ALKALOIDS AND FLAVONOIDS OF *ERYTHRINA MELANACANTHA*

A Thesis Presented to

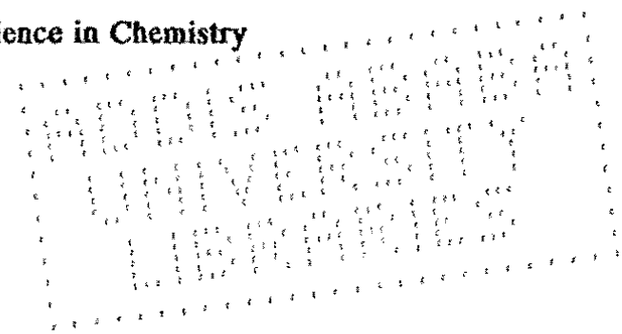
The School of Graduate Studies

Addis Ababa University

In Partial Fulfillment of

The Requirement for the Degree

Master of Science in Chemistry



By

Abraham Abebe

June, 1994

ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor, Dr. Ermias Dagne, for his constant guidance and encouragement during the course of this project.

I am indebted to Dr. Wendimagegn Mammo for his help particularly for searching on CD-ROM information on the genus *Erythrina*. Ato Abiy Yenesew and Ato Melaku Alemu are acknowledged for assistance in interpreting spectra. I am grateful to Ato Eyesuswork Reta and Ato Getachew Ashenafi for NMR spectral measurements and Ato Beniam Kebede for IR and UV spectra.

Last, but not least Ato Akalu Tefera is acknowledged for providing printing service throughout my study.

ABSTRACT

ALKALOIDS AND FLAVONOIDS OF *ERYTHRINA MELANACANTHA*

By: Abraham Abebe

Research Advisor: Dr. Ermias Dagne

The tropical genus *Erythrina,* which belongs to the family Leguminosae (sub-family Papilionoideae), locally known as "Korch" (Amharic) and "Wolensu" (Oromo) has folk medicinal uses such as in the treatment of stomach pain and female infertility. The seeds of this plant have been investigated before, whereas no previous phytochemical work has been reported from the stem bark of *E. melanacantha.*

The isolation and characterization of alkaloids and flavonoids from the seeds and stem bark, respectively, of this species constitute the subject of this study. As a result, the seeds and stem bark of *E. melanacantha* afforded eleven compounds, of which two are new natural products. From the seeds three known alkaloids, namely, erythraline (**11**), erymelanthine (**50**) and erysovine (**3**) have been identified. Chemical investigation of the stem bark resulted in the isolation of two new compounds characterized on the basis of spectroscopic data as 5-hydroxy-7,8-(hydroxyisopropyl dihydrofurano)flavanone (**152**) and a biflavanone (**159**). In addition, from the stem bark of this plant, five known flavonoids, namely, glabranin (**153**), obovatin (**155**), 7-hydroxy-8-prenylflavanone (**156**) 3'-O-methylorobol (**157**), 3'-methoxydaidzein (**158**) and a known cinnamate ester, erythrinasinate (**160**) have been isolated. Search in the chemical literature indicated that compounds **153**, **155**, **156** and **158** have not been reported before from the genus *Erythrina*.

Glabranin (**153**) is proposed as the biosynthetic precursor of the three compounds **152**, **155** and **156**, while compounds **152** and **153** serve as building blocks of the dimer **159**.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

LIST OF SCHEMES

LIST OF APPENDICES

APPENDIX PAGE

1. INTRODUCTION

The plant kingdom is a virtual gold mine of new chemical compounds, that show extreme structural diversity, waiting to be discovered. Secondary metabolites provide plants with a means of adjusting to changing circumstances and thus are measure of the fitness of the organism to survive [1]. These functions are a consequence of the bioactive properties of natural products which may be toxins, repellents, attractants, signal compounds, etc. Recently secondary metabolites are regarded as being essentially beneficial to the producer. Nevertheless, their role in the economy and survival of the plants is still controversial.

Of the 250,000 species of higher plants known to exist on earth, only a relatively handful have been studied phytochemically and for their potential therapeutic value in medicine. Since the beginning of the 19th century a large number of biologically active secondary metabolites of plant origin have been found to have commercial application as drugs, flavours, pesticides, etc [2]. It has been estimated that about 64% of the total population of the world is dependent on traditional medical practices for their primary health care needs. Higher plants are known to be the main source of drug therapy in traditional medicine [3].

The genus *Erythrina* (Leguminosae) comprising over 110 species, is widely distributed in tropical and subtropical regions of the world [4]. Various parts: roots, bark, leaves and wood of these plants are commonly used in African folk medicine [5] for the treatment of female infertility, stomach pain and gonorrhoea.

Studies of the chemistry of *Erythrina* species began in the early 1930s with studies of their alkaloids [6]. Originally alkaloids were defined as pharmacologically active nitrogen heterocyclic bases of plant origin. However, since the early 19th century the term alkaloid has been extended and includes most naturally occurring, nitrogen containing secondary metabolites of plant, microbial and even animal origin. Alkaloids have played a crucial part in the development of organic and medicinal chemistry. In recent years, particularly, alkaloids and related compounds have been used in cancer chemotherapy.

In addition to alkaloids, the bark and roots of plants belonging to this genus have been reported to be rich sources of cinnamate esters, pterocarpans and flavonoids.

Prior to the advent of modern techniques, flavonoids were the compounds of choice in early experiments on chemical genetics, because of their universal distribution in most aerial parts of plants and their ease of separation and identification. It is estimated that about 2% of carbon photosynthesized by plants is converted into flavonoids or closely

related compounds [7]. One of the functions of flavonoids in plants may be to protect them against diseases caused by microorganisms and to act as feeding deterrents to insects and other herbivorous animals [8]. Flavonoids exhibit a wide range of biological activities and currently are of particular interest as potential anticancer agents and as natural insecticides.

In the past few years, significant observation was made regarding biological functions of flavonoids which involves their action as signal molecules in the interaction between legumes and nodulating bacteria [9]. Various phenols, flavonoids, alkaloids, coumarins, diterpenes and other compounds, which inhibit the development of fungi and bacteria, may accumulate to high concentrations in epidermal tissues of higher plants.

E. melanacantha, native to East Africa [10], is a tree growing up to 20 m in height and is best characterized by its deciduous leaves, greyish black with dark hoacked spines, bright red flowers, red filaments, light brown anthers, branches bearing curved prickles and reddish brown seeds. The plant materials used in this study were collected in 1988 from from Negelle Borana (southern part of Ethiopia) near Melka Goba at an altitude of 980 m in *Acacia Comiphora* wood land habitat.

2. LITERATURE BACKGROUND

Erythrina species have long been known to produce alkaloids possessing curarizing properties [11]. Singh *et al.* [12] first reported some work on the non-alkaloidal constituents of some *Erythrina* species and Nakanishi and his co-workers [13] also carried out investigations on the neutral components of *E. abyssinica.*

2.1. ALKALOIDS OF *ERYTHRINA* SPECIES

Unlike many classes of naturally occurring substances which may be defined rather precisely, no entirely adequate definition of alkaloids is known because of the variety of alkaloid types in existence. Alkaloids, as nitrogenous bases, have been known for a long time as toxic principles [14] and attracted the attention of chemists due to their physiological activities. They have been classified into different groups by their origin (plant family or genus) or structural types. Alkaloids have not proved to be as generally useful in taxonomy as some other types of secondary metabolites. But Gomes and Gottlieb [15] categorized the Angiospermae into three major groups by their alkaloid distribution.

Erythrina species are known to elaborate a unique class of alkaloids known as erythrina alkaloids. Their structures have been derived from the tetracyclic spiroamine, erythrinane skeleton. There are now over seventy erythrina alkaloids of known structure and several more, the structures of which are yet to be assigned [16]. Over half of *Erythrina* species have been examined for their alkaloidal content. Most studies have concentrated on examination of the seeds, although alkaloids are proved to be found in the leaves, flowers, stem and stalks [17]. The alkaloidal profile often varies in the different parts of the plant. Some studies indicated that variation may result from the location of the plant or its age at harvest.

The curare-like action of those alkaloids in extracts of the seeds of *E. americana* was first recognized by Dominguez and Altamirano in 1877 and confirmed in later work by Ramirez and Rivero [18]. Plants of the genus *Erythrina* have several medicinal uses. These pharmacological activities seem to be due to the presence of the erythrina alkaloids [19].

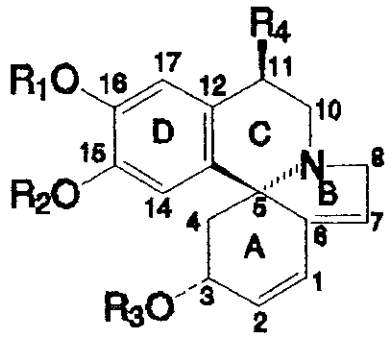
The names of the majority of erythrina alkaloids were coined from the prefixes, erythroi-, eryso- and erythra-, depending on their structure. The prefix erythroi- indicates that the ring-D is a lactone as in α- and ß-erythroidine (**52,53**); eryso- represent the presence of a phenolic group where oxygenation is common at carbons 3, 15 and 16 and rare elsewhere as in erysodine (**2**), erysovine (**3**), erysopine (**5**), etc. On the other hand, the term erythra- is used for the parent skeleton as in erythraline (**11**).

Phytochemical investigations of *Erythrina* species by various researchers described the occurrence and structures of a number of alkaloids. These structurally different compounds have been grouped as dienoid, alkenoid types and miscellaneous alkaloids. In addition to the ring A unsaturation, structural modifications such as the presence of hydroxyl, methoxyl and keto groups, exist in the different types of these alkaloids.

2.1.1. Dienoid type

These are compounds containing a conjugated diene system in the A and B rings (Table 1) and comprise the majority of erythrina alkaloids. Alkaloids such as, erysodine (**2**), erythraline (**11**), erysotrine (**1**) and erysovine (**3**) are the most abundant and occur in the majority of the species examined so far.

Table 1 Alkaloids of the dienoid type



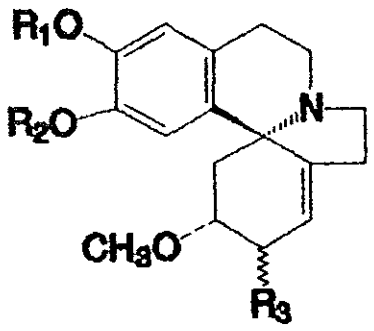
No/ Compound	R_1	R_2	R_3	R_4	Ref
1. Erysotrine	CH_3	CH_3	CH_3	H	20
2. Erysodine	H	CH_3	CH_3	H	21
3. Erysovine	CH_3	H	CH_3	H	21
4. Erythavine	CH_3	CH_3	H	H	22
5. Erysopine	H	H	CH_3	H	21
6. Erysonine	H	CH_3	H	H	23
7. Erysoline	CH_3	H	H	H	22
8. Erythristemine	CH_3	CH_3	CH_3	OCH_3	24
9. Erythrascine	CH_3	CH_3	CH_3	$OCOCH_3$	25
10. Erythrinine	$-CH_2-$		CH_3	OH	26
11. Erythraline	$-CH_2-$		CH_3	H	27
12. 11-Methoxyerythraline	$-CH_2-$		CH_3	OCH_3	28
13. 11-Oxoerysodine	H	CH_3	CH_3	=O	16
14. 11-Oxoerysovine	CH_3	H	CH_3	=O	16
15. 11-Oxoerysopine	H	H	CH_3	=O	16
16. 11-Oxoerythraline	$-CH_2-$		CH_3	=O	16
17. 11-Hydroxyerysodine	H	CH_3	CH_3	OH	16
18. 11-Hydroxyerysovine	CH_3	H	CH_3	OH	16
19. 11-Hydroxyerysotrine	CH_3	CH_3	CH_3	OH	16
20. 11-Methoxyerysodine	H	CH_3	CH_3	OCH_3	16
21. 11-Methoxyerysovine	CH_3	H	CH_3	OCH_3	16
22. 11-Methoxyerysopine	H	H	CH_3	OCH_3	16
23. 11,12-Dehydroerysodine	H	CH_3	CH_3	11,12db	16
24. 11,12-Dehydroerysovine	CH_3	H	CH_3	11,12db	16
25. Erysothiopine	OCH_3	a	CH_3	H	29
26. Glucoerysodine	b	CH_3	CH_3	H	16
27. Erysodinophorine	c	CH_3	CH_3	H	16
28. 15-Demethyleryso dinophorine	c	H	CH_3	H	16
29. Erysophorine	CH_3	c	CH_3	H	16
30. Isoerysopinophorine	H	c	CH_3	H	16
31. Erythrocarine	$-CH_2-$		H	H	16
32. Erysotrine-N-oxide					16
33. Erythrartine-N-oxide					16

where a: $R_2=HO_2CCH_2SO_2$, b: R_1O = 1-ß-glucosyl, c: hypaphorine ester.

2.1.2. Alkenoid type

This group includes alkaloids having a 1,6-double bond in the A ring of the basic skeleton. The common structural feature of these alkaloids is possession of hydrogen or methyl/methylenedioxy at C-15 and C-16 positions. In addition, methoxyl group is present at position C-3.

Table 2 Alkaloids of the alkenoid type



No. Compounds	R_1	R_2	R_3	Ref.
34. Dihydroerysotrine	CH_3	CH_3	H	30
35. Erythratidine	CH_3	CH_3	OH	31
36. Erythratidinone	CH_3	CH_3	=O	32
37. Erythramine	-CH_2-		H	33
38. Erythratine	-CH_2-		OH	27
39. Erythratinone	-CH_2-		=O	34
40. Dihydroerysovine	CH_3	H	H	16
41. Erysosalvine	CH_3	H	OH	22
42. Erysosalvinone	CH_3	H	=O	22
43. Dihydroerysodine	H	CH_3	H	16
44. Erysotine	H	CH_3	OH	22
45. Erysotinone	H	CH_3	=O	22
46. Erysopitine	H	H	OH	35
47. Erysoflorinone	H	H	=O	22

2.1.3. Miscellaneous

A number of alkaloids not belonging to the above two groups (Table 3) have also been isolated from various *Erythrina* species. Some of these such as orientaline (**65**), N-nororientaline (**66**), protosinomenine (**67**) and N-norprotosinomenine (**68**) are tetahydrobenzylisoquinoline type. In addition, hypaphorine (**69**), which has been reported to be present in almost every species studied, is probably ubiquitous. Those alkaloids in which the aromatic ring-D of the above two types has been replaced by an unsaturated lactone, as in compounds **52-56**, are also grouped under this category. Their corresponding structures are given in Appendix 5.

Table 3 Other alkaloids isolated from *Erythrina* species*

No	Compound*	Ref.
48.	Erybidine	16
49.	Erythrabine	16
50.	Erymelanthine	16
51.	Erysodinone	16
52.	α-Erythroidine	36
53.	ß-Erythroidine	36
54.	8-Oxo-α-erythroidine	16
55.	Dehydro-α-erythroidine	16
56.	8-Oxo-ß-erythroidine	37
57.	8-Oxo-erythraline	38
58.	8-Oxo-erythrinine	16
59.	11-Hydroxyerythratidine	16
60.	11-Methoxyerythratidine	16
61.	11-Hydroxyerythratine	16
62.	3-Demethoxyerythratidinone	16
63.	Erystotramidine	16
64.	Erythrocucculine	16
65.	Orientaline	25
66.	N-Nororientaline	26
67.	Protosinomenine	39
68.	N-Noroprotosinomenine	39
69.	Hypaphorine	16
161.	Demethoxycarbonylerymelanthine	105

* See Appendix 5 for their structures

The *Erythrina* species of Ethiopia have been subject of some phytochemical studies for their alkaloidal constituents. Thus, the seeds of *E. malnacantha* afforded the novel erythrina alkaloid, erymelanthine (**50**), containing a second nitrogen placed in the dienoid nucleus, together with erysovine (**3**) [41]. The related 8-oxo-alkaloid, melanacanthine, was later reported [42] along with erysodine (**2**), erysosalvine (**41**), erysotine (**44**) and erythratidine (**35**). Very recently, erythraline (**11**) and demethoxycarbonylerymelanthine (**161**) have been detected [105] from the seeds of *E. melanacantha* by GC-MS studies.

Seeds and flowers of *E. brucei* were reported to contain 8-oxoerythrinine (**58**) together with erythrinine (**10**), erythraline (**11**), erysodine (**2**) and 8-oxoerythraline (**57**) [43]. The alkaloids erysosalvine (**41**), erythraline (**11**), erythratidine (**52**) and erythrinine (**10**) have been shown to occur in the seeds of *E. burana* [44] whereas *E. abyssinica* was reported to produce erysodine (**2**), erysopine (**5**), erysotrine (**1**), erythraline (**11**), erythratidine (**35**), erythratine (**38**), erythristemine (**8**), glucoerysodine (**26**), 11-methoxyerysovine (**21**) and 11-oxoerysodine (**13**) [16].

2.1.4. BIOSYNTHESIS OF ERYTHRINA ALKALOIDS

Biogenetically the erythrinane skeleton is proposed to be derived from tyrosine units through oxidative coupling. Scheme 1 indicates the biogenetic routes responsible for the formation of erythraline (**11**) and erythroidine (**53**) [18]. The (+)-isomer of N-norprotosinomenine (**71**) is a better precursor of erythraline (**11**) than the (-)-isomer. Symmetrical intermediate dibenzazonine (**73**) is involved in the biosynthetic pathway. Experimentally it was confirmed that only the (-)-isomer of erysodienone (**75**) was a precursor of erythraline (**11**) and ß-erythroidine (**53**). The biosynthesis of α- and ß-erythroidine has been studied by tracer experiments [44]. This led to the proposal that it is derived from (-)-erysodienone.

The steps involved in the biosynthetic route shown in Scheme 1 are listed below:

A- Oxidative cyclization.

B- Oxidative *para-para* coupling with five membered ring formation.

C- Elimination and reduction.

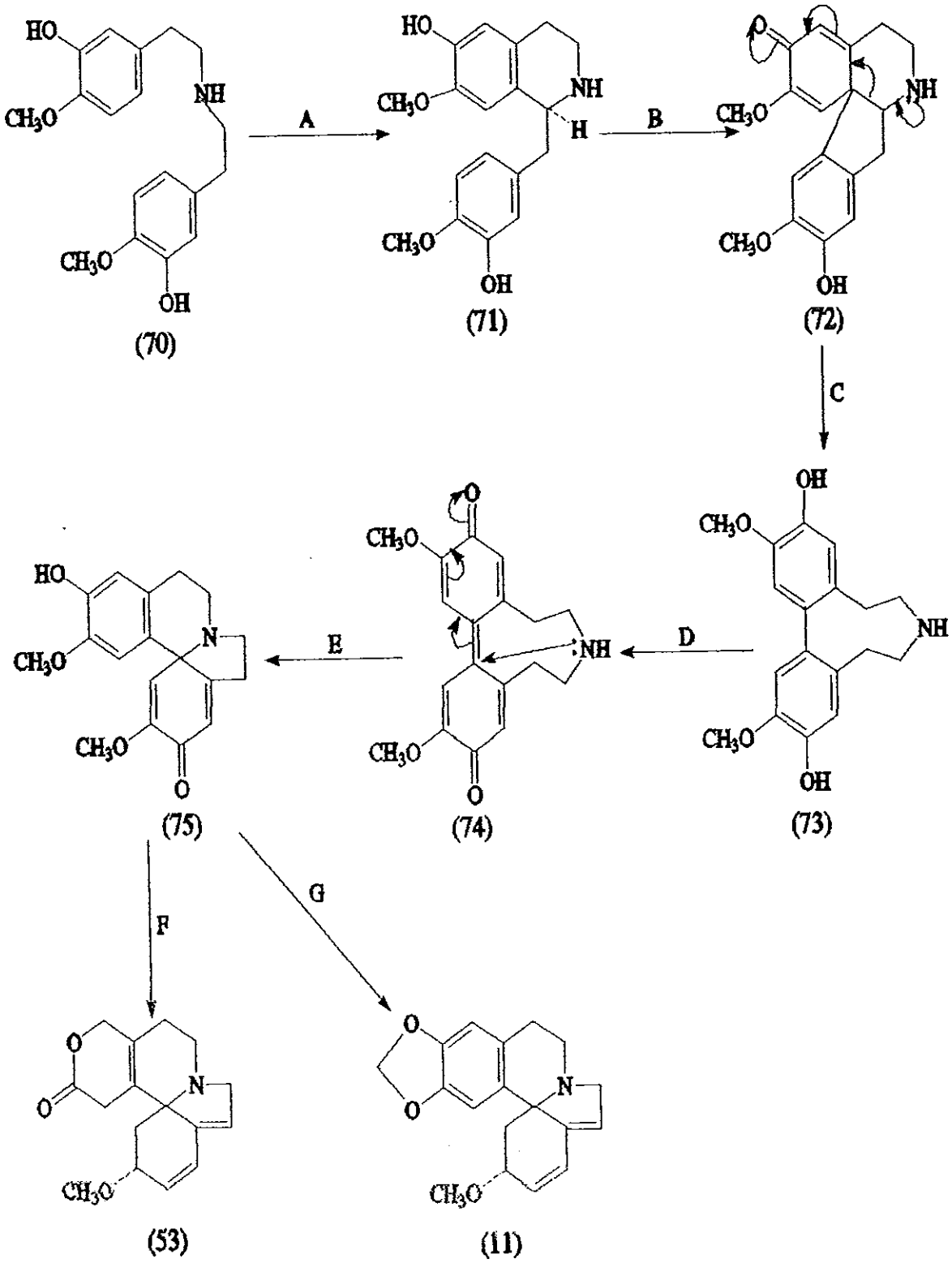
D- Oxidation of dibenzazonine (**73**) to a diphenoquinone (**74**).

E- Intramolecular attack of the nitrogen.

F- Lactonization of the aromatic ring.

G- 1,4-Elimination of water to form the diene alkaloid, erythraline (**11**).

Scheme 1 : Biosynthesis Out line Of Erythrina Alkaloids



2.1.5. SPECTRAL PROPERTIES OF ERYTHRINA ALKALOIDS

^{1}H and ^{13}C NMR spectroscopic techniques are essential means for structure identification of alkaloids. Furthermore, the analysis of alkaloids has been facilitated by the emergence of multiple stage mass spectroscopy. Boar and Widdowson [45] have studied the mass spectra of some erythrina alkaloids. The MS of dienes show similar fragmentation patterns which are initiated by 5,6-cleavage. The *cis*- and *trans*-erythrinanes may be distinguished by the chemical shifts of H-7 and H-11. In the *trans* series H-14 lies close to the axial protons at C-3 and C-1 and is consequently deshielded. The dienoid alkaloids show IR absorption signal at 1610 cm^{-1} and UV absorption maximum around 285 (dioxygenated aromatic ring) and 230-235 nm (diene). Alkenoids absorb in the UV around 225 nm, whereas the enone group usually shows UV absorption maximum around 230 nm and IR bands in the region of 1675-1698 cm^{-1} [17].

2.2. NON-ALKALOIDAL CONSTITUENTS OF *ERYTHRINA* SPECIES

Flavonoids occur widely in the plant kingdom and are especially common in leaves, flowers and pollens. They are also abundant in woody parts such as stems and roots. Plant flavonoids and isoflavonoids play an important ecological role; the compound may provide protection against diseases and herbivorous animals. It is generally assumed that external flavonoids act as UV-screen and play roles in the adaptation of plants to arid and alpine habitats [46], in other words they act as internal light filters for the protection of chloroplasts and other organelles from UV damage. Hence flavonoids occur in high concentrations in the vacuoles of epidermal cells as well as within chloroplasts. Since many isoflavonoids are produced by legumes as phytoalaxins after microbial attack, their role in plants may be to protect them against microorganisms. They are not only toxic to fungi and certain bacteria but also to other organisms. Simmonds *et al.* [47] assessed the insect antifeedant activity of a number of legume flavanones and chalcones on two species of army worms. They found that compounds containing methyl and prenyl substituents generally exhibited better anti-feedant activity than those containing only hydroxyl groups. Lane *et al.* [47] also tested many legume isoflavonoids for their feeding deterrent effects on the root-feeding larvae of the beetle *Castelytra zealandica* and found that compounds containing a dimethylchromene ring substitution showed the highest deterrence against this

beetle. Flavonoids have been reported to have a wide variety of biological activities. Certain flavonoids have been considered to display mutagenic and carcinogenic effects [48] whereas others, and sometimes the same flavonoids, have been reported to exert inhibition of tumor promotion.

In comparison to the various classes of compounds which have been examined, the flavonoids and related phenolic compounds have proved to be the most useful as taxonomic and evolutionary markers for the following reasons: they are widespread in lower and higher plants, they have high stability, they are easy to identify, their structural variability and known biosynthetic pathways [49]. The first real investigation on the use of flavonoids as systematic markers in higher plants was carried out in Sweden on *Pinus* species by Holger Erdtman and his colleagues starting in the 1940's. Later, Harborne and his co-workers have published several reports on the chemotaxonomic use of flavonoids [50].

Flavonoids of the genus *Erythrina* show a great structural diversity which arises from the ease with which the flavonoid nucleus is hydroxylated, methylated and prenylated. Prenylated flavonoids and isoflavonoids seem to be largely restricted to the subfamily Papilionoideae and they tend to occur in organs such as roots, stem, and heart wood rather than in leaves and seeds [51]. As a result, prenylation is a characteristic feature of erythrina flavonoids and isoflavonoids. Phytochemical investigation of a number of *Erythrina* species have shown the stem and roots to be rich sources of flavonoids, isoflavonoids, esters and triterpenoids. C-prenylated flavonoids are particularly common in the genus *Erythrina*. Many of the new flavonoids are positional isomers of one another, or cyclized derivatives thereof.

Especially, research groups in Cameroon and Nigeria are actively involved in the search for non-alkaloidal constituent of *Erythrina* species. These and other reports on the flavonoids in the genus *Erythrina* have indicated that only 15 *Erythrina* species have been studied for their non-basic chemical constituents. Most of these studies are restricted to the species found in West and Central Africa.

An attempt has been made here to review the flavonoids, cinnamate esters and triterpenes identified so far from the genus *Erythrina*. The names and structures of the compounds are given in Appendix 1-4 and 6-8. The *Erythrina* species which have so far been studied phytochemically are listed in Table-4.

Table 4 List of *Erythrina* species and their abbreviations

Erythrina species	Abbreviations
1. *E. abyssinica*	Ea
2. *E. berteroana*	Ebe
3. *E. x bidwillii*	Ebi
4. *E. burana*	Ebu
5. *E. cristagalli*	Ec
6. *E. eriotriocha*	Eer
7. *E. excelsa*	Eex
8. *E. fusca*	Ef
9. *E. gluca*	Eg
10. *E. mildbraedii*	Em
11. *E. sandwicensis*	Esa
12. *E. senegalensis*	Ese
13. *E. sigmoidea*	Esi
14. *E. suberosa*	Esu
15. *E. variegata*	Ev

2.2.1. FLAVANONES

Over nineteen flavanones have been isolated from six *Erythrina* species. These compounds are listed in Appendix 1. The main difference among the flavanones are: oxygenation pattern, position of prenyl substituents and cyclization of the prenyl group in different directions. Compounds such as, 2'-prenyleriodictyol (**93**), and sigmoidine A (**81**) and F (**86**) possess a unique 6'-prenylation pattern. Furthermore, 5,7,4'-trihydroxy-3'-methoxy-5'-prenylflavanone (**92**), which inhibited growth of *Cladosporium cucumerinum*, was identified as a component of *E. berteroana* [88].

2.2.2. ISOFLAVONES AND ISOFLAVANONES

Isoflavones with a variety of substitution patterns have been identified from seven species of *Erythrina*. In addition to oxygenation pattern, structural variations arise from the incorporation of prenyl substitutions into the ring system. Cyclization of an isoprenyl group on to a neighbouring phenol function results in the formation of further pyrano or furano ring systems and a variety of structures depending on the direction of ring fusion. Eriotriochin (**106**), a novel isoflavone which contains a glycol unit attached to the cyclized isoprene (furano ring system), was reported from *E. eriotriocha*. Lists of isoflavones and isoflavanones known to occur in *Erythrina* species and their structures are given in Appendix 2 and 7, respectively.

2.2.3. PTEROCARPANS, A CHALCONE AND A COUMESTANE

Appendix 3 lists the pterocarpans that have been characterized from *Erythrina* species. All of the erythrina pterocarpans have 6aR, 11aR absolute configuration.

A chalcone (abyssinone VI) was isolated from *E. abyssinica* and *E. sigmoidea*. A coumestane (4-hydroxycoumestrol) was obtained from *E. sigmoidea*. Appendix 8 shows the structures of pterocarpans, a chalcone and a coumestane isolated from *Erythrina* species.

2.2.4. ESTERS AND TRITERPENOIDS

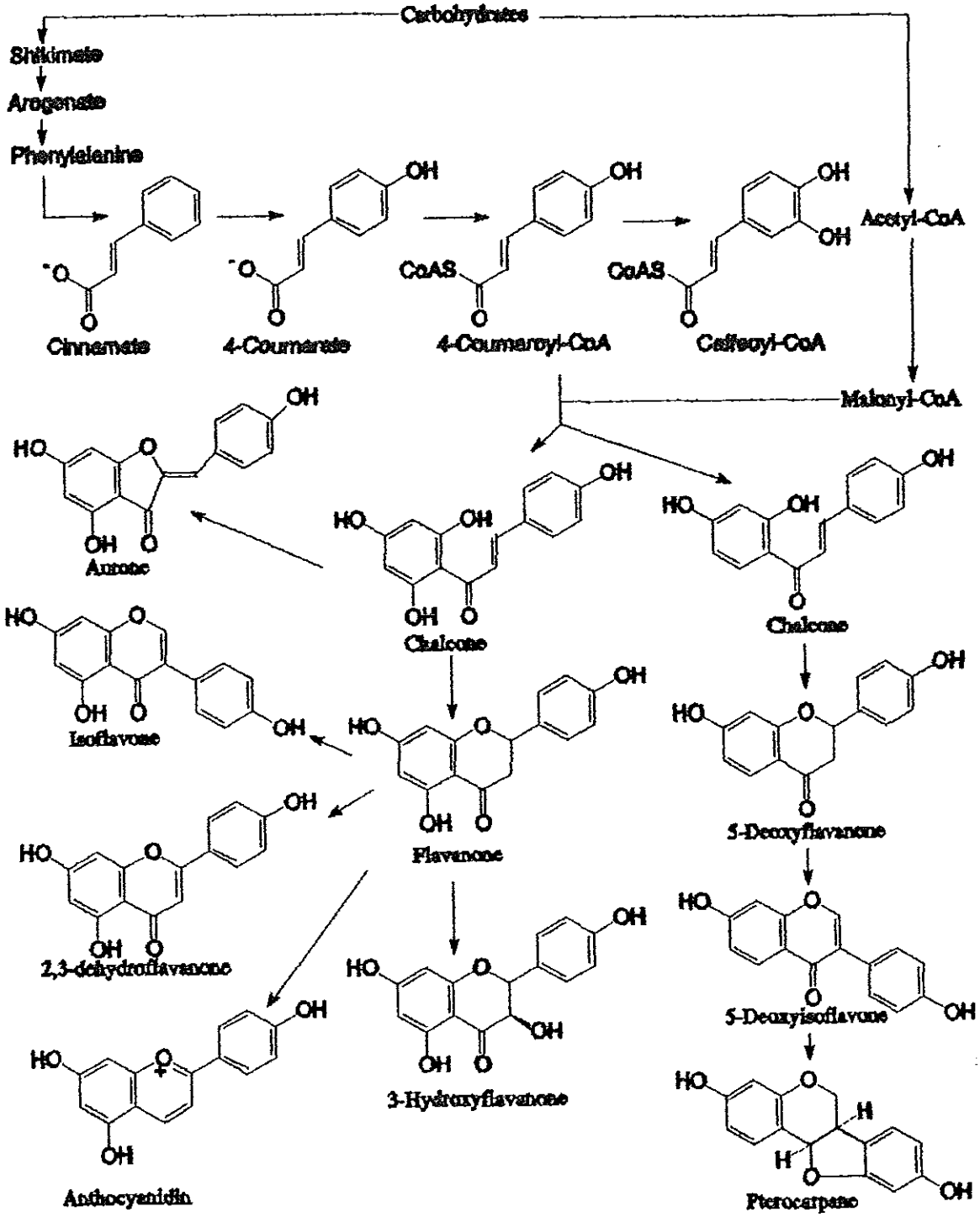
Few esters of cinnamic acid derivatives, which could be biogenetic precursors of flavonoids, have been reported from some species of *Erythrina*. They have characteristic hydroxylation or methoxylation pattern at C-4 as shown in Appendix 9. In addition to the known triterpenes, two novel triterpene saponins, sigmoisides A (**150**) and B (**151**), have been characterized from *E. sigmoidea* by Fomum *et al.* [5]. So far six triterpenes and four cinnamate esters have been identified from six different species of *Erythrina*.

2.2.5. BIOSYNTHESIS OF FLAVONOIDS

Coumaryl-CoA and malonyl-CoA of carbohydrate origion are the direct flavonoid precursors [52]. Malonyl-CoA is synthesized from the glycolysis intermediate acetyl-CoA carboxylase. As shown in Scheme 2, the supply of 4-coumaroyl-CoA involves the shikimate/arogenate pathway, which is the main route to the aromatic amino acids phenylalanine and tyrosine in higher plants. The central step in flavonoid biosynthesis is the condensation of three molecules of malonyl-CoA with a suitable hydroxycinnamic acid-CoA ester.

The B-ring and part of the heterocyclic ring of the flavonoid skeleton come from a suitable hydroxyl-cinnamic acid CoA ester, ordinarily 4-coumaroyl-CoA, whereas the A-ring originates from three acetate units *via* malonyl-CoA. The key enzyme for the formation of the flavonoid skeleton is chalcone synthase (CHS), which catalyses the stepwise condensation of three acetate units from malonyl-CoA with 4-coumaroyl-CoA to the C_{15} intermediate 2',4',6',4-tetrahydroxychalcone and 6'-deoxychalcone in action with

Scheme 2: Biosynthetic pathways leading to the major flavonoids classes



a NADPH-dependent reductase. Therefore, 6'-hydroxy and 6'-deoxychalcones are the immediate precursors for all flavonoid compounds [52].

All the simple flavonoids are derived biosynthetically from a common C_{15}-precursor, that is a chalcone intermediate, which is then modified in a variety of ways in a series of unit processes by oxidation, reduction, ring closure, methylation, etc. The stereospecific cyclization of the chalcone, catalysed by chalcone isomerase, provides a 2S-flavanone. Flavanones are the direct

precursors for the flavones, isoflavones and others. Isoflavones with different substituents may result in the corresponding pterocarpans, coumestans and rotenoids. Methylation and acylation of A- and B-ring hydroxyl groups are late events in the biosynthetic pathways. The metabolic pool of flavonoids is by no means static, but subject to turnover at widely varying rate. Scheme 2 indicates the biosynthetic routes for the formation of the most common flavonoids [52].

2.2.6. SPECTRAL PROPERTIES OF FLAVONOIDS

Spectroscopic techniques are most convenient means for structural determination of flavonoids. The main stages in the structural elucidation of flavonoids are recognition of the class to which the compound belongs and determination of the nature and orientation of substituent groups in the aromatic rings with the help of UV, NMR, IR, and MS techniques.

Flavonoids have very characteristic UV spectra and these give very valuable indication of the class of compound to which a particular flavonoid belongs. The substitution patterns of the A- and B- rings of these compounds can in part be determined by means of shift reagents. IR is used for the detection of functional groups in a molecule and the corresponding spectra of the flavonoids show absorption bands in the region 1500-1600 cm^{-1} due to aromatic rings along with a carbonyl bands at 1620-1670 cm^{-1} [53]. The presence of chelated OH is observed at shorter wave number than free OH. Moreover, the substitution pattern of the aromatic rings in the molecule could easily be deduced from the different types of signals in the finger print region of the spectrum.

^{1}H and ^{13}C NMR spectroscopic techniques are used to distinguish between the different classes of flavonoids, the oxygenation pattern and the number and structures of other functional groups. Flavonoids having a carbonyl group at C-4 are definitely identified

by their carbonyl resonance in the region 155-207 ppm and this value is strongly dependent on the molecular environment (conjugation and substitution). 2D NMR, homonuclear proton and hetronuclear carbon COSY, techniques are now commonly employed in the structural elucidation of flavonoids.

The isoflavones can be distinguished from other classes of flavonoids by UV and NMR spectroscopy. Most isoflavones have an intense UV absorption band at 255-275 nm and generally a less intense band or inflection at 310-330 nm. The low intensity absorption of the second band of isoflavones is a valuable diagnostic feature [54]. The ^{1}H NMR signal of the olefinic proton at C-2 in isoflavones appears as a characteristic down field singlet at δ 7.8-8.00. In view of the high value of the coupling constant (J = 12 Hz) between protons at C-2 and C-3 of ring-C, it has been concluded that all natural flavanones exist in the thermodynamically favoured conformation in which the proton at C-2 is axial and the phenyl ring equatorial. EIMS provides valuable information as to the substitution patterns of A- and B- rings and the molecular weight of flavonoids. Therefore, it gives characteristic fragments by fission of the molecular ion into A- and B-rings derived parts through the main RDA cleavage. The molecular ion may or may not appear as a major peak in the MS of flavonoid aglycones but should be an even mass number due to the presence of only oxygen, carbon and hydrogen atoms.

2.3. OBJECTIVES OF THIS STUDY

The present study was undertaken with the objectives of isolation and structural elucidation of secondary metabolites, mainly alkaloids and flavonoids from the seeds and stem bark, respectively, of one of the *Erythrina* species occurring in Ethiopia. Of the four *Erythrina* species known to occur in Ethiopia, *E. brucei* and *E. burana* are endemic while *E. melanacantha* and *E. abyssinica* are indigenous. We report here the results of investigations on the seeds and stem bark of *E. melanacantha*. In this study, emphasis is given to stem bark constituents, although flavonoids are estimated to be found in the other parts of the plant.

The higher plant *Erythrina* is a fast growing tree, whose trunk and roots are soft and easy to cut when it is wet, hard and strong when it is dry and light to handle. As a result there is a high production of timber commonly used in Ethiopia for making furniture, doors, window panes and other traditional household materials. Apart from these, the root bark of *E. abyssinica* is locally used in some part of our country in the

treatment of stomach pain. This is due to the presence of biologically active secondary metabolites, mainly flavonoids as indicated by Nakanishi *et al.* [13].

Knowledge of the types of compounds, their biosynthesis and pattern of distribution in *E. melanacantha* allows one to have more refined understanding of the plant which in turn may facilitate the utilization of this widely available plant resource.

3. RESULTS AND DISCUSSION

A broad range of chromatographic techniques, such as CC, TLC, PTLC, CPTLC and sephadex LH-20, have been employed for efficient and mild isolation of pure compounds. Structural elucidation of the compounds in this study were conducted mainly with the aid of NMR, UV, IR and MS spectroscopic techniques. The physical and spectral data of the known compounds isolated in this study have been compared with those reported in the literature.

In this study we have examined the seeds and stem bark of *E. melanacantha*. This is the first report on the chemical study of the stem bark. Chemical screening for alkaloids and flavonoids showed the bark to be devoid of alkaloids but rich in phenolic compounds. From the seeds, in addition to the two alkaloids reported earlier, namely, erymelanthine (**50**) and erysovine (**3**) [41], we have isolated a third alkaloid and identified it as the well known erythrina alkaloid, erythraline (**11**), based on its UV and ^{1}H NMR spectra. Its presence in the seeds of *E. melanacantha* was confirmed very recently by GC-MS studies [105].

3.1. CHARACTERIZATION OF ALKALOIDS OF *E. MELANACANTHA*.

3.1.1. Erythraline (11)

This compound was obtained from the $CHCl_3$ acidic extract as an amorphous brown sticky substance. Numerous attempts to induce crystallization failed. The structure of the compound was assigned mainly with the help of ^{1}H NMR spectrum. The two aromatic *para* protons appeared at δ 6.60 (H-14) and 6.75 (H-17) and the methoxyl protons appeared as a three-proton singlet at δ 3.32. The ^{1}H NMR data was in good agreement with that reported by Ito *et al.* [55] for erythraline. The UV absorption maxima at 233 and 290 nm are consistent with those of erythrina alkaloids possessing 1,6-diene system. Erythraline (**11**) was previously isolated from various *Erythrina* spp. including: *E. burana, E. abyssinica, E. gluca, E. fusca, E. variegata* [16] but this is the first report from the seeds of *E. melanacantha*. Erythraline (**11**) has been reported to cause curare-like paralysis in frogs [56].

3.1.2. Erymelanthine (50)

Compound **50** was isolated as pale brown amorphous substance after chromatographic separation of a mixture of librated alkaloids. Its ^{1}H NMR spectrum corresponds with the alkaloids of the 1,6-diene type. It was basically characterized by down field singlets at δ 8.00 and 8.50 which are due to H-14 and H-17, respectively. The coupling constant, J=1 Hz, between the aromatic protons indicated that these protons occupy α- and ß- positions of the aromatic ring. The singlets at at δ 3.31 and 3.96 are due to two methyl groups of the aromatic ether and ester functionalities, respectively. Compound **50** was characterised as erymelanthine based on its spectroscopic and physical data and by comparing these with those reported by Dagne and Steglich [41].

3.1.3. Erysovine (3)

Compound **3** was isolated as a sticky brown substance which showed two aromatic proton signals in its ^{1}H NMR spectrum at δ 6.50 (H-14) and 6.80 (H-17). The H-1, H-2 and H-7 proton resonances appeared as diffuse signals at δ 6.00, 6.40 and 5.70, respectively. Two methoxyl group singlets appeared at δ 3.30 and 3.90. Comparison of the ^{1}H NMR data with that reported for erysovine (**3**) [21] confirmed that the two compounds are identical.

3.2. CHARACTERIZATION OF FLAVONOIDS OF *E. MELANACANTHA*

The stem bark of *E. melanacantha* yielded seven flavonoids. Of these five turned out to be flavanones (**152**, **153**, **155**, **156**, and the dimeric biflavanone **159**). The remaining two are the known isoflavones **157** and **158**, identified from other genera of the same family. The structural assignments of these compounds are discussed below.

3.2.1. 5-hydroxy-7,8-(hydroxyisopropyl dihydrofurano) flavanone (152)

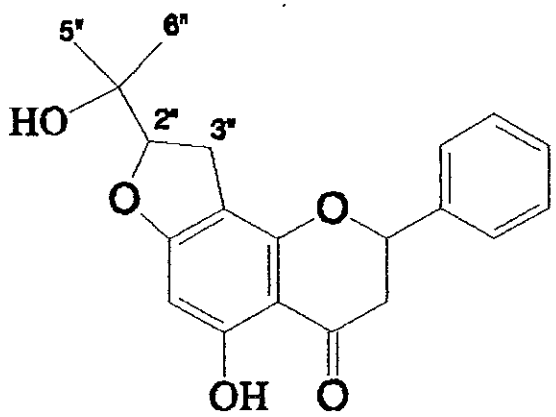
This compound displayed a molecular ion at m/z 340 (LRMS) which is consistent with a molecular formula $C_{20}H_{20}O_5$. The presence of a flavanone nucleus was confirmed by the occurrence in the ^{1}H NMR spectrum (Figure 1) of an ABX system centred at δ 2.86, 3.03 and 5.40 for the C-3 and C-2 protons [57]. Two sharp 3H singlets at δ 1.21 and 1.32 could be assigned to two methyl groups attached to an OH bearing carbon. The triplet at δ 4.70 was attributed to H-2", a methine proton next to an oxygen atom and bonded to hydroxyisopropyl group. The signals at δ 3.15 and 3.21 are assignable to the two

diastereotopic methylene protons at C-3" which are part of the dihydrofurano ring. The multiplet at δ 7.40 which appeared as a five-proton singlet indicated the absence of substituent in ring-B. The presence of a hydroxyisopropyl moiety was further confirmed by fragment ions in the MS (Figure 2) at m/z $[M-59]^+$ and 59. The MS fragmentation pattern of **152** is summerized in Scheme 3. The ion peak at m/z 104 (17%) originates from RDA fission at the C-ring and represents the B-ring moiety. The corresponding A-ring fragment ion signal was not visible.

Since C-2 of the molecule is a centre of asymmetry, two isomeric forms are possible. But the naturally occurring laevorotatory (-)-flavanone, which has the (2S)-configuration [8] is proposed based on its optical rotation.

The UV spectrum showed a maximum at 292 nm and an inflection at 338 nm, characteristic of a flavanone [58]. The occurrence of a 21 nm bathochromic shift on addition of $AlCl_3$ confirmed the presence of chelated OH at C-5, on the other hand absence of free hydroxyl group at C-7 was confirmed by lack of absorption shift on addition of NaOAc.

The IR spectrum exhibited bands at 3416 cm^{-1} (free OH), 1640 cm^{-1} (chelated and conjugated carbonyl group), 1390 and 1407 cm^{-1} (*gem*-dimethyl and/or isopropyl group). The presence of mono-substituted aromatic ring-B was derived from the band at 700 cm^{-1}.



(152)

The cyclization of the prenyl group observed in compound **152** appears to be unique in flavanones, but the closest analogue isoflavone was reported recently [59] from *E.*

eriotriocha. However, this kind of cyclization has been observed several times in coumarins and rotenoids [60,61]. Biosynthetically the furan ring might originate *via* oxidation of a prenyl double bond to a diol or epoxide followed by cyclization. Since glabranin (**153**) occurs in the stem bark of *E. melanacantha*, it is most likely the precursor of the cyclized product **152**. To our knowledge this is the first report of the occurrence of this compound in nature.

Figure-1 ^1H NMR spectrum of 5-Hydroxy-7,8-(hydroxyisopropyl-dihydrofurano) flavanone

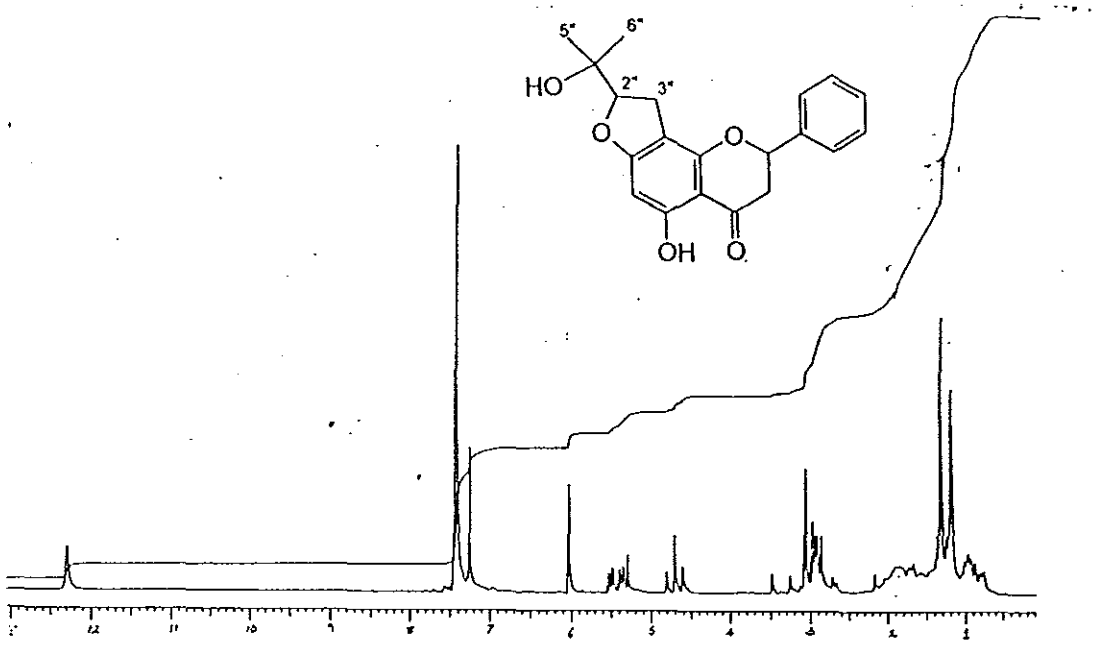
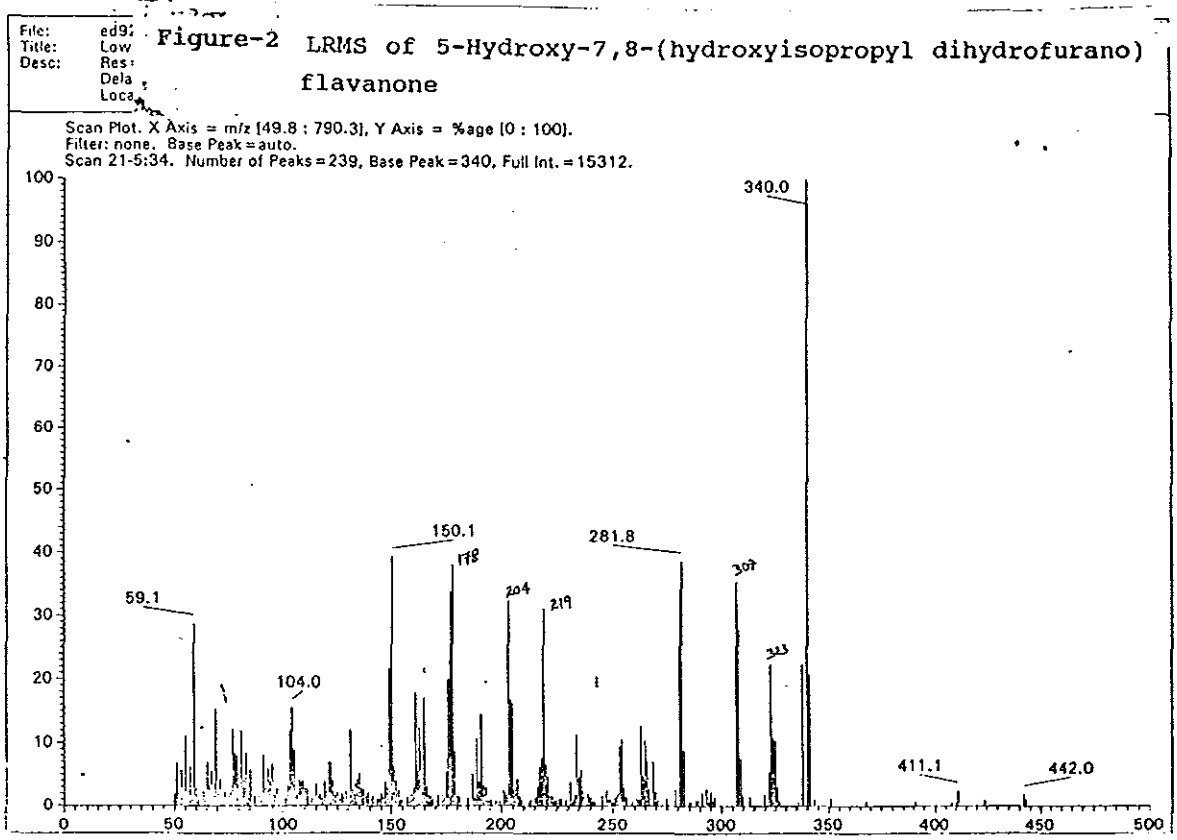
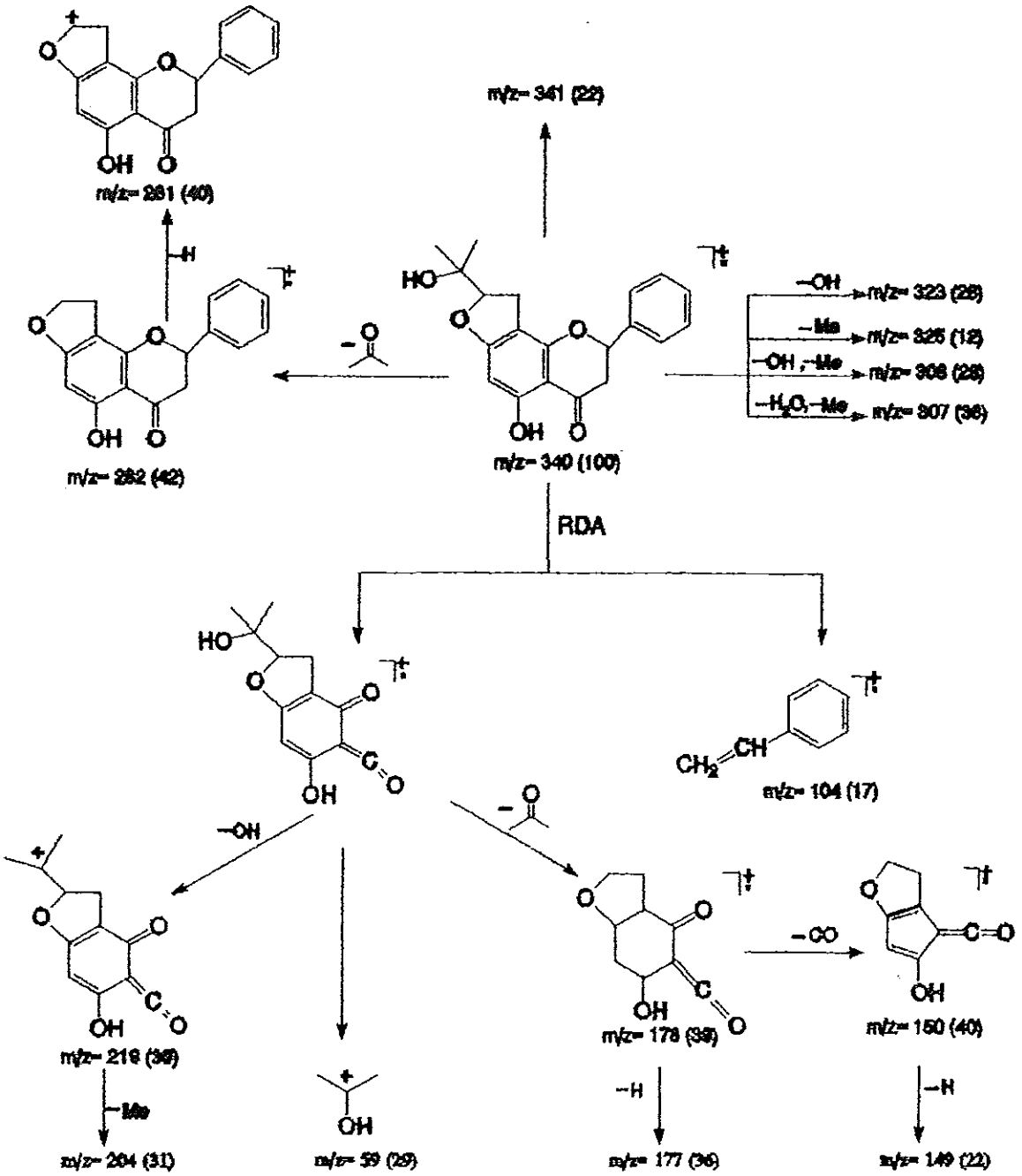


Figure-2 LRMS of 5-Hydroxy-7,8-(hydroxyisopropyl dihydrofurano) flavanone

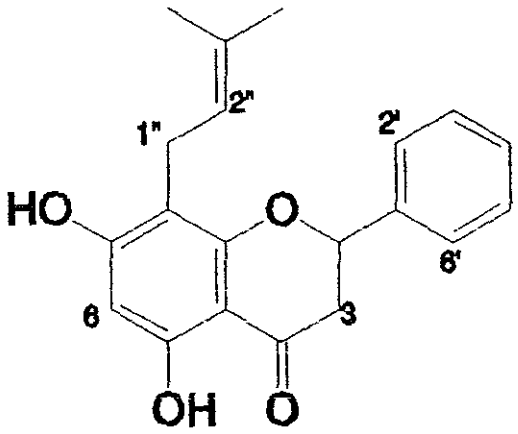


Scheme 3 : MS fragmentation pattern of 5-hydroxy-7,8-(hydroxyisopropyl dihydrofurano)flavanone (152)



3.2.2. Glabranin (153)

Compound **153** showed a UV absorption band at 293.3 nm (flavanone). Addition of $AlCl_3$ shifted the absorption maximum by 22 nm towards longer wavelength indicating the presence of 5-OH. However, there was no significant λ_{max} shift by addition of NaOAc and HCl. The IR spectrum revealed the presence of chelated-OH at 3353 cm^{-1} and chelated and conjugated carbonyl at 1645 cm^{-1}. Signals at 1436 and 1387 cm^{-1} suggest the presence of a *gem*-dimethyl group. The presence of a mono-substituted aromatic B-ring was derived from a band at 669 cm^{-1}. The ^1H NMR spectrum (Figure 3) was identical with that reported by Bohlmann and Abraham [62]. The mass spectrum gave a molecular ion peak at m/z 324 and a prominant ion at m/z 205 [63]. Further fragment ion peaks characteristic of flavanones were observed as depicted Scheme 4.



(153)

Additional structural evidence was obtained by chemical transformation of **153** to chroman **154**. Thus cyclization of **153** using HCO_2H and some drops of H_2SO_4 as described by Rao *et al.* [64] gave **154**. The formation of only one product confirmed that the prenyl group is at C-8 position. The presence of 5-OH in **154** was observed using $AlCl_3$ shift reagent which showed a 23.6 nm bathochromic shift. The spectral and chemical data gathered for **153** suggested that the compound is glabranin (5,7-dihydroxy-8-[3",3"-dimethyl allyl] flavanone).

Figure 3: ^1H NMR Spectrum of Glabranin (153)

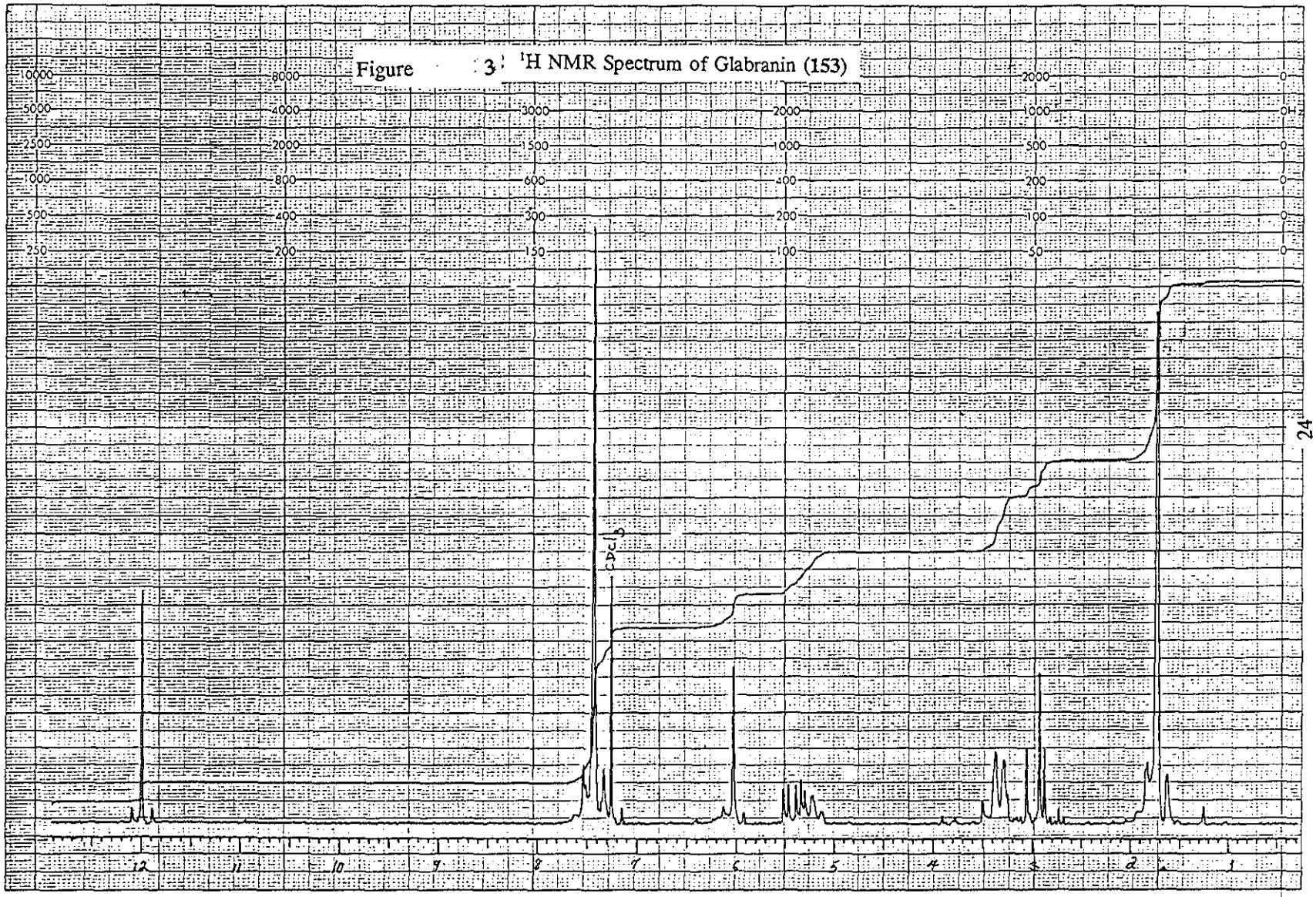
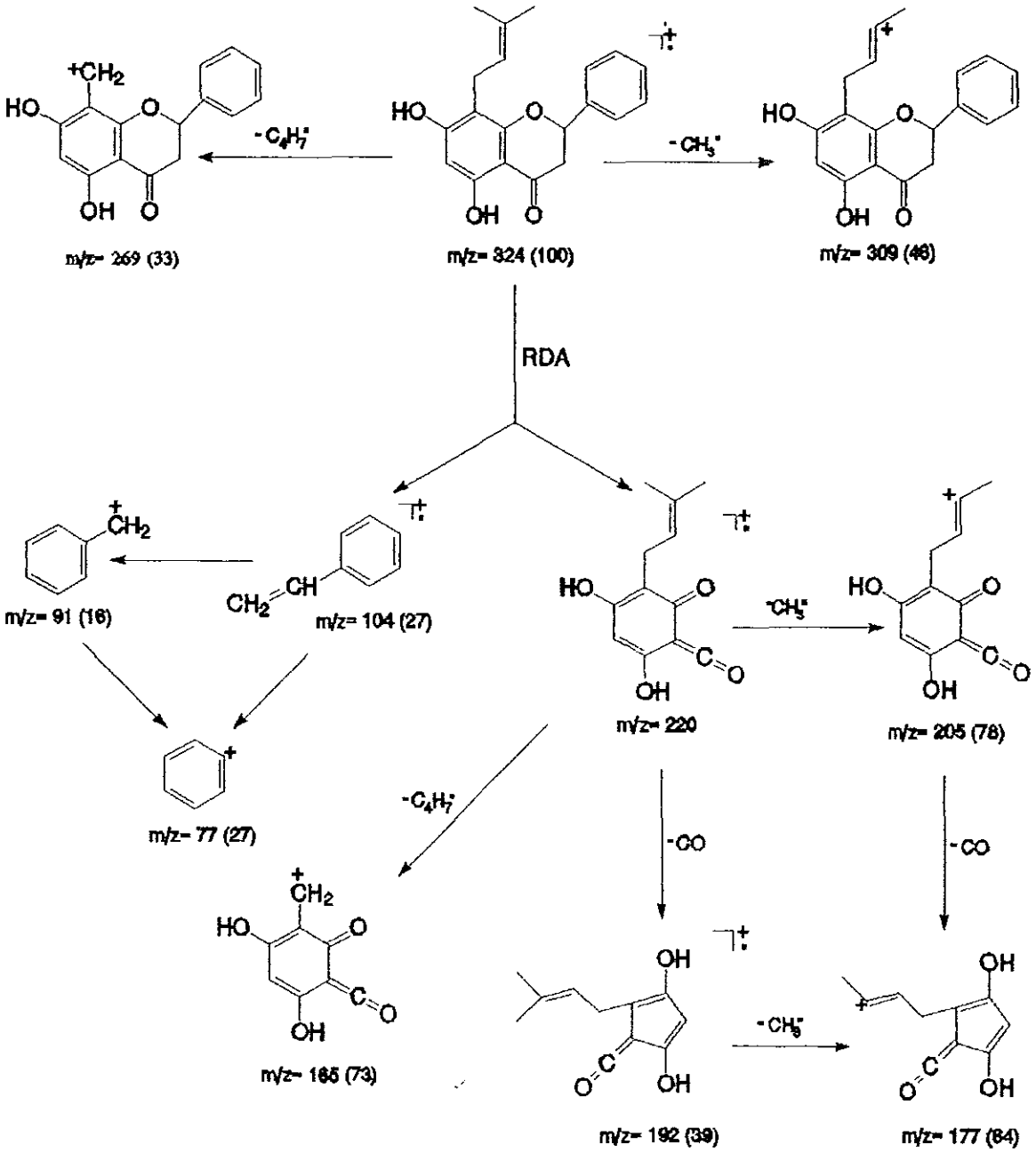


CHART NO. FX-5
PRINTED IN JAPAN

Scheme 4 : MS fragmentation pattern of glabranin (153)

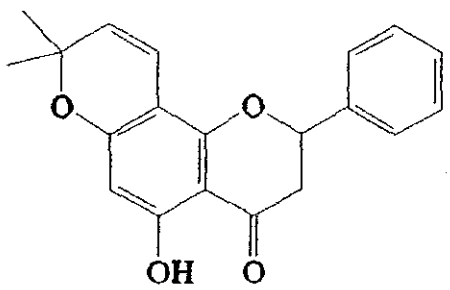


Glabranin was first reported by Bohlmann and Abraham [62] from *Helichrysum hypocephalum* (Compositae) and later from *Glycyrihiza lepidota* [65]. This, however, is the first report of its occurrence in the genus *Erythrina*. It was found to be active against *Staphylococcus aureus, Mycobacterium semegmatis, Candida albicans* and *Klebsiella pneumoniae* [13].

3.2.3. Obovatin (155)

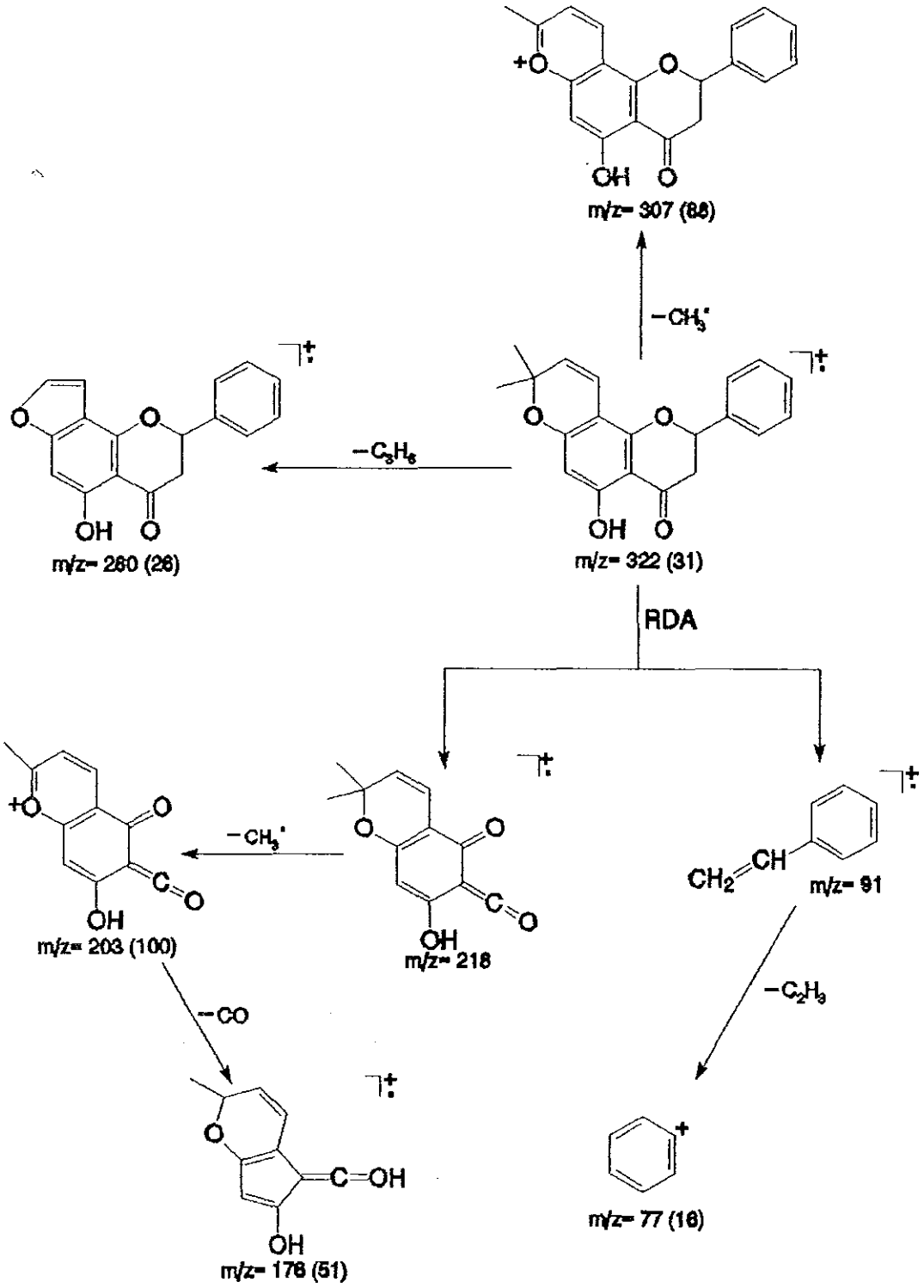
Obovatin was previously isolated from the roots of *Tephrosia pentaphylla* [66] and other genera of the family Leguminosae, however, this is the first report of its occurence in *Erythrina* species. Furthermore, obovatin, to our knowledge has not been found to co-occur with the preobovatin compound, glabranin.

Its UV absorption maximum at 270 nm suggested it to be a flavanone [58]. Addition of NaOAc powder to the methanolic solution showed no change in the absorption maximum indicating that the 7-OH is blocked. Bathochromic shift of the λ_{max} by 10.3 nm was indicative of the presence of a chelated 5-OH group. This was further confirmed by the presence of a singlet at δ 12.08 in the ^{1}H NMR spectrum. Furthermore, the presence of a *gem*-dimethylchromene ring was derived from the 6H singlet at δ 1.28 and an AB spin system at δ 5.50 (d, J = 10 Hz) and 6.55 (d, J = 10 Hz) was due to H-3" and H-4", respectively. The absence of substituents on ring-B was derived from the ^{1}H NMR spectrum. The MS showed a molecular ion peak at m/z 322 and the fragmentation pattern (Scheme 5) is consistent with structure **155**. Compound **155** was identified as obovatin based on the above spectroscopic data and by direct comparison (co-TLC) of **155** with authentic obovatin.



(155)

Scheme 5 : MS fragmentation pattern of obovatin (155)

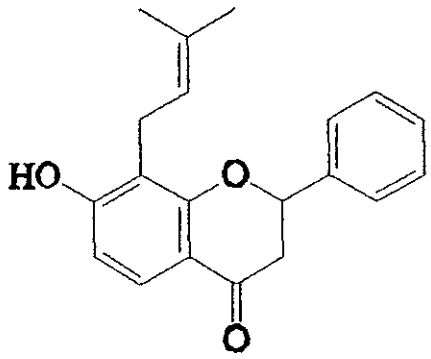


3.2.4. 7-Hydroxy-8-prenylflavanone (156)

The UV spectrum of compound **156** gave the anticipated bands for a flavanone [58]. The absence of a 5-hydroxyl substituent was derived from the absence of a bathochromic shift upon the addition of $AlCl_3$. The absorption maximum showed 11 nm red shift in the presence of NaOAc which is indicative of hydroxyl at C-7.

The LRMS showed a molecular ion peak at m/z 308 (25), which might corresponds with the molecular formula $C_{20}H_{20}O_3$, and it readily fragmented to yield a base peak at m/z 83. Prominent fragment ions appeared at m/z 204 (16) and 104 (19) and are in agreement with the fragmentation pattern expected for **156**. Other plausible fragment ions are shown in Scheme 6.

Its IR spectrum showed absorption bands at 3398 (free OH), 1646 (conjugated carbonyl) and 1597 cm^{-1} (aromatic ring). The presence of a *gem*-dimethyl group is indicated by the appearance of bands at 1442 and 1377 cm^{-1}. The ^1H NMR spectrum (Figure 4) showed an ABX splitting system due to C-2 and C-3 protons. The C-2 proton, the X part, appeared as a double of doublet at δ 5.45 (1H, J_{AX} = 13, J_{BX} = 3.5 Hz) while the C-3 protons, the AB part, appeared at δ 3.01 and 2.89 (J_{AB} = 17, J_{AX} = 13, J_{BX} = 3.5 Hz). The large value of J_{AX} (13 Hz) was indicative of an axial-axial coupling. Therefore, the C-2 hydrogen must be axial and ring-B must be equatorial.



(156)

The 5H multiplet at δ 7.42 and the two *ortho* coupled doublets at δ 7.75 and 6.54 (J = 9 Hz) showed lack of substitution in ring B and at 5 and 6 positions in ring A, respectively. The characteristic signals for the prenyl group at C-8 was observed in the ^1H NMR spectrum. Biogenetic considerations [67] support the placement of the free phenolic OH group at C-7 in ring-A while ring-B remains unsubstituted. Compound **156** was

therefore identified as 7-hydroxyl-8-prenylflavanone. The UV, ^{1}H NMR and MS data are in good agreement with those reported for 7-hydroxyl-8-prenylflavanone isolated from *Tephrosia falciformis* [68] and from the seeds of *Milletia ovalifolia* [69].

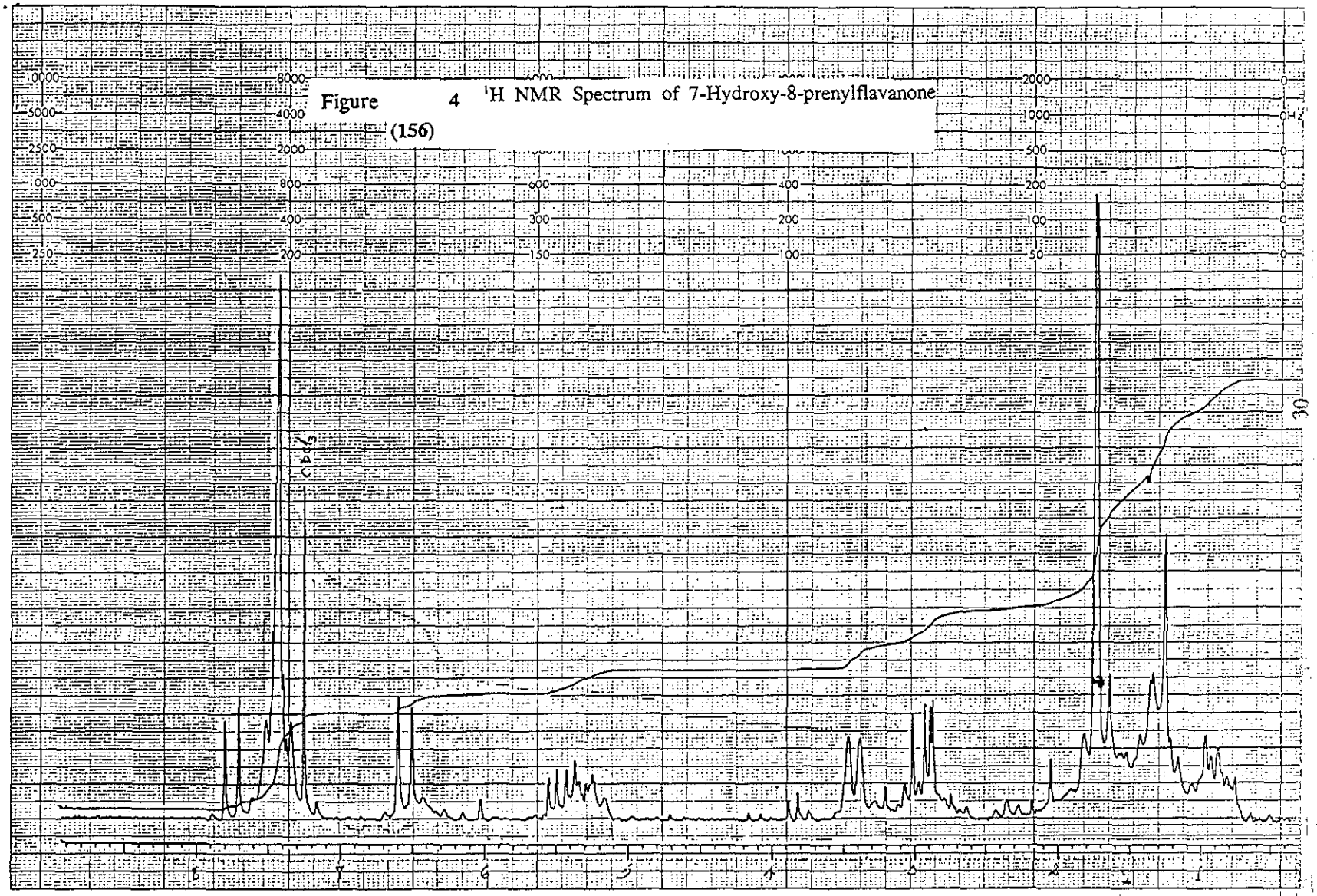
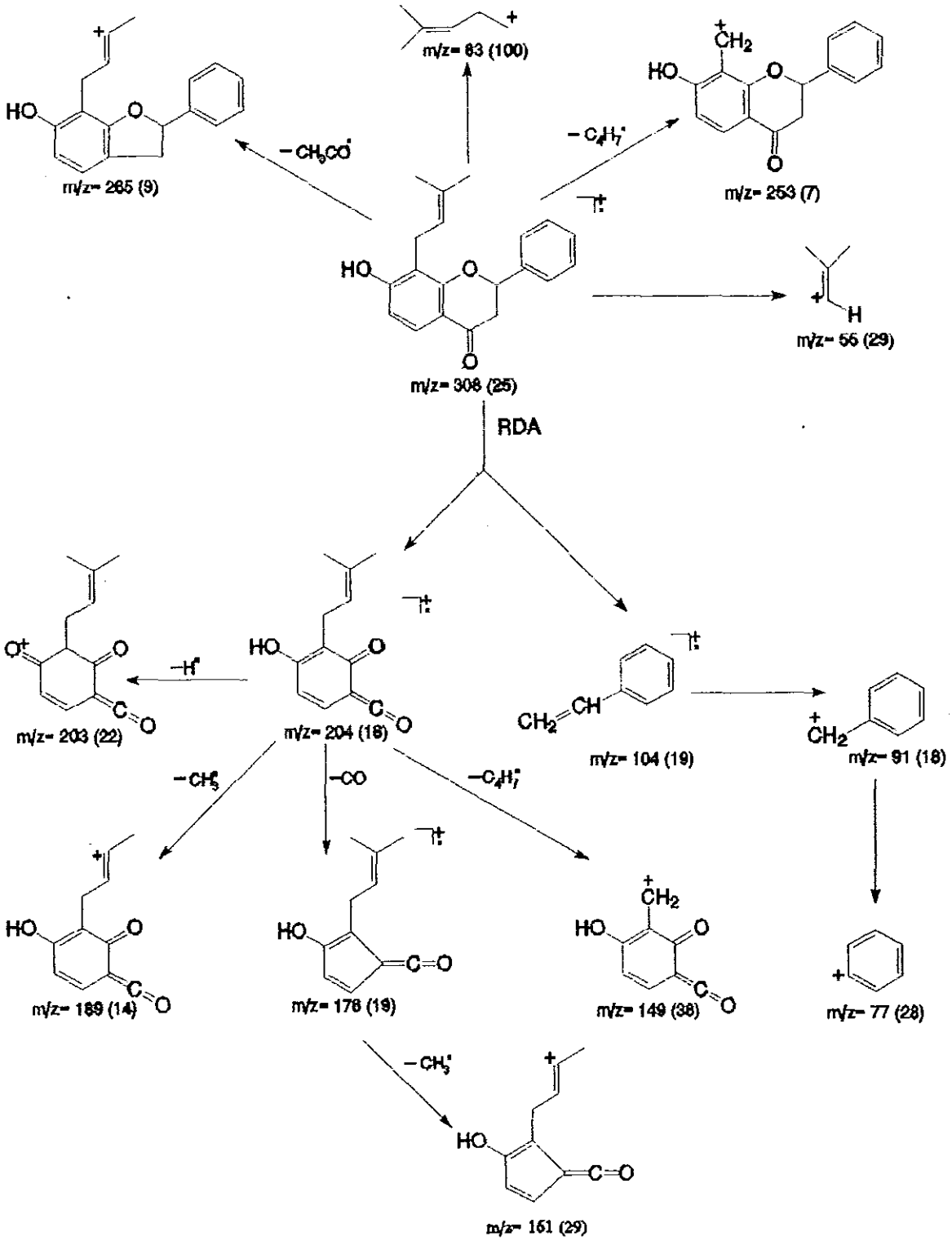


Figure 4 ^{1}H NMR Spectrum of 7-Hydroxy-8-prenylflavanone (156)

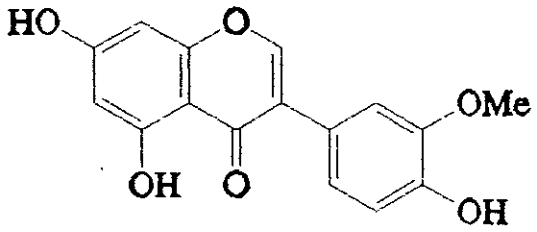
Scheme 6 : MS fragmentation pattern of 7-hydroxy-8-prenylflavanone (156)



3.2.5. 3'-O-methylorobol (157)

Compound **157** was obtained as a yellow crystalline solid, mp 210-213°. Its LRMS showed a molecular ion peak at m/z 300 consistent with the molecular formula $C_{16}H_{12}O_6$. Its UV (λ_{max} at 264 nm) and ^{1}H NMR (δ 7.86, 1H, H-2) spectrum were characteristic of an isoflavone. NaOAc (6 nm) and $AlCl_3$ (8 nm) induced shifts were observed in the UV spectrum of **157** suggesting that this compound possesses 7-OH and 5-OH groups. A red shift in the absorption maximum with increasing intensity of band I with NaOH indicated a hydroxyl group at C-4'.

The IR spectrum of **157** showed bands at 3244 (br) and 1656 cm^{-1} attributed to chelated hydroxyl and conjugated and chelated carbonyl groups, respectively. Absorption bands at 1579, 1518 and 1455 cm^{-1} are due to aromatic C=C in plane vibration and the band at 1198 cm^{-1} represents C-O streching of methoxyl group.



(157)

The downfield signal at δ 12.90 in the ^{1}H NMR spectrum indicated the presence of chelated hydroxyl group at C-5 position. The presence of -OMe group was shown by a 3H singlet at δ 3.94. The observation in the MS of an RDA fragment ion at m/z 149 allowed the placement of the OMe on ring B. The ion peak at m/z 153 arose from RDA cleavage followed by hydrogen transfer which resulted from the ring A moiety possessing 5,7-dihydroxyl groups.

The mass spectral fragmentation pattern (Scheme 7) together with ^{1}H NMR spectral evidence established that ring A had two hydroxyl groups and ring B a hydroxyl and methoxyl groups. The above data allowed the assignment of structure **157** to this compound. 3'-O-methylorobol (**157**) was first isolated from the wood of *Dalbergia inundata* (Leg.) [70] and later from *E. eriotriocha* [59]. The spectral data generated for **157** were similar with those reported for 3'-O-methylorobol (**157**) reported from the above two plants.

3.2.6. 3'-Methoxydaidzein (158)

Compound **158** was obtained as a white powder (mp 258-260°) and gave UV and ^{1}H NMR spectra typical of an isoflavone without hydroxyl at C-5. In the ^{13}C NMR spectrum (Figure 5) fifteen sp^2 carbon signals were observed of which seven are methine carbons and the remaining eight quaternary carbon atoms. Its LRMS showed a molucular ion at m/z 284 (100) in accord with an isoflavone containing two hydroxyl and one methoxyl groups.

Scheme 7: MS fragmentation pattern of 3′-o-methylorobol (157)

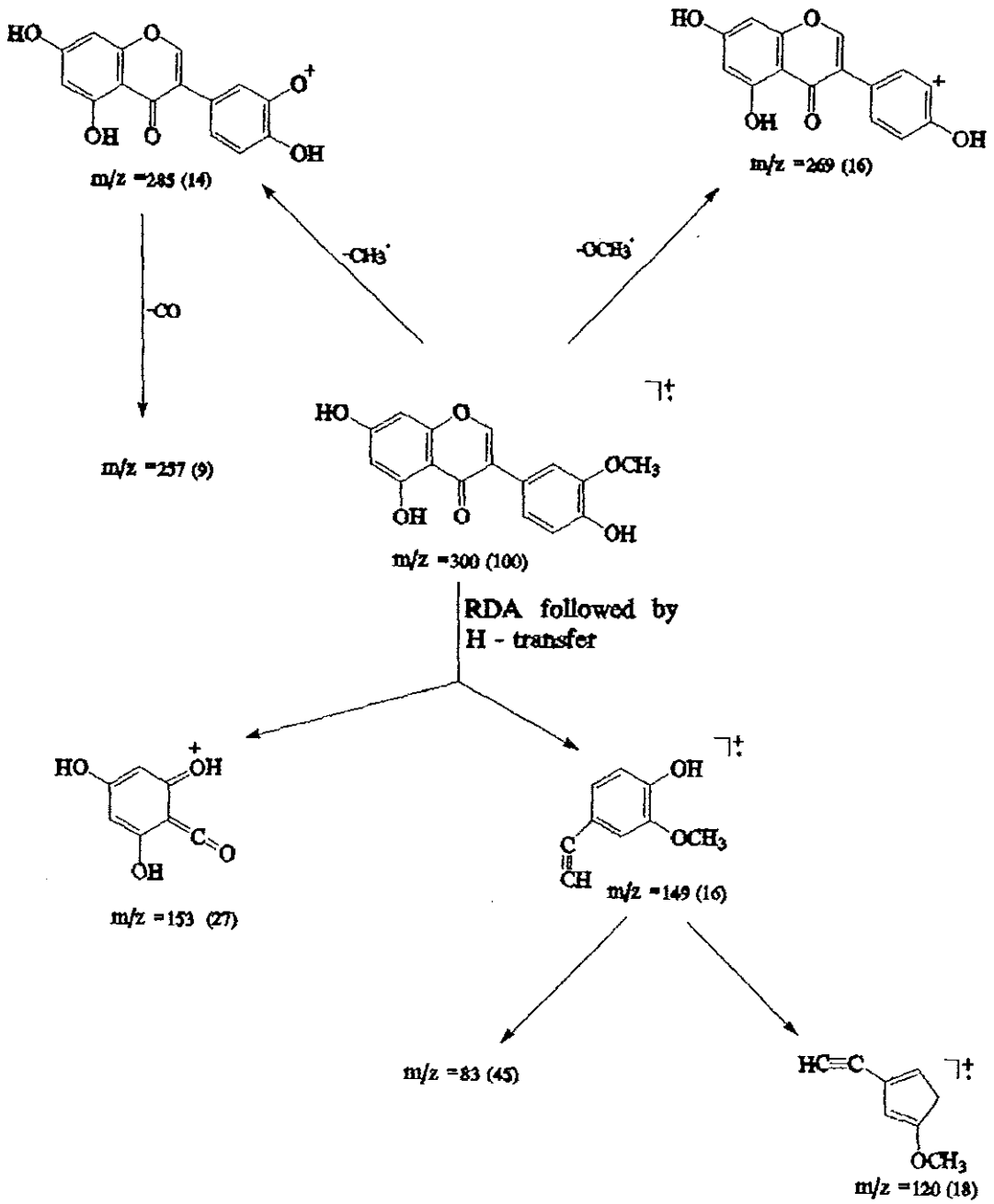
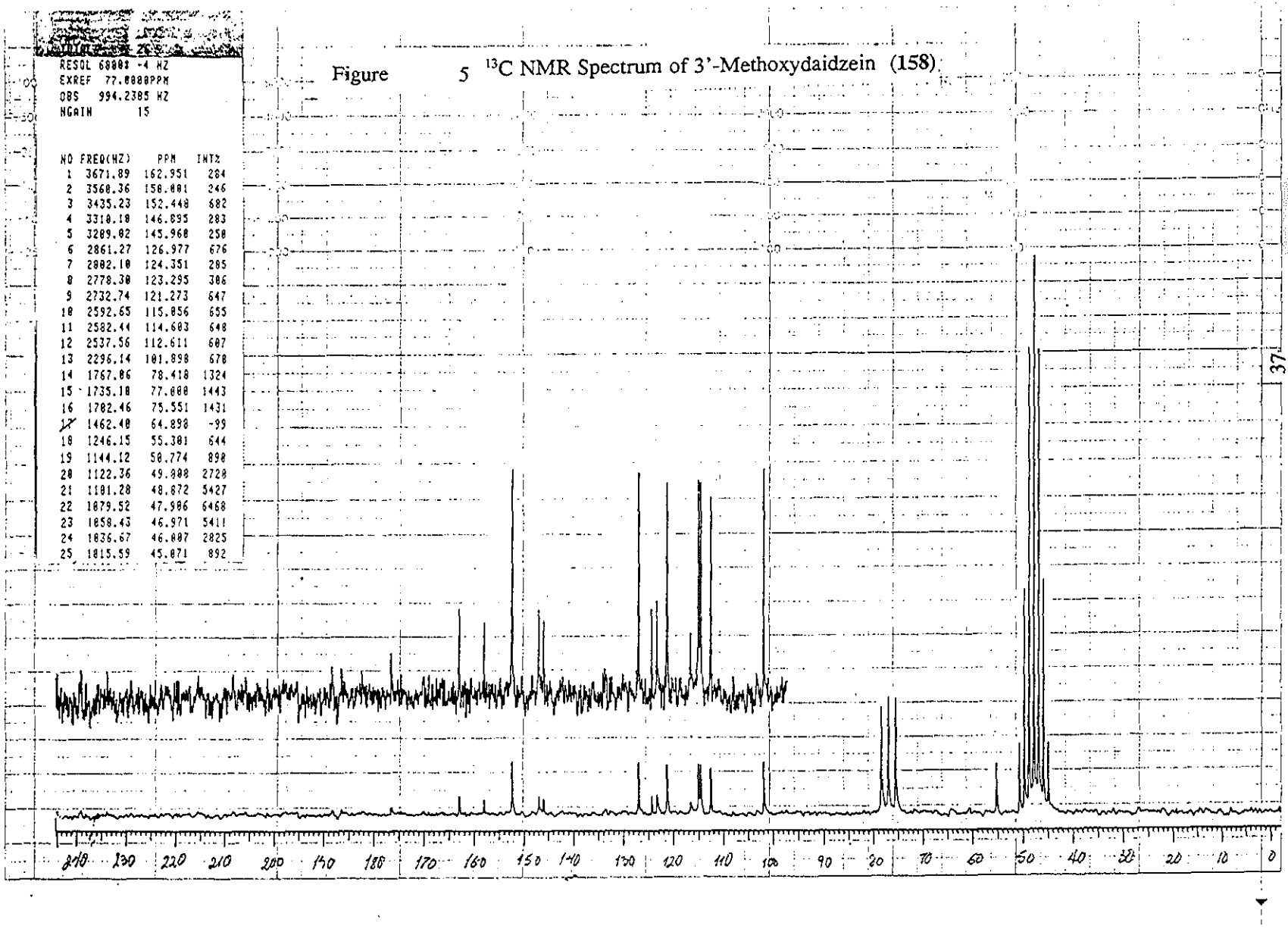


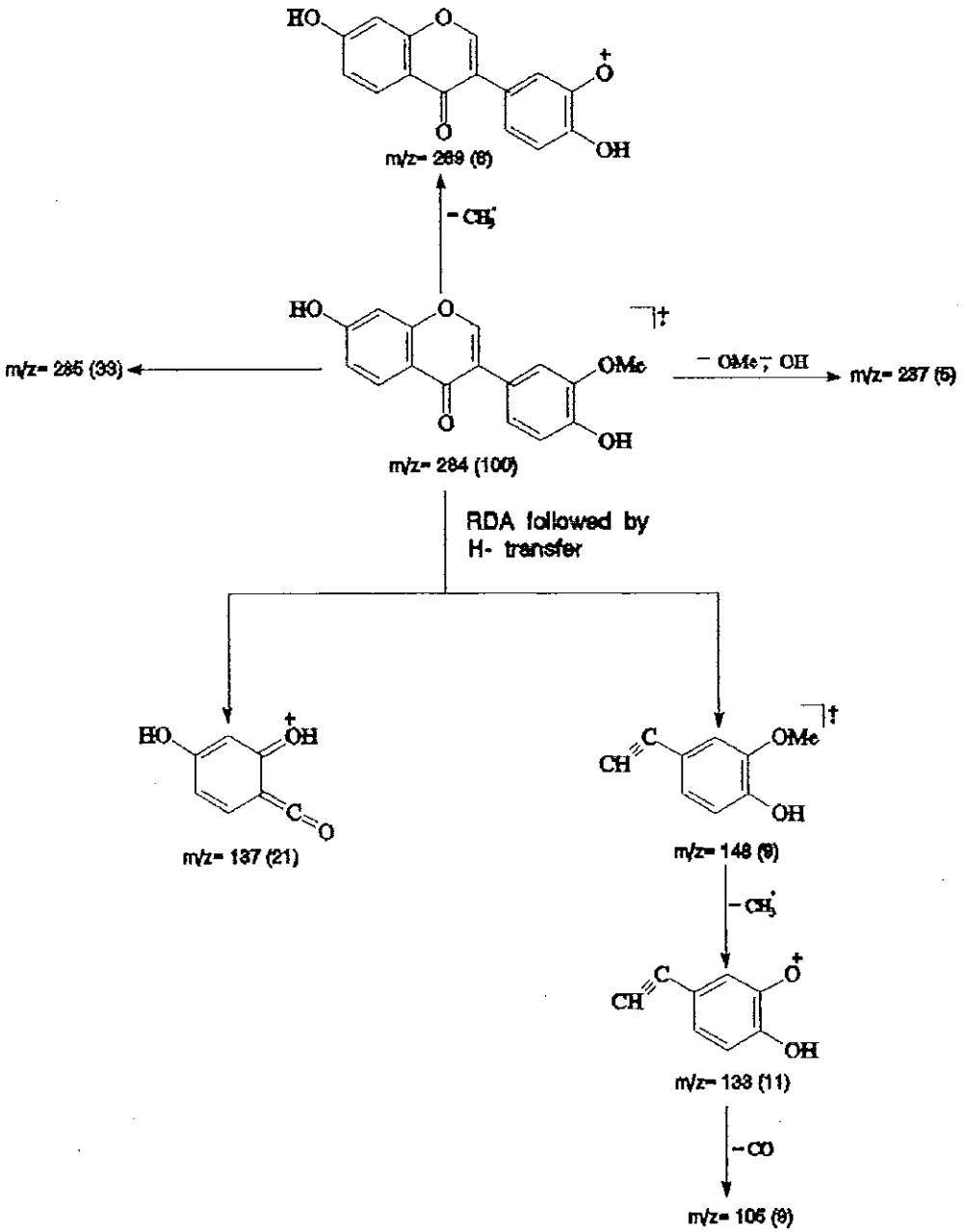
Table 5 Comparison of the ^{13}C NMR data of 158 [$CDCl_3$/ CD_3OD (1:1)] with that reported [72] for 3'-methoxydaidzein (acetone-d_6)

Carbon No	158 (δ in ppm)	3'-methoxydaidzein (δ in ppm)
2.	152.5	152.8
3.	123.3	124.7
4.	176.4	174.6
5.	127.0	127.2
6.	112.6	115.1
7.	163.0	162.5
8.	101.9	102.1
9.	158.0	157.4
10.	116.8	116.7
1'.	124.4	123.5
2'.	114.6	111.9
3'.	146.0	147.5
4'.	146.9	146.1
5'.	115.1	116.5
6'.	121.3	119.7
OMe	55.3	55.6

Figure 5 ^{13}C NMR Spectrum of 3'-Methoxydaidzein (158)



Scheme 8 : MS fragmentation pattern of 3′-methoxydaidzein (158)

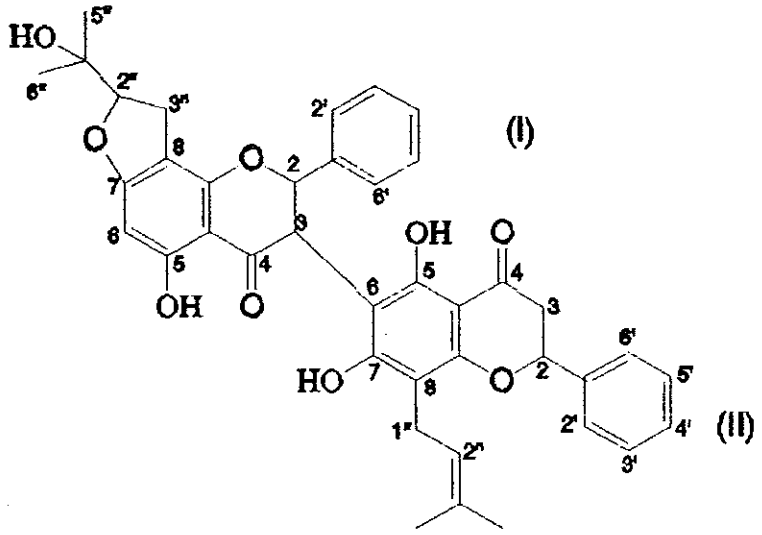


3.2.7. Biflavanone (159)

Compound **159** was isolated as a brown amorphous solid and its structure was established based on UV, ^{1}H NMR, IR and MS data. It showed a UV spectrum typical of a flavanone [58]. Lack of the expected bathochromic shifts of the shorter wavelength band on addition of NaOAc and $AlCl_3$ may be due to steric hindrance [73] caused by the dimeric linkage at C-6, which may reduce or prevent the acidity of the 7-OH. This suggests that at least one 5,7-dihydroxyflavanone system is found in the molecule. A broad band at 3413 cm^{-1} in its IR spectrum was ascribed to free OH groups, and bands at 1401 and 1253 correspond to *gem*-dimethyl and ether (C-O-C) stretchings, respectively. A characteristic C-H out-of-plane deformation of a mono-substituted aromatic ring was observed at 700 cm^{-1}. The downfield singlets at δ 11.75 and 12.20 in the ^{1}H NMR spectrum were assigned to two chelated OH groups.

The possible sight of dimerization was deduced as follows: first, the two-proton doublet at δ 6.57 (J = 1.5 Hz) corresponds to H-2 and H-3 of the flavanone I unit which indicated that C-3 of this flavanone unit is the most probable site of dimerization; second, the absence of any signal at δ 5.90-6.10, except at δ 6.00 which was assigned to H-6 of the flavanone I unit, established that there were no aromatic protons in ring A of the second flavanone unit (II). The above evidence, therefore, suggested that linkage between the two units took place at C-3 of I and C-6 of II. The coupling constant (1.5 Hz) between protons at C-2 and C-3 of I are indicative of the *trans* axial-equatorial orientation of these protons. As a result the flavanone unit II might occupy an axial position. It has been shown earlier that the chemical shifts of chelated OH groups of 6- alkyl substituted flavanones appeared more downfield than those of 6-unsubstituted flavanones [74]. In agreement with this, the signal at δ 11.75 can be attributed to the 5-OH of I while that at 12.20 may be due to the 5-OH of II.

Even though, the molecular ion peak was not clearly observed in the LRMS of **159** the fragment ion peaks for the monomeric units appeared at m/z 339 (42%) and 323 (47%). In addition, ion peaks discussed earlier for the suggested monomers glabranin (**153**) and 5-hydroxy-7,8-(hydroxyisopropyl dihydrofurano) flavanone (**152**) are also observed in the spectrum.



(159)

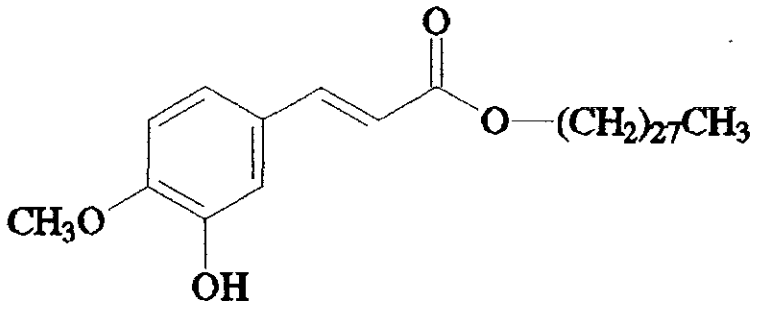
The most probable biogenetic pathway leading to the formation of the biflavanone (159) is the introduction of glabranin into a 5-hydroxy-7,8-(hydroxyisopropyl dihydrofurano) flavanone by condensation polymerization. This is perhaps the first example of a naturally occurring biflavanone having C-3 - C-6 linkage of two flavanone units.

A complete structural assignment of compound **159** requires HRMS (or FAB-MS) and ^{13}C NMR data.

3.3. CHARACTERIZATION OF THE CINNAMATE ESTER, ERYTHRINASINATE (160)

Erythrinasinate (160) was isolated as an amorphus solid and its structure was deduced from its spectral data. The ^{1}H NMR spectrum showed a triplet at δ 0.80 due to an aliphatic methyl group. The broad singlet at δ 1.25, integrating for 52 protons, suggests the presence of 26 CH_2 groups. In addition, the 2H triplet at δ 4.15 indicates the occurrence of an -OCH_2 group. The doublets (J = 16 Hz) at δ 6.25 and 7.60 are due to two *trans*-coupled olefinic protons at C-1' and C-2'. The aromatic proton resonances appeared at δ 6.85 (d), 6.95 (s) and 7.05 (d). The presence of an OCH_3 and a hydroxyl groups was evident from the signals at δ 3.80 (s) and 6.90 (s, exchangeable with D_2O). The presence of a cinnamoyl group attached to an aliphatic rest containing 28 carbons was evident from the ^{1}H NMR spectrum. This was further supported by the ^{13}C NMR sectrum (Table 6) which showed 17 carbon resonances. The signals at δ 144.6 and 109.5 are attributable to C-1' and C-2', respectively. Only 6 signals were observed for the 27

methylene carbons. The signals due to C-7'-C-28' overlap at δ 29.6 and appear as a broad singlet. The remaining carbon resonances are clearly observed in the spectrum and assignment of the signals is given in Table 6. Additional evidence for the presence of a substituted cinnamoyl portion in the molecule was furnished by the MS data which gave a base peak at 586 and a relatively strong peak at m/z 558. Fragment ion peaks at m/z 194 and 177 correspond to the loss of $C_{28}H_{57}$ and $OC_{28}H_{27}$, respectively, from the molecular ion.



(160)

Erythrinasinate was previously identified from three *Erythrina* spp. [75]. The identity of **160** was confirmed by direct comparison with an authentic sample earlier obtained from *E. abyssinica*.

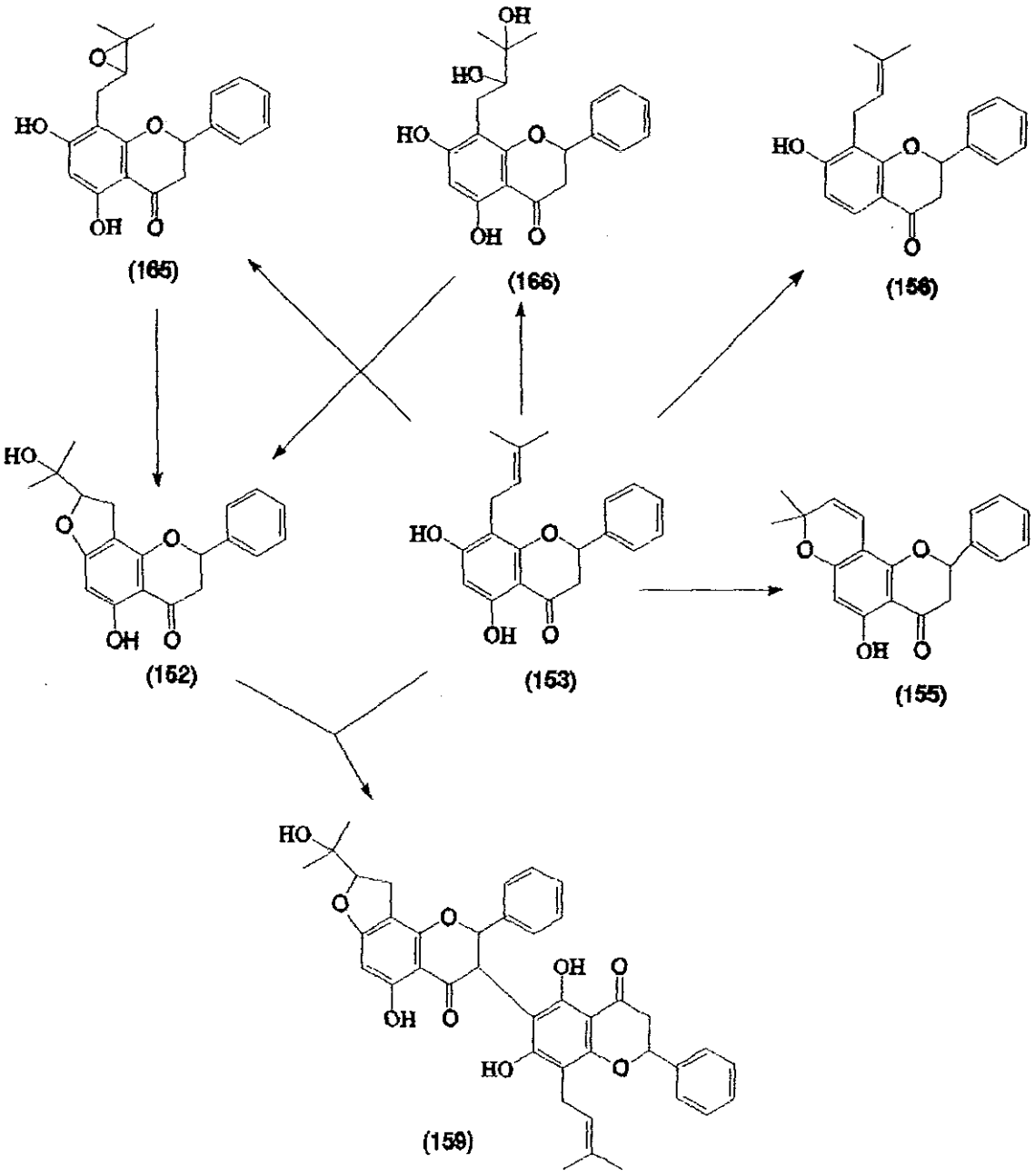
Table 6 ^{13}C NMR data for erythrinasinate (160)

Carbon No	Calculated (δ ppm)	160 (δ ppm)	Erythrinasinate (δ ppm)
1	131.7	127.0	127.0
2	113.5	114.5	115.7
3	147.4	147.0	147.9
4	150.5	148.0	146.7
5	117.0	117.9	114.7
6	120.2	122.9	123.1
OMe	54.0	56.0	55.9
1'	142.3	144.6	144.6
2'	118.3	109.5	109.3
3'	169.5	167.2	167.4
4'	67.5	64.6	64.6
5'	27.6	28.9	28.8
6'	25.3	26.0	26.0
7'-28'	29.9	29.6	29.7
29'	32.4	31.9	31.9
30'	23.0	22.7	22.7
31'	13.9	14.1	14.1

A further point to note with regards the isoflavonoids of *E. melanacantha* is the C-5 and C-7 hydroxylation of compound **157** whereas only C-7 hydroxylation of **158** with C-4' hydroxylation in both compounds. Such pattern is well known in the biosynthetic route of isoflavonoids [52] Compound **158** is expected to be the product of dehydroxylation of **157** or the reverse.

In addition to these, an unidentified isoflavone (based on ^{1}H NMR data) which requires generation of further spectroscopic data has been isolated from the stem bark of *E. melanacantha*.

Scheme 9: Proposed Biogenetic Relationships Amongst Flavanones Identified From The Stem bark Of E. melanacantha



5. EXPERIMENTAL

5.1. PLANT MATERIALS

The seeds and stem bark of *E. melanacantha* were collected in June, 1988 from Negelle Borana district near Melka Goba. The plant material was identified by Dr. Mesfin Tadesse, Addis Ababa University. A voucher specimen has been deposited under the cipher Mesfin 2600 at the National Herbarium.

5.2. MATERIALS AND APPARATUS

Melting points were determined on Thomas Hoover melting point apparatus and are uncorrected. Optical rotation was measured in a Perkin Elmer (model 241) spectropolarimeter. Si gel 60 $PF_{254+366}$ (Merck) and Si gel 60 (230-400 mesh ASTM) (Merck) were used for PTLC and CC, respectively. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Jeol FX 90Q instrument operating at 90 MHz and 22.5 MHz, respectively. FT-IR spectra were recorded using Perkin Elmer (model 1600) instrument with KBr pellets. UV spectra were obtained in MeOH with Spectronic 1001 Plus. LRMS were conducted on Finnigan Mat 1125 Spectrometer.

5.3. EXTRACTION AND ISOLATION

Table-7 List of solvent systems employed for CC, TLC, PTLC and CPTLC

Code	Solvent System	Ratio
A	Petrol/ $CHCl_3$	95:5
B	Petrol/ $CHCl_3$	9:1
C	Petrol/ $CHCl_3$	7:3
D	Petrol/ $CHCl_3$	1:1
E	$CHCl_3$/ MeOH	95:5
F	$CHCl_3$/ MeOH	1:1
G	Petrol/ EtOAc	9:1
H	C_6H_6/ Petrol/ EtOAc	6:18:1
I	C_6H_6/ Petrol/ EtOAc	3:9:1
J	C_6H_6/ Petrol/ EtOAc	3:4:3
K	Toluene/ EtOAC/ HOAc	90:19:1

5.3.1. Seeds of *E. melanacantha*

250 g of powdered seeds of *E. melanacantha* were defatted with petrol. The marc was then exhaustively extracted with MeOH and concentrated to give 2.2 g (0.88%) of crude extract. The MeOH extract was treated with 0.2 N HCl and successively partitioned with petrol (for efficient removal of the remaining fat) and $CHCl_3$. The $CHCl_3$ extract on TLC showed two spots which gave posetive result upon spraying with Dragondroff's reagent and yielded 76 mg (3.5%) of brown crude matter which was subjected to flash chromatoghraphy over Si gel (14 g) packed with solvent system D and eluted with petrol, petrol/$CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH and MeOH of increasing polarities. Fractions eluted with solvent system D and E were combined (TLC) and applied on PTLC using solvent system E to give 10 mg of compound **11**.

The aqueous solution fraction was basified to pH 8 by aqueous NH_3 solution and extracted three times with $CHCl_3$, dried over Na_2SO_4 and concentrated to yield 1.6 g (0.64%) of free alkaloidal fraction. This was then subjected to flash CC over 13 g of Si

gel and eluted with $CHCl_3$ and MeOH mixtures of increasing polarities. A total of nineteen fractions were collected. Fractions eluted with solvent system E afforded compound **50** (11 mg). The next fraction eluted with the same solvent system E resulted in 60 mg of brown red substance which was purified by CPTLC using solvent system B to give compound **3** (13 mg).

5.3.2. Stem bark of *E. melanacantha*

The ground stem bark of *E. melanacantha* (400 g) was successively extracted with petrol, $CHCl_3$, and MeOH. Concentration of the $CHCl_3$ extract gave a dark greenish residue (10 g) which was flash chromatoghraphed with 150 g of Si gel and a total of 15 fractions, 100 ml each, were collected while eluting with petrol, $CHCl_3$ and MeOH mixtures of increasing polarities. Fractions eluted with solvent system B were combined and subjected to sephadex LH-20 eluting with solvent system F and rechromatographed over Si gel. The petrol fractions of this CC afforded compound **159** (7 mg) and 60 mg of sticky amorphous solid was obtained at solvent system A. The later was again purified by PTLC using solvent system J and yielded 10 mg of compound **152**.

The fractions eluted with solvent system D were combined (TLC), concentrated and yielded 85 mg of pale yellowish substance which was applied on PTLC using solvent system H and resulted in compound **155** (9 mg) and compound **153** (40 mg). On the other hand, the fraction obtained from 100% $CHCl_3$ was first passed through sephadex LH-20 eluting with solvent system F and then applied on PTLC using solvent system I to give 130 mg of compound **160**.

Examination of the MeOH extract by TLC revealed the presence of phenolic compounds which fluoresce in the UV light at 254 nm and gave dark brown spots after spraying with fast blue salt B followed by 5% KOH reagent. The MeOH extract (12 g) was subjected to flash CC on 100 g of Si gel. Gradient elution was effected with petrol, EtOAC and MeOH mixtures and 200 ml each of the fractions were collected. The petrol fractions were combined (TLC) and concentrated to give 75 mg of yellowish solid which was further purified on a PTLC using solvent system I to give 35 mg of compound **156** Fractions eluted with solvent sysyem G were combined (TLC) to give 0.5 g of a greenish matter which was purified by PTLC using solvent system K and yielded compound **157** (13 mg) as crystalline solid. Lastly, the most polar fraction was eluted with solvent system C and was allowed to dissolve in solvent system E and the insoluble portion of it was

recrystallized from $CHCl_3$ to form compound **158** (55 mg).

5.4. PHYSICAL AND SPECTRAL IDENTITIES OF COMPOUNDS

5.4.1. Erythraline (11): brown amorphus solid; UV λ_{max} (MeOH) nm: 233, 290. ^{1}H NMR ($CDCl_3$) δ: 1.80 (1H, dd, J= 10, 12 Hz, H-4_{ax}), 2.50 (1H, dd, J= 5, 12 Hz, H-4_{eq}), 2.80 (1H, dd, J= 5, 14 Hz, H-10_{eq}) 2.85 (2H, m, H-11), 3.32 (3H, s, OMe), 3.40 (1H, dd, J= 5, 14 Hz, H-10_{ax}), 3.63 (2H, m, H-8), 3.80 (1H, m, H-3), 5.70 (1H, br s, H-7), 5.89 (2H, dd, J= 1.4 Hz, O-CH2-O), 6.0 (1H, d, J= 10 Hz, H-1), 6.50 (1H, dd, J= 2.5, 10 Hz, H-2), 6.60 (1H, s, H-14), 6.75 (1H, s, H-17).

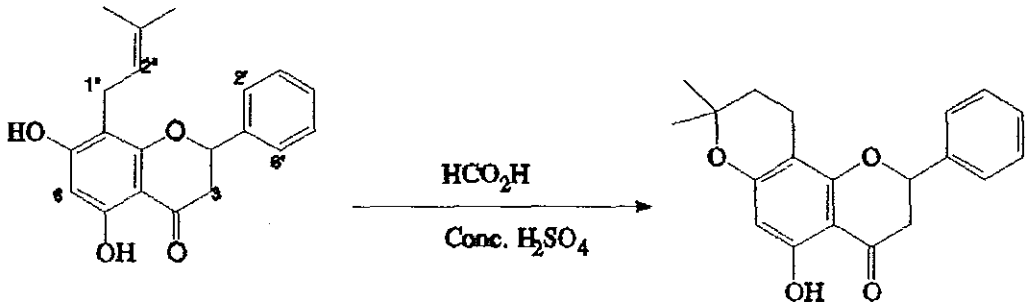
5.4.2. Erymelanthine (50): mp (lit. [41] 160-161°). ^{1}H NMR ($CDCl_3$) δ: 1.91 (1H, dd, J= 12 Hz, H-4_{ax}), 2.51 (1H, dd, J= 12 Hz, H-4_{eq}), 2.77 (1H, m, H-10_{eq}), 3.10 (2H, m, H-11), 3.31 (3H, s, OMe), 3.55 (2H, m, H-8), 3.75 (1H, m, H-10_{ax}), 3.96 (3H, s, CO_2CH_3), 4.00 (1H, m, H-3), 5.76 (1H, br s, H-7), 6.10 (1H, d, J= 10 Hz, H-1), 6.61 (1H, dd, J= 10 Hz, H-2), 8.00 (1H, s, H-14), 8.52 (1H, s, H-17).

5.4.3. Erysovine (3): amorphus, ^{1}H NMR ($CDCl_3$) δ: 1.80 (1H, dd, J= 10, 12 Hz, H-4_{ax}), 2.53 (1H, dd, J= 5, 12 Hz, H-4_{eq}), 2.34 (1H, dd, J= 5, 14 Hz, H-10_{eq}), 3.01 (2H, m, H-11), 3.33 (3H, s, OMe), 3.44 (1H, dd, J= 5, 14 Hz, H-10_{ax}), 3.81 (2H, m, H-8), 3.91 (3H, s, OMe), 4.01 (1H, m, H-3), 5.72 (1H, br s, H-7), 6.00 (1H, d, J= 10 Hz, H-1), 6.40 (1H, dd, J= 2.5, 10 Hz, H-2), 6.53 (1H, s, H-14), 6.81 (1H, s, H-17).

5.4.4. 5-Hydroxy-7,8-[hydroxyisopropyl dihydrofurano] flavanone (152): amorphous solid, $[\alpha]_D^{21}$ -53.1 (c= 0.33, $CHCl_3$), UV λ_{max}(MeOH) nm: 292, 338; UV λ_{max}(MeOH+$AlCl_3$) nm: 314, 385; UV λ_{max}(MeOH+NaOAc) nm: 293, 338. IR υ_{max} cm^{-1}: 3417, 1640, 1596, 1408, 1390, 1094, 700. ^{1}H NMR ($CDCl_3$) δ: 1.21 (3H, s, Me-5"), 1.32 (3H, s, Me-6"), 2.86 (1H, dd, J=12 and 16 Hz, H-3_{eq}), 3.03 (1H, dd, J=4 and 16Hz, H-3_{ax}), 3.16 (1H, dd, J=9 and 15 Hz, H-3"), 3.40 (1H, dd, J=9 and 15 Hz, H-3"), 4.70 (1H, t,J=8.5 Hz, H-2"), 5.40 (1H, dd, J=4 and 12 Hz, H-2), 6.00 (1H, s, H-6), 7.40 (5H, m, H-2'to 6') and 12.28 (1H, s, 5-OH). EIMS m/z (rel. int. %): 341 $[M+1]^+$ (22), 340 $[M]^+$ (100), 323 $[M-OH]^+$ (26), 307 $[M-33]^+$ (37), 282 $[M-58]^+$ (40), 281 $[M-59]^+$ (36), 219 (30), 204 (31), 178 (39), 177 (32), 150 (40), 104 $[B_1+2H]^+$ (16) and 59 (29).

5.4.5. Glabranin (153): mp 167-168° (lit. [63] 169-170°) UV λ_{max} (MeOH) nm: 293, 336; UV λ_{max}(MeOH+$AlCl_3$) nm: 315, 390; UV λ_{max}(MeOH+HCl) nm: 295, 337; UV λ_{max}(MeOH+NaOAC) nm: 292, 336. IR υ_{max} cm^{-1}: 3353, 2961, 2916, 2361, 1645, 1622, 1508, 1436, 1387, 1341, 1264, 1172,1071, 802, 699. ^{1}H NMR ($CDCl_3$) δ: 1.73 (6H, s, 2xMe), 2.90 (1H, dd, J= 12, 16 Hz, H-3ß), 3.05 (1H, dd, J= 4, 16 Hz, H-3α), 3.30 (2H, d, H-1"), 5.20 (1H, t, H-2"), 5.42 (1H, dd, J= 4, 12 Hz, H-2), 6.08 (1H, s, H-6), 7.45 (5H, s, H-2'to 6'), 12.00 (1H, s, D_2O exchangeable OH-5) (lit.[42]). EIMS m/z (rel. int. %): 324 [M, $C_{20}H_{20}O_4$]$^+$ (100), 309 [M-CH_3]$^+$ (46), 269 [M-C_4H_7]$^+$ (33), 205 [A-CH_3]$^+$ (78), 192 [A-CO]$^+$ (39), 177 (64), 165 (73), 104 (27), 91 (16),77 (27).

Cyclization of glabranin (**153**) to the flavanone **154**: 5 mg of **153** was taken up in 1 ml of HCO_2H and a few drops of conc. H_2SO_4 were added. The mixture was warmed for a few minutes while shaking until dissolved. The clear acid solution was allowed to stand at room temperature for 24 hr. It was then poured over crushed ice and gently shaken. The material was filtered, washed, dried and crystallized to give 3.8 mg (76%) of 5-hydroxyl-2",2"-dimethylchromaneflavanone (**154**) whose spectral data are given below.



(153) (154)

UV λ_{max}(MeOH) nm: 295.3; UV λ_{max}(MeOH+$AlCl_3$) nm: 318.9, 392.1. ^{1}H NMR ($CDCl_3$) δ: 1.35 (6H, s, 2xMe), 1.75 (2H, t, H-3"), 2.62 (2H, t, H-4"), 2.90 (1H, dd, J= 12, 16 Hz, H-3ß), 3.05 (1H, dd, J= 4, 16 Hz, H-3α), 5.42 (1H, dd, J= 4, 12 Hz, H-2), 5.97 (1H, s, H-6), 7.45 (5H, s, H-2'to 6').

5.4.6. Obovatin (155): amorphous (mp lit.[75] 126°), UV λ_{max}(MeOH) nm: 254, 271, 294, 353; UV λ_{max}(MeOH+$AlCl_3$) nm: 273, 281, 327, 410; UV λ_{max}(MeOH+NaOAc) nm: 254, 271, 294, 355. ^{1}H NMR ($CDCl_3$) δ: 1.28 (6H, s, 2xMe), 2.92 (1H, dd, J= 12, 16 Hz, H-3_{eq}) 3.02 (1H, dd, J= 4, 16 Hz, H-3_{ax}), 5.40 (1H, dd, J=

4, 12 Hz, H-2), 5.5 (1H, d, J= 10 Hz, H-3"), 6.00 (1H, s, H-6), 6.55 (1H, d, J= 10 Hz, H-4"), 7.41 (5H, s, H-2'to 6'), 12.08 (1H, s, D_2O exchangeable OH-5). EIMS m/z (rel. int. %): 322 [M, $C_{20}H_{18}O_4$]$^+$ (31), 307 [M-CH_3]$^+$ (88), 280 [M-C_3H_6]$^+$ (26), 203 (100), 176 (51), 77 (16).

5.4.7. 7-Hydroxy-8-prenylflavanone (156): UV λ_{max}(MeOH) nm: 237, 261sh, 287; UV λ_{max}(MeOH+$AlCl_3$) nm: 240, 286, 310sh; UV λ_{max}(MeOH+NaOAc) nm: 238, 259sh, 286, 298. IR υ_{max} cm^{-1}: 3398, 1646, 1597, 1443, 1377, 699. ^{1}H NMR ($CDCl_3$) δ: 1.71 (6H, s, 2xMe), 2.89 (1H, dd, J=12, 16 Hz, H-3_{eq}), 3.01 (1H, dd, J=4, 16 Hz, H-3_{ax}), 3.43 (2H, br d, H-1"), 5.25 (1H, t, H-2"), 6.54 (1H, d, J=9 Hz, H-6), 7.42 (5H, m, H-2'to 6'), 7.75 (1H, d, J= 9 Hz, H-5). EIMS m/z (rel. int. %): 308 [M]$^+$ (25), 265 [M-CH_3CO]$^+$ (9), 216 (15), 204 [A]$^+$ (16), 203 [A-H]$^+$ (22), 189 [A-CH_3]$^+$ (14), 176 [A-CO]$^+$ (19), 161 (29), 149 [A-C_4H_7]$^+$ (38), 104 [B]$^+$ (19), 91 (18), 83 (100), 77 (28), 55 [C_4H_7]$^+$ (29).

5.4.8. 3'-O-methylorobol (157): mp. 210-213° (lit.[62] 218-220°). UV λ_{max}(MeOH) nm: 264, 286, 332sh; UV λ_{max}(MeOH+$AlCl_3$) nm: 272, 311, 373; UV λ_{max}(MeOH+NaOAc) nm: 270, 289, 331sh; UV λ_{max}(MeOH+NaOH) nm: 255, 276, 324. IR υ_{max} cm^{-1}: 3244 (br), 1656, 1625, 1579, 1518, 1455, 1373, 1272, 1198, 1032, 777. ^{1}H NMR ($CDCl_3$) δ: 3.94 (3H, s, OMe), 6.32 (1H, d, J=2.0 Hz, H-6), 6.35 (1H, d, J=2.0 Hz, H-8), 6.95 (2H, d, J=1.4 and 8.0 Hz, H-5'and 6'), 7.13 (1H, d, J=1.4 Hz, H-2'), 7.86 (1H, s, H-2), 12.90 (1H, s, 5-OH). EIMS m/z (rel. int. %): 301 [M+1]$^+$ (21), 300 [M]$^+$ (100), 285 [M-CH_3]$^+$ (11), 269 [M-OMe]$^+$ (16), 257 (9), 229 (10), 153 [A+H]$^+$ (27), 148 [B_1]$^+$ (17), 120 (17), 83 (44) and 55 (18).

5.4.9. 3'-Methoxydaidzein (158): mp. 258-260° (lit.[72] 250-252°), UV λ_{max}(MeOH) nm: 250sh, 265, 290, 310sh; UV λ_{max}(MeOH+$AlCl_3$) nm: 250sh, 266, 286; UV λ_{max}(MeOH+NaOAc) nm: 265, 289, 310sh, 339sh; UV λ_{max}(MeOH+NaOH) nm: 261, 301, 326. IR υ_{max} cm^{-1}: 3413, 3269, 3069, 2530, 2431, 1627, 1576, 1519, 1375, 1292, 1238, 1120, 1020, 695. ^{1}H NMR ($CDCl_3$) δ: 3.89 (3H, s, OMe), 6.82 (1H, dd, J=2 and 9 Hz, H-6), 6.84 (1H, d, J=2 Hz, H-8), 6.87 (1H, d, J=9 Hz, H-5'), 6.93 (1H, dd, J=2 and 9 Hz, H-6'), 7.15 (1H, d, J=2 Hz, H-2'), 8.05 (1H, d, J=9 Hz, H-5), 8.16 (1H, s, H-2). For ^{13}C NMR ($CDCl_3$ +CD_3OD) see Table 4. EIMS m/z (rel. int. %): 285 [M+1]$^+$ (33), 284 [M]$^+$ (100), 269 [M-CH_3]$^+$ (6), 237 [M-OCH_3,-OH]$^+$ (5), 148 [B]$^+$ (9), 137

$[A+H]^+$ (21), 133 $[B\text{-}CH_3]^+$ (11), 112 (14), 105 (9) and 51 (10).

5.4.10. Biflavanone (159). Pale brown amorphous solid, UV λ_{max}(MeOH) nm: 256, 273sh, 290, 335; UV λ_{max}(MeOH+$AlCl_3$) nm: the same with decreasing intensities; UV λ_{max}(MeOH+NaOAc) nm: the same; UV λ_{max}(MeOH+NaOH) nm: 272, 289, 357 with increasing intensities. IR υ_{max} cm^{-1}: 3414, 1603, 1401, 1254, 1112, 700. ^1H NMR ($CDCl_3$) δ: 1.20 (3H, s, 5"-Me of I), 1.32 (3H, s, 6"-Me of I), 1.63 (6H, s, H-5" and 6" of II), 2.87 (1H, dd, H-$_{eq}$ of II), 2.98 (1H, d, H-$_{ax}$ of II), 3.28 (2H, dd, J=9 and 15 Hz, H-3" of I), 4.68 (1H, t, J=9 Hz, H-2" of I), 5.29 (1H, t, H-2" of II), 5.48 (1H, dd, J=4 and 12 Hz, H-2 of II), 6.00 (1H, s, H-6 of I), 6.57 (2H, d, J= 1.5 Hz, H-2 and H-3 of I), 7.40 (5H, s, H-2'to 6'of I), 7.44 (5H, s, H-2' to 6' of II), 11.75 (1H, s, 5-OH of I), 12.20 (1H, s, 5-OH of II). EIMS m/z (rel. int. %): 339 $[M (I)]^+$ (42), 323 $[M (II)]^+$ and $[M (I)\text{-}OH]^+$ (47), 307 $[M (I)\text{-}33]^+$ (25), 281 $[M (I)\text{-}59]^+$ (27), 268 $[M (II)\text{-}C_4H_7]^+$ (12), 219 (66), 204 (31), 176 (20), 165 (26), 164 (17), 150 (21), 85 (73), 82 (100) and 59 (11).

5.4.11. Erythrinasinate (160): mp 74-75° (lit. [76] 75-76°). UV λ_{max} (MeOH) nm: 252, 324. ^1H NMR ($CDCl_3$) δ: 0.80 (3H, t, Me), 1.25 (52H, s, $(CH_2)_{26}$), 3.80 (3H, s, OMe), 4.15 (2H, t, $OCH_2\text{-}CH_2R$), 6.25 (1H, d, J= 16 Hz, =CH), 6.85 (1H, d, J= 9 Hz, H-6), 6.90 (1H, s, exchangeable D_2O, OH), 6.95 (1H, s, H-2), 7.05 (1H, d, J= 9 Hz, H-5), 7.60 (1H, d, 16 Hz, CH=) (lit.[23]). For ^{13}C NMR information see Table 5. EIMS m/z (rel. int. %): 586 $[M, C_{38}H_{66}O_4]^+$ (100), 558 $[M\text{-}C_2H_5]^+$ (47), 194 $[M\text{-}C_{28}H_{57}]^+$ (26), 177 $[M\text{-}OC_{28}H_{57}]^+$ (26), 150 $[M\text{-}CO_2C_{28}H_{57}]^+$ (11), 137 $[M\text{-}CO_2C_{29}H_{58}]^+$ (22).

6. APPENDICES

APPENDIX 1. LIST OF FLAVANONES IDENTIFIED FROM *ERYTHRINA* SPECIES

NO.	Compound	MF	MW	Str. No.	Source	Ref.
1.	Abyssinone I	$C_{20}H_{18}O_4$	322	76	Ea[3]	13
2.	Abyssinone II	$C_{20}H_{20}O_4$	324	77	Ea[3]	13
3.	Abyssinone III	$C_{25}H_{26}O_4$	390	78	Ea[3]	13
4.	Abyssinone IV	$C_{25}H_{28}O_4$	392	79	Ea[3],Esi[2], Esi[1]	13,77 ,85
5.	Abyssinone V	$C_{25}H_{28}O_5$	408	80	Ea[3],Eer[1], Esi[1]	13,78 ,79
6.	Sigmidine A	$C_{25}H_{28}O_6$	424	81	Esi[1]	80
7.	Sigmoidine B	$C_{20}H_{20}O_6$	356	82	Esi[1]	80
8.	Sigmoidine C	$C_{20}H_{18}O_6$	354	83	Esi[1], Eer[1]	81,82 ,83
9.	Sigmoidine D	$C_{20}H_{20}O_7$	372	84	Esi[1]	84
10.	Sigmoidine E	$C_{25}H_{26}O_5$	406	85	Esi[1]	79
11.	Sigmoidine F	$C_{25}H_{26}O_6$	422	86	Esi[1]	85
12.	Sigmoidine G	$C_{20}H_{20}O_8$	388	87	Esi[1]	83
13.	3'-Prenylnaringenin	$C_{20}H_{20}O_5$	340	88	Eer[1]	78
14.	Eriotrinol	$C_{21}H_{20}O_7$	384	89	Eer[1]	83
15.	Erythrisenegalone	$C_{25}H_{26}O_5$	406	90	Ese[1]	86
16.	Senegalensein	$C_{25}H_{28}O_5$	408	91	Ese[1]	87
17.	5,7,4'-Trihydroxy-3'-methoxy-5'-prenylflavanone	$C_{21}H_{22}O_6$	370	92	Ebe[2], Esi[1]	88, 85
18.	2'prenyl eriodictyol	$C_{20}H_{20}O_6$	356	93	Esu[3]	89
19.	Lupinfolin	$C_{25}H_{26}O_5$	406	162	Ese[1]	106

APPENDIX 2. LIST OF ISOFLAVONES AND ISOFLAVANONES ISOLATED FROM *ERYTHRINA* SPECIES

NO	Compound	MF	MW	Str. No.	Source	Ref
1.	3'-O-methylorobol	$C_{16}H_{12}O_5$	284	94	Eer[1]	59
2.	2'-Hydroxy-5'-methoxybiochanin A	$C_{16}H_{12}O_6$	300	95	Eer[1]	78
3.	5,4'-dimethoxy-3'-prenylbiochanin A	$C_{22}H_{22}O_6$	382	96	Eer[1]	90
4.	Neobavaisoflavone	$C_{20}H_{18}O_4$	322	97	Esi[2]	78
5.	4'-O-methylalpinum isoflavone	$C_{21}H_{18}O_5$	350	98	Esi[1]	83
6.	Erythrinine A	$C_{20}H_{16}O_4$	320	99	Ev[1]	91
7.	Erythrinine B	$C_{20}H_{18}O_5$	338	100	Ev[1]	91
8.	Erythrinine C	$C_{20}H_{18}O_6$	354	101	Ev[1]	91, 82
9.	Oxyresveratrol	$C_{20}H_{18}O_6$	354	102	Ev[1]	91
10.	Alpinumisoflavone	$C_{20}H_{16}O_5$	336	103	Ev[1]	91
11.	Osajin	$C_{20}H_{16}O_5$	336	104	Ev[1]	91
12.	8-Prenyldaidzein	$C_{20}H_{18}O_4$	322	105	Ebi[2]	92
13.	Eriotriochin	$C_{27}H30O_7$	466	106	Eer[2]	59
14.	Sengalensis	$C_{25}H_{26}O_6$	422	107	Ese[1]	93
15.	Auriculatin	$C_{25}H_{24}O_6$	420	108	Eer[1], Ese[1], Ebi[2]	59,94, 92
16.	6,8-Diprenylgenistein	$C_{25}H_26O_5$	406	109	Ese[1], Ebi[2], Ef[3]	94,95, 98
17.	Warangalone (Scandenone)	$C_{25}H_{24}O_5$	404	110	Ese[1], Eer[1], Esi[1]	86,96, 78
18.	Auriculacin	$C_{25}H_{24}O_6$	420	111	Eer[1]	96
19.	Eryseneglensein D	$C_{25}H_{26}O_7$	438	112	Ese[1]	95
20.	Eryseneglensein E	$C_{25}H_{26}O_6$	422	113	Ese[1]	95
21.	8-Prenylluteon	$C_{25}H_{26}O_6$	422	114	Eer[1], Ese[1]	96, 95
22.	6,8-Diprenylorobol	$C_{25}H_{26}O_6$	422	115	Eer[1]	95
23.	Auriculatin-4'-O-glucoside	$C_{31}H_{34}O_{11}$	582	116	Eer[1]	82

Appendix 2. (Contd.)

NO.	Compound	MF	MW	Str. NO	Source	Ref.
24.	Genistein-7-O-glucoside	$C_{21}H_{20}O_9$	416	117	Ec[1]	97
25.	Daidzein-7-O-glucoside	$C_{21}H_{20}O_9$	416	118	Ec[1]	97
26.	Bidwillon A	$C_{25}H_{28}O_5$	408	119	Ebi[2]	92
27.	Bidwillon B	$C_{25}H_{26}O_5$	406	120	Ebi[2]	92
28.	5,7,3'-Trihydroxy-4'-methoxy-5'-prenylisoflavanone	$C_{21}H_{22}O_6$	369	121	Ebe ?	99
29.	Sigmoidine H	$C_{21}H_{20}O_5$	352	122	Esi[2]	78
30.	2,3-Dihydroauriculatin	$C_{25}H_{26}O_6$	422	123	Ese[1], Eer[1]	94, 59
31.	Erysenegalensein H	$C_{25}H_{26}O_7$	438	163	Ese[1]	106
32.	Erysenegalensein I	$C_{25}H_{26}O_7$	438	164	Ese[1]	106

APPENDIX 3. LIST OF PTEROCARPANS, A CHALCONE AND A COUMESTAN

NO.	Compound	MF	MW	Str.NO	Source	Ref.
1.	Erythrabyssin II	$C_{25}H_{28}O_4$	392	124	Em[3], Eab[3], Esi[2], Ec[1], Ebi[2], Ev[3]	100,13, 78, 95, 92,101
2.	Phaseollin	$C_{20}H_{18}O_4$	322	125	Eab[3], Esi[2],Ev[3]	13, 78, 101
3.	Phaseollidine	$C_{20}H_{20}O_4$	324	126	Eab[3], Ec[1], Ebu[1], Ev[3]	13, 95, 102,98
4.	Erythrabyssin I (Cristacarpin)	$C_{21}H_{22}O_5$	354	127	Eg[1], Eab[3], Ebu[1], Ec[4]	76, 13, 103
5.	Erybraedin A	$C_{25}H_{28}O_4$	392	128	Em[3]	100
6.	Erybraedin B	$C_{25}H_{26}O_4$	390	129	Em[3]	100
7.	Erybraedin C	$C_{25}H_{28}O_4$	392	130	Em[3]	100
8.	Isoneorautenol	$C_{20}H_{18}O_4$	322	131	Em[3]	100
9.	Erybraedin D	$C_{25}H_{26}O_4$	390	132	Em[3]	104
10.	Erybraedin E	$C_{22}H_{20}O_4$	348	133	Em[3]	104
11.	Erycristin	$C_{26}H_{30}O_4$	406	134	Ec[1]	95

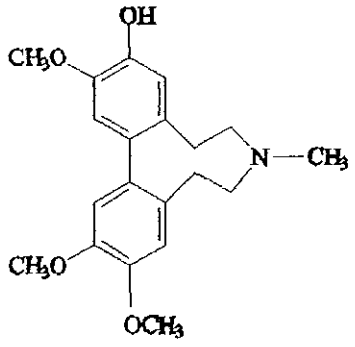
APPENDIX 3 (Contd.)

12.	Sandwicensin	$C_{21}H_{22}O_4$	338	135	Ec[1]	95
13.	Demethylmedicarpin	$C_{15}H_{12}O_4$	254	136	Ec[4], Esa ?	103,98
14.	Isomedicarpin	$C_{15}H_{14}O_4$	258	137	Esa ?	98
15.	Sandwicarpin	$C_{20}H_{20}O_5$	340	138	Esa ?	98
16.	3,6a,9-Trihydroxy pterocarpan	$C_{15}H_{12}O_5$	272	139	Esa ?	98
17.	Erycristagaline	$C_{25}H_{26}O_4$	390	140	Ec[2], Ev[3]	95,101
18.	Abyssinone VI	$C_{25}H_{28}O_4$	392	141	Eab[3], Esi[2]	13, 78
19.	4-Hydroxycoumestrol	$C_{15}H_8O_6$	284	142	Esi[5]	78

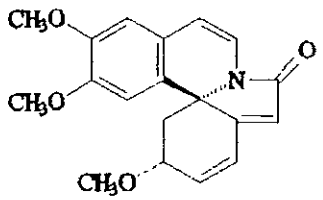
APPENDIX 4. ESTERS AND TRITERPENES ISOLATED FROM *ERYTHRINA* SPECIES

NO.	Compound	MF	MW	Str. NO	Source	Ref.
1.	Erythrinasinate	$C_{38}H_{66}O_4$	586	143	Eer[1],Ese[1] ,Eg[1],Em[1]	79,93 ,76
2.	Ester of- p-coumaric acid	$C_{39}H_{68}O_3$	584	144	Ese[1]	93
3.	Ester of ferulic acid	$C_{36}H_{62}O_4$	558	145	Eex[1]	93
4.	Ester of ferulic acid	$C_{38}H_{66}O_4$	586	146	Ese[1]	93
5.	Maniladiol	$C_{30}H_{50}O_2$	442	147	Eer[1],Esi[1]	90,5
6.	Serrat-14-ene-3ß,21α-diol	$C_{30}H_{50}O_2$	442	148	Eer[1]	90
7.	28-Acetoxy erythrodiol	$C_{32}H_{52}O_3$	484	149	Eer[1]	90
8.	Sigmoisides A	$C_{36}H_{60}O_7$	604	150	Esi[5]	5
9.	Sigmoisides B	$C_{36}H_{60}O_7$	604	151	Esi[1]	5
10.	Erythrodiol	$C_{30}H_{50}O_2$	442	---	Eer[1]	---

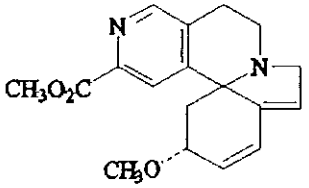
Appendix 5: Structures Of Misellaneous Erythrina Alkaloids



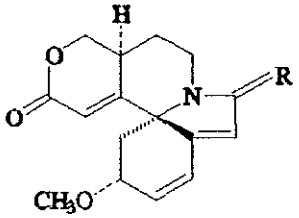
(48)



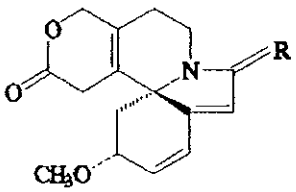
(49)



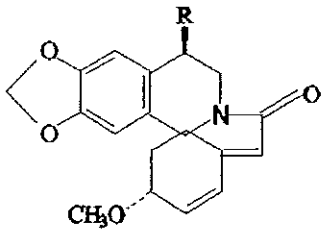
(50)



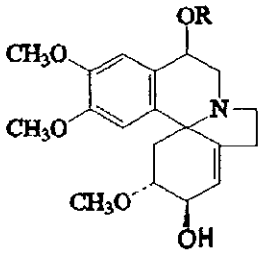
(52) R= H_2
(54) R= O



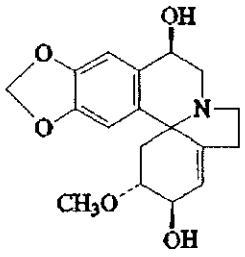
(53) R= H_2
(56) R= O



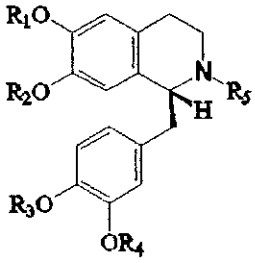
(57) R= H
(58) R= OH



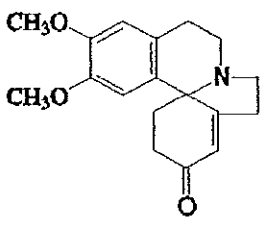
(59) R= H
(60) R= CH_3



(61)

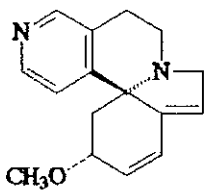


	R_1	R_2	R_3	R_4	R_5
(65) =	CH_3	H	H	CH_3	CH_3
(66) =	CH_3	H	H	CH_3	H
(67) =	H	CH_3	CH_3	H	CH_3
(68) =	H	CH_3	CH_3	H	H



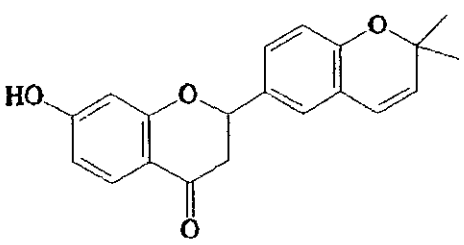
(62)

(69)

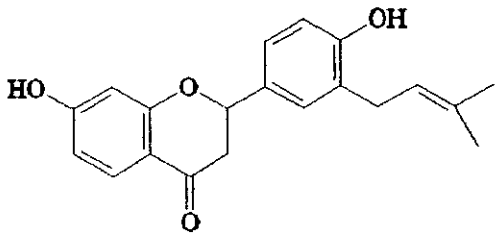


(161)

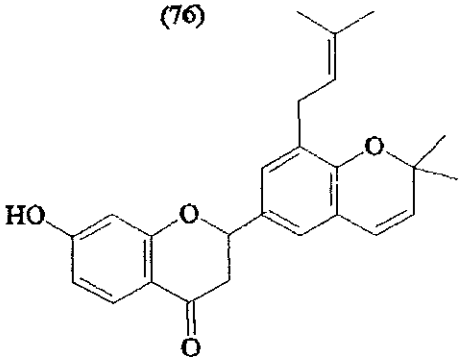
Appendix 6: Structures Of Flavanones Isolated From Erythrina Spec ies



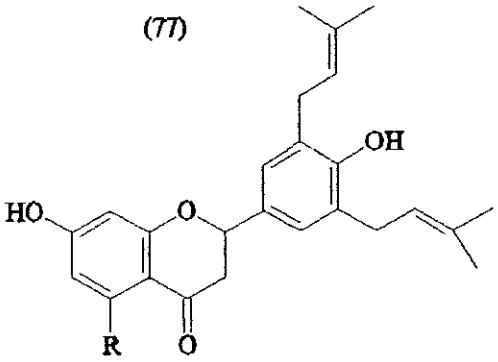
(76)



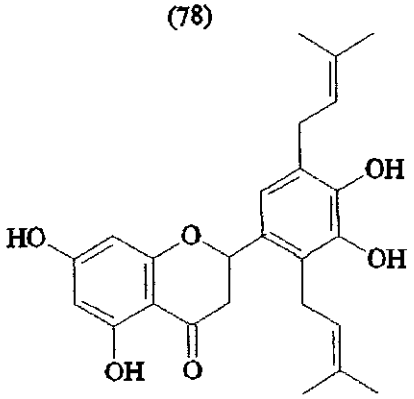
(77)



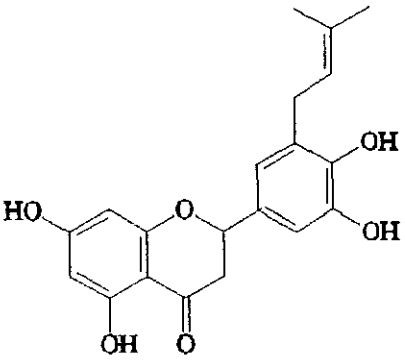
(78)



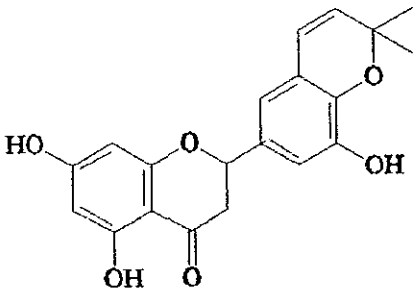
(79) R= H
(80) R= OH



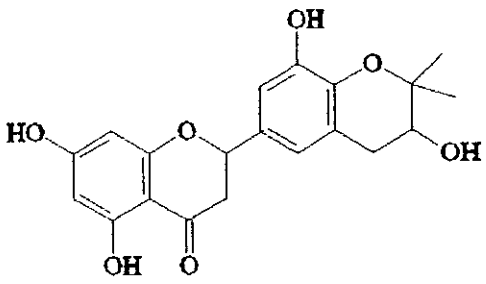
(81)



(82)

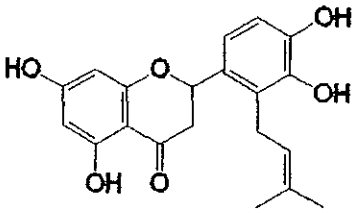


(83)

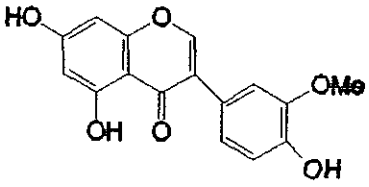


(84)

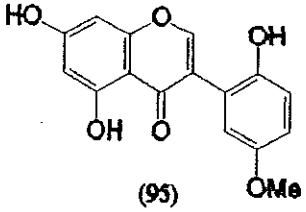
Appendix 7: Structures of 2'- Prenyleriodictyol and Isoflavones



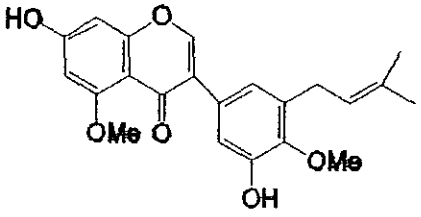
(93)



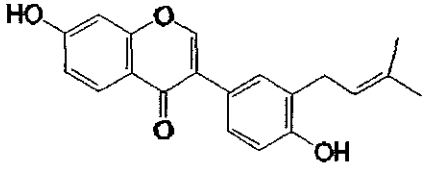
(94)



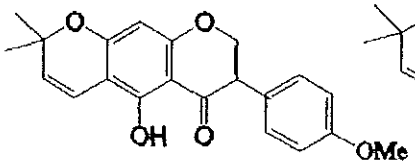
(95)



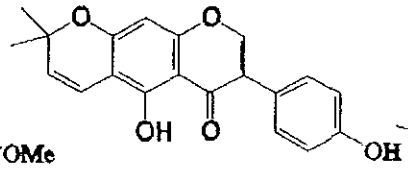
(96)



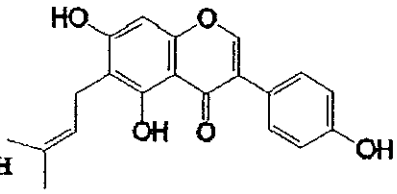
(97)



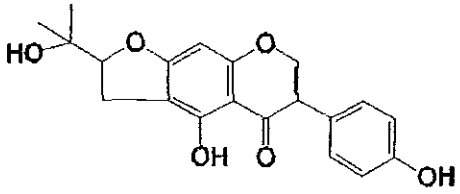
(98)



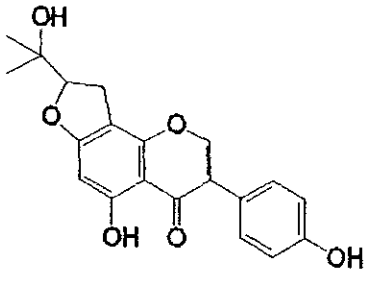
(99)



(100)

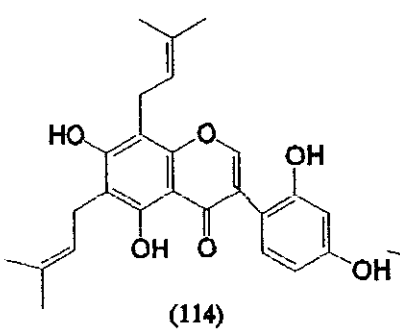


(101)

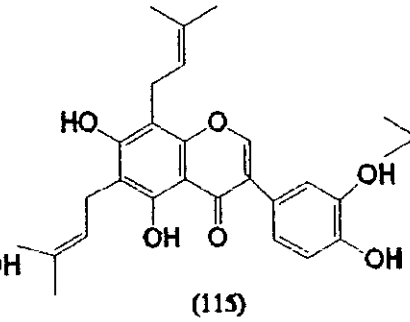


(102)

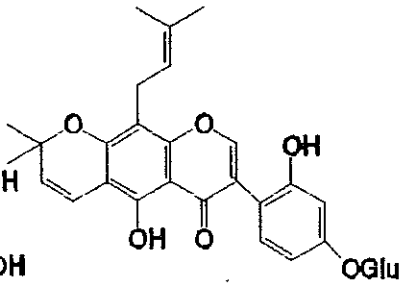
Appendix 7: (Contd.) and Isoflavanones



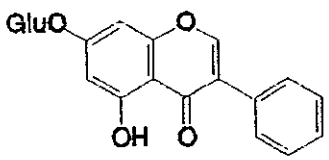
(114)



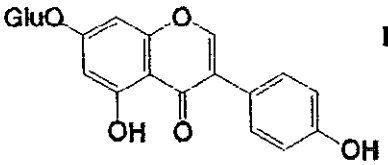
(115)



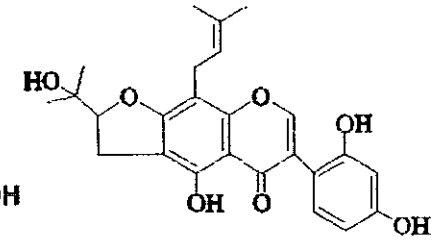
(116)



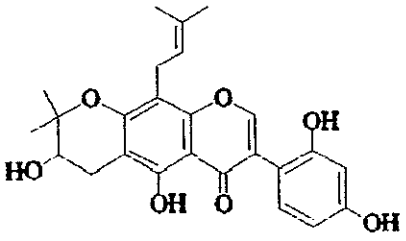
(117)



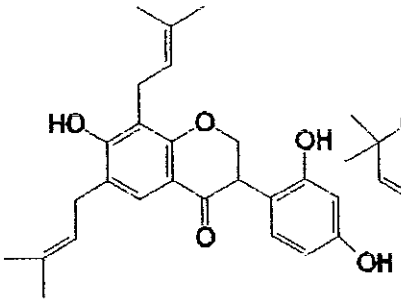
(118)



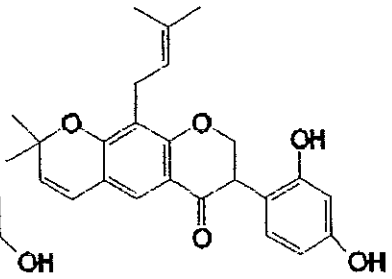
(163)



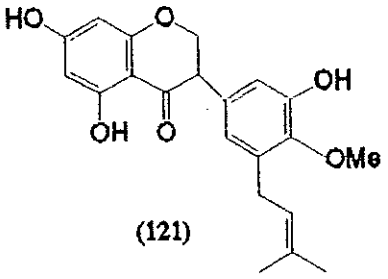
(164)



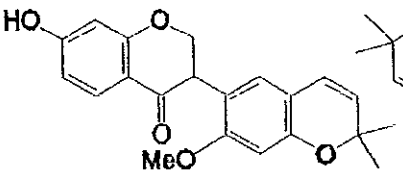
(119)



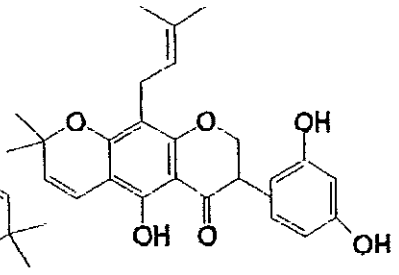
(120)



(121)

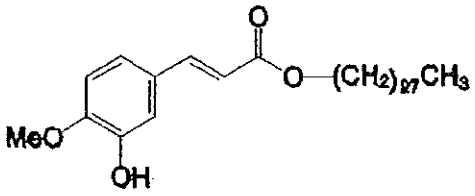


(122)

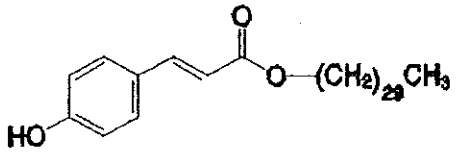


(123)

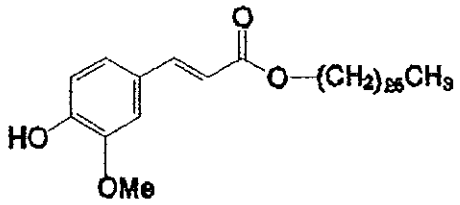
Appendix 9: Structures of Esters and Triterpenes



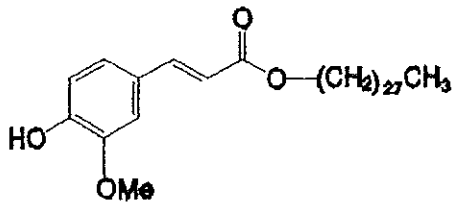
(143)



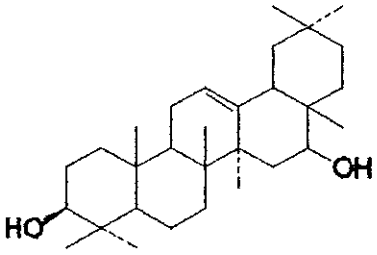
(144)



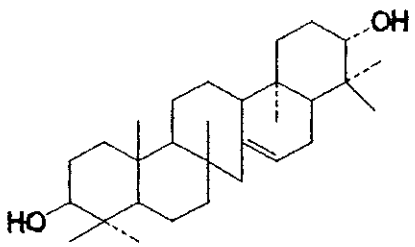
(145)



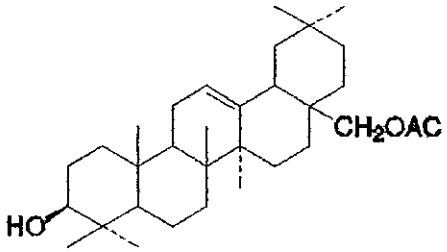
(146)



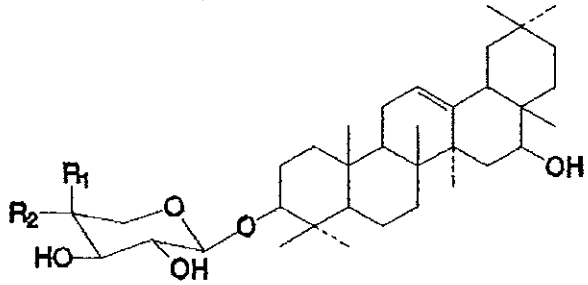
(147)



(148)



(149)



	R_1	R_2
(150) =	H	OH
(151) =	OH	H

7. REFERENCES

1. Williams, D. H., Stone, M. J., Hauck, P. R. and Rahman, S. K. (1989) *J. Nat. Prod.*, **52**, 1189.
2. Kinghorn, A. D. (1987) *J. Nat. Prod.*, **50**, 1009.
3. Farnsworth, N. R. (1990) *"The Role of Ethnopharmacology in Drug Development: Bioactive Compounds from Plants"*, John Wiley and Sons, West Sussex, p. 3.
4. Krukoff, B. A. and Barneby, R. C. (1974) *Lloydia*, **37**, 332.
5. Kouam, M. S., Nkengfack, A. E., Fomum, Z. T., Ubillas, R., Tempesta, M. S. and Meyer, M. (1991) *J. Nat. Prod.*, **54**, 1288.
6. Henry, T. A. (1949) *"The Plant Alkaloids"*, J. and A. Churchill Ltd., London, p. 386.
7. Smith, H. (1972) *"Phytochrome"*, Academic Press, New York, P. 433.
8. Grayer, R. J. (1989) *"Methods in Plant Biochemistry"*, ed. P. M. Dey and J. B. Harborne, Academic Press, London, Vol. 1, p. 286.
9. Zaat, S. A. J., Schrupsema, J., Wijffelman, C. A., Van Brussel, A. A. N. and Lugtenberg, B. J. J. (1989) *Plant Mol. Biol.*, **13**, 175.
10. Thulin, M. (1989) in *"Flora of Ethiopia-Pottosporaceae to Araliaceae"*, eds. I. Hedberg and S. Edwards, Addis Ababa, V. 3, p. 164.
11. Hill, R. K. (1967) in *"The Alkaloids: The Erythrina Alkaloids"*, ed. R. H. F. Manske, Academic Press, New York, Vol. 9, p. 513.
12. Singh, H., Chawla, A. S., Kapoor, V. K. and Kumar, N. (1981) *J. Nat. Prod.*, **44**, 526.
13. Kamat, V. S., Chuo, F. Y., Kubo, I. and Nakanishi, K. (1981) *Heterocycles*, **15**, 1163.
14. Ito, S. (1975) in *"Natural Products Chemistry: Alkaloids"*, ed. K. Nakanishi, T. Goto, S. Ito, S. Natori and S. Nozoe, Vol. 2, p. 256.
15. Gomes, C. M. R. and Gottlieb, O. R. (1980) *Biochem. Syst. Ecol.*, **8**, 81.
16. Southon, I. W. and Buckingham, J. (1989) *"Dictionary of Alkaloids"*, Chapman and Hall, London.

36. Aguilar, M. I, Giral, F. and Espejo, O. (1981) *Phytochemistry*, **20**, 2061.

37. Chawla, A. S., Jackson, A. H. and Ludgate, P. (1982) *J. Chem. Soc. Perkin Trans.* **I**, 2903.

38. Mantle, P. G., Laws, I. and Widdowson, D. A. (1984) *Phytochemistry*, **23**, 1336.

39. Ghosal, S., Majumdar, S. K. and Chakraborti, A. (1971) *Aust. J. Chem.*, **24**, 2733.

40. Games, D. E., Jackson, A. H., Khan, N. A. and Millington, D. S. (1974) *Lloydia*, **37**, 581.

41. Dagne, E. and Steglich, W. (1983) *Tetrahedron Letters*, **24**, 5067.

42. Jackson, A. H. (1985) in *"The Chemistry and Biology of Isoquinoline Alkaloids"*, ed. J. D. Phillipson, M. F. Robert and M. H. Zenk, Springer-verlag, Berlin.

43. Dagne, E. and Steglich, W. (1984) *Phytochemistry*, **23**, 449.

44. Leete, E. and Ahmad, A. (1966) *J. Am. Chem. Soc.*, **88**, 4722.

45. Boar, R. B. and Widdowson, D. A. (1970) *J. Chem. Soc. Sec.* **B**, 1591.

46. Valant-Vetschera, K. M. and Wollenweber, E. (1985) *"Flavonoids and Biflavonoids"*, eds., L. Farkas, M. Gabor and F. Kally, Elsevier, Netherlands.

47. Simmonds, M. S. J., Blaney, W. M., Delle Monache, F. and Marini Bettolo, G. B. (1990) *J. Chem. Ecol.*, **16**, 365.

48. Cody, V., Middleton, Jr, E. and Harborne, J. B. (1986) *"Plant Flavonoids in Biology and Medicine"*, Allen R. Liss, New York.

49. Saleh, N. A. M. (1989) in *"Proceedings of the Third NAPRECA Symposium on Natural Products and their Applications: Flavonoids and Chemosystematics"* ed. NAPRECA Tanzania, Dar es Salaam, p.148.

50. Swain, T. (1980) in *"Pigments in Plants: Flavonoids as Chemosystematic Markers in Plants"* ed. F. C. Cyzgan, G. Fischer, Strugard, p. 224.

51. Hegnauer, R. and Grayer-Barkmaijel, R. J. (1993) *Phytochemistry*, **34**, 3.

52. Heller, W. and Forkmann, G. (1994) in *"The Flavonoids: Advances in Research since 1986"*, ed. J. B. Harborne, Chapman and Hall, London, p. 502.

53. Agrawal, P. K. and Markham, K. R. (1989) *"Carbon-13 NMR of Flavonoids"*, ed. P. K. Agrawal, Elsevier, New York, p. 5.

54. Wong, E. (1974) in *"The Flavonoids"*, eds. J. B. Harborne, T. J. Mabry and H. Mabry, Chapman and Hall, London, p. 743.

55. Ito, K., Haruna, M., Jinno, Y. and Furakawa, H. (1976) *Chem. Pharm. Bull.*, **24**, 52.

56. Folkers, K. and Koniuszy, F. (1940) *J. Am. Chem. Soc.*, **62**, 1673.

57. Mabry, T. J., Markham, K. R. and Thomas, M. P. (1970) *"The Systematic Identification of Flavonoids"*, Springer, New York, p. 267.

58. Markham, K. R. (1982) *"Techniques of Flavonoids Identification"*, Academic Press, London, p. 39.

59. Nkengfack, A. E., Fomum, Z. T., Ubillas, R., Sanson, D. R. and Tempesta, M. S. (1990) *J. Nat. Prod.*, **53**, 509.

60. Adinarnyana, D. and Rao, J. R. (1975) *Indian J. Chem.*, **13**, 425.

61. Radhakrishiah, M. (1973) *Phytochemistry*, **12**, 3003.

62. Bohlmann, F. and Abraham, W. R. (1979) *Phytochemistry*, **18**, 1851.

63. Labbiento, L., Menichini, F. and Monache, F. D. (1986) *Phytochemistry*, **25**, 1505.

64. Rao, E. V., Venkataratnam, G. and Vilain, C. (1985) *Phytochemistry*, **24**, 2427.

65. Lester, A., Mitscher, G. S., Raghav Rao, Ishkhanna, T. V. and Drake, S. (1983) *Phytochemistry*, **22**, 573.

66. Dagne, E., Yenesew, A. and Watermann, P. G. (1987) *Phytochemistry*, **28**, 3207.

67. Ebel, E. and Hahlbrock, K. (1982) in *"The Flavonoids: Advances in Research Since 1975"*, ed. J. B. Harborne and J. T. Mabry, Chapman and Hall, New York, p. 649.

68. Khan, H. A., Chandrasekharan, I. and Ghanim, A. (1986) *Phytochemistry*, **25**, 767.

69. Gupta, R. K. and Krishamurti, M. (1976) *Phytochemistry*, **15**, 832.

70. De Almedia, M. E. L. and Gottlieb, O. R. (1974) *Phytochemistry*, **13**, 751.

71. Tori, K., Seo, S., Shimaoka, A. and Tomita, Y. (1974) *Tetrahedron Letters*, 4227.

72. Yahara, S., Ogata, T., Saijo, R., Konishi, R., Yamahara, J., Miyahara, K. and Nohara, T. (1989) *Chem. Pharm. Bull.*, **37**, 979.

73. Sherif, E. A., Gupta, R. K. and Krishna, M. M. (1980) *Tetrahedron Letters*, **21**, 641.

74. Fukai, T. and Nomura, T. (1991) *Heterocycles*, **32**, 499.

75. Venkataratnam, G., Venkata Rao, E. and Vilain, C. (1987) *Chem. Soc. Perkin. Trans.*, I, 2723.

76. Fomum, Z. T., Ayafor, J. F., Wandji, J., Fomban, W. G. and Nkengfack, A. E. (1986) *Phytochemistry*, **25**, 757.

77. Nkengfack, A. E., Kouam, J. Vouffo, T. W., Meyer, M., Tempesta, M. S. and Fomum, Z. T. (1994) *Phytochemistry*, **35**, 521.

78. Nkengfack, A. E., Sanson, D. R., Tempesta, M. S. and Fomum, Z. T. (1989) *J. Nat. Prod.*, **52**. 320.

79. Promsattha, R., Tempesta, M. S., Fomum, Z. T. and Mbafor, J. T. (1988) *J. Nat. Prod.*, **51**, 611.

80. Fomum, Z. T., Ayafor, J. F. and Mbafor, J. T. (1983) *Tetrahedron Letters*, **24**, 4127.

81. Fomum, Z. T., Ayafor, J. F., Mbafor, J. T. and Mbi, C. M. (1986) *J. Chem. Soc. Perkin Trans.* **I**, 33.

82. Nkengfack, A. E., Meyer, M., Tempesta, M. S. and Fomum, Z. T. (1991) *Planta Med.*, **57**, 488.

83. Nkengfack, A. E., Kouam, J., Vouffo, W. T., Fomum, Z. T., Dagne, E., Sterner, O., Browne, L. M. and Ji, G. (1993) *Phytochemistry*, **32**, 1305.

84. Promsattha, R., Tempesta, M. S., Fomum, Z. T. and Ayafor, J. F. (1986) *J. Nat. Prod.*, **49**, 932.

85. Promsattha, R., Mbafor, J. T., Tempesta, M. S. and Fomum, Z. T. (1989) *J. Nat. Prod.*, **52**, 1316.

86. Fomum, Z. T., Ayafor, J. F. and Wandji, J. (1985) *Phytochemistry*, **24**, 3075.

87. Fomum, Z. T., Ayafor, J. F., Wandji, J. (1987) *J. Nat. Prod.*, **50**, 921.

88. Maillard, M., Gupta, M. P. and Hostettmann, K. (1987) *Planta Med.*, 563.

89. Chauhan, P. and Saxena, V. K., (1987) *Planta Med.*, **55**, 221.

90. Nkengfack, A. E., Fomum, Z. T., Ubillas, R. and Tempesta, M. S. (1990) *J. Nat. Prod.*, **33**, 1552.

91. Deshpande, V. H., Pendse, A. D. and Pendse, R. (1977) *Indian J. Chem.*, **15B**, 205.

92. Iinuma, M., Tanaka, T., Mizuno, M., Yomamoto, H., Kobayashi, Y. and Yonemori, S. (1992) *Chem. Pharm. Bull.*, **40**, 2749.

93. Nkengfack, A. E., Fomum, Z. T., Ubillas, R., Killday, K. B. and Tempesta, M. S. (1990) *J. Nat. Prod.*, **53**, 1425.

94. Taylor, R. B., Corley, D. G., Tempesta, M. S., Fomum, Z. T., Ayafor, J. F., Wandji, J. and Ifeadike, P. N. (1986) *J. Nat. Prod.*, **49**, 670.

95. Mitscher, L. A., Gollapudi, S. R., Gerlach, D. C., Drake, S. D, Veliz, E. A. and Ward, J. A. (1988) *Phytochemistry*, 27, 381.

96. Nkengfack, A. E., Sanson, D. R., Fomum, Z. T. and Tempesta, M. S. (1989) *Phytochemistry*, **28**, 2522.

97. Imamura, H., Ito, M. and Ohashi, H. (1981) *Res. Bull. Fac. Agr. Gifu Univ.*, **45**, 77. [Chem. Abst. 1982, **96**, 196524].

98. Mitscher, L. A., Drake, S., Goliapudi, S. R. and Okwute, S. K. (1987) *J. Nat. Prod.*, **50**, 1025.

99. Maillard, M., Hamburger, M., Gupta, M. P. and Hostettmann, K. (1989) *Planta Med.*, **55**, 281.

100. Mitscher, L. A., Okwute, S. K., Gollapudi, S. R., Drake, S. and Ovona, E. (1988) *Phytochemistry*, **27**, 3449.

101. Telikepalli, H., Gollapudi, S. R., Keshavarz-Shokri, A., Velazquez, L., Sandmann, R. A., Veliz, E. A., Rao, K. V. J., Madhavi, A. S. and Mitscher, L. A. (1990) *Phytochemistry*, **29**, 2005.

102. Dagne, E., Alemu, M., Gunatilaka, A. A. L., Kingston, D. G. I., Hoffmann, G. and Johnson, R. K. (1993) *J. Nat. Prod.*, **56**, 1831.

103. Ingham, J. L. and Markham, K. R. (1980) *Phytochemistry*, **19**, 1230.

104. Mitscher, L. A., Okwute, S. K., Gollapudi, S. R. and Keshavarz-shokri, A. (1988) *Hetrocycles*, **27**, 2517.

105. Soto-Hernandez, M. and Jackson, A. H. (1994) *Planta Med.*, **60**, 175.

106. Wandji, J., Fomum, Z. T., Tillequine, F., Skaltsounis, A. L. and Koch, M. (1994) *Planta Med.*, **60**, 178.

107. Crombie, L., Rossiter, J. T., Bruggen, N. V. and Whiting, D. A. (1992) *Phytochemistry*, **31**, 451.

108. Tahara, S., Ingham, J. L. and Mizutani, J. (1989) *Phytochemistry*, **28**, 2079.