

PHYTOCHEMISTRY AND BIOACTIVITIES OF
SELECTED PLANTS BELONGING TO *ERYTHRINA*,
EUPHORBIA AND *SORBUS* GENERA



By

ABDUL LATIF

Dissertation submitted to the University of Peshawar in partial fulfillment of the
requirements for the Degree of Doctor of Philosophy in Chemistry

INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR, PESHAWAR
PAKISTAN
(APRIL, 2012)

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DECLARATIONS

This is to certify that this dissertation prepared by Mr. Abdul Latif entitled "Phytochemistry and Bioactivities of Selected Plants Belonging to Erythrina, Euphorbia and Sorbus Genera" is accepted in the present form by the Institute of Chemical Sciences, University of Peshawar as fulfilling this part of the requirements for the degree

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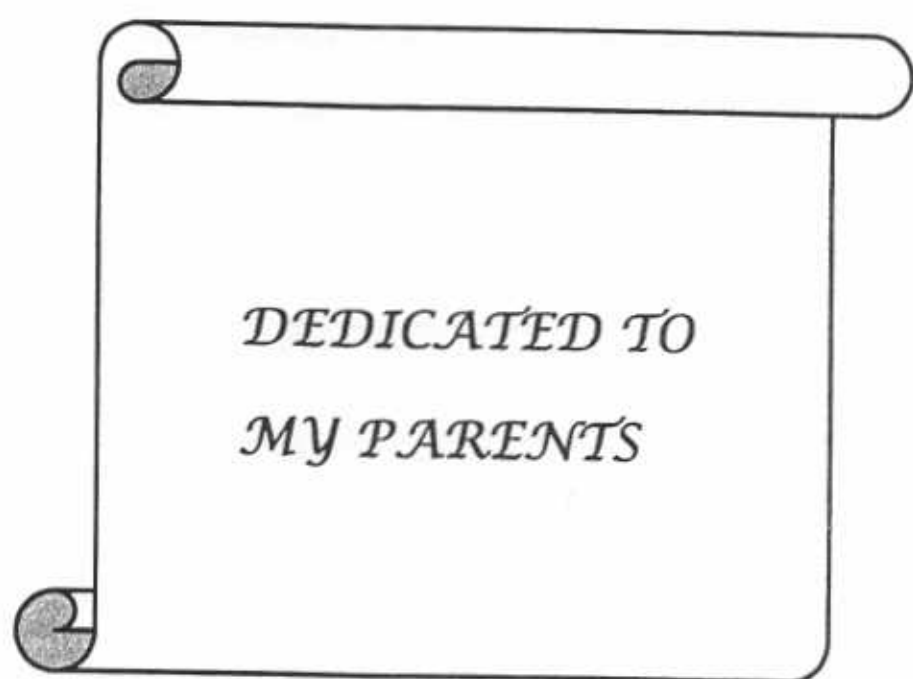
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CONTENTS

Acknowledgments	i
Abstract	iii
List of Abbreviations	vi
List of Schemes	viii
List of Tables	x
List of Figures	xii
Chapter 1 GENERAL INTRODUCTION.....	1
Chapter 2 BIOSYNTHESIS.....	5
2.1. Alkaloids	5
2.1.1. Biosynthesis of benzyloisoquinoline alkaloids	5
2.1.2. Biosynthesis of erythrina alkaloids	10
2.2. Terpenoids	12
2.2.1. Biosynthesis of diterpenoids and triterpenoids.....	12
2.2.1.1. Formation of isopentene unit	13
2.2.1.2. Formation of acyclic terpenoids from isopentene unit	15
2.2.1.3. Conversion of acyclic terpenoids to diterpenes	18
2.2.1.4. Conversion of acyclic terpenoids to triterpenes	22
2.2.1.4.1. Cyclization involving oxidation of the terminal part of squalene	22
2.2.1.4.2. Cyclization without oxidation of the terminal part of squalene	32
2.2.1.4.3. Cyclization at both terminal double bonds of squalene.....	33
2.3. Biosynthesis of lignans	34
Chapter 3 INTRODUCTION (Part-A)	38
3.1. Family Papilionaceae (Faboideae)	38
3.2. Genus <i>Erythrina</i>	38

3.3. <i>Erythrina suberosa</i> Roxb. (Gul-e-Nishtar).....	38
3.4. Pharmacological importance of genus <i>Erythrina</i>	39
3.5. Literature survey of genus <i>Erythrina</i>	41
Chapter 4	
RESULTS AND DISCUSSION (Part-A)	50
4.1. Structure elucidation of the isolated compounds from <i>E. suberosa</i>	51
4.1.1. Erysodine (89)	51
4.1.2. Erysotrine (90)	53
4.1.3. Erytharbine (91)	55
4.1.4. Erysotramidine (92)	57
4.1.5. Erythartine (93).....	59
4.1.6. Alpinumisoflavone (94)	61
4.2. Anxiolytic activity of erysodine (89) and erysotrine (90)	64
4.2.1. Elevated plus-maze (EPM)	64
4.2.2. Light dark transition model (LTDM)	64
4.2.3. Explanation of results obtained from EPM and LTDM	65
Chapter 5	
EXPERIMENTAL (Part-A)	69
5.1. General experimental conditions	69
5.1.1. Physical constants	69
5.1.2. Spectroscopic techniques	69
5.1.3. Chromatographic techniques	69
5.2. <i>Erythrina suberosa</i>	70
5.2.1. Plant material	70
5.2.2. Extraction and isolation	70
5.3. Characterization of known compounds from <i>Erythrina suberosa</i>	74
5.3.1. Erysodine (89).....	74
5.3.2. Erysotrine (90)	74
5.3.3. Erytharbine (91).....	75
5.3.4. Erysotramidine (92)	75
5.3.5. Erythartine (93).....	76
5.3.6. Alpinumisoflavone (94)	76
5.4. Anxiolytic activity	77
5.4.1. Subjects	77
5.4.2. Compound administration	77

5.4.3. Apparatus	77
5.4.4. Procedure	78
5.4.5. Statistics	78
Chapter 6	
INTRODUCTION (Part-B)	79
6.1. Family Euphorbiaceae	79
6.2. Genus <i>Euphorbia</i>	79
6.3. <i>Euphorbia neriifolia</i> Linn. (Hedge Euphorbia).....	80
6.4. Pharmacological importance of <i>Euphorbia neriifolia</i>	80
Chapter 7	
RESULTS AND DISCUSSION (Part-B)	88
7.1. Structure elucidation of new diterpenoids from <i>E. neriifolia</i>	89
7.1.1. <i>Ent</i> -3,4-seco-4,16 β ,17-trihydroxykauran-3-oic acid (95)	89
7.1.2. <i>Ent</i> -16 β -hydroxykauran-3,4-lactone (96)	92
7.1.3. 13 β ,19-Dihydroxy-3,15-dioxoatis-16-ene (97).....	96
7.1.4. 13 β -Hydroxy-3,15-dioxoatis-16-ene (98).....	99
7.1.5. 16 β ,17,19-Trihydroxy-3-oxo-atisane (99)	102
7.1.6. 4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid (100).....	105
7.1.7. 4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid methyl ester (101)	109
7.1.8. 1 α ,3 α -Dihydroxy- <i>ent</i> -abieta-8(14),13(15)-dien-16,12-olide (102)	112
7.2. Known compounds isolated from <i>E. neriifolia</i>	115
7.2.1. <i>Ent</i> -16 α ,17-Dihydroxyatisan-3-one (103)	115
7.2.2. (16 <i>R</i>)-16,17-Dihydroxy- <i>ent</i> -kauran-3-one (104).....	116
7.2.3. Ingenol (105).....	118
7.2.4. (23 <i>E</i>)-Eupha-8,23-diene-3 β ,25-diol-7,11-dione (106)	120
7.2.5. (23 <i>E</i>)-Eupha-8,23-diene-3 β ,25-diol-7-one (107).....	123
7.2.6. 3,7,12-Triacetyl-8-benzoylingol (108).....	125
7.2.7. (+)-Diasyringaresinol (109)	127
7.2.8. Palmarumycin JC2 (110)	128
7.2.9. (3 <i>R</i> ,5 <i>R</i> ,6 <i>R</i> ,7 <i>E</i>)3,5,6-Trihydroxy-7-megastigmen-9-one (111)	130
Chapter 8	
EXPERIMENTAL (Part-B)	133
8.1. General experimental conditions	133
8.1.1. Physical constants	133
8.1.2. Spectroscopic techniques	133
8.1.3. Chromatographic techniques	133

8.2. <i>Euphorbia neriifolia</i> Linn.....	134
8.2.1. Plant material	134
8.2.2. Extraction and isolation	134
8.3. Characterization of new compounds from <i>E. neriifolia</i>	137
8.3.1. <i>Ent</i> -3,4-seco-4,16 β ,17-trihydroxykauran-3-oic acid (95)	137
8.3.2. <i>Ent</i> -16 β -hydroxykauran-3,4-lactone (96)	137
8.3.3. 13 β ,19-Dihydroxy-3,15-dioxoatis-16-ene (97).....	138
8.3.4. 13 β -Hydroxy-3,15-dioxoatis-16-ene (98).....	138
8.3.5. 16 β ,17,19-Trihydroxy-3-oxo-atisane (99)	139
8.3.6. 4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid (100).....	139
8.3.7. 4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid methyl ester (101)	140
8.3.8. 1 α ,3 α -Dihydroxy- <i>ent</i> -abieta-8(14),13(15)-dien-16,12-olide (102)	140
8.4. Characterization of known compounds from <i>E. neriifolia</i>	141
8.4.1. <i>Ent</i> -16 α ,17-Dihydroxyatisan-3-one (103)	141
8.4.2. (16 <i>R</i>)-16,17-Dihydroxy- <i>ent</i> -kauran-3-one (104).....	141
8.4.3. Ingenol (105).....	141
8.4.4. (23 <i>E</i>)-Eupha-8,23-diene-3 β ,25-diol-7,11-dione (106)	142
8.4.5. (23 <i>E</i>)-Eupha-8,23-diene-3 β ,25-diol-7-one (107).....	142
8.4.6. 3,7,12-Triacetyl-8-benzoylingol (108).....	143
8.4.7. (+)-Diasyringaresinol (109)	143
8.4.8. Palmarumycin JC2 (110)	144
8.4.9. (3 <i>R</i> ,5 <i>R</i> ,6 <i>R</i> ,7 <i>E</i>)3,5,6-Trihydroxy-7-megastigmen-9-one (111)	144
8.5. Single-crystal X-ray structure determination of <i>ent</i> -3,4-seco-4,16 β ,17- trihydroxykauran-3-oic acid (95).....	144
8.6. X-ray single-crystal structure determination of 13 β ,19-dihydroxy-3,15- dioxoatis-16-ene (97).....	145
Chapter 9	
INTRODUCTION (Part-C)	146
9.1. Family Rosaceae	146
9.2. Genus <i>Sorbus</i>	146
9.3. <i>Sorbus lanata</i> (D. Don) Schauer	147
9.4. Pharmacological importance of genus <i>Sorbus</i>	147
9.5. Literature survey on <i>Sorbus lanata</i>	148
9.6. Structures of compounds isolated from genus <i>Sorbus</i>	149

Chapter 10

RESULTS AND DISCUSSION (Part-C)	152
10.1. Structure elucidation of new compounds from <i>S. lanata</i>	153
10.1.1. Sorlanin (112)	153
10.1.2. Peshawerin (113)	157
10.1.3. Sorbanin (114)	163
10.1.4. Sorbanolic acid (115)	167
10.2. Known compounds from <i>S. lanata</i>	173
10.2.1. Polystachyol (116)	173
10.2.2. Isolariciresinol (117)	175
10.2.3. Dihydrodehydrodiconiferyl alcohol (118)	177
10.2.4. Tuberculatin (119)	179
10.2.5. Ovafofinin E (120)	181
10.2.6. β -Pyrofuran (121)	183
10.2.7. Lyoniside (122)	186
10.2.8. 2'-Methoxyaucuparin (123)	188
10.2.9. Aucuparin (124)	189
10.2.10. Tetracosyl-3-(3,4-dihydroxyphenyl)acrylate (125)	191
10.2.11. 3 β ,23-Dihydroxy-lup-20(29)ene-28-oic acid-3 β -caffeate (126)	192
10.3. Diphenylpicrylhydrazyl (DPPH) radical scavenging activity	196

Chapter 11

EXPERIMENTAL (Part-C)	198
11.1. General experimental conditions	198
11.1.1. Physical constants	198
11.1.2. Spectroscopic techniques	198
11.1.3. Chromatographic techniques	199
11.2. <i>Sorbus lanata</i>	199
11.2.1. Plant Material	199
11.2.2. Extraction and isolation from the wood of <i>S. lanata</i>	200
11.3. Characterization of new compounds from <i>Sorbus lanata</i>	204
11.3.1. Sorlanin (112)	204
11.3.2. Peshawerin (113)	204
11.3.3. Sorbanin (114)	205
11.3.4. Sorbanolic acid (115)	205
11.4. Characterization of known compounds from <i>Sorbus lanata</i>	206
11.4.1. Polystachyol (116)	206
11.4.2. Isolariciresinol (117)	206

11.4.3. Dihydrodehydrodiconiferyl alcohol (118)	207
11.4.4. Tuberculatin (119)	207
11.4.5. Ovafolinin E (120)	208
11.4.6. β -Pyrofuran (121)	208
11.4.7. Lyoniside (122)	209
11.4.8. 2'-Methoxyaucuparin (123)	209
11.4.9. Aucuparin (124)	209
11.4.10. Tetracosyl-3-(3,4-dihydroxyphenyl)acrylate (125)	210
11.4.11. 3 β ,23-Dihydroxy-lup-20(29)ene-28-oic acid-3 β -caffeate (126)	210
11.5. Diphenylpicrylhydrazyl (DPPH) radical-scavenging assay	211
REFERENCES	212
LIST OF PUBLICATIONS	242

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ABSTRACT

The research work included in the present dissertation is mainly focused on the isolation and structure elucidation of pure chemical constituents from the selected plants belonging to *Erythrina*, *Euphorbia* and *Sorbus* genera. Moreover, biological activities have also been incorporated for the isolated compounds. There are three main parts of the dissertation:

Part-A: Phytochemistry and Bioactivity of Genus *Erythrina*

Part-B: Phytochemistry of Genus *Euphorbia*

Part-C: Phytochemistry and Bioactivity of Genus *Sorbus*

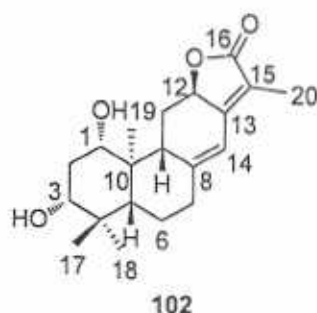
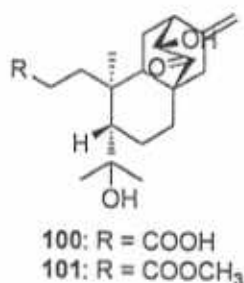
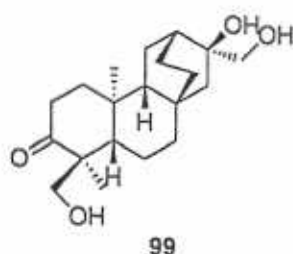
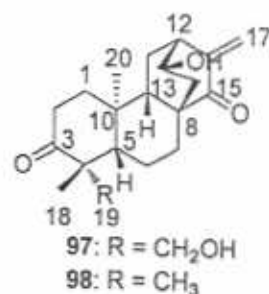
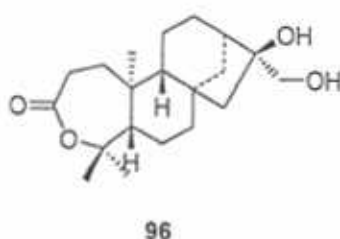
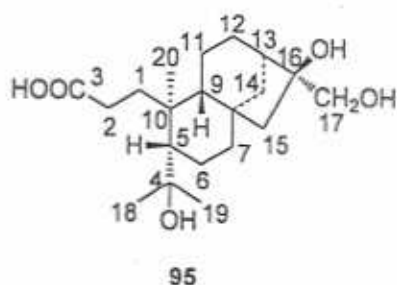
Part-A

This part describes the phytochemical investigation on *Erythrina suberosa* (Papilionaceae), a cultivated ornamental plant in Pakistan. Six known compounds including alkaloids (89-93) along with a known isoflavone (94) were isolated from the plant. Various spectroscopic techniques were used to characterize their structures. Alkaloids 89 and 90 were also tested for their anxiolytic properties (*Quim. Nova* 2011, 34, 808-811) in two well-known tests of anxiety-the elevated plus-maze (EPM) and light dark transition model (LDTM).

Part-B

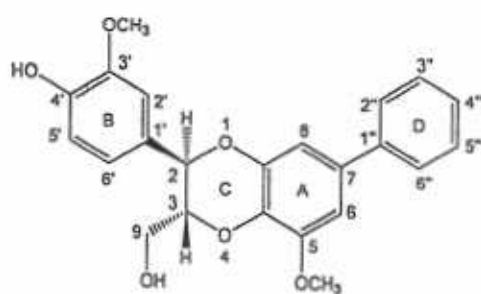
In this part of the work, the discussion is aimed at describing the isolation, purification and structure elucidation of eight new diterpenes (95-102, *Phytochemistry* 2012, 75, 153-158) and nine known compounds (103-111) from *Euphorbia neriifolia*

(Euphorbiaceae). Different physical characteristics and extensive spectroscopic studies were used for the structure elucidation of the isolated and purified compounds.

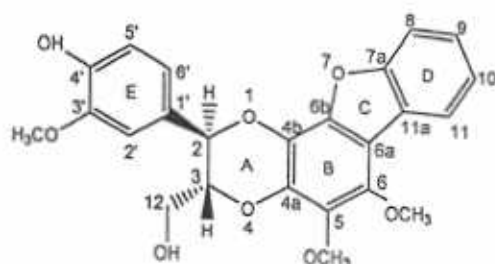


Part-C

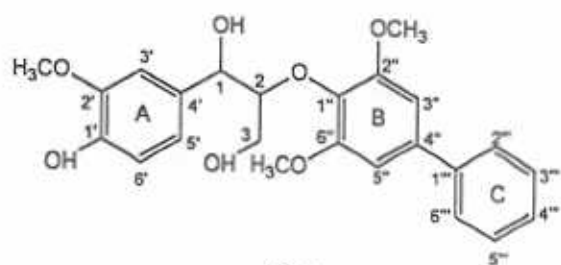
This is the last part of the dissertation, describing the phytochemistry of *Sorbus lanata* (Rosaceae). Four new compounds (112-115) and eleven known compounds (116-126) were isolated from the selected plant. Advanced spectroscopic techniques were used to determine the structures of these compounds. The isolated compounds were also evaluated for their radical scavenging activities in the diphenylpicrylhydrazyl (DPPH) assay using ascorbic acid as a standard.



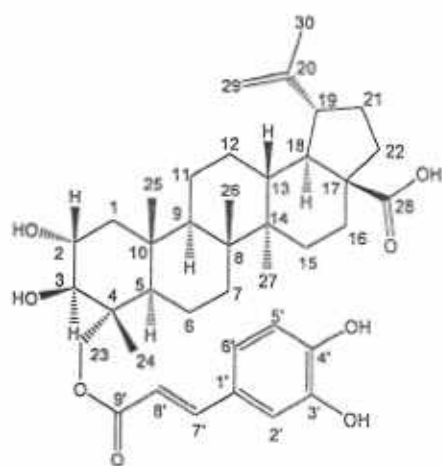
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LIST OF ABBREVIATIONS

Ar	Aryl
AcO	Acetate
BB	Broad Band
COSY	Correlation Spectroscopy
D	Deuterium
DCM	Dichloromethane
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic Acid
DPPH	Diphenylpicrylhydrazyl
EIMS	Electron impact mass spectrometry
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HSQC	Heteronuclear single quantum coherence
HRESIMS	High resolution electrospray ionization mass spectrometry
Hz	Hertz
¹ H-NMR	Hydrogen-1-nuclear magnetic resonance
¹³ C-NMR	Carbon-13-nuclear magnetic resonance
IC ₅₀	Inhibitory concentration 50 percent
IR	Infrared
J	Coupling constant
mM	Millimole
m/z	Mass to charge ratio
Me	Methyl
MeO	Methoxy
NOE	Nuclear overhauser effect

NOESY	Nuclear overhauser effect spectroscopy
<i>p</i>	Probability
<i>v</i>	Wave number
<i>p.o</i>	Per oral
i. p	Intraperitoneal
EPM	Elevated plus-maze
LDTM	Light dark transition model
DZP	Diazepam

LIST OF SCHEMES

Scheme-2.1: Formation of norlaudanosoline	7
Scheme-2.2: Formation of protopine, phthalideisoquinoline and benzophenanthridine alkaloids	8
Scheme-2.3: Isotopic labeling of different alkaloids using [α - ^{14}C] DL-tyrosine	9
Scheme-2.4: Biosynthesis of erythraline	11
Scheme-2.5: Formation of (<i>R</i>)-mevalonic acid	13
Scheme-2.6: Biosynthesis of DMAPP from (<i>R</i>)-mevalonic acid	14
Scheme-2.7: Formation of NPP and GPP	15
Scheme-2.8: Formation of citronellal in isotopic labeling experiment	16
Scheme-2.9: Formation of FPP, squalene and GGPP	17
Scheme-2.10: Formation of cembrene, verticillol, casbene, lathyrol and phorbol	19
Scheme-2.11: Conversion of GGPP to selerol, manool and labdanes	20
Scheme-2.12: Conversion of manool to abietadiene and cassaine	21
Scheme-2.13: Conversion of pimarane carbocation to beyerene, kaurene, atisirene, phyllocladane and pentacyclic trachylobane	23
Scheme-2.14: Conversion of kaurane to gibberellane, grayanotoxane and enmeane	24
Scheme-2.15: Conversion of squalene epoxide to dammerenediol	25
Scheme-2.16: Formation of euphol and tirucallol	26
Scheme-2.17: Formation of lupeol, taraxasterol and α -amyirin	27
Scheme-2.18: Formation of taraxerol, β -amyirin, friedeline and δ -amyirin	28
Scheme-2.19: Formation of lanosterol	29
Scheme-2.20: Formation of cycloartenol	30
Scheme-2.21: Formation of hopanone, arborinol and moretenol	31
Scheme-2.22: Formation of tetrahymanol and diploptene	32

Scheme-2.23: Formation of onocerin and ambrein	33
Scheme-2.24: Phenylpropanoid and monolignol pathway	34
Scheme-2.25: Biosynthetic pathway of podophyllotoxin and related lignans	36
Scheme-2.26: Lignans possibly derived from matairesinol	37
Scheme-5.1: Extraction of alkaloidal fraction from the crude extract of <i>E. suberosa</i>	72
Scheme-5.2: Isolation of alkaloid compounds (89-93)	73
Scheme-8.1: Extraction and fractionation from <i>E. neriifolia</i>	135
Scheme-8.2: Isolation of pure compounds from ethyl acetate fraction of <i>E. neriifolia</i>	136
Scheme-11.1: Extraction and fractionation from the wood of <i>S. lanata</i>	202
Scheme-11.2: Isolation of pure compounds (112-126) from ethyl acetate fraction of <i>S. lanata</i>	203

LIST OF TABLES

Table-3.1: Compounds isolated from genus <i>Erythrina</i>	41
Table-4.1: ^1H - and ^{13}C -NMR chemical shifts of (89)	53
Table-4.2: ^1H - and ^{13}C -NMR chemical shifts of (90)	55
Table-4.3: ^1H - and ^{13}C -NMR chemical shifts of (91)	57
Table-4.4: ^1H - and ^{13}C -NMR chemical shifts of (92)	59
Table-4.5: ^1H - and ^{13}C -NMR chemical shifts of (93)	61
Table-4.6: ^1H - and ^{13}C -NMR chemical shifts of (94)	63
Table-6.1: List of diterpenoids isolated from genus <i>Euphorbia</i>	81
Table-6.2: List of triterpenoids isolated from genus <i>Euphorbia</i>	85
Table-7.1: ^1H - and ^{13}C -NMR chemical shifts of (95)	91
Table-7.2: ^1H - and ^{13}C -NMR chemical shifts of (96)	95
Table-7.3: ^1H - and ^{13}C -NMR chemical shifts of (97)	98
Table-7.4: ^1H - and ^{13}C -NMR chemical shifts of (98)	101
Table-7.5: ^1H - and ^{13}C -NMR chemical shifts of (99)	104
Table-7.6: ^1H - and ^{13}C -NMR chemical shifts of (100)	108
Table-7.7: ^1H - and ^{13}C -NMR chemical shifts of (101)	111
Table-7.8: ^1H - and ^{13}C -NMR chemical shifts of (102)	114
Table-7.9: ^1H - and ^{13}C -NMR chemical shifts of (103)	116
Table-7.10: ^1H - and ^{13}C -NMR chemical shifts of (104)	118
Table-7.11: ^1H - and ^{13}C -NMR chemical shifts of (105)	120
Table-7.12: ^1H - and ^{13}C -NMR chemical shifts of (106)	122
Table-7.13: ^1H - and ^{13}C -NMR chemical shifts of (107)	124
Table-7.14: ^1H - and ^{13}C -NMR chemical shifts of (108)	126
Table-7.15: ^1H - and ^{13}C -NMR chemical shifts of (109)	128

Table-7.16: ^1H - and ^{13}C -NMR chemical shifts of (110)	130
Table-7.17: ^1H - and ^{13}C -NMR chemical shifts of (111)	132
Table-9.1: List of compounds isolated from genus <i>Sorbus</i>	148
Table-10.1: ^1H - and ^{13}C -NMR chemical shifts of (112)	157
Table-10.2: ^1H - and ^{13}C -NMR chemical shifts of (113)	162
Table-10.3: ^1H - and ^{13}C -NMR chemical shifts of (114)	167
Table-10.4: ^1H - and ^{13}C -NMR chemical shifts of (115)	171
Table-10.5: ^1H - and ^{13}C -NMR chemical shifts of (116)	175
Table-10.6: ^1H - and ^{13}C -NMR chemical shifts of (117)	177
Table-10.7: ^1H - and ^{13}C -NMR chemical shifts of (118)	179
Table-10.8: ^1H - and ^{13}C -NMR chemical shifts of (119)	181
Table-10.9: ^1H - and ^{13}C -NMR chemical shifts of (120)	183
Table-10.10: ^1H - and ^{13}C -NMR chemical shifts of (121)	185
Table-10.11: ^1H - and ^{13}C -NMR chemical shifts of (122)	187
Table-10.12: ^1H - and ^{13}C -NMR chemical shifts of (123)	189
Table-10.13: ^1H - and ^{13}C -NMR chemical shifts of (124)	190
Table-10.14: ^1H - and ^{13}C -NMR chemical shifts of (125)	192
Table-10.15: ^1H - and ^{13}C -NMR chemical shifts of (126)	194
Table-10.16: Antioxidant activity of compounds (112-126)	197

LIST OF FIGURES

Figure-4.1: Selected HMBC interactions of erysodine (89)	52
Figure-4.2: Selected HMBC interactions of alpinumisoflavone (94)	62
Figure 4.3: Effects of erysodine (89) (0, 3 or 10 mg/kg, <i>p.o.</i>), erysotrine (90) (0, 3 or 10 mg/kg, <i>p.o.</i>) and DZP (2 mg/kg, <i>i.p.</i>) on the behavior of mice in the EPM. Data are represented as mean $\pm$ SEM, N = 8-12. * <i>P</i> < 0.05 vs control group.....	67
Figure-4.4: Effects of erysodine (89) (0, 3 or 10 mg/kg, <i>p.o.</i>), erysotrine (90) (0, 3 or 10 mg/kg, <i>p.o.</i>) and DZP (2 mg/kg, <i>i.p.</i>) on the behavior of mice in the LDTM. Data are represented as mean $\pm$ SEM, N = 8-12. * <i>P</i> < 0.05 vs control group	68
Figure-7.1: X-ray single crystal structure of compound (95)	90
Figure-7.2: Selected HMBC correlations of compound (96)	94
Figure-7.3: Selected ROESY correlations of compound (96)	94
Figure-7.4: Selected HMBC correlations of compound (97)	97
Figure-7.5: X-ray single crystal structure of compound (97)	98
Figure-7.6: Selected HMBC correlations of compound (98)	100
Figure-7.7: Selected HMBC correlations of compound (99)	103
Figure-7.8: Selected HMBC correlations of compound (100)	107
Figure-7.9: ROESY correlations of compound (100).....	107
Figure-7.10: Selected HMBC correlations of compound (101)	110
Figure-7.11: Selected HMBC correlations of compound (102)	113
Figure-7.12: ROESY correlations of compound (102).....	114
Figure-10.1: Selected HMBC and H2BC correlations of sorlanin (112)	155
Figure-10.2: Mass fragmentation pattern of sorlanin (112)	155
Figure-10.3: Selected NOESY correlations of sorlanin (112)	156
Figure- 10.4: Mass fragmentation pattern of peshawerin (113)	160
Figure-10.5: Selected HMBC correlations of peshawerin (113)	161

Figure-10.6: Selected NOESY correlations of peshawerin (113)	161
Figure-10.7: Mass fragmentation pattern of sorbanin (114)	166
Figure-10.8: Selected HMBC correlations of sorbanin (114)	166
Figure-10.9: Selected HMBC correlations of sorbanolic acid (115)	170
Figure-10.10: Selected NOESY correlations of sorbanolic acid (115)	171
Figure-10.11: Crystal structure of compound (121)	185
Figure-10.12: DPPH radical scavenging activities of compounds (112-126)	197

Chapter: 1

GENERAL INTRODUCTION

The plant kingdom, a precious gift of almighty Allah, provides the basic needs of life such as food, medicines and shelter to the human beings. Today, various indigenous systems of medicines (Ayurveda, Chinese, Homeopathic, Allopathic and many more) are using plants as sources of drugs.

Modern drug system is based on medicinal plants and plays a vital role for the development of new drugs. A number of plants screening programs have been designed to discover the bioactive chemical constituents in medicinal plants. These molecules have been structurally characterized by various physical and chemical methods. The modern drug system uses these molecules in their recipes as active principles. Also, various synthetic projects are designed to build these biologically active constituents in the laboratories in order to make their availability easier and cheaper.

The uses of plants for the treatment of different diseases have been in practice in different cultures of the world. Even different animals have a tendency to treat themselves by utilizing various portions of some medicinal plants. For example, use of grasses by sick dogs for producing emesis and purgation and the use of *Aspilia* by female chimpanzees. *Aspilia* was later on found to contain kaurenic acid and grandifloric acid which are potent stimulators of uterine contractions.¹

The history of drugs is broadly divided into various stages. The Greek, Indian, Chinese, Sumerian, Egyptian and Assyrian civilizations mark the earlier stage followed by the Roman, Arabian, Medieval, and Modern periods. Rig Veda² written between 4500 and 1600 B.C. is considered to be the earliest record of medicinal uses of plants.

The practice of Greek medicines originated around 460 B. C. Hippocrates³ and Theophrastus⁴ did the major contributions to the Greek system of medicines followed by Dioscorides (60 A. D.), who furnished a highly important pharmacologic treatise of the Greek medicines. Furthermore the Greeks did an important pharmacological compilation of 37 volumes of "Natural History" by Pliny the elder (23-79 A. D.) and 30 volumes by Galen.⁵

The traditional system of Chinese medicines can be traced back to Chen Nung,^{6,7} the Chinese emperor in 3000 B. C. He pointed out many drugs and poisons, which were compiled in "Pen Tsao" meaning "The Great Herbal". He also recorded his observations about the diaphoretic and stimulatory effects of the drug "Ma-Huang". Nagai⁸ was the person who isolated alkaloid ephedrine from the same drug about 5000 years later.

Arabs gave another direction to the field of medicinal sciences. Islamic rules of hygienic life⁹ are mainly based on Al-Quran and Sunnah and are called Tibb-Al-Nabi. Greek system of medicines provided a base to the Arab medicines and a number of Muslim scientists were famous in this field. Rhazes had a great influence on this age. His work was published by the name "Alhvi Kabeer" or "Contentens of Rhazes". Avicenna another famous physician and philosopher explained 760 herbal drugs in his book "Canon of Medicines". This book remained as textbook of medicines in Europe till 17th century. The same book serves as the base of the "Greco-Arabic" system of medicines known as "Tibb-e-Unani". Another famous pharmacist Ibn Al-Baitar described the uses of a number of medicinal plants in his books "Kitab al-Mughni fial Adwiya al-Mufrada" and "Kitab al-Jami fial Adwiya al-Mufrada".

The development of chemotherapy at the start of this century led to an increasing change towards synthetic drugs but most recently, a considerable interest has been developed in natural products because of the importance of some constituents from medicinal plants for the treatment of cardiovascular diseases, mental disorders and cancers.

Lots of efforts have been done in Indo-Pakistan to organize the study of pharmacochemical properties of physiologically active constituents in plants. In this context, the first school of medicine was established in Calcutta by Colonel R. N. Chopra. Later on a research institute was established in 1930 under the supervision of great physician Hakim Ajmal Khan in Unani Tibbi College, Delhi. Dr. Salimuzzan Siddiqui did his research in this institute and isolated a large number of alkaloids from *Raulfia Serpenina* and *Holarrhena antidysentrica*. Amongst these, ajmaline series of alkaloids became famous for the treatment of cardiovascular diseases and mental disorders. This work brought a revival in the field of herbal medicines.

The discovery and importance of antibiotics such as cyclosporine A, mevinolin and ivermectin by natural products screening approaches stimulated an expansion of natural product-based drug discovery efforts in the pharmaceutical industry. The major function of natural products in drug discovery and development has been summarized in reviews presented by Clark¹⁰ in 1996 and Craig et al.¹¹ Some of the most recent publications demanding a progress in the field of natural products include “seeking drugs in natural products”,^{12,13} “new technologies and approaches in natural product drug discovery”¹⁴ and “natural products for the improvement of the quality of life”.¹⁵

The number of compounds isolated from plants ranges in thousands. These include secondary metabolites such as coumarins, flavonoids, isoflavonoids, lignans, saponins, glycosides, terpenoids, alkaloids, essential oils etc. Natural products chemists are mainly interested in exploring bioactive compounds with therapeutic effects. A very small number of plants out of 250,000-500,000 have been subjected to phytochemical investigation and biological screening. As each plant may contain thousands of compounds with different biological activities, an interest exists in screening plants for their therapeutic agents. For this purpose, it is required to isolate pure compounds from plants by different separation techniques such as thin layer chromatography (TLC), High Performance Liquid Chromatography (HPLC), Column Chromatography, Gas Liquid Chromatography (GLC) etc. and characterize them structurally. Also, chemical synthesis can be used to make those compounds which are in minor amounts to make them available for pharmaceutical industry. The combined effort of phytochemical investigations and synthetic chemistry led to the development of pharmaceutical industries all over the world.

Our continuous efforts are directed to investigate the indigenous flora enriched with valuable chemicals of medicinal importance, food, cosmetics and new materials. The research proposal of this PhD dissertation is focused on phytochemistry and bioactivity of some selected plants belonging to three genera namely *Erythrina*, *Euphorbia* and *Sorbus*.

Chapter: 2

BIOSYNTHESIS

2.1. Alkaloids

The word “alkaloid” was suggested by Meisner in 1819. The origin of this word lies in the Arabic word al-qali, the name of the plant from which soda was first obtained. Initially the word alkaloid was applied for all those organic compounds having basic properties. Alkaloids have highly complex molecular structures with most of them having significant pharmacological activities.

Alkaloids have been classified into the following categories:

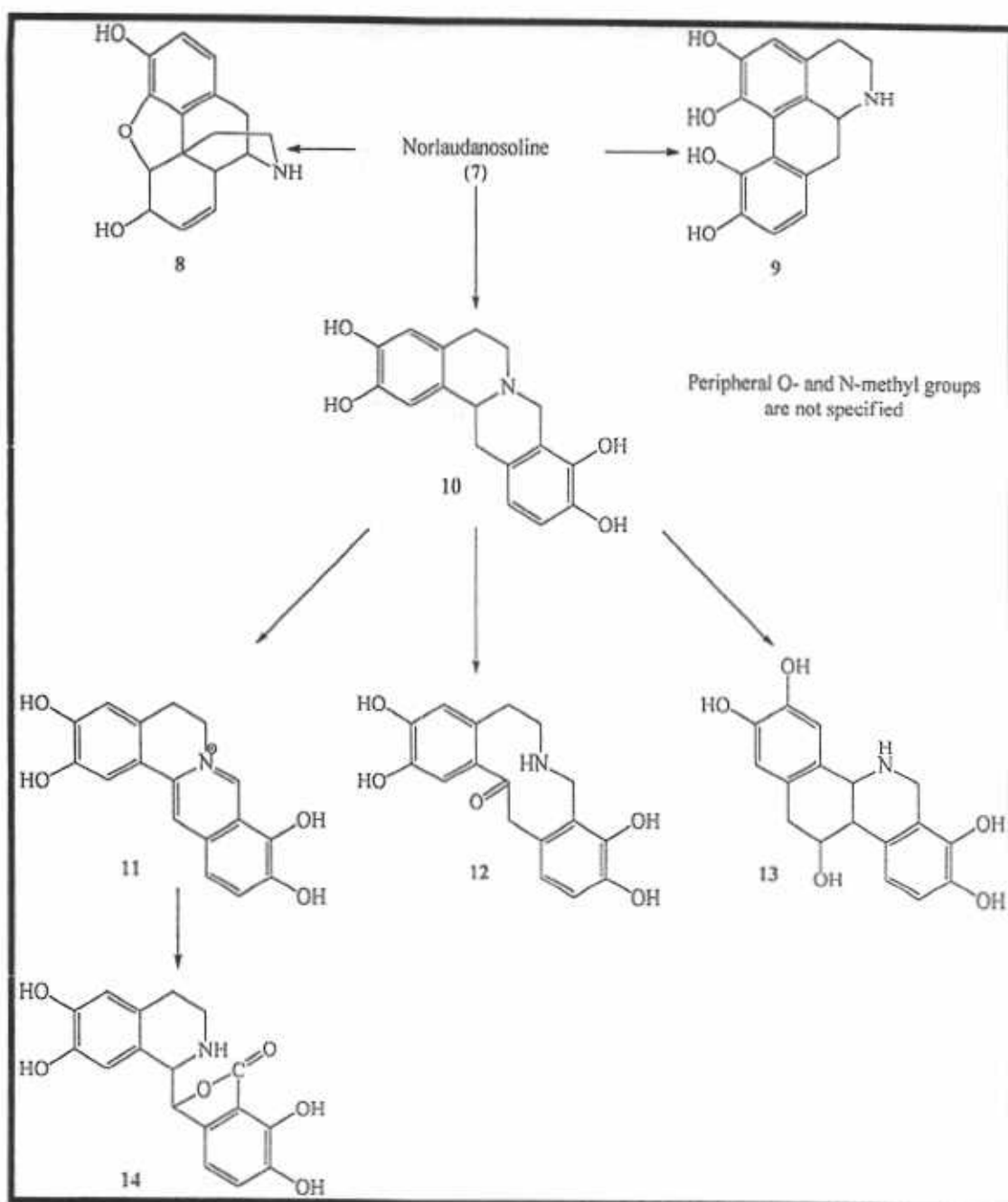
- Phenylethylamine
- Pyrrolidine group
- Piperidine group
- Indol group
- Pyridine-piperidine group
- Quinoline group
- Isoquinolinegroup
- Phenanthrene group
- Benzyloquinoline

2.1.1. Biosynthesis of benzyloquinoline alkaloids

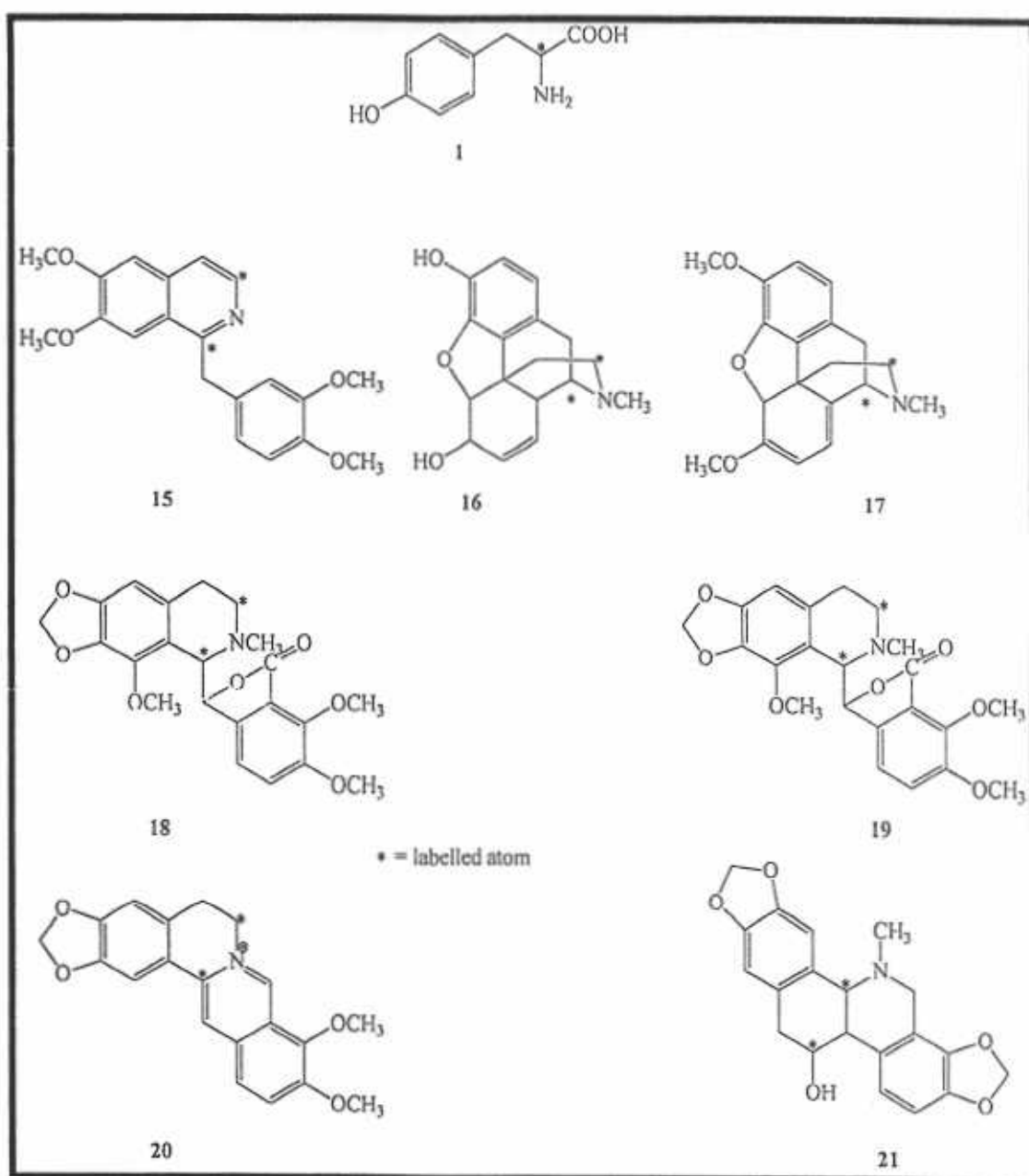
Winterstein and Trier¹⁶ proposed that norlaudanosoline (7) is the precursor for benzyloquinoline alkaloids. Norlaudanosoline can be derived from dopamine (4) and 3, 4-dihydroxyphenylpyruvic acid (3) or 3, 4- dihydroxyphenylacetaldehyde (5), which are derived from phenylalanine (6) or L-tyrosine (1) as shown in **scheme-2.1**.

Robinson¹⁷ highlighted the relationship between the structures of norlaudanosoline (7), hydrophenanthrene (8) and the aporphine (9) alkaloids (Scheme-2.2). He also proposed that the nucleus of tetrahydroprotoberberine (10) was related to the skeletons of protopine (12), phthalideisoquinoline (14) and benzophenanthridine (13) alkaloids.

The hypothesis of Winterstein and Robinson has been proved through several isotopic labeling experiments.¹⁸ As two C₆-C₂ units are derived from tyrosine; hence the radioactivity should enter two sites in the skeleton of these alkaloids. The predicted labeling pattern was shown when radioactive bases were isolated from [α -¹⁴C] DL-tyrosine fed plants. Radioactive samples of papaverine (15),¹⁹ morphine (16)²⁰ and narcotine²¹ isolated from *Papaver somniferum*; thebaine (17) from *Papaver bracteatum*;²² hydrastine (19)²³ and berberine (20)²⁴ from *Hydrastis Canadensis* and chlidonine (21)²⁵ from *Chelidonium majus* were degraded and in each case radioactivity was confined to marked (•) positions (Scheme-2.3).



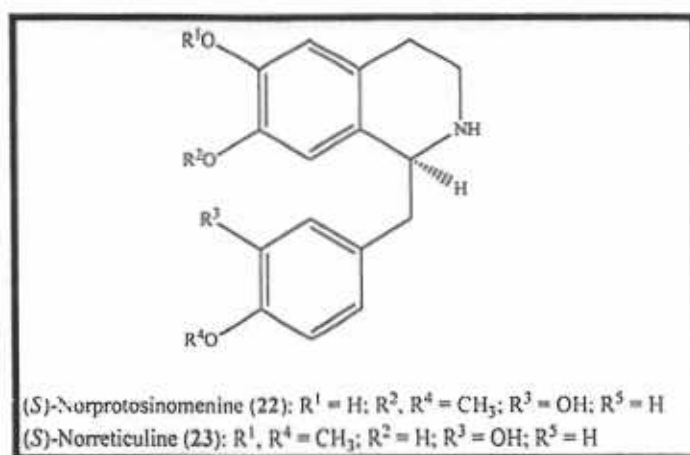
Scheme-2.2: Formation of protopine, phthalideisoquinoline and benzophenanthridine alkaloids

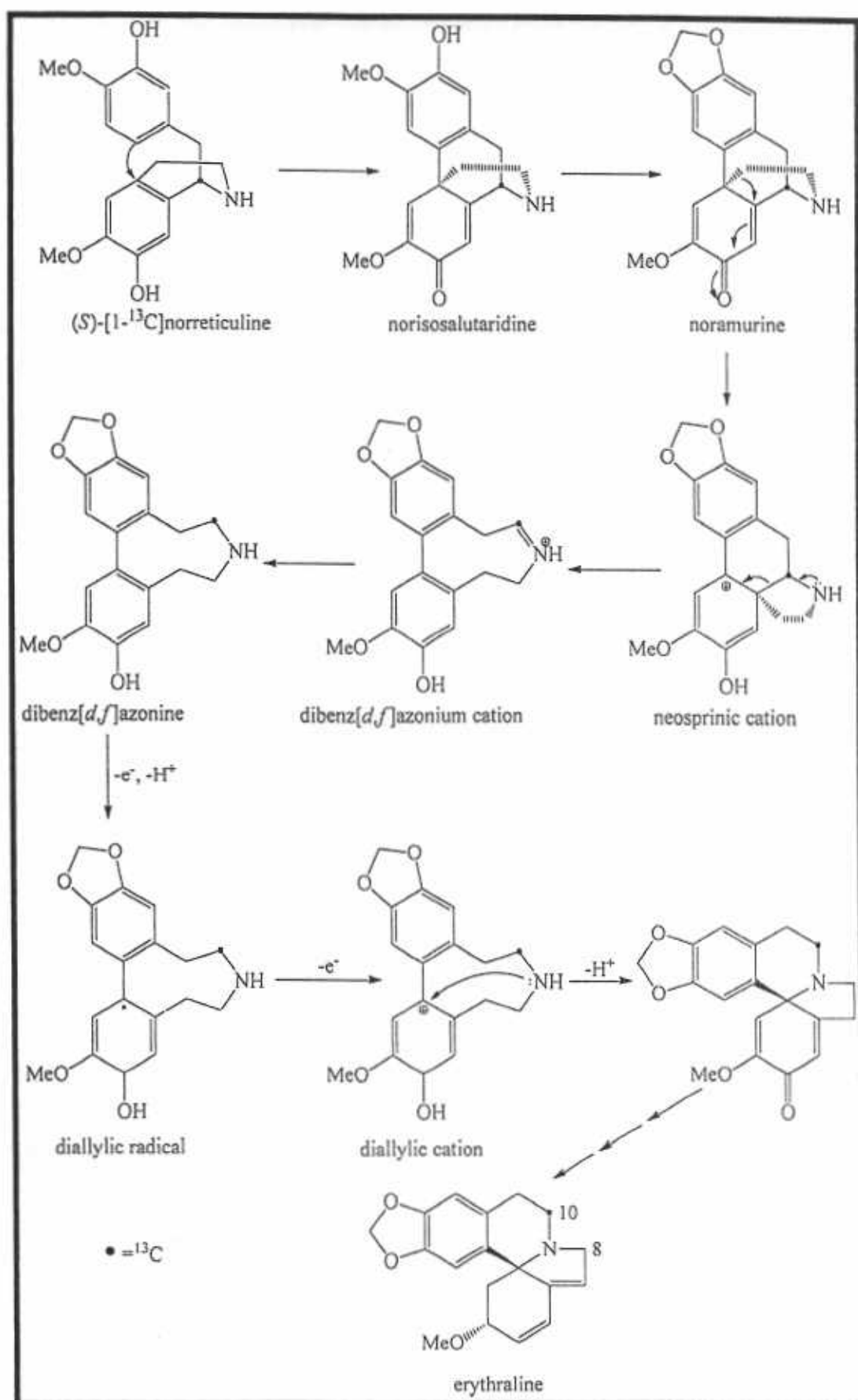


Scheme-2.3: Isotopic labeling of different alkaloids using $[\alpha\text{-}^{14}\text{C}]$ DL-tyrosine

2.1.2. Biosynthesis of erythrina alkaloids

Barton and Cohen proposed the first biosynthetic pathway for this class of alkaloids,²⁶ and observed by experiments that erythrina alkaloids are formed by the skeletal rearrangement of benzylisoquinoline alkaloids.²⁷ They also proposed that (*S*)-norprotosinomenine (22) is the precursor of the spirocyclic alkaloid erythraline in *E. crista-galli*. However, Frank and Teetz²⁸ demonstrated by biomimetic synthesis that (*S*)-norreticuline (23) a positional isomer of norprotosinomenine would more likely be a precursor in *erythrina* alkaloids biosynthesis, which was further supported by Ulrich *et al*²⁹ (Scheme-2.4) who carried out ¹³C-labeling studies to investigate the biosynthesis of such class of alkaloids and observed a non-random deposition of activity at position C-10 of the target molecule and interestingly no label was found at C-8 position, which would have been expected in the presence of norprotosinomenine as a precursor in the biosynthetic pathway. Therefore the existence of a symmetrical intermediate of diphenquinone type can be negated in the biosynthesis of erythrina alkaloids.





Scheme-2.4: Biosynthesis of erythraline

2.2. Terpenoids

The fragrance from many plants is mainly associated with volatile liquids known as essential or volatile oils. These oils are used in paint industry as solvent, in food industry as preservatives and as flavoring agent and to some extent in pharmaceuticals. The composition of these volatile oils came under extensive investigation in the 19th century. Their composition revealed that they are made up of a series of isomeric hydrocarbon units of formula $C_{10}H_{16}$. This unit was termed as terpene because of its association with turpentine. Earlier investigations regarding the structure of the terpene unit ($C_{10}H_{16}$) indicated it to be a dimer of the five carbon compound isoprene. A vast majority of terpenes isolated from plants possess formulae which are integral multiple of $C_{10}H_{16}$. Therefore, terpenoids can be defined as naturally occurring compounds containing a carbon framework based on two or more isoprene (C_5H_8) units. Terpenoids can be classified into hemiterpenoids ($C_5 = 1 \times C_5$), monoterpenes ($C_{10} = 2 \times C_5$), sesquiterpenes ($C_{15} = 3 \times C_5$), diterpenes ($C_{20} = 4 \times C_5$), sesterpenes ($C_{25} = 5 \times C_5$), triterpenes ($C_{30} = 6 \times C_5$), tetraterpenes ($C_{40} = 8 \times C_5$) and polyterpenes ($C_{40+n} = 8 + n \times C_5$) based on the number of isoprene units they used.

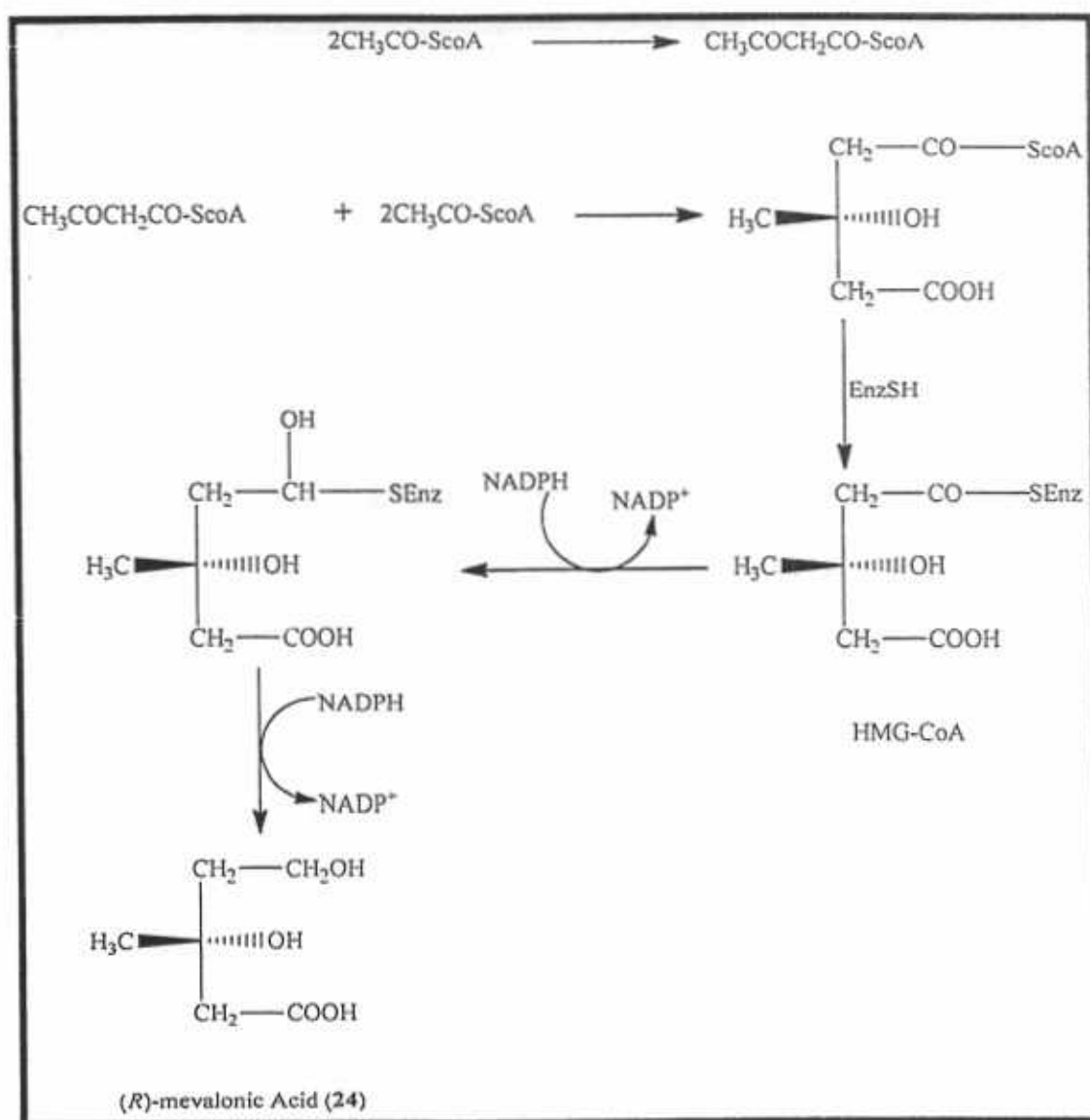
2.2.1. Biosynthesis of diterpenoids and triterpenoids

Terpenoids can be biosynthesized in three major steps:

- Formation of isopentene unit.
- Formation of acyclic Terpenoids from isopentene unit.
- Cyclization of acyclic terpenoids to form di- and tri-terpenes.

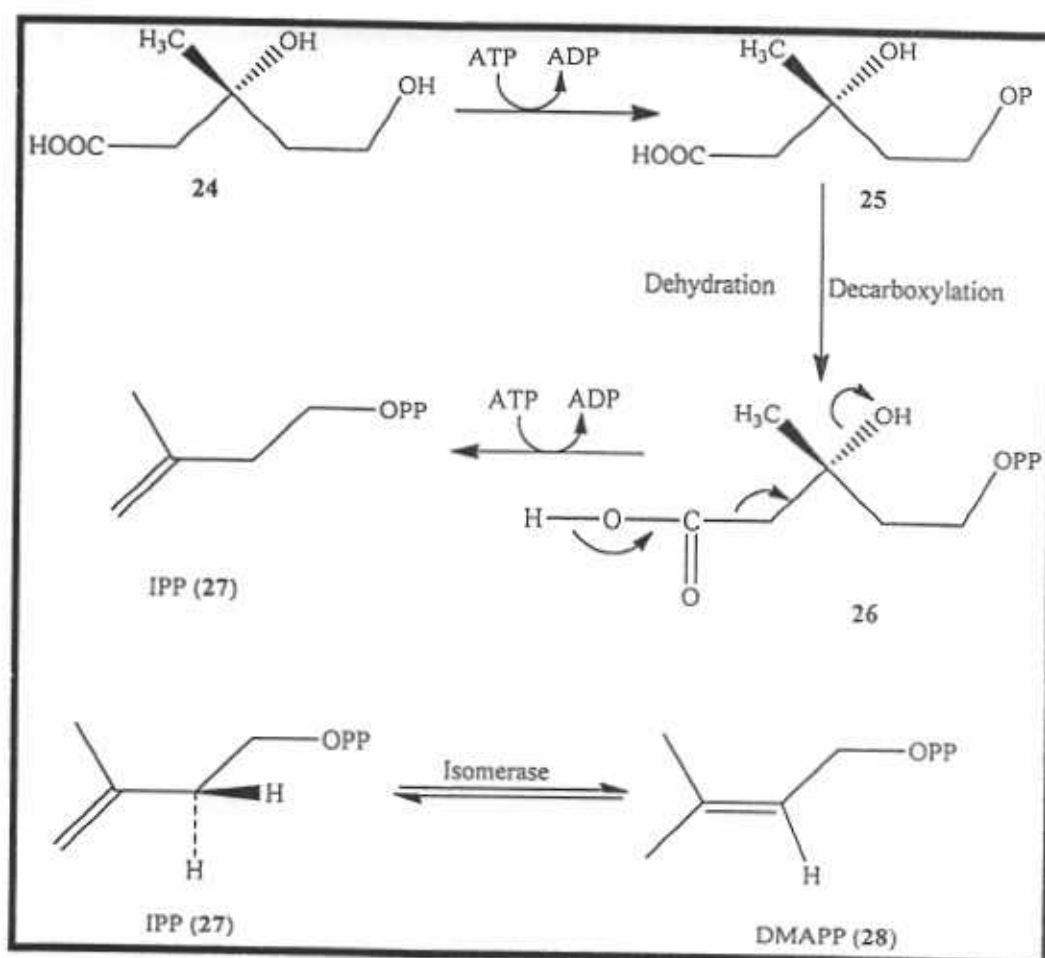
2.2.1.1. Formation of isopentene unit

Acetyl-coenzyme A derived from fats, carbohydrates or certain amino acids metabolism furnishes acetate ion which is converted into mevalonic acid through acetoacetyl coenzyme A and (*S*)-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA is reduced to (*R*)-mevalonic acid (24) by $\text{NADPH}_{\text{red}}$ (nicotinamid adenine dinucleotide phosphate; reduced form) (Scheme-2.5). The (*R*)-form of mevalonic acid is the only reactive species and can be used by the organism to produce terpenes whereas the *S*-form is metabolically inert.



Scheme-2.5: Formation of (*R*)-mevalonic acid (24)

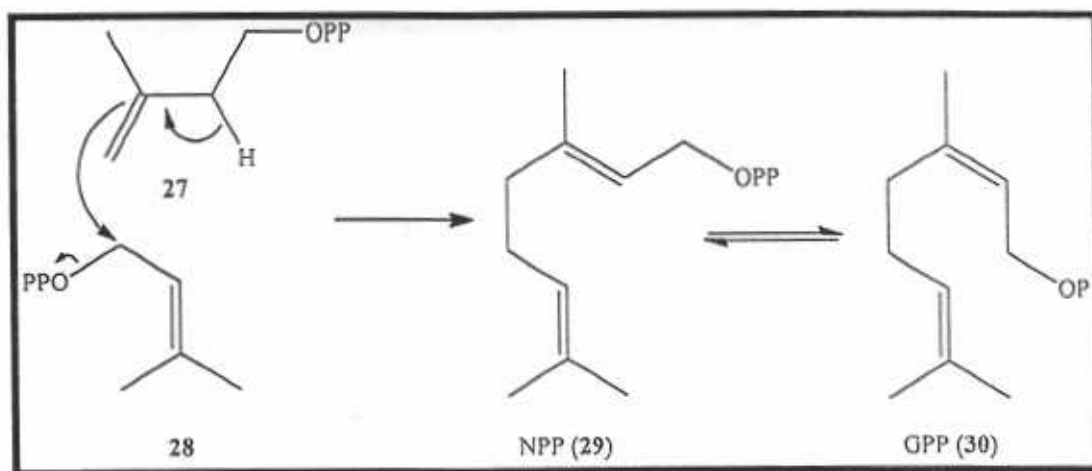
The isopentene unit is synthesized from (*R*)-mevalonic acid (**24**) through the pathway shown in (Scheme-2.6). This conversion needs the loss of one carbon atom and isotope labeling experiments showed that the carboxylic acid carbon atom is lost during the sequence.^{5,30-35} Mevalonic acid (**24**) first undergoes phosphorylation to produce mevalonic acid-5-phosphate (**25**) followed by further phosphorylation to give Mevalonic acid-5-pyrophosphate (**26**). Two molecules of ATP are utilized during the whole process and subsequent loss of water and CO₂ molecules produce 3-methylbut-3-enyl pyrophosphate or Δ^3 -isopentenyl pyrophosphate (IPP) (**27**). IPP can then undergo a stereospecific isomerization to form DMAPP (3, 3-dimethylallyl pyrophosphate) (**28**).³⁵ Consequently, a less reactive molecule is converted into a molecule which can easily attack on nucleophilic sites.



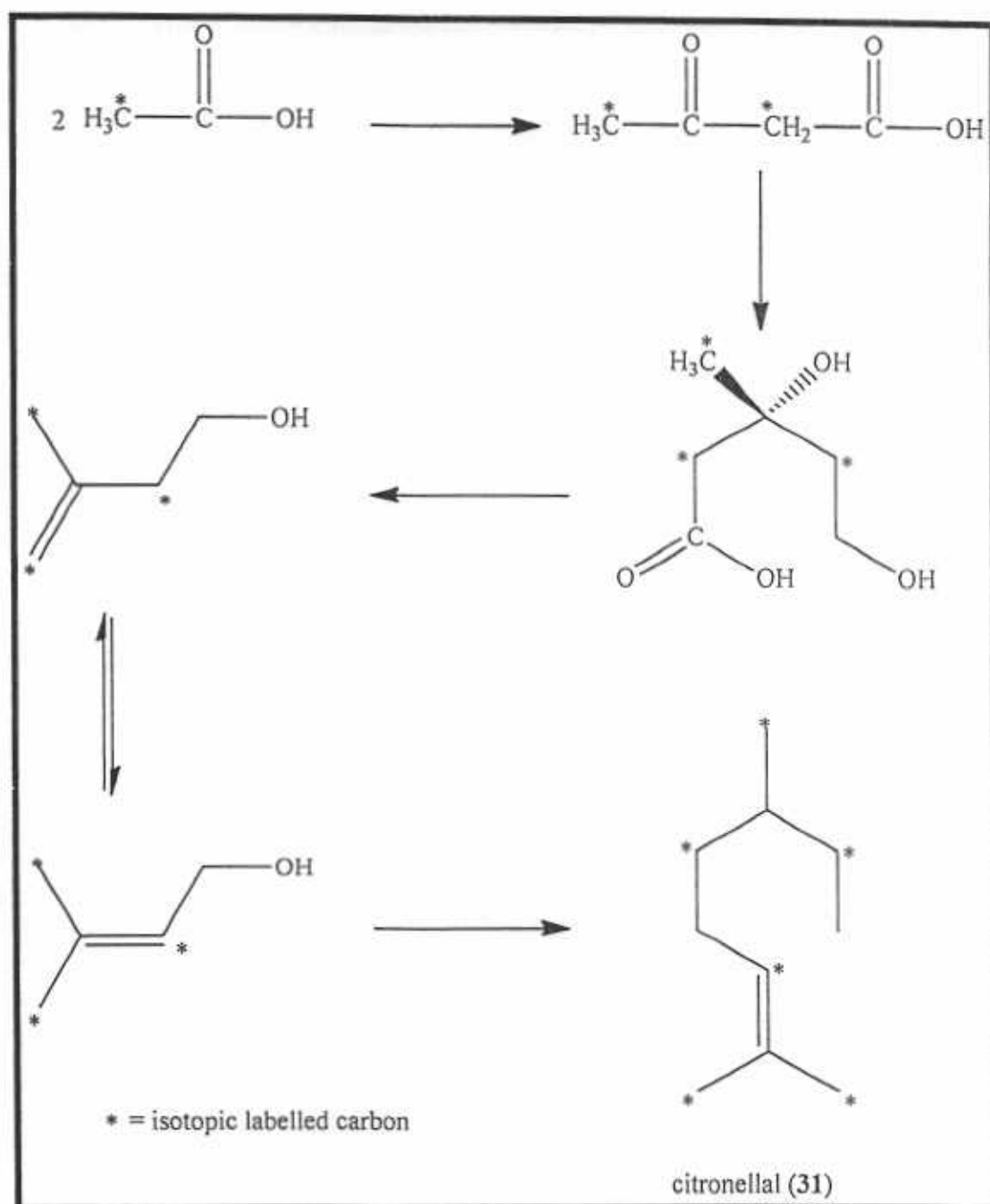
Scheme-2.6: Biosynthesis of DMAPP (28) from (*R*)-mevalonic acid (24)

2.2.1.2. Formation of acyclic terpenoids from isopentene unit

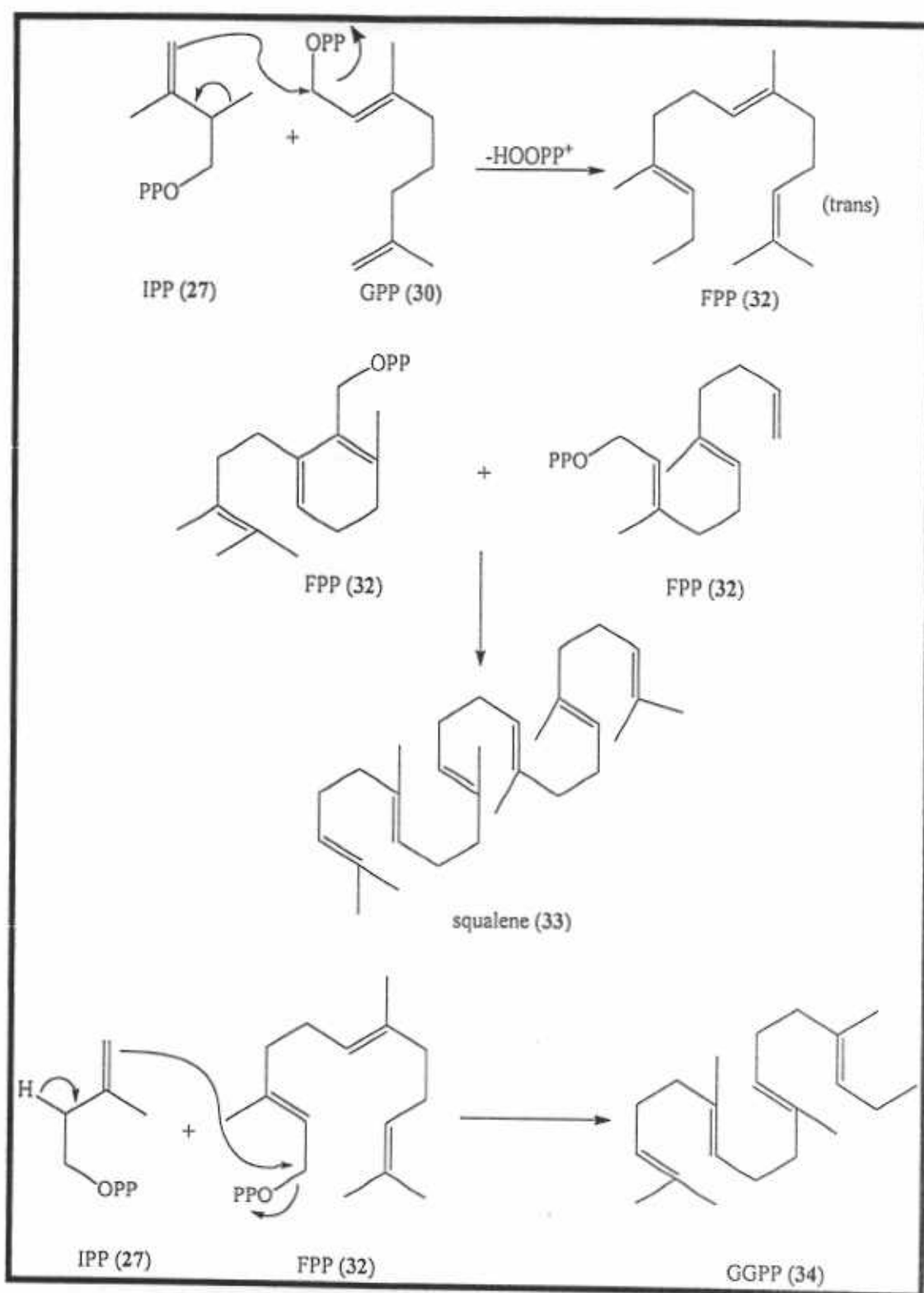
The IPP (27) and DMAPP (28) molecules undergo a condensation reaction in a head-to-tail fashion to form a unit of neryl pyrophosphate (NPP) (29) and geranyl pyrophosphate (GPP) (30) (Scheme-2.7). Isotopic labeling experiments with labeled acetate to give citronellal (31) (Scheme-2.8) confirm the validity of scheme-2.7. IPP (27) on further condensation with GPP (30) (Scheme-2.9) furnishes farnesyl pyrophosphate (FPP) (32) in all *trans*-configuration.³⁶⁻⁴⁰ FPP (32) is regarded as the immediate precursor of sesquiterpenes and its dimerization in a head-to-tail manner can give squalene (33)^{41,42} (Scheme-2.9), which is a precursor of triterpenoids. Farnesyl pyrophosphate (FPP) when adds on a C₅ unit, geranyl geranyl pyrophosphate (GGPP)⁴³ (34) is formed (Scheme-2.10), which is further converted into diterpenes by cyclization.



Scheme-2.7: Formation of NPP (29) and GPP (30)



Scheme-2.8: Formation of citronellal (31) in isotopic labeling experiment



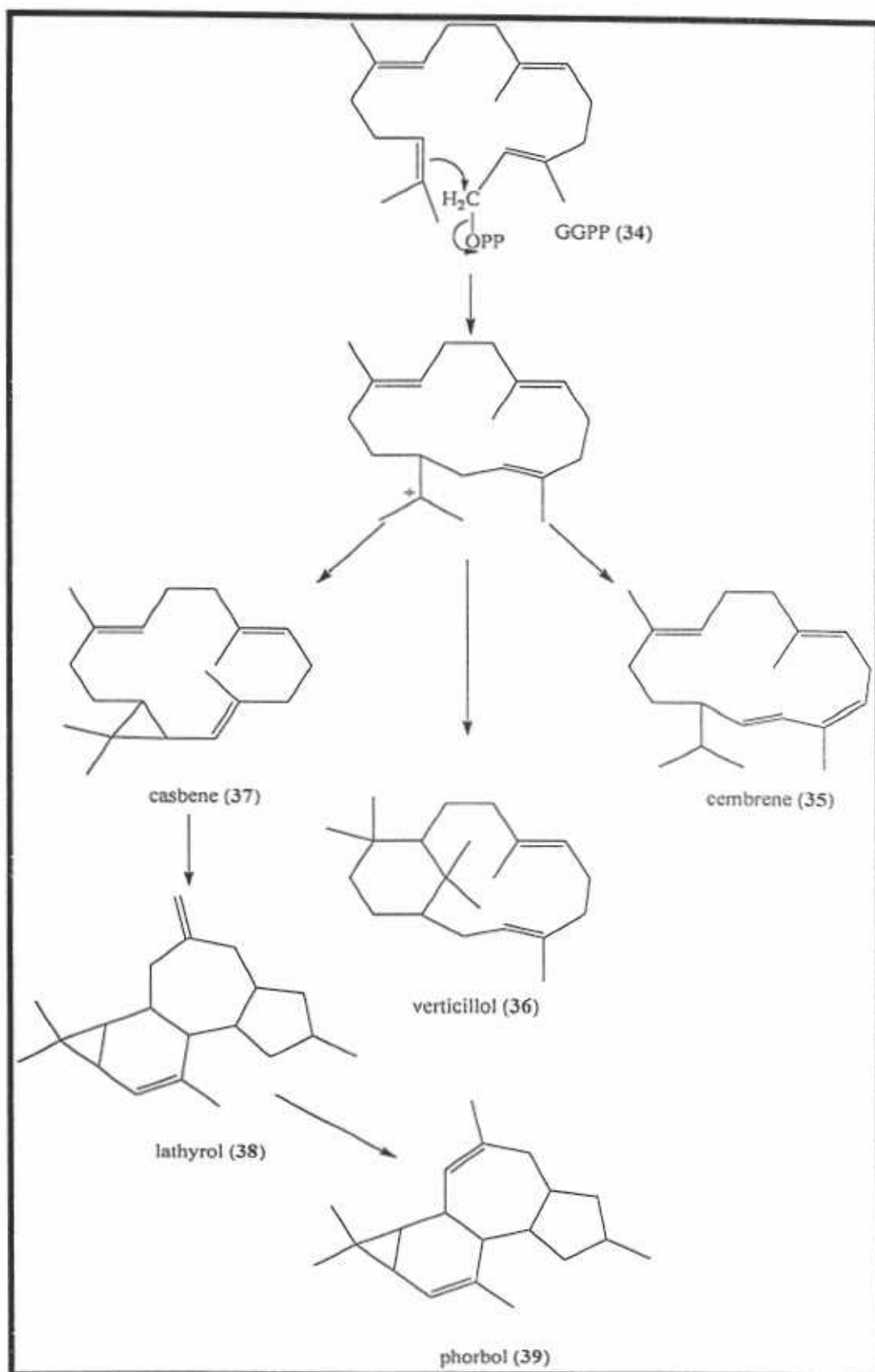
Scheme-2.9: Formation of FPP (32), squalene (33) and GGPP (34)

2.2.1.3. Conversion of acyclic terpenoids to diterpenes

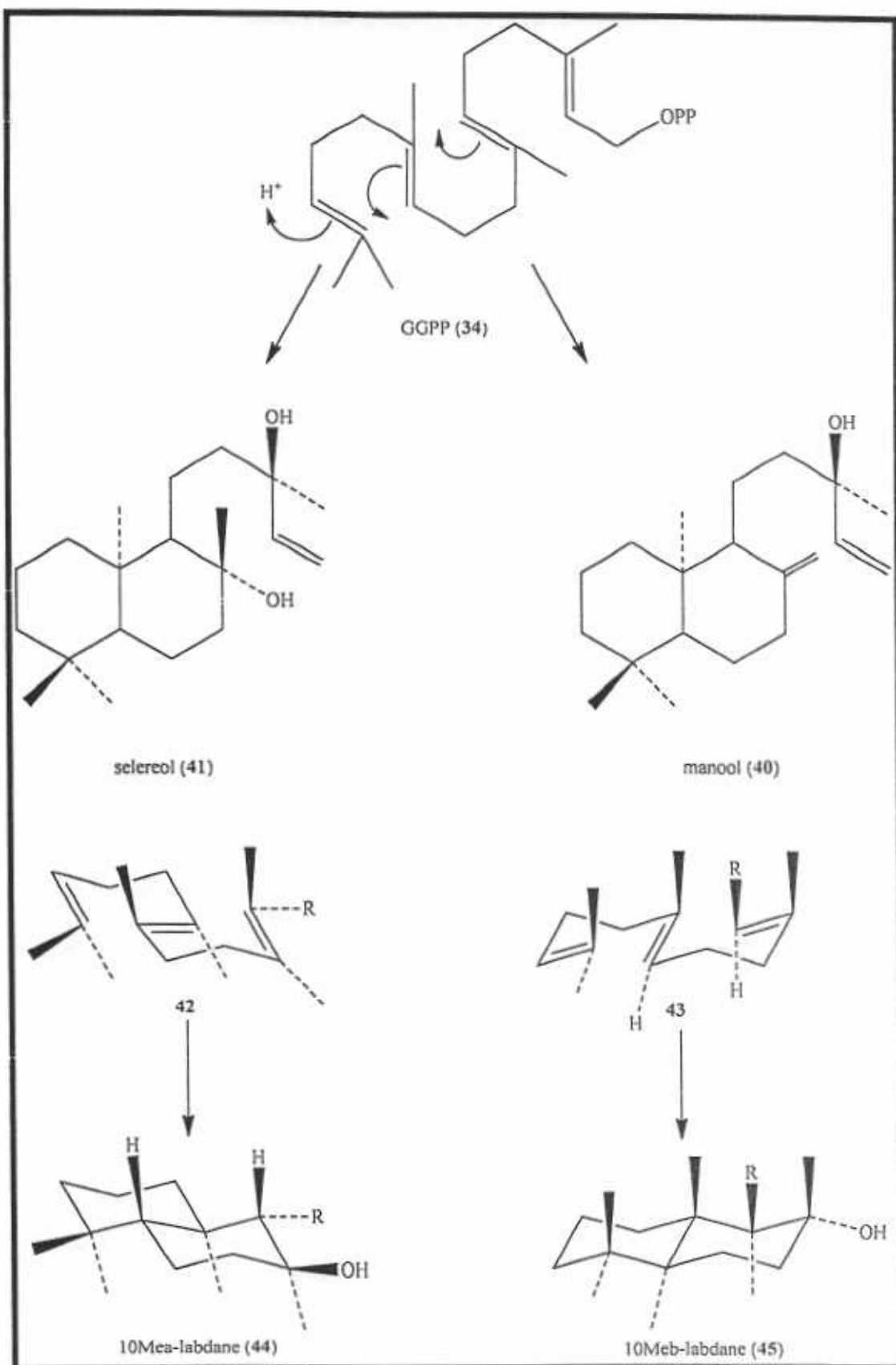
The biosynthesis of monocyclic diterpenes involve the heterolytic cleavage of $\text{CH}_2\text{-PP}$ part of GGPP (34) and a carbonium ion is generated which reacts with isopropylidene unit resulting in cyclization. Cemberene (35) has been suggested to arise from 34 in the same pathway. The macrocyclic ring can undergo further cyclization and rearrangement to form verticillol (36), casbene (37), lethyrol (38) and phorbol (39)⁴³⁻⁴⁵ (Scheme-2.10), while a direct cyclization of GGPP (34) furnishes bicyclic diterpenes such as manool (40), selerol (41) and isomeric labdanes (Scheme-2.11). The junction between ring A and ring B of all labdanes and diterpenes with more number of rings is always *trans*. Thus, the *trans* ring junction is a function of the relative orientation of the cyclizing double bonds in an open chain precursor on an enzyme surface.⁵

The polyenic chain retains a chair-chair conformation during cyclization process. This conformation may be found in two enantiomeric forms 42 and 43, which will ultimately give two enantiomeric labdane skeletons 44 and 45. As a number of tri-, tetra- and penta-cyclic diterpenes are derived from labdane diterpenes, hence such compounds are also distributed into two enantiomeric series.

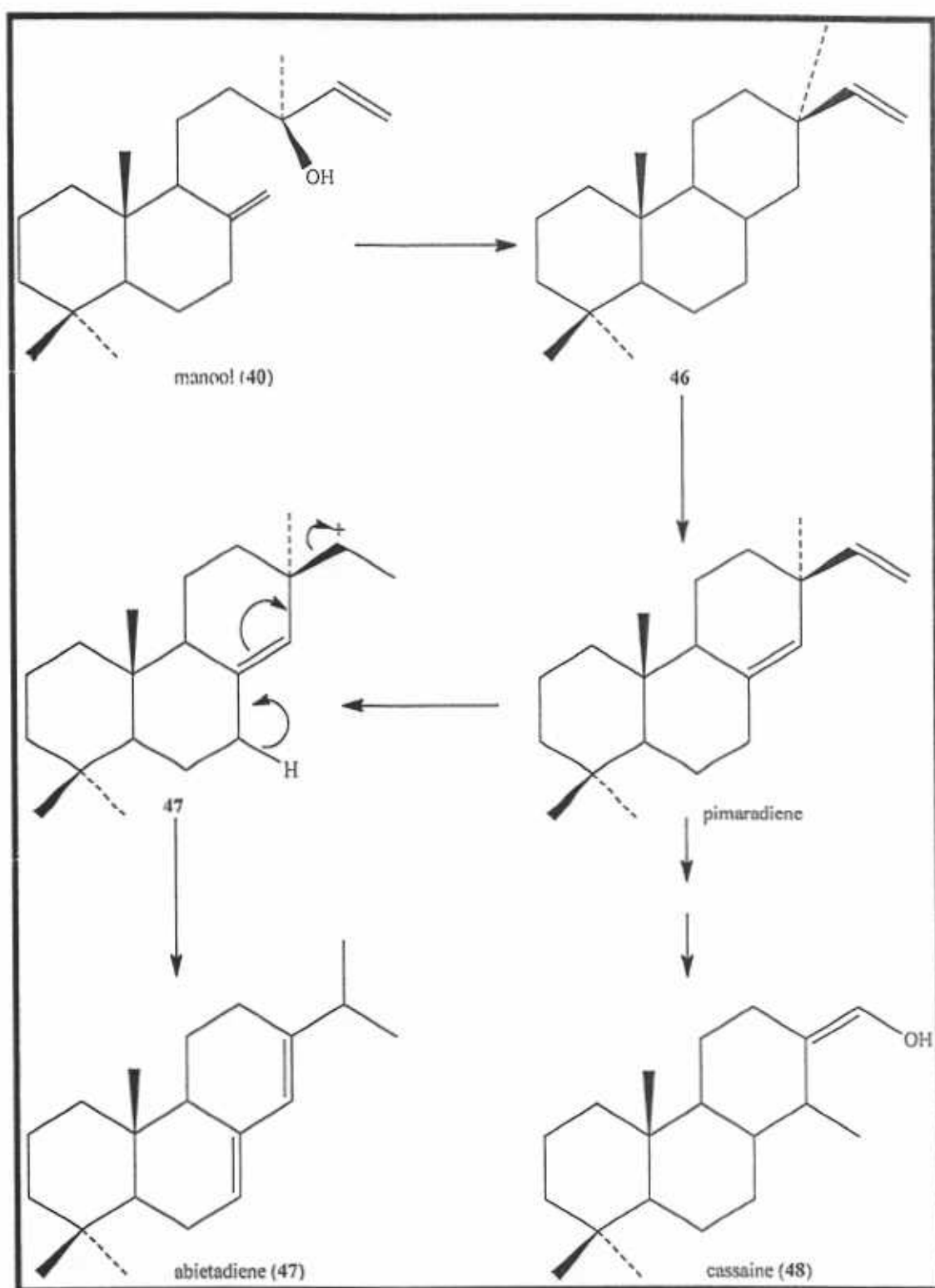
The tricyclic diterpenes such abietane (47) and cassaine (48) have pimarane skeleton, which are derived from manool (40) by further cyclization through the interaction between carbocation (47) and 8, 14-double bond via 46.⁴⁶ (Scheme-2.12)



Scheme-2.10: Formation of cembrene (35), verticillol (36), casbene (37), lathyrol (38) and phorbol (39)



Scheme-2.11: Conversion of GGPP (34) to sclereol (41), manool (40) and labdanes (44, 45)



Scheme-2.12: Conversion of manool (40) to abietadiene (47) and cassaine (48)

The tetra- and penta-cyclic diterpenes also belong to the pimarane series which are formed through a non-classical pimarane carbocation (48). This cation (48) can collapse in a number of ways to produce beyerene (49), kaurene (50), atisirene (51), phyllocladane (53) and pentacyclic trachylobane (52) skeletons⁴⁷ (Scheme-2.13). The kaurane skeleton can give rise to the tetracyclic series of diterpenes^{34,48,49} such as gibberllane (54), grayanotoxane (55) and enmeane (56) (Scheme-2.14).

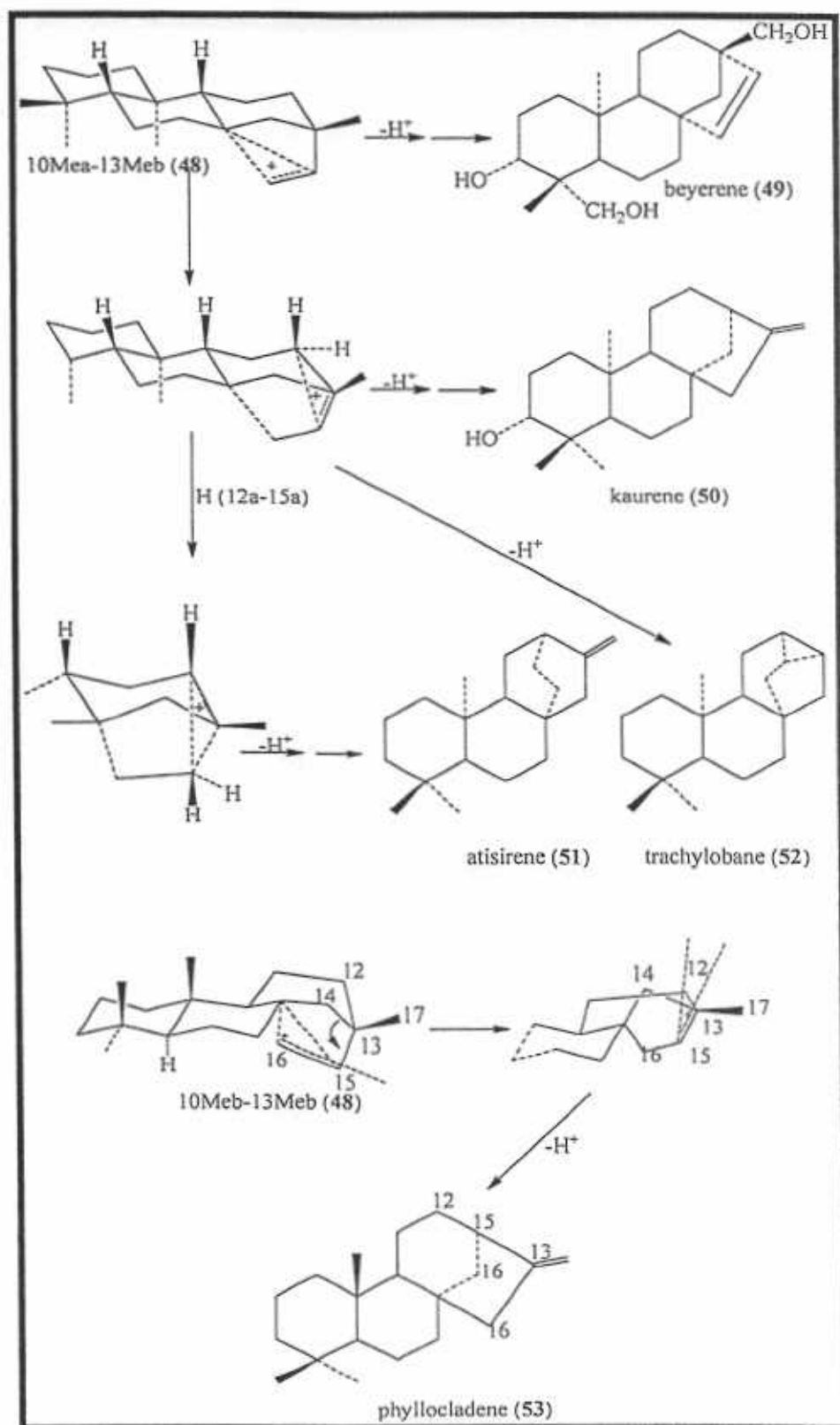
2.2.1.4. Conversion of acyclic terpenoids to triterpenes

The biological precursor of all triterpenes is hexa-ene all-*trans* squalene (33). The direct cyclization of squalene or its epoxide gives fundamental triterpenes. These triterpenes are then sub-divided into groups according to the cyclization path followed by them.

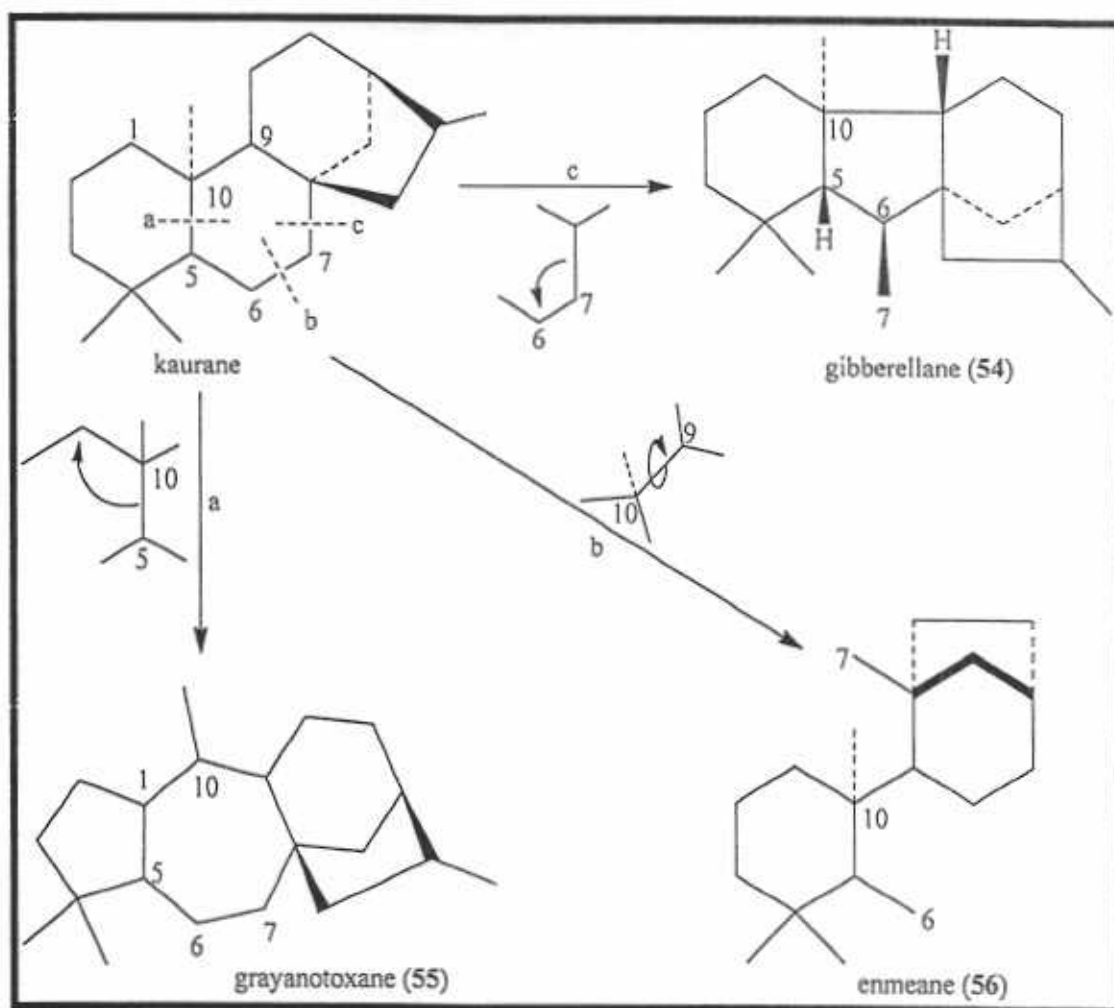
2.2.1.4.1. Cyclization involving oxidation of the terminal part of squalene

A number of tetra- and penta-cyclic triterpenes are derived from oxidative cyclization of 2,3-epoxy squalene (57) and classified according to squalene epoxide conformation. Thus, squalene epoxide conformation in chair-chair-chair-boat form give rise to tetracyclic triterpenes such as dammeranediol (59),^{50,51} euphol (60) and tirucallol (61)^{52,53} (Scheme-2.15 and 2.16) and pentacyclic triterpenoids such as lupeol (62), taraxasterol (63),⁵⁴ α -amyrin (64),⁵⁵ taraxerol (65),⁵⁶ β -amyrin (66),^{57,58} friedeline (67) and δ -amyrin (68)⁵⁸ (Scheme-2.17 and 2.18). A chair-boat-chair-boat conformation (69) produces lanosterol (71) and cycloartinol (72) (Scheme-2.19 and 2.20). Penta-cyclic triterpenoids such as hopanone (74), arborenol (76) and moretenol (78) are formed from squalene epoxide using conformations chair-chair-chair-chair

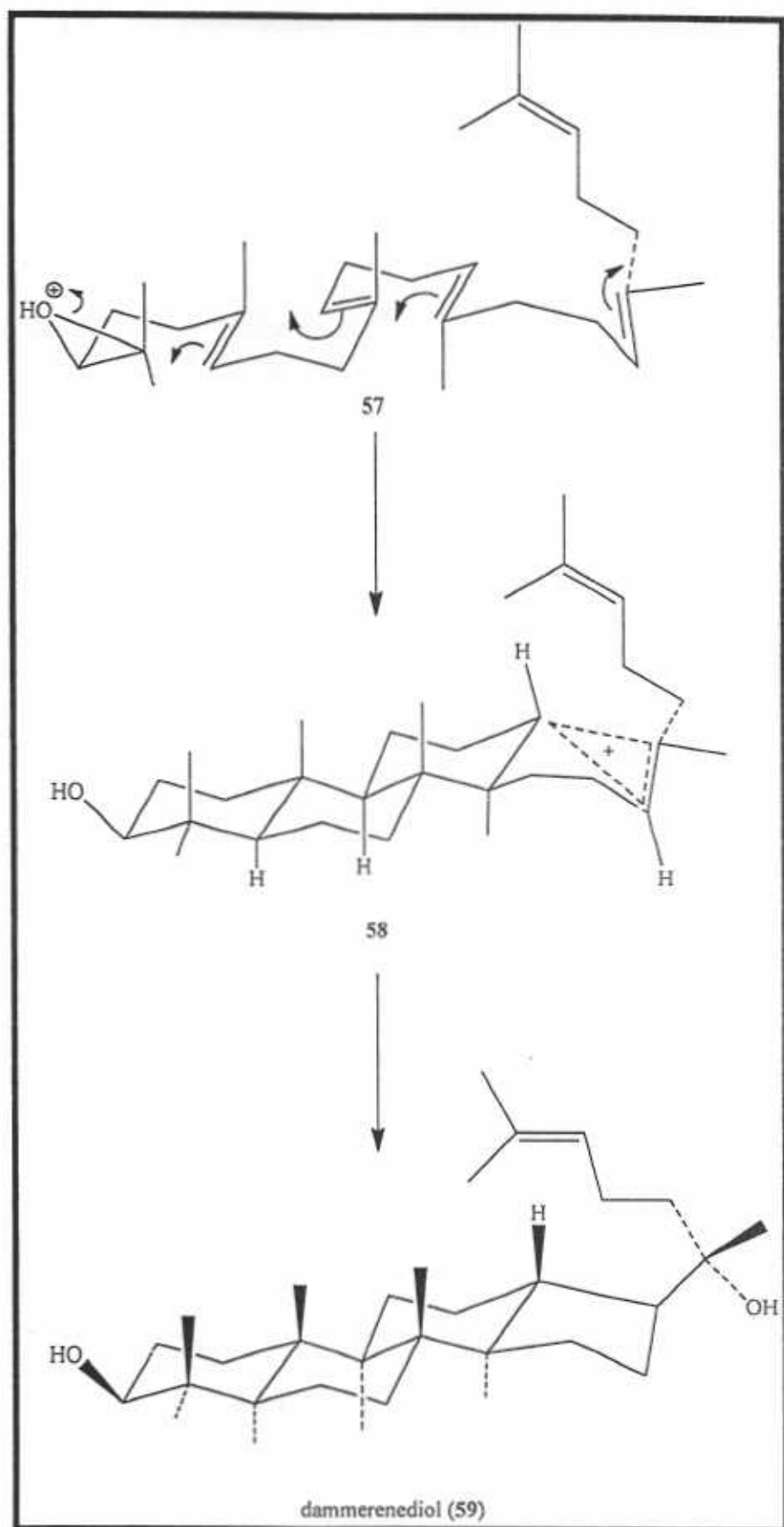
(73), chair-boat-chair-chair-boat (75) and chair-chair-chair-chair-boat (77), respectively (Scheme-2.21).



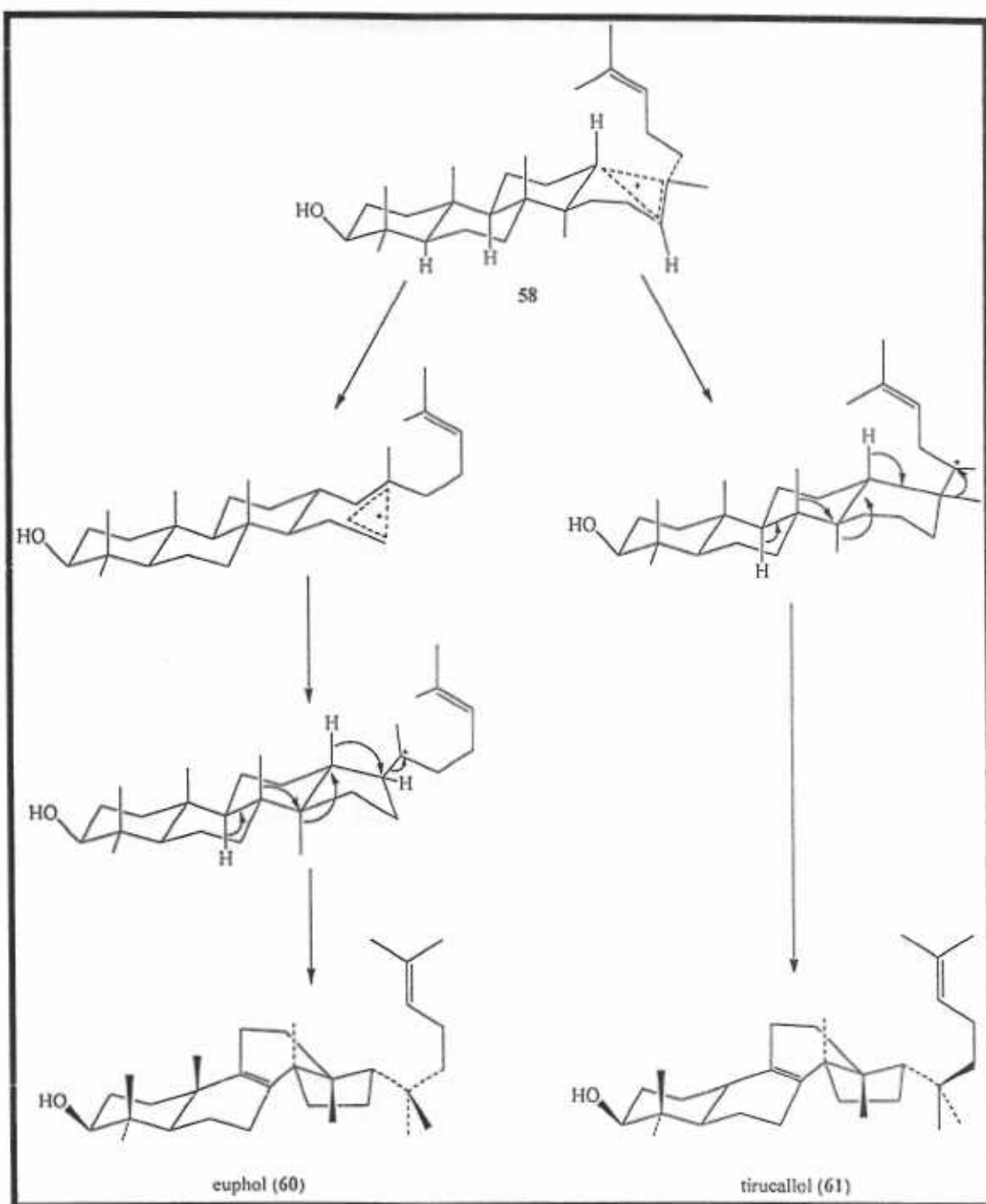
Scheme-2.13: Conversion of pimarane carbocation (48) to beyerene (49), kaurene (50), atisirene (51), phyllocladane (53) and pentacyclic trachylobane (52)



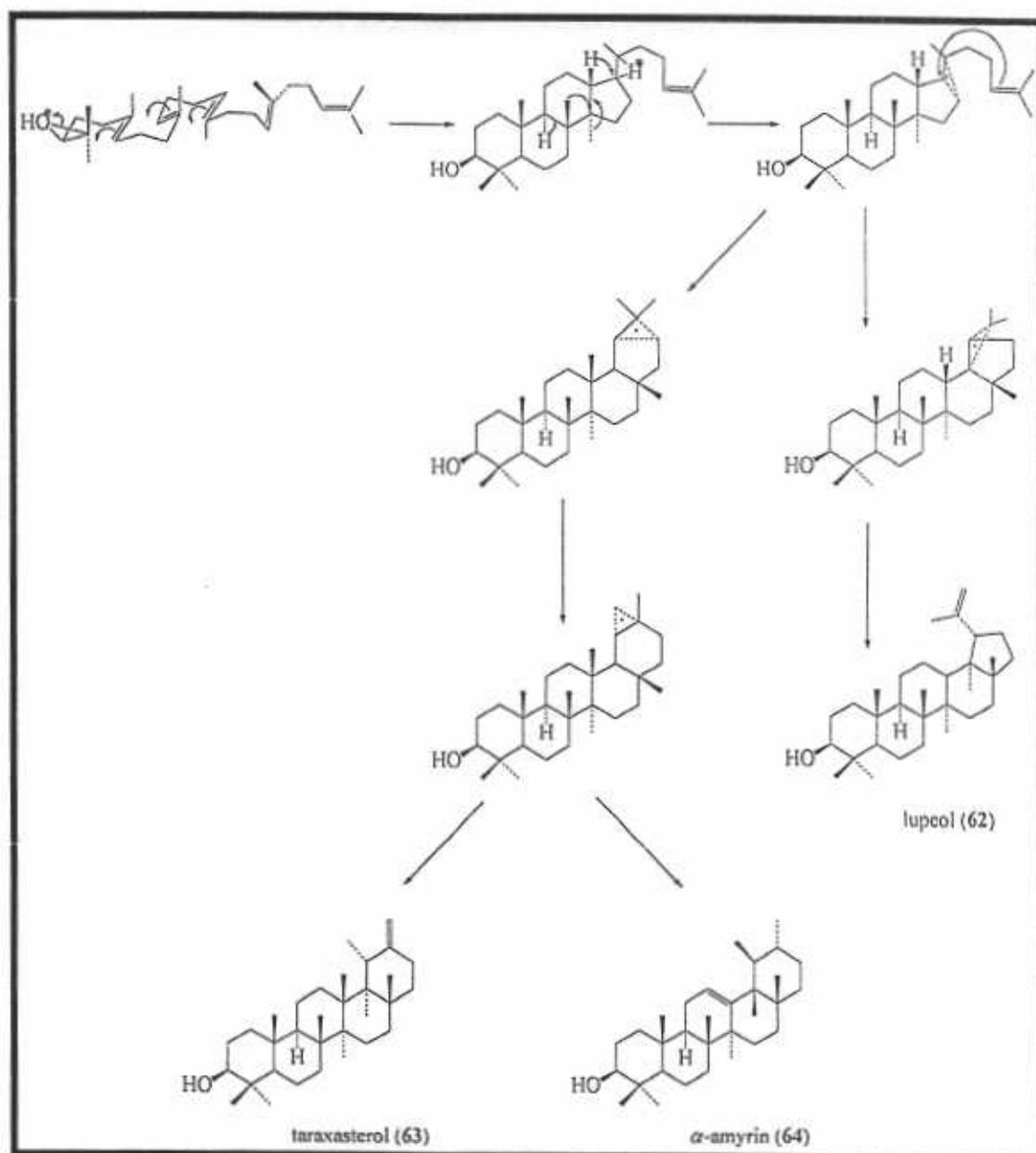
Scheme-2.14: Conversion of kaurane to gibberellane (54), grayanotoxane (55) and enmeane (56)



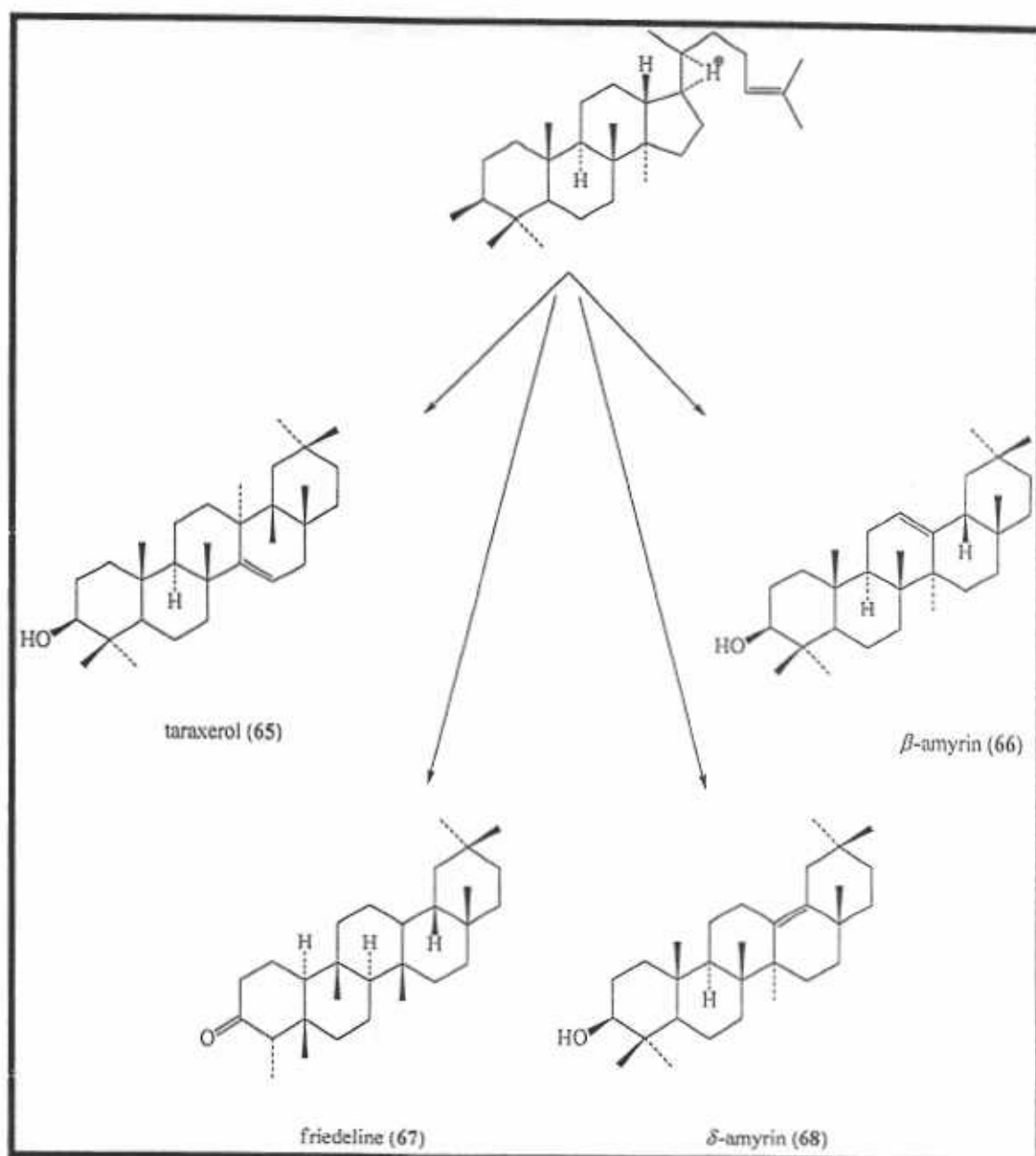
Scheme-2.15: Conversion of squalene epoxide (57) to dammerenediol (59)



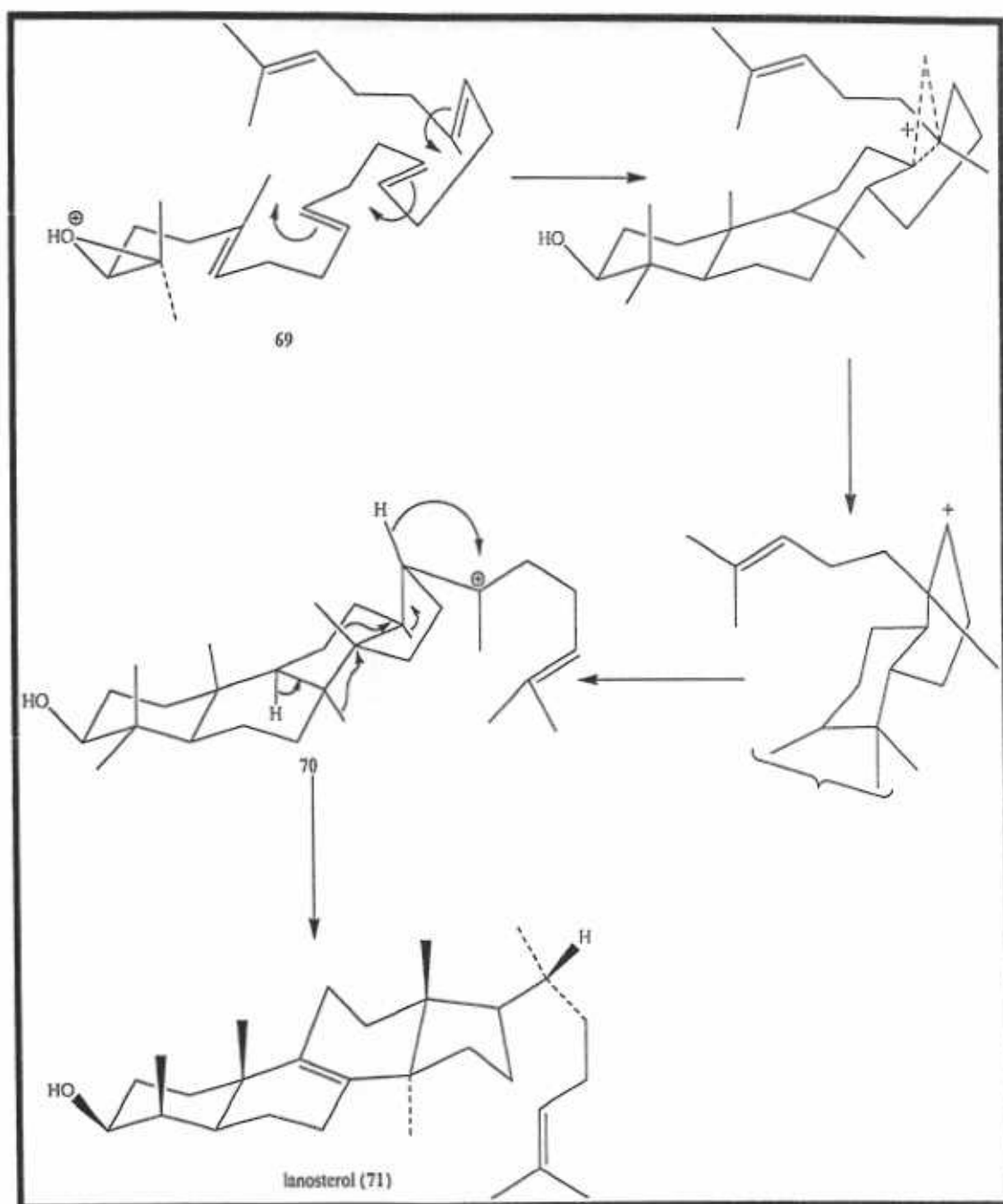
Scheme-2.16: Formation of euphol (60) and tirucallol (61)



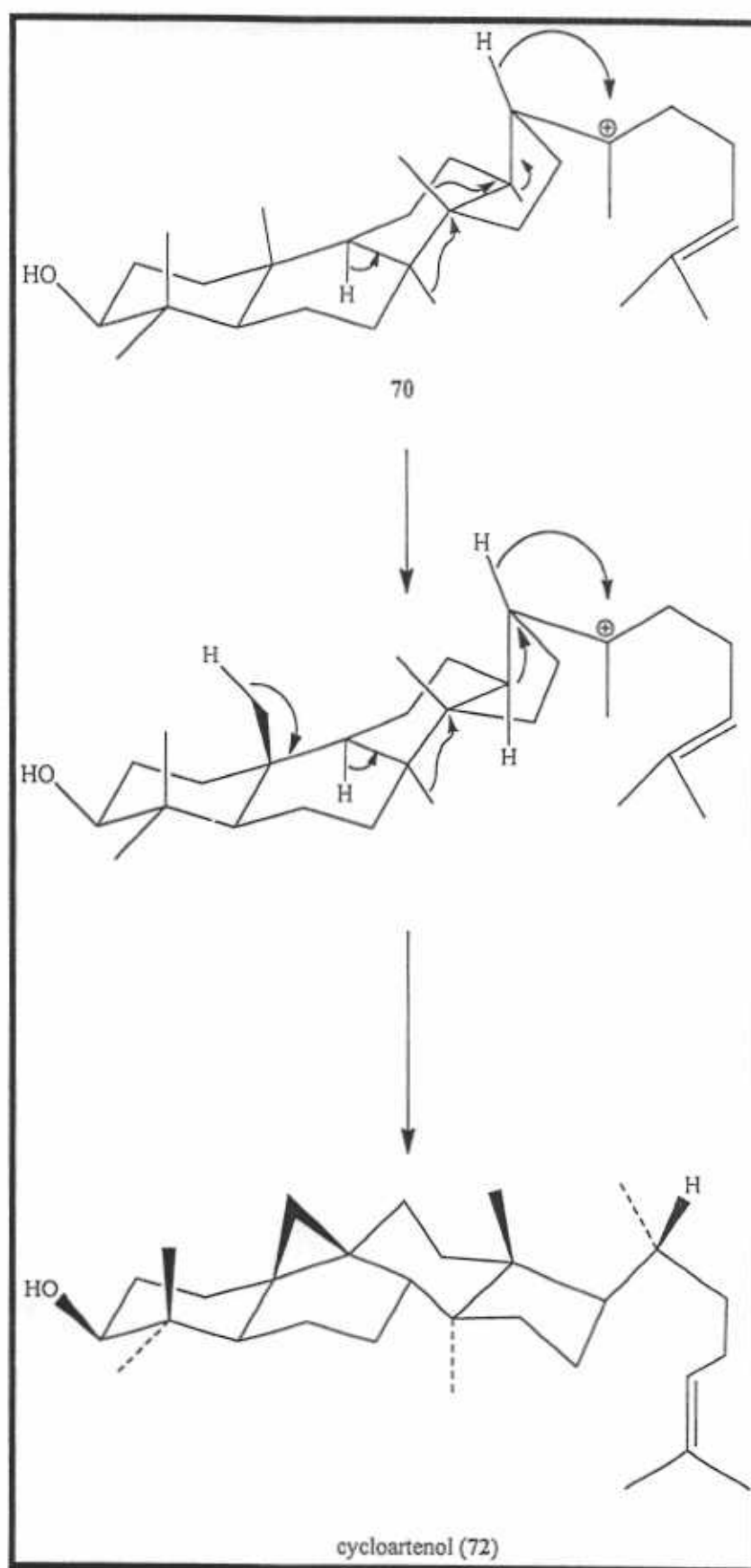
Scheme-2.17: Formation of lupeol (62), taraxasterol (63) and α -amyrin (64)



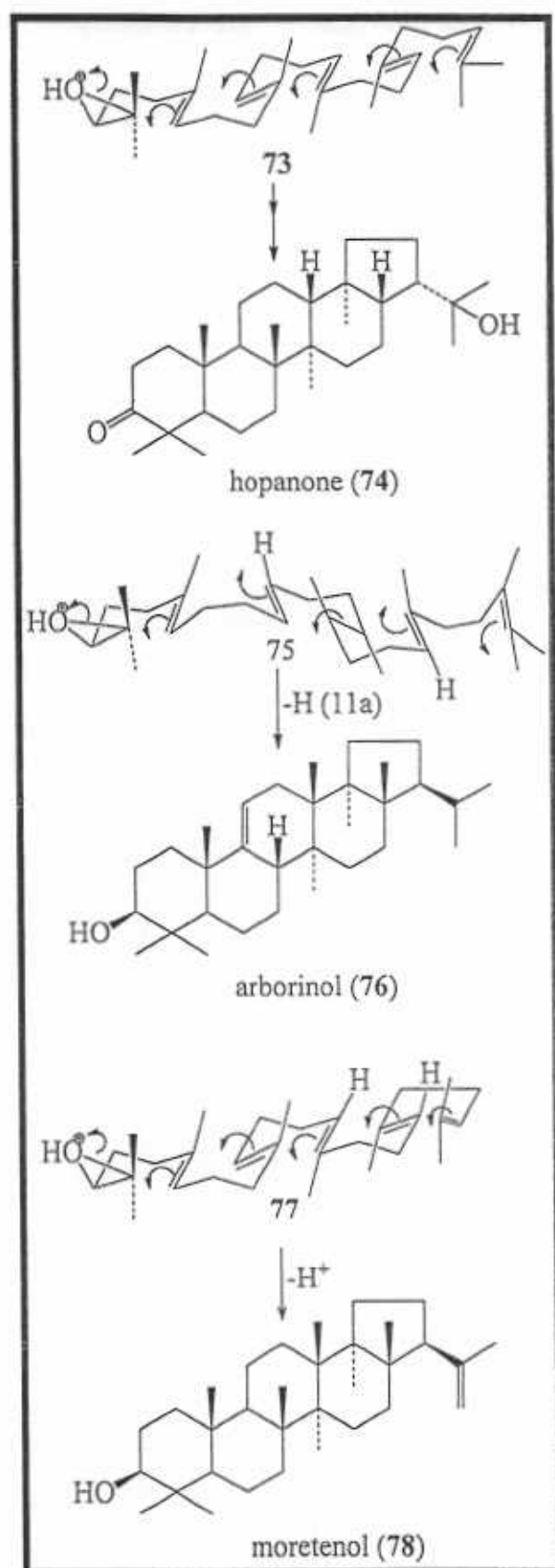
Scheme-2.18: Formation of taraxerol (65), β -amyrin (66), friedeline (67) and δ -amyrin (68)



Scheme-2.19: Formation of lanosterol (71)



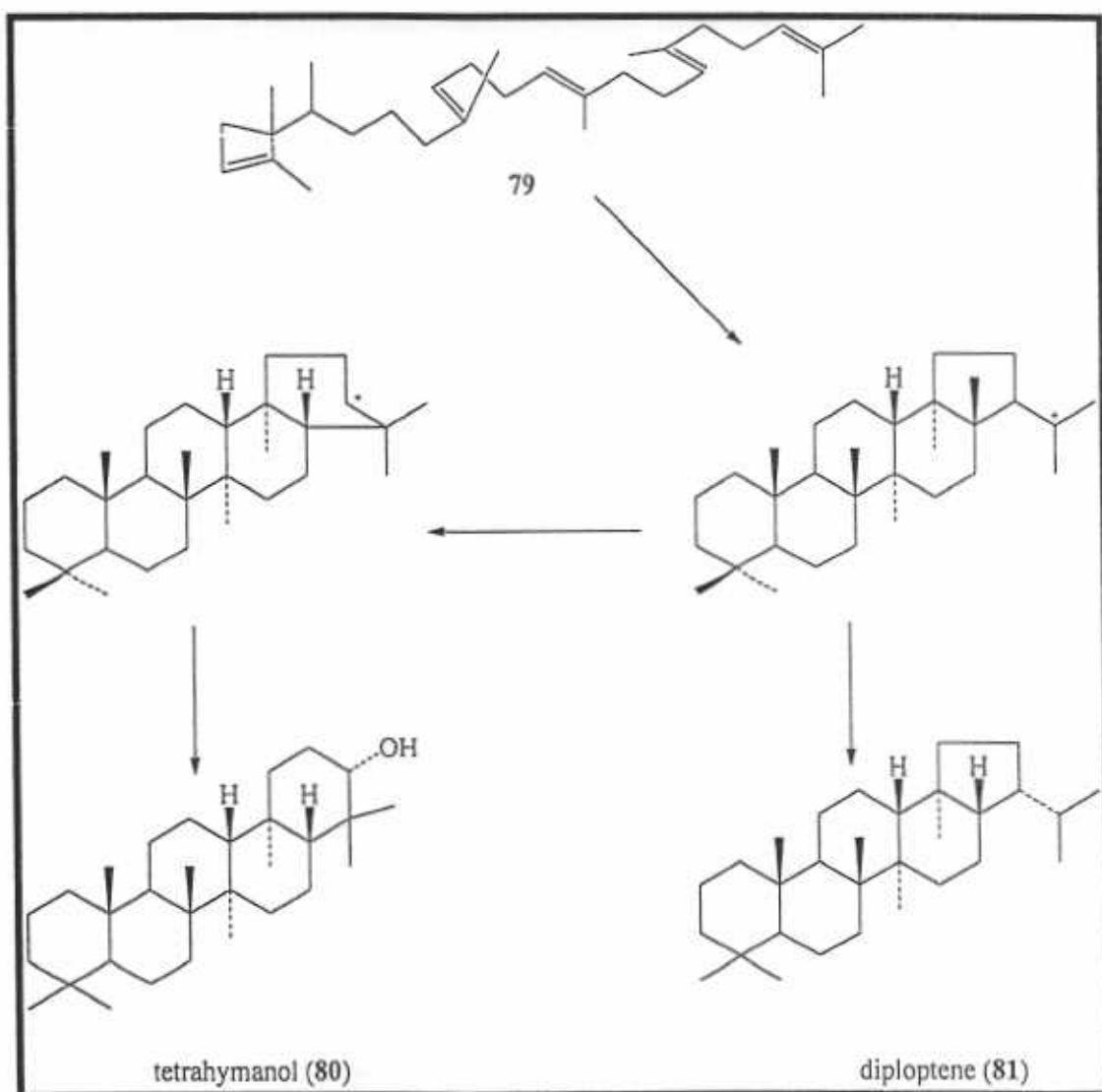
Scheme-2.20: Formation of cycloartenol (72)



Scheme-2.21: Formation of hopanone (74), arborinol (76) and moretenol (78)

2.2.1.4.2. Cyclization without oxidation of the terminal part of squalene

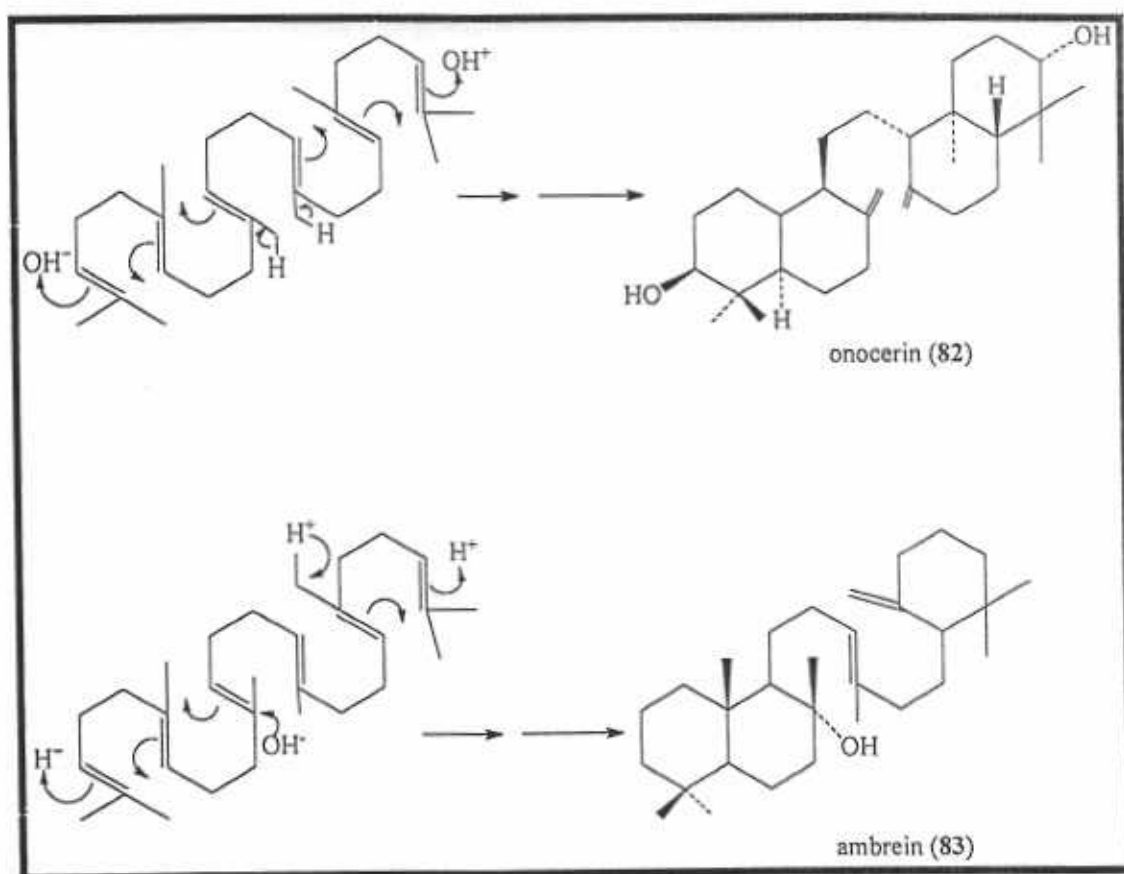
Triterpenes such as tetrahymanol (**80**)⁵⁹ and diploptene (**81**)⁶⁰ are formed by the non-oxidative cyclization of the terminals of squalene (**79**) type of cyclization which is less common than oxidative pathway (Scheme-2.22).



Scheme-2.22: Formation of tetrahymanol (**80**) and diploptene (**81**)

2.2.1.4.3. Cyclization at both terminal double bonds of squalene

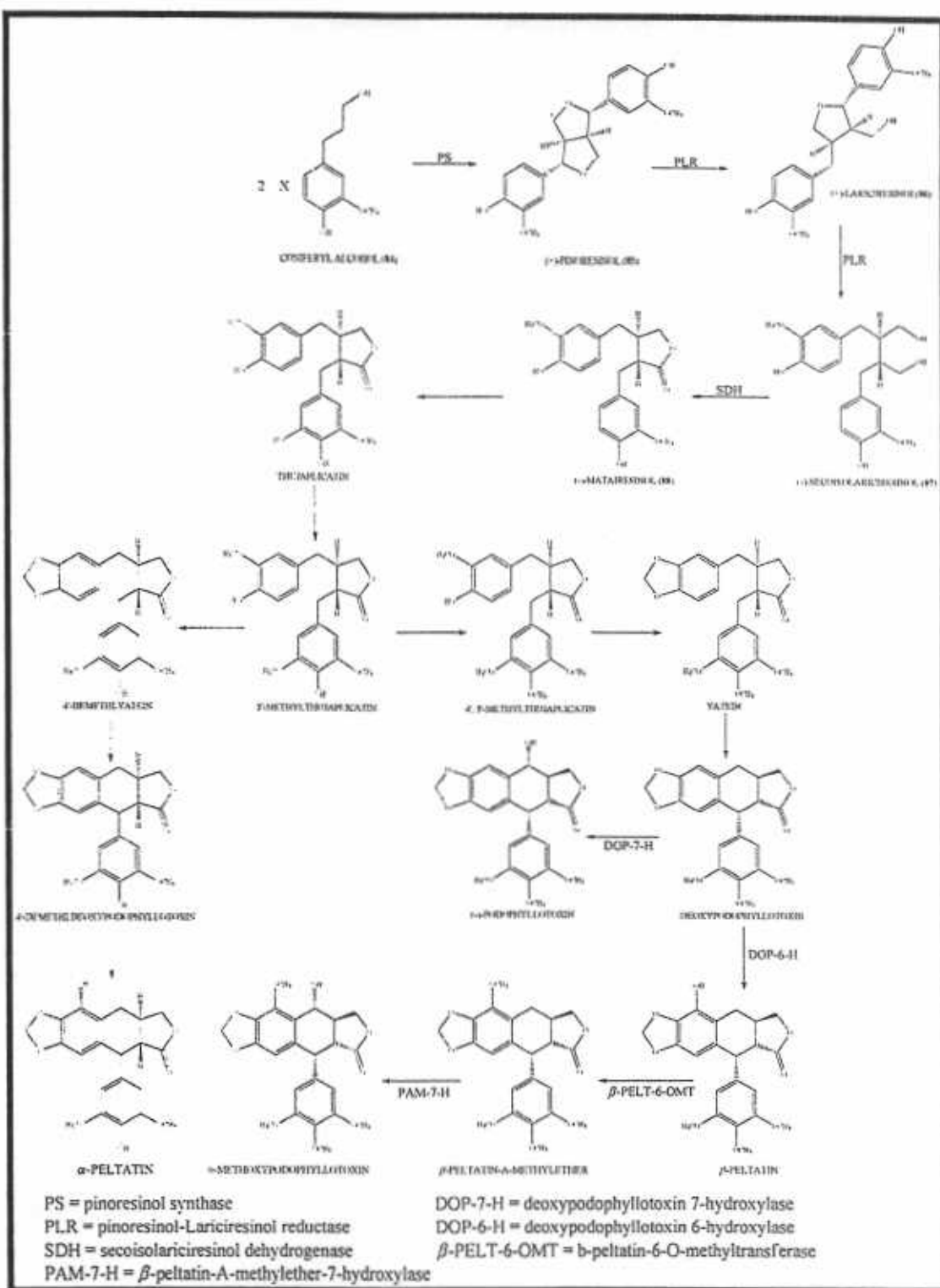
The biosynthetic pathway of onocerin (82) and ambrein (83) occurs in two separate electrophilic attacks on both ends of the squalene molecule. The two attacks are oxidation reactions for onocerin and no oxidation occurs in case of ambrein. Any information about the simultaneity of the two attacks is not known so far (Scheme-2.23).



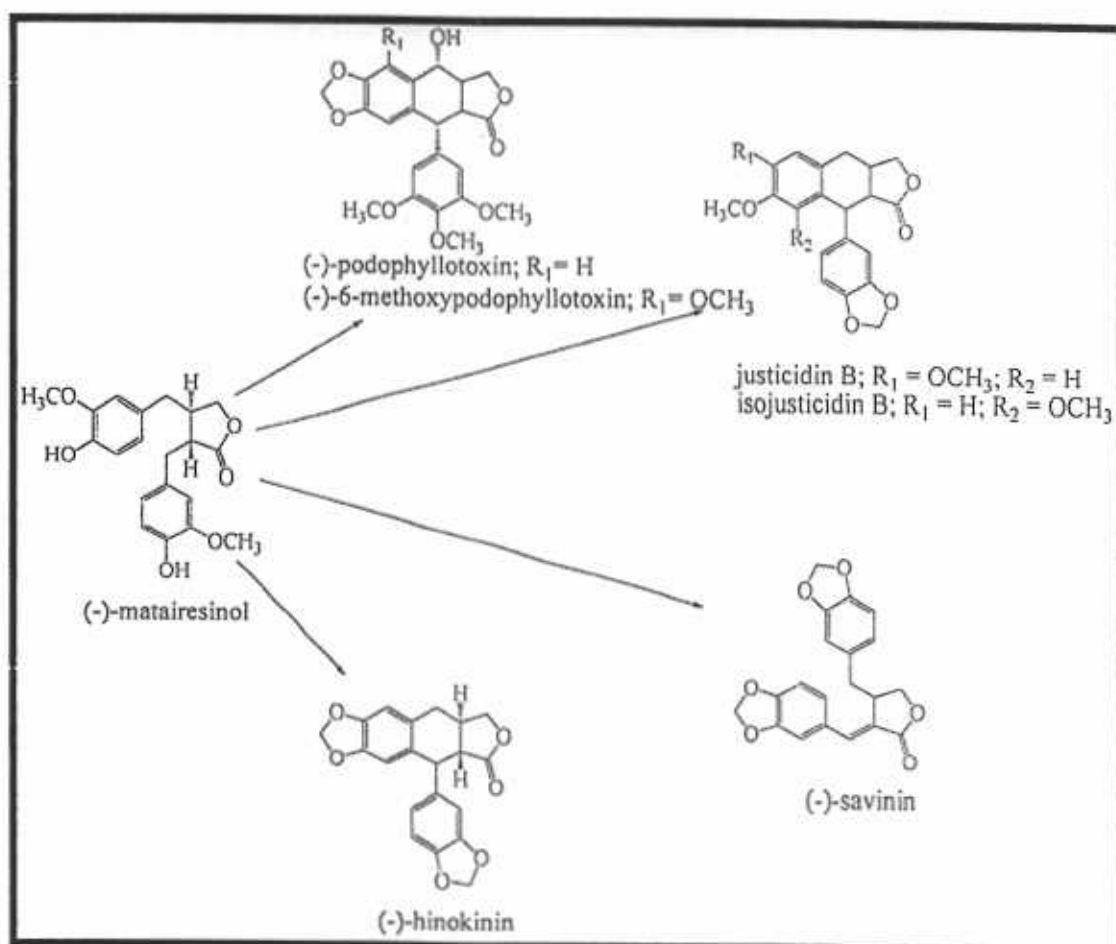
Scheme-2.23: Formation of onocerin (82) and ambrein (83)

Till today only cDNAs of the first biosynthetic steps have been cloned.⁶³ Coniferyl alcohol (84) (2 units) combines to form pinoresinol (85), a step facilitated by the so called dirigent protein.⁶⁴ The (+)-pinoresinol (85) is then reduced to (-)-secoisolariciresinol (87) through (+)-larisinal (86) by pinoresinol-lariciresinol reductase and subsequently oxidized to (-)-matairesinol (88).⁶⁵ The steps onwards 88 are mostly hypothetical because they were proposed after feeding of possible intermediates to *Anthriscus sylvestris*.⁶⁶

The biosynthetic pathways for the formation of other lignans are also believed to involve the same first steps of conversion of coniferyl alcohol to matairesinol. Matairesinol is considered to be a central intermediate involved in the formation of all diverse lignan structures (Scheme-2.26).



Scheme-2.25: Biosynthetic pathway of podophyllotoxin and related lignans



Scheme-2.26: Lignans possibly derived from matairesinol

Part-A

***Phytochemistry and Bioactivity of
Erythrina suberosa***



***Erythrina suberosa* Roxb.**

Chapter: 3

INTRODUCTION (Part-A)

3.1. Family Papilionaceae (Faboideae)

Family Papilionaceae is a large family of leguminous plants with 480 genera and 12000 species.⁶⁷ The plants of this family are mostly herbs, shrubs or trees occurring in all types of climatic conditions but mostly between and around the tropics and are more abundant in the Old World than in the New World. It is an extremely important family with regards to producing nutritious food, fiber, shelter, valuable medicines and also virulent poisons. The members of this family demonstrate a variety of properties, for example some are amylaceous (starchy), other oleaginous, many yield resins, balsams and dyes, a few are astringent, acrid and bitter, narcotic and poisonous, emetic and purging, tonic and restorative. The seeds are often anti periodic and the roots anthelmintic.

3.2. Genus *Erythrina*

The genus *Erythrina* is comprised of over 110 species having orange or red flowers, occurring as trees, shrubs and herbaceous plants. Coral tree is a collective name for the plants of this genus. The genus is found throughout the tropical and subtropical regions of the world. In Pakistan this genus is represented by 3 species which are cultivated as ornamental trees namely; *Erythrina suberosa*, *E. glaberescence* and *E. herbacea*.⁶⁸

3.3. *Erythrina suberosa* Roxb. (Gul-e-Nishtar)

E. suberosa is cultivated in Pakistan as ornamental plant. It is a tree of medium size with a height ranging from 13-17 m. The branches of the tree are armed with prickles and falls off after the third year. Leaves are trifoliate; flowers are red in color arranged

in fascicles of 1-3. Fruits are stipitate having a length of 12.5-15.0 cm and contain 2-5 seeds.

3.4. Pharmacological importance of genus *Erythrina*

The plants of genus *Erythrina* have been known for their pharmacological activities. South Americans used the extracts of these plants as arrow poisons, as antidote against strychnine or as hypnotic and antiepileptic.⁶⁹ The hydro-alcoholic extract of the seeds of *E. americana* was shown to have an activity similar to tubocurarine, when administered in different doses.⁷⁰ Erythroidine was the first crystalline pharmacologically active alkaloid isolated from *E. americana*⁷¹ and the name erythroidine was suggested by Altamirano for an unknown constituent of the same plant. Later on this constituent proved to be a mixture of two isomeric alkaloids; α - and β -erythroidines.⁷⁰

Several *Erythrina* species are used in traditional medicines as eyewashes, dressing of wounds, as relievers of joints pain, or as calming and relaxing agents.⁷² The leaf extract of *E. variegata* has been found to possess hypoglycemic activity in streptozotocin-induced diabetic rats,⁷³ thus sustaining the folk use of this plant in treating diabetes. The alkaloidal extracts of *E. variegata* showed depressed activity of the central nervous system (CNS) as well as anticonvulsive activity. They have also been found to increase pentobarbital hypnosis, and inhibit the acetylcholine-induced spasm. The ethanolic extract of the leaves of *E. indica*, which mainly consists of alkaloids, was shown to have antidiarrheal effects in animals,⁷⁴ thus supporting the traditional use of the plant for controlling the disease. The extracts of *E. velutina* and *E. variegata* were recorded for their spasmolytic and antineoplastic activities.^{75,76} Recent works by utilizing crude extracts and pure compounds of *E. americana*,

indicated a reduction in the aggressive behavior in rats. The factor responsible for these effects is an interaction between the cholinergic and GABAergic receptors. Amongst all the alkaloids tested, the α - and β -erythroidines were shown to have highest activities.⁷² The β -erythroidine and its more potent (2,7)-dihydroderivative have been in clinical trials as muscular relaxants,⁷⁰ an activity related to the antagonistic action of the dihydroderivative with the nicotinic receptors of acetylcholine.⁷⁷⁻⁷⁹ Erysodine, an alkaloid obtained from many *Erythrina* species, has been proven to be a competitive, reversible antagonist of nicotine-induced dopamine release and was found as potent as dihydro- β -erythroidine and can prove to be an important tool in characterizing nicotinic acetylcholinic receptors.⁸⁰ The hydro-alcoholic extracts of *E. mulungu* and purified compounds from the flowers of the plant were tested for their anxiolytic like properties using the elevated T-maze test, and the light dark transition model.⁸¹ A moderate activity was observed, which explains the folk use of this plant to calm agitation and for the treatment of other mental diseases. Recently, our collaborative research on the alkaloidal constituents of *E. suberosa* has shown that erysodine and erysotrine possess significant anxiolytic properties when subjected to the two tests of anxiety-the elevated plus-maze (EPM) and the light/dark transition model (LTDM).⁸²

In addition to pharmacological activities due to the presence of alkaloids, *Erythrina* plants also show biological properties due to glucoproteins or lectins having good agglutinant activity on human erythrocytes A, B, and O.^{83,84} Antimalarial saponins, such as sigmoidin C and D were isolated from the stem bark of *E. sigmoidea*⁸⁵ while sigmoiside E, a triterpenoid saponin obtained from the same plant showed antibacterial activity.⁸⁶ Petrocarpans such as cristacarpin, erystagallin and phaseollidine obtained from *E. crista-galli* act as phytoalexins and also show strong

antimicrobial activity against Gram-positive bacteria.⁸⁷ The erystagallin-A obtained from the stem bark of *E. variegata* has been found active in an anticancer assay against breast cancer T47D cell-lines *in vitro* and antimalarial activity against *Plasmodium falciparum* *in vitro*.⁸⁸ Erycristagallin, a petrocarpene isolated from *E. mildbraedii*,⁸⁹ was reported for its anti-inflammatory activity by inhibiting arachidonic acid metabolism through the 5-lipoxygenase pathway.

Cui and co-workers obtained and characterized four chalcones; abyssinones A-D from *E. abyssinica*. These compounds were observed with moderate cytotoxicity against Caco2 cell-lines of human colorectal cancer.⁹⁰

Several flavonoids,⁹¹⁻⁹³ isoflavonoids⁹⁴⁻⁹⁶ and petrocarpanes^{97,98} exhibit antimicrobial and antibacterial activities. The hepatoprotective properties of the *E. senegalensis* have been studied as because of the presence of prenylisoflavonoids, which act as novel HIV-I protease inhibitors.⁹⁹

3.5. Literature survey of genus *Erythrina*

Literature survey showed a great amount of work on genus *Erythrina*. The list of compounds provided in Table-3.1, indicates the variety of constituents found in the species of genus *Erythrina*.

Table-3.1: Isolated compounds from genus *Erythrina*

S. No	Name of Compound	Molecular Formula	Source
1	Abyssinoflavanone IV	C ₂₂ H ₂₀ O ₆	<i>E. abyssinica</i> ¹⁰⁰
2	Abyssinoflavanone V	C ₂₅ H ₂₈ O ₇	<i>E. abyssinica</i> ¹⁰⁰
3	Abyssinoflavanone VI	C ₂₅ H ₂₈ O ₇	<i>E. abyssinica</i> ¹⁰⁰
4	Abyssinone A	C ₂₀ H ₁₈ O ₅	<i>E. abyssinica</i> ¹⁰¹
5	Abyssinone B	C ₂₀ H ₂₀ O ₆	<i>E. abyssinica</i> ¹⁰¹
6	Abyssinone C	C ₂₀ H ₂₀ O ₇	<i>E. abyssinica</i> ¹⁰¹
7	Abyssinone D	C ₂₁ H ₂₂ O ₅	<i>E. abyssinica</i> ¹⁰¹

8	Abyssinone I	C ₂₀ H ₁₈ O ₄	<i>E. abyssinica</i> ¹⁰²
9	Abyssinone III	C ₂₅ H ₂₆ O ₄	<i>E. abyssinica</i> ¹⁰²
10	Alpinumisoflavone	C ₂₀ H ₁₆ O ₅	<i>E. caffra</i> ⁹²
11	Dihydroalpinumisoflavone	C ₂₀ H ₁₈ O ₅	<i>E. arborescens</i> ¹⁰³
12	Indicanin C	C ₂₁ H ₁₈ O ₅	<i>E. indica</i> ¹⁰⁴
13	Indicanin E	C ₂₂ H ₂₀ O ₆	<i>E. indica</i> ¹⁰⁵
14	Erysubin B	C ₂₀ H ₁₆ O ₆	<i>E. suberosa</i> ¹⁰⁶
15	Auriculasin	C ₂₅ H ₂₄ O ₆	<i>E. eriotricha</i> ¹⁰⁷
16	Auriculatin	C ₂₅ H ₂₄ O ₆	<i>E. senegalensis</i> ¹⁰⁸
17	Auriculatin 4'-glucoside	C ₃₁ H ₃₄ O ₁₁	<i>E. eriotricha</i> ¹⁰⁹
18	Erysenegalensein I	C ₂₅ H ₂₆ O ₇	<i>E. senegalensis</i> ¹¹⁰
19	2,3-Dihydro-2'-O-methylauriculatin	C ₂₆ H ₂₈ O ₆	<i>E. variegata</i> ¹¹¹
20	2,3-Dihydroauriculatin	C ₂₅ H ₂₆ O ₆	<i>E. addisoniae</i> ¹¹²
21	Erysenegalensein F	C ₂₅ H ₂₄ O ₇	<i>E. senegalensis</i> ¹¹³
22	Erysenegalensein L	C ₂₅ H ₂₄ O ₇	<i>E. senegalensis</i> ¹¹³
23	Bidwillon B	C ₂₅ H ₂₆ O ₅	<i>E. bidwilli</i> ¹¹⁴
24	Bidwillon C	C ₂₀ H ₁₆ O ₄	<i>E. bidwilli</i> ¹¹⁵
25	Burtinol A	C ₂₁ H ₂₂ O ₄	<i>E. burtii</i> ¹¹⁶
26	Burtinol D	C ₂₀ H ₂₀ O ₄	<i>E. burtii</i> ¹¹⁶
27	Crystamidine	C ₁₈ H ₁₅ NO ₄	<i>E. brucei</i> ¹¹⁷
28	3-Demethoxyerythratidinone	C ₁₈ H ₂₁ NO ₃	<i>E. subumbrans</i> ¹¹⁸
29	2,3-Dihydro-5,7-dihydroxy-2-(2,2,9,9-tetramethyl-2H,9H-benzo[2,1-b:3,4-b']dipyr-5-yl)-4H-1-benzopyran-4-one	C ₂₅ H ₂₄ O ₆	<i>E. abyssinica</i> ¹¹⁹
30	3,4-Dihydro-2',2'-dimethyl-7,7'-dihydroxy-8'-prenyl-3,6'-bi-2H-1-benzopyran	C ₂₅ H ₂₈ O ₄	<i>E. mildbraedii</i> ¹²⁰
31	Sigmoidin K	C ₂₅ H ₂₄ O ₅	<i>E. sigmoidea</i> ¹²¹
32	4'-O-Methylabyssinone IV	C ₂₆ H ₃₀ O ₄	<i>E. mildbraedii</i> ¹²²
33	7-Hydroxy-3'-(3-hydroxy-3-methyl-1-butenyl)-4'-methoxy-5'-(3-methyl-2-butenyl)flavanone	C ₂₆ H ₃₀ O ₅	<i>E. mildbraedii</i> ¹²²
34	4',7-Dihydroxy-3',5'-diprenylflavanone	C ₂₅ H ₂₈ O ₅	<i>E. addisoniae</i> ¹²³
35	Erysubin F	C ₂₅ H ₂₆ O ₄	<i>E. suberosa</i> ¹²⁴
36	Eryvarin S	C ₂₅ H ₂₆ O ₄	<i>E. variegata</i> ¹²⁵
37	Erythrabysin II	C ₂₅ H ₂₈ O ₄	<i>E. suberosa</i> ¹²⁶
38	Eryvarin J	C ₂₅ H ₂₈ O ₄	<i>E. variegata</i> ¹²⁷
39	Erybraedin A	C ₂₅ H ₂₈ O ₄	<i>E. burtii</i> ¹¹⁶
40	Erybraedin C	C ₂₅ H ₂₈ O ₄	<i>E. eriotricha</i> ¹²⁸
41	Erycristagallin	C ₂₅ H ₂₆ O ₄	<i>E. subumbrans</i> ⁹⁷
42	Eryvarin E	C ₂₆ H ₂₈ O ₄	<i>E. variegata</i> ¹²⁹
43	2-(3-Hydroxy-5-methoxyphenyl)-5-methoxy-6-benzofuranol	C ₁₆ H ₁₄ O ₅	<i>E. latissima</i> ¹³⁰
44	Dihydrooxyresveratrol	C ₁₄ H ₁₄ O ₄	<i>E. variegata</i> ¹³¹
45	Erythbidin E	C ₁₅ H ₁₂ O ₄	<i>E. × bidwillii</i> ¹³²
46	Eryvarinol A	C ₂₅ H ₂₆ O ₉	<i>E. variegata</i> ¹³³
47	Eryvarinol B	C ₃₀ H ₃₄ O ₉	<i>E. variegata</i> ¹³³

48	Addisofuran B	C ₂₀ H ₂₀ O ₄	<i>E. addisoniae</i> ¹³⁴
49	Erythrinassinate A	C ₃₈ H ₆₆ O ₄	<i>E. species</i> ¹³⁵
50	Erythrinassinate D	C ₃₆ H ₆₂ O ₄	<i>E. species</i> ¹³⁶
51	4',7-Dihydroxy-3'-prenylflavanone	C ₂₀ H ₂₀ O ₄	<i>E. abyssinica</i> ¹⁰²
52	Neobavaisoflavone	C ₂₀ H ₁₈ O ₄	<i>E. sigmoidea</i> ¹³⁷
53	4'-O-Methylneobavaisoflavone	C ₂₁ H ₂₀ O ₄	<i>E. sacleuxii</i> ¹³⁸
54	8-Prenyldaidzein	C ₂₀ H ₁₈ O ₄	<i>E. fusca</i> ¹³⁹
55	Addisofuran A	C ₂₀ H ₂₀ O ₄	<i>E. addisoniae</i> ¹³⁴
56	Bidwillol B	C ₂₀ H ₂₀ O ₄	<i>E. × bidwillii</i> ¹¹⁵
57	3-Hydroxy-10-(3-hydroxy-3-methylbutyl)-9-methoxypterocarpan	C ₂₁ H ₂₄ O ₅	<i>E. fusca</i> ¹⁴⁰
58	Sandwicensin	C ₂₁ H ₂₂ O ₄	<i>E. species</i> ¹⁴¹
59	Orientalol B	C ₂₁ H ₂₂ O ₄	<i>E. orientalis</i> ¹⁴²
60	Erypogin H	C ₂₀ H ₁₈ O ₄	<i>E. poeppigiana</i> ¹⁴³
61	Eryvarin D	C ₂₁ H ₂₀ O ₄	<i>E. variegata</i> ¹²⁹
62	Erypogin E	C ₂₀ H ₁₈ O ₄	<i>E. poeppigiana</i> ¹⁴⁴
63	Erysubin C	C ₁₇ H ₁₄ O ₅	<i>E. suberosa</i> ¹²⁴
64	Burtinol A	C ₂₁ H ₂₂ O ₄	<i>E. burtii</i> ¹¹⁶
65	Saclenone	C ₂₃ H ₂₄ O ₇	<i>E. sacleuxii</i> ¹⁴⁵
66	Eriotrinol	C ₂₁ H ₂₀ O ₇	<i>E. species</i> ¹⁴⁶
67	Erybidine	C ₂₀ H ₂₅ NO ₄	<i>E. species</i> ¹⁴⁷
68	Erybraedin B	C ₂₅ H ₂₆ O ₄	<i>E. mildbraedii</i> ¹⁴⁸
69	Erybraedin D	C ₂₅ H ₂₆ O ₄	<i>E. mildbraedii</i> ¹⁴⁹
70	Erybraedin E	C ₂₂ H ₂₀ O ₄	<i>E. mildbraedii</i> ¹⁴⁹
71	Erycristanol A	C ₂₀ H ₂₂ O ₃	<i>E. crista-galli</i> ¹⁵⁰
72	Erycristanol C	C ₂₀ H ₂₂ O ₄	<i>E. crista-galli</i> ¹⁵⁰
73	Erycristin	C ₂₆ H ₃₀ O ₄	<i>E. crista-galli</i> ¹⁵¹
74	Erymelanthine	C ₁₈ H ₂₀ N ₂ O ₃	<i>E. melanacantha</i> ¹⁵²
75	Decarbomethoxyerymelanthine	C ₁₆ H ₁₈ N ₂ O	<i>E. variegata</i> ¹⁵³
76	Melanacanthine	C ₁₈ H ₁₈ N ₂ O ₄	<i>E. melanacantha</i> ¹⁵⁴
77	Erypogin F	C ₂₁ H ₂₀ O ₅	<i>E. poeppigiana</i> ¹⁴³
78	Erypogin G	C ₂₂ H ₂₂ O ₆	<i>E. poeppigiana</i> ¹⁴³
79	Erypogin I	C ₂₁ H ₂₂ O ₆	<i>E. poeppigiana</i> ¹⁴³
80	Erypogin J	C ₂₆ H ₂₈ O ₅	<i>E. poeppigiana</i> ¹⁴³
81	Erysenegalensein C	C ₂₆ H ₂₈ O ₇	<i>E. senegalensis</i> ¹⁵⁵
82	Erysenegalensein J	C ₂₅ H ₂₄ O ₇	<i>E. senegalensis</i> ¹⁵⁶
83	Erysenegalensein K	C ₂₂ H ₁₈ O ₆	<i>E. senegalensis</i> ¹⁵⁶
84	Erysodienone	C ₁₈ H ₁₉ NO ₄	<i>E. lithosperma</i> ¹⁵⁷
85	Erythritol	C ₁₉ H ₂₃ NO ₄	<i>E. variegata</i> ¹⁵⁸
86	Erysodinophorine	C ₃₂ H ₃₈ N ₃ O ₄	<i>E. arborescens</i> ¹⁵⁹
87	Erysopinophorine	C ₃₁ H ₃₆ N ₃ O ₄	<i>E. arborescens</i> ¹⁶⁰
88	Erysophorine	C ₃₂ H ₃₈ N ₃ O ₄	<i>E. arborescens</i> ¹⁶¹
89	Isoerysopinophorine	C ₃₁ H ₃₆ N ₃ O ₄	<i>E. arborescens</i> ¹⁶²
90	Erysotramidine	C ₁₉ H ₂₁ NO ₄	<i>E. herbacea</i> ¹⁶³
91	8-Oxoerysodine	C ₁₈ H ₁₉ NO ₄	<i>E. fusca</i> ¹⁶⁴
92	Isococcolinine	C ₁₈ H ₁₉ NO ₃	<i>E. variegata</i> ¹⁶⁵
93	Erytharbine	C ₁₉ H ₁₉ NO ₄	<i>E. herbacea</i> ¹⁶³

94	11 β -Hydroxyerysotramidine	C ₁₉ H ₂₁ NO ₅	<i>E. lysistemom</i> ¹⁶⁶
95	11 β -Methoxyerysotramidine	C ₂₀ H ₂₃ NO ₅	<i>E. lysistemom</i> ¹⁶⁶
96	Erythromotidienone	C ₁₈ H ₁₉ NO ₃	<i>E. variegata</i> ¹⁶⁷
97	Erysotrine	C ₁₉ H ₂₃ NO ₃	<i>E. suberosa</i> ¹⁶⁸
98	Erysothiovine	C ₂₀ H ₂₃ NO ₇ S	<i>E. species</i> ¹⁶⁹
99	Glucoerysodine	C ₂₄ H ₃₁ NO ₈	<i>E. addisoniae</i> ¹⁷⁰
100	Rhamnoerysodine	C ₂₄ H ₃₁ NO ₇	<i>E. lysistemom</i> ¹⁷¹
101	Erysovine N-oxide	C ₁₈ H ₂₁ NO ₄	<i>E. addisoniae</i> ¹⁷⁰
102	Erythodine N-oxide	C ₁₈ H ₂₁ NO ₄	<i>E. velutina</i> ¹⁷²
103	Erysovine	C ₁₈ H ₂₁ NO ₃	<i>E. latissima</i> ¹⁷³
104	Erysodine	C ₁₈ H ₂₁ NO ₃	<i>E. latissima</i> ¹⁷³
105	Erythravine	C ₁₈ H ₂₁ NO ₃	<i>E. mulungu</i> ⁸¹
106	Erysothiopine	C ₁₉ H ₂₁ NO ₇ S	<i>E. species</i> ¹⁶⁹
107	15-Glucoerysopine	C ₂₃ H ₂₉ NO ₈	<i>E. latissima</i> ¹⁷⁴
108	16-Glucoerysopine	C ₂₃ H ₂₉ NO ₈	<i>E. latissima</i> ¹⁷⁴
109	Erysopine	C ₁₇ H ₁₉ NO ₃	<i>E. addisoniae</i> ¹⁷⁰
110	Erysoline	C ₁₇ H ₁₉ NO ₃	<i>E. species</i> ¹⁷⁵
111	10,11-Dioxoerysotrine	C ₁₉ H ₁₉ NO ₅	<i>E. herbacea</i> ¹⁶³
112	10-Hydroxy-11-oxoerysotrine	C ₁₉ H ₂₁ NO ₅	<i>E. herbacea</i> ¹⁶³
113	Erysotrine N-oxide	C ₁₉ H ₂₃ NO ₄	<i>E. herbacea</i> ¹⁶³
114	Erythagallin C	C ₂₀ H ₂₀ O ₅	<i>E. species</i> ^{87,176}
115	Erythbidin A	C ₂₀ H ₂₀ O ₄	<i>E. × bidwillii</i> ¹⁷⁷
116	Erythraline	C ₁₈ H ₁₉ NO ₃	<i>E. addisoniae</i> ¹⁷⁰
117	11-Methoxyerythraline	C ₁₉ H ₂₁ NO ₄	<i>E. lysistemom</i> ¹⁷⁸
118	8-Oxoerythraline	C ₁₈ H ₁₇ NO ₄	<i>E. brucei</i> ¹¹⁷
119	11 β -Methoxyerythraline N-oxide	C ₁₉ H ₂₁ NO ₅	<i>E. crista-galli</i> ¹⁷⁹
120	Erythbidine B	C ₁₈ H ₁₇ NO ₅	<i>E. × bidwillii</i> ¹⁸⁰
121	Erythrosotidienone	C ₁₇ H ₁₅ NO ₃	<i>E. variegata</i> ¹⁶⁷
122	10,11-Dioxoerythraline	C ₁₈ H ₁₅ NO ₅	<i>E. × bidwillii</i> ¹⁸¹
123	8-Oxoerythraline epoxide	C ₁₈ H ₁₇ NO ₅	<i>E. × bidwillii</i> ¹⁸¹
124	8-Oxoerythrinine	C ₁₈ H ₁₇ NO ₅	<i>E. brucei</i> ¹¹⁷
125	Erythrinine	C ₁₈ H ₁₉ NO ₄	<i>E. brucei</i> ¹¹⁷
126	Erythrocarine	C ₁₇ H ₁₇ NO ₃	<i>E. species</i> ¹⁸²
127	Erythartine	C ₁₉ H ₂₃ NO ₄	<i>E. mulungu</i> ¹⁸³
128	Erythrasine	C ₂₁ H ₂₅ NO ₅	<i>E. arborescens</i> ¹⁸⁴
129	11-Hydroxyerysovine	C ₁₈ H ₂₁ NO ₄	<i>E. stricta</i> ⁹⁸
130	11-Hydroxyerysodine	C ₁₈ H ₂₁ NO ₄	<i>E. stricta</i> ⁹⁸
131	11 α -Hydroxyerythravine	C ₁₈ H ₂₁ NO ₄	<i>E. mulungu</i> ⁸¹
132	Erythristemine	C ₂₀ H ₂₅ NO ₄	<i>E. lysistemom</i> ¹⁸⁵
133	11 β -Methoxyglucoerysovine	C ₂₅ H ₃₃ NO ₉	<i>E. lysistemom</i> ¹⁷¹
134	11 β -Methoxyglucoerysodine	C ₂₅ H ₃₃ NO ₉	<i>E. lysistemom</i> ¹⁷¹
135	Erythristemine N-oxide	C ₂₀ H ₂₅ NO ₅	<i>E. bidwillii</i> ¹⁸⁶
136	Erythartine N-oxide	C ₁₉ H ₂₃ NO ₅	<i>E. berteriana</i> ¹⁸⁷
137	Erythratidine	C ₁₉ H ₂₅ NO ₄	<i>E. falcata</i> ¹⁸⁸
138	Erysosalvine	C ₁₈ H ₂₃ NO ₄	<i>E. salviiflora</i> ¹⁸⁹
139	Erysotine	C ₁₈ H ₂₃ NO ₄	<i>E. salviiflora</i> ¹⁸⁹
140	Erysopitine	C ₁₇ H ₂₁ NO ₄	<i>E. variegata</i> ⁷³
141	10,11-Dioxoerythratidinone	C ₁₉ H ₁₉ NO ₆	<i>E. subumbrans</i> ¹⁹⁰

142	10,11-Dioxo-2-epierythratinine	C ₁₉ H ₂₁ NO ₆	<i>E. subumbrans</i> ¹⁹⁰
143	2-Epierythratinine	C ₁₉ H ₂₅ NO ₄	<i>E. fusca</i> ¹⁹¹
144	Erysosalvinone	C ₁₈ H ₂₁ NO ₄	<i>E. salviiflora</i> ¹⁸⁹
145	Erysotinine	C ₁₈ H ₂₁ NO ₄	<i>E. salviiflora</i> ¹⁸⁹
146	Erysoflorinone	C ₁₇ H ₁₉ NO ₄	<i>E. salviiflora</i> ¹⁸⁹
147	Erythratinone	C ₁₉ H ₂₃ NO ₄	<i>E. suberosa</i> ¹²⁶
148	11-Methoxyerythratinine	C ₂₀ H ₂₇ NO ₅	<i>E. species</i> ¹⁹²
149	Erythratinine	C ₁₈ H ₂₁ NO ₄	<i>E. species</i> ¹⁷⁵
150	Erythramine	C ₁₈ H ₂₁ NO ₃	<i>E. crista-galli</i> ¹⁹³
151	10,11-Dioxoerythratinine	C ₁₈ H ₁₇ NO ₆	<i>E. subumbrans</i> ¹⁹⁰
152	11-Methoxyerythratinine	C ₁₉ H ₂₃ NO ₅	<i>E. crista-galli</i> ¹⁷⁹
153	Erythratinine	C ₃₀ H ₃₆ N ₄ O ₅	<i>E. x bidwillii</i> ¹⁸⁰
154	α -Erythroidine	C ₁₆ H ₁₉ NO ₃	<i>E. breviflora</i> ¹⁹⁴
155	8-Oxo- β -erythroidine	C ₁₆ H ₁₇ NO ₄	<i>E. berteroana</i> ¹⁹⁵
156	8-Oxo- α -erythroidine	C ₁₆ H ₁₇ NO ₄	<i>E. berteroana</i> ¹⁹⁵
157	β -Erythroidine	C ₁₆ H ₁₉ NO ₃	<i>E. americana</i> ¹⁹⁶
158	8-Oxo- α -erythroidine epoxide	C ₁₆ H ₁₇ NO ₅	<i>E. poeppigiana</i> ¹⁷⁶
159	Eryvarietyrene	C ₂₀ H ₂₂ O ₂	<i>E. variegata</i> ¹⁹⁷
160	Erythidin C	C ₂₀ H ₂₄ O ₃	<i>E. x bidwillii</i> ¹³²
161	Erycristanol B	C ₂₀ H ₂₂ O ₃	<i>E. crista-galli</i> ¹⁵⁰
162	Eryvarin A	C ₂₁ H ₂₂ O ₆	<i>E. variegata</i> ¹⁹⁸
163	Eryvarin B	C ₂₅ H ₂₆ O ₆	<i>E. variegata</i> ¹⁹⁸
164	Eryvarin F	C ₂₅ H ₂₆ O ₆	<i>E. variegata</i> ¹⁹⁹
165	Eryvarin G	C ₂₅ H ₂₄ O ₆	<i>E. variegata</i> ¹⁹⁹
166	Eryvarin I	C ₂₁ H ₂₀ O ₄	<i>E. variegata</i> ¹²⁷
167	Eryvarin O	C ₂₁ H ₂₀ O ₅	<i>E. variegata</i> ²⁰⁰
168	Eryvarin Q	C ₂₅ H ₂₆ O ₅	<i>E. variegata</i> ²⁰⁰
169	Eryvarin R	C ₁₇ H ₁₆ O ₆	<i>E. variegata</i> ²⁰⁰
170	Eryzerin D	C ₂₅ H ₂₈ O ₄	<i>E. zeyheri</i> ²⁰¹
171	Euchrenone b ₁₀	C ₂₅ H ₂₆ O ₆	<i>E. vogelii</i> ²⁰²
172	Erysenegalensein H	C ₂₅ H ₂₆ O ₇	<i>E. senegalensis</i> ¹¹⁰
173	Eriotriochin	C ₂₇ H ₃₀ O ₇	<i>E. eriotriocha</i> ²⁰³
174	Folitenol	C ₂₅ H ₂₆ O ₄	<i>E. orientalis</i> ¹⁴²
175	Erysubin D	C ₂₅ H ₂₈ O ₅	<i>E. suberosa</i> ¹²⁴
176	Erysubin E	C ₂₅ H ₂₆ O ₅	<i>E. suberosa</i> ¹²⁴
177	5'-Formylpratensein	C ₁₇ H ₁₂ O ₇	<i>E. saculeuxii</i> ²⁰⁴
178	Orientanol C	C ₂₅ H ₂₆ O ₄	<i>E. orientalis</i> ¹⁴²
179	Erypogin B	C ₂₁ H ₂₀ O ₄	<i>E. poeppigiana</i> ¹⁴⁴
180	5-Deoxyglyasperin F	C ₂₀ H ₁₈ O ₅	<i>E. species</i> ¹⁴¹
181	Eryvarin U	C ₂₀ H ₁₈ O ₄	<i>E. variegata</i> ¹²⁵
182	1-Heptatriacontanol	C ₃₇ H ₇₆ O	<i>E. stricta</i> ²⁰⁵
183	1-Hexatriacontanol	C ₃₆ H ₇₄ O	<i>E. stricta</i> ²⁰⁵
184	8-(15-Hydroxypentadecyl)-7-methoxy-2H-1-benzopyran-2-one	C ₂₄ H ₃₆ O ₄	<i>E. stricta</i> ²⁰⁵
185	Hydroxycristacarpone	C ₂₁ H ₂₂ O ₆	<i>E. orientalis</i> ²⁰⁶
186	5-Hydroxy-9-(4-hydroxyphenyl)-2,2-dimethyl-2H,10H-benzo[1,2-b:3,4-b']dipyrans-10-one	C ₂₀ H ₁₆ O ₅	<i>E. lysisitemon</i> ²⁰⁷

187	α -Methoxy-N,N-dimethyl-1H-indole-3-propanamide, 9Cl. 3-(1H-Indol-3-yl)-2-methyl-N,N-dimethylpropanamide	$C_{14}H_{18}N_2O_2$	<i>E. burana</i> ²⁰⁸
188	14-Hydroxy-9,11-octadecadienoic acid	$C_{18}H_{32}O_3$	<i>E. burana</i> ²⁰⁸
189	Erythrasinate B	$C_{38}H_{66}O_4$	<i>E. variegata</i> ²⁰⁹
190	Erythrasinate C	$C_{24}H_{38}O_4$	<i>E. species</i> ¹³⁶
191	Eryvarin L	$C_{16}H_{14}O_5$	<i>E. variegata</i> ¹²⁷
192	Eryvarin P	$C_{17}H_{14}O_6$	<i>E. variegata</i> ²⁰⁰
193	Hypaphorine	$C_{14}H_{18}N_2O_2$	<i>E. velutina</i> ²¹⁰
194	Indicanin A	$C_{22}H_{20}O_6$	<i>E. indica</i> ²¹¹
195	Indicanin D	$C_{51}H_{78}O_7$	<i>E. indica</i> ¹⁰⁵
196	Erythrivarone B	$C_{25}H_{26}O_5$	<i>E. variegata</i> ²¹²
197	Erysubin A	$C_{20}H_{16}O_6$	<i>E. suberosa</i> ¹⁰⁶
198	Erythrinin C	$C_{20}H_{18}O_6$	<i>E. poeppigiana</i> ¹⁷⁶
199	5-Deoxylicoisoflavanone	$C_{20}H_{18}O_5$	<i>E. species</i> ¹⁴¹
200	Lonchocarpol D	$C_{25}H_{28}O_6$	<i>E. fusca</i> ²¹³
201	Eryvarin C	$C_{20}H_{20}O_4$	<i>E. variegata</i> ¹²⁹
202	1-Octatriacontanol	$C_{38}H_{78}O$	<i>E. stricta</i> ²⁰⁵
203	Sigmoiside A	$C_{36}H_{60}O_7$	<i>E. sigmoidea</i> ²¹⁴
204	Sigmoiside B	$C_{36}H_{60}O_7$	<i>E. sigmoidea</i> ²¹⁴
205	Sigmoiside E	$C_{36}H_{60}O_7$	<i>E. sigmoidea</i> ⁸⁶
206	α -Sophoradiol	$C_{30}H_{50}O_2$	<i>E. sigmoidea</i> ²¹⁵
207	28-Acetylerythrodol	$C_{32}H_{52}O_3$	<i>E. eriotriocho</i> ²¹⁶
208	Sigmoiside F	$C_{36}H_{60}O_8$	<i>E. sigmoidea</i> ²¹⁷
209	Sigmoiside C	$C_{36}H_{60}O_8$	<i>E. sigmoidea</i> ⁸⁵
210	Sigmoiside D	$C_{36}H_{60}O_7$	<i>E. sigmoidea</i> ⁸⁵
211	12-Oleanen-3-ol, propyl ether	$C_{33}H_{56}O$	<i>E. sigmoidea</i> ²¹⁷
212	Osajin	$C_{25}H_{24}O_5$	<i>E. orientalis</i> ²⁰⁶
213	Orientanol F	$C_{25}H_{26}O_5$	<i>E. orientalis</i> ²¹⁸
214	Erysenegalensein O	$C_{25}H_{26}O_7$	<i>E. senegalensis</i> ²¹⁹
215	Cristadine	$C_{18}H_{17}NO_4$	<i>E. crista-galli</i> ²²⁰
216	Erysenegalensein B	$C_{25}H_{28}O_7$	<i>E. senegalensis</i> ¹⁵⁵
217	2'-Hydroxy-5'-methoxybiochanin A	$C_{17}H_{14}O_7$	<i>E. eriotriocho</i> ²²¹
218	2',4',5,5',7-Pentahydroxyisoflavanone; 2',4',5'-Tri-Me ether	$C_{18}H_{18}O_7$	<i>E. latissima</i> ²²²
219	(R)-2,3-Dihydro-7-O-demethylrobustigenin	$C_{18}H_{18}O_7$	<i>E. saclexii</i> ¹⁴⁵
220	7-De-O-methylrobustigenin	$C_{18}H_{16}O_7$	<i>E. saclexii</i> ²⁰⁴
221	Phaseollin	$C_{20}H_{18}O_4$	<i>E. addisoniae</i> ²²³
222	Phomol	$C_{22}H_{36}O_7$	<i>E. crista-galli</i> ²²⁴
223	Erylatissin B	$C_{20}H_{16}O_5$	<i>E. latissima</i> ⁹¹
224	6'-Prenylsigmoidin C	$C_{25}H_{26}O_6$	<i>E. abyssinica</i> ¹¹⁹
225	Protosinomenine	$C_{19}H_{23}NO_4$	<i>E. lithosperma</i> ¹⁵⁷
226	Norprotosinomenine	$C_{18}H_{21}NO_4$	<i>E. lithosperma</i> ¹⁵⁷
227	Indicanin B	$C_{21}H_{18}O_6$	<i>E. indica</i> ¹⁰⁴
228	Scandenone	$C_{25}H_{24}O_5$	<i>E. fusca</i> ²²⁵

229	Erysenegalensein G	C ₂₅ H ₂₄ O ₆	<i>E. senegalensis</i> ¹¹³
230	Erysenegalensein M	C ₂₅ H ₂₄ O ₆	<i>E. senegalensis</i> ²²⁶
231	Scoulerine	C ₁₉ H ₂₁ NO ₄	<i>E. orientalis</i> ¹⁴⁷
232	Senegalensin	C ₂₅ H ₂₆ O ₆	<i>E. suberosa</i> ²²⁷
233	Sigmoidin C	C ₂₀ H ₁₈ O ₆	<i>E. sigmoidea</i> ²²⁸
234	Abyssinin I	C ₂₁ H ₂₀ O ₆	<i>E. abyssinica</i> ²²⁹
235	Abyssinoflavanone V	C ₂₀ H ₁₈ O ₅	<i>E. abyssinica</i> ²³⁰
236	Sigmoidin D	C ₂₀ H ₂₀ O ₇	<i>E. sigmoidea</i> ²³¹
237	Sigmoidin L	C ₂₅ H ₂₈ O ₈	<i>E. sigmoidea</i> ²³²
238	Sigmoidin G	C ₂₀ H ₂₀ O ₈	<i>E. sigmoidea</i> ¹⁴⁶
239	Sigmoidin E	C ₂₅ H ₂₆ O ₅	<i>E. sigmoidea</i> ²³³
240	Sigmoidin E; 3',4'-Dihydro, 3'ξ-hydroxy	C ₂₅ H ₂₈ O ₆	<i>E. abyssinica</i> ¹¹⁹
241	Sigmoidin E; 3',4'-Dihydro, 3'ξ,4'ξ-dihydroxy	C ₂₅ H ₂₈ O ₇	<i>E. abyssinica</i> ¹¹⁹
242	Sigmoidin F; 1'',2''-Dihydro, 1'ξ,2'ξ-dihydroxy	C ₂₅ H ₂₈ O ₈	<i>E. abyssinica</i> ¹¹⁹
243	2,2',3,3'-Tetrahydro-5,7,8'-trihydroxy-2',2'-dimethyl[2,6'-bi-4H-1-benzopyran]-4,4'-dione	C ₂₀ H ₁₈ O ₇	<i>E. abyssinica</i> ¹¹⁹
244	Sigmoidin H	C ₂₁ H ₂₀ O ₅	<i>E. sigmoidea</i> ²³⁴
245	Sigmoidin L	C ₂₀ H ₁₈ O ₇	<i>E. sigmoidea</i> ¹³⁶
246	Sigmoidin A	C ₂₅ H ₂₈ O ₆	<i>E. sigmoidea</i> ²²⁸
247	Abyssinin III	C ₂₅ H ₂₈ O ₆	<i>E. abyssinica</i> ²²⁹
248	Orientanol D	C ₂₅ H ₂₈ O ₆	<i>E. orientalis</i> ²¹⁸
249	Orientanol E	C ₂₅ H ₂₈ O ₆	<i>E. orientalis</i> ²¹⁸
250	Vogelin C	C ₂₅ H ₂₆ O ₆	<i>E. vogelii</i> ²³⁵
251	8-Prenylluteone	C ₂₅ H ₂₆ O ₆	<i>E. eriotricha</i> ¹⁰⁷
252	Erysenegalensein N	C ₂₅ H ₂₆ O ₇	<i>E. senegalensis</i> ²¹⁹
253	Erysenegalensein D	C ₂₅ H ₂₆ O ₇	<i>E. senegalensis</i> ²³⁶
254	6,8-Diprenylorobol	C ₂₅ H ₂₆ O ₆	<i>E. eriotricha</i> ¹⁰⁷
255	Eryvarin T	C ₁₇ H ₁₈ O ₅	<i>E. variegata</i> ¹²⁵
256	Eryvarin H	C ₁₇ H ₁₆ O ₅	<i>E. variegata</i> ¹²⁷
257	Eryvarin M	C ₁₇ H ₁₆ O ₆	<i>E. variegata</i> ²⁰⁰
258	2',3,4,4'-Tetrahydroxy-5-prenylchalcone	C ₂₀ H ₂₀ O ₅	<i>E. abyssinica</i> ²³⁷
259	Abyssinone D	C ₂₁ H ₂₂ O ₅	<i>E. abyssinica</i> ¹⁰¹
260	Sigmoidin B	C ₂₀ H ₂₀ O ₆	<i>E. sigmoidea</i> ²²⁸
261	Abyssinin II	C ₂₁ H ₂₂ O ₆	<i>E. abyssinica</i> ²²⁹
262	4'-O-Methylsigmoidin	C ₂₁ H ₂₂ O ₆	<i>E. berteriana</i> ²³⁸
263	8-Prenylluteolin; 3'-Me ether, 7-O-[β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside]	C ₃₂ H ₃₈ O ₁₅	<i>E. indica</i> ²³⁹
264	Erypogin C	C ₂₁ H ₂₂ O ₆	<i>E. poeppigiana</i> ¹⁴⁴
265	Vogelin A	C ₂₁ H ₂₂ O ₆	<i>E. vogelii</i> ²³⁵
266	Erypogin D	C ₂₂ H ₂₄ O ₆	<i>E. poeppigiana</i> ²⁴⁰
267	Sigmoidin J	C ₂₂ H ₂₄ O ₆	<i>E. sigmoidea</i> ¹²¹
268	Eryvarin N	C ₂₂ H ₂₄ O ₆	<i>E. variegata</i> ²⁰⁰
269	3',5,7-Trihydroxy-4'-methoxy-5'-	C ₂₁ H ₂₂ O ₆	<i>E. berteriana</i> ²³⁸

	prenylisoflavanone		
270	Vogelin F	C ₂₁ H ₂₀ O ₆	<i>E. vogelii</i> ²⁴¹
271	7- <i>O</i> -Methyluteone	C ₂₁ H ₂₀ O ₆	<i>E. burttii</i> ²⁴²
272	3',7-Dihydroxy-4',5-dimethoxy-5'-prenylisoflavone	C ₂₂ H ₂₂ O ₆	<i>E. eriotriocho</i> ²¹⁶
273	4'- <i>O</i> -Methylglycyrrhisoflavone	C ₂₁ H ₂₀ O ₆	<i>E. sacleuxii</i> ²⁰⁴
274	Abyssinone VI	C ₂₅ H ₂₈ O ₄	<i>E. abyssinica</i> ¹⁰⁰
275	4- <i>O</i> -Methylabyssinone VI	C ₂₆ H ₃₀ O ₄	<i>E. mildbraedii</i> ¹²²
276	Abyssinone V	C ₂₅ H ₂₈ O ₅	<i>E. eriotriocho</i> ²²¹
277	4'- <i>O</i> -Methylabyssinone V	C ₂₆ H ₃₀ O ₅	<i>E. burttii</i> ²⁴³
278	Burtinone	C ₂₆ H ₃₀ O ₆	<i>E. burttii</i> ²⁴³
279	Burtinonedehydrate	C ₂₆ H ₂₈ O ₅	<i>E. burttii</i> ²⁴²
280	Abyssinoflavanone VII	C ₂₅ H ₂₈ O ₆	<i>E. abyssinica</i> ²³⁰
281	Isoburtinone	C ₂₆ H ₃₀ O ₆	<i>E. addisoniae</i> ¹²³
282	Lonchocarpol A	C ₂₅ H ₂₈ O ₅	<i>E. fusca</i> ²¹³
283	Eryzerin C	C ₂₅ H ₃₀ O ₄	<i>E. zeyheri</i> ²⁰¹
284	2',4',7-Trihydroxy-3',6-diprenylisoflavanone	C ₂₅ H ₂₈ O ₅	<i>E. species</i> ¹⁴¹
285	Eryzerin A	C ₂₅ H ₂₈ O ₅	<i>E. zeyheri</i> ²⁰¹
286	Eryzerin B	C ₂₆ H ₃₀ O ₅	<i>E. zeyheri</i> ²⁰¹
287	Vogelin D	C ₂₆ H ₃₀ O ₅	<i>E. vogelii</i> ²⁴¹
288	Isoerysenegalensein E	C ₂₅ H ₂₆ O ₆	<i>E. lysistemom</i> ²⁴⁴
289	Erysenegalensein E	C ₂₅ H ₂₆ O ₆	<i>E. senegalensis</i> ²³⁶
290	1-Methoxyerythrabyssin II	C ₂₆ H ₃₀ O ₅	<i>E. subumbrans</i> ¹⁹⁰
291	Erytagallin A	C ₂₆ H ₃₀ O ₅	<i>E. subumbrans</i> ⁸⁷
292	Erytagallin B	C ₂₆ H ₃₀ O ₆	<i>E. subumbrans</i> ⁸⁷
293	Eryzerin E	C ₂₆ H ₃₀ O ₅	<i>E. zeyheri</i> ²⁰¹
294	Demethylvestitol	C ₁₅ H ₁₄ O ₄	<i>E. species</i> ²⁴⁵
295	Isovestitol	C ₁₆ H ₁₆ O ₄	<i>E. species</i> ²⁴⁵
296	Erylatissin C	C ₂₁ H ₂₂ O ₅	<i>E. latissima</i> ⁹¹
297	5-Deoxyabyssinin II	C ₂₁ H ₂₂ O ₅	<i>E. abyssinica</i> ²³⁷
298	Macatrichocarpin A	C ₂₁ H ₂₂ O ₅	<i>E. mildbraedii</i> ²⁴⁶
299	2'-Hydroxyneobavaisoflavanone	C ₂₀ H ₂₀ O ₅	<i>E. species</i> ¹⁴¹
300	Eryvellutinone	C ₂₁ H ₂₂ O ₅	<i>E. vellutina</i> ²⁴⁷
301	Sigmoidin I	C ₂₁ H ₂₂ O ₅	<i>E. sigmoidea</i> ¹³⁷
302	Bidwillol A	C ₂₁ H ₂₂ O ₄	<i>E. × bidwillii</i> ¹¹⁵
303	Erypogin A	C ₂₁ H ₂₂ O ₄	<i>E. poeppigiana</i> ¹⁴⁴
304	2',7-Dihydroxy-4'-methoxy-5'-prenylisoflavone	C ₂₁ H ₂₀ O ₅	<i>E. mildbraedii</i> ¹²⁰
305	Erylatissin A	C ₂₁ H ₂₀ O ₅	<i>E. latissima</i> ⁹¹
306	Vogelin E	C ₂₀ H ₁₈ O ₆	<i>E. vogelii</i> ²⁴¹
307	3'-Prenylbiochanin A	C ₂₁ H ₂₀ O ₅	<i>E. sacleuxii</i> ²⁰⁴
308	Erythrinin B	C ₂₀ H ₁₈ O ₅	<i>E. variegata</i> ²⁴⁸
309	Sandwicarpin	C ₂₀ H ₂₀ O ₅	<i>E. species</i> ²⁴⁵
310	Cristacarpin	C ₂₁ H ₂₂ O ₅	<i>E. species</i> ²⁴⁵
311	Eryvarin K	C ₂₁ H ₂₂ O ₅	<i>E. variegata</i> ¹²⁷
312	Erythbidin D	C ₁₇ H ₁₆ O ₅	<i>E. × bidwillii</i> ¹³²
313	Glycinol	C ₁₅ H ₁₂ O ₅	<i>E. species</i> ²⁴⁵

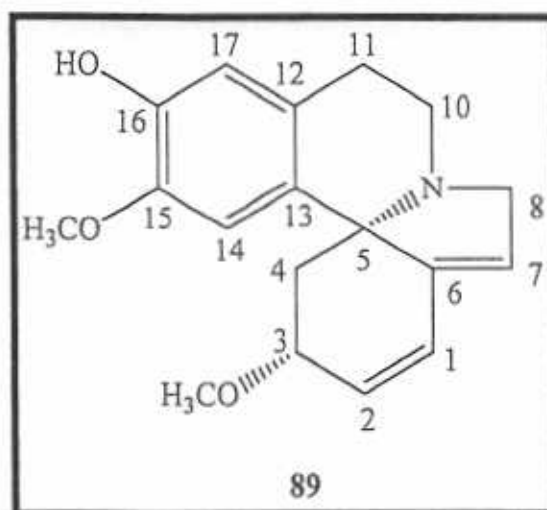
314	Erythrinarbine	$C_{13}H_{15}NO_3$	<i>E. arborescens</i> ²⁴⁹
315	N,N-Dimethyltryptophan	$C_{13}H_{16}N_2O_2$	<i>E. variegata</i> ⁷⁵
316	Vogelin G	$C_{25}H_{26}O_6$	<i>E. vogelii</i> ²⁴¹
317	Vogelin H	$C_{25}H_{26}O_6$	<i>E. vogelii</i> ⁹⁴
318	Vogelin I	$C_{25}H_{26}O_7$	<i>E. vogelii</i> ⁹⁴
319	Vogelin J	$C_{20}H_{16}O_5$	<i>E. vogelii</i> ⁹⁴

RESULTS AND DISCUSSION (Part-A)

This chapter describes the structure elucidation of five known alkaloids (89-93) together with the known isoflavone (94) from the flowers of *E. suberosa*. The dried and crushed flowers were extracted with 95% ethanol and alkaloidal fraction was separated through a standard procedure from the ethanolic extract. Pure alkaloids were obtained from the fraction through chromatographic techniques and their structures established with the help of advanced NMR and mass spectroscopic techniques. The alkaloids erysodine (89) and erysotrine (90) were tested for anxiolytic activity and were found to show significant anxiolytic effects in the two tests of anxiety-the elevated plus-maze (EPM) and the light dark transition model (LDTM).

4.1. Structure elucidation of the isolated compounds from *Erythrina suberosa*

4.1.1. Erysodine (89)



Compound 89 was obtained as white needles and showed positive reaction with Dragendorff reagent, indicating its alkaloidal nature. The UV spectrum showed absorption maxima at 233 and 284 nm, implying the existence of aromatic ring in the molecule. The IR spectrum indicated the absorption band for hydroxyl group at 3555 cm^{-1} . The molecular formula of 89 was determined as $\text{C}_{18}\text{H}_{19}\text{NO}_3$ on the basis of ^1H - and ^{13}C -NMR techniques and EIMS, where molecular ion peak was observed at m/z 299 $[\text{M}]^+$.

The NMR spectral data (Table-4.1) of compound 89 was similar to the well-known alkaloid erysodine.²²⁶ In ^1H -NMR of 89, the observed signals belonged to two aromatic protons at δ_{H} 6.63 (1H, s, H-14) and 6.73 (1H, s, H-17), protons of conjugated double bonds at δ_{H} 6.51 (1H, dd, $J = 10.0, 2.5$ Hz, H-1), 5.93 (1H, d, $J = 10.0$ Hz, H-2) and 5.66 (1H, br.s, H-7), two methoxide protons at δ_{H} 3.26 (3H, s, CH_3O -3) and 3.71 (3H, s, CH_3O -15), and nine aliphatic protons.

^{13}C -NMR (BB and DEPT) spectra of **89** showed eighteen carbon resonances for two methoxyl, four methylene, six methine, and six quaternary carbon atoms. The NMR assignments were confirmed with the help of two dimensional NMR techniques such as COSY, HMQC, HMBC and NOESY. Amongst important HMBC (Figure-4.1) correlations were those between protons at δ_{H} 3.26 and 3.71 to C-3 and C-15, respectively. The stronger correlation of H-17/C-15 and weaker correlation of H-14/C-15, helped in deciding the exact position of the methoxyl group to C-15. Thus the structure of compound **89** was unambiguously assigned as equal to that of erysodine.

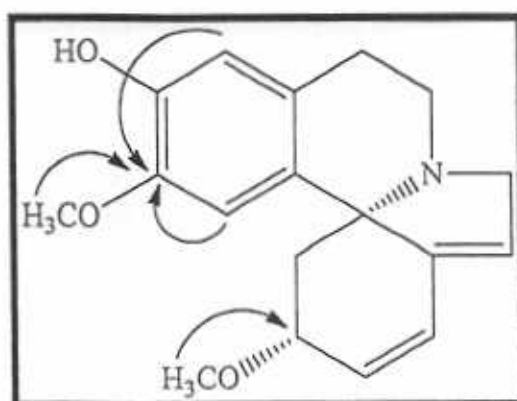


Figure-4.1: Selected HMBC interactions of erysodine (**89**)

Table-4.1: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of (89) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	125.4	CH	6.51, dd ($J = 10.0, 2.5$)
2	131.5	CH	5.93, d ($J = 10.0$)
3	76.7	CH	3.97, m
3-OCH ₃	55.7	CH ₃	3.26, s
4	41.3	CH ₂	2.46, m
			1.82, m
5	68.0	C	-
6	142.2	C	-
7	122.8	CH	5.66, br.s
8	57.1	CH ₂	3.48, m
			3.68, m
10	44.0	CH ₂	3.44, m
			2.80, m
11	23.9	CH ₂	2.85, m
			2.57, m
12	127.3	C	-
13	130.2	C	-
14	108.5	CH	6.73, s
15	144.8	C	-
15-OCH ₃	55.8	CH ₃	3.71, s
16	144.3	C	-
17	114.5	CH	6.63, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.1.2. Erysotrine (90)

Compound **90** was isolated as brown oil and gave positive result for alkaloid with Dragendorff reagent. The UV spectrum showed absorption at 230 and 280 nm, typical of erythrina alkaloids. The IR spectrum showed C=C stretching vibration at 1600 cm^{-1} . Molecular formula of **90** was estimated as $\text{C}_{19}\text{H}_{23}\text{NO}_3$ from ^1H - and ^{13}C -NMR data and EIMS analysis, where a molecular ion peak appeared at m/z 313 $[\text{M}]^+$.

A comparison of the NMR data (Table-4.2) of compound **90** with erysotrine,¹⁸³ a known alkaloid, clearly indicated the similarity between them. ^1H -NMR data of **90** (Table-4.2), consisted of signals for two aromatic protons at δ_{H} 6.76 (1H, s, H-14) and 6.56 (1H, s, H-17), protons of conjugated double bonds at δ_{H} 6.51 (1H, dd, $J =$

10.0, 2.0 Hz, H-1), 5.95 (1H, d, $J = 10.0$ Hz, H-2) and 5.66 (1H, br.s, H-7), three methoxide protons at δ_{H} 3.26, 3.69 and 3.79 (each 3H, s), and nine aliphatic protons. Nineteen carbon resonances were observed in ^{13}C -NMR spectrum of compound **90** as against that of **89**, where there were only two methoxyl groups. These carbon signals indicated three methoxys, four methylenes, six methines, and six quaternary carbons on the basis of DEPT spectral analysis. 2D-NMR techniques such as COSY, HMQC and NOESY were also used for structural assignments of compound **90**. All the NMR assignments were in perfect agreement to that of crysotrine.¹⁸³

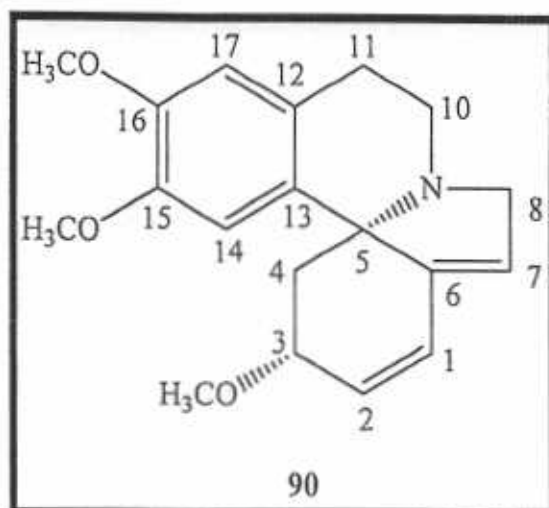


Table-4.2: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of (90) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	125.5	CH	6.51, dd ($J = 10.0, 2.0$)
2	131.1	CH	5.95, d ($J = 10.0$)
3	76.1	CH	4.01, m
3-OCH ₃	55.8	CH ₃	3.26, s
4	41.2	CH ₂	2.47, m
			1.82, m
5	68.0	C	-
6	142.2	C	-
7	122.7	CH	5.66, br.s
8	56.6	CH ₂	3.49, m
			3.65, m
10	43.5	CH ₂	3.51, m
			2.88, m
11	23.8	CH ₂	2.94, m
			2.57, m
12	126.3	C	-
13	131.1	C	-
14	109.4	CH	6.76, s
15	147.7	C	-
15-OCH ₃	55.9	CH ₃	3.79, s
16	147.1	C	-
16-OCH ₃	56.6	CH ₃	3.69, s
17	111.6	CH	6.56, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.1.3. Erytharbine (91)

Compound **91** was obtained from the alkaloidal fraction as yellowish brown solid. The UV spectrum indicated characteristic absorptions (266, 259 and 234 nm) of erythrina alkaloids. IR spectrum showed carbonyl function at 1690 cm^{-1} . A molecular ion peak at $m/z\ 325\ [\text{M}]^+$ was observed in the EIMS and molecular formula $\text{C}_{19}\text{H}_{19}\text{NO}_4$ was established through NMR spectral analyses in combination to EIMS data.

^1H -NMR data of compound **91** (Table-4.3) exhibited proton signals related to two aromatic protons singlets (δ_{H} 6.77 and 6.99), three proton of conjugated double bonds [δ_{H} 7.07 (1H, dd, $J = 10.0, 2.0$ Hz, H-1), 6.25 (1H, d, $J = 10.0$ Hz, H-2), and 6.08 (1H, br.s, H-7)], protons of a double bond conjugated to the aromatic ring [δ_{H} 6.83 and 6.25 (each 1H, d, $J = 7.2$ Hz, H-10, 11)], protons of three methoxy groups singlets (δ_{H} 3.24, 3.71, and 3.81), and three aliphatic protons. ^{13}C -NMR spectrum (Table-4.3) showed nineteen carbons resonances, which were identified as three methoxy, one methylene, eight methine and seven quaternary carbon atoms on the basis of DEPT spectral analysis. HSQC and HMBC NMR spectra were used to confirm the assigned structure. The spectral data of compound **91** was in complete agreement to the known alkaloid, erytharbine.¹⁶³

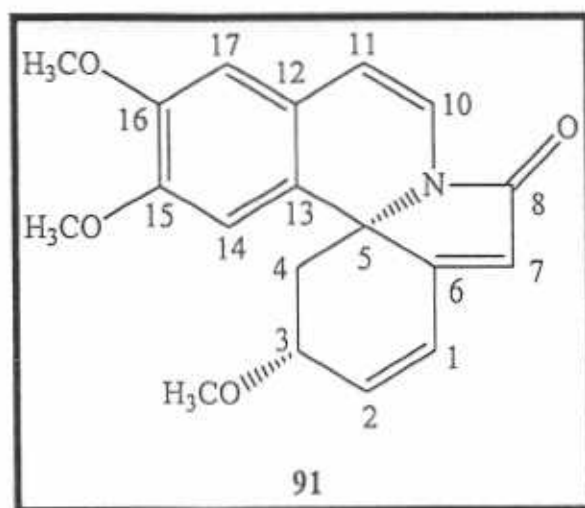


Table-4.3: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (91) in CD_3COCD_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	124.4	CH	7.07, dd ($J = 10.0, 2.0$)
2	138.8	CH	6.25, d ($J = 10.0$)
3	75.4	CH	3.71, m
3-OCH ₃	56.2	CH ₃	3.24, s
4	43.7	CH ₂	2.66, m
			1.36, m
5	66.4	C	-
6	157.1	C	-
7	120.4	CH	6.08, br.s
8	169.1	C	-
10	121.4	CH	6.83, d ($J = 7.2$)
11	113.6	CH	6.43, d ($J = 7.2$)
12	125.0	C	-
13	125.9	C	-
14	108.2	CH	6.77, s
15	149.1	C	-
15-OCH ₃	56.2	CH ₃	3.71, s
16	149.9	C	-
16-OCH ₃	56.2	CH ₃	3.81, s
17	111.8	CH	6.96, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.1.4. Erysotramidine (92)

Compound **92** was isolated as brown oily substance from the alkaloidal fraction. UV spectrum exhibited absorption maxima at 212, 236, 257 and 316 nm. IR spectrum displayed the presence of carbonyl function at 1665 cm^{-1} . EIMS gave a molecular ion peak at m/z 327 $[\text{M}]^+$, corresponding to the molecular formula $\text{C}_{19}\text{H}_{21}\text{NO}_4$.

The NMR spectra of **92** were similar to compound erytharbine (**91**), except for the absence of one double bond. The ^1H -NMR of **92** showed two aromatic protons at δ_{H} 6.86 (2H, s, H-14, 17), protons of conjugated double bond at δ_{H} 6.97 (1H, dd, $J = 10.0, 2.0\text{ Hz}$, H-1), 6.40 (1H, d, $J = 10.0\text{ Hz}$, H-2), and 5.95 (1H, br.s, H-7), protons of three methoxys singlets at δ_{H} 3.31, 3.68 and 3.78 and seven aliphatic protons.

^{13}C -NMR spectrum (Table-4.4) of 92 was found identical to the known alkaloid, erysotramidine.²⁵⁰

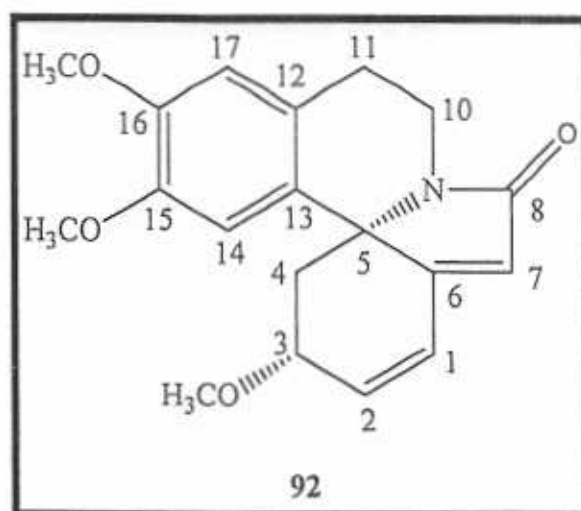


Table-4.4: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (92) in CD_3COCD_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	124.6	CH	6.97, dd ($J = 10.0, 2.0$)
2	137.7	CH	6.40, d ($J = 10.0$)
3	75.8	CH	3.82, m
3-OCH ₃	56.2	CH ₃	3.31, s
4	42.4	CH ₂	2.95, m
			1.58, m
5	66.7	C	-
6	158.8	C	-
7	120.4	CH	5.95, br s
8	171.0	C	-
10	37.8	CH ₂	3.54, m
			3.77, m
11	27.4	CH ₂	2.96, m
			3.10, m
12	124.6	C	-
13	127.7	C	-
14	110.0	CH	6.86, s
15	148.4	C	-
15-OCH ₃	56.2	CH ₃	3.68, s
16	149.8	C	-
16-OCH ₃	56.2	CH ₃	3.78, s
17	113.7	CH	6.86, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.1.5. Erythrartine (93)

Compound **93** was obtained as brown solid substance from the alkaloidal fraction. UV spectrum showed absorption maxima at 227 and 275 nm, indicating the presence of conjugated double bonds. IR spectrum exhibited absorption band for C=C stretching at 1607 cm^{-1} . The molecular formula of **93** was deduced as $\text{C}_{19}\text{H}_{23}\text{NO}_4$ through ESIMS (molecular ion peak at m/z 330 $[\text{M} + 1]^+$ in positive mode) and NMR analyses.

In comparison of ^1H -NMR data (Table-4.5), **93** was found identical to erythrartine,¹⁸⁶ a known alkaloid. The two aromatic protons singlets were observed at δ_{H} 6.85 (H-14),

and 7.00 (H-17). Protons of the conjugated double bonds resonated at δ_H 6.61 (1H, dd, $J = 10.0, 2.0$ Hz, H-1), 6.05 (1H, d, $J = 10.0$ Hz, H-2), and 5.75 (1H, br.s, H-7). The three methoxys singlets were observed at δ_H 3.32, 3.78, and 3.9. In addition to these, eight aliphatic proton signals were observed in ^1H -NMR spectrum of compound 93.

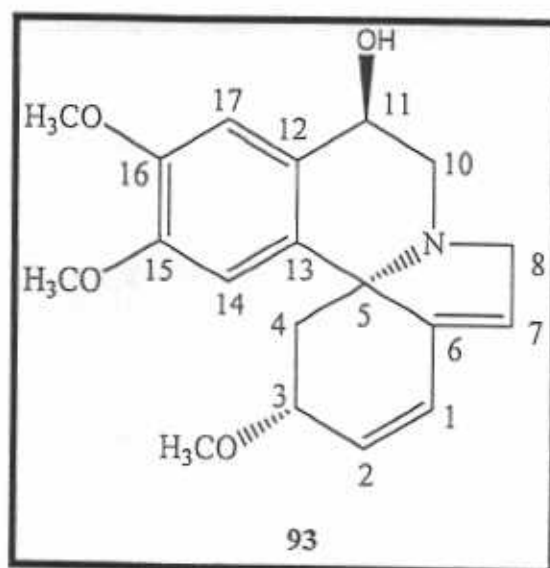
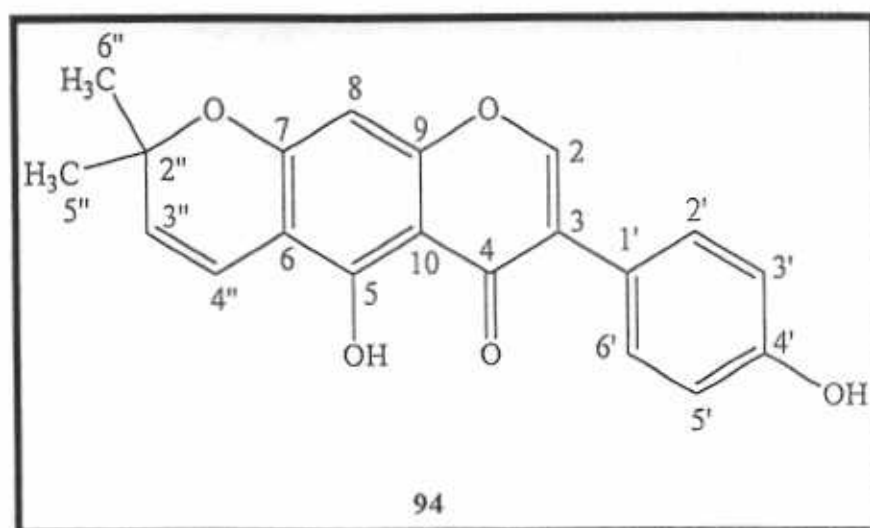


Table-4.5: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (93) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	125.3	CH	6.61, dd ($J = 10.0, 2.0$)
2	131.2	CH	6.05, d ($J = 10.0$)
3	75.6	CH	4.06, m
3-OCH ₃	55.8	CH ₃	3.32, s
4	40.5	CH ₂	2.42, m 1.88, m
5	66.2	C	-
6	141.7	C	-
7	123.3	CH	5.75, br.s
8	58.8	CH ₂	3.98, m 3.87, m
10	51.2	CH ₂	3.59, m 3.17, m
11	64.3	CH	4.77, m
12	128.3	C	-
13	129.4	C	-
14	108.6	CH	6.85, s
15	148.0	C	-
15-OCH ₃	55.8	CH ₃	3.78, s
16	148.2	C	-
16-OCH ₃	55.8	CH ₃	3.91, s
17	110.2	CH	7.00, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.1.6. Alpinumisoflavone (94)



Compound **94** was isolated as yellow needles. Its molecular formula was deduced from ESIMS (m/z 337 $[M + 1]^+$) and NMR spectral analyses. UV absorption maxima were recorded at 226, 280 and 340 nm, showing α,β -unsaturated ketone and aromatic ring in the molecule. IR absorption bands were detected at 3414 and 1722 cm^{-1} for hydroxyl and ketonic functions.

In ^1H -NMR spectrum of compound **94** (Table-4.6), the proton resonance at δ_{H} 7.83 (1H, s) was assigned to H-2, which is a characteristic peak for isoflavone skeleton. The proton signals at δ_{H} 5.63, 6.74 (each 1H, d, $J = 10.3$ Hz), and a six proton singlet at δ_{H} 1.48 were assignable to 2,2-dimethylpyranyl ring. Similarly, an A_2X_2 aromatic system could be established from chemical shift values at δ_{H} 7.38 (1H, dd, $J = 6.8, 2.4$ Hz) and 6.88 (1H, dd, $J = 6.8, 2.4$ Hz). The presence of a chelated hydroxyl group at δ_{H} 13.1 and one proton singlet at δ_{H} 6.35 implied the existence of a chromen ring fused to the aromatic ring in linear fashion. These characteristics of compound **94** in combination with ^{13}C -NMR spectral data and two dimensional NMR techniques such as HMBC (Figure-4.2) confirmed that **94** is a known isoflavone, alpinumisoflavone.²¹²

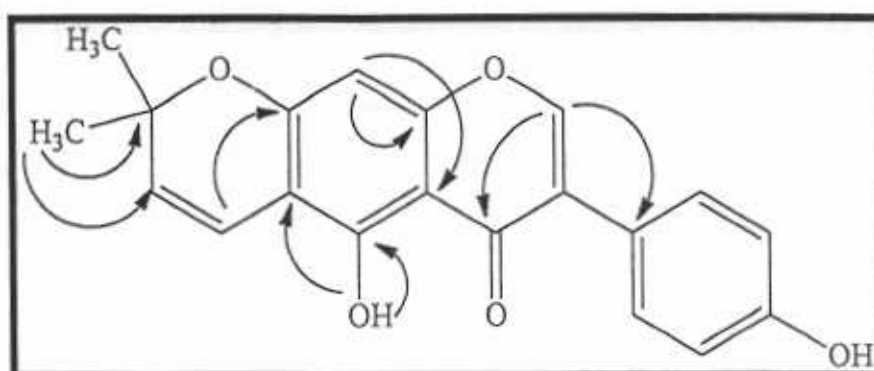


Figure-4.2: Selected HMBC interactions of alpinumisoflavone (**94**)

Table-4.6: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (94) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
2	152.6	CH	7.83, s
3	123.0	C	-
4	181.0	C	-
5	156.9	C	-
5-OH	-	-	13.1, s
6	105.6	C	-
7	159.6	C	-
8	95.0	CH	6.35, s
9	157.3	C	-
10	106.1	C	-
1'	123.5	C	-
2'	130.3	CH	7.38, dd ($J = 6.8, 2.4$)
3'	115.8	CH	6.88, dd ($J = 6.8, 2.4$)
4'	155.9	C	-
4'-OH	-	-	5.24, br s
5'	115.8	CH	6.88, dd ($J = 6.8, 2.4$)
6'	130.3	CH	7.38, dd ($J = 6.8, 2.4$)
2''	78.1	C	-
3''	128.2	CH	5.63, d ($J = 10.3$)
4''	115.5	CH	6.74, d ($J = 10.3$)
5''	28.3	CH_3	1.48, s
6''	28.3	CH_3	1.48, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

4.2. Anxiolytic activity of erysodine (89) and erysotrine (90)

The ethanolic extract was fractionated and subjected to chromatographic techniques, resulting in the isolation of alkaloid compounds **89** and **90**, which were identified as erysodine (**89**) and erysotrine (**90**) by comparison of spectral data (Table 4.1 & 4.2) with those reported in the literature. These two compounds were subjected to two tests of anxiety-the elevated plus-maze (EPM) and the light dark transition model (LDTM).

4.2.1. Elevated plus-maze (EPM)

Figure 4.3 shows the effects of erysodine (**89**) (0, 3 or 10 mg/kg, *p.o.*), erysotrine (**90**) (0, 3 or 10 mg/kg, *p.o.*) and diazepam (DZP) (2 mg/kg, *i.p.*) on the behavior of mice in the EPM. One-way ANOVA revealed significant differences of treatment on % open arms time [$F(5,55) = 12,21$; $p < 0.01$] and % open arms entries [$F(5,55) = 17,59$; $p < 0.01$]. Post hoc comparisons revealed that the significant differences were produced by DZP treatment in both parameters ($p < 0.01$) and by erysotrine (**90**) (10 mg/kg) in the % open arms entries ($p < 0.01$). One-way ANOVA followed by Duncan's test also revealed that DZP treatment significantly decreased frequency of closed arms entries [$F(5,55) = 2.78$; $p = 0.03$].

4.2.2. Light dark transition model (LDTM)

Figure 4.4 shows the effects of erysodine (**89**) (0, 3 or 10 mg/kg, *p.o.*), erysotrine (**90**) (0, 3 or 10 mg/kg, *p.o.*) and DZP (2 mg/kg, *i.p.*) on the behavior of mice in the LDTM. One-way ANOVA revealed significant differences of treatment on time spent in the illuminated compartment [$F(5,55) = 2,68$; $p = 0.03$] and transitions between both compartments [$F(5,55) = 3, 19$; $p = 0.01$]. Post hoc comparisons revealed that

DZP and erysodine (10 mg/kg) treated animals spent more time in the illuminated compartment ($p < 0.05$). Duncan's test also revealed significant differences of treatment on the number of transitions between both compartments of the LD TM for erysodine (89) (10 mg/kg, $p=0.02$) and erysotrine (90) (3 mg/kg, $p < 0.01$).

4.2.3. Explanation of results obtained from EPM and LTDM

The results of the present study demonstrated that acute per oral (*p.o.*) treatment with the *E. suberosa* alkaloids, erysodine (89) and erysotrine (90), produced anxiolytic-like effects in mice exposed to a two widely used anxiety tests, the elevated plus-maze (EPM) and the light-dark transition model (LTDM). In the EPM, only erysodine (10 mg/kg) increased percent of open arm entries and tended to increase percent of open arm time, the two conventional measures of anxiety in the EPM. Importantly, the anxiolytic-like effects of 89 were selective for anxiety measures, since this alkaloid did not change closed arms entries, the measure widely used as an index of general activity in the EPM.²⁵¹

In the LTDM, both erysodine (89) and erysotrine (90) produced anxiolytic-like effects. However, while erysodine (10 mg/kg) increased both time spent in the illuminated compartment and the number of transitions between compartments, erysotrine (3 mg/kg) increased the number of transitions only. Nevertheless, it must be emphasized that the lower dose of erysodine (3 mg/kg) and the two doses of erysotrine (3 and 10 mg) also tended ($p < 0.08$) to increase the time spent in the aversive place (i.e., the illuminated compartment). In addition, although both alkaloids have increased the number of transitions between compartments, which in turn could also be interpreted as an effect on locomotor behavior, neither erysodine

nor erysotrine changed locomotion (i.e., the number of closed arm entries) in the EPM.

Evidence suggesting that plants from genus *Erythrina* have anti-anxiety effects have been demonstrated elsewhere.^{81,226,252,253} For instance, recently Flausino *et al.*⁸¹ have reported that a new erythrinian alkaloid, (+)-11- α -hydroxyerythravine (OH-Ery), and the two known erythrinian alkaloids, erythravine (Ery) and (+)- α -hydroxyerysotrine (Eryso), isolated from *E. mulungu* impaired the inhibitory avoidance acquisition of the open arms in the elevated T-maze, an animal model of anxiety validated for rats²⁵⁴ and mice.²⁵⁵ Flausino *et al.*²⁵³ also observed that acute *p.o.* treatment with the alkaloids erythravine and (+)-11- α -hydroxyerythravine produced anxiolytic effects in the LDTM.

It is noteworthy that the benzodiazepine diazepam, a classic anxiolytic drug that was used as positive control group in the present study, also attenuated anxiety measures in both tests. These results strongly suggest that the alkaloids erysodine and erysotrine are markedly involved in the anti-anxiety activities of the *E. suberosa*.

Elevated Plus-Maze

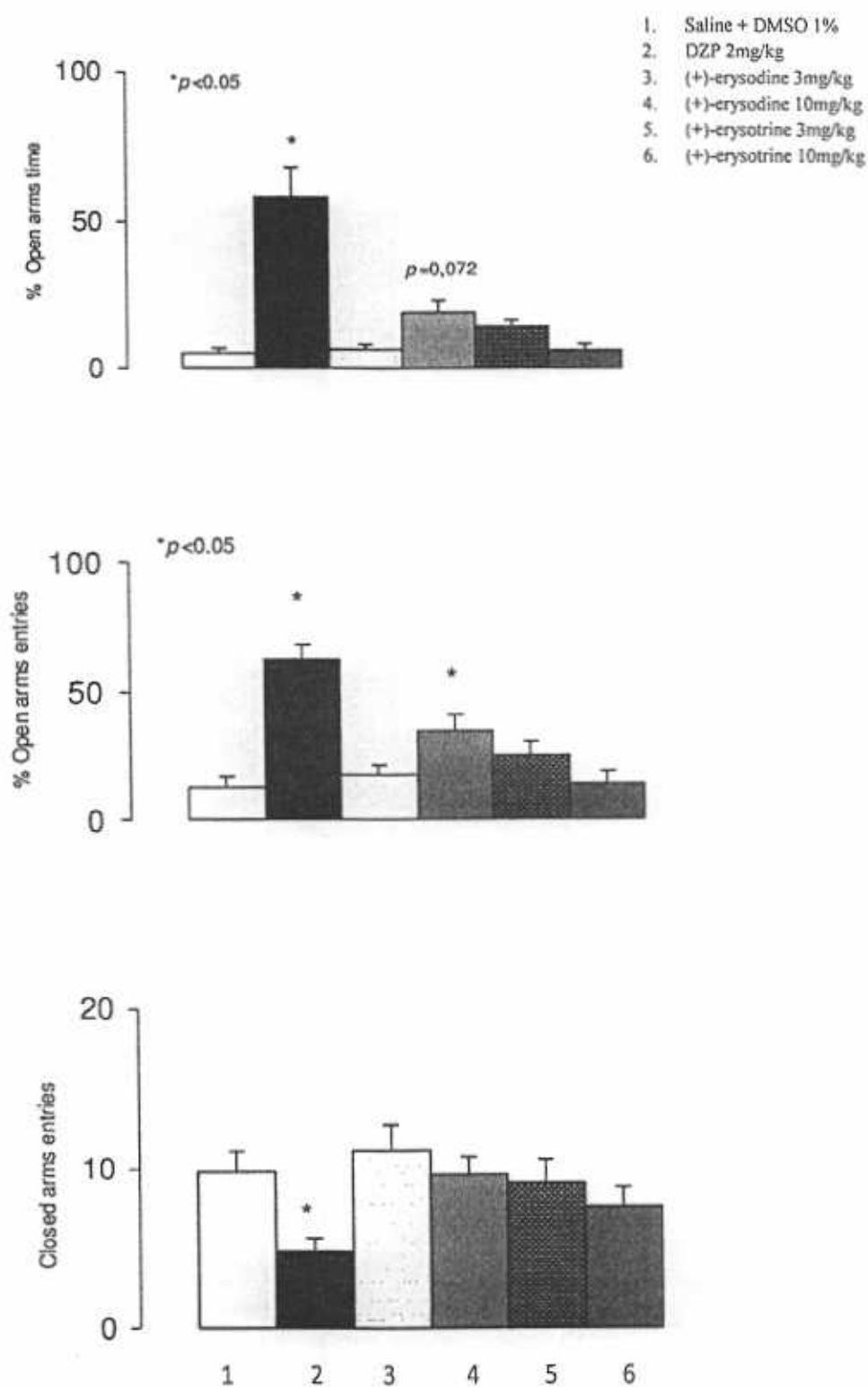


Figure 4.3: Effects of erysodine (89) (0, 3 or 10 mg/kg, *p.o.*), erysotrine (90) (0, 3 or 10 mg/kg, *p.o.*) and DZP (2 mg/kg, *i.p.*) on the behavior of mice in the EPM. Data are represented as mean $\pm$ SEM, N = 8-12. **P* < 0.05 vs control group.

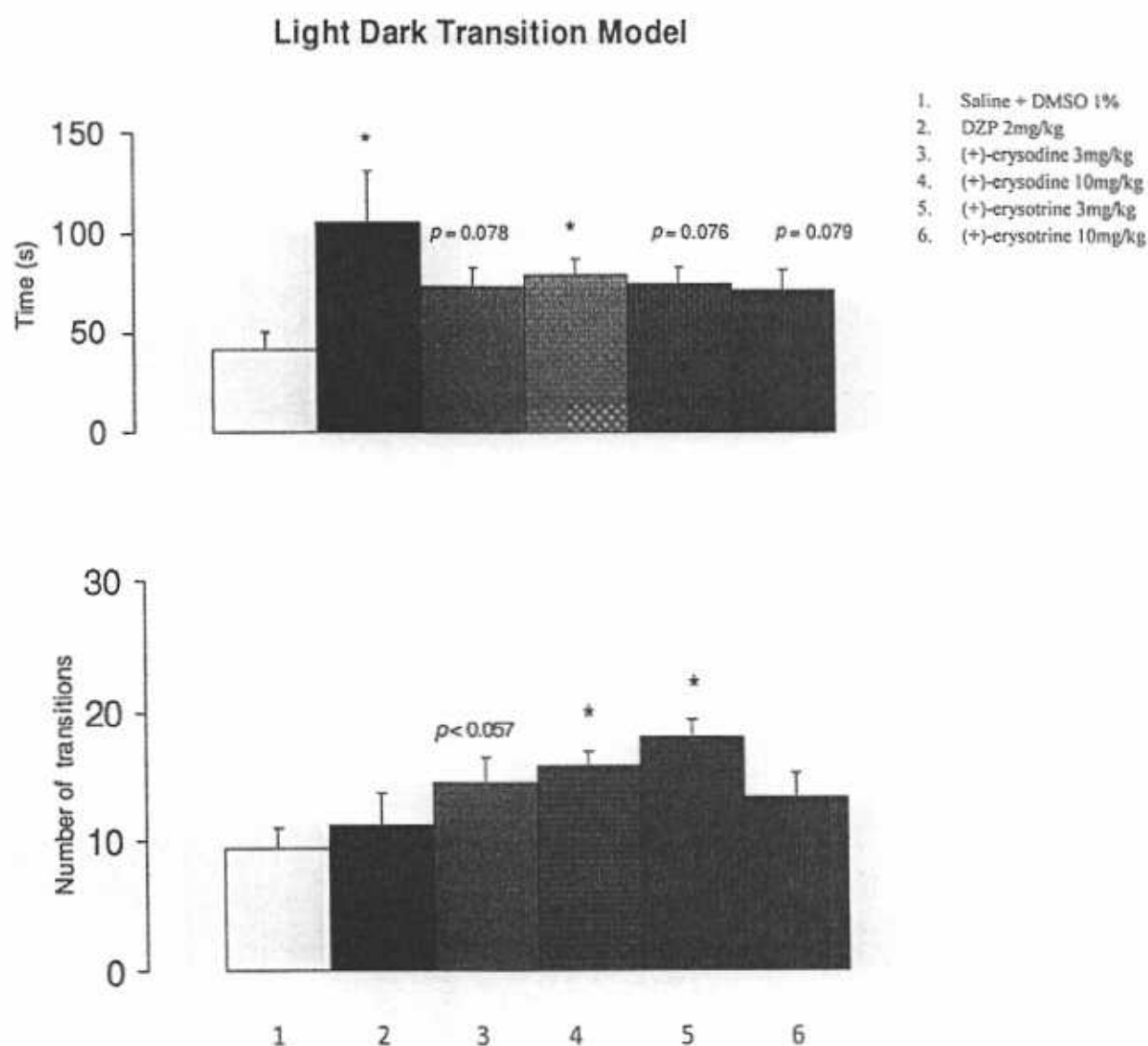


Figure 4.4: Effects of erysodine (89) (0, 3 or 10 mg/kg, *p.o.*), erysotrine (90) (0, 3 or 10 mg/kg, *p.o.*) and DZP (2 mg/kg, *i.p.*) on the behavior of mice in the LD TM. Data are represented as mean $\pm$ SEM, N = 8-12. **P* < 0.05 vs control group.

Chapter: 5

EXPERIMENTAL (Part-A)

5.1. General experimental conditions

5.1.1. Physical constants

Melting points (corrected and uncorrected) were measured in glass capillary tubes using Buchi 535 melting point apparatus. Optical rotations were determined on an ADP 220 polarimeter at 589 nm.

5.1.2. Spectroscopic techniques

Ultraviolet spectra (UV) were taken on Hitachi U-3200 spectrophotometer using methanol as solvent. IR spectra were obtained using a Perkin-Elmer FT-IR instrument for compounds mounted directly on the diamond cell. ^1H -, ^{13}C - and 2D-NMR techniques were recorded on Varian 400 MR, Varian 500 MR and Bruker AV 400. Low resolution electron impact ionization mass spectrometry (EIMS) was done with the help of JEOL JMS600 instrument. Electrospray ionization mass spectrometry (ESIMS) was done with Waters mass-directed autopurification system (Waters Quatro Micro), using solvent combinations of HPLC grade H_2O + 0.05% formic acid; Solvent B, HPLC grade CH_3CN).

5.1.3. Chromatographic techniques

Column chromatography was done on Merck silica gel (200 μm). Merck keisel gel 60 F254 pre-coated silica gel glass plates were used for analytical and preparative TLC. TLC Plates were visualized under UV light ($\lambda = 254 \text{ nm}$, 366 nm) or by heating after spraying with 15% aqueous sulfuric acid saturated with ceric sulfate. Dragendorff reagent was used as spraying reagent to visualize alkaloidal compounds. HPLC separations were performed on a Waters mass-directed autopurification system

consisting of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex LUNA column (5 μ , C₁₈, 100 Å, 10 x 250 mm) equipped with a Phenomenex Security Guard precolumn (Luna C₅ 300 Å) eluting at 4 mL/min. Solvent A, HPLC grade H₂O + 0.05% formic acid; Solvent B, HPLC grade CH₃CN + 0.045% formic acid or HPLC grade CH₃OH + 0.045% formic acid. The post-column flow was split (100:1) and the minority flow was made up with MeOH + 0.045% formic acid to 1 mL/min for simultaneous analysis by diode array (Waters 2998), evaporative light scattering (Waters 2424) and ESI mass spectrometry in positive and negative modes (Waters Quatro Micro).

5.2. *Erythrina suberosa*

5.2.1. Plant material

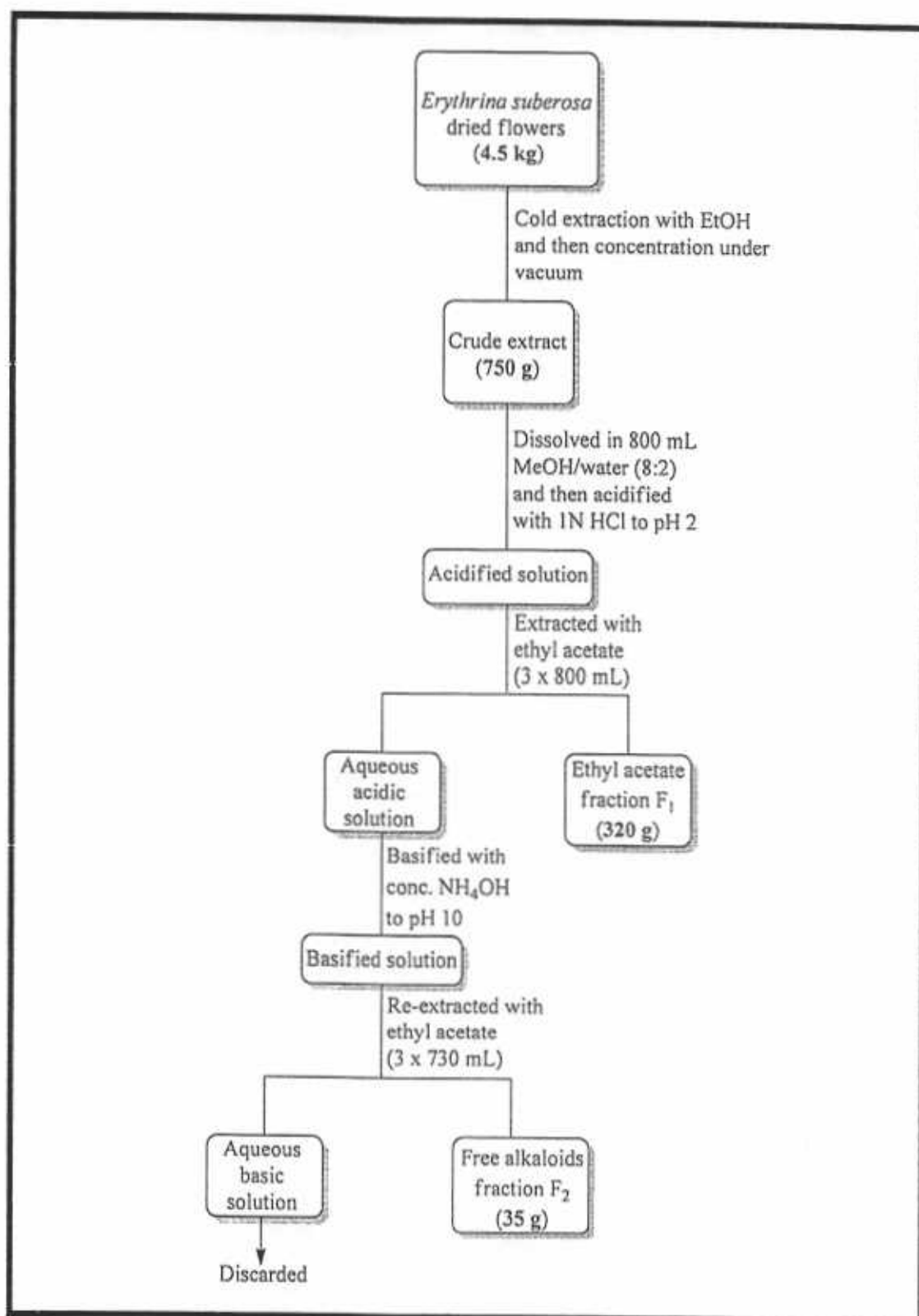
The flowers of *Erythrina suberosa* were collected in April 2007 from the campus of University of Peshawar, Peshawar, Pakistan where it is cultivated as an ornamental plant. A voucher specimen (ES-123) of the areal parts has been identified by Dr. Samin Jan, assistant professor, Islamia College University, Peshawar, Pakistan and deposited to the herbarium at Islamia College University, Peshawar, Pakistan.

5.2.2. Extraction and isolation

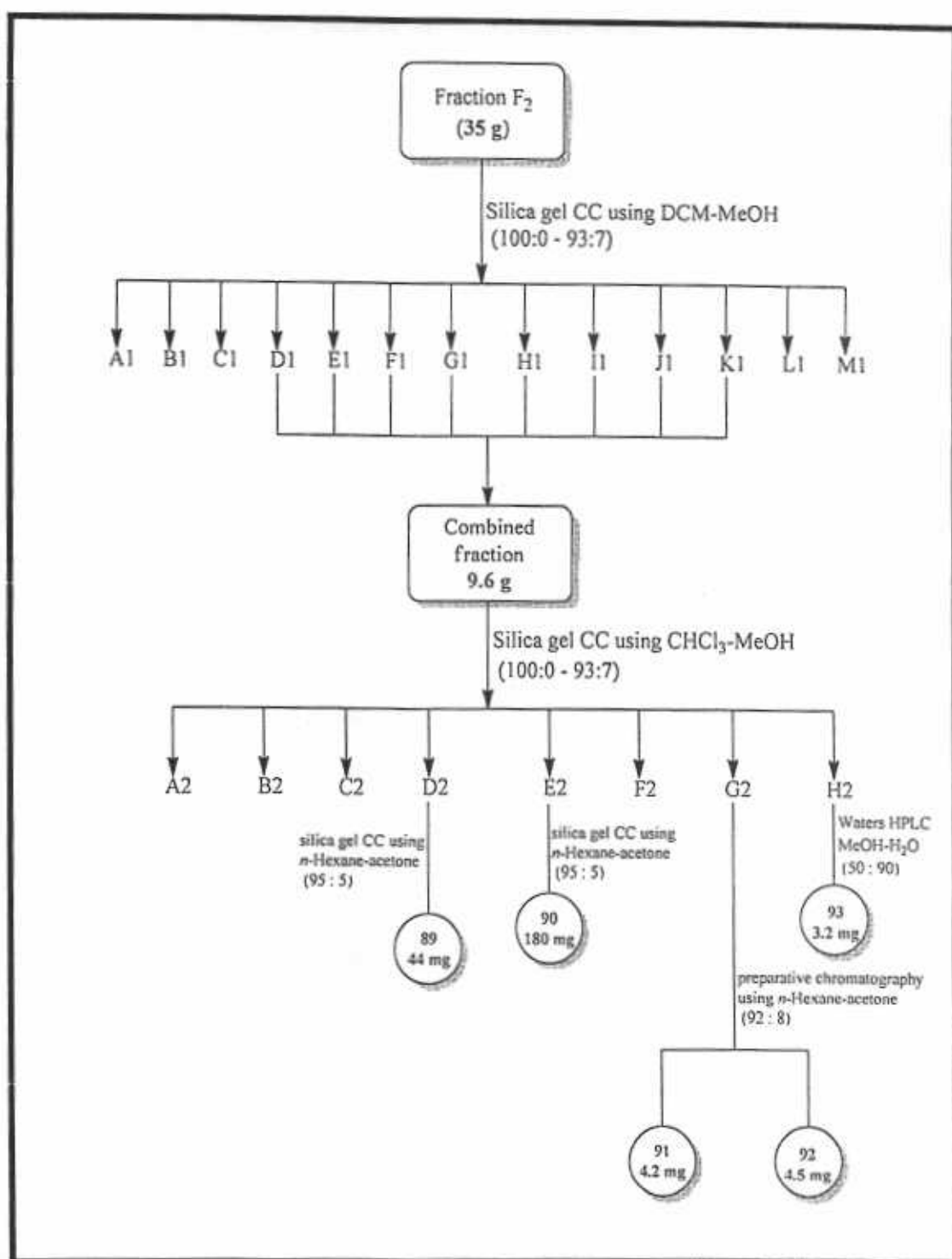
Dried flowers (4.5 kg) were extracted with ethanol using cold percolation method (Scheme-5.1). The solvent was removed under reduced pressure to afford a crude extract (715 g). The crude extract was then dissolved in 800 mL of methanol/water (8:2 v/v) and acidified to pH 2 with 1N HCl. The acidified solution was extracted with ethyl acetate (3 x 800 mL) to give fraction F₁ and the resultant hydrochloric acid solution was then made alkaline (pH 10) using concentrated NH₄OH. After that, it

was re-extracted with ethyl acetate (3×730 mL) to afford fraction F_2 which was a mixture of crude alkaloids (35 g).

The alkaloidal fraction F_2 (35 g) was applied to column chromatography (CC) (Scheme-5.2) over silica gel (200 μm , 25×7 cm) eluted with a gradient of dichloromethane-methanol (100:0 $\rightarrow$ 99.5:0.5 $\rightarrow$ 99:1 $\rightarrow$ 98.5:1.5 $\rightarrow$ 98:2 $\rightarrow$ 97.5:2.5 $\rightarrow$ 97:3 $\rightarrow$ 96.5:3.5 $\rightarrow$ 96:4 $\rightarrow$ 95.5:4.5 $\rightarrow$ 95:5 $\rightarrow$ 94:6 $\rightarrow$ 93:7) to yield 13 fractions (A1 to M1, 15 mL each). Fractions D1-K1 were pooled together (9.6 g) on the basis of their TLC profiles and then subjected to another column chromatography over silica gel (200 μm , 23×5 cm) with a gradient elution using chloroform-methanol (100:0 $\rightarrow$ 99:1 $\rightarrow$ 98:2 $\rightarrow$ 97:3 $\rightarrow$ 96:4 $\rightarrow$ 95:5 $\rightarrow$ 94:6 $\rightarrow$ 93:7) yielding 8 fractions (A2 to H2, 15 mL each). The fractions D2 and E2 were re-chromatographed on silica gel using a solvent combination of *n*-hexane-acetone (95:5), afforded compound 89 (44 mg) and 90 (180 mg), respectively. Fraction G2 was applied to preparative chromatography on silica gel pre-coated TLC glass plates and eluted in a solvent gradient of *n*-hexane-acetone (92:8), yielded compounds 91 (4.2 mg) and 92 (4.5 mg). Fraction H2 was purified through HPLC mass-directed preparative system using H_2O -MeOH as a mobile phase. 200 μl was injected during successive rounds of a 20 minutes program (0 min, 50% B; 13.00 min, 80% B; 17 min, 90% B; 20 min, 50% B). As a result compound 93 (3.2 mg) was obtained after drying the collected fractions under high vacuum. Compound 94 (12.5 mg) crystallized out as yellow needles in the sub-fractions collected after silica gel CC of fraction F_1 eluted with *n*-hexane-ethyl acetate (90:10) as solvent system.



Scheme-5.1: Extraction of alkaloidal fraction from the crude extract of *E. suberosa*



Scheme-5.2: Isolation of alkaloid compounds (89-93)

5.3. Characterization of known compounds from *Erythrina suberosa*

5.3.1. Erysodine (89)

Physical State: white needles

Yield: 44 mg

Melting point: 204 °C

UV $\lambda_{\max}$ in EtOH: 235 nm

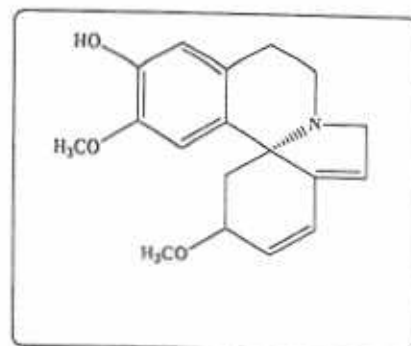
$[\alpha]_D^{23} = +243^\circ$ (c 0.5, EtOH)

IR (KBr) $\nu_{\max}$: 3555 cm^{-1}

EIMS: m/z 299 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-4.1

HSQC: See Table-4.1



5.3.2. Erysotrine (90)

Physical State: Brown oil

Yield: 180 mg

Melting point: 161 °C

UV $\lambda_{\max}$ in EtOH: 230, 280 nm

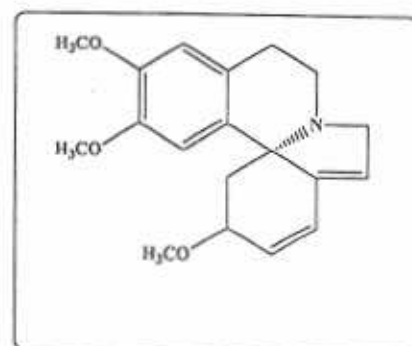
$[\alpha]_D^{23} = +140^\circ$ (c 0.4, EtOH)

IR (KBr) $\nu_{\max}$: 1600 cm^{-1}

EIMS: m/z 313 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-4.2

HSQC: See Table-4.2



5.3.3. Erytharbine (91)

Physical State: Yellowish brown solid

Yield: 4.2 mg

UV λ_{max} in EtOH: 266, 259 and 234 nm

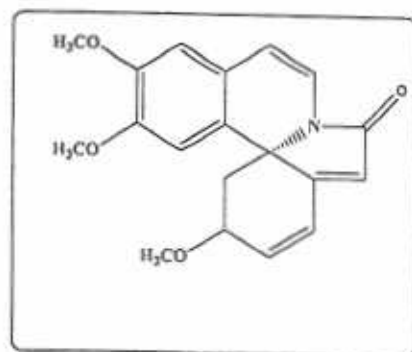
$[\alpha]_D^{23} = +561^\circ$ (c 0.1, CHCl_3)

IR (KBr) ν_{max} : 1690 cm^{-1}

EIMS: m/z 325 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-4.3

HSQC: See Table-4.3



5.3.4. Erysotramidine (92)

Physical State: Colourless oil

Yield: 4.5 mg

UV λ_{max} in EtOH: 212, 236, 257 and 316 nm

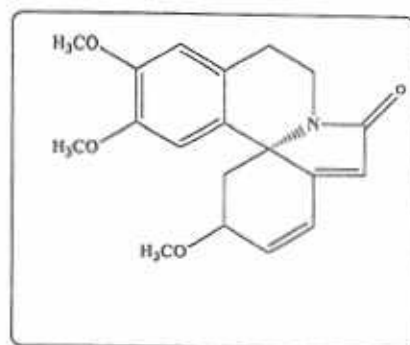
$[\alpha]_D^{23} = +140^\circ$ (c 0.4, EtOH)

IR (KBr) ν_{max} : 2930, 1665 and 1518 cm^{-1}

EIMS: m/z 327 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-4.4

HSQC: See Table-4.4



5.3.5. Erythrartine (93)

Physical State: Brown solid

Yield: 3.2 mg

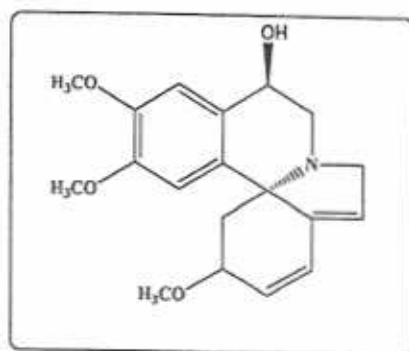
UV λ_{max} in EtOH: 227, 275 nm

IR (KBr) ν_{max} : 1607 cm^{-1}

ESIMS (+): m/z 330 $[\text{M} + 1]^+$

^1H - and ^{13}C -NMR: See Table-4.5

HSQC: See Table-4.5



5.3.6. Alpinumisoflavone (94)

Physical State: Yellow needles

Yield: 12.5 mg

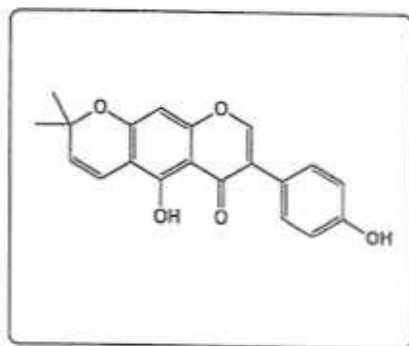
UV λ_{max} in EtOH: 226, 280, 340 nm

IR (KBr) ν_{max} : 3412, 2935, 1722, 1466, 1384 cm^{-1}

ESIMS: m/z 337 $[\text{M} + 1]^+$

^1H - and ^{13}C -NMR: See Table-4.6

HSQC: See Table-4.6



5.4. Anxiolytic activity

5.4.1. Subjects

Twelve groups (n=8-12/group) of Swiss male mice from São Paulo State University/UNESP, SP, Brazil, weighing 25-35 g, were housed in groups of 10 per cage (41 x 34 x 16 cm) and maintained under a normal 12-h light cycle (lights on 07:00 h) in a temperature/humidity controlled environment (23 ± 1 °C/ $55 \pm 5\%$) with free access to food and water. All animals were experimentally naive. Experimental procedures were in compliance with the US National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Local Ethical Committee (UNESP–Araraquara/SP, nr 35/2004).

5.4.2. Compound administration

Erysodine (89) and crysotrine (90) were suspended in saline (NaCl 0.9%) + 1% dimethyl sulphoxide (DMSO). Animals were orally treated by gavage with these compounds (3 and 10 mg/kg). These doses were based in the previous studies from our laboratory.^{81,253} A positive control group receiving intraperitoneal injection of diazepam (DZP, 2 mg/kg) was also included in the study. All compounds were administered 30 min before testing.

5.4.3. Apparatus

The EPM comprised two open arms (30 x 5 cm) and two closed arms (30 x 5 x 15 cm) that extended from a common central platform (5 x 5 cm). The apparatus was constructed from glass (clear walls) and wood (floor) and elevated 38.5 cm above floor level. The LDTM was a Plexiglas box (44.5 x 37 x 25 cm) divided by a barrier

with a door way (7 x 7.5 cm) through which mice could cross between two chambers, one made by white walls (27 x 37 x 25 cm) and illuminated by a white light (500 lux; illuminated compartment) and one made by black walls (17 x 37 x 25 cm) and illuminated by a red light (25 lux, dark compartment).

5.4.4. Procedure

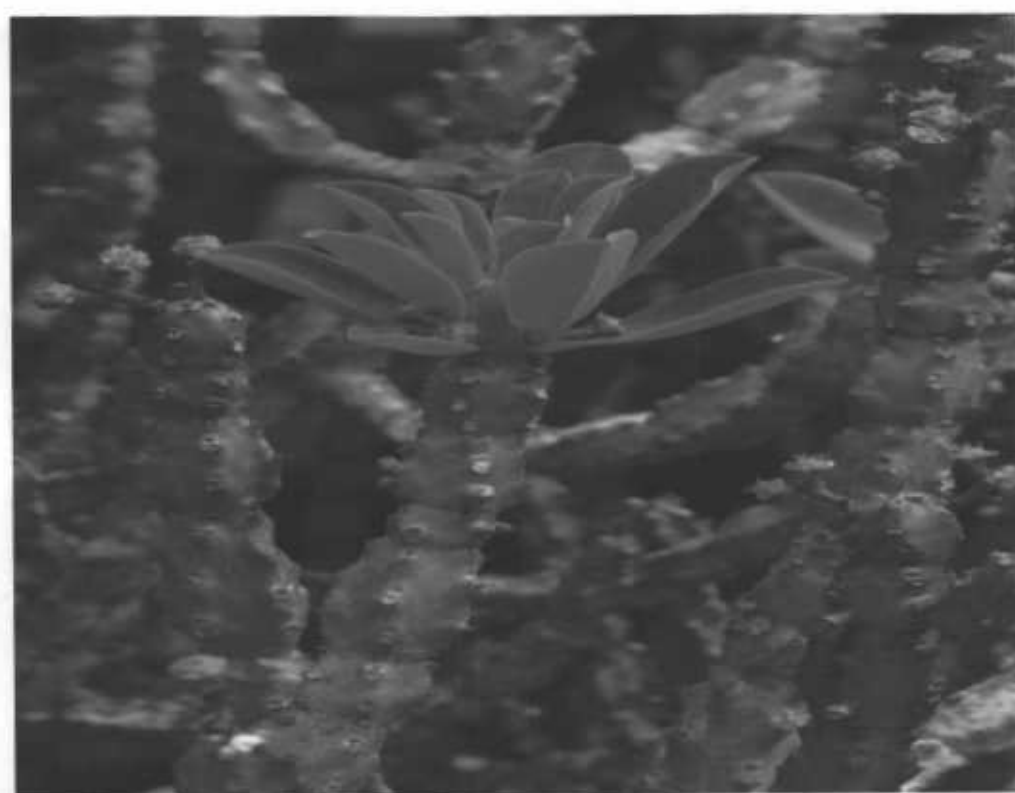
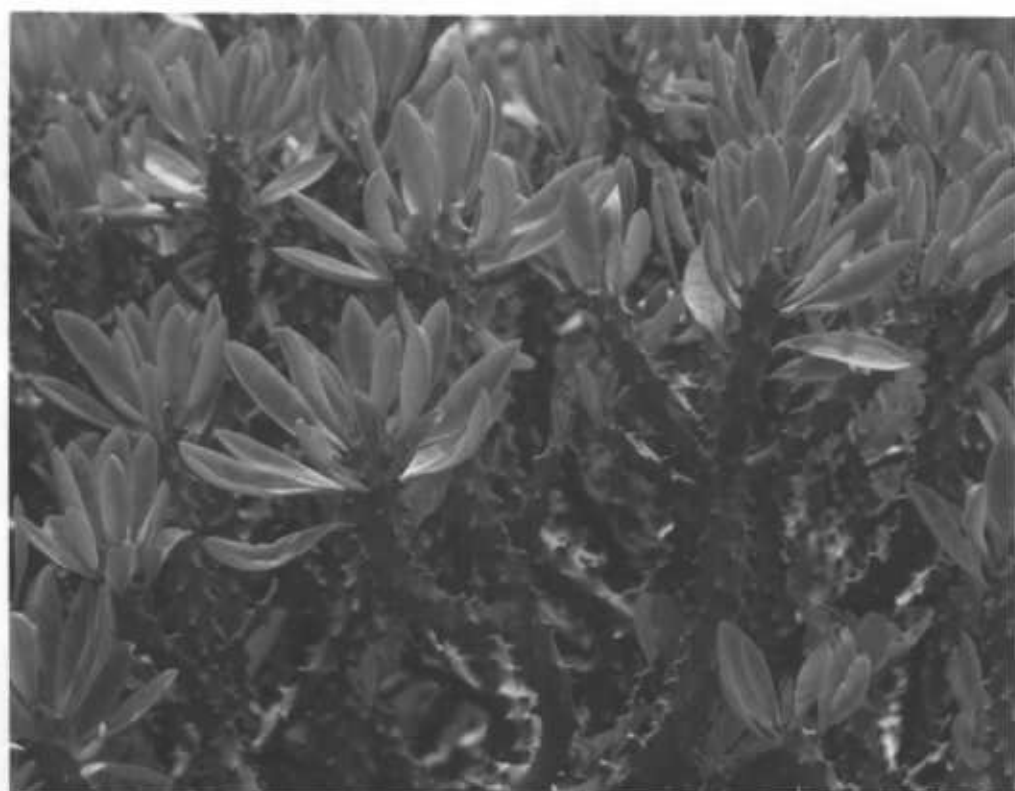
The mice were treated with erysodine (89) or erysotrine (90) (0, 3 or 10 mg/kg; p.o.) and 30 minutes later, they were individually exposed in the EPM or LDTM. **EPM:** Each animal was placed in the central square facing an open arm, and allowed to freely explore the EPM. The following spatio-temporal measures were recorded during a 5 min test: number of open and closed arm entries (arm entry: all four paws into an arm), % open entries $[(\text{open}/\text{total}) \times 100]$, and % open time $[(\text{time open}/300) \times 100]$. Percentage open arm entries and percentage open arm time were used as anxiety indices, and frequency of closed arms entries was used as locomotor activity. **LDTM:** Each animal was placed at the center of the illuminated compartment and, after the first passage to the dark compartment, the behavior of the animal was video recorded for 5 min to further analysis of the number of transition between compartments and the time spent in the illuminated compartment. Entry into a compartment was recorded when the animal crossed with all four paws the line that separated the two compartments.

5.4.5. Statistics

All results were submitted to one-way analysis of variance (ANOVA), followed by Duncan's Multiple Comparison Test when significant. In all cases a p value ≤ 0.05 was considered significant.

Part-B

***Phytochemistry of Euphorbia
neriifolia***



***Euphorbia neriifolia* Linn.**

Chapter: 6

INTRODUCTION (Part-B)

6.1. Family Euphorbiaceae

Family Euphorbiaceae is one of the largest families of the phylum Anthophyta comprising of 300 genera and 5000 species.²⁵⁶ The name of this family was coined by king Juba II of Mauritania (25 B. C), who discovered a succulent plant and named it Euphorbia in honour of his physician, Euphorbos.²⁵⁷ The plant species of the family have been known to be poisonous to human beings. They are often found involved in poisoning of livestock and used as constituents of arrow poisons. However, a wide range of pharmacological screenings carried out most recently have shown that some of them are quite useful for the treatment of a variety of ailments like cancer, rheumatism, neuralgia, asthma and bacterial infections.²⁵⁸ A number of species have also been in use in agriculture, as resources for certain technologies and ornamental plants in horticulture. These and many other uses of the species of this family have generated a wide interest in their phytochemistry and a voluminous work on the isolation and structural elucidation of different types of chemical constituents has so far been published.

6.2. Genus *Euphorbia*

Genus *Euphorbia* is the largest genus of the family Euphorbiaceae having more than 2000 species in the form of laticiferous herbs, shrubs and small trees, occurring in the tropical and temperate zones of Asia and other parts of the world. The chemical studies of *Euphorbia* species started as far back as 1834, when Rose and Fluckiger^{259,260} isolated a crystalline compound euphorbone from the lattices of some of its species. Bauer and Schenkel²⁶¹ obtained an unsaturated alcohol euphorbol from euphorbone in 1928. Later on, they found that this alcohol was a mixture α -euphorbol along with a low melting alcohol.²⁶² Subsequently, a large number of chemical

constituents, such as terpenoids, steroids and flavonoids have been isolated from the species of this genus. In this part of the thesis, we are mainly concerned with di- and tri-terpenes; hence a comprehensive review of the di- and tri-terpenes isolated so far from the species of genus *Euphorbia* is given in the Table 6.1 and 6.2.

6.3. *Euphorbia neriifolia* Linn. (Hedge Euphorbia)

E. neriifolia is an erect shrub with a height of up to 4 m. The stem tissues are fleshy and slightly succulent. The stem and branches have no articulation and bears 5 indistinct angles and spine-shields in 5 rows. The leaves are usually terminal.²⁶³

6.4. Pharmacological importance of *Euphorbia neriifolia*

E. neriifolia plant has been used in traditional medicines in different ways. For example, the latex is used to treat obstinate constipation and other abdominal conditions including jaundice and ascites. The latex is also used for the treatment of hemorrhoids, fistula-in-ano,²⁶⁴ obstinate skin diseases and urinary disorders including diabetes.²⁶⁵ The root mixed with black pepper is applied to cure snake bites.²⁶⁴ The aqueous extract of the latex is used for the healing of surgically produced cutaneous wounds whereby it increase the tensile strength, DNA content, epithelization and angiogenesis.²⁶⁶ The extract from the leaves of *E. neriifolia* has been shown to possess anti-anxiety, anti-psychotic and anticonvulsant activities in mice and rats.^{263,267} 70% v/v hydro-alcoholic extract of the leaves of the plant has been reported to show greater anti-inflammatory and analgesic effect than standard doses of indomethacin and diclofenac sodium.²⁶⁸ The ethyl acetate extract of *E. neriifolia* demonstrated significant cytotoxicities against the cell lines: Lewis lung carcinoma, B16F10 melanoma and SW480 human colon adenocarcinoma in a dose dependent

manner. The ethanolic extract of the leaves and petroleum ether extracts of the pods of *E. nerifolia* were shown to be effective in inhibiting the growth of *E. coli*.²⁶⁹

Table-6.1: List of diterpenoids isolated from genus *Euphorbia*

S. No	Name of Compound	Molecular Formula	Source
1	7-Acetoxy ingenol-5,20-diacetate-3-angelate	C ₃₁ H ₄₀ O ₉	<i>E. kamerunica</i> ²⁷³
2	15- <i>O</i> -Acetyl-3- <i>O</i> -benzoyl-characiol-5,6-oxide	C ₂₉ H ₃₆ O ₇	<i>E. characia</i> ²⁷⁰
3	15-Acetyl-3-benzoyl-jolkinol-5,6-oxide	C ₂₉ H ₃₆ O ₆	<i>E. characia</i> ²⁷¹
4	3- <i>O</i> -Acetyl-16- <i>O</i> -benzoyl-20- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenoyl]-16-hydroxy ingenol	C ₃₄ H ₄₀ O ₉	<i>E. canariensis</i> ²⁷²
5	20- <i>O</i> -Acetyl-3- <i>O</i> -(<i>Z</i> , <i>E</i> ,2 <i>E</i>)decadienoyl-ingenol	C ₃₄ H ₄₈ O ₇	<i>E. biglandulose</i> ²⁷³
6	20- <i>O</i> -Acetyl-3- <i>O</i> -(2 <i>E</i> ,4 <i>Z</i>)-2,4,6-decatrienoyl-ingenol	C ₃₂ H ₄₂ O ₇	<i>E. biglandulose</i> ²⁷³
7	15- <i>O</i> -Acetyl-5β-hydroxy-isocharaciol-3-benzoate	C ₂₉ H ₃₆ O ₇	<i>E. characia</i> ²⁷⁰
8	15- <i>O</i> -Acetyl-5β-hydroxy-isocharaciol-3-isobutyrate	C ₂₆ H ₃₈ O ₇	<i>E. characia</i> ²⁷⁰
9	15- <i>O</i> -Acetyl-5β-hydroxy-isocharaciol-3-liglate	C ₂₇ H ₃₈ O ₇	<i>E. characia</i> ²⁷⁰
10	20-Acetyl-ingenol-3-angelate	C ₂₇ H ₃₆ O ₇	<i>E. kamerunica</i> ²⁷⁴
11	20-Acetyl-ingenol-3-decadienoate	C ₃₂ H ₄₄ O ₇	<i>E. broteri</i> ²⁷⁵
12	20-Acetyl-ingenol-3-octenoate	C ₃₀ H ₄₂ O ₇	<i>E. kamerunica</i> ²⁷⁴
13	8-Acetyl-ingol-12-tigliate	C ₂₇ H ₃₈ O ₈	<i>E. kamerunica</i> ²⁷⁶
14	15-Acetyl-3-isobutyryl-jolkinol-5,6-oxide	C ₂₆ H ₃₆ O ₆	<i>E. characias</i> ²⁷¹
15	2-Acetyl-3-isobutyryl-15-nicotinoyl-2-hydroxyjolkinol-5,6-oxide	C ₃₂ H ₄₁ NO ₈	<i>E. characias</i> ²⁷¹
16	3- <i>O</i> -Acetyl-20- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenoyl]-ingenol	C ₂₇ H ₃₆ O ₇	<i>E. canariensis</i> ²⁷²
17	15-Acetyl-3-nicotinoyl-jolkinol-5,6-oxide	C ₂₈ H ₃₅ NO ₆	<i>E. characia</i> ²⁷¹
18	15- <i>O</i> -Acetyl-3- <i>O</i> -propionyl-characiol	C ₂₅ H ₃₆ O ₆	<i>E. characias</i> ²⁷⁰
19	15-Acetyl-3-propionyl-jolkinol-5,6-oxide	C ₂₅ H ₃₆ O ₆	<i>E. characias</i> ²⁷¹
20	20- <i>O</i> -Acetyl-resiniferonol-9,13,14-orthophenylacetate	C ₃₀ H ₃₄ O ₇	<i>E. poissonii</i> ²⁷⁷
21	15- <i>O</i> -Acetyl-3- <i>O</i> -tigloyl-characiol-5,6-oxide	C ₂₇ H ₃₈ O ₇	<i>E. characias</i> ²⁷⁰
23	3-Angelyl-ingenol	C ₂₅ H ₃₄ O ₆	<i>E. paralias</i> ²⁷⁸
24	3-Angelyl-20-deoxyingenol	C ₂₅ H ₃₄ O ₅	<i>E. paralias</i> ²⁷⁸
25	Caudicifoline	C ₂₀ H ₂₆ O ₄	<i>E. candellia</i> ²⁷⁹
26	3- <i>O</i> -(2 <i>E</i> ,4 <i>Z</i>)-decadienoyl-ingenol	C ₃₀ H ₄₂ O ₆	<i>E. esula</i> ²⁸⁰
27	3(2,4,6)decatrienolate-16-angelyl-ingenol	C ₃₅ H ₄₆ O ₈	<i>E. ingens</i> ²⁸¹

28	3(2,4,6)decatrienoate-ingenol	C ₃₀ H ₄₀ O ₆	<i>E. ingens</i> ²⁸⁰
29	3- <i>O</i> -(2 <i>E</i> ,4 <i>Z</i>)-2,1,6-decatrienoyl-20-deoxy-ingenol	C ₃₀ H ₄₀ O ₅	<i>E. biglandulose</i> ²⁷³
30	5- <i>O</i> -(2 <i>E</i> ,4 <i>Z</i>)-2,4,6-decatrienoyl-20-deoxy-ingenol	C ₃₀ H ₄₀ O ₅	<i>E. biglandulose</i> ²⁷³
31	3- <i>O</i> -n(Deca-2,4,6-trienoyl)-16- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenoyl]-16-hydroxy-ingenol	C ₃₅ H ₄₆ O ₈	<i>E. hermentiana</i> ²⁸²
32	12-Deoxy-16-hydroxy-phorbol-13-angelate-16-isobutyrate	C ₂₉ H ₄₀ O ₈	<i>E. cooperi</i> ²⁸³
33	12-Deoxy-16-hydroxy-phorbol-13-angelate-16-isobutrate-20-acetate	C ₃₁ H ₄₂ O ₉	<i>E. cooperi</i> ²⁸³
34	5-Deoxy-ingenol	C ₂₀ H ₂₈ O ₄	<i>E. myrsinites</i> ²⁸⁴
35	20-Deoxy-ingenol-3- <i>O</i> -angelate	C ₂₅ H ₃₄ O ₅	<i>E. peplas</i> ²⁷⁸
36	5-Deoxy-ingenol-3,20-diacetate	C ₂₄ H ₃₂ O ₆	<i>E. myrsinites</i> ²⁸⁴
37	12-Deoxy-phorbol	C ₂₀ H ₃₈ O ₅	<i>E. triangularis</i> ²⁸⁵
38	20-Deoxy-phorbol-13- <i>O</i> [<i>p</i> -acetoxypheylacetate]-20-acetate	C ₃₂ H ₃₈ O ₉	<i>E. poisonii</i> ²⁷⁷
39	12-Deoxy-phorbol-13-angelate	C ₂₅ H ₃₄ O ₆	<i>E. poisonii</i> ²⁸⁶
40	12-Deoxy-phorbol-13-angelate-20-acetate	C ₂₇ H ₃₆ O ₇	<i>E. resinifera</i> ²⁸⁶
41	12-Deoxy-4βOH-phorbol-13,20-diacetate	C ₂₄ H ₃₂ O ₇	<i>E. triangularis</i> ²⁸⁵
42	12-Deoxy-phorbol-13,20-diacetate	C ₂₄ H ₃₂ O ₇	<i>E. milli</i> ²⁸⁷
43	12-Deoxy-4βOH-phorbol-13-dodecanoate-20-acetate	C ₃₄ H ₅₂ O ₇	<i>E. coerleeseens</i> ²⁸⁸
44	12-Deoxy-phorbol-13-isobutyrate	C ₂₄ H ₃₄ O ₆	<i>E. poisonii</i> ²⁸⁶
45	12-Deoxy-phorbol-13-isobutyrate-20-acetate	C ₂₆ H ₃₆ O ₇	<i>E. resinifera</i> ²⁸⁹
46	12-Deoxy-phorbol-13-(2-methyl)butyrate	C ₂₅ H ₃₆ O ₆	<i>E. resinifera</i> ²⁸⁶
47	12-Deoxy-phorbol-13-(2-methyl)butyrate-20-acetate	C ₂₇ H ₃₈ O ₇	<i>E. poisonii</i> ²⁸⁶
48	12-Deoxy-4βOH-phorbol-13-octanoate-20-acetate	C ₃₀ H ₄₂ O ₇	<i>E. polyacantha</i> ²⁹⁰
49	4-Deoxy-phorbol-12-octa-2,3-dienoate-13,20-diacetate	C ₃₂ H ₄₂ O ₈	<i>E. biglandulose</i> ²⁹¹
50	4-Deoxy-phorbol-12-octa-2,3-dienoate-13-isobutyrate-20-acetate	C ₃₄ H ₄₆ O ₈	<i>E. biglandulose</i> ²⁹¹
51	4-Deoxy-phorbol-12-octa-2,3-dienoate-13-propanoate-20-acetate	C ₃₃ H ₄₄ O ₈	<i>E. biglandulose</i> ²⁹¹
52	12-Deoxy-phorbol-13-phenylacetate-20-acetate	C ₃₀ H ₃₆ O ₇	<i>E. resinifera</i> ²⁸⁶
53	12-Deoxy-phorbol-13-phenylacetate-20-acetate	C ₃₀ H ₄₀ O ₉	<i>E. tirucalli</i> ²⁹²
54	12-Deoxy-phorbol-13-tetradecanoate	C ₂₅ H ₅₄ O ₆	<i>E. triangularis</i> ²⁹³
55	12-Deoxy-phorbol-13-tiglate	C ₂₅ H ₃₄ O ₆	<i>E. triangularis</i> ²⁹⁴
56	15,17-Di- <i>O</i> -Acetyl-3- <i>O</i> -cinnamoyl-17-hydroxy-jloklinol	C ₃₃ H ₄₀ O ₇	<i>E. lathyrus</i> ²⁹⁵
57	3,7-Diacetyl-ingol-12-tiglate	C ₂₉ H ₄₀ O ₉	<i>E. kamerunica</i> ²⁷⁶
58	7,8-Diacetyl-ingol-12-tiglate	C ₂₉ H ₄₀ O ₉	<i>E. kamerunica</i> ²⁷⁶

59	3,12-Diacetyl-ingol-12-tigliate	C ₂₉ H ₄₀ O ₉	<i>E. kamerunica</i> ²⁹⁶
60	3,12-Di-O-acetyl-ingol-8-tigliate	C ₂₉ H ₄₀ O ₉	<i>E. lactea</i> ²⁹⁷
61	12,20-Dideoxy-phorbol-13-angelate	C ₂₅ H ₃₄ O ₅	<i>E. resinifera</i> ²⁹⁸
62	12,20-Dideoxy-phorbol-13-isobutyrate	C ₂₄ H ₃₄ O ₅	<i>E. resinifera</i> ²⁹⁸
63	12-Deoxy-phorbol-13-isobutyrate	C ₂₄ H ₃₄ O ₆	<i>E. triangularis</i> ²⁹³
64	3-O-(2,4,6,8)-Dodecatetraenoyl-ingol	C ₃₂ H ₄₂ O ₆	<i>E. esula</i> ²⁸⁰
65	6,20-Epoxy-lathyrol	C ₂₀ H ₃₂ O ₄	<i>E. lactea</i> ²⁹⁹
66	6,20-Epoxy-lathyrol-3-phenylacetate-15,5-diacetate	C ₃₂ H ₃₈ O ₇	<i>E. lactea</i> ²⁹⁹
67	Ester I ₃	C ₃₀ H ₃₈ O ₇	<i>E. lathyris</i> ³⁰⁰
68	Esulone A	C ₃₈ H ₄₂ O ₁₂	<i>E. esula</i> ³⁰¹
69	Esulone B	C ₄₀ H ₄₄ O ₁₂	<i>E. esula</i> ³⁰¹
70	Euphoheline A	C ₃₃ H ₄₄ O ₁₁	<i>E. helioscopia</i> ³⁰²
71	Euphoheline B	C ₃₁ H ₄₂ O ₁₀	<i>E. helioscopia</i> ³⁰²
72	Euphoheline C	C ₄₀ H ₄₈ O ₁₂	<i>E. helioscopia</i> ³⁰²
73	Euphoheline D	C ₃₁ H ₃₈ O ₁₀	<i>E. helioscopia</i> ³⁰²
74	Euphoheline E	C ₃₁ H ₄₀ O ₁₀	<i>E. helioscopia</i> ³⁰²
75	Euphorbia factor RL14	C ₂₈ H ₃₂ O ₆	<i>E. resinifera</i> ²⁹⁸
76	Euphorbiasteroid	C ₃₄ H ₄₀ O ₈	<i>E. lathyris</i> ³⁰³
77	Euphornin	C ₃₃ H ₄₄ O ₉	<i>E. madeni</i> ³⁰⁴
78	Euphornin A	C ₃₁ H ₄₂ O ₈	<i>E. helioscopia</i> ³⁰⁵
79	Euphornin B	C ₃₁ H ₄₂ O ₈	<i>E. helioscopia</i> ³⁰⁵
80	Euphornin C	C ₃₁ H ₄₈ O ₈	<i>E. helioscopia</i> ³⁰⁵
81	Euphoscopine A	C ₃₁ H ₄₀ O ₈	<i>E. helioscopia</i> ³⁰⁶
82	Euphoscopine B	C ₃₃ H ₄₂ O ₉	<i>E. helioscopia</i> ³⁰⁶
83	Helioscopinolide A	C ₂₀ H ₂₈ O ₃	<i>E. helioscopia</i> ³⁰⁷
84	Helioscopinolide B	C ₂₀ H ₂₈ O ₃	<i>E. helioscopia</i> ³⁰⁷
85	Helioscopinolide C	C ₂₀ H ₂₆ O ₃	<i>E. helioscopia</i> ³⁰⁷
86	20-Hexadecanoate-ingol	C ₃₆ H ₅₈ O ₆	<i>E. ingens</i> ³⁰⁸
87	3-Hexadecanoate-ingol	C ₃₆ H ₅₈ O ₆	<i>E. ingens</i> ³⁰⁸
88	3-Hexanoyl-20-deoxy-ingol	C ₂₆ H ₃₈ O ₅	<i>E. paralias</i> ²⁷⁸
89	16-Hydroxy-12-deoxy-phorbol-13-(2-methyl)2-butenate-16-isobutanoate	C ₂₉ H ₄₀ O ₈	<i>E. cooperii</i> ²⁷⁸
90	16-Hydroxy-12-deoxy-13-(2-methyl)2-butenate-16-isobutanoate-20-acetate	C ₃₁ H ₄₂ O ₉	<i>E. cooperii</i> ³⁰⁹
91	16-Hydroxy-ingol-3,5,16,20-tetraacetate	C ₂₈ H ₃₆ O ₁₀	<i>E. lactea</i> ²⁹⁷
92	16-Hydroxy-pseudjolkinolide B	C ₂₀ H ₂₈ O ₅	<i>E. paralias</i> ³¹⁰
93	Ingenol	C ₂₀ H ₂₈ O ₅	<i>E. lathyris</i> ²⁸⁴
94	Ingenol-3-decanoate	C ₃₀ H ₄₆ O ₆	<i>E. kamerunica</i> ²⁷⁴
95	Ingenol-3,20-dibenzoate	C ₃₄ H ₃₆ O ₇	<i>E. esula</i> ³¹¹
96	Ingenol-3,20-di-isobutyrate	C ₂₈ H ₄₀ O ₇	<i>E. cotinifolia</i> ³¹²
97	Ingenol-3-dodecanoate	C ₃₂ H ₅₀ O ₆	<i>E. kamerunica</i> ²⁷⁴
98	Ingenol-6-dodecanoate	C ₃₂ H ₅₀ O ₆	<i>E. esula</i> ³¹³
99	Ingenol-3-hexadecanoate	C ₃₆ H ₅₈ O ₆	<i>E. lathyris</i> ³¹⁴
100	Ingenol-5-hexadienoate	C ₂₆ H ₃₄ O ₆	<i>E. kamerunica</i> ²⁷⁴
101	Ingenol-20-isobutyrate	C ₂₄ H ₃₄ O ₆	<i>E. cotinifolia</i> ³¹²
102	Ingenol-12-octanoate	C ₂₈ H ₄₂ O ₆	<i>E. peplus</i> ³¹⁵

103	Ingenol-5-octenoate	C ₂₈ H ₄₂ O ₆	<i>E. kamerunica</i> ²⁷⁴
104	Ingenol-3-palmitate	C ₃₆ H ₅₈ O ₆	<i>E. serrata</i> ³¹⁶
105	Ingenol-3-propanoate-20-isobutyrate	C ₂₇ H ₃₈ O ₇	<i>E. cotinifolia</i> ³¹²
106	Ingenol-3-propanoate-20-(2-methyl)butanoate	C ₂₈ H ₄₀ O ₇	<i>E. cotinifolia</i> ³¹²
107	Ingenol-3-propanoate-4,5,20-triacetate	C ₃₁ H ₄₂ O ₁₁	<i>E. poisonii</i> ³¹⁷
108	Ingenol-6-tetradeca-2,4,6,8,10-pentenoate	C ₃₄ H ₄₄ O ₆	<i>E. esula</i> ³¹³
109	Ingenol-3-tetradeca-2,4,6,8,10-pentenoate	C ₃₄ H ₄₄ O ₆	<i>E. jolkin</i> ³¹⁸
110	Ingenol-3,5,20-triacetate	C ₂₆ H ₃₄ O ₈	<i>E. myrsinites</i> ²⁸⁴
111	Ingenol-7- <i>p</i> -(methoxy)phenylacetate-3,8,12-triacetate	C ₃₄ H ₄₂ O ₁₀	<i>E. resinifera</i> ³¹⁹
112	Ingenol-7-phenylacetate-3,8,12-triacetate	C ₃₄ H ₄₂ O ₁₀	<i>E. resinifera</i> ³¹⁹
113	Ingol-3,7,8,12-tetraacetate	C ₂₈ H ₃₈ O ₁₀	<i>E. kamerunica</i> ²⁷⁶
114	Ingol-3,8,12-triacetate-7-angelate	C ₃₁ H ₄₂ O ₁₀	<i>E. kamerunica</i> ³²⁰
115	Ingol-3,7,12-triacetate-8-nicotinate	C ₃₂ H ₃₉ NO ₁₀	<i>E. ingens</i> ²⁸¹
116	Ingol-3,8,12-triacetate-7-tiglate	C ₃₁ H ₄₂ O ₁₀	<i>E. kamerunica</i> ³²¹
117	12- <i>O</i> -isobutyl-phorbol-13-acetate-20-angelate	C ₃₁ H ₄₂ O ₉	<i>E. coerleseens</i> ³²²
118	Jolkinol A	C ₂₉ H ₃₆ O ₆	<i>E. lathyrus</i> ³²³
119	Jolkinol A	C ₂₆ H ₃₂ O ₆	<i>E. lathyrus</i> ³²³
120	Jolkinolide A	C ₂₀ H ₂₆ O ₃	<i>E. jolkin</i> ³²⁴
121	Jolkinolide B	C ₂₀ H ₂₆ O ₄	<i>E. jolkin</i> ³²⁵
122	Jolkinolide C	C ₁₉ H ₂₆ O ₃	<i>E. jolkin</i> ³²⁶
123	Jolkinolide D	C ₁₉ H ₂₆ O ₃	<i>E. jolkin</i> ³²⁶
124	Jolkinolide E	C ₁₉ H ₂₈ O ₂	<i>E. jolkin</i> ³²⁶
125	3- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenolyl]-20- <i>O</i> -acetyl-ingenol	C ₂₇ H ₃₆ O ₇	<i>E. hermentiana</i> ²⁸²
126	3- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenolyl]-16- <i>O</i> -acetyl-20-deoxy-16-hydroxy-ingenol	C ₂₇ H ₃₆ O ₇	<i>E. hermentiana</i> ²⁸²
127	3- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenolyl]-16- <i>O</i> -benzoyl-16-hydroxy-ingenol	C ₃₂ H ₃₈ O ₈	<i>E. canariensis</i> ²⁷²
128	3- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenolyl]-16,20-diacetyl-16-hydroxy-ingenol	C ₂₉ H ₃₈ O ₉	<i>E. hermentiana</i> ²⁸²
129	3- <i>O</i> -[(<i>Z</i>)-2-methyl-2-butenolyl]-5,16,20- <i>O</i> -triacetyl-16-hydroxy-ingenol	C ₃₁ H ₄₀ O ₁₀	<i>E. hermentiana</i> ²⁸²
130	8- <i>O</i> -methyl-ingol-12-acetate-7-angelate	C ₂₈ H ₄₀ O ₈	<i>E. kamerunica</i> ³²¹
131	8- <i>O</i> -methyl-ingol-3,12-diacetate-7-angelate	C ₃₀ H ₄₂ O ₉	<i>E. kamerunica</i> ³²¹
132	8- <i>O</i> -methyl-ingol-3,12-diacetate-7-benzoate	C ₃₂ H ₄₀ O ₆	<i>E. kamerunica</i> ³²¹
133	8- <i>O</i> -methyl-ingol-3,12-diacetate-7-tiglate	C ₃₀ H ₄₂ O ₉	<i>E. kamerunica</i> ³²⁷
134	Milliamine C	C ₄₃ H ₄₂ N ₃ O ₉	<i>E. milli</i> ³¹⁸
135	Milliamine H	C ₃₇ H ₄₀ N ₂ O ₉	<i>E. milli</i> ³²⁸
136	Milliamine I	C ₃₆ H ₄₀ NO ₁₀	<i>E. milli</i> ³²⁸
137	12- <i>O</i> -(2 <i>Z</i> ,4 <i>E</i>)Octadienyl-4-deoxyphorbol-13-acetate	C ₃₀ H ₄₀ O ₇	<i>E. tirucalli</i> ³²⁹

138	12- <i>O</i> -(2 <i>Z</i> ,4 <i>E</i>)Octadienyl-4-deoxyphorbol-13,20-diacetate	C ₃₂ H ₄₂ O ₈	<i>E. broteri</i> ²⁷⁵
139	12- <i>O</i> -(2 <i>Z</i> ,4 <i>E</i>)Octadienyl-4-phorbol-13,20-diacetate	C ₃₁ H ₄₂ O ₉	<i>E. broteri</i> ²⁷⁵
140	Phorbol	C ₂₀ H ₂₈ O ₆	<i>E. franckiana</i> ³³⁰
141	Proresiniferatoxine	C ₃₇ H ₄₂ O ₁₀	<i>E. resinifera</i> ²⁹⁸
142	Pseudojolkinolide A	C ₂₀ H ₂₆ O ₃	<i>E. paralias</i> ³¹⁰
143	Pseudojolkinolide B	C ₂₀ H ₂₆ O ₄	<i>E. paralias</i> ³¹⁰
144	Resiniferanol	C ₂₀ H ₂₈ O ₆	<i>E. resinifera</i> ²⁹⁸
145	Resiniferatoxine	C ₃₇ H ₄₀ O ₉	<i>E. resinifera</i> ²⁹⁸
146	3-Tetradecanoate-ingenol-5,20-diacetate	C ₃₈ H ₅₈ O ₈	<i>E. broteri</i> ²⁷⁵
147	20-Tetradecanoate-ingenol-3,5-diacetate	C ₃₈ H ₅₈ O ₈	<i>E. broteri</i> ²⁷⁵
148	5-Tetradecanoate-ingenol-3,20-diacetate	C ₃₈ H ₅₈ O ₈	<i>E. broteri</i> ²⁷⁵
149	3,7,12-Triacetyl-8-angelylingol	C ₃₁ H ₄₂ O ₁₀	<i>E. kamerunica</i> ³³¹
150	3,7,12- <i>O</i> -triacetyl-8- <i>O</i> -benzoyl-18-hydroxy-ingol	C ₃₂ H ₄₀ O ₁₀	<i>E. hermentiana</i> ³²⁷
151	5,15,17-tri- <i>O</i> -acetyl-3- <i>O</i> -benzoyl-17-hydroxy-isolathyrol	C ₃₃ H ₄₄ O ₉	<i>E. lathyrus</i> ²⁹⁵
152	3,7,12-triacetyl-8-benzoyl-ingol	C ₃₃ H ₄₀ O ₁₀	<i>E. kamerunica</i> ³³¹
153	3,7,8-triacetyl-ingol-12-tiglate	C ₃₁ H ₄₂ O ₁₀	<i>E. kamerunica</i> ³³²
154	2,5,8,15- <i>O</i> -triacetyl-nicotinoyl-2,5,8-trihydroxy-isocharaciol-3-benzoate	C ₃₉ H ₄₂ NO ₁₂	<i>E. chracias</i> ²⁷⁰
155	2,5,8,15- <i>O</i> -triacetyl-nicotinoyl-2,5,8-trihydroxy-isocharaciol-3-tiglate	C ₃₇ H ₄₅ NO ₁₂	<i>E. chracias</i> ²⁷⁰
156	3,7,12-triacetyl-8-tiglyl-ingol	C ₃₁ H ₄₂ O ₁₀	<i>E. kamerunica</i> ³³¹
157	Triaculetine	C ₂₀ H ₂₈ O ₂	<i>E. triaculeata</i> ³³³

Table-6.2: List of triterpenoids isolated from genus *Euphorbia*

S. No	Name of Compound	Molecular Formula	Source
1	α -Amyrin	C ₃₀ H ₅₀ O	<i>E. pillulifera</i> ³³⁴
2	β -Amyrin	C ₃₀ H ₅₂ O	<i>E. pillulifera</i> ³³⁴
3	β -Amyrin acetate	C ₃₂ H ₅₂ O	<i>E. ebracteolata</i> ³³⁵
4	δ -Amyrinone	C ₃₀ H ₄₈ O	<i>E. parviflora</i> ³³⁶
5	Balatol (Resiniferol)	C ₃₀ H ₅₀ O	<i>E. resinifera</i> ³³⁷
7	Betulinic acid	C ₃₀ H ₄₈ O ₃	<i>E. dracunculoides</i> ³³⁸
8	Butyrospermol	C ₂₀ H ₃₀ O	<i>E. sikkimensis</i> ³³⁹
9	Butyrospermolacetate	C ₃₂ H ₅₂ O	<i>E. regisjubae</i> ³³⁹
10	Campesterol	C ₂₈ H ₄₈ O	<i>E. pillulifera</i> ³⁴⁰
11	Citrostadienol	C ₃₀ H ₅₀ O	<i>E. splendens</i> ³⁴¹
12	Corollatadiol	C ₃₁ H ₅₂ O ₂	<i>E. corollata</i> ³⁴²
13	Cycloartanol	C ₃₀ H ₅₂ O	<i>E. trigona</i> ³⁴³
14	Cycloartenol	C ₃₀ H ₅₀ O	<i>E. moilli</i> ³⁴⁴
15	Cycloart-23-en-3 β -ol	C ₃₀ H ₅₀ O	<i>E. polygonifolia</i> ³⁴⁵
16	Cycloart-25-en-3 β -ol	C ₃₀ H ₅₀ O	<i>E. nivulia</i> ³⁴⁶
17	Cycloartenol acetate	C ₃₂ H ₅₂ O	<i>E. pallasii</i> ³⁴⁷
18	Cycloart-23-ene-3 β ,25-diol	C ₃₀ H ₅₀ O ₂	<i>E. cyparissias</i> ³⁴⁸

19	Cycloartenone	C ₃₀ H ₄₈ O	<i>E. balsimifera</i> ³⁴⁹
20	Cycloeucalenol	C ₃₀ H ₅₀ O	<i>E. royleana</i> ³⁵⁰
21	Cycloeuphornol	C ₃₁ H ₅₀ O	<i>E. tirucalli</i> ³⁵¹
22	Cyclolaudenol	C ₃₁ H ₅₂ O	<i>E. caducifolia</i> ³⁵²
23	3-Epicyclolaudenol	C ₃₁ H ₅₂ O	<i>E. caducifolia</i> ³⁵²
24	Cycloroylenol	C ₃₀ H ₅₀ O	<i>E. royleana</i> ³⁵³
25	(<i>E,Z</i>)2,4-Decadienoyl-lanosterol	C ₄₀ H ₆₄ O ₂	<i>E. biglandulose</i> ³⁴⁷
26	(<i>2E,2Z</i>)2,4,6-Deca-trienoyl-lanosterol	C ₄₀ H ₆₂ O ₂	<i>E. biglandulose</i> ³⁴⁷
27	β -Dihydro-fucusterol	C ₂₉ H ₅₀ O	<i>E. helioscopia</i> ³⁵⁴
28	Epigermanicol	C ₃₀ H ₅₀ O	<i>E. caducifolia</i> ³⁵⁵
29	Epifriede-linol	C ₃₀ H ₅₂ O	<i>E. antiqorum</i> ³⁵⁶
30	Epiteraxerol	C ₃₀ H ₅₀ O	<i>E. royleana</i> ³⁵⁷
31	Euphol	C ₃₀ H ₅₀ O	<i>E. tirucalli</i> ³⁴³
32	β , β -Euphol	C ₃₀ H ₅₀ O	<i>E. resinifera</i> ³⁵⁸
33	Euphorbinol	C ₃₁ H ₅₂ O	<i>E. trigona</i> ³⁵⁹
34	Euphorbol	C ₃₁ H ₅₂ O	<i>E. antiqorum</i> ³⁴⁴
35	β -Euphorbol	C ₃₁ H ₅₂ O	<i>E. candylocarpa</i> ³⁶⁰
36	δ -Euphorbol	C ₃₁ H ₅₂ O	<i>E. falcata</i> ³⁶¹
37	Euphorbol-hexacozonate	C ₅₇ H ₁₀₂ O ₂	<i>E. jaxartica</i> ³⁴⁴
38	Euphoron	C ₃₀ H ₄₈ O	<i>E. tirucalli</i> ³⁶²
39	Euphosterol	C ₂₅ H ₄₀ O	<i>E. pillulifera</i> ³⁶³
40	Friedelan-3 α -ol	C ₃₀ H ₅₂ O	<i>E. antiqorum</i> ³⁶⁴
41	Fiedelan-3 β -ol	C ₃₀ H ₅₂ O	<i>E. antiqorum</i> ³⁶⁴
42	Friedelan	C ₃₀ H ₅₀ O	<i>E. hirta</i> ³⁶⁵
43	Germanicol	C ₃₀ H ₅₀ O	<i>E. balsimifera</i> ³⁶⁶
44	Germanicone	C ₃₂ H ₄₈ O	<i>E. balsimifera</i> ³⁴⁹
45	Glut-5(6)-en-3 α -ol	C ₃₀ H ₅₀ O	<i>E. cyparissias</i> ³⁴⁸
46	Glut-5(6)-en-3 β -ol	C ₃₀ H ₅₀ O	<i>E. cyparissias</i> ³⁴⁸
47	Glut-5-en-3-one	C ₃₀ H ₄₈ O	<i>E. cyparissias</i> ³⁴⁸
48	Glut-5-(10)-en-1-one	C ₃₀ H ₄₈ O	<i>E. nerifolii</i> ³⁶⁷
49	Glut-5-en-3 β -ylacetate	C ₃₂ H ₅₂ O ₂	<i>E. royleana</i> ³⁶⁸
50	Handianol	C ₃₀ H ₅₀ O	<i>E. handiensis</i> ³⁶⁹
51	Hopenol-B	C ₃₀ H ₅₀ O	<i>E. supina</i> ³⁷⁰
52	Lanosterol	C ₃₀ H ₅₀ O	<i>E. latzi</i> ³⁷¹
53	Lanosteryl acetate	C ₃₂ H ₅₂ O ₂	<i>E. biglandulose</i> ³⁴⁷
54	Lupenone	C ₃₀ H ₄₈ O	<i>E. balsimifera</i> ³⁴⁹
55	Lupeol	C ₃₀ H ₅₀ O	<i>E. balsimifera</i> ³⁶⁴
56	Lupeol acetate	C ₃₂ H ₅₂ O ₂	<i>E. watandii</i> ³⁷²
57	Maculatol	C ₃₀ H ₅₀ O	<i>E. maculatta</i> ³⁷³
58	4-Methyl-cycloart-22-one-3 β ,25-diol	C ₂₉ H ₄₈ O ₂	<i>E. lactea</i> ³⁷⁴
59	24-Methylene-cycloartenol	C ₃₁ H ₅₂ O	<i>E. cyparissias</i> ³⁴⁴
60	Methyl-3-oxourcolate	C ₃₁ H ₄₈ O ₃	<i>E. caducifolia</i> ³⁵²
61	Moretenol	C ₃₀ H ₅₀ O	<i>E. laterifolia</i> ³⁷⁵
62	Moretenone	C ₃₀ H ₄₈ O	<i>E. laterifolia</i> ³⁷⁶
63	Nerifolol	C ₃₀ H ₅₀ O	<i>E. caltimondos</i> ³⁷⁵
64	Obtusifolol	C ₃₁ H ₅₂ O	<i>E. falcata</i> ³⁶¹
65	(<i>E,Z</i>)2,4-Octadienyl-cycloartenol	C ₃₈ H ₅₀ O ₂	<i>E. biglandulose</i> ³⁴⁷
66	(<i>E,Z</i>)-2,4-Octadienyl-tenosterol	C ₃₈ H ₆₀ O ₂	<i>E. biglandulose</i> ³⁴⁷

67	Olean-13(18)-en-3-one	C ₃₀ H ₄₈ O	<i>E. phosphora</i> ³⁷⁶
68	Oleanolic acid	C ₃₀ H ₄₈ O ₃	<i>E. paralias</i> ³⁷⁷
69	Spirosupinanonediol	C ₃₀ H ₅₀ O ₃	<i>E. supina</i> ³⁷⁸
70	Taraxerol	C ₃₀ H ₅₀ O	<i>E. hirta</i> ³⁶⁵
71	Taraxerone	C ₃₀ H ₄₈ O	<i>E. phosphora</i> ³⁷⁹
72	Taraxeryl acetate	C ₃₂ H ₅₂ O ₂	<i>E. royleana</i> ³⁶⁴
73	Taraxasterol	C ₃₀ H ₅₀ O	<i>E. watandii</i> ³⁸⁰
74	Taraxasterol acetate	C ₃₂ H ₅₂ O ₂	<i>E. granulata</i> ³⁸⁰
75	Tirucallol	C ₃₀ H ₅₀ O	<i>E. thymifolia</i> ³⁸¹
76	Uvaol	C ₃₀ H ₅₀ O ₂	<i>E. paralias</i> ³⁷⁷
77	Ursolic acid	C ₃₀ H ₄₈ O ₃	<i>E. paralias</i> ³⁷⁷

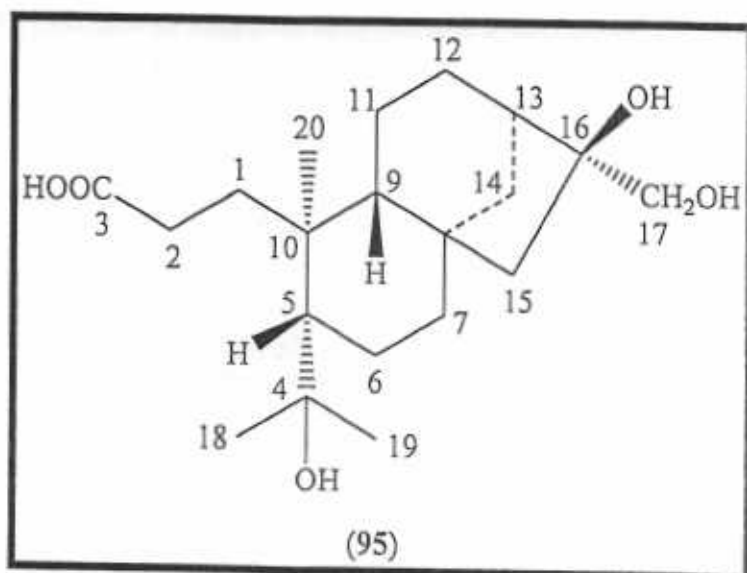
Chapter: 7

RESULTS AND DISCUSSION (Part-B)

This chapter describes our collaborative research work on the isolation, purification and structure elucidation of eight new diterpenoids (95-102)³⁸² and nine reported compounds (103-111) from the stem tissues of *Euphorbia neriifolia*. The stem tissues of the plant were shade dried under open air and then extracted with ethanol. The ethanolic extract was concentrated under vacuum. The combined extract was then fractionated into *n*-hexane, ethyl acetate and *n*-butanol fractions. The ethyl acetate fraction was subjected to a number of chromatographic techniques, resulting in the purification of compounds (95-111). The molecular structures of the purified compounds were determined with the help of advanced spectroscopic techniques.

7.1. Structure elucidation of new diterpenoids from *E. neriifolia*

7.1.1. *Ent*-3,4-seco-4,16 β ,17-trihydroxykauran-3-oic acid (95)



Compound **95** was obtained as colourless crystals. The molecular formula of **95** was deduced as $C_{20}H_{34}O_5$ from its HRESIMS ($[M+Na]^+$ m/z 377.2408; calcd 377.2404), indicating four degrees of unsaturation. The IR spectrum of **95** showed absorption bands of hydroxyl groups (3446 cm^{-1}) and a carboxylic acid (2929 and 1704 cm^{-1}).

The ^1H -NMR spectrum of **95** (Table-7.1) showed singlets for three tertiary methyls at δ_{H} 1.25, 1.30 and 1.18. The protons of the oxygenated methylene group appeared at δ_{H} 3.30 (d, $J = 10.5\text{ Hz}$, $\text{H}_a\text{-17}$) and 3.42 (d, $J = 10.5\text{ Hz}$, $\text{H}_b\text{-17}$). The ^{13}C -NMR (BB and DEPT) spectra (Table-7.1) indicated the presence of three methyls, nine methylenes (one oxygenated), three methines, and five quaternary carbons (one carboxyl acid). A comparison of the ^1H - and ^{13}C -NMR data (Table-7.1) of compound **95** with the known compound (5 β ,16 α)-4,16,17-trihydroxy-3,4-secohyalocladan-3-oic acid³⁸³ implied that they shared identical NMR spectral data. However the stereochemistry of **95** was exactly opposite to the reported compound which was confirmed through X-

rays single-crystal experiment (Figure-7.1). Also, the specific rotations were opposite i.e. $[\alpha]_D^{17}$ of **95** was equal to -49.0°C , while $[\alpha]_D^{26.7}$ of $(5\beta,16\alpha)$ -4,16,17-trihydroxy-3,4-secophyllocladan-3-oic acid reported as $+49.62^\circ\text{C}$.³⁸³ Thus, the structure of **95** was elucidated as *ent*-3,4-seco-4,16 β ,17-trihydroxykauran-3-oic acid.

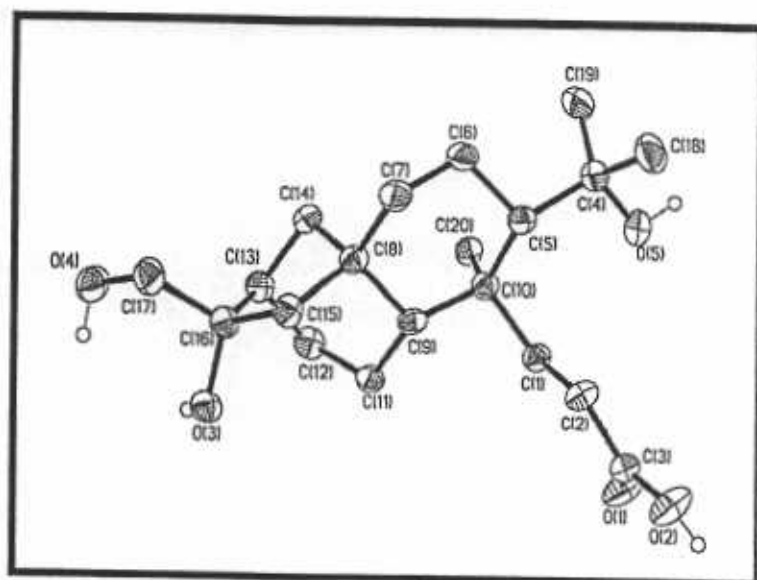
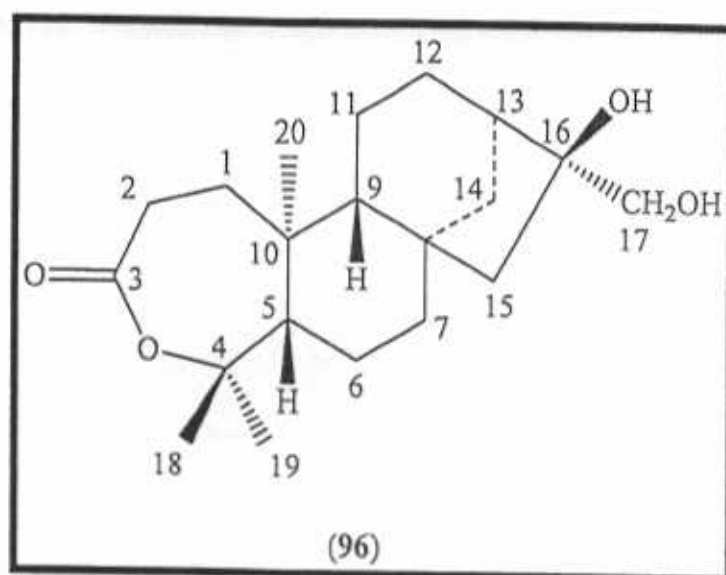


Figure-7.1: X-ray single crystal structure of compound (**95**)

Table-7.1 ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (95) in CD_3OD

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	35.5	CH_2	1.84, m 2.34, m
2	30.1	CH_2	2.16, m 2.42, m
3	179.1	C	-
4	76.2	C	-
5	52.6	CH	1.42, m
6	25.2	CH_2	1.52, m
7	42.2	CH_2	1.36, m
8	44.9	C	-
9	49.8	CH	1.29, m
10	43.7	C	-
11	19.8	CH_2	1.64, m 1.98, m
12	28.1	CH_2	1.82, m
13	42.1	CH	2.06, m
14	39.0	CH_2	1.18, m 1.94, m
15	52.9	CH_2	1.38, m
16	80.9	C	-
17	70.6	CH_2	3.30, d ($J = 10.5$ Hz) 3.42, d ($J = 10.5$ Hz)
18	28.4	CH_3	1.25, s
19	32.8	CH_3	1.30, s
20	22.6	CH_3	1.18, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.1.2. *Ent*-16 β -hydroxykauran-3,4-lactone (96)

The molecular formula of **96** was found to be $C_{20}H_{32}O_4$, based on HRESIMS ($[M+Na]^+$ m/z 359.2195; calcd 359.2198), indicating five degrees of unsaturation. The IR spectrum showed absorption peaks for hydroxyl groups (3486 and 3365 cm^{-1}) and a carbonyl group (1712 cm^{-1}).

The ^1H -NMR data (Table-7.2) of compound **96** consisted of proton resonances at δ_{H} 1.44, 1.36 and 1.20 (each 3H, s), for the three methoxyl groups at positions 18, 19 and 20, respectively. The protons of the hydroxymethylene group appeared at δ_{H} 3.45 and 3.36 (each 1H, d, $J = 10.8\text{ Hz}$). The protons of the methylenic group attached to the carbonyl group appeared at δ_{H} 2.61 and 2.45 (each 1H, ddd, $J = 13.8, 13.6, 4.8\text{ Hz}$).

The ^{13}C -NMR spectrum showed a total of twenty carbon signals which were classified into three methyls, nine methylenes, three methines and five quaternary carbons on the basis of DEPT spectral analysis. Three methyl groups resonated at δ_{C} 30.7 (C-18), 26.6 (C-19) and 20.5 (C-20). Furthermore, the NMR spectral data of **96** showed the presence of one carbonyl ester or acid (δ_{C} 175.4) and one CH_2OH group (δ_{H} 3.45, 1H, d, $J = 10.8\text{ Hz}$, H_a -17; 3.36, 1H, d, $J = 10.8\text{ Hz}$, H_b -17, δ_{C} 69.5). A

comparison of the ^1H - and ^{13}C -NMR data of **96** with those of **95** suggested that compound **96** was closely similar to **95**, except that compound **96** increased one degree of unsaturation and loss an eighteen molecular weight (H_2O molecule). In addition, the peak of C-4 (δ_{C} 86.0) was shifted downfield compared with the carbon signal at δ_{C} 76.2 in **95**, and the peak of C-3 (δ_{C} 175.4) was shifted upfield compared with the carbon at δ_{C} 179.1 in **95**. These revealed that compound **96** was a lactone between C-3 and C-4.

The HMBC NMR correlations (Figure-7.2) further confirmed the structural assignments. The existence of the lactone ring was confirmed from the HMBC cross peaks for H-5 to C-18 and C-19 and H-18 to C-5. Similarly, the protons of the oxygenated methylene at δ_{H} 3.45 and 3.36 showed HMBC correlations to C-13, C-15 and C-16.

The relative configuration of the hydroxymethylene was found to be α which was confirmed from the ROESY correlation (Figure-7.3) of H-13 to H-17, thus leaving a β -configuration for the OH group attached to C-16.

Therefore, based on the above arguments, compound **96** was characterized as *ent*-16 β -hydroxykauran-3,4-lactone.

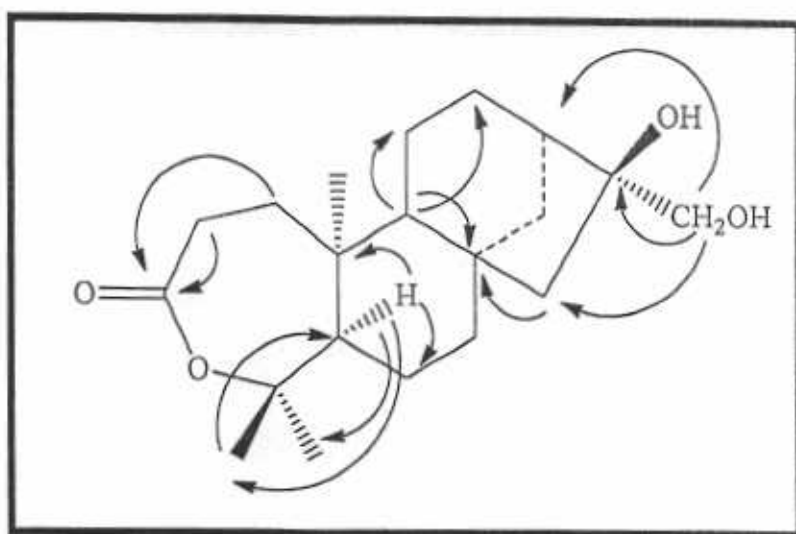


Figure-7.2: Selected HMBC correlations of compound (96)

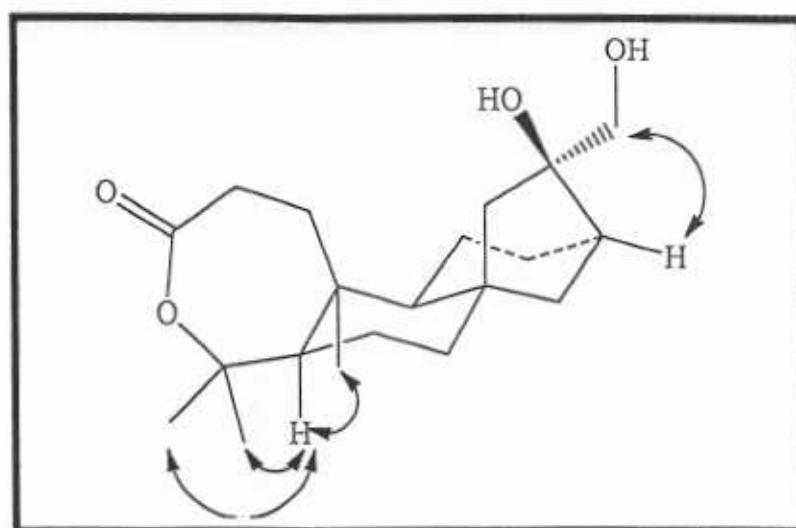


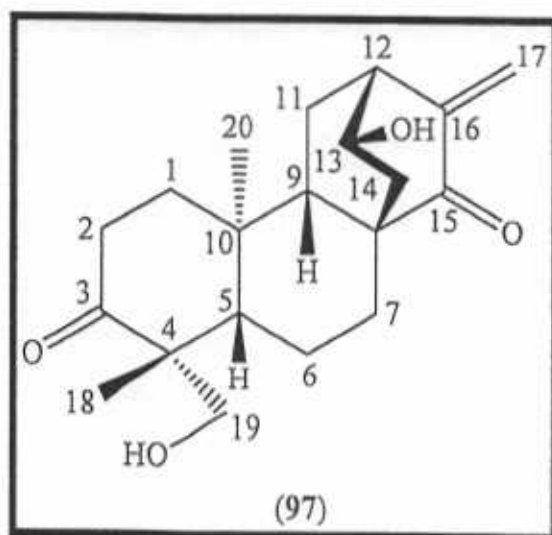
Figure-7.3: Selected ROESY correlations of compound (96)

Table-7.2: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (96) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	39.8	CH_2	1.57, m 1.87, m
2	32.3	CH_2	2.61, ddd ($J = 13.8$, 13.6, 4.8) 2.45, ddd ($J = 13.8$, 13.6, 4.8)
3	175.4	C	-
4	86.0	C	-
5	52.2	CH	1.79, dd ($J = 11.4$, 2.4)
6	24.9	CH_2	1.42, m
7	36.9	CH_2	1.88, m
8	43.3	C	-
9	56.4	CH	1.23, m
10	40.7	C	-
11	19.5	CH_2	1.52, m 2.02, m
12	26.4	CH_2	1.52, m 1.79, m
13	40.2	CH	2.09, m
14	39.9	CH_2	1.39, m 1.57, m
15	51.6	CH_2	1.38, m
16	79.4	C	-
17	69.5	CH_2	3.45, d ($J = 10.8$ Hz) 3.36, d ($J = 10.8$ Hz)
18	30.7	CH_3	1.44, s
19	26.6	CH_3	1.36, s
20	20.5	CH_3	1.20, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.1.3. 13 β ,19-Dihydroxy-3,15-dioxoatis-16-ene (97)



Compound **97** was isolated as colorless crystals. Its molecular formula was found as $C_{20}H_{28}O_4$ which was determined by HRESIMS ($[M + Na]^+$ m/z 355.1889; calcd 355.1885) and indicated seven degrees of unsaturation. The UV spectrum of **97** showed maximum absorption at 236 nm, indicating the presence of a conjugated double bond. IR spectrum revealed absorptions for hydroxyl groups (3544 and 3434 cm^{-1}), two carbonyl groups (1712 and 1673 cm^{-1}) and an olefin (1459 cm^{-1}).

The ^1H -NMR data (Table-7.3) of **97** exhibited proton signals for two methyls at δ_H 1.09 and 0.91, a hydroxymethylene at δ_H 3.42 (1H, d, $J = 11.4$ Hz, H_a -19) and 3.90 (1H, d, $J = 11.4$ Hz, H_b -19), a hydroxymethine at δ_H 3.83 (1H, d, $J = 3.0$ Hz, H -13) and a terminal olefinic methylene at δ_H 4.79 (1H, s, H_a -17) and 4.93 (1H, s, H_a -17).

In the ^{13}C -NMR (BB and DEPT) spectra of compound **97** (Table-7.3), twenty carbon resonances were observed which were assigned as two methyls, eight methylenes, four methines and six quaternary carbons. Six deshielded signals in the ^{13}C -NMR spectrum [δ_C 219.2 (C-15); 217.4 (C-3); 145.1 (C-16); 111.2 (C-17); 76.4 (C-13);

66.2 (C-19)] were assigned to two ketones, one terminal olefin, one oxygenated methine, and one oxygenated methylene. These observations indicated of the presence of four rings in 97. The comparison of ^{13}C -NMR data of 97 with those of the alboatisine A,³⁸⁴ showed it to be an atisane derivative.

The HMBC spectrum (Figure-7.4) of 97 showed correlations between H-1/C-3, H-2/C-3, Me-18/C-3, and between H-7/C-15, H-9/C-15, H-14/C-15, indicating that a ketone [δ_{C} 217.4 (C-3)] was located at C-3 and the other [δ_{C} 219.2 (C-15)] was located at C-15. Also, the correlations between H-5/C-19, Me-18/C-19, and H-11/C-13, H-13/C-16 proved that two hydroxyl groups were located at C-19 and C-13, respectively.

The relative stereochemistry of 97 was determined by X-ray single-crystal structure determination (Figure-7.5). Finally, the structure of 97 was identified as 13 β ,19-dihydroxy-3,15-dioxoatis-16-ene.

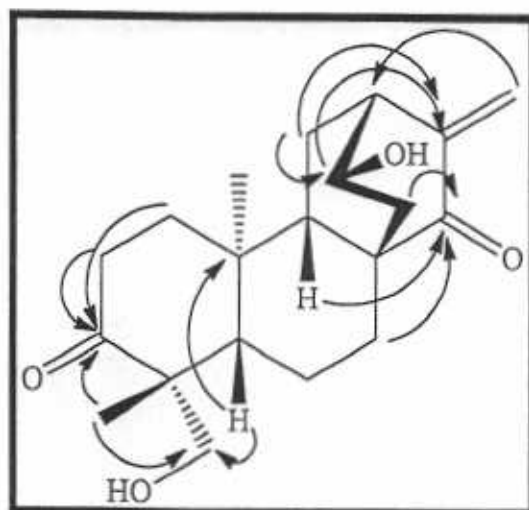


Figure-7.4: Selected HMBC correlations of compound (97)

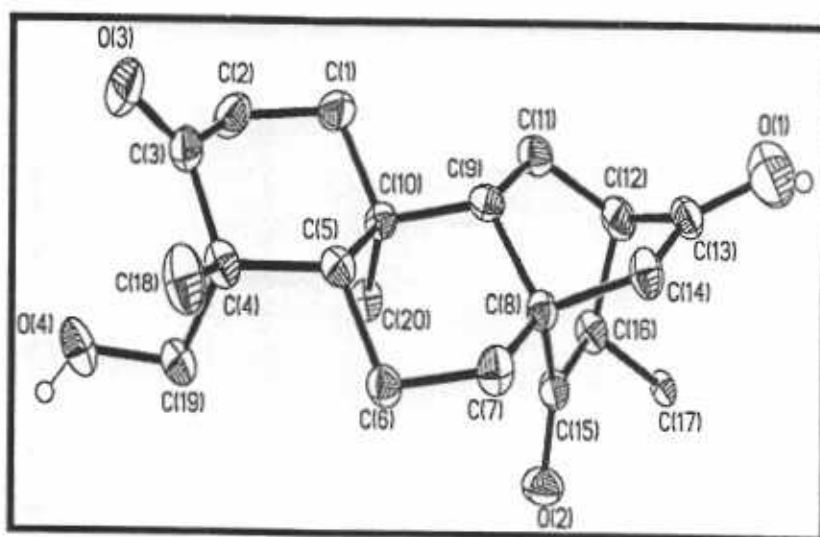
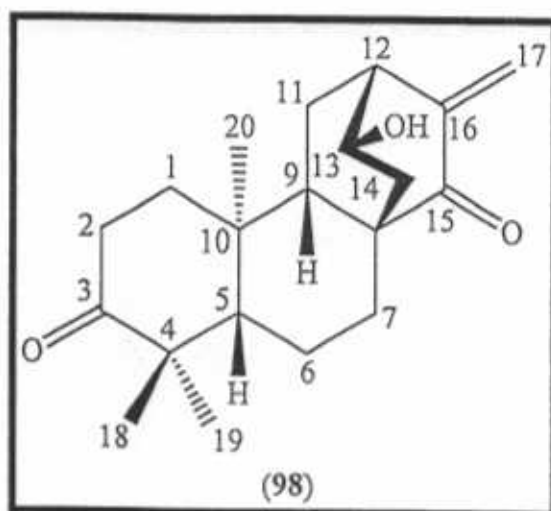


Figure-7.5: X-ray single crystal structure of compound (97)

Table-7.3: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (97) in CD_3OD

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	38.9	CH_2	1.38, m 1.92, m
2	36.6	CH_2	2.24, m 2.66, m
3	217.4	C	-
4	55.7	C	-
5	58.4	CH	1.40, m
6	21.5	CH_2	1.56, m
7	32.6	CH_2	0.71, m 2.32, m
8	48.7	C	-
9	52.9	CH	1.67, dd ($J = 11.2, 7.6$)
10	39.2	C	-
11	27.4	CH_2	1.81, m
12	47.6	CH_2	2.67, dd ($J = 11.7, 4.2$)
13	76.4	CH	3.83, d ($J = 3.0$)
14	44.6	CH_2	2.28, m
15	219.2	C	-
16	145.1	C	-
17	111.2	CH_2	4.79, s 4.93, s
18	21.5	CH_3	1.09, s
19	66.2	CH_2	3.42, d ($J = 11.4$) 3.90, d ($J = 11.4$)
20	15.0	CH_3	0.91, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.1.4. 13 β -Hydroxy-3,15-dioxoatis-16-ene (98)

Compound 98 was also obtained as colorless crystals and the molecular formula ($C_{20}H_{28}O_3$) was determined by HRESIMS ($[M + Na]^+ m/z$ 339.1940; calcd 339.1936). UV spectrum displayed maximum absorption at 235 nm, indicating the presence of a conjugated double bond. The IR spectrum consisted of absorptions for a hydroxyl group (3432 cm^{-1}), two carbonyl groups (1726 and 1697 cm^{-1}) and an olefin (1367 cm^{-1}).

The ^1H -NMR data (Table-7.4) of 98 showed similar proton signals as that of compound 97. The singlets of three methyls were observed at δ_H 1.08, 1.0 and 0.84 as compared to compound 97, which showed signals for only two methyl groups. Furthermore, the signals for a hydroxymethine at δ_H 3.84 (1H, d, $J = 3.0\text{ Hz}$, H-13) and a terminal olefinic methylene at δ_H 4.93 (1H, s, H_a -17) and 5.02 (1H, s, H_b -17) were also observed.

The ^{13}C -NMR (BB and DEPT) spectra (Table-7.4) of 98 showed twenty carbon atoms, which consisted of three methyls, seven methylenes, four methines and six

quaternary carbons. The ^{13}C -NMR spectrum of **98** showed carbon signals for two ketones at δ_{C} 216.3 (C-3) and 218.2 (C-15), one terminal olefin at δ_{C} 142.5 (C-16) and 111.3 (C-17) and one oxygenated methine at δ_{C} 75.3 (C-13). A comparison of the spectroscopic data of **98** with those of **97**, compound **98** was found very similar to compound **97**, except that the hydroxymethylene at C-19 (δ_{C} 66.2) in **97** was replaced by a methyl (δ_{C} 26.4) in **98**. All the assignments were confirmed through 2D-techniques such as HSQC and HMBC (Figure-7.6). Based on the spectral data, the structure of **98** was characterized as 13 β -hydroxy-3,15-dioxoatis-16-ene.

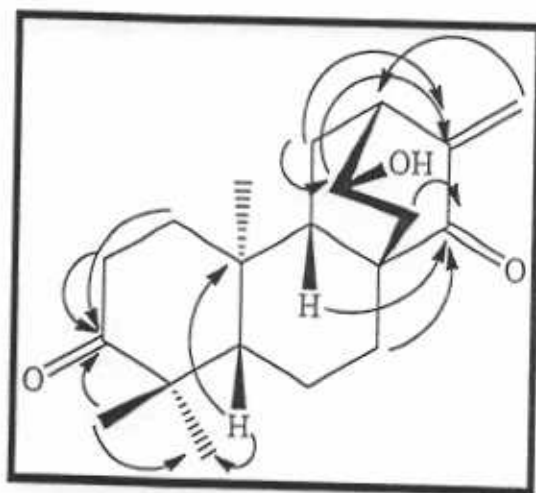
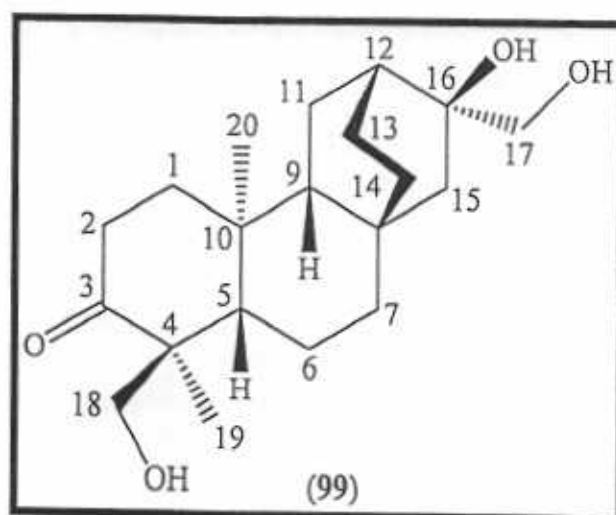


Figure-7.6: Selected HMBC correlations of compound (**98**)

Table-7.4: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (98) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	36.9	CH_2	1.32, m
2	34.3	CH_2	2.01, m
3	216.3	C	2.32, m
4	47.7	C	2.58, m
5	55.3	CH	-
6	20.2	CH_2	1.50, m
7	30.6	CH_2	1.62, m
8	47.5	C	0.87, m
9	51.3	CH	2.42, m
10	37.8	C	-
11	25.6	CH_2	1.74, dd ($J = 11.2, 7.6$)
12	45.0	CH_2	-
13	75.3	CH	1.84, m
14	43.9	CH_2	2.80, m
15	218.2	C	3.84, d ($J = 3.0$)
16	142.5	C	2.30, m
17	111.3	CH_2	-
18	22.1	CH_3	4.93, s
19	26.4	CH_3	5.02, s
20	13.9	CH_3	1.08, s
			0.84, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.1.5. 16 β ,17,19-Trihydroxy-3-oxo-atisane (99)

Compound **99** was isolated as a white powder and its formula was assigned as $C_{20}H_{32}O_4$ by HRESIMS ($[M + Na]^+ m/z$ 359.2193; calcd 359.2198). The IR spectrum showed absorption peaks of hydroxyl group at 3409 cm^{-1} and carbonyl group at 1704 cm^{-1} .

The $^1\text{H-NMR}$ spectrum (Table-7.5) of **99** displayed the presence of two methyl groups singlets at δ_H 1.12 and 1.24, a hydroxymethylene group at δ_H 3.48 (1H, d, $J = 10.8\text{ Hz}$, H_a -17) and 3.52 (1H, d, $J = 10.8\text{ Hz}$, H_b -17) and a second hydroxymethylene at δ_H 3.34 (1H, d, $J = 11.4\text{ Hz}$, H_a -18) and 4.04 (1H, d, $J = 11.4\text{ Hz}$, H_b -18).

The $^{13}\text{C-NMR}$ (BB and DEPT) spectra of compound **99** (Table-7.5), indicated the presence of two methyl, ten methylene, three methine and five quaternary carbon atoms. The methyl groups resonated at δ_C 20.6 and 14.8 and the presence of the two hydroxymethylene carbons was confirmed from their carbon shift values at δ_C 65.8 (C-17) and 69.7 (C-18). The downfield shift value at δ_C 217.8, established the presence of the ketonic carbon (C-3) in the molecule.

The NMR data of **99** was close to that of the atisane diterpene, *ent*-16 α ,17-dihydroxy-3-one (**103**),³⁸⁵ except for the presence of the hydroxymethylene group (C-18) in **99** instead of the methyl group at δ_{H} 1.03 in **103**. All the NMR assignments in compound **99** were verified with the help of COSY, HSQC and HMBC (Figure 7.7) NMR experiments. Thus, compound **99** was unambiguously assigned as 16 β ,17,19-trihydroxy-3-oxo-atisane.

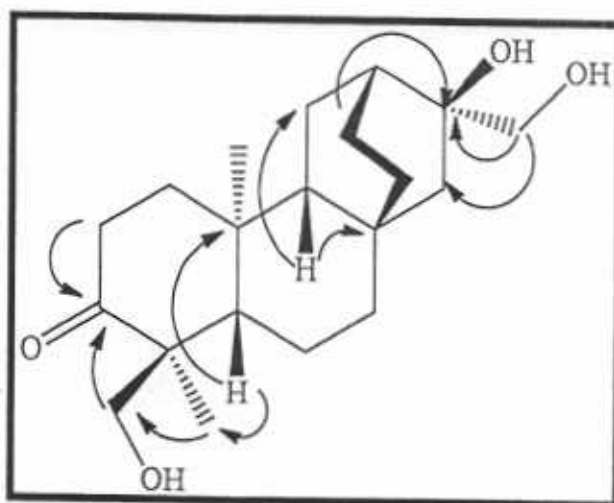
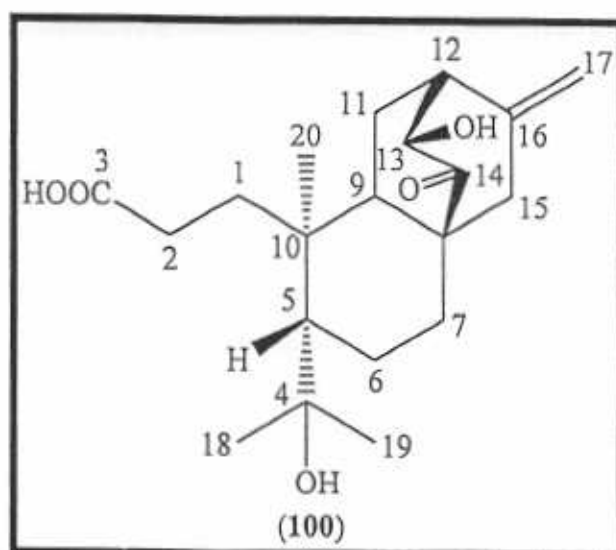


Figure-7.7: Selected HMBC correlations of compound (**99**)

Table-7.5: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (99) in CD_3OD

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	40.4	CH_2	1.32, m 1.96, m
2	36.0	CH_2	2.24, m 2.78, m
3	217.8	C	-
4	55.9	C	-
5	59.3	CH	1.34, m
6	20.9	CH_2	1.54, m
7	40.8	CH_2	1.12, m 1.42, m
8	33.9	C	-
9	52.8	CH	1.35, m
10	38.6	C	-
11	24.4	CH_2	1.48, m 1.66, m
12	33.2	CH	1.82, m
13	24.6	CH_2	1.22, m 2.06, m
14	28.5	CH_2	1.88, m
15	53.3	CH_2	1.01, dd ($J = 9.9, 3.3$) 1.34, m
16	75.2	C	-
17	65.8	CH_2	3.48, d ($J = 10.8$) 3.52, d ($J = 10.8$)
18	69.7	CH_2	3.34, d ($J = 11.4$) 4.04, d ($J = 11.4$)
19	20.6	CH_3	1.12, s
20	14.8	CH_3	1.24, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.1.6. 4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid (100)

Compound **100** was obtained as a white powder and its molecular formula was determined as $C_{20}H_{30}O_5$ by HRESIMS ($[M + Na]^+ m/z$ 373.2011; calcd 373.1991). The IR spectrum of **100** displayed absorption bands for hydroxyl groups (3423 cm^{-1}), a carboxylic acid (2939 and 1720 cm^{-1}) and an olefin (1438 cm^{-1}).

The ^1H -NMR data (Table-7.6) of compound **100** showed singlet peaks of three methyls at δ_{H} 1.27, 1.17 and 0.80, an olefinic methylene at δ_{H} 4.83 (1H, s, $H_{\text{a}}-17$) and 5.00 (1H, s, $H_{\text{b}}-17$) and a hydroxymethine at δ_{H} 3.84 (1H, s, H-13). The chemical shift values at δ_{H} 1.40 (1H, m) and 2.78 (1H, d, $J = 2.4\text{ Hz}$) were assigned to H-5 and H-12, respectively, on the basis of HSQC cross peaks for these protons to their corresponding carbon atoms.

The ^{13}C -NMR (BB and DEPT) spectra (Table-7.6) exhibited twenty carbon signals which were identified as three methyls, seven methylenes, four methines and six quaternary carbons. The upfield chemical shift values at δ_{C} 34.2, 27.5 and 17.9 were assigned to Me-18, Me-19 and Me-20, respectively. The olefinic methylene and

oxygenated methine were observed at δ_C 110.8 and 74.9, respectively. Similarly, the ketonic and carboxylic acid functions were identified by chemical shift values at δ_C 218.5 and 179.4, respectively.

The NMR data of 100 were comparable to those of antiquorin,³⁸⁶ except that the carbonyl group at δ_C 216.0 and a quaternary C-atom at δ_C 47.5 in antiquorin³⁸⁶ were replaced by the carboxylic carbonyl group at δ_C 179.4 and the oxygenated quaternary C-atom at δ_C 75.8 in 100. As no other unsaturated functions except for the COOH group (δ_C 179.4) appeared in the ^1H - and ^{13}C -NMR spectra, 100 should have a tricyclic structure, which means that ring A was cleaved. In the HMBC spectrum (Figure-7.8), cross peaks were observed between H-1, H-2 and COOH at δ_C 179.4, and between H-5 and the oxygenated quaternary C-atom at δ_C 75.8. These indicated that ring A is open between C-3 and C-4, C-3 being oxidized to the COOH group, and C-4 being the oxygenated quaternary C-atom. ROESY correlations (Figure-7.9) between H-9/H-5, as well as Me-20/Me-18 Me-20/Me-19 established the β -configuration of H-5 and the α -configuration of Me-20. Thus, compound 100 was assigned as 4,13 β -dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid.

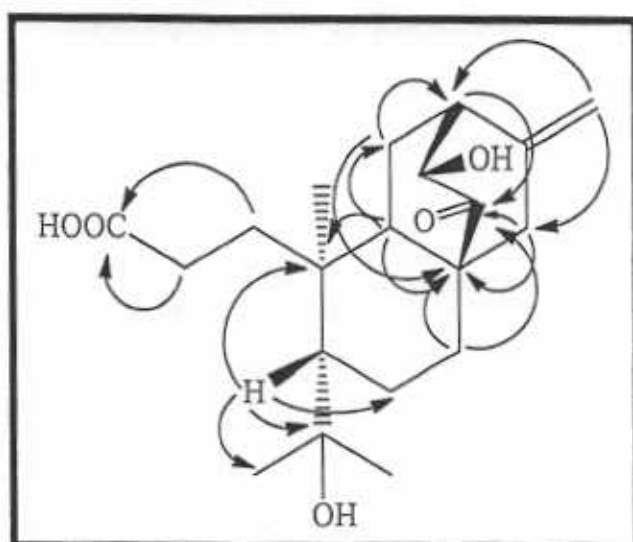


Figure-7.8: Selected HMBC correlations of compound (100)

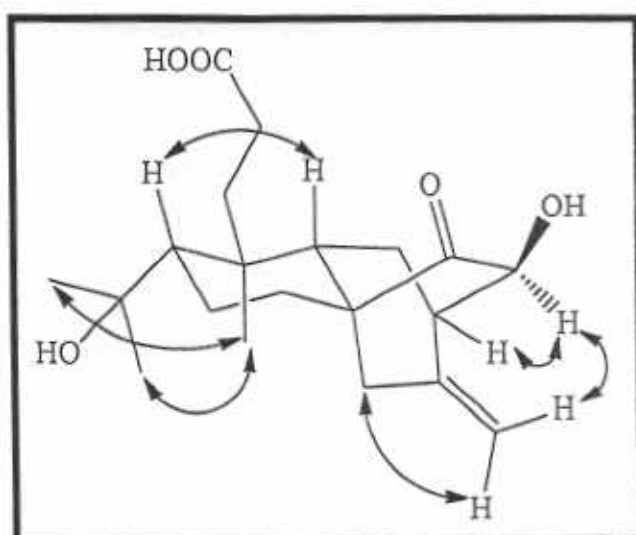


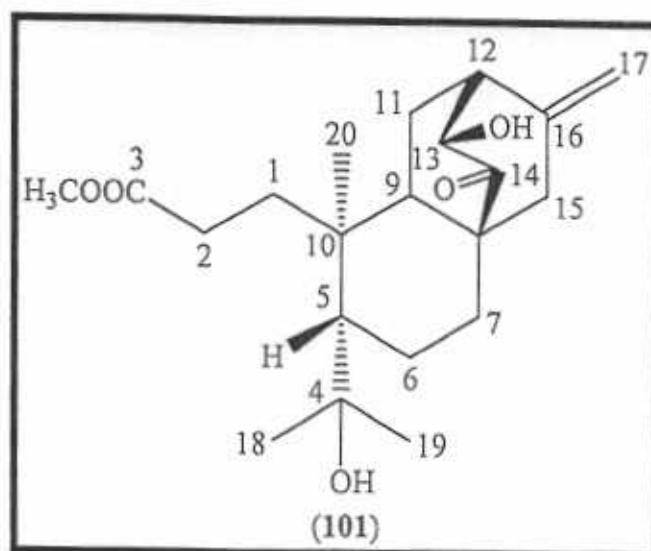
Figure-7.9: ROESY correlations of compound (100)

Table-7.6: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (100) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	32.3	CH_2	1.56, m 2.36, m
2	28.7	CH_2	2.21, m 2.60, m
3	179.4	C	-
4	75.8	C	-
5	51.2	CH	1.40, m
6	22.7	CH_2	1.49, m
7	30.4	CH_2	2.23, m
8	47.2	C	-
9	44.9	CH	1.74, m
10	41.3	C	-
11	25.0	CH_2	1.78, m 1.90, m
12	44.8	CH	2.78, d ($J = 2.4$)
13	74.9	CH	3.84, s
14	218.6	C	-
15	43.7	CH_2	2.28, s
16	142.2	C	-
17	110.8	CH_2	4.83, s 5.00, s
18	34.2	CH_3	1.27, s
19	27.5	CH_3	1.19, s
20	17.9	CH_3	0.80, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.1.7. 4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid methyl ester (101)



Compound **101** was obtained as a white powder and its molecular formula was determined as $C_{21}H_{32}O_5$ by HRESIMS ($[M + Na]^+ m/z$ 387.2150; calcd 387.2147). IR spectrum exhibited absorptions peaks at 3496 cm^{-1} (hydroxyl group), 1724 cm^{-1} (carbonyl group) and 1438 cm^{-1} (olefin).

The ^1H -NMR spectrum (Table-7.7) of **101** indicated the presence of four methyl groups at δ_{H} 1.26, 1.17, 0.80 (each 3H, s, Me-18, 19 and 20, respectively) and 3.64 (3H, s, CH_3O -3). The protons of the olefinic methylene were observed at δ_{H} 4.82 (1H, s, H_a -17) and 5.00 (1H, s, H_b -17), while that of oxygenated methine at a shift value of δ_{H} 3.84 (1H, d, $J = 2.7\text{ Hz}$).

The ^{13}C -NMR (BB and DEPT) spectra (Table-7.7) of **101** showed the presence of the carbons of four methyl groups at δ_{C} 34.5 (Me-18), 27.9 (Me-19), 18.1 (Me-20) and 51.8 (oxygenated). The presence of olefinic methylene carbon was evidenced at δ_{C} 110.8 (C-17), whereas the ester and ketonic carbonyls were observed at δ_{C} 175.2 (C-3) and 218.5 (C-14), respectively.

In comparison of its ^1H - and ^{13}C -NMR data with the spectral data of **100** indicated them to be similar, except that compound **101** had an additional methoxyl group at δ_{C} 51.8, and deduced the $-\text{COOCH}_3$ at C-2. All the NMR assignments were also confirmed by using 2D-techniques such as COSY, HSQC and HMBC (Figure- 7.10). Thus, the structure of **101** was assigned as 4,13 β -dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid methyl ester.

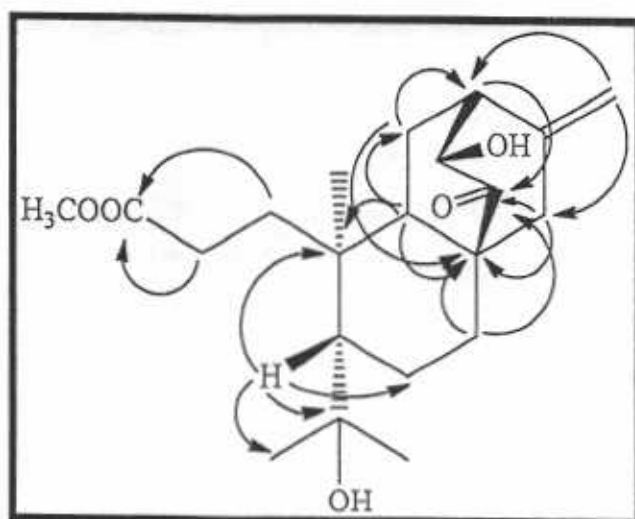
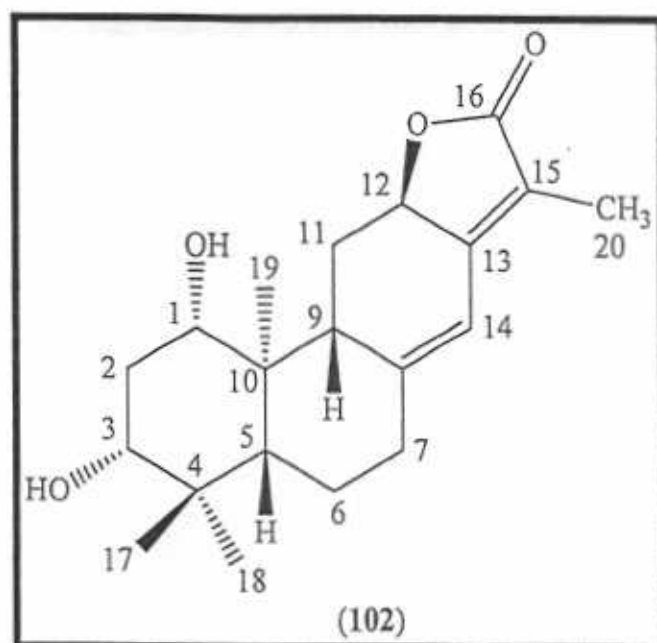


Figure-7.10: Selected HMBC correlations of compound (**101**)

Table-7.7: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (101) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	32.8	CH_2	1.56, m 2.36, m
2	28.9	CH_2	2.21, m 2.60, m
3	175.2	C	-
3-OCH ₃	51.8	CH_3	3.64, s
4	75.7	C	-
5	51.4	CH	1.40, m
6	23.1	CH_2	1.49, m
7	30.6	CH_2	2.23, m
8	47.4	C	-
9	45.0	CH	1.74, m
10	41.6	C	-
11	25.3	CH_2	1.78, m 1.90, m
12	45.2	CH	2.78, d ($J = 2.4$)
13	75.2	CH	3.84, d ($J = 2.7$)
14	218.5	C	-
15	44.1	CH_2	2.28, s
16	142.5	C	-
17	110.9	CH_2	4.82, s 5.00, s
18	34.5	CH_3	1.26, s
19	27.9	CH_3	1.17, s
20	18.1	CH_3	0.80, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.1.8. 1 α ,3 α -Dihydroxy-*ent*-abieta-8(14),13(15)-dien-16,12-olide (102)

Compound **102** was obtained as a white powder. Its molecular formula was established as $C_{20}H_{28}O_4$ by HRESIMS ($[M + Na]^+$ m/z 355.1881; calcd 355.1885). The UV spectrum showed absorption maximum at 291 nm, indicating the presence of conjugated double bonds in the molecule. The IR spectrum of **102** showed absorption bands of hydroxyl groups (3434 cm^{-1}), a carbonyl group (1727 cm^{-1}) and two carbon-carbon double bonds (1662 cm^{-1} and 1608 cm^{-1}).

Four tertiary methyl singlets at δ_H 1.89 (CH_3 -17), 1.24 (CH_3 -18), 1.23 (CH_3 -19) and 1.09 (CH_3 -20) were observed in the ^1H -NMR spectrum (Table-7.8) of **102**. The oxygenated methines resonated at δ_H 3.85 (1H, dd, $J = 11.1, 3.9\text{ Hz}$, H-1), 3.63 (1H, dd, $J = 12.0, 3.9\text{ Hz}$, H-3) and 5.23 (1H, dd, $J = 13.1, 4.5\text{ Hz}$, H-12) and an olefinic proton appeared at δ_H 6.30 (1H, s, H-14).

Twenty carbon resonances ($6 \times C$; $6 \times CH$; $4 \times CH_2$; $4 \times CH_3$) appeared in the ^{13}C NMR spectrum (Table 7.8). Eight signals in the ^{13}C NMR spectrum [δ_C 175.3 (C-16);

152.9 (C-8); 157.2 (C-13); 115.9 (C-14); 114.2 (C-15); 75.4 (C-1); 74.9 (C-3); 76.4 (C-12)] were assigned to one carbonyl group, two olefins, and three oxygenated CH groups. Comparison of the NMR data and molecular weight of **102** with those of the reported compound helioscopinolide A,³⁸⁷ indicated a similarity of spectral data, except for the presence of an additional hydroxyl group in **102**. The location of the additional hydroxyl group was assigned by HMBC correlations (Figure-7.11) and ROESY correlations at C-1 (Figure-7.12). The 1-OH was α -oriented on the basis of the ROESY correlations of H-1/H-9 and H-1/H-5, which was also supported by the coupling constants of H-1 ($J = 11.1, 3.9$ Hz).³⁸⁸ 3-OH was also α -oriented by the ROESY correlation of H-1/H-3 and H-5/H-3 (Figure-7.12) and the coupling constants of H-3 ($J = 12.0, 3.9$ Hz). Therefore, compound **102** was determined as 1 α ,3 α -dihydroxy-*ent*-abieta-8(14),13(15)-dien-16,12-olide.

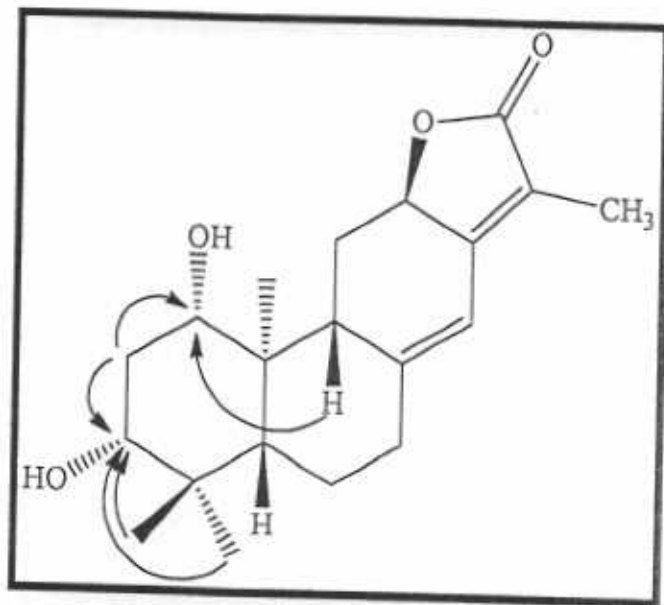


Figure-7.11: Selected HMBC correlations of compound (**102**)

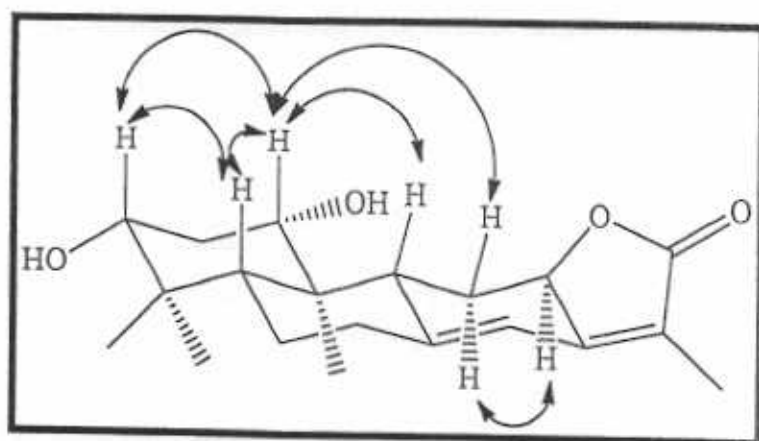


Figure-7.12: ROESY correlations of compound (102)

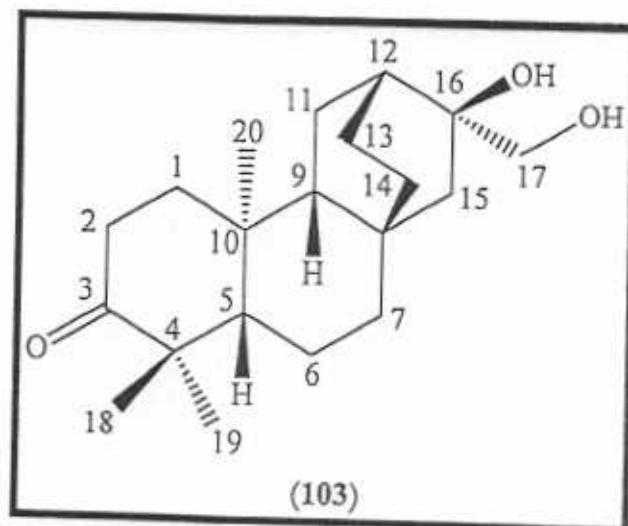
Table-7.8: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (102) in CD_3N

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	75.4	CH	3.85, dd ($J = 11.1, 3.9$)
2	39.4	CH ₂	2.27, m 2.42, m
3	74.9	CH	3.63, dd ($J = 12.0, 3.9$)
4	39.7	C	-
5	53.2	CH	1.16, dd ($J = 13.2, 2.4$)
6	23.5	CH ₂	1.82, m
7	37.0	CH ₂	2.14, ddd ($J = 14.4, 14.4, 5.1$) 2.44, m
8	152.9	C	-
9	52.8	CH	2.38, dd ($J = 11.7, 4.2$)
10	47.7	C	-
11	31.0	CH ₂	1.56, m 4.34, dd ($J = 12.3, 5.7$) 5.23, dd ($J = 13.1, 4.5$)
12	76.4	CH	-
13	157.2	C	-
14	115.9	CH	6.30, s
15	114.2	C	-
16	175.3	C	-
17	29.0	CH ₃	1.89, s
18	11.7	CH ₃	1.24, s
19	16.0	CH ₃	1.23, s
20	8.4	CH ₃	1.09, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.2. Known compounds isolated from *E. neriifolia*

7.2.1. *Ent*-16 α ,17-Dihydroxyatisan-3-one (103)



Compound **103** was separated as a white solid from the ethyl acetate fraction. The molecular formula of this compound was developed as $C_{20}H_{32}O_3$ by ^1H -, ^{13}C -NMR and ESIMS analysis which gave a molecular ion adduct at m/z 343.3 $[M + Na]^+$ in positive mode.

The NMR data (Table-7.9) of compound **103** was found identical to the known compound *ent*-16 α ,17-dihydroxyatisan-3-one.³⁸⁵ Important NMR assignments of **103** include three methyl protons singlets at δ_H 1.10, 1.06 and 1.03, doublets of the hydroxymethylene protons at δ_H 3.42 and 3.56 ($J = 10.8$ Hz, H_a -17 and H_b -17, respectively) and multiplets of three methines protons at δ_H 1.40 (H-5), 1.66 (H-9) and 1.86 (H-12). In ^{13}C -NMR, signals at δ_C 26.4, 21.8 and 13.7, were assigned to the three methyl groups. The ketone and hydroxymethylene carbons were found at carbon shift values of δ_C 217.7 and 69.2, respectively. All other spectral assignments were in agreement to *ent*-16 α ,17-dihydroxyatisan-3-one.³⁸⁵

Table-7.9: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (103) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	38.2	CH_2	1.32, m
2	34.2	CH_2	1.97, m
3	217.7	C	2.23, m
4	47.8	C	2.58, m
5	55.8	CH	-
6	19.8	CH_2	1.40, m
7	38.9	CH_2	1.56, m
8	33.1	C	0.78, m
9	51.1	CH	2.35, m
10	37.4	C	-
11	23.2	CH_2	1.66, m
12	32.3	CH	-
13	23.4	CH_2	1.81, m
14	27.4	CH_2	1.86, m
15	52.6	CH_2	1.24, m
16	74.3	C	2.05, m
17	69.2	CH_2	1.88, m
18	26.4	CH_3	0.98, m
19	21.8	CH_3	1.36, m
20	13.7	CH_3	-
			3.42, d ($J = 10.8$)
			3.56, d ($J = 10.8$)
			1.10, s
			1.06, s
			1.03, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.2.2. (16*R*)-16,17-Dihydroxy-*ent*-kauran-3-one (104)

Compound 104 was isolated from the ethyl acetate fraction as white powder. Its molecular formula ($\text{C}_{20}\text{H}_{32}\text{O}_3$) of was established by using NMR and ESIMS analysis (molecular ion adduct $[\text{M} + \text{Na}]^+$ at 343.3 in positive mode).

The ^1H -NMR spectrum (Table-7.10) of 104, displayed proton signals for three methyl groups at δ_{H} 1.06 (6H, s, CH_3 -18 and CH_3 -20) and 1.02 (3H, s, CH_3 -19). An AB pair of doublets ($J = 11.4$ Hz) was observed at δ_{H} 3.65 (H_a -17) and 3.77 (H_b -17).

The ^{13}C -NMR spectrum (Table-7.10) possessed twenty carbon signals. DEPT spectral analysis was used to characterize the twenty carbon signals, thus showing the presence of three methyls, nine methylenes, three methines and five quaternary carbons. The methyl groups were observed to resonate at δ_{C} 27.4, 21.1 and 18.0. The presence of the ketonic function in the molecule was detected at carbon shift value of δ_{C} 218.5 (C-3), whereas the hydroxymethylene carbon at δ_{C} 66.4 (C-17). The ^1H - and ^{13}C -NMR spectral data of **104** well matched with the reported compound, (16*R*)-16,17-dihydroxy-*ent*-kauran-3-one or abbeokutone.³⁸⁹

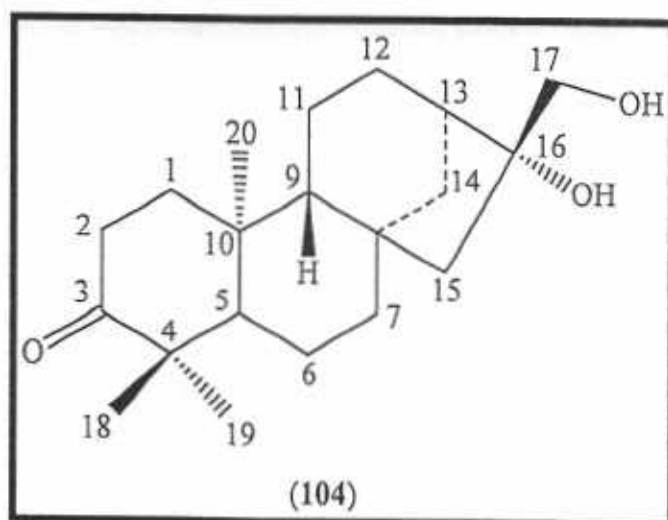


Table-7.10: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (104) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	39.4	CH_2	1.34, m 1.95, m
2	34.2	CH_2	2.46, dd ($J = 8.4, 6.3$)
3	218.5	C	-
4	47.3	C	-
5	54.4	CH	1.38, m
6	21.8	CH_2	1.42, m 1.49, m
7	41.0	CH_2	1.47, m 1.63, m
8	44.6	C	-
9	55.5	CH	1.08, m
10	38.6	C	-
11	19.0	CH_2	1.57, m
12	26.2	CH_2	1.50, m 1.60, m
13	45.4	CH	2.05, m
14	37.0	CH_2	1.61, m 1.87, m
15	52.8	CH_2	1.39, m 1.52, m
16	81.9	C	-
17	66.4	CH_2	3.65, d ($J = 11.4$) 3.77, d ($J = 11.4$)
18	27.4	CH_3	1.06, s
19	21.1	CH_3	1.02, s
20	18.0	CH_3	1.06, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.2.3. Ingenol (105)

Compound **105** was obtained as white solid from the ethyl acetate fraction. Molecular formula of this compound was found as $\text{C}_{20}\text{H}_{28}\text{O}_5$ with the help of ^1H -, ^{13}C -NMR and ESIMS analysis, where molecular ion adducts were detected at m/z 371.2 $[\text{M} + \text{Na}]^+$ in positive mode and at m/z 347.1 $[\text{M}-1]^-$ in negative mode. The ESIMS showed molecular ion peak at m/z 371.2 $[\text{M} + \text{Na}]^+$ in positive mode and at m/z 347.1 $[\text{M}-1]^-$

in negative mode. Corresponding to the mass (348 a.m.u), the molecular formula of **105** was deduced as $C_{20}H_{28}O_5$ through NMR spectral data.

From NMR data (Table-7.11) comparison, it was clearly revealed that compound **105** was similar to the well-known diterpene named ingenol³⁹⁰. Protons of the four methyls, one hydroxymethylene, two hydroxymethines and two olefins were found to resonate at [δ_H 1.05 (3H, s, CH₃-16), 1.11 (3H, s, CH₃-17), 0.93 (3H, d, J = 6.9 Hz, CH₃-18), 1.82 (3H, s, CH₃-19)], [δ_H 4.14 (2H, br.d, CH₂-20)], [δ_H 4.33 (1H, br.s, H-3), 3.78 (1H, br.s, H-5)] and [δ_H 5.85 (1H, br.s, H-1), 6.02 (1H, br.d, J = 4.2 Hz, H-7)], respectively. ¹³C-NMR spectrum (Table-7.11) of **105** showed signals for twenty carbon atoms. Amongst them were four methyls, two methylenes, eight methines and six quaternary carbons, based on DEPT spectral analysis. Carbon shift values at δ_C 28.7, 15.6, 17.7 and 15.7 were assigned to the four methyl carbons, whereas the shift values at δ_C 66.8, 80.2 and 75.1 belonged to one hydroxymethylene (C-20) and two hydroxymethines (C-3 and C-5), respectively. Two downfield carbon signals at δ_C 84.4 and 208.3 were observed due to an oxygenated quaternary carbon and a ketone carbonyl carbon, respectively.

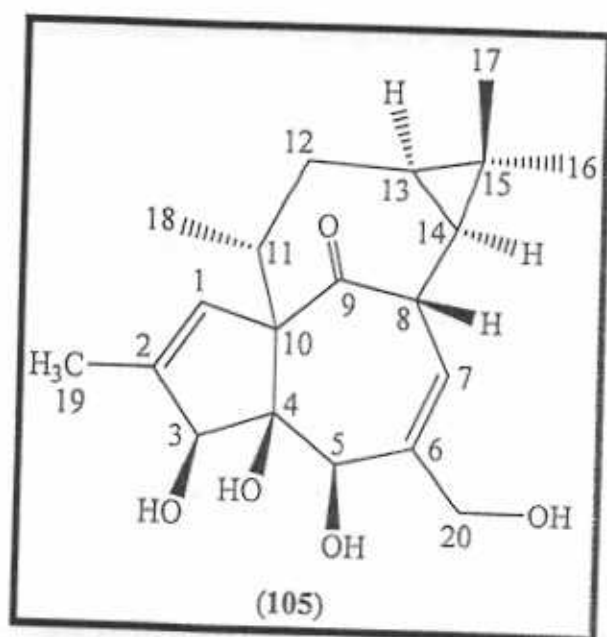


Table-7.11: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (105) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	129.2	CH	5.85, br.s
2	140.6	C	-
3	80.2	CH	4.33, br.s
4	84.4	C	-
5	75.1	CH	3.78, br.s
6	139.4	C	-
7	127.7	CH	6.02, br.d ($J = 4.2$)
8	44.2	CH	4.10, br.dd ($J = 4.2$)
9	208.3	C	-
10	72.8	C	-
11	39.5	CH	2.35, m
12	31.2	CH_2	2.24, m
13	23.5	CH	0.69, dd ($J = 15.0, 8.1$)
14	23.0	CH	0.88, m
15	24.0	C	-
16	28.7	CH_3	1.05, s
17	15.6	CH_3	1.11, s
18	17.7	CH_3	0.93, d ($J = 6.9$)
19	15.7	CH_3	1.82, s
20	66.8	CH_2	4.14, br.d

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.2.4. (23E)-Eupha-8,23-diene-3 β ,25-diol-7,11-dione (106)

Compound 106 was also obtained from the ethyl acetate fraction as white powder. The molecular formula $\text{C}_{30}\text{H}_{46}\text{O}_4$ was determined through NMR spectral analysis and ESIMS ($[\text{M} + \text{Na}]^+$ at 493.4 in positive mode).

The ^1H -NMR data (Table-7.12) of 106 exhibited resonances for the protons of a double bond at δ_{H} 5.59 (2H, m, overlapped), a secondary alcohol methine at δ_{H} 3.31 (1H, dd, $J = 10.5, 5.6$ Hz, H-3), a secondary methyl at δ_{H} 0.86 (3H, d, $J = 6.7$ Hz, CH_3 -21) and seven tertiary methyls at δ_{H} 0.91, 0.94, 1.02, 1.06, 1.31, 1.31 and 1.32 (each, 3H, s). The ^{13}C -NMR (BB and DEPT) spectra (Table-7.12) of 106 showed thirty carbon signals, which were divided into eight methyls, seven methylenes, six

methines and nine quaternary carbons atoms. The carbons of the disubstituted double bond appeared at δ_c 125.0 (C-23) and 140.0 (C-24), whereas the carbons involved in the formation of two conjugated ketones resonated at δ_c 200.1 (C-7), 149.9 (C-8), 154.9 (C-9) and 202.0 (C-11). In comparison of NMR spectral data, 106 was identified to be similar to the reported compound, (23*E*)-eupha-8,23-diene-3 β ,25-diol-7,11-dione.³⁹¹

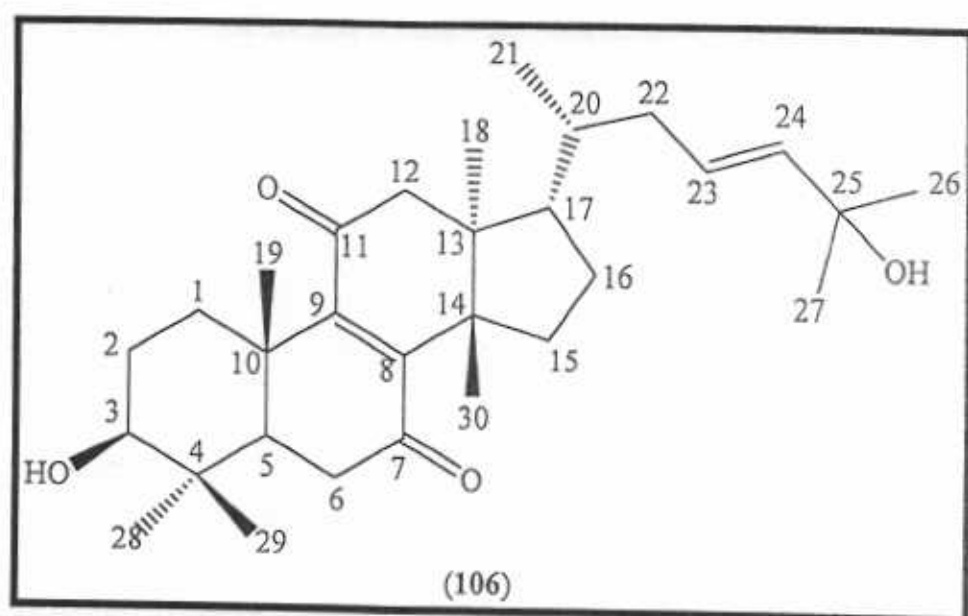
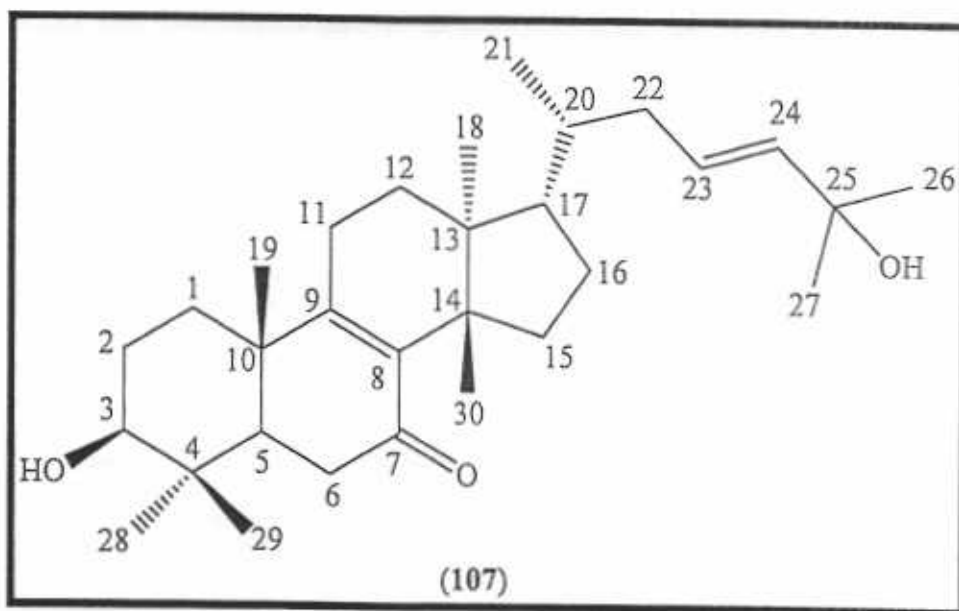


Table-7.12: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (106) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1a	34.0	CH_2	1.10, m
1b			2.55, m
2a	27.5	CH_2	1.76, m
2b			1.68, m
3	78.0	CH	3.31, dd ($J = 10.5, 5.6$)
4	38.7	C	-
5	48.6	CH	1.63, m
6a	35.9	CH_2	2.43, m
6b			2.52, m
7	200.1	C	-
8	149.9	C	-
9	154.9	C	-
10	38.2	C	-
11	202.0	C	-
12a	51.5	CH_2	2.44, m
12b			2.66, m
13	45.3	C	-
14	48.0	C	-
15a	31.9	CH_2	1.65, m
15b			2.16, m
16a	28.1	CH_2	1.42, m
16b			2.03, m
17	49.1	CH	1.64, m
18	18.8	CH_3	0.94, s
19	17.9	CH_3	1.32, s
20	36.2	CH	1.56, m
21	18.9	CH_3	0.86, d ($J = 6.7$)
22a	37.9	CH_2	1.75, m
22b			2.25, m
23	125.0	CH	5.59, m
24	140.0	CH	5.59, m
25	70.9	C	-
26	30.1	CH_3	1.31, s
27	30.2	CH_3	1.31, s
28	27.8	CH_3	1.02, s
29	15.2	CH_3	0.91, s
30	24.2	CH_3	1.06, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.2.5. (23E)-Eupha-8,23-diene-3 β ,25-diol-7-one (107)

Compound **107** was also isolated from the ethyl acetate fraction as white powder. The molecular formula $C_{30}H_{48}O_3$ was developed through NMR spectral analysis and ESIMS ($[M + Na]^+$ at 479.4 in positive mode).

The ^1H -NMR data (Table-7.13) of **107** showed signals for the protons of a double bond at δ_H 5.55 (2H, m, overlapped, H-23, 24), a secondary alcohol methine at δ_H 3.28 (1H, dd, $J = 11.5, 4.5$ Hz, H-3), a secondary methyl at δ_H 0.80, (3H, d, $J = 5.7$ Hz, CH_3 -21) and seven tertiary methyls at δ_H 0.71, 0.87, 0.96, 1.00, 1.02, 1.28, 1.28 (each, 3H, s). The ^{13}C -NMR data (Table-7.13) of **107** showed thirty carbon signals. These signals were divided into eight methyls, eight methylenes, six methines and eight quaternary carbons atoms on the basis of DEPT spectral analysis. The carbons of the disubstituted double bond resonated at δ_C 125.5 (C-23) and 139.5 (C-24), whereas the carbons involved in the formation of a conjugated ketone signaled at δ_C 198.5 (C-7), 138.9 (C-8) and 165.6 (C-9). In comparison of NMR data, compound

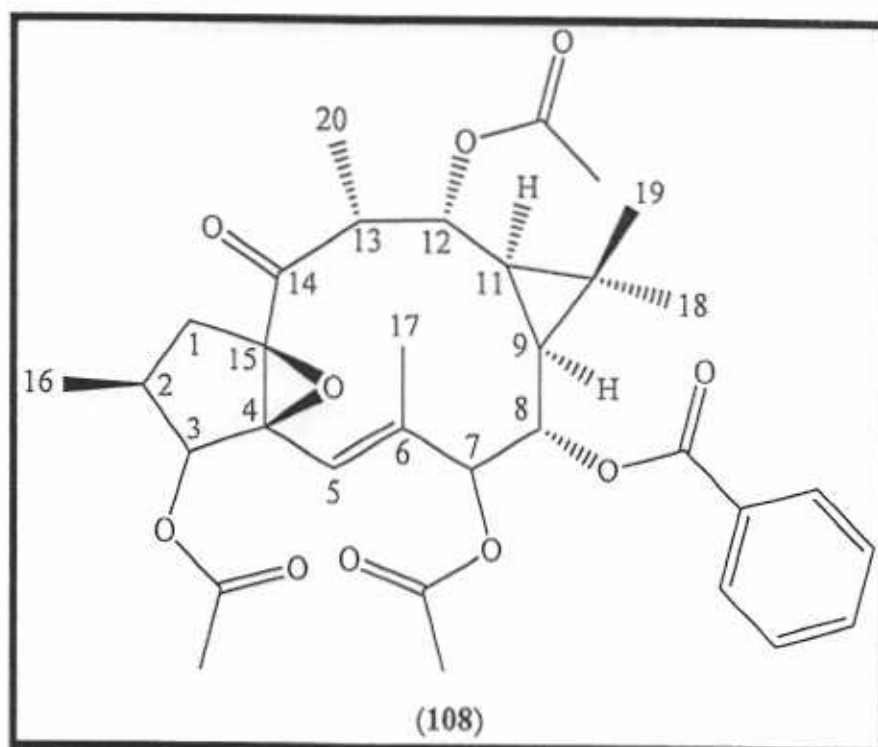
107 was found similar to the reported compound, (23*E*)-eupha-8,23-diene-3 β ,25-diol-7-one.³⁹¹

Table-7.13: ¹H-NMR (300 MHz) and ¹³C-NMR (75 MHz) chemical shifts of (107) in CDCl₃

C. No.	¹³ C-NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	¹ H-NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1a	34.7	CH ₂	1.45, m
1b			1.85, m
2a	27.5	CH ₂	1.75, m
2b			1.67, m
3	78.0	CH	3.28, dd ($J = 11.5, 4.5$)
4	39.0	C	-
5	48.2	CH	1.67, m
6a	35.9	CH ₂	2.41, m
6b			2.38, m
7	198.5	C	-
8	138.9	C	-
9	165.6	C	-
10	39.4	C	-
11a	23.8	CH ₂	2.37, m
11b			2.24, m
12a	30.0	CH ₂	1.76, m
12b			1.80, m
13	44.8	C	-
14	47.8	C	-
15a	31.5	CH ₂	1.54, m
15b			2.13, m
16a	28.6	CH ₂	1.35, m
16b			1.93, m
17	48.4	CH	1.57, m
18	16.0	CH ₃	0.71, s
19	18.7	CH ₃	1.02, s
20	36.1	CH	1.55, m
21	19.2	CH ₃	0.80, d ($J = 5.7$)
22a	38.4	CH ₂	1.82, m
22b			2.32, m
23	125.5	CH	5.55, m
24	139.5	CH	5.55, m
25	70.8	C	-
26	30.0	CH ₃	1.28, s
27	30.1	CH ₃	1.28, s
28	27.4	CH ₃	1.00, s
29	15.2	CH ₃	0.87, s
30	24.5	CH ₃	0.96, s

a. Broad Band; b. DEPT; c. ¹H-NMR; d. HSQC interactions

7.2.6. 3,7,12-Triacetyl-8-benzoylingol (108)



Compound **108** was obtained as white powder from the ethyl acetate fraction. NMR data in combination to ESIMS analysis (molecular ion adduct at m/z 619.3 $[M + Na]^+$ in positive mode) deduced a molecular formula $C_{33}H_{40}O_{10}$.

NMR data (Table-7.14) of **108**, when compared with a previously reported compound, 3,7,12-triacetyl-8-benzoylingol,³⁹² established that they are identical. The ^1H -NMR spectrum indicated the presence of three acetyl groups (δ_H 2.07, 2.13 and 2.21), one benzoyl group (δ_H 7.42, 7.57 and 8.00), a trisubstituted double bond (δ_H 5.63), four methines connected to ester carbonyl groups (δ_H 5.20, 5.36, 4.82 and 4.90), a vinylic methyl (δ_H 2.09), two tertiary methyls (δ_H 0.83 and 1.19) and two secondary methyls (δ_H 0.99 and 1.08). In ^{13}C -NMR spectrum (Table-7.14), thirty three carbon signals were detected. The multiplicities of these carbons were determined with the help of DEPT spectral analysis, thus indicating the presence of eight methyls (δ_C 13.7,

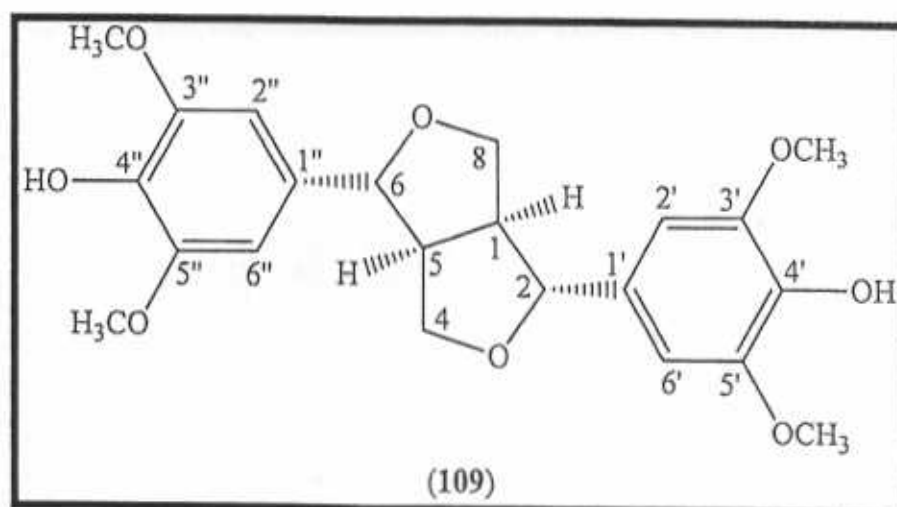
16.4, 17.2, 17.6, 20.8, 21.2, 21.3 and 29.4), one methylene (δ_C 31.6), nine methines (δ_C 25.3, 29.6, 31.2, 43.2, 70.9, 73.7, 76.5, 77.7 and 117.2) and five quaternary carbons (δ_C 19.8, 71.3, 72.2, 140.1 and 207.8). All the assignments were confirmed through 2D-techniques as well.

Table-7.14: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (108) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	31.6	CH_2	1.71, d ($J = 14.4$) 2.80, dd ($J = 15.0, 9.0$)
2	29.6	CH	2.47, m
3	77.7	CH	5.36, d ($J = 8.7$)
4	72.2	C	-
5	117.2	CH	5.63, s
6	140.1	C	-
7	76.5	CH	5.20, s
8	70.9	CH	4.82, dd ($J = 11.1, 2.1$)
9	25.3	CH	1.60, m
10	19.8	C	-
11	31.2	CH	1.20, m
12	73.7	CH	4.90, dd ($J = 10.8, 3.9$)
13	43.2	CH	2.98, m
14	207.8	C	-
15	71.3	C	-
16	16.4	CH_3	0.99, d ($J = 7.6$)
17	17.6	CH_3	2.09, s
18	29.4	CH_3	1.19, s
19	17.2	CH_3	0.83, s
20	13.7	CH_3	1.08, d ($J = 6.9$)
OAc ^e	170.0, 170.6, 170.8 20.8, 21.2, 21.3		2.07 (3H, s), 2.13 (3H, s) 2.21 (3H, s)
OBz ^f	128.7, 129.8, 130.0 133.4		7.42 (2H, m), 7.57 (1H, m) 8.0 (2H, m)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions; e. Acetyl group; f. Benzoyl group

7.2.7. (+)-Diasyringaresinol (109)



The ethyl acetate fraction yielded compound **109** in white amorphous powder form. On the basis of ESIMS analysis (molecular ion adduct at m/z 441.2 $[M + Na]^+$ in positive mode and at m/z 417.5 $[M - 1]^-$ in negative mode) followed by NMR data interpretation, the molecular formula of the compound was deduced as $C_{22}H_{26}O_8$.

In comparison of NMR data (Table-7.15), compound **109** was identified as a furofuranic-type lignan, known as diasyringaresinol (+),³⁹³ previously isolated from *Cassytha filiformis*. The ^1H -NMR spectrum clearly showed the existence of four methoxys [δ_H 3.88 (12H, s)], two hydroxys [δ_H 5.57 (2H, br.s)] and two pairs of equivalent aromatic protons [δ_H 6.58 (4H, d, $J = 6.0$ Hz)]. The proton chemical shifts in the range δ_H 3.00-5.00, implied the existence of bis-tetrahydrofuran ring of a 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane-type lignan. The *cis*-configuration of C-1 and C-5 was apparent from proton shift values at δ_H 3.08 (2H, m) for H-1 and H-5 and 4.72 (2H, d, $J = 4.2$) for H-2 and H-6. The two aromatic rings were positioned to C-2 and C-6 in axial/axial orientation, whereas the absolute configurations at C-1, C-2, C-5 and C-6 were assigned as *R*, as deduced from positive optical rotation, compound

109 possessed.

Table-7.15: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (109) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	54.5	CH	3.08, m
2	86.2	CH	4.72, d ($J = 4.2$)
4	71.9	CH ₂	4.28, dd ($J = 9.0, 6.9$) 3.91, dd ($J = 9.0, 3.3$)
5	54.5	CH	3.08, m
6	86.2	CH	4.72, d ($J = 4.2$)
8	71.9	CH ₂	4.28, dd ($J = 9.0, 6.9$) 3.91, dd ($J = 9.0, 3.3$)
1'	132.2	C	-
2'	102.8	CH	6.58, d ($J = 6.0$)
3'	147.3	C	-
3'-OCH ₃	56.5	CH ₃	3.88, s
4'	134.4	C	-
5'	147.3	C	-
5'-OCH ₃	56.5	CH ₃	3.88, s
6'	102.8	CH	6.58, d ($J = 6.0$)
1''	132.2	C	-
2''	102.8	CH	6.58, d ($J = 6.0$)
3''	147.3	C	-
3''-OCH ₃	56.5	CH ₃	3.88, s
4''	134.4	C	-
5''	147.3	C	-
5''-OCH ₃	56.5	CH ₃	3.88, s
6''	102.8	CH	6.58, d ($J = 6.0$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.2.8. Palmarumycin JC2 (110)

Compound 110 was obtained as a white solid. NMR spectral interpretation and ESIMS analysis (molecular ion adducts at m/z 335.1 $[\text{M} + 1]^+$ and 357.1 $[\text{M} + \text{Na}]^+$ in positive mode and at m/z 333.0 $[\text{M} - 1]^-$ in negative mode) established its molecular formula as $\text{C}_{20}\text{H}_{14}\text{O}_5$.

The NMR data (Table-7.16) of 110 were similar to a known deoxypreussomerin called palmarumycin JC2.³⁹⁴ Like palmarumycin JC2,³⁹⁴ compound 110 showed the presence of three aromatic systems. The protons of the aromatic rings were found to resonate in the range between δ_H 6.83-7.47. An AB pair of double doublets ($J = 18.0, 3.3$ Hz) resonated at δ_H 2.92 (H_a-2) and 3.22 (H_b-2). The presence of a hydroxymethine coupled to both H-2a and H-2b, was deduced from proton signal at δ_H 4.56 (1H, dd, $J = 6.6, 3.3$ Hz, H-3). The presence of a broad singlet at δ_H 12.3 was indicative of the existence of a chelated OH group in the molecule. In ^{13}C -NMR spectrum of compound 110, the carbons of the aromatic rings signaled in the range between δ_C 109.0-162.2. The carbon chemical shifts at δ_C 201.3, 41.5 and 67.2 were assigned to C-1, C-2 and C-3, respectively.

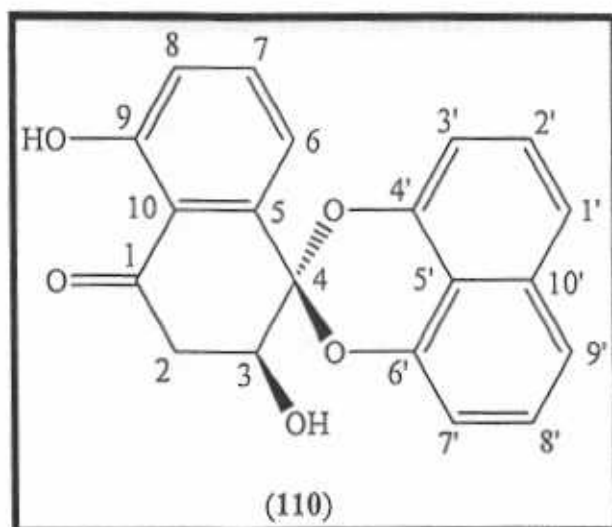


Table-7.16: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (110) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	201.3	C	-
2	41.5	CH ₂	2.92, dd ($J = 18, 3.3$) 3.22, dd ($J = 18, 3.3$)
3	67.2	CH	4.56, dd ($J = 6.6, 3.3$)
4	98.8	C	-
5	138.1	C	-
6	121.5	CH	7.26, dd ($J = 8.0, 1.0$)
7	137.3	CH	7.45, t ($J = 8.0$)
8	120.0	CH	7.00, dd ($J = 8.0, 1.0$)
9	162.2	C	-
10	121.2	C	-
1'	109.7	CH	7.47, dd ($J = 8.0, 1.0$)
2'	127.8	CH	7.40, t, ($J = 8.0$)
3'	118.1	CH	7.05, dd ($J = 8.0, 1.0$)
4'	146.4	C	-
5'	113.2	C	-
6'	147.2	C	-
7'	115.4	CH	6.83, dd, ($J = 8.0, 1.0$)
8'	127.8	CH	7.33, t ($J = 8.0$)
9'	109.0	CH	7.47, dd ($J = 8.0, 1.0$)
10'	134.3	C	-
OH	-	-	12.3 (br.s)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

7.2.9. (3*R*,5*R*,6*R*,7*E*)3,5,6-Trihydroxy-7-megastigmen-9-one (111)

Compound 111 was isolated from the ethyl acetate fraction as colourless oily substance. Its molecular formula ($\text{C}_{13}\text{H}_{22}\text{O}_4$) was assigned through NMR spectral data corresponding to a mass of 242 a.m.u (molecular ion adduct was observed at m/z 265.1 $[\text{M} + \text{Na}]^+$ in ESI positive mode).

A comparison of the NMR data (Table-7.17) of 111 with the known norisoprenoid, namely (3*R*,5*R*,6*R*,7*E*)3,5,6-Trihydroxy-7-megastigmen-9-one,^{395,396} established that they are the same. The ^1H -NMR spectrum of 111, indicated the presence of singlets for four methyls (δ_{H} 0.86, 1.17, 1.28 and 2.32), four aliphatic protons (δ_{H} 1.60-1.88,

m), a hydroxymethylene proton (δ_{H} 4.16, m, H-3) and two *trans*-olefinic protons (δ_{H} 6.37 and 7.28, each 1H, d, $J = 16.2$ Hz, H-7 and H-8, respectively). ^{13}C -NMR (BB and DEPT) spectra (Table-7.17) showed a total of thirteen carbon signals, which were identified as four methyls (δ_{C} 25.8, 26.8, 27.6 and 28.0), two methylenes (δ_{C} 45.4 and 45.6), a hydroxymethine (δ_{C} 64.4), two olefinic carbons (δ_{C} 131.3 and 149.0), three quaternary carbons (δ_{C} 40.3, 76.8 and 78.5) and one carbonyl carbon (δ_{C} 198.3).

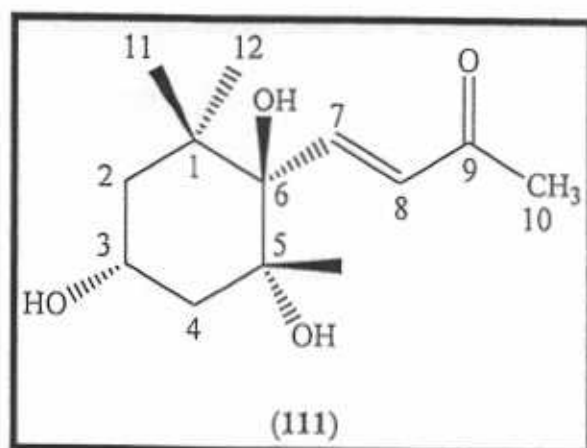


Table-7.17: ^1H -NMR (300 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (111) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	40.3	C	-
2	45.6	CH_2	1.60, m
3	64.4	CH	4.16, m
4	45.4	CH_2	1.80, m 1.88, m
5	76.8	C	-
6	78.5	C	-
7	149.0	CH	6.37, d ($J = 16.2$)
8	131.3	CH	7.28, d ($J = 16.2$)
9	198.3	C	-
10	25.8	CH_3	2.32, s
11	28.0	CH_3	0.86, s
12	27.6	CH_3	1.28, s
13	26.8	CH_3	1.17, s

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

Chapter: 8

EXPERIMENTAL (Part-B)

8.1: General experimental conditions

8.1.1: Physical constants

Optical rotations were determined on a Perkin-Elmer 341 polarimeter. X-ray diffraction data was acquired on Bruker diffractometer equipped with SMART APUX CCU area detector using Mo $K\alpha$ radiation, $\lambda = 0.71073 \text{ \AA}$.

8.1.2: Spectroscopic techniques

UV spectra were obtained on a Hitachi U-3200 IV spectrophotometer in methanol as solvent. IR spectra were obtained using a Nicolet FT-IR spectrophotometer. NMR spectra were recorded on a Bruker AMX 400 instrument using solvent peaks as references. High resolution electrospray ionization mass spectrometry (ESIMS) were recorded on a Micromass Q-Tif Global mass spectrometer and electrospray ionization mass spectrometry were performed on a Bruker Esquire 3000 Plus spectrometer.

8.1.3. Chromatographic techniques

Chromatographic separations were performed by using silica gel (200-300 mesh, Qingdao Marine Chemical Ltd., China) RP-18 (20-45 μm , Fuji Silysia Chemical Ltd.), sephadex LH-20 (20-100 μm , Pharmacia). Thin layer chromatography (TLC) was performed on GF254 silica gel plates (Yantai Huiyou Inc., China). All solvents used were of chemical grade and purchased from the Shanghai Chemical Plant, Shanghai, P. R. China.

8.2. *Euphorbia neriifolia* Linn.

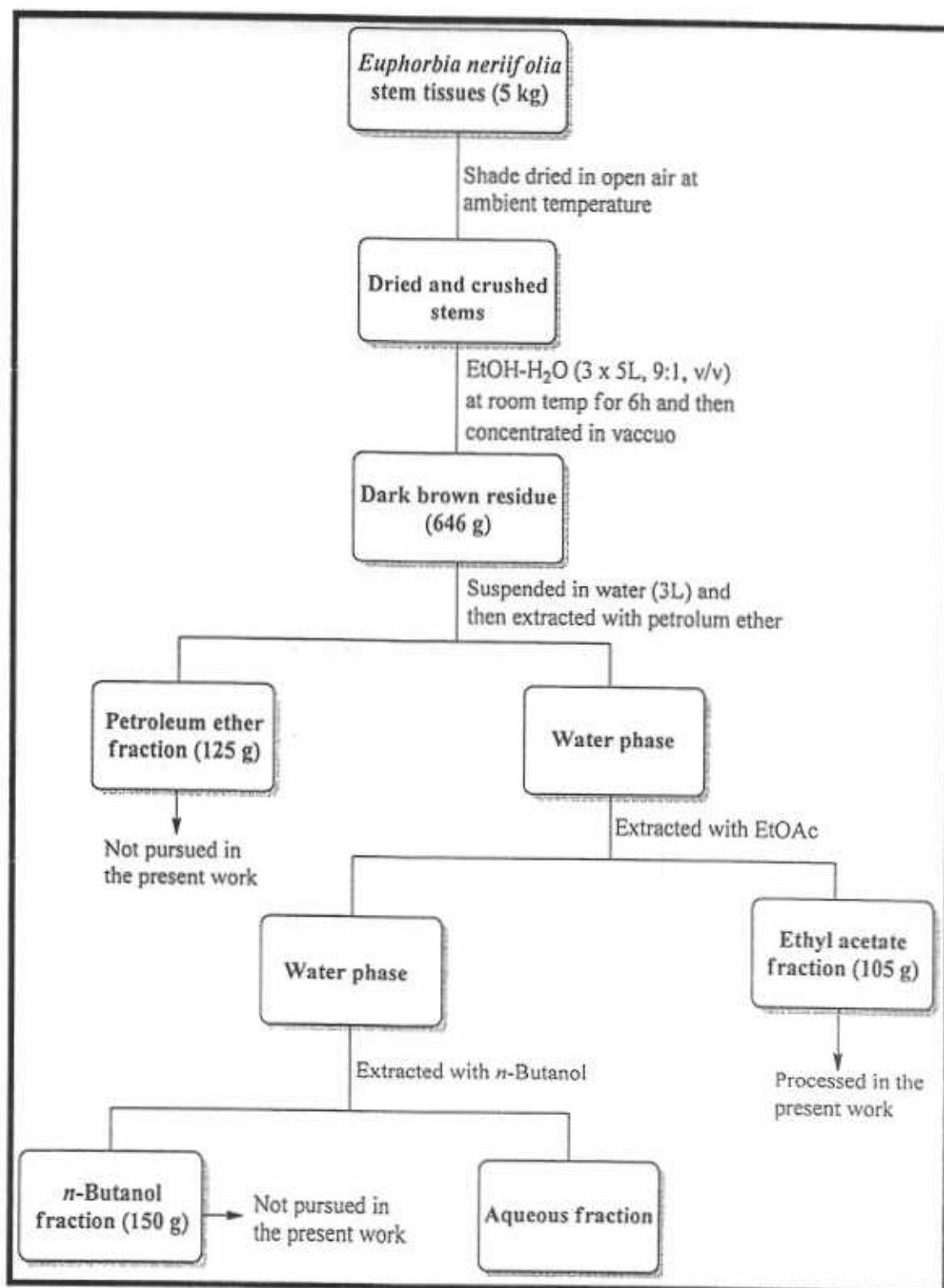
8.2.1. Plant material

The stems of *E. neriifolia* were collected from North Waziristan FATA Pakistan in July 2009. Taxonomic identification was done by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar., Pakistan. A voucher specimen number Latif-622(PU) was deposited in the herbarium at Department of Botany, University of Peshawar, Peshawar., Pakistan.

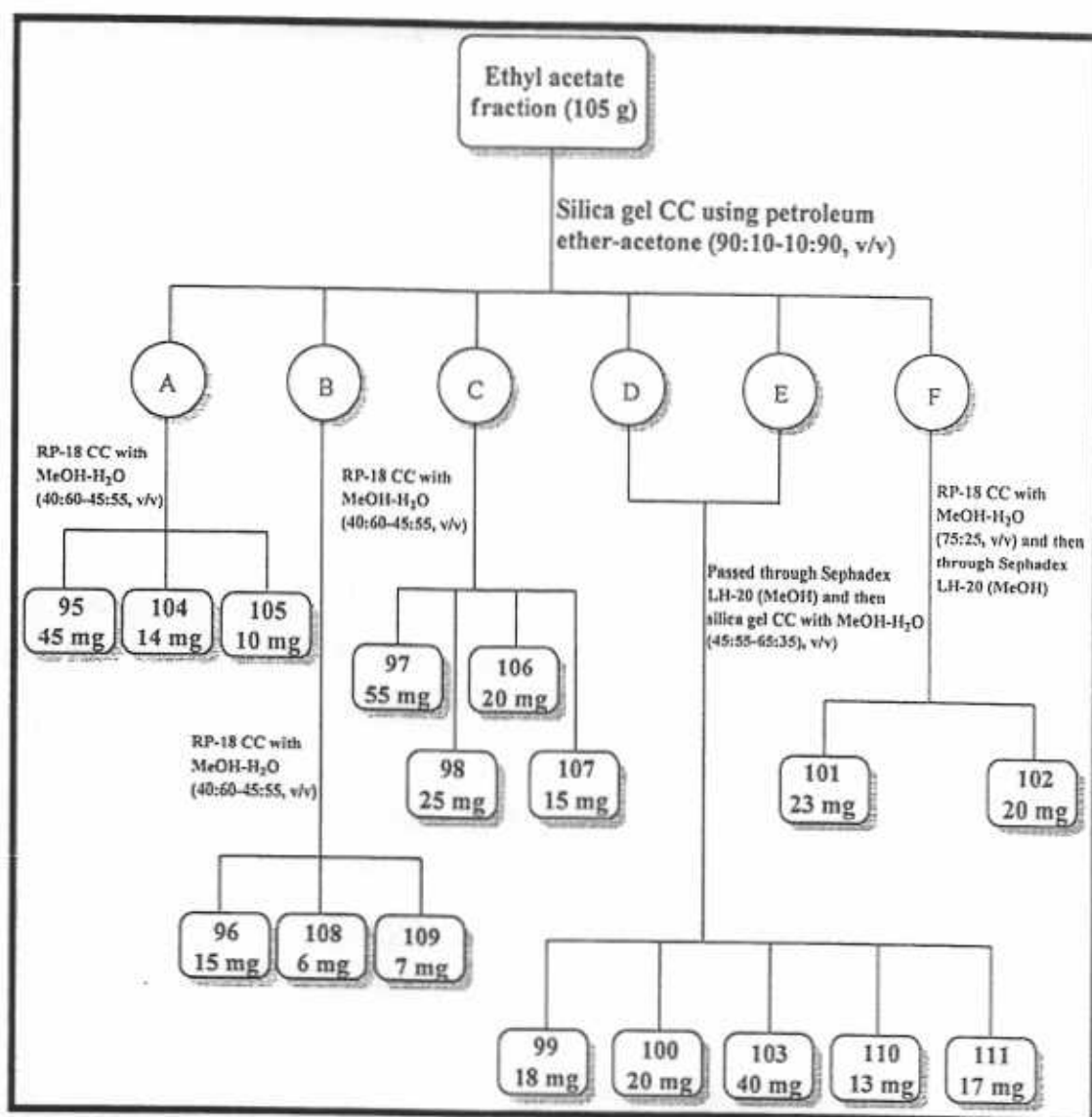
8.2.2. Extraction and isolation

The stem tissues (5 kg) were shade dried and crushed. The crushed dried stems were extracted three times with ethanol-water (3 x 5L) at room temperature. The combined extract was concentrated under vacuum to give a dark residue (646 g), which was suspended in water and then successively partitioned with petroleum ether, EtOAc, and *n*-BuOH to afford petroleum ether, EtOAc and *n*-BuOH soluble fractions (**Scheme-8.1**). The EtOAc fraction (105 g) was subjected to column chromatography (CC) over silica gel eluted with petroleum ether-acetone (90:10-10:90, v/v) to obtain six major fractions (A-F) (**Scheme-8.2**). A, B and C were further purified through flash column chromatography on a RP-18 column eluted with CH₃OH-H₂O (40:60-45:55, v/v), thus fraction A yielded **95** (45 mg), **104** (14 mg) and **105** (10 mg), fraction B yielded **96** (15 mg), **108** (6 mg) and **109** (7 mg), fraction C yielded **97** (55 mg), **98** (25 mg), **106** (20 mg) and **107** (15 mg). Fraction D and E were passed through a Sephadex LH-20 column (MeOH), and then subjected to silica gel CC MeOH-H₂O (45:55-65:35, v/v) to give **99** (18 mg), **100** (20 mg), **103** (40 mg), **110** (13 mg) and **111**(17 mg). Fraction F was first applied to an RP-18 column MeOH-

H₂O (75:25, v/v), followed by purification with a Sephadex LH-20 column (MeOH), to afford **101** (23 mg) and **102** (20 mg).



Scheme-8.1: Extraction and fractionation from *E. neriifolia*



Scheme-8.2: Isolation of pure compounds from ethyl acetate fraction of *E. neriifolia*

8.3. Characterization of new compounds from *E. neriifolia*

8.3.1. *Ent*-3,4-seco-4,16 β ,17-trihydroxykauran-3-oic acid (95)

Physical State: Colourless crystals

Yield: 45 mg

$[\alpha]_D^{17} = -49.0^\circ$ (*c* 0.45, MeOH)

IR (KBr) $\nu_{\max}$: 3446, 2929, 1704, 1675,
1452, 1278, 1199, 1022 cm^{-1}

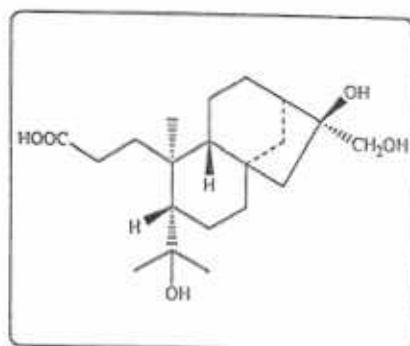
ESIMS: m/z 377 $[M + Na]^+$

HRESIMS: m/z 377.2408 $[M + Na]^+$

(calcd for $C_{20}H_{34}O_5Na$, 377.2404)

^1H - and ^{13}C -NMR: See Table-7.1

HSQC: See Table-7.1



8.3.2. *Ent*-16 β -hydroxykauran-3,4-lactone (96)

Physical State: Colourless powder

Yield: 15 mg

$[\alpha]_D^{24} = -38.0^\circ$ (*c* 0.055, MeOH)

IR (KBr) $\nu_{\max}$: 3486, 3365, 2937, 2869, 1712,
1446, 1375, 1288, 1112, 1022 cm^{-1}

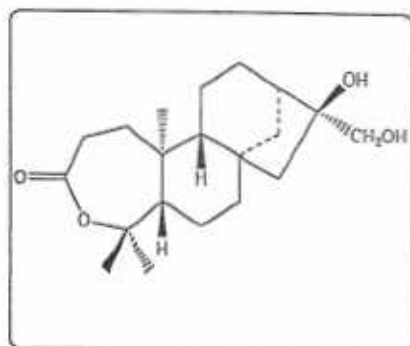
ESIMS: m/z 359 $[M + Na]^+$

HRESIMS: m/z 359.2195 $[M + Na]^+$

(calcd for $C_{20}H_{32}O_4Na$, 359.2198)

^1H - and ^{13}C -NMR: See Table-7.2

HSQC: See Table-7.2



8.3.3. 13 β ,19-Dihydroxy-3,15-dioxoatis-16-ene (97)

Physical State: Colourless crystals

Yield: 55 mg

UV $\lambda_{\max}$ in MeOH: 236 nm

$[\alpha]_D^{24} = +40.0^\circ$ (*c* 0.035, MeOH)

IR (KBr) $\nu_{\max}$: 3544, 3434, 2929, 2869,

1712, 1673, 1459, 1403, 1062, 540 cm^{-1}

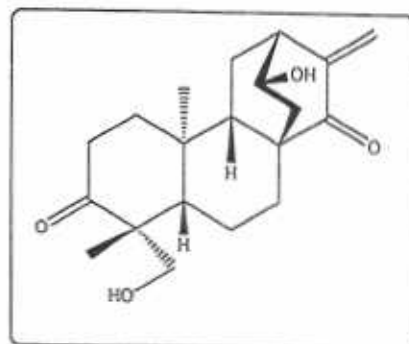
ESIMS: m/z 355 $[M + Na]^+$

HRESIMS: m/z 355.1889 $[M + Na]^+$

(calcd for $C_{20}H_{28}O_4Na$, 355.1885)

^1H - and ^{13}C -NMR: See Table-7.3

HSQC: See Table-7.3



8.3.4. 13 β -Hydroxy-3,15-dioxoatis-16-ene (98)

Physical State: Colourless crystals

Yield: 25 mg

UV $\lambda_{\max}$ in MeOH: 235 nm

$[\alpha]_D^{24} = +46.0^\circ$ (*c* 0.085, MeOH)

IR (KBr) $\nu_{\max}$: 3432, 2937, 2856, 1726, 1697,

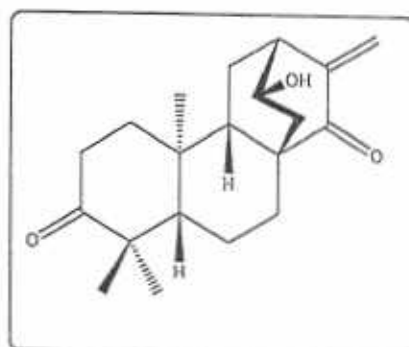
1608, 1367, 1114, 1072, 906 cm^{-1}

ESIMS: m/z 339 $[M + Na]^+$

HRESIMS: m/z 339.1940 $[M + Na]^+$

(calcd for $C_{20}H_{28}O_3Na$, 339.1936)

^1H - and ^{13}C -NMR: See Table-7.4



HSQC: See Table-7.4

8.3.5.16 β ,17,19-Trihydroxy-3-oxo-atisane (99)

Physical State: Colourless powder

Yield: 18 mg

$[\alpha]_D^{24} = +14.0^\circ$ (c 0.05, MeOH)

IR (KBr) $\nu_{\max}$: 3409, 2954, 2869, 1704,
1450, 1376, 1278, 1033 cm^{-1}

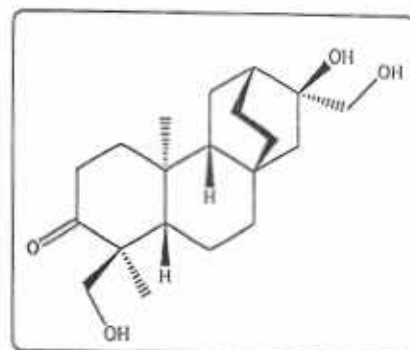
ESIMS: m/z 359 $[M + Na]^+$

HRESIMS: m/z 359.2193 $[M + Na]^+$

(calcd for $C_{20}H_{32}O_4Na$, 359.2198)

^1H - and ^{13}C -NMR: See Table-7.5

HSQC: See Table-7.5



8.3.6.4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid (100)

Physical State: Colourless powder

Yield: 20 mg

$[\alpha]_D^{24} = +10.0^\circ$ (c 0.115, MeOH)

IR (KBr) $\nu_{\max}$: 3423, 2939, 1720, 1438,
1195, 1070, 756 cm^{-1}

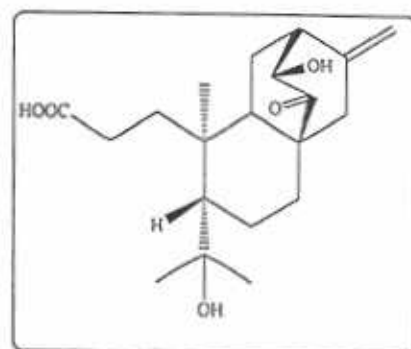
ESIMS: m/z 373 $[M + Na]^+$

HRESIMS: m/z 373.2011 $[M + Na]^+$

(calcd for $C_{20}H_{30}O_5Na$, 373.1991)

^1H - and ^{13}C -NMR: See Table-7.6

HSQC: See Table-7.6



8.3.7. 4,13 β -Dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid methyl ester (101)

Physical State: Colourless powder

Yield: 23 mg

$[\alpha]_D^{24} = +13.0^\circ$ (c 0.085, MeOH)

IR (KBr) $\nu_{\max}$: 3496, 2952, 1724, 1438,

1382, 1286, 1197, 1072 cm^{-1}

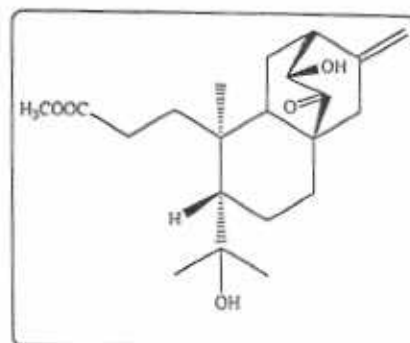
ESIMS: m/z 387 $[M + Na]^+$

HRESIMS: m/z 387.2150 $[M + Na]^+$

(calcd for $C_{21}H_{32}O_5Na$, 387.2147)

^1H - and ^{13}C -NMR: See Table-7.7

HSQC: See Table-7.7



8.3.8. 1 α ,3 α -Dihydroxy-*ent*-abieta-8(14),13(15)-dien-16,12-olide (102)

Physical State: White amorphous powder

Yield: 20 mg

UV $\lambda_{\max}$ in MeOH: 281 nm

$[\alpha]_D^{24} = +74.0^\circ$ (c 0.05, MeOH)

IR (KBr) $\nu_{\max}$: 3434, 2937, 2875, 1727,

1662, 1608, 1452, 1388, 1022 cm^{-1}

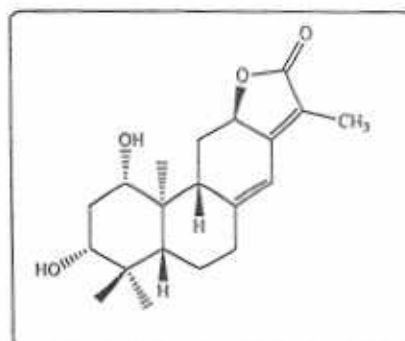
ESIMS: m/z 355 $[M + Na]^+$

HRESIMS: m/z 355.1881 $[M + Na]^+$

(calcd for $C_{20}H_{28}O_4Na$, 355.1885)

^1H - and ^{13}C -NMR: See Table-7.8

HSQC: See Table-7.8



8.4. Characterization of known compounds from *E. neriifolia*

8.4.1. *Ent*-16 β ,17-Dihydroxyatisan-3-one (103)

Physical State: White solid

Yield: 40 mg

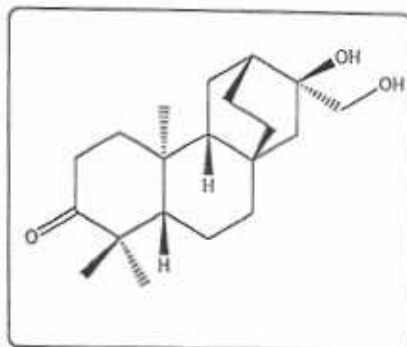
$[\alpha]_D^{25} = -37.9^\circ$ (*c* 0.5, CHCl₃)

IR: 3379, 1702 cm⁻¹

ESIMS: *m/z* 343.3 [M + Na]⁺

¹H- and ¹³C-NMR: See Table-7.9

HSQC: See Table-7.9



8.4.2. (16*R*)-16,17-Dihydroxy-*ent*-kauran-3-one (104)

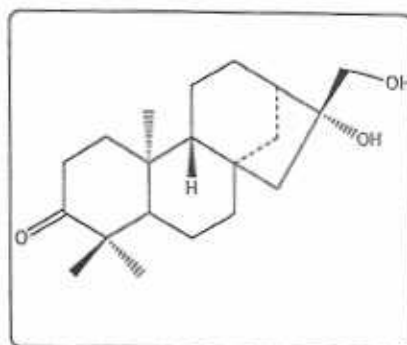
Physical State: White powder

Yield: 14 mg

ESIMS (+): *m/z* 343.3 [M + Na]⁺

¹H- and ¹³C-NMR: See Table-7.10

HSQC: See Table-7.10



8.4.3. Ingenol (105)

Physical State: White solid

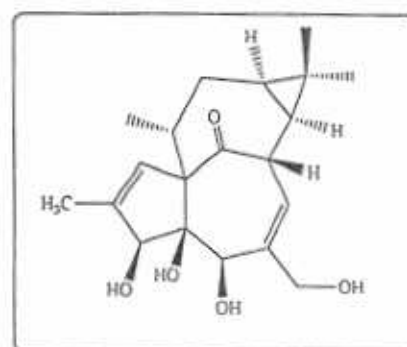
Yield: 10 mg

$[\alpha]_D^{25} = +40.9^\circ$ (*c* 0.1, MeOH)

IR (KBr) $\nu_{\max}$: 3352, 1710, 1623, 1460 cm⁻¹

ESIMS: *m/z* 371.2 [M + Na]⁺, 347.1 [M-1]⁻

¹H- and ¹³C-NMR: See Table-7.11



HSQC: See Table-7.11

8.4.4. (23*E*)-Eupha-8,23-diene-3 β ,25-diol-7,11-dione (106)

Physical State: White powder

Yield: 20 mg

UV λ_{max} in MeOH: 270, 202 nm

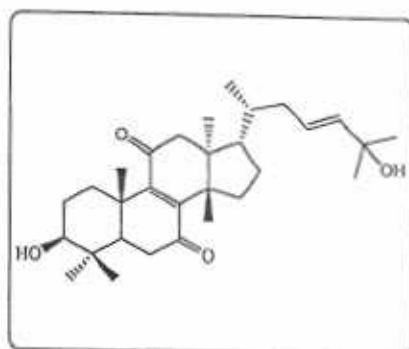
$[\alpha]_{\text{D}}^{25} = +6.8^{\circ}$ (*c* 0.15, MeOH)

IR: 3450, 1665, 1460, 1380 cm^{-1}

ESIMS (+): m/z 393.4 $[\text{M} + \text{Na}]^{+}$

^1H - and ^{13}C -NMR: See Table-7.12

HSQC: See Table-7.12



8.4.5. (23*E*)-Eupha-8,23-diene-3 β ,25-diol-7-one (107)

Physical State: White gummy solid

Yield: 15 mg

UV λ_{max} in MeOH: 260 nm

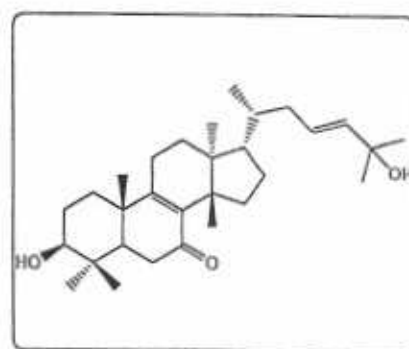
$[\alpha]_{\text{D}}^{25} = +14.8^{\circ}$ (*c* 0.21, MeOH)

IR (KBr) ν_{max} : 3432, 1652, 1456, 1378 cm^{-1}

ESIMS (+): m/z 479.4 $[\text{M} + \text{Na}]^{+}$

^1H - and ^{13}C -NMR: See Table-7.13

HSQC: See Table-7.13



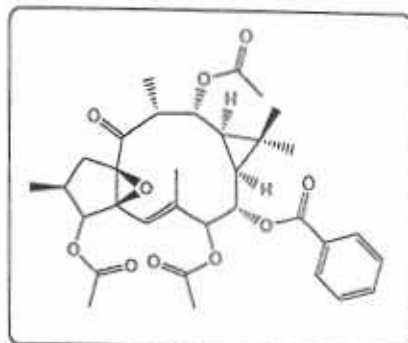
8.4.6. 3,7,12-Triacetyl-8-benzoylingol (108)

Physical State: White powder

Yield: 6 mg

 $[\alpha]_D^{25} = -62^\circ$ (c 0.5, CHCl_3)ESIMS (-): m/z 619.3 $[\text{M} + \text{Na}]^+$ ^1H - and ^{13}C -NMR: See Table-7.14

HSQC: See Table-7.14



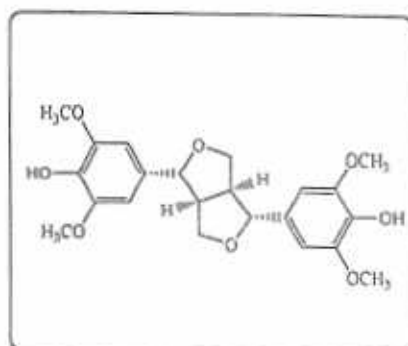
8.4.7. (+)-Diasyringaresinol (109)

Physical State: White amorphous powder

Yield: 7 mg

UV λ_{max} in MeOH: 210, 238 nm $[\alpha]_D^{25} = +110^\circ$ (c 0.1, CHCl_3)IR (KBr) ν_{max} : 3380, 1600, 1508 cm^{-1} ESIMS: 441.2 $[\text{M} + \text{Na}]^+$, m/z 417.5 $[\text{M} - 1]^-$ ^1H - and ^{13}C -NMR: See Table-7.15

HSQC: See Table-7.15



8.4.8. Palmarumycin JC2 (110)

Physical State: White solid

Yield: 13 mg

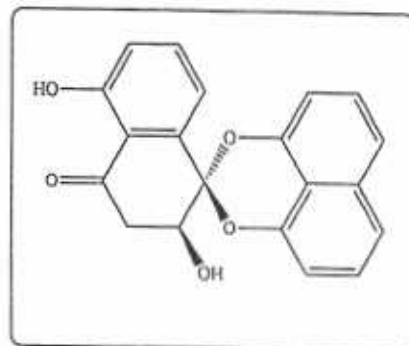
$[\alpha]_D^{25} = +131.9^\circ$ (c 0.5, CHCl_3)

IR: 3539, 1655, 1609, 1515, 1454 cm^{-1}

ESIMS: m/z 357.1 $[\text{M} + \text{Na}]^+$, 333.0 $[\text{M} - 1]^-$

^1H - and ^{13}C -NMR: See Table-7.16

HSQC: See Table-7.16



8.4.9. (3*R*,5*R*,6*R*,7*E*)3,5,6-Trihydroxy-7-megastigmen-9-one (111)

Physical State: colourless oil substance

Yield: 17 mg

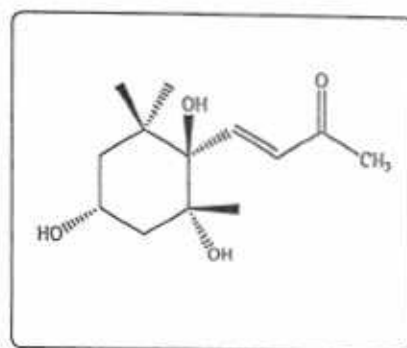
UV λ_{max} in MeOH: 232, 206 nm

IR (KBr) ν_{max} : 3330, 1723, 1467 cm^{-1}

ESIMS: m/z 365.1 $[\text{M} + \text{Na}]^+$

^1H - and ^{13}C -NMR: See Table-7.17

HSQC: See Table-7.17



8.5. Single-crystal X-ray structure determination of *ent*-3,4-seco-4,16 β ,17-trihydroxykauran-3-oic acid (95)

$\text{C}_{20}\text{H}_{34}\text{O}_5$, $M = 354.24$; orthorhombic, space group $P2_1$; $a = 7.259(3)$ Å, $b = 17.118(7)$ Å, $c = 18.863(8)$ Å, $\alpha = 90.00^\circ$, $\beta = 90.00^\circ$, $\gamma = 90.00^\circ$, $V = 2343.7(17)$ Å³, $Z = 4$, $d = 1.186$ g/cm³, crystal dimensions $0.25 \times 0.18 \times 0.17$ mm were used for measurement on a SHELXL-97 with a graphite monochromator, Mo K_α radiation. The total number of reflections measured was 11, 301, of which 5046 were observed, $I > 2\sigma(I)$. Final

indices: $R_1 = 0.0539$, $wR_2 = 0.1295$. The crystal structure of (95) was solved by direct method SHELXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHELXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of (95) have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 849726).

8.6. X-ray single-crystal structure determination of 13 β ,19-dihydroxy-3,15-dioxoatis-16-ene (97)

$C_{20}H_{28}O_4$, $M = 332.19$; orthorhombic, space group $P2_1$; $a = 10.64(2)$ Å, $b = 8.012(17)$ Å, $c = 13.12(3)$ Å, $\alpha = 90.00^\circ$, $\beta = 93.98(3)^\circ$, $\gamma = 90.00^\circ$, $V = 1116(4)$ Å³, $Z = 2$, $d = 1.341$ g/cm³, crystal dimensions $0.25 \times 0.20 \times 0.20$ mm were used for measurement on a SHELXL-97 with a graphite monochromator, Mo K_α radiation. The total number of reflections measured was 4156, of which 3603 were observed, $I > 2\sigma(I)$. Final indices: $R_1 = 0.0781$, $wR_2 = 0.2358$. The crystal structure of (97) was solved by direct chloroform SHELXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHELXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of (97) have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 849727).

Part-C

***Phytochemistry and Bioactivity of
Sorbus lanata***



***Sorbus lanata* (D. Don) Schauer**

Chapter: 9

INTRODUCTION (Part-C)

9.1. Family Rosaceae

Rosaceae are a medium sized family composed of about 3000 species and 95 genera. The family has a cosmopolitan distribution and represented in Pakistan by 27 genera and about 160 species, which are evergreen or deciduous trees, shrubs, sarmentous shrubs or perennial herbs.³⁹⁷ The family has been divided into four subfamilies namely Rosoideae, Spiraeoideae, Maloideae and Amygdaloideae. The largest genera of the family are: *Alchemilla* (270 species), *Sorbus* (260 species), *Crataegus* (260 species), *Cotoneaster* (260 species), *Rubus* (250 species)³⁹⁸ and *Prunus* (430 species). *Sorbus* is a well-known genus of Rosaceae family with many species finding their use in traditional medicines.³⁹⁹

9.2. Genus *Sorbus*

Sorbus is a large genus with about 260 species consisting of trees or shrubs. They are mainly distributed in the Northern Hemisphere with a circumboreal distribution (Europe, North Africa, Asia and northern North America).⁴⁰⁰ About 70 species of *Sorbus* have not been investigated for their chemical constituents, except for the species with well-known ethnomedicinal uses such as *S. cashmiriana*, *S. commixta*, *S. aucuparia*, *S. americana* and *S. decora*. *Sorbus* species have been reported for their antioxidative,⁴⁰¹⁻⁴⁰³ anti-atherogenic,⁴⁰⁴ anti-inflammatory,⁴⁰⁵ anti-diabetic,⁴⁰³ vasoprotective and vasorelaxant⁴⁰⁶ activities. These bioactivities are considered as due to the presence of phenolic constituents. Numerous species of the genus have been investigated for antioxidative capacities due to their total phenolic contents.^{401,402,407} These phenolics include simple phenolic acids, their esters with quinic acid (mostly caffeoylquinicacids), flavonols (quercetin, kaempferol and sexangularetin),

anthocyanins (cyaniding and pelargonidin derivatives) and tannin-type proanthocyanidins.^{401,402,408}

9.3. *Sorbus lanata* (D. Don) Schauer

Sorbus lanata also known as hairy rowan which is a small tree with white-woolly flower clusters, found in the Himalayas (Afghanistan to Nepal). The leaves are large, elliptic-obovate, with shallow acute and toothed lobes. The leaves of the plant are hairless above when mature, and grayish-white woolly below, or sometimes hairless. The flowers are white, scented, 1.3 cm across. Petals are hairless; styles are densely woolly in the lower half. The edible fruit is yellowish-red.

9.4. Pharmacological importance of genus *Sorbus*

The Genus *Sorbus* has wide ranging pharmacological applications. Various *Sorbus* species have been used for a wide variety of ethnomedical properties, such as anti-diarrhoeal, diuretic, anti-inflammatory, anti-diabetic, vasoprotective, broncho and vasorelaxant activities, and also as potent antioxidant agents.^{401,409-412} *S. commixta* has been reported for the treatment of cough, asthma and other bronchial disorders.⁴⁰⁹ *S. decora* has been used to treat symptoms associated with diabetes mellitus.⁴¹³ Bark tea of *S. cashmiriana* is used for the treatment of nausea and the berries are rich in vitamin C, hence used to cure scurvy.⁴¹⁴ The bark of *S. amurensis* is a remedy for cough and its fruits are used to treat stroke (especially heart diseases) and pain from severe wounds, while tea made from its bark is also used to treat nausea and to cleanse blood.³⁹⁹

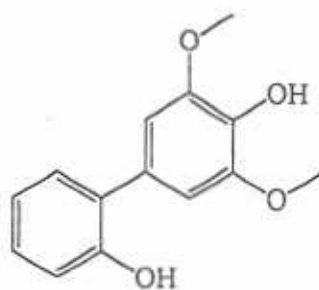
9.5. Literature survey on *Sorbus lanata*

As far as literature survey is concerned, no phytochemical study has been reported on *S. lanata*. As a matter of fact, the whole genus is little explored and up till now only 21 compounds have been isolated from the entire genus. An updated review of these compounds is provided in the Table-9.1.

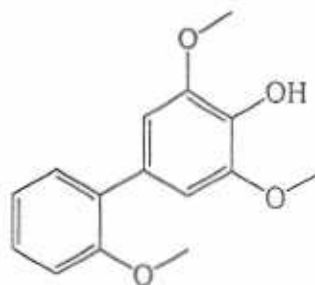
Table-9.1: List of compounds isolated from genus *Sorbus*

S. No	Name of Compound	Molecular Formula	Source
1	2,4'-Dihydroxy-3',5'-dimethoxybiphenyl	C ₁₄ H ₁₄ O ₄	<i>S. aucuparia</i> ⁴¹⁵
2	2,6-Dimethoxy-4-(2-methoxyphenyl)phenol	C ₁₅ H ₁₆ O ₄	<i>S. aucuparia</i> , <i>S. decora</i> ⁴¹⁵
3	4-Hydroxy-3,4',5-trimethoxybiphenyl	C ₁₅ H ₁₆ O ₄	<i>S. aucuparia</i> ⁴¹⁶
4	2-(3,5-Dimethoxyphenyl)phenol	C ₁₄ H ₁₄ O ₃	<i>S. aucuparia</i> ⁴¹⁵
5	3,5-Dimethoxy-4-biphenylol	C ₁₄ H ₁₄ O ₃	<i>S. aucuparia</i> ⁴¹⁷
6	5,6-Dihydro-6-methyl-2H-pyran-2-one	C ₆ H ₈ O ₂	<i>S. aucuparia</i> ⁴¹⁸
7	D-Glucitol	C ₆ H ₁₄ O ₆	<i>S. aucuparia</i> ^{419,420}
8	2,4-Hexadienoic acid-(2 <i>E</i> ,4 <i>E</i>)-form	C ₆ H ₈ O ₂	<i>S. aucuparia</i> ⁴²¹⁻⁴²³
9	L-Iditol	C ₆ H ₁₄ O ₆	<i>S. aucuparia</i> ⁴²⁴
10	Kaempferol-3-gentiobioside	C ₂₇ H ₃₀ O ₁₆	<i>S. pendula</i> ⁴²⁵
11	Sorboside	C ₃₃ H ₄₀ O ₂₁	<i>S. pendula</i> ⁴²⁶
12	Sorbinol B	C ₃₀ H ₄₈ O ₄	<i>S. cashmariana</i> ⁴²⁷
13	Sorbikortal-II	C ₃₀ H ₅₀ O ₃	<i>S. aucuparia</i> ⁴²⁸
14	Lyoniside	C ₂₇ H ₃₆ O ₁₂	<i>S. spp</i> ⁴¹⁷
15	7- <i>O</i> -β-D-Apiofuranoside	C ₂₀ H ₂₂ O ₁₀	<i>S. commixta</i>
16	7- <i>O</i> -β-D-Xylopyranosid	C ₂₀ H ₂₂ O ₁₀	<i>S. commixta</i>
17	3- <i>O</i> -[β-D-Glucopyranosyl-(1→2)-β-D-glucopyranoside]	C ₂₇ H ₃₀ O ₁₇	<i>S. aucuparia</i>
18	Parasorboside	C ₁₂ H ₂₀ O ₈	<i>S. aucuparia</i> ⁴²⁹
19	Sexangularetin-3-glucoside	C ₂₂ H ₂₂ O ₁₂	<i>S. spp</i> ⁴³⁰
20	3-(4-Hydroxy-3,5-dimethoxyphenyl)propanal	C ₁₁ H ₁₄ O ₄	<i>S. aucuparia</i> ⁴³¹
21	Sorbinol A	C ₃₀ H ₄₆ O ₃	<i>S. cashmariana</i> ⁴²⁷

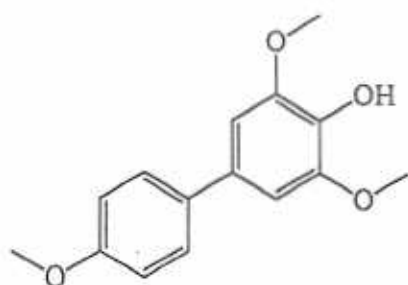
9.6. Structures of compounds isolated from genus *Sorbus*



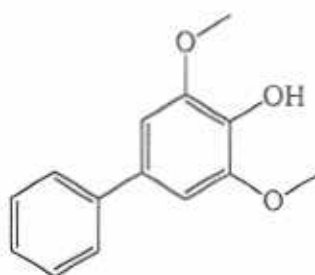
2,4'-Dihydroxy-3', 5'-dimethoxybiphenyl



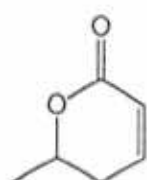
2,6-Dimethoxy-4-(2-methoxyphenyl)phenol



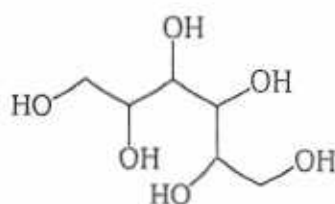
4-Hydroxy-3,4',5-trimethoxybiphenyl



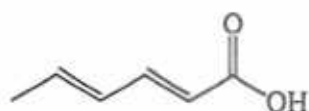
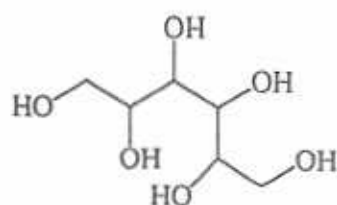
3,5-Dimethoxy-4-biphenylol



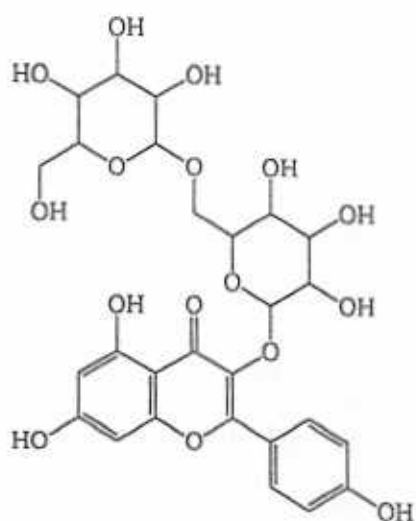
5,6-Dihydro-6-methyl-2H-pyran-2-one



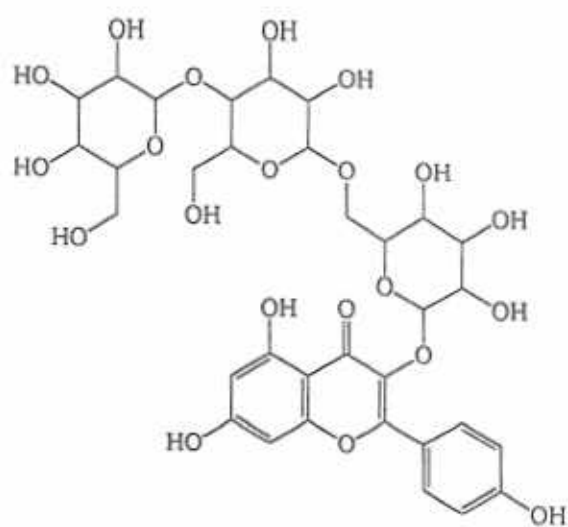
Glucitol-D-form

2,4-Hexadienoic acid-(2*E*,4*E*)-form

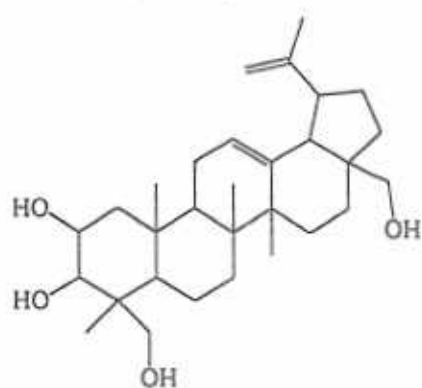
Iditol-L-form



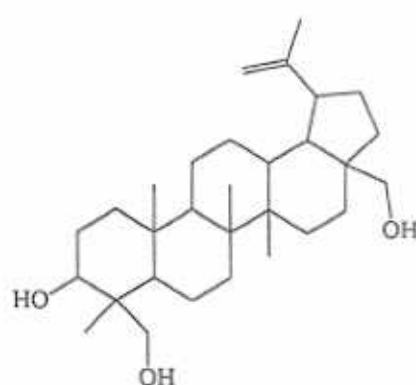
Kaempferol-3-gentiobioside



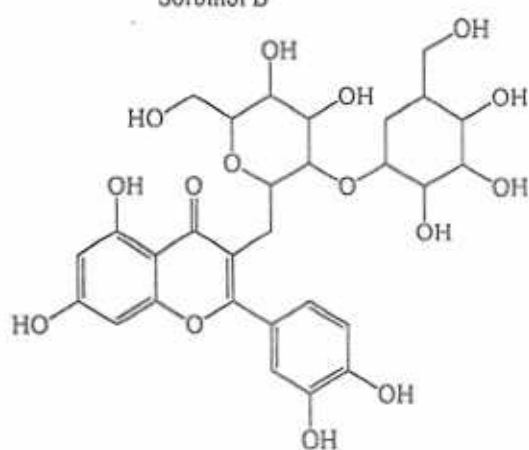
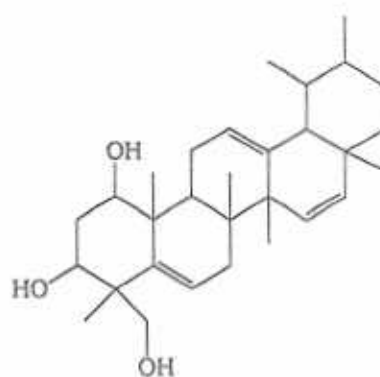
Sorboside



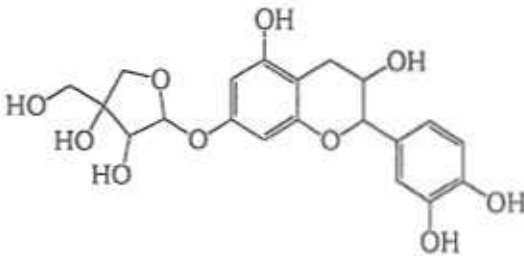
Sorbinol B



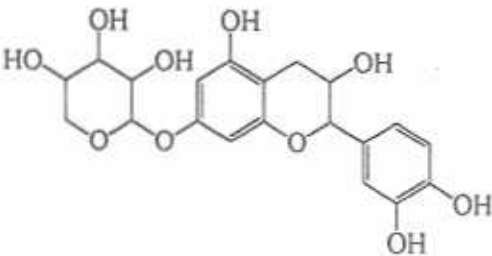
Sorbikortal-II

3-O-[\beta-D-Glucopyranosyl-(1 $\rightarrow$ 2)-\beta-D-glucopyranoside]

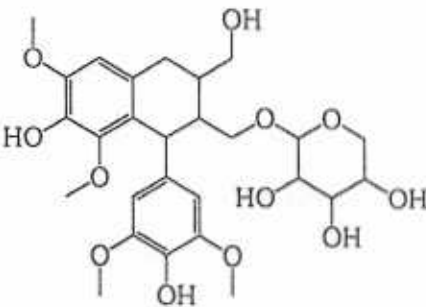
Sorbinol A



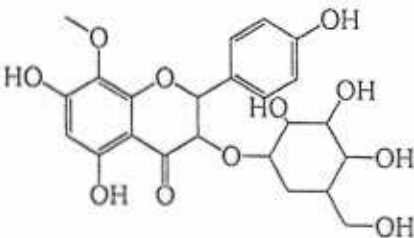
7-O-β-D-Apiofuranoside



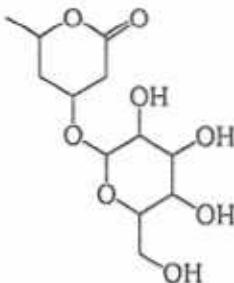
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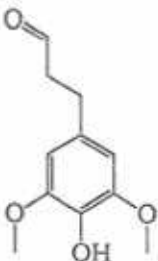
Lyoniside



Sexangularetin-3-glucoside



Parasorboside



3-(4-Hydroxy-3,5-dimethoxyphenyl)propanal

Chapter: 10

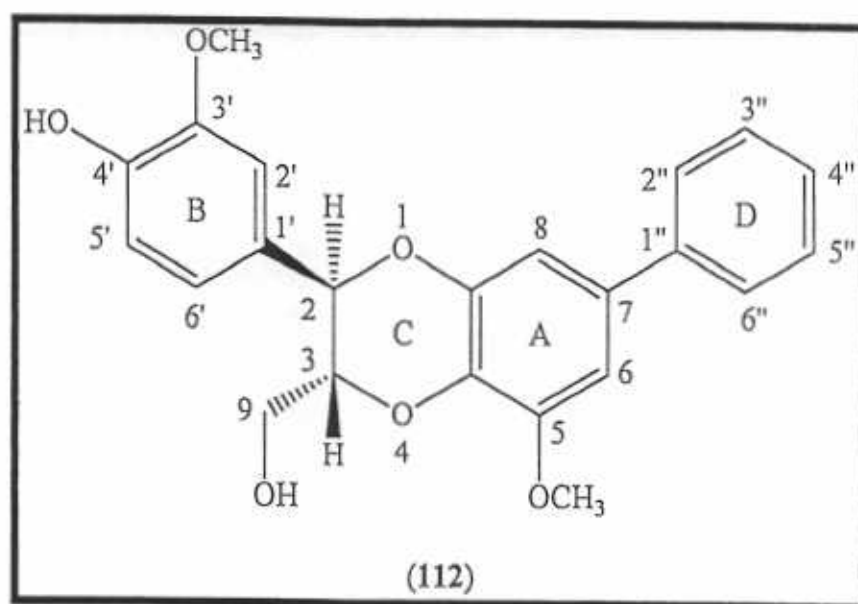
RESULTS AND DISCUSSION (Part-C)

This chapter describes the isolation, purification, structure elucidation and antioxidant evaluation of four new chemical constituents (112-115) along with eleven reported (116-126) compounds from the wood of *Sorbus lanata*. The reported compounds were isolated for the first time from the genus *Sorbus* with the exception of compounds (122-124).

The protocol followed for extraction from the powdered wood of *S. lanata* was based on standard extraction with methanol and partitioning of the resultant methanolic extract with hexane, chloroform, ethyl acetate and *n*-butanol. The ethyl acetate soluble fraction was then subjected to a variety of chromatographic methods for the isolation and purification of compounds. A number of advanced spectroscopic techniques were carried out to characterize the purified compounds. The purified compounds were also evaluated for their biological activity in the Diphenylpicrylhydrazyl (DPPH) radical scavenging assay.

10.1. Structure elucidation of new compounds from *S. lanata*

10.1.1. Sorlanin (112)



Compound 112 was obtained as yellowish gummy solid. Its molecular formula $C_{23}H_{22}O_6$ was based on the positive HRESIMS (m/z 417.1293 $[M + Na]^+$; calcd 417.1314), supported by ^1H -, ^{13}C -NMR spectral data. The UV spectrum of 112 showed maximum absorption band at 274 nm which is characteristic of conjugated double bonds. The IR spectrum showed absorption peaks at 3393, 2937 and 1598 cm^{-1} for OH, C-H aliphatic and C=C aromatic stretching vibrations respectively.

The ^1H -NMR spectrum (Table 10.1) of 112 showed the presence of an AX aromatic system (ring A) with two meta coupled protons at δ_H 6.78 (1H, d, $J = 2.0$ Hz, H-6) and δ_H 6.90 (1H, d, $J = 2.0$ Hz, H-8) including a methoxy group at δ_H 3.97 (3H, s, OCH_3 -5) and an AMX aromatic system (ring B) appeared at δ_H 6.96 (1H, d, $J = 2.0$ Hz, H-2') and δ_H 6.97 (2H, m, H-5', H-6') along with a methoxy group at δ_H 3.92 (3H, s, OCH_3 -3'). The ^1H -NMR spectrum further showed aromatic proton signals at δ_H 7.32 (1H, m,

H-4''), δ_{H} 7.41 (2H, m, H-3'') and δ_{H} 7.55 (2H, m, H-2'') indicating the presence of a phenyl group (ring D). An additional three carbon sequence; $\text{CH}(\text{O})\text{CH}(\text{O})\text{CH}_2\text{O}$ was deduced by the presence of a doublet resonating at δ_{H} 5.01 ($J = 8.0$ Hz), multiplets at δ_{H} 4.07, 3.59 and 3.90, which were assigned to H-2, H-3 and germinal methylene protons H-9, respectively.

Proton-proton correlation spectroscopy (^1H - ^1H -COSY), heteronuclear multiple bond correlations (HMBC) and heteronuclear 2-bond correlations (H2BC) spectra were helpful in establishing the presence of a phenyl propane⁴³² unit in 112. The two deshielded signals at δ_{H} 5.05, a typical benzylic methine and δ_{H} 4.07 coupled to each other, implying the existence of a 1, 4-dioxane moiety (ring C) between two phenyl rings.⁴³²⁻⁴³⁴

The ^{13}C -NMR (BB and DEPT) spectra (Table-10.1) of 112 showed two methyls (both oxygenated), one methylene (oxygenated), twelve methines (two oxygenated), and eight quaternary carbons (five oxygenated). Amongst these, notable assignments included the downfield signals at δ_{C} 61.7, 76.5 and 78.7 for the oxygenated carbons of propane moiety [$\text{CH}(\text{O})\text{CH}(\text{O})\text{CH}_2\text{O}$] and carbon shift values at δ_{C} 56.4 and 56.2 for the methoxys of ring A and B respectively.

The fragmentation (Figure-10.2) of compound 112 in the LC-ESIMS also gave some clues about the basic skeleton of the molecule. Although very few fragment ions were seen in the ESI mass spectrum, the observed fragments were supportive of the structure deduced from NMR spectroscopic data. The fragment ion at m/z 363 [$\text{M} - \text{H}_2\text{COH}$]⁻ supported the presence of the hydroxymethylene group in the compound while the fragment ion at m/z 375 [$\text{M} - 1 - \text{H}_2\text{O}$]⁻ was an indication of the loss of water molecule from the same hydroxymethylene group. Finally the mass peak at

m/z 179 $[M - 1 - C_{13}H_{12}O_3]^+$ implied a retro-Diels-Alder type of fragmentation in 1, 4-dioxane ring (ring A) of the predicted structure.

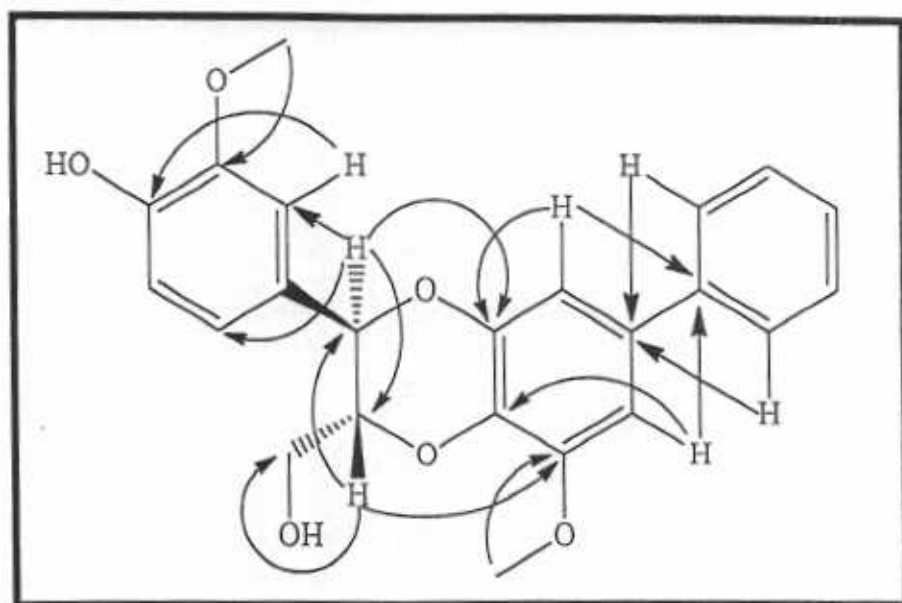


Figure-10.1: Selected HMBC and H2BC correlations of sorlanin (112)

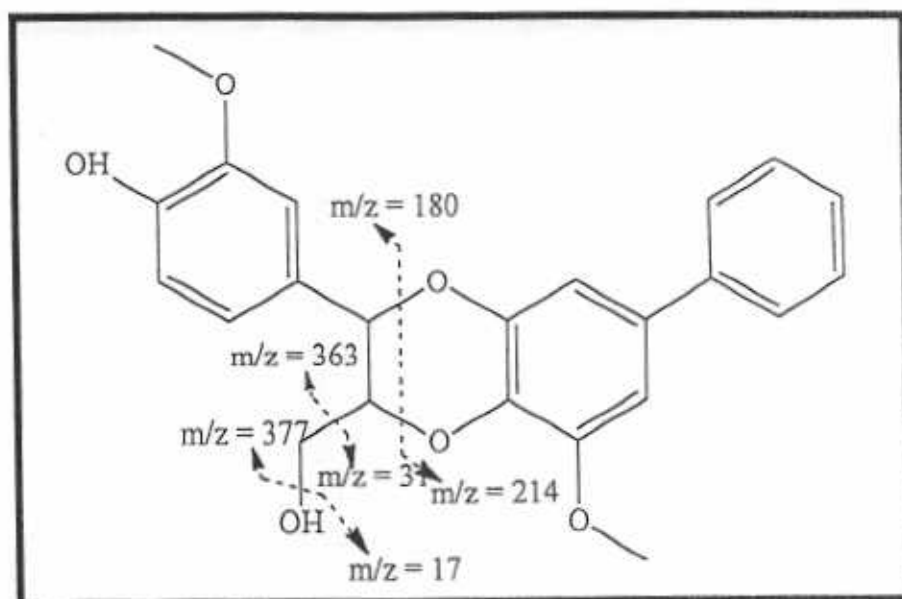


Figure-10.2: Mass fragmentation pattern of sorlanin (112)

The above observations summarize that 112 contained a phenyl group bridged to a phenylpropane unit through a 1, 4-dioxane moiety. The linkage between the two phenyl groups through a 1, 4-dioxane bridge was impossible to determine from an HMBC spectrum taken at normal J -value (8Hz). Therefore, the J value [$^2, ^3J(C, H)$] for a long-range coupling was reduced to 4 Hz and only then the HMBC cross-peaks for H-2/C-8a and H-3/C-5 were observed.⁴³⁵

The stereochemistry of H-2 and H-3 around the chiral centers of the dioxane ring was deduced to be trans using the value of coupling constant ($J = 8.0$ Hz) and then confirmed through a two dimensional Nuclear Overhauser Effect (NOE) NMR experiment.⁴³²⁻⁴³⁴ Thus the structure of compound 112 was decided to be 4-((2R,3R)-3-(hydroxymethyl)-5-methoxy-7-phenyl-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-2-methoxyphenol.

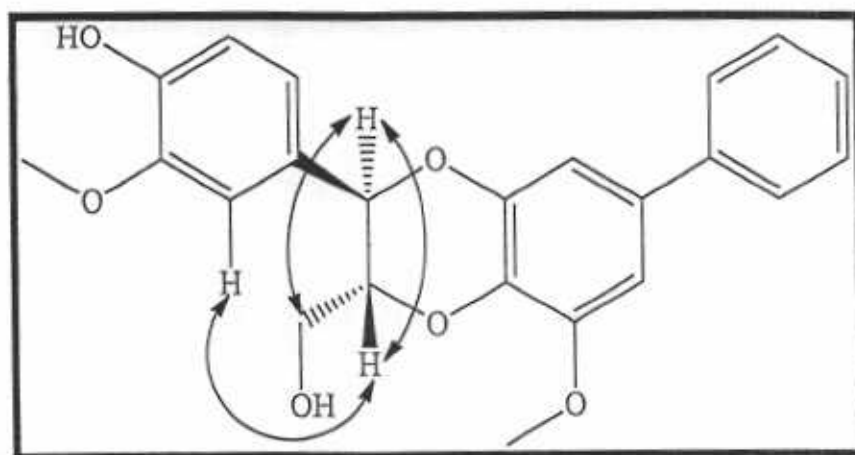


Figure-10.3: Selected NOESY correlations of sorlanin (112)

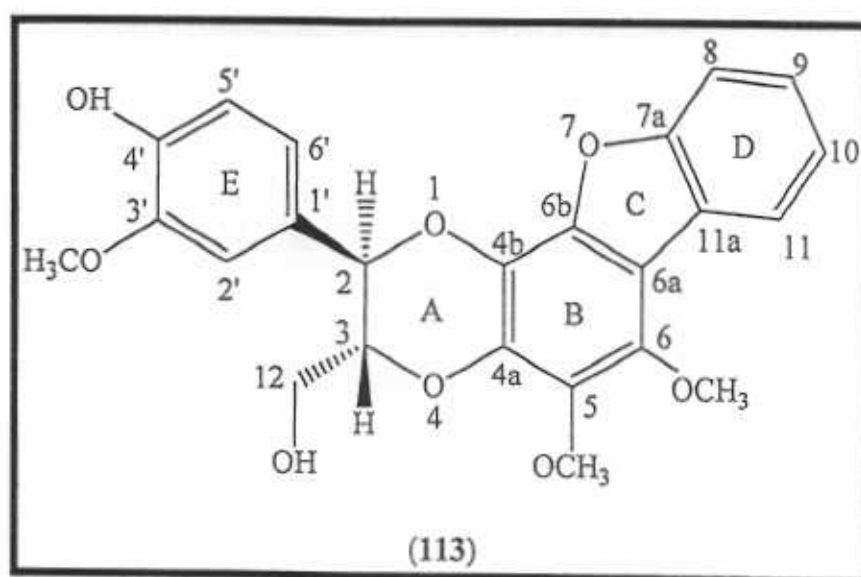
Table-10.1: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of sorlanin (112) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
2	76.5	CH	5.01, d ($J = 8.0$)
3	78.7	CH	4.07, m
4a	132.7	C	-
5	149.0	C	-
5-OCH ₃	56.4	CH ₃	3.97 (3H, s)
6	103.6	CH	6.78, d ($J = 2.0$)
7	134.2	C	-
8	108.8	CH	6.90, d ($J = 2.0$)
8a	144.8	C	-
9	61.7	CH ₂	3.59, m 3.90, m
1'	128.2	C	-
2'	109.8	CH	6.96, m
3'	147.0	C	-
3'-OCH ₃	56.2	CH ₃	3.92, s
4'	146.5	C	-
5'	114.8	CH	6.97, m
6'	120.9	CH	6.97, m
1''	140.9	C	-
2''	127.0 x 2	2 x CH	7.55, m
3''	128.9 x 2	2 x CH	7.41, m
4''	127.2	CH	7.32, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.1.2. Peshawerin (113)

Compound (113) was isolated as yellowish amorphous powder. Its molecular formula $\text{C}_{24}\text{H}_{22}\text{O}_8$ was deduced from HRESIMS ($[\text{M} + \text{Na}]^+$ at m/z 461.1202; calcd 461.1315), indicating fourteen degrees of unsaturation. The UV spectrum showed characteristic absorptions of dibenzofurans at 230, 262 and 291 nm.⁴³⁶ The IR spectrum indicated absorption maxima for the presence of hydroxyl (3401 cm^{-1}), C-H aliphatic (2935 cm^{-1}) and aromatic stretching vibrations (1605 cm^{-1}).



The ^1H -NMR data (Table-10.2) of 113 was quite similar to compound 112 with respect to the presence of signals for a phenylpropane moiety. The presence of an AMX aromatic system (ring E) was deduced from the proton chemical shifts at $\delta_{\text{H}} 6.96\text{--}7.01$ (3H, m, H-2', H-5' and H-6'), including methoxy group at $\delta_{\text{H}} 3.93$ (3H, s, $\text{OCH}_3\text{-}3'$). The three protons of the AMX system (ring E) appeared at very close chemical shift values and were assigned to their respective carbons on the basis of heteronuclear single-quantum correlation (HSQC). The presence of a three carbon sequence $[\text{CH}(\text{O})\text{CH}(\text{O})\text{CH}_2\text{O}]$ of a propane moiety was deduced by the observation of ^1H -NMR signals resonating at $\delta_{\text{H}} 5.06$ (1H, d, H-2), $\delta_{\text{H}} 4.10$ (1H, m, H-3), $\delta_{\text{H}} 3.65$ (1H, m, $\text{H}_a\text{-}12$) and $\delta_{\text{H}} 3.93$ (1H, m, $\text{H}_b\text{-}12$). Moreover, six additional signals appeared in the proton spectrum (Table-10.2) at $\delta_{\text{H}} 4.08$ (3H, s, $\text{OCH}_3\text{-}5$), $\delta_{\text{H}} 4.13$ (3H, s, $\text{OCH}_3\text{-}6$), $\delta_{\text{H}} 7.53$ (1H, m, H-8), $\delta_{\text{H}} 7.40$ (1H, m, H-9), $\delta_{\text{H}} 7.32$ (1H, m, H-10) and $\delta_{\text{H}} 8.06$ (1H, m, H-11). These signals were identical to the proton signals of β -pyrofuran, a dibenzofuran previously isolated from *Pyrus communis*.⁴³⁶

The ^{13}C -NMR (BB and DEPT) spectrum (Table-10.2) of compound 113 showed twenty four carbons including three methoxys, one hydroxymethylene, nine methines

(two oxygenated), and eleven quaternary carbons (eight oxygenated). The carbon signals at δ_C 61.9, 76.7 and 78.1 were assigned to the oxygenated carbons of propane moiety $[\text{CH}(\text{O})\text{CH}(\text{O})\text{CH}_2\text{O}]$. The downfield resonances at δ_C 142.4 (C-6b) and 156.2 (C-7a) were due the two carbons of the dibenzofuran bridged through an oxygen atom.

A close correlation was found between the ^{13}C -NMR shift values of 113 and 1,2, 4-trimethoxydibenzofuran-3-ol (β -pyrofuran)⁴³⁶ with two main differences in chemical shifts at δ_C 137.7 (C-4a) and δ_C 120.4 (C-4b). These two assignments require the carbons (both 4a and 4b) to be oxygenated in addition to the two adjacent carbons (C-5 and C-6) bearing the methoxy groups on the same ring.

The fragmentation pattern (Figure-10.4) of 113 was almost similar to compound 112. The fragment ion at m/z 407 $[\text{M} - 1 - \text{HCOH}]^-$ supported the presence of the hydroxymethylene group in the compound while the fragment ion at m/z 419 $[\text{M} - 1 - \text{H}_2\text{O}]^-$ was an indication to the loss of water molecule from the same hydroxymethylene group. Finally the mass peak at m/z 179 $[\text{M} - 1 - \text{C}_{14}\text{H}_{10}\text{O}_5]^-$ implied a retro-Diels-Alder type of fragmentation in the 1, 4-dioxane ring (ring A) of the predicted structure.

HMBC NMR experiment was used in confirming the presence of various fragments and their connectivity in the molecule. The HMBC correlations (Figure-10.5) of methylenic proton at δ_H 5.06 (H-2) to the aromatic ring (ring E) on one hand and to the carbons of the propane group on the other hand, helped in establishing the phenylpropane part of the molecule. Since it was not possible to establish the linkage of the dibenzofuran and phenylpropane units from a normal HMBC-NMR experiment carried out at $J = 8.0$ Hz. Therefore, the NMR experiment was run for the compound

113 after optimizing the J value for a long-range correlation to 4.0 Hz and a cross-peak for H-3/C-4a was observed. Also, long-range correlations were found for the protons of the two methoxys to C-5 and C-6 on ring B.

These observations led us to propose that compound 113 is a dibenzofuran-phenylpropane connected through a 1, 4-dioxane ring. As in case of 112, the *trans*-stereochemistry of the protons around the chiral centers of 1, 4-dioxane moiety was deduced from J -value comparison and 2D-NOESY NMR experiment (Figure-10.6). The coupling constant ($J = 8.2$ Hz) and the NOE correlation between H-3 and H-2' clearly shows the *trans*-configuration of the chiral centers of the 1, 4- dioxane ring.

The structure of peshawerin was thus characterized as 4-((2*R*,3*R*)-3-(hydroxymethyl)-5,6-dimethoxy-2,3-dihydrobenzo[*b*][1,4]dioxino[2,3-*g*]benzofuran-2-yl)-2-methoxyphenol. A large number of natural products have been found containing dioxane moiety.⁴³²⁻⁴³⁴ To our knowledge, peshawerin (113) is the first example of a dibenzofuran linked through a dioxane ring.

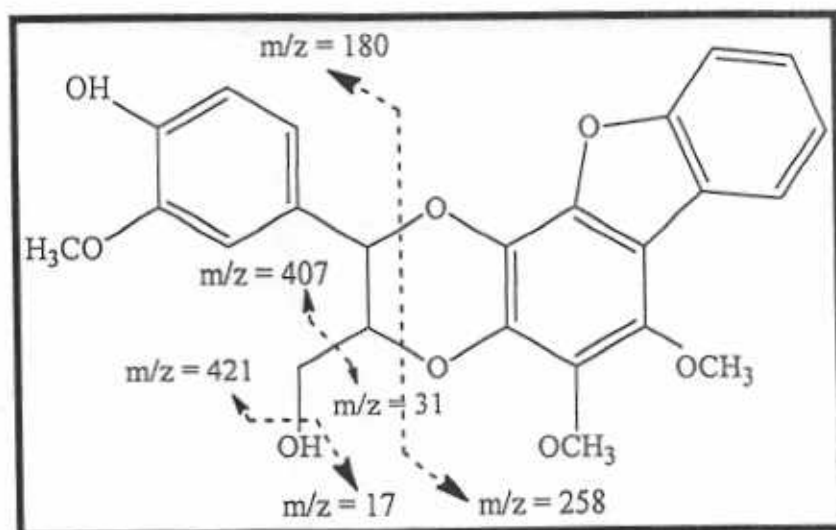


Figure- 10.4: Mass fragmentation pattern of peshawerin (113)

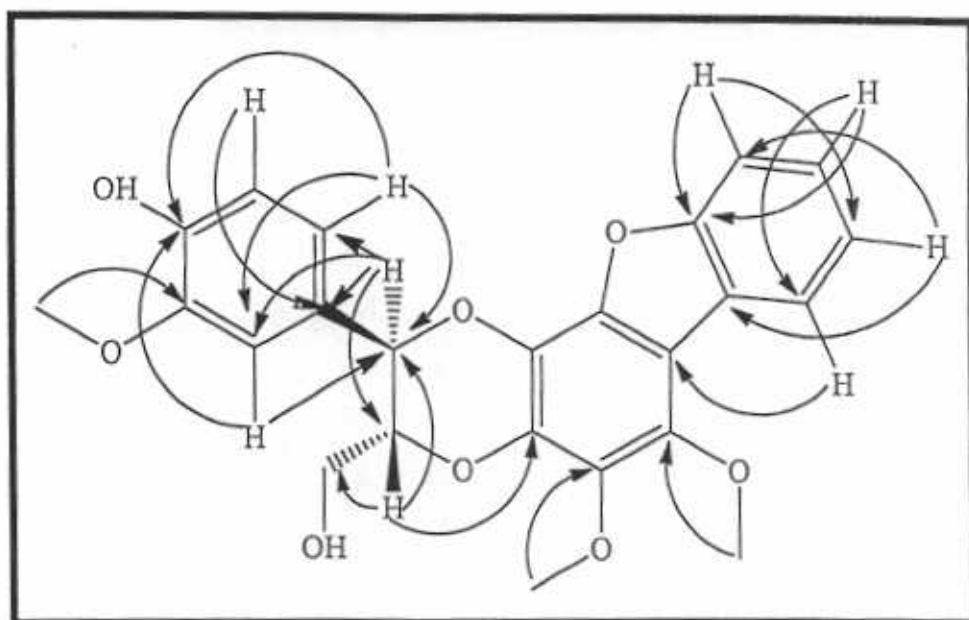


Figure-10.5: Selected HMBC correlations of peshawerin (113)

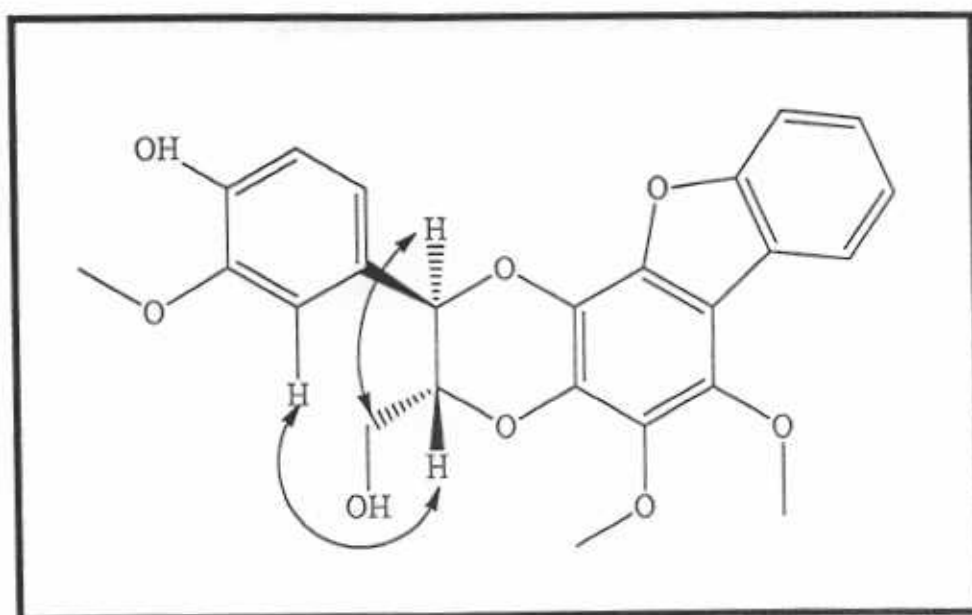


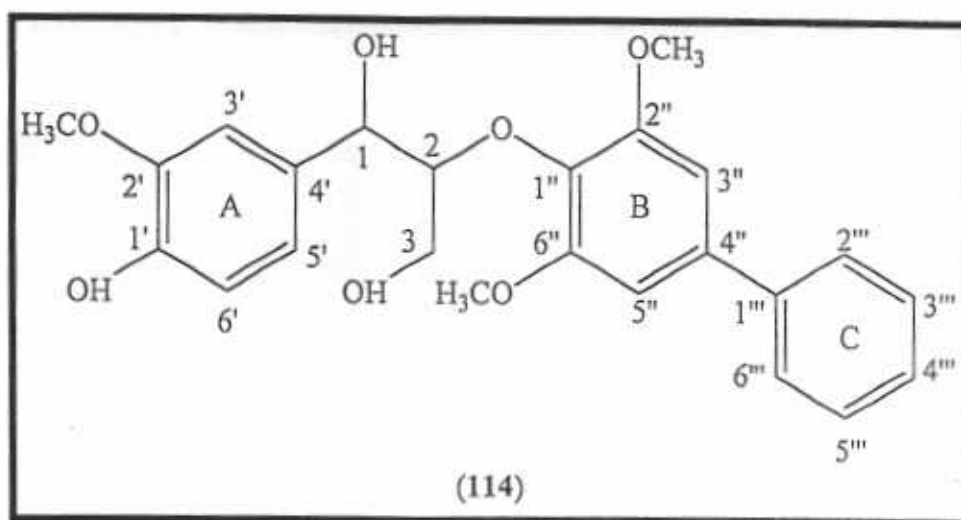
Figure-10.6: Selected NOESY correlations of peshawerin (113)

Table-10.2: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of peshawerin (113) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
2	76.7	CH	5.06, d ($J = 8.2$)
3	78.1	CH	4.10, m
4a	137.7	C	-
4b	120.4	C	-
5	137.8	C	-
5-OCH ₃	61.3	CH ₃	4.08 (3H, s)
6	130.4	C	-
6a	111.7	C	-
6b	142.4	C	-
6-OCH ₃	61.6	CH ₃	4.13 (3H, s)
7a	156.2	C	-
8	111.4	CH	7.53, m
9	126.2	CH	7.40, m
10	123.0	CH	7.32, m
11	122.3	CH	8.06, m
11a	123.6	C	-
12	61.9	CH ₂	3.65, m 3.93, m
1'	128.0	C	-
2'	109.9	CH	6.99, m
3'	147.0	C	-
3'-OCH ₃	56.2	CH ₃	3.93 (3H, s)
4'	146.6	C	-
5'	114.8	CH	6.96, m
6'	121.0	CH	7.01, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.1.3. Sorbanin (114)



Sorbanin (114) was obtained as a white amorphous solid with the molecular formula $C_{24}H_{26}O_7$ deduced from HRESIMS (m/z 449.1563 $[M+Na]^+$; calcd 449.1576) indicating twelve degrees of unsaturation. The UV spectrum of 114 showed maximum absorption of 267 nm, a characteristic of conjugated double bonds. The IR spectrum displayed absorptions at 3395, 2932 and 1600 cm^{-1} , which are characteristic for OH, C-H aliphatic and C=C aromatic stretching vibrations respectively.

The ^1H -NMR spectrum of 114 was rich in signals for aromatic protons. Three types of aromatic patterns could be deduced from the NMR data (Table-10.3) i.e. a phenyl group, an AMX type and the one with symmetrical substitution on it. The protons of the phenyl group showed multiplets resonating at δ_H 7.37 (1H, H-4'''), δ_H 7.45 (2H, H-3''', 5''') and δ_H 7.55 (2H, H-2''', 6''').⁴³⁷ The presence of an AMX aromatic system was rationalized from proton resonances at δ_H 6.90 (1H, d, $J = 8.0\text{ Hz}$, H-5'), δ_H 6.98 (1H, dd, $J = 8.0, 2.0\text{ Hz}$, H-6') and δ_H 7.00 (1H, d, $J = 2.0\text{ Hz}$, H-2') including an aromatic methoxy at δ_H 3.91 (3H, s, $\text{CH}_3\text{O}-3'$). The shielded isolated singlet at δ_H 6.82 (2H, s, H-3'', 5'') and the proton signals of two aromatic methoxyl groups resonating

as a singlet at δ_H 3.97 (6H, $\text{CH}_3\text{O}-2'', 6''$) implied the existence of a symmetrically substituted aromatic ring.⁴¹⁵ In addition to aromatic signals, non-aromatic resonances were also observed in the proton spectrum. These signals are δ_H 5.07 (1H, $d, J = 9.0$ Hz, H-1), δ_H 3.94 (1H, m, H-2), δ_H 3.35 (1H, m, H-3) and δ_H 3.63 (1H, m, H-3). The deshielded signal appearing at δ_H 5.07 (H-1), a typical of an oxygenated benzylic methine, was found coupled to the multiplet at δ_H 3.94 (H-2) which in turn was coupled to the geminal protons of an oxygenated methylene at δ_H 3.35 (H-3) and δ_H 3.63 (H-3). The intercoupling of these protons was confirmed from COSY spectrum as well. Thus the presence of a three carbon sequence $[\text{CH}(\text{O})\text{CH}(\text{O})\text{CH}_2\text{O}]$ similar to compounds 112 and 113 discussed in sections 10.1.1 and 10.1.2. The coupling constant between H-1 and H-2 was 9.0 Hz, demonstrating *trans*-oriented hydrogen atoms. The signals most probably arising from the protons of the hydroxyl groups in the molecule were observed at δ_H 4.35 and δ_H 5.59 in the ^1H -NMR spectrum.

The ^{13}C -NMR (BB and DEPT) spectra (Table-10.3) of 114 exhibited carbon resonances for three oxygenated methyls, one oxygenated methylene, twelve methines, and eight quaternary carbons. The carbon shift values appearing at δ_C 74.3, 89.4 and 60.8 were assigned to C-1, C-2 and C-3 of the propane moiety of the molecule based on HSQC NMR correlations to H-1, H-2 and H-3, respectively. The double intensity of carbon signals at δ_C 104.6 and 153.3 for the two pairs of carbons i.e. C-3'', C-5'' and C-2'', C-6'' respectively, was apparent from the ^{13}C -NMR spectrum. The carbons of a phenyl substituent were characterized by the chemical shift sequence of the downfield signals at δ_C 127.8 (C-4'''), δ_C 129.0 (C-3''', C-5''') and δ_C 127.2 (C-2''', C-6''') including a quaternary carbon at δ_C 141.1 (C-1''').⁴³⁷ The signals characteristic of 4-C-substituted-2-methoxyphenol included three aromatic

methines at δ_C 109.9 (C-3'), 120.6 (C-5') and 114.4 (C-6') along with three quaternary carbons at δ_C 145.6 (C-1'), 146.6 (C-2') and 132.1 (C-4').

The structure of 114 was substantiated by various mass fragments observed in ESIMS. The mass fragment ions at m/z 195 [$C_{10}H_{11}O_4$]⁺, 229 [$C_{14}H_{13}O_3$]⁺, 271 [$C_{16}H_{15}O_4$]⁺ and 409 [$C_{24}H_{25}O_6$]⁺ could be rationalized for the breakage of the molecule at various positions as shown in the **Figure-10.7**. The molecular formula of 114 as deduced from HRESIMS is $C_{24}H_{26}O_7$, which shows twelve degrees of unsaturation i.e. one degree less unsaturated than compound 112 discussed in section 10.1.1, thereby indicating one of the rings to be open. It would not be unreasonable to think of an open 1,4-dioxane ring. The 1,4-dioxane ring seemed to be broken between positions 3 and 4 (-O-CH₃) which can be rationalized from the presence of methoxys at C-2'' and C-6''.

The ^1H - ^1H COSY and HMBC NMR spectral data (**Figure-10.8**) confirmed the fragments and assignments. The symmetrical substitution pattern of syringyl system⁴¹⁵ (ring B) in the molecule was decided based on the strong correlations of the two protons singlet at δ_H 6.82 (H-3'', 5'') to the carbon at δ_C 134.9 (C-1'') and δ_C 104.6 (C-3'', 5'') and weak correlations to carbons at δ_C 138.4 (C-4'') and 153.3 (C-2'', 6''). The presence of a phenyl group as part of the symmetrically substituted aromatic ring (ring B) was validated by the HMBC cross-peak for the protons at δ_H 7.55 (2H, m, H-2''', 6''') with C-4'' (δ_C 138.4), whereas the two methoxys were fixed to C-2'' and C-6'' for a long-range correlation between the six protons singlet at δ_H 3.97 (CH₃O-2'', 6'') to the double intensity of carbon at δ_C 153.3 (C-2'', 6'').

From the normal ^1H - and ^{13}C -NMR (BB and DEPT) spectral data as well as the H-H and H-C connectivities observed in the COSY, NOESY and HMBC, the structure of

114 was characterized as 2-((3,5-dimethoxy-[1,1'-biphenyl]-4-yl)oxy)-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol.

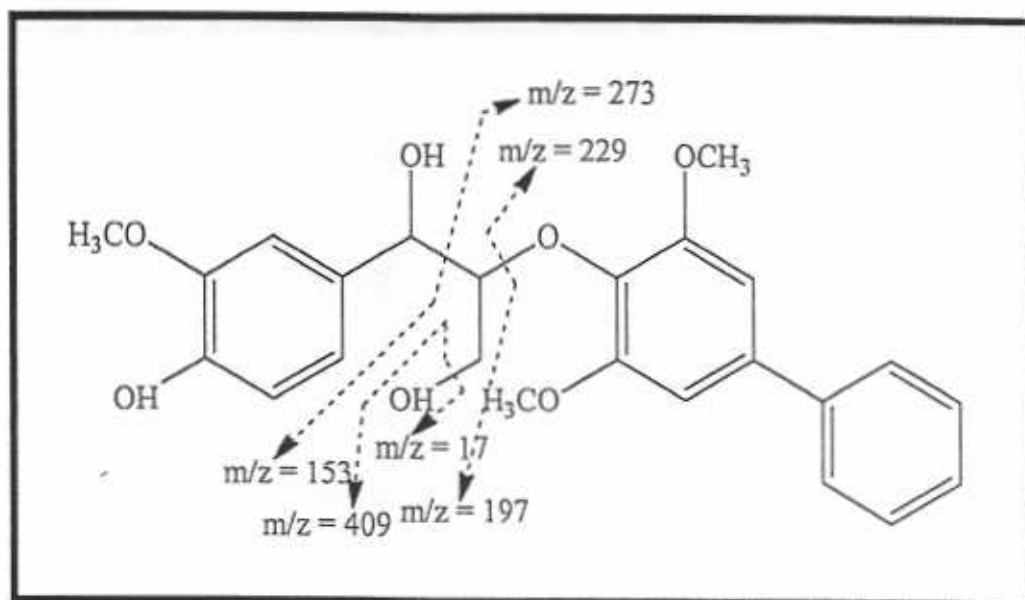


Figure-10.7: Mass fragmentation pattern of sorbanin (114)

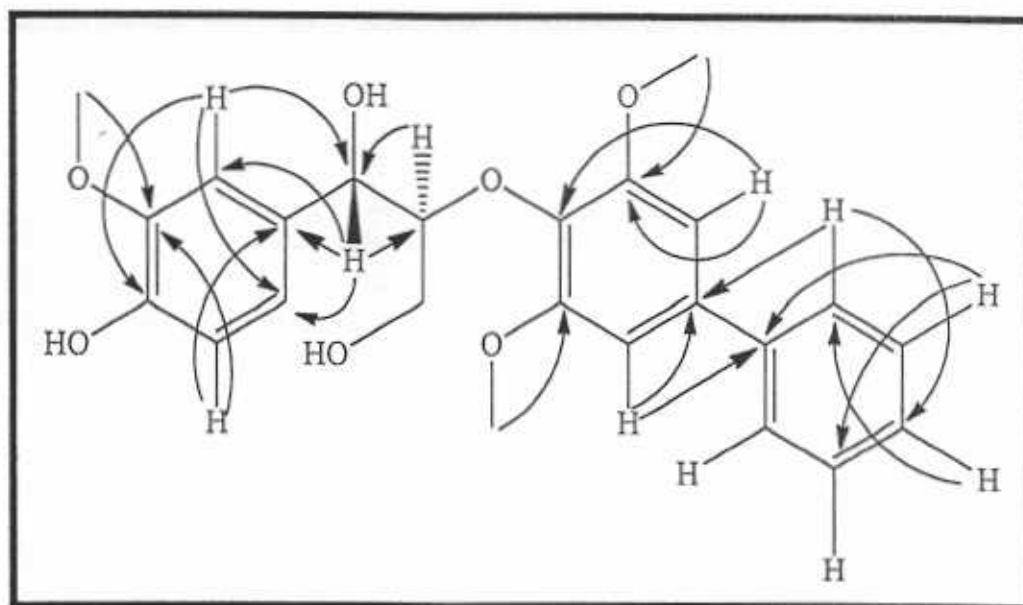


Figure-10.8: Selected HMBC correlations of sorbanin (114)

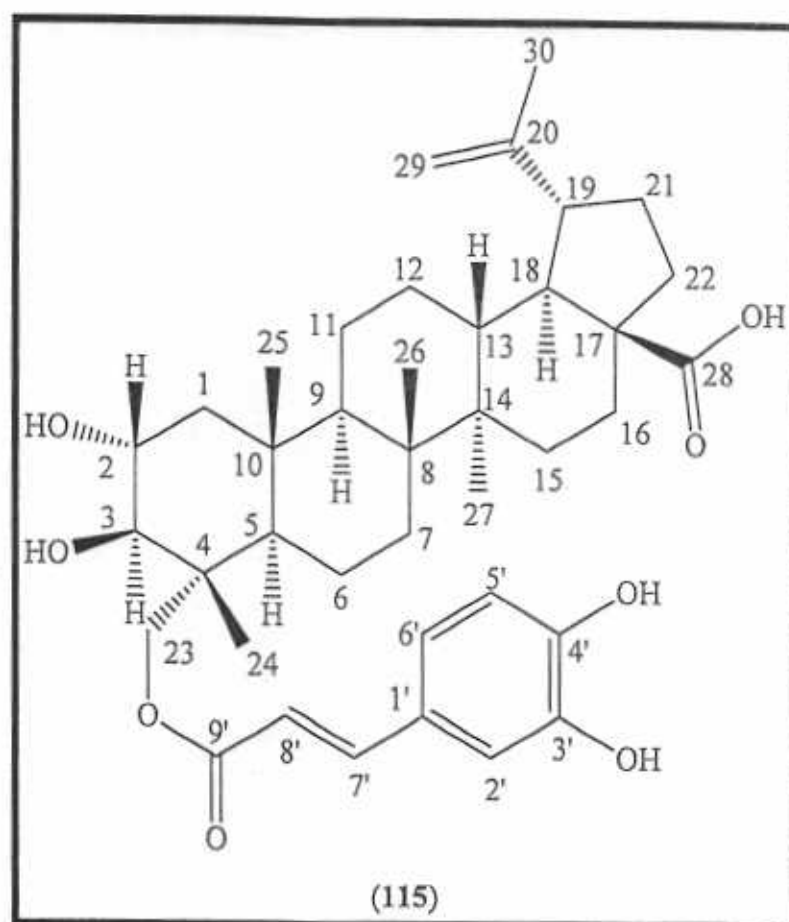
Table-10.3: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of sorbanin (114) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	76.7	CH	5.07, d ($J = 9.0$)
2	89.4	CH	3.94, m
3	61.9	CH ₂	3.35, m 3.63, m
1'	145.6	C	-
2'	146.6	C	-
2'-CH ₃ O	56.1	CH ₃	3.91, s
3'	109.9	CH	7.00, d ($J = 2.0$)
4'	132.1	C	-
5'	120.6	CH	6.98, dd ($J = 8.0$)
6'	114.4	CH	6.90, d ($J = 2.0$)
1''	134.9	C	-
2''	153.3	C	-
2''-CH ₃ O	56.4	CH ₃	3.97, s
3''	104.6	CH	6.82, s
4''	138.4	C	-
5''	104.6	CH	6.82, s
6''	153.3	C	-
6''-CH ₃ O	56.4	CH ₃	3.97, s
1'''	141.1	C	-
2'''	127.2	CH	7.55, m
3'''	129.0	CH	7.45, m
4'''	127.8	CH	7.37, m
5'''	129.0	CH	7.45, m
6'''	127.2	CH	7.55, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.1.4. Sorbanolic acid (115)

Compound 115 was obtained as a white amorphous powder. Its molecular formula was determined to be $\text{C}_{39}\text{H}_{54}\text{O}_8$ by positive mode HRESIMS ($[\text{M} + \text{Na}]^+$ m/z 673.3712; calcd 673.3716). The UV spectrum exhibited absorption maxima at 218 and 329 nm, suggesting the presence of an aromatic ring in the molecule, while the IR spectrum displayed characteristic absorptions bands caused by hydroxyl (3358 cm^{-1}), α,β -unsaturated carbonyl (1686 cm^{-1}), and aromatic (1602 and 1516 cm^{-1}) functionalities.



The ^1H -NMR spectrum (Table-10.4) of **115** indicated the presence of four tertiary methyls at δ_{H} 0.79 (CH_3 -24), 0.96 (CH_3 -25), 0.97 (CH_3 -26) and 0.99 (CH_3 -27), two hydroxymethine protons at δ_{H} 3.32 (1H, d, $J = 9.5$ Hz, H-3) and 3.70 (1H, m, H-2), a hydroxymethylene group at δ_{H} 4.00 and 4.09 (each 1H, d, $J = 11.2$ Hz, H-23). The proton signals appearing at δ_{H} 4.59 (1H, dd, $J = 2.3, 1.3$ Hz, H_{a} -29), 4.72 (1H, d, $J = 2.3$ Hz, H_{b} -29) and 1.71 (3H, s, CH_3 -30)⁴³⁸ in the ^1H -NMR spectrum (Table-10.4) and δ_{C} 110.0 (C-29) and δ_{C} 151.6 (C-20) in the ^{13}C -NMR indicated the presence of an isopropylidene moiety. Furthermore, three oxygen bearing carbons at δ_{C} 68.9 (C-2), 77.5 (C-3) and 66.1 (C-23) were observed in the ^{13}C -NMR spectrum, which clearly indicated that the triterpenoidal moiety was a derivative of betulinic acid. The presence of three extra aromatic protons signals at δ_{H} 7.04 (1H, dd, $J = 8.2, 2.1$ Hz, H-6'), δ_{H} 6.88 (1H, d, $J = 8.2$ Hz, H-5') and δ_{H} 7.17 (1H, d, $J = 2.1$ Hz, H-2') and *trans*-

olefinic protons signals at δ_H 6.32 (1H, d, $J = 15.9$ Hz, H-8') and δ_H 7.57 (1H, d, $J = 15.9$ Hz, H-7') were in agreement with signals of caffeic acid.

The ^{13}C -NMR (BB and DEPT) spectra (Table-10.4) of **115** showed 39 carbons indicating five methyls, eleven methylenes, twelve methines and eleven quaternary carbon atoms. The presence of two carbonyl carbons i.e. the ester and the acid, were confirmed from their signals resonating at δ_C 167.3 (C-9') and 177.5 (C-28), respectively, while the signals resonating at δ_C 151.6 (C-20) and 110.0 (C-29) implied the existence of the isopropylidene group in the molecule. The carbons resonating at downfield shift values of δ_C 68.9 (C-2) and 77.5 (C-3) were indicative of the presence of hydroxyl groups on them.

The NMR assignments and connectivities of **115** were done by COSY, HSQC, HMBC and NOESY spectral data analyses. The location of the *trans*-caffeoyl moiety at C-23 (δ_C 66.1) was deduced from long-range HMBC (Figure-10.9) correlations between the geminal protons at δ_H 4.00 (H_a-23) and 4.09 (H_b-23) to the carbonyl signal at δ_C 167.3, and to C-3, C-4 and C-5. The *trans*-olefinic protons at δ_H 6.32 (H-8') and δ_H 7.57 (H-7') also showed correlations to C-9'. The relative *trans*-configuration of H-2 and H-3, was supported by coupling constant ($J = 9.5$ Hz) and 2D-NOESY experiment wherein NOEs were seen between H-2 (δ_H 3.70) and H-24 (δ_H 0.79)/H-25 (δ_H 0.97), between H-3 (δ_H 3.32) and H-5 (δ_H 1.31). No NOE cross peak was observed between H-2 and H-3, which confirms that the two protons are fully staggered to each other. The stereochemistry around all the carbons atoms was done on the basis of 2D-NOESY NMR data (Figure-10.10). The spectral data (^1H and ^{13}C -NMR) as well as proton-proton and proton-carbon connectivities observed in the NOESY, COSY and HMBC spectra suggested that the structure of **115** as 2 α ,3 β ,23-

trihydroxy-lup-20(29)en-28-oic acid-23-caffeate, which is basically a caffeic acid ester of 23-dihydroxy-betulinic acid with one additional hydroxyl group at C-2.

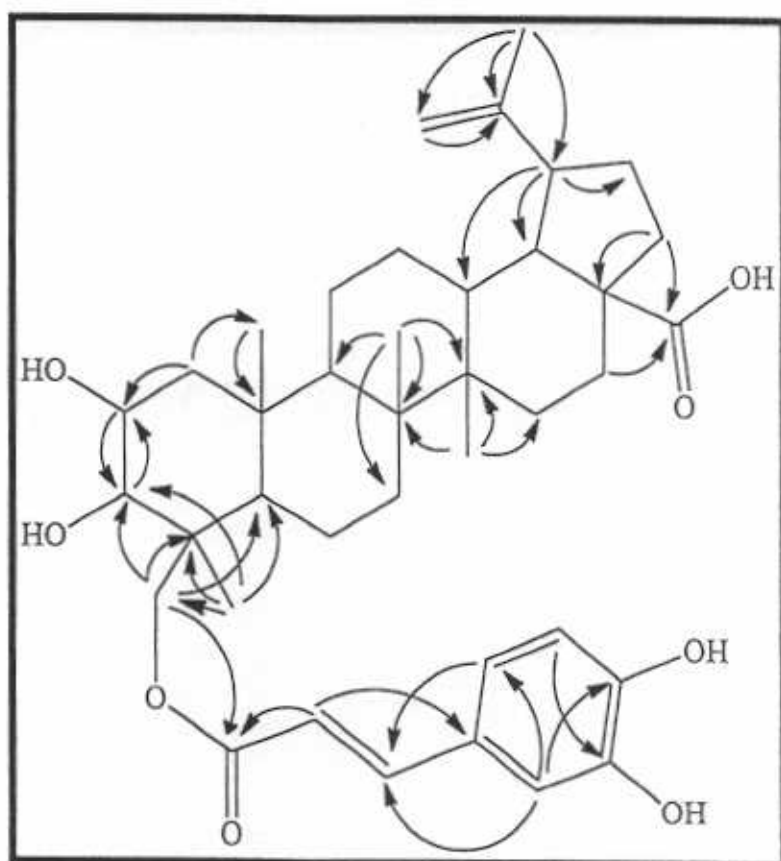


Figure-10.9: Selected HMBC correlations of sorbanolic acid (115)

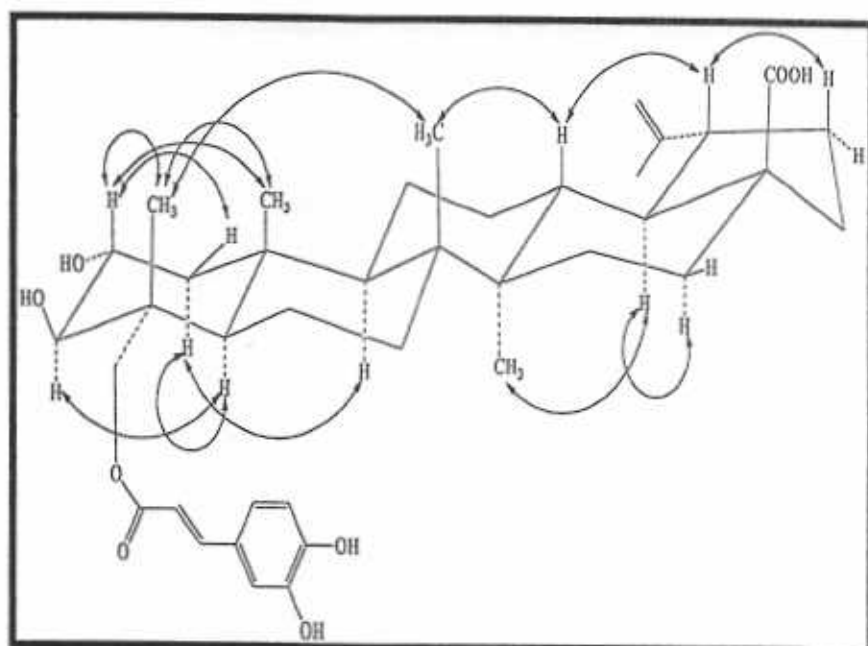


Figure-10.10: Selected NOESY correlations of sorbanolic acid (115)

Table-10.4: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of (115) in CD_3COCD_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	48.7	CH_2	2.01, dd ($J = 10.0, 5.0$) 0.88, m
2	68.9	CH	3.70, m
3	77.5	CH	3.32, d ($J = 9.5$ Hz)
4	43.1	C	-
5	48.8	CH	1.31, m
6	18.9	CH_2	1.46, m
7	34.9	CH_2	1.34, m 1.45, m
8	41.6	C	-
9	51.6	CH	1.46, m
10	38.9	C	-
11	21.9	CH_2	1.28, m 1.47, m
12	26.3	CH_2	1.08, m 1.74, m
13	39.0	CH	2.35, m
14	43.2	C	-
15	30.4	CH_2	1.14, m 1.52, m

Continued on next page

Table 10.4 continued.

16	32.8	CH ₂	1.43, m 2.22, m
17	56.8	C	-
18	49.9	CH	1.62, t ($J = 11.32$, 11.32)
19	47.9	CH	3.05, m
20	151.6	C	-
21	31.4	CH ₂	1.38, m 1.91, m
22	37.5	CH ₂	1.47, m 1.92, m
23	66.1	CH ₂	4.00, d ($J = 11.2$) 4.09, d ($J = 11.2$)
24	13.6	CH ₃	0.79, s
25	18.3	CH ₃	0.97, s
26	16.6	CH ₃	0.96, s
27	14.9	CH ₃	0.99, s
28	177.5	C	-
29	110.0	CH ₂	4.59, dd ($J = 2.4$, 1.4) 4.72, d ($J = 2.4$)
30	19.5	CH ₃	1.71, s
1'	127.6	C	-
2'	115.3	CH	7.17, d ($J = 2.2$)
3'	146.4	C	-
4'	148.8	C	-
5'	116.5	CH	6.88, d ($J = 8.2$)
6'	122.5	CH	7.04, dd ($J = 8.2$, 2.2)
7'	145.6	CH	7.57, d ($J = 15.9$)
8'	115.8	CH	6.32, d ($J = 15.9$)
9'	167.3	C	-

a. Broad Band; b. DEPT; c. ¹H-NMR; d. HSQC interactions

m) implied the existence of two hydroxymethylene groups i.e. (8-CH₂OH) and (8'-CH₂OH), respectively.

The ¹³C-NMR (BB and DEPT) data (Table-10.5) showed a double intensity of carbons at δ_C 148.4 (C-3 and C-5) and 107.3 (C-2 and C-6), which indicated them being a part of the symmetrically substituted aromatic ring. The carbon resonances at δ_C 147.7 and 147.2 were assigned to the methoxyls bearing carbons i.e. C-3' and C-5', respectively. The carbons at δ_C 64.0 (C-9) and 66.6 (C-9') were classified as hydroxy substituted methylenes based on DEPT spectral analysis.

The complete structure of compound 116 was arrived at, by using 2D-NMR techniques such as COSY, HSQC and HMBC and NOESY, in addition to the 1D-NMR experiments. A comparison of its spectral data (¹H- & ¹³C-NMR) and other physical constants, 116 was identified as polystachyol isolated from the plant *Aphanamixis polystachya*.⁴³⁹

Table-10.5: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (116) in CD_3COCD_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	139.4	C	-
2	107.3	CH	6.43, s
3	148.4	C	-
3-OCH ₃	56.8	CH ₃	3.73, s
4	135.0	C	-
5	148.4	C	-
5-OCH ₃	56.8	CH ₃	3.73, s
6	107.3	CH	6.43, s
7	42.7	CH	4.26, d ($J = 6.4$)
8	49.4	CH	1.96, m
9	64.0	CH ₂	3.57, m
1'	130.0	C	-
2'	107.2	CH	6.56, s
3'	147.7	C	-
3'-OCH ₃	56.4	CH ₃	3.83, s
4'	138.6	C	-
5'	147.2	C	-
5'-OCH ₃	59.5	CH ₃	3.35, s
6'	126.5	C	-
7'	33.9	CH ₂	2.63, m
8'	41.3	CH	1.64, m
9'	66.6	CH ₂	3.62, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.2. Isolariciresinol (117)

Compound 117 was obtained as white amorphous powder from the ethyl acetate fraction. EIMS showed a molecular ion m/z 360 $[\text{M}]^+$ corresponding to the molecular formula $\text{C}_{20}\text{H}_{24}\text{O}_6$. The ^1H -NMR spectrum (Table-10.6) of 117 was quite identical to compound 116 discussed in section 10.2.1, except for the absence of two methoxyl groups. An AMX aromatic system was characterized from the proton signals at δ_{H} 6.75 (1H, d, $J = 2.0$ Hz, H-2), 6.77 (1H, d, $J = 8.0$ Hz, H-5) and 6.62 (1H, dd, $J = 8.0$, 2.0 Hz, H-6), including a methoxy at 3.79 (3H, s, CH_3O -3). Two singlets at δ_{H} 6.65 (1H, s, H-2') and δ_{H} 6.19 (1H, s, H-5') were assigned to the AX aromatic ring in the

Table-10.6: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (117) in CD_3COCD_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	139.0	C	-
2	114.1	CH	6.75, d ($J = 2.0$)
3	148.9	C	-
3-CH ₃ O	56.7	CH ₃	3.79, s
4	144.9	C	-
5	116.0	CH	6.77, d ($J = 8.0$)
6	123.4	CH	6.62, dd ($J = 8.0, 2.0$)
7	48.6	CH	3.80, m
8	48.8	CH	1.81, m
9	61.8	CH ₂	3.41, m 3.68, m
1'	127.6	C	-
2'	112.5	CH	6.65, s
3'	145.5	C	-
3'-CH ₃ O	57.0	CH ₃	3.75, s
4'	144.4	C	-
5'	117.4	CH	6.19, s
6'	133.2	C	-
7'	34.3	CH ₂	2.75, m
8'	41.0	CH	1.98, m
9'	66.4	CH ₂	3.71, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.3. Dihydrodehydrodiconiferyl alcohol (118)

The ^1H -NMR data (Table-10.7) of compound 118 showed three aromatic protons signals at δ_{H} 6.88 (1H, d, $J = 8.0$ Hz, H-5), δ_{H} 6.92 (1H, dd, $J = 8.0, 2.0$ Hz, H-6) and δ_{H} 6.95 (1H, d, $J = 2.0$ Hz, H-2), which were assigned to the three protons in a 1, 3, 4-trisubstituted benzene ring, while two more aromatic singlets appearing at δ_{H} 6.68 (1H, s, H-2') and 6.69 (1H, s, H-6') were assigned to the two protons in a 1, 3, 4, 5-tetrasubstituted benzene ring. The chemical shift values at δ_{H} 5.55 and 3.61 appearing in the ^1H -NMR spectrum were assigned to the protons of an oxygenated (H-7) and non-oxygenated (H-8) methine, respectively. The coupling constant between H-7 and H-8 was 7.3 Hz, which indicated a *trans*-configuration of the two protons. The

hydroxymethylene protons were configured from signals at δ_H 3.92 (1H, m, H_a-9), 3.98 (1H, m, H_b-9) and 3.71 (2H, t, $J = 6.4$ Hz, H-9'). The two methoxyl groups i.e. CH₃O-3 and CH₃O-3' resonated at δ_H 3.87 and 3.89, respectively, while upfield region of the proton spectrum displayed two more methylene groups at δ_H 2.68 (2H, m, H-7') and 1.91 (2H, m, H-8').

The ¹³C-NMR (BB and DEPT) spectrum (Table-10.7) indicated two hydroxymethylene carbons at δ_C 63.9 (C-9) and 62.3 (C-9'), whereas an oxygenated methine carbon was found at resonance value of δ_C 87.9 (C-7). Furthermore, the two methylenic carbons i.e. C-7' and C-8' resonated at δ_C 32.0 and 34.6, respectively. The resonance peaks for methine carbon (C-8) and two methoxys at position 3 and 3' were observed at carbon shift values at δ_C 53.8, 55.9 and 56.0, respectively.

In combination to ¹H and ¹³C-NMR data, two dimensional techniques like COSY, HSQC, HMBC and NOESY were used to establish the connectivities of the different fragments. Finally, compound 118 was identified as the well-known neolignan, dihydrodehydrodiconiferyl alcohol by comparison of spectral data with the reported known compound in the literature.⁴⁴¹⁻⁴⁴³

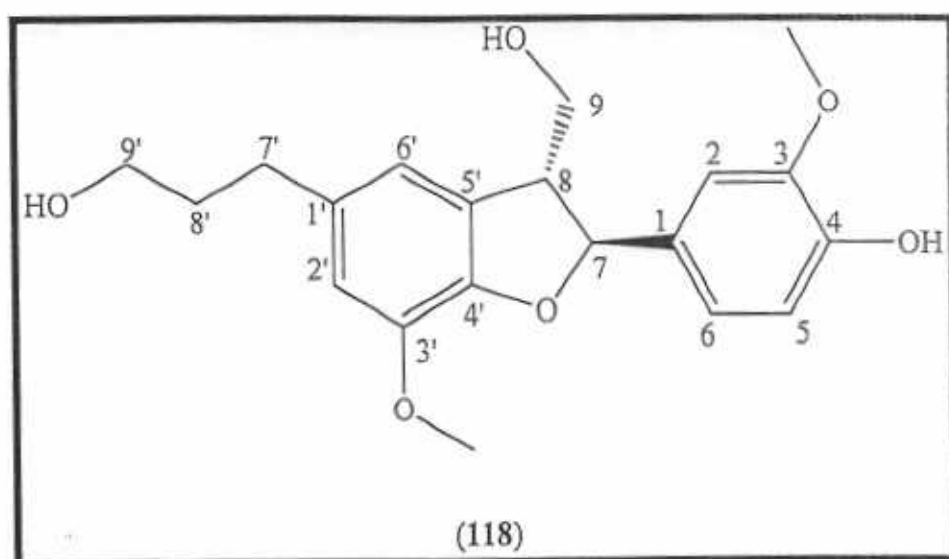


Table-10.7: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of (118) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	133.1	C	-
2	108.8	CH	6.95, d ($J = 2.0$)
3	146.6	C	-
3-OCH ₃	55.9	CH ₃	3.87, s
4	145.6	C	-
5	114.3	CH	6.88, d ($J = 8.0$)
6	119.4	CH	6.92, dd ($J = 8.0, 2.0$)
7	87.9	CH	5.55, d, ($J = 7.3$)
8	53.8	CH	3.61, m
9	63.9	CH ₂	3.92, m 3.98, m
1'	135.4	C	-
2'	112.4	CH	6.68, s
3'	144.2	C	-
3'-OCH ₃	56.0	CH ₃	3.89, s
4'	146.5	C	-
5'	127.7	C	-
6'	115.9	CH	6.69, s
7'	32.0	CH ₂	2.68, m
8'	34.6	CH ₂	1.91, m
9'	62.3	CH ₂	3.71, t ($J = 6.4, 6.4$)

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.4. Tuberculatin (119)

Compound 119 was obtained as a yellowish amorphous powder. Its molecular formula ($\text{C}_{26}\text{H}_{24}\text{O}_{11}$) was determined by ^1H - and ^{13}C -NMR analyses, and electrospray ionization mass spectrometry (ESIMS), which showed protonated molecular ion $[\text{M} + 1]^+$ at m/z 513 (ESI-positive) and deprotonated molecular ion $[\text{M} - 1]^-$ at m/z 511 (ESI-negative) and a fragment ion $[\text{M} - \text{pentose}]^-$ at m/z 379 in ESI-negative due to the loss of a pentose unit from the molecule.

The ^1H -NMR spectrum (Table-10.8) of 119 showed the AMX pattern of a piperonyl group at δ_{H} 6.88 (1H, d, $J = 2.0$ Hz, H-2'), δ_{H} 6.98 (1H, d, $J = 8.0$, Hz, H-5') and δ_{H}

6.82 (1H, d, $J = 8.0, 2.0\text{Hz}$, H-6'), while two aromatic singlets at δ_{H} 7.75 (1H, s, H-5) and 7.11 (1H, s, H-8) indicated a 1, 2, 4, 5-tetra substituted benzene ring. One proton multiplets resonated at δ_{H} 5.47 (H_a-9) and 5.54 (H_b-9) which were assigned to the methylene group of γ -lactone. These signals suggested the presence of an aryl naphthalide type of nucleus.⁴⁴⁴ The locations of two methoxy moieties (δ_{H} 3.74 and 4.01) on C-6 and C-7 were confirmed on the basis of HMBC spectral data. The presence of a sugar moiety was confirmed by the anomeric proton signal displayed at δ_{H} 5.59 (1H, t, $J = 2.9\text{ Hz}$, H-1''). The splitting pattern and coupling constants of the sugar moiety (Table-10.8) closely resembled to an β -D-apiosefuranosyl.⁴⁴⁵ The location of the sugar unit was confirmed from a long-range HMBC correlation between the anomeric proton δ_{H} 5.59 (H-1'') to C-4 (δ_{C} 146.4). All the spectral data was tantamount to the reported data of the known compound, tuberculatin.⁴⁴⁵

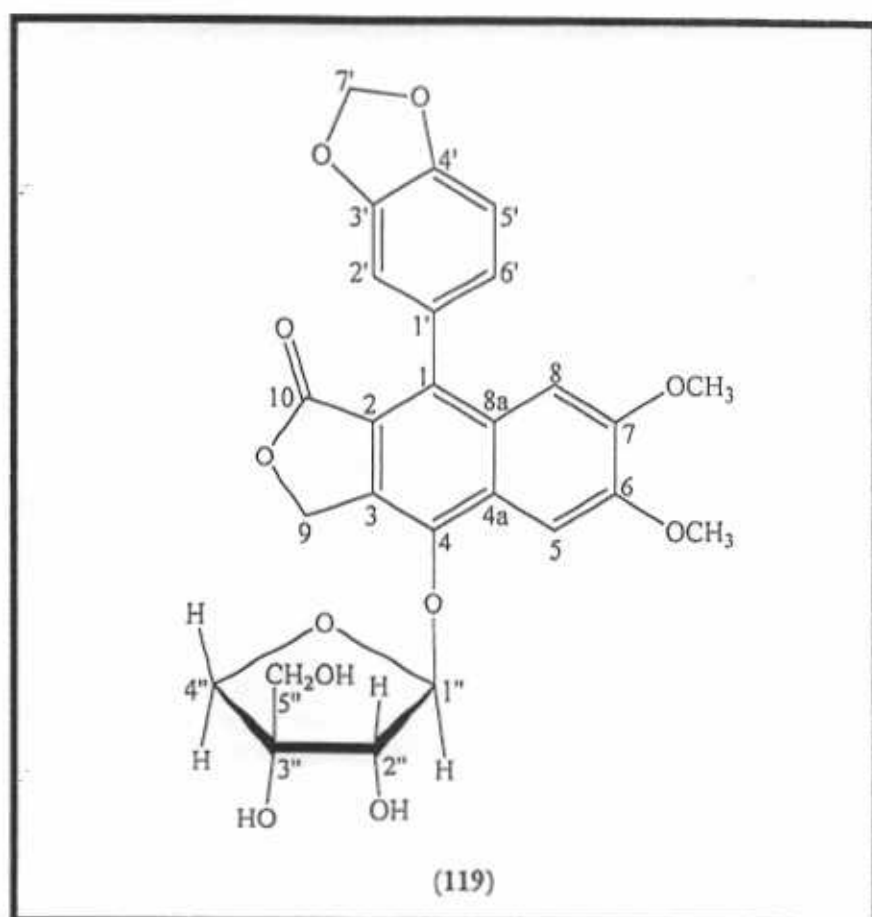


Table-10.8: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of (119) in CD_3COCD_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	136.4	C	-
2	120.7	C	-
3	130.1	C	-
4	146.4	C	-
4a	128.3	C	-
5	102.3	CH	7.75, s
6	153.5	C	-
6-OCH ₃	56.4	CH ₃	3.74, s
7	152.0	C	-
7-OCH ₃	56.8	CH ₃	4.01, s
8	107.5	CH	7.11, s
8a	131.8	C	-
9	68.1	CH ₂	5.47, m 5.54, m
10	170.3	C	-
1'	130.4	C	-
2'	112.4	CH	6.88, d ($J = 2.0$)
3'	148.9	C	-
4'	148.8	C	-
5'	109.2	CH	6.98, d ($J = 8.0$)
6'	125.2	CH	6.82, dd ($J = 8.0, 2.0$)
7'	102.7	CH ₂	6.10, dd ($J = 5.7, 1.1$)
1''	113.0	CH	5.59, t ($J = 2.9, 2.9$)
2''	78.9	CH	4.62, d ($J = 3.4$)
3''	80.4	C	-
4''	76.4	CH ₂	3.94, d ($J = 10.0$) 4.37, dd ($J = 10.0, 1.3$)
5''	64.9	CH ₂	3.73, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.5. Ovafolinin E (120)

Compound 120 was obtained as a yellowish amorphous powder. Its molecular formula ($\text{C}_{22}\text{H}_{24}\text{O}_8$) was determined by NMR spectral interpretation, and electrospray ionization mass spectrometry (ESIMS), which showed protonated molecular ion $[\text{M} + \text{H}]^+$ at m/z 417 (ESI-positive) and deprotonated molecular ion $[\text{M} - \text{H}]^-$ at m/z 415 (ESI-negative).

The ^1H -NMR spectrum (Table-10.9) of **120** showed resonances; one formyl [δ_{H} 9.56 (1H, s, H-9)], four olefinic [δ_{H} 6.25 (2H, s, H-2', 6'), 6.75 (1H, s, H-1) and 7.30 (1H, s, H-7)] and four methoxy [δ_{H} 3.76 (6H, s, CH_3O -3', 5'), 3.62 (3H, s, CH_3O -4) and 3.98 (3H, s, CH_3O -2)] protons. The ^{13}C -NMR spectrum (Table-10.9) displayed twenty two carbons including one formyl (δ_{C} 192.8), fourteen olefinic (δ_{C} 146.8, 146.7 $\times$ 2, 146.4, 145.6, 142.0, 136.1, 134.7, 133.4, 125.9, 123.1, 107.5, 104.4 $\times$ 2), one oxygenated methylene (δ_{C} 63.9), four methoxyl (δ_{C} 56.3 $\times$ 2, 56.4, 60.7) and two methine (δ_{C} 37.9, 42.4) carbons.

The NMR assignments were confirmed by 2D-techniques such as COSY, HSQC, and HMBC. A comparison of spectral data with reported compound ovafolinin E,⁴⁴⁶ confirmed the similarity of **120** to ovafolinin E.

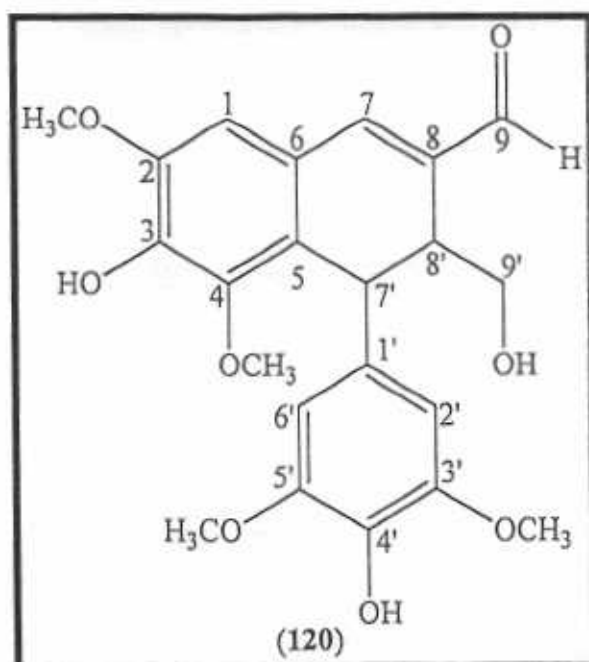


Table-10.9: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of (120) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	107.5	CH	6.75, s
2	146.8	C	-
2-OCH ₃	56.4	CH ₃	3.98, s
3	142.0	C	-
4	145.6	C	-
4-OCH ₃	60.7	CH ₃	3.62
5	125.9	C	-
6	123.1	C	-
7	146.4	CH	7.30, s
8	136.1	C	-
9	192.8	CH	9.56, s
1'	134.7	C	-
2'	104.4	CH	6.25, s
3'	146.7	C	-
3'-OCH ₃	56.3	CH ₃	3.76, s
4'	133.4	C	-
5'	146.7	C	-
5'-OCH ₃	56.3	CH ₃	3.76, s
6'	104.4	CH	6.25, s
7'	37.9	CH	4.68, s
8'	42.4	CH	3.30, m
9'	63.9	CH ₂	3.40, m
			3.61, m

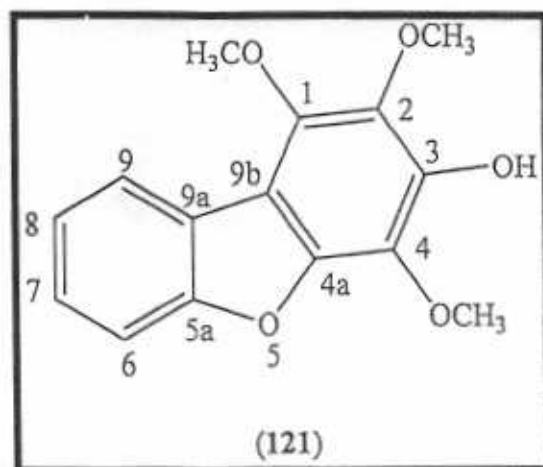
a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.6. β -Pyrofuran (121)

Compound 121 was isolated from the ethyl acetate fraction in colorless crystalline form. Its molecular formula ($\text{C}_{15}\text{H}_{14}\text{O}_5$) was deduced from EIMS corresponding to molecular ion peak at m/z 274 as well as from ^1H - and ^{13}C -NMR spectral data.

The ^1H -NMR was found to contain signals for four adjacent aromatic protons [δ_{H} 7.50 (1H, d, J = 8.0 Hz, H-6), δ_{H} 7.30 (1H, t, J = 7.5 Hz, H-7), δ_{H} 7.35 (1H, t, J = 7.5 Hz, H-8) and δ_{H} 8.00 (1H, d, J = 7.5 Hz, H-9)] and three methoxyl groups [δ_{H} 4.18 (3H, s, CH_3O -1), 3.99 (3H, s, CH_3O -2) and 4.05 (3H, s, CH_3O -4)], which were confirmed by HSQC NMR spectrum. The ^{13}C -NMR data (Table 10.10) of 121 was identical to the

previously reported dibenzofuran; β -Pyrofuran.⁴³⁶ Thus, showing the presence of twelve olefinic carbons at (δ_C 155.7, 143.9, 142.7, 141.5, 136.6, 128.9, 125.6, 123.5, 123.0, 121.9, 111.2 and 110.5) and three methoxy carbons at (δ_C 60.9, 61.2 and 61.5). The locations of methoxy groups were determined by HMBC-NMR cross peaks for long-range couplings between protons of the methoxyl groups to their corresponding carbons. Furthermore, the structure of this compound was also confirmed from single crystal X-rays crystallography (Figure 10.11).⁴⁴⁷



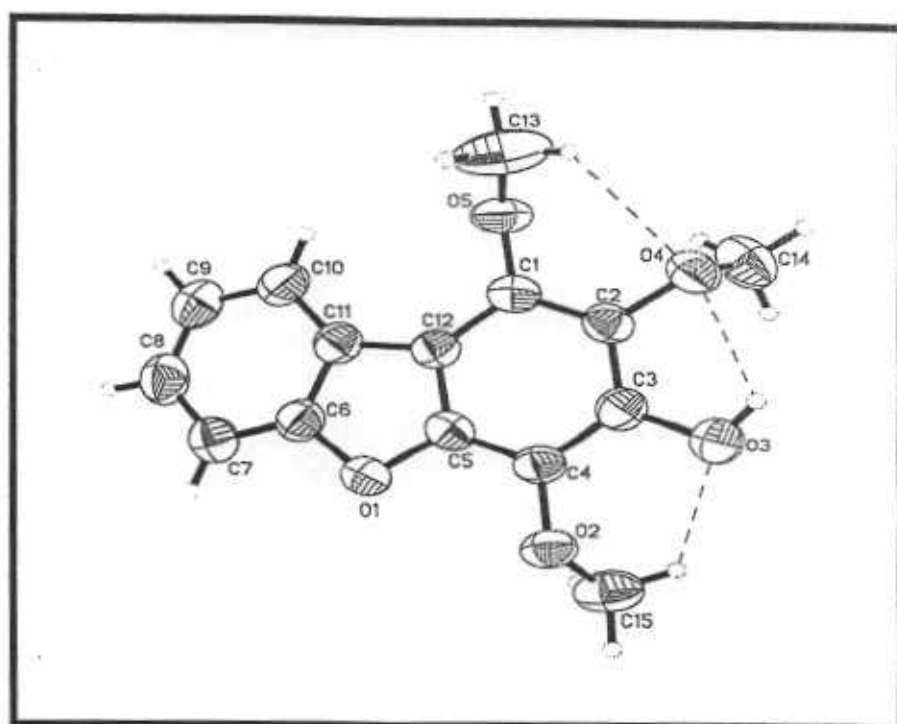


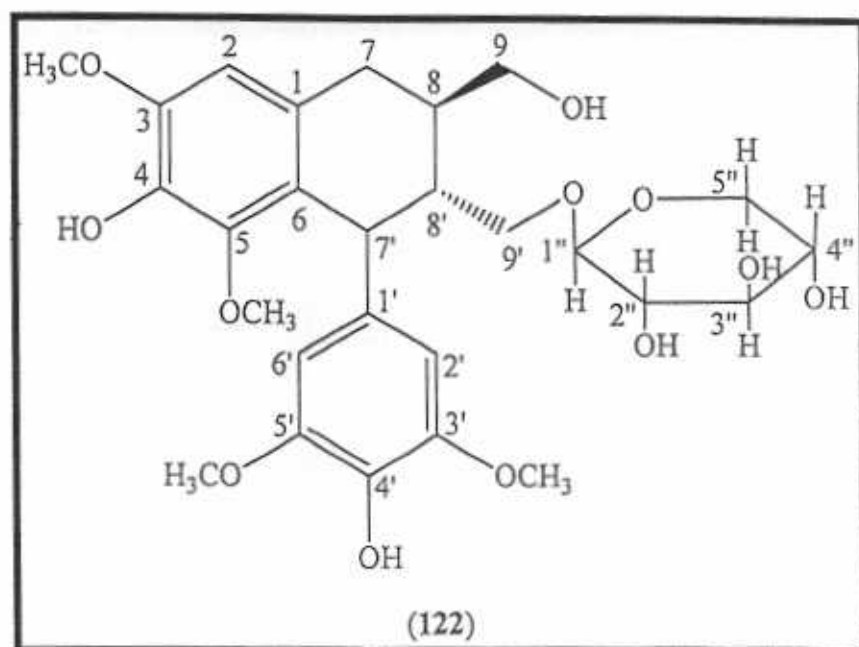
Figure-10.11: Crystal structure of compound (121)

Table-10.10: ^1H -NMR (500 MHz) and ^{13}C -NMR (75 MHz) chemical shifts of (121) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	128.9	C	-
1-OCH ₃	60.9	CH ₃	4.18, s
2	136.6	C	-
2-OCH ₃	61.2	CH ₃	3.99, s
3	141.5	C	-
4	142.7	C	-
4-OCH ₃	61.5	CH ₃	4.05
4a	143.9	C	-
5a	155.7	C	-
6	111.2	CH	7.50, d ($J = 8.00$)
7	125.6	CH	7.30, t ($J = 7.5, 7.5$)
8	123.0	CH	7.35, t ($J = 7.5, 7.5$)
9	121.9	CH	8.00, d ($J = 7.5$)
9a	123.5	C	-
9b	110.5	C	-

a. Broad Band; *b.* DEPT; *c.* ^1H -NMR; *d.* HSQC interactions

10.2.7. Lyoniside (122)



Compound **122** was isolated from the ethyl acetate fraction as white amorphous powder. Its formula ($C_{27}H_{36}O_{12}$) was deduced from ^1H - and ^{13}C -NMR data and EIMS analysis corresponding to molecular ion peak $[M]^+$ at m/z 552, while the fragment ion at m/z 419 $[M + H\text{-pentose}]^+$, indicating a pentose unit in the molecule.

The ^1H -NMR spectrum (Table-10.11) of compound **122** showed two aromatic singlets at δ_H 6.42 (2H, s, H-2', 6') and δ_H 6.56 (1H, s, H-2) and four aromatic methoxys at δ_H 3.74 (6H, s, CH_3O -3', 5'), δ_H 3.34 (3H, s, CH_3O -3) and 3.84 (3H, s, CH_3O -5). The methines of the aglycone part of the molecule were observed at δ_H 1.67 (1H, m, H-8), δ_H 4.35 (1H, d, $J = 6.4$, H-7') and δ_H 2.33 (1H, m, H-8'), while the protons of three methylenes (aglycone) were resonated at δ_H 2.65 (2H, m, H-7), δ_H 3.62 (2H, m, H-9) and δ_H 3.21 (2H, m, H-9'). The proton chemical shifts and coupling constants of the glycone part of the molecule confirmed the presence of a β -D-xylopyranosyl moiety in the molecule.⁴⁴⁸

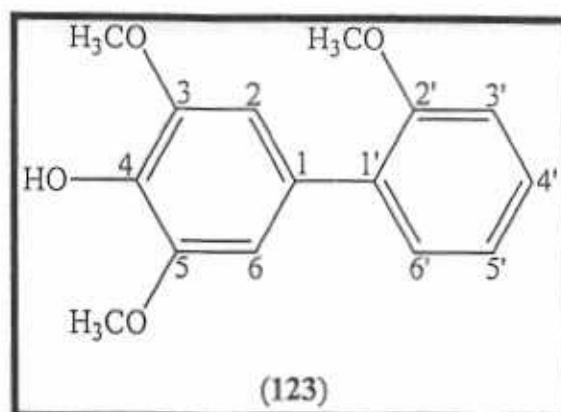
The ^{13}C -NMR data (Table-10.11) was in a perfect agreement to the literature data of the previously reported compound, Lyoniside.⁴⁴⁸ All the NMR assignments were also confirmed by COSY, HSQC and HMBC NMR experiments.

Table-10.11: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (122) in CD_3OD

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	130.1	C	-
2	107.8	CH	6.56, s
3	147.6	C	-
3-OCH ₃	60.0	CH ₃	3.34, s
4	139.9	C	-
5	148.6	C	-
5-OCH ₃	56.6	CH ₃	3.84, s
6	126.4	C	-
7	33.9	CH ₂	2.65, m
8	40.5	CH	1.67, m
9	66.0	CH ₂	3.62, m
1'	139.4	C	-
2'	106.9	CH	6.42, s
3'	148.9	C	-
3'-OCH ₃	56.8	CH ₃	3.74, s
4'	134.4	C	-
5'	148.9	C	-
5'-OCH ₃	56.8	CH ₃	3.74, s
6'	106.9	CH	6.42, s
7'	43.0	CH	4.35, d ($J = 6.4$)
8'	46.7	CH	2.33, m
9'	71.0	CH ₂	3.21, m
1''	105.5	CH	4.21, d ($J = 7.6$)
2''	74.9	CH	3.19, m
3''	78.0	CH	3.32, m
4''	71.2	CH	3.48, m
5''	66.9	CH ₂	3.19, m
			3.81, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.8. 2'-Methoxyaucuparin (123)



Compound **123** was obtained as white crystals from the ethyl acetate fraction. Its molecular formula ($C_{15}H_{16}O_4$) was determined through NMR spectral analyses in combination to ESIMS, which displayed protonated molecular ion $[M + 1]^+$ at m/z 361 in positive mode and deprotonated molecular ion $[M - 1]^-$ at m/z 359 in negative mode.

The ^1H -NMR spectrum (Table-10.12) of **123** exhibited a simple resonance pattern with close resemblance to the reported compound, 2',3,5-trimethoxy-[1,1'-biphenyl]-4-ol (2'-Methoxyaucuparin).⁴¹⁵ The shielded singlet at δ_H 6.78 (2H, s, H-2, 6) and six proton singlet at δ_H 3.93 (6H, s, CH_3O -3, 5) suggested a symmetrically substituted benzene ring. Moreover, an ABCD coupling pattern in the aromatic region was observed at δ_H 7.00 (1H, dd, $J = 8.7, 1.1$ Hz, H-3'), δ_H 7.31 (1H, m, H-4'), δ_H 7.03 (1H, td, $J = 7.5, 7.5, 1.0$ Hz, H-5') and δ_H 7.34 (1H, m, H-6') suggesting an *ortho*-substitution on one of the ring. Therefore, the methoxy group at δ_H 3.83 (3H, s, CH_3O -2') was placed at the 2'-position.

The ^{13}C -NMR data (Table-10.12) of **123** was consistent with the reported data of 2',3,5-trimethoxy-[1,1'-biphenyl]-4-ol(2'-Methoxyaucuparin).⁴¹⁵

Table-10.12: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (123) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	129.6	C	-
2	106.5	CH	6.78, s
3	146.4	C	-
3-OCH ₃	56.3	CH ₃	3.92, s
4	134.0	C	-
5	146.4	C	-
5-OCH ₃	56.3	CH ₃	3.92, s
6	106.5	CH	6.78, s
1'	131.0	C	-
2'	156.4	C	-
2'-OCH ₃	55.6	CH ₃	3.83, s
3'	111.3	CH	7.00, dd ($J = 8.7, 1.1$)
4'	128.3	CH	7.31, m
5'	120.8	CH	7.03, td ($J = 7.5, 7.5, 1.0$)
6'	130.7	CH	7.34, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.9. Aucuparin (124)

The ethyl acetate fraction afforded (124) as white crystalline solid. Molecular formula ($\text{C}_{14}\text{H}_{14}\text{H}_3$) of the compound was developed through ^1H - and ^{13}C -NMR analyses and ESIMS, where molecular ion adducts were observed as $[\text{M} + 1]^+$ at m/z 231 and $[\text{M} + \text{Na}]^+$ at 253 in positive mode and $[\text{M} - 1]^-$ at m/z 229 in negative mode.

The ^1H -NMR data (Table-10.13) of 124 was in complete agreement with that of aucuparin, a previously reported compound.⁴⁴⁹ A very simple resonance pattern indicating symmetrical substitution was observed. The two methoxys were resonate at δ_{H} 3.97 (6H, s, CH_3O -3, 5), whereas the shielded aromatic singlet at δ_{H} 6.81 (2H, s, H-2, 6) suggested that these two methoxys should be place at C-3 and C-5 rather than at C-2 and C-6. This substitution pattern was also confirmed from HMBC NMR correlations.

The ^{13}C -NMR data (Table-10.13) was also identical to aucuparin.⁴⁴⁹ In combination to ^1H - and ^{13}C -NMR were used COSY, HSQC and HMBC to confirm all the assignments. Similarity of the spectral data (1D- & 2D-NMR) with the known reported compound, aucuparin substantiated that the structure of 124 is identical to aucuparin.

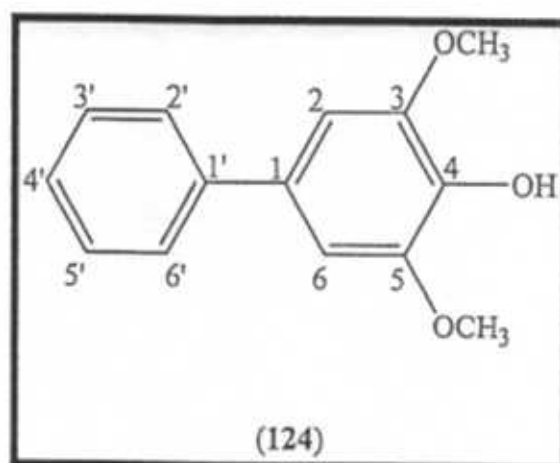
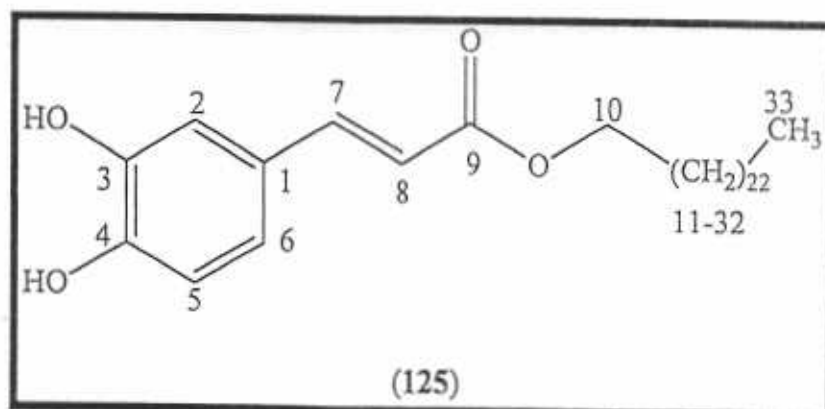


Table-10.13: ^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (124) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	132.9	C	-
2	104.1	CH	6.81, s
3	147.3	C	-
3-OCH ₃	56.4	CH ₃	3.97, s
4	134.4	C	-
5	147.3	C	-
5-OCH ₃	56.4	CH ₃	3.97, s
6	104.1	CH	6.81, s
1'	141.4	C	-
2'	126.9	CH	7.56, m
3'	128.7	CH	7.43, m
4'	126.9	CH	7.34, m
5'	128.7	CH	7.43, m
6'	126.9	CH	7.56, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.10. Tetracosyl-3-(3,4-dihydroxyphenyl)acrylate (125)



Compound **125** was obtained as a white precipitate insoluble in methanol. Its molecular formula ($C_{33}H_{56}O_4$) was deduced by EIMS (molecular ion peak $[M]^+$ at m/z 516) and NMR spectral data analyses.

The ^1H -NMR spectrum (Table-10.14) of compound **125** showed an AMX type of aromatic system with protons resonating at δ_H 6.98 (1H, d, $J = 2.0$ Hz, H-2), 6.88 (1H, d, $J = 8.0$ Hz, H-5) and 6.74 (1H, dd, $J = 8.0, 2.0$ Hz, H-6). The *trans* protons of the double bond were observed at δ_H 7.46 (1H, d, $J = 16.0$ Hz, H-7) and 6.15 (1H, d, $J = 16.0$ Hz, H-8), while a methyl multiplet appeared at δ_H 0.80 (3H, m, H-33).

The ^{13}C -NMR (BB and DEPT) spectra (Table-10.14) of compound **125** indicated the presence of one methyl, twenty three methylene, five methine and four quaternary carbons. The downfield carbon resonated at δ_C 168.0 was found due to α,β -unsaturated carbonyl carbon of the caffeic ester. The oxygen substituted methylene carbon (C-10) appeared at δ_C 64.6 in ^{13}C -NMR spectrum, whereas the terminal methyl displayed at δ_C 13.9. Compound **125** was identified as tetracosyl-3-(3, 4-dihydroxyphenyl)acrylate through comparison of spectral data with the literature of the known compound.⁴⁵⁰

Table-10.14: ^1H -NMR (500 MHz) and ^{13}C -NMR (100 MHz) chemical shifts of (125) in CDCl_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	126.9	C	-
2	114.8	CH	6.98, d ($J = 2.0$)
3	147.2	C	-
4	144.6	C	-
5	113.8	CH	6.88, d ($J = 8.0$)
6	121.8	CH	6.74, dd ($J = 8.0, 2.0$)
7	145.1	CH	7.46, d ($J = 16.0$)
8	115.0	CH	6.15, d ($J = 16.0$)
9	168.0	C	-
10	64.6	CH_2	4.10, m
11-32	22.6-31.89	CH_2	1.18-1.30, m
			1.00-1.10, m
33	13.9	CH_3	0.80, m

a. Broad Band; b. DEPT; c. ^1H -NMR; d. HSQC interactions

10.2.11. 3 β ,23-Dihydroxy-lup-20(29)ene-28-oic acid-3 β -caffeate (126)

Compound 126 was isolated as white amorphous powder. Its molecular formula ($\text{C}_{39}\text{H}_{54}\text{O}_7$) was obtained from NMR spectral interpretation and ESIMS, in which protonated molecular ion peak were noticed: $[\text{M}+1]^+$ at m/z 635 in ESI-positive and $[\text{M}-1]^-$ at m/z 633 in ESI-negative mode, suggesting a molecular mass of 634 a.m.u.

The ^1H -NMR spectrum (Table-10.15) of 126 revealed the presence of five tertiary methyl singlets at δ_{H} 0.79, 0.95, 0.98, 1.04, 1.70 and three aromatic protons at δ_{H} 7.02 (d, $J = 2.0$ Hz), 6.77 (d, $J = 8.3$ Hz) and 6.93 (dd, $J = 8.3, 2.0$ Hz) representing 1, 3, 4-substituted aromatic ring. In addition, the coupling constants of the protons at δ_{H} 7.51 and 6.22 was found 16.0 Hz, which indicated the presence of two *trans*-olefinic protons. From these observations we deduced an *O*-caffeoyl group in the molecule.⁴⁵¹

The ^{13}C -NMR spectrum (Table-10.15) of 126 displayed one ester carbonyl (δ_{C} 169.4), eight olefinic carbons (δ_{C} 115.2, 115.8, 116.6, 123.0, 127.9, 146.8, 146.9 and

149.7), five tertiary methyl groups (δ_C 13.9, 15.3, 16.8, 17.3 and 19.7), a carboxylic acid carbon (δ_C 180.2) and a terminal double bond (δ_C 110.3). In addition to these signals, twenty three other carbon resonances were found in ^{13}C -NMR spectrum. All these assignments were confirmed from 2D-techniques such as COSY, HSQC and HMBC. A comparison of these assignments with the reported known compound 3 β ,23-Dihydroxy-lup-20(29)ene-28-oic acid-3 β -caffeate),⁴⁵² indicated that the structure of **126** is identical to the known compound.

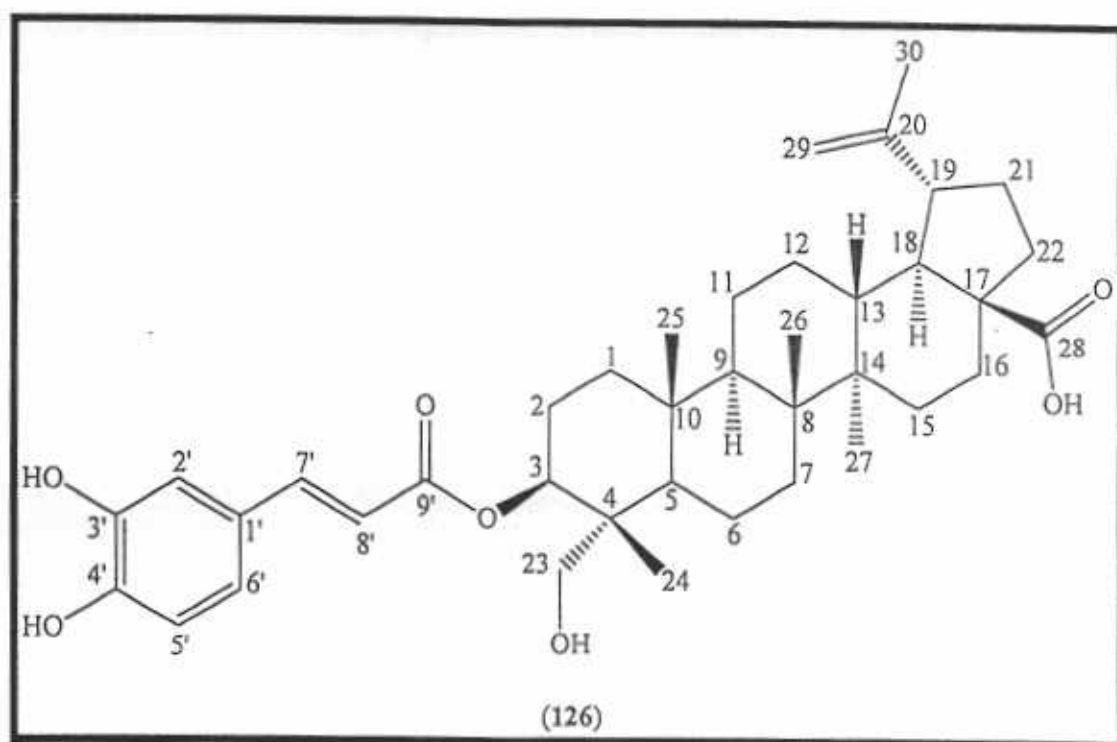


Table-10.15: ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) chemical shifts of (126) in CD_3COCD_3

C. No.	^{13}C -NMR (δ) ^a	Multiplicity (DEPT) ^{bd}	^1H -NMR (δ) Coupling Constants J_{HH} (Hz) ^{cd}
1	39.8	CH_2	1.03, m 2.33, m
2	24.4	CH_2	1.78, m
3	76.0	CH	4.95, m
4	43.2	C	-
5	48.2	CH	1.35, m
6	18.9	CH_2	1.44, m 1.50, m
7	35.0	CH_2	1.35, m 1.57, m
8	42.0	C	-
9	52.0	CH	1.44, m
1	39.8	CH_2	1.03, m 2.33, m
2	24.4	CH_2	1.78, m
3	76.0	CH	4.95, m
4	43.2	C	-
5	48.2	CH	1.35, m
6	18.9	CH_2	1.44, m 1.50, m
7	35.0	CH_2	1.35, m 1.57, m
8	42.0	C	-
9	52.0	CH	1.44, m
10	38.0	C	-
11	22.3	CH_2	1.28, m 1.47, m
12	27.0	CH_2	1.08, m 1.73, m
13	39.5	CH	2.35, m
14	43.8	C	-
15	30.9	CH_2	1.18, m 1.30, m
16	33.5	CH_2	1.42, m 2.24, m
17	57.6	C	-
18	50.6	CH	1.64, m
19	48.6	CH	3.02, m
20	152.1	C	-
21	31.9	CH_2	1.40, m 1.94, m
22	38.3	CH_2	1.45, m 1.91, m
23	64.8	CH_2	3.13, d ($J = 11.5$) 3.32, d ($J = 11.5$)

Continued on next page

Table-10.15 continued.

24	13.9	CH ₃	0.79, s
25	17.3	CH ₃	0.95, s
26	16.8	CH ₃	0.98, s
27	15.3	CH ₃	1.04, s
28	180.2	C	-
29	110.3	CH ₂	4.60, dd ($J = 2.2, 1.2$) 4.72, d ($J = 2.2$)
30	19.7	CH ₃	1.70, s
1'	127.9	C	-
2'	115.2	CH	7.02, d ($J = 2.0$)
3'	146.8	C	-
4'	149.7	C	-
5'	116.6	CH	6.77, d ($J = 8.3$)
6'	123.0	CH	6.93, dd ($J = 8.3, 2.0$)
7'	146.9	CH	7.51, d ($J = 16.0$)
8'	115.8	CH	6.22, d ($J = 16.0$)
9'	169.4	C	-

a. Broad Band; b. DEPT; c. ¹H-NMR; d. HSQC interactions

10.3. Diphenylpicrylhydrazyl (DPPH) radical scavenging activity

One of the well-known methods to evaluate the antioxidant potential is the DPPH radical scavenging assay. It is an organic free radical with purple colour and absorbs maximum UV radiations in the range of 515-528 nm. The absorption intensity is reduced when the radical accepts an electron or another free radical, thus changing colour from purple to yellow. The radical scavenging powers (Figure 10.12 & Table 10.16) of the pure compounds (112-126) isolated from the ethyl acetate fraction of *Sorbus lanata* and ascorbic acid taken as standard were studied against DPPH radical. All the compounds showed scavenging activities even at lowest concentration used in the assay. Compounds 115 ($IC_{50} = 24.2 \mu M$), 118 ($IC_{50} = 9.2 \mu M$), 122 ($IC_{50} = 21.2 \mu M$), 125 ($IC_{50} = 33.7 \mu M$), and 126 ($IC_{50} = 6.0 \mu M$) showed activities superior to ascorbic acid ($IC_{50} = 33.9 \mu M$). The scavenging activities of compounds 116, 117, 120, and 124 exceeded 50 % at the lowest concentration (5 μM) used. Compounds 112, 113, 114, 119, 121 and 123 were more reactive at lower concentrations and less reactive at higher concentrations. The radical scavenging activities of these compounds are due to their phenolic nature.

Table 10.16: Antioxidant activities of pure compounds isolated from the ethyl acetate fraction of *Sorbus lanata* showing % scavenging at different concentrations and 50% inhibitory concentrations (IC₅₀) in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay^a

Comp	% Scavenging ^c							
	5 ^b	15	30	60	125	250	500	IC ₅₀
112	44.5 ± 0.4	45.3 ± 0.2	47.4 ± 0.1	47.9 ± 0.05	49.2 ± 0.2	51.3 ± 0.1	56.4 ± 0.2	192.2 ± 2.9
113	45.5 ± 0.07	45.8 ± 0.1	45.6 ± 0.4	46.3 ± 0.1	46.6 ± 0.4	47.6 ± 0.3	50.5 ± 0.07	445.9 ± 4.0
114	46.1 ± 0.1	46.4 ± 0.06	46.5 ± 0.06	48.4 ± 0.1	52.4 ± 0.06	57.8 ± 0.1	68.5 ± 0.1	90.7 ± 0.5
115	44.6 ± 0.2	46.6 ± 0.4	52.1 ± 0.3	60.5 ± 0.4	79.4 ± 0.2	96.4 ± 0.2	96.7 ± 0.2	24.2 ± 1.1
116 ^f	52.3 ± 0.2	54.6 ± 0.1	58.6 ± 0.4	65.6 ± 0.4	75.9 ± 0.8	82.3 ± 0.2	88.5 ± 0.3	N.C
117 ^f	52.0 ± 0.4	55.4 ± 0.8	67.2 ± 0.2	74.5 ± 0.2	87.3 ± 0.2	93.5 ± 0.3	94.4 ± 0.2	N.C
118	49.5 ± 0.6	49.6 ± 0.1	55.3 ± 0.2	58.5 ± 0.2	65.5 ± 0.1	74.6 ± 0.3	84.7 ± 0.2	9.2 ± 1.5
119	49.7 ± 0.4	50.1 ± 0.1	51.3 ± 0.2	51.4 ± 0.2	51.9 ± 0.05	55.3 ± 0.2	62.3 ± 0.1	11.6 ± 2.7
120 ^f	50.5 ± 0.4	50.4 ± 0.3	55.3 ± 0.3	60.4 ± 0.3	67.7 ± 0.2	75.2 ± 0.2	82.3 ± 0.2	N.C
121	42.5 ± 0.5	43.4 ± 0.3	44.6 ± 0.4	46.3 ± 0.2	51.7 ± 0.3	58.4 ± 0.4	68.1 ± 0.3	114.4 ± 1.8
122	44.3 ± 0.3	48.5 ± 0.2	53.4 ± 0.2	60.5 ± 0.4	73.5 ± 0.6	85.3 ± 0.3	92.5 ± 0.06	21.2 ± 0.6
123	45.6 ± 0.3	48.5 ± 0.3	53.4 ± 0.5	55.6 ± 0.3	60.6 ± 0.2	62.3 ± 0.2	65.6 ± 0.2	23.0 ± 1.5
124 ^f	54.2 ± 0.2	54.6 ± 0.1	66.6 ± 0.1	69.3 ± 0.5	76.4 ± 0.1	79.2 ± 0.2	81.4 ± 0.2	N.C
125	44.2 ± 0.3	44.6 ± 0.2	49.5 ± 0.06	55.4 ± 0.4	70.5 ± 0.2	89.4 ± 0.2	96.2 ± 0.2	33.7 ± 0.5
126	50.3 ± 0.2	51.6 ± 0.4	59.5 ± 0.3	71.5 ± 0.2	94.4 ± 0.3	95.4 ± 0.1	96.1 ± 0.2	6.0 ± 0.7
Ascorbic acid ^d	43.2 ± 0.2	45.6 ± 0.4	46.6 ± 0.4	57.4 ± 0.3	73.7 ± 0.1	98.5 ± 0.4	98.4 ± 0.2	33.9 ± 0.9

^aValues are mean ± SD (n = 3). ^bConcentrations in μM. ^cScavenging % = 100 - (A sample / A control) × 100. ^dAscorbic acid used as positive control. ^eAbsorbance of sample and control measured at 517 nm. ^fN.C = not calculated.

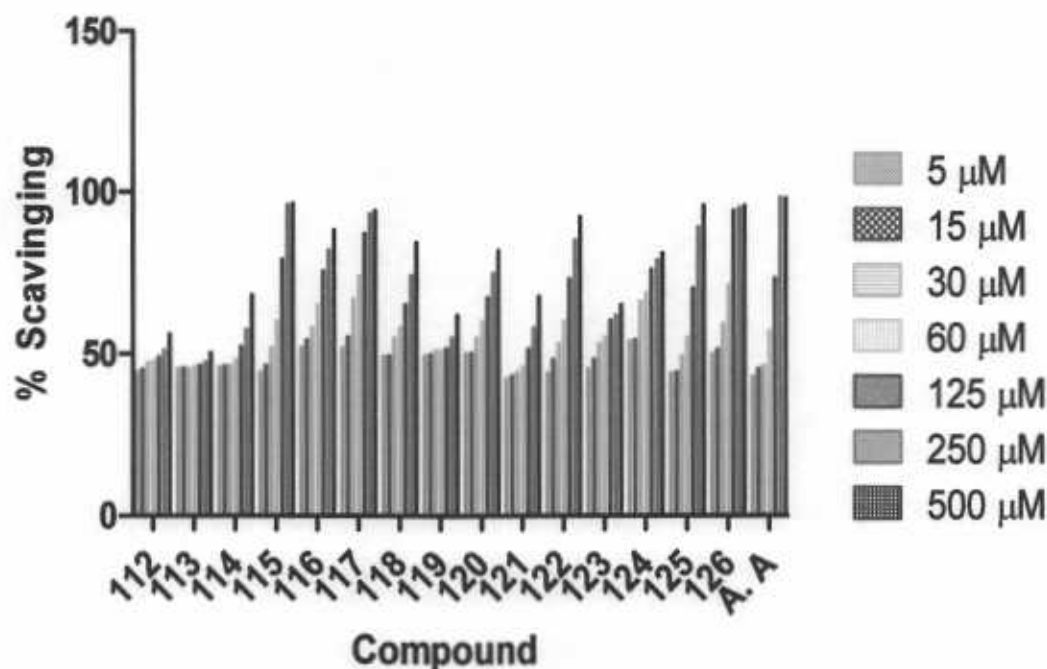


Figure-10.12: DPPH radical scavenging activities of compounds (112-126)

Chapter: 11

EXPERIMENTAL (Part-C)

11.1. General experimental conditions

11.1.1. Physical constants

Optical rotations were measured using an ADP 220 polarimeter at 589 nm. X-ray diffraction data was acquired on Bruker SMART APEX CCD area-detector diffractometer using Mo K_{α} radiation, $\lambda = 0.71073 \text{ \AA}$.

11.1.2. Spectroscopic techniques

UV spectra were obtained on Spectra Max 190 in a 96-well plate reader using methanol as solvent. IR spectra were obtained using a Perkin-Elmer FT-IR instrument for compounds mounted directly on the diamond cell. ^1H -NMR spectra were recorded in deuterated solvents such as CDCl_3 , CD_3OD and CD_3COCD_3 with TMS as internal standard at 300 MHz, 400 MHz and 500 MHz, on Bruker AVANCE-300, AVANCE-400, AVANCE-500, Varian-400 MR, Varian-S500 MR spectrometers, while the ^{13}C -NMR spectra were recorded in the same solvents at 75 MHz, 100 MHz and 125 MHz using the same instruments. The 2D spectra (COSY, HSQC, H2BC, HMBC and NOSEY) were taken at 400 MHz and 500 MHz using the above magnetic resonance spectrometers. Low-resolution electron impact ionization mass spectra were recorded on Finnigan MAT 311 with MASSPEC data system. HRESIMS were carried out on an APEX IV Fourier-transform ion cyclotron resonance instrument (Bruker Daltonics, Coventry, UK) using an Apollo ESI source. The nebulizer and drying gas was nitrogen at 180°C . Samples were infused from solution (50% MeOH/DCM) by syringe pump at 100 microlitres per hour. Accurate masses to within 5ppm were obtained using a broad range external calibration by a mixture of PEG polymer grades.

11.1.3. Chromatographic techniques

Silica gel (220-440 mesh) was used for column chromatography and Merck keisel gel 60 F254 pre-coated silica gel glass plates were used for analytical and preparative TLC. TLCs were visualized by UV light ($\lambda = 254$ nm, 366 nm) or by heating after spraying with 15% aqueous sulfuric acid saturated with ceric sulfate. Reverse phase HPLC was conducted using a Waters mass-directed autopurification system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex LUNA column (5 μ , C₁₈, 100 Å, 10 x 250 mm) equipped with a Phenomenex Security Guard precolumn (Luna C₅ 300 Å) eluting at 4 mL/min. Solvent A, HPLC grade H₂O + 0.05% formic acid; Solvent B, HPLC grade CH₃CN + 0.045% formic acid or HPLC grade CH₃OH + 0.045% formic acid. The post-column flow was split (100:1) and the minority flow was made up with MeOH + 0.045% formic acid to 1 mL/min for simultaneous analysis by diode array (Waters 2998), evaporative light scattering (Waters 2424) and ESI mass spectrometry in positive and negative modes (Waters Quatro Micro).

11.2. *Sorbus lanata*

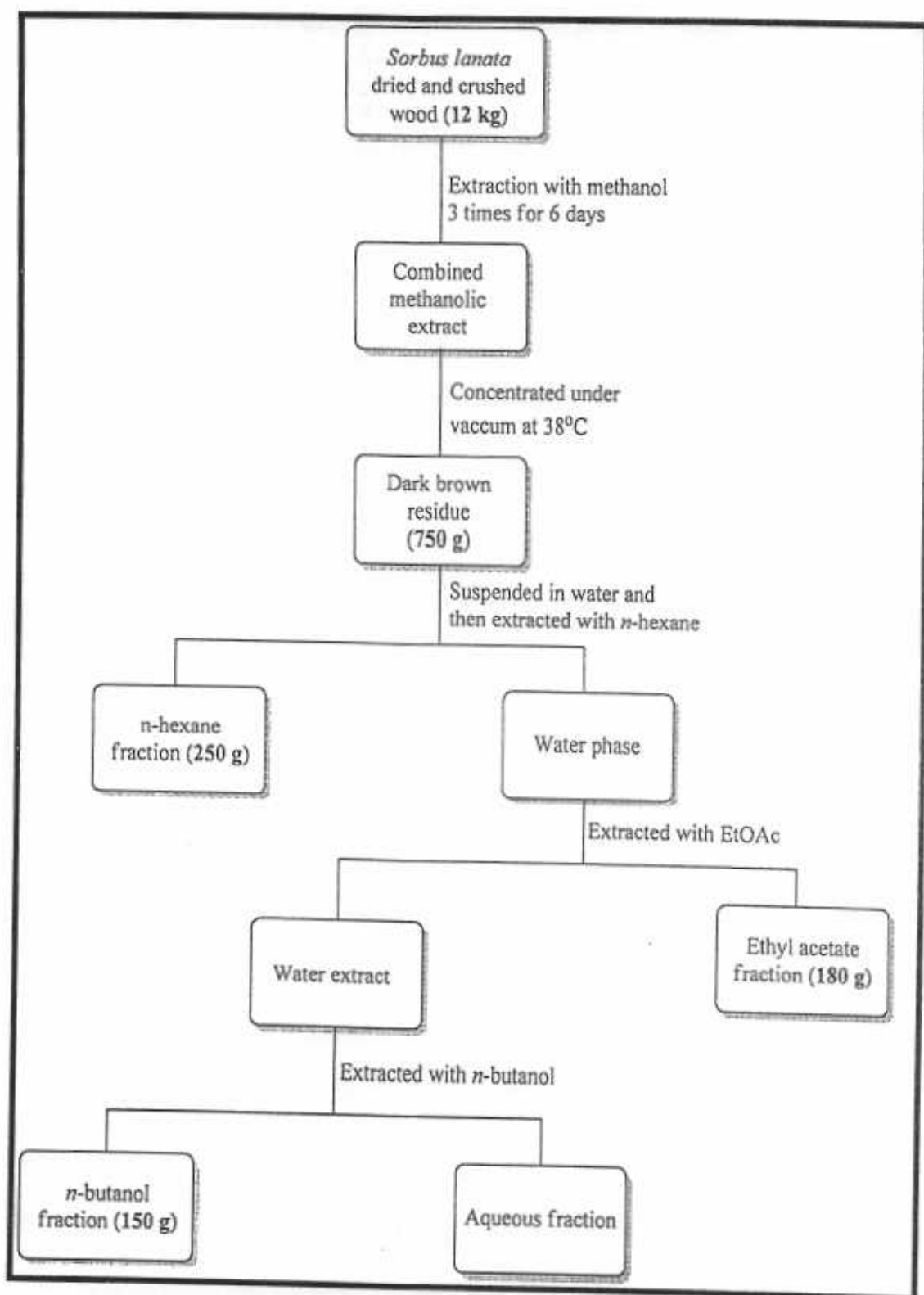
11.2.1. Plant Material

Aerial parts (stem, leaves and fruits) of *Sorbus lanata* were collected during fruiting session in August 2008 from Gilgit region in the north of Pakistan. Taxonomic identification was done by Mr. Naveed Ahmad, lecturer in Botany, department of Botany, University of Peshawar, Peshawar., Pakistan. A voucher specimen (JR-57/2008) was deposited in the herbarium at the Department of Botany, University of Peshawar, Peshawar., Pakistan.

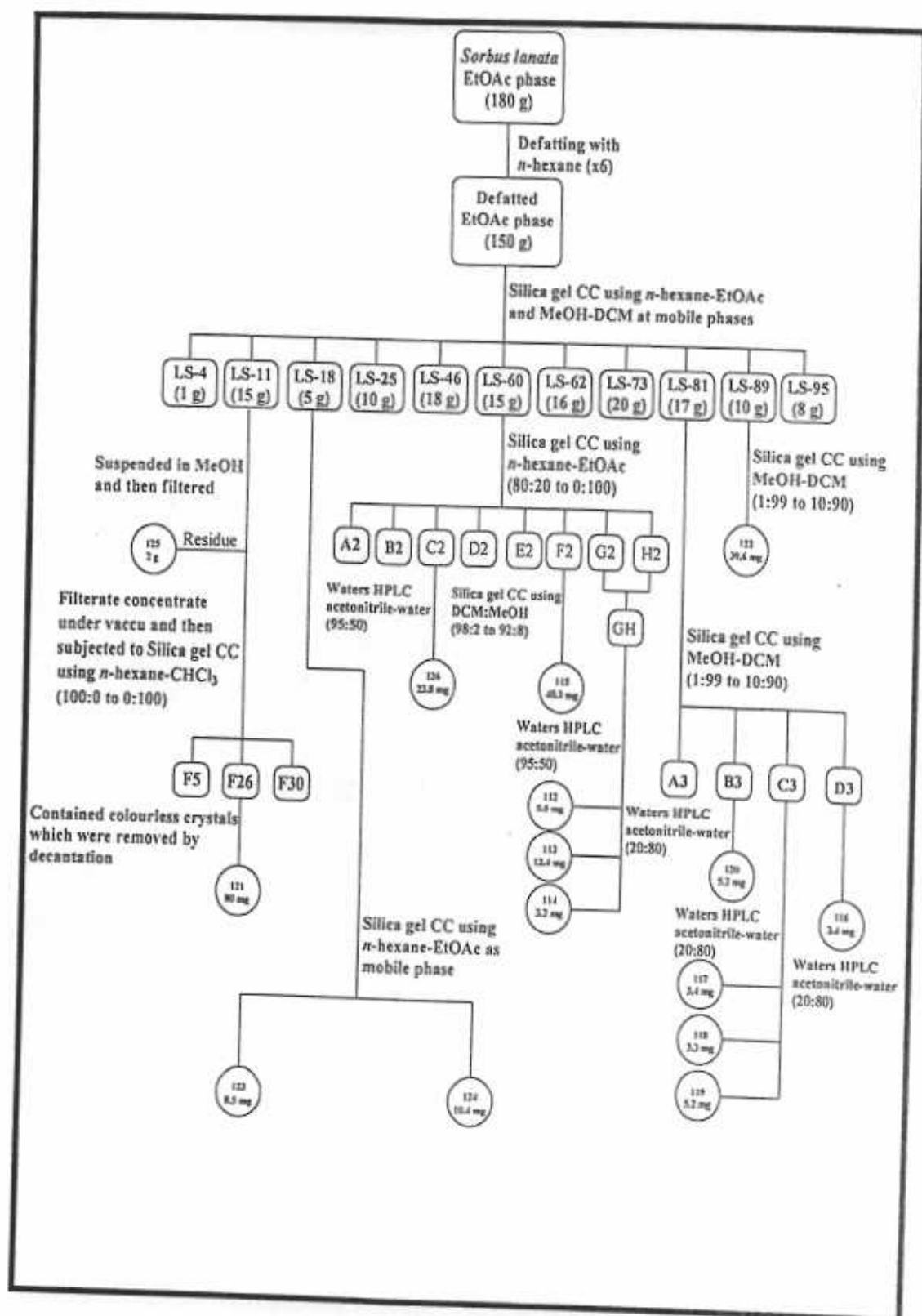
11.2.2. Extraction and isolation from the wood of *S. lanata*

The dried and crushed wood of *Sorbus lanata* (12 kg) was repeatedly ($\times 3$) extracted (Scheme-11.1) with methanol at room temperature. The combined methanolic extract was concentrated under vacuum to get a dark brown residue (750 g). The residue was suspended in water and successively partitioned in *n*-hexane, ethyl acetate and *n*-butanol soluble fractions. The ethyl acetate fraction (180 g) was further defatted with hexane for several times. The defatted ethyl acetate fraction (150 g) was subjected to column chromatography over silica gel. The column was first eluted with *n*-hexane-ethyl acetate (100:0 $\rightarrow$ 0:100) and then with dichloromethane (DCM)-methanol (MeOH) (98:2 $\rightarrow$ 90:10) as solvent systems. A total of 11 major fractions; LS-4, LS-11, LS-18, LS-25, LS-46, LS-60, LS-62, LS-73, LS-81, LS-89, and LS-95 were obtained according to compilation based on TLC profiles. Fraction LS-11 contained a white solid insoluble in methanol. The solid material was filtered and the filtrate was concentrated and further subjected to column chromatography over silica gel using *n*-hexane-CHCl₃ (100:0 $\rightarrow$ 0:100) as solvent system and as a result three major fractions; F-5, F-26 and F-30 were obtained. Fraction F-26 contained colourless crystals of various sizes which were separated from the solution by decantation. The crystals were washed with *n*-hexane ($\times 3$) to obtain pure crystals, and re-grown in a mixture of *n*-hexane-acetone (4:1) which furnished compound **121** as colourless needles (80 mg). The white residue removed from fraction LS-11 contained compound **125** (2g), which was further purified by washing with methanol ($\times 5$) and its purity was confirmed from thin layer chromatographic (TLC) analysis. Fraction LS-18 (5 g) was subjected to column chromatography (CC) over silica gel applying *n*-hexane-EtOAc (95:5) as mobile phase and yielded five sub-fractions (F-1, F-4, F-10, F-18, and F-20). Compounds **123** (8.5 mg) and **124** (10.4 mg) were obtained through

preparative TLC (95:5; *n*-hexane: EtOAc) from sub-fractions F-18 and F-20, respectively. Fraction LS-60 (15 g) was applied to CC on flash silica using a solvent system of *n*-hexane-EtOAc (80:20→0:100) and thus obtained eight sub-fractions (LS-60A2-H2). Compound 115 (40.3 mg) was furnished from fraction LS-60F2 by CC using (DCM-MeOH; 98:2→92:8). Fraction LS-60G2 and LS-60H2 were combined on the basis of liquid chromatography mass spectrometry to ultimately afford the combined fraction LS-60G2H2 which was purified into compounds 112 (5.5 mg), 113 (13.4 mg) and 114 (3.2 mg) by reverse phase HPLC (acetonitrile-water; 95:50), using a WATERS mass-directed autopurification system. The fraction LS-60C2 was also purified to give compound 126 (23.8 mg) by HPLC (acetonitrile-water; 95:50). Fraction LS-81 (17 g) was loaded to on flash silica (MeOH-DCM; 1:99→10:90). As a result four sub-fractions; LS-81A3-D3 were obtained. Reverse phase HPLC purification was performed on LS-81B3, LS-81C3 and LS-81D3 using the solvent system of acetonitrile-water (20:80) and thus afforded compound 120 (5.2 mg) from fraction LS-81B3, compounds 117 (3.4 mg), 118 (3.3 mg) and 119 (5.2 mg) from fraction LS-81C3 and compound 116 (3.4 mg) from LS-81D3. The fraction LS-89 (10 g) was applied to CC (MeOH-DCM; 1:99→10:90) giving compound 122 (39.6 mg).



Scheme-11.1: Extraction and fractionation from the wood of *S. lanata*



Scheme-11.2: Isolation of pure compounds (112-126) from ethyl acetate fraction of *S. lanata*

11.3. Characterization of new compounds from *Sorbus lanata*

11.3.1. Sorlanin (112)

Physical State: Yellowish gummy solid

Yield: 5.5 mg

UV λ_{max} in MeOH: 274 nm

IR ν_{max} cm^{-1} : 3393, 2937, 1598, 1576,

1516, 1488, 1456, 1435, 1416 cm^{-1}

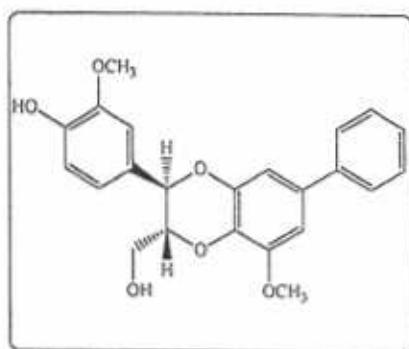
ESIMS (-): m/z 393 $[\text{M}-1]^-$, 375 $[\text{M}-1-\text{H}_2\text{O}]^-$,

363 $[\text{M}-\text{H}_2\text{COH}]^-$, 179 $[\text{M}-1-\text{C}_{13}\text{H}_{10}\text{O}_3]^-$

HRESIMS (+): m/z 417.1293 $[\text{M}+\text{Na}]^+$; calcd 417.1314

^1H - and ^{13}C -NMR: See Table-10.1

HSQC: See Table-10.1



11.3.2. Peshawerin (113)

Physical State: Yellowish white powder

Yield: 13.4 mg

UV λ_{max} in MeOH: 230, 262 and 291 nm

IR ν_{max} cm^{-1} : 3401, 2935, 1605, 1516,

1451, 1423 cm^{-1}

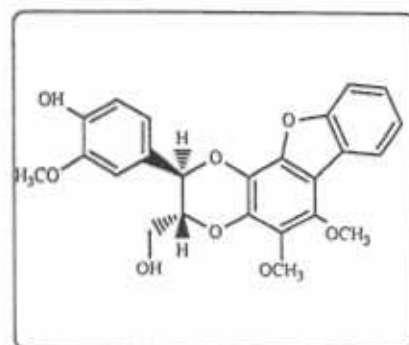
ESIMS (-): m/z 437 $[\text{M}-1]^-$, 419 $[\text{M}-1-\text{H}_2\text{O}]^-$,

407 $[\text{M}-1-\text{HCOH}]^-$, 179 $[\text{M}-1-\text{C}_{14}\text{H}_{10}\text{O}_5]^-$

HRESIMS (+): m/z 461.1202 $[\text{M}+\text{Na}]^+$; calcd 461.1315

^1H - and ^{13}C -NMR: See Table-10.2

HSQC: See Table-10.2



11.3.3. Sorbanin (114)

Physical State: White amorphous solid

Yield: 3.2 mg

UV $\lambda_{\max}$ in MeOH: 267 nm

IR $\nu_{\max}$ cm^{-1} : 3395, 2932 and 1600 cm^{-1}

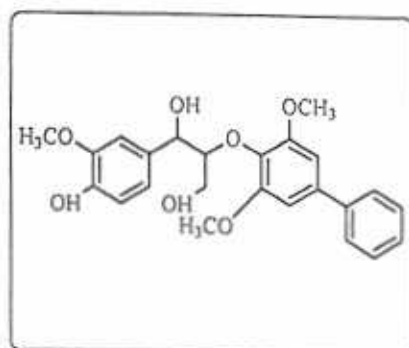
ESIMS (-): m/z 409 $[\text{M}-\text{H}_2\text{O}]^-$, 271 $[\text{C}_{16}\text{H}_{15}\text{O}_4]^-$,

229 $[\text{C}_{14}\text{H}_{13}\text{O}_3]^-$, 195 $[\text{C}_{10}\text{H}_{11}\text{O}_4]^-$

HRESIMS (+): m/z 449.1563 $[\text{M}+\text{Na}]^+$; calcd 449.1576

^1H - and ^{13}C -NMR: See Table-10.3

HSQC: See Table 10.3



11.3.4. Sorbanolic Acid (115)

Physical State: White amorphous powder

Yield: 40.3 mg

UV $\lambda_{\max}$ in MeOH: 218, 329 nm

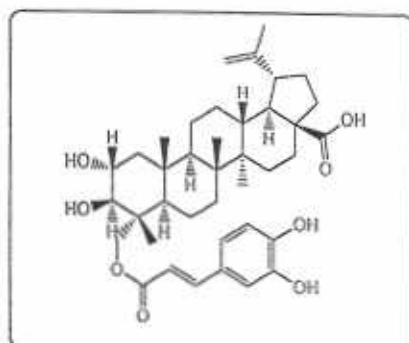
IR $\nu_{\max}$ cm^{-1} : 3358, 1686, 1602, 1516,
1447, 1378 cm^{-1}

ESIMS (-): m/z 649 $[\text{M}-\text{H}]^-$

HRESIMS (+): m/z 673.3712 $[\text{M}+\text{Na}]^+$; calcd 673.3716

^1H - and ^{13}C -NMR: See Table-10.4

HSQC: See Table-10.4



11.4. Characterization of known compounds from *Sorbus lanata*

11.4.1. Polystachyol (116)

Physical State: White amorphous powder

Yield: 3.4 mg

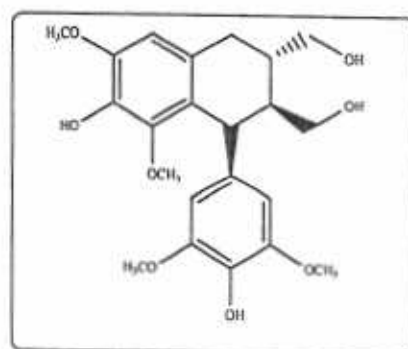
UV $\lambda_{\max}$ in MeOH: 277 nm

ESIMS (-): m/z 419 $[M-H]^-$

EI-MS: m/z 420 $[M]^+$

^1H - and ^{13}C -NMR: See Table 10.5

HSQC: See Table-10.5



11.4.2. Isolariciresinol (117)

Physical State: White amorphous powder

Yield: 3.4 mg

UV $\lambda_{\max}$ in MeOH: 283 nm

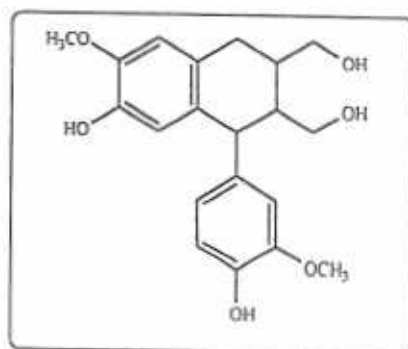
$[\alpha]_D = +22.1$ (MeOH; c 0.01)

IR $\nu_{\max}$ cm^{-1} : 3400, 2800, 2840, 1550, 1452 cm^{-1}

ESIMS (-): m/z 359 $[M-H]^-$

^1H - and ^{13}C -NMR: See Table-10.6

HSQC: See Table-10.6



11.4.3. Dihydrodehydrodiconiferyl Alcohol (118)

Physical State: colorless oily liquid

Yield: 3.3 mg

UV λ_{max} in MeOH: 237 nm

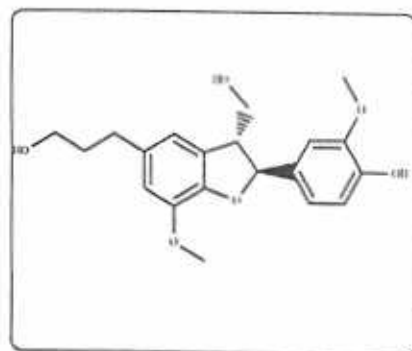
$[\alpha]_{\text{D}} = +58.0$ (MeOH; $c 0.1$)

IR ν_{max} cm^{-1} : 3450, 2944, 1702 cm^{-1}

EIMS (-): m/z : 360 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-10.7

HSQC: See Table-10.7



11.4.4. Tuberculatin (119)

Physical State: Yellowish white solid

Yield: 5.2 mg

$[\alpha]_{\text{D}} = -107.58$ (20% MeOH- CHCl_3 ; $c 1.112$)

UV λ_{max} in MeOH: 262, 316 nm

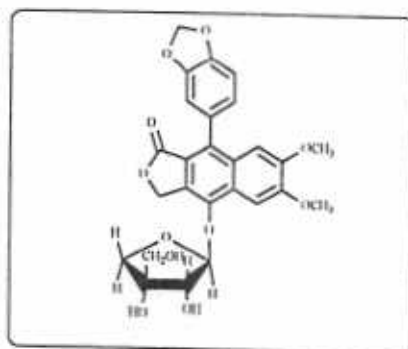
IR ν_{max} cm^{-1} : 3510, 1735, 1614, 932,

854, 827, 810 cm^{-1}

EIMS (-): m/z : 512 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-10.8

HSQC: See Table-10.8



11.4.5. Ovafolinin E (120)

Physical State: Yellowish white solid

Yield: 5.2 mg

$[\alpha]_D = +105.7$ (MeOH; c 0.16)

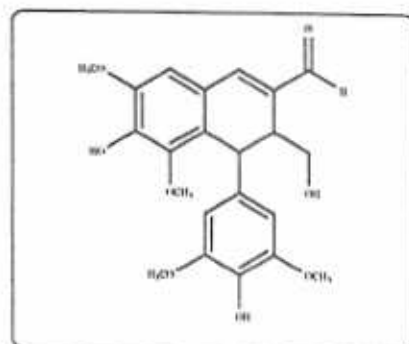
UV $\lambda_{\max}$ in MeOH: 254, 356 nm

IR $\nu_{\max}$ cm^{-1} : 3272, 2938, 1567 cm^{-1}

ESIMS (-): m/z : 415 $[\text{M}-1]^-$

^1H - and ^{13}C -NMR: See Table-10.9

HSQC: See Table-10.9



11.4.6. β -Pyrofuran (121)

Physical State: Colourless crystalline solid

Yield: 80 mg

UV $\lambda_{\max}$ in EtOH: 220, 229, 261, 289, 300 nm

IR $\nu_{\max}$ cm^{-1} : 3512, 3050, 3020, 3000,

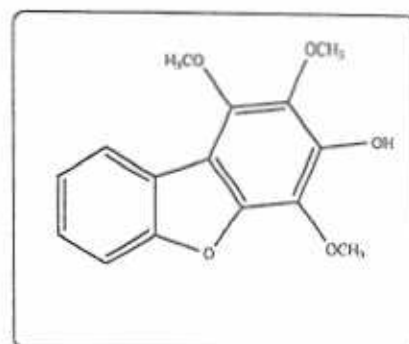
2932, 2830, 1630,

1600, 1500, 1460, 1448, 1410 cm^{-1}

EIMS: m/z 274 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-10.10

HSQC: See Table-10.10



11.4.7. Lyoniside (122)

Physical State: White amorphous powder

Yield: 39.6 mg

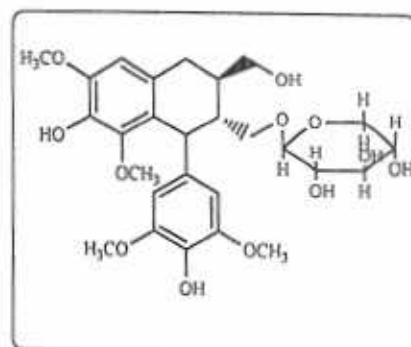
UV λ_{max} in EtOH: 215, 279 nm

$[\alpha]_{\text{D}} = +43.0$ (MeOH; c 0.8)

EIMS: m/z 552 $[M]^+$, 419 $[M + H - \text{xylose}]^+$

^1H - and ^{13}C -NMR: See Table-10.11

HSQC: See Table-10.11



11.4.8. 2'-Methoxyaucuparin (123)

Physical State: Colourless crystalline solid

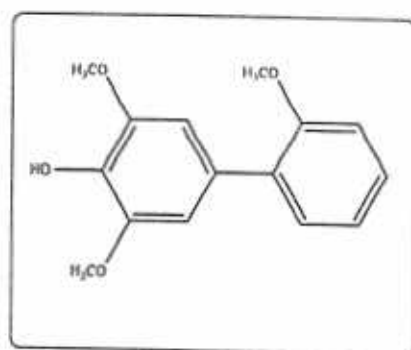
Yield: 8.5 mg

UV λ_{max} in EtOH: 268, 288 nm

EIMS: m/z 260 $[M]^+$

^1H - and ^{13}C -NMR: See Table-10.12

HSQC: See Table-10.12



11.4.9. Aucuparin (124)

Physical State: Colourless crystalline solid

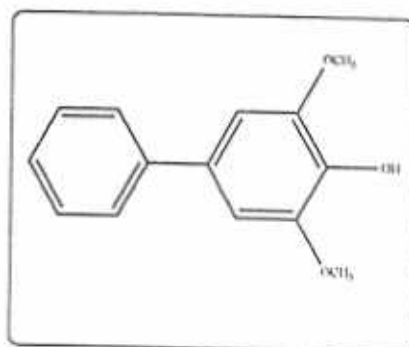
Yield: 10.4 mg

UV λ_{max} in EtOH: 228, 274 nm

EIMS: m/z 230 $[M]^+$

^1H - and ^{13}C -NMR: See Table-10.13

HSQC: See Table-10.13



11.4.10. Tetracosyl-3-(3, 4-dihydroxyphenyl)acrylate (125)

Physical State: White amorphous powder

Yield: 2000 mg

UV $\lambda_{\max}$ in CHCl_3 : 235, 325 nm

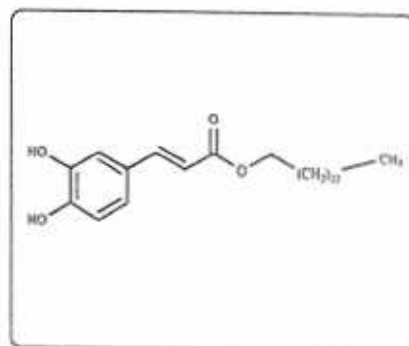
IR $\nu_{\max} \text{ cm}^{-1}$: 3500, 1700, 1670,

1600, 1510, 1460 cm^{-1}

EIMS: m/z 516 $[\text{M}]^+$

^1H - and ^{13}C -NMR: See Table-10.14

HSQC: See Table-10.14



11.4.11. 3β , 23-Dihydroxy-lup-20(29)ene-28-oic acid- 3β -caffeate (126)

Physical State: White amorphous powder

Yield: 23.8 mg

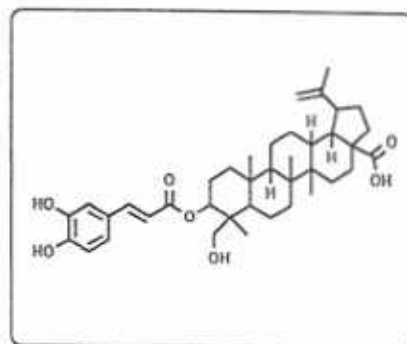
UV $\lambda_{\max}$ in MeOH: 328, 298, 246 nm

$[\alpha]_D = +32.9$ (Me_2CO ; $c0.9$)

ESIMS: m/z 633 $[\text{M}-1]^-$

^1H - and ^{13}C -NMR: See Table-10.15

HSQC: See Table-10.15



11.5. Diphenylpicrylhydrazyl (DPPH) radical-scavenging assay

The DPPH radical scavenging activity was performed in flat bottom polystyrene 96-well microtiter plates. The activities were measured by using a modified established methodology.^{453,454} To 100 μ L of each sample at different concentrations in MeOH were added 150 μ L of DPPH (0.25 mM) in MeOH. The mixture so obtained was shaken for a short time at room temperature and placed in the dark for 30 min. At the end of this period the absorbance of the mixture was measured at 517 nm by using a Spectra Max-190 96 well plate reader. Data was processed on SOFTmax pro 4.0 software. The percent DPPH scavenging from a sample at a given concentration was calculated from the absorbance readings as shown in the equation below.

$$\text{Scavenging \%} = 100 - (A_{\text{sample}} \times 100 / A_{\text{control}})$$

The assays were carried out in triplicate and the results are expressed as mean values $\pm$ standard deviations. The IC₅₀ values were calculated from the graph by using linear regression analysis.

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