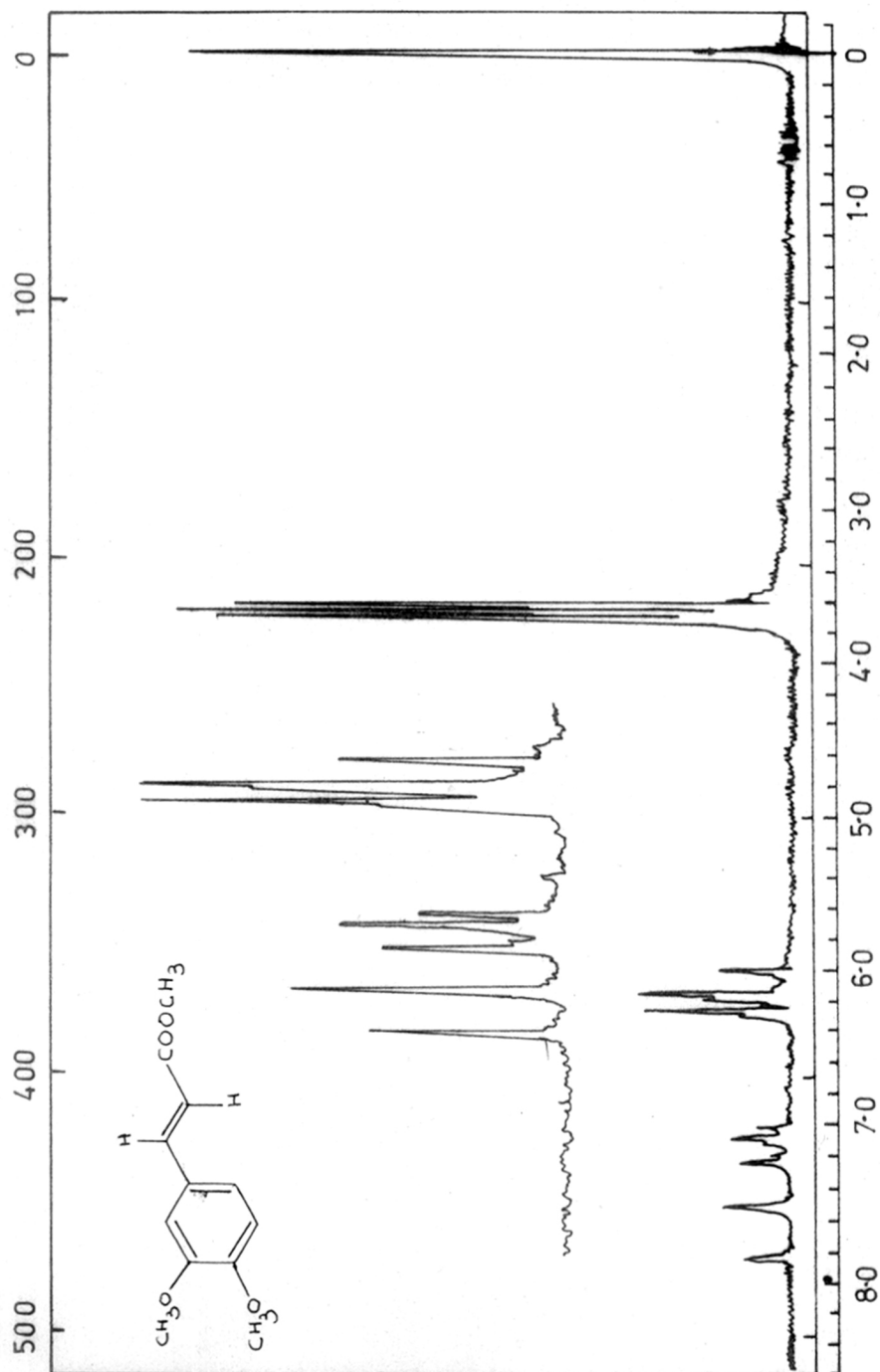
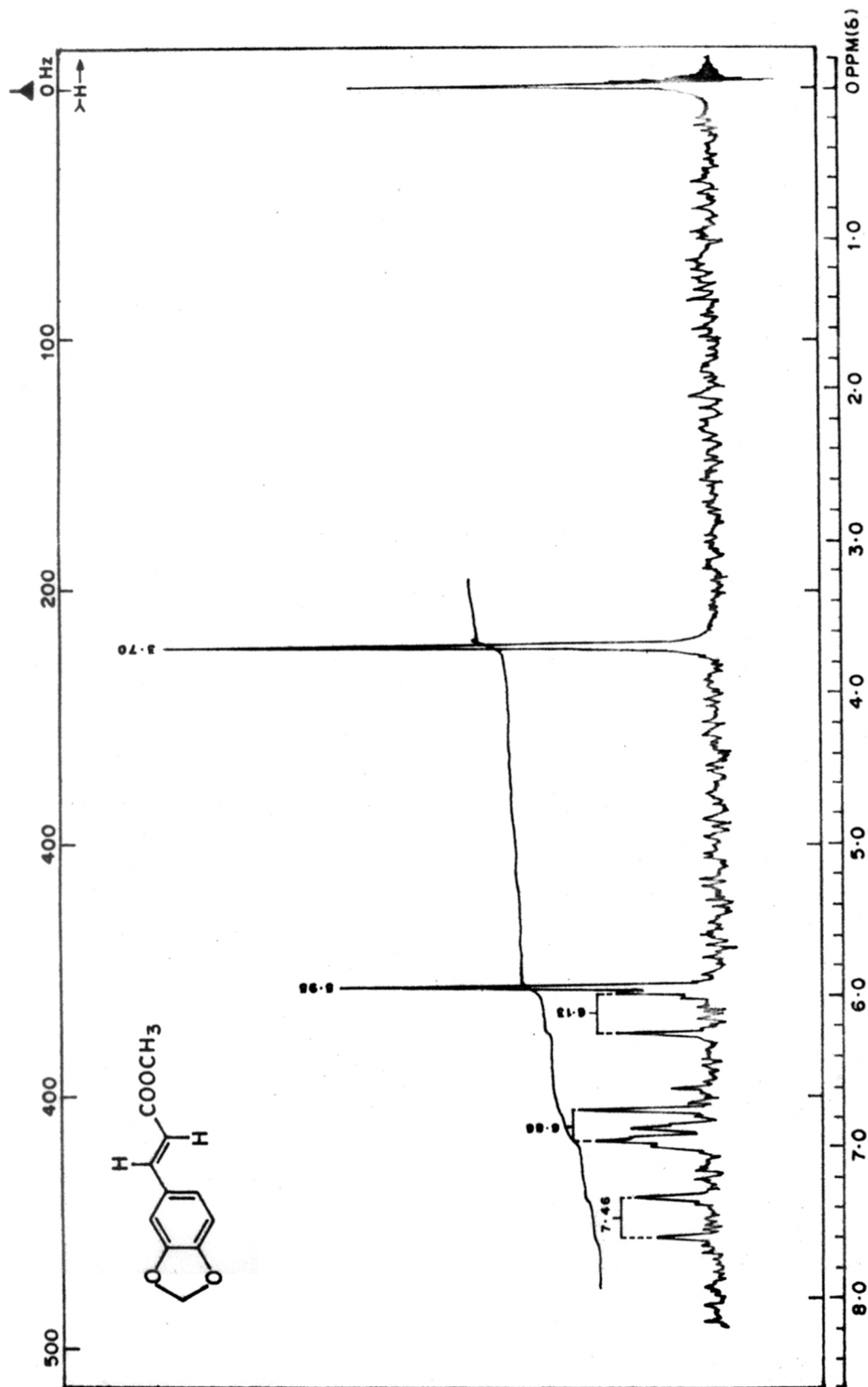


FIG. 9 ^1H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (V)

FIG 10: ^1H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (VI)



279

FIG.11 ^1H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (VII)

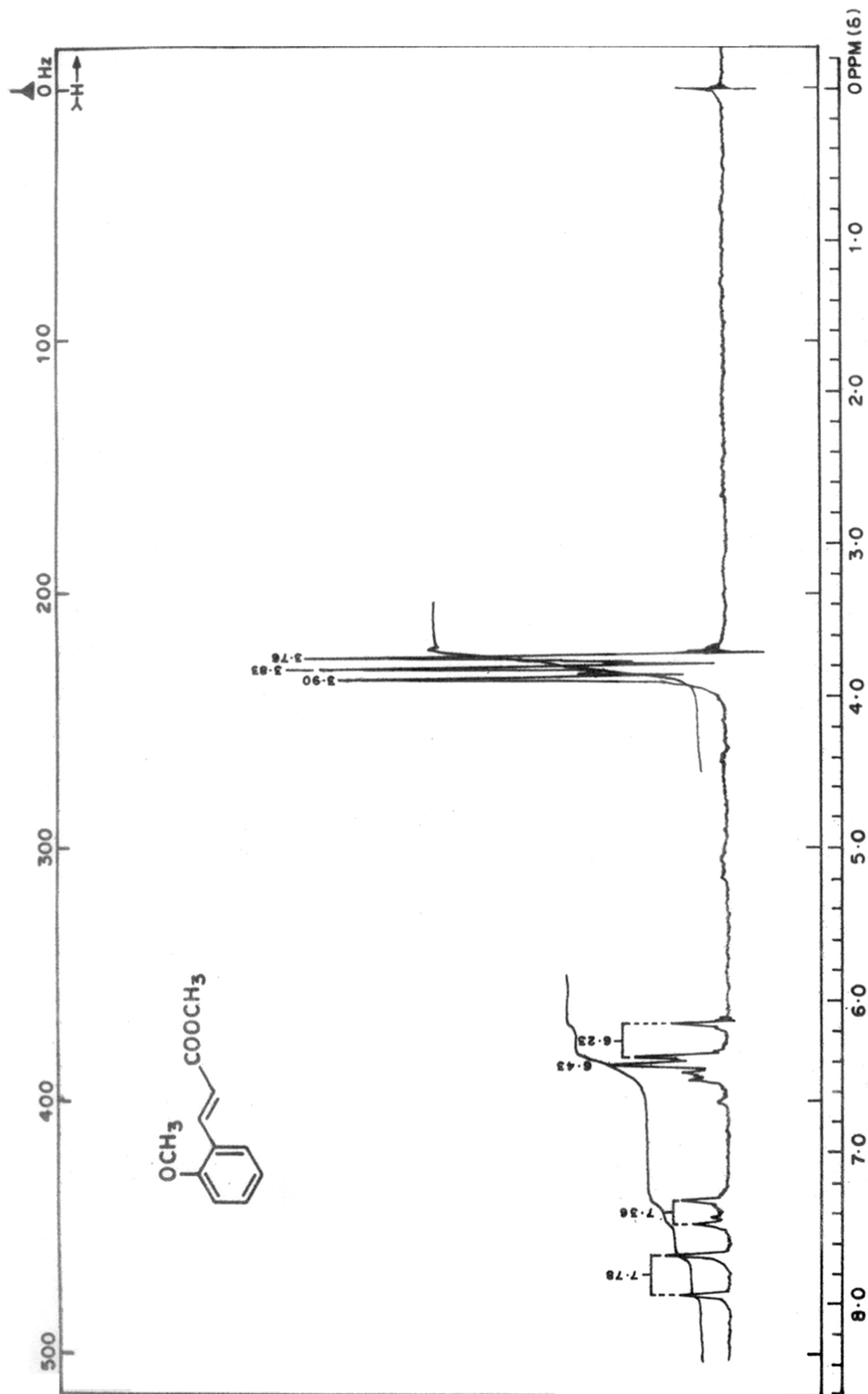


FIG. 12. ^1H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (VIII).

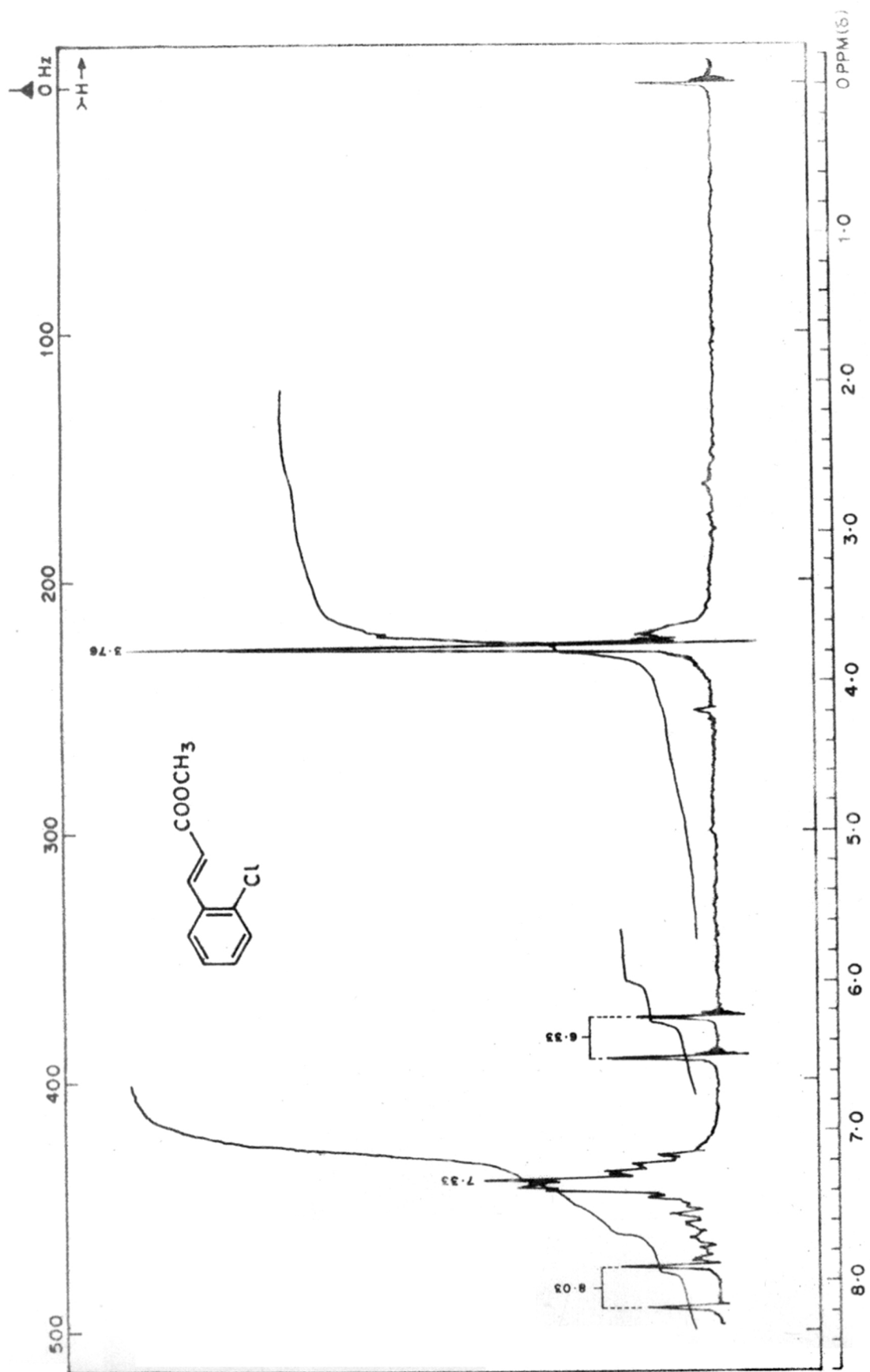


FIG. 13 ^1H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (IX)

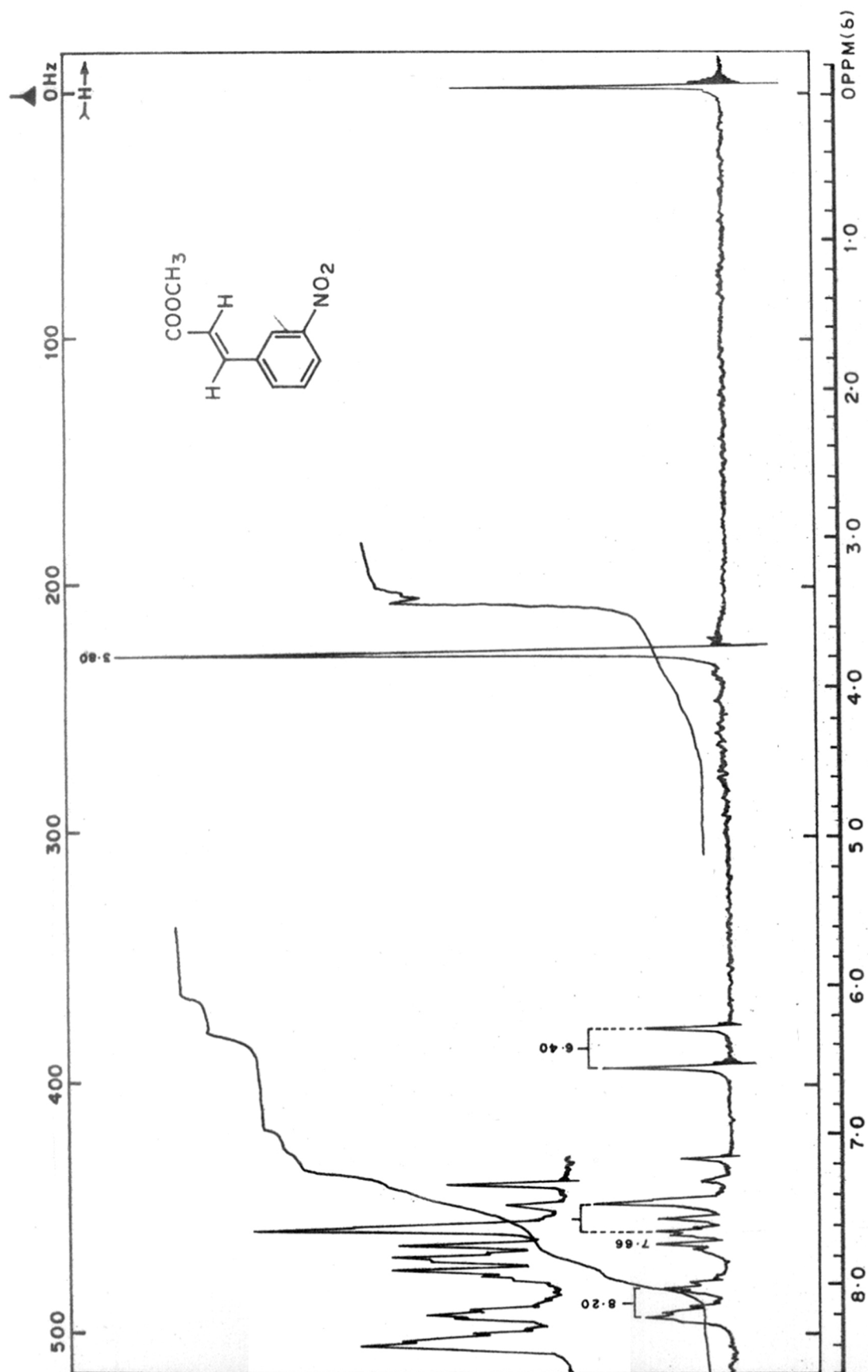
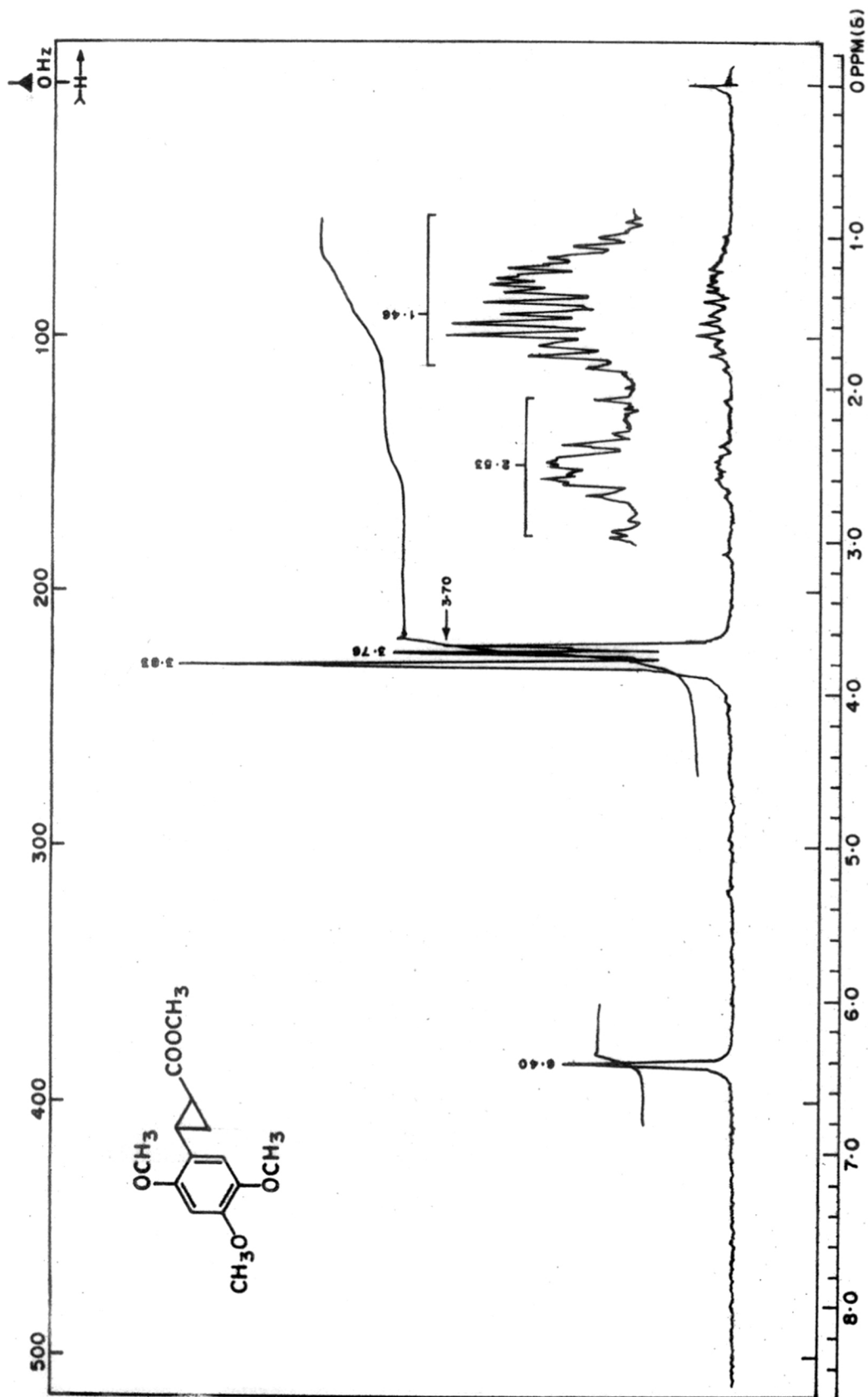
FIG. 14 ^1H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (X)

FIG. 15 ¹H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (XI)

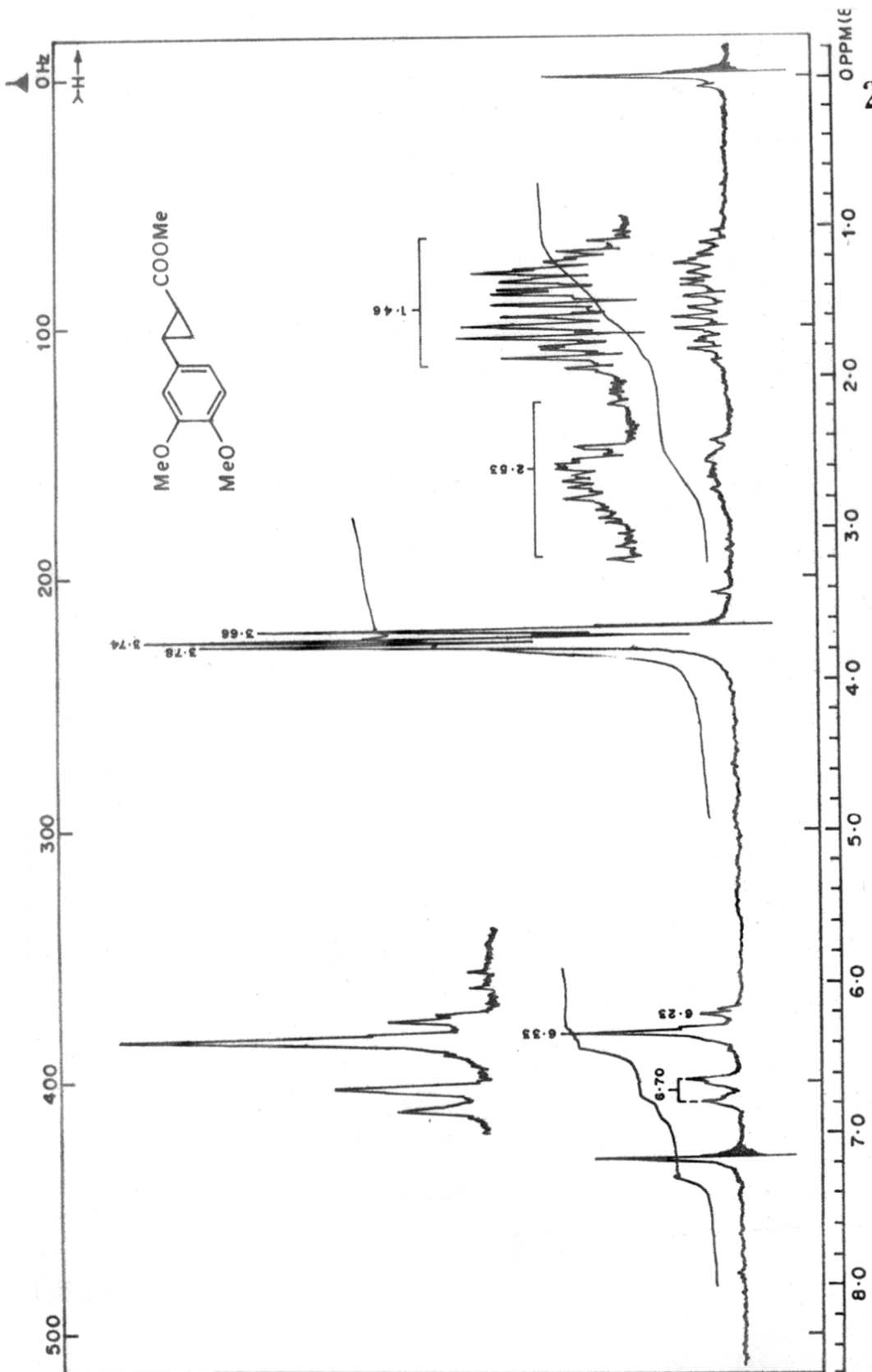


FIG. 15 ¹H NMR SPECTRUM OF METHYL ESTER OF COMPOUND (XII)

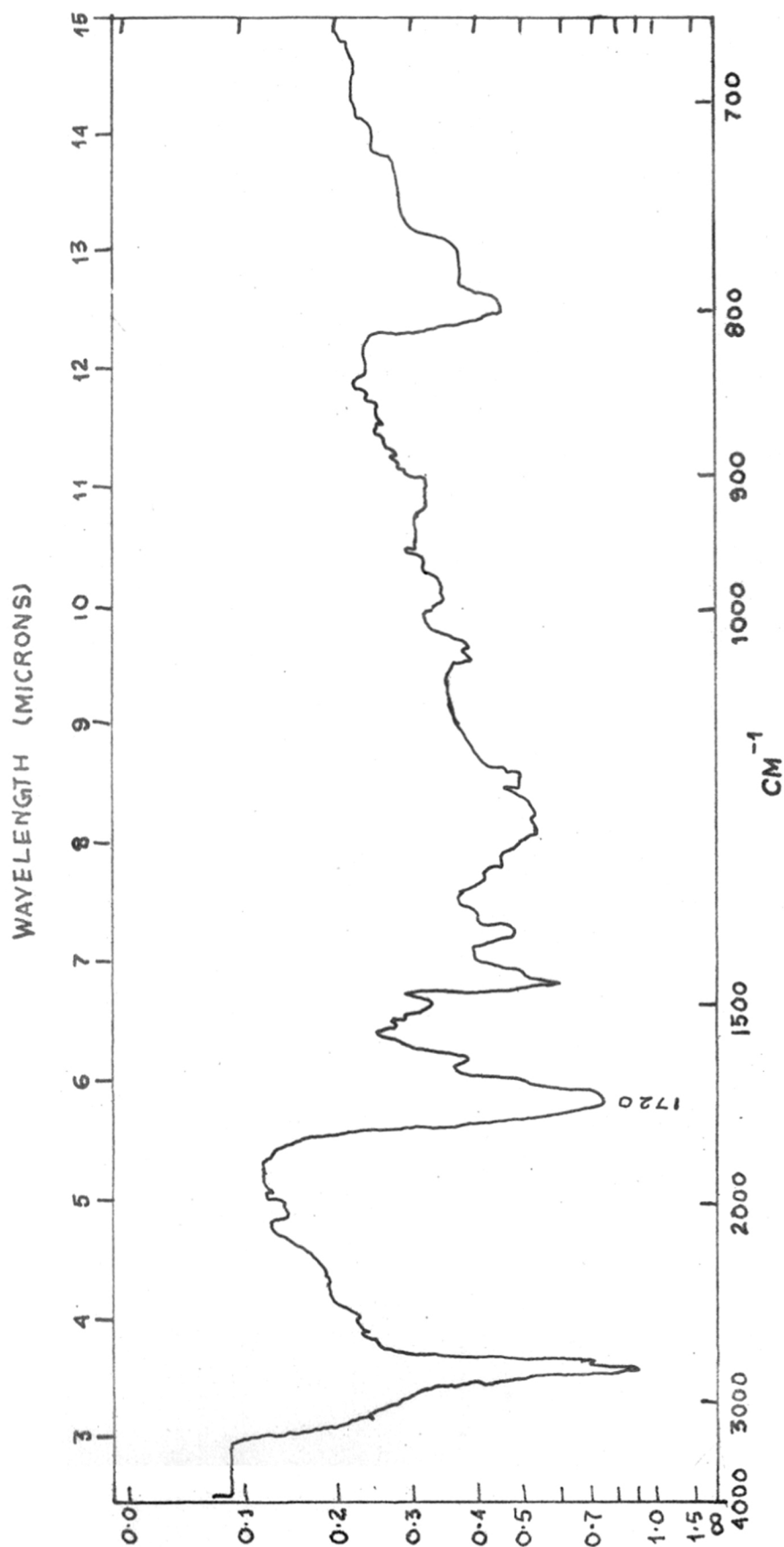
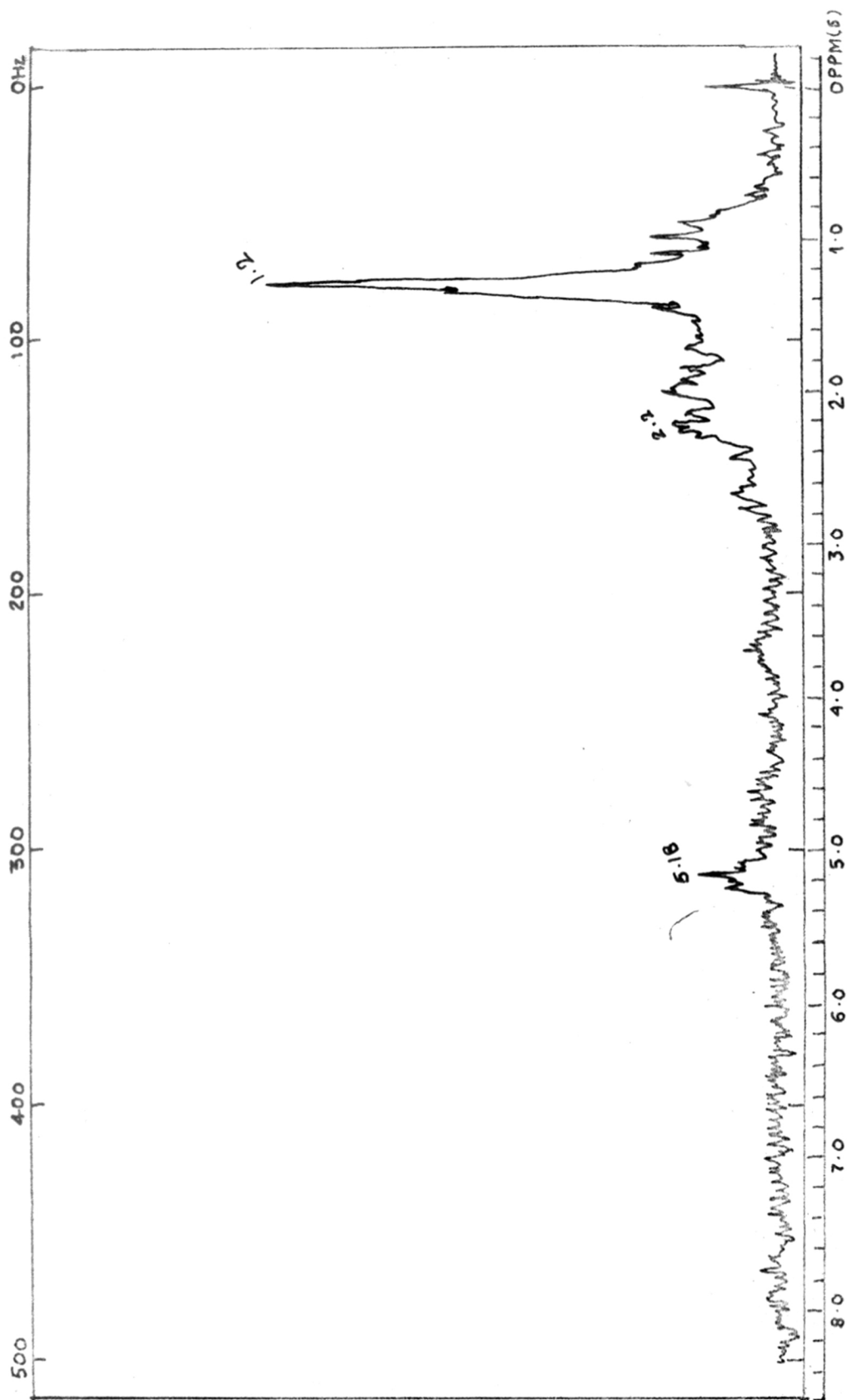


FIG 17: IR SPECTRUM OF COMPOUND. (JH. ACTIVE)

FIG. 18: ^1H NMR SPECTRUM OF CH. ACTIVE COMPOUND

ABSTRACT

A B S T R A C T

The thesis deals with the isolation, structure elucidation and chemical transformations of sesquiterpenoids, mainly sesquiterpene lactones. The thesis comprises of five chapters.

CHAPTER I - Sesquiterpene lactones of compositae

Sesquiterpene lactones are a class of naturally occurring plant terpenoids formed via head to tail condensation of three isoprene units and subsequent cyclization and oxidative transformations to a cis or trans fused lactones. Sesquiterpene lactones are characteristic constituents of Compositae, some of which are reported to be biologically active.

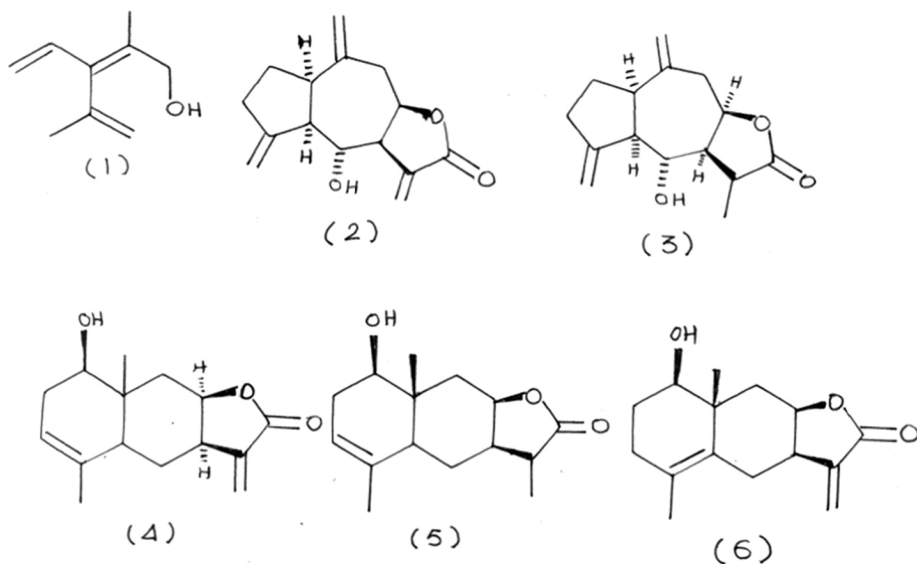
Chapter I attempts to give a brief review on the biogenesis, isolation methods, chemical transformations, physical methods for structure elucidation and biological activity of sesquiterpene lactones.

CHAPTER II - Chemical investigation of *Cyathocline purpurea*

So far only one species of the genus *Cyathocline* has been investigated chemically by Devangan et al.¹ A sesquiterpene alcohol lyratol (1) has been isolated from *Cyathocline lyrata*.

The chemical investigation of the acetone extract of *Cyathocline purpurea* resulted in isolation of four new sesquiterpene lactones (2-5), two guaianolides 2 and 3

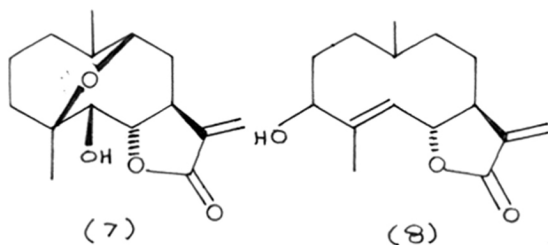
closely related to each other and two closely related eudesmanolides 4 and 5. Compounds 2 and 4 were isolated as major constituents whereas compounds 3 and 5 were present as minor constituents. In addition to these compounds (2-5) the extract also contained one more sesquiterpene lactone of known structure (6) as minor constituent.



The structure and stereochemistry of these compounds have been established from the extensive spectral studies of these compounds and transformation products, which are discussed in details.

CHAPTER III - Chemical investigation of *Cyathocline lutea*

The acetone extract of *Cyathocline lutea* on chemical investigation afforded two new germacranolides (7) and (8) the structure and stereochemistry of these lactones were established from their spectral data and chemical conversions. These aspects are discussed in detail.



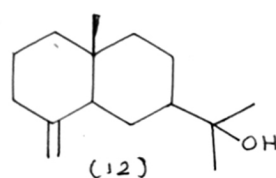
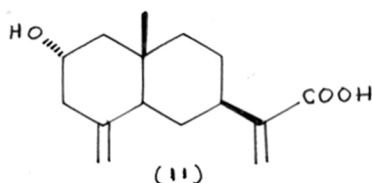
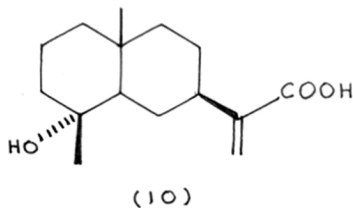
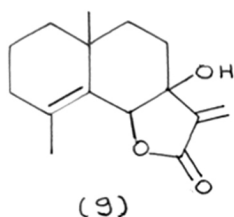
CHAPTER IV - Chemical investigation of *Sphaeranthus indicus*

Sphaeranthus indicus commonly called as gorakhmundi is a medicinally important plant found in various parts of India. It grows mainly in rice fields and is used in form of folk medicine.

The main constituents of the essential oil of *Sphaeranthus indicus* already reported are cadinene, α -lonone, β -caryophyllene, p-methoxycinnamaldehyde, eugenol, α -phelandrene, ocimene, citral and α -terpinene etc.² Besides the essential oil a bitter alkaloid has also been reported to occur in plant³.

The present chemical investigation of the acetone extract of plant resulted in isolation of a new sesquiterpene

(9) belonging to eudesmanolide class and two sesquiterpenic acids (10) and (11). In addition to these compounds the extract also contained a known sesquiterpene alcohol β -eudesmol (12) and acetylenic compound as minor constituents. Due to paucity of the acetylenic compound and its unstability, structure elucidation of acetylenic compound was not possible. The structures and stereochemistry of compound (9-11) were established from the extensive spectral studies of the compounds and their transformation products.



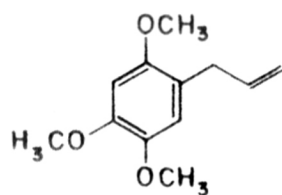
CHAPTER V - Structure activity relationship of insect antifeedant agents

Recently many insect antifeeding substances have been isolated from plants. In our studies directed towards the isolation and characterization of new insect control agents we screened a large number of plants belonging to family compositae.

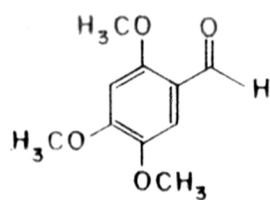
In the present study we isolated a known compound Y-asarone (13) from acetone extract of *Caesulia axillaris*^{4,5} which showed good antifeedant activity against larvae of *Spodoptera litura* and *Achea janata*.

Structure activity relationship study carried out with Y-asarone revealed that the presence of a $-CH_2-CH=CH_2$ in Y-asarone is possibly required for the antifeedant activity of Y-asarone as none of the compounds (14-17), in which the side-chain of Y-asarone was modified, showed any such activity.

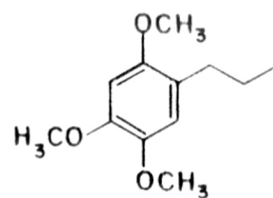
It is well documented in literature that the presence and position of o-methyl groups on aromatic ring play an important role in biological activity of such compounds. Cinnamic acid is known to show insecticidal activity against screw worms at 0.67% concentration. Hence keeping in view the above facts a number of substituted cinnamic acids (17-22) were prepared and tested for antifeedant activity against larvae of *Spodoptera litura* and *Musca domestica* adults. However, none of the compounds showed any appreciable activity. Cyclopropane analogues (23-25) of cinnamic acids also were prepared and tested for antifeedant activity. Here also none of the compounds showed any antifeedant activity. However, compounds (18) and (24) showed a good growth disruption activity against *Dystercus koenigii*.



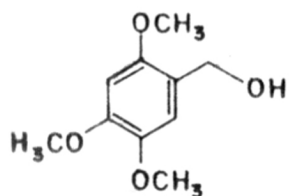
(13)



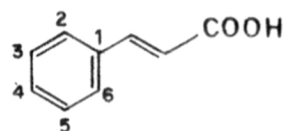
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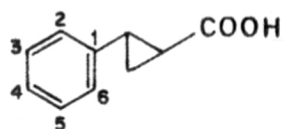
(15)



(16)



	2	3	4	5	6
(17)	OCH ₃	H	OCH ₃	OCH ₃	H
(18)	H	OCH ₃	OCH ₃	H	H
(19)	H	O-CH ₂ -O		H	H
(20)	OCH ₃	H	OCH ₃	H	H
(21)	H	H	H	H	Cl
(22)	H	H	H	H	NO ₂



	2	3	4	5	6
(23)	OCH ₃	H	OCH ₃	OCH ₃	H
(24)	H	OCH ₃	OCH ₃	H	H
(25)	H	O-CH ₂ -O		H	H

CHART (I)

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