# PHYTOCHEMICAL INVESTIGATION OF SURFACE EXUDATES OF *DODONAEA ANGUSTIFOLIA* AND *SENECIO ROSEIFLORUS* FOR BIOACTIVE PRINCIPLES

BY

LEONIDAH KERUBO OMOSA

A THESIS SUBMITTED IN FULFILMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF NAIROBI.

2009

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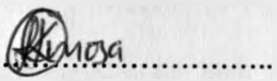

**LEONIDAH KERUBO OMOSA**

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**SUPERVISORS**

**PROF. J. O. MIDIWO**
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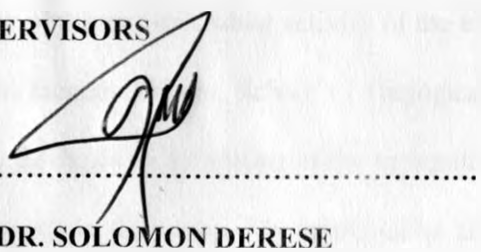

**DR. SOLOMON DERESE**
**DEPARTMENT OF CHEMISTRY**
**UNIVERSITY OF NAIROBI**

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*THIS THESIS IS DEDICATED TO MY HUSBAND BRIGHT KADENGE; SONS RYAN KADENGE AND EMMANUEL KADENGE; DAUGHTER KEISHA KADENGE; AND MY PARENTS*

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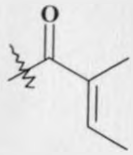
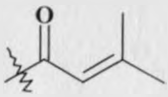
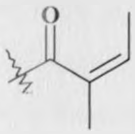
# LIST OF ABBREVIATIONS AND SYMBOLS

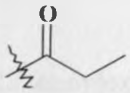
| | | | |
|---|---|---|---|
| APT | Attached proton test | IZ | Inhibition zone |
| *brs* | Broad singlet | *J* | Coupling constant |
| CC | Column chromatography | $LC_{50}$ | Concentration of 50% lethality |
| $CDCl_3$ | Deuterated chloroform | Lit. | Literature |
| $CH_2Cl_2$ | Dichloromethane | MeOH | Methanol |
| $^{13}$C-NMR | Carbon NMR | MHz | Mega Hertz |
| *d* | Doublet | MS | Mass Spectroscopy |
| *dd* | Doublet of doublet | *m* | Multiplet (multiplicity) |
| DMSO | Dimethyl sulphoxide | $[M]^+$ | Molecular ion |
| COSY | Correlation Spectroscopy | *m/z* | Mass to charge ratio |
| DEPT | Distortionless Enhancement by Polarization Transfer | NOE | Nuclear Overhauser Effect |
| DPPH | Diphenyl picryl hydrazine | NOESY | Nuclear Overhauser and Exchange Spectroscopy |
| EIMS | Electron Ionization Mass Spectroscopy | NMR | Nuclear Magnetic Resonance |
| δ | Chemical shift | PTLC | Preparative Thin Layer Chromatography |
| HPLC | High Perfomance Liquid Chromatography | λmax | Maximum wavelength of absorption |
| HPTLC | High Perfomance Thin Layer Chromatography | *s* | Singlet |
| HMBC | Heteronuclear Multiple Bond Correlation ($^2J_{CH}$, $^3J_{CH}$) | *t* | Triplet |
| HMQC | Heteronuclear Multiple Quantum Coherence ($^1J_{CH}$) | TLC | Thin Layer Chromatography |
| $^1$H NMR | Proton NMR | UV | Ultra Violent |
| Hz | Hertz | mp** | The melting point could not be determined due to the low yields of the compound |
| $IC_{50}$ | Concentration of 50% inhibition | | |

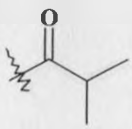
# LIST OF SUB-STRUCTURES

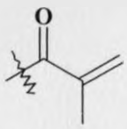
Ang = 

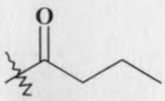
Sene = 

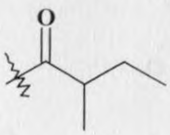
Tigl = 

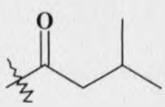
Prop = 

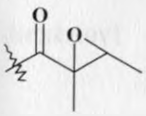
2-Meprop = 

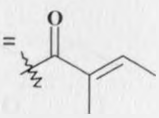
2-Mepropen = 

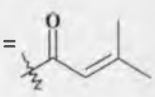
Bu = 

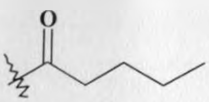
2-Mebu = 

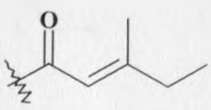
3-Mebu = 

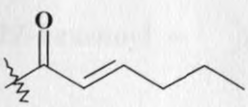
2,3-Epoxy-2-mebu = 

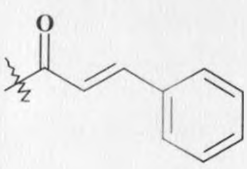
2-Me-2-buten = 

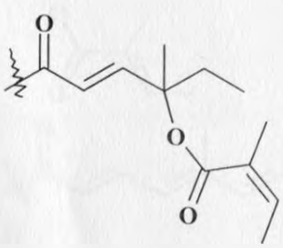
3-Me-2-buten = 

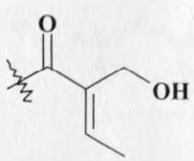
Pent = 

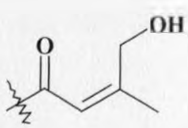
3-Me-2-penten = 

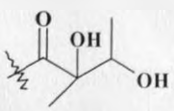
2Z-hexenoyl = 

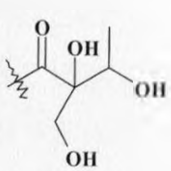
Cinn = 

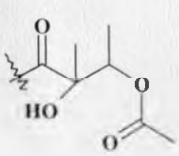
4-Tigloyloxy-4-methyl-2Z-hexenoyl = 

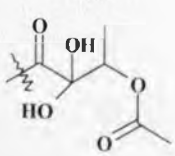
2-Hydroxyme-2-buten = 

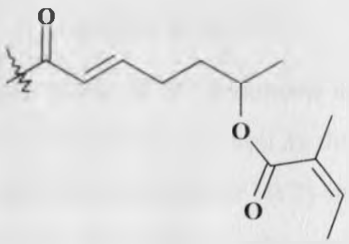
3-Hydroxyme-2-buten = 

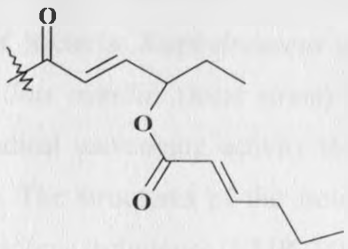
2,3-Dihydroxy-2-methylbu = 

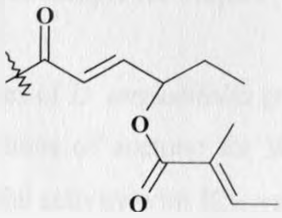
2,3-Dihydroxy-2-hydroxymebu = 

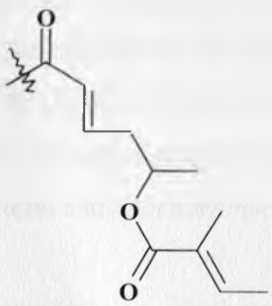
3-Acetoxy-2-hydroxy-2-mebu = 

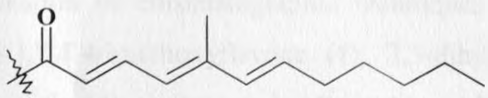
3-Acetoxy-2,2-dihydroxy-bu = 

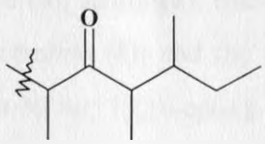
**5-Tigloyloxy-2Z-hexenoyl =** 

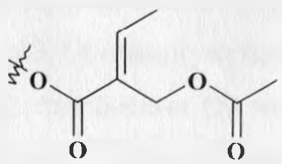
**4-Hex-2Z-enoyloxy-2Z-hexenoyl =** 

**4-Angeloyloxy-2Z-hexenoyl =** 

**5-Angeloyloxy-2Z-hexenoyl =** 

**5-Methyl-2E,4E,6E-dodecatrienoyl =** 

**4,5-Dimethyl-3-oxo-2-heptyl =** 

**O-2-(Acetoxymethyl)-2-buten) (Z) =** 

# ABSTRACT

The phytochemistry of the surface exudates of *Dodonaea angustifolia* (from two locations Ngong forest and Voi) and *Senecio roseiflorus*, as well as the antiplasmodial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strain of *Plasmodium falciparum*, for some of the isolated compounds, larvicidal activity against *Aedes aegypti* larvae, anti-microbial activity against three strains of bacteria: *Staphyloccocus aureus* (ATCC29737), *Escherichia coli* (ATCC25922) and *Bacillus pumilus* (local strain) and a local strain of fungus, *Saccharamyces cerevisiae* and radical scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) were carried out. The structures of the isolated compounds were determined using a combination of spectroscopic techniques [NMR, MS and UV].

The surface exudates of the fresh leaves of *D. angustifolia* growing in Ngong were extracted by successive dipping into fresh portions of acetone for short periods (15 seconds). The surface exudates showed anti-plasmodial activity with $IC_{50}$ values of 41.5 ± 3.9 µg/ml against chloroquine-sensitive (D6) strain of the *P. falciparum*. The larvicidal activity of this extract was not good, as its $LC_{50}$ value against the larvae of *Aedes aegypti* was > 60 µg/ml after 24 hours. The radical scavenging activity (RSA) of the extract towards DPPH was 54.6% at 11.4 µg/ml. The surface exudates showed activity against *Staphyloccocus aureus* (ATCC29737), *Escherichia coli* (ATCC25922) bacteria and *Saccharamyces cerevisiae* fungus.

The extract was subjected to a combination of chromatographic techniques leading to isolation of the flavonoids, 5-hydroxy-3,7,4′-trimethoxyflavone (**1**), 3,5-dihydroxy-7,4′,-dimethoxyflavone (**2**), kumatakenin (**3**), santin (**4**), rhamnocitrin (**5**), isokaempferide (**6**), 6-methoxykaempferol (**7**), and pinocembrin (**8**); and the diterpenoids, 2β hydroxyhardwickic acid (**9**), dodonic acid (**10**) and *ent*-3β,8α; 15,16-epoxy-13(16), 14-labdadiene-3,8-diol (**11**). All the compounds except santin (**4**) are reported from *D. angustifolia* for the first time from this plant. The flavonoids, 5-hydroxy-3,7,4′-trimethoxyflavone (**1**), 3,5-dihydroxy-7,4′,-dimethoxyflavone (**2**), kumatakenin (**3**), rhamnocitrin (**5**) were also isolated from the two collections of *D. angustifolia* (Taita hill near Voi town). However, the rest of the compounds were present only from the *D. angustifolia* (Ngong forest) reflecting geographical variability in this species.

Most of the isolated compounds showed moderate anti-plasmodial activity against the D6 strain of *Plasmodium falciparum* with $IC_{50}$ values between 7.6 ± 2.3 for kumatakenin and 18.4 ± 4.8 for 6-methoxykaempferol. Among the compounds tested for larvicidal activity

against *Aedes aegypti;* rhamnocitrin (**5**) and santin (**4**) showed good dose dependent activity with an $LC_{50}$ value of 1.75 and 5.1 μg/ml, respectively, after 24 hours. Some of the isolated flavonoids showed radical scavenging activity at 50 μM with rhamnocitrin (**5**) showing an activity of 96.2% followed by 3,5-dihydroxy-7,4′-dimethoxyflavone (2) with an activity of 25.5%.

Compounds **1, 3, 4, 5, 8, 9** and **10** were tested for anti-microbial activity. Compounds **5, 8, 9** and **10,** were active against *Staphylococcus aureus.* A number of compounds **4, 5, 8, 9** and **10** were identified as the active principles of the extracts against *Bacillus pumilus.* Compounds **4, 8, 9, 10** were active against the local strain of fungus, *Saccharomyces cerevisiae* with santin (**4**) being the most active having an inhibition zone of 11.15 mm at 31.25 μg/ml.

The fresh leaves of *D. angustifolia* growing in Voi were washed off the exudate in a similar way and tested for anti-plasmodial activity. The surface exudates showed moderate anti-plasmodial activity with $IC_{50}$ values of 56.3 ± 4.2 μg/ml against chloroquine-sensitive (D6) strain of the *P. falciparum.* This is comparable with the activity of the extract of the variety growing in Ngong forest. The radical scavenging activity (RSA) of the crude extract at 11.4 μg/ml was found to be 34.7% lower than the extract from *D. angustifolia* growing in Ngong forest. The surface exudates showed activity against all the three strains of bacteria and one strain of fungus.

Chromatographic separation of this extract gave the flavonoids **1**, **2**, **3** rhamnocitrin (**5**), penduletin (**12**), ayanin (**13**), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**), and kaempferol (**15**); the shikimate acid derivative 7-hydroxy-6-methoxycoumarin (**16**); and the diterpenoids hautriwaic acid (**17**), 15α-methoxy-*neo*-clerodan-3,13-diene-18,19:16,15-diolide (**18**), 15β-methoxy-*neo*-clerodan-3,13-diene-18,19:16,15-diolide (**19**) and *neo*-clerodane-3,13-dien-18,19:16,15-diolide (**20**).

The flavonoids, **12, 13, 14, 15**; the shikimate acid derivative 7-hydroxy-6-methoxycoumarin (**16**); and the diterpenoids **17**, 15α-methoxy-*neo*-clerodan-3,13-diene-18,19:16,15-diolide (**18**), 15β-methoxy-*neo*-clerodan-3,13-diene-18,19:16,15-diolide (**19**) and *neo*-clerodane-3,13-dien-18,19:16,15-diolide (**20**) were only isolated from *D. angustifolia* collected from Taita hills near Voi town.

Among the compounds isolated the flavonoids, **12, 13, 14, 15**: the coumarin **16** and the diterpenoid **17** have not been previously described from this plant. However, most of these compounds except **13** and **16** have been reported from other *Dodonaea* species. This is, however, the first report of **18, 19** and **20** in nature.

Hautriwaic acid (**17**) showed antiplasmodial activities against chloroquine-sensitive (D6) strains of *Plasmodium falciparum* with an $IC_{50}$ value of 10.2 μg/ml. Its activity was compared with that of its lactone, which had $IC_{50}$ values of 23.6 ± 2.6 and 23.0 ± 2.3 μg/ml against chloroquine-sensitive (D6) and resistant (W2) strains of *Plasmodium falciparum* respectively. ). Hautriwaic acid (**17**) showed good larvicidal activity ($LC_{50}$ 10.2 μg/ml, after 24 hours) against *Aedes aegypti* larvae, while its lactone had an $LC_{50}$ value > 100 μg/ml after 24 hours and therefore inactive. The radical scavenging activity of kaempferol (**15**) was found to be the highest with % RSA of 96.8 at 50 μM comparable to that of quercetin at this concentration.

Only two compounds **12** and **17** were tested for activity anti-microbial activity. Compound **12** showed activity against *Bacillus pumilus* (local strain) and *Saccharomyces cerevisiae* (local strain) Hautriwaic acid (**17**) showed activity against two strains of bacteria, *Staphylococcus aureus* (ATCC 29737), *Bacillus pumilus* (local strain) and one strain of *Saccharomyces cerevisiae* (local strain) no activity against *E. coli* was observed for these compounds.

The surface exudates of the leaves of *Senecio roseiflorus* was extracted similarly. The extract showed an antiplasmodial activity with $IC_{50}$ values of 90.0 ± 9.8 μg/ml against chloroquine-sensitive (D6) strain of *Plasmodium falciparum.* Anti-microbial activity against the three strains of bacteria and one strain of fungus was observed with the surface exudates.

The extract was then subjected to a combination of chromatographic techniques leading to the isolation of the flavonoids **1, 2, 3, 5. 6, 14**, 5,7-dihydroxy-3,4′-dimethoxyflavone (**21**), quercetin-3,4′-dimethyl ether (**22**), rhamnazin (**23**), retusin (**24**), 5,4′-dihydroxy-7-dimethoxyflavanone (**25**), 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**27**), 3,5-Dihydoxy-3′,4′,7-trimethoxyflavone (**28**) and the phenol, 4-hydroxy-methylbenzoate (**26**). All these compounds had not been isolated previously from this plant.

The flavanone, 5,4′-dihydroxy-7-methoxyflavanone(**25**) is the most potent against chloroquine-sensitive (D6) and resistant (W2) strains of *Plasmodium falciparum,* with $IC_{50}$

values of 3.2 ± 0.8 and 4.4 ± 0.01 μg/ml respectively. The other compounds showed moderate activities. The extract did not show good larvicidal activity against the larvae of Aedes *aegypti*, as its $LC_{50}$ values was >100 μg/ml after 24 hours. The flavonoids, **25** and **21** showed moderate and dose dependent activity with an $LC_{50}$ value of 14.3 and 15.5μg/ml respectively. The highest RSA activity was observed in quercetin-3,4′-dimethyl ether with % RSA of 77.1 at 50 μM. however, the activity of quercetin was higher.

Compound **22** showed activity against the three strains of bacteria; *Escherichia coli*, *Staphylococcus aureus* and *Bacillus pumilus* and no activity against the fungus. The flavanone, **5**, showed activity against *Staphylococcus aureus* (ATCC 29737), *Bacillus pumilus* (local strain) and *Saccharomyces cerevisiae* (local strain).

# CHAPTER ONE

# INTRODUCTION

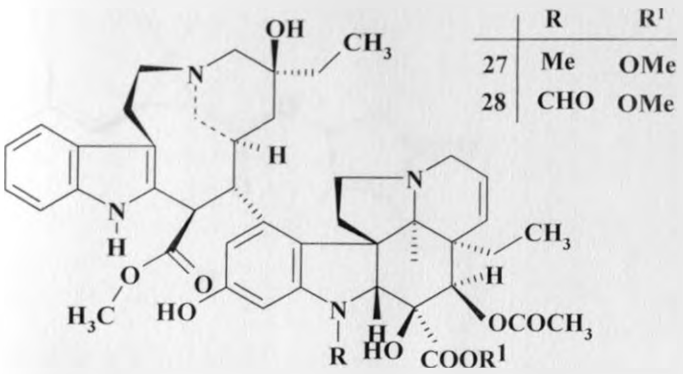
## 1.0 GENERAL

A substantial fraction of the world's population continues to use natural products, especially medical plant extracts to control infectious diseases and combat pests. Today, the pharmaceutical, food, beverage, flavour and fragrance companies have invested a lot of resources in the development of products that incorporate ingredients from plant sources, and have acquired considerable wealth from them.

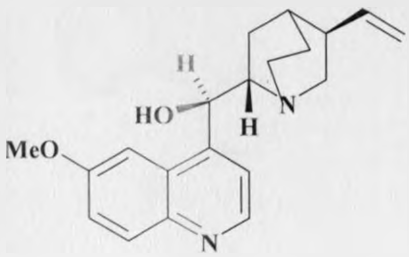
The use of plants for the treatment of disease goes back to early man. Before development of synthetic chemistry in the nineteenth century, nearly 80% of all medicines were obtained from plant materials [Farnsworth, 1985]. Most developing countries are endowed with vast resources of medicinal and aromatic plants. These plants have been used over the millennia for human welfare in the promotion of health and as drugs and fragrance materials. This close relationship between man and the environment continues even today since a large proportion of people in the developing countries still live in rural areas. Furthermore, these people are excluded from the luxury of access to modern therapy, mainly for economic and cultural reasons. In some countries where modern drugs are available, people still use the long traditional socio-cultural practices. Presently many people in developing countries, especially those in the sub-Saharan Africa, still depend on plant sources for their primary health care needs.

In a number of countries such as India, China, Sri Lanka and Australia, pharmaceutical companies are already marketing preparations of tablets, suspensions and capsules made directly from plant extracts for the treatment of specific diseases e.g. hepatitis, malaria, cancer, allergy and AIDS [Abrams, 1990; Decosterd *et al.*, 1991].

Plants and other organisms have been recognised as being the most efficient synthetic tools, capable of making a diversity of organic molecules (natural products that have complex structures and a variety of physical, chemical and biological properties). Most of these complex structures serve as templates (models) for the development of more effective synthetic agents. Some of these natural products may have complex chemical structures, and therefore may not be easily synthesised in the laboratory. For example, the two anti-cancer agents vinblastine (**27**) and vincristine (**28**) isolated from the Madagascan periwinkle, *Catharantus roseus* (Farnsworth, 1990; Cragg & Newman, 1997) are among the most widely used plant-derived natural products in the pharmaceutical industry and have not been substituted by new synthetic compounds. In fact, approximately 60 % of the available anti-cancer chemotherapeutic drugs are of plant origin [Kinghorn *et al.*, 1999]. Similarly 25% of the available modern anti-malarial drugs are of plant origin. Quinine (**29**) [Warhurst *et al.*, 2003], which was initially obtained from the species of *Cinchona* originating from South America, remains a vital drug in the treatment of malaria. Except for anti-folate anti-malarial drugs, all the other commonly used anti-malarial compounds are based plant derived compounds [Geoffrey, 1996]. Recent examples of development of drugs of plant origin include the new anti-malarial agent artemisin (**30**) [Mueller *et al.*, 2000] from *Artemisia annua* and the anti-cancer drug taxol (**31**) [Strobel *et al.*, 1992; Stierle *et al.*, 1993; Kim *et al.*, 1999; Kumaran *et al.*, 2008a; Kumaran *et al.*, 2008b] from some Yew species. Many of the structures discovered for the first time serve as models for the synthesis of biologically active compounds and have promoted research into the activity of analogous structures.




| | R | R¹ |
|---|---|---|
| 27 | Me | OMe |
| 28 | CHO | OMe |

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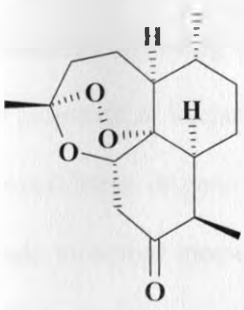


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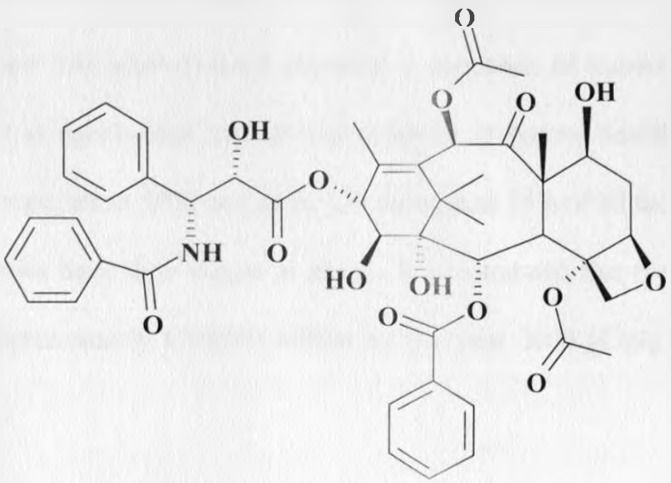


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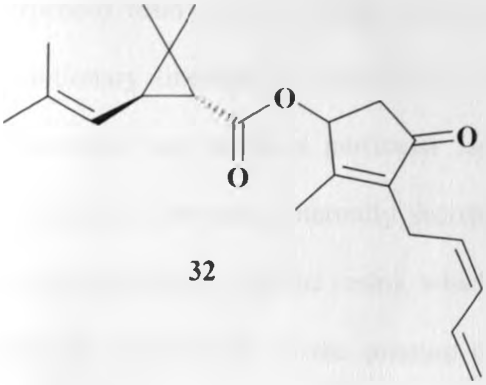
The development of scientific knowledge with the present day environmental awareness, selective and biodegradable compounds must replace the highly toxic and persistent chemicals in the environment. For this reason interest on insecticides and pesticides of plant origin is being revived. The most important pesticides of plant origin are pyrethrin (**32**), rotenone (**66**) and nicotine (**33**) [Curtis *et al.*, 1990].



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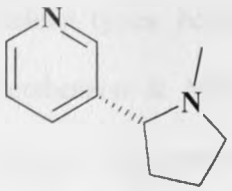


33

There is a trend in the World today to turn to natural substances due to various side effects by some synthetic drugs. Currently, about 200 plant-derived chemical compounds of known structures are being used as drugs or as agents that lead to improvement of human health [Farnsworth & Saejarto, 1991]. In Europe, about 50% and in the US more than 25% of all the prescriptions dispensed from pharmacies have their origin in plants. It is estimated that the trade in herbal medicines reached approximately US$500 billion by the year 2000 [Craig, 1999].

During the past decade, the World Health Organisation (WHO) and the governments in developing countries have been campaigning for the promotion and integration of herbal remedies in health care, as supplementary contribution to modern medical facilities especially in rural areas where modern health facilities and resources are inadequate or completely unavailable and if they are, they often prove to be too expensive.

### 1.1 Plant Resins

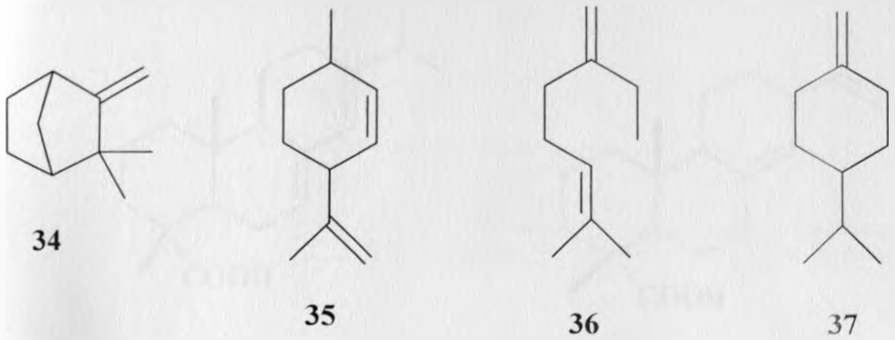
Plant resins, are lipid-soluble mixture of volatile and non-volatile terpenoid and/or phenolic secondary compounds that are usually secreted in specialized structures located either internally or on the surface of the plant and are of potential significance in ecological interactions.

Terpenoid resin occurs in most conifer families but is widely scattered among the major evolutionary lineages of Angiosperms. Specific terpenoid skeletal types, however, often characterize taxa such as particular families and genera [Gershenzon & Mabry, 1983]. Conifers only produce internally secreted terpenoid resins whereas Angiosperms produce both terpenoid and phenolic resins, which may be secreted internally or on the surface of the plant. The complexity of the mixture of compounds constituting a resin is important for ecological interactions. In general, among the 20–50 or more compounds that constitute a resin, only a few occur in high concentration. Surface resins are usually eliminated from the cellular sites of synthesis into different kinds of structures, and thence, exudation to the outside of the plant with or without injury. There are two types of extracellular resinous secretions, endogenous and exogenous secretions [Fahn, 1988]. Endogenous secretions accumulates in various internal structures (canals, pockets or cysts, cavities), which essentially are intercellular spaces surrounded by secretory cells. Normally, such material only exudes from the plant when it is injured. Exogenous secretion, on the other hand, occurs in various types of epidermal secretory cells (glandular hairs, bud trichomes) that may discharge the material to the outside of the organ either directly or first into a sub-cuticular space before further secretion. Resins that are secreted externally from these specialized structures usually coat the surfaces of stipules (which sheathe leaf buds), young leaves stems and /or the floral calyx.

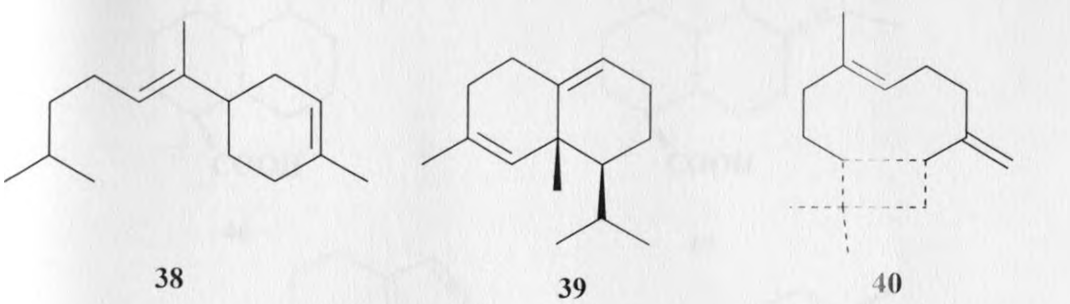
### 1.1.1 Terpenoid Resins

The volatile fraction of some angiosperm resins, usually consists of mono- and/or sesquiterpene hydrocarbons with some oxygenated forms and, occasionally, diterpene hydrocarbons. Structures of some of the most common volatile monoterpenes in various resins include campene (**34**), limonene (**35**), β-myrcene (**36**), β-phellandrene (**37**) and

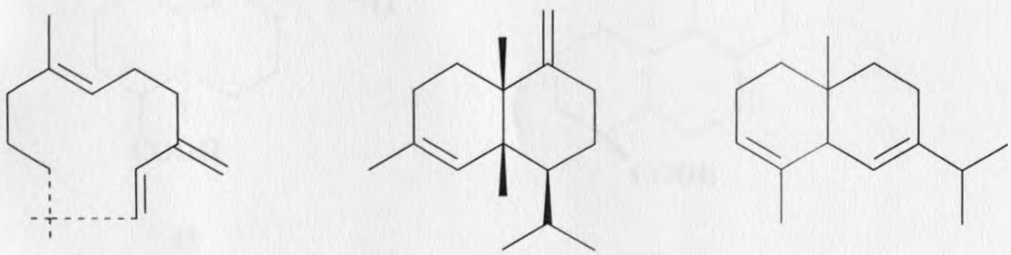
sesquiterpenes common in various resins include α-bisabolene (**38**), δ-cadinene (**39**), β-caryophyllene (**40**), γ-humulene (**41**), γ-muurolene (**42**) δ-selinene (**43**).



**34** **35** **36** **37**



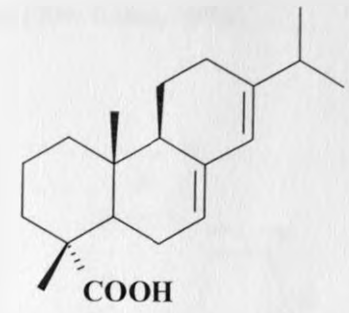
**38** **39** **40**



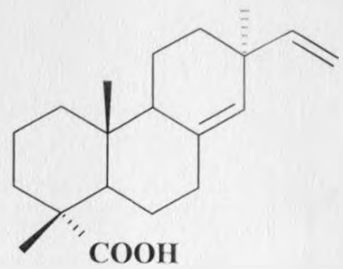
**41** **42** **43**

Diterpenes are the dominant components in the nonvolatile fraction of most angiosperm resins. They form the very hard copals used for varnishes because of the presence of labdadiene-type acids such as ozoic acid (**47**) (or alcohols). Angiosperm resins also contain numerous other diterpenoids such as the clerodane-type hardwickiic acid (**48**), labdane-type: communic acid (**46**) and ozoic acid (**47**), abietane-type: abietic acid (**44**), pimarane-type:

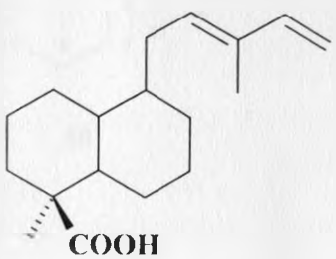
pimaric acid (**45**) and trachylobane-type: trachylobanic acid (**49**) [Richmond & Ghisalberti, 1994].



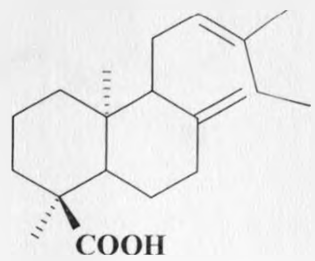

**44**



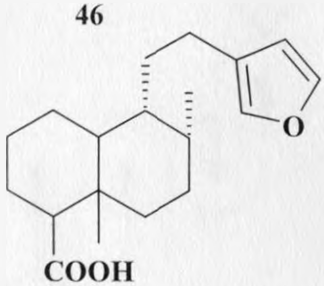

**45**



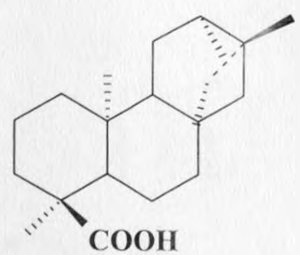

**46**




**47**



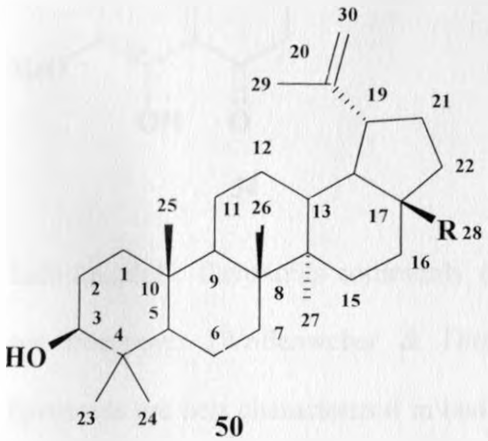

**48**



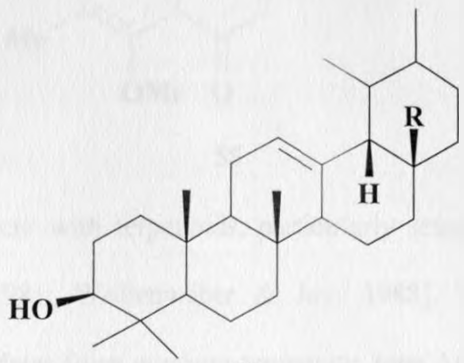

**49**

In some angiosperm families, triterpenes rather than diterpenes dominate the non-volatile composition of the resin. For example, triterpenes primarily with tetra- or pentacyclic skeletons characterize resins from the tropical families Burseraceae, Dipterocarpaceae, and Anacardiaceae. Resins from Burseraceae typically have pentacyclic lupane (**50**), ursane (**51**), and oleanane (**52**) triterpene skeletal types [Khalid, 1985]. Triterpenes with dammarane type skeleton such as dammaradienol (**53**) also characterize plant resin. The non-volatile fraction increases the viscosity of the resin, which can enhance the possibility of engulfing herbivores

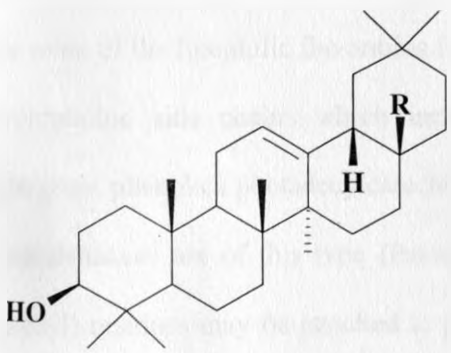
and other organisms visiting the tree. The relative proportion of volatile to nonvolatile compounds, which can vary even between species of the same genus, determines a resin's fluidity, viscosity, and polymerization rate. These in turn influence its ecological properties [Langenheim, 1994; Cates, 1996].



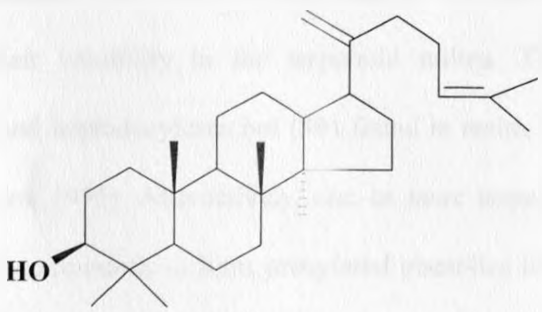

50




51




52

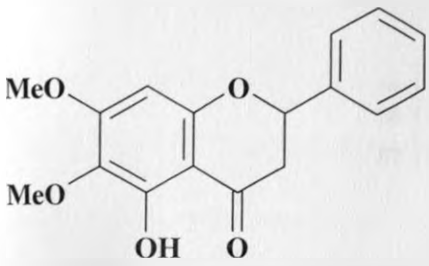



53

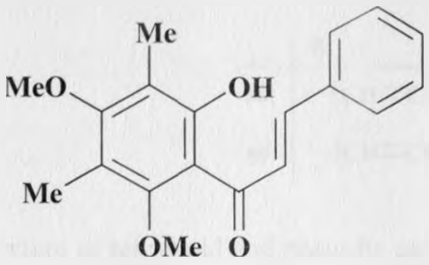
### 1.1.2 Phenolic Resins

Phenolic resins consist mostly of flavonoid aglycones with reduced number of hydroxyl substituents and a variable number of phenolic groups that are *O*-methyl substituted (methoxylated) or with methylenedioxy substituents. Penduletin (**12**) from *Dodonaea* species, 5-hydroxy-6,7-dimethoxyflavanone (**54**) found in farina of *Primula* species and myrigalon B

(**55**) found in the leaf exudates of *Comptonia peregrina* are such compounds (Harborne, 1980).

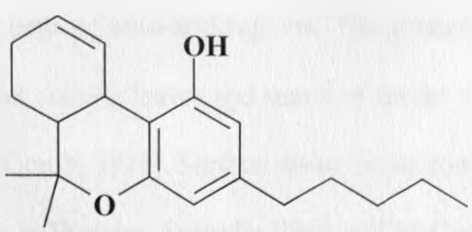



**54**

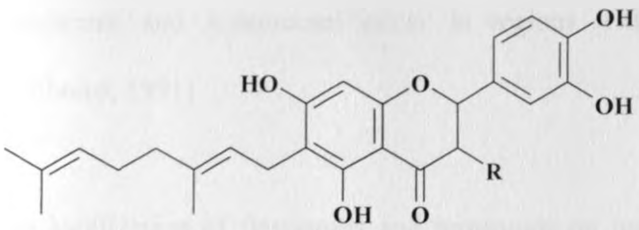


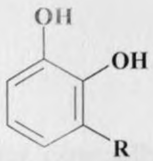

**55**

Such lipophilic flavonoids commonly co-occur with terpenoids, particularly sesquiterpenes and triterpenes [Wollenweber & Dietz, 1981; Wollenweber & Jay, 1988]. Lipophilic flavonoids are best characterized in bud exudates from northern temperate zone Angiosperm trees such as birches (*Betula*) and poplars *(Populus)* and in leaf resins of arid-zone shrubs like monkey flower (*Mimulus*) and yerba santa (*Eriodictyon*). Diplacone (**56**) and diplacol (**57**) are some of the lipophilic flavonoids from *Mimulus* (Hare, 2002). Some phenolic resins have hydrophobic side chains which increase their solubility in the terpenoid milleu. The allergenic phenolics pentadecylcatechol (**58**) and heptadecylcatechol (**59**) found in resins of Anacardiaceae are of this type (Paseshnichenko, 1995). Alternatively, one or more terpene (prenyl) residues may be attached to phenolic compounds to form prenylated phenolics like tetrahydrocannabinol (**60**) (Paseshnichenko, 1995).




**60**




| | R |
|---|---|
| 56 | H |
| 57 | OH |

| | R |
|---|---|
| 58 | $-(CH=CH)_4(CH_2)_6CH_3$ |
| 59 | $-(CH=CH)_6(CH_2)_4CH_3$ |

The surface resins consisting mostly of a mixture of terpenoid and phenolic compounds are a prominent feature of genera in numerous plant families, including Boraginaceae, Euphorbiaceae, Goodeniaceae, Lamiaceae, Myoporaceae, Sapindaceae, Scrophulariaceae, and Solanaceae in Australia, and the Asteraceae, Boraginaceae, Scrophulariaceae, and Zygophyllaceae (Wollenweber, 1981). Arid-zone shrubs of these plant families produce copious leaf surface resins that may constitute 17–30% of the dry weight of the leaf in western Australian shrubs [Dell & McComb, 1978].

Some of the genera of Asteraceae that produce terpenoid and phenolic surface resins, include, *Baccharis, Chrysothamnus, Grindelia, Gutierrezia, Balsamorhiza, Flourensia, Helianthus, Madia, Tagetes Haplopappus,* and *Xanthocephalum* [McLaughlin & Hoffmann, 1982]. Some genera such as *Grindelia*, have been studied extensively for economic use of their resins. Surface resins may be produced on plants growing in diverse habitats, but resin coatings are particularly prominent in plants of semi-arid regions. The greatest amount of resin produced by organ weight may be that coating leaves and stems of shrubs and some herbs in semi-arid to arid regions [Dell & McComb, 1978]. Surface resins occur commonly on shrubs and some herbs of diverse shrub taxa in Western Australia [Dell and McComb, 1978], in the deserts of the American Southwest [McLaughlin & Hoffmann, 1982], and in the chaparral of California. In European Mediterranean vegetation, the shrubs primarily produce essential oils rather than resins. In fact 49% of essential-oil producing genera (frequently in the families

Lamiaceae and Asteraceae) occur in regions with a Mediterranean climate [Ross & Sombrero, 1991].

The localization of flavonoids and terpenoids on leaf surface appear especially suited for protective mechanisms. The most evident role for this lipophilic material is an important reduction of water loss by cuticular transpiration [Rhoades, 1977b]. Another function for leaf resins, especially for desert plants, is partial reflection of light at the surface of these coatings, thus reducing excessive heating of the leaves. They also serve as screen against excess UV-radiation, due to the UV absorption by the dissolved flavonoids [Rhoades, 1977a]. Furthermore, the resin components, terpenoids as well as flavonoids possess anti-fungal, anti-bacterial, acti-viral activity [Chinou *et al.*, 1994] and also serve as insect deterrants when eaten or after penetration of the insect cuticle [Lincoln *et al.*, 1982]. For plants that fight for survival under extreme conditions it appears advantageous that micro-organisms are repelled at the leaf surface and that phytophagous insects that want to chew, suck or mine, will only eat a very small amount of leaf material before they realize a deterrent reaction [Finch, 1977]. Field observations at Aura Valley indicated that *Semiothisa colorata* (*Lepidoptera: Geometridae*), a moth larva and the grasshopper (*Iholacris parviceps Bruner* (*Orthoptera. Acrididae*), a more generalized feeder, preferably consumed mature leaves and avoided young leaves of *Larrea tridentata* cav of the high resin content of the young leaves. The high resinous phenolic constituents of creosatebush, *Larrea tridentata* cav. and *L. cuneifolia* cav (Zygophyllaceae) also deters herbivore grazing [Meyer & Karazov, 1989]. *Psiadia punctulata* is also avoided by browsing herbivores like giraffe and goats, even during severe drought [Midiwo *et al*, 1997]. This is due to the fact that the leaves, especially when young, are covered by gummy exudates which may be responsible for the deterrent effects. All the functions discussed would be of special importance for plants that fight for survival in extreme climatic conditions or xeric habitats.

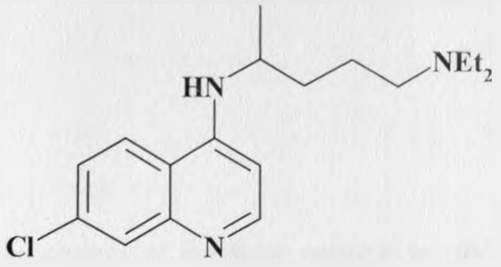
The leaf surfaces of *Dodonaea angustifolia* and *Senecio roseiflorus* are covered with sticky resinous exudate, which makes about 4-13% of the dry weight of the leaves [Ghisalberti, 1998]. In this study the crude extracts and some isolated pure compounds were tested for their activity against *Plasmodium falciparum*, *Aedes aegypti* larvae, *Escherichia coli* (ATCC25922), *Staphyloccocus aureus* (ATCC29737), *Bacillus pumilus* (local strain) and a local strain of fungus, *Saccharamyces cerevisiae*, and fungi. The extracts and pure compounds were also tested for anti-oxidant activity to determine their potential use as medicines. In this section the literature on the malaria burden, botanical larvicides, microbial drug resistance, oxidative stress and anti-oxidants will be reviewed.

**1.2 The Malaria burden**

Malaria is one of the most important infectious diseases, which affects more than a third of the world's population (about 2 billion people) who live in endemic areas. In Africa alone, there are an estimated 200–450 million cases infected with malaria parasites each year [Breman, 2001]. Estimates for annual malaria mortality range from 0·5 to 3·0 million people [Marsh, 1998]. The disease has probably accounted for more deaths and influenced the course of history more than any other disease [Roberts *et al.*, 2004]. It has had a disastrous effect on economic development throughout the world and continues to do so in some of the world's poorest developing countries. Most of the drugs used for treatment of malaria are of plant origin.

The first drug used for treatment of malaria, quinine (**29**), was obtained from the bark of a *Cinchona* tree. Quinine (**29**) still plays an important part in the treatment of malaria and in many countries is the drug of choice for complicated or severe malaria. Quinine (**29**) has strong unpleasant side effects and it is therefore often administered intravenously to hospitalised patients [Roberts *et al.*, 2004]. Up to 70% of patients who take quinine, for example, can experience tinnitus, vertigo and nausea that lasts throughout the dosage period

[Roberts *et al.*, 2004]. As a result of this chloroquine (**61**), which is structurally related quinine, was developed for a preferred prophylaxis and treatment of malaria.




**61**

Chloroquine (**61**) was initially preferred owing to the fact it was well tolerated and cheap to manufacture. However, the parasite developed resistance against chloroquine in the early 1960s in South East Asia and South America and has subsequently spread to most other malarial countries, which made the drug ineffective. Consequently, the malaria problem is more complicated now than before. The parasite has continued to develop resistance to current first-line anti-malarial drugs leading to escalating mortality rates [Trape *et al.*, 1998] imposing considerable pressures on health care systems.

As has been the case in the development of quinine and its derivatives, traditional medicinal plants continue to be the source of new anti-malarial drugs. In this connection artemisinin (**30**) was isolated from the Chinese traditional anti-malarial herb *Artemisia annua*. Currently, artemisinin (**30**) derivatives are used for the treatment of uncomplicated and severe forms of malaria. They reduce parasitaemia more rapidly than any other known anti-malarial compound, and are effective against multidrug-resistant parasites [Olliaro *et al.*, 2001].

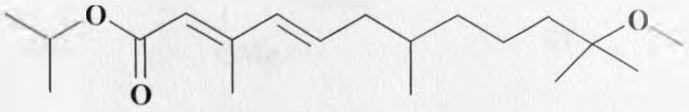
It is therefore necessary to continue to the search for new anti-malarial drugs to manage parasite resistance. The search for anti-malarial drugs from plants can be expedited by focusing on plants that have been used traditionally in the management of the disease or those

that produce compounds that are active against the malaria parasite or the symptoms caused by the parasite. In this vein this study looks for anti-plasmodial activity of the leaf surface exudates compounds of *D. angustifolia*-Ngong forest, *D. angustifolia* –Voi and S. *roseiflorous*.

**1.3. Botanical larvicides**

Larval control involves the control of mosquito populations they develop into adults. For most malaria vectors, reducing mosquito population densities by means of larvae eradiction is generally an efficient way of controlling malaria transmissions. Organophosphates, larvicidal oils, arsenical compounds, and larval development inhibitors have all been used with varying degrees of success [Gratz & Pal, 1988].

Constant applications of organophosphates such as methoprene (**62**) and temephos (**63**) and insect growth regulators such as diflubenzuron (**64**) and fenthion (**65**) are generally practised for the control of mosquito larvae [Yang *et al.*, 2002]. Although these insecticides are effective, their repeated use can disrupt natural biological control systems and result in the widespread development of resistance leading to large population of mosquitoes. The practice can also have undesirable effects on non-target organisms, creating environmental and human health concerns [Yang *et al.*, 2002]. These problems have highlighted the need for the development of new strategies for selective mosquito larval control. In the search for cost effective, environmental friendly alternatives for the control of disease vector insects, plant extracts and pure compounds have been screened for larvicidal activities [Mwangi & Mukiama, 1988; Mwangi & Rembold, 1988; Gikonyo *et al.*, 1998].

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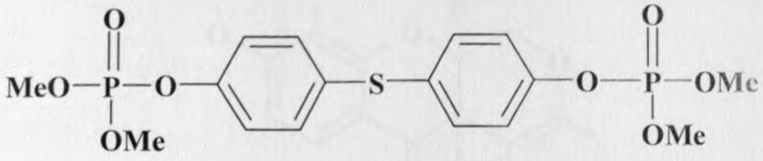

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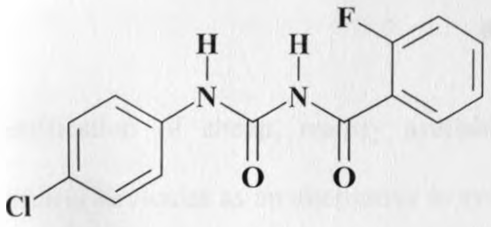

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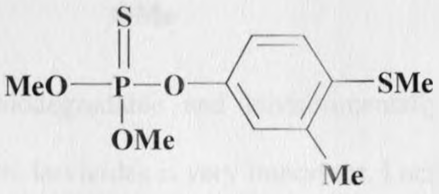
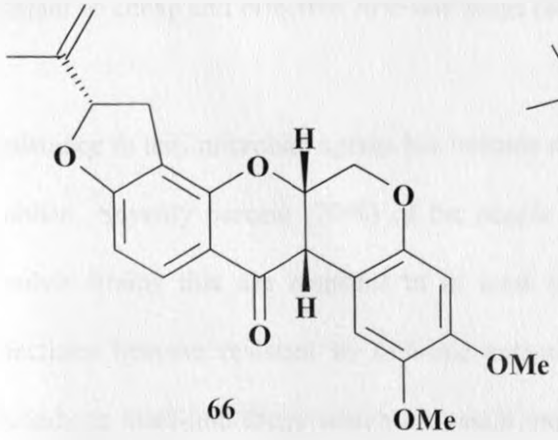


65

Rotenone (**66**), one of the most extensively used natural insecticide, active against fourth-instar larvae of *Aedes aegypti* L [Abe *et al.*, 1985]. The insecticidal activity of rotenone (**66**) and some other rotenoids including deguelin (**67**) and tephrosin (**68**) against a variety of insect species is well known (Dewick, 1994). Commercially, rotenone (**66**) is mainly extracted from roots of *Derris* species in Asia and *Lonchocarpus* species in South America. These and other rotenoids are also known to occur in the related genera including *Milletia* and *Tephrosia* [Dewick, 1994].



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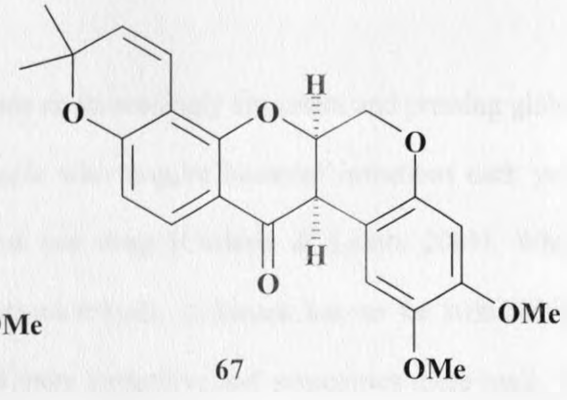

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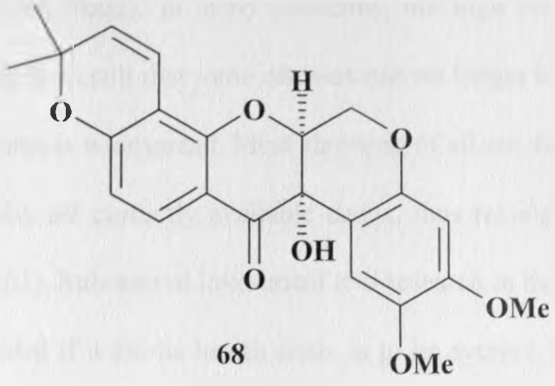


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Identification of cheap, readily available, biodegradable and environmentally friendly botanical larvicides as an alternative to synthetic larvicides is very important. Locally grown botanical larvicides could, not only be used, by local communities, for immediate control of mosquitoes but can also save precious foreign currency used on importation of synthetic insecticides. The identification of such botanical insecticides among the local flora may eventually lead to the cultivation and commercialization of these plants by the local farmers as an extra source of income.

**1.4. Microbial Drug Resistance**

Since their discovery during the 20th century, anti-microbial agents (antibiotics and related medicinal drugs) have substantially reduced the threat posed by infectious diseases [WHO, 2002]. There is a growing concern about the emergence and spread of microbes that are resistant to cheap and effective first-line drugs [Baron, 1982].

Resistance to anti-microbial agents has become an increasingly important and pressing global problem. Seventy percent (70%) of the people who acquire bacterial infections each year involve strains that are resistant to at least one drug [Cushnie & Lamb, 2005]. When infections become resistant to first-line anti-microbials, treatment has to be switched to second- or third-line drugs which are much more expensive and sometimes more toxic, as well. An example can be found in the drugs used to treat multidrug-resistant forms of tuberculosis which are over 100 times more expensive than the first-line drugs used to treat

non-resistant forms [WHO, 2002]. In many countries, the high cost of such replacement drugs is prohibitive, with the result that some diseases can no longer be treated in areas where resistance to first-line drugs is widespread. Most alarming of all are diseases where resistance is developing for virtually all currently available drugs, thus raising the spectre of a post-antibiotic era [WHO, 2002]. Substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted. Structural modification of anti-microbial drugs to which resistance has developed has proven to be an effective means of extending the lifespan of anti-fungal agents such as the azoles [Jeu *et al.*, 2003], anti-viral agents such as the non-nucleoside reverse transcriptase inhibitors [De Clerq, 2001], and various anti-bacterial agents including lactams and quinolones [Poole, 2001]. It is not surprising then, that in response to anti-microbial resistance, major pharmaceutical companies have concentrated their efforts on improving anti-microbial agents in established classes [Taylor *et al.*, 2002].

However, with the portfolio of chemotherapeutics currently available, it has been acknowledged that researchers are getting close to the end game in terms of parent structure alterations. A call has therefore been made for the development of new classes of drug that work on different target sites to those in current use [Kimberlin & Whitley, 1996]. Rational drug design does not always yield effective anti-microbials. Broad empirical screening of chemical entities for anti-microbial activity represents an alternative strategy for the development of novel drugs. Natural products have been a particularly rich source of anti-infective agents, yielding, for example, the penicillins in 1940, the tetracyclines in 1948 and the glycopeptides in 1955 [Silver & Bostian, 1990]. *Dodonaea* species are traditionally used for their anti-microbial properties in different parts of the world and have revealed several anti-microbial compounds, mostly flavonoids [Ahmed *et al.*, 1994; Rojas *et al.*, 1992; Sukkawala & Desai, 1962]. Increasingly, flavonoids are becoming the subject of medical research. They have been reported to possess many useful properties, including anti-

inflammatory, oestrogenic, enzyme inhibition, anti-microbial [Havsteen, 1983; Cushnie & Lamb, 2005], anti-allergic, anti-oxidant [Middleton & Kandaswami, 1993], vascular and cytotoxic anti-tumour activity [Harborne & Williams, 2000]. Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have been proposed for use against fungal pathogens of man [Harborne & Williams, 2000]. In this project the biological activity of the crude extracts and pure compounds of *D. angustifolia*-Ngong Forest, *D. angustifolia*-Voi and *Senecio roseiflorus* against bacteria and fungi were studied.

**1.5 Oxidative Stress and Anti-oxidants**

Under normal conditions, a dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the anti-oxidant capacity of the cell [Granot & Kohen, 2004; Stohs, 1995]. Oxidative stress is caused by an imbalance between the generation of ROS by endogenous/exogenous pro-oxidants and the defence mechanism against ROS by anti-oxidants. ROS are highly reactive molecules which are naturally occurring by-products of normal biological processes within the body or from exogenous factors. When anti-oxidants are not enough or during diseases, the amount of ROS increase and react with DNA, lipids and proteins causing cell death due to necrosis [Kannan & Jain, 2000].

Excess levels of ROS and the resulting oxidative stress have been implicated in a number of human diseases including diseases, cancer, atherosclerosis, asthma, arthritis, autism, cardiovascular and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease [Knight, 1998; Kehrer, 1993]. Increasing evidence suggests that aging may be a consequence of the normal, long-term exposure to ROS and the accumulation of oxidized, damaged molecules within the cell. A potent scavenger of free radicals (anti-oxidants) may serve as a possible preventative intervention for the diseases [Gyamfi *et al.*, 1999].

Anti-oxidants act as radical scavengers through the donation of one or more electrons thereby neutralizing the free radical and thus terminating the chain reactions, which could other wise

damage cells and tissues leading to diseases. Anti-oxidants can be classified into natural and synthetic. Natural anti-oxidants can be classified further into enzymatic (superoxide dismutase, ascorbate peroxidase, glutathione reductase, glutathione peroxidase, etc) and non-enzymatic anti-oxidants (glutathione, ascorbic acid, vitamin E, vitamin A) [Luis & Nombela, 1999]

The most commonly used synthetic anti-oxidants are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, which are incorporated in food products to stop the oxidation of polysaturated fatty acids that leads to the degradation of the food. However, these synthetic anti-oxidants have been implicated in liver damage and carcinogenesis in laboratory animals [Wang *et al.*, 2000]. Strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring anti-oxidants. Moreover, the synthetic anti-oxidants show low solubility and moderate anti-oxidant activity [Barlow, 1990; Branen, 1975]

Plant species with high amount of flavonoid and phenolic compounds such *Mellilotus officinalis* extract have shown higher potency than BHT in scavenging free radicals [Pourmorad *et al*, 2006].

Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as anti-oxidants, in reducing free radical induced tissue injury. Besides well known and traditionally used natural anti-oxidants from tea, wine, fruits, vegetables and spices, some natural anti-oxidant (rosemary and sage) are already exploited commercially either as anti-oxidant additives or a nutritional supplements [Schuler, 1990]. Also many other plant species have been investigated in the search for novel anti-oxidants [Chu, 2000; Koleva *et al.*, 2002;

Mantle *et al.*, 2000; Oke & Hamburger, 2002] but generally there is still a demand to find more information concerning the anti-oxidant potential of plant species.

The anti-oxidant activity of plants might be due to their phenolic compounds [Cook and Samman, 1996] which act as free radical terminators [Shahidi & Wanasundara, 1992]. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [Frankel, 1995]. Some evidence suggests that the biological actions of these compounds are related to their anti-oxidant activity [Gryglewski *et al.*, 1987]. The mechanisms of action of flavonoids are through scavenging or chelating process [Kessler *et al.*, 2003, Cook & Samman, 1996].

*Dodonaea* and *Senecio* plants have been known to elaborate flavonoids and phenolics some of which could have anti-oxidant activities. The crude extracts and some flavonoids from these plants were screened for their potential as anti-oxidants in the present work. The crude (or their active constituents) identified as having high levels of anti-oxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 BOTANICAL INFORMATION

### 2.1.1 THE FAMILY SAPINDACEAE

The genus *Dodonaea* belongs to the family Sapindaceae. Among flowering plants, the family Sapindaceae is one of the most widely distributed ones. It comprises between 140-150 genera and between 1,500-2,230 species, typically tropical and strongly represented in the American, African and Asian tropical zones, also in Japan and widespread in Australia. Members of the family are mostly trees and shrubs, or sometimes vines (cardiosperm) climbing with the help of simple or branched tendrills, which are modified influorescence axes, or lianas.

The East African genera of Sapindaceae belong to the following sub-families and tribes: Subfamily Dodonaeoideae with three tribes *Dodonaeeae* (*Dodonaea*), *Doratoxyleae* (*Filicium*) and *Harpullieae* (*Majidea*); and the subfamily Sapindoideae with eight tribes *Cupanieae (Aporriza*), *Nephelieae* (*Stadmania*), *Schleichereae* (*Macphersonia*), *Melicocceae* (*Tristiropsis*), *Lepisantheae* (*Lepisanthes*), *Sapindeae* (*Deinbollia*), *Thouinieae* (*Allophylus*) and *Paullinieae (Cardiospermum*) [Davis & Verdcourt., 1998].

In East Africa the family is represented by 61 species in 25 genera. Fourteen (14) of these species are endemic to the region. The 25 genera of East Africa are: *Dodonaea* (2 *sp*), *Filicium* (1 *sp*), *Zanha* (2 *spp*), *Majidea* (2 *spp*) *Aporrhiza* (1 *sp*), *Blighia* (2 *spp*), *Eriocoelum* (1 *sp*), *Haplocoelopsis* (1 *sp*), *Stadmania* (1 *sp*), *Pappea* (1 *sp*) *Macphersonia* (1 *sp*), *Lecaniodiscus* (2 *spp*), *Haplocoelum* (2 *spp*), *Camptolepis* (1 *sp*), *Lepisanthes* (1 *sp*), *Glenniea* (1 *sp*), *Placodiscus* (2 *spp*), *Pancovia* (6 *spp*), *Chytranthus* (4 *spp*), *Deinbollia* (3 *spp*), *Sapindus* (2 *spp*), *Allophylus* (18 *spp*) *Cardiospermum* (3 *spp*) *Paullinia* (1 *sp*). [Davis & Verdcourt, 1998]

Several species from this family have been cultivated in E. Africa for their edible fleshy fruits including several well known fruits such as *Litchi chinensis* (Lychee), which has been cultivated in Kenya (Kiambu & Kilifi district), Tanzania (Lushoto & Morogoro District) and Uganda (Mengo District). *Nephelium lappaceum* (rambutan) is grown for edible fruit in Tanzania (Lushoto District) and Uganda (Mengo District) while *Melicoccus bijugatus* also known as honey berry or Spanish lime, a native of S. America has been grown in Tanzania (Lushoto District) and Uganda (Entebbe). *Tristinopsis acuteangula*, a native of Malaysia to Queensland, Solomon Island, Guam and Palau Island is grown in Tanzania (Zanzibar) for timber [West, 1984].

#### 2.1.1.1 THE GENUS *DODONAEA*

The genus *Dodonaea* is predominantly Australian, comprising of 68 species of which 61 species are found in that sub-continent. Of the 61 species found in Australia, 59 are endemic. *Dodonaea palyndra* extends to New Guinea and *Dodonaea viscosa* is pantropical extending to Southern Africa and the Pacific. *D. viscosa* is a polymorphic species containing at least 7 sub-species [West, 1985]. All *Dodonaea* species are evergreen woody, perennials, most are erect, multi-stemmed shrubs 1-2 m in height but there is considerable variation in size. *Dodonaea humifosa* and *Dodonaea procumbens* are prostrate shrubs (10 cm high), whereas *Dodonaea viscosa* and *Dodonaea platyptera* can reach a height of 8m. In Australia, the genus is widespread especially in the inland regions [West, 1985]. Many of the western Australian species possess viscid resinous exudates on the leaves as is common in many other xerophitic species found in the region and elsewhere. It is widely grown for horticultural purposes in Australia, and in other countries it is used as a hedge plant, sand binder and for marshland reclamation [West, 1985]. In East Africa the genus *Dodonaea* is represented by two species *D. angustifolia* (Fig. 2.1) and *D. viscosa* (Fig. 2.2) [Beentje, 1994].

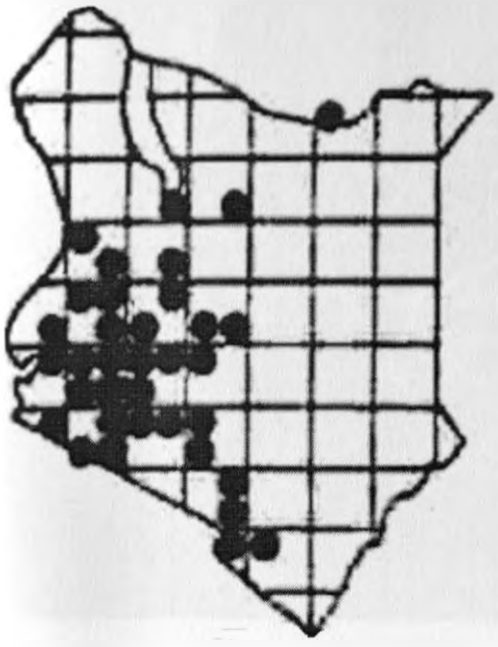


Figure 2.1: Distribution of *D. angustifolia* in Kenya [Beentje, 1994]

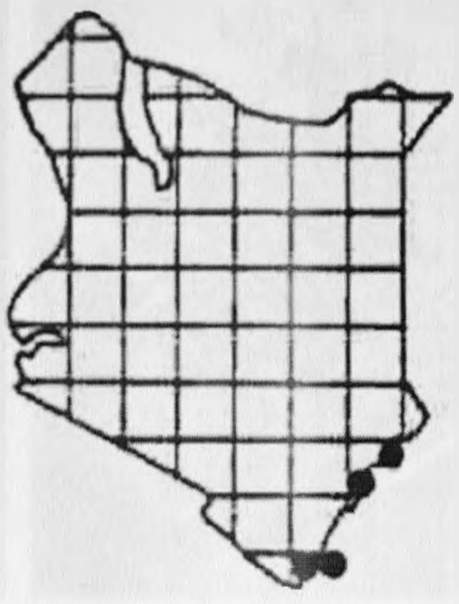


Figure 2.2: Distribution of *D. viscosa* in Kenya [Beentje, 1994]

#### 2.1.1.1.1 *D. VISCOSA*

*Dodonaea viscosa* is an extremely variable species throughout its natural range. There are many distinctive populations that have been described as separate species from *D. angustifolia* by some authorities. Wagner *et al* 1990 considers the Hawaiian *D. viscosa* populations as one species.

*Dodonaea viscosa* can be a small tree or shrub 1.5 to 4 m tall. Leaves are lance-shaped to elliptic (6 - 12.5 cm) long (1.8 - 4.2 cm) wide (mostly widest above the middle) and relatively broader. Flowers are whitish, mostly bisexual; scar of fallen sepals beneath fruit strongly bilobed. Fruits are white or brown to straw-coloured or greenish white, sometimes yellow or reddish, mostly two winged, (1.5 - 2.3 cm. long and 1.8 - 2.5 cm. wide. Seeds are sub-globose, 3 mm diameter, 2 - 3 mm thick and mostly rolling easily when dropped on a flat surface [Davies & Verdcourt, 1998].

Figure 2.3: *Dodonaea viscosa* female flowers [Carr, 1998]


Figure 2.4: *Dodonaea viscosa* male flowers [Carr, 1998]

#### 2.1.1.1.2 *DODONAEA ANGUSTIFOLIA*

*Dodonaea angustifolia* belonging to the Sapindaceae family is variously considered synonymous with, sub-species of, variety of or distinct species from *D. viscosa* [West, 1984]; [Vedcourt, 1994] and [Leenhouts, 1983] respectively depending on the particular authority. It is widely distributed in four continents - Australia, Africa, Asia and South America. In Africa it exists in Eastern, Southern and Western Africa. In Kenya, it exists along with *D. viscosa* which otherwise has exogenous origin in India [Beentje, 1994]. West (1984) revised the taxonomy of *Dodonaea* of Australia [ICRAF, 1991] and morphologically determined that *D. viscosa* had up to four sub-species existing in that country: *D. viscosa* ssp. *viscosa*, ssp. *burmanniana*, ssp. *angustifolia* and ssp. *spatulata*. No such work has been done for the African *D. angustifolia* other than the declaration that the coastal population is synonymous with *D. viscosa* from Australasia (Figure 2.2) [Leenhouts, 1983]. The main phytochemical feature in *D. angustifolia* is the leaf surface exudate (up to 13% dry leaf weight) composed of methylated flavones and flavonols in a diterpenoid (clerodane) milieu. In this work the phytochemical investigation of the upland (Ngong forest) and coastal (Voi) populations of *D.*

*angustifolia* was done. *Dodonaea angustifolia* can be a medium-sized shrub (Fig. 2.5) or small tree up to 9 m tall (Fig. 2.6), but most often 0.5-7.5 m in height. The plant may have one or several main trunks, which have reddish-brown to blackish - grey bark. There is a lot of variation in leaf size and shape, but the leaves are generally longer than they are wide and most often pointed (Lance-shaped to elliptic). Most often, the leaves are 1.5-13 cm. long, 0.6-3.5 cm. wide, narrowed gradually and rounded to an acute and weakly apiculate tip. They are usually glossy green and often have reddish midribs or stems. All the leaves are covered by a sticky substances but new leaves are stickier than the old ones. Generally, older leaves have a rough, sandpapery texture.

Figure 2.5: *Dodonaea angustifolia* shrub [Carr, 1998]


Figure 2.6: *Dodonaea angustifolia* tree [Carr, 1998]

The individual flowers are small, greenish yellow, 0.6 cm in diameter, and occur in branched clusters. They are mostly unisexual and only the female flowers develop into the decorative capsules. Some plants have both male and female flowers in which

Figure 2.7: Fruits of *D. angustifolia* [Carr, 1998]

case only a single plant is required to produce capsules [Bornhorst 1996; Koob, 2001; Obata, 1997; Rauch, 1997; Wagner, 1990]. Scar of fallen sepals beneath fruit usually annualar or slightly lobed. The fruit is red-tinged or mottled or crimson to purplish but can be white, brown or reddish green, predominantly two winged, but often with 3 or even 4 wings. Seeds compressed globose, ± 3 mm in diameter, 1.5 to 1.8 mm thick, with obtuse dorsal keel, not easily rolling when dropped on flat surface [Davies & Verdcourt, 1998]

### 2.1.1.2 MORPHOLOGICAL DIFFERENCES BETWEEN *D. VISCOSA* AND *D. ANGUSTIFOLIA*

From the morphological point of view it is not easy to distinguish the two species from each other because most features overlap (Table 2.1). In this study the phytochemistry of the *Dodonaea* populations from Ngong Forest representing *D. angustifolia* and from Taita hills near Voi town representing *D. viscosa* [Leenhouts, 1983; Davies & Verdcourt, 1998 ] was done to compare the compounds from the two populations and see if differences could be discerned.

Table 2.1: Morphological differences of *D.viscosa* and *D. angustifolia*

| *D. VISCOSA* | *D. ANGUSTIFOLIA* |
|---|---|
| Small tree or shrub 1.5 - 4 metres tall. | Medium-sized shrub or small tree up to 9 metres tall. |
| Leaves 6 - 12.5 cm. long, 1.8 - 4.2 cm wide. | Leaves are 1.5 - 13 cm. long and 0.6 to 3.5 cm. wide. |
| Flowers are whitish, mostly bisexual. | Flowers are greenish yellow mostly unisexual. |
| Fruit mostly two winged 1.5 - 2.3 cm. long and 1.8 - 2.5 cm. wide. | The fruit predominantly two winged, but often with 3 or even 4 wings |
| Seeds sub-globose, 3 mm diameter, 2 - 3 mm. thick, mostly rolling easily when dropped on a flat surface. | Seeds compressed globose, 2 - 3 mm diameter, 1.5 - 1.8 mm thick, with obtuse dorsal keel, not easily rolling when dropped on flat surface |

### 2.1.2 FAMILY ASTERACEAE

The genus *Senecio* belongs to the family Asteraceae or Compositae, known as the aster, daisy or sunflower family, the largest family of flowering plants in terms of number of species [Cronquist, 1981]. Plants in the Asteraceae family are mostly herbs, but some shrubs, trees and climbers do exist. According to the information from Royal Botanical Gardens of Kew, the family comprises more than 1,600 genera and 23,000 species [Heywood *et al.*, 1977]. The largest genera are *Senecio* (1,500 species), *Vernonia* (1,000 species), *Cousinia* (600 species), *Centaurea* (600 species). This family is characterized by having the flowers reduced and organized into an involucrate pseudanthium in the form of a head or capitulum [Stevens, 2007]. Asteraceae are cosmopolitan, but most common in the temperate regions and tropical mountains. The fruit of the Asteraceae has one seed per fruit, it may sometimes be flat, winged or spiny. The fruit morphology is often used to help determine plant relationships at the genus and species level [Heywood *et al.*, 1977]. The seeds usually have little or lack endosperm [Stevens, 2007].

The Asteraceae family is exceptionally rich in a range of secondary metabolites and also in the number of complex structures known one class [Heywood *et al.*, 1977]. These secondary metabolites include iso/chlorogenic acid, different classes of sesquiterpenoids lactones, pentacyclic triterpene alcohols, various alkaloids, acetylenes (cyclic, aromatic, with vinyl end

groups), flavonoids and tannins. They have terpenoid essential oils which never contain iridoids [Usher, 1966]. It is one of the most economically important families, for it is the source of food crops such as *Lactuca sativa* (lettuce), *Cichorium* (chicory), *Cynara scolymus* (globe artichoke), *Helianthus annuus* (sunflower), *Smallanthus sonchifolius* (yacón), *Carthamus tinctorius* (safflower) and *Helianthus tuberosus* (Jerusalem artichoke). A study of early herbals reveals that a surprisingly large number of Asteraceae were used for their curative properties [Hind *et al.*, 1995]. It is the source of medicinally important herbal teas such as chamomile tea from *Matricaria recutita* or *German chamomile* and the perennial *Chamaemelum nobile*, also called Roman chamomile. Other herbal teas include *Calendula*, (pot marigold), Echinacea (Echinacea purpurea), *Tagetes lucida* which is commonly grown and used as a tarragon substitute in climates where tarragon cannot survive. Finally, the wormwood genus *Artemisia* includes absinthe (*A. absinthium*) and tarragon (*A. dracunculus*). This family is an important source of medicine especially in areas where there is no access to Western medicine. Many members of the family are grown as ornamental plants for their flowers and some are important ornamental crops for the cut flower industry. Some examples are *Chrysanthemum, Gerbera, Calendula, Dendranthema, Argyranthemum, Dahlia, Tagetes, Zinnia* among many others. *Helianthus annuus* (domestic sunflower), and some species of *Solidago* (golden rod) are major "honey plants" for beekeepers. *Solidago* produces relatively high protein pollen, which helps honey bees over winter [Croat, 1972].

Some members of the Asteraceae which are economically important as weeds include the ragwort, *Senecio jacobaea*, groundsel *Senecio vulgaris* and *Taraxacum* (dandelion). The genera *Tanacetum*, *Chrysanthemum* and *Pulicaria* contain species with insecticidal properties. *Parthenium argentatum* (Guayule) is a source of hypoallergenic latex [Cornish &. Brichta, 2002].

### 2.1.2.1 THE GENUS *SENECIO*

*Senecio* is the largest genus of the Asteraceae family with over 1500 species distributed all over the World [Hind *et al.*, 1995]. A large number of these species are common perennial or annual weeds, but some are succulent and caudiciforms from tropical and sub-tropical areas. A number of succulent relatives have now been moved to the genus *Kleinia*. The flowers of *Senecio* are arranged in clusters at the top of the plants, they vary in color from white and yellow, to red and purple. Most succulent species tolerate no frost. Some species produce natural pesticides (especially alkaloids) to deter or even kill animals that would eat them. *Senecio* species are used as food plants by the larvae of some *Lepidoptera* species and as medicine by many communities.

#### 2.1.2.1.1 *SENECIO ROSEIFLORUS*

*Senecio roseiflorus* is an erect herb or weak shrub, densely glandular on all parts; leaves are oblong-lanceolate, lobed, stalkless, to 8 cm long; heads in a terminal corymb, with about 15 purple rays; phyllaries 8-10 mm long; achenes hairy. In Kenya *Senecio roseiflorus* is common in the drier alpine zone, 3100-4200 m [Agnew, 1994].

## 2.2 ETHNO-MEDICAL APPLICATION AND PHARMCOLOGICAL INFORMATION ON THE GENUS *DODONAEA* AND *SENECIO.*

### 2.2.1.1 ETHNO-MEDICAL APPLICATION OF *DODONAEA* SPECIES

Members of *Dodonaea* have been used for medicinal purposes by indigenous people in several continents and to a remarkable extent, for similar complaints. Table 2.2 gives a summary of different *Dodonaea* species, their origin and uses by different communities. The information given in the table is adapted from NAPRALERT, 2008 data base.

Table 2.2: Ethno-botanical uses of *Dodonaea* species

| **Species (Origin)** | **Plant part** | **Uses** | **Reference** |
|---|---|---|---|
| *Dodonaea viscosa* | | | |
| East Africa | Entire plant | Fish poison<br>Stomach pain | Hedberg *et al.*, 1983 |
| | Leaves | Hemorrhoids<br>Antipruritic in skin rashes<br>Febrifuge<br>Sore throat.<br>Antirheumatic<br>Hemorrhoids Stimulant<br>Anaesthetic Vermifuge<br>Dermatitis | Chhabra *et al.*, 1991a.<br>Hedberg *et al.*, 1983 |
| | Root | Increase lactation<br>Irregular menstruation | Kokwaro, 1976<br>Hedberg *et al.*, 1983 |
| Tanzania | Leaf | Antipruritic in skin rashes | Chhabra *et al.*, 1991a |
| | Root | Indigestion<br>Irregular menstruation<br>Peptic ulcer<br>Galactogogue in animals and humans<br>Indigestion<br>Peptic ulcers | Vasileva, 1969<br><br><br>Chhabra *et al.*, 1991a<br><br>Hedberg *et al.*, 1983 |
| Ethiopia | Leaves | Skin lesions<br>Fever<br>Malaria | Desta, 1993<br>Asres *et al.*, 2001 |
| Papua-New Guinea | Leaf | To make woman sterile.<br>For poultice | Holdsworth, 1989 |
| Guinea | Root | Augment lactation goats | Vasileva, 1969 |
| India | Fresh leaf | Wounds in cattle<br>Fractures in cattle<br>Swellings in cattle<br>Febrifuge<br>Rheumatism<br><br>Laxative<br>Menstruation | Davyt *et al.*, 1991<br>Reddy *et al.*, 1988<br><br>Hope *et al.*, 1993<br>Sukkawala & Desai, 1962 |
| Mexico | Entire plant | Stomach pain<br>Hepatic or splenic pain<br>Uterine colic | Rojas *et al.*, 1995 |
| | Leaves | Rheumatism<br>Wounds<br>Diarrhea<br>Skin infections<br>Fractures.<br>Used in a poultice<br>Postpartum recovery | Rojas *et al.*, 1996 |

Table 2.2: Ethno-botanical uses of *Dodonaea* species

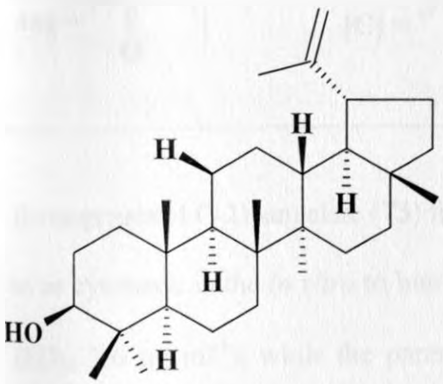
| Species (Origin) | Plant part | Uses | Reference |
|---|---|---|---|
| | Leaves | Menorrhagia<br>Hemorrhage<br>Infertility<br>Prevent miscarriage<br>Fever | Browner, 1985 |
| Uruguay | Fruit | Rheumatism | Gonzalez *et al.*, 1993 |
| | Dried leaf | Laxative | Davyt *et al.*, 1991 |
| | Dried leaf+ root | Astringent<br>Rheumatism | Gonzalez *et al.*, 1993 |
| | | Used as a febrifuge | Ramirez *et al.*, 1988 |
| Hawaii | Leaf | Asthma | Gonzalez *et al.*, 1993 |
| Peru | Dried leaf + stem | Increase lactation in cows | Ramirez *et al.*, 1988 |
| *Dodonaea viscosa* var. *angustifolia* | | | |
| Mexico | Dried aerial parts | Fevers | Dominguez *et al.*, 1980 |
| South Africa | | Pneumonia and tuberculosis | Watt & Breyer-Brandwijik, 1981 |
| Malaysia | Wood | Flatulence and cholic | |
| *Dodonaea viscosa* var. *angustissima* | | | |
| Australia | Leaves | Used to relieve fevers | Latz, 1995 |
| *Dodonaea lanceolata* | | | |
| Australia | Leaves | Pain and snake bite | Lassak *et al.*, 1990 |
| *Dodonaea madagascariensis* | | | |
| Madagascar | Leaves | Hypotensive and antispasmodic properties | Trotin *et al.*, 1972 |
| *Dodonaea physocarpa* | | | |
| Australia | Leaves+twigs | Alleviate symptoms of colds and flu | Barr, 1993 |
| *Dodonaea polyzyga* | | | |
| Australia | Leaves+twigs | Alleviate symptoms of colds and flu | Barr, 1993 |
| *Dodonaea triquetra* | | | |
| Australia | Root | Wounds and toothache | Dominguez *et al.*, 1980 |

## 2.3 BIOLOGICAL ACTIVITIES OF COMPOUNDS AND EXTRACTS FROM *DODONAEA* SPECIES

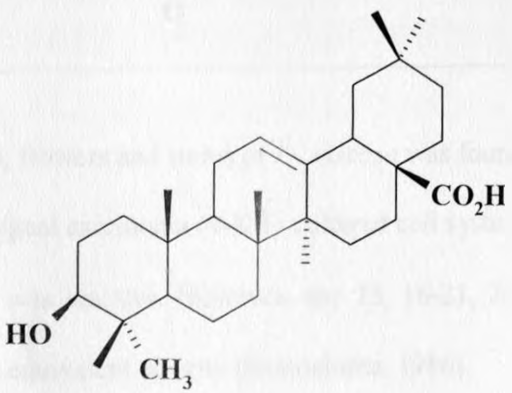
*Dodonaea* species extracts have been reported to have some bioactivities. An ethanolic extract of the leaves of *D. viscosa* showed anti-bacterial activity against various *Micrococcus, Bacillus, Salmonella* species and *Corynebacterium diphtheriae, Sarcina lutea and Escherichia coli* [Sukkawala and Desai, 1962]. In another study, a methanol extract of the aerial parts of *D. viscosa* collected in Mexico inhibited the growth of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* at

concentrations of 20 μg $ml^{-1}$ [Rojas *et al.*, 1992]. Extracts of the leaves of *D. madagascariensis* showed minimal anti-microbial activity [Trotin *et al.*, 1972]. The aqueous and ethanolic extracts obtained from the leaves of *D. viscosa* showed hypotensive properties [Sukkawala & Desai, 1962].

Many of the triterpenes present in *Dodonaea* have been shown to have bioactivity in separate studies. Lupeol (**69**) (25-250 mg $Kg^{-1}$) isolated from *D. attenuatta* var. *linearis* exerted anti-inflammatroy activity in a range of acute and chronic test models in rats. Oleonolic acid (**70**) obtained from the leaves of *D. madagascariensis* [Trotin, 1972] has been shown to have hepatoprotective, anti-hyperlipidemic and anti-inflammatory properties in laboratory animals [Lui, 1995].

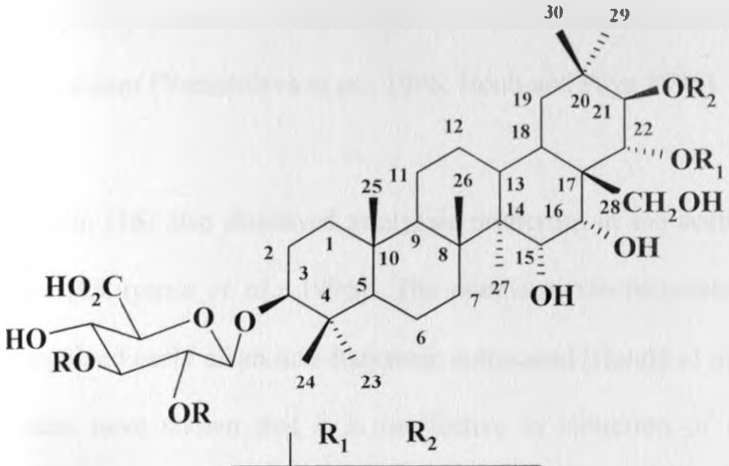



**69**

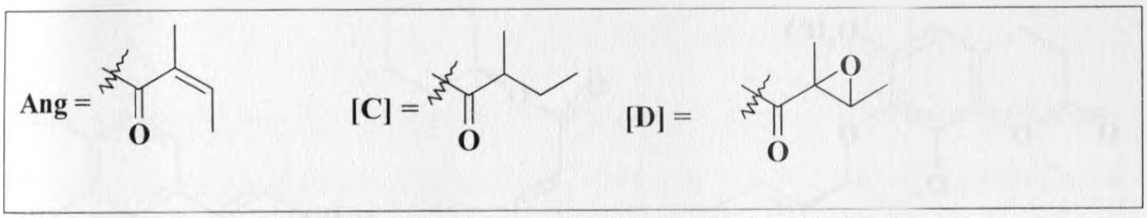



**70**

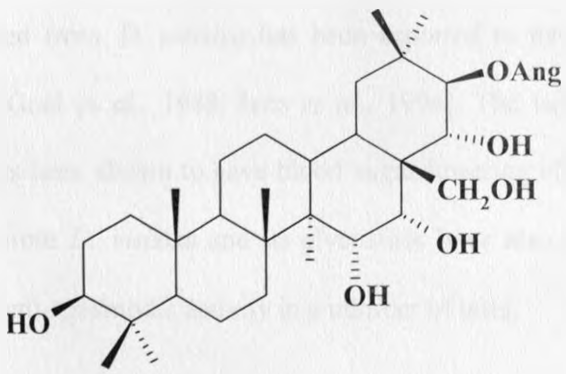
Triterpene saponins have long been known as fish poisons and the monodesmosidic saponins, with a free carboxylic acid at C-28, exhibit important molluscidal activity [Marston *et al.*, 1985]. Some saponins been tested for the control of schistosomiasis, a disease which affects millions of people living in Africa, Asia and South American countries. The saponins **71** and **72** isolated from *D. viscosa* showed 100% lethality at 25 ppm in the molluscicidal test using the bilharzia a vector snail *Biomphalaria glabrata* [Wagner *et al.*, 1987]. Many saponin preparations also have antitussive and expectorant properties as well as analgesic properties [Lacailledubois *et al.*, 1996].



| | $R_1$ | $R_2$ | |
|---|---|---|---|
| 71 | [C] | [D] | R = arabinosyl, galactosyl |
| 72 | Ang | [D] | |




Barringtogenol C-21-angelate (**73**) from the seeds, flowers and stems of *D. viscosa* was found to be cytotoxic in the *in vitro* to human nasopharygeal carcinoma (9-KB) cultured cell system ($ED_{50}$ 3.6 μg $ml^{-1}$), while the parent, triterpene was inactive. However, the 15, 16-21, 22-diacetonide from different *Dodonaea species* had equivalent activity [Konoshima, 1986].

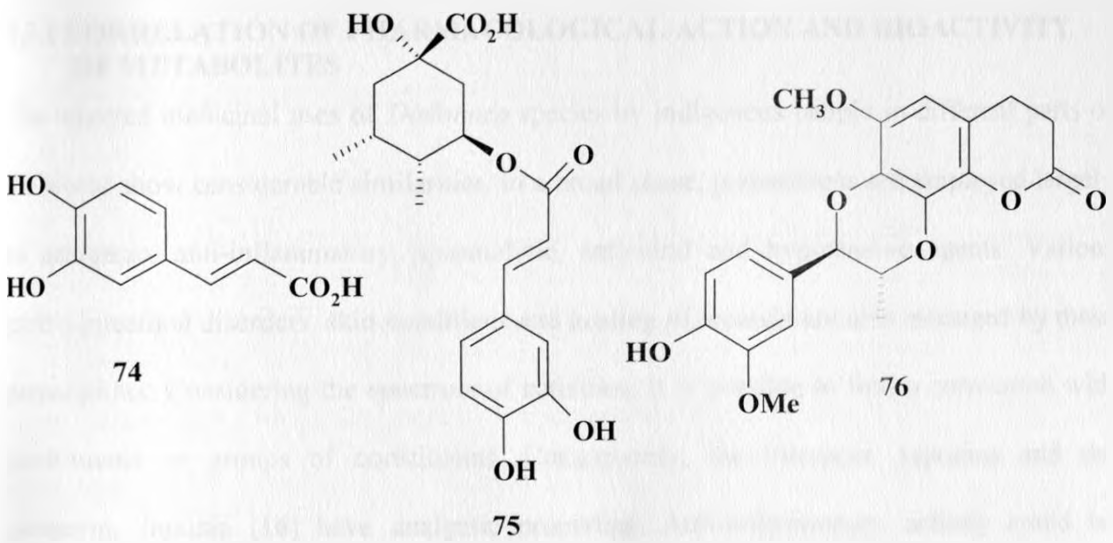


**73**

Hydroxycinnamic acids like caffeic acid (**74**), and chlorogenic acid (**75**) isolated from different *Dodonaea viscosa*, have anti-oxidant and tumour inhibiting activity [Johns *et al.*,

1995]. The coumarin, fraxetin (**16**) from *Dodonaea viscosa* has attracted some attention as an anti-oxidant [Yanishlieva *et al.*, 1996; Hoult and Paya 1996].

Fraxetin (**16**) also displayed analgesic properties in the acetic acid-induced writhing test on mice [Okuyama *et al.*, 1996]. The coumarin cleomiscosin A (**76**) from *D. viscosa* was recognized early as an anti-leukemic compound [Handa *et al.*, 1983]. However, more recent studies have shown that it is ineffective in induction of cell differentiation with human promyeolocytic leukemia (HL-60) cells [Luyengi *et al.*, 1996].

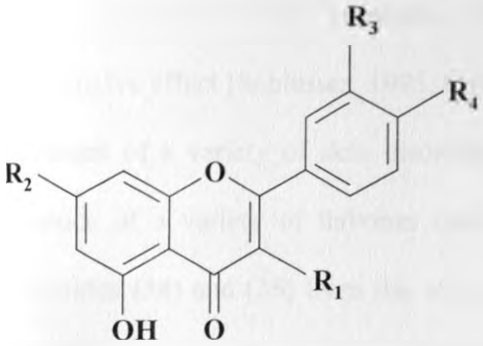



74 75 76

Kaempferol (**15**) isolated from *D. viscosa* has been reported to have anti-ulcer and anti-inflammatory activity [Goel *et al.*, 1988; Izzo *et al.*, 1994]. The isorhamnetin 3-glycoside (**77**) from *D. viscosa* has been shown to have blood sugar lowering effects [Zabroham *et al.*, 1986]. Quercetin (**78**) from *D. viscosa* and its glycosides have also been shown to exhibit anti-inflammatory and anti-spasmodic activity in a number of tests.

A number of 3-methoxyflavones, quercetin (**78**) and kaempferol (**15**) derivatives from *D. viscosa* have been shown to exhibit pronounced anti-viral activity and to be active against [Vlietinak *et al.*, 1995] picornavirus in tissue cultures. From a large screening programme,

some 3-methoxyflavones, including penduletin (**21**), from *D. viscosa* exhibited *in vitro* activity against polio and rhinovirus.

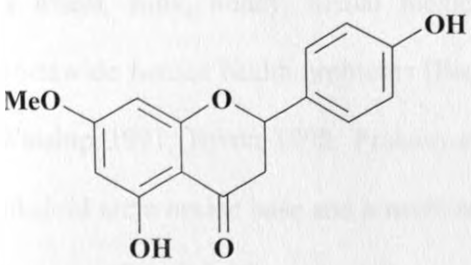



| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| 77 | ORut | OH | OH | OH |
| 78 | OH | OH | OH | OH |

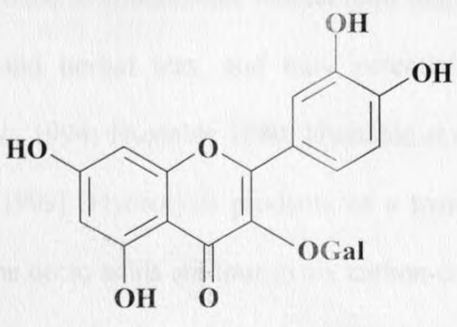
**Rut = rutinoside**

### 2.3.1 CORRELATION OF PHARMACOLOGICAL ACTION AND BIOACTIVITY OF METABOLITES

The reported medicinal uses of *Dodonaea* species by indigenous people in different parts of the world show considerable similarities. In a broad sense, preparations are employed largely as analgesic, anti-inflammatory, spasmolytic, anti-viral and hypotensive agents. Various gastro-intestinal disorders, skin conditions and healing of wounds are also managed by these preparations. Considering the spectrum of activities, it is possible to find a correlation with constituents or groups of constituents. Consequenty, the triterpene saponins and the coumarin, fraxetin (**16**) have analgesic properties. Anti-inflammatory activity could be associated with some of the diterpenes, lupeol (**69**), oleonolic acid (**70**), the saponins and the flavanoids. Spasmolytic activity could arise from the presence of some diterpenes, sakuranetin (**79**), quercetin (**78**) and rutin (**80**).




**79**




**80** **Gal = galactoside**

This activity could also explain the use of *Dodonaea* preparations to alleviate gastro-intestinal disorders. The 3-methoxyflavones could contribute to the anti-viral activity. The flavone hyperoside (**77**), quercetin (**78**) and rutin (**80**) have been shown to exert a hypotensive effect [Schlusser, 1995; Pathak, 1991]. The use of *Dodonaea* preparations in the treatment of a variety of skin disorders, ulcers and for wound healing is interesting. The presence of a variety of flavones including quercetin (**78**) from the leaves and oleanene glycosides (**34**) and (**35**) from the seeds of *D. viscosa* may contribute to the wound healing activity ascribed to preparations from these plants. In conclusion, there is significant circumstantial evidence for the pharmacological basis of the traditional medicinal uses of *Dodonaea* species. It seems likely that a number of compounds from these species may provide interesting leads for pharmacological evaluation.

### 2.2.1.2 ETHNO-MEDICAL APPLICATION OF THE GENUS *SENECIO*.

*Senecio* is known to elaborate pyrrolizidine alkaloids. Pyrrolizidine alkaloid-containing plants are widely distributed in many geographical regions in the world [Mattocks, 1986]. They exhibit hepatotoxic, mutagenic, carcinogenic and antitumor activities [Mattocks 1986; Rizk 1991; Wink, 1998].

Livestock are poisoned by grazing on plants containing pyrrolizidine alkaloids (PAs), causing livestock loss due to liver and pulmonary lesions [Roeder 1995; 2000; Smith & Culvenor 1981]. Pyrrolizidine alkaloids have also been found to contaminate human food sources, such as wheat, milk, honey, herbal medicines, and herbal teas, and may potentially cause worldwide human health problems [Betz *et al.*, 1994; Huxtable 1980; Huxtable *et al.*, 1983 Winship 1991; Byron 1998; Prakash *et al.*, 1999]. Hydrolysis products of a pyrrolizidine alkaloid are a necine base and a necic acid. The necic acids are four to six carbon-containing mono- or di-carboxylic acids, and are mostly branched-chained, unsaturated, hydroxylated, or epoxidized. Most of the pyrrolizidine alkaloids derived from esters of basic alcohols, the

necine bases, have been found to exhibit toxic effects. In most plants, they occur in their N-oxide form

PAs are a typical group of plant secondary compounds which are constitutively produced by plants as a defense against herbivores and insects [Hartmann & Ober, 2000]. They are part of a complex system of chemical ecological interactions between the plant and insect herbivores. Some adapted herbivores have even developed specific mechanisms to use these plant derived compounds for their own defense against predators [Ober, 2003; Hartmann, 2004].

Members of *Senecio* species has been used by indigenous people in several continents, to treat a number of ailments. Table 2.3 below summarizes the different *Senecio* species, their origin, plant parts and uses by different communities. The information given in Table 2.3 is adapted from NAPRALERT, 2006 data base.

Table 2.3: Ethno-medical application of *Senecio* species

| **Species (Origin)** | **Plant part** | **Use** | **Reference** |
|---|---|---|---|
| *S. abyssinicus* | | | |
| Nigeria | Roots | Treat rheumatism<br>Treat syphilis<br>Treat bruises<br>Used as a stomachic<br>Used as a blood purifier | Akah *et al.*, 1995 |
| *S. acanthifolius* | | | |
| Egypt | Flower + leaf | Treat amenorrhea | Dragendorff, 1898 |
| *S. albicaulis* | | | |
| Argentina | Aerial parts | Used to promote menstruation | Manfred, 1947 |
| *S. appendiculatus* | | | |
| Canary islands | Dried aerial parts | Used for dysmenorrhea | Darias *et al.*, 1989 |
| *S. aureus* | | | |
| USA | Aerial parts | Treat amenorrhea<br><br><br>Used as a diuretic<br>Used as a diaphoretic<br><br>Used as a tonic<br>Used as an emmenagogue | Schmid, 1976<br>"<br>"<br>Christopher, 1976<br><br><br>Krochmal and krochmal., 1973 |

Table 2.3: Ethno-medical application of *Senecio* species

| Species (Origin) | Plant part | Use | Reference |
|---|---|---|---|
| | Entire plant | Used to hasten delayed childbirth | Krochmal and krochmal, 1973 |
| | | Used as female regulator | ,, |
| | | Used for painful and spasmodic menstruation | Fielder, 1975 |
| | | Used to hasten childbirth | Lewis *et al.*, 1977 |
| India | Dried aerial part | Used for nervous disorders | Krochmal & krochmal, 1973 |
| England | Entire plant | Used for abortion | Speck, 1944 |
| | | Used ergot during parturiton | Shemluck, 1982 |
| | | Used as emmenagogue | Krag, 1976 |
| | ,, | Used as sedative | |
| | Rhizome | Used as diuretic<br>Used to promote menstrual discharge | Mausert, 1932 |
| | Root | Used for dysmenorrhea | Novitch & Schweiker, 1982 |
| *S. biafrae* | | | |
| Nigeria | Fresh leaf | Used for wound dressing<br>Used as an antiseptic<br>Used for indigestion | Akah, 1995 |
| *S. brasiliensis* | | | |
| Brazil | Dried entire plant | Treat fevers | Brandao *et al.*, 1985 |
| | Dried leaf | Treat malaria | Giberti, 1983 |
| Argentina | | Used as a febrifuge | |
| *S. candicans* | | | |
| Chile | Dried aerial parts | Said to be toxic | Urzua *et al.*, 1989 |
| *S. canicida* | | | |
| Mexico | Aerial parts | Used to kill dogs and coyotes | Mendez, 1937 |
| | ,, | Has been used in homeopathy to treat epilepsy | |
| *S. cannabifolius* | | | |
| Japan | Dried aerial parts | Eaten as a food | Hirono *et al.*, 1983 |
| *S. chenopodioides* | | | |
| Honduras | Dried entire plant | Used for aches and pains | Lentz *et al.*, 1998 |
| *S. chionogeton* | | | |
| Peru | Dried flowers | Used as a diaphoretic | Ramirez *et al.*, 1988 |
| | | Used as an expectorant<br>Used for bronchitis | |

Table 2.3: Ethno-medical application of *Senecio* species

| Species (Origin) | Plant part | Use | Reference |
|---|---|---|---|
| | Dried leaf | Used as an expectorant<br>Used for bronchitis<br>Used as a diaphoretic | |
| *S. chrysanthemoides* | | | |
| India | Dried entire plant | Used by physicians's as a medicine for debility | Koelz, 1979 |
| Nepal | Dried root | Used for indigestion | Manandhar, 1986 |
| *S. cineraria* | | | |
| South America | Entire plant | Used as an emmenagogue | Dragendorff, 1898 |
| France | Dried entire plant | Used as an emmenagogue | Benzanger-Beauquesne *et al*, 1980 |
| *S. cydoniifolius* | | | |
| Rwanda | Dried leaf | Used for diarrhea | Maikere-Faniyo *et al*, 1989 |
| *S. discifolius* | | | |
| East Africa | Entire plant<br>Leaf | Used to stimulate milk flow<br>Used as an anthelmintic | Kokwaro, 1976 |
| *S. discolor* | | | |
| Jamaica | Entire plant | Used to make a tea for fever | Asprey & Thornton, 1955 |
| *S. diversifolius* | | | |
| Nepal | Leaf juice<br>" | Used as a hemostatic.<br>Used as an anti-bacterial<br>Used for bleeding wounds and cuts as a hemostatic agent<br>Used for bleeding wounds and cuts as an antiseptic | Bhattarai, 1997 |
| *S. douglasii* | | | |
| USA | Aerial parts | Used as a cough medicine | Stillman *et al.*, 1977 |
| | | Used for pulmonary diseases | Huxtable, 1989; 1990 |
| | | Used for infected sores, or for cuts | Bocek, 1984 |
| | Dried entire plant | Used for a "cold in the idneys<br>Used for puerperal tetanus. | |
| *S. eriophyton* | | | |
| Bolivia | Dried leaf | Used as an emmenagogue. | Gonzalez & Silva, 1987 |
| Chile | " | " | " |
| *S. erosus* | | | |
| Peru | Leaf | Used for pains in the kidney. | Yelasco-Negueruela *et al.*, 1995 |

Table 2.3: Ethno-medical application of *Senecio* species

| Species (Origin) | Plant part | Use | Reference |
|---|---|---|---|
| *S. gigas* | | | |
| Ethiopia | Dried leaf | Treat typhus. | Desta, 1993 |
| | Dried root | Treat typhoid fever<br>Treat rheumatism | Asres *et al.*, 2001 |
| *S. glaucus* | | | |
| Kuwait | Entire plant | Used as an emmenagogue in case of amenorrhea | Alami *et al.*, 1975 |
| *S. graveolens* | | | |
| Argentina | Dried aerial | Used against diarrhea<br>Treat respiratory tract infections<br>Treat urinary tract infections | Perez & Anesini, 1994 |
| | | Treat stomach pains<br>Used as an antitussive<br>Used for altitude sickness | Giberti, 1983 |
| Chile | | Used for altitude sickness | Loyola *et al.*, 1985 |
| Bolivia | | Used as a tranquilizer for gastritis<br>Used as an expectorant for chronic cough | Bastien, 1983 |
| *S. grisebachii* | | | |
| Paraguay | Fresh inflorescence | Used to counteract misfortune, thought to be prevalent in August | Schmeda & Cespedes, 1986 |
| *S. hartwegii* | | | |
| Mexico | Fresh root | Rubbed on affected areas to kill lice and/or ticks | Ishikura, 1982 |
| *S. inornatus* | | | |
| Mexico | Dried aerial parts | Used for cardiac ailments<br>Used for respiratory ailments | Wiedenfeld *et al.*, 1996 |
| *S. integerrimus* | | | |
| USA | Entire plant | Used as a female regulator<br>Used as an emmenagogue | Fielder, 1975<br>Krag, 1976 |
| *S. integrifolius* | | | |
| China | Entire plant | Used as an anticancer agent | Duke and Ayensu, 1985 |
| *S. jacobaea* | | | |
| England | Aerial plant parts | If eaten by sheep their wool grows loose<br>Used as an emmenagogue<br>Used against cancerous lesions | Culpeper, 1650 |
| | Dried entire plant | Used as an emmenagogue | Dragendorff, 1898 |

Table 2.3: Ethno-medical application of *Senecio* species

| Species (Origin) | Plant part | Use | Reference |
|---|---|---|---|
| France | Dried entire plant | Used as an emmenagogue | Culpeper, 1650 |
| *S. kaempferi* | | | |
| Canary islands | Dried entire plant | Used for dysmenorrhea | Benzanger-Beauquesne *et al.*, 1980 |
| *S. latifolius* | | | |
| South Africa | Dried entire plant | Used for wounds<br>Used for sores | Simon & Lamla, 1991 |
| *S. linifolius* | | | |
| Spain | Dried aerial parts | Used in folks medicine. | Torres *et al.*, 1988 |
| *S. mannii* | | | |
| Rwanda | Dried leaf | Used for malaria | Hakizamungu, 1992 |
| *S. maranguensis* | | | |
| Tanzania | Dried entire plant | A Haya remedy for yaws and syphilis | Watt & Breyer-Brandwijk, 1962 |
| *S. moorei* | | | |
| Kenya | Fresh aerial parts | Suspected of being toxic to cows when eaten | Mugera, 1970 |
| *S. nemoralis ssp.fuchsii* | | | |
| Germany | Aerial parts<br>Dried entire plant | Used as a diabetic tea<br>Used for diabetes | Habs *et al.*, 1982a<br>Habs, 1982b |
| *S. nitidus* | | | |
| Colombia | Entire plant | Used to treat menstrual perturbations<br>Used as an emmenagogue | Garcia-Barriga, 1975<br>Gonzalez & Silva, 1987 |
| *S. nudicaulis* | | | |
| India | Dried entire plant<br>Fresh leaf juice<br>Root | Used to treat itching<br>Used for gonorrhea<br>Used as an anti-inflammatory agent | Jain & Puri, 1984<br>Jain *et al.*, 1994 |
| *S. obovatus* | | | |
| USA | Aerial parts | Used as an emmenagogue (hepatotoxic and/or carcinogenic) | Burlage, 1968 |
| *S. oreophyton* | | | |
| Chile | Aerial parts | Used as an emmenagogue | Gonzalez & Silva, 1987 |
| *S. oryzetorum* | | | |
| China | Entire plant | Used as an anticancer agent | Duke, 1985 |
| *S. oxyriaefolius* | | | |
| South Africa | Dried root | Used by a Mpondo female herbalist as a remedy for | Watt & Breyer-Brandwijk, 1962 |

Table 2.3: Ethno-medical application of *Senecio* species

| Species (Origin) | Plant part | Use | Reference |
|---|---|---|---|
| | | sterility in a married woman | |
| *S. palmatus* | | | |
| China | Dried entire plant | Used for amenorrhea<br>Used for abdominal distention and cramps. | Manual, 1974 |
| *S. pauperculus* | | | |
| USA-TX | Aerial parts | Used as an emmenagogue (hepatotoxic and/or carcinogenic) | Burlage, 1968 |
| *S. pseud-otites* | | | |
| Bolivia<br><br>Peru | Dried leaf | Used as an emmenagogue<br><br>Known as an abortive properties | Gonzalez & Silva, 1987<br>Debelmas, 1975 |
| *S. quinquelobus* | | | |
| India | Dried seed | Used for colic | Joshi *et al.*, 1982 |
| *S. rhizomatus* | | | |
| Peru | Dried leaf | Used as an astringent<br><br>Used as a diuretic<br>Used as a eupeptic<br>Used for pneumonia<br>Used in cases of sterility<br>Used for acne | Ramirez *et al.*, 1988 |
| *S. rudbeckiaefolius* | | | |
| Peru | Leaf | Used as an antitussive<br><br><br>Used to cure dislocations | Yelasco-Negueruela *et al.*, 1995 |
| *S. salignus* | | | |
| Mexico<br><br>Quatemala | Leaf<br><br>Dried leaf | Used as a tonic<br><br>Used for ringworm<br><br>Used for pimples and pustules<br>Used for conjunctivitis | Zamora-Martinez & Pola, 1992<br>Caceres *et al.*, 1987 |
| *S. scandens* | | | |
| India<br><br>China<br><br><br>China<br><br><br>India | Aerial part<br>„<br>Entire plant<br><br><br>Dried entire plant<br><br><br>Dried leaf | Used for jaundice<br>Used for malaria<br>Used as an anti-cancer agent<br><br>Used for fever<br>Used for ophthalmic disorders<br><br><br>Used for malaria<br>Used for eye troubles | Srivastava, 1993<br>„<br>Duke & Ayensu., 1985<br><br>Iam *et al.*, 2000 & Matsuda *et al.*, 1995<br><br>Manual, 1977<br>Srivastava, 1993 |
| *S. schimperi* | | | |

Table 2.3: Ethno-medical application of *Senecio* species

| **Species (Origin)** | **Plant part** | **Use** | **Reference** |
|---|---|---|---|
| Yemen | Dried leaf | Used medicinally | Fleurentin *et al.*, 1983 |
| *S. serratuloides* var.*serratuloides* | | | |
| South Africa | Dried aerial parts | Used for tuberculosis | Lall & Meyer, 1999 |
| *S. sonchifolius* | | | |
| Indonesia | Fresh root | Used for mild diarrhea. | Hirschhorn, 1983 |
| *S.* species | | | |
| Tanzania | Dried aerial parts | Used to treat fever<br>Used to treat sores.<br>Used to treat colic<br>Used to treat skin rashes | Chhabra & Uiso, 1991b |
| Yemen | | Used as bush tea<br>Used medicinally | Scott *et al.*, 1962<br>Fleurentin *et al.*, 1983 |
| *S. spegazzinii* | | | |
| Argentina | Dried aerial parts | Used to treat earache | Giberti, 1983 |
| *S. tenuifolius* | | | |
| India | Entire plant | Used for amenorrhea<br>Used for dysmenorrhea | Nagaraju & Rao, 1990 |
| *S. triangularis* | | | |
| India | Dried entire plant | Used as a sedative.<br>Used to treat chest pain | Rueger & Benn, 1983 |
| USA | Dried leaf + root | Used as a sedative | Hart, 1981<br>Shemluck, 1982 |
| *S. tussilaginis* | | | |
| Canary islands | Dried aerial parts | Used as an anti-tussive<br>Used for catarrh in children | Darias *et al.*, 1986 |
| *S. uspallatensis* | | | |
| Argentina | Dried root | Used as a substitute for mate infusion (ilex paraguariensis) | Pestchanker *et al.*, 1985b |
| *S. vaccinioides* | | | |
| Ecuador | Dried entire plant | Used as an emmenagogue. | Gonzalez & Silva, 1987 |
| *S. viridis* var.*viridis* | | | |
| Argentina | Dried leaf | Chewed to calm tooth pains | Giberti, 1983 |
| *S. volckmannii* | | | |
| Argentina | Dried aerial parts | Used to treat shock | Giberti, 1983 |
| *S.vulgaris* | | | |
| England | Aerial parts | Used for internal ulcer healing<br>Used for sciatica<br>Used as an antiepileptic | Culpeper, 1650<br>Dragendorff, 1898 |

Table 2.3: Ethno-medical application of *Senecio* species

| Species (Origin) | Plant part | Use | Reference |
|---|---|---|---|
| | | Used for menstrual troubles/complaints | ,, |
| | | | ,, |
| | | Used as an anthelmintic | ,, |
| | | Used as an emmenagogue(hepatotoxic and/or carcinogenic | Chopra *et al.*, 1949 |
| India | Sap | | |
| | | Used as an emmenagogue | Al-Rawi & Chakravarty, 1964 |
| Iraq | ,, | | |
| Italy | ,, | | De Feo *et al.*, 1992 |
| | | Used as a washing to treat varicose veins | ,, |
| ,, | Entire plant | Used to treat internal varices. | |
| Ecuador | ,, | Used as an emmenagogue. | Gonzalez & Silva, 1987 |
| Europe | ,, | Used for amenorrhea | Watt & Breyer-Brandwijk, 1962 |
| France | Dried entire plant | Used as an emmenagogue (hepatotoxic and/or carcinogenic) | Benzanger-Beauquesne *et al.*, 1980 |
| Tunisia | Dried entire plant | Used as an emmenagogue | Lemordant *et al.*, 1978 |
| USA | Root | Used as a uterine sedative | ,, |
| | | Used as an emmenagogue | ,, |
| | Dried entire plant | Used as an emmenagogue | Watt and Breyer-Brandwijk, 1962 |

## 2.4 PHYTOCHEMISTRY OF THE GENUS *DODONAEA*

Review on the chemical information of the *Dodonaea* species reveals that the most commonly reported secondary metabolites are diterpenes, flavonoids, triterpenes, and shikimate-derived metabolites. Others compounds include essential oils, carbohydrates and sterols. These classes of compounds are the main constituents of plant resins [Jefferies & Payne, 1973].

### 2.4.1 COMPOUNDS FROM *DODONAEA* SPECIES

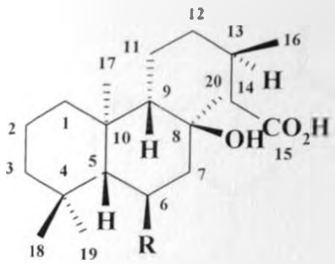
#### 2.4.1.1 DITERPENES FROM *DODONAEA* SPECIES

Many *Dodonaea* species possess viscid resinous exudate on the leaves surfaces. The resins are composed mainly of bicyclic diterpenes, with the occasional inclusion of flavones [Jefferies & Payne, 1973]. Bicyclic diterpenes (**69-87**) (Table 2.4) based on the *ent*-labdane

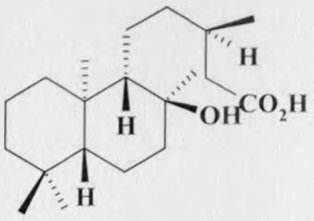
and *ent*-clerodane skeleton have been isolated from various *Dodonaea* species. From *D. viscosa*, the most widespread member of the genus, the diterpenoids, hautriwaic acid (**17**) [Mata *et al.*, 1991], methyl dodonate A (**92**) [Ortega *et al.*, 2001], methyl dodonate B (**93**) [Ortega *et al.*, 2001],, methyl dodonate C (**94**) [Ortega *et al.*, 2001], the *ent*-labdane furan (**76**) [Mata *et al.*, 1991] and the *ent*-clerodane furan (**82**) (Sadchev *et al.*, 1984) have been reported.

Table 2.4: Labdane and clerodane type diterpenoids from *Dodonaea* species

| Labdane diterpenoids | | |
|---|---|---|
| 6,8-Dihydroxy-15-labdanoic acid; (*ent*-6α,8α,13ξ)-form, 6-Ac (**81**) | *D. inaequifolia* | Dawson *et al.*, 1966 |
| 8-Hydroxy-15-labdanoic acid; (*ent*-6α,8α,13ξ)-form (**82**) | *D. lobulata*<br>*D. ptarmicaefolia* | Dawson *et al.*, 1966 |
| 6,8-Dihydroxy-15-labdanoic acid; (*ent*-6α,8α,13ξ)-form (**83**) | *D. lobulata*<br>*D. ptarmicaefolia* | Dawson *et al.*, 1966 |
| 7,8-Dihydroxy-15-labdanoic acid; (*ent*-6α,8α,13ξ)-form (**84**) | *D. lobulata*<br>*D. ptarmicaefolia* | Dawson *et al.*, 1966 |
| 15,16-Epoxy-3-hydroxy-8(17),13(16),14-labdatrien-18-oic acid; (*ent*-3β)-form, 3-Ac (**85**) | *D. petiolaris* | Jefferies *et al.*, 1981 |
| 7,13-Labdadiene-2,15-diol; 15-Carboxylic acid (**86**) | *D. microzyga* | Jefferies *et al.*, 1974 |
| 2,17-Dihydroxy-7,13-labdadien-15-oic acid; (*ent*-2α,13*E*)-form (**87**) | *D. microzyga* | Jefferies *et al.*, 1974 |
| 15,16-Epoxy-13(16),14-labdadiene-3,8-diol.(*ent*-3β,8α)-form (**11**) | *D. viscosa* | Dawson *et al.*, 1966<br>Mata *et al.*, 1991 |
| *ent*-Labdanolic acid (**91**) | *D. lobulata* | Dawson *et al.*, 1966 |
| Clerodane type diterpenoids from *Dodonaea* species | | |
| Hautriwaic acid (**17**) | *D. attenuata* var *linearis*<br>*D. viscosa* | Jefferies & Payne, 1967<br>Jefferies & Payne, 1973 |
| 7α-Hydroxy- hautriwaic lactone (**88**) | *D. attenuata* | Dawson *et al.*, 1966 |
| Hautriwaic lactone, 7-Ac (**89**) | *D. attenuata* | Dawson *et al.*, 1966 |
| Hautriwaic lactone (**90**) | *D. attenuata* var. *linearis* | Jefferies & Payne, 1967<br>Abdel-Mogib *et al.*, 2001 |
| 2β-Hydroxy hardwickiic acid (**9**) | *D. boroniifolia* | Jefferies *et al.*, 1973 |
| Dodonic acid (**10**) | *D. viscosa* | Sachdev *et al.*, 1984 |
| Methyl dodonate A (**92**) | *D. viscosa* | Ortega *et al.*, 2001 |
| Methyl dodonate A (**93**) | *D. viscosa* | Ortega *et al.*, 2001 |
| Methyl dodonate C (**94**) | *D. viscosa* | Ortega *et al.*, 2001 |
| Dodonolide (**95**) | *D. viscosa* | Ortega *et al.*, 2001 |



| | R |
|---|---|
| 81 | OAc |
| 83 | OH |



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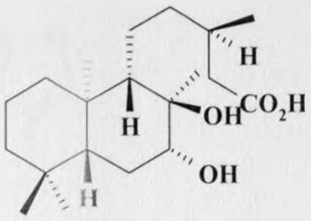

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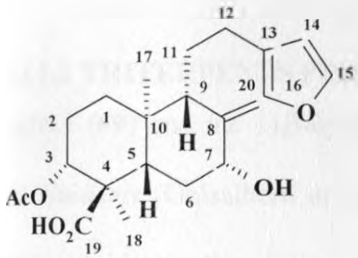

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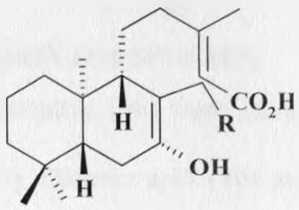
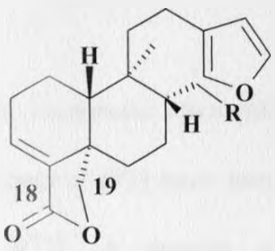
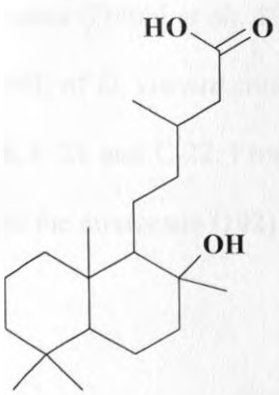


| | R |
|---|---|
| 86 | H |
| 87 | OH |



| | R |
|---|---|
| 88 | OH |
| 89 | OAc |
| 90 | H |



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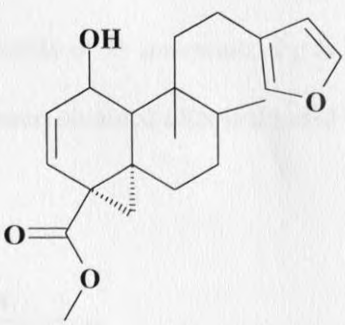

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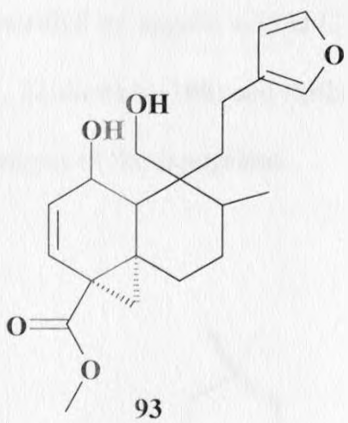

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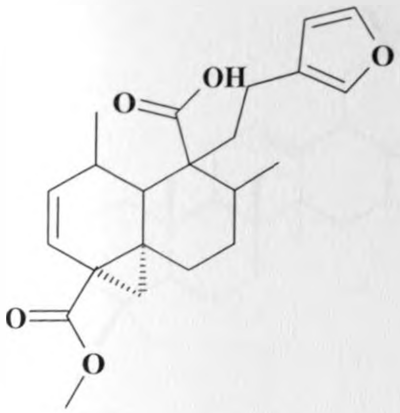


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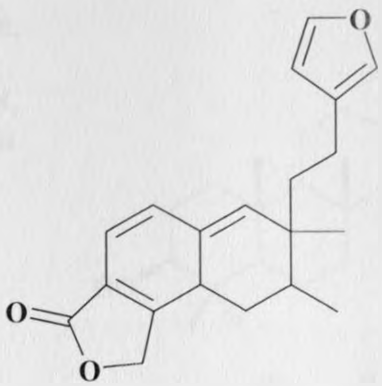


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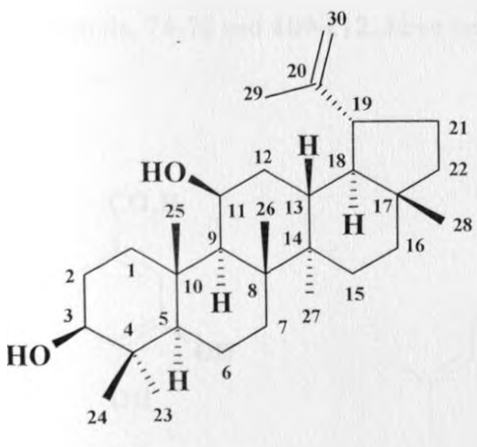
### 2.4.1.2 TRITERPENES FROM *DODONAEA* SPECIES.

Lupeol (**69**) and the 11β-hydroxy derivative (**96**) were isolated from *Dodonaea attenuatta* var. *linearis* [Ghisalberti *et al.*, 1973]. Oleonic acid (**70**) and hederogenol (**97**) have been obtained from the leaves of *D. madagascariensis* [Trotin, 1972]. A number of polyhydroxylate triterpenes containing the oleone skeleton have also been isolated. Among these jegosapogenol (**98**) and $R_1$-barrigenol (**99**) were obtained from the stem bark of *D. viscosa* [Dimbi *et al.*, 1985]. The seeds (Khan *et al.*, 1992), flowers and stems [Dimbi *et al.*, 1985] of *D. viscosa* contain derivatives of $R_1$-barrigenol (**99**) esterified by angelic acid at C-16, C-21 and C-22. From the seeds of *D. attenuata*, the two 21, 22-diesters (**100**) and (**101**) and the monoester (**102**) have been obtained after mild acid hydrolysis of the sapogenins.

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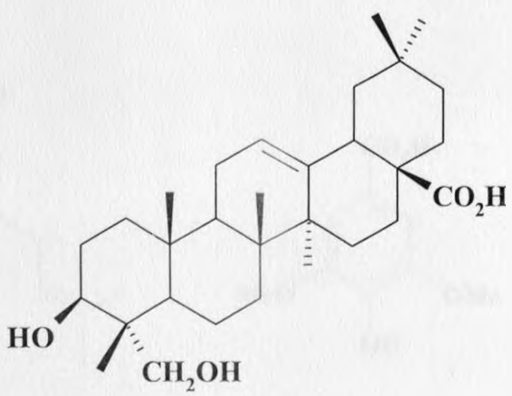


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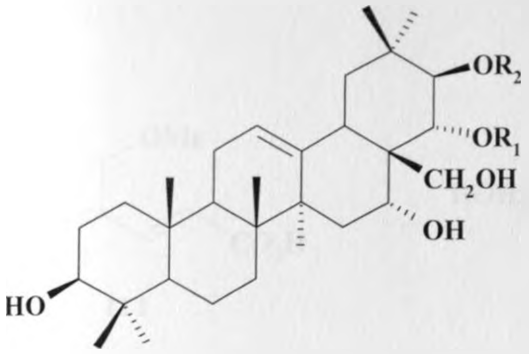
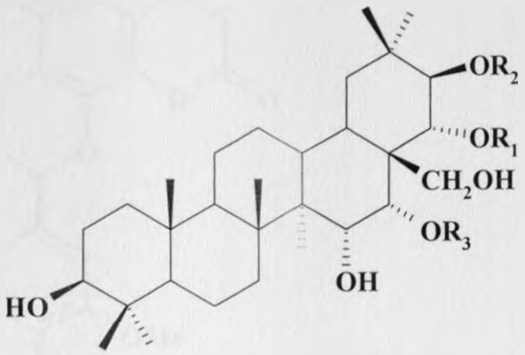
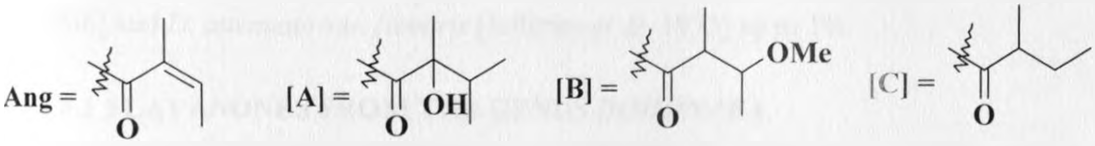



| | $R_1$ | $R_2$ |
|---|---|---|
| 98 | H | H |
| 99 | Ang | Ang |
| 103 | Ang | [A] |

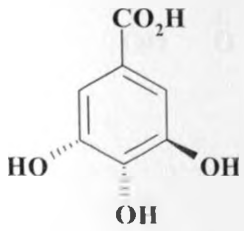



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 100 | H | [B] | H |
| 101 | Ang | [B] | H |
| 102 | Ang | [C] | H |
| 104 | H | H | H |
| 105 | Ang | Ang | H |
| 106 | Ang | [A] | H |
| 107 | H | H | Ang |

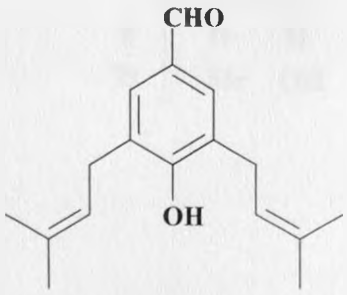



### 2.4.1.3 SHIKIMATE DERIVED METABOLITES FROM *DODONAEA* SPECIES

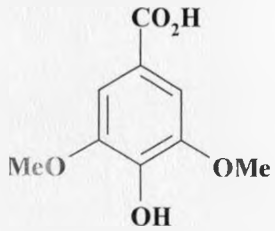
Shikimic acid (**108**) [Khan *et al.*, 1992] and a group of shikimic acid derived aromatic compounds, **74-76** and **109-112,** have been isolated from *Dodonaea* species.



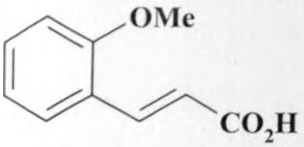

**108**




**109**




**110**

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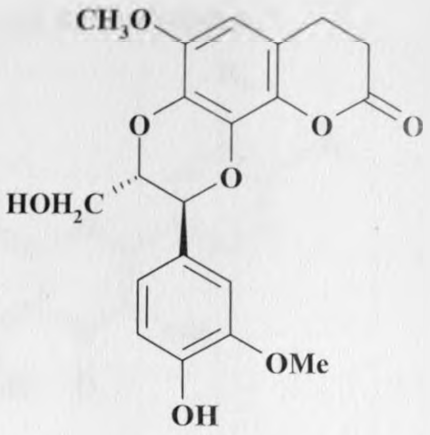


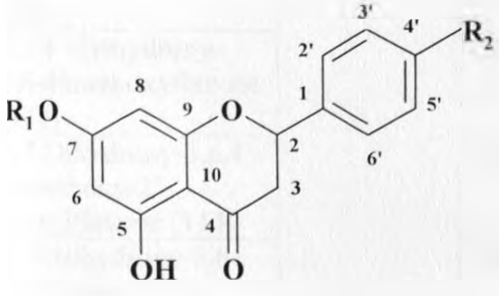

112

### 2.4.1.4. FLAVONOIDS FROM *DODONAEA* SPECIES

Many flavones **1**, **4-6**, **8**, **12**, **14-15**, **21**, **77-80** and **113-123** (Table 2.5), have also been isolated from the seeds, bark, flowers and leaves of *Dodonaea* species. A significant number contain a methoxy group at C-3 and C-6. In *D. madagascariensis*, flavonoids account for up to 3.3% of the dry weight of dry leaves [Trotin *et al.*, 1970] and in *D. lobulata* [Dawson *et al.*, 1966] and *D. attenuata* var. *linearis* [Jefferies *et al*, 1973] up to 1%.

#### 2.4.1.4.1 FLAVANONES FROM THE GENUS *DODONAEA*.

The flavanones 5,7-dihydroxyflavanone (**8**) [Sachdev & Kulshreshtha, 1983] and 5,4-dihydroxy 7-methoxyflavanone (**79**) [Mata *et al.*, 1991] have been isolated from *D.viscosa*.




| | $R_1$ | $R_2$ |
|---|---|---|
| **8** | **H** | **H** |
| **79** | **Me** | **OH** |

### 2.4.1.4.2 FLAVONES FROM THE GENUS *DODONAEA*

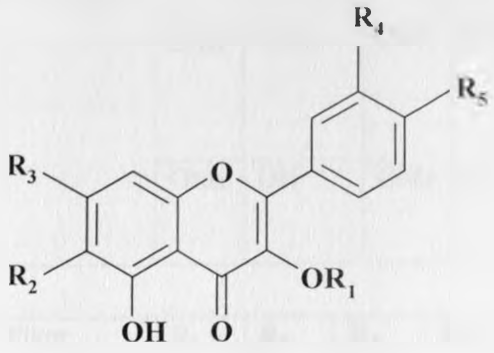



Table 2.5: Flavones from *Dodonaea* species

| 3-Methoxyflavones | | | | | | | |
|---|---|---|---|---|---|---|---|
| **3-methoxyflavone** | Plant source | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Reference |
| 5,7-Dihydroxy-3,6,4'-trimethoxyflavone (**4**) | D. *attenuata* var. *linearis* *D. viscosa* *D. viscosa* var. *angustifolia* | OMe | OMe | OH | H | OMe | Jefferies & Payne, 1973 |
| 5-Hydroxy-3,6,7,4'-tetramethoxyflavone (**14**) | *D. lobulata* *D. viscosa* | OMe | OMe | OMe | H | OMe | Sachdev & Kulshreshtha, 1983 |
| 5-Hydroxy-3,7,4'-trimethoxyflavone (**1**) | *D. viscosa* | OMe | H | OMe | H | OMe | Dreyer, 1978 |
| 5,7,4 -Trihydroxy-3-methoxyflavone (**6**) | | OMe | H | OH | H | OH | Wollenweber *et al.*, 1986 |
| 5,4'-Dihydroxy-3,6,7-trimethoxyflavone (**12**) | | OMe | OMe | OMe | H | OH | Khan *et al.*, 1992 |
| 5,7-Dihydroxy-3,4'-dimethoxyflavone (**21**). | | OMe | H | OH | H | OMe | |
| 5,7,4'-Trihydroxy-3,6-dimethoxyflavone (**113**) | | OMe | OMe | OH | H | OH | Sachdev & Kulshreshtha, 1983 |
| 5,7-Dihydroxy-3,6,4'-trimethoxy-2'-prenylflavone (**114**) | | OMe | OMe | OH | Pre | OMe | |
| 5, 7-Dihydroxy-3,6-dimethoxy-2'-(3-hydroxymethylbutyl) flavone (**115**) | | OMe | OMe | OH | [A] | OH | |
| 5, 7-Dihydroxy-3,6,4'-trimethoxy-2'-(3-hydroxymethylbutyl) flavone (**116**). | | OMe | OMe | OH | [A] | OMe | |
| 5,4'-Dihydroxy-3,6,7-trimethoxy-2'-(3-hydroxymethylbutyl) | | OMe | OMe | OMe | [A] | OH | |

Table 2.5: Flavones from *Dodonaea* species

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| flavone (**117**). | | | | | | | |
| 5-Hydroxy-3,6,7,4`-tetramethoxy-2`-(3-hydroxymethylbutyl) flavone (**118**). | | OMe | OMe | OMe | [A] | OMe | |
| 5,6,4`-Trihydroxy-7-methoxyflavone (**119**). | | OMe | OH | OMe | H | OH | Khan *et al.*, 1992 |

Flavonols

| Flavonol | Plant source | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Reference |
|---|---|---|---|---|---|---|---|
| 5,4`-Dihydroxy-7-methoxyflavonol (**5**) | *D. viscosa* | OH | H | OMe | H | OH | Wollenweber *et al.*, 1986 |
| 5,7,4`-Trihydroxyflavonol (**15**) | | OH | H | OH | H | OH | Paris & Nothis, 1970 |
| 5,7,2`,4`-Tetrahydroxyflavonol. (**78**) | | OH | H | OH | OH | OH | Khan *et al.*, 1992 |
| 5, 7,4`-trihydroxy-2`-methoxyflavonol.(**120**) | | OH | H | OH | OMe | OH | |

Glycosides

| Glycosides | Plant source | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Reference |
|---|---|---|---|---|---|---|---|
| [77] | *D. madagascariensis* *D. viscosa* | ORut | H | OH | OH | OH | Wollenweber *et al.*, 1986 |
| [80] | | Ogal | H | OH | OH | OH | Ramachandran & Subramanian, 1978 |
| [121] | *D. madagascariensis* | OGal | H | OGlu | OMe | OH | Trotin *et al.*, 1972 |
| [122] | *D. viscosa* | ORha-gal | H | OH | OMe | OH | Khan *et al.*, 1992 |
| [123] | | ORut | H | OH | OMe | OH | Ramachandran & Subramanian, 1978 |

[A] = 3-Hydroxymethylbutyl; Pre = prenyl; Ogal = galactoside; OGlu glucoside; ORha = rhamnoside; ORut = rutinoside.

### 2.4.2 GENERAL PHYTOCHEMICAL INFORMATION ON *SENECIO* SPECIES

A large variety of sesquiterpenoids [Bohlmann *et al.*, 1985; Dupre *et al.*, 1991], triterpenoids [Torres *et al.*, 1998], diterpenoids [Dong- Liang *et al.*, 1992], pyrrolizidine alkaloids [Bohlmann *et al.*, 1986b] and shikimic acid and its derivatives [Cardoso *et al.*, 1987] have been characterized from *Senecio* species [Ndom *et al.*, 2006].

### 2.4.2.1. PYRROLIZIDINE ALKALOIDS FROM *SENECIO* SPECIES

Pyrrolizidine alkaloids (PAs) are a class of phytochemicals found in several genera of the plant families, the occurrence of PAs is restricted to certain unrelated families within the angiosperm, particularly the three plant families, Boraginaceae, Compositae (Asteraceae), and Legumionsae (Fabaceae) and in more than 350 plant species, mainly the *Heliotroprium*, *Senecio*, *Crotalaria*, and *Symphytum* species.

The structures and numbering system of the four types of representative necine bases, platynecine (**124**), retronecine (**125**), heliotridine (**126**), and otonecine (**127**) are shown in Figure 2.9. The platynecine type pyrrolizidine alkaloids do not contain a double bond in the base, and retronecine and heliotridine are enantiomers. Because of their abundance and toxicities, including hepatotoxicity and carcinogenicity, the retronecine- and heliotridine-derived pyrrolizidine alkaloids have received the most attention.

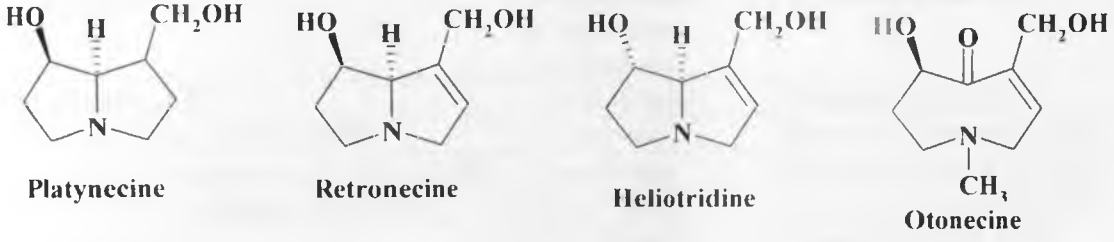



**Figure 2.8: Representative necine bases.**

Table 2.6: Pyrrolizidine alkaloids from *Senecio* species

| Alkaloid | Source | Reference |
|---|---|---|
| Bulgarsenine (**124**) | *S. abrotanifolius*<br>*S. doronicum*<br>*S. nemorensis* | Roeder *et al.*, 1984 |
| Platyphylline (**125**) | *S. adnatus*<br>*S. hygrophyllus*<br>*S. platyphyllus* | Koekemoer *et al.*, 1951 |
| Platyphylline *N*-oxide (**126**) | *S. adnatus*<br>*S. hygrophyllus*<br>*S. platyphyllus* | Koekemoer *et al.*, 1951 |
| Adonifoline (**127**) | *S. adonidifolius* | Witte *et al.*, 1992b |
| Erucifoline (**128**) | *S. aegypticus* | Witte *et al.*, 1992a; Roeder |

Table 2.6: Pyrrolizidine alkaloids from *Senecio* species

| Alkaloid | Source | Reference |
|---|---|---|
| | *S. erucifolius*<br>*S. erraticus*<br>*S. jacobaea*<br>*S. persoonii* | *et al.*,1993 |
| Jacozine (**129**) | *S. alpinus*<br>*S. jacobaea*<br>*S. incanus* | Klasek *et al.*, 1968 |
| Angularine (**130**) | *S. angulatus* | Porter *et al.*, 1962 |
| Rosmarinine (**131**) | *S. angulatus*<br>*S. braychypodus*<br>*S. hygrophyllus*<br>*S. pauciligulatus*<br>*S. taiwanensis*<br>*S. triangularis*<br>*S. rosmarinifolius* | Roitman, 1983 |
| 9-Angeloylhastanecine (**132**) | *S. aquaticus* ssp. *barbareifolius*<br>*S. chrysocoma* | Christov *et al.*, 2002a |
| Eruciflorine (**133**) | *S. argunensis*<br>*S. erucifolius*<br>*S. jacobaea* | Liu *et al.*, 1991 |
| 14,15-*trans*-Senaetnine (**134**) | *S. aucheri* | Bohlmann *et al.*, 1977; 1978c; 1979a |
| Dehydrosenaetnine (**135**) | *S. barbertonicus* | Bohlmann *et al.*, 1977; 1978c; 1979a |
| Jacobine (**136**) | *S. brasiliensis*<br>*S. cineraria*<br>*S. jacobaea* | Bradbury *et al.*, 1954 |
| Sencalenine (**137**) | *S. cacaliaster* | Roeder *et al.*, 1984 |
| 11-*O*-Acetylbulgarsenine (**138**) | *S. callosus* | Romo de Vivar *et al.*, 2007 |
| 11-*O*-Acetylbulgarsenine *N*-oxide (**139**) | *S. callosus* | Romo de Vivar *et al.*, 2007 |
| *N*-Chloromethylbulgarsenine (**140**) | *S. callosus* | Romo de Vivar *et al.*, 2007 |
| Callosine (**141**) | *S. callosus* | Perez-Castorena. *et al.*, 1998 |
| $O^2$-Senecioylmacronecine (**142**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| $O^9$-Senecioylmacronecine (**143**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| Retronecine 9-(2,3-dihydroxy-2-hydroxymethylbutanoate) 7-senecioate (**144**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| Retronecine 9-(2,3-dihydroxy-2-methylbutanoate) 7-senecioate *N*-oxide (**145**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| Retronecine 9-(3-acetoxy-2-hydroxy-2-methylbutanoate) 7-senecioate (**146**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| Retronecine 9-(3-acetoxy-2-hydroxy-2-methylbutanoate) 7-senecioate (**147**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| Senecicaudatin *O*-isopentanoate (**148**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| Senecicaudatin *O*-senecioate (**149**) | *S. caudatus* | Bohlmann *et al.*, 1986b |
| Senecicaudatinal hemiacetal (**150**) | *S. caudatus* | Bohlmann *et al.*, 1986b |

Table 2.6: Pyrrolizidine alkaloids from *Senecio* species

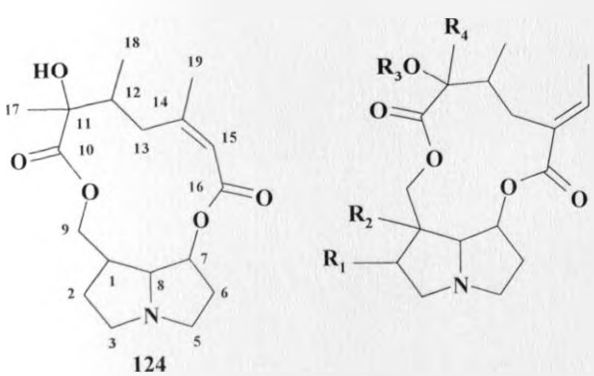
| Alkaloid | Source | Reference |
|---|---|---|
| Retronecine 9-sarracinate 7- senecioate *N*-oxide (**151**) | *S. caudatus*<br>*S. umgeniensis* | Bohlmann *et al.*, 1986b |
| Retronecine 9-sarracinate 7- senecioate (**152**) | *S. caudatus*<br>*S. triangularis*<br>*S. variabilis* | Bohlmann *et al.*, 1986b |
| $O^7$-Senecioylretronecine (**153**) | *S. caudatus*<br>*S. variabilis* | Bohlmann *et al.*, 1986b |
| 7-Hydroxy-1-methylenepyrrolizidine; (7*R*,7$\alpha$*R*)-form, Angeloyl (**154**) | *S. chrysocoma* | Grue and Liddell, 1993 |
| 7-Hydroxy-1-methylenepyrrolizidine; (7*S*,7$\alpha$*R*)-form, Angeloyl (**155**) | *S. chrysocoma* | Liddell *et al.*, 1993 |
| 7-Hydroxy-1-methylenepyrrolizidine; Angeloyl, *N*-oxide (**156**) | *S. chrysocoma* | Benn *et al.*, 1995 |
| Neosarracine (**157**) | *S. chrysocoma*<br>*S. hydrophyllus*<br>*S. kaschkarovii*<br>*S. mikanioides* | Stelljes *et al.*, 1991 |
| Rivularine. 7-Angeloylheliotridine (**158**) | *S. crispatis*<br>*S. rivularis* | Bohlmann *et al.*, 1986b |
| 2-Hydroxy-1-hydroxymethylpyrrolizidine; (1*R*,2*R*,7$\alpha$*S*)-form, $O^1$-Angeloyl, *N*-oxide (**159**) | *S. deferens* | Hirschmann *et al.*, 1988 |
| Doriasenine (**160**) | *S. doria* | Röder *et al.*, 1988 |
| Doronenine (**161**) | *S. doronicum* | Romo De Vivar *et al.*, 2007 |
| Riddelline (**162**) | *S. eremophilus*<br>*S. longiflorus*<br>*S. riddellii* | Adams *et al.*, 1957 |
| O-Acetylerucifoline (**163**) | *S. erucifolius*<br>*S. jacobacea* | Witte *et al.*, 1992a |
| Integerrimine *N*-oxide (**164**) | *S. erucifolius*<br>*S. nebrodensis*<br>*S. vulgaris* | Barrero *et al.*, 1991 |
| Sarracine (**165**) | *S. franchetii*<br>*S. mikanoides*<br>*S. rhombifolius*<br>*S. sarracenius*<br>*S. sylvaticus* | Kramov, 1967 |
| Sarracine *N*-oxide (**166**) | *S. chrysocoma*<br>*S. mikanoides*<br>*S. sarracenius* | Christov *et al.*, 2002b |
| FuchsiSenecionine (**167**) | *S. fuchsii* | Röeder *et al.*, 1977 |
| 8-Ethoxy-3-oxo-1,2-dehydroretrorsine (**168**) | *S. grisebachii* | Hirschmann *et al.*, 1985 |
| Hadiensine (**169**) | *S. hadiensis* | Were *et al.*, 1991 |
| 12-*O*-Acetylhadiensine (**170**) | *S. hadiensis* | Were *et al.*, 1991 |
| Petitianine (**171**) | *S. hadiensis* | Were *et al.*, 1991 |
| 12-*O*-Acetylneohadiensine (**172**) | *S. hadiensis* | Were *et al.*, 1991 |
| Neorosmarinine (**173**) | *S. hadiensis* | Were *et al.*, 1991 |
| 12*S*-Hydroxyretroisosenine (**174**) | *S. helodes* | Perez-Castorena *et al.*, |

Table 2.6: Pyrrolizidine alkaloids from *Senecio* species

| **Alkaloid** | **Source** | **Reference** |
|---|---|---|
| | *S. roseus* | 1997b |
| Hygrophylline (**175**) | *S. hygrophyllus* | Schlosser *et al.*, 1965 |
| Triangularicine (**176**) | *S. hydrophyllus*<br>*S. mikanoides* | Stelljes *et al.*, 1991 |
| Neosarranicine (**177**) | *S. hydrophyllus*<br>*S. mikanoides*<br>*S. serra* | Stelljes *et al.*, 1991 |
| Sarranicine (**178**) | *S. hydrophyllus*<br>*S. mikanoides*<br>*S. serra* | Stelljes *et al.*, 1991 |
| 1,7-Dihydroxy-1-hydroxymethylpyrrolizidine; (1*R*,7*R*,7$\alpha$*R*)-form, *N*-Me, $O^7$,$O^{1'}$-diangeloyl (**179**) | *S. integrifolius* | Roeder *et al.*, 1991 |
| Rivularine *N*-oxide (**180**) | *S. integrifolius* var. *fauriri* | Bohlmann *et al.*, 1986b |
| Aucherine (**181**) | *S. integrifolius* subsp. *aucheri* | Sener *et al.*, 1988 |
| Jacoline (**182**) | *S. jacobaea* | Bradbury *et al.*, 1954 |
| Jaconine (**183**) | *S. jacobaea* | Bradbury *et al.*, 1954 |
| Sceleratine *N*-oxide (**184**) | *S. latifolius* | Bredenkamp *et al.*, 1985b |
| Merenskine *N*-oxide (**185**) | *S. latifolius* | Bredenkamp *et al.*, 1985b |
| Merenskine (**186**) | *S. latifolius* | Gordon-Gray *et al.*, 1967 |
| Sceleratine (**187**) | *S. latifolius*<br>*S. sceleratus* | Bredenkamp *et al.*, 1985b |
| Diangeloylplatynecine (**188**) | *S. macedonicus* | Christov *et al.*, 2002a |
| 8-Episarracine (**189**) | *S. macedonicus* | Trendafilova *et al.*, 1995 |
| 8-Episarracine *N*-oxide (**190**) | *S. macedonicus* | Trendafilova *et al.*, 1995 |
| Macrophylline (**191**) | *S. macrophyllus* | Danilova *et al.*, 1955 |
| (*E*)-Seneciphylline epoxide (**192**) | *S. megaphyllus* | Bohlmann *et al.*, 1986b |
| Seneciphylline epoxide (**193**) | *S. megaphyllus*<br>*S. usgorensis* | Bohlmann et al., 1986b |
| Oxyretroisosenine (**194**) | *S. mulgediifolius* | Klasek *et al.*, 1973 |
| Mulgediifoline (**195**) | *S. mulgediifolius* | Klasek *et al.*, 1973 |
| 1,2-Dihydroxy-7-hydroxymethylpyrrolizidine; (1*R*,2*R*,7*R*,7$\alpha$*R*)-form, 2-Angeloyl (**196**) | *S. nemorensis* | Christov *et al.*, 2005 |
| Nemorensine (**197**) | *S. nemorensis* (several varieties) | Klasek *et al.*, 1973 |
| 1,2-Dehydrofuchsisenecionine (**198**) | *S. nemorensis* var. *fuchsii*<br>*S. variabilis* | Bohlmann *et al.*, 1986b |
| Oxynemorensine (**199**) | *S. nemorensis* var. *subdecurrens* | Klasek *et al.*, 1973 |
| Retroisosenine (**200**) | *S. nemorensis*<br>*S. mulgediifolius* | Klasek *et al.*, 1973 |
| Bisline (**201**) | *S. othonniformis*<br>*S. petasis*<br>*S. ruwenzoriensis* | Susag *et al.*, 2000<br>Coucourakis *et al.*, 1970, |
| Erucifoline *N*-Oxide (**202**) | *S. persoonii* | Roeder *et al.*, 1993 |

Table 2.6: Pyrrolizidine alkaloids from *Senecio* species

| Alkaloid | Source | Reference |
|---|---|---|
| Seneciphylline *N*-oxide (**203**) | *S. persoonii* | Roeder *et al.*, 1993 |
| Neoplatyphylline (**204**) | *S. platyphyllus*<br>*S. rhombifolius* | Jiang *et al.*, 2006<br>Cai and Wang, 1983 |
| Isorosmarinine (**205**) | *S. pterophorus* | Liddell *et al.*, 1993 |
| Seneciphyllinine (**206**) | *S. pterophorus* | Liddell *et al.*, 1993 |
| Racemocine (**207**) | *S. racemosus* | Ahmed *et al.*, 1991c |
| Racemonine (**208**) | *S. racemosus* | Roeder *et al.*, 1977 |
| Racemodine (**209**) | *S. racemosus* | Ahmed *et al.*, 1993 |
| 13*R*-Hydroxyretroisosenine (**210**) | *S. roseus* | Perez-Castorena *et al.*, 1997b |
| 7-Hydroxy-1-methylenepyrrolizidine; (7*R*,7$\alpha$*R*)-form, *N*-oxide (**211**) | *S. schweinfurthii* | Benn *et al.*, 1995 |
| 18-Hydroxyjaconine (**212**) | *S. selloi* | Krebs *et al.*, 1996 |
| Spartioidine (**213**) | *S. spartioides* | Adams *et al.*, 1957 |
| Dihydroretrorsine (**214**) | *S. subulatus* var. *erectus* | Pestchanker *et al.*, 1985a |
| Swazine (**215**) | S. swaziensis | Gordon-Gray *et al.*, 1974 |
| Triangularine (**216**) | *S. triangularis* | Röeder *et al.*, 1984 |
| Neotriangularine (**217**) | *S. triangularis* | Röeder *et al.*, 1984 |
| Usaramoensine (**218**) | *S. usaramoensis* | Adams *et al.*, 1953 |
| Uspallatine (**219**) | *S. uspallatensis* | Pestchanker *et al.*, 1985b |
| Senecivernine (**220**) | *S. vernalis* | Topuriya *et al.*, 1982 |
| Spartioidine *N*-oxide (**221**) | *S. vulgaris* | Roeder *et al.*, 1993 |
| Retrorsine (**222**) | *S.* spp | Roitman, 1985 |
| Retrorsine *N*-Oxide (**223**) | *S.* spp | Lock de Ugaz *et al.*, 1990 |
| Senaetnine (**224**) | *S.* spp | Bohlmann *et al.*, 1977; 1978c; 1979a |
| Senecionine (**225**) | *S.* spp | Barger *et al.*, 1936 |
| Integerrimine (**226**) | *S.* spp | Adams *et al.*, 1953 |
| Seneciphylline (**227**) | *S.* spp | Villarroel *et al.*, 1985 |

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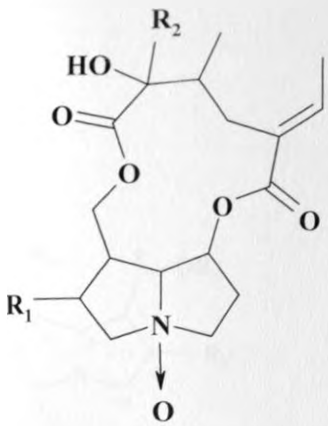
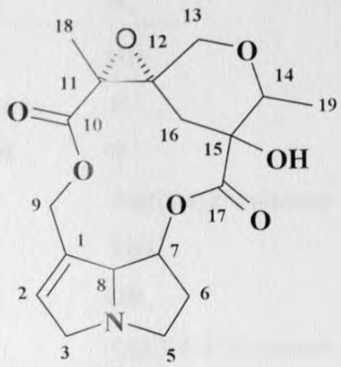
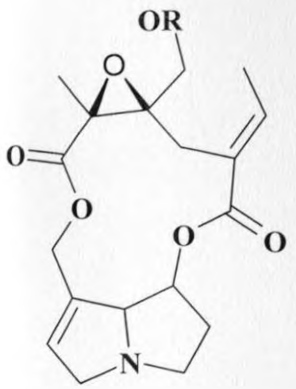
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| 125 | H | H | H | Me |
| 131 | OH | H | H | Me |
| 169 | H | OH | H | Me |
| 170 | H | OH | Ac | Me |
| 171 | H | OH | H | $CH_2OH$ |
| 172 | H | OH | Ac | Me (15*E*-isomer) |
| 173 | OH | H | H | Me (15*E*-isomer) |
| 204 | H | H | H | Me (15*E*-isomer) |
| 214 | H | H | H | $CH_2OH$ |



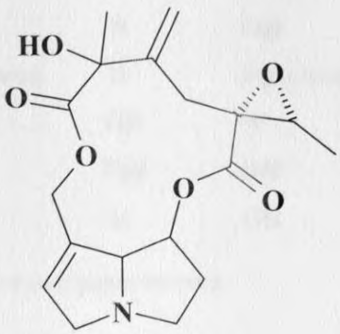
| | $R_1$ | $R_2$ |
|---|---|---|
| 126 | H | Me |
| 223 | OH | $CH_2OH$ |



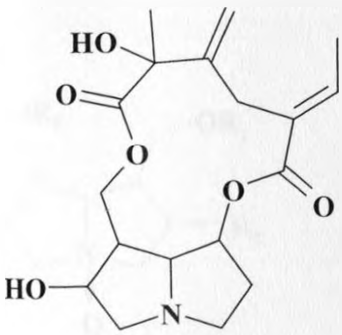
127



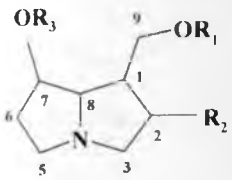
| | R |
|---|---|
| 128 | H |
| 163 | Ac |



129

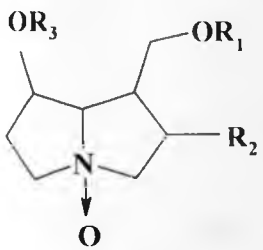


130



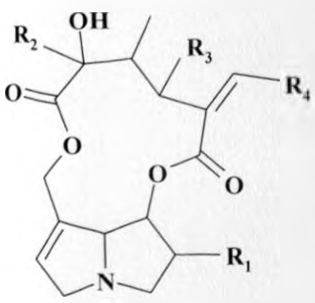

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 132 | Ang * | H | OH |
| 142 | H | Osene | H |
| 143 | Sene* | OH | H |
| 157 | 2-Hydroxyme-2-buten* | H | Tigl (2'*E*,2''*Z*)-isomer |
| 165 | 2-Hydroxyme-2-buten* | H | Tigl |
| 167 | Sene* | H | OH |
| 177 | 2-Hydroxyme-2-buten | H | Tigl (2'*E*,2''*E*)-isomer |
| 178 | 2-Hydroxyme-2-buten | H | Tigl (2'*Z*,2''*E*)-isomer |
| 188 | Tigl* | H | Tigl |
| 189 | 2-Hydroxyme-2-buten | H | Tigl (7a-epimer) |
| 191 | Tigl | OH | H |
| 196 | H | Tigl | OH |
| 207 | Tigl | H | OH |

* Abbreviations given on pages xvi-xvii

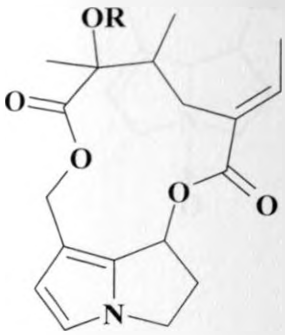



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 159 | Tigl | OH | H |
| 166 | 2-Hydroxyme-2-buten | H | Tigl |
| 190 | 2-Hydroxyme-2-buten | H | Ang * |

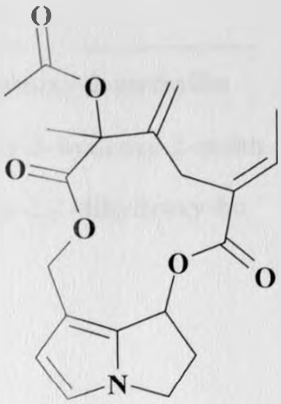
* Abbreviations given on pages xvi-xvii



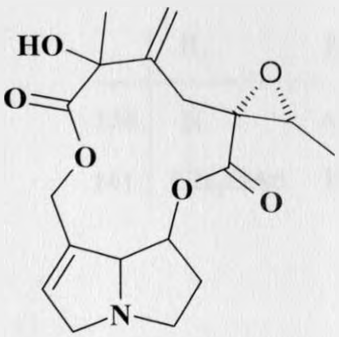

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| 133 | H | Me | H | $CH_2OH$ |
| 218 | H | Me | H | Me (15*E*-isomer, 12-epimer) |
| 219 | OH | Me | H | Me |
| 220 | H | Me | Me | H |
| 222 | H | $CH_2OH$ | H | Me |
| 225 | H | Me | H | Me |
| 226 | H | Me | H | Me (15*E*-isomer) |



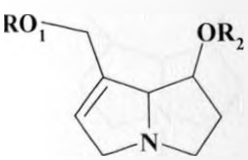

| | R |
|---|---|
| 134 | Ac (14,15-*trans*) |
| 224 | Ac |




135

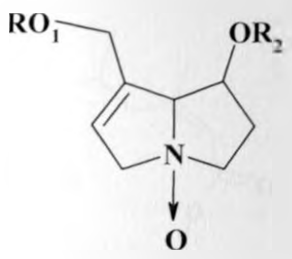



136

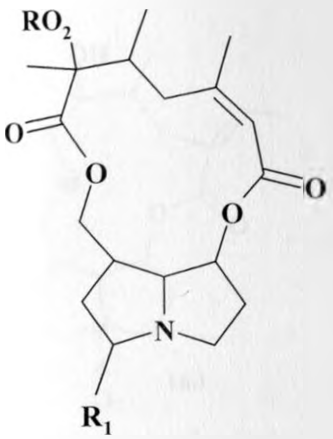



| | $R_1$ | $R_2$ |
|---|---|---|
| 137 | Tigl* | 3-Hydroxyme-2-buten |
| 144 | Sene | 2,3-Dihydroxy-2-hydroxymebu |
| 146 | Sene | 3-Acetoxy-2-hydroxy-2-mebu |
| 152 | Sene | 2-Hyroxymethyl-2-buten |
| 153 | H | Sene |
| 158 | H | 2-Me-2-buten |
| 160 | 2-Hyroxymethyl-2-buten | 3-Hyroxymethyl-2-buten |
| 176 | 2-Hyroxymethyl-2-buten | Tigl (2''*E*-isomer) |
| 198 | Sene | H |
| 216 | 2-Hyroxymethyl-2-buten | Tigl |
| 217 | 2-Hyroxymethyl-2-buten | Tigl (2'*E*-isomer) |

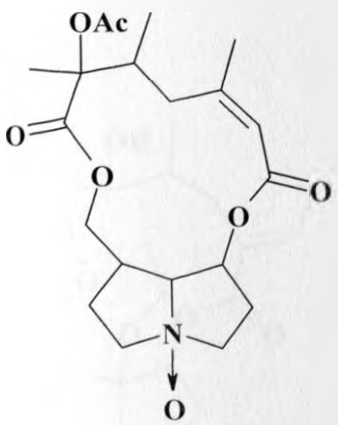
* Abbreviations in pages xvi-xvii



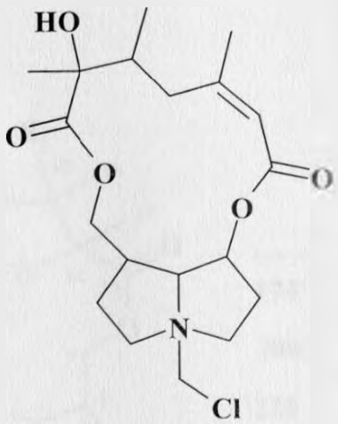

| | $R_1$ | $R_2$ |
|---|---|---|
| 145 | Sene | 2,3-Dihydroxy-2-methylbu |
| 147 | Sene | 3-Acetoxy-2-hydroxy-2-mebu |
| 151 | Sene | 3-Acetoxy-2,2-dihydroxy-bu |
| 180 | H | Tigl |



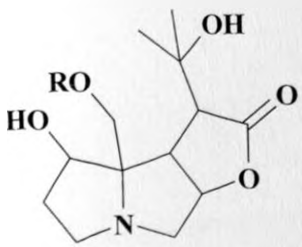

| | $R_1$ | $R_2$ |
|---|---|---|
| 138 | H | Ac |
| 141 | $CH_2OAc$ | H |



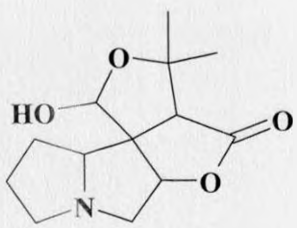

139



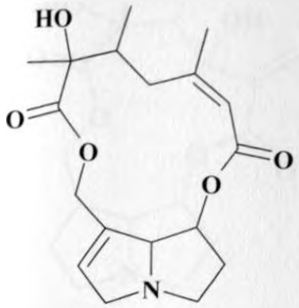

140



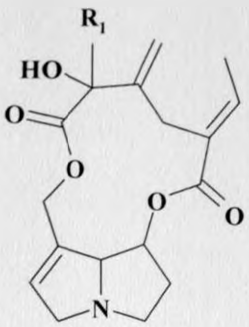

| | R |
|---|---|
| 148 | 3-Mebu |
| 149 | Sene |



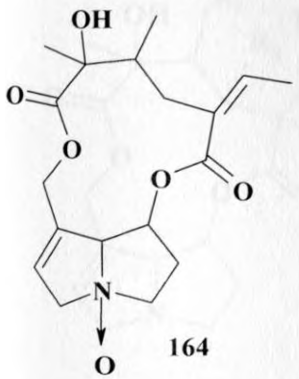

150



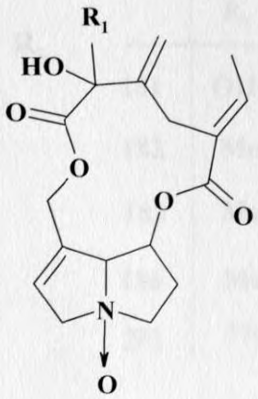
161



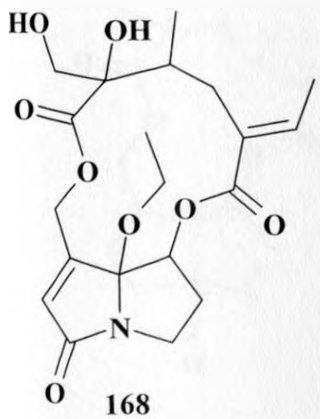
| | $R_1$ |
|---|---|
| 162 | $CH_2OH$ |
| 206 | Ac |
| 213 | Me (15*E*-isomer, 12-epimer) |
| 227 | Me |



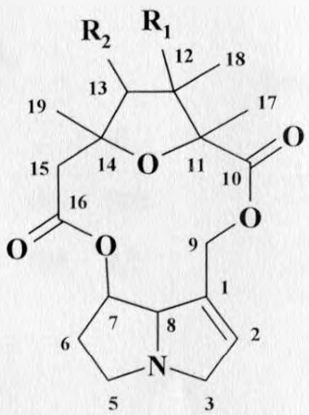
164



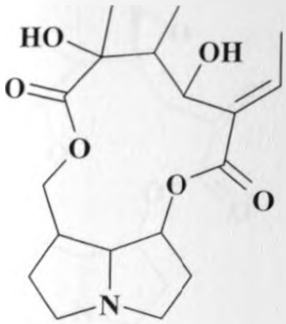
| | $R_1$ |
|---|---|
| 203 | Me |
| 221 | Me (15*E*-isomer, 12-epimer) |



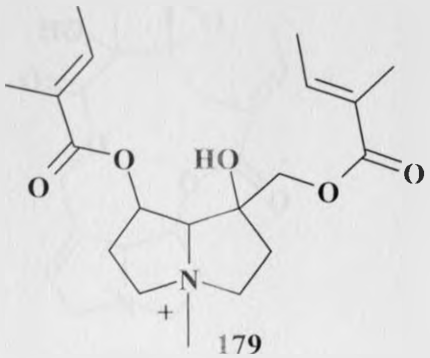
168



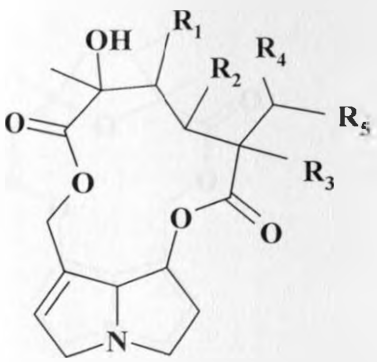
| | $R_1$ | $R_2$ |
|---|---|---|
| 174 | OH | H |
| 200 | H | H |
| 210 | H | Me |



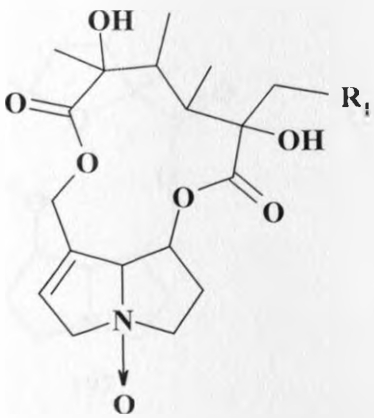
175



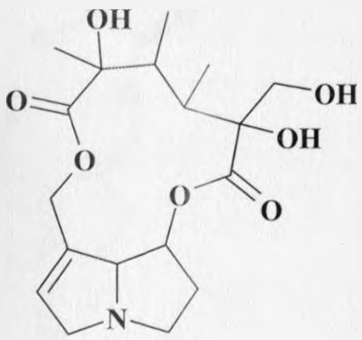
179



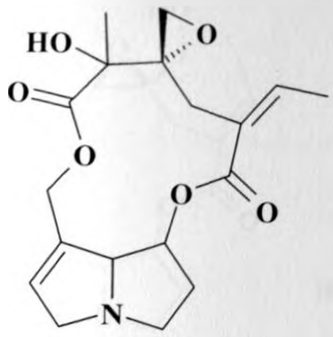
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| 181 | OH | H | H | Me | Me |
| 182 | Me | H | OH | OH | Me |
| 183 | Me | H | OH | Cl | Me |
| 186 | Me | Me | OH | H | Cl |
| 201 | Me | H | OH | H | Me |



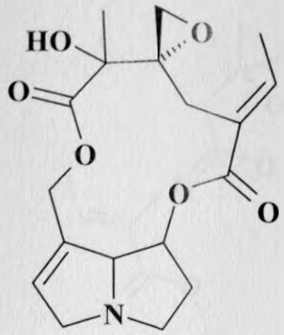
| | $R_1$ |
|---|---|
| 184 | OH |
| 185 | Cl |



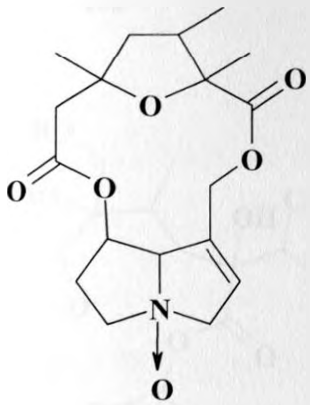
187



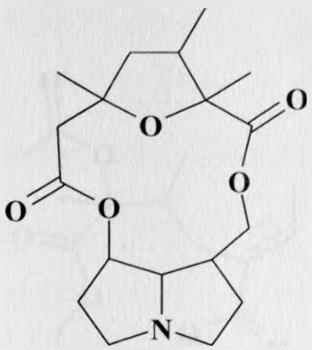
192



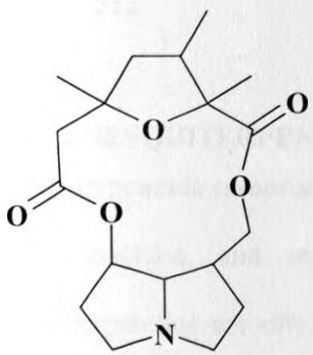
193



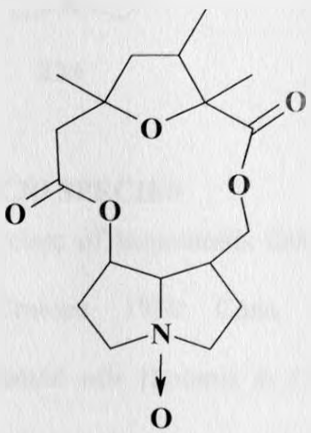
194



195 (11,14-diepimer)



197



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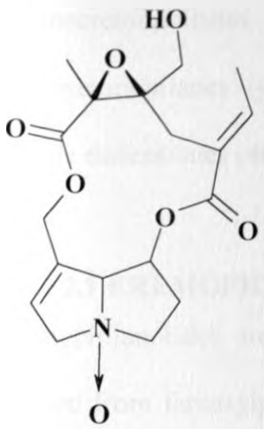
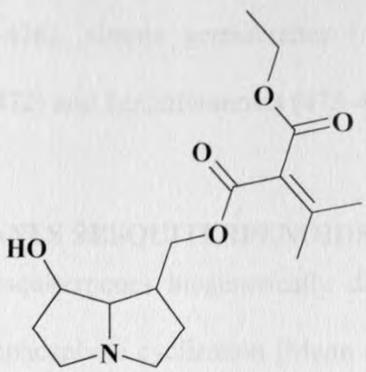
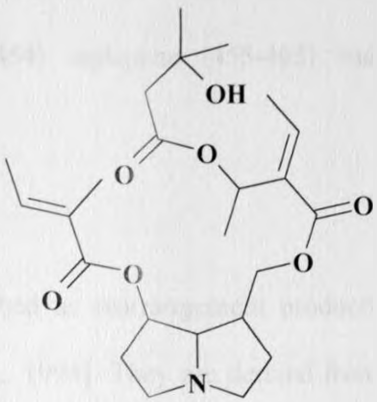


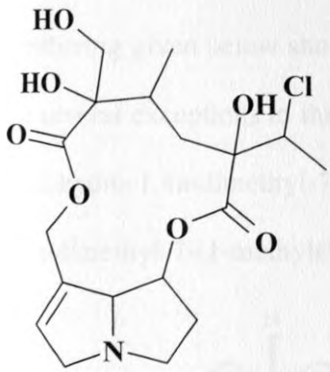

202



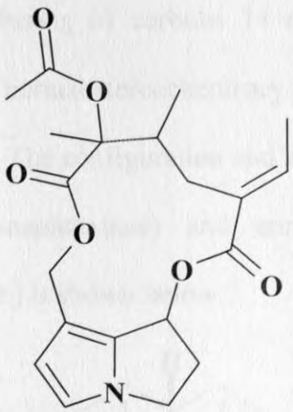

208




209




212




224

### 2.4.2.2 SESQUITERPENOIDS FROM *SENECIO* SPECIES

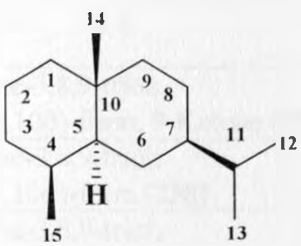
Sesquiterpenoids comprise a large and diverse class of isoprenoids found in plants, fungi, select bacteria, and insects [Loomis & Croteau, 1980; Cane, 1981]. In plants, sesquiterpenoids are often associated with essential oils [Loomis & Croteau, 1980], and except for a limited number of cases, such as the growth regulator abscisic acid [Wareing, 1978]. There is a large number of sesquiterpenoid carbon skeletons, which all, however, arise from the common precursor (farnesyl pyrophosphate) by various modes of cyclisations followed, in many cases, by skeletal rearrangement [Cane, 1981].

Different classes of sesquiterpenoids [Torres *et al.*, 1998], have been characterized from *Senecio* species [Ndom *et al.*, 2006] including eremophilanes (**228-293**),

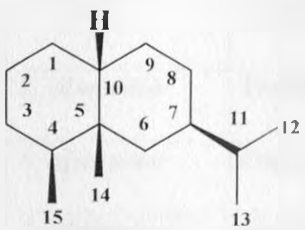
furanoeremophilanes (**299-351**), cacalol (**352-376**), bisabolanes (377-**406**), seco and abeoeremophilanes (**407-426**), simple germacranes (**427-454**), oplapane (**455-463**) and simple eudesmanes (**464-472**) and benzofuranoids (**473-485**).

### 2.4.2.2.1 EREMOPHILANES SESQUITERPENOIDS

Eremophilanolides are sesquiterpenes biogenetically described as rearrangement products derived from farnesylpyrophosphate cyclization [Mann *et al.*, 1994]. They are derived from eudesmanes by migration of the methyl group at C-10 to C-5 [Pinder, 1977]. There is confusion in the literature about the numbering of carbons 14 and 15. The biogenetic numbering given below should be used. The normal stereochemistry is shown, although there are several exceptions to this [Pinder, 1977]. The configuration and numbering of eudesmane (decahydro-1,4a-dimethyl-7-(1-methylethyl)-naphthalene) and eremophilane (decahydro-1,8a-dimethyl-7-(1-methylethyl)-naphthalene ) is shown below.




Eudasmane skeleton




Eremophilane skeleton

As with the other larger categories, eremophilanes can be classified further into simple eremophilanes, eremophilanolides and furanoeremophilanes, seco- and abeoeremophilanes and noreremophilanes [Pinder, 1977].

These secondary metabolites, along with pyrrolizidine alkaloids, are the most common natural products isolated from *Senecio* species [Bohlmann *et al.*, 1977; Rizk, 1991. Table 2.10 below lists some of eremophilanolides sesquiterpenoids isolated from this genus.

Table 2.7: Eremophilanoides of *Senecio* species

| **Eremophilane** | **Source** | **References** |
|---|---|---|
| 1,8-Dihydroxy-7(11),9-eremophiladien-12,8-olide; (1β,8α*OH*)-form **(228)** | *S. aegyptius* var. *discoideus* | Garduño-Ramírez *et al.*, 2001 |
| 1,8-Dihydroxy-7(11),9-eremophiladien-12,8-olide; (1β,8α*OH*)-form, 8-Me ether **(229)** | *S. aegyptius* var. *discoideus* | Garduño-Ramírez *et al.*, 2001 |
| 1-Hydroxy-7(11),9-eremophiladien-12,8-olide; (1β,8β)-form **(230)** | *S. aegyptius* var. *discoideus* | Garduño-Ramírez *et al.*, 2001 |
| 8-Hydroxy-1(10),7(11)-eremophiladien-12,8-olide; 8α*OH*-form, 1β,10β-Epoxide **(231)** | *S. aegypticus* var. *discoideus* | Garduño-Ramírez *et al.*, 2001 |
| 8-Hydroxy-1(10),7(11)-eremophiladien-12,8-olide; 8α*OH*-form, Me ether, 1β,10β-epoxide **(232)** | *S. aegypticus* var. *discoideus* | Garduño-Ramírez *et al.*, 2001 |
| 1,8-Dihydroxy-7(11)-eremophilen-12,8-olide; (1α,8β*OH*,10β)-form, 1-Tigloyl **(233)** | *S. almeydae* | Dupre *et al.*, 1991 |
| 3,9-Dihydroxy-7(11)-eremophilen-8-one; (3α,9α,10α)-form **(234)** | *S. almeydae* | Dupré *et al.*, 1991 |
| 3,9-Dihydroxy-7(11)-eremophilen-8-one; (3α,9α,10β)-form, 3-Angeloyl **(235)** | *S. almeydae* *S. sylvaticus* | Bohlmann *et al.*, 1977; 1978j; 1985 |
| 3,9-Dihydroxy-7(11)-eremophilen-8-one; (3α,9α,10β)-form, 3-Tigloyl **(236)** | *S. almeydae* | Bohlmann *et al.*, 1977; 1978j,; 1985 |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8α,9α,10β)-form, 9-Ketone **(237)** | *S. almeydae* | Dupré *et al.*, 1991 |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form **(238)** | *S. almeydae* | Dupré . *et al.*, 1991 |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form, 8-Ketone **(239)** | *S. almeydae* | Dupré *et al.*, 1991 |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form, 8-Ketone, 3-angeloyl **(240)** | *S. almeydae* *S. sylvaticus* | Dupré *et al.*, 1991 |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form, 8-Ketone, 3-tigloyl **(241)** | *S. almeydae* *S. sylvatic* | Dupré *et al.*, 1991 |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form, 8-Ketone, 3-(3-methyl-2-butenoyl) **(242)** | *S. almeydae* *S. sylvaticus* | Dupré *et al.*, 1991 |
| 1,10.,7,8-Diepoxy-3,6-dihydroxy-12, eremophilanolide; (1β,3β,6β,7α,8α,10β,11β*H*)-form, 6-(2-Methylbutanoyl **(243)** | *S. atratus* | Bohlmann *et al.*, 1986a |
| 1,10.,7,8-Diepoxy-3,6-dihydroxy-12,8 eremophilanolide; (1β,3β,6β,7α,8α,10β,11β*H*)-form, 6-(3-Methylbutanoyl) **(244)** | *S. atratus* | Bohlmann *et al.*, 1986a |
| 1,10-Epoxy-3,6,8-trihydroxy-7(11)-eremophilen-12,8-olide; (1β,3β,6β,8α*OH*,10β)-form, 6-(2-Methylbutanoyl) **(245)** | *S. atratus* | Zhao *et al.*, 1992 |

Table 2.7: Eremophilanoides of *Senecio* species

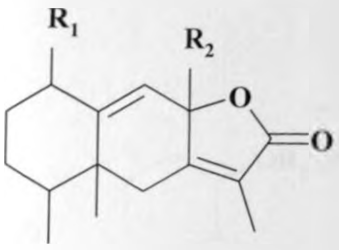
| **Eremophilane** | **Source** | **References** |
|---|---|---|
| 1,10-Epoxy-3,6,8-trihydroxy-7(11)-eremophilen-12,8-olide: (1β,3β,6β,8α*OH*,10β)-form, 6-(3-Methylbutanoyl) **(246)** | *S. atratus* | Zhao *et al.*, 1992 |
| 1,10-Epoxy-3,6,8-trihydroxy-7(11)-eremophilen-12,8-olide: (1β,3β,6β,8α*OH*,10β)-form, 6-Angeloyl **(247)** | *S. atratus* | Zhao *et al.*, 1992 |
| 7(11)-Eremophilen-12,8-olide; (8β,10α*H*)-form **(248)** | *S. aureus* | Goto *et al.*, 2001 |
| 8-Hydroxy-7(11)-eremophilen-12,8-olide; (8*R*,10α*H*)-form, 8-Et ether **(249)** | *S. aureus* | Zalkow *et al.*, 1979 |
| 1,8-Dihydroxy-7(11)-eremophilen-12,8-olide; (4α,5α,8α*OH*,10α)-form , 1-Ketone **(250)** | *S. bracteolatus* | Bohlmann *et al.*, 1986a |
| 1,8-Dihydroxy-7(11)-eremophilen-12,8-olide; (1α,8β*OH*,10β)-form, 8-Me ether, 1-angeloyl **(251)** | *S. cachinalensis*<br>*S. poepigii* | Reina *et al.*, 2006 |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form, 3-(4-Hex-2*Z*-enoyloxy-2*Z*-hexenoyl) **(252)** | *S. erubescens* var. *crepidifolius* | Bohlmann *et al.*, 1977; 1978d; 1982g; 1985 |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form, 3-(4-Angeloyloxy-2*Z*-hexenoyl) **(253)** | *S. erubescens* var. *crepidifolius* | Bohlmann *et al.*, 1977; 1978d; 1982g; 1985 |
| 11-Eremophilen-9-one; 10β-form **(254)** | *S. filaginoides* | Bohlmann *et al.*, 1986a |
| 8,12-Epoxy-8,12-dihydroxy-1,7(11)-eremophiladien-3-one; (8α*OH*,12α)-form, Di-Me ether **(255)** | *S. flavus* | Torres *et al.*, 1999 |
| 8,12-Epoxy-8,12-dihydroxy-1,7(11)-eremophiladien-3-one; (8α*OH*,12β)-form, Di-Me ether **(256)** | *S. flavus* | Torres *et al.*, 1999 |
| 8-Hydroxy-7(11),9-eremophiladien-12,8-olide; 8β*OH*-form **(257)** | *S. hieracioides*<br>*S. tsoongianus* | Zhao *et al.*, 2002 |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8β,9β,10β)-form, 8-Ketone, 3-angeloyl, 9-(3-methyl-2-butenoyl) **(258)**. | *S. gerardii* | Bohlmann *et al.*, 1977; 1978d,g,j; 1982g; 1985 |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8β,9β,10β)-form, 8-Ketone, 3,9-bis(3-methyl-2-butenoyl) **(259)** | *S. gerardii* | Bohlmann *et al.*, 1977; 1978d,g,j; 1982g; 1985 |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8β,9β,10β)-form, 8-Ketone, 3-tigloyl, 9-(3-methyl-2-butenoyl) **(260)** | *S. gerardii* | Bohlmann *et al.*, 1977; 1978d,g,j; 1982g; 1985 |
| 6-Hydroxy-1(10),7(11)-eremophiladien-12,8-olide; (6β,8α)-form **(261)** | *S. glaber* | Dupré *et al.*, , 1991 |
| 6-Hydroxy-1(10),7(11)-eremophiladien-12,8- | *S. glaber* | Pérez *et al.*, 1991 |

Table 2.7: Eremophilanoides of *Senecio* species

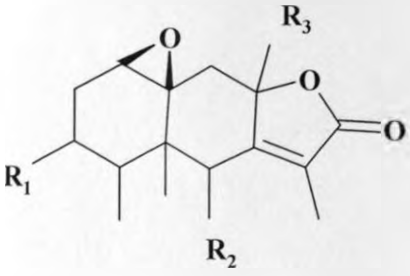
| **Eremophilane** | **Source** | **References** |
|---|---|---|
| olide; (6α,8β)-form (**262**) | *S. toluccanus* | |
| 6-Hydroxy-1(10),7-eremophiladien-12,8-olide; (6β,11β*H*)-form (**263**) | *S. glaber* | Dupré *et al.*, , 1991 |
| 6,8-Dihydroxy-1(10),7(11)-eremophiladien-12,8-olide; (6β,8α)-form (**264**) | *S. glaber*<br>*S. toluccanus* | Peréz *et al.*, 1991 |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8α,9β,10β)-form, 8-Ketone, 3-(4-angeloyloxy-2*Z*-hexenoyl) (**265**) | *S. glandulosopilosus* | Bohlmann *et al.*, 1977; 1978d,g,j; 1982g; 1985 |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8α,9β,10β)-form, 8-Ketone, 3-(5-angeloyloxy-2*Z*-hexenoyl) (**266**) | *S. glandulosopilosus* | Bohlmann *et al.*, 1977; 1978d,g,j; 1982g; 1985 |
| 1,10-Epoxy-6,8-dihydroxy-7(11)-eremophilen-12,8-olide; (1β,6β,8β,10β)-form, 6-(2-Methylpropenoyl) (**267**) | *S. isatideus* | Torres *et al.*, 1999 |
| 1,10-Epoxy-6,8-dihydroxy-7(11)-eremophilen-12,8-olide; (1β,6β,8β,10β)-form, 6-(2-Methylpropenoyl) (**268**) | *S. isatideus* | Zhao *et al.*, 1992 |
| 1,10-Epoxy-6,8-dihydroxy-7(11)-eremophilen-12,8-olide; (1β,6β,8β,10β)-form, 6-(3-Methyl-2-butenoyl) (**269**) | *S. isatideus* | Zhao *et al.*, 1992 |
| 1,10-Epoxy-6,8-dihydroxy-7(11)-eremophilen-12,8-olide; (1β,6β,8β,10β)-form, 6-Angeloyl (**270**) | *S. isatideus* | Zhao *et al.*, 1992 |
| 1,10-Epoxy-3,6,8-trihydroxy-7(11)-eremophilen-12,8-olide; (1β,3β,6β,8α*OH*,10β)-form, 6-(2-Methylpropanoyl), 1-Ac (**271**) | *S. mauricei* | Bohlmann *et al.*, 1978f |
| 1,10-Epoxy-3,6,8-trihydroxy-7(11)-eremophilen-12,8-olide; (1β,3β,6β,8α*OH*,10β)-form, 6-(2-Methylpropenoyl), 1-Ac (**272**) | *S. mauricei* | Bohlmann *et al.*, 1978f |
| 1,8-Dihydroxy-7(11)-eremophilen-12,8-olide; (1α,8β*OH*,10β)-form, 8-Me ether, 1-Ac (**273**) | *S. miser* | Reina *et al.*, 2001 |
| 3,9-Dihydroxy-7(11)-eremophilen-8-one; (3α,9β,10β)-form, 3-Tigloyl (**274**) | *S. ochoanus* | Bohlmann *et al.*, 1983 |
| 9,10-Epoxy-7(11)-eremophilen-8-one; (9α,10α)-form (**275**) | *S. oldhamianus* | Yang *et al.*, 2001 |
| 8,12-Epoxy-1(10),7(11),8-eremophilatriene-6,12-diol; (6β,12ξ)-form, 6-(2-Methylpropanoyl), 12-Me ether (**276**) | *S. pachyphyllos* | Ahmed *et al.*, 1991a,b |
| 8,12-Epoxy-1(10),7(11),8-eremophilatriene-6,12-diol; (6β,12ξ)-form, 6-Propanoyl, 12-Me ether (**277**) | *S. pachyphyllos* | Ahmed *et al.*, 1991a,b |
| 1,8-Dihydroxy-11-eremophilen-9-one; (1α,8α,10α)-form, 8-Angeloyl, 1-Ac (**278**) | *S. portalesianus* | Jakupovic *et al.*, 1991 |
| 1,8-Dihydroxy-11-eremophilen-9-one; (1α,8α,10α)-form, 8-Tigloyl, 1-Ac (**279**) | *S. portalesianus* | Reina *et al.*, 2001 |
| 1,10-Epoxy-11-eremophilen-8-ol ; (1β,8α,10β)-form, Angeloyl (**280**) | *S. portalesianus* | Jakupovic *et al.*, 1991 |
| 1,10-Epoxy-11-eremophilen-8-ol ; (1β,8α,10β)- | *S. portalesianus* | Jakupovic *et al.*, 1991 |

Table 2.7: Eremophilanoides of *Senecio* species

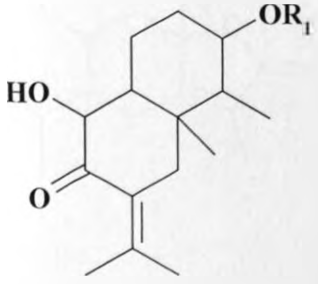
| **Eremophilane** | **Source** | **References** |
|---|---|---|
| form, Tigloyl (**281**) | | |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8β,9β,10β)-form, 8-Ketone (**282**) | *S. rhyncholaenus* | Bohlmann *et al.*, 1977; 1978d,g,j; 1982g; 1985 |
| 11-Eremophilene-3,8,9-triol; (3α,7α*H*,8β,9β,10β)-form, 8-Ketone, 3-(3-methyl-2-butenoyl), 9-tigloyl (**283**) | *S. rhyncholaenus* | Bohlmann *et al.*, 1977; 1978d,g,j; 1982g; 1985 |
| 1,10-Epoxy-4,6-dihydroxyfuranoeremophilan-9-one; (1β,4α,6β,10β)-form, 6-(2-Methylpropanoyl) (**284**) | *S. salignus* | Bohlmann *et al.*, 1976a |
| 12-Hydroxy-7(11),9-eremophiladien-8-one; 7(11)*E*-form, Ac (**285**) | *S. serratifolius* | Dupré *et al.*, 1991 |
| 12-Hydroxy-7(11),9-eremophiladien-8-one; 7(11)*Z*-form, Ac (**286**) | *S. serratifolius* | Dupré *et al.*, 1991 |
| 4'-Angeloyloxyisosenspeciosone (**287**) | *S. speciosus* | Bohlmann *et al.*, 1977; 1978j |
| 5'-Angeloyloxyisosenspeciosone (**288**) | *S. speciosus* | Bohlmann *et al.*, 1977; 1978j |
| 11-Eremophilene-3,8,9-triol; (3α,7β*H*,8α,9α,10α)-form, 8-Ketone, 3-(3-methyl-2-butenoyl), 9-(2-methylbutanoyl) (**289**) | *S. speciosus* | Bohlmann *et al.*, 1977; 1978j; 1982g; 1985 |
| 3,6,8-Trihydroxy-1(10),7(11)-eremophiladien-12,8-olide; (3α,6β,8α*OH*)-form, 3-Ac (**290**) Toluccanolide D | *S. toluccanus* | Pérez *et al.*, 1991 |
| 6,8-Dihydroxy-1(10),7(11)-eremophiladien-12,8-olide; (6β,8α)-form, 8-Et ether (**291**) | *S. toluccanus* | Morales *et al.*, 2000 |
| 1,6,10-Trihydroxy-7(11),8-eremophiladien-12,8-olide; (1β,6β,10α)-form, 6-Ac (**292**) | *S. tricephalus* | Bohlmann *et al.*, 1986a |
| 8-Hydroxy-1(10),7(11)-eremophiladien-12,8-olide; 8β*OH*-form, 1β,10β-Epoxide (**293**) | *S. tsoongianus* | Zhang *et al.*, 2005 |
| 8-Hydroxy-7(11),9-eremophiladien-12,8-olide; 8α*OH*-form (**294**) | *S. tsoongianus* | Zhao *et al.*, 2002 |
| 10-Hydroxy-7(11),8-eremophiladien-12,8-olide; 10α-form (**295**) | *S. tsoongianus* | Zhao *et al.*, 2002 |
| 1-Hydroxy-7(11)-eremophilen-12,8-olide; (1α,8α)-form, Angeloyl (**296**) | *S. viravira* | Bohlmann *et al.*, 1986a |
| 1-Hydroxy-7(11)-eremophilen-12,8-olide; (1α,8β)-form; Angeloyl (**297**) | *S. viravira* | Bohlmann *et al.*, 1986a |
| 6-Hydroxy-1(10),7(11),8-eremophilatrien-12,8-olide; 6β-form (**298**) | *S.* spp | Bohlmann *et al.*, 1977 |



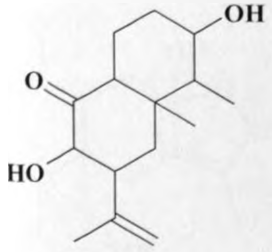

| | $R_1$ | $R_2$ |
|---|---|---|
| 228 | OH | OH |
| 229 | H | OMe |
| 230 | OH | H |
| 233 | OAng | OH |
| 257 | H | OH (8b OH) |
| 294 | H | OH (8a OH) |



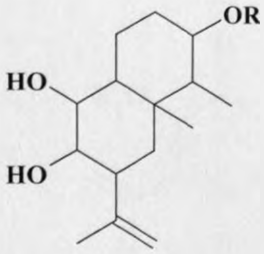

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 231 | H | H | OMe |
| 232 | H | H | OMe |
| 245 | OH | 3-Mebu | OH |
| 246 | OH | OAng | OH |
| 267 | H | 2-Me-2-propen | OH |
| 268 | H | OSene | OH |
| 269 | H | OTigl | OH |
| 270 | H | OAng | OH |
| 293 | H | H | OH |



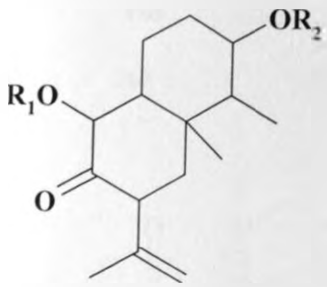

| | $R_1$ |
|---|---|
| 234 | H |
| 235 | Ang |
| 236 | Tig |
| 287 | 4-Tigloyloxy-4-methyl-2Z-hexenoyl |
| 288 | 5-Tigloyloxy-2Z-hexenoyl |



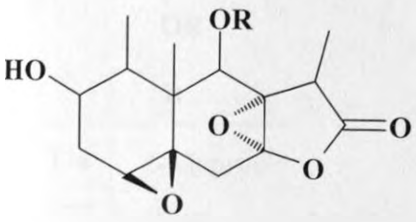

237



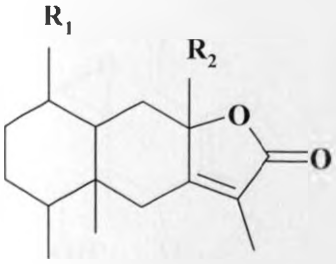

| | R |
|---|---|
| 238 | H |
| 252 | 4-Hex-2Z-enoyloxy-2Z-hexenoyl |
| 253 | 4-Angeloyloxy-2Z-hexenoyl |



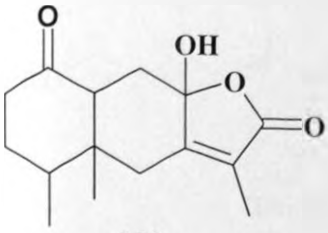

| | $R_1$ | $R_2$ |
|---|---|---|
| 239 | H | H |
| 240 | H | Ang |
| 241 | H | Tigl |
| 242 | H | Sene |
| 258 | Sene | Ang |
| 259 | Sene | Sene |
| 260 | Sene | Tigl |
| 265 | H | 4-Angeloyloxy-2Z-hexenoyl |
| 266 | H | 5-Angeloyloxy-2Z-hexenoyl |
| 282 | H | H |
| 283 | Tigl | Sene |
| 289 | H | Sene |



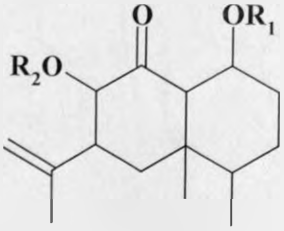

| | R |
|---|---|
| 243 | 2-Mebu |
| 244 | 3-Mebu |



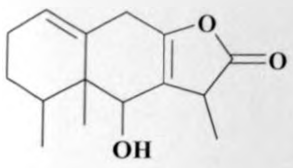

| | $R_1$ | $R_2$ | |
|---|---|---|---|
| 248 | H | H | |
| 249 | H | OEt | |
| 251 | OAng | OMe | |
| 273 | Ac | OMe | |
| 296 | OAng | H | (1α, 8α)-form |
| 297 | OAng | H | (1α, 8β)-form |




250




| | $R_1$ | $R_2$ |
|---|---|---|
| 254 | H | H |
| 278 | Ac | Ang |
| 279 | Ac | Tigl |



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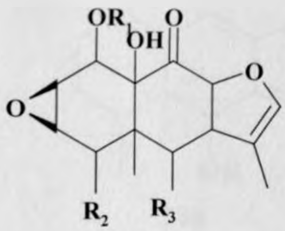
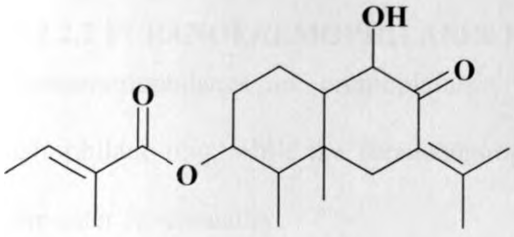


| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 271 | Ac | 2-Meprop | Me |
| 272 | Ac | H | 2-Mepropen |



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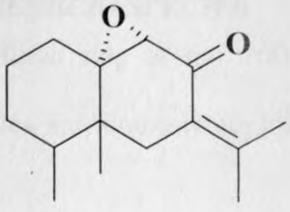

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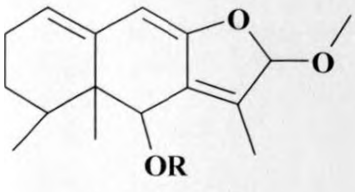
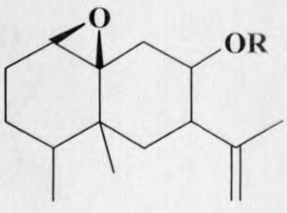
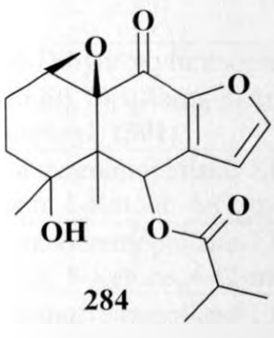


| | R |
|---|---|
| 276 | 2-Meprop |
| 277 | Prop |



| | R |
|---|---|
| 280 | Ang |
| 281 | Tigl |



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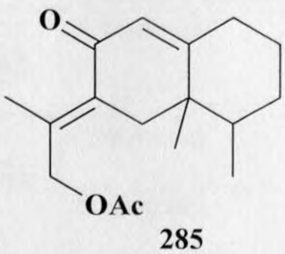

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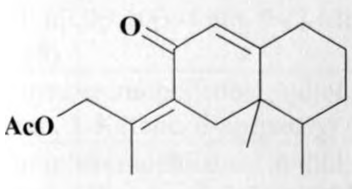

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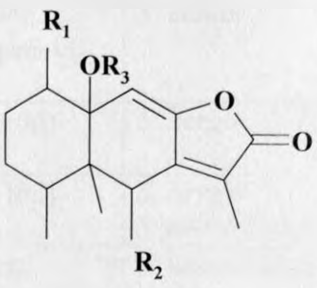
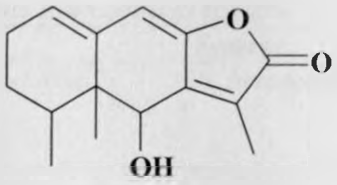


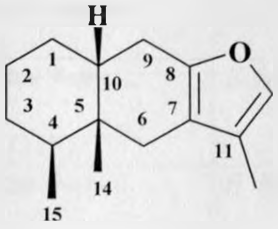
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 292 | OH | OAc | H |
| 295 | H | H | H |




**298**

### 2.4.2.2.2 FURANOEREMOPHILANES FROM *SENECIO* SPECIES

Furanoeremophilanes are eremophilanes with a furan ring at the 7(8) position in the eremophilane ring, while the furanoeremophilanoides are furanoeremophilanes with one or more ester functionality.




Furanoremophilane skeleton

Table 2.8: Furanoeremophilanoides from *Senecio* species.

| **Furanoeremophilanoides** | **Source** | **Reference** |
|---|---|---|
| Alloeophyllin (**299**) | *S. alloeophyllus* | Garrido *et al.*, 1995. |
| Sendarwin (**300**) | *S. alloeophyllus*<br>*S. darwinii*<br>*S. medley-woodii* | Garrido *et al.*, 1995 |
| 3,6-Dihydroxyfuranoeremophilan-9-one; (3α,6β,10β)-form, 3-Angeloyl, 6-(3-methyl-2-butenoyl) (**301**) | *S. andreuxii* | Bohlmann *et al.*, 1978a |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Ketone, 6-(2-methylpropanoyl) (**302**) | *S. auricula* | Torres *et al.*, 1998 |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Ketone, 6-(2-methylbutanoyl) (**303**) | *S. auricula* | Torres *et al.*, 1998 |
| Furanoeremophilane-1,6-diol; (1β,6β,10α)-form, 1-Ketone, 6-(2-methylbutanoyl) (**304**) | *S. auricula* | Torres *et al.*, 1998 |
| 1,10-Epoxyfuranoeremophilane-6,9-diol; (1β,6β,9β,10β)-form, 9-(2-Methylpropanoyl) (**305**) | *S. behnii* | Bohlmann *et al.*, 1981g ; Dupre *et al.*, 1991 |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Ketone, 6-propanoyl (**306**) | *S. bergii* | Torres *et al.*, 1998 |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Ketone, 6-tigloyl (**307**) | *S. bergii*<br>*S. bracteolatus* | Torres *et al.*, 1998 |
| 6,9-Dihydroxyfuranoeremophilan-1-one; (6β,9β,10β*H*)-form (**308**) | *S. bracteolatus* | Bohlmann *et al.*, 1986a |
| 6,9-Dihydroxyfuranoeremophilan-1-one; (6β,9β,10β*H*)-form, 6-Tigloyl (**309**) | *S. bracteolatus* | Bohlmann *et al.*, 1986a |

Table 2.8: Furanoeremophilanoides from *Senecio* species.

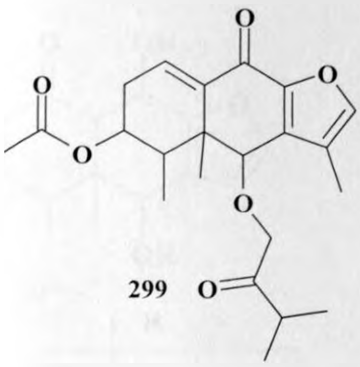
| Furanoeremophilanoides | Source | Reference |
|---|---|---|
| 6,9-Dihydroxyfuranoeremophilan-1-one; (6β,9β,10β*H*)-form, 6-Cinnamoyl (**310**) | *S. bracteolatus* | Bohlmann *et al.*, 1986a |
| 1,10-Epoxyfuranoeremophilan-6-ol; (1β,6β,10β)-, *O*-(2-Methylbutanoyl) (**311**) | *S. doria* | Bohlmann *et al.*, 1974; 1976a |
| 6,9-Dihydroxyfuranoeremophilan-1-one; (6β,9β,10β*H*)-form, 6-Pentanoyl (**312**) | *S. filaginoides* | Bohlmann *et al.*, 1986a |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form,1-Ketone, 6-(3-methylbutanoyl) (**313**) | *S. filaginoides* | Torres *et al.*, 1998 |
| Furanoeremophilane-1,6-diol; (1α,6β,10α)-form, 1-Ketone, 6-(2-methylpropanoyl) (**314**) | *S. heliopsis* | Torres *et al.*, 1998 |
| Furanoeremophilane-1,6-diol; (1β,6β,10α)-form, 1-Ketone, 6-(2-methylpropanoyl) (**315**) | *S. heliopsis* | Torres *et al.*, 1998 |
| 1,6-Dihydroxyfuranoeremophilan-9-one; (1α,6β,10α*H*)-form, 6-(3-Methylbutanoyl) (**316**) | *S. hypochoerideus* | Bohlmann *et al.*, 1978a |
| 1,6-Dihydroxyfuranoeremophilan-9-one; (1α,6β,10α*H*)-form, 6-(3-Methyl-2-butenoyl), 1-Ac (**317**) | *S. hypochoerideus* | Bohlmann *et al.*, 1978a |
| 1,6-Dihydroxyfuranoeremophilan-9-one; (1α,6β,10β*H*)-form (**318**) | *S. hypochoerideus* | Bohlmann *et al.*, 1978a |
| 1,6-Dihydroxyfuranoeremophilan-9-one; (1α,6β,10α*H*)-form, 6-Propanoyl (**319**) | *S. hualtaranensis* | Pestchanker *et al.*, 1996 |
| 1,10-Epoxy-6-hydroxy-2-furanoeremophilen-9-one; (1β,6β,10β)-form, 6-Methylpropanoyl) (**320**) | *S. mauricei* | Bohlmann *et al.*, 1978f |
| 1,10-Epoxy-6-hydroxy-2-furanoeremophilen-9-one; (1β,6β,10β)-form, 6-Methylpropenoyl) (**321**) | *S. mauricei* | Bohlmann *et al.*, 1978f |
| 6,10-Dihydroxy-2-furanoeremophilene-1,9-dione; (6β,10β)-form, 6-(2-Methylpropanoyl) (**322**) | *S. mauricei* | Bohlmann *et al.*, 1978f |
| 1,10-Epoxyfuranoeremophilane-3,6-diol; (1β,3β,6β,10β)-form, 6-(2-Methylpropanoyl) (**323**) | *S. nemorensis* ssp. *fuchsii* | Novotny *et al.*, 1973 |
| 1,10-Epoxyfuranoeremophilane-3,6-diol; (1β,3β,6β,10β)-form, 6-(2-Methylpropanoyl), 3-Ac (**324**) | *S. nemorensis* ssp. *fuchsii* | Novotny *et al.*, 1973 |
| 1,10-Epoxyfuranoeremophilane-3,6-diol; (1β,3β,6β,10β)-form, 6-(2-Methylbutanoyl) (**325**) | *S. nemorensis* ssp. *fuchsii* | Novotny *et al.*, 1973 |
| 1,10-Epoxyfuranoeremophilane-3,6-diol; (1β,3β,6β,10β)-form, 6-Angeloyl (**326**) | *S. nemorensis* ssp. *fuchsii* | Novotny *et al.*, 1973 |
| 1,10-Epoxyfuranoeremophilane-3,6-diol; (1β,3β,6β,10β)-form, 6-(3-Methylbutanoyl) (**327**) | *S. nemorensis* ssp. *subdecurens* | Jizba *et al.*, 1981 |
| 1,10-Epoxy-4,6-dihydroxyfuranoeremophilan- | *S. salignus* | Bohlmann *et al.*, |

Table 2.8: Furanoeremophilanoides from *Senecio* species.

| **Furanoeremophilanoides** | **Source** | **Reference** |
|---|---|---|
| 9-one; (4α,6β,10β)-form, 6-(2-Methylpropanoyl) (**328**) | | 1976a |
| 1,10-Epoxyfuranoeremophilane-6,9-diol; (1β,6β,9β,10β)-form, Diketone (**329**) | *S. smithii* | Bohlmann *et al.*, 1981g |
| Furanoeremophilane-1,3-diol; (1α,3α,10α*H*)-form, 3-(3-Methyl-2-butenoyl) (**330**) | *S. smithii* | Bohlmann *et al.*, 1981g |
| Furanoeremophilane-1,3-diol; (1α,3α,10α*H*)-form, 3-Angeloyl (**331**) | *S. smithii* | Bohlmann *et al.*, 1981g |
| Furanoeremophilane-1,3-diol; (1α,3α,10α*H*)-form, 3-(2-Methylpropanoyl) (**332**) | *S. smithii* | Bohlmann *et al.*, 1981g |
| Furanoeremophilane-3,9-diol; (3α,9α,10α)-form, 3-(5-Methyl-2*E*,4*E*,6*E*-dodecatrienoyl) (**333**) | *S. speciosus* | Bohlmann *et al.*, 1978j |
| 1,6-Dihydroxyfuranoeremophilan-9-one; (1α,6β,10α*H*)-form, 6-(Methylpropanoyl) (**334**) | *S. umbellatus* | Pestchanker *et al.*, 1996 |
| 6,9-Dihydroxyfuranoeremophilan-1-one; (6β,9β,10β*H*)-form, 6-Angeloyl, 9-(2-Methylpropanoyl) (**335**) | *S. viravira* | Bohlmann *et al.*, 1986a |
| Furanoeremophilan-1-ol; (1α,10β)-form, Angeloyl (**336**) | *S. viravira* | Bohlmann *et al.*, 1986a |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Angeloyl (**337**) | *S. viravira* | Torres *et al.*, 1998. |
| 1,10-Epoxyfuranoeremophilan-6-ol; (1β,6β,10β)-form, 6-Angeloyl (**338**) | *S.* spp. | Bohlmann *et al.*, 1974; 1976a |
| 6,10-Dihydroxyfuranoeremophil-1-en-3-one; (6β,10β)-form, 6-(2-Methylpropanoyl) (**339**) | *S.* spp. | Bohlmann *et al.*, 1977 |
| 6,10-Dihydroxyfuranoeremophil-1-en-3-one; (6β,10β)-form, 6-(2-Methylbutanoyl) (**340**) | *S.* spp. | Bohlmann *et al.*, 1977 |
| 6,10-Dihydroxyfuranoeremophil-1-en-3-one; (6β,10β)-form, 6-Angeloyl (**341**) | *S.* spp | Bohlmann *et al.*, 1977 |
| 1,10-Epoxyfuranoeremophilane-3,6-diol; (1β,3α,6β,10β)-form, 6-*O*-(3-Methyl-2-butenoyl) (**342**) | *S.* spp. | Bohlmann *et al.*, 1977. |
| 1,10-Epoxyfuranoeremophilane-3,6-diol; (1β,3α,6β,10β)-form, 6-*O*-(3-Methyl-2-butenoyl), 3-Ac (**343**) | *S.* spp. | Bohlmann *et al.*, 1977 |
| 1,6-Dihydroxyfuranoeremophilan-9-one; (1α,6β,10α*H*)-form, 6-Ac (**344**) | *S.* spp. | Bohlmann *et al.*, 1979b |
| 1,10-Epoxyfuranoeremophilane-6,9-diol; (1β,6β,9β,10β)-form, 6-Angeloyl (**345**) | *S.* spp. | Bohlmann *et al.*, 1981g |
| 1,10-Epoxyfuranoeremophilane-6,9-diol; (1β,6β,9β,10β)-form, 6-(3-Methyl-2-butenoyl) (**346**) | *S.* spp. | Bohlmann *et al.*, 1981g |
| 6,9-Dihydroxyfuranoeremophilan-1-one; (6β,9β,10β*H*)-form, 6-Angeloyl (**347**) | *S.* spp. | Bohlmann *et al.*, 1986a |
| 6,9-Dihydroxyfuranoeremophilan-1-one; | *S.* spp. | Bohlmann *et al.*, |

Table 2.8: Furanoeremophilanoides from *Senecio* species.

| **Furanoeremophilanoides** | **Source** | **Reference** |
|---|---|---|
| (6β,9β,10β*H*)-form, 6-(3-Methyl-2-butenoyl) (**348**) | | 1986a |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Ketone, 6-Ac (**349**) | *S.* spp. | Torres *et al.*, 1998 |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Ketone, 6-(3-methyl-2-butenoyl) (**350**) | *S.* spp. | Torres *et al.*, 1998 |
| Furanoeremophilane-1,6-diol; (1α,6β,10β)-form, 1-Ketone, 6-angeloyl (**351**) | *S.* spp. | Torres *et al.*, 1998 |



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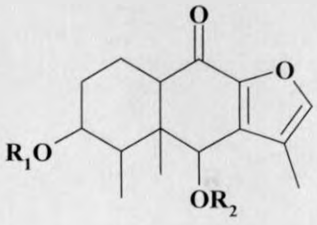
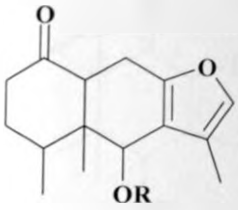
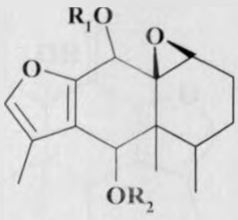


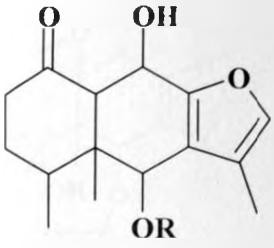
| | $R_1$ | $R_2$ |
|---|---|---|
| 300 | Ac | 2-Meprop |
| 301 | Ang | Sene |



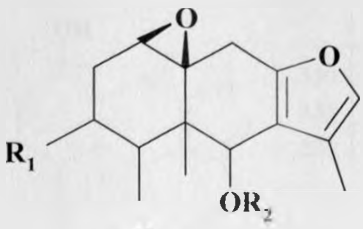
| | R |
|---|---|
| 302 | Meprop |
| 303 | Mebu-1α, 6β, 10β - form |
| 304 | Mebu-1β, 6β, 10α - form |
| 306 | Prop |
| 307 | Tigl |
| 313 | 3-Mebu |
| 314 | 2-Meprop 1α, 6β, 10α - form |
| 315 | 2-Meprop 1β, 6β, 10α - form |



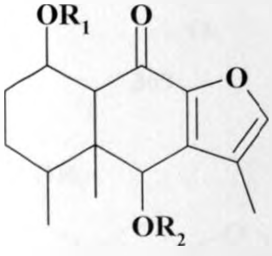
| | $R_1$ | $R_2$ |
|---|---|---|
| 305 | 2-Meprop | H |
| 345 | H | Ang |
| 346 | H | 3-Me-2-buten |



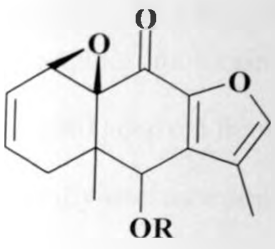
| | R |
|---|---|
| 308 | H |
| 309 | Tigl |
| 310 | Cinn |
| 312 | Pent |
| 347 | Ang |
| 348 | 3-Me-2-buten |



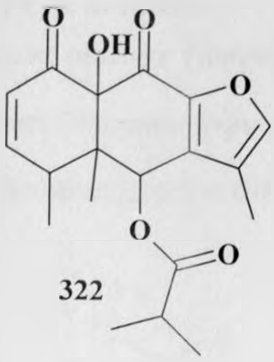
| | $R_1$ | $R_2$ |
|---|---|---|
| 311 | H | 2-Mebu |
| 323 | OH | 2-Meprop |
| 324 | Ac | 2-Meprop |
| 325 | OH | 2-Mebu |
| 326 | OH | Ang |
| 327 | OH | 3-Mebu |
| 338 | H | Ang |
| 342 | OH | 3-Me-2-buten |
| 343 | OAc | 3-Me-2-buten |



| | $R_1$ | $R_2$ |
|---|---|---|
| 316 | H | 3-Mebu |
| 317 | Ac | 3-Me-2-buten |
| 318 | H | H |
| 319 | H | Prop |
| 334 | H | 2-Meprop |
| 344 | H | Ac |



| | R |
|---|---|
| 320 | Meprop |
| 321 | Mepropen |



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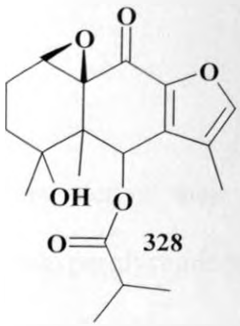

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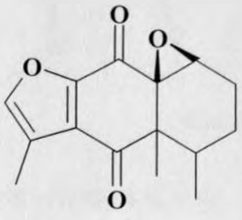

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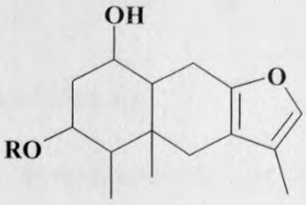
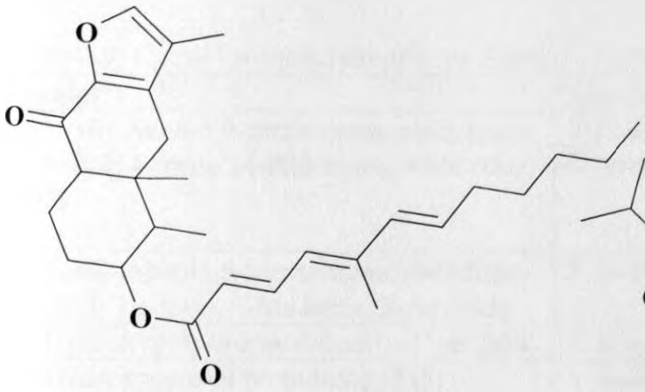


| | R |
|---|---|
| 330 | 3-Me-2-buten |
| 331 | Ang |
| 332 | Meprop |



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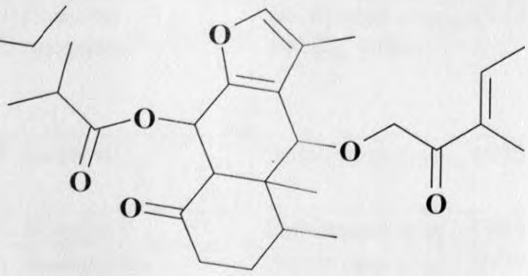

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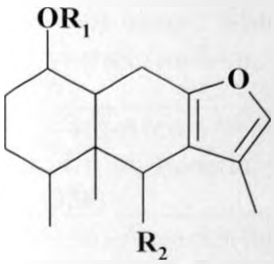
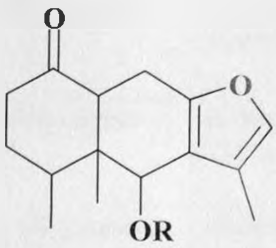


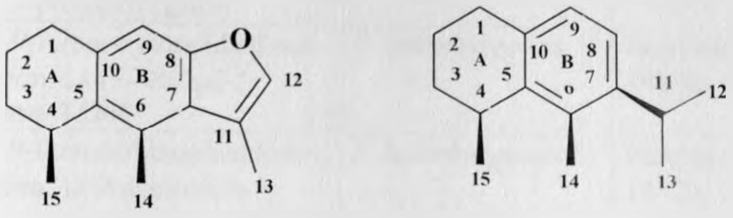
| | $R_1$ | $R_2$ |
|---|---|---|
| 336 | Ang | H |
| 337 | Ang | OH |



| | R |
|---|---|
| 349 | Ac |
| 350 | 3-Me-2-buten |
| 351 | Ang |

### 2.4.2.2.3 CACALOL SESQUITERPENES FROM *SENECIO* SPECIES

Cacalolides are biogenetic Wagner-Meerwein rearrangement products [Burgueno-Tapia *et al.*, 2001] derived from eremophilanes and furoeremophilanes [Burgueno Tapia *et al.*, 2001], typically with an aromatic ring B, in which carbon-14 has further migrated to C-6.




Cacalol skeletons

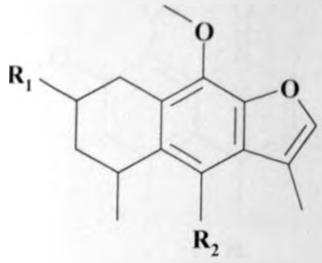
They derive their name from cacalol, a sesquiterpenoid that was isolated from the antihyperglycemic species *Cacalia decomposita* (Romo & Joseph-Nathan, 1964). Table 2.12 below lists some of cacalol sesquiterpenoids isolated from this genus.

Table 2.9: Cacalol sesquiterpenes from *Senecio* species

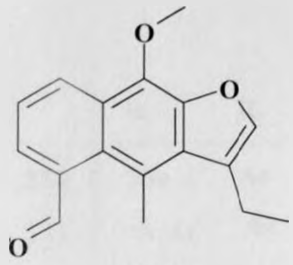
| **Cacalol** | **Source** | **Reference** |
|---|---|---|
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-9,14-diol; 4-form, 14-Aldehyde, 9-Me ether (**352**) | *S. fuertesii*<br>*S. picardae* | Bohlmann *et al.*, 1977; 1978c; 1990 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9-diol; 2α-form, 9-Me ether, 2-Ac (**353**) | *S. fuertesii* | Bohlmann *et al.*, 1990 |
| 13-Hydroxydehydrocacalohastin-15-al (**354**) | *S. heliopsis* | Bohlmann *et al.*, 1985 |
| 14-Hydroxycacalol propionate (**355**) | *S. inornatus* | Bohlmann *et al.*, 1977; 1978c; 1990 |
| 14-Acetoxycacalol propionate(**356**) | *S. inornatus* | Bohlmann *et al.*, 1977; 1978c; 1990 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 9-Propanoyl, 14-Ac (**357**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 9-Propanoyl, 2,14-di-Ac (**358**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 9,14-Dipropanoyl (**359**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 14-(2-Methylpropanoyl), 9-propanoyl (**360**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 2-(3-Methylbutanoyl), 9-propanoyl, 14-Ac (**361**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |

Table 2.9: Cacalol sesquiterpenes from *Senecio* species

| Cacalol | Source | Reference |
|---|---|---|
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 14-(2-Methylbutanoyl), 9-propanoyl (**362**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form,14-(3-Methylbutanoyl), 9-propanoyl (**363**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 14-(3-Methyl-2-butenoyl), 9-propanoyl (**364**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2α-form, 14-Angeloyl, 9-propanoyl (**365**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-2,9,14-triol; 2β-form, 9-Propanoyl, 2,14-di-Ac (**366**) | *S. lydenburgensis* | Bohlmann *et al.*, 1982b; 1985 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-9,13,14-triol; 9-Propanoyl, 13,14-di-Ac (**367**) | *S. lydenburgensis* | Bohlmann *et al.*, 1985; 1990 |
| 14(5→6)-Abeo-1,2-dihydroxy-1,3,5(10)-furanoeremophilatrien-9-one; 2-Me ether (**368**) | *S. madagascariensis* | Burgueno-Tapia *et al.*, 2001. |
| 14(5→6)-Abeo-1,2-dihydroxy-1,3,5(10)-furanoeremophilatrien-9-one; Di-Me ether (**369**) | *S. madagascariensis* | Burgueno-Tapia *et al.*, 2001 |
| 13-Acetoxycacalol methyl ether (**370**) | *S. picardae* | Bohlmann *et al.*, 1978e. |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-9,13,14-triol; 9-Me ether, 14-angeloyl, 13-Ac (**371**) | *S. picardae* | Bohlmann *et al.*, 1985; 1990 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-9,13,14-triol; 14-Aldehyde, 9-Me ether, 13-Ac (**372**) | *S. picardae* | Bohlmann *et al.*, 1985; 1990 |
| 14(5→6)-Abeo-5,9-furanoeremophiladiene-9,14-diol; 4β-form, 9-Me ether(**373**) | *S.* spp | Bohlmann *et al.*, 1977; 1978e; 1990 |
| 14(5→6)-Abeo-2,9-dihydroxy-2,5,9-furanoeremophilatrien-1-one; 2-Me ether (**374**) | *S.* spp. | Bohlmann *et al.*, 1977 |
| 14(5→6)-Abeo-3,9-dihydroxy-2,5,9-furanoeremophilatrien-1-one; 3-Me ether (**375**) | *S.* spp. | Bohlmann *et al.*, 1977 |
| 14(5→6)-Abeo-3,9-dihydroxy-2,5,9-furanoeremophilatrien-1-one; Di-Me ether (**376**) | *S.* spp. | Bohlmann *et al.*, 1977 |



| | $R_1$ | $R_2$ |
|---|---|---|
| 352 | H | =O |
| 353 | OAc | Me |



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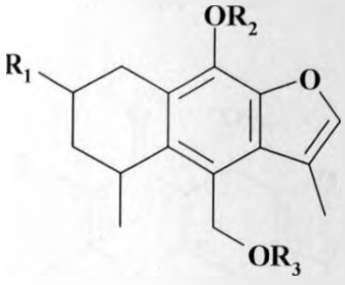
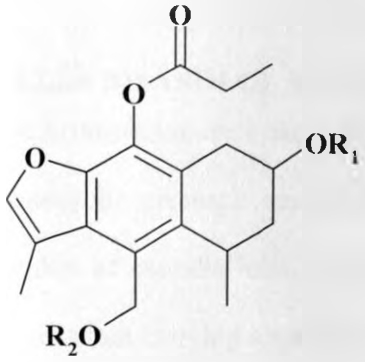
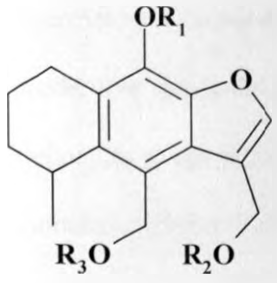


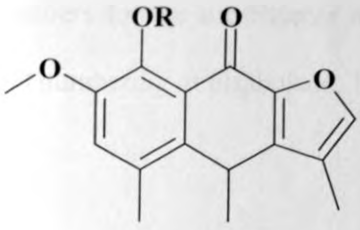
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 355 | H | Prop | OH |
| 356 | H | Prop | Ac |
| 357 | OH | Prop | Ac |
| 373 | H | Me | H |



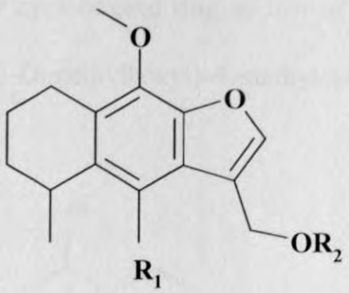
| | $R_1$ | $R_2$ | |
|---|---|---|---|
| 358 | Ac | Ac | (2α - form) |
| 359 | Ac | Prop | |
| 360 | H | 2-Meprop | |
| 361 | 3-Mebu | Ac | |
| 362 | H | 2-Mebu | |
| 363 | H | 3-Mebu | |
| 364 | H | 3-Me-2-buten | |
| 365 | H | Ang | |
| 366 | Ac | Ac | (2β -form) |



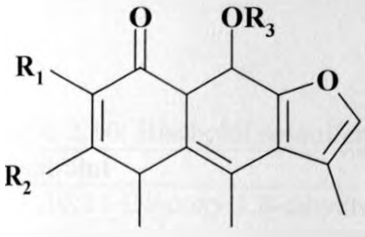
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 367 | Prop | Ac | Ac |
| 371 | Me | Ac | Ang |



| | R |
|---|---|
| 368 | H |
| 369 | Me |



| | $R_1$ | $R_2$ |
|---|---|---|
| 370 | Me | Ac |
| 372 | =O | Ac |



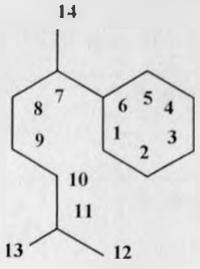
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 374 | OMe | H | H |
| 375 | H | OMe | H |
| 376 | H | OMe | Me |

### 2.4.2.2.4 BISABOLOL SESQUITERPENES FROM *SENECIO* SPECIES

The bisabolanes are a fairly large group mainly found as constituents of higher plants. The monocyclic aromatic sesquiterpenes of the bisabolane family are constituents of a large number of essential oils. Most of these compounds are characterized by a benzylic chiral center often carrying a methyl group at this position [Zhang & Rajabab, 2004] and have been isolated from natural sources in enantiomerically pure form [Fuganti *et al.*, 1999]. Diverse biological activities [Mayer *et al.*, 1998] exhibited by these compounds include anti-inflammatory, anti-viral, and anti-mycobacterial properties, and they have attracted considerable attention from synthetic chemists. Despite their rather simple structures, the stereocenter at the benzylic position [Cesati *et al.*, 2004] poses a significant challenge in the asymmetric synthesis of even the simplest of these molecules.

The numbering system used for bisabolanes is the same as the farnesane system. Since the cyclohexane ring has a plane of symmetry, substituents in this ring should be numbered where possible avoiding the compound locant, 1(6), for a double bond and keeping the

numbers for the substituents in the cyclohexane ring as low as possible. The configuration and numbering of bisabolane, 1-(1,5-Dimethylhexyl)-4-methylcyclohexane is shown below.



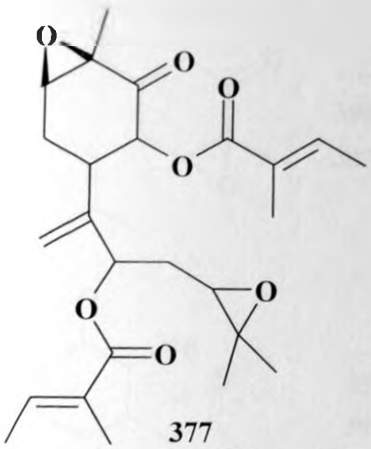

Bisabolane skeleton

Table 2.10: Bisabolol sesquiterpenes from *Senecio* species

| **Bisabolol** | **Source** | **Reference** |
|---|---|---|
| 3,4.,10,11-Diepoxy-1,8-dihydroxy-7(14)-bisabolen-2-one; (1α,3β,4β)-form, Diangeloyl (**377**) | *S. abrotanifolius* *S. fulgens* | Bohlmann *et al.*, 1981c |
| 3,4-Epoxy-7(14),10-bisaboladiene-1,2,8-triol; (1β,3α,4α,6ξ,8ξ)-form, 2-Ketone, 8-(2*Z*-hexenoyl), 1-Ac (**378**) | *S. erubescens* | Bohlmann *et al.*, 1981c; 1985 |
| 3,4-Epoxy-7(14),10-bisaboladiene-1,2,8-triol; (1β,2β,3β,4β,6ξ,8ξ)-form, 1,2-Diangeloyl, 8-Ac (**379**) | *S. fulgens* | Bohlmann *et al.*, 1981c; 1985 |
| 1,3,5,7(14),10-Bisabolapentaene-1,2,4,8-tetrol; 1,4-Quinone, 8-angeloyl (**380**) | *S. longifolius* | Bohlmann *et al.*, 1978i; 1985 |
| 3,7(14),10-Bisabolatrien-2-one (**381**) | *S. macroglossus* | Bohlmann *et al.*, 1985 |
| 1,3,5,7(14),10-Bisabolapentaene-1,2,4,8-tetrol; 8-Angeloyl (**382**) | *S. oxyodontus* | Gutierrez *et al.*, 1988 |
| 3-*O*-Angeloylsenecioodontol(**383**) | *S. oxyodontus* | Gutierrez *et al.*, 1988 |
| 1,3,5,7(14),10-Bisabolapentaene-1,2,4,8-tetrol; 2,4-Di-Me ether, 8-angeloyl (**384**) | *S. oxyodontus* | Bohlmann *et al.*, 1978h,i; 1985 |
| 1,3,5,7(14),10-Bisabolapentaene-1,2,4,8-tetrol; 1,4-Quinone, 2-Me ether, 8-angeloyl (**385**) | *S. oxyodontus* | Bohlmann *et al.*, 1978h,i; 1985 |
| 3,4-Epoxy-7(14),10-bisaboladiene-1,2,8-triol; (1α,3β,4β,6*S*)-form, 2,8-Diketone, 1-*O*-(3-methylbutanoyl) (**386**) | *S. pampeanus* | Bohlmann *et al.*, 1986a |
| 3,4-Epoxy-7(14),10-bisaboladiene-1,2,8-triol; (1α,3β,4β,6*S*)-form, 2,8-Diketone, 1-tigloyl (**387**) | *S. pampeanus* | Bohlmann *et al.*, 1986a |
| Cyclopampeanone; *O*-(3-Methylbutanoyl) (**388**) | *S. pampeanus* | Bohlmann *et al.*, 1986a |
| Cyclopampeanone; 7,10-Diepimer, *O*-tigloyl(**389**) | *S. pampeanus* | Bohlmann *et al.*, 1986a |
| Pampeanone *O*-Tigloyl (**390**) | *S. pampeanus* | Bohlmann *et al.*, 1986a |
| Pampeanone isovalerate (**391**) | *S. pampeanus* | Bohlmann *et al.*, 1986a |
| Pampeanone ; *O*-(3-Methylbutanoyl), 10- | *S. pampeanus* | Bohlmann *et al.*, 1986a |

Table 2.10: Bisabolol sesquiterpenes from *Senecio* species

| **Bisabolol** | **Source** | **Reference** |
|---|---|---|
| epimer (**392**) | | |
| 2,10-Bisaboladien-1-one; (6*S*,7*S*)-form (**393**) | *S. palmensis* | Reina *et al.*, 2002 |
| 3,4-Epoxy-1,11-dihydroxy-7(14),9-bisaboladiene-2,8-dione; (1α,3β,4β,9*E*)-form, 1-(3-Methylbutanoyl) (**394**) | *S. pompeanus* | Bohlmann *et al.*, 1986a |
| 3,4-Epoxy-1,11-dihydroxy-7(14),9-bisaboladiene-2,8-dione; (1α,3β,4β,9*E*)-form, 1-Tigloyl (**395**) | *S. pompeanus* | Bohlmann *et al.*, 1986a |
| 3,4-Epoxy-1,11-dihydroxy-7(14),9-bisaboladiene-2,8-dione; (1α,3β,4β,9*E*)-form, 11-Hydroperoxide, 1-(3-methylbutanoyl) (**396**) | *S. pompeanus* | Bohlmann *et al.*, 1986a |
| 3,4-Epoxy-1,11-dihydroxy-7(14),9-bisaboladiene-2,8-dione; (1α,3β,4β,9*E*)-form, 11-Hydroperoxide, 1-tigloyl (**397**) | *S. pompeanus* | Bohlmann *et al.*, 1986a |
| 3,4-Epoxy-1,11-dihydroxy-7(14),9-bisaboladiene-2,8-dione; (1α,3β,4β,9*E*)-form, 7α,14-Epoxide, 1-(3-methylbutanoyl) (**398**) | *S. pompeanus* | Bohlmann *et al.*, 1986a |
| 3,4-Epoxy-1,11-dihydroxy-7(14),9-bisaboladiene-2,8-dione; (1α,3β,4β,9*E*)-form, 7β,14-Epoxide, 1-tigloyl (**399**) | *S. pompeanus* | Bohlmann *et al.*, 1986a |
| 3,4-Epoxy-1,11-dihydroxy-7(14),9-bisaboladiene-2,8-dione; (1α,3β,4β,9*E*)-form, 7α,14-Epoxide, 11-hydroperoxide, 1-tigloyl (**400**) | *S. pompeanus* | Bohlmann *et al.*, 1986a |
| 1,3,5,7(14),10-Bisabolapentaene-1,2,4,8-tetrol; 2-Me ether, 8-angeloyl, 4-Ac (**401**) | *S. pubigerus* | Bohlmann *et al.*, 1978i; 1985 |
| 1,3,5,7(14),10-Bisabolapentaene-1,2,4,8-tetrol; 2-Me ether, 8-angeloyl, 1,4-di-Ac (**402**) | *S. pubigerus* | Bohlmann *et al.*, 1978i; 1985 |
| 1,3,5,7(14),10-Bisabolapentaene-1,2,4,8-tetrol; 1,4-Quinone, 2-angeloyl, 8-*O*-(2,3-epoxy-2-methylbutanoyl) (**403**) | *S. pubigerus* | Bohlmann *et al.*, 1978i; 1985 |
| 2,10-Bisaboladiene-1,12-diol; 1-Ketone, 12-Ac (**404**) | *S. smithii* | Bohlmann *et al.*, 1983 |
| Puliglutone (**405**) | *S. smithii* | Bohlmann *et al.*, 1983 |
| Sessquicineol (**406**) | *S. subrubriflorus* | Bohlmann *et al.*, 1982c |



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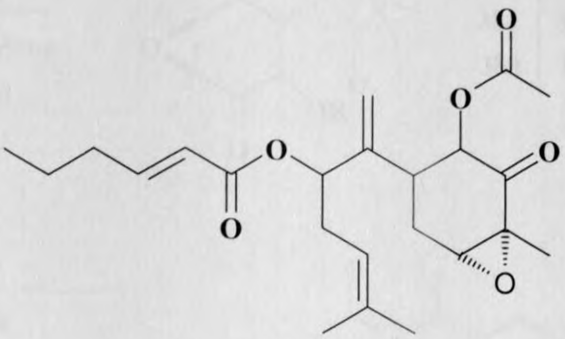

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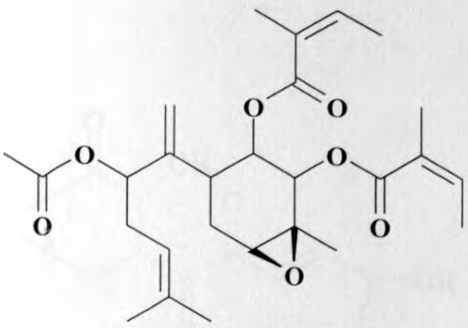
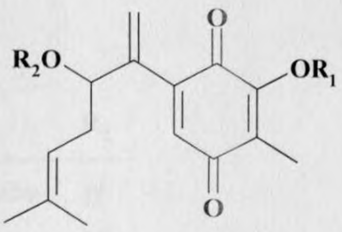
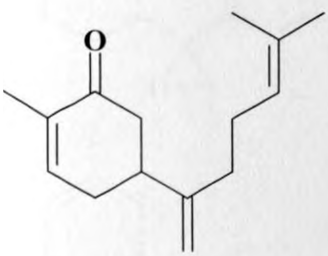


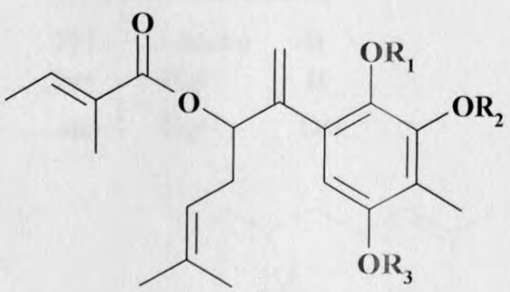
379



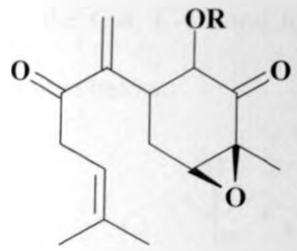
| | $R_1$ | $R_2$ |
|---|---|---|
| 380 | H | Ang |
| 385 | Me | Ang |
| 403 | Ang | 2,3-Epoxy-2-mebu |



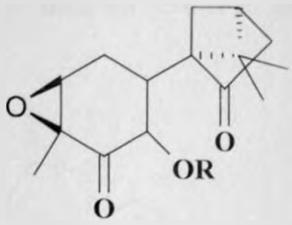
381



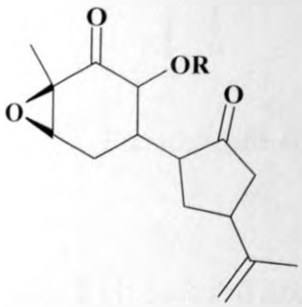
| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 382 | H | H | H |
| 383 | H | Ang | H |
| 384 | H | Me | Me |
| 401 | H | Me | Ac |
| 402 | Ac | Me | Ac |



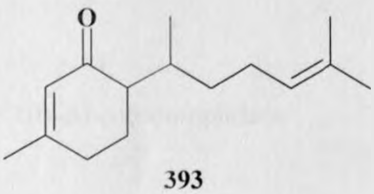
| | R |
|---|---|
| 386 | 3-Mebu |
| 387 | Tigl |



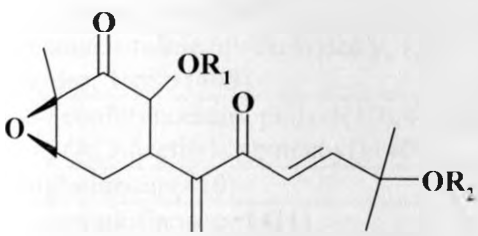
| | R |
|---|---|
| 388 | 3-Mebu |
| 389 | Tigl |



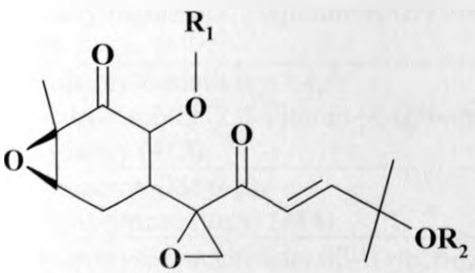
| | R |
|---|---|
| 390 | Tig |
| 391 | 3-Mebu |
| 392 | 3-Mebu; 10-epimer |



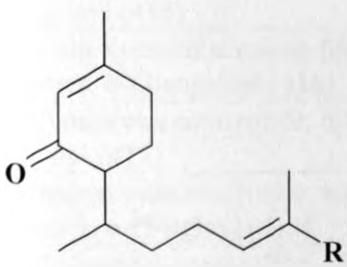
393



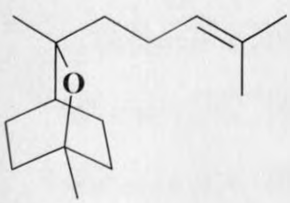
| | $R_1$ | $R_2$ |
|---|---|---|
| 394 | 3-Mebu | H |
| 395 | Tigl | H |
| 396 | 3-Mebu | OH |
| 397 | Tigl | OH |



| | $R_1$ | $R_2$ |
|---|---|---|
| 398 | 3-Mebu | H |
| 399 | Tigl | H |
| 400 | Tigl | OH |



| | R |
|---|---|
| 404 | OAc |
| 405 | =O |

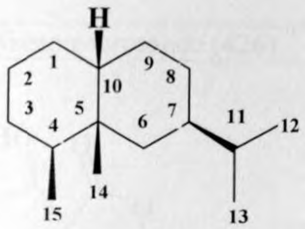


406

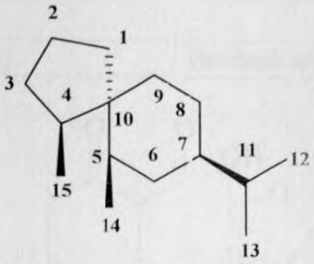
**2.4.2.2.5 SECO AND ABEOEREMOPHILANES**

Secoeremophilanes are derivatives of eremophilanes, formed by cleavage of the C-8, C-9 or C-9, C-10 bond followed by oxidation, while abeoeremophianes are formed by migration of one or more bonds in eremophilane or furanoeremophilane skeleton. The numbering of the eremophilane or furanoeremophilane skeleton is retained in the new structure. The migration

of the C-4, C-5 bond to C-10 in the eremophiane skeleton results to abeoeremophilane as shown below.




Eremophilane skeleton



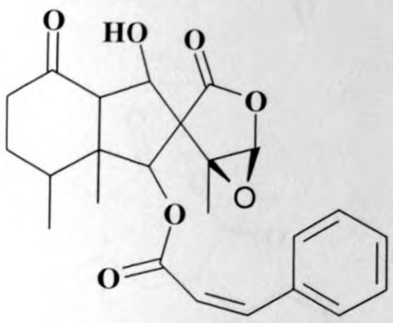

(10*R*)-4(5 → 10)-Abeoeremophilane

Table 2.11: Seco and Abeoeremophilanes sesquiterpenes from *Senecio* species

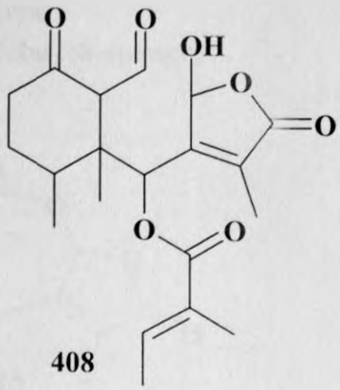
| **Seco and Abeoeremophilanes** | **Source** | **Reference** |
|---|---|---|
| Spirosenbergiolide (**407**) | *S. bergii* | Bohlmann *et al.*, 1986a. |
| Secomacrotolide 6β-Tigloyloxy, 1,10-dihydro, 1-oxo (**408**) | *S. bergii* | Trendafilova *et al.*, 1995 |
| 5,6-Secofuranoeremophila-1(10),4-dien-6-ol; *O*-(3-Methyl-2-butenoyl) (**409**) | *S. elegans* | Bredenkamp *et al.*, 1985a |
| Senglutinosin (**410**) | *S. glutinosus* | Bredenkamp *et al.*, 1985a |
| Norsecoglutinosone (**411**) | *S. glutinosus* | Zdero *et al.*, 1989a |
| 8-Hydroxy-6-methoxy-3,4,5-trimethylnaphtho[2,3-*b*]furan-9(4*H*)-one (**412**) | *S. linifolius* | Torres *et al.*, 1989 |
| 8-Hydroxy-6-methoxy-3,4,5-trimethylnaphtho[2,3-*b*]furan-9(4*H*)-one, 4-Hydroxy (**413**) | *S. linifolius* | Torres *et al.*, 1989 |
| Secomacrotolide 6-(2-Methylpropenoyloxy) (**414**) | *S. macedonicus* | Trendafilova *et al.*, 1995 |
| 6-Hydroxysecomacrolide; 6β-form, 6-*O*-Angeloyl (**415**) | *S. macrotis* | Bohlmann *et al.*, 1981e |
| 6-Hydroxysecomacrolide; 6β-form, 8-Epimer, 6-*O*-angeloyl (**416**) | *S. macrotis* | Bohlmann *et al.*, 1981e |
| 6-Hydroxysecomacrolide; 6β-form, 6-*O*-Tigloyl (**417**) | *S. macrotis* | Bohlmann *et al.*, 1981e |
| 6-Hydroxysecomacrolide; 6β-form, 8-Epimer, 6-*O*-tigloyl (**418**) | *S. macrotis* | Bohlmann *et al.*, 1981e |
| 6-Hydroxysecomacrolide; 6β-form, 6-(3-Methylbutanoyl) (**419**) | *S. macrotis* | Bohlmann *et al.*, 1981e |
| 6-Hydroxysecomacrolide; 6β-form, 8-Epimer, 6-(3-methylbutanoyl) (**420**) | *S. macrotis* | Bohlmann *et al.*, 1981e |
| Secomacrotolide 6β-(3-Methylbutanoyloxy) (**421**) | *S. macrotis* | Bohlmann *et al.*, 1981e |
| Secomacrotolide 6β-Angeloyloxy (**422**) | *S. macrotis* | Bohlmann *et al.*, 1981e. |
| Secomacrotolide 6β-Tigloyloxy (**423**) | *S. macrotis* | Bohlmann *et al.*, 1981e |

Table 2.11: Seco and Abeoeremophilanes sesquiterpenes from *Senecio* species

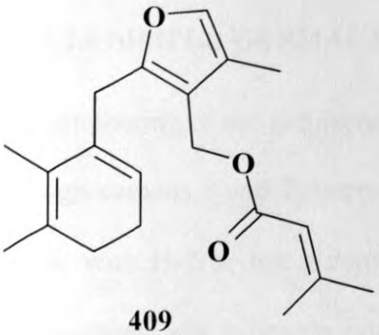
| Seco and Abeoeremophilanes | Source | Reference |
|---|---|---|
| Serratifolide A (**424**) | *S. serratifolius* | Dupré *et al.*, 1991 |
| Serratifolide B (**425**) | *S. serratifolius* | Dupré *et al.*, 1991 |
| 6-Acetoxysecomacrotolide (**426**) | *S.* spp. | Bredenkamp *et al.*, 1985a |



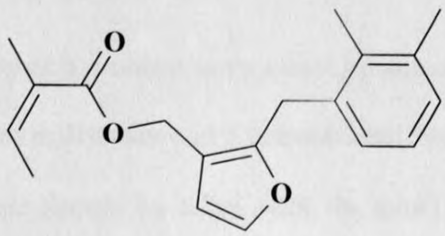
**407**



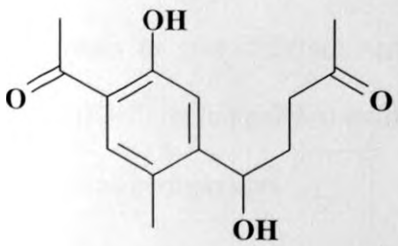
**408**



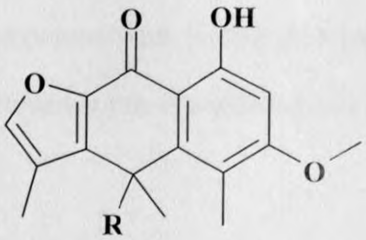
**409**



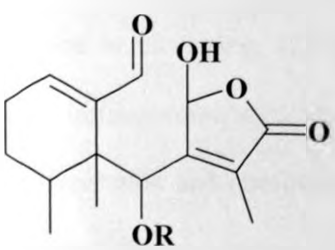
**410**



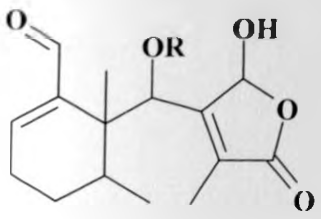
**411**



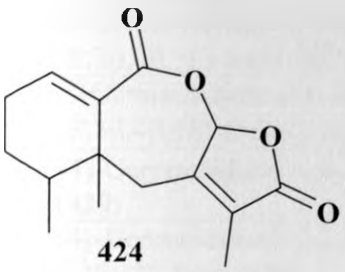
| | R |
|---|---|
| 412 | H |
| 413 | OH |



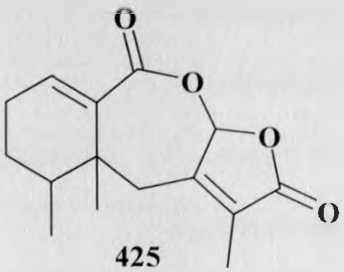
| | R |
|---|---|
| 414 | 2-Mepropen |
| 421 | 3-Mebu |
| 422 | Ang |
| 423 | Tigl |
| 426 | Ac |




| | R |
|---|---|
| 415 | Ang |
| 416 | Ang (8-epimer) |
| 417 | Tigl |
| 418 | Tigl (8-epimer) |
| 419 | 3-Mebu |
| 420 | 3-Mebu (8-epimer) |




424

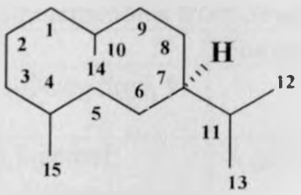



425

**2.4.2.2.6 SIMPLE GERMACRANE SESQUITERPENOIDS**

The numbering of the germacrane skeleton poses a problem since there is plane of symmetry through carbons 2 and 7. Germacranes are normally drawn in a conventional way as shown below with H-7 in the α-configuration. Care should be taken with the small number of germacranes with a double bond at C- 7 as the ring can be numbered in either direction. Germacranes frequently have double bonds in the 1(10) and 4 positions. There have been proposals to give different names to the skeletons with (1(10)*Z*,4*E*) (melampolides) and (1(10)*E*,4*Z*) (heliangolides) configurations. However this is confusing and all compounds are named as germacranes.

The large germacrane group is divided into simple germacranes, that is those without a lactone or furan ring, 12,6-germacranolides, 12,8-germacranolides, furanogermacranes, nor- and homogermacranes, secogermacranes, and cyclogermacranes [Fischer, 1990]. The configuration and numbering of germacrane (1, 7-Dimethyl-4-(1-methylethyl)-cyclodecane) is shown below.



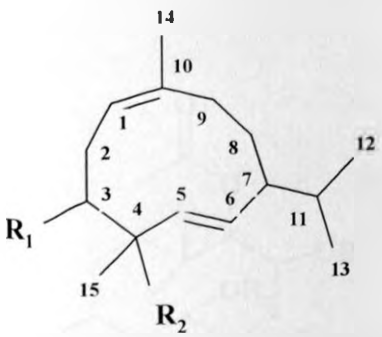

Germacrane

Table 2.12: Simple Germacrane sesquiterpenoids from *Senecio* species

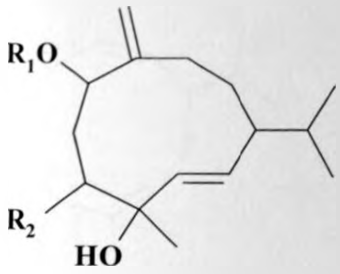
| **Simple Germacrane** | **Source** | **Reference** |
|---|---|---|
| 1(10),5-Germacradiene-3,4-diol; (1(10)*E*,3β,4β,5*E*)-form (**427**) | *S. adenophyllus* | Dupré *et al.*, 1991. |
| 1(10),5-Germacradiene-3,4-diol; (1(10)*E*,3β,4β,5*E*)-form, 3-Ac (**428**) | *S. adenophyllus* | Dupré *et al.*, 1991. |
| 5,10(14)-Germacradiene-1,4-diol; (1β,4β*OH*,5*E*)-form (**429**) | *S. adenophyllus* | Dupré *et al.*, 1991. |
| 5,10(14)-Germacradiene-1,3,4-triol; (1β,3β,4β,5*E*)-form (**430**) | *S. adenophyllus* | Dupré *et al.*, 1991. |
| 5,10(14)-Germacradiene-1,3,4-triol; (1β,3β,4β,5*E*)-form , 3-Ac (**431**) | *S. adenophyllus* | Dupré *et al.*, 1991. |
| 5,10(14)-Germacradiene-1,3,4-triol; (1β,3β,4β,5*E*)-form , 1-Hydroperoxide (**432**) | *S. adenophyllus* | Dupré *et al.*, 1991. |
| 4,5-Epoxy-1(10)-germacrene-3,6-diol; (1(10)*E*,3β,4α,5β,6β)-form, 3-Angeloyl (**433**) | *S. crassissimus*<br>*S. cylindricus*<br>*S. vitalis* | Bohlmann *et al.*, 1977; 1979d; 1980b. |
| 4,5-Epoxy-1(10)-germacrene-3,6-diol; (1(10)*E*,3β,4α,5β,6β)-form,3-Tigloyl (**434**) | *S. crassissimus*<br>*S. vitalis* | Bohlmann *et al.*, 1977; 1979d; 1980b. |
| 4,5-Epoxy-1(10)-germacrene-3,6-diol; (1(10)*E*,3β,4α,5β,6β)-form,3- (3-Methyl-2-butenoyl) (**435**) | *S. crassissimus*<br>*S. cylindricus*<br>*S. vitalis* | Bohlmann *et al.*, 1977; 1979d; 1980b. |
| 4,5-Epoxy-1(10)-germacrene-3,6-diol; (1(10)*E*,3β,4α,5β,6β)-form,3-Tigloyl, 6-Ac (**436**) | *S. ficoides* | Bohlmann *et al.*, 1977; 1979d; 1980b, |
| 1,10:4,5-Diepoxy-11(13)-germacrene-3,8,9-triol; (1β,3β,4α,5β,8β,9β,10α)-form, 3-Angeloyl, 8-(3-methyl-2-butenoyl) (**437**) | *S. galpinii* | Bohlmann *et al.*, 1978f; 1981a; 1982g. |
| 1,10:4,5-Diepoxy-11(13)-germacrene-3,8,9-triol; (1β,3β,4α,5β,8β,9β,10α)-form, 3-Angeloyl, 8-(3-methyl-2-butenoyl), 9-Ac (**438**) | *S. galpinii* | Bohlmann *et al.*, 1978f; 1981a; 1982g. |
| 1,10:4,5-Diepoxy-11(13)-germacrene-3,8,9-triol; (1β,3β,4α,5β,8β,9β,10α)-form, 3,8-Diangeloyl (**439**) | *S. galpinii* | Bohlmann *et al.*, 1978f; 1981a; |
| 1,10:4,5-Diepoxy-11(13)-germacrene-3,8,9-triol; (1β,3β,4α,5β,8β,9β,10α)-form, 3,9-Diangeloyl, 8-Ac (**440**) | *S. galpinii*<br>*S. rhomboideus* | Bohlmann *et al.*, 1978f; 1981a; 1982g. |
| 1,10-Epoxy-11-germacrene-3,4,5,8,9-pentol; (1β,3β,4α,5α,8β,9β,10α)-form, 3-Angeloyl, 8-(3-methyl-2-butenoyl), 9-Ac (**441**) | *S. galpinii* | Bohlmann *et al.*, 1982g. |
| 1,10-Epoxy-11-germacrene-3,4,5,8,9-pentol; (1β,3β,4α,5α,8β,9β,10α)-form, 3-Angeloyl, 8-(3-methyl-2-butenoyl), 5,9-di-Ac (**442**) | *S. galpinii* | Bohlmann *et al.*, 1982g. |
| 1,10-Epoxy-11-germacrene-3,4,5,8,9-pentol; | *S. galpinii* | Bohlmann *et al.*, |

Table 2.12: Simple Germacrane sesquiterpenoids from *Senecio* species

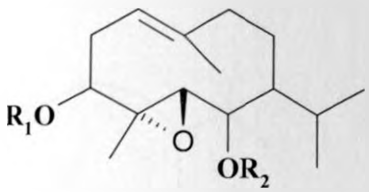
| Simple Germacrane | Source | Reference |
|---|---|---|
| (1β,3β,4α,5α,8β,9β,10α)-form, 3,8-Diangeloyl, 9-Ac (**443**) | | 1982g. |
| 1,10-Epoxy-11-germacrene-3,4,5,8,9-pentol; (1β,3β,4α,5α,8β,9β,10α)-form, 3,8-Diangeloyl, 5,9-di-Ac (**444**) | *S. galpinii* | Bohlmann *et al.*, 1982g. |
| 4(15),5,9-Germacratrien-1-ol; (1β,5*E*,9*Z*)-form (**445**) | *S. philippicus* | Jakupovic *et al.*, 1991. |
| 1,10:4,5-Diepoxy-11(13)-germacrene-3,8,9-triol; (1β,3β,4α,5β,8β,9β,10α)-form, 3,9-Diangeloyl (**446**) | *S. rhomboideus* | Bohlmann *et al.*, 1978f,g; 1981a; 1982f |
| 4,5-Epoxy-1(10)-germacrene-3,6-diol; (1(10)*E*,3β,4α,5β,6β)-form,3-(3-Methyl-2-butenoyl), 6-Ac (**447**) | *S. vitalis* | Bohlmann *et al.*, 1977; 1979d; 1980b. |
| 4,5-Epoxy-1(10),11-germacradiene-3,8,9-triol; (1(10)*E*,3β,4α,5α,8β,9β)-form, 3-Angeloyl, 8,9-di-Ac (**448**) | *S.* spp | Bohlmann *et al.*, 1977. |
| 4,5-Epoxy-1(10)-germacrene-3,6-diol; (1(10)*E*,3β,4α,5α,6β)-form, 3-Tigloyl (**449**) | *S.* sp. | Bohlmann *et al.*, 1977; 1979d; 1980b. |
| 4,5-Epoxy-1(10)-germacrene-3,6-diol; (1(10)*E*,3β,4α,5β,6β)-form,3-Angeloyl, 6-Ac (**450**) | *S.* spp | Bohlmann *et al.*, 1977; 1979b; 1980b. |
| 1(10),4-Germacradien-6-ol; (1(10)*E*,4*E*,6β)-form, Tigloyl (**451**) | *S.* spp. | Bohlmann *et al.*, 1977. |
| 1(10),4-Germacradien-6-ol; (1(10)*E*,4*E*,6β)-form, *O*-(3-Methyl-2-pentenoyl) (**452**) | *S.* spp. | Bohlmann *et al.*, 1977. |
| 1(10),4-Germacradien-6-ol; (1(10)*E*,4*E*,6β)-form, Angeloyl (**453**) | *S.* spp. | Bohlmann *et al.*, 1977. |
| 1(10),4-Germacradiene-3,6-diol; (1(10)*E*,3β,4*E*,6β)-form, 3-Angeloyl, 6-Ac (**454**) | *S.* spp | Bohlmann *et al.*, 1977. |



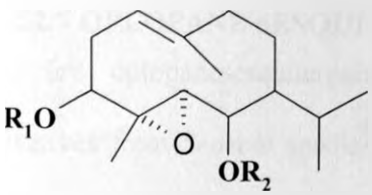

| | $R_1$ | $R_2$ |
|---|---|---|
| **427** | **OH** | **OH** |
| **428** | **OAc** | **OH** |



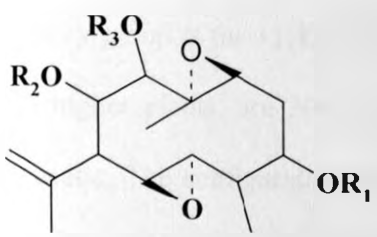

| | $R_1$ | $R_2$ |
|---|---|---|
| 429 | H | H |
| 430 | H | OH |
| 431 | H | OAc |
| 432 | OH | OH |



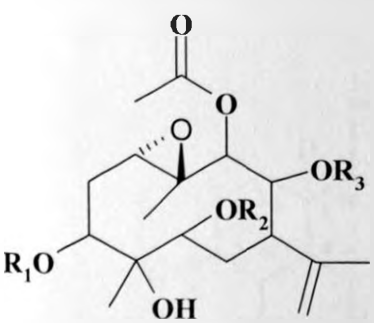

| | R | $R_2$ |
|---|---|---|
| 433 | Ang | H |
| 434 | Tigl | H (1(10)E,3β, 4α, 5β, 6β)-form. |
| 435 | Sene | H |
| 436 | Tigl | Ac |
| 447 | Sene | Ac |



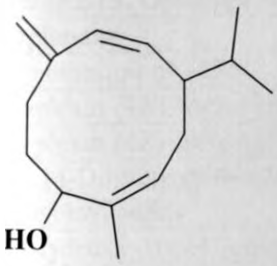

| | R | $R_2$ |
|---|---|---|
| 449 | Tigl | H (1(10)E,3β, 4α, 5α, 6β)-form. |
| 450 | Tigl | Ac |



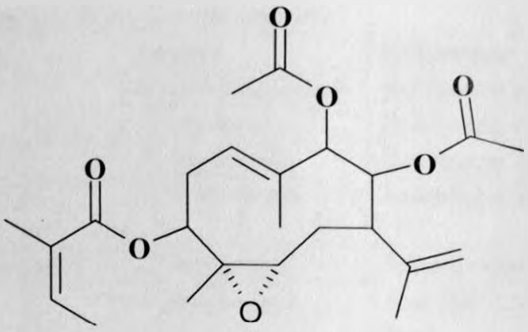

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 437 | Ang | Sene | H |
| 438 | Ang | Sene | Ac |
| 439 | Ang | Ang | H |
| 440 | Ang | Ac | Ang |
| 446 | Ang | H | Ang |



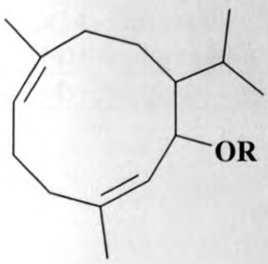

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 441 | Ang | H | Sene |
| 442 | Ang | Ac | Sene |
| 443 | Ang | H | Ang |
| 444 | Ang | Ac | Ang |
| 454 | Ang | Ac | Tigl |




445



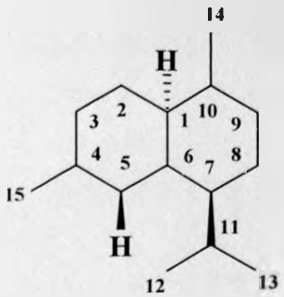

448



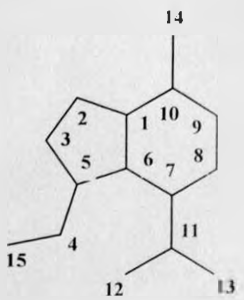

| | R |
|---|---|
| 451 | Tigl |
| 452 | 3-Me-2-penten |
| 453 | Ang |

### 2.4.2.2.7 OPLOPANE SESQUITERPENOIDS FROM *SENECIO* SPECIES.

Very few oplopanesesquiterpenes have been isolated from *Senecio* species. Oplopane derivatives from *Senecio* species usally possess a terminal double bond at C-10, an α, β-unsaturated ketone at C-3 or an hydroxyl group at the same position. Some compounds have an epoxy group at the 11(12) position, ester groups at C-8, C-9 or C-2 positions. Oplopanes, from higher plants, are 3(4 → 5)-abeocadinanes and the numbering system used here is biogenetic. The configuration and numbering of oplopane (1-ethyloctahydro-4-methyl-7-(1-methylethyl)-1H-indene) is shown below. Table 2.13 below gives a summary of all theoplopane sesquiterpenoids isolated from different *Senecio* species



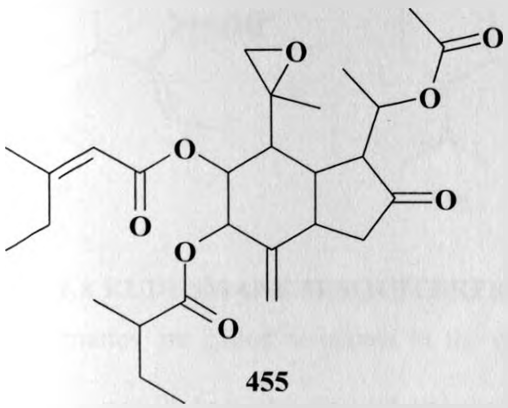

Cadinane skeleton



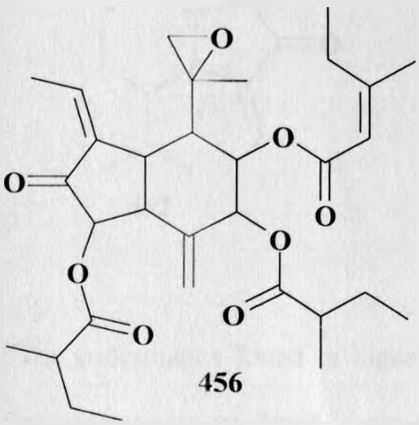

Oplopane skeleton

Table 2.13: Oplopane sesquiterpenoids from *Senecio* species

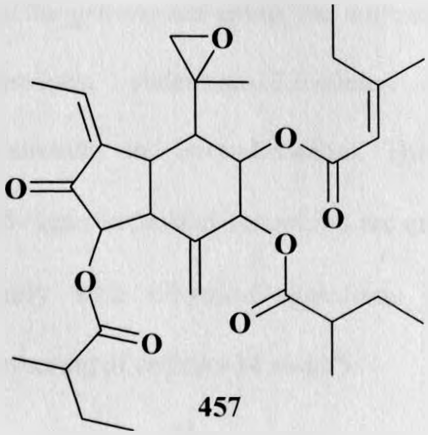
| **Oplopane** | **Source** | **Reference** |
|---|---|---|
| Abrotanifolone (**455**) | *S. abrotanifolius* | Bohlmann *et al.*, 1976b,c. |
| Implexin (4*E*)-form (**456**) | *S. implexus* | Bohlmann *et al.*, 1981a. |
| Implexin (4*Z*)-form (**457**) | *S. implexus* | Bohlmann *et al.*, 1981a. |
| 10,14-Dihydroxy-4-oplopanone; 14-Angeloyl (**458**) | *S. mexicanus* | Joseph-Nathan *et al.*, 1989b. |
| 4-Hydroxy-10(14)-oplopen-3-one (**459**) | *S. mexicanus* | Joseph-Nathan *et al.*, 1989b. |
| 4,10(14)-Oplopadien-3-ol (**460**) | *S. mexicanus* | Joseph-Nathan *et al.*, 1989a, b. |
| 4,10(14)-Oplopadien-3-ol; 3-Ketone (**461**) | *S. mexicanus* | Joseph-Nathan *et al.*, 1989a,b. |
| 4,10(14)-Oplopadien-3-ol; 3-Ketone, 10(14)*E*-isomer (**462**) | *S. mexicanus* | Joseph-Nathan *et al.*, 1989a,b. |
| 10(14)-Oplopen-3-one (**463**) | *S. mexicanus* | Joseph-Nathan *et al.*, 1990. |



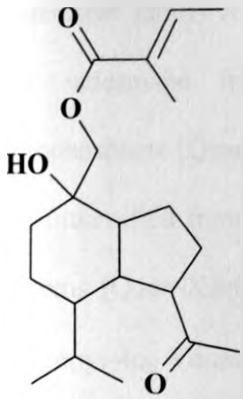
**455**



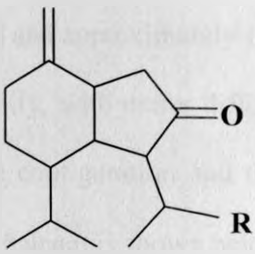
**456**



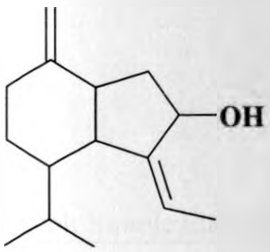
**457**



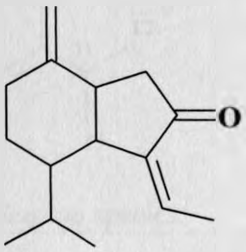

458



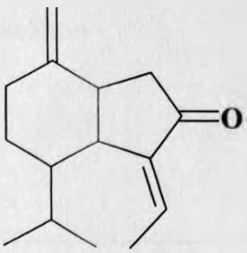

| | R |
|---|---|
| 459 | OH |
| 463 | H |




460



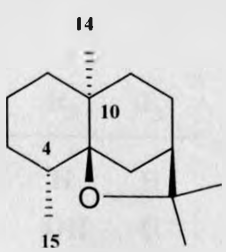

461




462

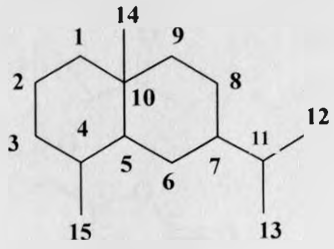
**2.4.2.2.8 EUDESMANE SESQUITERPENOID**

Eudesmanes are called selinanes in the older literature. The eudesmanes found in higher plants generally have the stereochemistry shown below. *Ent*-eudesmanes are found in some species of liverworts. As with the germacrane group, the eudesmanes are divided into groups comprising simple eudesmanes, eudesman-12,6-olides, eudesman-12,8-olides and furanoeudesmanes, secoeudesmanes, and noreudesmanes. There is also a large group of esters based on the dihydro-β- agarofuran skeleton which are grouped separately. Within the eudesmane group, particularly with dihydro-β-agarofuran derivatives, there is some confusion concerning the numbering of carbons 14 and 15.




Dihydro-β-agarofuran (2,2,5,9-Tetramethyl-2H-3, 9α-methano-1-benzoxepin)

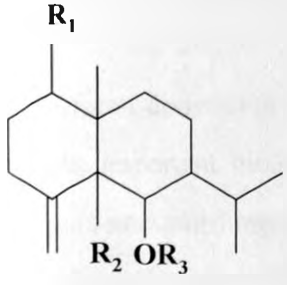
Asteraceae family is a rich source of sesquiterpenoid natural products, especially those with the eudesmane framework. The eudesmanoids are biosynthesised from farnesyl pyrophosphate [Quan-Xiang *et al.*, 2006] and approximately 1000 natural eudesmanoids have been identified from the Asteraceae family, with many different oxygenation and cleavage patterns [Quan-Xiang *et al.*, 2006]. The configuration and numbering of eudesmane (1, 2-isopropyl-4α, 8 dimethyldecahydronaphthalene) is shown below.



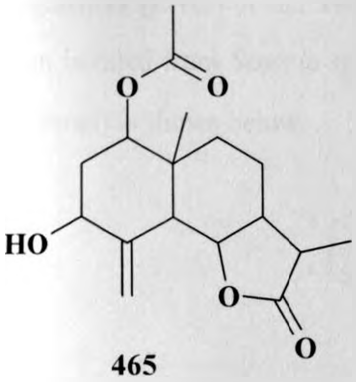

Eudesmane skeleton

Table 2.14: Simple Eudesmane from *Senecio* species.

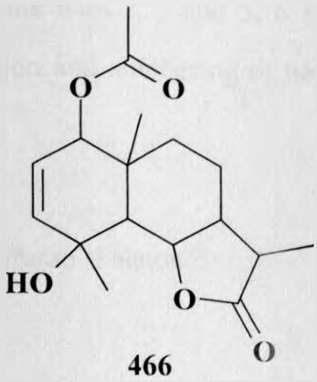
| **Eudesmane** | **Source** | **Reference** |
|---|---|---|
| 1-Hydroxy-4,11-eudesmadien-3-one; 1β-form(**464**). | *S. bracteolatus.* | Sanz *et al.*, 1990. |
| 1,3-Dihydroxy-4(15),11(13)-eudesmadien-12,6-olide; (1α,3α,6α)-form, 11β,13-Dihydro, 1-Ac (**465**) | *S. chrysanthemoides* | Mengi *et al.*, 1991 |
| 1,4-Dihydroxy-2-eudesmen-12,6-olide; (1α,4α,6α,11β*H*)-form, 1-Ac (**466**) | *S. chrysanthemoides* | Mengi *et al.*, 1991 |
| 1,2-Dihydroxy-3,11(13)-eudesmadien-12,8-olid-14-oic acid; (1α,2β,8β)-form, Di-Ac, 4,5-dimethyl-3-oxo-2-heptyl ester (**467**) | *S. flammeus* | Hu *et al.*, 1999 |
| 4(15)-Eudesmene-1,6-diol; (1β,5α,6α,10β)-form (**468**) | *S. microglossus* | Bohlmann *et al.*, 1980c; 1983b |
| 4(15)-Eudesmen-5-ol; 5α-form (**469**) | *S. rhyncholaenus* | Bohlmann *et al.*, 1978j |
| 4(15)-Eudesmen-6-ol; (5α,6α,7β,10β)-form, Tigloyl (**470**) | *S. rhyncholaenus* | Bohlmann *et al.*, 1978j; 1981f; 1982d |
| 7-Eudesmene-1,4-diol; (1β,4β)-form, 1-Angeloyl (**471**) | *S. spp* | Bohlmann *et al.*, 1977 |
| 7-Eudesmene-1,4-diol; (1β,4β)-form, 1-(3-Methyl-2-pentenoyl) (**472**) | *S. spp* | Bohlmann *et al.*, 1977 |



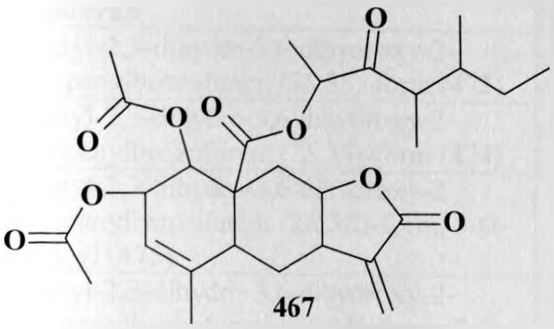

| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **464** | **OH** | **H** | **H** |
| **469** | **H** | **OH** | **H** |
| **470** | **H** | **H** | **Tigl** |



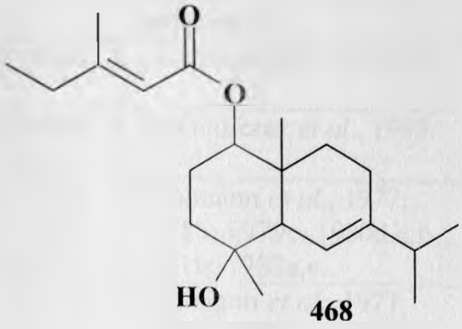

465



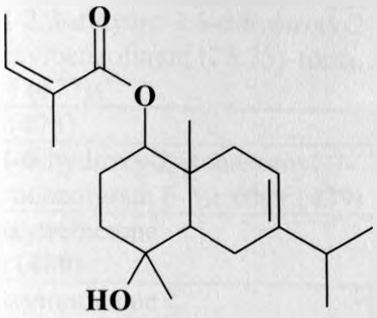

466



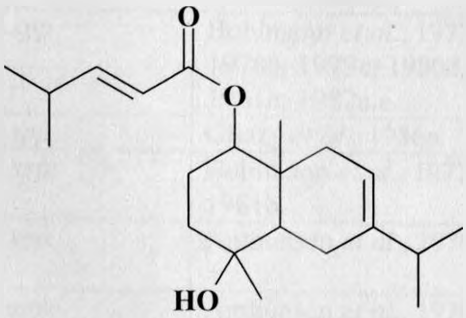

467




468




471



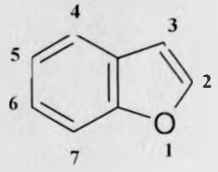

472

### 2.4.2.2.9 BENZOFURANOIDS FROM *SENECIO* SPECIES

Benzofurans are prominent natural products of many genera of the Asteraceae and are especially common in the tribes Astereae, Eupatorieae, Heliantheae, Inuleae and Senecioneae [Proksch & Rodriguez, 1983]

Benzofuran derivatives are an important class of heterocyclic compounds that are known to possess important biological properties. Substituted benzofurans find application as anti-oxidants and anti-fungal, anti-tumor a variety of drugs and in other fields of chemistry and agriculture [Gundogdu-Karaburun *et al.*, 2006]. In addition, benzofurans are used in cosmetic formulations [Sharifi *et al.*, 2008] and have the application as synthetic precursors for optical

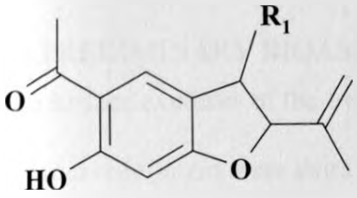
brighteners [Elvers *et al.*, 1999]. Simple benzofurans with 2, 3 and 5, 6 substituents have been isolated from *Senecio* species. The configuration and numbering of benzofuran (benzo [β] furan) is shown below.



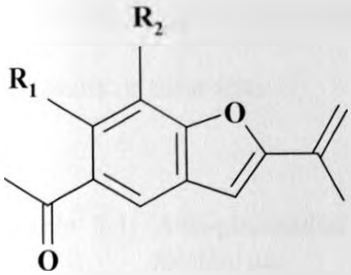
Benzofuran skeleton

Table 2.15: Benzofuranoids from *Senecio* species.

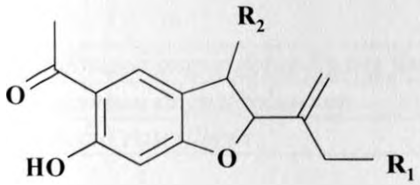
| **Benzofuran** | **Source** | **Reference** |
|---|---|---|
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2*S*,3*S*)-form **(473)** | *S. desfontainei* | De Guttierez *et al.*, 1995. |
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2*S*,3*S*)-form **(474)** | *S. desfontainei* | De Guttierez *et al.*, 1995. |
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2*R*,3*R*)-form, 3-O-Angeloyl **(475)** | *S. spp* | Bohlmann *et al.*, 1977; 1978b; 1979c; 1980d,e,f; 1981b; 1982a,e. |
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2*R*,3*R*)-form, 3-O-[2-(Acetoxymethyl)-2-butenoyl](*Z*-) **(476)** | *S. spp* | Bohlmann *et al.*, 1977; 1978b; 1979c; 1980d,e,f; 1981b; 1982a,e. |
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2*R*,3*S*)-form, 3-O-Angeloyl **(477)** | *S. spp* | Bohlmann *et al.*, 1977; 1978b; 1979c; 1980d,e,f; 1981b; 1982a,e. |
| Euparin **(478)** | *S. spp.* | Ghazy *et al.*, 1986a. |
| 5-Acetyl-6-hydroxy-2-isopropenyl-7-methoxybenzofuran; 6-Me ether **(479)** | *S. spp.* | Bohlmann *et al.*, 1977; 1981b. |
| 6-Hydroxytremetone (*S*)-form **(480)** | *S. spp.* | Anthonsen *et al.*, 1970. |
| 6-Hydroxytremetone (*S*)-form; 3'-Hydroxy **(481)** | *S. spp.* | Anthonsen *et al.*, 1970. |
| 6-Hydroxytremetone (*S*)-form, 3'-Acetoxy **(482)** | *S. spp.* | Anthonsen *et al.*, 1970. |
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2*R*,3*R*)-form, 3-O-Angeloyl **(483)** | *S. spp* | Bohlmann *et al.*, 1977; 1978b,k,l; 1979c; 1980; 1981b, d; 1982a,e. |
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2R,3R)-form, 3-O-[2-(Acetoxymethyl)-2-butenoyl](*Z*-) **(484)** | *S. spp* | Bohlmann *et al.*, 1977; 1978b,k,l; 1979c; 1980d,e,f; 1981b,d; 1982a,e. |
| 5-Acetyl-2,3-dihydro-3,6-dihydroxy-2-isopropenylbenzofuran; (2*R*,3*S*)-form, 3-O-Angeloyl **(485)** | *S. spp* | Bohlmann *et al.*, 1977; 1978b,k,l; 1979c; 1980d,e,f; 1981b,d; 1982a,e. |




| | $R_1$ |
|---|---|
| 473 | OH (2*S*,3*S*)-form |
| 474 | OH (2*R*,2*R*)-form |
| 475 | OAng (2*R*,3*R*)-form |
| 476 | O-2-(Acetoxymethyl)-2-buten) (*Z*) |
| 477 | OAng (2*S*,3*S*)-form |
| 480 | H |
| 483 | OAng (2*R*,3*R*)-form |
| 485 | OAng (2*R*,3*S*)-form |




| | $R_1$ | $R_2$ |
|---|---|---|
| 478 | OH | H |
| 479 | OMe | OMe |




| | $R_1$ | $R_2$ |
|---|---|---|
| 481 | OH | H |
| 482 | OAc | H |
| 484 | OH | O-2-(Acetoxymethyl)-2-buten) (*Z*) |

# CHAPTER THREE

# RESULTS AND DISCUSSION

## 3.0 PRELIMINARY BIOASSAY TEST RESULTS

The surface exudates of the fresh leaves of *D. angustifolia*-Ngong, *D. angustifolia*-Voi and *Senecio roseiflorus* were extracted by successive dipping into fresh portions of acetone for short periods (15 seconds). The crude extracts were screened for anti-plasmodial activities against chloroquine-senstive (D6) strain of *Plasmodium falciparum*. Table 3.1 summarizes the results of these tests.

Table 3.1: Anti-plasmodial activity of plant extracts against D6 strain of *Plasmodium falciparum*.

| **Species** | **Plant part** | **$IC_{50}$ values in µg/ml** |
|---|---|---|
| | | **D6** |
| *Dodonaea angustifolia-Ngong forest.* | Fresh leaves | 44.5 ± 3.5 |
| *Dodonaea angustifolia-Voi* | Fresh leaves | 56.0 ± 4.2 |
| *Senecio roseiflorus* | Fresh leaves | 90.0 ± 9.8 |

The crude extract of fresh leaves of *Dodonaea angustifolia*-Ngong forest, *Dodonaea angustifolia*-Voi and *Senecio roseiflorus* were also screened for larvicidal activity against the larvae of *Aedes aegypti*. The surface exudates showed minimal activity ($LC_{50}$) with values greater than 60 µg/ml after 24 hours. The extract of fresh leaves of *Dodonaea angustifolia*-Ngong forest, *Dodonaea angustifolia*-Voi and *Senecio roseiflorus* were further tested for radical scavenging activity to determine their potential as anti-oxidants. The results showed that poor radical scavenging activities even at 20 µg/ml.

The extract of fresh leaves of *Dodonaea angustifolia*-Ngong forest, *Dodonaea angustifolia*-Voi and *Senecio roseiflorus* were also tested for anti-bacterial activity against three strains of bacteria: *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 25922) and *Bacillus pimilus* (local strain); and anti-fungal activity against one local strain of

*Saccharamyces cerevisiae* fungus. The three crude extracts showed activity against bacteria and fungi.

Table 3.12: Anti-microbial activities of the crude extracts of *D. angustifolia* from different geographical locations, *Senecio roseiflorus* and some pure compounds aagingt 3 bacteria and one fungus species.

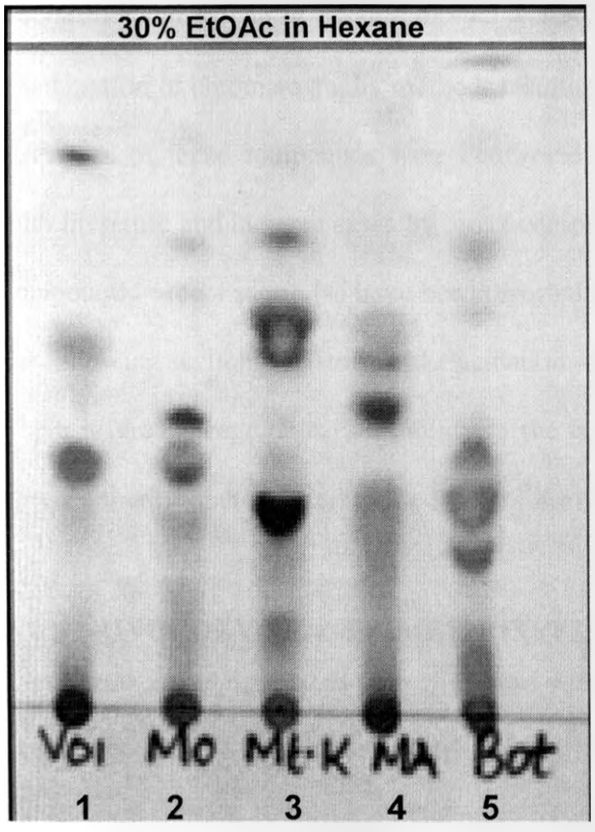
| **Sample** | **µg/disc** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|
| **Crude extracts** | | | | | |
| Surface exudate of *D. angustifolia* (leaves)-Ngong forest | 2500 | 18.86[a] | 20.05[a] | 19.42[a] | 10.79[a] |
| Surface exudate of *D. angustifolia* (leaves)-Voi | 2500 | 17.58 | 19.21 | 18.85 | 12.45 |
| Surface exudate of *D. angustifolia* (leaves)-Kilifi. | 2500 | 19.06 | 18.89 | 18.60 | 14.40 |
| Surface exudate of *D. angustifolia* (leaves)-Garborone (Botswana) | 2500 | 16.33 | 18.94 | 16.25 | 12.18 |
| Surface exudate of *D. angustifolia* (leaves)-Madagascar | 2500 | 17.87 | 21.68 | 18.43 | 11.45 |
| Surface exudate of *Senecio roseiflorus* (leaves) | 2500 | 18.66 | 19.15 | 18.95 | 11.80 |

*Microorganisms*: 1=*Escherichia coli* (ATCC 25922), 2=*Staphylococcus aureus* (ATCC 29737). 3=*Bacillus pumilus* (local strain), 4=*Saccharomyces cerevisiae* (local strain).
"-" Not active.
[a] Inhibition zone in mm.

In this study, therefore, the chromatographic separation and structural elucidation of the crude extracts was undertaken to characterize the bioactive principles present in these extracts and to confirm their *in vitro* bioactivity.

TLC analyses of the extracts obtained from fresh leaves *Dodonaea angustifolia*-Ngong, *Dodonaea angustifolia*-Voi and *Senecio roseiflorus* showed the presence of UV (254 nm) and iodine active compounds. From chemotaxonomic data most of these compounds were assumed to be flavonoids and terpenoids (Dawson *et al.*, 1966; Payne & Jefferies, 1973; Jefferies, 1979; Jefferies & Payne, 1967; Jefferies *et al.*, 1973, 1974, 1981). The TLC analyses of extracts obtained from fresh leaves *Dodonaea angustifolia*-Ngong, *Dodonaea angustifolia*-Voi were compared and the flavonoid and diterpenoid profiles of the material collected from Ngong, representing the upland region, found not to completely match those

from in Taita Hills near Voi town, which is more of a coastal location (Figure 3.1). The TLC profile below shows variation in the *D. angustifolia* exudates components collected from Voi, Kilifi (MO), Mt. Kenya (Mt.K), Gaborone (Bot), Madagascar (MA). The flavonoids and terpenoids do not completely match any of the local ones under study. From the above overview, different *Dodonaea angustifolia* populations elaborate different flavonoids and terpenoids.




*Dodonaea angustifolia* collections from:

**1.** Taita Hills near Voi. Kenya.

**2.** Kilifi, Kenya.

**3.** Ngong Forest. Kenya.

**4.** Madagascar, Madagascar.

**5.** Garborone, Botswana.

Figure 3.1: TLC profiles of extracts of *D. angustifolia* from different populations

The extracts of Dodonaea angustifolia from Ngong Forest and Voi were subjected to chromatographic separation to give twenty-eight compounds. Three of these compounds have not been reported before. The isolated compounds were characterized using UV, $^{1}$H-NMR, $^{13}$C-NMR, MS. Some of these compounds were tested for anti-plasmodial, larvicidal, anti-oxidant and anti-microbial activity. In the following sections the isolation, structural elucidation and biological activity of the isolated compounds will be discussed.

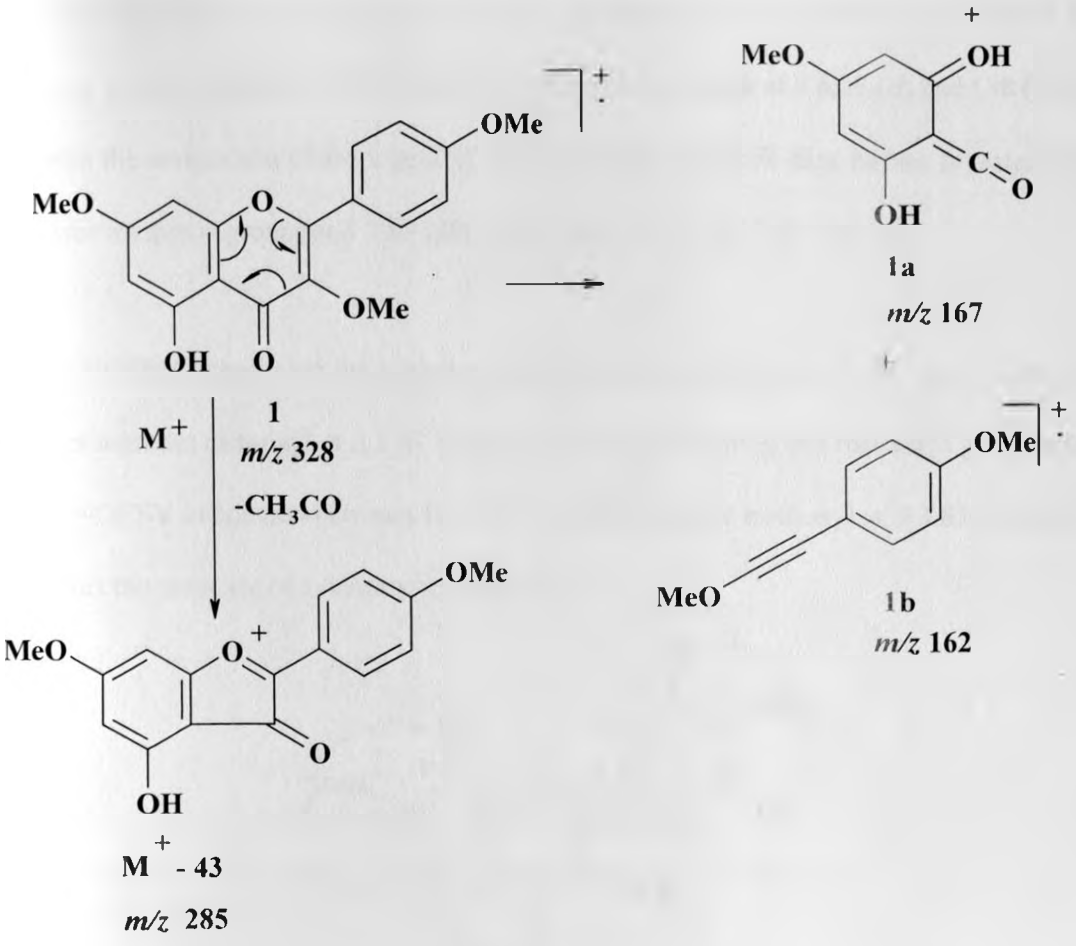
## 3.1 COMPOUNDS FROM *DODONAEA ANGUSTIFOLIA*- NGONG FOREST.

The extraction of the surface exudates on the aerial parts (mainly leaves) was done after plucking out the flowers. The surface exudates of the leaves was extracted by two successive dipping into fresh portions of acetone for short periods (15 seconds) to yield the crude extract, thus avoiding the extraction of the internal tissue components. The extract was tested for anti-plasmodial activity against chloroquine-senstive (D6) strain of *Plasmodium falciparum* and had an $IC_{50}$ value of 44.5 ± 3.5 µg/ml. The crude extract was subjected to a combination of chromatography methods resulting in the isolation of eleven compounds. The structures of these compounds were confirmed by comparison of their spectroscopic data with literature and in some cases by direct comparison (co-TLC) with authentic samples. All compounds except santin (**4**) have been reported here for the first time from this plant and in the following sections the structural elucidation of these compounds will be discussed.

There is great geographical variability in the composition for this substance in *Dodonaea* species, therefore the interest in the study of Kenyan species.

### 3.1.1. 5-HYDROXY-3,7,4'-TRIMETHOXYFLAVONE (1)

Compound **1** was isolated as yellow crystals with melting point of 145-147 °C. It appears as yellow spot (in $R_f$ 0.3 [20% n-$C_6H_{12}$ $CH_2Cl_2$]) which intensified on exposure to ammonia vapour indicating it is phenolic. The mass spectrum showed molecular ion peak at *m/z* 328 [$M^+$] corresponding to the formula $C_{18}H_{14}O_6$ and also an intense peaks at 285 [$M^+$ - 43] (Scheme 3.1) due to the standard C-ring collapse [Harborne, 1994] for 3-methyl ether flavone.




Scheme 3.1: MS fragmentation pattern of 15-hydroxy-3,7,4'-trimethoxyflavone (**1**)

The UV peak at [$\lambda_{max}$ 268.5 and 346.5 nm] [Mabry *et al.*, 1970], $^{1}$H-NMR signal at (δ 12.63 (chelated OH)) plus the $^{13}$C-NMR at δ 157.1 (C-2), 139.4 (C-3) and 179.0 (C-4) NMR are consistent with a 5-hydroxyflavonol derivative [Agrawal, 1989].

The $^{1}$H-NMR (Table 3.2) indicated the presence of two *meta* coupled aromatic protons at δ 6.42 and 6.33 (1H, *d*, *J* = 2.0 Hz) due to ring A and an AA'BB' spin system at δ 8.05 (2H, *d*, *J* = 9.0 Hz) and 7.00 (2H, *d*, *J* = 9.0 Hz) assigned to H-2' and H-3' in ring B. With oxygenations at C-5 and C-7 (biogenetically expected), the *meta* coupled protons at δ 6.33 (*d*, *J* = 2 Hz) and 6.42 (*d*, *J* = 2 Hz) are assigned to H-6 and H-8 respectively. HMBC correlation between the chelated hydroxyl proton (δ 12.63) with C-6 (δ 98.1) and HMQC correlation

between the proton at δ 6.33 and C-6 (δ 98.1) confirmed the assignment of the signals at δ 6.33 (*d*) to H-6. Similarly, HMBC correlation between the peak at δ 6.33 (*d*) and C-8 (δ 92.4) allowed the assignment of the signal at δ 6.42 to H-8. $^{1}$H-NMR data further revealed peaks for three methoxyl groups at δ 3.87 (3H, *s*), δ 3.85 (3H, *s*), and 3.84 (3H, *s*).

In the NOESY experiment the signals at 6.33 (*d*, H-6) and 6.42 (*d*, H-8) were observed to interact with the methoxyl at δ 3.87 (Figure 3.2) thus confirming this methoxyl group at C-7, while NOESY interaction between H-3′/H-5′ (δ 7.00) and the methoxyl at δ 3.85 (Figure 3.2) indicates the presence of a methoxyl group at C-4′.

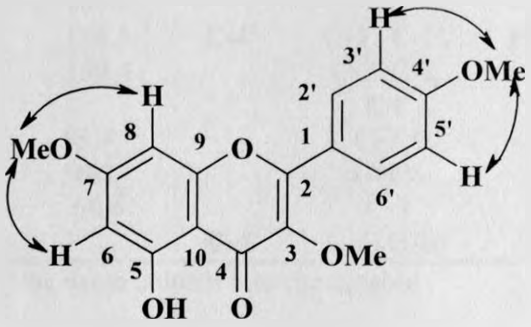



Figure 3.2: NOESY interactions in 5-hydroxy-3,7,4′-trimethoxyflavone (**1**)

The $^{13}$C-NMR of the third methoxyl group (δ 60.4) is consistent with di-*ortho* arrangement which suggests the presence of this group at C-3.

Based on the spectroscopic data and comparison with literature information compound **1** was identified as 5-hydroxy-3,7,4′-trimethoxyflavone (**1**) [Rossi *et al.*, 1997]. This compound has previously been reported from *Dodonaea viscosa* [Wollenweber *et al.*, 2004], but is being reported the first time from *Dodonaea angustifolia.*

Table 3.2: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 5-Hydroxy-3,7,4'-trimethoxyflavone (**1**)

| *C* | $\delta_{1H}$ (*m*, *J* (Hz)) | $\delta_{13C}$ | *HMBC* $^2J$ | *HMBC* $^3J$ | *NOESY* |
|---|---|---|---|---|---|
| 2 | | 157.1 | | | |
| 3 | | 139.4 | | | |
| 4 | | 179.0 | | | |
| 5 | | 162.3 | | | |
| 6 | 6.33 (*d*, 2.0) | 98.10 | C-5, C-7 | C-8,C-10 | H-6, OMe-7 |
| 7 | | 165.7 | | | |
| 8 | 6.42 (*d*, 2.0) | 92.4 | C-7, C-9 | C-10, C-6 | H-8, OMe-7 |
| 9 | | 156.2 | | | |
| 10 | | 106.3 | | | |
| 1′ | | 123.1 | | | |
| 2′ | 8.05 ( *d*, 9) | 130.4 | | C-6′,C-2, C4′ | |
| 3′ | 7.00 ( *d*, 9) | 114.3 | C-4′ | C-5′, C-1′, | H-3′, OMe-4′ |
| 4′ | | 161.9 | | | |
| 5′ | 7.03 ( *d*, 9) | 114.3 | C-4′ | C-3′, C-1′, | H-5′, OMe-4′ |
| 6′ | 8.08 ( *d*, 9) | 130.4 | | C-2′,C-2, C4′ | |
| OMe | 3.87 (*s*) * | 55.7 * | | C-7 * | |
| OMe | 3.85 (*s*) * | 56.0 * | | C-4′* | |
| OMe | 3.84 (*s*) | 60.4 | | C-3 | |
| OH | 12.63 (*s*) | | C-5 | C-6, C-10 | |

* Chemical shift values in the same column interchangeable

### 3.1.2. 3,5-DIHYDROXY-7,4′,-DIMETHOXYFLAVONE (2)

Compound **2** was isolated as yellow needle-like crystals with melting point of 182-184 °C. It appears as yellow spot ($R_f$ 0.3 [20% n-$C_6H_{12}$ - $CH_2Cl_2$]) which intensified on exposure to ammonia vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 314 corresponding to the formula $C_{17}H_{14}O_6$.

The UV peaks at [$\lambda_{max}$ 268.5 and 346.5 nm] [Mabry *et al.*, 1970], $^1$H-NMR signal at δ 11.74 (chelated OH) and $^{13}$C-NMR peak at δ 145.7 (C-2), 135.7 (C-3) and 175.2 (C-4) [Agrawal 1989] indicates that this compound is a 5-hydroxyflavonol derivative

The $^1$H-NMR (Table 3.3) indicated the presence of two *meta* coupled aromatic protons at δ 6.50 and 6.38 (1H, *d*, *J* = 2.1 Hz) due to ring A and an AA′BB′ spin system at δ 8.19 and 7.05

(2H, *d*, $J$ = 9.3 Hz) assigned to H-2′, H-6′, H-3′, H-5′ respectively, of a para substituted ring B. With oxygenations at C-5 and C-7 (biogenetically expected), the *meta* coupled protons are assigned to H-6 and H-8. HMBC correlation between the chelated hydroxyl proton (δ 11.74) with C-6 (δ 97.9) and HMQC correlation between the proton at δ 6.38 and C-6 (δ 97.9) led to the assignment of the peak at δ 6.38 (*d*) to H-6. Similarly, HMBC correlation between the signal at δ 6.38 (*d*) and C-8 (δ 92.2) allowed the placement of the proton resonating at δ 6.50 to H-8.

Table 3.3: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 3,5-dihydroxy-7,4′-dimethoxyflavone (**2**)

| C | $\delta_{1H}$ ($m$, $J$ (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 145.7 | | |
| 3 | | 135.7 | | |
| 4 | | 175.2 | | |
| 5 | | 160.9 | | |
| 6 | 6.38 *(d, 2.4)* | 97.9 | C-5, C-7 | C-8,C-10 |
| 7 | | 165.7 | | |
| 8 | 6.50 *(d, 2.4)* | 92.2 | C-7, C-9 | C-10, C-6 |
| 9 | | 156.9 | | |
| 10 | | 104.0 | | |
| 1′ | | 123.2 | | |
| 2′ | 8.18 *(d, 9.3)* | 129.4 | | C-6′,C-2, C4′ |
| 3′ | 7.06 *(d, 9.3)* | 114.1 | C-4′ | C-5′, C-1′, |
| 4′ | | 161.2 | | |
| 5′ | 7.05 *(d, 9.9)* | 114.1 | C-4′ | C-3′, C-1′, |
| 6′ | 8.19 *(d, 9.9)* | 129.4 | | C-2′,C-2, C4′ |
| OMe | 3.90 *(s)* | 55.4 | | C-7 |
| OMe | 3.89 *(s)* | 55.8 | | C-4′ |
| OH | 11. 74 *(s)* | | C-5 | C-6, C-10 |
| OH | 6.57 *(s)* | | C-4′ | |

Furthermore, $^1$H-NMR revealed peaks for two methoxyl groups at δ 3.90 and 3.89 (3H, *s*). One of the methoxyl groups was placed at C-7 and the other at C-4′ but not at C-3 since they resonate at δ 55.4 and 55.8 which is typical for isolated methoxyl groups. If either was at position C-3 then it would have resonated *ca.* δ 60 in the $^{13}$C-NMR. The positions of the methoxyl groups were confirmed by HMBC correlations of the peak at δ 3.90 to C-7 (δ 165.7) and δ 3.89 to C-4′ (δ 161.2).

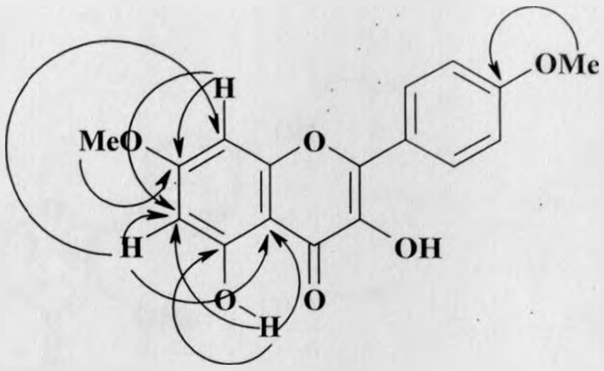
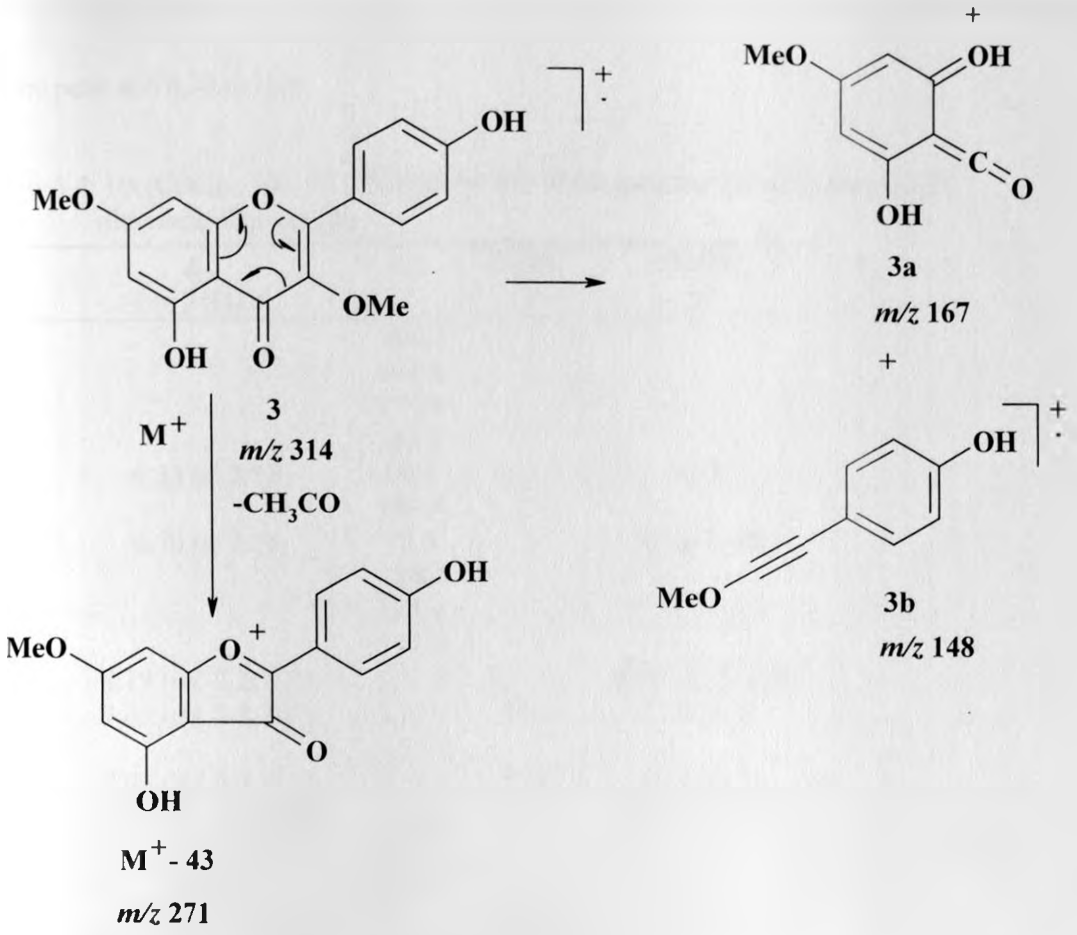



Figure 3.3: HMBC and HMQC of 3,5-dihydroxy-7,4′-dimethoxyflavone (**2**).

Based on these spectroscopic data, compound **2** was identified as 3,5-dihydroxy-7,4′-dimethoxyflavone (**2**). This compound has been previously isolated from *Dodonaea viscosa* [Wollenweber *et al.*, 2004], but is being reported for the first time from *Dodonaea angustifolia*.

### 3.1.3. KUMATAKENIN (3)

Compound **3** was isolated as yellow needle- like crystals with melting point of 253-254 °C. It appears as yellow spot ($R_f$ 0.5 [2% MeOH - $CH_2Cl_2$]) which intensified on exposure to ammonia vapour indicating it is phenolic. The mass spectrum showed molecular ion peak at *m/z* 314 [$M^+$] corresponding to the formula $C_{17}H_{14}O_6$ and an intense peaks at 271 [$M^+$ - 43] (Scheme 3.2) due to the standard C-ring collapse for 3-methyl ether flavone [Harborne, 1994].




Scheme 3.2: Fragmentation pattern of kumatakenin (3)

The UV peak [$\lambda_{max}$ 269.0 and 348.0 nm] $^1$H-NMR signal at (δ 12.76 (chelated OH) and $^{13}$C-NMR peak δ 148.0 (C-2), 137.6 (C-3) and 177.4 (C-4) is consistent with a 5-hydroxyflavonol derivative [Mabry *et al.*, 1970; Agrawal 1989].

The $^1$H-NMR (Table 3.4) indicated the presence of two *meta* coupled aromatic protons at δ 6.70 and 6.33 (1H, *d*, *J* = 2.1 Hz) which due to ring A and an AA'BB' spin system at δ 8.19 (2H, *dd*, *J* = 2.1, 9.9 Hz) and δ 7.03 (2H, *dd*, *J* = 2.1, 9.9 Hz) assigned to C-4' substituted ring B protons. With oxygenations at C-5 and C-7 (biogenetically expected), the *meta* coupled protons are assigned to H-6 and H-8. HMBC correlation between the chelated hydroxyl proton (δ 12.76) with C-6 (δ 99.1) and HMQC correlation between the proton at δ 6.33 and C-6 (δ 99.1) (Figure 3.4) led to the assignment of the doublet at δ 6.33 to H-6. Similarly,

HMBC correlation between the signal at δ 6.33 (*d*) and C-8 (δ 93.4) allowed the confirmation of the peak at δ 6.70 to H-8.

Table 3.4: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 5,4′-dihydroxy-3,7-dimethoxyflavone **(3)**

| *C* | $\delta_{1H}$ (*m*, *J* (Hz)) | $\delta_{13C}$ | *HMBC* $^2J$ | *HMBC* $^3J$ |
|---|---|---|---|---|
| 2 | | 148.0 | | |
| 3 | | 137.6 | | |
| 4 | | 177.4 | | |
| 5 | | 162.5 | | |
| 6 | 6.33 (*d*, 2.1) | 99.1 | | C-8 |
| 7 | | 167.4 | | |
| 8 | 6.70 (*d*, 2.1) | 93.4 | | C-6, C-10 |
| 9 | | 158.4 | | |
| 10 | | 105.6 | | |
| 1′ | | 124.0 | | |
| 2′ | 8.19 (*dd*, 2.2, 9.9) | 131.2 | | C-6′, C-2, C4′ |
| 3′ | 7.03 (*dd*, 2.2, 9.9) | 117.1 | C-4′ | C-5’, C-1’ |
| 4′ | | 161.0 | | |
| 5′ | 7.03 (*dd*, 2.2, 9.9) | 117.1 | C-4′ | C-3′, C-1′, |
| 6′ | 8.19 (*dd*, 2.2, 9.9) | 131.2 | | C-2′, C-2, C4′ |
| OMe | 3.95 *(s)* | 57.1 | | C-7 |
| OMe | 3.88 *(s)* | 60.9 | | C-3 |
| OH | 12.76 *(s)* | | C-5 | C-6 |

The $^1$H-NMR also displayed peaks for two methoxyl groups at δ 3.95 and 3.88 (3H, *s*). HMBC correlation of the methoxy at δ 3.95 with 167.4 confirmed the methoxyl at C-7 in ring A.

The second methoxyl was confirmed at C-3 and not at C-4′ due to the fact that it resonates at δ 60.9 which is typical for a di-*ortho* substituted methoxyl groups.

Thus based on this and correlation with literature [Vieira *et al.*, 1997], compound **3** was identified as 5,4′-dihydroxy-3,7-dimethoxyflavone **(3)** (kumatakenin). This compound has been previously isolated from several plant species including the aerial parts of *Achillea ageratum* [Vieira *et al.*, 1997] and *Solanum paludosum* [Sarmento *et al.*, 2002], but is being reported for the first time from the genus *Dodonaea*.

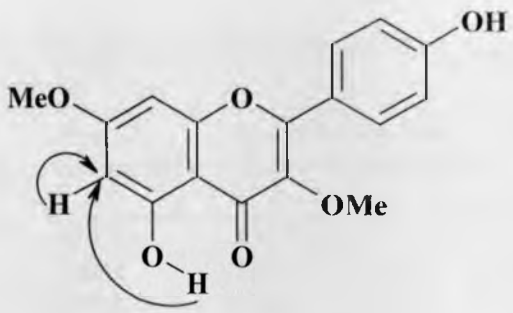



Figure 3.4: HMBC and HMQC correlations for kumatakenin (**3**).

**3.1.4. SANTIN (4)**

Compound **4** was isolated as yellow needle like crystals with a melting point 159-161 °C. It appears as yellow spot ($R_f$ 0.4 [2% MeOH - $CH_2Cl_2$]) which intensified on exposure to ammonia vapour indicating it is phenolic.The UV peak [$\lambda_{max}$ (MeOH) 272.0 and 337.0 nm], $^1$H-NMR signal δ 11.76 (chelated OH) and $^{13}$C-NMR peak δ 156.0 (C-2), 138.3 (C-3) and 179.2 for (C-4) is consistent with a 5-hydroxyflavonol derivative [Mabry *et al.*, 1970; Agrawal, 1989].

The $^1$H-NMR (Table 3.5) displayed an AA′BB′ system resonating at δ 8.11 and 7.12 (2H, *d*, *J* = 8.8 Hz) corresponding to a *para* substituted ring B and a singlet at δ 6.60 (1H) assigned to a tri- substituted ring A.

The $^1$H-NMR (Table 3.5) further displayed signals for three methoxyls at δ 3.91 3.87 and 3.86, two of which are di-*ortho* substituted (δ 60.0 and 59.6 in $^{13}$C NMR). The location of one of the methoxyl group was assigned to C-4′ due to NOE interaction between the aromatic protons at C-3′/C-5′ (δ 7.12) with the methoxyl group at δ 3.91. This led to two possible structures **4a** and **4b**.

Table 3.5: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 5,7-dihydroxy-3, 6, 4′-trimethoxyflavone (**4**)

| C | $\delta_{1H}$ (*m*, *J* (Hz)) | $\delta_{13C}$ of compound **4** δ | $\delta_{13C}$ of Santin (**4a**) (Barbera *et al.*, 1986) δ | $\delta_{13C}$ of **4b** (Roitman & James., 1985). δ |
|---|---|---|---|---|
| 2 | | 156.0 | 156.2 | 155.4 |
| 3 | | 138.3 | 138.7 | 137.5 |
| 4 | | 179.2 | 179.6 | 178.0 |
| 5 | | 152.5 | 151.8 | 155.9 |
| 6 | | 131.3 | 130.1 | 98.8 |
| 7 | | 157.4 | 152.2 | 156.9 |
| 8 | 6.36 (*d*, 2.0) | 93.9 | 93.1 | 127.5 |
| 9 | | 153.0 | 155.9 | 148.5 |
| 10 | | 105.6 | 106.2 | 104.0 |
| 1′ | | 123.0 | 123.1 | 120.7 |
| 2′ | 8.11 (*d*, 8.8) | 130.4 | 130.2 | 129.9 |
| 3′ | 7.12 (*d*, 9.0) | 114.3 | 114.1 | 115.7 |
| 4′ | | 162.1 | 160.2 | 160.2 |
| 5′ | 7.12 (*d*, 9.0) | 114.3 | 114.1 | 115.7 |
| 6′ | 8.11 (*d*, 8.8) | 130.4 | 130.2 | 129.9 |
| OMe-3 | 3.91 *(s)* | 60.0 | 60.1 | |
| OMe-6 | 3.87 *(s)* | 59.6 | 61.8 | |
| OMe-4′ | 3.86 *(s)* | 55.2 | 55.4 | |
| OH | 11.76. *(s)* | | | |
| OH | 3.08 *(brs)* | | | |

However, the spectral data (Table 3.5) of this compound **4** is in close agreement with that reported in literature [Barbera *et al.*, 1986] (Table 3.5) for santin (**4a**) and not for 5, 7-dihydroxy-3, 8, 4′-trimethoxyflavone [Roitman & James, 1985] (Table 3.5) (**4b**) especially the $^{13}$C-NMR for C-6, C-8 and C-9. Based on this the compound was identified as 5, 7-dihydroxy-3, 6, 4′-trimethoxyflavone (santin) (**4a**) and not 5, 7-dihydroxy-3, 8, 4′-trimethoxyflavone (**4b**). This compound has been previously isolated from the leaf extract of three *Dodonaea* species including *Dodonaea attenuate* var. *linearis* [Jefferies & Payne, 1973], *Dodonaea viscosa* [Sachdev & Kulshreshtha,1982; Wollenweber *et al.*, 1986; Abdel-Mogib *et al.*, 2001] and *Dodonaea angustifolia* [Sachdev & Kulshreshtha., 1984].

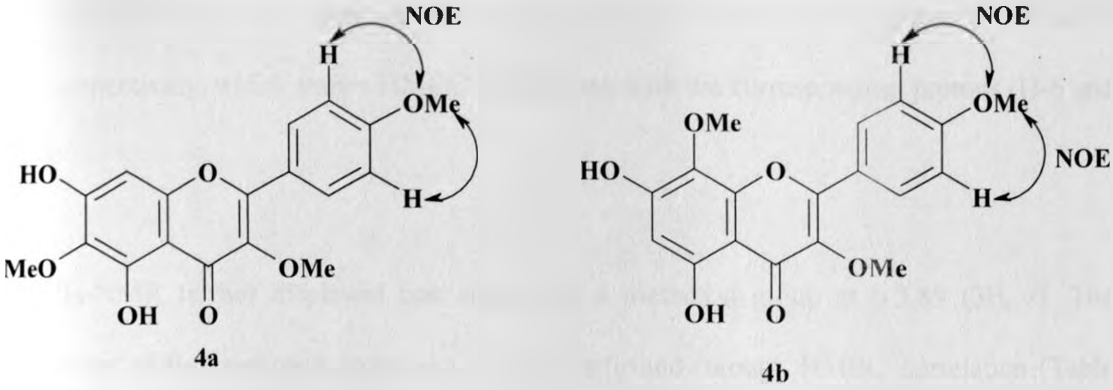



Figure 3.5: NOE correlation of santin (**4**)

### 3.1.5. RHAMNOCITRIN (5)

Compound **5** was isolated as yellow crystals with melting point of 221-223 °C. It appears as yellow ($R_f$ 0.4 (2% MeOH in $CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating that it is phenolic.

The UV ($\lambda_{max}$ 266.0 and 364.0 nm) [Mabry *et al.*, 1970], $^1H$ (δ 12.18 (for chelated hydroxyl group)) and $^{13}C$ (δ 147.2.2 for C-2, 136.3 for C-3 and 176.5 for C-4) NMR spectra [Agrawal, 1989] is consistent with the flavonol derivative. EI-MS analysis of compound **5** showed a molecular ion peak *m/z* 300 corresponding to $C_{16}H_{12}O_6$.

The $^1H$-NMR (Table 3.6) indicated the presence of two *meta* coupled aromatic protons at δ 6.41 and 6.20 (1H, *d*, $J$ = 2.1 Hz) which were assigned to a di-substituted ring A and an AA'BB' spin system centered at δ 6.92 and 8.10 ($J$ = 9.0 Hz) which were assigned to 4'-substituted ring B protons. The assignment of two sets of doublets to C-6 and C-8 was confirmed from HMBC correlation of the proton at δ 6.20 with C-5 (δ 161.7), C-7 (δ 164.7), C-8 (δ 93.6), C-10 (δ 103.7) and the proton at δ 6.41 with C-6 (δ 98.4). C-7 (δ 164.7), C-9 (δ 157.4), and C-10 (δ 103.7). C-5 (δ 161.7

The $^{13}$C-NMR spectrum (Table 3.6) showed signals for C-6 and C-8 of ring A at δ 98.4 and δ 93.6 respectively, which shows HMQC correlations with the corresponding protons (H-6 and H-8).

The $^{1}$H-NMR further displayed one singlet for a methoxyl group at δ 3.89 (3H, *s*). The placement of the methoxyl group at C-7 was confirmed through HMBC correlation (Table 3.6) of the protons at δ 3.89 with C-7 (δ 164.7). The location of the methoxyl group was confirmed by NOE experiment which exhibited NOE interactions between the methoxyl at δ 3.89 (C-7) and the of *meta* coupled aromatic protons at δ 6.41 (C-8) and δ 6.20 (C-6).

Thus based on these spectroscopic data compound **5** was identified as 5,4′-dihydroxy-7-dimethoxyflavonol (**5**) (rhamnocitrin). The data is in close agreement with that for rhamnocitrin (**5**) previously isolated from the aerial parts of *Dodonaea viscosa* [Ghisalberti, 1998].

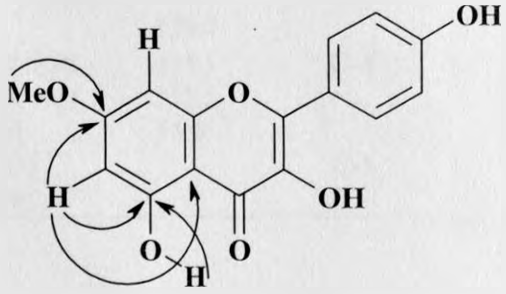



Figure 3.6: HMBC correlations of 5,4′-dihydroxy-7-dimethoxyflavonol (**5**)

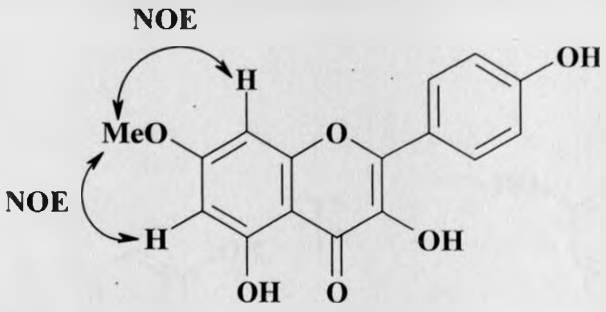



Figure 3.7: NOE correlations of the methoxy group of 5,4′-dihydroxy-7-dimethoxyflavonol (**5**)

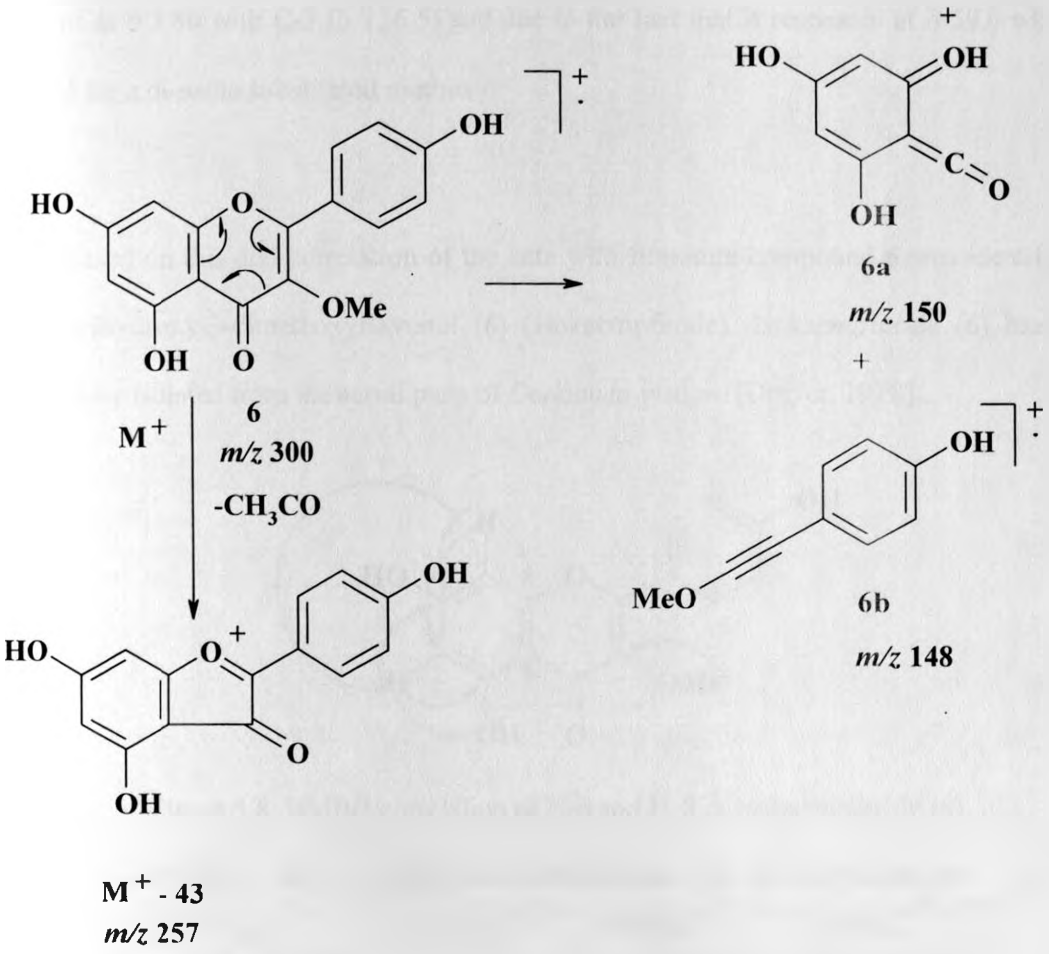
Table 3.6: ID ($CD_3OD$: 300, 75.5 MHz) and 2D NMR data for 5,4′-dihydroxy-7-dimethoxyflavonol (**5**)

| C | $\delta_{1H}$ (*m*, *J* (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 147.2 | | |
| 3 | | 136.3 | | |
| 4 | | 176.5 | | |
| 5 | | 161.7 | | |
| 6 | 6.20 (*d*, 2.1) | 98.4 | C-5, C-7 | C-8, C-10 |
| 7 | | 164.7 | | |
| 8 | 6.41 (*d*, 2.1) | 93.6 | C-9, C-7 | C-6, C-10 |
| 9 | | 157.4 | | |
| 10 | | 103.7 | | |
| 1′ | | 122.9 | | |
| 2′ | 8.10 (*d*, 9.0) | 129.8 | | C-6′,C-2, C4′ |
| 3′ | 6.92 (*d*, 9.0) | 115.5 | C-4′ | C-5′, C-1′ |
| 4′ | | 159.7 | | |
| 5′ | 6.92 (*d*, 9.0) | 115.5 | C-4′ | C-3′, C-1′, |
| 6′ | 8.10 (*d*, 9.0) | 115.5 | | C-2′,C-2, C4′ |
| OMe | 3.89 (*s*) | 55.6 | | C-7 |
| OH | 12.18 (*s*) | | C-5 | C-6, C-10 |
| OH | 9.2 (*brs*) | | | |

### 3.1.6. ISOKAEMPFERIDE (6).

Compound **6** was isolated as yellow crystals with melting point of 299-302 °C. It appears as yellow ($R_f$ 0.3 (2% MeOH in $CH_2Cl_2$) which intensifies on exposure to ammonia vapour indicating that it is phenolic. The $^{13}C$ (δ 147.6 for C-2, 136.5 for C-3 and 176.7 for C-4) NMR is consistent with a flavonol derivative [Agrawal 1989]. The mass spectrum showed molecular ion peak at *m/z* 300 [$M^+$] corresponding to $C_{16}H_{12}O_6$ and also an intense peak at

257 [M$^{+}$- 43] (Scheme 3.3) corresponding to the C-ring collapse for 3-methyl ether flavone [Harborne, 1994].




Scheme 3.3: Fragmentation pattern of isokaempferide (6)

The $^{1}$H-NMR (Table 3.7) indicated the presence of two *meta* coupled aromatic protons at δ 6.41 and 6.20 (1H, $J$ = 2.0 Hz) which were assigned to a di-*ortho* substituted ring A and an AA'BB' spin system centered at δ 6.92 and 8.10 ($J$ = 9.0 Hz)which were assigned to 4'-substituted ring B protons. The assignment of two sets of doublets to C-6 and C-8 was confirmed from HMBC correlation of δ 6.20 with C-7 (δ 165), C-8 (δ 93.6), C-10 (δ 104.0), C-5 (δ 161.4) and δ 6.41 with C-6 (δ 98.5), C-7 (δ 165), C-9 (δ 157.7), and C-10 (δ 104.9).

The $^1$H-NMR displayed one singlet for a methoxyl group at δ 3.80 (3H, *s*). The placement of the methoxyl group at C-3 was confirmed through HMQC correlation (Figure 3.8) of the protons at δ 3.80 with C-3 (δ 136.5) and due to the fact that it resonates at δ 59.0 which is typical for a di-*ortho* substituted methoxyl.

Thus based on this and correlation of the data with literature compound **6** was identified as 5,7,4′-trihydroxy-3-dimethoxyflavonol (**6**) (Isokaempferide). Isokaempferide (**6**) has been previously isolated from the aerial parts of *Dodonaea viscosa* [Dreyer, 1978].

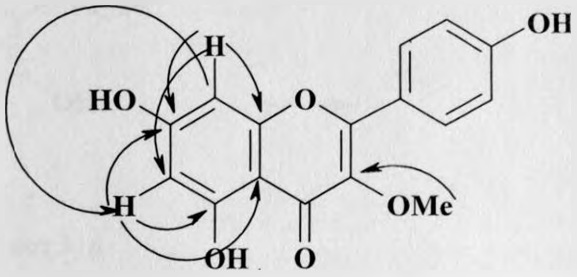


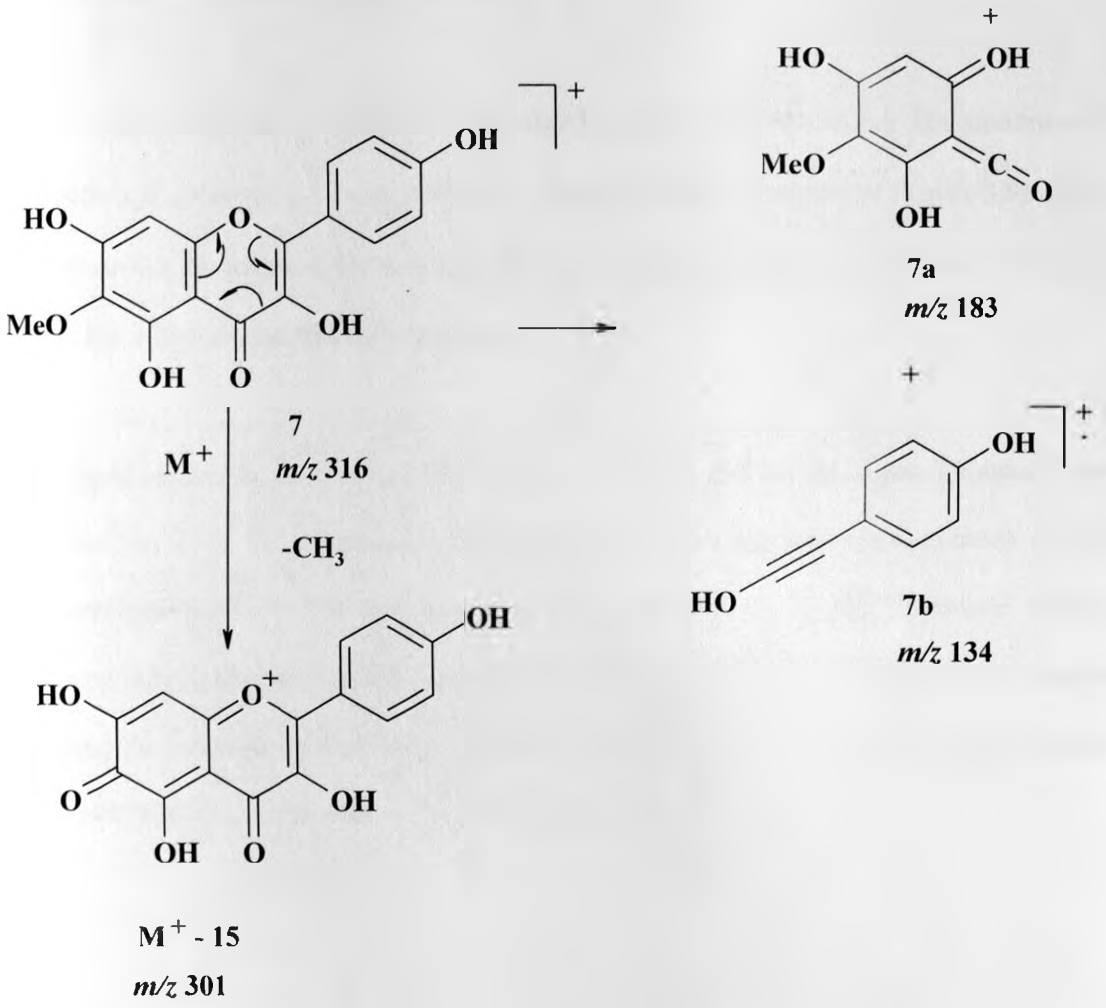

Figure 3.8: HMBC correlation of H-6 and H-8 of isokaempferide (**6**).

Table 3.7: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for isokaempferide (**6**)

| C | $\delta_{1H}$ (*m*, *J* (Hz)) | $\delta_{13C}$ | HMBC | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 147.6 | | |
| 3 | | 136.5 | | |
| 4 | | 176.7 | | |
| 5 | | 161.4 | | |
| 6 | 6.20 (*d*, 2.0) | 98.5 | C-5, C-7 | C-8, C-10 |
| 7 | | 164.9 | | |
| 8 | 6.41 (*d*, 2.0) | 93.6 | C-9, C-7 | C-6, C-10 |
| 9 | | 157.7 | | |
| 10 | | 104.0 | | |
| 1′ | | 122.0 | | |
| 2′ | 8.12 (*d*, 9.0) | 129.9 | | C-6′,C-2, C4′ |
| 3′ | 6.92 (*d*, 9.0) | 115.7 | C-4′ | C-5′, C-1′ |
| 4′ | | 159.8 | | |
| 5′ | 6.92 (*d*, 9.0) | 115.7 | C-4′ | C-3′, C-1′, |
| 6′ | 8.12 (*d*, 9.0) | 129.9 | | C-2′,C-2, C-4′ |
| OMe | 3.80 *s* | 59.0 | | C-3 |

### 3.1.7. 6-METHOXYKAEMPFEROL (7).

Compound 7 was isolated as yellow needle like crystals with melting point of 253-254 °C. It appears as yellow spot ($R_f$ 0.5 (4% MeOH in $CH_2Cl_2$), which intensified on exposure to ammonia, vapour indicating it is phenolic. The mass spectrum showed molecular ion peak at *m/z* 316 [$M^+$] corresponding to $C_{16}H_{12}O_7$ and an intense peak at 301 [$M^+$-15] (Scheme 3.4) due to the 6-$OCH_3$ flavonol fragmentation [Markham, 1982].




Scheme 3.4: Fragmentation pattern of 6-methoxykaempferol (7)

The $^1$H-NMR peak at δ 12.34 (chelated hydroxyl group) and a $^{13}$C-NMR peak at δ 148.0 (C-2), 137.0 (C-3) and 177.6 (C-4) NMR is consistent with a 5-hydroxyflavonol derivative [Agrawal, 1989].

The $^{1}$H-NMR (Table 3.8) displayed the presence of a singlet at δ 6.63 (1H, *s*) which was assigned to a tri- substituted ring A and an AA′BB′ spin system centered at δ 7.02 and 8.17 (*dd*, *J* = 2.1, 9.0 Hz) which were assigned to 4′-substituted ring B protons. The assignment of the singlet to C-8 was confirmed from HMBC correlation between the chelated hydroxyl proton at δ 12.34 with the C-6 at δ 132.4 and not with the carbon at δ 95.2 which has an HMQC correlation with the singlet at δ 6.63.

The $^{1}$H-NMR displayed one singlet for a methoxyl group at δ 3.96 (3H, *s*). The placement of the methoxyl group at C-3 was confirmed through HMBC correlation (Table 3.8) of the protons at δ 3.96 with C-6 (132.4) and due to the fact that it resonates at δ 61.4 which is typical for a di-*ortho* substituted methoxyl.

Thus based on the $^{1}$H, $^{13}$C-NMR, COSY, NOESY, HMQC and HMBC data compound 7 was identified as 3, 5, 7, 4′-tetrahydroxy-6-methoxyflavonol (6-methoxykaempferol) (7). 6-methoxykaempferol (7) has not been previously reported from the *Dodonaea* species. However, this compound has been isolated from the aerial parts of a number of plant species including *Heteranthemis viscidihirta* [Valant-Vetschera *et al.*, 2003], *Centaurea inca* [Akkal *et al.*, 1997] and *Carthamus tinctorius* [Hattori *et al.*, 1992].

Table 3.8: ID ($CD_3OD$: 300, 75.5 MHz) and 2D NMR data for 6-methoxykaempferol (7)

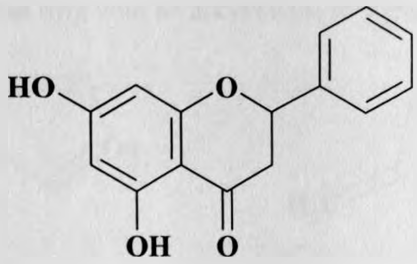
| C | $\delta_{1H}$ (*m*, *J* (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 148.0 | | |
| 3 | | 137.0 | | |
| 4 | | 177.6 | | |
| 5 | | 153.3 | | |
| 6 | | 132.4 | | |
| 7 | | 158.6 | | |
| 8 | 6.63 (*s*) | 95.2 | C-9, C-7 | C-6, C-10 |
| 9 | | 153.8 | | |
| 10 | | 105.3 | | |
| 1′ | | 123.0 | | |
| 2′ | 8.17 (*dd*, 2.1, 9.0) | 131.2 | | C-6′,C-2, C4′ |
| 3′ | 7.02 (*dd*, 2.1, 9.0) | 117.0 | C-4′ | C-5′, C-1′ |
| 4′ | | 160.9 | | |
| 5′ | 7.02 (*dd*, 2.1, 9.0) | 117.0 | C-4′ | C-3′, C-1′, |
| 6′ | 8.17 (*dd*, 2.1, 9.0) | 131.2 | | C-2′,C-2, C4′ |
| OMe | 3.96 (*s*) | 61.4 | | C-6 |
| OH | 12.34 (*s*) | | C-5 | C-10, C-6 |
| OH | 9.09 (*brs*) | | | |

### 3.1.8. PINOCEMBRIN (8).

Compound **8** was isolated as white crystals with a melting point 192-193°C. It appears as yellow spot ($R_f$ 0.5 (1% MeOH in $CH_2Cl_2$), which intensified on exposure to ammonia, vapour indicating it is phenolic. The UV peak at $\lambda_{max}$ (MeOH) 289.0 [Mabry *et al.*, 1970], $^1$H-NMR at δ 12.14 (chelated hydroxyl), an ABX spin system at δ 5.55 (*dd*, *J* = 3.0, 13.6 Hz for H-2) assigned to a methine proton attached to an oxygen , δ 2.77 (*dd*, *J* = 3.4, 13.6 Hz for H-3) , δ 3.16 (*dd*, *J* = 13.0, 17.40 Hz for H-3) and $^{13}$C-NMR signals at δ 79.9 (C-2), δ 43.6 (C-3) and δ 196.5 (C-4) is consistent with a 5-hydroxyflavanone derivative [Agrawal, 1989].

The $^1$H-NMR exhibited *meta* coupled protons resonating at δ 5.96 and δ 5.95 (*d*, *J* = 2.0 Hz) which were assigned to ring A protons and another two sets of peaks at δ 7.53 (2H) and δ 7.43 (3H) which were assigned to an unsubstituted ring B protons. Using the above spectroscopic data and comparison with literature [Sachdev & Kulshreshtha, 1983]

compound **8** was identified as 5,7-dihydroxyflavanone (pinocembrin). Pinocembrin (**8**) has been previously isolated from *Dodonaea viscosa* [Sachdev & Kulshreshtha, 1983].




Pinocembrin (**8**)

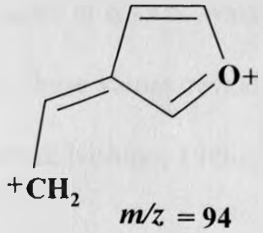
Table 3.9: $^{1}H$ ($(CD_3)_2CO$, 200 MHz) and $^{13}C$ (200 MHz) NMR for pinocembrin (**8**)

| C | $\delta_{1H}$ (*m*, *J* (Hz)) | $\delta_{13C}$ |
|---|---|---|
| 2 | | 79.91 |
| 3 | | 43.64 |
| 4 | | 196.53 |
| 5 | | 163.91 |
| 6 | 5.95 (*d*, *J* = 2.0) | 96.86 |
| 7 | | 167.32 |
| 8 | 5.94 (*d*, *J* = 2.0) | 95.84 |
| 9 | | 165.04 |
| 10 | | 103.05 |
| 1′ | | 139.87 |
| 2′ | | 129.33 |
| 3′ | 7.53 *m* | 127.18 |
| 4′ | 7.43 *m* | 129.27 |
| 5′ | 7.53 *m* | 127.18 |
| 6′ | 8.20 (*d*, *J* = 8.8) | 129.33 |
| OH-5 | 12.14 *s* | |

### 3.1.9. 2β- HYDROXYHARDWICKIIC ACID (9).

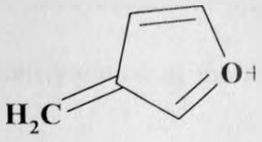
Compound **9** was obtained as white crystals from $CH_2Cl_2$/MeOH. $R_f$ 0.4 (3% MeOH in $CH_2Cl_2$) and was UV (254 nm) active.

The EI-MS of compound **9** provided the $M^+$ at *m/z* 332 indicating the molecular formula $C_{20}H_{28}O_4$. The peaks at *m/z* 95 and 81 as shown in the fragments **9a** and **9b** respectively suggested the presence of furan ring with an alkyl chain in **9** [Spanevello & Vila, 1994].




*m/z* = 94

**9a**




*m/z* = 81

**9b**

Typical downfield shifted broad singlets in the $^1$H-NMR spectrum of compound **9** (Table 3.10) at δ 6.37, 7.37, and 7.44 were attributed to the H-14, H-16 and H-15 protons, respectively, suggesting the presence of a β-substituted furan ring. The $^1$H-NMR further showed, a broad singlet at δ 6.62 which was assigned to a β olefinic proton conjugated to a carboxyl group, a three proton doublet at δ 0.85 ($J$ = 6.6 Hz) attributed to the secondary methyl, a three proton singlet at δ 0.80 and δ 1.32 attributed to the tertiary methyl groups typical of clerodane-type diterpenes [Givovich *et al.*, 1986; Luteijn *et al.*, 1982; San-Martin *et al.*, 1986; Sharma *et al.*, 1984]. The $^1$H-NMR spectrum of **9** showed a signal at δ 4.35 (*m*, 1 H) indicating that one of the two protons at C-2 had been substituted by an OH group [Fang *et al.*, 1988].

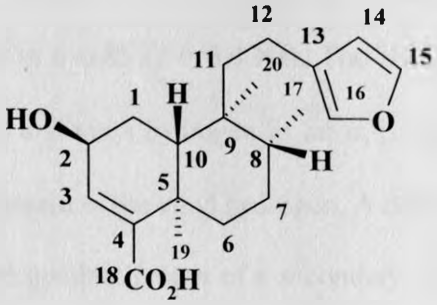
The $^1$H-$^1$H COSY experiment showed coupling between the methyl at δ 0.85 and the H-8 proton at δ 1.52. It also showed coupling between the proton at δ 6.37 with the proton at δ 2.40 assigned to H-12 and between the proton at δ 7.37 and the H-12 methylene protons at δ 2.40 and δ 2.30. In addition there were coupling between the protons at δ 6.37, δ 7.37 and δ 7.44.

The $^{13}$C-NMR spectrum (APT) (Table 3.10) corroborated the presence of three methyl groups, five methylenes, seven methines and five quaternary carbon atoms. The $^{13}$C-NMR chemical shift of methyl at C-19 was observed at δ 21.6 and the β-positioned axial methyl group at C-20 appeared at δ 19.5, while the β-positioned equatorial methyl group at C-17 resonated at δ 17.0. These values reveal the *trans* configuration at the A/B ring junction of compound **9** [Manabe & Nishino, 1986].

The $^{13}$C-NMR showed the downfield shift of the C-2 signal at δ 69.7 due to the OH group. The position of the OH group at C-2 was further confirmed by HMBC experiments, which showed cross peaks between H-2 proton (δ 4.35) and C-1(δ 29.3*/28.6*), C-10 (δ 46.8), C-3 (δ 141.8) and C-4 (δ 43.3). Similarly, olefinic proton at H-3 (δ 6.62) showed HMBC correlations to C-4 (δ 143.6), C-2 (δ 69.7), C-18 (δ 169.2) and C-5 (δ 40.4). The proton at δ 6.37 assigned to H-14 showed cross peak correlations to the C-13 (δ 127.2), C-15 (δ 144.3), C-16 (δ 140.2) and C-12 (δ 19.4*/δ 19.5*). The methyl protons at C-19 (δ 1.32) showed HMBC correlation with C-4 (δ 143.6), C-5 (δ 40.4), C-6 (δ 37.2) and C-10 (δ 46.8). In addition there was HMBC cross peaks between H-10 proton (δ 1.59) and C-1 (δ 18.1), C-8 (δ 37.6), C-9 (δ 40.1), C-11 (δ 40.0) and C-4 (δ 43.3).

The stereochemistry was confirmed on the basis of NOESY cross peaks observed between H-20/H-17 and H-20/H-19. However, there were no cross peaks between H-20/H-17/H-19 and H-10. Further, confirmation of the structure of **9** was provided by NOESY experiment, which showed cross peaks between H-2 and H-10 establishing the spatial proximity of H-2 and H-10, thus placing the hydroxyl group at C-2 to β-position. These results can be rationalized only if C-20, C-17, C-19 and HO-C (2) are on the same face of the molecule while H-10 and H-2 are on another face of the molecule The $^{1}$H- and $^{13}$C-NMR, HMBC, and NOESY data were consistent with the structure of 2β-hydroxy-15,16-epoxy-5β.8β,9β,10α-cleroda-

3,13(16),14-trien-18-oic acid (2β-Hydroxyhardwickiic acid) for compound **9**. 2β-Hydroxyhardwickiic acid (**9**) has been previously isolated from *Dodonaea boroniifolia* [Jefferies *et al*., 1973] and *Duranta repens* [Anis *et al*., 2001]. However, this is the first report of this compound from *D. angustifolia*.




2β-Hydroxyhardwickiic acid (**9**)

Table 3.10: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 2β-hydroxyhardwickiic acid (**9**)

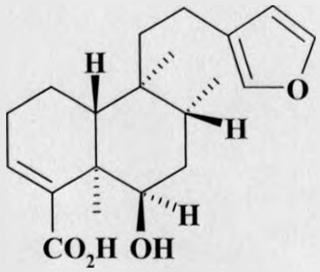
| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ |
|---|---|---|
| 1 | 29.3 | 2.08 (*m*), 2.00 (*m)* |
| 2 | 69.7 | 4.35 (*m*) |
| 3 | 141.8 | 6.63 (*brs)* |
| 4 | 143.6 | - |
| 5 | 40.8 | - |
| 6 | 37.2 | 2.42 (*m*), 1.18 (*m*) |
| 7 | 28.8 | 1.50 (*m*), 1.38 (*m*) |
| 8 | 37.4 | 1.63 (*m*) |
| 9 | 39.9 | - |
| 10 | 46.8 | 1.48 (*m*) |
| 11 | 39.6 | 1.57 (*m*) |
| 12 | 19.4* | 2.40 (*m*), 2.30 (*m*) |
| 13 | 127.2 | - |
| 14 | 112.6 | 6.37 (*brs*) |
| 15 | 144.3 | 7.44 (*brs*) |
| 16 | 140.2 | 7.37 (*brs*) |
| 17 | 17.0 | 0.85 (*d*, 6.6 Hz) |
| 18 | 169.2 | - |
| 19 | 21.6 | 1.32 (*s*) |
| 20 | 19.5* | 0.80 (*s*) |

* Interchangeable

### 3.1.10. DODONIC ACID (10)

Compound **10** was obtained as white crystals from $CH_2Cl_2$/MeOH with melting point of 105-107 °C. $R_f$ 0.5 (2% MeOH/$CH_2Cl_2$) and was UV (254 nm) active.

The $^1$H-NMR of **10** exhibited signals for two tertiary methyl groups at δ 0.75 and 1.28 and one secondary methyl group at δ 0.85 ($J$ = 6.6 Hz). The $^1$H-NMR further dispayed broad singlet at δ 6.87 assigned to a β-vinyl hydrogen in an α, β-unsaturated carbonyl grouping having a methylene group adjacent to the vinyl hydrogen. A double doublet at δ 3.60 ($J$ = 5.6, 10.4 Hz) was assigned to the geminal proton of a secondary hydroxyl group at C-6, which was coupling with the two protons at C-7. Typical downfield signals in the $^1$H-NMR spectrum of compound **10** at δ 6.35, 7.35 and 7.43 were attributed to H-14, H-16 and H-15, respectively, suggesting the presence of a 3-substituted furan ring. The peaks between δ 1.82 and 1.55 are due to the methylene groups. The $^{13}$C-NMR (DEPT) corroborated the presence of three methyl groups, five methylenes, seven methines and five quaternary carbon atoms.The $^{13}$C-NMR chemical shift of the methyls were observed at δ 16.1, 17.1 and 18.1. These values reveal the *trans* configuration at the A/B ring junction of compound **9** [Manabe & Nishino, 1986]. From the above spectroscopic studies and comparison of the spectral data with that in literature [Sachdev & Kulshreshtha, 1984; Van Heerden *et al.*, 2000] compound **10** was deduced to be dodonic acid. Dodonic acid (**10**) has been isolated before from *Dodonaea angustifolia* [Van Heerden *et al.*, 2000] and *Dodonaea viscosa* [Sachdev & Kulshreshtha, 1984]




**10**

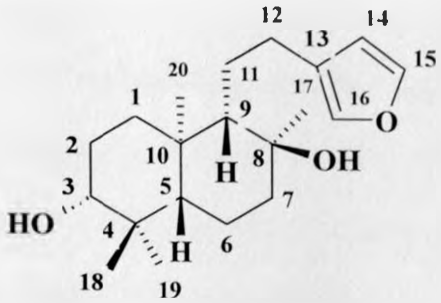
### 3.1. 11. *ENT*-3β, 8α; 15,16-EPOXY-13(16), 14-LABDADIENE-3, 8-DIOL (11)

Compound **11** was isolated as white crystals from $CH_2Cl_2$/MeOH. Its spot on TLC had an $R_f$ value of 0.4 (1% MeOH in $CH_2Cl_2$) and was UV (254 nm) inactive but turned brown on exposure to iodine vapour.

The $^1$H-NMR of this compound displayed signals at δ 6.29, 7.23 and 7.34 attributed to the two α and one β-proton of a β-substituted furan ring. The $^1$H-NMR further displayed a distorted quartet at δ 3.23 ($J$ = 4.8, 10.6 Hz) which was assigned to the geminal proton of a secondary hydroxyl group at C-3, which was coupling with the two protons at C-2. The $^1$H-NMR spectrum of **11** showed the signals of four tertiary methyl groups at δ 0.76, 0.81 and 0.98 and 1.14 characteristic of a labdane skeleton. The multiplet between δ 2.47 and 1.73 was attributed to the methylene protons.

The $^{13}$C-NMR displayed 20 signals, assigned to a diterpene skeleton. The $^{13}$C-NMR (DEPT) corroborated the presence of four methyl groups, six methylenes, three methines and three quaternary carbon atoms. The $^{13}$C-NMR chemical shift of the methyls were observed at δ 16.4, 16.8, 21.2 and 24.8. These values reveal the *trans*-configuration at the A/B ring junction of compound **11** [Manabe & Nishino, 1986].

From the above spectroscopic studies and comparison of the spectral data with that in literature [Mata *et al.*, 1991] compound **11** was deduced to be *ent*-3β, 8α; 15, 16-Epoxy-13(16), 14-labdadiene-3, 8-diol. *ent*-3β, 8α; 15, 16-epoxy-13(16), 14-labdadiene-3, 8-diol (**11**) has been isolated previously from *Dodonaea viscosa* [Dawson *et al.*, 1966].




Dodonic acid (**11**)

### 3.2 COMPOUNDS FROM *DODONAEA ANGUSTIFOLIA*-VOI

The extraction of the surface exudates on the aerial parts (mainly leaves) was done after plucking out the flowers. The surface exudates of the leaves was exhaustively extracted by successive dipping into fresh portions of acetone for short periods (less than 15 seconds) to yield the crude extract, thus avoiding the extraction of the internal tissue components. The extract was tested for anti-plasmodial activity against chloroquine-senstive (D6) strain of *Plasmodium falciparum* with $IC_{50}$ of 56.3 ± 4.2 μg/ml. The rest of the crude extract was then subjected to gravity column chromatography on silica gel eluting with mixtures of n-hexane/dichloromethane and then with dichloromethane/methanol. Further purification of compounds was done using further chromatography on silica gel and Sephadex LH 20 and finally crystallization leading to the isolation of eleven compounds. The structures of these compounds was confirmed by comparison of their spectroscopic data with literature and in some cases by direct comparison (co-TLC) with authentic samples.

5-Hydroxy-3,7,4′-trimethoxyflavone (**1**), 3,5-dihydroxy-7,4′-dimethoxyflavone (**2**), kumatakenin (**3**), rhamnocitrin (**5**) were also isolated from *D. angustifolia* growing in Ngong forest and therefore their structure elucidation are not described again. Among the compounds isolated the flavonoids, penduletin (**12**), ayanin (**13**), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**), kaempferol (**15**), the coumarin 7-hydroxy-6-methoxycoumarin (**16**) and the diterpenoid hautriwaic acid (**17**) have not been previously described from this

plant species. However, most of these compounds except ayanin (13) and 7-hydroxy-6-methoxycoumarin (**16**) have been reported from different *Dodonaea* species. This is the first report of *neo*clerodan-3,13-dien-16,15: 18,19-diolide (**18**), 15α-methoxy-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**19**), 15β-methoxy-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**20**). The structure of these compounds was determined using a combination of spectroscopic techniques. In this section the structural elucidation of these compounds will be discussed

### 3.2.1. PENDULETIN (12).

Compound **12** was isolated as yellow crystals with melting point of 220-222 °C. It appears as yellow spot ($R_f$ 0.5 (4% MeOH in $CH_2Cl_2$), which intensified on exposure to ammonia, vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 344 corresponding to $C_{17}H_{14}O_6$.

The UV ($\lambda_{max}$ MeOH 272.0 and 341.5 nm) [Mabry *et al.*, 1970], $^1$H (δ 12.73 (for chelated hydroxyl group)) and $^{13}$C (δ 148.0 for C-2, 137.0 for C-3 and 177.6 for C-4) NMR [Agrawal 1989] is consistent with a 5-hydroxyflavonol derivative.

The $^1$H-NMR (Table 3.11) displayed the presence of a singlet at δ 6.63 (1H, *s*) which was assigned to a tri-substituted ring A and in AA'BB'spin system centered at δ 7.03 and 8.06 (*dd*, *J* = 2.1, 9.0 Hz) which were assigned to 4'-substituted ring B protons. The assignment of the singlet to C-8 was confirmed from HMBC correlation between the chelated hydroxyl proton at δ 12.73 with the carbon at δ 133.8 and not with the carbon at δ 92.4 which has an HMQC correlation with the singlet at δ 6.63.

The $^1$H-NMR (Table 3.11) displayed three peaks for three methoxyl group at δ 3.98, 3.88 and 3.80.

The placement of the methoxyl group at C-3, C-6 and C-7 was confirmed through HMBC correlation (Table 3.11) of the protons at δ 3.98 with C-6 (δ 133.8), δ 3.88 with C-3 (δ 139.8) and C-7 (δ 160.8).

The $^{13}$C-NMR for the methoxyl groups at δ 60.9 and δ 61.2 requires that the methoxyl groups are di-*ortho* substituted. The $^{13}$C-NMR of the other methoxyl group was δ 57.5 which is typical of an isolated or mono substituted methoxyl group

Thus based on this and comparison of the data with literature compound **12** was identified as 3,5,7,4′-tetrahydroxy-6-methoxyflavonol (**12**) (penduletin or candirone). Penduletin (**12**) has been previously isolated from the *Dodonaea viscosa* [Sachdev & Kulshreshtha, 1986].

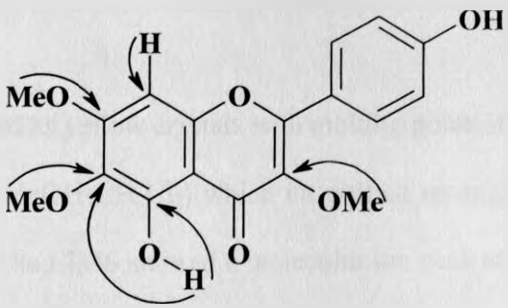



Figure 3.9: HMBC correlations in 3,5,7,4′-tetrahydroxy-6-methoxyflavonol (**12**).

Table 3.11: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for penduletin (**12**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 157.7 | | |
| 3 | | 139.8 | | |
| 4 | | 180.5 | | |
| 5 | | 154.3 | | |
| 6 | | 133.8 | | |
| 7 | | 160.8 | | |
| 8 | 6.63 (*s*) | 92.4 | C-9, C-7 | C-6, C-10 |
| 9 | | 153.9 | | |
| 10 | | 107.7 | | |
| 1′ | | 123.4 | | |
| 2′ | 8.06 (*dd*, 2.1, 9.0) | 131.9 | | C-6′,C-2, C4′ |
| 3′ | 7.03 (*dd*, 2.1, 9.0) | 117.1 | C-4′ | C-5′, C-1′ |
| 4′ | | 161.7 | | |
| 5′ | 7.03 (*dd*, 2.1, 9.0) | 117.1 | C-4′ | C-3′, C-1′, |
| 6′ | 8.06 (*dd*, 2.1, 9.0) | 131.9 | | C-2′,C-2, C4′ |
| OMe | 3.98 (*s*) | 57.5 | | C-6 |
| OMe | 3.88 (*s*) | 60.9 | | |
| OMe | 3.80 (*s*) | 61.2 | | |
| OH | 12.73 *s* | | C-5 | C-10, C-6 |

### 3.2.2. AYANIN (13).

Compound **13** was isolated as yellow crystals with melting point of 173-174 °C. It appears as yellow spot ($R_f$ 0.5 (2% MeOH-$CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 344 corresponding to $C_{18}H_{16}O_7$ and an intense peak at 301[$M^+$- 43] corresponding to standard flavonol C-ring collapse [Harborne, 1994].

The $^1$H (δ 12.77 (for chelated hydroxyl group)) and $^{13}$C (δ 157.6 for C-2, 140.3 for C-3 and 180.3 for C-4) NMR [Agrawal 1989] is consistent with a 5-hydroxyflavonol derivative.

The $^1$H-NMR (Table 3.12) indicated the presence of two *meta* coupled aromatic protons at δ 6.67 and 6.32 which were assigned to ring A and an AXY spin system at δ 7.05 (1H, *d*, *J* = 8.7 Hz), 7.72 (1H, *dd*, *J* = 2.1 and 8.7 Hz) and 7.80 (1H, *dd*, *J* = 2.1 Hz) assigned to C-3' and C-4' substituted ring B. With oxygenations at C-5 and C-7 (biogenetically expected), the *meta* coupled protons are assigned to H-6 and H-8. HMBC correlation between the chelated

hydroxyl proton (δ 12.77) with C-6 (δ 99.1) and HMQC correlation between the proton at δ 6.32 and C-6 (δ 99.1) led to the assignment of the doublet at δ 6.32 to H-6. Similarly, HMBC correlation between the doublet at δ 6.32 and C-8 (δ 93.7) allowed the placement of the doublet at δ 6.67 to H-8.

The $^{1}$H-NMR (Table 3.12) also displayed peaks for three methoxyl groups at δ 3.95, 3.92 and 3.90. One of the methoxyl groups was placed at C-3 due to the fact that it resonates at δ 60.9 in $^{13}$C-NMR which is typical for a di-*ortho* substituted methoxyl. The placement of the other two methoxyl groups at C-7 and C-4′ position was confirmed by the HMBC correlation between the methoxyl group at δ 3.95 with C-7 (δ 167.3) and that at δ 3.92 with C-4′ (δ 151.2). HMBC correlation of the carbon at δ 151.2 (C-4′) with the three protons in the AXY spin system was also observed.

Thus based on this and correlation of the data with literature compound **13** is identified as 5,3′-dihydroxy-3,4′,7-trimethoxyflavone (**13**) (ayanin). Ayanin (**13**) has not been isolated from *Dodonaea* species but from several other plant species including the aerial parts of *Bahia glandulosa* [Perez-Castorena *et al.*, 1997a] *Psiadia dentate* [Jakobsen *et al.*, 2001].

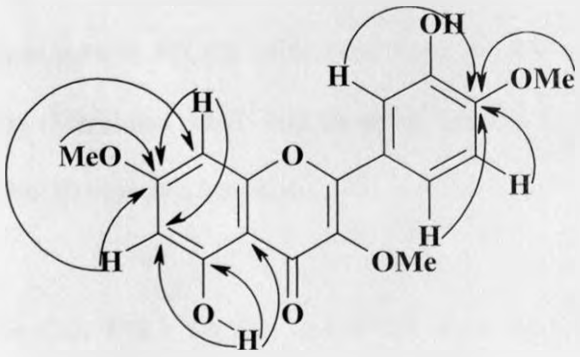



Figure 3.10: HMBC correlations of ayanin (**13**)

Table 3.12: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for ayanin (**13**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 157.6 | | |
| 3 | | 140.3 | | |
| 4 | | 180.3 | | |
| 5 | | 163.6 | | |
| 6 | 6.32 (*d*, 2.4) | 99.1 | C-5 | C-8, C-10 |
| 7 | | 167.3 | | |
| 8 | 6.67 (*d*, 2.4) | 93.7 | C-9, C-7 | C-6, C-10 |
| 9 | | 158.4 | | |
| 10 | | 107.3 | | |
| 1′ | | 123.5 | | |
| 2′ | 7.72 (*dd*, 2.1, 8.7) | 124.1 | C-1′ | C-2, C4′ |
| 3′ | 7.05 (*dd*, 2.1, 8.7) | 116.8 | C-2′ | C-5′ |
| 4′ | | 151.2 | | |
| 5′ | | 148.6 | C-4′ | C-3′, C-1′, |
| 6′ | 7.80 (*d*, 2.1) | 113.4 | | C-2′, C-2, C4′ |
| OMe | 3.90 (*s*) | 60.9 | | C-3 |
| OMe | 3.95 (*s*) | 57.1 | | C-7 |
| OMe | 3.92 (*s*) | 57.1 | | C-4′ |
| OH | 12.77 (*s*) | | C-5 | C-10, C-6 |
| OH | 8.89 (*brs*) | | | |

### 3.2.3. 5-HYDROXY-3,6,7,4′-TETRAMETHOXYFLAVONE (14)

Compound **14** was isolated as yellow crystals with melting point of 178-180 °C. It appears as yellow spot $R_f$ 0.5 (4% MeOH/$CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 358 corresponding to $C_{19}H_{18}O_7$ and an intense peak at 343 [$M^+$ -$CH_3$] and 315 [$M^+$ - 43] corresponding to 6-$OCH_3$ flavonol fragmentation (Markham, 1982) and standard flavonol C-ring collapse (Harborne, 1994) for 3-methyl ether flavone respectively.

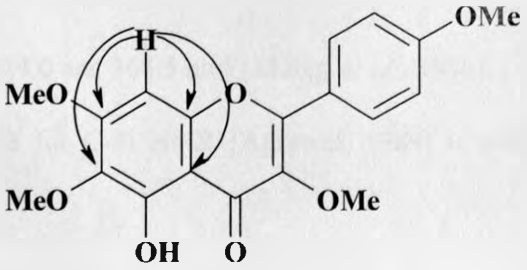
The $^{13}C$ (δ 156.0 for C-2, 138.7 for C-3 and 178.9 for C-4) NMR [Agrawal, 1989] is consistent with a flavonol derivative.

The $^1H$-NMR (Table 3.13) displayed the presence a singlet at δ 6.51 (1H, *s*) which was assigned to a tri-substituted ring A and an AA′BB′ spin system centered at δ 7.03 and 8.08

(*dd*, $J$ = 2.1, 9.0 Hz) which were assigned to 4′-substituted ring B protons. With the biogenetically expected oxygenations at C-5 and C-7 the singlet at δ 6.51 was assigned to either H-6 or H-8. The peak at δ 6.51 was assigned to H-8 due to HMBC correlations with C-6 (δ 132.3), C- 7 (δ 158.7), C-9 (δ 152.3) and C-10 (δ 106.6).

The $^1$H-NMR also displayed peaks for four methoxyl groups at δ 3.96, 3.93 and 3.90 and 3.87. From the HMQC correlations, two of the methoxyl groups were placed at C-3 and C-6 due to the fact that they resonated at δ 60.1 and δ 60.9, respectively, which is typical for di-*ortho* substituted methoxyl groups. The other two methoxyl groups were placed at C-7 and at C-4′. The placement of the methoxyl group at δ 3.96 and 3.90 to C-7 and C-4′ position respectively, was confirmed by the HMBC correlation between the methoxyl group at δ 3.96 and C-7 (δ 158.7) and C-4′ (δ 161.7), respectively.

Thus based on this and correlation of the data with literature compound **14** was identified as 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**). Compound **14** has been previously isolated from the *Dodonaea viscosa* [Sachdev & Kulshreshtha, 1983] and *Dodonaea lobulata* [Dawson *et al.*, 1966]. However, this compound has never been isolated from *Dodonaea angustifolia*.




**14**

Figure 3.11: HMBC of 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**)

Table 3.13: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 5-hydroxy-3,6,7,4'-tetramethoxyflavone (**14**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 156.0 | | |
| 3 | | 138.7 | | |
| 4 | | 178.9 | | |
| 5 | | 152.8 | | |
| 6 | | 132.3 | | |
| 7 | | 158.7 | | |
| 8 | 6.51 *s* | 90.3 | C-9, C-7 | C-6, C-10 |
| 9 | | 152.3 | | |
| 10 | | 106.6 | | |
| 1′ | | 122.8 | | |
| 2′ | 8.08 (*dd*, 2.1, 9.0) | 130.1 | | C-6′, C-4′,C-2 |
| 3′ | 7.03 (*dd*, 2.1, 9.0) | 114.1 | C-4′ | C-5′,C-1′ |
| 4′ | | 161.7 | | |
| 5′ | 7.03 (*dd*, 2.1, 9.0) | 114.1 | C-4′ | C-3′, C-1′, |
| 6′ | 8.08 (*dd*, 2.1, 9.0) | 130.1 | | C-2′,C-2, C-4′ |
| OMe | 3.87 *s* | 60.1 | | C-3 |
| OMe | 3.96 *s* | 55.3 | | C-7 |
| OMe | 3.93 *s* | 60.9 | | C-8 |
| OMe | 3.90 *s* | 56.0 | | C-4′ |

### 3.2.4. KAEMPFEROL (15).

Compound **15** was isolated as yellow crystals with melting point of 276-278 °C. It appears as yellow spot ($R_f$ 0.5 (4% $MeOH/CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 286 corresponding to $C_{15}H_{10}O_6$.

The UV ($\lambda_{max}$ MeOH 267.0.0, 324.0 and 364.5 nm) [Mabry *et al.*, 1970], $^{13}C$ (δ 148.0 for C-2, δ 137.0 for C-3 and δ 177.3 for C-4) NMR [Agrawal. 1989] is consistent with a 5-hydroxyflavonol derivative.

The $^1H$-NMR (Table 3.14) indicated the presence of two *meta* coupled aromatic protons at δ 6.54 and 6.27 (1H, *d*, *J*=2.1 Hz) which were assigned to ring A and an AA′BB′ spin system at δ 8.17 and 7.02 (2H, *dd*, *J* = 2.1 and 9.0 Hz) assigned to C-4′ substituted ring B. With

oxygenations at C-5 and C-7 (biogenetically expected) the *meta* coupled protons are assigned to H-6 and H-8.

The assignment of two sets of doublets to C-6 and C-8 was confirmed from HMBC correlation of δ 6.20 with C-7 (δ 165), C-8 (δ 93.6), C-10 (δ 104.0), C-5 (δ 161.4) and δ 6.41 with C-6 (δ 98.5), C-7 (δ 165), C-9 (δ 157.7), and C-10 (δ 104.9), respectively.

The $^{13}$C-NMR (Table 3.14) further showed signals for C-6 and C-8 of ring A at δ 98.4 and 93.6, respectively, which shows HMQC correlations with the corresponding protons at H-6 and H-8.

Thus based on this and correlation of the data with literature compound **15** was identified as 5,7,4'-tetrahydroxy-flavonol (**15**) (kaempferol etc.). Kaempferol (**15**) has been previously isolated from the *Dodonaea viscosa* [Khan *et al.*, 1992].

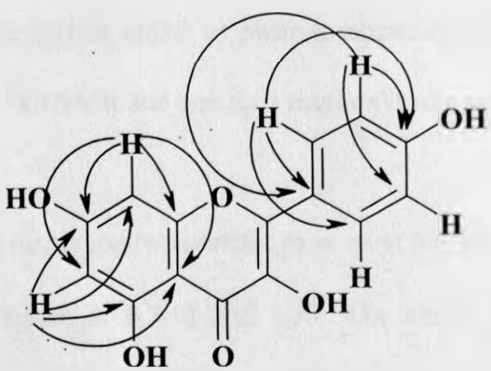



Figure 3.12: HMBC correlations of kaempferol (**15**)

Table 3.14: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for kaempferol (**15**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 141.7 | | |
| 3 | | 137.3 | | |
| 4 | | 177.3 | | |
| 5 | | 163.0 | | |
| 6 | 6.27 (*d*, 2.1) | 99.9 | C-5 | C-8, C-10 |
| 7 | | 165.7 | | |
| 8 | 6.54 (*d*, 2.1) | 95.2 | C-9, C-7 | C-6, C-10 |
| 9 | | 158.5 | | |
| 10 | | 104.9 | | |
| 1′ | | 124.0 | | |
| 2′ | 8.17 (*dd*, 2.1, 9.0) | 131.2 | C-1′ | C-6′, C4′ |
| 3′ | 7.02 (*dd*, 2.1, 9.0) | 117.0 | | C-4′, C-5′,C-1′ |
| 4′ | | 160.9 | | |
| 5′ | 7.02 (*dd*, 2.1, 9.0) | 117.0 | | C-3′, C-1′, C-4′ |
| 6′ | 8.17 (*dd*, 2.1, 9.0) | 131.2 | C-1′ | C-2 , C-4′ |
| OH | 9.2 (*brs*) | | | |

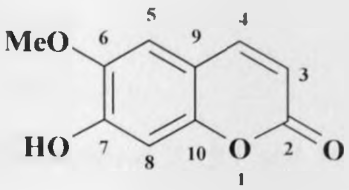
### 3.2.5. SCOPOLETIN (16).

Compound **16** was isolated as white needles with melting point of 203-204°C. It showed strong blue fluorescence with UV (254 nm) and had an $R_f$ 0.3 ($CH_2Cl_2$). The $^{13}$C-NMR (Table 3.15) displayed nine carbon atoms of phenylpropanoid, one of which is for an ester carbonyl *ca* δ 160.7 in the $^{13}$C-NMR and one for a methoxyl substituent at *ca* δ 56.0.

The $^1$H-NMR (Table 3.15) displayed two olefinic protons at δ 6.16 ($J$ = 9.2 Hz) and 7.84 ($J$ = 9.2 Hz), two aromatic singlets at δ 7.18 and 6.79. The above data is consistent with a coumarin derivative. The protons at δ 7.18 and 6.76 were assigned to H-5 and H-8 on the coumarin skeleton. The $^1$H-NMR further exhibited a three proton singlet at δ 3.89 attributed to a methoxyl group. The position of the methoxyl group was confirmed by NOESY experiment, which showed cross peaks between the methoxyl group and H-5 (δ 7.18) singlet.

From these spectral data and comparison with literature [Abyshev *et al.*, 1980] this compound was identified as 7-hydroxy-6-methoxycoumarin (**16**) (scopoletin). Scopoletin (**16**) has been previously isolated from *Dodonaea viscosa*, and other plant species such as

*Haplophyllum vulcanicum* [Ayhan and Mehmet, 2008] but this is the first report of this compound from *Dodonaea angustifolia*.




7-hydroxy-6-methoxycoumarin (**16**)

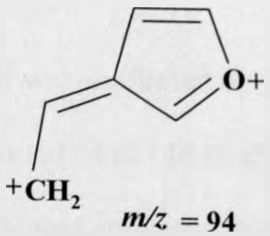
Table 3.15: $^{1}H$ (Acetone-$d_6$ at 300 MHz) and $^{13}C$ (75 MHz) NMR chemical shift data for 7-hydroxy-6-methoxycoumarin (**16**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ |
|---|---|---|
| 2 | - | 160.7 |
| 3 | 6.16 (*d*, 9.4) | 112.4 |
| 4 | 7.84 (*d*, 9.2) | 144.1 |
| 5 | 7.18 (*s*) | 109.2 |
| 6 | - | 150.5 |
| 7 | - | 151.6 |
| 8 | 6.79 (*s*) | 103.0 |
| 9 | - | 145.4 |
| 10 | - | 111.2 |
| OMe | 3.89 (*s*) | 56.0 |

### 3.2.6. HAUTRIWAIC ACID (17)

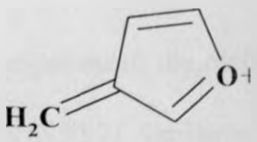
Compound **17** was obtained as white crystals from $CH_2Cl_2$/MeOH with melting point of 183-184 °C. $R_f$ 0.4 (3% MeOH/$CH_2Cl_2$) and was slightly UV (254 nm) active and turned brown on exposure to iodine vapours.

The EI-MS of compound **17** provided the molecular ion peak at *m/z* 332 indicating the molecular formula $C_{20}H_{28}O_4$. The $^{13}C$-NMR spectrum (APT) corroborated the presence of two methyl groups, seven methylenes and six methines and five quaternary carbon atoms. The peaks at *m/z* 95 and 81 as shown in the fragments **17a** and **17b** respectively suggested the presence of furan ring with an alkyl chain in **17** [Spanevello & Vila, 1994]. These results indicated that compounds **17** is a diterpene with a furan ring.




*m/z* = 94

**17a**




*m/z* = 81

**17b**

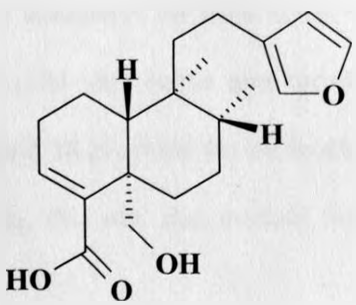
The $^{13}$C-NMR spectrum exhibited signals at δ 19.1 and 16.7 due to tertiary and secondary methyl groups at C-9 and C-8, respectively, in agreement with the data of compounds having both of these substituents as alpha on a *trans*-clerodane skeleton [Givovich *et al.*, 1986; San-Martin *et al.*, 1986; Sharma *et al.*, 1984]. In *trans*-clerodanes, C-19 carbons resonates between δ 11-19 whereas in *cis*-clerodanes appears at about δ 25. Moreover, C-20 in *trans*-clerodanes resonates at higher field (δ 17-19) than in *cis*-clerodanes (δ 21-29) [Manabe & Nishino, 1986].

The $^{1}$H-NMR spectrum of compound **17** (Table 3.16) displayed a broad singlet at δ 6.28, 7.26 and 7.37 attributed to the H-14, H-16 and H-15 protons of the β substituted furan ring. $^{1}$H-NMR further showed an AB spin system at δ 3.74 and 4.13 ($J$ = 12 Hz) attributed to the H-19 hydroxyl methylene. In addition, a broad one proton singlet at δ 6.63 was assigned to a β-olefinic proton conjugated to a carboxyl group, a three proton doublet at δ 0.87 ($J$ = 6.6 Hz) was attributed to the secondary methyl and a three proton singlet at δ 0.79 attributed to the tertiary methyl group typical of clerodane-type diterpenes.

The COSY experiment showed coupling between the methyl at δ 0.87 and the H-8 proton at δ 1.63. Furthermore, the COSY experiment showed coupling between the proton at δ 6.28 with the proton at δ 2.42 assigned to H-12 and between the proton at δ 7.26 and the H-12 methylene protons at δ 2.42 and δ 2.20. There were also coupling between the protons at δ 6.28, 7.26 and 7.34.

The structure of **17** was confirmed by the HMBC experiment, the olefinic proton at δ 6.63 showed correlations to C-4 (δ 138.9), C-5(43.3), C-2 (δ 27.7). Similarly, the proton at δ 6.28 assigned to H-14 showed cross peak correlations to the C-13 (δ 126.7), C-15 (δ 144.0), C-16 (δ 139.7) and C-12 (δ 18.1*/δ 19.1*). In addition there was HMBC cross peaks between the hyroxymethylene protons at δ 3.74, and C-5 (δ 43.3), C-6 (δ 32.9) and C-10 (δ 47.8).

The stereochemistry was confirmed on the basis of NOESY cross peaks observed between H-20/H-17 and H-20/H-19 (the two protons). However, there were no cross peaks between H-20/H17/H-19 (the two protons) and H-10. These results can be rationalized only if C-20, C-17, C-19 are on the same face of the molecule and H-10 on another face of the molecule. All the data are in agreement with compound **17** being hautriwaic acid. Hautriwaic acid (**17**) has been previously isolated from *Dodonaea viscosa* [Hsu *et al.*, 1971] and *Dodonaea attenuata* [Jefferies & Payne, 1967, 1973].




Hautriwaic acid (**17**)

Table 3.16: $^{1}H$ (MeOD at 300 MHz) and $^{13}C$ (75 MHz) NMR chemical shift data for hautriwaic acid (**17**)

| C | $\delta_{13C}$ | $\delta_{1H}$ (*m*, (Hz)) |
|---|---|---|
| 1 | 19.1* | 1.74 (*m*), 1.69 (*m*) |
| 2 | 27.7* | |
| 3 | 138.2 | 6.63 (*brs*) |
| 4 | 138.9 | - |
| 5 | 43.3 | - |
| 6 | 32.9 | - |
| 7 | 28.1* | 1.55 (*m*), 1.45 (*m*) |
| 8 | 37.6 | 1.63 (*m*) |
| 9 | 40.1 | - |
| 10 | 47.9 | 1.59 (*m*) |
| 11 | 40.0 | 1.57 (*m*) |
| 12 | 18.1* | 2.40 (*m*), 2.20 (*m*) |
| 13 | 126.7 | - |
| 14 | 111.9 | 6.28 (*brs*) |
| 15 | 144.0 | 7.37 (*brs*) |
| 16 | 139.7 | 7.26 (*brs*) |
| 17 | 16.2 | 0.87 (*d*, 6.6) |
| 18 | - | - |
| 19 | 66.2 | 4.13 (*d*, 1.2), 3.74 (*d*, 1.2) |
| 20 | 19.1 | 0.79 (*s*) |

### 3.2.7. *NEO*CLERODAN-3,13-DIEN-16,15: 18,19-DIOLIDE (18)

Compound **18** was obtained as colourless oil from $CH_2Cl_2$/MeOH. $R_f$ 0.2 (1% MeOH in $CH_2Cl_2$) and was slightly UV (254 nm) active and turned brown on exposure to iodine vapours. The EI-MS of compound **18** provided the molecular ion peak at *m/z* 330 indicating the molecular formula $C_{20}H_{26}O_4$, this was also evident from the $^{13}C$-NMR which showed resonance for twenty carbons.

The clerodane skeleton of compound 18 was identified by its unique $^{1}H$ and $^{13}C$-NMR signals (Table 3.17), in which a tertiary methyl group appeared as a singlet at $\delta_H$ 0.62 and $\delta_C$ = 17.5 assigned to C-20 and a secondary methyl group as a doublet at $\delta_H$ = 0.86 (*J* = 6.6 Hz) and $\delta_C$ = 15.5 assigned to C-17. The presence of two α,β-unsaturated γ-lactone moiety is evident in this compound from the $^{1}H$-NMR signals at δ 6.76 (*dd*, *J* = 7.4, 2.0 Hz) and 7.14 (*t*, *J* = 1.5 Hz) for olefinic β-protons, δ 4.30 (*d*, *J* = 8.1 Hz), 3.92 (*dd*, *J* = 8.0, 2.0 Hz) and δ 4.79 (*d*, *J* =

1.8 Hz) for oxymethylenes at C-19 and C-15, respectively. The corresponding carbons in the $^{13}$C-NMR for the lactone moiety appeared at δ 169.3 and 174.2 for C=O; δ 71.7 and 70.2 for oxymethylenes and the olefinic carbons resonated at δ 135.8, 138.4, 143.9 and 134.3.

The methylene protons at C-19 had an AB spin system. The *pro*-19S diastereotopic proton of this group (δ 3.92) was also ω-coupled ($^4J$ = 2.0 Hz) with the H-6β proton, indicating an α-axial orientation for C-19 [Bruno *et al.*, 1981; Esquivel *et al.*, 1986a, 1986b and Stapel., 1980].

In the $^1$H-NMR the *pro*-19R proton resonated at δ 4.30 which is in agreement with lack of a substituent at C-7 position in this compound. The presence of a substituent at C-7 position usually affects the chemical shift value of *pro*-R proton but has no effect on the *pro*-19S proton. The *pro*-R proton is usually downfield shifted by a factor of about 0.95 ppm when an α–axial hydroxyl group is present at C-7 [Herz *et al.*, 1977; Ohsaki *et al.*, 1986; Zdero *et al.*, 1989b] or by a factor of 0.50 ppm when an α–axial acetate group is bound to C-7. Change in hybridization at C-7 also influences the chemical shift of *pro*-R proton, so an average of δ 4.0 is observed [Herz *et al.*, 1977; Esquivel *et al.*, 1988] in oxo-derivatives. In the absence of the above mentioned factors, an average of δ 4.35 is expected for the *pro*-19R proton, as observed in compound **18**.

The COSY spectrum clearly indicates that the proton at δ 7.14 (*t*, *J* = 1.5 Hz), assigned to H-14, is coupled with the oxymethylene protons at C-15. The triplet is characteristic of a proton on the β-carbon of an α-substituted butenoid ring.

The $^{13}$C-NMR (Table 3.17) signals at δ 17.5 and 15.5 due to tertiary and secondary methyl groups at C-9 and C-8 respectively are in agreement with the data of compounds having both of these substituents as alpha on a *trans*-clerodane skeleton [Givovich *et al.*, 1986; Luteijn *et*

*al.*, 1982; San-Martin *et al.*, 1986; Sharma *et al.*, 1984]. This was further confirmed by the ω-coupling shown by the *pro*-19S in compound **18** (δ 3.92, *dd*, $J$ = 8.1 and 2.0 Hz) indicating an α-axial configuration of the C-19 methylene group in agreement with *trans*-clerodanes having an axial methyl group at C-5 and an axial H-6 [Gambaro *et al.*, 1986; Givovich *et al.*, 1986; San-Martin *et al.*, 1986]

In *trans*-clerodanes C-19 resonates between δ 11-19 whereas in *cis*-clerodanes this carbon appears at about δ 25. Moreover, C-20 in *trans*-clerodanes resonates at higher field (δ 17-19) than in *cis*-clerodanes (δ 21-29) [Manabe & Nishino 1986]. In the $^{13}$C-NMR of compound **18** the C-20 carbon resonated at δ 17.5, confirming the *trans* stereochemistry.

The stereochemistry of compound **18** was further confirmed on the basis of NOESY cross peaks observed between H-20/H-17, H-19 (*pro*-S, *pro*-R)/H-20 and H-6β/H-10. However, there were no cross peaks between H-20/H-17/H-19 (the two protons) and H-10. These results can be rationalized only if C-20, C-17, C-19 are on the same face of the molecule and H-10 on the other face.

The olefinic proton at C-3 (δ 6.76) showed HMBC cross peak with the carbons at C-16 (δ 169.3), C-5 (δ 45.5), C-2 (δ 27.6) and C-1 (δ 19.5). The other olefinic proton at C-14 (δ 7.14) showed HMBC cross peaks with C-16 (δ 174.2), C-5 (δ 70.2), C-13 (δ 134).

All the data are in agreement with compound being a *neo*-clerodane skeleton. Diterpenoids with α-substituted butenolide moieties have not been isolated from *Dodonaea* species before. This is the first report of this compound in nature.

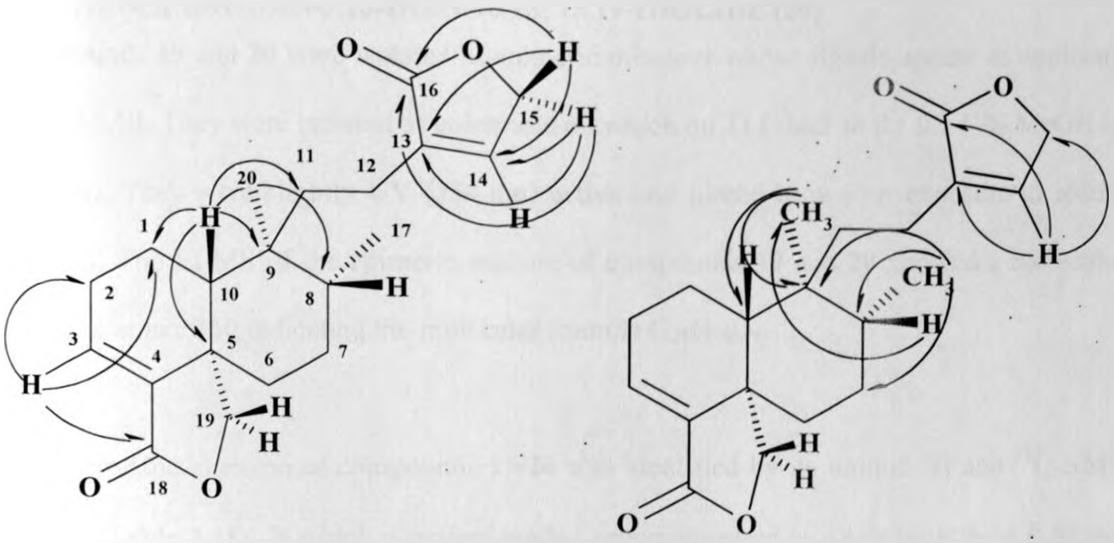


Figure 3.13: HMBC of compound *neo*clerodan-3,13-dien-16,15: 18,19-diolide (**18**).

Table 3.17: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for *neo*clerodan-3,13-dien-16,15: 18,19-diolide (**18**).

| C | $\delta_{13C}$ | $\delta_{1H}$ (*m*, (Hz)) |
|---|---|---|
| 1 | 19.5 | 1.77 (*m*), 1.07 (*m*) |
| 2 | 27.6 | 2.40 (*m*), 2.23 (*m*) |
| 3 | 135.8 | 6.76 (*dd*, 7.4; 2.0 Hz) |
| 4 | 138.4 | - |
| 5 | 45.5 | - |
| 6 | 34.4 | 1.93 (*m*), 1.25 (*m*) |
| 7 | 27.7 | 1.62 (*m*), 1.51 (*m*) |
| 8 | 36.5 | 1.68 (*m*) |
| 9 | 38.7 | - |
| 10 | 48.0 | 1.75 (*m*) |
| 11 | 35.2 | 1.60 (*m*) |
| 12 | 18.9 | 2.24 (*m*), 2.04 (*m*) |
| 13 | 134.3 | - |
| 14 | 143.9 | 7.14 (*t*, 1.5) |
| 15 | 70.2 | 4.79 (*d*, 1.8). |
| 16 | 174.2 | - |
| 17 | 15.5 | 0.86 (*d*, 6.6) |
| 18 | 169.3 | - |
| 19 | 71.7 | *pro*-R 4.30 (*d*, 8.1), *pro*-S 3.92 (*dd*, 8.0, 2.0) |
| 20 | 17.5 | 0.62 (*s*) |

### 3.2.8. 15β-*NEO*CLERODAN-3,13-DIEN-16,15: 18,19-DIOLIDE (19) AND 15α-*NEO*CLERODAN-3,13-DIEN-16,15: 18,19-DIOLIDE (20)

Compounds **19** and **20** were isolated as epimeric mixtures whose signals appear as duplicate in the NMR. They were isolated as colourless oil which on TLC had an $R_f$ 0.1 (1% MeOH in $CH_2Cl_2$). They were slightly UV (254 nm) active and turned brown on exposure to iodine vapours. The EI-MS of the epimeric mixture of compounds **19** and **20** showed a molecular ion peak at *m/z* 360 indicating the molecular formula $C_{21}H_{28}O_5$.

The clerodane skeleton of compounds **19/20** was identified by its unique $^1$H and $^{13}$C-NMR signals (Table 3.18), in which a tertiary methyl group appeared as a singlet at $\delta_H$ = 0.62 and $\delta_C$ = 17.5 assigned to C-20 and a secondary methyl group as a doublet at $\delta_H$ = 0.85/0.86 (*J* = 6.6 Hz) and $\delta_C$ = 15.5 to C-17. The presence of two α,β-unsaturated γ-lactone moiety in the epimeric mixtures is evident from the $^1$H-NMR signals at δ 6.76 (*m*) and 6.79 (*m*) for olefinic β-protons; δ 4.30 (*d*, *J* = 8.1 Hz) and 3.92 (*dd*, *J* = 8.0, 2.0 Hz) for geminal oxymethylene protons at C-19; δ 5.74 (*m*) for an acetal proton and δ 3.58 (*s*) for a methoxy group at C-15. The corresponding carbons in the $^{13}$C-NMR appeared at δ 169.3 and 171.2 for C=O, δ 71.7 for the oxymethylene at C-19; δ 102.5 and δ 57.1/57.2 for an acetal and methoxy groups at C-15, respectively; for and the four olefinic carbons resonated at δ 135.7/135.8 (C-3), 138.7 (C-4), δ 138.4/138.5 (C-13), and δ 141.5/141.6 (C-14).

The *pro*-S (δ 3.92) diastereotopic proton of at C-19 was ω-coupled ($^4J$ = 2.0 Hz) with H-6β proton, indicating an α-axial orientation for C-19 [Bruno *et al.*, 1981; Esquivel *et al.*, 1986a, 1986b; Stapel, 1980].

The multiplet at δ 6.79 (*m*) (H-14) is coupled with the doublet at δ 5.74 (*m*) (H-15) as evident in the COSY spectra. The methoxy protons (δ 3.58) showed HMBC correlations to C-15 (δ

102.5). Furthermore, the substitution at this lactone ring was confirmed by HMBC cross peaks of H-15 with the carbons at C-13 (δ 138.4/138.4), C-14 (δ 141.6), C-16 (δ 171.2) and the methoxy at δ 57.2/57.1 (Figure 3.19).

The placement of the methoxy at C-15 was confirmed from the COSY and 2D-NOESY experiments which showed correlations of this group with H-15 (δ 5.74), H-14 (δ 6.79) which in turn showed long-range allylic coupling with $CH_2$-12 (δ 2.29 and δ 2.02).

The shielded $^{13}$C-NMR (Table 3.18) signals at δ 17.5 and 15.5 due to tertiary and secondary methyl groups at C-9 and C-8, respectively, are in agreement with the data of compounds having both of these substituents as alpha on a *trans*-clerodane skeleton [Givovich *et al.*, 1986; Luteijn *et al.*, 1982; San-Martin *et al.*, 1986; Sharma *et al.*, 1984]. This *trans* stereochemistry was further confirmed by the ω-coupling shown by the *pro*-19S in **19/20** (δ 3.92, *dd*, $J$ = 8.0 and 2.0 Hz) with H-6 protons, [Gambaro *et al.*, 1986; Givovich *et al.*, 1986; San-Martin *et al.*, 1986]

In the HMBC experiment of compound **19/20** (Figure 3.15), the olefinic proton at δ 6.76 showed correlations to C-4 (δ 135.8/135.7), C-18 (δ 169.3), C-5 (δ 45.5), C-2 (δ 27.7/27.6) confirming its placemnt at C-3. Similarly, the other olefinic proton (δ 6.79) showed cross peak correlations to C-13 (δ 138.5/138.4), C-16 (δ 171.2), C-15 (δ 102.5) and C-12 (δ 18.91/18.87) which confirms its location at C-14.

The relative configuration of compounds **19/20** was confirmed by 2D-NOESY experiment which showed cross peaks between H-20, H-17, H-19 and H-6$\beta$. However, there were no cross peaks between H-20/H-17/H-19 (the two protons) and H-10 and therefore the decalin

ring junction was deduced to be in *trans* configuration. This is the first report of this epimeric mixture in nature.

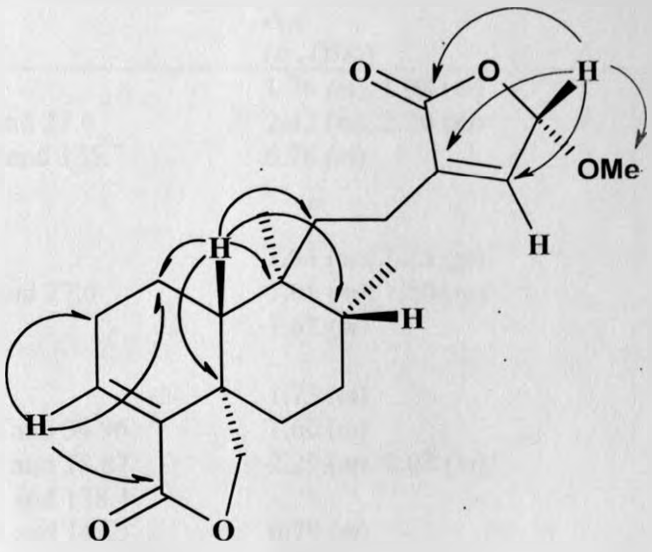



Figure 3.14: HMBC correlations of 15β-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**19**)

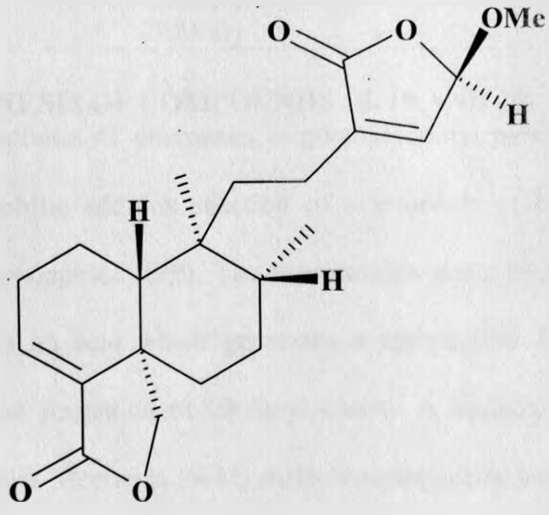


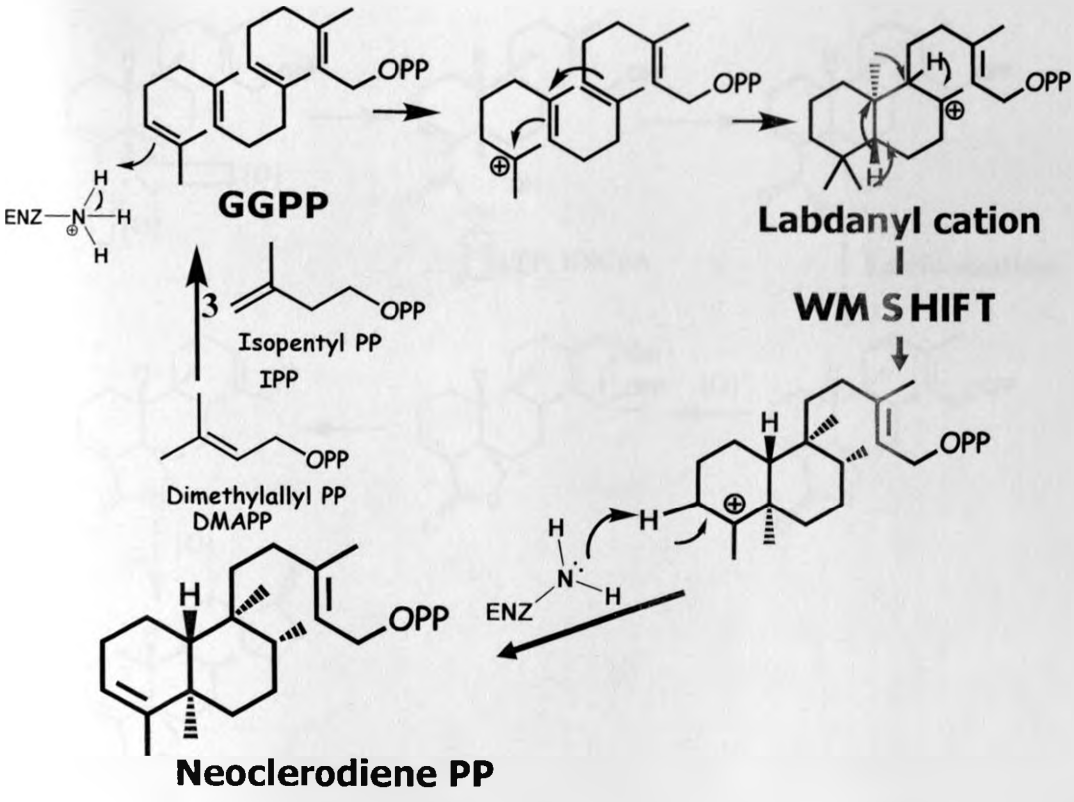

Figure 3.15: HMBC correlations of 15α-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**20**)

Table 3.18: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for the epimeric mixture of 15β-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**19**) and 15α-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**20**)

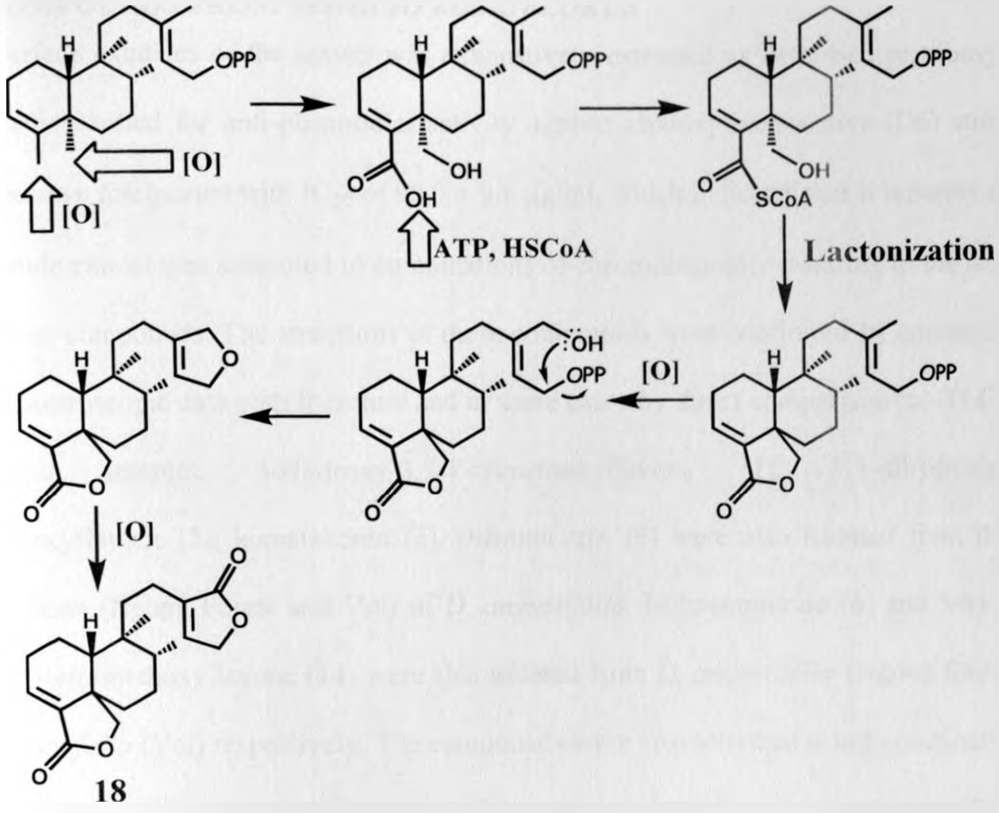
| C | $\delta_{13C}$ | $\delta_{1H}$ (*m*, (Hz)) |
|---|---|---|
| 1 | 19.5 | 1.76 (*m*), 1.08 (*m*) |
| 2 | 27.7 and 27.6 | 2.42 (*m*), 2.20 (*m*) |
| 3 | 135.8 and 135.7 | 6.76 (*m*) |
| 4 | 138.7 | - |
| 5 | 45.5 | - |
| 6 | 34.4 | 1.94 (*m*), 1.25 (*m*) |
| 7 | 27.7 and 27.6 | 1.65 (*m*), 1.50 (*m*) |
| 8 | 36.6 | 1.67 (*m*) |
| 9 | 38.8 | - |
| 10 | 48.1 | 1.73 (*m*) |
| 11 | 35.02 and 34.96 | 1.60 (*m*) |
| 12 | 18.91 and 18.87 | 2.29 (*m*), 2.02 (*m*) |
| 13 | 138.5 and 138.4 | - |
| 14 | 141.6 and 141.5 | 6.79 (*m*) |
| 15 | 102.5 | 5.74 (*m*) |
| 16 | 171.2 | - |
| 17 | 15.5 | 0.86 (*d*, 6.6) and 0.85 (*d*, 6.6) |
| 18 | 169.3 | - |
| 19 | 71.7 | *pro*-R 4.30 (*d*, 8.0), *pro*-S 3.92 (*dd*, 8.0, 2.0) |
| 20 | 17.5 | 0.62 (*s*) |
| OMe | 57.2 and 57.1 | 3.58 (*s*) |

### 3.2.9. PROPOSED BIOGENESIS OF COMPOUNDS 18, 19 AND 20

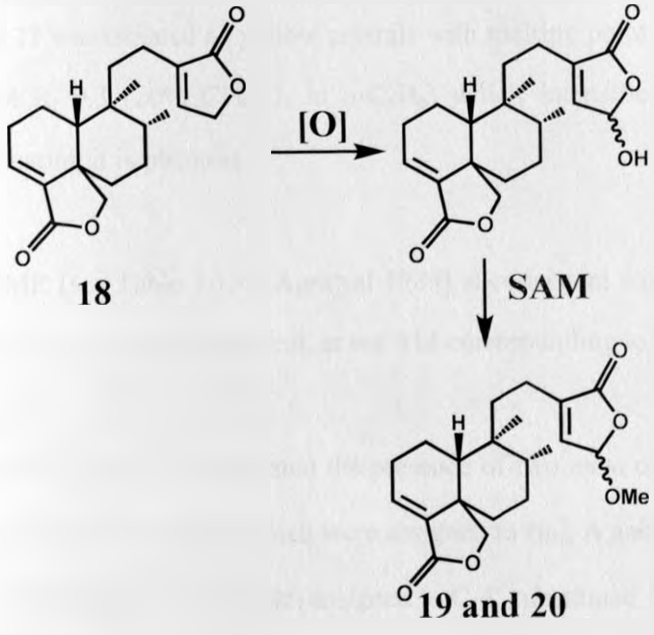
The precursor for the biosynthesis of diterpenes is geranylgeranyl pyrophosphate (GGPP) which is formed by electrophilic addition reaction of a molecule of DMAPP with three molecules of isopentyl pyrophosphate (IPP). The biosynthesis starts by the reaction of the double bond on GGPP with an acid which generates a carbocation. Further elctrophilic addition reactions lead to the formation of labdanyl cation. A sequence of concerted 1,2 hydride and 1,2 methyl Wagner Meerwein (WM) shifts/reaarangement leads to the formation of neoclerodiene pyrophosphate, which is a precursor for clerodane diterpenes.




Oxidation of the methyl groups at C-4 and C-5 to a carboxylic acid and an alcohol respectively, in the intermediate. The hydroxyl group in the carboxylic acid is a bad leaving group and therefore reacts with adenosine triphosphate (ATP) to give COOP which after reaction with coenzyme A yields the most reactive intermediate which can easily lactonize. Further, oxidation of the methyl at C-16 to an alcohol and its subsequent reaction yields compound **18** which is one of the new compounds isolated in this study.




Oxidation of **18** at C-15 and reaction with S-adenosinemethiopnine (SAM) leads to the epimeric mixtures **19** and **20**.



## 3.3 COMPOUNDS FROM *SENECIO ROSEIFLORUS*

The surface exudates of the leaves was exhaustively extracted as described previousy. The extract was tested for anti-plasmodial activity against chloroquine-senstive (D6) strains of *Plasmodium falciparum* with $IC_{50}$ of 90.0 ± 9.8 μg/ml, which indicates that it is barely active. The crude extract was subjected to combinations of chromatography resulting to the isolation of eleven compounds. The structures of these compounds were confirmed by comparison of their spectroscopic data with literature and in some cases by direct comparison (co-TLC) with authentic samples. 5-Hydroxy-3,7,4′-trimethoxyflavone (**1**), 3,5-dihydroxy-7,4′,-dimethoxyflavone (**2**), kumatakenin (**3**), rhamnocitrin (**5**) were also isolated from the two populations (Ngong Forest and Voi) of *D. angustifolia*. Isokaempferide (**6**) and 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**) were also isolated from *D. angustifolia* (Ngong forest) and *D. angustifolia* (Voi) respectively. The compounds were characterized using combinations of spectroscopic techniques. In this section the structure elucidation of compounds **21**-**27** is discussed.

### 3.3.1. 5, 7-DIHYDROXY-3,4′ -DIMETHOXYFLAVONE (21)

Compound **21** was isolated as yellow crystals with melting point of 233-235 °C. It appears as yellow spot $R_f$ 0.3 (20% $CH_2Cl_2$ in n-$C_6H_6$) which intensified on exposure to ammonia vapour indicating it is phenolic.

The $^{13}$C-NMR (see Table 3.19) [Agrawal 1989] is consistent with a flavonol derivative. The EI-MS showed a molecular ion peak at *m/z* 314 corresponding to $C_{16}H_{14}O_6$.

The $^1$H-NMR (Table 3.19) indicated the presence of two *meta* coupled aromatic protons at δ 6.43 and 6.23 (*d*, *J* = 2.1 Hz) which were assigned to ring A and an AA′BB′ spin system at δ 8.09 and 7.04 (*dd*, *J* = 2.1, 9.0 Hz) assigned to C-4′ substituted ring B. With oxygenations at C-5 and C-7 (biogenetically expected) the *meta* coupled protons are assigned to H-6 and H-8.

The $^{1}$H-NMR also displayed peaks for two methoxyl groups at δ 3.91 and 3.81. The NOE interactions between H-6 and H-8 and the methoxyl at δ 3.91 places one of the methoxyl group at C-7, while the $^{13}$C-NMR of the other methoxyl group (δ 59.7) indicates that this group is di-*ortho* substituted which is in agreement with the placement of this group at C-3. The placement of the methoxyl group at C-3 and C-7 was confirmed from the HMBC correlation of the methoxyl group at δ 3.81 and 3.91 with C-3 (δ 138.5) and C-7 (δ 156.2), respectively.

Thus based on this and comparison of the data with literature information compound **21** was identified as 5,7-dihydroxy-3,4′-dimethoxyflavone (**21**). This compound has been previously isolated from *Dodonaea viscosa* [Wollenweber *et al.*, 1986]. However, this is the first report of the isolation of the compound from *Senecio roseiflorus*.

Table 3.19: ID ($CD_3OD$: 300, 75.5 MHz) and 2D NMR data for 5,7-dihydroxy-3, 4′-dimethoxyflavone (**21**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^{2}J$ | HMBC $^{3}J$ |
|---|---|---|---|---|
| 2 | | 157.6 | | |
| 3 | | 138.5 | | |
| 4 | | 177.0 | | |
| 5 | | 162.2 | | |
| 6 | 6.23 (*d*, 2.1) | 99.0 | | |
| 7 | | 165.2 | | |
| 8 | 6.43 (*d*, 2.1) | 93.9 | | |
| 9 | | 157.5 | | |
| 10 | | 102.0 | | |
| 1′ | | 123.0 | | |
| 2′ | 8.09 (*d*, 9.0) | 130.4 | | C-6′, C-4′,C-2 |
| 3′ | 7.10 (*d*, 9.0) | 114.3 | C-4′ | C-5′,C-1′ |
| 4′ | | 162.3 | | |
| 5′ | 7.03 (*dd*, 2.1, 9.0) | 114.3 | C-4′ | C-3′, C-1′, |
| 6′ | 8.08 (*dd*, 2.1, 9.0) | 130.4 | | C-2′,C-2, C4′ |
| OMe | 3.81 (*s*) | 59.7 | | C-3 |
| OMe | 3.91 (*s*) | 55.1 | | C-7 |

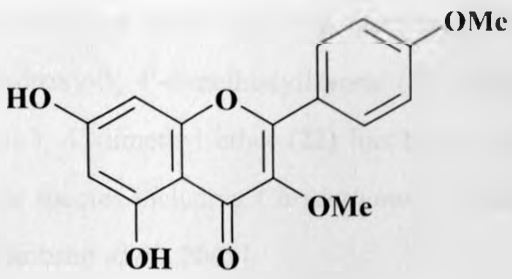



Figure 3.16: 5,7-dihydroxy-3,4′-dimethoxyflavone (21)

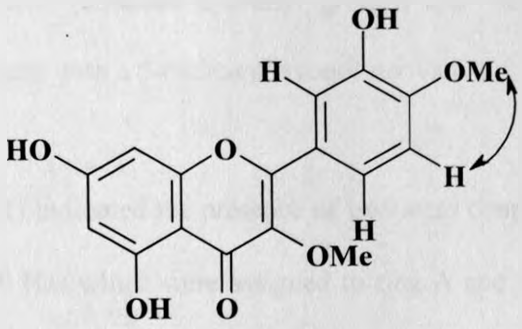
### 3.3.2. QUERCETIN-3, 4'-DIMETHYL ETHER (22)

Compound **22** was isolated as yellow crystals with melting point of 235-236 °C. It appears as yellow spot $R_f$ 0.5 (2% MeOH in $CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 330 corresponding to $C_{17}H_{14}O_7$. The $^{13}$C-NMR (see Table 3.20) indicated that compound **22** is a flavone (Agrawal, 1989).

The $^1$H-NMR (Table 3.20) indicated the presence of two *meta* coupled aromatic protons at δ 6.39 and 6.20 (*d*, *J* = 2.0 Hz) which were assigned to ring A and an AXY spin system at δ 7.06 (*d*, *J* = 8.5 Hz), 7.60 (*d*, *J* = 2.0) and 7.80 (*dd*, *J* = 2.0 and 8.5 Hz) assigned to a di-sustituted ring B.

With oxygenations at C-5 and C-7 (biogenetically expected) the *meta* coupled protons are assigned to H-6 and H-8. The $^1$H-NMR also displayed peaks for two methoxyl groups at δ 3.94 and 3.80. One methoxyl group was placed at C-3 due to the fact that it resonates at δ 60.7 which is typical for a methoxyl group at this position. The other methoxy group ($\delta_H$ 3.94, $\delta_C$ 56.6) was placed in ring B due to its NOE interaction with ring B protons and not ring A protons. The $^{13}$C-NMR singlet for the oxygenated aromatic carbons are shielded (at δ 151.9 and 147.9) indicating that these carbons are *ortho* to each other [Markham, 1982], for if this was not the case they would have resonated at *ca* 160 ppm. This implies that the methoxy group could be placed at either C-3′ or C-4′. However, the NOE interactions between this methoxyl group and the *ortho*-substituted aromatic proton at δ 7.06 confirms this methoxyl group at C-4′. The HMBC data also confirms the same (see Table 3.20).

Thus based on this and comparison of the data with literature information the compound is identified as 3', 5, 7-trihydroxy-3, 4'-dimethoxyflavone (**22**) (trivial name quercetin-3, 4'-dimethyl ether). Quercetin-3, 4'-dimethyl ether **(22)** has been previously isolated from the aerial parts of several plant species including *Chrysothamnus viscidiflorus* [Sepulveda *et al.*, 1994]; *Psiadia dentate* [Jakobsen *et al.*, 2001].




**22**

Figure 3.17: NOE interactions of compound quercetin-3, 4'-dimethyl ether (**22**)

Table 3.20: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for quercetin-3, 4'-dimethyl ether (**22**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 157.7 | | |
| 3 | | 140.0 | | |
| 4 | | 180.2 | | |
| 5 | | 158.6 | | |
| 6 | 6.39 (*d*, 2.0) | 95.0 | C-5, C-7 | C-8, C-10 |
| 7 | | 166.2 | | |
| 8 | 6.20 (*d*, 2.0) | 100.0 | C-9, C-7 | C-6, C-10 |
| 9 | | 163.3 | | |
| 10 | | 106.0 | | |
| 1' | | 123.0 | | |
| 2' | 7.63 (*dd*, 2.0 8.5) | 122.3 | | C-6, C-4',C-2 |
| 3' | 7.06 (*d*, 8.5) | 112.5 | C-4' | C-1', C-5' |
| 4' | | 151.9 | | |
| 5' | | 147.9 | | |
| 6' | 7.60 (*d*, 2.0) | 116.3 | C-5' | C-2', C-4', C-2 |
| OMe | 3.80 (*s*) | 60.7 | | C-3 |
| OMe | 3.94 (*s*) | 56.6 | | C-4' |
| OH | | | | |

### 3.3.3. RHAMNAZIN (23)

Compound **23** was isolated as yellow crystals with melting point of 216-218°C. It appears as yellow spot $R_f$ 0.5 (2% MeOH in $CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 330 corresponding to $C_{17}H_{14}O_7$. The $^1H$ (δ 12.13 (chelated hydroxyl group)) and $^{13}C$-NMR (see Table 3.21) [Agrawal 1989] is consistent with a 5-hydroxyflavonol derivative.

The $^1H$-NMR (Table 3.21) indicated the presence of two *meta* coupled aromatic protons at δ 6.72 and 6.33 (*d*, *J* = 2.0 Hz) which were assigned to ring A and an AXY spin system at δ 7.02 (*d*, *J* = 8.7 Hz), δ 7.85 (*dd*, *J* = 2.1 and 8.7 Hz) and δ 7.92 (*d*, *J* = 2.0 Hz) assigned to C-3′ and C-4′ substituted ring B. With oxygenations at C-5 and C-7 (biogenetically expected) the *meta* coupled protons are assigned to H-6 and H-8.

The $^1H$-NMR also displayed peaks for two methoxyl groups at δ 3.93 and 3.90. One methoxyl groups at C-7 while the other could be at C-3′ or C-4′ due to the fact that they both resonate at δ 55.7 which is typical for isolated methoxyl groups. The placement of the methoxyl group at C-7 was confirmed by the HMBC correlation between the methoxyl group at δ 3.90 and C-7 (δ 165.9).

The NOE correlation between the methoxy group at δ 3.93 with the *meta* coupled proton at δ 7.92 places the other methoxy group at C-3′.

Thus based on this and correlation of the data with literature compound **23** is identified as 3,5,4′-trihydroxy-7,3′-dimethoxyflavone (**23**) (trivial name rhamnazin). Rhamnazin (**23**) has been isolated previously from the aerial parts *Polygonum punctatum* [Marin *et al.*, 2001], and *Grindelia nana* [Wollenweber *et al.*, 1997a; 1997b].

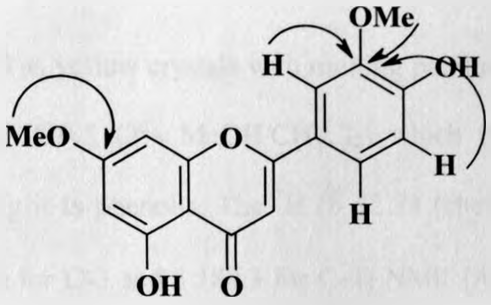



Figure 3.18: HMBC correlations of rhamnazin (**23**)

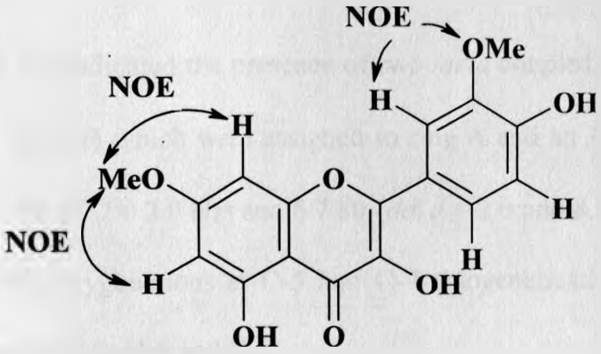



Figure 3.19: NOE correlations of rhamnazin (**23**)

Table 3.21: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 3,5,4′-trihydroxy-7,3′-dimethoxyflavone (**23**)

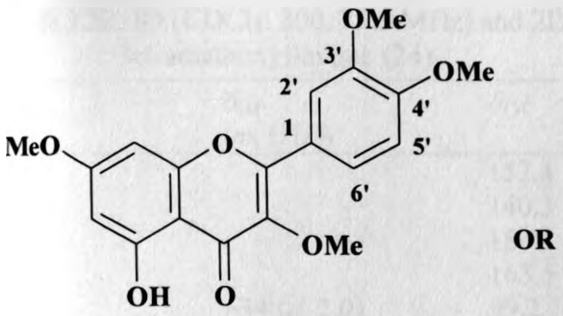
| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 148.0 | | |
| 3 | | 136.0 | | |
| 4 | | 178.0 | | |
| 5 | | 162.0 | | |
| 6 | 6.33 (*d*, 2.0) | 97.6 | C-5, C-7 | C-8, C-10 |
| 7 | | 165.9 | | |
| 8 | 6.72 (*d*, 2.0) | 92.1 | C-9, C-7 | C-6, C-10 |
| 9 | | 158.0 | | |
| 10 | | 106.0 | | |
| 1′ | | 123.0 | | |
| 2′ | 7.92 (*d*, 2.1) | 122.1 | | C-4′, C-6′, C-2 |
| 3′ | 7.02 (*d*, 8.7) | 115.3 | C-2′, C-4′ | |
| 4′ | | 151.0 | | |
| 5′ | | 148.0 | | |
| 6′ | 7.85 (*dd*, 2.1, 8.4) | 111.3 | | C-2′, C-4′, C-2 |
| OMe | 3.90 (*s*) | 55.7 | | C-7 |
| OMe | 3.93 (*s*) | 55.7 | | C-5′ |
| OH | 12.13 (*s*) | | | |

### 3.3.4. RETUSIN (24)

Compound **24** was isolated as yellow crystals with melting point of 235-236°C. It appears as yellow spot on $R_f$ value of 0.5 (2% $MeOH/CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The $^1H$ (δ 12.74 (chelated hydroxyl) and $^{13}C$ (δ 157.4.0 for C-2, δ 140.3.0 for C-3 and δ 180.3 for C-4) NMR [Agrawal 1989] is consistent with a 5-hydroxyflavonol derivative.

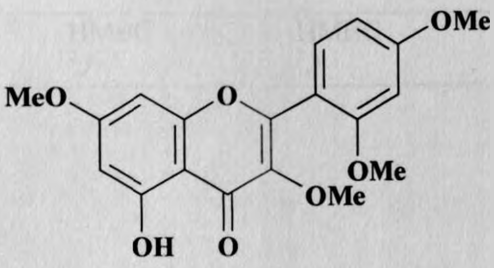
The $^1H$-NMR (Table 3.22) indicated the presence of two *meta* coupled aromatic protons at δ 6.70 and 6.34 (*d*, *J* = 2.0 Hz) which were assigned to ring A and an AXY spin system at δ 7.16 (*d*, *J*=8.5 Hz), δ 7.77 (*d*, *J* = 2.0 Hz) and δ 7.80 (*dd*, *J* = 2.0 and 8.5Hz) assigned to a di-substituted ring B. With oxygenations at C-5 and C-7 (biogenetically expected) the *meta* coupled protons are assigned to H-6 and H-8.

The $^1H$-NMR also displayed peaks for four methoxyl groups at δ 3.931, 3.928, 3.918 and 3.909. The $^{13}C$-NMR (Table 3.22) further showed signals for methoxyl groups at δ 61.0, 57.0, 57.1 and 56.8 which shows HMQC correlations with the corresponding protons δ 3.928, 3.918, 3.909 and 3.931, respectively. This gives two possible structures for this compound as 24**a** or 24**b**, based on the $^{13}C$-NMR chemical shift values of these oxygenated aromatic carbons. Oxygenated aromatic carbons with oxygenation at *ortho* or *para* position resonate at *ca* 150 ppm (as in **24b**), while those with no oxygenation at either position resonate at *ca* 160 ppm (as in **24b**) [Markham, 1982].



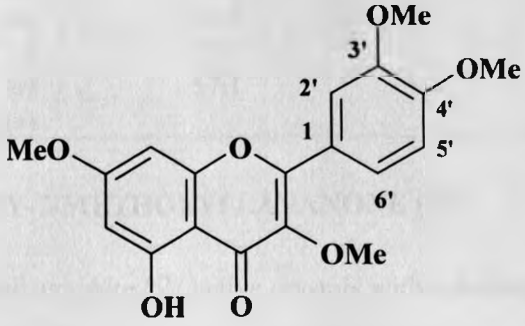

24a

OR




24b

Thus based on this and correlation of the data with literature compound **24** is identified as 5-hydroxy-3,7,3',4'-tetramethoxyflavone (**24**) (retusin). Retusin (**24**) has been isolated from *Artemisia rupestris* [Valant-Vetschera *et al.*, 2003] and *Mirabilis viscosa* [Wollenweber & Dorr, 1996]




Retusin (**24**)

Table 3.22: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 5-hydroxy-3,7,3',4'-tetramethoxyflavone (**24**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 157.4 | | |
| 3 | | 140.3 | | |
| 4 | | 180.3 | | |
| 5 | | 163.5 | | |
| 6 | 634 (*d*, 2.0) | 99.2 | | |
| 7 | | 167.3 | | |
| 8 | 6.70 (*d*, 2.0) | 93.6 | | |
| 9 | | 158.4 | | |
| 10 | | | | |
| 1' | | 124.3 | | |
| 2' | 7.77 (*d*, 2.0) | 123.7 | | C-4' |
| 3' | 7.16 (*d*, 8.5) | 112.9 | | C-1', C-5' |
| 4' | | 153.6 | | |
| 5' | | 150.7 | | |
| 6' | 7.80 (*dd*, 2.0, 8.5) | 113.2 | | C-4', C-2 |
| OMe | 3.931 (*s*) | 56.8 | | C-7 |
| OMe | 3.928 (*s*) | 61.0 | | C-3 |
| OMe | 3.918 (*s*) | 57.0 | | C-4' |
| OMe | 3.909 (*s*) | 57.1 | | C-3' |
| OH | 12.74 (*s*) | | | |

### 3.3.5. 5, 4'-DIHYDROXY-7-METHOXYFLAVANONE (25).

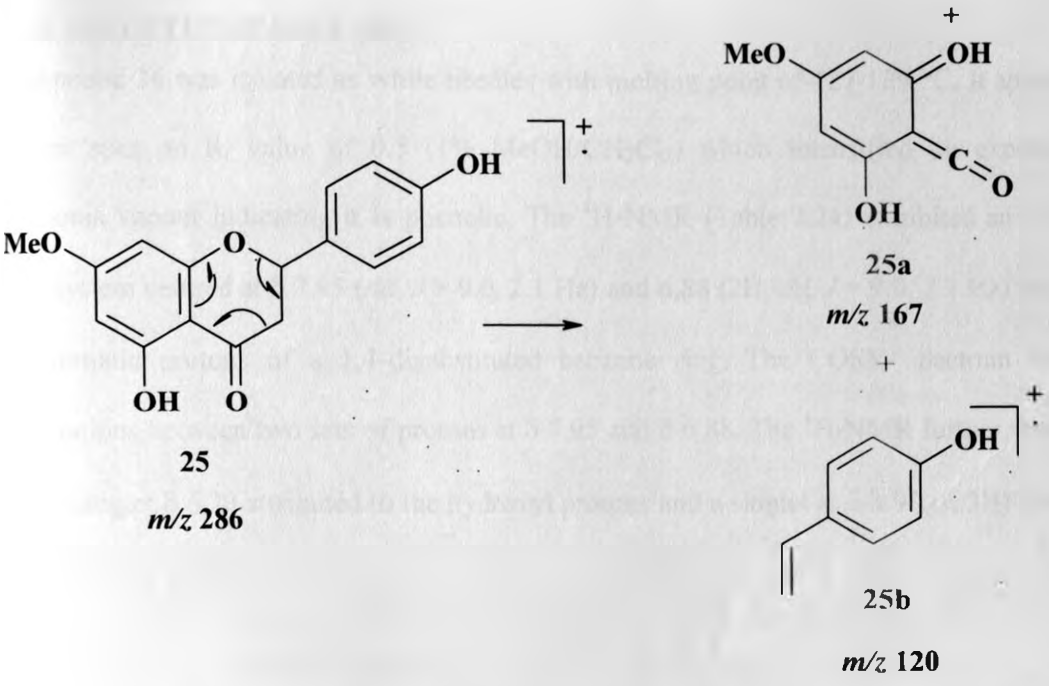
Compound **25** was isolated as white UV active crystals with a melting point of 152-154 °C. It appears as yellow spot on $R_f$ value of 0.4 (1% MeOH/$CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The UV ($\lambda_{max}$ (MeOH) 283.0 nm [Mabry *et al.*, 1970], $^1H$ (δ 12.02 (for chelated hydroxyl proton), δ 5.36 (*dd*, $J$ = 3.0 and 13.0 Hz for H-2), a methine proton attached to an oxygen, δ 2.79 (*dd*, $J$ = 3.0 and 17 Hz for H-3) and δ 3.09 (*dd*, $J$ = 13.0 and 17. 0 Hz for H-3) and $^{13}C$ (δ 79.2 for C-2, δ 43.4 for C-3 and δ 196.3 for C-4) NMR [Agrawal, 1989] is consistent with a 5-hydroxyflavanone derivative. The EIMS showed a molecular ion peak at *m/z* 286 (Scheme 3.5) corresponding to $C_{16}H_{14}O_5$.

The $^1H$-NMR (Table 3.23) indicated the presence of two *meta* coupled aromatic protons at δ 6.07 and δ 6.06 (*d*, $J$ = 2.0 Hz) which were assigned to a *di*- substituted ring A and AA'BB'spin system centered at δ 6.89 and 7.34 (*d*, $J$ = 8.5 Hz) which were assigned to 4'-

substituted ring B protons. In the MS the presence of a fragment ion at *m/z* 167 (**25a**) and *m/z* 120 (**25b**) (Scheme 3.5), resulting from a retro-Diels Alder cleavage of ring C, would place one hydroxyl group at C-5′ and one methoxyl at C-7 in ring A, and hence rings B and C should contain the other hydroxyl group.

With oxygenations at C-5 and C-7 (biogenetically expected) the *meta* coupled protons are assigned to H-6 and H-8. HMBC correlation between the chelated hydroxyl proton (δ 12.02) with C-6 (δ 95.0) and HMQC correlation between the proton at δ 6.06 and C-6 (δ 95.0) led to the assignment of the doublet at δ 6.06 to H-6. Similarly, HMBC correlation between the doublet at δ 6.07 and C-8 (δ 94.0) allowed the placement of the doublet at δ 6.07 to H-8. The $^{1}$H-NMR displayed one singlet for a methoxyl group at δ 3.81 (3H, *s*). The location of the methoxyl group at C-7 was further confirmed using HMBC (Table 3.23) which showed correlations between the protons at δ 3.81 with C-7 (δ 164.4) and due to the fact that it resonates at δ 55.9, which is typical for an isolated substituted methoxyl group.

Thus based on this and correlation of the data with literature compound 25 is identified as 5, 4′-dihydroxy-7-dimethoxyflavanone (**25**). 5,4′-Dihydroxy-7-methoxyflavanone (**25**) has been previously isolated from the aerial parts of *Dodonaea viscosa* [Mata *et al.*, 1991]. However, it has not been isolated from *Senecio roseiflorus*.




Scheme 3.5: Fragmentation pattern of 5,4′-dihydroxy-7-methoxyflavanone (**25**).

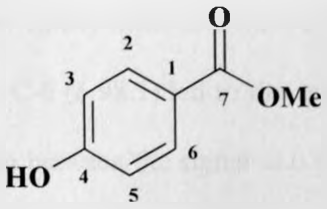
Table 3.23: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 5,4′-dihydroxy-7-methoxyflavanone (**25**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | 5.36 (*dd*, 3.0,13.0) | 79.2 | C-1' | C-3', C-4 |
| 3 | 3.09 (*dd*, 13.0, 17.0), 2.79 (*dd*, 3.0, 17.0) | 43.4 | C-2, C-4 | C-1' C-10 |
| 4 | | 196.3 | | |
| 5 | | 163.0 | | |
| 6 | 6.06 (*d*, 2.0) | 95.0 | | |
| 7 | | 164.4 | | |
| 8 | 6.07 (*d*, 2.0) | 94.0 | C-9, C-7 | C-6, C-10 |
| 9 | | 168.30 | | |
| 10 | | 103.4 | | |
| 1′ | | 128.2 | | |
| 2′ | 7.34 (*d*, 8.5) | 130.8 | C-3′ | C-6′, C-4′,C-2 |
| 3′ | 6.89 (*d*, 8.5) | 115.9 | C-4′,C-2′ | C-5′ |
| 4′ | | 156.2 | | |
| 5′ | 6.89 (*d*, 8.5) | 115.9 | C-4′, 6′ | C-5′ |
| 6′ | 7.34 (*d*, 8.5) | 130.8 | C-5′ | C-2′,C-2, C-4′ |
| OMe | 3.81 (*s*) | 55.9 | | C-7 |
| OH | 12.02 (*s*) | | C-5 | C-6, C-10 |
| OH | 5.12 (*s*) | | C-4′ | C-3′ |

### 3.3.6. METHYLPARABEN (26).

Compound **26** was isolated as white needles with melting point of 127-129 °C. It appears as yellow spot on $R_f$ value of 0.5 (1% $MeOH/CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The $^1$H-NMR (Table 3.24) exhibited an AA'BB' spin system centred at δ 7.95 (*dd*, *J* = 9.0, 2.1 Hz) and 6.88 (2H, *dd*, *J* = 9.0, 2.1 Hz) assigned to aromatic protons of a 1,4-disubstituted benzene ring. The COSY spectrum showed correlations between two sets of protons at δ 7.95 and δ 6.88. The $^1$H-NMR further showed a broad singlet δ 6.20 attributed to the hydroxyl protons and a singlet at δ 3.90 (*s*, 3H) assigned to a methoxy group.

The $^{13}$C-NMR spectrum (APT) corroborated the presence of one methoxy group, four methines and three quaternary carbon atoms. The $^{13}$C-NMR (Table 3.24) exhibited a signal at δ 167.3 for an ester group and at δ 52.0 for a carbomethoxy group.This data is consistent with a *p*-hydroxy benzoic acid skeleton for compound 26. C-2/C-6 at δ 131.9 and C-3/C-5 at δ 115.3. The HMQC experiment, showed cross peaks between the signal at δ 7.95 in the $^1$H-NMR and δ 131.9 (C-2/C-6), the signal at δ 6.88 and δ 115.3 (C-3/C-5) and the signal at δ 3.90 with δ 52.0. The structure of this compound was confirmed by the HMBC correlations between δ 7.95 (H-2/H-6) and the C-3 (δ 115.3), C-2/C-6 (δ 131.9), C-4 (δ 160.3) and C-7 (δ 167.3). In addition HMBC cross peaks were observed between the protons at δ 6.88 (H-3/H-5) and C-3 (δ 115.3), C-1 (δ 122.4) and C-4 (δ 160.3). There was HMBC correlation between the methoxy protons and the carbonyl carbon at δ 167.3. Based on these spectral data and comparison with literature information [Yoshioka *et al.*, 2004], compound **26** was identified as methyl-4-hydroxybenzoate (**26**) (trivial name methylparaben). This compound has been isolated from the fruit and leaf of *Vitex rotundifolia* [Yoshioka *et al.*, 2004].




Methylparaben (**26**)

Table 3.24: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 4-hydroxybenzoic acid methyl ester (**26**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | $^2J$ | $^3J$ |
|---|---|---|---|---|
| 1 | | 122.4 | | |
| 2 | 7.95 (*dd*, 9.0, 2.1) | 131.9 | C-1, C-3 | C-7, C-4 |
| 3 | 6.88 (*dd*, 9.0, 2.1) | 115.3 | C-2, C-4 | C-1, C-5 |
| 4 | | 160.2 | | |
| 5 | 6.88 (*dd*, 9.0, 2.1) | 115.3 | C-4, C-6 | C-3, C-1 |
| 6 | 7.95 (*dd*, 9.0, 2.1) | 131.9 | C-5,C-1 | C-4, C-7 |
| 7 | | 167.3 | | |
| OH | 6.20 (*s*) | | | |
| OMe | 3.90 (*s*) | 52.0 | | C-7 |

### 3.3.7. 5,4′-DIHYDROXY-3,7,3′-TRIMETHOXYFLAVONE (27)

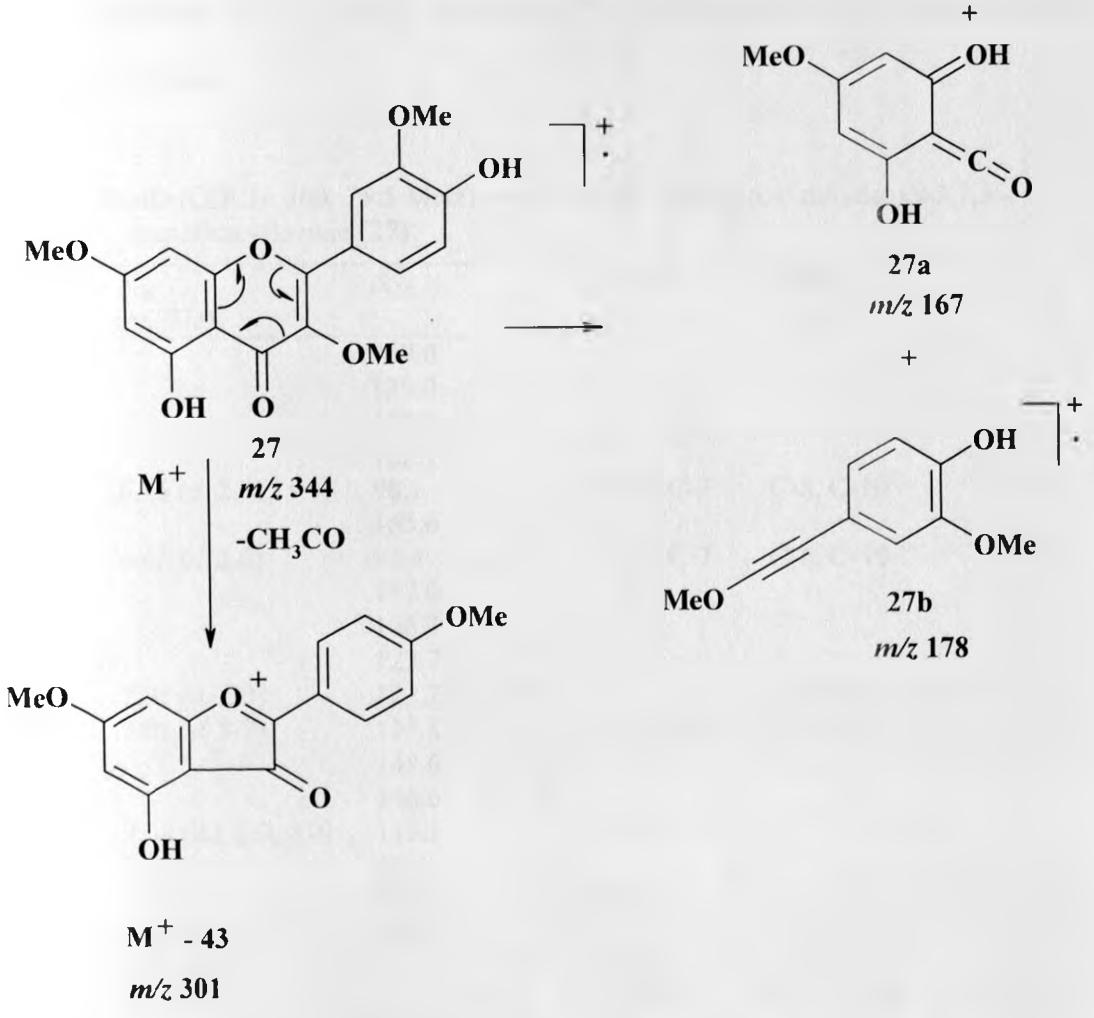
Compound **27** was isolated as yellow crystals with melting point of 235-236 °C. It appears as yellow spot $R_f$ 0.3 (2% MeOH/$CH_2Cl_2$) which intensified on exposure to ammonia vapour indicating it is phenolic. The EIMS showed a molecular ion peak at *m/z* 344 corresponding to $C_{18}H_{16}O_7$. The EIMS an intense peak at 301 [$M^+$-43] (Scheme 3.6) corresponding to standard flavonol C-ring contraction [Harborne, 1994] for 3-methyl ether flavone.

The $^1H$ (δ 12.64 (for chelated hydroxyl group) and $^{13}C$ (δ 157.0 for C-2, 139.0 for C-3 and 179.0 for C-4) NMR [Agrawal 1989] is consistent with a flavonol derivative.

The $^1H$-NMR (Table 3.2) indicated the presence of two *meta* coupled aromatic protons at δ 6.45 and 6.36 (*d*, $J$ = 2.0 Hz) which were assigned to ring A and and an AXY spin system at δ 7.05 (*d*, $J$ = 8.7 Hz), δ 7.68 (*dd*, $J$ = 2.0 and 8.0 Hz) and δ 7.71 (*d*, $J$ = 2.0 Hz) assigned to C-3′ and C-4′ substituted ring B. With oxygenations at C-5 and C-7 (biogenetically expected), the *meta* coupled protons are assigned to H-6 and H-8. HMBC correlation between

the chelated hydroxyl proton (δ 12.64) with C-6 (δ 98.10) and the HMQC correlation between the proton at δ 6.36 and C-6 (δ 98.1) led to the assignment of the peak at δ 6.32 to H-6. Similarly, HMBC correlation between the signal at δ 6.36 (*d*) and C-8 (δ 92.4) allowed the placement of the doublet at δ 6.45 (*d*) to H-8.

The $^{1}$H-NMR (Table 3.25) also displayed peaks for three methoxyl groups at δ 3.99, 3.88 and 3.86. In the MS spectra the presence of a fragment ion at *m/z* 167 (**27a**) (Scheme 3.6), resulting from a retro-Diels Alder cleavage of ring C, would place one hydroxyl group at C-5 and one methoxyl group at C-7 in ring A, and hence rings B and C should contain the other hydroxyl and two methoxyl groups.




Scheme 3.6: Fragmentation pattern of 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**27**)

One of the remaining methoxyl was placed at C-3 due to the fact that it resonates at δ 60.4 which is typical for a di- *ortho* substituted methoxyl and the other at either C-3′or C-4′.

NOESY correlations between the methoxyl at δ 3.99 with the *meta* proton at δ 7.71 confirmed its location at C-3′ position. In addition HMBC cross peaks were observed between the proton at δ 7.71 and C-1′(122.9*/122.7*), C-2′(122.9*/122.7*), C-3′(146.6) and C-4′(148.6), the proton at δ 7.68 and C-6′(112.1), C-4′(148.6), C-2 (157.0). There was HMBC cross peaks observed between the *ortho* coupled proton at δ 7.05 and C-1′(122.9*/122.7*), C-2′(122.9*/122.7*), C-3′(146.6) and C-4′(148.6) (Figure 3.20).

Based on these spectral data and comparison of the data with literature information [Likhitwitayawuid *et al.*, 2006], compound **27** was identified as 5,4′-dihydroxy-3,7,3′-trimethoxyflavone.

Table 3.25: ID ($CDCl_3$: 300, 75.5 MHz) and 2D NMR data for 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**27**)

| C | $\delta_{1H}$ (*m*, (Hz)) | $\delta_{13C}$ | HMBC $^2J$ | HMBC $^3J$ |
|---|---|---|---|---|
| 2 | | 157.0 | | |
| 3 | | 139.0 | | |
| 4 | | 179.0 | | |
| 5 | | 162.7 | | |
| 6 | 6.36 (*d*, 2.0) | 98.1 | C-5, C-7 | C-8, C-10 |
| 7 | | 165.6 | | |
| 8 | 6.45 (*d*, 2.0) | 92.4 | C-9, C-7 | C-6, C-10 |
| 9 | | 157.0 | | |
| 10 | | 106.3 | | |
| 1′ | | 122.7*/122.9* | | |
| 2′ | 7.71 (*d*, 2.0) | 122.7*/122.9* | | C-4′, C-6′, C-2 |
| 3′ | 7.05 (*d*, 8.7) | 114.8 | C-2′, C-4′ | C-1′, C-5′ |
| 4′ | | 148.6 | | |
| 5′ | | 146.6 | | |
| 6′ | 7.68 (*dd*, 2.0, 8.0) | 112.1 | C-1′, C-5′ | C-2′, C-4′, C-2 |
| OMe | 3.86 (*s*) | 60.4 | | C-3 |
| OMe | 3.88 (*s*) | 56.0 | | C-7 |
| OMe | 3.99 (*s*) | 56.3 | | C-5′ |
| OH | 5.98 (*s*) | | C-4′ | C-3′, C-5′ |
| OH | 12.6 (*s*) | | C-5 | C-6, C-10 |

* The δ values in the same column can be interchangeable.

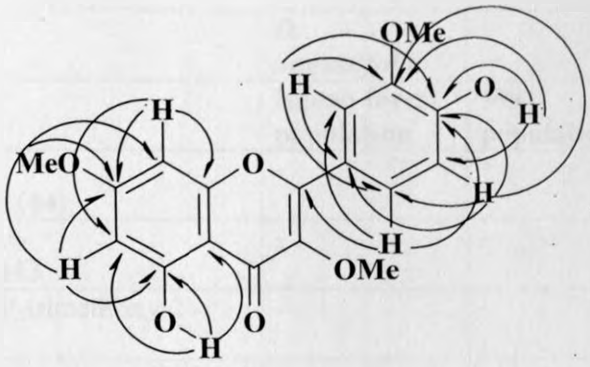



Figure 3.20: HMBC correlations of 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**27**)

## 3.4 CHEMOTAXONOMIC SIGNIFICANCE OF THE ISOLATED COMPOUNDS

### 3.4.1 *DODONAEA ANGUSTIFOLIA* (FROM NGONG FOREST AND VOI)

From this work a total of 11 compounds were isolated (7 flavones, 1 flavanone, 2 clerodane type diterpenes and 1 labdane type diterpene) from the surface exudates of *D. angustifolia* from Ngong forest. Table 3.26 below summarizes the flavonoids and terpenoids from this population of *D. angustifolia*. All of these compounds except 5,7-dihydroxy-3,6,4′-trimethoxyflavone (**4**) have not been previously reported from this plant but they have been reported from other species of the genus *Dodonaea*.

Table 3.26: Distribution of flavonoids, diterpenoids and shikimates of *D. angustifoila* (Ngong Forest and Voi) and D. viscosa

| **TYPE COMPOUND** | ***D. angustifolia*** | | ***D.viscosa*** |
|---|---|---|---|
| | **Ngong forest population** | **Voi population** | |
| **3-Methoxyflavones** | | | |
| 5-Hydroxy-3,7,4′-trimethoxyflavone (**1**) | + | + | + |
| 5,4′-Dihydroxy-3,7-dimethoxyflavone (**3**) | + | + | - |
| 5,7-Dihydroxy-3,6,4′-trimethoxyflavone (**4**) | + | - | + |
| 5,7,4′,-T rihydroxy-3-methoxyflavone (**6**) | + | - | + |
| 5,4′-Dihydroxy-3,6,7-trimehtoxyflavone (**12**) | - | + | + |
| 5,3′-Dihydroxy-3, 4′, 7-trimethoxyflavone (**13**) | - | + | - |

Table 3.26: Distribution of flavonoids, diterpenoids and shikimates of *D. angustifoila* (Ngong Forest and Voi) and D. viscosa

| TYPE COMPOUND | *D. angustifolia* | | *D.viscosa* |
|---|---|---|---|
| | **Ngong forest population** | **Voi population** | |
| 5-Hydroxy-3,6,7,4′-tetramethoxyflavone (**14**) | - | + | + |
| 5,7,4′-Trihydroxy-3,6-dimethoxyflavone (**113**) | - | - | + |
| 5,7-Dihydroxy-3,6,4′-trimethoxy-2′-prenylflavone (**114**) | - | - | + |
| 5, 7-Dihydroxy-3,6-dimethoxy-2′-(3-hydroxymethylbutyl) flavone (**115**) | - | - | + |
| 5,7-Dihydroxy-3,6,4′-trimethoxy-2′-(3-hydroxymethylbutyl) flavone (**116**). | - | - | + |
| 5,4′-Dihydroxy-3,6,7-trimethoxy-2′-(3-hydroxymethylbutyl) flavone (**117**). | - | - | + |
| 5-Hydroxy-3,6,7,4′-tetramethoxy-2′-(3-hydroxymethylbutyl) flavone (**118**). | - | - | + |
| 5,6,4′-Trihydroxy-7-methoxyflavone (**119**). | - | - | + |
| **Flavonols** | | | |
| 3,5-Dihydroxy-7,4′,-dimethoxyflavone (**2**) | + | + | - |
| 3,5,4′-Trihydroxy-7-methoxyflavone (rhamnocitrin) (**5**) | + | + | + |
| 3,5,7,4′-Tetrahydroxy-6-methoxyflavone (**7**) | + | - | - |
| 3,5,7,4′-Tetrahydroxyflavone (**15**) | - | + | + |
| 5,7,2′,4′-Tetrahydroxyflavonol (**78**) | - | - | + |
| 5,7,4′-Trihydroxy-2`-methoxyflavonol (**120**) | - | - | + |
| **Flavanone** | | | |
| 5,7-Dihydroxyflavanone (**8**) | + | - | + |
| 5,4′-Dihydroxy-7-methoxyflavanone (**79**) | - | - | + |
| **Coumarin** | | | |
| 7-Hydroxy-6-methoxycoumarin (**16**) | - | + | + |
| **Clerodane type diterpenoids** | | | |
| 2β-hydroxzyhardwickic acid (**9**) | + | - | + |
| Dodonic acid (**10**) | + | - | + |
| Hautriwaic acid (**17**) | - | + | + |
| *neo*clerodan-3,13-dien-16,15: 18,19-diolide (**18**) | - | + | - |
| 15β- Methoxy-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**19**) | - | + | - |
| 15α- Methoxy-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**20**) | - | + | - |
| Methyl dodonate A (**92**) | - | - | + |
| Methyl dodonate B (**93**) | - | - | + |

Table 3.26: Distribution of flavonoids, diterpenoids and shikimates of *D. angustifoila* (Ngong Forest and Voi) and D. viscosa

| **TYPE COMPOUND** | ***D. angustifolia*** | | ***D.viscosa*** |
|---|---|---|---|
| | **Ngong forest population** | **Voi population** | |
| Methyl dodonate C (**94**) | - | - | + |
| Dodonolide (**95**) | - | - | + |
| **Labdane type diterpenoid** | | | |
| 15,16-epoxy-13(16), 14-labdadiene-3, 8-diol; *ent*-3β, 8α form (**11**) | + | - | + |

A total of 13 compounds were isolated (8 flavones, 1 coumarin and 4 clerodane type diterpenes) from the surface exudates of *D. angustifolia* population from Voi. Table 3.26 below summarizes the flavonoids and terpenoids from this population of *D. angustifolia*. All of these compounds have not been reported previously from this plant but they have been reported from other species of the genus *Dodonaea*. The new compounds from *D. angustifolia* population from Voi are *neo*clerodan-3,13-dien-16,15: 18,19-diolide (**18**), 15β-methoxy-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**19**) and 15α-methoxy-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**20**).

The two populations of *D. angustifolia* are closely related in that they both elaborate the same class of compounds; flavonoids and terpenoids. These are the main compounds found on plant exudates. The flavonoids are usually methylated in order to increase their solubility in the terpenoid milleu. However, only four flavonoids (two 3-methoxyflavones and two flavonols) out of sixteen flavonoids isolated from the two populations are shared between them. The terpenoids isolated from the two populations are mainly the clerodane type and they are not shared between the two populations.

The two populations of *Dodonaea* in Kenya (Ngong Hills and Voi) do not have the same set of surface flavones / flavonols and diterpenoids in both quality and quantity proportions of the exudates (Table 3.26). All flavonoids from the two populations of *D. angustifolia* except 5,3′-dihydroxy-3,4′,7-trimethoxyflavone (ayanin) (**13**) are kaempferol methyl ethers. The

composition of the surface exudates of *D. angustifolia* was compared with that of *D. viscosa* because of the controversy that exists between them. *Dodonaea angustifolia* is variously considered synonymous with, sub-species of or distinct genus from *D. viscosa* depending on the particular authority. In Kenya *D. angustifolia* was declared synonymous with *D. viscosa* from Australasia.

The oxygenation pattern of some of the flavonoids isolated from both *D. angustifolia* and *D. viscosa* is similar as shown in the flavones **1, 3-6, 12, 14, 15, 113,** and **119** and the flavanones **8** and **79**. However, prenylation at C-2′ is evident in compounds **78, 114-118** and **120** from *Dodonaea viscosa* which is lacking in compounds from *D. angustifolia*. Prenylation at C-2′ can serve as a chemotaxonomic marker for *Dodonaea viscosa*. Clerodane and only one labdane type diterpenes were isolated from the two species of *Dodonaea*. The diterpenoid profile of *D. angustifolia* consists of 2β-hydroxyhardwickic acid (**9**), dodonic acid (**10**), 15,16-epoxy-13(16), 14-labdadiene-3, 8-diol; *ent*-3β, 8α form (**11**) from the Ngong Forest population and hautriwaic acid (**17**), *neo*clerodan-3,13-dien-16,15: 18,19-diolide (**18**), 15β-methoxy-*neo*clerodan-3,13-dien-16,15: 18,19-diolide (**19**), 15α-methoxy-*neo*clerodan-3,13-dien-16,15:18,19-diolide (**20**) from Voi population. Compounds **9**, **10** and **11** could serve as chemotaxonomic markers for *D. angustifolia* from Ngong Forest while hautriwaic acid (**17**) and the three new clerodane diterpenes from *D. angustifolia* could also serve as chemotaxonomic markers for *D. angustifolia* species from Voi. The two *D. angustifolia populations* are also qualitatively different, in their terpenoid profile, from *D. viscosa* (Table 3.26) which seems to produce methyl dodonate diterpenoids [Ortega., 2001] which could be genetically advanced structures from dodonic and hautriwaic acids observed in *D. angustifolia* from Ngong Forest and Voi, respectively. These compounds could also be used as chemotaxonomic markers for *D. viscosa*.

From these phytochemical work a sound conclusion can be made that different populations of *D. angustifolia* and *D. viscosa* have different flavonoid and diterpenoid profiles in their

exudates. The two populations of *D. angustifolia* and *D. viscosa* present three different chemotypes, and probably taxonomically distinct varieties or species. However, the HPLC/HPTLC profiles of these populations and other populations in Kenya need to be determined before a definite conclusion can be made.

### 3.4.2 *SENECIO ROSEIFLORUS*.

This study led to isolation of fourteen compounds, all of which were phenolic (thirteen flavonoids and one carbomethoxy phenol), from the surface exudates of *S. roseiflorus*. Table 3.27 summarizes the compounds isolated from the exudates of *S. roseiflorus* and those shared with the two populations of *D. angustifolia*.

Table 3.27: Distribution of flavonoids, diterpenoids in *S. roseiflorus* and the two populations of *D. angustifoila* from Ngong Forest and Voi

| CLASS<br>**COMPOUND** | ***S. roseiflorus*** | ***D. angustifolia*** | |
|---|---|---|---|
| | | **Ngong Forest** | **Voi** |
| **3-Methoxyflavones** | | | |
| 5-Hydroxy-3,7,4'-trimethoxyflavone (**1**) | + | + | + |
| 5,4'-Dihydroxy-3,7-dimethoxyflavone (**3**) | + | + | + |
| 5,7,4',-T rihydroxy-3-methoxy flavone (**6**) | + | + | |
| 5,3'-Dihydroxy-3,4',7-trimethoxyflavone (**13**) | + | | + |
| 5,7-Dihydroxy-3, 4'-dimethoxyflavone (**21**) | + | | |
| 3',5,7-Trihydroxy-3,4'-dimethoxyflavone (**22**) | + | | |
| 5-Hydroxy-3,7,3',4'-tetramethoxyflavone (**24**) | + | | |
| **Flavonols** | | | |
| 3,5-Dihydroxy-7,4',-dimethoxyflavone (**2**) | + | + | + |
| 3,5-Dihydroxy-7,4',-dimethoxyflavone (**5**) | + | + | + |
| 3,4',5-Trihydroxy-3',7-dimethoxyflavone (**23**) | + | | |
| **Flavanone** | + | | |
| 5,4'-Dihydroxy-7-dimethoxyflavanone (**25**) | + | | |
| **Phenol** | + | | |
| 3-Carbomethoxyphenol (**26**) | + | | |

Four flavonoids from *S. roseiflorus* were similar to those isolated from the two populations of *D. angustifolia*. Terpenoids and methyl ethers of the widespread flavonoids, apigenin, kaempferol, quercetin occurs scattered within the Angiosperms as well as in farinose exudate of gymnogrammoid ferns (Wollenweber *et al*, 1982). We sometimes find in one plant the

whole series of possible methyl derivative (normally with the exception of 5-methyl ethers) of a distinct basic skeleton. The flavonoid and terpenoid profiles of plant resins overlap in some plant species, belonging to totally different genera as is the case with *D. angustifolia* and *S. roseiflorus*. In such cases the flavonoid and terpenoid patterns between different genera alone cannot be used for chemotaxonomic purposes. However, the patterns of these compounds within some genera such as *Notholaena* (Wollenweber, 1975) are, used to typify species, recognized varieties, and even chemical races.

This study and previous studies have revealed that flavonoids and terpenoids are characteristic compounds on surface exudates, that being the reason why some methylated flavonoids were common in *S. roseiflorus* and the two populations of *D. angustifolia* studied. The flavonoids are usually methylated or have hydrophobic side chains to increase their solubility in the terpenoid milleu. The localization of these compounds (flavonoids and diterpenoids) on leaf surface is specially suited for ecological protection.

### 3.5 BIOLOGICAL ACTIVITIES.

#### 3.5.1 ANTIPLASMODIAL TESTS

The extract of fresh leaves of *Dodonaea angustifolia*-Ngong forest, *Dodonaea angustifolia*-Voi, and *Senecio roseiflorus* were tested for anti-plasmodial activities against *Plasmodium falciparum*. The tests were done against two different strains of *Plasmodium falciparum* parasites. These strains are the chloroquine-senstive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) that are commonly used in drug sensitivity assays. The most commonly used anti-malarial drugs chloroquine, quinine and mefloquine are used as positive control. Some of the compounds isolated were also tested for anti-plasmodial activities and most of them showed potent and dose dependent activities. In this section the bioassay results of this study are discussed.

### 3.5.1.1 ANTI-PLASMODIAL ACTIVITY OF *D. ANGUSTIFOLIA*-NGONG FOREST

The acetone extract of the fresh leaves (surface exudates) of *D. angustifolia*-Ngong Forest showed mild anti-plasmodial activity ($IC_{50}$ 41.5 ± 3.9 µg/ml) against chloroquine-sensitive (D6) strain of the *P. falciparum*. The pure compounds from the surface exudates had moderate activity ($IC_{50}$ 7.60-18.40 µg/ml) as listed in Table 3.28. All the eight flavonoids were moderately active and their activity was considerably more than that of the crude extract and hence the need to test the rest of the compounds from this plant. The anti-plasmodial activities of some of the compounds isolated from this plant are summarized in Table 3.28.

Table 3.28: In vitro activity ($IC_{50}$) of compounds from *D. angustifolia*-Ngong Forest against D6 strains of *Plasmodium falciparum*.

| | **$IC_{50}$ in µg/ml** |
|---|---|
| **Tested compound** | **D6** |
| **Flavonoids** | |
| 5-Hydroxy- 3,7,4′-trimethoxyflavone (**1**) | 13.8 ± 4.2 |
| 3,5-Dihydroxy-7,4′-dimethoxyflavone (**2**) | 13.0 ± 2.4 |
| 5,4′-Dihydroxy-3,7-dimethoxyflavone (Kumatakenin) (**3**) | 7.6 ± 2.3 |
| 5,7 Dihydroxy-3,6,4′-trimethoxyflavone (Santin) (**4**) | 8.6 ± 1.7 |
| 3,5,4′-Trihydroxy-7-methoxyflavone (Rhamnocitrin) (**5**) | 17.4 ± 3.9 |
| 5,7,4′-Dihydroxy-3-methoxyflavone (Isokaempferide) (**6**) | 11.1 ± 4.0 |
| 3,5,7,4′-tetrahydroxy-6-methoxyflavone (6-methoxykaempferol) (**7**) | 18.4 ± 4.8 |
| 5,7-Dihydroxyflavanone (Pinocembrin) (**8**) | 10.7 ± 1.3 |
| **Terpenoids** | |
| 2β–Hydroxyhardwickiic acid (**9**) | 10.8 ± 2.2 |
| Dodonic acid (**10**) | 9.7 ± 2.8 |
| Chloroquine | 0.003 ± 0.001 |
| Quinine | 0.063 ±0.003 |
| Mefloquine | 0.002 ± 0.001 |

### 3.5.1.2 ANTI-PLASMODIAL ACTIVITIES OF *D. ANGUSTIFOLIA*-VOI

The acetone extract of the fresh leaves of *D. angustifolia*-Voi showed very mild anti-plasmodial activity ($IC_{50}$ values of 56.3 ± 4.2 µg/ml) against chloroquine-sensitive (D6) strain of *Plasmodium falciparum*. Hautriwaic acid (**17**) showed moderate antiplasmodial activity ($IC_{50}$ 23.6 ± 2.6 µg/ml and 23.0 ± 2.3 µg/ml) against chloroquine-sensitive (D6) and resistant (W2) strains of *Plasmodium falciparum*, respectively. The crude extract of the two populations of *D. angustifolia* did not have good anti-plasmodial activity. However, their

activities were comparable. The anti-plasmodial activities of other compounds isolated from this plant were not determined.

### 3.5.1.3 ANTI-PLASMODIAL ACTIVITIES OF *SENECIO ROSEIFLORUS*

The acetone extract of the fresh leaves of *Senecio roseiflorus* showed no anti-plasmodial activity ($IC_{50}$ 90.0 ± 9.8 µg/ml) against chloroquine-sensitive (D6) strain of *Plasmodium falciparum*.

The anti-plasmodial activity of some of the compounds isolated from this plant are summarized in Table 3.29. The flavanone 5, 4′-dihydroxy-7-dimethoxyflavanone (**25**) is the most potent among the flavonoids tested. The other flavonoids showed moderate activity. The activity of all the compounds tested against the two strains of *Plasmodium falciparum* were comparable, with no significant differences. It would be of interest to screen a wide range of flavanones to ascertain their importance as lead structures for clinically useful products.

Table 3.29: In vitro activity ($IC_{50}$) from compounds of *Senecio roseiflorus* against D6 and W2 strains of *Plasmodium falciparum*.

| | $IC_{50}$ in µg/ml | |
|---|---|---|
| Tested compound | D6 | W2 |
| **Flavone** | | |
| 5,4′-Dihydroxy-3,6,7-trimethoxyflavone (**12**) | 18.2 ± 3.5 | 28.9 ± 1.0 |
| 5,7-Dihydroxy-3,4′-dimethoxyflavone (**21**) | 8.9 ± 1.7 | 8.5 ± 1.4 |
| 3′,5,7-Trihydroxy-3,4′-dimethoxyflavone (quercetin-3, 4′-Dimethyl Ether)(**22**) | 18.2 ± 3.5 | 28.9 ± 2.3 |
| 3,4′5-Trihydroxy-3′,7-Dimethoxyflavone (rhamnazin) (**23**) | 18.6 ± 7 | 12.1 ± 3.3 |
| 5-Hydroxy-3,7,3′,4′-tetramethoxyflavone (retusin) (**24**). | 10.7 ± 5.7 | 9.4 ± 3.7 |
| 5,4′-Dihydroxy-3,7,3′-trimethoxyflavone (**27**) | 10.9 ± 2.1 | - |
| **Flavanone** | | |
| 5, 4′-Dihydroxy-7-Dimethoxyflavanone (**25**) | 3.2 ± 0.8 | 4.4 ± 0.01 |
| Chloroquine | 0.43 ± 0.002 | 0.51 ± 0.004 |
| Quinine | 0.069 ± 0.001 | 0.073 ±0.002 |
| Mefloquine | 0.004 ± 0.001 | 0.002 ± 001 |

### 3.5.4 ANTI-MICROBIAL ACTIVITY

The tests were carried out on the acetone extracts of *D. angustifolia* from five different geographical locations for comparison and *Senecio roseiflorus*. Some of the pure compounds from *D. angustifolia* (Ngong Forest and Voi) and *S. roseiflorus* were also tested for activity.

Evaluation of anti-microbial activity of extracts and pure compounds was accomplished using the agar well-diffusion method [Bauer *et al*., 1966]. The extracts and pure compounds were tested for activity against three strains of bacteria; *Staphyloccocus aureus* (ATCC29737), *Escherichia coli* (ATCC25922) and *Bacillus pumilus* (local strain) and a local strain of fungus, *Saccharamyces cerevisiae*. The inhibition zones were measured in millimeter and the results obtained are presented in Table 3.34. All the extracts showed activity against the organisms tested. The anti-microbial activity of *D. angustifolia* extracts from the five different geographical locations were similar. All compounds except 3,4′,5-trihydroxy-3′,7-dimethoxyflavone (quercetin-3,4′-dimethyl ether) (**22**) were inactive against *Escherichia coli* which is a virulent strain of bacteria. The fact that compound **22** was active and 3, 4′,5-trihydroxy-7-dimethoxyflavone (**5**), with a similar substitution pattern except for the absence of a methoxyl substituent at the C-3′, was inactive implies that the presence of a methoxyl group at C-3′ in compound **22** appears to be important for activity against *Escherichia coli*. The methylated flavonoids **5, 8, 9, 10, 17, 22** and **25** were active against *Staphylococcus aureus*. Previous investigations have shown that such lipophilic flavonoids display anti-microbial activity. It was argued that this property was due to their ability to penetrate biological membranes [Harborne, 1983].

Although it is not possible to establish a general structure-activity relationship, some trends can be observed. For a good biological activity against *Staphylococcus aureus* a 3,5,4′ trihydroxy substitution as encountered in compounds **5** and **22** seems to be important for the flavones. It is not clear whether it is the presence of just three hydroxyl groups or the substitution pattern of three hydroxyl groups on the flavonoid skeleton that determines activity. The activity of the two flavanones 5,7-dihydroxyflavanone (pinocembrin) (**8**) and 5,4′-dihydroxy-7-dimethoxyflavanone (**25**) is almost identical. For activity against *Staphylococcus aureus* a minimum of three hydroxyl groups in flavones and may be two in flavanones seems to be necessary. The flavonoids, 5-hydroxy-3,7,4′-trimethoxyflavone (**1**)

5,7-dihydroxy-3,6,4′-trimethoxyflavone (santin) (**4**) and 5,4′-dihydroxy-3,6,7-trimethoxyflavone (penduletin) (**12**), with three methoxy groups were inactive even at 500 μg/ml against this strain of bacteria. While the phenolic groups may interact with biological structures through hydrogen bonding [McClure, 1975] a certain degree of lipophilicity is apparently required for the biological activity of the flavonoid.

A number of compounds **4, 5, 8, 9, 10, 12, 17, 22** and **25** were found to be active against *Bacillus pumilus*. 5,7-Dihydroxy-3,6,4′-trimethoxyflavone (santin) (**4**), 3,5,4′-trihydroxy-7-methoxyflavone (rhamnocitrin) (**5**) and 3,5,4′,-trihydroxy-3′,7-dimethoxyflavone (quercetin-3,4′-dimethyl ether) (**22**) were active against this local strain of bacteria, with inhibition zones of 9.89 mm at 250 μg/ml, 11.73 mm at 125 μg/ml and 8.97 mm at 31.25 μg/ml, respectively. As was observed with *Staphylococcus aureus* methoxy substitution at the C-3′ is important for activity against this bacteria. The two flavanones, 5,7-dihydroxyflavanone (pinocembrin) (**8**) and 5,4′-dihydroxy-7-dimethoxyflavanone (**25**) were active against this bacteria, but their structure activity relationships could not be established.

Compounds **4, 8, 9, 10, 12, 17, 22** and **25** and hautriwaic acid lactone were active against the local strain of fungus, *Saccharomyces cerevisiae*. Santin (**4**) was the most active flavone with an inhibition zone of 11.15 mm at 31.25 μg/ml while 5,4′-dihydroxy-3,6,7-trimethoxyflavone (penduletin) (**12**) was less active with an inhibition zone of 11.50 mm at 500 μg/ml. The presence of 5,7-dihydroxy substitution seems to be important for activity of flavones against *Saccharomyces cerevisiae* fungus. This is evident from the observation that **4** with a 5,7-dihydroxy substituents was more active than **2** with 5,4′-dihydroxy substituents. The flavones, 5-hydroxy-3,7,4′-trimethoxyflavone (**1**) and 5,4′-dihydroxy-3,7-dimethoxyflavone (kumatakenin) (**3**) were inactive even at 500 μg/ml. The flavanones, 5,7-dihydroxyflavanone (pinocembrin) (**8**) and 5,4`-dihydroxy-7-dimethoxyflavanone (**25**), were both active against *Saccharomyces cerevisiae*. 3,5,4′-Trihydroxy-7-methoxyflavone (rhamnocitrin) (**5**)with three hydroxyl groups had no activity while **22** had minimum activity (IZ of 10.80 mm at 500

μg/ml). The presence of two polar groups (hydroxyl), but not more, and a minimum of three methoxy groups, appears to be necessary for good activity while complete methylation except for C-5 removes activity as encountered in 5-hydroxy-3,7,4'-trimethoxyflavone (**1**). Compared to the anti-bacterial flavones, the anti-fungal compounds tend to be more lipophilic.

Hautriwaic acid lactone showed activity with an inhibition zone of 10.34 mm (31.25 μg/ml): almost as active as santin (**4**), the most active compound, probably due to its lipophilic nature. The other diterpenoids, 2β-hydroxyhardwickic acid (**9**), dodonic acid (**10**) and hautriwaic acid (**17**) showed low activity (IZ of 10.80, 10.10, 9.65 mm at 125 μg/ml, 62.5 and 500 μg/ml, respectively). The three diterpenoids **9**, **10**, **17** have the same carbon skeleton and one hydroxyl substituent each, but differ in the position of the hydroxyl group on the ring. The hydroxyl group is at C-2, C-6 and C-19 in compounds **9**, **10**, **17**, respectively. Consequently, the activity of these diterpenoids is determined by the position of the hydroxyl group. The order of activity depends on the position of hydroxyl group: C-6 (**10**) > C-2 (**9**)> C-19 (**17**).

Although substituents were found to be important in determining whether or not compounds were fungitoxic, an unambiguous relationship between structure and activity was not revealed. Thus for both flavonoids and diterpenoids, with different structural features, the anti-fungal activity may depend on some common physicochemical attribute, perhaps lipophilicity and ability to penetrate fungal membranes, rather than a common structure.

Table 3.34: Anti-microbial activity of the acetone extracts of *D. angustifolia* from different geographical locations, *Senecio roseiflorus* and some pure compounds

| **Sample** | **μg/disc** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|
| **Crude extracts** | | | | | |
| Surface exudate of *D. angustifolia* (leaves)-Ngong forest | 2500 | 18.86[a] | 20.05[a] | 19.42[a] | 10.79[a] |
| Surface exudate of *D. angustifolia* (leaves)-Voi | 2500 | 17.58 | 19.21 | 18.85 | 12.45 |
| Surface exudate of *D. angustifolia* (leaves)-Kilifi. | 2500 | 19.06 | 18.89 | 18.60 | 14.40 |

Table 3.34: Anti-microbial activity of the acetone extracts of *D. angustifolia* from different geographical locations, *Senecio roseiflorus* and some pure compounds

| **Sample** | **µg/disc** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|
| Surface exudate of *D. angustifolia* (leaves)-Garborone (Botswana) | 2500 | 16.33 | 18.94 | 16.25 | 12.18 |
| Surface exudate of *D. angustifolia* (leaves)-Madagascar | 2500 | 17.87 | 21.68 | 18.43 | 11.45 |
| Surface exudate of *Senecio roseiflorus* (leaves) | 2500 | 18.66 | 19.15 | 18.95 | 11.80 |
| **Compounds** | | | | | |
| 5-Hydroxy-3,7,4'-trimethoxyflavone (**1**) | 500 | - | - | - | - |
| 5,4`-Dihydroxy-3,7-dimethoxyflavone (Kumatakenin) (**3**) | 500 | - | - | - | - |
| 5,7-Dihydroxy-3,6,4'-trimethoxyflavone (Santin) (**4**) | 500 | - | - | 10.08 | 11.92 |
| | 250 | - | - | 9.89 | 11.89 |
| | 125 | - | - | - | 11.60 |
| | 62.50 | - | - | - | 11.22 |
| | 31.25 | - | - | - | 11.15 |
| 3,5,4`-Trihydroxy-7-methoxyflavone (Rhamnocitrin) (**5**) | 500 | - | 11.95 | 12.68 | - |
| | 250 | - | 11.68 | 11.83 | - |
| | 125 | - | 11.40 | 11.73 | - |
| | 62.50 | - | 10.80[a] | - | - |
| | 31.25 | - | - | - | - |
| 5,7-Dihydroxyflavanone (Pinocembrin) (**8**) | 500 | - | 13.88[a] | 13.42[a] | 14.72[a] |
| | 250 | - | 12.90 | 13.12 | 14.66 |
| | 125 | - | 11.53 | 11.49 | 14.08 |
| | 62.50 | - | 9.49 | 9.87 | 11.92 |
| | 31.25 | - | - | - | - |
| 2β-hydroxyhardwickic acid (**9**) | 500 | - | 11.83 | 11.71 | 10.95 |
| | 250 | - | 10.17 | 10.47 | 10.82 |
| | 125 | - | - | - | 10.80 |
| Dodonic acid (**10**) | 500 | - | 13.74 | 12.74 | 10.94 |
| | 250 | - | 11.13 | 10.71 | 10.90 |
| | 125 | - | - | - | 10.40 |
| | 62.50 | - | - | - | 10.10 |
| 5,4`-Dihydroxy-3,6,7-trimethoxyflavone (penduletin) (**12**) | 500 | - | - | 9.38 | 11.50 |
| Hautriwaic acid (**17**) | 500 | - | 11.77 | 12.27 | 9.65 |
| | 250 | - | 9.93 | 11.07 | - |
| | 125 | - | - | 9.48 | - |
| Hautriwaic acid lactone | 500 | - | - | - | 10.90 |
| | 250 | - | - | - | 10.89 |
| | 125 | - | - | - | 10.84 |
| | 62.50 | - | - | - | 10.63 |
| | 31.25 | - | - | - | 10.34 |
| 3,5,4'-Trihydroxy-3',7-dimethoxyflavone (quercetin-3, 4`-dimethyl ether) (**22**) | 500 | 12.76 | 11.72 | 10.57 | 10.8 |
| | 250 | 12.47 | 9.68 | 9.72 | - |
| | 125 | 11.97 | 9.58 | 9.42 | - |

Table 3.34: Anti-microbial activity of the acetone extracts of *D. angustifolia* from different geographical locations, *Senecio roseiflorus* and some pure compounds

| Sample | µg/disc | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| | 62.50 | 10.66 | 9.37 | 9.20 | - |
| | 31.25 | 9.08 | - | 8.97 | - |
| 5,4'-Dihydroxy-7-methoxyflavanone (**25**) | 500 | - | 13.20 | 10.84 | 12.44 |
| | 250 | - | 12.12 | 10.53 | 12.21 |
| | 125 | - | 12.04 | 10.18 | 12.04 |
| | 62.50 | - | 11.32 | - | 11.48 |
| | 31.25 | - | - | - | 10.59 |
| Gentamicin | 30 | 12.39 | 24.74 | 30.34 | - |
| Nystatin | 25 | - | - | - | 25.6 |

*Microorganisms*: 1=*Escherichia coli* (ATCC 25922), 2=*Staphylococcus aureus* (ATCC 29737). 3=*Bacillus pumilus* (local strain), 4=*Saccharomyces cerevisiae* (local strain).
"-" Not active.
[a] Inhibition zone in mm.

### 3.5.2 LARVICIDAL ACTIVITY

#### 3.5.2.1 LARVICIDAL ACTIVITY OF *D. ANGUSTIFOLIA*-NGONG FOREST AND ITS COMPOUNDS

The acetone extract of the fresh leaves of *Dodonaea angustifolia*-Ngong Forest and some of its compounds were tested against the larvae of *Aedes aegypti*. The objective of this study is to identify botanical insecticides for the control of disease vector insects. Rotenone was usd as the standard insecticide.

The extracts, compounds 5-hydroxy-3,7,4'-trimethoxyflavone (**3**) and 5,7-dihydroxyflavanone (**8**) did not show significant activities even at 20 µg/ml, ($LC_{50}$ > 60 µg/ml after 24 hours) (Figure 3.21).

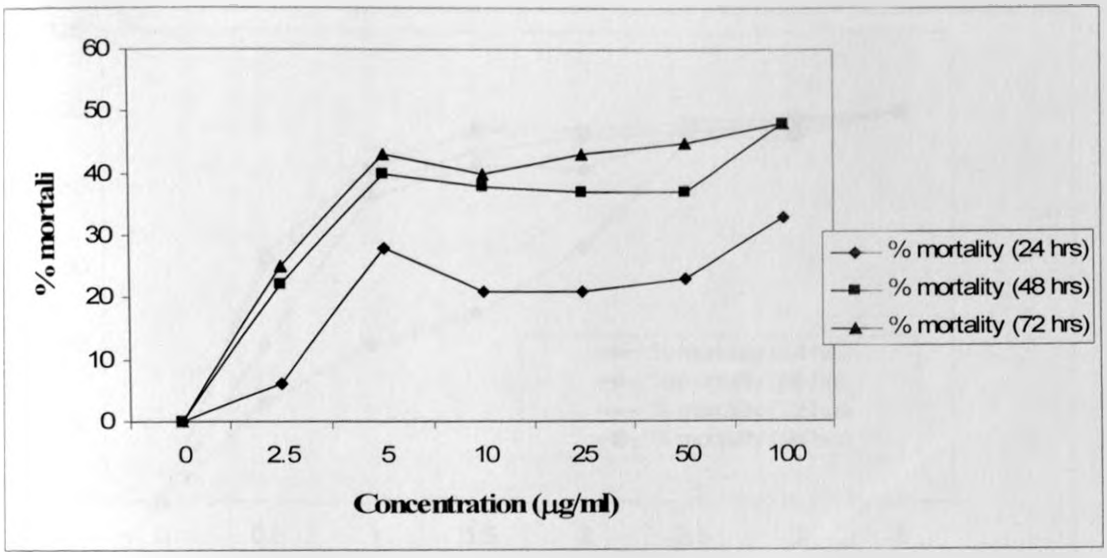



Figure 3.21: Larvicidal activity of the extracts of *Dodonaea angustifolia*-Ngong Forest on the second instar *Aedes aegypti* larvae

The results of the tests are summarized in Table 3.30. 3,5,4'-Trihydroxy-7-methoxyflavone (**5**) and 5,7 dihydroxy-3,6,4'-trimethoxyflavone (**4**) showed good and dose dependent activity ($LC_{50}$ 1.75 µg/ml and 5.1 µg/ml respectively, after 24 hours) (Table 3.30). 3,5,4'-Trihydroxy-7-methoxyflavone (**5**) which was the most active, caused 100% mortality at 6.5 µg /ml (Figure 3.22) after 24 hours.

Table 3.30: Larvicidal activity ($LC_{50}$) of compounds from *Dodonaea angustifolia*-Ngong Forest on second instar *Aedes aegypti* larvae

| **Compound** | **$LC_{50}$ in µg/ml** |
|---|---|
| **Flavonoids** | |
| 5-Hydroxy- 3,7,4'-trimethoxyflavone (**1**) | 75.0 |
| 3,5-Dihydroxy-7,4'-dimethoxyflavone (**2**) | 20.0 |
| 5,4'-Dihydroxy-3,7-dimethoxyflavone (**3**) | 17.5 |
| 5,7- Dihydroxy-3,6,4'-trimethoxyflavone (Santin) (**4**) | 5.1 |
| 3,5,4'-Trihydroxy-7-methoxyflavone (**5**) | 1.75 |
| 5,7-Dihydroxyflavanone (Pinocembrin) (**8**) | >100 |
| **Diterpenoid** | |
| Dodonic acid (**10**) | >100 |
| **Rotenone** | 0.68 |

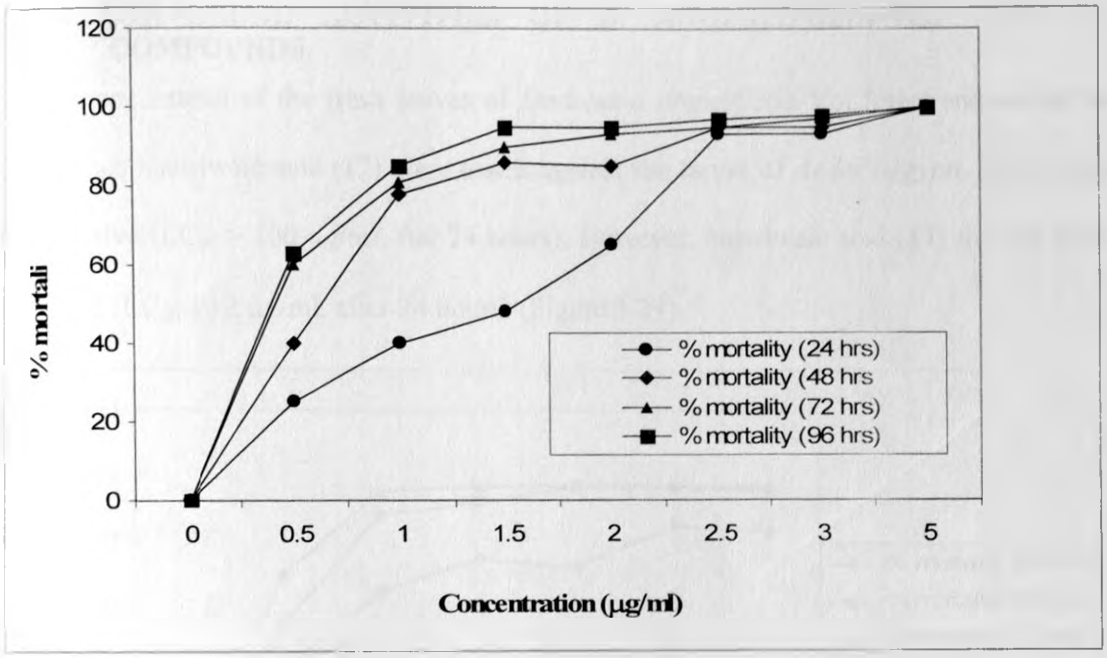



Figure 3.22: Larvicidal activity of 3,5,4'-trihydroxy-7-methoxyflavone (**5**) on the second instar *Aedes aegypti* larvae

3,5-Dihydroxy-7,4'-dimethoxyflavone (**2**) was inactive ($LC_{50}$ 20 µg/ml, after 24 hours). All the active compounds were less potent than rotenone ($LC_{50}$ 0.68 µg/ml, after 24 hours). The larvicidal activity of the rude extract of *D. angustifolia* (Ngong Forest) and some compounds after 24 hours is summarized in figure 3.23.

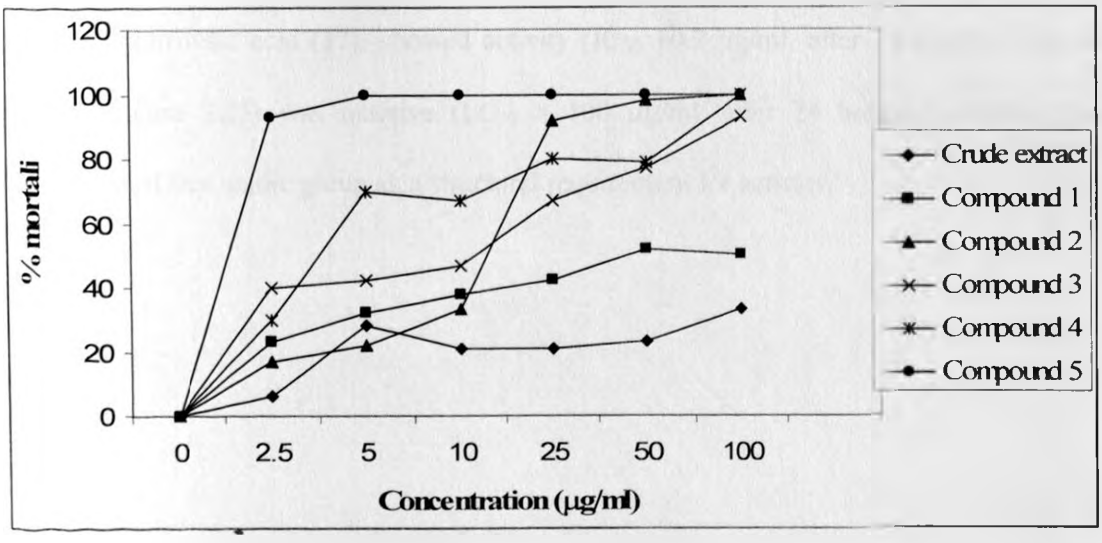



Figure 3.23: Larvicidal activity of the extracts of *D. angustifolia* (Ngong Forest) and some of its compounds after 24 hours

### 3.5.2.2 LARVICIDAL ACTIVITIES OF *D. ANGUSTIFOLIA*-VOI AND ITS COMPOUNDS.

The acetone extract of the fresh leaves of *Dodonaea angustifolia*-Voi forest and one of its compounds hautriwaic acid (**17**) were tested against the larvae of *Aedes aegypti*. The extract was inactive ($LC_{50}$ > 100 µg/ml, fter 24 hours). However, hautriwaic acid (**17**) showed good larvicidal ($LC_{50}$ 10.2 µg/ml, after 24 hours) (Figure 3.24).

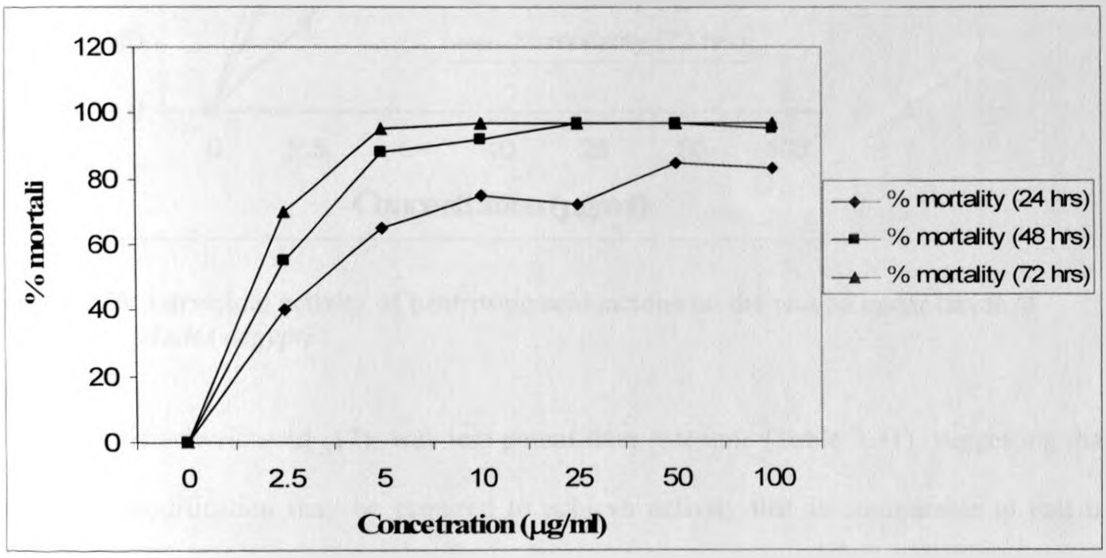



Figure3.24: Larvicidal activity of hautriwaic acid on the second instar *Aedes aegypti* larvae

The activity of hautriwaic acid (**17**) was compared with that of its lactone. It is interesting to note that hautriwaic acid (**17**), showed activity ($IC_{50}$ 10.2 µg/ml, after 24 hours) while its lactone (Figure 3.25) was inactive ($LC_{50}$ > 100 µg/ml, after 24 hours), indicating the importance of free acidic group as a structural requirement for activity.

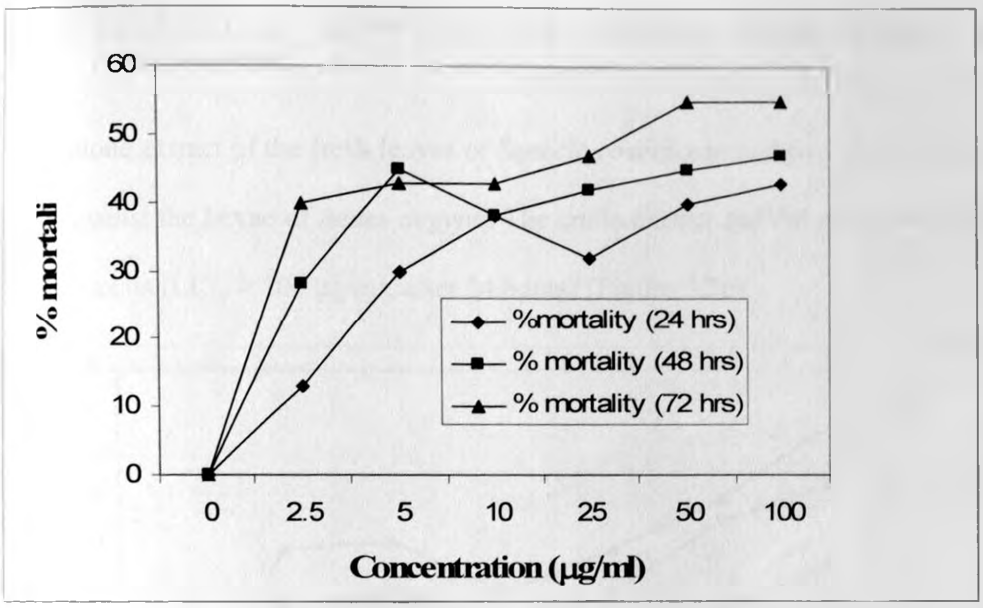



Figure 3.25: Larvicidal activity of hautriwaic acid lactone on the second instar larvae of *Aedes aegypti*

However, hautriwaic acid (**17**), was less potent than rotenone (Table 3.31), suggesting that structural modification may be required to achieve activity that is comparable to that of rotenone.

Table 3.31: Mosquito larvicidal activities ($LC_{50}$) against *Aedes aegypti* of the hautriwaic acid and its lactone

| Tested compound | $LC_{50}$ in µg/ml |
|---|---|
| **Diterpenoids** | |
| Hautriwaic acid (**17**) | 10.2 |
| Hautriwaic acid lactone | > 100 |
| **Rotenone** | 0.68 |

### 3.5.2.3 LARVICIDAL ACTIVITIES OF *SENECIO ROSEIFLORUS* AND ITS COMPOUNDS.

The acetone extract of the fresh leaves of *Senecio roseiflorus* and two of its compounds were tested against the larvae of *Aedes aegypti*. The crude extract and did not show good larvicidal activity, as its ($LC_{50}$ > 100 µg/ml, after 24 hours) (Figure 3.26).

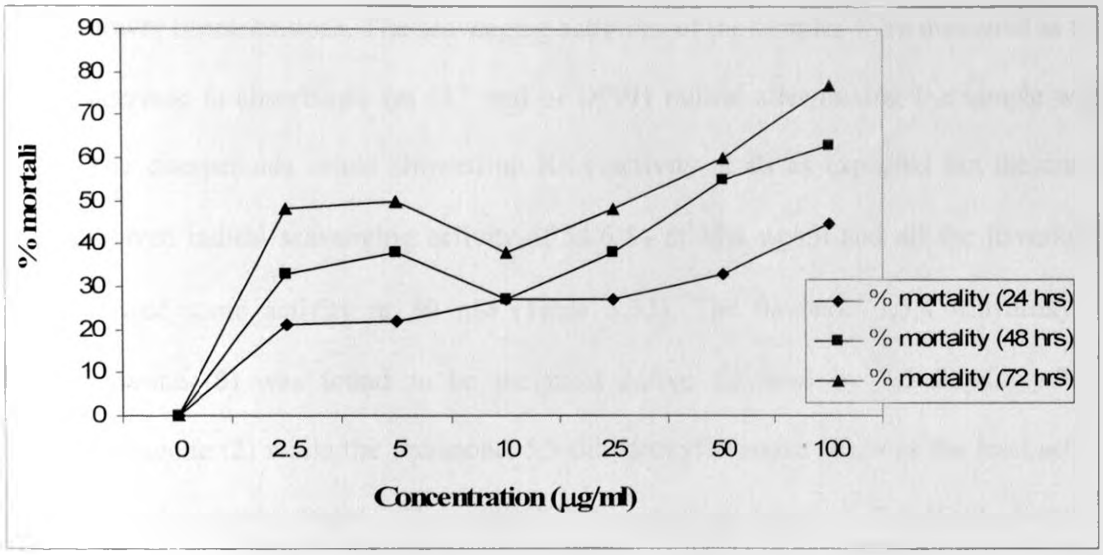



Figure 3.26: Larvicidal activity of extracts of *Senecio roseiflorus* on the second *Aedes aegypti* instar larvae

Compounds 5,4'-dihydroxy-7-methoxyflavanone (**25**) and 5,7-dihydroxy-3,4'-dimethoxyflavone (**21**) showed moderate and dose dependent activity ($LC_{50}$ 14.3 µg/ml and 15.5 µg/ml after 24 hours), respectively. The isolated compounds seem to have better activity than the crude extracts in all the three plant species. Consequently, there is need to test all the pure compounds isolated from this plant for their activity to establish their use to control malaria vectors.

### 3.5.3 ANTI-OXIDANT ACTIVITIES

### 3.5.3.1 ANTI-OXIDANT ACTIVITIES OF *DODONAEA ANGUSTIFOLIA*-NGONG FOREST.

Preliminary radical scavenging activities, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as a spray reagent on TLC plates, of the acetone extract of fresh leaves of *D. angustifolia*-Ngong Forest indicated that this extract contains compounds with radical

scavenging activities. Some of the compounds isolated from this plant were tested for activity. Radical scavenging activities were observed in the extract and some compounds (Table 3.32). Using spectrophotometric method, the radical scavenging activity of the acetone extract of *D. angustifolia* was tested at 11.4 μg/ml while the pure compound were tested at 50 μM. The compounds that had comparable activity to quercetin at that concentration were tested at lower concentrations. The scavenging activities of the samples were measured as the percent decrease in absorbance (at 517 nm) of DPPH radical after mixing the sample with DPPH. The diterpenoids tested showed no RSA activity at all as expected but the crude extract showed radical scavenging activity of 54.6 % at 11.4 μg/ml and all the flavonoids tested showed some activity at 50 μM (Table 3.32). The flavonol, 3,5,4′-trihydroxy-7-methoxyflavone (**5**) was found to be the most active followed by 3,5-dihydroxy-7,4′-dimethoxyflavone (**2**) while the flavanone, 5,7-dihydroxyflavanone (**8**), was the least active of all the flavonoids tested. The radical scavenging activity of 3,5,4′-trihydroxy-7-methoxyflavone (**5**) was ascertained at lower concentrations and found to be lower than to that of quercetin (Figure 3.27).

Table 3.32: Radical scavenging activities of flavonoids of *D. angustifolia*-Ngong forest

| **COMPOUND** | **TLC ASSAY RESULTS** | **% RSA (50 μM)** |
|---|---|---|
| Quercetin | + | 96.7 |
| **Flavonols** | | |
| 3,5-Dihydroxy-7,4′-dimethoxyflavone (**2**) | + | 25.4 |
| 3,5,4′-Trihydroxy-7-methoxyflavone (**5**) | + | 96.2 |
| **3-methoxyflavones** | | |
| 5,4′--Dihydroxy-3,7-dimethoxyflavone(Kumatakenin) (**3**) | + | 18.4 |
| 5,7- Dihydroxy-3,6,4′-trimethoxyflavone (Santin) (**4**) | - | 6.67 |
| 5,7,4′-Dihydroxy-3-methoxyflavone (**6**) | + | 11.2 |
| **Flavanone** | | |
| 5,7-Dihydroxyflavanone (**8**) | - | 2.31 |

### 3.5.3.2 ANTI-OXIDANT ACTIVITIES OF *DODONAEA ANGUSTIFOLIA*-VOI

Some of the compounds isolated from *Dodonaea angustifolia*-Voi were tested for radical scavenging activity. The diterpenoid tested showed no RSA activity while the flavonoids tested were active at 50 μM. The flavonol, kaempferol (**15**) was found to be the most active with RSA of 96.8% at 50 μM. 5,4'-Dihydroxy-3,6,7-trimethoxyflavone (12) had RSA of only 2.55% at 50 μM. The activity of kaempferol (**15**) is comparable to that of quercetin at 50 μM. However, at lower concentrations the activity of quercetin is higher than that of kaempferol (**15**). The activity of 3,5,4'-trihydroxy-7-methoxy flavone (5) and kaempferol (**15**) are comparable at all concentrations. Flavonols had appreciably good anti-oxidant activity compared to the 3-methoxyflavones, indicating the importance of the the hydroxyl group at C-3.

### 3.5.3.3 ANTI-OXIDANT ACTIVITIES OF *SENECIO ROSEIFLORUS*.

The acetone extract and two compounds isolated from *Senecio roseiflorus* were tested for radical scavenging activity. The extract had minimal activity with 9.25 % RSA at 11.4 μg/ml, which is higher than that of 5, 4'-dihydroxy-7-dimethoxyflavanone (25) (Table 3.33). The highest activity was observed in quercetin-3, 4'-dimethyl ether (**22**). However, the activity of quercetin was higher than that of its derivative (Table 3.33).

Table 3.33: Radical scavenging activities of quercetin and flavonoids of *S. roseiflorus*.

| **COMPOUND** | **TLC ASSAY RESULTS** | **% RSA (50 μM)** |
|---|---|---|
| Quercetin | + | 96.7 |
| **3-methoxyflavone** | | |
| Quercetin-3,4'-dimethyl ether (**22**) | + | 77.1 |
| **Flavanone** | | |
| 5, 4'-Dihydroxy-7-methoxyflavanone (**25**) | - | 1.22 |

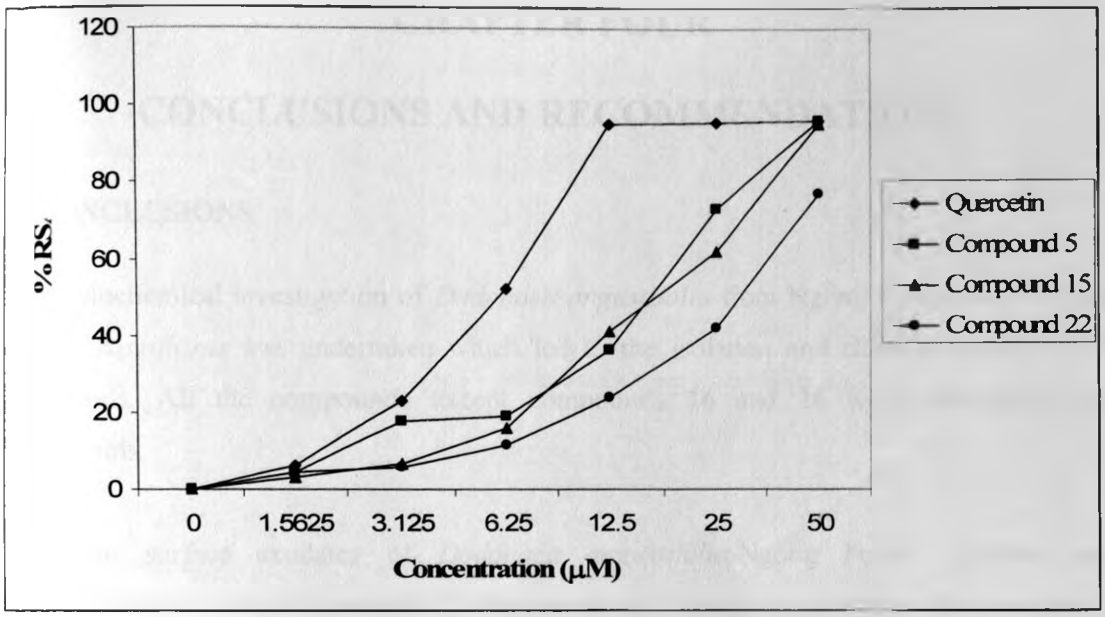



Figure 3.27: Radical scavenging activity of the standard (quercetin), rhamnocitrin (**5**), kaempferol (**15**) and quercetin-3, 4'-dimethyl ether (**22**).

# CHAPTER FOUR

# CONCLUSIONS AND RECOMMENDATIONS

## 4.1 CONCLUSIONS

The phytochemical investigation of *Dodonaea angustifolia* from Ngong Forest and Voi and *Senecio roseiflorus* was undertaken which led to the isolation and characterization of 27 compounds. All the compounds except compounds **16** and **26** were flavonoids and diterpenoids.

From the surface exudates of *Dodonaea angustifolia*-Ngong Forest isolation and characterization of eight flavonoids (7 flavones and 1 flavanone) and three diterpenoids (2 *ent*-clerodane and one *ent*-labdane type) was achieved.

The phytochemical study on the surface exudates of *Dodonaea angustifolia*-Voi led to the isolation and characterization of a total of 8 flavonoids, a coumarin and four diterpenoids (all *ent*-clerodane). Three of these compounds *neo*-clerodan-3,13-dien-16,15: 18,19-diolide (**18**), 15β-*neo*-clerodan-3,13-dien-16,15: 18,19-diolide (**19**) and 15α-*neo* clerodan-3,13-dien-16,15: 18,19-diolide (**20**) are new.

Additional chemotaxonomic information that could help solve the taxonomical controversy between *Dodonaea angustifolia*-Ngong Forest and *Dodonaea angustifolia*-Voi, which are morphologically similar, was achieved. The two populations of *D. angustifolia* are of different chemotypes and may not be different species.

The surface exudates of *Senecio roseiflorus* led to the isolation and characterization of a total of 10 flavonoids (seven flavones and one flavanone) and one benzene derivative. This is the first phytochemical report of this plant.

The presence of a chelated hydroxyl group at C-5 position was identified as the main structural feature of all flavonoids isolated from the surface exudates of *Dodonaea angustifolia* and *Senecio roseiflorus*. The flavonoids from these plants are mainly kaempferol and quercetin methyl ether.

The anti-plasmodial activity of the crude extracts, some of the flavonoids and diterpenoids of these plants were tested. The results showed that among the flavonoids tested the flavones had moderate activity and the highest anti-plasmodial activity was exhibited by flavanone (**25**), while the diterpenoids had the least activity. Earlier studies (Andayi, 2005) have shown that flavanones have good anti-plasmodial activity against both chloroquine sensitive (D6) and chloroquine resistant strain (W2). This group of compounds can be potential candidates for use as lead compounds in developing drugs to combat chloroquine resistant malaria.

The surface exudates of the *D. angustifolia*-Ngong Forest and *Senecio roseiflorus* screened for anti-oxidant activity at 11.4 μg/ml showed 54.6 and 9.25% RSA respectively. The two plant species elaborate surface exudates consisting mainly of terpenoids and flavonoids. The RSA of the surface exudate is due to the flavonoids. The difference in RSA of the two plant species could be in the qualitative and quantitative composition of the exudate. The surface exudates of *D. angustifolia* could be richer in flavonoids that have higher radical scavenging activity, in this case the flavonol, as compared to *Senecio roseiforus*. The pure compounds from the two plant species were also tested for anti-oxidant activities and some of the flavonoids showed RSA activity. The potential use of the surface exudates and flavonoids from the surface exudates of the two plants species as radical scavenger was established. The structure-activity relationship of the active flavonoids showed that flavonols had appreciable activity as compared to 3-methoxyflavones isolated from the surface exudates.

The crude extracts of the plant extracts and some pure compounds were tested for larvicidal activity against *Aedes aegypti*. The results indicated that flavonoids **5**, **4**, **25**, **21** showed good and dose dependent activity after 24 hours. Compound **5**, being the most active, caused 100% mortality at 6.5 μg /ml after 24 hours. The diterpenoid, hautriwaic acid (**17**) showed good larvicidal after 24 hours. The activity of this compound (**17**) was compared with that of its lactone. It is interesting to note that hautriwaic acid (**17**), showed activity while its lactone was inactive ($LC_{50}$ > 100 μg/ml, after 24 hours), indicating the importance of free acidic group as a structural requirement for activity. These compounds could have potential use for small scale control of mosquitoes in rural communities in East Africa where mosquito transmitted diseases such as malaria is endemic. The isolated compounds seem to have better activity than the crude extracts in all the three plant species. Consequently, there is need to test all the pure compounds isolated from this plant for their activity to establish their use to control malaria vectors.

The surface exudates of fresh leaves of *Dodonaea angustifolia*-Ngong forest, *Dodonaea angustifolia*-Voi and *Senecio roseiflorus* showed anti-bacterial activity against three strains of bacteria *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 25922) and *Bacillus pimilus* (local strain) but minimum anti-fungal activity against one local strain of fungus *Saccharamyces cerevisiae*. Compounds **1, 3, 4, 5, 8, 9, 10, 12, 17, 22** and **25** isolated from this plant were tested and only **4, 8, 9, 10, 22** and **25** were found to be active against at least one bacteria and fungi.

## 4.2 RECOMMENDATIONS.

HPLC (High Perfomance Liquid Chromatography) profiles of *Dodonaea angustifolia*-Ngong Forest, *Dodonaea angustifolia*-Voi and *Dodonaea angustifolia* from different geographical locations within Kenya should be compared in order to unequivocally determine their taxonomic relationships.

The oil from the surface exudates of the plants should be studied using HPLC to isolate all the constituent compounds. The internal tissue compounds of the plants should also be investigated.

The *in vitro* activity of all the flavonoids isolated should be tested against the two strains of *P. falciparum* and then other strains as well. The *in vivo* activity of the flavonoids isolated should be tested against the two strains of *P. falciparum*. The bioactivy of the new compounds against different bacteria strains, the two strains of *Plasmodium falciparum* (D6 and W2) and larvicidal activity against the 2$^{nd}$ instar stage of *Aedes aegypti* should be done.

Comprehensive structure-activity relationship studies should be carried out on the active compounds to determine the properties responsible for anti-plasmodial, mosquito larvicidal and anti-microbial activities.

Methylation of 5-OH of the isolated flavonoids should be done and the 5-methyl ethers flavonoids subjected to anti-fungal activities to establish their activities because previous investigations have shown that methylation of 5-OH is the structural feature essential for good anti-fungal activity [Tomas-Barberan *et al.*, 1988]. The anti-viral activity of different 3-methoxyl flavonoids should be established, as these compounds have been reported to exhibit anti-viral activity [Van Hoof *et al.*, 1984].

Structural modifications of the isolated compounds should be carried out and screened for bioactivity in order to determine the functional groups that are necessary for activity.

DNA or genetical profiling of *Dodonaea* species for taxonomic identification should be included, besides essential oil (GC) and polar compound profiles (HPLC).

# CHAPTER FIVE

# EXPERIMENTAL

## 5.0 GENERAL

The NMR spectra were recorded on a Varian-Mercury 200MHz and Brucker 300, 500 and 600 MHz instruments. Chemical shifts were measured in ppm in δ values relative to the internal standard tetramethylsilane (TMS). COSY, NOESY, DEPT, APT, HMQC and HMBC spectra were acquired using the standard Bruker software. EIMS spectra were recorded on 70 eV SSq 710 Finnigan MAT spectrometer. UV values were obtained using SP8 150 UV/VIS spectrophotometer. Melting points were recorded using a Gallenkamp melting point apparatus with capillary tubes.

Column chromatography was carried out using silica gel 40 (Merck, 70-230 mesh) and Sephadex LH 20. Analytical thin layer chromatography and preparative thin layer chromatography (PTLC) were done using Merck pre-coated 60 $F_{254}$ and Merck 60 $PF_{254}$.

## 5.1 PLANT MATERIALS

### 5.1.1 *DODONAEA ANGUSTIFOLIA (NGONG FOREST)*

The fresh leaves of *Dodonaea angustifolia* were collected from Ngong Forest, on 20th November 2001. The plant was identified by Mr. S.G. Mathenge of the University of Nairobi Herbarium, School of Biological Sciences (SBS), where a voucher specimen is kept.

### 5.1.2 *DODONAEA ANGUSTIFOLIA (VOI)*

The fresh leaves of *Dodonaea angustifolia* were collected from Voi on 30th August 2002. It was identified by Mr. S. G. Mathenge of the School of Biological Sciences (SBS), Herbarium, University of Nairobi, where a voucher specimen is deposited.

### 5.1.3 *SENECIO ROSEIFLORUS*

The fresh leaves of *Senecio roseiflorus* were collected from Mt. Kenya Forest, Meru, at about 1300-1500ft, on 30th August 2002 with the assistance of Mr. S.G. Mathenge of the School of

Biological Sciences (SBS), Herbarium, University of Nairobi, where the specimen is deposited.

## 5.2 EXTRACTION AND ISOLATION OF COMPOUNDS

### 5.2.1 *DODONAE ANGUSTIFOLIA (NGONG FOREST)*

#### 5.2.1.1 EXTRACTION AND ISOLATION OF COMPOUNDS FROM FRESH LEAVES (AERIAL PARTS)

The leaves of this plant were shinny and gummy indicating that they were coated with resin. Extraction of the surface exudates on the aerial parts (mainly leaves) was done after plucking out the flowers. The surface exudates of the leaves, 450 g, was washed out into solvent by successive dipping into fresh portions of acetone for short periods (< 15 seconds) to yield 52 g of extract, thus avoiding the extraction of the internal tissue components.

A portion of the extract (7 g) was kept aside for bioassays. The rest of the extract, 45 g, was adsorbed on silica gel (45 g) and subjected to column chromatography silica gel (450 g) under $CH_2Cl_2$-*n*-$C_6H_{14}$ in the ratio of 1:1. Separation was carried out by stepwise gradient elution with mixtures of $CH_2Cl_2$-*n*-$C_6H_{14}$ and MeOH/$CH_2Cl_2$ in increasing polarities. Yellow armophous solids of 3,5-dihydroxy-7,4′-dimethoxyflavone (**2**) (30 mg) [Drayer, 1978] precipitated out of the fraction eluted in 50% $CH_2Cl_2$-*n*-$C_6H_{14}$. The fraction eluted in 60 % $CH_2Cl_2$-*n*-$C_6H_{14}$ afforded yellow needles of 5-hydroxy-3, 7,4′-trimethoxyflavone **(1)** (204 mg) [Dreyer, 1978]. 5,7-dihydro-3,6,4′-trimethoxyflavone (santin) (**4**) (350 mg) [Sachdev & Kulshreshtha, 1982; Wollenweber *et al.*, 1986; Abdel-Mogib *et al.*, 2001] precipitated out of the fraction eluted with 90% $CH_2Cl_2$-*n*-$C_6H_{14}$ and neat $CH_2Cl_2$. Purification of the mother liquor of the fraction eluted in 90% $CH_2Cl_2$-*n*-$C_6H_{14}$ and neat $CH_2Cl_2$ using PTLC ($SiO_2$, $CH_2Cl_2$ multiple development) afforded *ent*-3β, 8α-15,16-epoxy-13(16), 14-labdadiene-3, 8-diol (**11**) (10 mg) [Dawson *et al.*, 1966]. The fraction eluting in 1 % MeOH-$CH_2Cl_2$ after column chromatography on sephadex LH 20 (MeOH-$CH_2Cl_2$ (1:1)) gave dodonic acid (**10**) (500 mg) [Van Heerden *et al.*, 2000; Sachdev & Kulshreshtha, 1984], while that eluting in 2 % MeOH-$CH_2Cl_2$ after column chromatography on sephadex LH 20 (MeOH-$CH_2Cl_2$ (1:1))

gave 5.7-dihydroxyflavanone (pinocembrin) **(8)** (120 mg) [Sachdev & Kulshreshtha, 1983]. The fraction eluted in 3 % MeOH-$CH_2Cl_2$ afforded 5,4'-dihydroxy-3,7-dimethoxy flavone (kumatakenin) **(3)** (200 mg) [Vieira *et al.*, 1997; Sarmento *et al.*, 2002] and 3,5,4'-trihydroxy-7-methoxyflavone (rhamnocitrin) **(7)** (184 mg) [Valant-Vetschera *et al.*, 2003; Akkal *et al.*, 1997; Hattori *et al.*, 1992]. The fraction eluted in 4% MeOH-$CH_2Cl_2$ after purification with column chromatography on sephadex LH 20 (MeOH-$CH_2Cl_2$ (1:1)) afforded 2β-hydroxyhardwickiic acid **(9)** (778 mg) [Jefferies *et al.*, 1973; Anis *et al.*, 2001], 3,5,4'-trihydroxy-7-methoxyflavone (rhamnocitrin) **(5)** (60 mg) [Wollenweber *et al.*, 1986] and 5,7,4'-trihydroxy-3-methoxyflavone (isokaempferide) **(6)** (40 mg) [Dreyer, 1978]. The fraction eluted in 5% MeOH/$CH_2Cl_2$ yieded 3,5,7,4'-tetrahydroxy-6-methoxyflavone (7, 60 mg) [Valant-Vetschera *et al.*, 2003; Akkal *et al.*, 1997; Hattori *et al.*, 1992].

### 5.2.1.2 PHYSICAL AND SPECTROSCOPIC PROPERTIESOF COMPOUNDS OF *D. ANGUSTIFOLIA* FROM NGONG FOREST.

***5-Hydroxy-3,7,4'-trimethoxyflavone (1)***

Yellow crystals ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 145-147 °C lit. mp 145-147 °C [DNP]; $R_f$ 0.3 [20% n-$C_6H_{12}$ $CH_2Cl_2$]: molecular formular $C_{18}H_{16}O_6$; % yield 0.45; UV: $\lambda_{max}$ (MeOH) 268.5 and 346.5 nm; $^1H$ (see Table 3.2); $^{13}C$-NMR (see Table 3.2); EIMS *m/z* (rel. int.): 328 $[M]^+$ (49), 327 $[M-H]^+$ (47), 285 $[M-43]^+$ (26), 167 (14).

***3,5-Dihydroxy-7,4',-dimethoxyflavone (2)***

Yellow crystals ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 180-181 °C lit. mp 178-180 °C [DNP]; $R_f$ 0.4 [20% n-$C_6H_{12}$ $CH_2Cl_2$]; molecular formular $C_{17}H_{14}O_6$; % yield 0.07; UV: $\lambda_{max}$ (MeOH) 269 and 348 nm;$^1H$ (Table 3.3); $^{13}C$-NMR (see Table 3.3); EIMS *m/z* (rel. int.): 314 $[M]^+$ (18.6), 167(12.9).

***5,4'-Dihydroxy-3,7-dimethoxyflavone (kumatakenin) (3)***

Yellow crystals ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 253-255 °C lit. mp 253-254 °C [DNP]; $R_f$ 0.5 [2% MeOH - $CH_2Cl_2$]; molecular formular $C_{17}H_{14}O_6$; % yield 0.44; UV: $\lambda_{max}$ (MeOH) 268.5 and 346.5 nm; $^1H$ (Table 3.4); $^{13}C$-NMR (see Table 3.4); EIMS *m/z* (rel. int.): 314 $[M]^+$ (35), 271 $[M-OMe]^+$ (25), 167 (10).

***5,7-Dihydroxy-3,6,4'-trimethoxyflavone (santin) (4).***

Yellow needle like crystals ($CH_2Cl_2$-*n*-$C_6H_{14}$), 159-161 °C lit. mp 159-161 °C [DNP]; $R_f$ 0.4 [2% MeOH - $CH_2Cl_2$]; molecular formular $C_{18}H_{16}O_7$; % yield 0.78; UV: $\lambda_{max}$ (MeOH) 272.0 and 337.0 nm; $^1H$ (Table 3.5); $^{13}C$-NMR (see Table 3.5).

*3,5,4'-Trihydroxy-7-methoxyflavone (rhamnocitrin) (5)*

Yellow crystals (MeOH-$CH_2Cl_2$), mp 221-222 °C lit. mp 221-223°C [DNP]; $R_f$ 0.4 [2% MeOH - $CH_2Cl_2$]; molecular formular $C_{16}H_{12}O_6$; % yield 0.13; UV $\lambda_{max}$ (MeOH) 266.0 and 364.0 nm; $^1H$ (Table 3.6); $^{13}C$-NMR (Table 3.6); EIMS *m/z* (rel. int.): 300 $[M]^+$ (10), 167 (4).

*5,7,4',-Trihydroxy-3-methoxy flavone (isokaempferide) (6).*

Yellow crystals (MeOH-$CH_2Cl_2$), mp >300 °C lit. mp 299-302 °C [DNP]; $R_f$ 0.3 [2% MeOH in $CH_2Cl_2$]; molecular formular $C_{16}H_{12}O_6$; % yield 0.09; $^1H$ (Table 3.7); $^{13}C$-NMR (Table 3.7); EIMS *m/z* (rel. int.): 300 $[M]^+$ (100), 257 $[M - OMe]^+$ (12.3), 150 (31.1).

*3,5,7,4'-tetrahydroxy-6-methoxyflavone (6-methoxykaempferol) (7).*

Yellow armophous solid (MeOH-$CH_2Cl_2$), 265-266 °C lit. mp 268-270 °C [DNP]; $R_f$ 0.5 [4% MeOH in $CH_2Cl_2$]; molecular formular $C_{16}H_{12}O_7$; % yield 0.41; $^1H$ (Table 3.8); $^{13}C$-NMR (Table 3.8); EIMS *m/z* (rel. int.): 316 $[M]^+$ (87.0), 301 $[M - Me]^+$ (14.0).

*5,7-Dihydroxyflavanone (pinocembrin) (8).*

White crystals ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 192-193 °C lit. mp 192-193 °C [DNP]; $R_f$ 0.5 [1% MeOH in $CH_2Cl_2$]; molecular formular $C_{17}H_{12}O_4$; % yield 0.27; UV $\lambda_{max}$ (MeOH) 289 nm; $^1H$ (Table 3.9); $^{13}C$-NMR (Table 3.9).

*2β-Hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic acid. 2β-hydroxyhardwickiic acid (9).*

White needles ($CH_2Cl_2$-*n*-$C_6H_{14}$). mp**; $R_f$ 0.4 [3% MeOH in $CH_2Cl_2$]; molecular formular $C_{20}H_{28}O_4$; % yield 1.73; $^1H$ (Table 3.10); $^{13}C$-NMR (Table 3.10); EIMS *m/z* (rel. int.): 332 $[M]^+$ (7), 95 $[C_6H_7O]^+$ (15), 81 $[C_5H_5O]^+$ (23), 54 (41), 46 (100).

*15,16-epoxy-6-hydroxy-3,13(16),14-clerodatrien-18-oic acid. (Dodonic acid) (10)*

Colourless crystals (MeOH-$CH_2Cl_2$), mp 105-107 °C lit. mp 105-107 °C [DNP]; $R_f$ 0.5 [2% MeOH/$CH_2Cl_2$]; molecular formular $C_{20}H_{28}O_4$; % yield 1.11; $^1H$-NMR (200 MHz, $CDCl_3$): δ 7.39 (*d, J* = 16.6 Hz) (H-15), δ 6.86 (distorted *t, J* =4.8 Hz, H-16) and 6.35 (*s*, H-14), 3.60 (*dd, J* = 5.6, 10.4 Hz, H-6), 1.28 (*s*, Me-19), 0.85 (*d, J* = 6.6 Hz, Me-17), 0.75 (*s*, Me-20); $^{13}C$-NMR (50 MHz, $CDCl_3$): δ 143.5 (C-4), 142.2 (C-15), 140.9 (C-3), 139.3 (C-16), 126.3 (C-13), 111.6 (C-14), 75.0 (C-6), 18.1 (Me-20), 17.1 (Me-17), 16.1 (Me-19).

*ent-3β, 8α-15,16-Epoxy-13(16), 14-labdadiene-3, 8-diol (11)*

White crystals (MeOH-$CH_2Cl_2$), mp**; $R_f$ 0.4 [1% MeOH in $CH_2Cl_2$]; molecular formular $C_{20}H_{32}O_3$; % yield 0.02; $^1H$-NMR (200MHz, $CDCl_3$): δ 7.34 (*s*, H-15), 7.23 (*s*, H-16) and 6.29 (*s*, H-14), 3.23 (*dd, J* = 4.8, 10.6 Hz, H-3), 1.14 (*s*, Me-17), 0.98 (*s*, Me-20), 0.81 (*s*, Me-18) and δ 0.76 (*s*, Me-19). $^{13}C$-NMR (50 MHz, $CDCl_3$): δ 143.5 (C-14), 139.5 (C-16), 126.2 (C-13), 111.8 (C-14), 79.6 (3), 74.8 (8), 61.9 (C-9), 55.8 (C-5), 28.9/29.1 (Me-17), 24.8 (Me), 16.6 (Me), 16.4 (Me).

### 5.2.2 *DODONAE ANGUSTIFOLIA (VOI)*

#### 5.2.2.1 EXTRACTION AND ISOLATION OF COMPOUNDS FROM FRESH LEAVES (AERIAL PARTS)

The leaves of this plant were shinny and gummy indication that they were coated with resin. Extraction of the surface exudates on the aerial parts (leaves) was done after plucking out the flowers. The surface exudates of the leaves (143 g) was extracted by successive dipping into fresh portions of ethyl acetate for short periods (< 15 seconds) to yield 143 g of crude extract, thus avoiding the extraction of the internal tissue component.

#### 5.2.2.2 ISOLATION AND PURIFICATION OF SURFACE COMPOUNDS FROM *DODONAEA ANGUSTIFOLIA* (VOI)

A portion of the extract (5 g) was kept aside for various bioassays. The rest of the extract (138 g) was subjected to column chromatography on silica gel (1.4 kg) eluting with different mixtures of $CH_2Cl_2$-*n*-$C_6H_{14}$ followed by MeOH-$CH_2Cl_2$ in increasing polarities. Yellow crystals of 5-hydroxy-3,7,4′-trimethoxyflavone (**1**) (206 mg) [Dreyer, 1978] precipitated out of the fraction eluted in 50% $CH_2Cl_2$-*n*-$C_6H_{14}$ mixture. The mother liquor was purified by column chromatography on Sephadex LH- 20 (eluting with MeOH/$CH_2Cl_2$; 1:1) to yield 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**) (46 mg) [Wollenweber *et al.*,1986] and 3,5-dihydroxy-7,4′,-dimethoxyflavone (**2**) (1 g) [Dreyer, 1978]. The fraction eluted in 60 % $CH_2Cl_2$/n-hexane afforded crystals of 5,4′-dihydroxy-3,7-dimethoxyflavone (kumatakenin) (**3**) (1.5 g) [Vieira *et al.*, 1997] [Sarmento *et al.*, 2002]. The mother liquor was separated by PTLC ($SiO_2$) ($CH_2Cl_2$-*n*-$C_6H_{14}$; 1:1) severally times to give yellow crystals of 5,4′-dihydroxy-3,6,7-trimethoxyflavone (penduletin) (**12**) (108 mg) [Sachdev& Kulshreshtha., 1986]. Colourless crystals of hautriwaic acid (**17**, 2.53 g) [Jefferies & Payne, 1967; 1973; Hsu *et al.*, 1971] precipitated out of the fraction eluted in neat $CH_2Cl_2$. The mother liquor of this fraction was concentrated in *vacuo* and subjected to recrystallization to yield a second crop of hautriwaic acid (**17**) (277 mg). The fraction eluted in 1 % MeOH-$CH_2Cl_2$ after

purification by PTLC ($SiO_2$) 20 % EtOAc-*n*-$C_6H_{14}$ with multiple development, afforded 5,3'-dihydroxy-3, 4', 7-trimethoxyflavone (ayanin) (**13**) (36 mg) [Perez-Castorena *et al.*, 1997a; Jakobsen *et al.*, 2001]. The mother liquor was purified by PTLC developing severally in ($SiO_2$, $CH_2Cl_2$) to yield 7-hydroxy-6-methoxycoumarin (**16**) (22 mg) [Andrianova *et al.*, 1975] as the major band.

The fraction eluted in 2 % MeOH-$CH_2Cl_2$ after purification by column chromatography (sephadex LH 20, MeOH-$CH_2Cl_2$; 1:1) and preparative TLC (1 % MeOH-$CH_2Cl_2$ x 2) afforded *neo*-clerodan-3,13-dien-16,15:18,19-diolide (**18**) (60 mg), 15α-methoxy-*neo*-clerodan-3,13-dien-16,15:18,19-diolide (**19**) (78 mg), and 15β-methoxy-*neo*-clerodan-3,13-dien-16,15:18,19-diolide (**20**) (78 mg). The fraction eluted in 3 % MeOH-$CH_2Cl_2$ after purification by column chromatography (sephadex LH 20, MeOH/$CH_2Cl_2$; 1:1) yielded 3,5,4'-trihydroxy-7-methoxyflavone (rhamnocitrin) (**5**) (33 mg) [Wollenweber *et al.*, 1986] and 3,5,7,4'-tetrahydroxy-flavone (kaempferol) (**15**) (57 mg) [Khan *et al.*, 1992].

### 5.2.2.3 PHYSICAL AND SPECTROSCOPIC PROPERTIES OF COMPOUNDS FROM *D. ANGUSTIFOLIA* FROM VOI.

***5,4'-Dihydroxy-3,6,7-trimehtoxyflavone (penduletin) (12)***

Yellow armophous ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 220-222 °C lit. mp 221-222 °C [DNP]; $R_f$ 0.5 [4% MeOH in $CH_2Cl_2$]; molecular formular $C_{18}H_{16}O_7$; % yield 0.08; UV: $\lambda_{max}$ (MeOH) 272.0 and 341.5 nm; $^1H$ (Table 3.11); $^{13}C$-NMR (Table 3.11); EIMS *m/z* (rel. int.): 344 $[M]^+$ (55.0), 329 $[M\text{-}Me]^+$ (31.5), 301 $[M\text{-}]^+$ (12.0).

***5,3'-Dihydroxy-3, 4', 7-trimethoxyflavone (ayanin) (13)***

Yellow crystals (MeOH-$CH_2Cl_2$), mp 173-174 °C lit. mp 173-174°C [DNP]; 0.5 (2% MeOH-$CH_2Cl_2$]; molecular formular $C_{18}H_{16}O_7$; % yield 0.03; $^1H$ (Table 3.12); $^{13}C$-NMR (Table 3.12); EIMS *m/z* (rel. int.): 344 $[M]^+$ (66.0), 329 $[M\text{-}Me]^+$ (35.0), 301 $[M\text{-}OMe]^+$ (35.0).

***5-Hydroxy-3,6,7,4'-tetramethoxyflavone (14)***

Yellow crystals (MeOH-$CH_2Cl_2$), mp 178-180 °C lit. mp 178-180 °C [DNP]; $R_f$ 0.5 [4% MeOH/$CH_2Cl_2$]; molecular formular $C_{19}H_{18}O_7$; % yield 0.03; $^1H$ (see Table 3.13); $^{13}C$-NMR (see Table 3.13); EIMS *m/z* (rel. int.): 358 $[M]^+$ (62.0), 343 $[M\text{-}Me]^+$ (32.0), 315 $[M\text{-}OMe]^+$ (14.0).

*3,5,7,4'-Tetrahydroxy-flavone (kaempferol) (15)*

Yellow armophous ($MeOH$-$CH_2Cl_2$), mp 276-278 °C lit. mp 276-278°C [DNP]; $R_f$ 0.5 [4% $MeOH/CH_2Cl_2$]; molecular formular $C_{15}H_{10}O_6$; % yield 0.04; UV: $\lambda_{max}$ (MeOH) 267.0, 324.0 and 364.5 nm; $^1H$ (Table 3.14); $^{13}C$-NMR (Table 3.14); EIMS *m/z* (rel. int.): 286 $[M]^+$ (100.0), 329 $[M\text{-}Me]^+$ (31.5), 301 $[M\text{-}OMe]^+$ (12.0).

*7-Hydroxy-6-methoxycoumarin (16)*

Colourless crystals ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 201-202 °C lit. mp 203-204 °C [DNP]; $R_f$ 0.3 ($CH_2Cl_2$); molecular formular $C_{10}H_8O_4$; % yield 0.2; $^1H$ (Table 3.15); $^{13}C$-NMR (Table 3.15).

*ent-15,16-Epoxy-19-hydroxy-3,13(16),14-clerodatrien-18-oic acid (hautriwaic acid) (17)*

Colourless needles ($MeOH$-$CH_2Cl_2$), mp 179-180 °C lit. mp 183-184°C [DNP]; $R_f$ 0.4 [3% $MeOH/CH_2Cl_2$]; molecular formular $C_{20}H_{28}O_4$; % yield 2.03; $^1H$ (Table 3.16); $^{13}C$-NMR (Table 3.16); EIMS *m/z* (rel. int.): 333 $[MH]^+$ (32), 332 $[M]^+$ (23), 284 $[C_{19}H_{24}O_2]^+$ (45), 220 $[C_{14}H_{20}O_2]^+$ (23), 219 $[C_{14}H_{19}O_2]^+$ (13), 189 $[C_{13}H_{17}O]^+$ (27), 95 $[C_6H_7O]^+$ (95), 81 $[C_6H_7O]^+$ (99).

*Neoclerodan-3,13-dien-16,15: 18,19-diolide (18)*

Colourless oil ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp**; $R_f$ 0.2 [1% MeOH in $CH_2Cl_2$]; molecular formular $C_{20}H_{26}O_4$; % yield 0.04; $^1H$ (Table 3.17); $^{13}C$-NMR (Table 3.17); HR-EIMS: *m/z* = 331.1894 $[MH]^+$: EIMS *m/z* (rel. int.): 330 $[M]^+$ (13), 300 $[M\text{-}CH_2O]^+$ (13), 219 $[C_{14}H_{19}O_2]^+$ (53), 189 (100), 161 (15), 105 (20), 91 (30), 84 (28).

*15β-Neoclerodan-3,13-dien-16,15: 18,19-diolide (19) and 15α-neoclerodan-3,13-dien-16,15: 18,19-diolide (20)*

Colourless oil ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp**; $R_f$ 0.1 [1% MeOH in $CH_2Cl_2$]; molecular formular $C_{21}H_{28}O_5$; % yield 0.06; $^1H$ (Table 3.18); $^{13}C$-NMR (Table 3.18). HR-EIMS: *m/z* = 360.8609 $[M]^+$: EIMS *m/z* (rel. int.): 361 $[MH]^+$, 342 $[M\text{-}H_2O]^+$ (18), 330 $[CH_2O]^+$ (36), 329 (54), 310 (52), 283 (52), 189 (32), 91 (43), 86 (59), 84 (100).

## 2.3 *SENECIO ROSEIFLORUS*

### 5.2.3.1 EXTRACTION AND ISOLATION FROM FRESH LEAVES (AERIAL PARTS)

Extraction of the surface exudates on the aerial parts (mainly leaves) of *Senecio roseiflorus* was carried out on fresh plant material, by successive dipping into fresh portions of ethyl acetate after short periods (< 15 seconds). Whenever the colour of the solvent become intense yellow, it was changed. The extracts were combined, filtered and concentrated in *vacuo*. In the process of removing the solvent, a white precipitate was formed which was determined to be an inorganic material, NaCl.

This was filtered out to give 15 g of material. Further removal of solvent of the filtrate resulted in a pale brown gummy solid (223 g). The remaining aerial parts of the plant were spread on a bench to dry out.

### 5.2.3.2 ISOLATION AND PURIFICATION OF SURFACE COMPOUNDS FROM *SENECIO ROSEIFLORUS*.

A portion of the extract (15 g) was kept aside for various bioassays. The rest of the extract (50 g) was adsorbed on silica gel 50 g and subjected to column chromatography ($SiO_2$, 500 g $CH_2Cl_2$-*n*-$C_6H_{14}$ 1:1. Separation was carried out by stepwise gradient elution using mixtures of $CH_2Cl_2$-*n*-$C_6H_{14}$ followed by MeOH-$CH_2Cl_2$ in increasing polarities. The fraction eluted in 60 % $CH_2Cl_2$-*n*-$C_6H_{14}$ was purified by CC (sephadex-LH 20, MeOH-$CH_2Cl_2$; 1:1) to give 5-hydroxy-3,7,4′-trimethoxyflavone (**1**) (33 mg) [Dreyer, 1978], 3,5-dihydroxy-7,4′-dimethoxyflavone (**2**) (54 mg) [Dreyer, 1978], 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**27**) (103 mg) and 3,5-dihydoxy-3′,4′,7-trimethoxyflavone (**28**) (21 mg). Yellow crystals of 3,4′,5-trihydroxy-3′,7-dimethoxyflavone (rhamnazin) (**23**) (39 mg) [Marin *et al.*, 2001] precipitated out from the fraction eluted in 80 % $CH_2Cl_2$/n-hexane. The mother liquor was separated by CC (sephadex-LH 20; MeOH- $CH_2Cl_2$; 1:1) to yield yellow crystals of 3,5,4′-trihydroxy-7-methoxyflavone (rhamnocitrin) (**5**) (24 mg) [Wollenweber *et al.*, 1986]. White crystals of 5,4′-dihydroxy-7-dimethoxyflavanone (**25**, 1.480 g) [Mata *et al.*, 1991] precipitated out of the fraction eluted in neat $CH_2Cl_2$. The mother liquor was purified by CC (sephadex-LH 20; MeOH- $CH_2Cl_2$; 1:1) coupled with PTLC ($SiO_2$), 90 % $CH_2Cl_2$-*n*-$C_6H_{14}$ multiple development to yield 20 mg of 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**14**) [Wollenweber *et al.*,1986] and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (retusin) (**24**) (28 mg) [Valant-Vetschera *et al.*, 2003; Wollenweber & Dorr., 1996].

The fraction eluted in 2 % $CH_2Cl_2$ -MeOH after purification by CC ($SiO_2$, 1% MeOH-$CH_2Cl_2$ ) followed by PTLC ($SiO_2$, $CH_2Cl_2$) multiple development yielded 3,5,4′-trihydroxy-7-methoxyflavone (rhamnocitrin) (**5**) (15 mg) [Wollenweber *et al.*, 1986] and 5,7,4′,-

trihydroxy-3-methoxy flavone (isokaempferide) (**6**) (24 mg) [Dreyer., 1978]. The fraction eluted with 3 % $CH_2Cl_2$-MeOH afforded 104 mg of 5, 7-dihydroxy-3,4'-dimethoxyflavone (**21**) [Wollenweber *et al.*, 1986] and 53 mg of 3',5,7-trihydroxy-3,4'-dimethoxyflavone (quercetin-3,4'-dimethyl ether) (**22**) [Sepulveda *et al.*, 1994; Jakobsen *et al.*, 2001]. The mother liquor was purified by preparative TLC ($SiO_2$, $CH_2Cl_2$ multiple development) to yield 3,4',5-trihydroxy-3',7-dimethoxyflavone (rhamnazin) (**23**) (35 mg) [Marin *et al.*, 2001] and 4-hydroxy-methylbenzoate (**26**) (22 mg) [Yoshioka *et al.*, 2004].

### 5.2.3.3 PHYSICAL AND SPECTROSCOPIC PROPERTIES OF COMPOUNDS FROM *SENECIO ROSEIFLORUS.*

***5,7-Dihydroxy-3,4'-dimethoxyflavone (21)***

Yellow armophous ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 233-235 °C; $R_f$ 0.3 [20% $CH_2Cl_2$ in n-$C_6H_6$]; molecular formular $C_{17}H_{14}O_6$; % yield 0.21; $^1H$ (Table 3.19); $^{13}C$-NMR (Table 3.19); EIMS *m/z* (rel. int.): 314 $[M]^+$ (100.0), 271 $[M\text{-}OMe]^+$ (75.0).

***3',5,7-Trihydroxy-3,4'-dimethoxyflavone (quercetin-3,4'-dimethyl ether) (22)***

Yellow armophous ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 232-234 °C lit. mp 235-236°C [DNP]; $R_f$ 0.5 [2% MeOH in $CH_2Cl_2$]; molecular formular $C_{17}H_{14}O_7$; % yield 0.11; $^1H$ (Table 3.20); $^{13}C$-NMR (Table 3.20). EIMS *m/z* (rel. int.): 330 $[M]^+$ (100.0), 287 $[M\text{-}OMe]^+$ (12.3), 167 (18.5).

***3,4',5-Trihydroxy-3',7-dimethoxyflavone (rhamnazin) (23)***

Yellow armophous ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 216-218 °C lit. mp 216-218 °C [DNP]; $R_f$ 0.5 [2% MeOH in $CH_2Cl_2$]; molecular formular $C_{17}H_{14}O_7$; % yield 0.07; $^1H$ (Table 3.21); $^{13}C$-NMR (Table 3.21); EIMS *m/z* (rel. int.): 330 $[M]^+$ (100.0), 287 $[M\text{-}OMe]^+$ (12.2), 167 (16.7).

***5-Hydroxy-3,7,3',4'-tetramethoxyflavone (retusin) (24)***

Yellow armophous (MeOH-$CH_2Cl_2$), mp 160-162 °C lit. mp 159-160°C [DNP]; $R_f$ 0.5 [2% MeOH/$CH_2Cl_2$]; molecular formular $C_{19}H_{18}O_7$; % yield 0.06; $^1H$ (Table 3.22) and $^{13}C$-NMR (Table 3.22).

***5,4'-Dihydroxy-7-methoxyflavanone (25)***

White crystals ($CH_2Cl_2$-*n*-$C_6H_{14}$), mp 151-153°C lit. mp 152-154 °C [DNP]; $R_f$ 0.4 [1% MeOH in $CH_2Cl_2$]; molecular formular $C_{16}H_{14}O_5$; % yield 2.96; UV $\lambda_{max}$ (MeOH) 283.0 nm); $^1H$ (Table 3.23) and $^{13}C$-NMR (Table 3.23). EIMS *m/z* (rel. int.): 286 $[M]^+$ (43.7), 167 (100.0), 120 (34.4).

***4-Hydroxybenzoic acid methyl ester (26)***

Colourless oil, mp 127-129 °C literature 127-129 °C [DNP]; $R_f$ 0.5 [1% MeOH/$CH_2Cl_2$]; molecular formular $C_8H_8O_3$; % yield 0.04; $^1H$ (Table 3.24); $^{13}C$-NMR (Table 3.24).

## 5.4 BIOLOGICAL ACTIVITY STUDIES

### 5.4.1 *IN VITRO* ANTI-PLASMODIAL TEST

The extracts and the pure compounds were assayed using an automated micro-dilution technique to determine 50% growth inhibition of cultured parasites [Chulay *et al.*, 1983; Desjardins *et al.*, 1979]. Two strains of *Plasmodium falciparum* parasites, from the Walter Reed Army Institute of Research, that are commonly used in drug sensitivity assays were cultured. The chloroquine sensitive Sierra Leone I (D6) and chloroquine- resistant Indo-china I (W2) strains were grown in continuous culture supplemented with mixed gas (90% nitrogen, 5% oxygen, 5% carbon dioxide), 10 % human serum, and 6% hematocrit of A+ red blood cells. Once cultures reach a parasitemia level of 3% with at least a 70% ring stage development, parasites were transferred to a 96 well microtiter plate with wells pre-coated with sample. The samples were serially diluted across the plate to provide a range of concentration used to accurately determine $IC_{50}$ values. Plates were incubated in a mixed gas incubator for 24 hours. Following the specified incubation time, [$^3$H]-hypoxanthine was added and parasites allowed to grow for an additional 18 hours. Cells were processed with a plate harvester (Tom Tec) onto a filter paper and washed to eliminate unincorporated [$^3$H]-hypoxanthine. Filters were measured for activity in a microtiter plate scintillation counter (Wallac). Data from the counter was imported into a Microsoft Excel spreadsheet which isand subsequently imported into an Oracle database/program to determine ($IC_{50}$) values.

### 5.4.2 LARVICIDAL ACTIVITY ASSAY

The eggs of Aedes aegypti L. (Diptera: Culicidae) were obtained from the Department of Zoology, University of Nairobi. The eggs were flooded with 0.08% NaCl solution and left to hatch at 28 °C. Twenty second instar larvae were transferred into a petri-dish containing 10ml of 0.08% NaCl solution. The larvae were treated with the test extracts and pure compounds according to Mwangi and Rembold [1998]. Twenty milligrams of test samples were dissolved in 2 ml of DMSO. From the stock solution different concentrations were prepared

by serial dilution and the larvae were tested for mortality at 20, 10, 5, 2.5, 1.25 and 0.75 µg/ml of sample solutions. Control larvae in all cases received 50µl of DMSO as in test larvae. Each experiment was run in triplicate.Mortality were checked after 24, 48 and 96 hours. $LC_{50}$ values were calculated from the average of three observations for each concentration using Finney's probit analysis for quantal data [Mwangi *et al.*, 1988, McLaughlin et al., 1991].

### 5.4.3 RADICAL SCAVENGING TEST USING DPPH

To a MeOH solution (3 ml) of DPPH (100 uM), 0.5 ml of test compounds at 50 µM (10 µg/ml for crude extract) were added and the mixture was shaken and left to stand for 30 min. The Radical Scavenging Activities were estimated as the percentage decrease of absorbance of DPPH (100 µM) at 517 nm [Ohinishi *et al.*, 1994]. For 3,5,4'-trihydroxy-7-methoxyflavone (rhamnocitrin) (**5**), 5,7,4',-trihydroxy-3-methoxy flavone (isokaempferide) (**6**), and quercetin etc, tests were done at six different concentrations ( 50.25, 12.5, 3.13 and 1.56 µM). In all cases the mean values were used from triplicate experiments. $EC_{50}$ values were calculated using Finney's probit analysis for quantal data [McLaughlin *et al.*, 1991].

### 5.4.4 ANTI-MICROBIAL TEST

Evaluation of anti-microbial activity of extracts and pure compounds was accomplished using the agar well-diffusion method [Bauer *et al.*, 1966]. The extracts and pure compounds were tested for activity against three strains of bacteria: *Staphyloccocus aureus* (ATCC29737), *Escherichia coli* (ATCC25922) and *Bacillus pumilus* (local strain) and a local strain of a fungus, *Saccharamyces cerevisiae*. The bacterial test organisms were cultured on tryptone soya agar while the fungus was cultured in Saboraud's dextrose agar.

Seven cylindrical plugs were removed from the solidified nutrient agar plates at equidistant points, using a sterile cork borer, to produce wells (5 mm diameter, 2 mm depth). The seven wells were used for six different plant extract at 2500 µg/ml and a control.

The extracts 50 mg and pure compounds (10 mg) were dissolved in 1 ml of solvent (DMSO) to give the stock solution of 50 mg/ml for extract and 10 mg/ml for pure compounds which were 31.25, 62.5, 125, 250 and 500 µg/disc subjected to anti-microbial test for the crude plant extracts. However, for the pure compounds, different concentrations were prepared by serial dilution of the stock solution. Only 50 µl of the solution of extract, pure compound, standard drug (Gentamicin 0.3 mg/ml, nystatin 0.25 mg/ml) or solvent (DMSO) were filled in each well. All experiments were done in triplicate. The inoculated petri dishes prepared as described above were left for 30 minutes for diffusion and then incubated overnight (18 hours) at 37 °C and 25 °C for bacteria and fungi, respectively. The anti-microbial activity was recorded as the width (mm) of the clear zone of inhibition surrounding the agar well after 18 hrs of incubation for the bacteria and fungi.

The minimum inhibitory concentration (MIC) was determined by the agar well-diffusion method. MIC of a compound is defined as the lowest concentrations of the compounds that visually inhibits growth compared with growth in control wells.

# CHAPTER SIX

## 6.1 REFERENCES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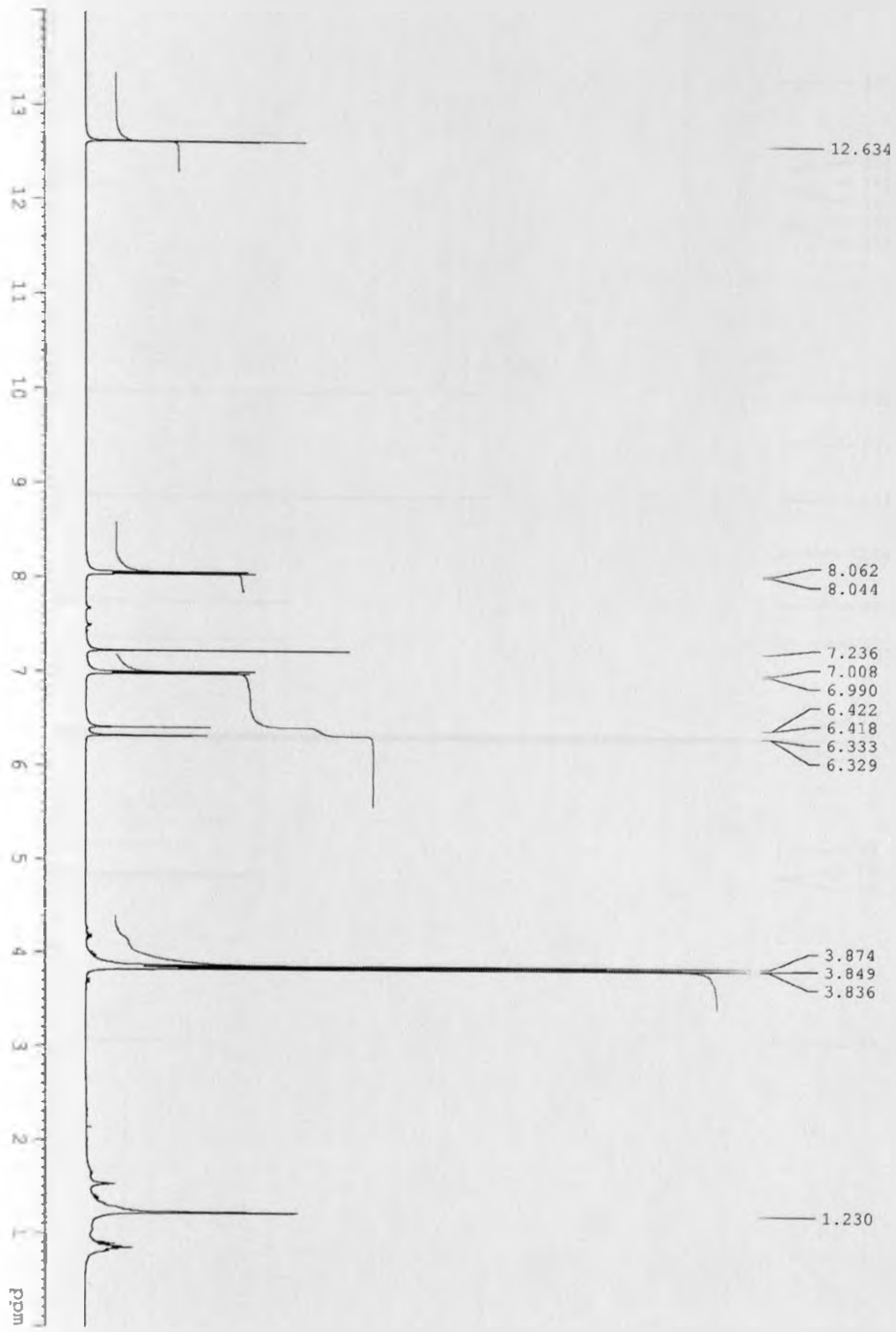
# APPENDIX I: SPECTRA FOR COMPOUND 1

# $^{1}$H-NMR SPECTRUM FOR COMPOUND 1



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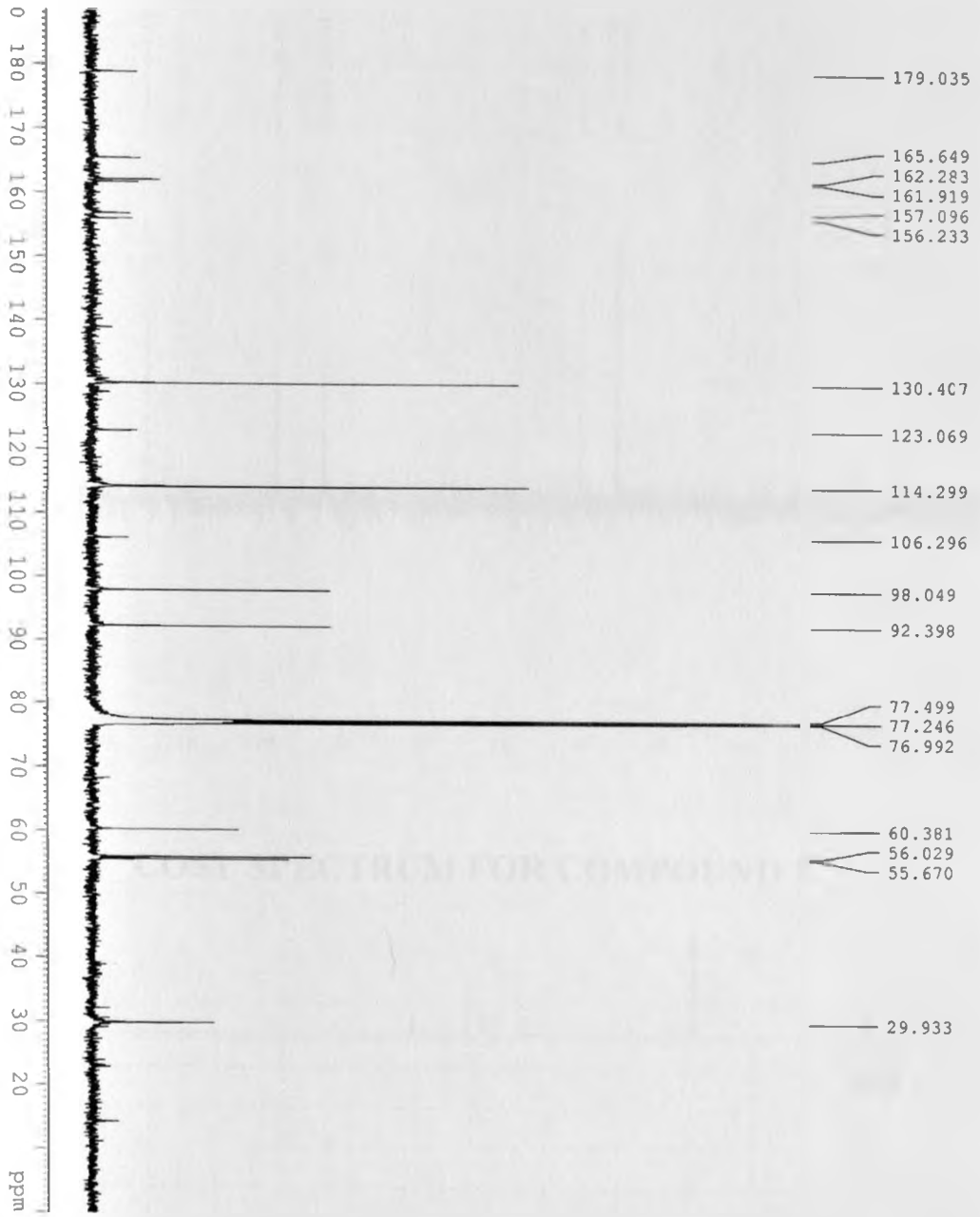

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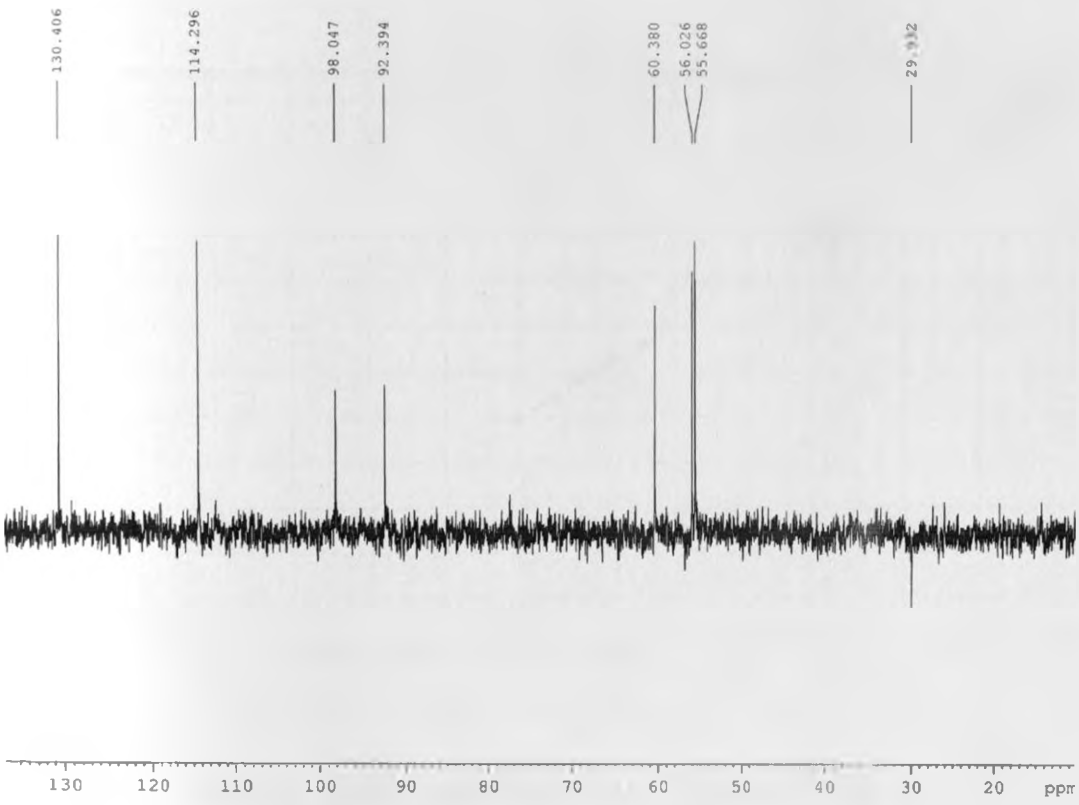


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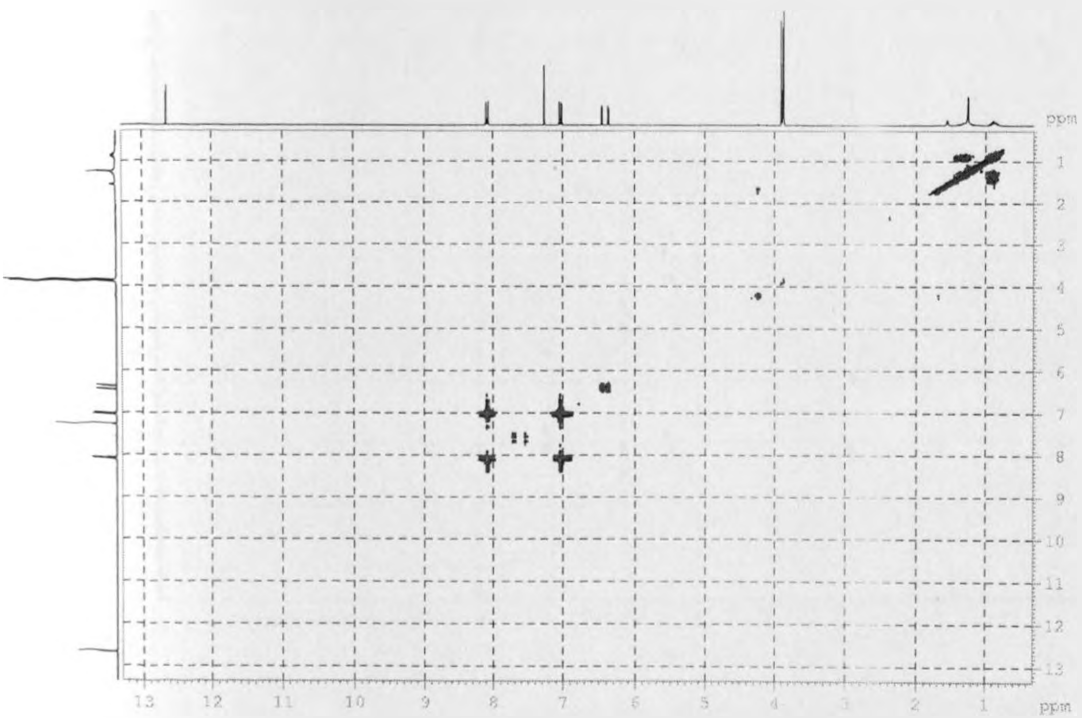

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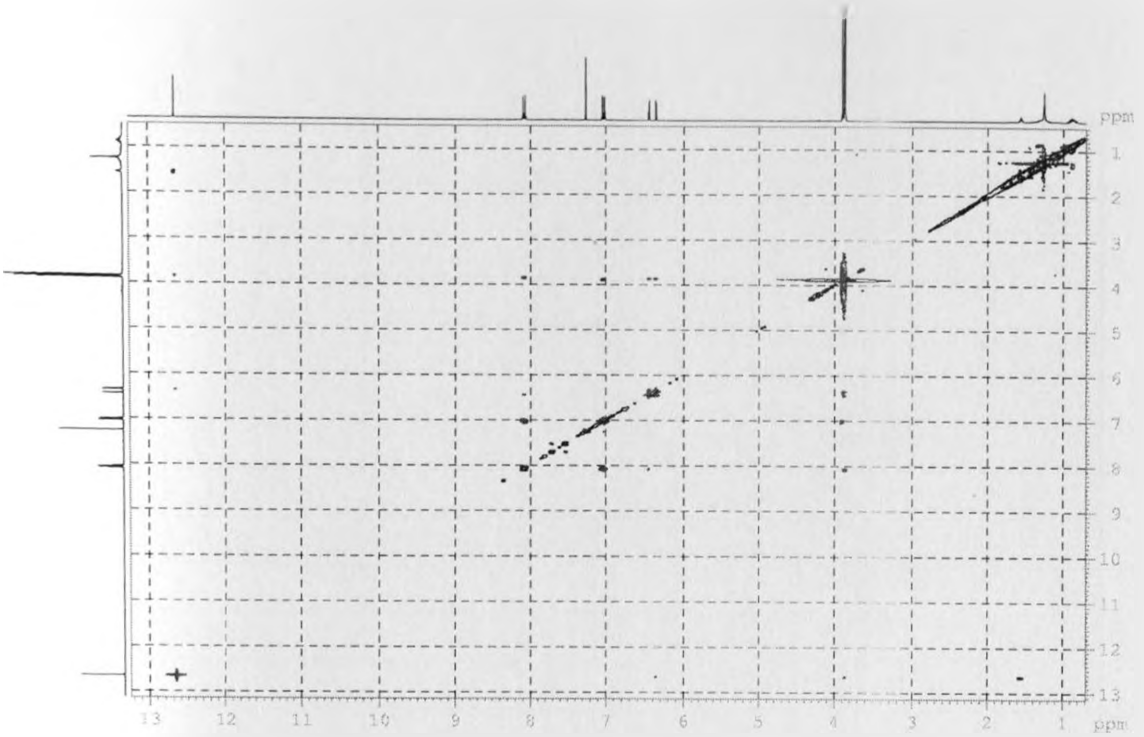


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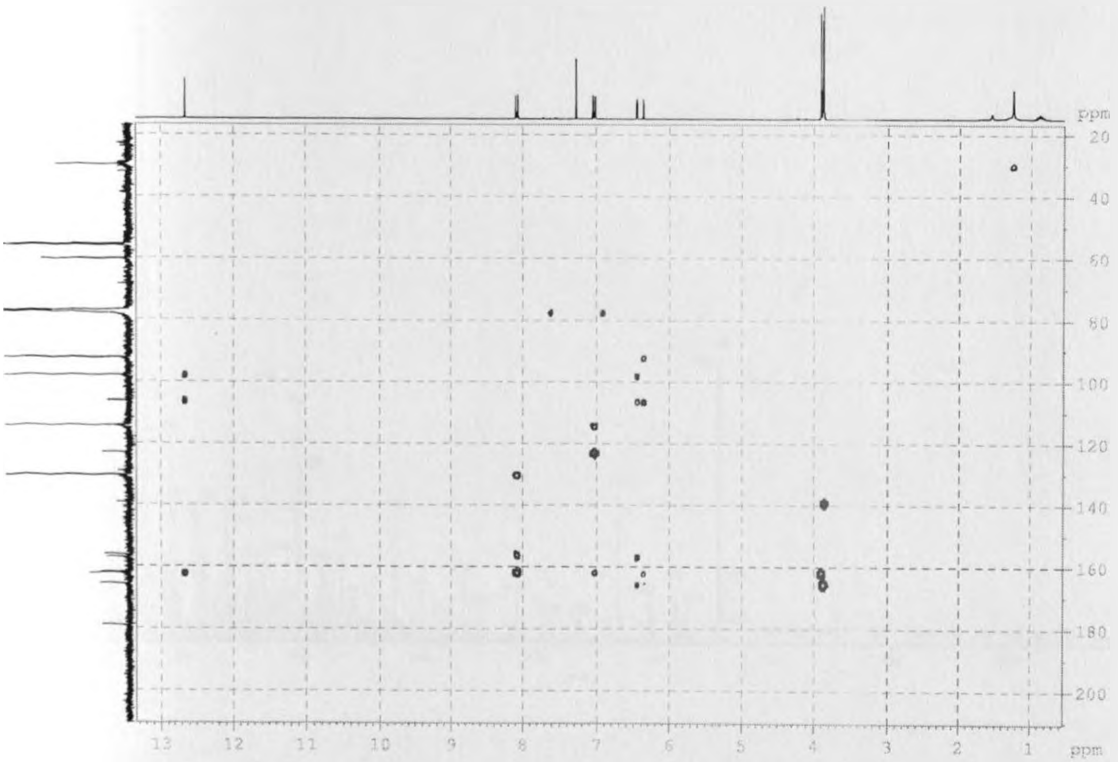

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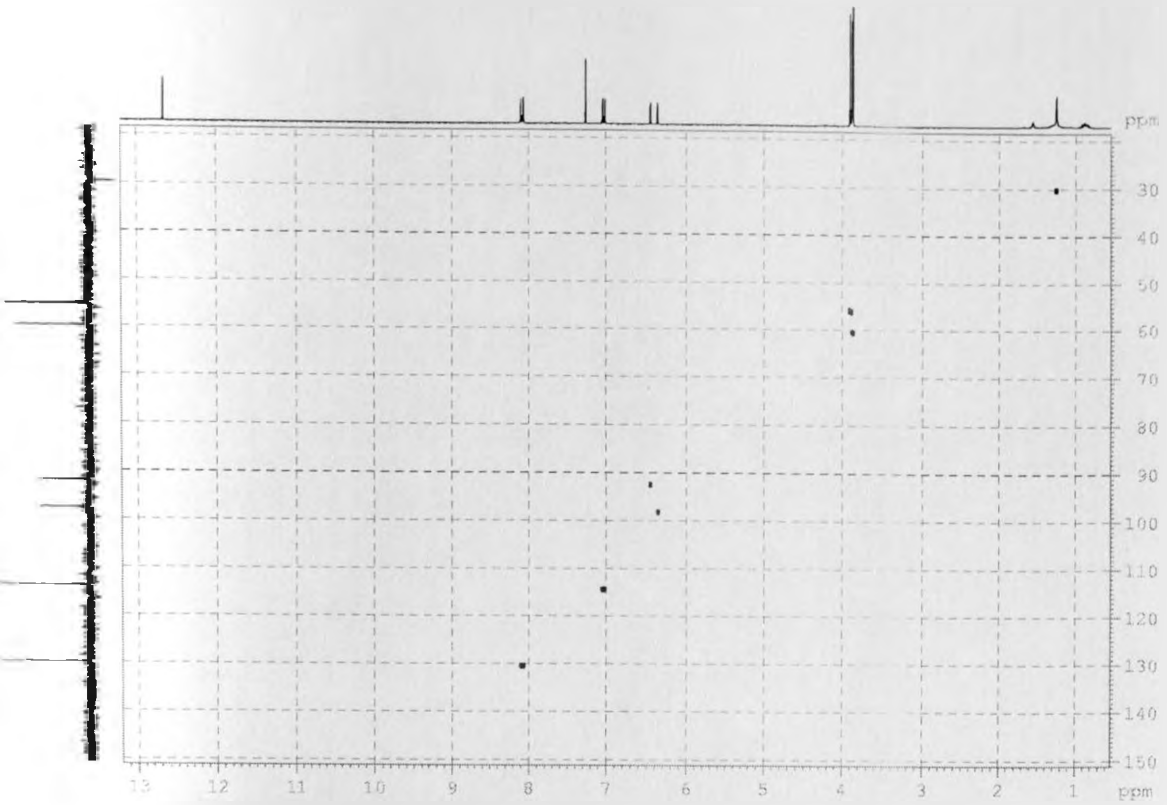


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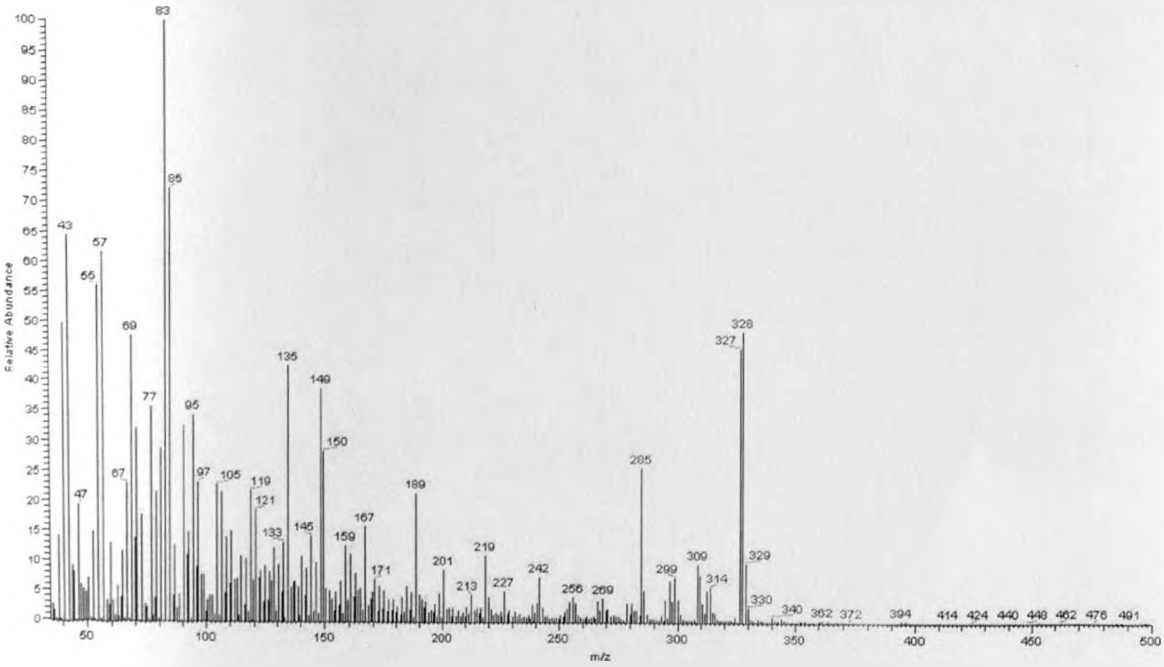

# APPENDIX II: SPECTRA FOR COMPOUND 2

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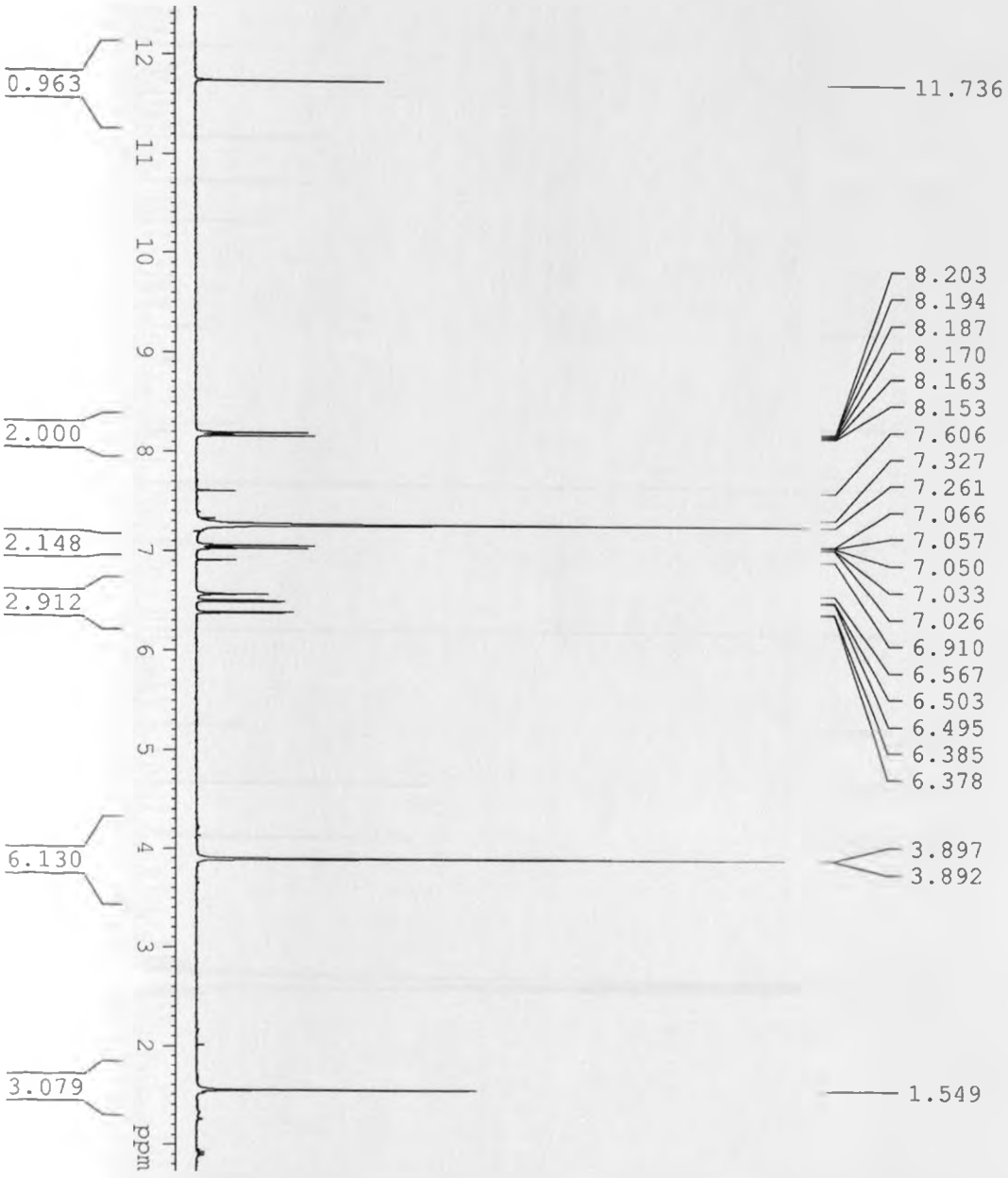

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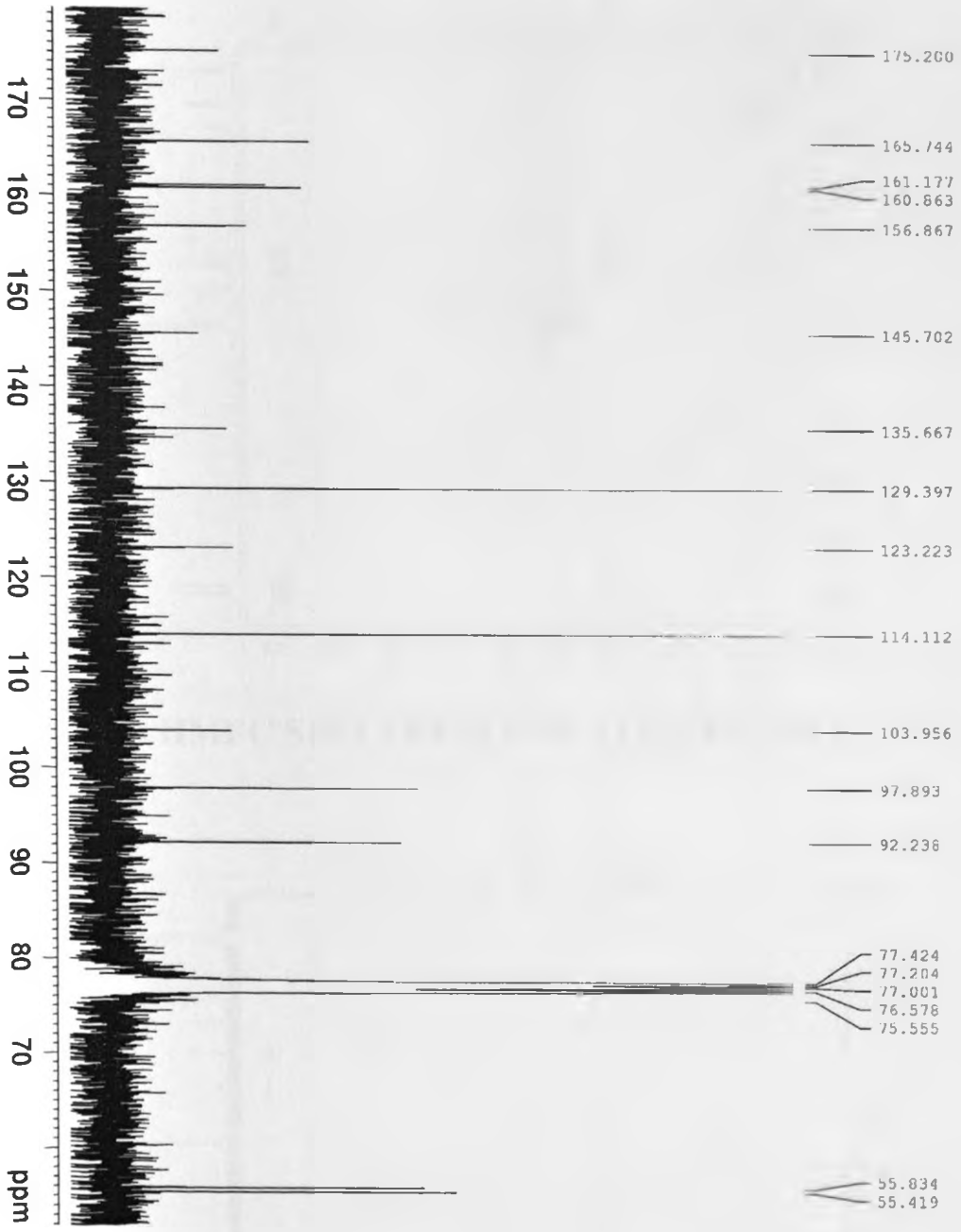

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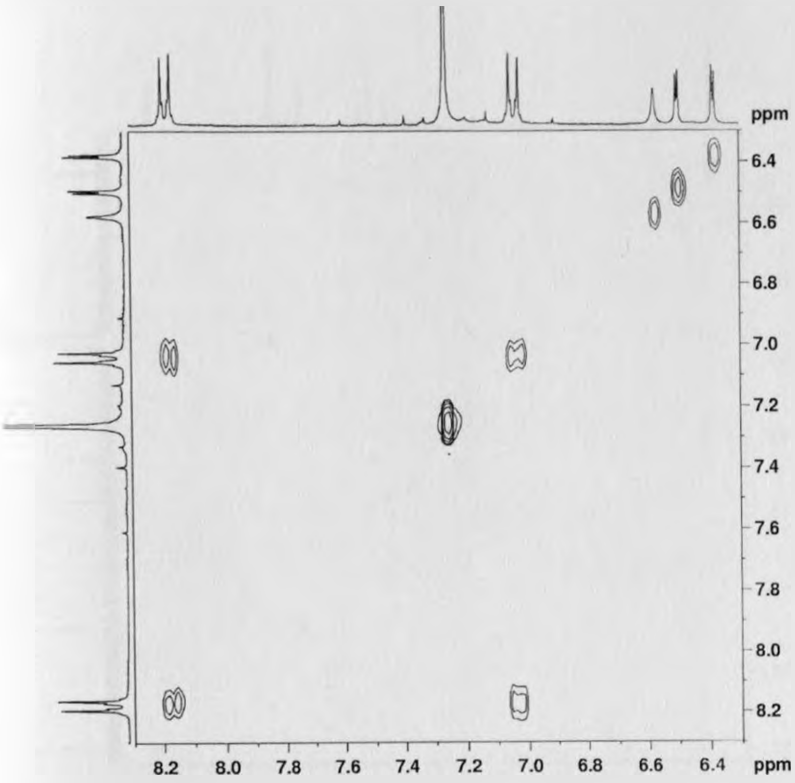


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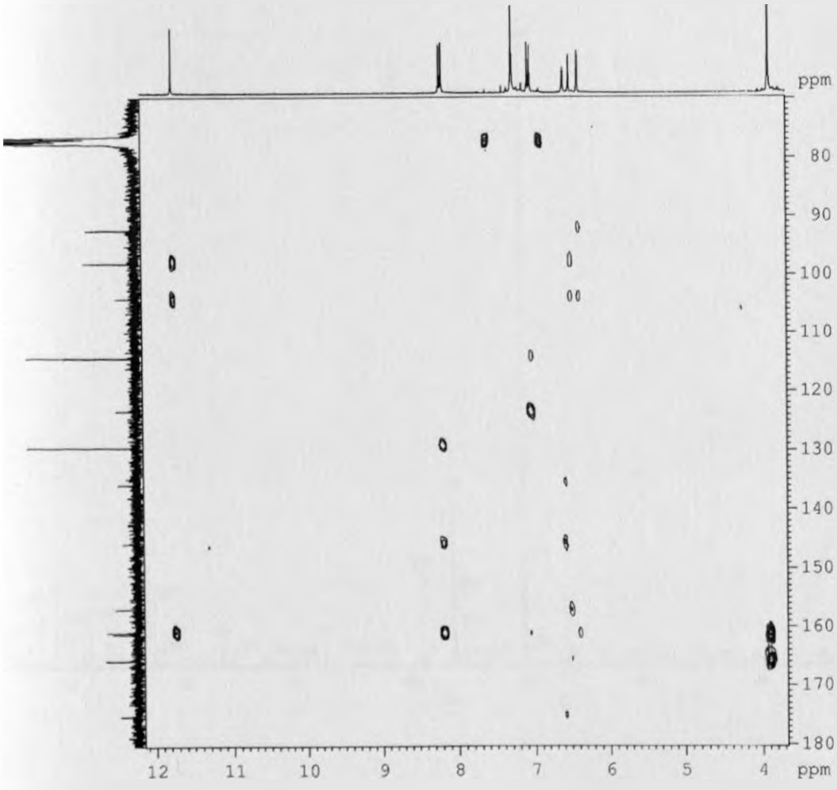

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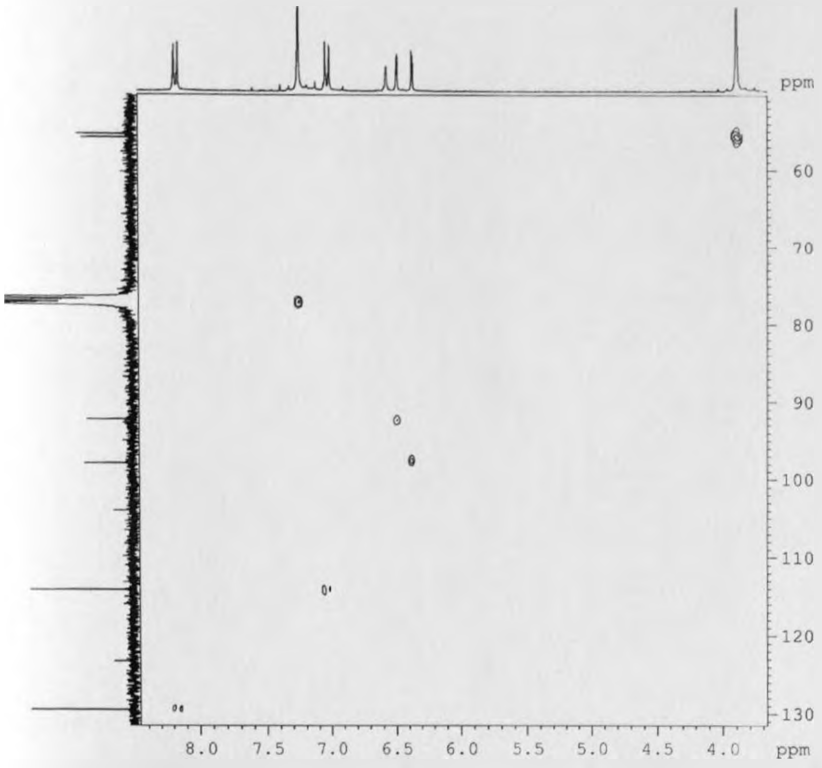


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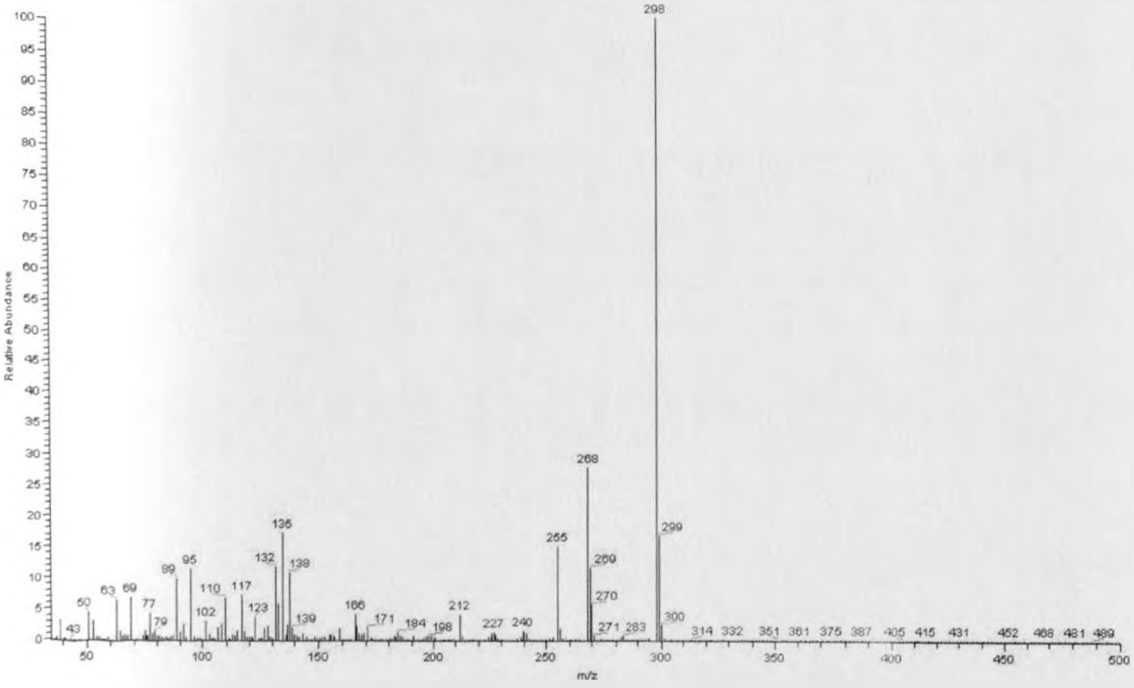

# APPENDIX III: SPECTRA FOR COMPOUND 3

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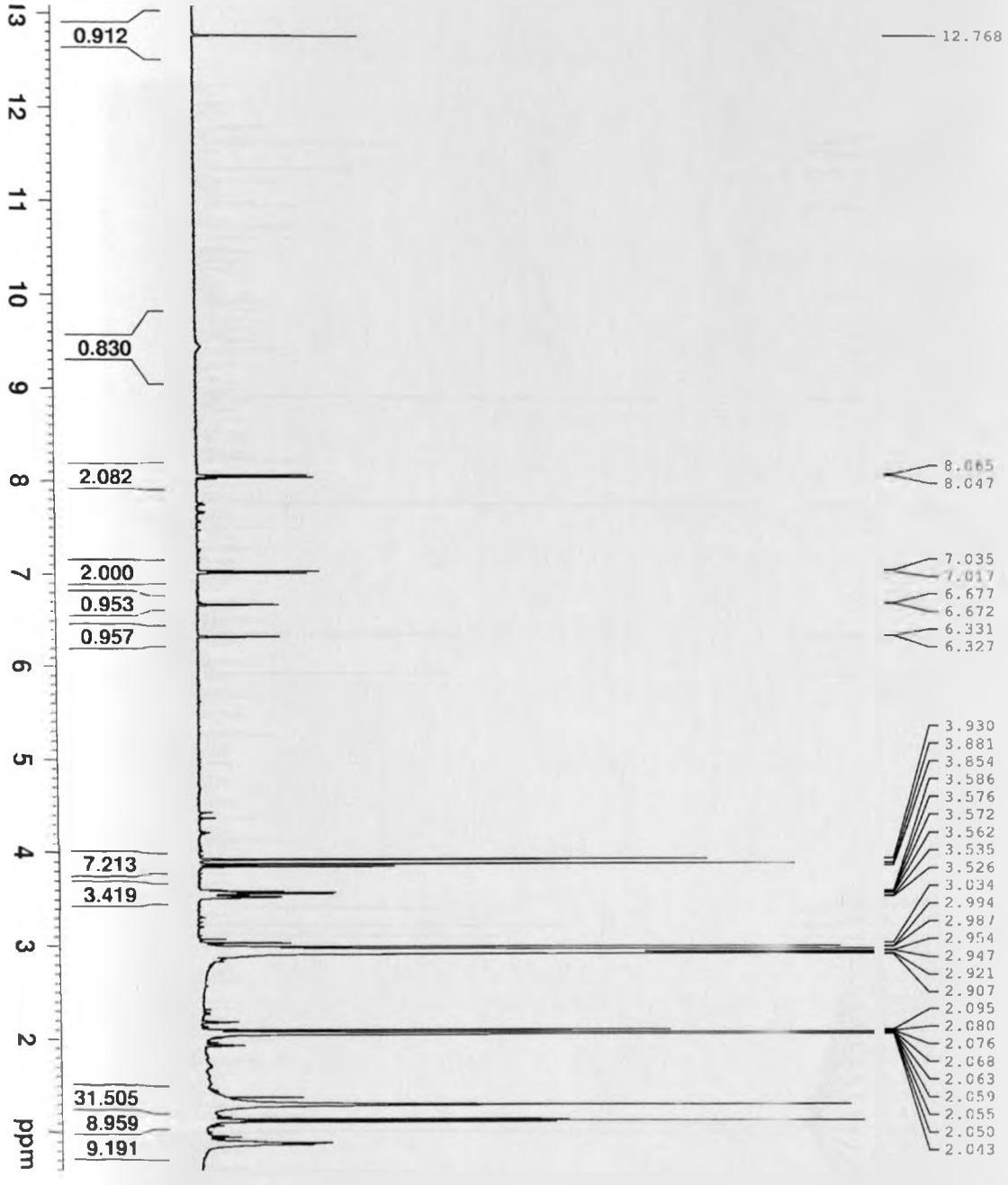

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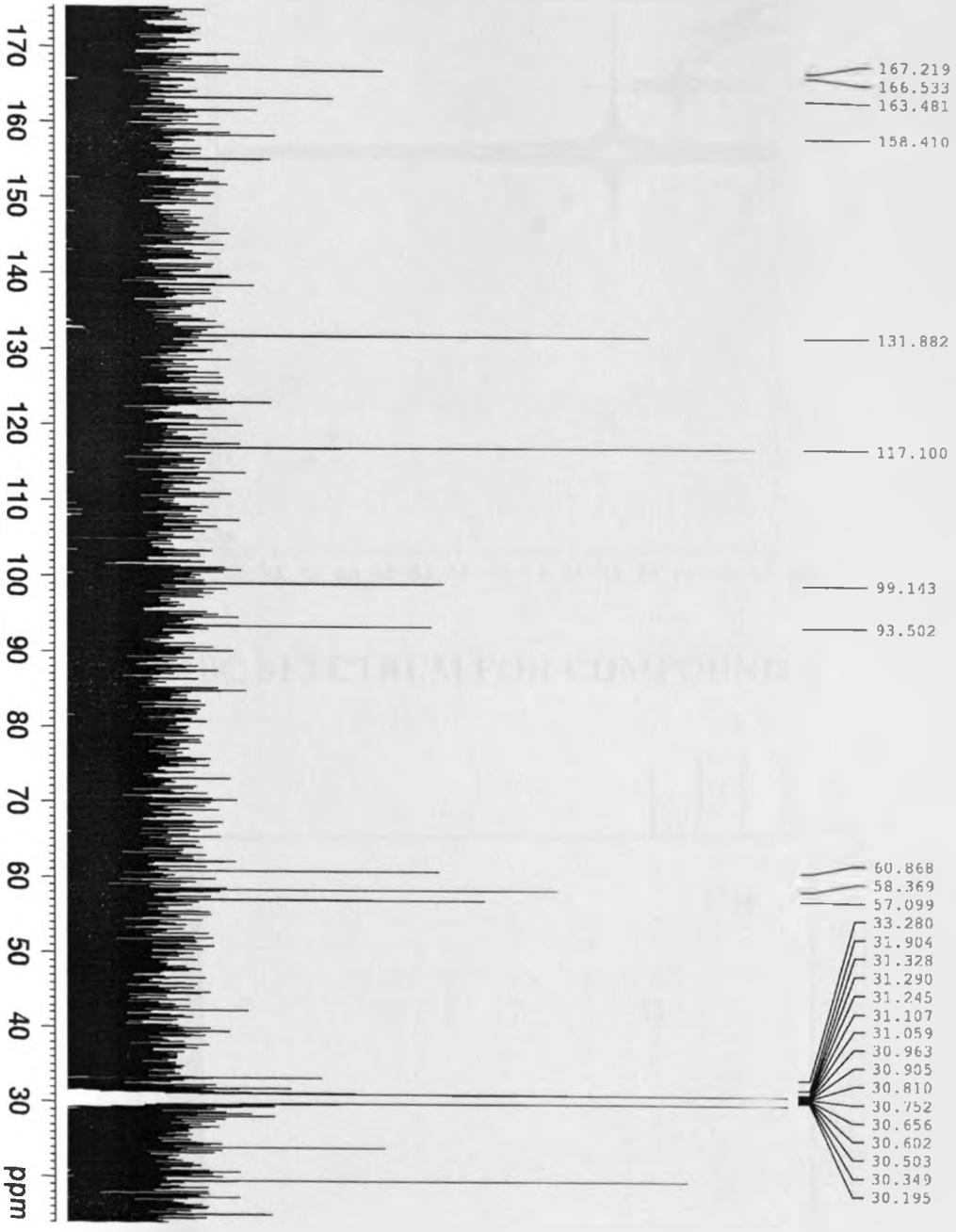
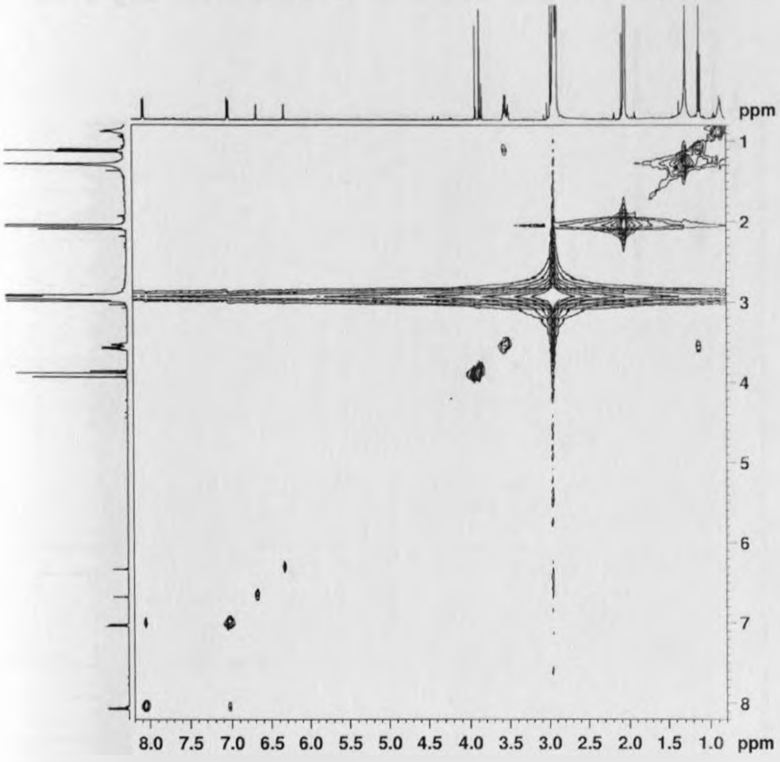


## $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 3

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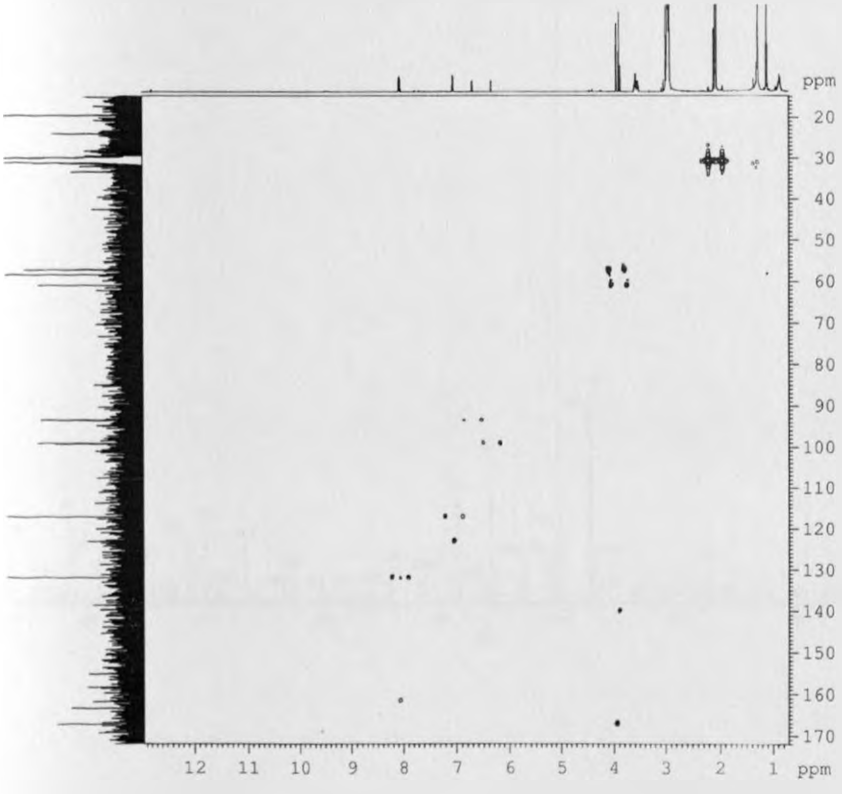

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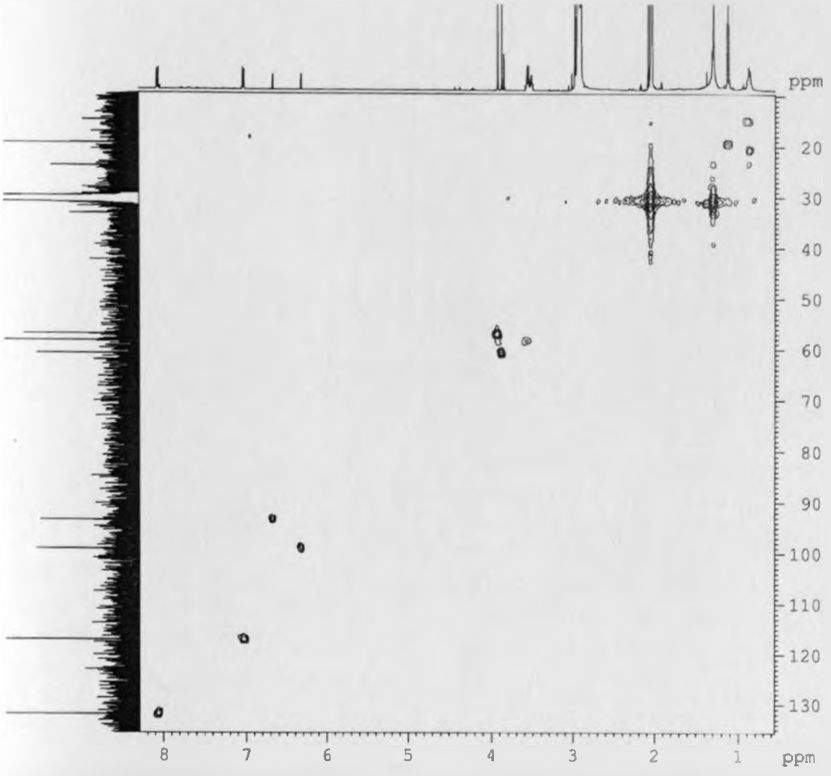


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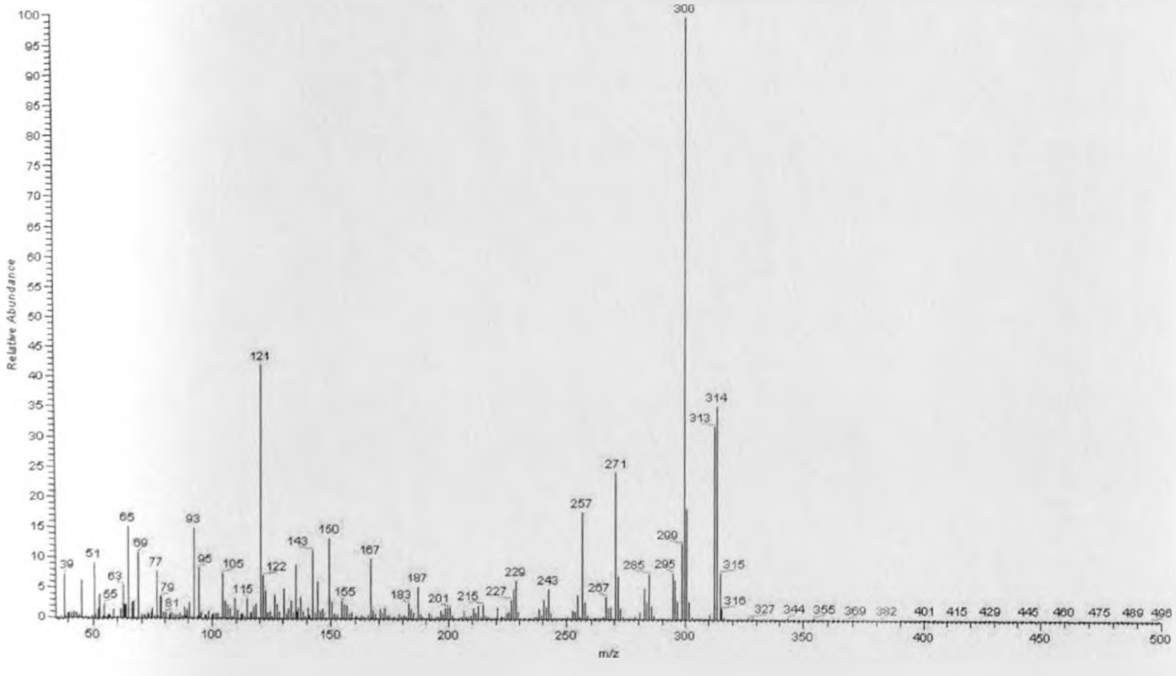

# APPENDIX IV: SPECTRA FOR COMPOUND 4

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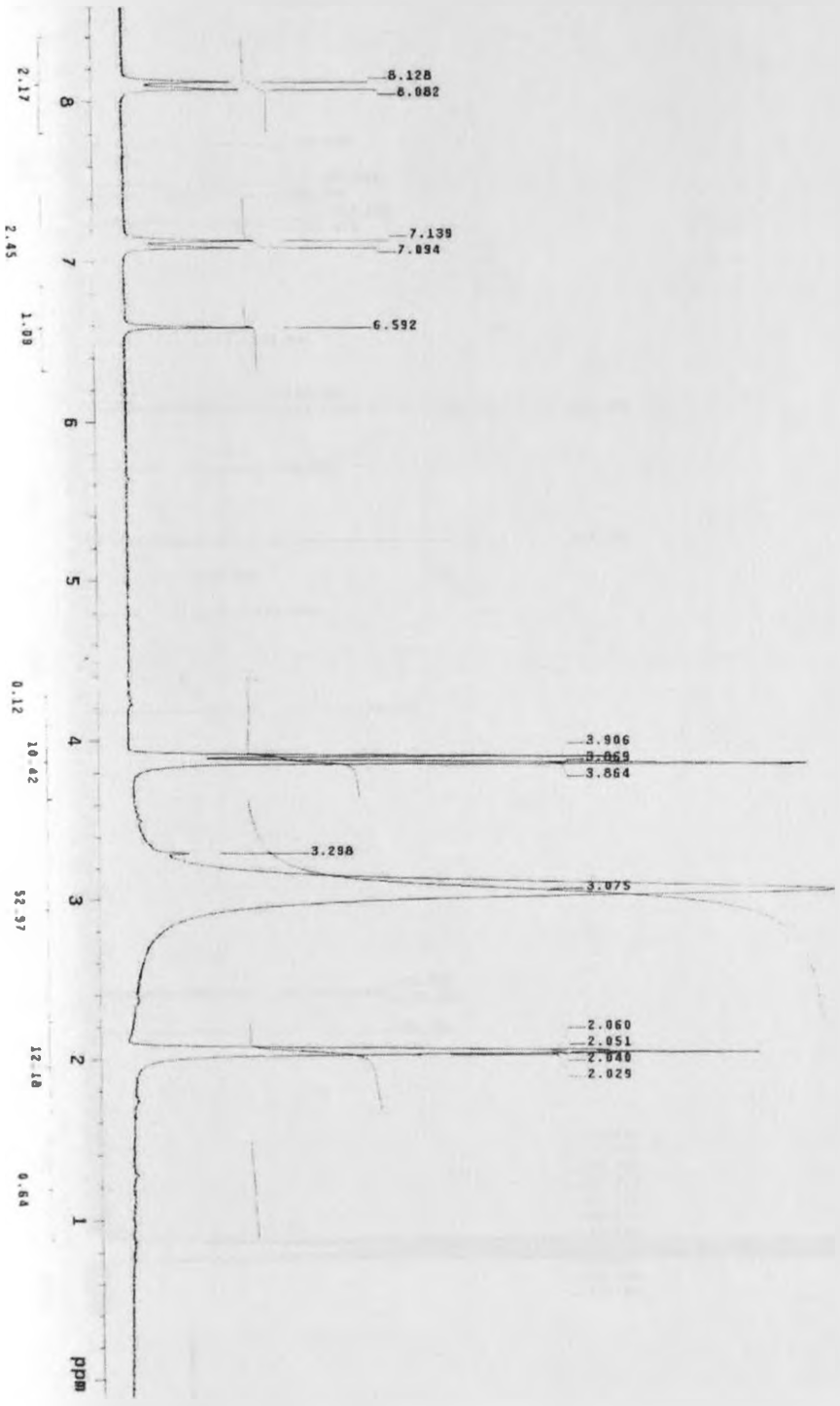

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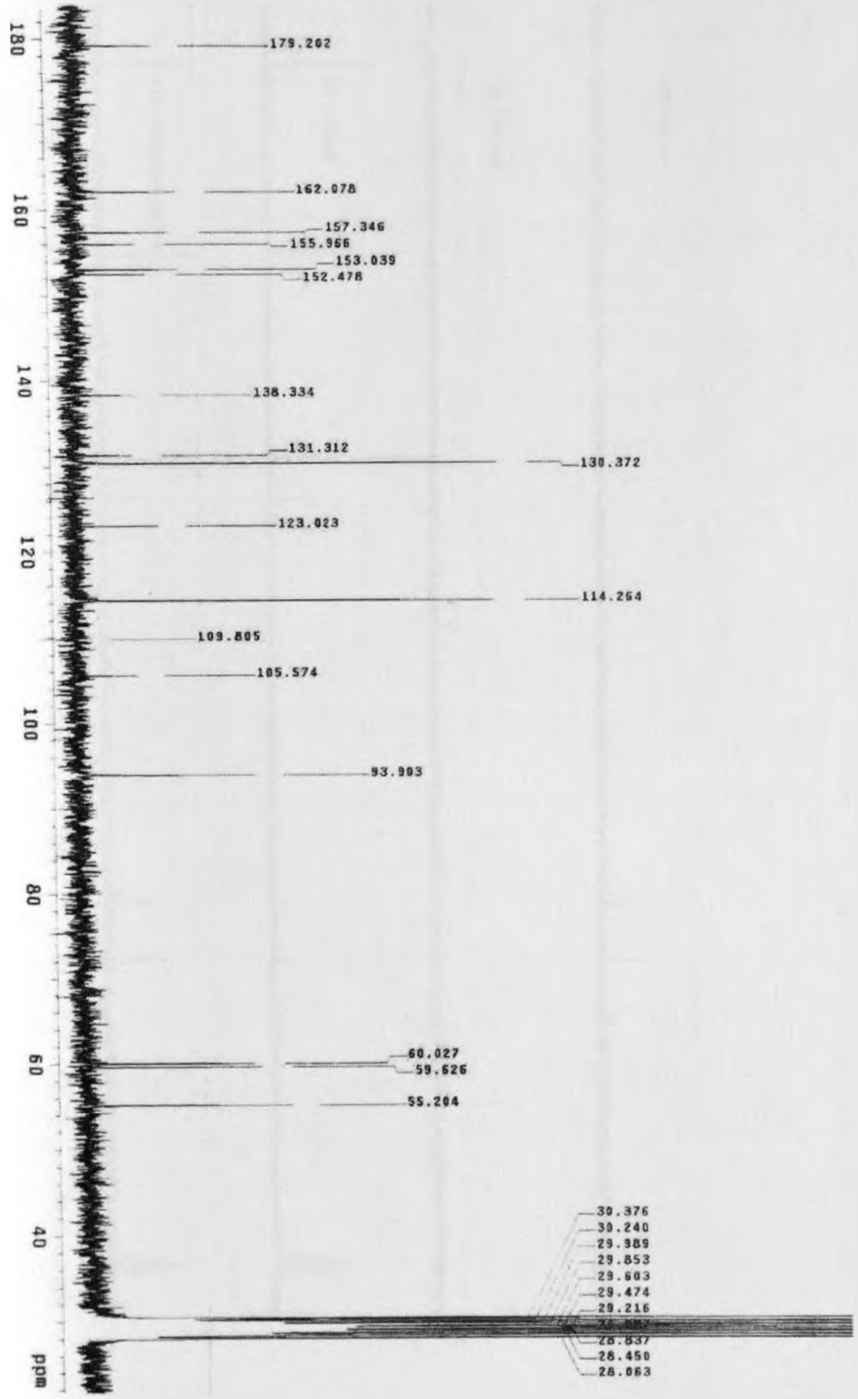

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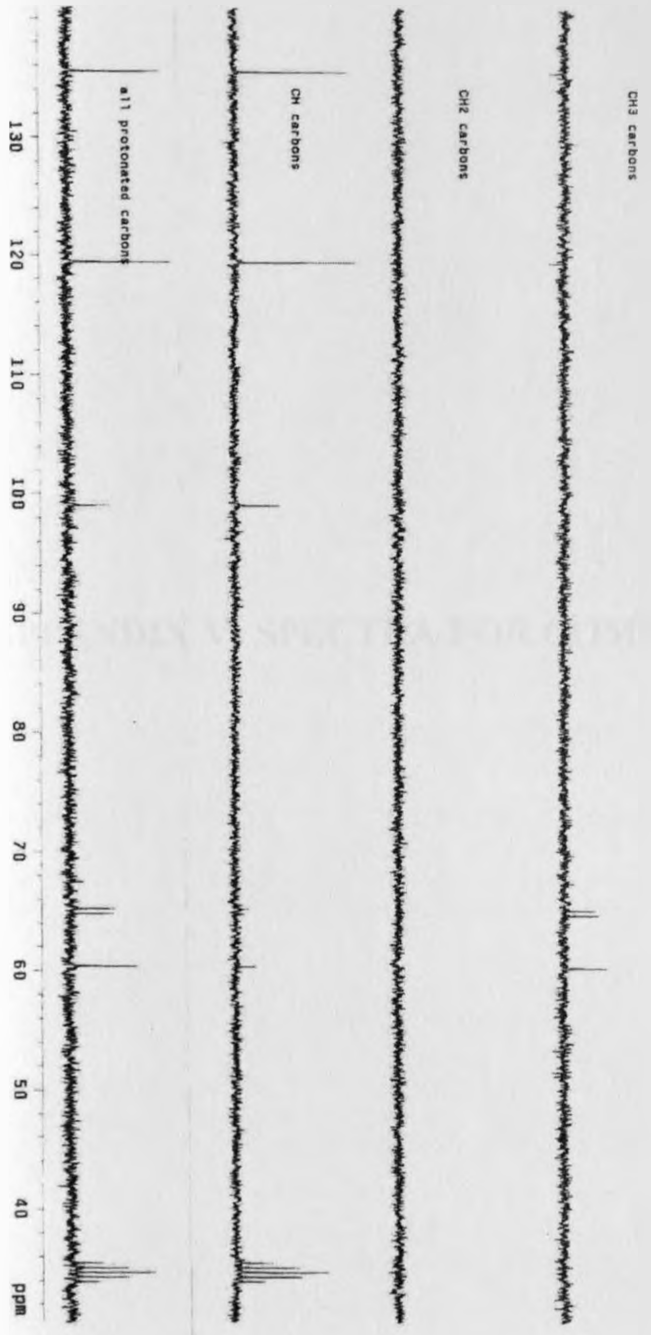
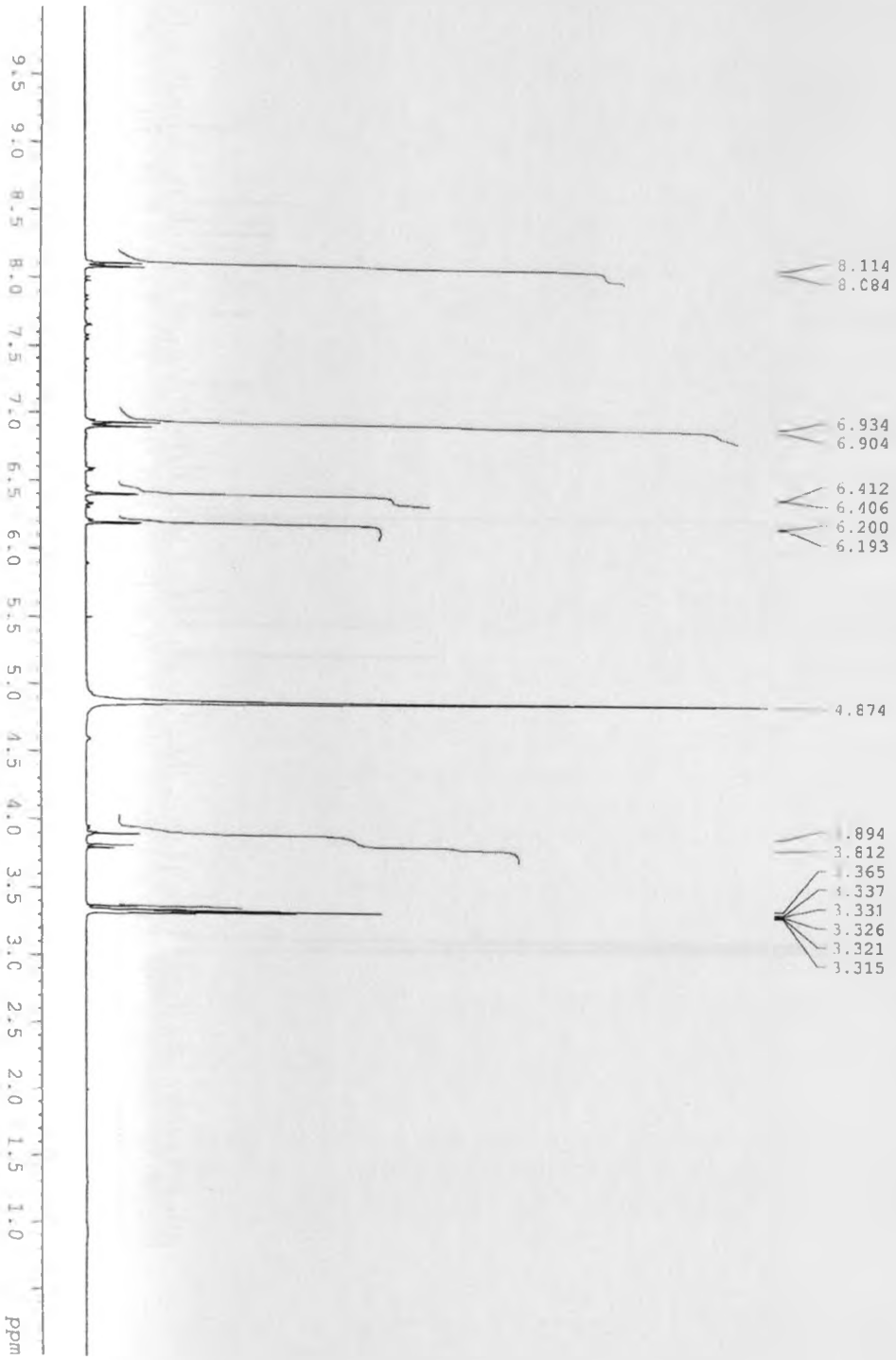
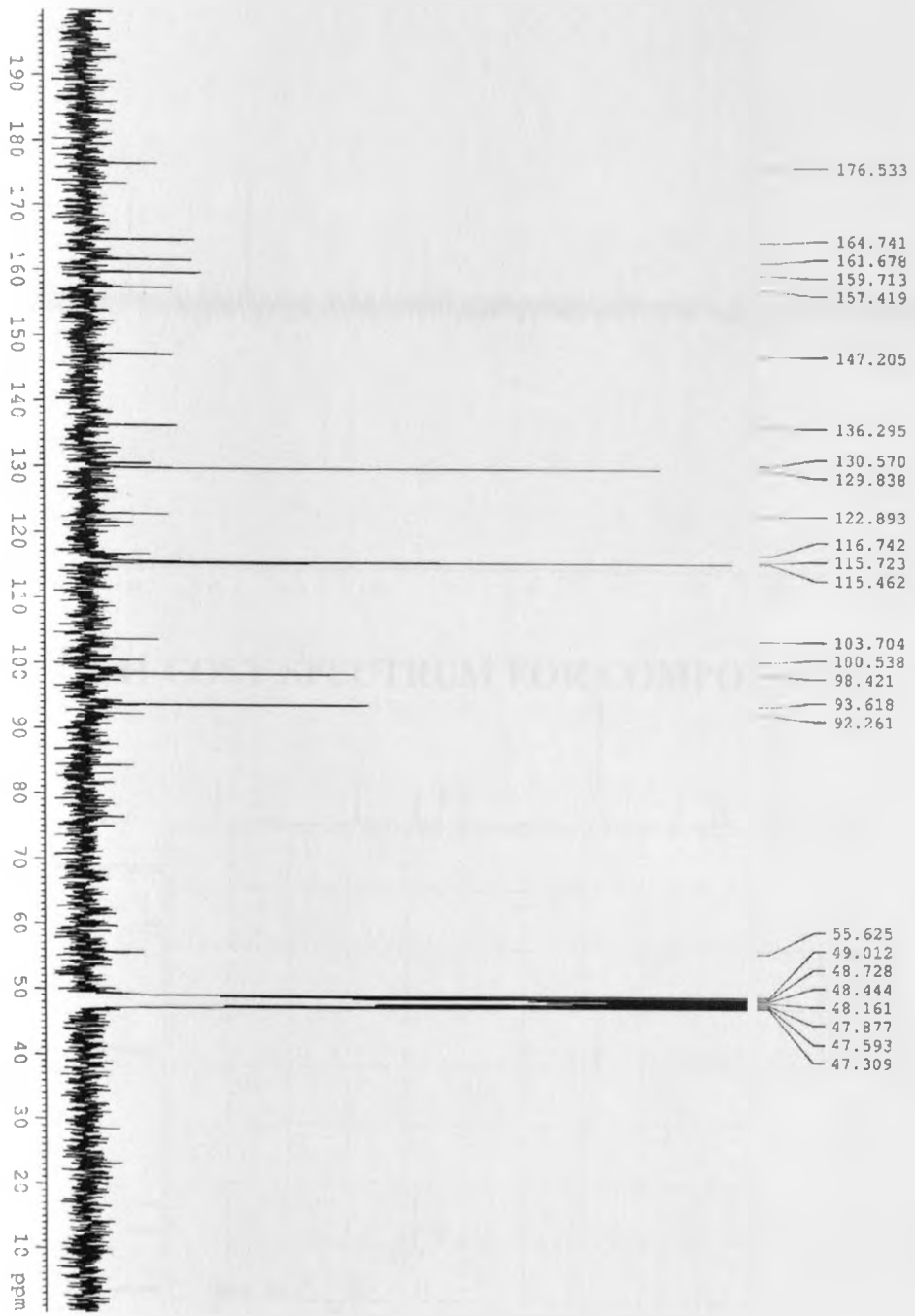


# APPENDIX V: SPECTRA FOR COMPOUND 5

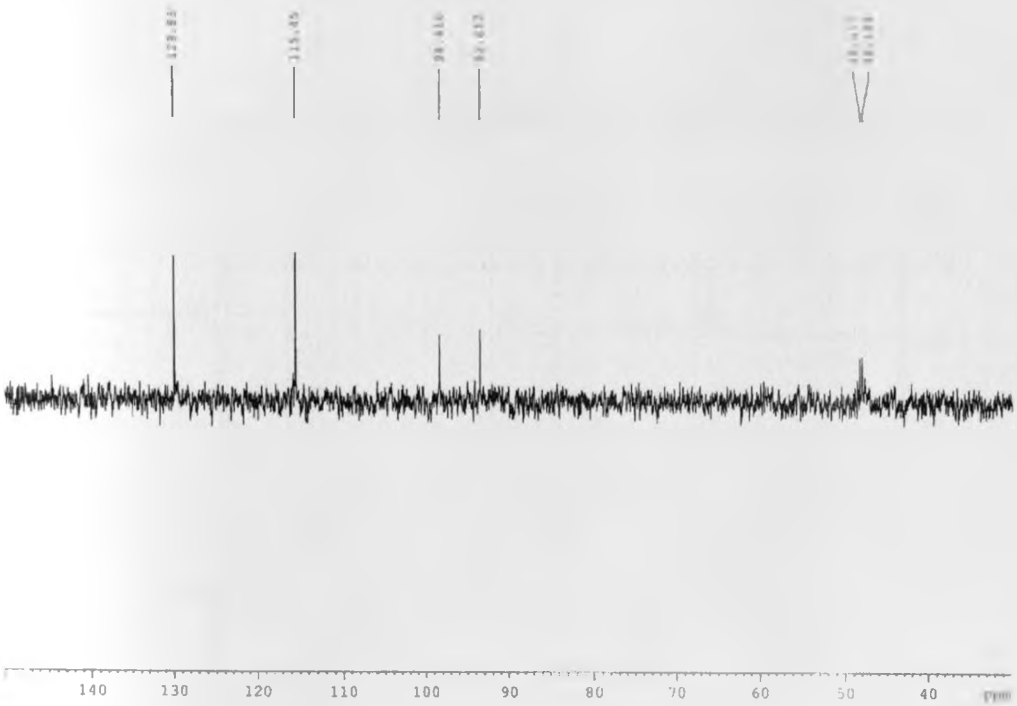
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 5



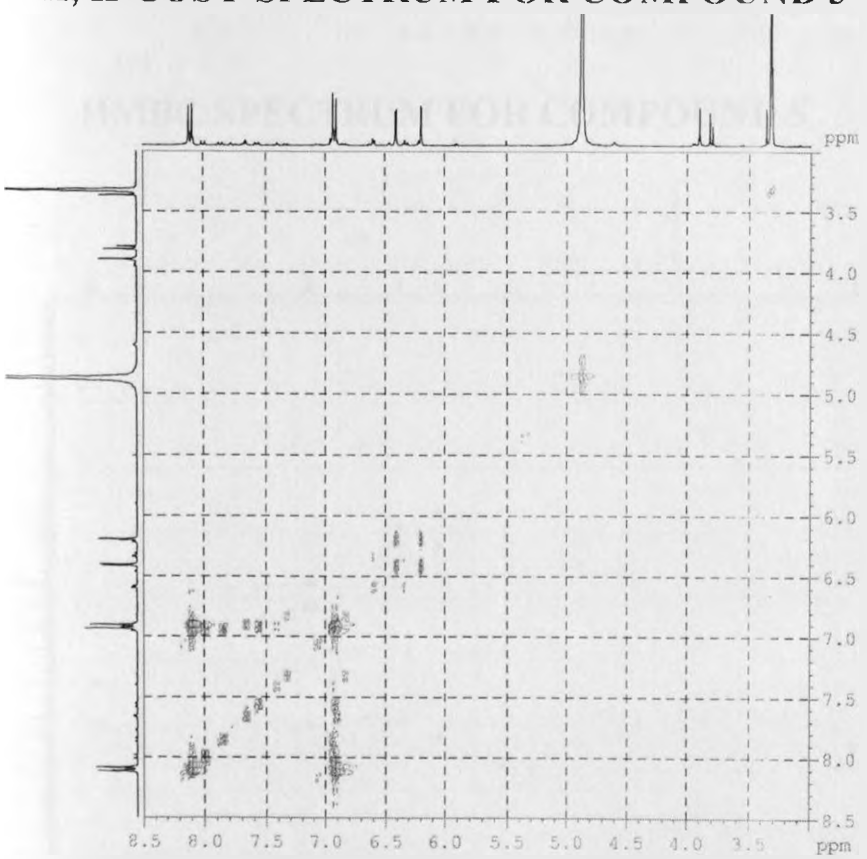
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 5



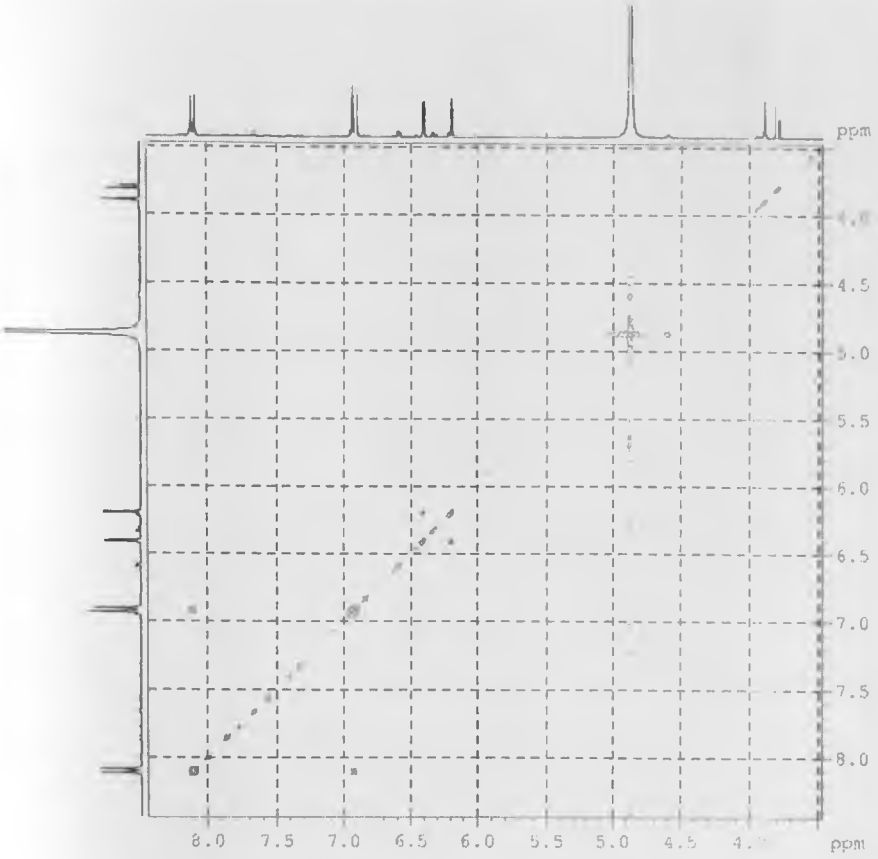
# DEPT SPECTRUM FOR COMPOUND 5



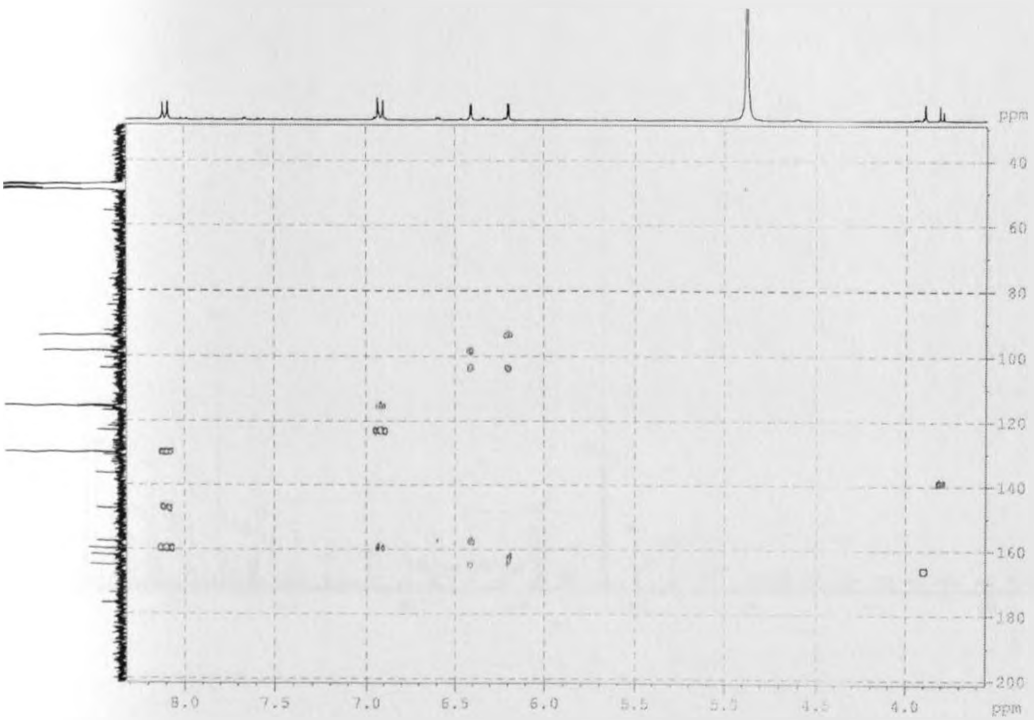

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 5



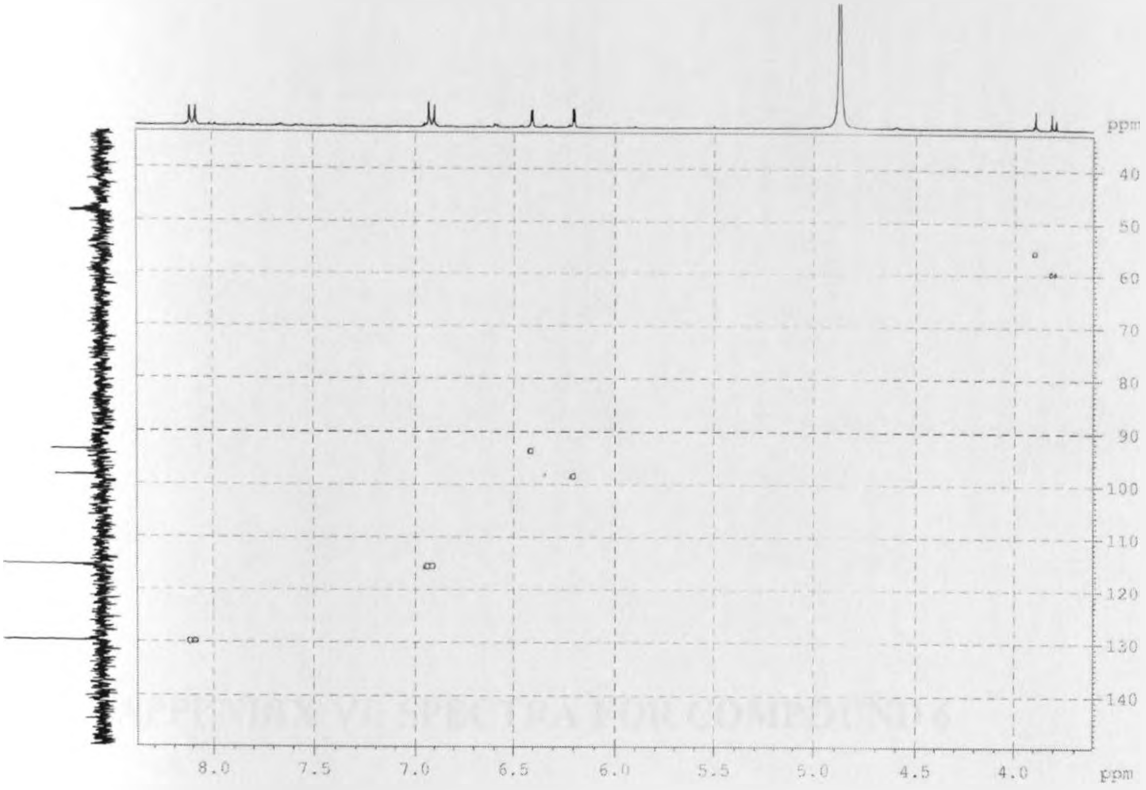
## NOESY SPECTRUM FOR COMPOUND 5



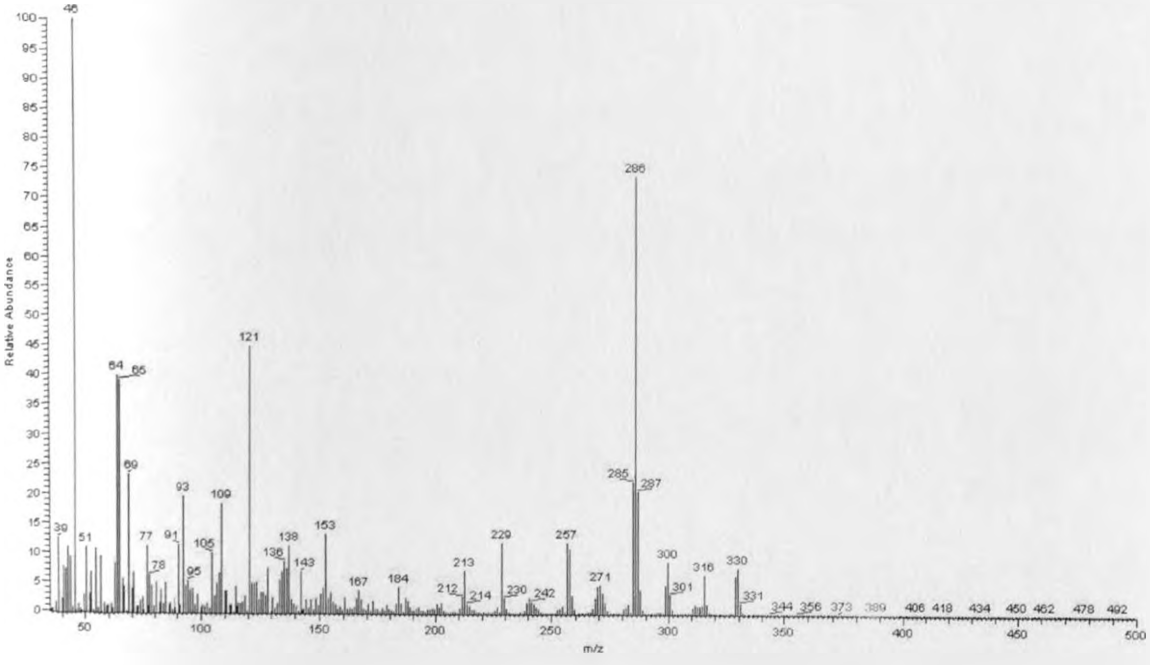

## HMBC SPECTRUM FOR COMPOUND 5



# HSQC-DEPT SPECTRUM FOR COMPOUND 5

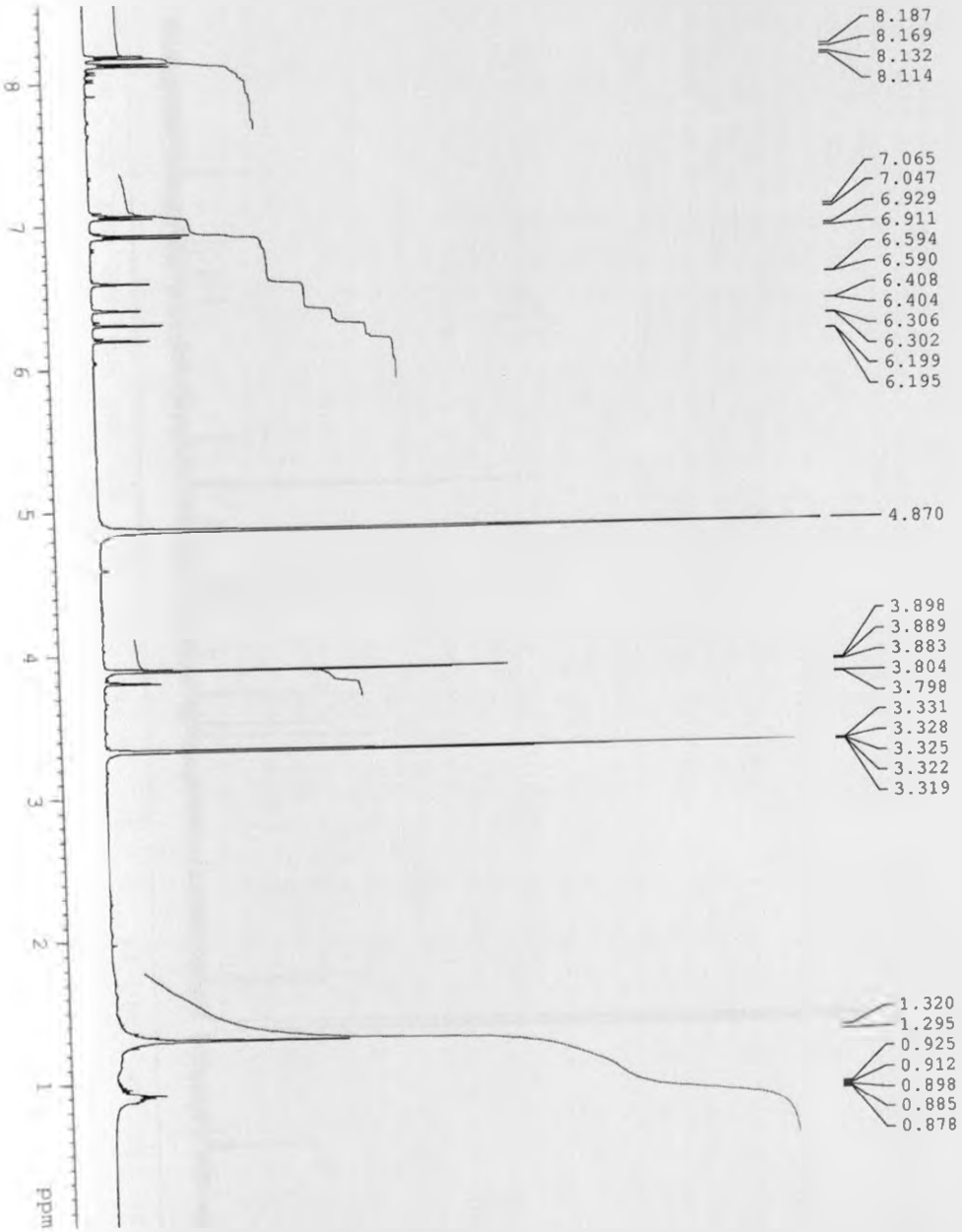



# MASS SPECTRUM FOR COMPOUND 5

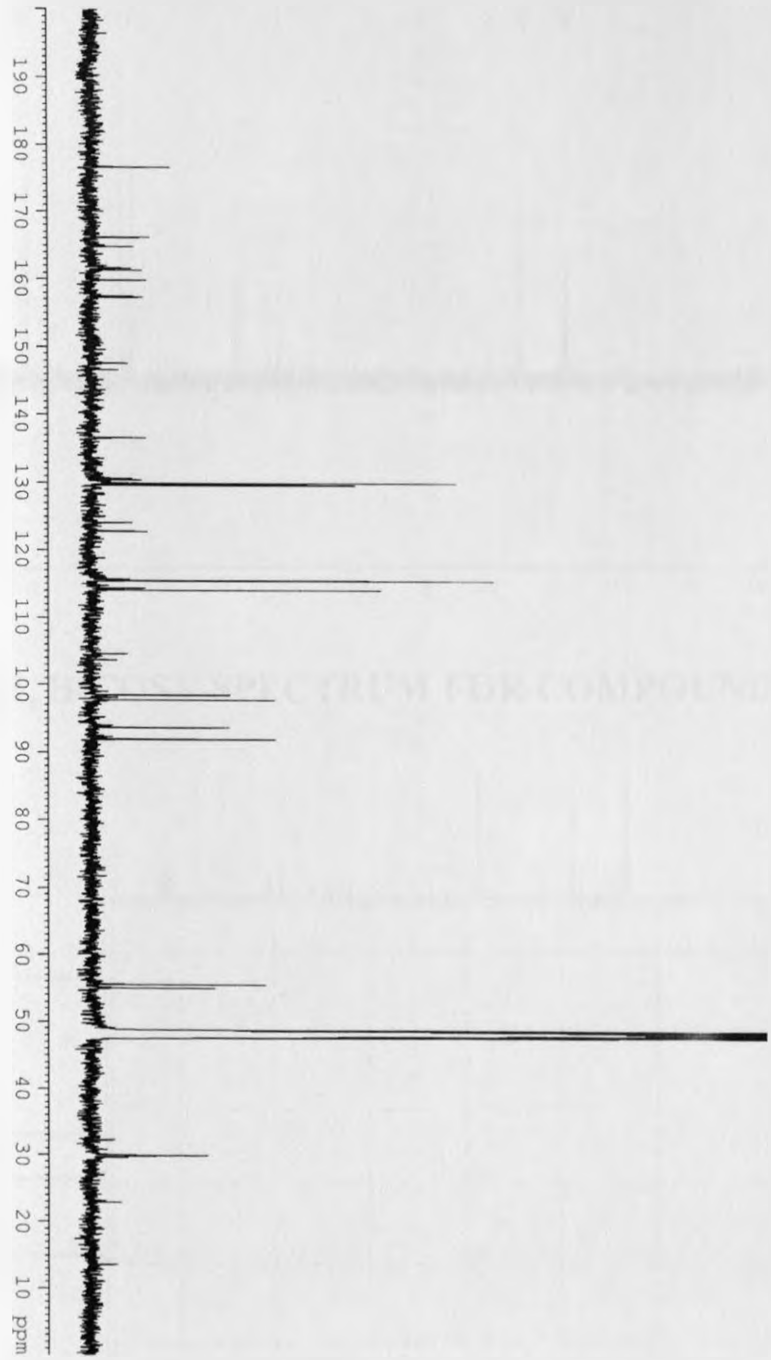


# APPENDIX VI: SPECTRA FOR COMPOUND 6

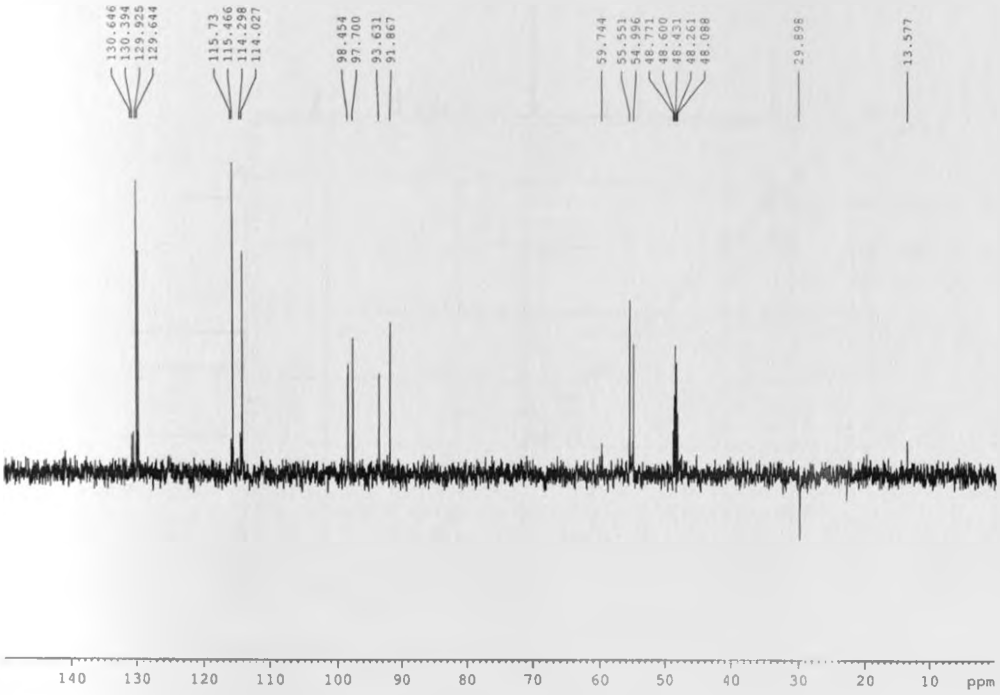
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 6



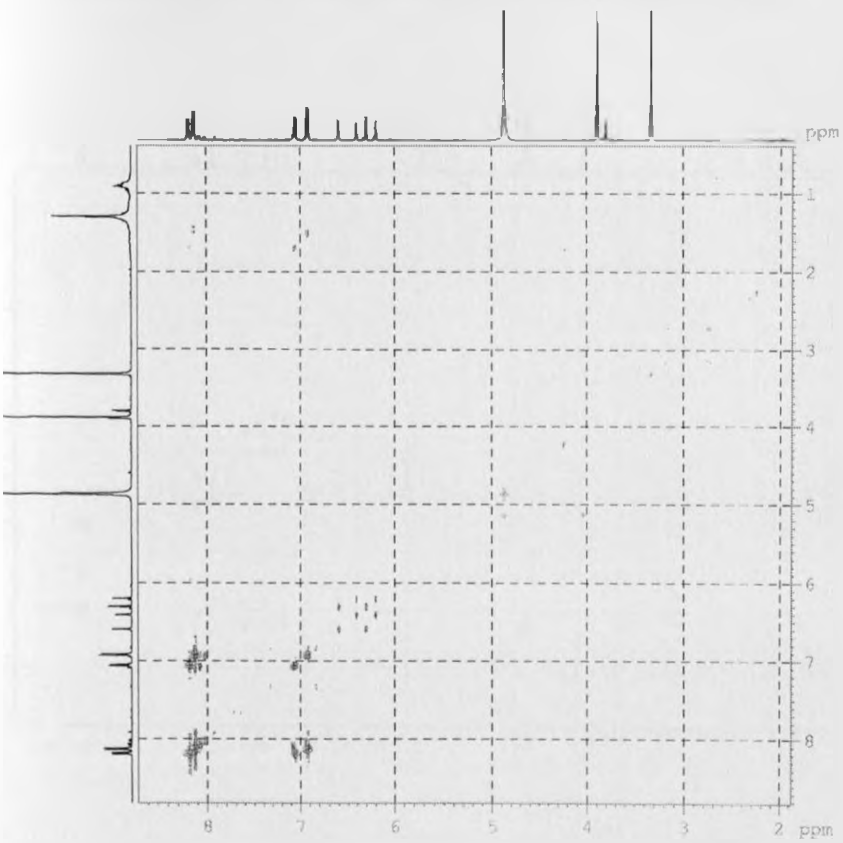
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 6



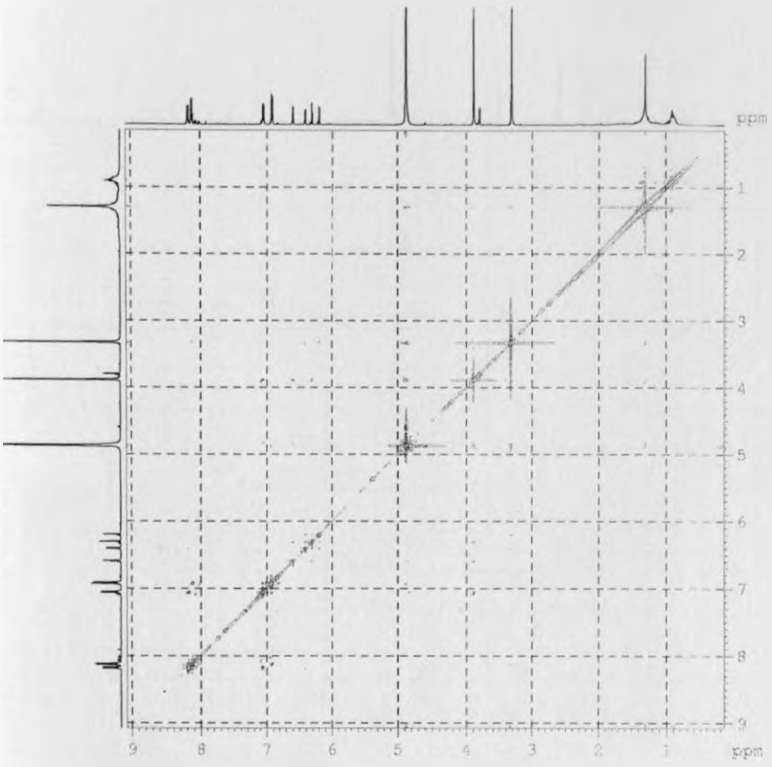
# DEPT SPECTRUM FOR COMPOUND 6



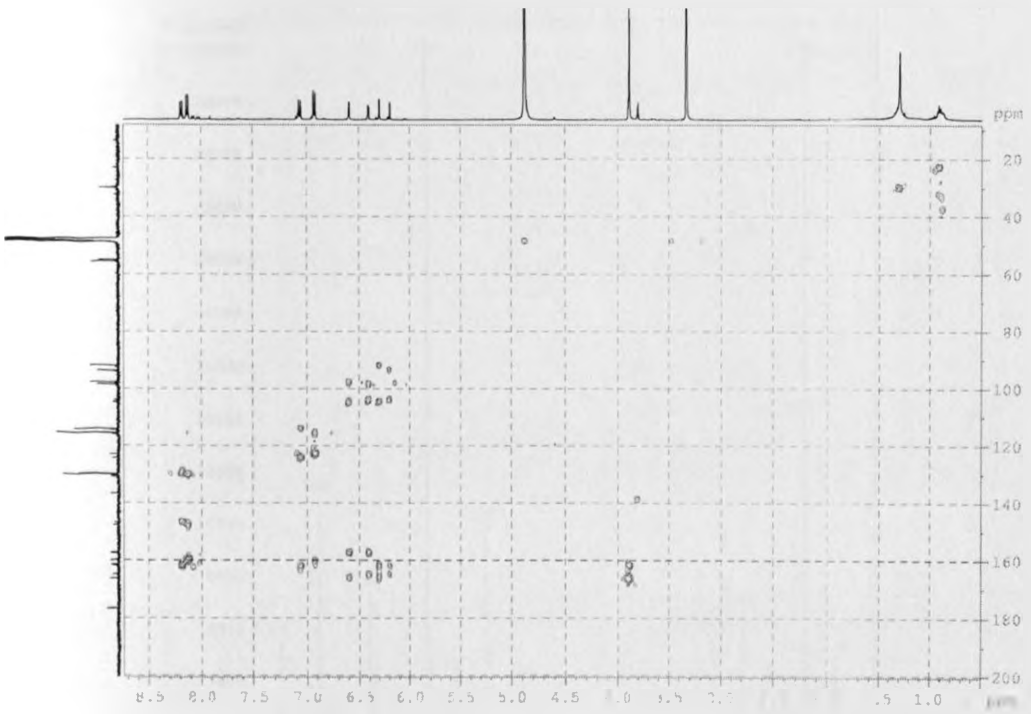

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 6



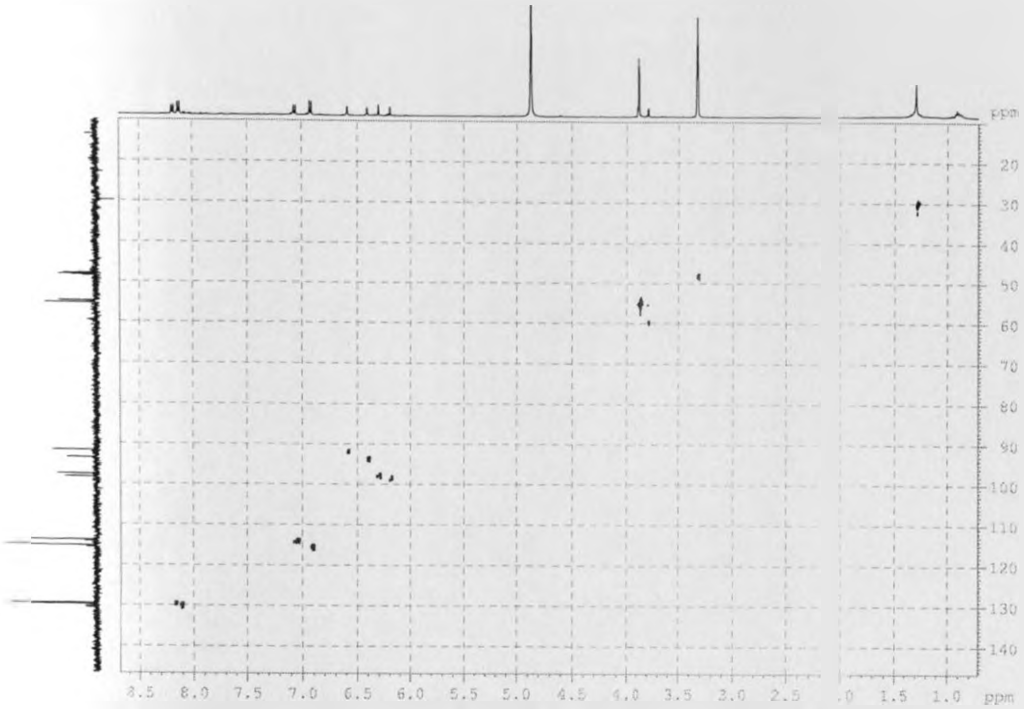
## NOESY SPECTRUM FOR COMPOUND 6



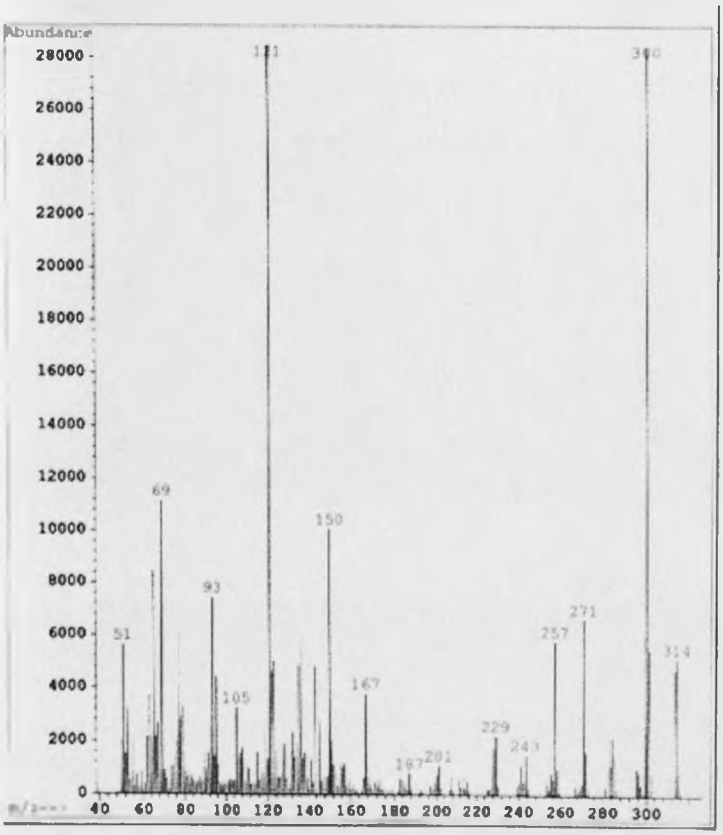

## HMBC SPECTRUM FOR COMPOUND 6



# HSQC-DEPT SPECTRUM FOR COMPOUND 6

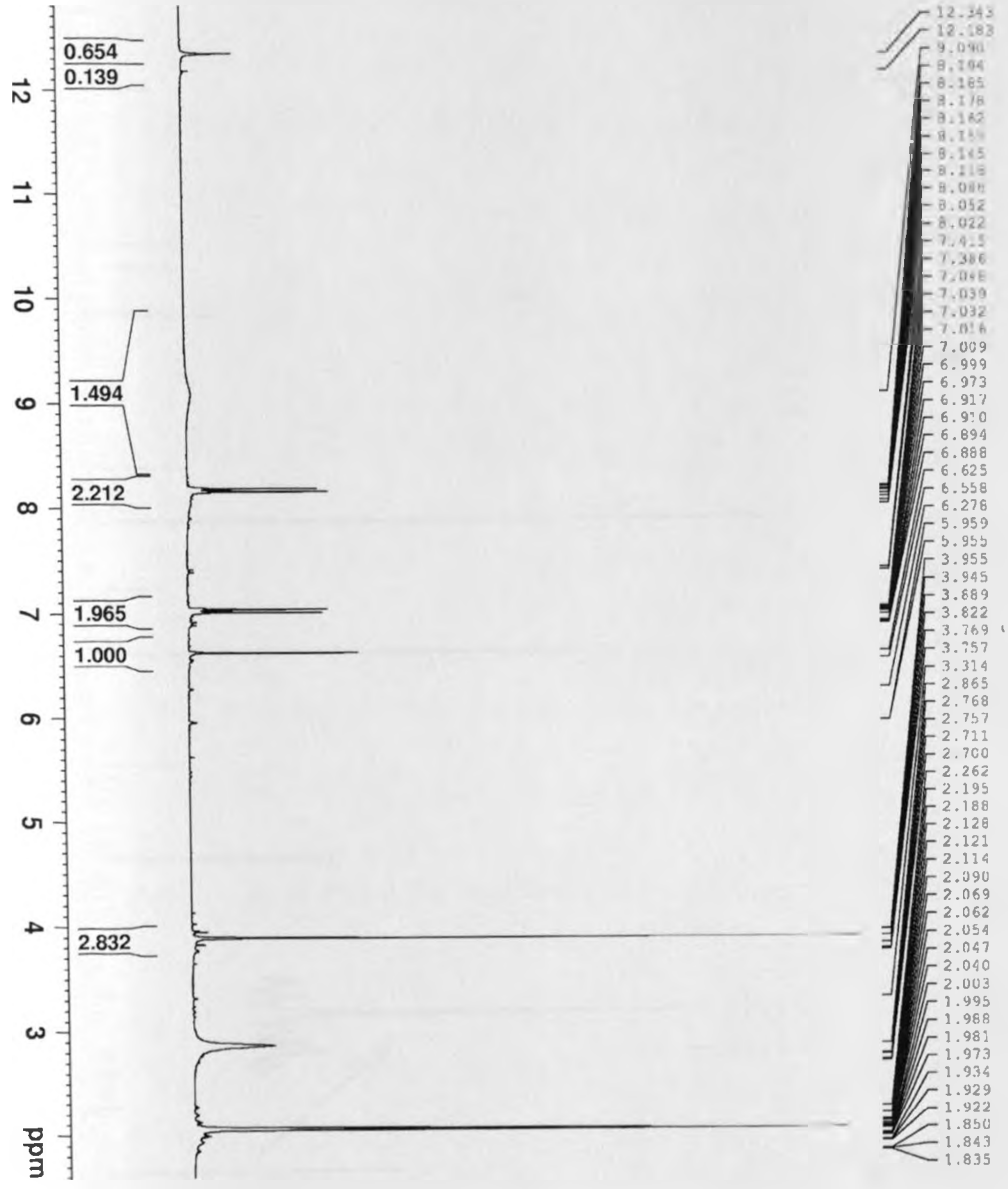



# MASS SPECTRUM FOR COMPOUND 6

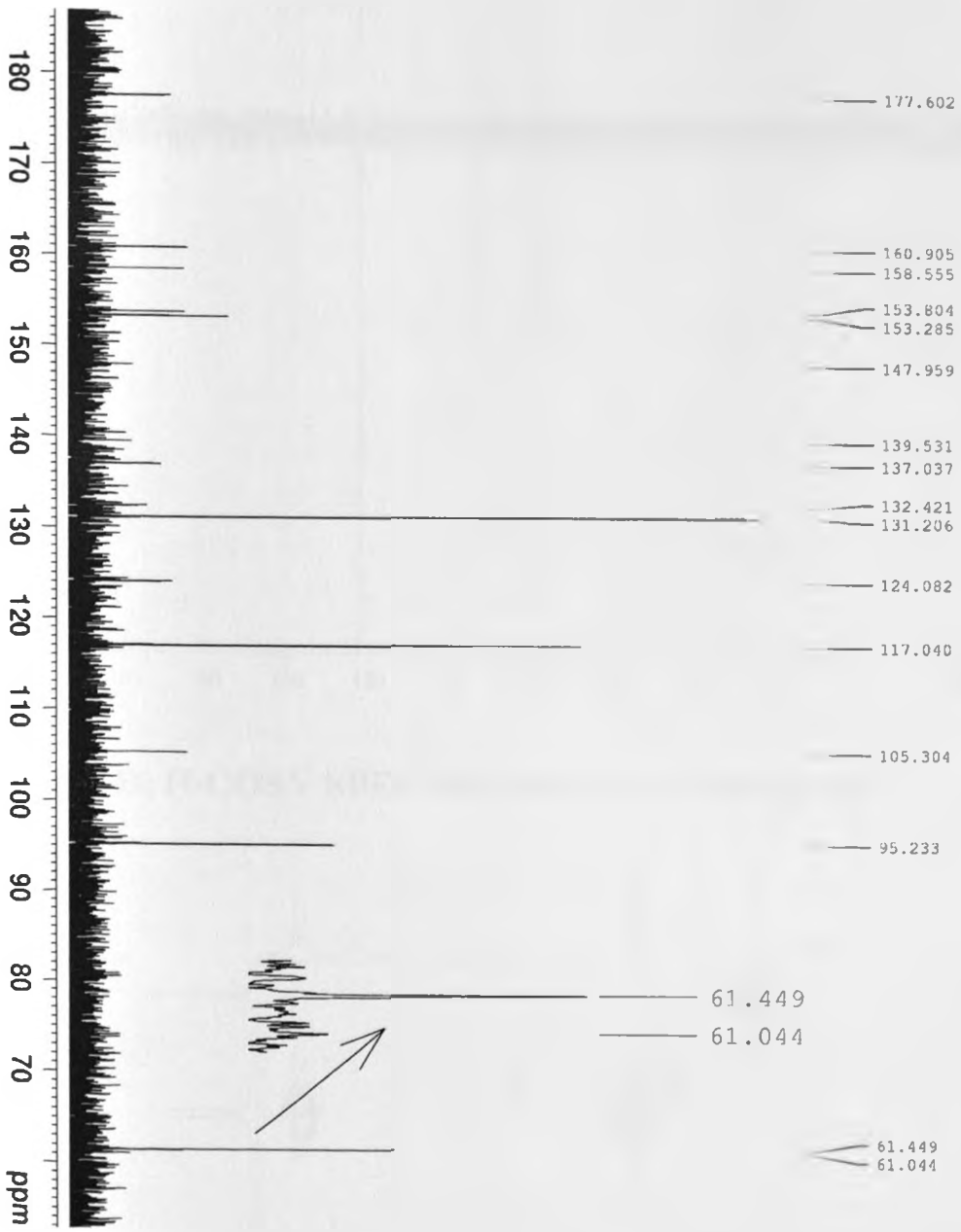


# APPENDIX VII: SPECTRA FOR COMPOUND 7

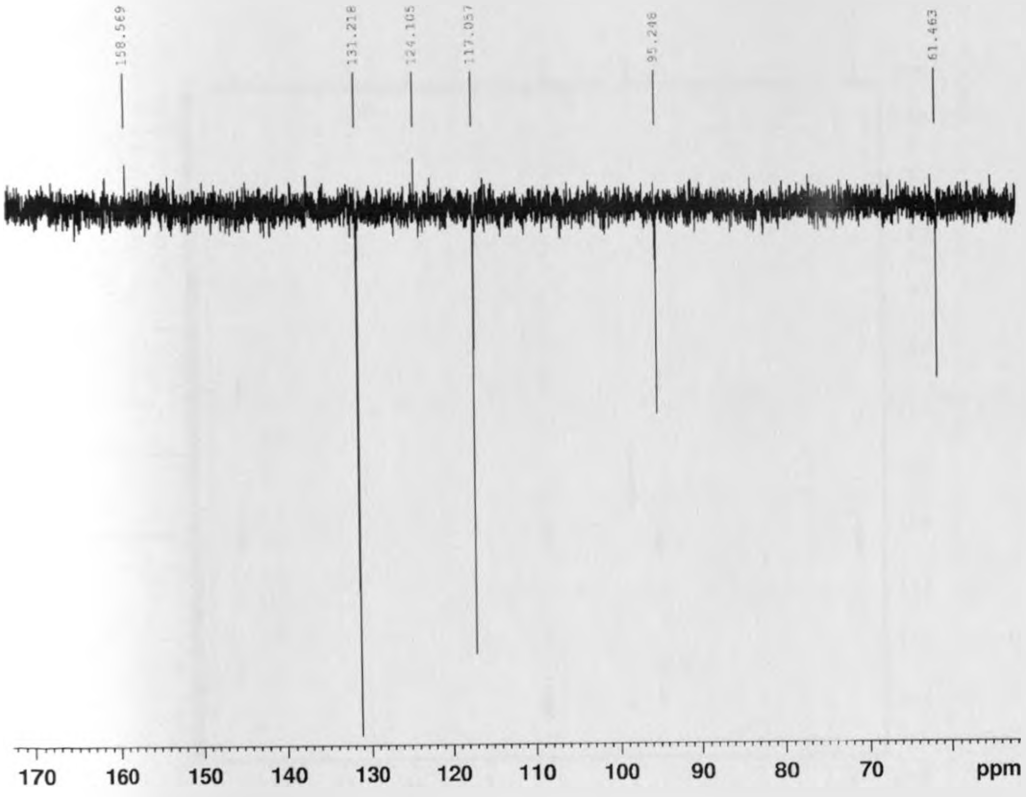
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 7



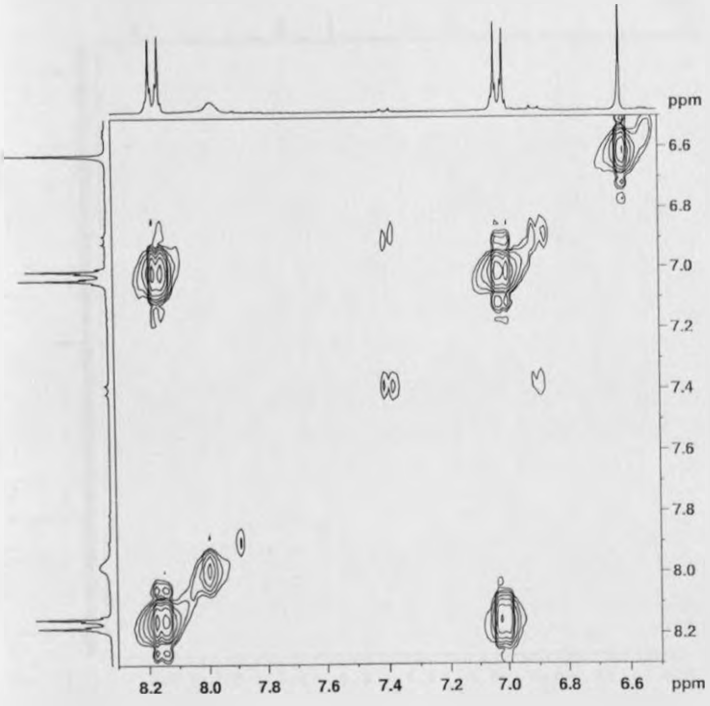
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 7



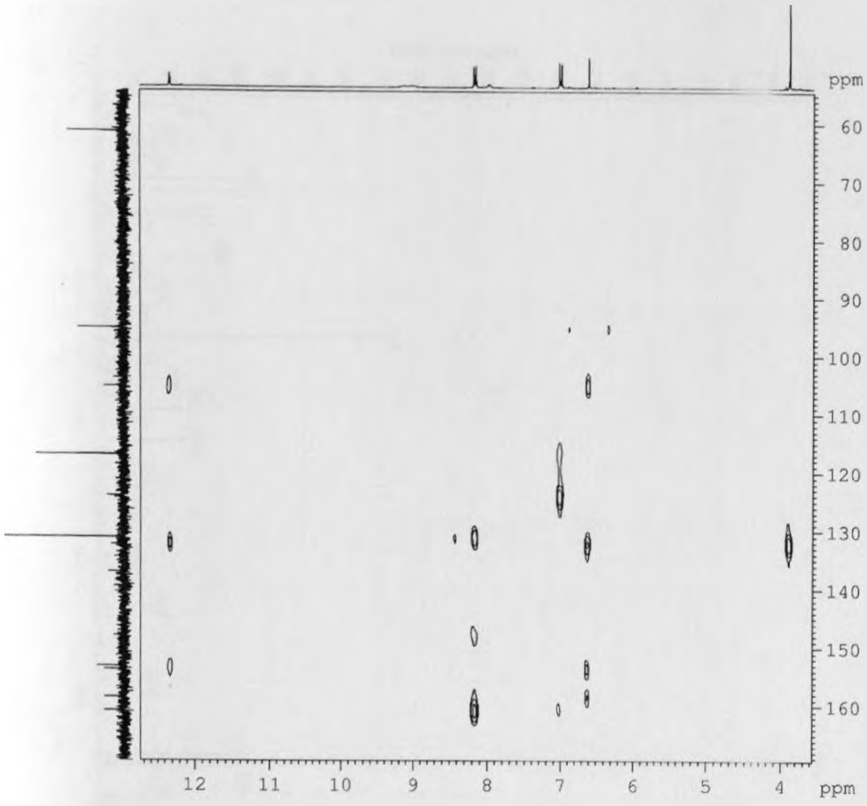
# APT SPECTRUM FOR COMPOUND 7



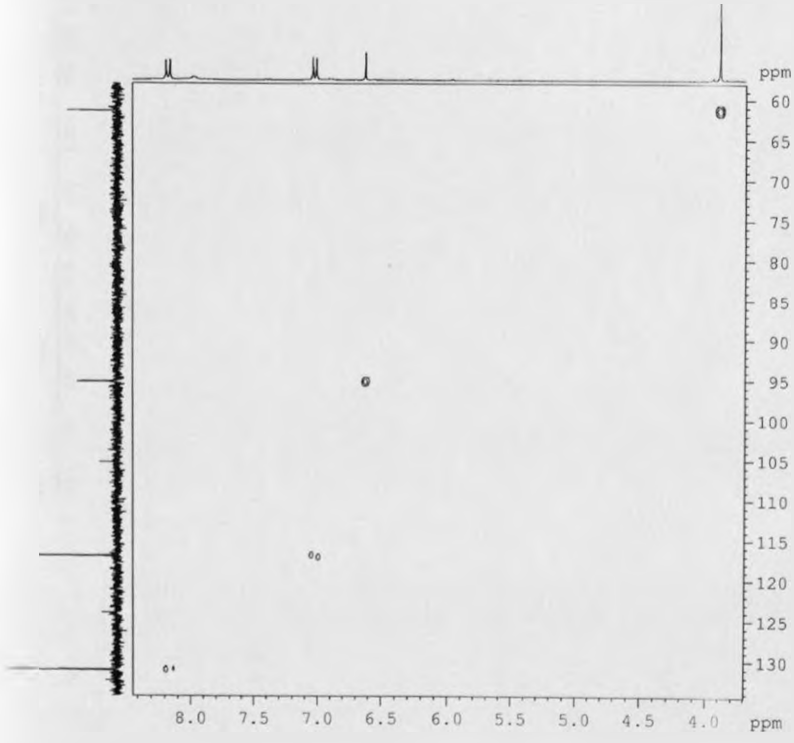

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 7



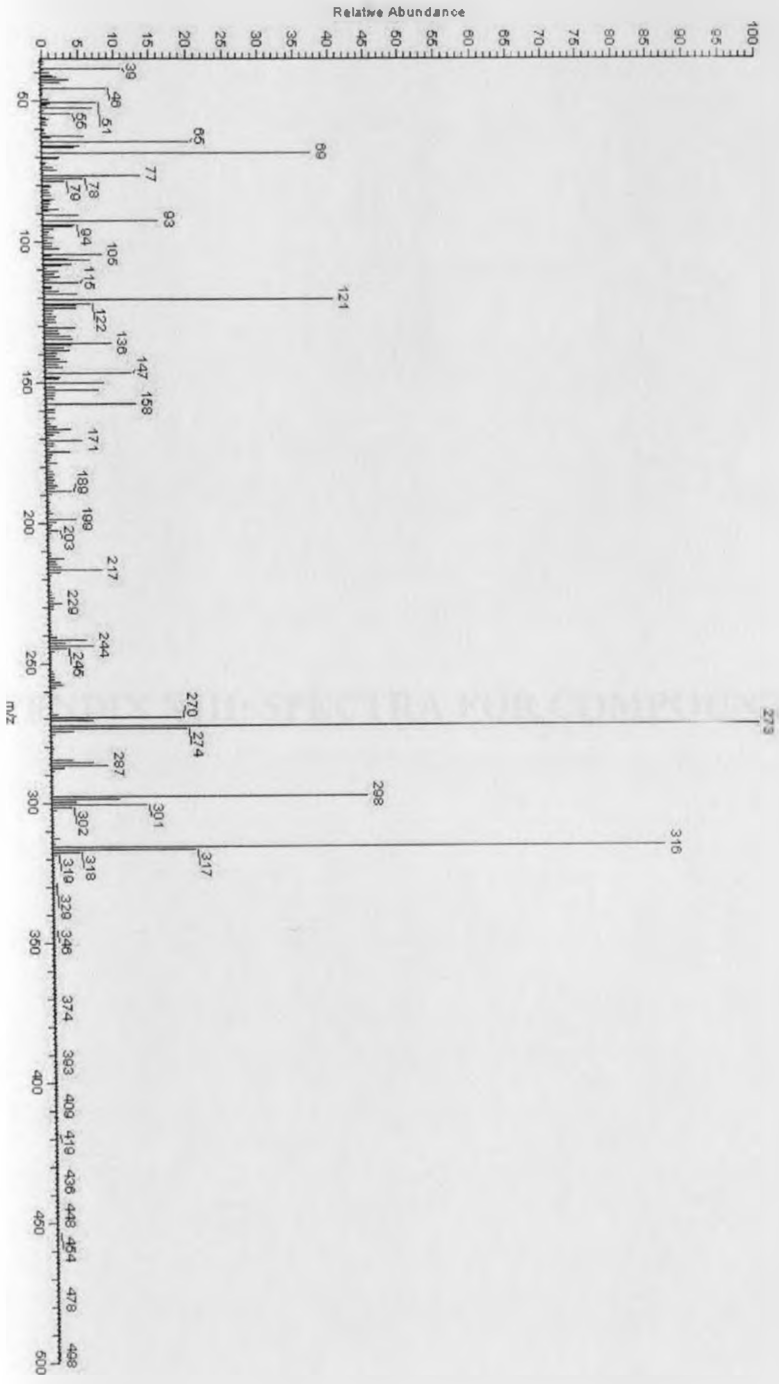
# HMBC SPECTRUM FOR COMPOUND 7



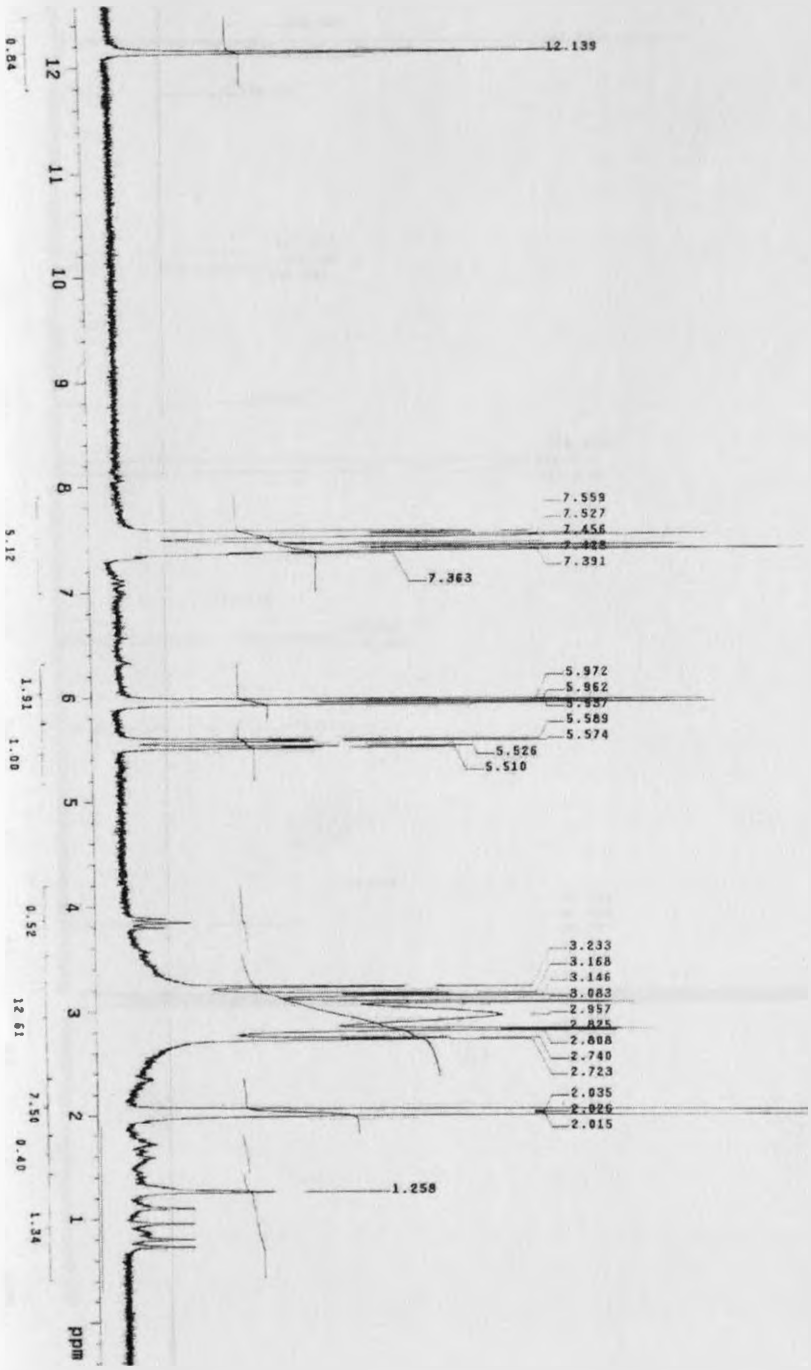

# HMQC SPECTRUM FOR COMPOUND 7



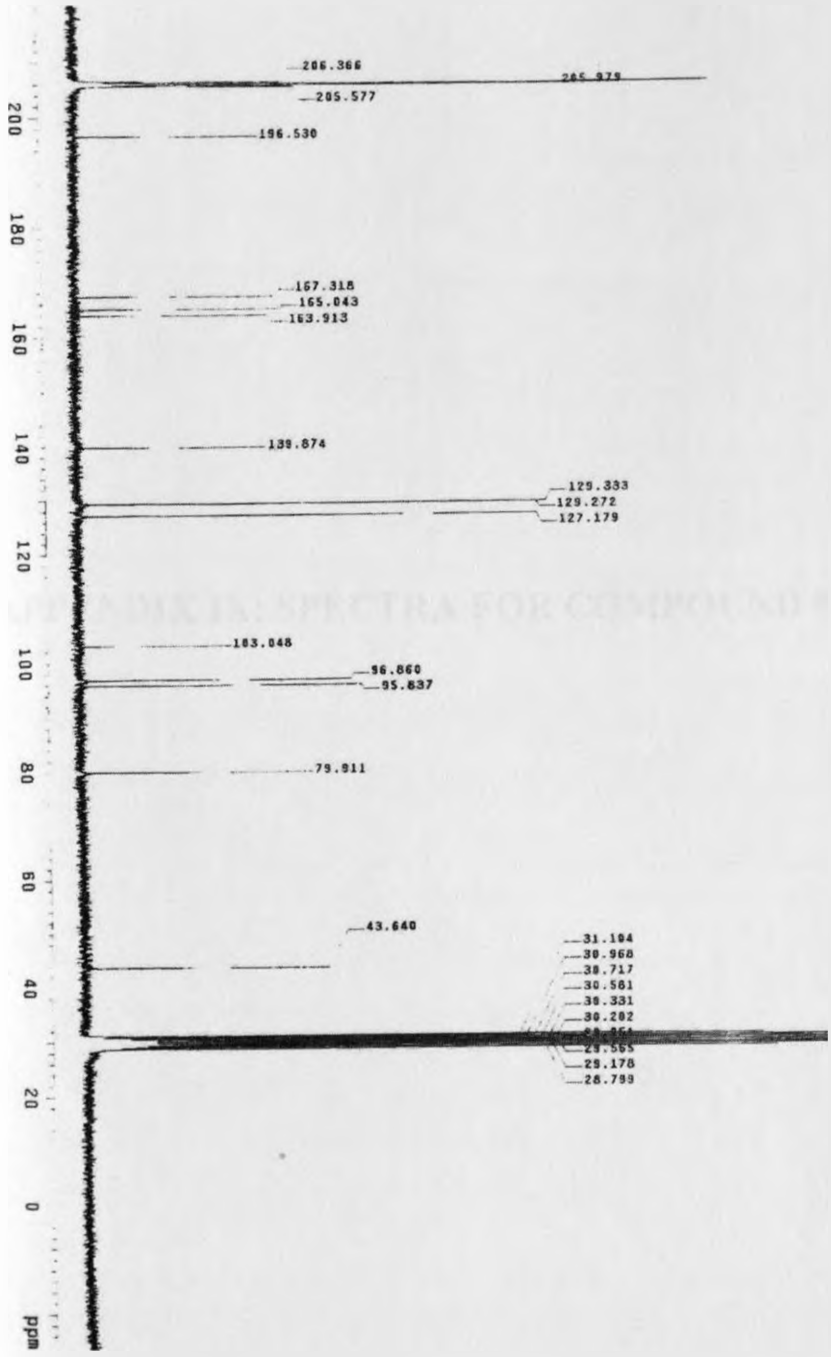
# MASS SPECTRUM FOR COMPOUND 7



# $^{1}$H-NMR SPECTRUM FOR COMPOUND 8

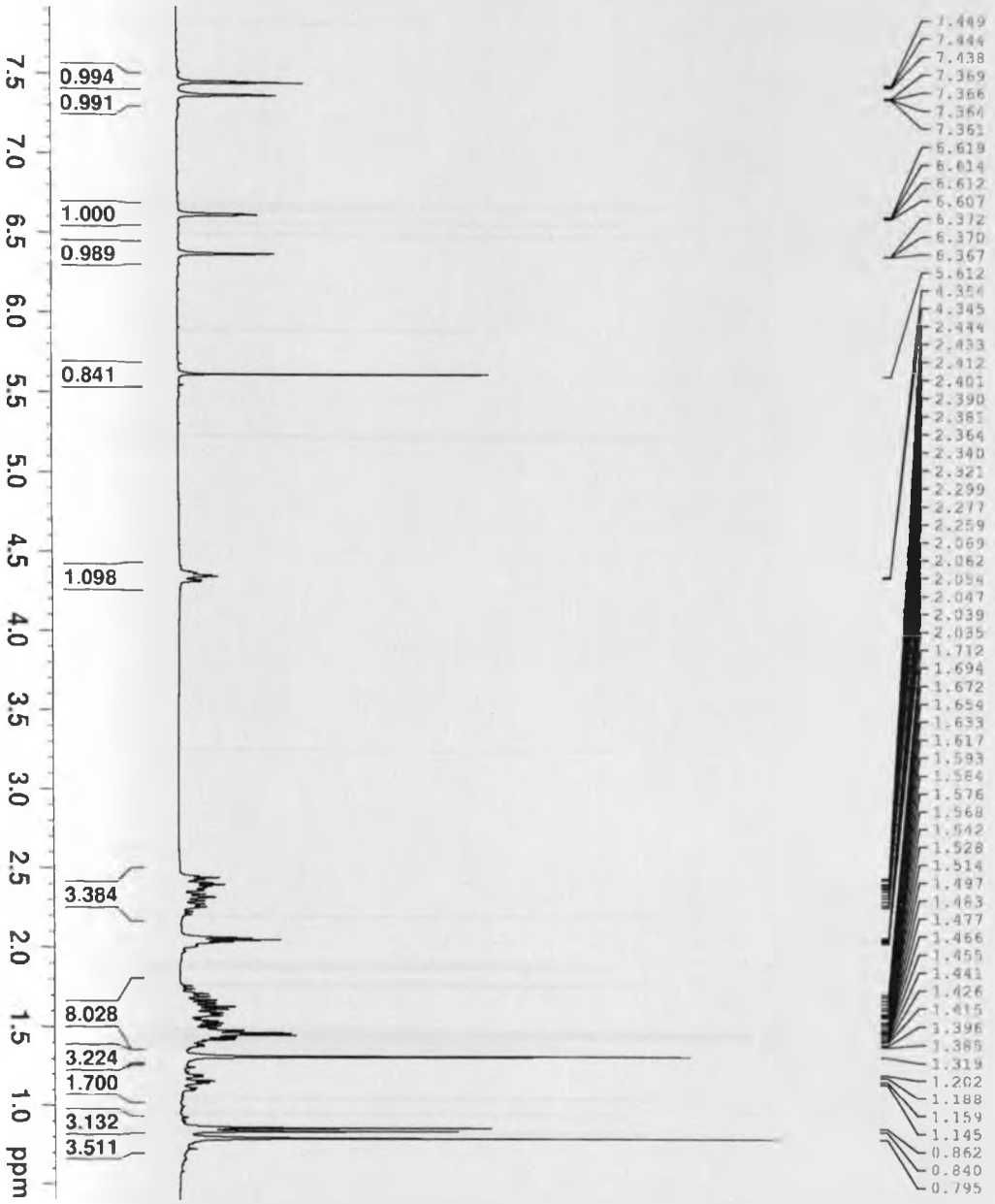


# $^{13}$C-NMR SPECTRUM FOR COMPOUND 8

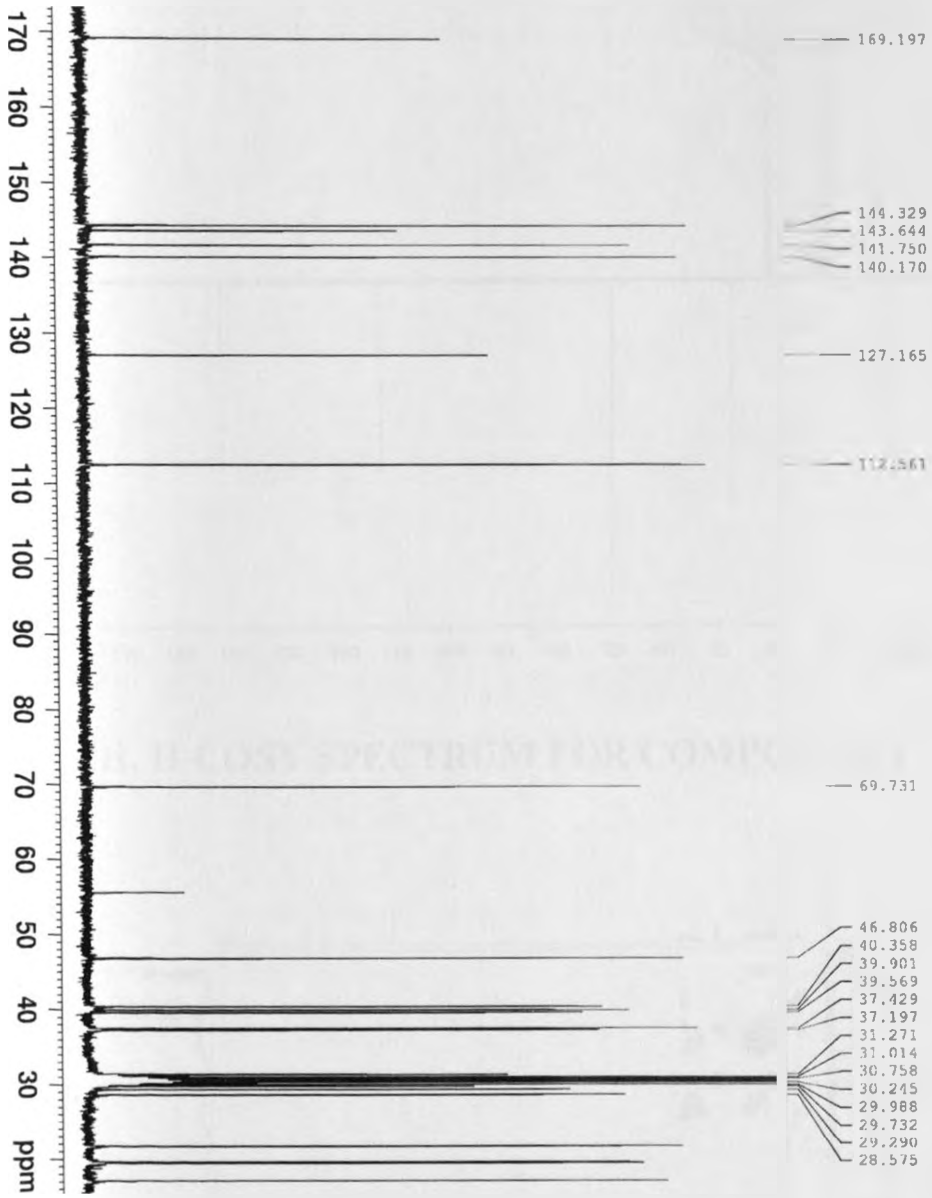


# APPENDIX IX: SPECTRA FOR COMPOUND 9

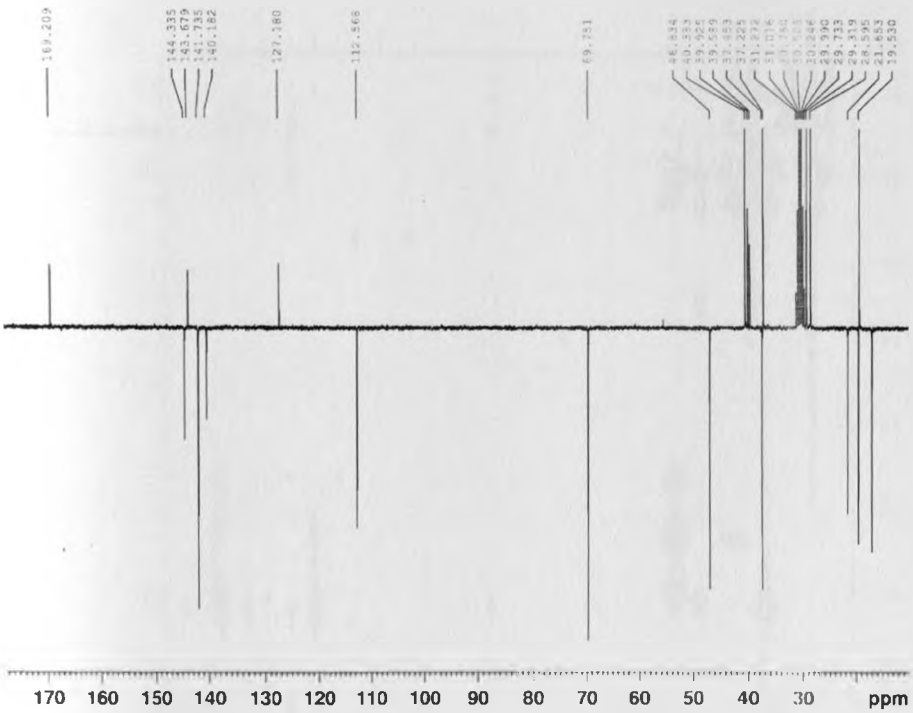
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 9



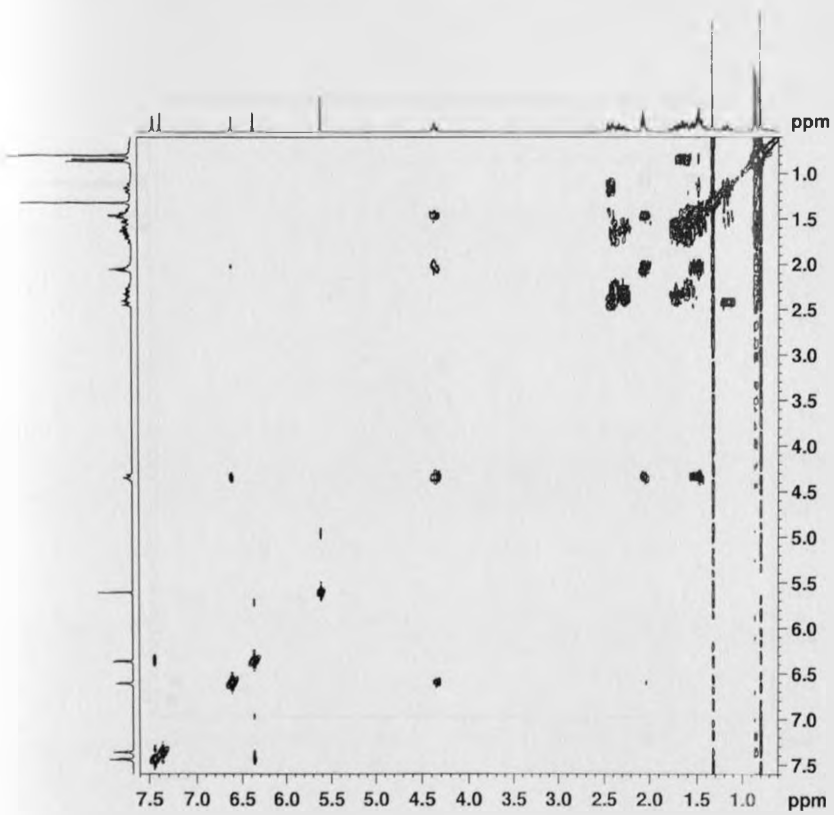
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 9



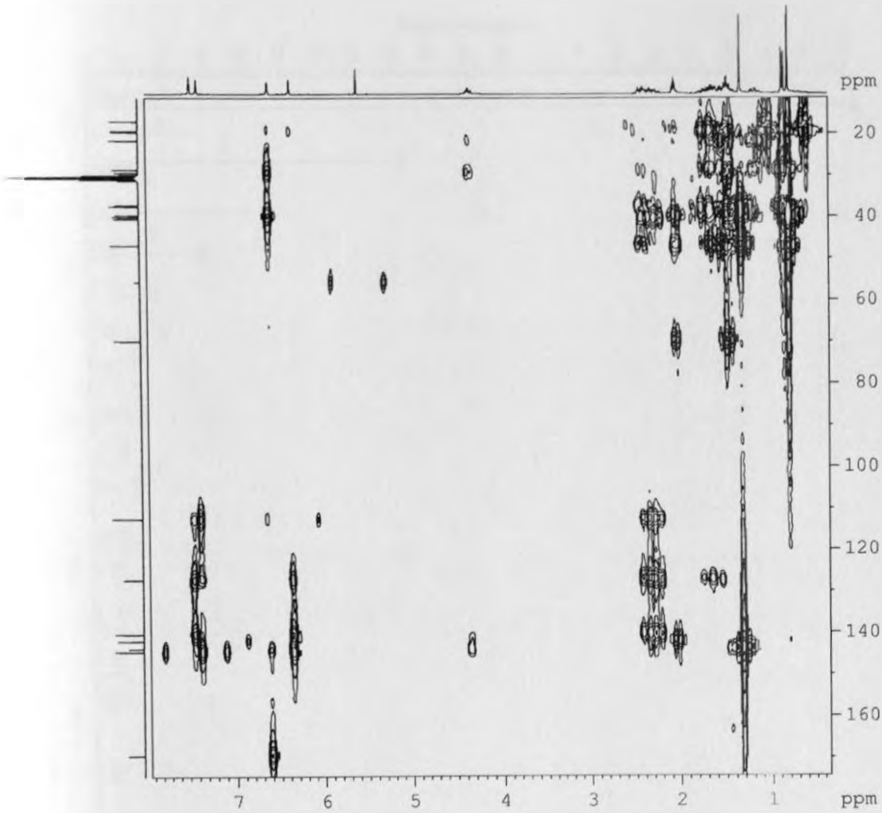
# APT SPECTRUM FOR COMPOUND 9



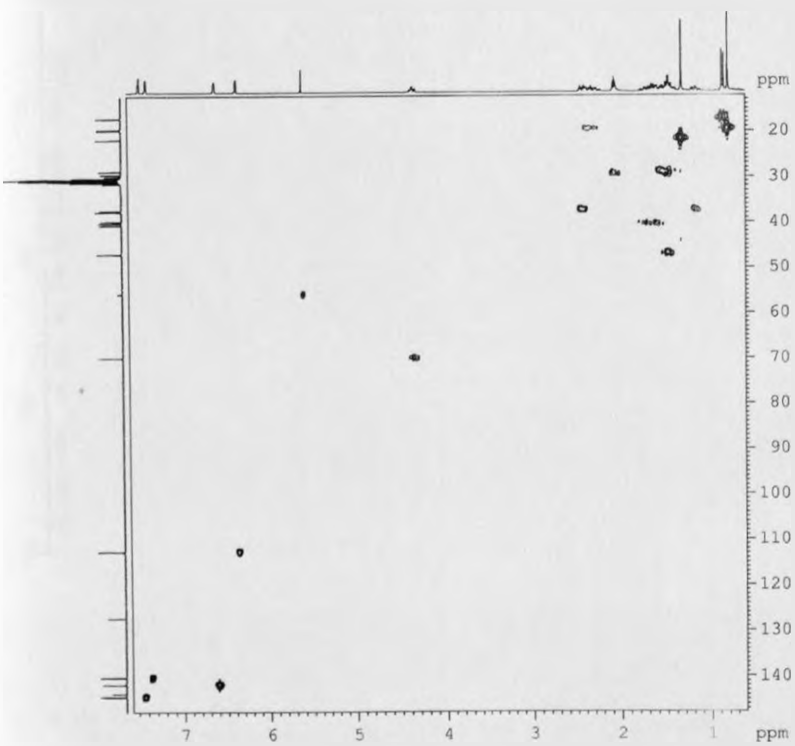

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 9



## HMBC SPECTRUM FOR COMPOUND 9



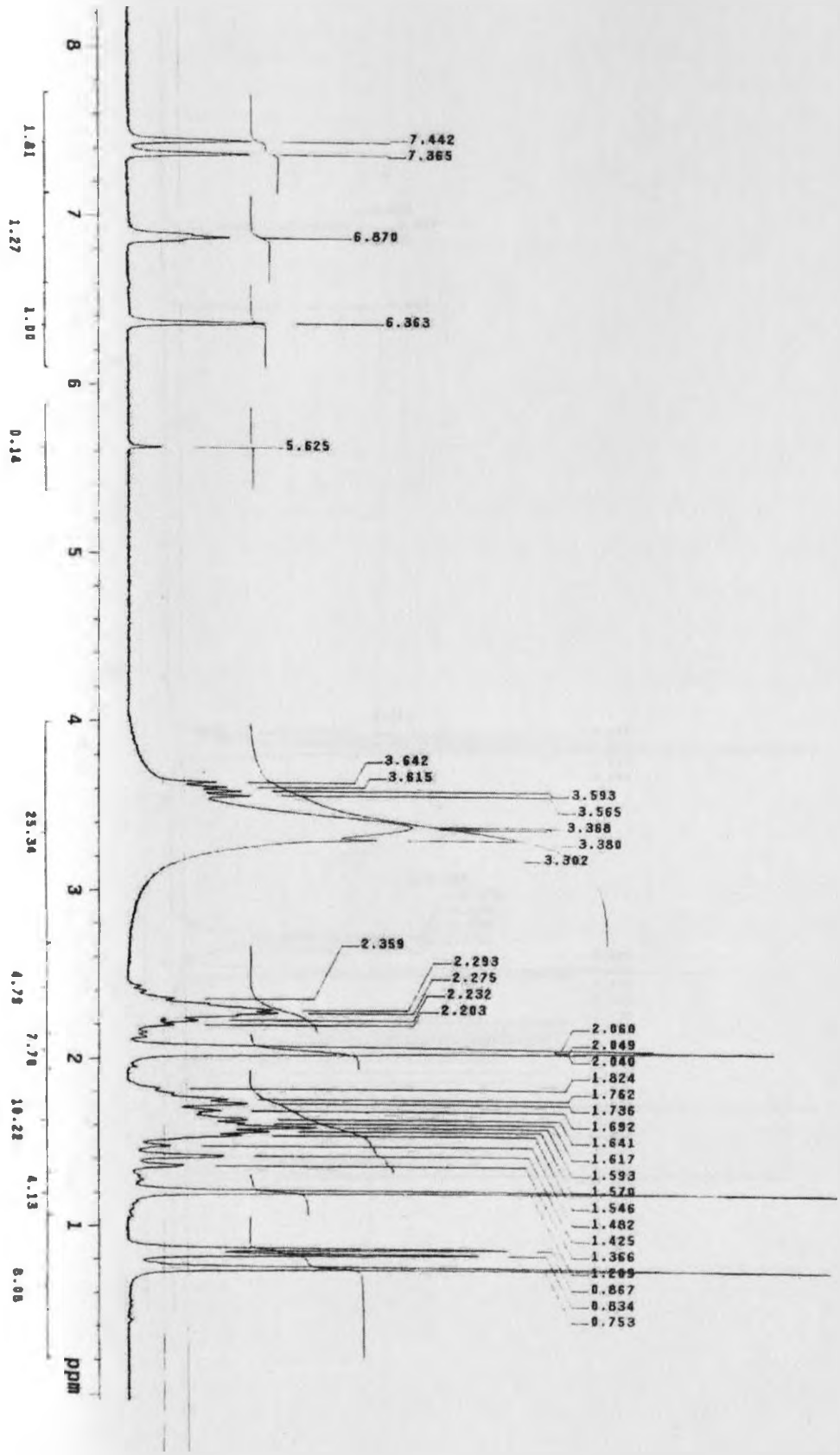

## HMQC SPECTRUM FOR COMPOUND 9



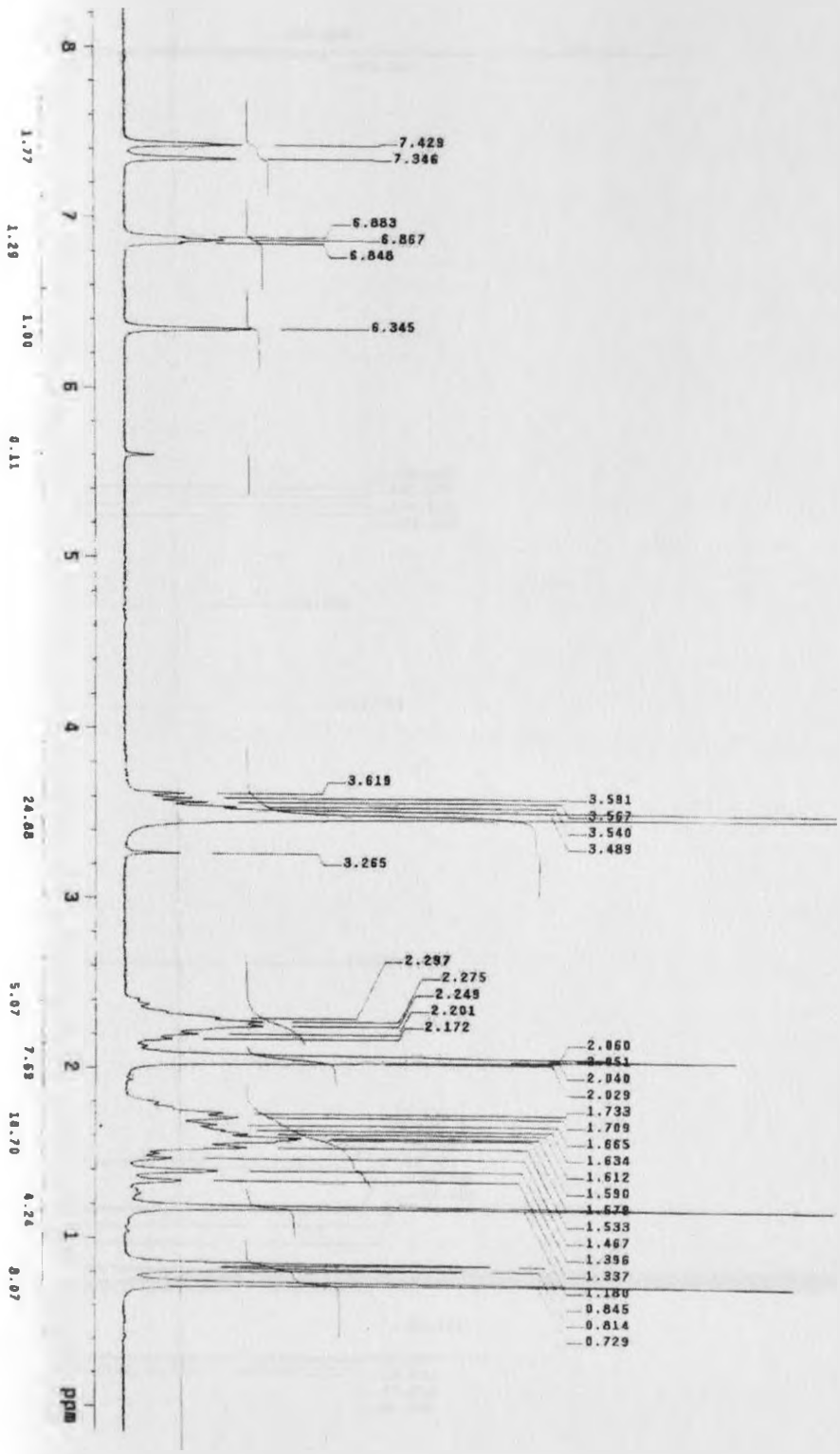
# MASS SPECTRUM FOR COMPOUND 9

# APPENDIX X: SPECTRA FOR COMPOUND 10

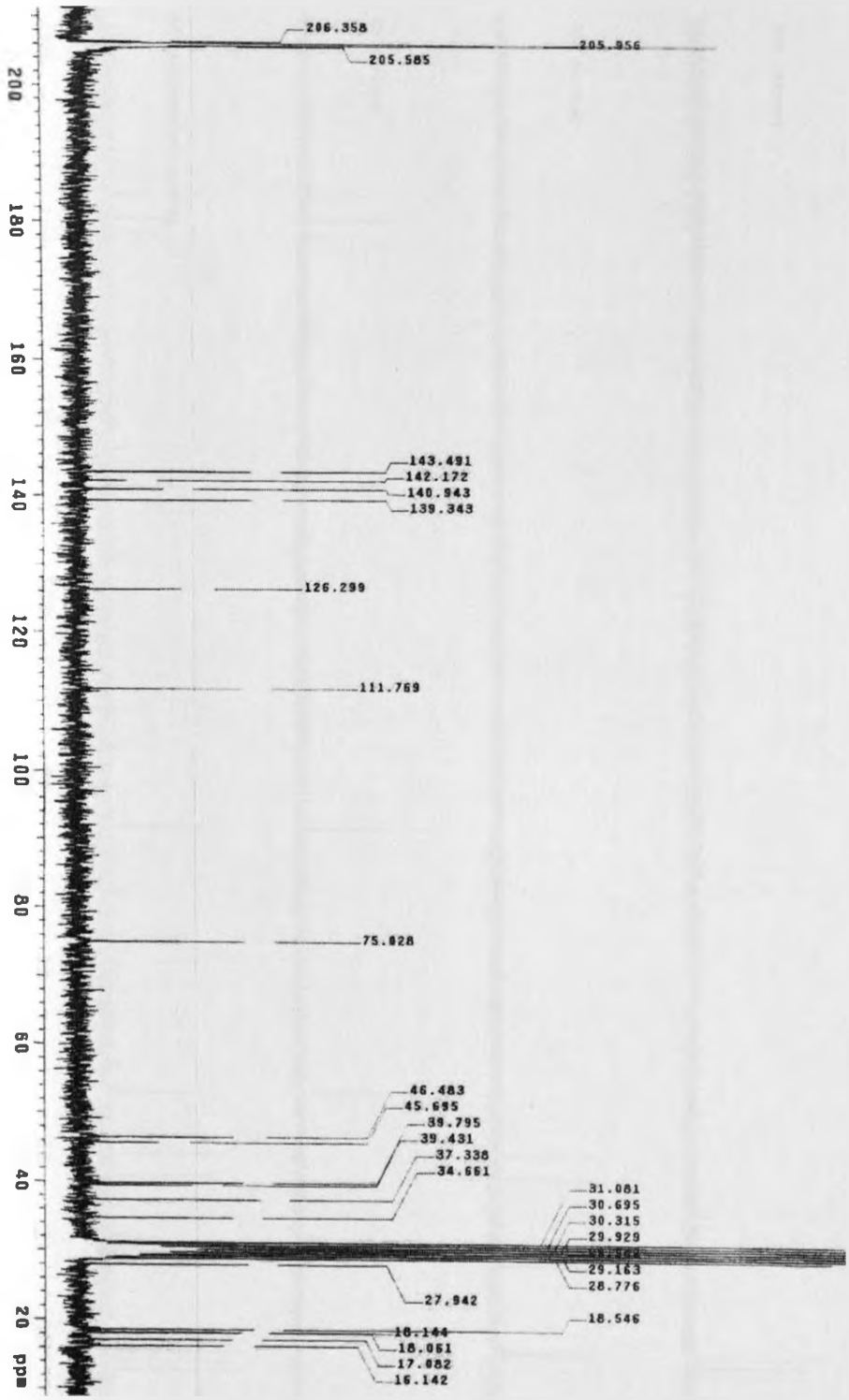
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 10



# $^{1}$H-NMR SPECTRUM FOR COMPOUND 10

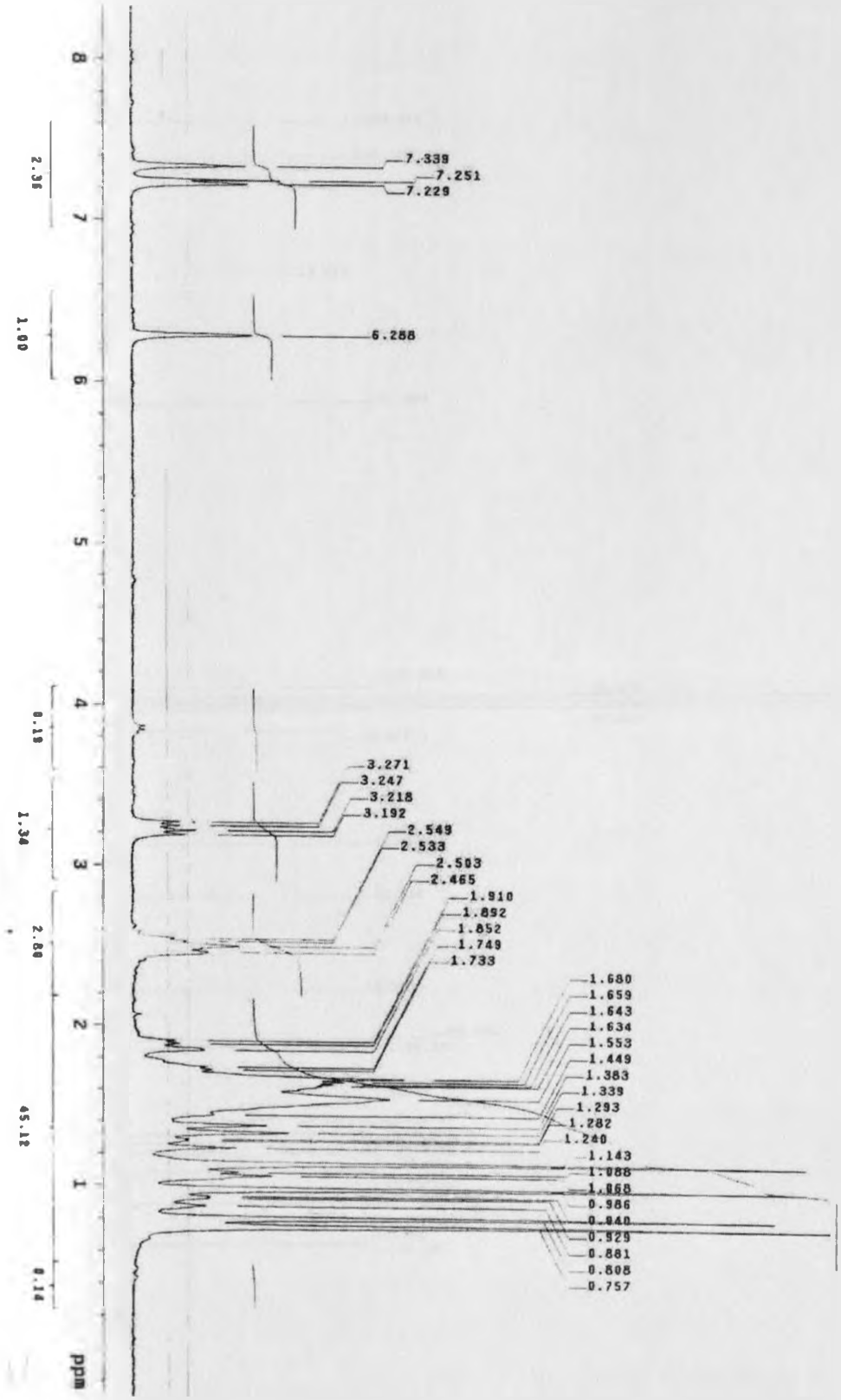


# $^{13}$C-NMR SPECTRUM FOR COMPOUND 10

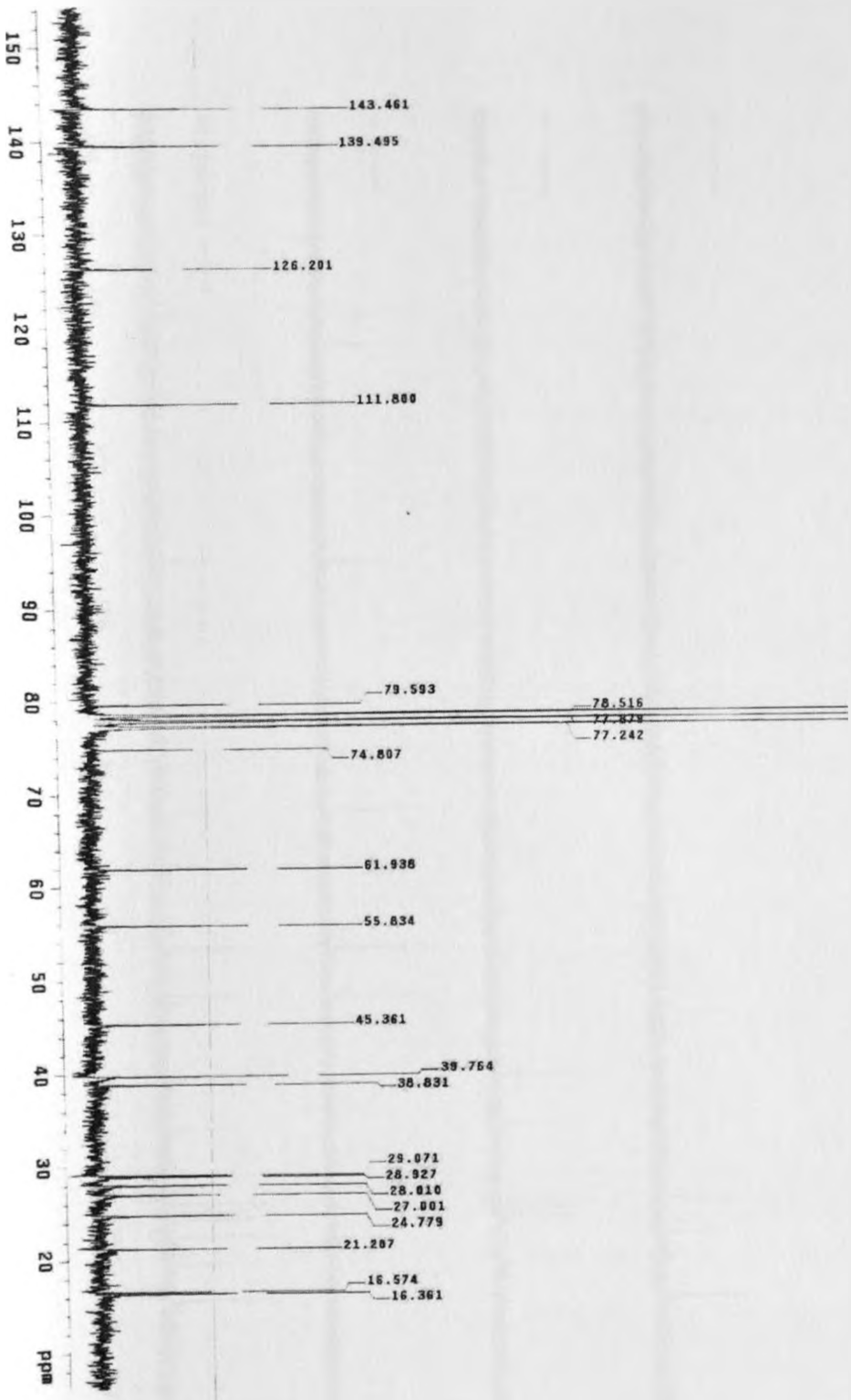


# APPENDIX XI: SPECTRA FOR COMPOUND 11

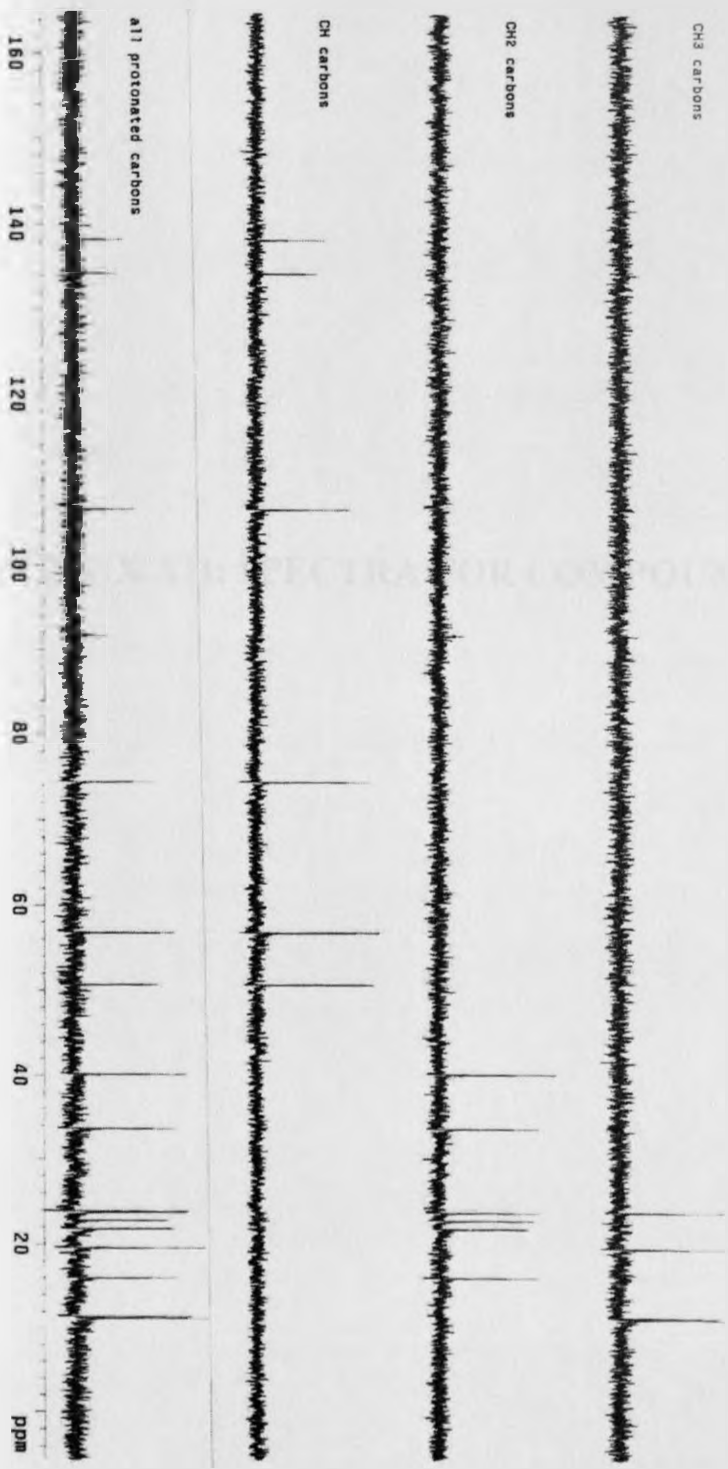
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 11



# $^{13}$C-NMR SPECTRUM FOR COMPOUND 11

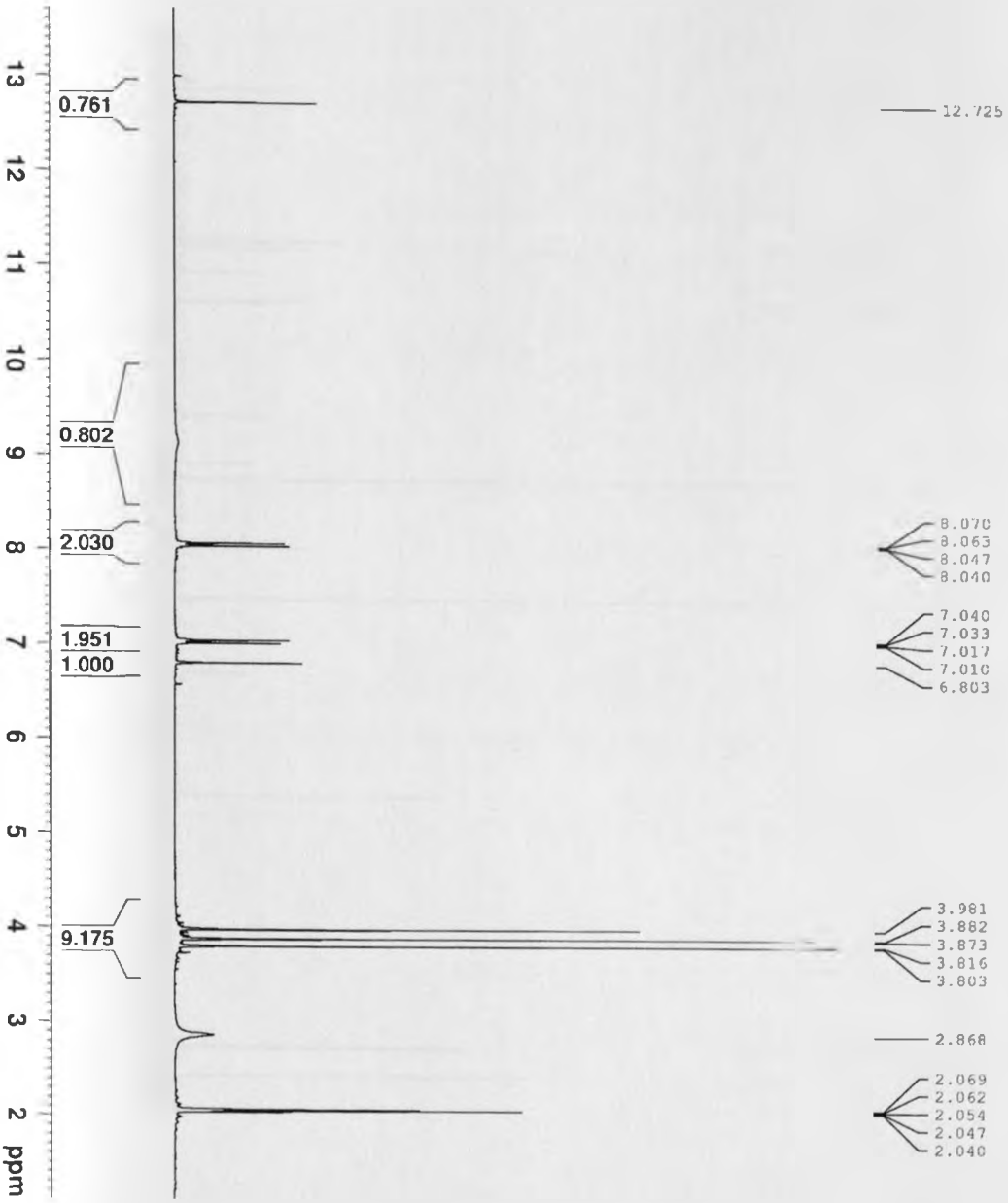


# DEPT SPECTRUM FOR COMPOUND 11

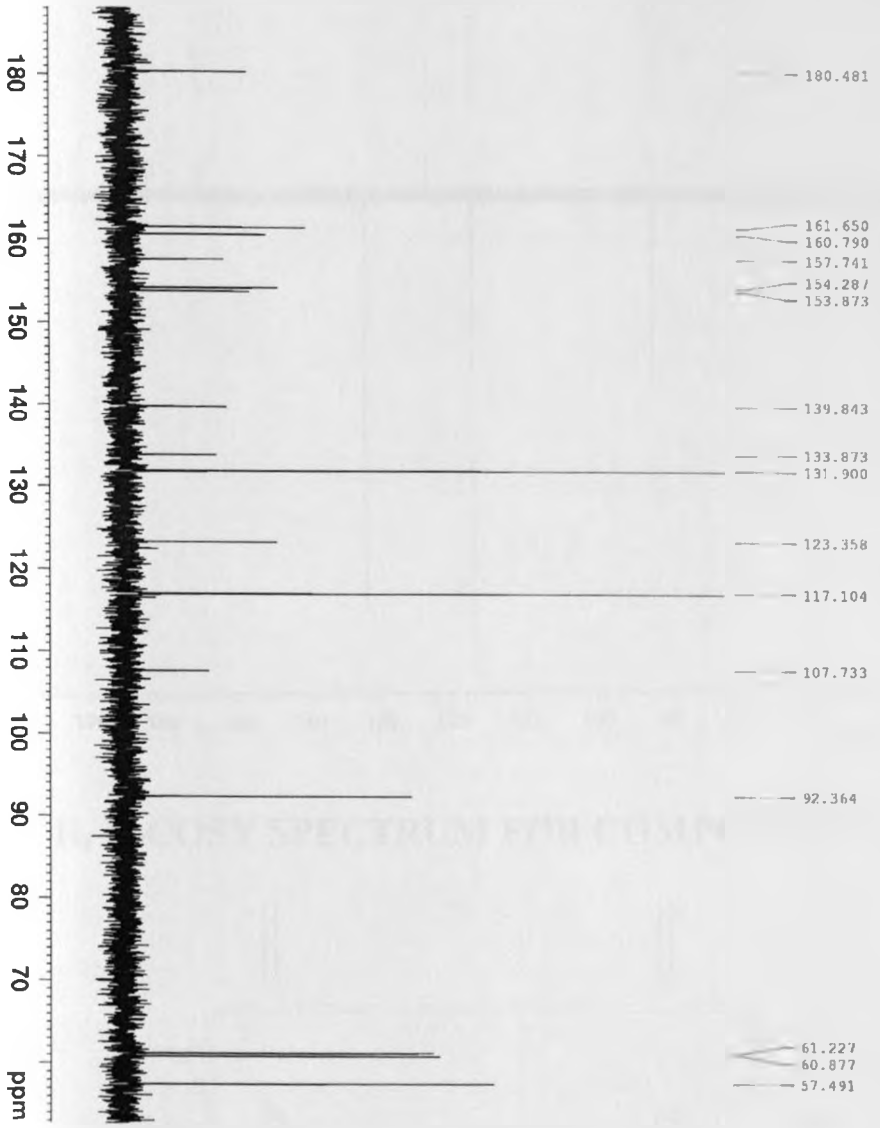


# APPENDIX XII: SPECTRA FOR COMPOUND 12

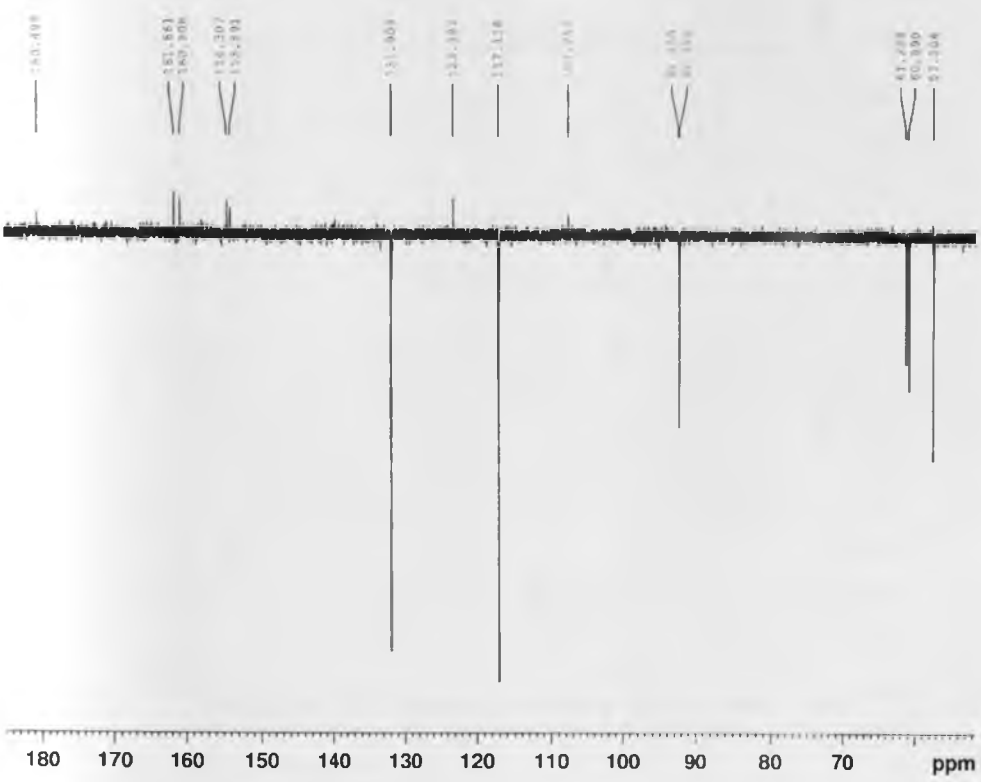
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 12



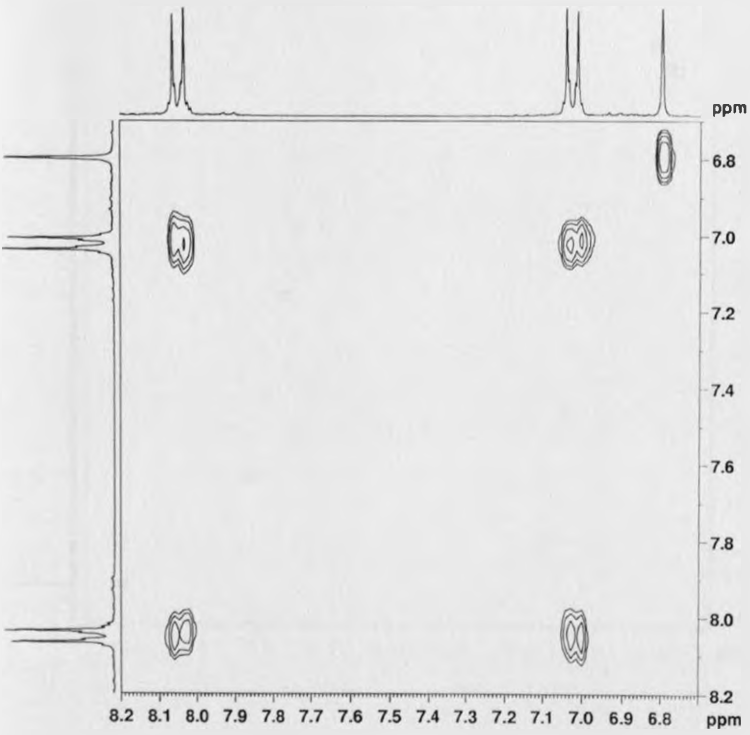
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 12



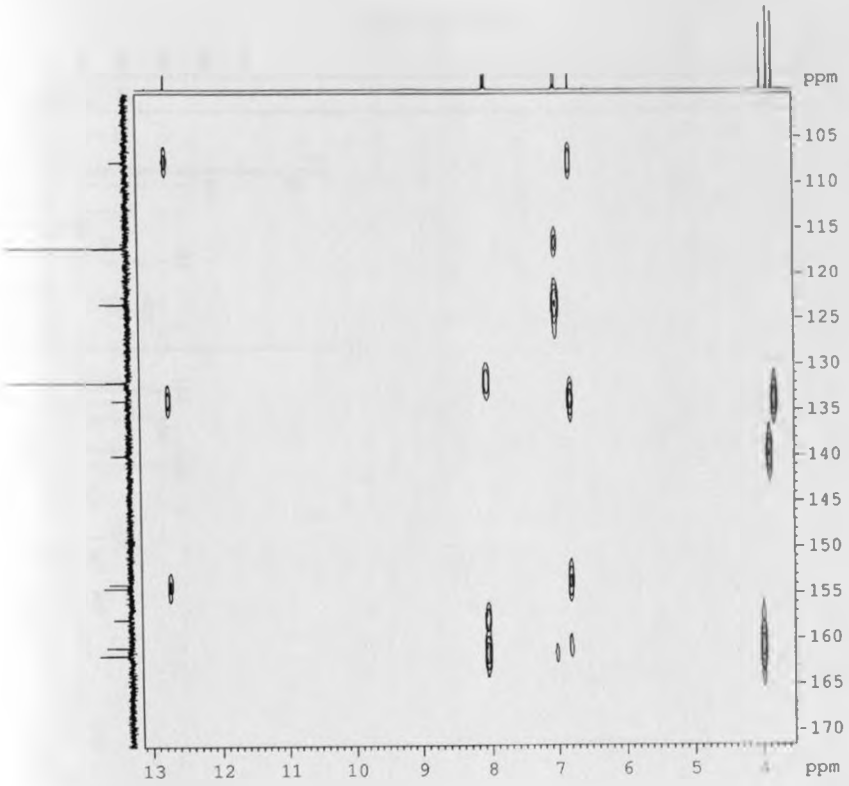
# APT SPECTRUM FOR COMPOUND 12



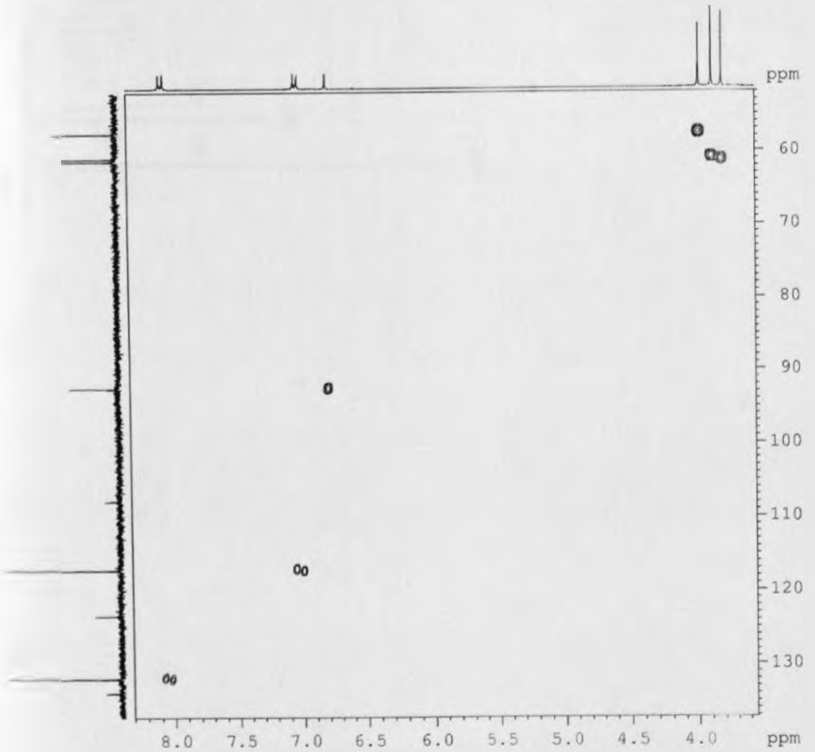

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 12



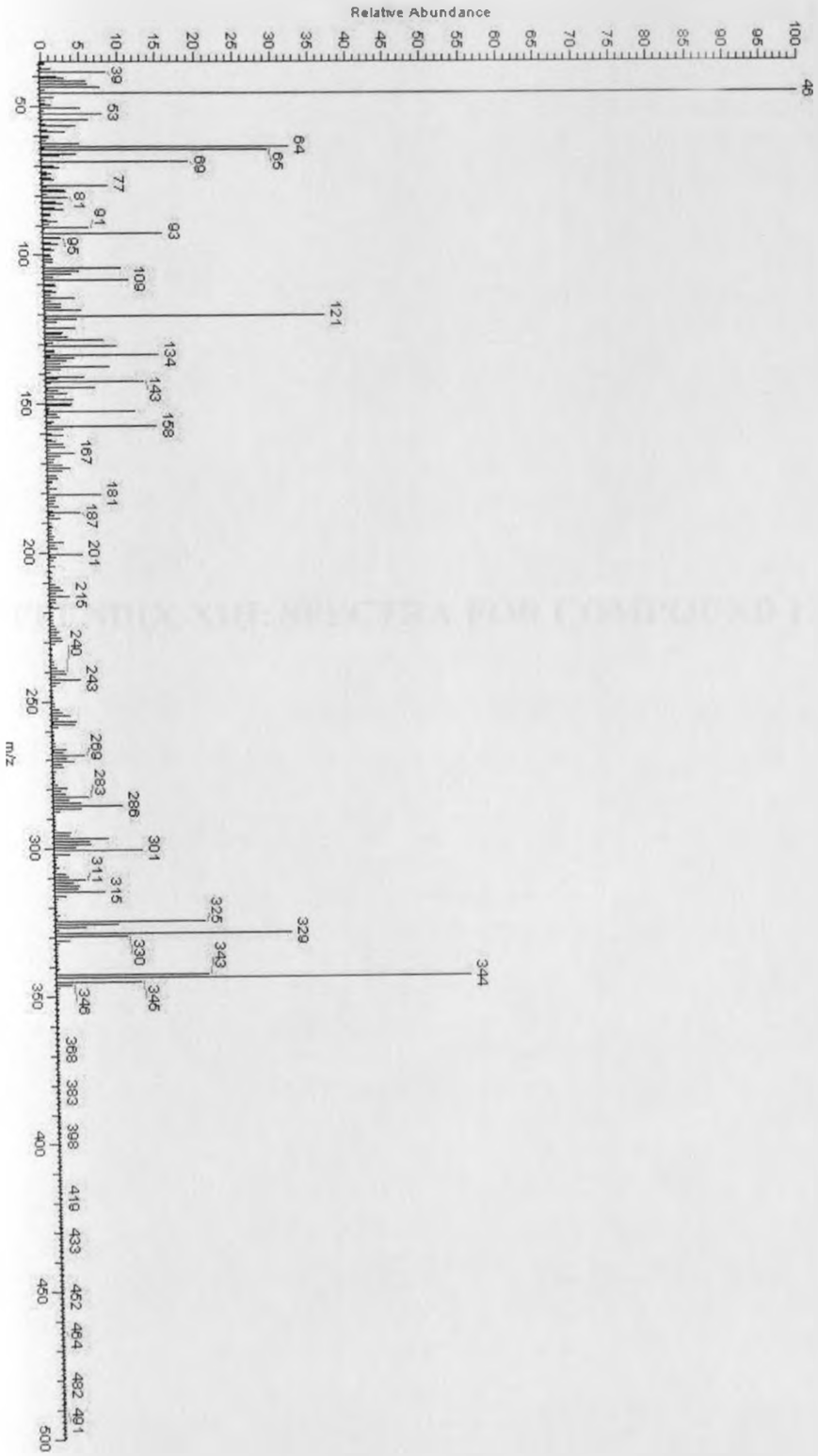
# HMBC SPECTRUM FOR COMPOUND 12




# HMQC SPECTRUM FOR COMPOUND 12

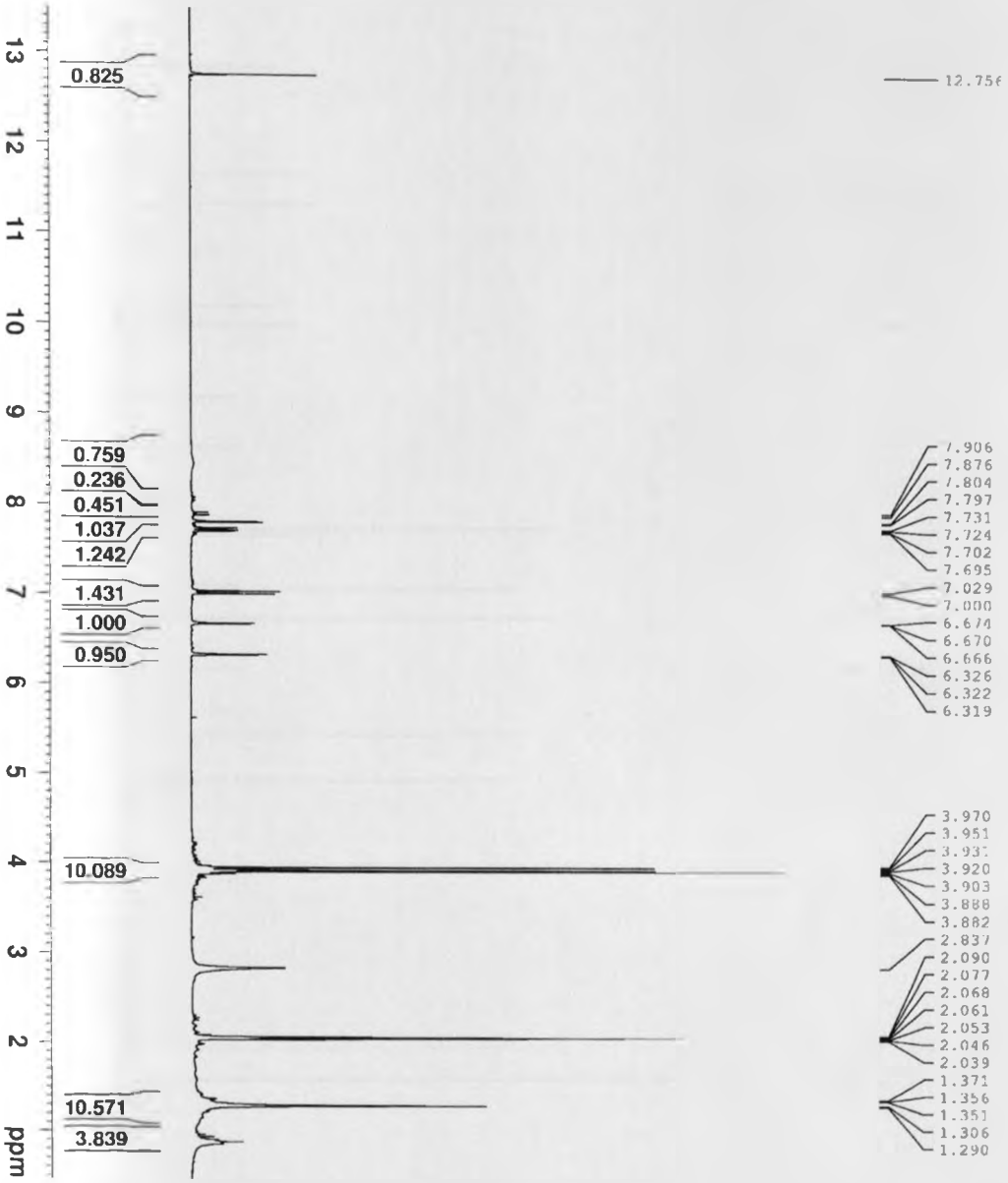


# MASS SPECTRUM FOR COMPOUND 12

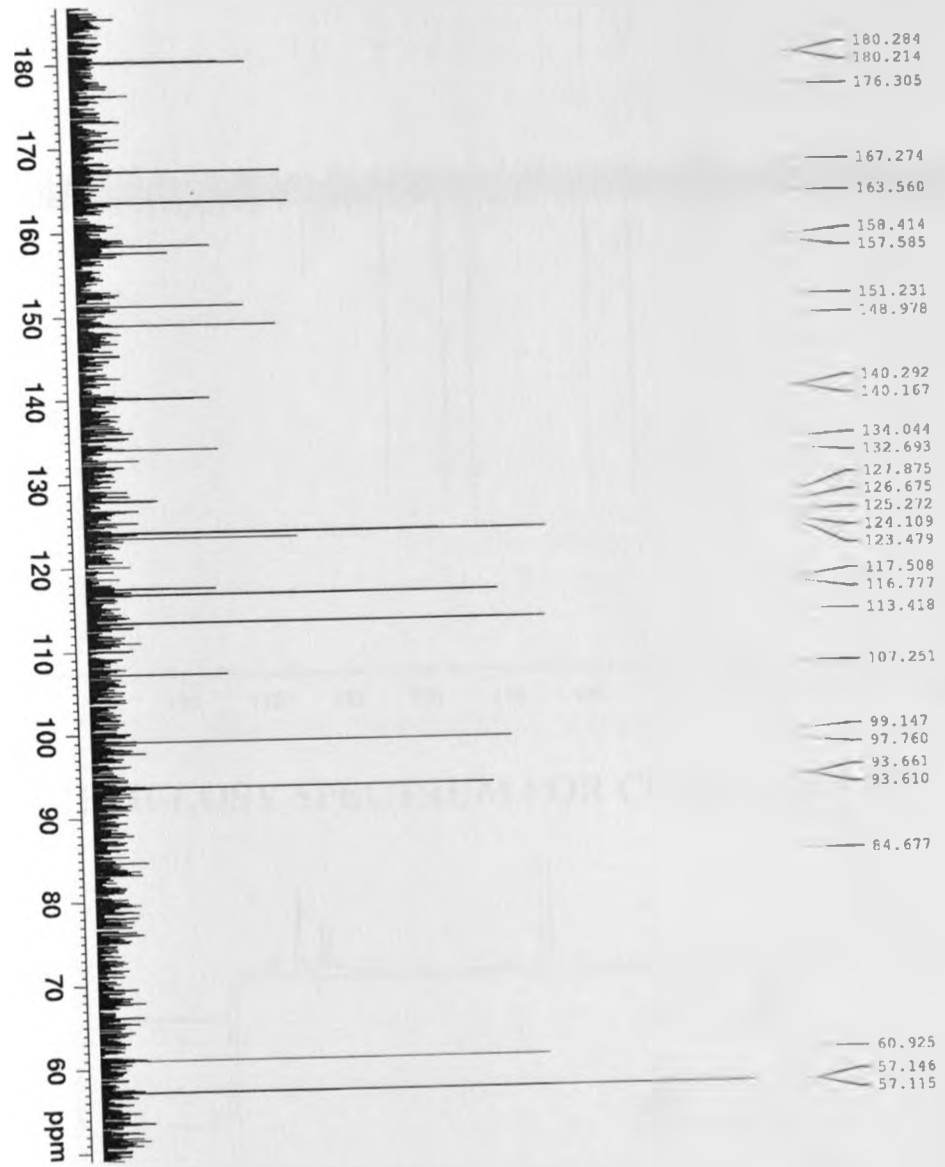


# APPENDIX XIII: SPECTRA FOR COMPOUND 13

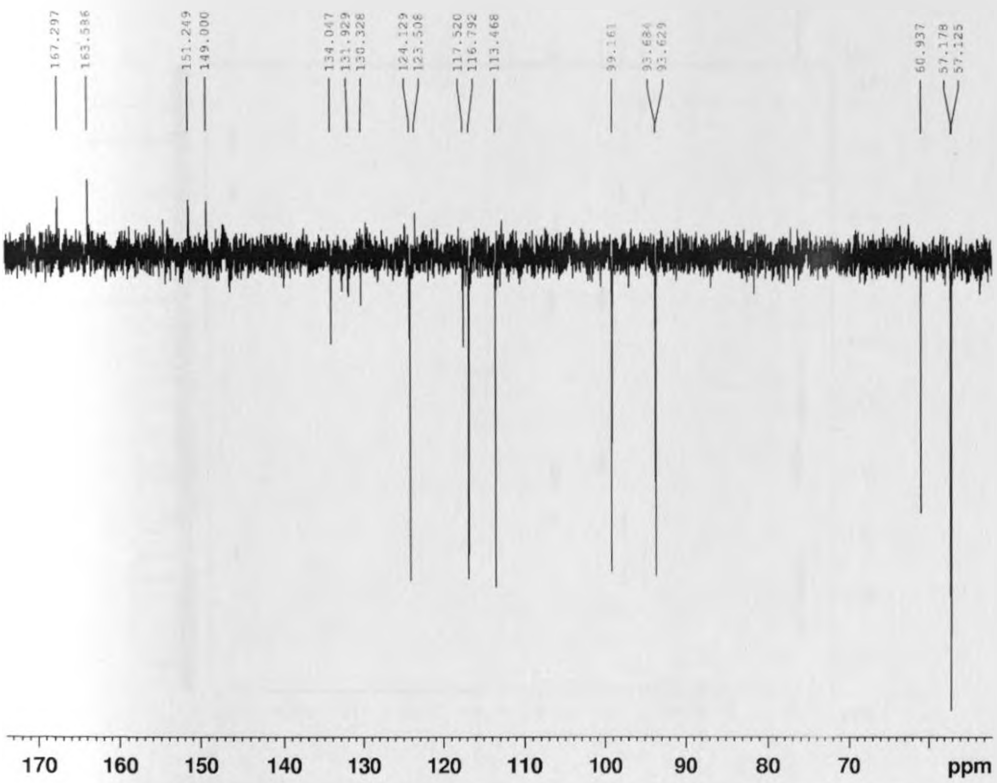
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 13



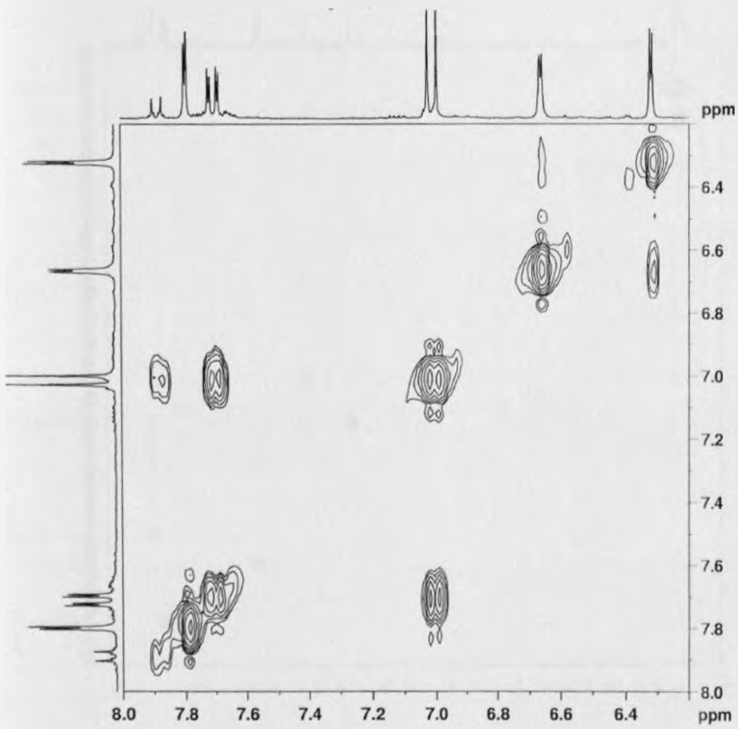
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 13



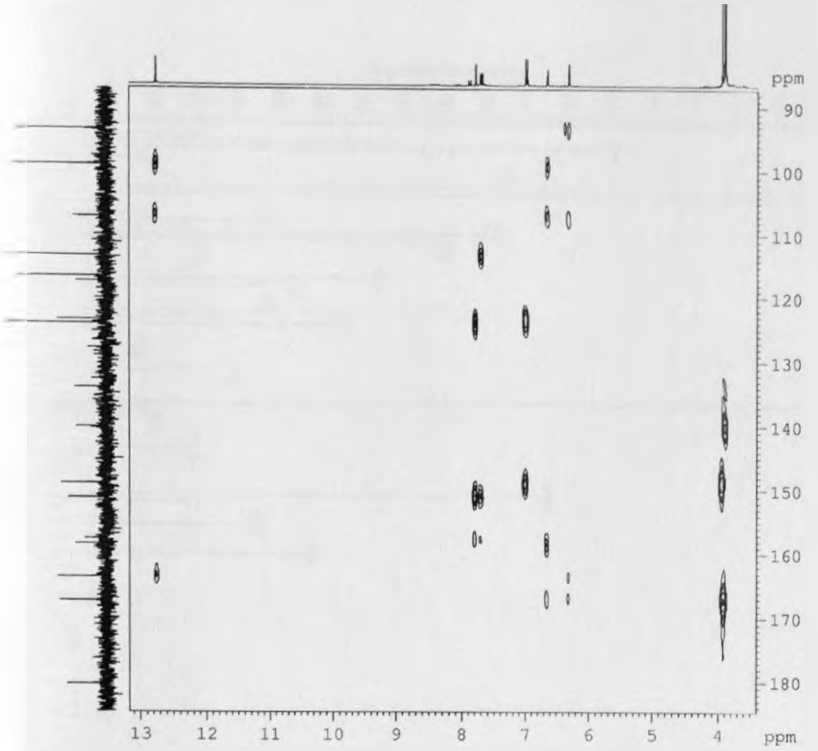
# APT SPECTRUM FOR COMPOUND 13



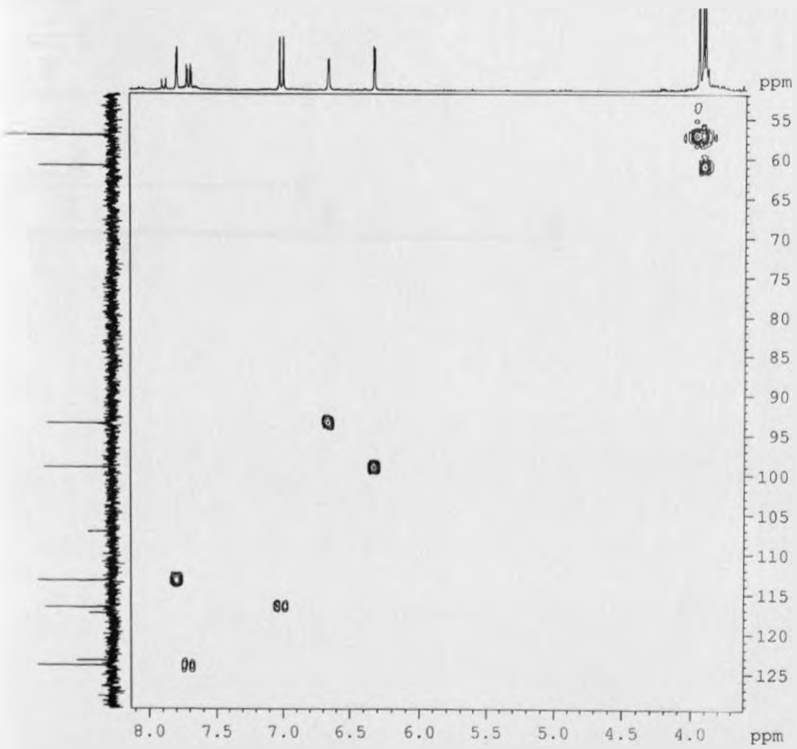

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 13



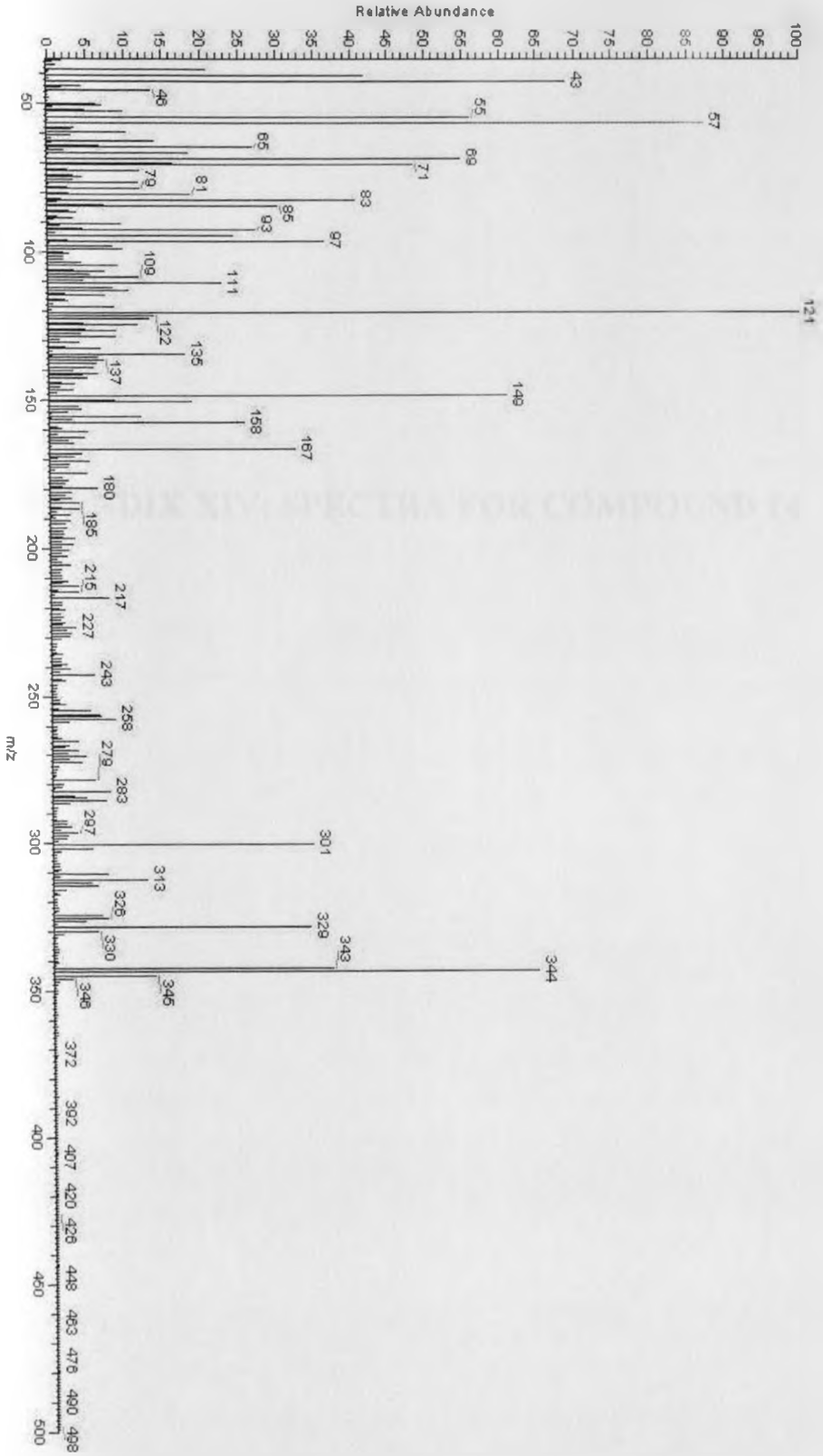
# HMBC SPECTRUM FOR COMPOUND 13




# HMQC SPECTRUM FOR COMPOUND 13

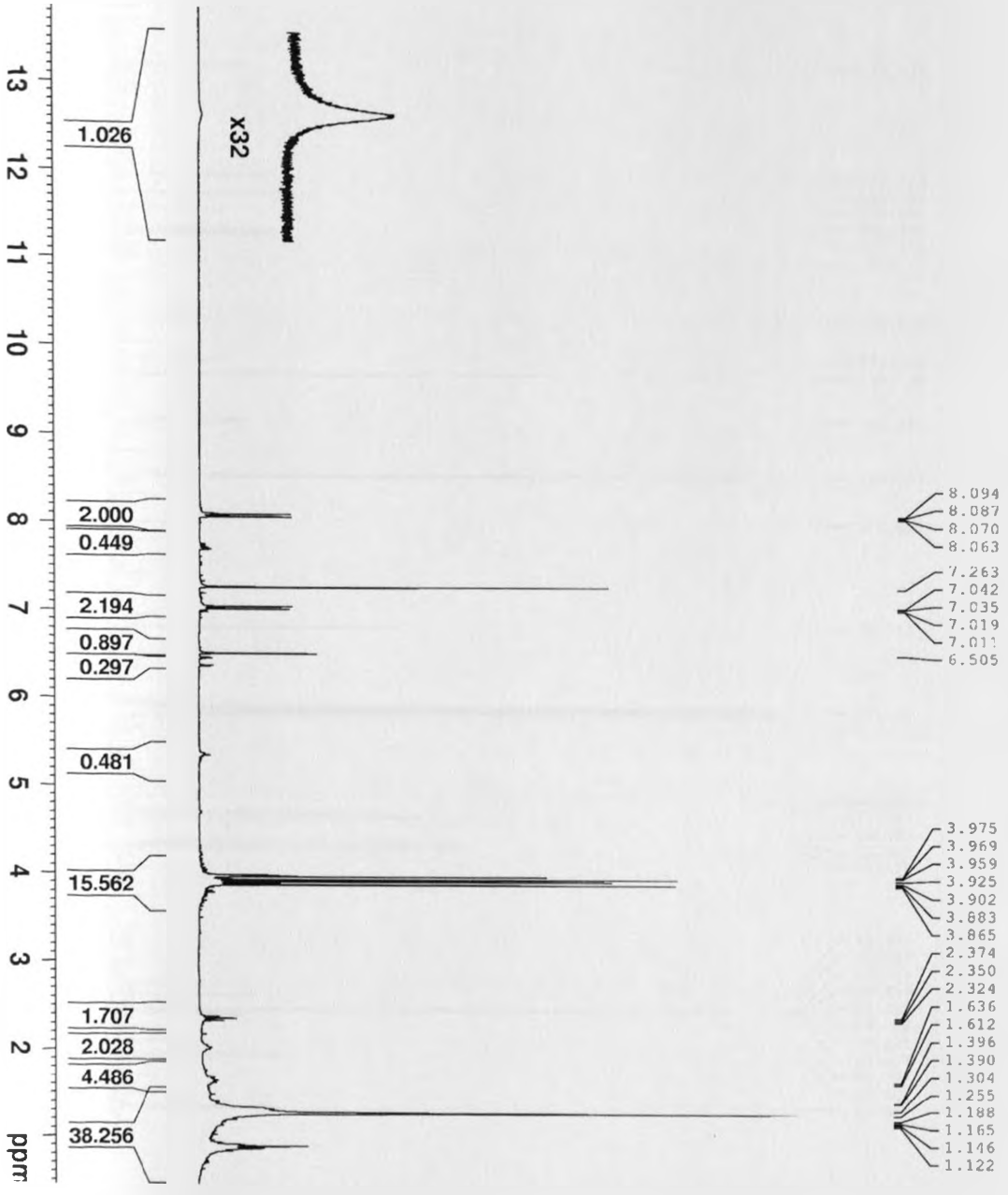


# MASS SPECTRUM FOR COMPOUND 13

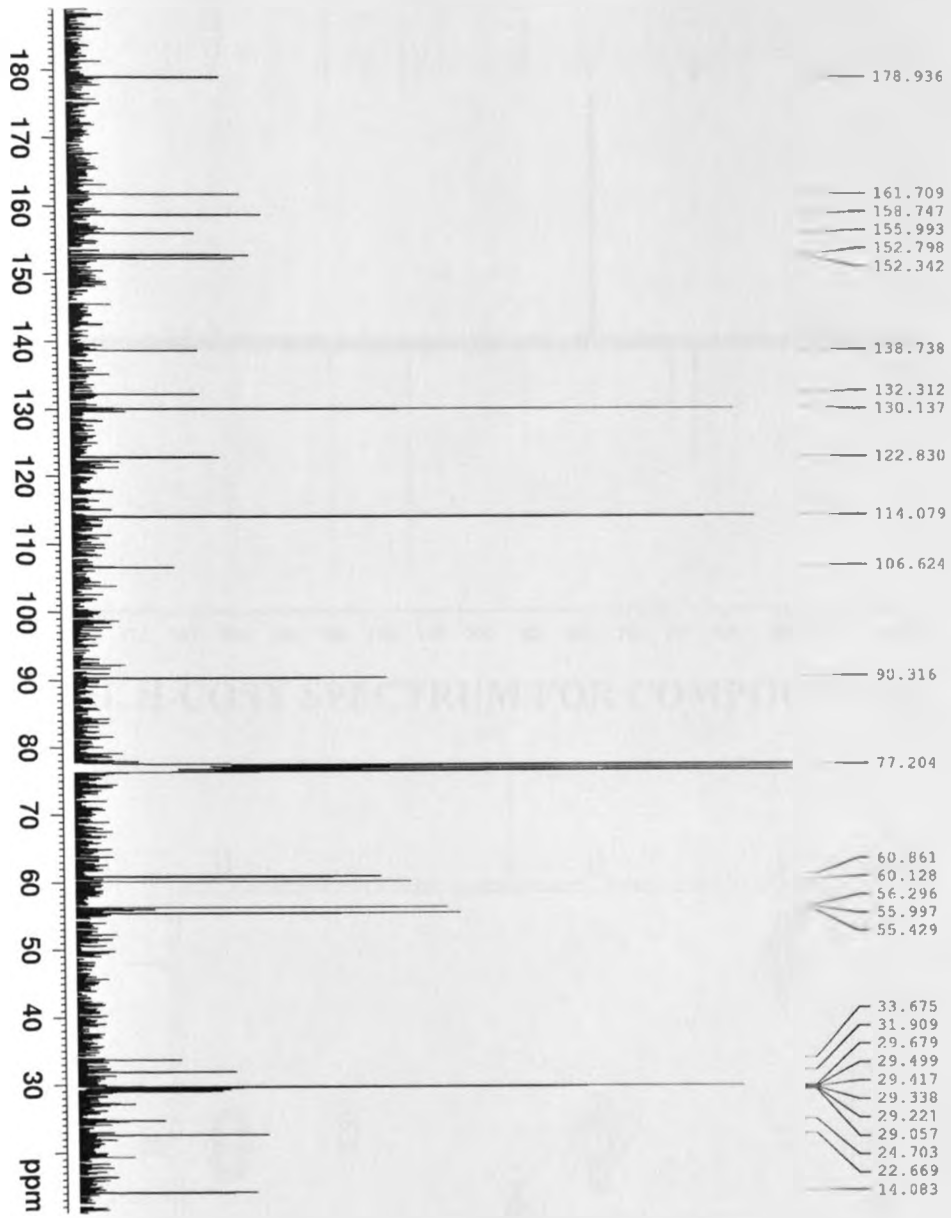


# APPENDIX XIV: SPECTRA FOR COMPOUND 14

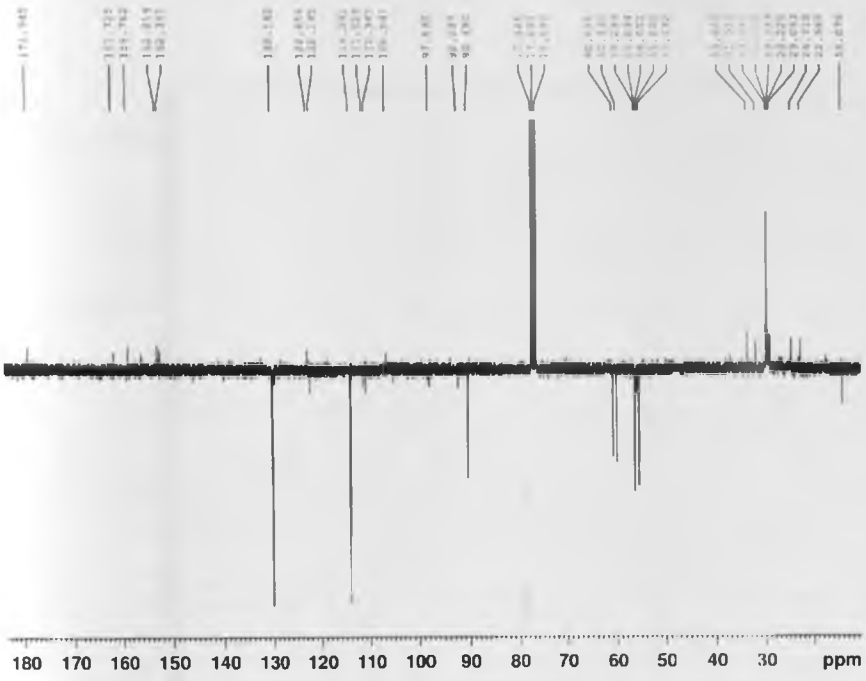
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 14



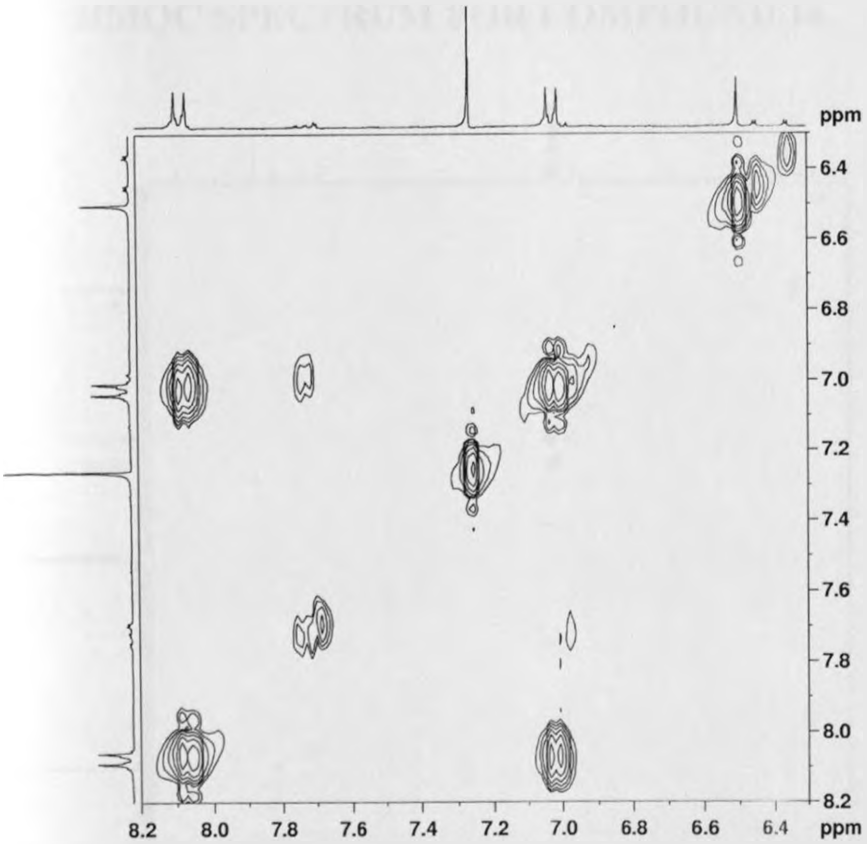
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 14



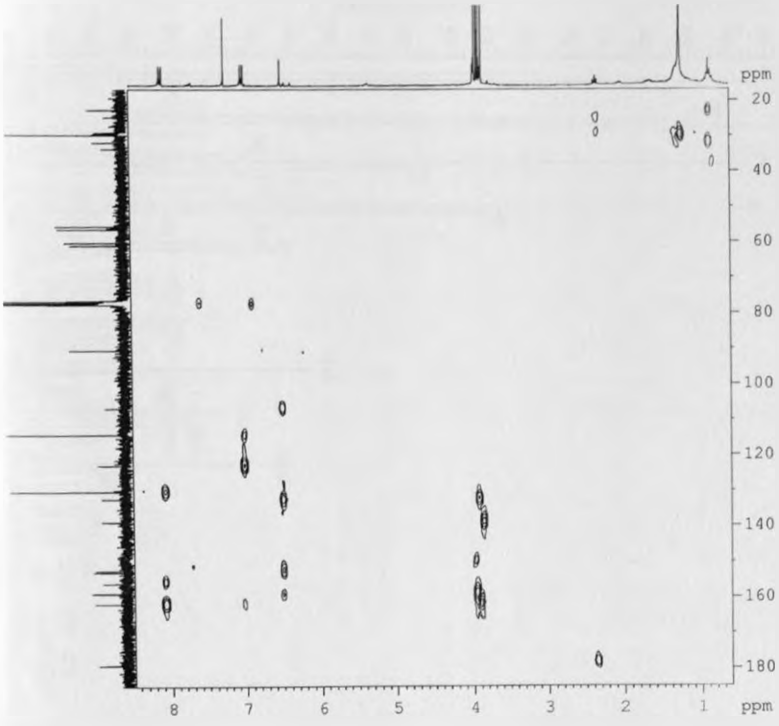
## APT SPECTRUM FOR COMPOUND 14



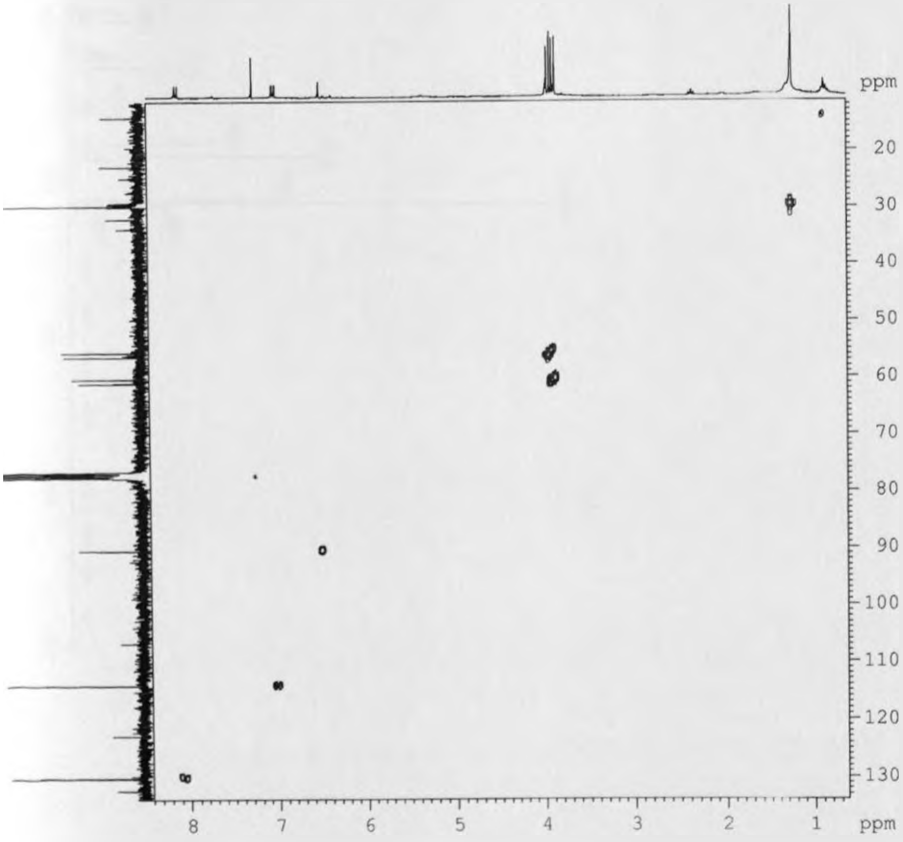

## $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 14



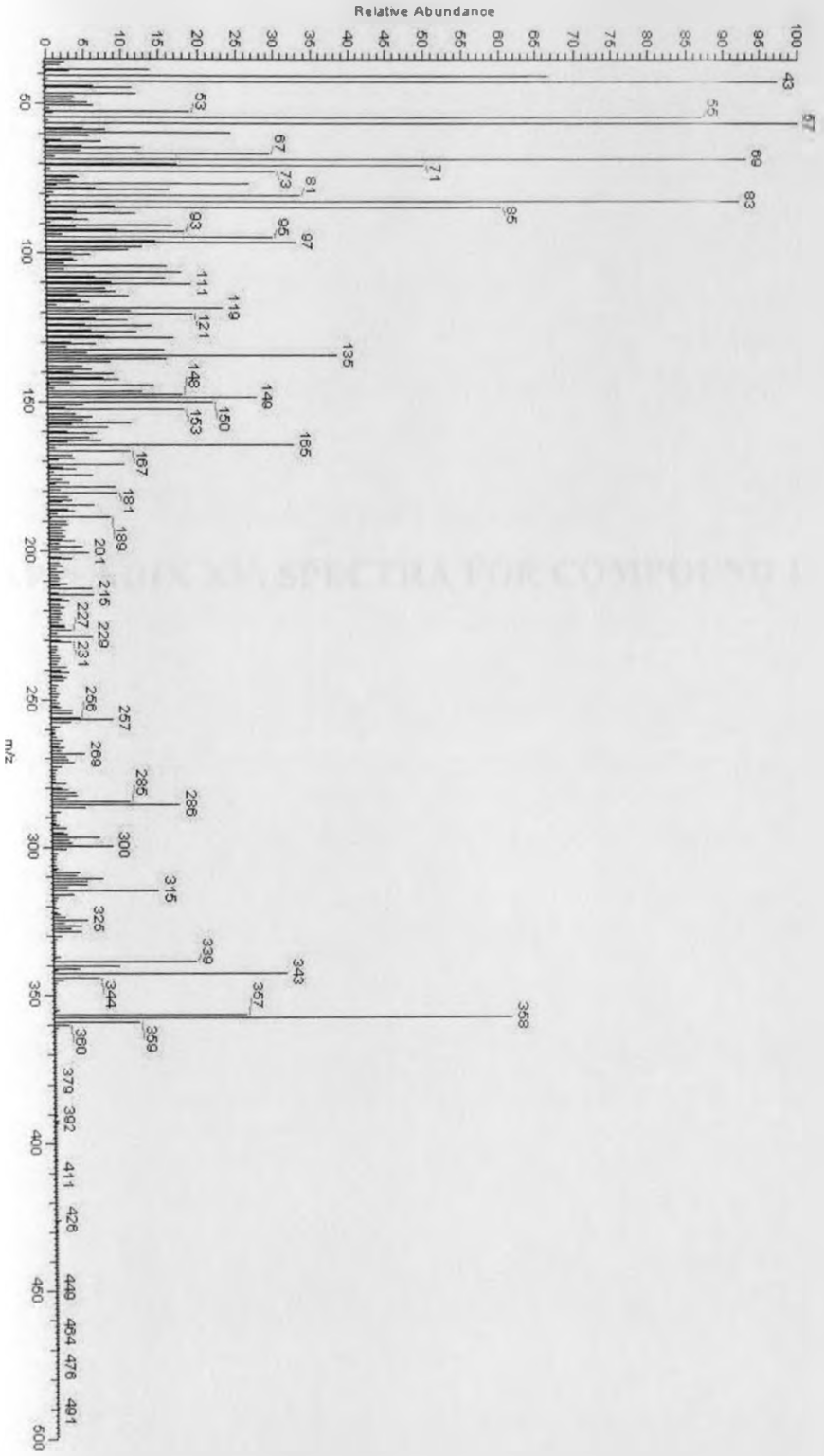
## HMBC SPECTRUM FOR COMPOUND 14




## HMQC SPECTRUM FOR COMPOUND 14

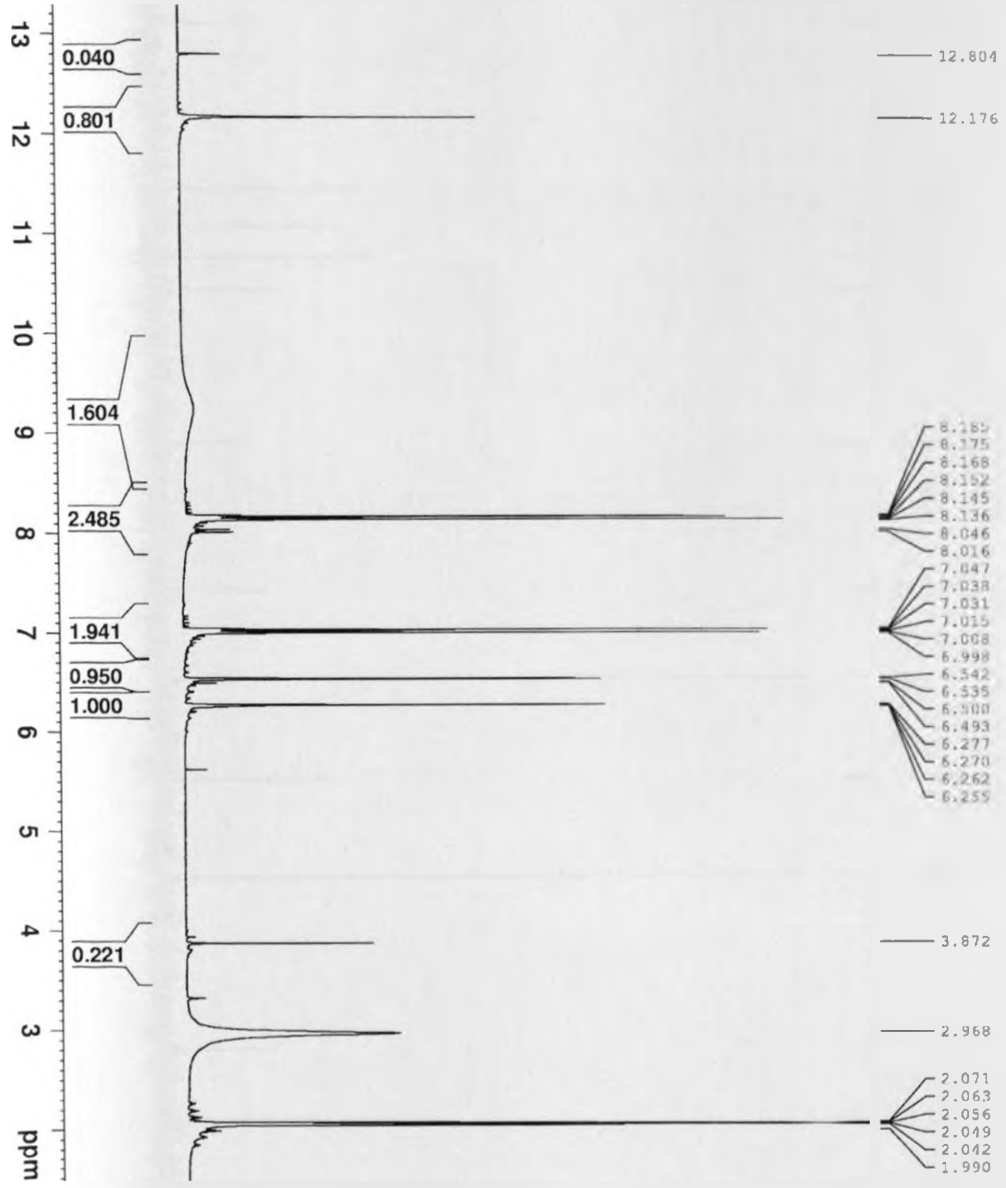


# MASS SPECTRUM FOR COMPOUND 14

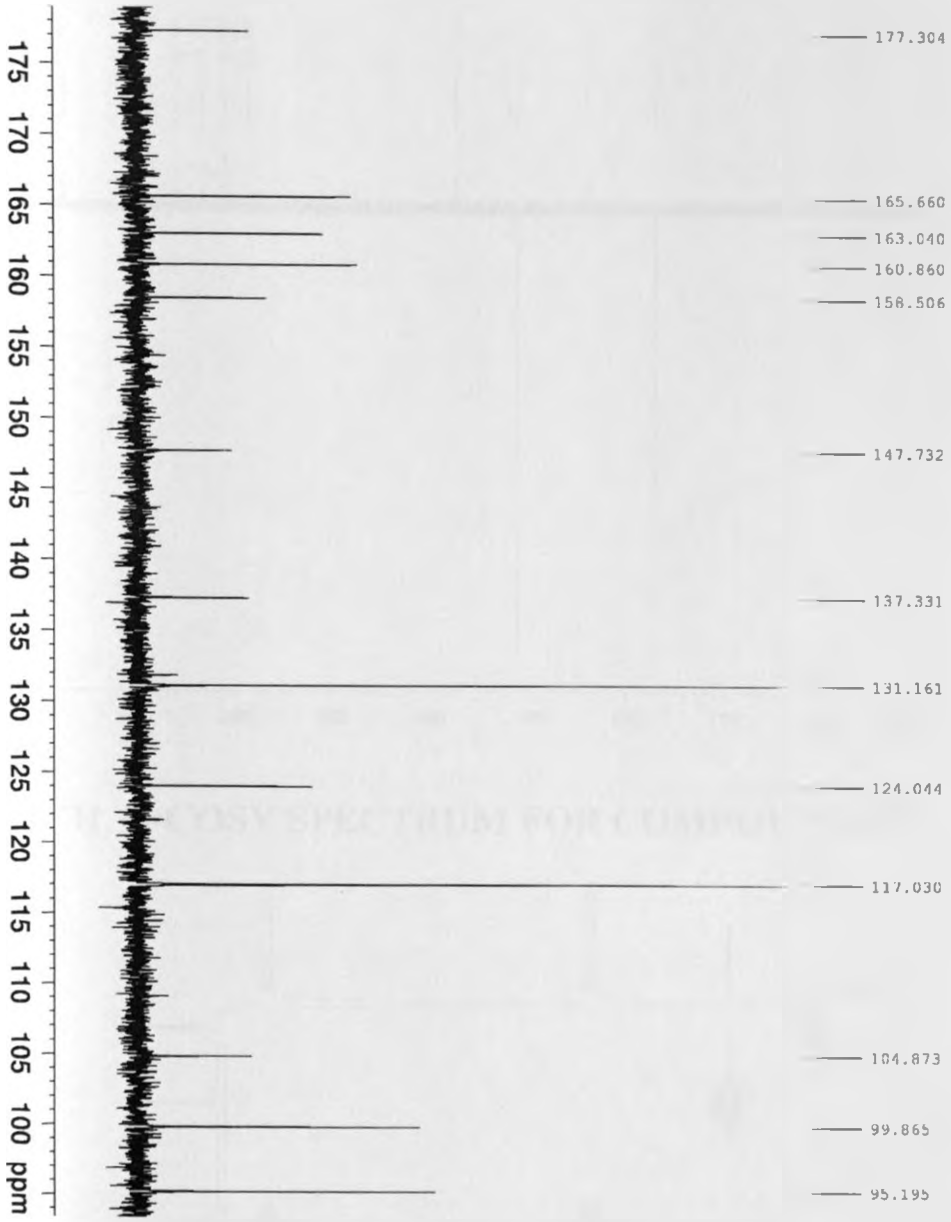


# APPENDIX XV: SPECTRA FOR COMPOUND 15

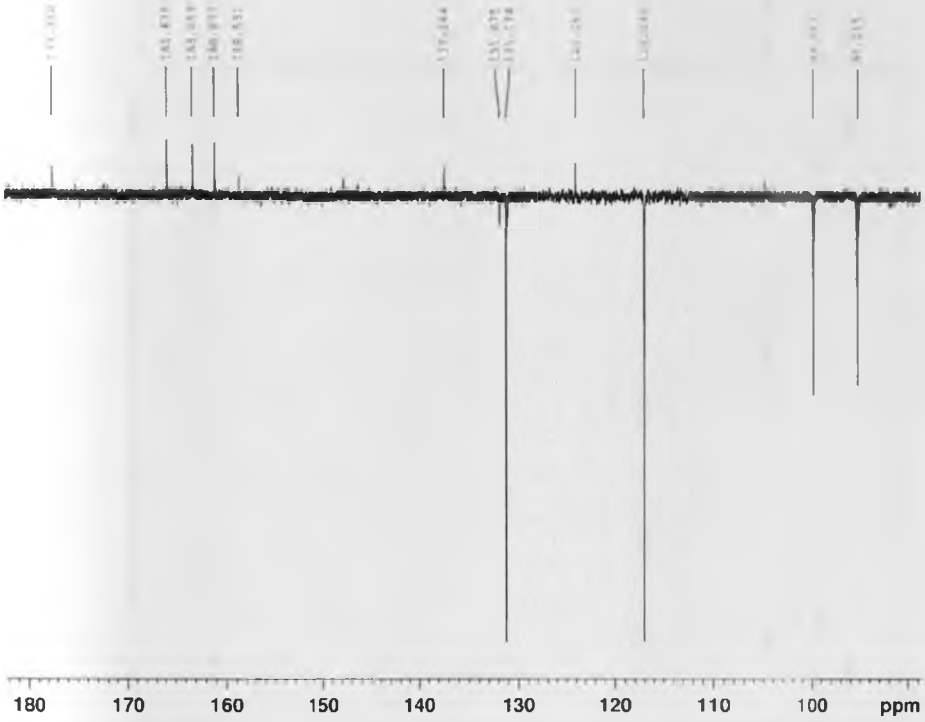
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 15



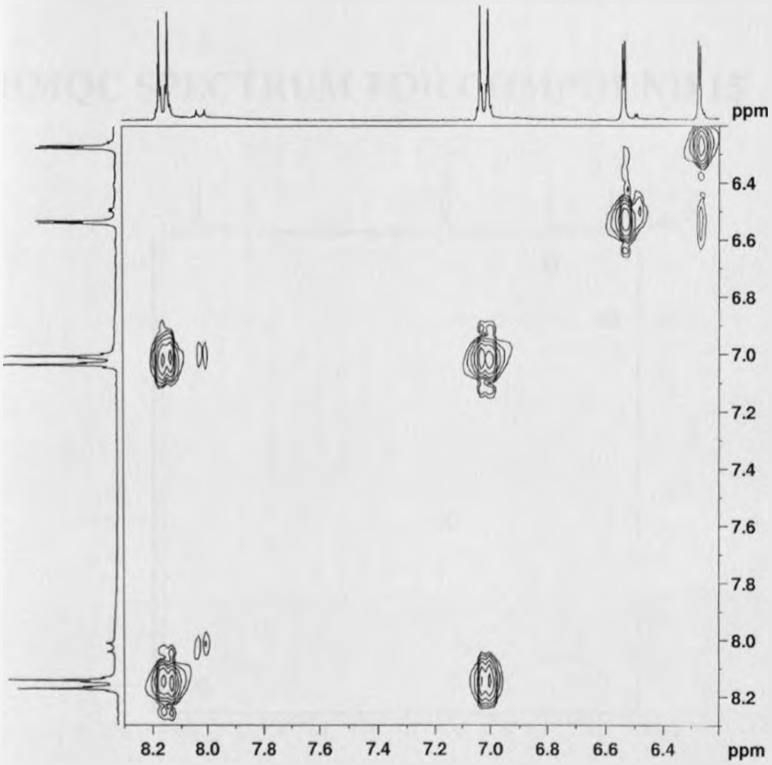
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 15



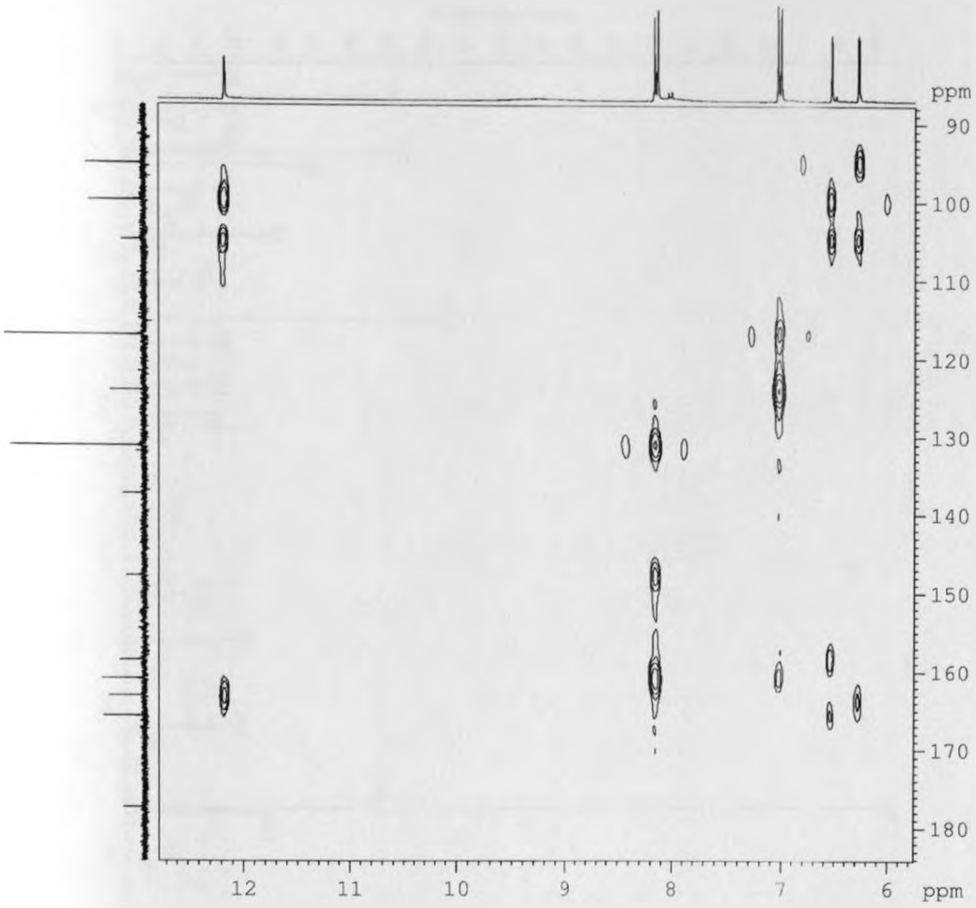
# APT SPECTRUM FOR COMPOUND 15



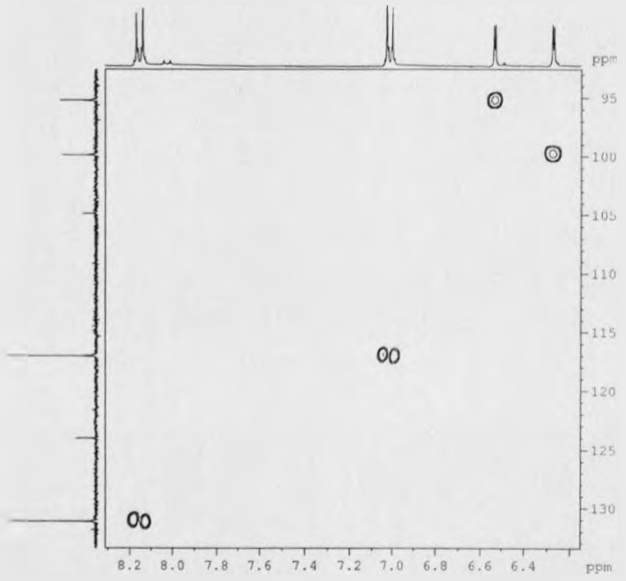

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 15



# HMBC SPECTRUM FOR COMPOUND 15



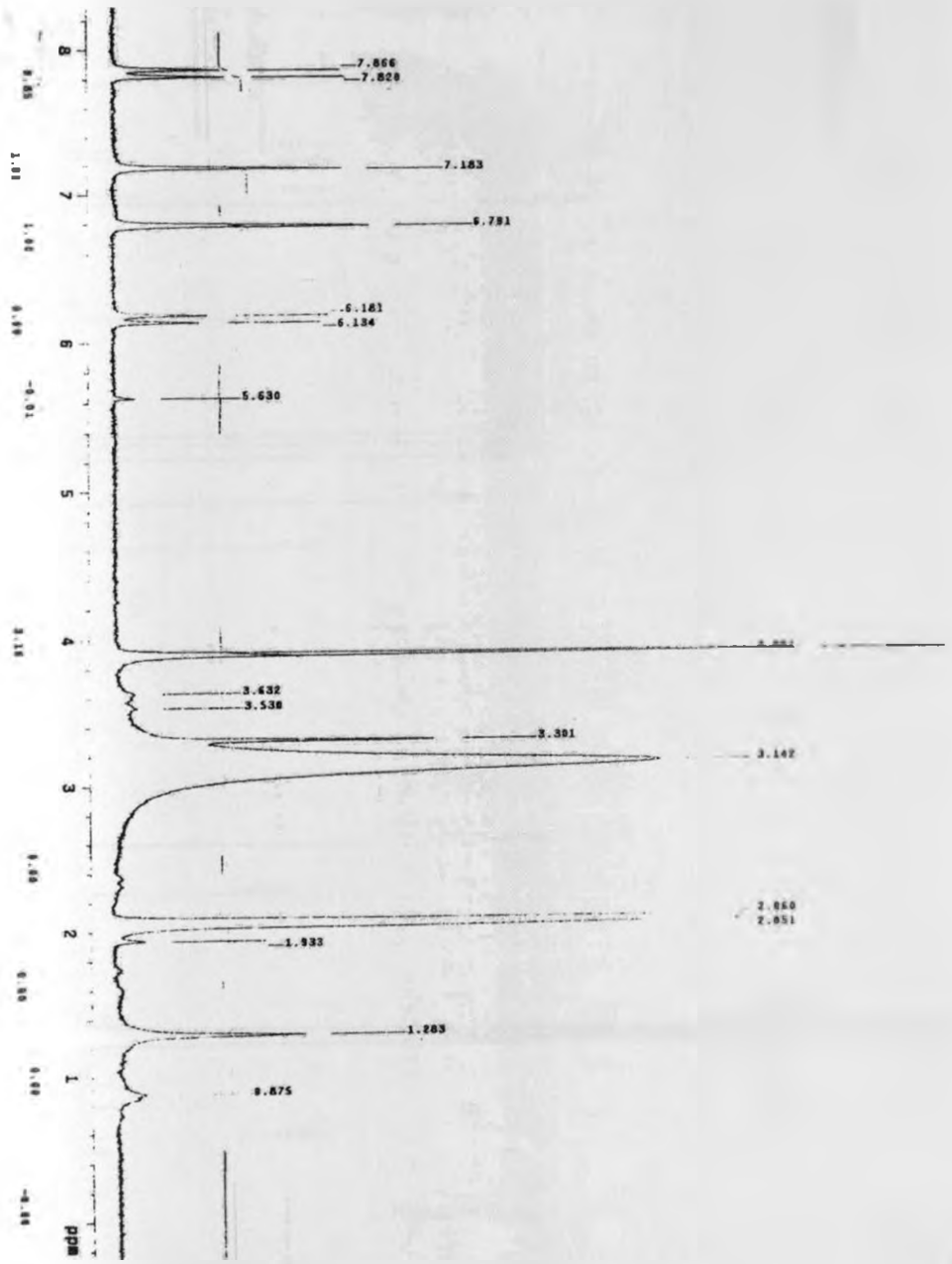

# HMQC SPECTRUM FOR COMPOUND 15



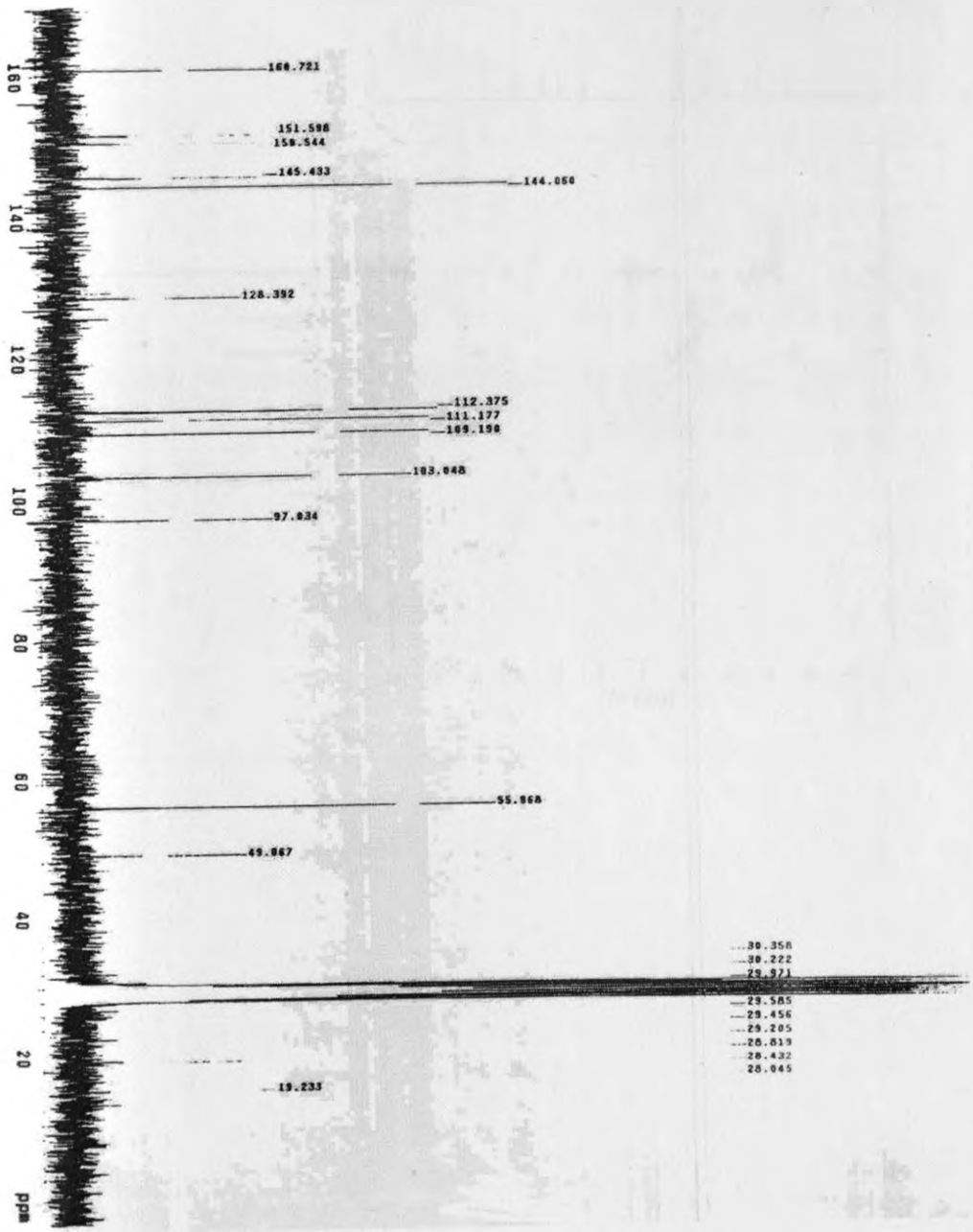
# MASS SPECTRUM FOR COMPOUND 15

# APPENDIX XVI: SPECTRA FOR COMPOUND 16

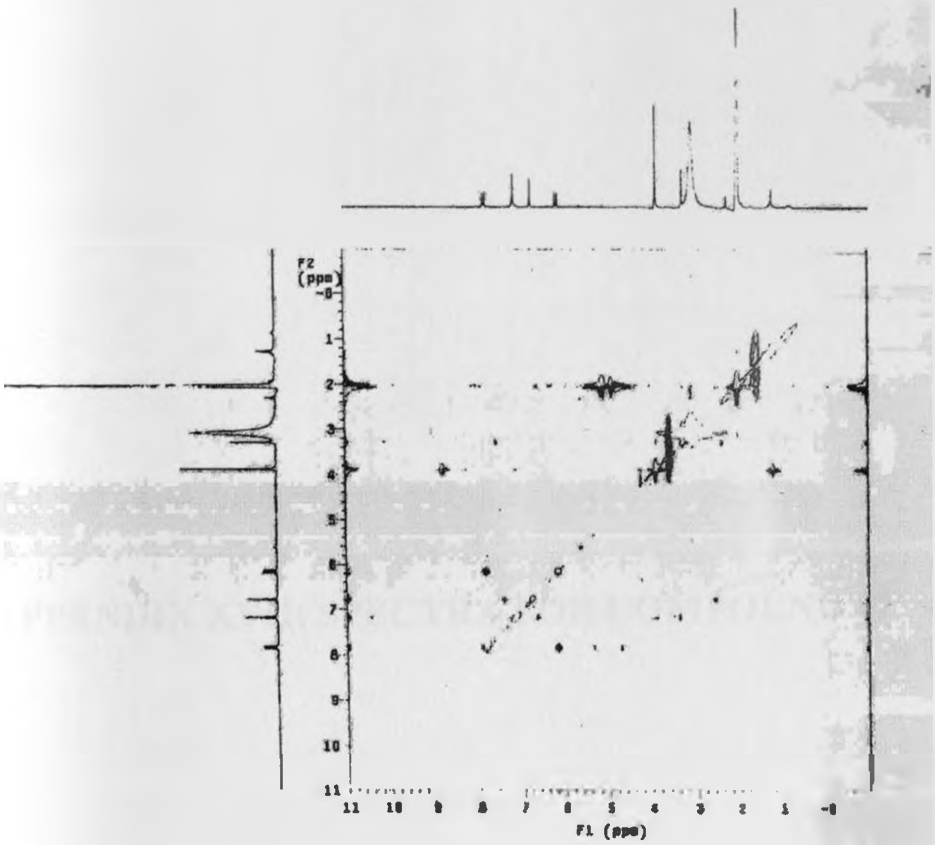
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 16



# $^{13}$C-NMR SPECTRUM FOR COMPOUND 16

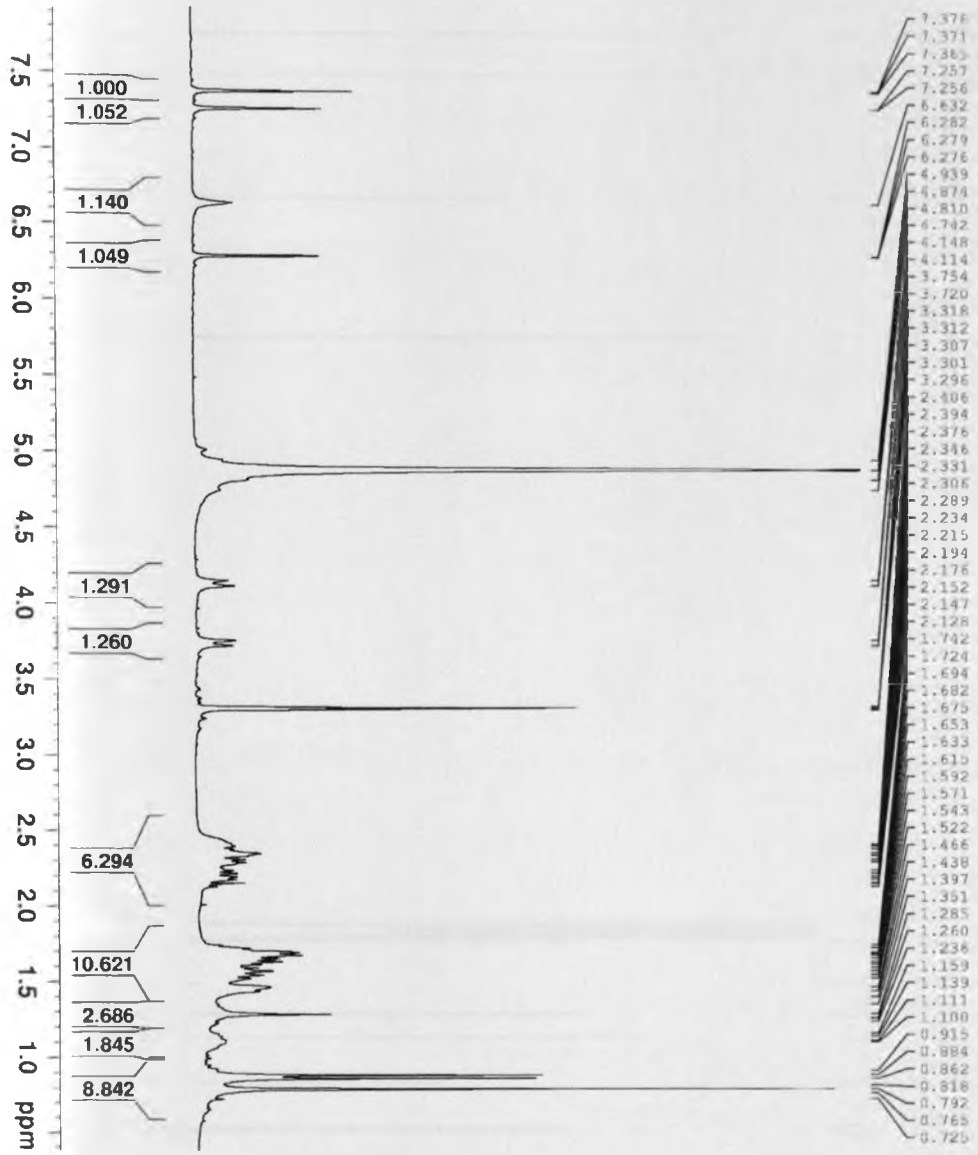


# NOESY SPECTRUM FOR COMPOUND 16

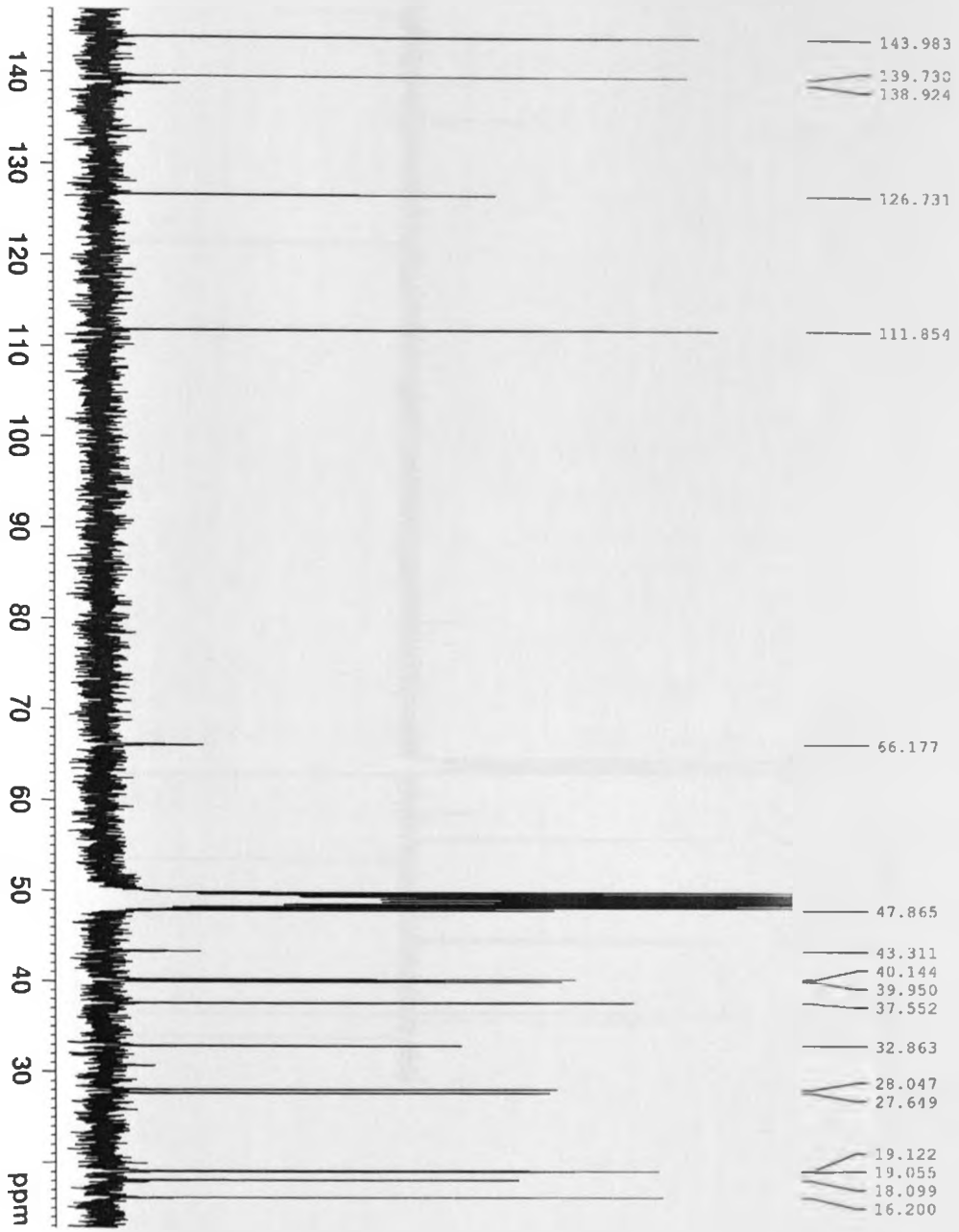


# APPENDIX XVII: SPECTRA FOR COMPOUND 17

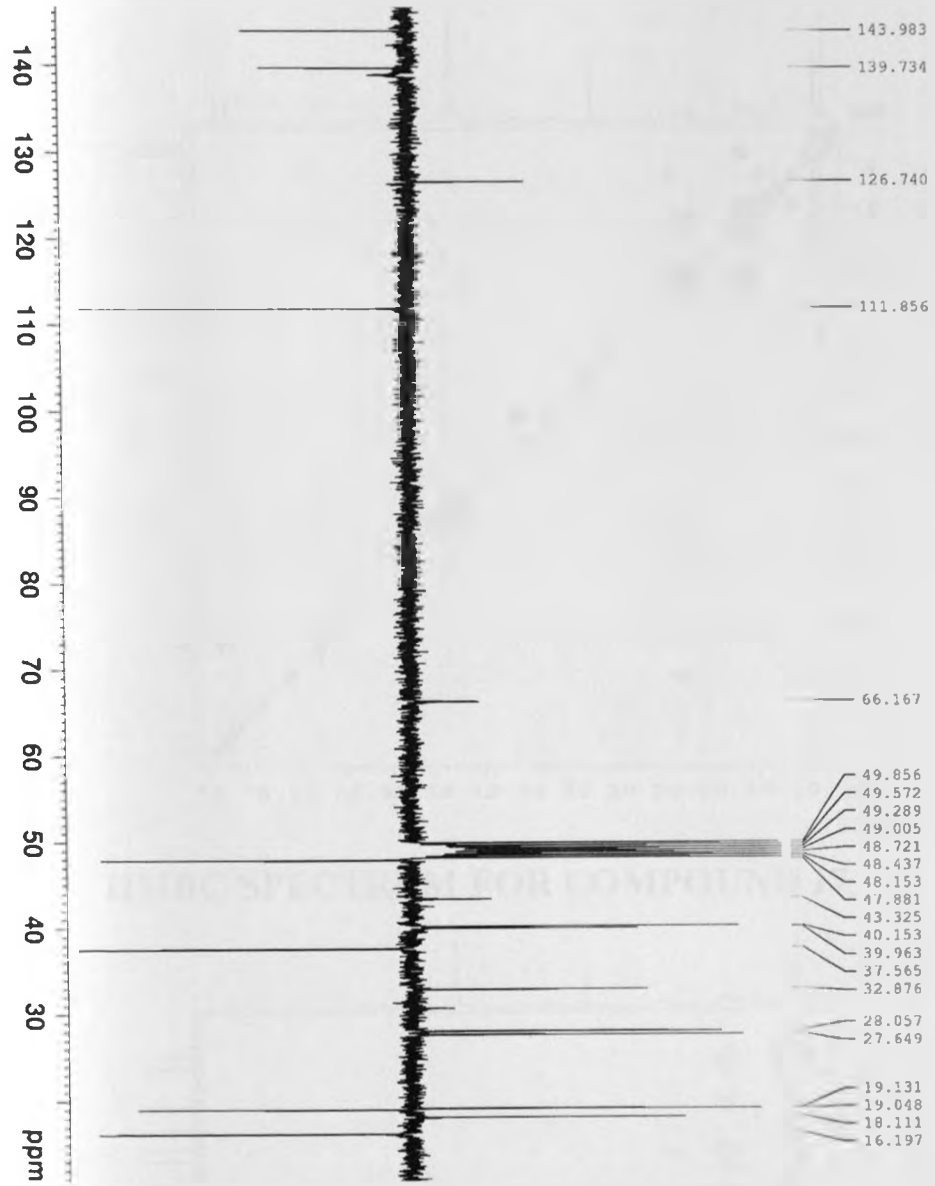
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 17



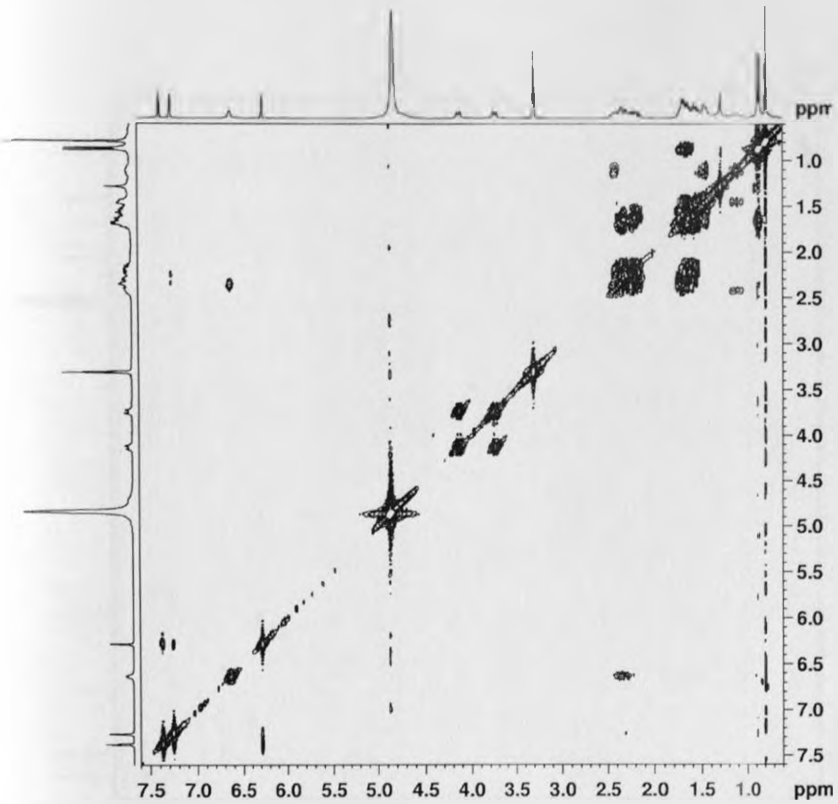
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 17



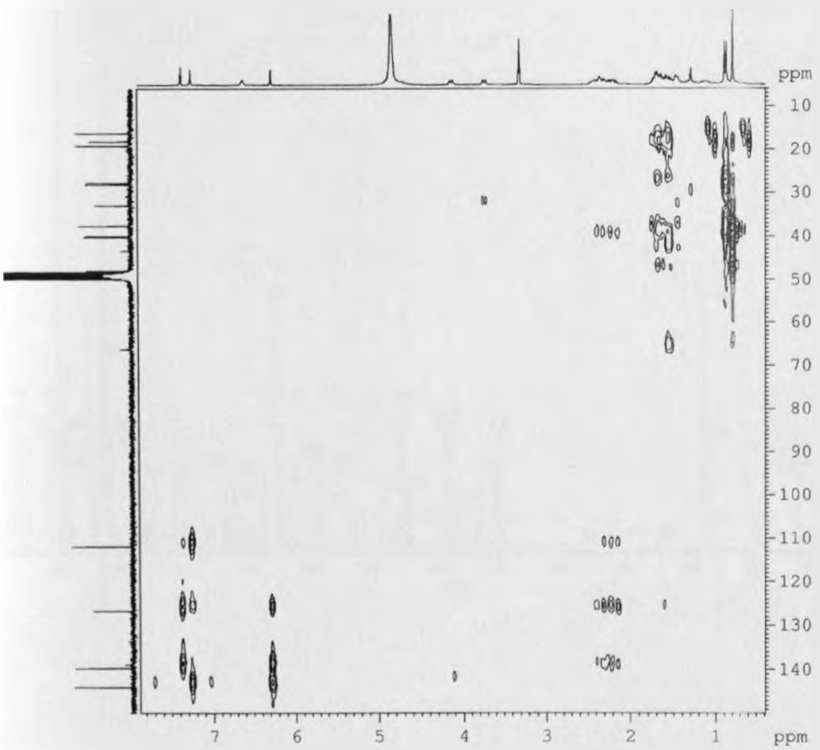
# APT SPECTRUM FOR COMPOUND 17



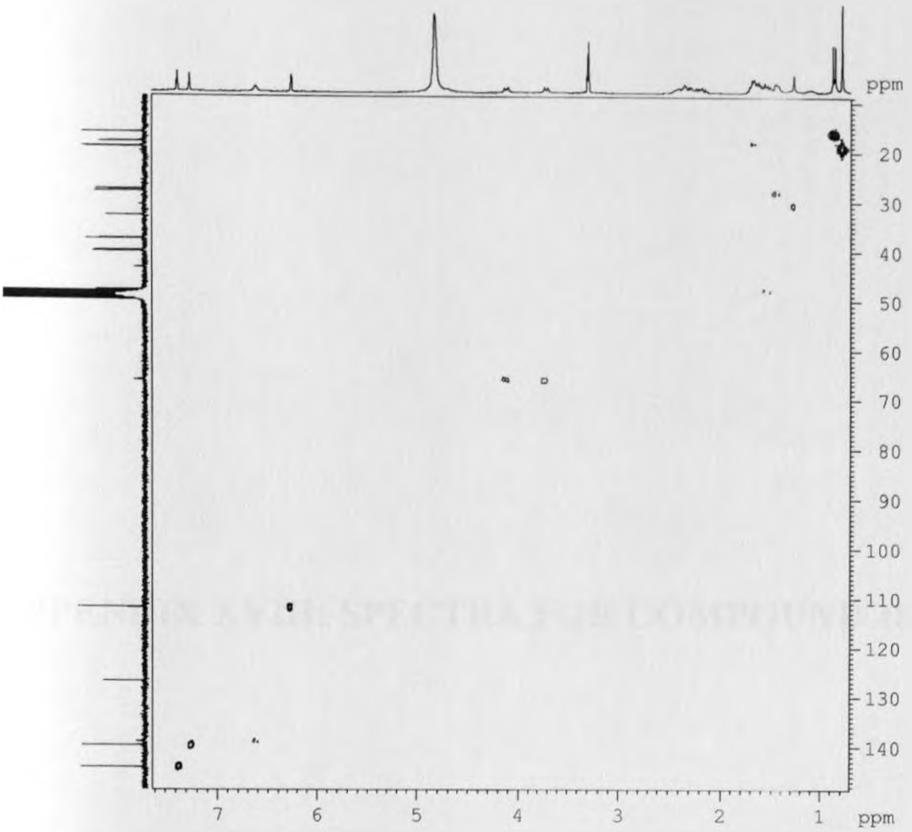
## $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 17



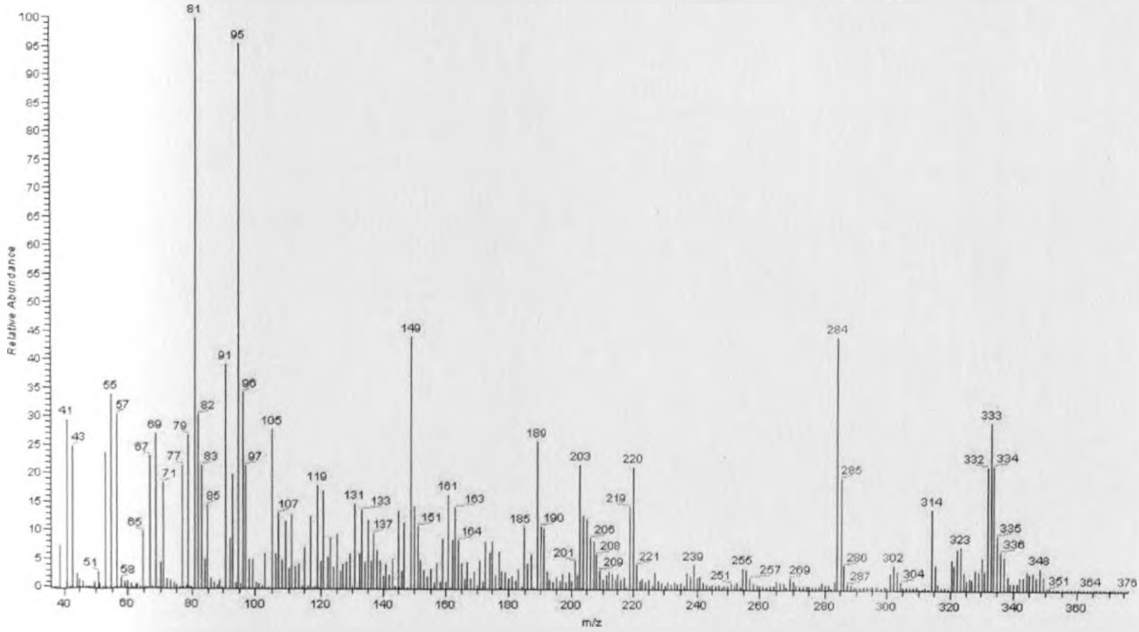

## HMBC SPECTRUM FOR COMPOUND 17



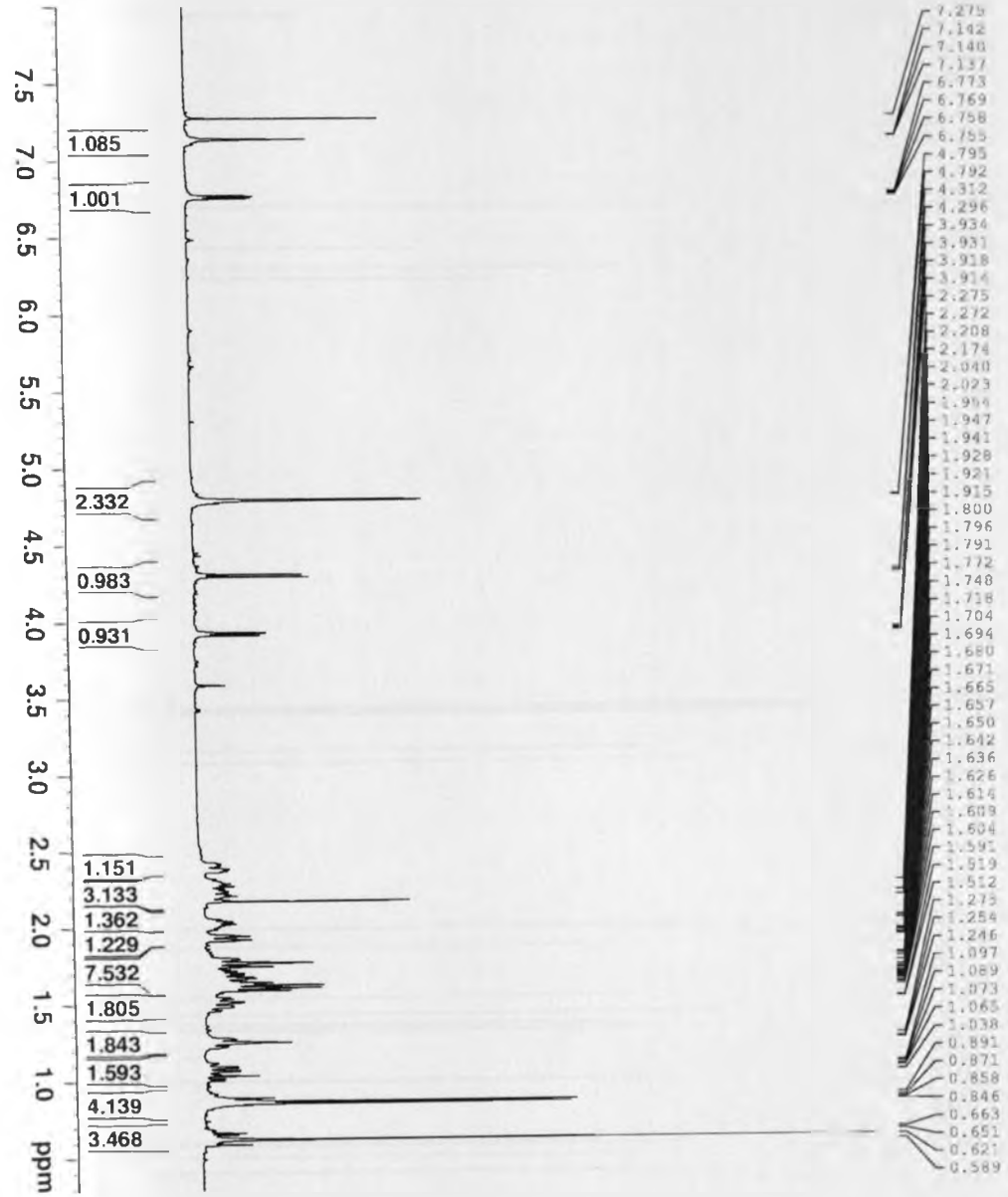
# HMQC SPECTRUM FOR COMPOUND 17



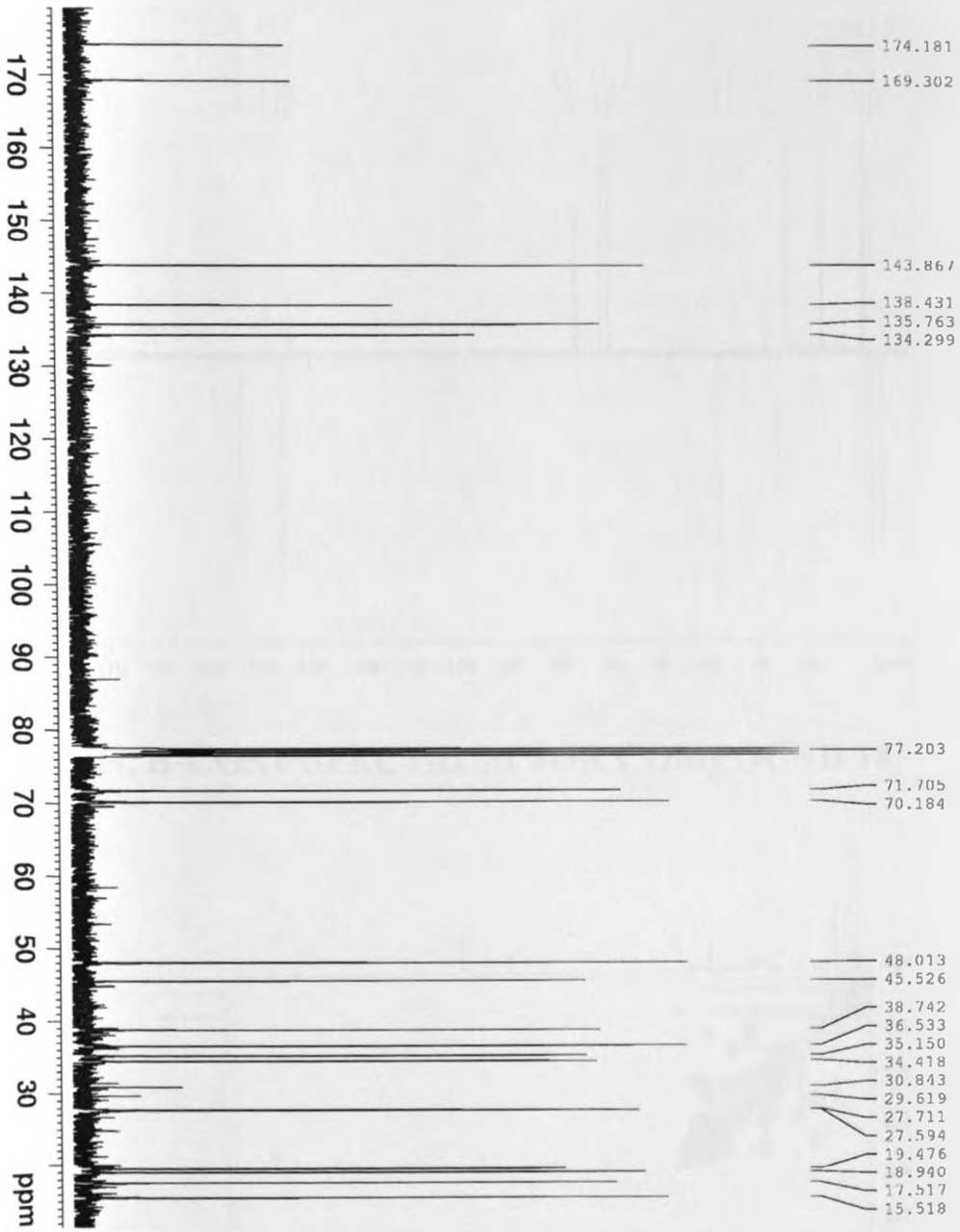

# MASS SPECTRUM FOR COMPOUND 17



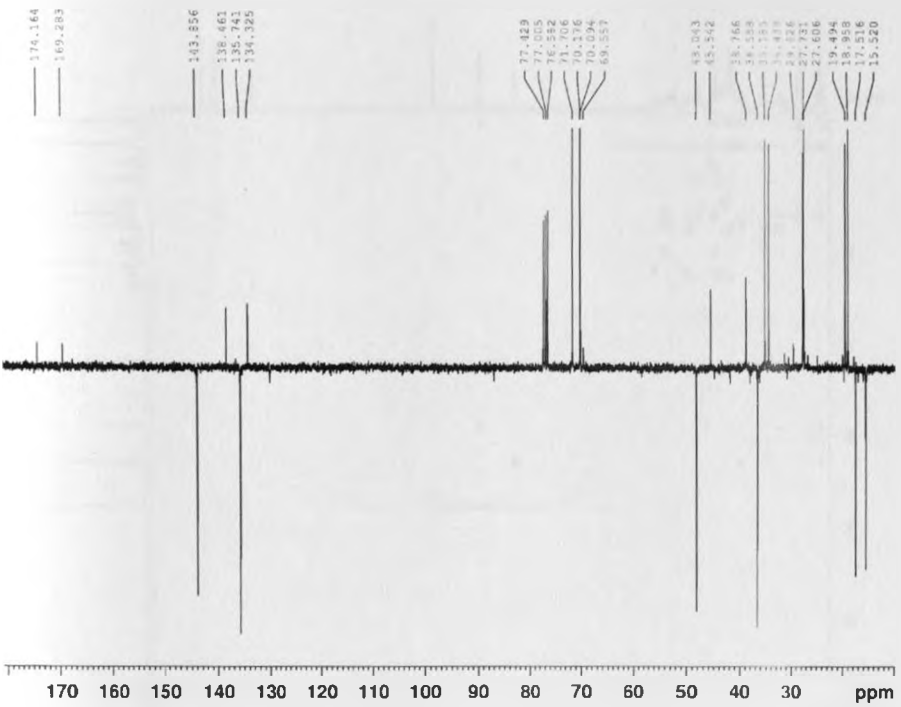
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 18



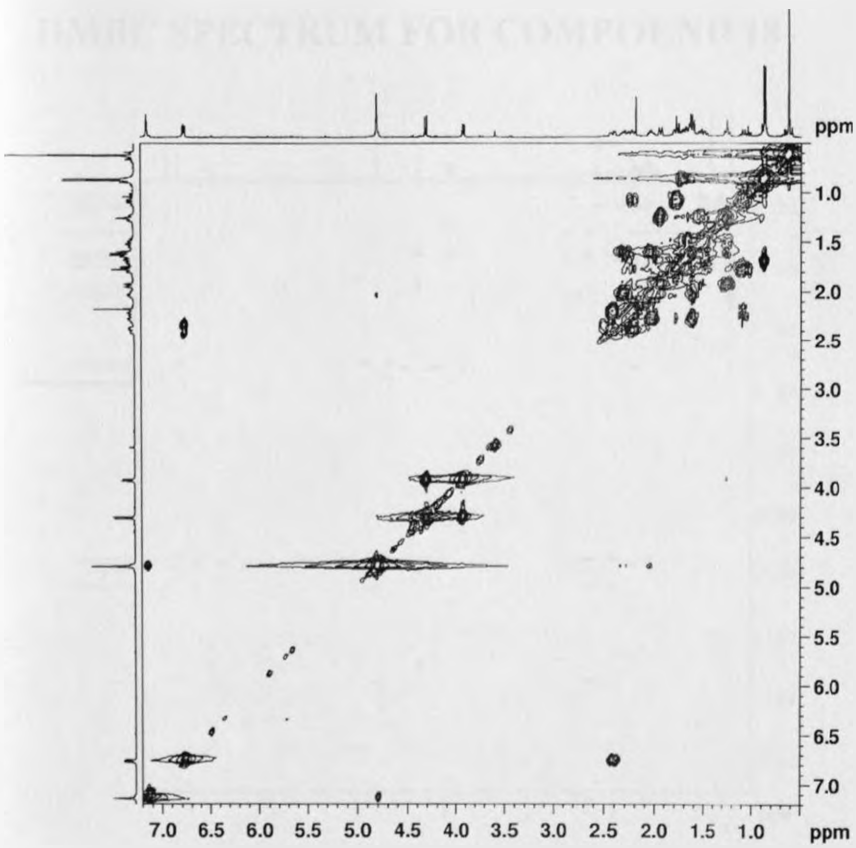
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 18



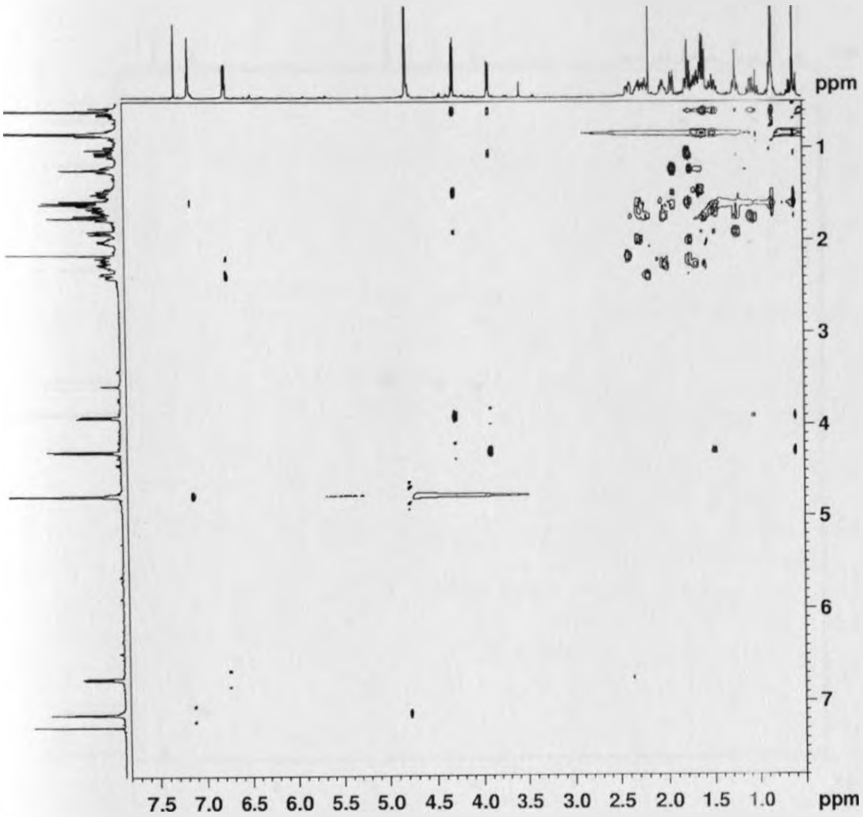
# APT SPECTRUM FOR COMPOUND 18



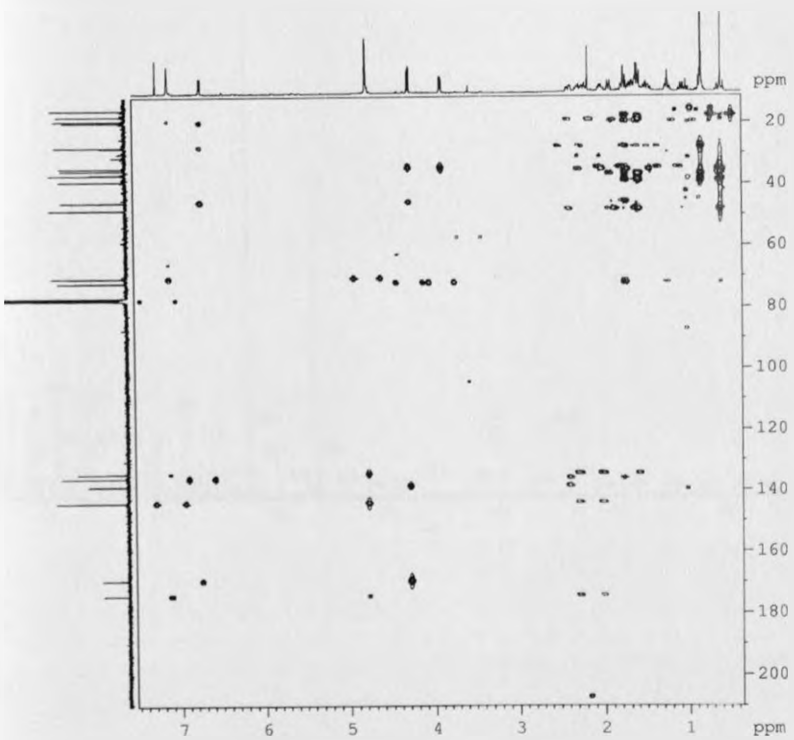

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 18



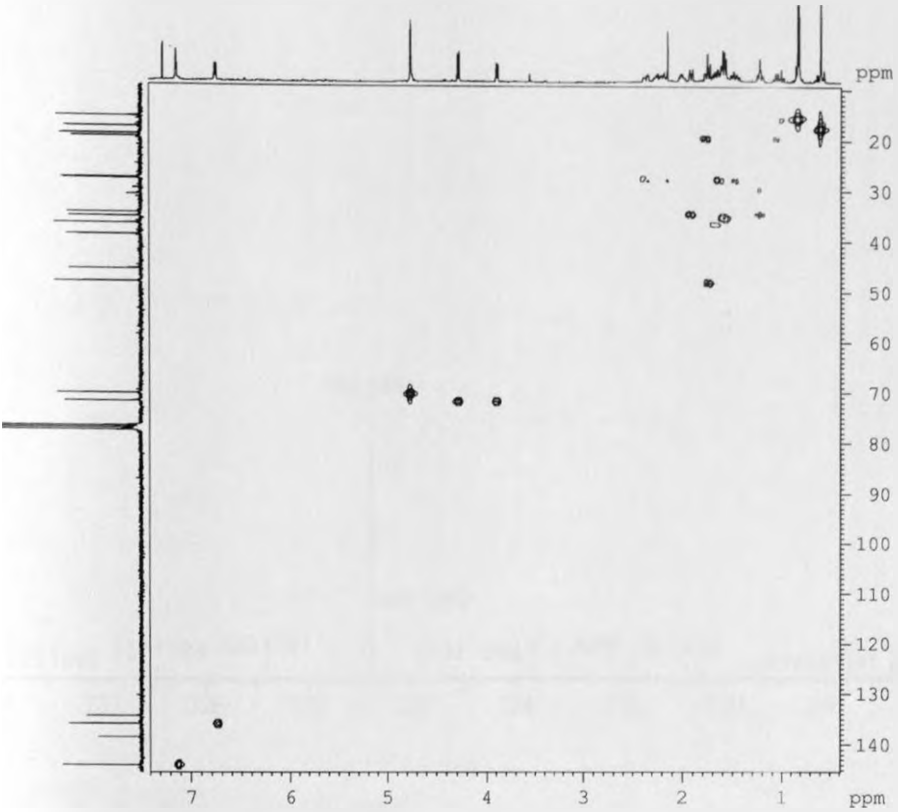
## NOESY SPECTRUM FOR COMPOUND 18



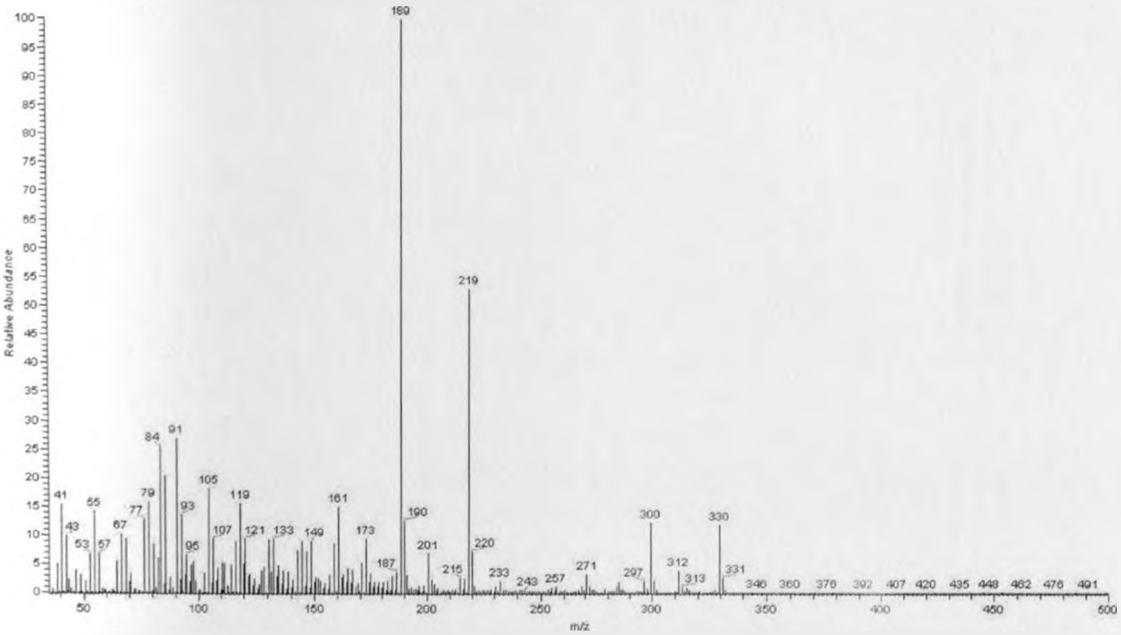

## HMBC SPECTRUM FOR COMPOUND 18



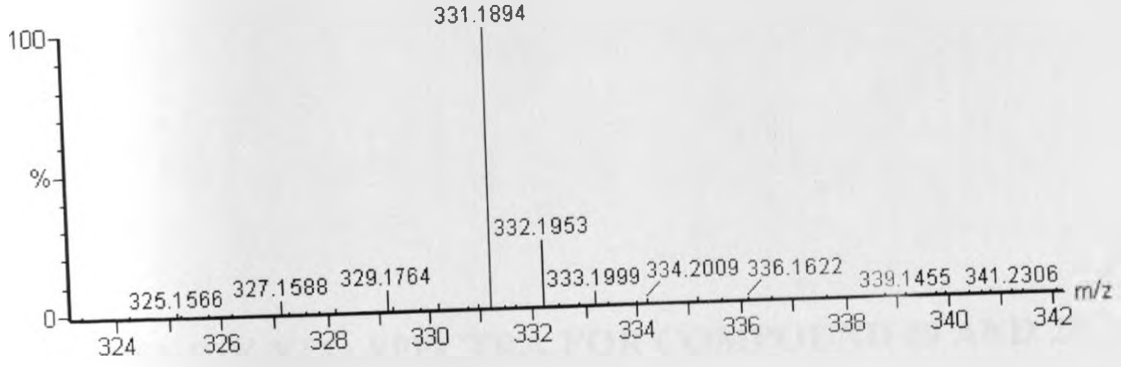
# HMQC SPECTRUM FOR COMPOUND 18




# MASS SPECTRUM FOR COMPOUND 18

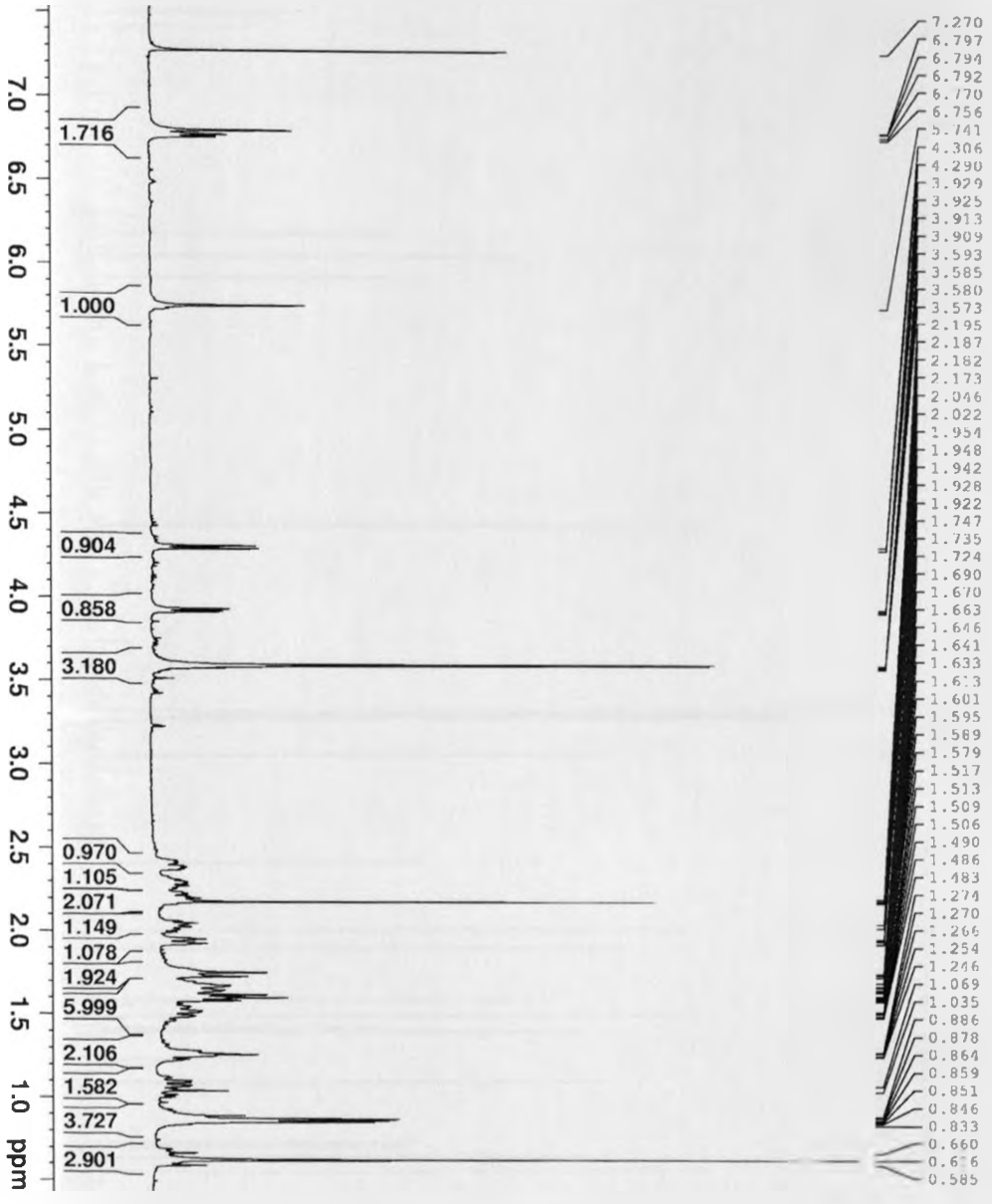


# HIGH RESOLUTION MASS SPECTRUM FOR COMPOUND 18

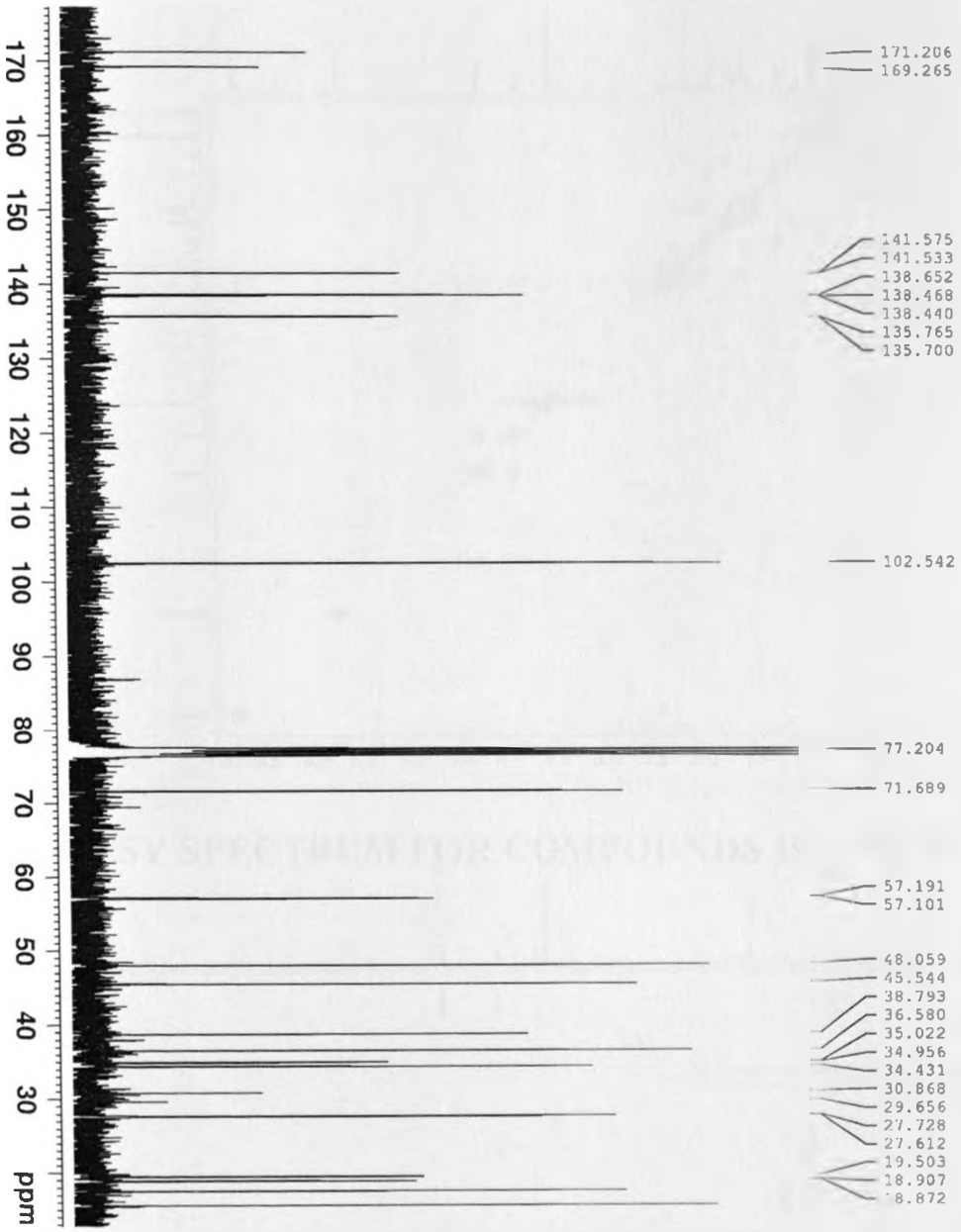


# APPENDIX XIX: SPECTRA FOR COMPOUND 19 AND 20

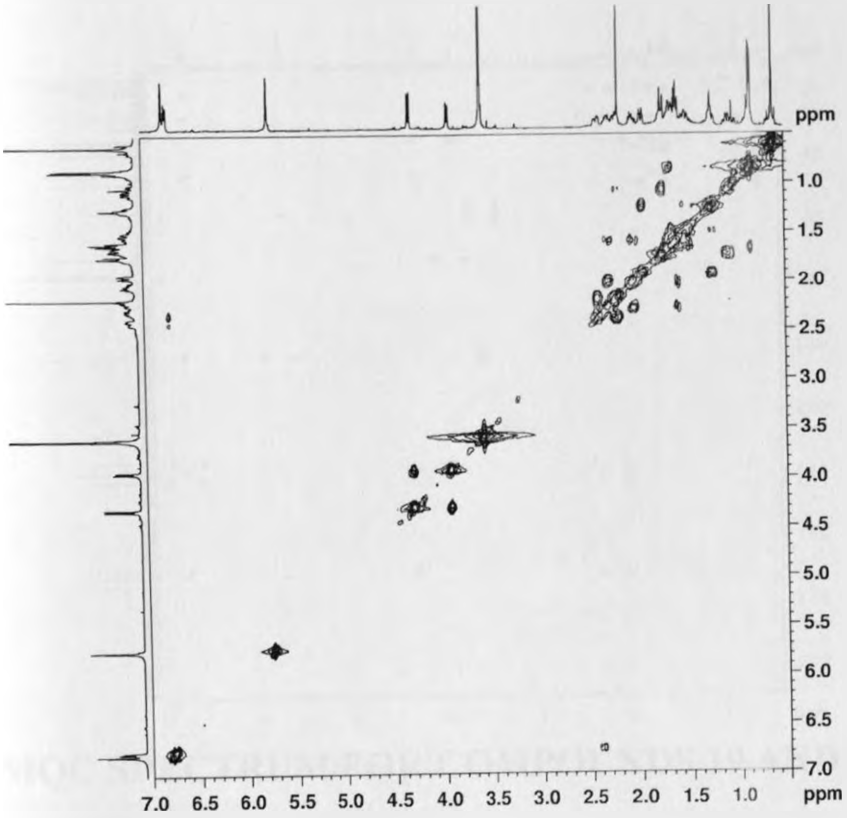
# $^1$H-NMR SPECTRUM FOR COMPOUNDS 19 AND 20



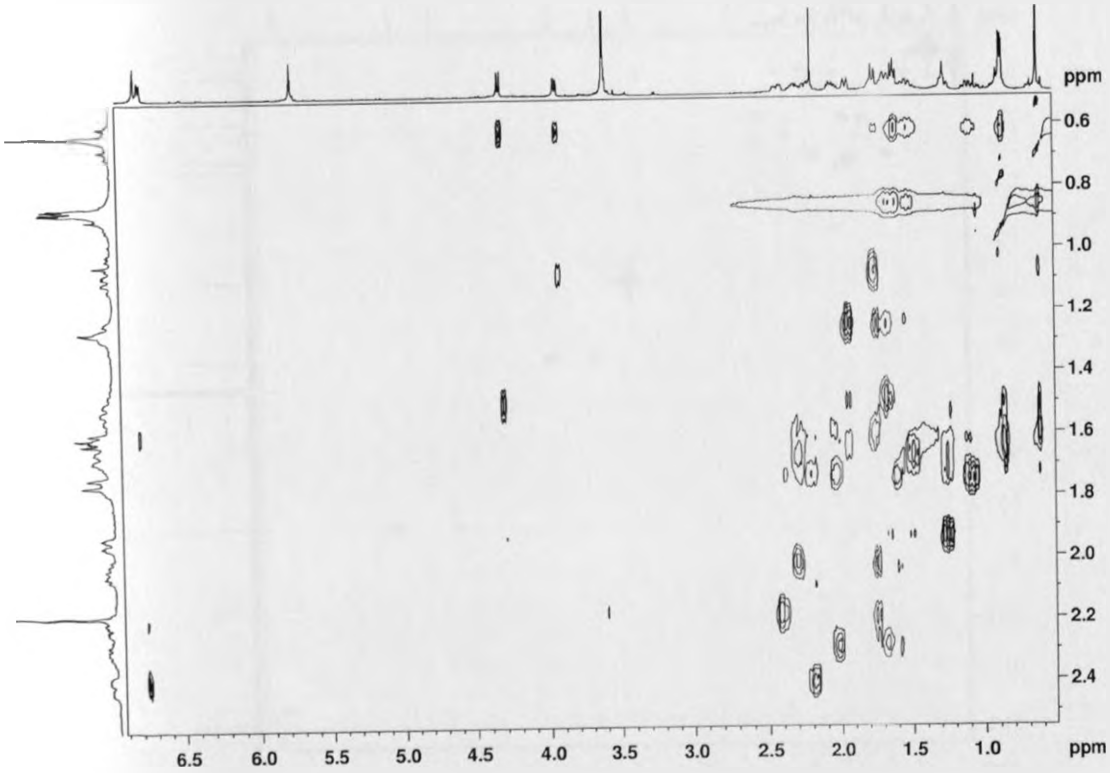
# $^{13}$C-NMR SPECTRUM FOR COMPOUNDS 19 AND 20



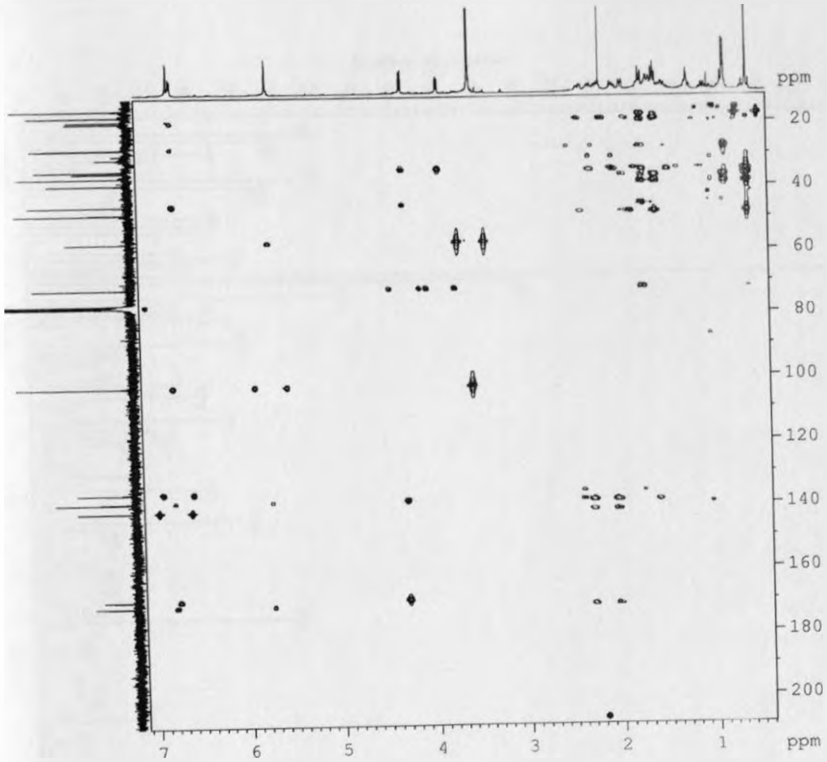
## $^1$H, H-COSY SPECTRUM FOR COMPOUNDS 19 AND 20



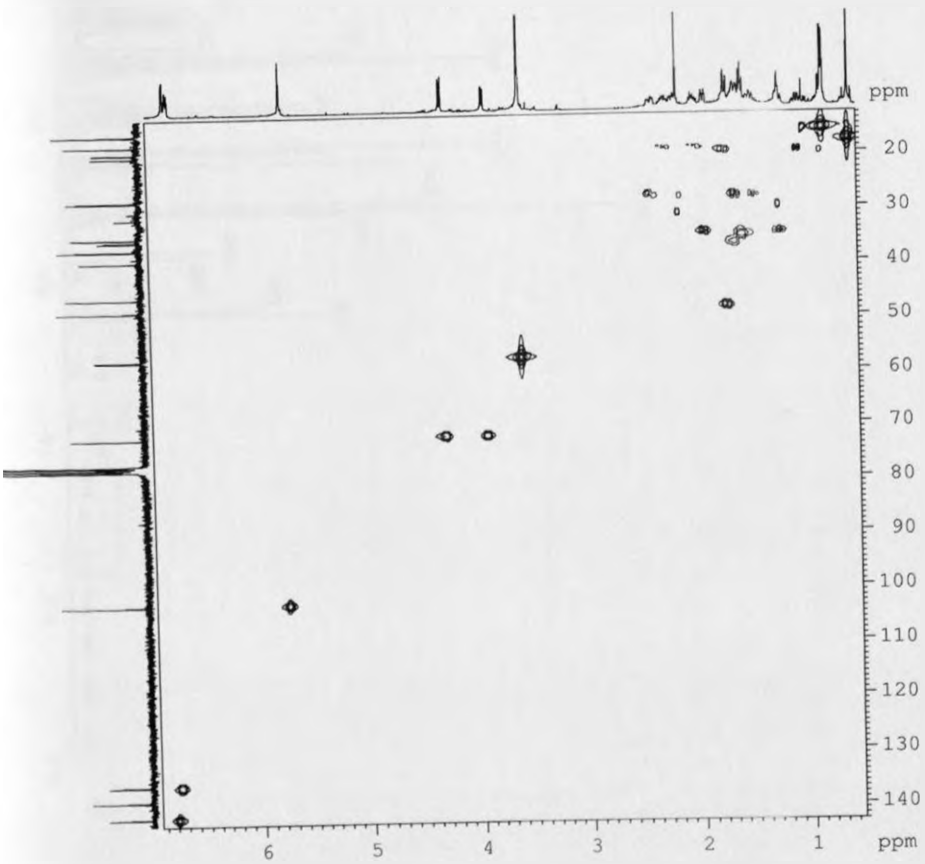

## NOESY SPECTRUM FOR COMPOUNDS 19 AND 20



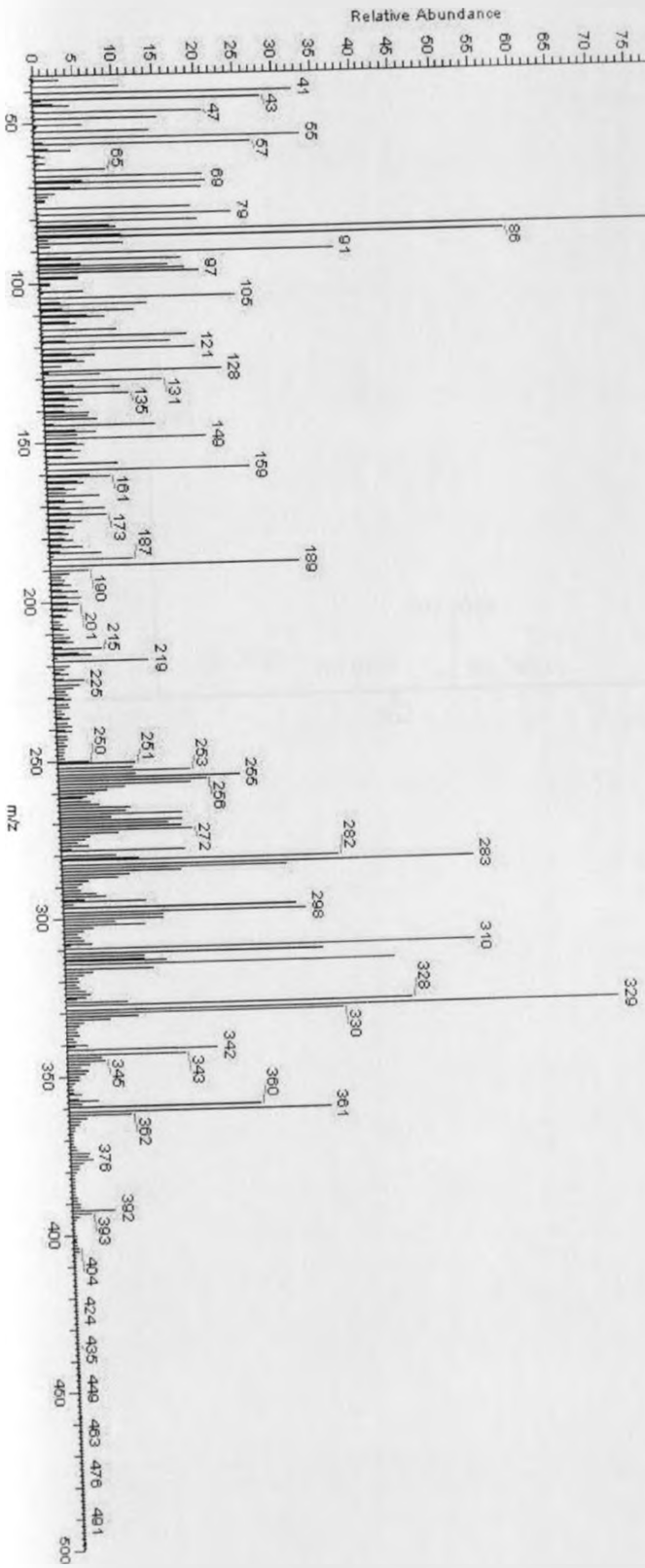
## HMBC SPECTRUM FOR COMPOUNDS 19 AND 20



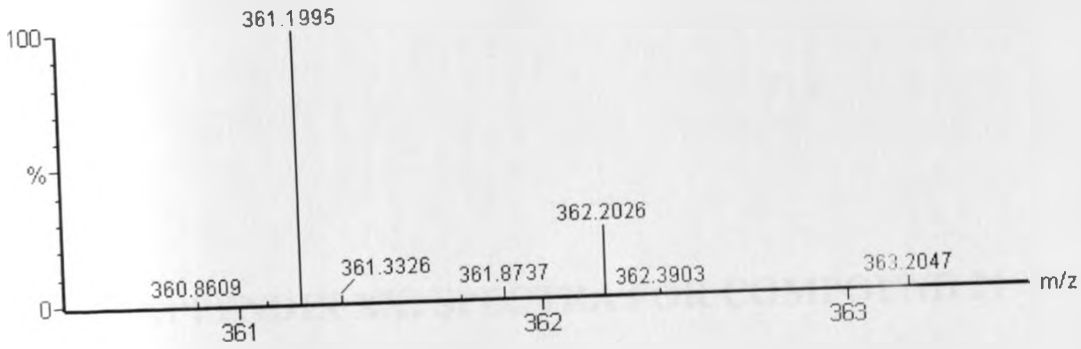

## HMQC SPECTRUM FOR COMPOUNDS 19 AND 20



# MASS SPECTRUM FOR COMPOUNDS 19 AND 20

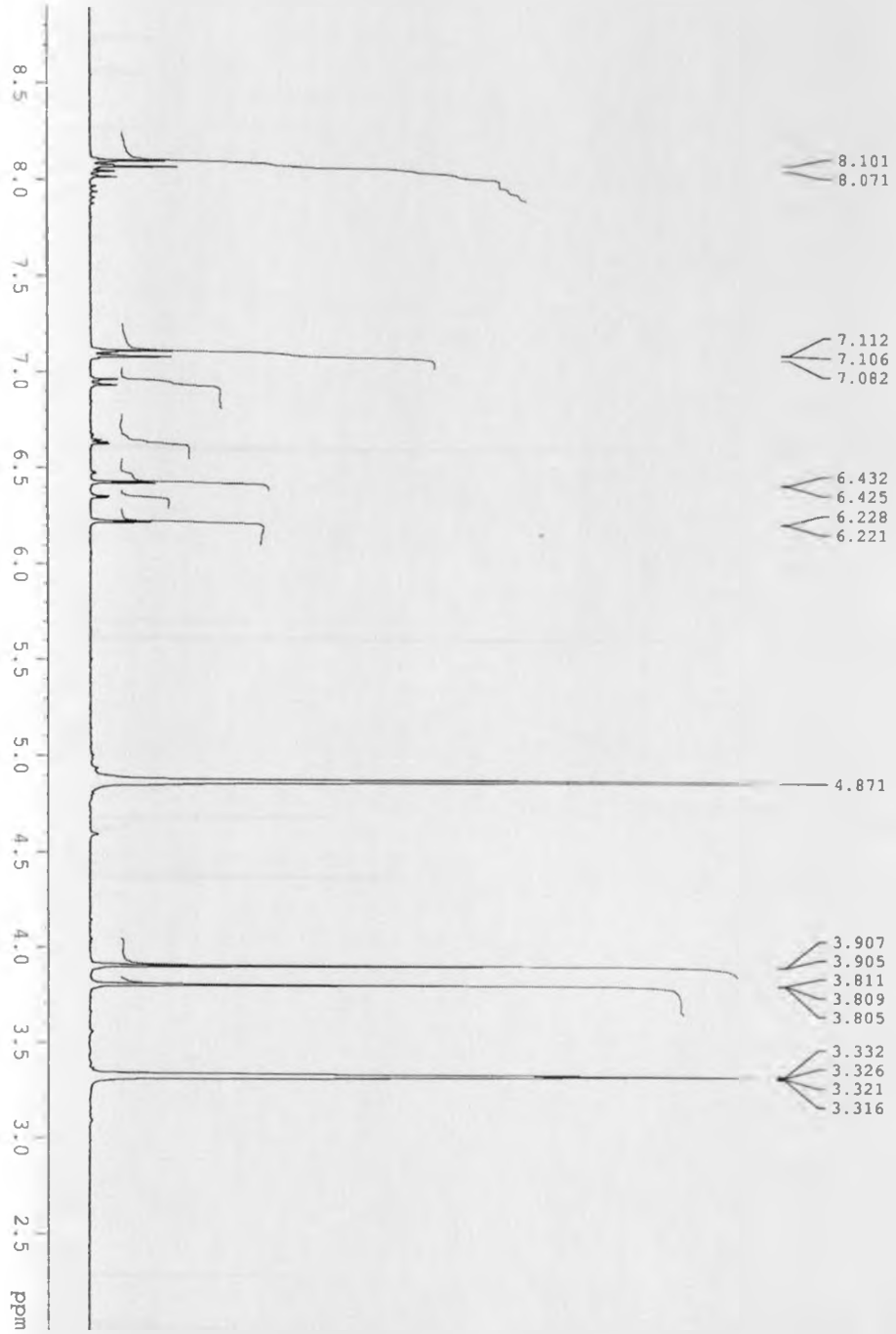


# HIGH RESOLUTION MASS SPECTRUM FOR COMPOUND 19 & 20

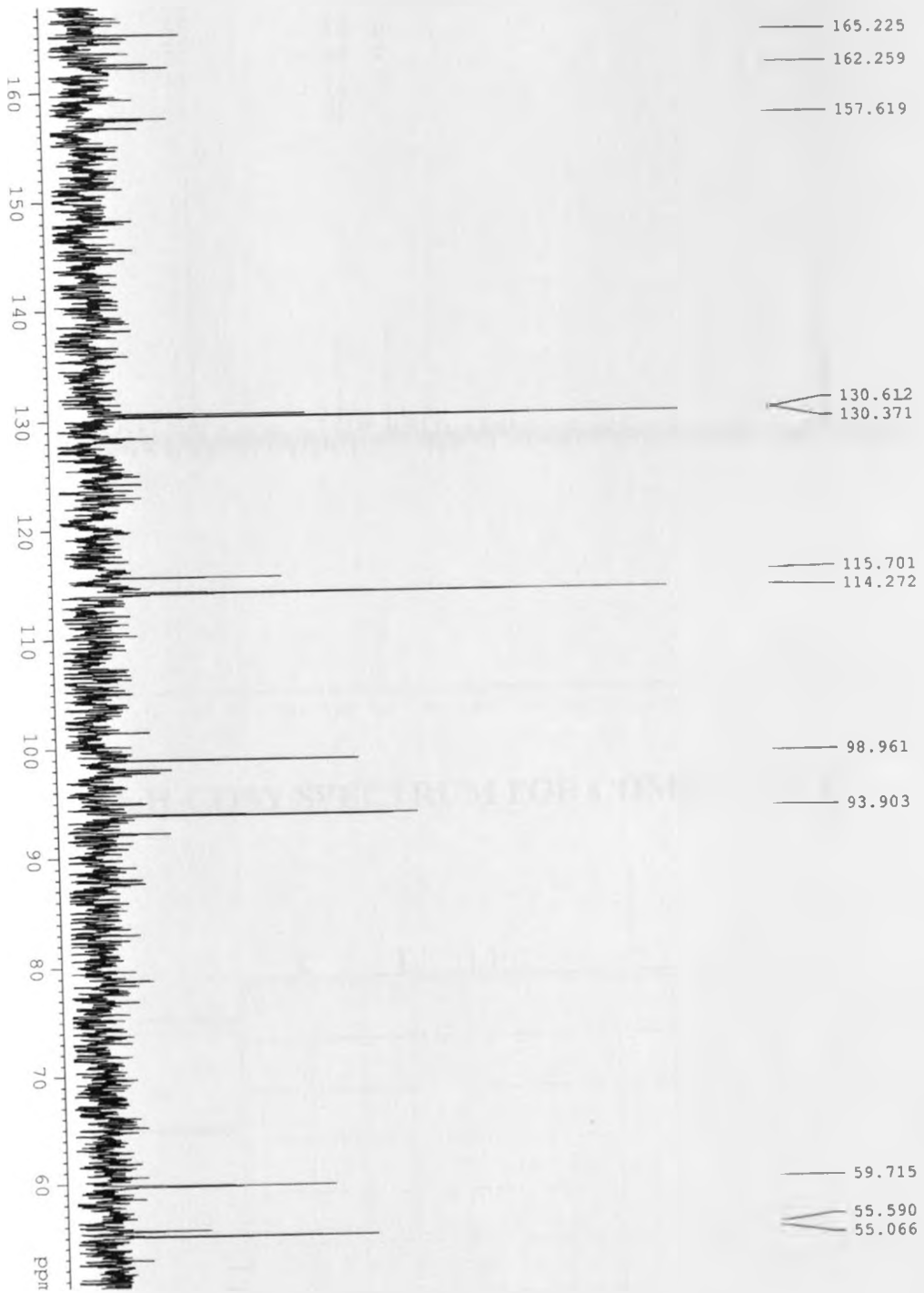


# APPENDIX XX: SPECTRA FOR COMPOUND 21

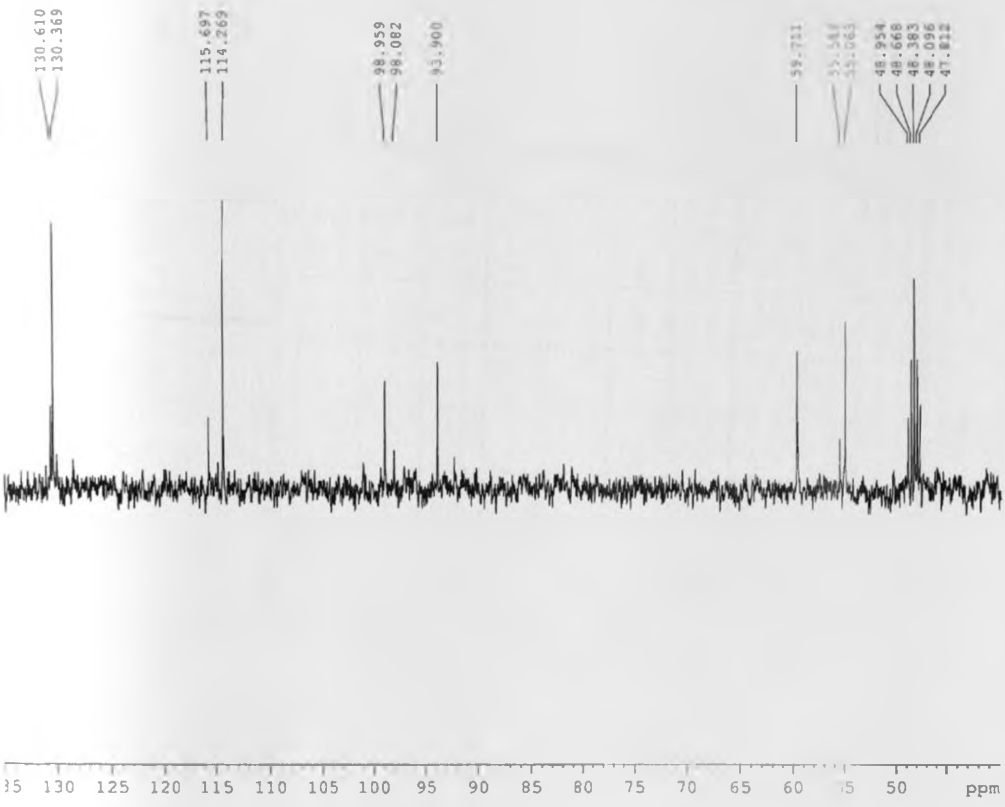
# $^1$H-NMR SPECTRUM FOR COMPOUND 21



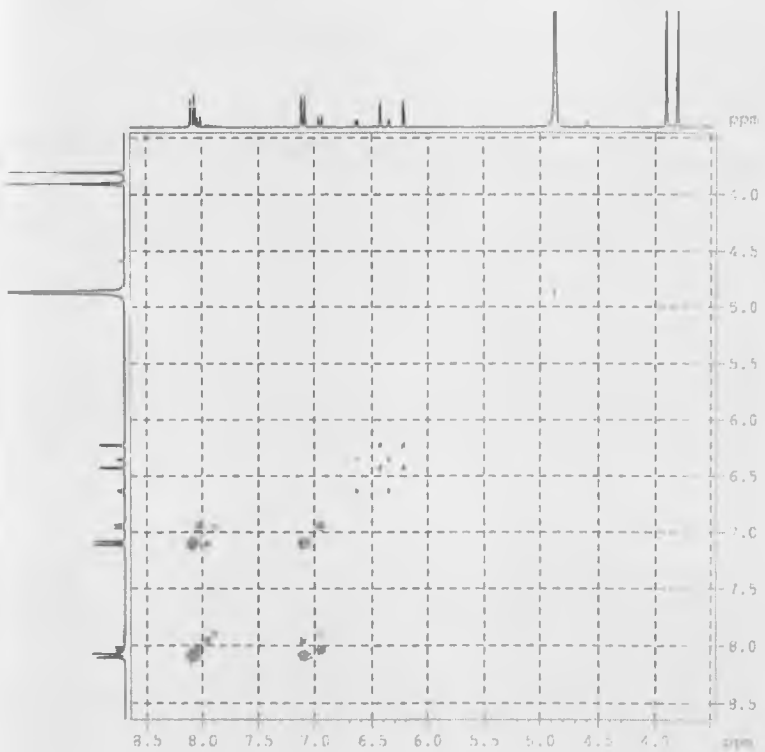
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 21



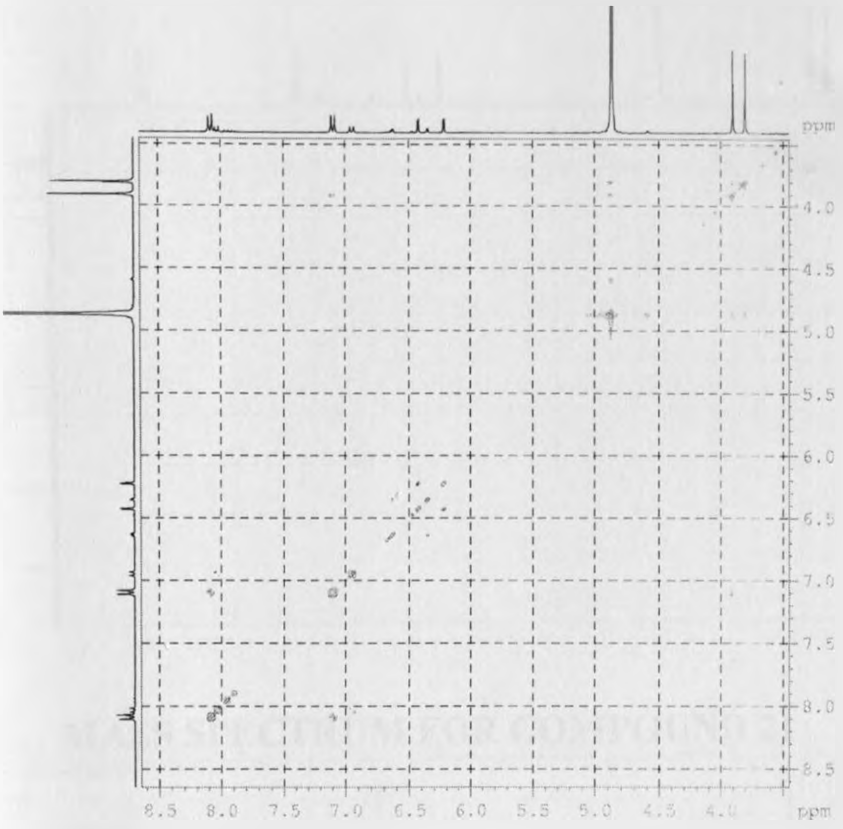
# DEPT SPECTRUM FOR COMPOUND 21



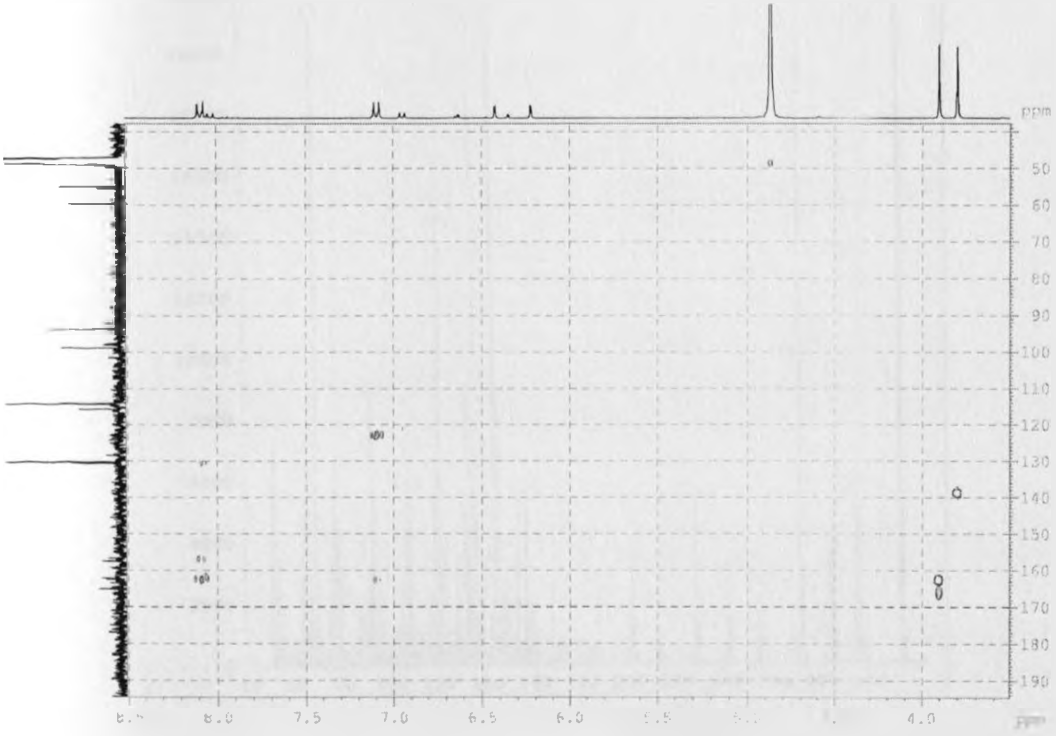

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 21



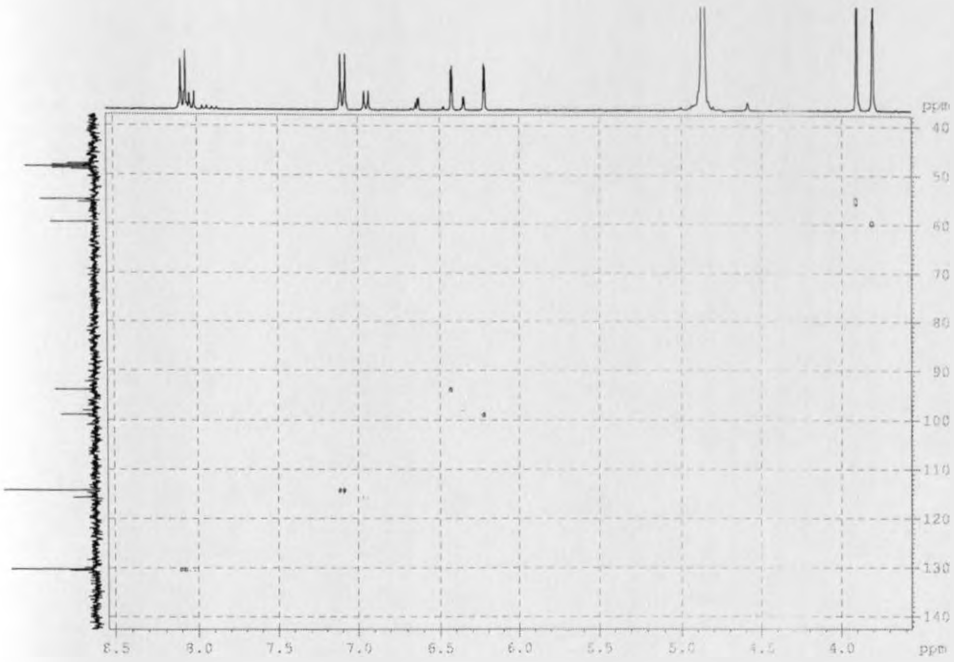
## NOESY SPECTRUM FOR COMPOUND 21



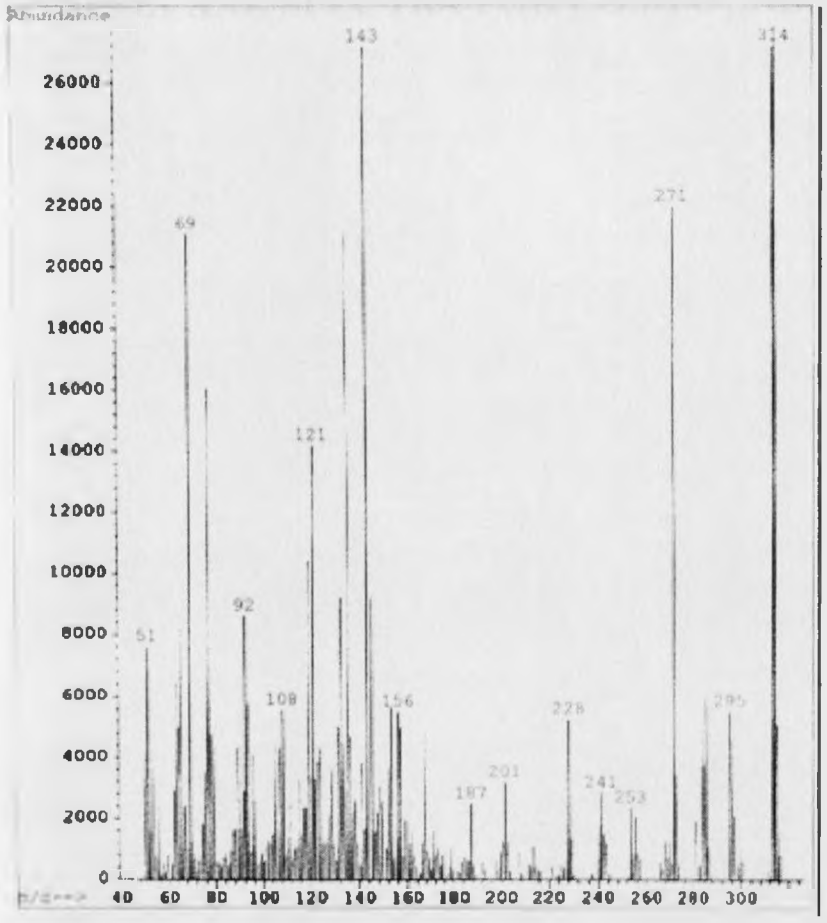

## HMBC SPECTRUM FOR COMPOUND 21



# HSQC-DEPT SPECTRUM FOR COMPOUND 21

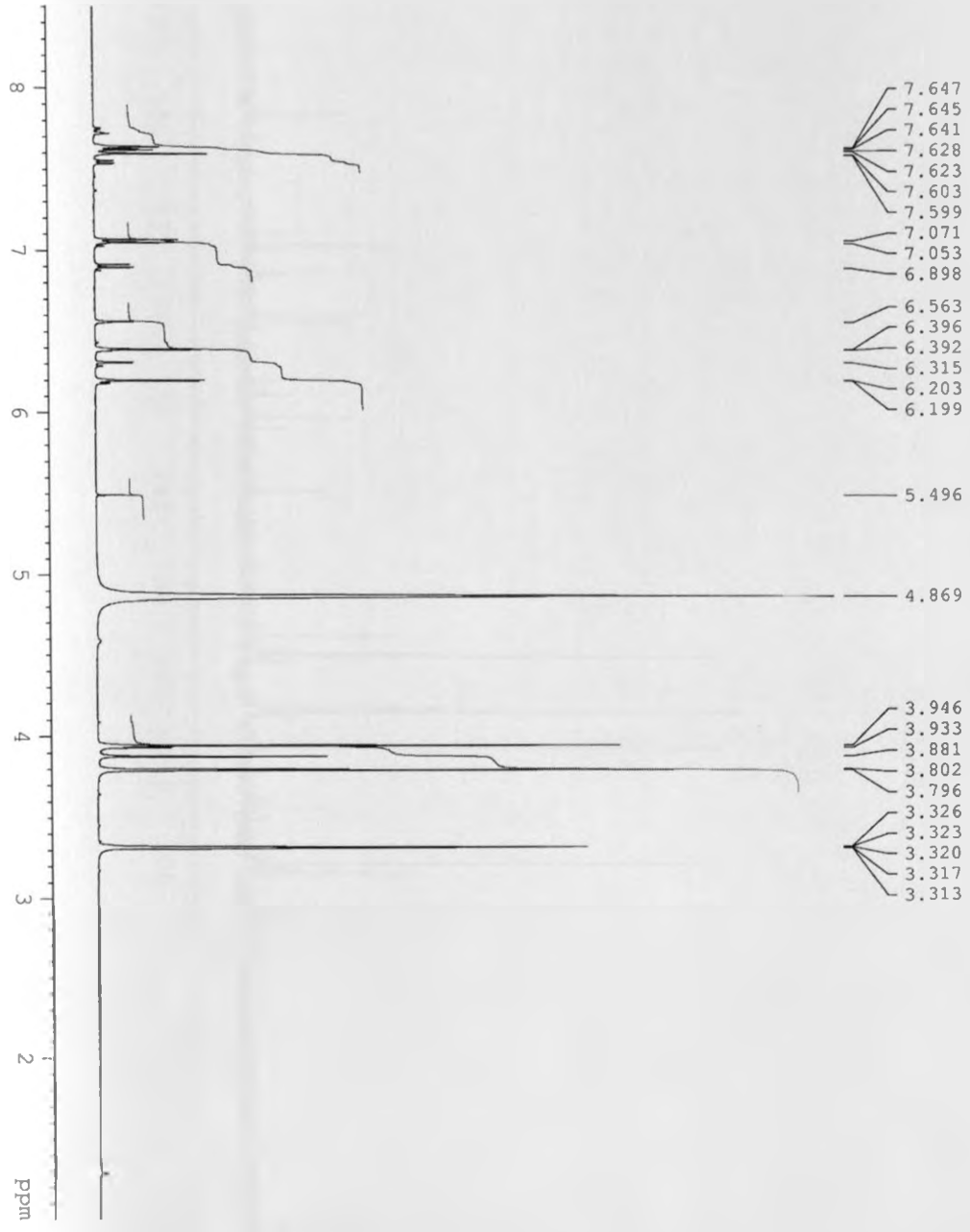



# MASS SPECTRUM FOR COMPOUND 21

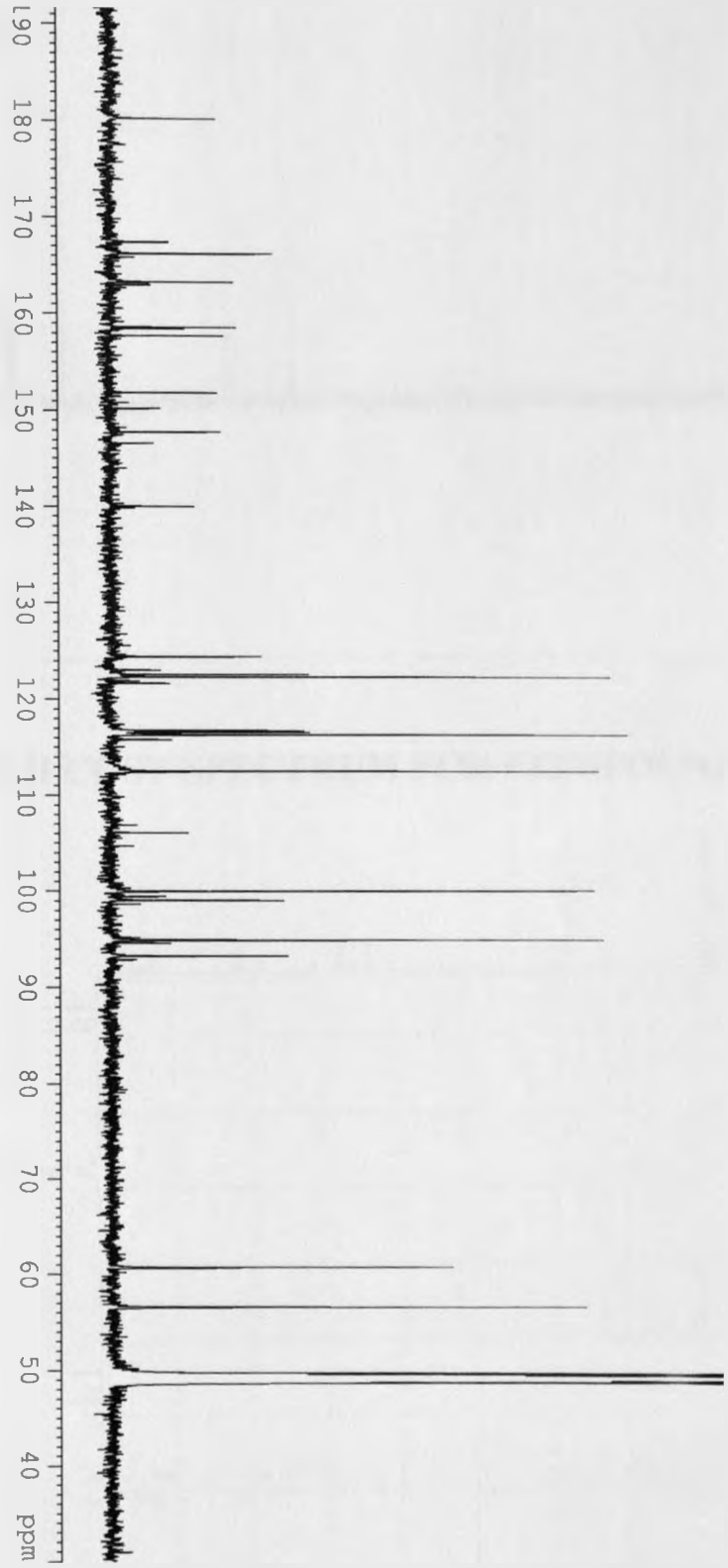


# APPENDIX XXI: SPECTRA FOR COMPOUND 22

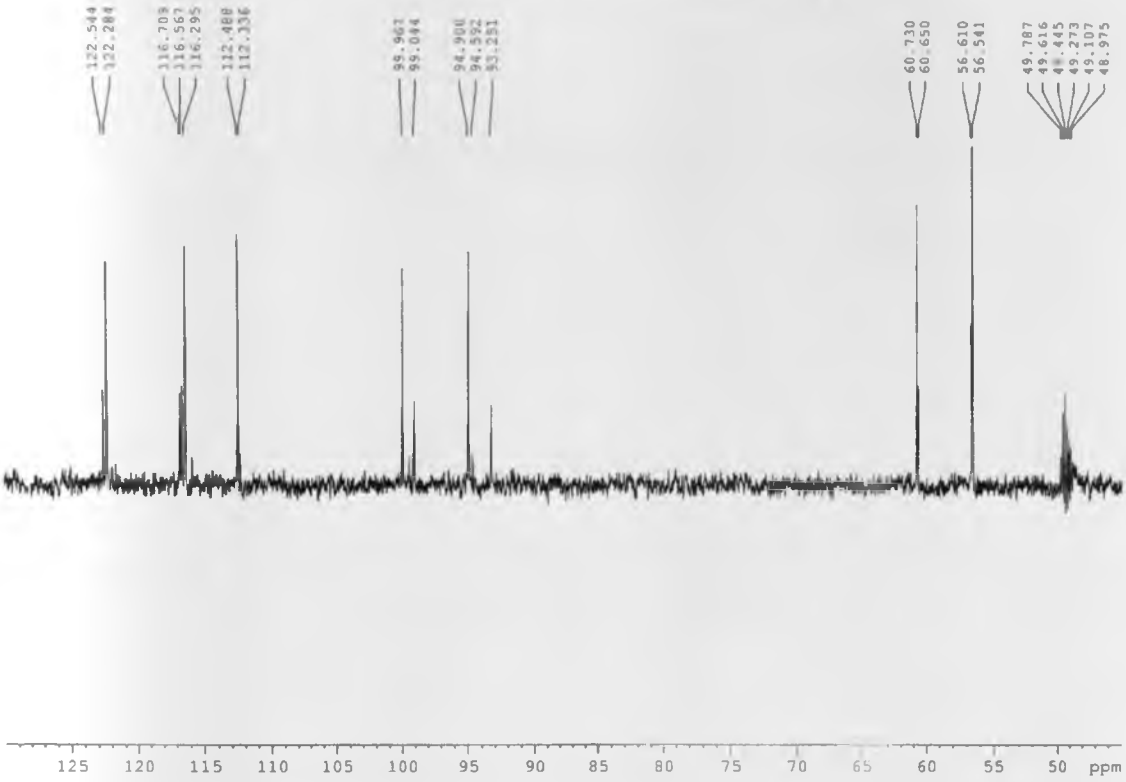
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 22



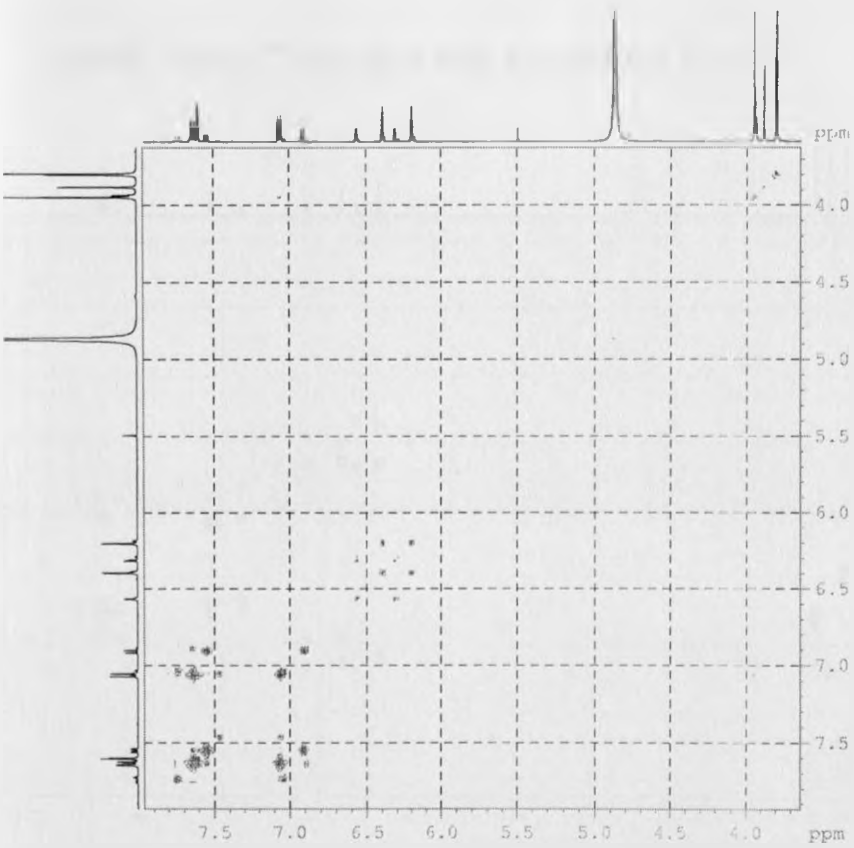
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 22



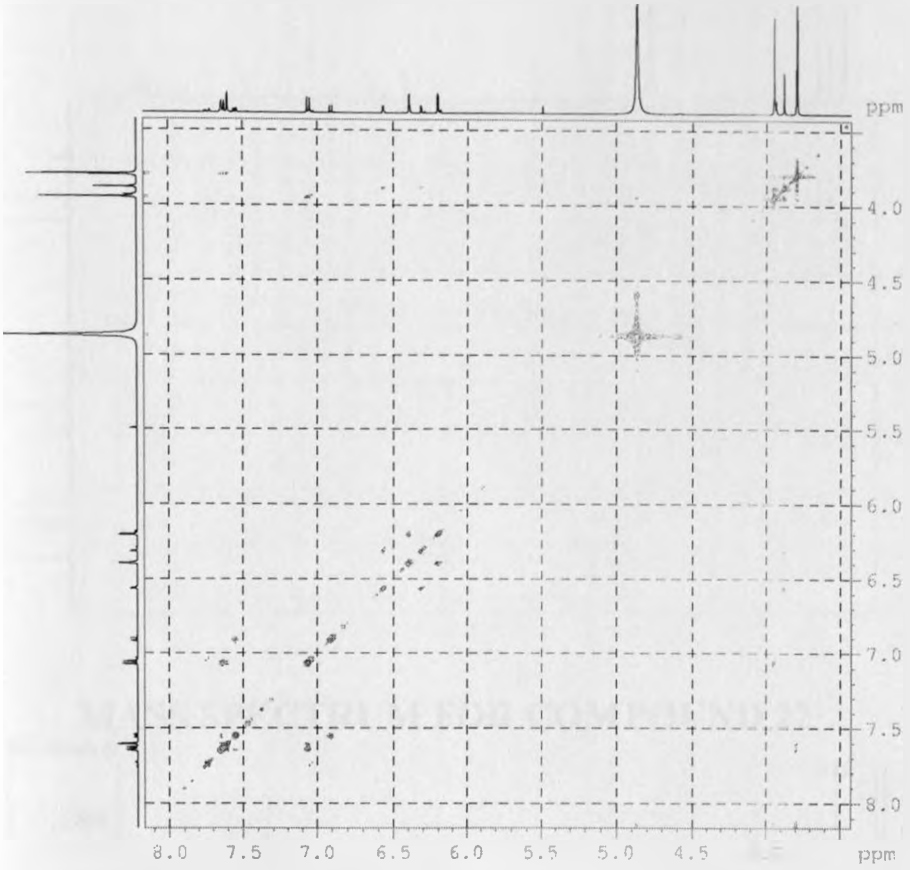
# DEPT SPECTRUM FOR COMPOUND 22



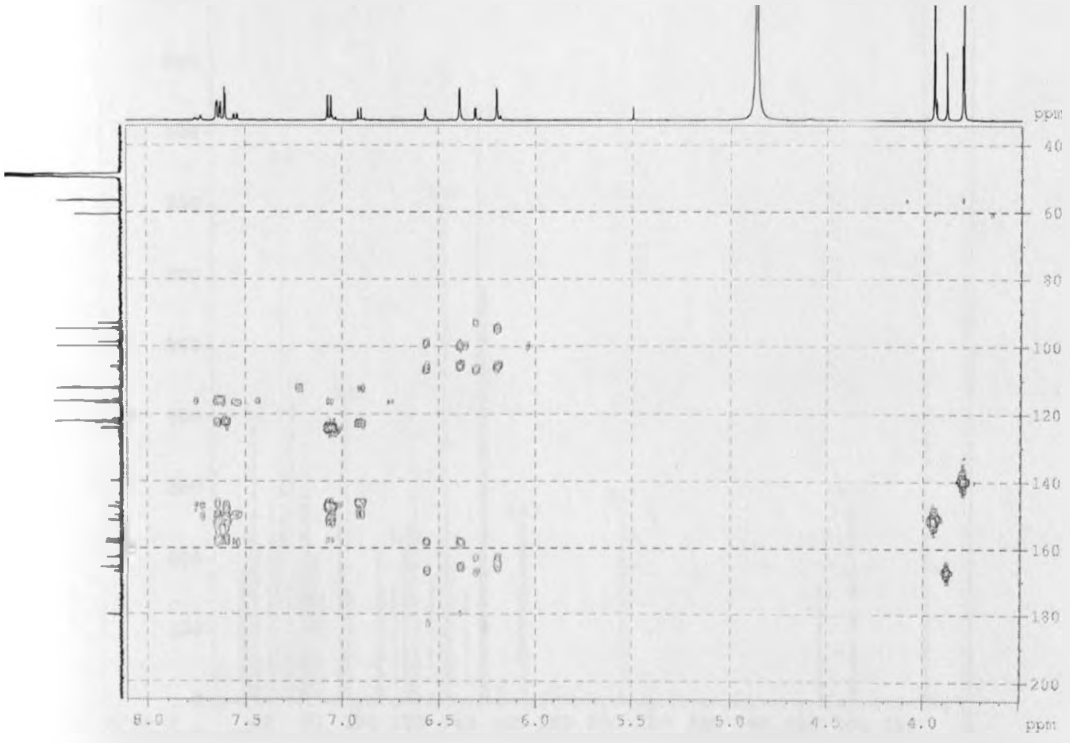

# $^{1}$H, H COSY SPECTRUM FOR COMPOUND 22



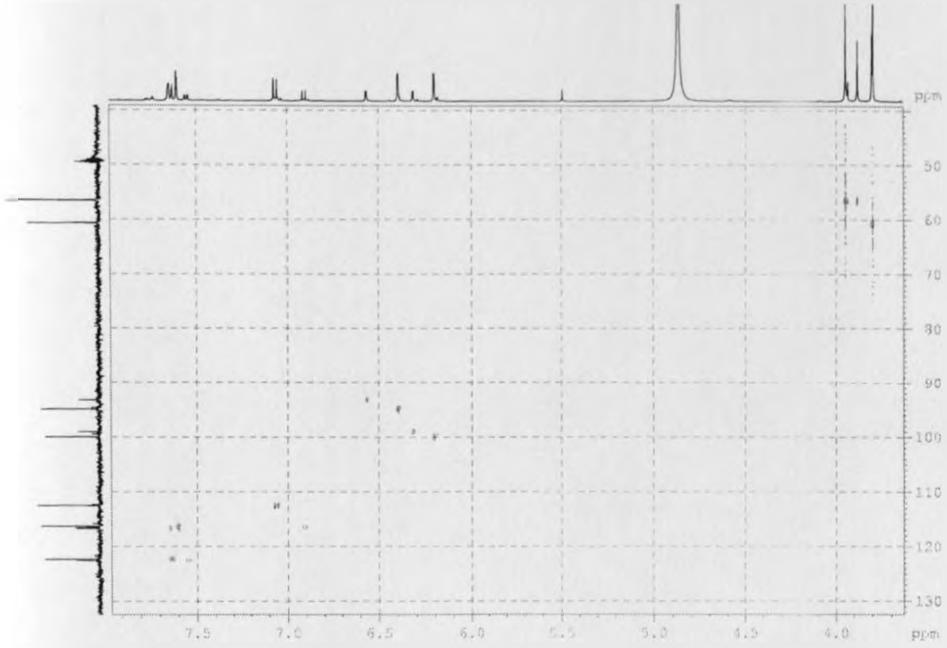
## NOESY SPECTRUM FOR COMPOUND 22



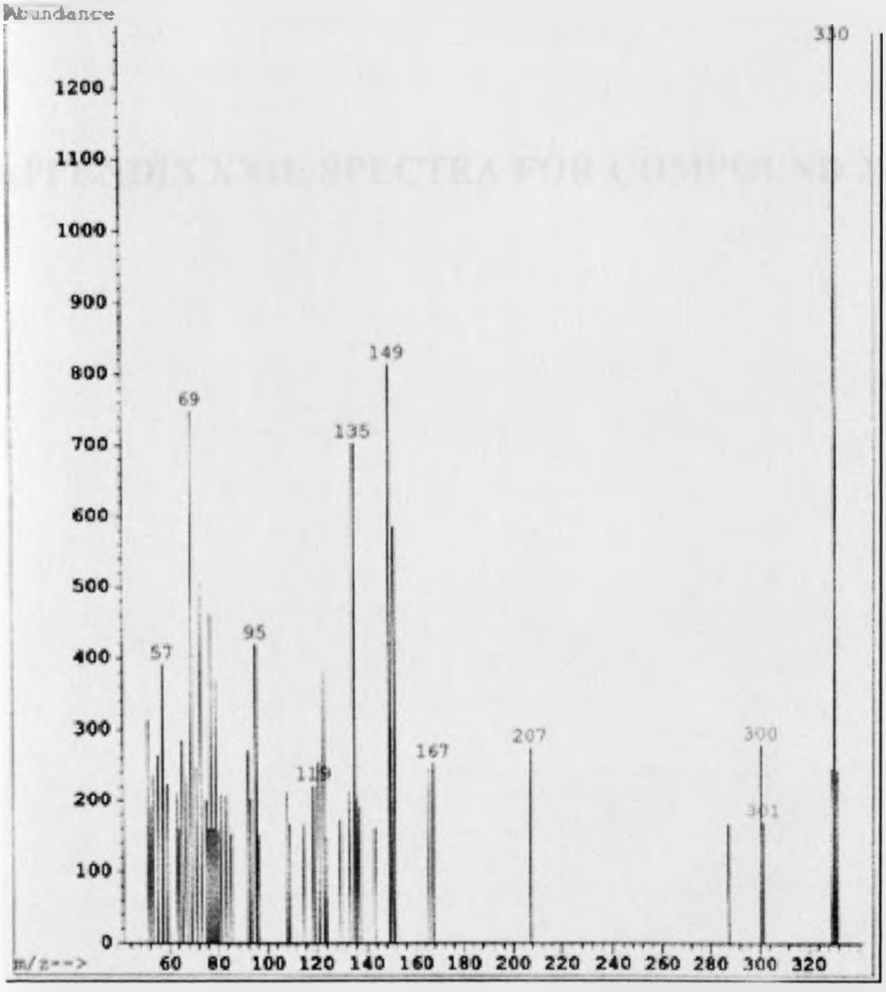

## HMBC SPECTRUM FOR COMPOUND 22



# HSQC-DEPT SPECTRUM FOR COMPOUND 22

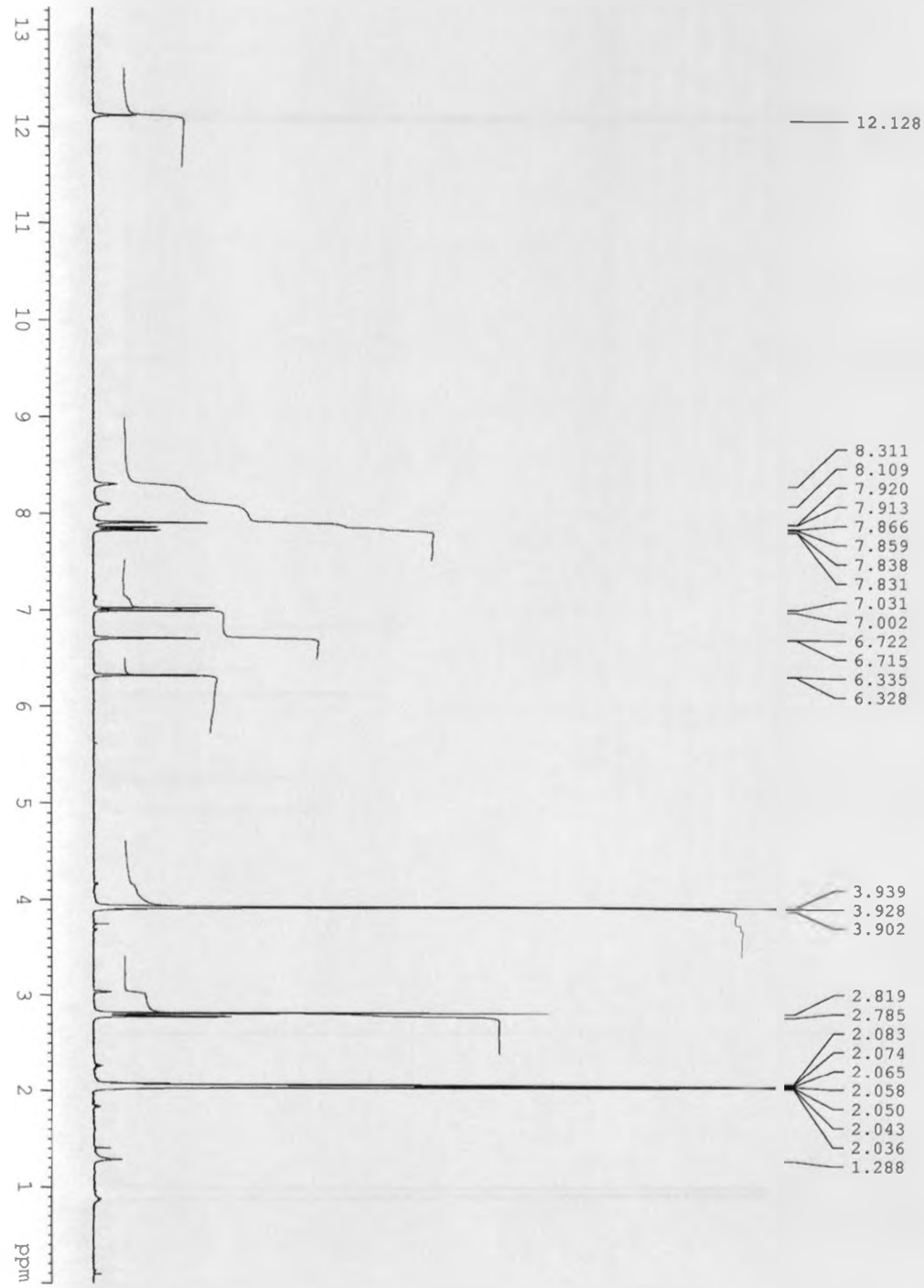



# MASS SPECTRUM FOR COMPOUND 22

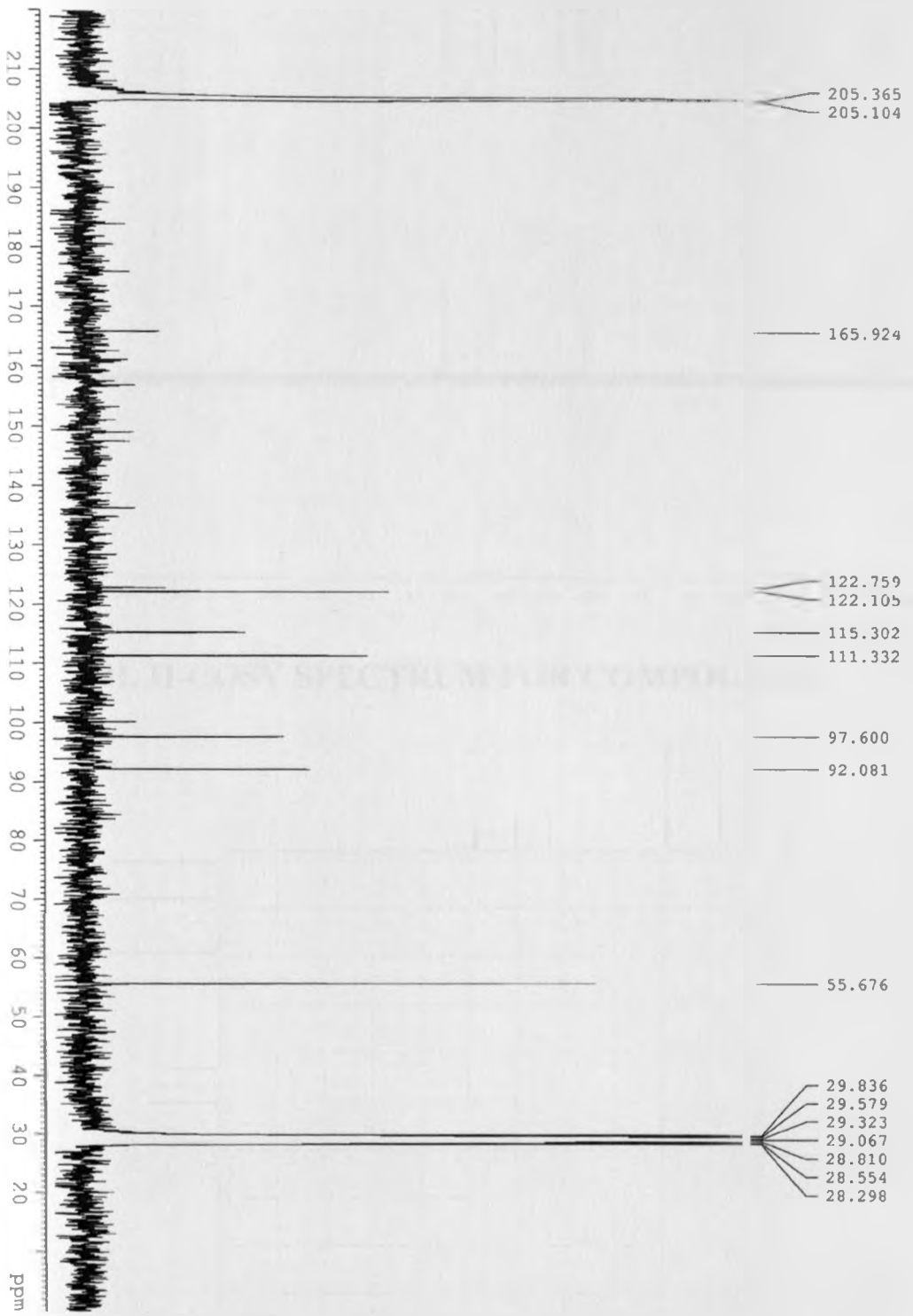


# APPENDIX XXII: SPECTRA FOR COMPOUND 23

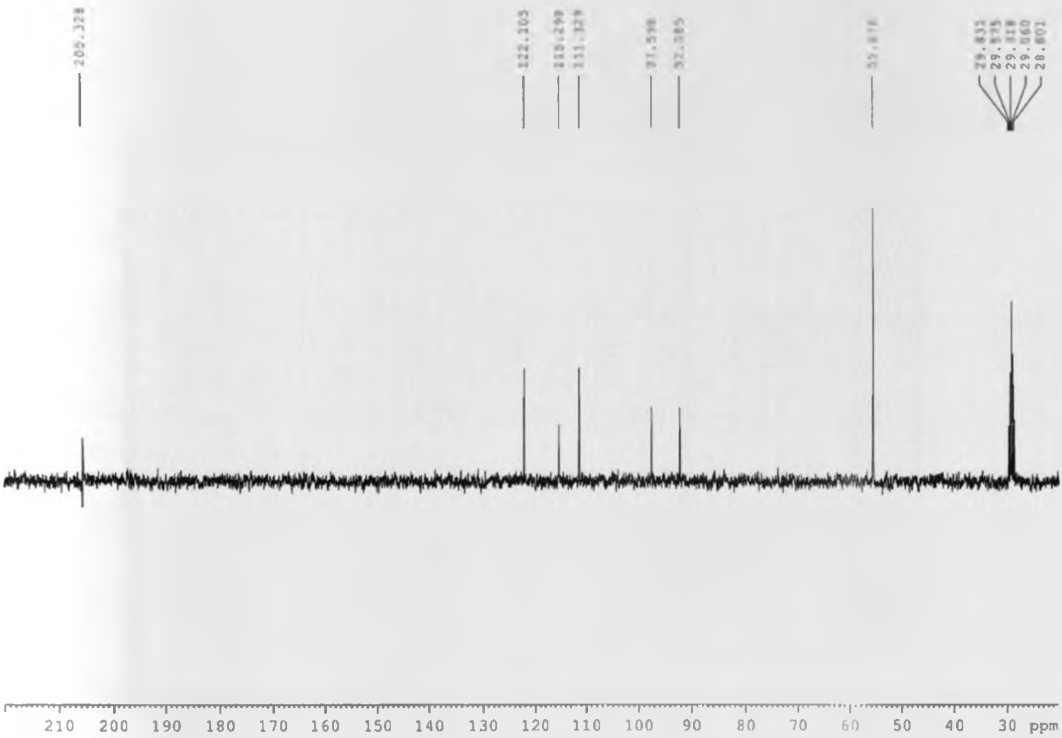
# $^{1}$H-NMR SPECTRUM FOR COMPOUD 23



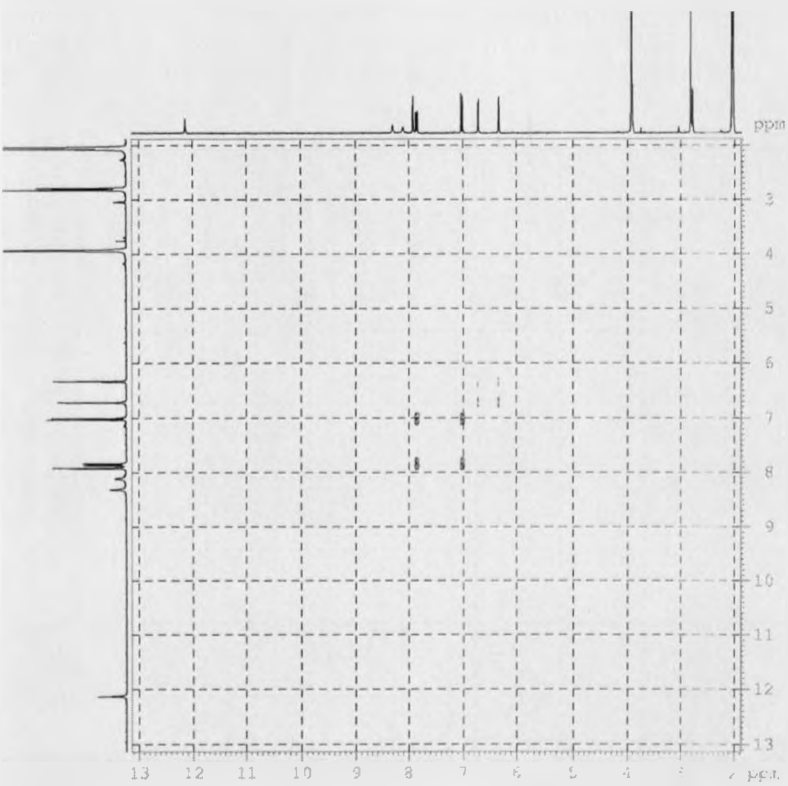
# $^{13}$C-NMR SPECTRUM FOR COMPOUD 23



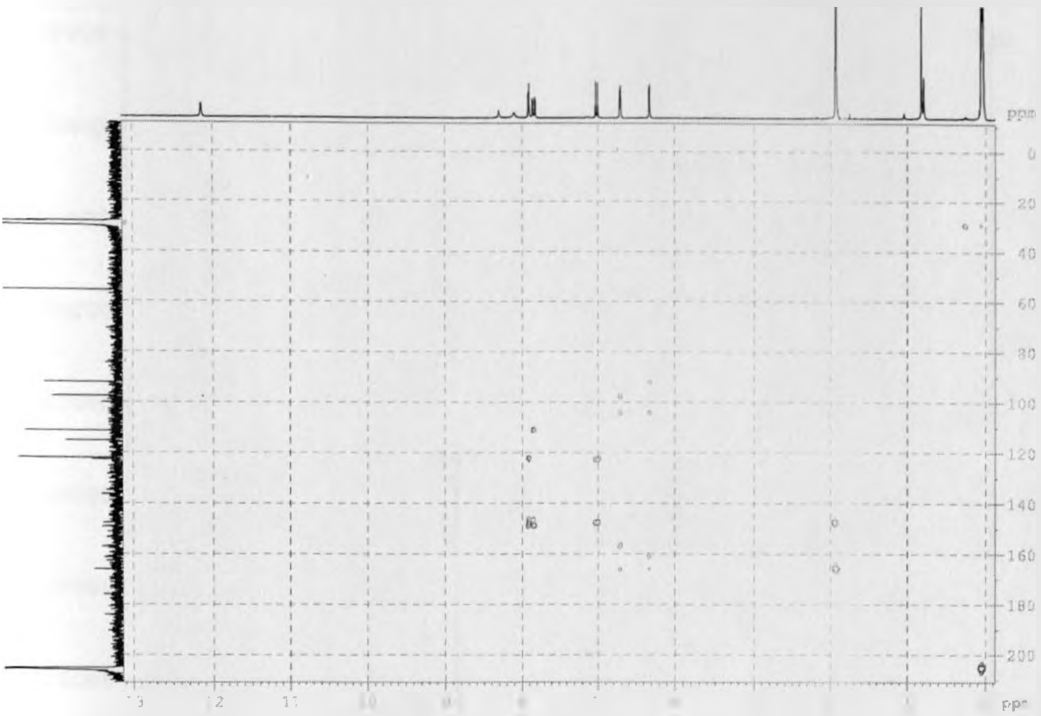
# DEPT SPECTRUM FOR COMPOUD 23



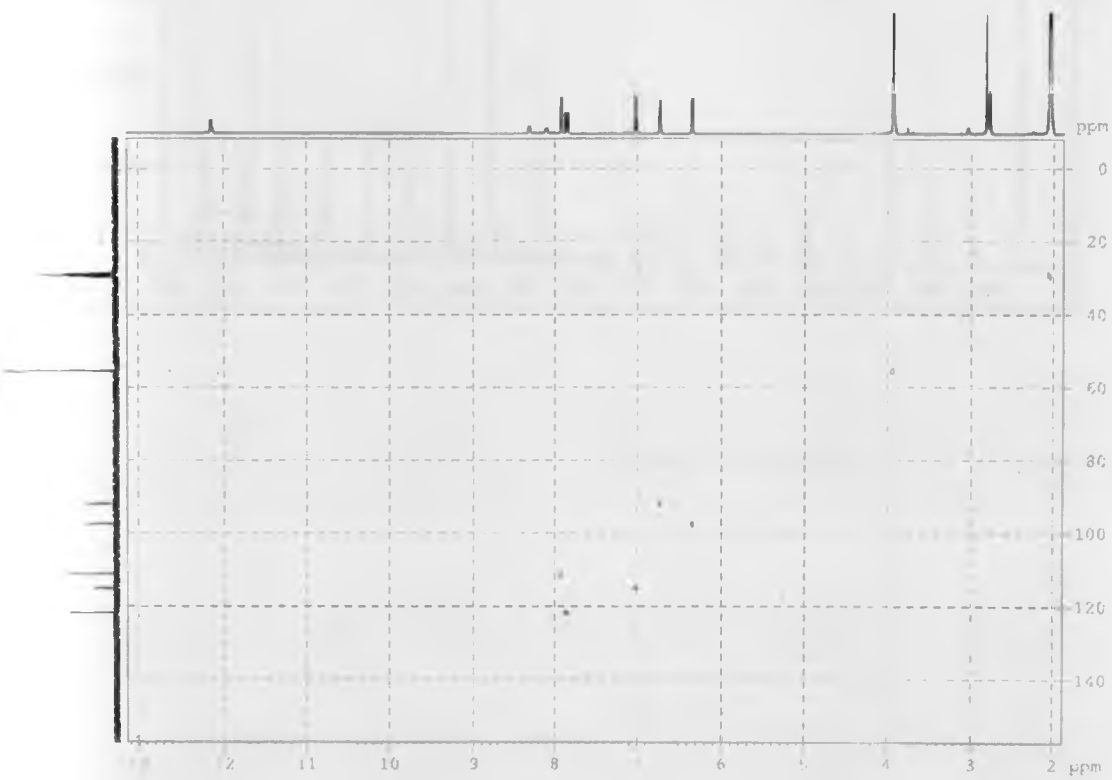

# $^{1}$H, H-COSY SPECTRUM FOR COMPOUD 23



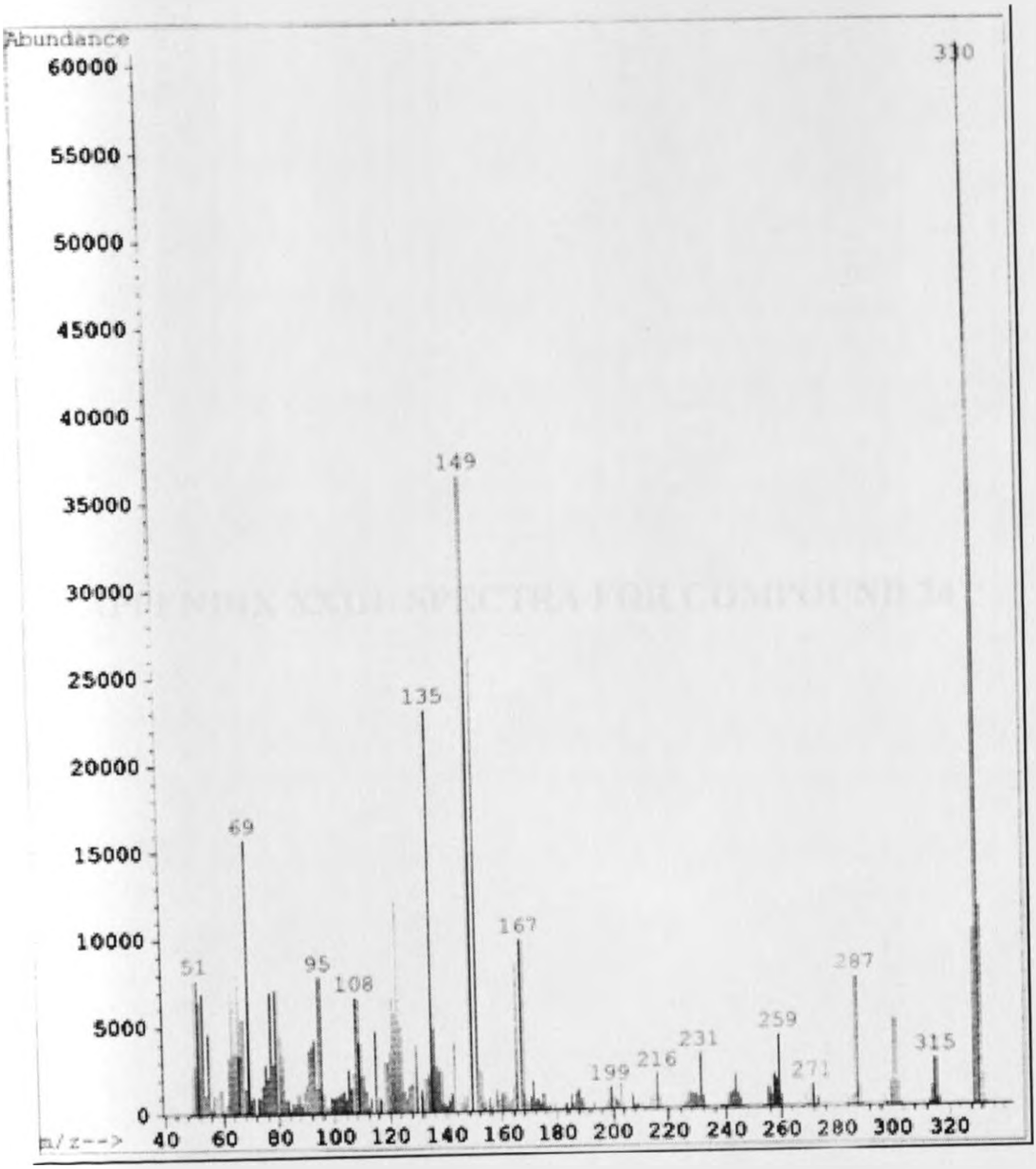
## HMBC SPECTRUM FOR COMPOUD 23




## HSQC-DEPT SPECTRUM FOR COMPOUD 23

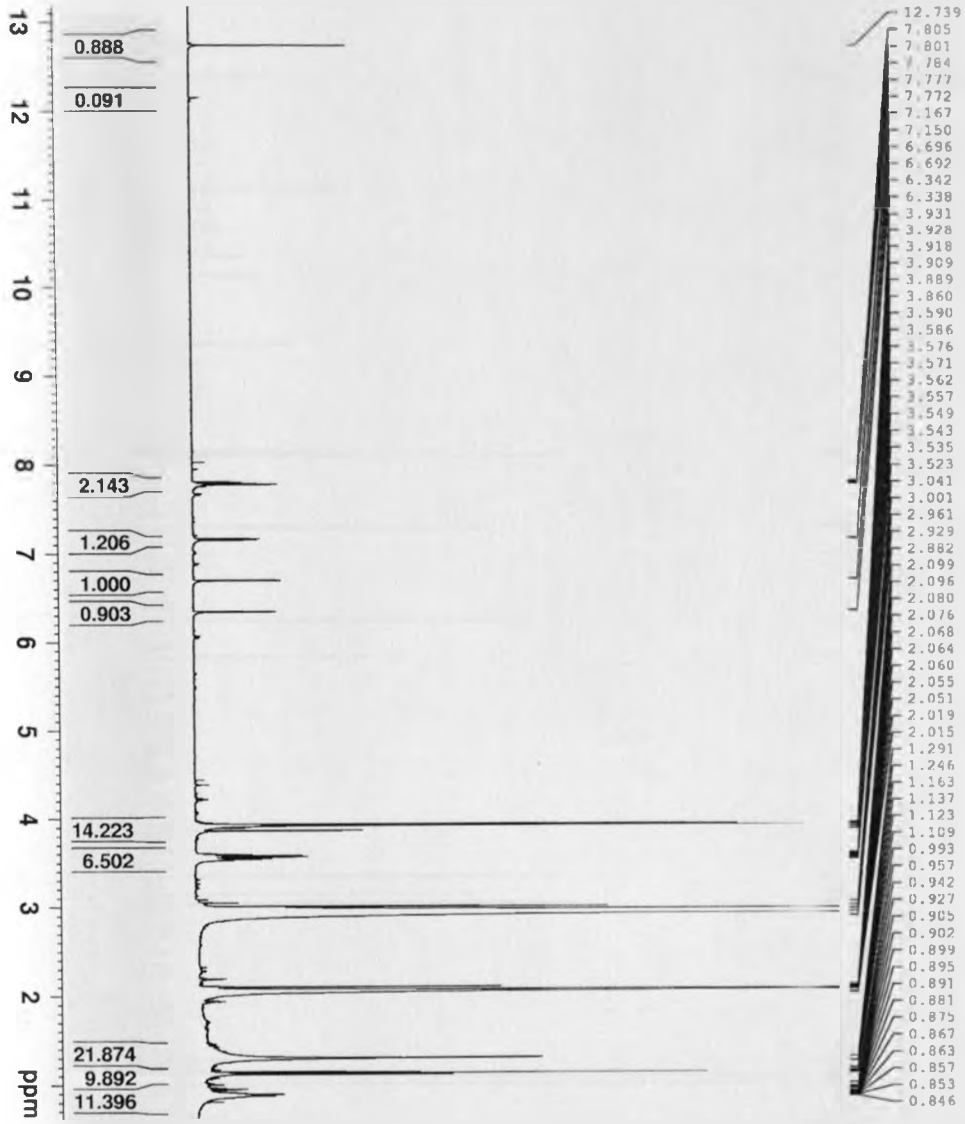


# MASS SPECTRUM FOR COMPOUD 23

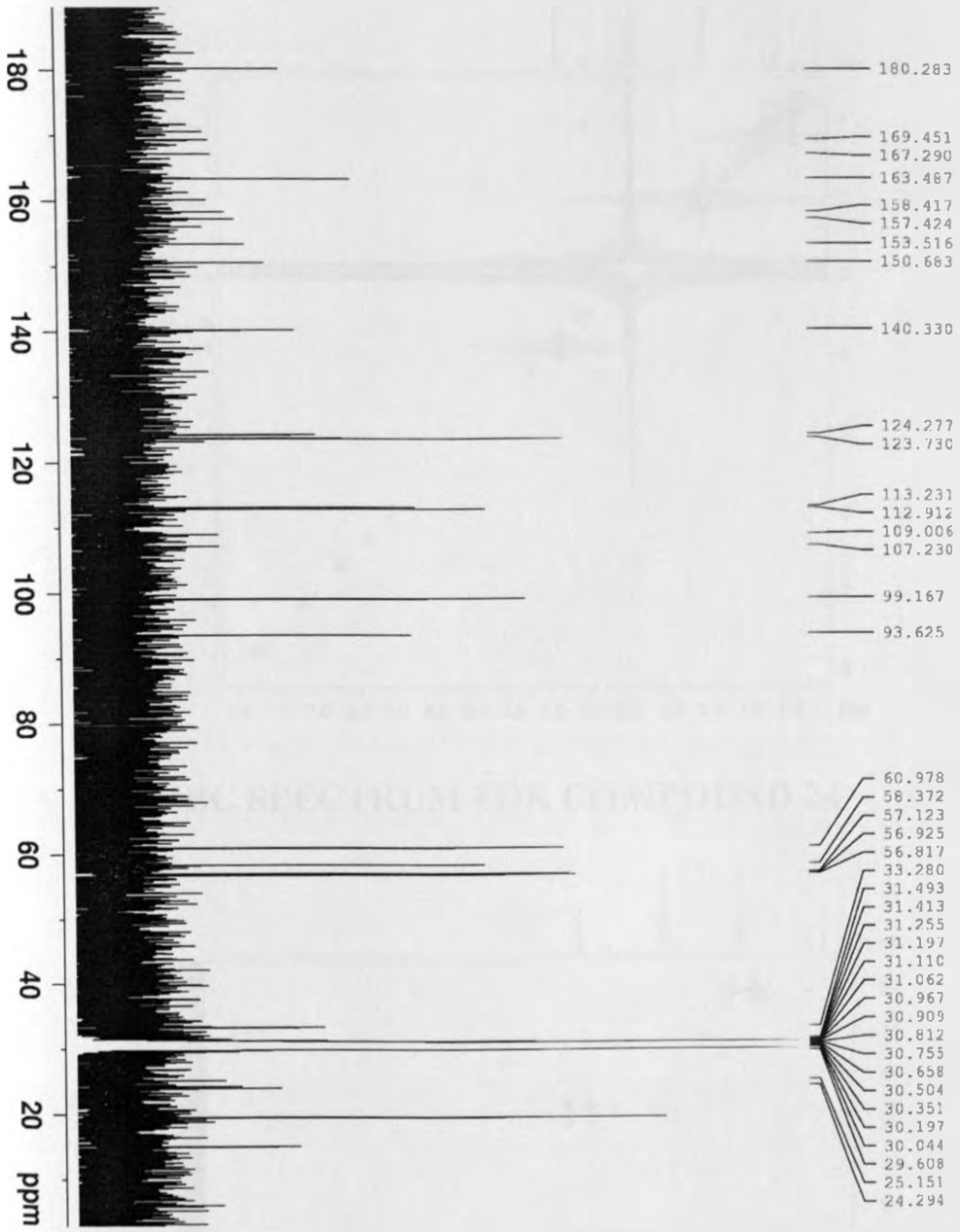


# APPENDIX XXIII: SPECTRA FOR COMPOUND 24

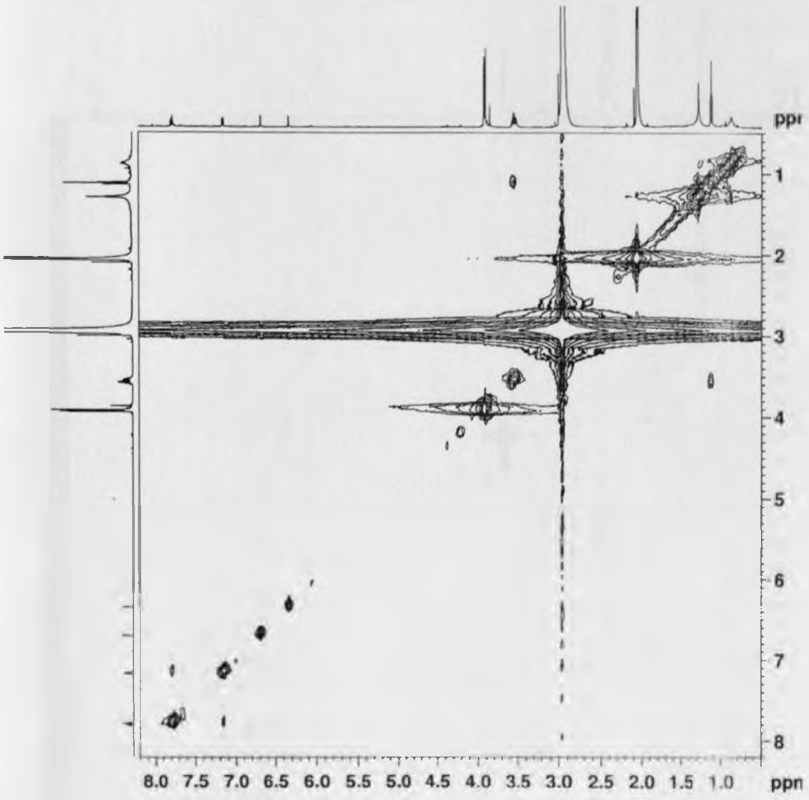
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 24



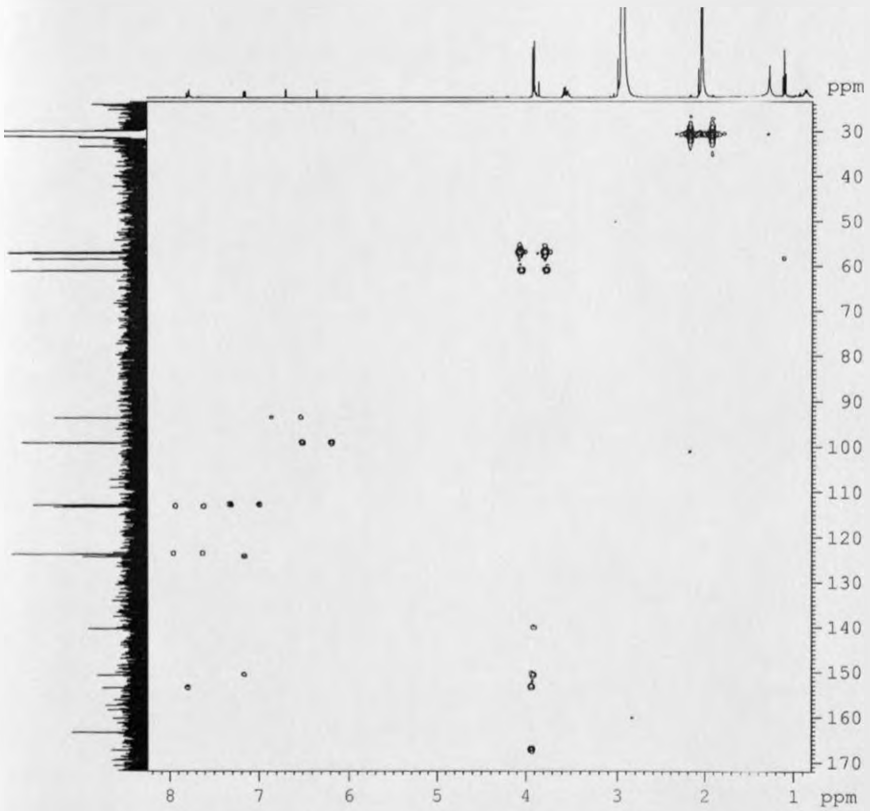
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 24



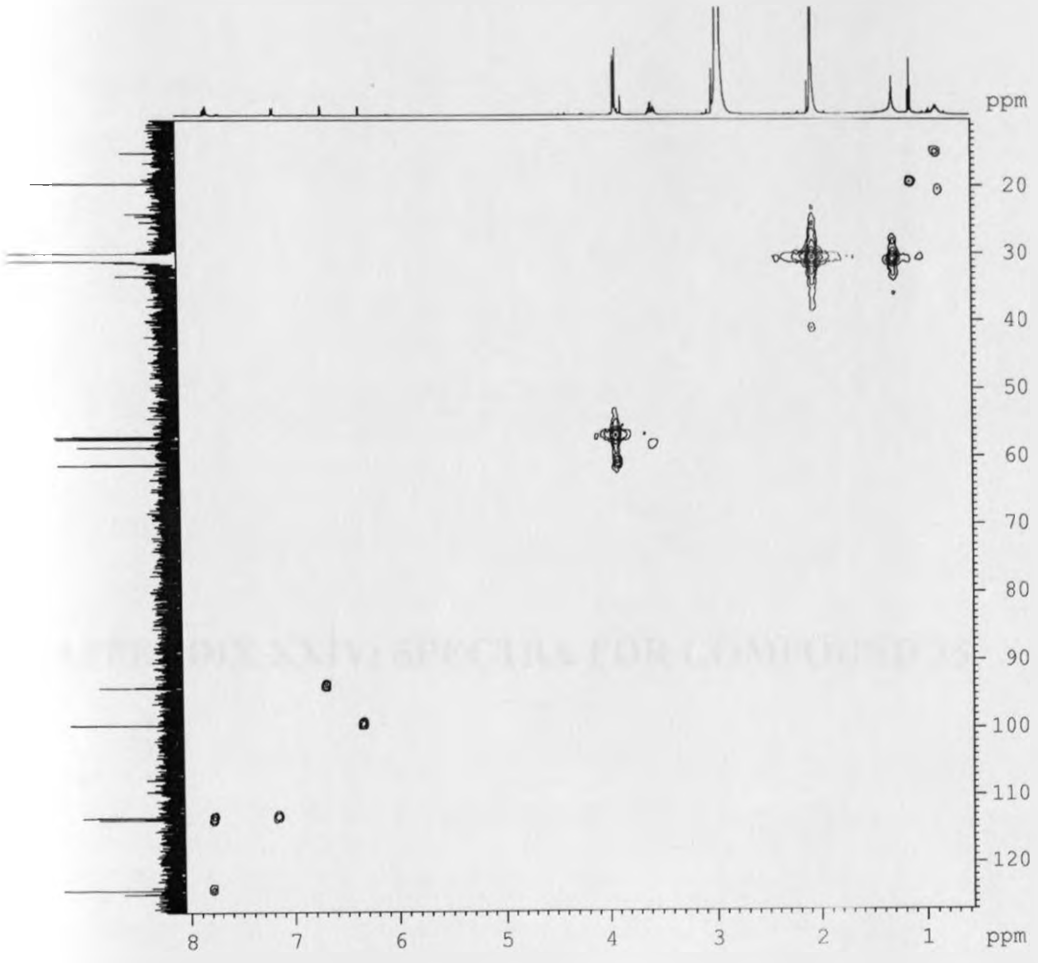
## $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 24




## HMBC SPECTRUM FOR COMPOUND 24

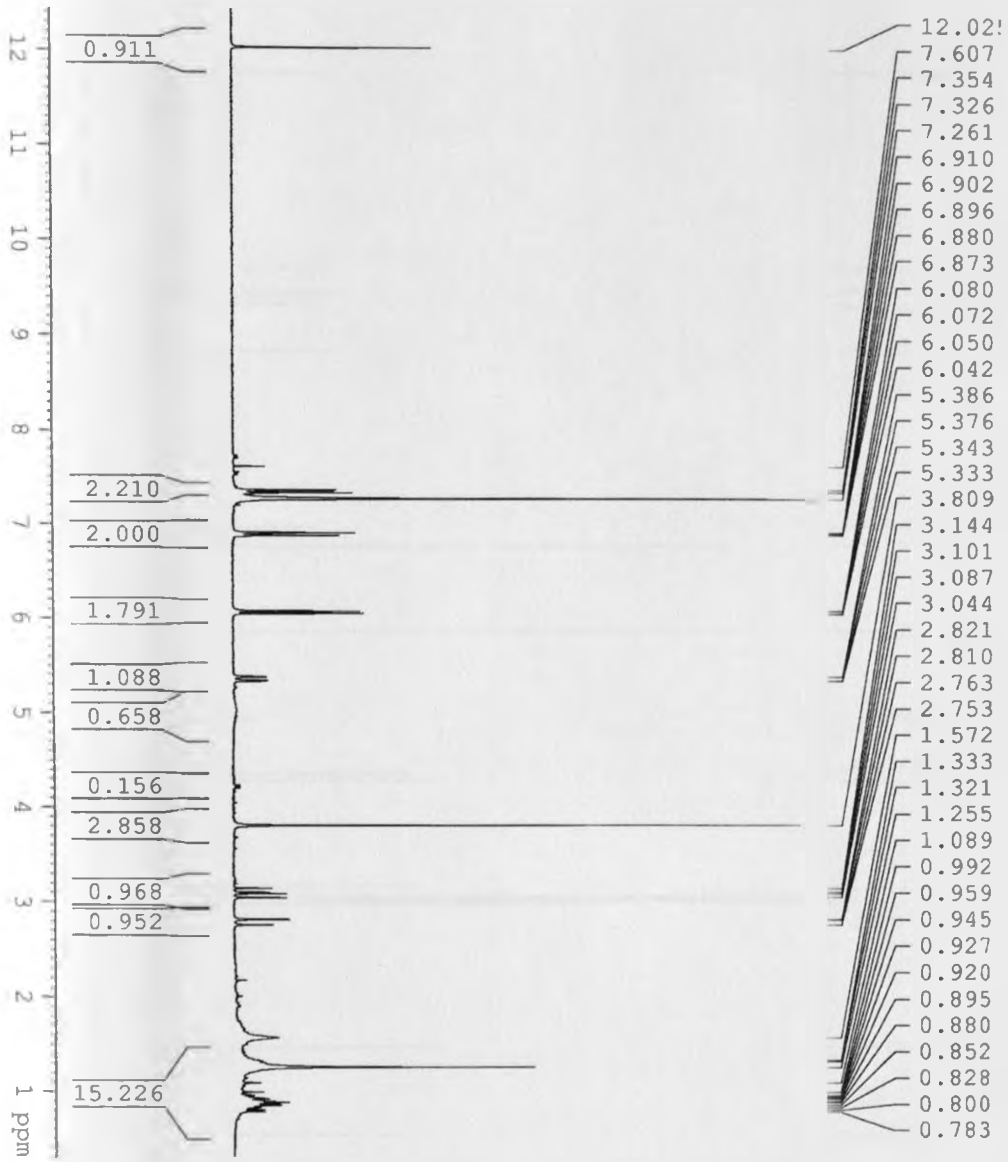


# HMQC SPECTRUM FOR COMPOUND 24

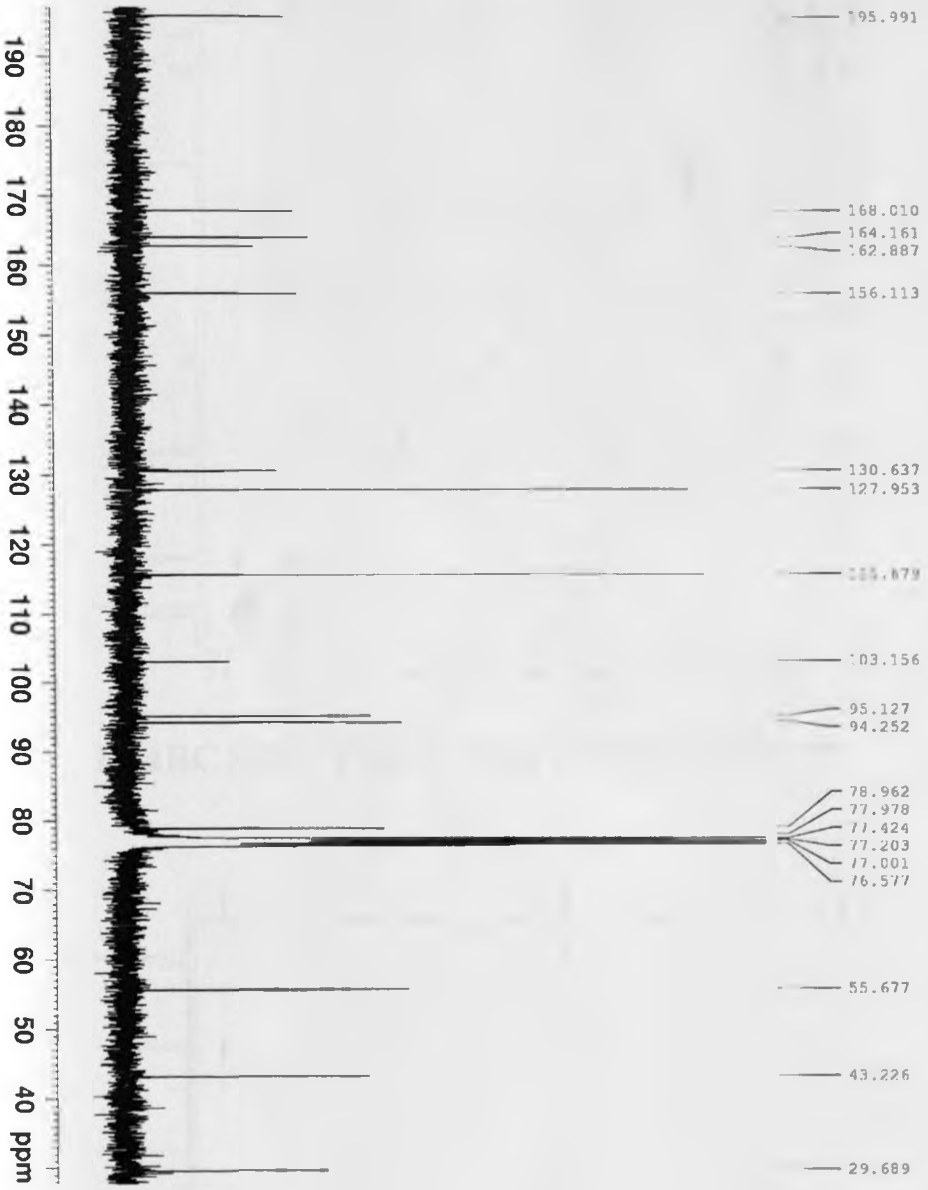


# APPENDIX XXIV: SPECTRA FOR COMPOUND 25

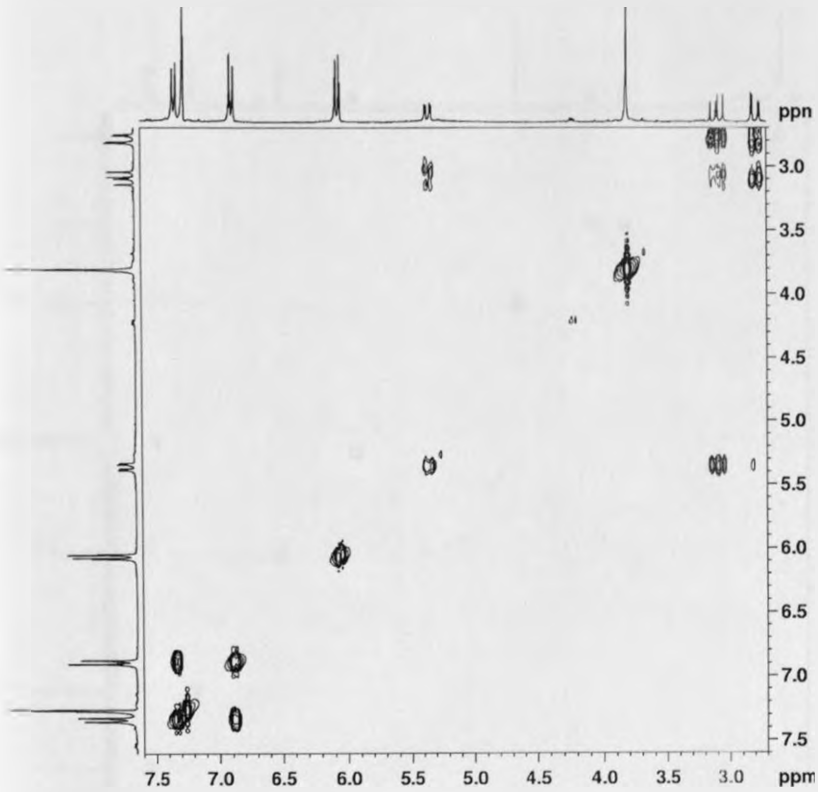
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 25



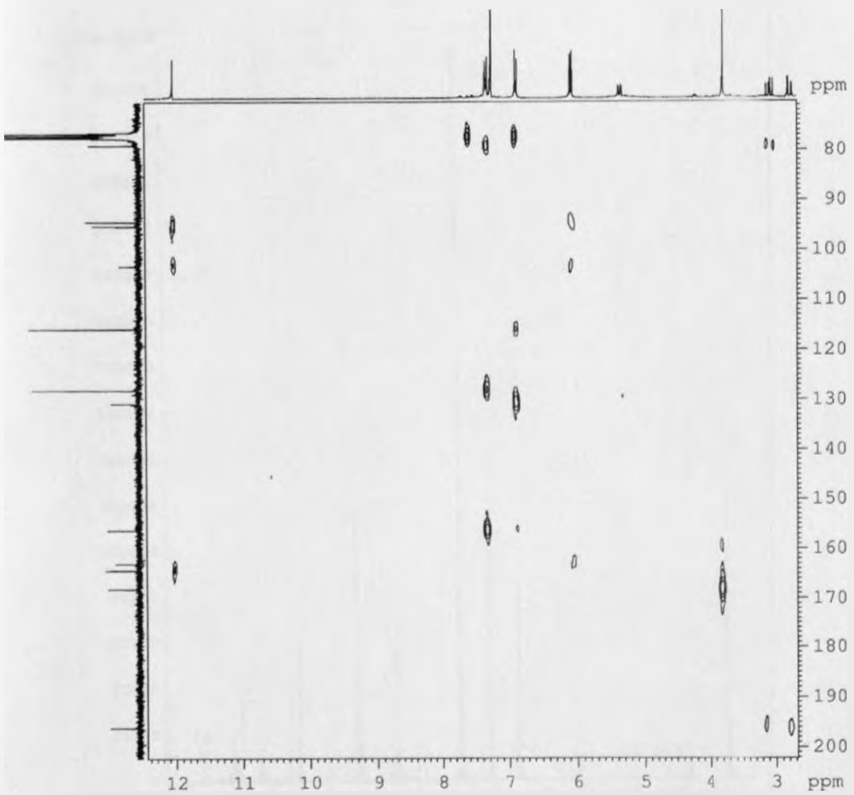
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 25



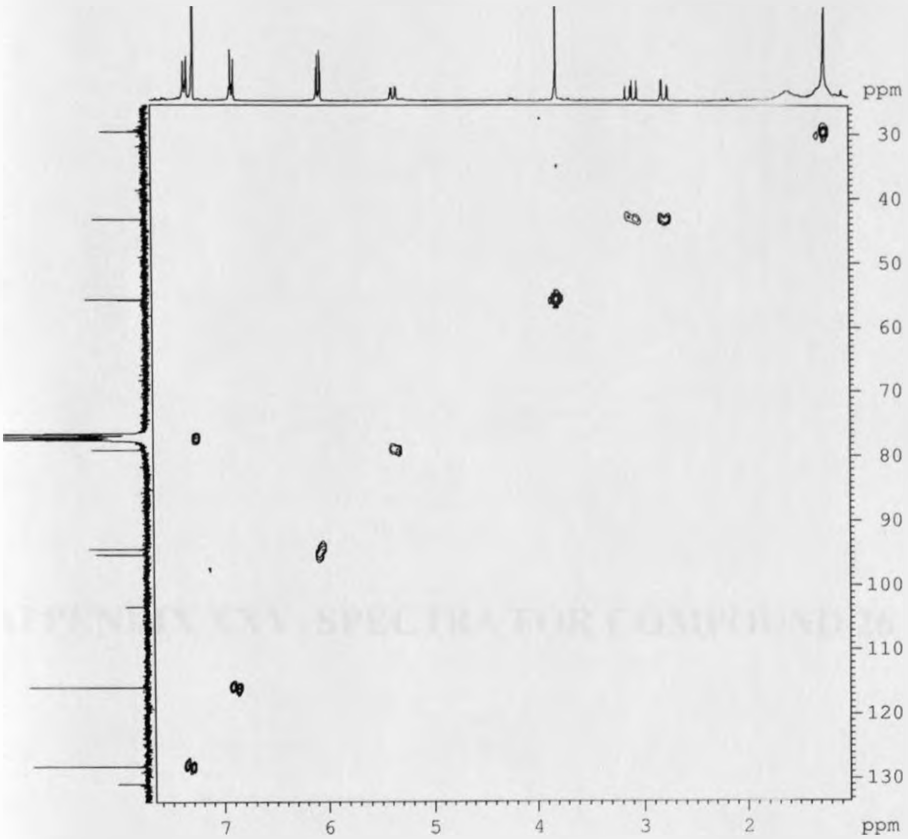
## $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 25



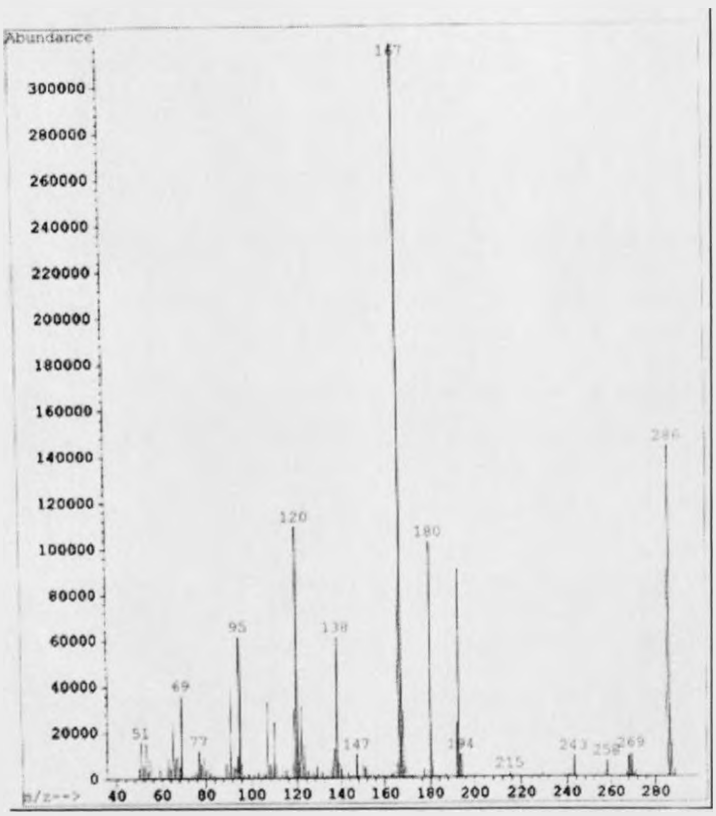

## HMBC SPECTRUM FOR COMPOUND 25



# HMQC SPECTRUM FOR COMPOUND 25

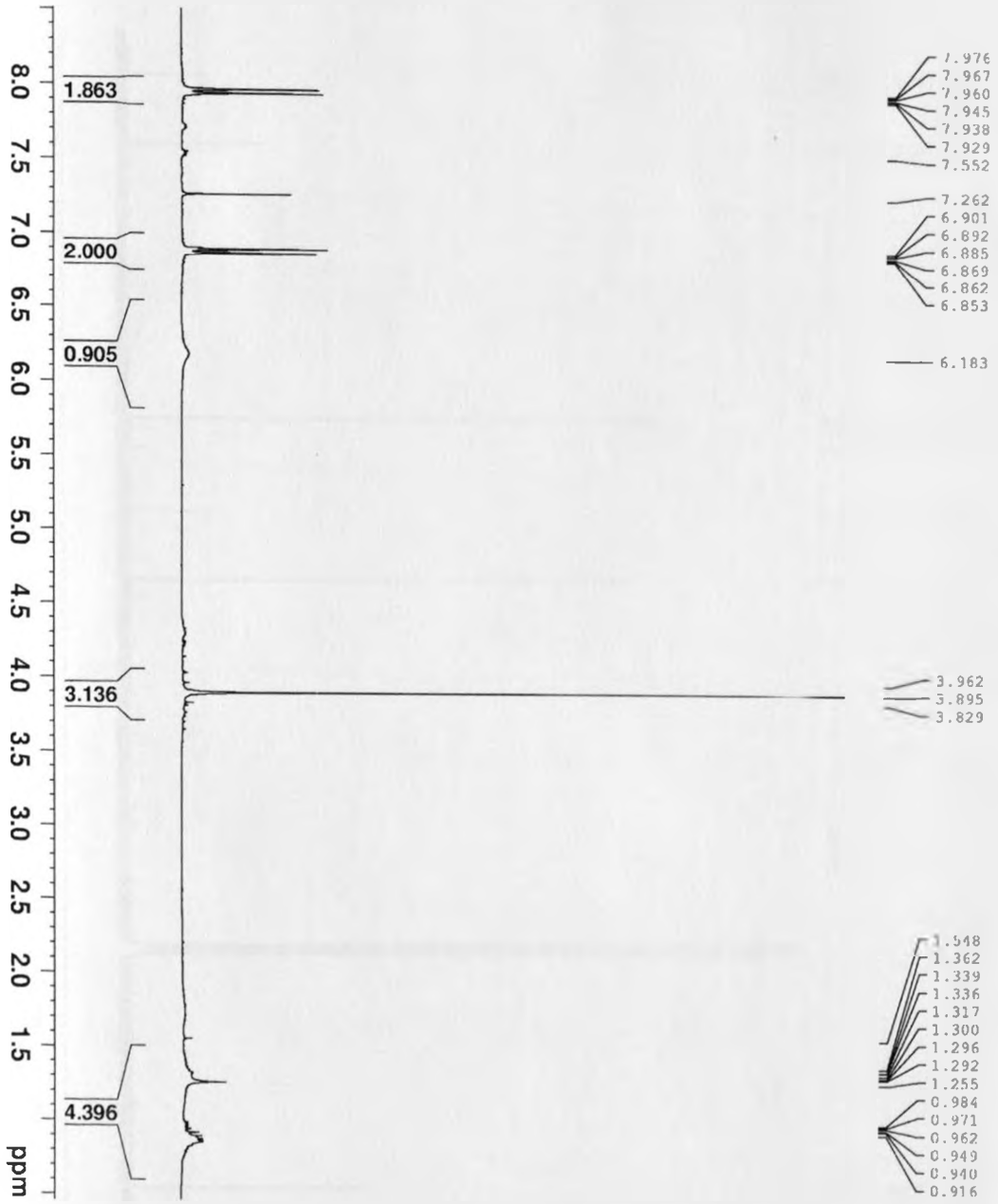



# MASS SPECTRUM FOR COMPOUND 25

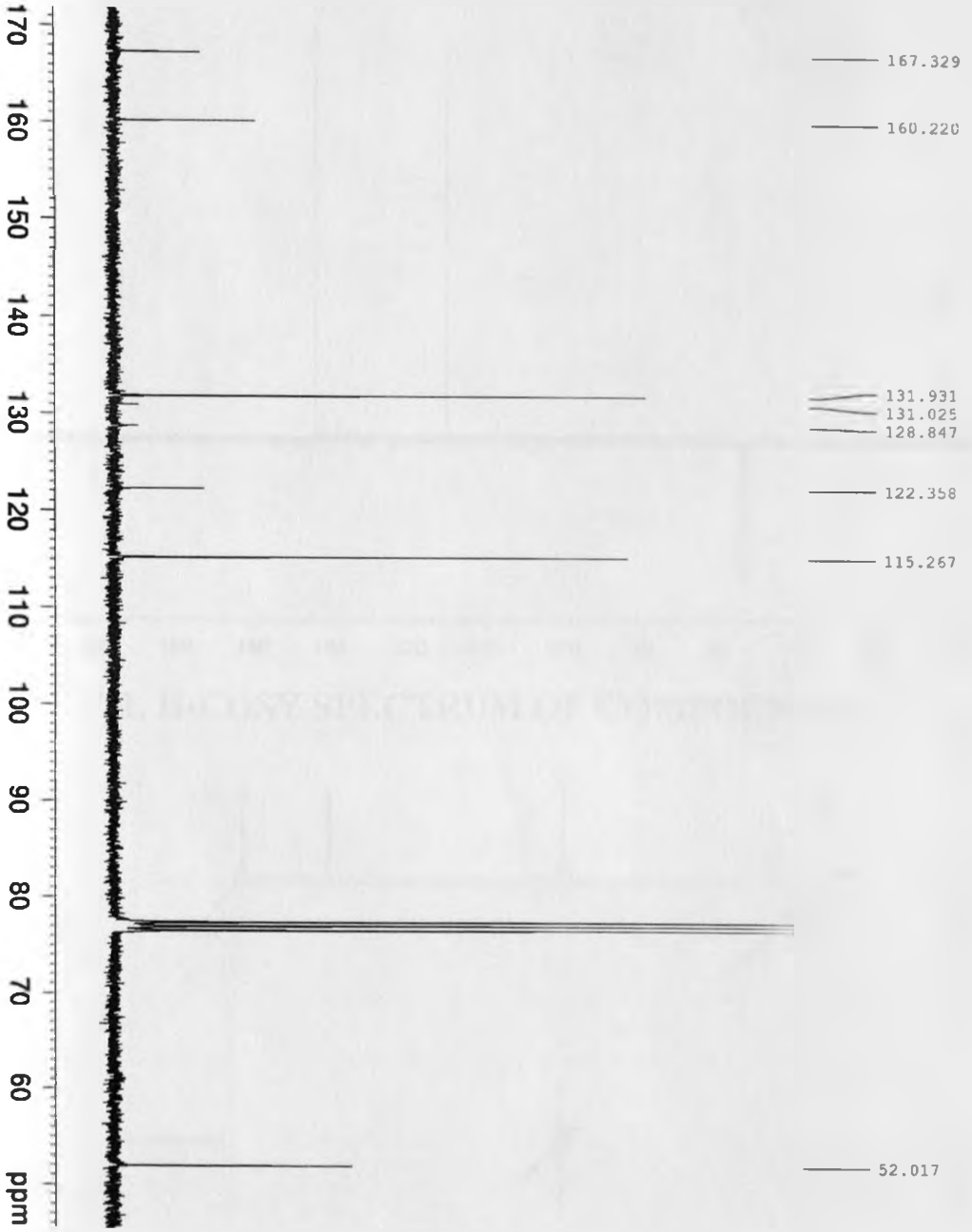


# APPENDIX XXV: SPECTRA FOR COMPOUND 26

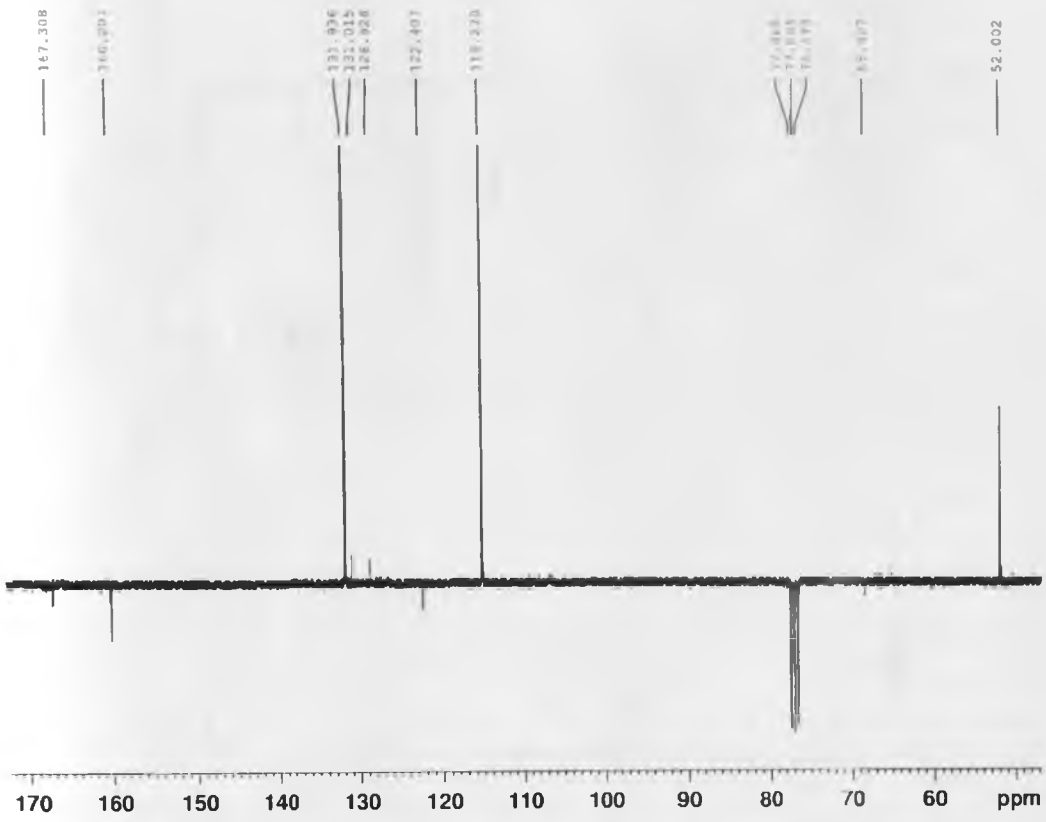
# $^{1}$H-NMR SPECTRUM OF COMPOUND 26



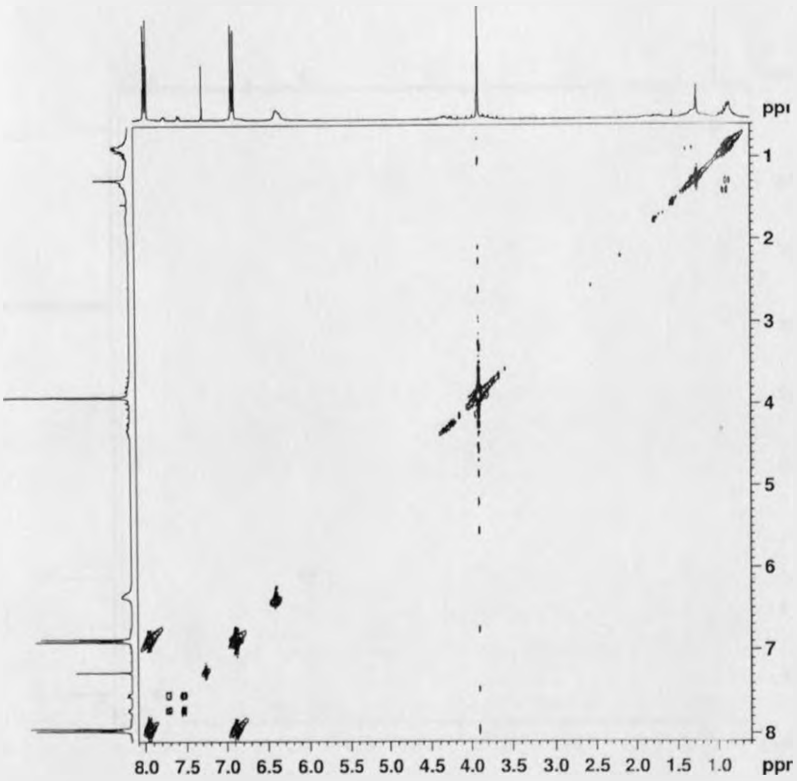
# $^{13}$C-NMR SPECTRUM OF COMPOUND 26



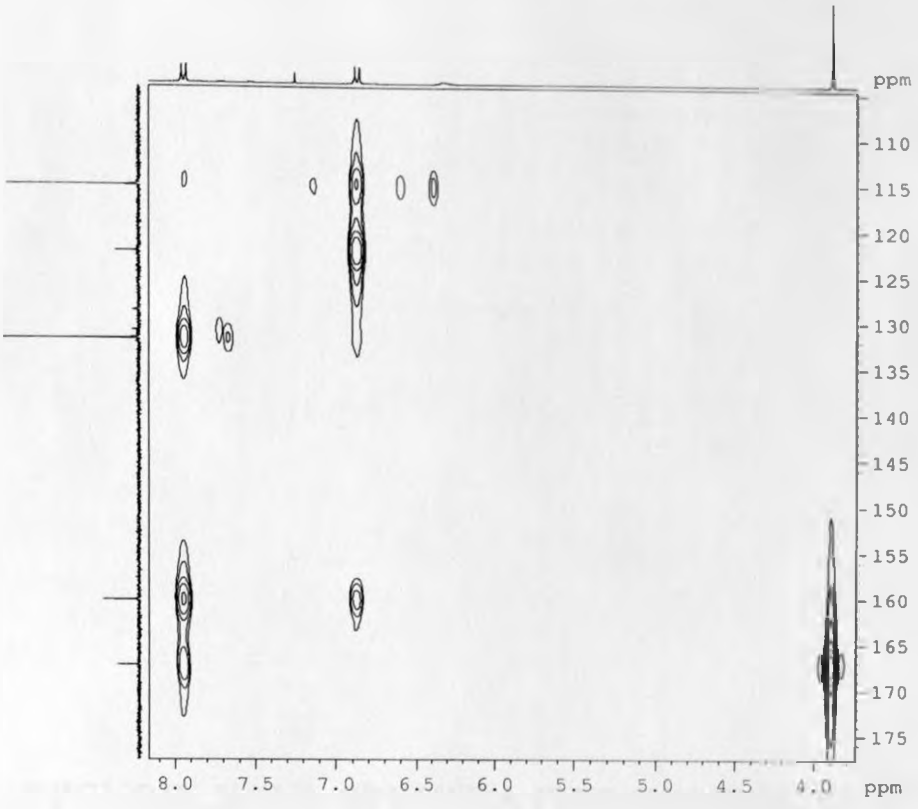
## APT SPECTRUM OF COMPOUND 26



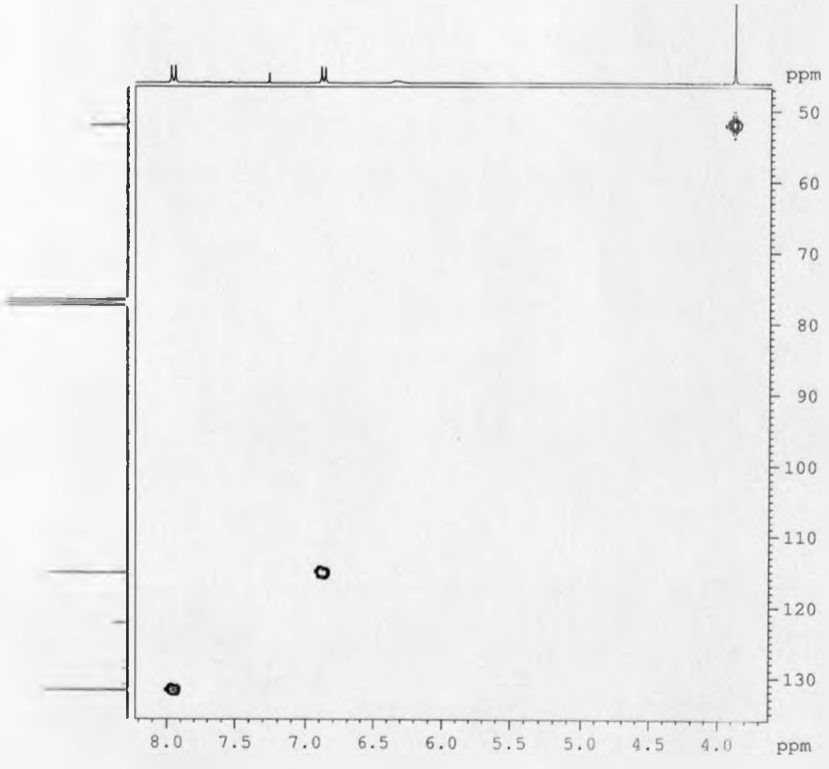

## $^{1}$H, H-COSY SPECTRUM OF COMPOUND 26



# HMBC SPECTRUM OF COMPOUND 26

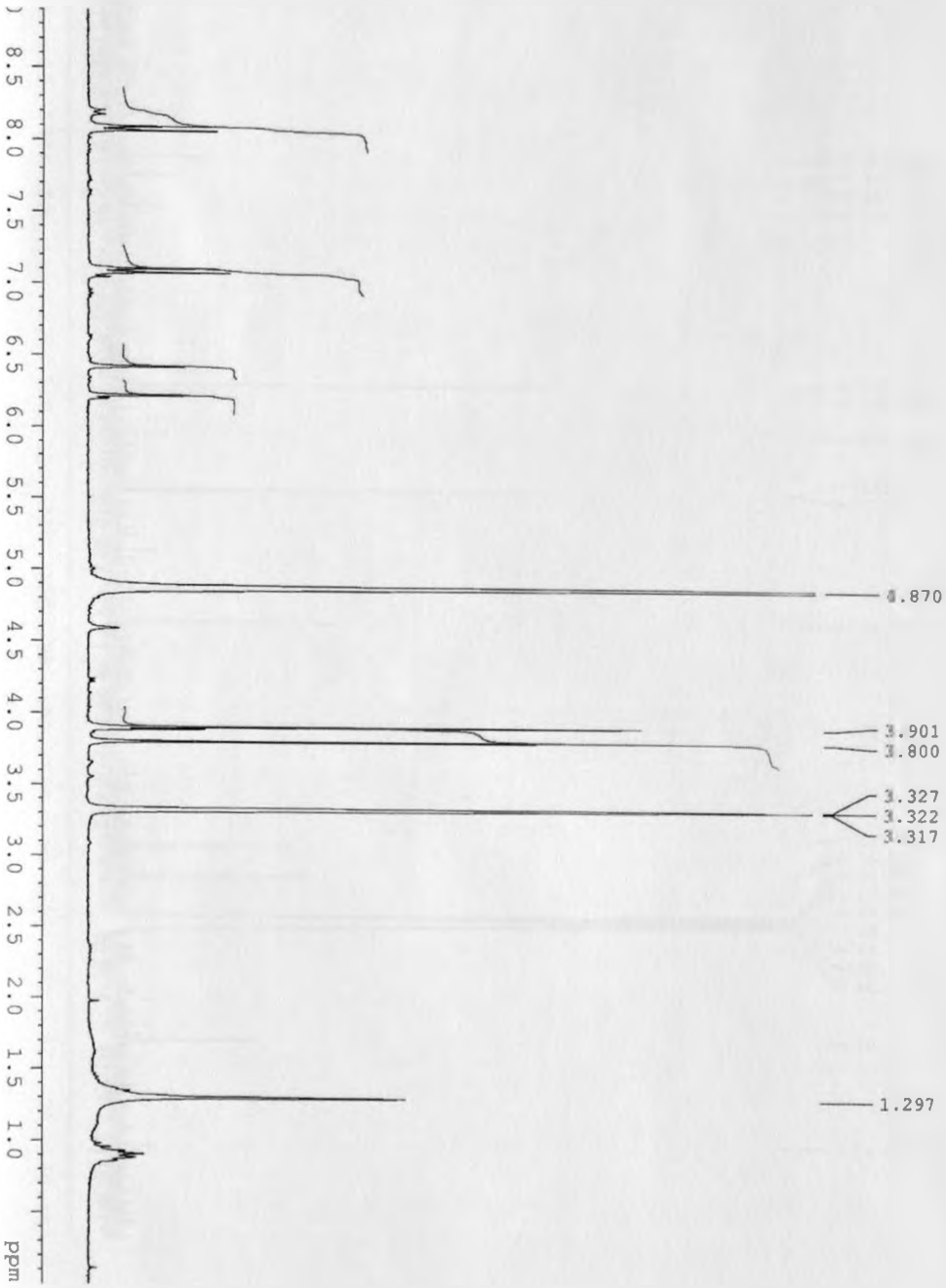



# HMQC SPECTRUM OF COMPOUND 26

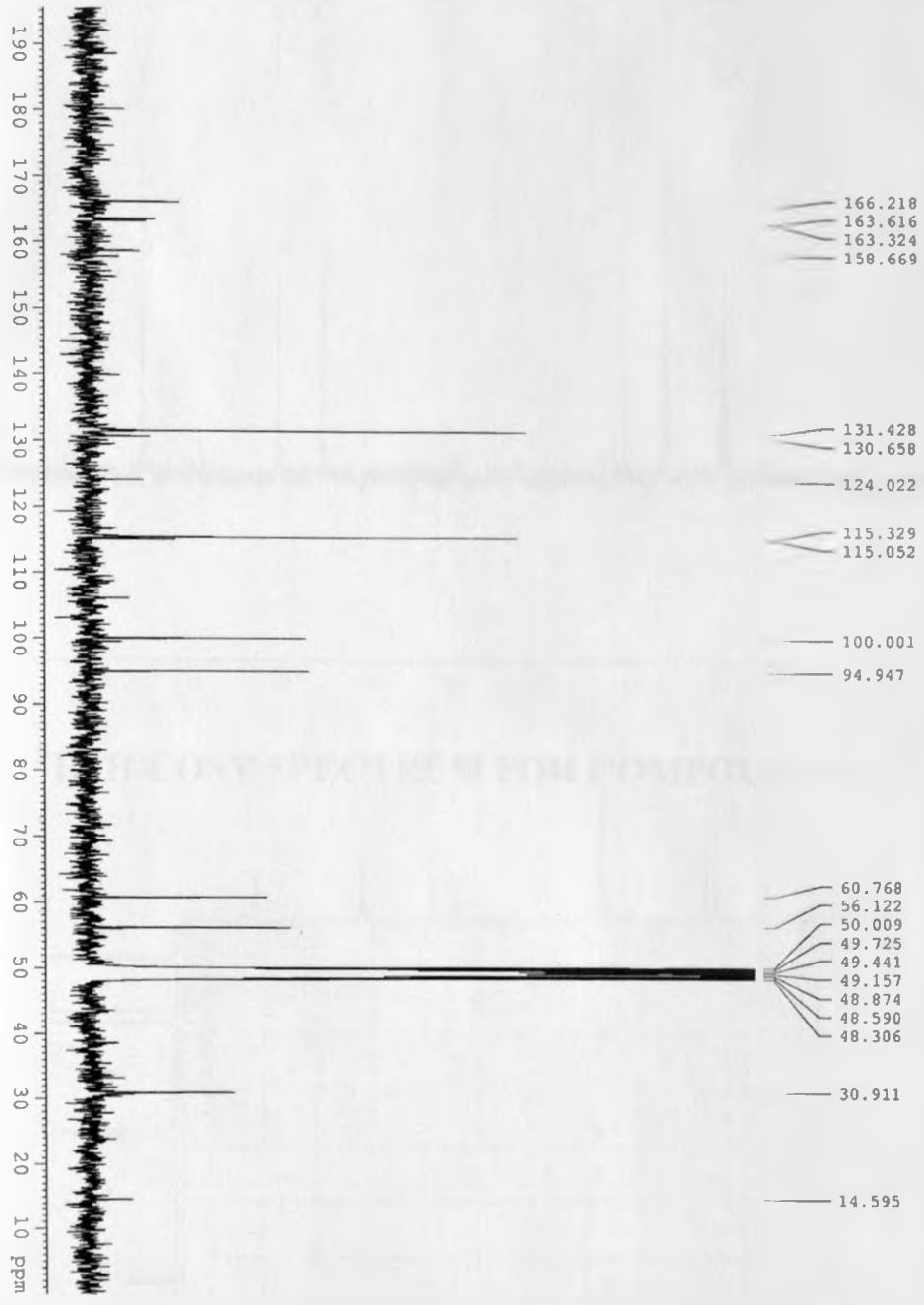


# APPENDIX XXVI: SPECTRA FOR COMPOUND 27

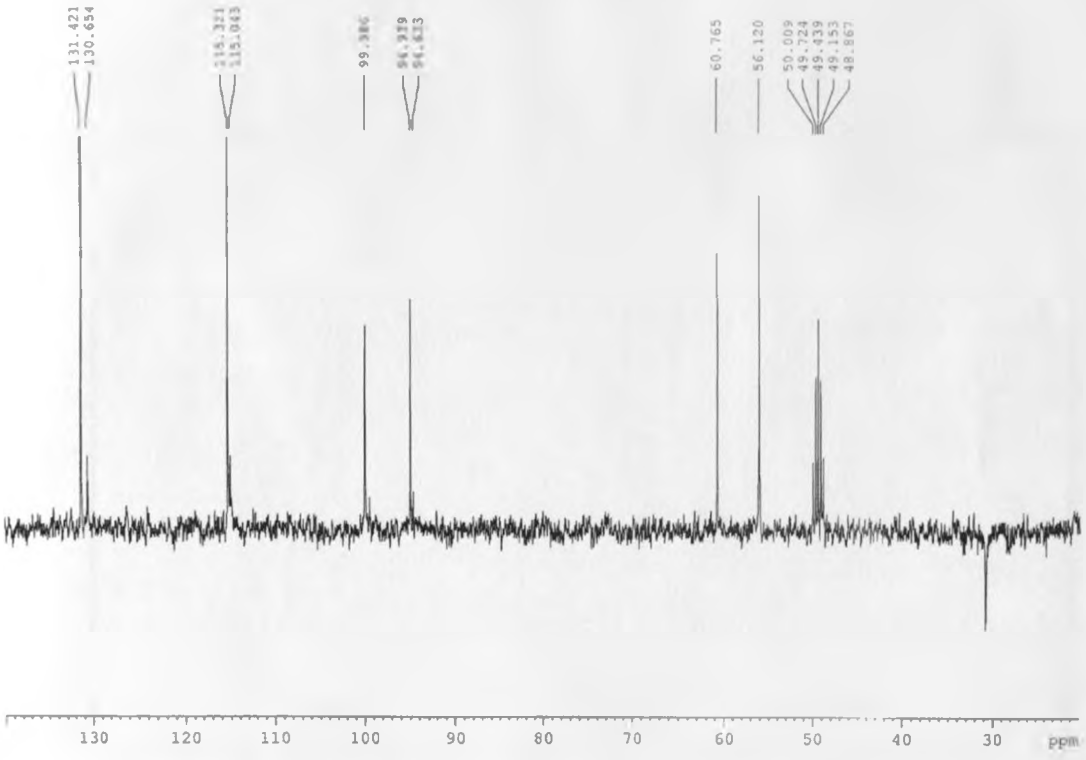
# $^{1}$H-NMR SPECTRUM FOR COMPOUND 27



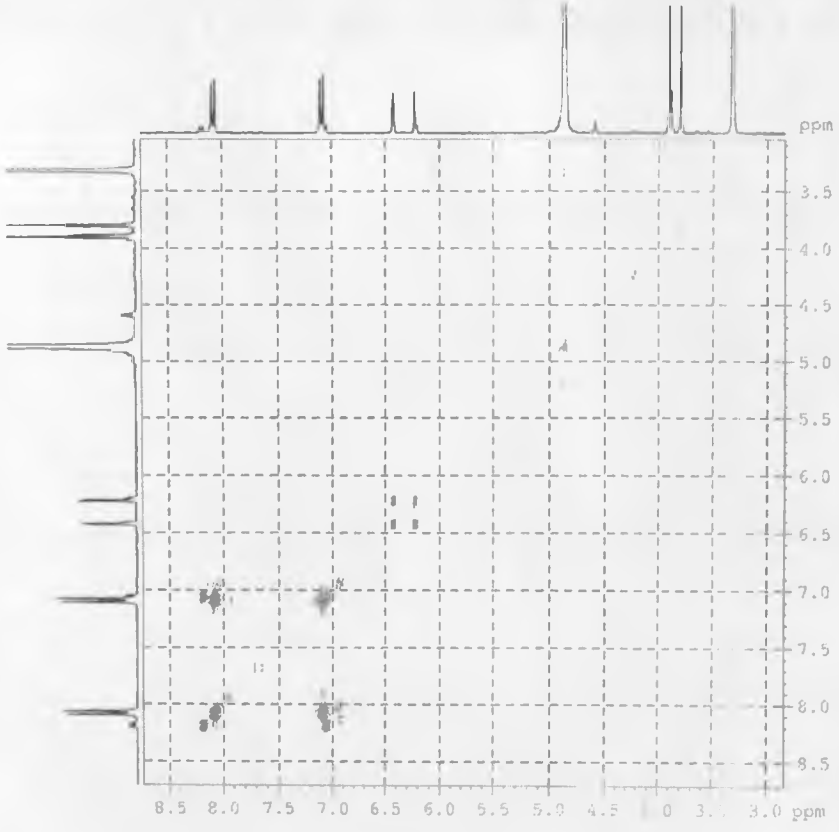
# $^{13}$C-NMR SPECTRUM FOR COMPOUND 27



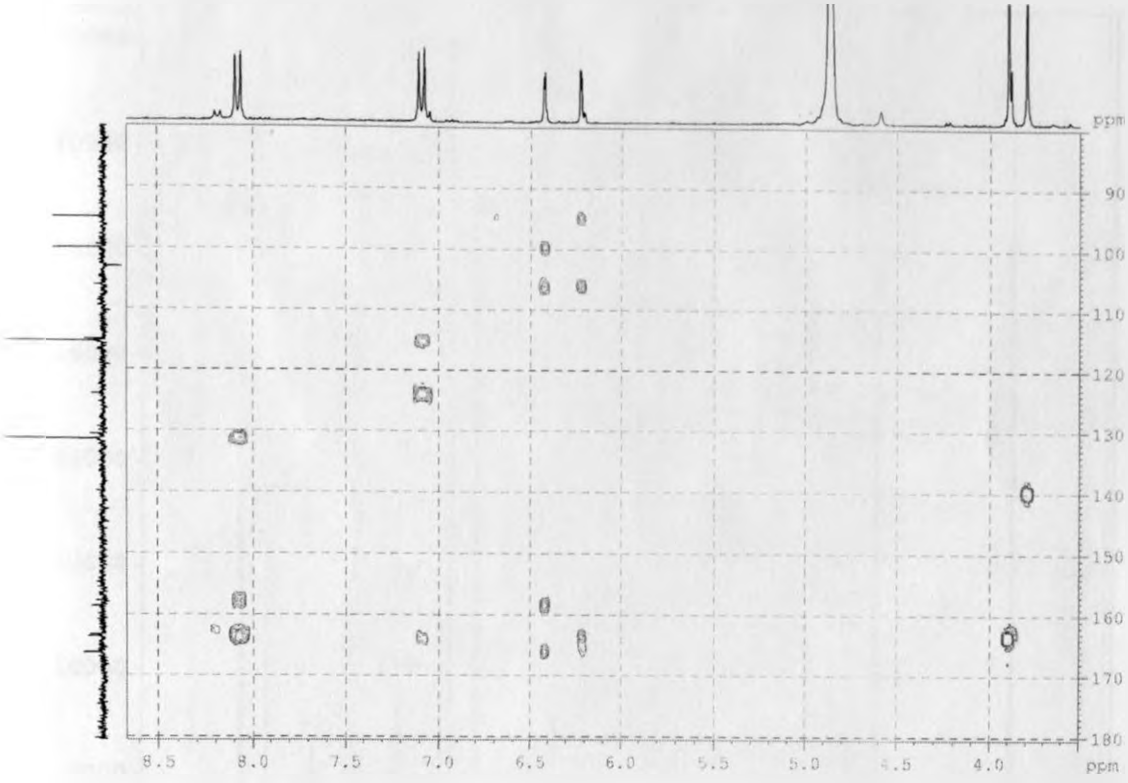
## DEPT SPECTRUM FOR COMPOUND 27



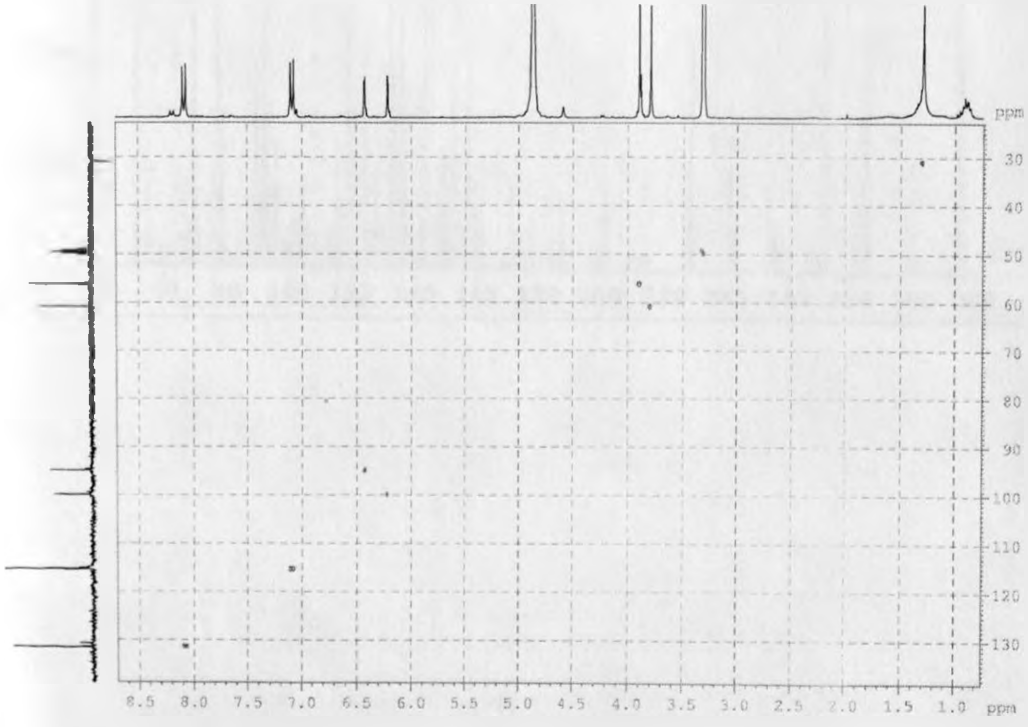

## $^{1}$H, H-COSY SPECTRUM FOR COMPOUND 27



## HMBC SPECTRUM FOR COMPOUND 27




## HSQC-DEPT SPECTRUM FOR COMPOUND 27



# MASS SPECTRUM FOR COMPOUND 27