UNIVERSITY OF NAIROBI

COLLEGE OF PHYSICAL AND BIOLOGICAL SCIENCES

DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF THE ROOTS OF *MILLETTIA USARAMENSIS* SUBSPECIES *USARAMENSIS* FOR ANTIPLASMODIAL PRINCIPLES

BY

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DECLARATION

THIS THESIS IS MY ORIGINAL WORK AND HAS NEVER BEEN PRESENTED FOR A DEGREE IN ANY UNIVERSITY

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THIS WORK IS DEDICATED TO MY FAMILY MEMBERS

ACKNOWLEDGEMENTS

I am deeply indebted to Prof. Abiy Yenesew and Dr. Martin Mbugua for their supervision and continous guidance, inspiration and support during this research work. I wish to extend my acknowledgements to Dr. Matthias Heydenreich, University of Potsdam for generating the spectroscopic data.

I sincerely thank the University of Nairobi for awarding me a scholarship to pursue my MSc studies. Many thanks to the academic and technical staff of the Department of Chemistry, University of Nairobi for their assistance in various ways. I want to appreciate Mr Hoseah Akala of the United States Army Medical Research Unit-Kenya for carrying out antiplasmodial tests of some of the isolated compounds in this work.

I would wish to express my sincere thanks to all postgraduate and undergraduate students who were carrying out research in the natural products laboratory, for their cooperation and advice during the course of my research work.

My appreciation is extended to my family members, friends and colleagues for their understanding, encouragement and support.

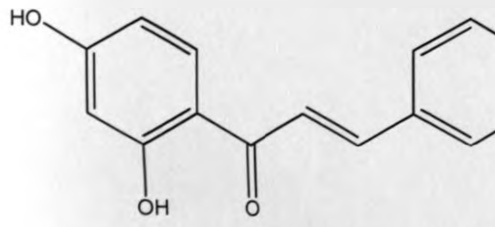
Abstract

The family Fabaceae is the third largest family of flowering plants with about 20,000 species in 650 genera. The family is subdivided into three sub-families, namely Caesalpinoideae, Mimosoideae and Papilionoideae sub-families. The genus *Millettia* belongs to the sub-family Papilionoideae which is kwown to elaborate prenylated flavonoids and isoflavonoids. These compounds possess a wide range of biological activities, the most prominent being anti-inflammatory, anti-microbial and anti-plasmodial activities. In this study the anti-plasmodial activities of crude extracts and pure compounds obtained from the root bark of *Millettia usaramensis* have been investigated.

The dried and ground root bark of *Millettia usaramensis* was exhaustively extracted with CH_2Cl_2/MeOH (1:1) by cold percolation at room temperature. The extract was subjected to a combination of chromatographic techniques leading to the isolation of ten compounds. These were identified as the rotenoids, usararotenoid-A (**6**), usararotenoid-C (**9**), 12a-epimillettosin (**8**), 12-dihydrousararotenoid-A (**7**), 12-dihydrousararotenoid-B (**2**), 12-dihydrousararotenoid-C (**3**); the chalcones, 4'-*O*-geranylisoliquiritigenin (**10**), 4-*O*-geranylisoliquiritigenin (**1**); the flavanone, 4'-geranyloxy-7-hydroxyflavanone (**4**) and the cinnamyl alcohol 4-geranyloxycinnamyl alcohol (**5**).

The chalcone, 4-*O*-geranylisoliquiritigenin (**1**), the flavanone, 4'-geranyloxy-7-hydroxyflavanone (**4**) and the dihydrorotenoids, 12-dihydrousararotenoid-B (**2**) as well as 12-dihydrousararotenoid-C (**3**) are new compounds. The cinnamyl alcohol derivative, 4-geranyloxycinnamyl alcohol (**5**) is reported here for the first time from this genus. The identification of these compounds was based on spectroscopic evidence including ^{1}H NMR, ^{13}C NMR, HMBC, HMQC, COSY and MS.

The *in-vitro* antiplasmodial activities of some of the isolated compounds was tested against the chloroquine-resistant (W2) strain of *Plasmodium falciparum*. The rotenoid **8** together with the flavanone **4** and the chalcone **10** showed moderate antiplasmodial activities with IC_{50} values of 3.1, 4.1 and 1.6 µg/ml respectively.



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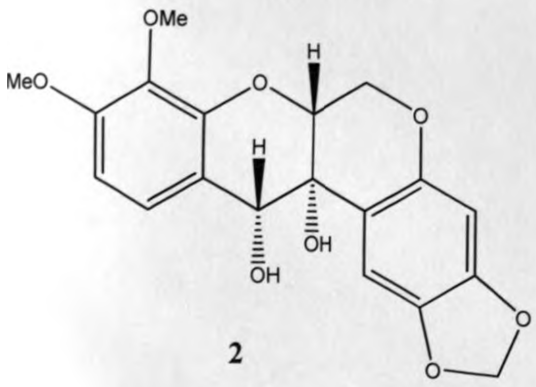

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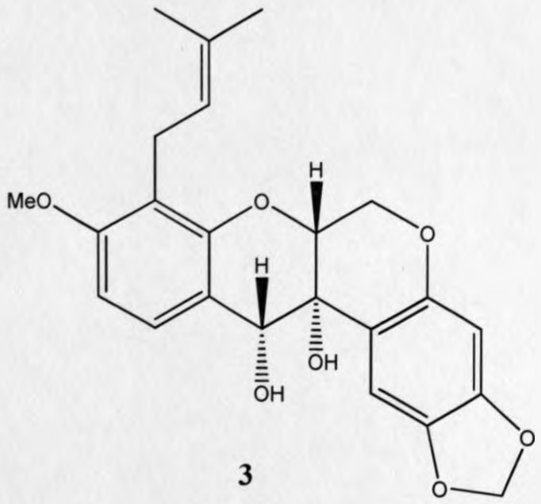

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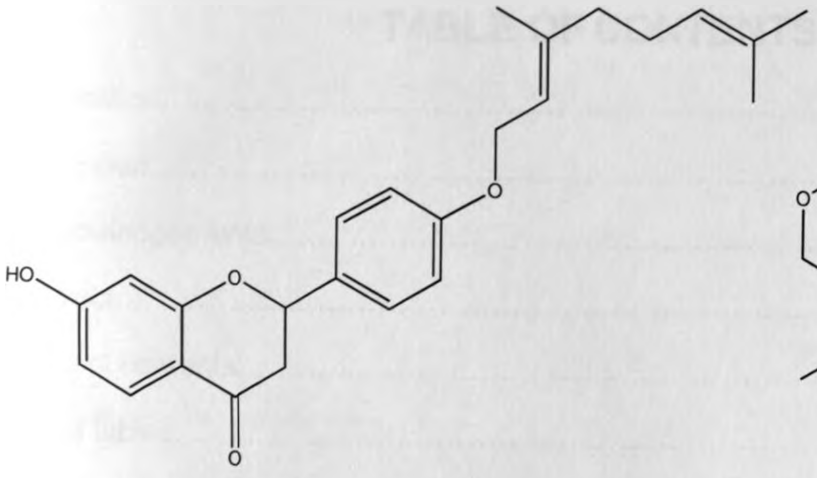

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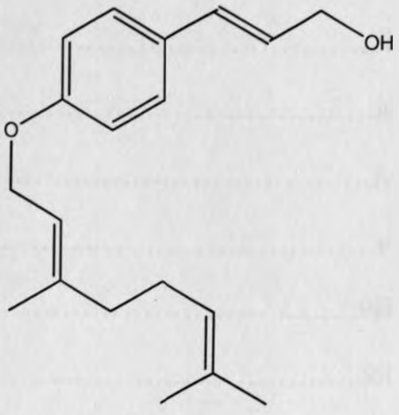


5

TABLE OF CONTENTS

LIST OF TABLES

LIST OF SPECTRA

LIST OF ABBREVIATIONS AND SYMBOLS

IC_{50}	Concentration of 50% inhibition	*dd*	Doublet of a doublet
LC_{50}	Concentration of 50% lethality	*s*	Singlet
mpt	Melting point	*m*	Multiplet
MS	Mass Spectroscopy	*d*	Doublet
$[M]^+$	Molecular ion	*t*	Triplet
EIMS	Electron Ionization Mass Spectroscopy	AP	Aerial Parts
m/z	Mass to charge ratio	FL	Flowers
NMR	Nuclear Magnetic Resonance	HW	Heart Wood
d	Chemical shift	LF	Leaves
Hz	Hertz	NA	Not Active
NOE	Nuclear Overhauser Effect	NS	Not Specified
HMBC	Heteronuclear Multiple Bond Correlation ($^2J_{CH}$,	NT	Not Tested
COSY	Correlated Spectroscopy	RB	Root Bark
HMQC	Heteronuclear Multiple Quantum Coherence ($^1J_{CH}$)	RT	Root
DEPT	Distortionless Enhanced Polarization Transfer	RW	Root Wood
NOESY	Nuclear Overhauser and Exchange Spectroscopy	SB	Stem Bark
PTLC	Preparative Thin Layer Chromatography	SD	Seeds
TLC	Thin Layer Chromatography	SDP	Seed Pods
HRMS	High Resolution Mass Spectroscopy	ST	Stem
		VS	Vine Stems
RF	Retention Factor	WD	Wood

CHAPTER ONE

INTRODUCTION

1.1 GENERAL

Medicinal plants are of great importance to the health of individuals and the society at large. Natural products have served as an important source of drugs since ancient times. In sub-Saharan Africa, including East Africa, herbal remedies continue to be more accessible and affordable than conventional drugs that are often beyond the reach of the poor. Hence, up to 80% of the population rely on medicinal plants as remedy against infectious diseases (Kokwaro, 1993). These medicinal plants are selected not on the basis of their chemical constituent, but on their perceived ability to restore patients' condition to normal (Stephen *et al.*, 2009). The medicinal value of these plants lies in chemical substances present that produce a definite physiological action on the human body (Trease and Evans, 2002). Consequently, the use of herbs is best justified by the isolation of compounds that are active against the causative agents of diseases.

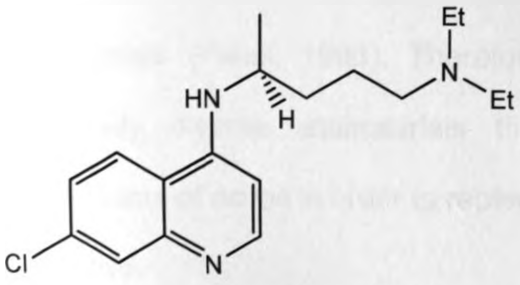
More than half of the pharmaceuticals in use today are derived from natural sources (Corrando, 2004). Thus interest in natural products still remains strong. This is attributed to several factors, including unmet therapeutic needs that drive new drug discovery, the remarkable diversity of both chemical structures and biological activities of naturally occurring secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes, the development of novel and sensitive techniques

to detect biologically active chemotypes from nature, improved techniques to isolate, purify and structurally characterize these active constituents and the success of herbal remedies in the global market place (Corrando, 2004).

Malaria is one of the most prevalent Killer diseases in the tropical and sub-tropical regions. It affects over three hundred million people annually, causing two million deaths among the affected persons (WHO, 2010). The malaria burden is strongly experienced in sub-Saharan Africa, where about 90% of cases and deaths occur. It is also a serious health problem in south east Asia and South America (Mishra, 1999). It is caused by the protozoa *Plasmodium* species and transmitted by female *Anopheles* mosquito which takes a blood meal containing parasites from an infected person. The parasites are injected together with the saliva of the mosquito the next time it bites a human victim. While in the human body the parasites invade the parenchymal cells of the liver.

The major problem associated with the prevention and treatment of malaria is the spreading of resistant strains of *Plasmodium* species to the available first-line anti-malarial drugs such as chloroquine (**11**) and development of resistant mosquito to conventional insecticides. Therefore, development of new drugs or drug combination therapy is required for the prevention and treatment of this infectious disease, preferably, drugs with a unique mode of action or with different chemical composition from those currently in use.

Most drugs used in the treatment of malaria are derived from plants used in different parts of the world to treat malaria. The bark of the South American 'fever tree" *Cinchona succiruba*, was effective in controlling malaria (Robbers *et al.*, 1996). Quinine (**12**) was ultimately identified as the active anti-malarial constituent of the *Cinchona* bark. The newest anti-malarial artemisinin (**13**), which has been used traditionally in China for treatment of malaria is isolated from *Artemisia annua*. These active plant constituents have served as molecular templates for the development of synthetic antimalarials that are safe and more effective than the mother molecules. There is still a great potential for plants in the development of new drugs.



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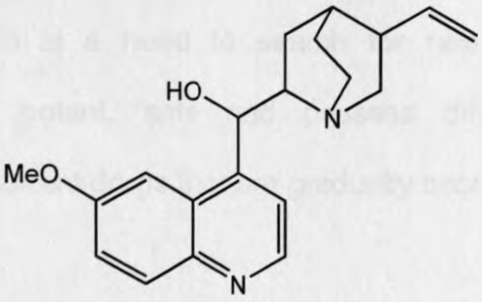

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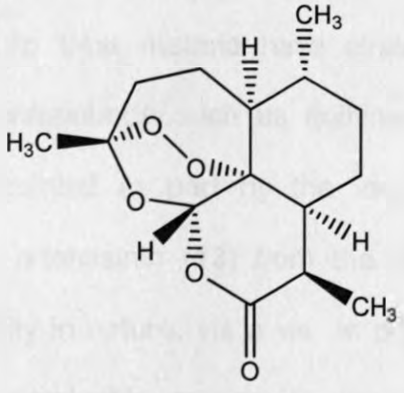


13

1.2 PROBLEM STATEMENT

Infectious diseases including malaria account for approximately one-half of all deaths in tropical countries. It is estimated that about 80% of all clinical cases of malaria occur in tropical African countries (Kitua and Malebo, 2004). In Kenya, malaria is the greatest contributor to the rising rate of morbidity and mortality of all infectious diseases. Despite the progress made in understanding the malaria parasite and their control, incidents of epidemics due to drug resistance pose enormous public health concerns. These negative health trend calls for renewed strategies in treatment and prevention of malaria. The proposed solution to the malaria pandemic is the multi-pronged approach including prevention (such as vaccination), improved monitoring and the development of new treatments. It is this last solution that would encompass the development of new antimalarials (Fauci, 1998). Therefore, there is a need to search for new and structurally diverse antimalarials that are potent, safe and possess different mechanisms of action in order to replace the current drugs that are gradually becoming ineffective.

1.3 JUSTIFICATION OF THE RESEARCH

Medicinal plants used to treat malaria have already provided valuable leads for potential anti-malarial compounds such as quinine (**12**). The renewed interests in plants have been stimulated in part by the identification of the anti-plasmodial sesquiterpene lactone, artemisinin (**13**) from the medicinal plant *Artemisia annua*. Therefore, chemodiversity in nature, vis a vis in plants, microorganisms and marine organisms, still offers a valuable source for discovery of potent anti-malarial lead structures. Phytochemical information available in literature indicate that flavonoids

exhibit anti-plasmodial activity including those isolated from *Millettia* species. Therefore, it is worthwhile to isolate the constituents of the roots of *Millettia usaramensis* and investigate their antiplasmodial activities.

1.4 OBJECTIVES OF THE RESEARCH

The general objective of this research was to isolate and characterize compounds from the root bark of *Millettia usaramensis* subspecies *usaramensis* with anti-plasmodial activities.

The specific objectives of the research were:-

1. To establish the anti-plasmodial activity of the root bark of *Millettia usaramensis* subspecies *usaramensis*.
2. To isolate and characterize the constituents of the root bark of *M. usaramensis* subspecies *usaramensis*.
3. To determine the antiplasmodial activity of the isolated compounds.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE MALARIA BURDEN

Malaria is estimated to kill between 1.5 and 2.7 million persons a year out of 300 million people who contract malaria (Mishra, 1999). Most of the deaths occur to children living in sub-Saharan Africa; the disease accounts for 20% of all childhood deaths (WHO, 2010). It has been estimated that a single bout of malaria costs a sum equivalent to over 10 working days. In 1987 the total economic cost of malaria for health care, treatment and lost production was estimated to be US$ 800 million for tropical Africa. This figure is expected to rise in the coming years (Corrado, 2004).

In Kenya, malaria is the third greatest contributor to the rising rate of morbidity and mortality of all infectious diseases. The high risk group are those who have not developed immunity (children under five years of age, travelers and immigrants) and those with diminished immunity (pregnant women, immuno-compromised patients and people from endemic areas who are exposed to re-infection) (Kirira *et al.*, 2006).

Malaria in humans is caused by four species of parasites belonging to the genus *Plasmodium; P. falciparum, P. malaria, P. ovale* and *P. vivax*. Of these *P. falciparum* is the parasite causing most deaths. It is worth to note that, an intensive use of insecticides, predominantly DDT, and the use of the drug chlroquine, has reduced malaria from a large part of tropical world and almost completely eradicated it from the

non-tropical world (Day, 1998; Jayaraman, 1997). However development of resistance to DDT amongst the mosquitoes, together with resistance of the parasites against quinoline and sulfonamide drugs has dramatically aggrevated this situation. Today malaria has again become one of the three most fatal diseases in the world together with HIV/AIDS and lower respiratory infections (WHO, 2008).

Although artemisinin-based combination therapies (ACTs) have been developed to enhance clinical efficacy and to treat malaria caused by resistant parasites, these drugs are not yet easily accessible nor affordable to most Africans. In addition, if resistance to ACTs develops and spreads to large geographical areas, as has happened with chloroquine and sulfadoxine-pyrimethamine (SP), the public health consequences could be dire, as there are no alternative antimalarial medicines available in the near future (WHO, 2009).

Therefore the need for structurally diverse efficacious anti-malarial drugs can not be over emphasized. During drug design and development, the problem of drug resistance can be dealt with either by identifying new targets which are critical to the disease progress or essential for the survival of the parasites (Vishnu *et al.*, 2000). Natural products remain the chief source of identification of lead structures. Structural modification of such compounds can result in highly efficacious chemotherapeutic agents with reduced toxicity and side effects.

2.2 LIFE CYCLE OF THE MALARIA PARASITE

When a mosquitoe takes blood from an infected person it swallows some female or male gametocytes of the parasite. The gametocytes undergo sexual reproduction in the digestive tract of the mosquito. Ultimately, the sporozoites are injected with the saliva of the mosquito the next time it bites a human victim. While in the human body the sporozoites invade the parenchymal cells of the liver. During development in the liver the patient remains asymptomatic but after a variable period of time, 6–8 days for *P. vivax*, 9 days for *P. ovale*, 12–16 days for *P. malaria*, and 5–7 days for *P. falciparum*, merozoites are released from the liver and invade the erythrocytes, where they feed on the haemoglobin. After proliferation the erythrocyte ruptures and the liberated merozoites invade other erythrocytes. Some parasites might differentiate into a dormant stage (hypnozoites), providing a reservoir that can be activated for up to five years after the initial infection. (Casteel, 1997).

2.3 MALARIA CONTROL STRATEGIES

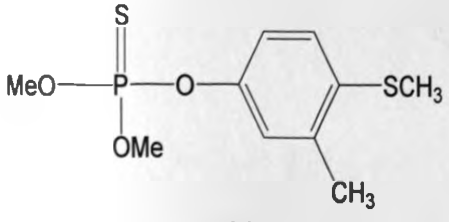
Over the years, several strategies of combating malaria have been developed which include, the use of insecticides in vector control, the use of anti-malarial drugs, the development of an anti-malarial vaccine and the use of genetic engineering to come up with non-vector species of mosquitoes (Phillipe and Miller, 2002). The current malaria control strategy calls for the selection of those control measures which are most appropriate to local circumstances, capabilities and malaria risk.

2.3.1 VECTOR CONTROL

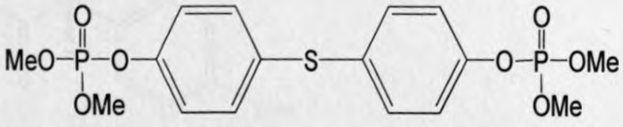
Vector control remains the most generally effective measure for reduction of malaria morbidity and mortality by reducing the levels of malaria transmission. It involves the use of insecticides to kill the mosquito at one of its developmental stages. Other methods involve the use of mosquito repellants such as mosquito coils and insecticide treated mosquitoe nets (Phillipe and Miller, 2002).

2.3.1.1 LARVAL CONTROL

The deterioration of indoor residual spraying programmes led to the resurgence of malaria. Eventually spurring renewed interest in larval and personal protection measures for reduction of malaria transmission. It is worth to note that, organophosphates, larvicidal oils, arsenical compounds and development inhibitors of mosquito larvae have all been used with varying degrees of success as larval control measures (Gratz and Pal, 1988). Organophosphates such as fenthion (**14**) and temephos (**15**) as well as insect growth regulators such as diflubenzuron (**16**) and methoprene (**17**) have been used for the control of mosquito larvae (Yang *et al.*, 2002).



14



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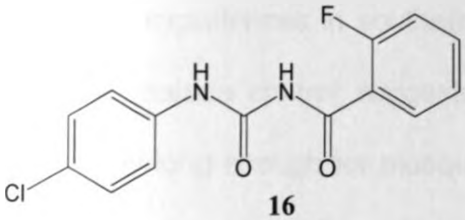

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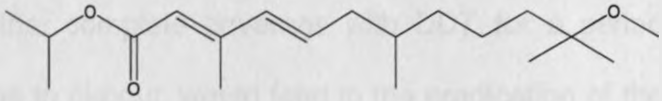
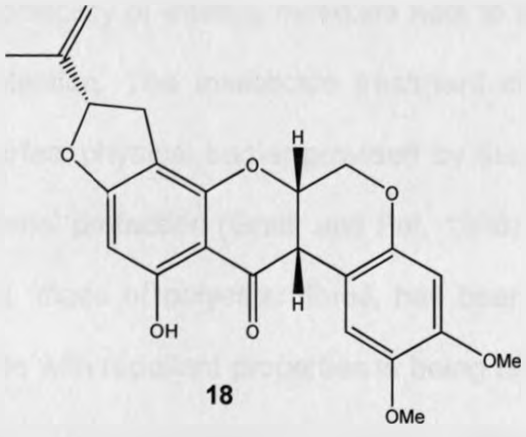


17

The need to discover cost effective and environmentally friendly alternative insecticides, has resulted in plant extracts as well as pure compounds being tested for larvicidal activity (Mwangi and Rembold, 1998). Rotenone (**18**) extracted from the roots and stems of several tropical and subtropical plants belonging to the genera *Lonchocarpus* and *Derris,* is one of the most extensively used natural insecticide. In addition rotenone (**18**) has also been reported to be highly active against the fourth-instar larvae of *Aedes aegypti L* (Abe *et al.*, 1985).



18

2.3.1.2 ADULTICIDES

The early experiences in southern Europe, North America and Taiwan on the use of DDT for malaria control suggested that complete coverage with DDT for a period sufficiently long enough for mosquitoes to die out, would lead to the eradication of the disease. Indoor residual spraying with DDT, and later with other residual insecticides, became the backbone of the malaria eradication campaign. However, the success of indoor residual spraying depends largely on the mosquitoes resting indoors before or after feeding. Not all species do this naturally and the excito-repellency of DDT and pyrethroids may dissuade mosquitoes from resting long on sprayed surfaces. The use of insecticides especially the halogen-based such as DDT have been associated with vector resistance and environmental bioaccumulation (Valule *et al.*, 1994).

2.3.1.3 BED NETS AND REPELLANTS

The development of pyrethroids with long residual action and very low mammalian toxicity suggested the possibility of treating mosquito nets to add an insecticidal effect to their mechanical protection. The insecticide treatment of nets adds a chemical barrier to the often imperfect physical barrier provided by the net and thus, improving its effectiveness in personal protection (Gratz and Pal, 1988). Recently a long-lasting deltamethrin-treated tent, made of polyester fibres, has been developed. In addition, permethrin, an insecticide with repellant properties is being used to treat blankets and bed sheets (WHO, 2004). The use of plants as natural repellants or insecticides has been documented from many areas (Curtis *et al.*, 1990), but most of the products from these plants have not been carefully characterised. Citronella products are used in

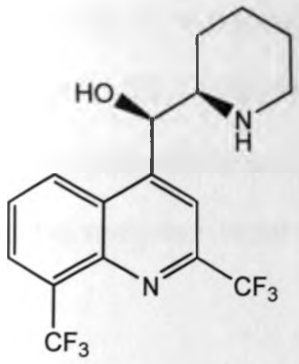
India and are effective against anopheline mosquitoes but their protective effects do not last for long. In China, an extract of *Eucalyptus maculata* (lemon eucalyptus) is widely used as a topical ointment (Lawless, 1995).

2.3.2 DRUGS AVAILABLE FOR TREATMENT OF MALARIA

At the present, a number of drugs are available for the treatment of malaria (Casteel, 1997; Murray and Perkins, 1996). Some of these are presented below according to their mechanism of action.

2.3.2.1 HAEM DETOXIFICATION

The haem remaining after digestion of the protein part of haemoglobin is toxic to the parasites, which appears to be due to the strong reducing power of iron (II)-haem complex (Ridley, 1997). Even after oxidation to iron (III)-haem, the complex remains toxic as it is membrane-interactive and potentially lytic (Ridley, 1997). To avoid the toxic effects, the parasites convert haem to the polymeric haemozoin also known as ß-haematin or malaria pigment. Inhibition of haem polymerisation is believed to be the mechanism of action of antimalarials such as quinine (**12**), chloroquine (**11**), mefloquine (**19**), primaquine (**20**) and halofantrine (**21**) (Bray *et al.*, 1998; Casteel, 1997; Fitch and Chou, 1997; Ridley, 1997).



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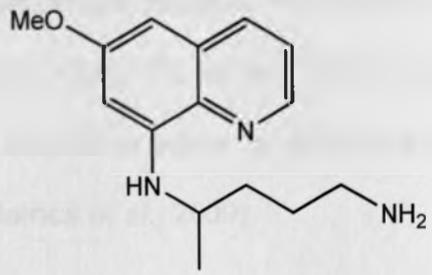

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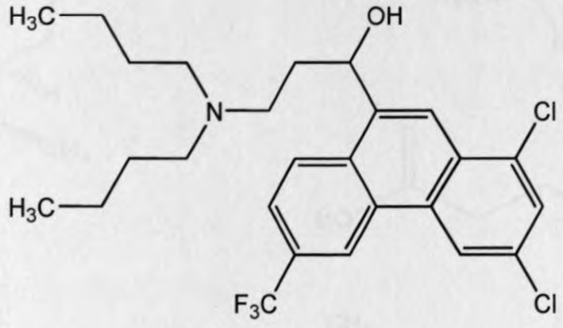
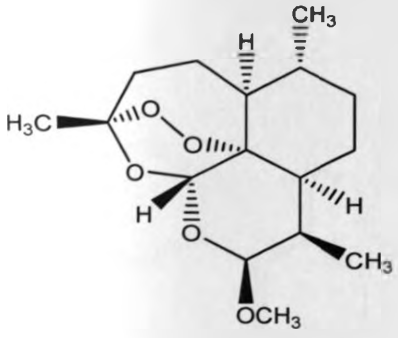


21

In addition, the peroxide antimalarials such as artemisinin (**13**) appear to be able to inhibit this polymerisation by alkylating the haem (Posner, 1997). It is suggested that, the iron (II) in the haem remaining after proteolysis of the protein part of haemoglobin, cleaves the peroxide bridge forming a reactive radical which, after some rearrangements in the molecule, alkylates one of the pyrazole rings in the porphyrin nucleus of haem. This alkylation is believed to inhibit the haemazoin formation. In the body this reaction sequence can only be induced by degraded haemoglobin.

Artemisinin is often used in combination with other antimalarials such as mefloquine (**19**) or tetracyclines (Casteel, 1997). Programmes undertaken to produce artemisinin

derivatives with more desirable pharmaceutical properties, resulted in the development of artemether (**22**), artesunate (**23**) and arteether (**24**) (Wu *et al.*, 1995; Klayman, 1985). Currently artemether is the artemisinin derivative which is approved for the treatment of malaria in most parts of the world (Barnes *et al.*, 2009).



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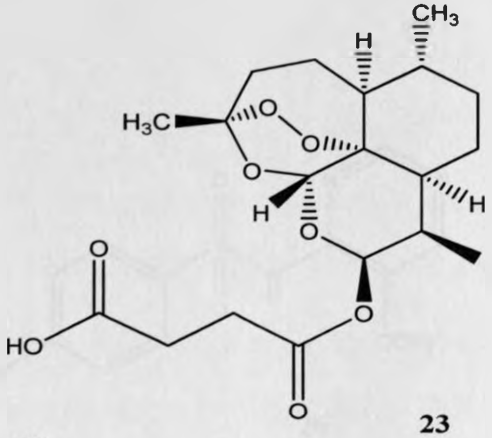

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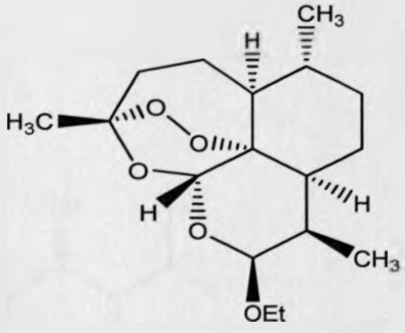
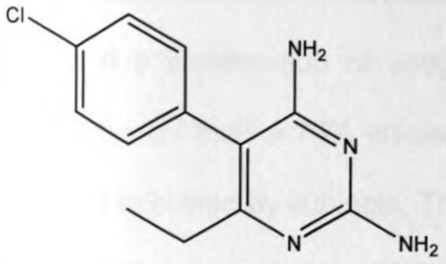


24

2.3.2.2 FOLATE METABOLISM

Some of the most widely used antimalarials are mixtures of sulfonamides and pyrimethamine. Sulfonamides prevent formation of dihydropterate and pyrimethamine is an inhibitor of dihydrofolate reductase. Both types of compounds thus inhibit the formation of dihydrofolate, which is necessary in the biosynthesis of pyrimidines. A frequently used drug is fansidar, which is a mixture of the antifolate pyrimethamine (**25**)

and sulfadoxine (**26**) (Casteel, 1997). Another combination of antifolates introduced recently is the biguanide chlorproguanil (**27**) with the sulfone drug dapsone (**28**) (Casteel, 1997). Besides being cheaper than fansidar, it is safe and effective in places with uncomplicated *P. falciparum* malaria (Casteel, 1997). However, it can neither be used for prophylaxis nor for treatment of malaria in multidrug resistant areas (Casteel, 1997).



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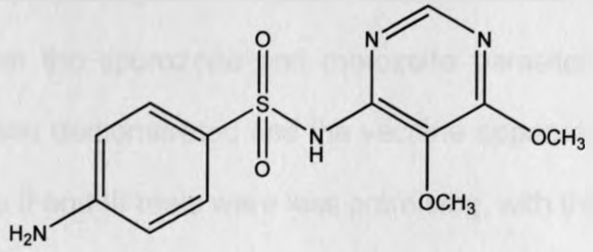

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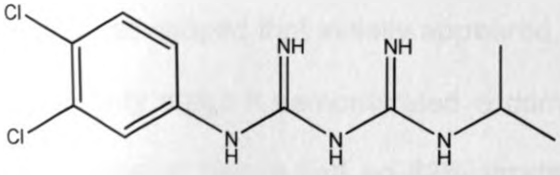

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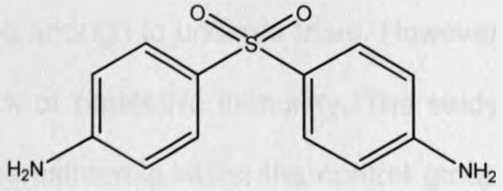

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2.3.3 VACCINE DEVELOPMENT

Vaccines are often the most efficient and cost-effective tools for public health. They have historically contributed to a reduction in the spread and burden of infectious diseases. Developing a vaccine against malaria would be critical in eradicating the disease since it would complement existing control and treatment interventions.

The first malaria vaccine developed that had undergone field trials, was the SPf66. It presented a combination of antigens from the sporozoite and merozoite parasites. During phase I trials a 75% efficacy rate was demonstrated and the vaccine appeared to be well tolerated by subjects. The phase II and III trials were less promising, with the efficacy falling to between 38.8% and 60.2%. It is still not known how the SPf66 vaccine confers immunity; therefore remaining an unlikely solution to malaria (Moorthy *et al.*, 2004). The CSP, also based on the circumsporoziote protein was the next vaccine developed that initially appeared promising enough to undergo trials. However at an early stage it demonstrated a complete lack of protective immunity. The study group used in Kenya had an 82% incidence of parasitaemia whilst the control group only had an 89% incidence (Moorthy *et al.*, 2004).

The NYVAC-Pf7 multistage vaccine attempted to use different technology, incorporating seven *P. falciparum* antigenic genes. These came from a variety of stages during the life cycle. CSP and sporozoite surface protein 2 were derived from the sporozoite phase. The liver stage antigen 1, three from the erythrocytic stage and one sexual stage antigen were included. Despite demonstrating cellular immune

responses in over 90% of the subjects had very poor antibody responses. In 1995 a field trial involving [NANP]19-5.1 proved to be very successful. Out of 194 children vaccinated none developed symptomatic malaria in the 12 week follow-up period and only 8 failed to have higher levels of antibody present. The vaccine consisted of the schizont export protein and the sporozoite surface protein [NANP] (Moorthy *et al.*, 2004).

RTS,S is the most recently developed recombinant vaccine and consists of the *P. falciparum* cirumsporozoite protein from the pre-erythrocytic stage. The CSP antigen causes the production of antibodies capable of preventing the invasion of hepatocytes and additionally elicits a cellular response enabling the destruction of infected hepatocytes. Whereas the CSP vaccine presented problems in trials due to its poor immunogenicity. The RTS,S attempted to avoid these by fusing the protein with a surface antigen from Hepatitis B, hence creating a more potent and immunogenic vaccine (Abdulla *et al.*, 2008). RTS,S, is the most clinically advanced malaria vaccine candidate, currently undergoing Phase III testing, often the last phase of testing prior to licensure. This is a major achievement in the field of malaria vaccine development, and if all goes well, RTS,S will become the first ever vaccine approved for protection against malaria.

2.4 BOTANICAL INFORMATION

2.4.1 THE FAMILY FABACEAE

The family Fabaceae, also referred to as the Leguminosae, is the third largest family of flowering plants comprising of 730 genera and over 19,400 species, most of which are shrubs but also include trees found in both temperate and tropical areas. It is also commonly known as the legume, pea or bean family referring to the typical fruit of these plants (Schrire *et al.*, 2005). Legumes include a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fiber, fuel, timber and medicinal production (Lewis *et al.*, 2005). Leguminous plants are known for their ability to fix atmospheric nitrogen thus replenishing nitrogen deficient soils.

The largest genera in this family include *Astragalus* with more than 2,000 species, *Acacia* with more than 900 species, and *Indigofera* with around 700 species. The leaves of Fabaceae species are imparipinnately compound while the leaflets are stipulate and opposite. The flowers are perigynous and commonly in racemes, spikes, or heads of white, rose or purple colour. The petals are basically distinct except for variable connation of the two lowermost ones called the keel petals. The pistil is simple, comprising a single style and stigma, and a superior ovary with one locule containing marginal ovules while the fruit is usually a legume (Schrire *et al.*, 2005).

The Fabaceae family is subdivided into three sub-families, namely Papilinoideae, Caesalpinoideae, Mimosoideae. Papilionoideae is the largest sub-family having 14,000

species in 500 genera (ALCAF, 1995). The Papilionoideae sub-family is divided into 32 tribes. The tribes Tephroseae and Phaseoleae are known to produce prenylated flavonoids and isoflavonoids, some of which posses important biological activities (Hegnauer and Grayer-Barkmeijer, 1993).

2.4.1.1 THE GENUS *MILLETTIA*

The genus *Millettia* comprises of about 200 species of trees, shrubs and woody climbers widely distributed in tropical and subtropical Africa, Asia and Australia (Geesink, 1981). It belongs to the Papilionoideae sub-family and Tephrosiae tribe. The genus is further sub-divided into two sub-genera, namely: sub-genus *Millettia* and sub-genus *Otosema*. In Kenya, six species represent the genus *Millettia*, these are *M. dura, M. leucantha, M. usaramensis, M. oblata, M. lasiantha and M. tanaensis*. All the six species belong to the sub-genus *Millettia* (Beentje, 1994).

2.4.1.1.1 *MILLETTIA USARAMENSIS*

M. usaramensis Taub. is a shrub approximately 7 metres high and has purple flowers. The leaves have 4-6 pairs of leaflets and its pods are reddish-brown. There are two known sub-species of *M. usaramensis*, sub-species *usaramensis* and sub-species *australis*. The sub-species *usaramensis* has two varieties, these are, *var. usaramensis* and *var. parvifolia*. Whereby *Var. usaramensis* occurs in Kenya and Tanzania; while *var. parvifolia* is found only in Tanzania. *M. usaramensis* sub-species *australis* is found in Zimbabwe, Malawi and Mozambique (Gillet *et al.*, 1971).

Figure 1: *Millettia usaramensis* (Photo by Mark Hyde)

2.5 ETHNO-MEDICAL AND PHARMACOLOGICAL INFORMATION

2.5.1. ETHNO-BOTANICAL USES OF THE GENUS *MILLETTIA*

Plants of the genus *Millettia,* form part of the medicinal flora and have been used traditionally for the treatment of various ailments. Table 2.1 summarizes the traditional medicinal uses of some *Millettia* species.

Table 2.1: Ethno-botanical uses of the *Millettia* species

Species	Plant part	Uses	Reference
M. auriculata	Leaves	Male infertility	Choudhary *et al.*, 1990
	Roots	Fish poison Pesticide Vermicide	Jain *et al.*, 1994
M. caerulea	Leaf+stem	Reduce infection in cuts and burns	Anderson, 1986
M. dielsiana	Vine	Improve circulation and dissolve blood clots	Pong *et al.*, 1981
M. dura	Entire plant	Fish poison	Teesdale, 1954
M. elongatistyla	Roots	Treat schistosomiasis	Hostettmann, 1984
M. extensa	Roots	Treat stomach pain	Singh *et al.*, 1994
	Root bark	Prevent conception	Singh *et al.*, 1994

Table 2.1: Ethno-botanical uses of the *Millettia* species cont....

Species	Plant part	Uses	Reference
M. ferruginea	Roots	Treat gonorrhea	Desta, 1993
M. kitanja	Leaves	Treat diabetes	Mueller-O *et al.*, 1971
M. lasiantha	Roots	Aphrodisiac	Kokwaro, 1993
M. leptobotrya	Roots	Treat wounds	Pei, 1985
M. makondesis	Leaves	Treat toothache	Kokwaro, 1976
M. oblate	Bark	To treat stomachache, cough	Kokwaro, 1976
	Roots	To treat swollen body	
M. pachycarpa	Roots	Treat swelling	Pei, 1985
	Roots	Fish poison	Ramanujan *et al.*, 1980
	Seeds	Fish poison	Mukerjee *et al.*, 1956
M. pervilleana	Seeds	Fish poison	Galeffi *et al.*, 1997
M. usaramensis	Roots	Treat snake bite	Kokwaro, 1976

2.5.2 BIOLOGICAL ACTIVITY OF *MILLETTIA* SPECIES

In addition to its wide use as an anti-inflammatory agent, the genus *Millettia* has shown a wide range of biological activities which are listed in table 2.2. It is worth to note that the extract of the stem bark of *Millettia usaramensis* has shown antiplasmodial activity (Yenesew *et al.*, 2003).

Table 2.2: Biological activity of some species of *Millettia*

Plant species	Plant part	Biological ctivity	Reference
M. brandisiana	Aerial parts	Antimicrobial	Tippawan *et al.*, 2005
		Antioxidant	
		Antiinflammatory	Pancharoen *et al.*, 2008
M. conraui	Stem bark	a-Glucosidase Inhibitors	Alembert *et al.*, 2007
M. erythrocalyx	N/S	Antiviral	Likhitwitayawuid *et al.*, 2005
M. griffoniana	Root bark	Antiinflammatory	Yankep *et al.*, 2003
M. Laurentii	Stem bark	Insecticidal	Kamnaing *et al.*, 1994
M. leucantha	Stem bark	Anti-inflammatory	Ampai *et al.*, 2003
M. pachycarpa	Seeds	Insecticidal	Singhal *et al.*, 1983
M. racemosa	Stem bark	Antibacterial	Rao and Krupadanam, 1994
M. taiwaniana	Stem	Antitumor	Ito *et al.*, 2004
M. thonningii	Seeds	Antischistosomal	Lyddiard *et al.*, 2002
M. usaramensis	Stem bark	Antiplasmodial	Yenesew *et al.*, 2003
M. versicolor	Aerial part	Anti-inflammatory	Fotsing *et al.*, 2003
	Root	Anthelminthic	Kasonia *et al.*,1989

2.6 PHYTOCHEMICAL INVESTIGATION OF THE FABACEAE FAMILY

Chemical investigation of species of the family Fabaceae has resulted in the isolation of anthraquinones, alkaloids, terpenoids and flavonoids among others; the flavonoids are the most comprehensively investigated. In contrast with the parent class of flavonoids, the distribution of the isoflavonoid in the plant kingdom is relatively limited, probably owing to the sporadic occurrence of isoflavone synthase. Isoflavonoids have been mostly found in the subfamily Papilionoideae of the Fabaceae family (Botta *et al.*, 2009). However the common emphasis of the fact that isoflavonoids are characteristic metabolites of leguminous plants sometimes leads to overlooking that the presence of isoflavonoids has also been reported in other families. The spectrum of isoflavonoid producing taxa includes the representatives of four classes of multicellular plants, namely the Bryopsida, the Pinopsida, the Magnoliopsida and the Liliopsida (Botta *et al.*, 2009).

It is also worth to note that, isoprenoid-substituted isoflavonoids are expressed from a smaller number of plants including those of the Fabaceae, as a result of the restricted distribution of prenyltransferase. Previous studies of extracts of *Millettia* species have led to the isolation of flavones, flavanones, chalcones, rotenoids and isoflavones.

2.6.1 COMPOUNDS ISOLATED FROM THE GENUS *MILLETTIA*

2.6.1.1 ISOFLAVONES FROM THE GENUS *MILLETTIA*

Isoflavones constitute the largest percentage of naturally occuring isoflavonoids. Among the isoflavonoids of this genus, isoflavones are the most predominant secondary metabolites. The isoflavones reported from the genus *Millettia* are listed in table 2.3.

Table 2.3: Isoflavones of *Millettia*

Isoflavone	**Source (plant part)**	**Reference**
Auricularin (**29**)	*M. auriculata* (RT)	Shabbir and Zaman, 1970
Auriculasin (**30**)	*M. auriculata* (LF) *M. auriculata* (SD) *M. taiwaniana* (SB)	Minhaj *et al.*, 1976 Raju and Srimannarayana, 1978 Ito *et al.*, 2004
Auriculatin (**31**)	*M. auriculata* (RT) *M. auriculata* (SD)	Shabbir and Zaman, 1970 Raju and Srimannarayana, 1978
Auriculin (**32**)	*M. auriculata* (RB)	Shabbir and Zaman, 1970
Aurmillone (**33**)	*M. auriculata* (SD)	Raju and Srimannarayana, 1978
2'-Deoxyisoauriculatin(**34**)	*M. auriculata* (RT)	Shabbir and Zaman, 1970
Isoauriculasin (**35**)	*M. auriculata* (LF)	Minhaj *et al.*, 1976
Isoauriculatiin (**36**)	*M. auriculata* (RB)	Shabbir and Zaman, 1970
Isoaurmillone (**37**)	*M. auriculata* (SDP)	Gupta *et al.*, 1983

Table 2.3: Isoflavones of *Millettia* cont....

Isoflavone	Source (plant part)	Reference
2'-O-Methylisoauriculatin(**38**)	*M. auriculata* (RB)	Shabbir and Zaman, 1970
Conrauinones A (**39**)	*M. conraui* (SB)	Fuendjiep *et al.*, 1998a
Conrauinones B (**40**)	*M. conraui* (SB)	Fuendjiep *et al.*, 1998a
Conrauinones C (**41**)	*M. conraui* (SB)	Fuendjiep *et al.*, 1998b
Conrauinones D (**42**)	*M. conraui* (SB)	Fuendjiep *et al.*, 1998b
7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone (**43**)	*M. conraui* (SB)	Fuendjiep *et al.*, 1998b
5-Methoxydurmillone (**44**)	*M. conraui* (SB) *M. ferruginea* (SB)	Fuendjiep *et al.*, 1998b Dagne *et al.*,1989
Afromosin (**45**)	*M. reticulata* (SB) *M. nitida* (VS)	Chen *et al.*, 1983 *Xiang et al.*, 2009
Biochanin A (**46**)	*M. nitida* (VS)	Feng *et al.*, 2007
8-O-Methylretusin (**47**)	*M. reticulata* (SB)	Chen *et al.*, 1983
Odoratin (**48**)	*M. griffoniana* (RB)	Yankep *et al.*, 1997
Calopogoniumisoflavone (**49**)	*M. dura* (SB) *M. ferruginea* (SB)	Yenesew *et al.*, 1996 Dagne *et al.*, 1990a
Calopogoniumisoflavon A,6-methoxy (**50**)	*M. dura* (SDP)	Yenesew *et al.*, 1997b
6-Demethyldurallone (**51**)	*M. dura* (SDP)	Yenesew *et al.*, 1996

Table 2.3: Isoflavones of *Millettia* cont....

Isoflavone	Source (plant part)	Reference
7,2'-Dimethoxy-4',5'-Methylenedioxyisoflavone(**52**)	*M. dura* (SB) *M. griffoniana* (RB) *M. puguensis*	Dagne *et al.*, 1991 Yankep *et al.*, 1997 Kapingu *et al.*, 2006
Durallone (**53**)	*M. dura* (SDP)	Yenesew *et al.*, 1996
Durlettone (**54**)	*M. dura* (SD) *M. dura* (SD)	Ollis *et al.*, 1967 Dagne *et al.*, 1991
Durlmillone (**55**)	*M. dura* (SD) *M. ferruginea* (SB) *M. rubiginosa* (RB) *M. griffonianone* (RB)	Ollins *et al.*, 1967 Dagne *et al.*, 1989 Desai *et al.*, 1977 Yankep *et al.*, 1997
Isoerythrin A, 4'-(3-methylbut-2-enyl ether (**56**)	*M. dura* (SDP)	Yenesew *et al.*, 1996
Jamaicin (**57**)	*M. dura* (SD) *M. ferruginea* (SB) *M. usaramensis* (SB) *M. griffonianone* (RB)	Yenesew *et al.*, 1997b Dagne *et al.*, 1989 Yenesew *et al.*, 1998c Yankep *et al.*, 1997
Maximaisoflavone B (**58**)	*M. dura* (SB)	Dagne *et al.*, 1991
Maximaisoflavone D (**59**)	*M. dura* (SB)	Yenesew *et al.*, 1996
Maximaisoflavone H (**60**)	*M. dura* (SB) *M. dura* (SB)	Dagne *et al.*, 1991 Yenesew *et al.*, 1996
Milldurone (**61**)	*M. dura* (SB)	Ollis *et al.*, 1967

Table 2.3: Isoflavones of *Millettia* cont....

Isoflavone	Source (plant part)	Reference
Predurallone (**62**)	*M. dura* (SDP)	Yenesew et al., 1996
Barbigerone (**63**)	*M. ferruginea* (SD) *M. usaramensis* (SB) *M. taiwaniana*	Dagne *et al.*, 1990a Yenesew *et al.*, 1998c Ito *et al.*, 2004
Calopogonium isoflavone B (**64**)	*M. ferruginea* (SB) *M. griffonianone* (RB)	Dagne *et al.*, 1989 Yankep *et al.*, 1997
Ferrugone (**65**)	*M. ferruginea* (SD)	Dagne *et al.*, 1991
7-*O*-Geranylformononetin (**66**)	*M. ferruginea* (RB) *M. griffonianone* (RB)	Dagne *et al.*, 1990b Yankep *et al.*, 1997
7-Hydroxy-5,6-dimethoxy-3',4'-methylenedioxyisoflavone (**67**)	*M. ferruginea* (SB)	Dagne *et al.*, 1989
Ichthynone (**68**)	*M. ferruginea* (SB) *M. rubiginosa* (RB)	Dagne *et al.*, 1989 Desai *et al.*, 1977
Isojamaicin (**69**)	*M. ferruginea* (SB) *M. usaramensis* (SB) *M. griffoniana* (SD)	Dagne *et al.*, 1989 Yenesew *et al.*, 1998c Ngamga *et al.*, 2005
Nordurlettone (**70**)	*M. ferruginea* (SB)	Dagne *et al.*, 1990a
Prebarbigerone (**71**)	*M. ferruginea* (SB) *M. griffoniana* (SD)	Dagne *et al.*, 1990a Ngamga *et al.*, 2005

Table 2.3: Isoflavones of *Millettia* cont....

Isoflavone	Source (plant part)	Reference
Predurmillone (**72**)	*M. ferruginea* (SB)	Dagne *et al.*, 1990a
Preferrugone (**73**)	*M. ferruginea* (SB)	Dagne *et al.*, 1990a
Pre-5-methoxydurmillone (**74**)	*M. ferruginea* (SB)	Dagne *et al.*, 1989
Griffonianone B (**75**)	*M. griffonianone* (RB)	Yankep *et al.*, 2001
Griffonianone C (**76**)	*M. griffonianone* (RB)	Yankep *et al.*, 2001
7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone (**77**)	*M. griffonianone* (RB)	Yankep *et al.*, 2001
3',4'-Dihydroxy-7-*O*-[(E)-3,7-dimethylallyl-2,6-octadienyl]isoflavone (**78**)	*M. griffonianone* (RB)	Yankep *et al.*, 1998
4'-Methoxy-7-*O*-[(E)-3-methyl-7-hydroxy-2,6-octadienyl]isoflavone(**79**)	*M. griffonianone* (RB)	Yankep *et al.*, 1998
7-O-Geranylpseudobaptigenin (**80**)	*M. griffonianone* (RB)	Yankep *et al.*, 1997
Odorantin (**81**)	*M. griffonianone* (RB)	Yankep *et al.*, 1997
Maximaisoflavone (**82**)	*M. griffonianone* (RB) *M. usaramensis* (SB)	Yankep *et al.*, 2001 Yenesew, 1997a
Pyrano[5'',6:6'',7]isoflavone,2',4',5'-trimethoxy-2'',2''-dimethyl (**83**)	*M. ichthyochtona* (LF)	Kamperdick *et al.*, 1998
Gliricidin (**84**)	*M. laurentii* (HW)	Kamnaing *et al.*, 1999
85	*M. pachycarpa* (LF)	Singhal *et al.*,1981

Table 2.3: Isoflavones of *Millettia* cont....

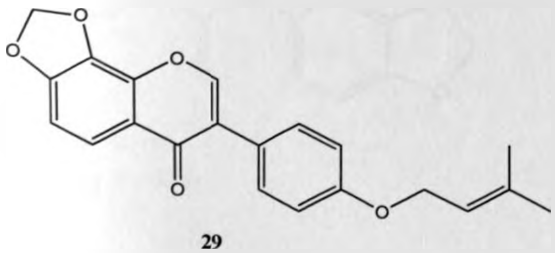
Isoflavone	Source (plant part)	Reference
86	*M. pachycarpa* (LF)	Singhal *et al.*,1981
87	*M. pachycarpa* (LF)	Singhal *et al.*,1981
88	*M. pachycarpa* (LF)	Singhal *et al.*,1981
4'-*O*-methylderrone (**89**)	*M. pachycarpa* (SD)	Singhal *et al.*,1981
6,8-Diprenylorobol (**90**)	*M. pachycarpa* (AP)	Singhal *et al.*,1981
5,7,4'-Trihydroxy-6,3'-diprenylisoflavone (**91**)	*M. pachycarpa* (AP)	Singhal *et al.*,1983
6,8-Diprenylgenistein (**92**)	*M. pachycarpa* (AP)	Singhal *et al.*,1983
6,8-Diprenylpratensin (**93**)	*M. pachycarpa* (SD)	Singhal *et al.*,1983
Pomiferin (**94**)	*M. pachycarpa* (SD)	Singhal *et al.*,1983
2'-Hydroxylupalbigenin (**95**)	*M. pulchra* (AP)	Baruah *et al.*,1984
2'-Methoxylupalbigenin (**96**)	*M. pulchra* (AP)	Baruah *et al.*,1984
Alpinumisoflavone (**97**)	*M. thonningii* (SD) *M. taiwaniana*	Olivares, 1982 Ito *et al.*, 2004
O,*O*-Dimethylalpinumisoflavone (**98**)	*M. thonningii* (RB)	Asoamaning *et al.*, 1999
3'-Hydroxy-4'-methoxy alpinumisoflavone (**99**)	*M. thonningii* (SD)	Olivares *et al.*, 1982
5- Methoxyalpinumisoflavone (**100**)	*M. thonningii* (RW)	Asoamaning *et al.*, 1999

Table 2.3: Isoflavones of *Millettia* cont....

Isoflavone	Source (plant part)	Reference
4'-Methoxyalpinumisoflavone (**101**)	*M. thonningii* (SD)	Khalid *et al.*, 1983
5-*O*-Methyl-4'-*O*-(3-methyl-2-butenyl)-alpinumisoflavone (**102**)	*M. thonningii* (SD)	Asoamaning *et al.*, 1995
Robustone (**103**)	*M. thonningii* (SD)	Khalid *et al.*, 1983
Thonninginisoflavone (**104**)	*M. thonningii* (RB)	Asoamaning *et al.*, 1995
Norisojamaicin (**105**)	*M. usaramensis* (SB)	Yenesew, 1997a
Toxicaroliisoflavone (**106**)	*M. usaramensis* (SB) *M. brandisiana* (LF)	Yenesew, 1997a Pancharoen *et al.*, 2008
Robustigenin (**107**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
Brandisianin A (**108**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
7, 4'-Di-*O*-prenylgenistein (**109**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
Millewanins A (**110**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004
Millewanins B (**111**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004
Millewanins C (**112**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004
Millewanins D (**113**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004
Millewanins E (**114**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004
Warangalone (**115**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004
8-γ,γ-Dimethylallylwighteone (**116**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004
5.7.4'-Trihydroxy-3',5'-di-methylallylisoflavone (**117**)	*M. taiwaniana* (ST)	Ito *et al.*, 2004

Table 2.3: Isoflavones of *Millettia* cont....

Isoflavone	Source (plant part)	Reference
Viridiflorin (**118**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
Hirsutissimiside B (**119**)	*M. nitida* (VS)	Xiang *et al.*, 2009
Sphaerobioside (**120**)	*M. nitida* (VS)	Xiang *et al.*, 2009
3'-*O*-Methylorobol (**121**)	*M. nitida* (VS)	Feng *et al.*, 2007
122	*M. puguensis*	Kapingu *et al.*, 2006
123	*M. puguensis*	Kapingu *et al.*, 2006



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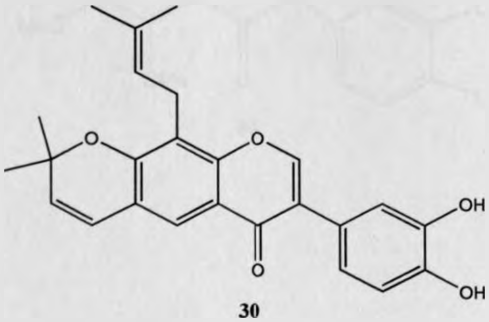

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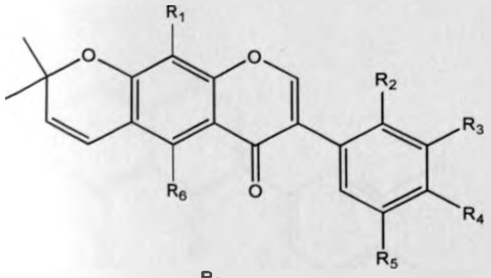
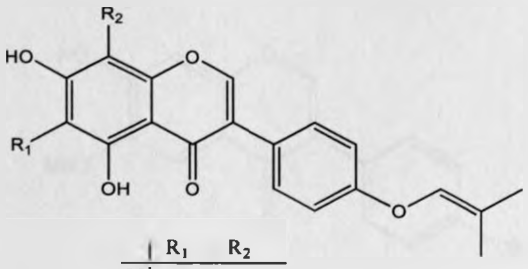
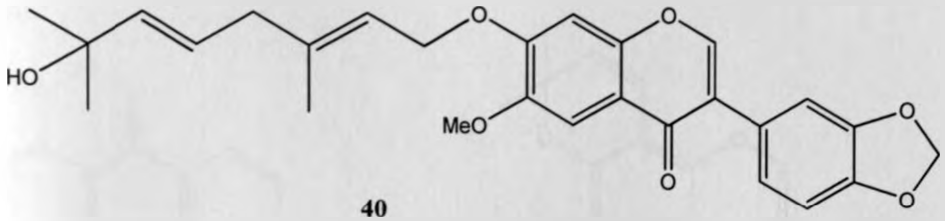


	R_1	R_2	R_3	R_4	R_5	R_6
31	Prenyl	H	H	OH	H	OH
32	Prenyl	H	OH	OMe	H	H
34	H	H	H	*O*-Prenyl	H	OH
35	H	OH	H	*O*-Prenyl	H	OH
36	H	H	O-Prenyl	H	OH	H
38	H	CH_3	H	O-Prenyl	H	OH



	R_1	R_2
33	H	OMe
37	OMe	H



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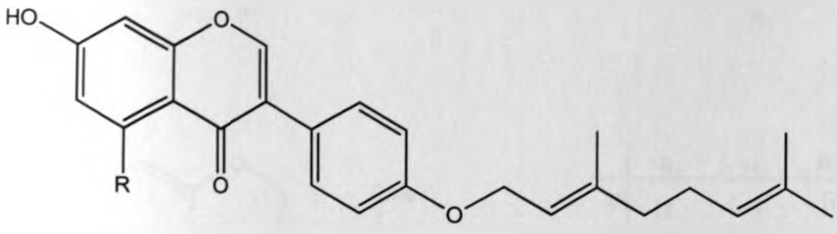
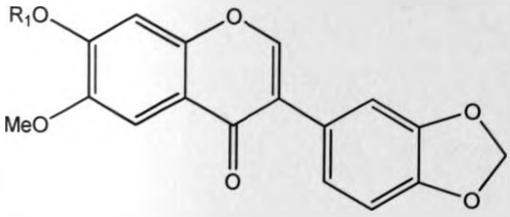
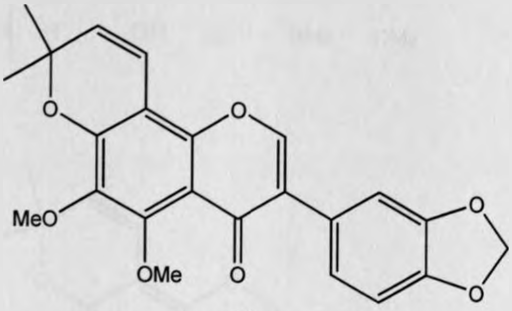


41 R=H
42 R=OH



43 R_1=H
122 R_1=CH_3



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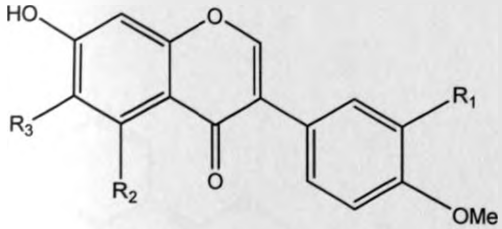
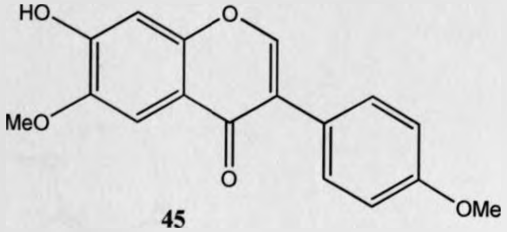


	R_1	R_2	R_3
46	H	OH	H
48	OH	H	OMe



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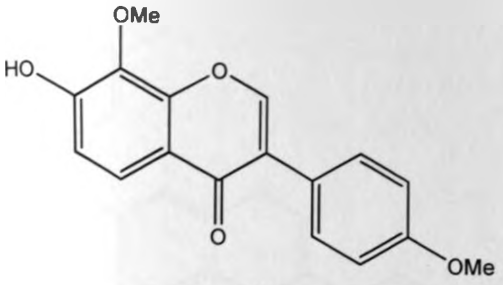

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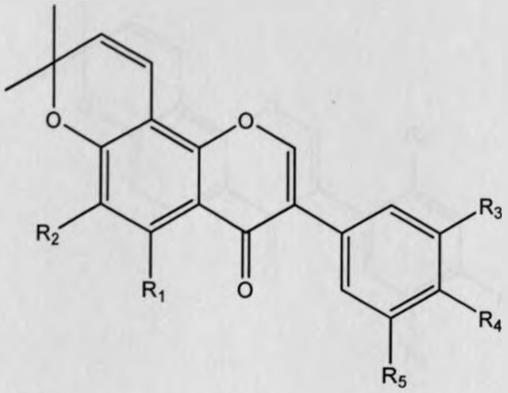
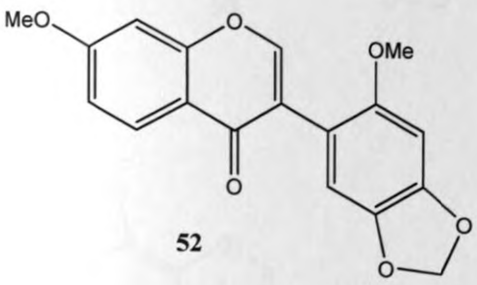


	R_1	R_2	R_3	R_4	R_5
49	H	H	H	OMe	H
50	H	OMe	H	OMe	H
53	H	OMe	OMe	OMe	H
54	H	OH	H	OMe	OMe



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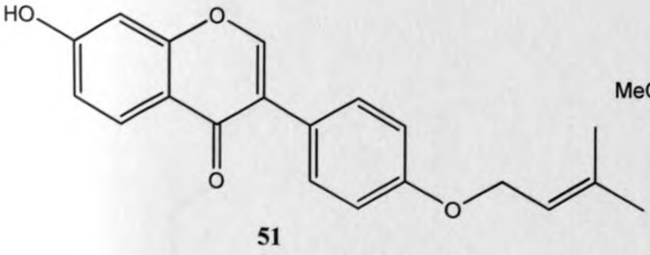

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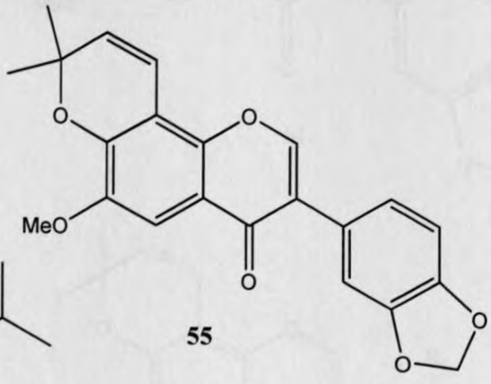

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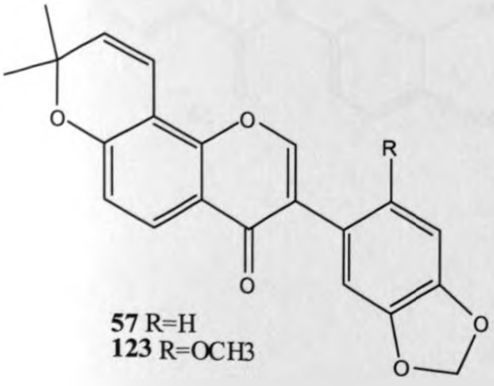
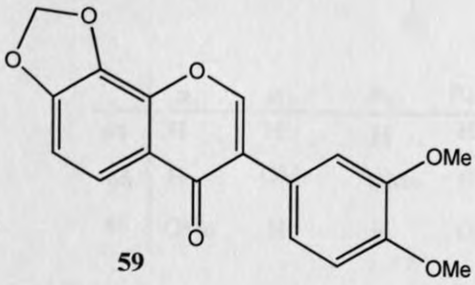


57 R=H
123 R=OCH3



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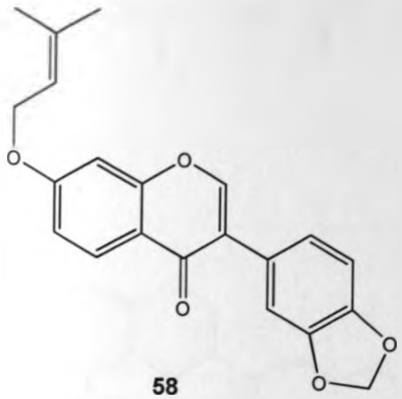

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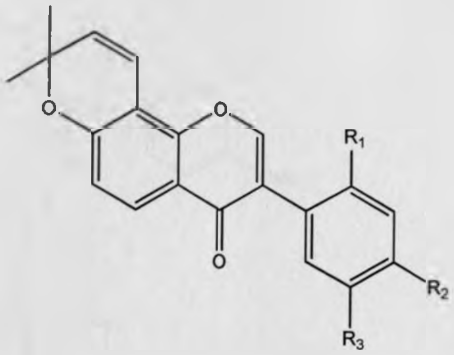
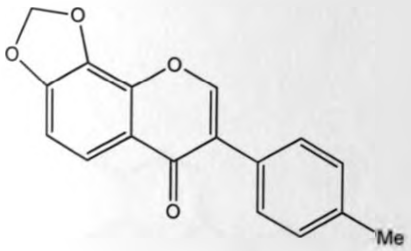


	R_1	R_2	R_3
56	H	O-Prenyl	H
63	OMe	OMe	OMe



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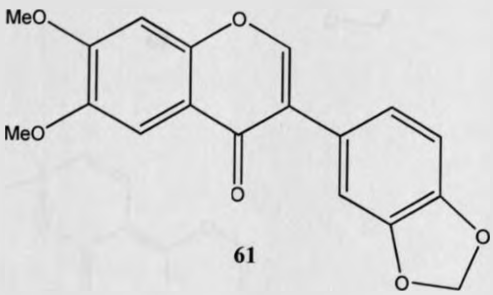

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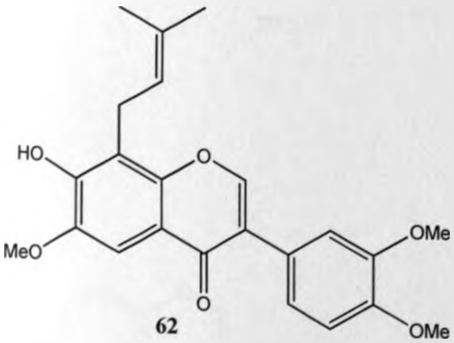

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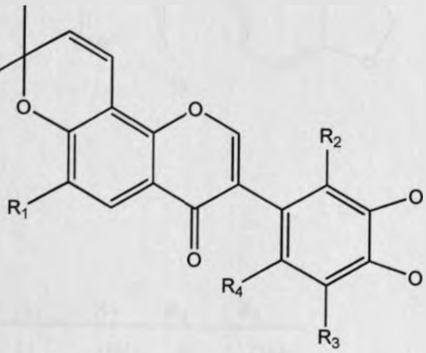
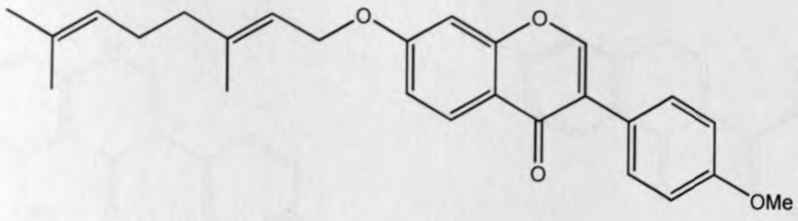


	R_1	R_2	R_3	R_4
64	H	H	H	H
65	H	OMe	OMe	H
68	OMe	H	H	OMe



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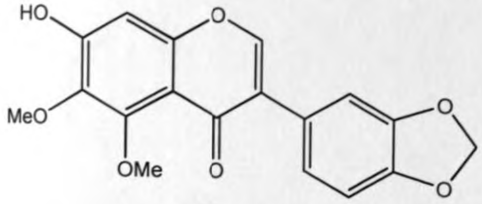

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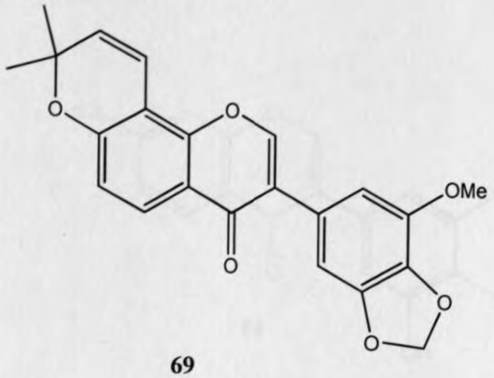
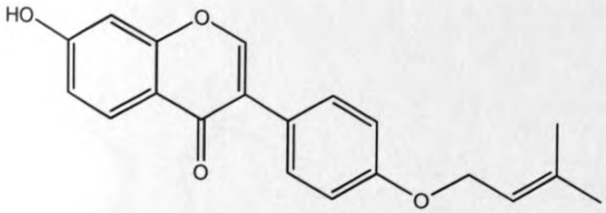


69



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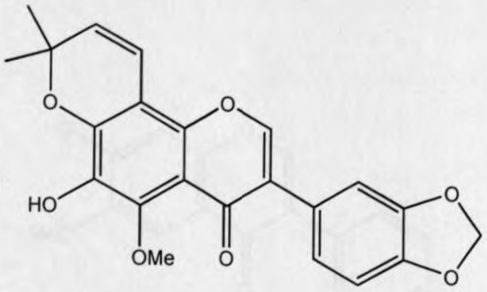

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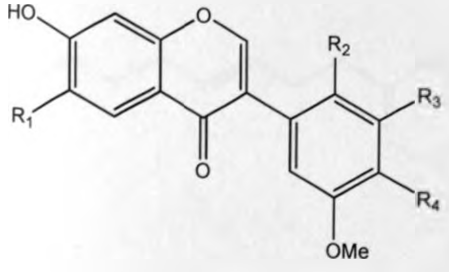
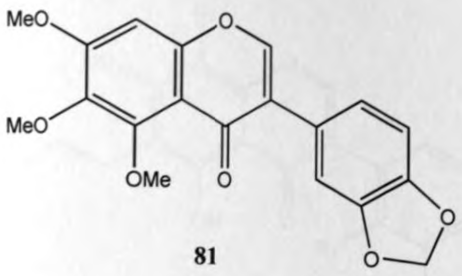


	R_1	R_2	R_3	R_4
71	H	OMe	H	OMe
72	OMe	H	OCH_2O	
73	H	OMe	OCH_2O	



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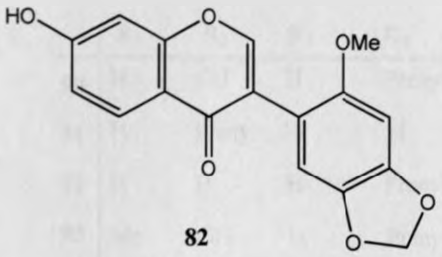

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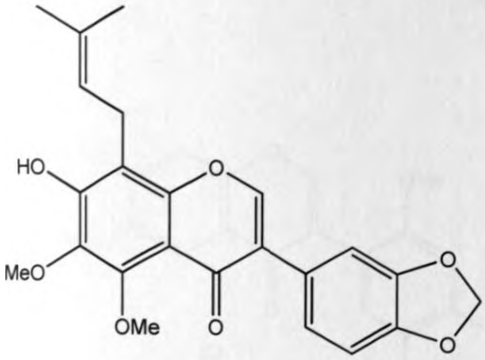

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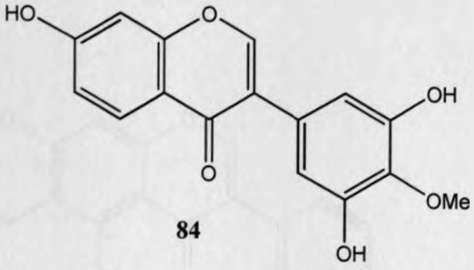

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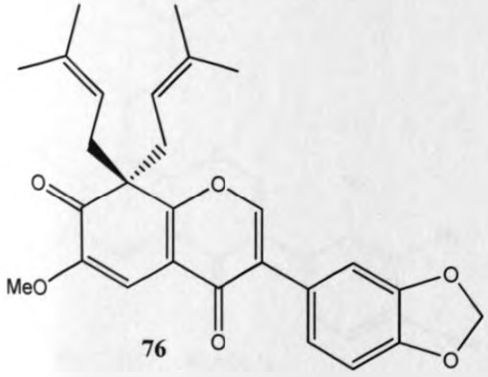

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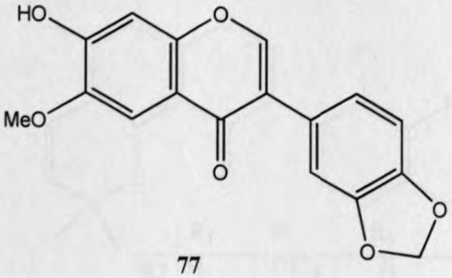

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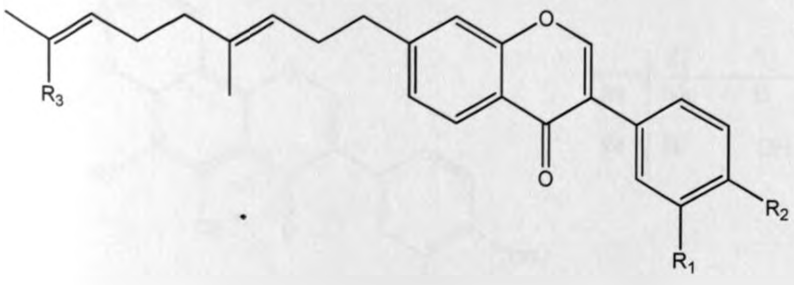
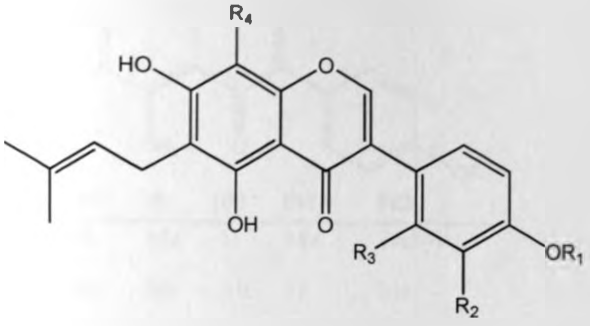
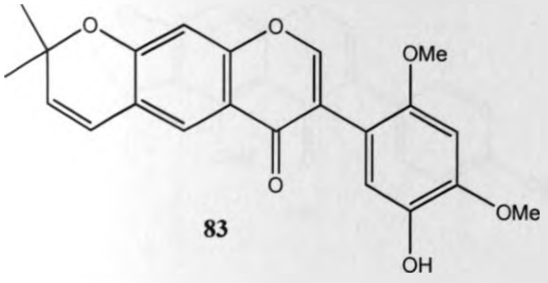


	R_1	R_2	R_3
78	OH	OH	Me
79	H	OMe	CH_2OH
80	OCH_2O		H



	R_1	R_2	R_3	R_4
90	H	OH	H	Prenyl
91	H	Prenyl	H	H
92	H	H	H	Prenyl
93	Me	OH	H	Prenyl
95	H	Prenyl	OH	H
96	H	Prenyl	OMe	H



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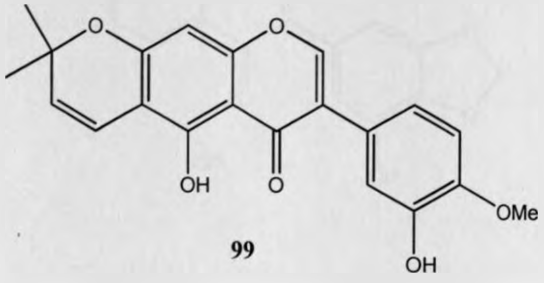

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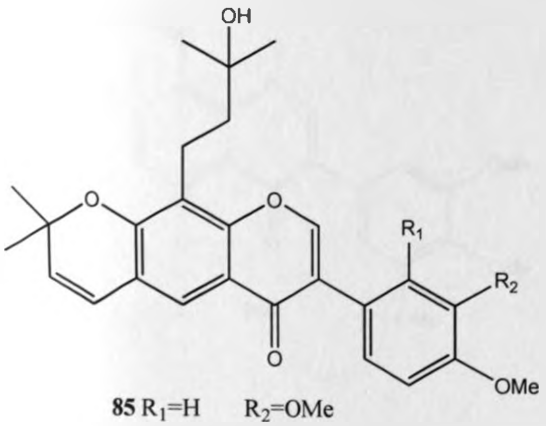
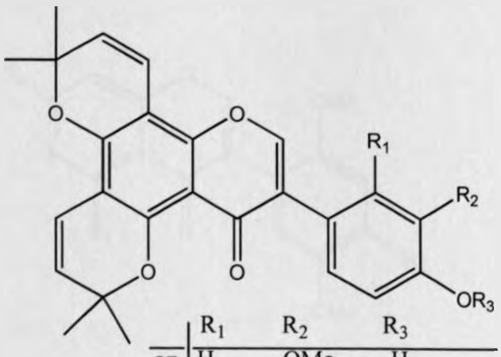
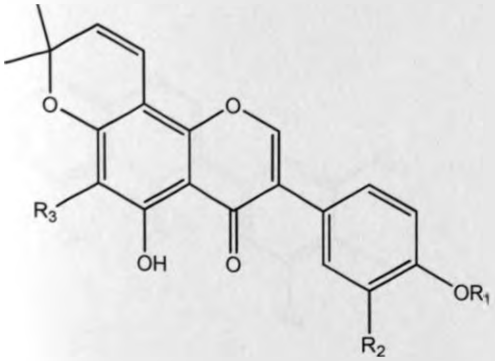


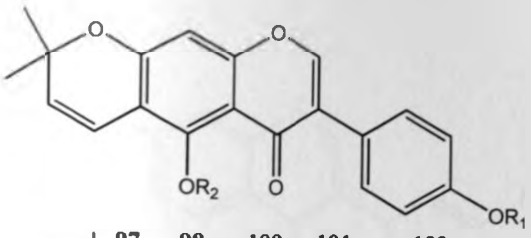
85 R_1=H R_2=OMe
86 R_1=OMe R_2=H



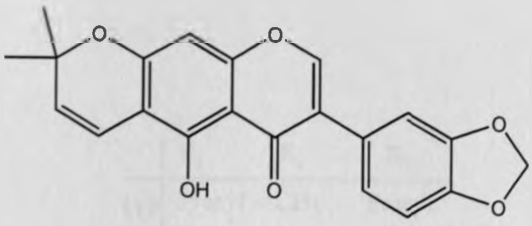
	R_1	R_2	R_3
87	H	OMe	H
88	OH	H	Me



	R_1	R_2	R_3
89	Me	H	H
94	H	OH	Prenyl



	97	98	100	101	102
R_1	H	Me	H	Me	Prenyl
R_2	H	Me	Me	H	Me



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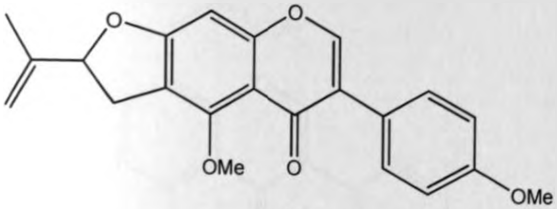

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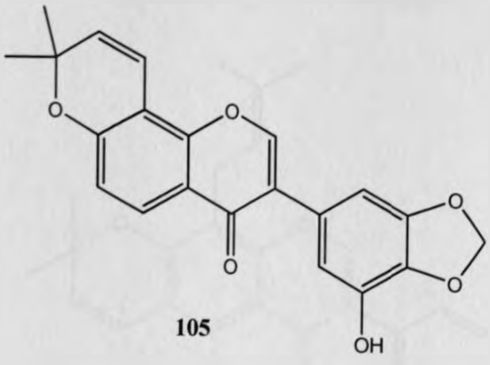

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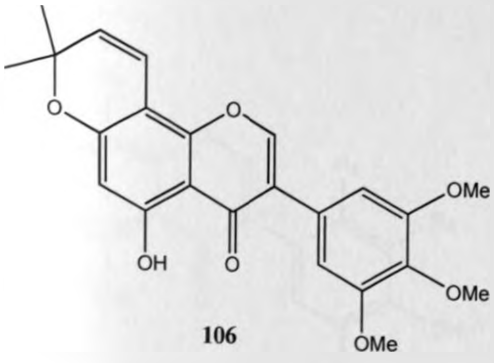

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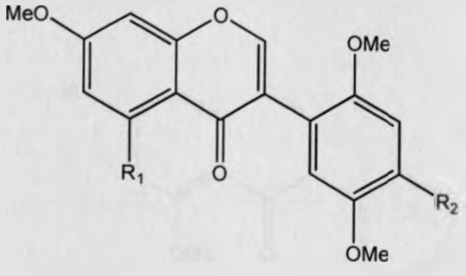
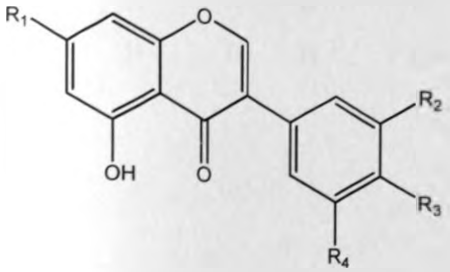
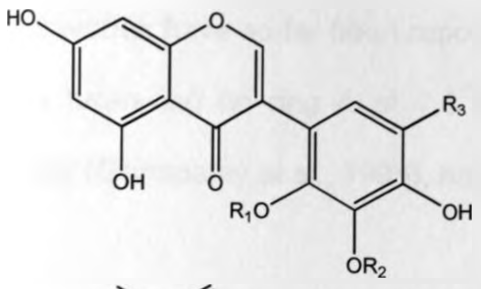


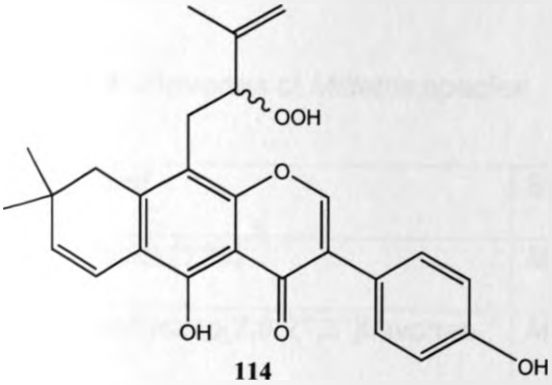
	R_1	R_2
107	OH	OMe
108	OMe	*O*-Prenyl



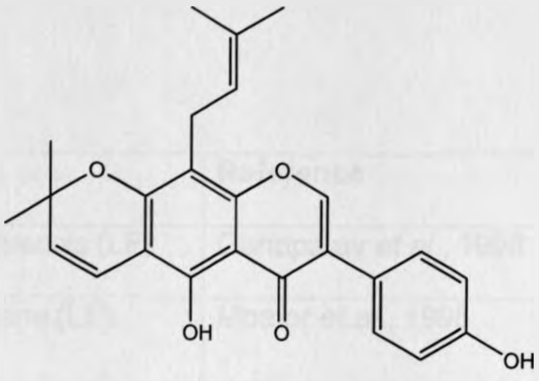
	R_1	R_2	R_3	R_4
109	O-Prenyl	H	O-Prenyl	H
113	OH	Geranyl	OH	Prenyl



	R_1	R_2	R_3
110	Prenyl	CH_3	Prenyl
111	Prenyl	CH_3	Geranyl
112	Prenyl	H	Geranyl



114



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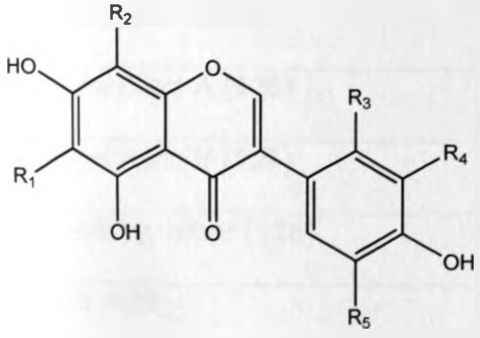
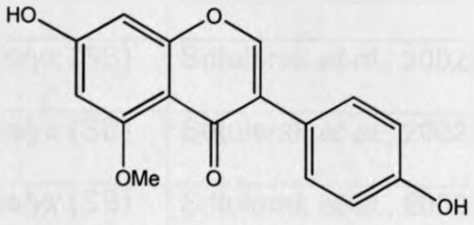


	R_1	R_2	R_3	R_4	R_5
116	prenyl	Prennyl	H	H	H
117	H	H	H	O-Prenyl	Prenyl
118	Prenyl	H	OMe	H	OMe
120	H	H	H	H	H
121	H	H	H	H	OH



119

2.6.1.2 FLAVONES AND ANTHOCYANINS OF *MILLETTIA*

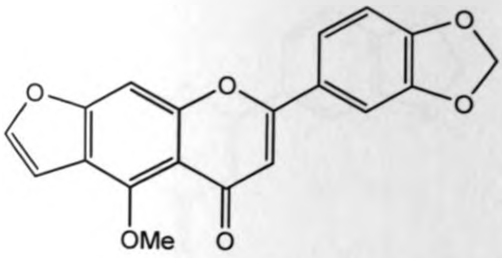
Several flavones have so far been reported from the genus *Millettia* and most of them posses a furan-ring on ring A at 7,8 position. However, pongamol (**122**), from *M. penguensis* (Ganapatay *et al.*, 1998), has the furan-ring at 6, 7-position.

Table 2.4: Flavones of *Millettia* species

Compound	**Species**	**Reference**
Pongaglabol (**123**)	*M. penguensis* (LF)	Ganapatay *et al.*, 1998
5-Methoxyfurano[7,8:2",3"]flavones (**124**)	*M. sangana* (LF)	Mbafor *et al.*, 1995
Millettocalyxin C (**125**)	*M. erythrocalyx* (SB)	Sritularak *et al.*, 2002a
Miilletttocalyxins A (**126**)	*M. erythrocalyx* (SB)	Sritularak *et al.*, 2002a
Miilletttocalyxins B (**127**)	*M. erythrocalyx* (SB)	Sritularak *et al.*, 2002a
Pongol methyl ether (**128**)	*M. erythrocalyx* (SB)	Sritularak *et al.*, 2002a
Ovalifolin (**129**)	*M. erythrocalyx* (SB)	Sritularak *et al.*, 2002a
Pongaglabrone (**130**)	*M. erythrocalyx* (SB)	Sritularak *et al.*, 2002a
Karanjone (**131**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1976a
Karanjin (**132**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1976a
Lanceolatin (133)	*M. ovalifolia (SD)* *M. nitida (VS)*	Gupta et al., 1976a Xiang et al., 2009
3,6-Dimethoxyfuranol[7,8:2",3"] flavones (134)	*M. ichthyochtona(LF)*	Kamperdick et al., 1998

Table 2.4: Flavones of *Millettia* species cont....

Compound	Species(plant part)	Reference
Astragalin (**135**)	*M. Zechiana* (AP)	Parvez and Ogbide, 1990
3-Hydroxy-4'-methoxyflavone (**136**)	*M. Zechiana* (AP)	Parvez and Ogbide, 1990
3-0-a-L-rhamnosekampferol (**137**)	*M. Zechiana* (AP)	Parvez and Ogbide, 1990
Quercitrin (**138**)	*M. Zechiana* (AP)	Parvez and Ogbide, 1990
Isoquercitrin (**139**)	*M. Zechiana* (AP)	Parvez and Ogbide, 1990
7-O-ß-D-glucoside-8-hydroxyquercetin (**140**)	*M. Zechiana* (FL)	Ogbeide and Parvez, 1992
3-Methyletherquercetin (**141**)	*M. Zechiana* (FL)	Ogbeide and Parvez, 1992
Laurentinol (**142**)	*M. laurenti* (FL)	Kamnaing *et al.*, 1999
3',5'-dimethoxy-[2",3":7,8]-furanoflavone (**143**)	*M. erythrocalyx* (LF)	Kittisak *et al.*, 2005
6,3'-dimethoxy-[2",3":7,8]-furanoflavone (**144**)	*M. erythrocalyx* (SD)	Sritularak *et al.*, 2006
Anthocyanins		
Cyanin (**145**)	*M. zechiana (AP)*	Parvez and Ogbide, 1990
3,5-Di-O-ß-D-glucosidemalvidin(**146**)	*M. zechiana (AP)*	Parvez and Ogbide, 1990
3-0-a-L-rhamnosepelargonidin (**147**)	*M. zechiana (AP)*	Parvez and Ogbide, 1990



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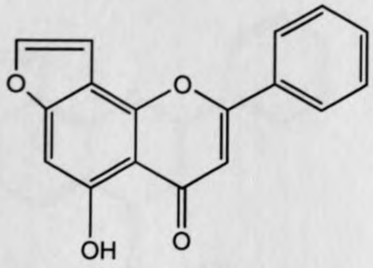

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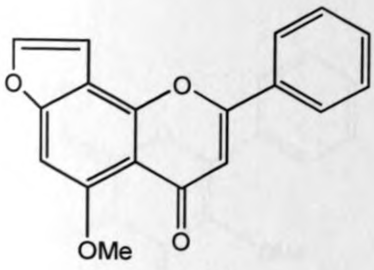

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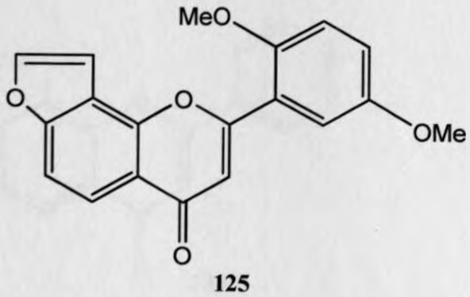

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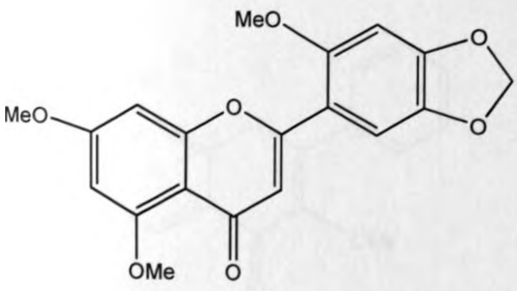

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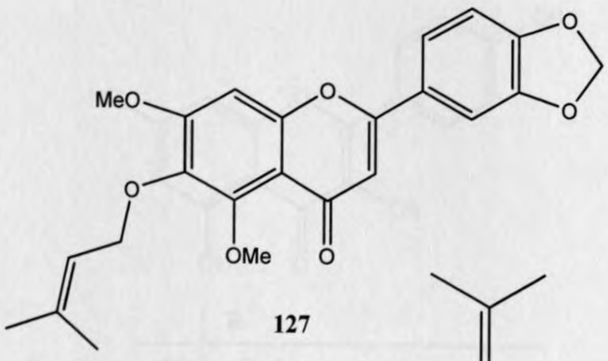

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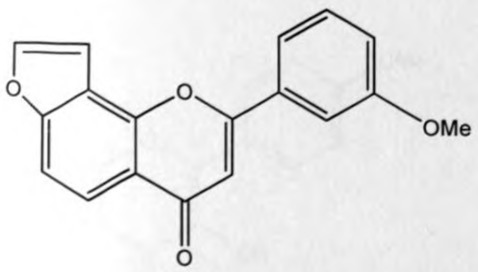

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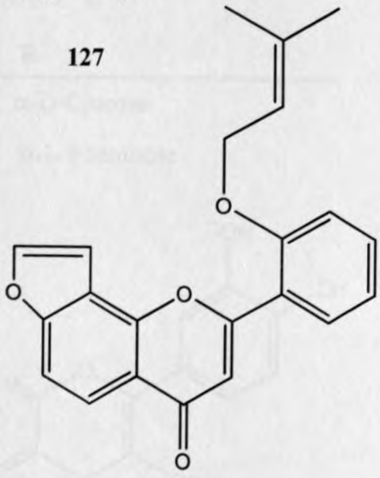

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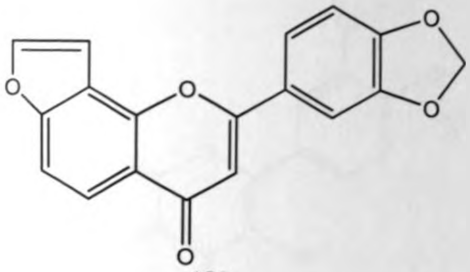

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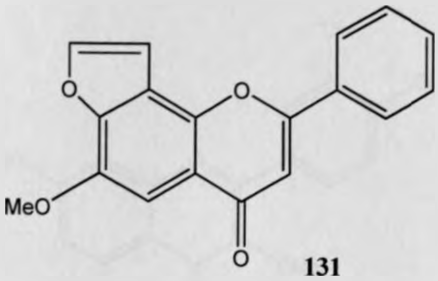

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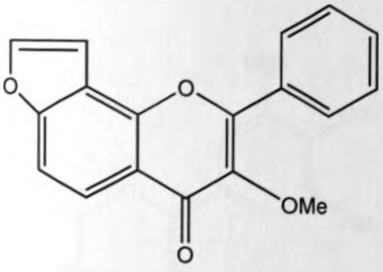

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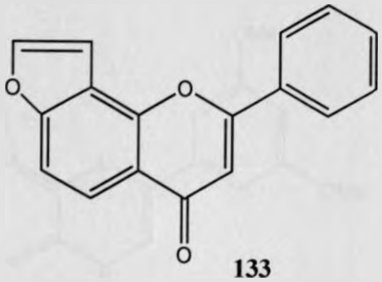

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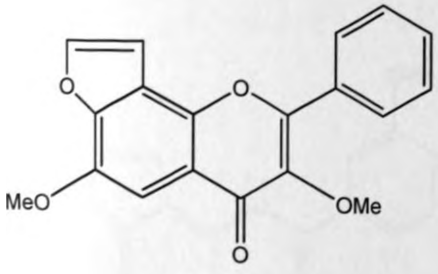

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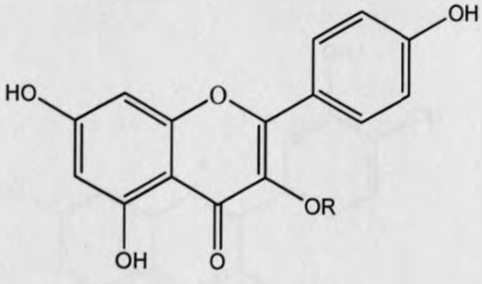
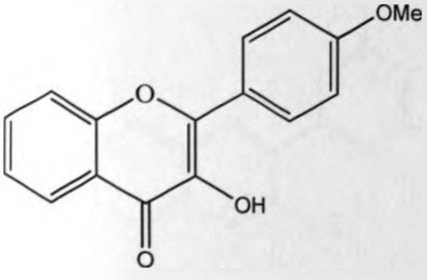


	R
135	α-D-Glucose
137	α-L-Rhamnose



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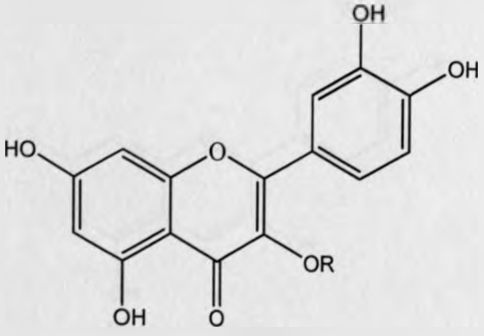
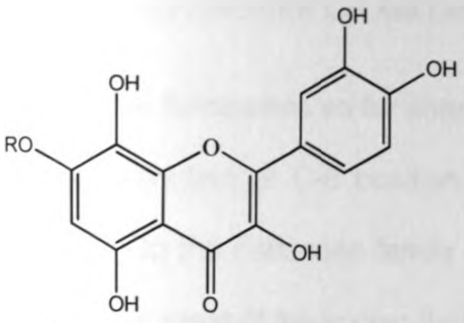
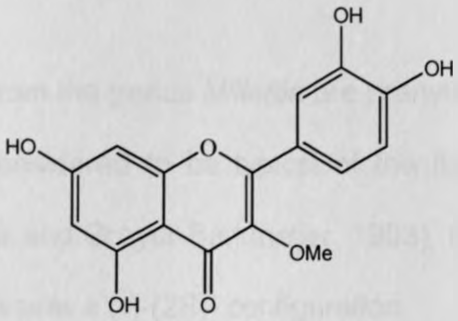


138 R=α-L-Rhamnose
139 R=β-D-Glucose



140 R=β-D-Glucose



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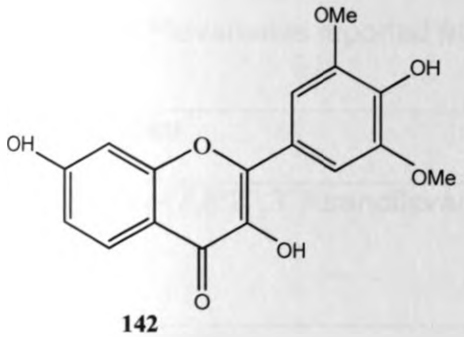

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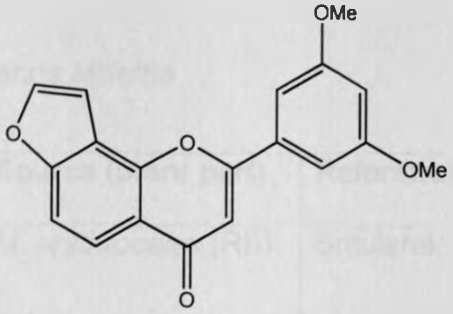

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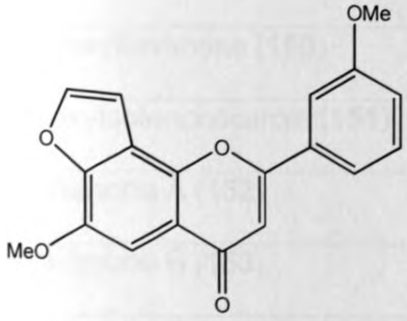

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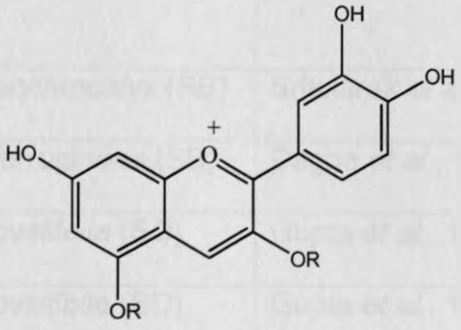
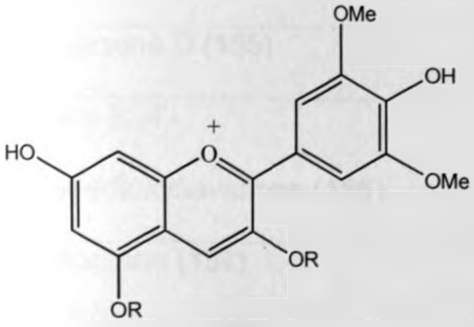
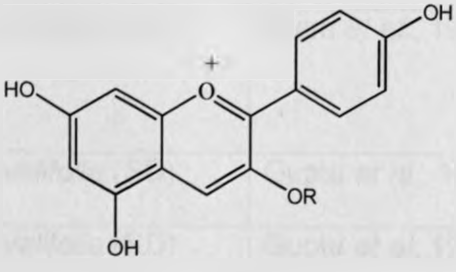


145 R=β-D-Glucose



146 R= β-D-Glucose



147 R=α-L-Rhamnose

2.6.1.3 FLAVANONES OF *MILLETTIA*

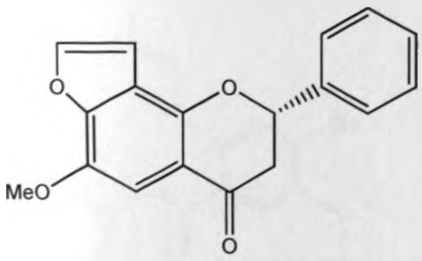
Most of the flavanones so far characterized from the genus *Millettia* are prenylated and lack oxygenation at C-5 position. This is considered to be typical of the flavonoids belonging to the Fabaceae family (Hagnaeuer and Grayer-Barkmeijer, 1993). It's worth to note that most of the known flavanones possess a (-)-(2S)- configuration.

Table 2.5: Flavanones reported from the genus *Millettia*

Flavanones	**Source (plant part)**	**Reference**
6-Methoxy-[7,8:2'',3'']furanoflavanone (**148**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002a
Ponganone (**149**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002a
7-Prenyloxyflavanone (**150**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002a
4'-Hydroxyisolonchocarpin (**151**)	*M. ferrugineae* (SB)	Dagne *et al.*, 1989
Ovaliflavanone A (**152**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1976a
Ovaliflavanone B (**153**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1976a
Ovaliflavanone C (**154**)	*M. ovalifolia* (SD)	Islam *et al.*, 1980
Ovaliflavanone D (**155**)	*M. ovalifolia* (SD)	Islam *et al.*, 1980
7-Hydroxy-3',4'-methylenedioxyflavanone (**156**)	*M. ovalifolia* (SD)	Islam *et al.*, 1980
Ovalichromene (**157**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1976b
Ovalichromene A (**158**)	*M. ovalifolia* (SD)	Gupta *et al*, 1976b

Table 2.5: Flavanones reported from the genus *Millettia* cont....

Flavanones	Source (plant part)	Reference
Ovalichromene B (**159**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1976b
Milletenin A (**160**)	*M. ovalifolia* (LF)	Khan *et al.*, 1974
Milletenin B (**161**)	*M. ovalifolia* (LF)	Khan *et al.*, 1974
Isolonchocarpin (**162**)	*M. ovalifolia (SD)*	Krishnamuruti *et al.*, 1987
Sophoranone (**163**)	*M. pulchra (AP)*	Baruah *et al.*, 1984
(-)-(2*S*)-6,3',4'-trimethoxy-[2",3":7,8]-furanoflavanone (**164**)	*M. erythrocalyx* (SD)	Sritularak *et al.*, 2006
Eriodictyol (**165**)	*M. duchesnei* (AP)	François *et al.*, 2008



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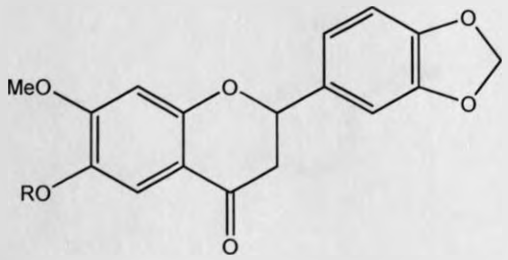
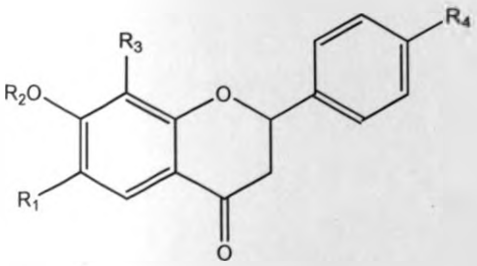
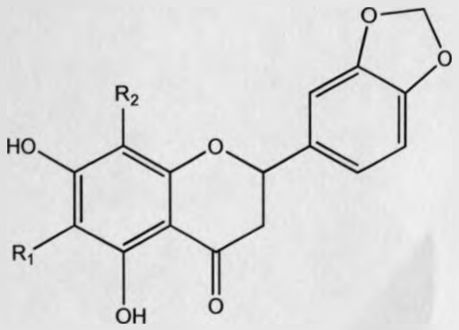


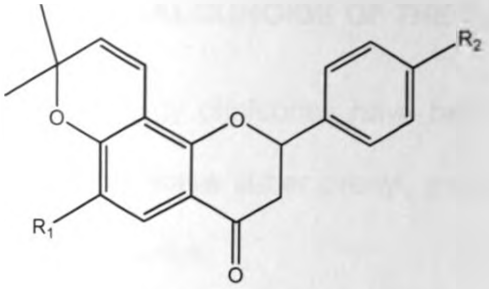
149 R=Prenyl



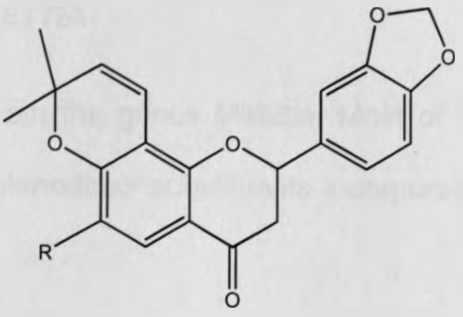
	R_1	R_2	R_3	R_4
150	H	Prenyl	H	H
152	Prenyl	H	Prenyl	H
153	H	H	Prenyl	H



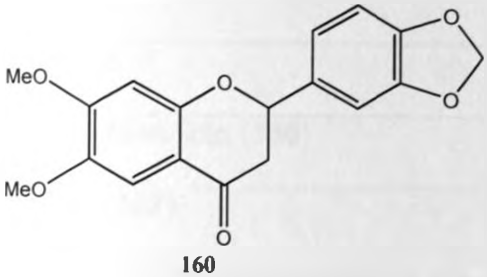
	154	**155**	**156**
R_1	H	Prenyl	H
R_2	Prenyl	Prenyl	H



	151	157	162
R_1	H	Prenyl	H
R_2	OH	OH	H



	R
158	OMe
159	H



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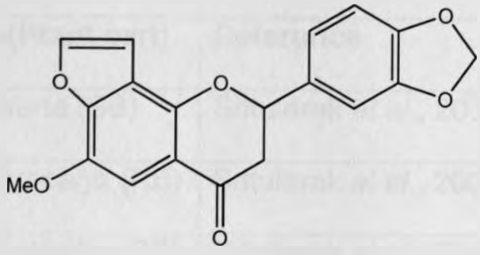

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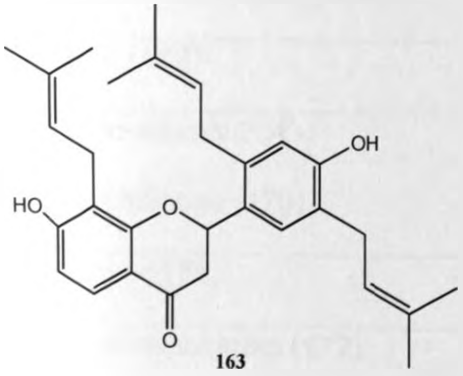

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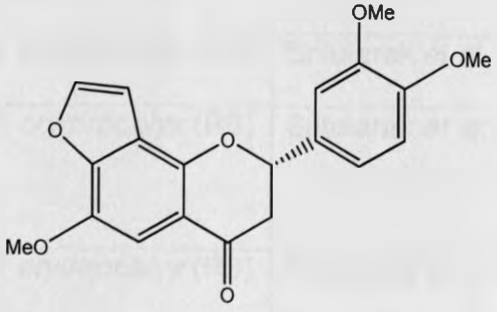

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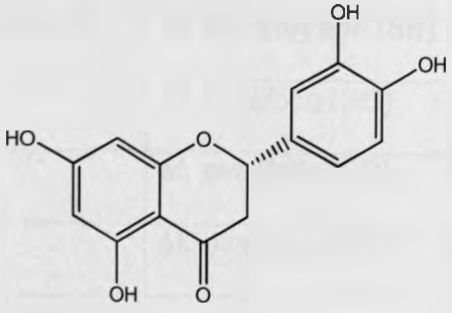


165

2.6.1.4 CHALCONOIDS OF THE GENUS *MILLETTIA*

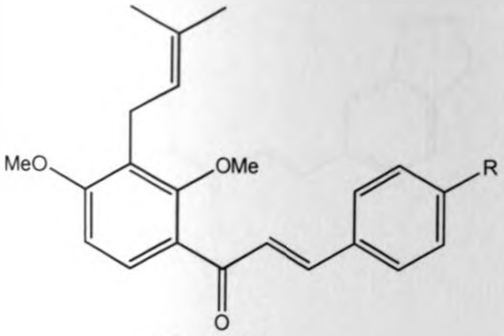
About twenty chalcones have been reported from the genus *Millettia*. Most of these chalcones have either prenyl, geranyl or methylenedioxy substituents incorporated in their structures.

Table 2.6: Chalcones of *Millettia* species

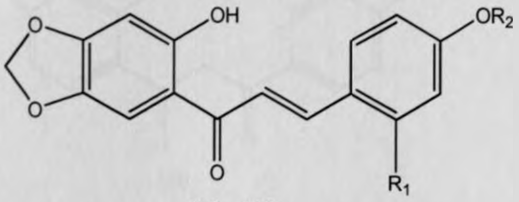
Chalcones	**Source(Plant part)**	**Reference**
4-Hydroxyderricidin (**166**)	*M. dielsiana* (SB)	Sritularak *et al.*, 2002a
Derricidin (**167**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002a
2'-Hydroxy-3,4-methylenedioxy-4'-γ,γ-dimethylallyloxychalcone (**168**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002a
Ponganone (**169**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002a
3,4-methylenedioxy-2',4'-dimethoxychalcone (**170**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002b
Purperenone (**171**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002b
4'-Hydroxylonchocarpin (**172**)	*M. ferruginea* (SB)	Dagne *et al.*, 1989
Dihydromilletenone, methylether (**173**)	*M. hemsleyana* (SB)	Mahmoud *et al.*, 1985
Dihydroisomilletenone,methylether(**174**)	*M. hemsleyana* (SB)	Mahmoud *et al.*, 1985
Ovalichalcone (**175**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1977a
Ovalichalcone A (**176**)	*M. ovalifolia* (SD)	Islam *et al.*, 1980
Ovalitenin A (**177**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1977b

Table 2.6: Chalcones of *Millettia* species cont....

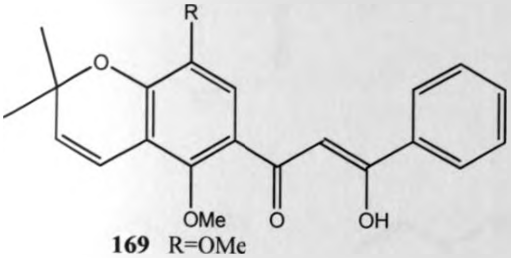
Chalcones	**Source(Plant part)**	**Reference**
Ovalitenin B (**178**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1977b
Ovalitenin C (**179**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1980
Ovalitenone (**180**)	*M. ovalifolia* (SD)	Gupta *et al.*, 1977b
Pongamol (**181**)	*M. ovalifolia* (RB)	Saxena *et al.*, 1987
Milletenone (**182**)	*M. ovalifolia* (SD)	Saxena *et al.*, 1987
2'-Hydroxy-4-methoxylonchocarpin (**183**)	*M. pachycarpa* (SD)	Singhal, 1983
4'-*O*-Geranylisoliquiritigenin (**184**)	*M. ferruginea* (RB) *M. griffoniana* (RB) *M. usaramensis* (SB)	Dagne *et al.*, 1990b Yankep *et al.*, 1997 Yenesew, 1997a
4'-Geranyloxy-a,4,2'-trihydroxydihydrochalcone (**185**)	*M. usaramensis* (SB)	Yenesew, 1997a
2'-Hydroxy-3,4-dimethoxy-[2",3":4',3']-furanochalcone (**186**)	*M. erythrocalyx* (SDP)	Sritularak and Kittisak, 2006
2',3-Dihydroxy-4-methoxy-4'-γ,γ-dimethylallyloxychalcone (**187**)	*M. erythrocalyx* (SDP)	Sritularak and Kittisak, 2006



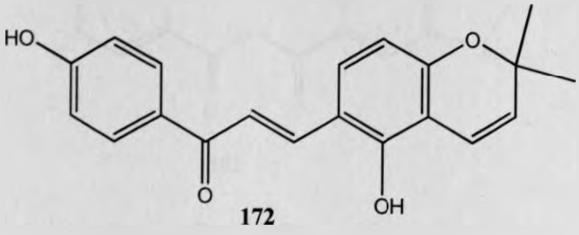
166 R=OH
167 R=H



	168	**170**
R_1	H	Prenyl
R_2	OMe	H



169 R=OMe
171 R=H



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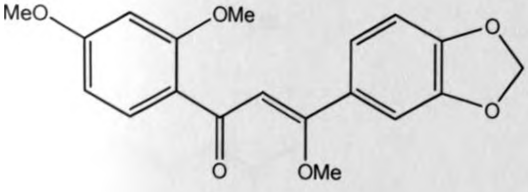

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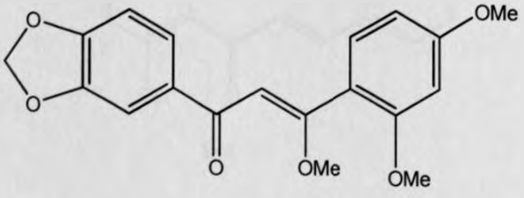

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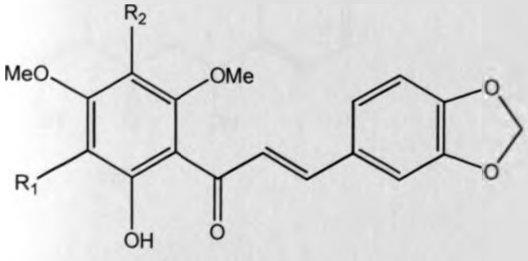
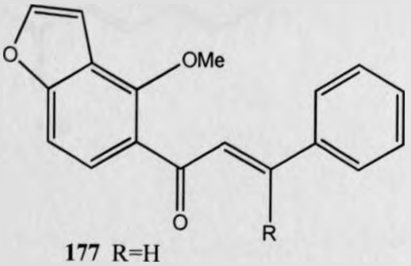
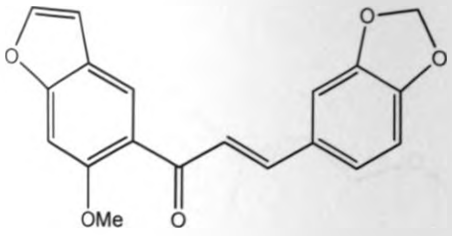


	R_1	R_2
175	H	Prenyl
176	Prenyl	H



177 R=H
178 R=OMe



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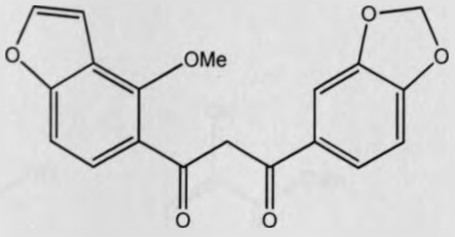

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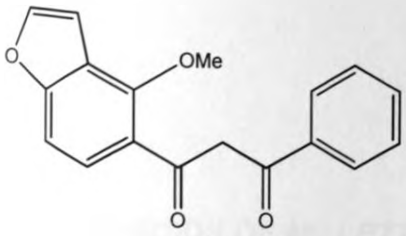

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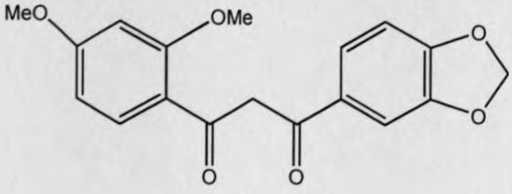

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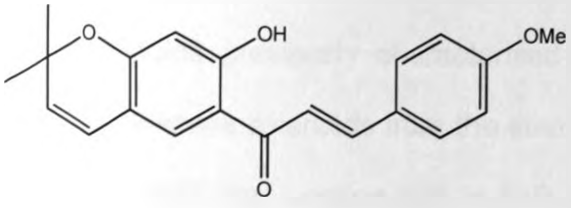

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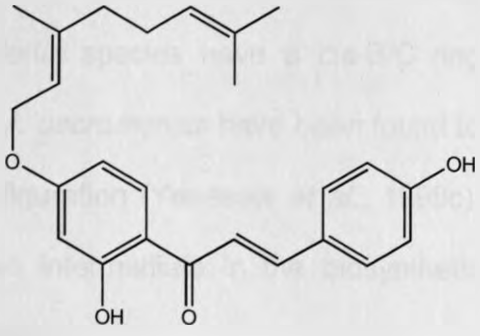

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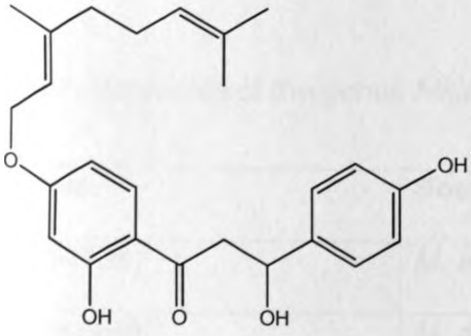

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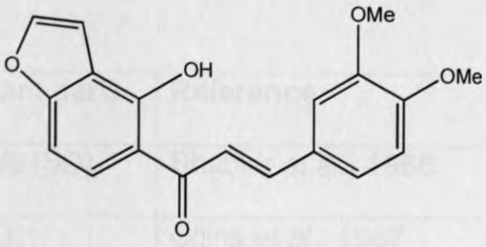

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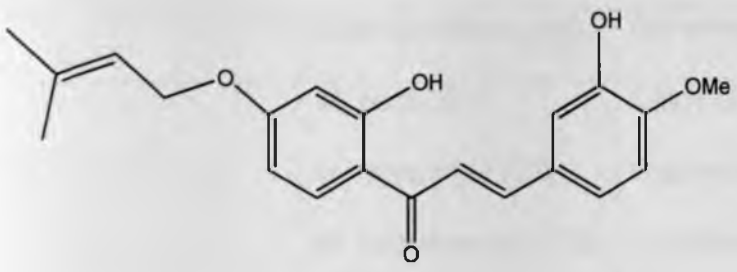


187

2.6.1.5 ROTENOIDS OF *MILLETTIA*

The presence of rotenoids in some *Millettia* species has been reported (Table 2.7). Most rotenoids previously characterised from *Millettia* species have a *cis*-B/C ring junction. However rotenoids from the stem bark of *M. usaramensis* have been found to have *trans*-B/C ring junction with a 6aR,12aS configuration (Yenesew *et al.*, 1998c). Rot-2'-enoic acid (**188**) has been shown to be an intermediate in the biosynthetic pathway of other rotenoids (Crombie *et al.*, 1979; 1982).

Table 2.7: Rotenoids of the genus *Millettia*

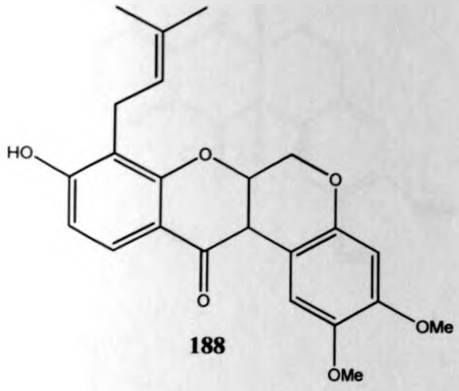
Rotenoids	**Source (plant part)**	**Reference**
Sumatrol (**189**)	*M. auriculata* (SD)	Shabbir *et al.*, 1968
Millettone (**190**)	*M. dura* (SD)	Ollins *et al.*, 1967
Millettosin (**191**)	*M. dura* (SD)	Ollins *et al.*, 1967
6a, 12a-Dehydromillettone (**192**)	*M. usaramensis* (SB)	Yenesew *et al.*, 2003
Stemonal (**193**)	*M. brandiasa* (LF)	Pancharoen *et al.*, 2008

Table 2.7: Rotenoids of the genus *Millettia* cont....

Rotenoid	Source (plant part)	Reference
Deguelin (**194**)	*M. dura* (SD)	Ollins *et al.*, 1967
	M. ferruginea (SD)	Dagne *et al.*, 1991
	M. usaramensis (SD)	Yenesew *et al.*, 1997b
	M. pachycarpa (SD)	Haoyu *et al.*, 2008
	M. taiwaniana	Ito *et al.*, 2004
Rotenone (**195**)	*M. dura* (SD)	Ollins *et al.*, 1967
	M. ferruginea (SD)	Dagne *et al.*, 1991
	M. pachycarpa (SD)	Singhal *et al.*, 1982
6a,12a-Dehydrodeguelin (**196**)	*M. dura* (SD)	Ollins *et al.*, 1967
	M. duchesnei (AP)	François *et al.*, 2008
	M. pachycarpa (SD)	Haoyu *et al.*, 2008
Tephrosin (**197**)	*M. dura* (SD)	Ollins *et al.*, 1967
	M. ferruginea (SD)	Dagne *et al.*, 1991
	M. usaramensis (SD)	Yenesew *et al.*, 1997b
	M. pachycarpa (SD)	Haoyu *et al.*, 2008
	M. griffoniana (SD)	Ngamga *et al.*, 2005
	M. taiwaniana	Ito *et al.*, 2004
Griffonianone A (**198**)	*M. griffoniana* (RB)	Yankep *et al.*, 2001
12a-Hydroxyrotenone (**199**)	*M. dura* (SD)	Ollins *et al.*, 1967
Rot-2'-enoic acid (**200**)	*M. pachycarpa* (SD)	Singhal *et al.*, 1982

Table 2.7: Rotenoids of the genus *Millettia* Cont....

12a-Hydroxy Rot-2'-enoic acid, *cis* (**201**)	*M. pachycarpa* (SD)	Singhal *et al.*, 1982
12a-Epimilletosin (**202**)	*M. usaramensis*(SB)	Yenesew *et al.*, 1998c
(+)-Usararotenoid A (**203**)	*M. usaramensis*(SB)	Yenesew *et al.*, 1998c
(+)-12-Dihydrousararotenoid A (**204**)	*M. usaramensis*(SB)	Yenesew *et al.*, 1998c
(+)-Usararotenoid B (**205**)	*M .usaramensis* (SB)	Yenesew *et al.*, 1998c
Elliptol (**206**)	*M. duchesnei* (AP)	François *et al.*, 2008
12-Deoxo-12a-methoxyelliptone (**207**)	*M. duchesnei* (AP)	François *et al.*, 2008
6-methoxy-6a,12a-dehydrodeguelin (**208**)	*M. duchesnei* (AP)	François *et* al., 2008
a-Toxicarol (**209**)	*M. brandisiana* (LF) *M. taiwaniana* (ST)	Pancharoen *et al.*, 2008 Ito *et al.*, 2004
Sermundone (**210**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
12a-Hydroxy-a-toxicarol (**211**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
6-Deoxyclitoriacetal (**212**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
6a,12a-Dehydro-a-toxicarol (**213**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
6a,12a-Dehydrosermundone (**214**)	*M. brandisiana* (LF)	Pancharoen *et al.*, 2008
6-Oxo-6a,12a-dehydrodeguelin (**215**)	*M. duchesnei* (AP)	François *et al.*, 2008
Elliptone (**216**)	*M. duchesnei* (AP)	François *et al.*, 2008
12a-Hydroxyelliptone (**217**)	*M. duchesnei* (AP)	François *et al.*, 2008
Usararotenoid C (**218**)	*M. usaramensis* (SB)	Yenesew *et al.*, 2003



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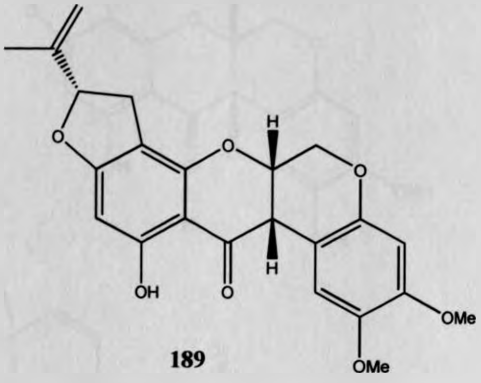

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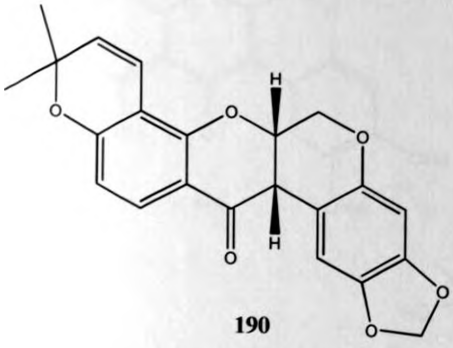

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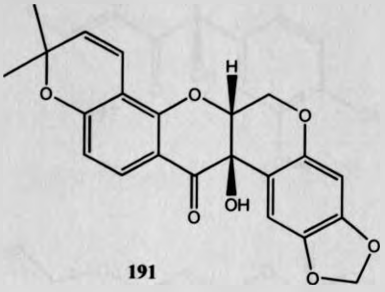

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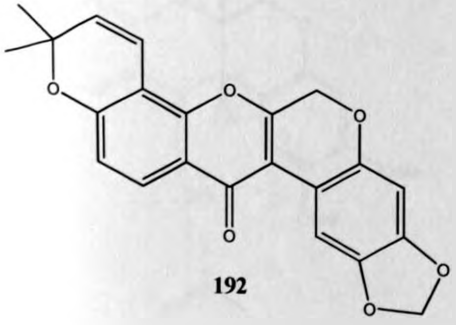

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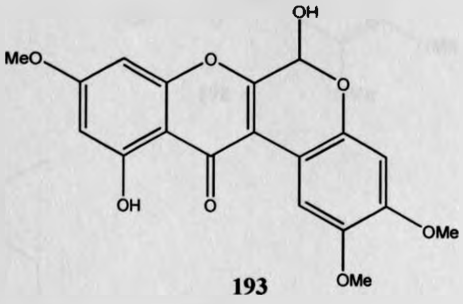

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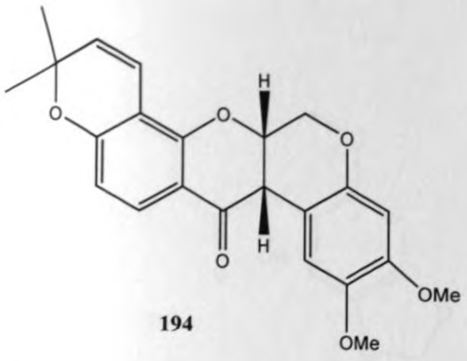

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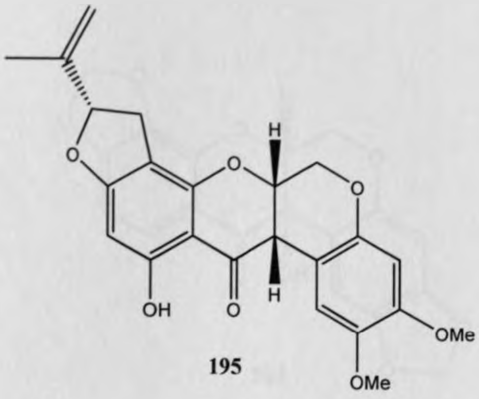

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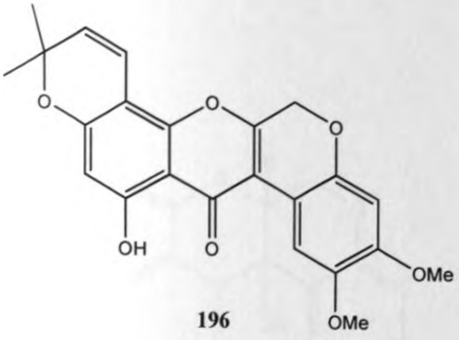

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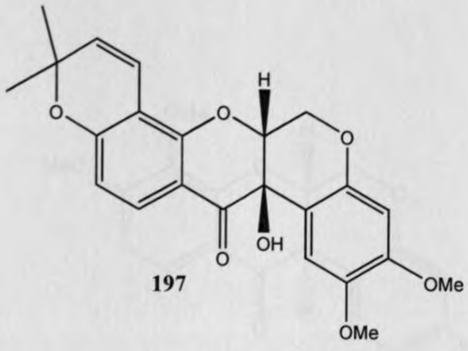

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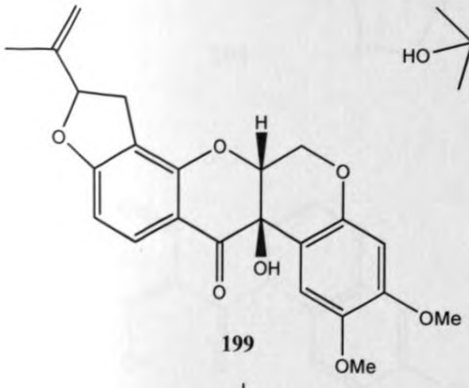

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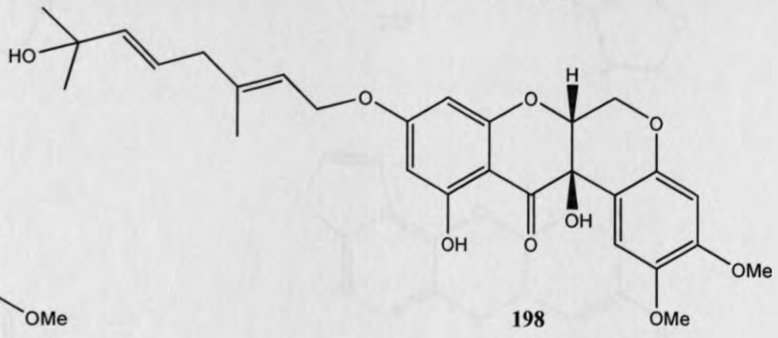

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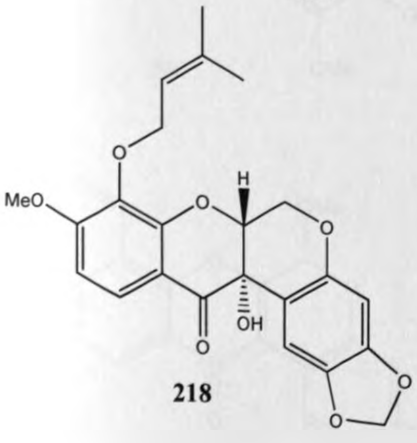

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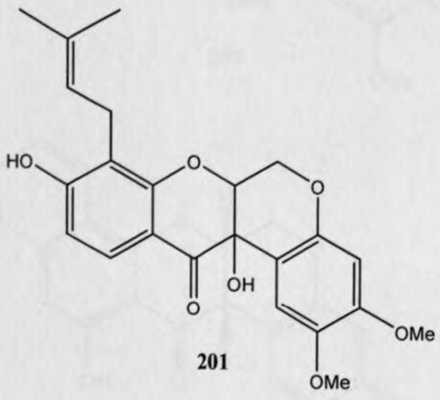

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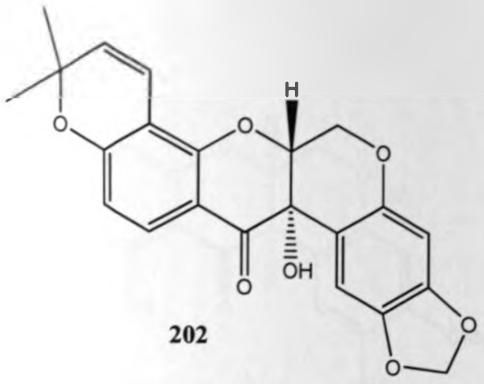

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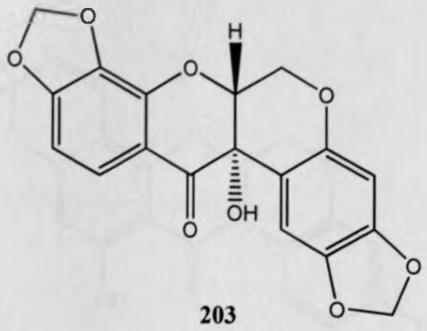

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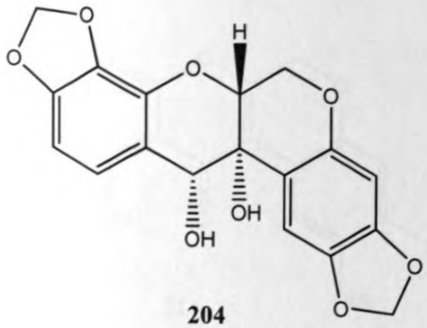

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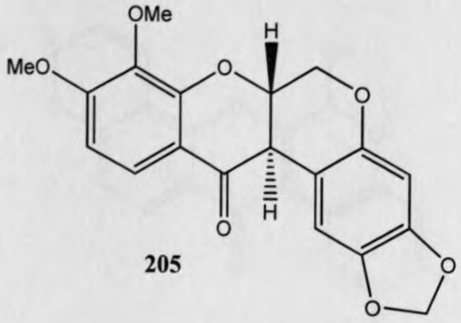

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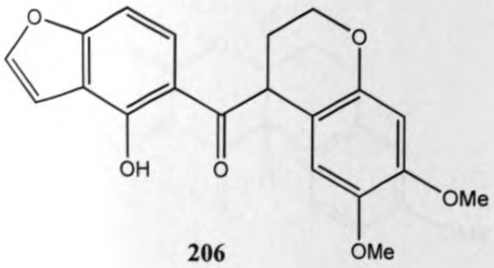

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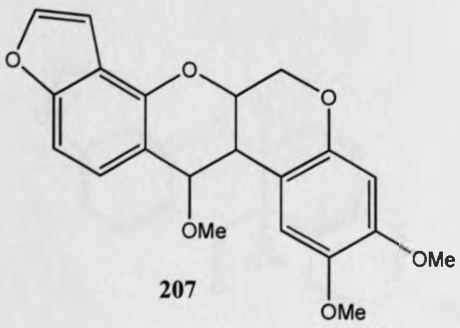

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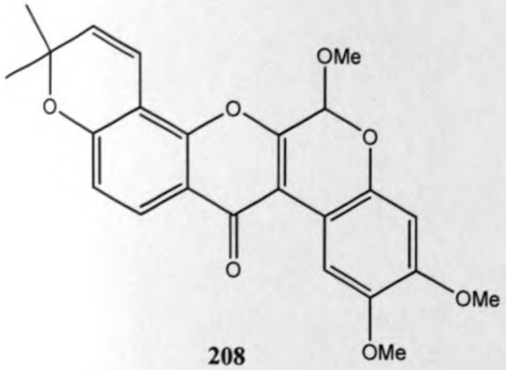

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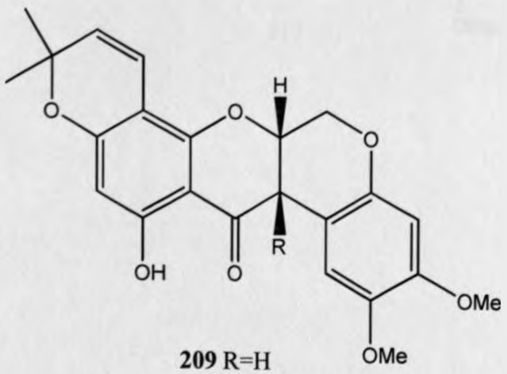
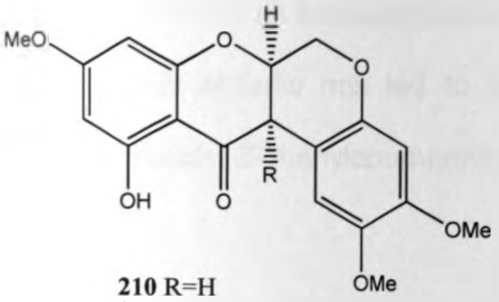
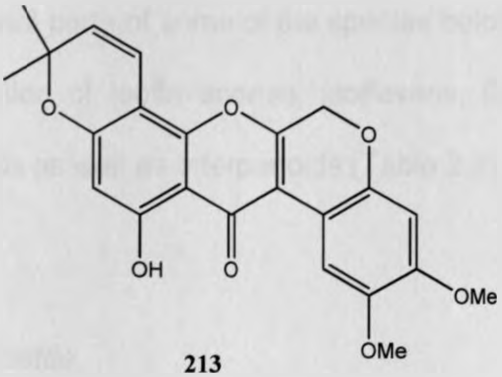


209 R=H
211 R=OH



210 R=H
212 R=OH



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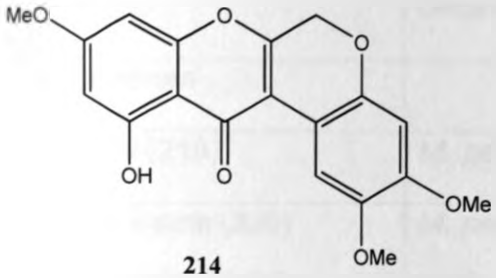

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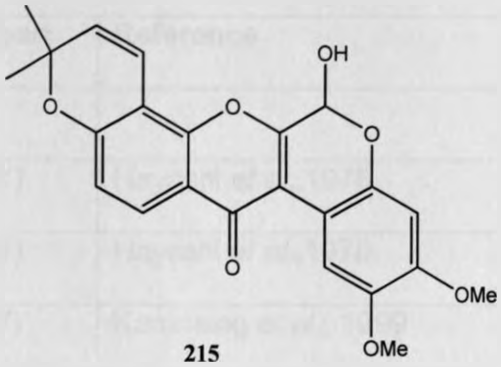

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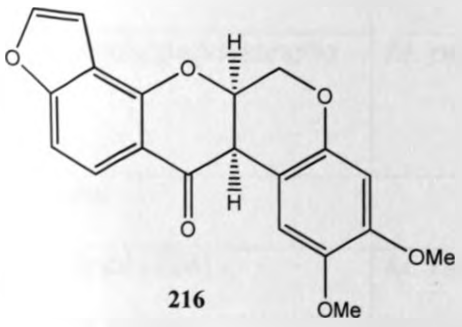

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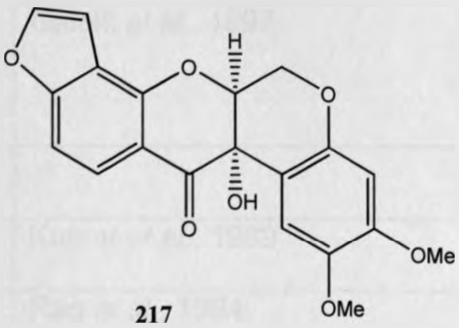


217

2.6.1.6 MINOR COMPOUNDS FROM THE GENUS *MILLETTIA*

Work done mostly on the heart wood and aerial parts of some of the species belonging to the genus *Millettia* has led to the isolation of isoflavanones, isoflavans, flavan, pterocarpanoids, 3-phenylcoumarins, alkaloids as well as triterpenoids (Table 2.8).

Table 2.8: Minor compounds of the genus *Millettia*

Compound	Source (plant part	Reference
Isoflavanones		
Pendulone (**219**)	*M. pendula* (HW)	Hayashi *et al.*,1978
Claussequinone (**220**)	*M. pendula* (HW)	Hayashi *et al.*,1978
Laurentiquinone (**221**)	*M. laurentii* (HW)	Kamnaing *et al.*, 1999
Pervilleanone (**222**)	*M. pervilleana* (RB)	Galeffi *et al.*, 1997
3'-O-Demethylpervilleanone (**223**)	*M. pervilleana* (RB)	Galeffi *et al.*, 1997
Isoflavans		
Cyclomillinol (**224**)	*M. racemosa* (HW)	Kumar *et al.*, 1989
Isomillinol B (**225**)	*M. racemosa* (HW)	Rao *et al.*, 1994
Laxifloran (**226**)	*M. racemosa* (HW)	Rao *et al.*, 1994
Millinol (**227**)	*M. racemosa* (HW)	Kumar *et al.*, 1989
Millinol B (**228**)	*M. racemosa* (HW)	Kumar *et al.*, 1989
Millinolol (**229**)	*M. racemosa* (HW)	Rao *et al.*, 1996

Table 2.8: Minor compounds of the genus *Millettia* cont....

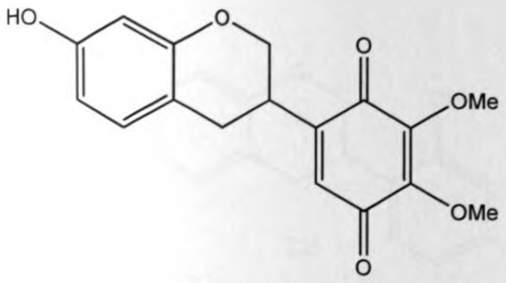
Compound	Source (plant part	Reference
Neomillinol (**230**)	*M. racemosa* (HW)	Rao *et al.*, 1996
Flavan		
2,5-Dimethoxy-4-hydroxy-(2'',3'':7,8)-furanoflavan (**231**)	*M. erythrocalyx* (RB)	Sritularak *et al.*, 2002b
Pterocarpanoids		
Flemichapparin B (**232**)	*M. ferruginea* (SB)	Dagne *et al.*, 1989
Emoroidocarpan (**233**)	*M. pervilleana* (RB)	Palazzino *et al.*, 2003
Pervilline (**234**)	*M. pervilleana* (RB)	Palazzino *et al.*, 2003
Pervillinine (**235**)	*M. pervilleana* (RB)	Palazzino *et al.*, 2003
Maackiain (**236**)	*M. pulchra* (AP) *M. Puguensis* (RT)	Baruah *et al.*, 1984 Kapingu *et al.*, 2006
6-Methoxyhomopterocarpin (**237**)	*M. pulchra* (AP)	Baruah *et al.*, 1984
6-Methoxypterocarpin (**238**)	*M. pulchra* (AP)	Baruah *et al.*, 1984
3-Phenylcoumarins		
Thonningine A (**239**)	*M. thonningii* (RW)	Khalid *et al.*, 1983
Thonningine B (**240**)	*M. thonningii* (RW)	Khalid *et al.*, 1983
Thonningine C (**241**)	*M. thonningii* (RW)	Asomaning *et al.*, 1995
Robustic acid (**242**)	*M. thonningii* (RW)	Khalid *et al.*, 1983
Pervilleanine (**243**)	*M. pervilleana* (RB)	Palazzino *et al.*, 2003

Table 2.8: Minor compounds of the genus *Millettia* Cont....

Compound	Source (plant part)	Reference
4-Hydroxy-5,6,7-trimethoxy-3-(3',4'-methylenedioxy) phenylcoumarin (**244**)	*M. griffoniana (RB)*	Yankep *et al.*, 1998
Alkaloids		
Millaurine (**245**)	*M. laurentii*	Ngamga *et al.*, 1993
O-acetylmillaurine (**246**)	*M. laurentii*	Ngamga *et al.*, 1993
5a,9a-dihydro-5a-hydroxymillaurine (**247**)	*M. laurentii*	Ngamga *et al.*, 1994
Millettonine (**248**)	*M. laurentii*	Kamnaing *et al.*, 1994
Steroids		
Stigmasterol (**249**)	*M. versicolor* (LF)	Ongoka *et al.*, 2008
24-methylenecycloartan-3ß-ol (**250**)	*M. versicolor*(LF)	Ongoka *et al.*, 2008
22,23-dihydrostigmasterol (**251**)	*M. versicolor* (LF)	Ongoka *et al.*, 2008
Stigmastan-3-ol (**252**)	*M. versicolor* (LF)	Ongoka *et al.*, 2008
ß-sitosterol (**253**)	*M. brandiasa* (LF)	Pancharoen *et al.*, 2008
3-*O*-[ß-D-glucopyranosyl]-sitosterol (**254**)	*M. brandiasa* (LF)	Pancharoen *et al.*, 2008
Tri terpenes		
Lupeol (**255**)	*M. versicolor* (LF) *M. puguensis* (RT)	Alphonse *et al.*, 2006 Kapingu *et al.*, 2006
Taraxasterol (**256**)	*M. versicolor* (LF)	Alphonse *et al.*, 2006

Table 2.8: Minor compounds of the genus *Millettia* cont....

Compound	Source (plant part)	Reference
ß-amyrin (**257**)	*M. versicolor* (LF)	Alphonse *et al.*, 2006
Others		
Ononin (**258**)	*M. nitida*	Xiang *et al.*, 2009



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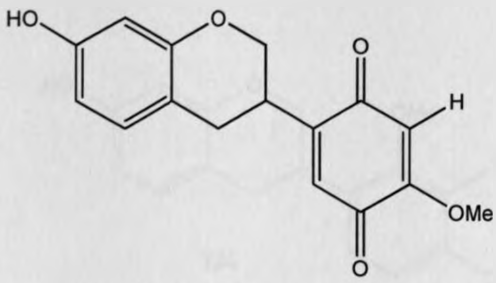

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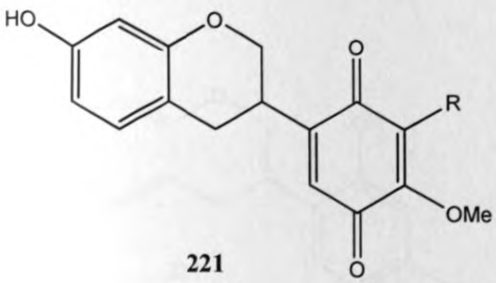

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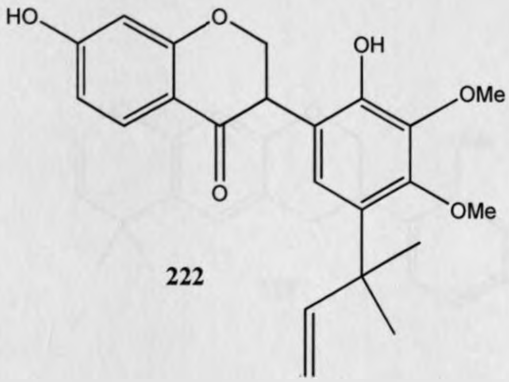

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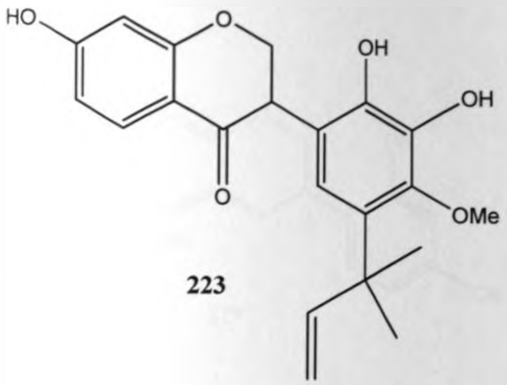

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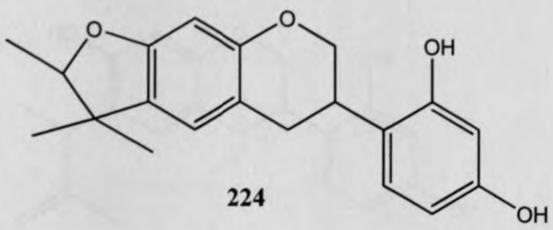

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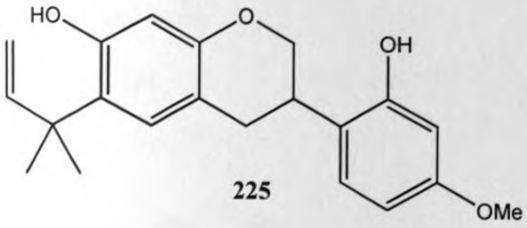

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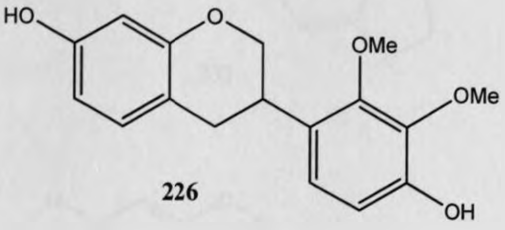

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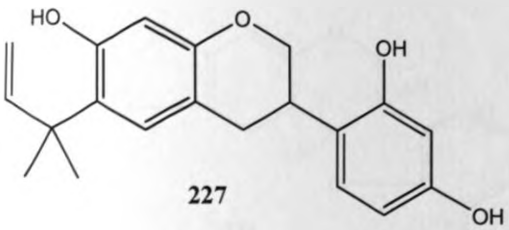

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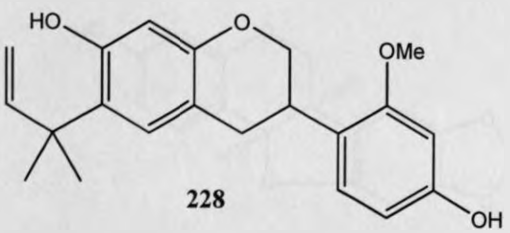

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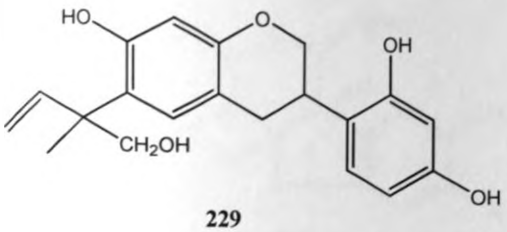

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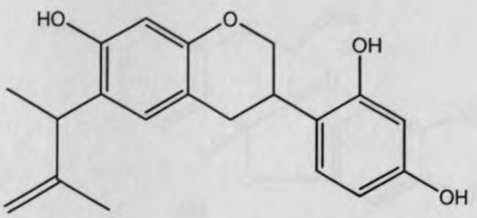

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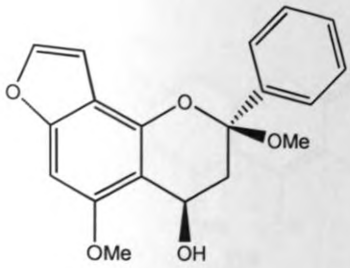

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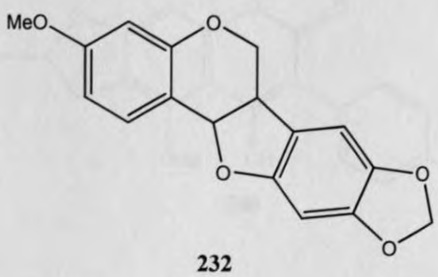

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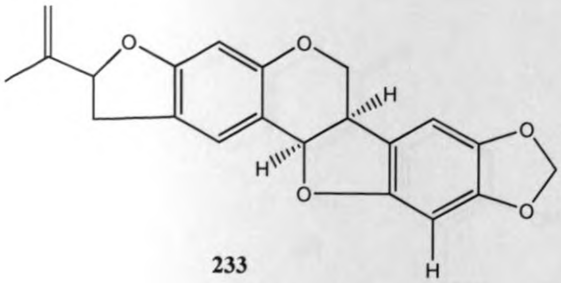

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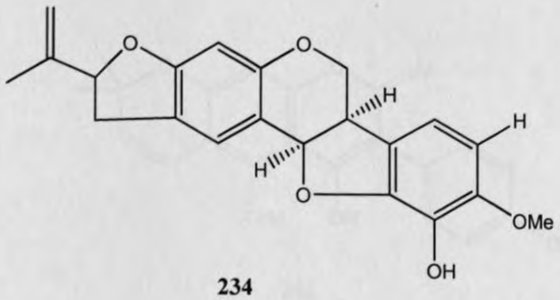

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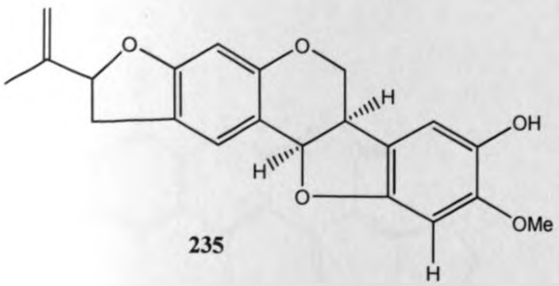

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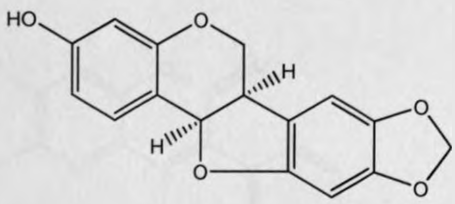

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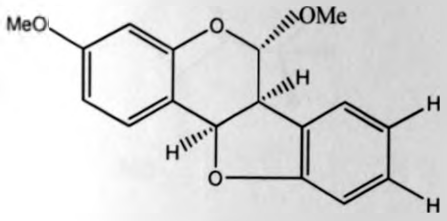

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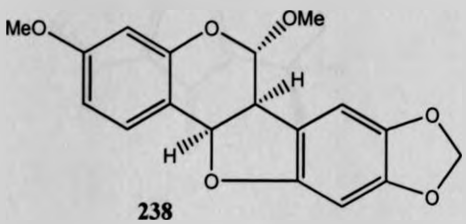

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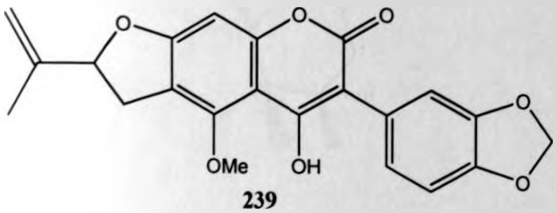

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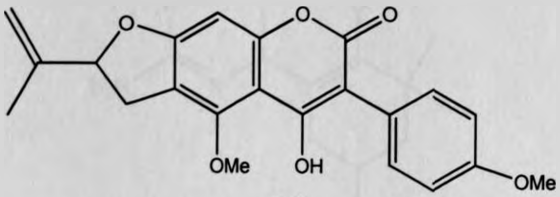

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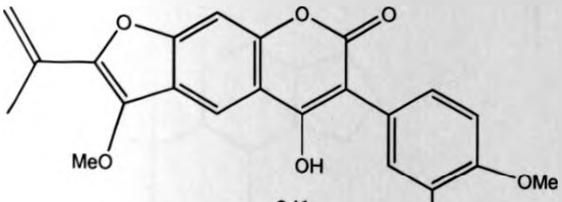

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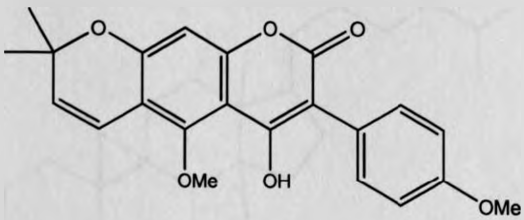

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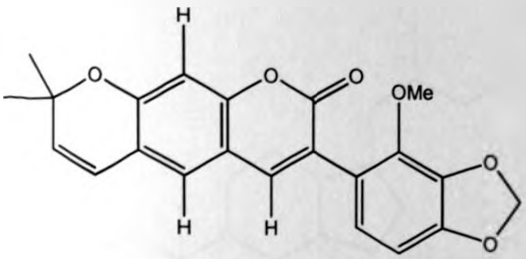

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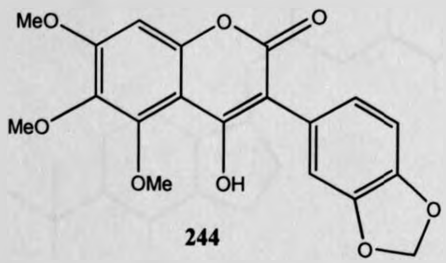

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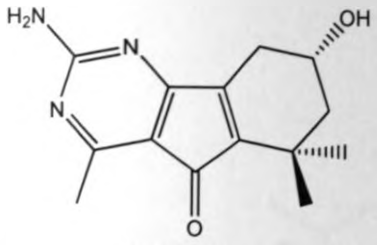

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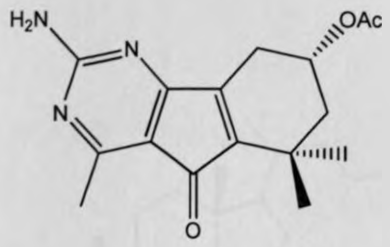

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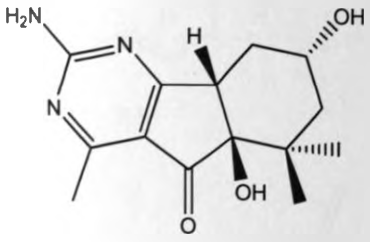

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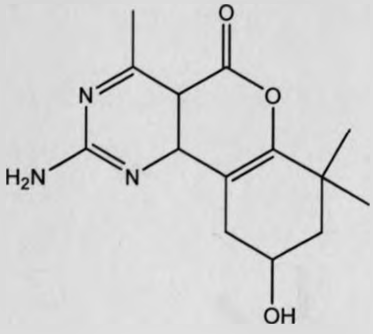

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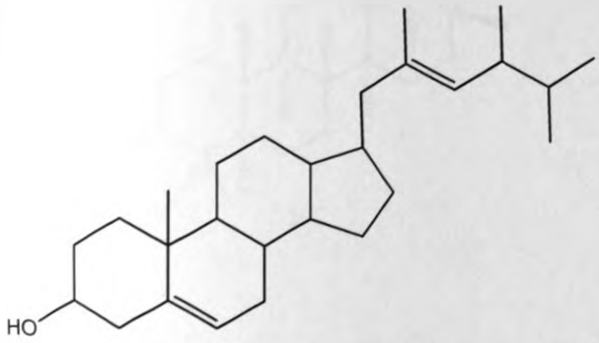

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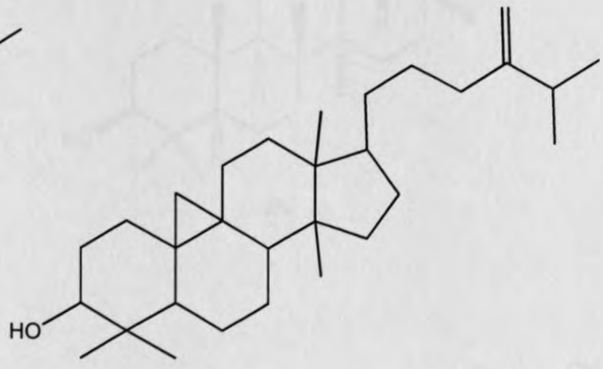

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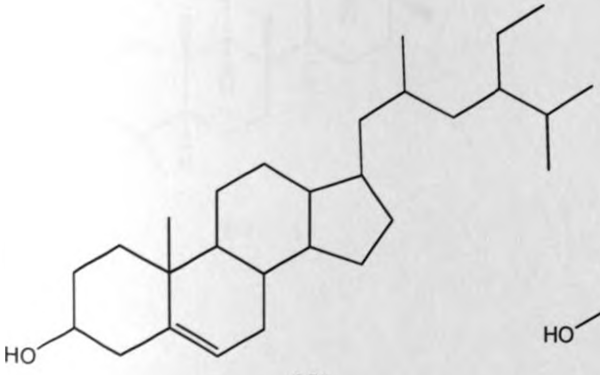

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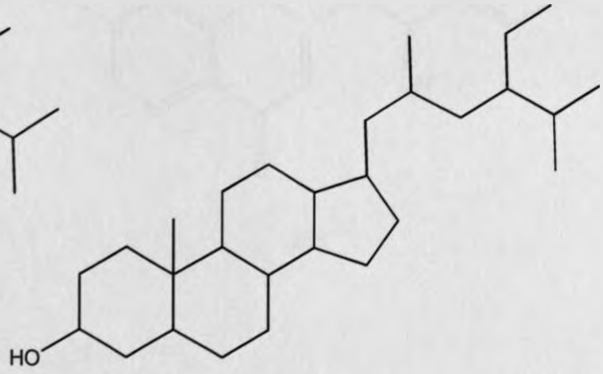

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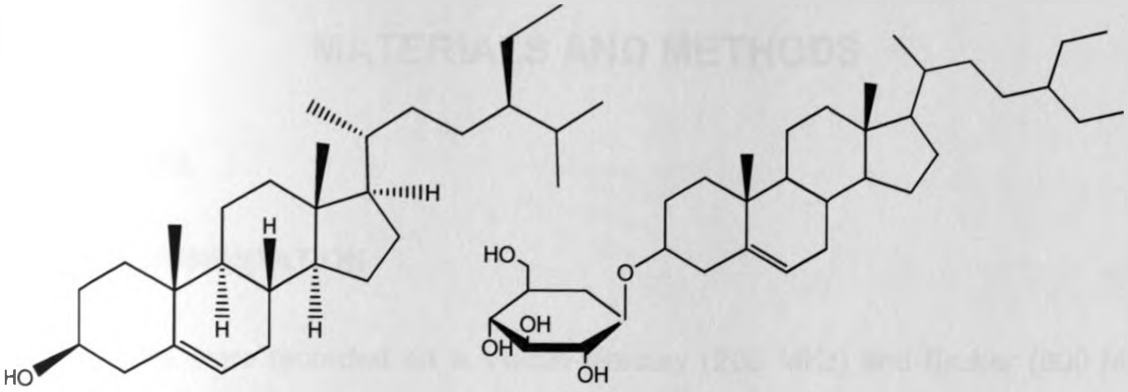

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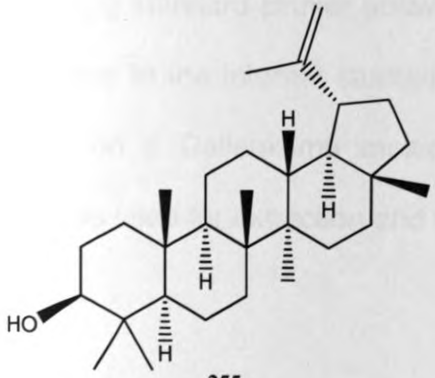

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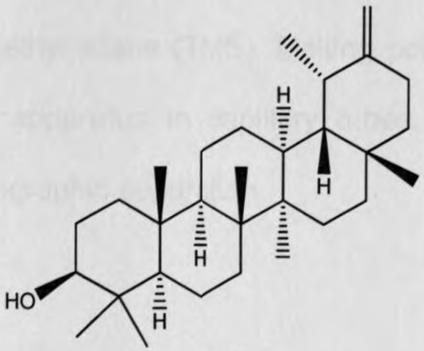

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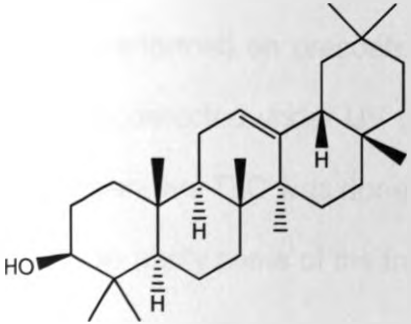

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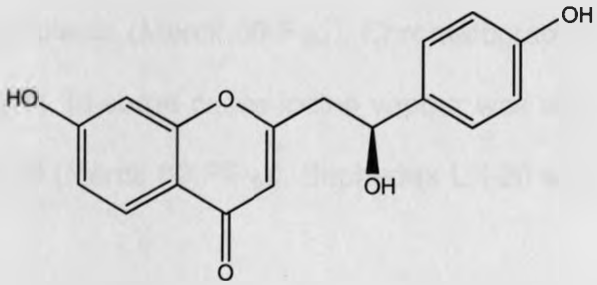

258

CHAPTER THREE

MATERIALS AND METHODS

3.1 GENERAL

3.1.1 INSTRUMENTATION

NMR spectra were recorded on a Varian-Mercury (200 MHz) and Bruker (600 MHz) instruments. Two dimensional experiments (COSY, HMQC and HMBC spectra) were acquired using standard Bruker software. Chemical shifts were measured in ppm in d values relative to the internal standard tetramethyl silane (TMS). Melting points were determined on a Gallenkamp melting point apparatus in capillary tubes. Distilled solvents were used for extraction and chromatographic separation.

3.1.2 CHROMATOGRAPHIC CONDITION

Column chromatography was done on Silica gel (Merck 60, 70-230 mesh). Analytical TLC was performed on precoated Silica gel plates (Merck 60 F_{254}). Chromatographic zones were detected under UV (254 nm) light. In some cases iodine vapour was also used. Preparative TLC was done on Silica gel (Merck 60 PF_{254}). Sephadex LH-20 was also used to purify some of the fractions.

3.1.3 TLC SOLVENT SYSTEM

Hexane/ ethylacetate; 4:1

Dichloromethane (100%)

Dichloromethane/ ethylacetate; 9:1

3.2 PLANT MATERIALS

The root bark of *Millettia usaramensis* subspecies *usaramensis* was collected in Jadini forest, Coast province in February 2008. The plant was identified by Mr S. G. Mathenge of the Herbarium, Botany Department, University of Nairobi where voucher specimen was deposited.

3.3 EXTRACTION AND ISOLATION OF COMPOUNDS

3.3.1 ISOLATION OF COMPOUNDS FROM THE ROOT BARK OF *M. USARAMENSIS*

Dried and ground root bark (1.5 Kg) of *Millettia usaramensis* was extracted with dichloromethane/methanol (1:1) by cold percolation. The extract was evaporated under reduced pressure to yield 60 g of crude extract. About 40 g of the extract was subjected to column chromatography on Silica gel (300 g) eluting with hexane containing increasing amounts of ethylacetate.

The fraction eluting at 2% EtOAc in n-hexane was subjected to MPLC using n-hexane and increasing amounts of dichloromethane to yield 4-geranyloxycinnamyl alcohol (**5**, 82 mg). The fraction eluted with 3% EtOAc in n-hexane was purified by crystallization from methanol to yield 12a-epimillettosin (**8**, 40 mg) (Yenesew *et al.*, 1998c). The mother liquor of fraction eluting with 3% EtOAc in n-hexane was subjected to column chromatography using n-hexane containing increasing amounts of ethyl acetate. The fractions obtained were further separated on Sephadex LH-20 column (eluent CH_2Cl_2/MeOH, 1:1) yielding 12-dihydrousararotenoid-C (**3**, 19 mg). The fraction which was eluted with 4% EtOAc in n-hexane was purified by crystallization from methanol to give crystals of usararotenoid-A (**6**, 30 mg) (Yenesew *et al.*, 1998c). The Mother liquor of fraction eluted with 4% EtOAc in n-hexane was subjected to MPLC using n-hexane containing increasing amounts of dichloromethane. This gave 4'-*O*-geranylisoliquiritigenin (**10**, 95 mg) (Yenesew et al., 1998c), 4-*O*-geranylisoliquiritigenin (**1**, 68 mg) and a mixture of compound **8** and usararotenoid-C (**9**).

The separation of 12-dihydrousararotenoid-B (**2**, 15 mg) was achieved after subjecting the fractions eluted with 8% EtOAc in n-hexane to column chromatography on Sephadex LH-20 (eluent; CH_2Cl_2/MeOH, 1:1). The fractions which were eluted with 9% and 10% EtOAc in n-hexane were combined and subjected to MPLC separation using n-hexane containing increasing amounts of dichloromethane to give 4'-geranyloxy-7-hydroxyflavanone (**4**, 31 mg). The fraction eluted with 11% EtOAc in n-hexane after purification by crystallization (methanol) afforded 12-dihydrousararotenoid-A (**7**, 80 mg) (Yenesew *et al.*, 1998c).

3.4 BIOLOGICAL TESTING

3.4.1 ANTIPLASMODIAL TEST

The 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 different strains of *Plasmodium falciparum* parasites were cultured that are commonly used in drug sensitivity assays. The chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) strains were grown in a continuous culture supplemented with mixed gas (90% nitrogen, 5% oxygen and 5% carbondoixide), 10% human serum, and 6% hematocrit of A+ red blood cells. Once cultures reach a parasitemia of 3% with at least a 70% ring developmental stage present, parasites were transferred to a 96 well microtiter plate with wells pre-coated with compound. 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 (TomTec) onto filter paper and washed to eliminate unincorporated isotope. Filters were measured for activity in a microtiter plate scintillation counter (Wallac). Data from the counter was imported into Microsoft Excel spreadsheet, which was then imported into an Oracle database/program to determine IC_{50} values.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF ISOLATED COMPOUNDS

4.1.1 COMPOUNDS FROM *MILLETTIA USARAMENSIS*

The air dried and ground root bark of *M. usaramensis* was extracted with CH_2Cl_2/MeOH (1:1). The crude extract obtained was subjected to column chromatography using n-hexane containing increasing amounts of ethylacetate as the eluent. From this ten compounds were isolated. The identification and characterization of these compounds is discussed below.

4.1.1.1 4-*O*-GERANYLISOLIQUIRITIGENIN (1)

Compound **1** was isolated as a yellow oil with an R_f value of 0.52 (5% EtOAc in CH_2Cl_2). HRMS analysis of compound **1** showed a molecular ion peak at *m/z* 392.1968 corresponding to the molecular formula $C_{25}H_{28}0_4$. The occurrence of two *trans*–oriented olefinic protons at d 7.48 (*d*, *J* = 15.6 Hz) and 7.85 (*d*, *J* = 15.6 Hz) in the ^{1}H NMR spectrum was suggestive of a chalcone moiety. This was further supported by the presence of a peak at d_C 192.3 for carbonyl, d_C 119.3 for C-a and d_C 144.7 for C-ß in the ^{13}C NMR spectrum. The fragment ion at *m/z* 137 and *m/z* 255 in the mass spectrum suggested the presence of a carbonyl group. The ^{1}H NMR spectrum revealed a chelated hydroxyl proton at d 13.51 which places an hydroxyl group at the C-2' position of ring B. An ABX pattern with doublets at d 6.40 (*J* = 2.4 Hz) and 7.83 (*J* = 8.4

Hz) and a double doublet at d 6.45 (J = 8.4, 2.4 Hz) indicating a C-2' (with OH) and C-4' substituted ring B. The occurrence of a AA'XX' spin system at d 6.95 (d, J = 8.4 Hz) and 7.62 (d, J = 8.4 Hz), confirms that ring A is substituted at C-4.

The ^{1}H NMR further displayed the presence of three methyl groups (at d 1.61, 1.67 and 1.75), three methylenes (of which two appear at d 2.07-2.15 as a multiplet and the other as a doublet at d 4.60 (J = 6.6 Hz)) and two olefinic protons (centred at d 5.10 (m) and 5.47 (t, J = 6.6 Hz)), suggesting the presence of either a geranyl or neryl substituent. It has been shown by Kozawa *et al.*, (1977), that ^{13}C NMR data, particularly chemical shifts values of C-10" and C-4", aid in distinguishing a geranyl from a neryl side chain. The chemical shifts at d_C 16.7 for C-10" and 39.7 for C-4" is in agreement with a geranyl side chain in compound **1**. The attachment of the geranyl group on the oxygen at C-4 was confirmed by a NOESY experiment which indicated interaction between the methylene protons at C-1" and H-3/H-5. This was further supported by an HMBC study which showed a ^3J correlation between H-1" and C-4. On the basis of these evidences the compound was identified as 4-*O*-geranylisoliquiritigenin (**1**), which is a new compound.

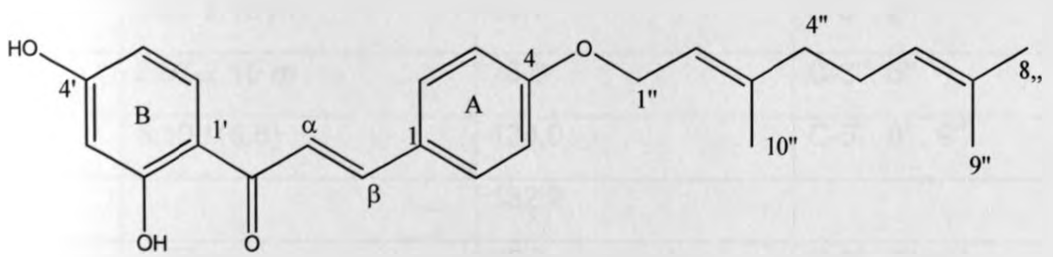

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Table 4.1: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **1** (CD_2Cl_2)

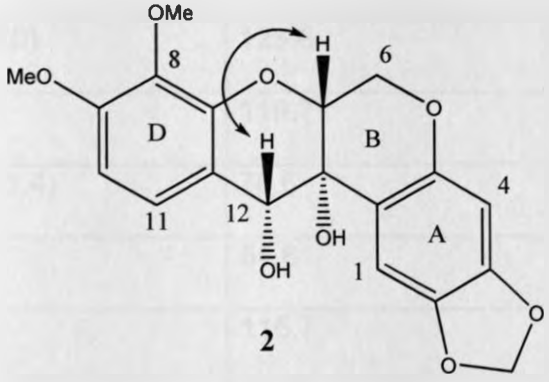
Position	d_H (*J* in Hz)	d_C	HMBC
1		127.6	
2	7.62 *d* (8.4)	130.7	C- ß, 4, 6
3	6.95 *d* (8.4)	115.4	C-1, 4
4		161.6	
5	6.95 *d* (8.4)	115.4	C-1, 4
6	7.62 *d* (8.4)	130.7	C- ß, 2, 4
1'		114.7	
2'-OH	13.51 *s*		C-1', 2', 3'
C-2'		163.2	
3'	6.40 *d* (2.4)	103.7	C-1', 2', 4', 5'
4'		166.6	
5'	6.45 *dd* (8.4, 2.4)	107.9	C-1', 4'
6'	7.83 *d* (8.4)	132.1	C-1', 2', 3', 4', 5'
H-a	7.48 *d* (15.6)	119.3	C- ß, 1
H-ß	7.85 *d* (15.6)	144.7	C-2, 6
C=O		192.3	
1"	4.60 *d* (6.6)	65.4	C-4, 2", 3"
2"	5.47 *t* (6.6)	117.9	
3"		141.9	
4"	2.07-2.15 *m*	39.7	C-3", 2"
5"	2.07-2.15 *m*	26.6	C-3", 6"
6"	5.10 *t* (6.6)	124.0	C-5", 8", 9"
7"		132.2	
8"	1.67 *s*	25.7	C-6", 7", 9"
9"	1.61 *s*	17.7	C-6", 7", 8"
10"	1.75 *s*	16.7	C-2", 3", 4"

4.1.1.2 12-DIHYDROUSARAROTENOID-B (2)

Compound **2** was isolated as colourless amorphous solid with a melting point greater than 215°C. The HRMS mass spectrum showed a molecular ion peak at *m/z* 374.0999 corresponding to the molecular formula of $C_{19}H_{18}O_8$. In the ^1H NMR spectrum, the occurrence of an ABX spin system centred at d 4.28 (*dd*, *J* = 10.8, 4.8 Hz), 4.34 (*dd*, *J* = 11.4, 9.6 Hz) and 4.39 (*dd*, *J* = 10.2, 4.8 Hz) for 6a, 6a and 6ß protons respectively, indicated that compound **2** is a 12a-hydroxyrotenoid (Yenesew *et al.*, 1998c). Furthermore, the presence of two methoxyl (d_H 3.79 and 3.84, d_C 60.9 and 56.3) and a methylenedioxy (d_H 5.92, d_C 101.9) substituents were evident from NMR. The ^{13}C NMR did not show a carbonyl signal as in natural rotenoids. However, the appearance of a doublet at d_H 4.89 (*J* = 11.4 Hz) for H-12 and d_C 70.6 for C-12 was consistent with the occurrence of an oxymethine at C-12. In the COSY spectrum, the doublet at d_H 4.89 showed correlation with a doublet at d_H 2.78 (*J* = 11.4 Hz). Upon addition of drops of D_2O, the signal at d_H 2.78 disappeared while the doublet at d_H 4.89 collapsed into a singlet, which is consistent with a hydroxyl group at C-12.

The presence of two *ortho*-coupled aromatic protons appearing at d 7.27 and 6.68 (*d*, *J* = 9.0 Hz), in the ^1H NMR spectrum, would place either the methylenedioxy or the methoxyl groups at C-8/9. However the HMBC experiment showed ^3J correlation of the methoxyl protons with C-8 and C-9 clearly placing the methoxyl groups at these carbon atoms. The ^1H NMR also showed the presence of two isolated aromatic protons at d_H 7.77$_{\alpha}$(*s*) for H-1 and 6.39 (*s*) for H-4, allowing the placement of the methylenedioxy group at C-2/C-3. This is supported by the occurrence of a fragment ion at *m/z* 192 in the mass spectrum, indicating the placement of the methylenedioxy group in ring-A (

Yenesew *et al.*, 1998). H-1 is strongly deshielded, indicating a *trans*-B/C ring junction (Oberholser *et al.*, 1974; Dewick, 1994, Yenesew *et al.*, 1997, 2003). Furthermore, the large coupling constant between H-6a and one of the C-6 protons (J = 11.4 Hz) was again indicative of a 1,2-*trans*-diaxial relationship. The NOESY spectrum showed NOE interaction between H-6a and H-12, requiring a 1,3-diaxial relationship, hence the relative orientation of H-12 should be ß as in H-6a. This new compound was therefore identified as the 12-dihydro derivative of usararotenoid-B (Yenesew et al., 1997) for which the trivial name 12-dihydrousararotenoid-B (**2**) is suggested.



2

Table 4.2: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **2** (CD_2Cl_2)

Position	d_H (J in Hz)	d_C	HMBC
1	7.77 *s*	107.2	C-2, 3, 4a, 12a, 12b
2		142.7	
3		147.4	
4	6.39 *s*	98.4	C-2, 4a, 12b

Table 4.2: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **2** cont....

Position	d_H (*J* in Hz)	d_C	HMBC
4a		149.4	
6a	4.34 *dd* (11.4, 9.6)	62.6	C-12, 12a
6ß	4.39 *dd* (10.2, 4.8)		
6a	4.28 *dd* (10.8, 4.8)	73.4	C-12a
7a		149.9	
8		136.5	
9		153.3	
10	6.68 *d* (9.0)	106.6	C-8, 9, 11a
11	7.27 *d* (9.0)	123.6	C-9, 12
11a		119.7	
12	4.89 *d* (11.4)	70.6	C-11, 11a, 12b
12a		64.6	
12b		115.7	
8-OMe	3.79 *s*	60.9	C-8
9-OMe	3.84 *s*	56.3	C-9
2-OCH_2O-3	5.92 *d* (1.2)	101.9	C-2, 3
	5.93 *d* (1.2)		
12-OH	2.78 *d* (11.4)		C-12, 12a
12a-OH	2.50 *s*		C-6a, 12, 12a, 12b

4.1.1.3 12-DIHYDROUSARAROTENOID-C (3)

Compound **3** was isolated as a colorless amorphous solid with an R_f value of 0.58 (5% EtOAc in CH_2Cl_2). The HRMS mass spectrum showed a molecular ion peak at *m/z* 412.1520 ($C_{23}H_{24}O_7$). As in compound **2** the ^1H NMR spectrum revealed an ABX spin system centred at d_H 4.37 (*dd*, *J* = 10.2, 3.6 Hz)), 4.34 (*dd*, *J* = 13.8, 10.2 Hz) and 4.26 (*dd*, *J* = 10.8, 4.8 Hz) which is typical of the 6ß, 6a and 6a protons of 12a-hydroxyrotenoids (Yenesew *et al.*, 1998c). As in compound **2** a carbonyl peak was absent in the ^{13}C NMR spectrum and was replaced with an oxymethine peak for C-12 resonating at d_C 73.0 ppm. This peak showed HMQC correlation with the signal at d_H 4.91. In the COSY spectrum signal at d_H 4.91 (*d*, *J* = 10.8 Hz) showed correlation with the signal at d_H 2.78 (*d*, *J* = 10.8 Hz). When D_2O was added to the sample, the doublet at d_H 2.78 disappeared while the doublet at d_H 4.91 collapsed into a singlet. This confirmed that the signal at d_H 4.91 is for H-12 and the exchangeable proton is 12-OH.

A methoxyl (d_H 3.85 and d_C 55.8), a methylenedioxy (d_H 5.94 and d_C 101.4) and a 3,3-dimethylallyl (3.36, *m* for H-1'; 5.25 ,*t*, *J*=7.2 Hz for H-2'; 1.68, *s* for H-4'; 1.78, *s* for H-5') substituents on the 12,12a-dihydroxyrotenoid skeleton were evident from the ^1H and ^{13}C NMR spectra (Table 3.3). The presence of two aromatic singlets at d 7.81 (H-1) and 6.45 (H-4), in the ^1H NMR spectrum, and the chemical shift values of the ring A carbon atoms suggest the placement of the methylenedioxy group at C-2/C-3. In ring D, two *ortho*-coupled aromatic protons at d_H 6.67 and 7.42 (*J* = 9.0 Hz) were assigned to H-10 and H-11, respectively. This allows the placement of the 3,3-dimethylallyl group at C-8 and the methoxyl at C-9, and was confirmed by NOESY (which showed

interaction between the methoxyl protons (d_H 3.85) and the aromatic proton at C-10) and HMBC (^3J correlation between the methoxyl protons and C-9) experiments. The chemical shift value for H-1 (d_H 7.81) is strongly deshielded when compared to the value observed for rotenoids with *cis*-B/C ring junction (d_H 6.4-6.8) indicating that the B/C ring junction has a *trans*-geometry (Oberholser *et al.*, 1974; Dewick, 1994). Therefore, this compound was therefore identified as 12-dihydrousararotenoid-C (**3**), which is a new compound.

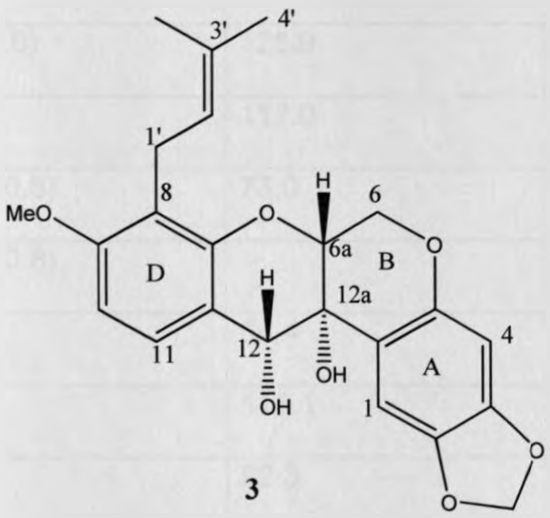


Table 4.3: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **3** ($CDCl_3$)

Position	d_H (*J* in Hz)	d_C	HMBC
1	7.81 *s*	107.2	C-2, 3, 4, 4a, 12b
2		142.5	
3		149.1	
4	6.45 *s*	98.3	C-1, 2, 3, 4a
4a		149.6	

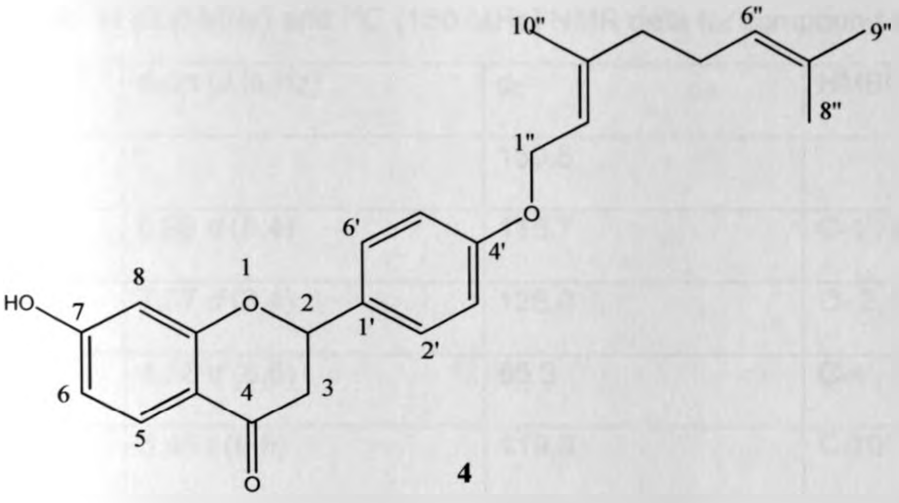
Table 4.3: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **3** cont....

Position	^{1}H d_H (*J* in Hz)	d_C	HMBC
6a	4.34 *dd* (13.8, 10.2 Hz)	62.3	C-4a, 12, 12a
6ß	4.37 *dd* (10.2, 3.6 Hz)		
6a	4.26 *dd* (10.8, 4.8 Hz)	70.7	C-4a, 12a
7a		151.2	
8		118.0	
9		157.8	
10	6.67 *d* (9.0)	105.3	C-7a, 8, 9, 11a
11	7.42 *d* (9.0)	126.9	C-7a, 9, 10, 11a
11a		117.0	
12-H	4.91 *d* (10.8)	73.0	C-7a, 11a, 12b
12-OH	2.78 *d* (10.8)		
12a		64.4	
12b		115.1	
1'	3.36 *m*	22.3	C-7a, 8, 9, 2', 3'
2'	5.25 *t* (7.2)	122.2	C-1', 4', 5'
3'		131.5	
4'	1.68 *s*	17.8	C-2', 3'
5'	1.78 *s*	25.8	C-2', 3'
2-OCH_2O-3	5.93	101.4	C-2', 3'
	5.95		
9-OMe	3.85 *s*	55.8	C-9

4.1.1.4 4'-GERANYLOXY-7-HYDROXYFLAVANONE (4)

Compound **4** was isolated as a colourless oil with R_f value of 0.46 (5% EtOAc in CH_2Cl_2). It analysed for $C_{22}H_{32}O_6$ by EI mass spectroscopy showing a molecular ion peak at *m/z* 392. The presence of an AXY spin system centred at d 2.78 (*J* = 16.8, 2.4 Hz), 3.06 (*J* = 16.8, 13.2) for CH_2-3, 5.40 (*J* = 13.2, 2.4) for H-2 and carbon resonances at 191.9 (C=O), 79.9 ppm (C-2) and 44.1 ppm (C-3) in the ^1H and ^{13}C spectra were consistent with a flavanone skeleton. Furthermore in the ^1H NMR spectrum an AXY spin system with doublets at d 7.79 (*J*=8.4 Hz) and 6.48 (*J*=2.4 Hz) and a double doublet at d 6.57 (*J*=8.4, 2.4 Hz) is consistent with the oxygenation of C-7 of ring-A.

The ^1H NMR further revealed the presence of an AA'XX' spin system centred at d 7.37 (*d*, *J*=8.4 Hz) and 6.93 (*d*, *J*=8.4 Hz) that requires oxygenation at C-4' of ring-B. The ^1H NMR (d 4.56, *d*, *J*=6.6 Hz; 5.46, *t*, *J*=6.6 Hz; 2.11, *m*; 5.10, *m*; 1.60, s; 1.67, *s*; 1.73, *s*) signals further revealed the presence of a geranyloxy moiety (Dagne *et al.*, 1990b). The HMBC experiment showed a ^3J correlation peak between CH_2-1'' of the geranyloxy group and C-4' thus placing the geranyloxy group at C-4'. This was confirmed from NOESY spectrum which showed NOE between methylene protons at C-1'' and H-3'/H-5'. On this basis the isolate was identified as 4'-geranyloxy-7-hydroxyflavanone (**4**) which is a new compound.



4

Table 4.4: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **4** (CD_2Cl_2)

Position	d_H (*J* in Hz)	d_C	HMBC
2	5.40 *dd* (13.2, 2.4)	79.9	C-1', 3, 2', 6'
3	3.06 *dd* (16.8, 13.2)	44.1	C-1', 2
3	2.78 *dd* (16.8, 2.4)		
4		191.9	
4a		114.9	
5	7.79 *d* (8.4)	129.4	C-8a, 7
6	6.57 *dd* (8.4, 2.4)	110.9	C-4a, 7, 8
7		164.2	
8	6.48 *d* (2.4)	103.6	C-4a, 6, 7, 8a
8a		164.0	
1'		130.9	
2'	7.37 *d* (8.4)	128.0	C- 2, 3', 4', 6'
3'	6.93 *d* (8.4)	115.7	C-1', 4', 5'

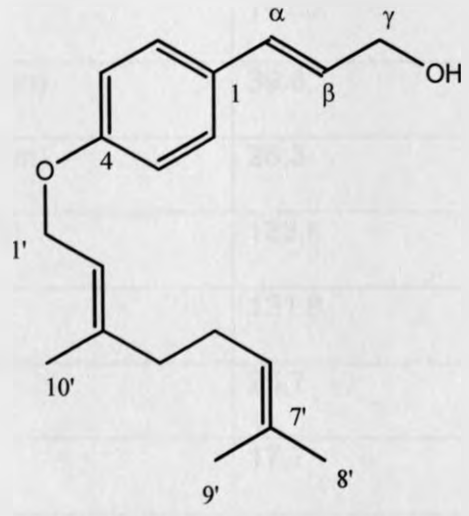
Table 4.4: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **4** cont....

Position	d_H *m* (*J* in Hz)	d_C	HMBC
4'		159.5	
5'	6.93 *d* (8.4)	115.7	C-1', 4', 3'
6'	7.37 *d* (8.4)	128.0	C- 2, 2', 4', 5'
1"	4.56 *d* (6.6)	65.3	C-4', 2", 3"
2"	5.46 *t* (6.6)	119.6	C-10", 4"
3"		141.6	
4"	2.11 *m*	39.8	C-2", 6"
5"	2.11 *m*	26.6	C-6", 7", 4"
6"	5.10 *m*	124.0	C-8", 9"
7"		132.0	
8"	1.60 s	17.7	C-6", 9"
9"	1.67 s	25.7	C-6", 8"
10"	1.73 s	16.6	C-4"

4.1.1.5 4-*O*-GERANYLOXYCINNAMYL ALCOHOL (5)

Compound **5** was isolated as a colourless amorphous solid with an R_f value of 0.47 (5% EtOAc in CH_2Cl_2). HRMS analysis of compound **5** showed a molecular ion peak at *m/z* 286.1931 ($C_{19}H_{26}O_2$). The ^{1}H NMR spectrum, indicated the presence of an AA'XX' spin system at d 7.34 (*d*, *J* = 8.4 Hz for H-2/6) and 6.89 (*d*, *J* = 8.4 Hz for H-3/5) which is the characteristic pattern of 1, 4-disubstituted benzene. The chemical shifts of H-3/5

(d 6.89) and that of C-4 (d 158.7) in the ^{1}H and ^{13}C NMR spectrum, respectively, were indicative of oxygenation in this ring. The ^{1}H NMR also showed two *trans*-oriented olefinic protons H-a (d 6.57, *d*, *J* = 15.6 Hz) and H-ß (d 6.26, *dt*, *J* = 15.6, 6.0 Hz) adjacent to an oxymethylene group (d 4.32, *J* = 6.0 Hz) which was consistent with a 4-oxycinnamyl alcohol skeleton. As in compounds **1** and **4** the presence of a geranyl group was clearly evident from the NMR spectra, and its attachment on the oxygen at C-4 is shown by a NOESY experiment which indicated interaction between the methylene protons at C-1' and the aromatic protons at C-3 and C-5 positions. This was further supported by HMBC ^{3}J correlation between CH_2-1' with C-4. Based on these evidences the compound was identified as colenemol (**5**), previously isolated from *Coleonema pulchellum* (Gunter *et al.*, 1997). This is however the first report on the occurrence of compound **5** in the genus *Millettia.*



5

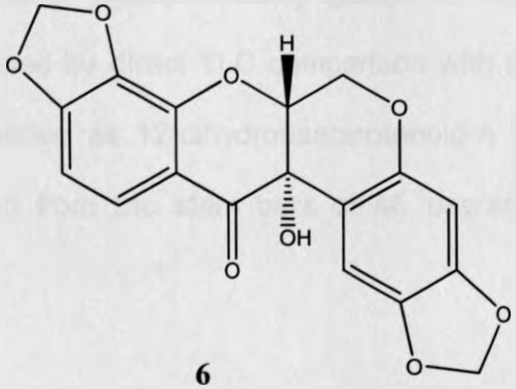
Table 4.5: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **5** ($CDCl_3$)

Position	d_H (*J* in Hz)	d_C	HMBC
1		129.3	
2	7.34 (2H, *d*, 8.4)	127.7	C-3, 4, 6, a
3	6.89 (2H, *d*, 8.4)	114.8	C-1, 2, 4, 5
4		158.7	
5	6.89 (2H, *d*, 8.4)	114.8	C-1, 3, 6
6	7.34 (2H, *d*, 8.4)	127.7	C- 4, 5, a
a	6.57 (1H, *d*, 15.6)	131.1	C- β, γ
ß	6.26 (1H, *dt*, 15.6, 6.0)	126.1	C- α, γ, 1
γ	4.32 (2H, *d*, 6.0)	64.0	C- a, ß
1'	4.56 (2H, *d*, 6.0)	64.9	C-2', 3', 4
2'	5.51 (1H, *t*, 6.0)	119.4	C-1', 4', 10'
3'		141.3	
4'	2.09-2.16 (4H, *m*)	39.6	C-2', 3', 5', 6', 10'
5'	2.09-2.16 (4H, *m*)	26.3	C-3', 4', 6',7'
6'	5.12 (1H, *t*, 6.0)	123.8	C-5', 8', 9'
7'		131.8	
8'	1.70 (3H, *s*)	25.7	C-6', 7', 9'
9'	1.63 (3H, *s*)	17.7	C-6', 7', 8'
10'	1.76 (3H, *s*)	16.7	2', 3', 4'

4.1.1.6 USARAROTENOID-A (6)

Compound **6** was isolated as colourless crystals with an R_f value of 0.44 (5% EtOAc in CH_2Cl_2). The ^1H and ^{13}C NMR showed that, the compound was a 12a-hydroxyrotenoid derivative (Table 3.6) having two methylenedioxy groups. The ^1H NMR spectrum also indicated the presence of two isolated aromatic protons d_H 7.59 for H-1 and d 6.42 for H-4, allowing the placement of one of the methylenedioxy group at C-2/C-3.

The second methylenedioxy group was placed at C-8/C-9, due to the presence of two *ortho*-coupled aromatic protons appearing at d_H 6.70 (J = 9.0 Hz) for H-10 and d 7.62 (J = 8.4 Hz) for H-11 in the ^1H NMR spectrum. H-1 is strongly deshielded (d_H 7.59) indicating a *trans*-B/C ring junction (Oberholser *et al.*, 1974; Dewick, 1994). The structure of compound **6** was confirmed by comparison with published data and by direct TLC comparison with authentic sample. Compound **6** has been reported from the stem bark of *M. usaramensis* (Yenesew *et al.*, 1998c).

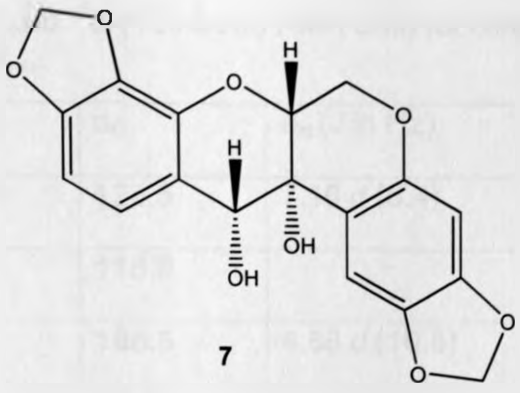


6

4.1.1.7 12-DIHYDROUSARAROTENOID-A (7)

Compound **7** was isolated as colourless amorphous solid from methanol with an R_f value of 0.25 (5% EtOAc in CH_2Cl_2). The ^1H and ^{13}C NMR data (Table 3.6) suggested that this compound had a 12-dihydrorotenoid skeleton (Abe *et al.*, 1985). This was supported by the appearance of a doublet at d_H 4.88 (*d*, *J* = 10.8 Hz) for H-12, which is consistent with the occurrence of an oxymethine at C-12 resonating at d_C 71.9, unlike for compound **6** where C-12 resonates at d_C 186.8 (C=O). Otherwise compound **7** has identical substitution pattern to compound **6**, being substituted with two methylenedioxy groups.

Thus in the ^1H NMR spectrum (Table 3.6) the presence of two *ortho*-coupled aromatic protons appearing at d_H 6.56 (*d*, *J* = 8.4 Hz) for H-10 and 7.10 (*d*, *J* = 8.4 Hz) for H-11, would place one of the methylenedioxy groups at C-8/C-9. The presence of two *para*-oriented isolated aromatic protons in ring A (d_H 7.91 for H-1 and 6.36 for H-4) allowed the placement of the other methylenedioxy group at C-2/C-3. The structure of compound **7** was confirmed by direct TLC comparison with authentic sample. Hence the compound was identified as 12-dihydrousararotenoid-A (**7**). This compound has previously being isolated from the stem bark of *M. usaramensis* (Yenesew *et al.*, 1998c).



7

Table 4.6: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compounds **6** and **7** (CD_2Cl_2)

	Compound **6**		Compound **7**	
Position	d_H (*J* in Hz)	d_C	d_H (*J* in Hz)	d_C
1	7.59 *s*	109.5	7.91 *s*	109.0
2		142.6		143.5
3		149.8		150.1
4	6.42 *s*	98.6	6.36 *s*	99.2
4a		151.0		151.0
6a	4.41	61.8	4.37 *dd* (11.4, 5.4)	63.0
6ß	4.40		4.28 *dd* (10.8, 4.8)	
6a	4.69 *dd* (9.0, 7.2)	77.1	4.42 *dd* (10.8, 4.8)	74.9
7a		143.8		139.9
8		134.5		134.9
9		154.6		149.6
10	6.70 *d* (8.4)	104.4	6.56 *d* (8.4)	103.4

Table 4.6: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compounds **6** and **7** (CD_2Cl_2) cont....

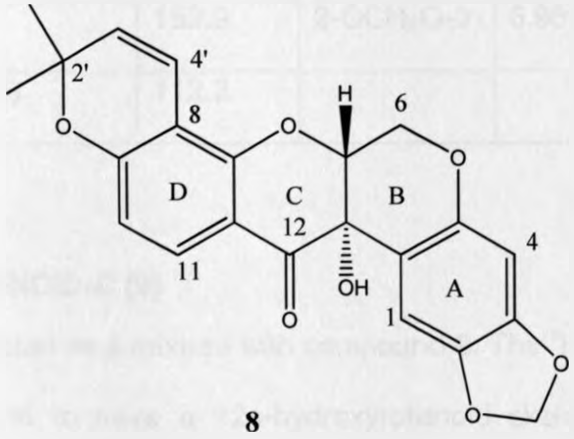
Position	d_H (*J* in Hz)	d_C	d_H (*J* in Hz)	d_C
11	7.62 *d* (8.4)	124.5	7.10 *d* (8.4)	122.9
11a		116.9		124.5
12		186.8	4.88 *d* (10.8)	71.9
12a		67.3		65.5
12b		110.7		118.5
2-OCH_2O-3	5.96	103.4	5.96	103.0
8-OCH_2O-9	6.08	102.1	6.00	102.9
	6.13			

4.1.1.8 12a-EPIMILLETOSIN (8)

Compound **8** was isolated as colourless crystals with an R_f value of 0.65 (5% EtOAc in CH_2Cl_2). The ^{1}H and ^{13}C NMR data (Table 3.7) suggested this compound to be a 12a-hydroxyrotenoid. The presence of a 2,2-dimethylpyrano and a methylenedioxy (d_H 5.95) substituents was evident from ^{1}H NMR spectrum and their placement at C-8/C-9 and at C-2/C-3 was indicated from the ^{1}H and ^{13}C NMR data (Table 3.7).

The chemical shift value for H-1 (d_H 7.65) was strongly deshielded when compared to the value observed for rotenoids with *cis*-B/C ring junction (d 6.4-6.8) indicating that the B/C ring junction has a *trans*-stereochemistry (Oberholzer *et al.*, 1974; Messana *et al.*,

1986; Dewick, 1994). There are two possible stereoisomers with *trans* B/C ring junction, which are defined by the relative configuration of H-6a to the two H-6 protons, in which H-6a can be described as equatorial or axial. In this case the presence of a large coupling constant (J = 10.8 Hz) between H-6a and one of the C-6 protons requires the presence of a 1,2-transdiaxial relationship (Fukami and Nakajima, 1971). The structure of compound **8** was confirmed by comparison with published data (Yenesew *et al.*, 1998c) and by direct TLC comparison with authentic sample. Thus the compound was identified as 12a-epimilletosin (**8**). This compound has been reported from the stem bark of *M. usaramensis* (Yenesew *et al.*, 1998c)



8

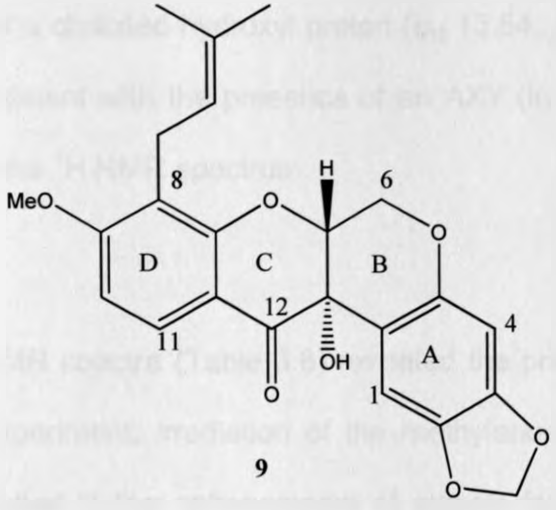
Table 4.7: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **8** (CD_2Cl_2)

Position	d_H (*J* in Hz)	d_C	Position	d_H (*J* in Hz)	d_C
1	7.65 *s*	109.6	11	7.75 *d* (8.4)	129.9
2		142.6	11a		111.0
3		149.7	12		187.4
4	6.41 *s*	98.6	12a		66.7
4a		151.0	12b		113.9
6a	4.41 (10.8, 10.2)	62.0	2'		78.1
6ß	4.38 (10.2, 5.4)		3'	5.67 *d* (10.2)	129.7
6a	4.63 (10.8, 4.8)	77.0	4'	6.65 *d* (10.2)	115.4
7a		156.0	5'	1.56 *s*	28.5
8		109.3	6'	1.47 *s*	28.1
9		159.9	2-OCH_2O-3	5.95 *s*	102.0
10	6.56 *d* (8.4)	112.2			

4.1.1.9 USARAROTENOID-C (9)

Compound **9** was isolated as a mixture with compound **8**. The ^{1}H NMR data (Table 3.8) showed this compound to have a 12a-hydroxyrotenoid skeleton. The presence of methoxy, prenyl and methylenedioxy substituents on the 12a-hydroxyrotenoid skeleton was evident from the ^{1}H NMR data (Table 3.8). In the ^{1}H NMR spectrum, the presence of two *ortho*-coupled aromatic protons appearing at d_H 6.71 (*d*, *J* = 9.0 Hz for H-10) and 7.84 (*d*, *J* = 9.0 Hz for H-11) would place the methoxyl and prenyl substituent at C-9 and C-8.

In ring A the ^1H NMR spectrum further showed the presence of two isolated aromatic singlets at d_H 7.63 for H-1 and 6.40 for H-4. This allows the placement of the methylenedioxy group at C-2/C-3. As in other 12a-hydroxyrotenoids, H-1 (d_H 7.63) is strongly deshielded, indicating a *trans*-B/C ring junction. Compound **9** has been previously isolated from the stem bark of *Millettia usaramensis* (Yenesew *et al.*, 1983c)



9

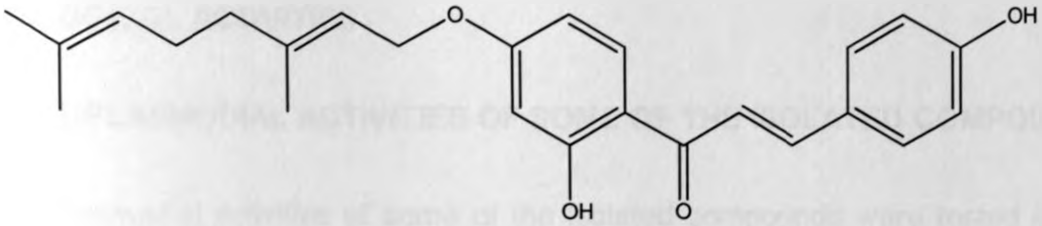
Table 4.8: ^1H (600 MHz) NMR data for compound **9** (CD_2Cl_2)

Position	^1H d_H (*J* in Hz)	Position	^1H d_H (*J* in Hz)
1	7.63 *s*	1'	3.36 *d* (6.6)
4	6.40 *s*	2'	5.18 *t* (6.6)
6a	4.43 *dd* (10.0, 5)	4'	1.67 *s*
6ß	4.37 *dd* (6.0, 5.0)	5'	1.78 *s*
6a	4.60 *dd* (10.0, 5.0)	2-OCH_2O-3	5.94 *s*
10	6.71 *d* (9.0)	9-OMe	3.90 *s*
11	7.84 *d* (9.0)		

4.1.1.10 4'-*O*-GERANYLISOLIQUIRITIGENIN (10)

Compound **10** was isolated as a yellow oily substance with an R_f value of 0.63 (5% EtOAc in CH_2Cl_2). Evidence that this compound was a chalcone was available from the ^1H NMR spectrum which showed signals for two *trans*-oriented olefinic protons, H-a (d 7.45, *d*, *J* = 15.6 Hz) and H-ß (d 7.84, *d*, *J* = 15.6 Hz). The corresponding ^{13}C NMR signals for C-a and C-ß were at d_C 118.7 and 144.4 respectively, while the carbonyl group resonated at 192.1 ppm (Andrei *et al.*, 2000). The ^1H NMR (Table 3.9) further showed the presence of a chelated hydroxyl proton (d_H 13.54, *s*). Oxygenation at C-4, C-2' and C-4' was consistent with the presence of an AXY (in ring B) and AA'XX' (in ring A) spin systems in the ^1H NMR spectrum.

Both the ^1H and ^{13}C NMR spectra (Table 3.8) revealed the presence of a geranyloxy group. In a NOESY experiment, irradiation of the methylene protons at C-1'' of the geranyl side-chain resulted in the enhancement of signals for H-3' and H-5', clearly placing the geranyloxy group at C-4'. On this basis the compound was identified as 4'-*O*-geranylisoliquiritigenin (**10**). This is the first report on the isolation of 4'-*O*-geranylisoliquiritigenin from the root bark of *M. usaramensis.* However it has previously being reported from the stem bark of *M. usaramensis* (Yenesew *et al.*, 1998c), root bark of *M. ferruginea* (Dagne *et al.*, 1990b) and *M. griffoniana* (Yankep *et al.*, 1997).



10

Table 4.9: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **10** (CD_2Cl_2)

Position	d_H *m* (*J* in Hz)	d_C	Position	d_H *m* (*J* in Hz)	d_C
1		127.9	H-ß	7.84 *d* (15.6)	144.4
2	7.56 *d* (8.4)	130.8	C=O		192.1
3	6.88 *d* (8.4)	116.3	1"	4.59 *d* (6.6)	65.5
4		158.4	2"	5.48 *t* (6.6)	118.1
5	6.88 *d* (8.4)	116.3	3"		142.5
6	7.56 *d* (8.4)	130.8	4"	2.10 *m*	39.8
1'		114.2	5"	2.10 *m*	26.5
2'-OH	13.54 *s*		6"	5.10 *m*	123.9
2'		165.7			
3'	6.47 *d* (2.2)	102.0	7"		132.2
4'		166.8	8"	1.67 *s*	25.9
5'	6.50 *dd* (2.2, 9.8)	108.5	9"	1.61 *s*	17.9
6'	7.82 *d* (9.8)	131.3	10"	1.75 *s*	17.0
H-a	7.45 *d* (15.6)	118.7			

4.2 BIOLOGICAL ACTIVITIES

4.2.1 ANTIPLASMODIAL ACTIVITIES OF SOME OF THE ISOLATED COMPOUNDS

The antiplasmodial activities of some of the isolated compounds were tested *in vitro* against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* (Table 3.10). This included the chalcones, 4'-*O*-geranylisoliquiritigenin (**10**) and the flavanone 4'-geranyloxy-7-hydroxyflavanone (**4**) and the rotenoid 12a-epimillettosin (**8**). Compound **10** showed higher activity than its isomeric counterpart 4-*O*-geranylisoliquiritigenin (**1**) indicating that the position of the geranyloxy substituent could be an influencing factor. The rotenoid 12a-epimillettosin (**8**) showed moderate antimalarial activity unlike other rotenoids with trans-B/C ring junction.

The dihydrochalcones, diuvaretin and uvaretin have also been shown to be potent antimalarial leads (Nkunya *et al.*, 1991). It is interesting to note that licochalcone A, a retrochalcone has been identified as a potential antimalarial agent and its potential as a drug is currently under investigation (Chen *et al.*, 1994). Chalcones bartericin A, stipulin and 4-hydroxylonchocarpin isolated from *Dorstenia barteri* were found to be active *in vitro* against *P. falciparum*, demonstrating potencies with relatively low IC_{50} values (2.15 µM, 5.13 µM and 3.36 µM respectively).

Table 4.10:. *In-vitro* antiplasmodial activity of some compounds from *M. usaramensis*

Sample	Chloroquine-sensitive (D6) IC_{50} (µg/ml)	Chloroquine-resistant (W2) IC_{50} (µg/ml)
12a-Epimilletosin (**8**)	2.7 ± 0.3	3.1 ± 1.1
4'-O-Geranylisoliquiritigenin (**10**)	4.5 ± 1.9	1.6 ± 0.6
Usararotenoid-C (**6**)/epimilletosin	NT	3.0 ± 0.3
4-Geranyloxy-a,2',4'-trihydroxydihydrochalcone (**4**)	11.4 ± 0.9	4.1 ± 0.6
Reference drugs		
Chloroquine	0.07 ± 0.01	0.012 ± 0.006
Mefloquine	0.002 ± 0.004	0.038± 0.004

NT=not tested

4.3 PHYSICAL AND SPECTROSCOPIC DATA OF THE ISOLATED COMPOUNDS

4.3.1 4-*O*-GERANYLISOLIQUIRITIGENIN (1)

Yellowish oil. $[M]^+$ *m/z* 392.1968. ^1H NMR (CD_2Cl_2, 600 MHz): d 7.62 (2H, *d*, *J* = 8.4 Hz, H-2, 6), 6.95 (2H, *d*, *J* = 8.4 Hz, H-3, 5), 6.40 (1H, *d*, *J* = 2.4 Hz, H-3'), 6.45 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-5'), 7.83 (1H, *d*, *J* = 8.4 Hz, H-6'), 7.48 (1H, *d*, *J* = 15.6 Hz, H-a), 7.85 (1H, *d*, *J* = 15.6 Hz, H-ß), 4.60 (2H, *d*, *J* = 6.6 Hz, H-1''), 5.47 (1H, *t*, *J* = 6.6 Hz, H-2''), 2.07-2.15 (4H, *m*, H-4'', H-5''), 5.10 (1H, *t*, *J* = 6.6 Hz, H-6''),1.67 (Me, *s*, H-8''), 1.61 (Me, *s*, H-9''), 1.75 (Me, *s*, H-10''). ^{13}C NMR (CD_2Cl_2, 150 MHz): d 127.6 (C-1), 130.7 (C-2, C-6), 115.4 (C-3, C-5), 161.6 (C-4), 114.7 (C-1'), 163.2 (C-2'), 103.7 (C-3'), 166.6 (C-4'), 107.9 (C-5'), 132.1 (C-6'), 192.3 (C=O), 119.3 (C-a), 144.7 (C-ß), 65.4 (C-1''),

117.9 (C-2''), 141.9 (C-3''), 39.7 (C-4''), 26.6 (C-5''), 124.0 (C-6''), 132.2 (C-7''), 25.7 (C-8''), 17.7 (C-9''), 16.7 (C-10'').

4.3.2 12-DIHYDROUSARAROTENOID-B (2)

Colourless amorphous solid, mp. > 215°C. $[M]^+$ *m/z* 374.0999. ^1H NMR (CD_2Cl_2, 600 MHz): d 7.77 (1H, *s*, H-1), 6.39 (1H, *s*, H-4), 4.34 (1H, *dd*, *J* = 11.4, 9.6 Hz, H-6a), 4.39 (1H, *dd*, *J* = 10.2, 4.8 Hz, H-6ß), 4.28 (1H, *dd*, *J* = 10.8, 4.8 Hz, H-6a), 6.68 (1H, *d*, *J* = 9.0 Hz, H-10), 7.27 (1H, *d*, *J* = 9.0 Hz, H-11), 4.89 (1H, *d*, *J* = 11.4 Hz, H-12), 3.79 (-OMe, *s*), 3.84 (-OMe, *s*), 5.92 (-OCH_2O-, *d*, *J*=1.2 Hz), 5.93 (-OCH_2O-, *d*, *J*=1.2 Hz), 2.78 (OH-12, *d*, *J* = 11.4 Hz) 2.50 (OH-12a, *s*). ^{13}C NMR (CD_2Cl_2, 150 MHz): d 107.2 (C-1), 142.7 (C-2), 147.4 (C-3), 98.4 (C-4), 149.4 (C-4a), 62.6 (C-6), 73.4 (C-6a), 149.9 (C-7a), 136.5 (C-8), 153.3 (C-9), 106.6 (C-10), 123.6 (C-11), 119.7 (C-11a), 70.6 (C-12), 64.6 (C-12a), 115.7 (C-12b),) 60.9 (-OMe, *s*), 56.3 (-OMe, *s*), 101.9 (-OCH_2O-, *d*).

4.3.3 12-DIHYDROUSARAROTENOID-C (3)

Colourless amorphous solid. $[M]^+$ *m/z* 412.1520. ^1H NMR ($CDCl_3$, 600 MHz): d 7.81 (1H, *s*, H-1), 6.45 (1H, *s*, H-4), 4.34 (1H, *J* = 13.8, 10.2 Hz, H-6a), 4.37 (1H, *J* = 10.2, 3.6 Hz, H-6ß), 4.26 (1H, *J* = 10.8, 4.8 Hz, H-6a), 6.67 (1H, *d*, *J* = 9.0 Hz, H-10), 7.42 (1H, *d*, *J* = 9.0 Hz, H-11), 4.91 (1H, *d*, *J* = 10.8 Hz, H-12), 3.36 (1H, *m*, H-1'), 5.25 (1H, *t*, *J* = 7.2 Hz, H-2'), 1.68 (Me, *s*, H-4'), 1.78 (Me, *s*, H-5'), 3.85 (-OMe, *s*), 5.93 (-OCH_2O-, *s*), 5.95 (-OCH_2O-, *s*), 2.78 (-OH, *d*, *J* = 10.8). ^{13}C NMR ($CDCl_3$, 150 MHz): d 107.2 (C-1), 142.5 (C-2), 149.1 (C-3), 98.3 (C-4), 149.6 (C-4a), 62.3 (C-6), 70.7 (C-6a),

151.2 (C-7a), 118.0 (C-8), 157.8 (C-9), 105.3 (C-10), 126.9 (C-11), 117.0 (C-11a), 73.0 (C-12), 64.4 (C-12a), 115.1 (C-12b), 22.3 (C-1'), 122.2 (C-2'), 131.5 (C-3'), 17.8 (C-4'), 25.8 (C-5'), 55.8 (-OMe), 101.4 (-OCH_2O-).

4.3.4 4'-GERANYLOXY-7-HYDROXYFLAVANONE (4)

Colourless oil. $[M]^+$ *m/z* 392. ^1H NMR (CD_2Cl_2, 600 MHz): 5.40 (1H, *dd*, *J* = 13.2, 2.4 Hz, H-2), 3.06 (1H, *dd*, *J* = 16.8, 13.2 Hz, H-3), 2.78 (1H, *dd*, *J* = 16.8, 2.4 Hz, H-3), 7.79 (1H, *d*, *J* = 8.4 Hz, H-5), 6.57 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-6), 6.48 (1H, *d*, *J* = 2.4 Hz, H-8), d 7.37 (2H, *d*, *J* = 8.4 Hz, H-2', 6'), 6.93 (2H, *d*, *J* = 8.4 Hz, H-3', 5'), 4.56 (2H, *d*, *J* = 6.6 Hz, H-1''), 5.46 (1H, *t*, *J* = 6.0 Hz, H-2''), 2.11 (4H, *m*, H-4'', H-5''), 5.10 (1H, *m*, H-6''),1.60 (Me, *s*, H-8''), 1.67 (Me, *s*, H-9''), 1.73 (Me, *s*, H-10''). ^{13}C NMR (CD_2Cl_2, 150 MHz): d 79.9 (C-2), 44.1 (C-3), 191.9 (C-4), 114.9 (C-4a), 129.4 (C-5), 110.9 (C-6), 164.2 (C-7), 103.6 (C-8), 164.0 (C-8a), 130.9 (C-1'), 128.0 (C-2', 6'), 115.7 (C-3', 5'), 159.5 (C-4'), 65.3 (C-1''), 119.6 (C-2''), 141.6 (C-3''), 39.8 (C-4''), 26.6 (C-5''), 124.0 (C-6''), 132.0 (C-7''), 17.7 (C-8''), 25.7 (C-9''), 16.6 (C-10'').

4.3.5 4-GERANYLOXYCINNAMYL ALCOHOL (5)

Colourless solid. $[M]^+$ *m/z* 286.1931. ^1H NMR ($CDCl_3$, 600 MHz): d 7.34 (2H, *d*, *J* = 8.4 Hz, H-2, 6), 6.89 (2H, *d*, *J* = 8.4 Hz, H-3, 5), 6.57 (1H, *d*, *J* = 15.6 Hz, H-a), 6.26 (1H, *dt*, *J* = 15.6, 6.0 Hz, H-ß), 4.32 (2H, *d*, *J* = 6.0 Hz, H-γ), 4.56 (2H, *d*, *J* = 6.0 Hz, H-1'), 5.51 (1H, *t*, *J* = 6.0 Hz, H-2'), 2.09-2.16 (4H, *m*, H-4', H-5'), 5.12 (1H, *t*, *J*=6.0, H-6'), 1.70 (Me, *s*, H-8'), 1.63 (Me, *s*, H-9'), 1.76 (Me, *s*, H-10'). ^{13}C NMR ($CDCl_3$, 150 MHz):

d 129.3 (C-1), 127.7 (C-2, C-6), 114.8 (C-3, C-5), 158.7 (C-4), 131.1 (C-a), 126.1 (C-ß), 64.0 (C-γ), 64.9 (C-1'), 119.4 (C-2'), 141.3 (C-3'), 39.6 (C-4'), 26.3 (C-5'), 123.8 (C-6'), 131.8 (C-7'), 25.7 (C-8'), 17.7 (C-9'), 16.7 (C-10').

4.3.6 USARAROTENOID-A (6)

Colourless crystals. ^{1}H NMR (CD_2Cl_2, 600 MHz): d 7.59 (1H, *s*, H-1), 6.42 (1H, *s*, H-4), 4.41 (1H, H-6a), 4.40 (1H, H-6ß), 4.69 (1H, *dd*, *J* = 9.0, 7.2 Hz, H-6a), 6.70 (1H, *d*, *J* = 8.4 Hz, H-10), 7.62 (1H, *d*, *J* = 8.4 Hz, H-11), 5.96 (2H, -OCH_2O-), 6.08 (2H, -OCH_2O-). ^{13}C NMR (CD_2Cl_2, 150 MHz): d 109.5 (C-1), 142.6 (C-2), 149.8 (C-3), 98.6 (C-4), 151.0 (C-4a), 61.8 (C-6), 77.1 (C-6a), 143.8 (C-7a), 134.5 (C-8), 154.6 (C-9), 104.4 (C-10), 124.5 (C-11), 116.9 (C-11a), 186.8 (C-12), 67.3 (C-12a), 110.7 (C-12b), 103.4 (-OCH_2O-), 102.1 (-OCH_2O-).

4.3.7 12-DIHYDROUSARAROTENOID-A (7)

Colourless amorphous solid. ^{1}H NMR (CD_2Cl_2, 600 MHz): d 7.91 (1H, *s*, H-1), 6.36 (1H, *s*, H-4), 4.42 (1H, *dd*, *J* = 11.4, 5.4 Hz, H-6a), 4.28 (1H, *dd*, *J* = 10.8, 4.8 Hz, H-6ß), 4.37 (1H, *dd*, *J* = 10.8, 4.8 Hz, H-6a), 6.56 (1H, *d*, *J* = 8.4 Hz, H-10), 7.10 (1H, *d*, *J* = 8.4 Hz, H-11), 4.88 (1H, *d*, *J* = 10.8 Hz, H=12) 5.96 (2H, -OCH_2O-), 6.00 (2H, -OCH_2O-). ^{13}C NMR (CD_2Cl_2, 150 MHz): d 109.0 (C-1), 143.5 (C-2), 150.1 (C-3), 99.2 (C-4), 151.0 (C-4a), 63.0 (C-6), 74.9 (C-6a), 139.9 (C-7a), 134.9 (C-8), 149.6 (C-9), 103.4 (C-10), 122.9 (C-11), 124.5 (C-11a), 71.9 (C-12), 65.5 (C-12a), 118.5 (C-12b), 103.0 (-OCH_2O-), 102.9 (-OCH_2O-).

4.3.8 12a-EPIMILLETTOSIN (8)

Colourless crystals. ^{1}H NMR (CD_2Cl_2, 600 MHz): d 7.65 (1H, *s*, H-1), 6.41 (1H, *s*, H-4), 4.41 (1H, *dd*, *J* = 10.8, 10.2 Hz, H-6a), 4.38 (1H, *dd*, *J* = 10.2, 5.4 Hz, H-6ß), 4.63 (1H, *dd*, *J* = 10.8, 4.8 Hz, H-6a), 6.56 (1H, *d*, *J* = 8.4 Hz, H-10), 7.75 (1H, *d*, *J* = 8.4 Hz, H-11), 5.67 (1H, d, *J* = 10.2 Hz, H-3'), 6.65 (1H, d, *J* = 10.2 Hz, H-4'), 5.95 (-OCH_2O-, *s*), 1.56 (Me, *s*, H-5'), 1.47 (Me, *s*, H-6'). ^{13}C NMR (CD_2Cl_2, 150 MHz): d 109.6 (C-1), 142.6 (C-2), 149.7 (C-3), 98.6 (C-4), 151.0 (C-4a). 62.0 (C-6), 77.0 (C-6a), 156.0 (C-7a), 109.3 (C-8), 159.9 (C-9), 112.2 (C-10), 129.9 (C-11), 111.0 (C-11a), 187.4 (C-12), 66.7 (12a), 113.9 (-12b), 78.1 (C-2'), 129.7 (C-3'), 115.4 (C-4'), 28.5 (C-5'), 28.1 (C-6'), 102.0 (-OCH_2O-).

4.3.10 USARAROTENOID-C (9)

Colourless oil. ^{1}H NMR (CD_2Cl_2, 600 MHz) d 7.63 (1H, *s*, H-1), 6.40 (1H, *s*, H-4), 4.43 (1H, *dd*, *J* = 10.0, 5.0 Hz, H-6a), 4.37 (1H, *dd*, *J* = 10.0, 5.0 Hz, H-6 ß), 4.60 (1H, *dd*, *J* = 10.0, 5.0 Hz, H-6a), 6.71 (1H, *d*, *J* = 9.0 Hz, H-10), 7.84 (1H, *d*, *J* = 9.0 Hz, H-11), 3.36 (2H, *d*, *J* = 6.6 Hz H-1'), 5.18 (1H, *t*, *J* = 6.6 Hz, H-2'), 1.67 (Me, *s*, H-4'), 1.78 (Me, *s*, H-5'), 3.90 (-OMe, *s*), 5.94 (-OCH_2O-, *s*).

4.3.9 4'-*O*-GERANYLISOLIQUIRITIGENIN (10)

Yellow oil. ^{1}H NMR (CD_2Cl_2, 600 MHz): d 7.56 (2H, *d*, *J* = 8.4 Hz, H-2, 6), 6.88 (2H, *d*, *J* = 8.4 Hz, H-3, 5), 6.47 (1H, *d*, *J* = 2.2 Hz, H-3'), 6.50 (1H, *dd*, *J* = 9.8, 2.2 Hz, H-5'),

7.82 (1H, *d*, *J* = 9.8 Hz, H-6'), 7.45 (1H, *d*, *J* = 15.6 Hz, H-a), 7.84 (1H, *d*, *J* = 15.6 Hz, H-ß), 4.59 (2H, *d*, *J* = 6.6 Hz, H-1''), 5.48 (1H, *t*, *J* = 6.6 Hz, H-2''), 2.10 (4H, *m*, H-4'', H-5''), 5.10 (1H, *m*, H-6''),1.67 (Me, *s*, H-8''), 1.61 (Me, *s*, H-9''), 1.75 (Me, *s*, H-10''). ^{13}C NMR ($CDCl_3$, 50 MHz): d 127.9 (C-1), 130.8 (C-2, C-6), 116.3 (C-3, C-5), 158.3 (C-4), 114.2 (C-1'), 165.7 (C-2'), 102.0 (C-3'), 166.8 (C-4'), 108.5 (C-5'), 131.3 (C-6'), 192.1 (C=O), 118.7 (C-a), 144.4 (C-ß), 65.5 (C-1''), 118.1 (C-2''), 142.5 (C-3''), 39.8 (C-4''), 26.5 (C-5''), 123.9 (C-6''), 132.2 (C-7''), 25.9 (C-8''), 17.9 (C-9''), 17.0 (C-10'').

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- From the root bark of *M. usaramensis* ten compounds have been isolated and characterized as usararotenoid-A, usararotenoid-C, 12a-epimillettosin, 12-dihydrousararotenoid-A, 12-dihydrousararotenoid-B, 12-dihydrousararotenoid-C, 4'-*O*-geranylisoliquiritigenin, 4-*O*-geranylisoliquiritigenin, 4'-geranyloxy-7-hydroxyflavanone and 4-geranyloxycinnamyl alcohol.
- Of these 4-*O*-geranylisoliquiritigenin, 4'-geranyloxy-7-hydroxyflavanone, 12-dihydrousararotenoid-B and 12-dihydrousararotenoid-C are new compounds while 4-geranyloxycinnamyl alcohol is reported here for the first time in the genus *Millettia*.
- 4'-Geranyloxy-7-hydroxyflavanone, the mixture of usararotenoid-A/12a-epimillettosin and the chalcone, 4'-*O*-geranylisoliquiritigenin showed good antiplasmodial activity against the chloroquine-resistant (W2) strain of *Plasmodium falciparum*.

RECOMMENDATIONS

- Further phytochemical investigation of the root bark of *M. usaramensis* should be carried out in order to determine the complete phytochemical profile of this plant.
- Structure-activity relationship studies should be carried out to determine the properties responsible for observed activities in the compounds **4**, **8**, and **10**.
- Toxicity studies of the extract and compounds is necessary in order to establish their safety and efficacy.
- *In vivo* antiplasmodial activity tests should be carried out on the crude extracts and isolated compounds from this plant.
- Investigation on the phytochemistry of the other subspecies of *M. usaramensis* will shade some light on the chemical inter-relationship between the two sub-species.

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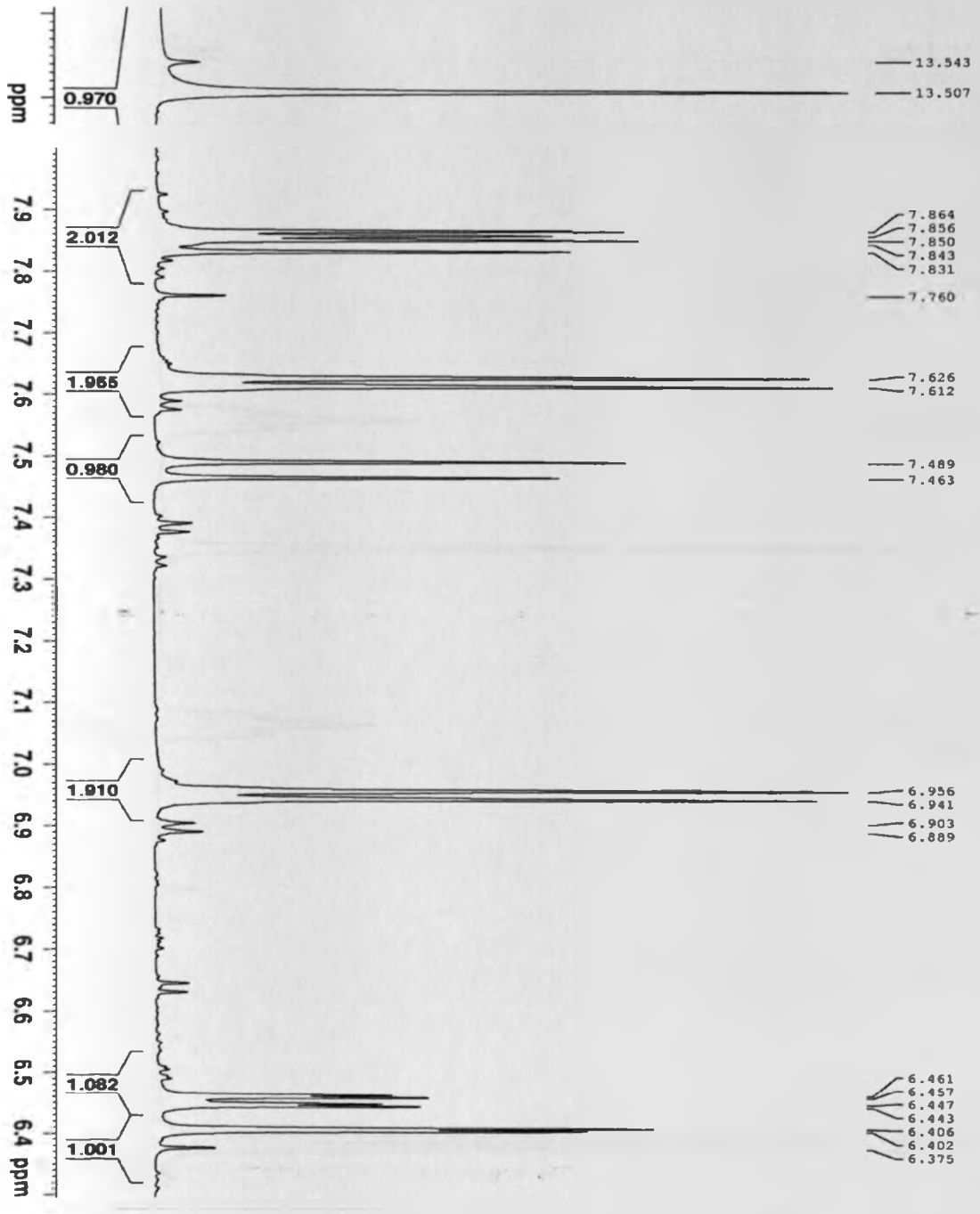
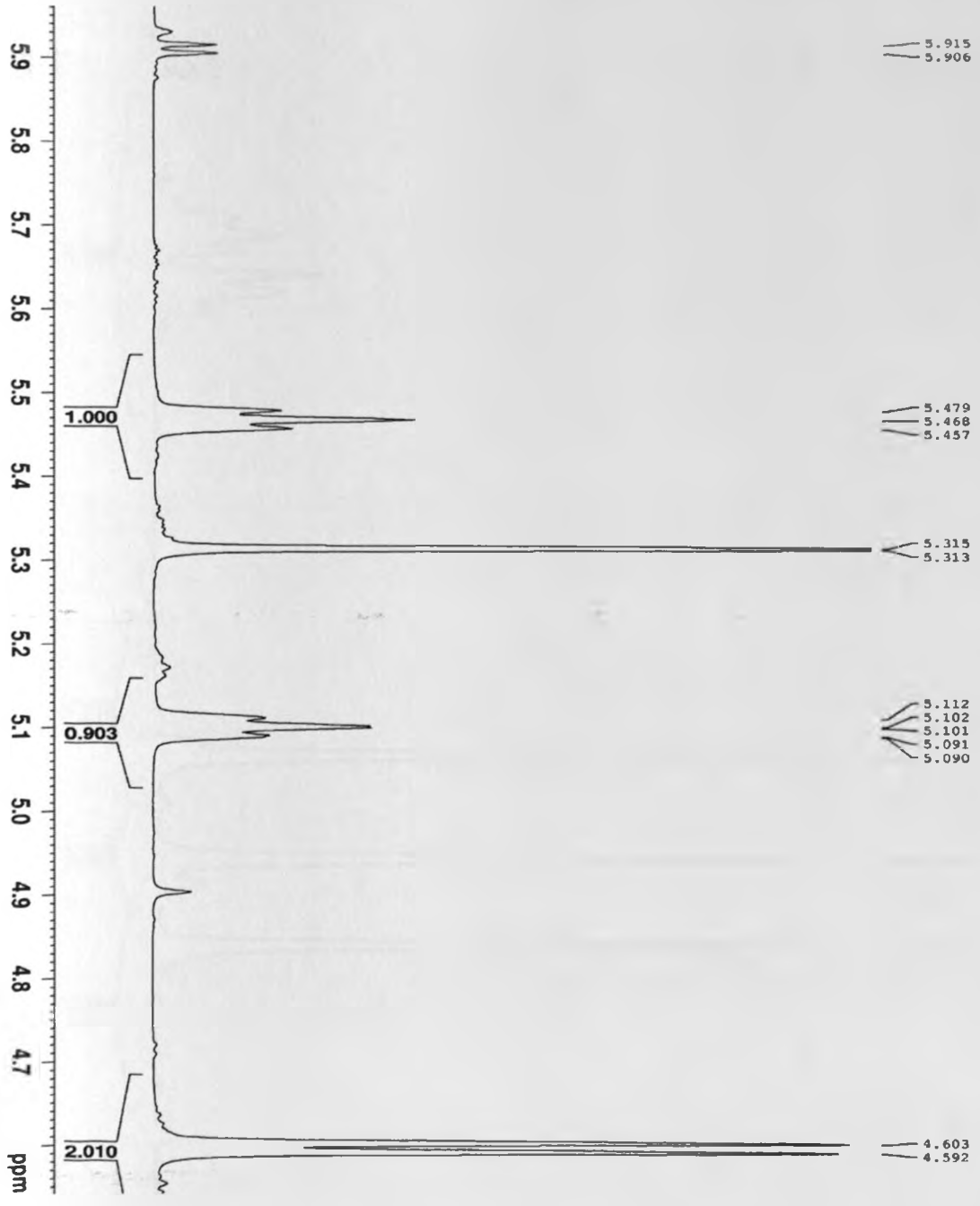
APPENDIX A: SPECTRA FOR COMPOUND **1**

^{1}H NMR SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



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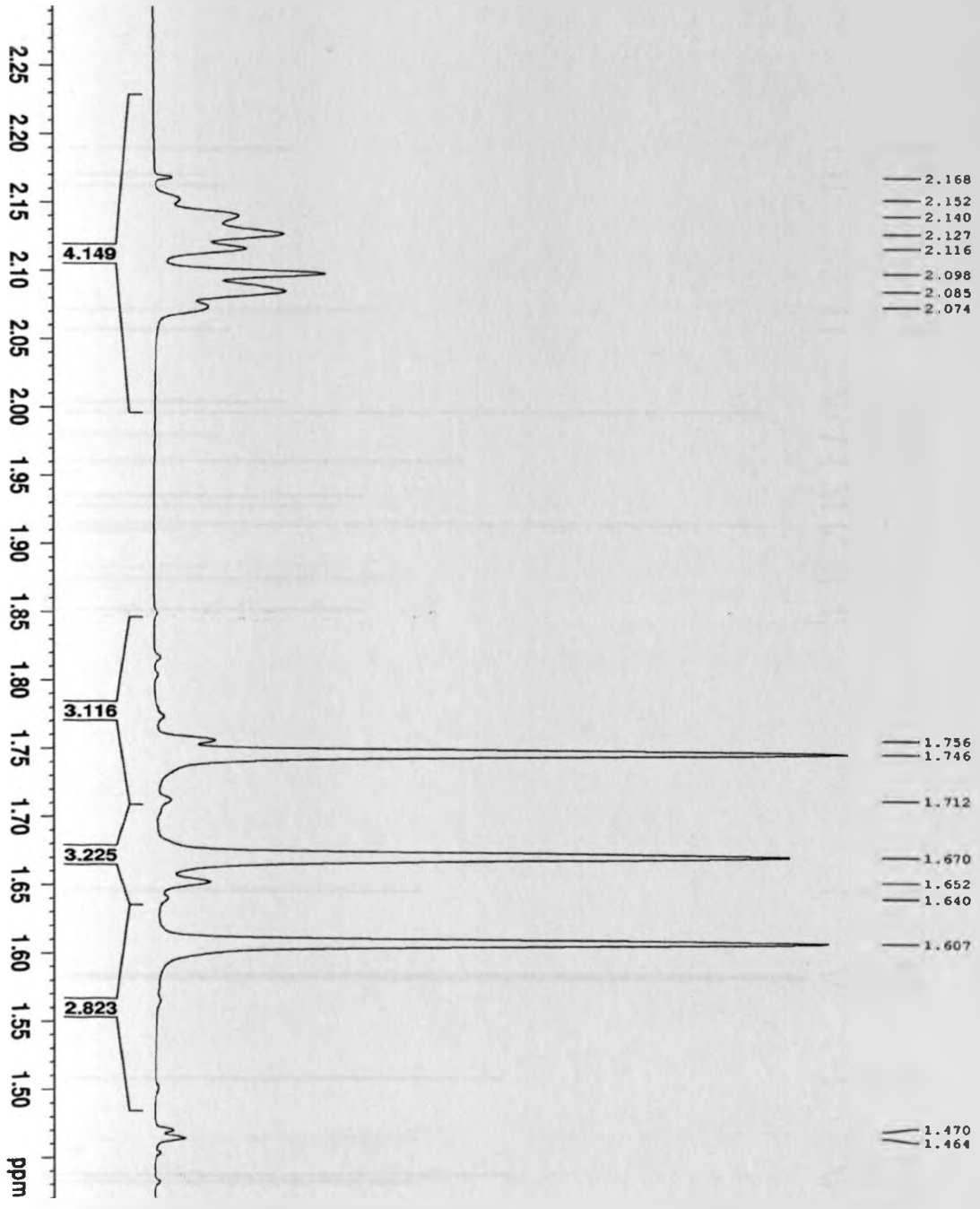
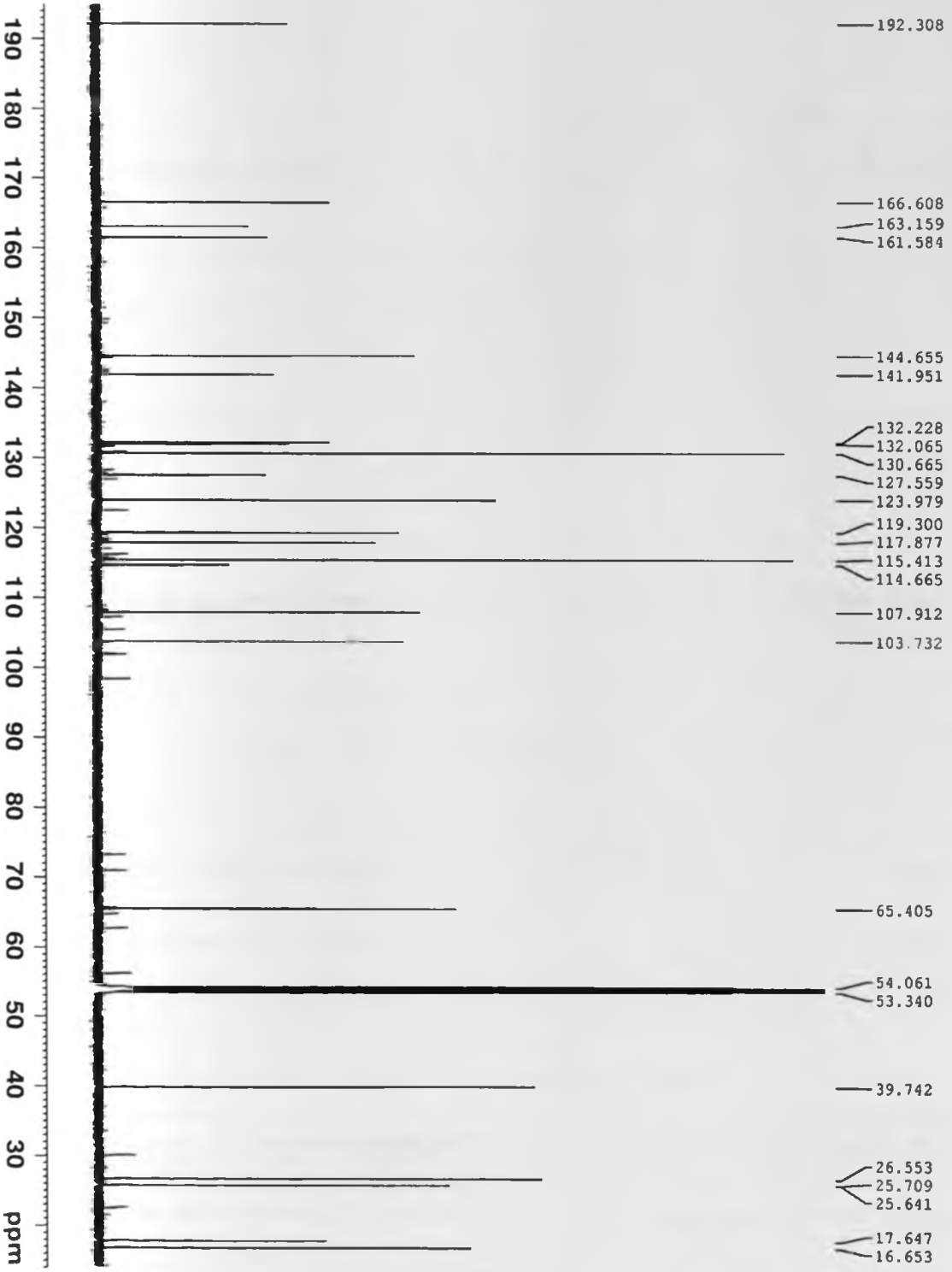


^{13}C NMR SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



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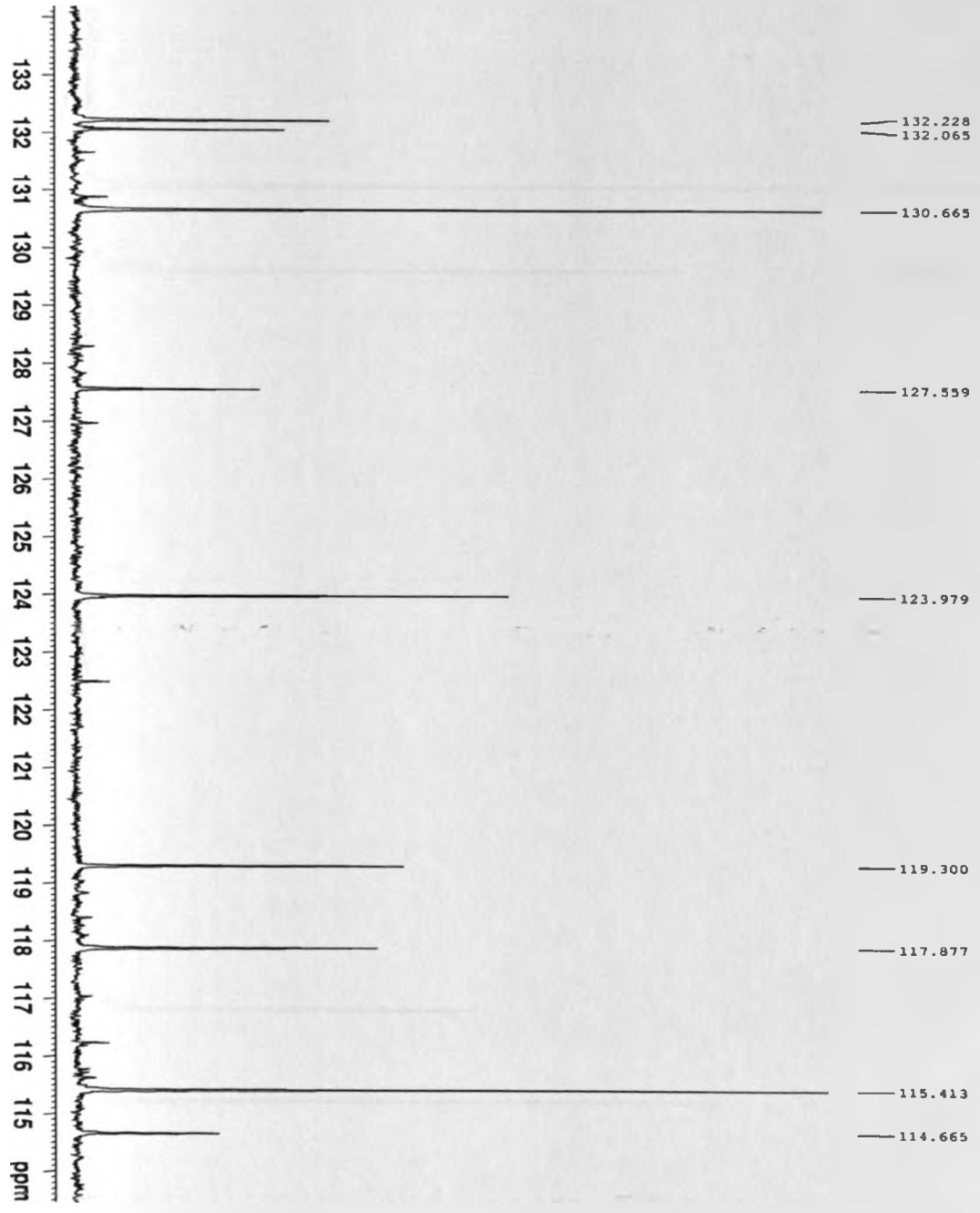
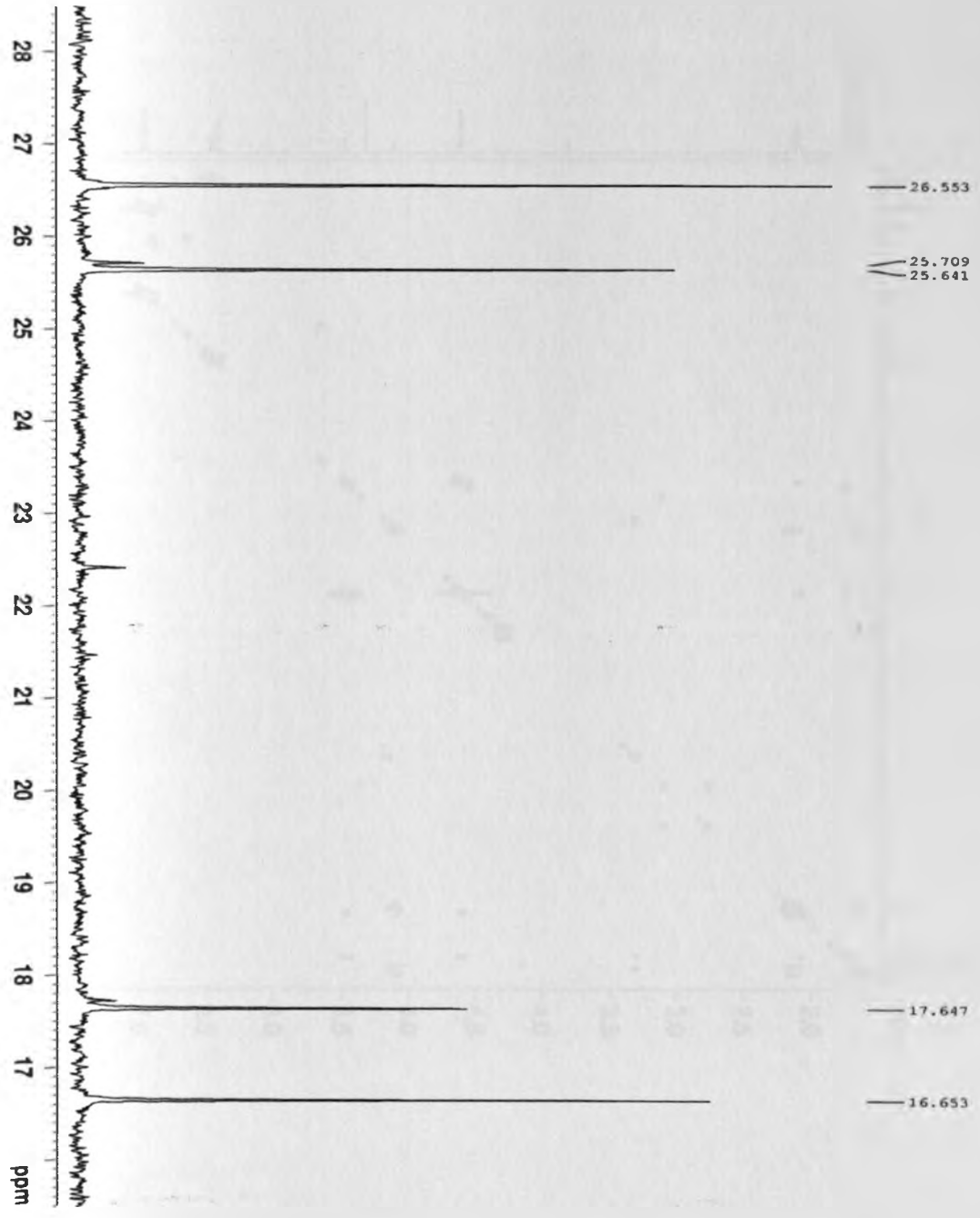
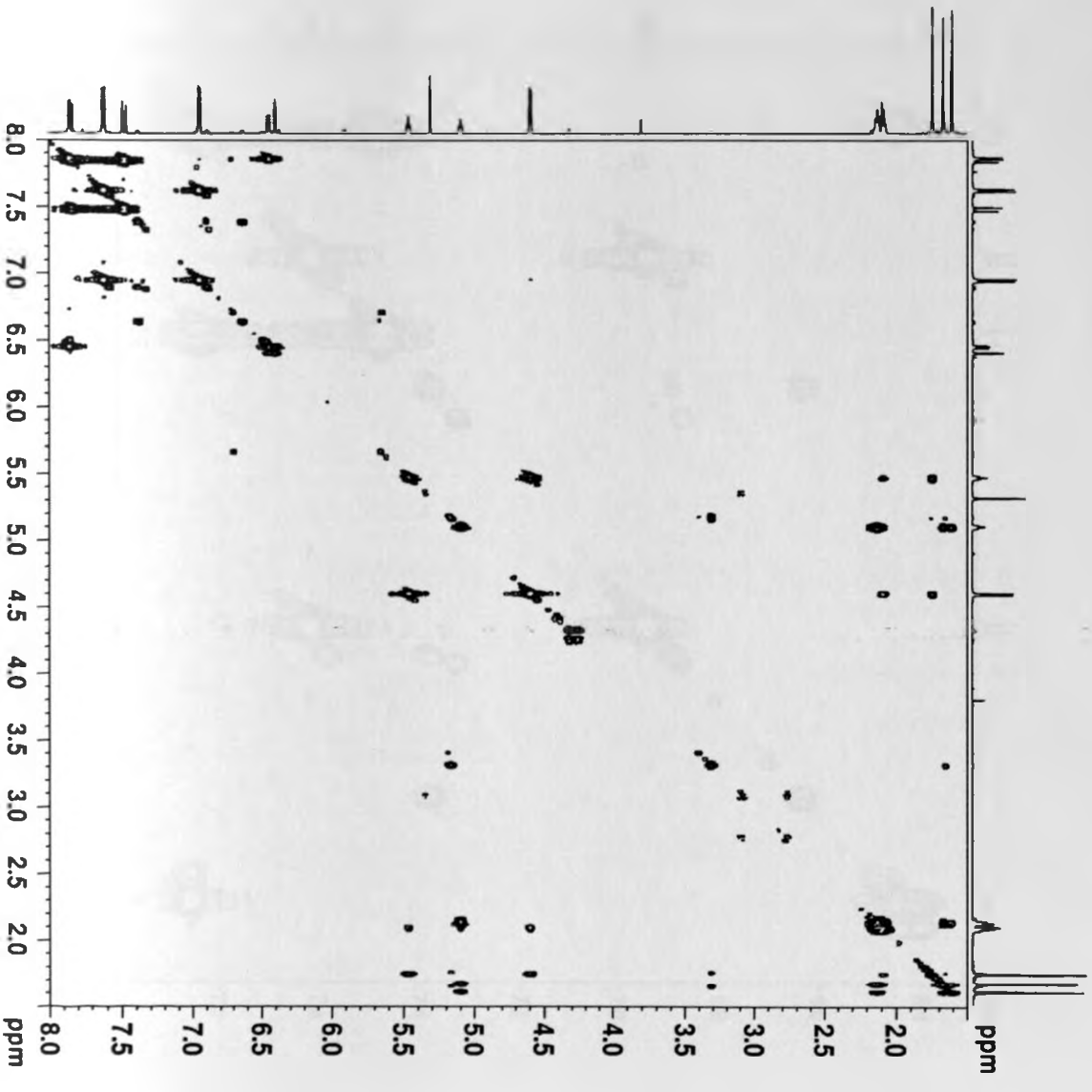


^{13}C NMR SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



COSY SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



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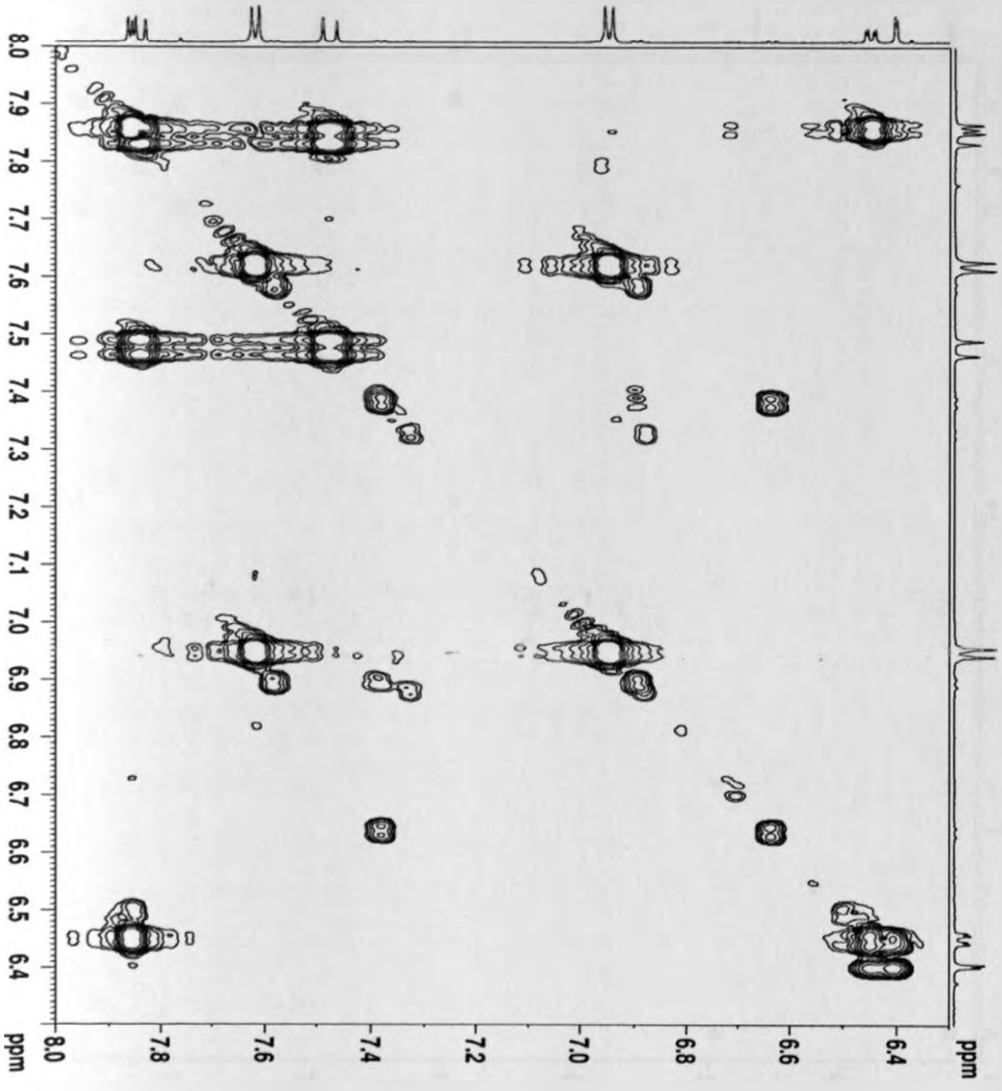

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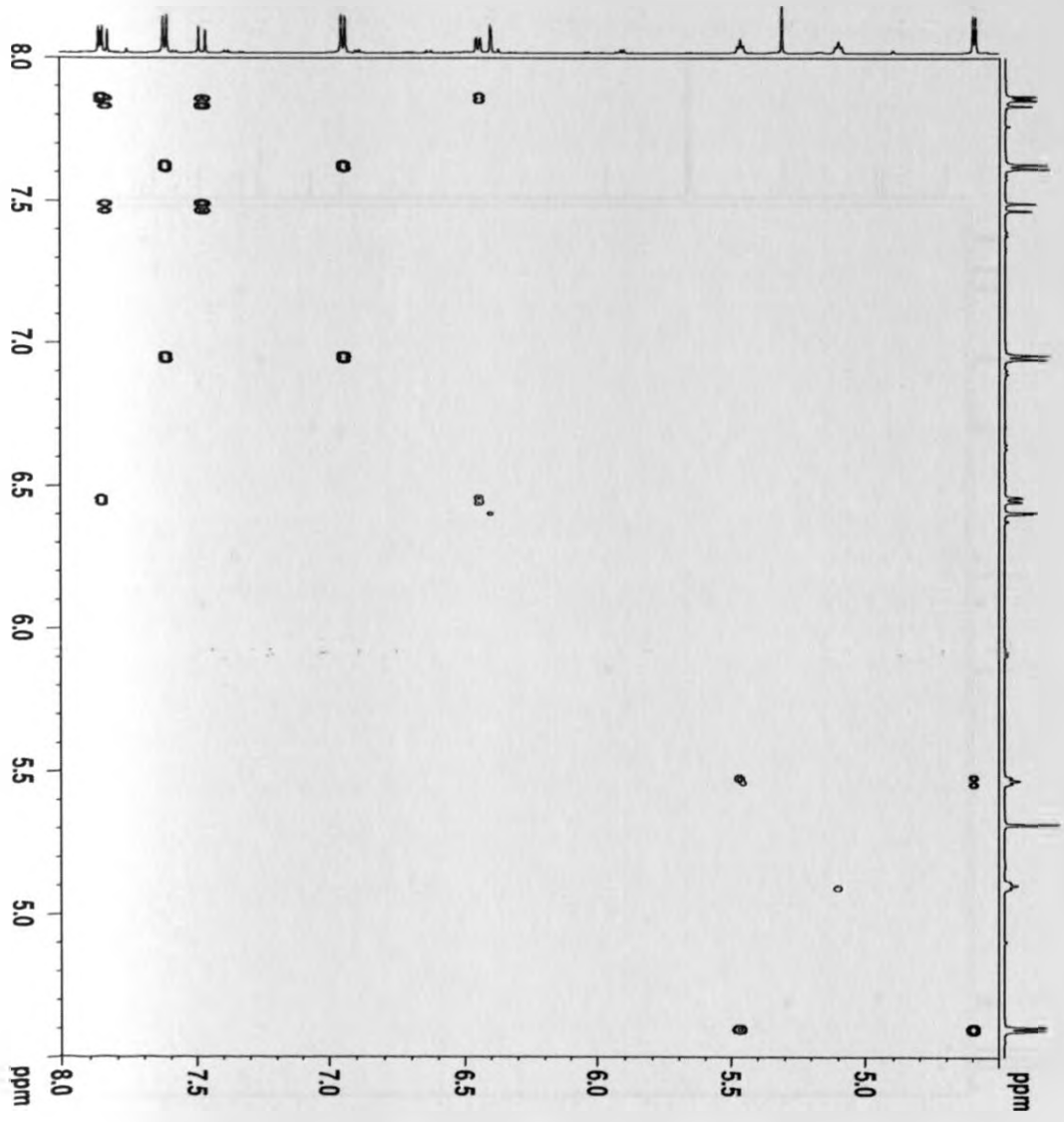
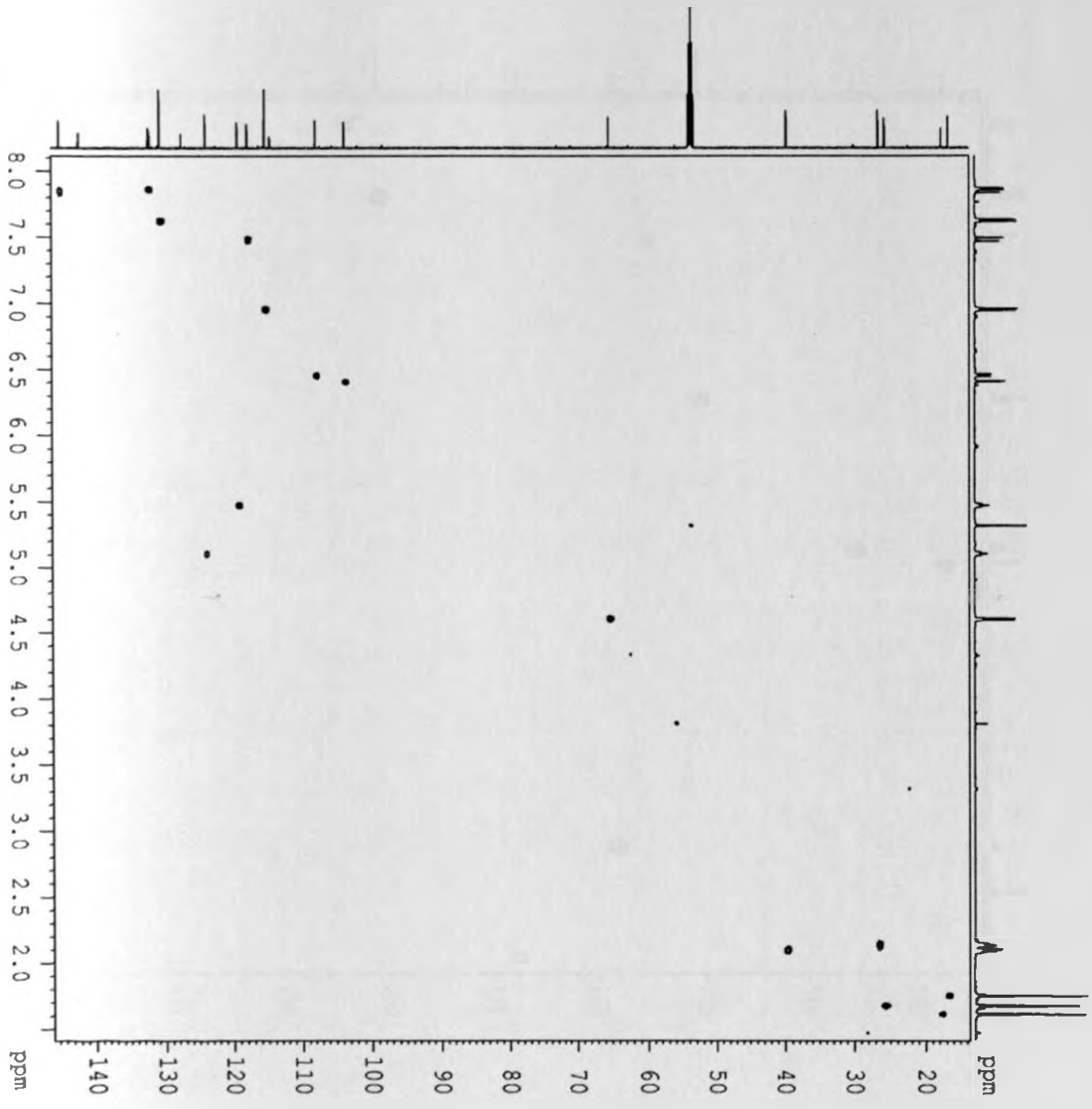
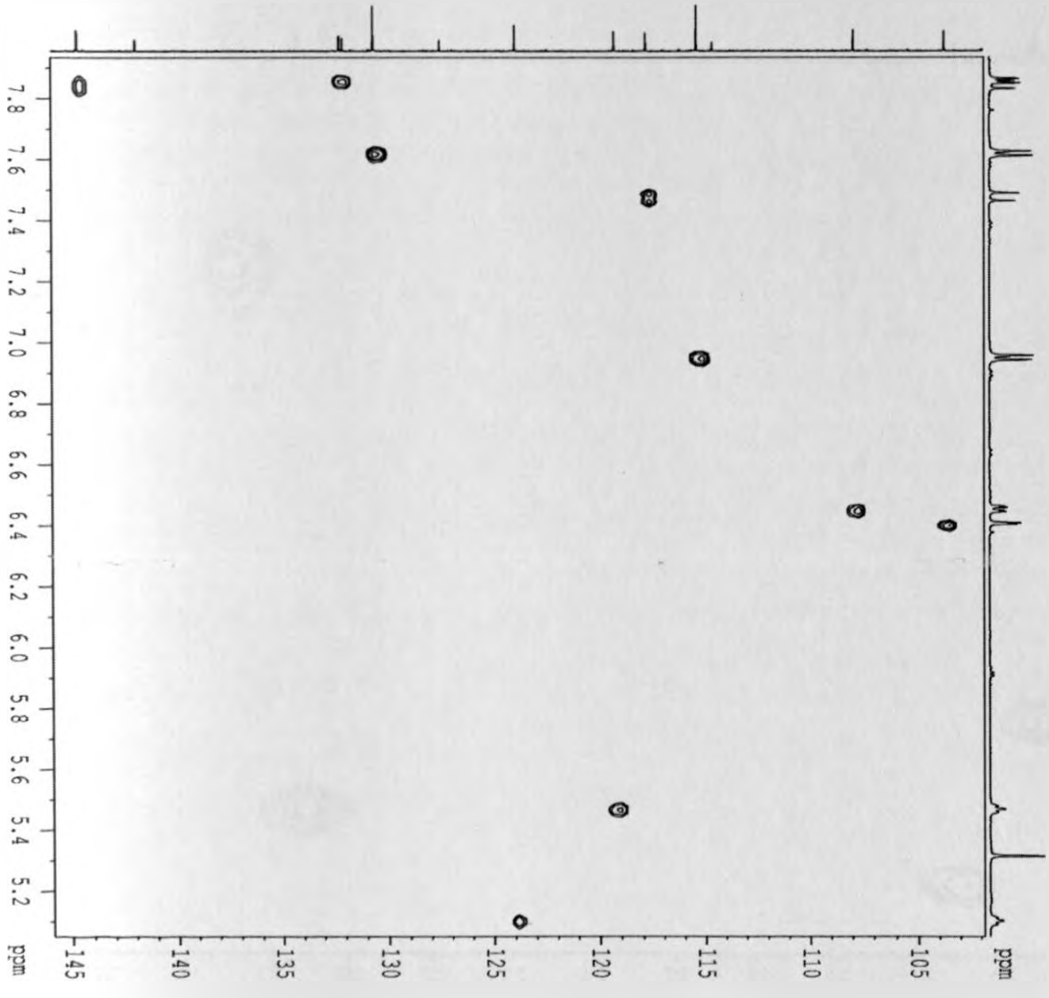


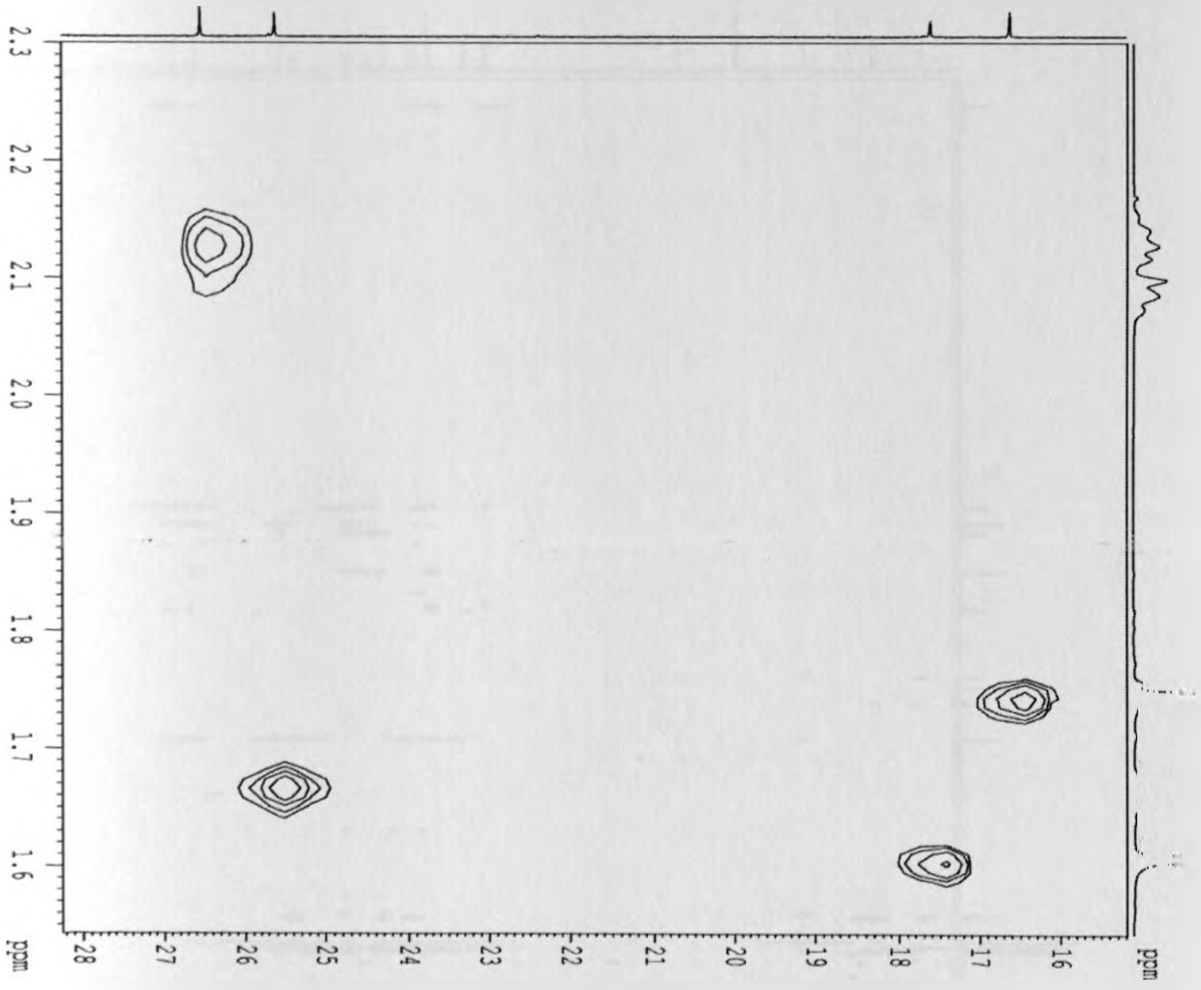
HMQC SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



HMQC SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



HMQC SPECTRUM FOR COMPOUND **1** (CD_2CL_2, 600 MHz)



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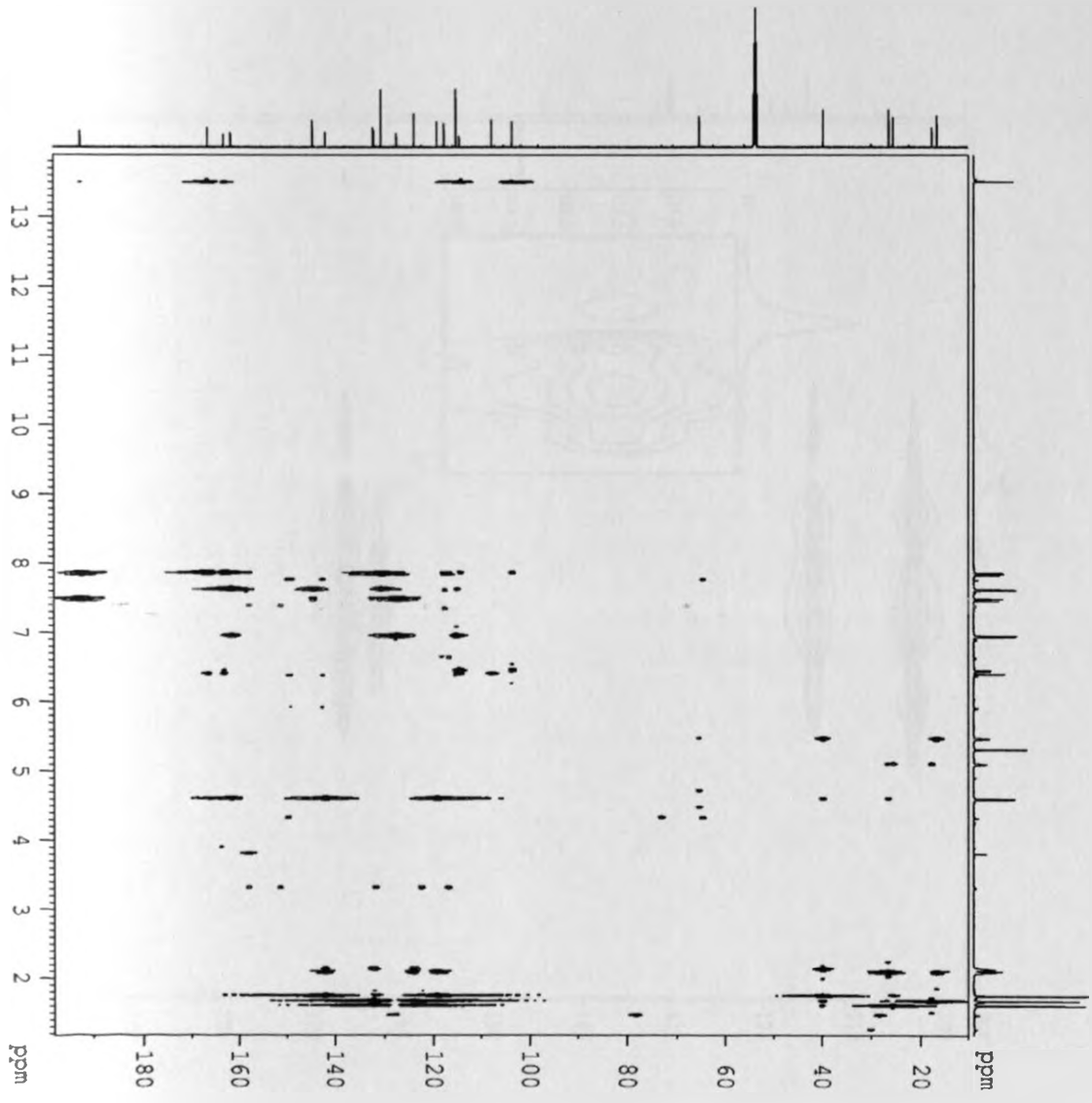

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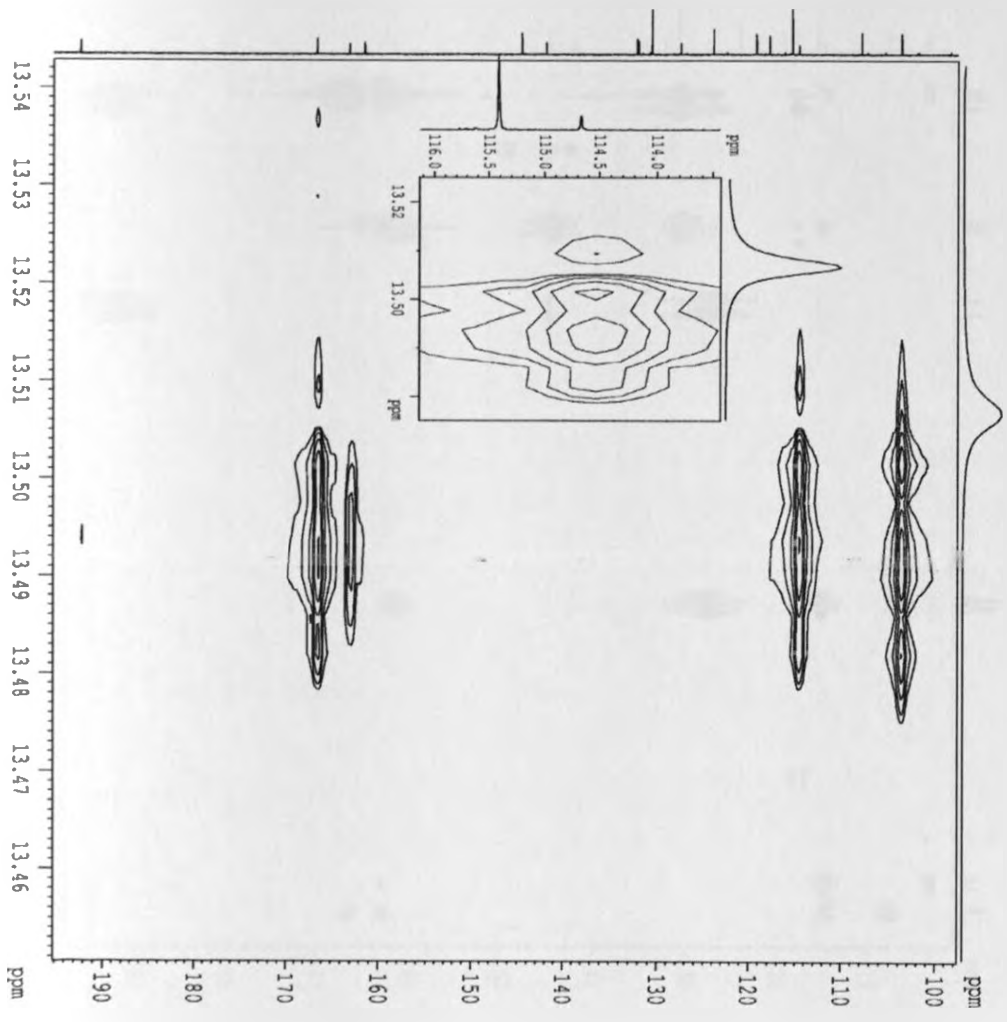

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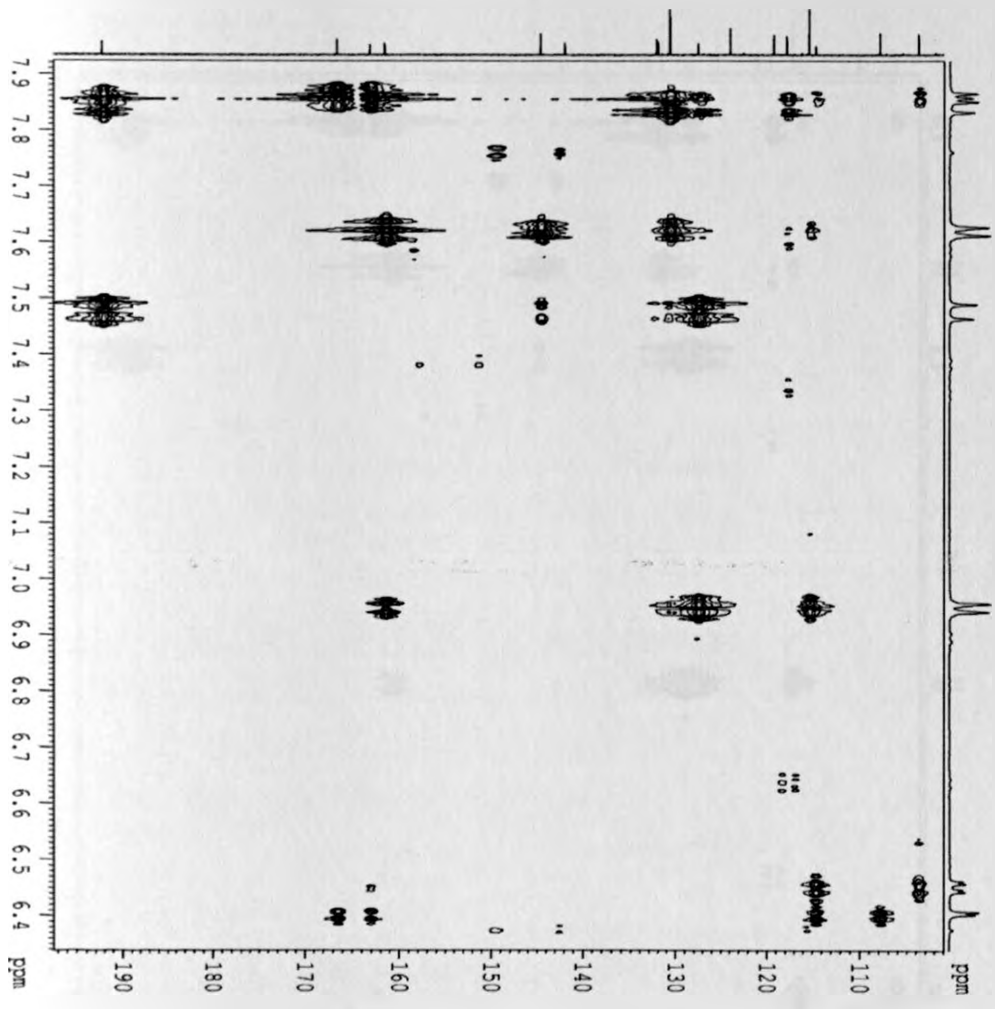

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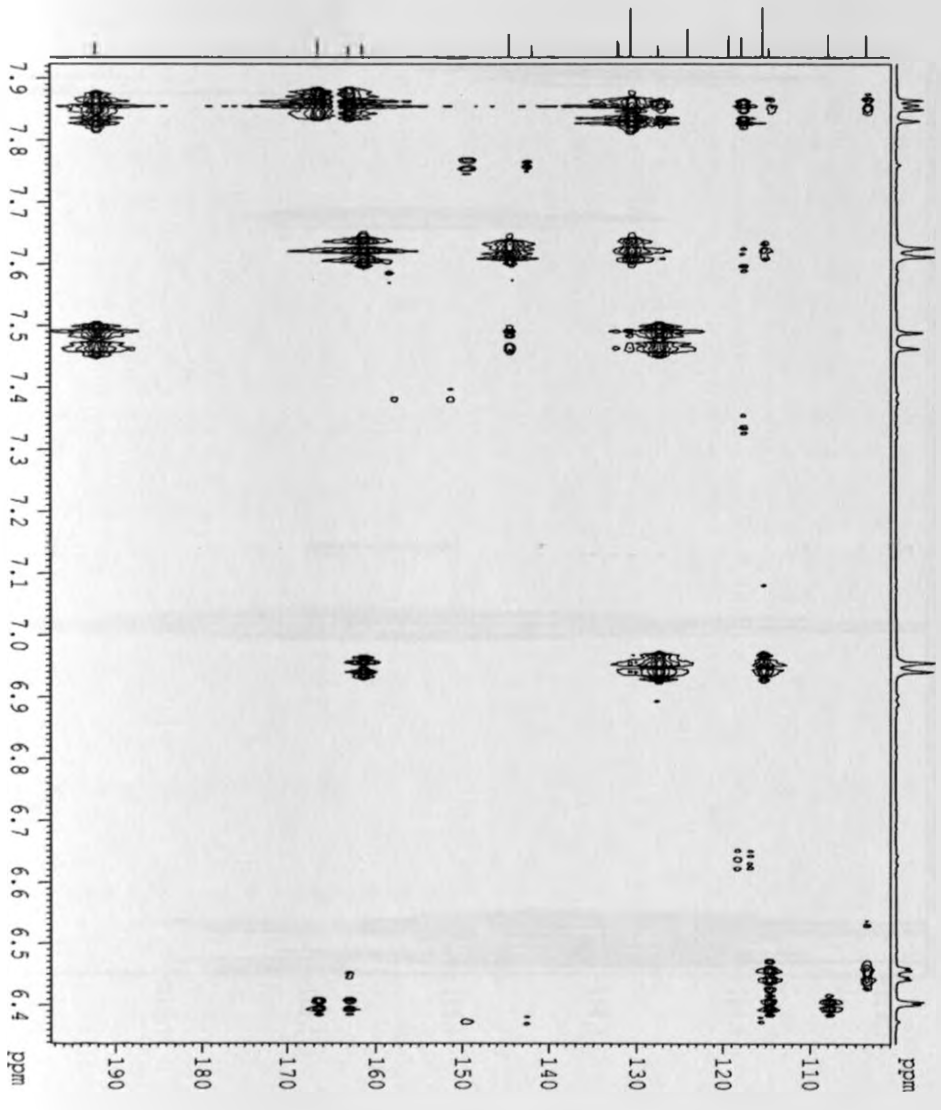

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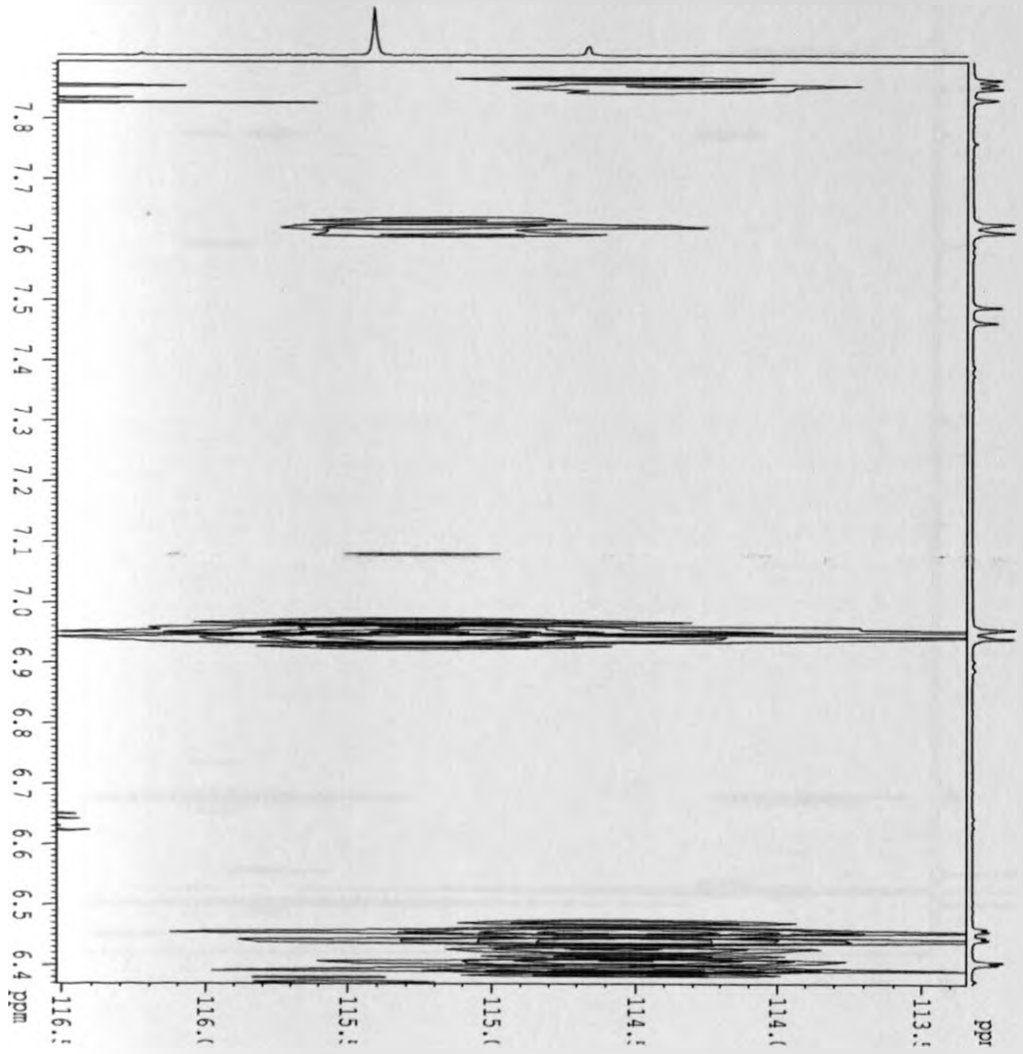

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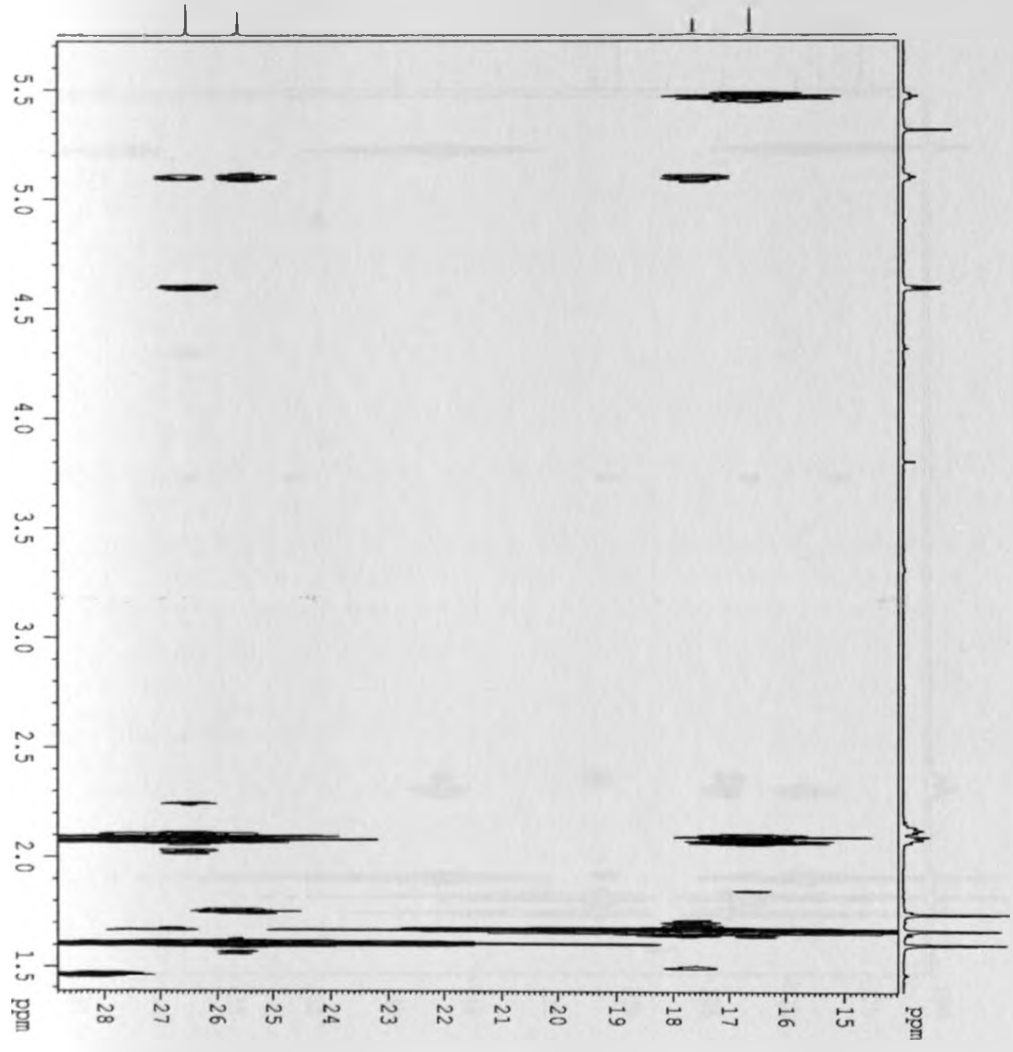

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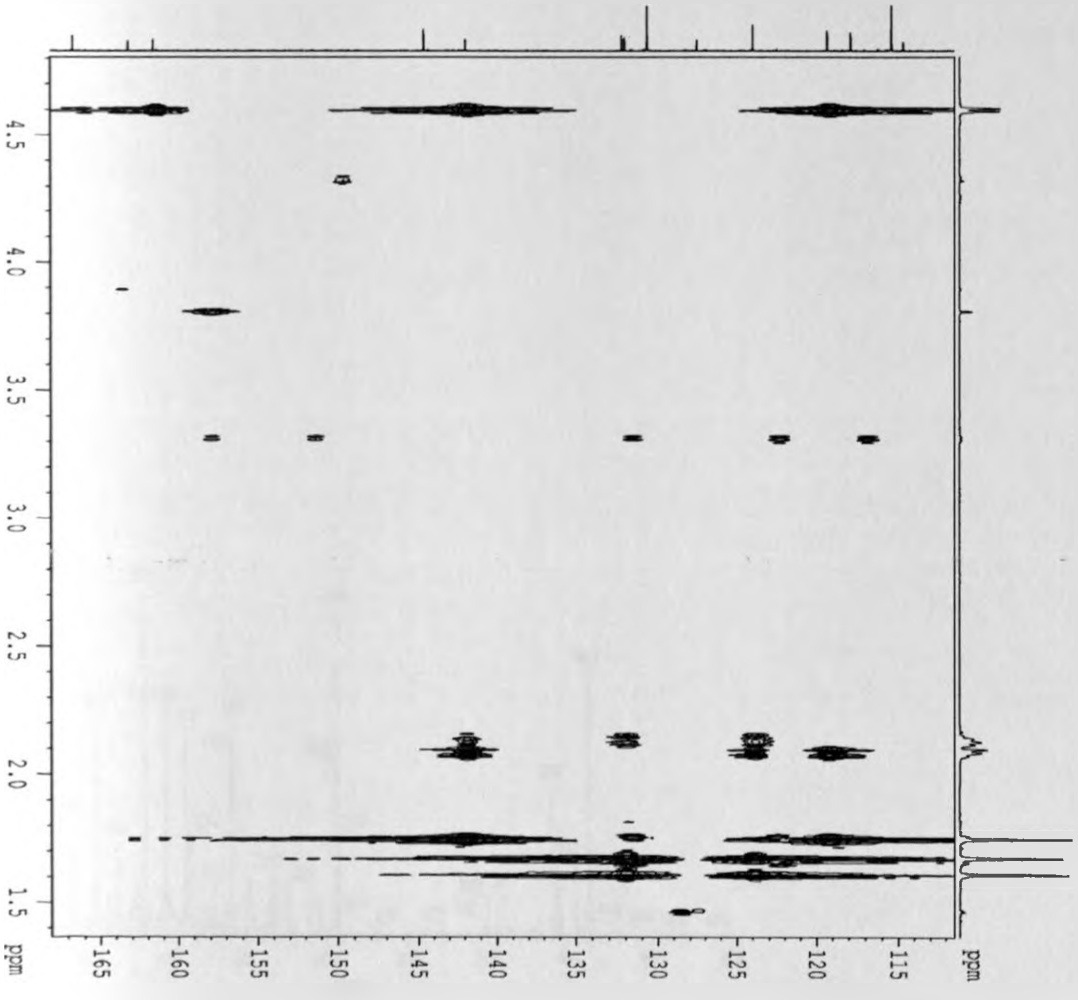
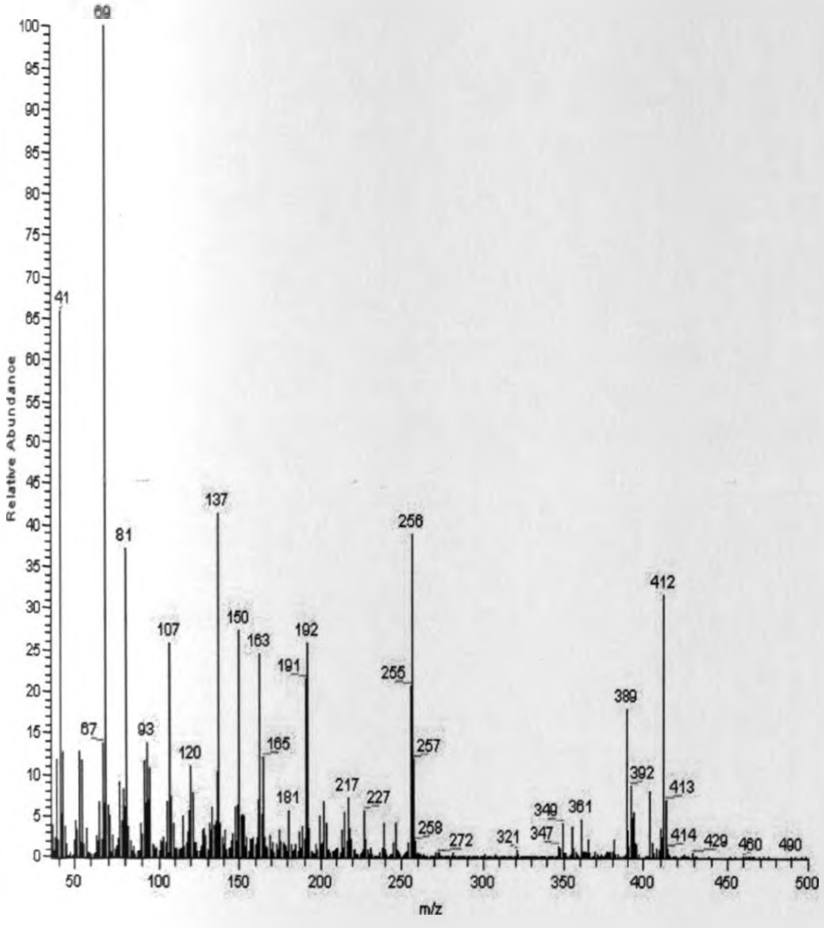
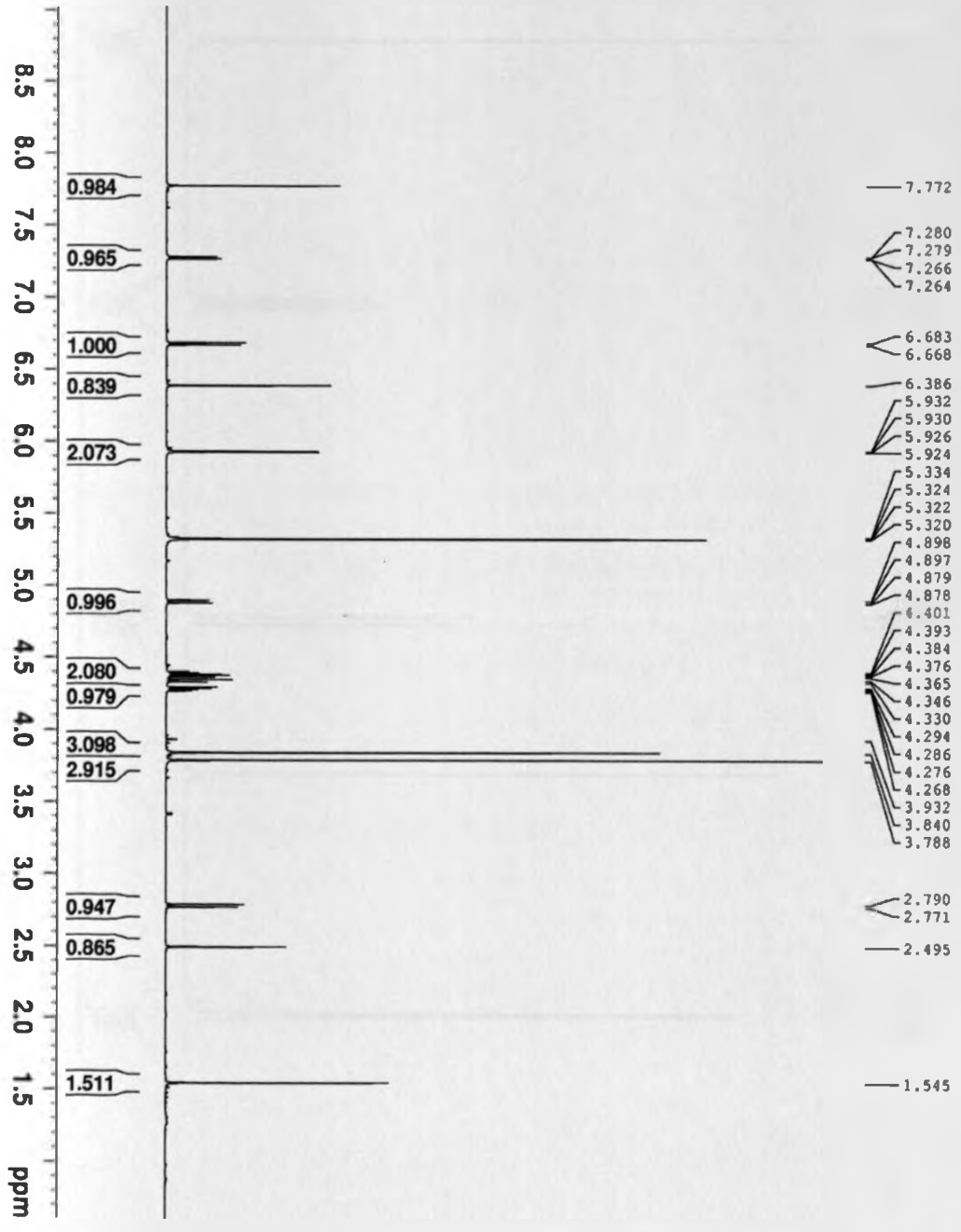


MASS SPECTRUM FOR COMPOUND **1**

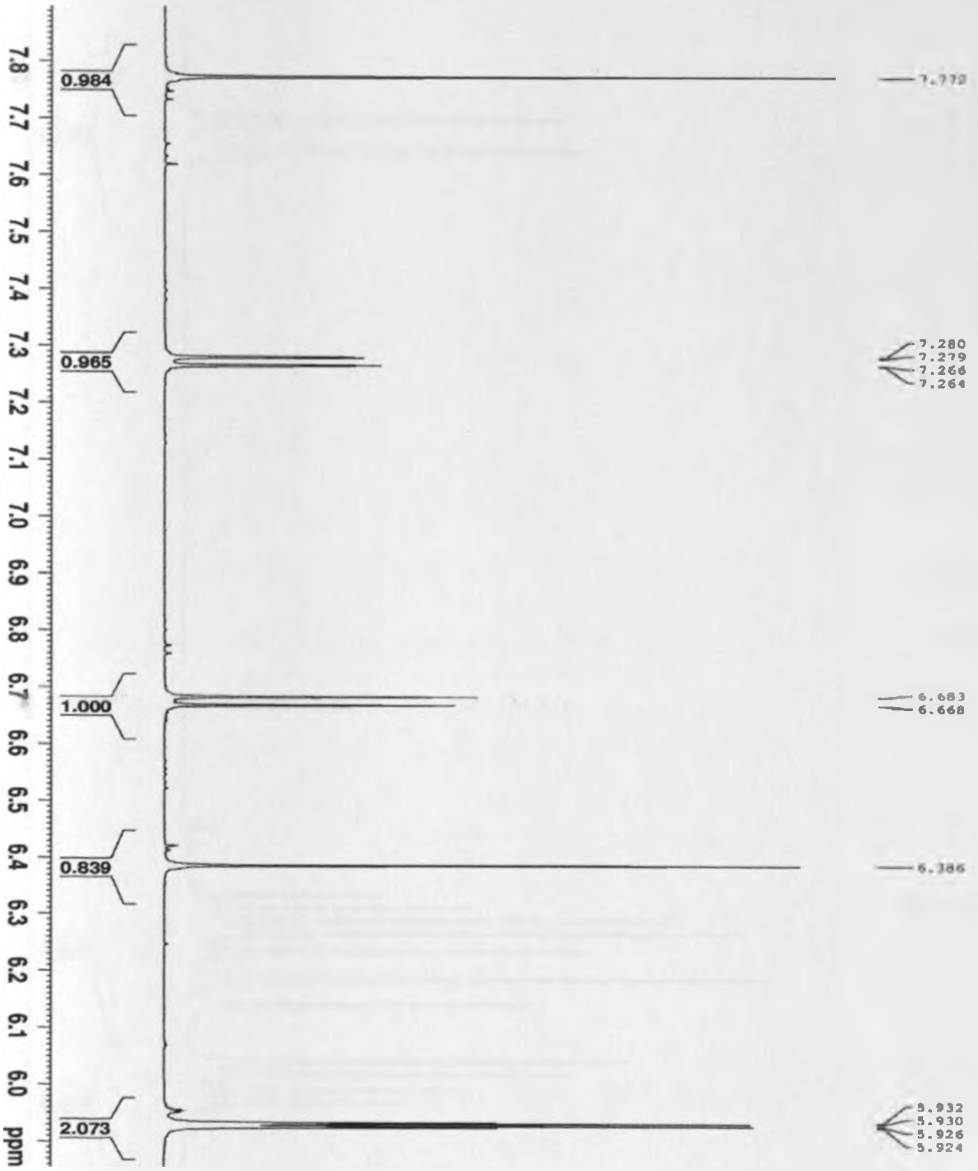


APPENDIX B: SPECTRA FOR COMPOUND **2**

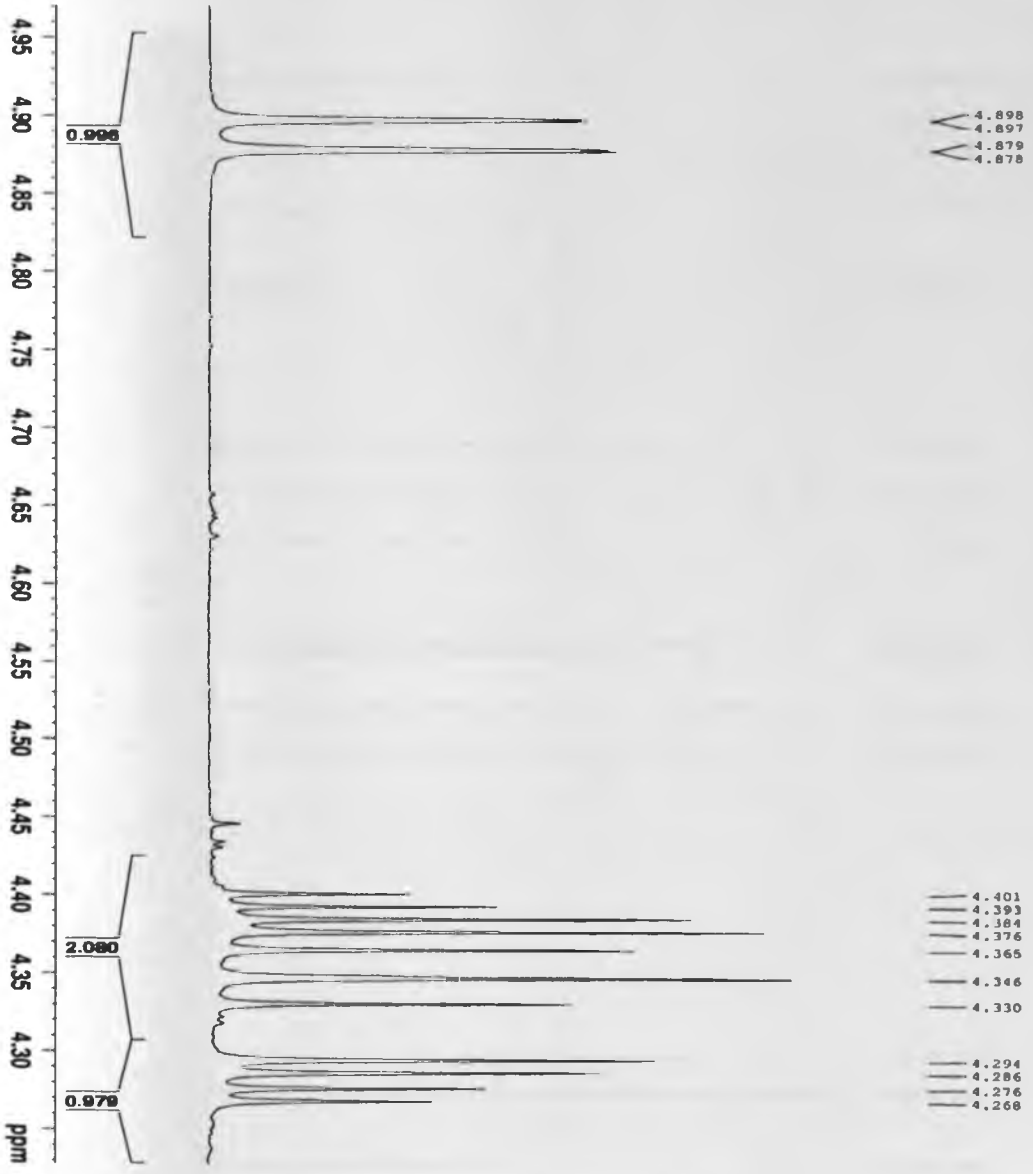
1H NMR SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



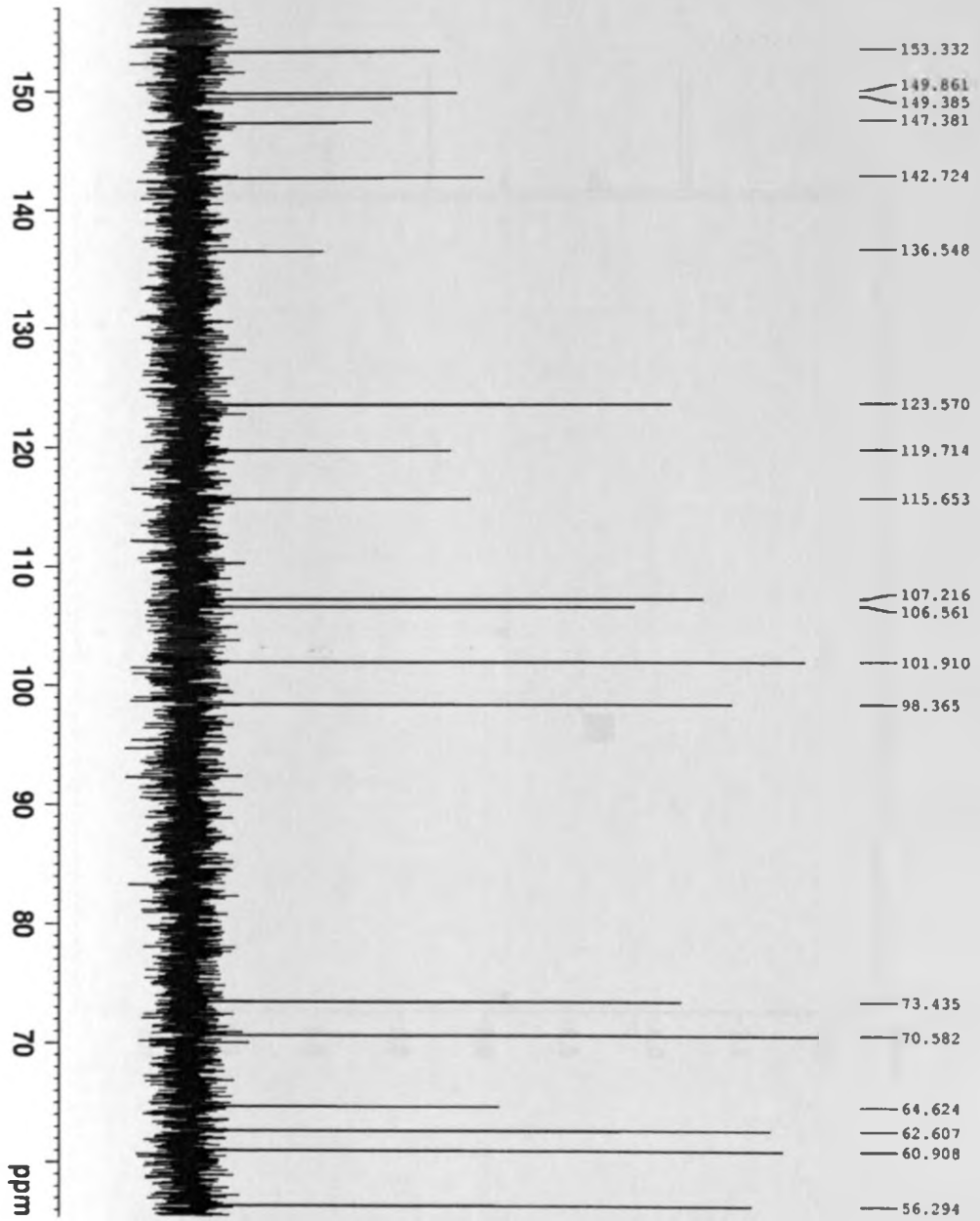
^{1}H NMR SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



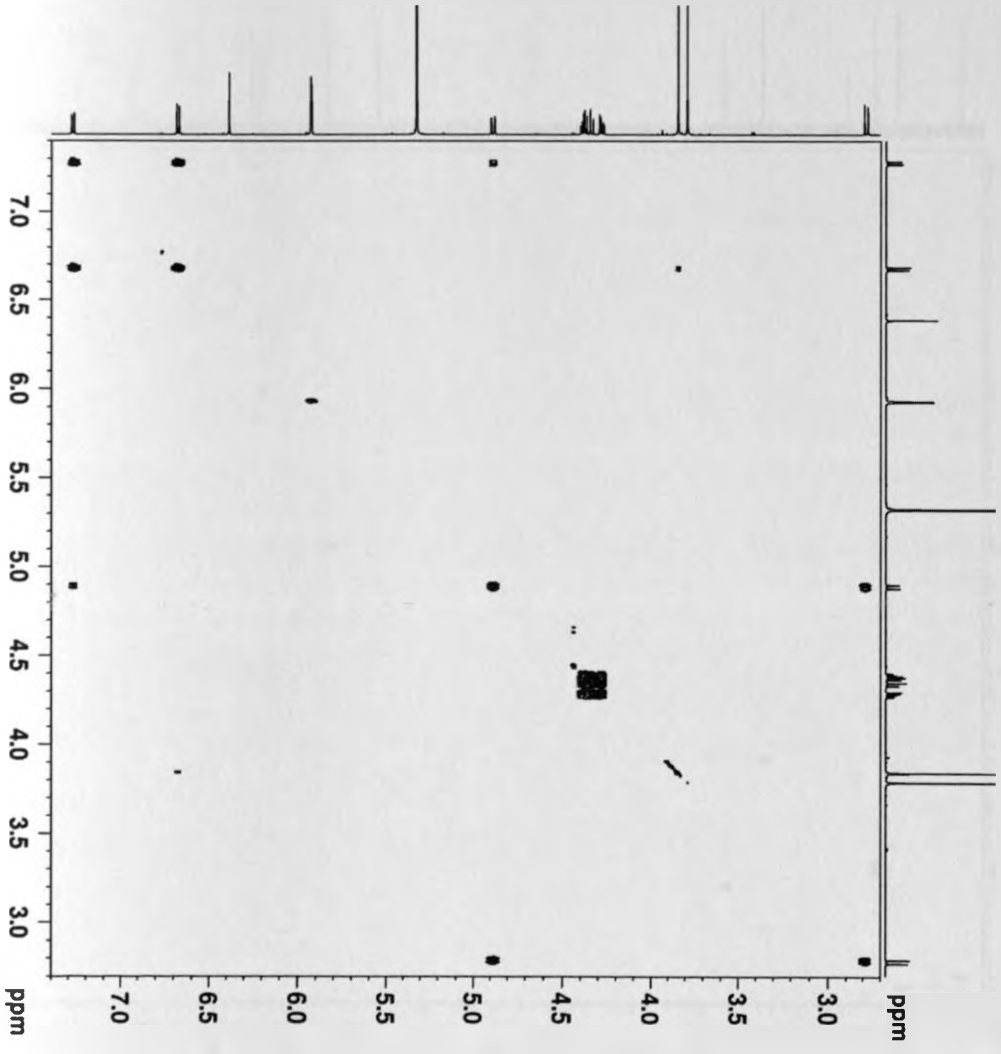
^{1}H NMR SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



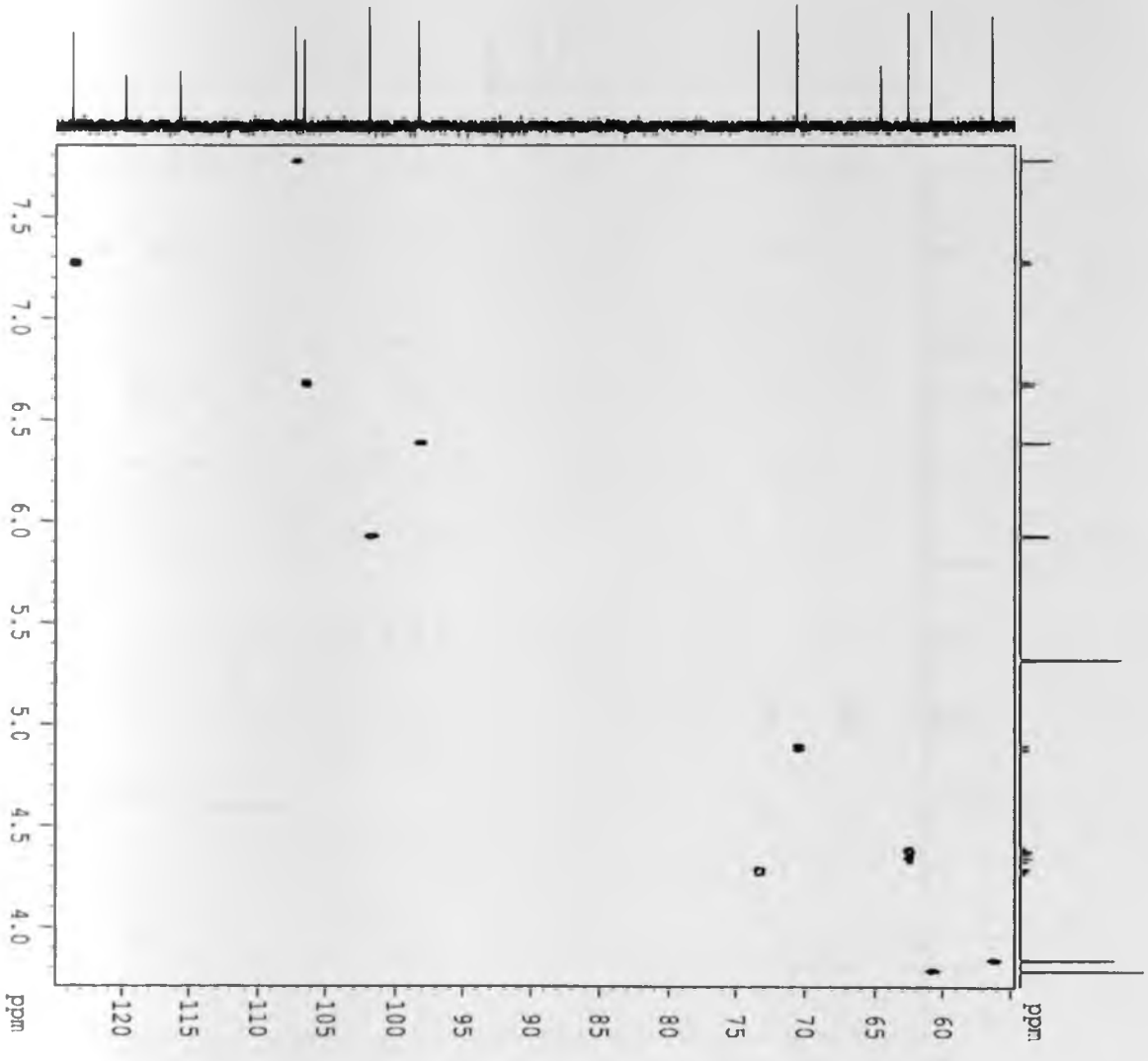
^{13}C SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



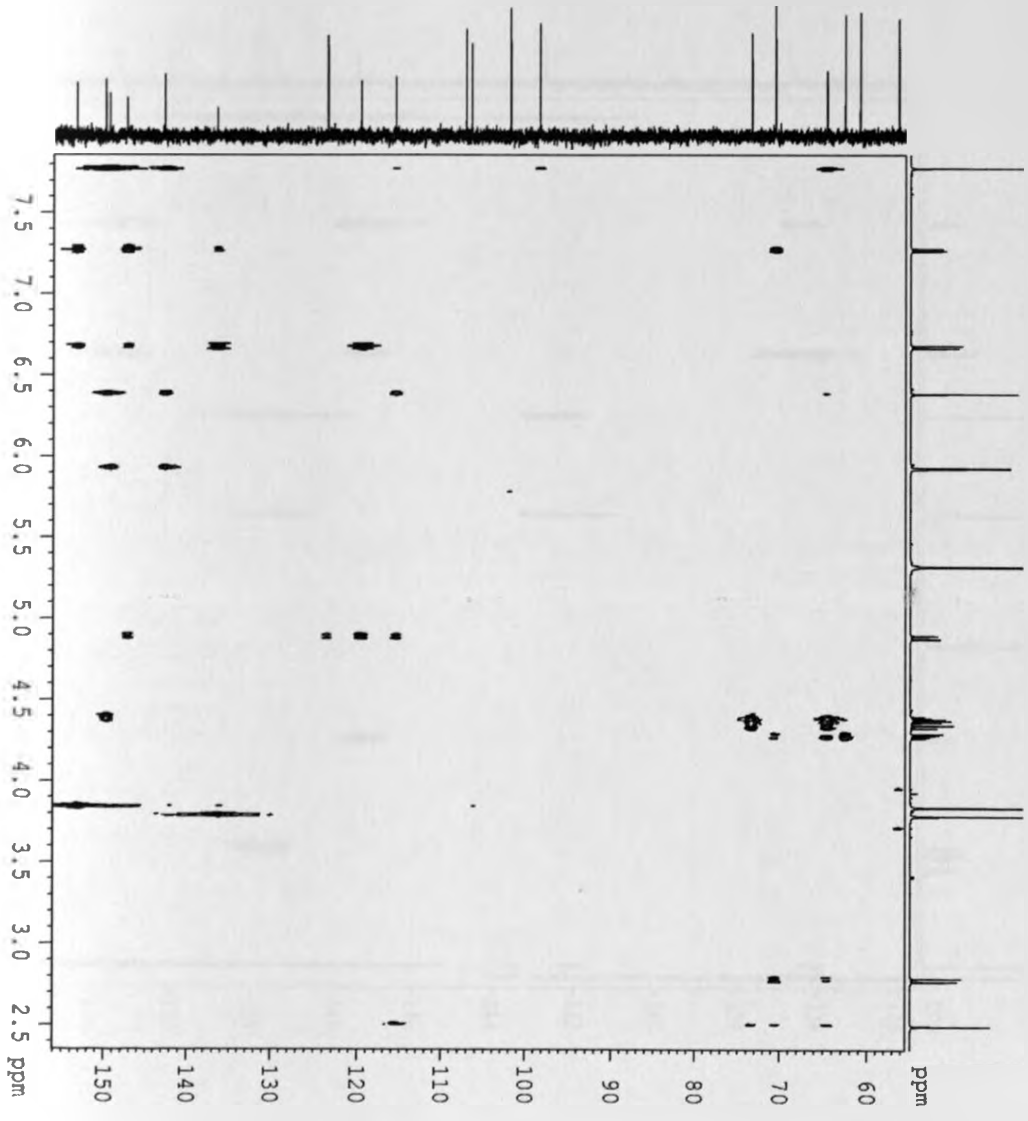
COSY SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



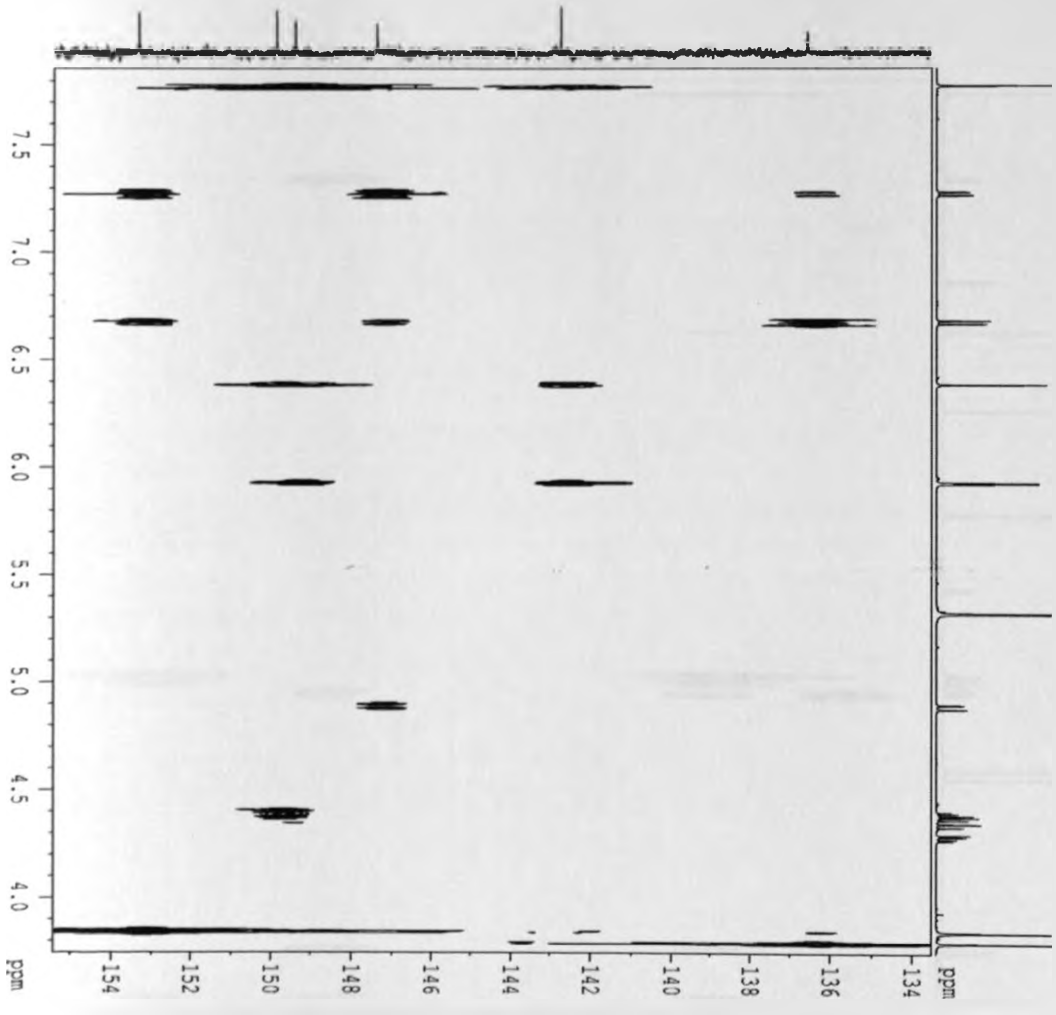
HMQC SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



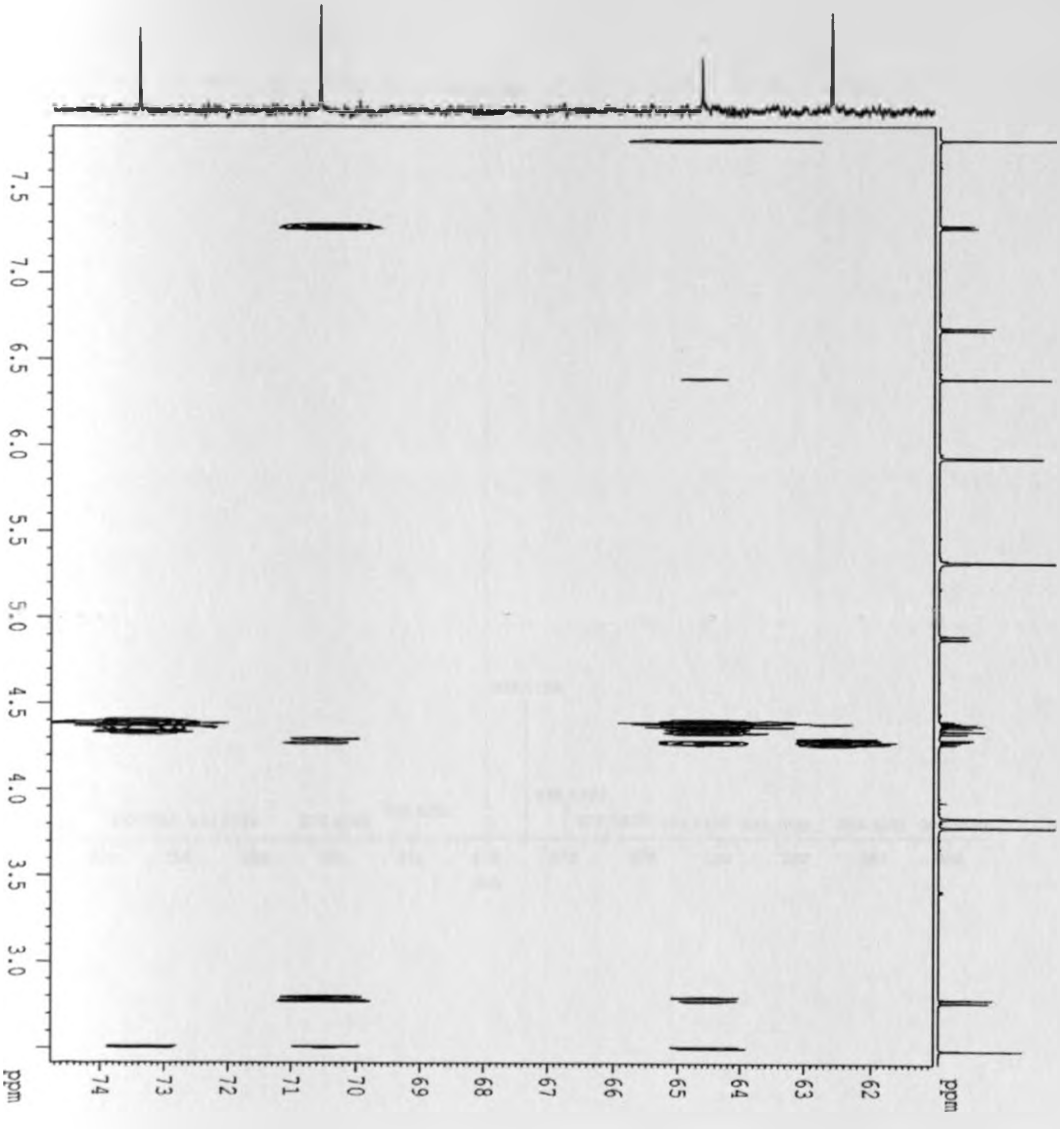
HMBC SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



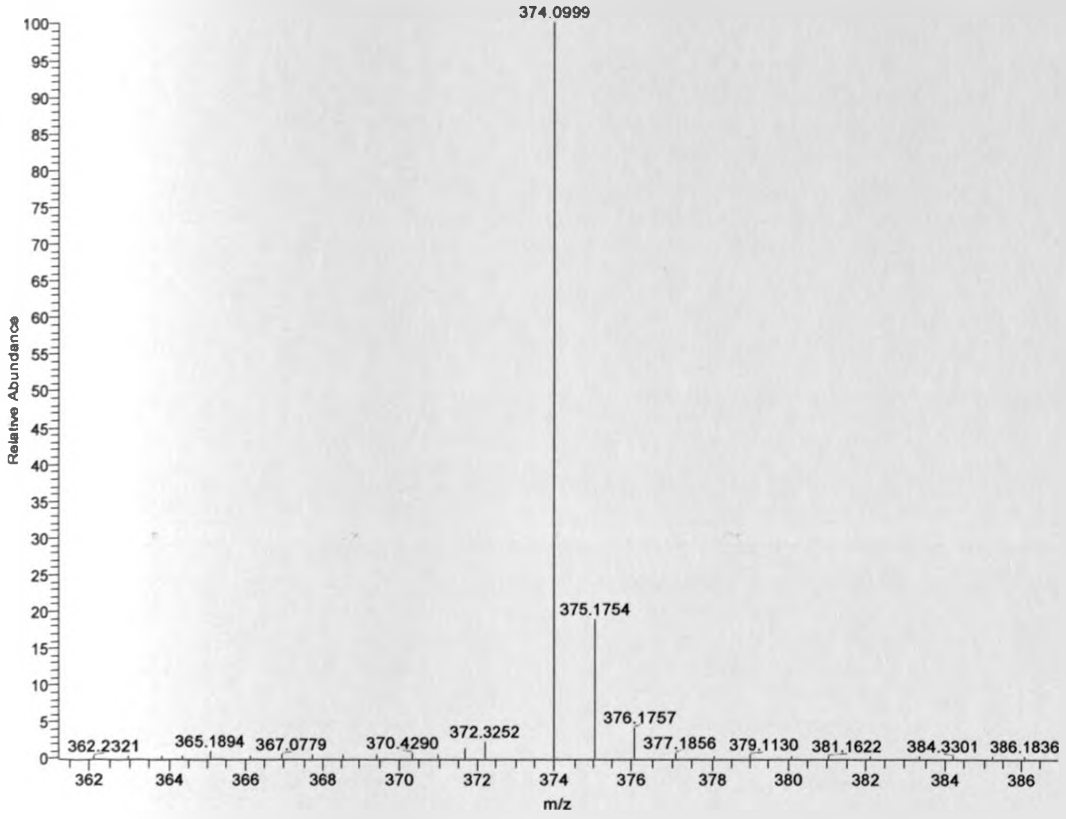
HMBC SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)



HMBC SPECTRUM FOR COMPOUND **2** (CD_2CL_2, 600 MHz)

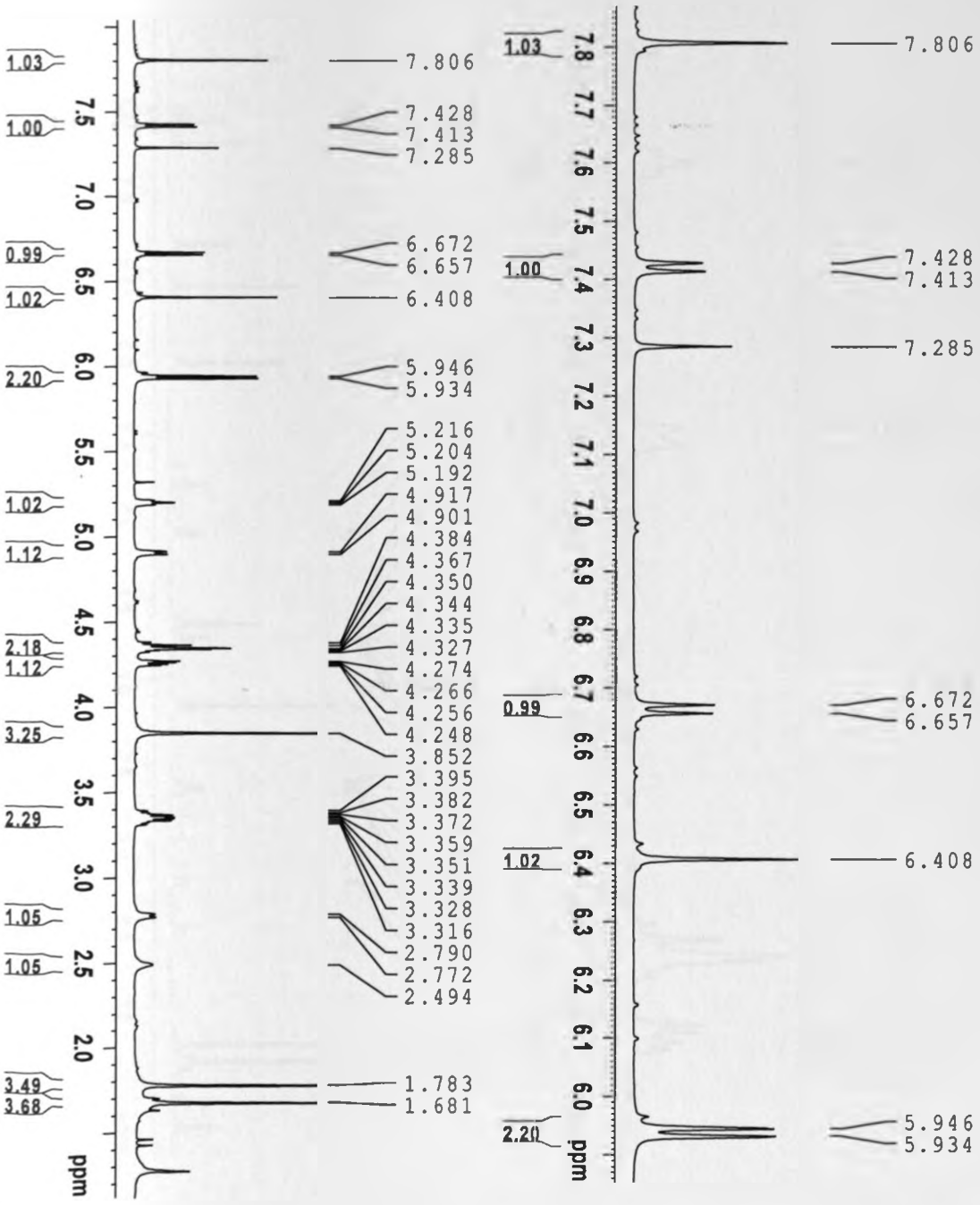


MASS SPECTRUM FOR COMPOUND **2**

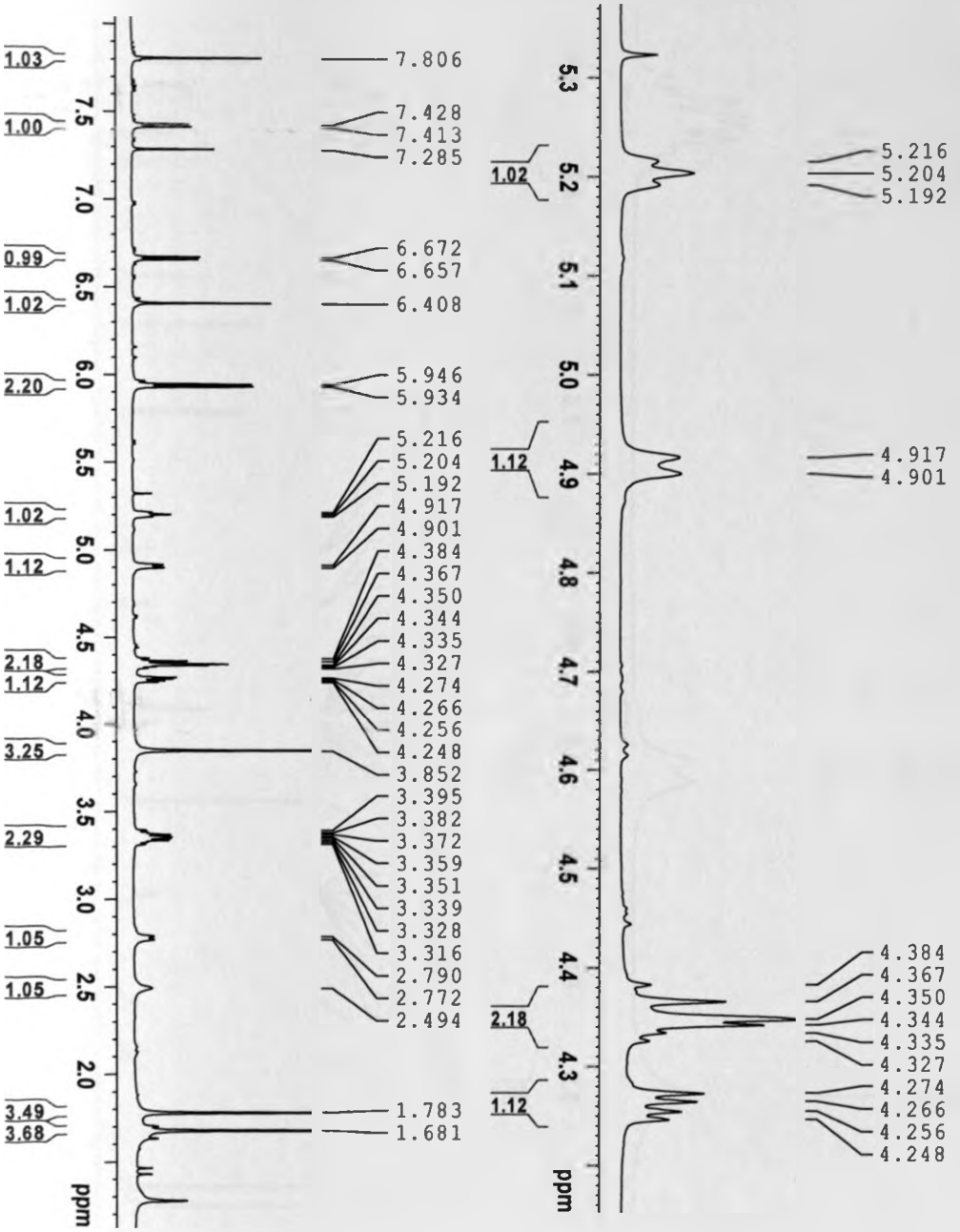


APPENDIX C: SPECTRA FOR COMPOUND **3**

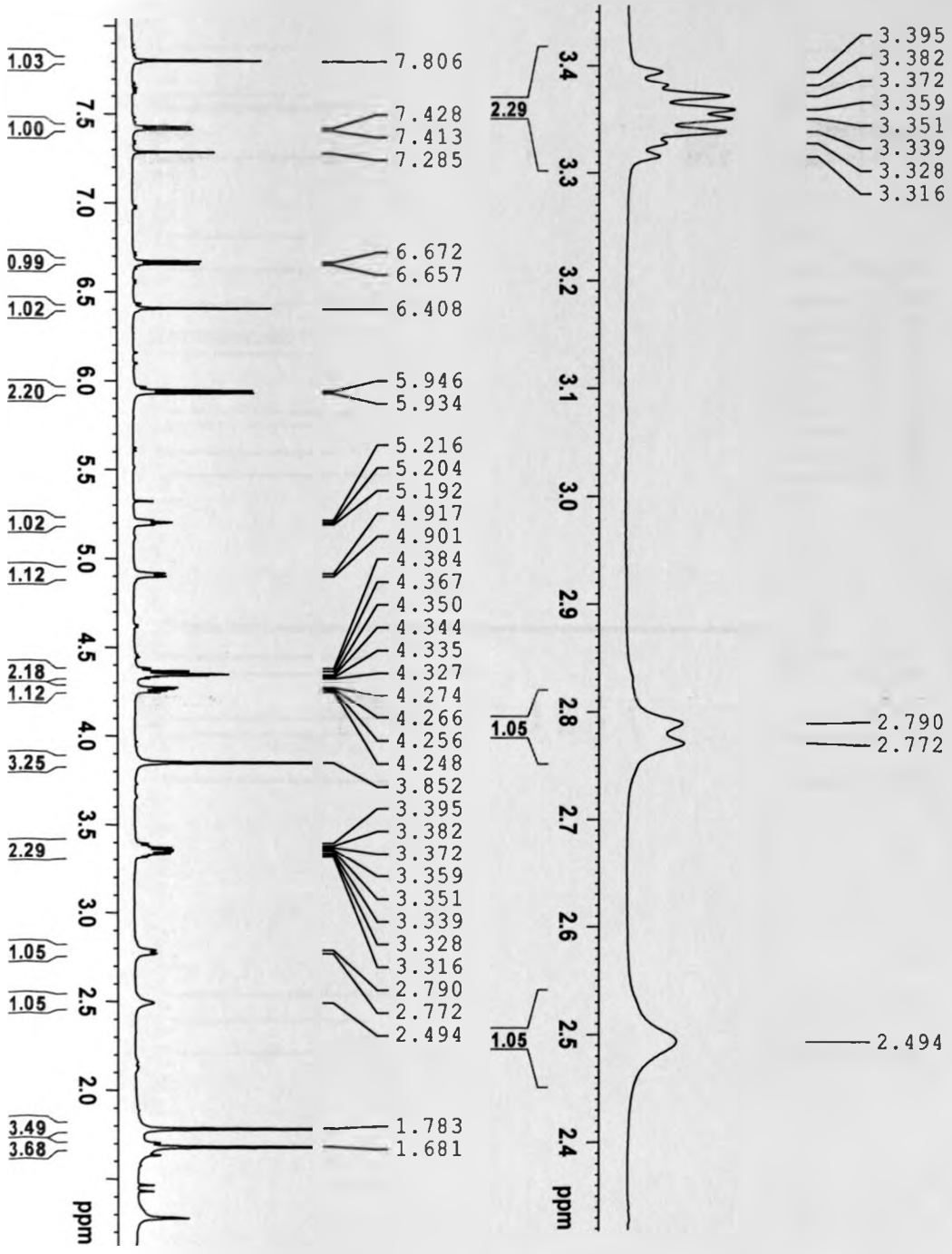
^{1}H NMR SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



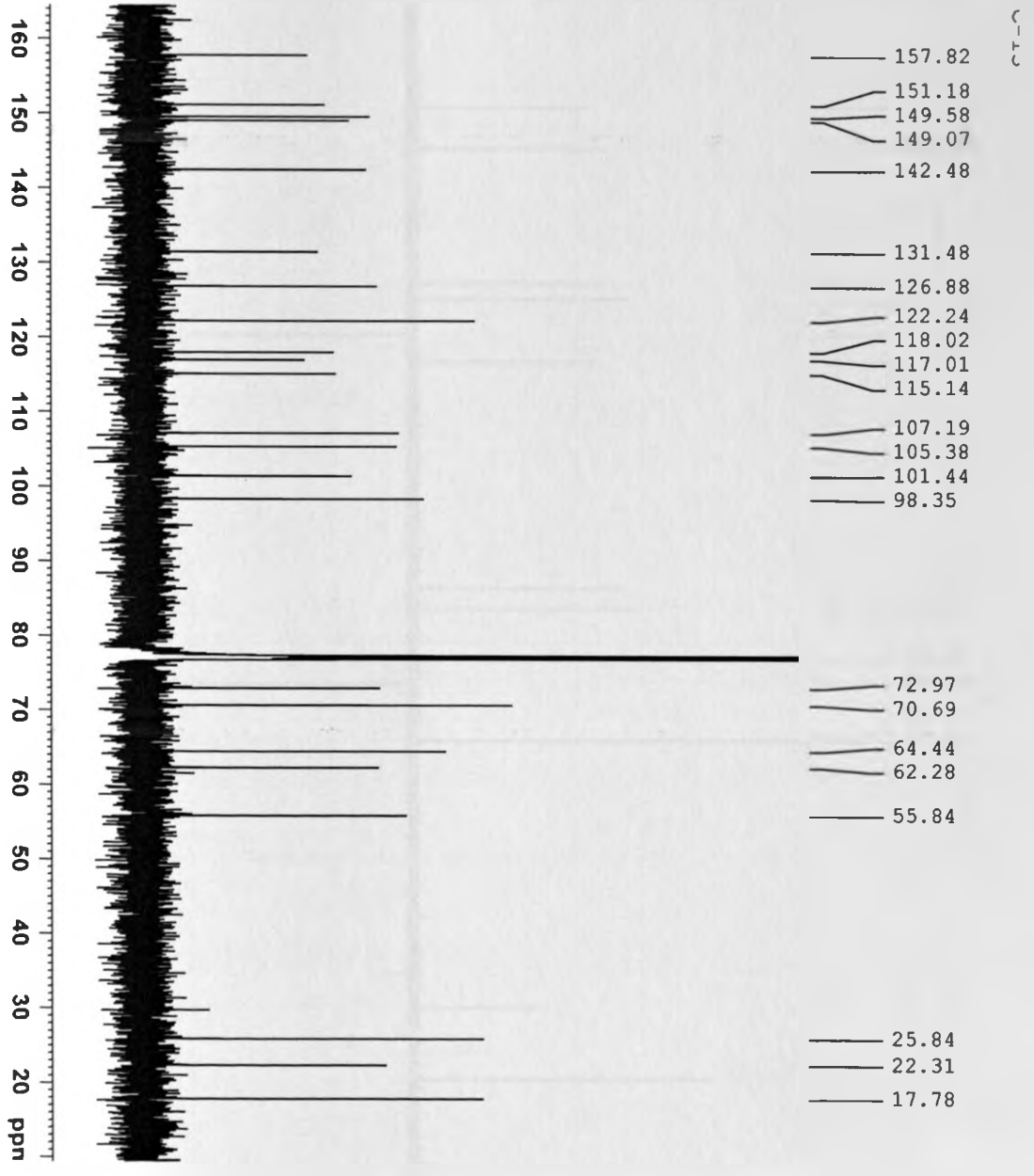
^{1}H NMR SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



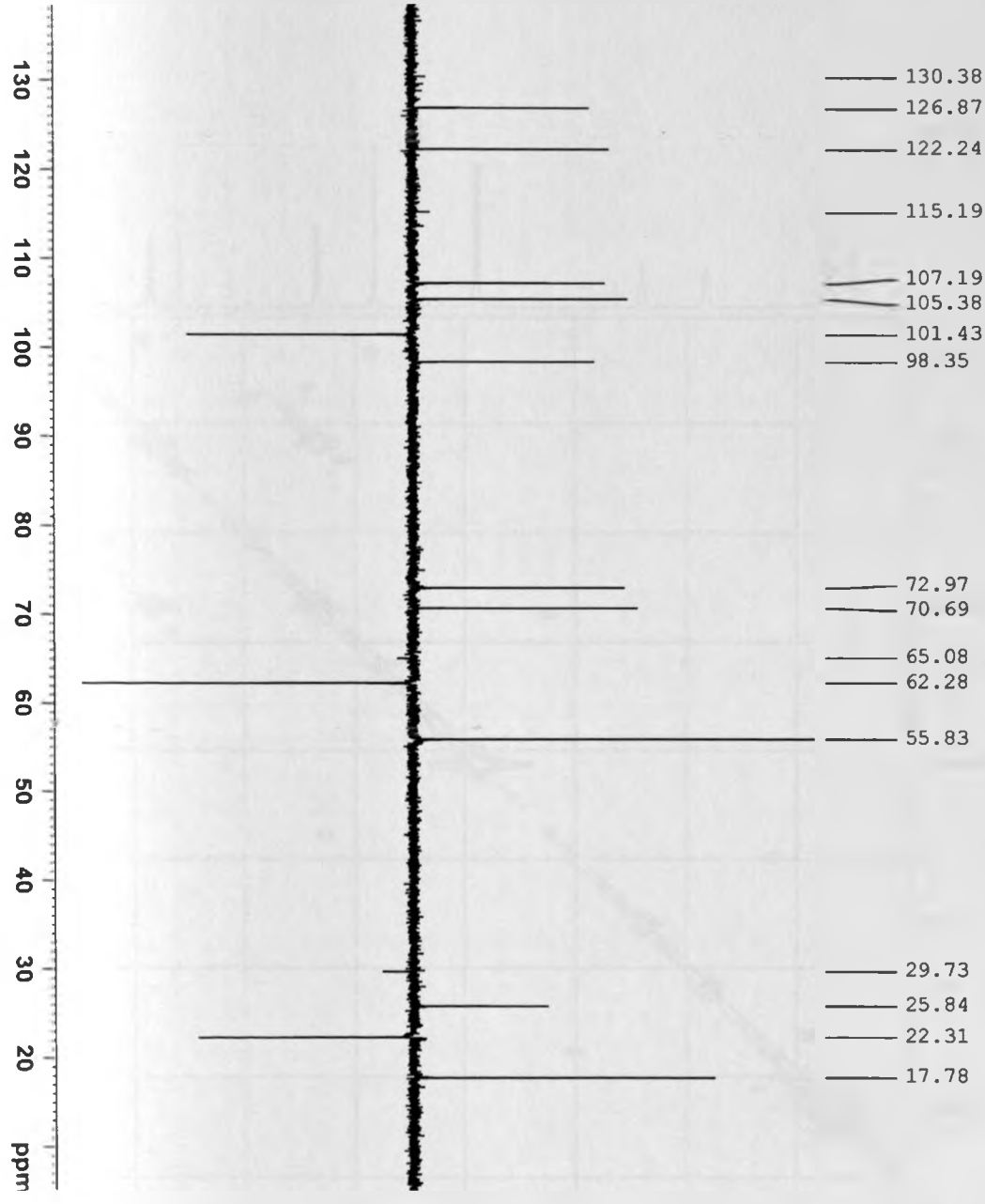
^{1}H NMR SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



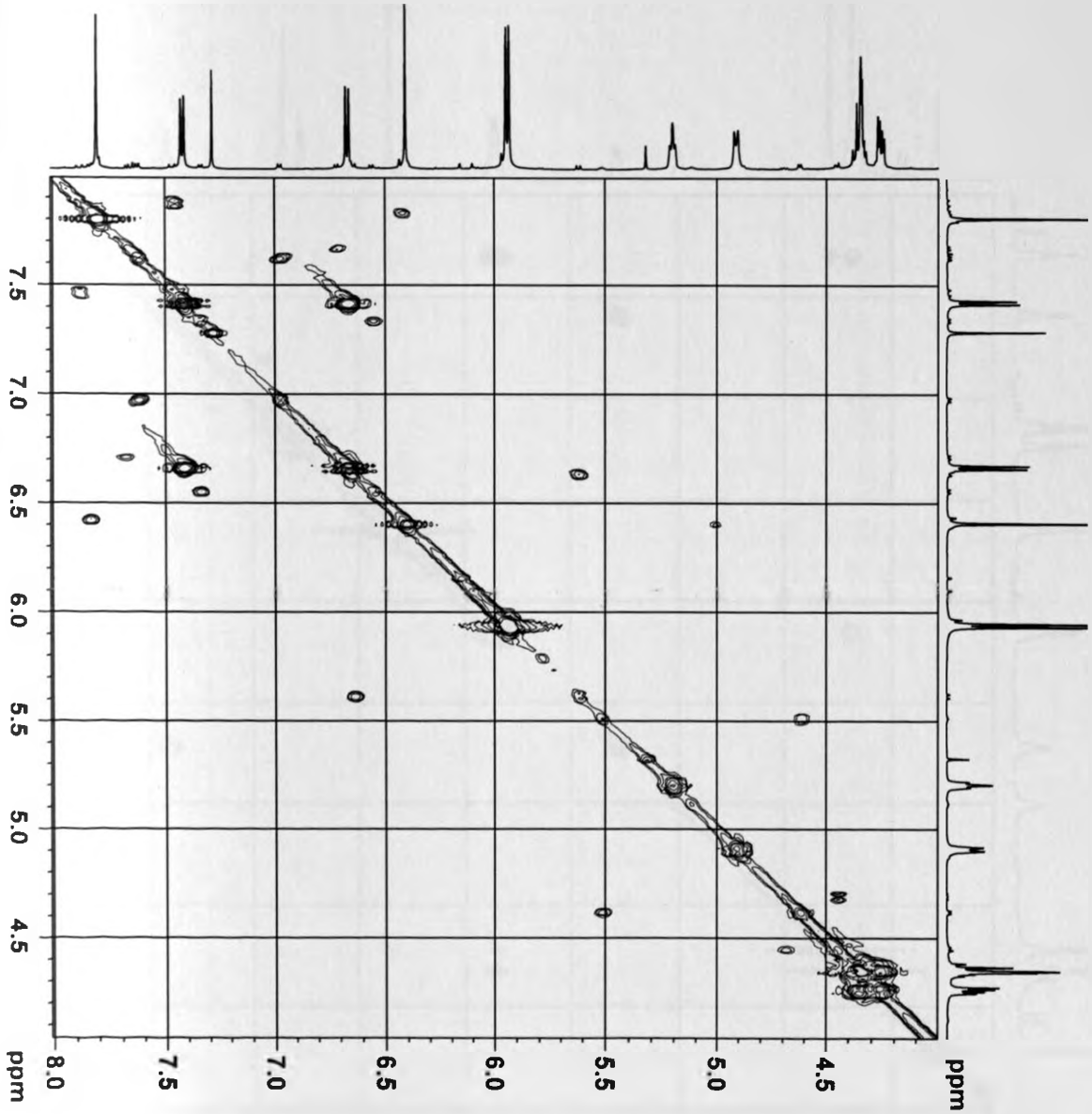
^{13}C NMR SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



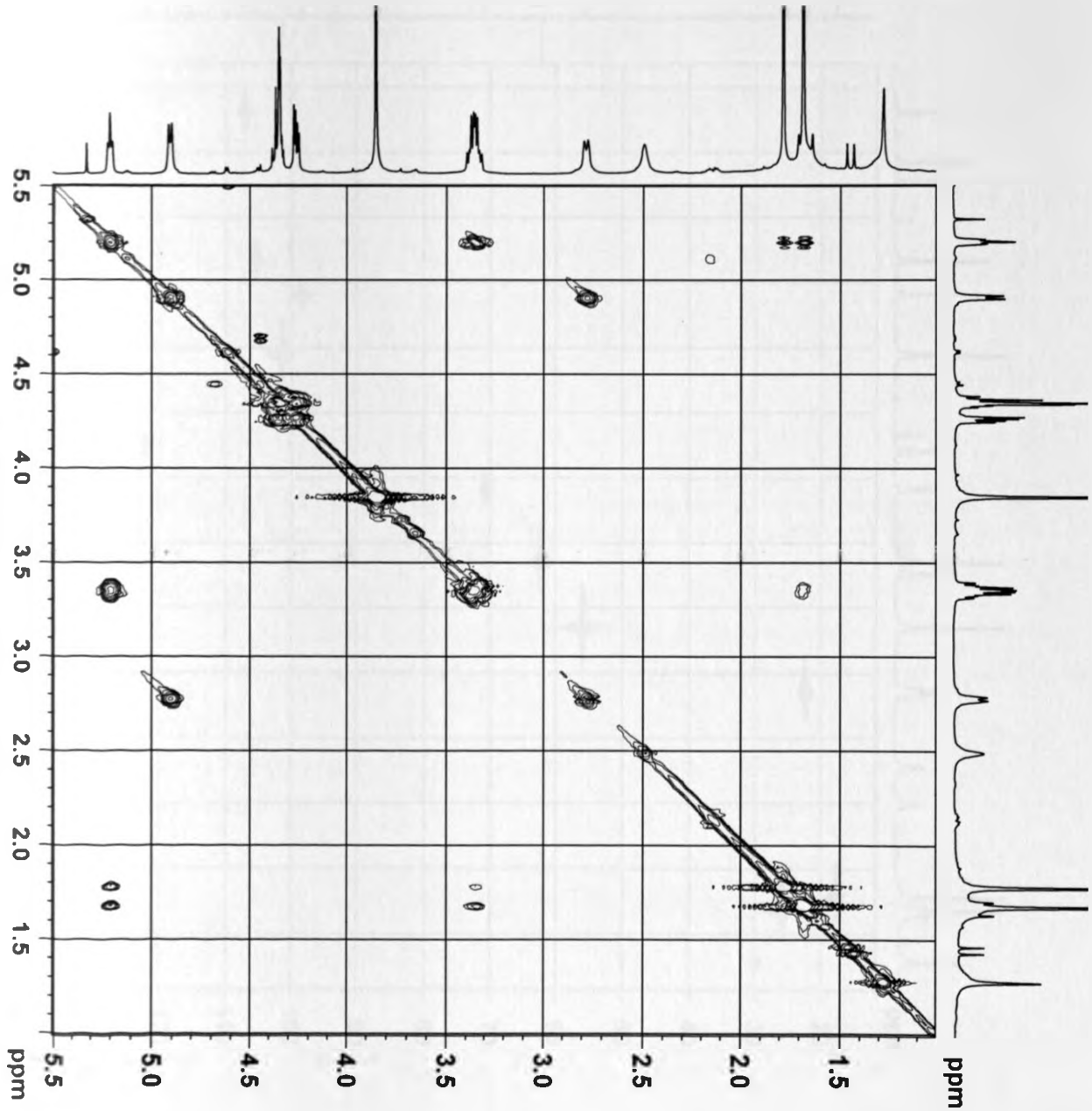
^{13}C NMR SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



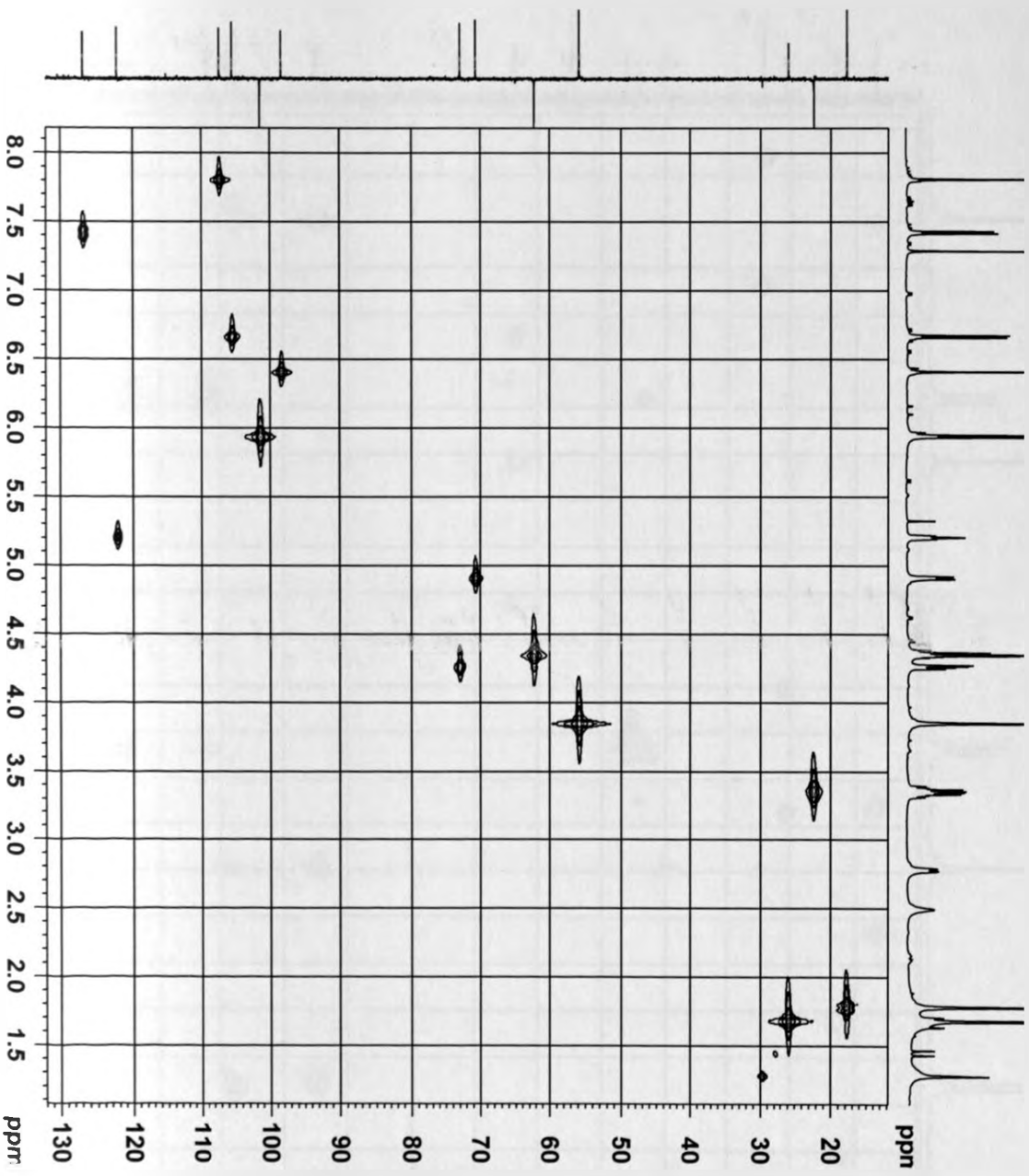
COSY SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



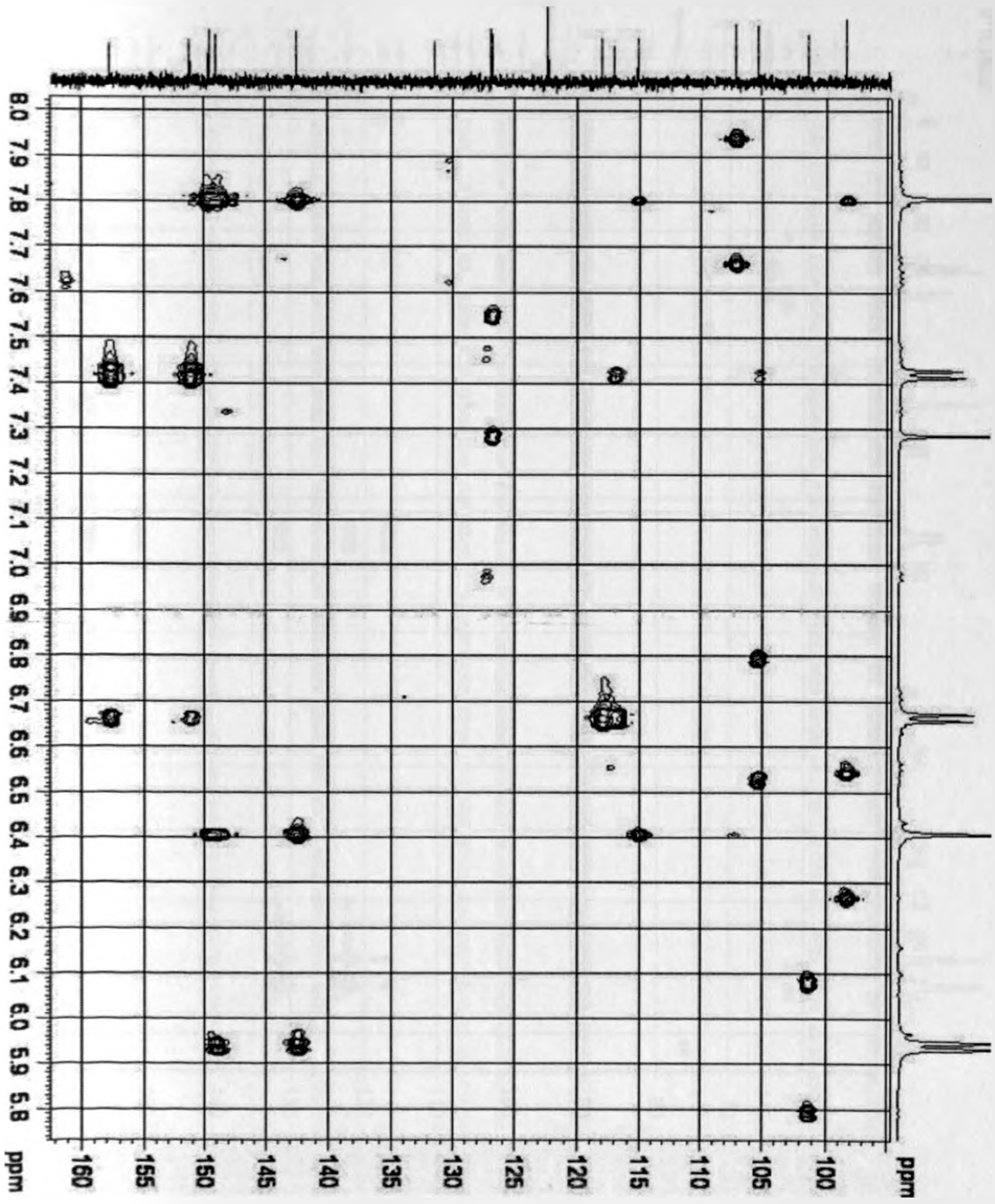
COSY SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



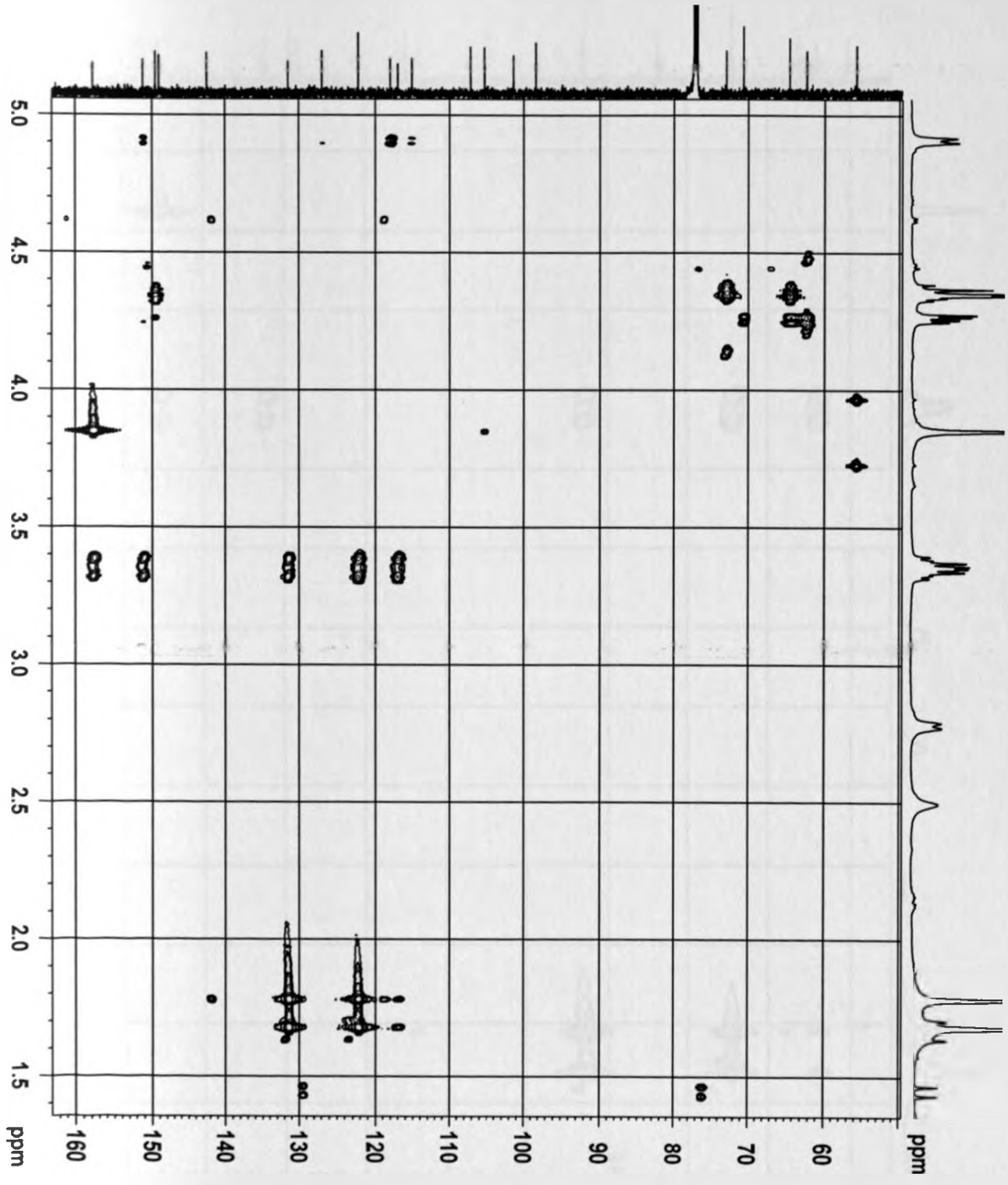
HMQC SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



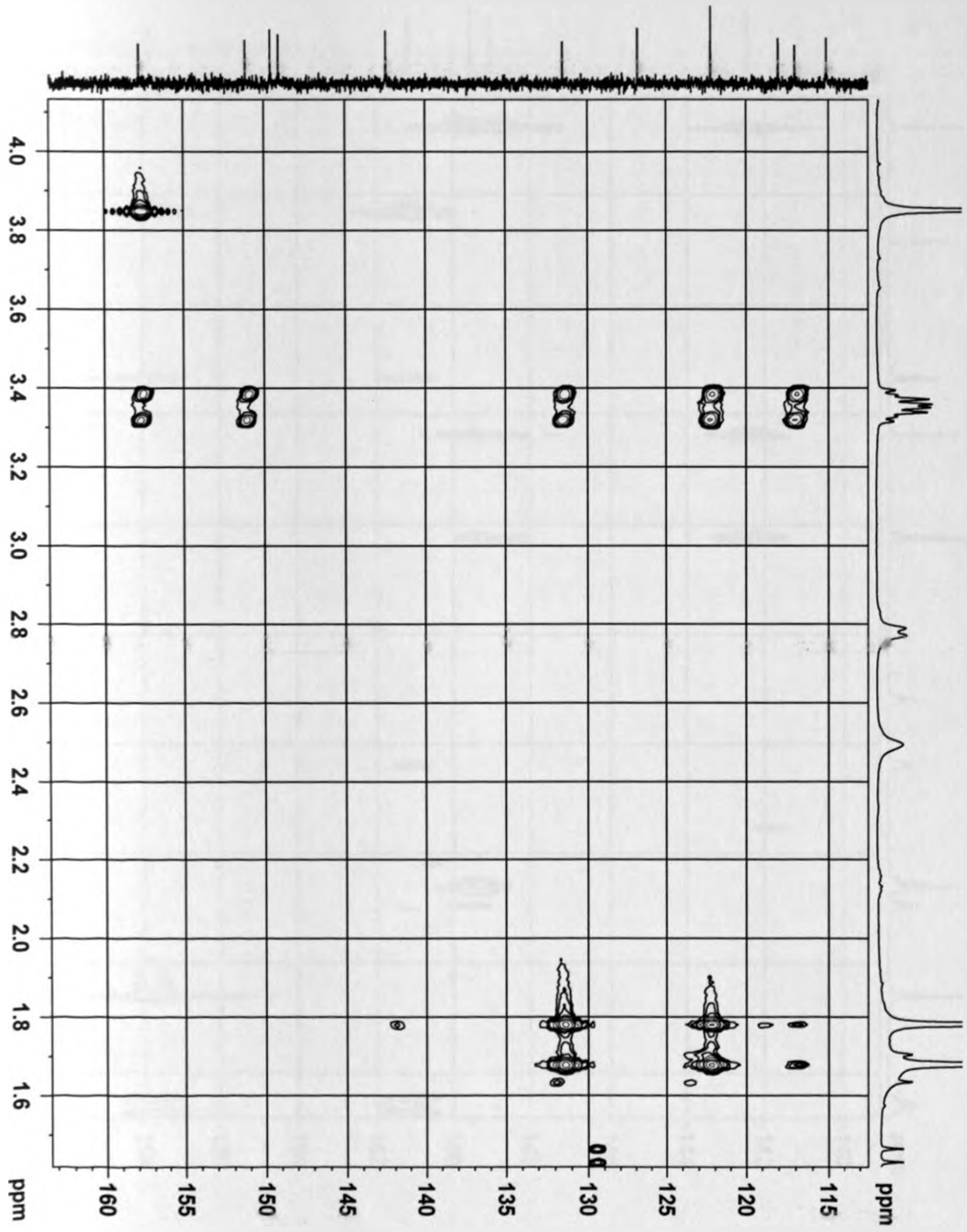
HMBC SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



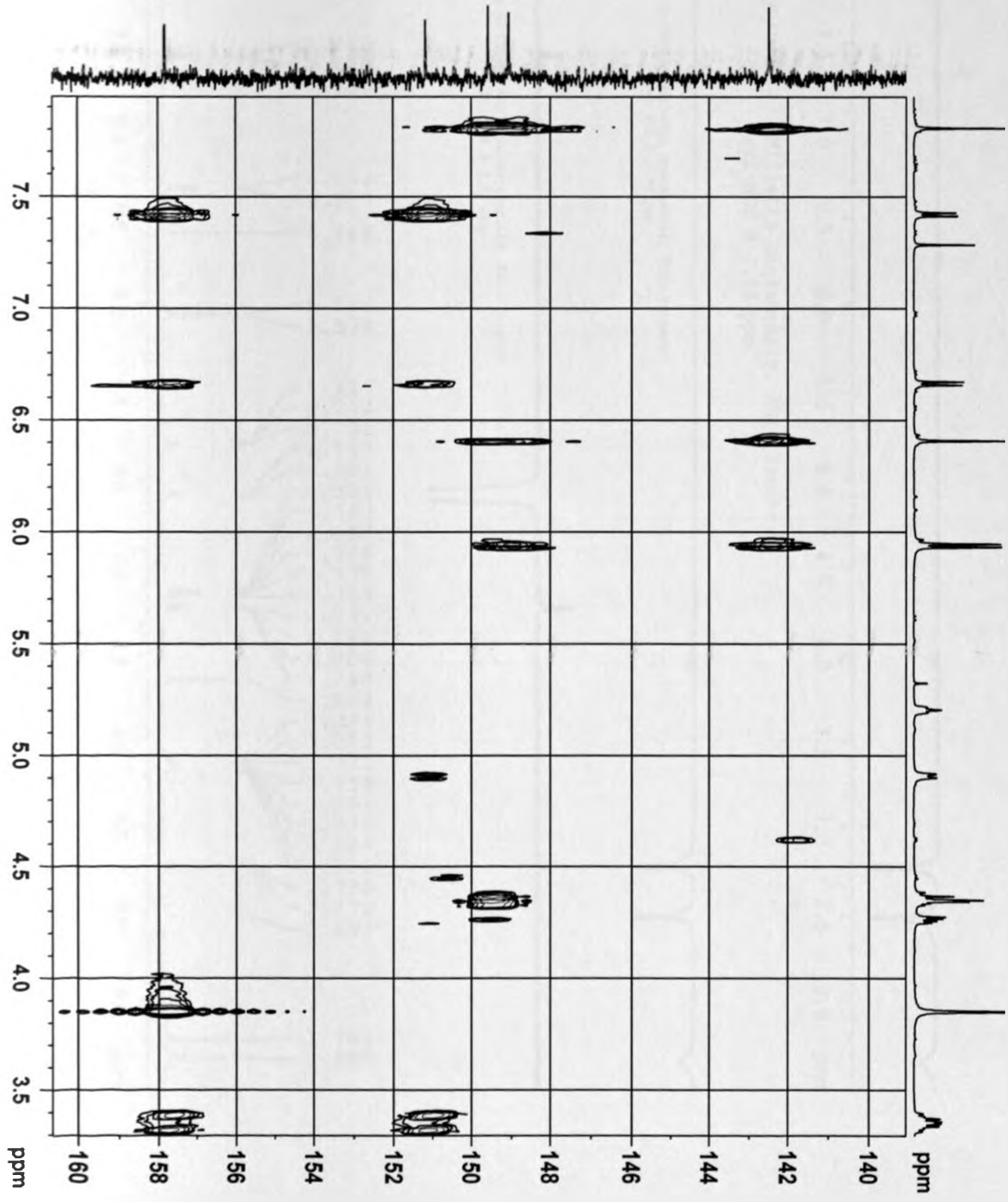
HMBC SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



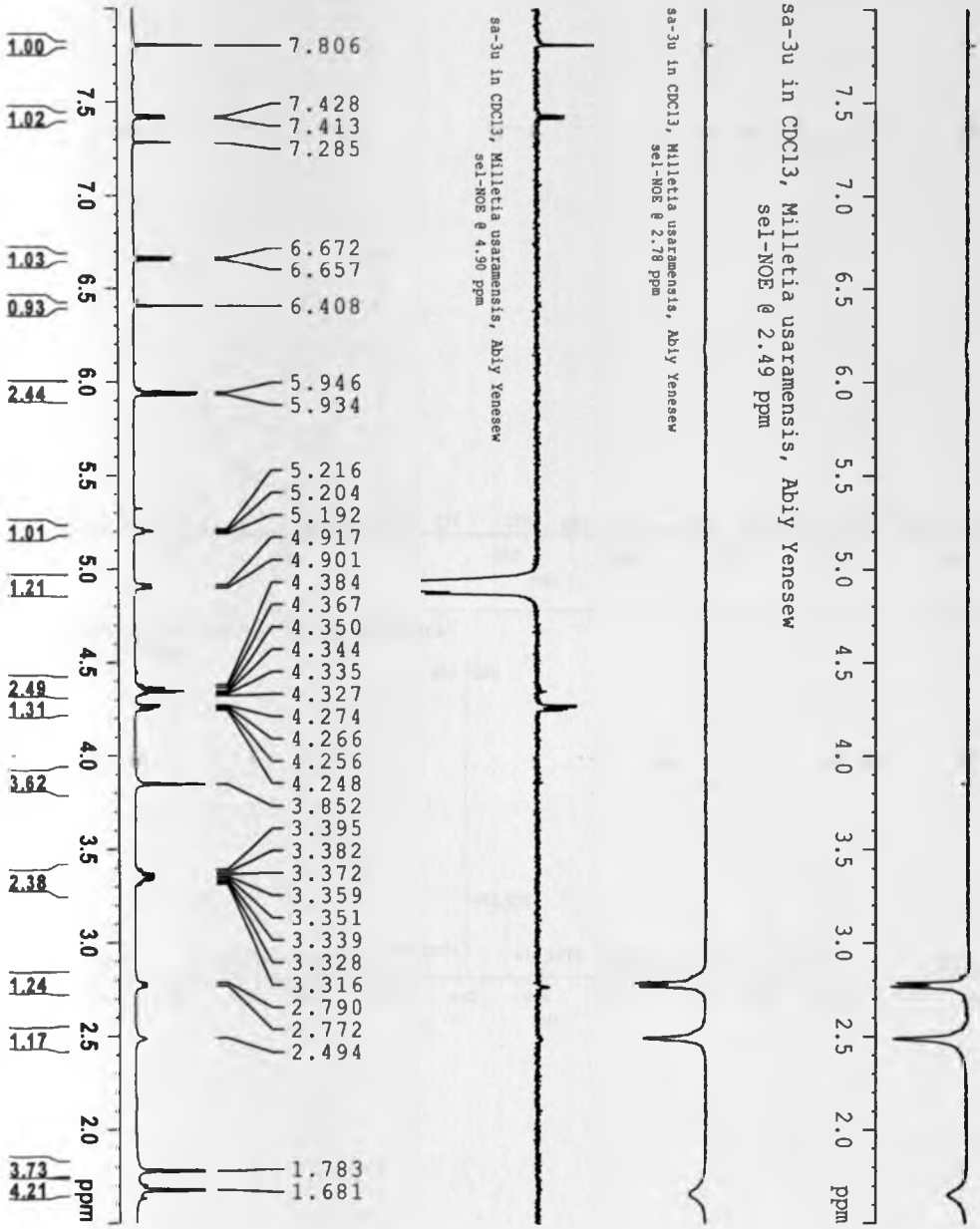
HMBC SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



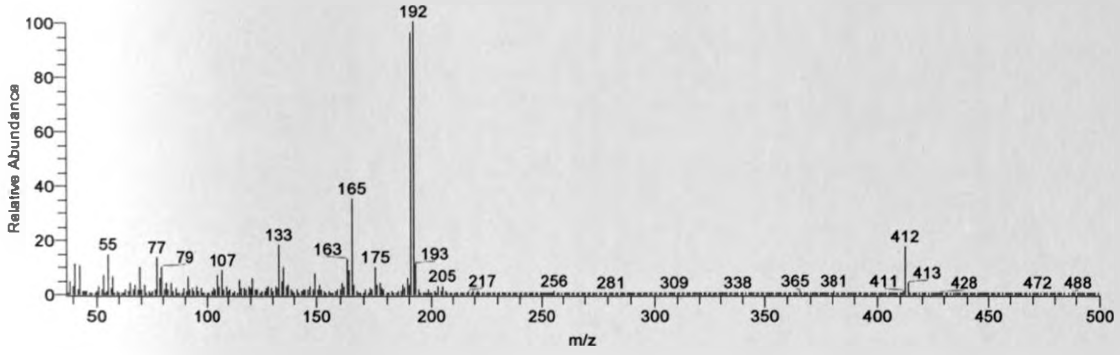
HMBC SPECTRUM FOR COMPOUND **3** ($CDCL_3$, 600 MHz)

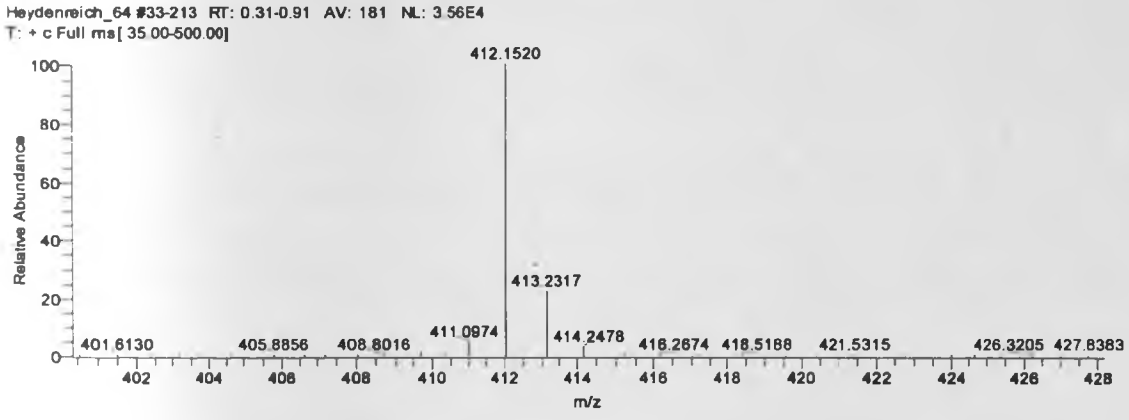


NOE EXPERIMENT FOR COMPOUND **3** ($CDCL_3$, 600 MHz)



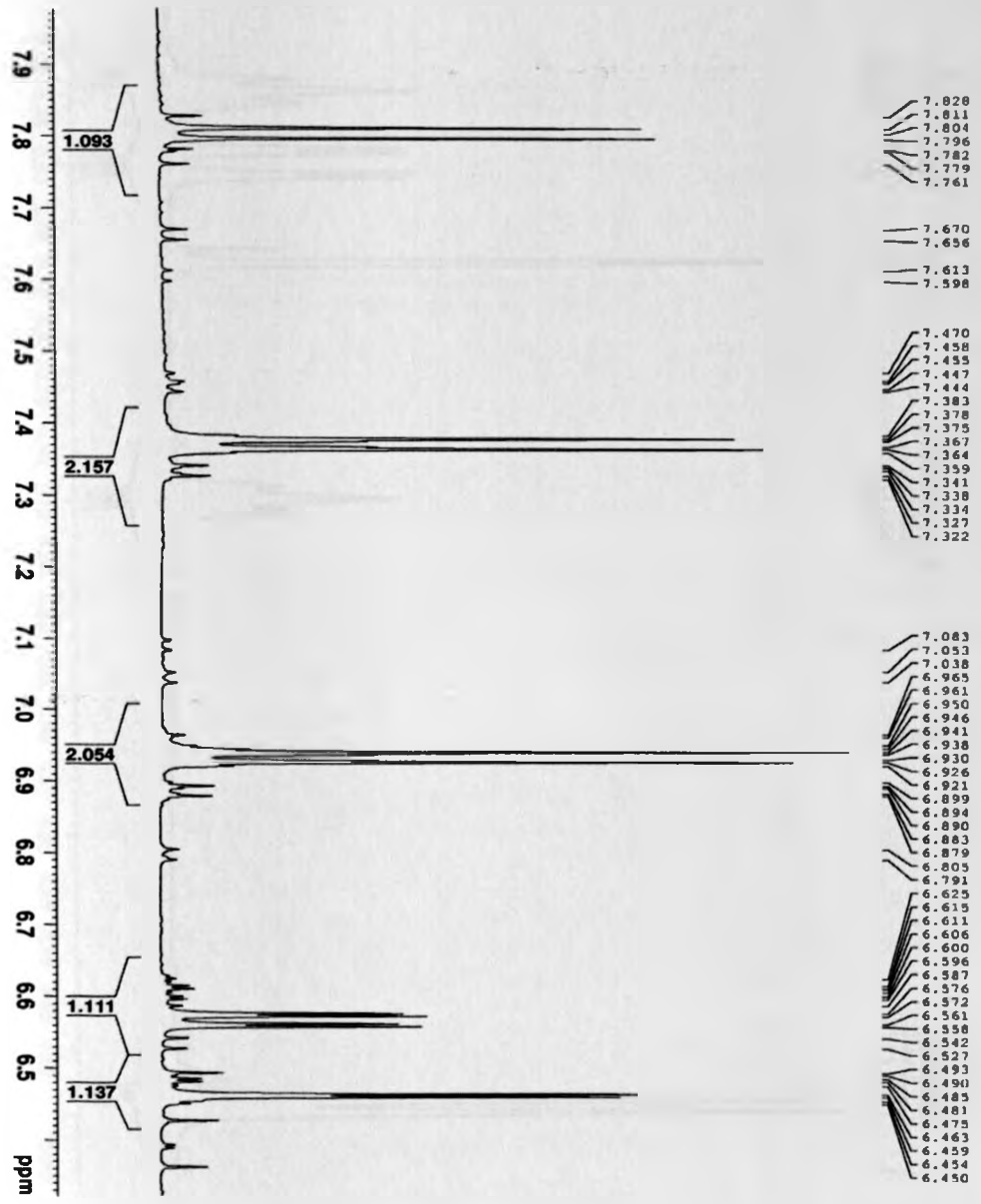
MASS SPECTRUM FOR COMPOUND **3**



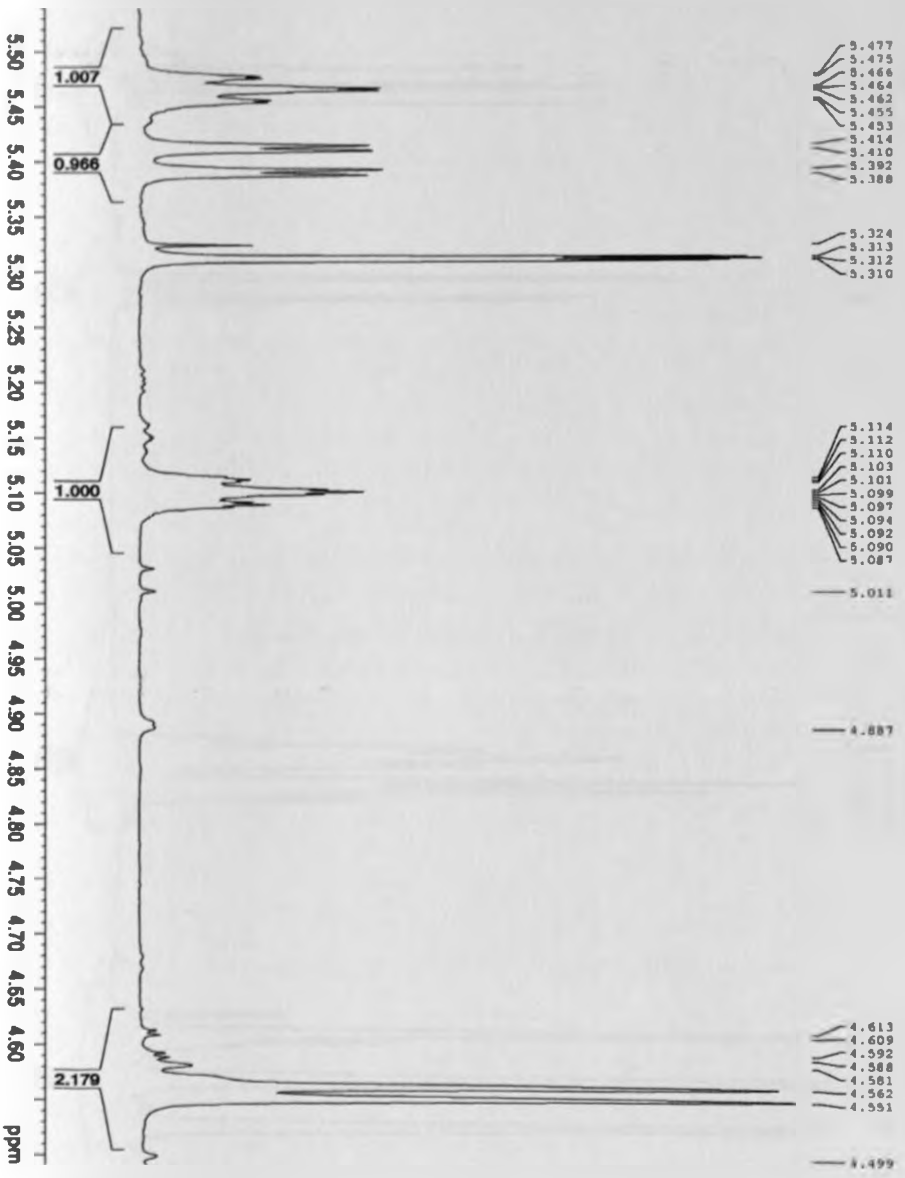


APPENDIX D: SPECTRA FOR COMPOUND **4**

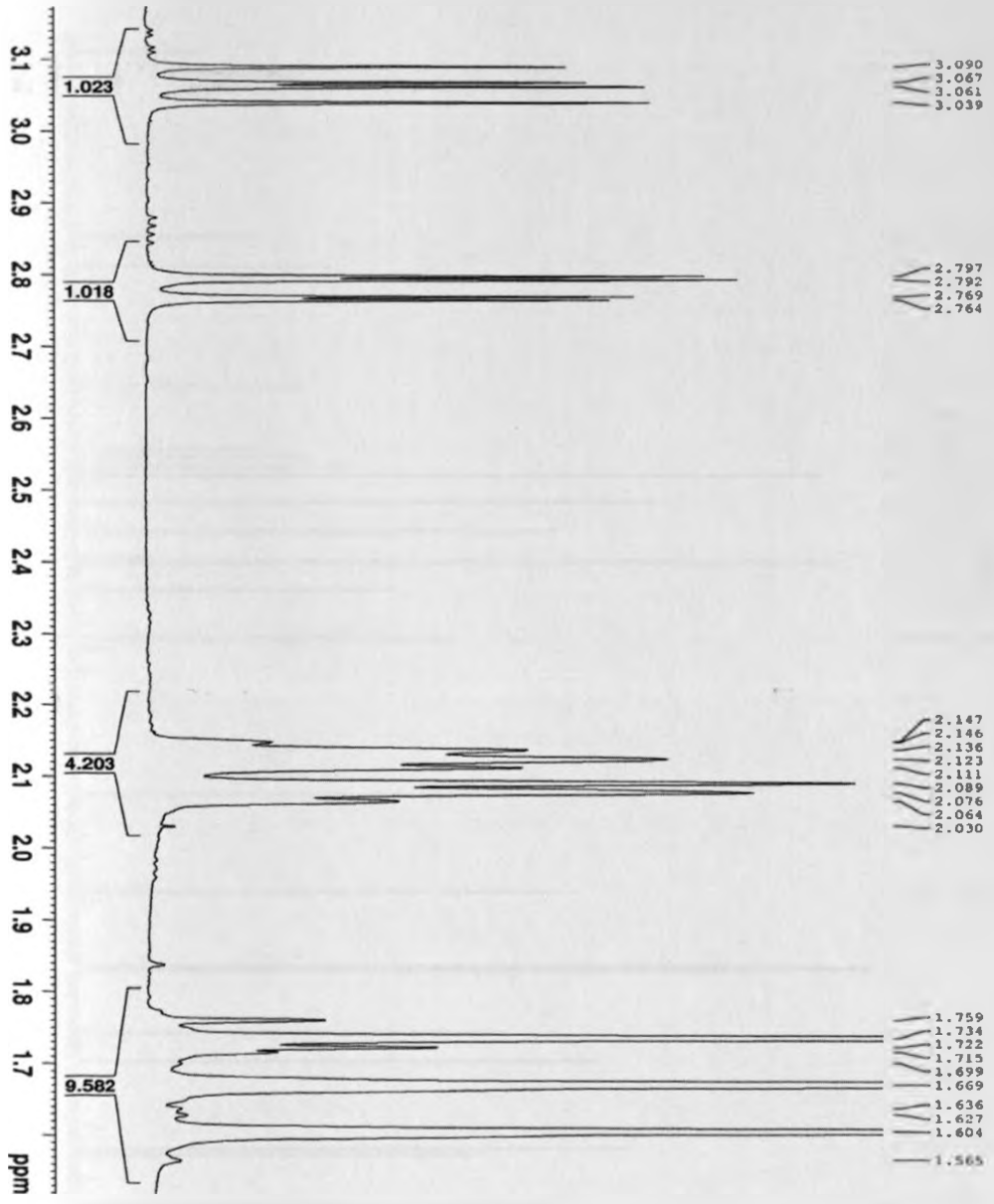
^{1}H NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



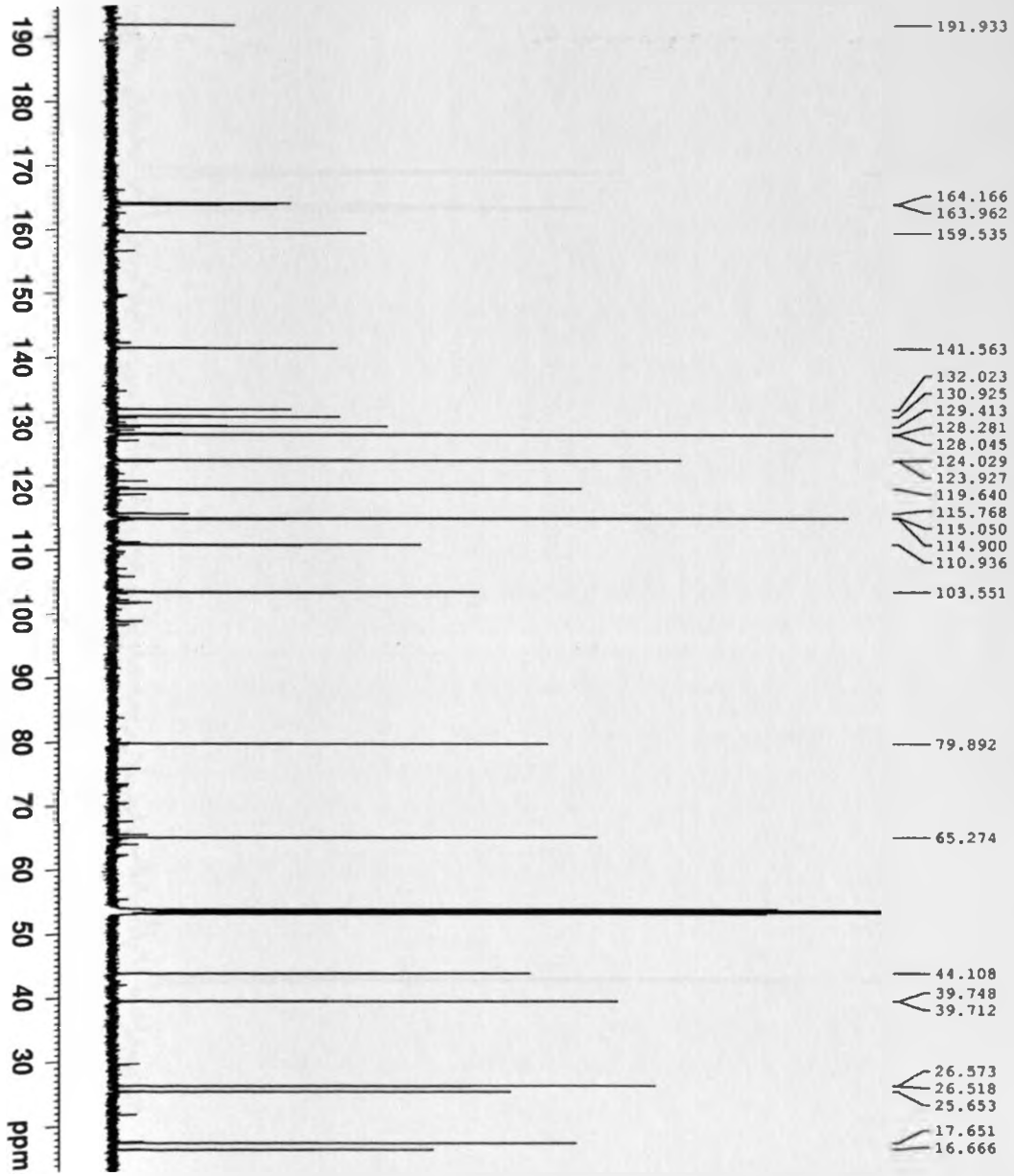
^{1}H NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



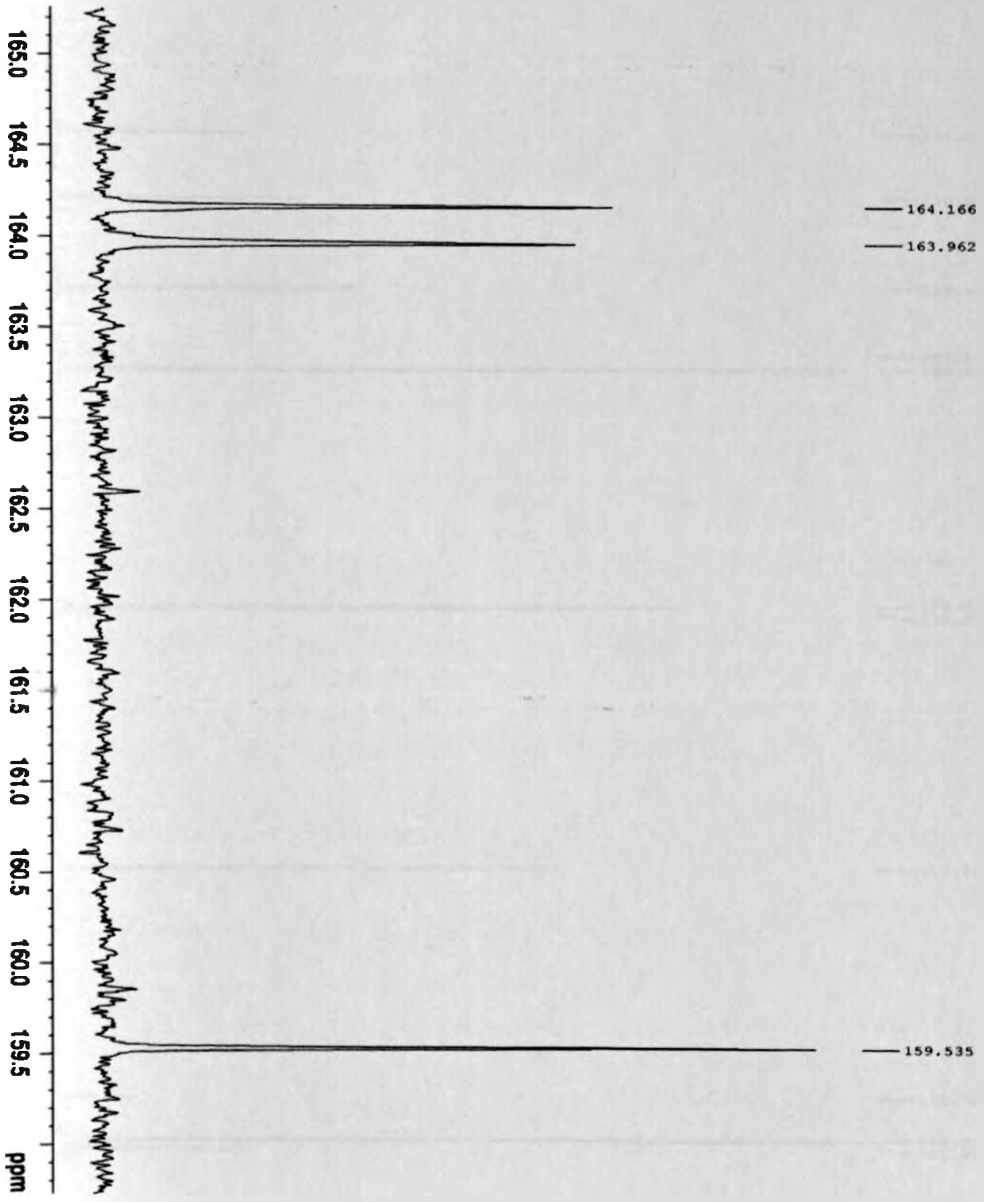
^{1}H NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)

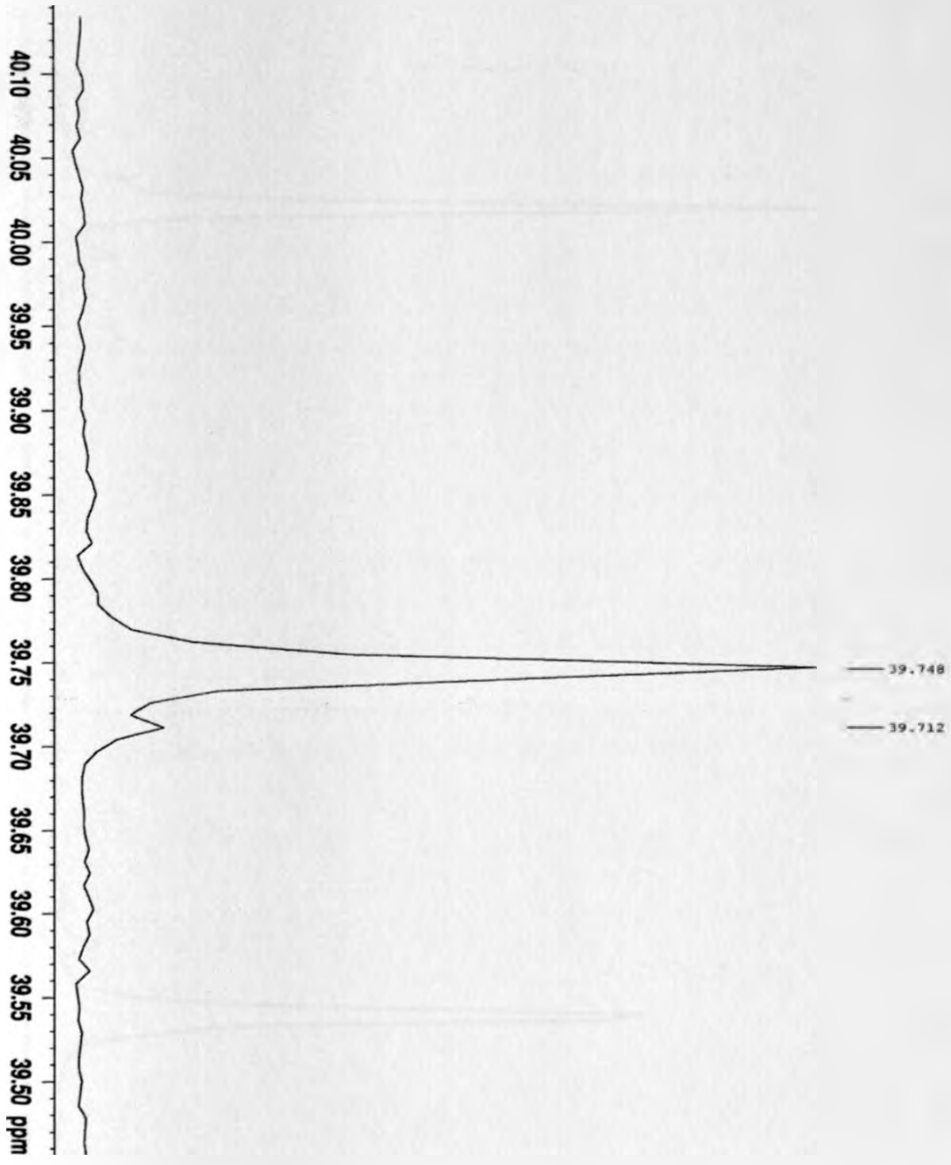


^{13}C NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)

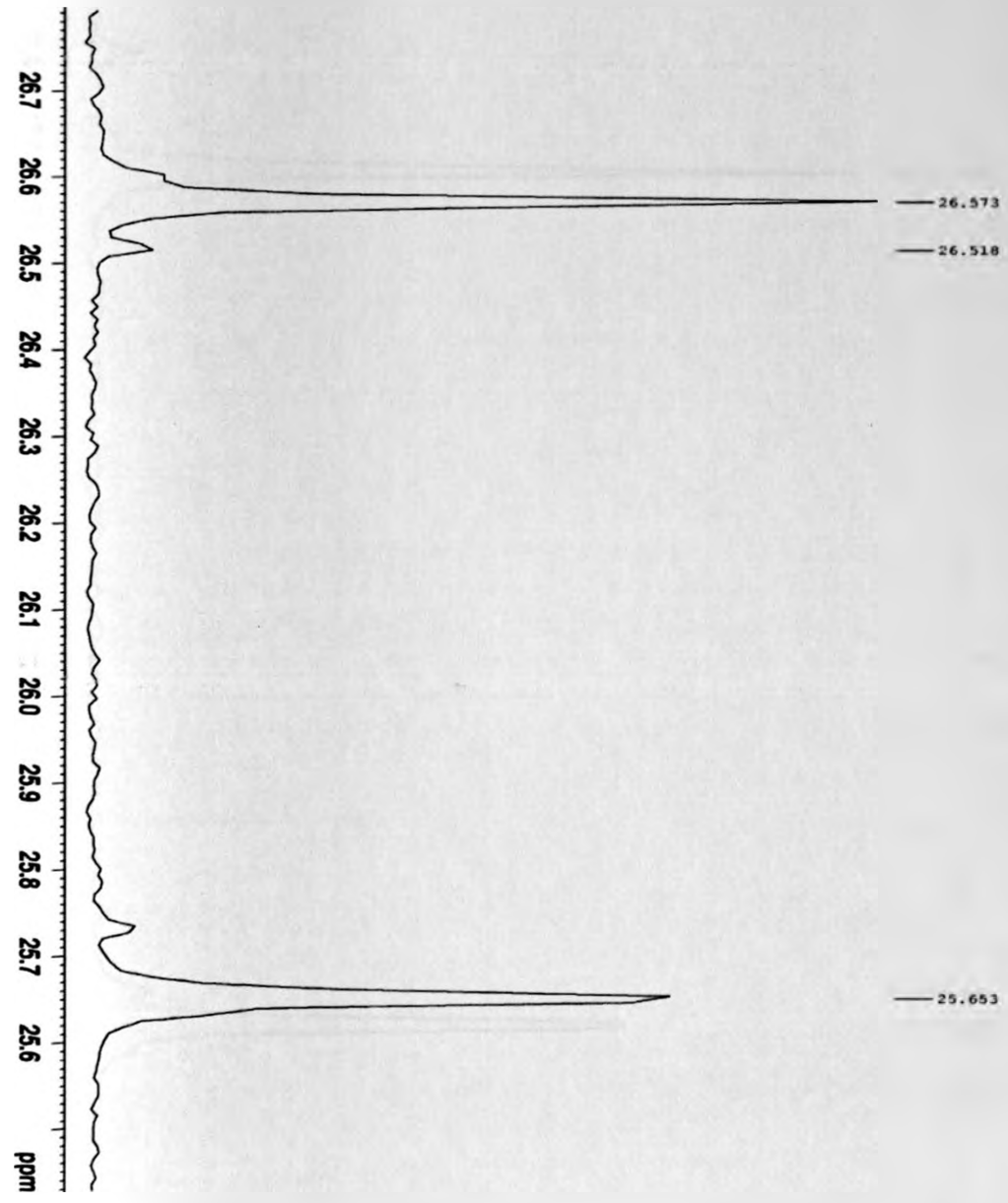


^{13}C NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)

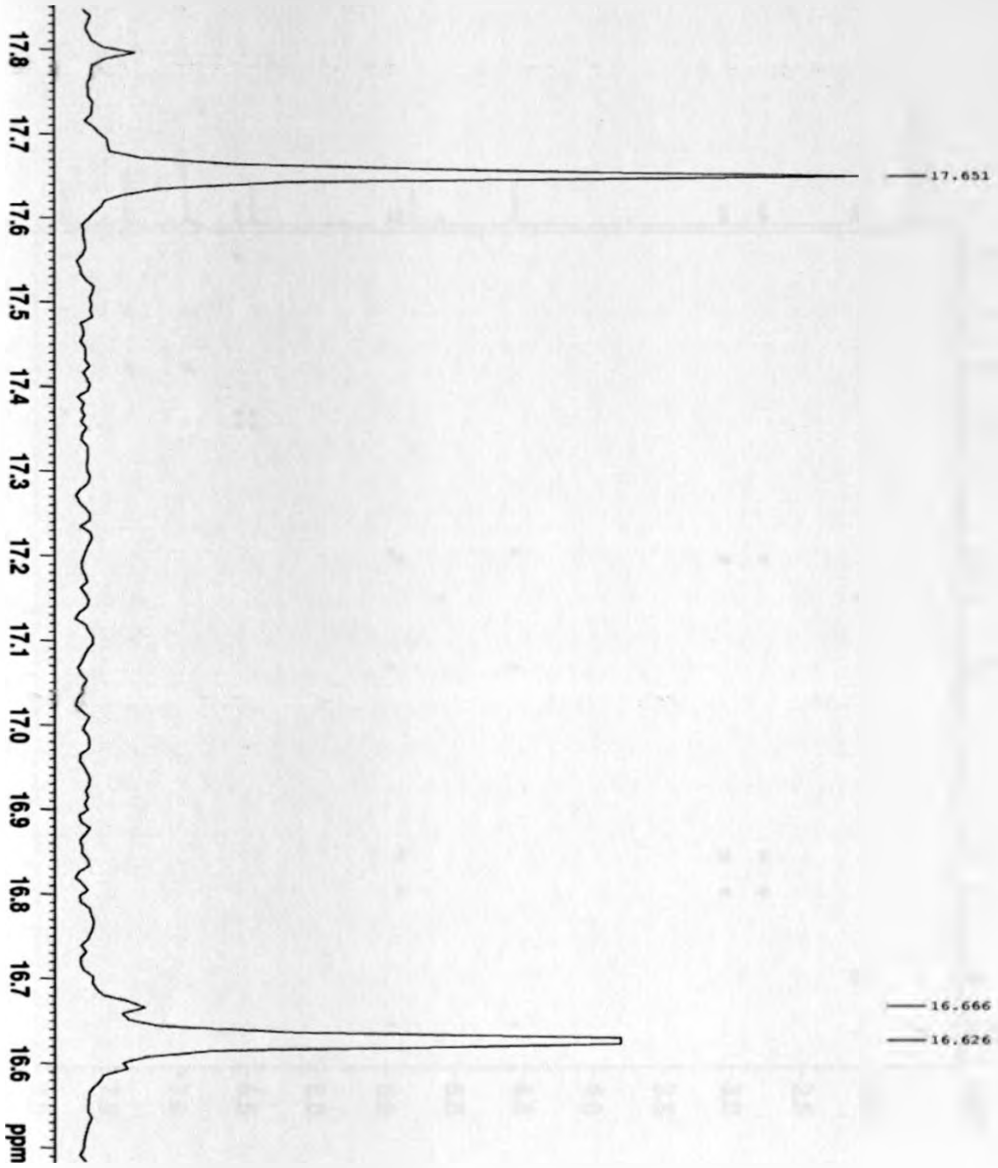
^{13}C NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



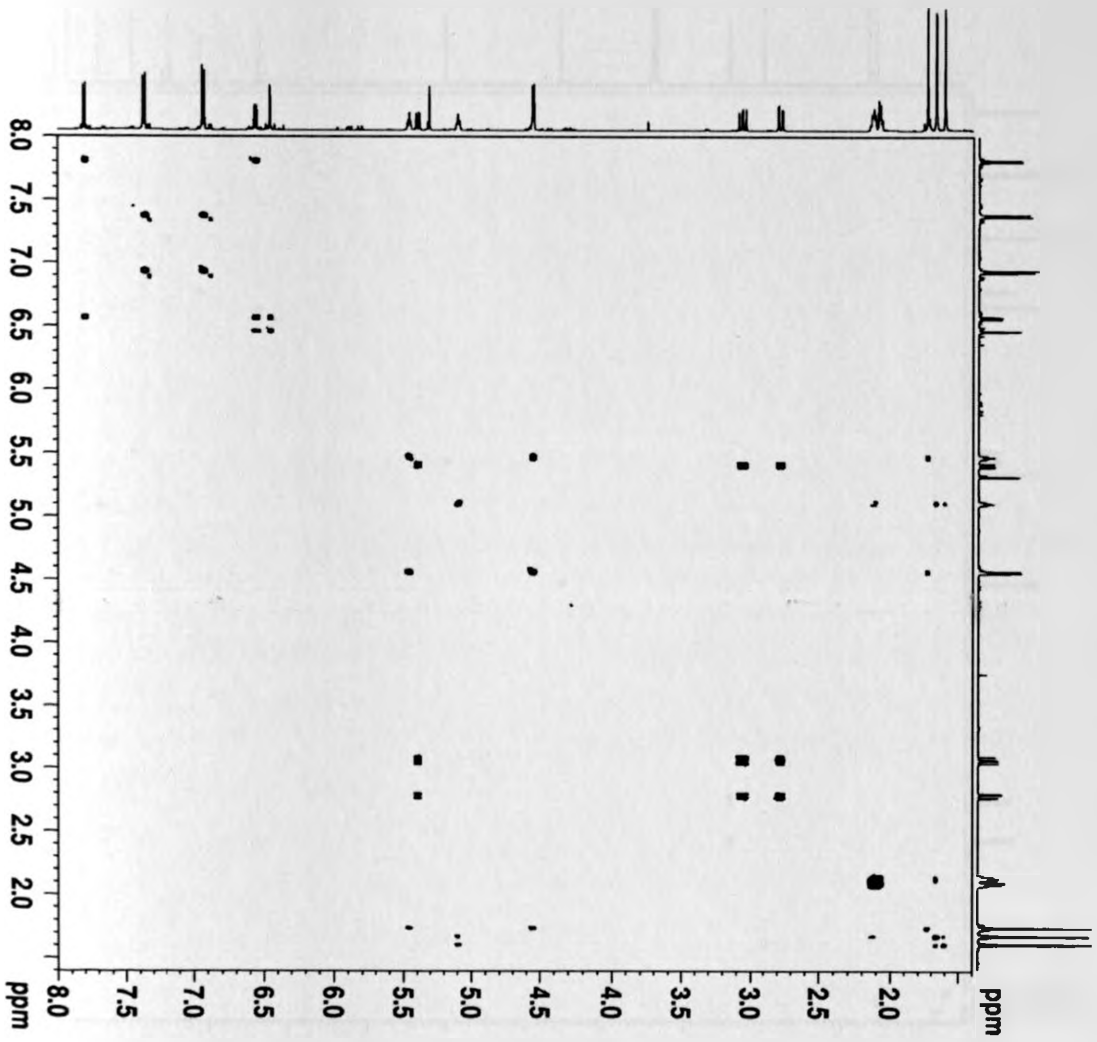
^{13}C NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



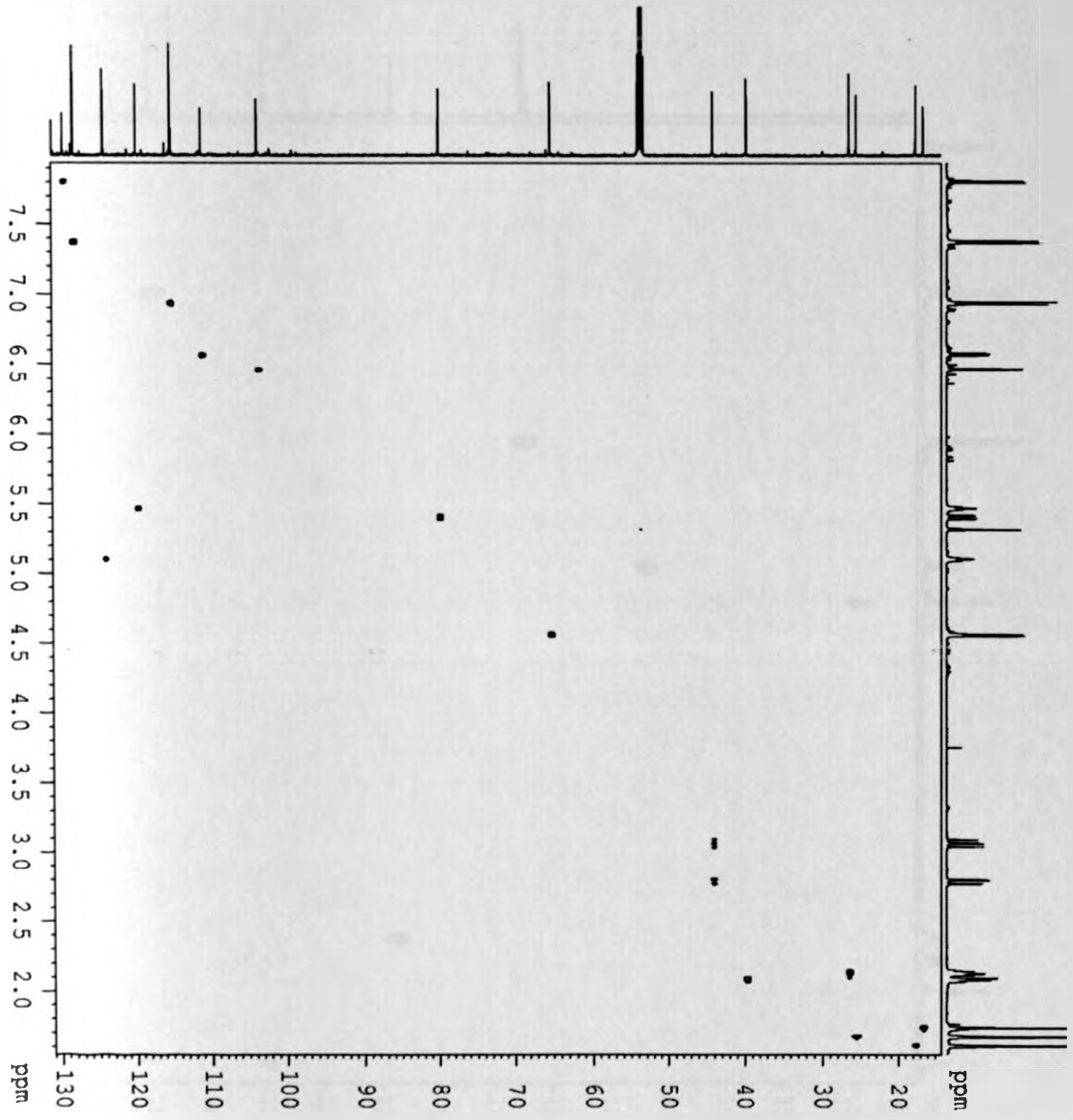
^{13}C NMR SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



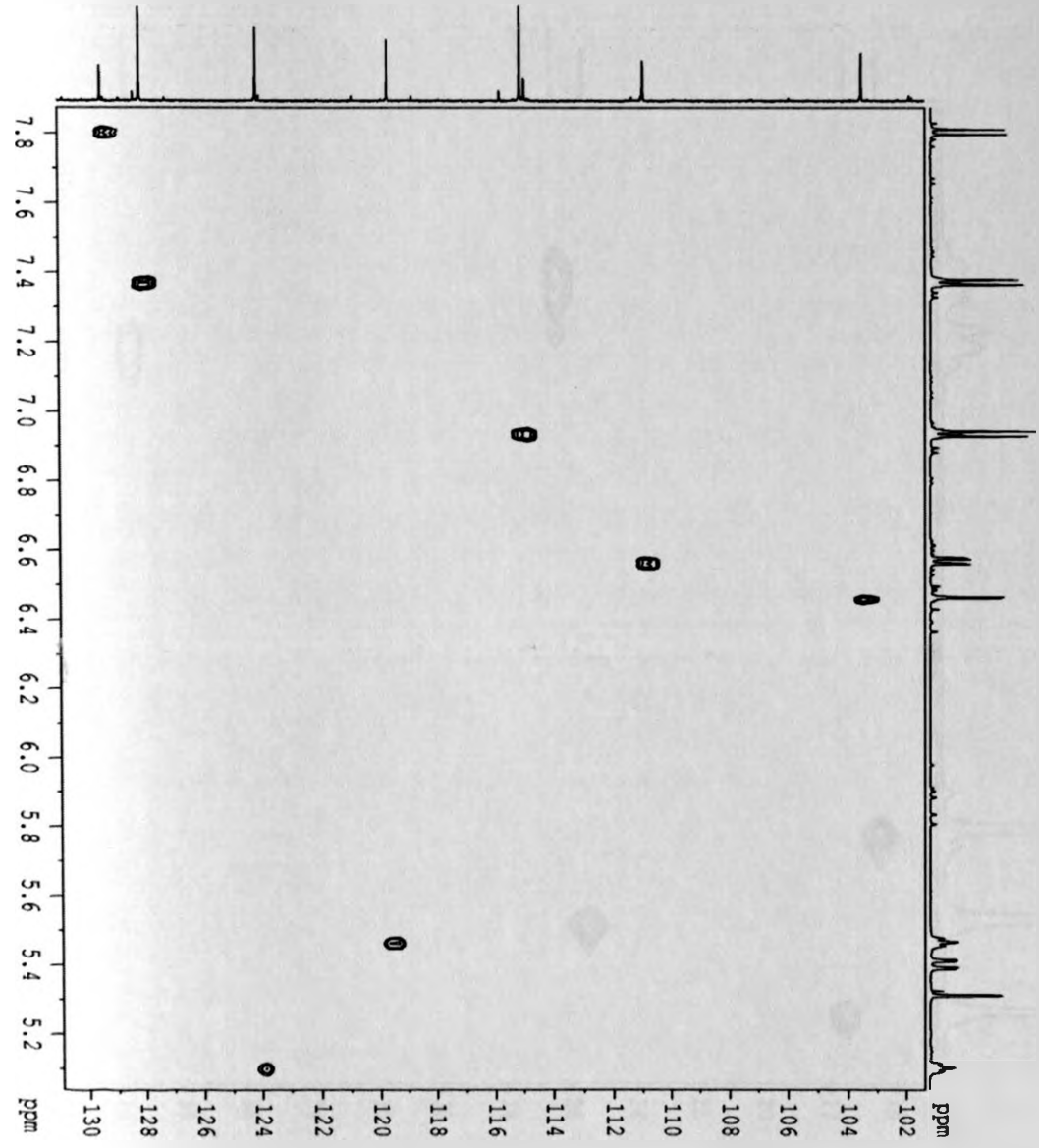
COSY SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



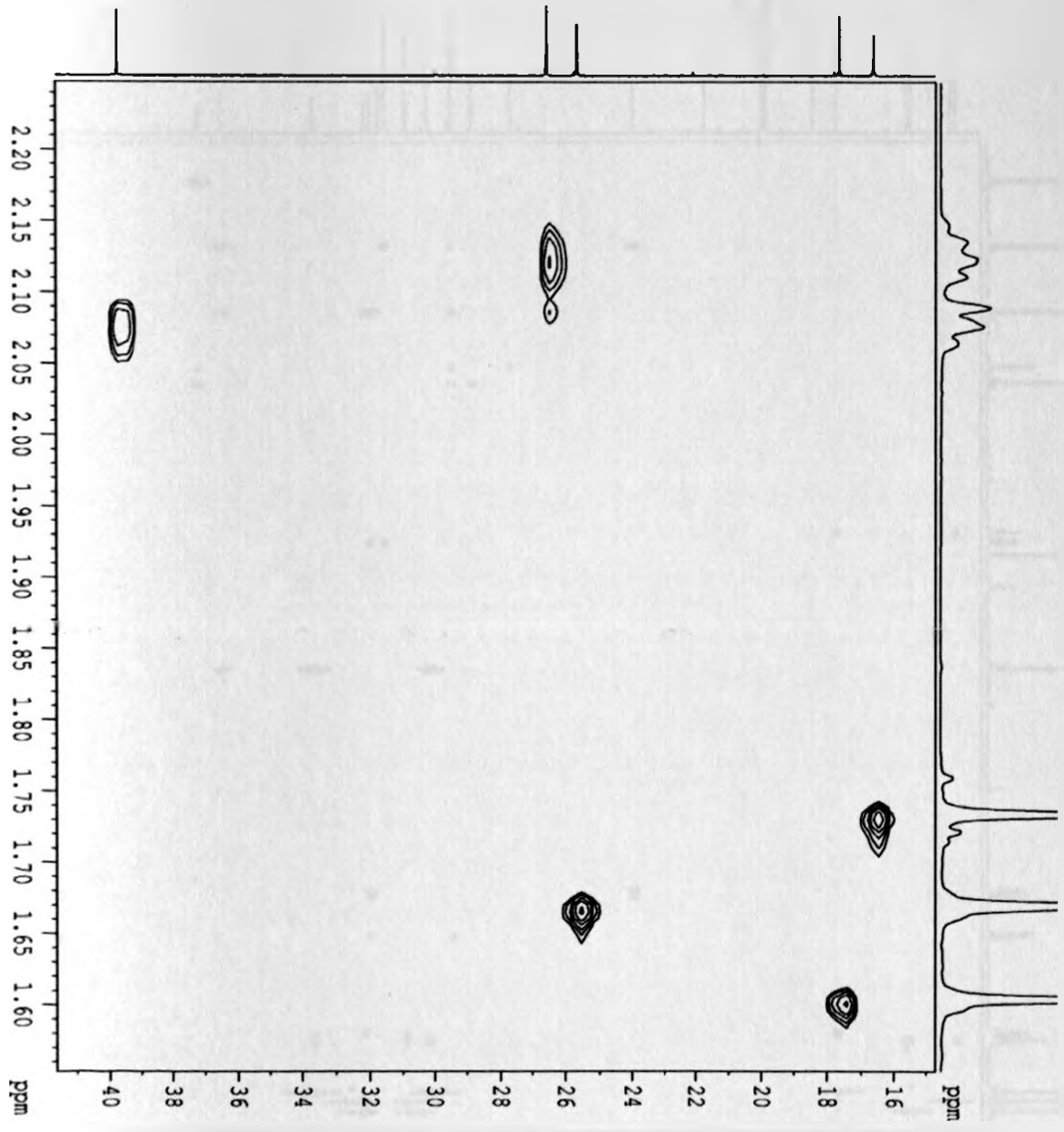
HMQC SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



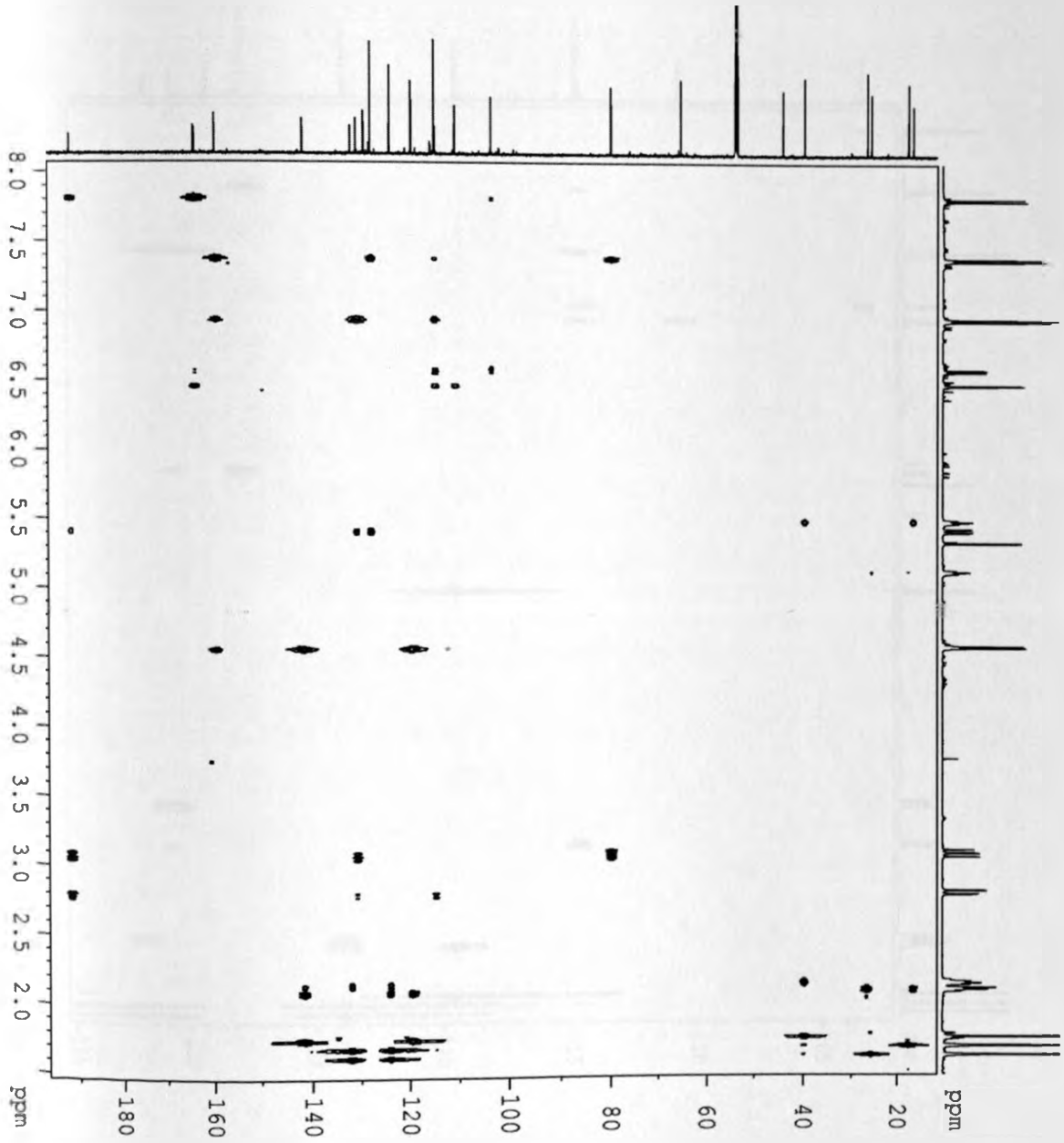
HMQC SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



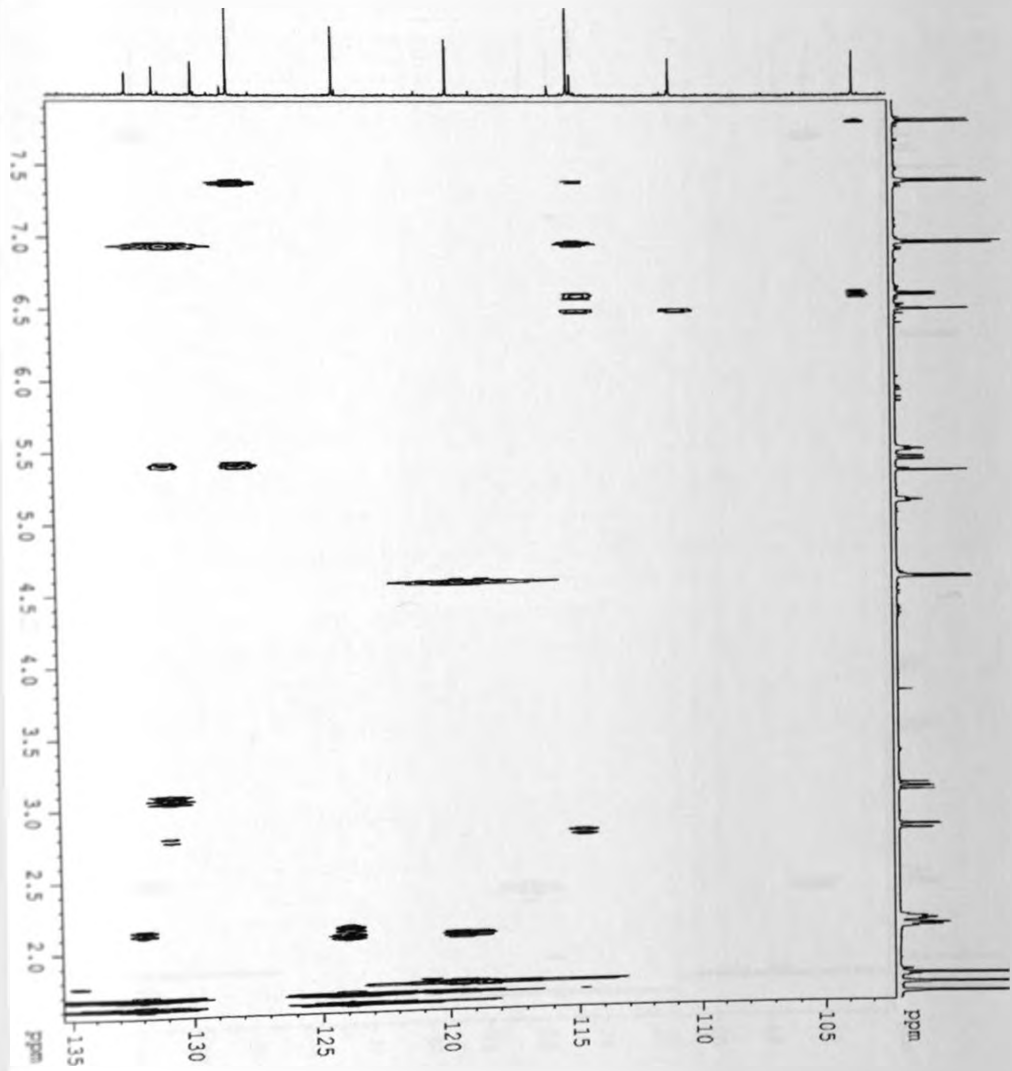
HMQC SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



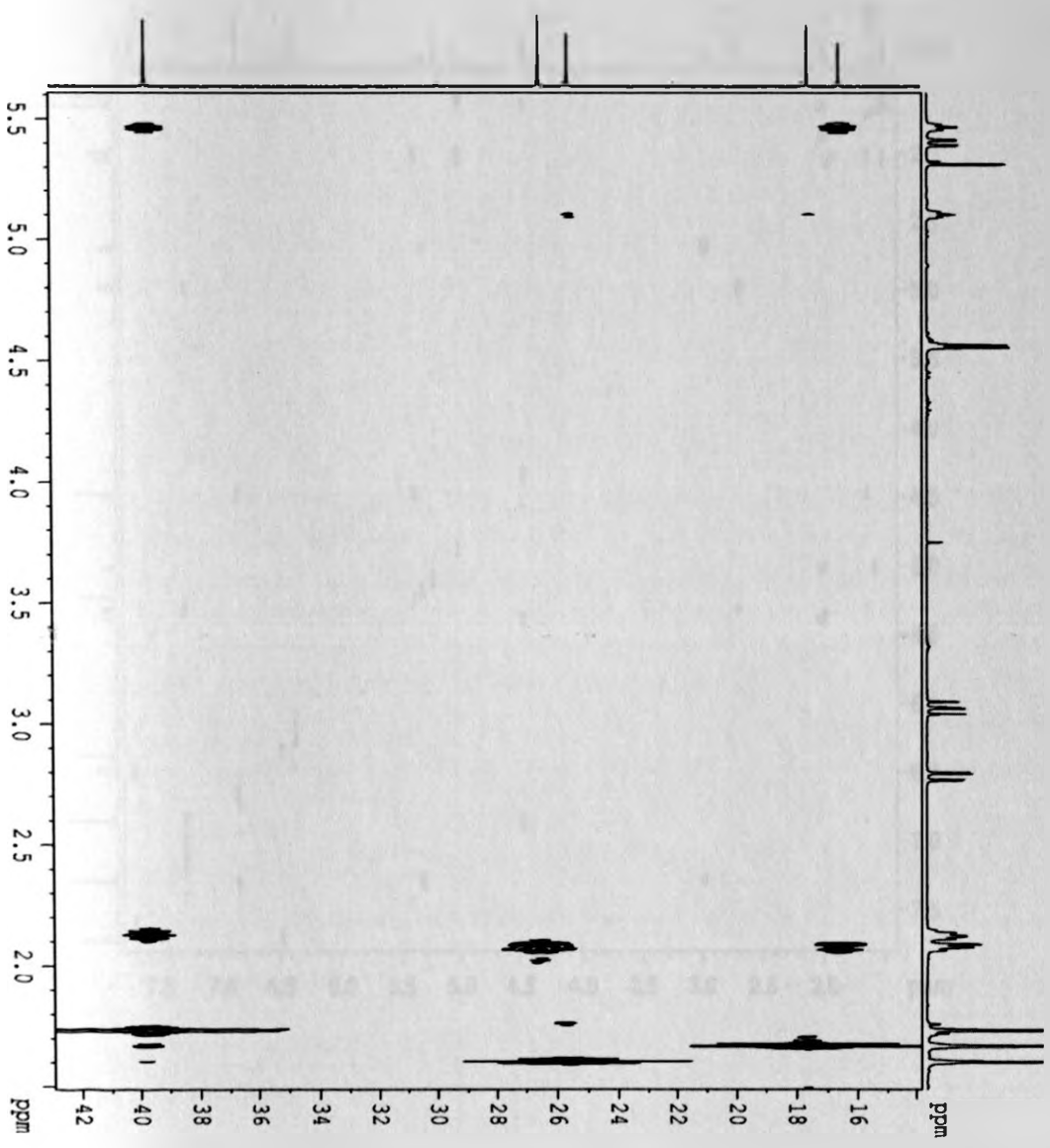
HMBC SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



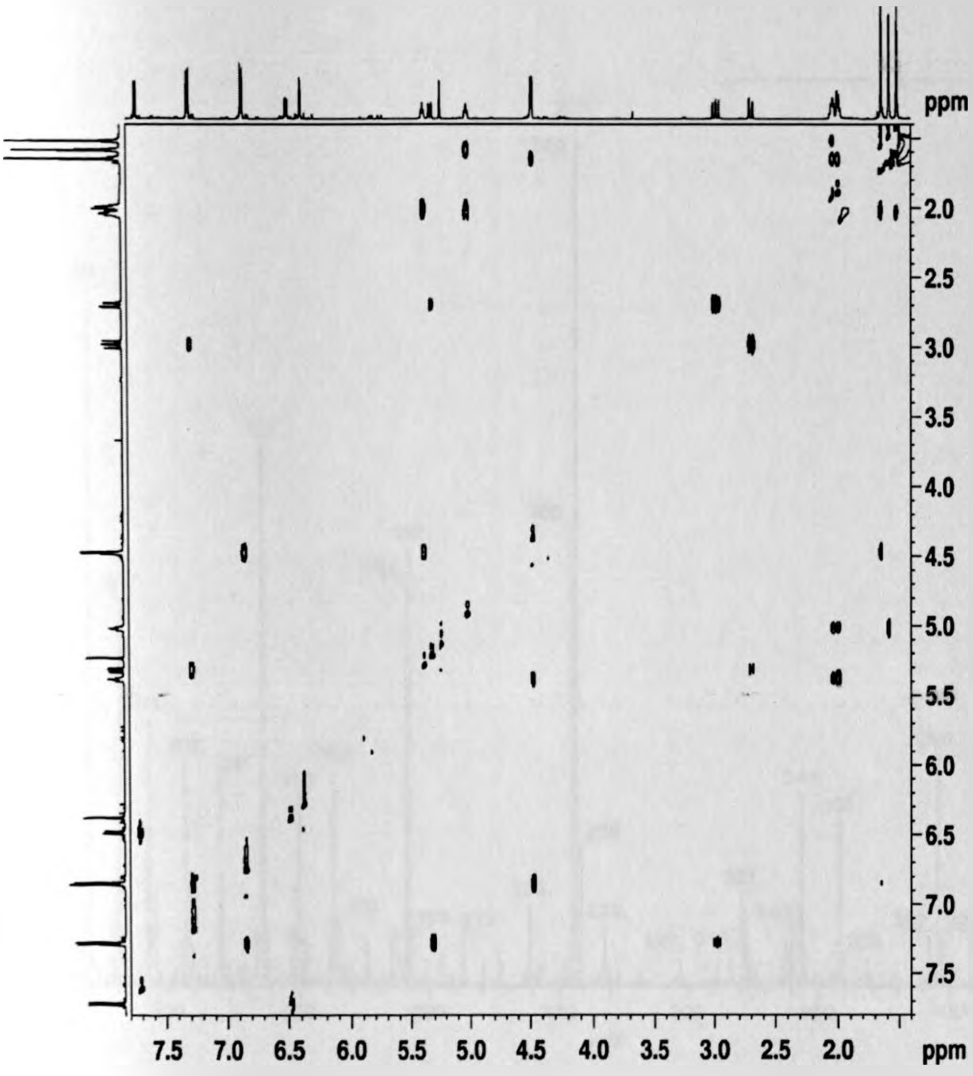
HMBC SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



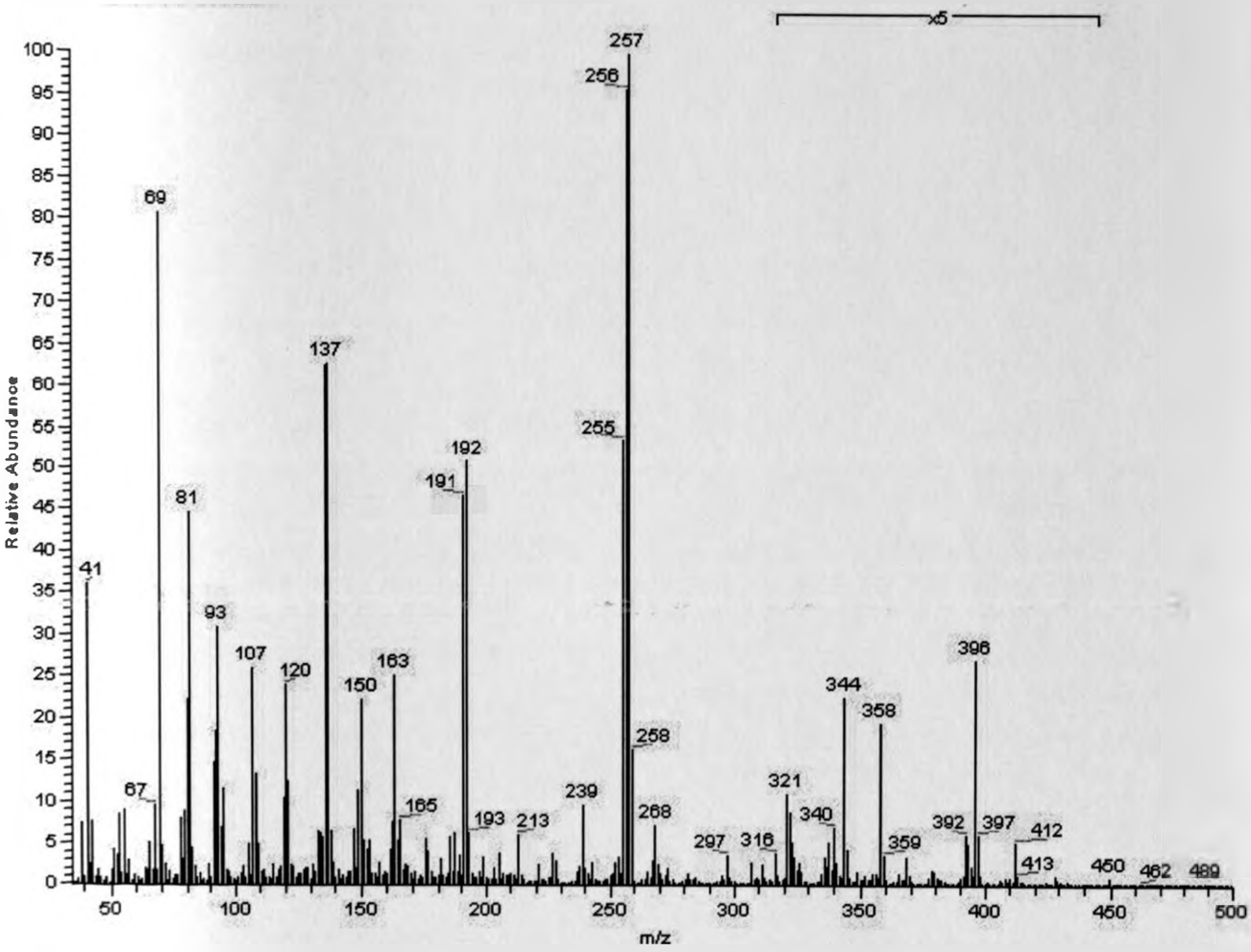
HMBC SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



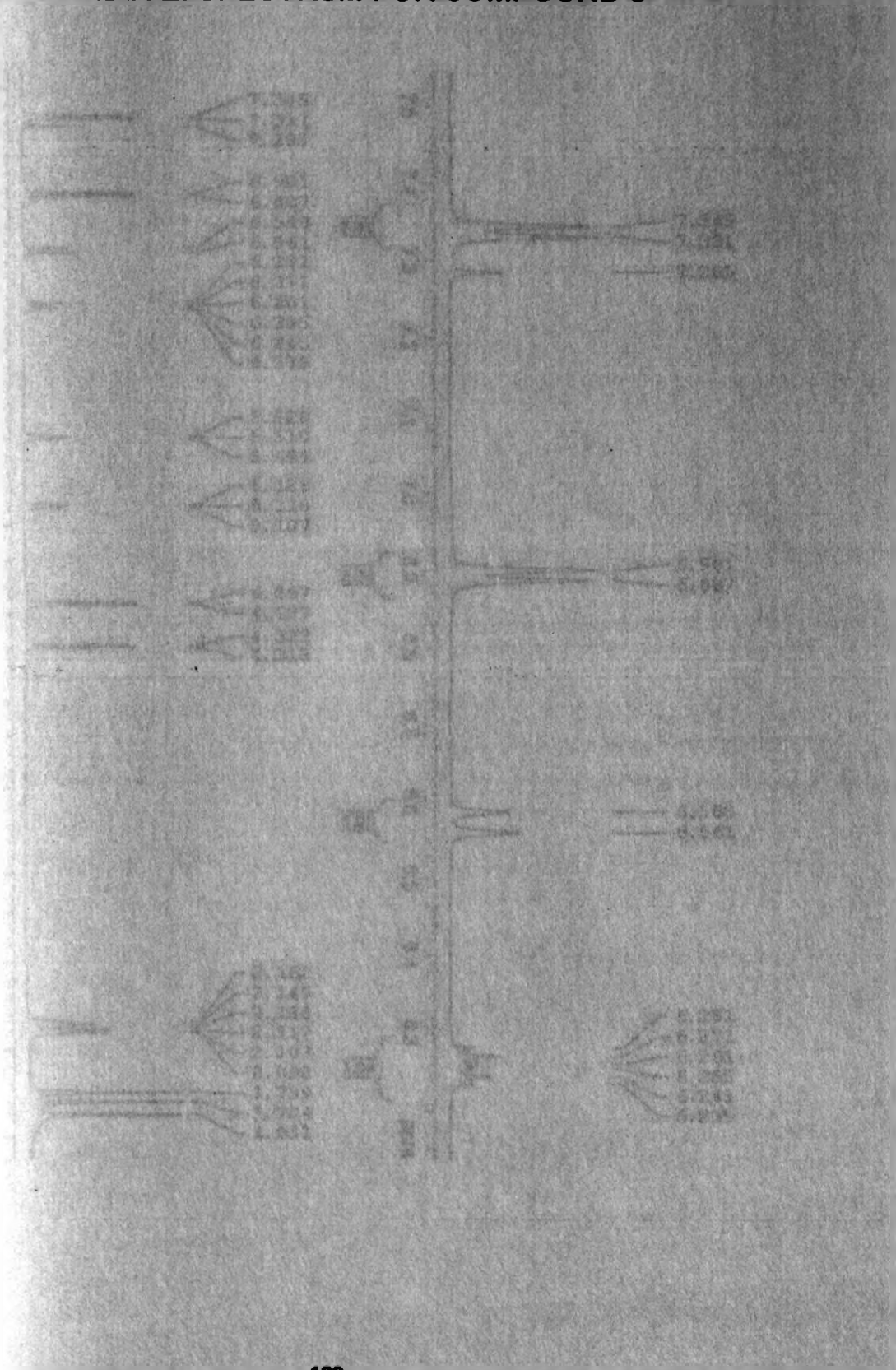
NOESY SPECTRUM FOR COMPOUND **4** (CD_2CL_2, 600 MHz)



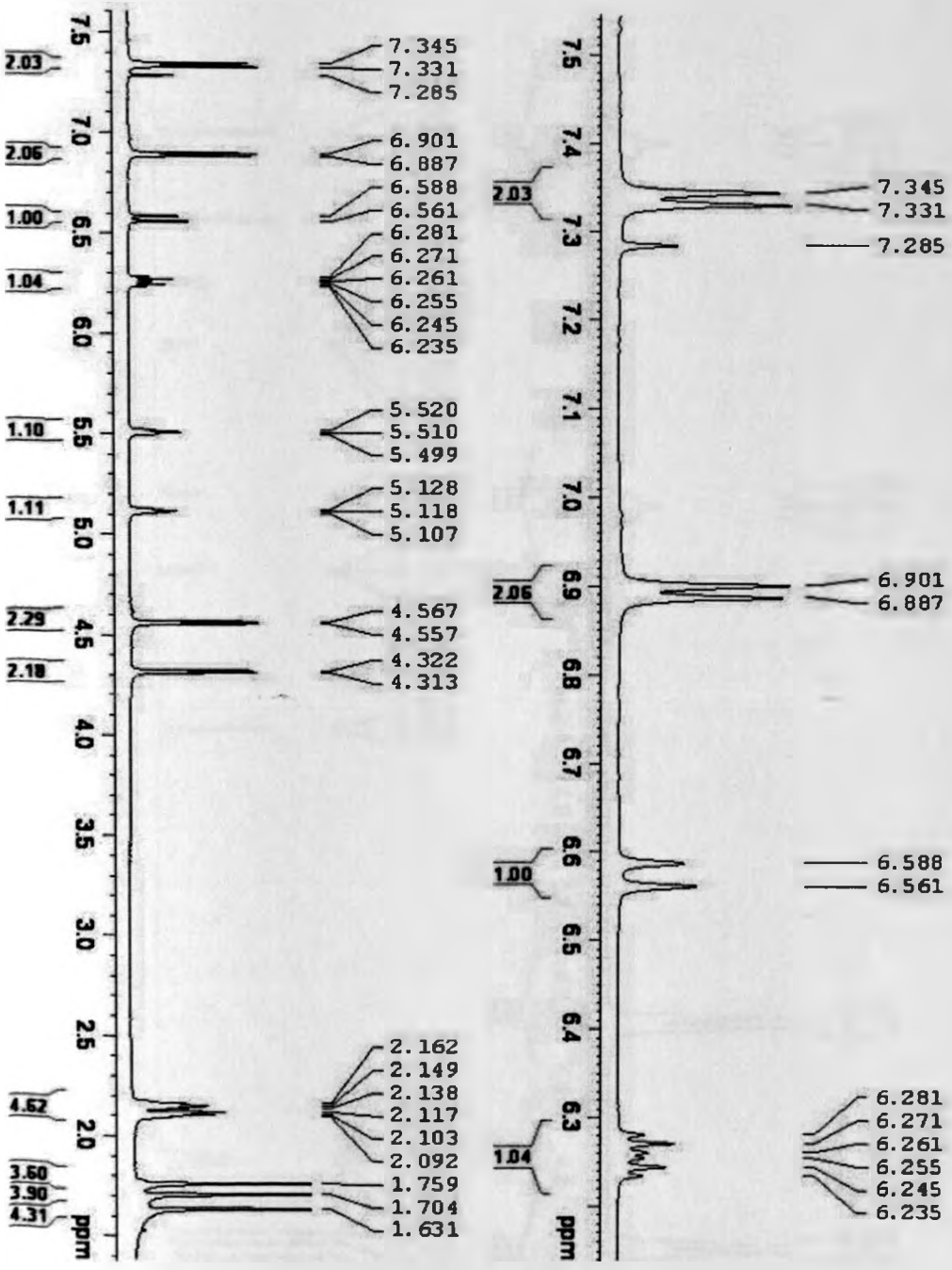
MASS SPECTRUM FOR COMPOUND **4**



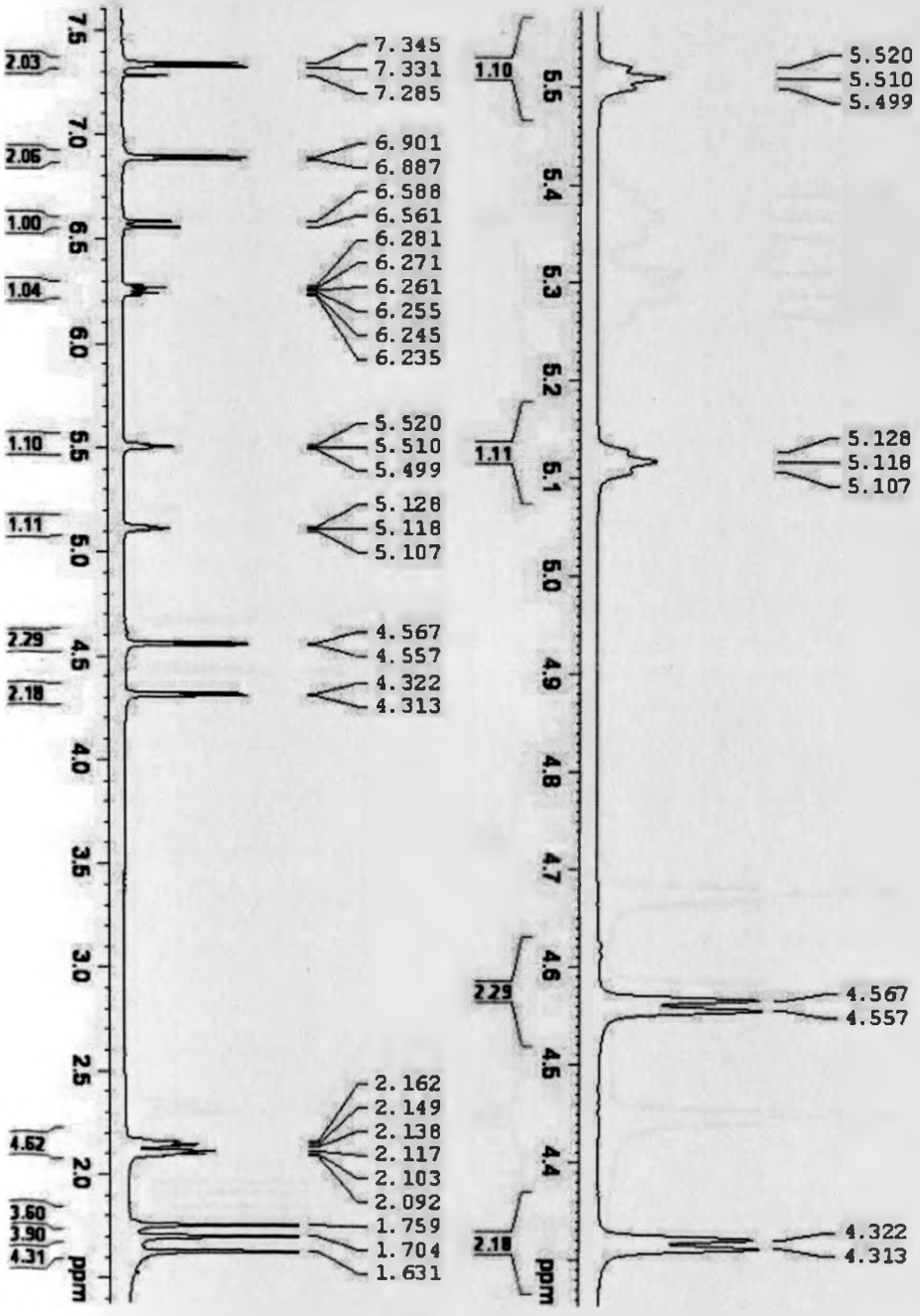
APPENDIX E: SPECTRUM FOR COMPOUND 6



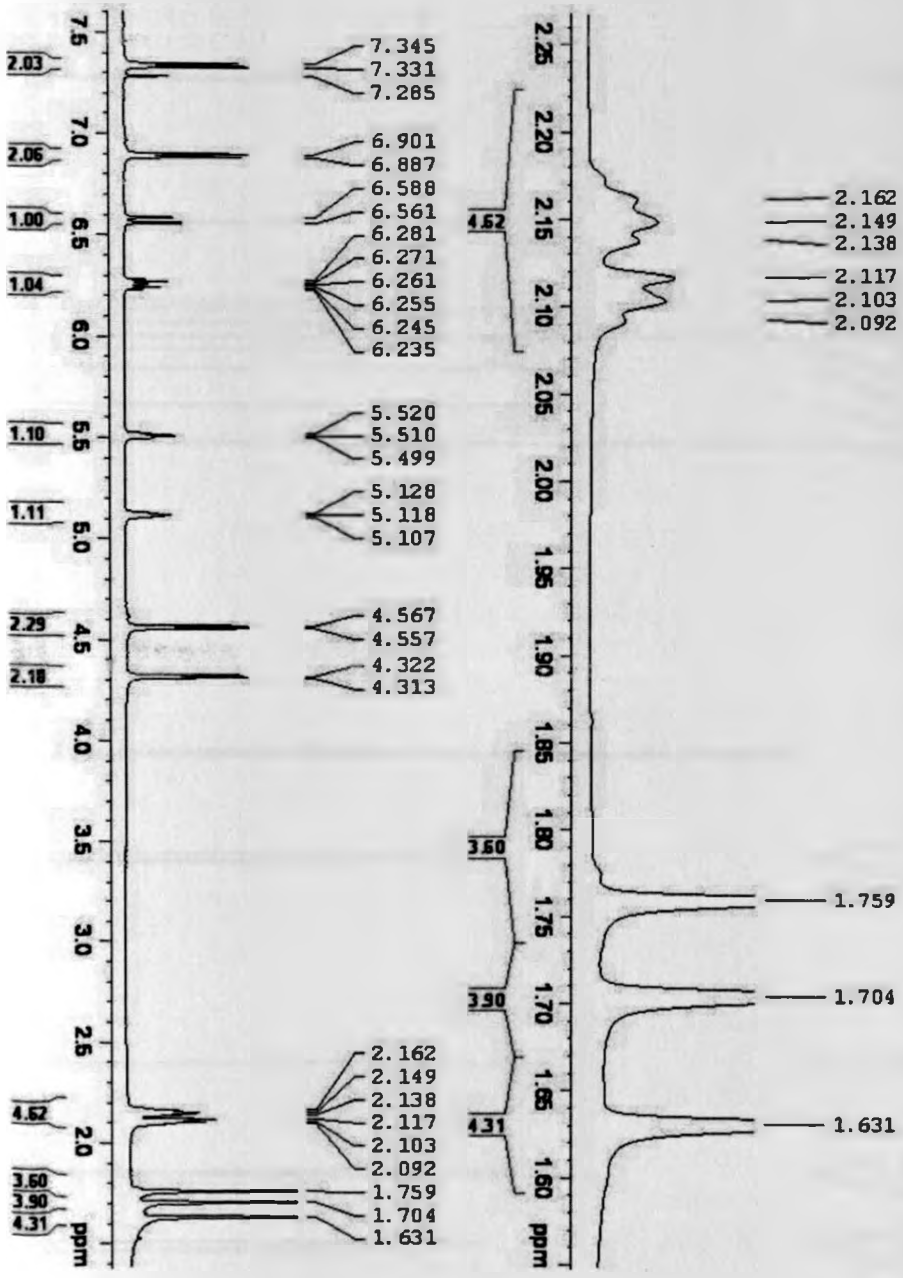
^{1}H NMR SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



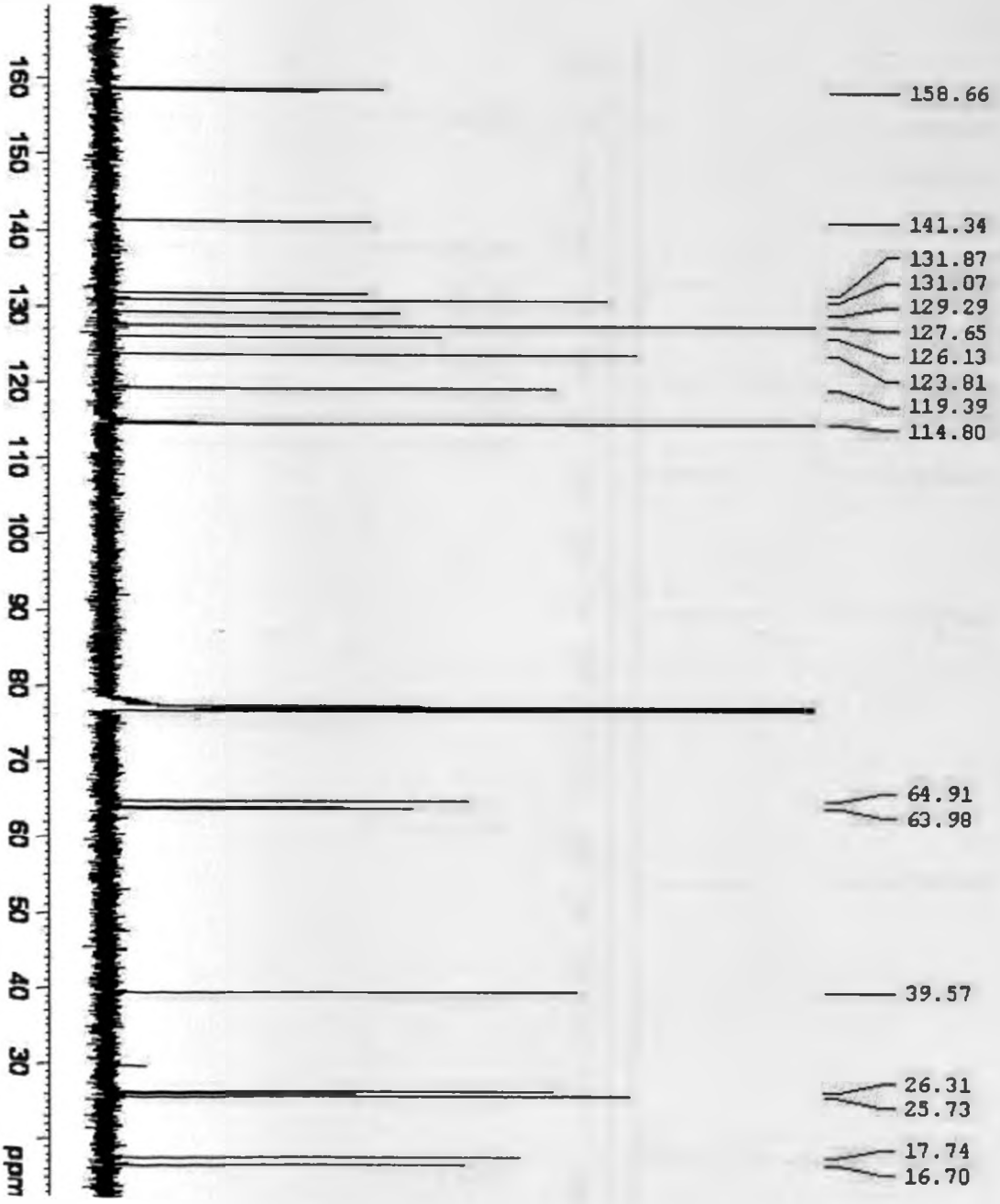
^{1}H NMR SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



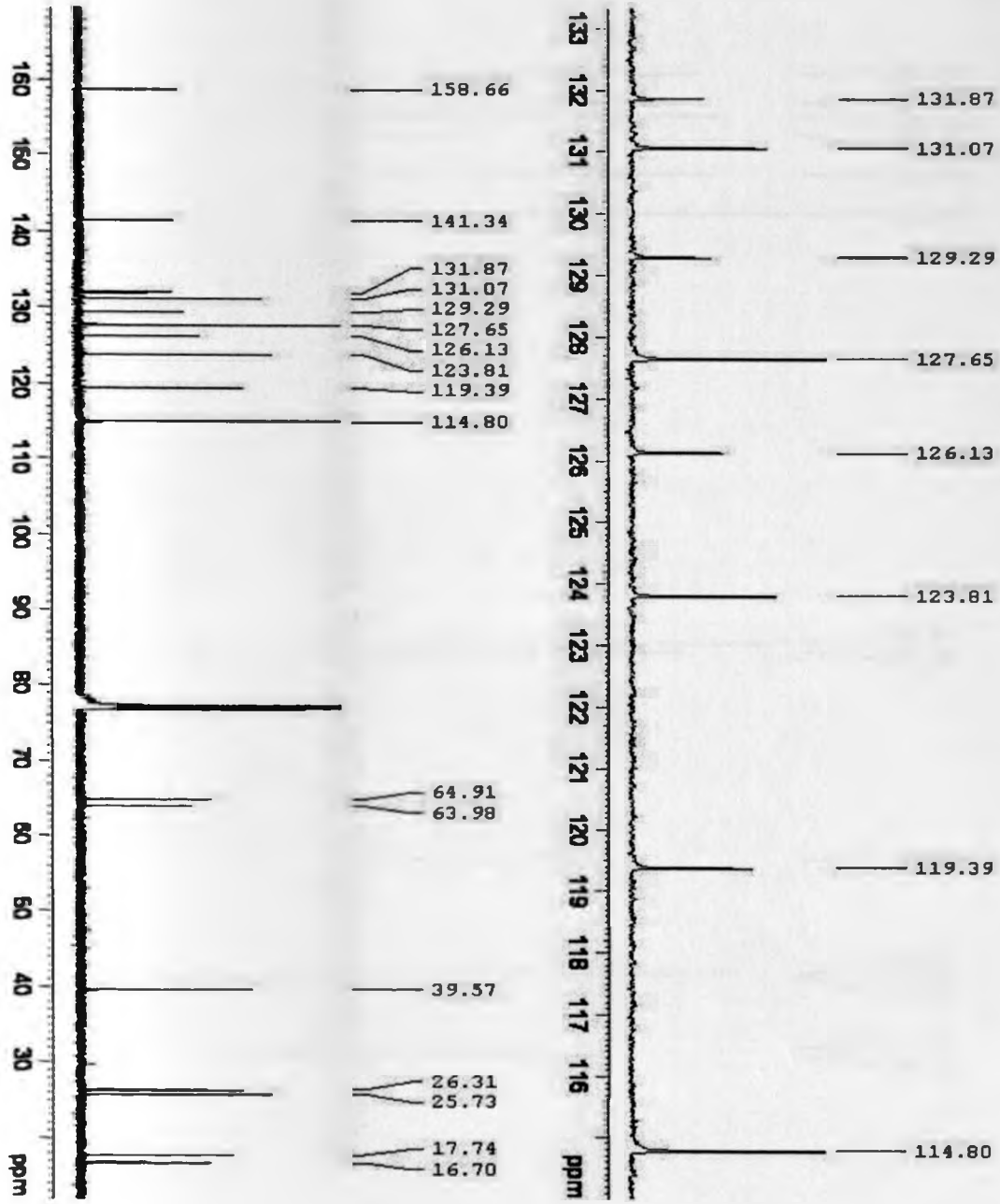
^{1}H NMR SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



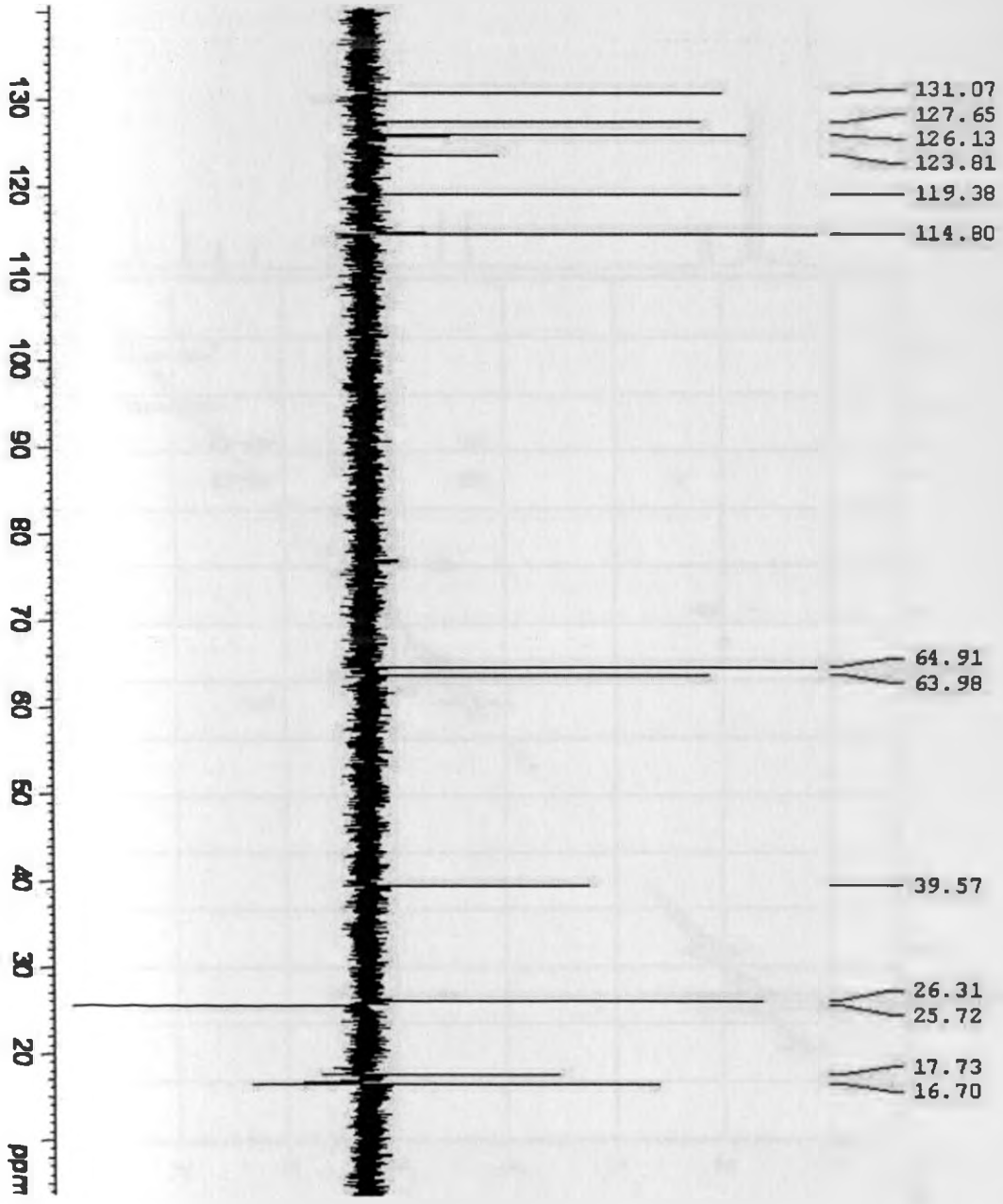
^{13}C NMR SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



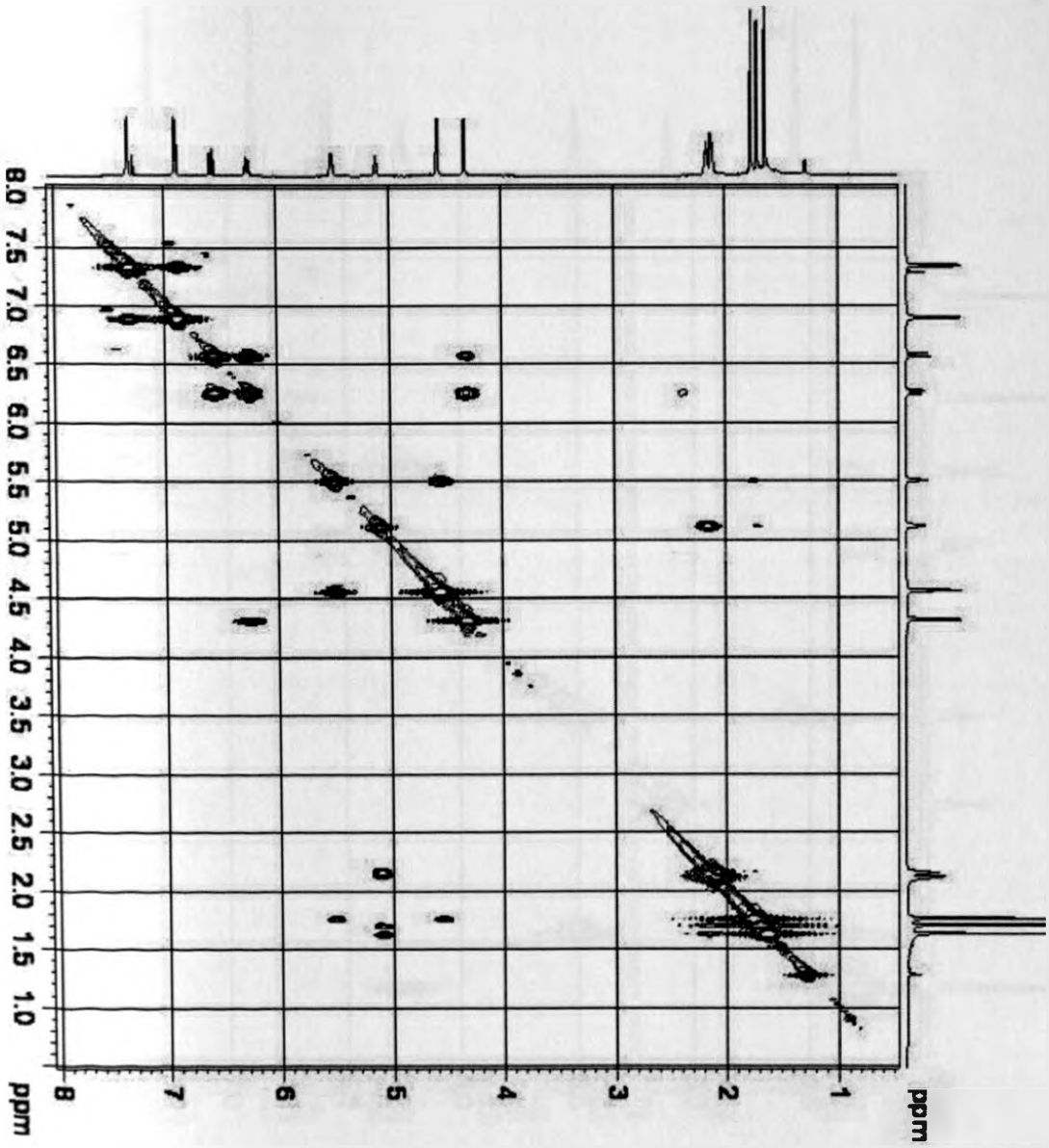
^{13}C NMR SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



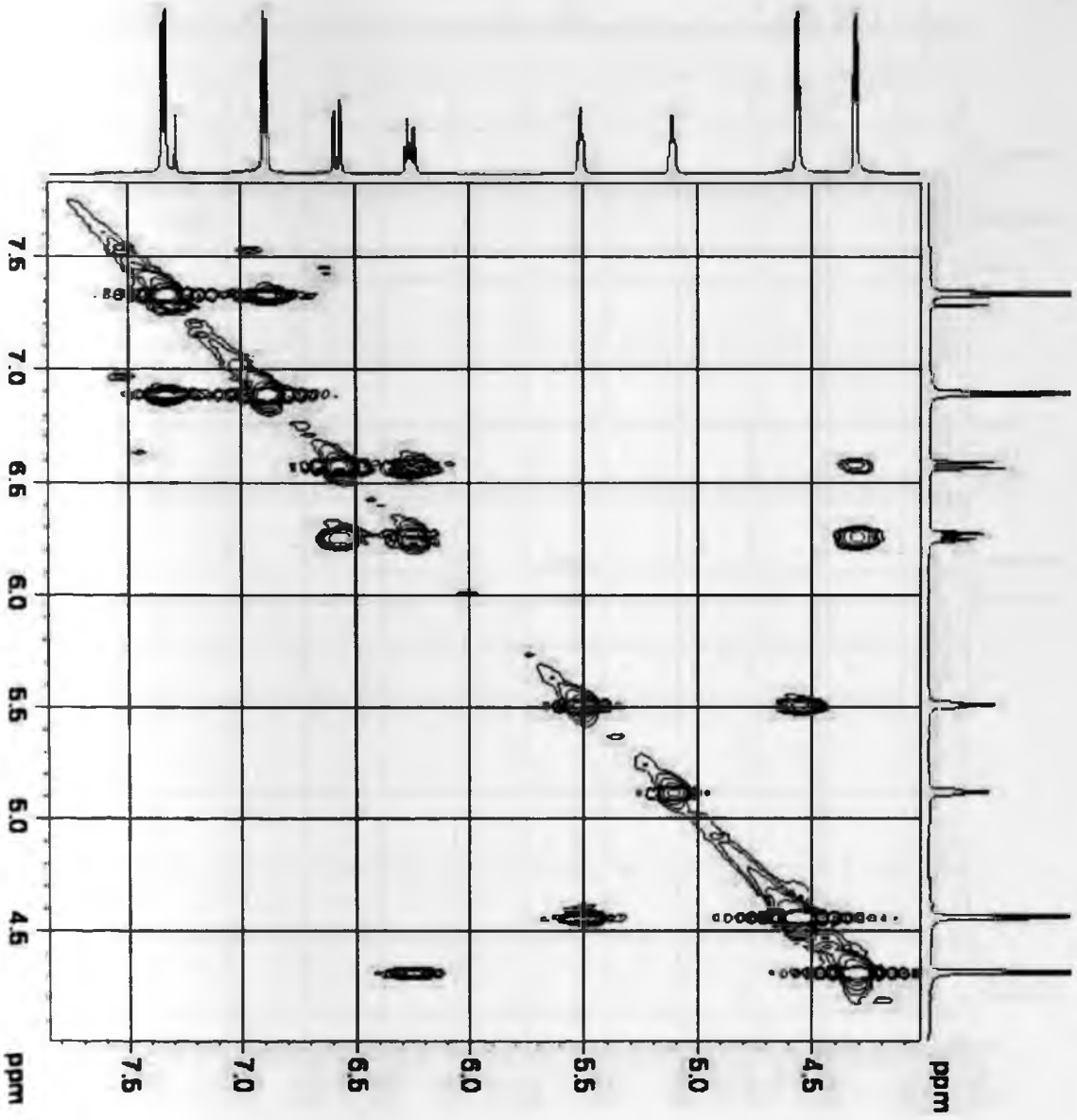
DEPT SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



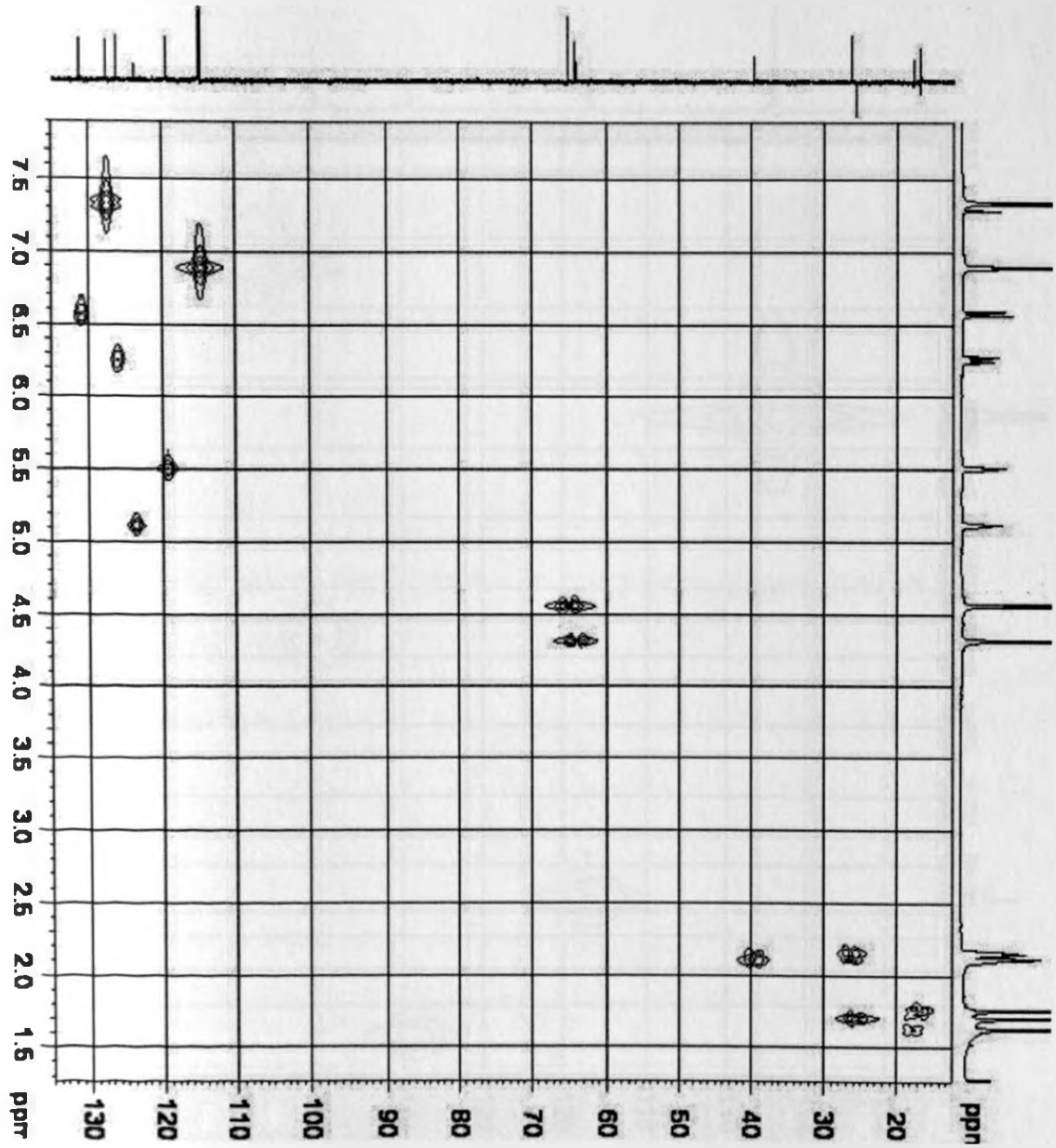
COSY SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



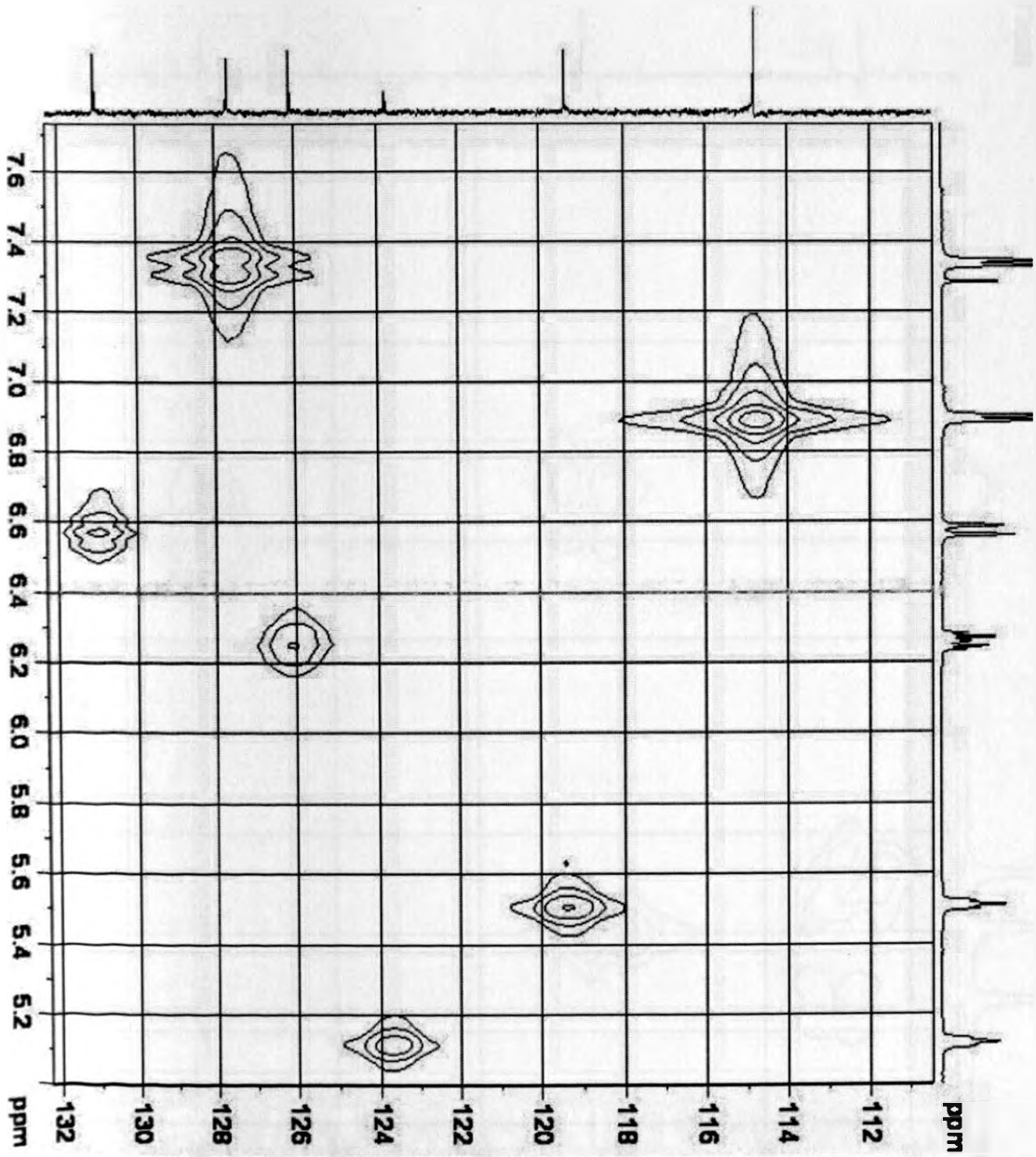
COSY SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



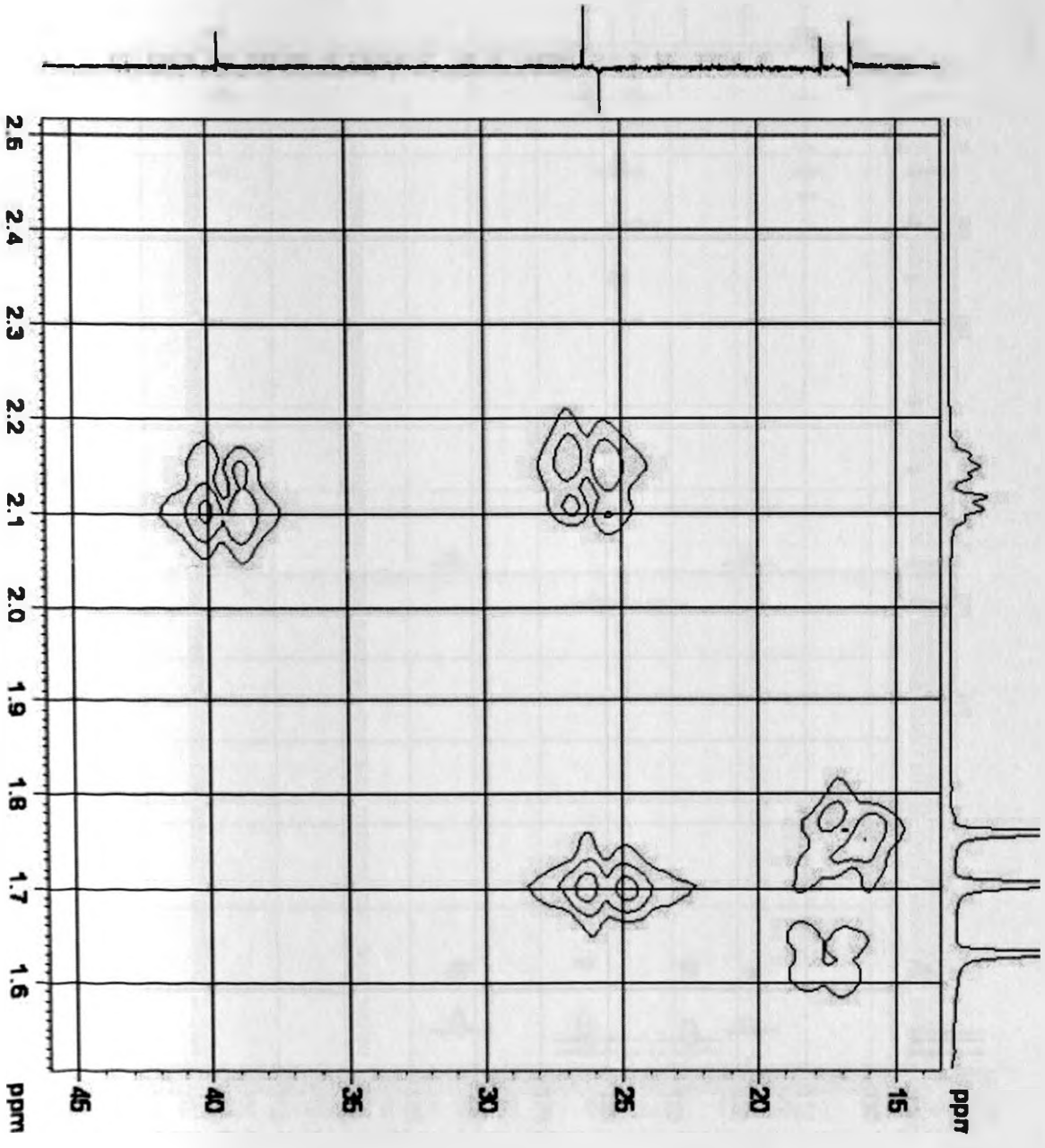
HMQC SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



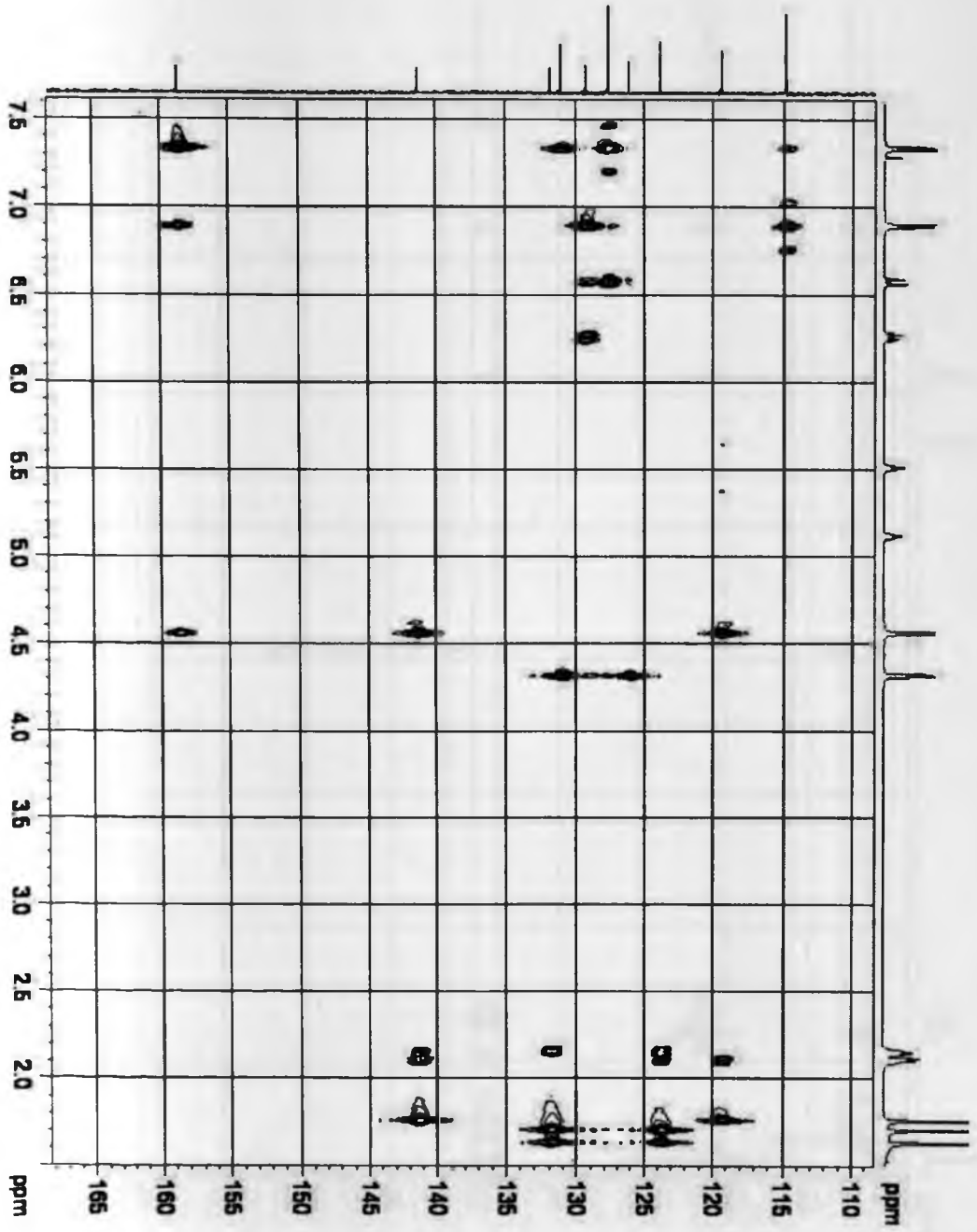
HMQC SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



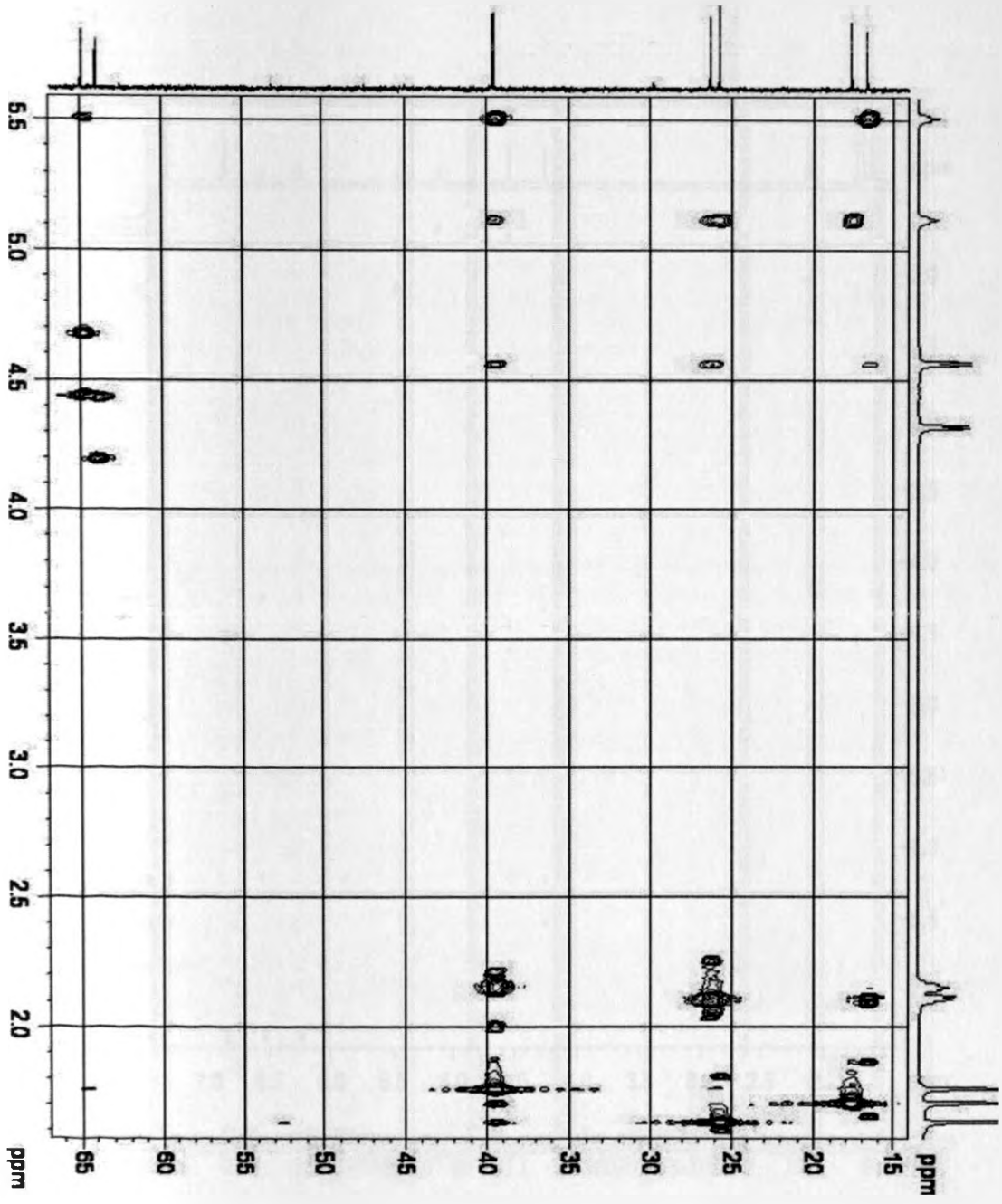
HMQC SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



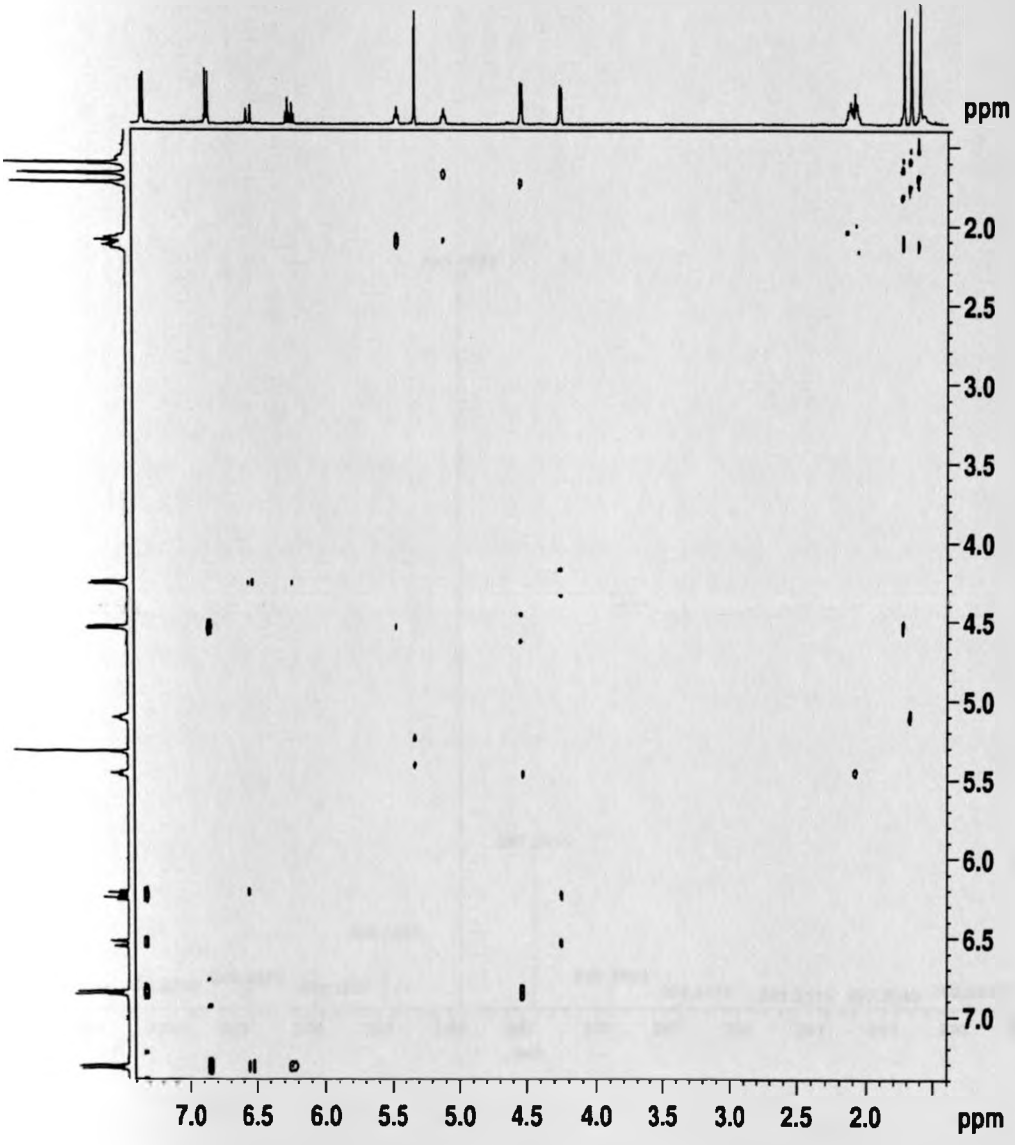
HMBC SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



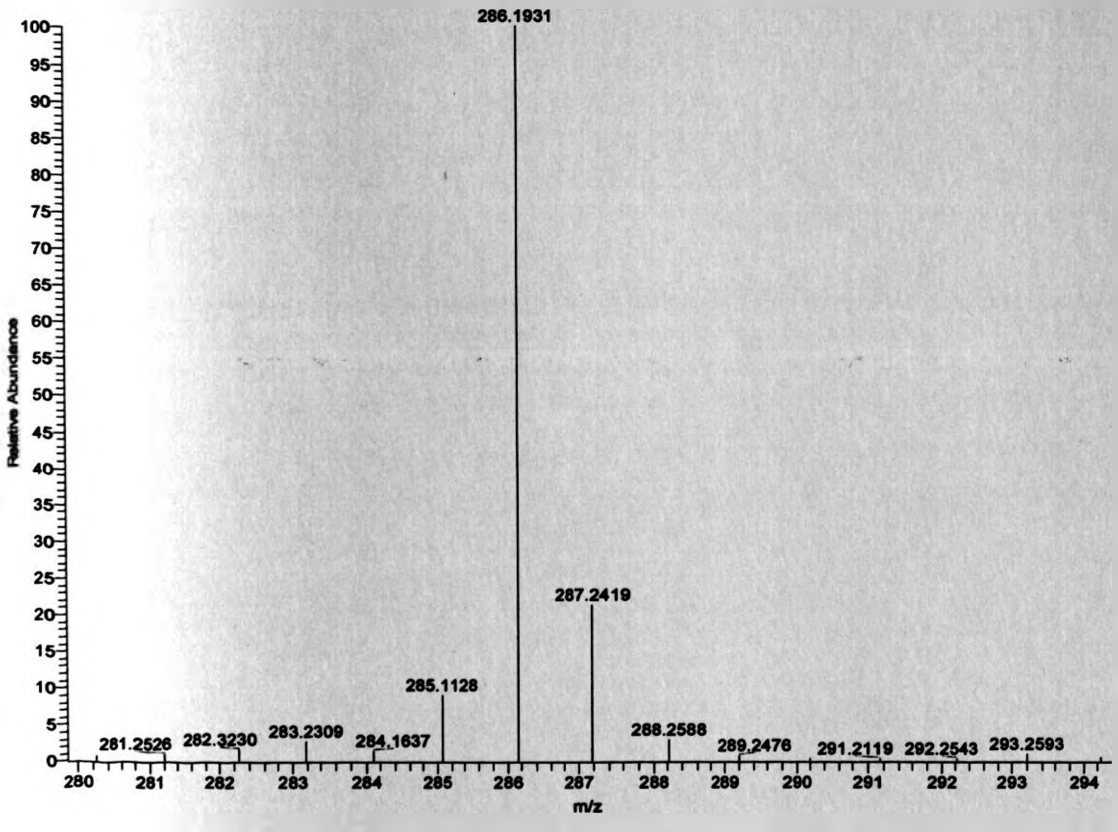
HMBC SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)



NOESY SPECTRUM FOR COMPOUND **5** ($CDCL_3$, 600 MHz)

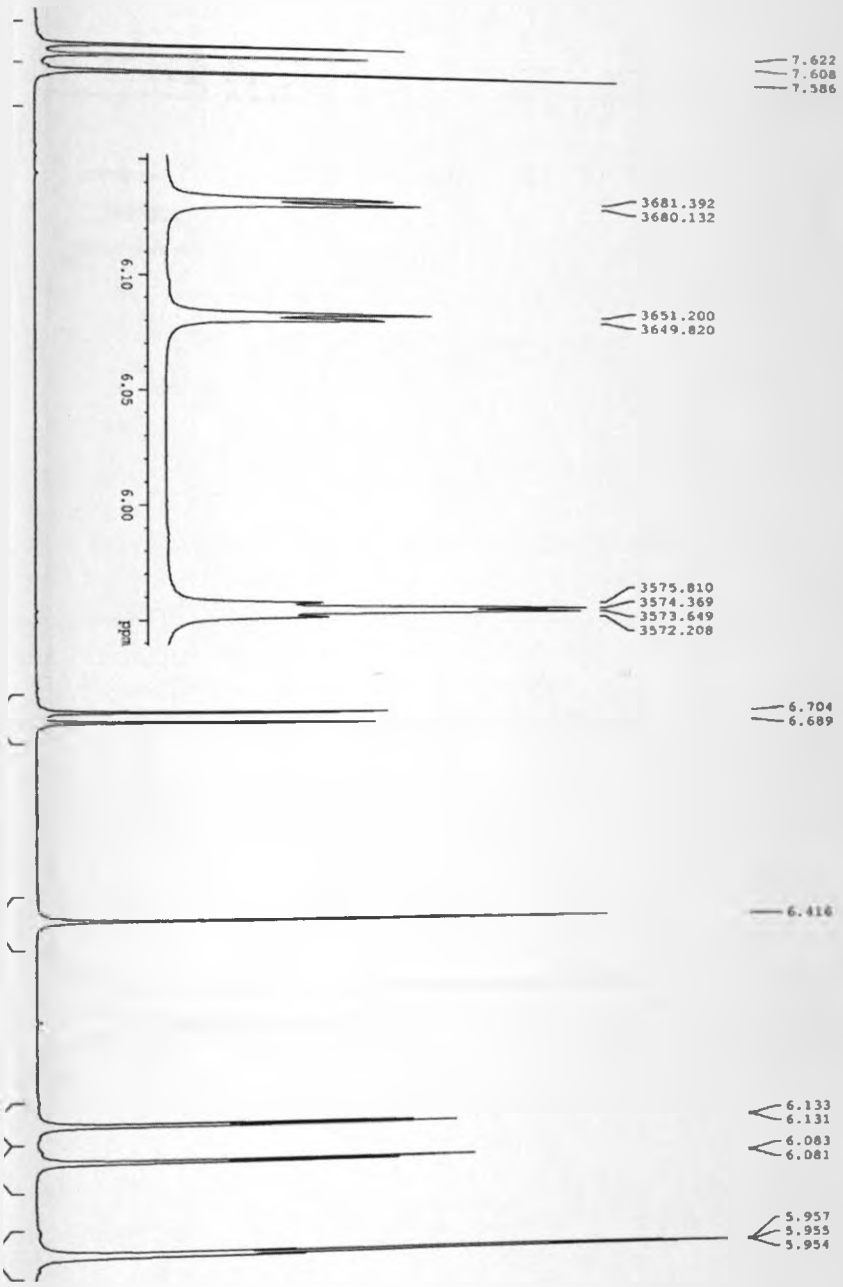


MASS SPECTRUM FOR COMPOUND **5**

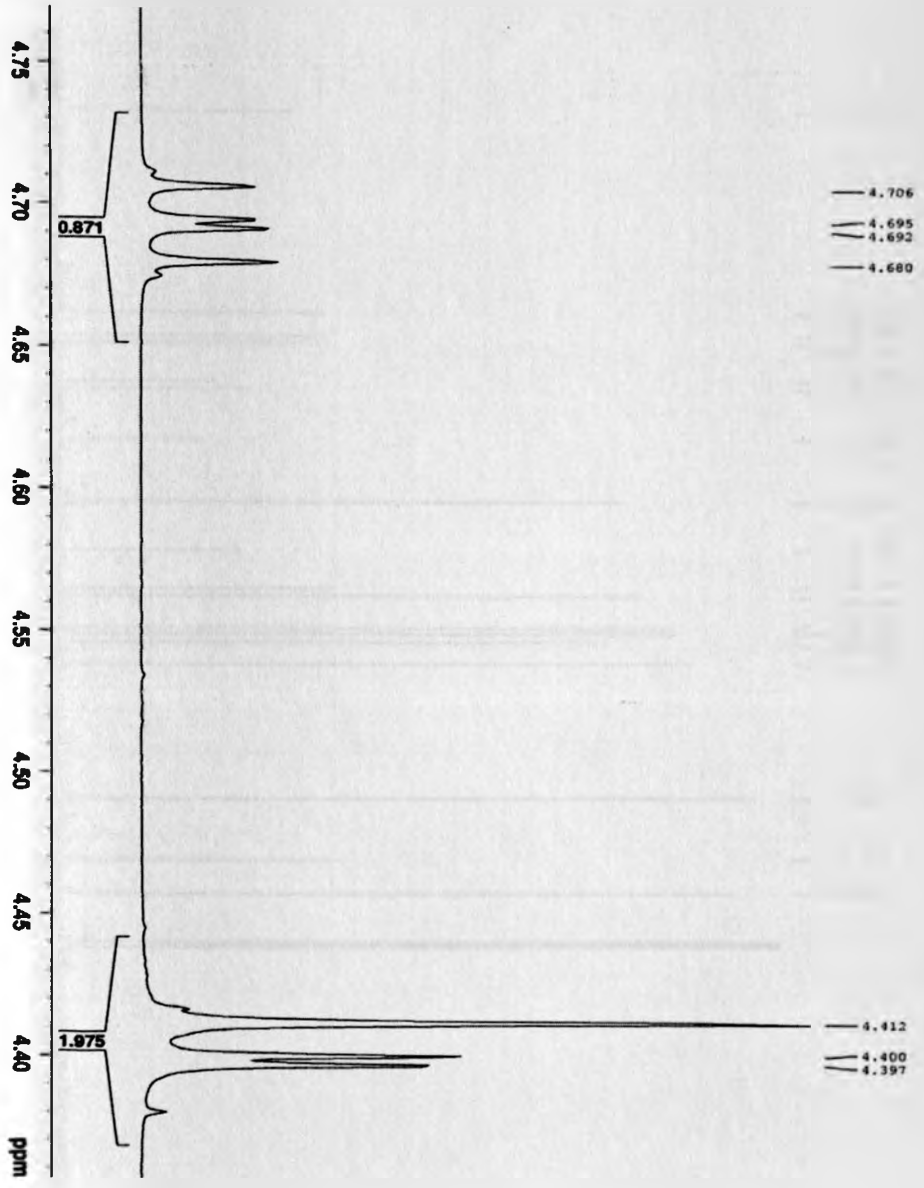


APPENDIX F: SPECTRA FOR COMPOUND **6**

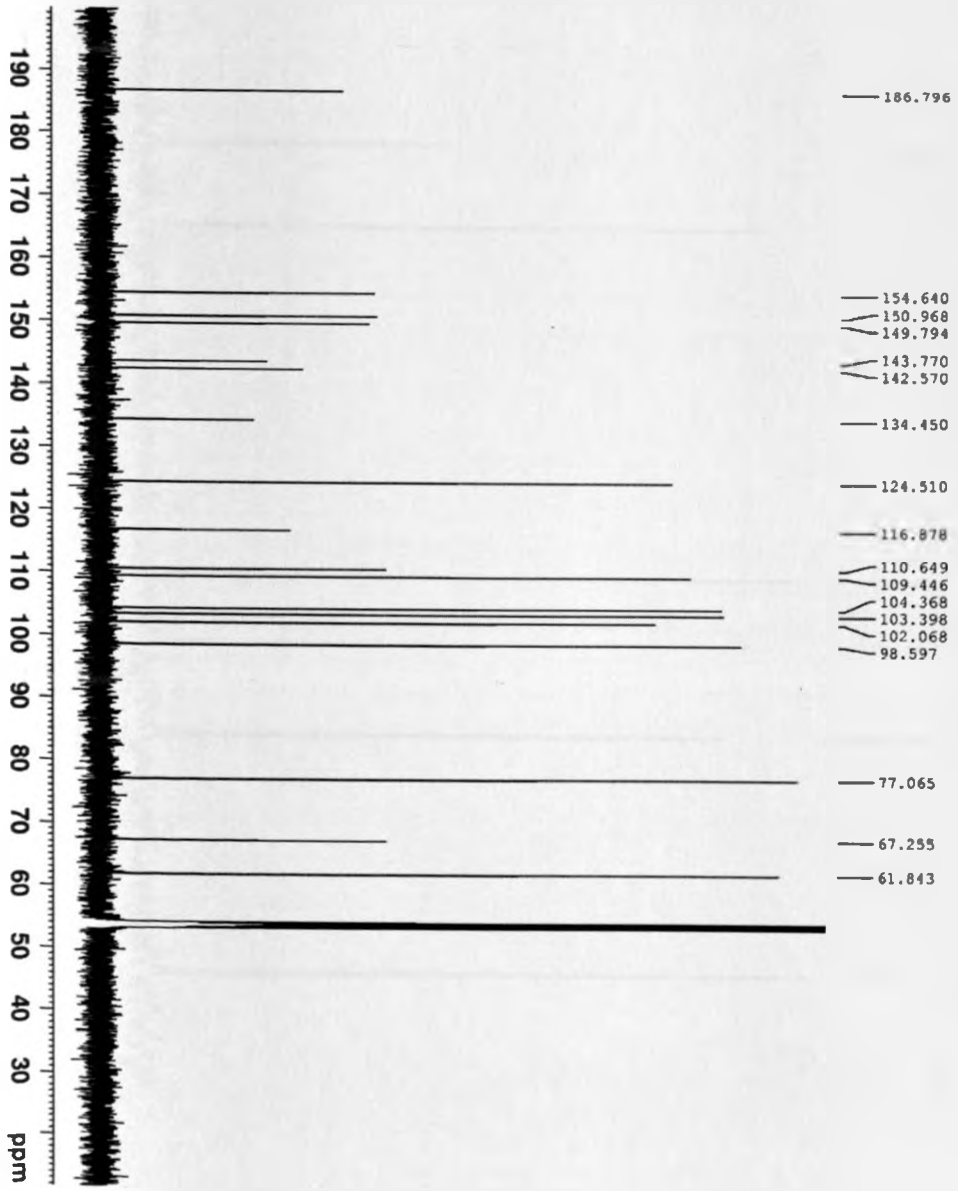
ECTRUM FOR COMPOUND **6** (CD_2CL_2, 600 MHz)



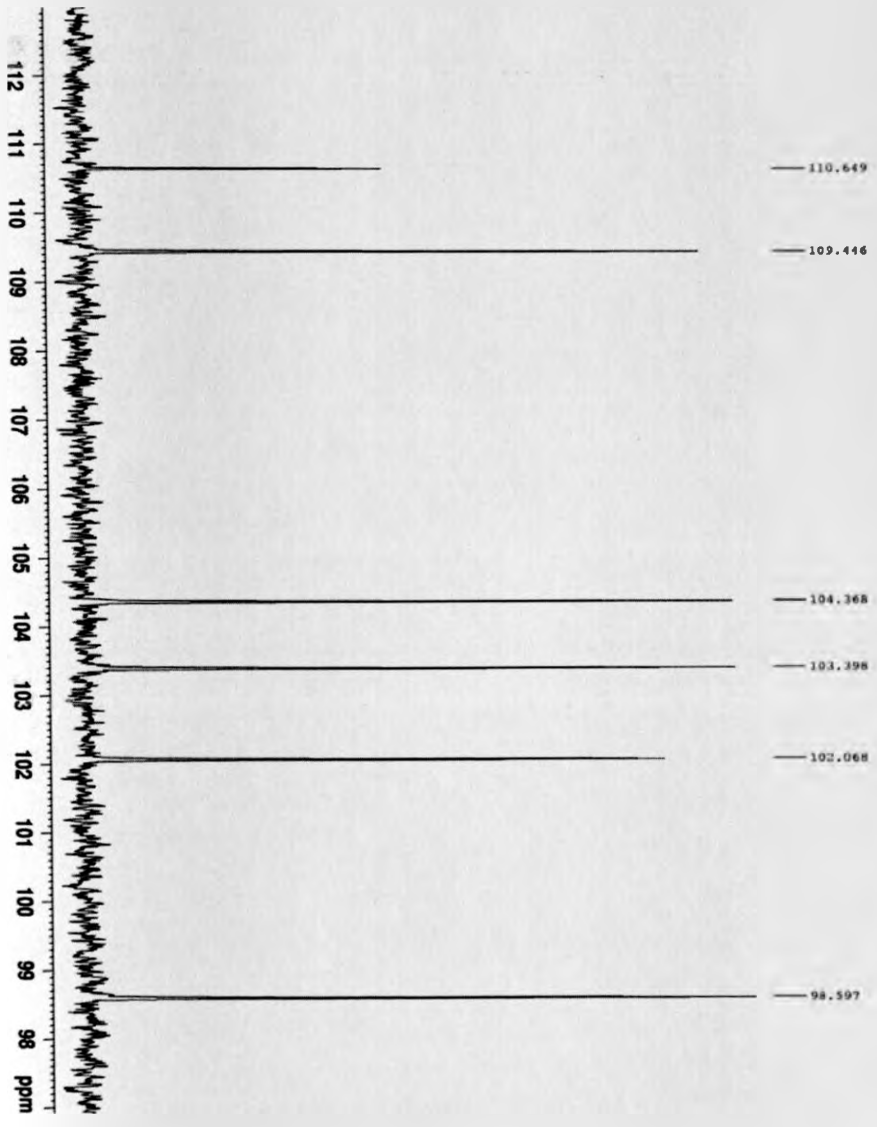
^{1}H NMR SPECTRUM FOR COMPOUND **6** (CD_2CL_2, 600 MHz)



^{13}C SPECTRUM FOR COMPOUND **6** (CD_2CL_2, 600 MHz)

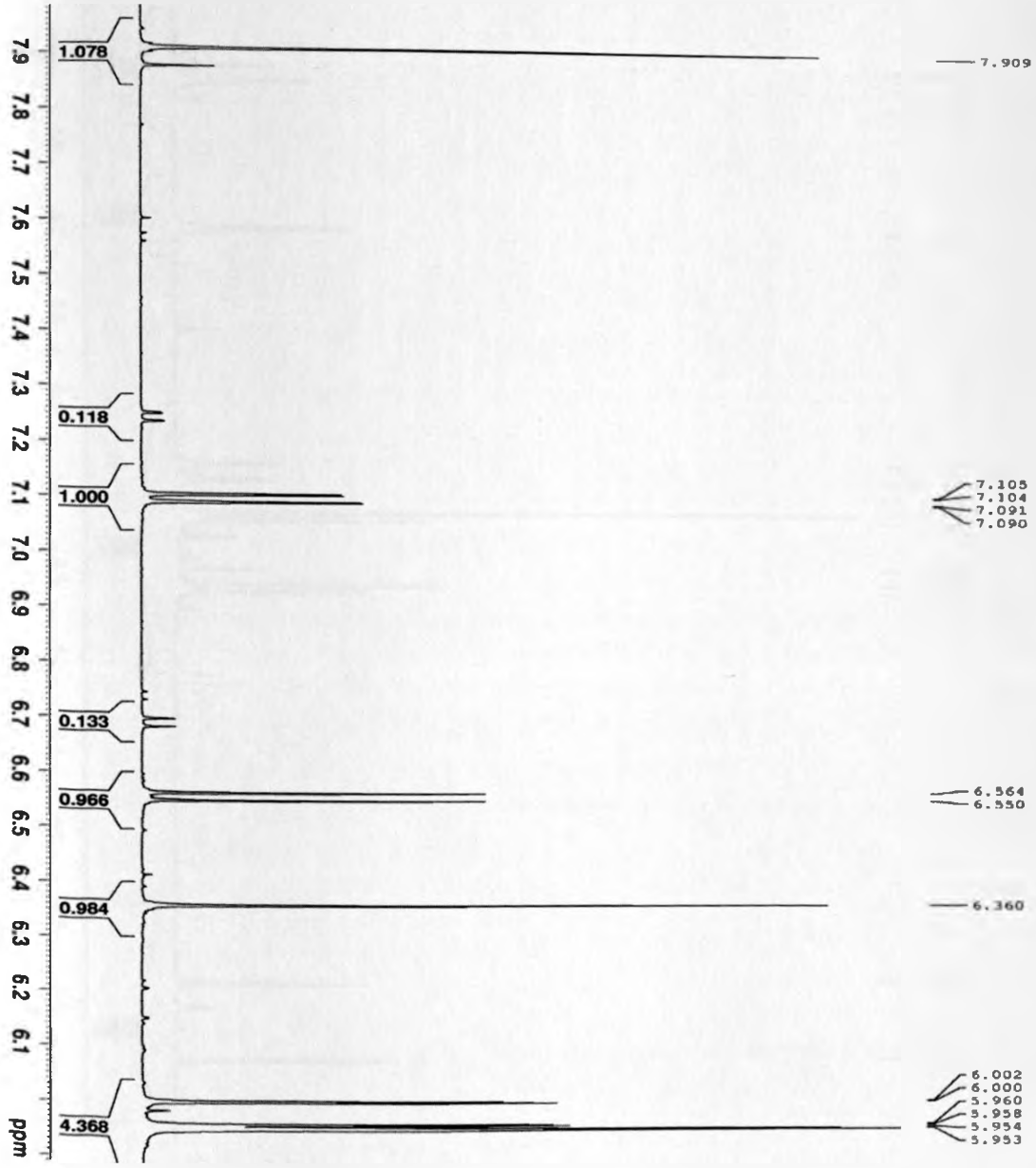


^{13}C SPECTRUM FOR COMPOUND **6** (CD_2CL_2, 600 MHz)

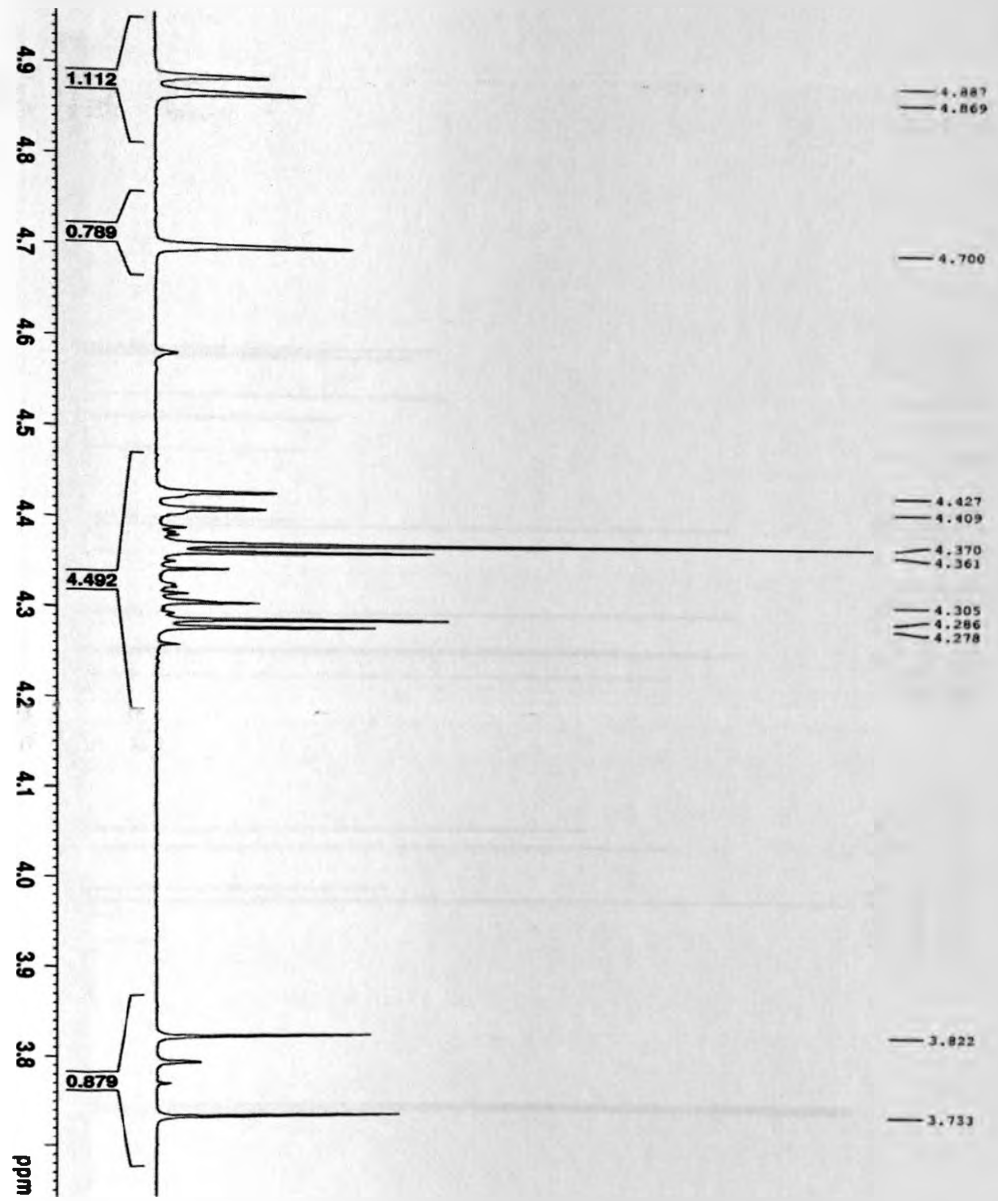


APPENDIX G: SPECTRA FOR COMPOUND **7**

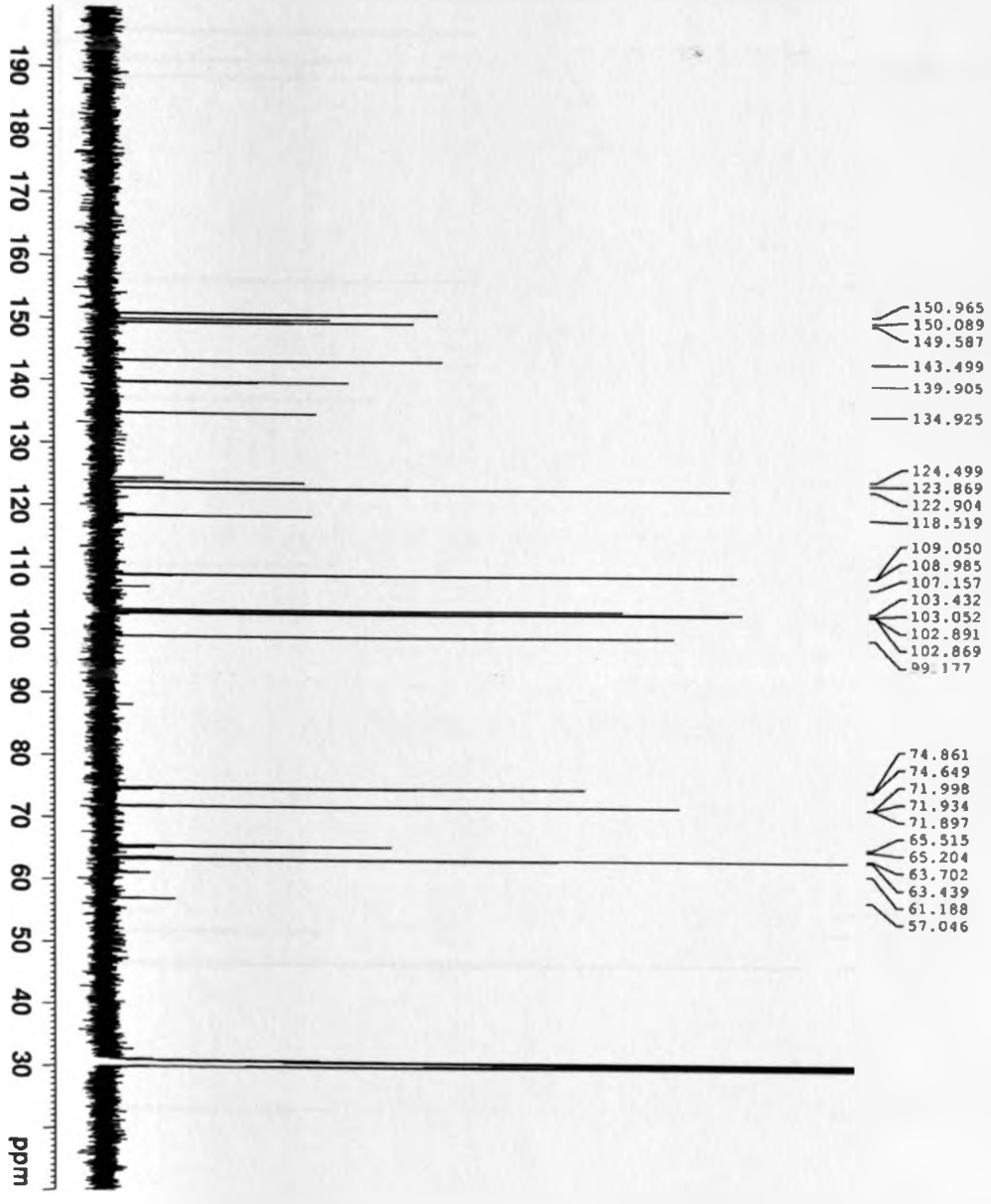
^{1}H NMR SPECTRUM FOR COMPOUND **7** (CD_2CL_2, 600 MHz)



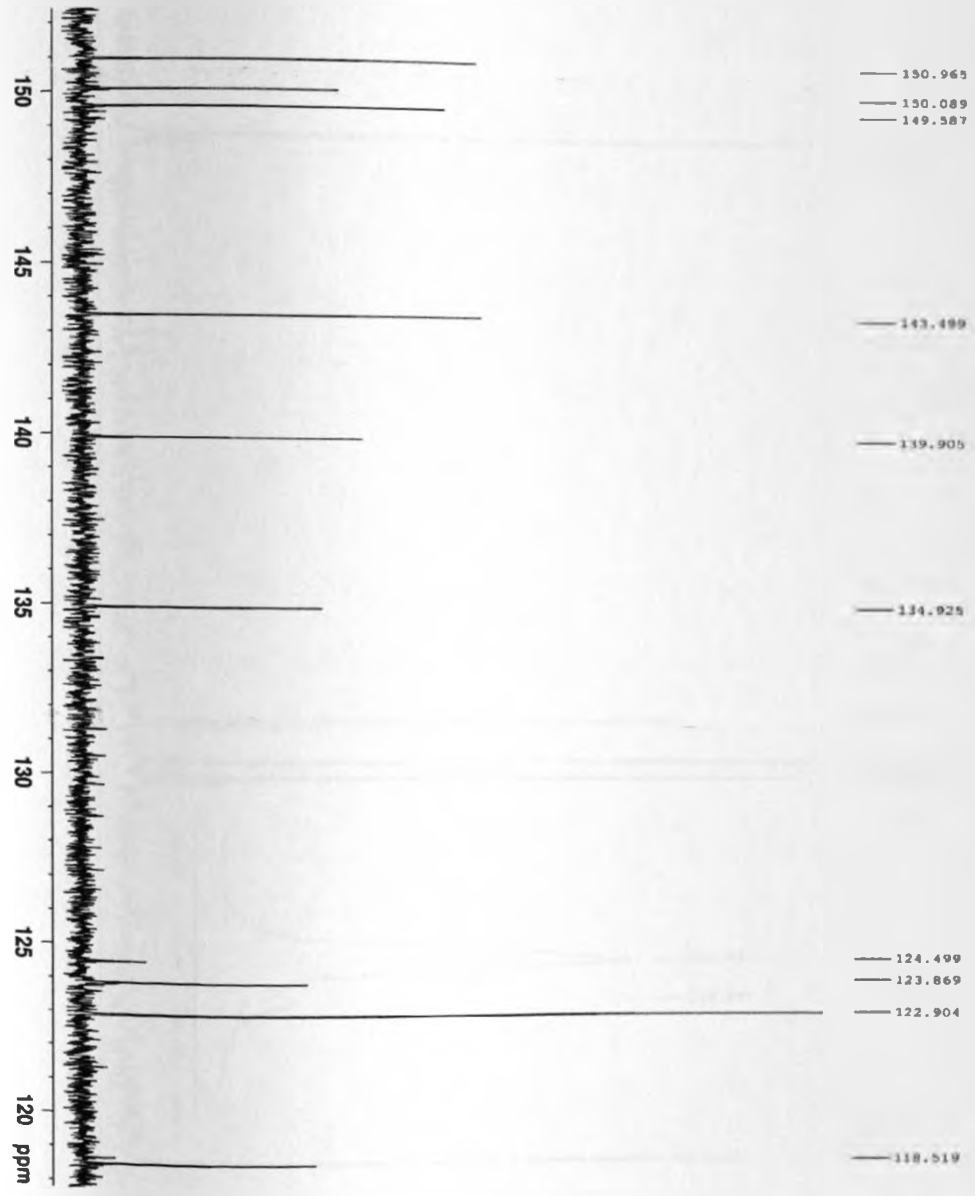
^{1}H NMR SPECTRUM FOR COMPOUND **7** (CD_2CL_2, 600 MHz)



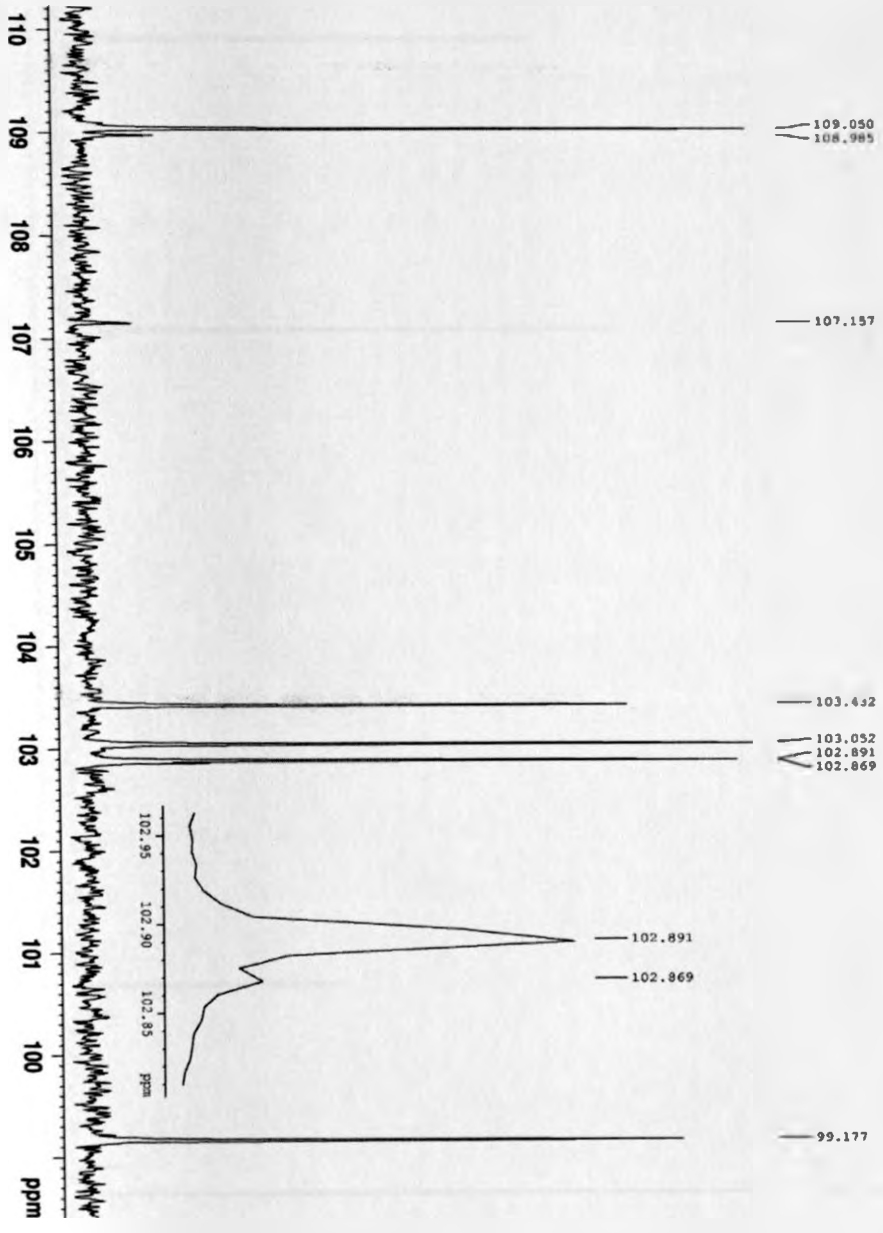
^{13}C NMR SPECTRUM FOR COMPOUND **7** (CD_2CL_2, 600 MHz)



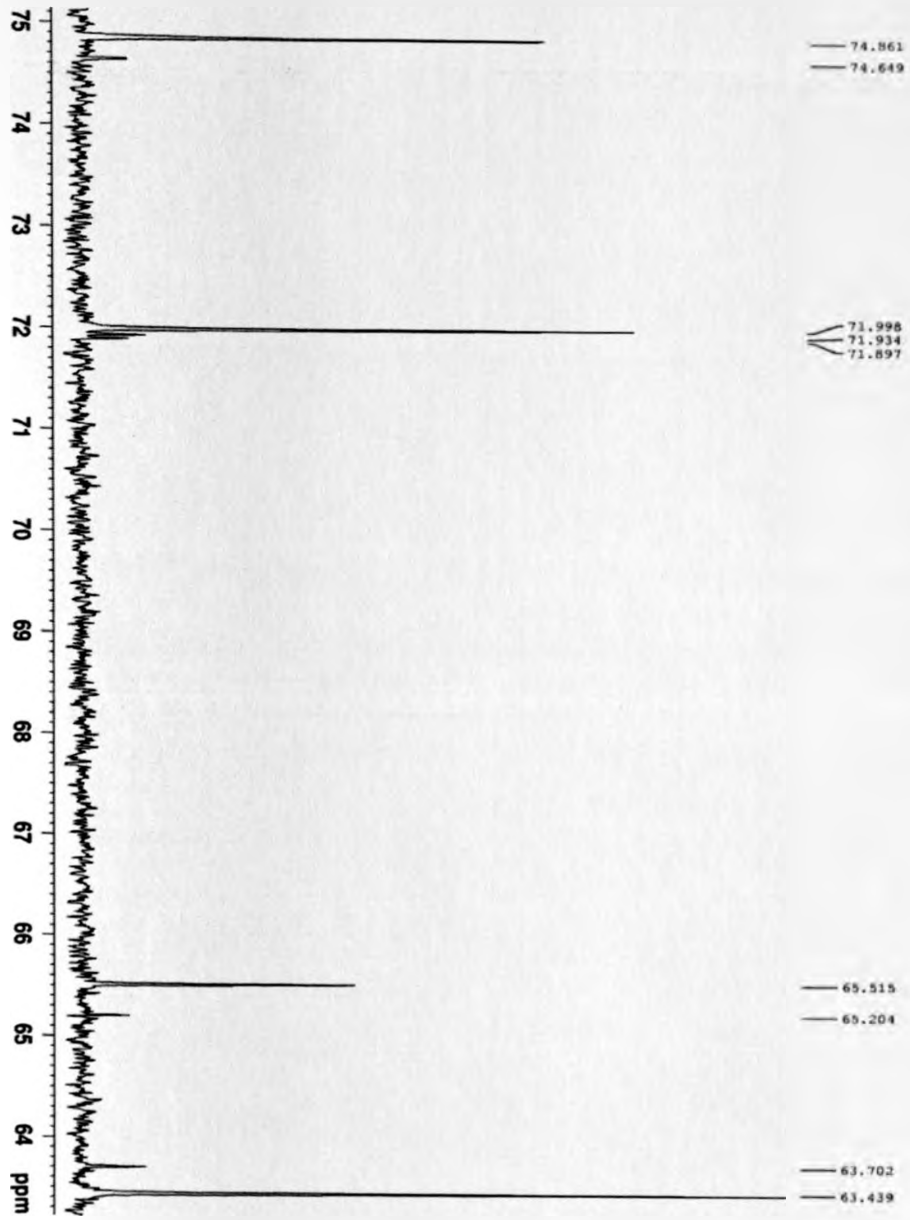
^{13}C NMR SPECTRUM FOR COMPOUND **7** (CD_2CL_2, 600 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND **7** (CD_2CL_2, 600 MHz)

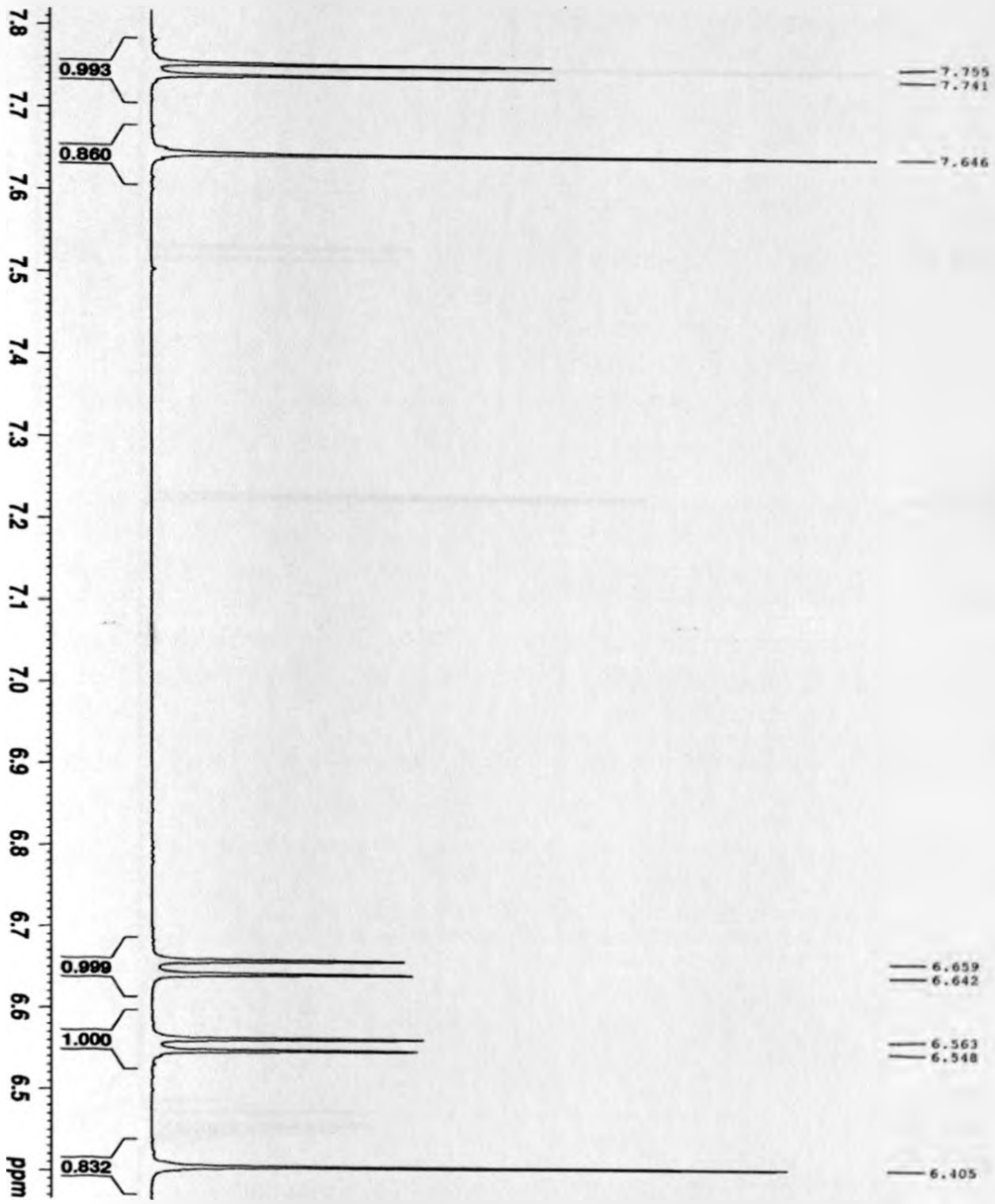


^{13}C NMR SPECTRUM FOR COMPOUND **7** (CD_2CL_2, 600 MHz)

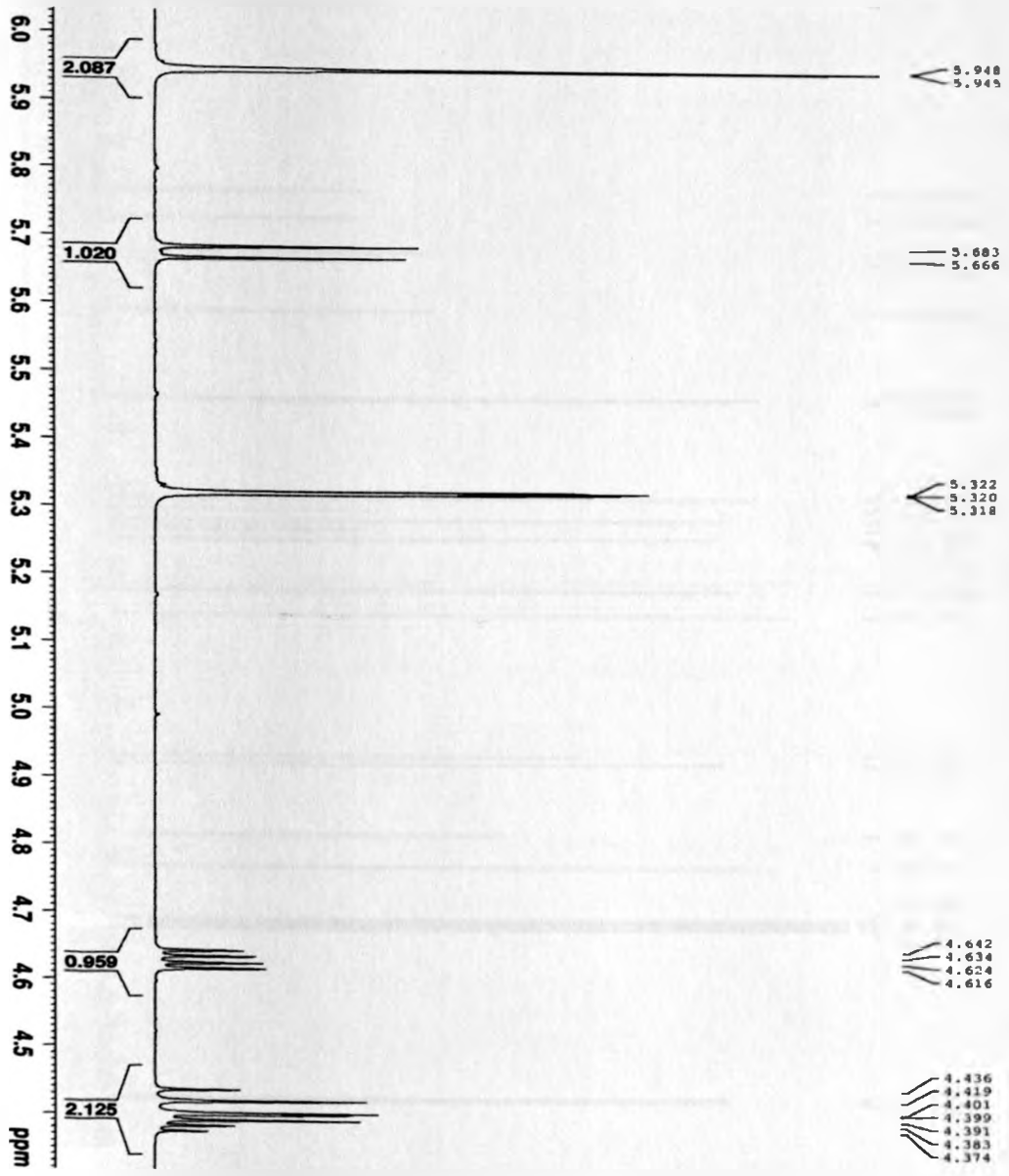


APPENDIX H: SPECTRA FOR COMPOUND **8**

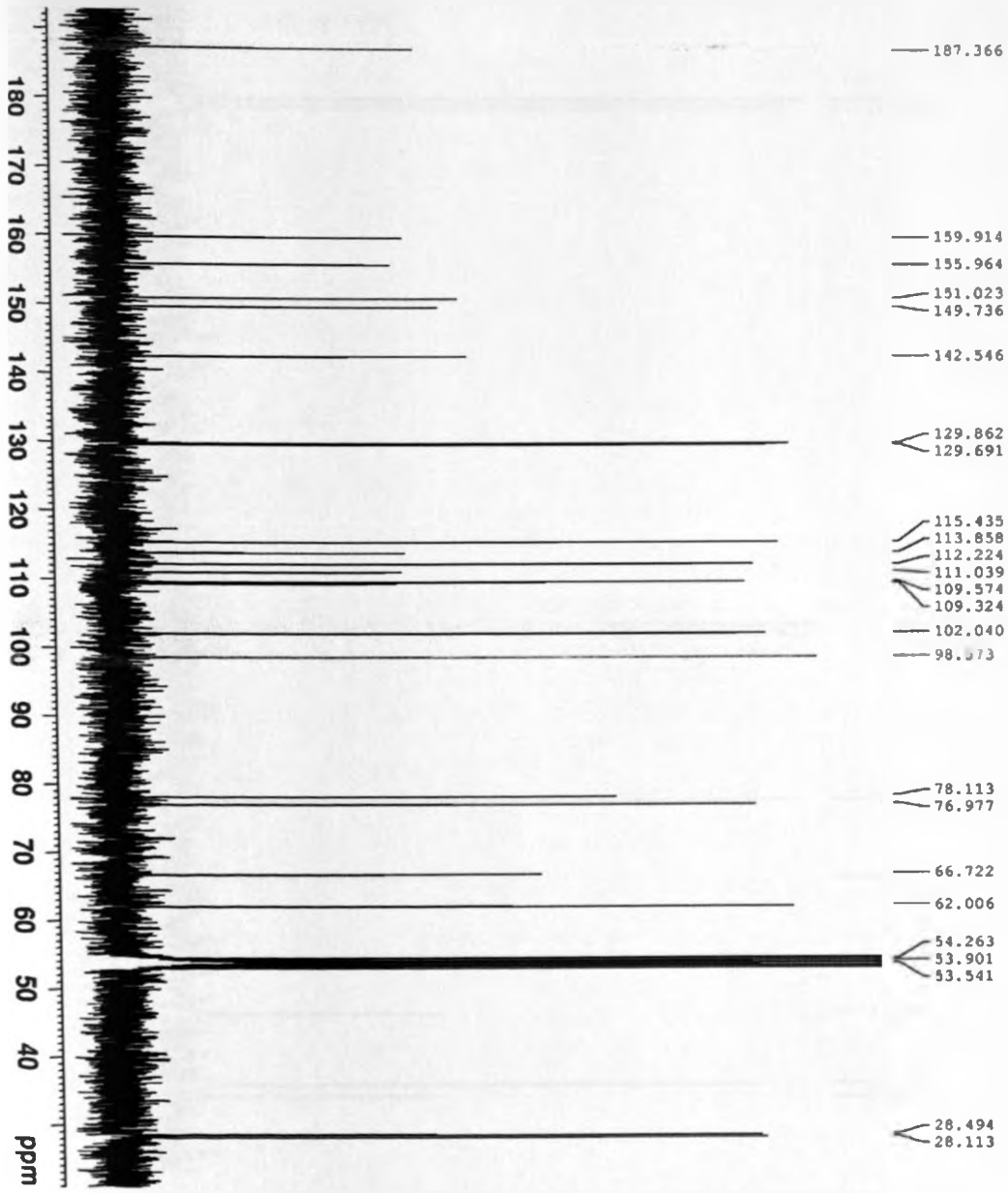
^{1}H NMR SPECTRUM FOR COMPOUND **8** (CD_2CL_2, 600 MHz)



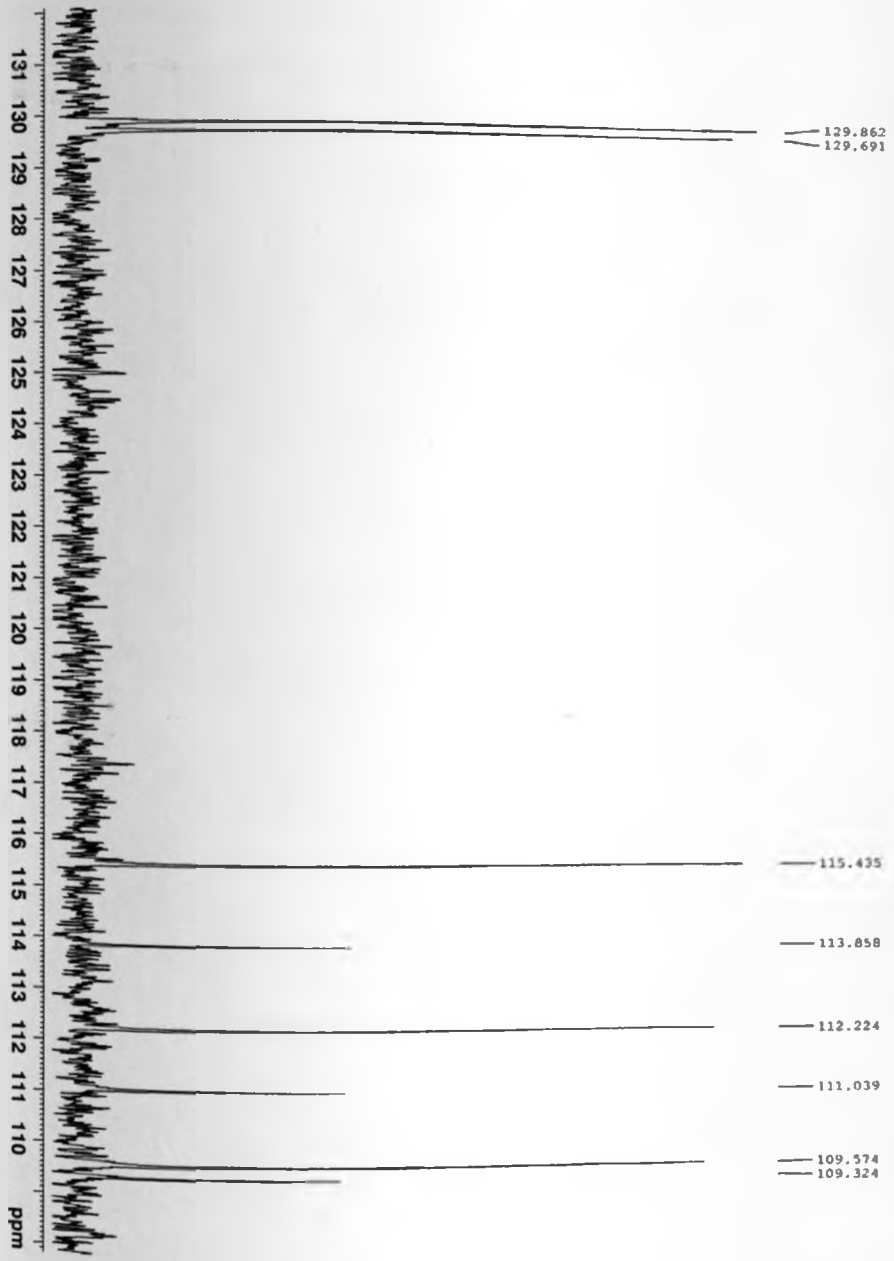
^{1}H NMR SPECTRA FOR COMPOUND **8** (CD_2CL_2, 600 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND **8** (CD_2CL_2, 600 MHz)

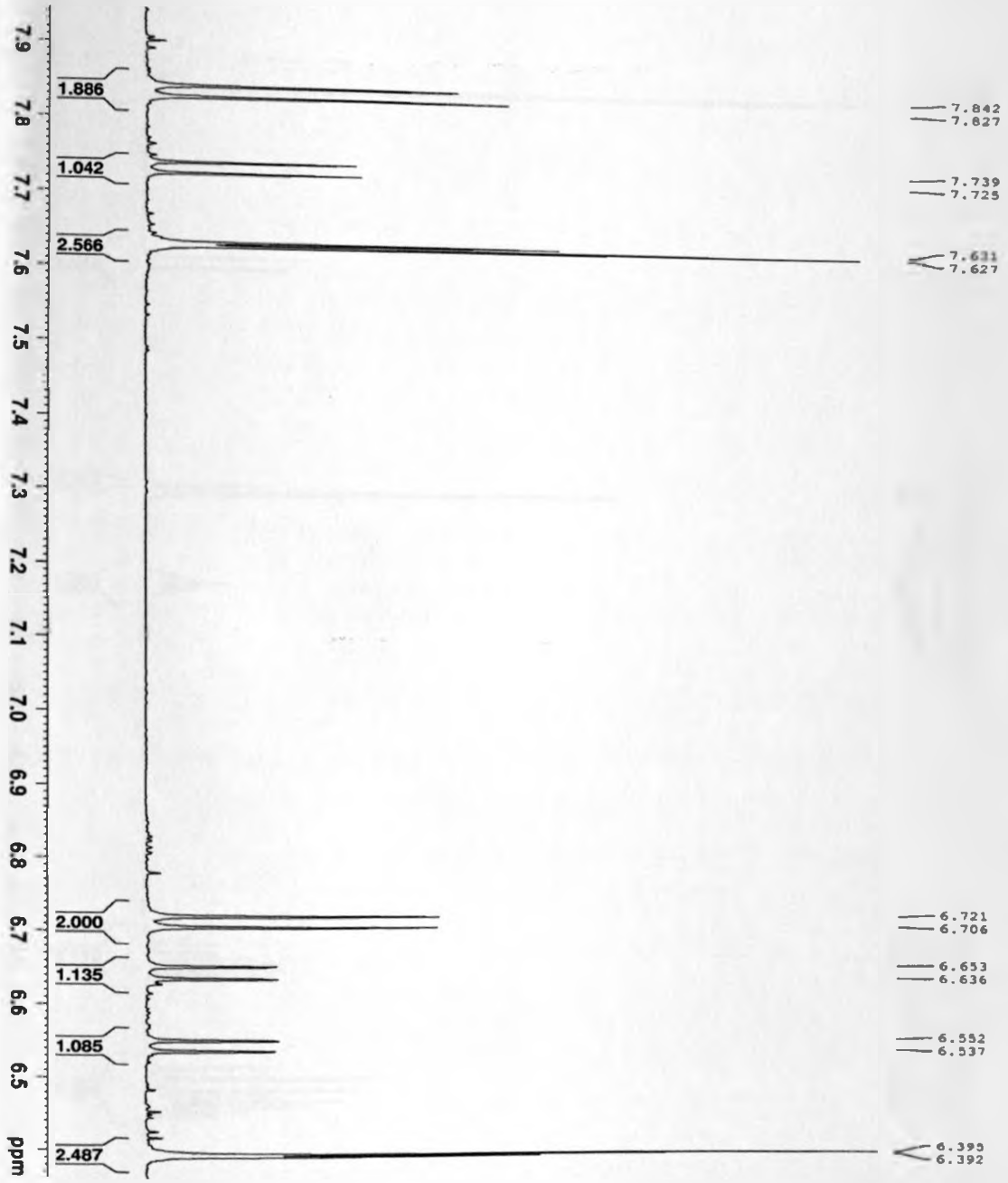


^{13}C NMR SPECTRUM FOR COMPOUND **8** (CD_2CL_2, 600 MHz)

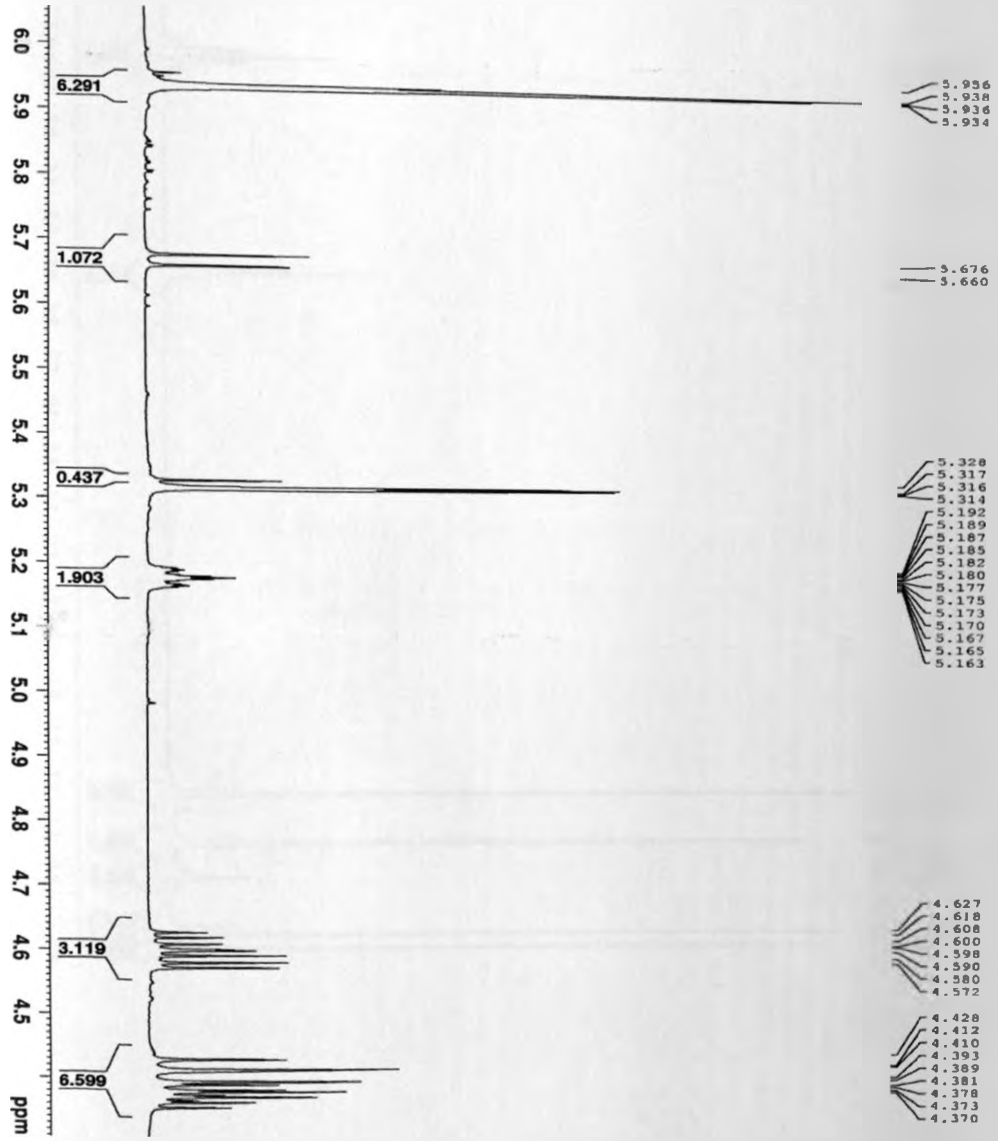


APPENDIX I: SPECTRA FOR COMPOUND **9**

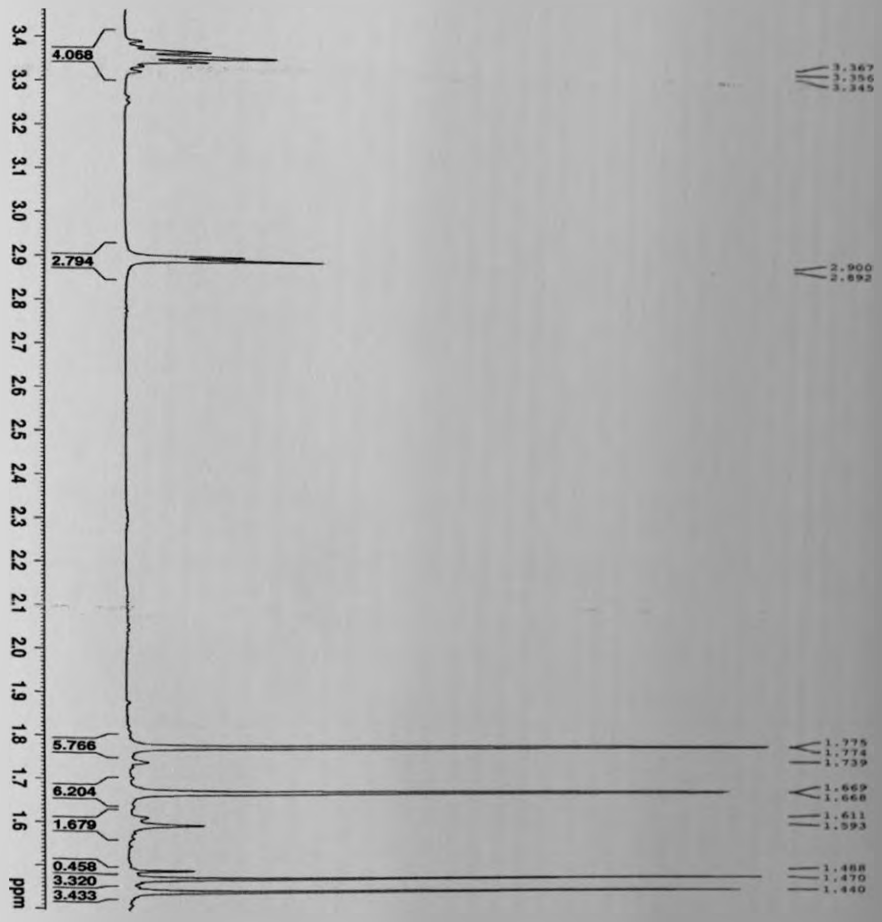
NMR SPECTRUM FOR COMPOUND **8** AND **9** (CD_2CL_2, 600 MHz)



^{1}H NMR SPECTRUM FOR COMPOUND **8** AND **9** (CD_2CL_2, 600 MHz)

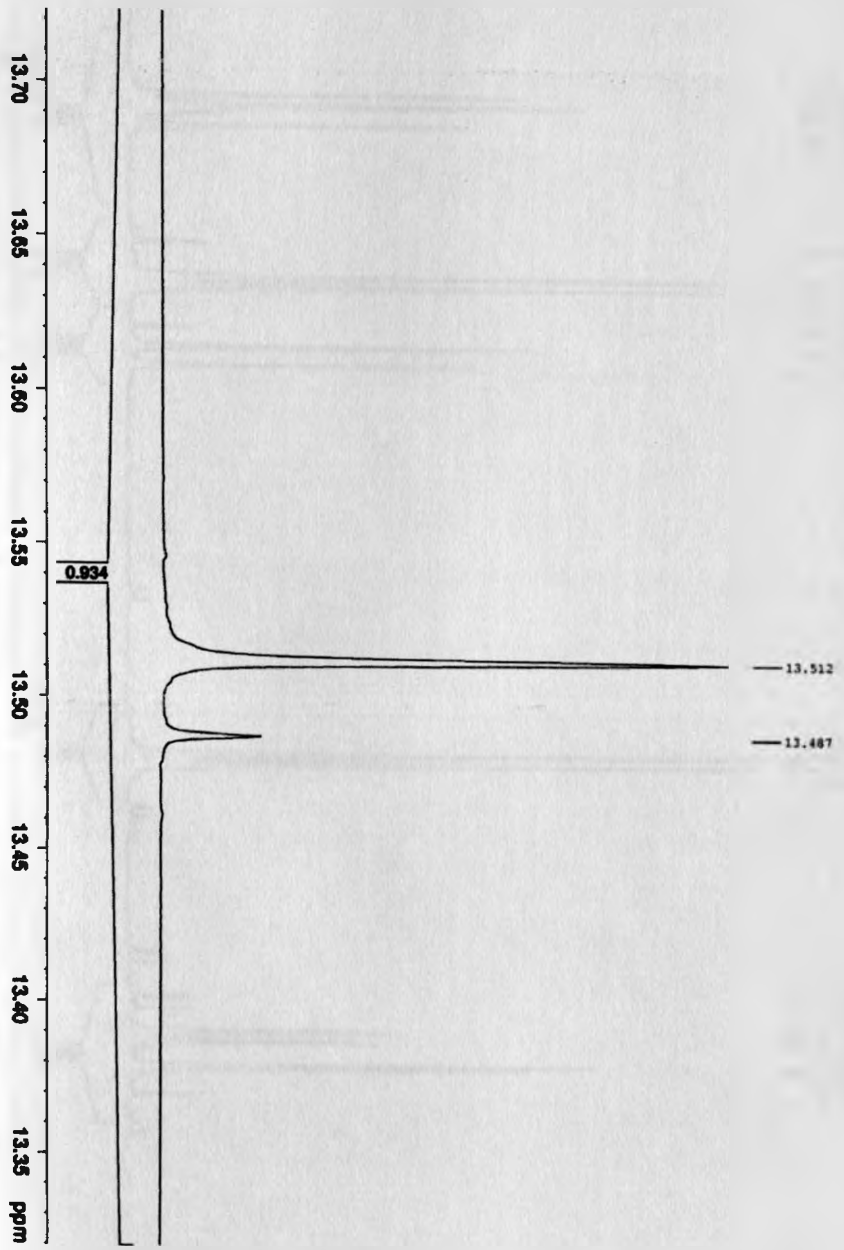


^{1}H NMR SPECTRUM FOR COMPOUND **8** AND **9** (CD_2CL_2, 600 MHz)

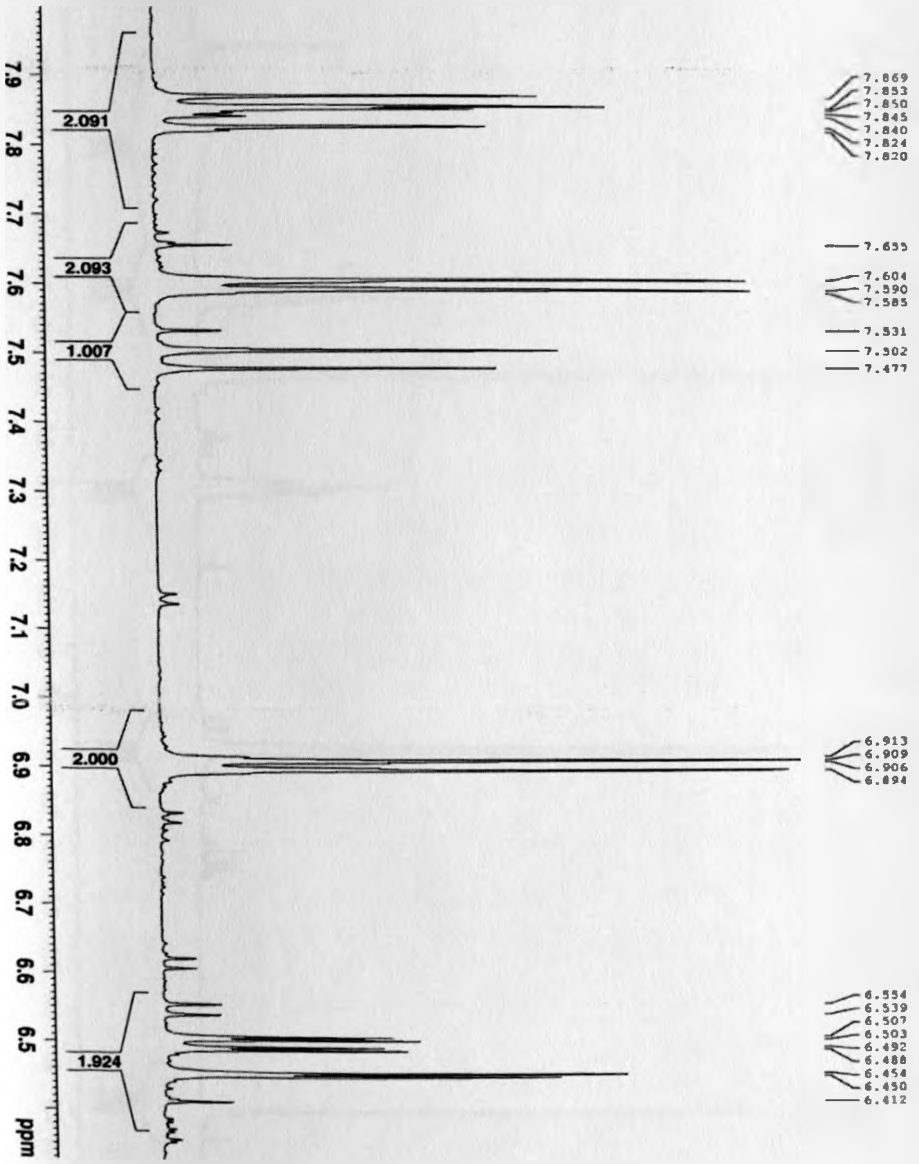


APPENDIX J: SPECTRA FOR COMPOUND **10**

^{1}H NMR SPECTRA FOR COMPOUND **10** (CD_2CL_2, 600 MHz)



^{1}H NMR SPECTRA FOR COMPOUND **10** (CD_2CL_2, 600 MHz)



^{1}H NMR SPECTRA FOR COMPOUND **10** (CD_2CL_2, 600 MHz)