CHEMICAL INVESTIGATION

OF

VERNONIA FILICERA

A THESIS

SUBMITTED TO

THE SCHOOL OF GRADUATE STUDIES

ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF SCIENCE IN CHEMISTRY

BY

ANNAH WAMBUI KEIGE

JUNE 1990

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

CHEMICAL INVESTIGATION OF *VERNONIA FILIGERA*

by

Annah Wambui Keige

Chemistry Department

Science Faculty

Approved by:

Dr. Berhanu Abegaz
Advisor

Prof. Y. Kashman
External Examiner

Dr. Ermias Dagne
Examiner

Dr. Tarekegne Gebreyesus
Examiner

Dr. Dirshaye Menberu
Examiner

DEDICATION

To my parents, Teresah and Joseph Keige, for understanding my need for education and making it possible.

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ACKNOWLEDGEMENTS

I would like to express my gratitude to my advisor Dr. Berhanu Abegaz for his advise and assistance in the course of this work.

My appreciation and thanks also go to Dr. Ermias Dagne and Dr. Tarekegn Gebreyesus who were assisting me when my advisor went on sabbatical leave abroad, and to Dr. Mesfin Tadesse for identifying the plant material.

I also wish to express my thanks to Dr. Gezachew Alemayu for translating a German text for me. I am also grateful to Dr. Wandimagen Mamo, w/t Yordanos Woldu, Ato Naod Kebede and all those who assisted me in various ways in the course of this work.

Lastly, I wish to acknowledge and thank the DAAD/NAPRECA Scholarship Programme for giving me the scholarship that enabled me to pursue this research and for their financial assistance.

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ABSTRACT

Chemical Investigation

of

Vernonia filigera

by

Annah Wambui Keige

Research advisor: Dr. Berhanu Abegaz

Chemical investigation of the aerial parts of *Vernonia filigera* resulted in the isolation of 3-methyl quercetin, vernolepin and vernodalin. These compounds have been previously isolated from other species of the genus.

Structures were established using IR, ^{1}H NMR, ^{13}C NMR, and mass spectral interpretation and comparison with literature values.

^{13}C NMR of vernolepin and vernodalin is reported here for the first time.

The genus *Vernonia* is the largest genus of the tribe Vernonieae (Asteraceae=Compositae) and one of the larger genera of flowering plants.[1] The tribe Vernonieae has about 1500 species with more than 1000 belonging to the genus *Vernonia*.[2] *Vernonia* is mainly tropical and is found in the warmer regions of both hemispheres, with many of it's species growing in Brazil and tropical Africa.[2] There are more than 500 species in Africa and Asia at least 300 in Mexico, Central and South America and about 16 in the United States.[3]

Division of the tribe Vernonieae into subtribes and genera is still very uncertain, with problems in *Vernonia* from specific upto generic level.[2,4]

Chemical evidence especially the sesquiterpene lactones and flavonoids support the hypothesis that the genus has two centres of origin, one in Africa and the other in South America.[4] It can also be used to distinguish between the new and the old world species.[2,3]

Chromosome count also supports and amplifies these conditions -new world n=17, old world n=9, 10.[4] The cytological difference was used to divide the genus into two subgenera, one each for the new and the old world.[5] Palynological studies also support this distinction. However, the flavonoid and sesquiterpene lactones provide evidence linking the new and old world species.

Several *Vernonia* species have been found to be of various economic importance. Some are troublesome as weeds, including *V. baldwinii* of North America and *V. cinerea*, a pan tropical weed.[1]

Two species, *V. anthelmintica* and *V. galamensis* have been examined as possible

oil seed crop plants.[2] Both contain vernolic acid present as a triglyceride, trivernolin, which can be used as a plasticizer in paint and coating industries. Further experimental and breeding work is still being carried out on *V. galamensis* which was found to have a better seed retention and a higher oil content.

A number of *Vernonia* species have been widely used in traditional medicine. *V. anthelmintica* has been used as an anthelmintic and for treatment of asthma and kidney troubles.[3] *V. amygdalina* has been used in treatment of fevers, stomach upsets, schistosomiasis and also as food.[7] *V. hindebrantii* was reportedly used for arrow poisoning.[7] Other species have been used for treatment of malaria, cough, snake-bites and abdominal pains.[8]

Some of the species of *Vernonia* have been shown to contain active compounds which possess cytotoxic and antitumor activity,[9,10,11] antiviral activity,[12] plant growth regulatory activity and insect and herbivore feeding deterrents.[13,14,15] Most of these activities are attributed to sesquiterpene lactones (eg. Vernolepin,[9,10] vernodalin,[11] glaucolides[13,14]) and some to the presence of flavonoids (eg. 3-methyl quercetin[12]).

Chemical studies on several species have revealed that together with sesquiterpene lactones which are the major constituents, other compounds are also present including flavonoids, triterpenes, sterols, acetylenes and vernolic acid.[16]

1.1 OBJECTIVE OF THE PROJECT

A survey of literature on *Vernonia* showed that most of the chemical investigation on the genus has taken place over the last two decades. However, out of the 1000 species, less than 150 have been thoroughly examined. Interest in the genus was sparked off by isolation of antitumor agents. Due to the complexity of the genus and interest in their sesquiterpene lactones, our laboratory set out to study the chemistry of the *Vernonia* of Ethiopia. A series of such studies are currently underway.

There are over 60 species listed for the genus *Vernonia* in Ethiopia. They are found from sea level to an altitude of 3000 m.[17,18] Very little chemical investigation has been carried out on the *Vernonia* of Ethiopia.

V. filigera is distinctly endemic of Ethiopia.[19] It is found between 2000-3000 m altitude.[20] Todate, no chemical investigation has been reported on this plant.

This project deals with the investigation of *V. filigera*. The objective is to undertake chemical screening of *V. filigera* for it's chemical constituents especially the sesquiterpene lactones and flavonoids.

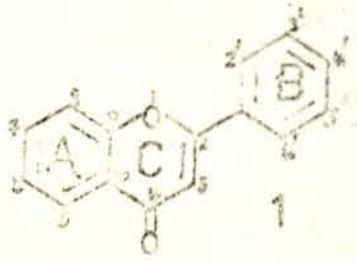
2. BACKGROUND

2.1 Flavonoids

Flavonoids make up one of the largest group of naturally occuring phenolic compounds.[21] They occur mainly in flowering plants and ferns.[22] In plants they are found as aglycones, O- and C-glycosides, sulfates and biflavonoids.[23]

2.1.1. Classification

Flavonoids contain fifteen carbon atoms in their basic nucleus which are arranged in a C_6-C_3-C_6 manner i.e. two aromatic rings connected by a three-carbon unit which may or may not form a ring.[23,24] The rings are labelled as A, B and C and the carbon atoms are numbered as shown in 1. A modified numbering system is used for chalcones as in 2.



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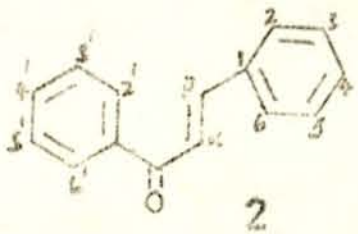

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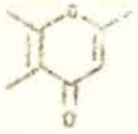
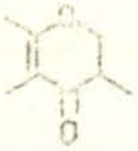
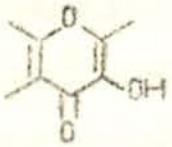
2.1.2 Structural variation

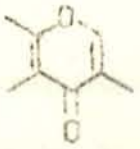
Various structural variations in flavonoid aglycones are caused by hydroxylation of the aromatic rings, methylation of the hydroxyl groups or of the flavonoid nucleus, glycosylation, prenylation, dimerisation and bisulfate formation.[23]

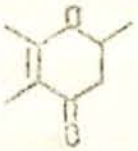
The oxidation state of the three carbon link forms the basis for classification of flavonoids. The major classes are listed below

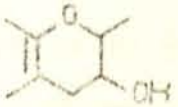
Flavone 

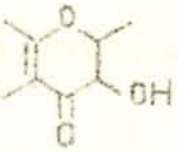
Isoflavanone 

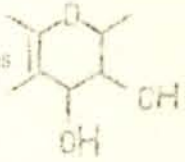
Flavonol 

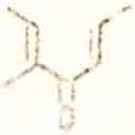
Isoflavone 

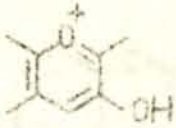
Flavanone 

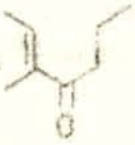
Catechins (flavane-3-ols) 

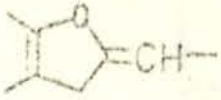
Flavane-3,4-diols (dihydroflavonols) 

Leucoanthocyanidins (flavan-3,4-diols) 

Chalcones 

Anthocyanidins 

Dihydrochalcones 

Aurones 

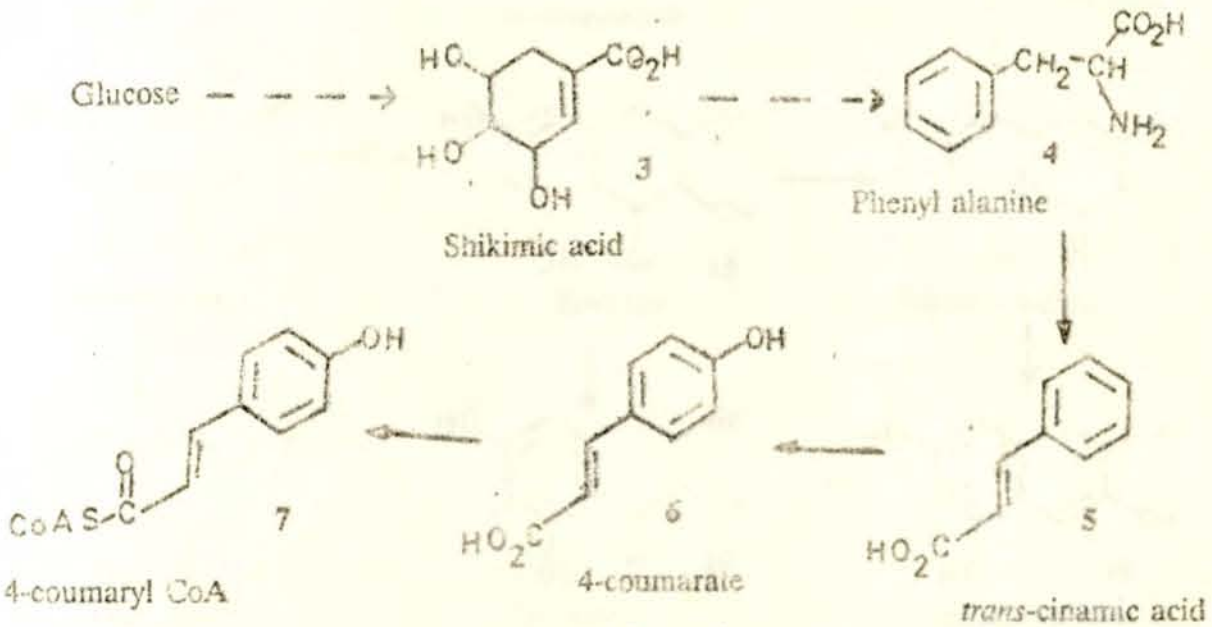
2.1.3 Biological activity

Several flavonoids have been found to have some interesting biological activity. Some known biological activities of flavonoids include activites such as nector guide components,[25,26,2] anti-inflamatory and anti-oedima,[29] cardiovascular,[12, 36] inhibition of aldose reductase,[30] phytoalexins,[31] antimicrobial,[32, 33, 34] anticancer[35] and antifungal.[12, 36]

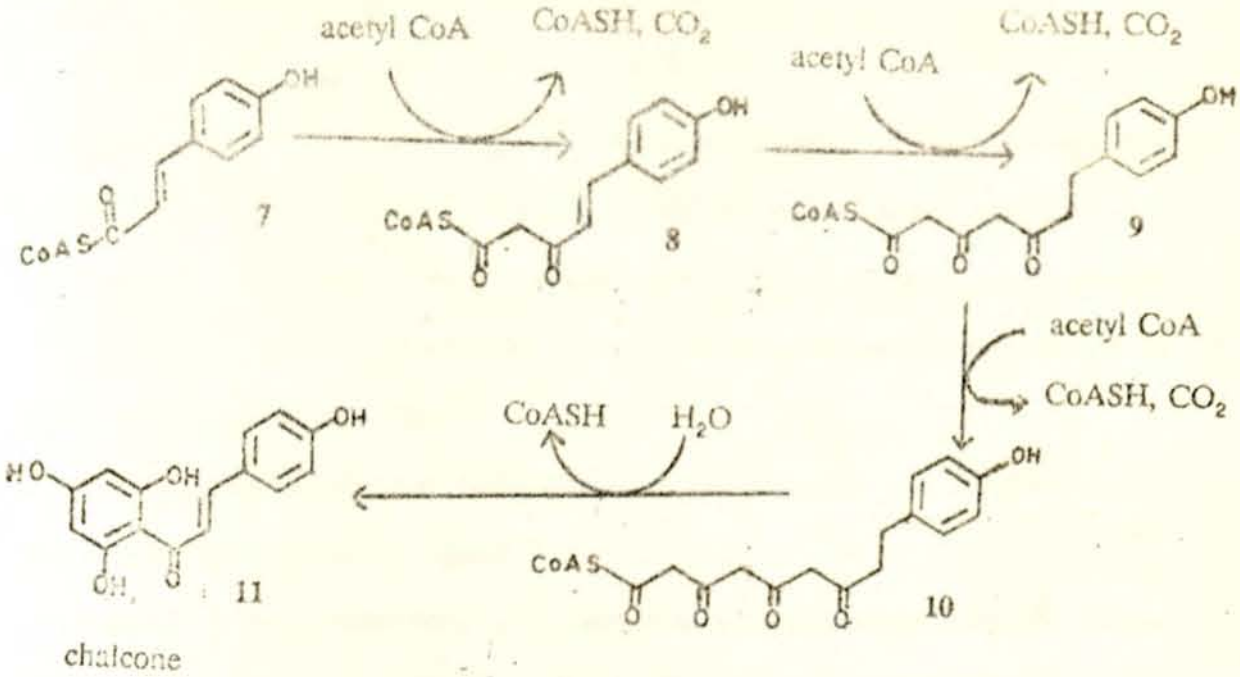
2.1.4 Biosynthesis of flavonoids

All classes of flavonoids are biogenetically related by a common biosyntetic pathway which incorporates precursors from both the "shikimate" and the "acetate-malonate" pathways.[22,23] The C_6-C_3 unit of ring B and ring C, for which phenylalanine is the precursor, is derived from the shikimate pathway.[38] The C_6 unit of ring A is formed from the head-tail condensation of acetyl CoA, the precursor of the acetate-malonate pathway.[38] The first intermediate formed immediately after the confluence of the two pathways is thought to be a chalcone, **11**.

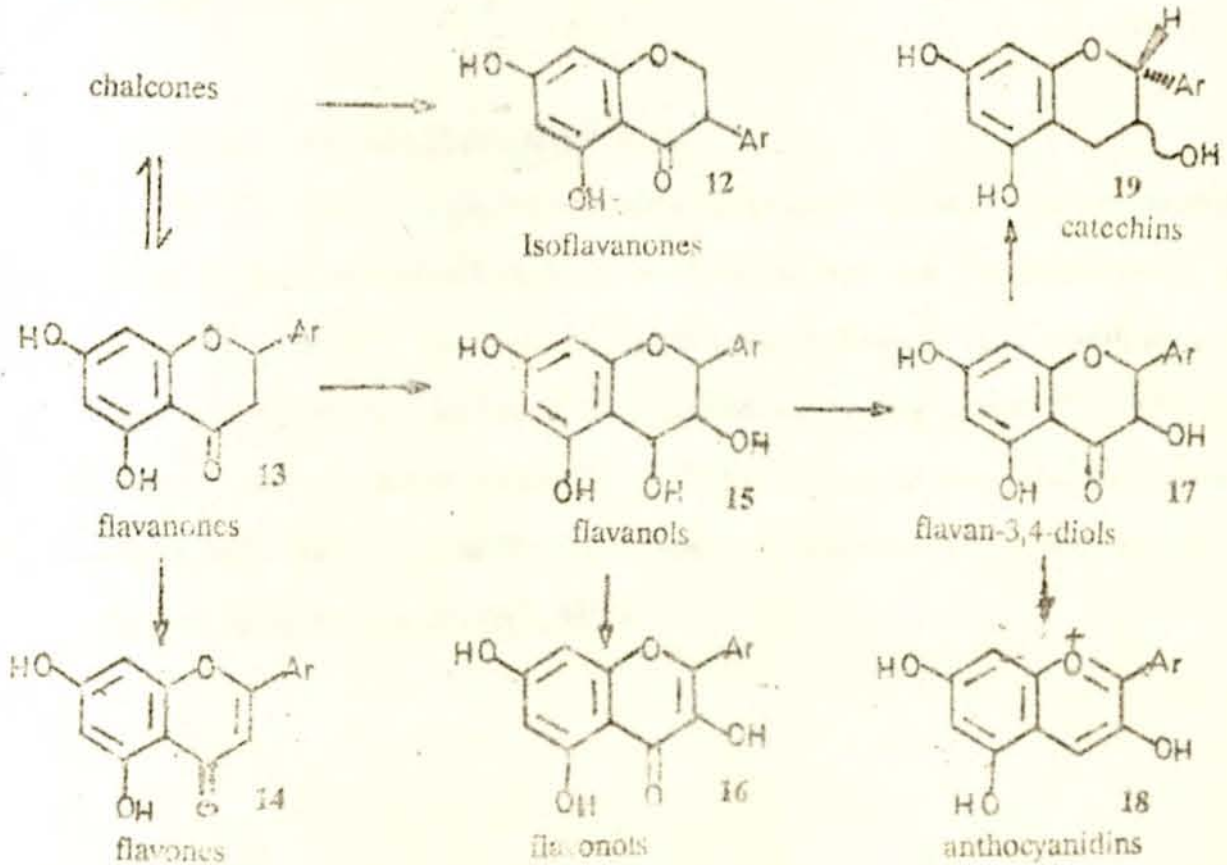
Scheme 1: Formation of coumaryl CoA



Scheme 2: Biosynthesis of chalcone



Scheme 3 Biogenetic relationships of flavonoids



2.1.5 Identification

2.1.5.1 Colour reactions

Flavonoid colour reactions offer only a broad indication of the flavonoid type because within any given class, the colour produced depends on the oxygenation pattern.[22]

Exposure of flavonoid TLC to ammonia fumes causes marked colour changes especially when viewed under UV light. Relation between the spot appearence and the flavonoid structure can be made.[39]

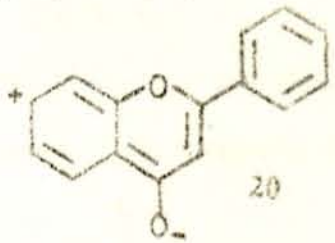
Some flavonoids give characteristic colours on reduction with magnesium and hydrochloric acid (Shinoda's reagent).[22]

When an extract is made alkaline by addition of aqueous sodium hydroxide, various colour changes are observed which can provide information about the basic structure of the flavonoid. However, in flavonoid mixtures changes due to one might be masked by those of another.[22,23]

2.1.5.2 Ultra-violet visible spectroscopy

UV-Visible spectroscopy is a very useful technique in flavonoid structural analysis. It aids both in the identification of the flavonoid type and the defination of the oxygenation pattern.[30] The spectra is usually ran in methanol.[23] Use of shift reagents helps to establish the location of the unsubstituted hydroxyl groups.[23,30] The shift reagents used are sodium methoxide (NaOMe), sodium acetate (NaOAc), sodium acetate/boric acid ($NaOAc/H_3BO_3$), aluminium chloride ($AlCl_3$) and aluminium chloride/hydrochloric acid ($AlCl_3/HCl$)

The flavonoid spectrum consists of two absorption maxima in the region 240-550 nm.[22,23] These are referred to as band I (300-550 nm) and band II (240-285 nm).[23] Band I is associated with the B-ring cinnamoyl system (21) and band II with A-ring benzoyl system (20).[22]



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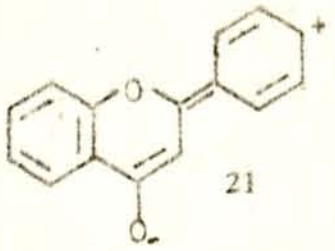

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The position and intensity of the maxima varies with the relative resonance contributions of these systems. Changes in the A ring substitution are reflected in band II absorption while changes in substitution of B- and C-ring are reflected in band I absorption. Increasing oxygenation in a ring causes a bathochromic shift in the relevant band. Methylation and glycosylation causes a hypsochromic shift.

2.1.5.3 Proton NMR spectroscopy

Proton NMR is very useful in the stucture elucidation of flavonoids. The proton signals generally occur in the range of 0-9 ppm.[39]

A-ring protons

For flavones, flavonols and isoflavones with 5,7-dihydroxyl groups, the C-6 and C-8 protons give rise to two doublets (J = 2.5 Hz) in the range 6.0-6.5 ppm. The C-6 doublet occurs at consistently higher field than C-8 due to the ortho effect of the hydroxyl groups. If a sugar is attached to the C-7 hydroxyl group, both signals are shifted downfield.[23,39]

If C-5 is not oxygenated, the proton absorbs near 8.0 ppm due to the deshielding by the C-4 keto group. It appears as a doublet (J = 9 Hz) due to ortho coupling between C-5 and C-6 protons.[38]

B-ring protons

B-ring protons usually appear in the range 6.7-7.9 ppm. The coupling pattern of these protons is characteristic of the substitution pattern of the ring.[25,39]

C-ring protons

In flavones and flavonols C-3 proton appears as a singlet near 6.3 ppm and is therefore distinguished from C-6 and C-8 protons which occur in the same region.[39]

In isoflavones, the C-2 proton is at β -position to the C-4 keto group and occurs in the range 7.6-7.8 ppm.

For chalcones, the H-α and H-β protons appear as doublets (J = 17 Hz), H-α in the range 6.7-7.4 ppm and H-β in the the range 7.3-7.7 ppm.

The aurone benzylic protons appear as a singlet at 6.5-6.7 ppm.[39]

In flavanones the C-2 proton appears as a double-doublet near 5.2 ppm while C-3 protons occur as two overlaping double-doublets near 2.8 ppm. The C-2 proton of dihydroflavonols occur as a doublet (J = 11 Hz) near 5.2 ppm while C-3 proton doublet appears near 4.3 ppm.

2.1.5.4 ^{13}C NMR Spectroscopy

^{13}C NMR of flavonoids can be useful in special cases. Although the resonances of the aromatic carbon atoms cannot be used to distinguish between the different types of flavonoids, the chemical shifts of the three carbons of ring C are usually quite distinct

for the different classes. Assignment of the aromatic carbon signals is based on the basic absorption of the flavone and additive parameters taking into account the oxygenation pattern.

Table 1: ^{13}C NMR resonances of ring C[26]

Flavonoid type	C-2	C-3	C-4
chalcones	136.9-145.4 d	116.6-128.7	188.6-194.5s
flavanone	75.0-80.3 d	42.8-44.6 t	189.5-195.5 s
flavones	160.5-165.0 s	103.0-111.8 d	176.3-184.0 s
flavonols	145-150	136-139	172-177
Isoflavones	149.8-155.4 d	122.3-125.9	174.5-181.0 s
aurones	146.1-147.7 s	111.6-111.9 d	182.5-182.7
pterocarpans	66.4-66.5 t	39.5-40.2 d	78.4-78.5 d

2.1.5.5 Mass spectroscopy

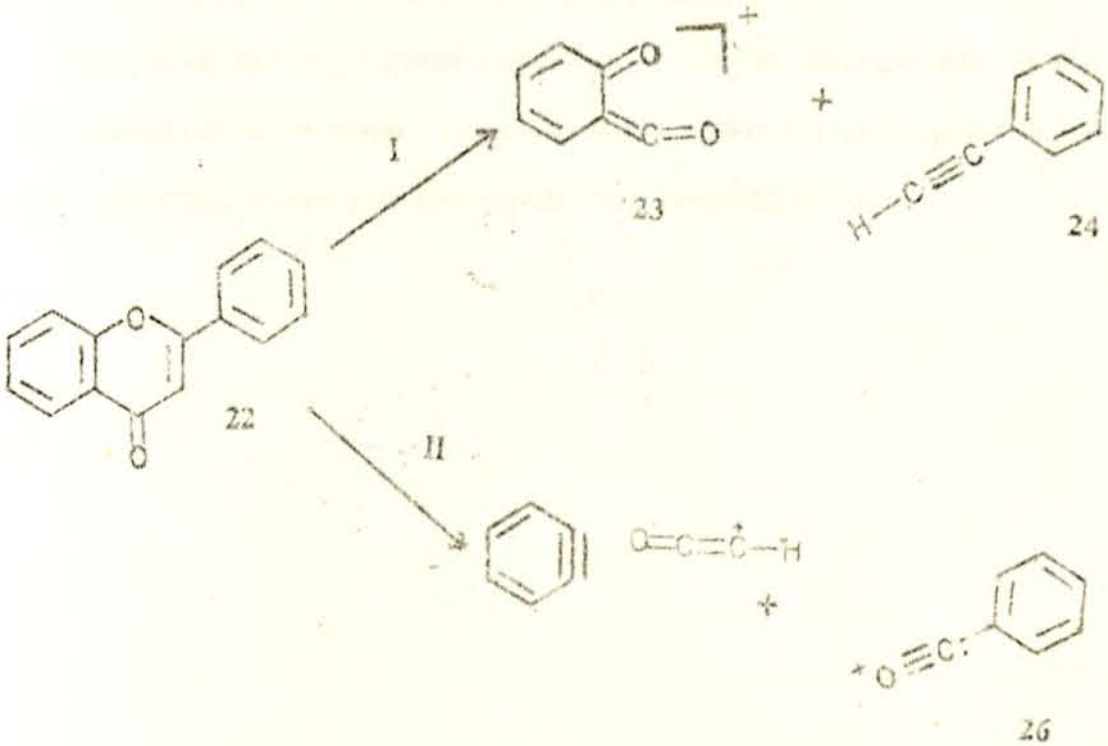
The first objective in interpretation of mass spectrum is the identification of the unfragmented molecular ion. Other major fragments are related to the molecular ion by rationalising their loss in molecular weight using recognisable fragmentation pathways.[28] In the mass spectra of aglycones, the molecular ion appears as a major peak. It's measurement permits the calculation of it's elemental composition.

A and B-ring fragmentation

The fragmentation of the molecular ion into A- and B-ring containing fragments provide useful structural information. Fragmentation follows one of the two competing pathways [23,40]

I: Retro Diels-Alder

II:Route II



2.1.6 Flavonoids of the genus *Vernonia*

Most of the flavonoids isolated from the genus *Vernonia* are based on the flavones (apigenin and luteolin types) and the flavonols (kaempferol and quercetin types).[3] The compounds include the aglycones, their methylated derivatives and their O- and C-glycosides. Exceptions include the flavanone hesperitin isolated from *V. brevifolia*[3] and

trien isolated from *V. reticiflora*.[41]

Flavonoids have been used in assisting the classification of the genus.[2,3] It appears that a complex flavonoid profile might represent a primitive character within the genus. It has been observed that species which are recognized as more "advanced" on other than chemical grounds (e.g. morphology), contain a relatively small number of flavonoids (1-4). A large number of flavonoids appear as a primitive character. This reduction trend in evolution has been observed in several cases.[2,3,42]

In addition, new world species show the most complex flavonoid patterns while old world species show the simplest flavonoid pattern. Old world species give only flavones while new world species give either flavones or flavonols, or both.[2,3]

2.2 TERPENOIDS

Terpenoids form a large class of natural products which are widely distributed in living systems, including plants, fungi, marine organisms and insects.[43] They have a common biosynthetic origin based on the mevalonic acid-derived isopentenyl pyrophosphate.[44] Their carbon skeleton is built up from the union of two or more of the isopentenyl units which are usually linked in a head-tail manner, with some notable exceptions.[45]

Studies of terpenoids have been stimulated by their wide range of biological activities. These include activities such as allergenic agents,[46,47] cytotoxic and antitumor agents,[10,11,48] regulators of plant growth,[49,50] antischistosomal agents,[51] antimicrobial agents and accessory pigments in photosynthesis.[49] Monoterpenes and sesquiterpenes are also major components of essential oils.[52]

2.2.1 Classification

Terpenoids are classified on the basis of the number of isoprene units in their assembly.[51] They fall mainly into the classes of hemiterpenoids (C_5), monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), sesterpenoids (C_{25}), triterpenoids and steroids (C_{30}), carotenoids (C_{40}) and polyterpenoids ($C > 40$).

The sesquiterpenoids provide a remarkable diversity of the carbon-skeleton compared to the other classes.[54] They are also the most important class of terpenoids in the Asteraceae (=Compositae) family.

2.2.2 Structural diversity

The aliphatic precursor in terpenoid biosynthesis is converted to cyclic terpenes through one or more cyclisation steps.[47] Other structural modifications involving rearrangements, oxidation, ring cleavage, loss of side chains or combination with units of other biosynthetic origin provide a wide range of structural diversity.[48]

Isomerisation is also common among terpenoids. In addition, they are usually alicyclic and since the cyclohexane ring is usually twisted in the "chair" form, different geometrical conformers are possible depending on the substitution around the ring.[49] Stereochemistry of cyclic terpenoids is therefore highly involved and often difficult to determine.[49] This has been especially the case in sesquiterpene lactones where the stereochemistry of several compounds had to be modified recently.[56, 57,58,59,60]

2.2.3 Characterisation

Detection of terpenoids is usually difficult since all of them are colourless except carotenoids. There is no universal chromogenic reagent specific for terpenoids.[49]

Detection is usually carried out by spraying sulfuric acid or vanillin/sulfuric acid on TLC plates and heating.

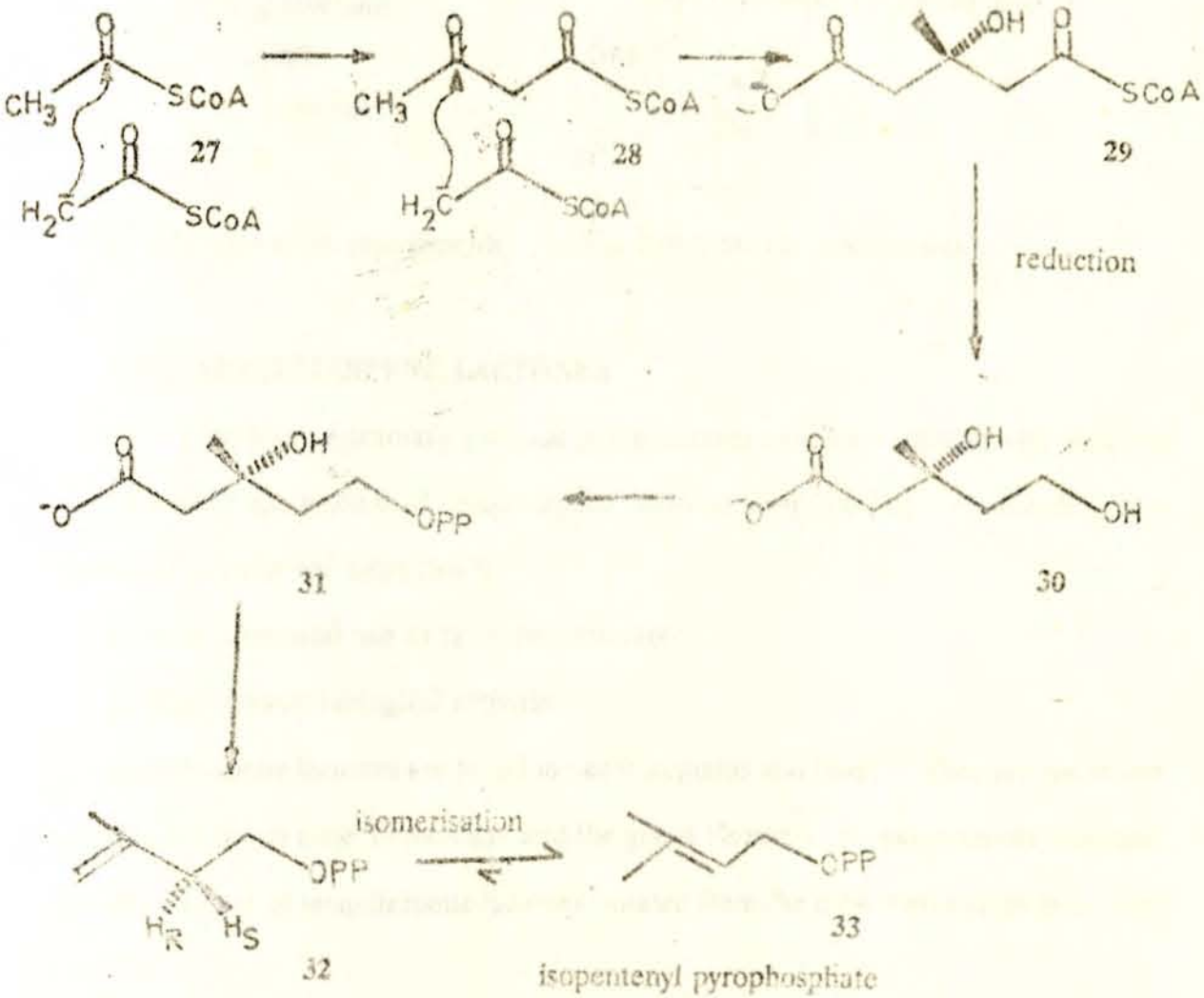
Other methods of detection that can be used include spraying with 5% antimony chloride in chloroform or 0.2% aqueous potassium permaganate. Double bond containing compounds can be detected using iodine while 2,4-dinitrophenylhydrazine is used for ketone groups.[49]

Structure elucidation is carried out using spectroscopic techniques, namely IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy together with biogenetic considerations. X-ray crystallography, although not a routine and simple technique, provides unequivocal proof of structure including stereochemistry.[49]

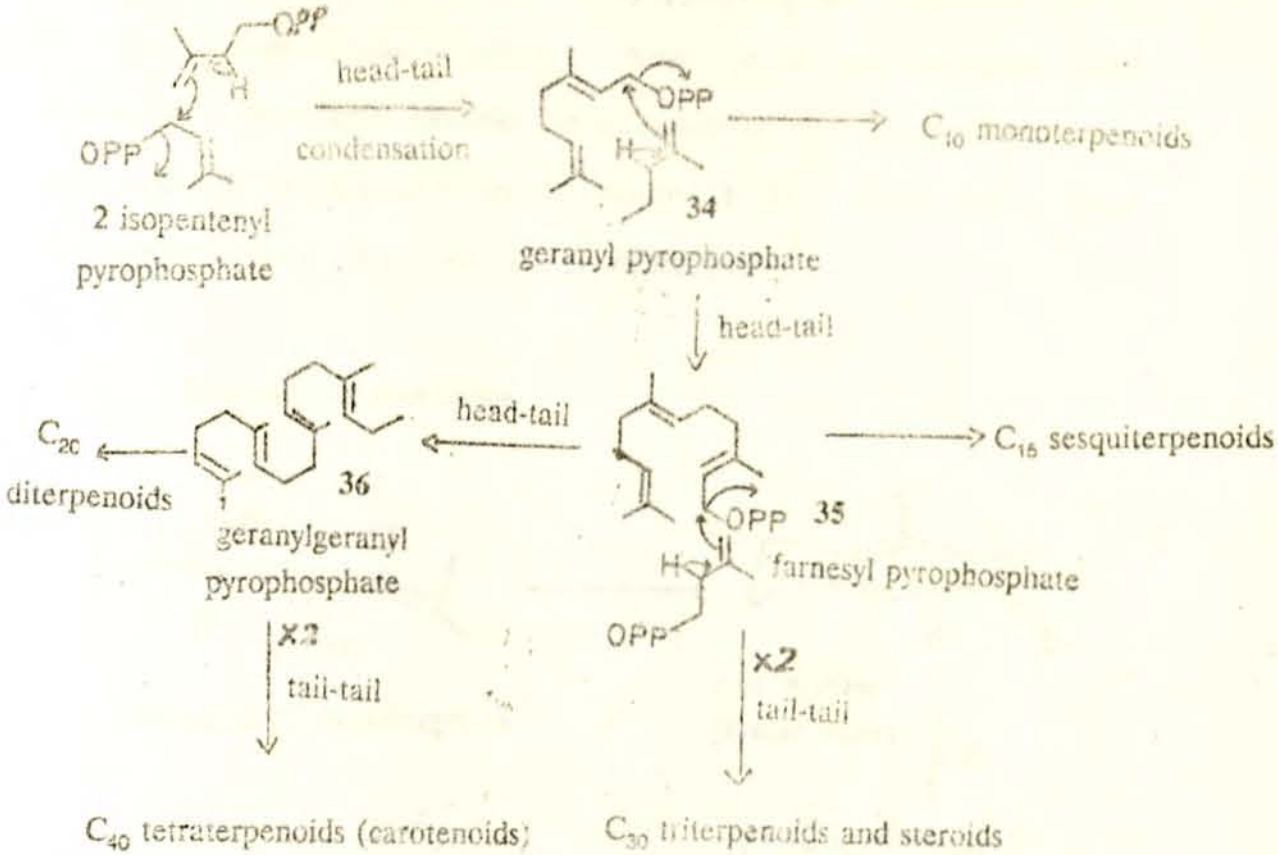
2.2.4 Biosynthesis of terpenoids

The invivo precursor in biogenesis of terpenoids is not isoprene but isopentenyl pyrophosphate.[49] This is formed from acetyl CoA via mevalonic acid.[51] The linking of isopentenyl pyrophosphate gives rise to the various classes of terpenoids. Most terpenoids are cyclic with one or more functional groups. This implies that the final steps in the biosynthesis involves cyclisation and oxidation or other structural modifications which introduce functional groups.[49,55]

Scheme 4: Biosynthesis of Isopentenyl pyrophosphate



Scheme 5: Terpenoid biosynthesis



2.2.5 SESQUITERPENE LACTONES

There has been a dramatic increase in the number of reports dealing with isolation and structure elucidation of sesquiterpene lactones over the last two decades. This increased interest has been due to:[51]

a) their successful use as taxonomic markers

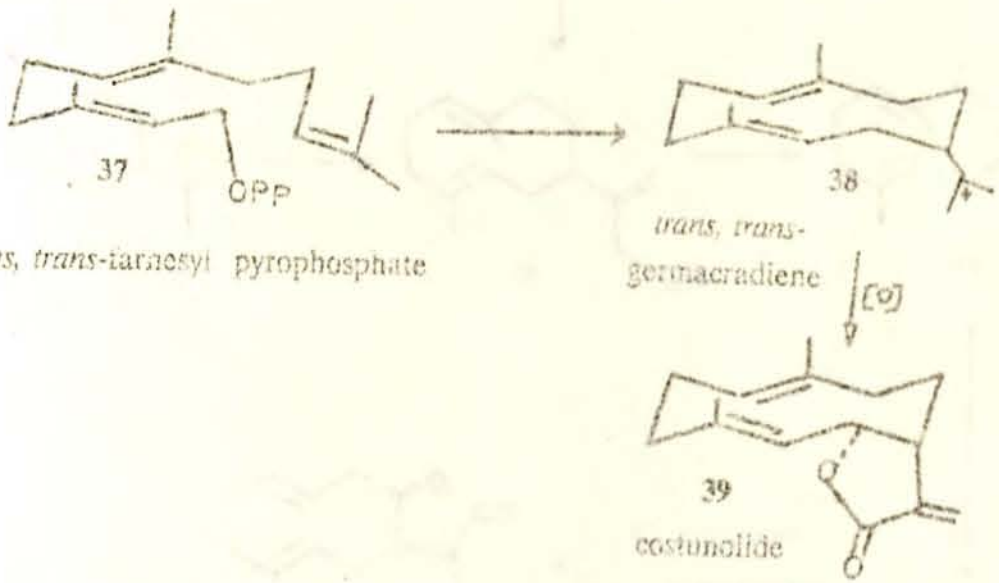
b) their various biological activities.

Sesquiterpene lactones are found to occur in plants and fungi.[54] They are the major constituents of the tribe Vernonieae and the genus *Vernonia*. A recent report indicated that the number of sesquiterpene lactones isolated from the tribe Vernonieae goes upto 236.[56]

2.2.6 Biogenesis of sesquiterpene lactones

Sesquiterpenoids are derived from farnesyl pyrophosphate.[54] Cyclisation of *trans, trans*-farnesyl pyrophosphate results in the formation of *trans, trans*-germacradiene intermediate. Enzymatic oxidative modification of this intermediate provides germacranolides, the simplest of which is costinolide 39.[21] Other skeletal types of sesquiterpene lactones are derived from germacradiene.

Scheme 6: Formation of costinolide



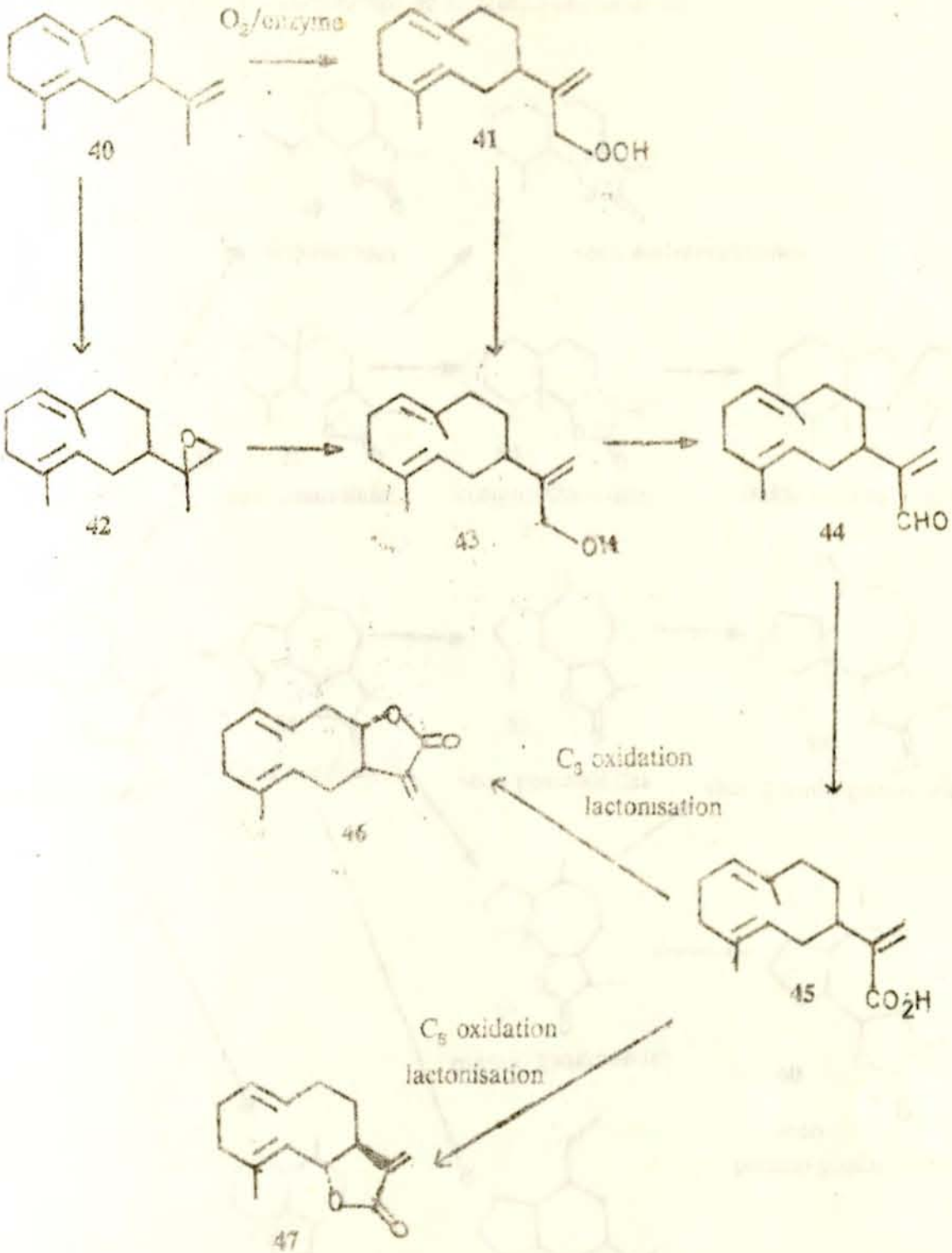
trans, trans-farnesyl pyrophosphate

trans, trans-germacradiene

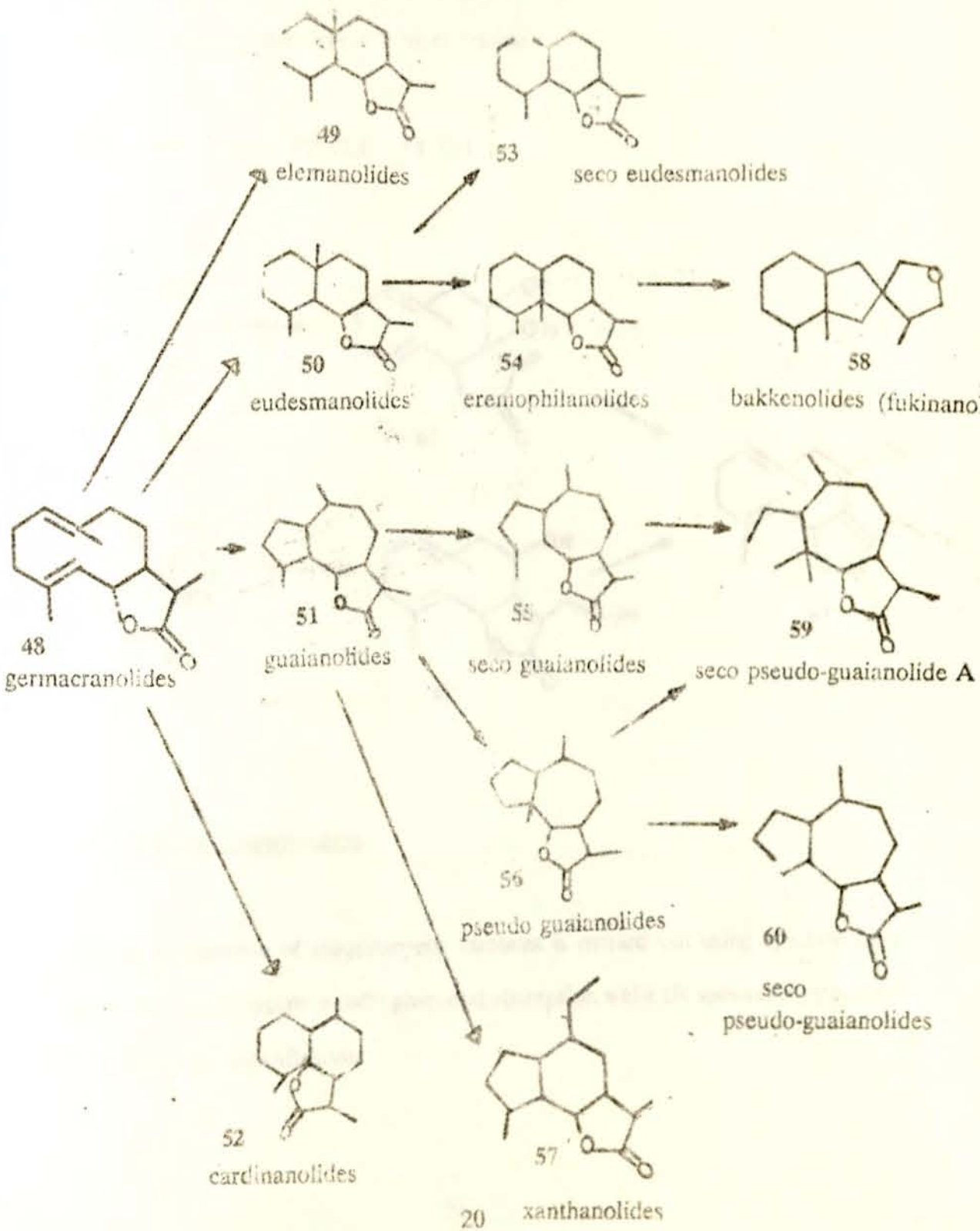
costunolide

Two possible routes have been proposed for the formation of the lactone ring, resulting in the formation of 6,12 as well as 8,12 connected lactone rings.[55] The C-6 connected lactones are formed predominantly because position 6 is allylic to a double bond making it more favourable to hydroxylation compared to C-8.

Scheme 7. Formation of the lactone ring



Scheme 8: Biogenetic relationships of sesquiterpene lactones

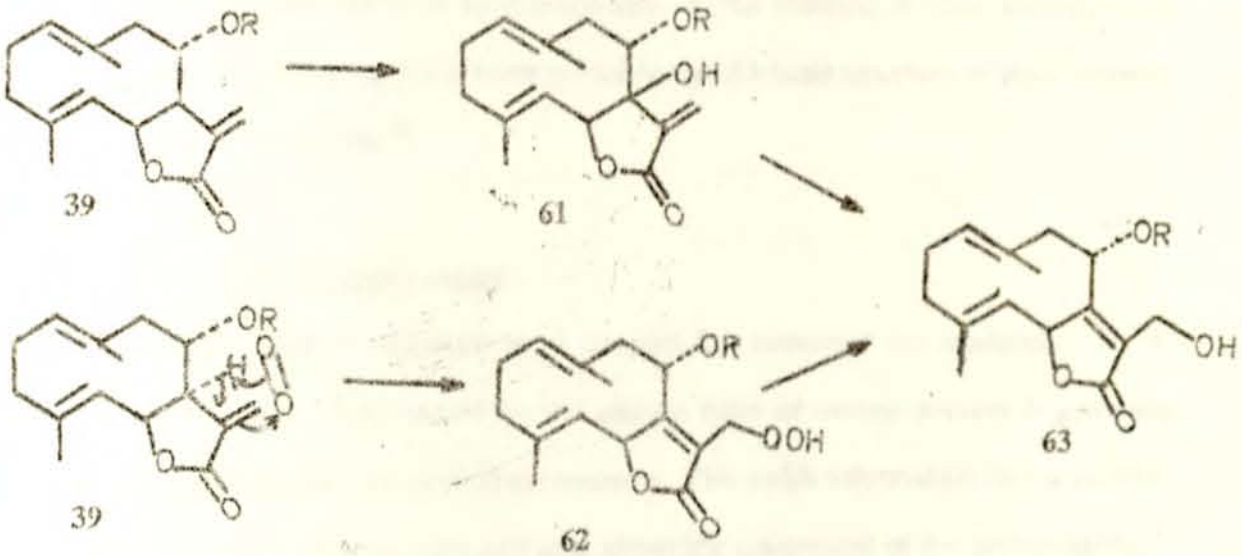


Isomerisation of Δ 11,13 to Δ 7,11 in germacranolides is accompanied by oxidation to produce glaucolide-type germacranolides.[56] This can occur through two possible routes,

1) allylic oxidation followed by rearrangement

2) reaction with oxygen followed by reduction

Scheme 9: Isomerisation of Δ 11,13 to Δ 7,11



2.2.7 Structure determination

Structure elucidation of sesquiterpene lactones is carried out using spectroscopic techniques. UV spectroscopy usually gives end absorption while IR spectroscopy is used for functional group identification.

2.2.7.1 Proton NMR spectroscopy

NMR spectroscopy is the most commonly used technique in structure elucidation of sesquiterpene lactones. A lot of structure information is obtained from ^{1}H NMR spectra together with double resonance experiments. In addition ^{1}H NMR of derivatives such as acetates and use of shift reagents assist in spectra interpretation.

NOE difference spectroscopy has also been applied in structure determination and has proved especially useful in stereochemistry. It has resulted in some configuration reassignment and in laying out of some guidelines on the basic structure of sesquiterpene lactones from Vernonieae.[58]

2.2.7.2 ^{13}C NMR spectroscopy

The requirement of relatively large samples has restricted the availability of ^{13}C NMR spectral data. Information on the various types of carbon present is generally obtained in proton-noise decoupled experiments. This yields information on the number of hydrogen at each carbon atom and also allows the assignment of the carbon atoms.[61]

2.2.7.3 Mass spectroscopy

Mass spectroscopy is usually restricted to finding the parent ion and detection of the ester side chain attached to the main skeleton. In compounds containing hydroxyl or ester functions, the parent ion peak is usually missing due to loss of water or the ester side chain by McLafferty rearrangement.[61] In such cases, the ester group is observed as an intense peak.

2.2.8 Sesquiterpene lactones of the genus *Vernonia*

Sesquiterpene lactones are widely distributed in the Asteraceae family. They have proven to be of considerable taxonomic value in understanding the evolutionary relationship among population, species and genera of this family.[3,56]

Germacranolide type lactones are the largest class of sesquiterpene lactones found in the genus *Vernonia*. Glaucolide-type lactones, which contain an endocyclic Δ7,(11) α,β-unsaturated lactone in which C-13 is oxidized and usually bears an acetate group are characteristic of this genus.[62]

Among the new world species examined so far, there are many reports of glaucolides (Δ4 trans) and hirsutinolides (glaucolides with Δ5 and a 1,4 ether linkage).[62] Guaianolides have also been reported, but non-glaucolide type germacranolides are rare. They are reported in only six out of the 53 of the new world species producing sesquiterpene lactones.[57,62,63,64,65]

By contrast, both glaucolide and non-glaucolide type germacranolides have been reported from the old world species, in addition to eudesmanolides, guaianolides and elemanolides.[62]

It had been observed that primitive species contain germacranolides such as glaucolide A or B, while more recently evolved species contain structurally simpler compounds or lack sesquiterpene lactones.[2,64] Recently minute quantities were isolated from species which had earlier been reported as lacking sesquiterpene lactones.[66] This may be an indication that, for chemotaxonomic purposes, a more careful re-investigation may be necessary in species where no lactones have been reported.

Although sesquiterpene lactones of the new world species had been reported to be sufficiently different from those of the old world species,[2,3] investigation of several South

African species have shown a close relation to the American species.[66,67,68,69] Species from Malawi however gave sufficiently different sesquiterpene lactones.[70]

The big diversity in the genus *Vernonia* and the pronounced difference in the chemistry of different species may be explained by the degree of evolution in the genus.[58]

3. RESULTS AND DISCUSSION

3.1 General extraction

The aerial parts of V. filigera were collected from Gefersa near the water reservoir in November 1989. 200 gm of the air dried plant material was extracted by soaking in petroleum ether: ethyl acetate: methanol (1:1:1) for five days in a fridge. 200 ml methanol was added to the dried extract, shaken overnight and filtered off to remove the fatty materials. After evaporation some benzene was added, swirled and evaporated to remove water.

Application of vacuum liquid chromatography (VLC),column chromatography, preparative thin layer chromatography (pTLC), and centrifugal thin layer chromatography resulted in the isolation of 3-methyl quercetin, vernolepin and vernodalin as detailed in the experimental section.

3.2 Characterisation of 3-methyl quercetin

3-methyl quercetin was isolated as yellow crystals of melting point 262-4°. The UV spectrum (MeOH) gave two peaks assigned as band II, 258 nm and band I, 360 nm.

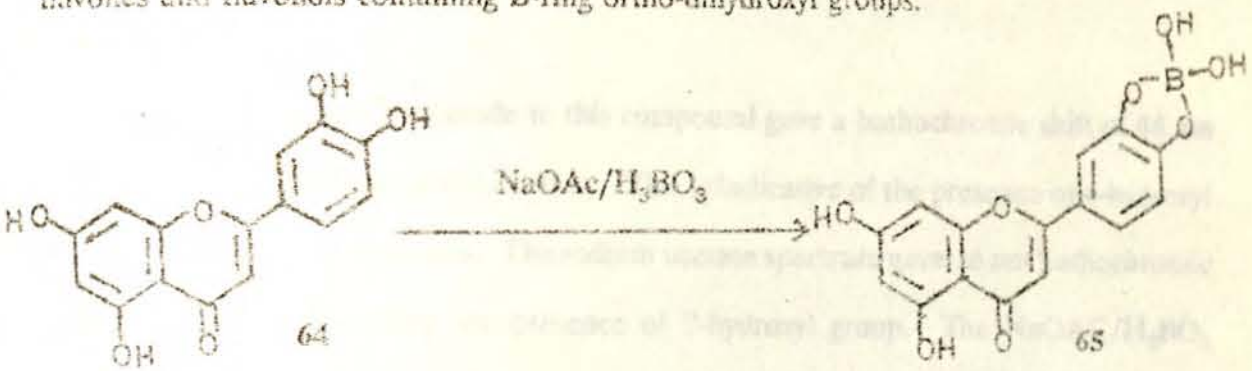
Shift reagents are very useful in assisting the determination of the oxygenation pattern of flavonoids. The reagents used include NaOMe, NaOAC, $NaOAC/H_3BO_3$, $AlCl_3$, $AlCl_3/HCl$.[23,39]

NaOMe is a strong base and ionoses to some extent all phenolic hydroxyl groups.[23] It is therefore a good "fingerprint" indicator of the hydroxylation pattern of the flavonoid. For flavones and flavonols, a bathochromic shift of 40-65 nm in band I is diagnostic of the presence of 4^1-hydroxyl group.[39]

Degradation of the spectrum with time is a good indicator of alkali sensitive groups.[23]

NaOAc is a weaker base than NaOMe and ionises only the most acidic of the flavonoid hydroxyl groups (3, 7 or 4'-OH). It is used primarily for the detection of 7-hydroxyl groups. In flavones and flavonols with 7-hydroxyl groups, it causes a bathochromic shift of 5-20 nm in band II.

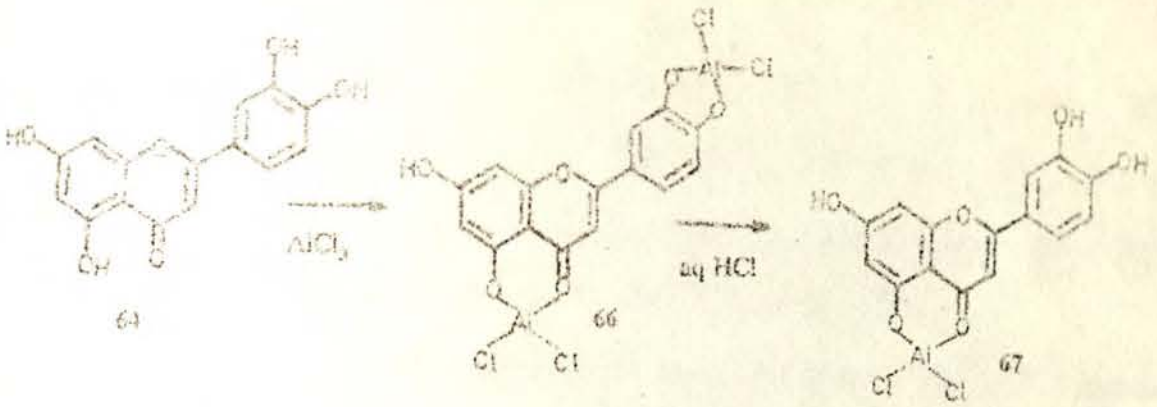
Boric acid chelates two ortho-hydroxyl groups in presence of NaOAc, resulting in a bathochromic shift. A bathochromic shift of 12-30 nm in band I is observed for flavones and flavonols containing B-ring ortho-dihydroxyl groups.



64 65

Aluminium chloride forms acid-labile complexes with ortho-dihydroxyl groups and acid stable complexes with hydroxyl groups at C-3 or C-5.[23,39] An $AlCl_3$ spectrum therefore represents the total effect of all the complexes while the $AlCl_3$/HCl spectrum represents only the effects of the hydroxyl-keto complex due to the acid stability of the complex between the C-4 keto function and C-3- or C-5-hydroxyl group.[39]

A hypsochromic shift of the $AlCl_3$ spectrum (of about 30-40 nm) is observed in band I due to the decomposition of the ortho-dihydroxyl complex when HCl is added. Any shift remaining after the addition of HCl is due to the presence of 3- or 5-hydroxyl groups. Otherwise the original methanol spectrum should be obtained.[39]



Addition of sodium methoxide to this compound gave a bathochromic shift of 48 nm in band I without changing the intensity. This is indicative of the presence of 4-hydroxyl group in the flavonoid nucleus. The sodium acetate spectrum gave 16 nm bathochromic shift of band II indicating the presence of 7-hydroxyl group. The $NaOAC/H_3BO_3$ spectrum showed a bathochromic shift of 18 nm suggesting the presence of ortho-dihydroxyl groups in ring B. Comparison of the $AlCl_3$, $AlCl_3$/HCl and methanol spectra showed presence of 5-hydroxyl group and ortho-dihydroxyl groups in ring B.

The IR spectrum (KBr) showed peaks at 3440 and 3200 cm^{-1} indicative of the presence of hydroxyl groups. A peak at 1660 cm^{-1} was attributed to the presence of a chelated keto group, confirming the presence of 5-hydroxyl group which is chelated to the C-4 keto group. The aromatic peaks were observed at 1610, 1560,1500 and 1450 cm^{-1}.

The 90 MHz ^1H NMR spectrum was taken in acetone-d_6/MeOD mixture. This showed broad singlets at 6.18 and 6.4 ppm intergrating for one proton each. This corresponds to H-6 and H-8 of flavones respectively. A one proton doublet at 6.9 ppm corresponds to H-5^1 while the one-proton doublet at 7.53 ppm was assigned to H-6^1.

A broad singlet at 7.65 ppm overlapping the doublet at 7.53 ppm was assigned to H-2^1. A three-proton broad singlet at 3.8 ppm was attributed to the presence of a methoxy group.

A 300 MHz ^{1}H NMR spectrum ran in $CDCl_3$/DMSO-d_6 gave a one-proton singlet at 12.5 ppm confirming the presence of 5-OH. Other signals were found to agree with the above observations as follows, 7.45 (d, 1H, H-2^1) , 7.32 (dd, 1H, H-6^1), 6.75 (br d, 1H, H-5^1), 6.18(d, 1H, H-8), 6.05 (d, 1H, H-6), 3.62 (s, 3H, OCH_3).

The ^{13}C NMR of this compound gave 16 carbon atoms. The DEPT spectrum showed presence of 5 methine groups (CH), two of which are equivalent, one methoxy (OCH_3) and 10 quartenary carbons.

The ^{13}C assignment of the carbon shifts of flavonoids is based on the use of flavone as a model and additive substituent parameters for benzene. Presence of a C-3 methoxy was supported by the ^{13}C in which the methoxy signal appeared downfield at 60.5 ppm. Methoxy carbons usually resonate at 55-56.5 ppm. However a downfield shift to the range 59.5-63.0 ppm is observed when the methoxy group is di-ortho substituted by a bulky substituent like -OH, -OMe or a ring junction.

The ^{1}H NMR and ^{13}C NMR support the structure of 3-methyl quercetin 68.

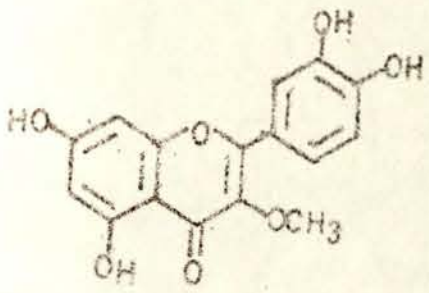


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Table 2: ^{1}H NMR spectrum for 3-methyl quercetin (300 MHz, $CDCl_3$/DMSO-d_6

	ppm	
H-5	12.5	s,1H
H-6	6.05	d,1H
H-8	6.18	d,1H
H-2^1	7.45	d,1H
H-5^1	6.75	br d,1H
H-6^1	7.32	dd,1H
OCH_3	3.62	s,1H

Table 3: ^{13}C NMR Chemical shift assignment for 3-methyl quercetin (90 MHz, acetone d_6/MeOD)

	ppm
C-2	158.2
C-3	139.7
C-4	179.9
C-5	163.0
C-6	99.8
C-7	165.5
C-8	94.8
C-9	157.5
C-10	106.1
C-1^1	123.2
C-2^1	116.6
C-3^1	146.3
C-4^1	149.6
C-5^1	116.6
C-6^1	122.4
OCH_3	60.5

3.3 Characterization of vernolepin

Vernolepin was isolated as colourless crystals of melting point 178-179° (lit.179-180). It gave optical rotation $[\alpha]_D$ +87 (c 1.15, acetone). UV spectrum (MeOH) gave an end absorption at 218 nm.

Infrared spectrum (KBr) exhibited bands at 3580 and 3490 cm^{-1} characteristic of hydroxyl groups (OH), 2940 cm^{-1} attributed to unsaturated methylene (=CH_2), 1760 cm^{-1} indicative of ɣ- lactone and 1720 cm^{-1} attributed to a δ-lactone. A peak at 1640 cm^{-1} was attributed to a carbon-carbon double bond.

The ^{13}C NMR showed presence of fifteen carbon atoms indicating the compound to be a sesquiterpene. Two of these are carbonyl carbons. Distortionless enhancement by polarization transfer (DEPT) spectrum indicated that there were 3 quartenary carbons, 5 methine (CH) and 5 methylene (CH_2) groups. These were shown to include 3 terminal methylenes (=CH_2), one double bonded methine (=CH) and two double bonded quartenary carbons.

Chemical ionization mass spectra (CIMS) gave a molecular ion peak of m/z 277 $(M+1)^+$ corresponding to the molecular formular $C_{15}H_{15}O_5$. No major fragments were observed since this is a low energy fragmentation.

The 300 MHz ^1H NMR spectrum exhibited a pair of doublets, one proton each, at 6.2 and 6.01 ppm (J=3.3 Hz). These are indicative of the presence of a methylene group exocyclic to a -lactone. A pair of broad singlets at 6.7 and 5.92 ppm, each for one proton was attributed to the presence of a second methylene group exocyclic to another lactone.

A typical feature of ^1H NMR spectrum of sesquiterpene lactones containing an α-methylene-δ-lactone moiety is the appearence of two doublets (J=1-4 Hz), usually

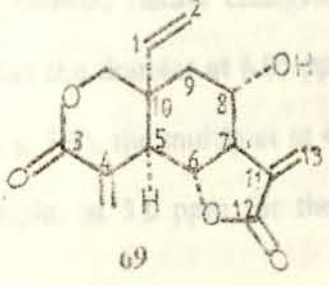
one below and the other above 6.0 ppm.[61] These are due to the two C-13 methylene protons. The low field absorption is due to the proton oriented towards the lactone carbonyl group. The coupling is due to allylic interaction between the protons at C-7 and the C-13 methylene protons.

Geminal coupling (J=0.7-2.0 Hz) between the two C-13 protons together with a paramagnetic shift of H-13a to below 6.0 ppm is observed in germacranolides which contain an α-hydroxyl group at C- 8 in 6,12-lactonised compounds. These are attributed to Van der waals proximity effects of the hydroxyl group upon the bonding orbitals of H-13a. The effects can be helpful in stereochemical and conformational assignments.[61]

Other olefinic proton signals were exhibited at 5.71 ppm (m, 1H), and 5.26 ppm (m, 2H). These were attributed to the presence of an ethylene group. Other proton signals were exhibited at 4.38 (d, 1H), 4.19 (dd, 1H), 4.04 (m, 1H), 3.91 (t,1H) 3.15 (d,1H), 2.65 (tt,1H), 2.24 (d,1H), 1.95 (dd,1H), and 1.64 (m,2H) ppm.

Comparison of 90 MHz ^{1}H NMR spectrum of this compound in deuterated chloroform with that reported by Kupchan et al[8,10] and Laekeman et al[71] for vernolepin 69, at 100 MHz using the same solvent gave a good agreement except for the two protons at C-15. These are reported appearing as a doublet (J=1 Hz) for each proton while in this work they appeared as broad singlets.

The 300 MHz ^{1}H NMR spectrum was also in good agreement with the above two, but gave better resolution for each individual proton, although it also gave singlets for the C-15 protons.



69

Some decoupling experiments were also undertaken. Irradiation of H-8 (4.04 ppm) showed it to be coupled to the triplet at 2.65 ppm (H-7) which was reduced to a doublet, the doublet at 2.24 ppm (OH) which was reduced to a singlet, the double doublet at 1.95 ppm (H-9a) which was reduced to a doublet and the multiplet at 1.64 ppm (H-9b) which was reduced to a triplet.

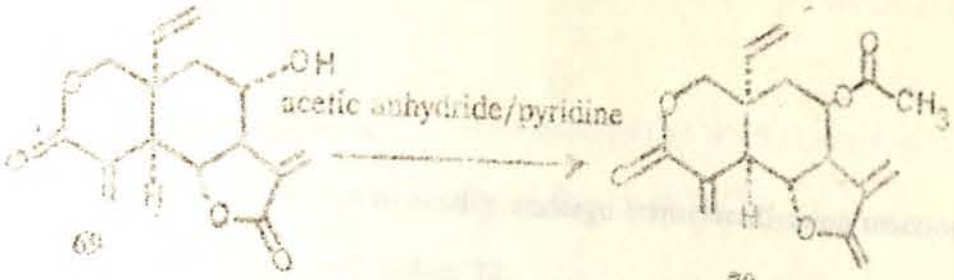
Irradiation of H-5 (3.15ppm) showed it to be coupled to the triplet at 3.91 ppm (H-6) which was reduced to a doublet.

Irradiation of H-9a (1.95 ppm) showed it to be coupled to the multiplet at 4.04ppm (H-8), the doublet at 2.24 ppm (OH) and the multiplet at 1.64 ppm (H-9b)

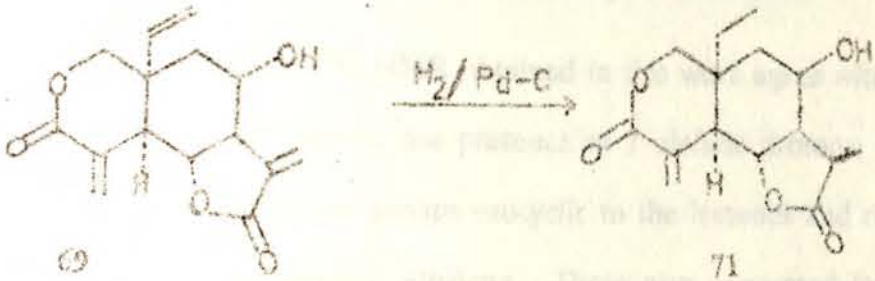
Kupchan et al[9,10] showed vernolepin to be a sesquiterpene dilactone by carring out the lactone-titration experiment. A solution of the lactone in 1.0 N aqueous sodium hydroxide is evaporated to dryness under reduced pressure and the residue dissolved in water and titrated with acid.[37] Vernolepin consumed 2 mole equivalent of base, indicating presence of two lactone functional groups.

Kupchan et al[9,10] also reported that acetylation of vernolepin resulted in a product (70) whose 100 MHz ^{1}H NMR gave a multiplet at 4.5- 4.0 ppm integrating for 3 protons (compared to 4 for vernolepin) while a new one proton multiplet was observed at 5.1 ppm for the proton on the acetate bearing carbon. The doublet at 6.03 ppm (H-13a) also shifted to 5.62 ppm and the acetate peak appeared as a singlet (3H) at 2.12 ppm. There was no D_2O exchangeable signals as had been observed for vernolepin.

Acetylation in this work showed similar changes. The 90 MHz ^{1}H NMR of the acetylated product showed that the doublet at 6.05 ppm shifted to 5.6 ppm, an acetate peak was observed (2.1 ppm, s, 3H), the multiplet at 4.2 ppm intergrated for 3 protons while there was a new multiplet at 5.0 ppm for the proton on the acetate bearing carbon.



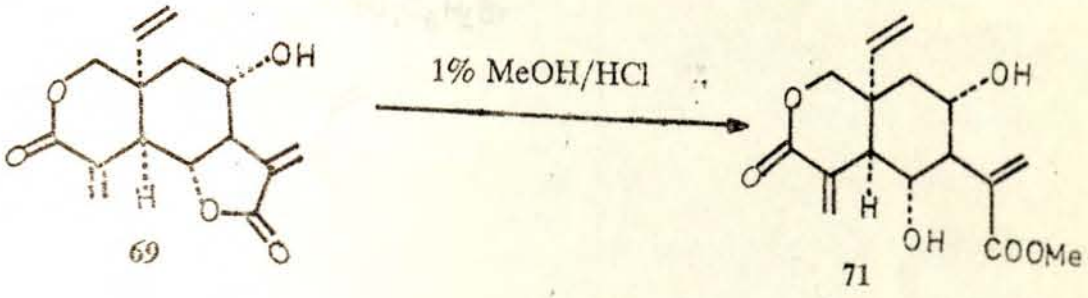
Hydrogenation of vernolepin by Kupchan et al [9, 10] with 10% palladium-on-charcoal catalyst was terminated after the rapid uptake of 2 mole equivalent of hydrogen to give hydrovernolepin **71.**



The ^{1}H NMR of 71 showed signals for only two protons in the vinyl region (d, J = 1 Hz, 6.7 and 5.9 ppm) indicating that 71 had only the double bond exocyclic to the second lactone (the δ-lactone). The doublets for the methylene conjugated to the γ-lactone were replaced by a 3 proton doublet at 1.39 ppm (J = 7 Hz) for the newly formed methyl group. Disappearance of the 3 proton multiplet at 5.4 ppm was interpreted as indicative of the presence of a monosubstituted double bond. This was supported by the appearance of a distorted 3 proton triplet (J = 8 Hz) at 0.92 ppm, indicative of an ethyl group. This implied the presence of a monosubstituted ethylene in vernolepin,

suggesting an elemane skeleton.

Vernolepin was also found to readily undergo transesterification reaction in acidic methanol to give the methanol adduct 72.



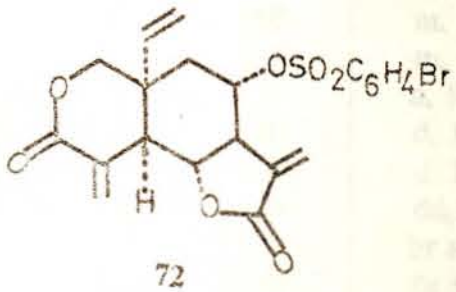
69 71

The ^{1}H NMR and the ^{13}C NMR obtained in this work agree with the vernolepin structure. The ^{1}H NMR showed the presence of 7 olefinic protons; 4 of these were assigned to the two methylene groups exocyclic to the lactones and the other 3 were assigned to the monosubstituted ethylene. These also accounted for the 3 olefinic methylene and one olefinic methine signals in the ^{13}C NMR. A doublet at 4.38 ppm (1H, J=2.09 Hz) was assigned to H-14a while a doublet at 4.19 ppm (1H, J=2 Hz) was assigned to H-14b. This coupling pattern results from the two protons coupling with each other.

From the irradiation experiments the signal at 4.04 ppm (m, 1H) was assigned to H-8. This appears as a multiplet due to interaction with the two protons at C-9 and the proton at C-7. The triplet at 3.91 ppm was assigned to H-6. H-6 occurs as a triplet due to coupling with H-5 and H-7 which are equivalent. H-7 appears as a triplet of a triplet (2.65 ppm) due to coupling with H-6 and H-8 and the spartial influence of the C-13 methylene protons.

The ^{13}C NMR data of vernolepin is reported here for the first time. Assignment of the carbon shifts was done by comparison with the ^{13}C NMR of other non-glaucolide germacranolides.

The stereochemistry of vernolepin was astablished by McPhail and Sim using X-ray crystallography of vernolepin bromobenzenesulfonic acid **73**.[93]



72

This was prepared by treatment of vernolepin with p-bromobenzenesulfonyl chloride in pyridine at room temperature.

Vernolepin has previously been isolated from *V. hymenolepis*,[9,10] *V. amygdalina*,[12] and *V. guineensis*.[72] It has also been reported to have cytotoxic and antitumor activity.[9,10,12]

Table 4: ^{1}H NMR spectral data for vernolepin (300 MHz, $CDCl_3$)

	ppm	
H-1	5.71	m, 1H
H-2	5.26	m, 2H
H-5	3.15	d, 1H
H-6	3.91	t, 1H
H-7	2.65	tt,1H
H-8	4.04	m, 1H
H-9a	1.95	m, 1H
H-9b	1.64	m, 1H
H-13	6.2	d, 1H
H-13	6.01	d, 1H
H-14	4.38	d, 1H
H-14	4.19	dd,1H
H-15	6.7	br s,1H
H-15	5.92	br s,1H

Table 5: ^{13}C NMR spectral data for vernolepin (300 MHz, $CDCl_3$)

	ppm
C-1	140.2
C-2	116.7
C-3	163.4
C-4	130.2
C-5	46.7
C-6	78.3
C-7	53.3
C-8	66.9
C-9	43.5
C-10	41.1
C-11	136.5
C-12	169.0
C-13	121.7
C-14	70.9
C-15	135.7

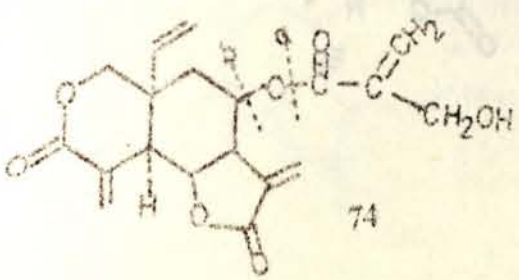
3.4 Characterization of vernodalin

Vernodalin was isolated as a colourless amorphous solid. It gave optical rotation $[\alpha]_D$ +118 (c 0.055, $CHCl_3$). UV gave an end absorption at 212 nm.

The infrared spectra ($CHCl_3$) exhibited bands at 3600 cm^{-1} characteristic of hydroxyl group (OH), 3070 cm^{-1} and 1640 cm^{-1} attributed to carbon-carbon double bond, a peak at 1790 cm^{-1} suggestive of an α,β-unsaturated γ-lactone and at 1740 cm^{-1} suggestive of the presence of a second lactone. The IR spectrum was similar to that of vernolepin.

The ^{13}C NMR indicated the presence of 19 carbon atoms, suggesting that the basic skeleton is that of a sesquiterpene. It showed 3 of the carbons as carbonyl groups. The DEPT spectrum showed the presence of 7 methylene (CH_2), 5 methine (CH) and 3 quaternary carbons. Four of the methylene carbons were found to be terminal methylene ($=CH_2$), one methine (=CH-) and three quaternary carbons are also double bonded.

CIMS gave a molecular ion peak at m/z 361 $(M+1)^+$, which was also the base peak. This is consistent with the molecular formular $C_{19}H_{20}O_7$. Other peaks included; M+1/z (relative intensity) 277 (40%), 103 (10%), and 85 (5%). The peak at 277 was taken to represent the loss of an ester side chain ($C_4H_4O_2$) leaving an ion similar to vernolepin. This was confirmed by the presence of a peak at m/z 85 according to fragmentation a. A second peak at m/z 103 was attributed to the loss of $C_4H_7O_3$ according to fragmentation b.

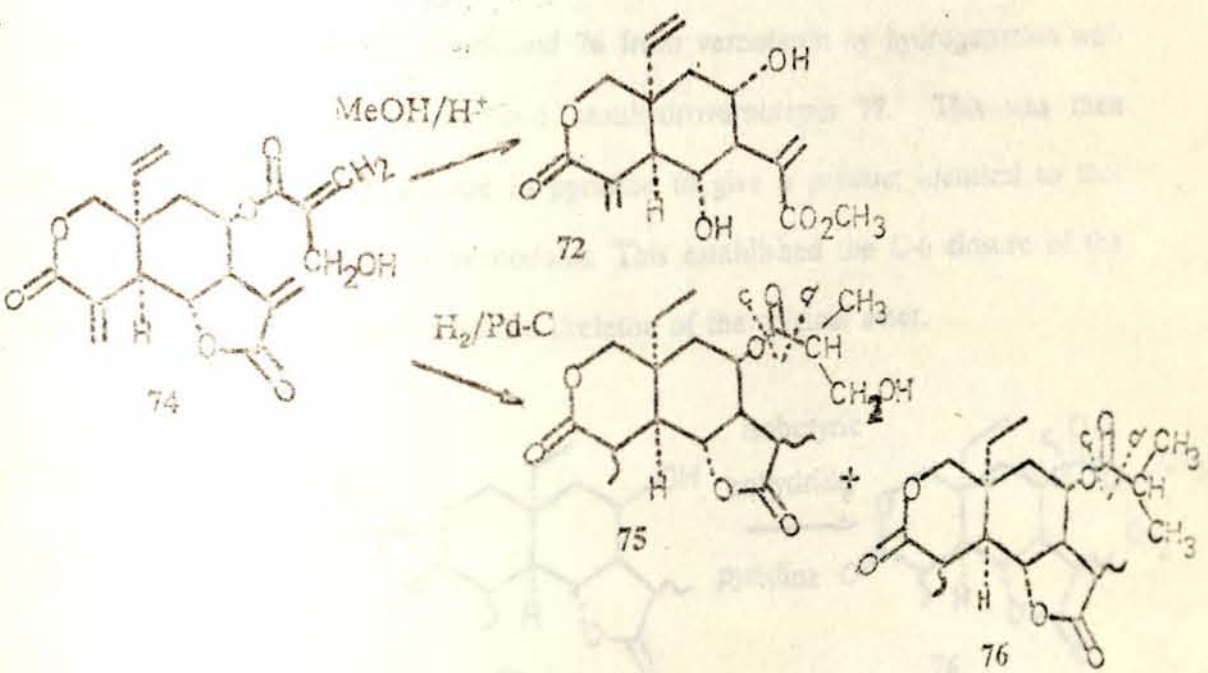


74

The 300 MHz ^{1}H NMR gave a pair of doublets , one proton each, at 6.21 and 5.63 ppm (J=3.2 Hz) indicating the presence of a methylene exocyclic to a γ-lactone. A pair of broad singlets at 6.7 and 5.9 ppm, one proton each, showed the presence of a methylene conjugated to a second lactone.

Comparison of the ^{1}H NMR obtained for vernodalin 74 with that obtained for vernolepin 69 suggested that both had similar sesquiterpene lactone skeleton. Vernodalin was suspected to be an ester of vernolepin. Vernodalin spectrum showed extra peaks at 6.3 ppm (br s, 1H), 5.92 ppm (d, 2H, one of which was assigned to H-15), 4.37 ppm (d, 2H) and a triplet at 2.2 ppm attributed to a hydroxyl group. These signals correspond to those of hydroxymethacrylate side chain. This was confirmed by the presence of a peak at m/z 85 in the mass spectrum.

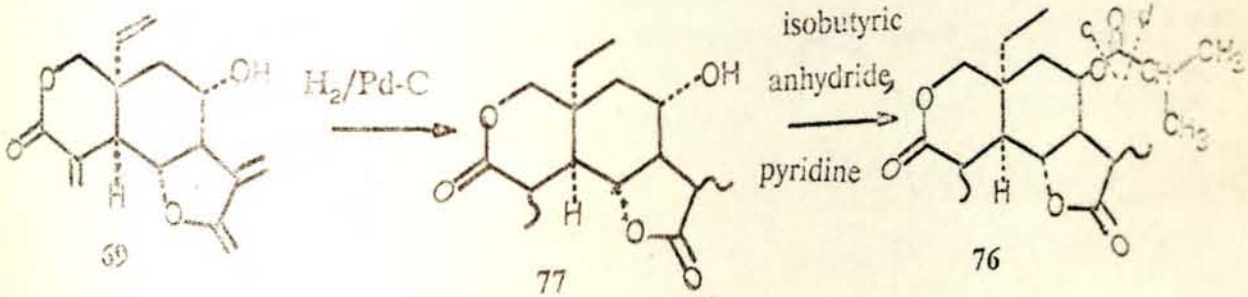
Kupchan et al [11] observed that hydrolysis of vernodalin with acidic methanol yielded an adduct 72 identical with that obtained from the hydrolysis of vernolepin. This confirmed the suspected similarity of their sesquiterpene lactone skeletones.



Kupchan et al[71] and Toubiana et al[82] showed that hydrogenation of vernodalin in methanol with 10% palladium-on-charcoal yielded a mixture of two products 75 and 76. The major product was the octahydro-derivative 75. It's ^1H NMR showed no signals for olefinic protons but had new signals at higher field. A triplet at 0.92 ppm (3H) similar to that of tetrahydrovernolepin 71 indicated an ethyl group between C-1 and C-2. Three-proton doublets at 1.40, 1.28 and 1.18 ppm corresponded to secondary methyl groups formed by reduction of the two double bonds exocyclic to the lactones and that present on the ester. The mass spectrum of 75 gave peaks at m/z 59 and 87 due to fragmentations c and d, confirming the change in the hydroxymethacrylate side chain.

The second hydrogenation product 76 gave peaks in the mass spectrum at m/z 43 and 71 corresponding to fragmentation c and d. These together with a molecular ion peak at m/z 352 indicated that the hydroxyl group of the side chain had been hydrogenolysed to give an isobutyrate ester. Similar hydrogenation results are also reported by Toubiana et al.[72]

Kupchan et al[71] prepared compound 76 from vernolepin by hydrogenation with palladium-on-charcoal catalyst to yield hexahydrovernolepin 77. This was then acetylated with isobutyric anhydride in pyridine to give a product identical to that obtained from hydrogenation of vernodalin. This established the C-6 closure of the lactone in vernodalin and confirmed the skeleton of the original ester.



59 77 76

Comparison of ^1H NMR and evidence of ^{13}C show that the compound is vernodalin. Vernodalin has been previously isolated from *V. amygdalina*[11] and *V. guineensis*.[72] It has been reported to have anti-tumor activity.

Table 6: ^1H NMR spectrum for vernodalin (300 MHz, $CDCl_3$)

	ppm	
H-1	5.74	m, 1H
H-2	5.33	m, 2H
H-5	3.0	m, 2H
H-6	4.04	t, 1H
H-7	3.0	m, 2H
H-8	5.18	m, 1H
H-9a	2.0	t,1H
H-9b	1.65	m, 1H
H-13a	6.22	d, 1H
H-13b	5.63	d, 1H
H-14	4.48	d, 1H
H-14	4.28	dd,1H
H-15	6.7	br s 1H
H-15	5.95	m, 2H
H-3'	4.37	d, 2H
H-3'	4.37	d, 2H
H-4'	6.3	br s,1H
H-4'	5.95	m, 2H
OH	2.2	t, 1H

Table 7: ^{13}C NMR spectral assignment for vernodalin (90 MHz, $CDCl_3$)

	ppm
C-1	140.1
C-2	116.7
C-3	163.3
C-4	130.7
C-5	47.0
C-6	78.0
C-7	50.4
C-8	69.0
C-9	38.9
C-10	41.1
C-11	139.5
C-12	168.4
C-13	121.0
C-14	70.9
C-15	134.8
C-1'	165.1
C-2'	136.0
C-3'	61.7
C-4'	126.5

4. EXPERIMENTAL

4.1 Plant material

Vernonia filigera is a plant distinctly endemic of Ethiopia. It has been reported to occur in Shoa, Welega, Gondar and Godjam administrative regions. The aerial parts of *V. filigera* were collected from Gefersa near the water reservoir, about 80 Km south of Addis Ababa, in November 1989. The plant was identified by Dr. Mesfin Tadesse and Mr. Mike Gilbert at the National Herberium, Addis Ababa University. A voucher specimen is deposited at the Herberium. After collection, the plant material was air dried and then extracted.

4.2 Materials

The melting points were determined on Karl Kolb D-6072 rapid melt and are not corrected. UV was recorded on UV Beckman DU-65 spectrophotometer. IR was recorded on a Perkin-Elmer 727B infrared spectrophotometer. Optical rotation was determined on a Perkin-Elmer 241 polarimeter. Proton NMR and ^{13}C NMR were determined on a Joel 90 MHz spectrophotometer and also on a 300 MHz. Mass spectrum was recorded on a chemical ionization mass spectrophotometer.

Analytical TLC was done on 0.2 mm thick layer of silica gel 60 F_{254} precoated sheets (Merck). Detection was done using vanillin/sulfuric acid and heat or using UV. Preparative thin layer chromatography (pTLC) was carried out using 2 mm thick layer of silica gel $HF_{254+366}$ (Merck) and detection under UV. Column chromatography was carried out using silica gel 60 (70-230 mesh) and also sephadex LH 20. Centrifugal thin layer chromatography (chromatotron) was carried out using 2 mm thick layer of silica gel 60 PF_{254} (Merck).

4.3 Extraction and purification

The air dried plant material (200 g) was extracted by soaking in 3 litres of petrol: Diethyl ether: methanol (1:1:1) in a fridge for five days. The extract was evaporated under vacuum. Methanol (200 ml) was added and shaken overnight. The mixture was filtered and the filtrate evaparated under reduced pressure. Benzene (25 ml) was added, swirled and evaporated. This yielded 14 g of the crude extract.

The crude extract (8 g) was adsorbed on 30 g silica gel. This was applied to 300 g silica gel and vacuum liquid chromatography (VLC) carried out using petrol: ethyl acetate (9:1), (1:1), ethyl acetate (100%) and ethyl acetate: methanol (9:1). A total of 25 fractions of 200 ml each were collected. These were combined on the basis of TLC to yield 6 fractions. Fractions 1,2,3 and 6 were not analysed further.

Table 8: Solvent systems used for column chromatography, preparative and centrifugal thin layer chromatography

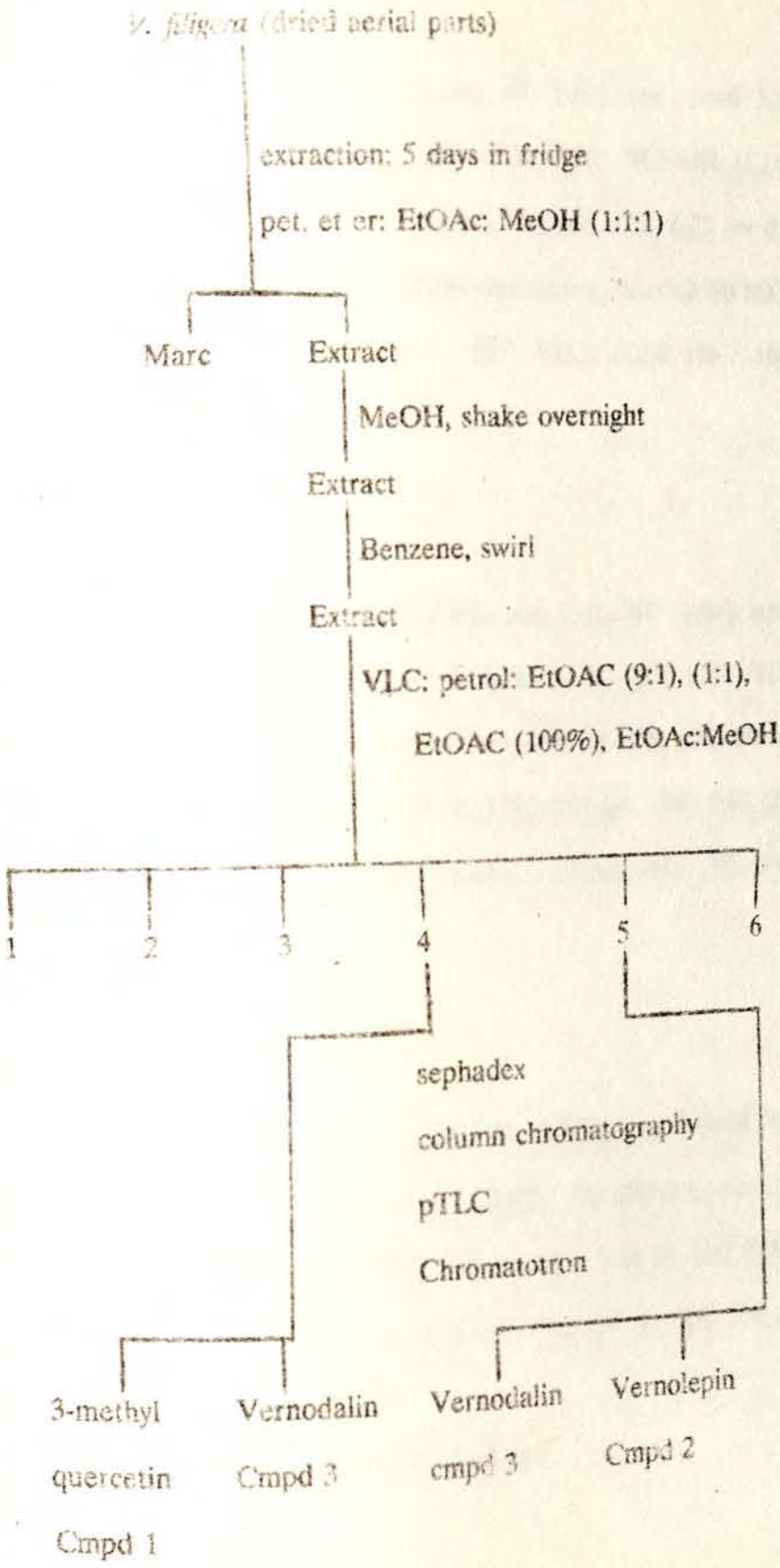
No	solvent systems	ratio
1	chloroform : methanol	2:1
2	chloroform : methanol	9:1
3	petrol: ethyl acetate	1:1
4	benzene : ethyl acetate	3:2
5	benzene : acetone	4:1
6	dichloromethane : ethyl acetate	4:1

Fraction 4 was passed through sephdex LH-20 column using solvent system 1. It yielded 4 fractions. The first of these fractions, AK-31-6, was passed twice through a silica gel column using solvent system 5 and then through sephadex LH-20 using solvent system 1 to give AK-38-11. This was passed through chromatotron twice using solvent system 2 to give 70 mg of compound 3 which was identified as vernodalin.

The second fraction obtained after sephadex LH 20 elution of fraction 4, AK-31-9, was passed through sephadex a second time to give a terpene fraction and a flavonoid fraction, AK-32-14. The terpene fraction was similar to AK-38-11. AK-32-14 was purified by preparative TLC using solvent system 2 to give compound 1 which was identified as 3-methyl quercetin.

Fraction 5 was passed through sephadex LH-20 using solvent system 1 to yield 3 fractions. The first of these, AK-31-11, was eluted through a silica gel column using solvent system 3 to give AK-32-4 and AK-32-17. Purification of AK-32-4 by several column chromatographic steps on silica gel and pTLC yielded AK-37-2 which was similar to compound 3. Purification of AK-32-17 using similar procedures yielded compound 2 which was identified as vernolepin.

OUTLINE OF THE EXTRACTION



4.4 Physical and data

Compound 1

M.P. 262-4°. UV (MeOH): 258, 360 nm; IR (KBr): cm^{-1}; 3440, 3200, 1660, 1610, 1560, 1510, 1500, 1450, 1370, 1310, 1280, 1220, 1180; ^1H NMR: ($CDCl_3$/DMSO-d_6, 300 MHz), ppm; 12.5 (s, 1H), 7.45 (d,1H),7.32 (dd, 1H), 6.75 (br d, 1H), 6.18 (d, 1H), 6.05 (d,1H), 3.62 (s, 3H); ^{13}C NMR (acetone-d_6/MeOD, 90 MHz), ppm; 179.9, 165.5, 163.0, 158.2, 157.5, 149.6, 146.3, 139.7, 123.2, 122.4, 116.7, 106.1, 99.8, 94.8, 60.5,

Compound 2

M.P. 178-9°, lit. 179-180°; UV (MeOH): nm, 218; IR: (KBr), cm^{-1}; 3580, 3490, 2940, 1760, 1720, 1640, 1490, 1410, 1370, 1280, 1170, 1050, 970; ^1H NMR: ($CDCl_3$ 300, MHz), ppm; 6.7 (br s, 1H), 6.2 (d, 1H), 6.01 (d, 1H), 5.92 (br s, 1H), 5.71 (m, 1H), 5.26 (m, 2H), 4.38 (d, 1H), 4.19 (d, 1H), 4.04 (m, 1H), 3.91 (t,1H), 2.95 (d, 1H), 2.65 (t, 1H) 2.24 (t, 1H), 1.95 (dd,1H), 1.64 (m, 1H); ^{13}C NMR (300, MHz, $CDCl_3$): See Table 9.

Compound 3

Amorphous solid; UV (MeOH),nm, 212; IR: ($CHCl_3$), cm^{-1}, 3600, 3050, 1790, 1740, 1640, 1410, 1300, 1260,1170; ^1H NMR: ($CDCl_3$, 300 MHz), ppm; 6.7 (br s, 1H), 6.3 (br s,1H), 6.21 (d, 1H), 5.95 (m, 2H), 5.74 (m, 1H), 5.63 (d, 1H), 5.33 (m, 2H), 5.18 (m, 1H), 4.48 (d, 1H), 4.37, (d,2H), 4.28 (dd,1H), 4.04 (t, 1H), 3.0, (m, 2H), 2.2 (t, 1H) 2.0 (t, 1H), 1.65 (m,1H)

^{13}C NMR: see Table 10 CIMS: M+1/Z 361

4.5 Acetylation of compound 2

10 mg of compound 2 was dissolved in 20 ml of acetic anhydride and 2 drops of pyridine were added. The mixture was stirred at room temperature for 6 hours. TLC of the mixture showed a higher RF (solvent system 3).

The mixture was poured into crushed ice, shaken and left overnight to hydrolyse the acetic anhydride. Sodium hydrogen carbonate ($NaHCO_3$) was added until bubbling ceased. The mixture was extracted 3 times with chloroform. The chloroform extracts were combined and evaporated under vacuum. The residue was applied on pTLC and developed using solvent system 3. This yielded the acetylated product.

1H NMR: (CDCl3, 90 MHz), 6.7 (br s, 1H), 6.25 (d, 1H), 6.05 (d,1H), 5.95 (br s,1H), 5.4 (m, 4H), 4.2 (m, 3H), 2.95 (m, 2H), 2.1 (s,3H), 1.6 (m, 2H).

4.6 Demethylation of compound 1

5 mg of compound 1 was mixed with 10 ml of 48% hydrobromic acid. This was refluxed for three hours. TLC spotting using solvent system 2 showed decreased RF. Seperation of the product proved difficult since the mixture was miscible with ethyl acetate. Some water was added and the mixture was extracted with ethyl acetate. This was dried with sodium sulphate and then evaporated. Co-spotting the product with quercetin gave similar RF using solvent system 2.

Fig. 1 ^{1}H NMR spectrum of vernodalin

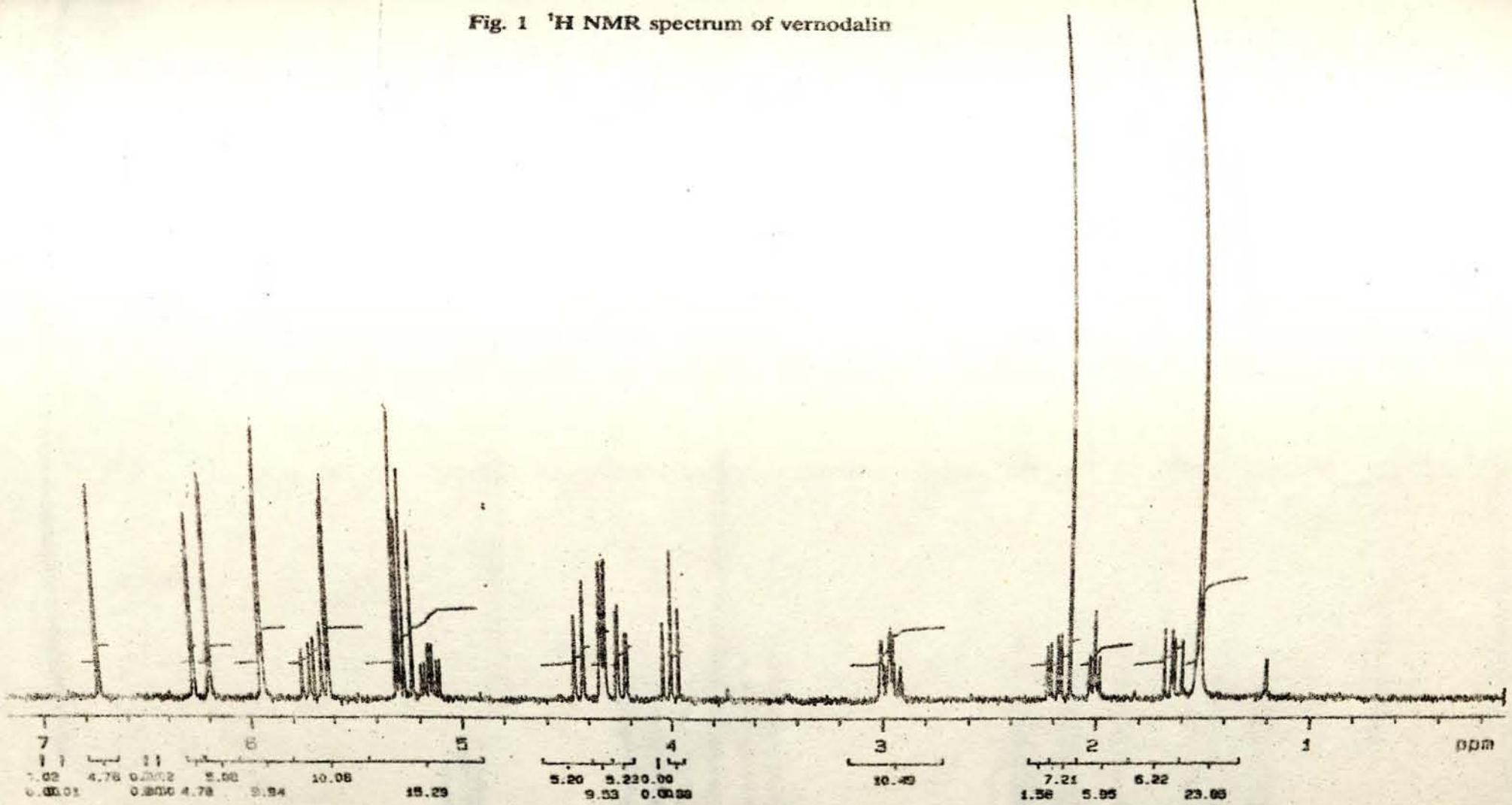
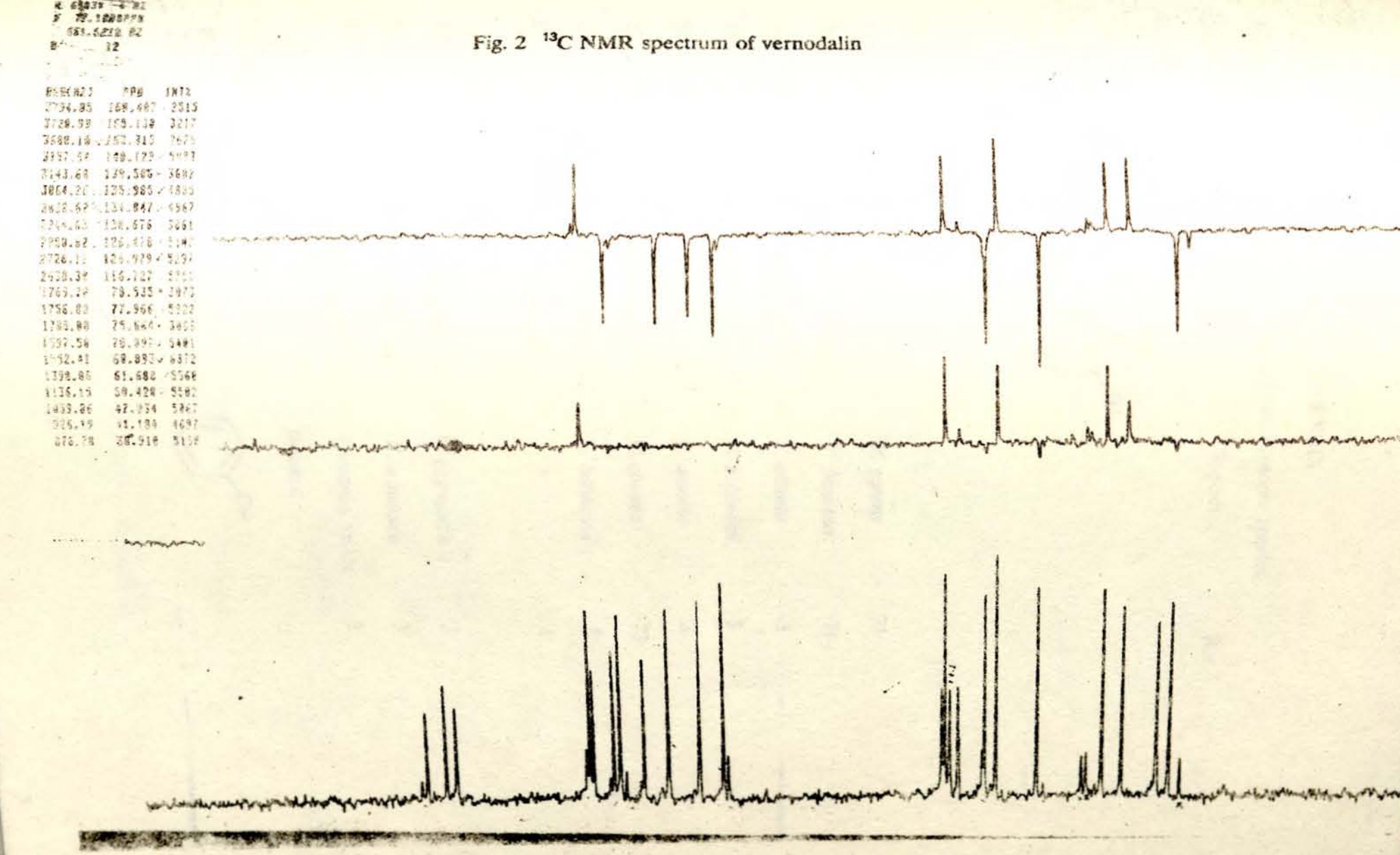
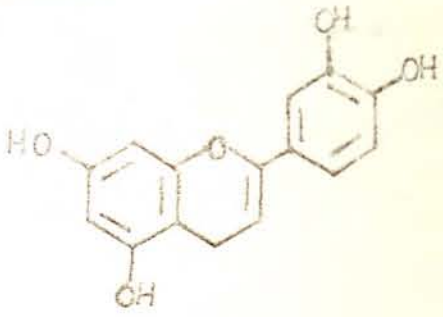
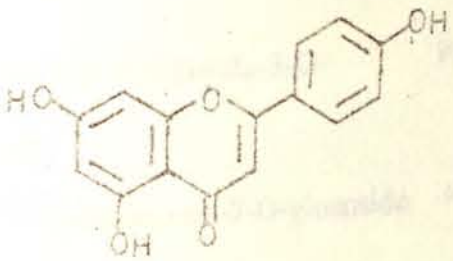


Fig. 2 ^{13}C NMR spectrum of vernodalin



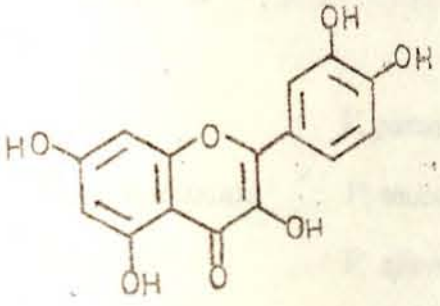
APPENDIX

Appendix 1: Some flavonoids isolated from *Vernonia* species

Cmpd no.	Cmpd name	Species	Ref.
Luteolin derivatives			
			
64	Luteolin	*V. patens*	73
78	Luteolin-7,3^1-dimethyl ether	*V. flexuosa*	74
79	Luteolin-7,3,-dimethyl ether	*V. acaulis*	3
80	Luteolin-4-methyl ether	*V. baldwinii*	3
81	Luteolin-7-methyl ether	*V. acaulis*	3
82	Luteolin-7-O glucoside	*V. cinerea*	73
83	Luteolin-7-O-arabinoglucoside	*V. baldwinii*	3
84	Luteolin-4^1-O-glucoside-7-O-galactoside	"	3
85	Luteolin-7-O-galactoglucoside	*V. capreaefolia*	3
86	Luteolin-7-O-glucoglucosode	*V. arbuscula*	3
87	Luteolin-6- and 8-C glucosides	*Vernonia*, series *flexuosa*	3
Apigenin derivatives			

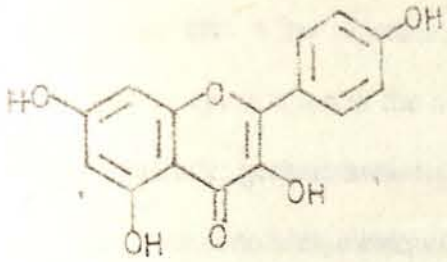
88	Apigenin	*V. angustifolia*	3
89	Apigenin-7-methyl ether	*V. arkansana*	92
90	Apigenin- 7-O-glucoside	*V. fasciculata*	3
91	Apigenin-7-methyl ether-4-glucogalactoside	"	75

Quercetin derivatives



92	Quercetin	*V. patens*	73
68	Quercetin-3-methyl ether	"	73
93	Quercetin-7,3,4^1-trimethyl ether	*V. brefolia*	3
94	3-O-acyl quercetin-7,3^1-dimethyl ether	"	3
95	Quercetin-3-O-glucoside	*V. cinerea*	3
96	Quercetin-3-O-rhamnoglucoside	*V. afromanta*	3
97	Quercetin-7-O-glucoside-3-O-rhamnoside	*V. abyssinica*	3
98	Quercetin-7-O-diglucoside-3-O-glucoside	*V. brevifolia*	3
99	Quercetin-7-O-diglucoside-3-O-diglucoside	*V. brevifolia*	3
100	3,3^1-O-diacylquercetin-7-O-glucoside	*V. nudiflora*	3

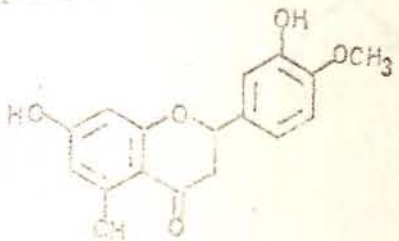
Kaempferol derivatives



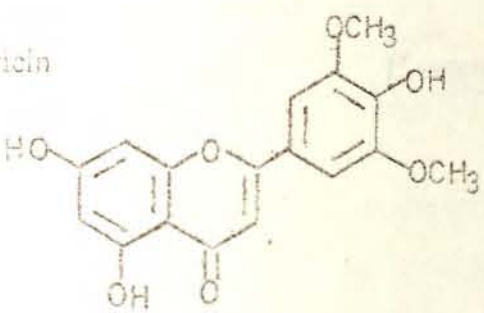
55	Kaempferol	*V. patens*	73
56	Kaempferol-3-O-rhamnoglucoside	*V. unicana*	3
57	Kaempferol-3-O-glucoside	*V. afromontana*	3
58	Kaempferol-3-O-galactoside	*V. eleagnifolia*	76

Other

59	Hesperetin	*V. brevifolia*	3



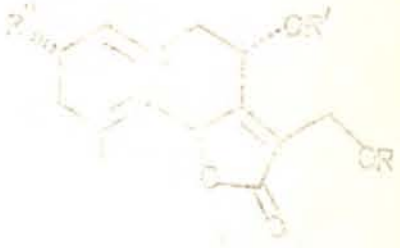
60	Tricin	*V. remotiflora*	41



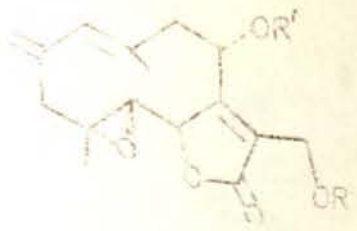
Appendix 2: Some sesquiterpene lactones isolated from the genus *Vernonia*

Since there [illegible] skeletal variation within any class of sesquiterpene lactones isolated from the genus *Vernonia*, only a few representative ones have been presented here. The variation [illegible] to epoxidation of the double bonds or allylic oxidation. The classes represented here include; germacranolides (glaucolides, hirsutinolides and non-glaucolides), guaianolides, pseudoguaianolides, bourbonenolides, elemanolides, eudesmanolides and allylic germacranolides.

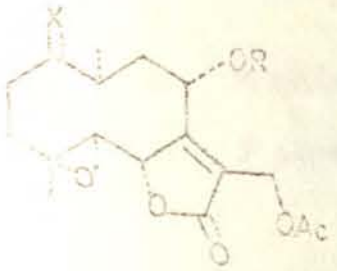
npd No.	Compound	Species	locality	Ref.
	<u>Germacranolides</u>			
	<u>Glaucolides</u>			



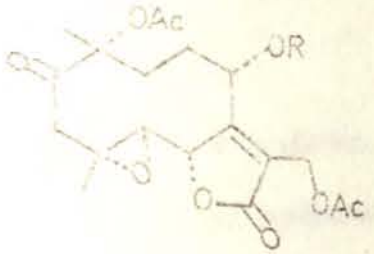
	R	R^1	R^2			
7	Ac	[illegible]	O	*V. natalensis*	S. Africa	58
8	Ac	[illegible]	[illegible]	"	"	"
9	Ac	Ac	[illegible]	*V. cotoneaster*	Brazil	65
0	H	H	[illegible]	"	"	77



	R	R'	X			
111	Ac	Tigl	H_2	*V. marginata*	U.S.	73
112	Ac	Mecr	H_2	*V. arkansana*	Brazil	79
113	Ac	Mecr	α-OAc, H	*V. natalesis*	S. Africa	58
114	Ac	Epmecr	O	*V. suthelandii*	"	68

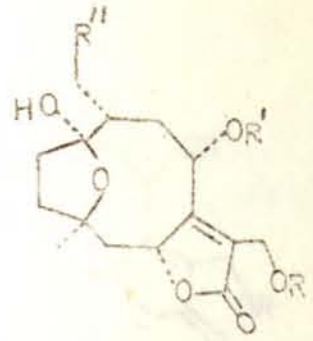


	R	X			
115	Ac	α-OAc	*V. cenferta*	N. America	60
116	Mecr	O	*V. oligocephala*	S. Africa	63

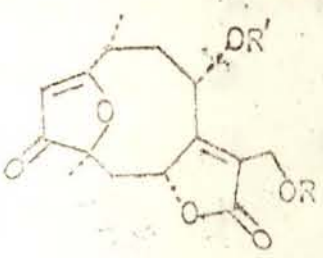


	R			
117	Mecr	*V. glauca*	N. America	81
118	Ac	*V. baldwinii*	"	82
119	Ac	*V.lanuginesa*	Brazil	57

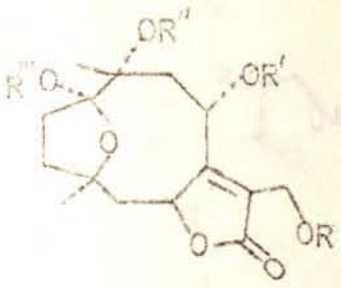
Hirsutinolides



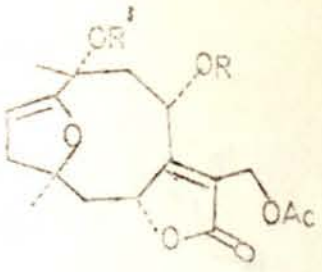
	R	R^1	R^{11}			
120	Ac	Meacr	H	*V. angulifolia*	S. Africa	67
121	Ac	Emeacr	H	"	"	"
122	H	Meacr	H	"	"	"
123	H	Emeacr	H	"	"	"
124	H	Emeacr	H	"	"	"
125	Ac	OHmeacr	H	*V. hirsuta*	"	"
126	Ac	meacr	OH	*V. oligocephala*	"	"



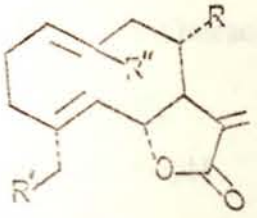
	R	R^1			
127	Ac	4-Hysen	*V. poskeana*	S. Africa	69
128	Ac	Sen	var. *poskeana*	"	"
129	Ac	Meacr	"	"	"
130	H	4-Hysen	"	"	"
131	Ac	5-Acang	*V. poskeana*	"	"
132	Ac	5-Hysen	var. *chlololepsis*	"	"



	R	R^{I}	R^{II}	R^{III}			
133	Ac	Ac	H	H	*V. saltansis*	Bolivia	82
134	Ac	Ac	H	H	"	"	"
135	Ac	Pro	H	Ac	"	"	"
136	Ac	Pro	H	Ac	*V. scorpiodes*	"	"
137	Ac	Pro	H	Me	"	"	"
138	Ac	Ac	H	ival	*V. venosissima*	Brazil	64
139	Ac	Meacr	Ac	Me	*V. polyanthes*	"	90
140	Ac	Meacr	H	H	*V. cinerea*	Costa Rica	70
141	Ac	Tigl	H	H	"	"	"
142	Ac	Ang	H	H	*V. jalcana*	Peru	77
143	Me	Ac	H	H	"	"	"
144	Me	Meacr	H	H	"	"	"
145	Me	Ac	AC	Me	"	"	"
146	Ac	AC	Ac	Ac	"	"	"

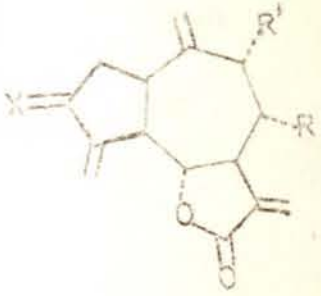


	R	R^1			
147	Meacr	H	*V. novoberacensis*	Guatemala	67
148	Ac	H	*V. venosissima*	Brazil	64

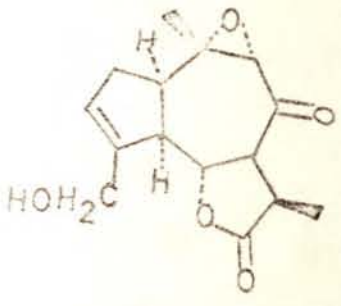


	R	R^1	R^{11}			
149	Omeacr	OH	CH_2OH	*V. pectoralis*	–	83
150	H	H	Me	*V. hirsuta*	S. Africa	67
151	H	OSen	Me	var. *flanaganii*	"	"
152	H	OIVal	Me	"	"	"
153	H	H	Me	*V. arkansana*	Brazil	64
154	Omeacr	OH	CHO	*V. pectoralis*	"	84
155	OHmeacr	OH	Me	*V. neocorymbosa*	S. Africa	69

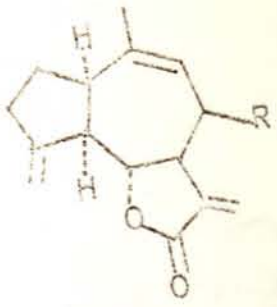
Guaianolides



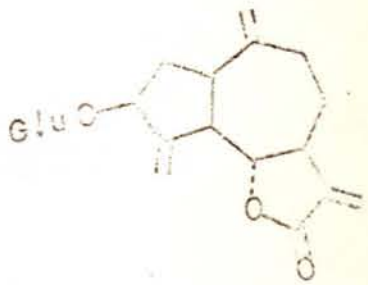
	R	R^1	X			
156	H	H	ß-glu, H	*V. Flexuosa*	–	91
157	H	H	ß-Omeacr,H	"		91
158	Osen,H	H	H_2	*V. nudiflora*	U.S.A.	85
159	H	H	ß-OH	*V. anisochaetolides*	S. Africa	77
160	H	H	α-Osen	*V. hirsuta* var.	"	"
161	H	H	α-OH	*flanaganii*	"	"
162	OH	H	O	*V. novobracensis*	Guatemala	"
163	H	OH	O	*V. scorpiodes*	Bolivia	82
164	H	H	H_2	*V. arkansana*	Brazil	79
165	H	H	ß-OSen,H	"	"	"
166	Tigl	H	ß-OH	*V. nitidula*	"	87
167	Ang	"	"	"	"	"



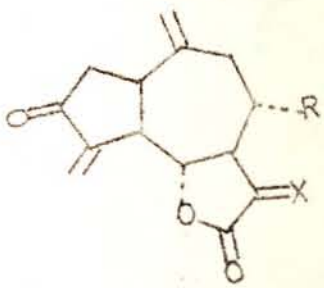
168 *V. natalansis* S. Africa 69



	R			
169	H	*V. hirsuta*	S. Africa	67
170	OSen	*V. oligocephal.*	"	"

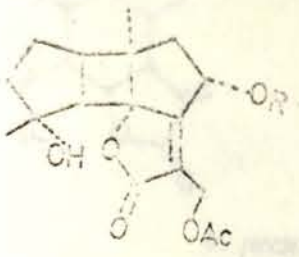


171		*V. flexuosa*	–	85

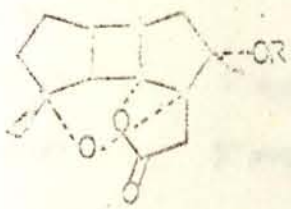


	R	X			
172	H	CH2	*V. profuga*	–	79
173	OH	H,α-Me	"	"	"
174	OH	CH2	"	"	"

Bourbonalides

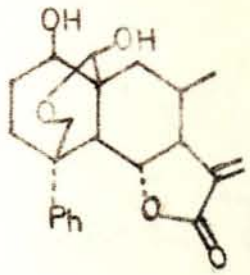


	R			
175	Ac	*V. arkansana*	Brazil	7
176	Tigl	"	"	"
177	Meacr	"	"	"



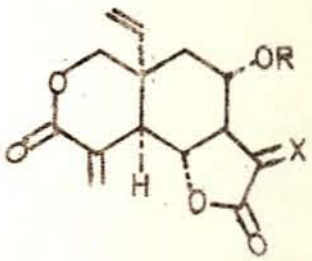
	R			
178	Tigl	*V. arkansana*	Brazil	64
179	Meacr	"	"	"

Eudesmanolides

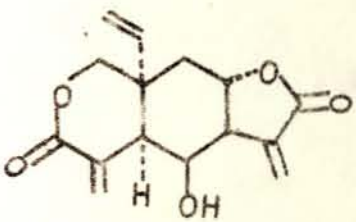


180	*V. pectoralis*	–	88

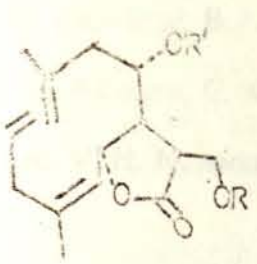
Elemanolides



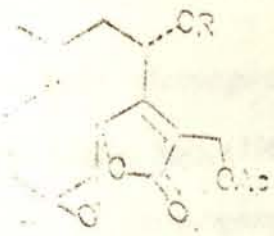
	R	X			
69	H	CH2	*V. hymenolepis*	Ethiopia	9,10
74	Hmeacr	CH2	*V. amygdalina*	"	11
181	Hmeacr	α-CH_3,H	"	Kenya	89



182	*V. hymenolepis*	Ethiopia	9,10



	R	R^1			
183	Ac	Ac	*V. cotoneaster*	Peru	77
184	H	H	"	"	"
185	Ac	H	"	"	"



	R			
186	Ac	*V. lilacina*	Brazil	77

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DECLARATION

I the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

Name: Annah Wambui Keige

Signature: _____

This thesis has been submitted for examination with my approval as University Advisor.

Name: Dr. Berhanu Abegaz

Signature: _____

Place and date of submission: Chemistry Department,

Addis Ababa University

June 1990