PHYTOCHEMICAL INVESTIGATION OF THE STEM BARK OF *MILLETTIA OBLATA* SSP. *TEITENSIS* FOR ANTIPLASMODIAL AND LARVICIDAL PRINCIPLES

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university. The research is carried out in the Department of Chemistry of the University of Nairobi.

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

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This thesis is dedicated to my late beloved mum; Esther Nyongesa. Thank you for your great love and for giving me direction in life. My love for you is immeasurable.

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LIST OF ABBREVIATIONS

m/z	Mass to charge ratio	$[M]^+$	Molecular ion
CHS	Chalcone synthase	CHI	Chalcone isomerase
PKR	Polyketide reductase	EIMS	Electron ionization mass spectroscopy
IC_{50}	Concentration of 50% inhibition	LC_{50}	Concentration of 50% lethality
COSY	Correlated spectroscopy	DEPT	Distortionless enhanced polarization transfer
HMBC	Heteronuclear multiple bond correlation ($^2J_{CH}$, $^3J_{CH}$)	HMQC	Heteronuclear multiple quantum coherence ($^1J_{CH}$)
NMR	Nuclear magnetic resonance	λ_{max}	Maximum wavelength of absorption
UV	Ultra violet	δ	Chemical shift
MS	Mass spectroscopy	*J*	Coupling constant
Hz	Hertz	MHz	Mega hertz
d	Doublet	*S*	Singlet
t	Triplet	*M*	Multiplet (multiplicity)
dd	Doublet of a doublet	TLC	Thin layer chromatography
AP	Aerial parts	FL	Flowers
HW	Heart wood	L	Leaves
RB	Root bark	RW	Root wood
SB	Stem bark	SD	Seeds
SDP	Seedpods	WD	Wood
DCM	Dichloromethane	Mp	Melting point

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ABSTRACT

The genus *Millettia* is rich in flavonoids and isoflavonoids. In the search for compounds with antiplasmodial and larvicidal activities from medicinal plants, the stem barks of *Millettia oblata* ssp. *teitensis* were analysed. The dried and ground stem barks were extracted with the CH_2Cl_2/MeOH (1:1) by cold percolation for 24 hours at room temperature. The crude extracts showed significant antiplasmodial activities with IC_{50} values of 10.0±2.3 and 12.0±1.2 µg/ml against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum*, respectively. Chromatographic separation of the extract led to the isolation of eight compounds. These were identified as durmillone (**1**), 4'-prenyloxyderrone (**2**), maximaisoflavone H (**3**), 8-*O*-methylretusin (**4**), maximaisoflavone J (**5**), maximaisoflavone B (**6**), tephrosin (**7**) and lupeol (**8**). 4'-Prenyloxyderrone (**2**) is a novel compound. The remaining compounds are reported here for the first time from *Millettia oblata* ssp. *teitensis*. The identification of these compounds was based on spectroscopic techniques (^{1}H NMR, ^{13}C NMR, HMBC, HMQC, COSY, DEPT, UV and MS).

The novel compound, 4'-prenyloxyderrone (**2**) showed antiplasmodial activity with IC_{50} of 6.0±0.9 and 5.4±1.0 µg/ml against (W2) and (D6), respectively. Durmillone (**1**) showed activity against (W2) and (D6) strains of *Plasmodium falciparum* with IC_{50} value of 9.8±0.1 and 6.0±1.2 µg/ml, respectively. Lupeol (**8**) showed antiplasmodial activity with IC_{50} value of 13.9±0.9 µg/ml against (D6). Tephrosin (**7**) showed antiplasmodial activity with IC_{50} value of 11.5±0.3 and 12.7±0.9 µg/ml against (W2) and (D6) respectively. A mixture of Maximaisoflavone J (**5**) and Maximaisoflavone B (**6**) showed activity against (W2) and (D6) strains of *Plasmodium falciparum* with IC_{50} value of 16.5±3.5 and 4.6±0.5 µg/ml, respectively.

In addition to anti-plasmodial activity tests, the methanol extracts of the stem barks, seeds and seedpods were tested against second instar larvae of the mosquito, *Aedes aegypti* for larvicidal activities. The stem bark extract showed activities with the LC_{50} values of 11.2±1.9 μg/ml after 24 hours. The seed extract showed potent larvicidal activity with LC_{50} value of 1.4±0.2 μg/ml after 24 hours. The seedpod extracts were inactive and after 10 days the larvae turned into adult mosquitoes. The observed activity of the seeds and the stem barks of *Millettia oblata* ssp. *teitensis* should mainly be due to the presence of rotenoids in these extracts.

CHAPTER ONE

1.0 INTRODUCTION

1.1 GENERAL

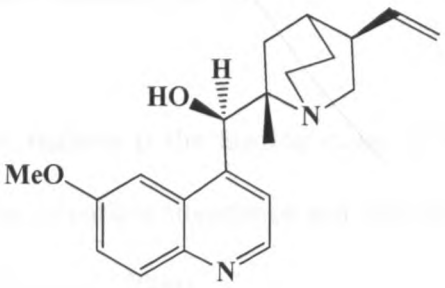
Plants have been used and are still in use by many people for the treatment of various diseases including malaria. The medicinal properties of plants depend upon the presence of certain active principles which vary from plant to plant. The use of medicinal plants varies from species to species, from disease to disease, from tribe to tribe and even from person to person (Kokwaro, 2009).

Although the use of bioactive natural products as herbal drug preparations dates back hundreds of years ago, their application as isolated and characterized compounds to modern drug discovery and developments started in the 19^{th} century, the dawn of chemotherapy era (Bankova, 2007). The searches for new biologically active compounds are most often based on hints coming from ethnobotany but there are still a huge number of unstudied plants (Bankova, 2007).

There is still a great potential for plants in the development of new drugs especially from African plants, because the continent has an immensely rich biodiversity and knowledge in using plants to treat various ailments. In fact the WHO estimates that 80% of Africans below the Sahara depend solely on traditional medicine from plants for their primary health care needs (Bankova, 2007). These resources, however, are hardly scientifically investigated.

Some African scientists have made efforts to document medicinal plants and their mode of use; for example, Kokwaro (2009) and Sofowora (1982) have listed some of the traditional medicinal plants used in East and West Africa, respectively. Kokwaro (2009) listed close to 1400 East African medicinal plants and the diseases they treat. Among those listed by the author to have wide traditional medicinal uses are *Millettia* species. They have various traditional medicinal uses in many communities including anti-malarial uses. Investigation of the phytochemistry of some of these plants indicates that the plants contain mainly isoflavonoids most of which are prenylated. Prenylated isoflavonoids have been shown to have various biological activities including anti-plasmodial, anti-microbial, anti-cancerous and anti-oxidant (Yenesew, 1997 and Derese, 2004).

Pharmaceutical research in natural products represents a major strategy for discovering and developing new drugs. The discovery of quinine (**9**) from *Cinchona succiruba* Vahl. (Rubiaceae) (Rang *et al.*, 2003; Trease and Evans, 2002) and artemisinin (**10**) from *Artemisia annua* L. (Asteraceae) followed by their subsequent development as antimalarial drugs provided impetus to the management of malaria (Taylor and Triggle, 2007; Rang *et al.*, 2003).



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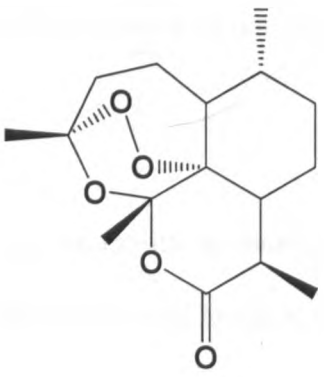

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1.2 Malaria

Malaria is a vector-borne infectious disease caused by protozoan parasites. It is spread in tropical and sub-tropical regions. Each year, there are approximately 350-500 million cases of malaria, killing between one and three million people, the majority being young children in Sub-Saharan Africa (Johnson *et al.,* 2007; Taylor and Triggle, 2007). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development.

The disease is caused by protozoan parasites of the genus *Plasmodium*. Five species of the *Plasmodium* parasite can infect humans. The most deadly form of the disease is caused by *Plasmodium falciparum*. The parasite is transmitted to humans via the bites of infected female mosquitoes. In the human body, the parasite multiplies in the liver, and then infects red blood cells.

Malaria transmission can be reduced by the use of mosquitoe nets and insect repellents to prevent mosquitoe bites, or by mosquito control measures such as spraying insecticides inside houses and draining standing water where mosquitoes lay their eggs. Prevention by

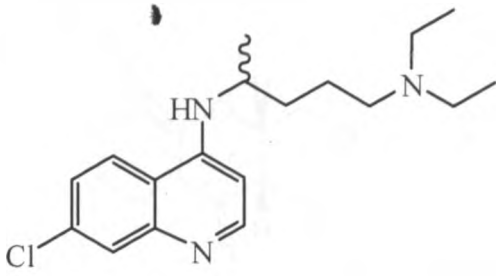
vaccine is theoretically possible; however, work on the development of malaria vaccines has not been successful so far.

In Kenya, malaria is the leading cause of morbidity and mortality, accounting for 30-50% of the outpatient attendance and 20% of all admissions to health facilities (Kigondu, 2007; Kirira *et al.*, 2006).

Kenya has revised its treatment policy by adopting artemisinin **(10)** combination therapy (ACT) as the first line drugs for treatment of uncomplicated malaria. Spread of multidrug resistant (MDR) strains of *Plasmodium* and the adverse side effects of the existing antimalarial drugs have necessitated the search for novel, well tolerated and more efficient antimalarial drugs. Indigenous plants are important sources of biologically active compounds that have potential for the development into novel antimalarial drugs (Yenesew, 1997 and Derese, 2004).

The increasing prevalence of strains of *Plasmodium falciparum* that are resistant to chloroquine **(11)**, a blood schizontocide which had been efficacious, safe, accessible and affordable, poses a serious problem for malaria control, predisposing Africa to an unprecedented situation since the only affordable treatment options are rapidly losing therapeutic efficacy (Frankish, 2002). Drug resistant strains of *Plasmodium falciparum* are endemic in many areas of the world and the majority of conventional antimalarial drugs have been associated with treatment failure (Johnson *et al.,* 2007). These developments and the difficulty of creating efficient vaccines, coupled with adverse

reactions to chemotherapy, underline the urgent need for novel, cheap, safe and efficacious antimalarial drugs. It is estimated that 80% of people worldwide use herbal remedies, due to limited access to modern medicine because of low income and the shortage of efficient health care facilities. There is a dearth of evidence on the efficacy and safety of these remedies, despite the fact that validation of traditional practices could lead to innovative strategies in malaria control (Vishnu *et al.*, 2000).



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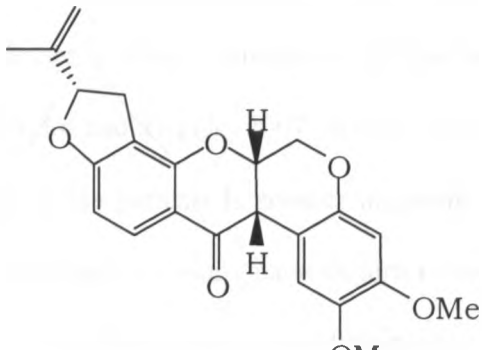
Plants of the genus *Millettia* are used traditionally in different cultures globally. These plants have a wide range of biological activities such as anti-tumoral, anti-flammatory, anti-viral, bactericidal and insecticidal. Phytochemical investigations of roots, stems, leaves, seeds and seedpods have been done on *Millettia dura* and *Millettia usaramensis* (Yenesew, 1997 and Derese, 2004). Flavonoids from these *Millettia* species have shown appreciable anti-plasmodial activities.These plants are known to contain several classes of isoflavonoids. In this work the anti-malarial activity of the crude extract of the stem and the isoflavonoids of *Millettia oblata* ssp. *teitensis* were investigated in order to determine their potential use as anti-malarials.

1.3 Biodegradable larvicides

Rotenone (**12**) is one of the extensively used natural insecticides (Abe *et al.*, 1985). The insecticidal activities of rotenone and other rotenoids against a variety of insect species

are well known, (Derese, 2004). Commercially, rotenone (**12**) is mainly extracted from the *Derris* and *Lonchocarpus* species from Asia and South America respectively. *Millettia* plants have not been exploited commercially as a source of rotenoids, even if the seeds of these plants are known in traditional practice for their insecticidal and piscicidal properties (Dagne *et al.,* 1991).



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In the search for biodegradable compounds with larvicidal and pesticidal activities from plants, the larvicidal activities of rotenoids isolated from the seeds of *Millettia usaramensis* ssp *usaramensis* have been reported (Yenesew *et al.*, 2003). Insect resistance to most conventional insecticides have resulted in high prevalence of mosquito transmitted diseases in Africa. Aiming for the discovery of cost effective alternatives for the control of vector disease insects, extracts from plants have been tested for larvicidal activities, (Yenesew *et al.*, 2003). In this study, the larvicidal activities of the extract of *Millettia oblata* ssp. *teitensis* were tested against the larvae of *Aedes aegpti.*

1.4 Problem statement

Malaria is a major contributor to the global burden of disease and a significant impediment to socio-economic development in poor countries. Malaria remains one of the deadliest diseases on this planet, which accounts for 350-500 million clinical cases and up to 2.7

million deaths each year (Taylor and Triggle, 2007). It is the leading cause of the rising morbidity and mortality rates in most countries in Sub-Saharan Africa. At least one child dies of malaria every 40 seconds in the world and this shows the devastating effect of this disease (Geissbühler *et al.,* 2007; Taylor and Triggle, 2007). Efforts to combat the disease are hampered by growing resistance of malaria parasites to the available drugs. Furthermore the cost of drugs is a sizeable proportion of the total health expenditure in most developing countries. Drug related expenses in these countries account for between 30-50% of the total cost of healthcare needs (Taylor and Triggle, 2007; WHO, 2007). Toxicity of the available antimalarial drugs to many of the patients is another important issue yet to be addressed conclusively. Because of these reasons, many patients turn to herbal medicines which may end up being more detrimental to their health, since little or no scientific research has been done on these herbal remedies to establish their safety and efficacy. Due to the increasing prominence of herbal remedies, additional contributions describing scientific investigations of a rigorous nature are most welcomed. In addition, continuous research is desperately needed in order to come up with compounds which can be developed into new, cheap, less toxic and more efficacious antimalarial drugs for combating the disease.

1.5 Justification of the Research

Various attempts have been made in the control of malaria including, selective application of vector control, early diagnosis and effective and prompt treatment of malarial disease and early detection or forecasting of epidemics and rapid application of control measures. Since malaria is transmitted by the female anopheles mosquito, a major strategy of control is to attack the vector with adulticides and larvicides. Extended use, however, has led to emergence of insecticide-resistant mosquitoes (Campbell, 1997). The use of *Millettia*

species of Kenyan origin for control of mosquitoes at larvae stage may provide a useful deterrent against proliferations of these disease vectors. Previous investigations of the phytochemical profile of the genus *Millettia* have resulted in the isolation and identification of compounds such as isoflavonoids, chalcones, rotenoids among many others. Rotenone **(12)** and tephrosin **(7)** have showed potent larvicidal activity against 2nd instar larvae of *Aedes aegypti* (Yenesew *et al*., 2005). The study of *Millettia oblata* ssp. *teitensis* may lead to the isolation, identification and characterization of cheap, less toxic and more efficacious natural larvicidal compounds. Furthermore, the antiplasmodial activities of several flavonoids have been reported (Yenesew 1997 and Derese 2004).

The identification of antiplasmodial and larvicidal compounds, which are biodegradable and environmentally friendly, that can be used as an alternative to synthetic antimalarial drugs and larvicides will contribute significantly to safe guarding the health of the people especially those living in malaria endemic rural areas. It is worth to note that the medicinal plant industry plays a critical role in empowering large numbers of rural population in many African countries (Anthonia and Benjamin, 2003).

Thus, in this study, the antiplasmodial principles of the crude extracts and isolated compounds from the stem bark of *Millettia oblata* ssp. *teitensis* were tested against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum.* Furthermore the larvicidal principles of the crude extracts of the stem bark, seeds and seedpods were tested against the larvae of *Aedes aegypt,* in order to establish the potential use of the *Millettia oblata* ssp. *teitensis* in the treatment and control of malaria.

1.6.0 Objectives

1.6.1 General Objective

To isolate, identify and characterize the compounds of *Millettia oblata* ssp. *teitensis* and establish their antiplasmodial and larvicidal activity.

1.6.2 Specific Objectives

- To determine the antiplasmodial activities of the crude extracts of the stem bark of *Millettia oblata* ssp. *teitensis*
- To determine the larvicidal activities of the crude extracts of the stem barks, seeds and seedpods of *Millettia oblata* ssp. *teitensis*
- To isolate and characterize the constituents of the stem barks of *Millettia oblata* ssp. *teitensis*
- To determine the antiplasmodial activities of the isolated compounds.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botanical information

2.1.1 The family Fabaceae

The genus *Millettia* belongs to the family Fabaceae (also known as Leguminosae). This family comprises of 657 genera and about 20,000 species of trees, shrubs and herbs. These plants are known for their ability of nitrogen fixation in the soil. They are widely distributed in the temperate as well as tropical regions of the world (Heguaurer and Grayer-Barkmeijer, 1993).

The family is divided into three sub-families; Mimosoideae, Papilionoideae and Caesalpinioideae. The genus *Millettia* belongs to the sub-family Papilionoideae.

2.1.1.1 The sub-family Papilionoideae

Papilionoideae is the largest of the three sub-families with over 630 genera and 18,000 species of trees, shrubs and herbs. This sub-family has 32 tribes and is distinguished from the two other subfamilies by the presence of papilionoid flowers, a hilar valve of seeds and by their ability to synthesize quinolizidine alkanoids and isoflavonoids (Polhill, 1981).

2.1.1.2 The genus *Millettia*

The genus *Millettia* belongs to the tribe Tephrosiae that is known to synthesize prenylated flavonoids and isoflavonoids, (Derese, 2004). The genus has over 323 species

worldwide and found widely distributed in tropical Africa, Asia and Australia. *Millettia* plants are trees, shrubs or lianas, or rarely semi-herbaceous plants with woody rootstocks, (Derese, 2004).

In Kenya, the genus *Millettia* is represented by six species, which are *Millettia dura, Millettia lasiantha, Millettia leucantha, Millettia oblata, Millettia tanaensis* and *Millettia usaramensis,* (Beentje, 1994).

2.1.1.2.1 *Millettia oblata* Dunn.

Millettia oblata Dunn is distributed in Kenya and Tanzania. There are five subspecies of *Millettia oblata* Dunn namely; *Millettia oblata* ssp. *teitensis* J. B. Gillett, *Millettia oblata* ssp. *intermedia* J. B. Gillett, *Millettia oblata* ssp. *stolzii* J. B. Gillett, *Millettia oblata* ssp. *oblata* Dunn and *Millettia oblata* ssp. *burtii* J. B. Gillett, (Catalogue of Life, 2009). *Millettia oblata* ssp. *teitensis* J. B. Gillett is found in Kenya. Table 2.1 gives a summary on distribution of *Millettia oblata* Dunn subspecies.

Table 2.1: Distribution of *Millettia oblata* Dunn subspecies

Subspecies	**Distribution**	**Reference**
teitensis J. B. Gillett	Threatened small tree with perennial lifespan found only from Taita Hills forest, Kenya.	Catalogue of Life, 2009
oblata Dunn	A small tree found in tropical areas of Tanzania, with perennial lifespan	Catalogue of life, 2009
intermedia J. B. Gillett	A small tree distributed in tropical areas of Tanzania, with perennial life span	Catalogue of life, 2009
stolzii J. B. Gillett	A small tree distributed in tropical areas of Tanzania, with perennial life span	Catalogue of life, 2009
burttii J. B. Gillett	A shrub or small tree distributed in tropical areas of Tanzania, with perennial lifespan	Catalogue of life, 2009

Figure 2.1: *Millettia oblata* ssp. *teitensis* (Catalogue of life, 2009)

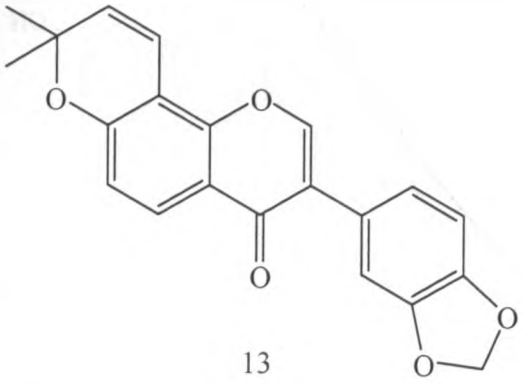
2.1.2 The genus *Millettia* as potential sources of anti-malarials.

Research on the Kenyan *Millettia* species as potential anti-malarial drugs has been going on since late 1990's at the University of Nairobi, Kenya. Phytochemical investigations of roots, stems, leaves, seeds and seedpods have been done widely on *Millettia dura* and

Millettia usaramensis, (Yenesew, 1997 and Derese, 2004). Flavonoids from these *Millettia* species have shown appreciable anti-plasmodial activities. However, there are no records of the use of these plants as anti-malarials in traditional medicine. Table 2.2 gives a summary of anti-plasmodial activities of flavonoids from Kenyan *Millettia* species.

Table 2.2: Anti-plasmodial activities of flavonoids from Kenyan *Millettia* species

	IC_{50} in (μM)		
Flavonoids	**W2**	**D6**	**Reference**
Maximaisoflavone H **(3)**	38.7±0.6	45.6±0.1	Derese 2004
Maximaisoflavone B **(6)**	58.9±1.5	33.3±1.7	"
Jamaicin **(13)**	45.6±2.3	46.6±1.2	"
7,2'-Dimethoxy-4',5'-methylenedioxyisoflavone **(14)**	49.4±0.2	51.5±0.7	"
Mildurone **(15)**	40.7±2.2	50.4±0.8	"
Calopogoniumisoflavone A **(16)**	81.4	51.8	"
Durmillone **(1)**	25.1±1.6	37.3±1.8	"
Isoerythrin A 4'-(3-methyl-2-butenyl)ether **(17)**	21.8±0.6	24.7±0.8	"
Isojamaicin **(18)**	39.0±0.8	48.7±1.1	"
Nordurlettone **(19)**	51.4±1.7	20.8±1.5	"
7,3'-Dimethoxy-4',5'-methylendioxyisoflavone **(20)**	56.3±0.8	42.8±0.6	"
Durallone **(21)**	49.9±2.4	32.7±0.4	"
6-Methoxycalopogonium isoflavone A **(22)**	35.4±1.9	53.3±1.7	"
Deguelin **(23)**	21.1	13.8±4.5	"
Millettone **(24)**	64.1	48.9±12.9	"
Usararotenoid-A **(25)**	66.6	60.7	Yenesew et al, 2003
12a-Epimillettosin **(26)**	22.2	19.4	
4'-O –Geranyloxyisoliquiritigenin **(27)**	8.7	10.6	"
Barbigerone **(28)**	27.0	27.3	"
Usararotenoid C **(29)**	25.8	70.1	"
6a,12a-Dehydromillettone **(30)**	33.3	39.1	"
Chloroquine **(11)**	0.094	0.009	"
Quinine **(9)**	0.209	0.044	"



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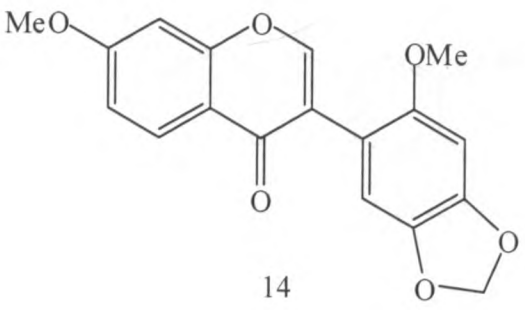

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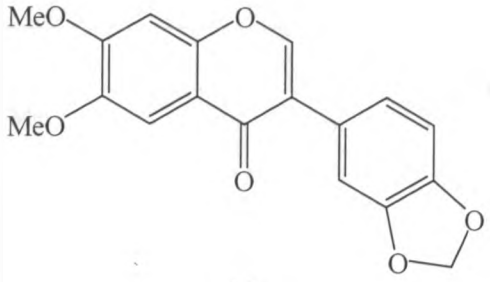

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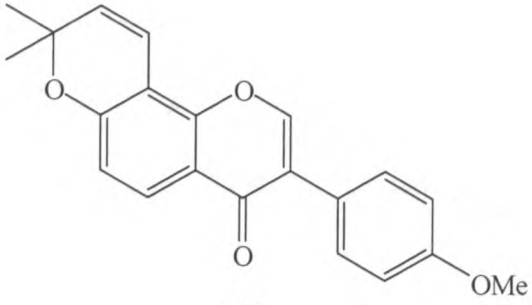

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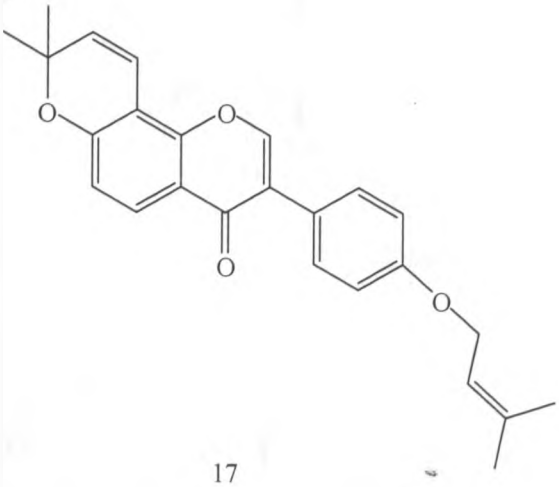

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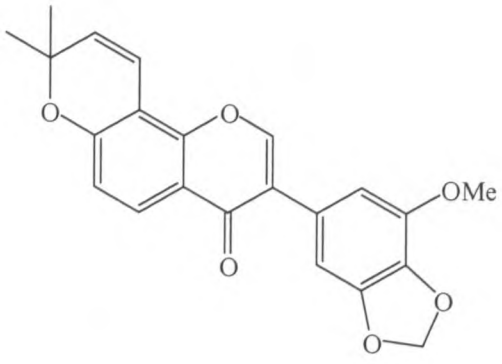

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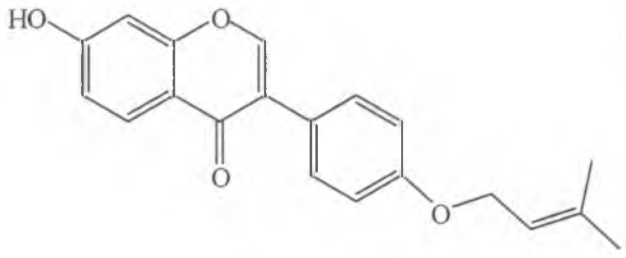

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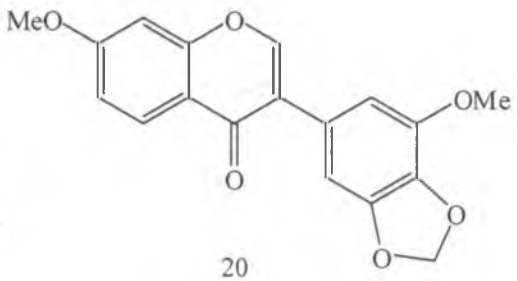

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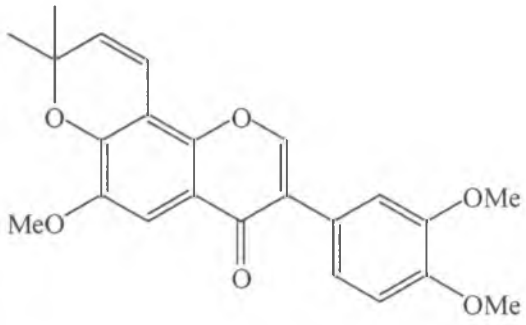

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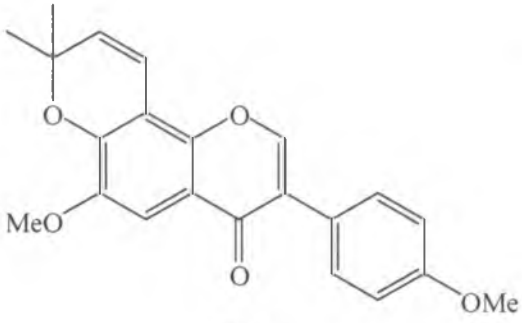

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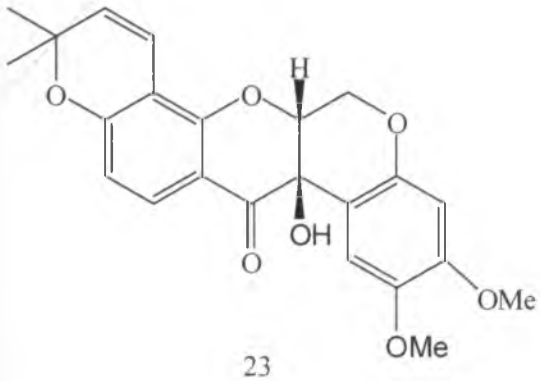

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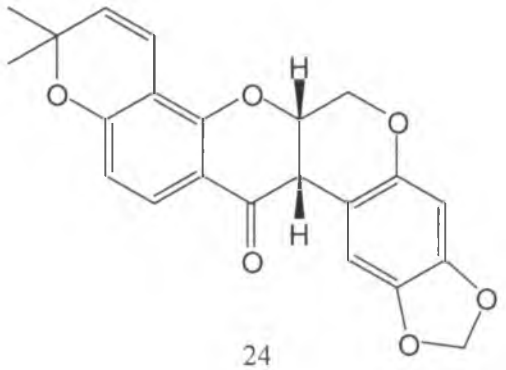

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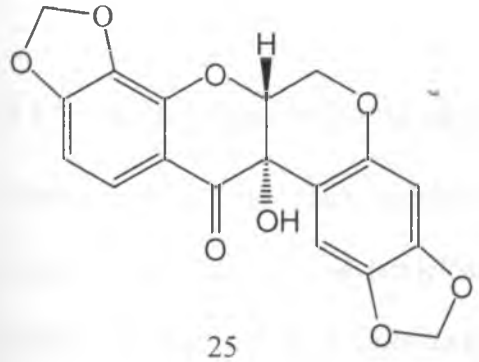

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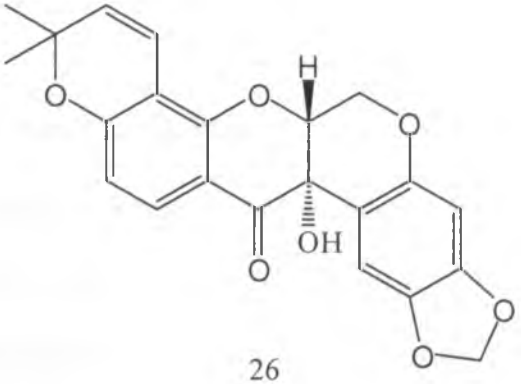

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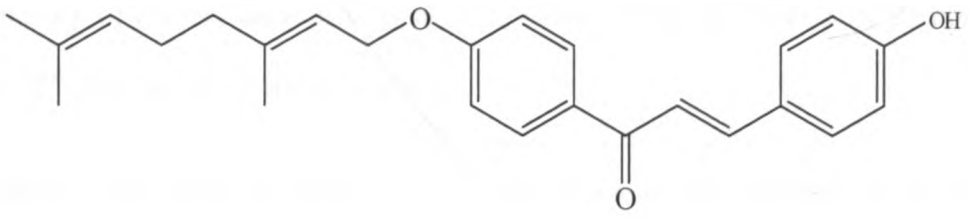

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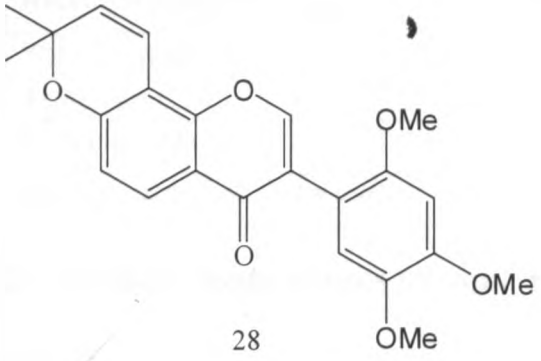

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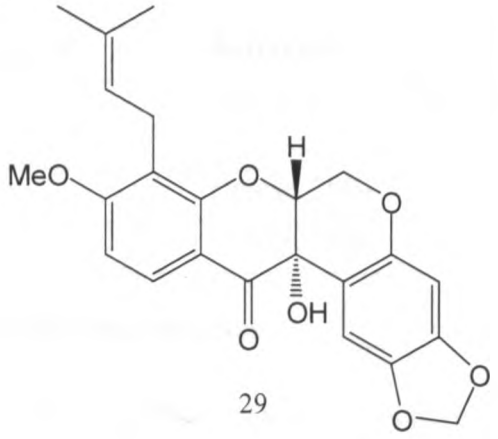

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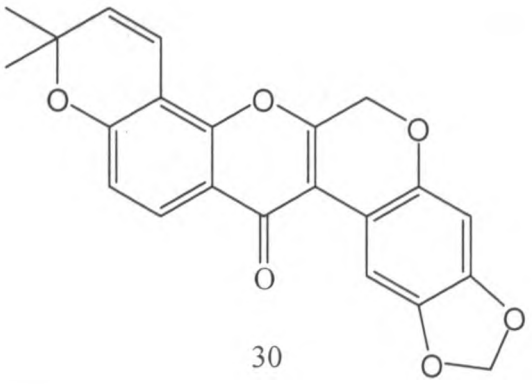

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2.1.3 The Kenyan *Millettia* plants as potential larvicides

Research on *Millettia* plants species at the University of Nairobi has revealed that the seeds of these plants show larvicidal activity. The phytochemical investigation of the seeds of *Millettia dura* and *Millettia usaramensis* have led to the isolation of rotenoids as the main components of the seeds, (Yenesew *et al.*, 2003, Derese, 2004). The rotenoids

are believed to be responsible for the larvicidal activity. Table 2.3 below gives the summary of the larvicidal activity as LC_{50} values of the rotenoids after 24 hours against the 2nd instar larvae of *Aedes aegypti*.

Table 2.3: The larvicidal activity, LC_{50}, of the rotenoids after 24 hours on 2nd instar larvae of *Aedes aegypti*.

Rotenoid/ Crude Extract	**LC_{50} in µg/ml**	**Reference**
Rotenone (**12**)	0.47	Yenesew *et al.*, 2003
Tephrosin (**7**)	1.40	"
Deguelin (**23**)	1.60	Derese, 2004
Seed-extract of *Millettia dura*	0.90	Derese, 2004

2.1.4 Ethno-medical uses of some Kenyan *Millettia* species

Millettia species are used traditionally in different cultures globally. These plants have a wide range of biological activities such as anti-tumoral, anti-flammatory, anti-viral, bactericidal and insecticidal. Table 2.4 below summarizes the reported uses of the Kenyan *Millettia* species (Gillet *et al.*, 1971).

Table 2.4: Traditional uses of some Kenyan *Millettia* species.

Species	Plant part	Uses	Reference
M. lasiantha	Roots	Decoctions of roots drunk as aphrodisiac (Kenya).	Gillet *et al.*, 1971
M. oblata	Barks	Treat stomach-aches Remedy against cough	Gillet *et al.*, 1971
M. usaramensis	Roots root pulp	Fish poison in Kenya Treatments of snake bite	Gillet *et al.*, 1971
M. dura	Roots	Decoctions employed to treats swollen parts of the body. Treats bladder problems	Gillet *et al.*, 1971

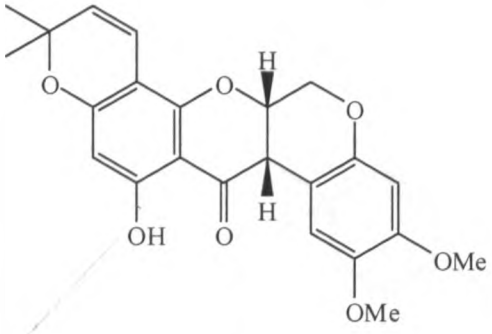
2.2 Phytochemistry of the genus *Millettia*

Previous studies of extracts of *Millettia* species have led to the isolation of alkaloids, flavones, chalcones, rotenoids, isoflavones and coumarins among others. Phytochemical investigation of the Kenyan *Millettia* species at the University of Nairobi have led to the isolation of flavanones, chalcones, rotenoids, isoflavones and terpenes, among others, (Yenesew, 1997, Derese, 2004), of which isoflavonoids are the most reported.

2.2.1 Rotenoids of the genus *Millettia*

Rotenoids mainly occur in the seeds of *Millettia* plants. These compounds, especially rotenone (**12**), are considered to be responsible for insecticidal and piscicidal activities observed in the *Millettia* species, (Ollis *et al.*, 1967, Yenesew *et al.*, 2003). Most of the rotenoids previously characterized from these plants have a *cis*-B/C junctions as in rotenone. However, rotenoids of the stem bark of the Kenyan *Millettia usaramensis* have a novel *trans*-B/C ring junction with a 6a*R*, 12a*S* configuration (Derese, 2004).

A recent investigation in Thailand isolated four rotenoids from the flowers of *Millettia brandisiana* Kurz (Orasa *et al.*, 2007). These four rotenoids had not been isolated previously from other *Millettia* species but from different leguminous plants; *Tephrosia* species and *Clitoria* species (Orasa *et al.*, 2007). These four rotenoids are; α - toxicarol (**31**), 12a – hydroxyl – α – toxicarol (**32**), 6 – deoxyclitoriacetal (**33**) and 6a, 12a – dehydro – α – toxicarol (**34**).



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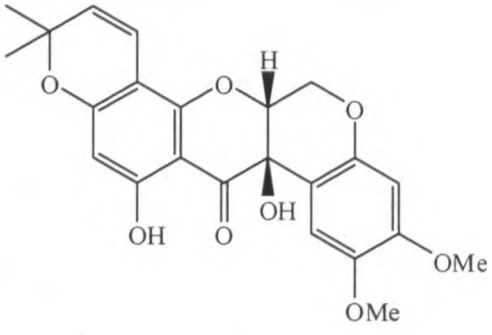

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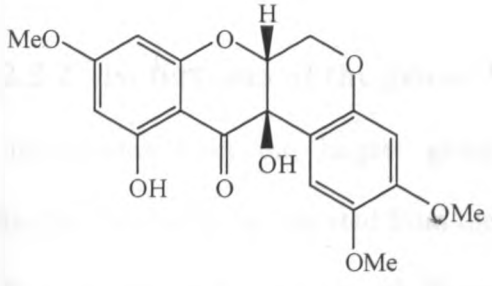

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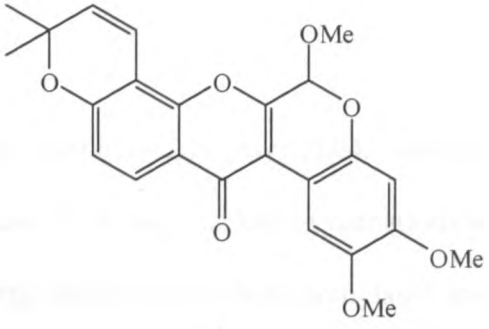

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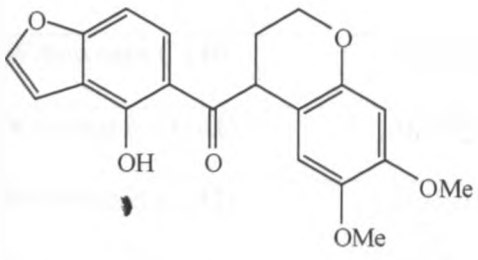
From the *Millettia duchesnei* plant, three new rotenoids have been reported from the twigs (Ngandeu *et al.*, 2008). These are; elliptol (**35**), 12–deoxo-12α–methoxyelliptone (**36**) and 6–methoxy–6a, 12a–dehydrodeguelin (**37**).



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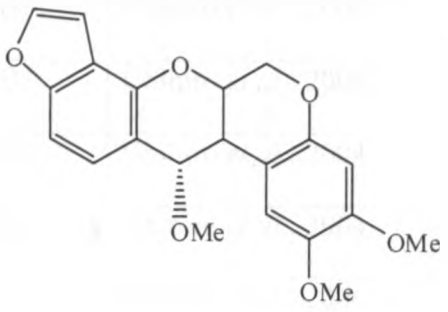

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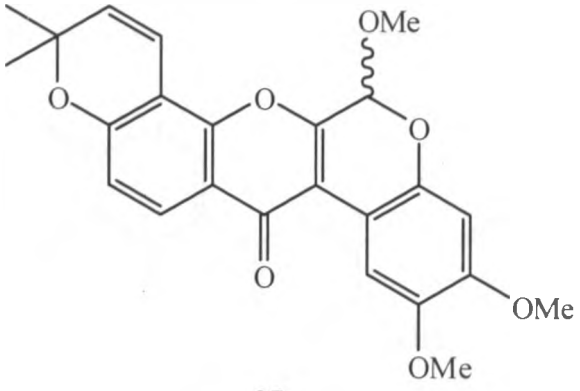

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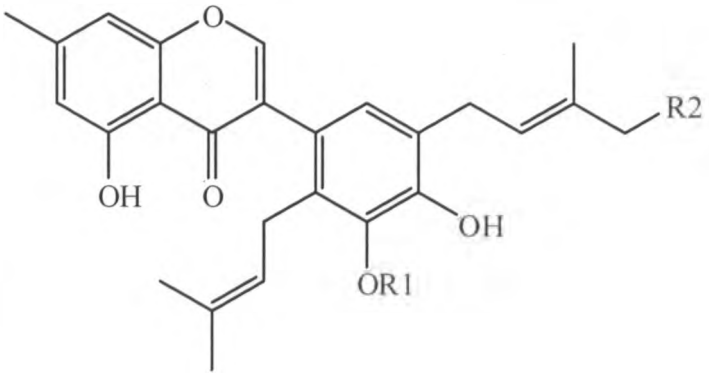
2.2.2 Isoflavones of the genus *Millettia*

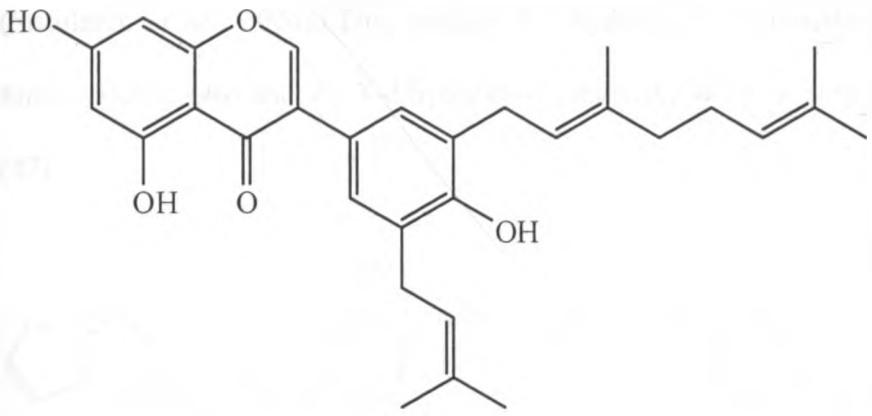
Isoflavones form the largest group of natural isoflavonoids. By 2004, seventy isoflavones had been reported from the genus. About 37 of them, lacked oxygenations at C-5, 54 were prenylated or with furanol/pyrano ring while 28 were both prenylated and C-5 deoxygenated, (Derese, 2004). Eight new isoflavones have been reported, (Chihiro *et al.*, 2004 and 2006). Table 2.5 gives the summary of isoflavones reported.

Table 2.5: A summary of isoflavones of *Millettia* reported.

Isoflavone	**Source (plant part)**	**Reference**
Willewanin A (**38**)	*Millettia taiwaniana* (SB)	Chihiro *et al.*, 2004
Willewanin B (**39**)	*Millettia taiwaniana* (SB)	Chihiro *et al.*, 2004
Willewanin C (**40**)	*Millettia taiwaniana* (SB)	Chihiro *et al.*, 2004
Willewanin D (**41**)	*Millettia taiwaniana* (SB)	Chihiro *et al.*, 2004
Willewanin E (**42**)	*Millettia taiwaniana* (SB)	Chihiro *et al.*, 2004
Willewanin G (**43**)	*Millettia pachycarpa* (L)	Chihiro *et al.*, 2006
Willewanin H (**44**)	*Millettia pachycarpa* (L)	Chihiro *et al.*, 2006
Furowanin B (**45**)	*Millettia pachycarpa* (L)	Chihiro *et al.*, 2006



	R_1	R_2
38	Me	H
39	Me	Prenyl
40	H	Prenyl



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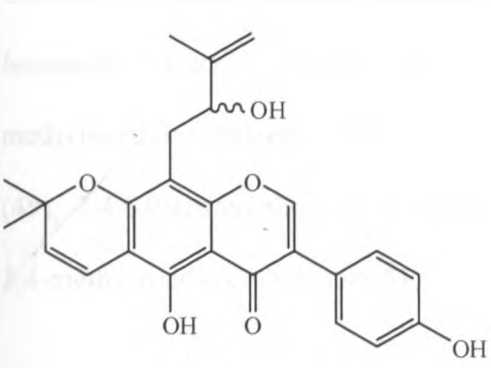

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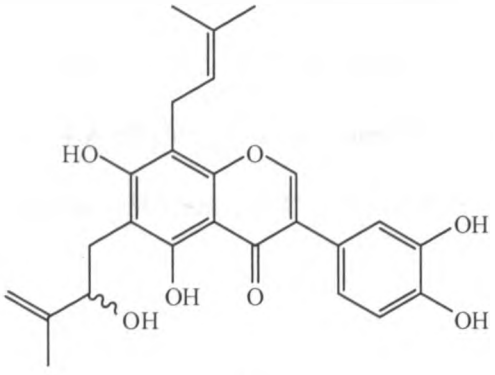

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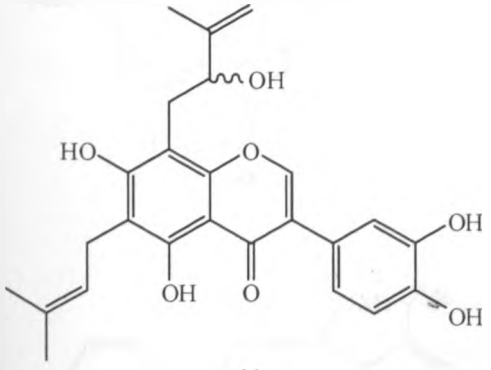

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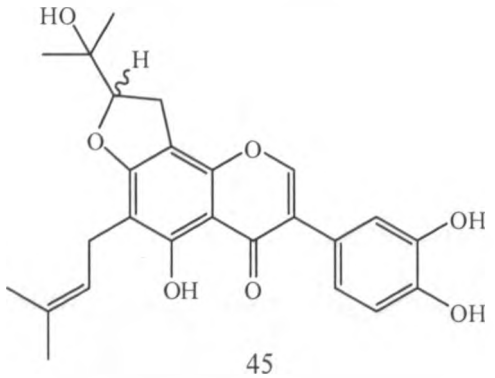

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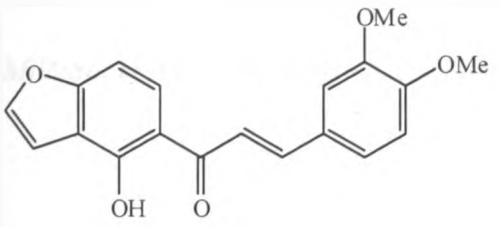
2.2.3 Chalcones of the genus *Millettia*

By 2004, 18 chalcones had been isolated from the genus *Millettia*, (Derese, 2004). Two new chalcones have been reported from the seedpods of *Millettia erythrocalyx* Gagnep

(Sritularak *et al.*, 2006). This include 2'– hydroxy–3, 4–dimethoxy–[2", 3": 4', 3']–furaochalcone (**46**) and 2', 3–dihydroxy–4– methoxy–4'–γ, γ–dimethylallyloxychalcone (**47**).



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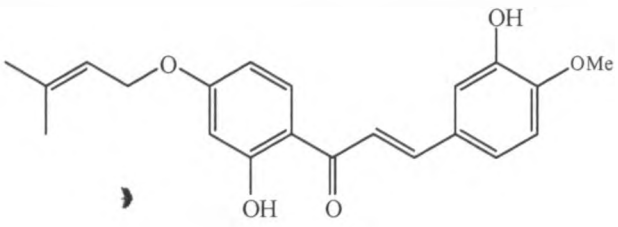

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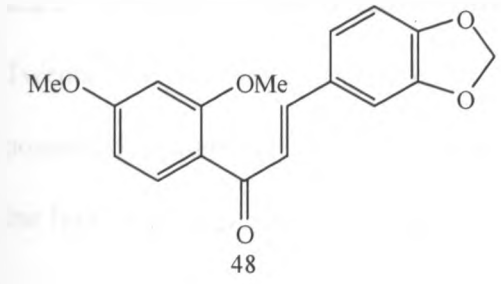
Also four chalcones derivatives have been reported from the stem bark of *Millettia leucantha* Kurz, (Ampai *et al.*, 2003). These are; 2',4'-dimethoxy-3,4-methylenedioxychalcone (**48**), 2',4',6'-trimethoxy-3,4-methylenedioxydihydrochalcone (**49**), 2,4,6,β-tetramethoxy-3',4'-methylenedioxychalcone (**50**) and 2',4',6'-trimethoxy-3,4-methylenedioxychalcone (**51**).



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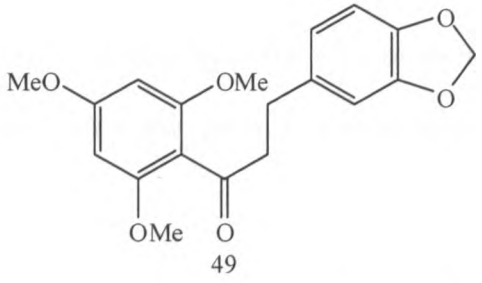

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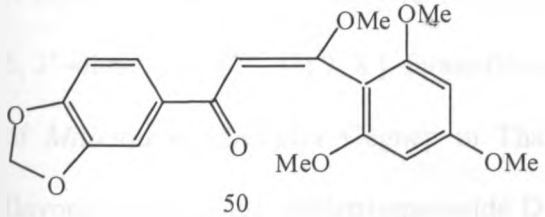

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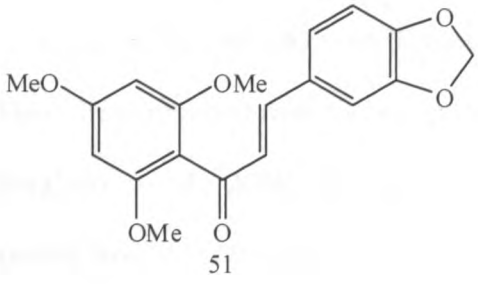

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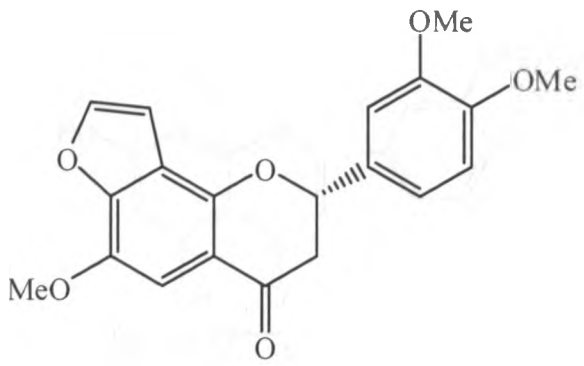
2.2.4 Flavanones of the genus *Millettia*

By 2004, all the flavanones that had been characterized were prenylated and lacked oxygenation at C – 5 positions (Derese, 2004). A new flavanone, (-) –(2*S*)–6, 3', 4'–trimethoxy – [2", 3": 7, 8]–furanoflavanone (**52**) has been reported from the seedpods of *Millettia erythrocalyx* Gagnep, (Sritularak *et al.*, 2006).



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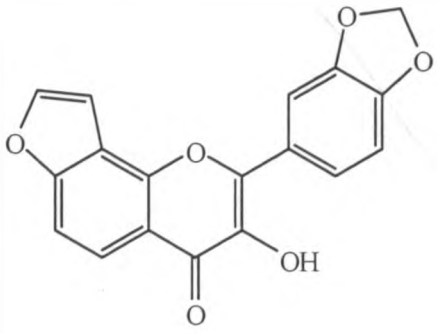
2.2.5 Flavones and Anthocyanins of the genus *Millettia*

Twenty five flavones had been isolated from the genus *Millettia* by 2004. 60% of them possessed a furan-ring, which is not a common substituent in the genus. In all the cases, the furan-ring is on ring A (Derese, 2004).

A novel flavonol; 3', 4'–methylenedioxy–[2", 3": 7, 8]-furanoflavonol (**53**) and flavone; 6, 3'–dimethoxy-[2", 3": 7, 8]–furanoflavone (**54**) have been reported from the seedpods of *Millettia erythrocalyx* Gagnep in Thailand (Sritularak *et al.*, 2006) and one new flavonol triglycoside; millettiaspecoside D (**55**) reported from *Millettia speciosa* Champ in China (Ting *et al.*, 2010).



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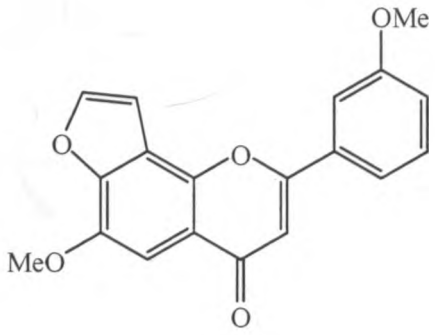

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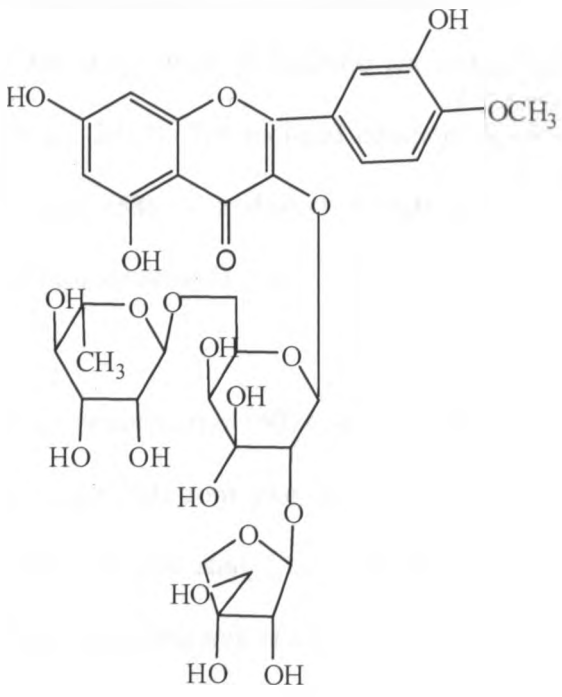

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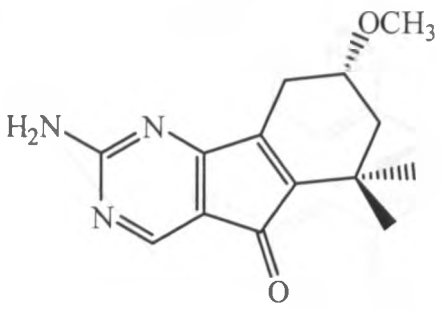
2.2.6 Alkaloids of the genus *Millettia*

Apart from flavonoids, alkaloids have also been reported from this genus. They are reported only from the species *Millettia laurentii* (Derese, 2004). Millaurine A (**56**), a new guanidine alkaloid has been isolated from a Camerounian plant *Millettia laurentii* (Ngamga *et al.*, 2007).



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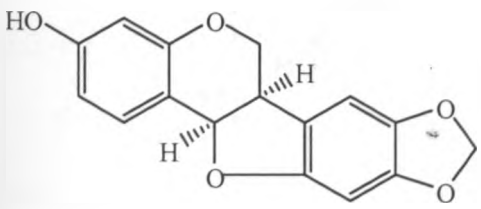
2.2.7 Other Phenolic compounds of the genus *Millettia*

Phenolic compounds from the stem wood of *Millettia leucantha* Kurz in Thailand have been reported (Sritularak *et al*, 2010). The isolated compounds were identified as (-)–maackiain (**57**), syringic acid (**58**), 4–hydroxyl–3–methoxybenzoic acid (**59**), (-)–balanocarpol (**60**) and (+)–diptoindonesin D (**61**).

It should be noted that both (-)–balanocarpol (**60**) and (+)–diptoindonesin D (**61**) could be considered as biogenetically derived from two units of the stilbene resveratrol (**62**) (Sritularak *et al*, 2010). Prior to this study, no stilbene – related compounds were identified from the genus *Millettia* (Sritularak *et al*., 2010).



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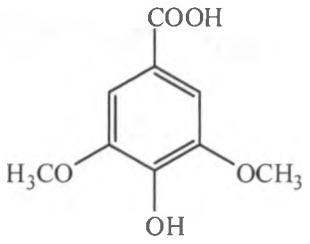

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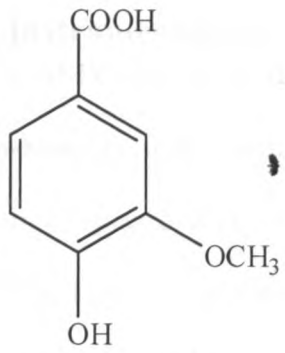

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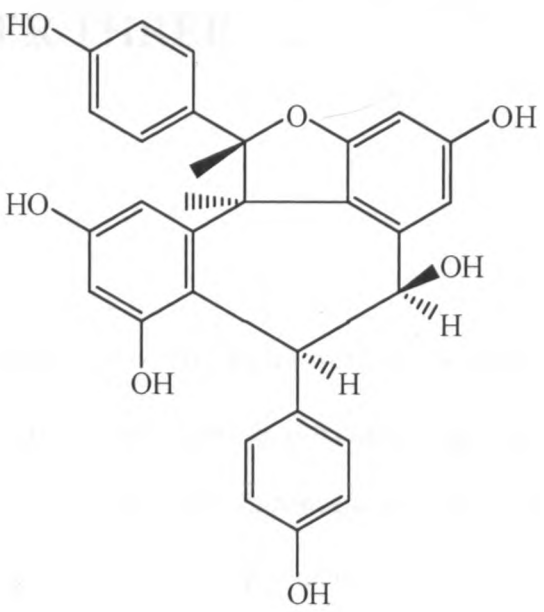

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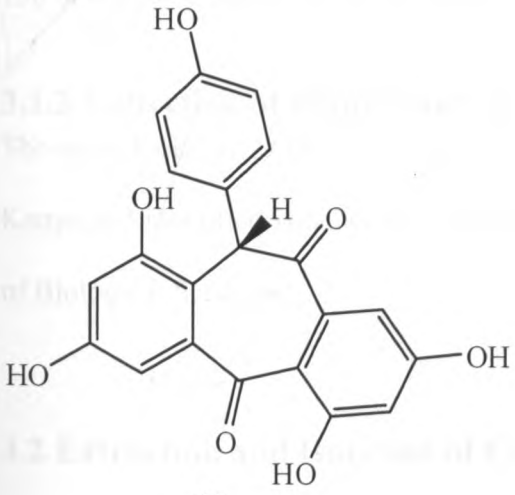

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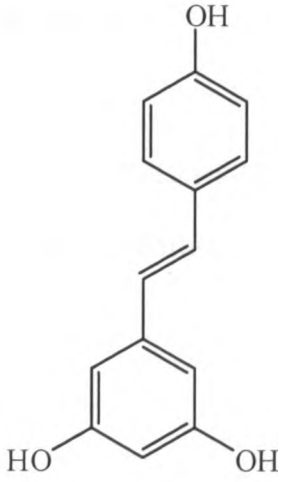

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CHAPTER THREE

3.0 METHODOLOGY

3.1 General

3.1.1 Instrumentation

The ^{13}C NMR (125 or 50 MHz) and ^{1}H NMR (300 or 200 MHz) were run on Bruker or Varian-Mercury spectrometers using residual solvent signal as reference. Homonuclear correlation spectroscopy (COSY), Nuclear Overhauser Enhancement spectroscopy (NOESY), Heteronuclear correlation spectroscopy (HETCOR) including HMBC ($^{2}J_{CH}$, $^{3}J_{CH}$) and Heteronuclear multiple quantum coherence [HMQC ($^{1}J_{CH}$)] were acquired using standard Bruker software. UV/VIS spectra were recorded using a Pye-Unicam SPS-150 Spectophotometer. The plant material was grounded using Willymill.

3.1.2 Collection of Plant Material

The stem barks of *Millettia oblata* ssp. *teitensis* were collected from Taita Hill forest, Kenya and identified voucher specimen is deposited at the University Herbarium, School of Biological Sciences.

3.2 Extraction and Isolation of Compounds

Air dried and ground stem bark of *Millettia oblata* ssp. *teitensis* (449 g) were extracted with CH_2Cl_2/MeOH (1:1) by cold percolation. The extract was evaporated under reduced pressure to yield 19.9 g (4.4 %) crude extract. The 19.9 g extract was subjected to column chromatography on Silica gel (230 g) eluting with hexane containing increasing

percentage of ethyl percentage (1%, 3%, 5%, 7%, 9%, 12%, 15%, 20%, 25%, 30% and 40%, ethyl acetate in hexane each of *ca* 1L). 26 fractions labelled A to Z were collected.

Crystallization of the combined fractions A to D (which was eluted with 3%EtOAc in hexane) in dichloromethane/methanol gave rise to a new compound 1, 4'-prenyloxyderrone (56 mg). Crystallization of the combined fractions E to H (which were eluted with 12% EtOAc in hexane) in dichloromethane/methanol gave rise to compound 2, durmillone (400 mg) (Ollis *et al.*, 1967). Crystallization of the combined fractions I to K in dichloromethane/methanol gave rise to compound 3, lupeol (40 mg) (Furukawa *et al.*, 2002). Crystallization of the combined fractions L to N (which were eluted with 20%EtOAc in hexane) in dichloromethane/methanol gave rise to a mixture of two compounds (4) and (5); maximaisoflavone J, this is the first report on this plant and maximaisoflavone B (180 mg) (Dagne *et al*, 1991).

Combined fractions of P to Q (which were eluted with 30%EtOAc in hexane) was subjected to Sephadex LH-20 [CH_2Cl_2/CH_3OH (1:1)] to give rise to compound 6, maximaisoflavone H (10 mg) (Dagne *et al.*, 1991 and Yenesew *et al.*, 1996).

Combined fractions of R to V (which were eluted with 30%EtOAc in hexane) was subjected to small column chromatography on Silica gel (27g) using hexane containing increasing amounts of ethyl acetate to realize compound 7, tephrosin (78 mg) (Ollis *et al.*, 1967) and compound 8, 8-*O*-methylretusin (5 mg) (Chen *et al.*, 1983 and Rui *et al.*, 1989)

3.3 Physical and Spectroscopic Data for the Isolated Compounds.

3.3.1 DURMILLONE (1)

White crystals; R_f=0.4 (n-hexane/EtOAc, 7:3), mp 179 – 181 ° C. ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 7.95 (1H, *s*, H–2), 7.49 (1H, *s*, H-5), 7.08 (1H, *d*, *J*=1.8 Hz, H-2'), 6.97 (1H, *dd*, *J*=1.8, *J*=7.8 Hz, H-6'), 6.87 (1H, *d*, *J*=7.8 Hz, H-5'), 6.82 (1H, *d*, *J*=9.6 Hz, H-4''), 5.99 (2H, *s*, O-CH_2-O), 5.77 (1H, *d*, *J*=9.6 Hz, H-3''), 3.93 (3H, *s*, OMe-6), 1.64 (3H, *s*, Me-2'') and 1.52 (3H, *s*, Me-2''). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 175.7 (C-4), 152.0 (C-2), 147.9 (C-4'), 147.8 (C-3'), 147.6 (C-8a), 147.5 (C-7), 147.4 (C-6), 130.6 (C-3''), 126.1 (C-1'), 124.5 (C-3), 122.6 (C-6'), 117.8 (C-4a), 115.4 (C-4''), 110.4 (C-8), 110.0 (C-2'), 108.6 (C-5'), 105.3 (C-5), 101.4 (OCH_2O), 78 (C-2''), 56.6 (6-OMe) and 28.2 (2''-Me_2). (Appendix A).

3.3.2 4'-PRENYLOXYDERRONE (2)

White crystals; R_f =0.4 (n-hexane/EtAOc, 7:3), mp 130 – 132 ° C, EIMS (m/z 404, $C_{25}H_{24}O_5$); ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 12.94 (*s*, 5-OH), 7.94 (1H, *s*, H-2), 7.44 (2H, *d*, *J*=9.0 Hz, H-2' and H-6'), 6.96 (2H, *d*, *J*=9.0 Hz, H-3' and H-5'), 6.71 (1H, *d*, *J*=10.2 Hz, H-4''), 6.25 (1H, *s*, H-6), 5.62 (1H, *d*, *J*=10.2 Hz, H-3''), 5.49 (1H, *t*, J=6.6 Hz, H-2'''), 4.55 (2H, *d*, *J*=6.6 Hz, H-1'''), 1.80 (3H, *s*, Me-3'''), 1.76 (3H, *s*, Me-3'''), 1.53 (3H, *s*, Me-2'') and 1.47 (3H, *s*, Me-2''). ^{13}C NMR (CD_2Cl_2, 150 MHz): δ 181.3 (C-4), 162.5 (C-5), 159.8 (C-4'), 159.4 (C-7), 152.9 (C-2), 152.5 (C-8a),138.4 (C-3'''), 130.4 (C-2' and C-6'), 127.8 (C-3''), 123.8 (C-3), 123.1 (C-1'), 119.9 (C-2'''), 114.9 (C-3' and C-5'), 114.7 (C-4''), 106.3 (C-4a), 101.5 (C-8), 100.2 (C-6), 78.4 (C-2''), 29.9 (2''-Me), 28.2 (2''-Me), 25.7 (3'''-Me) and 18.2 (3'''-Me). (Appendix B).

3.3.3 MAXIMAISOFLAVONE H (3)

White crystals; R_f =0.7(n-hexane/EtAOc, 7:3), mp 190-192 ° C, ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 7.92 (1H, *s*, H-2), 7.83 (1H, *d, J*=8.4 Hz, H-5), 7.46 (2H, *d, J*=9.0 Hz, H-2' and H-6'), 6.98 (2H, *d, J*=9.0 Hz, H-3' and H-5'),6.96 (1H, *d, J*=8.4 Hz, H-6), 6.21 (2H, *s*, -OCH_2O-) and 3.84 (3H, *s*, 4'-OMe). (Appendix C).

3.3.4 8-*O*-METHYLRETUSIN (4)

White crystals; R_f =0.7(n-hexane/EtAOc, 6:4), mp 230-232°C ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 8.00 (1H, *s*, H-2), 7.91 (1H, *d, J*=9.0 Hz, H-5), 7.49 (2H, *d, J*=8.4 Hz, H-2' and H-6'), 7.04 (1H, *d, J*=9.0 Hz, H-6), 6.97 (2H, *d, J*=8.4 Hz, H-3' and H-5'), 4.07 (3H, *s*, 8-OCH_3) and 3.83 (3H, *s*, 4'-OCH_3). ^{13}C NMR (CD_2Cl_2, 150 MHz): δ 175.7 (C-4), 159.8 (C-4'), 153.1 (C-6), 150.3 (C-5), 150.1 (C-7 and C-8a), 134.2 (C-8), 130.3 (C-3' and C-5'), 124.5 (C-3), 124.3 (C-1'), 119.0 (C-4a), 113.8 (C-2' and C-6'), 56.6 (8-OMe) and 56.6 (4'-OMe).(Appendix D).

3.3.5 MAXIMAISOFLAVONE J (5)

White crystals; R_f =0.5 (n-hexane/EtAOc, 7:3), mp 138-140 ° C; ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 8.10 (1H, *d, J*=8.4 Hz, H-5), 7.92 (1H, *s*, H-2), 7.47 (2H, *d, J*=8.4 Hz, H-2' and H-6'), 6.96 (2H, *d, J*=8.4 Hz, H-3' and H-5'), 6.87 (1H, *d, J*=2.4 Hz, H-8), 5.49 (1H, *t, J*=6.6 Hz, H-2''), 4.62 (2H, *d, J*=6.6 Hz, H-1''), 3.83 (3H, *s*, 4'-OMe), 1.81 (3H, *s*, 3''-Me) and 1.77 (3H, *s*, 3''-Me).^{13}C NMR (CD_2Cl_2, 150 MHz): δ 175.8 (C-4), 163.6 (C-5), 159.8 (C-7 and C-4'), 158.2 (C-8a), 152.6 (C-2), 139.4 (C-3''), 130.4 (C-2' and C-6'), 125.0 (C-3), 124.7 (C-1'), 118.9 (C-2''), 115.2 (C-3' and C-5'), 109.9 (C-4a and C-8),

101.6 (C-6), 65.8 (C-1''), 55.5 (4'-OMe), 25.7 (3''-Me) and 18.2 (3''-Me). (Appendix E).

3.3.6 MAXIMAISOFLAVONE B (6)

White crystals; R_f =0.5 (n-hexane/EtAOc, 7:3), mp 126-128 ° C; ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 8.10 (1H, *d, J*=8.4 Hz, H-5), 7.92 (1H, *s*, H-2), , 7.10 (1H, *d, J*=2.4 Hz, H-2'), 6.96 (1H, *dd, J*=2.4, 8.4 Hz, H-6'), 6.87 (1H, *d, J*=2.4 Hz, H-8), 6.86 (1H, *d, J*=8.4 Hz, H-5'), 5.99 (2H, *s*, OCH_2O), 5.49 (1H, *t, J*=6.6 Hz, H-2''), 4.62 (2H, *d, J*=6.6 Hz, H-1''), 1.81 (3H, *s*, 3''-Me) and 1.77 (3H, *s*, 3''-Me).^{13}C NMR (CD_2Cl_2, 150 MHz): δ 175.8 (C-4), 163.6 (C-5), 159.8 (C-7 and C-4'), 158.2 (C-8a), 152.6 (C-2), 147.9 (C-4'), 147.8 (C-3'), 139.4 (C-3''), 125.0 (C-3), 124.7 (C-1'), 122.6 (C-6'), 118.9 (C-2''), 109.9 (C-2', C-4a and C-8), 108.4 (C-5'), 101.1 (OCH_2O), 101.6 (C-6), 65.8 (C-1''), 25.7 (3''-Me) and 18.2 (3''-Me). (Appendix F).

3.3.7 TEPHROSIN (7)

Yellow oil; R_f =0.5(n-hexane/EtAOc, 6:4), mp 197-198 ° C ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 7.79 (1H, *d, J*=8.4 Hz, H-11), 7.31 (1H, *s*, H-1), 6.56 (1H, *d, J*=10.4 Hz, H-4'), 6.53 (1H, *d, J*=8.4 Hz, H-10), 6.01 (1H, *s*, H-4), 5.51 (1H, *d, J*=10.4 Hz, H-3'), 4.70 (1H, *dd, J*=2.4, 12.0 Hz, H-6a), 4.65 (2H, *dd, J*=2.4, 12.0 Hz, H-6), 3.85 (3H, *s*, OMe), 3.76 (3H, *s*, OMe), 1.49 (3H, *s*, 2'-Me) and 1.42 (3H, *s*, 2'-Me). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 191.7 (C-12), 161.0 (C-9), 156.9 (C-7a), 151.4 (C4a), 148.7 (C-2), 144.2 (C-3), 129.1 (C-11), 128.8 (C-3'), 115.7 (C-4'), 112.2 (C-1), 111.4 (C-11a), 109.7 (C-10), 109.4 (C-8), 108.9 (C-12b), 101.4 (C-4), 78.3 (C-2'), 76.5 (C-6a), 67.7 (C-12a), 64.1 (C-6), 56.6 (OMe), 56.1 (OMe), 28.8 (2'-Me) and 28.6 (2'-Me). (Appendix G).

3.3.8 LUPEOL (8)

White crystals; R_f=0.7 (n-hexane/EtAOc, 9:1), mp 213-215 °C ^{1}H NMR (CD_2Cl_2, 600 MHz), δ 0.91 (H-1a), 1.68 (H-1e), 1.54 (H-2a), 1.61 (H-2e), 3.18 (H-3), 0.69 (H-5), 1.39 (H-6a), 1.54 (H-6e), 1.41 (H-7), 1.28 (H-9), 1.25 (H-11a), 1.42 (H-11e), 1.07 (H-12a), 1.68 (H-12e), 1.67 (H-13), 1.01 (H-15a), 1.71 (H-15e), 1.38 (H-16a), 1.49 (H-16e), 1.37 (H-18), 2.39 (H-19), 1.33 (H-21), 1.93 (H-21), 1.20 (H-22), 1.42 (H-22), 0.98 (Me-23), 0.77 (Me-24), 0.84 (H-25), 1.04 (Me-26), 0.97 (Me-27), 0.79 (Me-28), 4.56 (H-29), 4.69 (H-29), 1.69 (H-30). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 38.9 (C-1), 28.2 (C-2), 79.2 (C-3), 39.1 (C-4), 55.5 (C-5), 16.2 (C-6), 34.5 (C-7), 41.1 (C-8), 50.7 (C-9), 37.4 (C-10), 19.5 (C-11), 25.4 (C-12), 38.3 (C-13), 43.1 (C-14), 27.7 (C-15), 35.8 (C-16), 43.2 (C-17), 48.5 (C-18), 48.2 (C-19), 151.2 (C-20), 30.0 (C-21), 40.0 (C-22), 21.2 (C-23), 21.2 (C-24), 15.6 (C-25), 18.2 (C-26), 14.8 (C-27), 16.3 (C-28), 109.5 (C-29), 18.5 (C-30). (Appendix H).

3.4 Biological Activity Assays

3.4.1 *In-vitro* antiplasmodial activity assays

The crude extract and pure compounds were assayed using a non radioactive assay technique (Smilkstein *et al.*, 2004) with modifications to determine 50% growth inhibition of cultured parasites. This is an accepted method for assaying *in-vitro* drug susceptibility using the fluorochrome called ''SYBR Green I'', a non-radioactive intercalating DNA marker that accurately depicts *in vitro* parasite replication. This test replaces the older, ^{3}H-hypoxanthine uptake assay, is fully endorsed by the WHO.

Briefly, two different strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), of *Plasmodium falciparum* were grown as described in the literature [Johnson *et al.*, 2007]. Concurrently, twofold serial dilutions of the drugs chloroquine (1.953 to 1,000 ng/ml), mefloquine (0.488 to 250 ng/ml) and test sample (97.7-50,000 ng/ml) were prepared on a 96 well plate. The culture-adapted *Plasmodium falciparum* were added on to the plate containing dose range of drugs and incubated in gas mixture (5% CO_2, 5% O_2, and 90% N_2) at 37°C. The assay was terminated 72 hrs later by freezing at -80°C.

After thawing, lysis buffer containing SYBR Green I (1x final concentration) were added directly to the plates and gently mixed by using the Beckman Coulter Biomek 2000 automated laboratory workstation (Beckman Coulter, Inc., Fullerton, CA). The plates were incubated for 5 – 15 minutes at room temperature in the dark. Parasite growth inhibition was quantified by measuring the per-well relative fluorescence units (RFU) of SYBR Green I dye using the Tecan Genios Plus (Tecan US, Inc., Durham, NC) with excitation and emission wavelengths of 485 nm and 535 nm, respectively, and with the gain set at 60. Differential counts of relative fluorescence units (RFUs) were used in calculating IC_{50}'s for each drug using prism 4.0 software for Windows (Graphpad Software, San Diego, CA). A minimum of three separate determinations was carried out for each sample. Replicates had narrow data ranges hence presented as mean ± SD.

3.4.2 Larvicidal activity assays

The eggs of *Aedes aegypti* L. (Diptera: Culicidae) were obtained from the Department of Zoology, University of Nairobi. The eggs were flooded with 0.08% NaCl solution and

left to hatch at 28°C. 20 2nd instar larvae were transferred into a Petri-dish containing 500ml of 0.08% NaCl solution. The larvae were treated with the test extracts according to Mwangi and Rembold (1998). Every 20 milligrams of test samples were dissolved in 2 ml of DMSO. From the stock solution different concentrations were prepared by serial dilution and the larvae were tested for mortality at 20, 10, 5, 2.5 and 1.25 µg/ml of sample solutions. Control larvae in all cases received 50µl of DMSO as in the test larvae. The rotenone (**12**) was used as the standard. Mortality was checked after 24 hours. LC_{50} values were calculated from the average of three observations for each concentration using Finney's probit analysis for quantal data (McLaughlin *et al.*, 1991: Finney, 1971)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 PRELIMINARY TEST RESULTS

TLC analyses of the crude extracts obtained from the stem bark of *Millettia oblata* ssp. *teitensis* showed the presence of UV (254 nm) active compounds. From biogenetic considerations most of these compounds were considered to be isoflavonoids. The extracts were subjected to chromatography which led to the isolation of eight compounds. The isolated compounds were characterized using 1D (^{1}H and ^{13}C), 2D (COSY, HMBC and HMQC) NMR, and MS. In the following sections the isolations, structural elucidation and biological activities of compounds of this plant will be discussed.

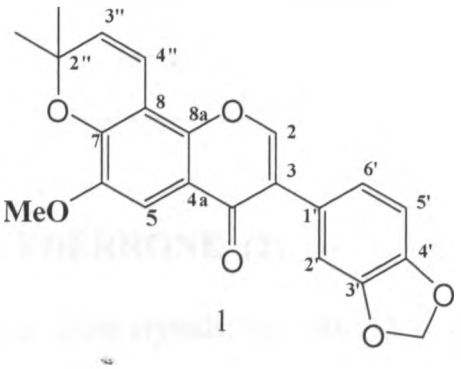
4.1 COMPOUNDS FROM THE STEM BARK OF *MILLETTIA OBLATA* SSP. *TEITENSIS*

4.1.1 DURMILLONE (1)

Compound **1** was isolated as white crystals. The ^{1}H (δ 7.95 for H-2) and ^{13}C (δ 152.0 for C-2, 124.5 for C-3 and 175.7 for C-4) NMR spectra (Table 4.1) indicated that compound **1** is an isoflavone derivative (Yenesew *et al.,* 1996). Furthermore, the ^{1}H and ^{13}C NMR spectra showed the presence of a methylenedioxy (δ 5.99, *s*, in ^{1}H and 101.4 in ^{13}C NMR) and a methoxyl (δ 3.93 in ^{1}H and 56.6 in ^{13}C NMR) groups. The ^{1}H NMR spectrum also showed a *cis*-olefinic protons consisting of two doublets at δ 6.82 and 5.77 (*d*, J = 9.6 Hz), which together with two singlets at δ 1.52 and 1.64 each integrating for three protons, suggesting the presence of a 2, 2-dimethylpyrano substituent.

The ^{1}H NMR spectrum in addition showed a deshielded aromatic proton at δ 7.49 and an AMX spin system aromatic protons [δ 7.08 (*d, J* = 1.8), 6.87 (*d, J* = 7.8), 6.97 (*dd, J* = 1.8, 7.8 Hz)].

The placement of the methylenedioxy group at C-7/C-8 will result in two highly shielded oxygenated aromatic carbons (C-7 and C-8) which normally resonate at δ_C 130 (Agrawal 1989). In compound **1** these carbons are resonating at around δ 147 and therefore the methylenedioxy group is at C-3'/C-4' position and hence the pyran ring at C-7/C-8 position. The deshielded aromatic proton at δ 7.49 is assigned to H-5 of ring A and hence the methoxyl is at C-6. Based on these and by comparison of the data with literature, compound **1** was identified as durmillone (**1**). Durmillone has been previously isolated from *Millettia dura* (Ollis *et al.*, 1967). This is, however, the first report of durmillone from this plant.



1

Table 4.1: ^{1}H (CD_2Cl_2, 600 MHz) and ^{13}C ($CDCl_3$, 50 MHz) - NMR Chemical Shift Values for Compound **1**

Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm	Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm
2	7.95 (*s*)	152	4'		147.9
3		124.5	5'	6.87 (*d*, *J*=7.8)	108.6
4		175.7	6'	6.97 (*dd*, *J*=1.8, 7.8)	122.6
4a		117.8	1''		
5	7.49 (*s*)	105.3	2''		78.4
6		147.4	2''-Me_2	1.52 (*s*)	28.2
7		147.5		1.64 (*s*)	28.2
8		110.4	3''	5.77 (*d*, *J*=9.6)	130.6
8a		147.6	4''	6.82 (*d*, *J*=9.6)	115.4
1'		126.1	6-OMe	3.93 (*s*)	56.6
2'	7.08 (*d*, *J*=1.8)	110.0	-OCH_2O-	5.99 (*s*)	101.4
3'		147.8			

4.1.2 4'-PRENYLOXYDERRONE (2)

Compound **2** was isolated as white crystals; mp 130-132 °C. The HRMS of compound **2** showed a $[M]^+$ at *m/z* 404.1603 corresponding to the molecular formula $C_{25}H_{24}O_5$ The ^{1}H (δ 7.93 for H-2) and ^{13}C (δ 152.9 for C-2, 123.8 for C-3 and 181.3 for C-4) NMR spectra (Table 4.2) indicated that compound **2** is an isoflavone derivative (Yenesew *et al.*, 1996).

The ^{1}H NMR spectrum showed a *cis*-olefinic system consisting of two doublets at δ 6.71 and 5.62 (*d, J* = 10.2Hz), which together with 6H singlet at δ 1.47 and 1.53 suggested the presence of a 2, 2-dimethylpyrano substituent. The ^{1}H NMR spectrum further indicated the presence of a prenyloxy group (δ 5.49, 1H, *t*, *J* = 6.6 Hz for H-2'''; 4.55, 2H, *d*, *J* = 6.6 Hz for H-1'''; 1.76, 3H, *s*, for H-4''' and 1.80, 3H, *s*, for H-5'''). The corresponding carbons resonated at δ 65.1 (C-1'''), 119.9 (C-2'''), 138.4 (C-3'''), 18.2 (C-4''') and 25.7 (C-5''') in the ^{13}C NMR, respectively. This was further confirmed by the presence in the EIMS of an intense peak at *m/z* 321 $[(M-15)-C_5H_9]^+$ due to the loss of a prenyl (**Figure 4.1**).

The ^{1}H NMR revealed that the presence of an AA'XX' spins system centred at δ 7.44 and 6.96 (*d, J* = 9 Hz) indicating that ring B is substituted at C-4' with the prenyloxy group. The presence of the fragment at *m/z* 203 (**2b in Figure 4.1**) in the HRMS resulting from retro-Diels-Alder cleavage of ring C is consistent with the placement of the prenyloxy group in ring-B and, the hydroxyl and 2, 2-dimethyl group in ring A. A singlet aromatic proton at δ 6.25 and a chelated OH (δ 12.94) in ring A require that the pyran group is placed at either C-5/C-6 or C-7/C-8 positions. HMBC correlation of the singlet proton at δ 6.25 with C-5 (**Figure 4.2**) places this proton at H-6 and hence the pyran group at C-7/C-8 positions. On this basis, compound **2** was characterized as 7-hydroxy-7,8-(2,2-dimethylpyrano)-4'-prenyloxyisoflavone for which the trivial name 4'-prenyloxyderrone is suggested. This is the first report of this compound in nature. The identity of this new compound was further confirmed through HMQC and HMBC experiments.

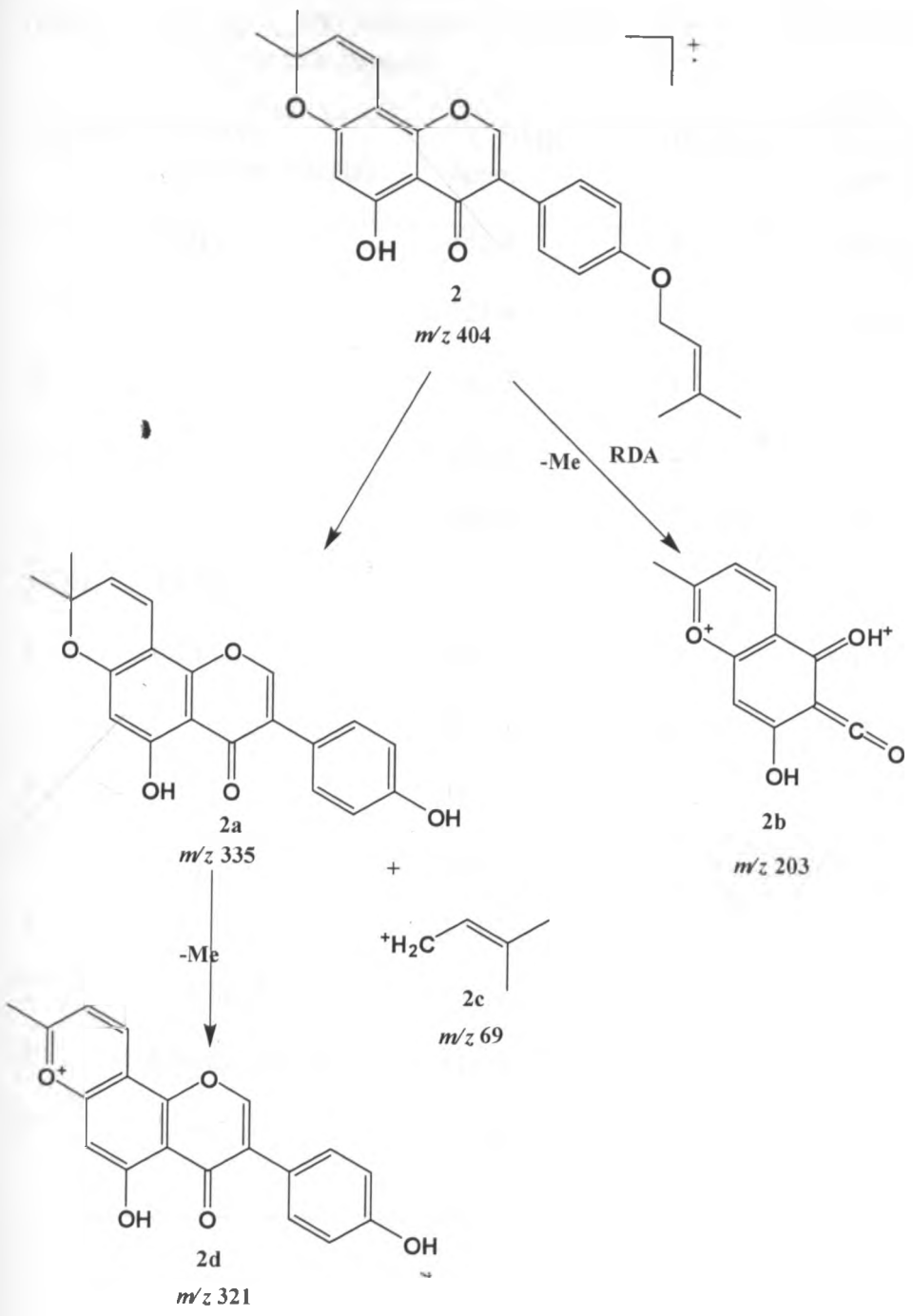


Figure 4.1: EIMS fragmentation pattern of compound 2

Table 4.2: ^{1}H (CD_2Cl_2, 600 MHz) and ^{13}C (CD_2Cl_2, 150 MHz) -NMR Chemical Shift Values for Compound **2**

Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm	Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm
2	7.94 (*s*)	152.9	5'	6.96 (*d*, *J*=9.0)	114.9
3		123.8	6'	7.44 (*d*, *J*=9.0)	130.4
4		181.3	1''		
4a		106.3	2''		78.4
5		162.5	2''-Me_2	1.47 (*s*)	28.2
5-OH	12.94 (*s*)			1.53 (*s*)	29.9
6	6.25 (*s*)	100.2	3''	5.62 (*d*, *J*=10.2)	127.8
7		159.4	4''	6.71 (*d*, *J*=10.2)	114.7
8		101.5	1'''	4.55 (*d*, *J*=6.6)	65.1
8a		152.5	2'''	5.49 (*d*, *J*=6.6)	119.9
1'		123.1	3'''		138.4
2'	7.44 (*d*, *J*=9.0)	130.4	4'''-Me	1.80 (*s*)	18.2
3'	6.96 (*d*, *J*=9.0)	114.9	5'''-Me	1.76 (*s*)	25.7
4'		159.8			

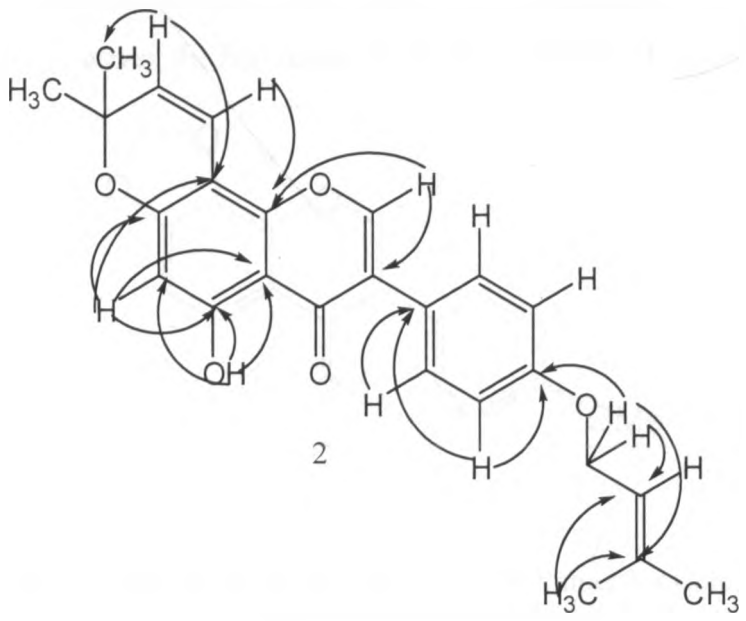


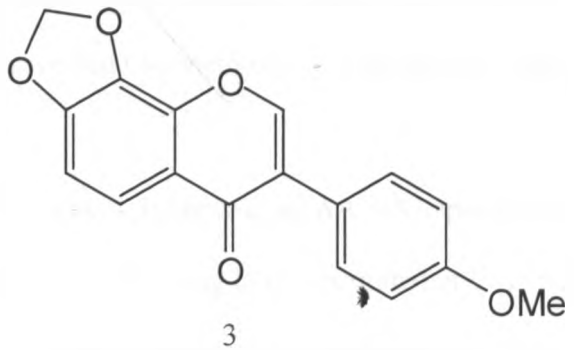
Figure 4.2: HMBC Correlation in Compound 2

4.1.3 MAXIMAISOFLAVONE H (3)

Compound **3** was isolated as white crystals. The ^{1}H (δ 7.92 for H-2) NMR specta (Table 4.4) reveal that compound **3** is an isoflavone derivative (Yenesew *et al.*, 1996). The ^{1}H NMR spectrum further revealed the presence of a methylenedioxyl (δ 6.21, *s*,) and a methoxyl (δ 3.84, *s*,) groups.

The ^{1}H NMR spectrum showed an AA'XX' spin system centred at δ 7.46 and 6.98 (*d, J* = 9.0 Hz), indicating that ring B is substituted at C-4' with the methoxyl group. In ring A, the methylenedioxy group was placed at C-7/C-8 position because of AX spins system at δ 7.83 and 6.96 (*d, J* = 8.4 Hz) for H-5 and H-6. Based on these and by comparison of the data with literature, compound **3** was identified as maximaisoflavone H (**3**), a compound

which has been previously isolated from *Millettia dura* (Dagne *et al.*, 1991; Yenesew *et al.*, 1996). This is, however, the first report of maximaisoflavone H from this plant.



3

Table 4.3: ^{1}H (CD_2Cl_2, 600 MHz) -NMR Chemical Shift Values for Compound **3**

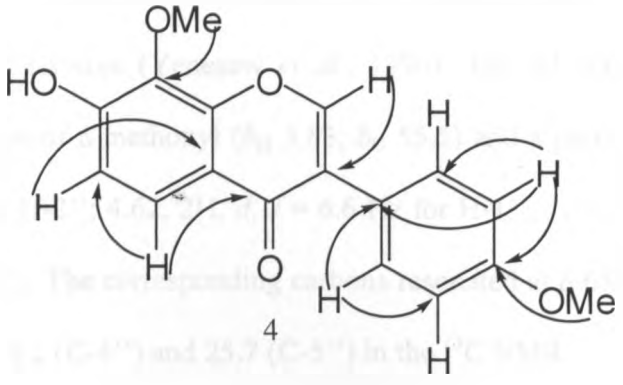
Position	^{1}H NMR δppm (*m*, *J* in Hz)	Position	^{1}H NMR δppm (*m*, *J* in Hz)
2	7.92 (*s*)	1'	
3		2'	7.46 (*d*, *J*=9.0)
4		3'	6.98 (*d*, *J*=9.0)
4a		4'	
5	7.83 (*d*, *J*=8.4)	5'	6.98 (*d*, *J*=9.0)
6	6.96 (*d*, *J*=8.4)	6'	7.46 (*d*, *J*=9.0)
7		4'-OMe	3.84 (*s*)
8		-OCH_2O-	6.21 (*s*)
8a			

4.1.4: 8 – *O* – METHYLRETUSIN (4)

Compound **4** was isolated as white crystals. The ^{1}H (δ 8.00 for H-2) NMR specta (Table 4.5) reveal that compound **4** is an isoflavone derivative (Yenesew *et al.*, 1996). The ^{1}H NMR spectrum further revealed two methoxyl (δ 3.83 and 4.07 ppm) substituents.

The ^{1}H NMR spectrum (Table 4.5) showed an AA'XX' spin system centred at δ 7.49 and 6.97 (*d, J* = 9.0 Hz), indicating that ring B is substituted at C-4' with either a methoxyl or a hydroxyl group. The ^{1}H NMR spectrum also revealed an AX spins system at δ 7.91 and 7.04 (*d, J* = 9.0 Hz) which were readily assigned to H-5 and H-6.

From the HMBC spectrum (**Figure 4.3**), the methoxyl group at δ 4.07 was correlating with C-8 and the methoxyl at δ 3.83 correlating with C-4'. The hydroxyl group was then placed at C-7. Based on these and by comparison of the data with literature, compound **4** was identified as 8-*O*-methylretusin (**4**), a compound which has been previously isolated from *Millettia dielsiana* (Rui *et al.*, 1989) and *Millettia reticulata* (Chen *et al.*, 1983). This is, however, the first report of 8-*O*-methylretusin from this plant.



4

Figure 4.3: HMBC Correlation in Compound 4

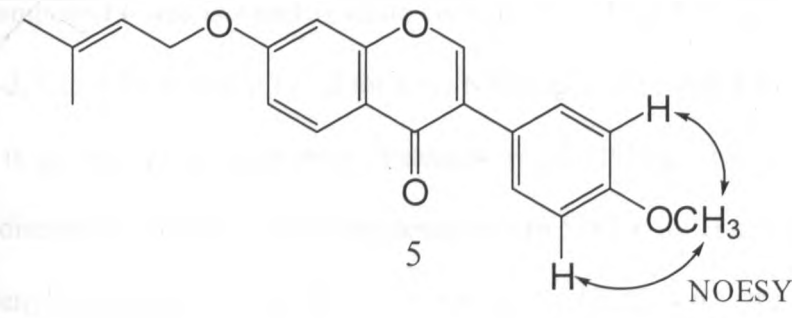
Table 4.4: ^{1}H (CD_2Cl_2, 600 MHz)-NMR Chemical Shift Values for Compound **4**

Position	^{1}H NMR δppm (*m*, *J* in Hz)		Position	^{1}H NMR δppm (*m*, *J* in Hz)
2	8.00 (*s*)		8-OMe	4.07 (*s*)
3			1'	
4			2'	7.49 (*d*, *J*=8.4)
4a			3'	6.97 (*d*, *J*=8.4)
5	7.91 (*s*, *J*=9.0)		4'	
6	7.04 (*s*, *J*=9.0)		5'	6.97 (*d*, *J*=8.4)
7			6'	7.49 (*d*, *J*=8.4)
8			4'-OMe	3.83 (*s*)
8a				

4.1.5: MAXIMAISOFLAVONE J (5)

Compound **5** was isolated as white crystals The ^{1}H (δ 7.92 for H-2) and ^{13}C (δ 152.6 for C-2, 125.0 for C-3 and 175.8 for C-4) NMR spectra (Table 4.7) indicated that compound **5** is an isoflavone derivative (Yenesew *et al.*, 1996). The ^{1}H NMR spectrum further indicated the presence of a methoxyl (δ_H 3.83; δ_C 55.5) and a prenyloxy group (δ 5.50, 1H, *t*, *J* = 6.6 Hz for H-2''; 4.62, 2H, *d*, *J* = 6.6 Hz for H-1''; 1.77, 3H, *s*, for H-4'' and 1.81, 3H, *s*, for H-5''). The corresponding carbons resonated at δ 65.8 (C-1''), 118.9 (C-2''), 139.4 (C-3''), 18.2 (C-4'') and 25.7 (C-5'') in the ^{13}C NMR.

The ^{1}H NMR revealed an AA'XX' spins system centred at δ 7.47 and 6.96 (*d*, *J* = 8.4 Hz) indicating that ring B is substituted at C-4' with either methoxyl or prenyloxy group. The position of methoxyl group was determined from NOESY interactions between the methoxyl group at δ 3.83 and the doublet at δ 6.96, hence the methoxyl group was placed at C-4' and prenyloxyl group at C-7 of monosubstituted ring A where H-5, H-6 and H-8 appeared at δ_H 8.10 (*d*, *J* = 8.4 Hz, H-5), 6.96 (*d*, *J* = 8.4 Hz, H-6) and 6.87 (*d*, *J* = 2.4 Hz, H-8). NOE interaction of CH_2-1'' with H-6 and H-8 is consistent with the placement of the prenyloxy group at C-7. Based on these and by comparison of the data with literature (Rao *et al.*, 1994), compound **5** was identified as maximaisoflavone J (**5**). This is the first report of maximaisoflavone J from the genus *Millettia*.



5

Table 4.5: ^{1}H (CD_2Cl_2, 600 MHz) and ^{13}C (CD_2Cl_2, 150 MHz) -NMR Chemical Shift Values for Compound **5**

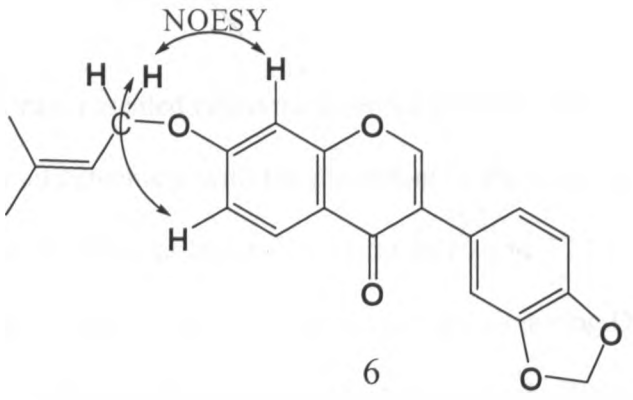
Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm	Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm
2	7.92 (*s*)	152.6	3'	6.96 (*d*, *J*=8.4)	115.2
3		125.0	4'		159.8
4		175.8	5'	6.96 (*d*, *J*=8.4)	115.2
4a		109.9	6'	7.47 (*d*, *J*=8.4)	130.4
5	8.10 (*d*, *J*=8.4)	163.6	1''	4.62 (*d*, *J*=6.6)	65.8
6	6.96 (*d*, *J*=8.4)	101.6	2''	5.49 (*t*, *J*=6.6)	118.9
7		159.8	3''		139.4
8	6.87 (*d*, *J*=2.4)	109.9	4''-Me	1.77 (*s*)	18.2
8a		158.2	5''-Me	1.81 (*s*)	25.7
1'		124.7	6-OMe	3.83 (*s*)	55.5
2'	7.47 (*d*, *J*=8.4)	130.4			

4.1.6: MAXIMAISOFLAVONE B (6)

Compound **6** was isolated as white crystals The ^{1}H (δ 7.92 for H-2) and ^{13}C (δ 152.6 for C-2, 125.0 for C-3 and 175.8 for C-4) NMR spectra (Table 4.8) indicated that compound **6** is an isoflavone derivative (Yenesew *et al.*, 1996). The ^{1}H NMR spectrum further indicated the presence of a methylenedioxy (δ 5.99, *s*, in ^{1}H and 101.1 in ^{13}C NMR) and a prenyloxy group (δ 5.50, 1H, *t*, *J* = 6.6 Hz for H-2''; 4.62, 2H, *d*, *J* = 6.6 Hz for H-1''; 1.77, 3H, *s*, for H-4'' and 1.81, 3H, *s*, for H-5''). The corresponding carbons of the prenyloxy group resonated at δ 65.8 (C-1''), 118.9 (C-2''), 139.4 (C-3''), 18.2 (C-4'') and 25.7 (C-5'').

The ^{1}H NMR spectrum in addition showed an AMX spin system for aromatic protons [δ 7.10 (*d*, *J* = 2.4, H-2'), 6.86 (*d*, *J* = 8.4, H-5'), 6.96 (*dd*, *J* = 8.4, 2.4, H-6')] in ring B. The position of the prenyloxyl group was determined from NOESY interactions between the methylene protons at δ 4.62 and the doublet at δ 6.87 (*d*, *J* = 2.4, H-8) and δ 6.96 (*d*, *J* =

8.4, H-6) in ring A, hence the prenyloxyl group was placed at C-7 and methylenedioxy group at C-3'/C-4' in agreement with the biogenetic expectaction of oxygenation at C-7 and C-4'. Based on these and by comparison of the data with literature (Rao *et al.*, 1984), compound **6** was identified as maximaisoflavone B (**6**), a compound which has been previously isolated from *Millettia dura* (Dagne *et al.*, 1991). This is the first report of maximaisoflavone B from this plant.



6

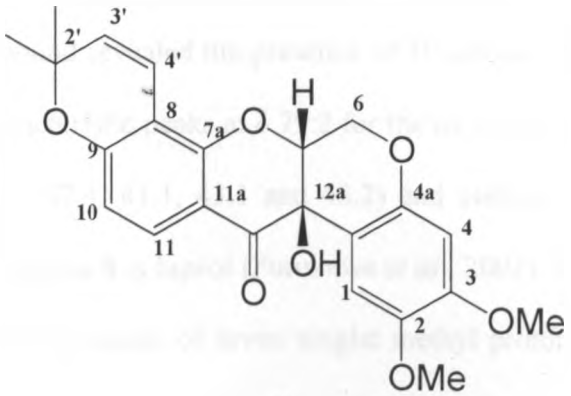
Table 4.6: ^{1}H (CD_2Cl_2, 600 MHz) and ^{13}C (CD_2Cl_2, 150 MHz) -NMR Chemical Shift Values for Compound **6**

Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm	Position	^{1}H NMR δppm (*m*, *J* in Hz)	^{13}C NMR δppm
2	7.92 (*s*)	152.6	3'		147.8
3		125.0	4'		147.9
4		175.8	5'	6.86 (*d*, *J*=8.4)	108.4
4a		109.9	6'	6.96 (*dd*, *J*=2.4, 8.4)	122.6
5	8.10 (*d*, *J*=8.4)	163.6	1''	4.62 (*d*, *J*=6.6)	65.8
6	6.96 (*d*, *J*=8.4)	101.6	2''	5.49 (*t*, *J*=6.6)	118.9
7		159.8	3''		139.4
8	6.87 (*d*, *J*=2.4)	109.9	4''-Me	1.77 (*s*)	18.2
8a		158.2	5''-Me	1.81 (*s*)	25.7
1'		124.7	$-OCH_2O-$	5.99 (*s*)	101.1
2'	7.10 (*d*, *J*=2.4)	109.9			

4.1.7 TEPHROSIN (7)

Compound 7 was isolated as yellow oil. The ^{1}H-NMR [δ 4.65 (*dd*, *J* = 12.0, 2.5 Hz, H-6), 4.58 (*dd*, *J* = 2.5, 0.8 Hz, H-6), 4.70 (*dd*, *J* = 12.0, 0.8 Hz, H-6a)] and ^{13}C-NMR [δ 64.1 (C-6), 76.5 (C-6a)] showed peaks which are characteristic of a 12a-hydroxyrotenoid skeleton (Yenesew *et al.*, 2003). The ^{1}H-NMR (Tabe 4.7) further revealed two methoxy (δ 3.77, 3.66) and a 2, 2-dimethylpyran δ 1.42 (2'-Me), 1.49 (2'-Me), 5.51 (d, *J* = 10.4 Hz, H-3'), 6.56 (d, *J* = 10.4 Hz, H-4') substituents.

In the ^{1}H-NMR spectrum revealed two *para*-oriented aromatic protons at δ 7.31 (H-1) and 6.47 (H-4) which are in agreement with the placement of the methoxyls at C-2 and C-3 in ring A. The presence of AX doublets at δ 6.51 for H-10 and 7.77 for H-11, would place the 2,2-dimethylpyran group at C-8/C-9, with oxygen at C-9 in ring D. Based on these and by comparison of the data with literature (Yenesew *et al.*, 2003), compound 7 was identified as tephrosin (7) (Ollis *et al.*, 1967) which has earlier been isolated from the seeds of *Millettia dura* (Yenesew *et al.*, 2003). This is the first report of tephrosin from this plant.



7

Table 4.7: ^{1}H (CD_2Cl_2, 600 MHz) and ^{13}C ($CDCl_3$, 50 MHz) -NMR Chemical Shift Values for Compound **7**

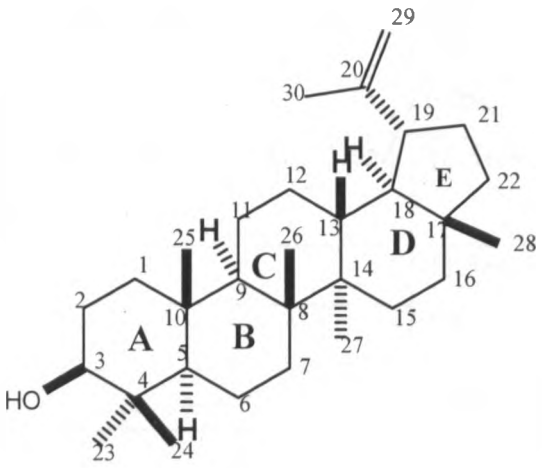
Position	^{1}H NMR , δppm (*m*, *J* in Hz)	^{13}C NMR , δppm
1	7.31 (*s*)	112.2
1a		108.9
2		148.7
3		144.2
4	6.01 (*s*)	101.4
4a		151.4
6	4.65 (*dd*, *J*=2.4, 12.0)	64.1
	4.58 (*dd*, *J*=0.8, 2.4)	
6a	4.70 (*dd*, *J*=0.8, 12.0)	76.5
7a		156.9
8		109.4
9		161.0
10	6.53 (*d*, *J*=8.4)	109.7
11	7.79 (*d*, *J*=8.4)	129.1
11a		111.4
12		191.7
12a		67.7
2'		78.3
3'	5.51 (*d*, *J*=10.4)	128.8
4'	6.56 (*d*, *J*=10.4)	115.7
2'-Me_2	1.49 (*s*)	28.8
	1.42 (*s*)	28.6
OMe-2	3.77 (*s*)	56.6
OMe-3	3.66 (*s*)	56.1

4.1.8 LUPEOL (8)

Compound **8** was obtained as non-UV active white crystals (from MeOH). The ^{13}C NMR and DEPT of this compound revealed the presence of 30 carbons indicating that it could be a triterpene. The characteristic peaks at δ 79.2 for the oxygenated C-3, the quarternary carbon peaks (at δ 39.1, 37.4, 41.1, 43.1 and 43.2) and olefinic carbons (δ 151.2 and 109.5) showed that compound **8** is lupeol (Furukawa *et al.*, 2002). In agreement with this, the ^{1}H NMR revealed the presence of seven singlet methyl protons (δ 0.98, 0.77, 0.84,

1.04, 0.97, 0.79, and 1.69) and methylene protons (δ 4.56 and 4.69) as well as a double doublet at δ 3.18.

In the ^{1}H-NMR spectrum, a double doublet at δ 3.18 was assigned to H-3, 0.98 for Me-23, 0.77 for Me-24 and 0.84 for Me-25 in ring A. In ring B, the singlet methyl protons δ 1.04 was assigned Me-26 and 0.97 for Me-27 in ring C. In ring D, the singlet methyl protons δ 0.79 was assigned Me-28 and 1.69 for me-30 in ring E. The methylene protons (δ 4.56 and 4.69) were assigned at H-29a and H-29b. This is the first report of Lupeol from this plant.



8

4.2 Biological Activities of the Isolated Compounds

4.2.1 Anti-plasmodial activities of compounds from the stem bark of *Millettia oblata* ssp. *teitensis*

The crude extract (CH_2Cl_2/MeOH, 1:1) of the stem bark of *Millettia oblata* ssp. *teitensis* was tested for antiplasmodial activities against two different strains of the malaria parasite. Moderate antiplasmodial activities against both W2 and D6 strains of

Plasmodium falciparum was observed with an IC_{50} value of 10.0±2.3 and 12.0±1.2 μg/ml respectively. The crude extract of the stem bark of *Millettia oblata* ssp. *teitensis* yielded eight compounds, of which five were tested against the W2 and D6 strains of *Plasmodium falciparum,* and showed anti-plasmodial activities. The results are summarised in Table 4.8.

Table 4.8: *In vitro* IC_{50} values of some compounds of *Millettia oblata* ssp. *teitensis* against W2 and D6 strains of *Plasmodium falciparum*.

Tested compound	IC_{50} in μg/ml (± SD)	
	W2	D6
Durmillone (**1**)	9.8±0.1	6.0±0.6
4'-Prenyoxyderrone (**2**)	6.0±0.9	5.4±1.0
A mixture of maximaisoflavone J (**5**) and maximaisoflavone B (**6**)	16.5± 3.5	4.6± 0.5
Tephrosin (**7**)	11.5±0.3	12.7±0.9
Lupeol (**8**)	NT	13.9±0.9
Chloroquine	0.0699±0.0102	0.0139±0.0012
Mefloquine	0.0024±0.0004	0.0321±0.0027

NT- Not tested

The activities observed for the crude extract is within the range reported for isoflavones (Yenesew, 1997 and Derese, 2004). It is worth noting that the crude extract is more active against the chloroquine-resistant strain than the chloroquine-sensitive strain.

Durmillone (**1**) showed good antiplasmodial activity with the activity being more against the chloroquine-sensitive D6 stains than the chloroquine-resistant W2 strains. The new compound 4'-prenyloxyderrone (**2**) showed good activity against both strains. Lupeol (**8**)

showed moderate antiplasmodial activity, while tephrosin (**7**) and the mixture of maximaisoflavone J (**5**) and maximaisoflavone B (**6**) showed some appreciable amount of antiplasmodial activity.

4.2.2 Larvicidal activity tests

The MeOH/CH_2Cl_2 (1:1) extracts of the stem, seeds and seedpods of *Millettia oblata* ssp. *teitensis* were partitioned between hexane and methanol. The purpose of partitioning was to remove the oil part which is the hexane layer. The methanol layer of the stem, seeds and seedpods were tested against the second instar larvae of *Aedes aegypti*. The stem bark extract showed larvicidal activity with the LC_{50} value of 11.2±1.9 μg/ml after 24 hours. The seed extract showed potent larvicidal activity with LC_{50} value of 1.4±0.2 μg/ml after 24 hours. The seedpod extracts were inactive even at 20.0 μg/ml concentration of the sample and after 10 days the larvae turned into adult mosquitoes. The observed activity of the seeds and the stem bark of *Millettia oblata* ssp. *teitensis* could be due to the presence of rotenoids in their composition.

Rotenoids are known to occur widely in some genera of the family leguminosae, such as *Millettia*, *Lonchocarpus*, *Tephrosia* and *Derris* (Dewick, 1994). These plants are distributed in tropical region of the world where there are mosquitoes. Utilization of such plants as larvicidal agents will decrease the population of malaria vector and help to control epidermic caused by the vector. Isoflavones are reported to be inactive for larvicidal activity (Yenesew, 1997), that is why larvicidal activities of the pure compounds were not carried out.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

In this study, the stem bark of *Millettia oblata* ssp. *teitensis* was investigated and eight compounds were isolated and characterized. The conclusions drawn from this study are outlined below:

1. The phytochemical study of the stem bark of *Millettia oblata* ssp. *teitensis* led to the isolation and characterization of eight compounds. These include six isoflavones: durmillone (**1**), 4'-prenyloxyderrone (**2**), maximaisoflavone-H (**3**), 8–*O*–methylretusin (**4**), maximaisoflavone J (**5**) and maximaisoflavone B (**6**), one rotenoid tephrosin (**7**) and a triterpene lupeol (**8**). Of these, the isoflavone 4'-prenyloxyderrone is a new compound.

2. The antiplasmodial activities of the crude extracts and some of the isolated compounds were tested against chloroquine-resistant strain (W2) and the chloroquine-sensitive strain (D6) of *Plasmodium falciparum* parasite for malaria. The results showed that some compounds had moderate antiplasmodial activities with the new compound showing the highest activity with IC_{50} values of 6.0±0.9 for W2 and 5.4±1.0 for D6. The crude extract of stem, seeds and seedpods were tested for larvicidal activities and the seed extract had the highest larvicidal activity with LC_{50} value of 1.4±0.2 µg/ml after 24 hours.

5.2 RECOMMENDATIONS

Although the results of the study demonstrated moderate antiplasmodial activities of the extract and compounds isolated from the stem bark of *Millettia oblata* ssp. *teitensis*, more information on toxicity and efficacy is required as a means of developing them into therapies for human use. In order to fulfill some of these requirements, the following recommendations are put forward:

1. Further phytochemical investigation of the stem bark, root, seeds and seedpods of *Millettia oblata* ssp. *teitensis* should be carried out in order to determine fully all the major and isolable compounds that are biosynthesized by this medicinal plant.

2. *In vivo* antiplasmodial activity tests should be carried out on the extract and isolated compounds from this medicinal plant in order to establish their potency and efficacy.

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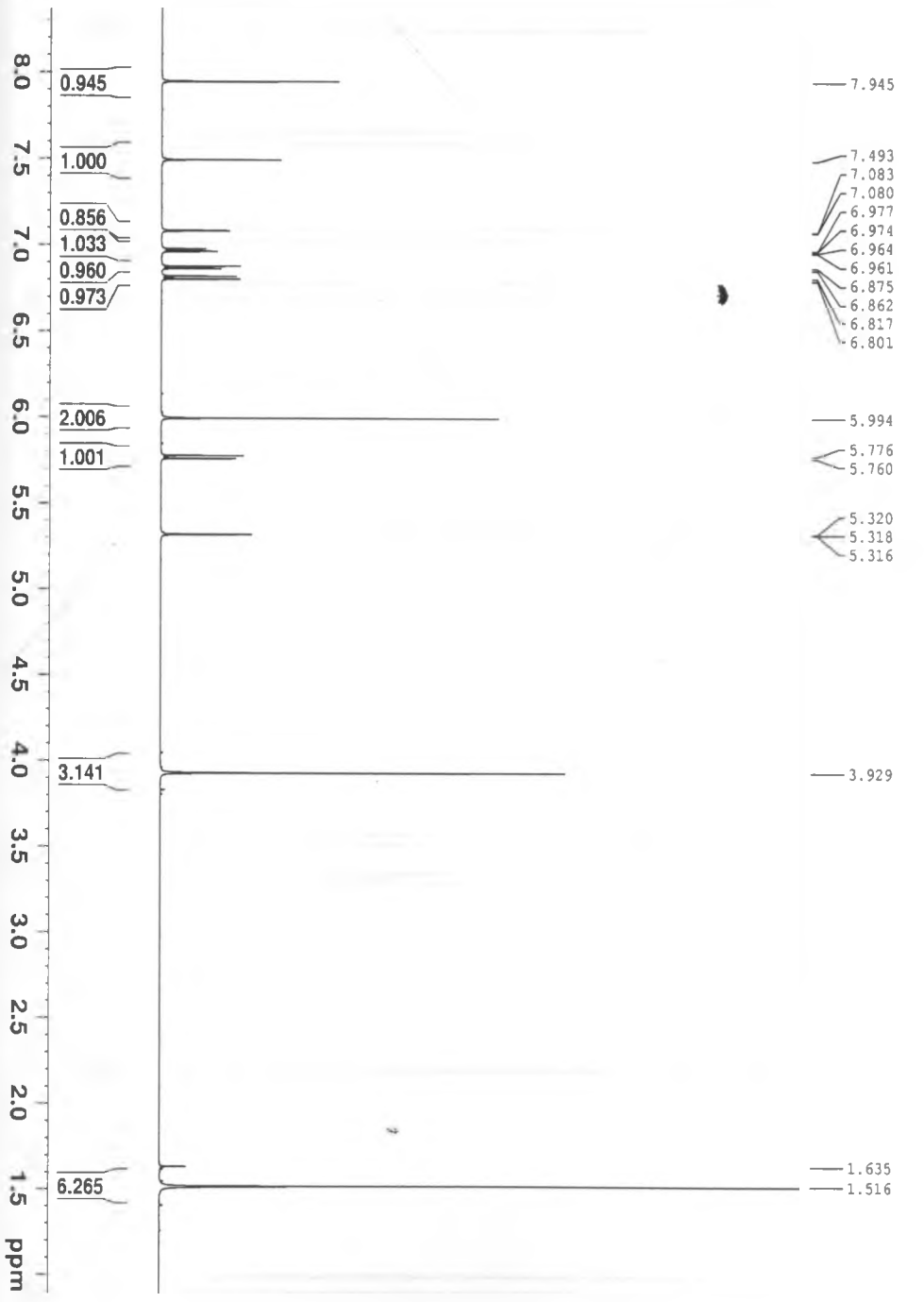
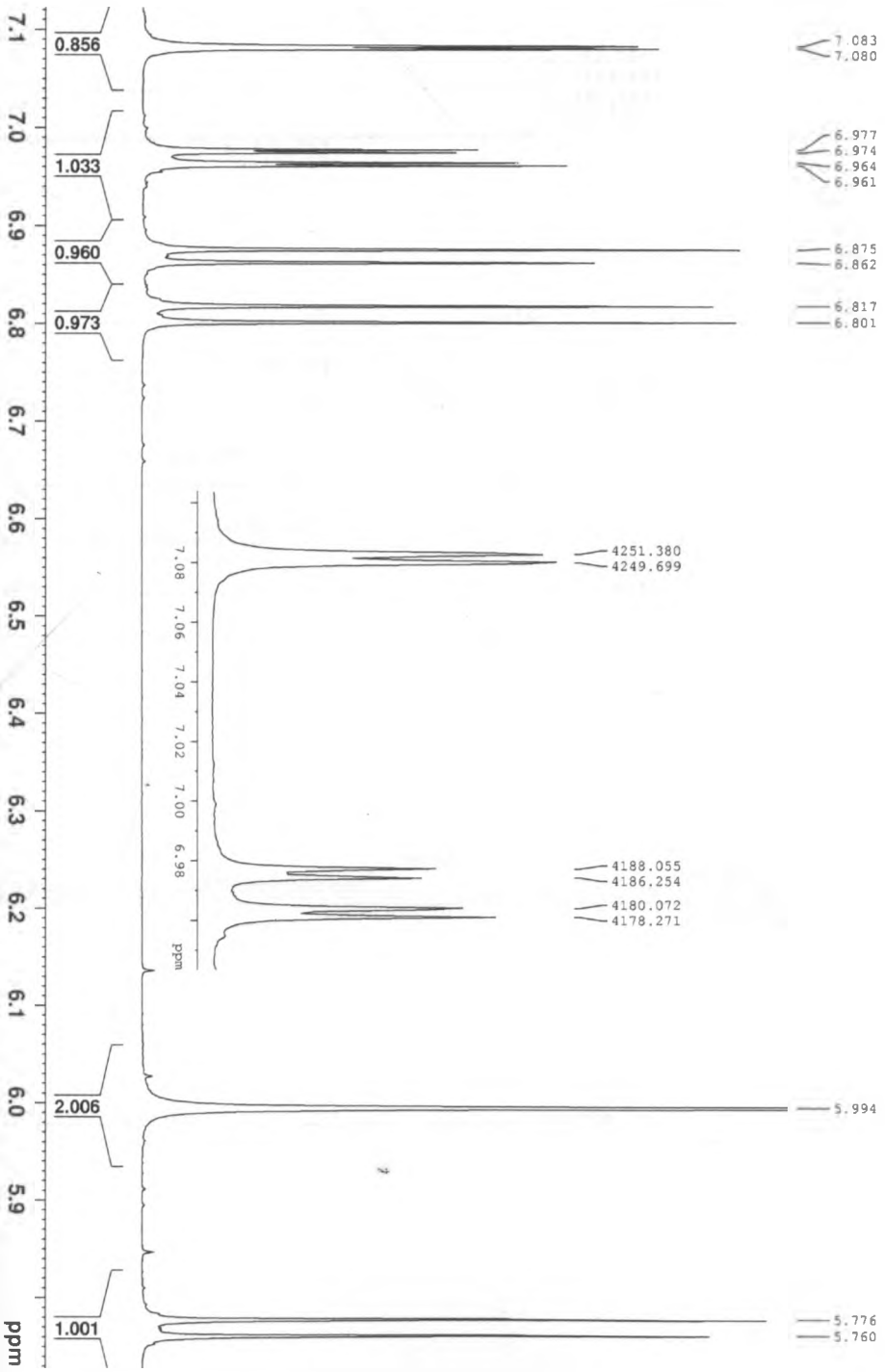
APPENDICES

APPENDIX A: SPECTRA FOR COMPOUND **1**

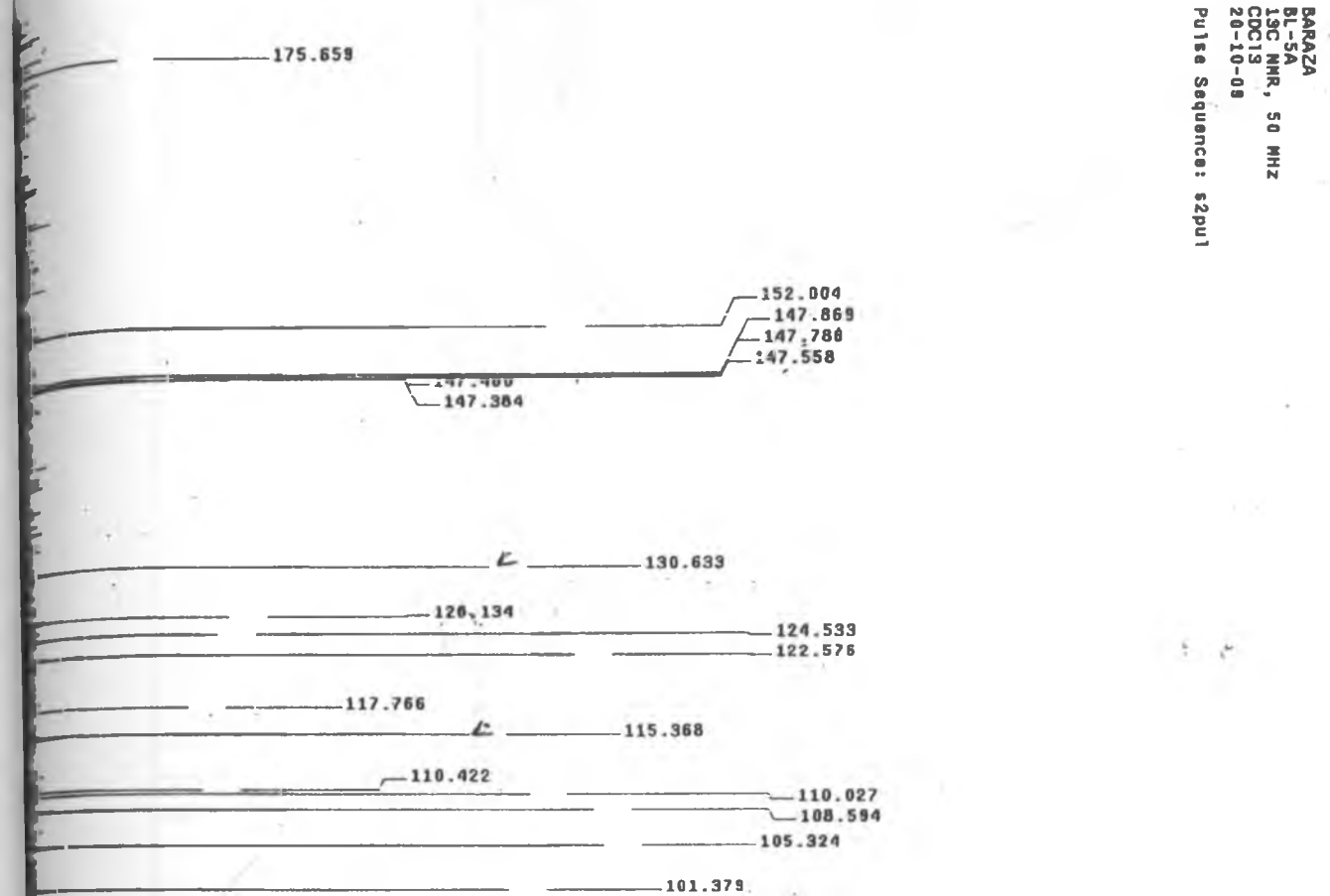
^{1}H NMR SPECTRUM FOR COMPOUND 1 (600 MHz, CD_2Cl_2)

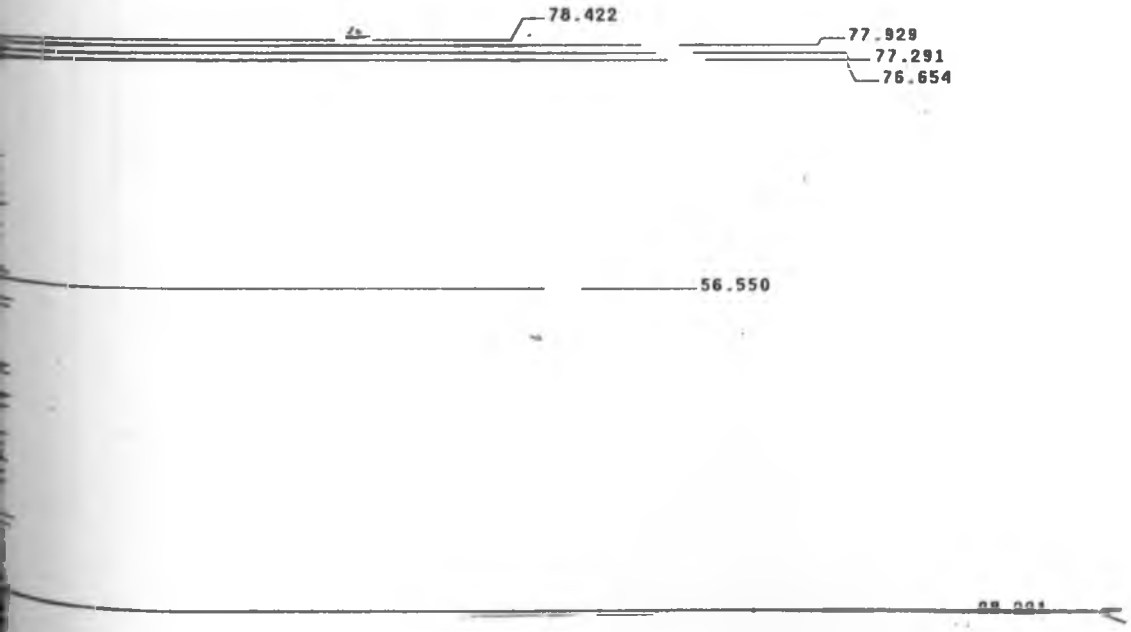


^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 1

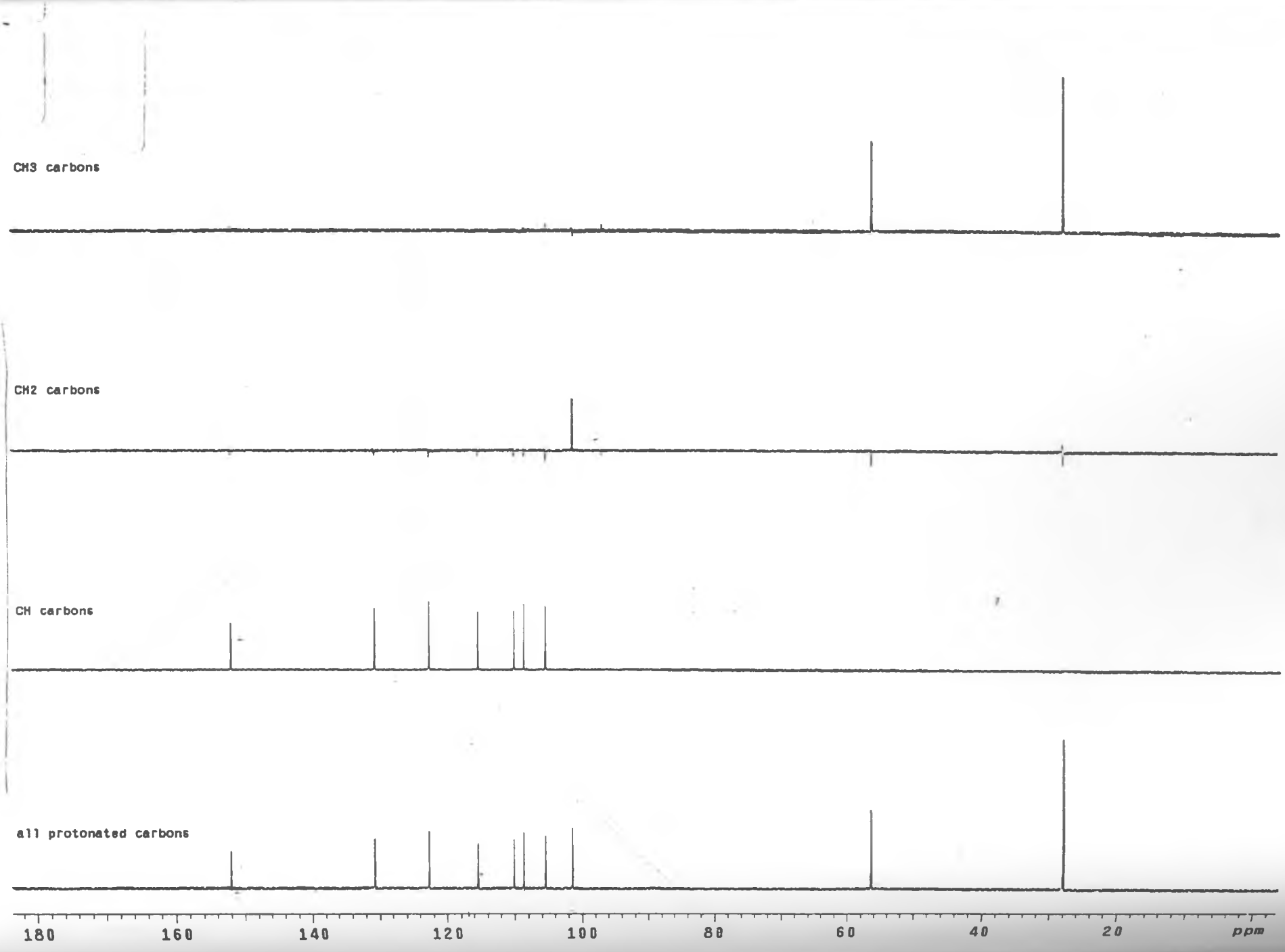


^{13}C NMR SPECTRUM FOR COMPOUND 1 (50 MHz, $CDCl_3$)



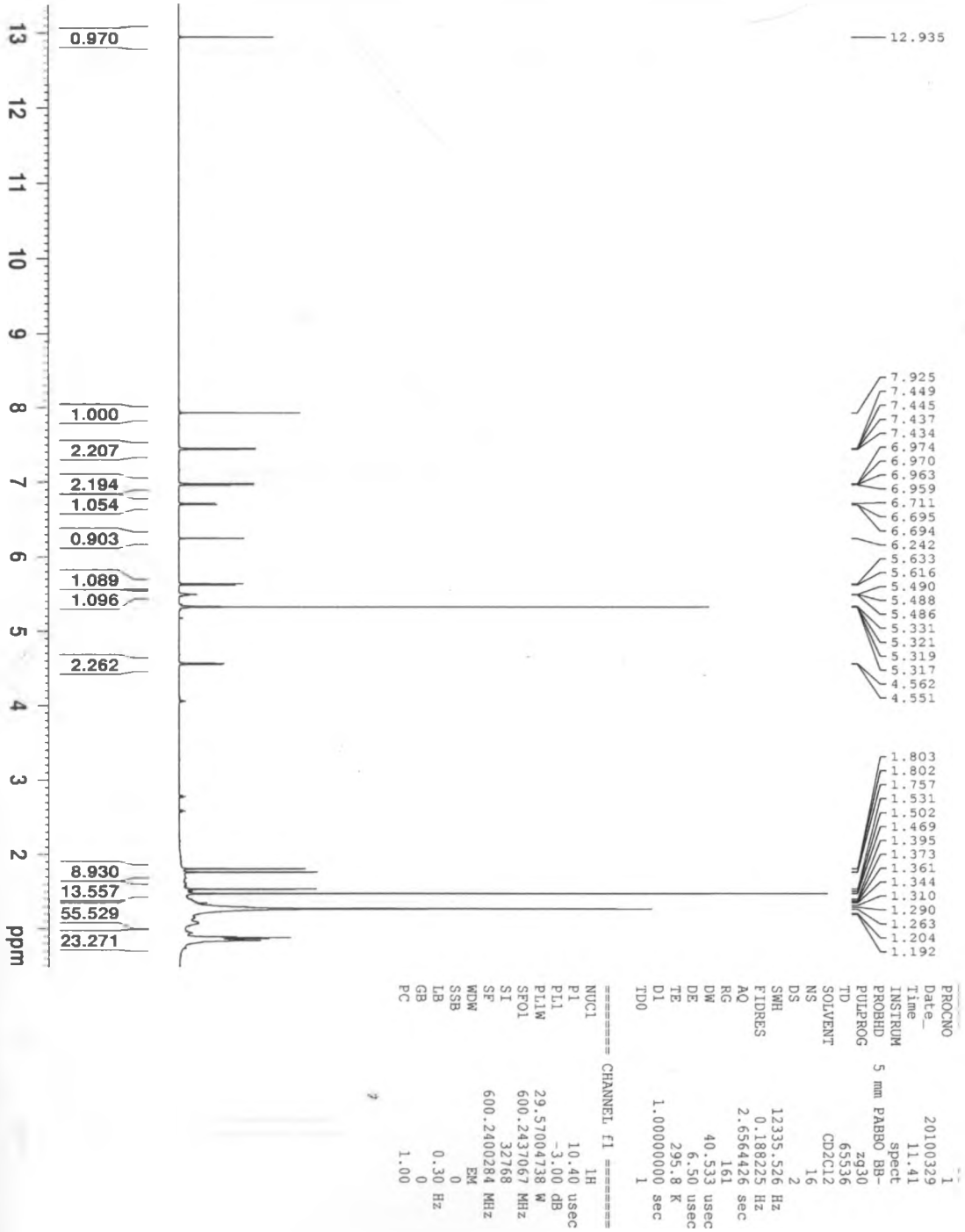

78.422
77.929
77.291
76.654
56.550

DEPT NMR SPECTRUM FOR COMPOUND 1

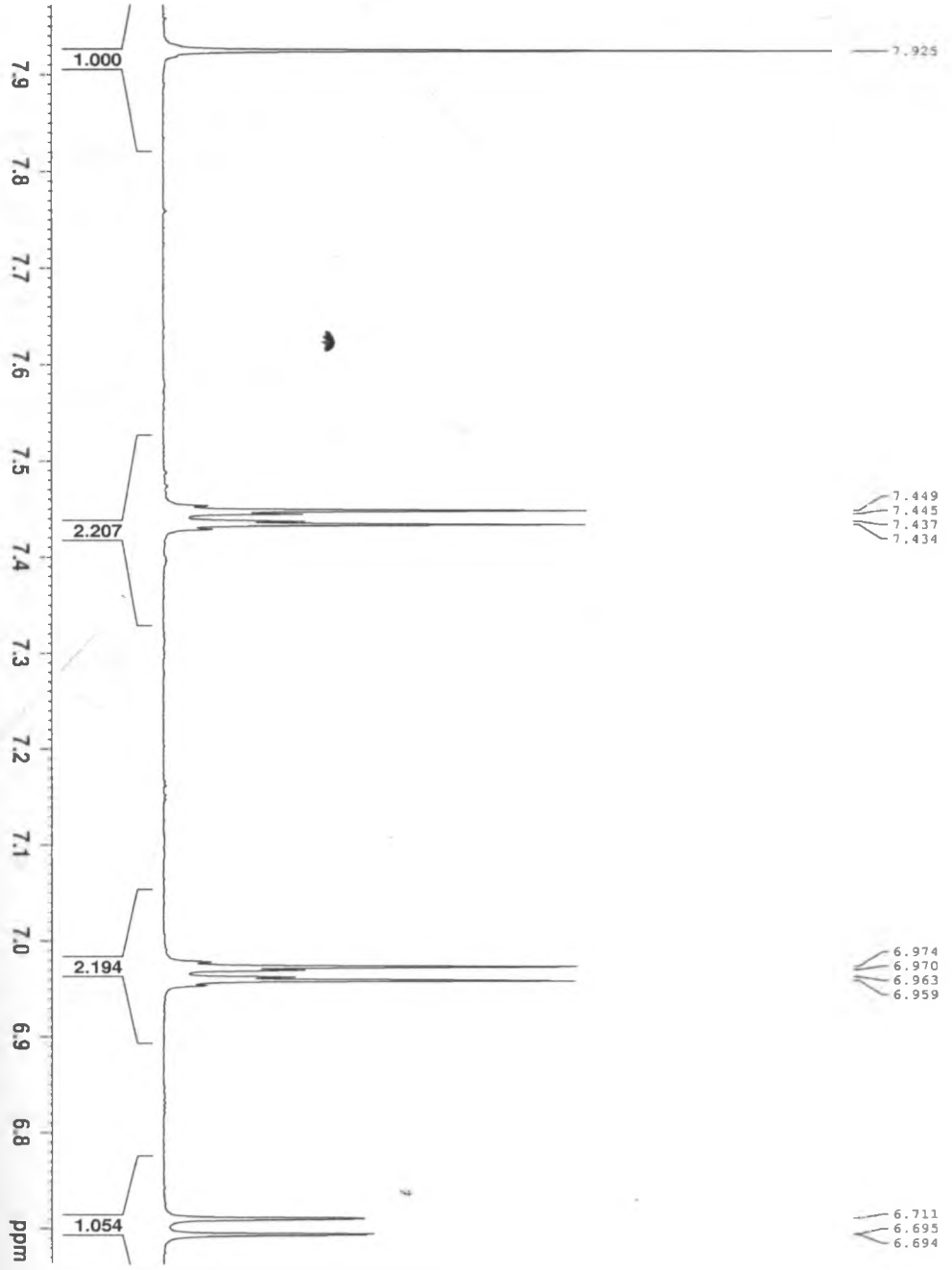


APPENDIX B: SPECTRA FOR COMPOUND **2**

^{1}H NMR SPECTRUM FOR COMPOUND 2 (600 MHz, CD_2Cl_2)



^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 2



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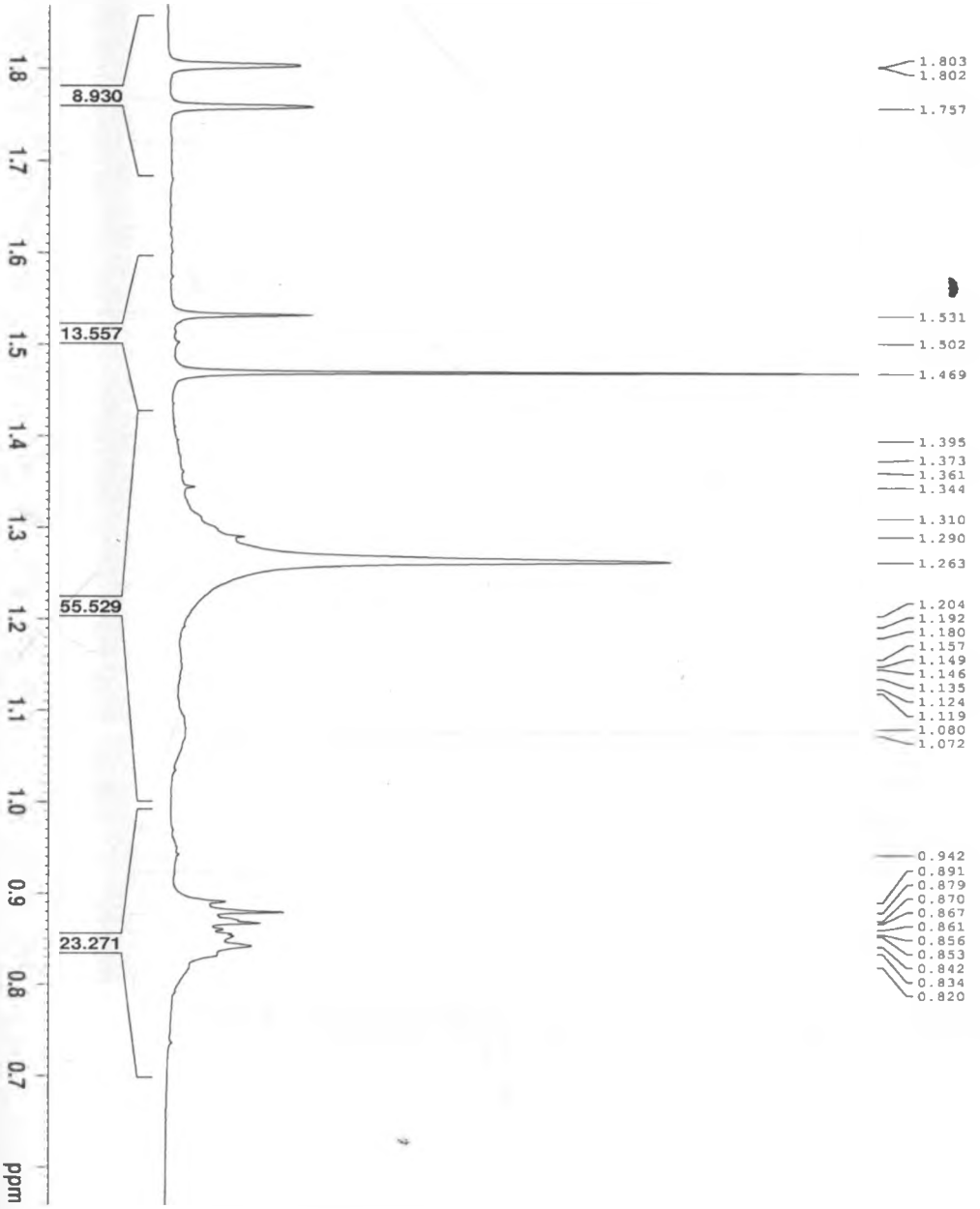
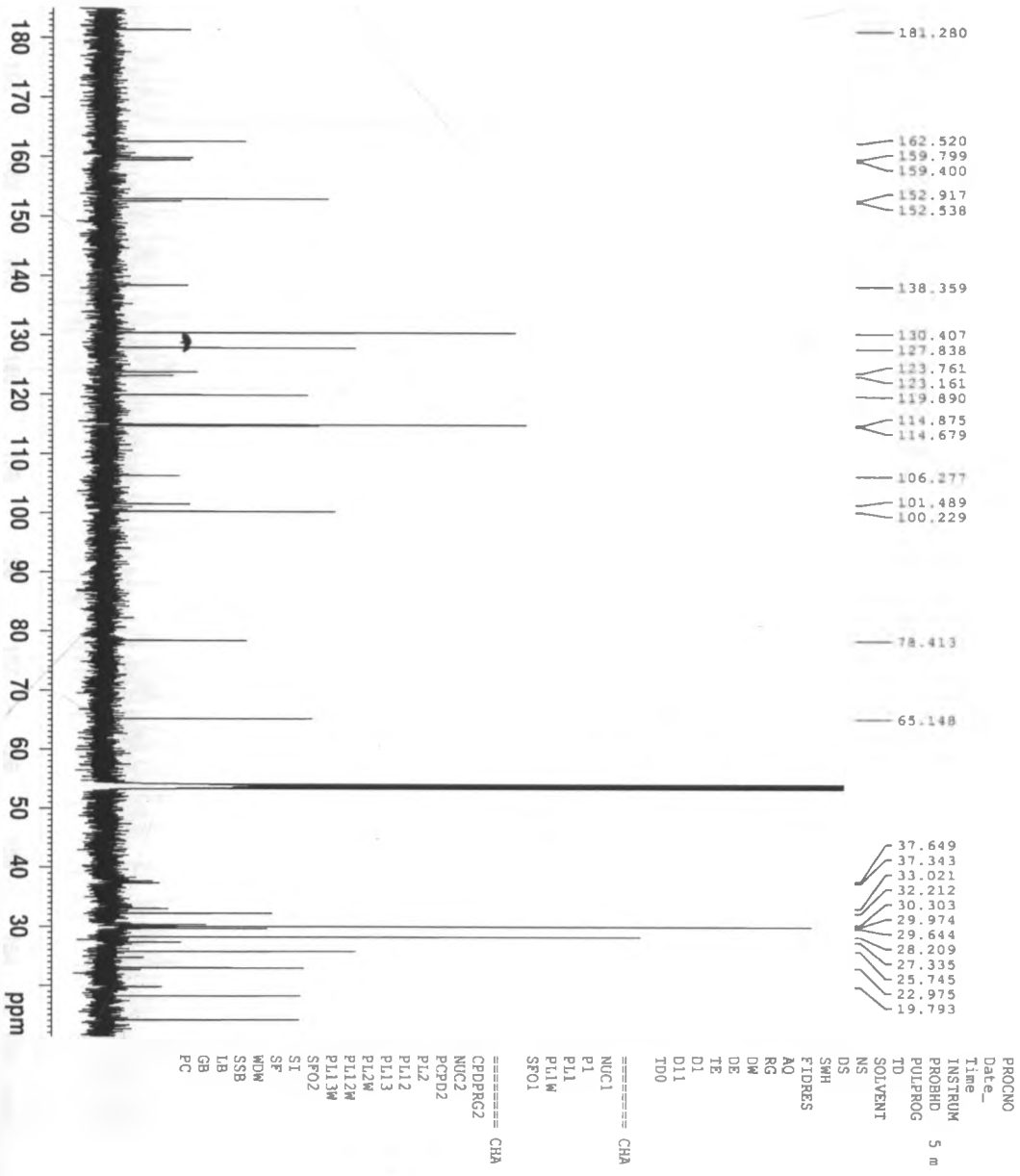
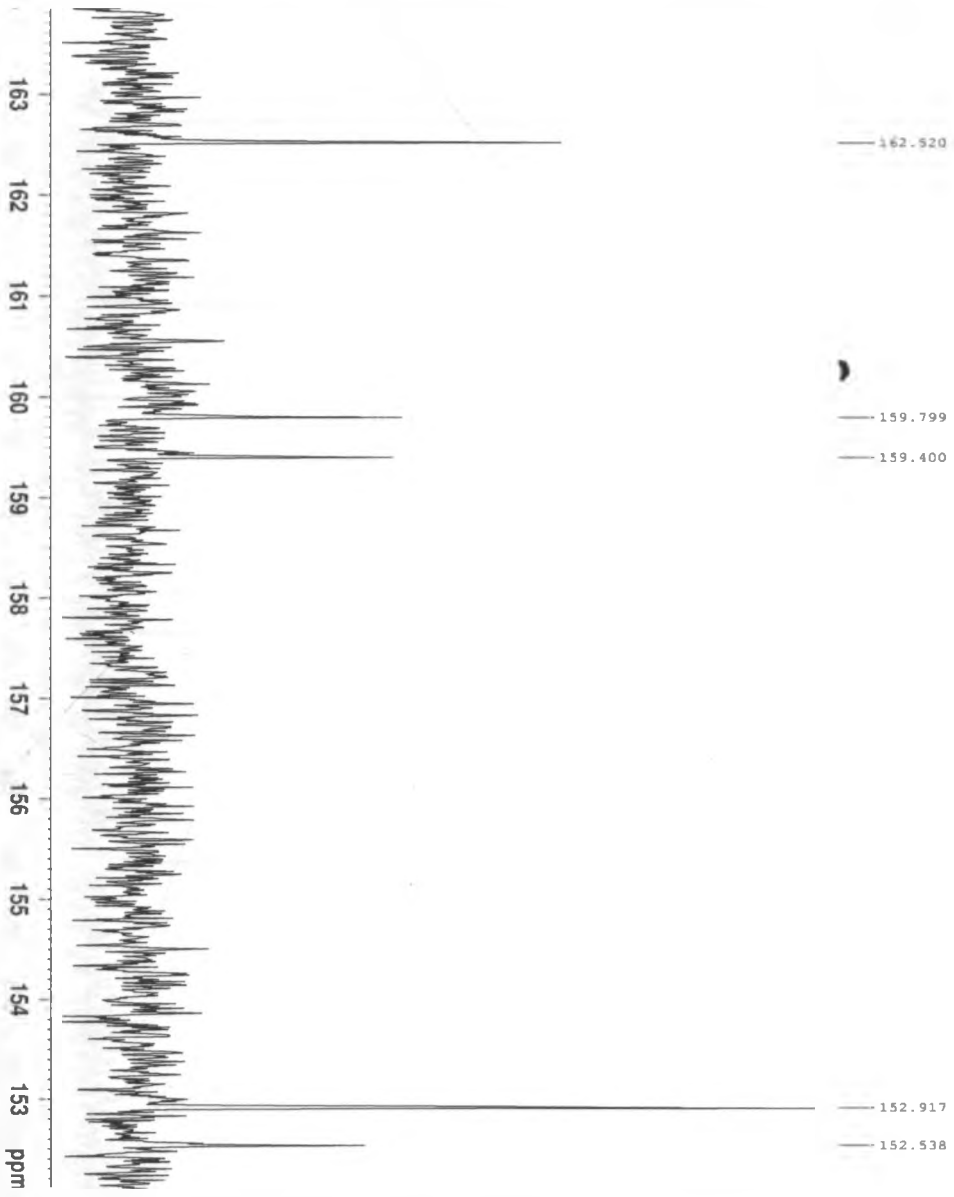


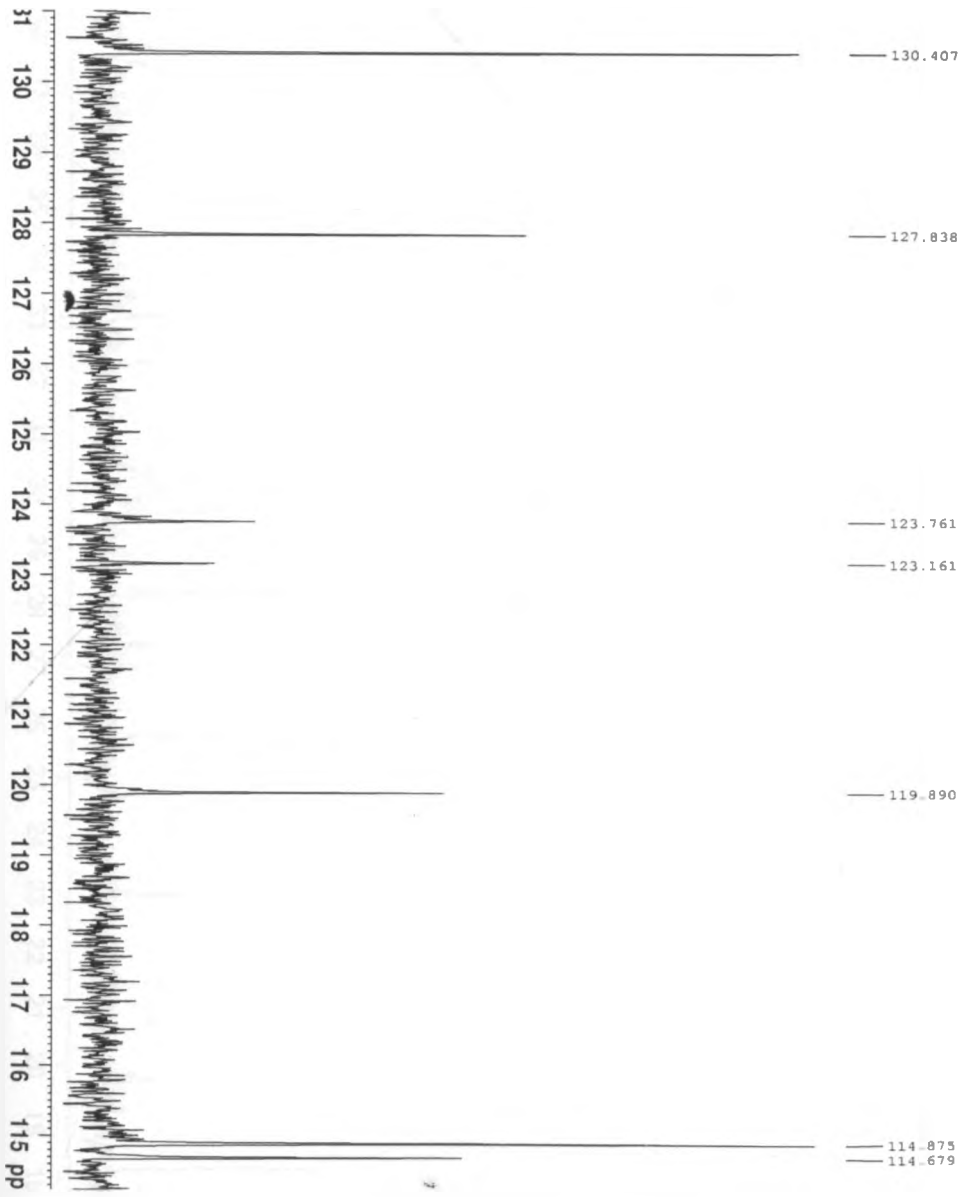
^{13}C NMR SPECTRUM FOR COMPOUND 2 (150 MHz, CD_2Cl_2)



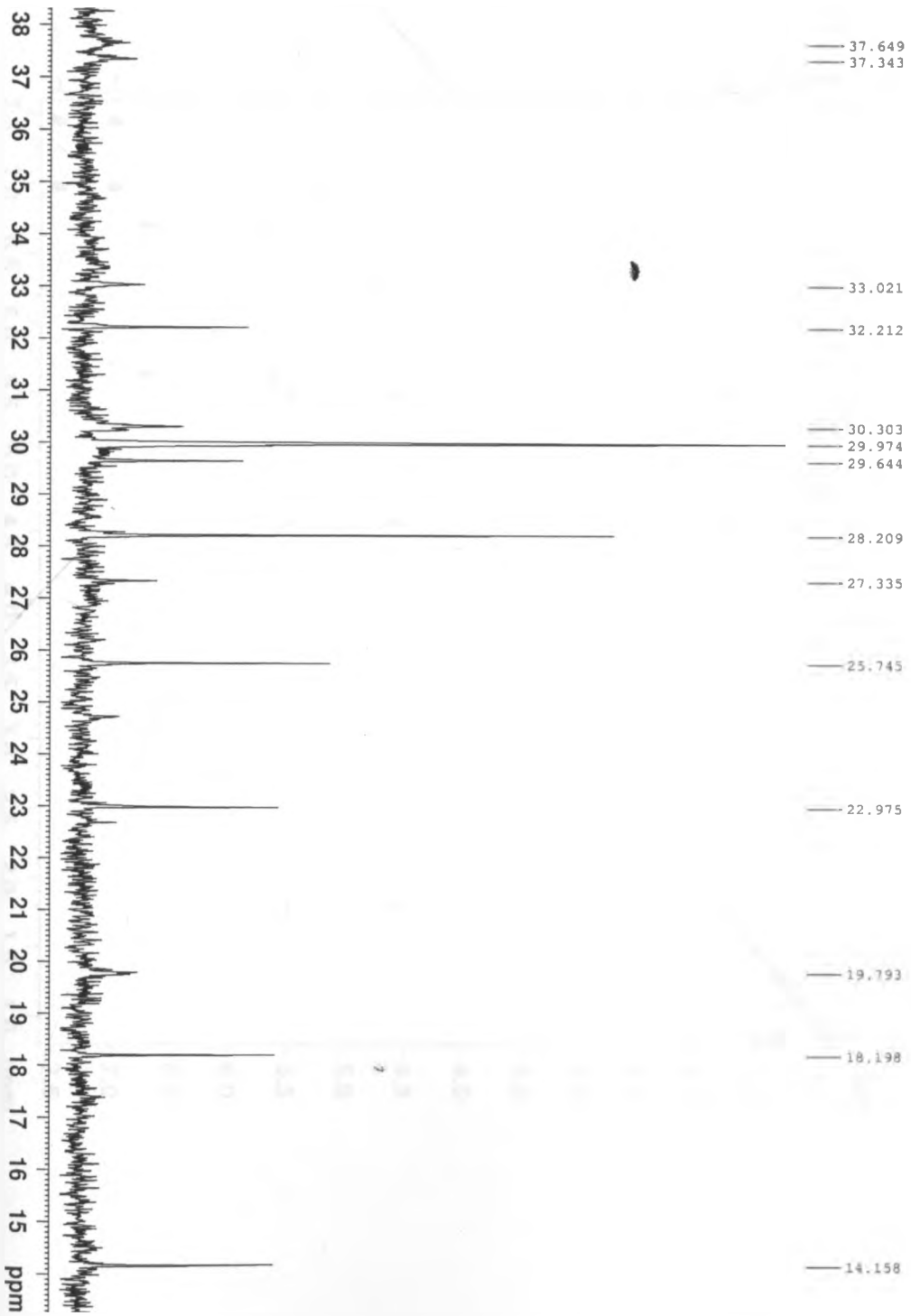
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 2



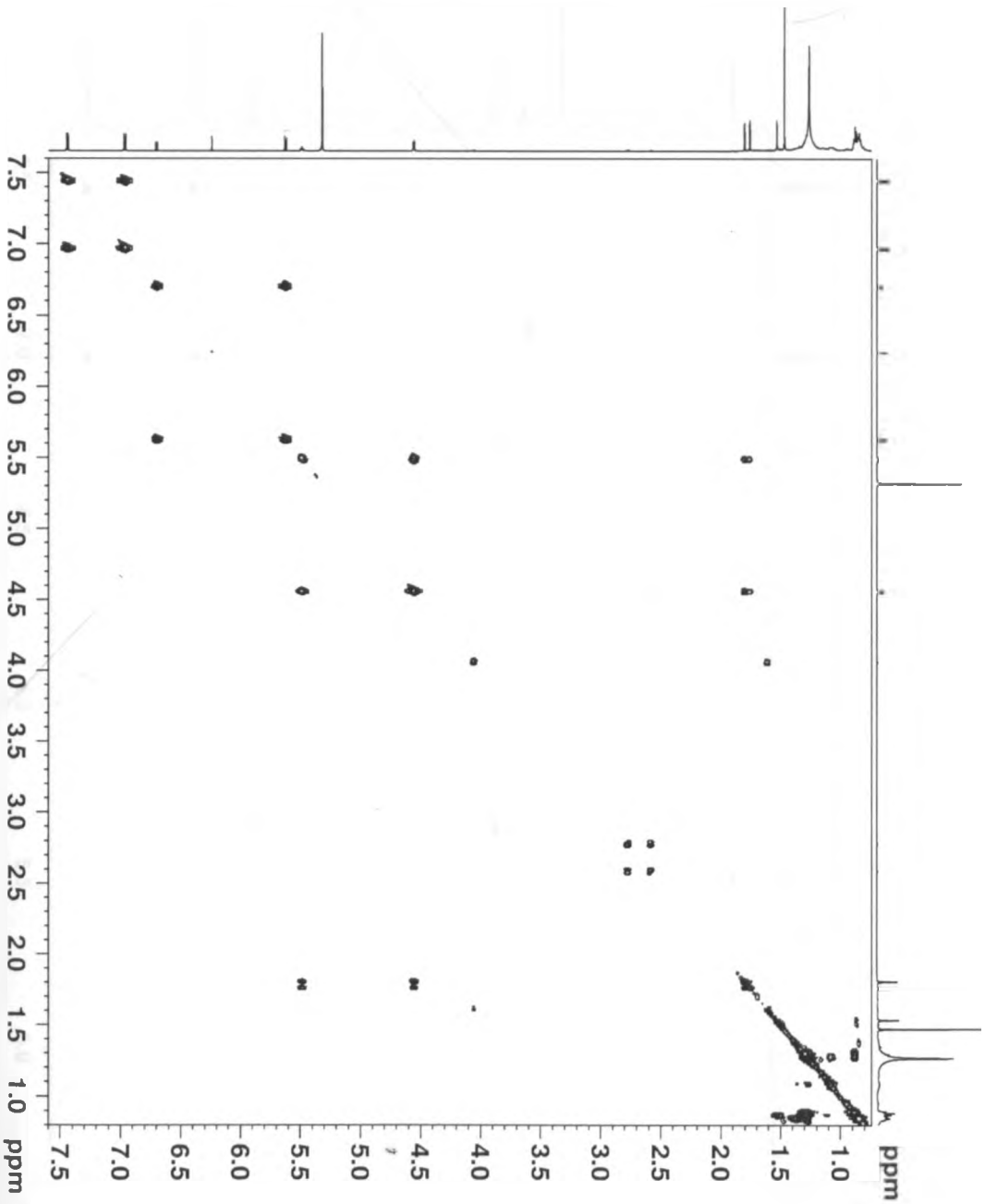
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 2



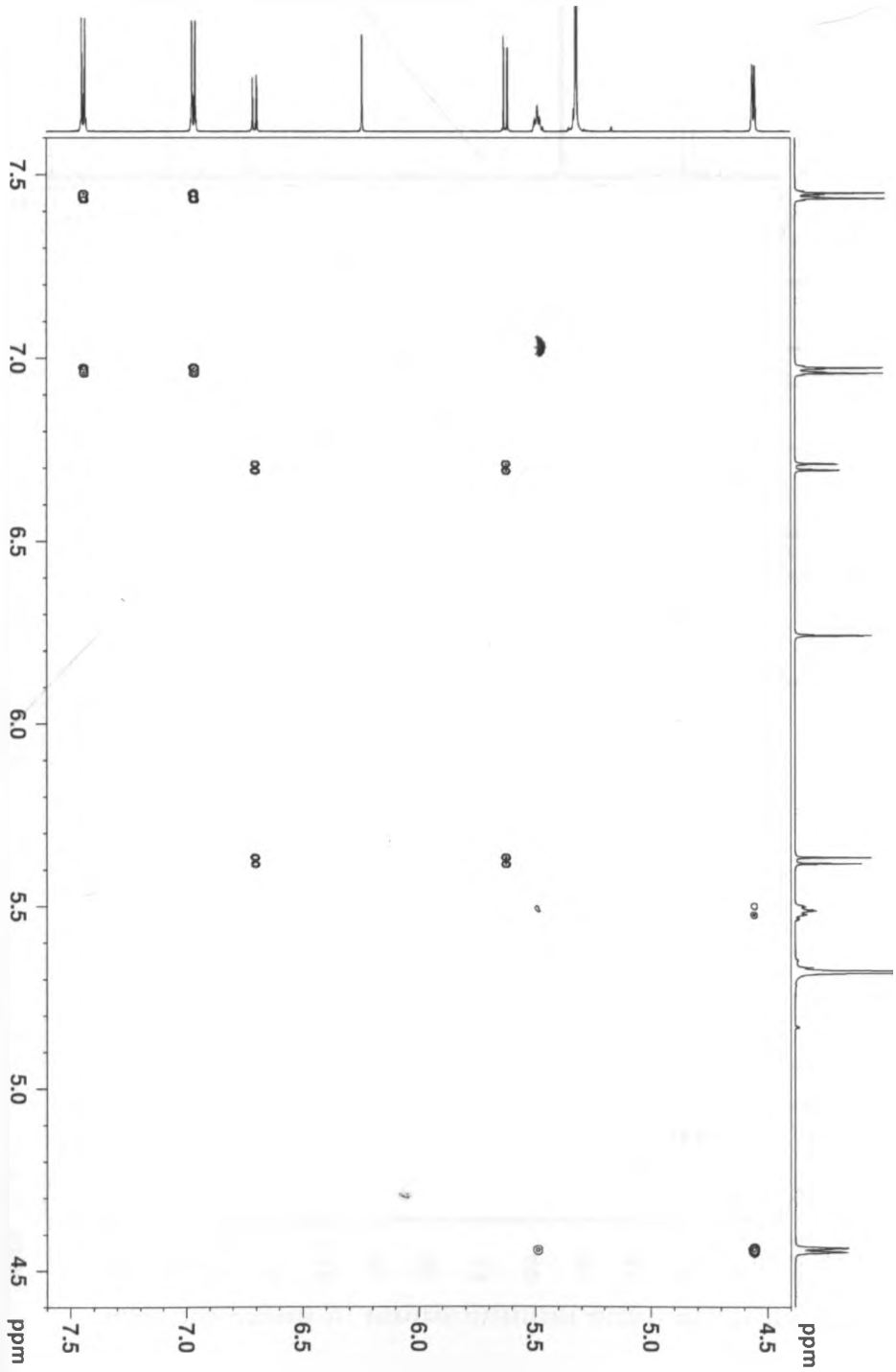
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 2



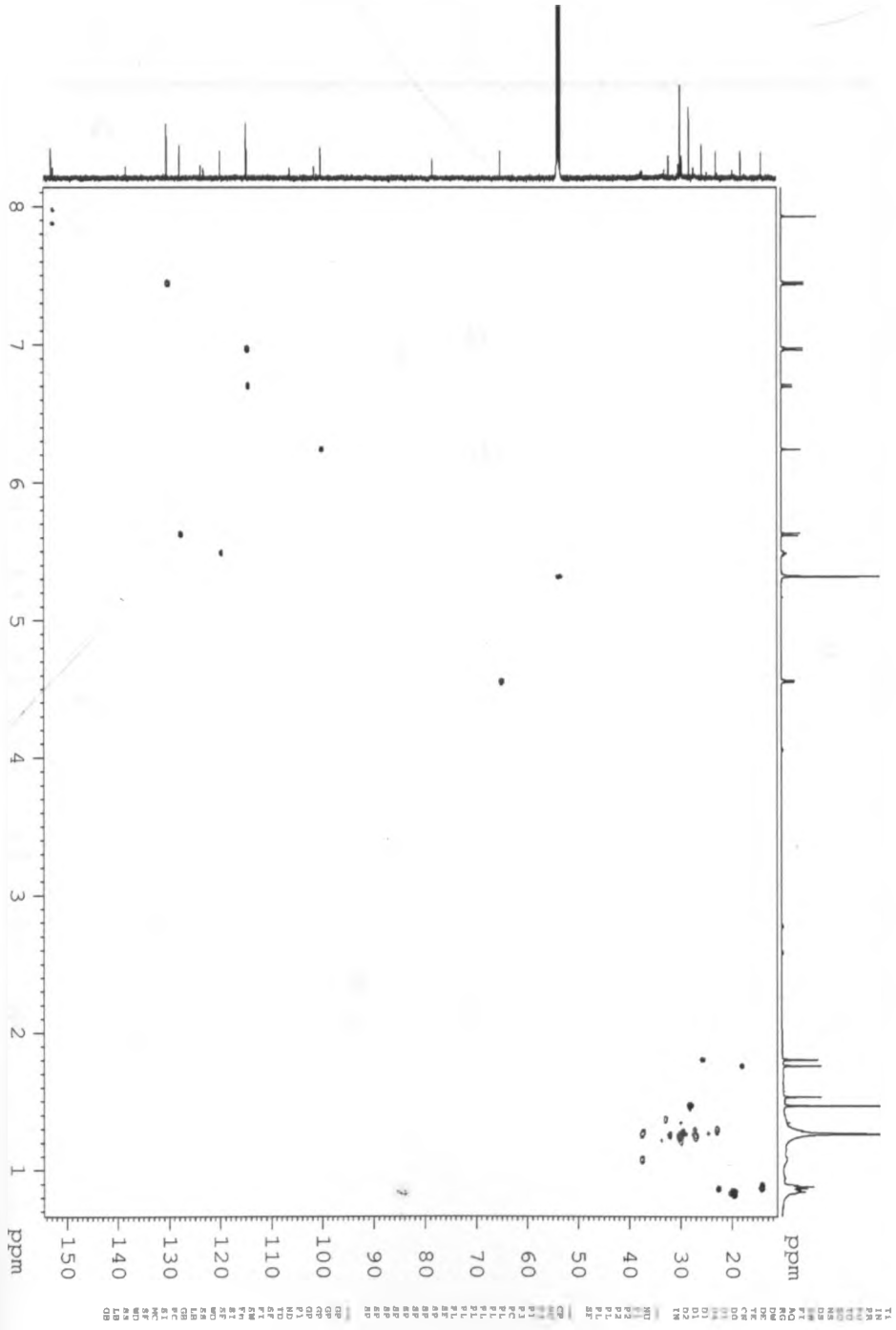
$^{1}H - {}^{1}H$ COSY SPECTRUM FOR COMPOUND 2



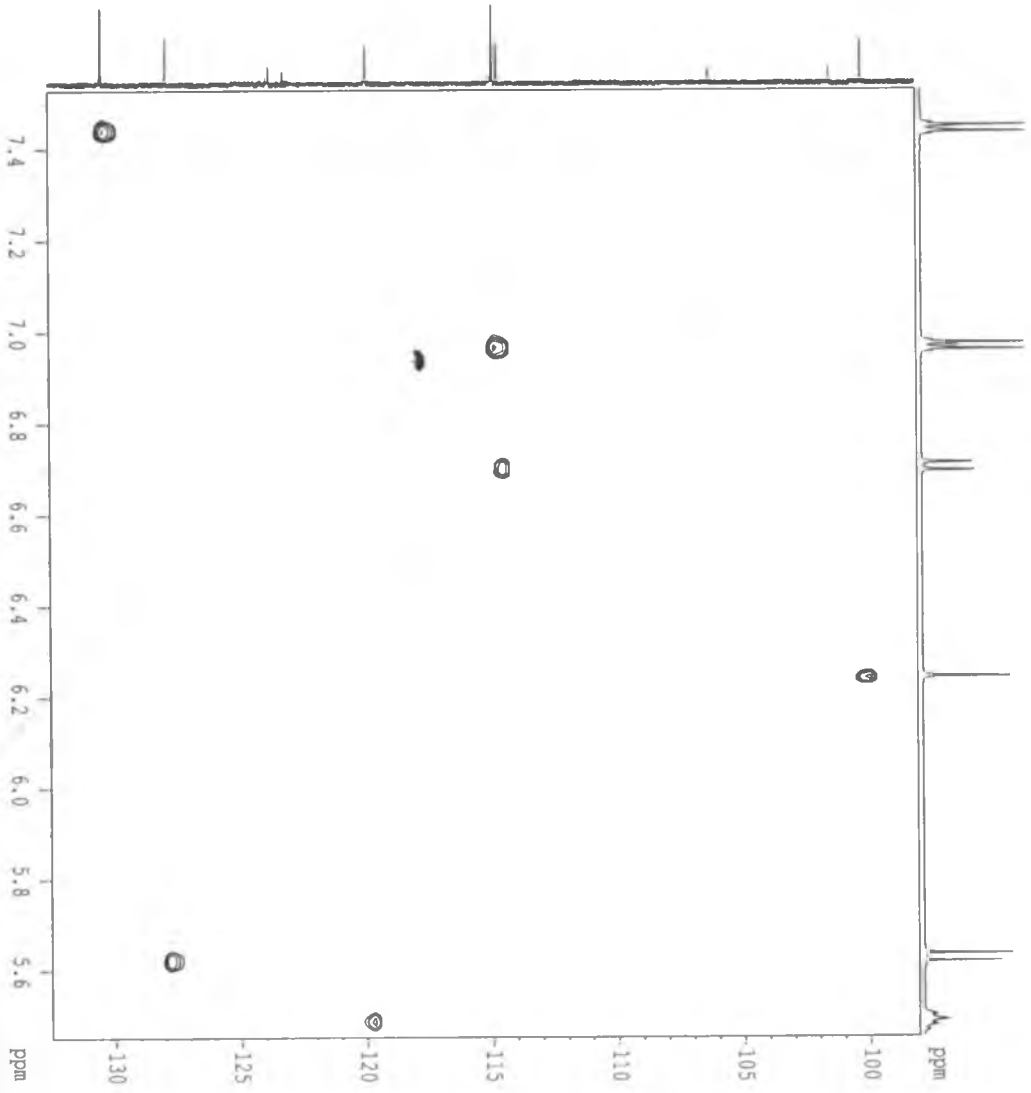
$^{1}H - {}^{1}H$ COSY SPECTRUM EXPANSION FOR COMPOUND 2



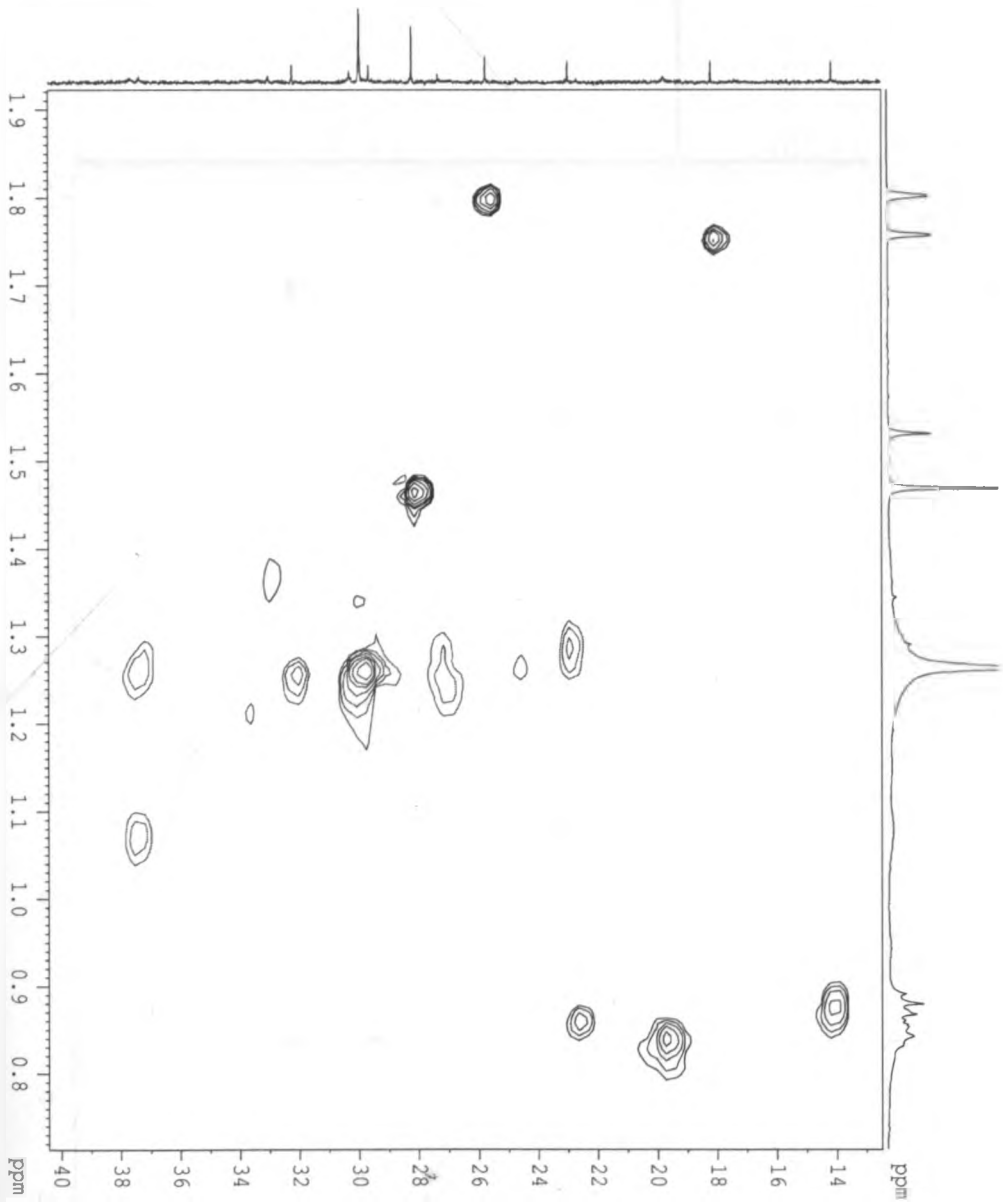
HMQC SPECTRUM FOR COMPOUND 2



HMQC SPECTRUM EXPANSION FOR COMPOUND 2



HMQC SPECTRUM EXPANSION FOR COMPOUND 2



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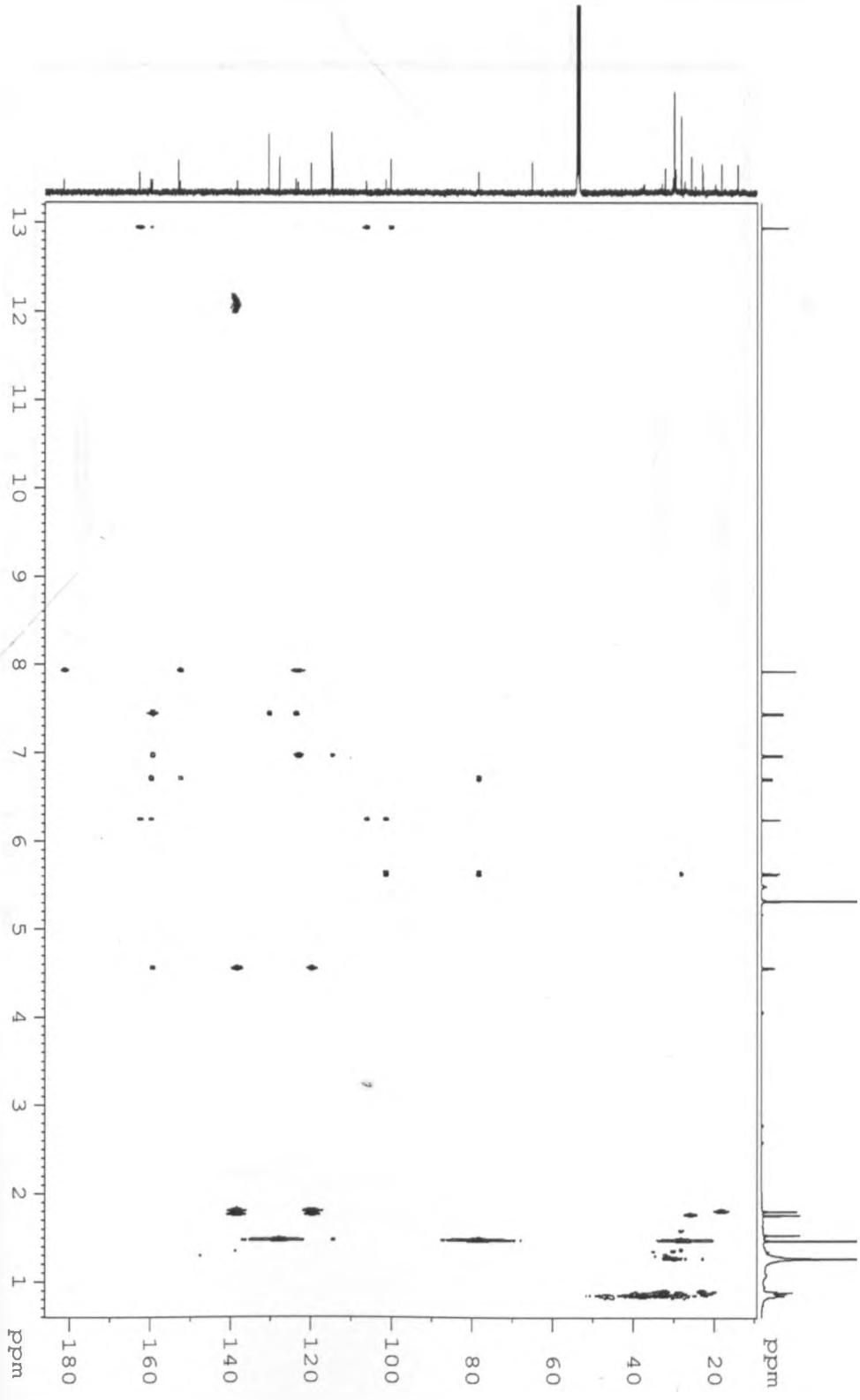

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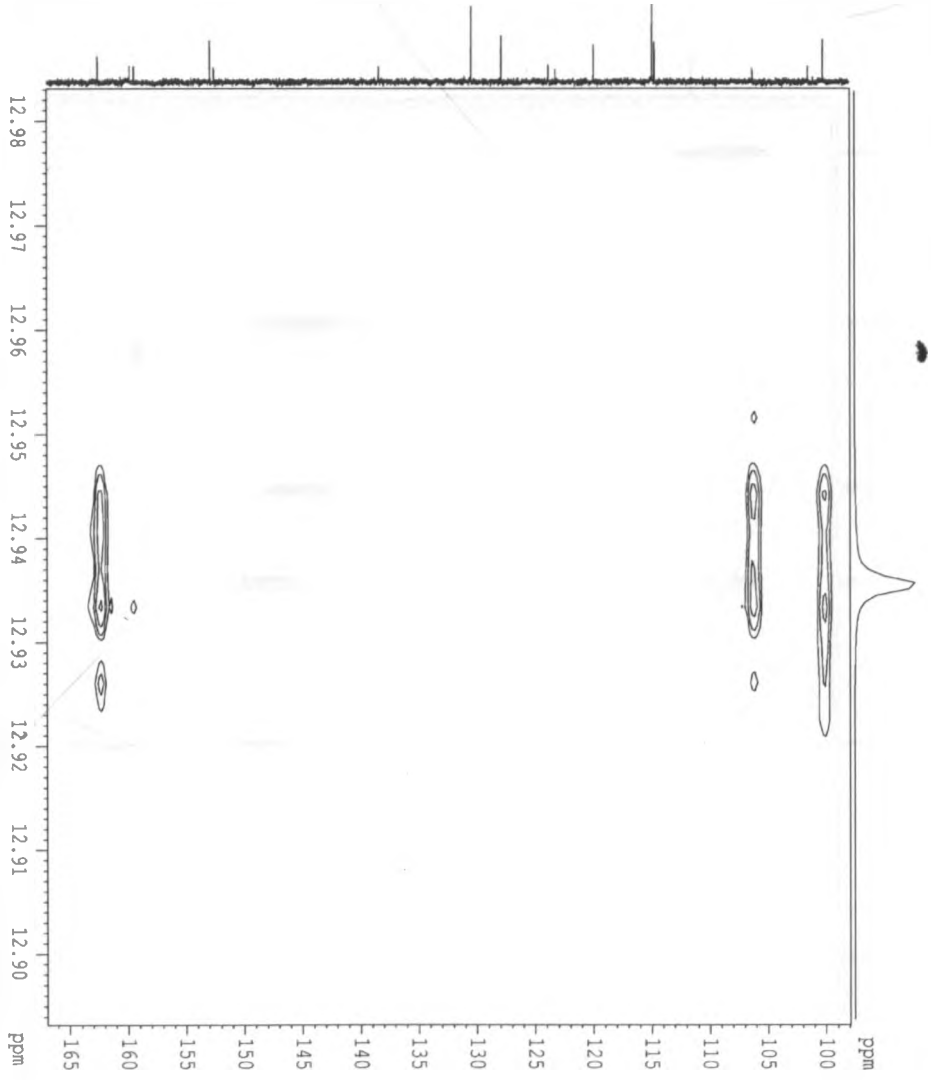

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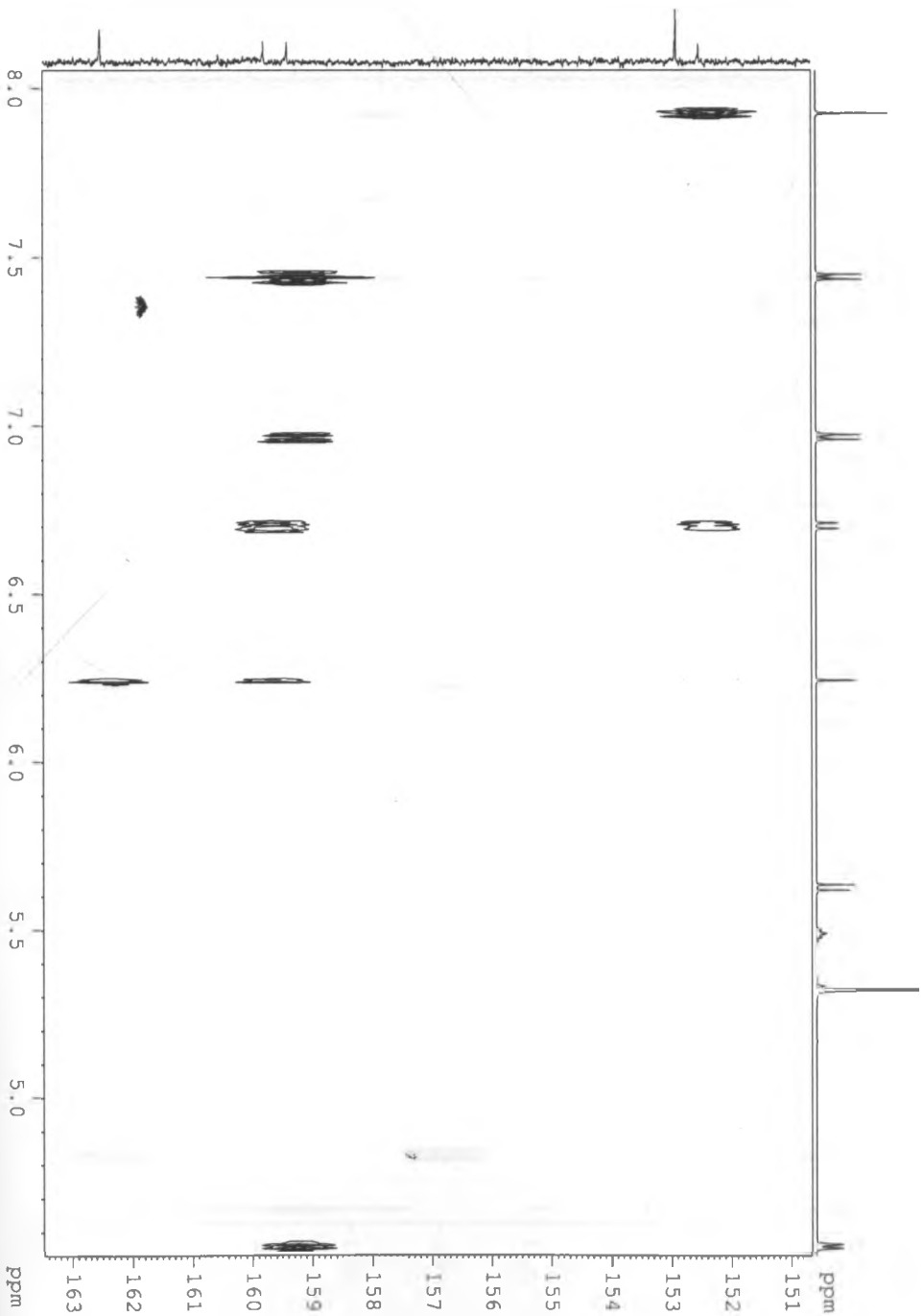

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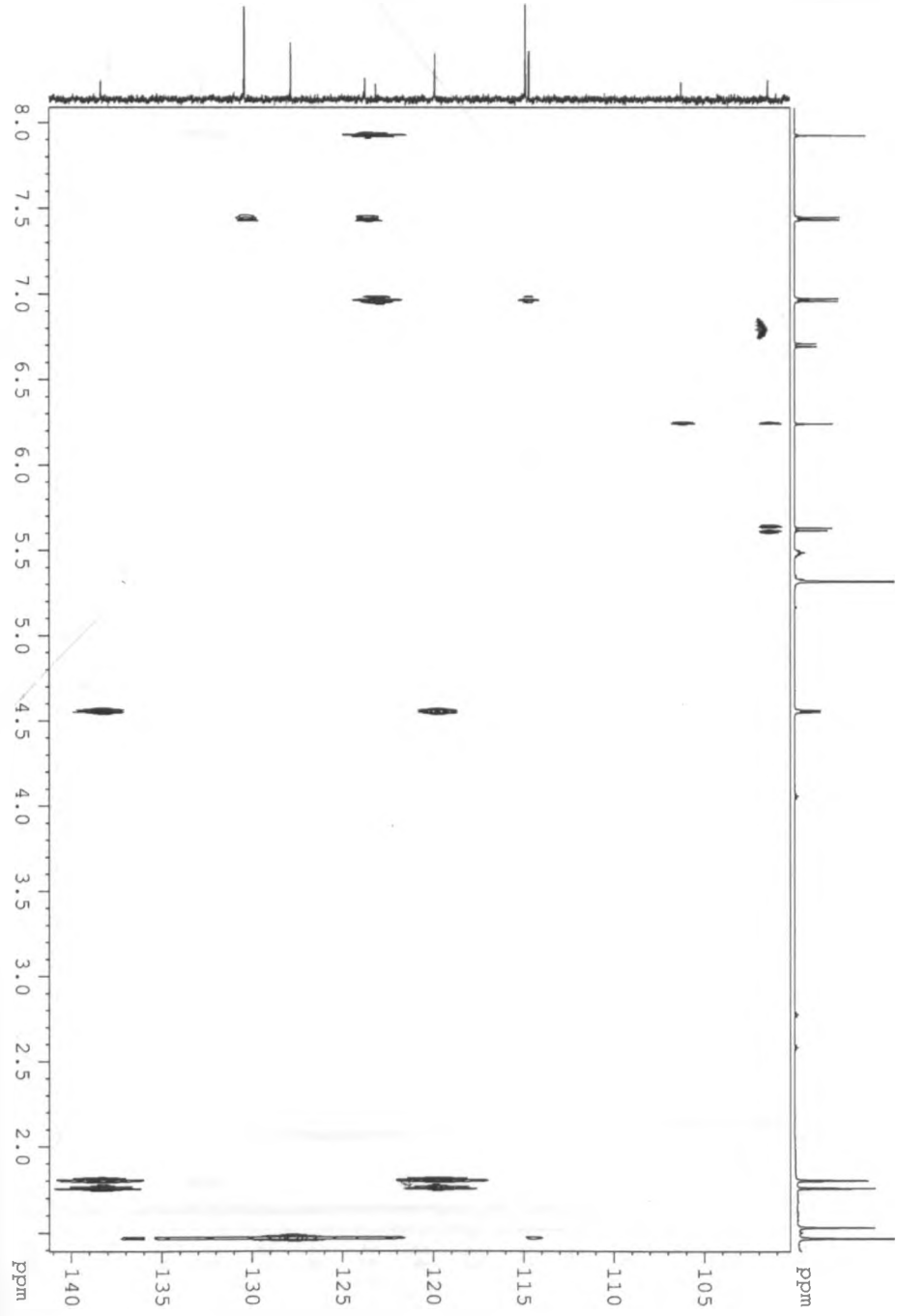

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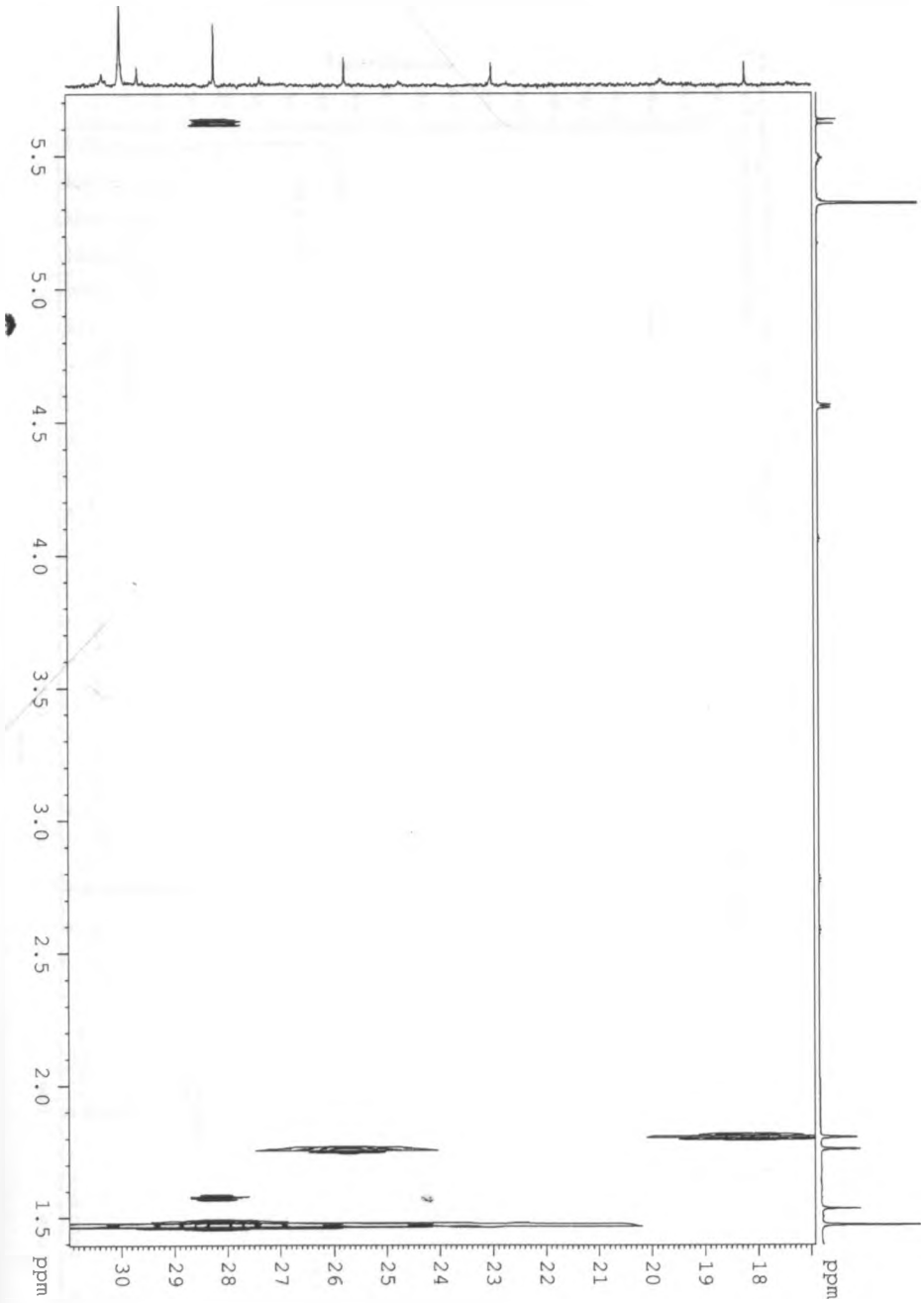
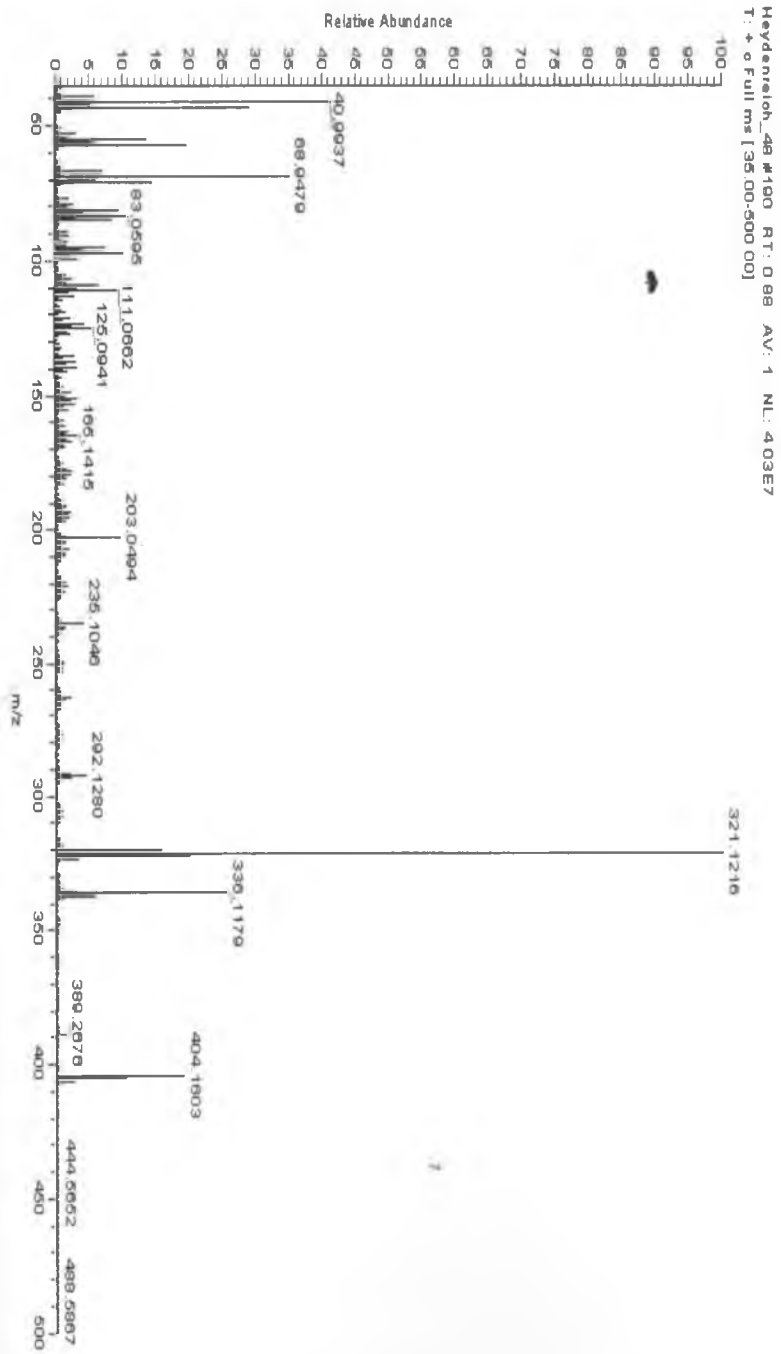
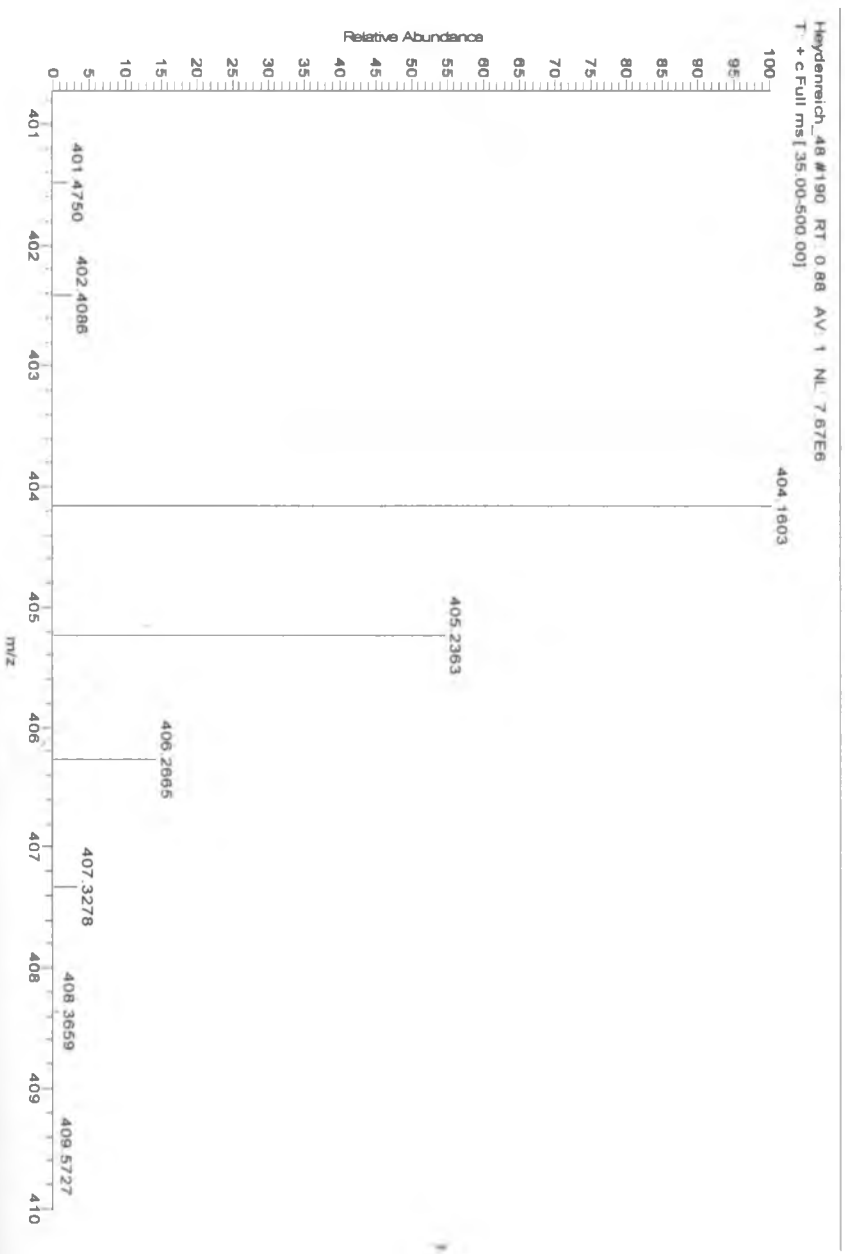


EI-MS SPECTRUM FOR COMPOUND 2

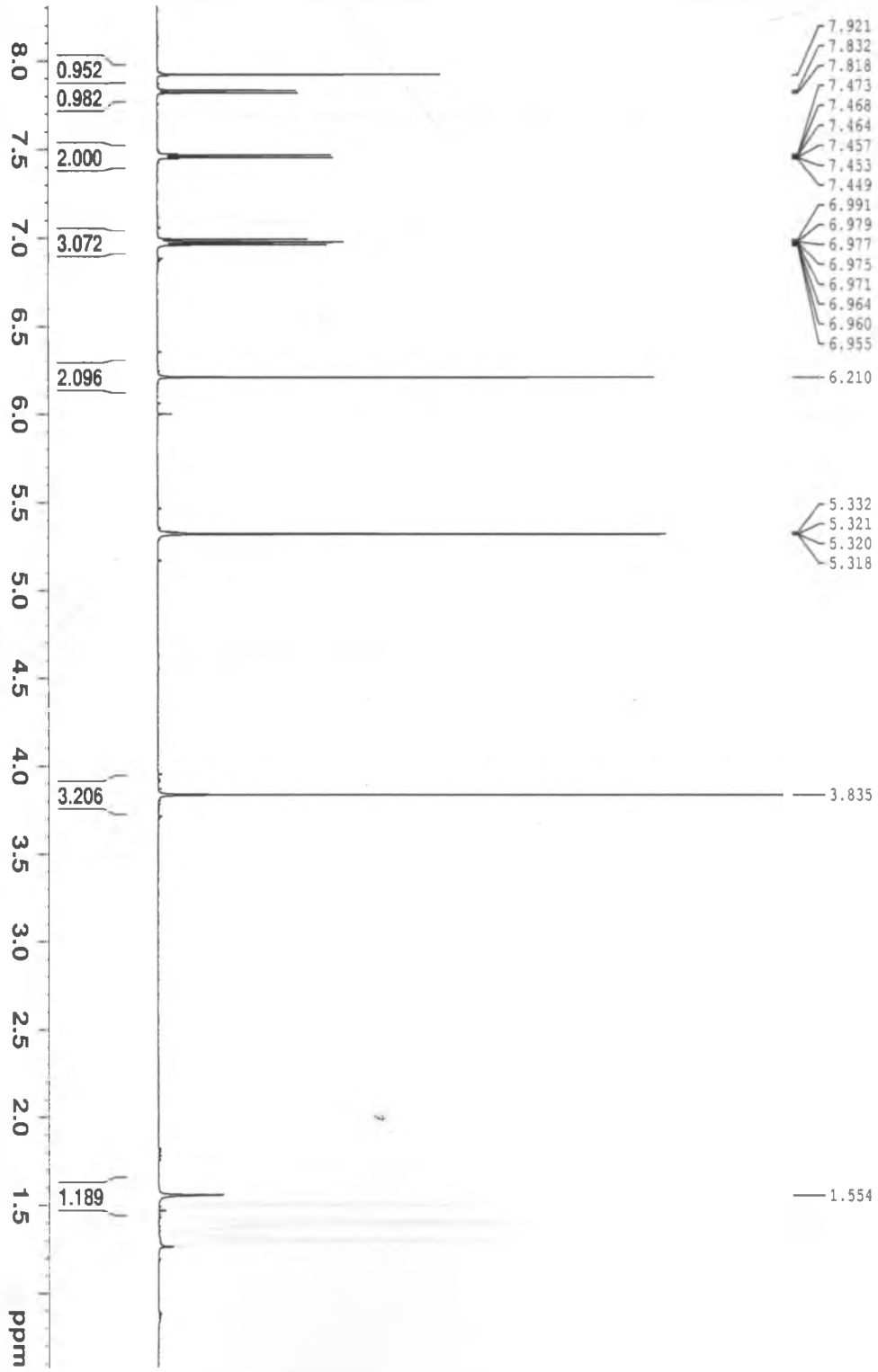


HR-MS SPECTRUM FOR COMPOUND 2

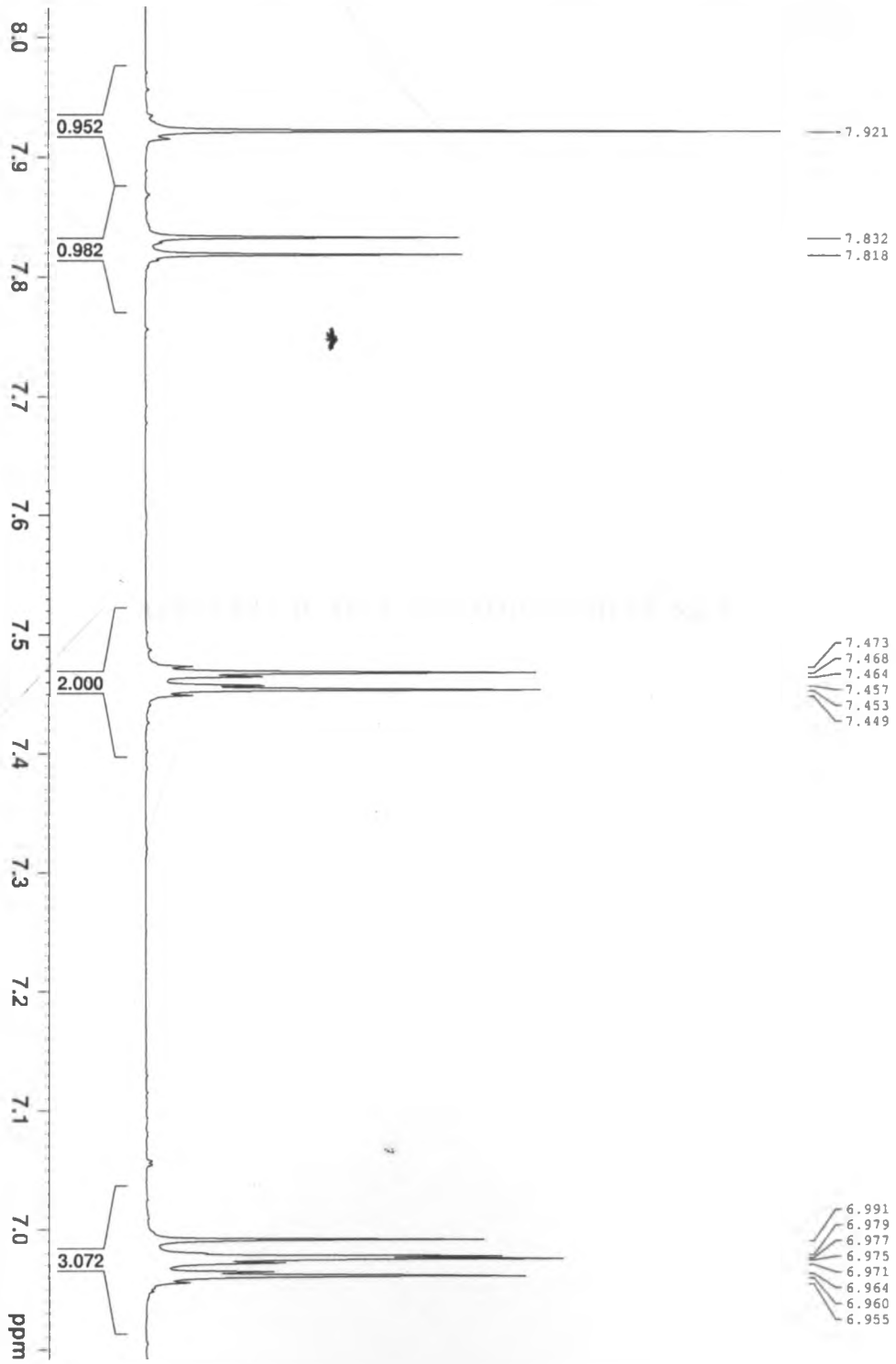


APPENDIX C: SPECTRA FOR COMPOUND 3

^{1}H NMR SPECTRUM FOR COMPOUND 3 (600 MHz, CD_2Cl_2)

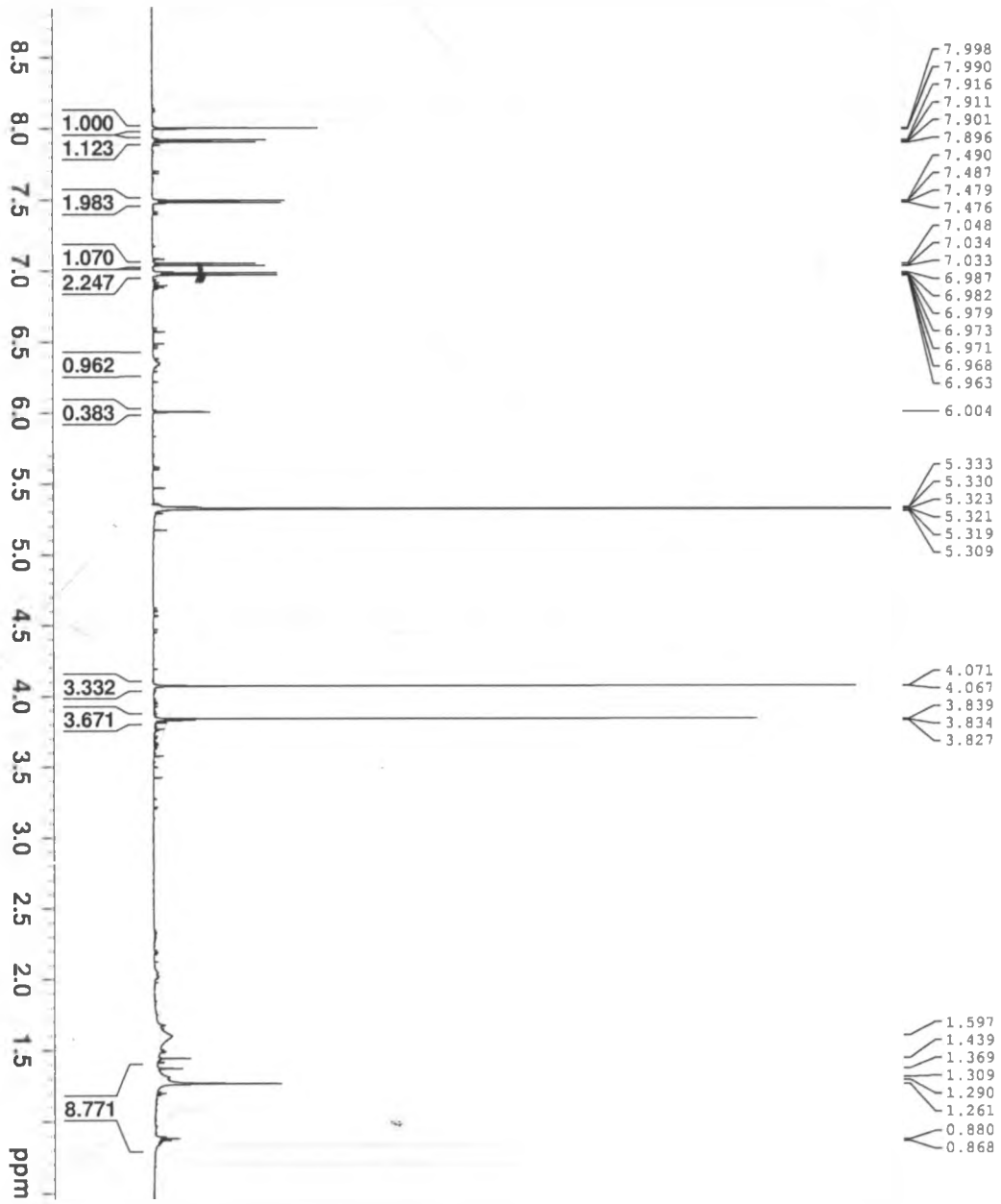


^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 3

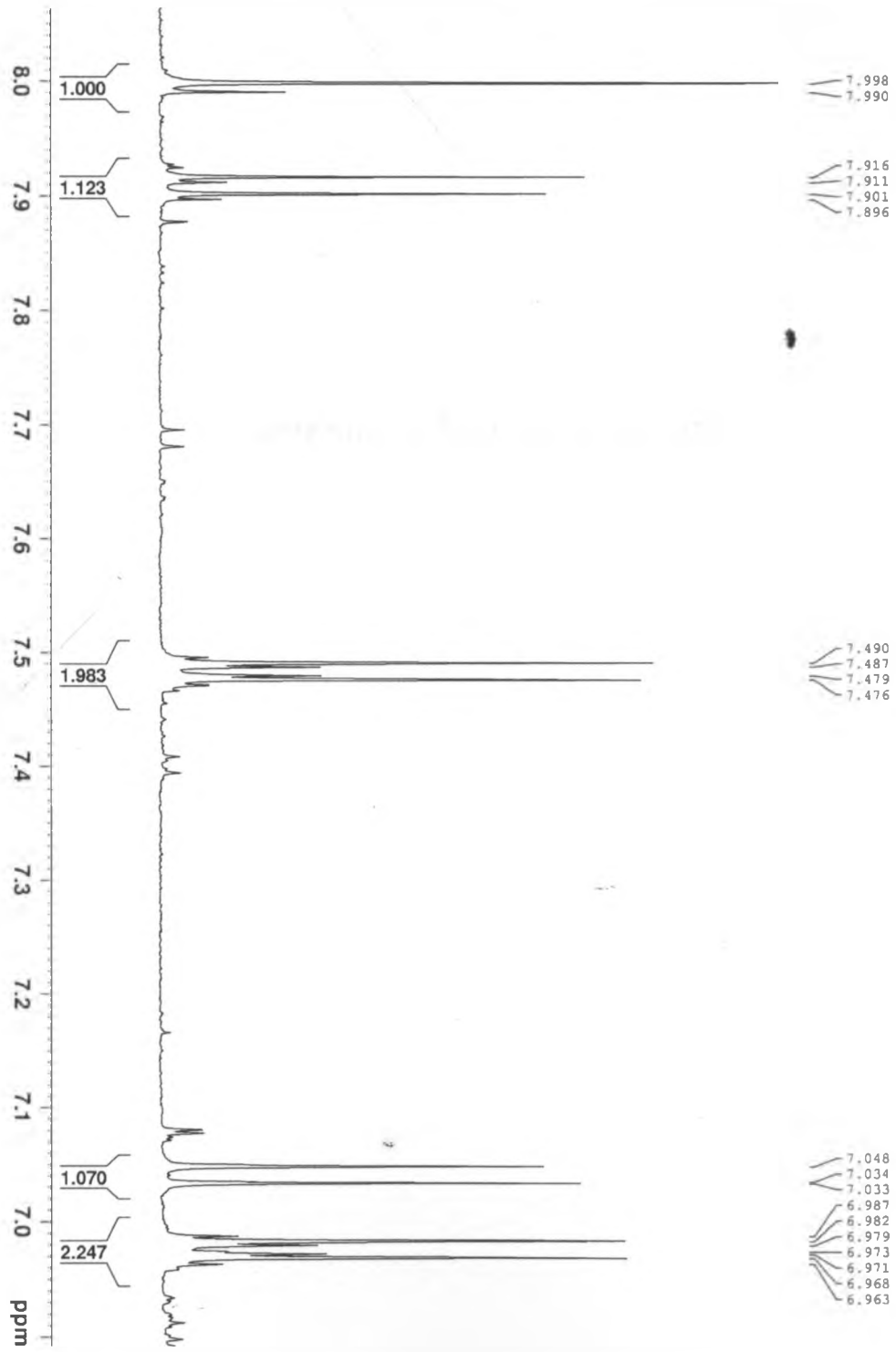


APPENDIX D: SPECTRA FOR COMPOUND 4

^{1}H NMR SPECTRUM FOR COMPOUND 4 (600 MHz, CD_2Cl_2)

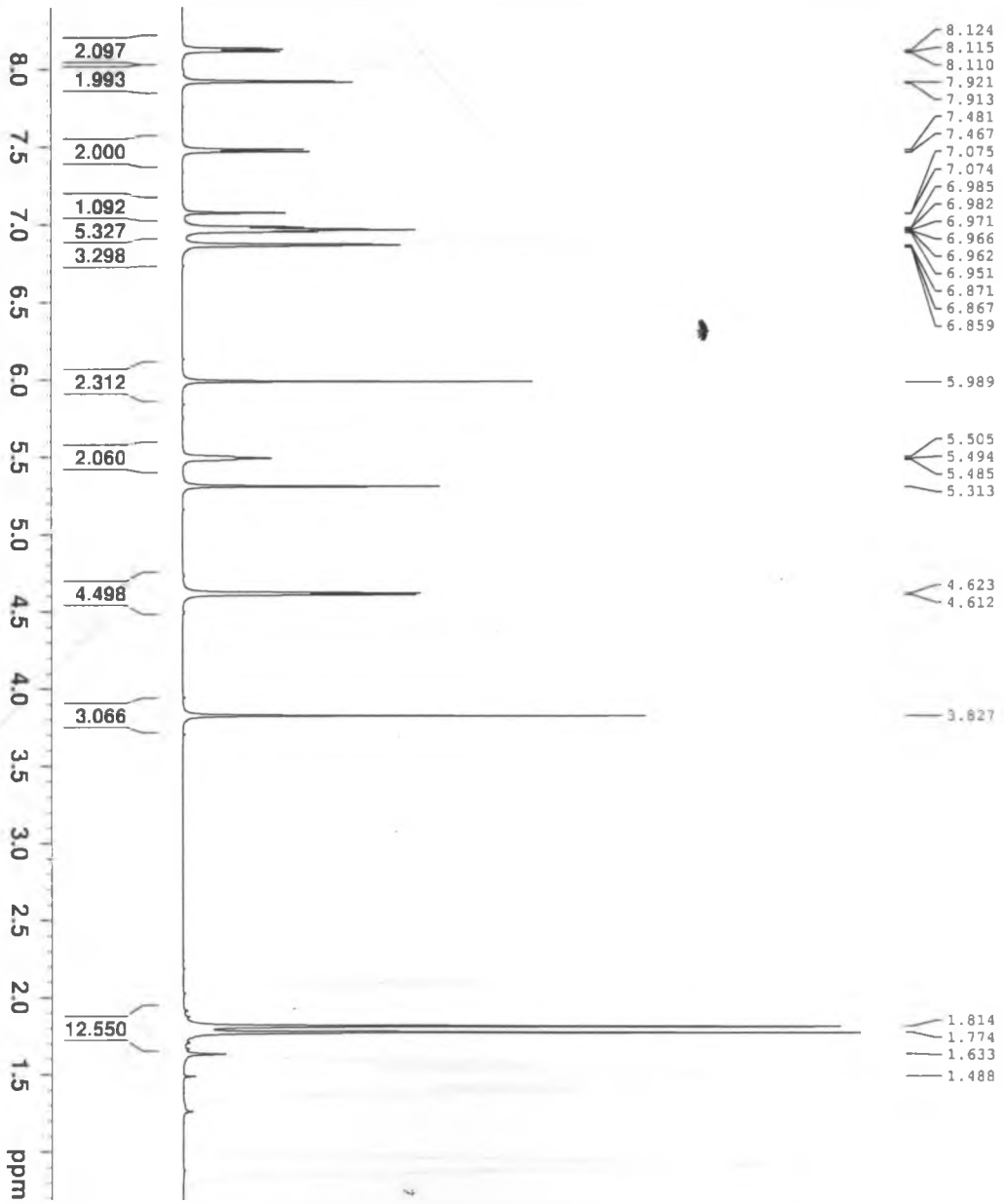


^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 4

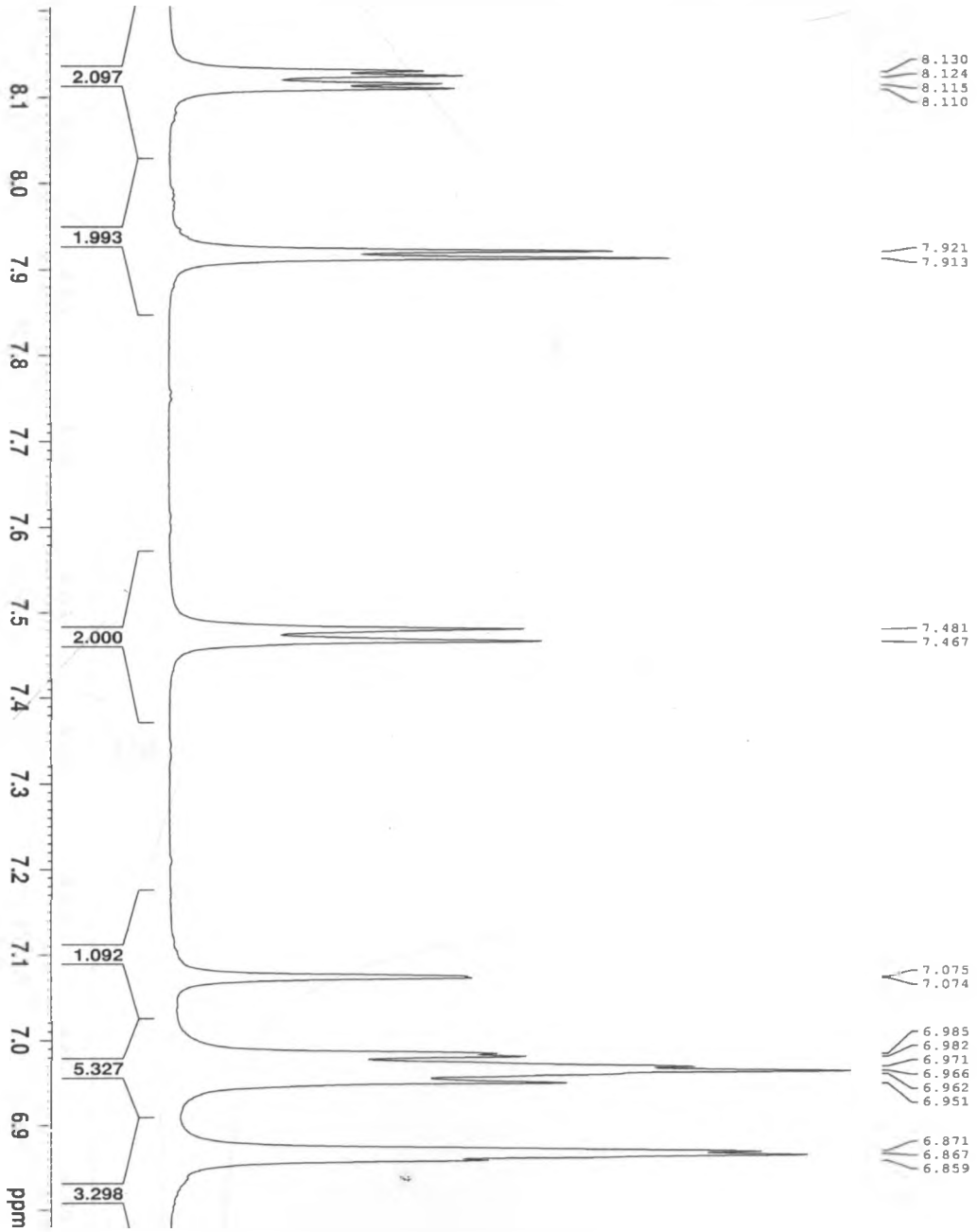


APPENDIX E: SPECTRA FOR COMPOUND 5 AND 6

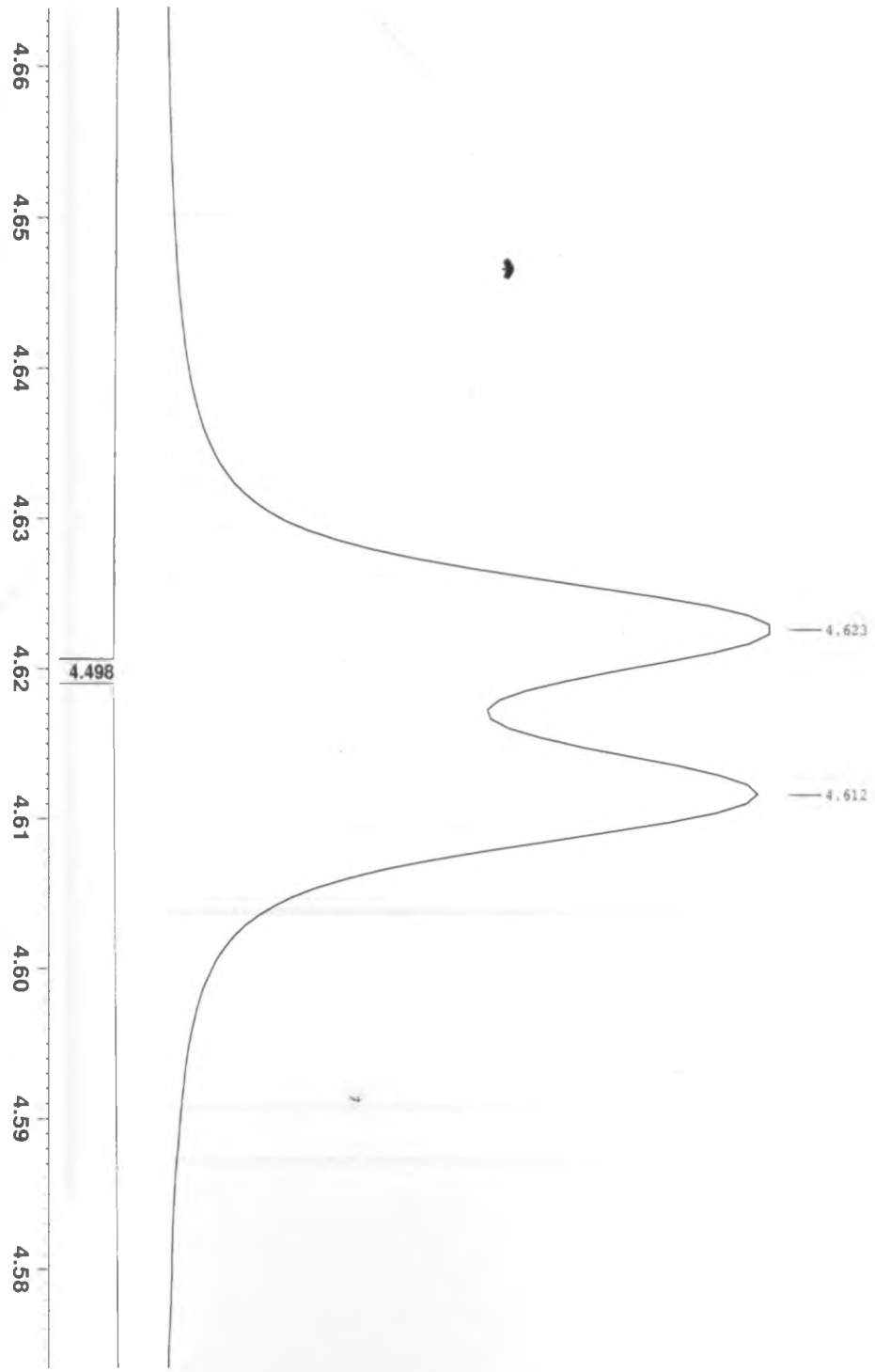
^{1}H NMR SPECTRUM FOR COMPOUND 5 AND 6 (600 MHz, CD_2Cl_2)



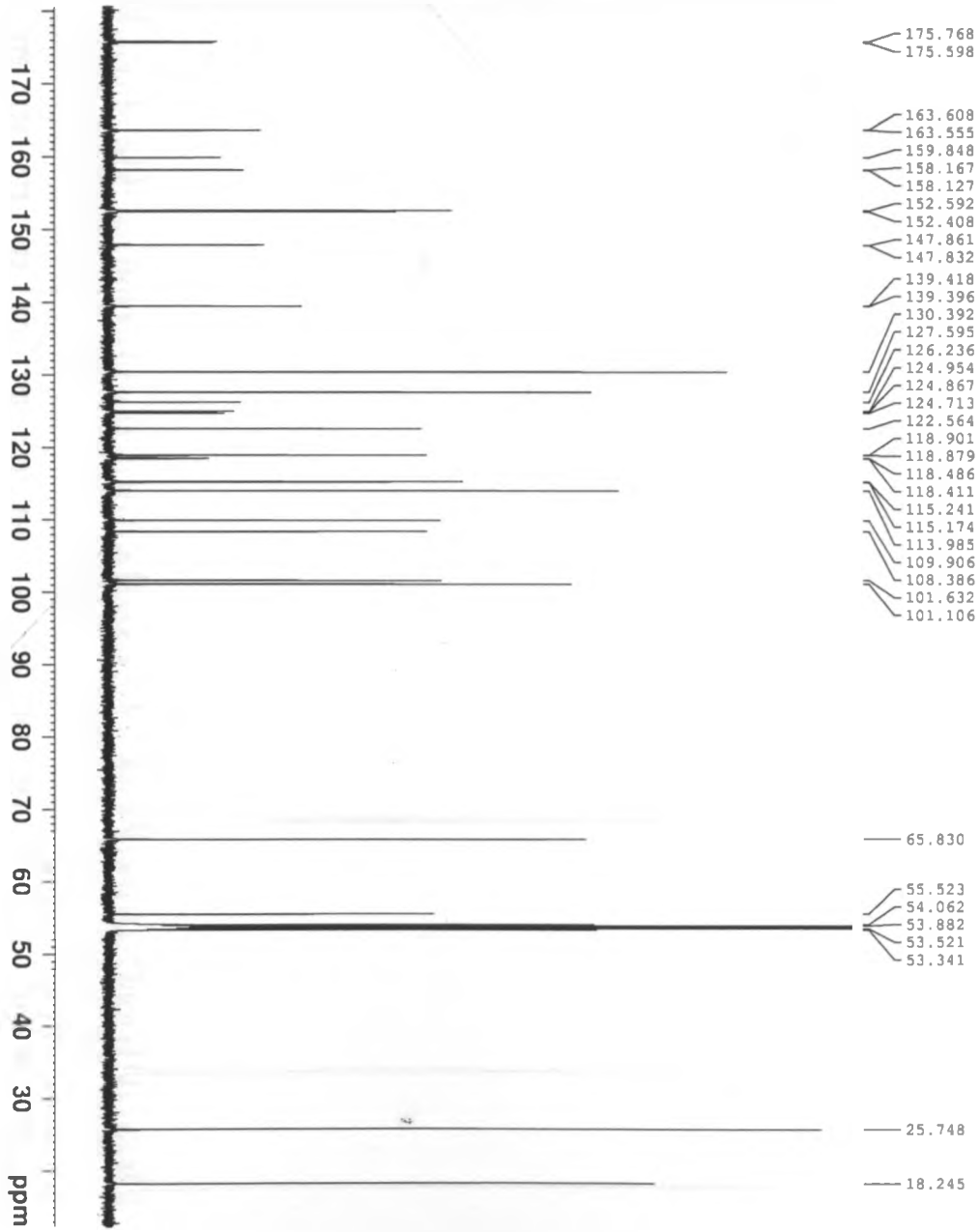
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



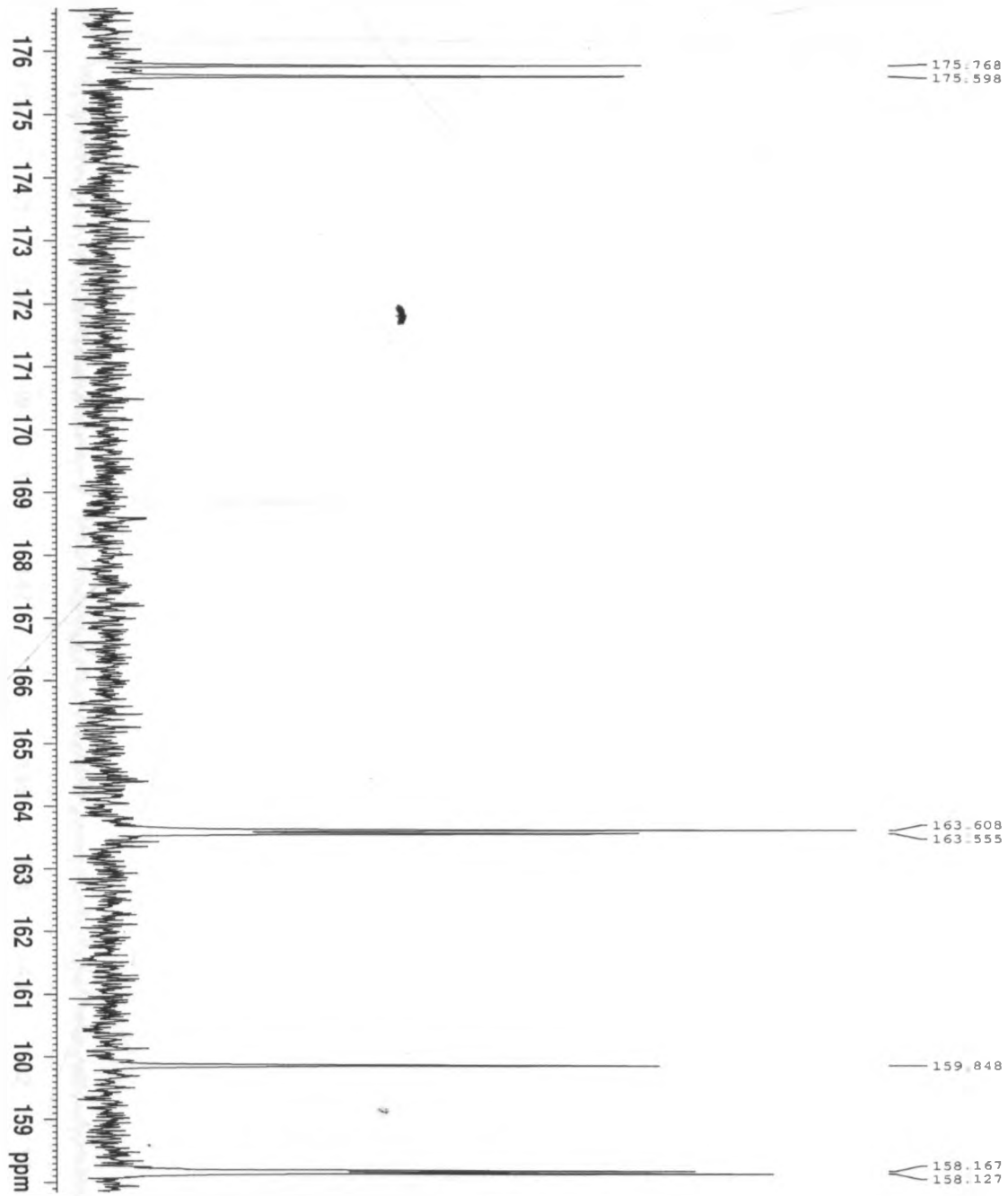
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



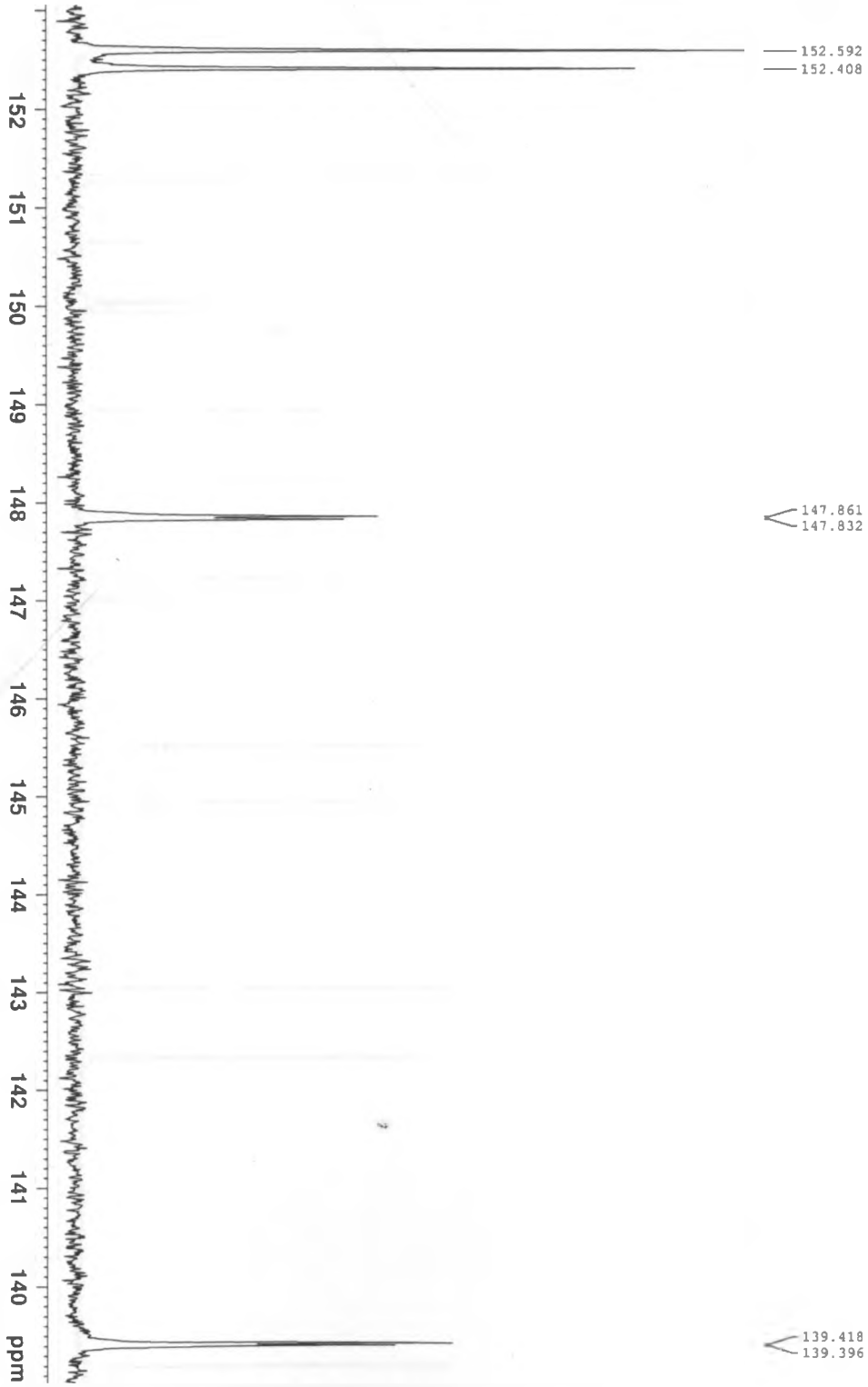
^{13}C NMR SPECTRUM FOR COMPOUND 5 AND 6 (150 MHz, CD_2Cl_2)



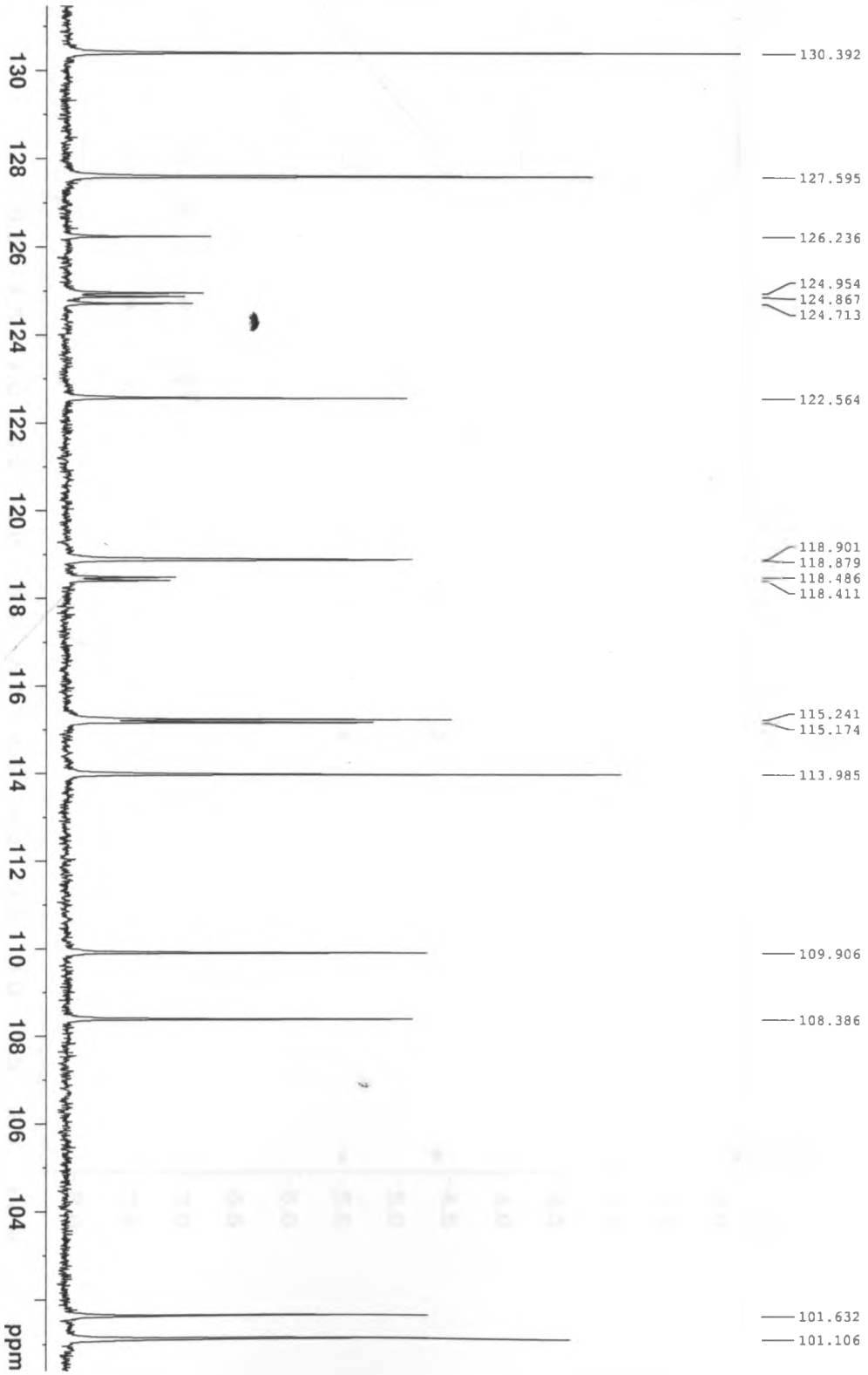
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



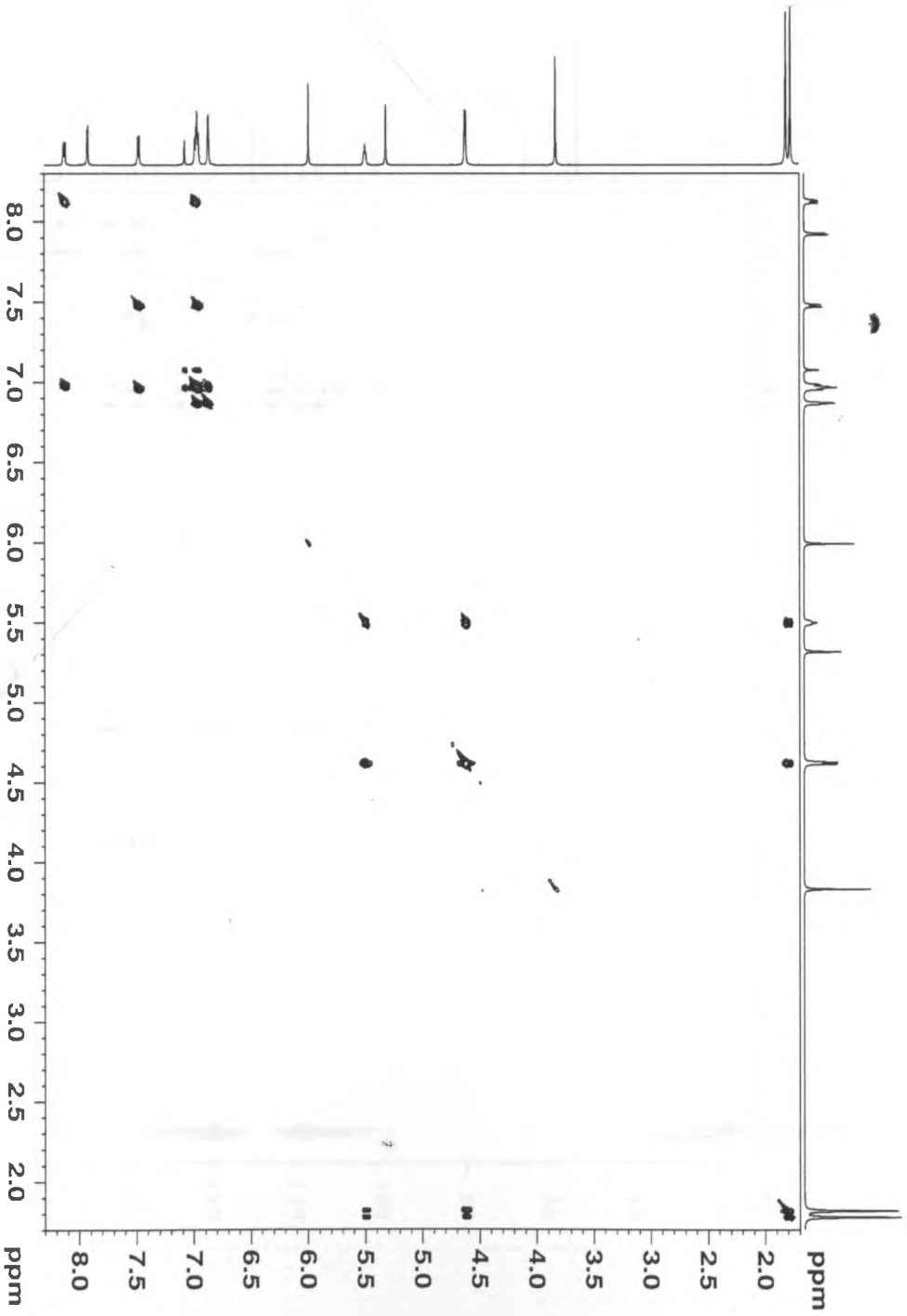
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



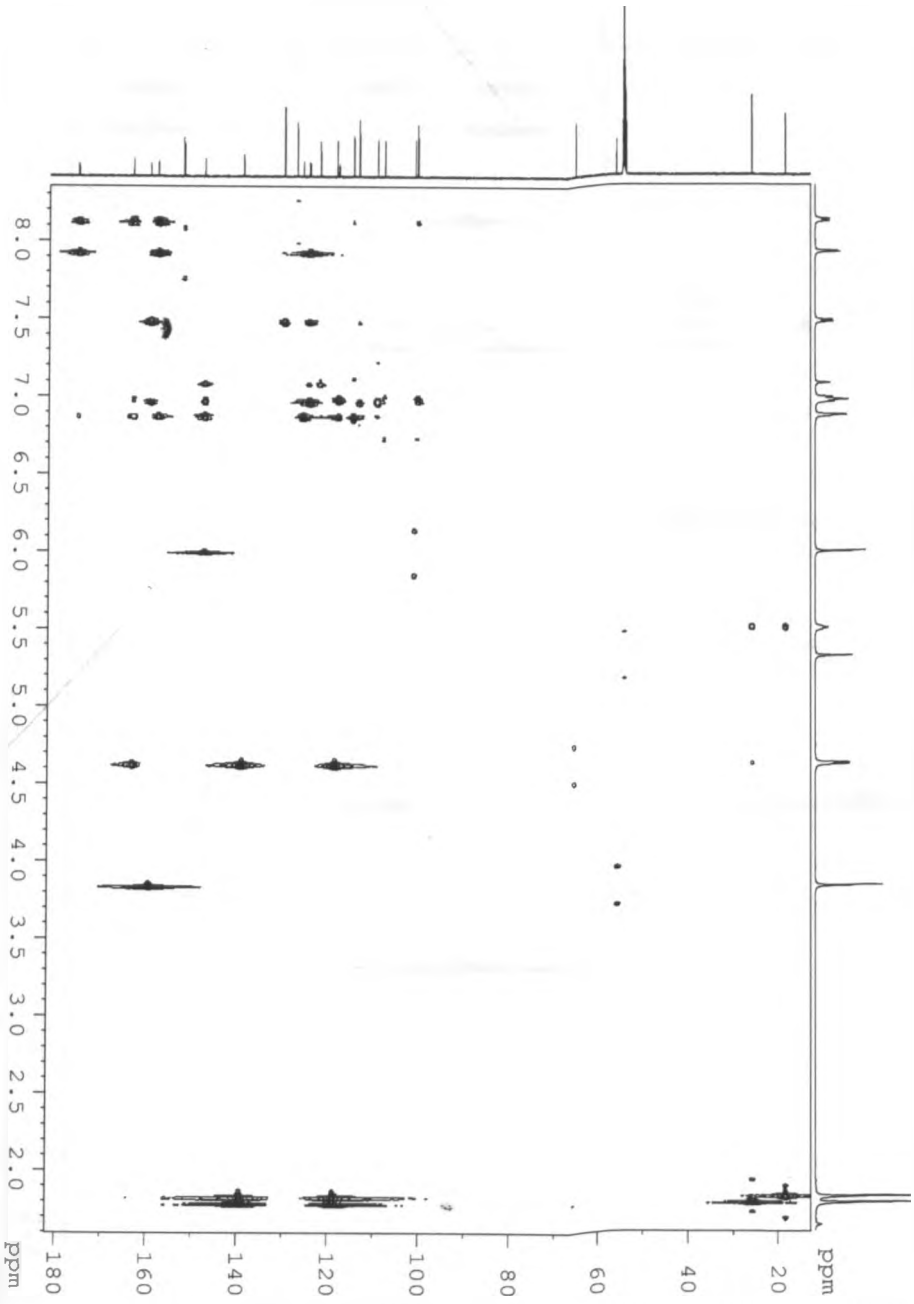
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



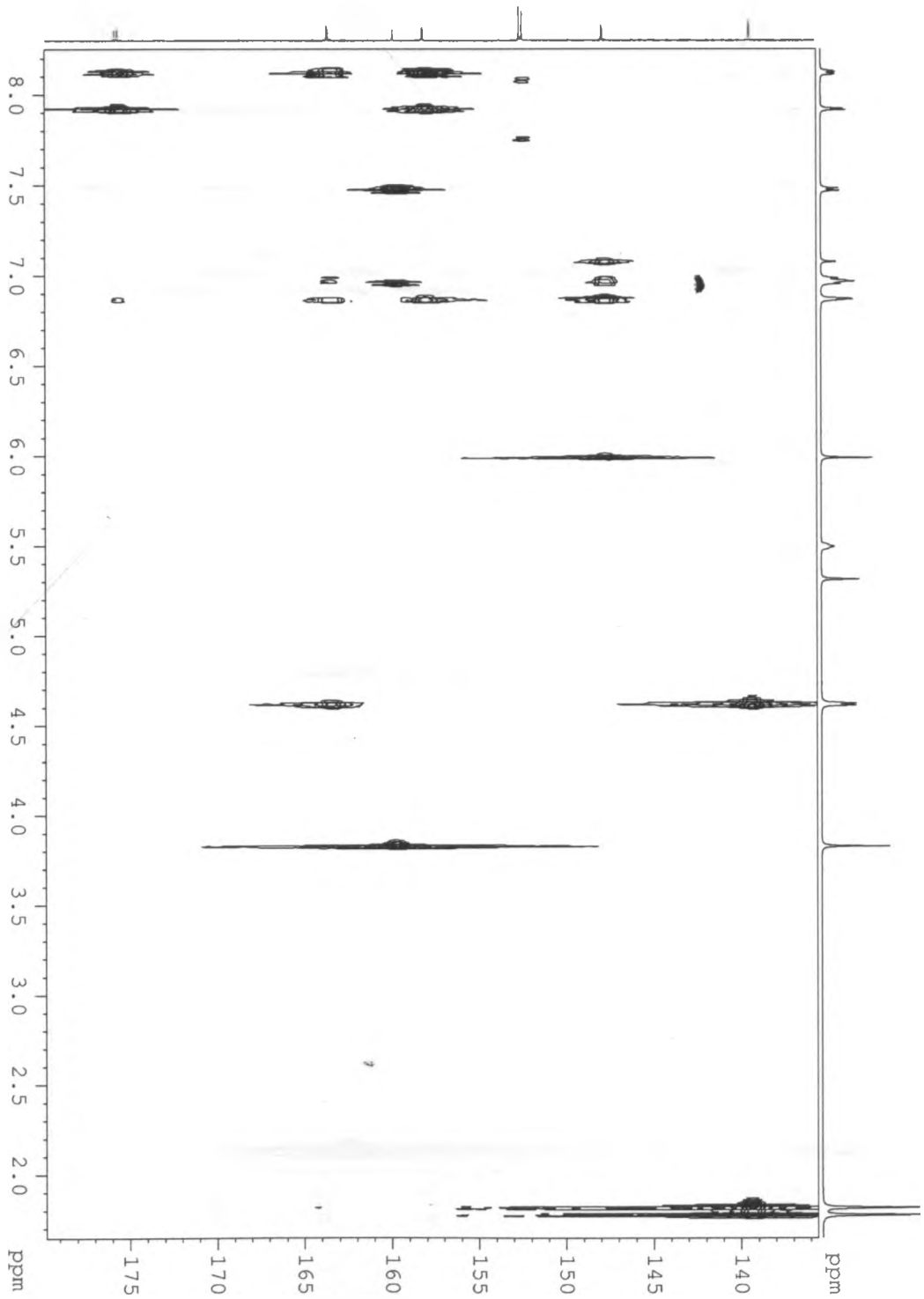
$^{1}H - {}^{1}H$ COSY SPECTRUM FOR COMPOUND 5 AND 6



HMBC SPECTRUM FOR COMPOUND 5 AND 6



HMBC SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



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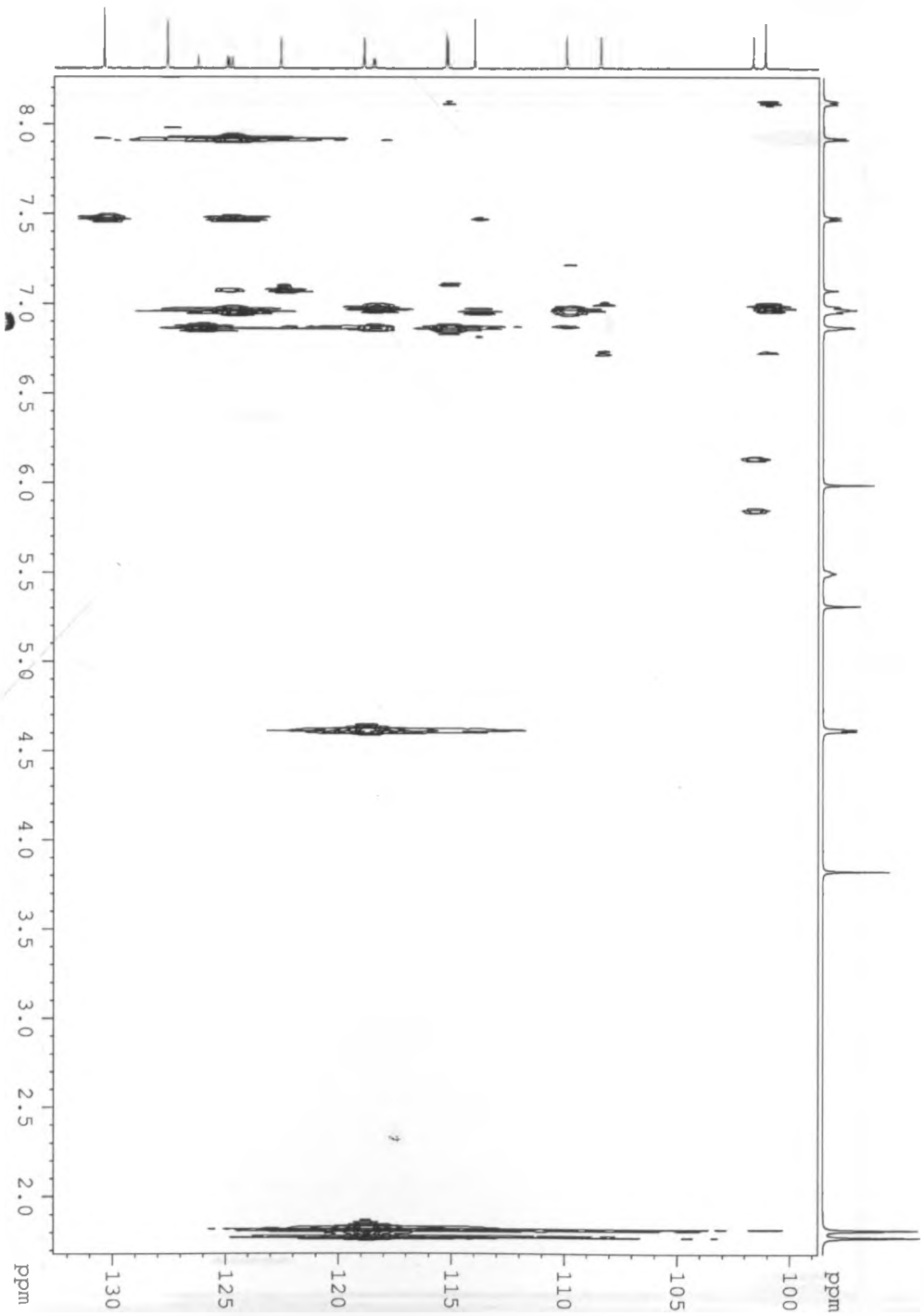
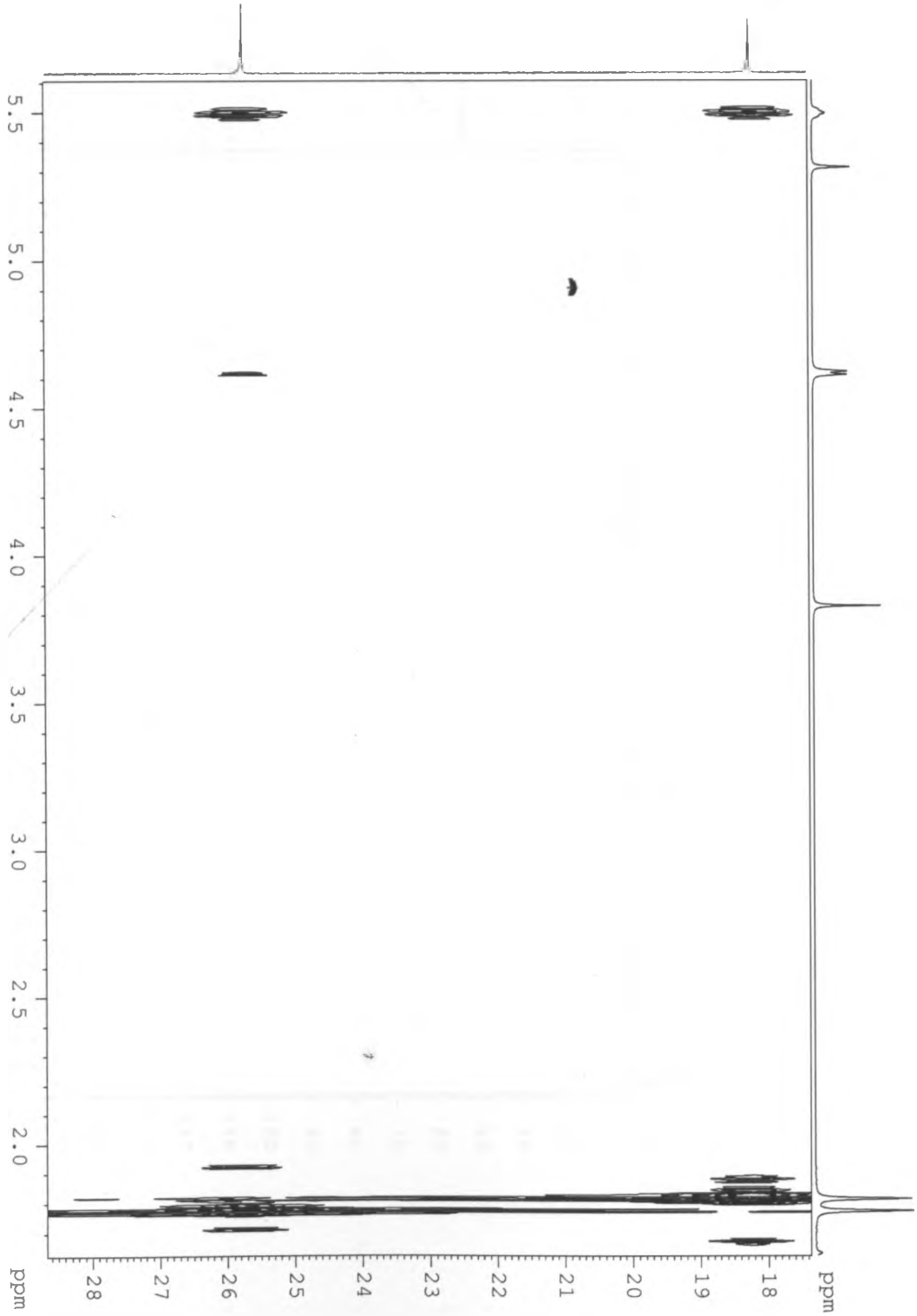
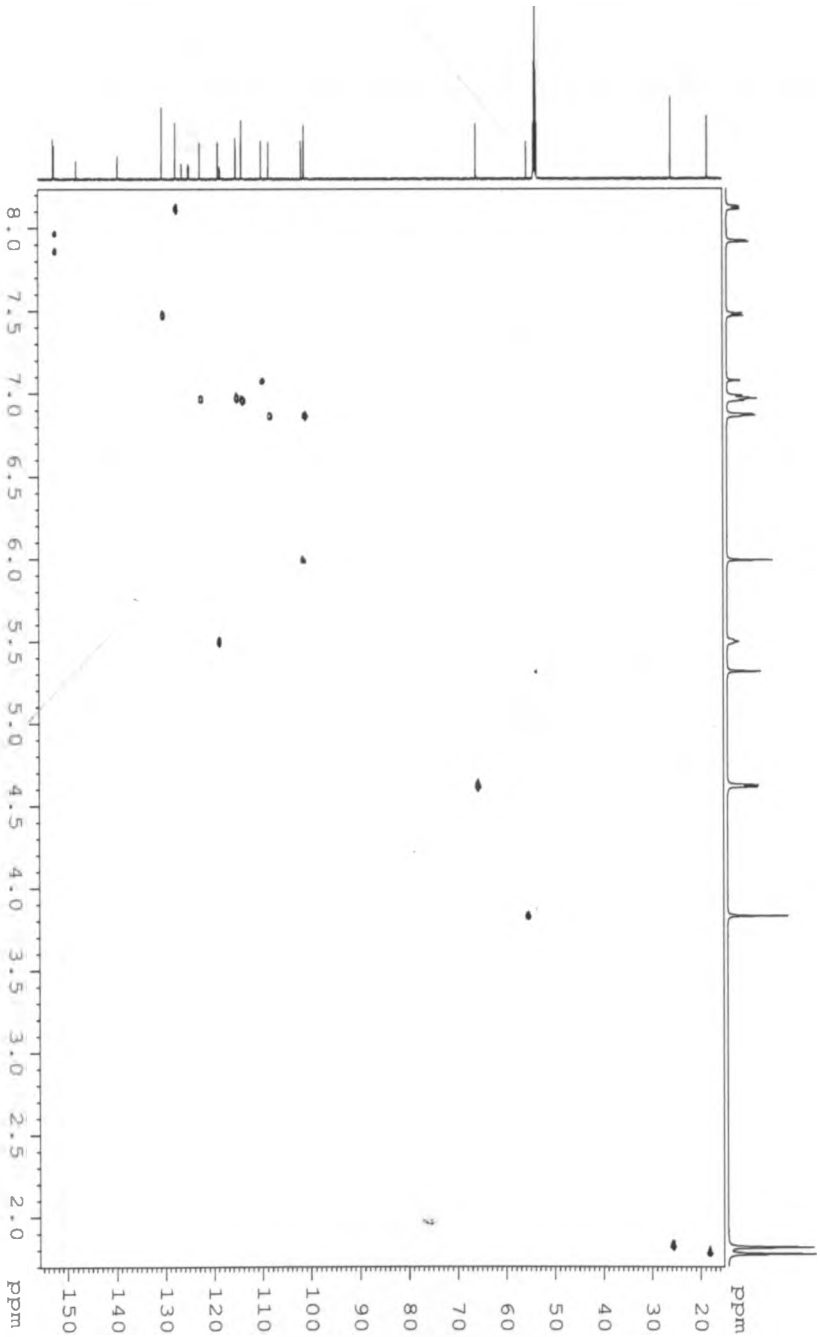


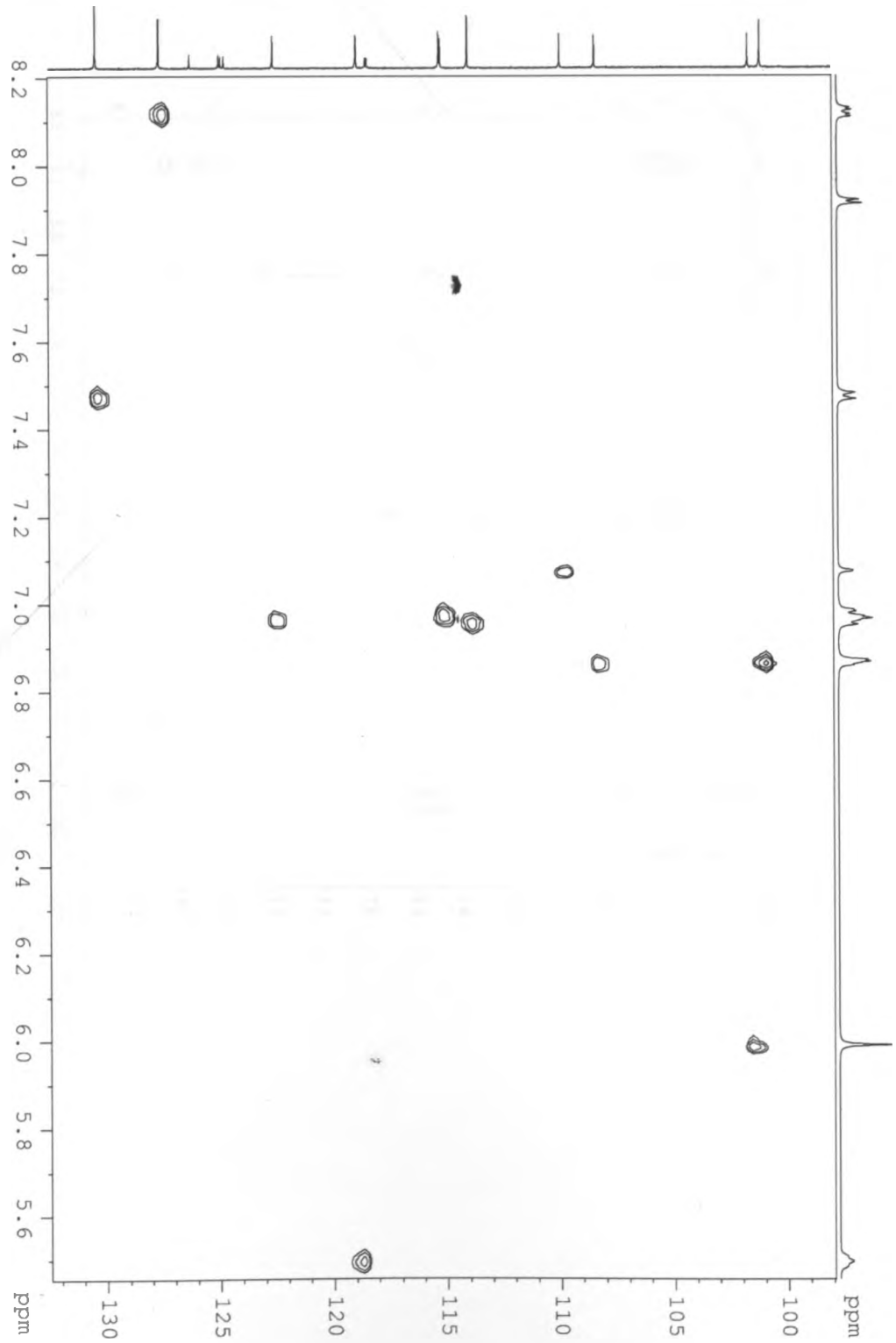
HMBC SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



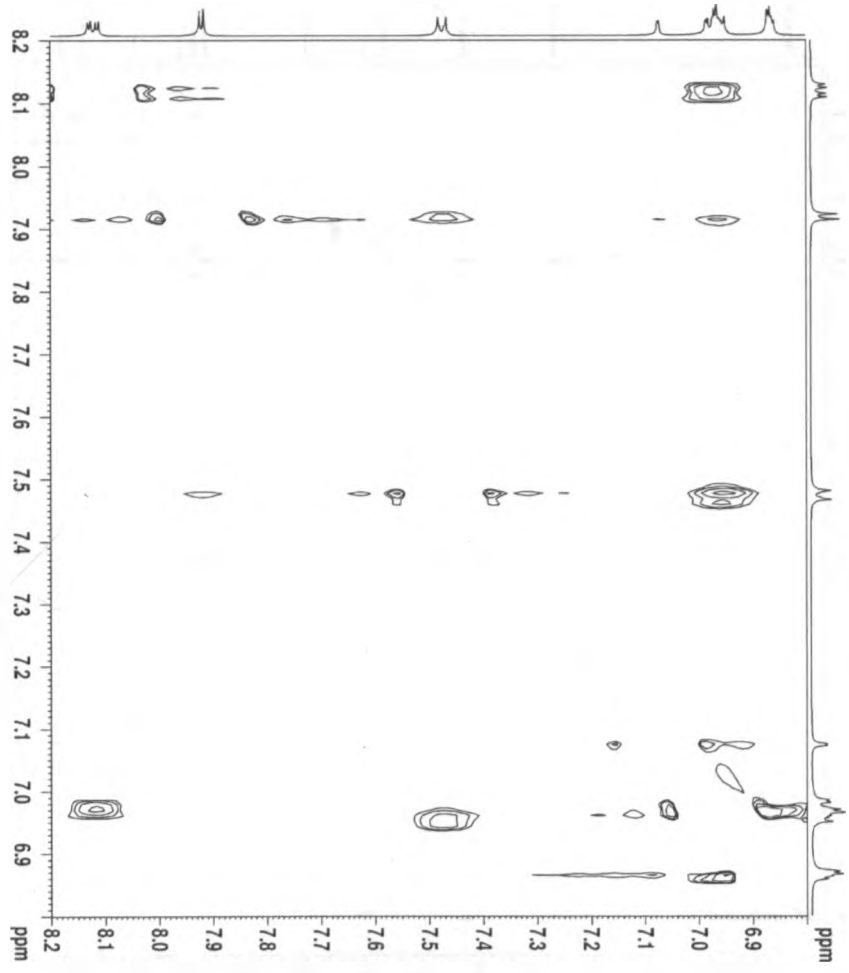
HMQC SPECTRUM FOR COMPOUND 5 AND 6



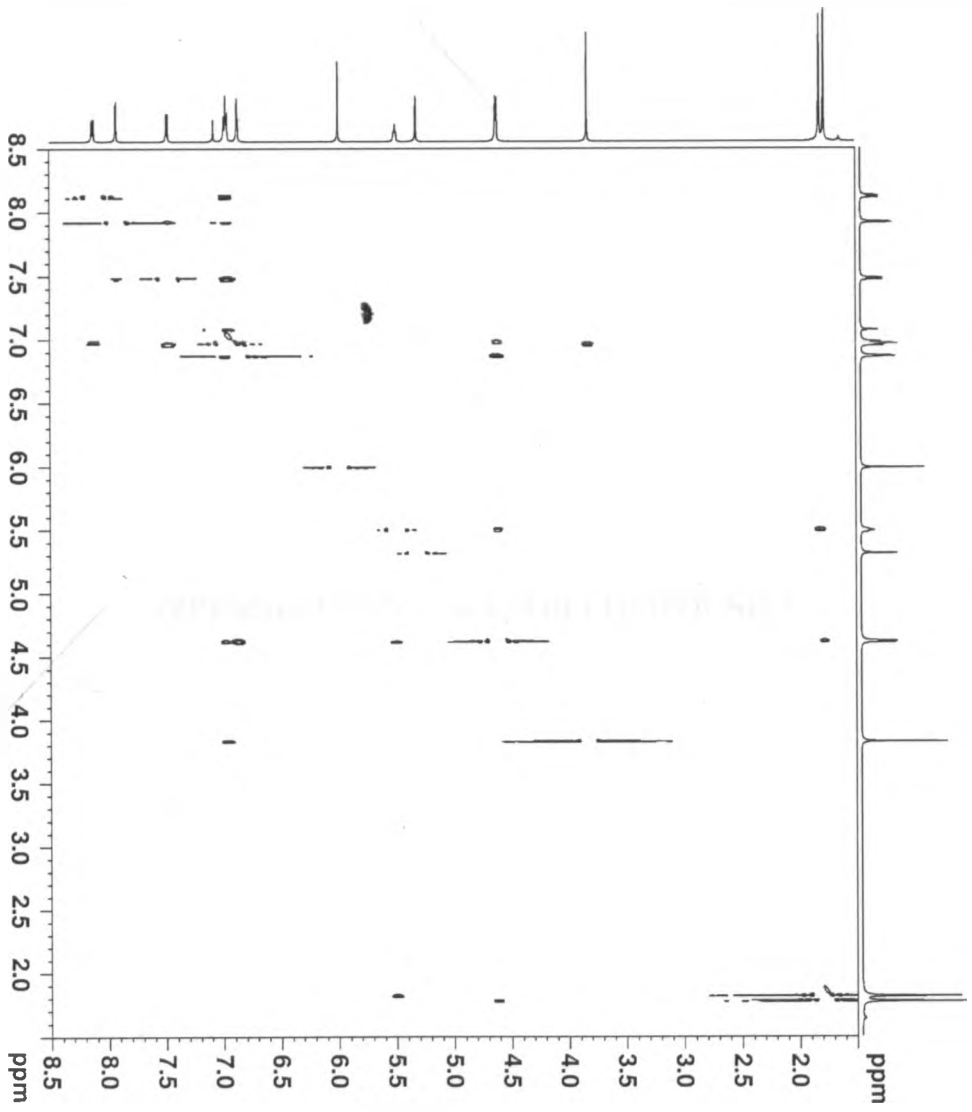
HMQC SPECTRUM EXPANSION FOR COMPOUND 5 AND 6



NOESY SPECTRUM FOR COMPOUND 5 AND 6

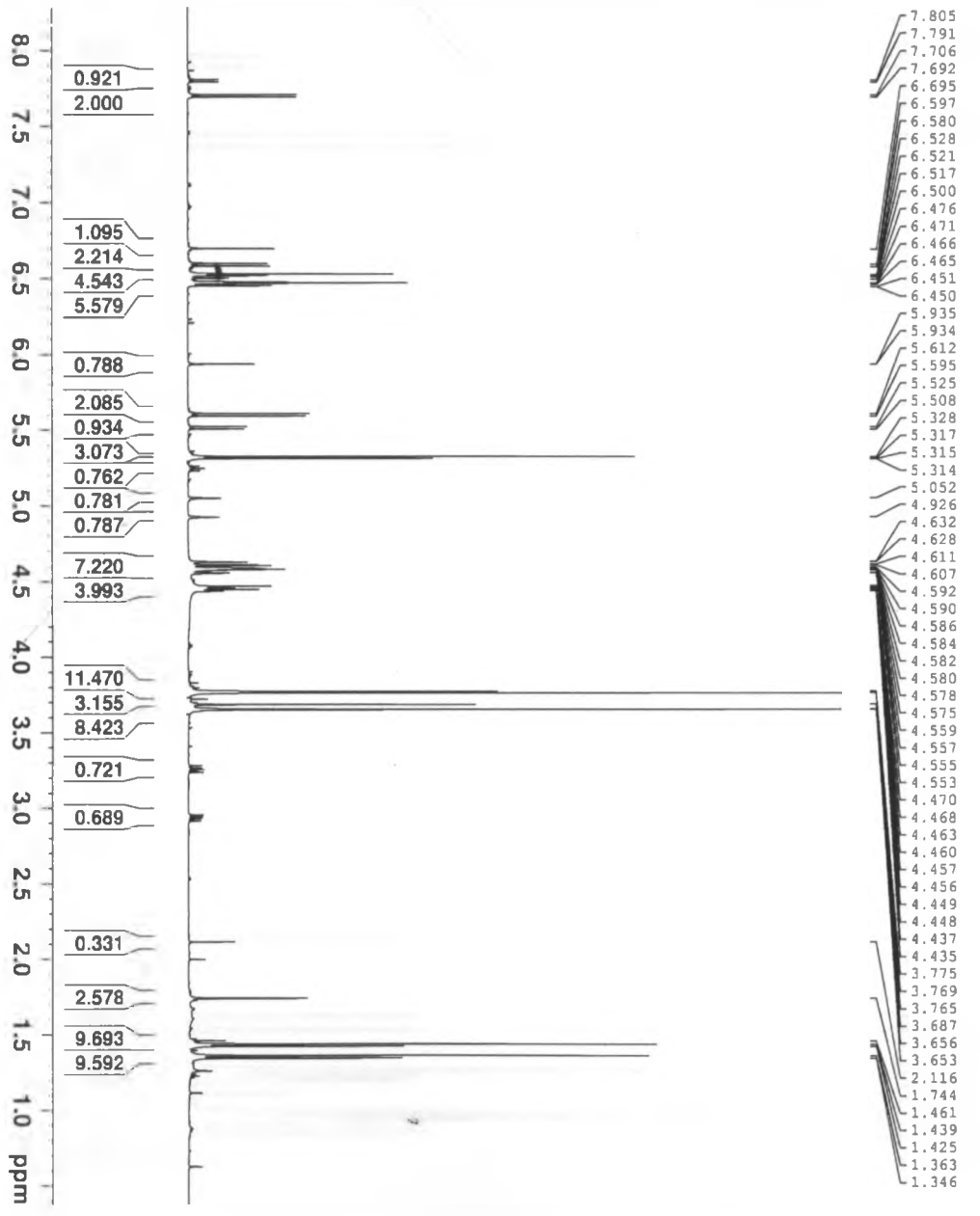


NOESY SPECTRUM FOR COMPOUND 5 AND 6

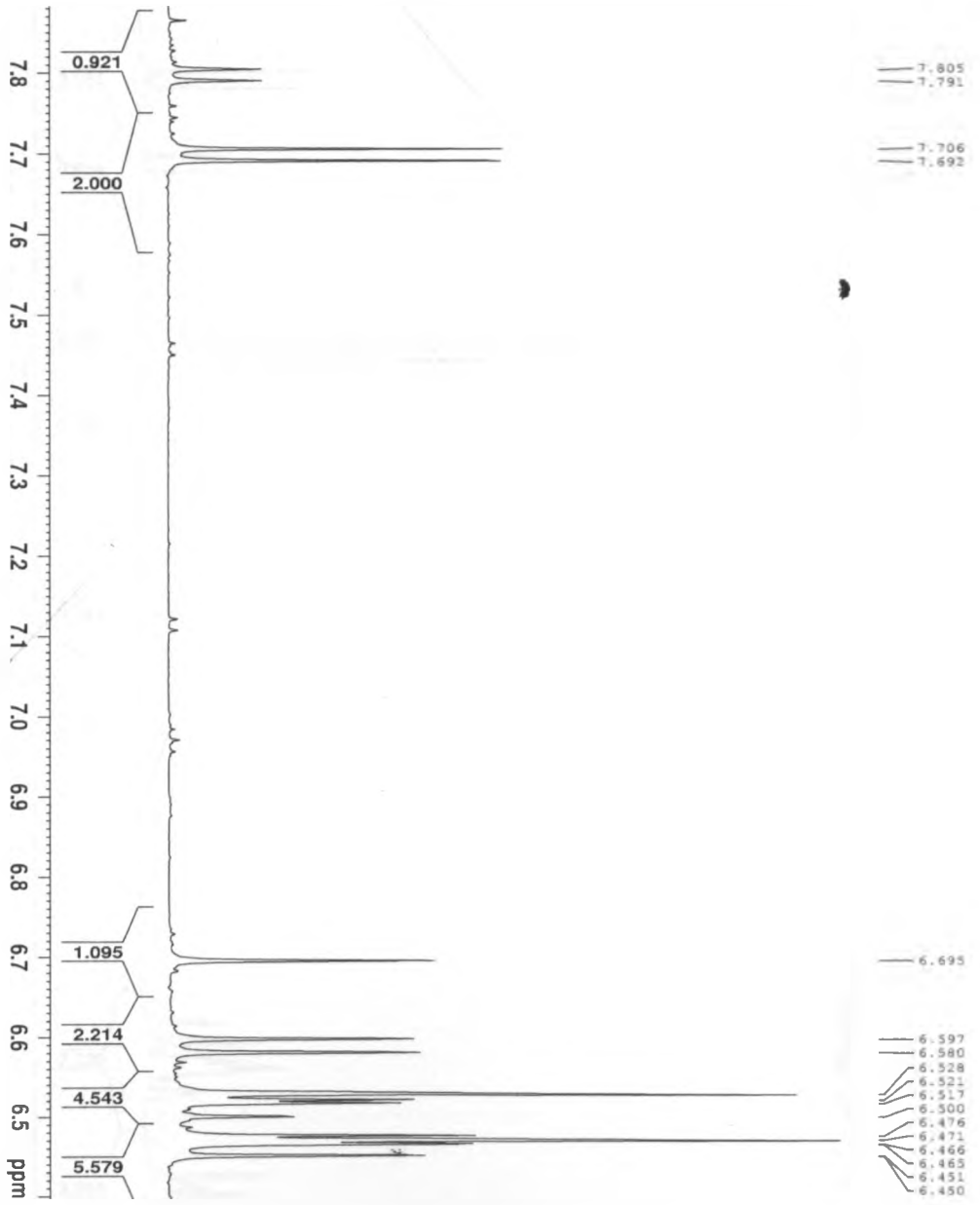


APPENDIX F: SPECTRA FOR COMPOUND 7

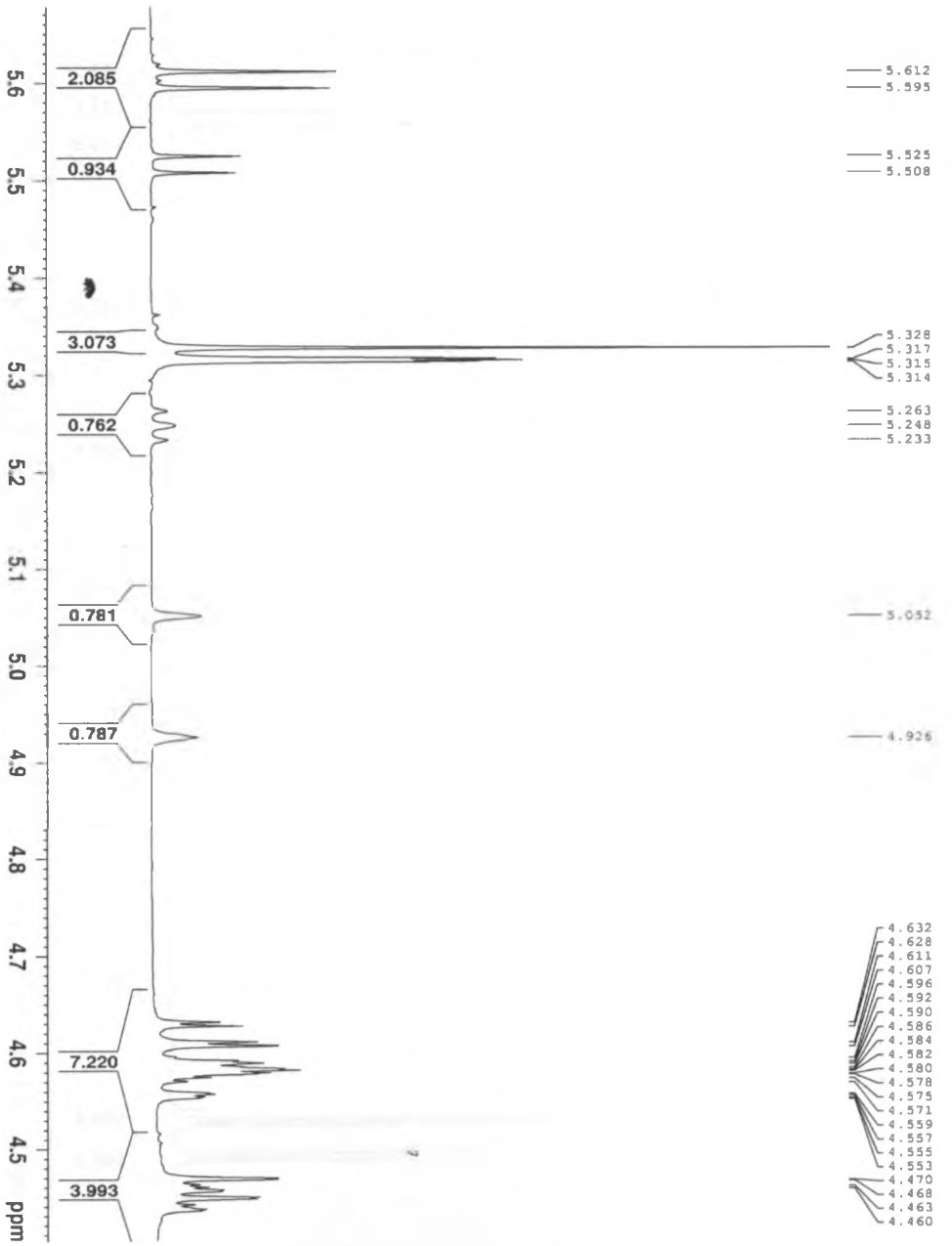
^{1}H NMR SPECTRUM FOR COMPOUND 7 (600 MHz, CD_2Cl_2)



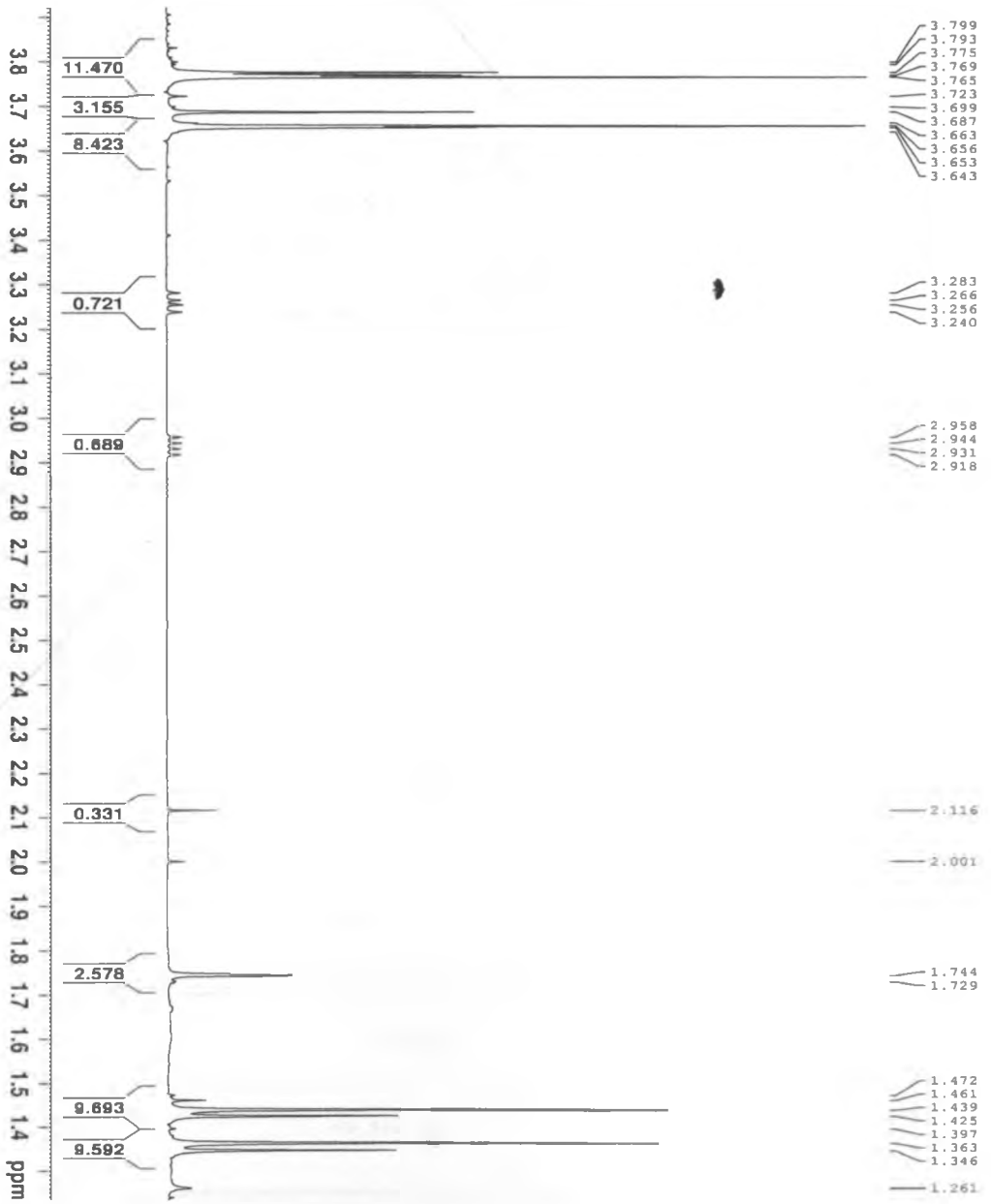
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 7



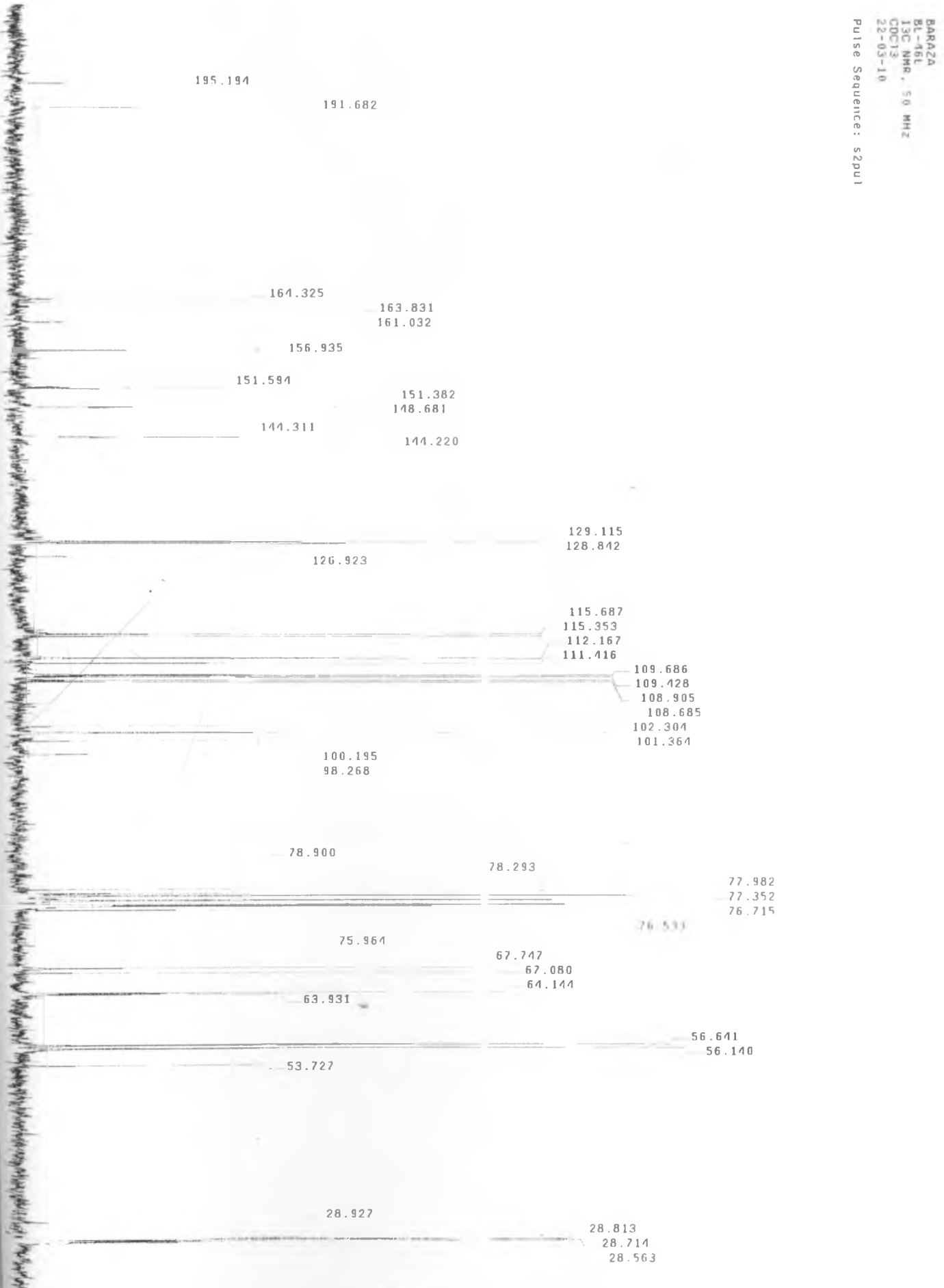
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 7



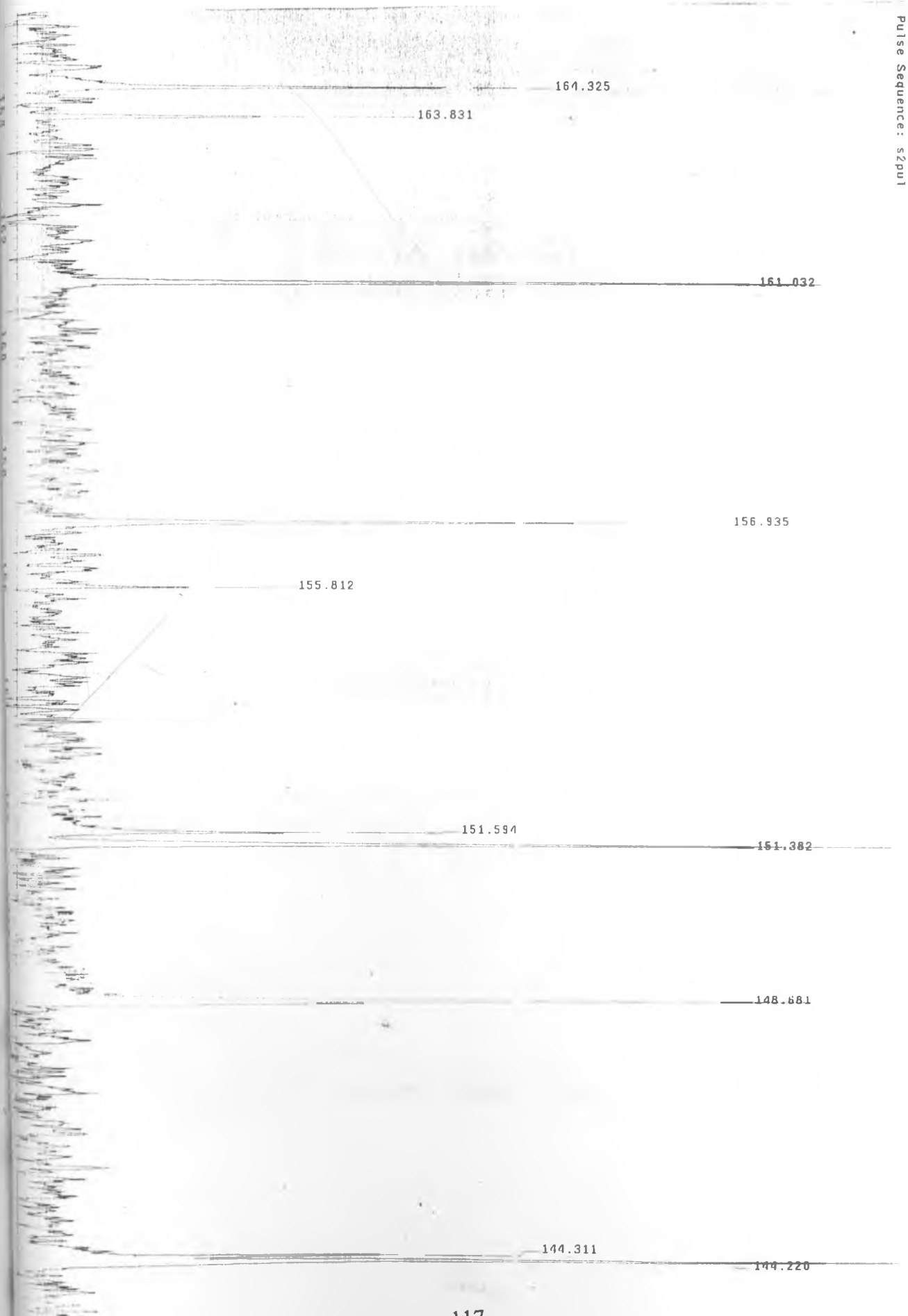
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 7



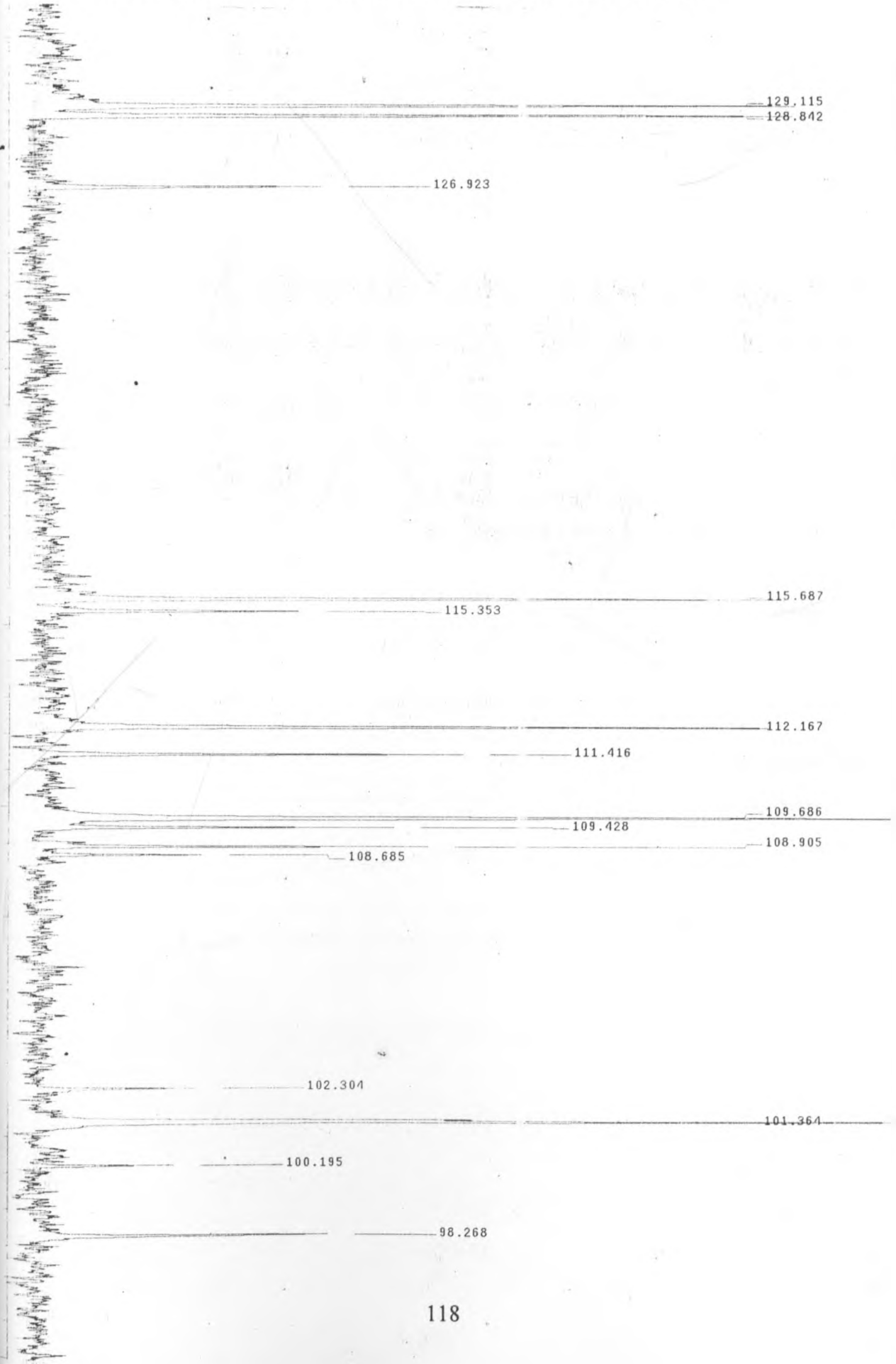
^{13}C NMR SPECTRUM FOR COMPOUND 7 (50 MHz, $CDCl_3$)



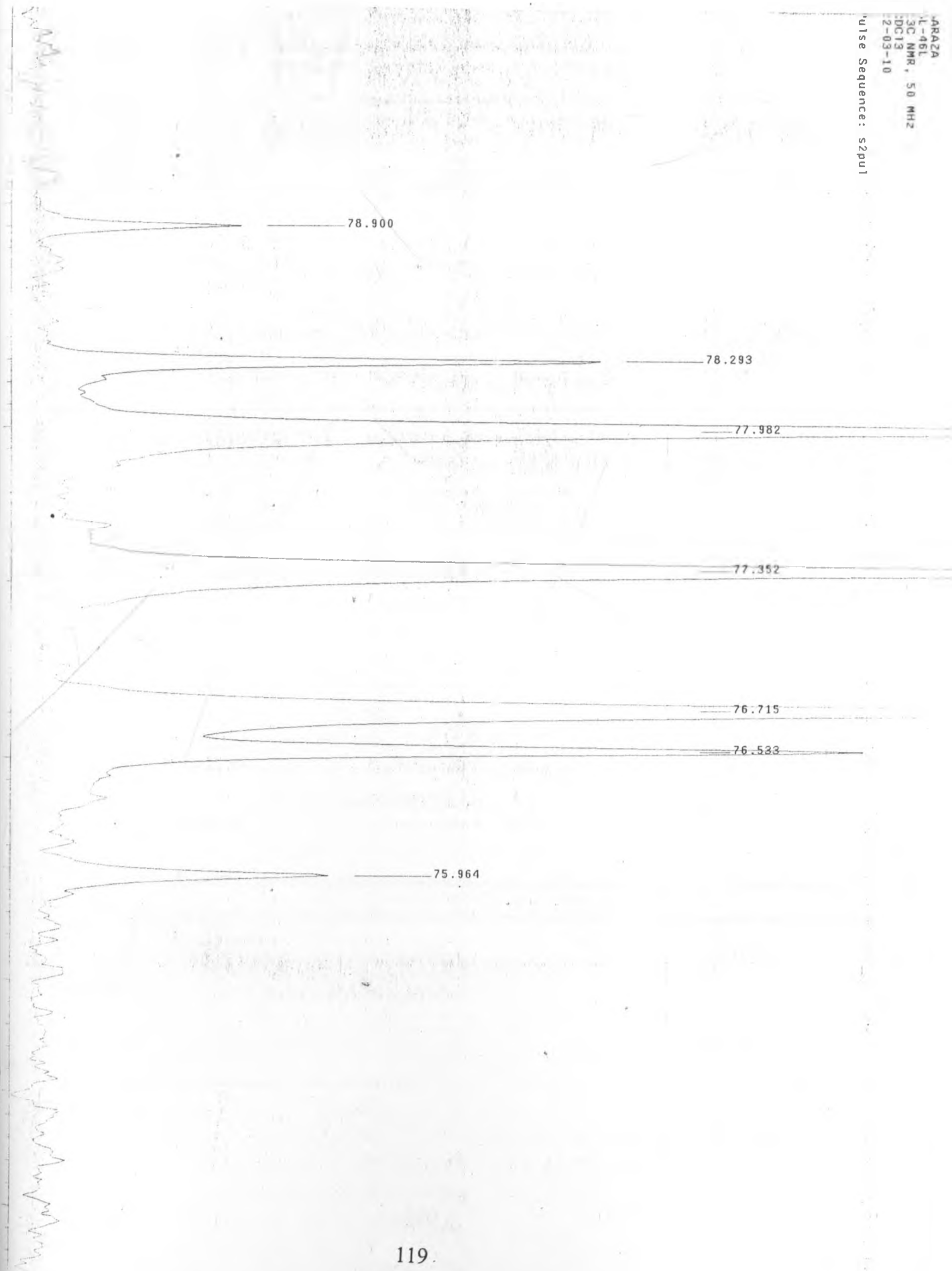
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 7



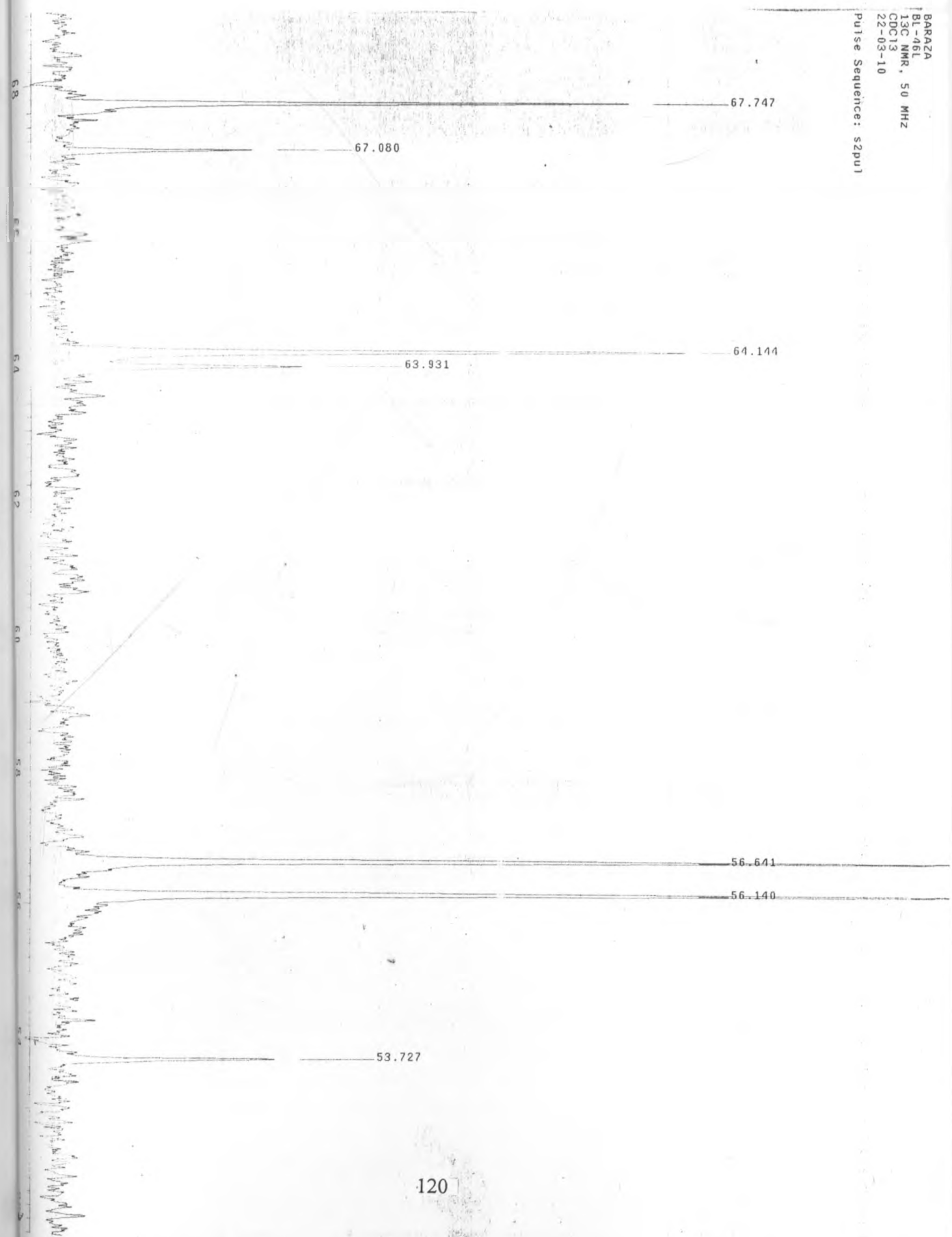
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 7



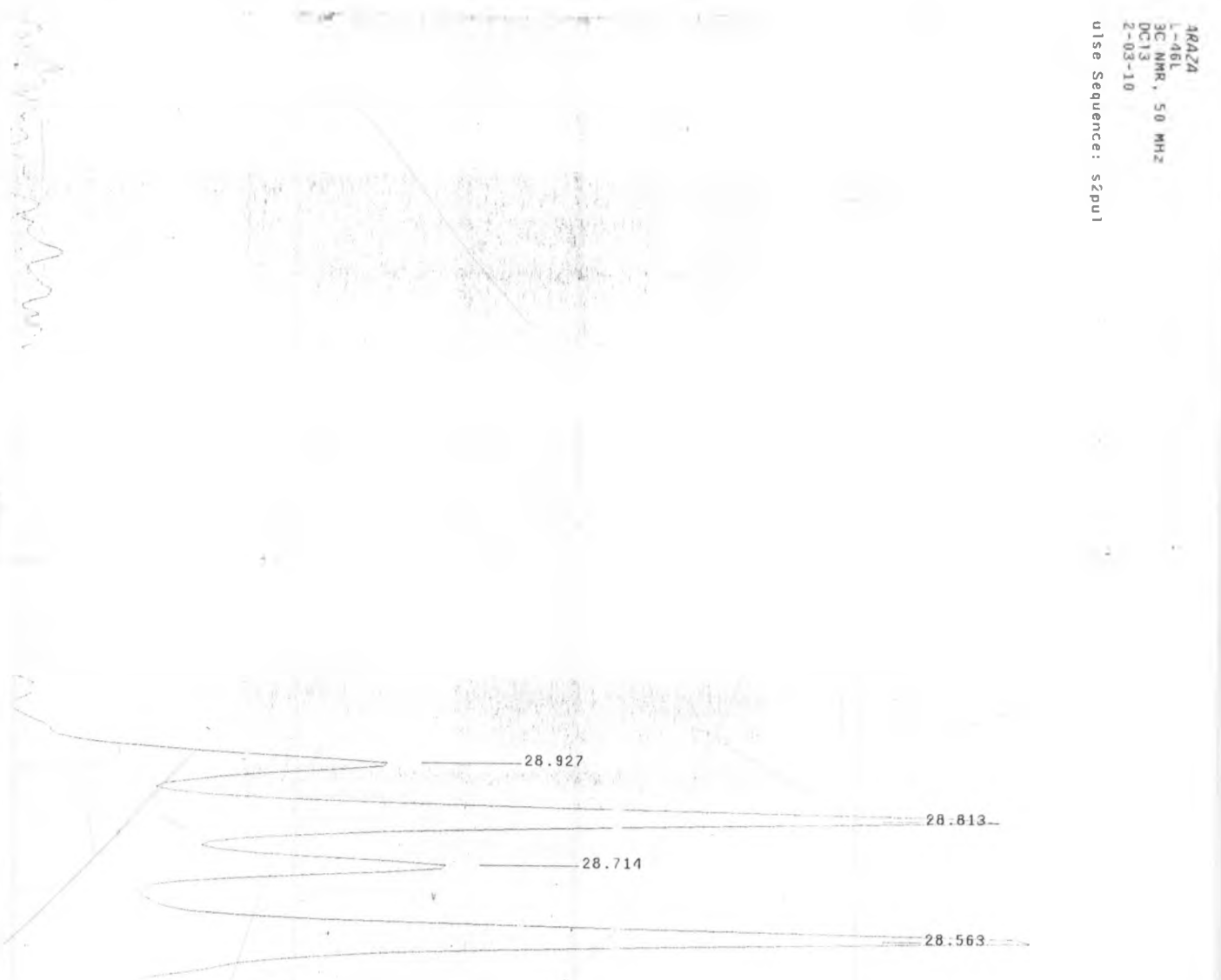
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 7



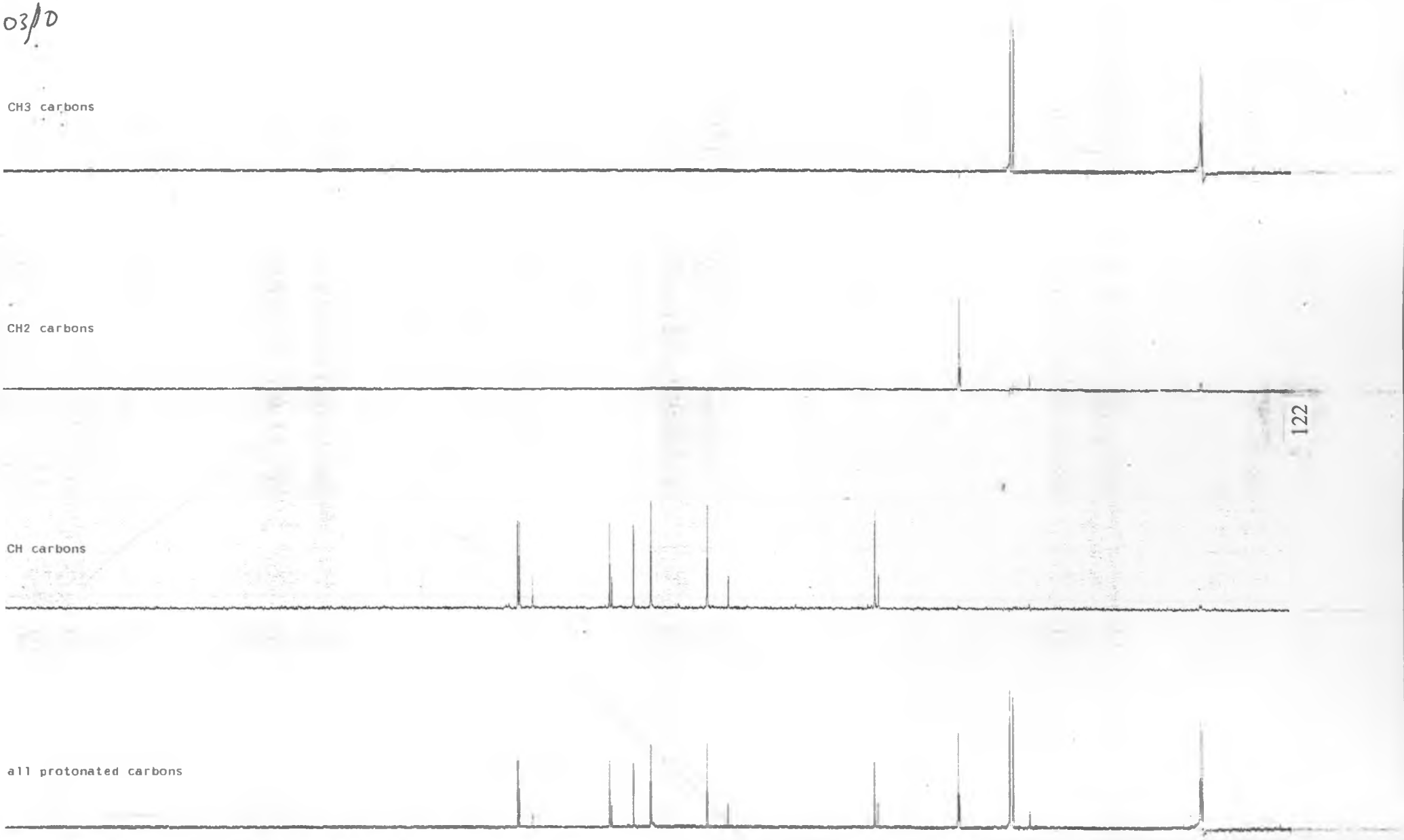
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 7



^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 7

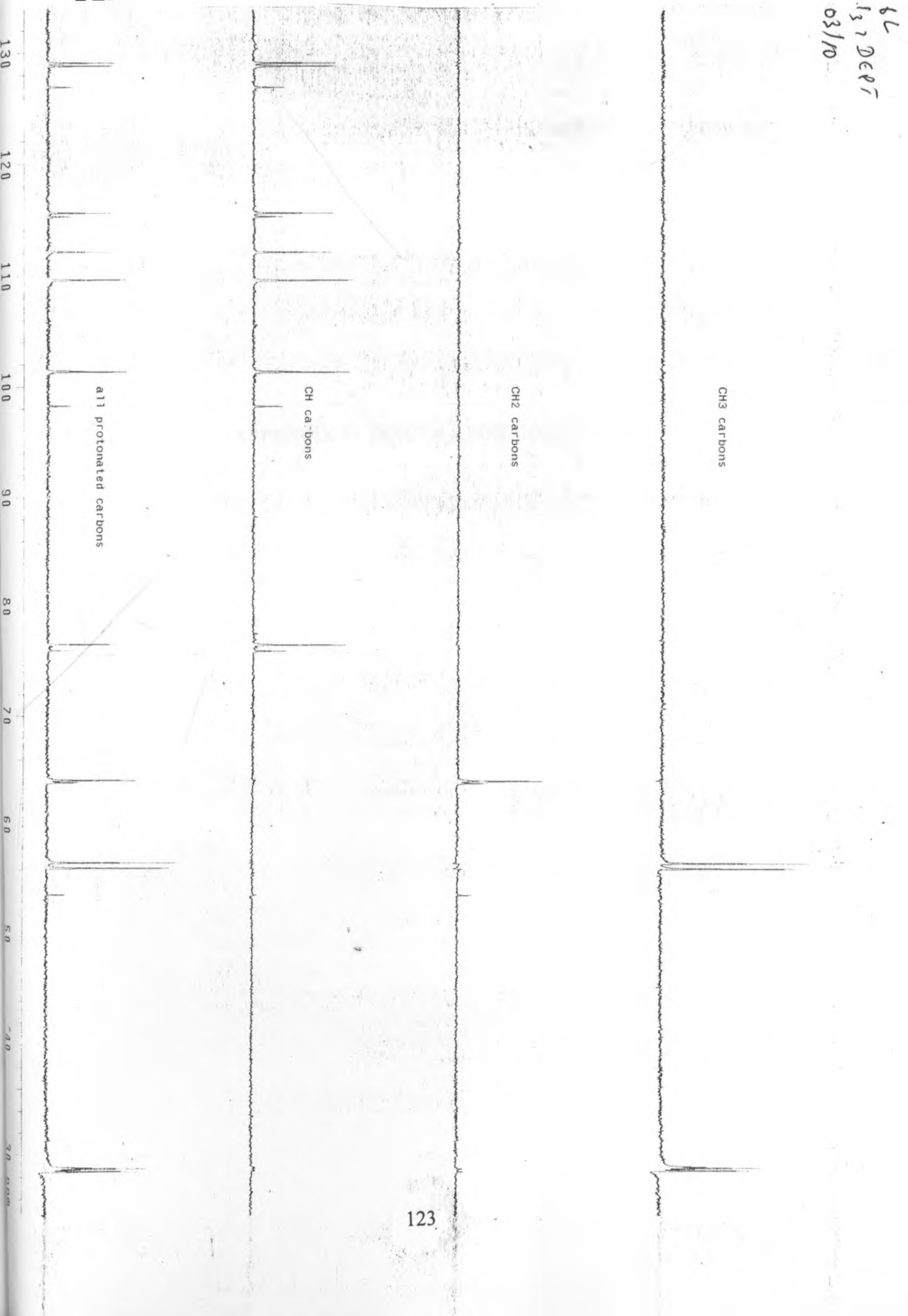


Sa
46 L
$CDCl_3$
PT
03/10



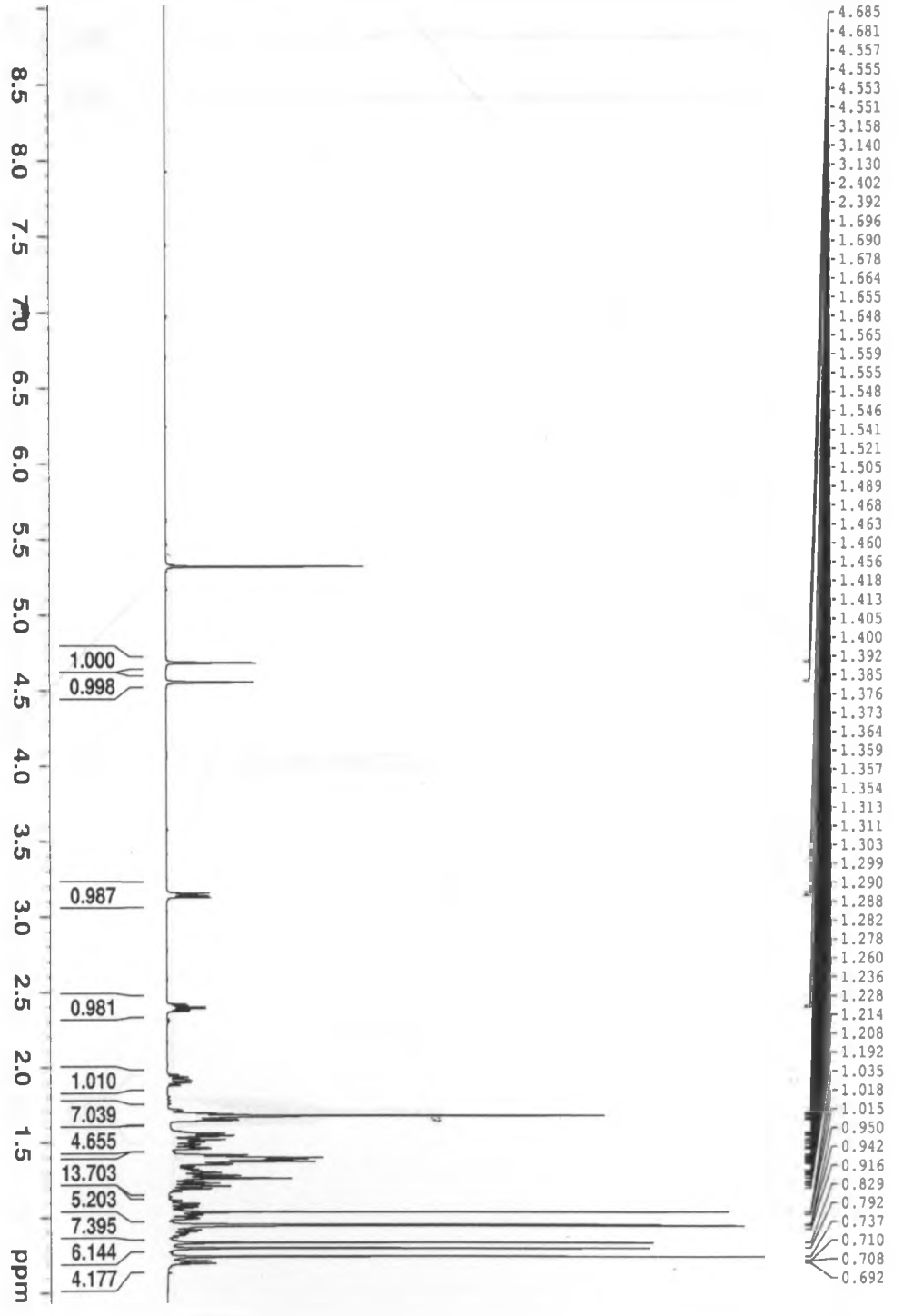
DEPT NMR SPECTRUM FOR COMPOUND 7

DEPT NMR SPECTRUM FOR COMPOUND 7

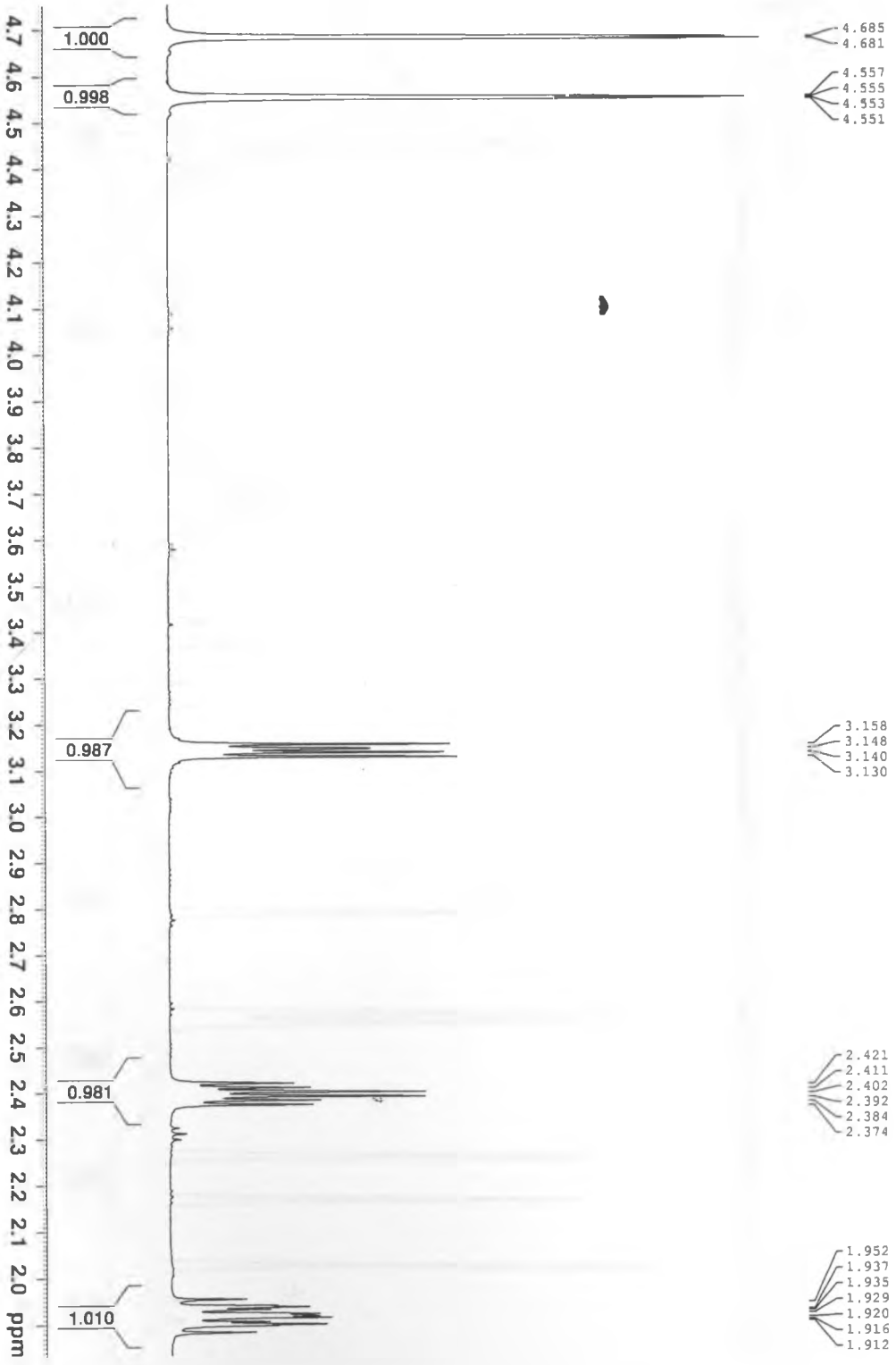


APPENDIX G: SPECTRA FOR COMPOUND 8

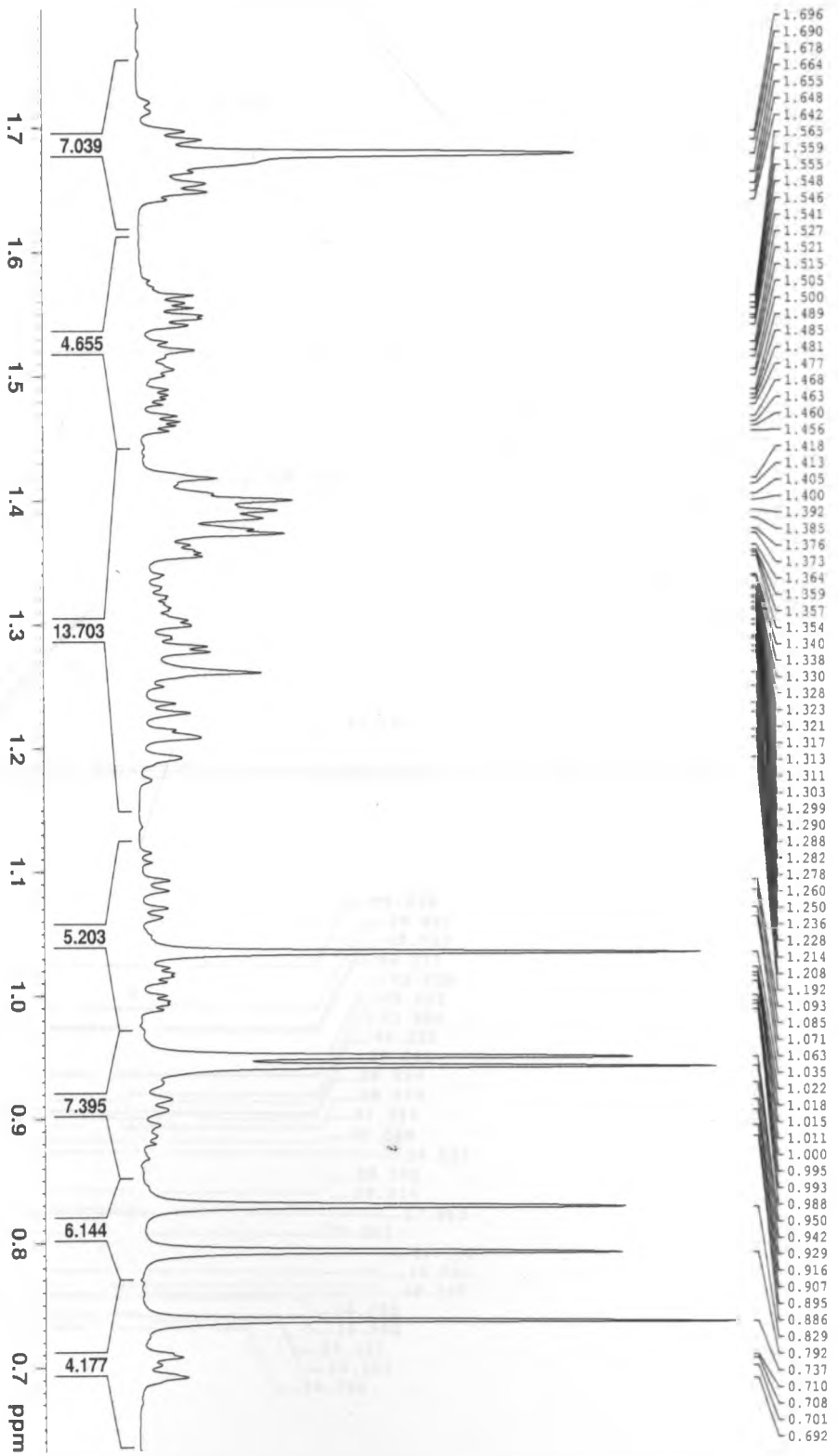
^{1}H NMR SPECTRUM FOR COMPOUND 8 (600 MHz, CD_2Cl_2)



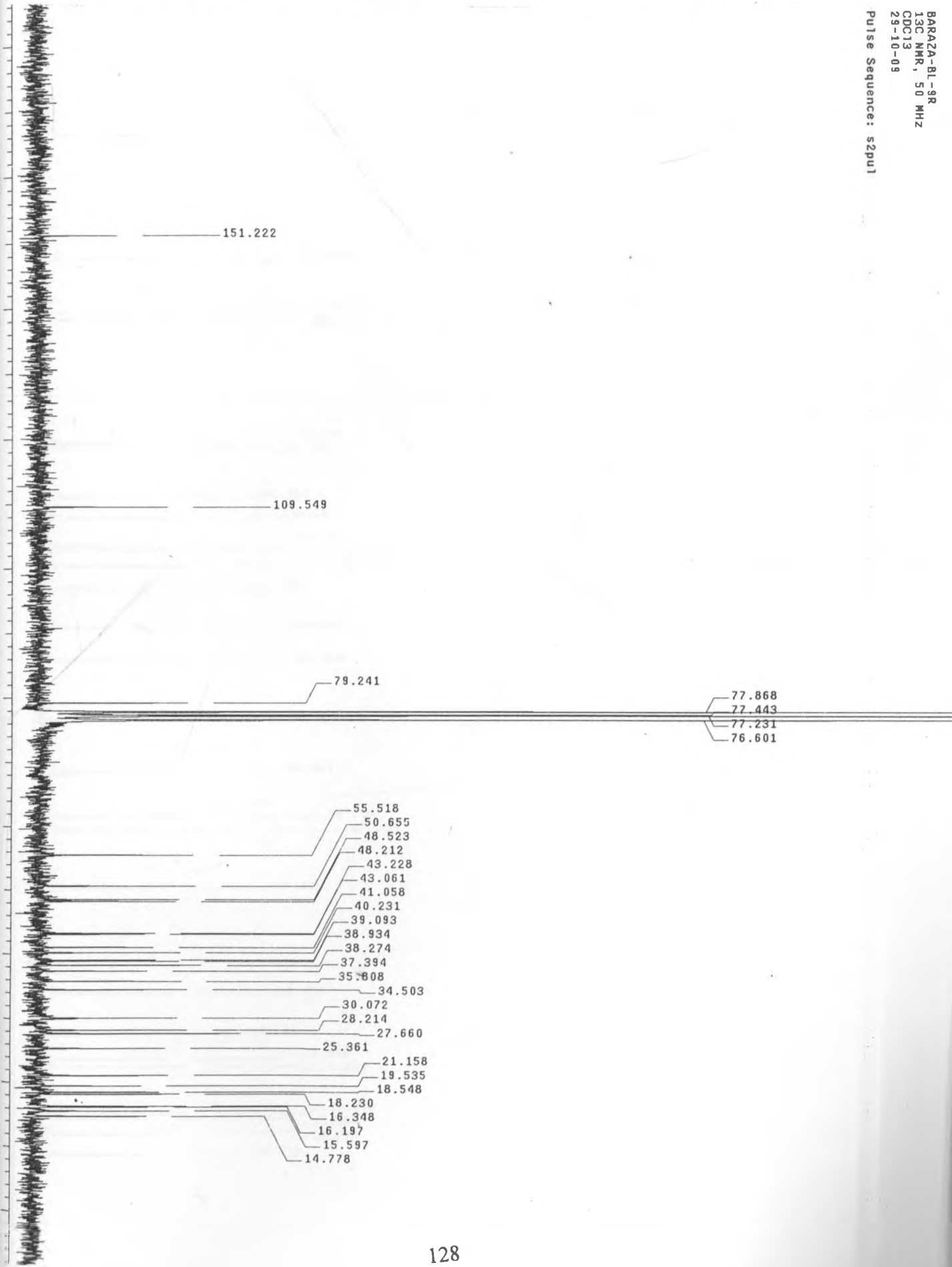
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 8



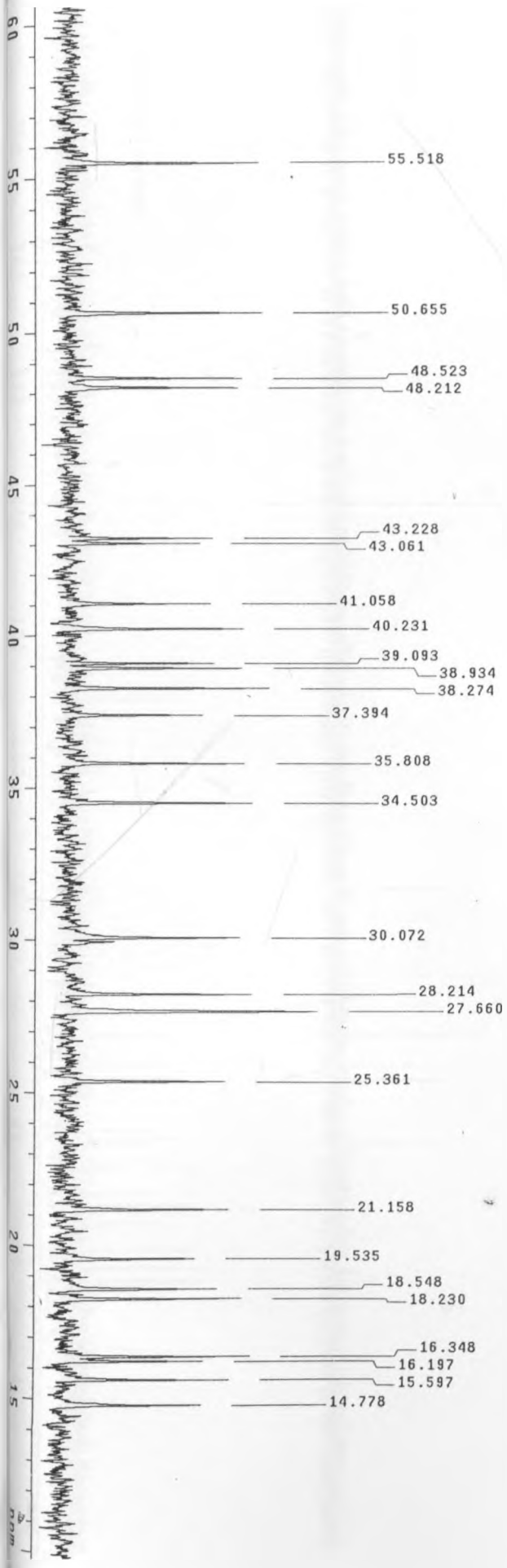
^{1}H NMR SPECTRUM EXPANSION FOR COMPOUND 8



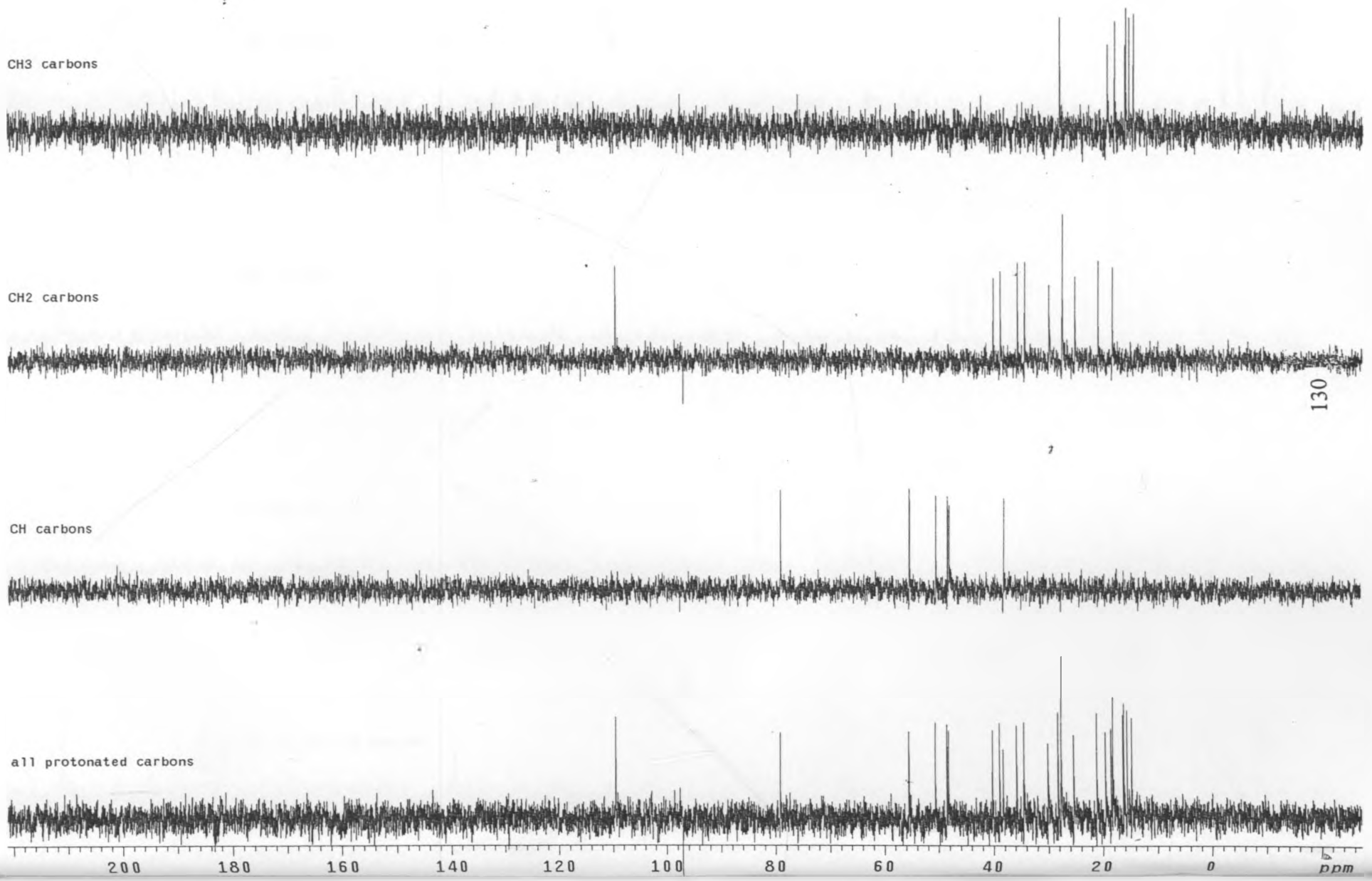
^{13}C NMR SPECTRUM FOR COMPOUND 8 (50 MHz, $CDCl_3$)



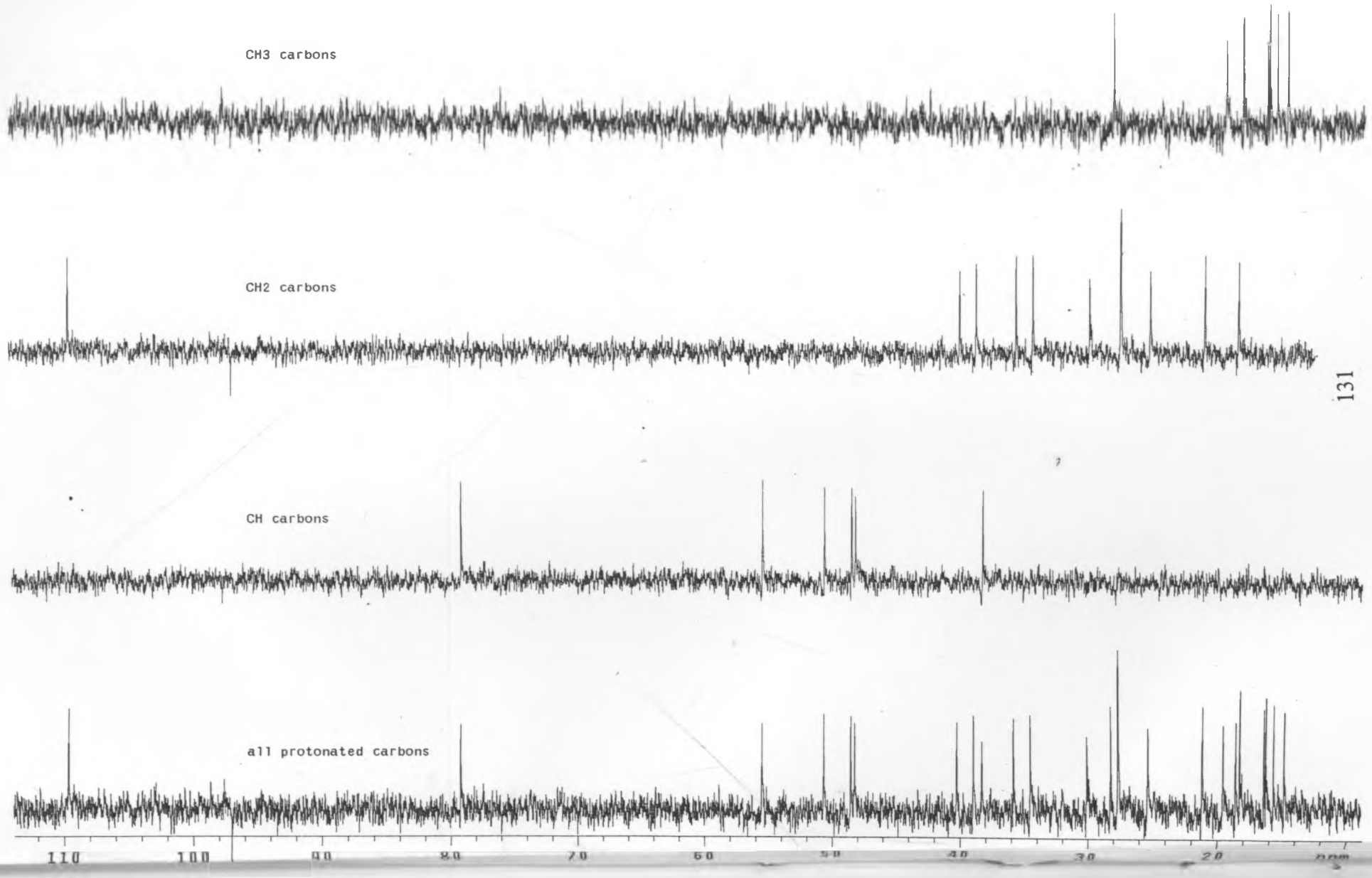
^{13}C NMR SPECTRUM EXPANSION FOR COMPOUND 8



DEPT NMR SPECTRUM FOR COMPOUND 8



8a
-9R
PT
11/10/05



DEPT NMR SPECTRUM FOR COMPOUND 8