CHEMICAL INVESTIGATION OF *PULICARIA WIGHTIANA*, *TURRAEA VILLOSA*, *GRANGEA MADERASPATANA* AND SOME CHEMICAL TRANSFORMATIONS OF α-PINENE

A thesis submitted to the
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By

Y.G. CHIPLUNKAR
Organic Chemistry : Technology
National Chemical Laboratory
Pune 411 008, India

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DEDICATED TO

MY PARENTS AND IN-LAWS

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CERTIFICATE

Certified that the work incorporated in the thesis entitled "**Chemical investigation of *Pulicaria wightiana, Turraea villosa, Grangea maderaspatana* and some chemical transformations of α-Pinene**" submitted by **Ms.Yamini Ganesh Chiplunkar**, was carried out by her at the National Chemical Laboratory, Pune, under my supervision for the degree of Doctor of Philosophy in Chemistry. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

July 1995
Pune

Dr.B.A.Nagasampagi
Research Guide

C O N T E N T S

CHAPTER IV Asymmetric Synthesis of B-seco Taxanoids from α-Pinene

ABBREVIATIONS

Ac	Acetyl
Bz	Benzoyl
Cin	Cinnamoyl
CSA	chlorosulphonic acid
DABCO	1,4-Diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DET	Diethyltartarate
DMAP	N,N-Dimethylamino pyridine
DHP	Dihydropyran
DME	Dimethoxy ethane
g.	gram/s
h.	hour/s
imid.	imidazole
MCPBA	m-chloroperbenzoic acid
Me	Methyl
PCC	Pyridinium chlorochromate
PDC	Pyridinium dichromate
Ph	Phenyl
Piv	Pivaloyl
TBAF	Tetrabutylammonium fluoride
TBCO	2,4,4,6-tetrabromocyclohexa-2,5-dienone
TBDMS	tert-Butyldimethylsilyl
TBHP	tert-Butyl hydroperoxide
p-TsOH	*p*-toluenesulfonic acid
Tig	Tigloyl
TMEDA	N,N,N',N'-Tetramethylenediamine
W-M Ketone	Wieland-Miescher ketone

GENERAL REMARKS

1. The figure numbers, chart numbers, scheme numbers and reference numbers, etc. given in each Chapter refer to that particular Chapter only.

2. All melting points (m.p.) and boiling points (b.p.) are uncorrected (°C). The boiling points in case of liquids refer to the oil bath temperatures.

3. All solvents and reagents were purified and dried using standard procedures. See: Perrin,D.D.; Armarego,W.L.F.; Perrin,D.R. "Purification of Laboratory Chemicals", Second Edition, Pergamon Press, Oxford, 1980.

4. Silica gel used for column chromatography was 60-120 mesh.

5. The thin layer chromatography (tlc) and preparative tlc (ptlc) plates were preared by spreading an aqueous suspension of silica gel (200-300 mesh, containing 13% $CaSO_4$ as binder) uniformly over glass plates using an applicator.

6. Layer thickness: tlc plates: 0.2mm, preparative tlc plates: 1.15mm. After initial drying at room temperature, the plates were activated at 100° for one hour before use. The solvent systems are given in parantheses. (For example, Acetone:Pet.ether, 10:90).

7. After development, the spots on tlc plates were visualized by exposing them to iodine vapour and/or by spraying with a mixture of H_2SO_4:HNO_3 (1:1), followed by charring in an oven at 150-160°C. In case of ptlc, the bands of compounds were visualized by spraying a dilute solution of iodine in chloroform only to the sides of plate covering the major portion with a glass plate.

8. In general, all reactions requiring anhydrous conditions were carried out under dry, oxygen free, nitrogen atmosphere.

9. Usual work-up refers to extraction of the reaction mixture with a suitable organic solvent (which is specified in the individual experiments), washing the organic layer with Na_2CO_3, water, followed by brine and drying over anhydrous Na_2SO_4.

10. Optical rotations were measured using $CHCl_3$ solutions unless otherwise mentioned, using sodium light (5893) as the source on a JASCO DIP-181 digital polarimeter.

11. UV absorption spectra were recorded on Shimadzu UV-VIS recording spectrometer, UV-260.

12. IR spectra were recorded on a Perkin-Elmer Infracord model 137-E. λmax are reported in reciprocal centimeters (cm^{-1}).

13. ^{1}H-NMR spectra were recorded on Varian T-60, FT-80A, Bruker FT-90, MSL-200 or MSL-300 instruments. The ^{13}C-NMR spectra were recorded on MSL-200 at 50.13 MHz or on MSL-300 at 75.48 MHz. The multiplicity of ^{13}C signals has been assigned by INEPT/DEPT experiments. All spectra were taken in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. ^{1}H-NMR data, using standard notations, are presented in the following order. Chemical shift (δ) (splitting pattern, J=coupling constant, relative proton ratio, assignment). The following abbreviations have been used while presenting the NMR data: s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet and br-broad.

14. ^{1}H-^{1}H COSY experiments were carried out on MSL-300 spectrometer.

15. Mass spectra (MS) were recorded on Finnigan Mat-1020 using a direct inlet system, at an ionization potential of 70 eV.

16. Elemental analyses (Anal.) were carried out using empty tube combustion method on Hoslis rapid carbon hydrogen analyzer.

17. Analytical GLC of the starting materials and reaction products has been carried out on a Hewlett Packard Gas Chromatograph 5793, with the following columns:

(i) OV-101 (5%, 6' x 1.8" O.D., Aluminium column)

(ii) SE-30 (10%, 6' x 1.8" O.D., Aluminium column).

ABSTRACT

Title: **Chemical investigation of *Pulicaria wightiana, Turraea villosa, Grangea maderaspatana* and some chemical transformations of α-Pinene**

The thesis comprises of four chapters -

CHAPTER I: Pregnane Steroids from Plants: A Review

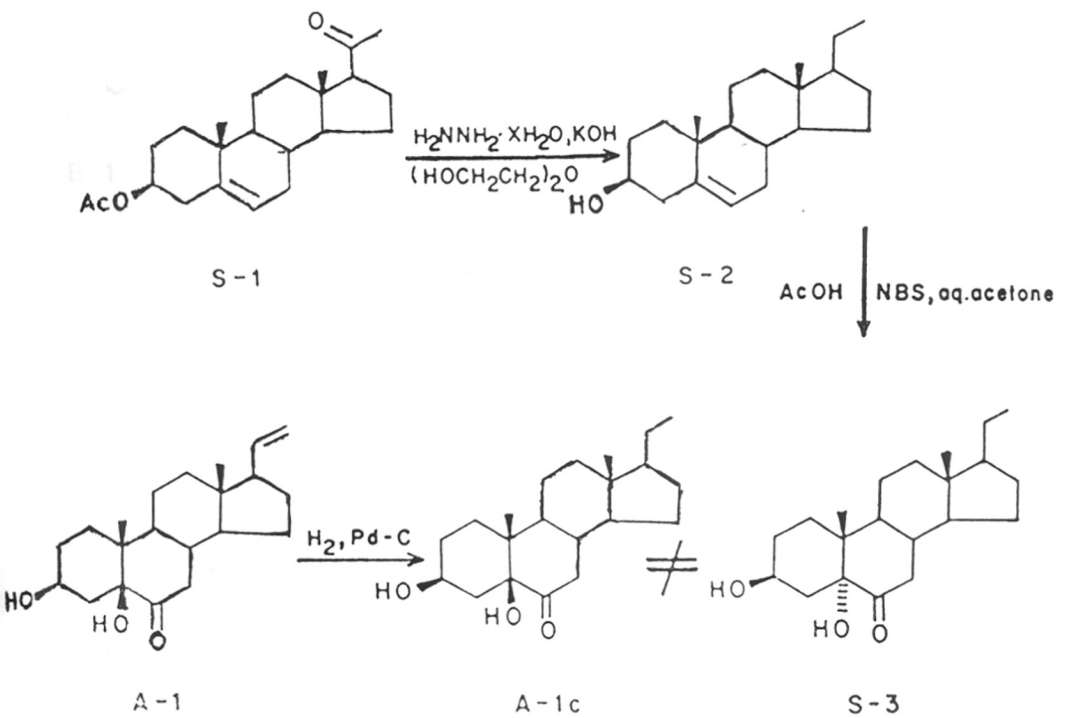
The chapter consists of a review of naturally occurring pregnanes from terrestrial plants. The review covers 172 new pregnane glycosides and aglycones reported during the period 1988-1993. The compounds have been classified based on the extent and sites of oxygenation on the carbon framework. The structures of pregnane glycosides have been given along with the sugar moieties. Biological activity is also included wherever reported.

CHAPTER II: Chemical investigation of *Turraea villosa*

The structure elucidation of a new sterol Villosterol[1] **A-1** is discussed in this chapter. The structure has been assigned based on spectroscopic studies and on the attempted partial synthesis of 20,21-dihydro villosterol starting from the known steroid, pregnenolone acetate [Scheme-1].

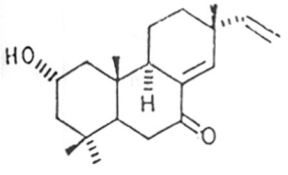
Pregnenolone acetate **S-1** on Huang-Minlon reduction yielded **S-2** which was converted to **S-3**, with NBS, aq. acetone; the reaction known to give 5α-hydroxy, 6-keto steroid[2]. Villosterol on hydrogenation was expected to give the same compound, but spectroscopic studies revealed **A-1c** to be 3β,5β-dihydroxy-pregnane-6-one. Based on this, stereochemistry of 5-hydroxy group in villosterol was assigned as 'β' which was further confirmed by X-ray analysis.

SCHEME - 1

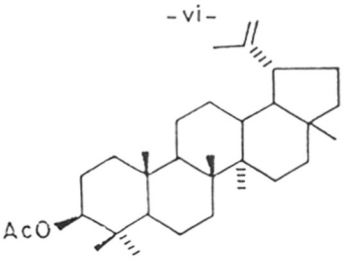


CHAPTER III: Chemical investigation of *Pulicaria wightiana* and *Grangea maderaspatana*

This chapter includes the isolation and characterization of two new diterpenoids, one each from *P.wightiana* and *G.maderaspatana*. A new pimarane diterpenoid **B-1** is reported from *P.wightiana* along with two known triterpenoids **B-2** and **B-3**. From *G.maderaspatana*, isolation of a new labdane diterpenoid **C-1** and four known compounds **C-2** to **C-5** has been reported. Structures of the new compounds **B-1** and **C-1** have been elucidated[3a,b] based on the spectroscopic studies and chemical correlation.



B-1



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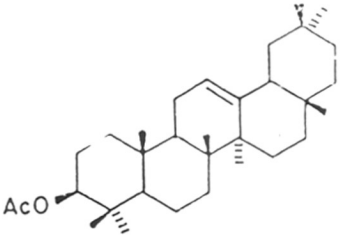

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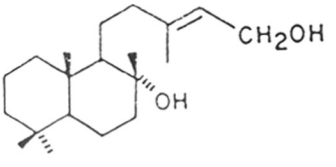
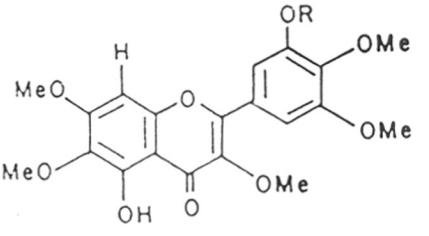
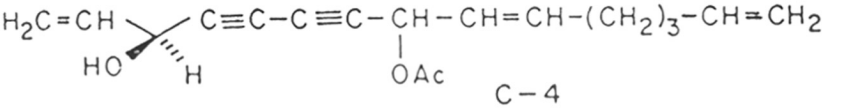


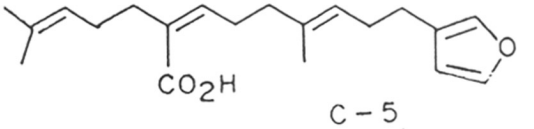
C-1



C-2 --- R = H
C-3 --- R = Me



C-4



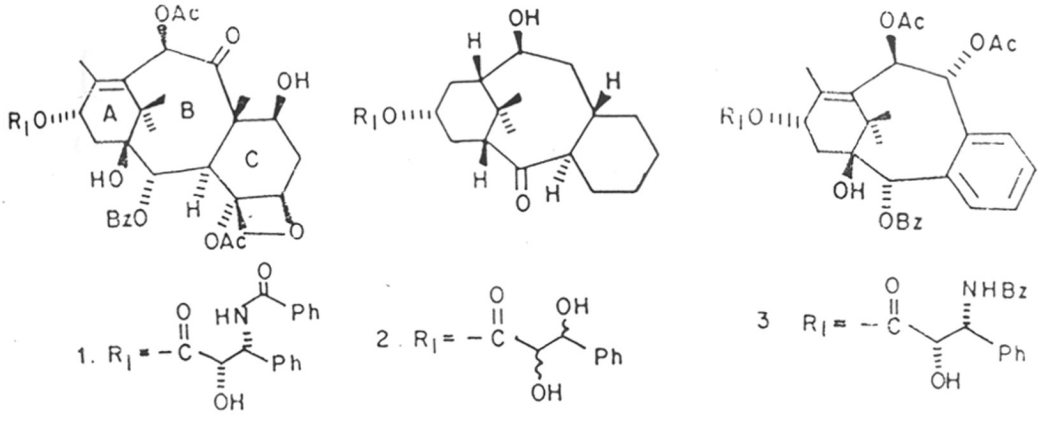
C-5

CHAPTER IV: Asymmetric Synthesis of B-seco-Taxanoids from α-Pinene

Taxol **1**, a complex, highly functionalized diterpenoid is currently the most exciting anticancer drug possessing activity against breast and ovarian cancer.[4] The total synthesis of **1** has been recently reported independently by the groups of Nicolaou[5] and Holton.[6] Currently, the major efforts are directed towards the synthesis of structurally simpler taxanoids as anticancer agents through the Structure-Activity Relationship (SAR) studies. One approach is to transform **1** into less

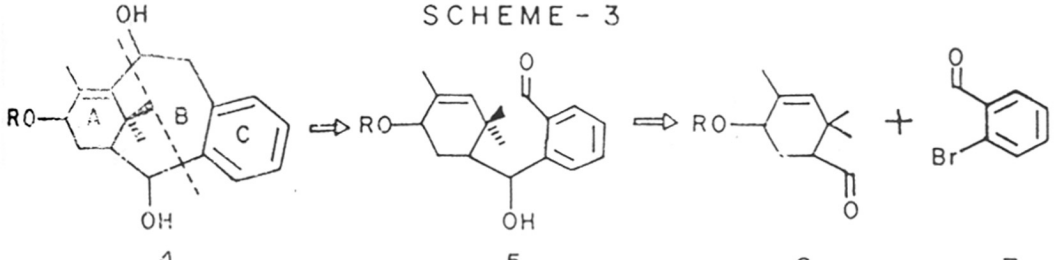
functionalized taxanoids, while the other one is to develop efficient strategies for their asymmetric synthesis. For example, the synthetic analogues of **1**, such as **2**, **3** prepared by Blechert[7] and Nicolaou[8] have been shown to be of great promise [Scheme-2].

SCHEME - 2



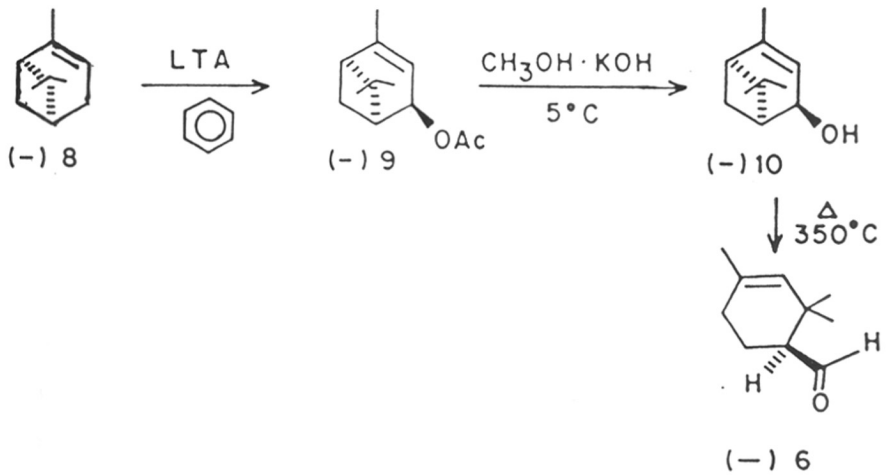
The present chapter describes a novel and efficient approach for the asymmetric synthesis of C-aromatic seco-B taxanoid of the type **4** using α-Pinene **8** as the starting point; based upon the retrosynthetic analysis, as depicted in [Scheme-3].This strategy therefore demands the synthesis of the key precursor **5** to reach the target molecule **4**.

SCHEME - 3



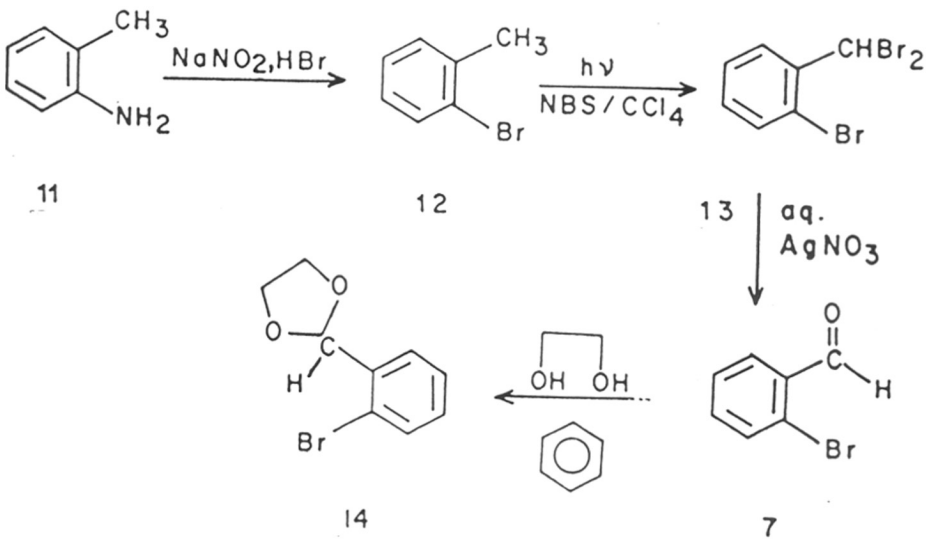
Although several syntheses of A-ring subunits of the type **6** are reported in the literature,[9] an easy accessibility particularly to the chiral A-ring still remains problematic. We have now prepared[10] **6** from α-Pinene **8** as shown in [Scheme-4].

SCHEME - 4



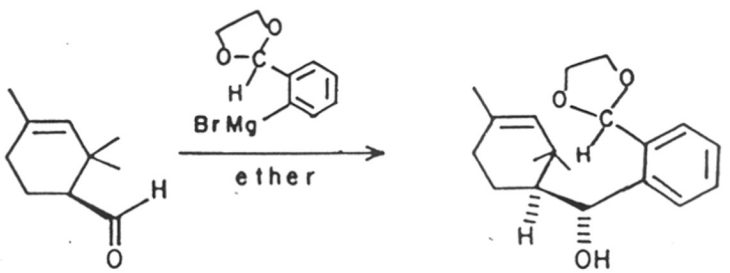
The C-ring unit **7** was procured from O-toluidine[11] **11** [Scheme-5].

SCHEME - 5



The two units **6** and **7** were then readily coupled using the Grignard reaction. [Scheme-6].

SCHEME - 6



(−) 6 15

The structure of **15** was thoroughly established by its spectral properties and confirmed by the X-ray analysis. The transformation of **5** into **4** by the standard homologation procedure, followed by the application of one of the known protocols i.e. McMurry coupling[9] for the construction of central B-ring cyclooctane is underway.

In summary, a new approach based on use of α-Pinene, which is readily available in both the enantiomeric forms, has been developed.

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CHAPTER I

Pregnane Steroids from Plants : A Review

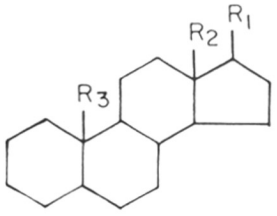
1.1 : Introduction

Sterols (derived from the Greek word "Stereos" meaning solid) are solid alcohols widely distributed in plant and animal kingdom. The steroid family encompasses a wide variety of medicinally important compounds such as bile acids, vitamin D, various antibiotics, insect moulting hormones and adrenocortical hormones.

The study of naturally occurring steroids dates back to 1812 when Michel Eugene Chevreul first differentiated between saponifiable and non-saponifiable lipids leading to discovery of cholesterol.[1] Thereafter, isolation of a large number of similar compounds such as bile acids, ergosterol etc.from animal and plant origin was reported. However, the systematic study on the structure of cholesterol began after almost sixty years, which was taken up by Diels and Windaus in 1903[2].

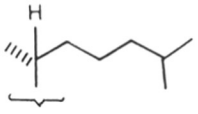
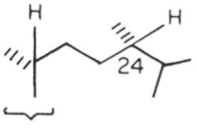
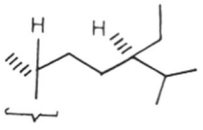
The real expansion of studies on the natural steroids started when the sex hormones and adrenocortical hormones were isolated and identified during 1929-1940[3]. The earlier studies were limited only to the abundantly available steroids occurring as major compounds, the structures of which were mostly given based on the exhaustive degradation of the natural compounds. Soon with the advent of spectroscopic techniques such as UV-VIS, IR, Mass and especially the ^{1}H-NMR, chemists relied more on the combination of chemical correlation with an already known compound and spectral data for structure elucidation. A dramatic rise in the number of naturally occurring steroids was observed only after sixties when the Fourier transform (FT) techniques were introduced in the analytical instruments, especially the NMR. This made it possible for a chemist to characterize even a few milligram of the sample leading to its structure elucidation. The research in this area then picked up, as seen by the ever increasing number of new natural steroids appearing in the literature every year.

The basic skeleton of steroids can be represented as **1**; which is a perhydrocyclopentenophenanthrene framework with substituents at R1, R2 and R3.



1

Table-1 : Classes of steroids

Class	Substituents		
	R_1	R_2	R_3
Gonane	H	H	H
Estrane	H	Me	H
Androstane	H	Me	Me
Cholestane		Me	Me
Ergostane		Me	Me
Stigmastane		Me	Me
Pregnane	Et	Me	Me

Based on these substituents, steroids are classified into different classes such as gonane, estrane, androstane, cholestane, ergostane, stigmastane and pregnane (Table-1).

In this review, we restrict ourselves to the class of naturally occurring pregnanes from terrestrial plants. The class of pregnanes inclusive of sex hormones like gestogens, corticosteroids and a number of other compounds of immense biological significance, possesses a great potential with respect to chemistry and pharmacology. Pregnanes are also supposed to be the biological precursors of cardenolides[4]; the compounds known for a powerful heart stimulating activity.

Upto 1960, very few pregnanes with relatively simple skeletons were isolated every year. However, after seventies, the number of natural pregnanes isolated is continuously increasing along with the complexity of their structures. The latest review covering the reports of natural pregnanes up to 1987 has appeared in Phytochemistry 1989[5] and every year new compounds are being added to the number of natural pregnanes. For example, in the last six years, almost a hundred and fifty new compounds have been reported in this area. Therefore, it was felt logical to review the natural pregnanes reported in the literature after 1987 till date. Since the pregnanes differ greatly in the position and number of oxygenated sites, the compounds have been classified based on the extent of oxygenation on the skeletal framework. This enabled us to categorize them as di-, tri-, tetra-oxygenated pregnanes, etc. Those which possess exceptional structural features have been grouped together as unusual pregnanes.

The natural pregnanes occur as pregnane glycosides as well as aglycones. It is well known that different sugar moieties can affect the solubility behaviour and other physico-chemical properties which may in turn can play an important role in the biological activity of the compounds. Therefore, in this review, for the first time we are reporting the pregnanes occurring as glycosides along with the structures of their sugar moieties. Biological activity of the compounds has also been included wherever possible.

CLASSIFICATION OF PREGNANES

1.2 A : Dioxygenated pregnanes

This class includes the compounds with oxygenations at C_3 and either at C_{16} or C_{20} [Chart-1].

A-1 :Compounds with oxygenation at C_3 and C_{16}

The aglycones **1,2,3,4** from this class were isolated from the ethyl acetate soluble fraction of *Commiphora mukul* gum resin. The fraction is reported to have significant hypocholestremic/hypolipaemic activity[6,7]. Some of these dioxygenated pregnanes **2,3** gain further importance as they exist as probable intermediates in the proposed pathway for catabolism of cholesterol occurring in plants[8].

A-2 :Compounds with oxygenation at C_3 and C_{20}

Two new glycosides **5,6** from antitumor fraction of *Periploca sepium* root barks[9] belong to this class. The sugar linkage in these compounds is present at C_{20}, which is an unusual position. Two aglycones from *C.mukul*[6] **7,8** also find place in this group.

1.3 B : Trioxygenated pregnanes

The pregnanes belonging to this class have been further subdivided into four groups, B-1,B-2,B-3 and B-4 based on the oxygenation sites [Chart-1].

B-1 :Compounds with oxygenation at C_3, C_{20} and C_{12}

The group includes compounds with characteristic double bond positions viz. $C_{4(5)}$, $C_{6(7)}$ and $C_{16(17)}$. Neridienone A **9**, first isolated from *Nerium odorum*[10a] is a pregnane frequently ocurring in diferrent species of the family Apocynaceae[10b]. Its various derivatives such as 6,7 dihydro, 12 keto Neridienone A (**10-14**) have been isolated from *N. odorum* and *Anodendron affine* [10a,b,c,11]. A similar compound in the same series, 21-hydroxypregna-4,6-diene-3,20-dione

15 has been reported by Abe *et al*[12]. Neridienone-B **16** which is a 20,21-dihydroxypregna-4,6-diene-3,12 dione[10c] is structurally similar to these compounds and hence, though tetraoxygenated, it has been included in this subgroup. The compounds **9,12,13** exhibit piscicidal activity such as arresting the movement of goldfish[10,11].

B-2 :Compounds with oxygenation at C_3, C_{20} and C_{14}

All the compounds belonging to this class have been isolated from different species of the family Asclepiadaceae. Although the parent aglycones of some of the compounds are reported in the literature, various new glycosides which have been isolated recently deserve special mention.

Glycosides belonging to this subgroup are sioraside **17** from *Streblus asper*[13] and Calogenin glycosides indicine **18** and hemidine **19**[14]. Oxysine **20** from *Oxystelma esculentum*[15] and calocinin **21** isolated by Sethi *et al.* from *Periploca calophylla*[16] are also included in the same group.

B-3 :Compounds with oxygenation at C_3, C_{20} and C_{16}

This group includes the aglycones lycopersiconolide **22**[17], lycopersiconol **23**[18] and 20S-hydroxyvespertilin **24**[19] isolated from different species of the family Solanaceae and two diastereomeric alcohols toosendansterols A and B **25,26** from *Melia toosendan* (Meliaceae)[20]. Pregnane steroids are not commonly found in these families and very few such as the obove mentioned are reported in the literature. The glycosides **27,28,29** from the antitumor fraction of *Periploca sepium*[9] also find place in this group.

B-4 :Compounds with oxygenation at C_3, C_{20} and C_{17}

The glycosides teikasides and periplocosides form a major portion of this group. Abe and Yamauchi in 1981 reported the isolation of teikaside A, later renamed as teikaside A-IIIa **35** from *Trachelospermum asiaticum*[21]. In continuation of this work, several glycosides viz. teikasides A-Ia,A-Ib...**30-38**[22], C-O, C-IIa....**39-45**[23] were isolated from the same source. Similar glycosides such as teikasides AL-Ic...**46-48** were isolated from another species of

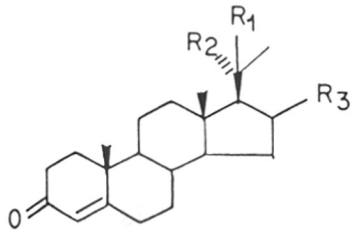
Trachelospermum, T.liukiuense[24]. The common aglycone in all these compounds, which was named Teikagenin, possessed a double bond at a rather unusual position; $C_{6(7)}$. Out of the C_3, C_{20} and C_{17} oxygenation, sugar moieties were attached only at C_3 and C_{20}. Teikaside A-IIIa is structurally similar to glycosides K, H_1 and E isolated from *Bei-Wujiapi* (a Chinese crude drug from *P.sepium*)[25,26a,b]. These glycosides from the drug were found to cause potentiation of nerve fibre outgrowth mediated by NGF (nerve growth factor). Teikaside A-IIIa was, therefore, expected to show similar activity[21].

Four more new glycosides of teikagenin namely basicosides A,B,C and D **49-52** were isolated from the roots of *Apocynum venetum*[12]. The antitumor fraction of *P.sepium* when hydrolysed, yielded the compound S-2A **53**[27] named periplocogenin[27]. From the unhydrolyzed fraction of the same plant, a number of new glycosidal compounds namely periplocosides A,B,C **54-56**[28] and periplocosides J,K,F,O **57-60**[29] were isolated by Itokawa *et al.* The common aglycone in all these compounds is similar to teikagenin, except for the double bond position . The double bond in periplocosides is at $C_{5(6)}$. The hexosulose and heptulopyranose sugars in the glycoside portion are characteristic features of the compounds. Partial hydrolysis of periplocoside A yielded the glycosides, periplocosides D,E,L,M and N[30].

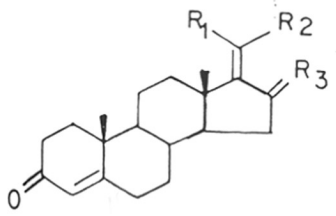
Chart-1: Di- and trioxygenated Pregnanes

Class A: Dioxygenated pregnanes		
Sub-class	Sites of oxygenation	Compound Numbers
A-1	C_3, C_{16}	1, 2, 3, 4
A-2	C_3, C_{20}	5, 6, 7, 8
Class B: Trixoygenated pregnanes		
B-1	C_3, C_{20}, C_{12}	9, 10-14, 15, 16
B-2	C_3, C_{20}, C_{14}	17, 18, 19, 20, 21
B-3	C_3, C_{20}, C_{16}	22, 23, 24, 25, 26, 27-29
B-4	C_3, C_{20}, C_{17}	30-38, 39-45, 46-48, 49-52, 53, 54-56, 57-60

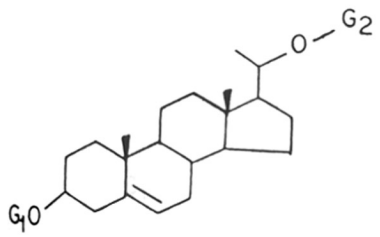
Chart.1 : Structures



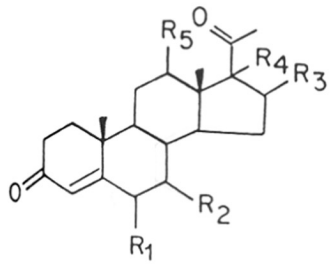
	R_1	R_2	R_3
1.	H	H	α-OH
7.	H	OH	H
8.	OH	H	H



	R_1	R_2	R_3
2.	H	CH_3	O
3.	H	CH_3	β-OH
4.	CH_3	H	O

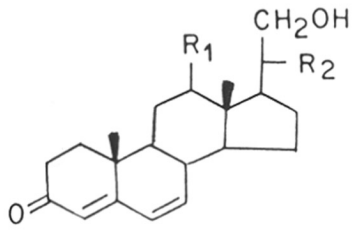


	G_1	G_2
5.	dgtl(1→4)-cym	glu(1→6)-glu(1→2)-dgtl
6.	2-OAc-dgtl(1→4)-cym	glu(1→6)-glu(1→2)-dgtl

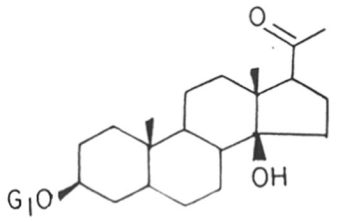


	R_1	R_2	R_3	R_4	R_5
9.	=		=		β-OH
10.	=		H	H	β-OH
11.	H	H	H	H	β-OH
12.	H	H	=		β-OH
13.	=		=		= O
14.	H	H	=		= O

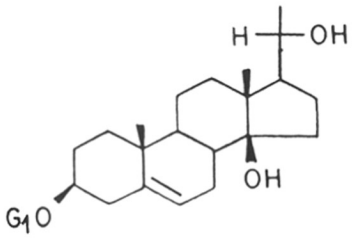
Chart.1 : Structures (contd.)



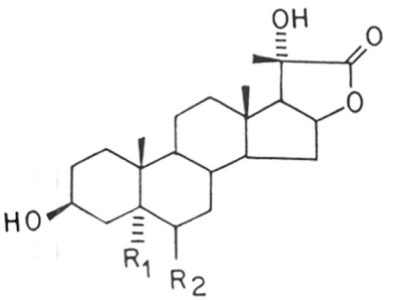
	R_1	R_2
15.	H	=O
16.	=O	β-OH



17. G_1 = 3-O-Me-glu

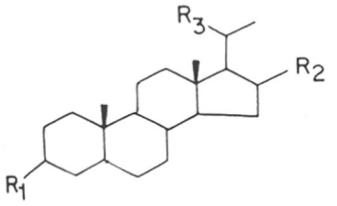


	G_1
18.	digit
19.	boiv
20.	olean(1→4)-thev(1→4)-cym(1→4)-digit
21.	β-L-2,6-dideoxyfuco

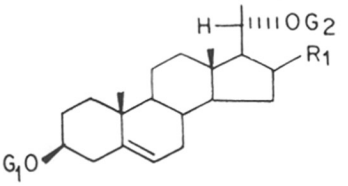


	R_1	R_2
22.	H	H
24.	=	

Chart.1 : Structures (contd.)

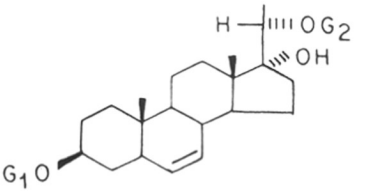


	R_1	R_2	R_3
23.	β-OH	β-OH	=O
25.	β-OH	=O	β-OH
26.	α-OH	=O	β-OH



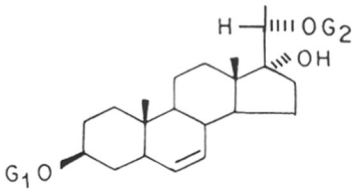
	R_1	G_1
27.	β-OH	2-OAc-dgtl(1→4)-cym
28.	β-OH	H
29.	α-OH	2-OAc-dgtl(1→4)-cym

For 27,28,29: G_1 = glu(1→6)-glu(1→2)-dgtl



	G_1	G_2	Teikaside
30.	dgtl	can	A-Ia
31.	dgtl	olean	A-Ib
32.	dgtl	glu(1→4)-digin	A-IIa
33.	dgtl	glu(1→4)-olean	A-IIb
34.	dgtl	glu(1→4)-can	A-IIc
35.	dgtl	glu(1→4)-sar(1→4)-sar	A-IIIa

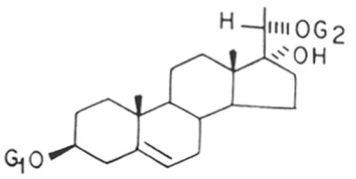
Chart.1 : Structures (contd.)



	G_1	G_2	Teikaside
36.	dgtl	glu(1→4)-olean(1→4)-sar	A-III b
37.	dgtl	glu(1→4)-olean(1→4)-olean	A-III c
38.	dgtl	glu(1→4)-digin(1→4)-sar	A-III d
39.	4-OAc-α-L-sar(1→4)-dgtl	H	C-O
40.	4-OAc-α-L-sar(1→4)-dgtl	glu(1→4)-digin	C-II a
41.	4-OAc-α-L-sar(1→4)-dgtl	glu(1→4)-olean	C-II b
42.	4-OAc-α-L-sar(1→4)-dgtl	glu(1→4)-can	C-II c
43.	4-OAc-α-L-sar(1→4)-dgtl	glu(1→4)-olean(1→4)-olean	C-III a
44.	4-OAc-α-L-sar(1→4)-dgtl	glu(1→4)-cym(1→4)-olean(1→4)-olean	C-IV a
45.	α-L-sar(1→4)-dgtl	glu(1→4)-cym(1→4)-olean(1→4)-olean	B-IV a
46.	dgtl	dgtl	AL-Ic
47.	dgtl(1→4)-dgtl	dgtl	BL-Ic
48.	dgtl	glu(1→4)-dgtl	AL-II d

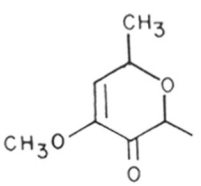
		Basicoside
49.	fuco	A
50.	3'-OAc-fuco	B
51.	fuco	C
52.	fuco	D

Chart. I : Structures (contd.)



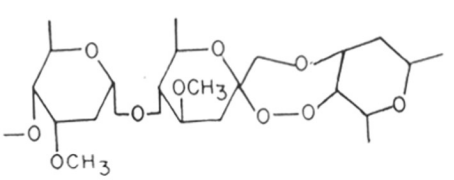
	G_1	G_2	Periplocoside
53.	I	H	
54.	I	2-OAc-dgtl(1→4)-cym(1→4)-cym(1→4)-cym-II	A
55.	I	cym(1→4)-cym-II	B
56.	I	cym-II	C
57.	H	dgtl(1→4)-cym(1→4)-can(1→4)-digit-II	J
58.	I	dgtl(1→4)-cym(1→4)-can(1→4)-digit-II	K
59.	H	dgtl(1→4)-cym(1→4)-cym(1→4)-cym-II	F
60.	I	3-O-methoxymethyl-can	O

For 53 – 60 :

I = 

4',6'-dideoxy-3'-OMe-Δ^3-D-2'-hexosulose

I

II = 

II {(1→5)-3,7-dideoxy-4-OMe-α-D-gluco-2-heptulopyranosyl(2→4)-dioxy(1→3)-β-D-c an}

1.4 C : Tetraoxygenated pregnanes

Pregnanes with oxygenations at C_3, C_{12}, C_{14} and C_{20} constitute a major part of this class. The remaining compounds possess oxygen functions at various positions including the rare ones such as C_2, C_4 and C_{16} [Chart-2]. Some of these compounds exhibit unique structural features such as C_{17} ethyl group and uncommon site of sugar linkage, C_2.

C-1 :Compounds with oxygenation at C_2, C_3, C_4 and C_{16}

Ketwaru *et al.* in 1993 reported the isolation of two diastereomeric aglycones **61,62** from roots and leaves of *Trichilia schomburgkii*[31]. The compounds possess hydroxyl groups at uncommon positions such as C_2, C_3 and C_4 along with a ketone at C_{16}. The C_{17} ethyl group is another characteristic feature of the compound, rarely observed in pregnanes from terrestrial plants[6,32].

C-2 :Compounds with oxygenation at C_2, C_3, C_{20} and C_{16}/C_{12}

Very few pregnanes belong to this subclass of compounds. Stizophyllin **63** from *Stizophyllum riparium*[33] is a novel pregnane with three nonconjugated double bonds at $C_{4(5)}$, $C_{7(8)}$ and $C_{16(17)}$. Its 16,17-dihydro analogue **64** has also been reported from the same species[34]. Compound **63** exhibits cytotoxicity against P-388 lymphocytic leukemia test system[33,34]. Nakanishi *et al.* have reported the isolation of toosendanoside **65** from *Melia toosendan* (Meliaceae)[35]. The compound is one of the rare meliaceaous pregnane glycosides and possesses a sugar linkage at an uncommon site i.e. C_2.

C-3 :Compounds with oxygenation at C_3, C_{12}, C_{14} and C_{20}

This class includes boucerosides from *Boucerosia aucheriana*. Some of them consist of a known genin boucerin isolated in 1967 by Nikaido and co-workers[36]. However, these compounds namely boucerosides A-I,A-II,B-I,B-II **66-69**[37], ADC, ADO, BDO, BDC **70-73**[38] have

been included in the review for their characteristic glycosidal linkages. The new glycosides of 5,6 dihydro boucerin with different ester groups named as boucerosides-ANC,ANO etc.**74-79** have been reported by Tanaka *et al.*[38].

1.5 D : Pentaoxygenated pregnanes

The group consists of pregnane glycosides mainly from the family Asclepiadaceae and has been subdivided into four subgroups D-1, D-2, D-3 and D-4 [Chart-2].

D-1 :Compounds with oxygenation at C_3, C_{12}, C_{14}, C_{17} and C_{20}

Ito *et al.* have reported the isolation of a utendin glycoside MF-D **80** from *Marsdenia formosana* along with the glycoside MF-C **81** of pergularin which is C_{20} oxidized utendin. The same plant has yielded another glycoside MF-A **82** with the genin dehydrotomentosin[39]. Two more utendin glycosides viz. cynanformosides A,B **83,84** were reported from *Cynanchum formosanum*[40]. A tomentogenin glycoside, dregeoside C **85** from *Dregea lanceolata* also finds place in this group[41].

D-2 :Compounds with oxygenation at C_3, C_{11}, C_{12}, C_{14} and C_{20}

Cynafosides A,B **86, 87**, two novel glycosides from *Cynanchum africanum* were reported in 1985 by Tsukamoto *et al.*[42]. The aglycone cynafogenin with two ester linkages at C_{11} and C_{12} was later characterized completely eliminating the ambiguities regarding the ester positions[43]. In continuation with this work, two more cynafogenin glycosides cynafosides C,D **88, 89** were isolated from the same source[44]. In the sugar linkage of these compounds both D-and L-cymarose are observed, which is an uncommon feature. Working on the same lines, cynafosides E,F,G,H **90-93** were reported by Steyn *et al* from *C. africanum*. The compounds **88-90, 92, 93** produce symptoms of cynanchosis in guinea pigs[45]. Various dregeosides **94-104** isolated from *Dregea volubilis*[41,46a,b] are also included in the same group. The stem of *D.volubilis* is used as an antifebrile and emetic in south east Asia[46a]. The dregeosides Apl and Ao1 exhibited antitumor activity against

Ehrlich carcinoma (solid type). Aol also showed activity against melanoma B-16[46a,b]. The dregeosides consisted of different types of aglycone moieties such as drevogenin A, drevogenin D and drebyssogenin.

D-3 :Compounds with oxygenation at C_3, C_8, C_{12}, C_{14} and C_{20}

Calotroposides A, D, F, G **105-108** with lineolon as the aglycone belong to this subgroup. The roots of *Calotropis gigantea* from which the calotroposides have been isolated, are used in the folk medicine of Indonesia as an antiscabetic and against snake bite[47a,b].

D-4 :Compounds with oxygenation at C_3, C_{14}, C_{17}, C_{20} and C_{21}

Two new and one known methoxy pregnanes (**109**, **110** and **111** respectively) with the above mentioned rare oxygenation pattern form this small group. These novel compounds have been reported by Xu *et al* from *P. sepium*[48].

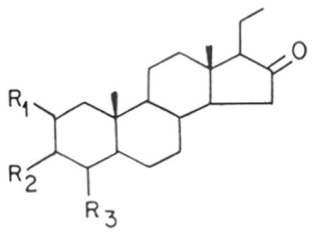
Chart-2 : Tetra- and pentaoxygenated pregnanes

Class C: Tetraoxygenated pregnanes		
Sub-class	Sites of oxygenation	Compound Numbers
C-1	C_2, C_3, C_4, C_{16}	**61, 62**
C-2	C_2, C_3, C_{20}, C_{16}/C_{12}	**63, 64, 65**
C-3	C_3, C_{12}, C_{14}, C_{20}	**66-69, 70-73, 74-79**
Class D: Pentaoxygenated pregnanes		
D-1	C_3, C_{12}, C_{14}, C_{17}, C_{20}	**80, 81, 82, 83, 84, 85**
D-2	C_3, C_{11}, C_{12}, C_{14}, C_{20}	**86, 87, 88, 89, 90-93, 94-104**
D-3	C_3, C_8, C_{12}, C_{14}, C_{20}	**105-108**
D-4	C_3, C_{14}, C_{17}, C_{20}, C_{21}	**109, 110, 111**

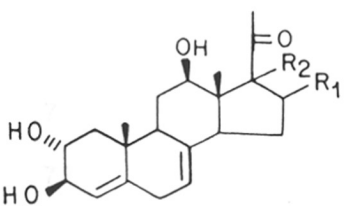
1.6 E : Hexaoxygenated pregnanes

The compounds have been subdivided into two groups E-1 and E-2 [Chart-3].

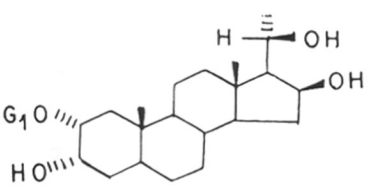
Chart. 2. Structures



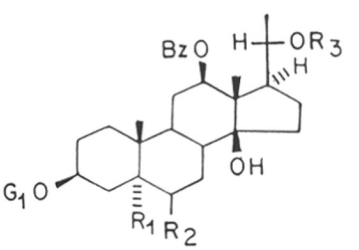
	R_1	R_2	R_3
61.	β-OH	β-OH	β-OH
62.	α-OH	α-OH	β-OH



	R_1	R_2
63.	=	
64.	H	H

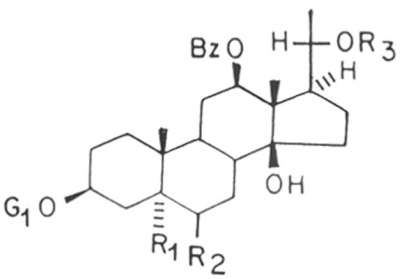


65. G_1 = glu



	R_1	R_2	R_3	G_1	Bouceroside
66.	H	H	Bz	G_b	A I
67.	=		Bz	G_b	A II
68.	H	H	Bz	G_a	B I
69.	=		Bz	G_a	B II
70.	=		H	G_a	ADC
71.	=		H	G_b	ADO

Chart.2 : Structures (contd.)



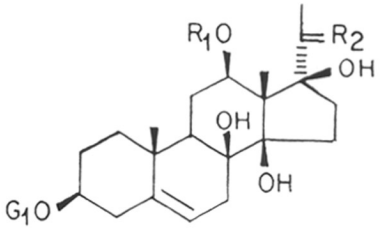
	R_1		R_2	R_3	G_1	Bouceroside
72.		=		Ac	G_b	BDO
73.		=		Ac	G_a	BDC
74.	H		H	H	G_a	ANC
75.	H		H	H	G_b	ANO
76.	H		H	Ac	G_b	BNO
77.	H		H	Ac	G_a	BNC
78.	H		H	Bz	G_b	CNO
79	H		H	Bz	G_b	CNC

For 66 – 79 :

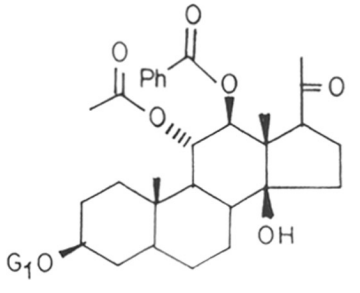
G_a = 3-OMe-6-deoxy-allo(1→4)-cym(1→4)-cym

G_b = 3-OMe-6-deoxy-allo(1→4)-olean(1→4)cym

Chart.2 : Structures (contd.)

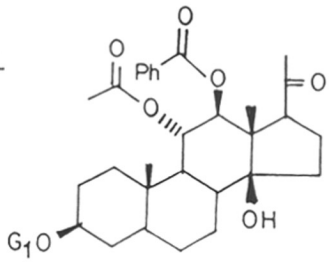


	R_1	R_2	G_1	
80.	H	β-OH	cym	
81.	H	=O	cym	
82.	Tig	β-OAc	cym	
83.	H	β-OH	olean	Cynanformoside A
84.	H	β-OAc	olean	B
85.	Ac	β-OBz	olean(1→4)-cym(1→4)-cym	Dregeoside C

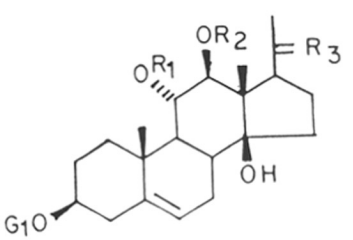


	G_1	Cynafoside
86.	glu(1→4)-α-L-cym(1→4)-cym(1→4)-cym	A
87.	glu(1→4)-α-L-cym(1→4)-cym(1→4)-digit	B
88.	glu(1→4)-α-L-cym(1→4)-olean(1→4)-digit(1→4)-cym	C

Chart 2. Structures

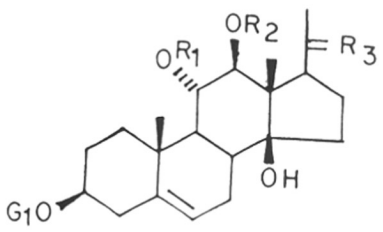


	G_1	Cynafoside
89.	glu(1→4)-α-L-cym(1→4)-olean(1→4)-digit(1→4)-digit	D
90.	α-L-cym(1→4)-cym(1→4)-digit	E
91.	α-L-cym(1→4)-olean(1→4)-digit(1→4)-digit	F
92.	olean(1→4)-digit(1→4)-digit	G
93.	glu(1→4)-glu(1→4)-α-L-cym(1→4)-cym(1→4)-cym	H



	R_1	R_2	R_3	G_1	Dregeoside
94.	$-COCH_3$	-Isoval	O	G_a	Apl
95.	$-COCH_3$	-Isoval	O	G_b	Ao1
96.	$-COCH_3$	-Isoval	O	G_c	Aa1
97.	$-COCH_3$	-Isoval	O	G_d	A_{11}
98.	-Cin	$-COCH_3$	O	G_d	C_{11}

Chart. 2: Structures (contd)



	R_1	R_2	R_3	G_1	Dregeoside
99.	H	H	β-O-Isoval	G_a	K_{pl}
100.	H	H	β-O-Isoval	G_c	K_{al}
101.	H	H	β-OH	G_a	D_{pl}
102.	H	H	β-OH	G_c	D_{al}
103.	$-COCH_3$	-Isoval	β-OH	G_a	G_{pl}
104.	$-COCH_3$	-Isoval	β-OH	G_c	G_{al}

For 94 – 104 :

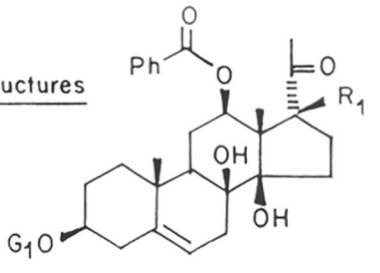
G_a = 3-OMe-6-deoxy-allo(1→4)-olean(1→4)-cym(1→4)-cym

G_b = glu(1→4)-3-O-Me-6-deoxy-allo(1→4)-olean(1→4)-cym(1→4)-cym

G_c = 3-O-Me-6-deoxy-allo(1→4)-cym(1→4)-cym(1→4)

G_d = glu(1→4)-3-OMe-6-deoxy-allo(1→4)-cym(1→4)-cym

Chart. 2 : Structures



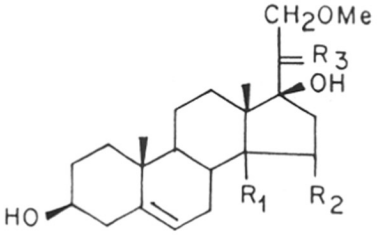
	R_1	G_1	Calotroposide
105.	H	G_a	A
106.	H	G_b	D
107.	H	G_c	F
108.	H	olean(1→4)-cym(1→4)--cym	G
140.	OH	3-O-Me-6-deoxy--allo(1→4)-cym(1→4)-cym	
			Calotroposide
141.	OH	G_a	B
142.	OH	G_b	C
143.	OH	G_c	E

For 105 – 108, 140 – 143 :

G_a = cym(1→4)-olean(1→4)-olean(1→4)-cym(1→4)-cym

G_b = olean(1→4)-olean(1→4)-olean(1→4)-cym(1→4)-cym

G_c = olean(1→4)-olean(1→4)-cym(1→4)-cym



	R_1	R_2	R_3
109.	OH	H	β-OH
110.	=		β-OH
111.	OH	H	= O

E-1 :Compounds with oxygenation at C_3, C_8, C_{11}, C_{12}, C_{14} and C_{20}

Two new pregnane glycosides drelin **112** and ceolin **113** with a new genin have been reported from *Dregea lanceolata*[49]. The genin possesses a peculiar, hitherto unknown position of double bond at $C_{3(4)}$. In the continued research on the same species, Krishna *et al.* isolated another new genin lanceogenin **114** along with its glycoside lanceolin **115**[50]. The glycosides of marsectahexol and marsdenin **116, 117, 118** have also been reported from the same source[51]. Dregeoside H **119** from *Dregea volubilis* [46b] with a similar oxygenation pattern is also included in this group.

E-2 :Compounds with oxygenation at C_3, C_8, C_{12}, C_{14}, C_{17} and C_{20}

Thirteen wilfosides isolated from *Cynanchum wilfordi* constitute a major part of this group. Tsukamoto *et al.* in 1985 first reported the isolation of wilfosides C3N,C2G....**120-125**[52a]. In the continued chemical investigation of the plant, the same group isolated seven more glycosides wilfosides D1N, F1N...**126-132**[52b]. These wilfosides are composed of various aglycones such as wilforidine, caudatin, cynanforidine and kidjoranine. The sugar linkages in some of them include both D-and L-cymarose.

Recently, cynaricuosides A, B, C **133-135** have been reported from roots of *Cynanchum auriculatum*[53] along with wilfosides C3N, C1N, C1G and K1N. The compounds possess aglycones similar to those of wilfosides.

Marsdekoiside A **136**[54]; the glycoside of 12-cinnamoyl 5α -dihydrosarcostin and other glycosides of similar aglycone skeletons with different substituents at C_{12} and C_{21} named dregeoside **137**[55], dregeoside A,B **138, 139**[56,45c] belong to this subgroup. Also included are folotsoside A **140** and calotroposides B,C,E **141-143**; the glycosides of 12-benzoyl deacetyl metaplexigenin from *Folotsoside sarcostemmoides*[57] and *Calotropis gigantea*[46a,b]. One more glycoside of 12-cinnamoyl deacetyl metaplexigenin **144** is reported from *Oxystelma esculentum* [58] by Trivedi *et al.*

1.7 F : Heptaoxygenated pregnanes

F-1 :Compounds with oxygenation at C_3, C_8, C_{11}, C_{12}, C_{14}, C_{17} and C_{20}

From *Oxystelma esculentum*, Trivedi *et al.* isolated a new heptaoxygenated pregnane named esculentin **145**[59]. The genin part is C_{20} oxidized stephanol,first reported in 1968 by Fukuoka *et al*[60] [Chart-3].

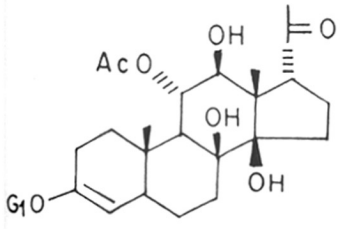
Chart-3: Hexa- and heptaoxygenated pregnanes

Class E: Hexaoxygenated pregnanes		
Sub-class	Sites of oxygenation	Compound Numbers
E-1	C_3, C_8, C_{11}, C_{12}, C_{14}, C_{20}	**112, 113, 114, 115, 116-118, 119**
E-2	C_3, C_8, C_{12}, C_{14}, C_{17}, C_{20}	**120-125, 126-132, 133-135, 136, 137, 138, 139, 140, 141-143, 144**
Class F: Pentaoxygenated pregnanes		
F-1	C_3, C_8, C_{11}, C_{12}, C_{14}, C_{17}, C_{20}	**145**

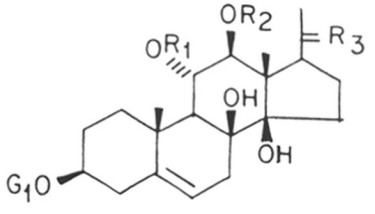
1.8 G : Pregnanes with unusual skeletons [Chart-4]

The advancements in spectroscopic techniques, especially the NMR experiments such as NOESY, COSY together with X-ray crystallography have made it possible for a chemist to characterize novel and interesting skeletons of natural products. This fact is reflected in isolation and characterization of unusual pregnanes. Earlier, the uncommon skeletons were mostly the pregnanes with unusual positions of oxygen functions or ether linkages[5]. However, the isolation of novel compounds like glaucogenin and atratogenin glycosides with seco-pregnane skeletons has changed the very basic concept of "perhydrocyclopentenophenanthrene" framework for

Chart.3: Structures

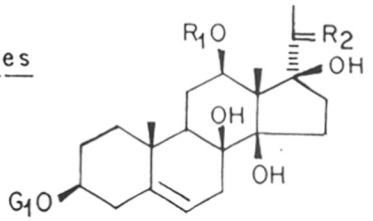


	G_1
112.	boiv(1→4)-cym(1→4)-cym
113.	cym(1→4)-3-O-Me-6-deoxy-allo(1→4)-olean



	R_1	R_2	R_3	G_1
114.	$-COCH_3$	H	β-OH	H
115.	$-COCH_3$	H	β-OH	α-L-digin(1→4)-α-L-digin(1→4)-cym(1→4)-olean
116.	H	H	=O	cym(1→4)-olean
117.	H	H	β-OH	cym(1→4)-cym
118.	$-COCH_3$	H	=O	α-L-digin(1→4)-α-L-digin(1→4)-cym(1→4)-olean
119.	H	H	β-OH	3-O-Me-6-deoxy-allo(1→4)-cym(1→4)-digit

Chart.3: Structures



	R_1	R_2	G_1	Wilfoside
120.	-Ikem	O	G_a	C 3 N
121.	-Ikem	O	G_b	C 1 N
122.	-Ikem	O	G_c	C 2 N
123.	-Ikem	O	G_d	C 3 G
124.	-Ikem	O	glu(1→4) – G_b	C 1 G
125.	-Ikem	O	glu(1→4) – G_c	C 2 G
126.	-COPh	O	G_b	D 1 N
127.	-Cin	O	G_b	K 1 N
128.	H	O	G_b	M 1 N
129.	-Cin	β-O-Ikem	G_b	F 1 N
130.	-Cin	β-O-Tig	G_b	W 1 N
131.	-Cin	β-O-Tig	G_a	W 3 N
132.	-Cin	β-O-Nic	glu(1→4)-G_b	G 1 G
				Cynauricuoside
133.	-Cin	O	glu(1→4)-G_b	A
134.	$-COCH_3$	O	G_b	B
135.	-Ikem	O	glu(1→4)-glu(1→4)	C

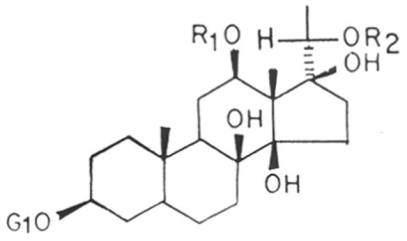
For 120 – 135 : G_a = cym(1→4)-α-L-digin(1→4)-cym

G_b = α-L-cym(1→4)-cym(1→4)-α-L-digin(1→4)-cym

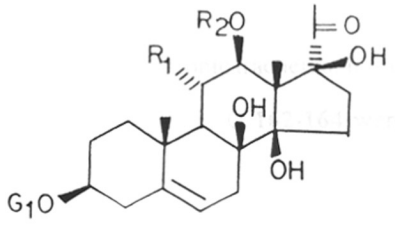
G_c = α-L-cym(1→4)-cym(1→4)-α-L-digin(1→4)-digit

G_d = glu(1→4)-cym(1→4)-α-L-digin(1→4)-cym

Chart. 3 Structures (contd.)



	R_1	R_2	G_1
136.	$-COCH=CH-Ph$	H	3-O-Me-6-deoxy-allo(1→4)-olean(1→4)-cym
137.	$-COPh$	H	olean(1→4)-cym(1→4)-cym
138.	$-COCH_2CHMe_2$	$COCH_3$	6-deoxy-3-O-Me-allo(1→4)-olean(1→4)-cym
139.	$-COCH_2CHMe_2$	H	glu(1→4)-6-deoxy-3-O-Me-allo(1→4)--olean(1→4)-digit



	R_1	R_2	G_1
144.	H	$-COCH=CH-Ph$	cym(1→4)-thev(1→4)-cym(1→4)-digit
145.	OH	H	thev(1→4)-cym(1→4)-olean

pregnanes. The glaucogenin glycosides **146-150** were first isolated in 1982-83 from *Pai-chien*[61]; a popular Chinese drug made from dried roots of *Cynanchum glaucescenes.* The drug is used as an antitussive and expectorant[61]. The basic skeleton of glaucogenin consists of a 13,14; 14,15 diseco pregnane system with two in-built epoxide linkages and a lactone moiety. The class is further divided into three different genin types namely glaucogenin A,B and C based on the presence of hydroxyl groups at C_2, C_3 and C_{11}. These naturally occurring aglycones have also been isolated from" *Pai chien*"[62]. A number of such pregnanes, glaucosides F,G,H,I,J**(151-155)** have been reported from the same source[63,64].

Working on the same lines, Zhang *et al.* in 1985 carried out the chemical investigation of another Chinese crude drug "*Pai-Wei*", dried roots of *Cynanchum atratum.* The investigation was done because of the confusion in the therapy and nomenclature between the two drugs "*Pai-Wei*" and "*Pai-Chien*". The isolated compounds were named as cynatratosides A-F **156-161** and all possessed glaucogenin-C as the aglycone[65,66].

Later on in 1986, another Chinese crude drug "*Xu-Chang-Qing*" was chemically investigated. The crude drug, which is dried whole plant of *Cynanchum paniculatum* is used in China mainly as an anodyne and for therapy of chronic tracheitis. It is used as a substitute for "*Pai-Wei*". Three new glycosides, cynapanosides A, B, C **162-164** were isolated from this drug[67]. The glycosides are 7β-hydroxylated derivatives of cynatratosides A, B, C. During this investigation, the authors revised the previously reported structure of glaucogenin-B. The 11β-OH of this aglycone was actually found to be 7 β-OH[67]. In the continued research on the same drug, neocynapanoside-A **165**, possessing a new genin neocynapanogenin A was isolated by the same authors[68]. Very recently, a new aglycone, 17-hydroxylated glaucogenin-A named as hancogenin-B **166** has been isolated by Lou *et al.* from the roots of *Cynanchum hancockianum*[69] along with other known aglycones and glycosides.

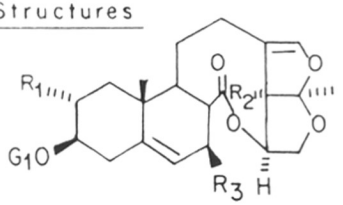
In 1988, atratosides A, B, C, D **167-170** with yet another new skeleton were isolated from *Cynanchum atratum*[70]. The 14-oxo, 14,15 seco pregnane skeleton consists of a 15,20 epoxide and three olefinic bonds at $C_{5(6)}$, $C_{15(16)}$ and $C_{17(20)}$. The glycosides belonging to this class consist of three different aglycones namely atratogenin A,B and cynajapogenin A. Atratogenin A possesses a hydroxyl at C_2 and a C_{18} methyl group in the seco-pregnane skeleton. The C_2 oxidation product of this genin is atratogenin B while cynajapogenin A shows absence of the C_{18} methyl group. According to the authors, the skeleton must have been derived biogenetically from a pregnane type precursor by conversion to the furan derivative via the oxidative cleavage between C_{14}-C_{15} bond and dehydrative condensation of C_{15} and C_{20} oxo groups[70].

Velutinol A **171** isolated from *Mandevilla velutina* (Apocynaceae) forms one more example of unusual pregnane with three characteristic ether linkages in the C ring. This oxypregnane has been reported to be a potent antiinflammatory agent[71]. In 1993, we have reported the isolation of another unusual pregnane villosterol **172** from *Turraea villosa* (Meliaceae)[32b]. The aglycone consists of *cis*-fused A,B ring system, rare oxygenation sites at C_5, C_6 and a vinyl group at C_{17}, uncommon in plant pregnanes. The compound is the first example of a pregnane with *cis*-fused A,B rings isolated from terrestrial plants.

Chart-4: Unusual pregnanes

Sub-class	Compound Numbers
Glaucosides	**146-150, 151-155.**
Cynatratosides	**156-161**
Cynapanosides	**162-164**
Atratosides	**167-170**
Others	**165, 166, 171, 172**

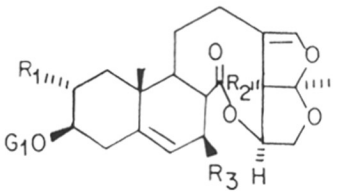
Chart. 4 Structures



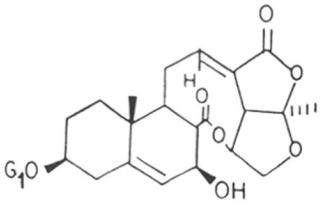
	R_1	R_2	R_3	G_1	Glaucoside
146.	OH	H	H	olean	A
147.	OH	H	H	α-L-cym(1→4)-cym(1→4)-cym	B
148	OH	H	H	α-L-cym(1→4)-digit(1→4)-cym	C
149	OH	H	H	α-L-cym(1→4)-digit(1→4)-olean	D
150.	H	H	H	α-L-cym(1→4)-β-L-cym(1→4)-thev	E
151.	OH	H	H	α-L-cym(1→4)-β-L-cym(1→4)-olean	F
152.	H	H	H	α-L-cym(1→4)-digit(1→4)-thev	G
153.	OH	H	H	glu(1→4)-α-L-cym(1→4)-digit(1→4)-cym	H
154.	OH	H	H	glu(1→4)-α-L-cym(1→4)-β-L-cym(1→4)-β-L-cym	I
155.	H	H	OH	glu(1→4)-α-L-cym(1→4)-digit(1→4)-olean	J

					Cynatratoside
156.	H	H	H	olean	A
157.	H	H	H	α-L-cym(1→4)-digit(1→4)-olean	B
158	H	H	H	α-D-olean(1→4)-digit(1→4)-α-D-olean	C
159.	H	H	H	glu(1→4)-α-L-cym(1→4)-α-D-digit(1→4)-olean	D
160.	H	H	H	α-D-glu(1→4)-olean(1→4)-digit(1→4)-α-D-olean	E
161.	H	H	H	cym(1→4)-α-L-digin(1→4)-cym	F

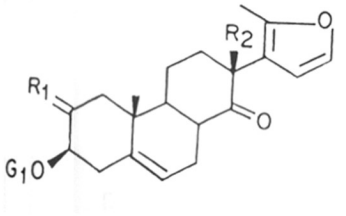
Chart. 4 : Structures (contd.)



	R_1	R_2	R_3	G_1	Cynapanoside
162.	H	H	OH	olean	A
163.	H	H	OH	α-L-cym(1→4)-digit(1→4)-olean	B
164.	H	H	OH	α-D-olean(1→4)-digit(1→4)-α-D-olean	C
166.	OH	OH	H	H	Hancogenin-B

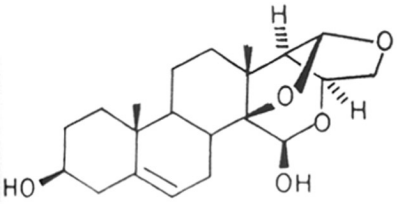


	G_1
165.	α-L-cym(1→4)-digit(1→4)-olean

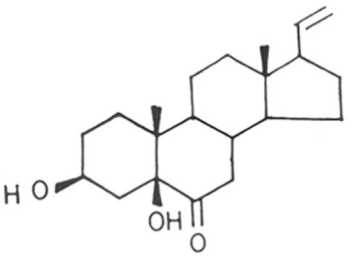


	R_1	R_2	G_1
167.	α-OH	Me	cym(1→4)-α-L-digin(1→4)-cym
168.	α-OH	Me	glu(1→4)-cym(1→4)-α-L-digin(1→4)cym
169.	=O	Me	glu(1→4)-cym(1→4)-α-L-digin(1→4)cym
170.	α-OH	H	α-D-olean(1→4)-digit(1→4)-cym

Chart.4 : Structures (contd.)

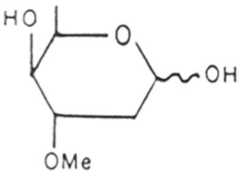


171

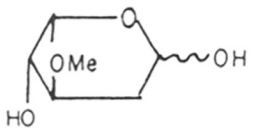


172

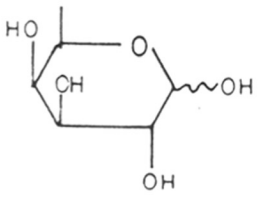
Sugars :



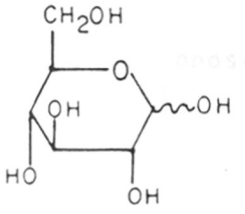
D-sarmentopyranose
(sar)



L-sarmentopyranose
(L-sar)

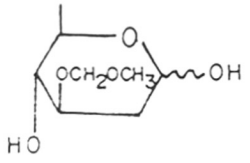


D-fucopyranose
(fuco)

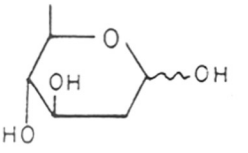


D-glucopyranose
(glu)

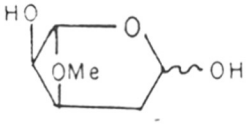
Sugars. (contd.)



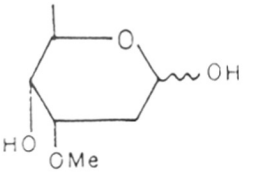
3-O-methoxymethyl D-canaropyranose (3-O-methoxymethyl D-can)



D-canaropyranose (can)



L-cymaropyranose (L-cym)



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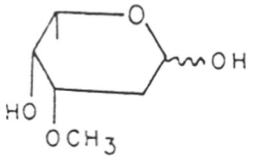
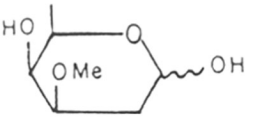


L-diginopyranose (L-digin)



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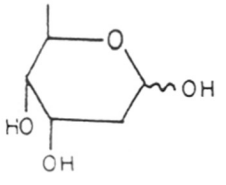
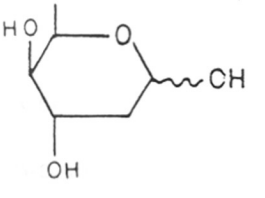
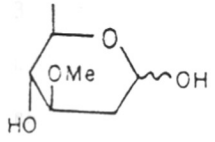


D-digitoxopyranose (digit)

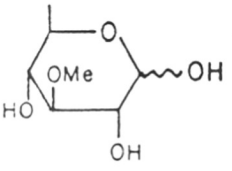


D-boivinopyranose (boiv)

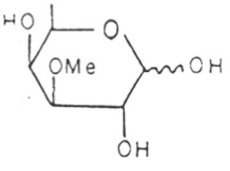
Sugars.(contd.)



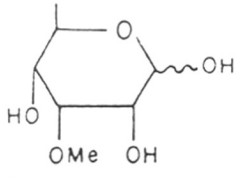
D-oleandropyranose
(olean)



D-thevetopyranose
(thev)



D-digitalopyranose
(dgtl)



6-deoxy-3-O-methyl
D-allopyranose
(6-deoxy-3-O-Me-D-allo)

The abbreviations are as follows:

cym, β-D-cymaropyranose; α-L-cym, α-L-cymaropyranose; dgtl, β-D-digitalopyranose; glu, β-D-glucopyranose; digit, β-D-digitoxopyranose; boiv, β-D-boivinopyranose; olean, β-D-oleandropyranose, thev, β-D-thevetopyranose; fuco, β-D-fucopyranose; digin, β-D-diginopyranose; α-L-digin, α-L-diginopyranose, can, β-D-canaropyranose; sar, β-D-sarmentopyranose, α-L-sar, α-L-sarmentopyranose, allo, β-D-allopyranose.

1.9 : Conclusion

While reviewing the reports of naturally occurring plant pregnanes published during 1988-1993 it was observed that oxygenations at C_{12}, C_{14} and C_{20} are quite common in natural pregnanes. Almost all pregnanes invariably possessed oxygenation at C_3. Although the pregnanes with oxygen functions at C_8, C_{11}, C_{14}, C_{16} and C_{17} were reported, the one at C_9 was hardly observed. In pregnanes occurring as glycosides,sugars such as cymarose, oleandrose, glucose, digitalose were quite common. In some of the pregnane glycosides such as cynafosides, cynatratosides both the optical forms of a certain sugar,(e.g.D-and L-cymarose) were present in the same linkage. Characteristic features of the pregnane skeleton were observed in the class of unusual pregnanes. These included bond breakage at C_{13}-C_{14} and/or C_{14}-C_{15} and cis-fusion of A,B rings as well as uncommon oxygenation patterns.

Pregnanes from almost all groups exhibited different types of biological activity such as antitumor, piscicidal, antiinflammatory properties. Some crude drugs made from the species like *Cynanchum glaucescenes*, *C. paniculatum* from which a number of pregnane glycosides have been isolated, find use as antitussive, expectorant mixtures. The species thus gain further importance as potential sources of new natural product based drugs.

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CHAPTER II

Chemical Investigation of *Turraea villosa*

The work presented in this Chapter has been published in the form of a research paper. Chiplunkar,Y.G., Nagasampagi,B.A., Tavale,S.S. and Puranik,V.G. (**1993**), *Phytochemistry*, **33**, 901.

2.1 Introduction

The genus *Turraea* (Linn.) belonging to family Meliaceae is a large genus consisting of sixty to seventy species. It is placed in the tribe Turraeae, subfamily Meliodeae. The species, mostly occurring as shrubs or small trees are spread in the tropical and sub-tropical regions of Africa, Asia and Australia.

2.2 Biological activity of *Turraea* species

Various species of the genus *Turraea* are reported to exhibit a wide range of biological activities. *T.floribunda, T.robusta* are some of the prominent species of this genus. These species are native of the African countries and have been employed in the African folk medicines. The preparation from root and bark of *T.floribunda* is used in the treatment of rheumatism, dropsy and heart diseases[1]. In traditional medicine of Tanzania, tea prepared from roots of *T.robusta* is used for curing stomach pains and diarrhoea[2]. The roots of *T.villosa* are used in the treatment of leprosy and fistulae[3].

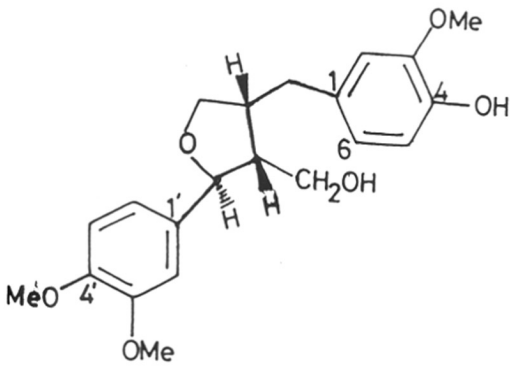
Apart from the usage in folk medicines, a systematic study of biological activity of some *Turraea* species has also been carried out. The infused leaves and stem of *T.casimiriana* are reported to affect capillary permeability[4]. The total extract of leaves of *T.nilotica* was found to exhibit cytotoxicity. Chemical separation of the major cytotoxic fraction yielded a new lignan **1** in pure form but the pure compound was inactive in KB cell culture assay[5]. The cytotoxicity thus could be due to some other compounds present in the fraction.

Chemical investigation of *Turraea* species

2.3 Previous Work

Inspite of the fact that some sixty species belong to this genus, very few of them have been investigated chemically. This may be attributed to the scarcity and infrequent occurrence of the species.

T.obtusifolia gave prieurianin **2**[6a] as a major compound which is a characteristic limonoid of the genera *Trichilia* and *Gaurea*. Thus, isolation of **2** establishes taxonomic relationship of *T.obtusifolia* with the above mentioned genera. Compound **2**, which is also obtained from *Nymania* species belonging to subfamily Meliodeae, serves as a useful taxonomic marker[6a]. *T.floribunda* when investigated chemically, yielded compounds **3**, **4** and **5**[6a]. These compounds possess skeletons similar to the typical limonoids of genus *Trichilia*, namely havanensin, heudelottin and hirtin[6b]. The compounds are probably the intermediates enroute to prieurianin **2**, and thus confirm the taxonomical linkage between *Trichilia* and *Turraea*. Mzikonone **6**, a new tetranortriterpenoid was isolated by Rajab *et* al. from *T.robusta*[2]. This compound with azadirone **8** skeleton possesses oxygen function in C ring and that too only at C_{12} which is uncommon in meliacins. In continuation of this work, Bentley and co-workers isolated Mzikonol **7** from root bark of *T.robusta* along with the known limonoids azadirone **8**, 1,2-dihydroazadirone **9** and nimbolinin B **10**. A new tetranortriterpene lactone turranolide **11** and a known triterpene butyrospermol **12** were also isolated from the same source[7]. These compounds are at much less oxidation levels than prieurianin and other limonoids from *Turraea* species. This indicates that oxygenation pattern of the limonoid should be applied very carefully as a criterion for taxonomic relationship between various species. Very recently, three new compounds named turraflorin A (**13**), B (**14**) and C (**15**) have been isolated from *T.robusta*[1]. These compounds possess skeletal framework similar to prieurianin, but are at lower oxidation levels than it. *T.nilotica* when investigated chemically gave three new protolimonoids **16**, **17**, **18** in contrast to other species of *Turraea* which are reported to contain mainly limonoids. Niloticin **16** was found to be the major compound amongst the three protolimonoids[8]. Mention must be made here about a report regarding the major cytotoxic fraction of *T.nilotica* which yielded a new lignan, lariciresinol 4'-monomethyl ether **1** along with the known fatty acids namely arachidic, behenic and lignoceric acid[9]. The hexane solubles of alcoholic extract of the leaves of the same plant revealed presence of C_{19}-C_{37} hydrocarbons, α- and β-amyrins, β-sitosterol as well as lauric, myristic, palmitic, stearic, oleic, linoleic and arachidic acids[10].



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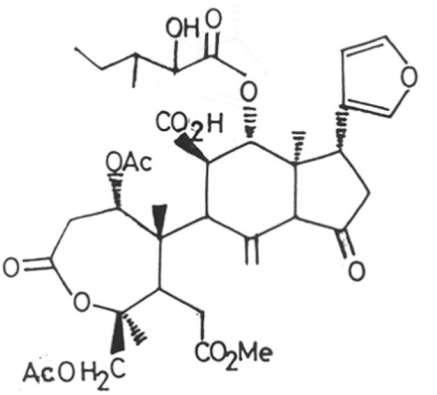

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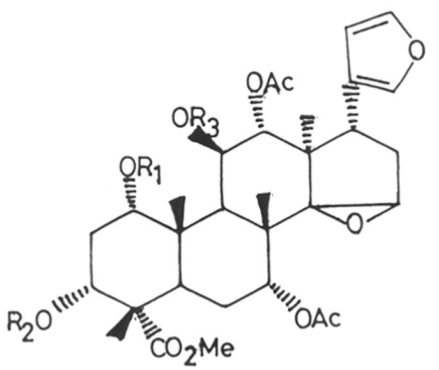
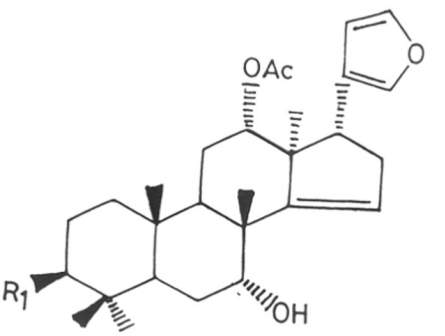
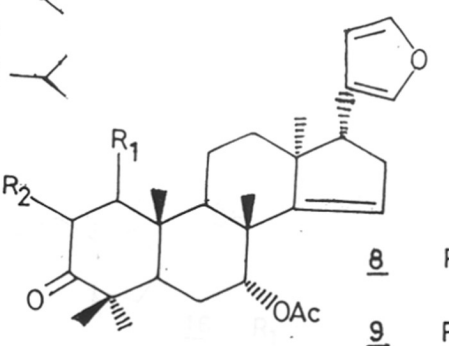


	R_1	R_2	R_3
3	Ac	H	H
4	Ac	H	$-C(=O)-CH(CH_3)_2$
5	H	Ac	$-C(=O)-CH(CH_3)_2$



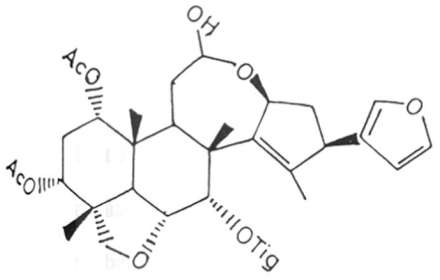
6 R_1 = =O

7 R_1 = OH



8 R_1R_2 = double bond

9 $R_1 = R_2 = H$



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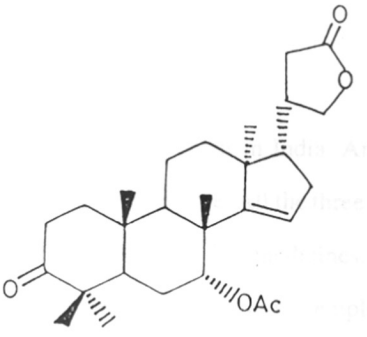

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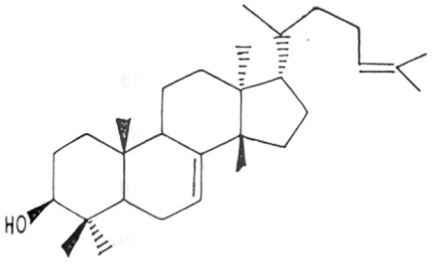

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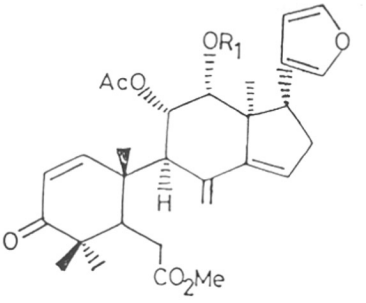
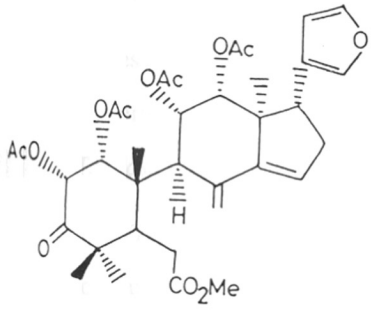


13 R_1 = Ac

14 R_1 = H



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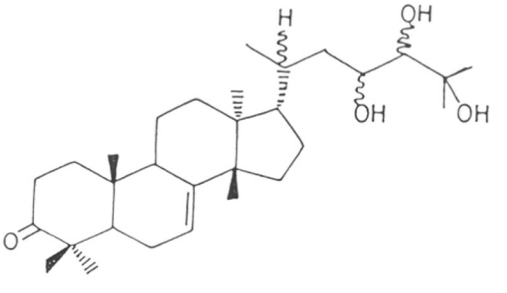

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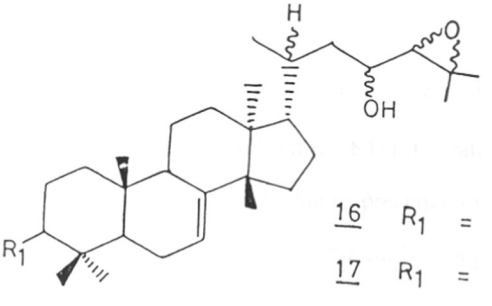


16 R_1 = =O

17 R_1 = β-OH

2.4 Present Work

Two species of the genus *Turraea* viz. *T.villosa* and *T.virens* occur in India. An exotic plant *T.obtusifolia* has been found to be introduced in India as a garden tree. All the three species are distributed mainly in Gujarat, Maharashtra, Kerala and find use in the folk medicines. Leaves and bark of *T.obtusifolia* are reported to be drastically purgative while *T.virens* is employed in treatment of fits[3].

T.villosa (Benn.) (Called as *Pandre* or *Kapurbhendi* in Marathi) is a small, deciduous tree with elliptic or ovate leaves and white, sweet scented flowers. The flowering season is April-May. It is found from Gujarat to Kerala, both on the hilly and coastal areas. In the Annamalai hills, it is found upto an altitude of 1200m. In Maharashtra, it occurs in the Western Ghat region, southwards from Mahabaleshwar. The roots of *T.villosa* are used in leprosy and in applications for fistulae[3].

The meliaceous plants are known to be rich in terpenoids and limonoids and many of them possess significant biological activity. Considering these facts and the reported medicinal properties of *Turraea* species, the work on chemical investigation of this hitherto unexplored *T.villosa* was undertaken.

The shade dried leaves of *T.villosa* were extracted with acetone. The extract was subjected to column chromatography to obtain four broad fractions A, B, C, D. Fraction C on repeated column and preparative thin layer chromatography (ptlc) yielded compound **A-1**.

Structure elucidation of A-1

2.4.1 Functional groups and probable molecular formula

Compound **A-1**, white crystalline solid, m.p. 192-193°C, $[\alpha]_D$ -35.29° ($CHCl_3$; c 0.17) showed in its UV spectrum λmax at 207 nm (ε 1841) indicating the absence of any conjugated system. In the IR spectrum (Fig.1), bands due to presence of hydroxyl (3480 cm^{-1}), an unconjugated carbonyl (1720 cm^{-1}) and olefinic (1650 cm^{-1}) groups were observed. In its ^{1}H-NMR

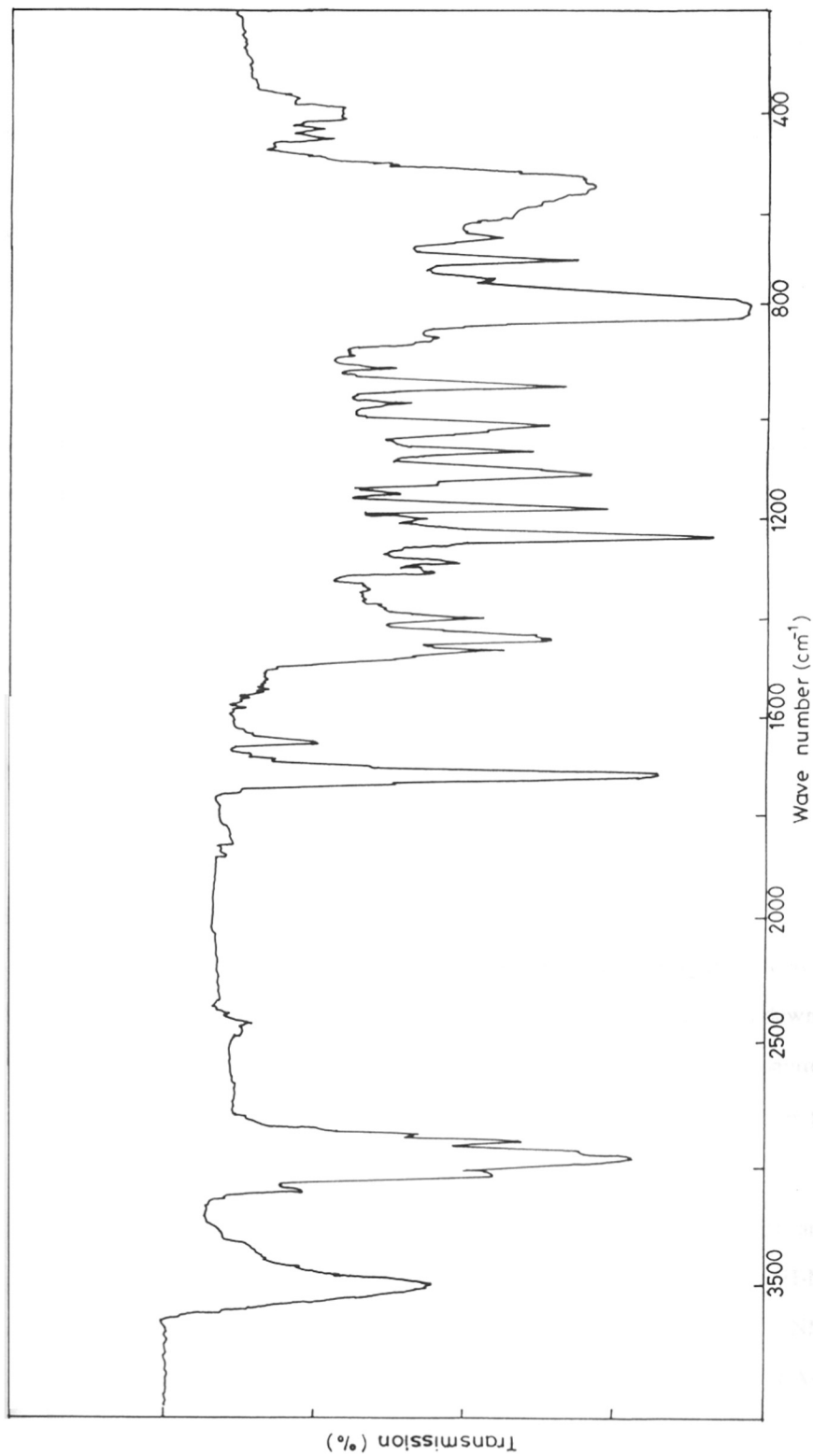


Fig.1 : IR spectrum of compound A-1.

spectrum (Fig.2), **A-1** exhibited only two singlets at δ 0.50 and 0.68 assignable to two tertiary methyl groups. Three multiplets at δ 4.09 (1H), 4.90 (2H) and 5.67 (1H); the latter two probably due to olefinic moieties, were also observed in addition to two sharp singlets at δ 4.25 and 4.40 for exchangeable protons.

The molecular ion peak $[M]^+$ in the mass spectrum of **A-1** (Fig.3), was observed at m/z 332. Based on it, the tentative molecular formula could either be $C_{20}H_{28}O_4$ or $C_{21}H_{32}O_3$, which in turn suggested a diterpenoid skeleton for **A-1**. The fragment peak at 314 (M^+-18) due to elimination of water indicated presence of hydroxyl group.

2.4.2 Nature of functional groups and skeleton

In order to understand the nature of oxygen functionalities other than carbonyl in **A-1**, the compound was subjected to acetylation at room temperature. The acetylated product **A-1a** in its IR spectrum (Fig.4), exhibited the presence of a hydroxyl group (3480 cm^{-1}) and an extra carbonyl (1733 cm^{-1}). The band at 3480 cm^{-1} in **A-1a** indicated the presence of a tertiary hydroxyl group in the parent compound **A-1** which failed to undergo acetylation under normal conditions. The signal at 314 in the mass spectrum of **A-1a** (Fig.5), was attributable to the (M^+-60) ion formed due to elimination of CH_3COOH from the monoacetate. The carbinyl proton (C<u>H</u>OAc) in the ^{1}H-NMR spectrum of acetate (Fig.6) was observed at δ 4.99 (1H). The same signal was present at δ 4.09 (1H) in the NMR spectrum of the parent hydroxyl compound (Fig.2). This downfield shift of 0.90 δ on acetylation, together with the integration confirmed the secondary nature of hydroxyl group in **A-1**. The other signals in the ^{1}H-NMR of **A-1a** were very close to those present in the parent compound **A-1**.

Two quartets in the DEPT ^{13}C-NMR spectrum of **A-1a** (Fig.7) attributable to angular methyl groups confirmed the presence of two methyl groups in **A-1** as observed in the ^{1}H-NMR (Fig.2). Further, one of the two methyl groups appeared as upfield as δ 0.50 in the ^{1}H-NMR spectrum which was suggestive of either an unusual diterpenoid or a steroid skeleton for **A-1**.

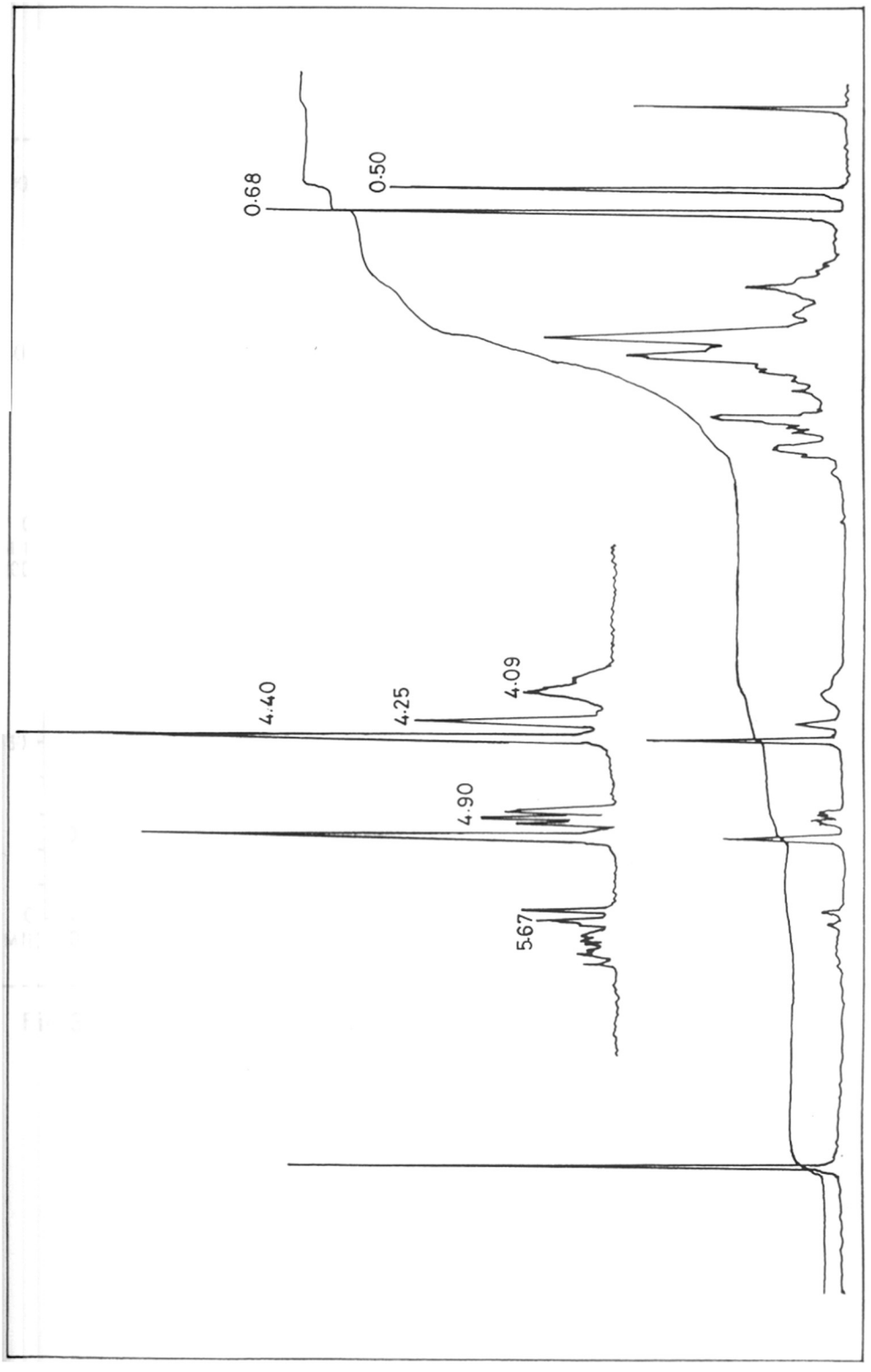


Fig.2 : ^{1}H NMR spectrum of compound A-1.

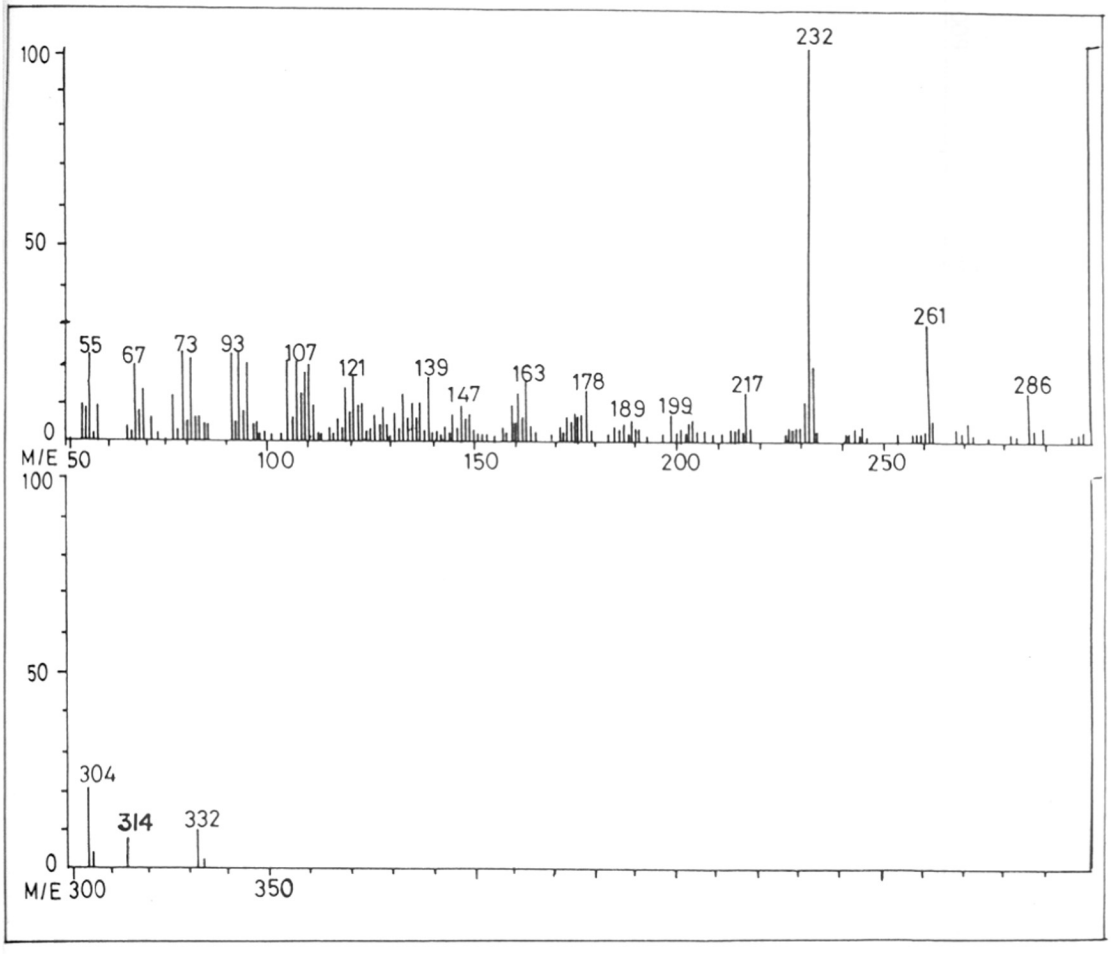


Fig.3 : Mass spectrum of compound A-1.

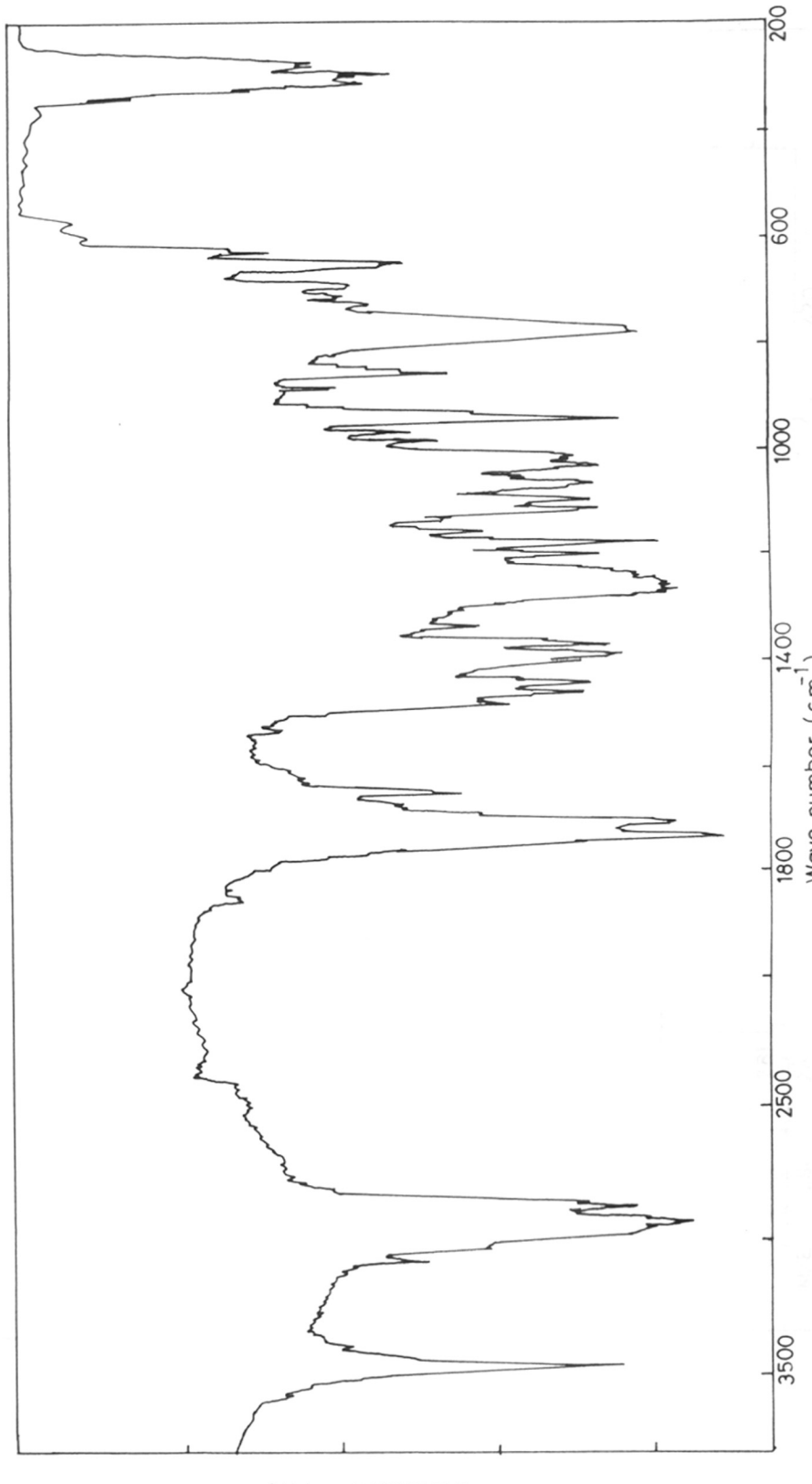


Fig.4 : IR spectrum of compound A-1a.

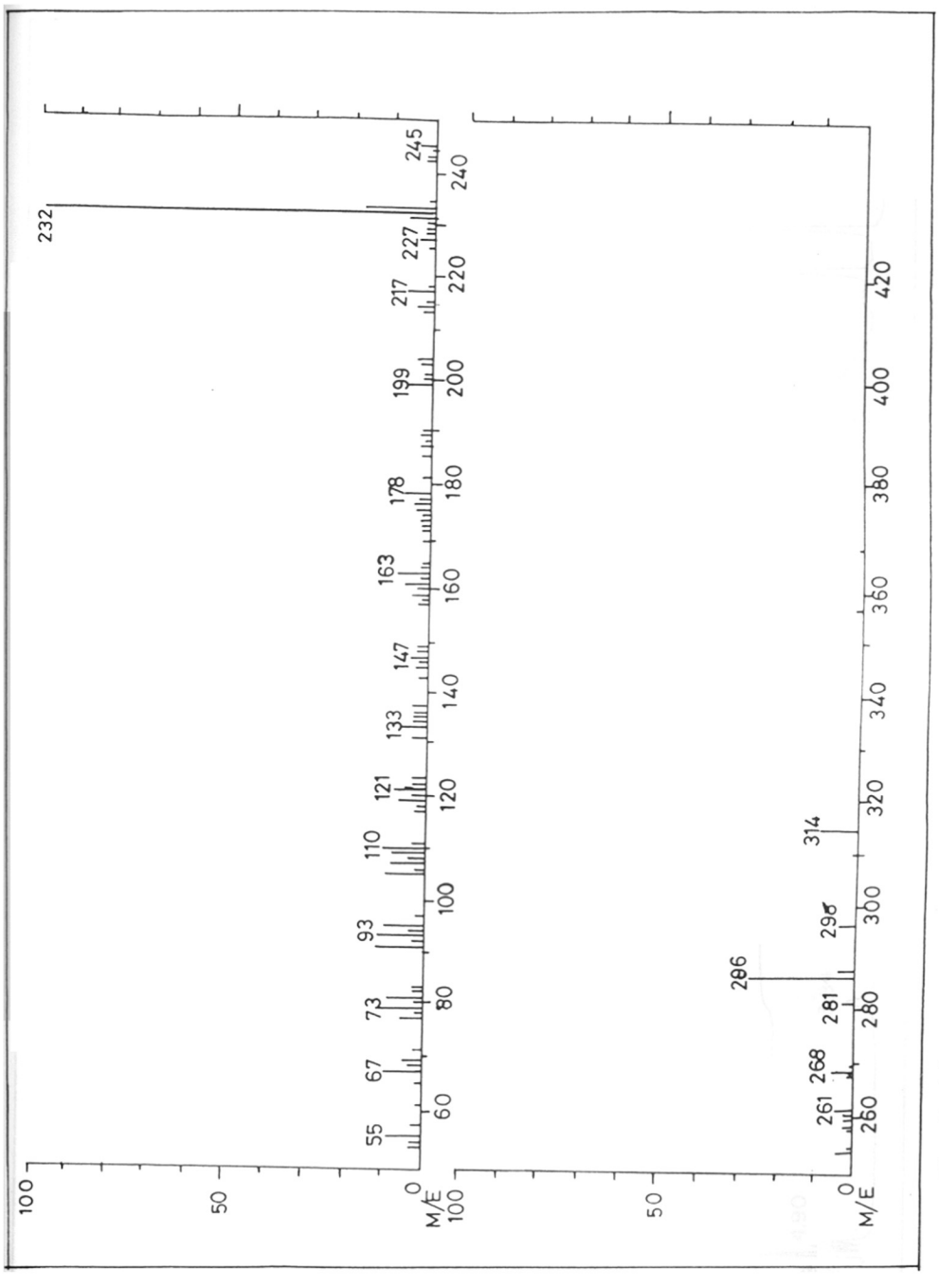


Fig.5 : Mass spectrum of compound A-1a.

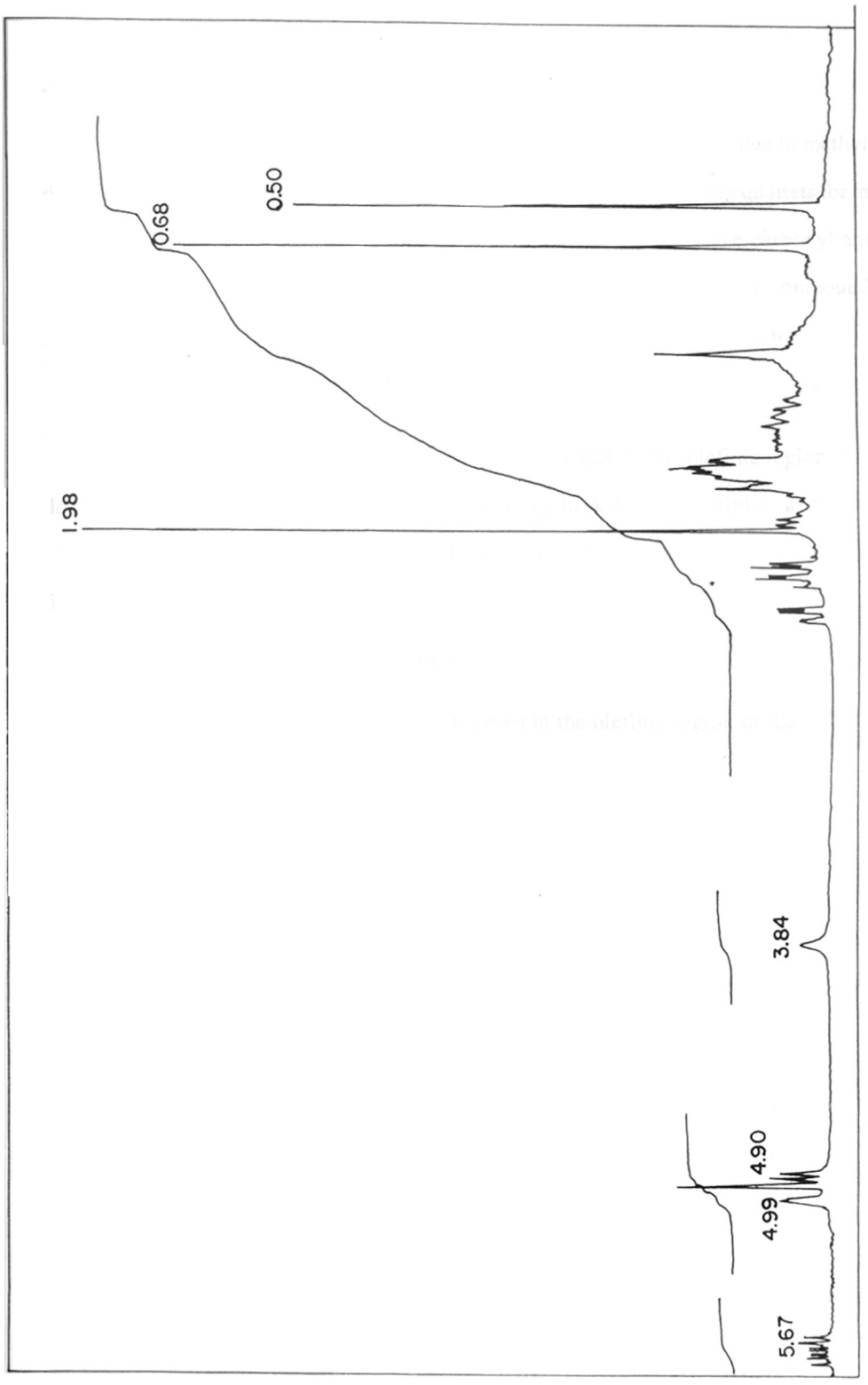


Fig. 6 : ^{1}H - NMR spectrum of compound A-1a.

2.4.3 ^{13}C-NMR

The acetate **A-1a** in its DEPT ^{13}C-NMR (Fig.7) exhibited nine triplets due to methylenes, six doublets for methine carbons, two singlets for quaternary carbons and three quartets for methyl groups. It also showed the presence of two carbonyl groups. Excluding one carbonyl and one methyl signal due to acetate group, the remaining signals belonged to the parent compound **A-1**. Apart from the methyl groups, the ^{13}C-NMR provided the following information:

a. Presence of vinyl group

Appearance of only one triplet (-CH_2) and a doublet (-CH) in the olefinic region (110-150 δ) clearly showed presence of a vinyl group (-CH=CH_2) in **A-1**. The multiplets at δ 4.90 (2H) and 5.67 (1H) in the ^{1}H-NMR were thus easily accounted for an exo-methylene group and an olefinic proton respectively.

b. Absence of tetrasubstituted double bond

Any quaternary carbon signal was not observed in the olefinic region of the ^{13}C-NMR of **A-1a** which ruled out the possibility of a tetrasubstituted double bond in **A-1**.

c. Carbonyl on ring

Presence of only one carbonyl in the parent compound **A-1** was evident from the ^{13}C-NMR of **A-1a**. Its chemical shift of δ 213 revealed that it was present on the ring itself and not as a part of acid or ester group on the skeleton. It is well known that ring carbonyls appear much downfield (190-200 δ) than the carbonyls of acids or esters (160-180 δ)[11].

d. Absence of ether linkage or epoxide group

Only two signals, a doublet at δ 67 (CH-OAc) and a singlet at δ 80 assignable to the carbon bearing the tertiary hydroxyl group were observed in the region (50-90 δ) in the ^{13}C-NMR of

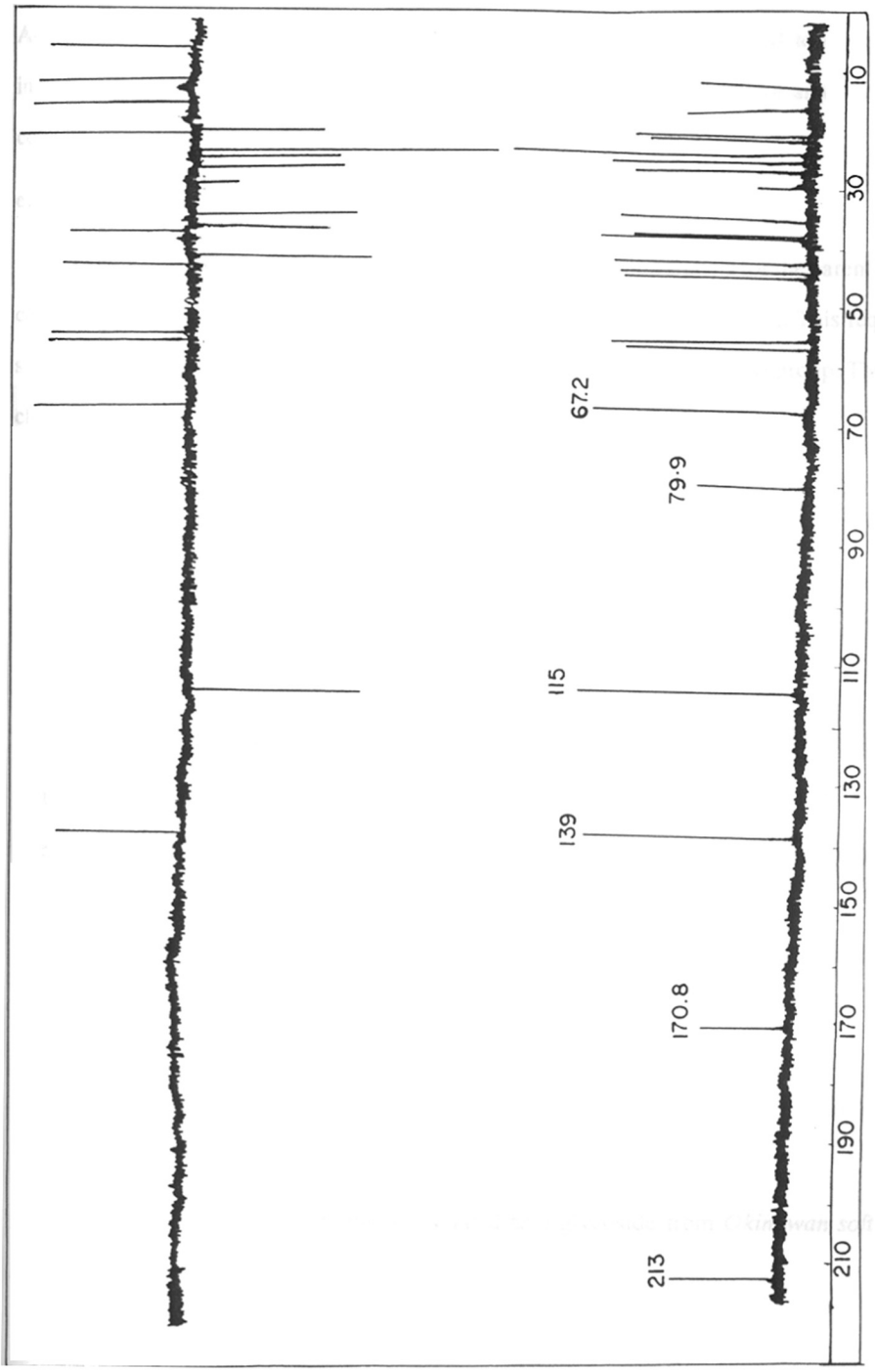


Fig. 7 : ^{13}C-NMR spectra of compound A-1a.

A-1a. Absence of further signals in this region typical of carbon attached to oxygen atoms indicated that **A-1** neither possessed any ether linkage (CH_2-O or CH-O) nor an epoxide group connecting two quaternary carbons.

e. Correct molecular formula

Taking into account the aforesaid facts along with 21 carbon signals for the parent hydroxyl compound, it turned out that **A-1** possessed the molecular formula $C_{21}H_{32}O_3$. This required six sites of unsaturation, two of which were due to a vinyl group and a carbonyl group. Thus, it was clear that we were dealing with a tetracyclic compound with -

i) 21 carbon atoms

ii) A secondary and a tertiary hydroxyl group

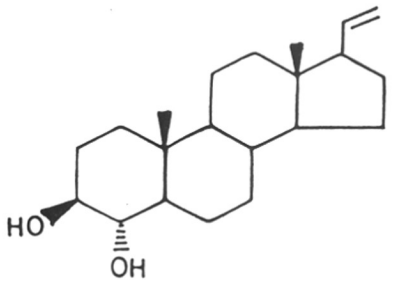
iii) A carbonyl on the ring

iv) A vinyl group

v) Two angular methyl groups

The previous reports on various species of *Turraea* revealed the presence of limonoids, protolimonoids and tetranortriterpenoids[6,7,8]. Further, in the family Meliaceae, degraded triterpenoids are known to occur widely, but the presence of steroids other than common phytosteroids (such as stigmasterol, sitosterol) and diterpenoids is not very common[12]. Keeping this in view, literature search of a number of diterpenoids, degraded triterpenoids and steroids was carried out. From the survey, it was clear that though a few examples of tetracyclic diterpenoids with upfield methyls around δ 0.6 are known[13], the presence of methyls in the upfield region is a characteristic feature of the steroid skeleton. This prompted us to think compound **A-1** as a possible C_{21}-steroid.

A report of a pregnane steroid **19** isolated as a glycoside from *Okinawan soft coral of Alcyonium sp.*[14] supported this view.



19

A comparison of methyl chemical shifts and other prominent signals in the ^{1}H-NMR of **A-1** and **19** led us to the conclusion that **A-1** possessed a pregnane skeleton with a vinyl group at C_{17}. A close look at the ^{13}C-NMR spectra of **A-1a** and **19** made it clear that for C_{11} to C_{21} in **19**, there existed corresponding signals in **A-1a** matching well with respect to chemical shift and signal multiplicity (Table-1). This was evident from the DEPT ^{13}C-NMR of **A-1a**. Thus **A-1**, as in **19** possessed C and D rings without any substituent. The remaining signals in the ^{13}C-NMR spectrum consisted of four triplets at δ 24.5, 29.6, 34.8, 41.8; three doublets at δ37.4, 43.1, 67.2; and two singlets at δ 43.9 and 79.9. The functional groups in **19** were present at C_3 and C_4 on the A ring. The differences in the chemical shifts of C_1 to C_{10} of **19** and **A-1a** suggested that the groups in **A-1** were located at positions different than those of **19**.

Table-1

^{13}C Chemical shifts of compound **19** and **A-1a** (only for C_{11}-C_{21})

Carbon No. (Signal Multiplicity)	Compound **19**	Compound **A-1a**	Carbon No. (Signal Multiplicity)	Compound **19**	Compound **A-1a**
11(t)	20.9	21.1	17(d)	55.6	55.0
12(t)	37.8	37.0	18(q)	13.1	12.7
13(s)	43.8	44.0	19(q)	13.9	16.9
14(d)	55.8	56.0	20(d)	140.1	139.0
15(t)	25.0	25.5	21(t)	114.7	115.0
16(t)	27.5	26.9			

2.4.4 Assignment of -OH and -C=O positions

A powerful feature of ^{13}C-NMR is that, within a family of compounds, a given substituent produces remarkably similar effects on the carbons in its close vicinity. These shielding or deshielding effects on the site of substitution (α-carbon), adjacent to the substituted carbon (β-carbon) and its neighborhood (γ-carbon) are specific and conclusive[15]. This consistency in the influence of substituent over α, β and γ carbons was utilized for assigning the positions of secondary, tertiary hydroxyl and carbonyl groups on A and B rings.

a. Position of secondary hydroxyl

It is known that all phytosteroids originate from cycloartenol[16] possessing a secondary hydroxyl at C_3. Considering this biogenesis for plant steroids, it was logical to place the secondary hydroxyl group in **A-1** at C_3. Further, the ^{1}H-^{1}H COSY spectrum of **A-1a** (Fig.8) indicated coupling of the carbinyl proton at δ 4.99 with two vicinal ring methylenes resonating at δ 1.75 and 2.3. The COSY picture was thus in support of the C_3-hydroxyl group. Although majority of natural sterols possess 3β-hydroxyl group, there are reports in literature describing the presence of naturally occurring 3α-hydroxylated steroids[17]. Thus, the secondary hydroxyl in **A-1** could either be C_3-α or C_3-β which appeared as a doublet at δ 67.2 in the ^{13}C-NMR spectrum.

b. Position of tertiary hydroxyl

As the substituents in **A-1** were located only on A and B rings, the tertiary hydroxyl could be at C_5, C_8 or C_9. In the ^{13}C-NMR spectrum, the corresponding carbon appeared at δ 79.9. In case of 5α-pregnanes, the downfield doublets due to C_5 and C_9 normally appear around δ 47 and 55 respectively (Table-2)[15]. In **A-1a**, amongst $C_{1\text{-}10}$, the doublet at δ 37.4 fitted well with the ^{13}C chemical shift value for unsubstituted C_8 (δ 35.7). Thus, C_8 in **A-1** probably did not carry a hydroxyl group.

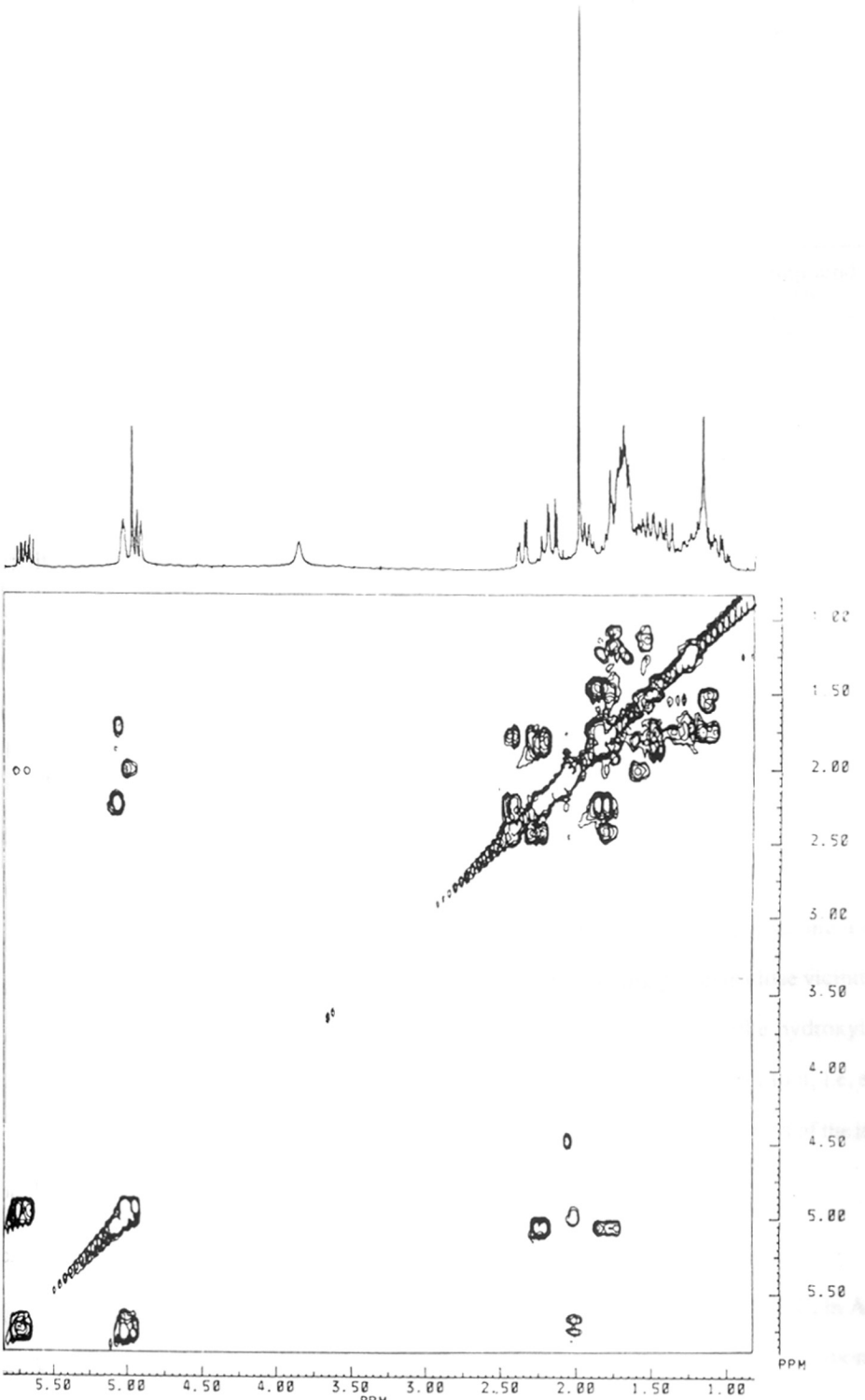


Fig.8 : $^{1}H-^{1}H$ COSY spectrum of compound A-1a.

Table-2

^{13}C Chemical Shifts of 5α-pregnanes and compound **19** (only for C_1-C_{10})

Carbon No.	5α-pregnane	Compound **19**	Carbon No.	5α-pregnane	Compound **19**
1	38.9	37.0	6	29.2	23.5
2	22.3	29.7	7	32.4	32.3
3	26.9	76.7	8	35.7	35.5
4	29.2	75.4	9	55.3	55.2
5	47.2	51.8	10	36.4	37.5

The ^{13}C chemical shift for unsubstituted C_9 in 5α-pregnanes is around δ 55. The hydroxyl substitution causes a downfield shift of minimum 30 δ[15,18] which would take C_9 to approximately δ 85 on hydroxylation. Further, in naturally occurring pregnanes, hydroxyl is hardly observed at C_9 and C_8-hydroxyl is normally accompanied by a tertiary hydroxyl at C_{14}[19,20]. All these facts suggested that no tertiary hydroxyl group was present either at C_8 or C_9.

C_5 resonates around δ 47 in 5α-pregnanes (Table-2) and the increment for hydroxyl substitution is 26 δ[15]. The hydroxyl substituted C_5 would then appear around δ 73[18]. In ^{13}C-NMR of **A-1a**, the singlet due to carbon bearing tertiary hydroxyl was observed at δ 79, a value close to δ 73. As it has been already deduced, the hydroxyl could not be placed at C_8 or C_9 and therefore, it would be at C_5. Further, it is possible that an electron withdrawing group in close vicinity could be responsible for its downfield shift of 6 δ than the normal value. Thus, the hydroxyl in **A-1** could be at C_5 with the possible presence of a carbonyl functionality adjacent to it, i.e. either at C_4 or at C_6. The hydroxyl group could either be α or β oriented. However, no report of the isolation of 5β-hydroxylated natural pregnane has so far appeared in the literature.

c. Position of carbonyl group

As already seen from the ^1H-^1H COSY (Fig.8), the acetate bearing carbon C_3 in **A-1a** was flanked by two vicinal ring methylenes at C_2 and C_4. Further, the presence of carbonyl at C_4 would have caused a downfield shift of approximately 14 δ on C_3 in the ^{13}C-NMR spectrum[15].

In **A-1a**, C_3 at δ 67 was already little upfield than the normal value (δ 70) in 3-acetyl steroids[15,18]. Taking into consideration all these facts, carbonyl was placed at C_6. ^{13}C-chemical shift of C_7 in pregnanes without a carbonyl at C_6 is reported around δ 32 (Table-2)[15]. Thus, considering the deshielding due to the adjacent C_6-carbonyl, a triplet at δ 41.8 observed in the ^{13}C-NMR of **A-1a** could easily be assigned for C_7.

Thus, compound **A-1** was tentatively assigned the structure: 3ξ, 5ξ-dihydroxy-20-pregnen-6-one. However, the following points required more clarification.

i) In **A-1**, C_{19} methyl resonated at δ 16.9. This downfield shift of 4-5 δ from the usual value for C_{19} in pregnanes (δ12-13)[15,18] could not be explained.

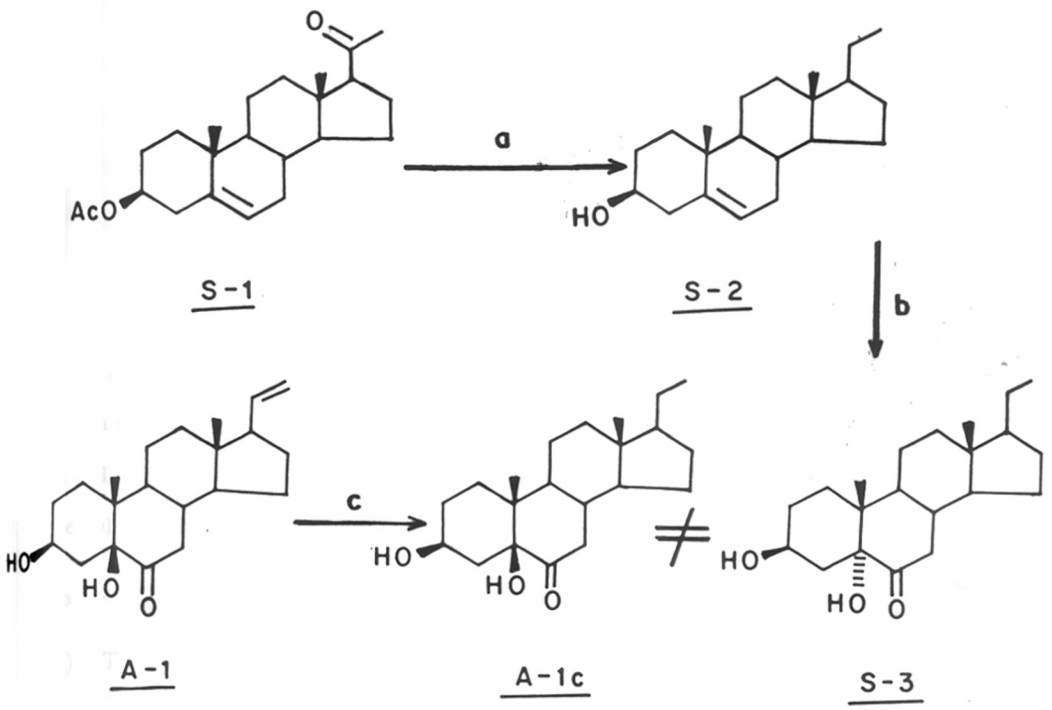
ii) The carbon bearing the acetate group in steroid acetates resonates around δ70-72[15,18]. The same carbon appeared at δ67.2 in the ^{13}C-NMR spectrum of **A-1a**. The cause of this deviation of 3δ was not clear.

iii) A carbonyl at C_6 is reported to induce a downfield shift of 11.7δ and 14.5δ on C_5 and C_7 respectively[15]. ^{13}C values assignable to these carbons in **A-1a** did not match with the reported deshielding effects of carbonyl.

2.4.5 Chemical evidence

To clarify the above mentioned points, **A-1a** was first converted to the 20,21-dihydro derivative **A-1c**and a simple synthetic correlation starting from known 3β-acetyl pregnane **S-1** to arrive at a common 3β,5α dihydroxy pregnane was undertaken as per the following Scheme-1.

Pregnenolone acetate **S-1** when subjected to Huang-Minlon reduction[21] with hydrazine hydrate and KOH in ethylene glycol yielded the C_{20} reduced compound **S-2**. The treatment of **S-2** with N-bromo succinimide in aqueous acetone; the reaction known to give 5α-hydroxy, 6-keto steroids[22] yielded the desired product **S-3**. Compound **A-1** on hydrogenation yielded **A-1c** which was expected to be same as **S-3**.



Reagents: (a) $H_2NH_2.xH_2O$, KOH, $(OHCH_2CH_2)_2O$; (b) NBS, aq. acetone, AcOH; (c) H_2, Pd-C.

Scheme-1

However, it was found that the two compounds were not identical as there existed a small difference in their Rf values on tlc plate. A close look at the ^{1}H-NMR spectrum of **S-3** revealed that the $\underline{H}$-C_3-OH at δ 4.16 appeared as a broad multiplet spreading over 8-10 Hz, whereas the corresponding multiplet in **A-1** at δ 4.09 was a narrow one, with a width of 3-4 Hz. This difference could easily be attributed to the axial and equatorial nature of the respective protons. The axial $\underline{H}$-C_3-OH in **S-3** experiences both axial-axial (a,a) and axial-equatorial (a,e) coupling with the methylene protons at C_2 and C_4 (Fig.9a).

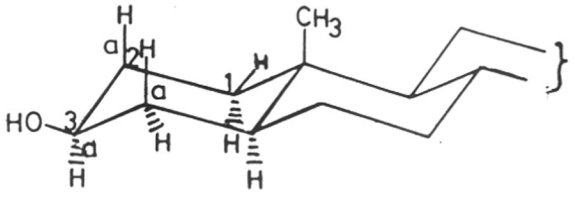


Fig.9a:(a,a) and (a,e) coupling of $\underline{H}-C_3-OH$

The broad multiplet observed was due to the *trans*-diaxial coupling which is reported to have a magnitude of 5-10 Hz[23]. A narrow multiplet for carbinyl proton in **A-1** indicated that H_3 in **A-1** was equatorial undergoing only (e,e) and (e,a) coupling with the adjacent C_2 and C_4 methylenes, the magnitude of which is small (2-3 Hz)[24].

H_3 in a steroid skeleton could be equatorial if:

(a) The hydroxyl at C_3 is α-oriented (Fig.9b) or

(b) The hydroxyl at C_3 is β-oriented, but A,B ring fusion is *cis* (Fig.9c).

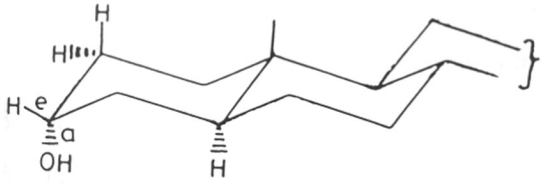


Fig.9b: α-oriented C_3-OH

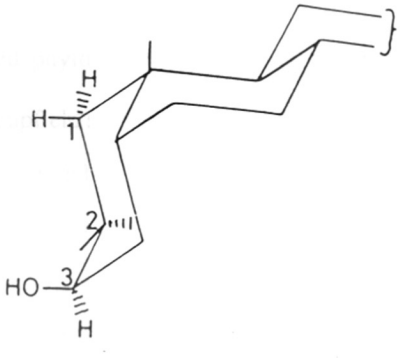
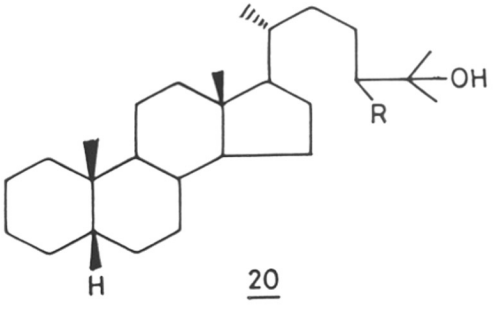


Fig.9c: β-oriented C_3-OH in cis-fused A,B ring system.

Although, there are reports of natural pregnanes possessing 3α-hydroxyl group[17], the discrepancies in the ^{13}C chemical shifts of **A-1a** were observed for C_3 as well as C_5, C_6 and C_{19}, where the carbon atoms on the A,B ring junction were involved. The ^{13}C chemical shift values of the aforementioned carbons differed from those reported for *trans* A,B ring fused pregnanes. This implied that **A-1** possibly possessed a *cis*-fused A,B ring system.

In the literature, no pregnane from the terrestrial plant source with *cis*-A,B ring fusion has been reported so far. However, *cis* fusion of A and B rings is a characteristic feature of the ecdysone skeleton **20**[25].



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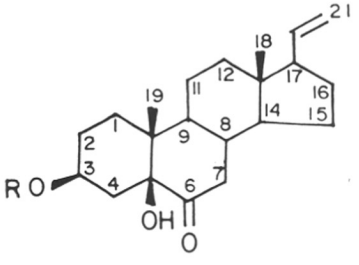
The study of ^{13}C-NMR spectra of 5β-hydroxylated phytoecdysones revealed that the β-hydroxyl at C_5 shields the angular methyl C_{19} causing an upfield shift of 5-6 δ.[26] In *cis* A,B ring fused 5β-H pregnanes, C_{19} normally appears around δ23[15], which in presence of 5β-hydroxyl would resonate around δ17. This value was well in agreement with that observed for C_{19} in **A-1a** (δ16.9). This shielding behavior of 5β-hydroxyl served as a strong evidence for the presence of *cis* A,B ring junction as well as C_5-hydroxyl in **A-1**. Comparison of the ^{13}C chemical shifts of C_5 of 5-hydroxylated ecdysones with that of **A-1a** was carried out. In all the compounds, C_5 resonated around δ 79[26], whereas same carbon in **A-1a** was observed at δ 79.9.

The compound **A-1** was thus assigned the structure 3β,5β-dihydroxy-20pregnen-6-one. This steroid isolated from *T. villosa* was named as 'villosterol'. Pregnane steroids with *trans*-fused A,B rings or a double bond at $C_{4(5)}$ or $C_{5(6)}$ have been reported from marine sources[14,27] and terrestrial species[28], but villosterol is the first example of the pregnane with *cis* A,B ring fusion isolated from a plant source.



A−1 R=H

A-1a R=Ac

The structure was further confirmed by X-ray crystallography.

2.4.6 X-ray data of compound **A-1**

The structure of **A-1** was solved by single crystal X-ray diffraction studies. The crystals belong to orthorhombic space group $P2_12_12_1$ with a=6.523(1), b=13.474(2) and c=20.948(3)Å, V=1841.139Å^3, z=4. The data was collected on an Enraf Nonius CAD-4F-11 L/M single crystal X-ray diffractometer using MoK$_\alpha$ radiation (λ=0.7107Å). The structure has been refined to R=0.039, R_w=0.043. A perspective view of the molecule **A-1** along with the crystallographic numbering is shown in Fig.10. The atomic coordinates and equivalent thermal parameters for non-hydrogen atoms are given in Table-3.

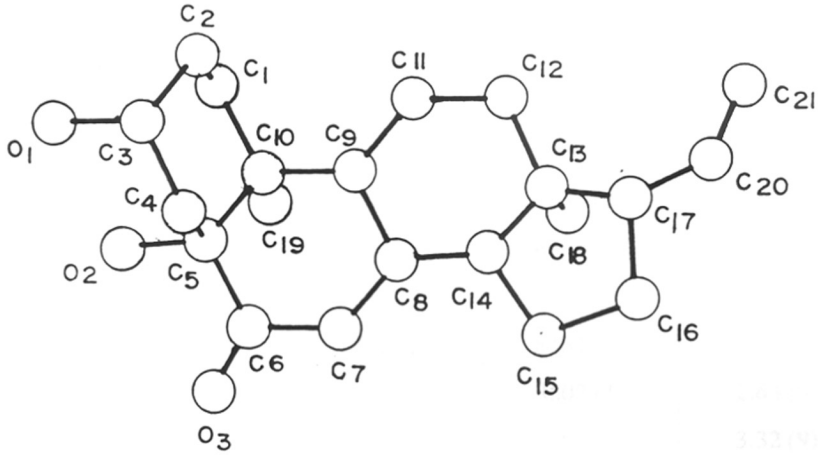


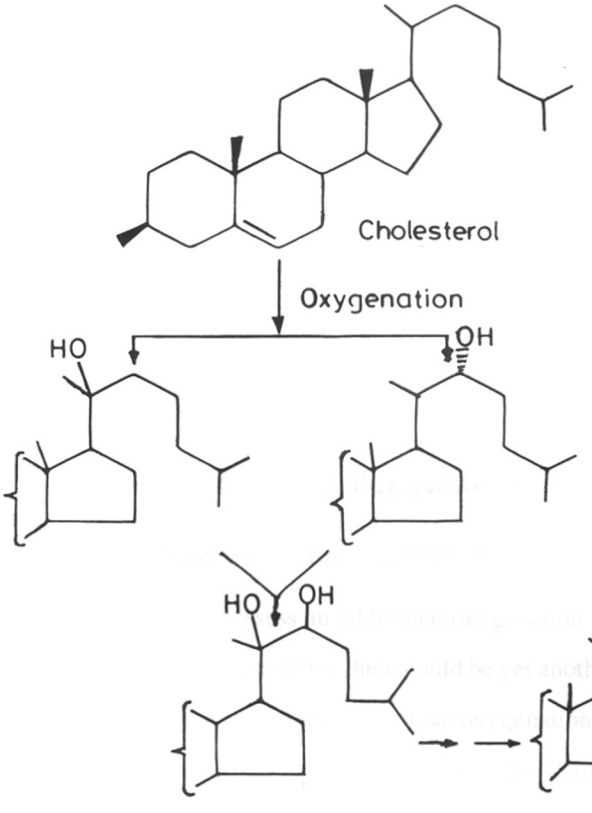
Fig. 10: A perspective view of the enantiomer of compound A-1

Table-3: Atomic coordinates ($x10^4$) and equivalent isotropic thermal parameters for non-hydrogen atoms with E.S.D.S. In parentheses

Atom	X	Y	Z	$B_{eq}(\mathring{A}^2)$
O-1	12721 (4)	6419 (2)	4930 (1)	4.20 (7)
O-2	9782 (4)	7120 (2)	5796 (1)	4.51 (8)
O-3	9686 (7)	8650 (2)	6577 (1)	9.29 (12)
C-1	11856 (5)	5357 (2)	6204 (1)	3.10 (9)
C-2	13890 (5)	5487 (2)	5860 (1)	3.49 (9)
C-3	13972 (5)	6454 (2)	5490 (1)	3.44 (9)
C-4	13425 (5)	7328 (2)	5916 (1)	3.55 (9)
C-5	11362 (5)	7181 (2)	6269 (1)	3.19 (9)
C-6	11002 (7)	8058 (2)	6699 (1)	4.70 (11)
C-7	12349 (7)	8147 (2)	7283 (1)	5.01 (11)
C-8	12373 (5)	7172 (2)	7663 (1)	3.13 (9)
C-9	12861 (4)	6291 (2)	7230 (1)	2.53 (7)
C-10	11328 (4)	6201 (2)	6665 (1)	2.69 (8)
C-11	13097 (6)	5312 (2)	7605 (1)	3.35 (9)
C-12	14554 (5)	5405 (2)	8181 (1)	3.35 (9)
C-13	13978 (4)	6270 (2)	8607 (1)	2.63 (9)
C-14	13945 (5)	7214 (2)	8202 (1)	3.32 (9)
C-15	13822 (8)	8052 (3)	8695 (2)	5.55 (13)
C-16	15099 (8)	7652 (3)	9268 (2)	5.76 (13)
C-17	15618 (5)	6558 (2)	9109 (1)	3.67 (13)
C-18	11918 (5)	6050 (3)	8942 (2)	3.99 (9)
C-19	9119 (5)	6023 (3)	6913 (2)	4.70 (10)
C-20	15715 (5)	5896 (3)	9682 (1)	4.07 (9)
C-21	17093 (6)	5204 (3)	99772 (2)	5.46 (11)

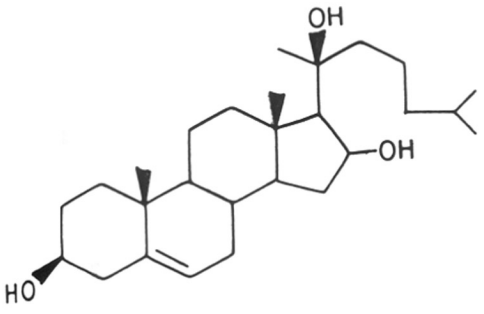
2.5 Biogenesis

It has now been accepted that the pregnane phytosteroids originate from catabolism of cholesterol occurring in plants[29,30]. The cleavage of the isooctyl side chain in cholesterol takes place via the C_{20}, C_{22} dihydroxylated cholesterols [Scheme-2].

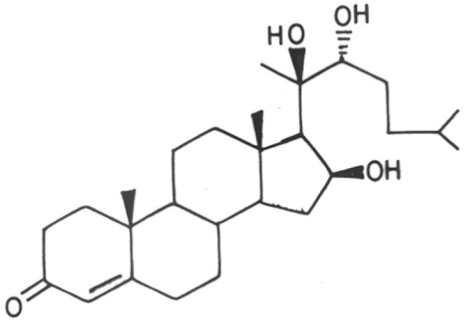


Scheme 2 : Catabolism of Cholesterol

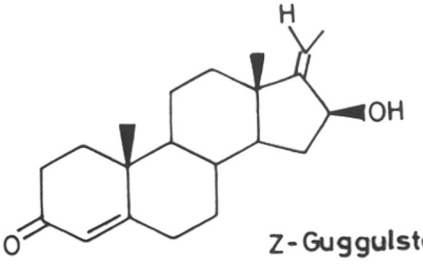
The steroids isolated from *Commiphora mukul*[30] parallel this cholesterol to pregnenolone sequence [Scheme-3].



Guggulsterol - II



Guggulsterol - I

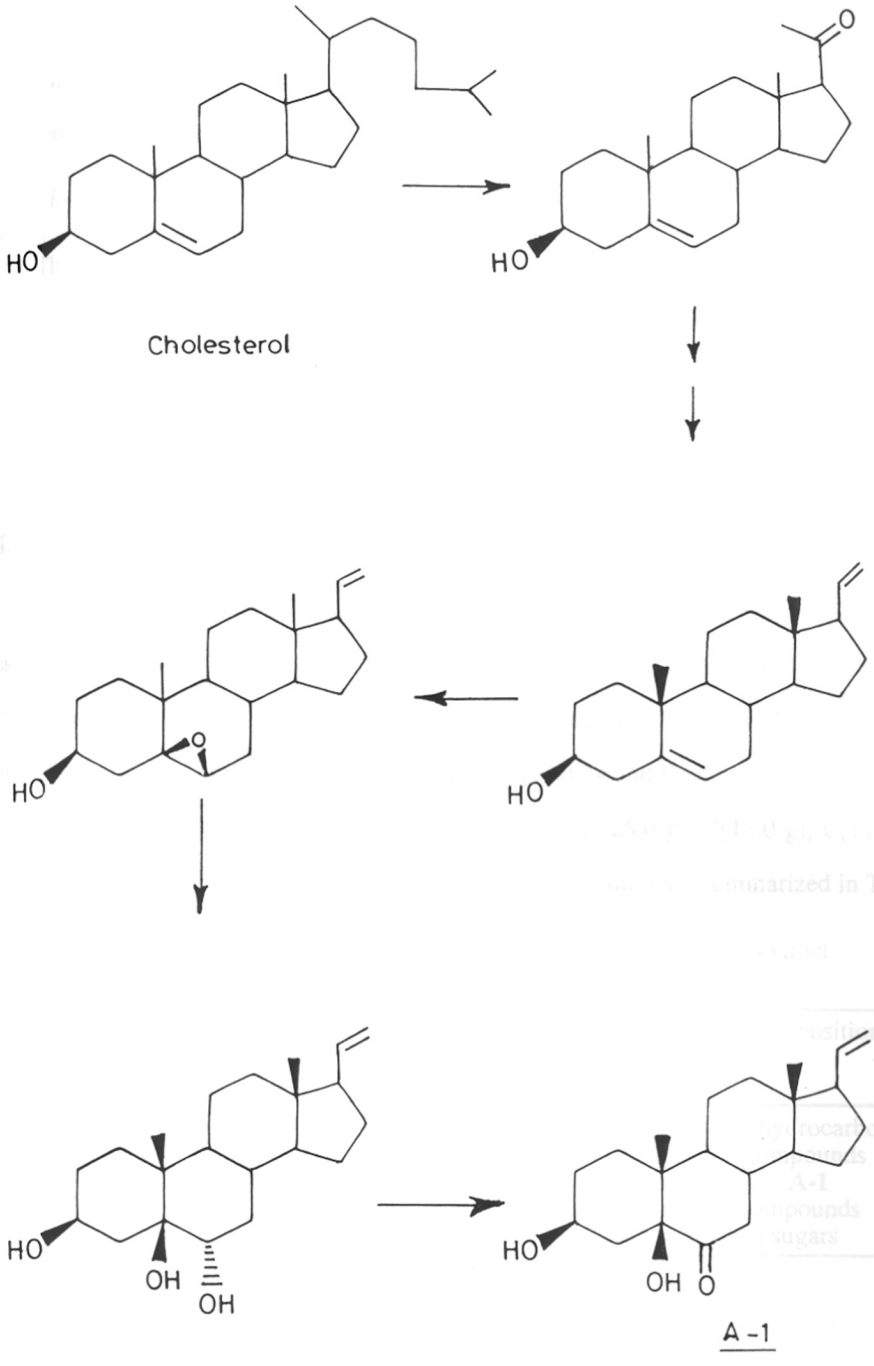


Z-Guggulsterol

Scheme-3 Steroids from C. mukul

The only difference is, all these steroids possess an additional oxygenation at C_{16}. Isolation of villosterol **A-1** without C_{16} oxygenation suggests that there could be yet another pathway for the catabolism of cholesterol operating in plants, proceeding without oxygenation at C_{16}. The oxygen functions at C_5 and C_6 could have arisen from the β-epoxidation of the double bond at $C_{5(6)}$ in cholesterol, followed by the epoxide ring opening giving 5,6 dihydroxylated compound. This compound would further undergo oxidation yielding a C_6-keto compound [Scheme-4].

To summarize the work, the chapter describes the isolation and structure elucidation of a novel pregnane sterol, villosterol. The steroid possesses *cis*- fused A,B rings, rarely observed in natural pregnanes. The structure has been assigned based on spectroscopic and X-ray crystallographic studies. Chemical evidence for structure determination and probable biogenetic path for the compound have also been discussed.



A-1

Scheme-4 probable biogenesis of A-1.

2.6 Experimental

Plant material

The plant *Turraea villosa* identified by authorities of the Botanical Survey of India, was collected from Kashele, near Karjat (Maharashtra), during November 1987.

Extraction

The aerial parts of the plant were shade dried, coarsely powdered (1.8 Kg) and extracted with acetone exhaustively by continuous extraction process. The end point of extraction was determined by the test sample (100 mL) yielding negligible weight of residue on evaporation of solvent. The acetone extract was concentrated at reduced pressure to obtain a gummy mass (82 g).

2.6.1 *Chromatographic separation*

The total extract (80.0 g.) was subjected to column chromatography over silica gel (60-120 mesh, 1.5 Kg). The elution was started with petroleum ether and continued with mixture of pet.ether and acetone with successive increase in the percentage of acetone. The separation was monitored by tlc. All fractions were concentrated separately and the fractions showing similar tlc pattern were combined to obtain four broad fractions A(26.0 g), B(18.0 g), C(17.0 g) and D(14.0 g). The details of the column chromatographic separation are summarized in Table-I.

Table-I: Column chromatographic separation of the acetone extract

	Eluent Pet.ether: Acetone	Total volume collected	Final fraction	Net wt. in gm.	Approximate composition
1	100:00	6x500 mL	A	26.0	Straight chain hydrocarbons
2	85:15	4x500 mL	B	18.0	Unidentified compounds
3	70:30	6x500 mL	C	17.0	Compound **A-1** and unidentified compounds
4	00:100	8x500 mL	D	14.0	Plant acids and sugars

2.6.2 *Rechromatography of fraction C*

Tlc examination of fraction C (17.0 g) revealed that it was a mixture of minimum six compounds having close Rf values (tlc system, ethyl acetate: benzene, 78:22).

The fraction was rechromatographed over silica gel (60-120 mesh, 400 g.), details of which are given in Table-II. The elution started with pet.ether:acetone (92:8) with successive increase in the proportion of acetone. Fractions having similar composition were combined to obtain four major fractions I (5.3 g.), II (3.6 g.), III (1.5 g.) and IV (6.6 g.).

Table-II: Rechromatography of Fraction C

	Eluent Pet.ether: Acetone	Total volume collected	Final fraction	Net wt. in gm.	Approximate composition
1	92:8	4x250 mL	I	5.3	Straight chain compounds
2	84:16	4x250 mL	II	3.6	Complex mixture of unidentified compounds
3	76:24	5x250 mL	III	1.5	Compound **A-1** + complex mixture of unidentified steroids
4	68:32	2x250 mL	IV	6.6	Complex mixture of compounds

2.6.3 *Isolation of compound A-1*

Fraction III obtained from chromatography of fraction C gave a gummy mass (1.5 g.). It was further purified by repeated preparative thin layer chromatography (ptlc) (chloroform : methyl cyanide 15:3) to give compound **A-1** (12 mg).

Compound **A-1**, white crystalline solid, m.p. 192-193°C (acetone-pet.ether, crystals); $[\alpha]_D$ -35.29°C ($CHCl_3$; c 0.17).

IR (Fig.1), $CHCl_3$: cm^{-1} 3480, 1720, 1650 and 1240.

^{1}**H-NMR** (Fig.2), (90 MHz): δ 0.50 (s, 3H), 0.68 (s, 3H), 4.09 (m, 1H), 4.25 (s, 1H, exch.), 4.4 (s, 1H, exch.), 4.90 (m, 2H), 5.67 (m, 1H).

MS (Fig.3), m/z (rel.int.): 332 $[M]^+$(9), 314(3), 304(22), 286(12), 271(5), 261(30), 232(100), 217(11).

Acetate A-1a of compound A-1

A solution of 8 mg. of compound **A-1** in 0.5 mL of pyridine and 0.5 mL of acetic anhydride was left overnight at room temperature and worked up in the routine manner. The acetylated product **A-1a** was obtained as a white solid which was further purified by recrystallization in acetone, pet.ether mixture.

Compound **A-1a**, m.p. 152-155°C (acetone-pet.ether, crystals).

IR (Fig.4), $CHCl_3$: cm^{-1} 3480, 1733, 1715 and 1650.

^{1}H-NMR (Fig.6), (300 MHz): δ 0.50 (s, 3H), 0.68 (s, 3H), 1.98 (s, OCOMe), 3.84 (br s, exch.), 4.90 (m, 2H), 4.99 (m, 1H), 5.67 (m, 1H).

^{13}C-NMR (Fig.7), (75.48 MHz): δ 29.6(t, C_1), 24.5(t, C_2), 67.2 (d, C_3), 34.8 (t, C_4), 79.9 (s, C_5), 213.0 (CO, C_6), 41.8 (t, C_7), 37.4 (d, C_8), 43.1 (d, C_9), 43.9 (s, C_{10}), 21.1 (t, C_{11}), 37.0 (t, C_{12}), 44.0 (s, C_{13}), 56.0 (d, C_{14}), 25.5 (t, C_{15}), 26.9 (t, C_{16}), 55.0 (d, C_{17}), 12.7 (q, C_{18}), 16.9 (q, C_{19}), 139.0 (d, C_{20}), 115.0 (t, C_{21}), 170.8 (OCOMe), 21.5 (q, OCOMe).

MS (Fig.5), m/z (rel.int.): 314 (10, $[M-60]^+$), 296(5), 286(28), 281(3), 268(7), 253(5), 232(100), 217(7).

2.6.4 Scheme-1

Commercially available pregnenolone acetate **S-1** was used as a starting material for *Scheme-1*.

3β-hydroxy-5-pregnene, *S-2*:A mixture of **S-1** (800 mg, 2.2 mmol), sodium hydroxide (290 mg, 7.2 mmol) and hydrazine hydrate (85%) (0.5 mL) in diethylene glycol (8 mL) in a round bottom flask fitted with air condenser was heated on a sand bath for one hour. The temperature

of sand bath was maintained just sufficient to reflux the reaction mixture. The condenser was removed after one hour and the reaction mixture was heated further for two and half hours at 200-250°C. The reaction mixture was then diluted with water, acidified with HCl and extracted with chloroform (3x30mL). The organic layer on routine work-up yielded the crude product which was purified by preparative thin layer chromatography (ethyl acetate : pet.ether 8:92) to give **S-2** (324 mg), yield 48%.

IR $CHCl_3$: cm^{-1} 3460, 1630, 1210.

^{1}H-NMR (90 MHz): δ 0.58 (s, 3H), 1.02 (s, 3H), 1.05 (ill-defined t, 3H), 3.53 (m, 1H) and 5.36 (m, 1H).

MS m/z (rel.int.): 302(78), 284(42), 269(61), 217(58), 191(65), 105(88), 91(100).

3β,5α-dihydroxypregnane-6-one, S-3

Compound **S-2** (300 mg, 1 mmol) was mixed with acetone (14 mL), water (2 mL), N-bromo succinimide (200 mg, 1.25 mmol) and acetic acid (0.2 mL) and the reaction mixture was left at room temperature for twenty hours. The solution was then diluted and extracted with chloroform (4x15 mL). Standard work up of organic layer and concentration followed by preparative tlc (ethyl acetate : pet.ether, 10:90) furnished compound **S-3** (83 mg), yield 25%.

IR $CHCl_3$: cm^{-1} 3452, 1715, 1518.

^{1}H-NMR (90 MHz): δ 0.56 (s, 3H), 0.82 (s, 3H), 1.05 (ill defined t, 3H), 4.16 (m, 1H, 8-10 Hz).

MS m/z (rel.int.): 334(20), 316 (100), 301(10), 283(6), 273(8), 234(18), 219(15), 205(10), 163(15).

3β,5β-dihydroxypregnane-6-one, A-1c

Compound **A-1** (10 mg) on hydrogenation with H_2, 10% Pd-C yielded compound **A-1c** (8mg).

IR $CHCl_3$: cm^{-1} 3465, 1720, 1235.

^{1}H-NMR (90 MHz, $CDCl_3$): δ 0.55 (s, 3H), 0.78 (s, 3H), 1.1 (ill defined t, 3H), 4.22 (m, 1H, 4 Hz).

MS m/z (rel.int.): 334(10), 316(28), 298(10), 288(50), 270(10), 234(100, base peak), 219(25), 163(20).

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CHAPTER III

Chemical Investigation of *Pulicaria wightiana* and *Grangea maderaspatana* : Isolation of two new diterpenoids

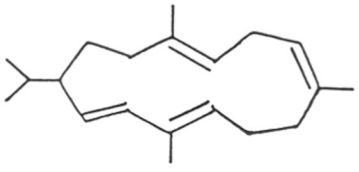
The work presented in this Chapter has been published in the form of research papers:

i) Chiplunkar, Y.G.and Nagasampagi, B.A.
(1992) *J.Nat.Pro. 55*, 1328.

ii) Rojatkar, S.R., Chiplunkar, Y.G. and Nagasampagi, B.A.
(1994) *Phytochemistry 37*, 1213.

3.1 Introduction

Diterpenoids are C_{20} compounds formed by the combination of four isoprene ($-C_5H_8$) units. Plants produce a vast number of diterpenoids with diversified skeletal frameworks. This class includes monocyclic compounds such as cembrene **1**, 1,6,10-duvatriene **2**, simple bicyclic diterpenoids like labdanes, clerodanes as well as tricyclic, tetracyclic and pentacyclic diterpenes.



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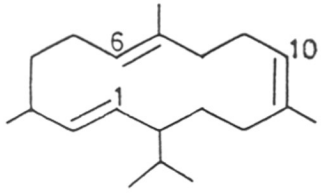

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The exact role of diterpenoids or in general the secondary metabolites in the host metabolism is not yet known. Many scientists are of the view that they could be the stress compounds evolutionally retained due to continuous environmental stress. The stress could be due to the presence of insects, drought conditions, excess of sunlight, etc. Thus, the stress compounds are better understood as defence chemicals such as insect antifeedants, growth inhibitors or insect repellents. Certain non-stress metabolites performing definite roles in the physiological activities are also synthesized by plants. Natural diterpenoids are known to occur as stress as well as non-stress metabolites. Gibberelins as plant growth regulators[1], oryzalexin D, a phytoalexin from blast-infected rice leaves[2], anthocyanins for attracting the pollinating insects are a few examples.

Besides this, a number of diterpenoids with significant biological activity have been isolated from plants. For example, ferruginol from *Podocarpus ferrugineus* resin[3] possesses antifungal properties while taxol from *Taxus brevifolia*[4] is a well known anti-cancer drug.

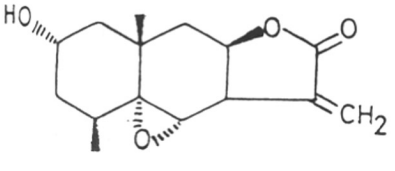
Thus, diterpenoids constitute a major portion of the secondary metabolites synthesized by the plants. In this chapter, we report the isolation and structure elucidation of two new diterpenoids, one each from *Pulicaria wightiana* and *Grangea maderaspatana*. One of them represents a sandaracopimarane skeleton, while the other one is a labdane diterpenoid.

3A : Chemical Investigation of Pulicaria wightiana

The genus *Pulicaria* belongs to the tribe Inuleae, subtribe Inulinae, family Compositae. It is a large genus consisting of a number of annual or perennial herbs distributed in Asia, Europe and Africa. The chemical investigation of more than thirteen species of *Pulicaria* has been carried out so far yielding a vast number of new and novel terpenoids as well as aromatic compounds.

3.2 Biological activity of *Pulicaria* species

Some of the species of *Pulicaria* possess significant medicinal properties. For example, *P.paludosa* is used in the ointment for skin disorders[5]. 2α-hydroxy-5α,6α-epoxyalantolactone **3** isolated from *P.crispa* exhibited antineoplastic activity. Axillarin **4** from the same species is reported to be cytotoxic and is a potential cancer chemopreventive agent[6]. The extract of leaves of *P.dysenterica* causes an antihistaminic effect on the ileum of guinea pig, rat uterus and isolated tracheal chains of guinea pigs. Antiserotonin effect on rat fundus preparation is also reported for the same[7].



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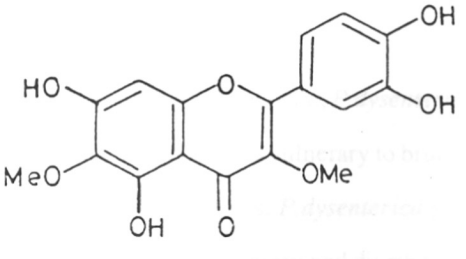

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3.3 Previous work on *Pulicaria* species

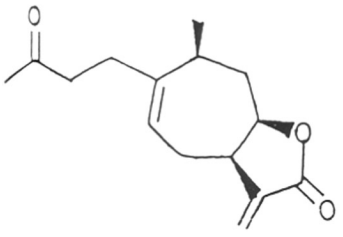
The genus is not chemically homogeneous and different types of secondary metabolites have been isolated from different species. The compounds include sesquiterpenoids, diterpenoids, aromatic compounds and polyacetylenes.

The sesquiterpenoids isolated from *Pulicaria* exhibit a wide variety of skeletal types. For example, xanthanolides **5, 6, 7** have been isolated from *P.sicula*, *P.undulata*[8,9], while bisabolene type sesquiterpenes **8, 9** occur in *P.glutinosa*[10] along with the germacrane derivatives such as **10, 11**[11]. *P.undulata* has also yielded eudesmanolides like **12**, norguaianolide **13** and pseudo-guaianolide **14**[9], whereas guaianolides exemplified by **15, 16** are reported from *P.sicula*[8] and *P.glutinosa*[10]. Caryophyllene derivatives form a major part of the sesquiterpenes from *Pulicaria* and have been reported from various species viz. *P.dysenterica*[12,13], *P.scabra*[14], *P.arabica*[15] and *P.paludosa*[16]. The compounds **17, 18** and **19** are some of the representative compounds of this class. In addition to this, novel sesquiterpenes such as pulicaral **20**[17] and hydroxyisocomene **21**[13] have also been reported from some of the *Pulicaria* species.

Diterpenoids from *Pulicaria*[18,19,20] include clerodanes **22, 23** and kauranes like **24**[20] isolated from the species *P.crispa*, *P.glutinosa*, *P.anguistifolia* and *P.gnaphalodes*. Further, the genus is known to contain polyacetylenes like **25**[21], aromatic compounds such as **26**[22,23] and thymol derivatives of the type **27**[13-15,23].

3.4 Present Work

Eight species of the genus *Pulicaria* are found in India, out of which *P.crispa*, *P.dysenterica* and *P.foliolosa* are the prominent ones. Dried herb of *P.crispa* is applied as a vulnerary to bruises and sores of bullocks. Decoction of the plant is taken for febrile conditions. *P.dysenterica* possesses tonic, astringent, diuretic properties and is used in treatment of dysentery and diarrhoea[24].



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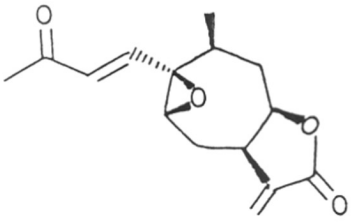

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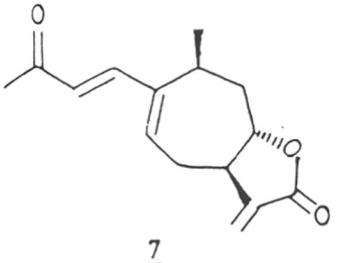

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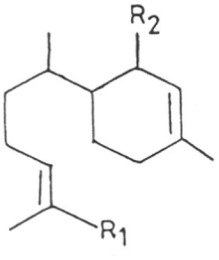
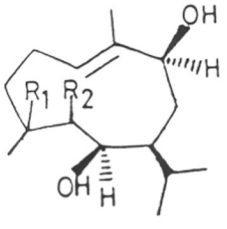
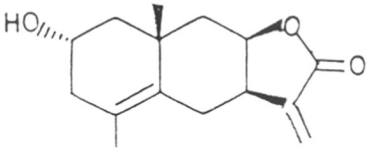


	R_1	R_2
8	CH_2OH	OH
9	CHO	=O



10 R_1R_2 = double bond

11 R_1R_2 = β-epoxide



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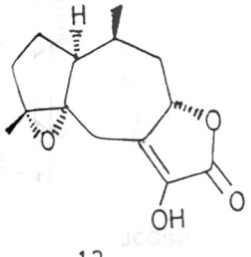

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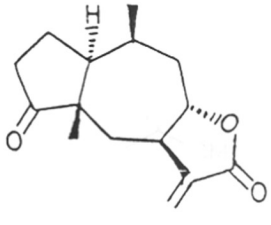

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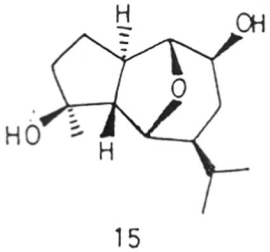

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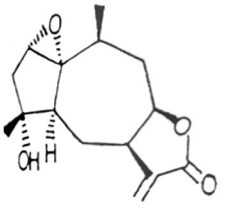

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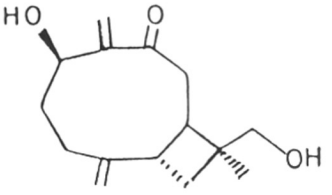

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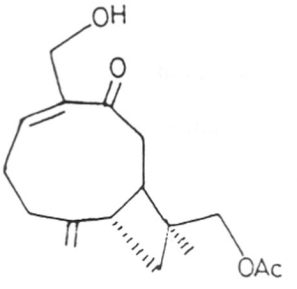

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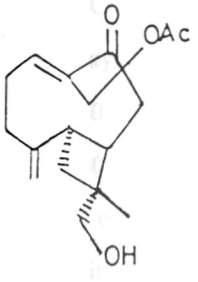

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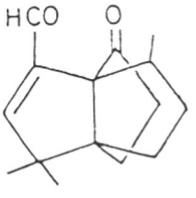

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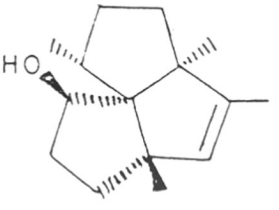

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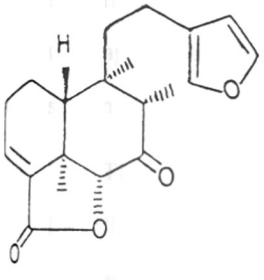

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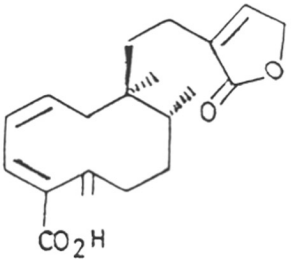

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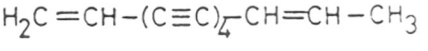

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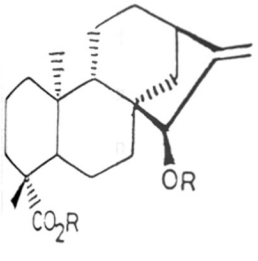
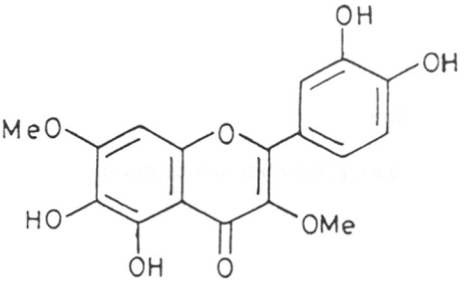


R = glucose

24



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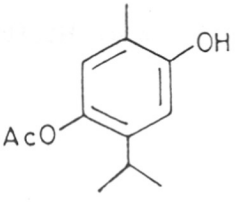

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The acetone extract of the shade dried herb *P.wightiana* was subjected to chromatographic separation to give three broad fractions A, B and C. Fraction B on repeated column chromatography and preparative thin layer chromatography yielded compound **B-1** (19 mg), m.p. 155-157℃ $[\alpha]_D$ - 9.09^0.

3.4.1 Functional groups of **B-1**

Compound **B-1** in its IR spectrum (Fig.1) displayed a broad band at 3400 cm^{-1} due to hydroxyl group, an intense band at 1690 cm^{-1} for the α,β unsaturated carbonyl and a sharp band at 1640 cm^{-1} for the olefinic stretching. In the UV spectrum of **B-1**, the observed λ_{max} at 251 nm (ε 2980) was in support of the presence of conjugated carbonyl. The low ε value indicated the cisoid conformation of the conjugated system[25]. It is known that the ratio of integrated band intensities of the C=O and C=C stretching vibrations in the IR spectrum lies between 0.6 to 3.5 for cisoid and is more than 6 for transoid ketones[26]. Although such a quantitative estimation was not carried out for **B-1**, the ratio of band heights of C=O and C=C in the IR spectrum was observed to be approximately 1.04 (Fig.1). The ratio corroborated the presence of cisoid ketone-olefin system.

The mass spectrum of **B-1** exhibited the molecular ion peak $[M]^+$ at m/z 302 (Fig.2), which led to the probable molecular formula $C_{20}H_{30}O_2$ for **B-1**. The peak at m/z 284, (M^+-18) supported the presence of hydroxyl group in **B-1**.

The ^{1}H-NMR spectrum of **B-1** (Fig.3) contained three singlets at δ 0.87 (3H), 0.91 (6H) and 1.09 (3H) for four quaternary methyls. A multiplet centered at δ 3.91 (1H) probably due to the carbinyl proton (C<u>H</u>OH) and a downfield doublet at δ 6.71 (J=2Hz) for the proton at β-position of an α,β-unsaturated ketone were also observed. Further, multiplets at δ 4.93 (2H) and δ 5.82 (1H) in the ^{1}H-NMR of **B-1** were assignable to the vinyl group.

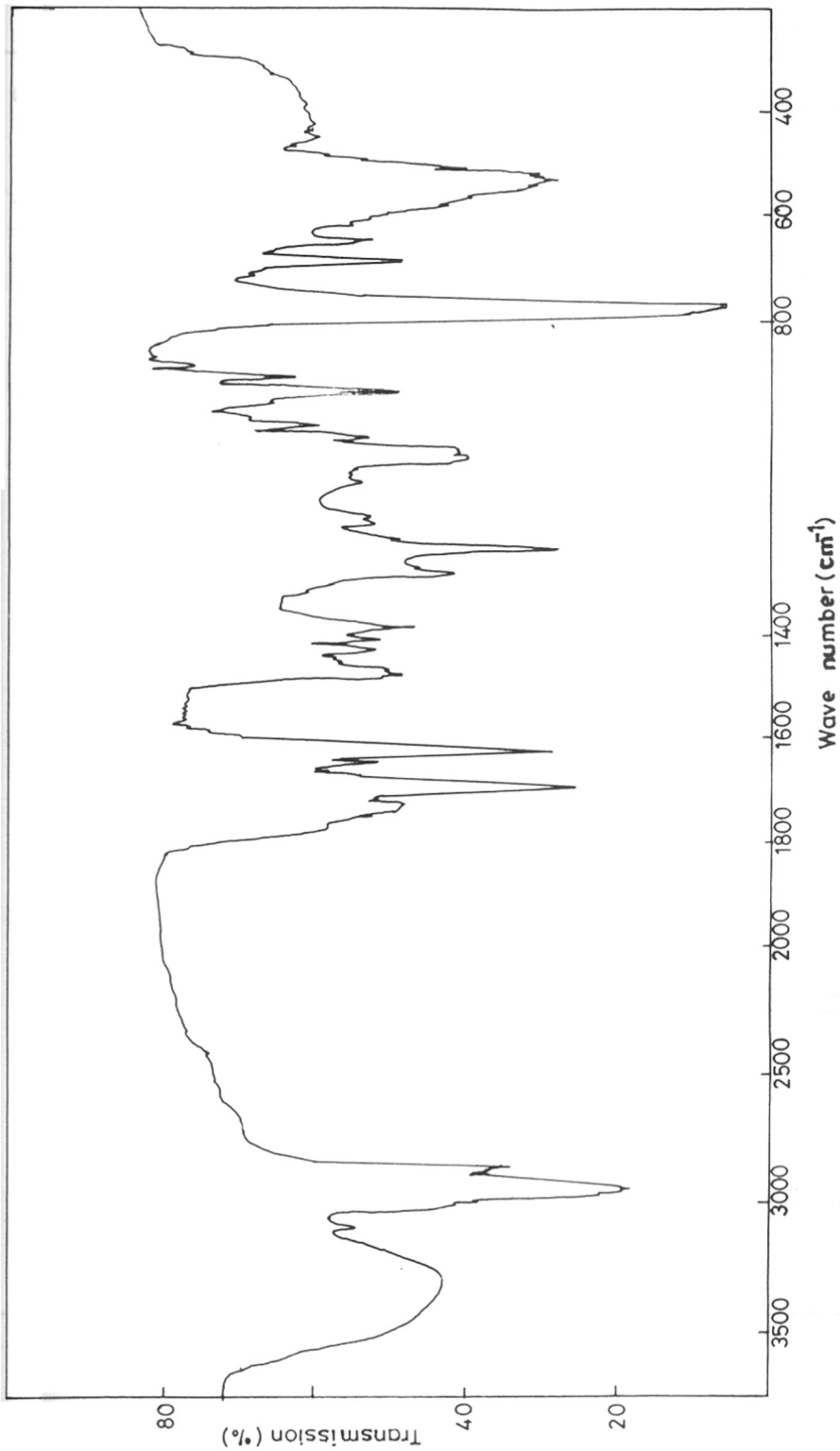


Fig.1 : IR spectrum of compound B-1.

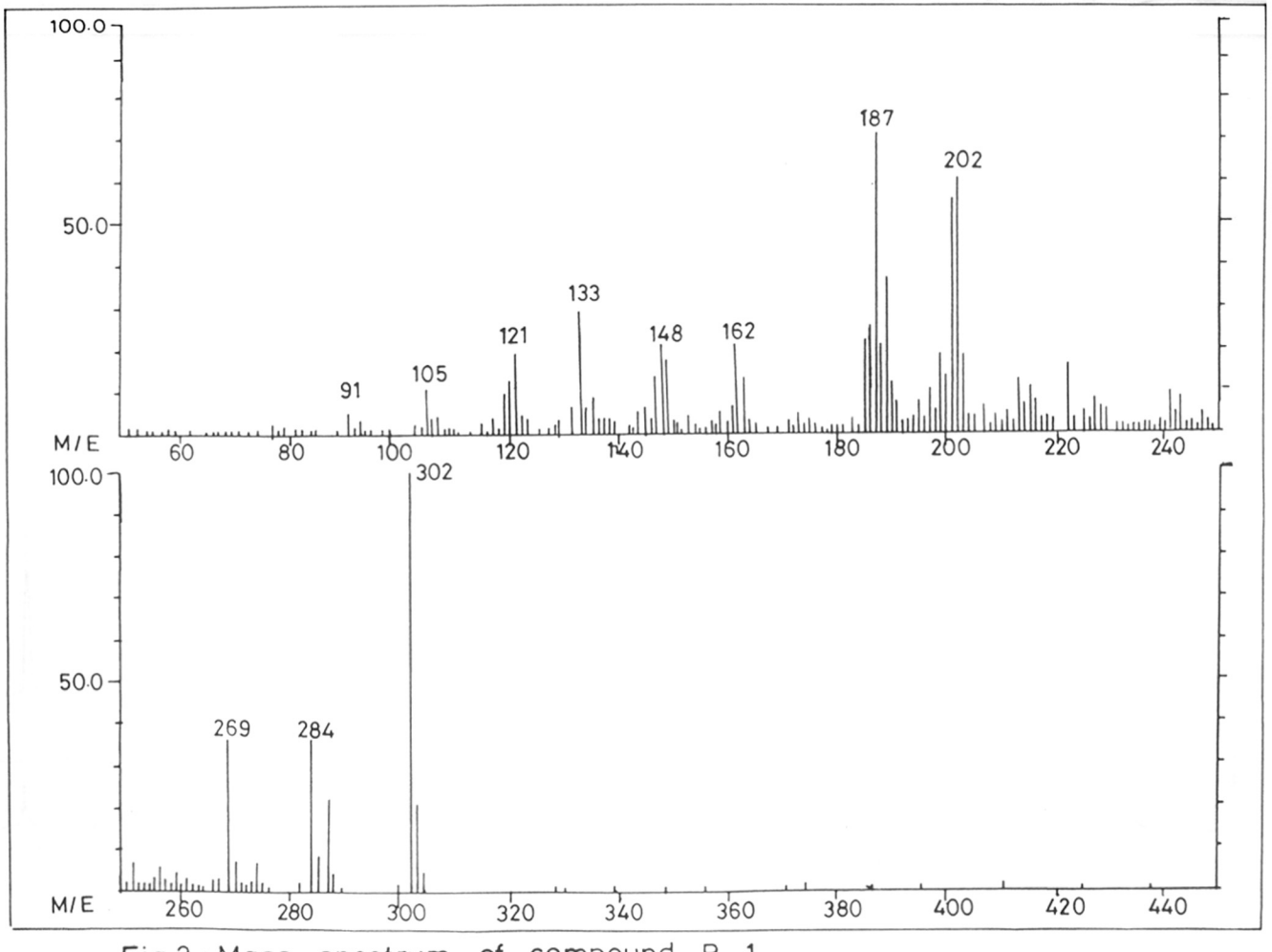


Fig.2 : Mass spectrum of compound B-1.

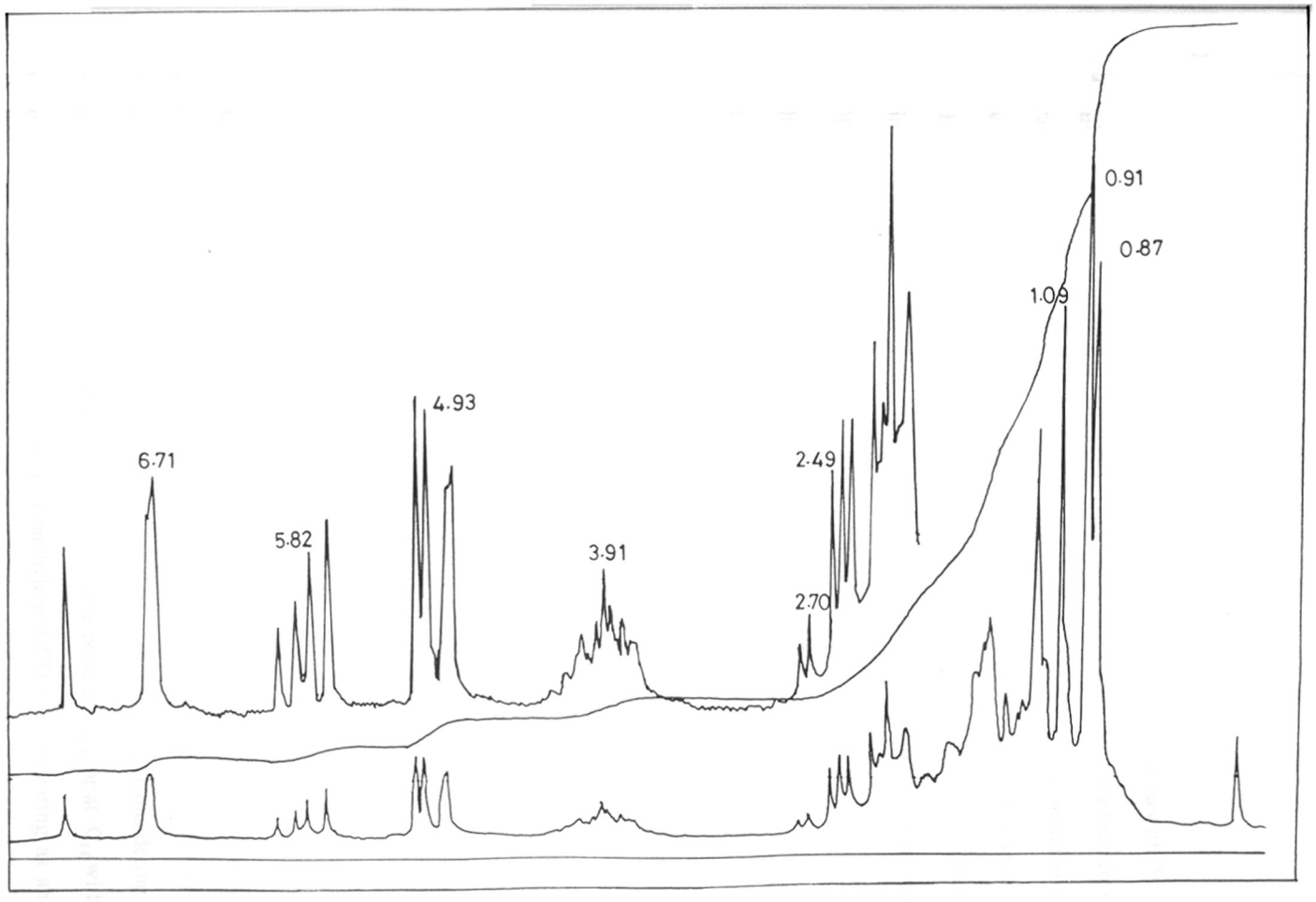


Fig.3 : ^{1}H-NMR spectrum of compound B-1.

3.4.2 Nature of the hydroxyl group in **B-1**

Compound **B-1** on acetylation at room temperature yielded a gummy mass **B-1a** with $[M]^+$at m/z 344 ($C_{22}H_{32}O_3$). The IR spectrum of **B-1a** (Fig.4) showed the absence of band due to hydroxyl and an extra carbonyl at 1730 cm^{-1}. In the ^{1}H-NMR spectrum of **B-1a** (Fig.5), a multiplet at δ 4.20 (1H) was observed as a result of the shift of the signal at δ 3.91 (1H) in **B-1**. The remaining signals in the ^{1}H-NMR spectrum of **B-1a** were identical to those of **B-1**. Thus, the shift (0.3 δ) of the carbinyl proton on acetylation confirmed the presence of secondary hydroxyl group in the parent compound.

All the above data revealed that we were dealing with a pimarane type diterpenoid (Fig.6) possessing a secondary hydroxyl and an α, β- unsaturated cisoid ketone.

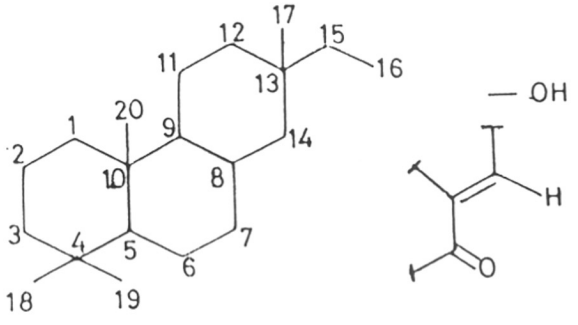


Fig.6 : Pimarane skeleton

Biogenetically, pimaranes either contain a hydroxyl at C_8 or the consequent unsaturation at that position due to the elimination of H_2O[27a]. The compound **B-1** possessed a cisoid carbonyl and the ^{1}H-NMR spectrum revealed presence of a proton β- to carbonyl group. Considering this, the carbonyl group could be placed either at C_7 with a double bond at $C_{8(14)}$ or at C_{14} with the conjugated olefin at $C_{7(8)}$. However, an isolated methylene ($-CH_2CO$) appearing as an AB quartet[27b] at δ 2.49 and 2.70 was observed in the ^{1}H-NMR spectrum of **B-1**(Fig.3). This implied that the carbonyl group in **B-1** was probably situated at C_7.

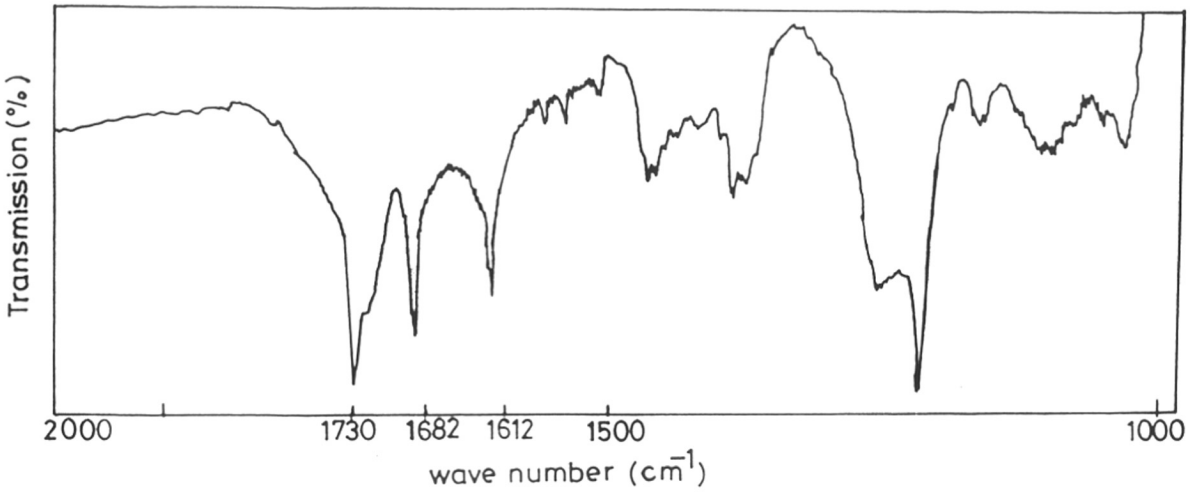


Fig.4: IR spectrum of compound B-1a.

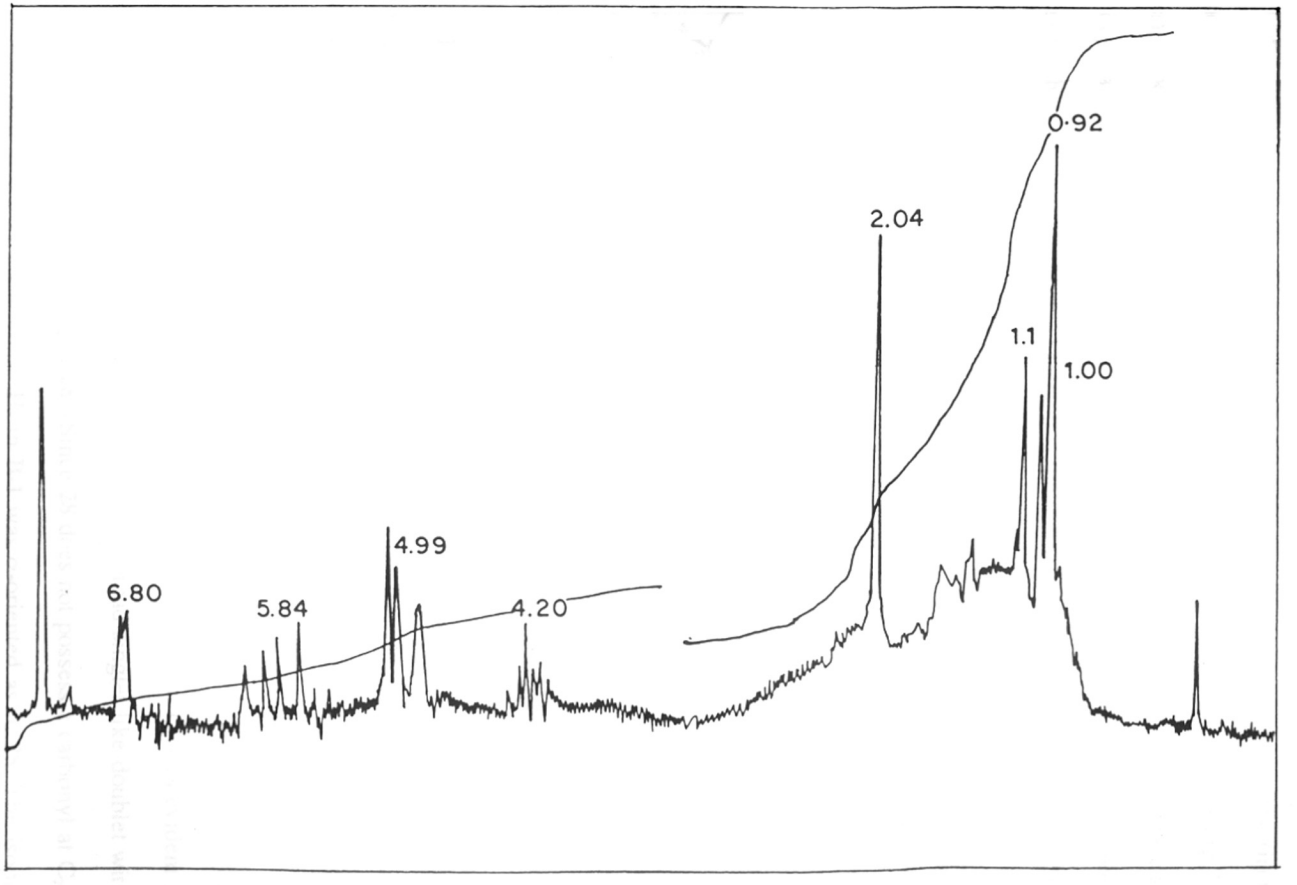


Fig. 5 : ^{1}H - NMR spectrum of compound B-1a

3.4.3 Skeleton of **B-1**

Pimarane-type compounds are divided into three sub-classes (i) pimaranes, (ii) isopimaranes and (iii) sandaracopimaranes based on the stereochemistry at C_9 and C_{13} (Fig.7). (There seems to be some confusion in the literature regarding the identification of pimarane skeletons as isopimaranes, sandaracopimaranes etc. Convention in Ref. [28] has been followed in this chapter. Thus, sandaracopimarane skeleton is the one with α-oriented C_9-proton and C_{13}-vinyl group).

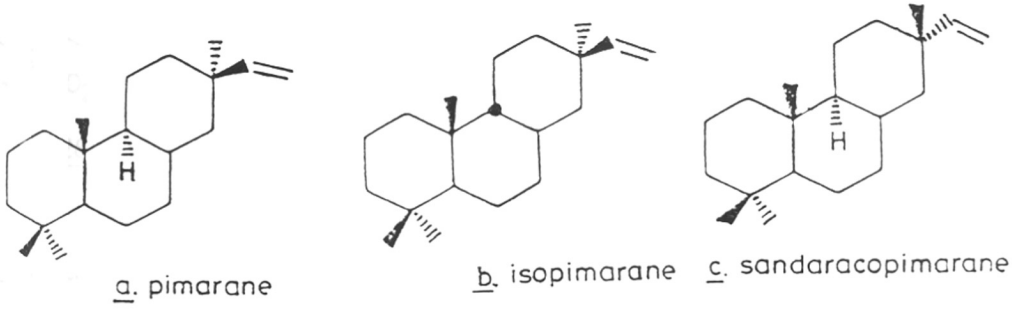


Fig.7 : Different types of pimaranes

The stereochemistry and consequently, the skeletal type of **B-1** was fixed based on the comparison of the ^{1}H-NMR spectra of B-1 and other compounds belonging to the three subclasses. Careful observation of ^{1}H-NMR of **B-1** revealed its close resemblance to that of methyl sandaracopimarate, **28**[28]. The splitting patterns of multiplets due to the olefinic and exo-methylene protons of the vinyl group were found to be identical (Fig. 8a,b). The vinyl group at C_{13} in **B-1**, therefore, could be α-oriented. The stereochemistry of the proton at C_9 was evident from the ^{1}H-NMR signal for H_{14} (δ 6.71) in **B-1**. Nature of the broad singlet like doublet with J=2Hz (Fig.8b) was similar to that of H_{14} in **28**. (Since **28** does not possess a carbonyl at C_7, the H_{14} signal appeared at δ 5.17). Therefore, H_9 in **B-1** was α-oriented as in **28**. The isopimaranes

(Fig.7b) and rimuene possessing β-oriented H_9, on the other hand displayed clean doublet or ill-defined multiplet for H_{14}. Thus, **B-1** possessed a sandaracopimarane skeleton with α-oriented H_9 and C_{13} vinyl group.

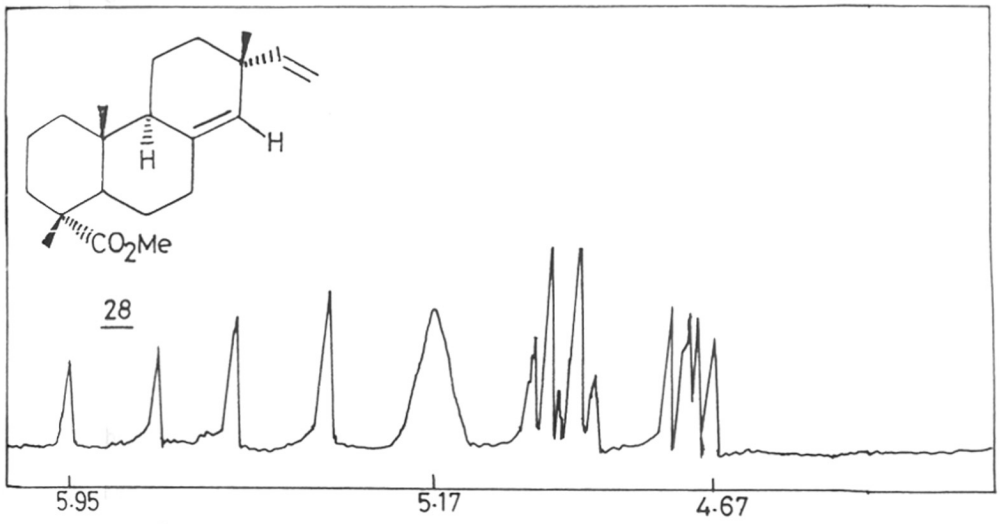


Fig. 8(a): ^{1}H-NMR spectrum of methyl sandaracopimarate in the region δ 4.6 to 6.0.

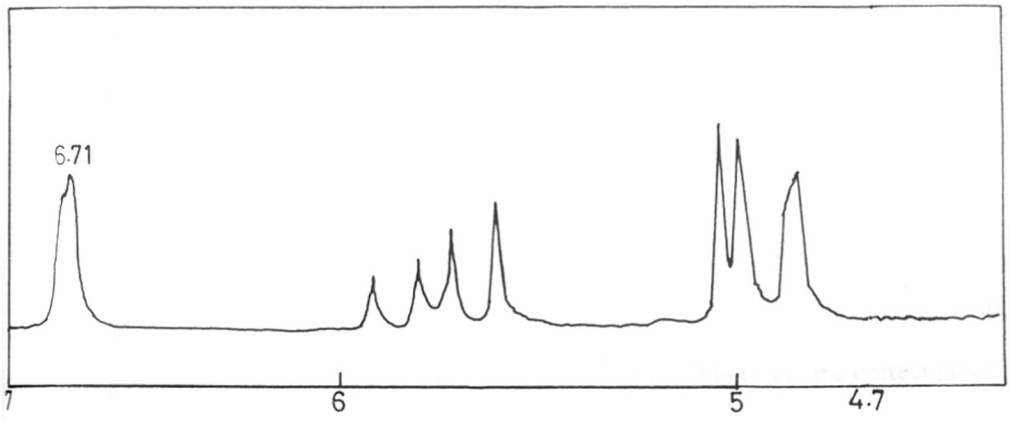
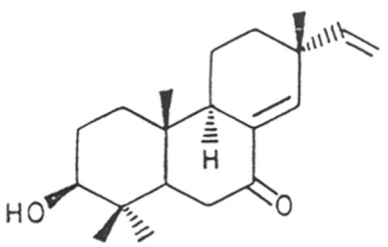


Fig. 8(b): ^{1}H-NMR spectrum of compound B-1 in the region δ 4.7 to 6.8.

3.4.4 Positions of functional groups

The position of α, β-unsaturated carbonyl in **B-1** was fixed based on the comparison of ^{1}H and ^{13}C chemical shifts of **B-1** with those of reported sandaracopimarane **29**[29]. The ^{1}H-NMR spectrum of **B-1** when compared with that of linifoliol **29**; an aglycone from *Leucas linifolia*[29], brought out striking similarities in the two.



29

The only difference observed was in the chemical shift of multiplet due to carbinyl proton. In **29**, it resonated at δ 3.32 as against the value of δ 3.91 in **B-1**. The ^{13}C-NMR spectra of **B-1** (Fig.9) and **29** also matched well except for the A-ring carbon atoms C_1.C_4, C_{18} and C_{19}. A difference of almost 14δ was observed in the ^{13}C chemical shifts of the carbinyl carbons in **B-1** and **29** (Table-1).

From these evidences, it could be concluded that (i) **B-1** possessed a structure very close to **29** with the carbonyl at C_7 and the double bond at $C_{8(14)}$; (ii) Position of secondary -OH in **B-1** differed from that of **29**.

For the position of hydroxyl group, various pimarane alcohols[30] were checked for spectral properties (^{1}H and ^{13}C-NMR). The reported chemical shift of carbinyl proton in ^{1}H-NMR spectrum of **30**: 8(14), 15-sandaracopimaradiene 2α,18-diol[31] led us to the correct placement of hydroxyl group in **B-1**.

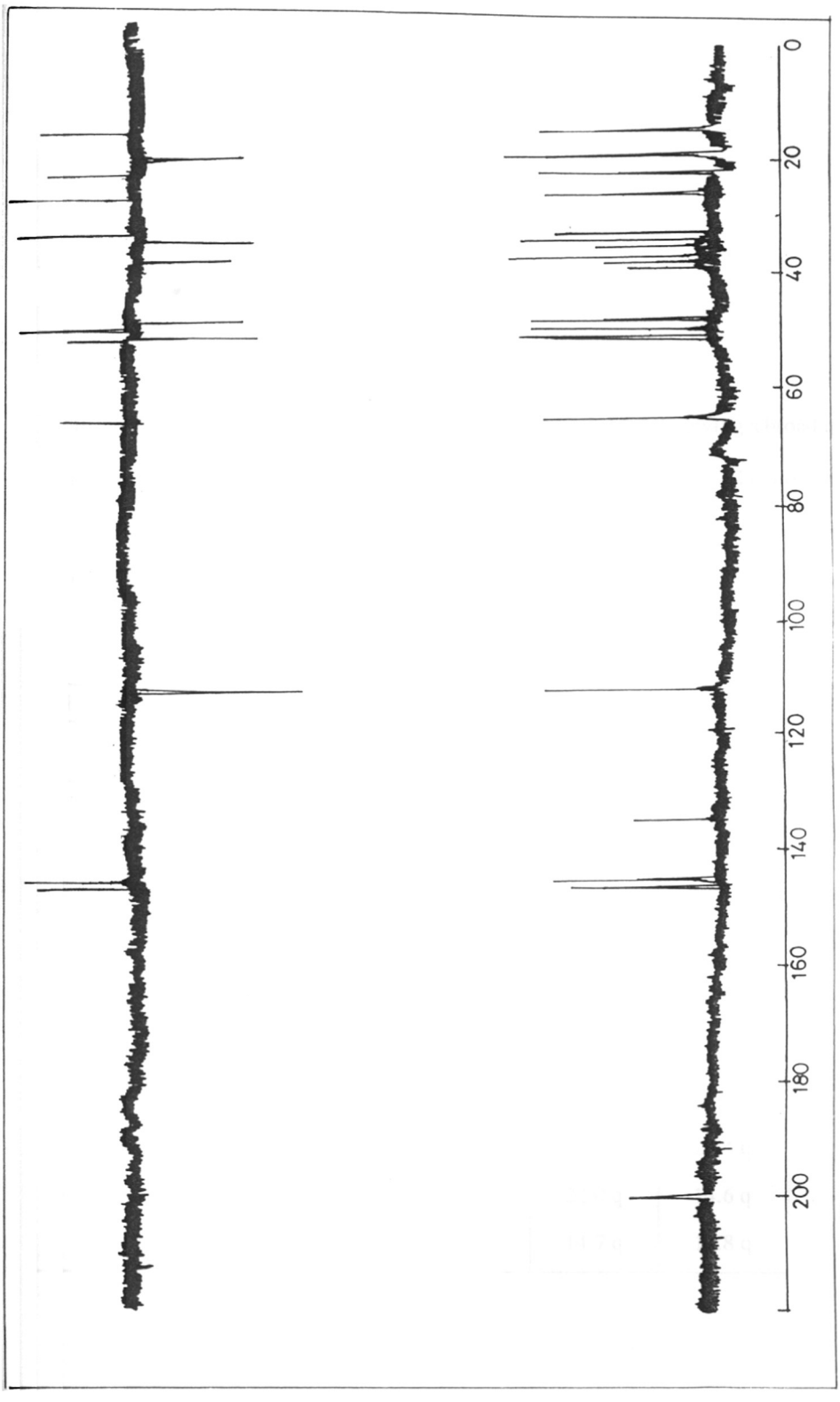
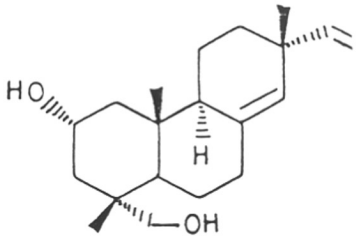


Fig. 9 : ^{13}C- NMR spectrum of compound B-1.



30

In the ^{1}H-NMR of **30**, the β-oriented H-C_2-OH appeared at δ 3.92 giving a broad multiplet and the corresponding carbon resonated at δ 65.3 in the ^{13}C-NMR spectrum. The broad multiplet at δ 3.91 in the ^{1}H-NMR spectrum and a doublet at 64.8 in the ^{13}C-NMR spectrum of **B-1** enabled us to place the hydroxyl group at C_2, with α-orientation.

Table-1: ^{13}C-NMR data of compounds **B-1** and **29**

Carbon No.	Compound		Carbon No.	Compound	
	B-1	**29**		**B-1**	**29**
1	47.8 t	36.8 t	11	19.0 t	19.1 t
2	64.8 d	27.3 t	12	36.9 t	35.8 t
3	50.7 t	78.4 d	13	37.6 s	38.6 s
4	34.7 s	38.8 s	14	144.8 d	144.5 d
5	50.9 d	50.6 d	15	146.1 d	146.2 d
6	33.9 t	34.0 t	16	111.7 t	111.8 t
7	199.8 co	200.2 co	17	25.7 q	25.8 q
8	134.5 s	134.7 s	18	32.5 q	27.4 q
9	49.3 d	49.5 d	19	22.0 q	14.6 q
10	38.5 s	38.6 s	20	14.7 q	13.8 q

For a rigid chair conformation of A-ring in pimaranes, the α-hydroxyl at C_2 would be equatorial making the carbinyl proton axially oriented (Fig.10). The corresponding multiplet at δ 3.91 in ^{1}H-NMR spectrum of **B-1** was spread over 18-20Hz. The nature of the multiplet assignable to the axial-axial (a,a) and axial-equatorial (a,e) coupling experienced by H_2 thus supported the proposed α orientation for the hydroxyl group.

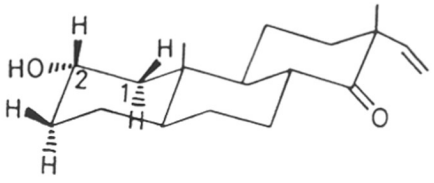
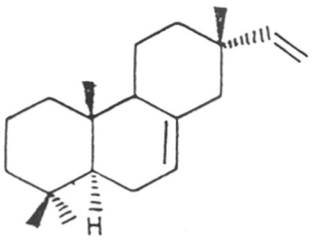


Fig.10 : Chair conformation of pimaranes.

Further, the ^{13}C chemical shifts of A-ring carbons in **B-1** were compared with those reported for sandaracopimarane **31** without any substituent on the A-ring[32] (Table-2).

Table-2: ^{13}C Chemical shifts of compounds **B-1** and **31**

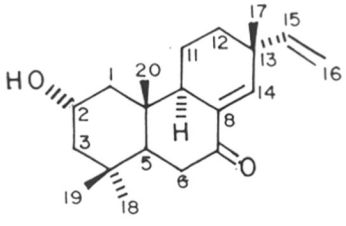
Carbon No.	Compound **B-1**	Compound **31**
1	47.8 t	40.1 t
2	64.8 d	19.0 t
3	50.7 t	42.5 t
4	34.7 s	33.1 s
5	50.9 d	50.5 d
18	32.5 q	33.9 q
19	22.0 q	22.6 q



31

The observed downfield shift of 7-8 δ for C_1 and C_3 in the ^{13}C-NMR spectrum of **B-1** was in agreement with the assigned position C_2 for the hydroxyl group.

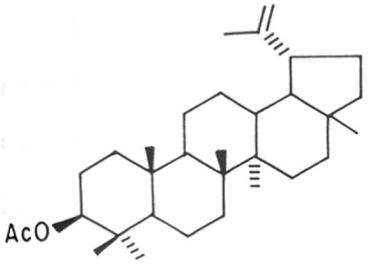
Thus, the structure of **B-1** was elucidated as sandaracopimara-8(14),15-diene-7-keto-2α-ol.



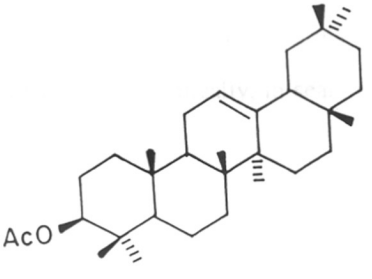
B-1 R = H

B-1a R = Ac

Compounds **B-2** and **B-3** obtained from fraction A of the extract by repeated column chromatography with silver nitrate impregnated silica gel were identified as β-amyrin acetate and lupeol acetate respectively. The identification was done based on direct comparison of their physical characteristics and spectral data with those reported[33,34].



B-2



B-3

3B : Chemical Investigation of Grangea maderaspatana

The genus *Grangea* is a small genus of herbs distributed in tropical and subtropical parts of Africa and Asia. Only two species of this genus occur in India, of which *G.maderaspatana* is a commonly found weed spread throughout India.

G.maderaspatana (Marathi: *Mashipatri*) is a procumbent, hairy annual with spreading stems and alternate, sessile, pinnately lobed leaves. The leaves have a characteristic odour. The flowerheads are globose and yellow in colour.

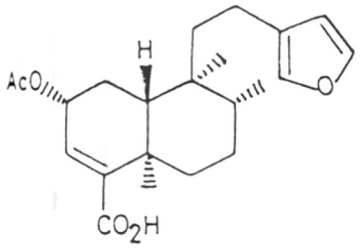
3.5 Medicinal activity of Grangea species

Leaves of *G.maderaspatana* are reported to be stomachic, deobstruent, antispasmodic and are prescribed in infusion or electuary. They find use in antiseptic and anodyne fomentations. The juice of leaves is employed as an instillation for ear-ache[35]. The crude chloroform extract of *G.maderaspatana* is reported to exhibit strong cytotoxic activity[36]. A mixture of flavonoids obtained from the extract of this species causes estrogenicity and antiimplantational activity in mouse[37].

Although a number of compounds have already been isolated from *G.maderaspatana*, the reported medicinal activity of the plant prompted us to investigate it chemically, in search of some more new compounds.

3.6 Previous Work on Grangea maderaspatana

Diterpenoids constitute a major portion of the various compounds isolated from this species. A number of clerodane derivatives such as **32, 33**, nor-clerodanes like **34** and nor-seco-clerodanes of the type **35** have been reported from this plant[38]. Sesquiterpene lactones **36, 37, 38**[36] and stigmasteroids such as chondrillasterone **39**[39] have also been isolated from the same in addition to phenyl alanine derivative **40**[40].



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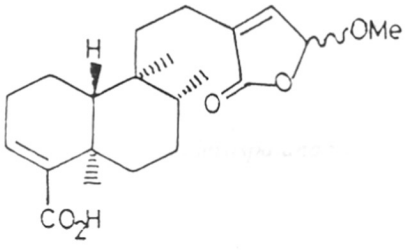

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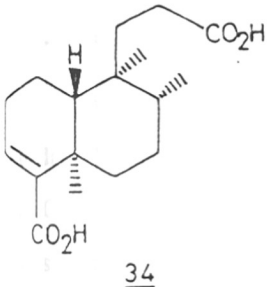

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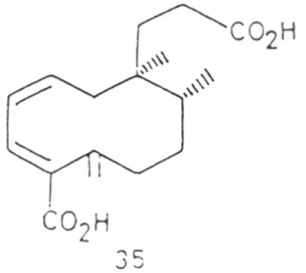

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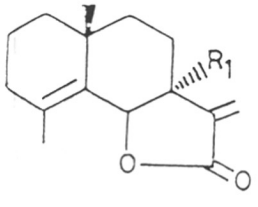
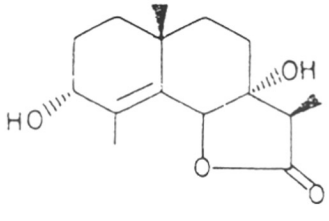


	R_1
36	H
37	OH



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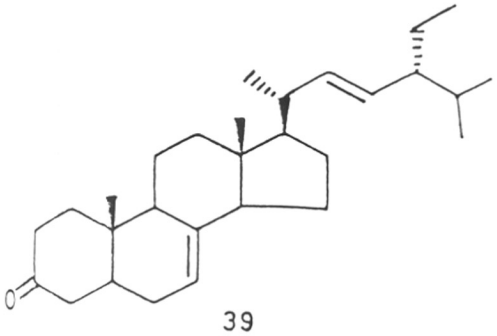

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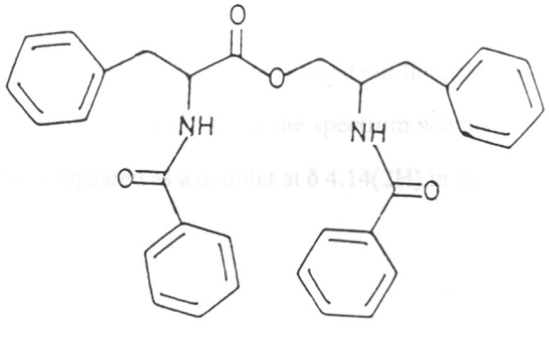

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3.7 Present Work

In this section, the isolation of a new labdane diterpenoid **C-1** from *G.maderaspatana* along with four known compounds **C-2**, **C-3**, **C-4** and **C-5** is reported.

The shade dried aerial part of *G.maderaspatana* was extracted with acetone. The extract was subjected to column chromatography and six broad fractions (A-F) were collected. Fraction D on repeated chromatographic separation yielded compound **C-1** (65 mg) as a viscous oil.

3.7.1 Spectral studies of **C-1**

Compound **C-1**, in its IR spectrum (Fig.11) showed a strong, broad band at 3510 cm^{-1} for hydroxyl group and a weak band at 1660 cm^{-1} for olefinic carbon stretching. In the mass spectrum of **C-1**, the molecular ion peak $[M]^+$ was observed at m/z 308, based on which compound **C-1** was tentatively assigned the molecular formula $C_{20}H_{36}O_2$.

The ^{1}H-NMR of **C-1** (Fig.12) displayed four singlets at δ 0.80 (6H), 0.90 (3H), 1.14 (3H) and 1.71 (3H) corresponding to five methyl groups. One of the methyls (δ 1.71) was probably due to the vinylic methyl group. The spectrum also showed a triplet at δ 5.42 (1H) for the olefinic proton and a doublet at δ 4.14 (2H).

^{13}C-NMR of **C-1**

The DEPT ^{13}C-NMR spectrum of **C-1** (Fig.13,Table-3) exhibited five quartets ($-CH_3$), eight triplets ($-CH_2$), three doublets (-CH) and four singlets (-C-) thus confirming the suggested molecular formula. Appearance of only two signals in the olefinic region of ^{13}C-NMR;δ 123.2(d) and δ 140.8(s) revealed the presence of a trisubstituted double bond and also ruled out the possibility of a tetrasubstituted olefin in **C-1**. A downfield triplet at δ 59.2 in the spectrum was in support of the primary hydroxyl group CH_2OH which appeared as a doublet at δ 4.14(2H) in the ^{1}H-NMR.

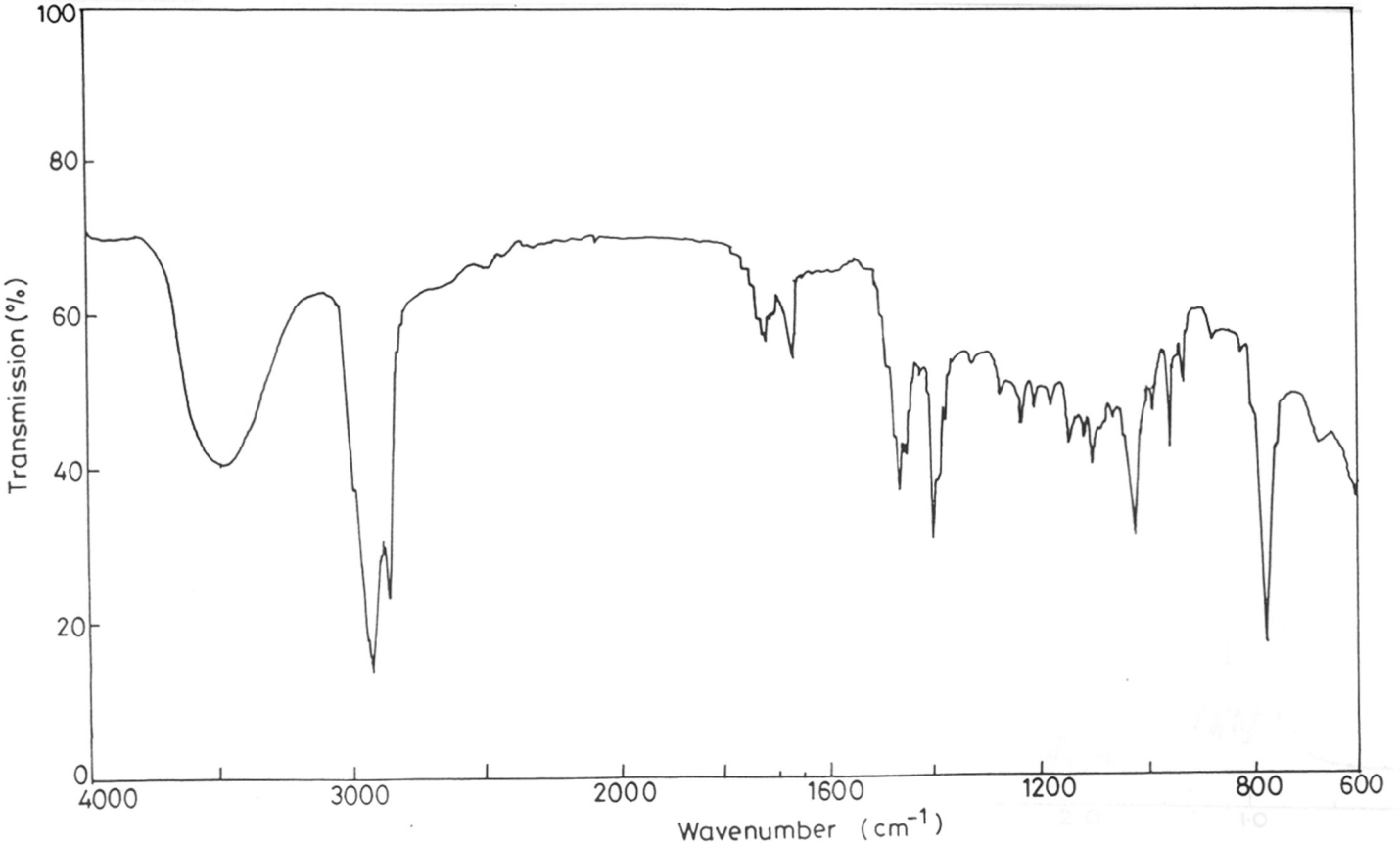


Fig.11: IR spectrum of compound C-1.

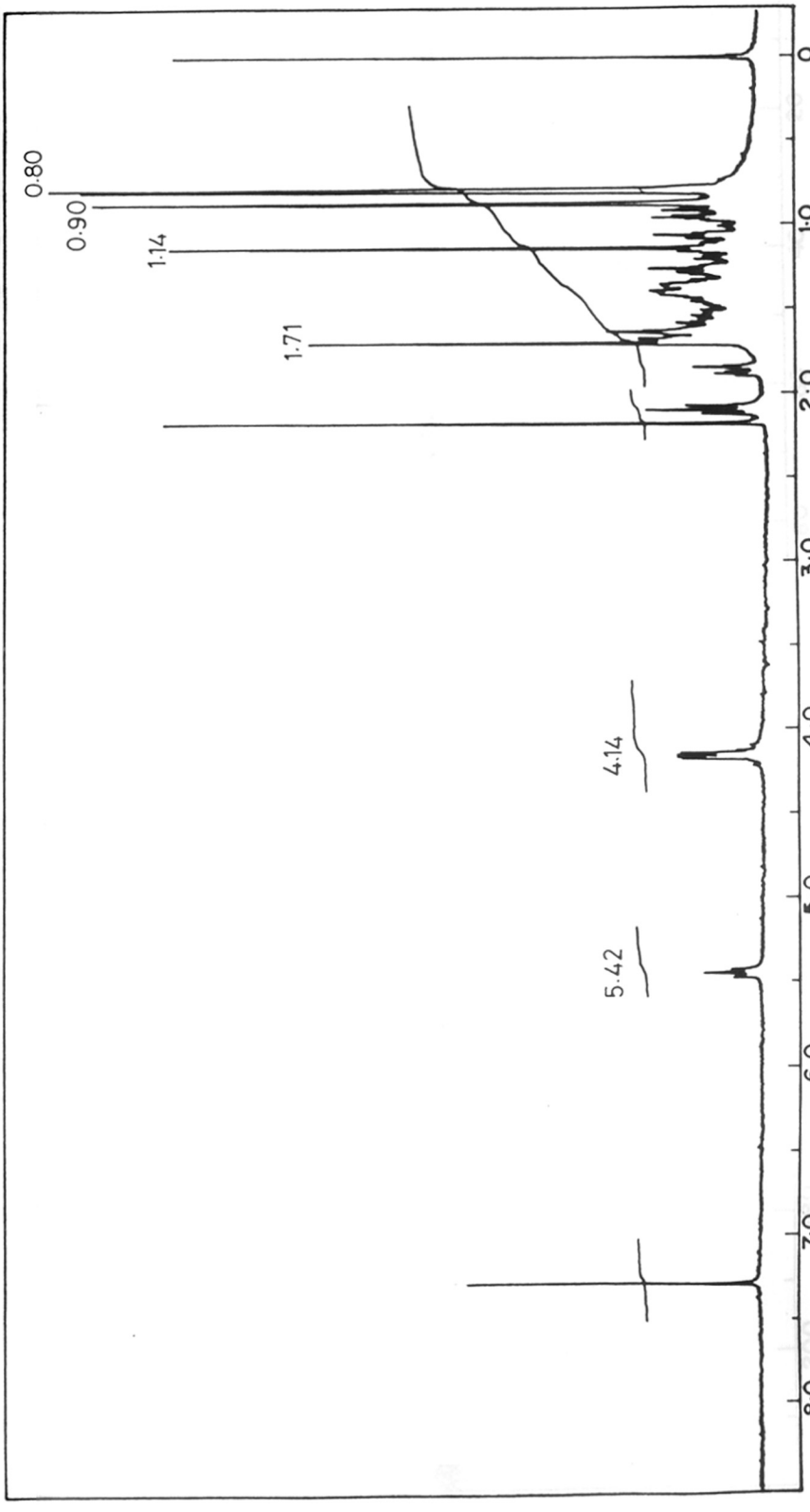


Fig.12 : ^{1}H-NMR spectrum of compound C-1.

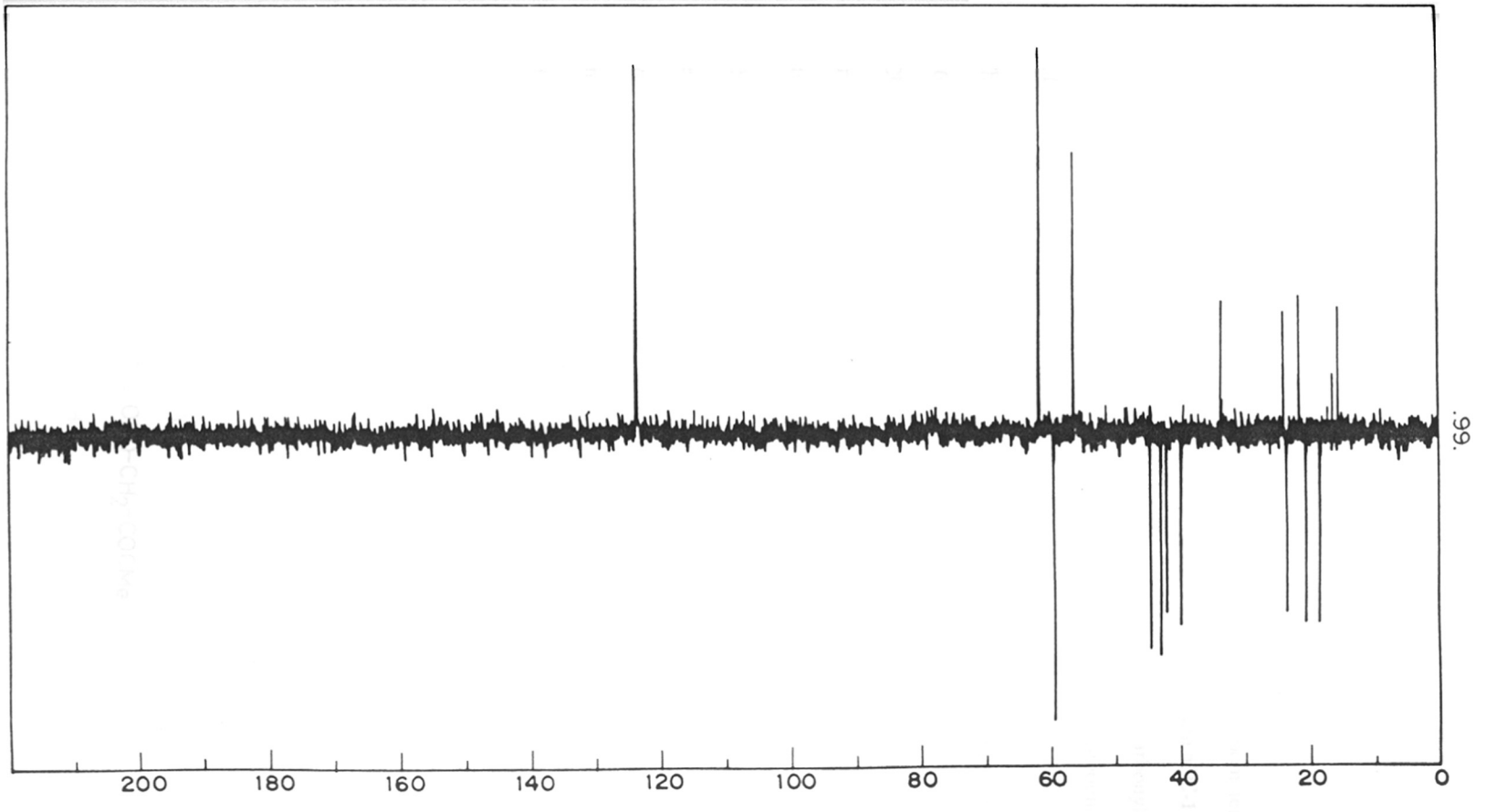


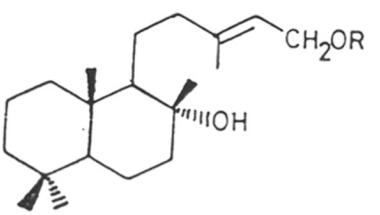
Fig.13 : DEPT ^{13}C-NMR spectrum of compound C-1.

3.7.2 Acetylation of **C-1**

Compound **C-1** on treatment with pyridine and acetic anhydride at room temperature yielded the monoacetate **C-1a** with $[M]^+$ at m/z 350 ($C_{22}H_{38}O_3$). The IR spectrum of **C-1a** (Fig.14) displayed bands at 3500 cm^{-1} due to hydroxyl group, 1730 cm^{-1} for the acetate carbonyl and 1650 cm^{-1} for the olefinic moiety. The presence of tertiary hydroxyl in the parent compound **C-1** was evident from the band at 3500 cm^{-1} in the IR spectrum of **C-1a**.

The ^{1}H-NMR spectrum of **C-1a** (Fig.15) was similar to that of **C-1** except for the doublet at δ 4.56 (2H). The doublet, obtained due to the shift of signal at δ 4.14 in **C-1**, was assignable to the $C\underline{H}_2OCOCH_3$ protons. The primary acetate group was further supported by the triplet at δ 61.3 in the DEPT ^{13}C-NMR spectrum of **C-1a** (Fig.16). The monoacetate **C-1a** thus accounted for the two oxygen functions in **C-1** as a primary and a tertiary hydroxyl group.

The molecular formula $C_{20}H_{36}O_2$ for **C-1** requiring three sites of unsaturation; one of which was due to a trisubstituted olefin, thus represented a bicyclic diterpenoid. The literature search for bicyclic diterpenoids possessing labdane, clerodane and chettaphanane skeletons[41] brought to our notice, the labdane derivative **41**. The compound **41** reported by Urones *et al.* from *Parentucellia latifolia* was obtained by the acetylation of the hydrolysis product of 8-hydroxy-13E-labden-15-yl-methyl malonic acid diester **42**[42].



41. R = Ac

42. R = OCO–CH_2–COCMe

C1. R = H

C1a. R = Ac

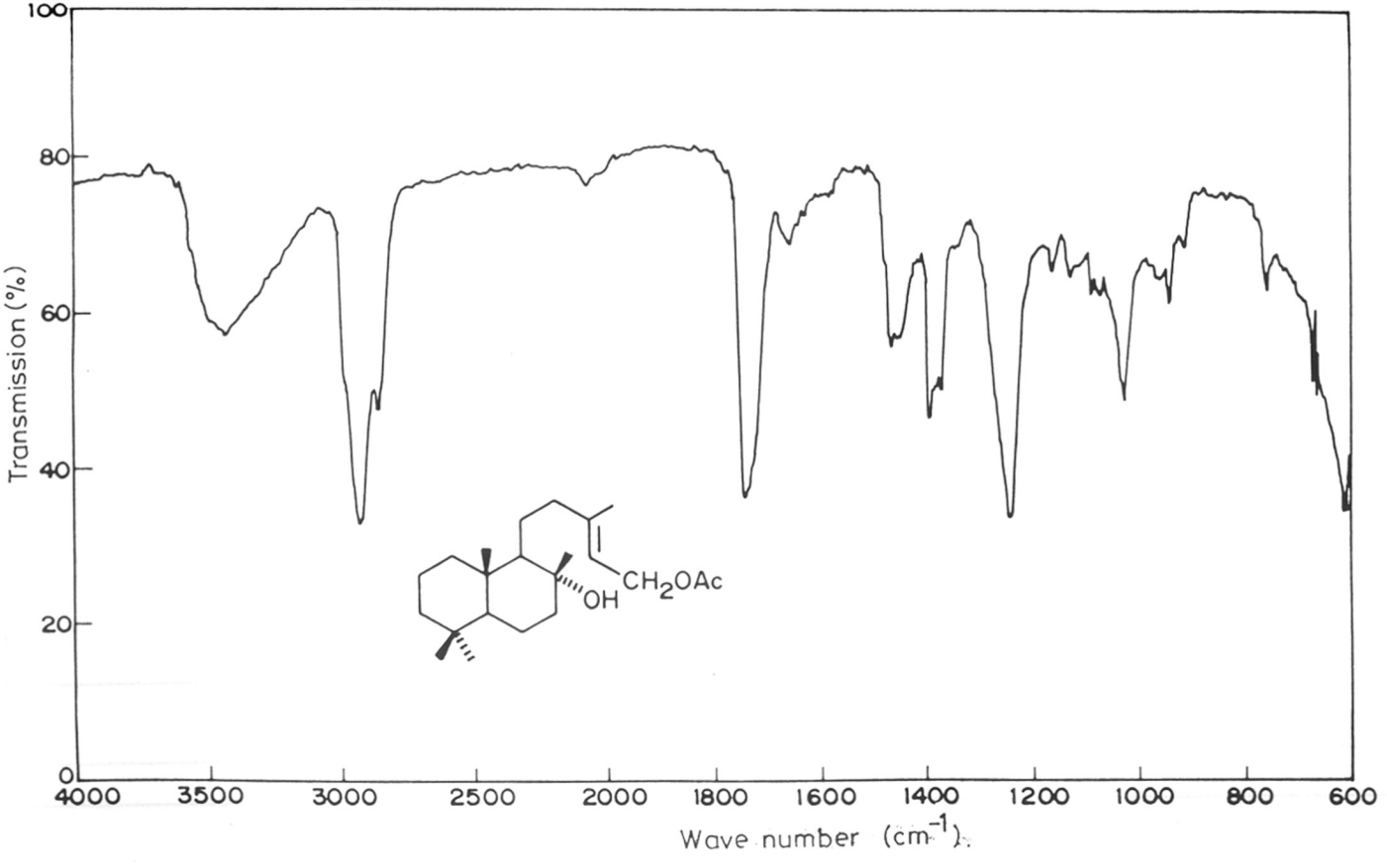


Fig.14 : IR spectrum of compound C-1a.

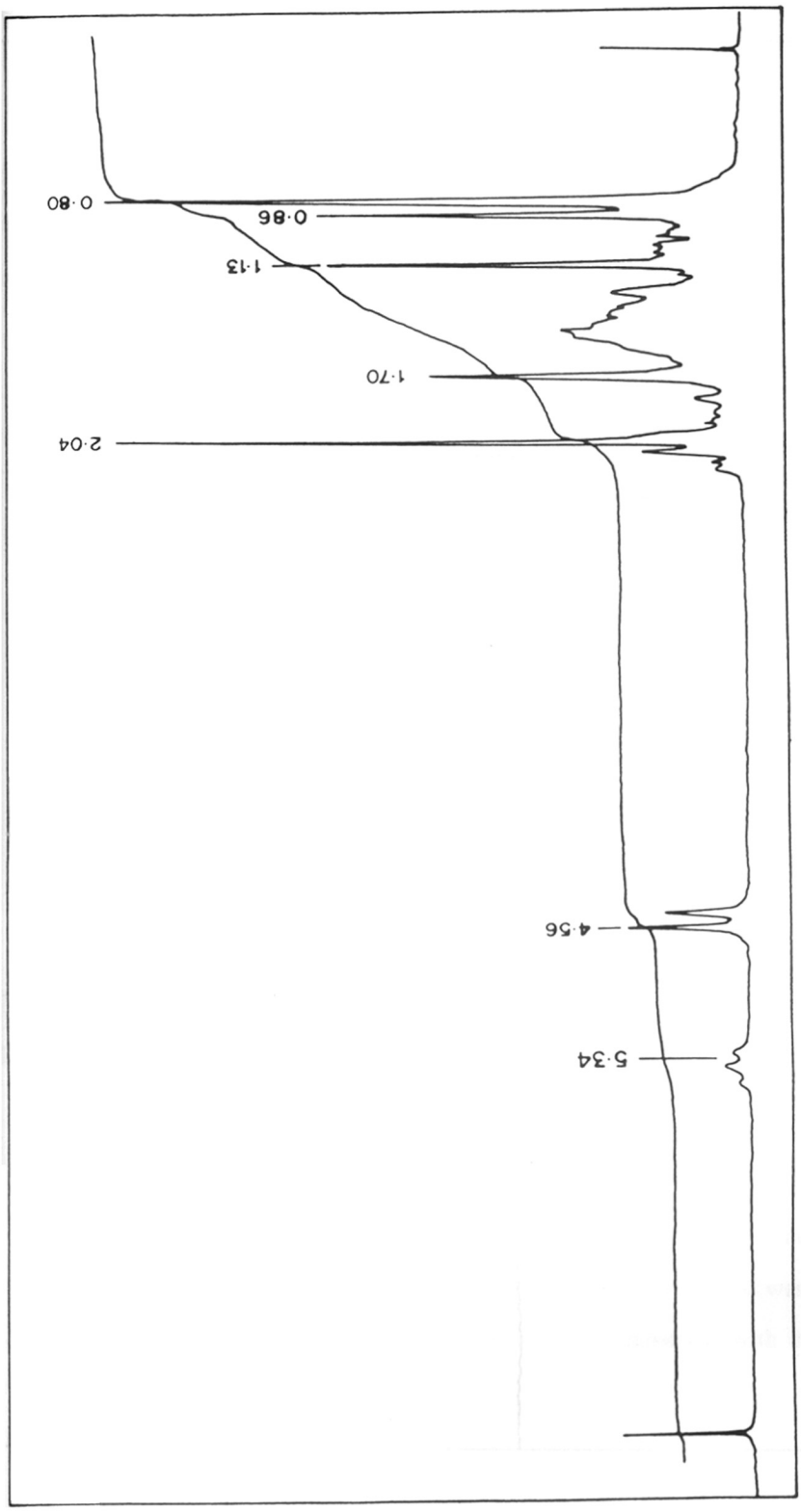


Fig.15 : ^{1}H-NMR spectrum of compound C-1a.

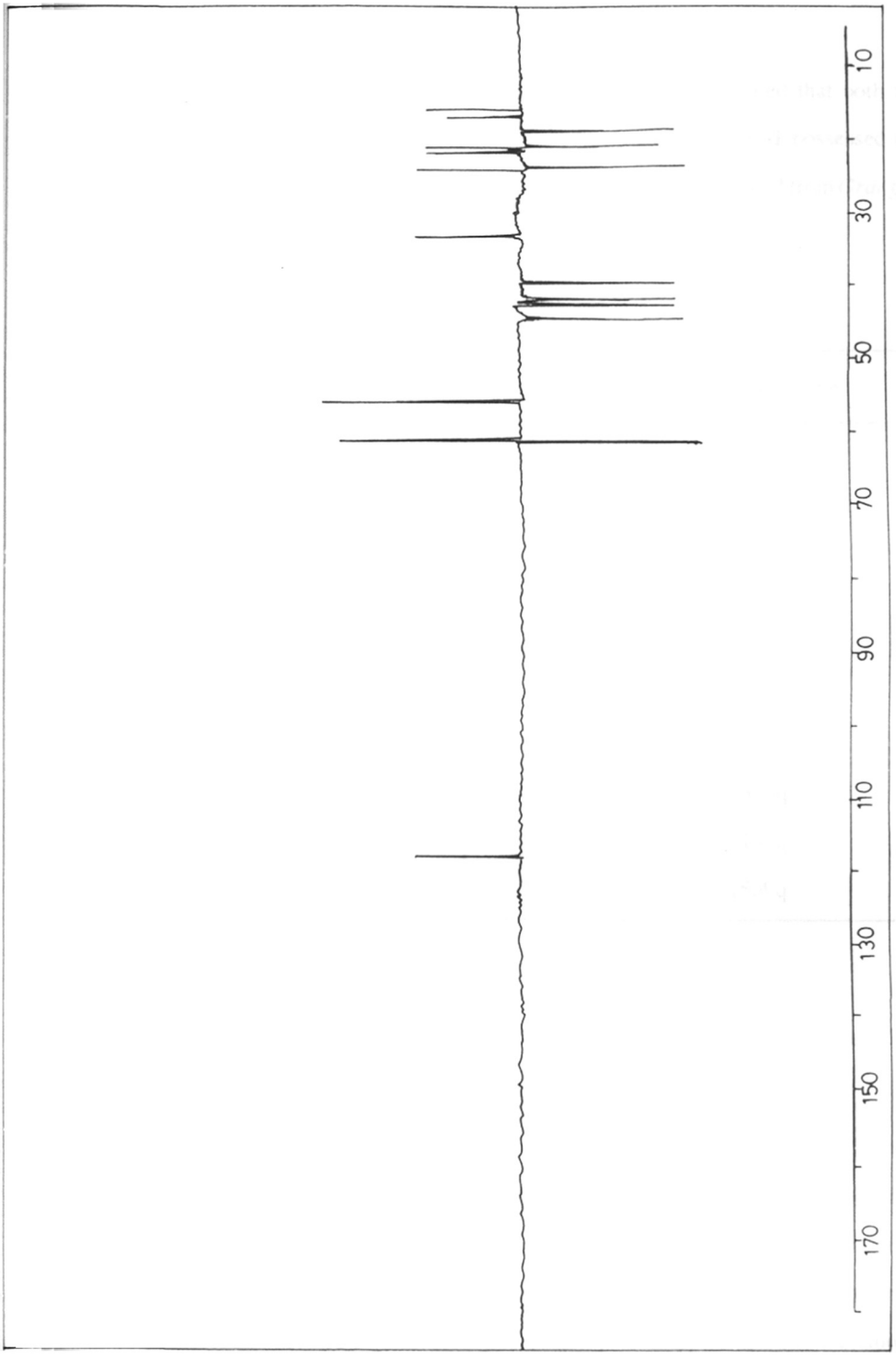


Fig.16: DEPT ^{13}C-NMR spectrum of compound C-1a.

The similarity in the ^{1}H and ^{13}C NMR spectra of **41** and **C-1a** revealed that both the compounds were identical [Table-3]. Thus, the parent hydroxy compound **C-1** possessed the structure 8-hydroxy-13E-labden-15-ol. **C-1** is the first labdane diterpenoid isolated from *Grangea maderaspatana*.

Table-3: ^{13}C-NMR of compound **C-1**

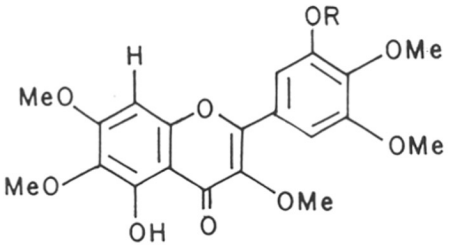
Carbon No.	Chemical Shift δ	Carbon No.	Chemical Shift δ
1	39.2 t	11	23.9 t
2	18.4 t	12	42.2 t
3	42.8 t	13	140.8 s
4	33.3 s	14	123.2 d
5	56.1 d	15	59.2 t
6	20.5 t	16	16.5 q
7	44.5 t	17	24.0 q
8	74.0 s	18	33.2 q
9	61.1 d	19	21.4 q
10	39.2 s	20	15.4 q

3.7.3 Characterization of C-2 to C-5

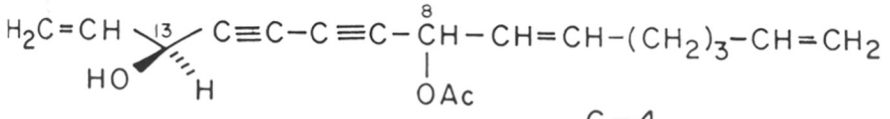
The compounds **C-2**, **C-3**, **C-4** and **C-5** were characterized based on their spectral data and were found to be known compounds.

From the ^{1}H-NMR spectra of compounds **C-2** and **C-3**, it was clear that both were polymethoxy flavonoids with five and six methoxy groups respectively. **C-2** was identified as 5,3'-dihydroxy-3,6,7,4',5'-pentamethoxy flavone based on comparison with the compound reported from *G.maderaspatana*[43a,b]. Compound C-3 in its ^{1}H-NMR spectrum exhibited two singlets at δ 6.4(1H) and 7.51(2H) assignable to H_8, $H_{2'}$, $H_{6'}$respectively, along with six methoxy

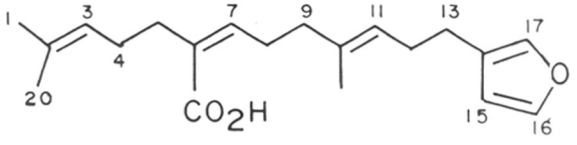
groups(Fig.17). The spectrum was found to be well in agreement with that of the flavonoid reported by Henrick and Jefferies[44] and structure for **C-3** was assigned as 5-hydroxy-3,6,7,3',4',5'-hexamethoxy flavone.(The same compound has been isolated from *G.maderas-patana* by Pandey and co-workers. However, the ^{1}H-NMR spectrum reported by the authors consisted of two doublets at δ 7.42 and 7.48 due to H_2 and H_6 and a singlet at δ 6.43 for H_8[43].) Compound **C-5** was identified as centipedic acid[43] while **C-4** as 8-Acetoxy-pentadeca-1,6(cis),14-triene-9,11 diyne-13-ol[43] based on the comparison of their ^{1}H-NMR and mass spectra.



C-2 R=H
C-3 R=Me



C-4



C-5

3.8 Biogenesis of labdane and pimarane diterpenoids

The proposed biogenetic path responsible for the biosynthesis of the abovementioned diterpene classes is depicted in the following schemes.

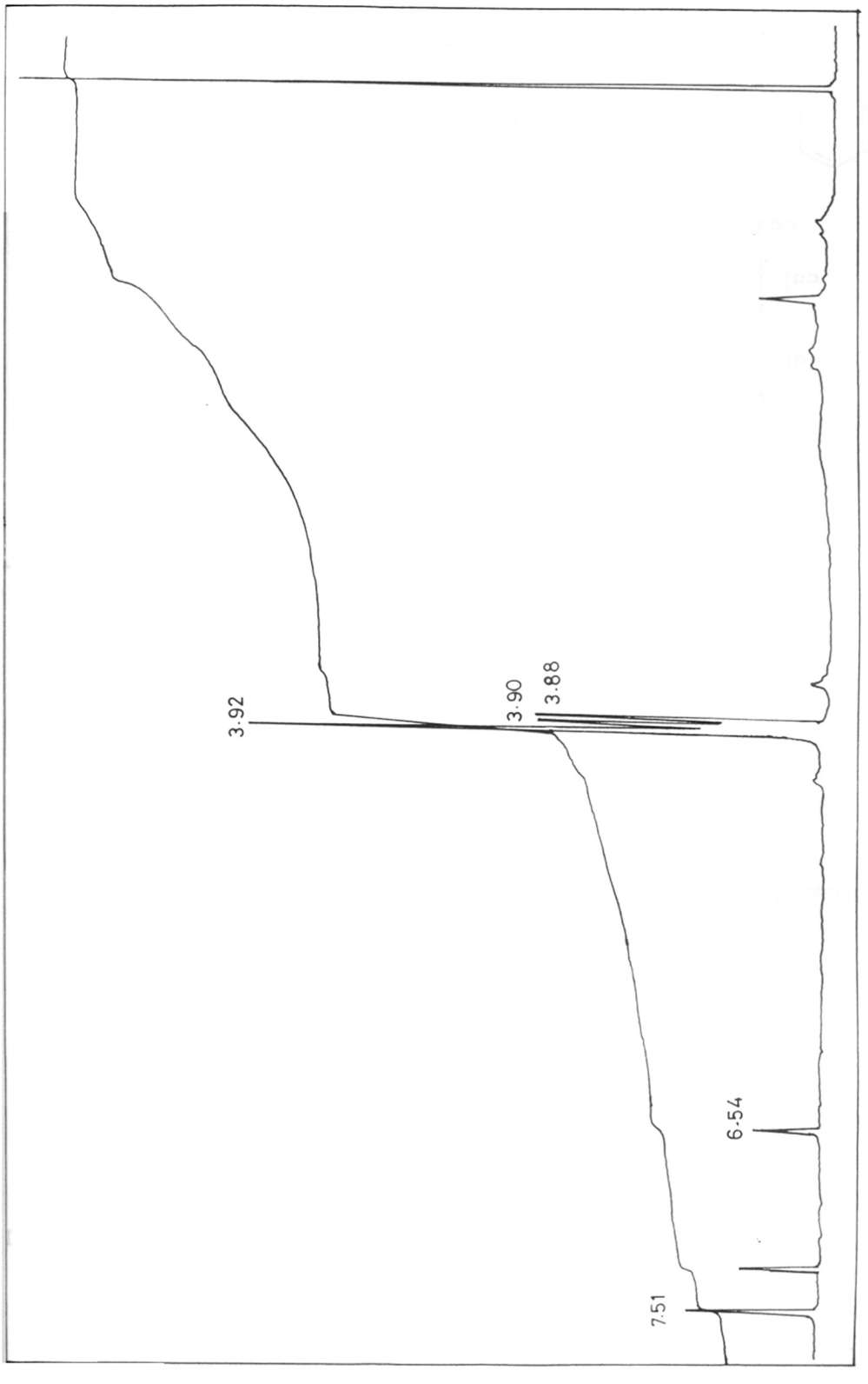
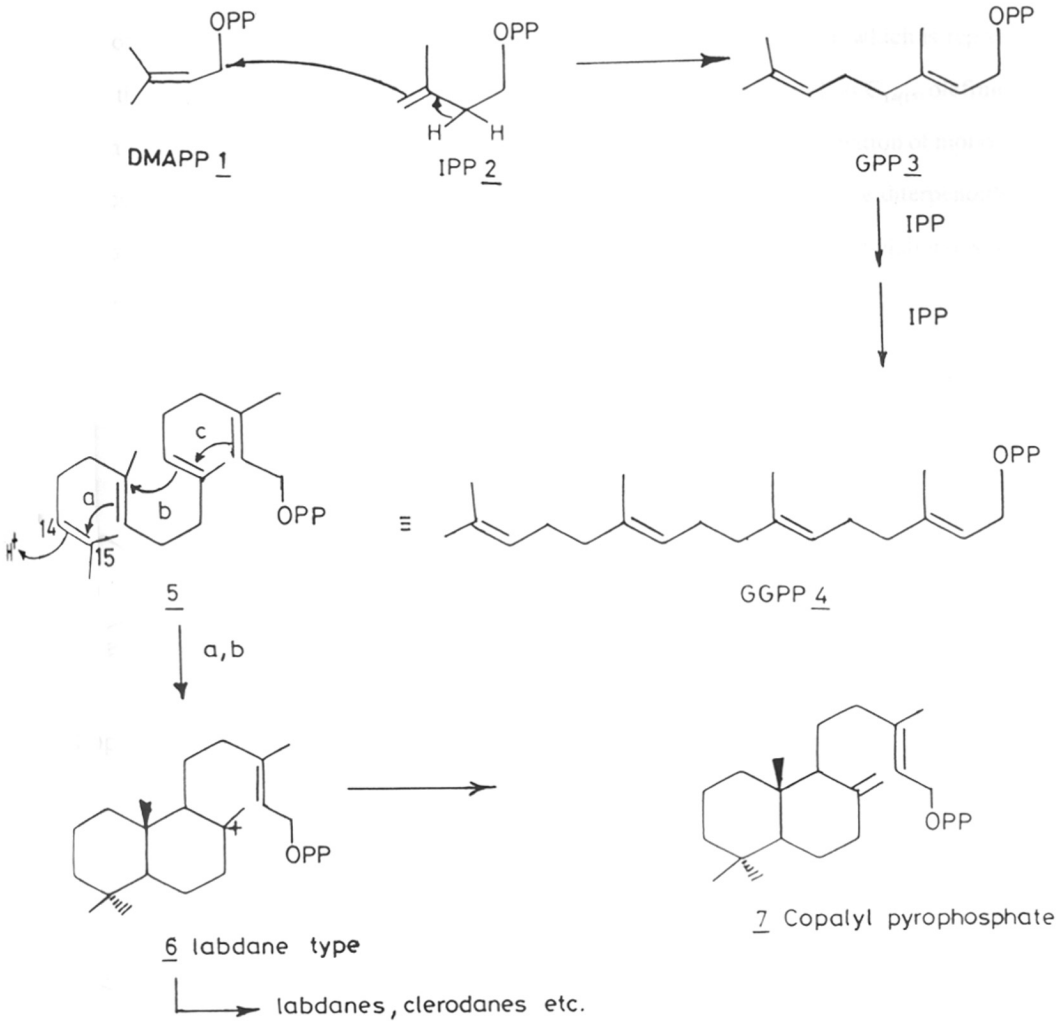


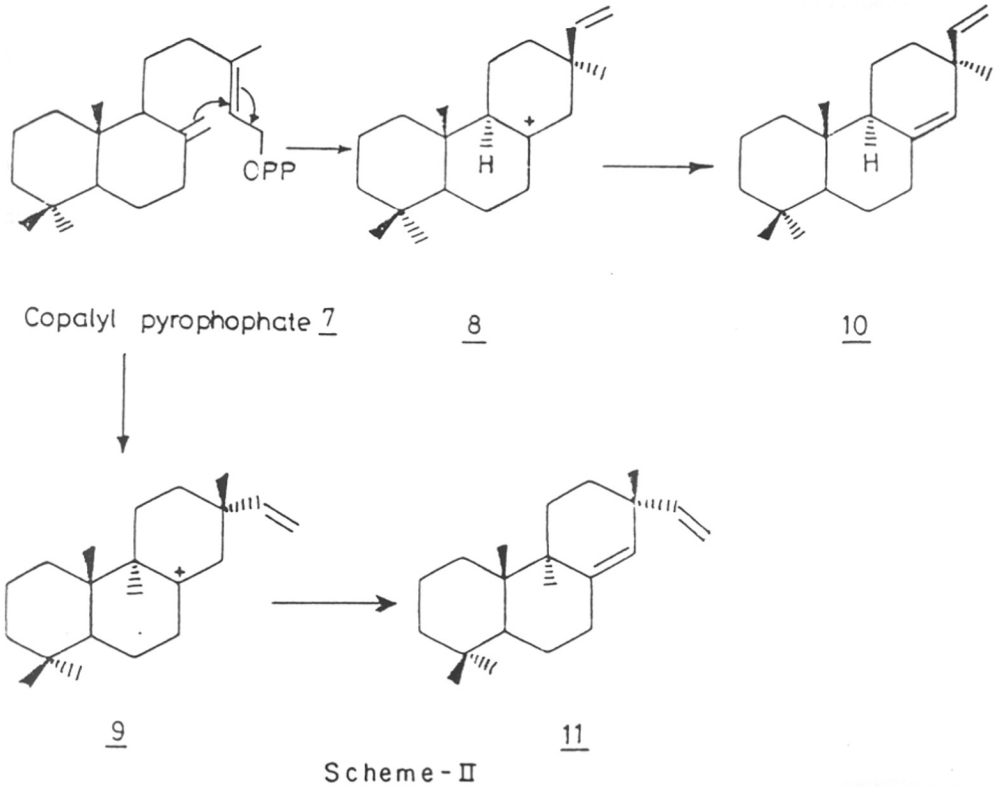
Fig. 17 : ^{1}H - NMR spectrum of compound C-3.



Scheme – I : Biogensis of diterpenoid skeleton.

As shown in Scheme-I, condensation of the two active forms of isoprene ($-C_5H_8$) unit; DMAPP **1** and IPP **2** in the head to tail fashion leads to Geranyl pyrophosphate, GPP **3**. Further

addition of two IPP units to **3** yields Geranyl geraniol pyrophosphate GGPP **4**, which is represented in the folded form as **5**. The attack of an electrophilic species, such as H^+ at $C_{14(15)}$ olefinic linkage in **5** can trigger the various cyclization processes responsible for the formation of mono-, bi- or tricyclic diterpenoids. One of such processes (a,b) leads to the labdane type diterpenoids **6**. The copalyl pyrophosphate **7** derived from the labdane structure, then further elaborates to various tri- and tetracyclic diterpenoid skeletons.



Scheme - II

Formation of pimaranes and pimaradienes from **7** is shown in Scheme-II. The copalyl pyrophosphate **7** which is an intermediate in the formation of pimaranes, undergoes cyclization probably proceeding *via* the ionization of the pyrophosphate moiety followed by the attack of

$C_{8(17)}$ double bond. In this process, a new asymmetric centre C_{13} is generated. As the attack of double bond on C_{13} can take place in both the directions, pimaranes of both the types with α- as well as β-oriented vinyl group **8, 9** occur in nature. The fact that naturally occurring pimaranes almost invariably possess either a hydroxyl or an unsaturation at C_8 supports the prediction of intermediacy of **8** or **9** in the biogenetic path.

In summary, the chapter includes chemical investigation of *P.wightiana* and *G.maderas-patana*. Structure elucidation of new pimarane diterpenoid, sandaracopimara-8(14),15-diene-7-keto-2α-ol (**B-1**) along with the known triterpenoids β-amyrin acetate **B-2** and lupenyl acetate **B-3** from *P.wightiana* is described. Isolation and characterization of a new labdane diterpenoid 8-hydroxy-13E-labden-15-ol (**C-1**) and four known compounds **C-2** to **C-5** from *G.maderas-patana* is also discussed. The structures of the new compounds have been elucidated based on their spectral properties.

3.9 Experimental

A : Isolation of compounds from ***P.wightiana***

Plant material

The plant *Pulicaria wightiana*, identified by authorities of the Botanical Survey of India, was collected from Pune during October 1988.

Extraction

The herb was shade dried, coarsely powdered and the powdered material (1.5 Kg) was extracted with acetone exhaustively by continuous extraction process. The test sample (100 mL) yielding negligible weight of the residue on evaporation of solvent determined the end point of extraction. The acetone extract was concentrated at reduced pressure to obtain a viscous mass (70.0 g.).

3.9.1 *Chromatographic separation*

The extract (65.0 g.) was subjected to column chromatography over silica gel (60-120 mesh, 1.6 Kg). The elution was started with petroleum ether and continued with the mixture of pet.ether and acetone with successive increase in the percentage of acetone. The separation was monitored by tlc. All fractions were concentrated separately and those showing similar tlc pattern were combined. Three major fractions, A(25.0 g.), B(10.0 g.) and C(27.0 g.) were collected. Details of the column chromatographic separation are summarized in Table-I.

3.9.2 *Rechromatography of fraction B*

The tlc examination of fraction B (10.0 g.) revealed that it was a complex mixture of five to six compounds with close Rf values. The fraction was, therefore, rechromatographed over silica gel (60-120 mesh, 300g.), using ethyl acetate, pet.ether as elution gradient. The elution started with ethyl acetate, pet.ether 15:85 and continued with increase in percentage of ethyl acetate. The details of the separation are given in Table-II. Fractions showing similar pattern on tlc were combined to obtain three broad fractions **I** (3.2g.), **II** (3.0g.) and **III** (3.8g.).

Table-I: Column chromatographic separation of acetone extract of *P.wightiana*

No.	Eluent Acetone : Pet.ether	Total volume collected	Final fraction	Net Wt. in gm.	Approximate Composition
1	15:85	8 x 500 mL	A	25.0	Compounds B-2, B-3, straight chain hydrocarbons, unidentified compounds
2	25:75	6 x 500 mL	B	10.0	Compound **B-1** and unidentified compounds
3	35:65 and MeOH	7 x 500 mL 2 x 500 mL	C	27.0	Unidentified compounds, acids and sugars

Table II: Column chromatographic separation of fraction B

No.	Eluent Ethyl Acetate : Pet.ether	Total volume collected	Final fraction	Net Wt. in gm.	Approximate Composition
1	10:90	4x250 mL	I	3.2	Unidentified compounds
2	22:78	5x250 mL	II	3.0	Compound **B-1** and complex mixture of terpenoids
3	35:65	4x250 mL	III	3.8	Unidentified alcohols and other compounds

3.9.3 *Isolation of Compound B-1*

Column purification and repeated preparative thin layer chromatography (ptlc) of fraction II (3.0g.) obtained from rechromatography of fraction B yielded compound **B-1** (19 mg). [Solvent system for ptlc, MeCN : $CHCl_3$, 2.5:15].

Compound **B-1**, light yellow crystalline solid, m.p. 155-157°C (MeOH), $[\alpha]_D$ - 9.09°, ($CHCl_3$; c 0.07).

UV, λ_{max} (MeOH): 251 nm (ε 2980).

IR (Fig.1), $CHCl_3$: cm^{-1} 3400, 1690, 1620.

^{1}**H-NMR** (Fig.3),(90 MHz): δ 0.87 (s, 3H), 0.91 (s, 6H), 1.09 (s, 3H), 3.91 (m, 1H), 4.93 (m,

2H), 5.82 (dd, J=10,18Hz, 1H), 6.71 (d, J=2Hz, 1H).

^{13}C-NMR (Fig.9),(75.48 MHz): Table-1.

MS (Fig.2), m/z (rel.int.): 302 ([M]$^+$, 100), 284(37), 274(7), 269(38), 202(62).

Acetylation of compound **B-1**

Compound **B-1** (10 mg) was mixed with acetic anhydride (0.2 mL), pyridine (0.2 mL) and the reaction mixture was left overnight at room temperature. Ether extraction of the reaction mixture and standard work up of the organic layer furnished compound **B-1a** (7 mg) as a gummy mass.

IR (Fig.4),$CHCl_3$: cm^{-1} 1730, 1682, 1216.

^{1}H-NMR (Fig.5),(80 MHz): δ 0.92 (s, 6H), 1.00 (s, 3H), 1.1 (s, 3H), 2.04 (s, OCOMe), 4.20 (m, 1H), 4.99 (m, 2H), 5.84 (dd, J=10,18Hz, 1H), 6.80 (d, J=2Hz, 1H).

MS m/z (rel.int.): 344 ([M]$^+$, 100), 329(15), 298(12), 284(75), 269(42), 202(38).

3.9.4 *Isolation of compounds B-2 and B-3*

Both the compounds were isolated by column chromatographic separation of fraction A (25.0 g.) using tlc grade silver nitrate impregnated silica-gel(100-200 mesh).

Compound **B-2**, (***β-amyrin acetate***): White crystalline solid, m.p. 237-238°C (lit. 241-242.5)[33]

^{1}H-NMR, (90 MHz): δ 0.86 (s, 3H), 0.91 (s, 12H), 0.97 (s, 6H), 1.14 (s, 3H), 2.10 (s, 3H, $CO\underline{CH_3}$), 4.59 (m, 1H), 5.24 (t, 1H).

Compound **B-3**, (***lupenyl acetate***): White crystalline solid, m.p. 216-218°C (lit. 218.5-219.5°C)[34b]

^{1}H-NMR, (90 MHz): δ 0.81 (s, 3H), 0.85 (s, 9H), 0.92 (s, 3H), 1.01 (s, 3H), 1.75 (br s, 3H), 2.09 (s, 3H, $CO\underline{CH_3}$), 4.65 (ill-defined d, 2H), 4.72 (m, 1H).

B : Isolation of compounds from G.maderaspatana

Plant material

The plant *G.maderaspatana* was collected during September 1987 near Lonavala, Maharashtra. The species was identified by the authorities of Maharashtra Association of Cultivation of Science, Pune.

Extraction

The aerial part of *G.maderaspatana* was shade dried and powdered. The powder (1.0 Kg) was extracted with acetone by continuous extraction process. End point of extraction was determined by the test sample (100 mL), giving negligible weight of residue on evaporation of solvent. Concentration of the extract at reduced pressure yielded a gummy mass (42.0 g).

3.9.5 Chromatographic separation

The acetone extract (39.0 g) of *G.maderaspatana* was column chromatographed over silica gel (60-120 mesh, 400g.). The eluent used was acetone/petroleum ether, polarity of which was increased successively by increasing the percentage of acetone. The chromatographic separation was monitored by tlc and the fractions were concentrated separately and combined based on tlc pattern. Six broad fractions, **A** (12.7 g.), **B** (7.4 g.), **C** (3.5 g.), **D** (5.0 g.), **E** (3.2 g.) and **F** (5.1 g.) were collected. Details of the separation are given in Table-III.

3.9.6 Rechromatography of Fraction **D**

From the tlc pattern, it was clear that fraction D was a complex mixture of terpenic compounds. Rechromatography of the fraction (5.0 g.) was therefore, carried out over silica gel (60-120 mesh, 155 g.). Details of the chromatographic separation are given in Table-IV.

Table III: Column chromatographic separation of acetone extract of *G.maderaspatana*

No.	Eluent Acetone Pet.ether	Total volume collected	Final fraction	Net Wt in gm.	Approximate Composition
1	5:95	6x250 mL	A	12.7	Straight chain hydrocarbons, Compounds **C-4**, **C-5** and unidentified compounds
2	15:85	4x250 mL	B	7.4	Unidentified compounds
3	25:75	3x250 mL	C	3.5	Complex mixture of terpenoids
4	30:70	5x250 mL	D	5.0	Compound **C-1** and mixture of unidentified compounds
5	35:65	4x250 mL	E	3.2	Compounds **C-2**, **C-3** and other unidentified compounds
6	45:55 and MeOH	4x250 mL 2x250 mL	F	5.1	Sugar glycosides, acids

Table IV: Column chromatographic separation of fraction **D**.

No.	Eluent Ethyl acetate: Pet.ether	Total volume collected	Final fraction	Net Wt.in gm.	Approximate Composition
1	15:85	3x250 mL	I	0.8	Complex mixture of terpenoids
2	24:76	4x250 mL	II	1.1	Mixture of three to four diterpenoids and **C-1**
3	30:70	4x250 mL	III	1.5	Unidentified compounds
4	38:62	4x250 mL	IV	1.6	Complex mixture of unidentified terpenes

The elution starting with ethyl acetate / pet.ether (15:85) mixture was continued with the successive increase in polarity of eluent. Four fractions **I** (0.8g.), **II** (1.1g.), **III** (1.5g.) and **IV** (1.6g.) were obtained.

3.9.7 *Isolation of compound* **C-1**

Fraction II (1.1g.) was subjected to ptlc with EtOAc:Benzene:Pet.ether (30:35:35) system. Compound **C-1** (65 mg) was obtained as a viscous oil.

IR (Fig.11), $CHCl_3$: cm^{-1} 3510, 1660, 1400, 780.

^{1}H-NMR (Fig.12), (300 MHz): δ 0.80 (s, 6H), 0.90 (s, 3H), 1.14 (s, 3H), 4.14 (d, J=6.8Hz, 2H), 5.42 (br t, J=6.8Hz, 1H).

^{13}C-NMR (Fig.13), (75.48 MHz): Table-3.

MS m/z (rel.int.): 308 $[M]^+$ (1), 290(5), 275(4), 192(55), 177(80), 109(60), 95(85), 81(100), 69(91).

Acetylation of compound **C-1**

Compound **C-1** (25 mg) was dissolved in pyridine (2.5 mL) and acetic anhydride (2.5 mL) and the mixture was left overnight at room temperature. Routine work-up yielded the acetate **C-1a** as an oil.

IR (Fig.14), $CHCl_3$: cm^{-1} 3470, 1745, 1650, 1240.

^{1}H-NMR (Fig.15), (90 MHz): δ 0.80 (s, 6H), 0.86 (s, 3H), 1.13 (s, 3H), 1.70 (br s, 3H), 4.56 (d, J=6.8Hz, 2H), 5.34 (br t, J=6.8Hz, 1H) and 2.04 (s, 1H, $OCO\underline{CH_3}$).

^{13}C-NMR (Fig.16), (75.48 MHz): δ 39.1 (t, C_1), 18.4 (t, C_2) 42.7 (t, C_3), 33.3 (s, C_4), 56.0 (d, C_5), 20.4 (t, C_6), 44.6 (t, C_7), 73.9 (s, C_8), 61.2 (d, C_9), 39.7 (s, C_{10}), 23.7 (t, C_{11}), 41.9 (t, C_{12}), 143.4 (s, C_{13}), 118.0 (d, C_{14}), 61.3 (t, C_{15}), 16.5 (q, C_{16}), 23.3 (q, C_{17}), 33.1(q,C_{18}), 21.4(q,C_{19}), 15.4 (q, C_{20}), 171.0 ($O\underline{CO}CH_3$), 20.9 (q, $OCO\underline{CH_3}$).

MS m/z (rel.int.): 350 $[M]^+$ (1), 290(17), 272(21), 257(20), 204(22), 192(72), 177(100), 137(23), 123(24), 109(22), 95(16), 81(12).

3.9.8 *Isolation of compounds* **C-2** to **C-5**

Compounds **C-2** and **C-3** were obtained by column chromatographic separation of fraction E (3.0 g.) [Silica gel 60-120 mesh, 90g.] of the total extract of *G.maderaspatana*.

Compound **C-2**, (*5,3'-dihydroxy-3,6,7,4',5'-pentamethoxy flavone*): Yellow crystals,(35 mg), m.p.206-208°C (lit.[45] 217-218°C).

^{1}H-NMR (90 MHz): δ 3.87 (s, 3H), 3.89 (s, 3H), 3.91 (s, 6H), 3.98 (s, 3H), 6.4 (s, 1H), 7.43 (dd, 2H).

Compound **C-3**, (*5-hydroxy-3,6,7,3',4',5'-hexamethoxy flavone*): Yellow crystals,(50 mg), m.p.172-174°C (lit.[45] 176-178°C).

^{1}H-NMR (Fig.17), (90 MHz): δ 3.87 (s, 3H), 3.92 (s, 3H), 3.92 (s, 12H), 6.4 (s, 1H), 7.51 (s, 2H).

Compounds **C-4** and **C-5** were isolated from the column chromatography of fraction **A** (12.0 g.) of the total extract of *G.maderaspatana*.

Compound **C-4**, (*centipedic acid*)[43b]:

^{1}H-NMR (200 MHz): δ 1.62 (br s, 6H, H_1, H_{18}), 5.12 (m, 1H, H_3), 6.05 (br t, 1H, H_7), 5.24 (m, 1H, H_{11}), 6.27 (br s, 1H, furan proton, H_{15}), 7.18-7.34 (m, 2H, furan protons, H_{16}, H_{17}), 1.70 (br s, 3H, H_{20}).

Compound **C-5**, (*8-Acetoxy-pentadeca-1,6-(cis), 14-triene-9,11-diyne-13-ol*)[43a]:

^{1}H-NMR (90 MHz): δ 5.22 (m, H_1 *cis*), 5.50 (m, H_1 *trans*), 5.95 (m, H_2), 4.89 (br d, H_3), 6.15 (br d, H_8), 5.45 (m, H_9), 5.62 (m, H_{10}), 5.78 (m, H_{14}), 5.01 (m, H_{15}), 2.05 (s, 3H, OCO$\underline{CH_3}$).

MS (m/z, rel.int.): 272(10), 255(2), 217(5), 203(6), 175(8), 161(20), 157(30).

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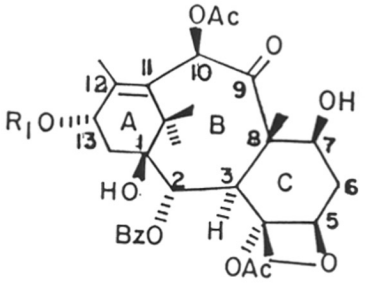
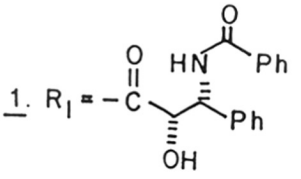
CHAPTER IV

Asymmetric Synthesis of B-seco Taxanoids from α-Pinene

Taxol **1**, a complex, highly functionalized diterpenoid is currently the most exciting anticancer drug exhibiting activity against breast and ovarian cancer[1]. The term, B-seco-taxanoid encompasses the compounds possessing the carbon framework similar to taxol, with central cyclooctane B-ring opened structures.





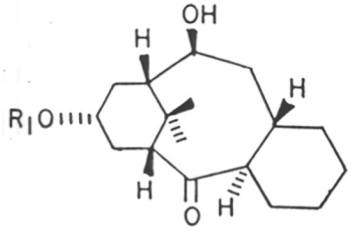
4.1 Taxol: Historical background

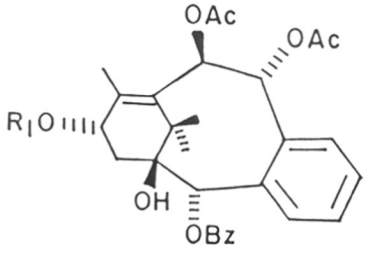
The bark of pacific yew tree, *Taxus brevifolia* from Oregon forest was collected in 1962 by the National Cancer Institute, USA, as a part of the programme of exploring new potential anticancer agents. Initial screening of the crude alcohol extract of the stem bark exhibited strong cytotoxicity. This remarkable activity led to the isolation of the active component, Taxol after almost a decade. The novel structure of this compound, present as a minor constituent in the species, was elucidated in 1971 by X-ray crystallography[2]. Between 1960 and 1981, more than 1,10,000 compounds from 35,000 plant species were screened for biological activity and taxol proved to be the most potent amongst them[3]. Nevertheless, further investigation of taxol languished for almost ten years mainly for three reasons; (i) difficulties in extraction, (ii) problems associated with the screening systems and (iii) the notion that taxol would be another microtubule destabilizing agent similar to the known anticancer drugs. In 1979, work carried out by Horwitz and co-workers brought out the unique mode of action of taxol on the cell system[4]. Unlike the other antitumor drugs such as colchicine and vinca alkaloids which act by microtubule destabilization, taxol interacts with microtubules in a manner that catalyzes their formation from

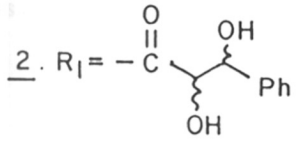
tubulin and stabilizes the resulting structures. The microtubules thus form stable bundles making the cell unable to assemble a normal mitotic spindle[5]. This discovery of the mechanism of action of taxol gave momentum to the research on taxol, a highly valuable drug in cancer therapeutics.

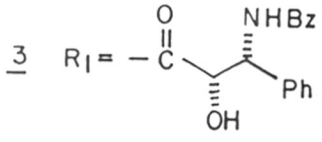
4.2 Previous work on taxol

The use of such a promising medicine is, however, hampered by its limited availability as a natural product. At present, isolable quantity of taxol can only be obtained by a tedious extraction of bark of very slow growing species *T. brevifolia*, where it is present in very small amounts (0.01%). The isolation process requires removal of bark which is fatal to the tree. Such a harvest of the trees endangers the old growth forests and consequently causes a threat to the eco-system. To meet the increasing demands for taxol, alternative sources for the drug utilizing biosynthetic, cell culture, botanical, semi-synthetic and total synthetic approaches[6] have been investigated thoroughly in the last ten years. Recently, two groups headed by Nicolaou[7] and Holton[8] have published the total synthesis of this novel anticancer drug. However, due to the large number of steps involving the use of highly specific reagents, the synthesis does not seem to be economically viable. On the other hand, during the process of development of simpler synthetic analogues of taxol, the compounds such as **2** and **3** [Scheme-1] independently prepared by Bletchert,[9] Nicolaou[10] have been found to exhibit antitumor properties.





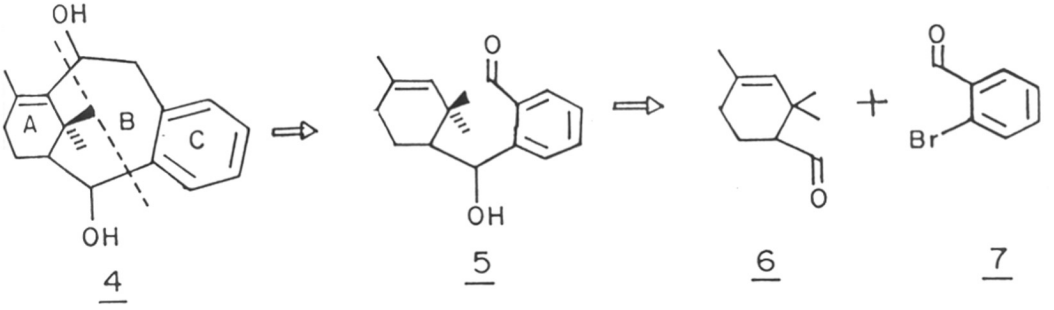




Scheme-1

One of the diastereomers of compound **2** lacking some characteristic features of taxol such as the oxetane D-ring, oxygenation at C_7 and C_9, etc. is reported to inhibit the depolymerization of tubulin when subjected to an *in-vitro* tubulin test[9]. It is thus the first taxol analogue possessing activity similar to taxol. Compound **3** with C-aromatic taxol framework exhibited significant cytotoxicity against a variety of tumor cell lines[10]. These compounds **2** and **3** therefore, hold a great promise as potential anti-tumor drugs. Their syntheses consequently have set a trend for concentrated efforts in the area of developing taxanoid frameworks or simplified taxane analogues. These compounds would be more readily available than taxol circumventing the problem of its limited resources and, at the same time would retain its interesting biological activity.

Considering the biological activity exhibited by the C-aromatic taxanoid **3**, we planned a convergent strategy for the synthesis of C-aromatic taxol framework **4**. The retrosynthetic analysis forming the basis of the strategy is depicted in Scheme-2



Scheme-2

The present chapter describes a novel and efficient approach towards synthesis of B-seco,C-aromatic taxanoids. Use is made of the abundantly available natural product α-pinene, as a starting material for development of the A-ring subunit.

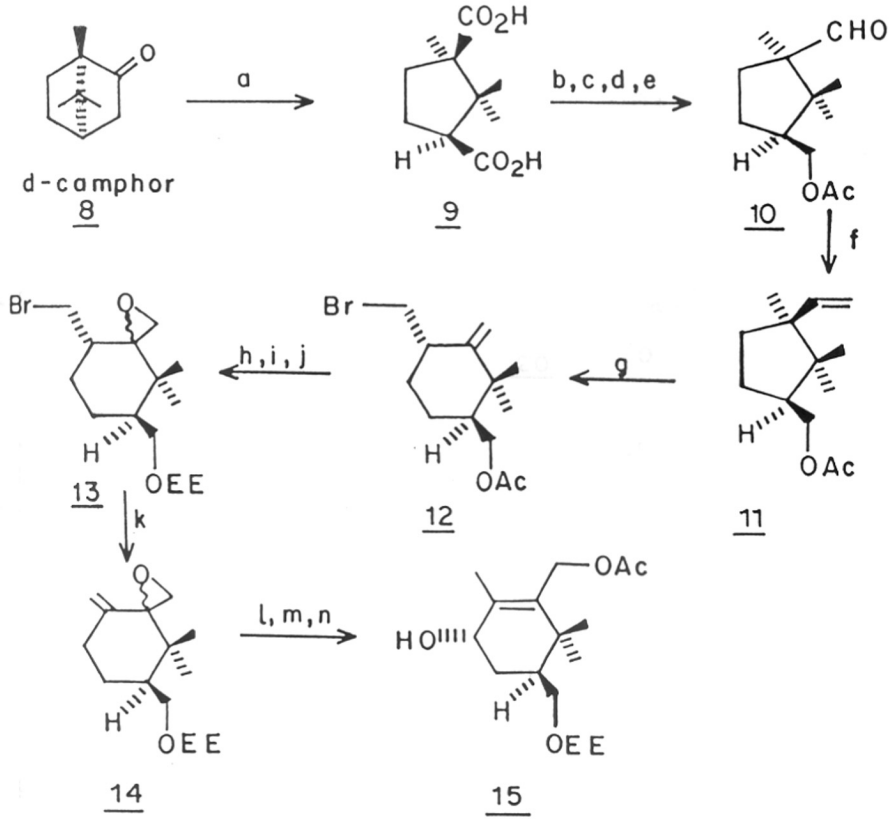
α-Pinene is the most widely distributed natural monoterpene. It occurs in various species of pines. It is also a major component of industrial solvents such as turpentine. It consists of ten carbon atoms out of twenty required in the taxol framework and is available naturally in both the enantiomeric forms. It also contains an inbuilt gem-dimethyl group which is a characteristic feature of **1**. All these facts make α-Pinene an attractive starting material which on suitable transformations would lead to the A-ring equivalent **6** in the synthesis of taxol framework. As given in Scheme-2, *ortho*-bromobenzaldehyde would form the C-ring equivalent.

A large number of approaches for the synthesis of A-ring unit have been reported in the literature[11]. However, very few of them are devoted towards their chiral syntheses. A brief summary of these chiral syntheses indicating some of their salient features is presented in the following Schemes 3, 4, 5, 6, 7 and 8. Scheme-8 describes the approach suggested by Wender *et al.* The Wender's group was the first one to use (+) α-Pinene **43** as the starting material for synthesis of C-aromatic taxanoids[17a].

In this scheme, the air oxidation product of α-Pinene, verbenone **44**[17b] on reaction with the dibromide **45** yielded the C-alkylated verbenone **46**. The photochemical conversion of **46** to **47** based on the well known 1,3-alkyl shift of verbenone giving chrysanthenone[18] is the key step in the strategy.

The scheme brought out the shortest route for developing a suitably functionalized A-ring structure of taxol molecule from α-Pinene. However, the photochemical step in the scheme demanded for a particular chromophoric system inherently reducing the flexibility. This in turn restricted the versatility of the scheme in the preparation of a wide variety of taxol analogues. We, therefore, planned our strategy for developing the B-seco taxanoid skeleton avoiding the photochemical reaction. The retrosynthetic analysis has already been given in Scheme-2.

1. Kitagawa et. al.[12] (1984)



Reagents: (a) HNO_3, Hg_2SO_4; (b) CH_2N_2; (c) $LiAlH_4$; (d) Ac_2O, AcONa; (e) PCC, CH_2Cl_2; (f) Ph_3PCH_3Br, NaH, DMSO; (g) TBCO; (h) KOH-MeOH; (i) CH_2=CHOEt, p-TsOH.H_2O; (j) MCPBA, CH_2Cl_2; (k) tert.AmONa, THF-DMSO; (l) Na/liq. NH_3; (m) Ac_2O, pyridine; (n) SeO_2, dioxane, pyridine

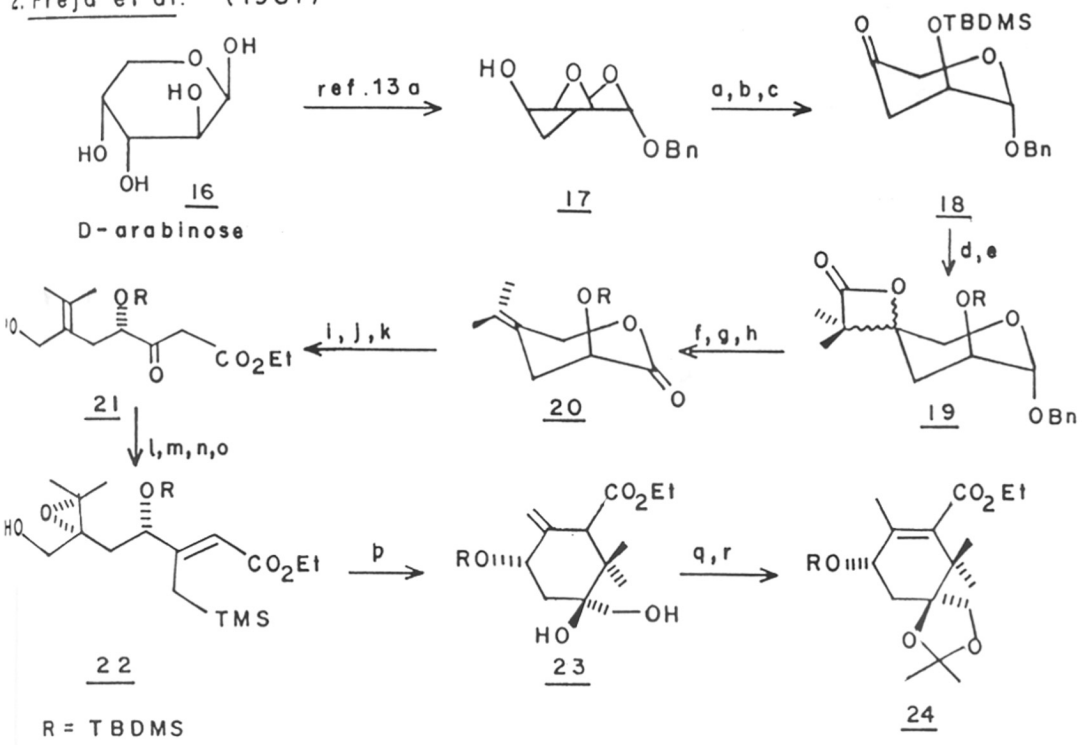
EE = ethoxyethyl, tert.AmONa = sodium tertiary amylate.

Scheme – 3

Number of steps : Sixteen

Key step : ring enlargement of **11** using TBCO.

2. Frejd et al.[13] (1987)



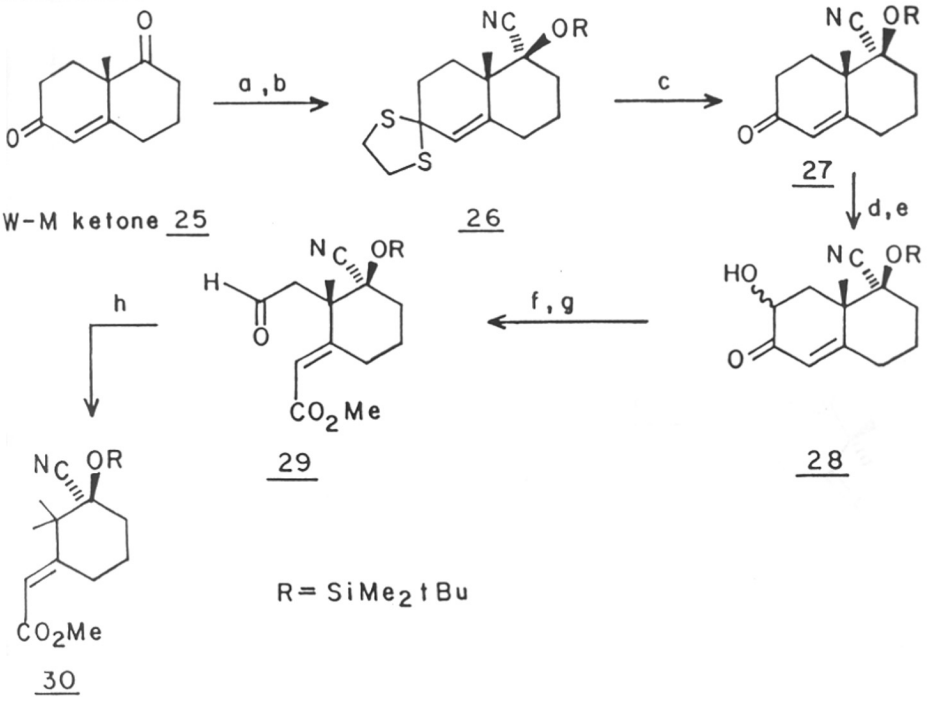
Reagents: (a) DMSO, $(COCl_2)_2$, NEt_3; (b) NaI, acetone, HOAc, NaOAc; (c) TBDMS-Cl, imid.; (d) LDA, isobutyric acid; (e) $PhSO_2Cl$, pyridine; (f) Pd-C, H_2, HOAc; (g) PDC, Ac_2O, CH_2Cl_2; (h) 170°C; (i) $Ti(OiPr)_4$; (j) DHP, pyridinium tosylate; (k) $(TMS)_2NLi$; TMEDA, EtOAc; (l) KOtBu, $ClPO(OEt)_2$; (m) $TMSCH_2MgCl$, 5% $Ni(acac)_2$; (n) pyridinium tosylate, iPrOH; (o) $Ti(OiPr)_4$, (-)DET, TBHP; (p) $BF_3.OEt_2$, CH_2Cl_2, 0°C; (q) $BF_3.OEt_2$, acetone, CH_2Cl_2; (r) DBU, 185°C.

Scheme-4

Number of steps : > Twenty

Key step : Lewis acid promoted cyclization of **22**.

3. Watt et. al.[14] (1993)



Reagents: (a) $HSCH_2CH_2SH$, p-TsOH, AcOH; (b) TBSCN, ZnI_2, CH_2Cl_2; (c) $Tl(NO_3)_2$, aq. MeOH, $CHCl_3$, THF; (d) $Pb(OAc)_4$, benzene, reflux; (e) K_2CO_3, MeOH; (f) $NaIO_4$, aq. t-BuOH; (g) CH_2N_2; (h) $RhCl(PPh_3)_3$, benzene.

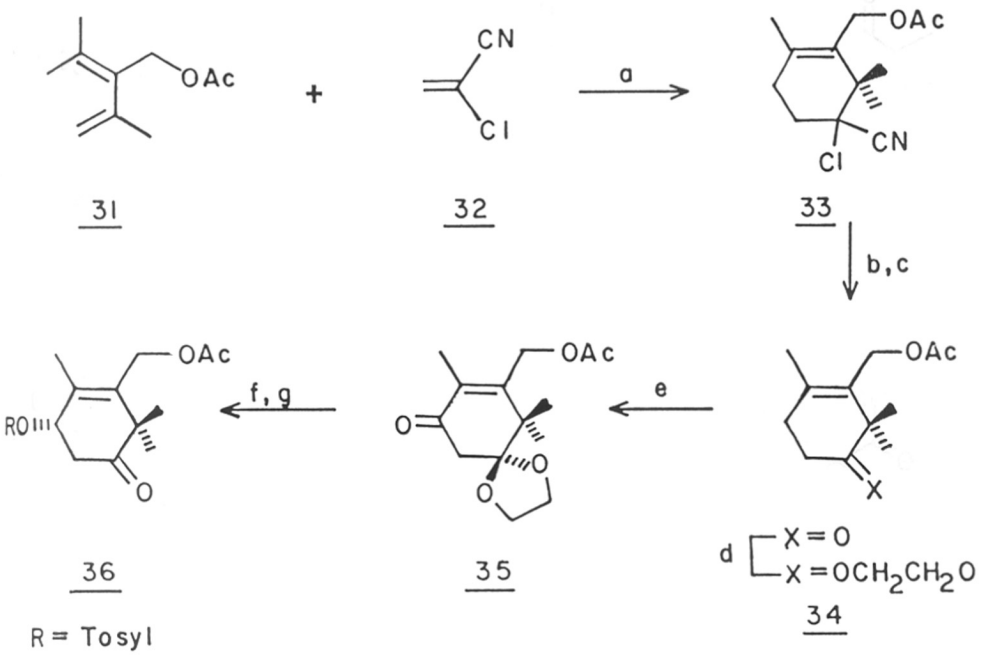
Scheme - 5

Costly starting material

Number of steps : Nine

Key step : α-hydroxylation leading to **28** and its successive periodate cleavage.

4. Nicolaou et.al.[15] (1992)



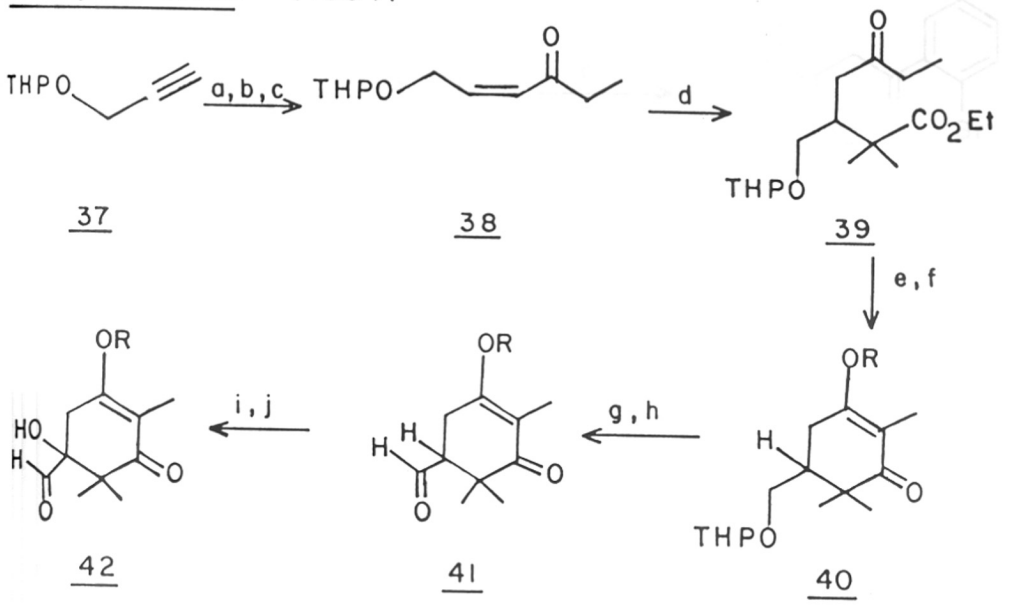
Reagents: (a) 135°C, 96 h.; (b) KOH, t-BuOH, 70°C; (c) Ac_2O, DMAP, CH_2Cl_2; (d) ethylene glycol, CSA, benzene, 70°C; (e) SeO_2, 1,4-dioxane, 100° then PCC, 4A° molecular sieves, CH_2Cl_2; (f) Corey's (R)-Oxazaborolidine, catecholborane, toluene, -78° to 0°C; (g) TsOH, acetone-H_2O.

Scheme – 6

Number of steps : Seven

Key step : Diels Alder reaction of **31** and **32**.

i. Kuwajima et. al.[16] (1994)



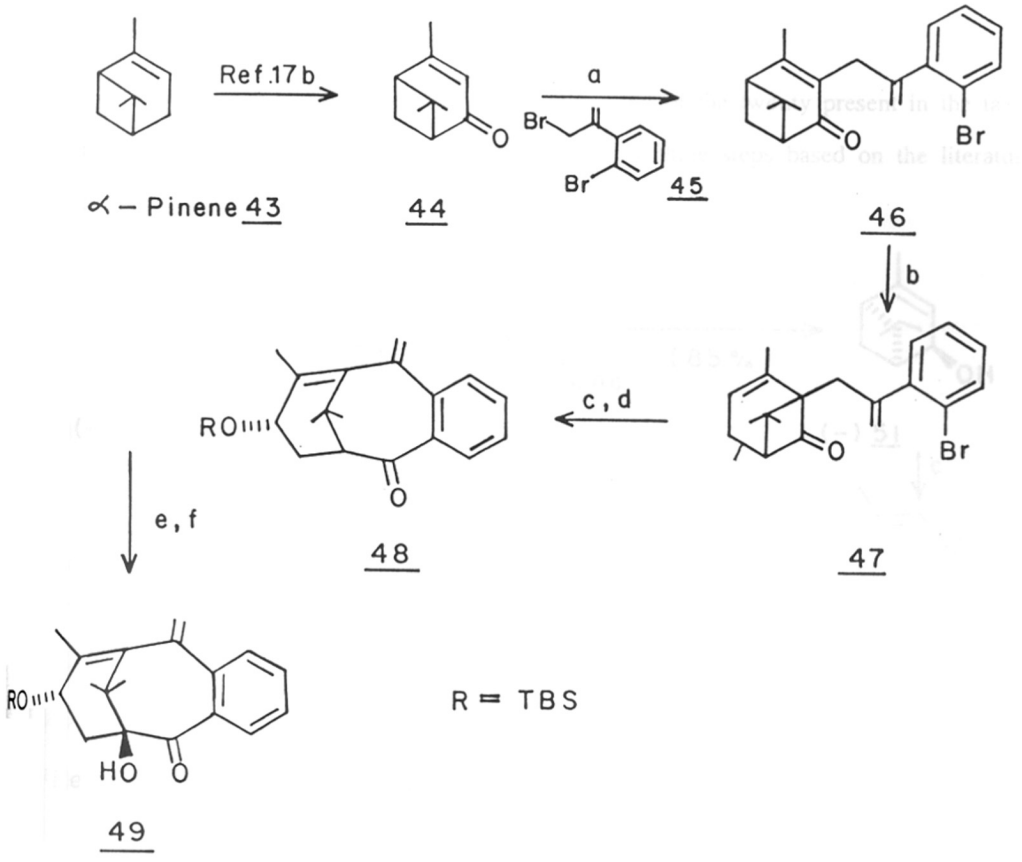
Reagents: (a) n-BuLi, CH_3CH_2CHO; (b) H_2/lindlar catalyst; (c) $(COCl_2)_2$, DMSO, NEt_3; (d) >=<$^{OEt}_{OEt}$, (e) t-BuOK; (f) Ac_2O, Et_3N; (g) Cat. p-TsOH; (h) $(COCl_2)_2$, DMSO, NEt_3; (i) R_3Si-OTf, DBU-DMAP (R_3Si=TBS); (j) OsO_4, L*, $K_3Fe(CN)_6/K_2CO_3$, t-BuOH/H_2O, (L*=DHQ-PHN, chiral ligand).

Scheme – 7

Number of steps : Ten

Key step : Diekmann condensation of **39**.

6. Wender et. al.[17] (1992)



Reagents: (a) KOtBu, DME; (b) hv, C_6H_{12}; (c) t-BuLi, TMEDA, THF; (d) i. $Ti(OiPr)_4$, t-BuOOH, CH_2Cl_2, ii. DABCO, CH_3CN; (e) TBSCl, imid., DMF; (f) KOtBu, O_2, DMSO, THF.

Scheme – 8

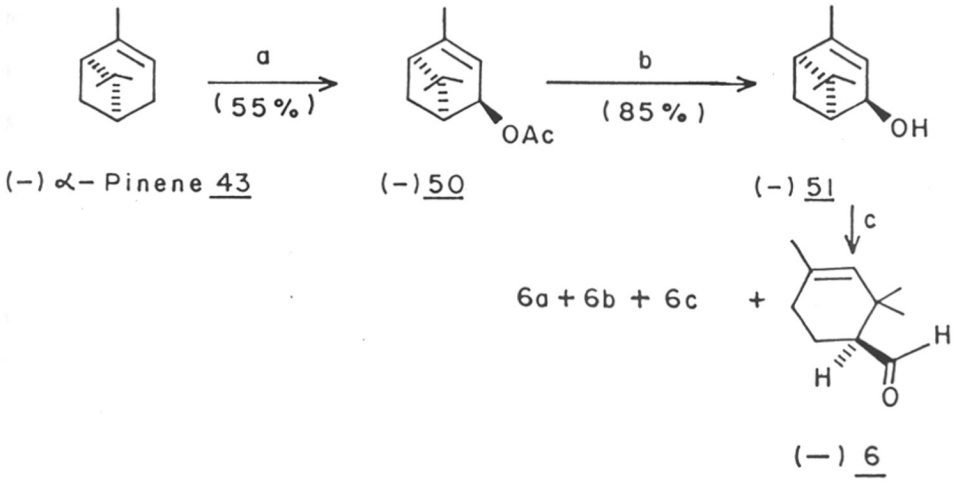
Starting material inexpensive

Number of steps : Seven for total framework of taxol.

Key step : Photochemical transformation of **46** to **47**.

4.3 Present Work

The synthon **6** consisting of ten carbon atoms out of the twenty present in the taxol framework was synthesized from α-Pinene **43** in only three steps based on the literature reports[19,20]. [Scheme-9].



Reagents: (a) $Pb(OAc)_4$, benzene; (b) KOH, MeOH, 5°C; (c) Δ, 350°C.

Scheme - 9

4.3.1 The salient features of Scheme-9

a) Use of abundantly available, inexpensive starting material, α-Pinene

b) The natural occurrence of α-Pinene in both the enantiomeric forms : an added advantage.

c) Short and convenient synthesis of large quantities of aldehyde **6** required for further reaction sequences, as the main step in the synthesis is the thermolytic rearrangement.

d) The shortest and practical route for developing the chiral A-ring equivalent of taxol framework.

e) Elaboration of compound **6** possessing two active functional groups (an olefin and a carbonyl) possible in a number of different ways leading to various taxane analogues.

It may first be pointed out that between the two enantiomeric forms of α-pinene, the (+)-(1R,5R)-α-pinene is necessary in order to generate the taxanoid possessing absolute stereochemistry at C_1 identical to that of the naturally occurring taxol **1**. Since the desired isomer was not available to us, the readily available one viz. (-)-(1S,5S)-α-pinene was employed in the present study.

4.3.2 Preparation of (-)-(S)-2,2,4-trimethyl-3-cyclohexene-1-carbaldehyde, **6**

The (-)-(1S,5S)-α-pinene **43** on oxidation with lead tetraacetate (LTA) gave the trans-verbenyl acetate **50**, which on hydrolysis with KOH in methanol yielded the *trans*-verbenol **51**[19] in 47% overall yield. Compounds **50** and **51** were identified based on the comparison of spectral data with that of reported[19]. *Trans*-verbenol **51** was subjected to thermolysis[20] at varied temperatures ranging from 300°C to 370°C. The optimum temperature for pyrolysis was fixed as 350°C based on the maximum formation of aldehyde **6** (38%) as shown by the GC analysis of crude pyrolysates (See Table-1).

Table-1: Pyrolysis of *trans*-verbenol **51** giving **6**

		GLC Pattern (RRT)*, %								
Expt	Temp (°C)	**6** (6.4)	**51** (6.9)	(8.1-8.3)	(8.8-9.0)	(9.5-9.6)	(10.2-10.3)	Other peaks		
1	300	--	72.0	4.4	2.0	3.6	3.8	--	--	--
2	320	5.5	67.9	3.2	2.9	3.5	2.6	--	--	--
3	330	28.0	22.5	11.8	2.6	9.0	--	8.5	--	--
4	350	38.0	3.5	14.4	11.1	3.5	--	9.5	3.4	--
5	370	37.2	3.8	12	15.0	8.6	5	4.4	2.0	9.0

*The numbers in brackets denote RRTs

The required aldehyde **6** was obtained by chromatographic separation of the pyrolyzed material and was fully characterized. Its IR spectrum (Fig.1) showed the presence of carbonyl group (1720 cm^{-1}), the olefinic moiety (1670 cm^{-1}), while the characteristic band of an aldehyde due to HC=0 stretching was observed at 2710 cm^{-1}. The ^{1}H-NMR spectrum of **6** (Fig.2) exhibited three singlets at δ 0.97, 1.13 and 1.68 each integrating for three protons due to three methyl groups. The multiplet due to olefinic proton was observed at δ 5.08 and a doublet for aldehyde proton appeared at δ 9.80. The chemical shift values were well in agreement with those reported in literature[20].

The chromatographic separation of the non-aldehydic fraction of the pyrolysate was also attempted. The work was carried out with a view to isolate and characterize the intermediates formed during the pyrolysis reaction which could be of mechanistic as well as synthetic value. A careful analysis of GLC pattern of the pyrolysate revealed that it contained three major components appearing as closely spaced spots on the tlc plate. Based upon the increasing order of the relative retention times (RRTs) in GLC, these side products have been indicated as 6a, b and c in Scheme-9. Out of the three, only **6b** was isolated with 80% purity (GLC).

Characterization of **6b**

The IR spectrum of **6b** (Fig.3) indicated the presence of hydroxyl (3430 cm^{-1}) as well as olefinic (1660 cm^{-1}) groups. Compound **6b** in its ^{1}H-NMR spectrum (Fig.4) exhibited two singlets at δ 1.67 (3H) and 1.71 (3H) suggesting the presence of two vinylic methyl groups. The multiplet at δ 4.85 (2H) could be assigned for an exo-methylene group in **6b**. The other two multiplets at δ 5.43 (1H) and 4.10 (1H) were concluded to be due to the olefinic proton and the carbinyl proton, CHOH respectively. The literature survey revealed that ^{1}H-NMR spectrum of **6b** showed a close resemblance to that of limonen-5-ol **52**[21a,b] based on which the structure for **6b** was proposed.

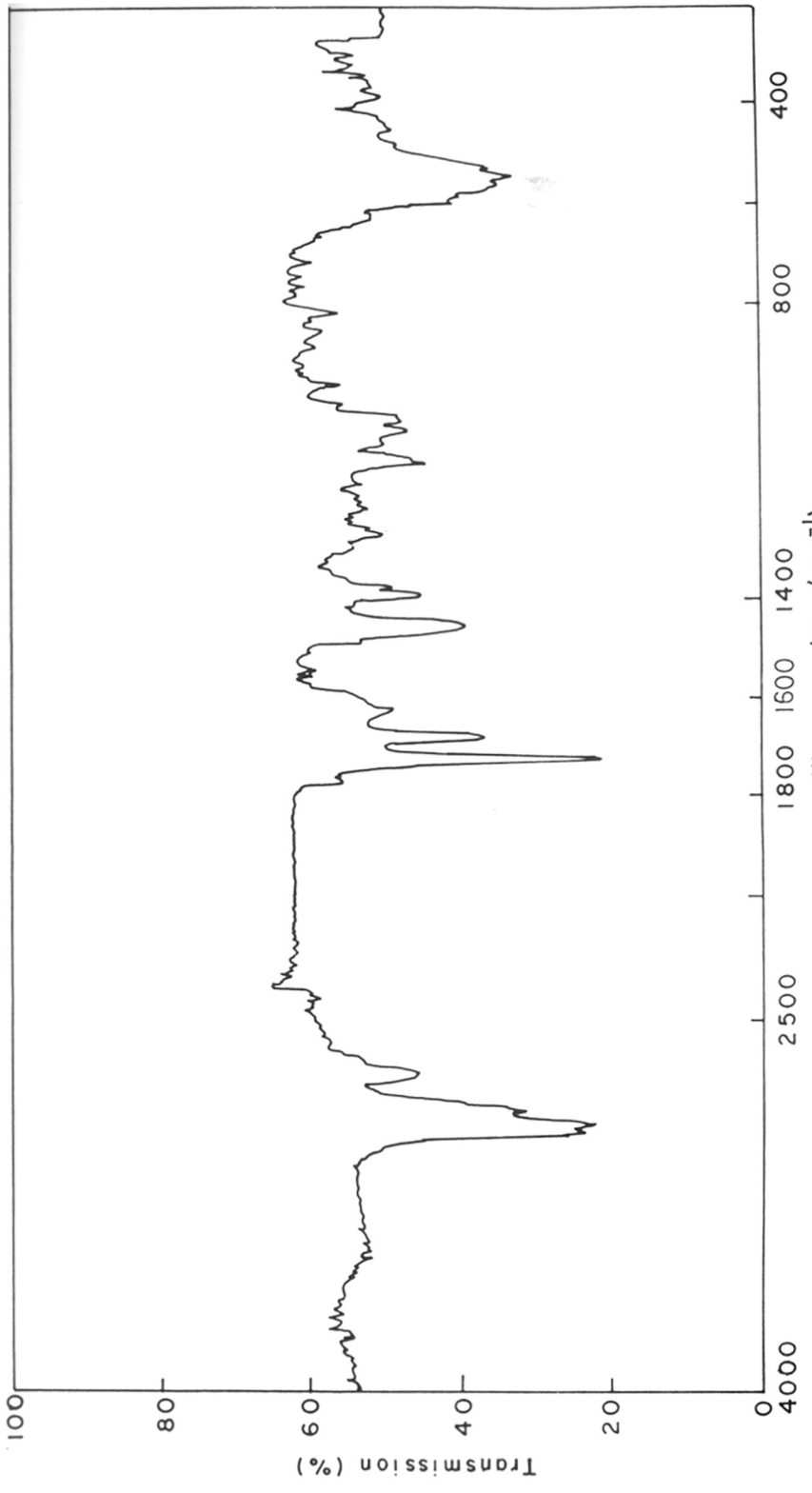


Fig.1 : IR spectrum of compound 6.

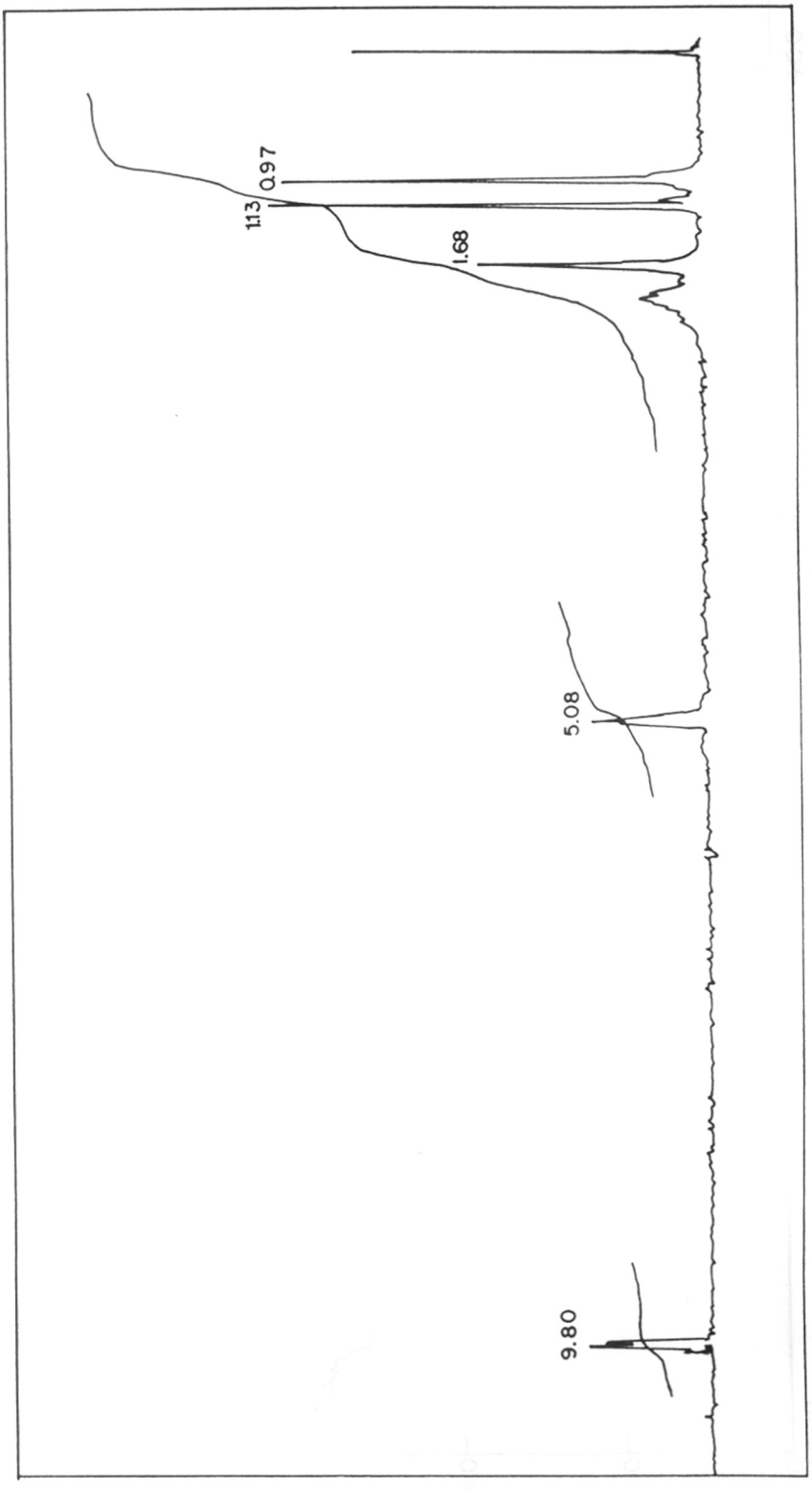


Fig.2: ^{1}H-NMR spectrum of compound 6.

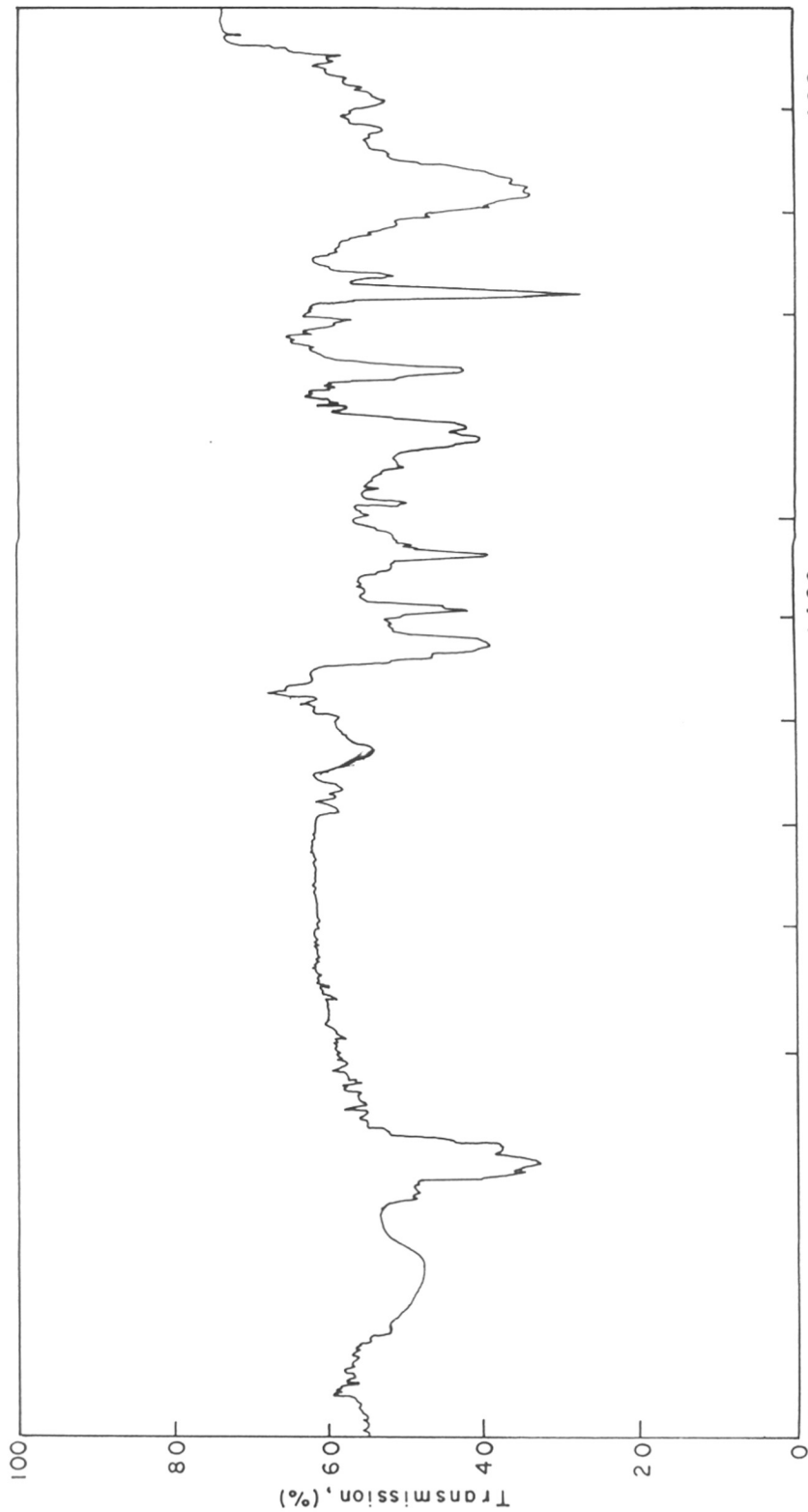


Fig.3 : IR spectrum of compound of 6b.

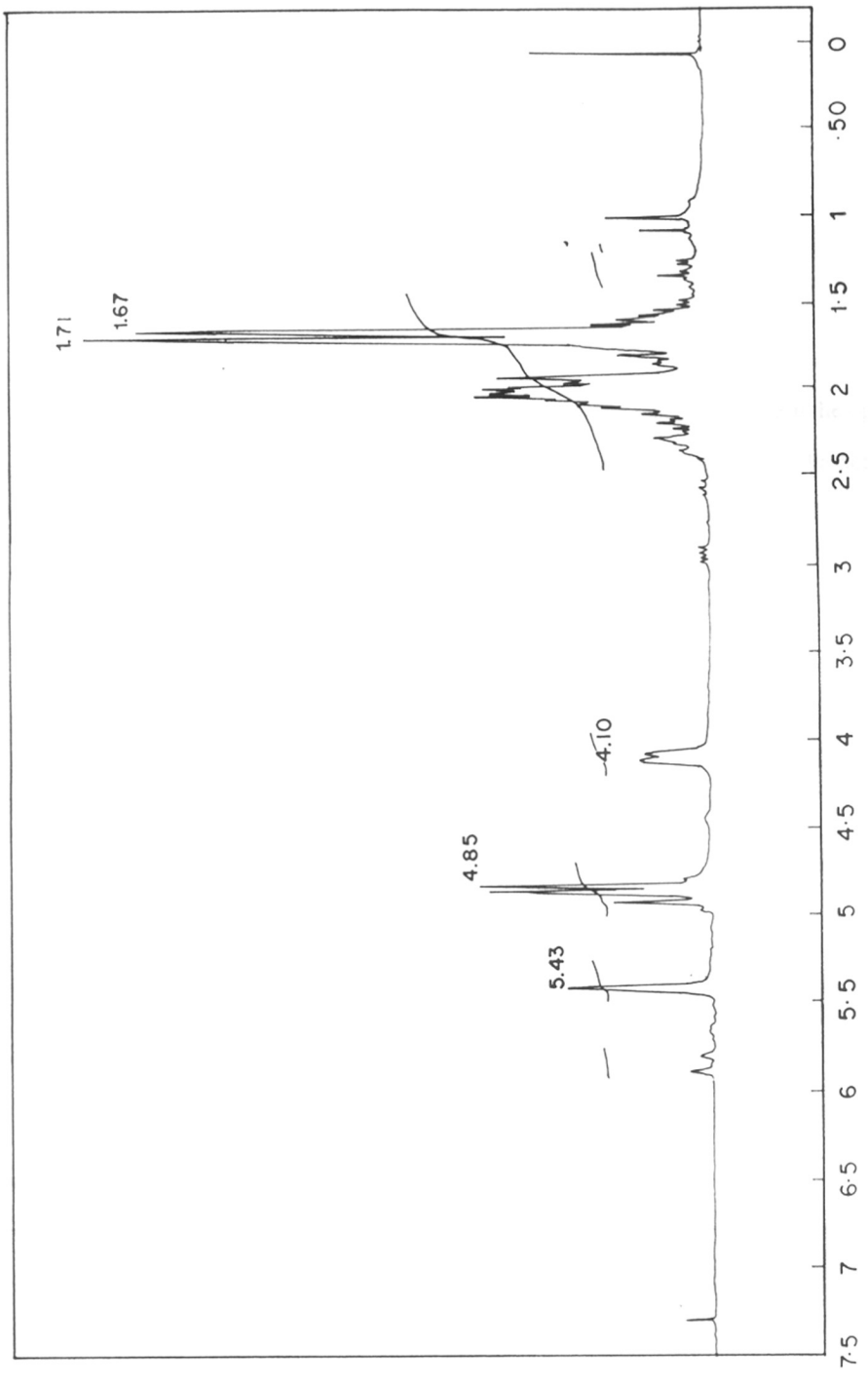
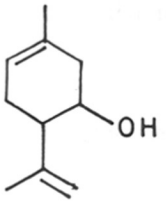
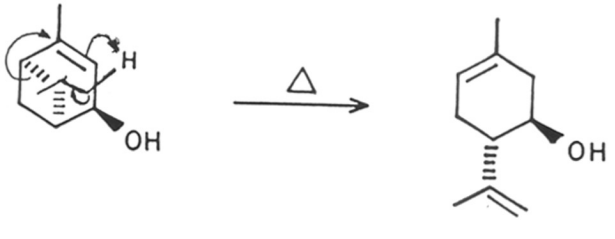


Fig. 4 : ^1H – NMR spectrum of compound 6b



52

The compound **6b** has probably arisen from the retro-ene reaction wherein the opening of the cyclobutane ring in trans-verbenol occurs as indicated in Scheme-10. The characterization of the other two side products **6a** and **6c** remained incomplete since those could not be obtained in sufficiently pure form to enable the structure elucidation.



51 6b

Scheme - 10

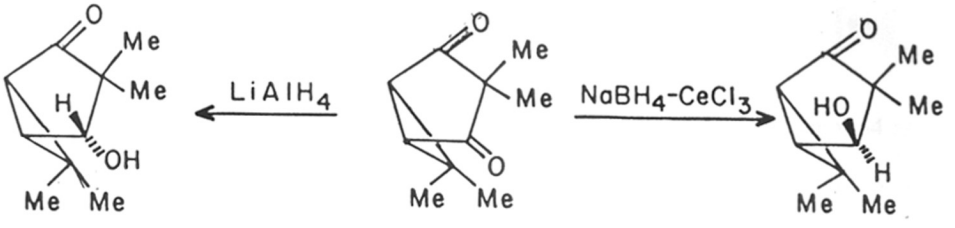
4.3.3 Different methods attempted to prepare *trans*-verbenol

The Scheme-9, yielding the required aldehyde **6** involved the allylic oxidation of α--pinene with LTA in benzene. Although it is the only route known in the literature for the preparation of *trans*-verbenol, the method suffers from two major disadvantages. One is the problem of disposal of large quantities of reduced LTA viz. lead diacetate, another being the use of harmful solvent like benzene in large amounts. It was therefore considered worthwhile to

device a better synthetic method to prepare *trans*-verbenol **51** obviating the above mentioned drawbacks. This was attempted by the stereoselective reduction of verbenone **44**, obtained by the oxidation of *trans*-verbenol[17c,d].

1. Reduction of verbenone with Luche's reagent ($CeCl_3$-$NaBH_4$)[22]

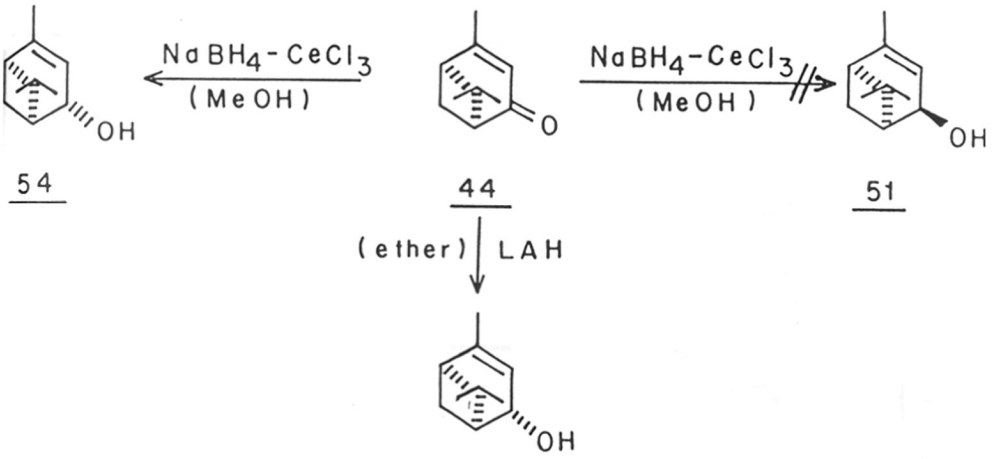
The lanthanides, especially the cerium compounds have been reported to alter the stereochemical course of reductions of various ketones such as enediones, cage diketones[23] and bicyclo[3.1.0] hexanone **53**[24] compared to their reductions with common reducing agents *viz.* $NaBH_4$, $LiAlH_4$, etc.



endo-ol 53 exo-ol

In case of **53**, the usual reducing agents such as lithium aluminium hydride are known to give predominantly the endo-alcohol[24], whereas the Luche's reagent ($CeCl_3$-$NaBH_4$) yields exclusively the exo-alcohol. It has been hypothesized that the lanthanide salts complex with the ketone carbonyl causing steric hindrance at the exo-face, thus forcing the attack of hydride from the endo side.

Verbenone **44**, when reduced with $NaBH_4$ or $LiAlH_4$ predominantly gives *cis*-verbenol **54**,[25,26] [Scheme-11] wherein the reagent attacks the carbonyl group from less-hindered face. The Luche's reagent is known for its high propensity of reacting with the carbonyl from the most hindered face. The reagent was thus expected to attack the carbonyl group of verbenone from the side of gem-dimethyl group giving the required *trans*-verbenol **51**.



Scheme – 11

However, **44** on treatment with $CeCl_3$-$NaBH_4$ in methanol furnished *cis*-verbenol **54** as evident from its ^{1}H-NMR spectrum (Fig.5). In case of verbenone, the inversion of stereochemistry in reduction reaction by Luche's reagent was not observed. The results of the reduction carried out under various conditions are given in Table-2.

Table-2: Reduction of Verbenone

				GLC % [RRT]				
No	Reagent	Temp. °C	Solvent	Alcohol	Verbe-none	Other Peaks		NMR Inference for product
1.	$NaBH_4$	0	MeOH	53 [3.10]	24 [4.47]	4 [5.27]	5 [2.76]	Mixture
2.	$NaBH_4$	RT	i-PrOH	38 [3.24]	28 [4.43]	25 [4.22]	4 [5.20]	Mixture
3.	$NaBH_4$+ $CeCl_3$-$7H_2O$	0	MeOH	73 [3.44]	9 [4.37]	6 [5.17]	--	*Cis*-Verbenol
4.	LAH	0	Ether	80 [3.37]	13 [4.38]	6 [5.23]	--	*Cis*-Verbenol

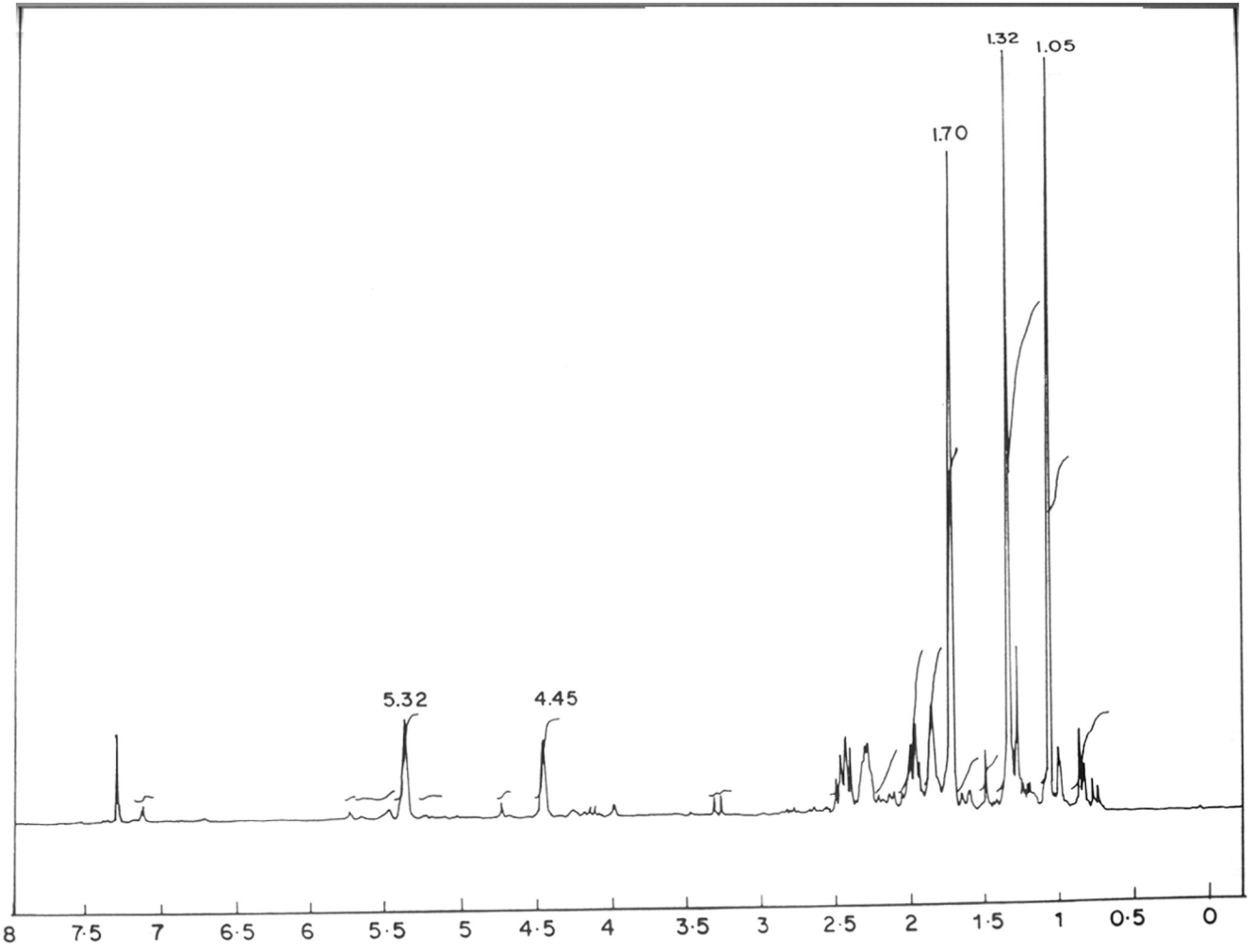
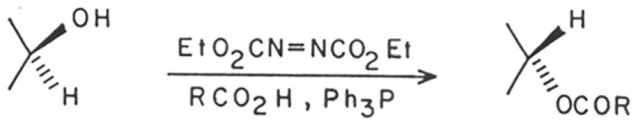


Fig. 5 : ^{1}H NMR spectrum of compound 54.

We then focussed our attention on inverting the stereochemistry of *cis*-verbenol.

2. Inversion of configuration of hydroxyl group in cis-verbenol **54**

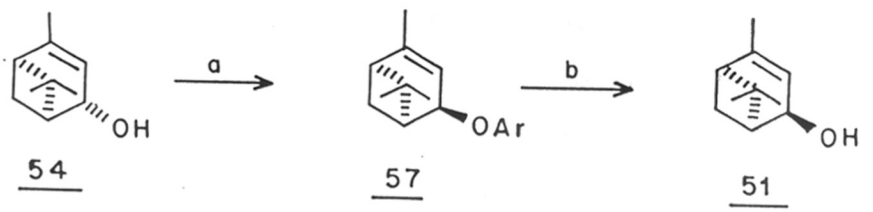
The Mitsunobu reaction utilizing triphenyl phosphine and diethylazodicarboxylate, DEAD ($EtO_2CN{=}NCO_2Et$)[27] is known to be an important synthetic tool for inverting the alcohol stereochemistry [Scheme-12].



55 56

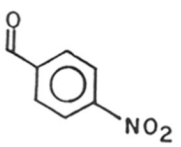
Scheme – 12

The procedure involved mild and almost neutral reaction conditions and has proved useful in the synthesis and transformation of various classes of natural products.[28,29] The same method was attempted to prepare *trans*-verbenol by inversion of configuration of *cis*-alcohol **54** [Scheme-13].



54 57 51

Ar =



Reagents: (a) Ph_3P, DEAD, *p*-nitrobenzoic acid; (b) KOH, MeOH.

Scheme – 13

The *cis*-verbenol **54** when subjected to the reaction with DEAD-Ph_3P and *p*-nitrobenzoic acid gave the corresponding *p*-nitrobenzoate **57** (Fig.6, 7, 8) which on hydrolysis furnished the *trans*-verbenol **51**. However, because of the difficulties in the separation of the desired product and poor yields (~20%), the method was far away from practical utility.

Thus, failure to obtain *trans*-verbenol by a route avoiding the use of LTA compelled us to use the method depicted in Scheme-9 for the synthesis of *trans*-verbenol; from which the required aldehyde **6** was obtained by pyrolysis.

4.3.4 Preparation of C-ring equivalent of taxol framework

While aiming at the synthesis of simplified taxol analogues, a number of authors have chosen the aromatic C-ring equivalents in the convergent synthesis. Approach by Kuwajima and co-workers[30] is described in Scheme-14.

Kuwajima *et al.* used compound **58**, metallated 2-dimethoxymethyl-6-methoxybenzene as C-ring equivalent of taxane framework. The intramolecular, $TiCl_4$ mediated cyclization of **60** was explored in terms of diastereoselectivity of the reaction.

Almost simultaneously, Nicolaou *et al.*[10] reported the C-aromatic taxanoids based upon the McMurry reductive coupling in an intramolecular fashion [Scheme-15].

The taxanoid **67** was obtained as a mixture of diastereomers, which on chromatographic separation afforded **67a** and **67b**. Interestingly, only one of the diastereomers **67a** was found to possess antitumor properties.

As already discussed in Scheme-8, Wender *et al.*[17] utilized the bromoalkyl benzene derivative **45** as the C-ring subunit. The C-alkylation of verbenone **44** with the aromatic derivative followed by rearrangement and successive reactions led to the taxol framework [see Scheme-8].

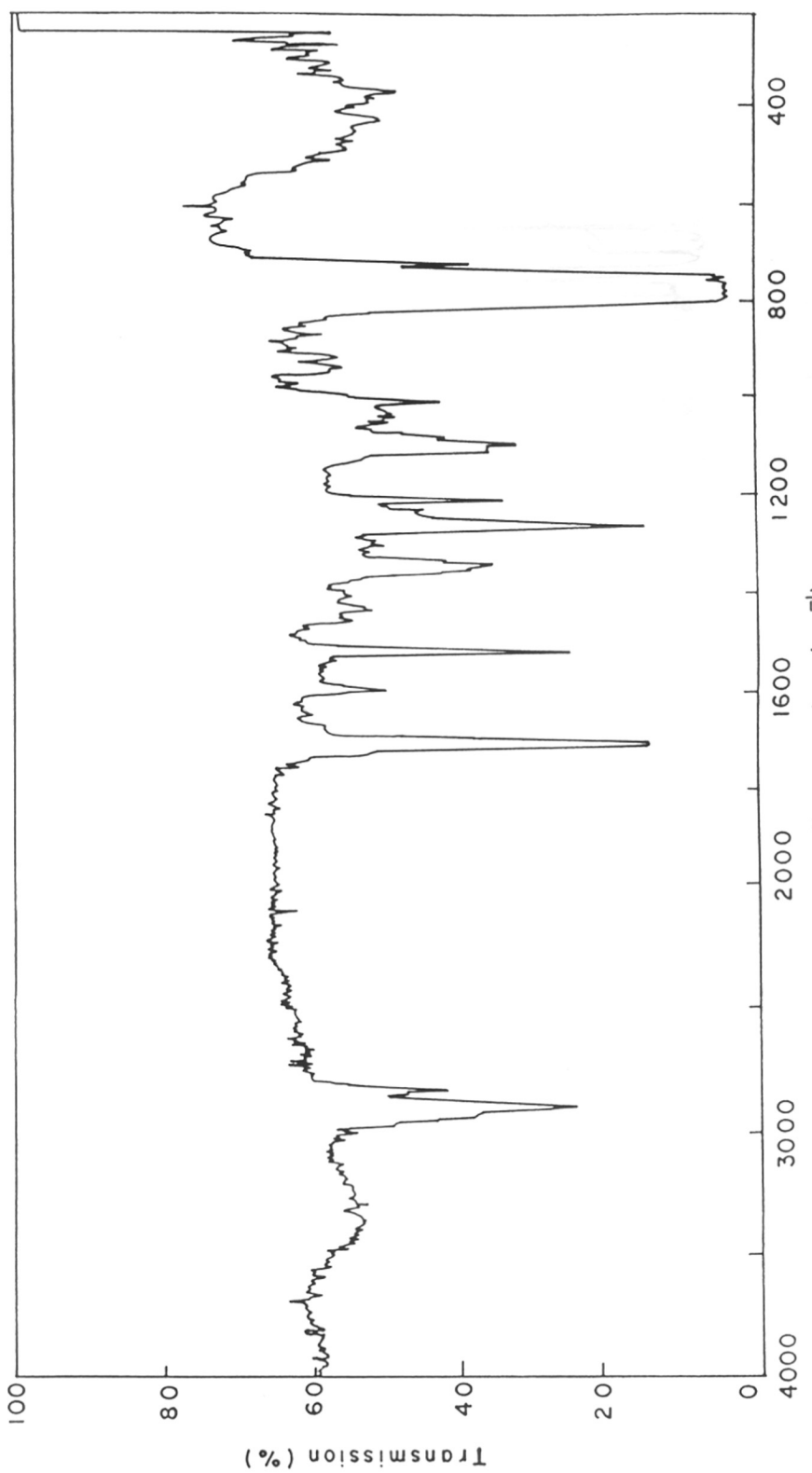


Fig. 6: IR spectrum of compound 57.

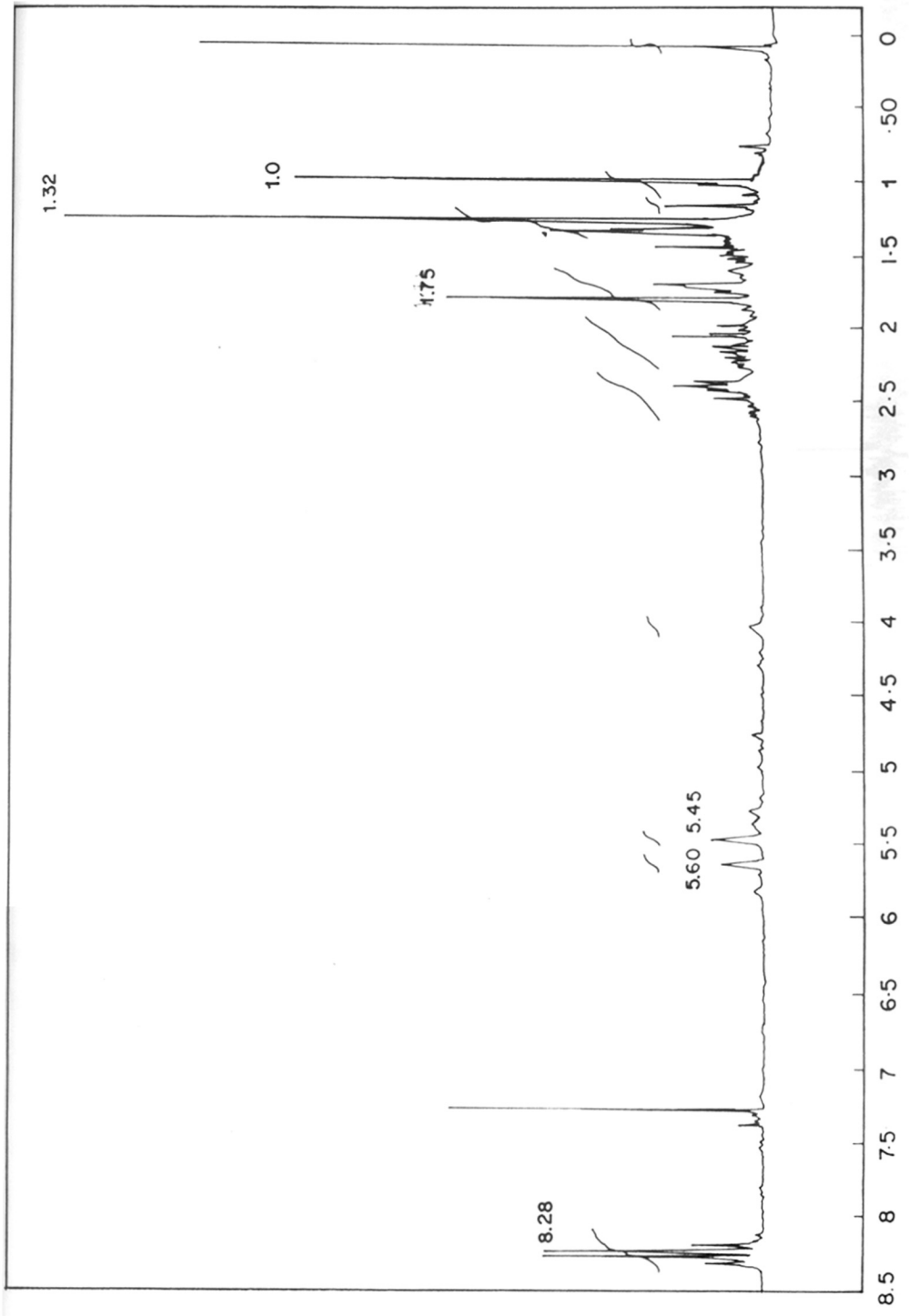


Fig.7 : ^{1}H-NMR spectrum of compound 57.

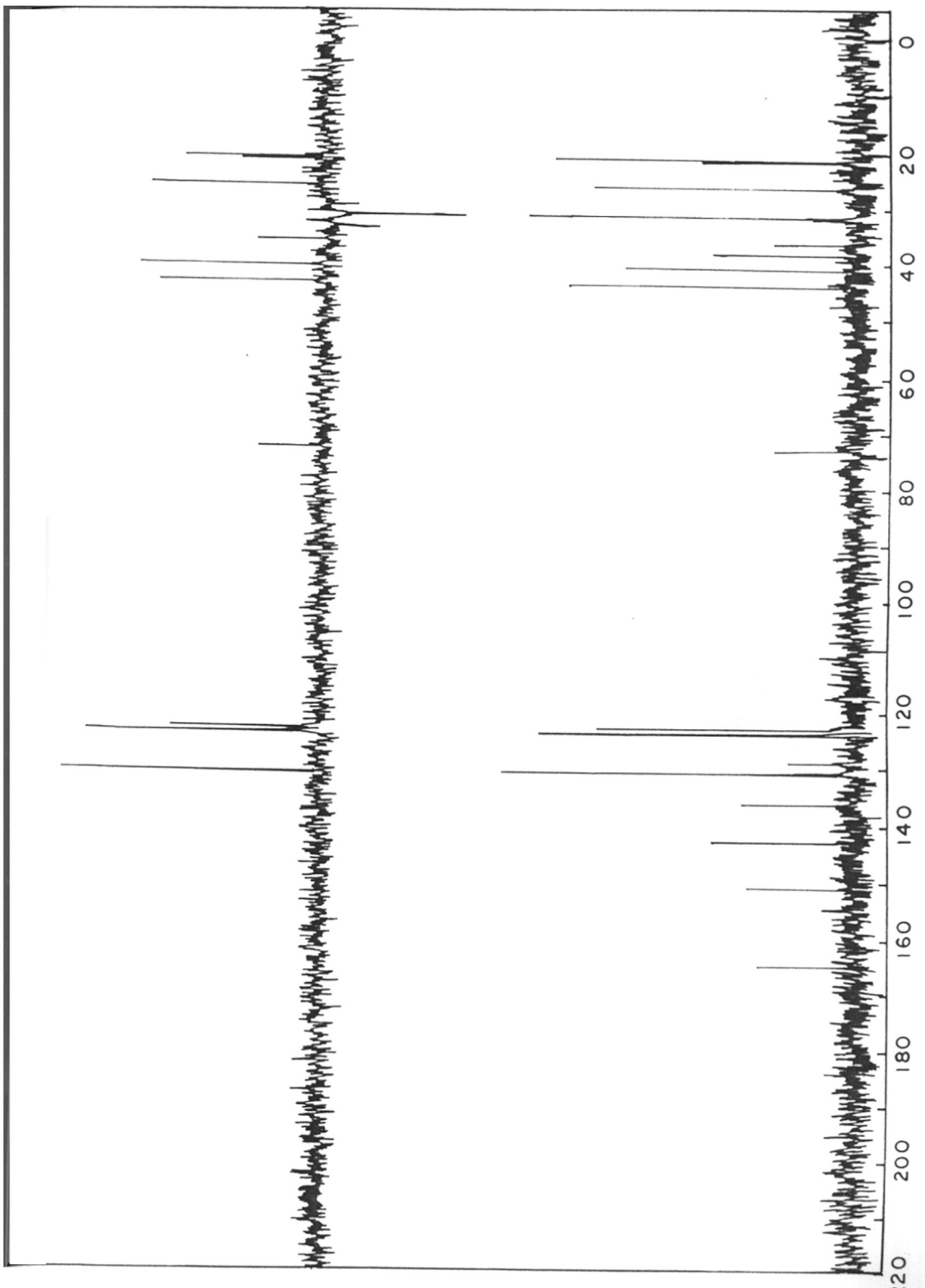
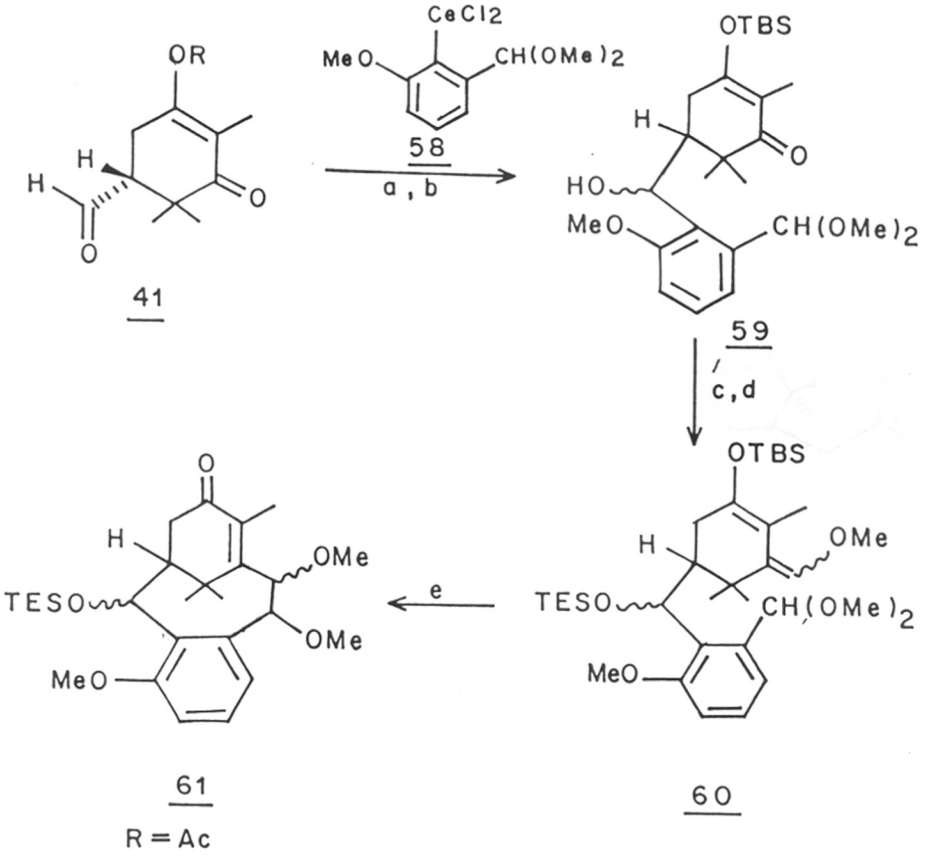


Fig.8 : ^{13}C-NMR spectra of compound 57.

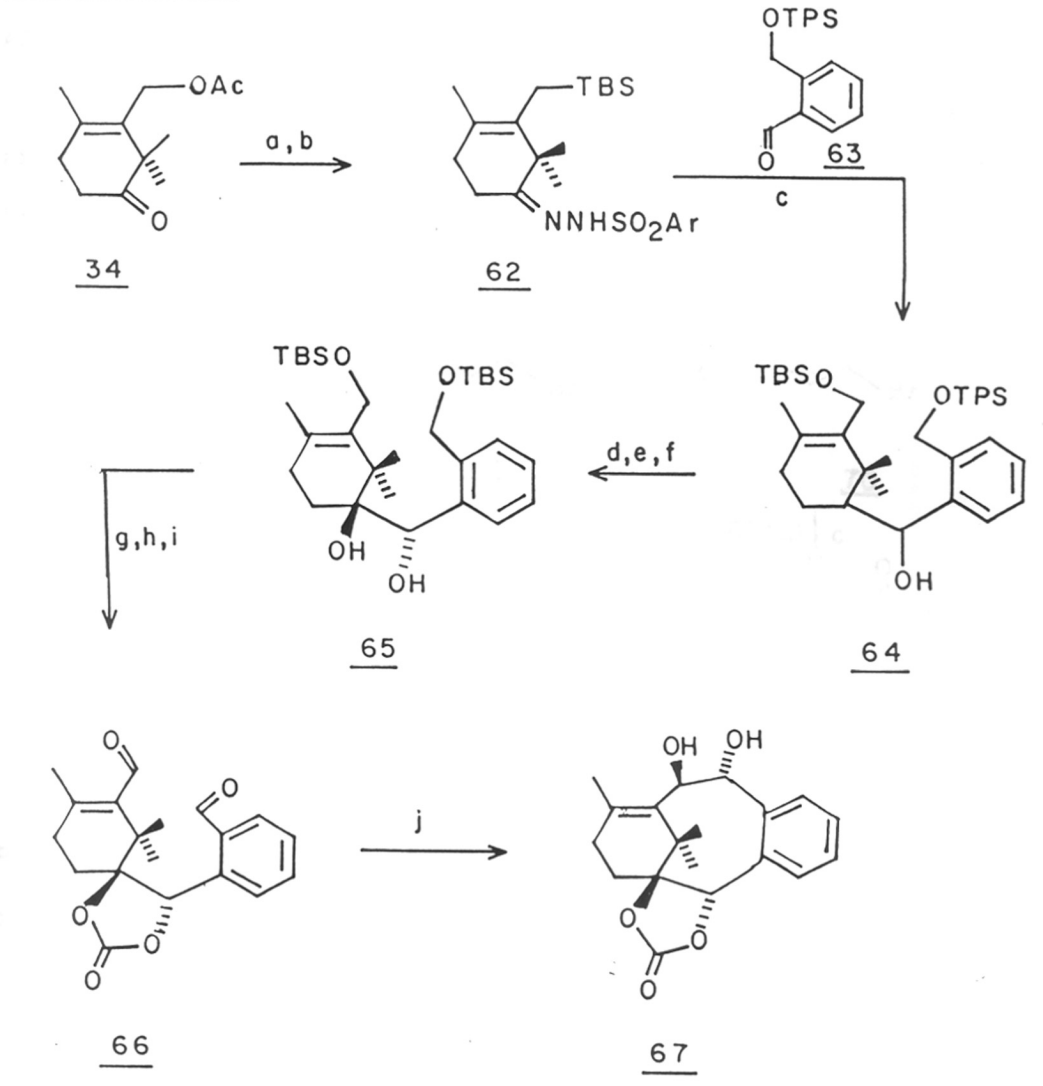
Kuwajima et. al.[30] (1994)



Reagents: (a) m-anisaldehyde dimethyl acetal, $CeCl_3$, n-BuLi, THF; (b) pyrrolidine, TBSCl, Et_3N; (c) TESCl, imidazole; (d) TMS(MeO)CHLi, t-BuOK; (e) $TiCl_4$, CH_2Cl_2.

Scheme – 14

Nicolaou et. al.[10] (1994)

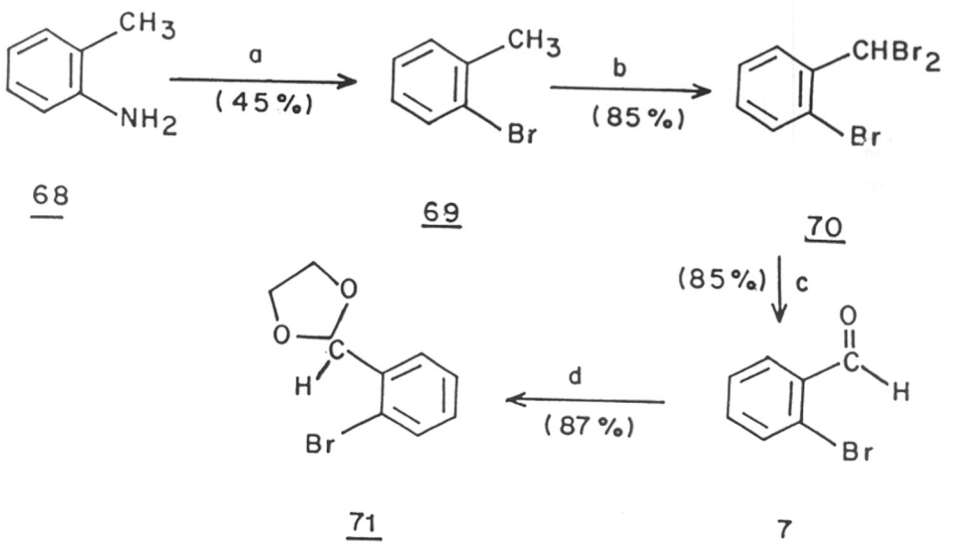


Reagents: (a) t-BuMe$_2$SiOTf, 2,6 lutidine; (b) 2,4,6-triisopropylbenzene sulfonyl hydrazide, MeOH (Ar = 2,4,6-triisopropylbenzene); (c) n-BuLi, THF, -78° to 25°C, cool to 0°C and then **63**; (d) t-BuOOH, Vo(acac)$_2$, benzene; (e) LAH, Et$_2$O, reflux; (f) TBS-Cl, imidazole, CH_2Cl_2; (g) carbonyldiimidazole, acetonitrile, reflux; (h) TBAF, THF; (i) TPAP, NMO, CH_2Cl_2; (j) $TiCl_3(DME)_{1.5}$, Zn-Cu, DME, reflux, then **66**.

Scheme – 15

4.3.5 Synthesis of aromatic C-ring unit **71**

The choice of *ortho*-bromobenzaldehyde **7**, as the C-ring equivalent in our convergent approach has already been indicated in Scheme-2. Since the material was rather costly and was not readily available, it was synthesized following the reported procedure[31], as given in Scheme-16.



Reagents: (a) $NaNO_2$, HBr; (b) NBS, hν, CCl_4; (c) aq. $AgNO_3$, acetone; (d) $HOCH_2CH_2OH$, benzene, PTSA.

Scheme – 16

The diazotization of *ortho*-toluidine **68** in presence of HBr yielded *ortho*-bromotoluene **69** in 45% yield[31a]. Compound **69** was converted to the dibromo derivative **70** by the photoinduced benzylic bromination with N-bromosuccinimide[31b]. The dibromo compound **70** on treatment with aqueous silver nitrate was transformed into **7** in 32% overall yield based on *o*-toluidine[31b]. Protection of the aldehyde carbonyl with ethylene glycol[31c] yielded its acetal **71** (Fig.9) in 87% yield.

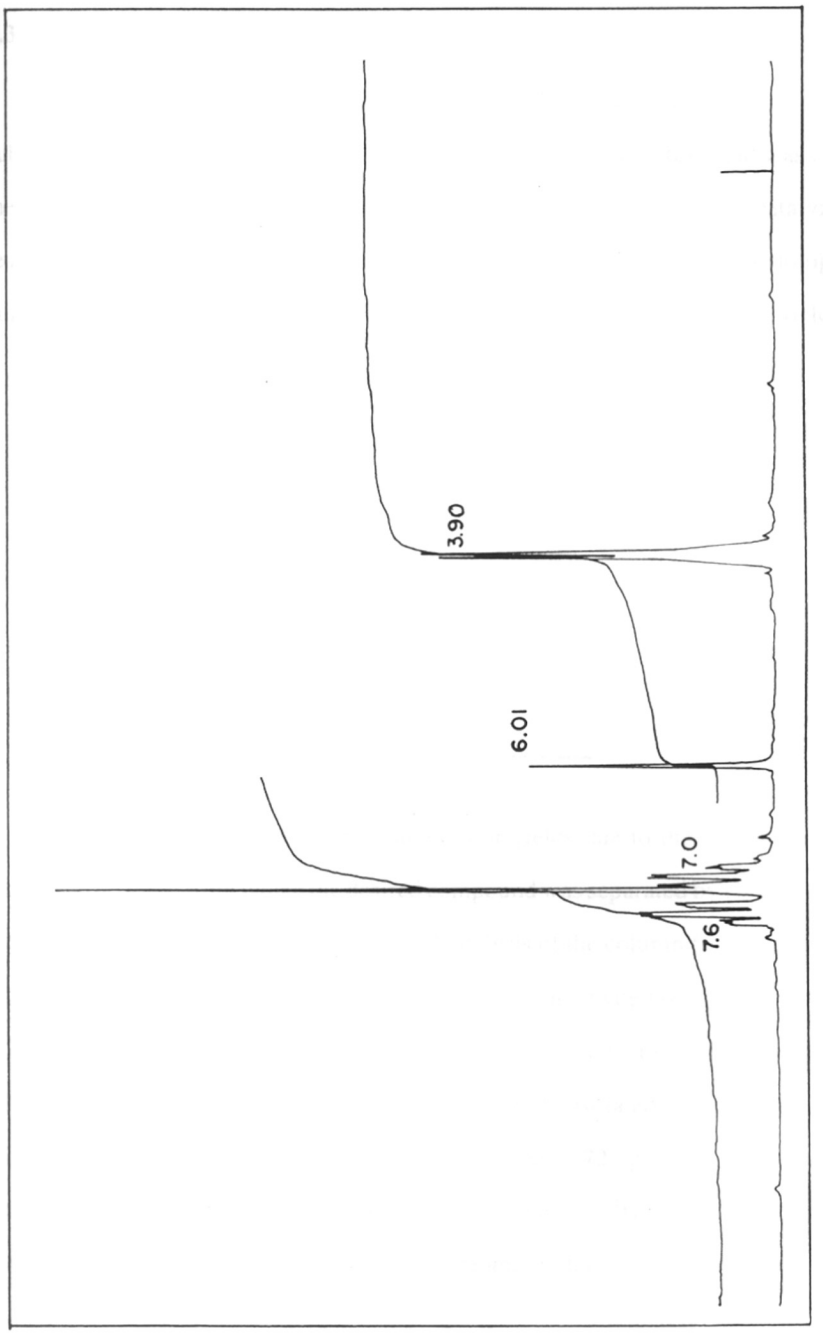
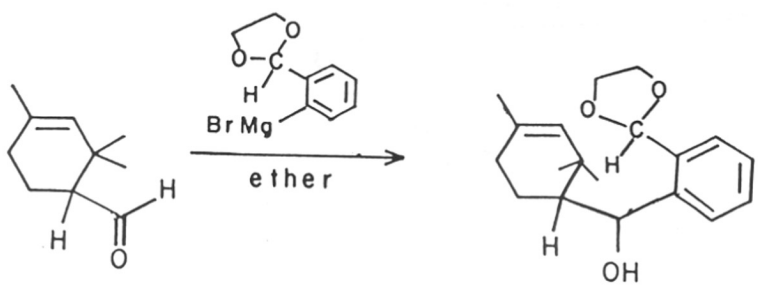


Fig. 9 : ^{1}H-NMR spectrum of compound 71.

4.3.6 Grignard coupling of **6** and **71**

The Grignard reagent prepared from the acetal **71** was successfully reacted with the A-ring subunit **6** using THF as solvent. Initially, the synthesis of B-seco-taxanoid was carried out using the racemic aldehyde (±6)*. The aldehyde was prepared by BF_3.etherate catalyzed Diels Alder reaction of 2,4-dimethyl 1,3-pentadiene and acrolein; employing the methodology developed in our group. The B-seco-taxanoid **72** (Fig.10, 11, 12, 13) was obtained in 25% yield. [Scheme-17].



6 72

Scheme-17

Compound **72** was obtained in rather poor yields due to the formation of side products during the reaction from which the desired compound was separated by column chromatography, followed by recrystallization. The ^{1}H-NMR analysis of the column fractions containing the side products indicated that deprotection of the aromatic aldehyde taking place during the reaction as well as formation of the diastereomer of **72** could be the factors complicating the isolation of **72**. Another diastereomer of **72**, however, could not be isolated in pure form from the reaction mixture.The column purified, recrystallized compound **72** appeared homogeneous by tlc and HPLC (Fig.14). This fact coupled with the homogeneity of ^{1}H and ^{13}C-NMR spectra suggested that **72** was obtained as a predominant diastereomer in the Grignard reaction.

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*I am thankful to Mr.A.Ramani for making available the racemic aldehyde.

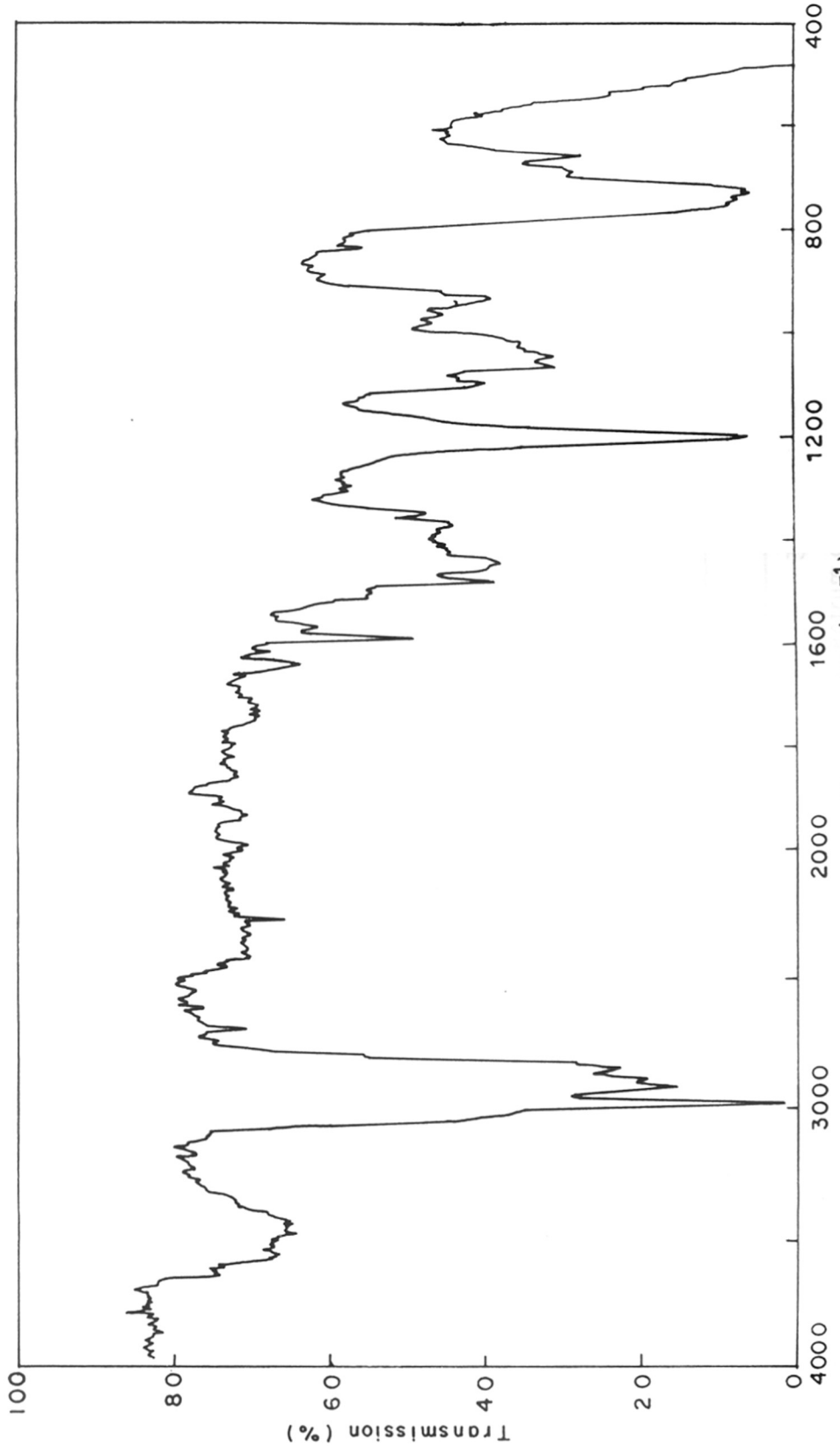


Fig.10 : IR spectrum of compound 7.2

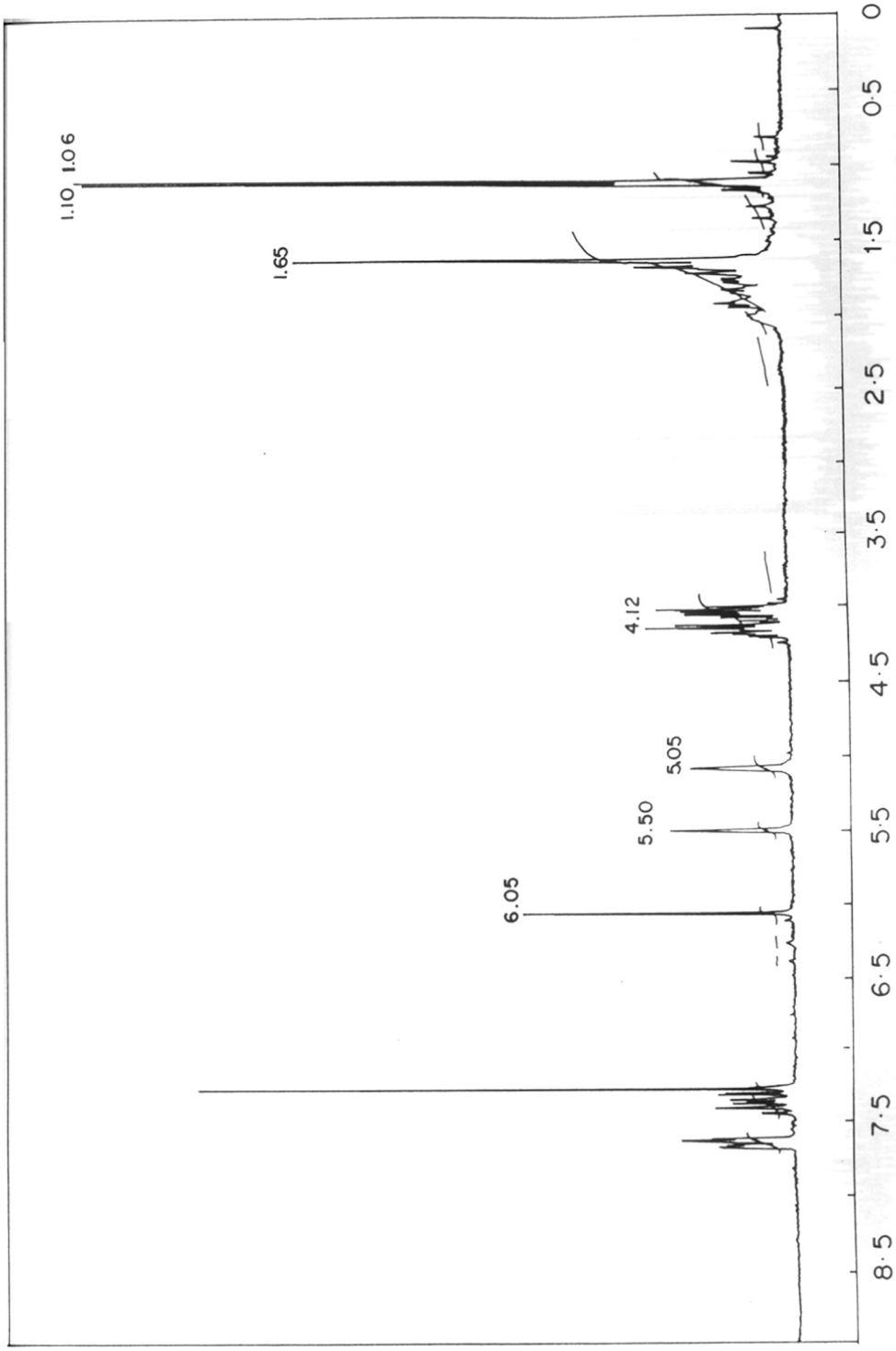


Fig. 11 : ^{1}H- NMR spectrum of compound 72

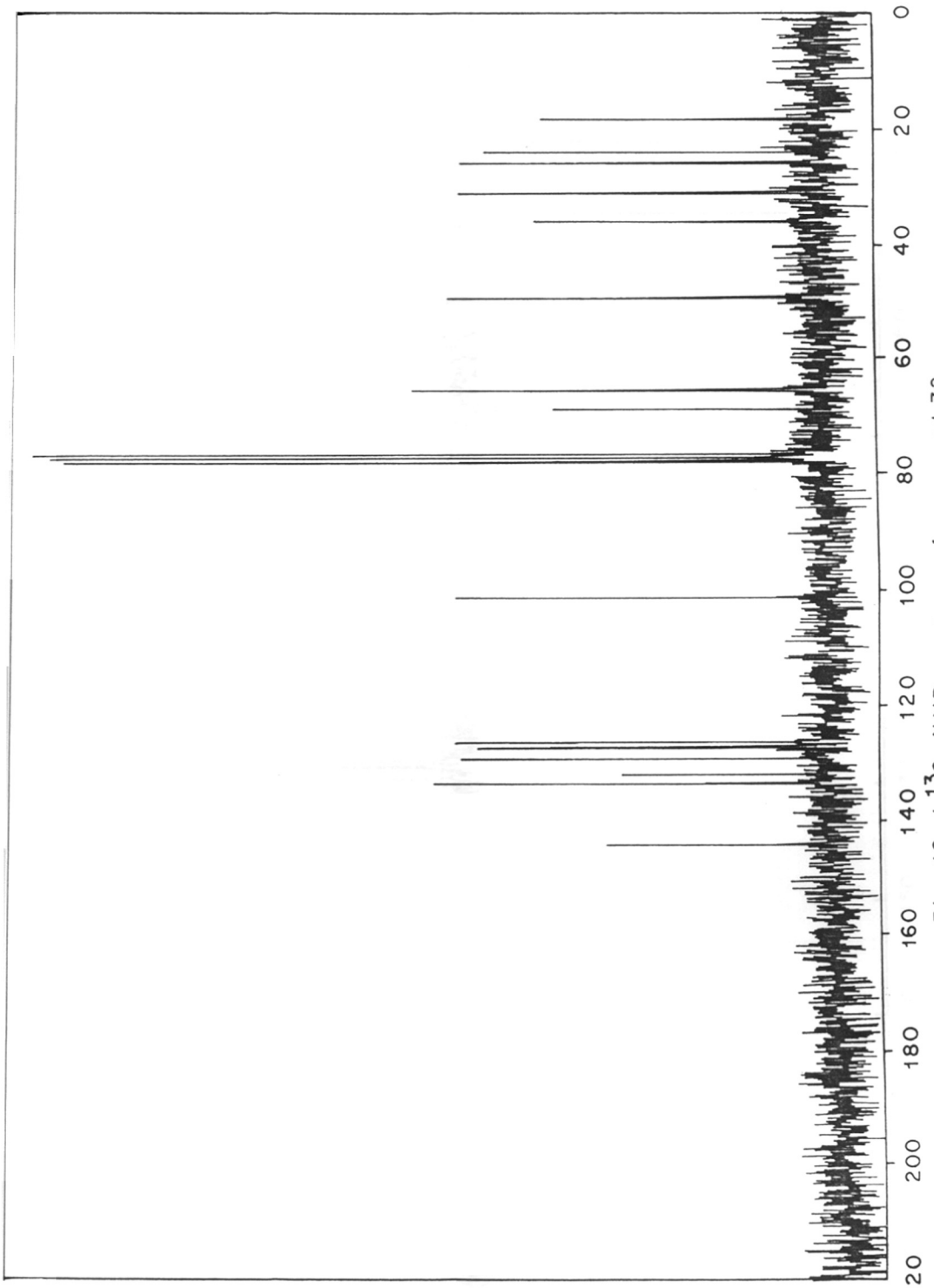


Fig. 12 a: ^{13}C-NMR spectrum of compound 72

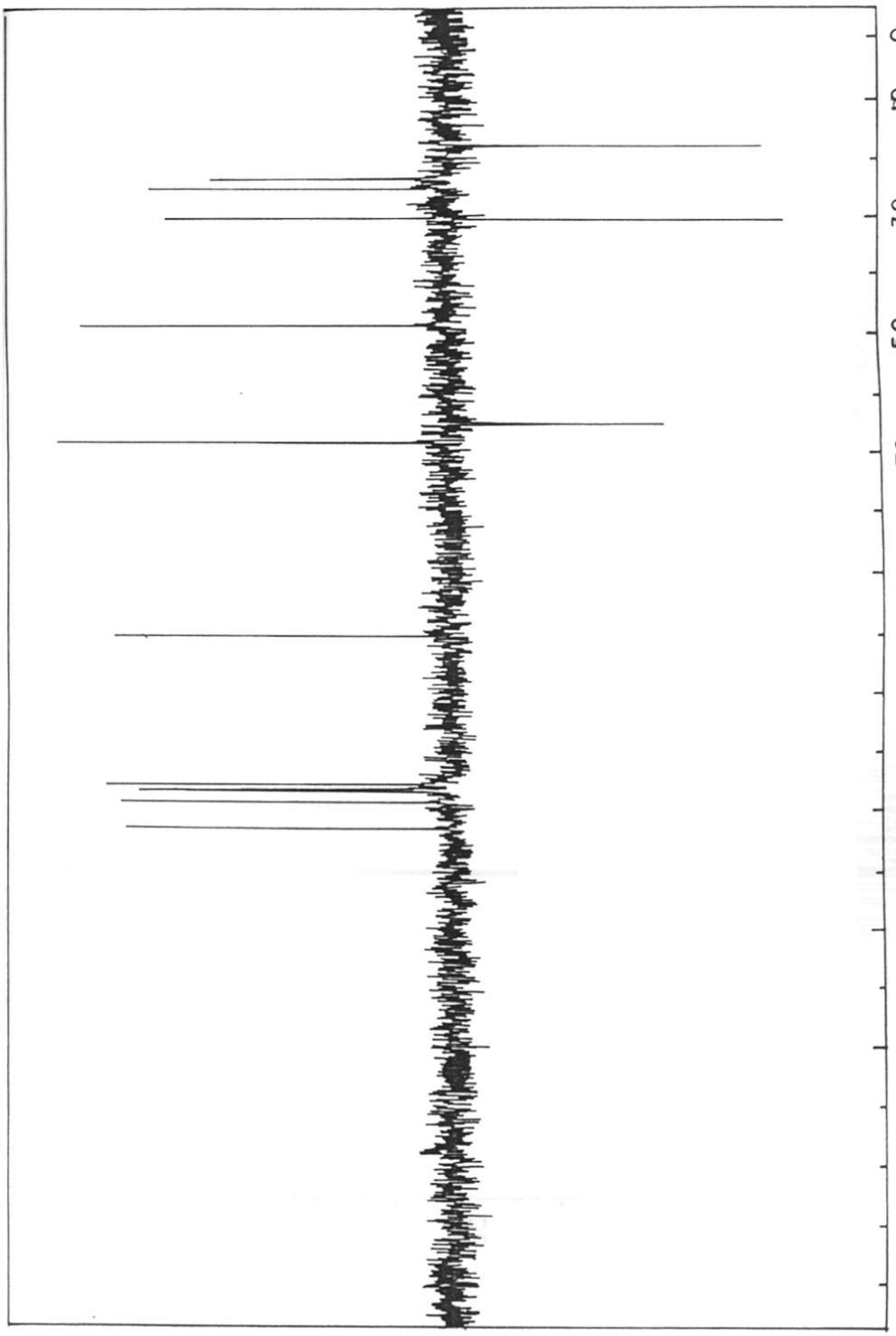


Fig.12b: DEPT-^{13}C-NMR spectrum of compound 72

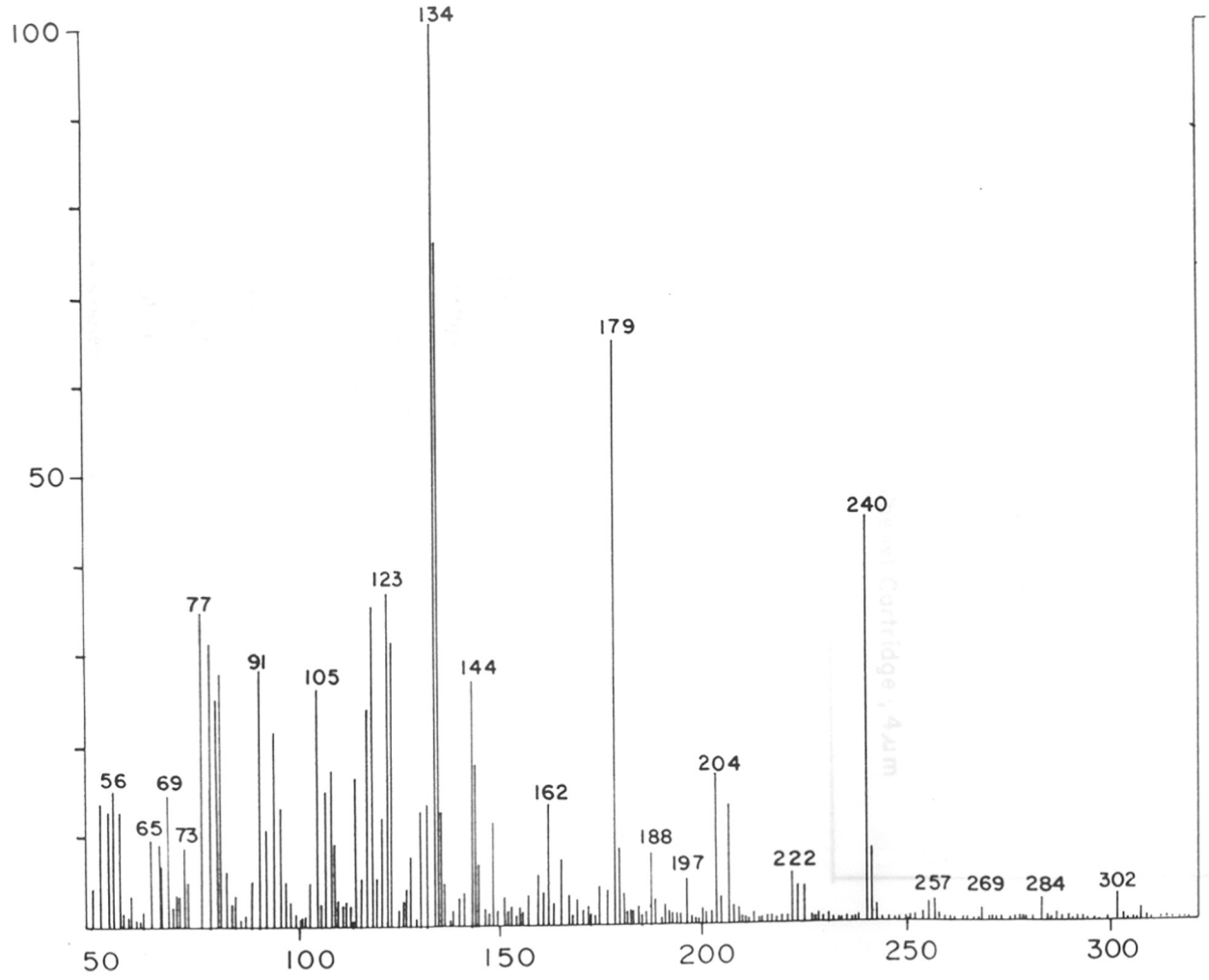


Fig.13 : Mass spectrum of compound 72.

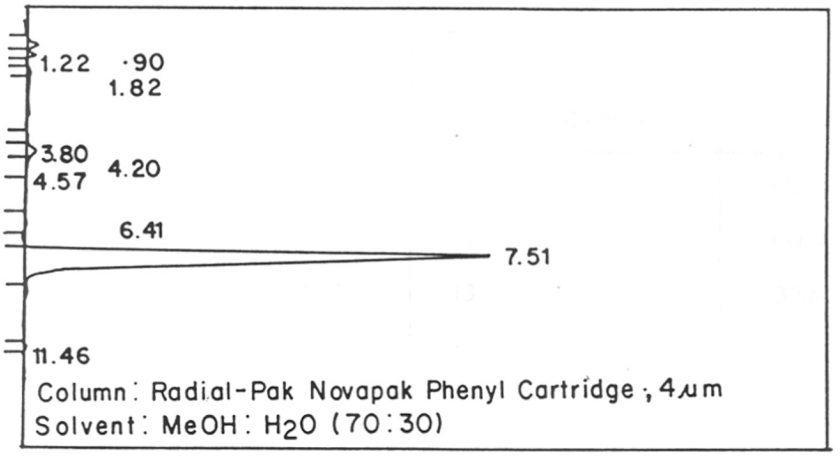


Fig.14 : HPLC chart for compound 72.

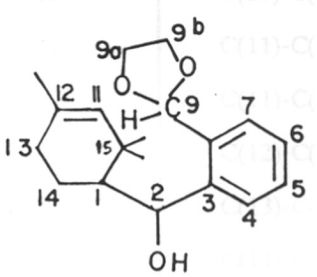
4.3.7 Spectroscopic studies of **72**

Compound **72** in its IR spectrum (Fig.10) showed the presence of bands due to hydroxyl group (3480 cm^{-1}), olefinic moiety (1630 cm^{-1}) and benzene ring (1600). The ^{1}H-NMR spectrum of **72** (Fig.11) exhibited two singlets at δ 1.06 and 1.10 (3H each) for the gem-dimethyl group and a singlet at δ 1.65 for the vinylic methyl group. The protons of 1,3 dioxolane ring appeared at δ 4.12 (m, 4H), and the corresponding methine proton resonated at δ 6.05 (s, 1H). The carbinyl proton C<u>H</u>OH in **72** was observed at δ 5.05, as a doublet with coupling constant of less than 1Hz, while the olefinic proton was located at δ 5.50. The aromatic ring protons were observed in the region δ 7.25 to 7.70. In the DEPT ^{13}C-NMR spectrum (Fig.12b, Table-3), three singlets for four quaternary carbons, eight doublets, four triplets and three quartets were observed and in the mass spectrum (Fig.13), $[M]^+$ was observed at m/z 302 supporting the structure assigned to **72**.

Table-3: ^{13}C-NMR spectrum of **72**					
*Carbon No.	**Signal Multiplicity	Chemical Shift (δ)	*Carbon No.	**Signal Multiplicity	Chemical Shift (δ)
1	d	49.0	11	d	126.3
2	d	68.7	12	s	131.9
3	s	144.0	13	t	30.6
4	d	133.5	14	t	18.0
5	d	127.2	15	s	35.7
6	d	127.0	16	q	23.6
7	d	129.1	17	q	25.4
8	s	144.0	18	q	30.4
9	d	100.8	9a	t	65.5
10	-	---	9b	t	65.4

* Taxol numbering followed

**Signal multiplicity evident by DEPT ^{13}C-NMR spectrum



72

The structure of **72** was further confirmed by X-ray crystallography.

4.3.8 X-ray crystallographic data of **72**

The compound **72**, $C_{19}H_{26}O_3$ crystallized in the monoclinic space group P_{21}/n with a=8.069(1), b=17.558(3), c=35.929(4)Å, β=90.17(1)°, V=5090.2(12)Å^3 and z=12. A crystal size 0.22x0.4x1.2 mm was chosen for single crystal X-ray diffraction studies using M_oK_α radiation (λ = 0.7107Å) on p.c. controlled CAD-4 Enraf Nonius X-ray diffractometer. The structure has been solved by direct methods using MULTAN. Three independent molecules with similar conformation were present in the asymmetric unit. The cyclohexene ring possessed distorted chair conformation while the 1,3-dioxalane ring was in envelope conformation. The PLUTO diagram of one of the three molecules in the asymmetric unit of **72** along with crystallographic numbering is shown in Fig.15. The bond length and bond angle data for compound **72** is given in Tables 4, 5.

Table-4: Bond length data for compound **72**

Bond	Bond length (Å)	Bond	Bond length (Å)
O(1)-C(1)	1.415	C(8)-C(9)	1.363
O(1)-C(3)	1.434	C(9)-C(10)	1.529
O(2)-C(2)	1.413	C(10)-C(11)	1.537
O(2)-C(3)	1.392	C(11)-C(12)	1.520
O(3)-C(10)	1.417	C(11)-C(16)	1.555
C(1)-C(2)	1.500	C(12)-C(13)	1.504
C(3)-C(4)	1.517	C(13)-C(14)	1.484
C(4)-C(5)	1.395	C(14)-C(15)	1.341
C(4)-C(9)	1.395	C(14)-C(19)	1.515
C(5)-C(6)	1.398	C(15)-C(16)	1.499
C(6)-C(7)	1.360	C(16)-C(17)	1.500
C(7)-C(8)	1.394	C(16)-C(18)	1.532

Table 5: Bond angle data for compound **72**

Bond	(°)	Bond	(°)
C-1 - O-1 - C-3	107.8	O-3 - C-10 - C-9	111.3
C-2 - O-2 - C-3	106.8	O-3 - C-10 - C-11	107.7
O-1 - C-1 - C-2	105.3	C-9 - C-10 - C-11	114.4
O-2 - C-2 - C-1	103.6	C-10 - C-11 - C-12	111.0
O-1 - C-3 - O-2	106.1	C-10 - C-11 - C-16	115.3
O-1 - C-3 - C-4	110.5	C-12 - C-11 - C-16	111.2
O-2 - C-3 - C-4	111.8	C-11 - C-12 - C-13	113.6
C-3 - C-4 - C-5	116.9	C-12 - C-13 - C-14	111.9
C-3 - C-4 - C-9	122.0	C-13 - C-14 - C-15	121.0
C-5 - C-4 - C-9	121.0	C-13 - C-14 - C-19	118.2
C-4 - C-5 - C-6	120.0	C-15 - C-14 - C-19	120.8
C-5 - C-6 - C-7	119.3	C-14 - C-15 - C-16	127.9
C-6 - C-7 - C-8	119.4	C-11 - C-16 - C-15	109.4
C-7 - C-8 - C-9	123.4	C-11 - C-16 - C-17	108.0
C-4 - C-9 - C-8	116.8	C-11 - C-16 - C-18	108.7
C-4 - C-9 - C-10	123.6	C-17 - C-16 - C-18	109.6
C-8 - C-9 - C-10	119.5		

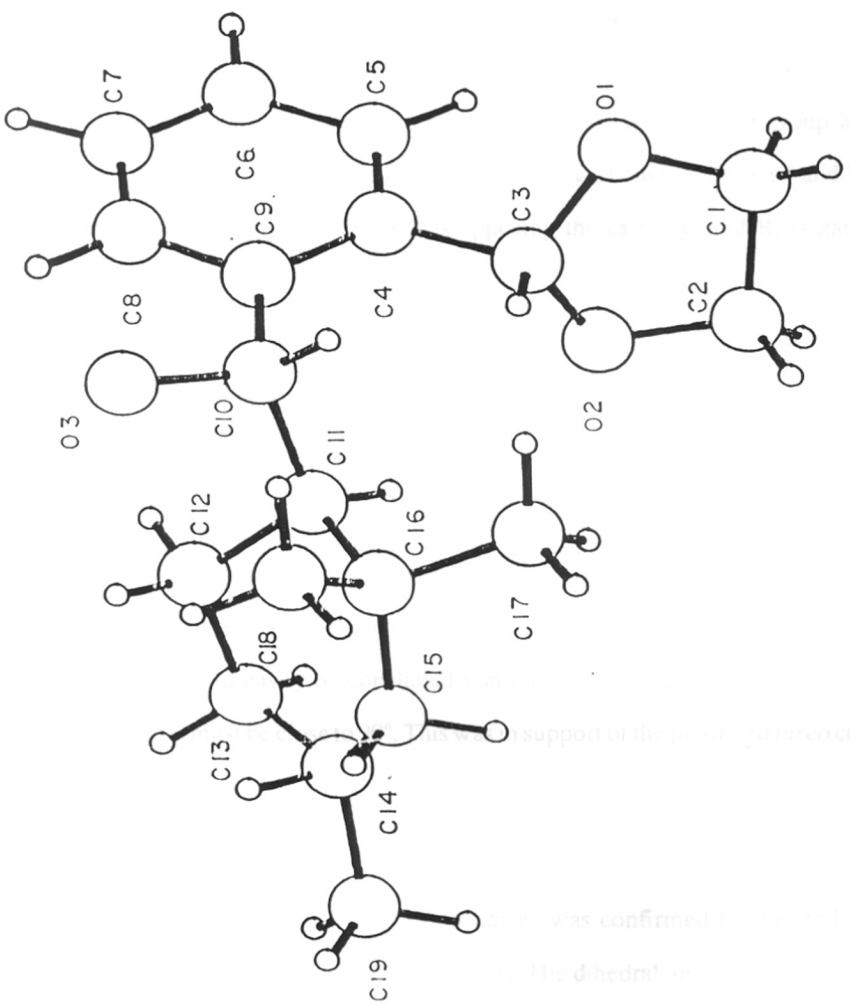


Fig.15: PLUTO diagram of compound 72 with crystallographic numbering.

4.3.9 Stereochemical study of **72**

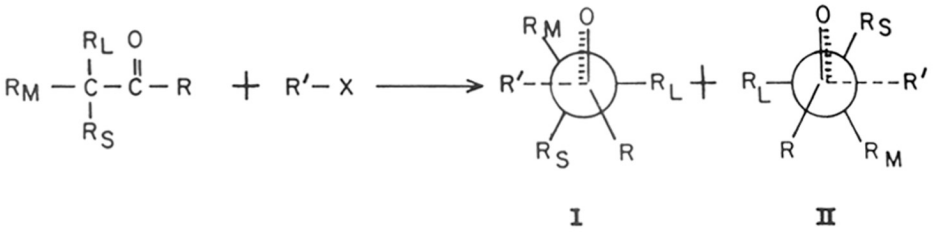
The stereochemical outcome of the Grignard reaction of **6** and **71** was predicted on the basis of Felkin-Anh model[32,33] for addition reactions to carbonyl compounds. In this model, the transition state is considered to be comprised of two reactive conformations I and II [Scheme-18]. In both the conformations, the largest or the most electron withdrawing group at Cα in the carbonyl compound; (R_L) is placed at right angle to the carbonyl. Between I and II, state I in which the medium sized group at α; (R_M) is opposing the carbonyl and R_S is gauche to R is usually preferred.

Application of the model to the reaction under consideration revealed that the aldehyde **6** would predominantly yield the R,S-diastereomer of compound **72**. Using *threo erythro* notation, the diastereomer could be called as *threo* isomer [Scheme-19].

Evidence from 1H-NMR:Careful observation of the ^{1}H-NMR of **72** revealed that the carbinyl proton H-C_2-OH at δ 5.05 appeared as a doublet with very low (< 1Hz) coupling constant. Applying the Karplus equation[34] which correlates the coupling constants in NMR with the dihedral angles, it could easily be concluded that the torsion angle between the two adjacent protons on C_1 and C_2 must be close to 90°. This was in support of the predicted *threo* configuration for **72**.

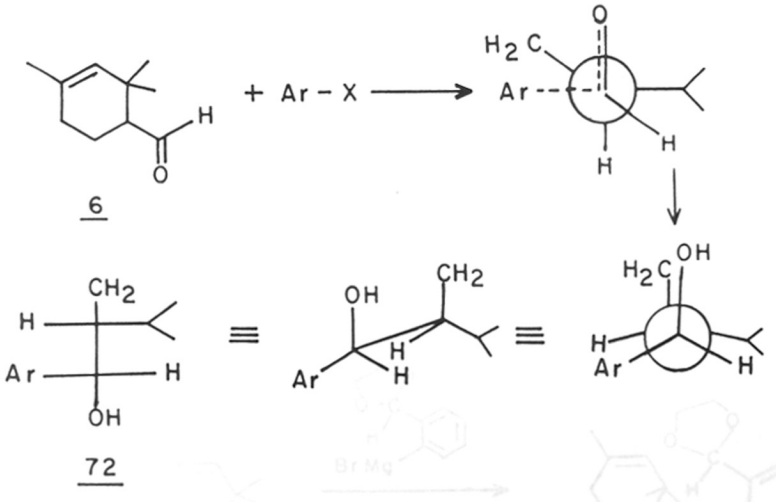
4.3.10 Study of dihedral angles

The prediction of configuration for **72** as *threo* was confirmed by the study of dihedral angles between the respective atoms. [See Table-6]. The dihedral angle data was obtained from the X-ray diffraction studies. The molecular model prepared as per the dihedral angles clearly revealed the *threo* configuration for **72**.



I II

Scheme – 18



6

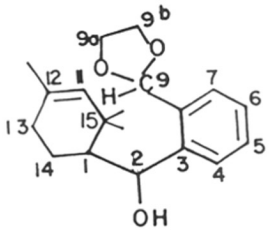
72

Ar =

CH_2, From cyclohexene ring

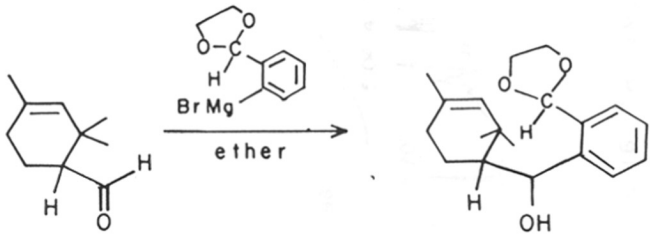
Scheme – 19

Table-6: Dihedral angles at C_1 and C_2 in compound **72**		
Atoms under consideration	Angle (°)	
H_1-C_1-C_2-H_2	79.3	
H_1-C_1-C_2-HO	161.1	
H_1-C_1-C_2-C_3	40.7	
C_{14}-C_1-C_2-OH	41.5	
C_{14}-C_1-C_2-C_3	78.9	
C_{14}-C_1-C_2-H_2	161.1	
C_{15}-C_1-C_2-OH	81.0	
C_{15}-C_1-C_2-H_2	38.6	
C_{15}-C_1-C_2-C_3	158.6	



72

Preparation of optically active B-seco taxanoid was carried out following exactly the procedure given for racemic **72**. However, the optically active compound **72** was obtained as a diastereomeric mixture as evident by the ^{1}H-NMR spectrum (Fig.16).



(–)**6** **72**

Further work for making available more quantities of **72** and its chromatographic purification is in progress.

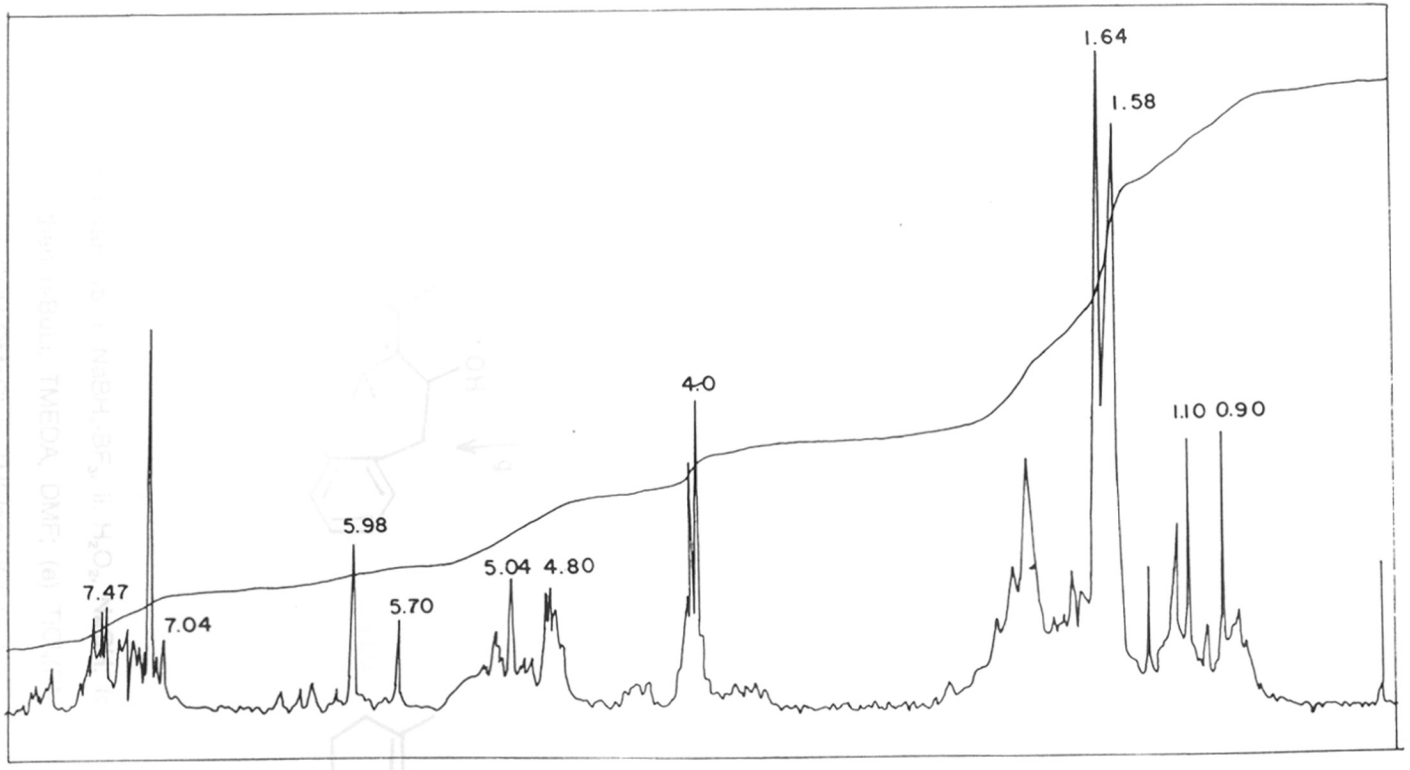
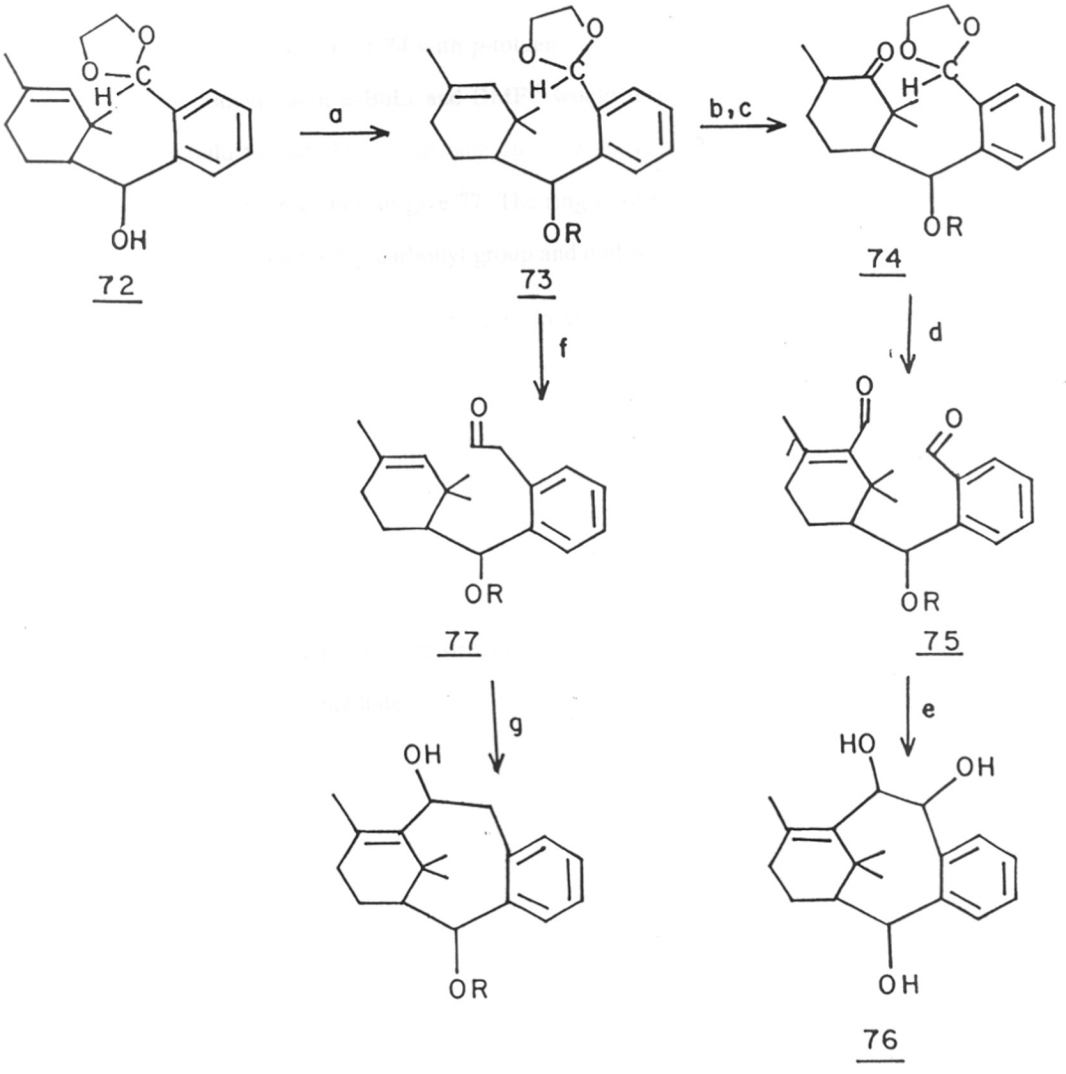


Fig. 16: ^{1}H-NMR spectrum of compound 72

4.4 Future Plans



Reagents: (a) CH_3I/NaH; (b) i. $NaBH_4$-BF_3, ii. H_2O_2, NaOH; (c) Collin's oxidation; (d) p-$TsNHNH_2$, EtOH, then n-BuLi, TMEDA, DMF; (e) $TiCl_3$(DME), Zn-Cu, DME; (f) i. CH_3SOCH_2Li/THF, ii. Ph_3PCH_2OMeCl, iii. THF/aq. HCl; (g) $SnCl_4$.

Scheme – 20

As given in Scheme-20, it is planned to protect the secondary hydroxyl group in **72** with a suitable group giving **73** which then would be subjected to hydroboration and subsequent oxidation to yield **74**. Reaction of **74** with *p*-toluene sulphonohydrazide and treatment of the resulting tosylhydrazone with *n*-BuLi and DMF[35] would lead to **75**. McMurry coupling[10] of compound **75** would furnish the cyclooctane **76**. Alternatively, **73** would be subjected to the standard homologation procedure to give **77**. The ring closure would then be carried out with the ene reaction[36] between the C_{10} carbonyl group and olefin at C_{11}.

According to the plan, protection of the hydroxyl group in **72** has been carried out using CH_3I and NaH giving the 2-methoxy compound **73**. Further work is underway.

In summary, this chapter describes an easy and convenient access to the C_{10} aldehyde **6** as A-ring subunit of taxol. Preparation of the aldehyde has been carried out employing an inexpensive starting material, α-pinene. The aldehyde **6** has been utilized in the construction of B-seco-C-aromatic taxanoid **72**. The methodology now provides a new avenue in generating variety of taxanoids for the purpose of structure-activity relationship (SAR) studies using the B-seco taxanoid as key intermediate.

4.5 Experimental

Bicyclo [3.1.1] hept-3-en-2-ol, 4,6,6-trimethyl (trans-verbenol) [19], **51**

1. LTA oxidation of α-pinene

Compound **51** was prepared employing a slight modification of the reported procedure which involved addition of α-pinene to a slurry of lead tetraacetate (LTA) in benzene.

(i) *verbenyl acetate* **50**:(-)-α-pinene $[\alpha]_D$-42.6 (neat), (35.0g, 0.26 mol) was added dropwise (30 min.) to a stirred suspension of LTA (102.0g, 0.23 mol) in dry benzene (150 mL) at 60-70°C. The mixture was further stirred for 1 h, cooled to room temperature and filtered. Water (4-5 mL) was added with shaking to the filtrate and dried over Na_2SO_4. The residue obtained on evaporation of solvent was digested in acetic acid (130 mL) at room temperature for 1 h. Dilution with water followed by repeated extraction with pet.ether (4x200 mL) afforded an extract which on a standard work-up yielded verbenyl acetate **50**. b.p. 110-120°/7mm, 27.5g, 55%.

(ii) *trans*-verbenol **51** : Aqueous KOH (11.9g. in 60 mL H_2O) was added to a methanolic solution of the acetate **50** (26.0g, 0.13 mol in 60 mL methanol). The mixture was left in refrigerator for two days. The residue obtained on evaporation of methanol was taken in water and extracted repeatedly with pet.ether (4x150 mL). A standard work-up of the combined organic extract furnished **51** as an oily product. b.p. 110-115°/9mm, 17.3g, 85%, $[\alpha]_D$ - 102.8 ($CHCl_3$, c 2.2). Overall yield based on α-pinene, 47%.

IR, neat: cm^{-1} 3340, 1660, 1380, 940, 860.

^{1}H-NMR (200 MHz): δ 0.82 (s, 3H), 1.3 (s, 3H), 1.74 (s, 3H), 4.26 (m, 1H), 5.35 (m, 1H) [lit.[19]].

2. Mitsunobu reaction of *cis*-verbenol

(i) Diethylazadicarboxylate DEAD (2.26 mL, 14.5 mmol) was added to the mixture of **54** (0.45g, 2.96 mmol), triphenylphosphine (3.80g, 14.5 mmol), *p*-nitrobenzoic acid (2.1g, 12.7

mmol) in dry benzene (60 mL) and stirred at room temperature for 14 h. Removal of the volatile components under reduced pressure yielded a residue which was purified by column chromatography.

The residue (0.95g.) adsorbed on silica gel (60-120 mesh, 3g.) was loaded on a column packed with column grade silica gel (40 g). Progress of the separation was followed by tlc. Details of the chromatographic separation are given in Table-I.

Table-I: Chromatographic separation of compound **57**

Eluent Ethyl acetate:Pet.ether	Total volume collected	Final fraction	Approximate composition
2:98	4x50 mL	A	Less polar impurities
4:96	4x50 mL	B	Unidentified compounds
6:94	8x50 mL	C	Desired compound **57**, unreacted Ph_3P and unidentified compounds
10:90	8x50 mL	D	Unidentified compounds

Fraction C containing the required compound **57** on further chromatographic purification yielded **57** (80% pure by NMR), m.p. 70-72°C, 0.18g, 20%.

IR (Fig.6), $CHCl_3$: cm^{-1} 1720, 1615, 1540, 1365, 1300.

^{1}H-NMR (Fig.7), (200 MHz): δ 1.0 (s, 3H), 1.32 (s, 3H), 1.75 (s, 3H), 5.45 (m, 1H), 5.60 (br s, 1H), 8.28 (m, 4H).

^{13}C-NMR (Fig.8), (50.13 MHz): δ 44.0 (d, C_1), 150.8 (s, C_2), 122.9 (d, C_3), 72.8 (d, C_4), 41.0 (d, C_5), 38.4 (s, C_6), 31.7 (t, C_7), 26.4 (q, C_8), 21.0 (q, C_9), 21.4 (q, C_{10}), 130.8, 123.8 (aromatic C-H), 142.7, 136.1 (aromatic -C-), 165 (s, CO).

(ii) Compound **57** (0.15g, 0.5 mmol) in 4 mL methanol was treated with aqueous KOH (0.05g. in 4 mL water) and the reaction mixture was kept in refrigerator for two days. Evaporation of methanol yielded a residue which was diluted with water and extracted with pet.ether (4x6 mL). Standard work-up of the combined organic extract followed by preparative thin layer

chromatography (ptlc) [ethyl acetate:pet.ether, 15:25] furnished *trans*-verbenol **51**; 40 mg, overall yield 10% based on *cis*-verbenol. The product was identified by comparison of the spectral data with that of known compound.

(-)-(S)-2,2,4-trimethyl-3-cyclohexene-1-carbaldehyde, 6 : *Trans*-verbenol **51** on thermolysis, followed by column chromatographic separation furnished **6**. The thermolysis unit consisted of (i) a quartz column of length 100.0 cm, i.d. 1.2 cm packed with quartz helices, (ii) a dropping funnel fitted on the top of the column along with a nitrogen inlet and (iii) a collecting trap at the bottom of the column kept in ice-salt mixture. In a typical experiment, a 2% solution of *trans*-verbenol **51** (10.0g.) in dry pet.ether was dropped slowly over the quartz column at 350°C in a slow current of N_2. Progress of the reaction was monitored by GLC. [SE-30 10%, 90°C (6 min) $\xrightarrow{10°/min}$ 150°C (5 min)]. Column chromatographic separation of the crude pyrolysate yielded the aldehyde **6**.

Concentrated pyrolysate (9.0g.) was loaded on a column of silica gel (60-120 mesh, 270g.). The chromatography was monitored by tlc, details of which are given in Table-II.

Table-II: Chromatographic separation of crude pyrolysate

Eluent, Ethyl acetate: pet.ether	Total volume collected	Final fraction	Approximate composition
00:100	4x100 mL	A	Straight chain compounds
1:99	4x100 mL	B	Compound **6a**; less polar than the required aldehyde and other compounds
1.5:98.5	6x100 mL	C	Required aldehyde **6**.
2:98	4x100 mL	D	Compound **6b**; more polar than **6** and other compounds.
4:96	6x100 mL	E	Compound **6c** and other compounds

Fraction C on evaporation of the solvent yielded **6**, 2.5g, 25% isolated yield.

IR (Fig.1), neat: cm^{-1} 2710, 1720, 1670.

^{1}H-NMR (Fig.2), (80 MHz): δ 0.97 (s, 3H), 1.13 (s, 3H), 1.68 (s, 3H), 5.08 (m, 1H), 9.80 (d, 1H) [lit.[20]].

Limonen-5-βol, 6b: A rechromatographic separation of fraction D (Table-II) yielded **6b**, 80 mg (GLC purity 80%).

IR (Fig.3), $CHCl_3$: cm^{-1} 3430, 3070, 1660, 890, 800.

^{1}H-NMR (Fig.4), (200 MHz): δ 1.67 (s, 3H), 1.71 (s, 3H), 4.10 (m, 1H), 4.85 (m, 2H), 5.43 (m, 1H).

Bicyclo [3.1.1] hept-3-en-2-one, 4,6,6-trimethyl (verbenone), 44:

Trans-verbenol **51** on oxidation furnished verbenone. Brown's reagent[17c] (4.8 mL, 10 mmol) [prepared by mixing 10.0g. $Na_2Cr_2O_7$, 30 mL water, 13.6g. H_2SO_4 (97%) and diluting the solution to 50 mL] was added dropwise to the stirred solution of **51** (1.2g, 8 mmol) in 80 mL ether at 0°C. The ether layer was separated on stirring the solution for 1 h. and combined with ether washings (4x15 mL) of the aqueous layer. Usual work-up of the ether extract yielded verbenone **44** b.p. 170-180°/20mm, 0.65g, 55%.

IR, neat: cm^{-1} 1690, 1630.

^{1}H-NMR (200 MHz): δ 0.97 (s, 3H), 1.4 (s, 3H), 1.9 (s, 3H), 5.64 (m, 1H). [lit.[17d]].

***Cis*-verbenol, 54**

(i) Reduction of verbenone with LAH[25]

Verbenone **44** (0.45g, 3.0 mmol) in dry ether (10 mL) was added dropwise (~ 10 min) to the suspension of LAH (0.06g., 1.6 mmol) in dry ether (25 mL) at 0°C and stirred for 2 h. To it were added sequentially water (1 mL), 15% aq. NaOH (1 mL) and water (3 mL) and stirred further for 1.5 h. Filtration of the reaction mixture and routine work-up of the filtrate furnished **54**. m.p. 64-66°C, 0.340g, 75% [Lit.[25]].

IR, $CHCl_3$: cm^{-1} 3340, 1660, 1400, 1020, 960.

^{1}H-NMR (Fig.5), (200 MHz): δ 1.05 (s, 3H), 1.32 (s, 3H), 1.70 (s, 3H), 4.45 (br s, 1H), 5.32 (br s, 1H).

(ii) Reduction of verbenone with Luche's reagent ($CeCl_3$-$NaBH_4$)[22]

$CeCl_3.7H_2O$ (1.58g., 4.2 mmol) was added to the solution of **44** (0.64g., 4.2 mmol) in methanol (40 mL) at 0°C, followed by addition of $NaBH_4$ (0.16g, 4.2 mmol) in methanol (30 mL). After stirring the reaction mixture for 2 h, water (2-3 mL) was added. Extraction with ether and standard work-up of the organic layer yielded **54**. m.p. 63-65°C, 0.55g, 85%. [lit.[25]].

Ortho-bromobenzaldehyde, 7[31]

(i) *Ortho*-bromotoluene, **69**: A solution of 9.4g. (0.088 mol) of *ortho*-toluidine **68** in 40% aq. HBr (51 mL) at 0-5°C was treated with $NaNO_2$ (6.9g, 0.1 mol), added in small portions maintaining the temperature below 10°C. After the addition, Cu-powder (0.3g.) was added and the reaction mixture was heated cautiously on a steam bath for 1.5 h. Extraction of the cooled reaction mixture with CH_2Cl_2 (4x150 mL), followed by a standard work-up of the organic layer yielded **69**. b.p. 100-108°/10mm, 6.7g, 45%.

(ii) *Ortho*-bromobenzylidene dibromide, **70**: A mixture of **69** (6.25g, 0.037 mol), powdered N-bromosuccinimide (13.4g, 0.075 mol) and benzoyl peroxide (100 mg) in dry CCl_4 (120 mL) was heated under reflux for 28 h. while irradiating with a tungsten filament lamp, (500 Watt). The progress of the reaction was monitored by ^{1}H-NMR and its completion was ensured by the disappearance of the singlet at δ 4.50 ($\underline{CH_2}Br$) in ^{1}H-NMR spectrum. Filtration of the reaction mixture, followed by standard work-up of the organic filtrate yielded **70**. b.p. 110-120°/0.5mm, 10.2g, 85%.

(iii) *Ortho*-bromobenzaldehyde, **7**: Compound **70** (10.0g., 0.03 mol) in acetone (64 mL) was mixed with a solution of $AgNO_3$ (10.2g., 0.06 mol) in water (18 mL). The mixture after stirring for 40 min. was filtered. Extraction of the filtrate with ether (4x150 mL), followed by routine

work-up of the ether layer afforded **7**. b.p. 75-85°/0.5mm, 4.7g., 85%. The overall yield based on *ortho*-toluidine was 32%.

IR, neat: cm^{-1} 2820, 2710, 1680, 1605, 830, 760.

^{1}H-NMR (90 MHz): δ 7.25-8.1 (m, 4H), 10.3 (s, 1H).

Compound **7** (4.6g, 0.025 mol) in dry benzene (25 mL), ethylene glycol (1.9g, 0.03 mol) and *p*-toluenesulphonic acid (100 mg) were heated in a Dean-Stark apparatus for 10 h. and the reaction mixture was left at 25°C for 12 h. It was then washed successively with 2N NaOH (3x12 mL) and brine (3x12 mL). The organic layer yielded the acetal **71**. b.p. 120-130°/0.4mm, 4.90g., 87%. **^{1}H-NMR** (Fig.9) (80 MHz) δ 3.90 (m, 4H), 6.01 (s, 1H), 7.0-7.6 (m, 4H).

Compound 72: In a 100 mL three necked flask equipped with a N_2 inlet and a reflux condenser were placed Mg turnings (0.21g, 8.8 mmol), a pinch of iodine and dry ether (25 mL). Compound **71** (1.0g, 4.4 mmol) was added in portions along with a small quantity of 1,2-dibromoethane as initiator. The reaction mixture was gradually warmed and refluxed for 30 min. Ether was then evaporated, followed by slow addition (~ 10 min) of compound (±) **6** (0.55g., 3.6 mmol) in 30 mL dry THF. The reaction mixture was stirred for 10 h. at room temperature, treated with aq. NH_4Cl solution and extracted with ether. Standard work-up of the ether layer furnished the residue (1.1g.) which was purified by column chromatography. The residue was loaded on a column of silica gel (60-120 mesh, 45g.) and chromatographed using acetone:pet.ether as eluent, polarity of which was increased successively. Details of the chromatographic separation are given in Table-III.

Fraction D (Table III) on evaporation of solvent furnished a solid which was crystallised to yield **72** (0.270g.), m.p. 152-154°C, isolated yield 25%

IR (Fig.10), $CHCl_3$: cm^{-1} 3480, 1630, 1600.

^{1}H-NMR (Fig.11), (200 MHz): δ 1.06 (s, 3H), 1.10 (s, 3H), 1.65 (s, 3H), 4.12 (m, 4H), 5.05 (d, 1H, J=<1 Hz), 5.50 (s, 1H), 6.05 (s, 1H), 7.25-7.70 (m, 4H).

^{13}C-NMR (Fig.12a,b), (50.13 MHz): Table-3

MS (Fig.13), m/z (rel.int.): 302(4), 284(4), 240(45), 204(18), 179(65), 134(100), 123(38), 77(36).

Anal. Found: Carbon (74.58%), Hydrogen (8.95%)

Calculated: Carbon (75.44%), Hydrogen (8.62%)

Table-III: Chromatographic separation of compound **72**

Eluent Acetone: pet.ether	Total volume collected	Final fraction	Approximate composition
00:100	4x50 mL	A	Mixture of two to three compounds
2:98	5x50 mL	B	Unidentified compounds
4:96	6x50 mL	C	Complex mixture
6:94	8x50 mL	D	Compound **72**
8:92	4x100 mL	E	Unidentified compounds

Preparation of optically active 72: Grignard reaction of (-) **6** (0.300g, 2.0 mmol) and **71** (0.550g, 2.4 mmol) was carried out following the procedure as given for **72** from racemic aldehyde **6**. The crude reaction mixture (0.85g.) on column chromatographic separation yielded a liquid, 0.130g, 22% isolated yield.

^{1}H-NMR (Fig.16), (90 MHz): δ 0.90 (s, 3H), 1.10 (s, 3H), 1.58, 1.64 (s, 3H), 4.0 (m, 4H), 4.80 (m) and 5.04 (s) (1H), 5.70, 5.98 (s, 1H), 7.04-7.47 (m, 4H).

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