UNIVERSITY OF NAIROBI

COLLEGE OF PHYSICAL AND BIOLOGICAL SCIENCES

DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF THREE *ERYTHRINA* SPECIES AND *TECLEA NOBILIS*

BY

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DECLARATION

This thesis is my original work and has never been presented for a degree in any university.

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DEDICATION

This thesis is dedicated to my beloved parents; Mr. Peter Kenduiwo, Mrs. Mary Kenduiwo and my beloved son Gideon Kipngetich. Thank you for your great love, support and for giving me direction in life.

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LIST OF ABBREVIATIONS AND SYMBOLS

HMQC	Heteronuclear Multiple Quantum Coherence ($^{1}J_{CH}$)
HMBC	Hetronuclear Multiple Bond Correlation ($^{2}J_{CH}$, $^{3}J_{CH}$)
COSY	Correlated Spectroscopy
NOESY	Nuclear Overhauser and Exchange Spectroscopy
NMR	Nuclear Magnetic Resonance
1D	One Dimensional analysis
2D	Two Dimensional analysis
UV	Ultra Violet
MHz	Mega Hertz
Hz	Hertz
J	Coupling constant
S	Singlet
d	Doublet
dd	Doublet of a doublet
t	Triplet
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
IC_{50}	Concentration of 50% Inhibition
RB	Root bark
SB	Stem bark
F	Flower

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ABSTRACT

The acetone extract of the root bark of *Erythrina burttii* showed *in vitro* antiplasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 0.97 ± 0.2 and 1.73 ± 0.5 µg/ml respectively. The extract also had radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with EC_{50} value of 12.5 µg/ml and antimicrobial activity against a range of organisms. Previously reported isoflav-3-enes; burttinol-A and burttinol-C, and 2-arylbenzofuran derivative burttinol-D were identified as the most active antiplasmodial (IC_{50} < 10 µM) and free radical scavenging (EC_{50} *ca.* 10 µM) principles. In addition, three more compounds were isolated and characterized as bidwillon A (**1**), 5-hydroxy-2-methoxybenzaldehyde (**2**) and erythrinasinate (**3**). The 2-arylbenzofuran derivative burttinol-D was identified as a potent antibacterial and antifungal compound of the root bark of *Erythrina burttii*.

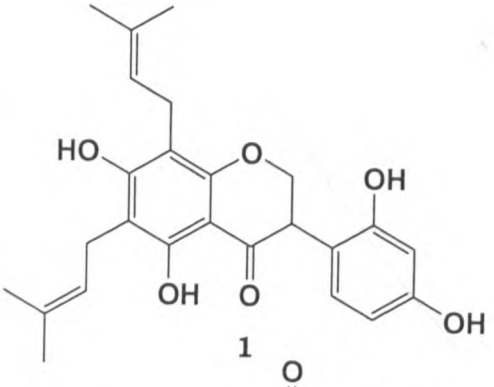
The acetone extract of the roots of *Erythrina abyssinica* showed antiplasmodial [IC_{50} 9.7 ± 1.1 µg/ml (against the D6) and 5.3 ± 0.7 µg/ml (W2) strains] and radical scavenging [against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical with EC_{50} value of 18.62 µg/ml] activities. Chromatographic separation resulted in the isolation and identification of erycristagallin (**4**) as one of the active principles among other compounds. The acetate derivative (**4a**) was completely inactive showing the importance of free phenolic groups for both antiplasmodial and radical scavenging activities.

Extending the interest on the phytochemistry and biological activity of this genus, the stem bark of *Erythrina brucei* was extracted with CH_2Cl_2 and MeOH (1:1) by cold percolation. The extract was subjected to chromatographic separation, which led to isolation of four alkaloids (**5-8**). The

structures of the compounds were determined using NMR (^{1}H, ^{13}C, NOESY, COSY). Compound (**8**), is a new alkaloid and the trivial name 10-oxo-eryhtraline has been assigned. The other three known alkaloids were identified as crystamidine (**5**), 8-oxo-erythraline (**6**), and erythraline (**7**). Besides this document, there has been only one other report on the occurrence of alkaloids in the stem bark of *Erythrina* species which otherwise elaborate flavonoids. The isolated compounds from *Erythrina brucei* were tested for radical scavenging activities; however none of them showed significant activity.

Phytochemical investigation of *Teclea nobilis* (stem bark), another alkaloid containing plant with wide traditional uses, led to isolation of five compounds (**9-13**). The structures of the compounds were again determined using NMR (^{1}H, ^{13}C, NOESY, COSY). These include four alkaloids [maculine (**9**), flindersiamine (**10**), 4,7-dimethoxyfuro [2,3-b] quinolin-6-ol (**11**), 7-(3-methylbuta-1,3-dienylloxy)-4,6-dimethoxyfuro [2,3-b] quinoline (**12**)] and a triterpene derivative Lupeol (**13**). Among these, compound **12** is a new alkaloid.

The crude extract and the isolated compounds; maculine (**9**) and flindersiamine (**10**) isolated from the stem bark of *Teclea nobilis* were tested against certain bacteria (*Staphylococcus aureus* and *Escherichia coli*) and fungi (*Candida albicans*, *Aspergillus niger*). The crude extract and the isolated compounds did not show any activity against the four pathogens.



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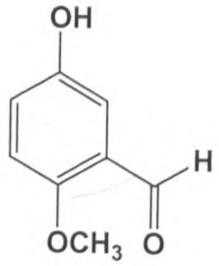

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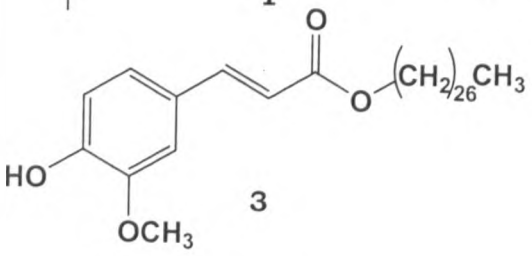

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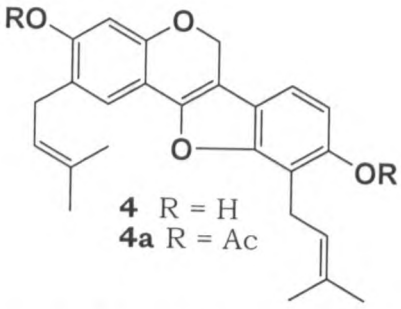

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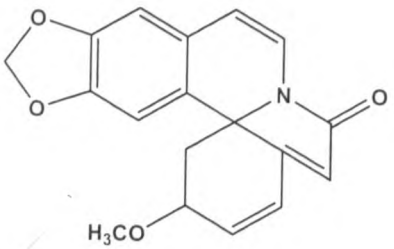
4a R = Ac



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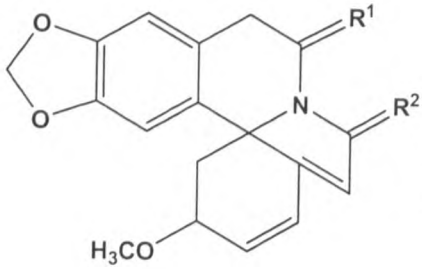
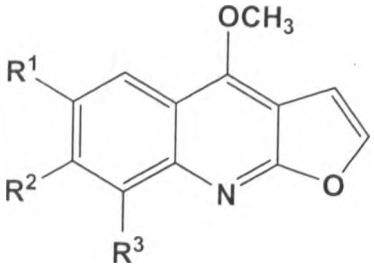
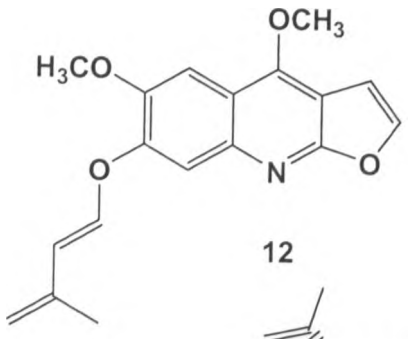


6 $R^1 = O, R^2 = H_2$
7 $R^1 = R^2 = H_2$
8 $R^1 = H_2 = R^2 = O$



9 R^1-R^2 = -OCH_2O-, R^3 = H
10 R^1-R^2 = -OCH_2O-, R^3 = OCH_3
11 R^1 = OH, R^2 = OCH_3, R^3 = H



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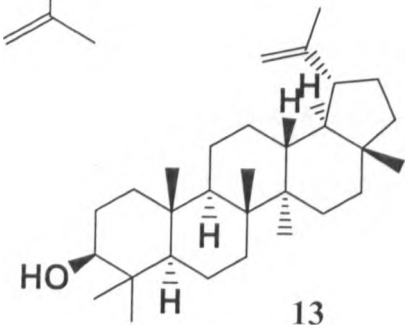

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CHAPTER ONE

INTRODUCTION

1.1 General

Herbal remedies have been considered to be an important source for identification of lead compounds; this is due to their use in the treatment of different ailments since antiquity. However, only about 20% of the plants with claimed ethnomedical uses have been subjected to bioassay screening (Houghton, 2001). In addition to providing templates for development of modern medicine, traditional medicine continues to offer health coverage for over 80% of the world population, especially in the developing world (WHO, 2002). Even in Europe, Asia, and America, traditional remedies still find some use as cure of 'mind affecting diseases' and herbal medicines are often considered to be gentle and safe alternatives to synthetic drugs.

In Africa, traditional healers have for centuries been the main providers of primary health care (Kala *et al.*, 2004). In East Africa, including Kenya, plants are still widely used in treatment of various ailments. Many plants derived natural products have been reported to exhibit activities against parasitic protozoa (Wright and Phillipson, 1990). These studies show that bioactive products of these plants may be useful in modern medicine. *Erythrina* species have received considerable attention recently (Prozesky *et al.* 2001). The flavonoids of *Erythrina* species have been associated with anti-infective activities, including antimicrobial and antiplasmodial activities. The crude extracts and flavonoids isolated from two *Erythrina* species, *E. abyssinica* and *E. burttii*, have been investigated for *in vivo* an *in vitro* antiplasmodial, anti-oxidant and antimicrobial activities. Despite alkaloids being one of the major metabolites of this genus, they

have not received much attention of late, except for antioxidant and antimicrobial activities reported for the alkaloids isolated from *Erythrina mulungu*.

Although alkaloids have been traditionally isolated from plants, an increasing number are found in animals, insects, and marine invertebrates and microorganisms. Many alkaloids have been used for hundreds of years in medicine and some are still prominent drugs today. Hence, this group of compounds has had great prominence in many fields of scientific endeavor and continues to be of great interest today (Roberts, M. 1998). Two alkaloid bearing plants with wide traditional uses, *Erythrina brucei* and *Teclea nobilis* have been phytochemically investigated.

1.2 Problem statement

Infectious diseases are a major problem in developing countries with malaria and other microbial infections being among the leading causes of deaths in Africa (WHO, 2002). Controlling these infectious diseases is more difficult today because of the emergence of drug resistance. At the same time the burden of infectious diseases is high and patients with a resistant infection may be unable to obtain or afford any of the available drugs.

1.3 Justification

Traditional medicine continues to offer health coverage for over 80% of the world population, especially in the developing world. Natural products have made an important contribution in identification of lead compounds; this is due to their use in the treatment of different ailments since antiquity. However, only about 20% of the plants with claimed ethnomedical uses have been subjected to bioassay screening.

It can be appreciated that the plants of genus *Erythrina* are sources of secondary metabolites with unusual chemical structures such as alkaloids and flavonoids. *Erythrina* species have received

considerable attention recently (Prozesky *et al.* 2001). The flavonoids of *Erythrina* species have been associated with anti-infective activities, including antimicrobial and antiplasmodial activities. The crude extracts and flavonoids isolated from *Erythrina* species have been investigated for antiplasmodial, anti-oxidant and antimicrobial activities.

Despite alkaloids being one of the major metabolites of this genus, they have not received much attention although many of them have been used for hundreds of years in medicine and some are still prominent drugs today.

Alkaloids have had great prominence in many fields of scientific endeavor and continue to be of great interest today (Roberts, 1998). Alkaloids are commonly isolated from the seeds and flowers of *Erythrina* species. Preliminary TLC analysis of the stem bark of *Erythrina brucei* showed the presence of alkaloids unexpectedly. There is only one report on the occurrence of alkaloids on the stem bark of *Erythrina* species. This research project, focused at isolating the constituents of the stem bark of *Erythrina brucei* and *Teclea nobilis*.

1.4 Objectives

1.4.1 General objectives

To isolate and characterize antimalarial, antioxidant, and antimicrobial compounds from *Erythrina burttii*, *Erythrina abyssinica*, *Erythrina brucei* and *Teclea nobilis*.

1.4.2 Specific objectives

1. To isolate compounds from the stem bark and root bark of *Erythrina abyssinica, Erythrina brucei*, *Erythrina burttii* and *Teclea nobilis*.
2. To characterize the structures of the isolated compounds.
3. To establish the anti-plasmodial, antimalarial, antioxidant, antimicrobial activities of crude extracts and isolated compounds.

CHAPTER TWO

2.0 LITERATURE REVIEW

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents. Today, natural products (including their derivatives and analogs) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing 25% of the total (Balandrin *et al.*, 1993). The World Health Organization estimates that 80% of the people in developing countries rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts (WHO, 2002). This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (De Smet, 1997). Further evidence of the importance of natural products is provided by the fact that almost half of the world's 25 best selling pharmaceuticals are either natural products or their derivatives (Pezzuto, 1997).

Conservative estimates suggest that there are more than 250,000 species of higher plants existing on this planet, and only a very small percentage of these have been exhaustively studied for their potential value as a source of drugs. Obviously natural products will continue to be important as sources of medicinal agents (Pezzuto, 1997).

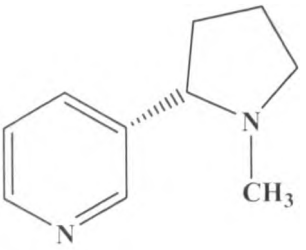
In addition to the natural products which have found direct medicinal application as drug entities, many others can serve as chemical models or templates for the design, synthesis, and semi-synthesis of novel substances for treating humankind's diseases.(Faccini, 2001). These substances embrace some of the most exciting new therapeutic agents currently available for use in clinical setting. Although there are some new approaches to drug discovery, such as

combinatorial chemistry and computer-based molecular modeling design, none of them can replace the important role of natural products in drug discovery and development (Pezzuto, 1997). Among all classes of natural products, alkaloids are among the most used as drugs directly or serve as templates for more active synthetic analogues (Pezzuto, 1997).

2.1 Alkaloids

Alkaloids include those natural products that contain nitrogen, usually as part of a cyclic system. These alkaloids have been divided into three major classes depending on the precursors and the final structure (Waterman, 1993). They include true-alkaloids, *pseudo*-alkaloids and *proto*-alkaloids. The true-alkaloids are derived from amino acids. They are basic and contain nitrogen in a heterocyclic ring, for example, nicotine (**14**) and atropine (**15**). Common alkaloid ring structures include the pyridines, pyrroles, indoles, pyrrolidines, iso-quinolines and piperidines. The *pseudo*-alkaloids are basic but are not derived from amino acids, for example, caffeine (**16**). While the *proto*-alkaloids are basic and are derived from amino acids, but the nitrogen is not in a heterocycle, for example, phenylethylamine-derived alkaloid such as mescaline (**17**).

Alkaloids occur widely in plants and are perhaps best known for their pharmacological properties (Herbert, 2001). Many common drugs are alkaloid-based. Examples with mild physiological effects include mescaline (**17**) and nicotine. (**14**) More potent examples include cocaine (**18**) and morphine (**19**).



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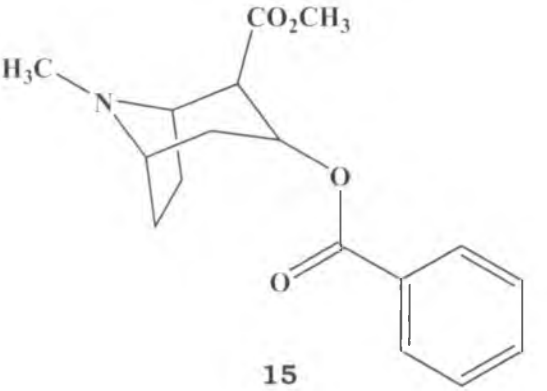

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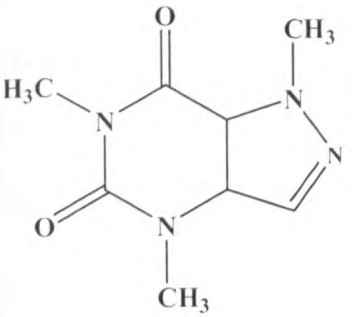

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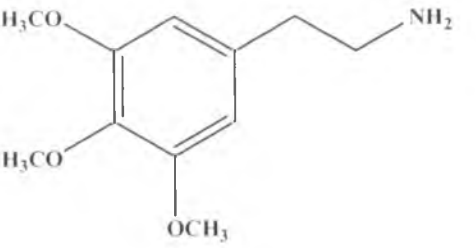

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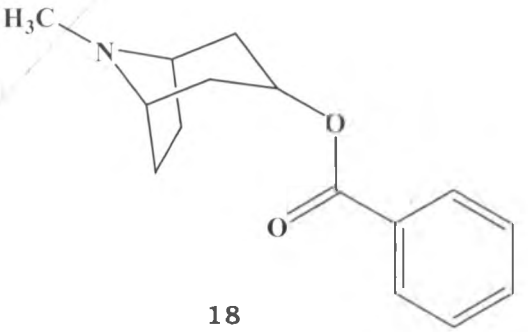

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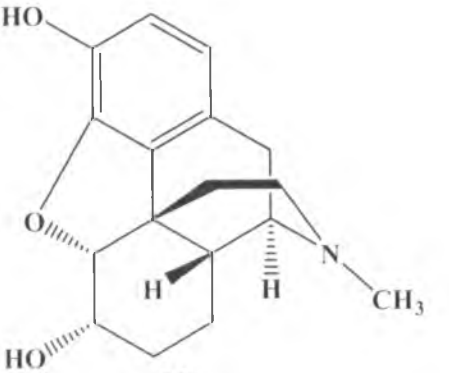

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2.2 BOTANICAL INFORMATION

2.2.1 THE FAMILY FABACEAE

The family Fabaceae, also known as Leguminosae, is the second largest family of dicotyledons after Asteraceae. It is widely distributed worldwide (Hegnauer and Barkmeijer, 1993) and is the third largest family of flowering plants after Oichidiaceae and Asteraceae.

Fabaceae comprises of some 650 genera and 20,000 species of herbs, trees, shrubs and lianas. The one character that members of this family share in common is the pod otherwise technically known as the legume. Members of this family are also recognized for their ability to support nitrogen fixation through symbiosis (Harborne *et al.*, 1971).

It is indicated that the family Fabaceae is one of the few families where investigation for flavonoids is most comprehensive (Hegnaur and Grayer Barkmeijer, 1993). The main reason for phytochemical interest in this family is the diversity of flavonoid metabolism. In addition to flavones and flavonols, which are commonly found in these plants, this family is also known for the production of chalcones and 2,3-dihydroflavonoids such as flavanones. Furthermore, this family elaborate isoflavonoids which are almost entirely restricted to this family (Hegnaur and Grayer Barkmeijer, 1993; Dewick, 1994).

2.2.2 The genus *Erythrina*

The genus *Erythrina* comprises more than 100 species that are widely distributed in tropical and subtropical regions of the world (Cui *et al.*, 2008). In Africa, it is represented by about 30 species, five of these are found in Kenya. These are *E. abyssinica, E. burttii, E. excelsa, E. malancantha* and *E. sacleuxii* (Beentje., 1994).

2.2.2.1 *Erythrina abyssinica*

Erythrina abyssinica is a tree of about 15 m tall with a deciduous bark that is yellow-brown, thick and corky, fissured, usually with thick spines. Its mature leaves are usually velvety-hairy beneath. It is found in Western and Central regions of Kenya (Beentje, 1994). Its wood is used for making doors, stools and beehives. The Maasai brew a tonic tea from the roots.

Figure 1: Picture of *Erythrina abyssinica*

2.2.2.2 *Erythrina burttii*

Erythrina burttii is a flat-topped tree 5-17 m tall. Its branches are spiny, which bear the leaves and flowers which are straight and grow horizontally above the larger branches. It often flowers when leafless. Its flowers and seeds are red. It grows in wooded or scattered tree grassland (Beentje, 1994).

Figure 2: Picture of *Erythrina burttii*

2.2.2.3 *Erythrina brucei*

Erythrina brucei is a tree growing to 15-20 m with colorful flowers which is widely distributed only on the highlands of Ethiopia (Thulin, 1983). The plant has been used in Ethiopian medicine folk among other things for the treatment of ear infections (Games *et al*, 1974).

Figure 3: Picture of *Erythrina brucei*

2.2.3 Ethnomedical information on the genus *Erythrina*

The genus *Erythrina* has a long history of use in indigenous medicinal practices for the treatment of various diseases worldwide (Mitscher *et al.*, 1987). For example, it was known long ago that the seed extracts of *E. americana* produce a strong curare-like action. Thus, the use of such extract was suggested as a substitute for curare, which has been used therapeutically against tetanus and other convulsions. On the other hand, the stem bark of *E. abyssinica* has been used in folk medicines for the treatment of trachoma, malaria and elephantiasis and the roots have been used in the treatment of syphilis by traditional African healers (Kokwaro, 1993). The natives of South America used concentrated extracts of these species as arrow poisons, as an antidote against strychnine or as a hypnotic and antiepileptic (Folkers and Unna, 1938). Ethnomedical uses of some *Erythrina* species are shown in table 2.1.

Table 2.1: Ethnomedical uses of some *Erythrina* species

Species (Plant part)	Use	Reference
Erythrina abyssinica		
Stem bark	Trachoma, elephantiasis, malaria	Ichimaru *et al.*, 1996
Root bark	Syphilis	Ichimaru *et al.*, 1996
Leaf	Leprosy	Boily and van Puyvelde, 1986
Erythrina americana		
Entire plant	Malaria	Hastings, 1990
Stem bark	Contraception	
Flowers	Insomnia	
Fruit	Anti-inflammation	
Leaves	Abscesses	
Seed	Similar in effect to curare	
Erythrina crista-galli		
Stem bark	Diarrhoea, respiratory and urinary tract infection	Perez and Anesini, 1994
Leaf	Anti-haemorrhoidal	Bandoni *et al.*, 1976
Erythrina folkersii		
Stem bark	Treat inflammation of the womb	Zamora-Martinez and Pola , 1992
Entire plant	Relieves appendicitis	Hastings, 1990
Erythrina indica		
Stem bark	Astringent, febrifuge, emenagogue	Khan *et al.*, 1994
Leaves	Laxative	Chopra and Ghosh, 1935
Erythrina mildbraedii		
Stem bark	Aphrodiasac	Vasileva, 1969
Erythrina mulungu		
Stem bark	Reduce fever	Brandao *et al.*, 1985
Erythrina sacleuxii		
Root bark	Malaria	Gessler *et al.*, 1994
Leaf	Malaria	Gessler *et al.*, 1994
Erythrina senegalensis		
Stem bark	Serious wounds, jaundice, bronchial infections, leprosy, venereal disease, dysentery	Le Grand, 1989
Root bark	Malaria	Etkin, 1997
Erythrina variegata var. orientalis		
Stem Bark	Analgesic, rheumatism	Duke and Ayensu 1985
Leaves	Emmenagogue	Saha *et al.*, 1961

2.2.4 Biological activities of *Erythrina* species

When the alcoholic extract of seeds of *Erythrina americana* was applied to dogs at different doses, it provoked a similar activity to that of tubocurarine (muscle relaxant) (Lozoya and Lozoya, 1982). Pharmacological assays performed with the alkaloids of *E. americana* have shown anticonvulsant, hypnotic and analgesic effects (Garín-Aguilar *et al.*, 2000). *E. glauca* and *E. lysistemon* have been reported to possess antiviral, antibacterial, and estrogenic activity (Ito, 1999; Tanee *et al.*, 2007). Furthermore, analgesic and anti-inflammatory effects were observed for an aqueous extract of the stem bark of *E. senegalensis* (Saidu *et al.*, 2000).

The biological activity of *Erythrina mulungu* is associated to alkaloids (Ozawa *et al.* 2009). Many of these alkaloids have demonstrated anti-inflammatory, cardioactive, narcotic, and sedative activities (Parsons and Palframan, 2010). The alkaloids erythravine (**143**) and 11-hydroxy erythravine isolated from the flowers of *E. mulungu* have inhibited chemically induced seizures in rats (Faggion *et al.* 2011). *E. mulungu* has also demonstrated antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and antimycobacterial activity against *Mycobacterium fortuitum* and *M. smegmatis* (Virtuoso *et al.*, 2005; Ferreira de Lima *et al.*, 2006).

From the bark of *E. abyssinica*, coumestans and benzofurans have been isolated with stimulatory effects on AMP-activated protein kinase (AMPK) (Nguyen *et al.*, 2010). These have been proposed as a therapeutic target for the treatment of metabolic syndrome including obesity and Type-2 diabetes.

Erymildbaedin A and B isolated from the stem bark of *E. mildbraedi* have strongly inhibited the growth of human breast, prostate, and endometrial adenocarcinoma cell lines (Tchokouaha *et al.*

2010). The pterocarpans Erybraedin A (**109**), B (**110**) and C (**111**) isolated from *E. mildbraedii* showed potent anti-bacterial activities against *Staphylococcus aureus* and *Mycobacterium smegmatis* (Mitscher *et al.*, 1988a). Biseryvarin A from the roots of *E. variegata* showed low antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) while erybacin B isolated from the roots of *E. herbacea* has showed a potent bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Tanaka *et al.*, 2010b).

The methanol extract of the bark of *E. variegata* showed significant antimalarial activity toward *Plasmodium falciparum in vitro* using the lactate dehydrogenase assay, while the ethyl acetate fraction showed the most activity, exhibiting equipotency against both strains of parasite with IC_{50} of 23.8 µg/ml against 3D7 and 9.3 µg/ml against K1 (Herlina *et al.*, 2009).

Furthermore, by using the antimalarial activity to follow separation, the ethyl acetate fraction was separated by combination of column chromatography to yield an active compound warangalone (**101**). Which showed antiplasmodial activity against both strains of parasite used with IC_{50} of 4.8 µg/ml against 3D7 and 3.7 µg/ml against K1 respectively (Herlina *et al.*, 2009). Pterocarpan-type flavonoids with antibacterial, antiplasmodial and cytotoxic activities were isolated from the stems of *E. fusca* (Innok *et al.*, 2010).

From the acetone extracts of *Erythrina abyssinica* (Yenesew *et al.* 2003) antiplasmodial activity was reported due to the presence of flavonoids and isoflavonoids rather than to alkaloids. A pterocarpene from *E. mildbraaaiii* has shown anti-oxidant and anti-inflammatory activities (Njamen *et al.*, 2003).

2.3 Phytochemical information

2.3.1 Compounds reported from the genus *Erythrina*

The genus *Erythrina* is very rich in secondary metabolites belonging to various classes. The presence of alkaloids in seeds of several species of *Erythrina* has been known since 1930 and it has been shown that close botanical relationship between members of the genus *Erythrina* is paralleled by the presence of a series of isoquinoline alkaloids (Jackson, 1985, Dyke and Quessy, 1981). The other secondary metabolites present in this genus are flavanones, flavonols, chalcones, cinnamoylphenols, stilbenoids, isoflavones, isoflavans, isoflavanones, pterocarpans, isoflav-3-enes, 3-phenoxychromones, coumastans, 3-phenylcoumarins, lignans, cinnamate esters, simple phenolics, triterpenes, sesquiterpenes, long chain carboxylic acids, and long-chain alcohols (Majinda *et al.*, 2005). Flavonoids are the most prevalent and have been reported from almost all *Erythrina* species so far investigated.

2.3.1.1 Flavonoids

Flavonoids are widely distributed in higher plants and have been known as natural plant compounds for a long time. In spite of this, the interest on biological activities of these compounds has been limited. However, there is considerably increased interest on plant flavonoids as human dietary components, as pharmacological agents and as having significant activity in a variety of isolated animal cell systems (Harborne *et al.*, 1986). The term "flavonoid" is generally used to describe a broad collection of natural products that have a C6-C3-C6 carbon framework, or more specifically phenyl benzopyran functionality. Depending on the position of the linkage of the aromatic ring (Fig 4) to the benzopyrano (chromano) moiety, this group of natural products may be divided into three sub-classes: flavonoids (2-phenylbenzopyrans, **21**),

isoflavonoids (3-benzopyrans, **22**), and neoflavonoids (4-benzopyrans, **23**). All the three groups share a common chalcone precursor, and therefore are biogenetically and structurally related (Marais *et al.*, 2006).

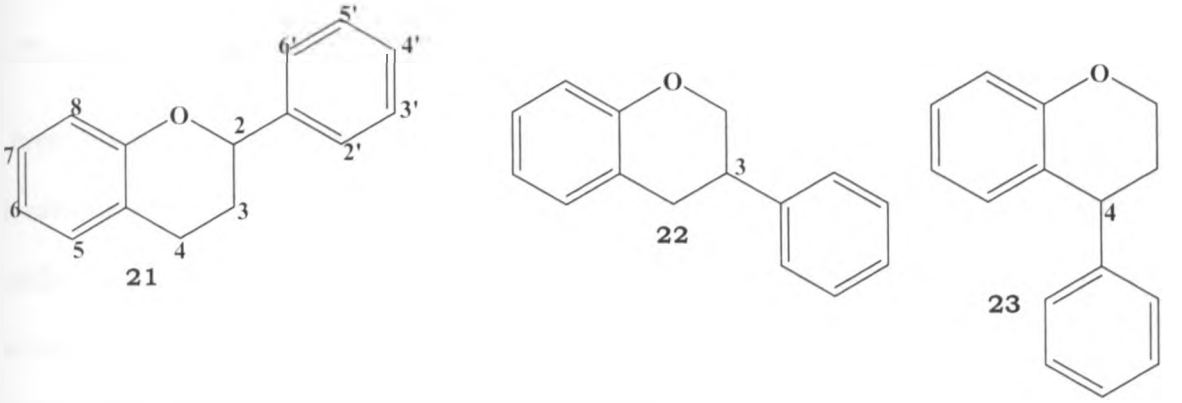


Figure 4: The skeletal structures of flavonoid sub-classes

Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into different subgroups including flavans, flavanones and flavones (Fig 5). Among the flavonoids of *Erythrina*, the flavanones are the most widely distributed and these are listed in Table 2.2.

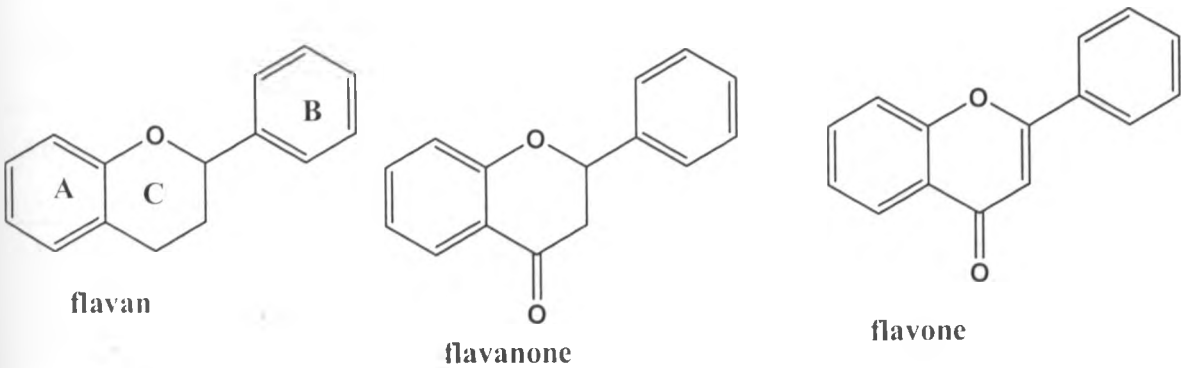


Figure 5: Some flavonoid subclasses

2.3.1.1.1 Flavanones of *Erythrina*

The basic flavanone of this genus is 5,7,4'-tri-oxygenated, with some exceptions where loss of oxygenation at C-5 has been observed; some examples are abyssinone II (**46**), III (**34**) and IV (**35**) isolated from *E. abyssinica*.

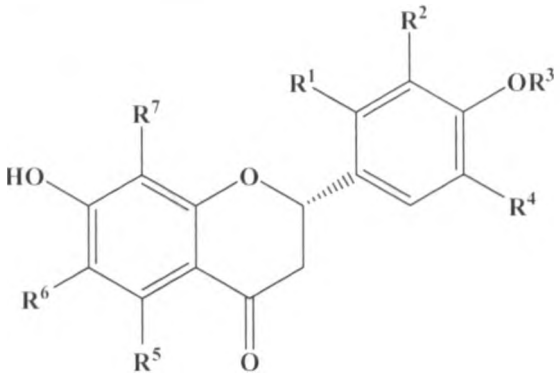
Prenylation is a common feature among flavanones; it usually occurs in the ring B and is mostly located at C-3' and/or at C-5'. In some cases prenylation may occur in ring A, an example is citflavanone (**49**). All flavanones are *C*-prenylated, with the exception of sigmoidin L (**41**), which is *O*-prenylated.

O-methylation is observed in ring B at C-3' and C-4', although it is not a common feature of these compounds. Some exceptions are abyssinin II (**57**).and burttinone (**48**).

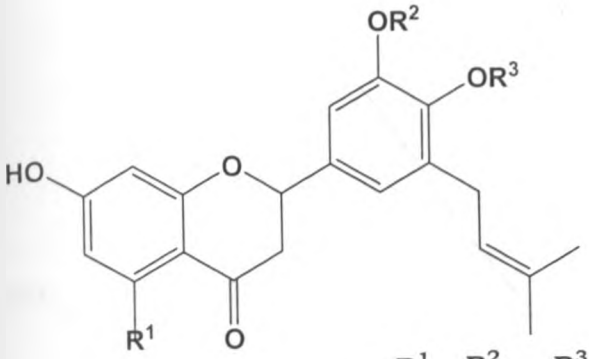
Table 2.2: Flavanones of *Erythrina*

Flavanones	**Source (plant part)**	**Reference**
5-Deoxyabyssinin II (**30**)	*E. abyssinica* (SB)	Induli, 2006
Sigmoidin B (**31**)	*E. sigmoidea* (AP)	Fomum *et al.*, 1986c
Abyssinone IV (**32**)	*E. abyssinica* (RB)	Kamat *et al.*, 1981
	E. sigmoidea (SB)	Nkengfack *et al.*, 1994b
	E. sacleuxii (SB)	Yenesew *et al.*, 2000
Abyssinone V (**33**)	*E. abyssinica* (RB)	Kamat *et al.*, 1981
	E. burttii (SB)	Yenesew *et al.*, 1998a
Abyssinin III (**34**)	*E. abyssinica* (SB)	Ichimaru *et al.*, 1996
Sigmoidin A (**35**)	*E. sigmoidea* (AP)	Fomum *et al.*, 1986c
Sigmoidin C (**36**)	*E. eriotriocha* (SB)	Nkengfack *et al.*, 1991
	E. sigmoidea (AP)	Fomum *et al.*, 1986c
Sigmoidin D (**37**)	*E. sigmoidea* (AP)	Promsatha *et al.*, 1987
Sigmoidin E (**38**)	*E. sigmoidea* (AP)	Promsatha *et al.*, 1988

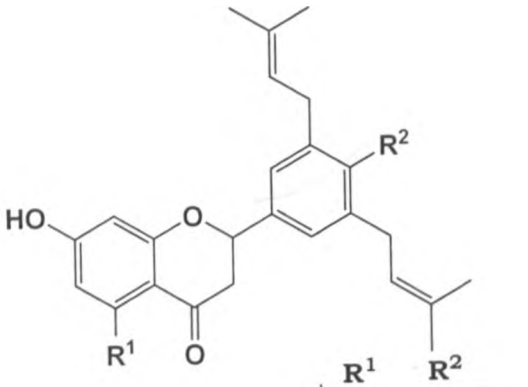
Table 2.2: Flavanones of *Erythrina*		
Sigmoidin F(**39**)	*E. sigmoidea* (AP)	Promsatha *et al.*, 1989
Sigmoidin G (**40**)	*E. sigmoidea* (AP)	Nkengfack *et al.*, 1993
Sigmoidin L (**41**)	*E. sigmoidea* (SB)	Nkengfack *et al.*, 1997
Abyssinoflavanone IV (**42**)	*E. abyssinica* (SB)	Moriyasu *et al.*, 1998
Abyssinoflavanone V(**43**)	*E. abyssinica* (SB)	Moriyasu *et al.*, 1998
Abyssinoflavanone VI (**44**)	*E. abyssinica* (SB)	Moriyasu *et al.*, 1998
Abyssinone I (**45**)	*E. abyssinica* (RB)	Kamat *et al.*, 1981
Abyssinone II(**46**)	*E. abyssinica* (RB)	Kamat *et al.*, 1981
Abyssinone-V-4'-methyl ether (**47**)	*E. burttii* (SB)	Yenesew *et al.*, 1998a
	*E. lysistemon (*SB*)*	El-Masry *et al.*, 2002
	E. sacleuxii (SB)	Yenesew *et al.*, 2000
Burttinone (**48**)	*E. burttii* (SB)	Yenesew *et al.*, 1998a
	E. lysistemon (SB)	El-Masry *et al.*, 2002
Citflavanone (**49**)	*E. eriotriocha* (SB)	Ito *et al.*, 1988
Eriotrinol (**50**)	*E. eriotriocha* (SB)	Nkengfack *et al.*, 1993
Erythrisenegalone (**51**)	*E. senegalensis* (SB)	Fomum *et al.*, 1985
Isobavachin (**52**) (**78**)	*E. variegata* (RB)	Telikepalli *et al.*, 1990
Lupinifolin (**53**)	*E. senegalensis* (SB)	Wandji *et al.*, 1994c
Senegalensein (Lonchocarpol A) (**54**)	*E. senegalensis* (SB)	Fomum *et al.*, 1987
Sigmoidin B-3'-methyl ether (**55**)	*E. abyssinica* (SB)	Ichimaru *et al.*, 1996
	E. berteroana (SB)	Maillard *et al.*, 1987
	E. sigmoidea (AP)	Promsatha *et al.*, 1986
Sigmoidin B-4'-methyl ether (**56**)	*E. abyssinica* (SB)	Ichimaru *et al.*, 1996
	E. burttii (SB)	Yenesew *et al.*, 1998a
Abyssinin II (**57**)	*E. abyssinica(*SB*)*	Kamat *et al.*, 1981



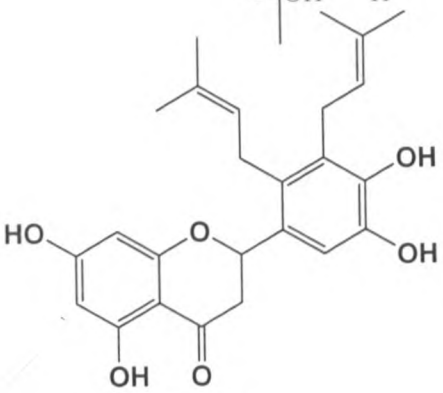
	R^1	R^2	R^3	R^4	R^5	R^6	R^7
46	H	H	H	Prenyl	H	H	H
54	H	H	H	H	OH	Prenyl	Prenyl
57	H	OMe	H	Prenyl	OH	H	H
55	H	Prenyl	H	OMe	OH	H	H
56	H	Prenyl	Me	OH	OH	H	H
47	H	Prenyl	Me	Prenyl	OH	H	H



	R^1	R^2	R^3
30	OH	Me	H
31	OH	H	H



	R^1	R^2
32	H	OH
33	OH	OH



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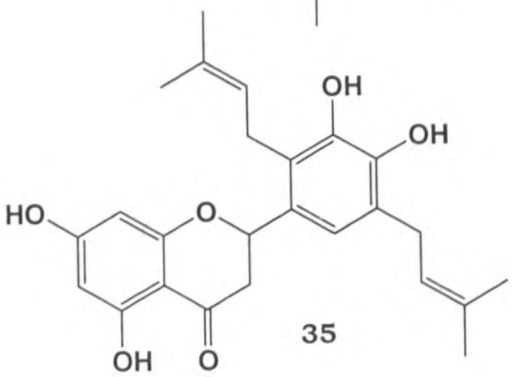

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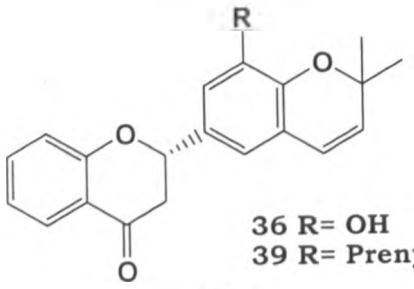
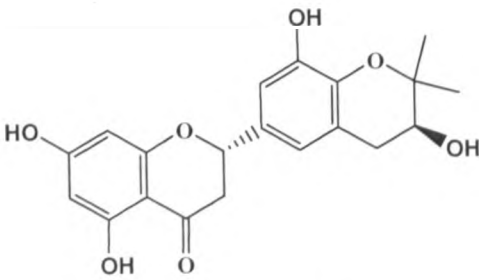


36 R= OH
39 R= Prenyl



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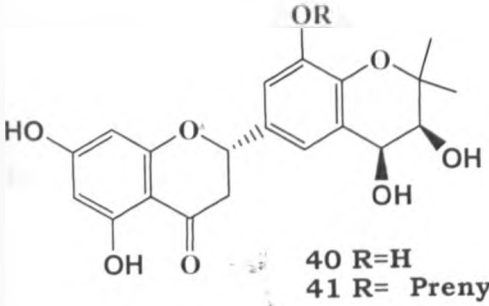
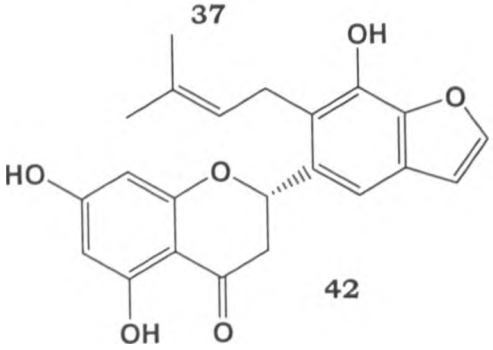


40 R=H
41 R= Prenyl



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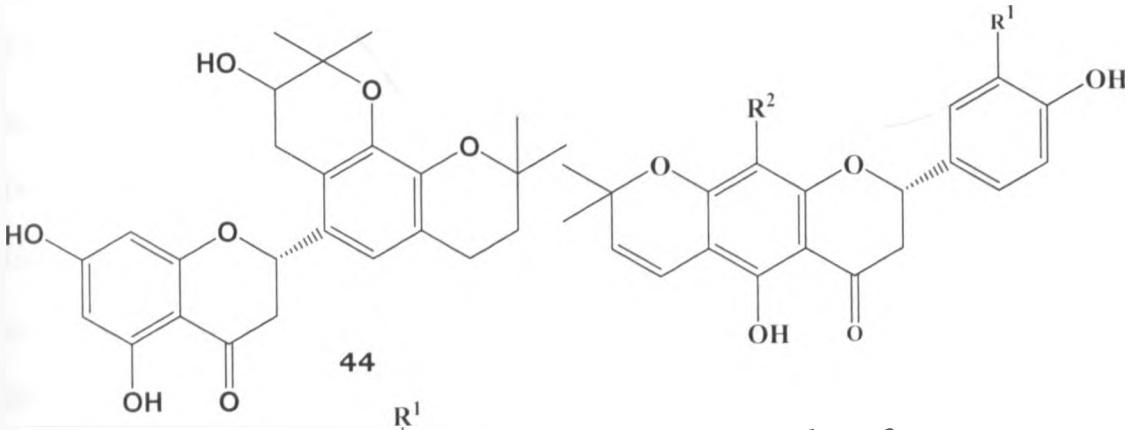

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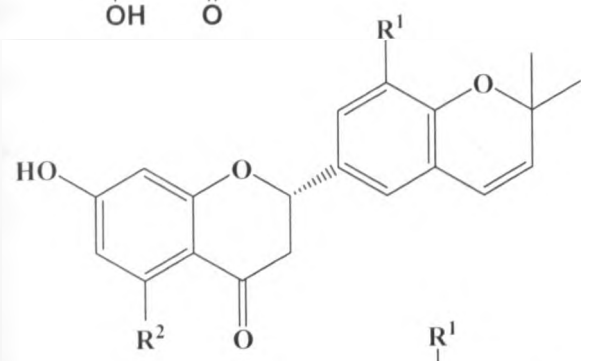
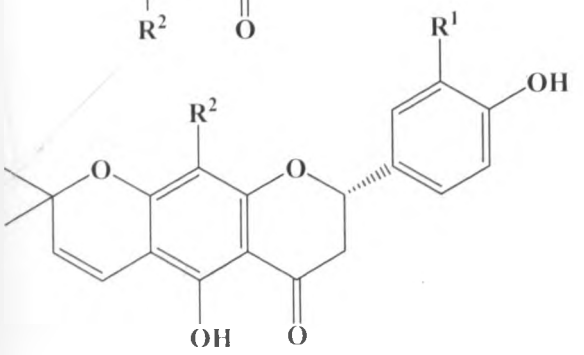
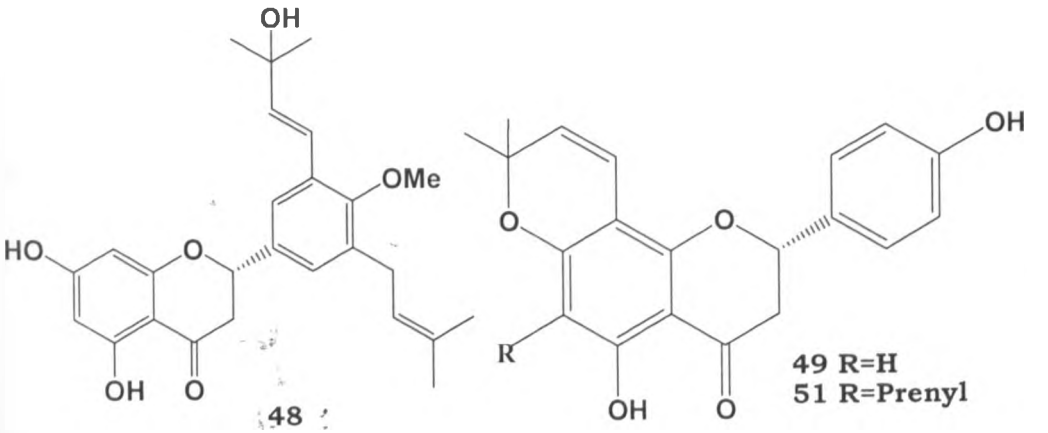


	R^1	R^2
11	Prenyl	H
45	H	H
59	Prenyl	H
66	OMe	OH



	R^1	R^2
50	OMe	H
53	H	Prenyl



48

49 R=H
51 R=Prenyl

2.3.1.1.2 Isoflavonoids of *Erythrina*

The isoflavonoids are a distinctive subclass of the flavonoids possessing a 3-phenylchroman skeleton, biogenetically derived by 1,2-aryl migration from a 2-phenylchroman precursor. Despite their limited distribution in the plant kingdom, isoflavonoids are remarkably diverse as far as structural variations are concerned (Marais *et al.*, 2006). This arises not only from the number and complexity of substituents on the basic 3-phenylchroman system, but also from the different oxidation levels and presence of additional heterocyclic rings (Marais *et al.*, 2006). The most commonly found isoflavonoids (Figure 6) of this genus are isoflavones (Table 2.5), isoflavanones (Table 2.3), isoflavans (Table 2.4), isoflav-3-enes (Table 2.6) and pterocarpanoids (Table 2.7).

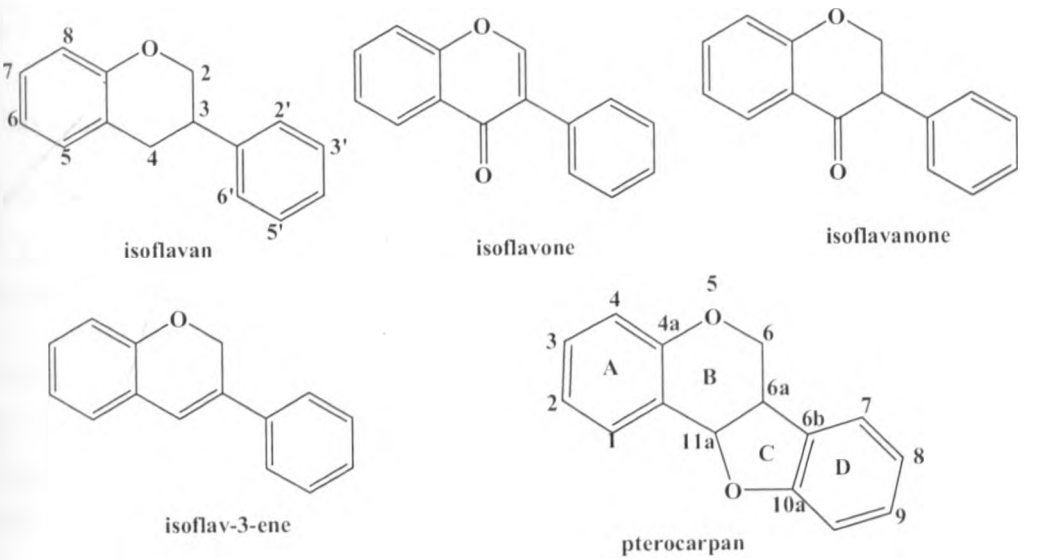


Figure 6: Commonly found isoflavonoids subclasses of *Erythrina*

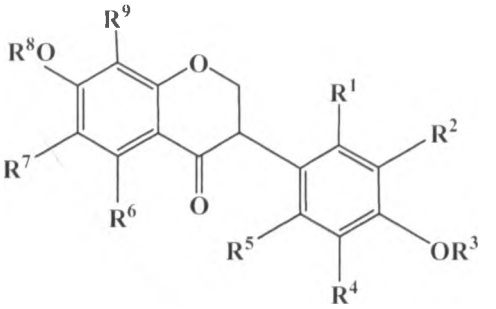
2.3.1.1.3 Isoflavanones of *Erythrina*

Isoflavanones are biosynthetic intermediates between isoflavones and pterocarpans which mainly accumulate when the plants are challenged by micro-organisms and hence are phytoalexins in most cases [Dewick, 1994]. Prenylation is common and tends to occur at C-6 and /or C-8 of ring A, or at C-3' or C-5' of ring B. The only exception to this is 2',5,7-trihydroxy-3'-methoxy-4'-prenylisoflavanone (**60**) which has a prenyl group at C-4' position of ring B. The common cyclization product involving a prenyl group and an adjacent hydroxyl has been observed to be the dimethylpyran substituent. Methylation also occurs and is mostly observed in ring B. The common configuration is usually R at C-3 among *Erythrina* isoflavanones.

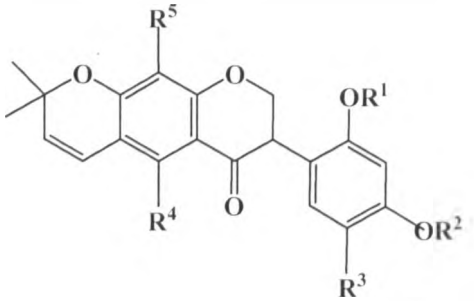
Table 2.3: Isoflavanones of *Erythrina*

Isoflavanone	**Source (plant part)**	**Reference**
2, 3-Dihydroauriculatin (**58**)	*E. senegalensis* (SB) *E. eriotriocha* (SB)	Taylor *et al.*, 1986 Nkengfack *et al.*, 1990a
2',4',7-Trihydroxy-3',6-diprenylisoflavanone (**59**)	*E. lysistemon* (RB)	McKee *et al.*, 1997
2',5,7-Trihydroxy-4'-methoxy-5' prenylisoflavanone (**60**)	*E. berteroana* (SB)	Maillard *et al.*, 1989
2'-Hydroxyneobavaisoflavanone (**61**)	*E. lysistemon* (RB)	McKee *et al.*, 1997
5,7-Dihydroxy-2',4',5'-trimethoxyisoflavanone (**62**)	*E. latissimia* (SB)	Wanjala & Majinda, 2000
5-Deoxyglyasperin F (**63**)	*E. lysistemon* (RB)	Wanjala & Majinda, 2000
5-Deoxylicoisoflavanone (**64**)	*E. lysistemon* (RB)	Wanjala & Majinda, 2000
Bidwillon A (Eriotrichin-B) (**1**)	*E. bidwillii* (RB) *E. burttii* (SB) *E. eriotricha* (RB) *E. orientalis* (RB)	Iinuma *et al.*, 1992 Yenesew *et al.*, 1998a Nkengfack *et al.*, 1995 Tanaka *et al.*, 1998b

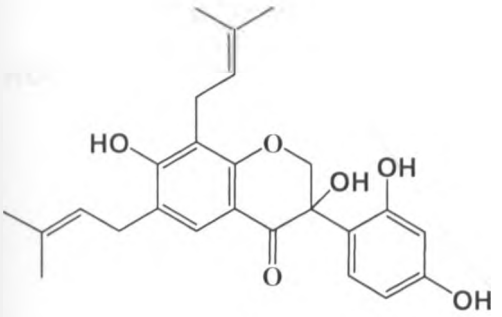
Bidwillon B (**66**)	*E. bidwillii* (RB)	Iinuma *et al.*, 1992
Erypoegins C (**67**)	*E. poeppigiana* (RB)	Tanaka *et al.*, 2002a
Erypoegins D (**68**)	*E. poeppigiana* (RB)	Tanaka *et al.*, 2002a
Erysenegalensein B (**69**)	*E. senegalensis* (SB)	Wandji *et al.*, 1995c
Erysenegalensein C (**70**)	*E. senegalensis* (SB)	Wandji *et al.*, 1995c
Erysenegalensein I (**71**)	*E. senegalensis* (SB)	Wandji *et al.*, 1994c
Eryvellutinone (**72**)	*E. vellutina* (SB)	Da-cunha *et al.*, 1996
Orientanol D (**73**)	*E. orientalis* (RB)	Tanaka *et al.*, 1998c
Orientanol E (**74**)	*E. orientalis* (RB)	Tanaka *et al.*, 1998c
Orientanol F (**75**)	*E. orientalis* (RB)	Tanaka *et al.*, 1998c
R-2,3-Dihydro-7-*O*-demethylrobustigenin (**76**)	*E. sacleuxii* (SB)	Tanaka *et al.*, 1998c
R-Saclenone (**77**)	*E. sacleuxii* (SB)	Yenesew *et al.*, 2000
Sigmoidin H (**78**)	*E. sigmoidea* (RB)	Nkengfack *et al.*, 1994b
Sigmoidin I (**79**)	*E. sigmoidea* (RB)	Nkengfack *et al.*, 1994a
Sigmoidin J (**80**)	*E. sigmoidea* (RB)	Nkengfack *et al.*, 1994c
Vogelin A (**81**)	*E. vogelli* (RB)	Queiroz *et al.*, 2002
Vogelin B (**82**)	*E. vogelli* (RB)	Queiroz *et al.*, 2002
Vogelin C (**83**)	*E. vogelli* (RB)	Queiroz *et al.*, 2002
Vogelin D (**84**)	*E. vogelli* (RB)	Queiroz *et al.*, 2002



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9
60	OH	H	Me	Prenyl	H	OH	H	H	H
76	OMe	H	Me	OMe	H	OH	H	H	H
64	OH	Prenyl	H	H	H	H	H	H	H
69	OH	H	H	OH	H	H	Prenyl	H	Prenyl
72	OH	H	H	H	H	H	Prenyl	H	Me
79	OMe	H	H	H	Prenyl	H	H	H	H
80	OMe	H	H	OMe	H	H	Prenyl	H	H
59	OH	Prenyl	H	H	H	H	Prenyl	H	H
62	OMe	H	Me	OMe	H	OH	H	H	H
67	OMe	H	H	Prenyl	H	OH	H	H	H
68	OMe	H	H	Prenyl	H	OH	H	H	Me
74	OH	Prenyl	H	H	H	OH	Prenyl	H	H
81	OH	H	Me	Prenyl	H	OH	H	H	H
82	OMe	H	H	Prenyl	H	OH	H	H	H
83	OMe	H	H	Prenyl	H	OH	H	H	Prenyl
84	H	Prenyl	Me	H	H	OH	Prenyl	H	H



	R^1	R^2	R^3	R^4	R^5
66	H	H	H	H	Prenyl
58	H	H	H	OH	Prenyl
70	H	H	OMe	OH	Prenyl
77	Me	Me	OMe	OH	H



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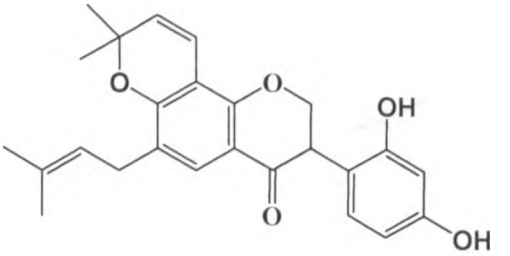

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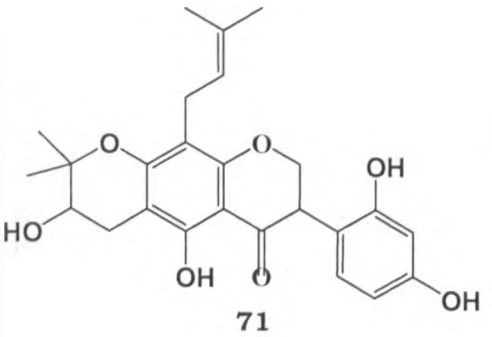

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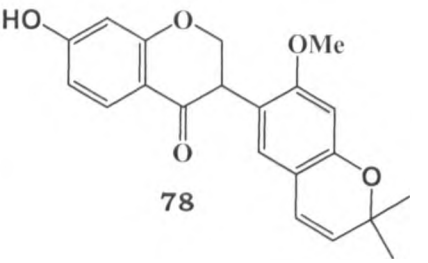

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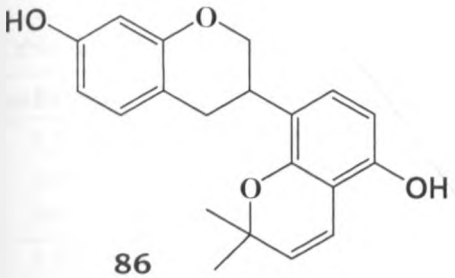
23.1.1.4 Isoflavans of *Erythrina*

Isoflavans are another subclass of isoflavonoids, most of them function as phytoalexins in legumes (Dewick, 1994).

Table 2.4: Isoflavans of *Erythrina*

Isoflavans	**Source (plant part)**	**Reference**
Demethylvestitol (**85**)	*E. sandwicensis* (LF)	Ingham, 1980
Erythbidin A (**86**)	*E. X bidwilli* (WD)	Tanaka *et al.*, 1998d
Eryvarin C (**87**)	*E. variegata* (RB)	Tanaka *et al.*, 2001c
Isovestitol (**88**)	*E. sandwicensis* (LF)	Ingham, 1980
Phaseollinisoflavan (**89**)	*E. X bidwilli* (WD)	Tanaka *et al.*, 1998d



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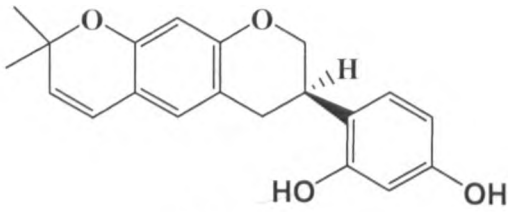

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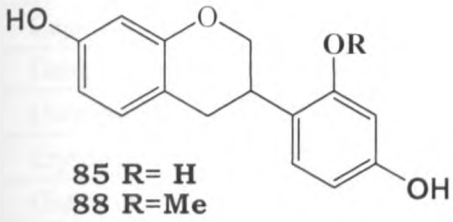
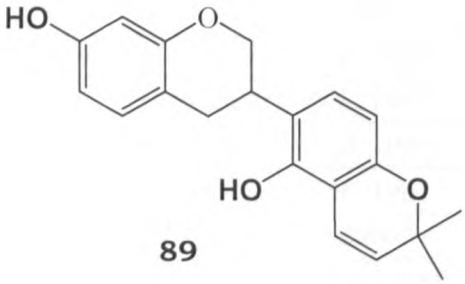


85 R= H
88 R=Me



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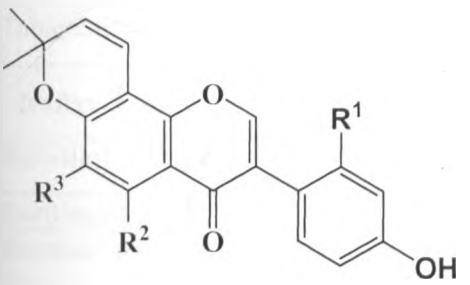
2.3.1.1.5 Isoflavones of *Erythrina*

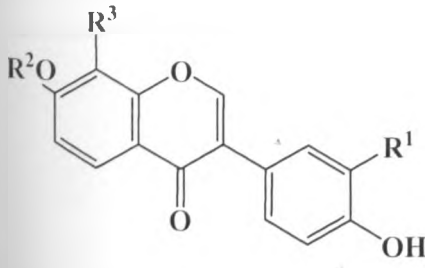
Isoflavones represent the largest group of natural isoflavonoids (Dewick, 1994). Isoflavones of this genus are also 4',5,7 tri-oxygenated, with some exceptions where loss of oxygenation at C-5 has been observed *vis* bidwillon C (**93**) and 8-prenyldaidzein (**91**).

Prenylation is a common feature of *Erythrina* isoflavones as in the flavanones of this genus. However, unlike the flavanones of this genus, prenylation in the isoflavones is mostly in ring A, at C-6 and/or C-8 positions. When prenylation is observed in ring B it is usually at C-3' position.

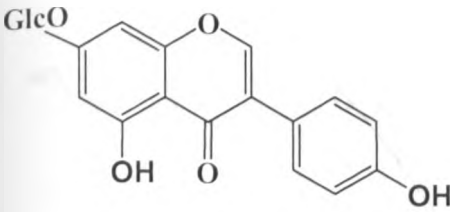
Table 2.5: Isoflavones of *Erythrina*		
Isoflavone	**Source (plant part)**	**Reference**
7,3'-Dihydroxy-5,4'-dimethoxy-5'-prenylisoflavone (5,4'-Dimethoxy-3'-prenylbiochanin-A) (**90**)	*E. eriotriocha* (SB)	Nkengfack *et al.*, 1990b
8-Prenyldaidzein (**91**)	*E. X bidwillii* (RB)	Iinuma *et al.*, 1992
Auriculatin-4'-*O*-glucoside (**92**)	*E. eriotriocha* (SB)	Nkengfack *et al.*, 1991
Bidwillon C (Isoerythrinin A) (**93**)	*E. X bidwillii* (RB)	Iinuma *et al.*, 1994
Cajanin (**94**)	*E. indica* (RB)	Waffo *et al.*, 2000
Daidzein 7-O-glucoside (Daidzin) (**95**)	*E. cristal-galli* (SB)	Imamura *et al.*, 1981
Derrone (**96**)	*E. cristagalli* (RB)	Mitscher *et al.*, 1987
Erythinin A (**97**)	*E. variegata* (SB)	Despande *et al.*, 1977
Genstein 7-*O*-glucoside (**98**)	*E. cristal-galli* (SB)	Imamura *et al.*, 1981
Indicanin C (**99**)	*E. indica* (RB)	Waffo *et al.*, 2000
Indicanine E (**100**)	*E. indica* (SB)	Nkengfack *et al.*, 2001
Warangalone (Scandenone) (**101**)	*E. eriotriocha* (SB)	Nkengfack *et al.*, 1989b
	E. variegata (WD)	Tanaka *et al.*, 2000



	R^1	R^2	R^3
93	H	H	H
94	H	OH	Prenyl
96	OH	OH	H



	R^1	R^2	R^3
91	H	H	Prenyl
95	H	Glc	H



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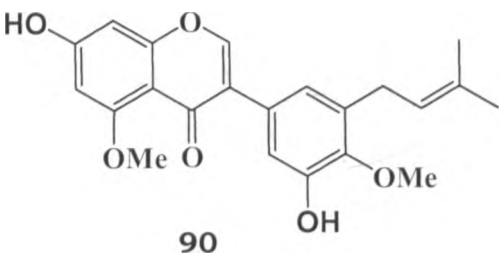

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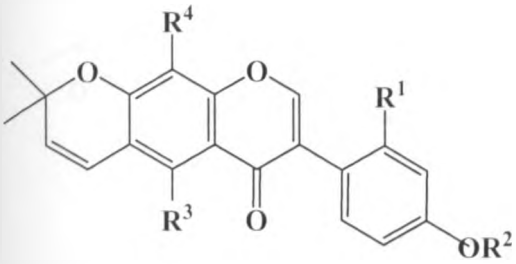


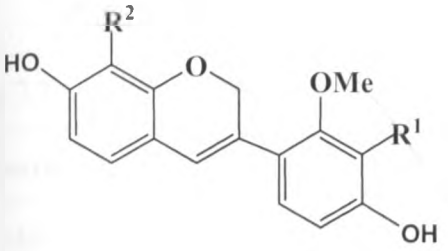
	R¹	R²	R³	R⁴
97	H	H	H	H
92	OH	Glc	OH	Prenyl
99	H	H	OMe	H
100	OH	Me	OMe	H
101	H	H	OH	Prenyl

2.3.1.1.6 Isoflav-3-enes of *Erythrina*

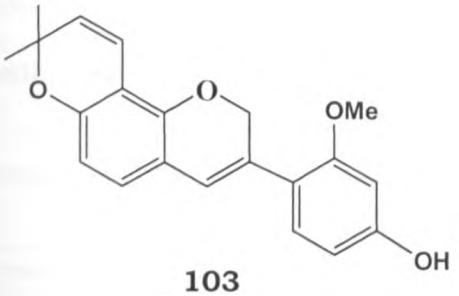
Isoflav-3-enes are also classified among the rare isoflavonoids.

Table 2.6: Isoflav-3-enes of *Erythrina*

Isoflav-3-enes	Source (plant part)	Reference
Bidwillol A (**102**)	*E. X bidwilli* (RB)	Iinuma *et al.*, 1994
Burttinol A (**103**)	*E. burttii* (RB)	Yenesew *et al.*, 2002
Burttinol B (Erypoegin B) (**104**)	*E. burttii* (RB) *E. poeppigiana* (RB)	Yenesew *et al.*, 2002 Tanaka *et al.*, 2002a
Burttinol C (Erypoegin A) (**105**)	*E. burttii* (RB) *E. poeppigiana* (RB)	Yenesew *et al.*, 2002 Tanaka *et al.*, 2002a



	R1	R2
102	Prenyl	H
105	H	Prenyl



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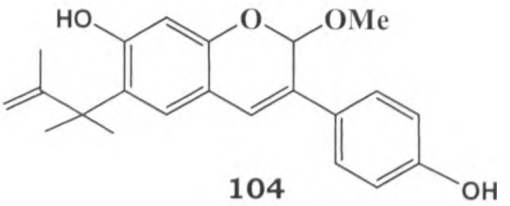


104

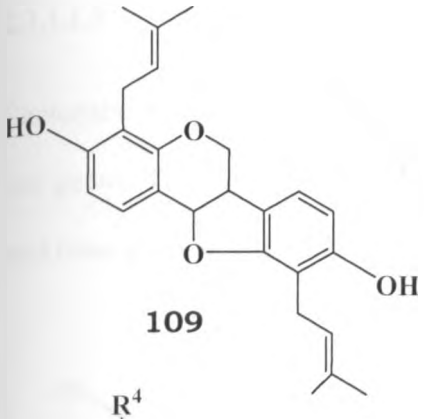
2.3.1.1.7 Pterocarpanoids of *Erythrina*

Pterocarpanoids are the second largest group of isoflavonoids (Dewick, 1994). Several pterocarpanoids tend to be phytoalexins, which are toxic compounds produced by plants following microbial infections, constituting a natural defense mechanism against microorganisms (Ingham, 1980). They are conveniently subdivided into pterocarpans, 6a-hydroxypterocarpans and pterocarpenes.

Prenylation is again a common feature among the pterocarpanoids of this genus, it usually occurs at C-2 and C-10 except in some cases where prenylation is at C-4 and/or C-8, e.g. erybraedin A (**109**). The prenyl group is modified in some cases to give a furano- or pyrano-ring.

Table 2.7: pterocarpanoids of *Erythrina*

Pterocarpans	**Source (plant part)**	**Reference**
1-Methoxyphaseollidin (**106**)	*E. vogellii* (RB)	Queiroz *et al.*, 2002
Calopocarpin (**107**)	*E. burttii* (SB)	Yenesew *et al.*, 1998a
Demethylmedicarpan (**108**)	*E. crista-galli* (SD)	Ingham and Markham, 1980
	E. sandwicensis (LF)	Ingham, 1980
Erybraedin A (**109**)	*E. burttii* (RB)	Yenesew *et al.*, 2002
	E. eriotricha (RB)	Nkengfack *et al.*, 1995
	E. midbraedii (RB)	Mitscher *et al.*, 1988b
Erybraedin B (**110**)	*E. midbraedii* (RB)	Mitscher *et al.*, 1988b
Erybraedin C (**111**)	*E. midbraedii* (RB)	Mitscher *et al.*, 1988b
Erybraedin D (**112**)	*E. midbraedii* (RB)	Mitscher *et al.*, 1988b
Erybraedin E (**113**)	*E. midbraedii* (RB)	Mitscher *et al.*, 1988b
Erycristin (**114**)	*E. crista-galli* (SB)	Mitscher *et al.*, 1988b
Erythrabysin II (**115**)	*E. abyssinica* (RB)	Kamat *et al.*, 1981
	E. X bidwilli (RB)	Iinuma *et al.*, 1992
	E. burttii (RB)	Yenesew *et al.*, 2002
	E. crista-galli (SB)	Mitscher *et al.*, 1988a
	E. orientalis (RB)	Tanaka *et al.*, 1998b
	E. poeppigiana (RB)	Tanaka *et al.*, 2002a
	E. sigmoidea (RB)	Nkengfack *et al.*, 1994c
	E. suberosa (RB)	Tanaka *et al.*, 2001b
	E. variegata (RB)	Telikepalli *et al.*, 1990
Pterocarpenes		
3-Hydroxy-9-methoxy-10-(3,3-dimethylally) pterocarpene (**116**)	*E.abyssinica* (RB)	Yenesew *et al.*, 2009
Erypoegin E (**117**)	*E. poeppigiana* (RB)	Tanaka *et al.*, 2002a
Eryvarin D (**118**)	*E. variegata* (RB)	Tanaka *et al.*, 2001c
Eryvarin E (**119**)	*E. variegata* (RB)	Tanaka *et al.*, 2001c



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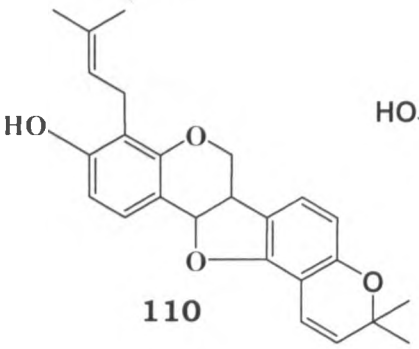

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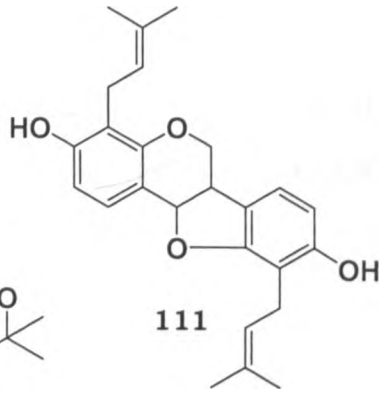

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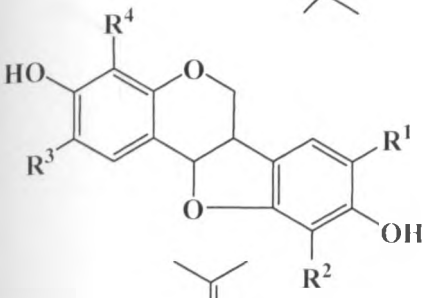
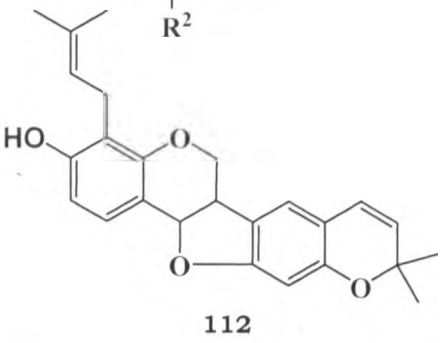


	R^1	R^2	R^3	R^4
107	H	H	Prenyl	H
108	H	H	H	H
106	H	Prenyl	OMe	H



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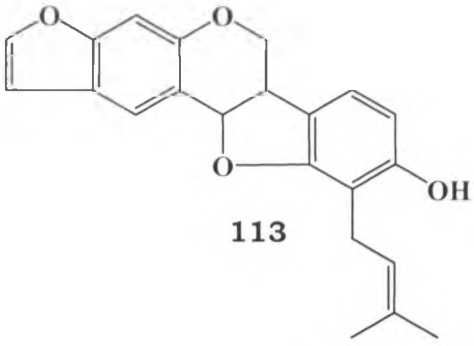

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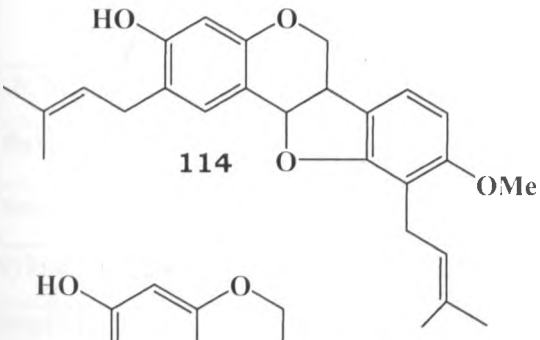

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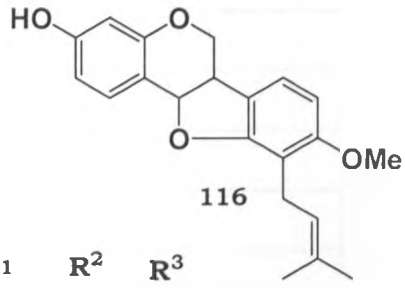

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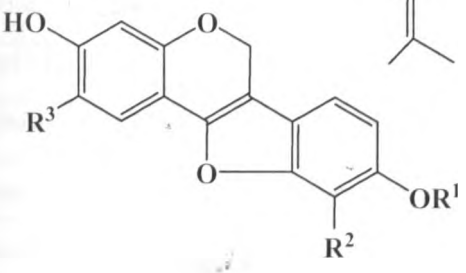


	R^1	R^2	R^3
117	H	Prenyl	H
118	Me	Prenyl	H
119	Me	Prenyl	Prenyl

2.3.1.1.8 Other minor flavonoids of *Erythrina*

Chalcones, 2-arylbenzofurans and 3-phenylcoumarins are classified as the minor flavonoids of this genus. They are listed in table 2.8. Natural chalconoids which also contain a C6-C3-C6 backbone are subdivided into chalcones, dihydrochalcones and retrochalcones (Fig. 7).

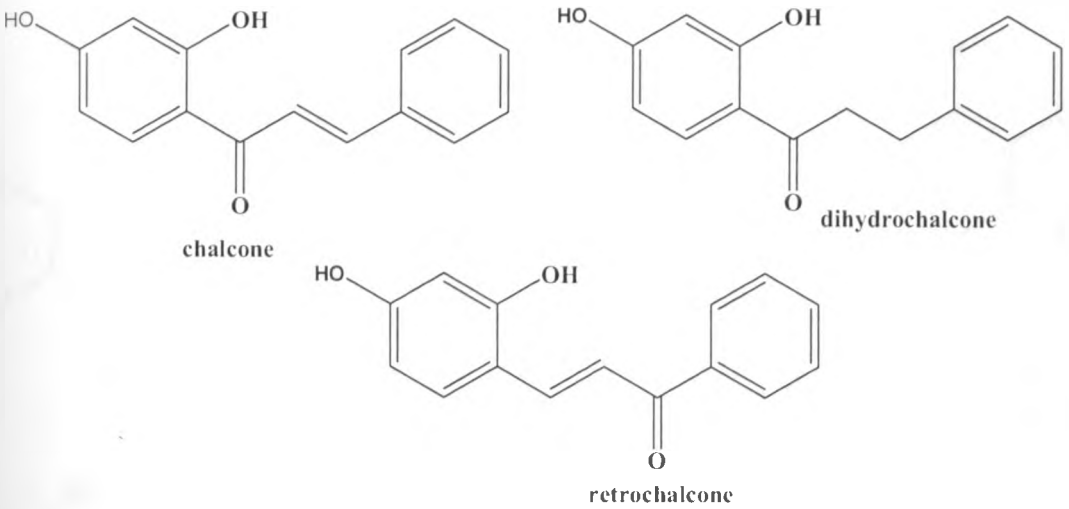
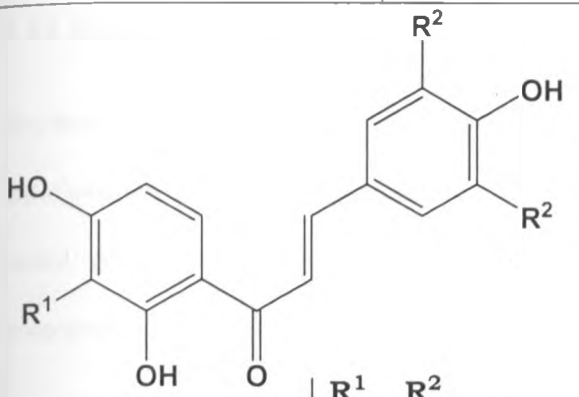


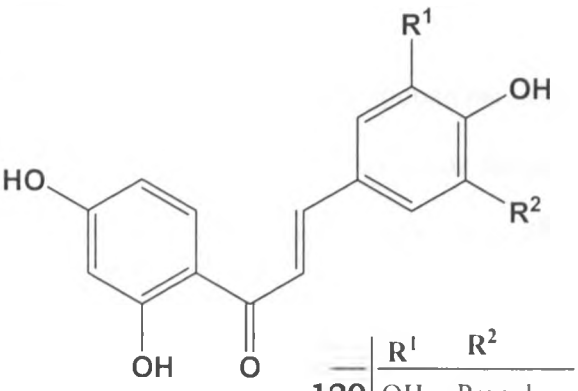
Figure 7: Basic skeletal structures of natural chalconoids

Table 2.8; Minor flavonoids of *Erythrina*		
Minor flavonoids	**Source**	**Reference**
Chalcones		
5'-Prenylbutein **(120)**	*E. abyssinica* (SB)	Induli, 2006
Homobutein **(121)**	*E. abyssinica* (SB)	Induli, 2006
Licoagrochalcone **(122)**	*E. abyssinica* (SB)	Induli,2 006
Abyssinone VI **(123)**	*E. abyssinica (RB)*	Kamat *et al.*, 1981
Erycristanol C **(124)**	*E. crista-galli (HW)*	Iinuma *et al.*, 1994a
Isobavachalcone **(125)**	*E. burttii (RB)*	Yenesew *et al.*, 1998a

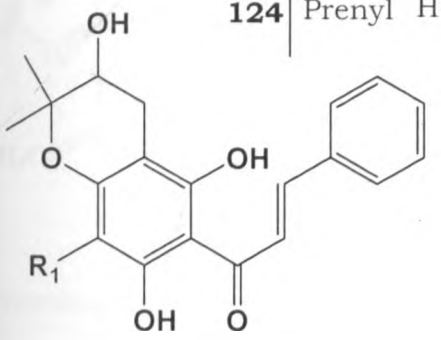
2-Arylbenzofurans		
Bidwillol B (**126**)	*E. bidwilli* (WD)	Iinuma *et al.*, 1994
Burttinol D (**127**)	*E. burttii* (RB)	Yenesew *et al.*, 2002
3-Phenylcoumarins		
Indicanin A (**128**)	*E. indica* (RB)	Nkengfack *et al.*, 2000
Indicanin B (**129**)	*E. indica* (RB)	Waffo *et al.*, 2000



	R^1	R^2
123	H	Prenyl
124	Prenyl	H



	R^1	R^2
120	OH	Prenyl
121	OMe	H
122	H	Prenyl



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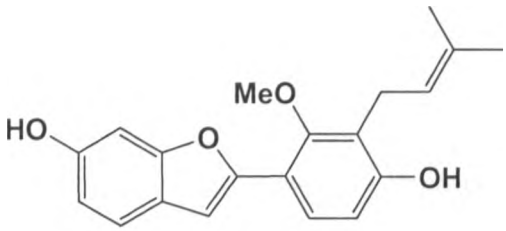

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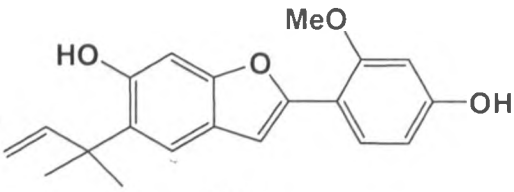

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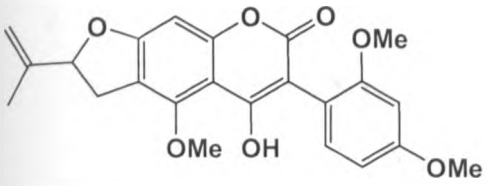

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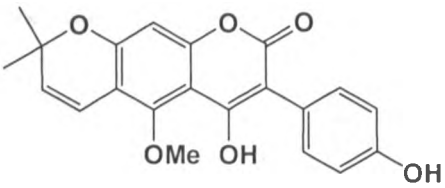

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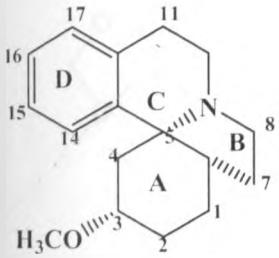
2.4 Alkaloids

2.4.1 Structural classification of *Erythrina* alkaloids

Erythrina alkaloids are characterized by their unique skeleton of a tetracyclic spiroamine. They are classified into two main groups: alkaloids possessing a 6,5,6,6 indoloisoquinoline skeleton called erythrinane (**130**) and those with a skeleton of 6,5,7,6 indolobenzazepine called homoerythrinane (**131**) alkaloids.



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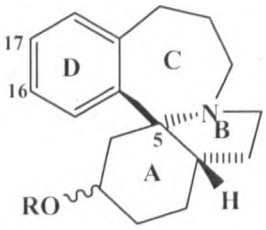

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Depending on the nature of D-ring both groups can be subdivided into aromatic and non-aromatic erythrinan alkaloids.

2.4.1.1 Aromatic erythrinan alkaloids

Aromatic erythrinan alkaloids can be further divided into:

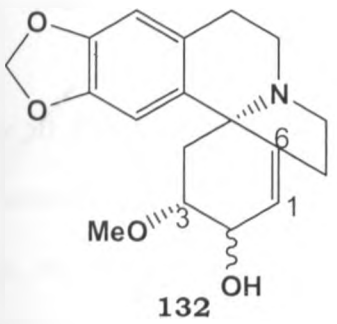
1. Alkenoid alkaloids (Fig 8) which possess an aromatic D-ring and a double bond at position 1, e.g. erythratine (**132**).

2. Dienoid alkaloids which possess an aromatic D-ring and a 1,6-diene system, e.g. erythraline (**133**) and erysonine (**134**).

2.4.1.2 Non-aromatic erythrinan alkaloids

The group non-aromatic erythrinan alkaloids (Fig 9) includes the presence of oxa-compounds in the D-ring, which are called lactonic alkaloids, Examples include, erythroidine (**135**) and cocculolidine (**136**).

Alkaloids with a pyridinium group instead of a phenyl group (Fig.10) have been isolated in both series (aromatic and non-aromatic), and these are called heteroaromatics; examples include erymelanthine (**137**), holidine (**138**).and selaginoidine (**139**).



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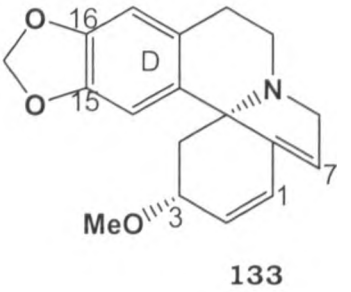
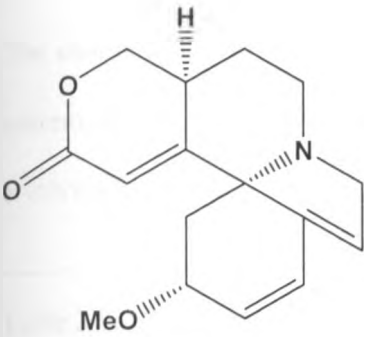


133

Figure 8: Examples of aromatic erythrinan alkaloids, alkenoids (e.g., 132) and dienoid (e.g. 133)



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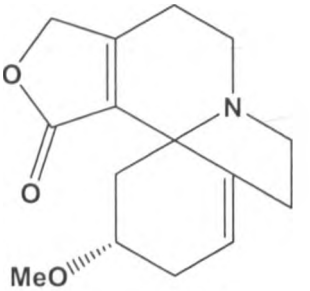

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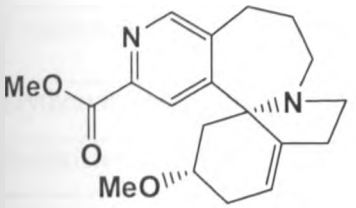
Figure 9: Examples of non-aromatic erythrinan alkaloids



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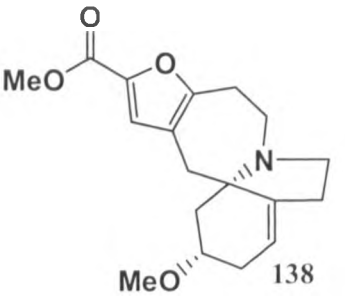

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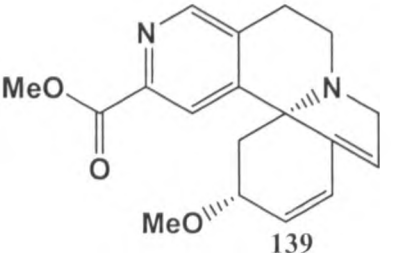

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Figure 10: Examples of hetro-aromatic erythrinan alkaloids

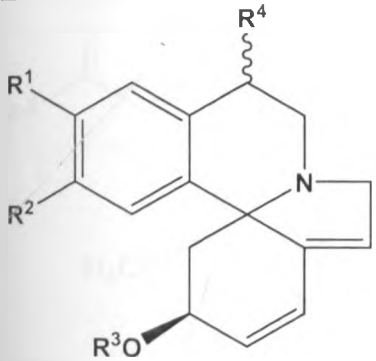
Both aromatic-erythrinane and homo-erythrinane alkaloids show a characteristics substitution pattern, *vis* the presence of oxygenation at C-3, C-15 and C-16. In some of the alkaloids deoxygenation at C-16 has also occurred. In both aromatic- and non-aromatic series of alkaloids the A/B ring fussion is *cis*-configured (Mondon, *et al.*, 1970; Reiman and Ettmayr, 2004). Generally alkaloids of *Erythrina* are dextrorotatory, and the absolute configuration at C-3 is *R* while that of C-5 is *S* (Amer, *et al.*, 1991) the only exception is wilsonine (**149**), which is reported to have *R* configuration at C-5.

The presence of alkaloids in the genus *Erythrina* has been known for a long time. Alkaloids belonging to this genus have attracted wide interest because of their remarkable physiological action.

The characteristic structural feature involves the presence of a tetracyclic {6-5-6-6} spiroamine system, which is known as Erythrinan. This makes these alkaloids be generally referred to as erythrinan alkaloids.

Table 2.9: Dienoid erythrinan alkaloids from Kenyan *Erythrina* species

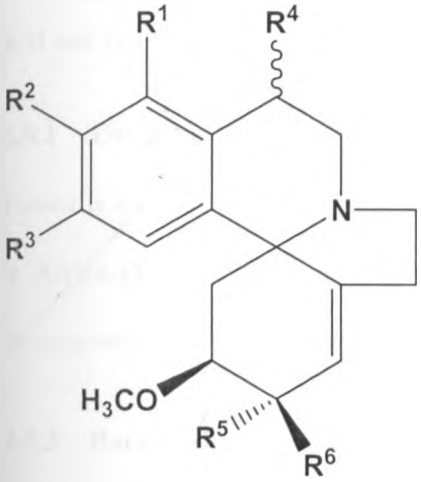
Alkaloid	**Plant source (Part)**	**Reference(s)**
Erysonine (**140**)	*E. abyssinica* (SD)	Willaman and Schubert, 1961
Erysopine (**141**)	*E. melanacantha* (SD)	Hernandez and Jackson, 1994
Erysotrine (**142**)	*E. melanacantha* (SD)	Hernandez and Jackson, 1994
Erythravine (**143**)	*E. abyssinica* (SD)	Barakat *et al.*, 1977
Erythristemine (**144**)	*E. abyssinica* (SD)	Ghosal *et al.*, 1972



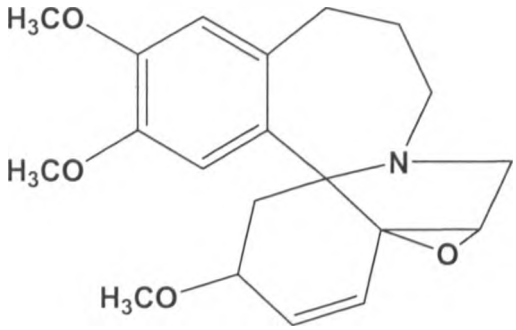
	R^1	R^2	R^3	R^4
140	OH	OMe	H	H
141	OH	OH	Me	H
142	OMe	OMe	Me	H
143	OMe	OMe	H	H
144	OMe	OMe	Me	OMe

Table 2.10: Alkenoid *Erythrinian* alkaloids from *Erythrina* species

Alkaloid	Plant source (Part)	Reference(s)
Erysoflorinone (**145**)	*E. melanacantha* (SD)	Jackson *et al.*, 1982
Erysosalvinone (**146**)	*E. melanacantha* (SD)	Jackson *et al.*, 1982
Erysotine (**147**)	*E. melanacantha* (SD)	Jackson *et al.*, 1982
Erythratine (**148**)	*E. americana* (F)	Aguilar *et al.*, 1981
Wilsonine (**149**)	*E. americana* (F)	Aguilar *et al.*, 1981



	R^1	R^2	R^3	R^4	R^5	R^6
145	H	OH	OH	H	O	
146	H	OMe	OH	H	O	
147	H	OH	OMe	H	OH	H
148	H	$-OCH_2O-$		H	OH	H



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2.5 The family rutaceae

The Rutaceae, a large family of mostly trees, shrubs and woody vines, contain 161 genera and 1,815 species of mostly tropical and subtropical distribution (Chase *et al.*, 1999). The family is known for its economic importance: edible fruits, timber and aromatic oils (Scott *et al.*, 2000). In the systematic treatment of Rutaceae, Engler (1931) recognized seven subfamilies: Rhabdodendroideae, Aurantioideae, Flindersioideae, Spanthelioideae, Rutoideae, Dictyolomatoideae and Toddalioideae. The family is well known for the large number and diversity of its secondary metabolites, most of which are the alkaloids derived from anthranilic acid and tyrosine, limonoids and cuomarins (Waterman 1973).

2.5.1 The genus *teclea*

Teclea is a genus in the subfamily toddaliea of the family Rutaceae. There are about 30 species in Africa (Victor, 2000), some Kenyan species include; *Teclea ameniensis*, *T. grandifolia*, *T. hanangesis*, *T. nobilis*, *T. trichocarpa* and *T. simplicifolia* (Beentje 1994).

2.5.3 Botanical information on *Teclea nobilis*

Teclea nobilis is a shrub or understory tree 4-18m high, which is evergreen, with a grey brown bark and finely grooved leaves. It is widely distributed in tropical Eastern Africa, namely Ethiopia, Sudan, Somalia, Kenya, Uganda, Tanzania and also in Arabia. This plant is used in folk medicine of many African societies (Beentje 1994).

Figure 11: Picture of *Teclea nobilis*

2.5.4 Ethnomedical information on the genus *Teclea*

Teclea nobilis is a plant used in folk medicine as an analgesic and antipyretic agent. In South Africa, the bark of *T. nobilis* is reported to be a gonorrhoea remedy while in Tanzania, the leaves are used as cure for fever (Watt and Breyer-Brandwijk, 1962). Similarly in Ethiopian folk medicine the leaves are used to control pain (Mascolo *et al.*, 1988). *T. trichocarpa* is used by traditional healers belonging to the Akamba tribe of East Africa for malaria treatment, as an anti-helmintic and the vapour is inhaled as a cure for fever (Watt and Breyer-Brandwijk, 1962). The various parts of the plant including leaves and stem bark are said to be a remedy for gonorrhea and pain (Watt & Breyer-Brandwijk, 1962). *T. ouabanguiensis* is used as a remedy for coughs and asthma in Cameroon (Watt and Breyer-Brandwijk, 1962).

2.5.5 Biological activities of the genus *Teclea*

Antipyretic and analgesic activities of the ethanol extract of *Teclea nobilis* have been reported (Mascolo *et al*, 1988). Further pharmacological studies indicated that quinoline alkaloids are responsible for the observed analgesic and antipyretic activities of this plant (Yenesew and Dagne, 1988). Antifungal, antibacterial and *in vitro* anti-plasmodial activities of *Teclea trichocarpa* have also been reported (Lwande *at al.*, 1983). Other biological studies of this plant revealed potent insect anti-feedant activity against the African armyworm, *Spodoptera exempta* (Muriithi *et al.*, 2002).

2.6 Compounds reported the genus *Teclea*

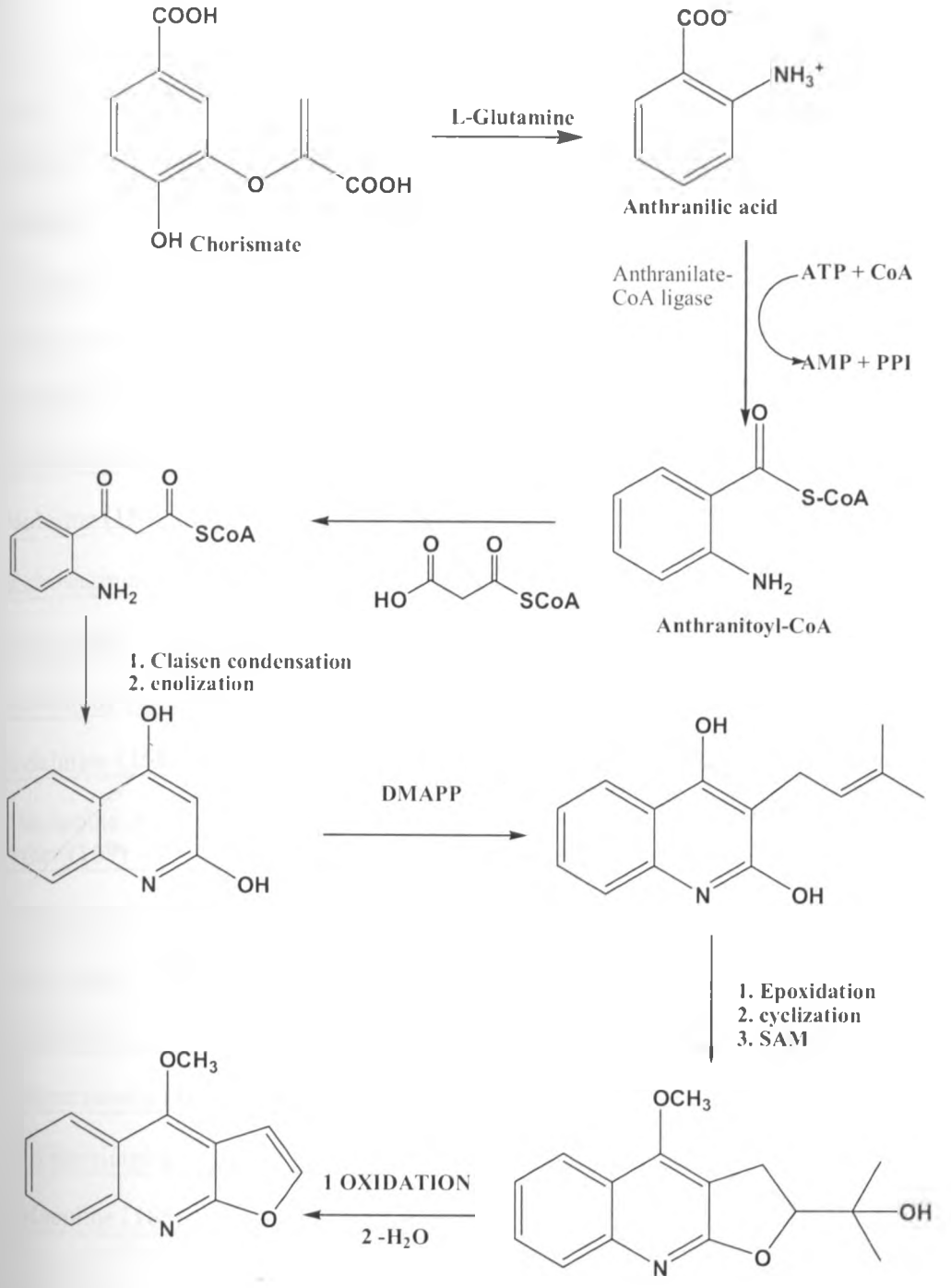
Phytochemical investigations on this genus have revealed the presence of quinoline and furoquinoline alkaloids, while limonoids, tetranortriterpenes, triterpenes, alkaloids, and flavonoid glucosides were isolated from *T. ouabanguiensis, T. grandifolia, T. verdoorniana*, and *T. sudanica*, respectively (Dagne *et al.*, 1988).

Some chlorinated compounds have been isolated from the genus. Chlorodesnkolbisine (**153**) isolated from the aerial parts of *Teclea nobilis* (Al-Rehaily *et al.*, 2002) has been reported. These chlorinated compounds have not been reported from any other source, hence there is a possibility that the plant was not correctly identified or chlorination could be as the result of environmental factors. Also it is reported that the compound was isolated from the aaerial parts, this need to be verified because *Teclea nobilis* is a big tree not a herb.

This genus *Teclea* is well known for alkaloids derived from anthranilic acid, limonoids, coumarins and triterpene derivative such as lupeol (**13**) (Waterman, 1973). The alkaloids found in this genus are classified into two: acridone and furoquinoline alkaloids.

2.6.1 Furoquinoline alkaloids

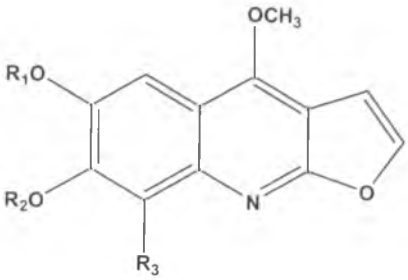
Furoquinoline alkaloids are the most widespread type of alkaloid in the genus *Teclea* (Table 2.11) as well as the family Rutaceae. They are planar aromatic compounds where a benzene ring is fused to quinoline and furan rings (Mester, 1983). Furoquinolines are biosynthesized from anthranilic acid, which is formed from chorismate and L-glutamine (Scheme 2.1).

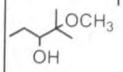
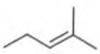


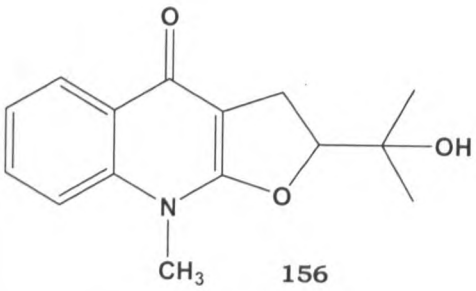
Scheme 2.1: Biosynthesis of furoquinoline alkaloids (Cordell, 1981)

Table 2.11: Furoquinoline alkaloids from *Teclea* species

Alkaloid	**Plant source**	**Reference**
Tecleaoxine (**150**)	*T. nobilis* (AE)	Al-Rehaily *et al.*, 2002
Isotecleoxine (**151**)	*T. nobilis* (AE)	Al-Rehaily *et al.*, 2003
Methylnkolbisine (**152**)	*T. nobilis* (AE)	Al-Rehaily *et al.*, 2003
Chlorodesnkolbisine (**153**)	*T. nobilis* (AE)	Al-Rehaily *et al.*, 2003
Nobiline (**154**)	*T. nobilis* (AE)	Yenesew and Dagne, 1988
Kukosaginine (**155**)	*T. nobilis* (AE)	Pusset *et al.*, 1991
Isoplatydesmine (**156**)	*T. nobilis* (L)	Yenesew and Dagne, 1988
Ribalinine (**157**)	*T. nobilis* (L)	Yenesew and Dagne, 1988
Edulinine (**158**)	*T. nobilis* (L)	Higa *et al.*, 1974
Haplopine-3,3'dimethylallyl ether (**159**)	*T. nobilis* (AE)	Bessonova *et al.*, 1974
Anhydroevoxine (**160**)	*T. nobilis* (AE)	Bessonova *et al.*, 1982
8-Methoxy-flindersine (**161**)	*T. nobilis* (AE)	Campbell *et al.*,1990
Montrifoline (**162**)	*T. nobilis* (L, F)	Ayafor *et al.*, 1982
Skimmianine (**163**)	*T. nobilis* (L, F)	Al-shama *et al.*, 1979
Flindersiamine (**164**)	*T. nobilis* (L, F)	Ayafor *et al.*, 1982
Maculine (**165**)	*T. nobilis* (L, F)	Fish *et al.*, 1976



	R_1	R_2	R_3
150		CH_3	H
151	CH_3		H
152		CH_3	H
153		CH_3	H
154	CH_3		H
155	CH_3	CH_3	H



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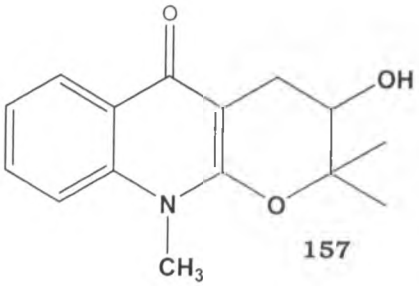

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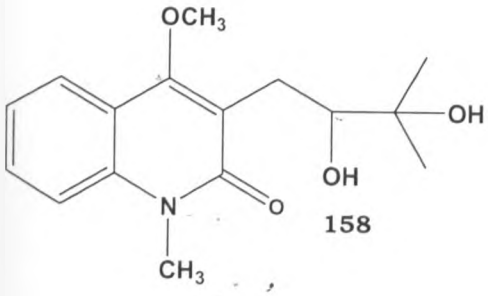

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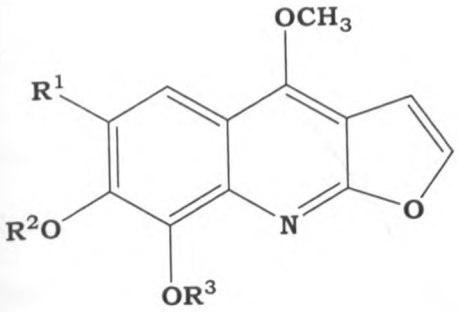
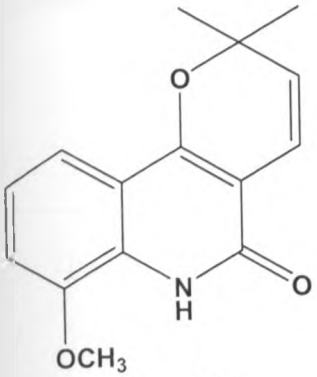


	R1	R2	R3
159	H	Prenyl	CH_3
160	H		CH_3



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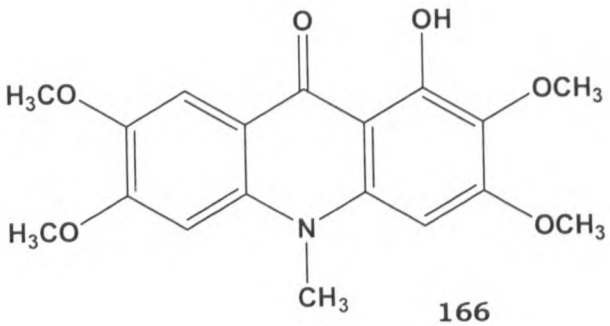

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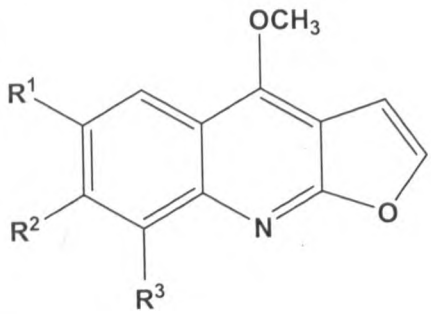


	R1	R2	R3
162	$(Me)_2COHCHOHCH_2O$-	OMe	H
163	H	OMe	OMe
164	OCH_2O		OMe
165	OCH_2O		H

2.6.2 Acridone alkaloids of the genus *Teclea*

Acridone alkaloids are found in natural plant sources, particularly plants belonging to Rutaceae, including *Teclea* species. Acridones have unique molecular structure of two benzene rings bridged by nitrogen and a keto group (Fig. 12). These compounds are biosynthesized as shown in Scheme 2.2. The distribution of acridone alkaloids in the genus *Teclea* are shown in Table 2.12.

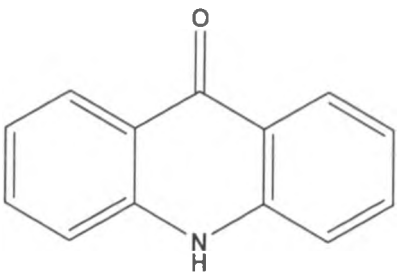
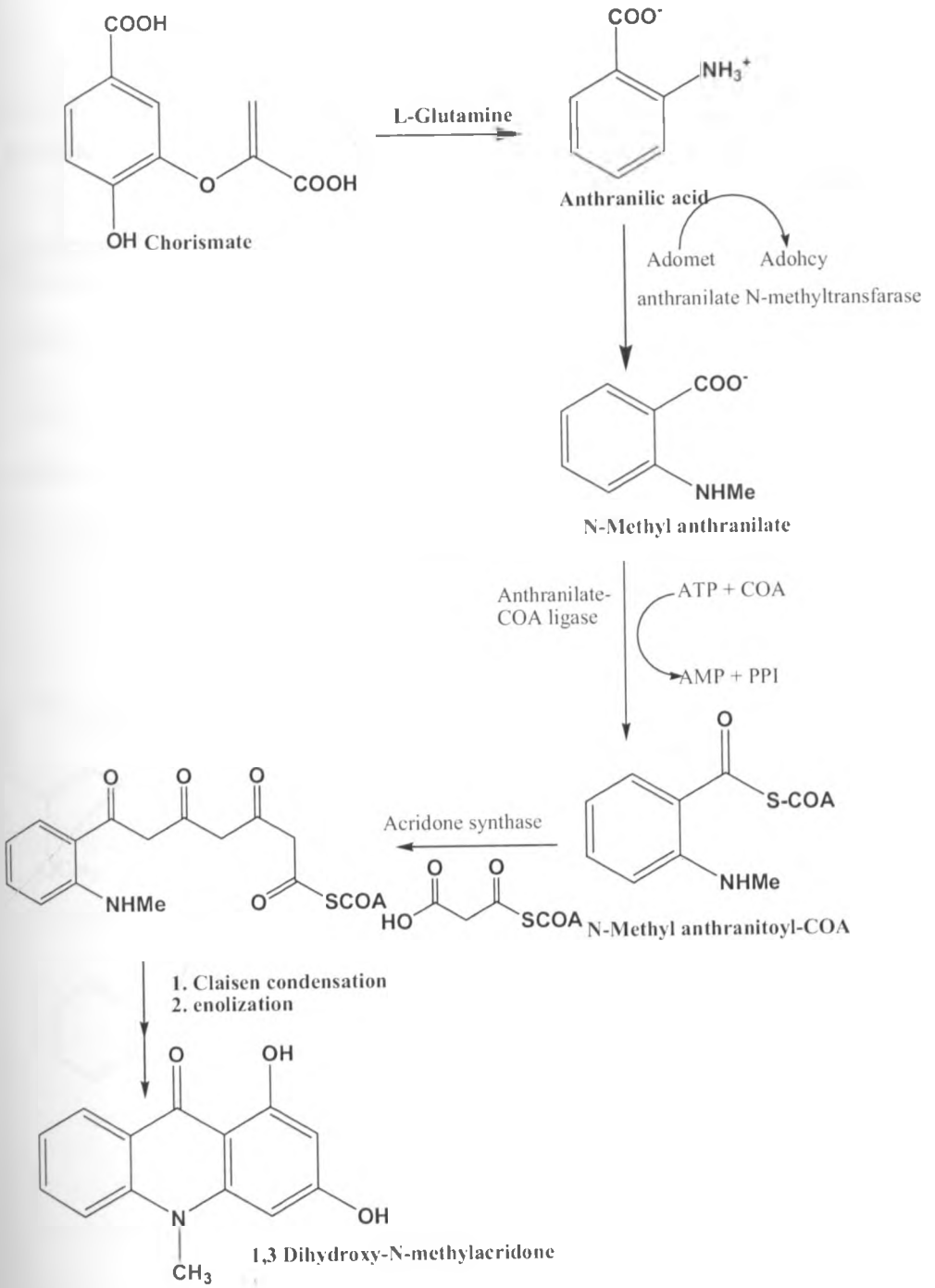


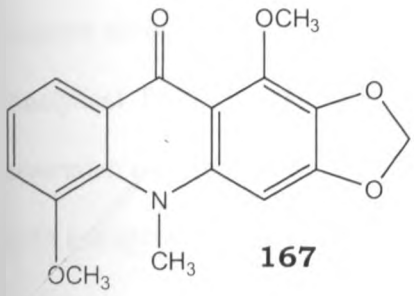
Figure 12: The acridone skeleton



Scheme 2.2: Biosynthesis of acridone alkaloids (Girighav *et al.*, 2010)

Table 2.12: Acridone alkaloids from the genus *Teclea*

Alkaloid	Plant source (Plant part)	Reference
Arborinine (**166**)	*T. nobilis* (AE)	Bergenthal *et al.*, 1979
Tecleanthine(**167**)	*T. bolviniane* (L)	Vaquette, 1978
6-methoxytecleanthine(**168**)	*T. bolviniane* (L)	Vaquette, 1978
1,3,4-trimethoxy-N-methyl acridone (**169**)	*T. bolviniane* (L)	Vaquette, 1978
Evoxanthine (**170**)	*T. bolviniane* (L)	Vaquette, 1978



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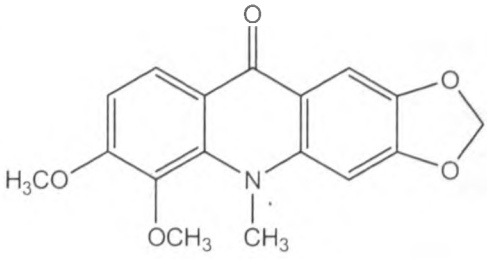

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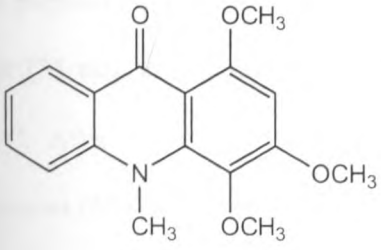

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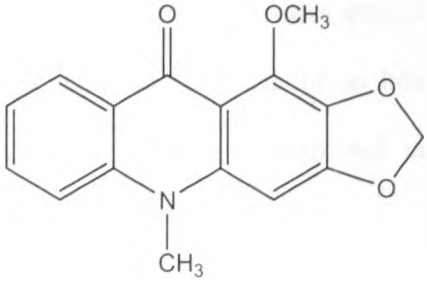

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CHAPTER THREE

3.0 EXPERIMENTAL

3.1. Instrumentation

The ^{1}H (200 or 600 MHz) and ^{13}C (50 or 150 MHz) were acquired using Varian-Mercury and Bruker instrument using residual solvent signals as reference. Homonuclear Correlation Spectroscopy (COSY), Hetronuclear Multiple Quantum Correlation (HMQC) and Hetronuclear Multiple Bond Correlation (HMBC) spectra were obtained using the standard Bruker software.

3.2 Chromatographic methods

Column chromatography was on silica gel 60G (Merck, 70-230 mesh) and Sephadex LH-20. Analytical TLC using silica gel 60 F_{254} (Merck) pre-coated plates were used to monitor the separation of compounds. For qualitative work, the TLC plates were visualized under ultraviolet (254 and 366 nm) light, exposure to Iodine vapor or spraying with Dragendorff reagent.

Each preparative TLC plate (20×20 cm) was prepared form a slurry containing silica gel (13 g), water (33 ml). The plates were dried at room temperature and then activated for an hour at 110°C. After developing the plate with a suitable solvent system, it was examined under ultraviolet (254 and 366 nm) light for detection of the bands.

3.3 Plant material

3.3.1 *Teclea nobilis*

The stem bark of *Teclea nobilis* was collected from Kakamega forest, Kenya, in July 2010. The plant was identified at the University Herbarium, School of Biological Sciences, University of Nairobi.

3.3.2 *Erythrina brucei*

The stem bark of *Erythrina brucei* was collected from Ethiopia. The plant was identified at the University Herbarium, Jimma University, Ethiopia.

3.3.3 *Erythrina burttii*

The root bark of *Erythrina burttii* was collected from Kakamega forest. The plant was identified at the University Herbarium, Botany Department, University of Nairobi, where voucher specimen was deposited.

3.1.3.4 *Erythrina abyssinica*

The roots of *Erythrina abyssinica* was collected from Kakamega forest. The plant was identified at the University Herbarium, Botany Department, University of Nairobi, where voucher specimen was deposited.

3.2 Extraction and isolation of compounds

3.2.1 Extraction and isolation of compounds from *Erythrina burttii*

The air dried and ground root bark (900 g) of *Erythrina burttii* was extracted thrice using acetone by cold percolation. The crude extract (48 g) was subjected to column chromatography on oxalic acid impregnated silica gel (500 g). Gradient elution with n-hexane containing increasing amount of ethyl acetate and finally washed with 100% MeOH afforded fourteen major fractions (labeled A- N).

Fraction B (eluted with n-hexane in ethyl acetate) was further separated on Sephadex LH-20 (eluted with CH_2Cl_2/MeOH; 1:1), and further subjected to column chromatography on oxalic acid impregnated silica gel (eluting with 2% ethyl acetate in n-hexane) and crystallized (from n-hexane/CH_2Cl_2) which yielded compound **1**(12 mg), **2** (8 mg) and **3** (6 mg).

3.2.2 Extraction and isolation of compounds from *Erythrina brucei*

The dried and ground stem bark (700 g) of *Erythrina brucei* was extracted thrice using CH_2Cl_2/MeOH (1:1) by cold percolation. The crude extract (20 g) was subjected to column chromatography on silica gel (300 g). Gradient elution with n-hexane containing increasing amount of ethyl acetate and finally washed with 100% MeOH afforded twenty three major fractions (labeled A-V).

Fractions J-M (4% ethyl acetate in hexane) were combined and subjected to further column chromatography on silica gel (eluting with n-hexane-CH_2Cl_2-acetone in the ratio of 98-1-1), and purification using preparative TLC yielded compound **5** (14 mg).

Fractions N-V (6% ethyl acetate in n-hexane) was combined, purified by CC on Sephadex LH-20 (CH_2Cl_2/MeOH (1:1), and further purification of fractions using preparative TLC (solvent; 5% MeOH in CH_2Cl_2) yielded compounds **6** (15 mg), **7** (14 mg) and **8** (12 mg).

3.2.3 Extraction and isolation of compounds from the stem bark of *Teclea nobilis*

The dried and ground stem bark (3.2 kg) of *Teclea nobilis* was extracted thrice using CH_2Cl_2/MeOH (1:1) by cold percolation. The crude extract (80 g) was subjected to column chromatography on silica gel (600 g). Gradient elution with n-hexane containing increasing amount of ethyl acetate and finally washed with MeOH afforded twenty major fractions (labeled A-T).

Fraction B eluted with n-hexane was crystallized (from n-hexane/CH_2Cl_2) to yield compound **13** (10 g). Fraction G eluted with (4% ethyl acetate in n-hexane) was crystallized (from n-hexane/CH_2Cl_2) to yield compound **9** (200 mg), similar treatment of fraction M eluted with (6% ethyl acetate in n-hexane) yielded compound **10** (8 mg).

Fraction I eluted with (8% ethyl acetate in n-hexane) was further purified by column chromatography on silica gel (50 g), eluting with 50 to 100% CH_2Cl_2 in n-hexane) and crystallization (from n-hexane/CH_2Cl_2) yielded compound **11** (9 mg). Fraction M (eluted with 55% CH_2Cl_2 in n-hexane) was similarly treated to yield compound **12** (24 mg).

3.3 Biological activity tests

3.3.1 Preliminary radical scavenging test

A 12 mg portion of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was dissolved in 50 ml of analar grade MeOH and placed in a dark spray bottle. The isolates and crude extracts were spotted on TLC plate and developed using appropriately solvent. The TLC plate was then dried in open air and the spots visualized under UV light at 254 nm and marked with Pencil. Finally the dried plates were sprayed with DPPH reagent.

3.3.2 Quantitative radical scavenging test

Crude extracts and pure compounds were tested for radical scavenging activity using a the method describes by Ohnishi *et al.* (1994). Standard solutions (140 µg/ml, 70 µg/ml, 35 µg/ml, 17.5 µg/ml, 8.75 µg/ml and 4.375 µg/ml) of the test samples were prepared in double distilled methanol. From each concentration, 0.5 ml of the test compound was added to 3 ml of 0.1 mM DPPH dissolved in methanol solution. The mixture was shaken and left to stand for 30 min. The radical scavenging activities were estimated as the percent decrease of the absorbance of DPPH (0.1 mM) at 517 nm (Ohnishi *et al.*, 1994). In all cases the mean values were used from triplicate experiments. Effective concentration (EC_{50}) values were calculated using Finney's probit analysis for quantal data (McLaughlin *et al.*, 1991).

3.3.3 *In vitro* antiplasmodial activity test

The crude extract and pure compounds were assayed using a non-radioactive assay technique (Smilkstein *et al.*, 2004) with modifications (Johnson *et al.*, 2007) to determine 50% growth inhibition of cultured parasites. Briefly, two different strains, chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), of *P. falciparum* were grown as described (Johnson *et al.*, 2007). Drugs and compounds were dissolved in 99.5% dimethylsulfoxide (DMSO) and diluted in complete Roswell Park Memorial Institute 1640 series of Cell Culture Medium (RPMI 1640) prepared as described by Akala *et al.* (2011). Briefly, the basic culture medium was prepared from RPMI 1640 powder (10.4 g; Invitrogen, Inc. augmented with 2 g glucose and 5.95 g of HEPES, dissolved to homogeneity in 1 liter of de-ionized water and sterilized with a 0.2 µM filter. Complete RPMI 1640 media, used for all parasite culture and drug dilutions, consisted of basic RPMI 1640 media with 10% (vol/vol) human ABO pooled plasma, 3.2% (vol/vol) sodium bicarbonate and 4 µg/ml hypoxanthine. Complete RPMI 1640 media was stored at 4°C and used within 2 weeks.

Concurrently, two-fold serial dilutions of chloroquine (1.953 to 1,000 ng/ml), mefloquine (0.488 to 250 ng/ml) and test sample (97.7 to 50,000 ng/ml) were prepared on a 96-well plate, such that the amount of DMSO was equal or less than 0.0875%. The culture-adapted *P. falciparum* at 2% hematocrit and 1% parasitemia, 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 hours later by freezing at -80°C and parasite growth inhibition quantified as mean ± standard deviation (Mean IC_{50} ± SD) as described (Johnson *et al.*, 2007).

3.3.4 *In vivo* antiplasmodial tests

The crude extract of *Erythrina abyssinica* and *Erythrina burttii* was assessed for *in vivo* activity in a four-day suppressive test against *Plasmodium berghei* infections in mice (peters, 1980). All test mice were infected on day zero. The dosage used was 1000 mg. test groups of mice (five mice each of extract and dose) were immediately treated orally and given a single dose of test samples. Two control groups of mice were used. One group was given chloroquine, which was the treatment drug of choice (10 mg/kg body weight) and the other group was given placebo, in this case water (negative control) for four days. On the fifth day, a thin blood film stained with Giemsa was prepared for each mouse and the parasitemia was determined microscopically. The percentage of parasitemia was calculated by the comparison of the parasitemia of the treated mice with the parasitemia of the control group (negative control).

3.3.5 Antimicrobial testing

The microbial assays were carried out at the Kenya Medical Research Institute (KEMRI) according to Biyiti *et al* (1988), with variety of micro-organisms. Bacterial and fungal strains were used to carry out microbial assays. Pathogenic bacteria; Gram-positive bacteria (*staphylococcus aureus*) and gram-negative bacteria (*Pseudo aeruginosa* and *Escherichia coli*)

Pathogenic fungi; Yeast (*Candida albicans*) and dermatophytes (*Cryptococcus neoformans*, *microsporum gypseum* and *Trichophyton mentagrophytes*).

Weighed aliquots of each dry sample was dissolved in acetone and placed in a 6 mm Whatman paper disc to give different concentrations. Discs of chloramphenical (standard) were also used for comparison. The discs were air dried and placed on agar plates seeded with micro-organisms

and, after incubation the zones of inhibition were measured. The incubation conditions depend on the micro-organism tested. Bacteria were incubated for 4 hrs at 37°C on Mueller Hinton agar. Fungi were grown on Sabourand dextrose agar for 3 days at 37°C (for yeast) and 24 hrs at 28°C (for dermatophytes).

3.4.1 Physical and spectroscopic data for the isolated compounds from the root bark of *Erythrina burttii*

Bidwillon A (1)

Colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.56 (2H, m, CH_2-2), 4.16 (1H, dd, J = 6.4, 10.8, H-3), 7.69 (1H, s, H-5), 6.96 (1H, d, J = 2.4, H-2'), 7.01 (1H, dd, J = 2.4, 7.8, H-4'), 7.25 (1H,d, J = 7.8, H-5'), 3.24 (2H, d, J = 6.2, H-1''), 5.17 (2H, m, CH_2-2''), 1.68 (3H, s, C-4''), 1.73 (3H, s, H-5''), 3.24 (2H, d, J = 7.0, H-1'''), 5.10 (2H, m, CH_2-2'''), 1.66 (3H, s, C-4'''), 1.73 (3H, s, C-5'''). ^{13}C NMR (50 MHz, $CDCl_3$): δ 71.2(C-2), 47.5(C-3), 190.9(C-4), 126.1(C-5), 121.1(C-6), 150.5(C-7), 121.2(C-8), 153.5(C-9), 124.7(C-10), 119.7(C-1'), 134.0(C-2'), 116.9(C-3'), 149.5(C-4'), 119.2(C-5'), 128.2(C-6'), 29.9(C-1''), 123.6(C-2''), 132.7 (C-3''), 20.3 (C-4''), 25.9 (C-5''), 23.6 (C-1'''), 123.6 (C-2'''), 130.6 (C-3'''), 18.0 (C-4'''), 21.3 (C-5''').

5-hydroxy-2-methoxybenzaldehyde (2).

White solids. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.45 (1H, *d*, *J* = 1.4 Hz, H-3), 7.01 (1H, *dd*, *J* = 1 4, 7.4 Hz, H-4), 7.48 (1H, *d*, *J* = 2.0 Hz, H-6), 3.93 (1H, *s*, OCH_3), 9.83 (1H, *s*, H-1'). ^{13}C NMR (50 MHz, $CDCl_3$): δ 130.4 (C-1), 153.3(C-2), 115.6 (C-3), 110.5(C-4), 148.7(C-5), 126.7 (C-6), 55.9(OCH_3), 190.7(C-1').

Erythrinasinate (3)

White amorphous powder. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.04 (1H, *d*, *J* = 1.6 Hz, H-2), 6.92 (1H, *d*, *J* = 8.0 Hz, H-5), 7.07 (1H, *dd*, *J* = 1.6, 8.0 Hz, H-6), 3.93 (3H, *s*, OMe), 7.61 (1H, *d*, *J* = 16.0 Hz, H-1'), 6.29 (1H, *d J* = 16.0 Hz, H-2'), 4.20 (*t*, *J* = 7.0 Hz, H-1"), 1.70 (*brs*, H-2"and 0.88 (3H, *t*, *J* = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 127.3 (C-1), 115.9 (C-2), 148.1 (C-3), 147.0 (C-4), 114.9 (C-5), 123.3 (C-6), 56.2 (OMe), 144.8 (C-1'), 109.5 (C-2'),167.6 (C-3'), 64.9 (C-1"), 29.0 (C-2"), 26.2 (C-3"), 29.9 (C-4''- C-25''), 32.1 (C-26"), 14.3 (C-27").

3.4.2 Physical and spectroscopic data for the isolated compounds from the root bark of *Erythrina abyssinica*

Erycristagallin (4)

^{1}H NMR (200 MHz, $CDCl_3$): δ7.33 (1H, *s*, H-1), 6.63(1H, *s*, H-4), 7.19 (1H, *d*, *J* = 8.4, H-7), 6.95 (1H, *d*, *J* = 8.4, H-8), 3.56/3.2 (1H, *d*, *J* = 7.4, H-1'/H-1''), 5.23/5.28 (2H, *m*, H-2'/H-2''), 1.73/ 1.87 (1H, *s*, H-4'/4''), 1.71/1.78 (1H, *s*, H-5'/5'')^{13}C NMR (50 MHz, $CDCl_3$): δ 108.3 (C-1a), 126.8 (C-1), 123.6(C-2), 154.6(C-3), 118.7(C-4), 152.9(C-4a), 65.6(C-6), 114.7(C-6a), 118.5(C-6b), 121.8(C-7), 121.0(C-8), 149.6(C-9), 121.6(C-10), 148.3(C-10a), 146.1(C-11a), 28.3/25.9 (C-1'/C-1''), 116.3/110.9 (C-2'/C-2''), 133.0/133.5 (C-3'/C-3''), 18.1/18.0(C-4'/C-4''), 23.9/21.1(C-5'/C-5'')

3.4.3 Physical and spectroscopic data for the isolated compounds of the stem bark of *Erythrina brucei*

Crystamidine (5)

Yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.93 (1H, *dd, J* = 2.2, 10.0 Hz, H-1), 6.32 (1H, *br d, J* = 10.2 Hz, H-2), 3.68 (1H, *m*, H-3), 2.69 (1H, *m*, H-4a), 1.39 (1H, *m*, H-4eq), 6.12 (1H, *s*, H-7), 6.88 (1H, *d, J* = 7.2 Hz, H-10), 6.07 (1H, *d, J* = 7.2 Hz, H-11), 6.68 (1H, *s*, H-14), 6.72 (1H, *s*, H-17), 5.97 (1H, *d, J* = 1.4 Hz, OCH_2O), 5.92 (1H, *d, J* = 1.4 Hz, OCH_2O), 3.35 (3H, *s*, OMe). ^{13}C NMR (50 MHz, $CDCl_3$): δ 120.7 (C-1), 120.4(C-2), 74.8 (C-3), 29.9 (C-4), 66.2 (C-5), 113.6 (C-7), 155.7 (C-8), 138.2 (C-10), 123.8 (C-11), 126.6 (C-12), 125.7 (C-13), 107.9 (C-14), 147.4 (C-15), 146.8 (C-16), 104.2 (C-17), 101.6 (OCH_2O), 56.6 (OMe).

8-oxo-erythraline (6)

Colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.86 (1H, *br, d, J* = 2.4, 10.2 Hz, H-1), 6.30 (1H, *d, J* =10.2 Hz, H-2), δ 3.79 (1H, *m*, H-3), 1.68 (1H, *dd* , *J* = 10.4, 11.2 Hz, H-4a), 2.78 (1H, *dd, J* = 10.4, 11.2 Hz, H-4e), 5.96 (1H, *br, s*, H-7) ,3.75 (1H, *m*, H-10), 3.60 (1H, *m*, H-10), 3.13 (1H, *m*, H-11), 2.93 (1H, *m*, H-11), 6.75 (1H, *s*, H-14), 6.72 (1H, *s*, H-17), 5.91 (1H, *d, J* = 1.6 Hz, OCH_2O), 5.88 (1H, *d, J* = 1.6 Hz, OCH_2O), 3.31 (3H, *s*, OMe). ^{13}C NMR (50 MHz, $CDCl_3$): δ 136.8 (C-1), 123.8 (C-2), 75.1 (C-3), 27.5 (C-4), 67.0 (C-5), 157.5 (C-6), 120.3 (C-7), 171.1 (C-8), 41.6 (C-10), 38.0 (C-11), 130.4 (C-12), 128.3 (C-13), 109.4 (C-14), 147.2 (C-15), 146.2 (C-16), 105.6 (C-17), 101.6 (OCH_2O), 56.6 (OMe).

Erythraline (7)

Colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.52 (1H, *dd*, *J* = 2.2, 10.2 Hz, H- 1), 5.97 (1H, *br d*, *J* = 9.6 Hz, H-2), 3.47 (1H, *m*, H-3), 1.86 (1H, *t*, *J* = 10.6 Hz, H-4a), 2.49 (1H, *dd*, *J* = 5.6, 11.8 Hz, H-4e), 5.71 (1H, *br s*, H-7), 3.94 (1H, *br d*, *J* = 11.4 Hz, H-8), 3.76 (1H, *dd*, *J* = 2.8, 14.2 Hz, H-8), 2.92 (1H, *m*, H-10), 2.84 (1H, *m*, H-10), 2.65 (1H, *m*, H-11) 2.70 (1H, *m*, H-11), 6.62 (1H, *s*, H-14), 6.75 (1H, *s*, H-17), 5.87 (1H, *d*, *J* = 1.4 Hz, OCH_2O),5.90 (1H, *d*, *J* = 1.6 Hz, OCH_2O), 3.33 (3H, *s*, OMe). ^{13}C NMR (50 MHz, $CDCl_3$): δ 125.3 (C-1), 131.8(C-2), 76.2(C-3), 29.9(C-4), 67.8(C-5), 132.4(C-6), 122.4(C-7), 57.8(C-8), 44.7(C-10), 41.7 (C-11), 132.4 (C-12), 128.0 (C-13), 108.9 (C-14), 146.4 (C-15), 146.1 (C-16), 106.4 (C-17), 100.9 (OCH_2O), 56.2 (OMe).

10-oxo-erythraline (8)

Colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.63 (1H, *dd*, *J* = 1.8, 10.2 Hz, H-1), 6.00 (1H, *br s*, H-2), 3.70 (1H, *br s*, H-3), 2.57 (1H, *dd*, *J* = 5.6, 11.4 Hz, H-4a), 1.93 (1H, *t*, *J* = 11.4 Hz, H-4e), 5.73 (1H, *br s*, H-7), 4.39 (2H, *br s*, H-8), 5.25 (1H, *br s*, H-11) ,4.01 (1H, *br s*, H-11), 6.87 (1H, *s*, H-14), 7.21 (1H, *br s*, H-17), 5.93 (1H, *d*, *J* = 1.4 Hz,OCH_2O), 5.97 (1H, *d*, *J* = 1.4 Hz, OCH_2O), 3.29 (3H, *s*, OMe). ^{13}C NMR (50 MHz, $CDCl_3$): δ 124.3 (C-1), 131.9 (C-2), 76.2 (C-3), 29.9 (C-4), 71.9 (C-5), 138.8 (C-6), 120.1 (C-7), 67.9 (C-8), 173.0 (C-10), 39.7 (C-11), 131.4 (C-12), 129.9 (C-13), 106.3 (C-14), 147.7 (C-15), 146.8 (C-16), 104.1 (C-17), 101.6 (OCH_2O), 56.6 (OMe).

3.4.4 Physical and spectroscopic data for the isolated compounds from the stem bark of *Teclea nobilis*

Maculine (9)

White amorphous powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.29 (1H, *d*, *J* = 3 Hz, H-2), 6.94 (1H, *d*, *J* = 3 Hz, H-3), 7.23 (1H, *s*, H-5), 7.41 (1H, *s*, H-8), 4.33 (3H, *s*, OCH_3), 6.03 (2H, *s*, OCH_2O). ^{13}C NMR (50 MHz, $CDCl_3$): δ 142.7 (C-2), 104.7 (C-3), 102.6 (C-3a), 156.1 (C-4), 114.4 (C-4a), 98.2 (C-5), 150.9 (C-6), 146.2 (C-7), 102.6 (C-8), 143.9 (C-8a), 163.3 (C-9a), 59.1 (OMe), 101.8 (OCH_2O).

Flindersiamine (10)

White amorphous powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.50 (1H, *d*, *J* = 3 Hz, H-2), 6.93 (1H, *d*, *J* = 3 Hz, H-3), 7.15 (1H, *s* H-5), 4.31 (3H, *s*, 4-OCH_3), 4.20 (3H, *s*, 8-OCH_3), 5.99 (2H, *s*, OCH_2O). ^{13}C NMR (50 MHz, $CDCl_3$): δ 143.1 (C-2), 104.6 (C-3), 102.9 (C-3a), 156.2 (C-4), 115.1 (C-4a), 92.6 (C-5), 138.2 (C-6), 137.8 (C-7), 146.2 (C-8), 136.9 (C-8a), 162.8 (C-9a), 60.8 (4-OMe), 59.1 (8-OMe), 101.7 (OCH_2O).

4, 7-dimethoxyfuro[2,3-b]quinolin-6-ol (11)

White amorphous powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.52 (1H, *d*, *J* = 3.0 Hz, H-2), 7.05 (1H, *d*, *J* = 3.0 Hz, H-3), 7.42 (1H, *s*, H-5), 7.15 (1H, *s*, H-8), 4.39(3H, s, 4-OCH_3), 3.96(3H, *s*, 6-OCH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 141.7 (C-2), 108.1 (C-3), 101.4 (C-3a), 155.9 (C-4), 112.2 (C-4a), 104.5 (C-5), 150.3 (C-6), 146.9 (C-7), 100.1 (C-8), 141.6 (C-8a), 162.6 (C-9a), 58.4 (4-OMe), 55.3 (6-OMe).

7-(3-methylbuta-1,3-dienylloxy)-4,6-dimethoxyfuro[2,3-b]quinoline (12)

White amorphous powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.58 (1H, *d*, J= 2.8 Hz, H-2), 7.05(1H, *d*, J= 2.8 Hz, H-3), 7.52 (1H, s, H-5), 7.35 (1H, s, H-8), 4.45(3H, *s*, 4-OCH_3), 4.02(3H, *s*, 6-OCH_3), 6.85(1H, *d*, *J*= 12.2 Hz, H-1'), 6.32 (1H, *d*, *J*= 12.2 Hz, H-2'), 4.94 (1H, *br s*, H-4'), 4.89(1H, *br s*, H-4), 1.91 (3H, *s*, H-5'). ^{13}C NMR (50 MHz, $CDCl_3$): δ 142.9 (C-2), 111.6 (C-3), 102.8 (C-3a), 150.1 (C-4), 109.7 (C-4a), 101.2 (C-5), 141.9 (C-6), 147.7 (C-7), 104.6 (C-8), 138.6 (C-8a), 155.6 (C-9a), 58.9 (4-OMe), 56.1 (6-OMe), 142.3 (C-1'), 115.3 (C-2'), 114.5 (C-3'), 118.9 (C-4'), 18.9 (C-5')

Lupeol (13)

White amorphous powder. ^{1}H NMR (200 MHz,$CDCl_3$), 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), 1.08 (H-12a), 1.68 (H-12e), 1.67 (H-13), 1.01 (H-15a), 1.74 (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), 16.1 (C-6), 34.5 (C-7), 41.0 (C-8), 50.6 (C-9), 37.4 (C-10), 19.5 (C-11), 25.3 (C-12), 38.3 (C-13), 43.0 (C-14), 27.6 (C-15), 35.8 (C-16), 43.2 (C-17), 48.5 (C-18), 48.2 (C-19), 151.5 (C-20), 30.1 (C-21), 40.2 (C-22), 21.1 (C-23), 21.2 (C-24), 15.6 (C-25), 18.2 (C-26), 14.7 (C-27), 16.3 (C-28), 109.6 (C-29), 18.5 (C-30).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Compounds isolated from *Erythrina burttii*

The air dried and ground root bark of *Erythrina burttii* was extracted with acetone by cold percolation. The acetone extract was then subjected to *in vitro* antiplasmodial activity tests. The results obtained indicated potent antiplasmodial activities against the D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum*, with IC_{50} values of 0.97 ± 0.2 and 1.73 ± 0.5 μg/ml respectively.

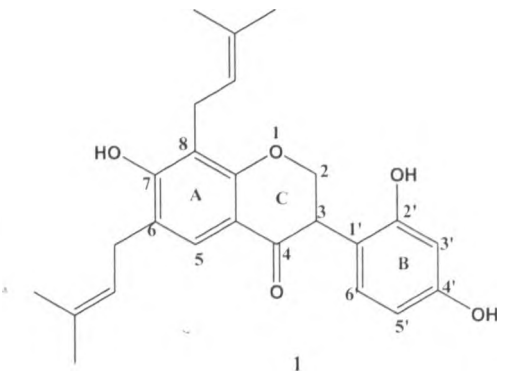
The crude extract was also tested for antioxidant activities using 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical. The extract had radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with EC_{50} value of 12.5 μg/ml. Previously reported (Yenesew *et al.*, 2002) isoflav-3-enes; burttinol-A (**90**) and burttinol-C (**93**), and the 2-arylbenzofuran derivative burttinol-D (**123**) were identified as the most active antiplasmodial ($IC_{50} < 10$ μM) and free radical scavenging (EC_{50} *ca.* 10 μM) principles.

In addition, three more compounds were isolated and characterized as bidwillon A (**1**), 5-hydroxy-2-methoxybenzaldehyde (**2**) and erythrinasinate (**3**). The structures of these compounds were determined on the basis of spectroscopic evidence and by comparison with literature where necessary.

4.1.1 Bidwillon A (1)

Compound **1** was obtained as colorless oil. Evidence that **1** has an isoflavanone skeleton was observed from ^{1}H NMR, (δ_H 4.56 for CH_2 at C-2 and 4.16, *dd*, *J*=6.4, 10.8 Hz for H-3), and ^{13}C NMR (δ_C 71.5 for C-2, δ_C 47.5 for C-3 and δ_C 190.9 for C-4). Ring A is tri-substituted, the only deshielded aromatic proton resonating at δ_H 7.69 (*s*) was assigned to H-5-being *peri* to the carbonyl at C-4. One of substituents in this ring is hydroxyl, placed at C-7 from biogenetic considerations. The ^{1}H and ^{13}C NMR spectra (Table 4.1) showed that there are two 3,3-dimethylally substituent at located and were C-6 and C-8, ortho to oxygen as biolgenetically expected.

In ring B, there were three mutually coupled aromatic protons resonating at δ_H 6.96 (1H, *d*, *J*=2.4 Hz), 7.01 (1H, *d*, *J*=2.4, 7.8 Hz) and 7.8 (1H, *d*, *J*=7.8 Hz), which were assigned to H-3', H-5' and H-6' respectively. The assignment was consistent with biologically expected oxygenation (hydroxyl) at C-2' and C-4'. Therefore this compound was characterized as 2',4',7-trihydroxy-6,-8-di(3',3'-dimethylallyl) isoflavanone, trivial name bidwillon A (**1**) previously reported from the root bark of *E. orientalis* (Tanaka *et al.*, 1998).



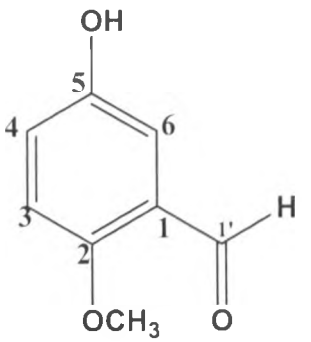
1

Table 4.1: ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data for compound **1** in $CDCl_3$

Position	δ_H (J in Hz)	δ_C
1		
CH_2-2	4.56 *m*	71.2
3	4.16 (1H, *dd*, *J*=6.4, 10.8)	47.5
4		190.9
5	7.69 (*s*)	126.1
6		121.1
7		150.5
8		121.2
9		153.5
10		124.7
1'		119.7
2'		134.0
3'	6.96 (1H, *d*, *J*=2.4)	116.9
4'		149.5
5'	7.01 (1H, *dd*, *J*=2.4, 7.8)	119.2
6'	7.25 (1H,*d*, *J*=7.8)	128.2
1''	3.24 (2H, *d*, *J*=6.2)	29.9
2''	5.17 (*m*)	123.6
3''		132.7
4''	1.68 (3H, *s*)	20.3
5''	1.73 (3H, *s*)	25.9
1'''	3.24 (2H,*d*, *J* = 7.0)	23.6
2'''	5.10 (*m*)	123.6
3'''		130.6
4'''	1.66 (3H, *s*)	18.0
5'''	1.73 (3H, *s*)	21.3

4.1.2 5-Hydroxy-2-methoxybenzaldehyde (2).

The ^{1}H NMR spectrum of compound **2** showed the presence of three aromatic protons resonating at δ_H 7.45 (*d*, *J*=1.4 Hz), 7.01 (*dd*, *J*=1.4, 7.4 Hz) and 7.48 (*d*, *J*=2.0 Hz) of a tri-substituted benzene ring. The presence of an aldehydic proton, resonating at δ_H 9.83 (δ_C 190.7), a methoxyl group, resonating at δ_H 3.93 (δ_C 55.9), and hydroxyl ((δ_C 148.7) was also evident. Since there was no evidence of chelation, the hydroxyl and methoxyl substituents were placed at C-5 and C-2 positions respectively. This compound was characterized as 5-hydroxy-2-methoxybenzaldehyde.



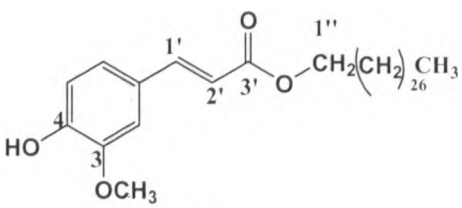
2

Table 4.2: ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data for **2** in $CDCl_3$

Position	δ_H (J in Hz)	δ_C
1		130.4
2		153.3
3	7.45 (1H, *d*, *J*=1.4 Hz)	115.6
4	7.01 (1H, *dd*, *J*=1.4, 7.4 Hz)	110.5
5-OH		148.7
6	7.48 (1H, *d*, *J*=2.0 Hz)	126.7
OCH_3	3.93 (1H, *s*)	55.9
1'	9.83 (1H, *s*)	190.7

4.1.3 Erythrinasinate B (3)

The ^{1}H NMR spectrum of compound **3** revealed the presence of three aromatic protons with an AXY spin system (δ_H 7.07, *dd*, *J*=2.6, 8.0 Hz, 7.04, *d*, *J*=2.6 Hz and 6.91, *d*, *J*=8 Hz), two *trans*-oriented olefinic protons(resonating at δ_H 7.60 *d*, *J*=15.6 Hz and 6.29 *d*, *J*=2.6 Hz) and an oxymethylene protons (resonating at δ_H 4.25, *t*, *J*=6.6 Hz), terminal methyl (resonating at δ_H 0.87, *t*, *J*=5.4 Hz) of a long alkyl chain δ_H [1.75 for H-2'', 1.22, *brs* for $(CH_2)_{25}$] and methoxyl proton resonating at δ_H 3.93. The ^{13}C NMR data indicated a presence of an ester carbonyl carbon resonating at δ_C 177.0 in addition to signals for the aromatic ring, olefinic carbons and the alkyl chain carbon atoms. This suggested that this compound is a long chain cinnamyl ester derivative with an hydroxyl and methoxyl substituents at C-3 and C-4. This compound was therefore identified as erythrinasinate B (**3**), a compound previously reported from some *Erythrina* species (Nkengfack *et al.*, 2001; Yenesew, 1997a).



3

4.2 Compounds isolated from the roots of *Erythrina abyssinica*

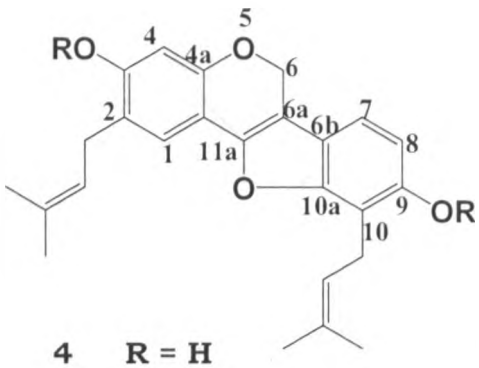
The dried and ground roots of *Erythrina abyssinica* were extracted with acetone by cold percolation. The acetone extract showed potent antiplasmodial [IC_{50} 9.7 ± 1.1 µg/ml (against the D6) and 5.3 ± 0.7 ug/ml (W2) strains], and radical scavenging [against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with EC_{50} value of 18.62 µg/ml] activities. Chromatographic separation resulted in the isolation and identification of erycristagallin (**4**) as one of the active principles among other compounds. The acetate derivative (**4a**) is completely inactive showing the importance of free phenolic groups for both antiplasmodial and radical scavenging activities. The characterization of this compound is discussed below.

4.2.1 Erycristagallin diacetate (4a)

Compound **4** was isolated as white crystals of the diaceteate **4a**. From the ^{1}H NMR spectrum, a two proton resonance at δ_H 5.56, for methylene protons at C-6 with the corresponding carbon resonating at δ_C 65.6, δ_C 114.7 for C-6a and δ_C 146.1 for C-11a were evidence of a pterocarpene skeleton for compound **4a**. From the ^{1}H and ^{13}C NMR spectra the presence of two prenyl and two acetate groups was evident (Table 4.4). Biogenetically oxygenation is expected at C-3 and at C-9 and the acetates could only be placed here, with the prenyl groups expected to be *ortho* to these oxygen substituents.

There are four aromatic protons (two singlets and two *ortho*-coupled protons) in this compound of which the most deshielded signal δ_H 7.33 (s) was assigned to H-1 which would place one of the prenyl groups at C-2 and consequently the second singlet (δ_H 6.63) to H-4 with acetoxy at C-3. Then the two *ortho*-coupled protons resonating at δ_H 7.10 (*d*, *J*=8.4 Hz) and 6.84 (*d*, *J*=8.4 Hz) were assigned to H-7 and H-8 respectively, with the second prenyl to C-10 and the acetoxy in

this ring at C-9. Consequently **4a** was therefore characterized as 3,9-diacetoxy-2,10-di(3',3'-dimethylallyl) pterocarpene. The parent compound erycristagallin (**4**) has been reported from the roots of this plant and is partly responsible for the antioxidant and antiplasmodial activity. The acetate derivative, though inactive in *in vitro* antioxidant and antiplasmodial assays, was isolated as white stable crystals and is a good way of storing the compound for an *in vivo* test as it can hydrolysed releasing the active phenolic parent molecule **4**.



4 **R = H**
4a **R = Ac**

Table 4.3: ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data for (**4**) in $CDCl_3$

Position	δ_H (J in Hz)	δ_C
1a		108.3
1	7.33 *s*	126.8
2		123.6
3		154.6
4	6.63 *s*	118.7
4a		152.9
5		
6		65.6
6a		114.7
6b		118.5
7	7.19 (*d*, *J*=8.4)	121.8
8	6.95 (*d*, *J*=8.4)	121.0
9		149.6
10		121.6
10a		148.3
11		
11a		146.1
1'	3.56 (*d*, *J*=7.4)	28.3
2'	5.23 *m*	116.3
3'		133.0
4'	1.73 *s*	18.1
5'	1.71 *s*	23.9
1'	3.2 (*d*, *J*=7.2)	25.9
2'	5.28 *m*	110.9
3'		133.5
4'	1.87 *s*	18.0
5'	1.78 *s*	21.1
OAC	2.34 *s*	170.1
OAC	2.30 *s*	169.4

4.3 Compounds from the stem bark of *Erythrina brucei*

The air dried and ground stem bark of *Erythrina brucei* was extracted with CH_2Cl_2/MeOH (1:1) by cold percolation. The crude extract was subjected to chromatographic separation, with gradient elution of n-hexane containing increasing amounts of ethyl acetate which led to isolation of four alkaloids. The structures of these compounds were determined on the basis of spectroscopic evidence and by comparison with literature where necessary.

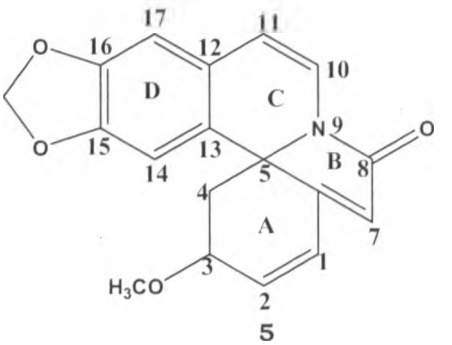
4.3.1 Crystamidine (5)

Compound **5** was isolated as colorless oil with R_f value of 0.44 (30% acetone in n-hexane). The spot turned orange when sprayed with dragendorff reagent, which is an indication that compound **5** is an alkaloid. The ^{1}H NMR spectrum (Table 3.1) revealed the presence of three vinylic protons at δ_H 6.32 (*dd*, *J*=10.2, 2.2 Hz) , 6.93 (*br d*, *J*=10.2 Hz) and 6.12 (*s*) which are characteristic of dienoid protons of an Erythrinan alkaloid, corresponding to protons at C-1, C-2 and C-7 respectively, with corresponding carbon atoms appearing at δ_C 138.2 (C-1), 123.8(C-2), 120.4 (C-7). Other two vinylic protons resonated at δ_H 6.88 (*d*, *J*=7.2 Hz) and 6.07 (*d*, *J*=7.2 Hz), which was assigned to H-10 and H-11 respectively, with corresponding carbon resonating at δ_C 120.7 (C-10) and 113.6(C-11).

The presence of a methylenedioxy group was evident from the ^{1}H NMR spectrum which showed an AB doublets resonating at δ_H 5.97 (*d*, *J*=1.4 Hz) and 5.92 (*d*, *J*=1.4 Hz) and the corresponding carbon appearing at δ_C 101.6. This group was placed between C-15 and C-16 so as to explain two *para*-oriented aromatic protons at C-14 and C-17, [δ_H 6.68 (*s*) and 6.72 (*s*)] with corresponding carbon appearing at δ_C 107.9 (C-14) and 104.2 (C-17).

Three aliphatic protons in the A ring [δ 1.39 (*dd*, *J*=10.2, 11.4), 2.69 (*ddd*, *J*=1.8, 6.6, 11.4), and 3.68 *m*] and a methoxy group attached to an sp^3 carbon (δ_H 3.35, δ_C 56.6). The methoxyl group was placed to C-3 which is the case in all erythrinan alkaloids (Masouda *et al.*, 1991).

The ^{13}C NMR spectrum revealed the presence of eighteen non-equivalent carbons, including an amidic carbonyl resonating at high field value of δ_C 168.8, which was assigned to C-8, the upfield shift of this carbonyl is explained by the fact that it is part of an extended conjugated system in addition to being amidic. The *spiro*-center (C-5) resonated at δ_C 66.2 as in other erythrinan alkaloids (Masouda *et al.*, 1991). Thus compound **5** was identified as crystamidine, a compound previously reported from the leaves of *E. crista-galli* (Mantle *et al.*, 1984.). Although the configuration at the two stereo-centres has not been determined, it is worth to mention that the configuration is usually is *S* at C-5 and *R* at C-3 among *Erythrina* alkaloids.



5

Table 4.4: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for crystamidine (**5**) in $CDCl_3$

Position	δ_H (J in Hz)	δ_C	^{2}J, ^{3}J
1	6.32 (1H, *br d*, *J*=10.2)	138.2	
2	6.93 (1 H, *dd*, *J*=2.2,10.0)	123.8	C-3
3	3.68 (1H, *m*)	74.8	
4	2.69 (1H, *ddd*, *J*=1.8, 6.6, 11.4), 1.39 (1H, *dd*, *J*=10.2, 11.4)	42.8	C-5, C-3,
5		66.2	
6		155.7	
7	6.12 (1H, *s*)	120.4	C-5, C-6, C-8
8		168.8	
10	6.88 (1H, *d*, *J*=7.2)	120.7	C-5
11	6.07 (1H, *d*, *J*=7.2)	113.6c	C-12
12		126.6	
13		125.7	
14	6.68 (1H, *s*)	107.9	C-15, C-16, C-13
15		147.4	
16		146.8	
17	6.72 (1H, *s*)	104.2	C-15, C-16, C-12, C-11
OCH_2O-	5.97 (1H, *d*, *J*=1.4),5.92 (1H, *d*, *J*=1.4)	101.6	
OCH_3	3.35 (3H, *s*)	56.6	C-3

4.3.2 8-oxo-erythtraline (6)

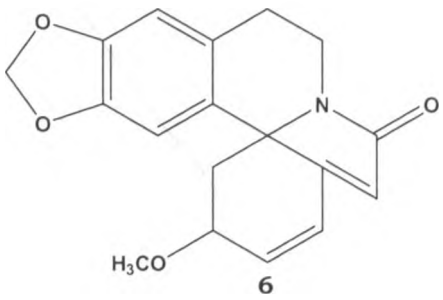
Compound **6** was isolated as colorless oil with R_f value of 0.31 (n-hexane/CH_2Cl_2 1:1). The spot turned orange when sprayed with dragendorff reagent which is an indication of an alkaloid. As in compound **5**, the ^1H NMR spectrum revealed three vinylic protons at δ_H 6.30 (*br d*, *J*=10.2 Hz), 6.86 (*dd*, *J*=10.2, 2.4 Hz) and 5.96 (*s*) corresponding to protons at C-1, C-2 and C-7, with corresponding carbon appearing at δ_C 136.8 (C-1), 123.8 (C-2), 120.3 (C-7).

The presence of a methoxy group (at δ_H 3.13 (*s*), δ_C 56.6) at C-3 was also apparent as the other *Erythrina* alkaloids (Masouda *et al.*, 1991). Three aliphatic protons in ring A at δ_H 1.68 (*dd*, *J*=11.4, 10.8), 2.78 (*dd*, *J*=11.4, 5.0), and 3.79(*m*), were assigned to CH_2-4 and H-3.

Ring D is identical to that of compound **5** with the methylenedioxy protons appearing as AB doublets [at δ_H 5.88(*d*, *J*=1.8 Hz) and 5.91 (*d*, *J*=1.8 Hz)] and the corresponding carbon appearing at δ_C 101.6, and were placed at C-15 and C-16 with the two *para*-oriented aromatic protons (H-14 and H-17) resonating at δ_H 6.75 and 6.72, with the corresponding carbon resonating at δ_C 105.6 (C-17) and δ_C 109.4 (C-14). In fact the only difference between compounds **5** and compound **6** is in C ring; the two olefinic protons in compound **5** (H-10 and H-11) are now replaced with four aliphatic protons at δ_H 3.75 (*m*), 3.60 (*m*), 3.13 (*m*) and 2.97 (*m*) corresponding to two methylene groups showing that compound **6** is a dihydro-derivative of 5.

As in compound **5**, **6** also has a carbonyl functionality at C-8 which is both amidic and part of an extended conjugated system resulting in upfield shifted carbonyl resonance (δ_C 171.1) The ^{13}C NMR spectrum revealed the presence of eighteen non-equivalent carbons, including the signal for the *spiro*-center at C-5 resonated at δ_C 67.0, it is worth to mention that the configuration is usually is *S* at C-5 and *R* at C-3 among *Erythrina* alkaloids. Consequently compound **6** was

identified as 8-oxo-erythtraline, a compound previously reported from the leaves of *Erythrina crista-galli* (Mantle, *et al.*, 1984).



6

Table 4.5 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for 8-oxo-erythraline **(6)** in $CDCl_3$

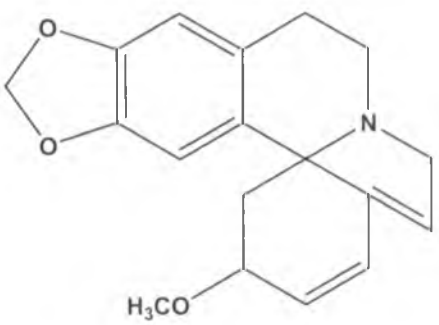
Position	**δ_H (J in Hz)**	**δ_C**
1	6.30 (1H, *br d*, *J*=10.2 Hz)	136.1
2	6.28 (1H, *dd*, *J*=2.4, 10.2 Hz)	123.8
3	3.79 (1H, *m*)	75.1
CH_2-4	1.68 (1H, *dd*, *J*=10.8, 11.4 Hz),2.78 (1H, *dd*, *J* = 5.0, 11.4 Hz)	27.5
5		67.0
6		157.2
7	5,96 (1H, *br*, *s*)	120.3
8		171.1
10	3.75 (1H, *m*), 3.60 (1H, *m*)	41.6
11	3.13 (1H, *m*), 2.93 (1H, *m*)	38.0
12		130.4
13		128.3
14	6.75 (1H, *s*)	109.4
15		147.2
16		146.2
17	6.72 (1H, *s*)	105.2
-OCH_2O-	5.91 (1H, *d*, *J*=1.6 Hz), 5.88 (1H, *d*, *J*=1.6 Hz)	101.6
OCH_3	3.33 (3H, *s*)	56.6

4.3.3 Erythraline (7)

Compound **7** was isolated as colorless oil with R_f value of 0.42 (50% CH_2Cl_2 in n-hexane). The spot turned orange when sprayed with dragendorff reagent, which is an indication of an alkaloid. As in compound **6**, the ^1H NMR spectrum revealed three vinylic protons at δ_H5.97 (*br d, J*=9.6 Hz), 6.52 (*dd, J*=10.2, 2.2 Hz) and 5.71 (*s*) which are characteristic of dienoid protons of an erythrinan alkaloid, corresponding to protons at C-1, C-2 and C-7, with corresponding carbon appearing at δ_C 131.8 (C-1), 125.3 (C-2), 122.9 (C-7).

The presence of a methoxyl group (at δ_H 3.33, δ_C 56.2) at C-3 was also apparent as the other *erythrinan* alkaloids (Masouda *et al.*, 1991). Three aliphatic protons in the ring A at δ_H 1.86 (*t, J* =10.6 Hz), 2.49 (*dd, J*=5.6, 11.8 Hz) and 3.47 (*m*), were assigned to CH_2-4 and H-3. Ring D is identical to that of compound **6** with methylenedioxy protons appearing as an AB doublets [at δ_H 5.90 (*d, J*=1.4 Hz) and 5.87 (*d, J*=1.4 Hz)] and the corresponding carbon at δ_C 100.9, and were placed at C-15 and C-16 with the two *para*-oriented aromatic protons (H-14 and H-17), resonating δ_H 6.75 (δ_C 106.4) and 6.62 (δ_C108.9), with corresponding carbon resonating at (C-14) and (C-17). Ring C is also identical to that of compound **6,** with four aliphatic protons at δ_H 2.65 (*m*), 2.70 (*m*), 2.84 (*m*), 2.92 (*m*) corresponding to two methylene groups.

In fact the only difference between compounds **6** and **7** is in ring B; carbonyl functionality at C-8 is now replaced with methylene group [at δ_H 3.94 (*br d, J*=11.4 Hz) and 3.76 (*dd, J*=2.8, 14.2 Hz)] with corresponding carbon at appearing at δ_C 57.8. The ^{13}C NMR spectrum revealed the presence of eighteen non-equivalent carbons, including the signal for the *spiro*-center at C-5 resonated at δ_C 67.8. Consequently compound **7** was identified as erythtraline, a compound previously reported from the leaves of *Erythrina crista-galli* (Mantle, *et al.*, 1984).



7

Table 4.6: ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data for erythraline (**7**) in $CDCl_3$

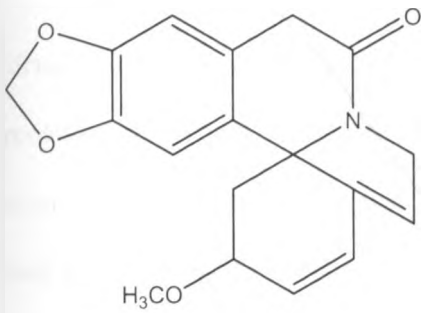
Position	**δ_H (J in Hz)**	**δ_C**
1	6.52 (1H, *dd*, *J*=2.2, 10.2)	125.3
2	5.97 (1H, *br d*, *J*=9.6)	131.8
3	3.47 (1H, *m*)	76.2
4	1.86 (1H, *t*, *J*=10.6),2.49 (1H, *dd*, *J*=5.6, 11.8)	29.9
5		67.8
6		132.4
7	5.71 (1H, *br s*)	122.4
8	3.94 (1H,*brd*, *J*=11.4), 3.76 (1H, *dd*, *J*=2.8, 14.2)	57.8
10	2.92 (1H, *m*), 2.84 (1H, *m*)	44.7
11	2.70 (1H, *m*), 2.65 (1H, *m*)	41.7
12		132.4
13		128.0
14	6.62 (1H, *s*)	106.4
15		146.4
16		146.1
17	6.75 (1H, *s*)	108.9
$-OCH_2O-$	5.87 (1H,*d*, *J*=1.4), 5.90 (1H, *d*, *J*=1.6)	100.9
OCH_3	3.33 (3H, *s*)	56.2

4.3.4 10-oxo-erythtraline (8)

Compound **8** was isolated as colorless oil with R_f value of 0.40 (50% CH_2Cl_2 in n-hexane). The spot turned orange when sprayed with dragendorff reagent. As in compound **7**, the ^1H NMR spectrum revealed the presence of three vinylic protons at δ_H 6.63 (*dd*, *J*=10.2, 1.8 Hz), 6.63 (*brs*) and 5.73 (*brs*) corresponding to protons at C-1, C-2 and C-7, with corresponding carbon atoms appearing at δ_C 131.9 (C-1), 124.3 (C-2), 120.1 (C-7).

The presence of a methoxyl group at (δ_H 3.29, δ_C 56.6) was also apparent as the other *Erythrina* alkaloids (Masouda *et al.*, 1991). Three aliphatic protons in the ring A at δ_H 1.93 (*t*, *J*=11.4 Hz), 2.57 (*dd*, *J*=5.6, 11.4 Hz) and 3.70 (*m*), were assigned to CH_2-4 and H-3. Ring D is identical to that of compound **7**, with methylenedioxy signal appearing as AB doublets [at δ_H 5.93(*d*, *J*=1.4 Hz) and 5.97 (*d*, *J*=1.4 Hz)] and the corresponding carbon at δ_C 101.6, and were placed at C-15 and C-16 with the two *para* aromatic protons H-14 and H-17, resonating at δ_H 6.8 7 (δ_C 104.1) and 7.21 (δ_C 106.3), respectively. The only difference between compound **7** and **8** is in ring C where the methylene group at C-10 is replaced in compound **8** with carbonyl functionality which is amidic resulting in upfield shifted carbonyl resonance at δ_C 173.0.

Related compound which places the carbonyl at C-11 has been reported and the data was comparable, however based on COSY and NOEDIFF experiment results, its points to C-10 oxygenation for compound **8**. Thus irradiation of H-17 causes enhancement of proton signals for CH_2-11 and vice versa. Therefore this compound was characterized 10-0xo-erythraline (**8**), which is a new compound. This new compound unfortunately decomposed before further analysis could be done.



8

Table 4.7: ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data for compound **8** in $CDCl_3$

Position	**δ_H (J in Hz)**	**δ_C**
1	6.63 (1H, *dd*, *J*=1.8, 10.2)	124.3
2	6.00 (1H, *br s*)	131.9
3	3.70 (1H, *br s*)	76.2
4	2.57 (1H,*dd*, *J*=5.6, 11.4),1.93 (1H, *t*, *J*=11.4)	29.9
5		71.9
6		138.8
7	5.73 (1H, *br s*)	120.1
8	4.39 (2H, *br s*)	67.9
10		173.0
11	5.25 (1H,*br s*), 4.01 (1H, *br s*)	39.7
12		131.4
13		129.9
14	6.87 (1H, *s*)	104.1
15		147.7
16		146.8
17	7.21 (1H, *br s*)	106.3
$-OCH_2O-$	5.93 (1H, *d*, *J*=1.4), 5.97 (1H, *d*, *J*=1.4)	101.6
OCH_3	3.29 (3H, *s*)	56.6

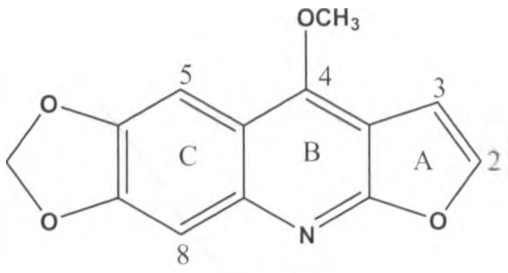
4.4 Compounds from *Teclea nobilis*

The air dried and ground stem bark of *Teclea nobilis* was extracted with CH_2Cl_2/MeOH (1:1) by cold percolation. The crude extract was then subjected to chromatographic separation which led to isolation of four alkaloids and one terpenoid. The structures of these compounds were determined on the basis of spectroscopic evidence and by comparison with literature where necessary.

4.4.1 Maculine (9)

Compound **9** was isolated as white feathery like crystals with R_f value of 0.33 (1% MeOH in CH_2Cl_2). The spot turned orange when sprayed with dragendorff reagent which is an indication of an alkaloid. The ^{1}H NMR spectrum revealed the presence of a pair of AB doublets at δ_H 7.49 (*d*, *J*=3 Hz, H-2) and 6.94 (*d*, *J*=3 Hz, H-3) corresponding to the two furan protons of furoquinoline alkaloids, and the corresponding carbon atoms resonating at δ_C 142.7 (C-2) and 104.7 (C-3). A downfield shifted methoxyl group resonating at δ_H 4.33 (δ_C 59.1), is also characteristic of a methoxyl group at C-4 for furoquinoline alkaloids (Ayafor and Okogun, 1982).

The presence of a methylenedioxy substituent in the furoquinoline skeleton was evident from the NMR spectra [δ_H 6.03 and the corresponding carbon at δ_C 101.6] and this was placed between C-6/C-7 due to the presence of two *para*-oriented singlets resonating at δ_H 7.23 and 6.41 which could only be assigned to H-5 and H-8, respectively; the corresponding carbon atoms resonating at δ_C 102.6 (C-5) and 98.2 (C-8). Therefore this compound was assigned structure **9**, trivial name masculine, previously isolated from the leaves of *Teclea nobilis* (Yenesew and Dagne, 1988). However this is the first report from the bark.



9

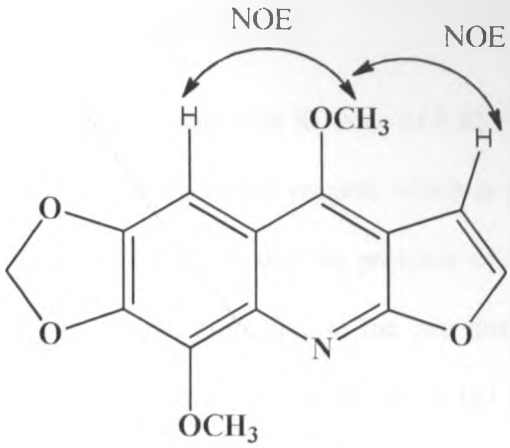
Table 4.8: ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data for maculine (**9**) in $CDCl_3$

position	**δ_H (J in Hz)**	**δ_C**
2	7.29 (1H, *d*, *J*=3)	142.7
3	6.94 (1H, *d*, *J*=3)	104.7
3a		102.6
4		156.1
4a		114.4
5	7.41 (1H, *s*)	102.6
6		150.9
7		146.2
8	7.23 (1H, *s*)	98.2
8a		143.9
9a		163.3
$-OCH_3$	4.33 (3H, *s*)	59.1
$-OCH_2O-$	6.03 (2H, *s*)	101.8

4.4.2 Flindersiamine (10)

Compound **10** was isolated as needle-like crystals with R_f value of 0.46 (2% MeOH in CH_2Cl_2). The spot turned orange when sprayed with dragendorff reagent which is an indication of an alkaloid. As in compound **9**, the ^1H NMR spectrum revealed the presence of a pair of AB doublets at δ_H 7.50 (J=3Hz) and 6.93 (J=3Hz) corresponding to the two furan protons of furoquinoline alkaloids, and the corresponding carbon atoms resonating at δ_C 143.1(C-2) and 104.6 (C-3). A downfield shifted methoxyl group resonating at δ_H 4.31 (δ_C 60.8), is also characteristic of a methoxyl group at C-4 for furoquinoline alkaloids (Ayafor and Okogun, 1982).

The presence of a methylenedioxy substituent in the furoquinoline skeleton was evident from the NMR spectra [δ_H 5.99 and the corresponding carbon at δ_C 101.7]. In fact the only difference between compounds **9** and **10** is in ring C; there is only one singlet aromatic proton resonating at δ_H 7.15, with corresponding carbon at δ_H 92.6, the other singlet is now replaced with a downfield shifted methoxyl resonance [δ_H 4.20 and the corresponding carbon resonating δ_C 59.1]. This indicates that it is di-*ortho*-substituted hence it is placed at C-8. The placement of the second methoxyl at C-8 was confirmed by NOEDIFF experiment. Thus irradiation of methoxyl at C-4 causes enhancement of H-3 and H-5. Therefore this compound was assigned structure **10**, trivial name flindersiamine previously isolated from the leaves of *Teclea nobilis* (Yenesew and Dagne, 1988). Again this is the first report from the stem bark of *T. nobilis*.



10

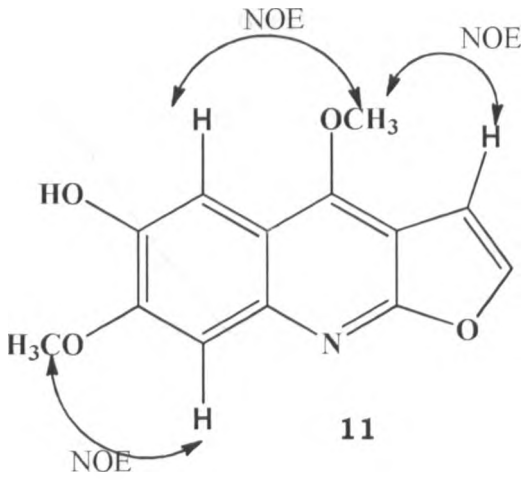
Table 4.9: ^{1}H (200 MHz) and ^{13}C (50 MHz) data for flindersiamine (**10**) in $CDCl_3$

position	**δ_H (J in Hz)**	**δ_C**
2	7.50 (1H, *d*, *J*=3)	143.1
3	6.93 (1H, *d*, *J*=3)	104.6
3a		102.9
4		156.2
4a		115.1
5	7.15 (1H, *s*)	92.6
6		138.2
7		137.8
8		146.2
8a		136.9
9a		162.8
4-OCH_3	4.31 (3H, *s*)	60.8
8-OCH_3	4.20 (3H, *s*)	59.1
-OCH_2O-	5.99 (2H, *s*)	101.7

4.4.3 4, 7-dimethoxyfuro [2,3-b]quinolin-6-ol (11)

Compound **11** was isolated as yellow crystals with R_f value of 0.42 (3% MeOH in CH_2Cl_2). The spot turned orange when sprayed with dragendorff reagent, which is an indication of an alkaloid. As in compound **9**, the ^1H NMR spectrum revealed the presence of a pair of AB doublets at δ_H 7.52 (J=2.8 Hz) and 7.05 (J=2.8 Hz) corresponding to the two furan protons of furoquinoline alkaloids, and the corresponding carbon atoms resonating at δ_C 141.7 (C-2) and 108.1 (C-3). A downfield shifted methoxyl group resonating at δ_H 4.39 (δ_C 58.4), is also characteristic of a methoxyl group at C-4 for furoquinoline alkaloids (Ayafor and Okogun, 1982).

The presence of two *para*-oriented singlets at δ_H 7.42 and 7.15 were assigned to H-5 and H-8 protons respectively (the corresponding carbon atoms resonating at δ_C 104.5 (C-5) and 100.1 (C-8) with C-6 and C-7 being substituted. The only difference between compound **9** and **11** is in C ring; the signal for methylenedioxy group in compound **9** is now replaced with a methoxyl [δ_H 3.96 and corresponding carbon appearing at δ_C 55.3] and hydroxyl group. This compound appears to be precursor to compound **9** where oxidation and cyclization involving the methoxyl and hydroxyl groups at C-6 and C-7 in compound **11** to produce the methylenedioxy group in **9**. The methoxyl group was placed at C-7 based on NODIFF experiment, where irradiation of the methoxyl at C-4 showed enhancement of the signal for H-5. Similarly irradiation of the methoxyl at C-7 showed enhancement of the signal for H-8. Therefore this compound was characterized as 4,7-dimethoxyfuro[2,3-b]quinolin-6-ol (**11**), previously isolated from *Monnieria trifolla* (Rutaceae) (Bhattachary *et al.*, 1984).



11

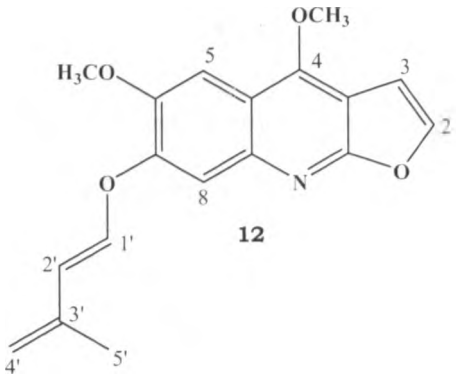
Table 4.10 ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR data for **11** in $CDCl_3$

position	**δ_H (J in Hz)**	**δ_C**
2	7.52 (1H, *d*, *J*=3.0)	141.7
3	7.05 (1H, *d*, *J*=3.0)	108.1
3a		101.4
4		155.9
4a		112.2
5	7.42 (1H,*s*)	104.5
6-OH		150.3
7		146.9
8	7.15 (1H,*s*)	100.1
8a		141.6
9a		162.6
4-OCH_3	4.39	58.4
7-OCH_3	3.96	55.3

4.4.4 7-(3'-methylbut-1',3'-dienyloxy)-4,6-dimethoxyfuro[2,3-b]quinoline (12)

Compound **12** was isolated as yellow oil with R_f value of 0.5 (1% MeOH in CH_2Cl_2). The spot turned orange when sprayed with dragendorff reagent which is an indication of an alkaloid. The ^1H NMR spectrum as the other alkaloids of this plant revealed the presence of a pair of AB doublets corresponding to the two furan protons (H-2 and H-3) of furoquinoline alkaloids along with a downfield shifted methoxyl which is also characteristic of a methoxyl group at C-4 for furoquinoline alkaloids (Ayafor and Okogun, 1982). The ^{1}HNMR spectrum further showed a second methoxyl group resonating at δ_H 4.02, with corresponding carbon resonating at δ_C 56.1 The presence of two singlet aromatic protons at 7.52 (H-5, δ_C 104.6) and 7.32 (H-8, δ_C 101.2) is consistent with C-6 and C-7 substituted C ring. One of these substituents being methoxyl (δ_H 4.02, δ_C 56.1) groups and was placed at C-6 based on NODIFF experiment. Irradiation of methoxyl at C-4 showed interaction with H-3 and H-5, and irradiation of the methoxyl group resonating at δ_H 4.02 ppm (6-OMe) showed interaction with H-5.

The substituent at C-7 is 3-methylbut-1,3-dienyloxy, as evidenced by the presence in the ^1H NMR spectrum of a pair of doublets resonating at δ_H 6.85 (J=12.2 Hz, for H-1'), and 6.32 (J=12.2 Hz, H-2'), terminal methylene protons resonating at δ_H 4.94 (1H, *br s*) and 4.89 (1H, *br s*), and a methyl group at δ_H 1.91 for Me-5). The corresponding carbon atoms of this group appeared at δ_C 142.3 (C-1'), 115.3 (C-2'), 114.5 (C-3'), 118.9 (C-4') and 18.9 (C-5'). Therefore this compound was characterized as 7-(3-methylbuta-1,3-dienylloxy)-4,6-dimethoxyfuro[2,3-b]quinoline (**12**), which is a new compound. This new compound unfortunately decomposed before further analysis could be done.



12

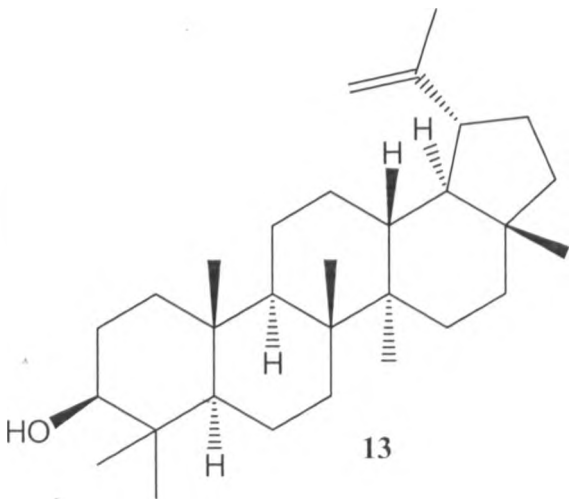
Table 4.11 ^{1}H (200 MHz) and ^{13}C (50 MHz) data for (**12**) in $CDCl_3$

Position	δ_H (J in Hz)	δ_C
2	7.58 (1H, *d*, *J*=2.8)	142.9
3	7.05(1H, *d*, *J*=2.8)	111.6
3a		102.8
4		150.1
4a		109.7
5	7.52 (1H, s)	101.2
6		141.9
7		147.7
8	7.35 (1H, s)	104.6
8a		138.6
9a		155.6
4-OCH_3	4.45(3H,*s*)	58.9
6-OCH_3	4.02(3H, *s*)	56.1
1'	6.85(1H, *d*, *J*=12.2)	142.3
2'	6.32 (1H, *d*, *J*=12.2)	115.3
3'		114.5
4'	4.94 (1H, *br s*) 4.89(1H, *br s*)	118.9
5'	1.91 (3H,*s*)	18.9

4.4.5 Lupeol (13)

Compound **13** was obtained as non-UV active white crystals. The ^{13}C NMR of this compound revealed the presence of 30 carbons of a triterpene derivative where seven are methyl carbons (δ_C 28.2, 16.1, 15.6, 18.2, 14.7, 16.3 and 19.5) with the characteristic peaks at δ_C 79.2 for the oxygenated C-3, the quarternary carbon peaks (at δ_C 39.1, 37.4, 41.0, 43.0 and 43.2) and olefinic carbons (δ_C 151.2 and 109.5), the remaining carbons corresponding to ten methylene carbons (δ_C 18.5, 21.1, 25.3, 27.6, 29.9, 30.1, 34.5, 35.8, 38.9 and 40.2) and five methine carbons (δ_C 48.2, 48.5, 50.6, 55.5 and 38.3). The data showed that compound **13** is lupeol (**13**)

Further the ^1H NMR confirmed the presence of seven singlet methyl protons (at δ 0.95 for Me-23, 0.77 for Me-24, 0.81 for Me-25, 1.01 for Me-26, 0.93 for Me-27, 0.77 for Me-28, and 1.66 for Me-30) and methylene protons (at δ 4.55 for H-29a and 4.68 for H-29b) as well as a double doublet at δ 3.17 for H-3. Based on these and by comparison of the data with literature, compound **13** was identified as lupeol (**13**) [Furukawa *et al.*, 2002]. A compound which widely occur in plants.



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4.5 Biological activities

4.5.1 Radical scavenging activity test

Preliminary tests for radical scavenging activities using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as a spray reagent on TLC plates, of the extracts from *Erythrina abyssinica, E. burttii* and *E. brucei* showed that *E. abyssinica* and *E. burttii* were active while *E. brucei* did not show significant activity.

4.5.2 Quantitative radical scavenging activity test

The acetone extract of the root bark of *Erythrina burttii* showed radical scavenging activity against 1, 1-diphenyl-1-picrylhydrazyl (DPPH) radical with EC_{50} value of 12.5 μg/ml. The previously reported isoflav-3-enes; burttinol-A (**90**) and burttinol-C (**93**), and the 2-arylbenzofuran derivative burttinol-D (**123**) were identified as the most active free radical scavenging principles (Table 4.13).

The acetone extract of the roots *Erythrina abyssinica* showed radical scavenging activity against 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical with EC_{50} value of 18.62 μg/ml. The compounds responsible for the radical scavenging activity of the roots of *E. abyssinica* have been identified as the pterocarpenes erycristagallin (**4**) and 3-hydroxy-9-methoxy-10-(3,3-dimethylally)-pterocarpene (**103**), among other flavonoids (Table 4.13) (Yenesew *et al.*, 2009). However these are unstable, hence acetylation was done to stabilize them for storage. Interestingly the acetate derivatives prepared from these compounds were completely inactive showing the importance of free phenolic groups for activity.

4.5.3 *In vitro* antiplasmodial activities

The acetone extract of the root bark of *Erythrina burttii* showed *in vitro* antiplasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 0.97 ± 0.2 and 1.73 ± 0.5 µg/ml respectively (Table 4.13). The previously reported isoflav-3-enes; burttinol-A (**90**) and burttinol-C (**93**), and the 2-arylbenzofuran derivative burttinol-D (**123**) were identified as the most active antiplasmodial principles ($IC_{50} < 10$ µM) against both strains (Table 4.13). The crude extract is more active than any of the pure compounds, which suggests that additional antiplasmodial compounds may exist within the roots of *E. burttii*, or the high activity of the crude extract may also be due to synergistic effect of multiple compounds.

The acetone extract of the roots of *Erythrina abyssinica* was also tested and showed *in vitro* antiplasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 9.7 ± 1.1 and 5.3 ± 0.7 µg/ml respectively. Erycristagallin (**4**) was identified as one of the active principles among other flavonoids previously characterized. It showed antiplasmodial activity against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* with IC_{50} values of 19.0 ± 0.9 and 20.1 ± 3.6 µg/ml respectively (Yenesew *et al.*, 2003). When the phenolic groups are completely blocked through acetylation, the acetate derivative; erycristagallin diacetate (**4a**) is completely inactive. This clearly showed the importance of free phenolic groups for antiplasmodial activity.

Table 4.12: *In vitro* antiplasmodial and Radical Scavenging Activities (RSA) of crude extracts and isolates from *Erythrina burttii* and *Erythrina abyssinica*

Sample	Antiplasmodial activity [IC_{50} (μM)]		RSA [EC_{50} (μM)]
	D6	W2	DPPH
Crude extracts			
E. burttii (root bark)	0.97 ± 0.2	1.73 ± 0.5	12.5
E. abyssinica (roots)	9.7 ± 1.1	5.3 ± 0.7	18.62
Burttinol-A (**90**)	7.6 ± 0.3	8.5 ± 0.6	9.8
Burttinol-C (**93**)	9.3 ± 0.9	9.1 ± 1.2	10.6
Burttinol-D (**123**)	4.9 ± 0.3	6.1 ± 1.5	9.9
erycristagallin (**4**)	19.0 ± 0.9	20.1 ± 3.6	8.2
3-Hydroxy-9-methoxy-10-(3,3-dimethylally) pterocarpene (**103**)	-	-	10.8
Reference drugs			
Chloroquine	0.009 ± 0.001	0.08 ± 0.001	NT
Quinine	0.04 ± 0.02	0.21 ± 0.02	NT
Quercetin	NT	NT	5.4

4.5.4 *In vivo* antiplasmodial activities

The crude extract of the roots of *Erythrina burttii*, the roots and stem bark of *E. abyssinica* were tested in an *in vivo* 4-days *Plasmodium berghei* ANKA suppressive test (Peters *et al.*, 1975), at 800 mg/kg/day. All the extracts showed weak to moderate parasite suppression activities (Table 4.14).

Table 4.13: *In vivo* antimalarial activity of *Erythrina* species against *P. berghei* ANKA suppressive test

Sample	Dosage (mg/kg/day)	% Average *parasitaemia* on day 4	% *Chemosupression*
E. abyssinica (stem bark)	800	20.17 ± 2.8	44.2
E. abyssinica (root bark)	800	18.2 ± 2.3	49.7
E. burttii (root bark)	800	17.17 ± 2.7	52.5
Chloroquine	10	0.977 ± 0.4	97.3

4.5.5 Antimicrobial activities

The crude acetone extract of the root bark of *Erythrina burttii* showed moderate activity against Methicillin resistant *Staphylococcus aureus* (clinical isolate), *Trichophyton mentagrophytes* and *Candida albicans* (inhibition zone of 10 mm) with an MIC value of 130.13 µg/disc.

Burttinol-D showed significant activity against *Trichophyton mentagrophytes* (inhibition zone of 28 mm) with an MIC value of 13.0µg/disc. Also it showed potent *in-vitro* antifungal and antibacterial activity against gram positive and gram negative, yeast and fungi namely *candida albicans*, *Cryptococcus neoformans*, *Staphylococcus aureus*, *MRSA* and *microsporum gypseum*.

The crude extract of *Erythrina abyssinica* showed moderate activity against Methicillin resistant *Staphylococcus aureus* (inhibition zone of 7 mm), *Staphylococcus aureus* (inhibition zone of 8 mm) and *Pseudo aeruginosa* (inhibition zone of 7 mm) with an MIC value of 310.0µg/disc.

Previously isolated compound erythrabyssin-I showed significant activity against *Staphylococcus aureus* and *bacillus subtilis* with MIC value of 12.5 and 6.25µg/disc respectively (Kamat *et al.*, 1981).

Table 4.14; Antimicrobial activity of the crude extract of the roots of *E. abyssinica, E. burttii* and burttinol-D

Sample	*E. abyssinica* (crude)		*E. burttii* (crude)		Burttinol D	
Amount (Mg)	Zone of inhibition in mm at 0.31 mg/disc	MIC (mg/disc)	Zone of inhibition in mm at 0.31 mg/disc	MIC (mg/disc)	Zone of inhibition in mm at 0.2 mg/disc	MIC (mg/disc
ACTIVITY						
Staphylococcus aureus	7	0.31	7	0.26	11	0.025
Methicillin resistant *Staphylococcus aureus*(clinical isolate)	8	0.31	10	0.13	12	0.025
Trichophyton mentagrophytes	-		10	0.13	28	0.013
Pseudo aeruginosa	7	0.31	-		10	0.05
Candida albicans	-		10	0.13	11	0.02
Cryptococcus neoformans	-		-		7	0.2
Microsporum gypseum.	-		-		15	0.025

MIC; Minimum Inhibitory Concentrations (mg/disc)

Antibacterial and antifungal tests were carried out on the crude extracts and some of the isolated compounds against gram positive bacteria (*Staphylococcus aureus*), gram negative bacteria (*Escherichia coli*) and fungi (*Candida albicans*, *Cryptococcus neoformans*, *Staphylococcus aureus*, *MRSA*, *Aspergillus niger* and *microsporum gypseum*).

The crude extract and the isolated compounds maculine (**9**) and flindersiamine (**10**) of *Teclea nobilis* tested did not show any activity against bacteria (*Staphylococcus aureus* and *Escherichia coli*) and fungi (*Candida albicans*, *Aspergillus niger*).

4.6 CONCLUSION

The crude acetone extracts of the root bark and stem bark of *E. burttii* demonstrated significant *in vitro* antiplasmodial, radical scavenging, antimicrobial and antifungal activities. The isoflav-3-enes; burttinol-A (**90**) and burttinol-C (**93**), and the 2-arylbenzofuran derivative burttinol-D (**123**) along with erythrinabyssine IV have been identified as the most active antiplasmodial and radical scavenging principles of the root extract. Burttinol-D was also identified as the most potent antimicrobial and antifungal agent of the roots of *E. burttii.*

From the root bark of *Erythrina burttii*, bidwillon A (**1**), 5-hydroxy-2-methoxybenzaldehyde (**2**) and erythrinasinate (**3**) were also identified.

The acetone extract of the roots of *Erythrina abyssinica* also showed moderate antiplasmodial activity [IC_{50} 9.7 ± 1.1 μg/ml (against the D6) and 5.3 ± 0.7 μg/ml (W2) strains] and potent radical scavenging activity against DPPH radical. Erycristagallin among other flavonoids is responsible for these activities. Erycristagallin diacetate is inactive (IC_{50} > 100 M). This clearly shows the importance of free phenolic groups for antiplasmodial and radical scavenging activities of erycristagallin. In an *in vivo* assay both the roots of *E. burttii* and *E. abyssinica* showed weak antimalarial activities. However none of the pure compounds were tested.

From the stem bark of *Erythrina brucei* four compounds were isolated. Four of these were identified as crystamidine (**5**), 8-oxo-erythraline (**6**), Erythraline (**7**), while the fourth compound, named here as 10-oxo-erythraline (**8**). is a new compound; *Erythrina* alkaloids occur in flowers and seeds; this is the second report of isolation of *Erythrina* alkaloids from the stem bark. In antimicrobial assay, neither the crude extract nor the pure compounds showed significant activities.

Phytochemical investigation of the stem bark of *Teclea nobilis* led to the isolation of five compounds. Four of these were identified as the alkaloids maculine (**9**), flindersiamine (**10**), 4, 7-dimethoxyfuro [2, 3-b] quinolin-6-ol (**11**), and the triterpene derivative Lupeol (**13**). The fifth isolate is a new alkaloid and was characterized as 7-(3-methylbuta-1, 3-dienylloxy)-4, 6-dimethoxyfuro [2, 3-b] quinoline (**12**). Three compounds did not show significant antimicrobial activities.

4.7 RECOMMENDATIONS

1. Semi-purified fractions and major compounds isolated from *E. burttii* and *E. abyssinica* which also showed *in vitro* antiplasmodial activity should be tested for *in vivo* antimalarial activities.
2. Combination of the various compounds and extracts obtained from *E. burttii* and *E. abyssinica* also with standard drugs should be done to evaluate possible synergistic effects.
3. More comprehensive phytochemical work should be done on different parts of *Erythrina brucei* and *Teclea nobilis*.

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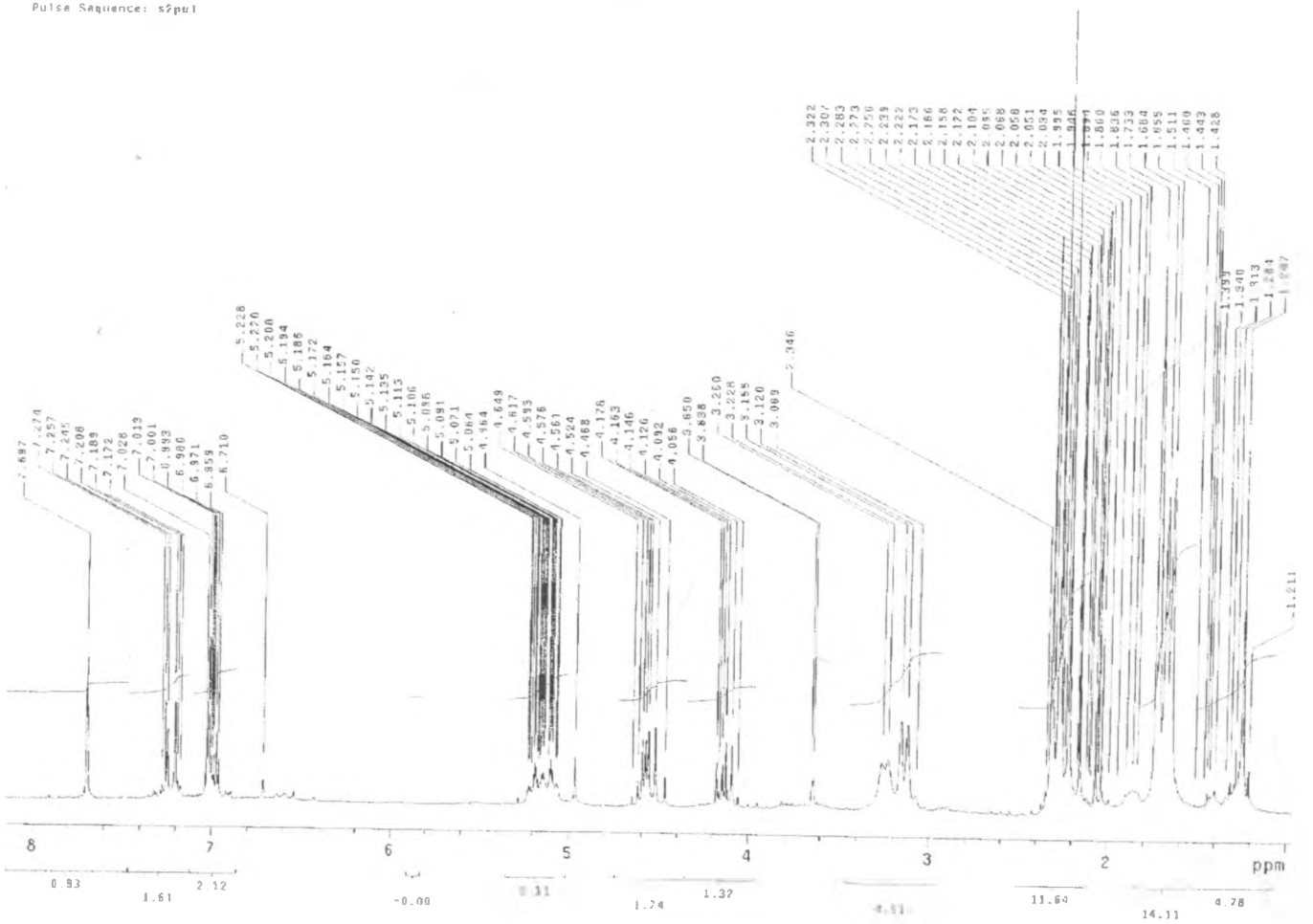

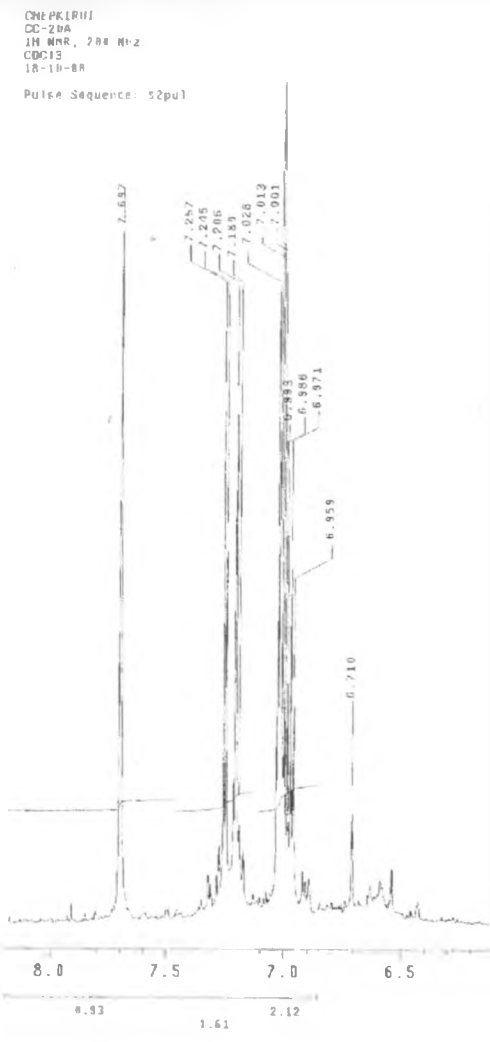
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1H NMR, 200 MHz
CDCl3
18-10-08
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7.297
7.295
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7.028
7.013
7.001
6.993
6.986
6.971
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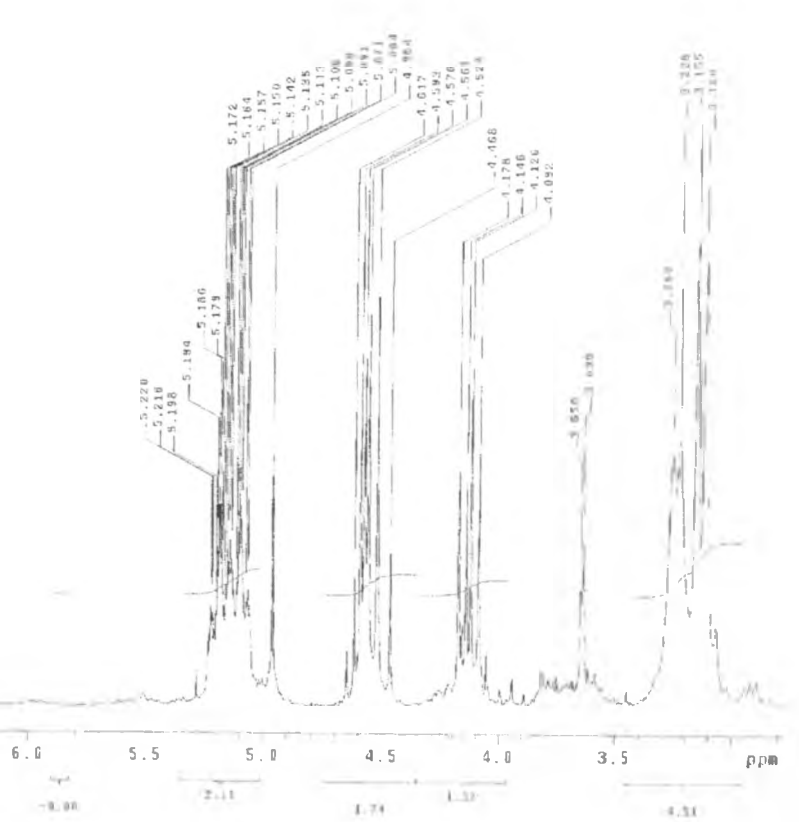
8.0
7.5
7.0
6.5
0.93
2.12
1.61

^{1}H NMR SPECTRUM FOR COMPOUND 1



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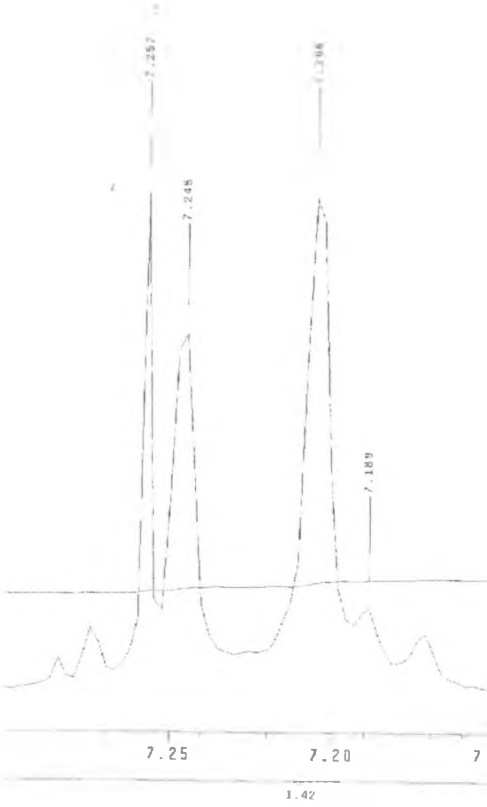

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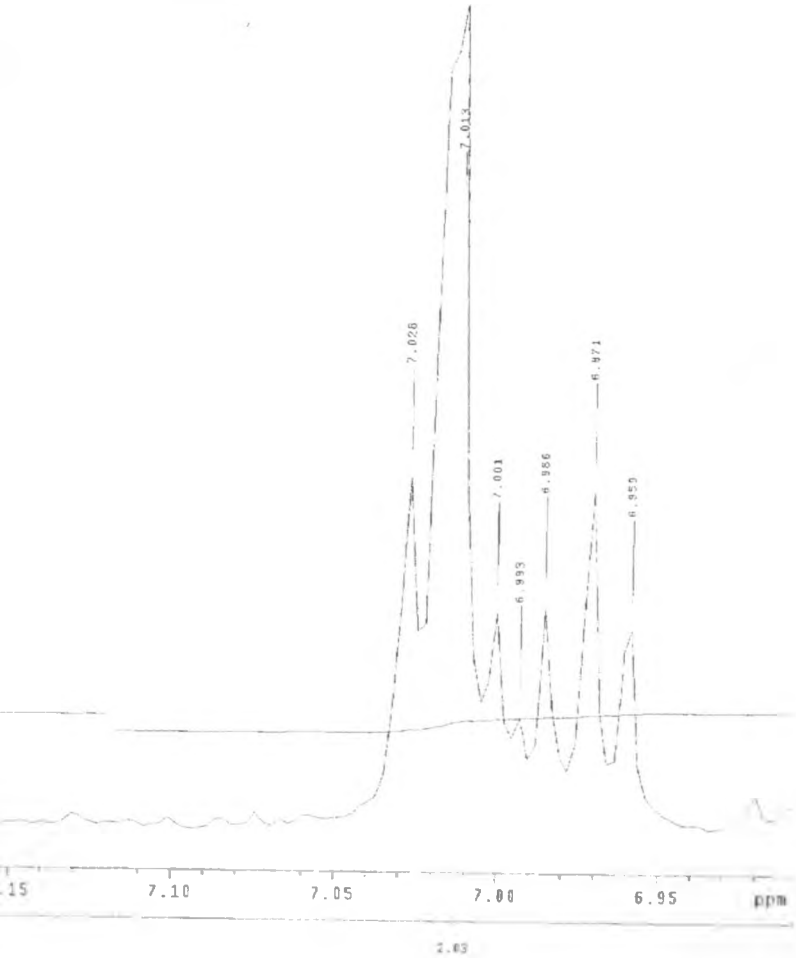

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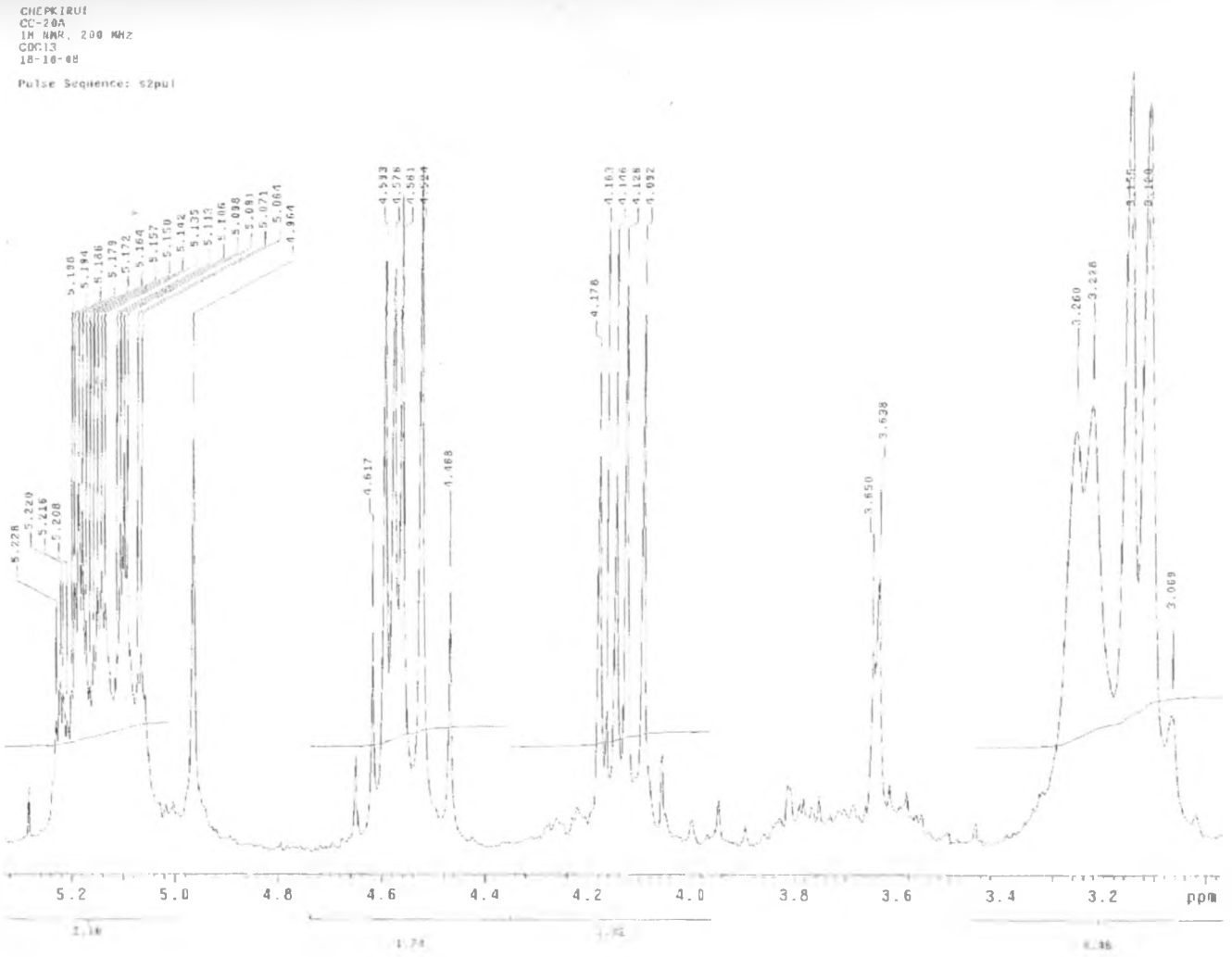

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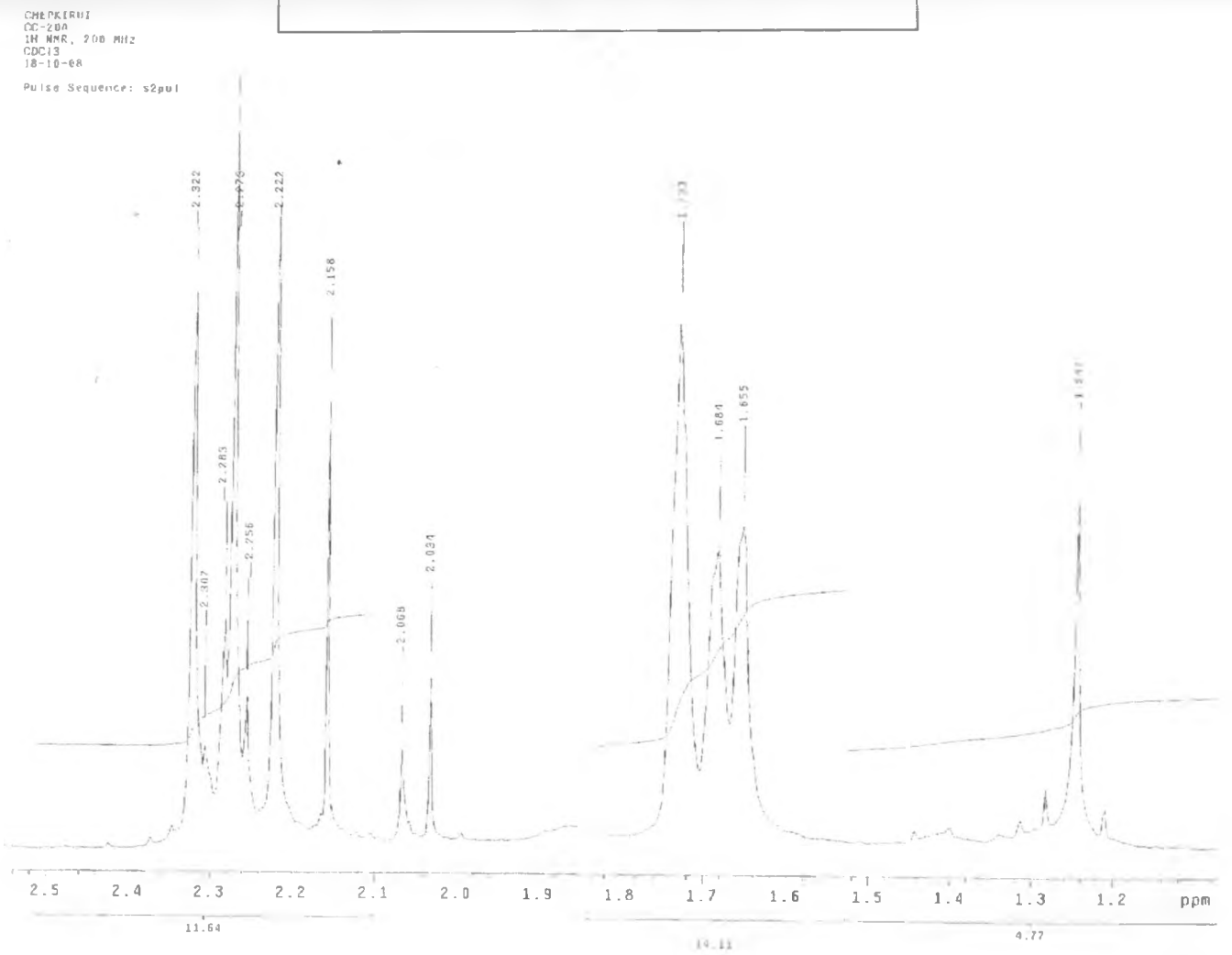

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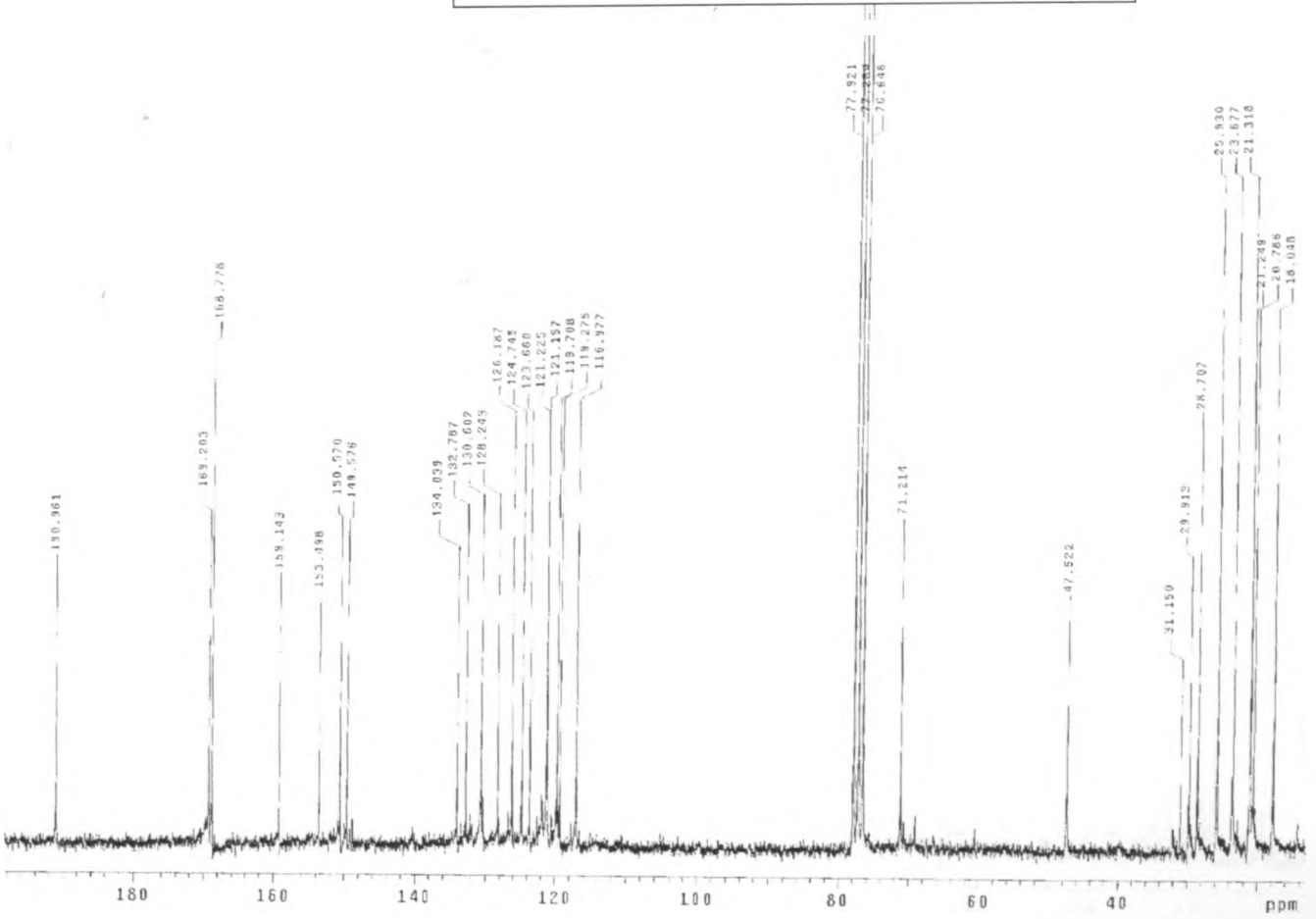
19-10-0A

Pulse Sequence: s2pul

^{13}C NMR SPECTRUM FOR COMPOUND 1



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19-10-08

Pulse Sequence: s2pul

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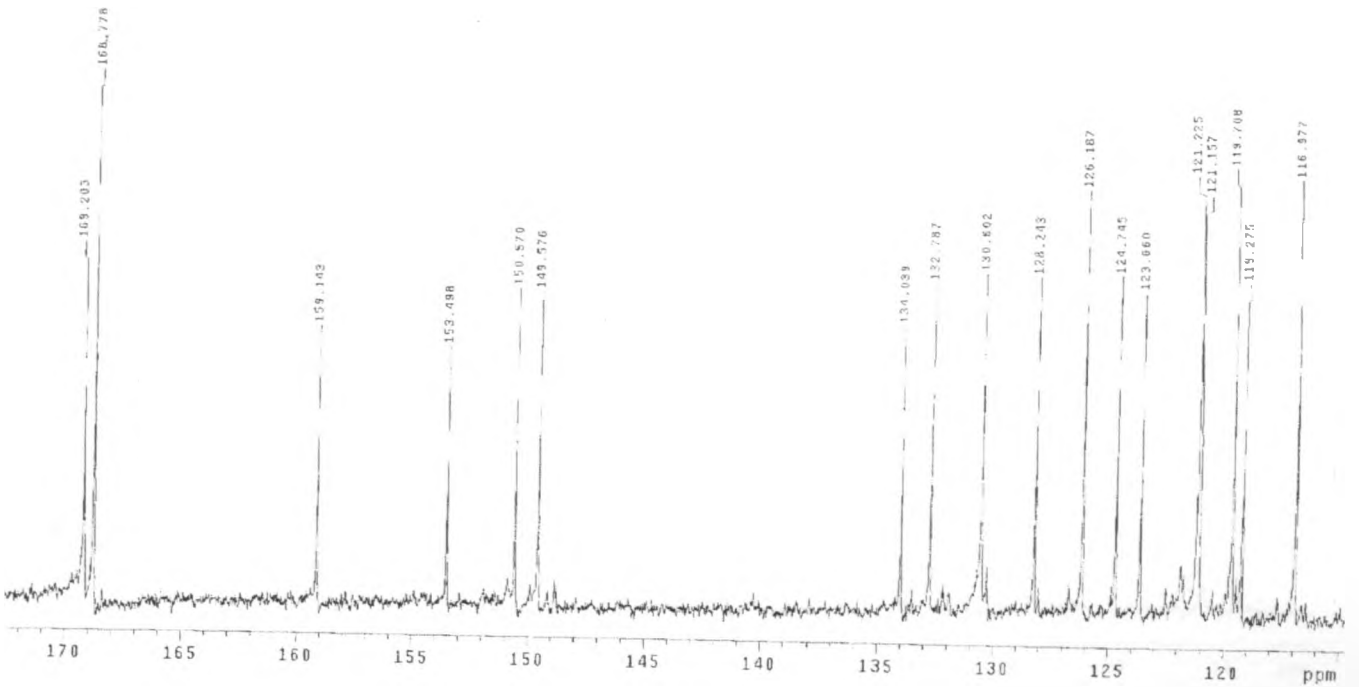

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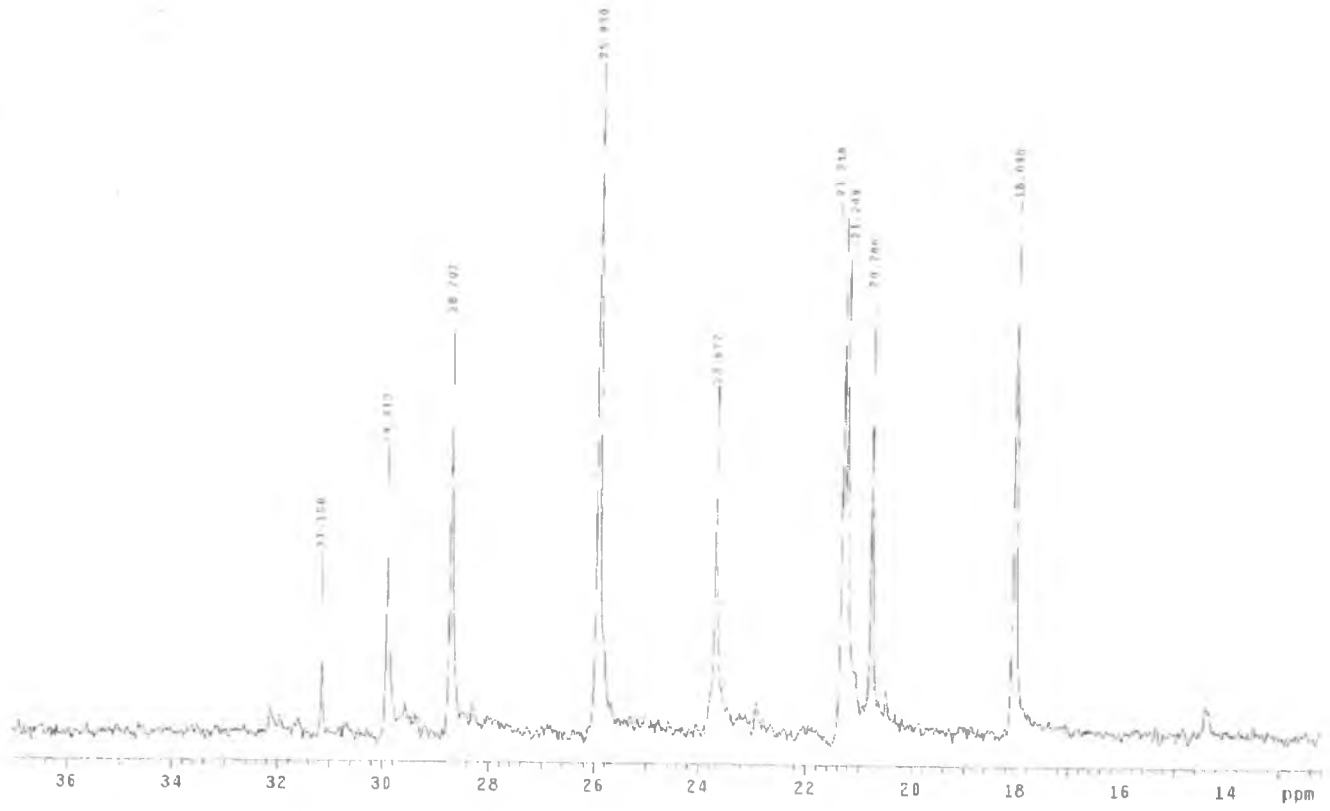
Pulse Sequence: s2pul

^{13}C NMR SPECTRUM FOR COMPOUND 1



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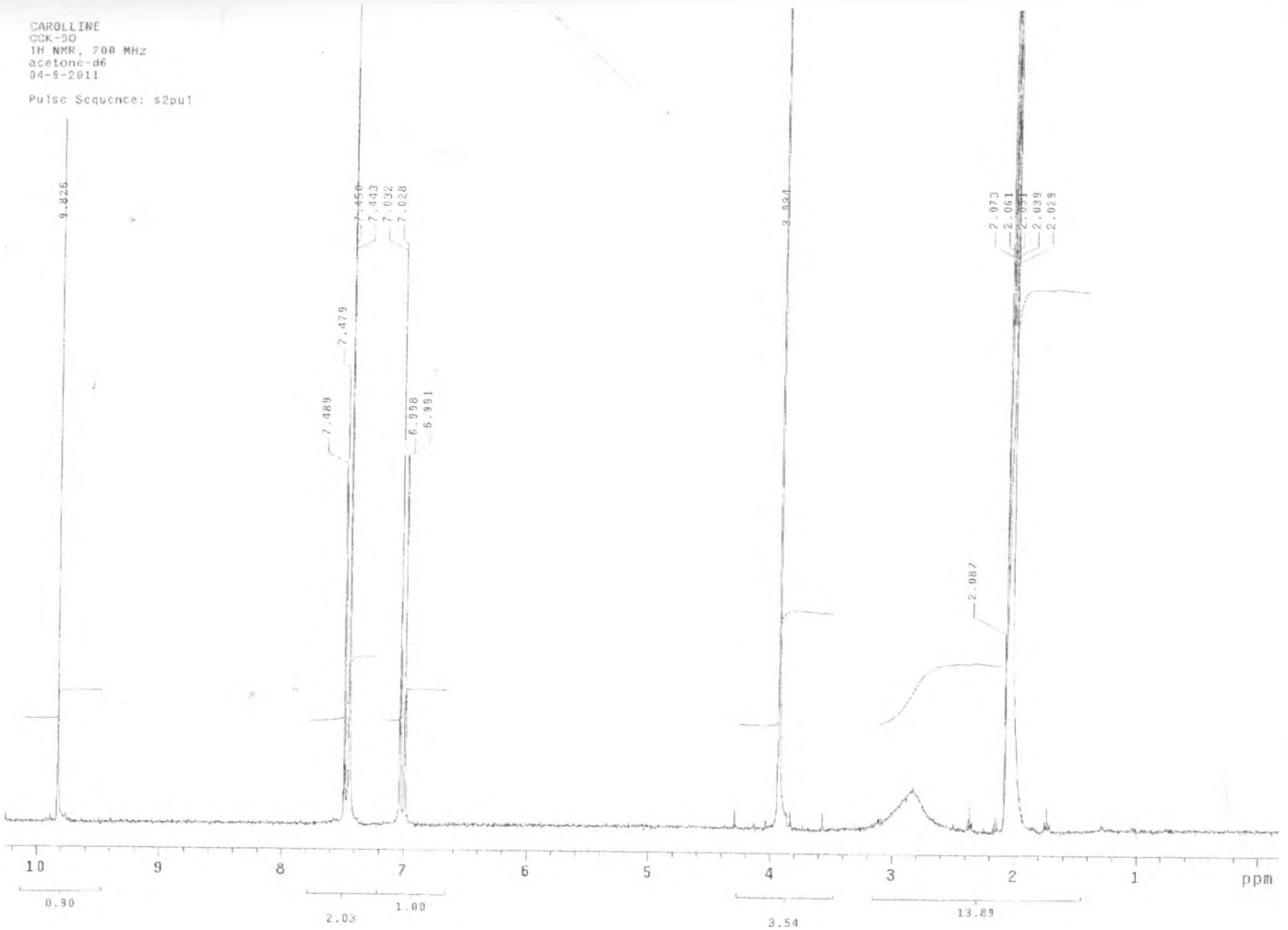

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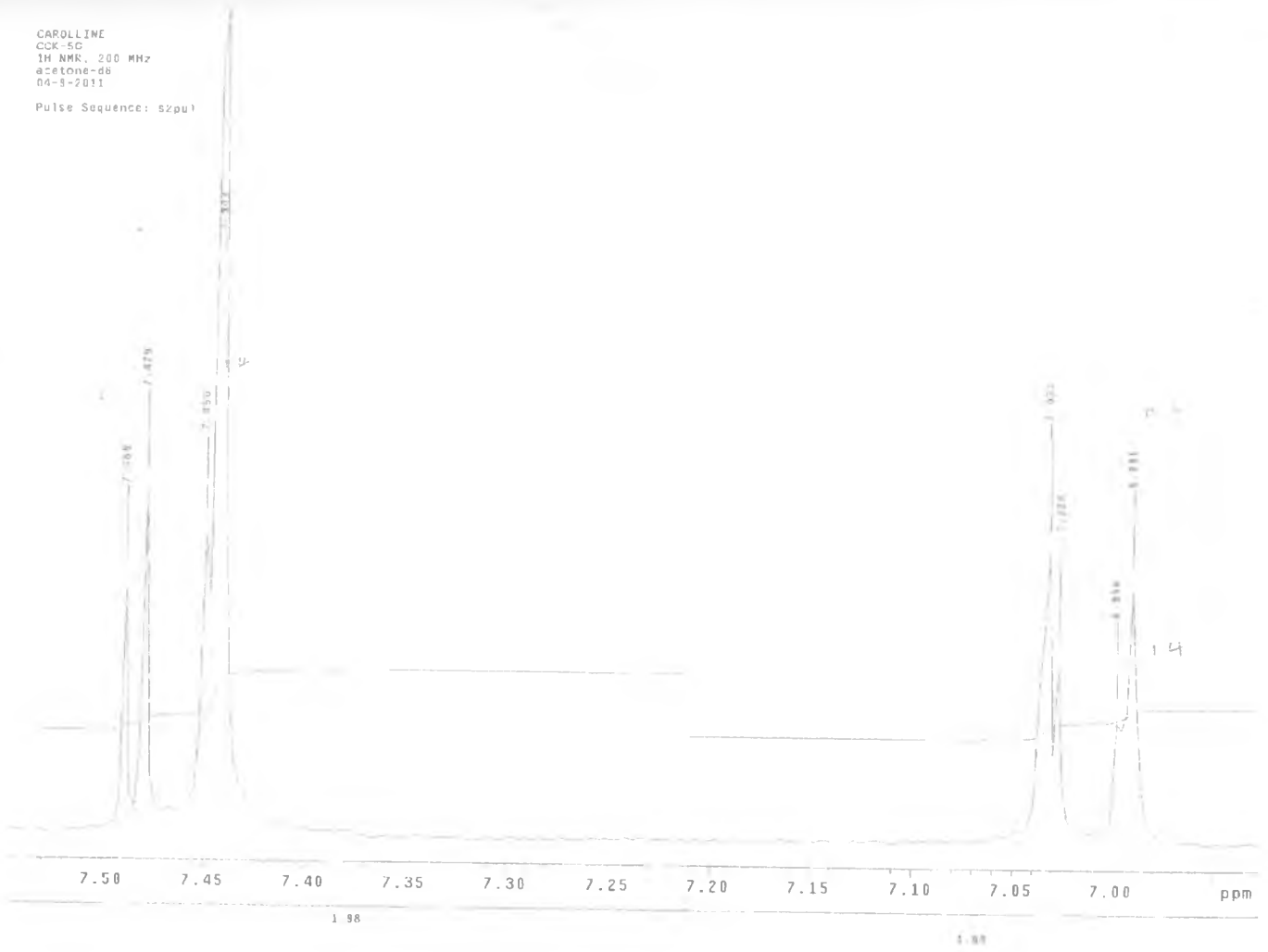

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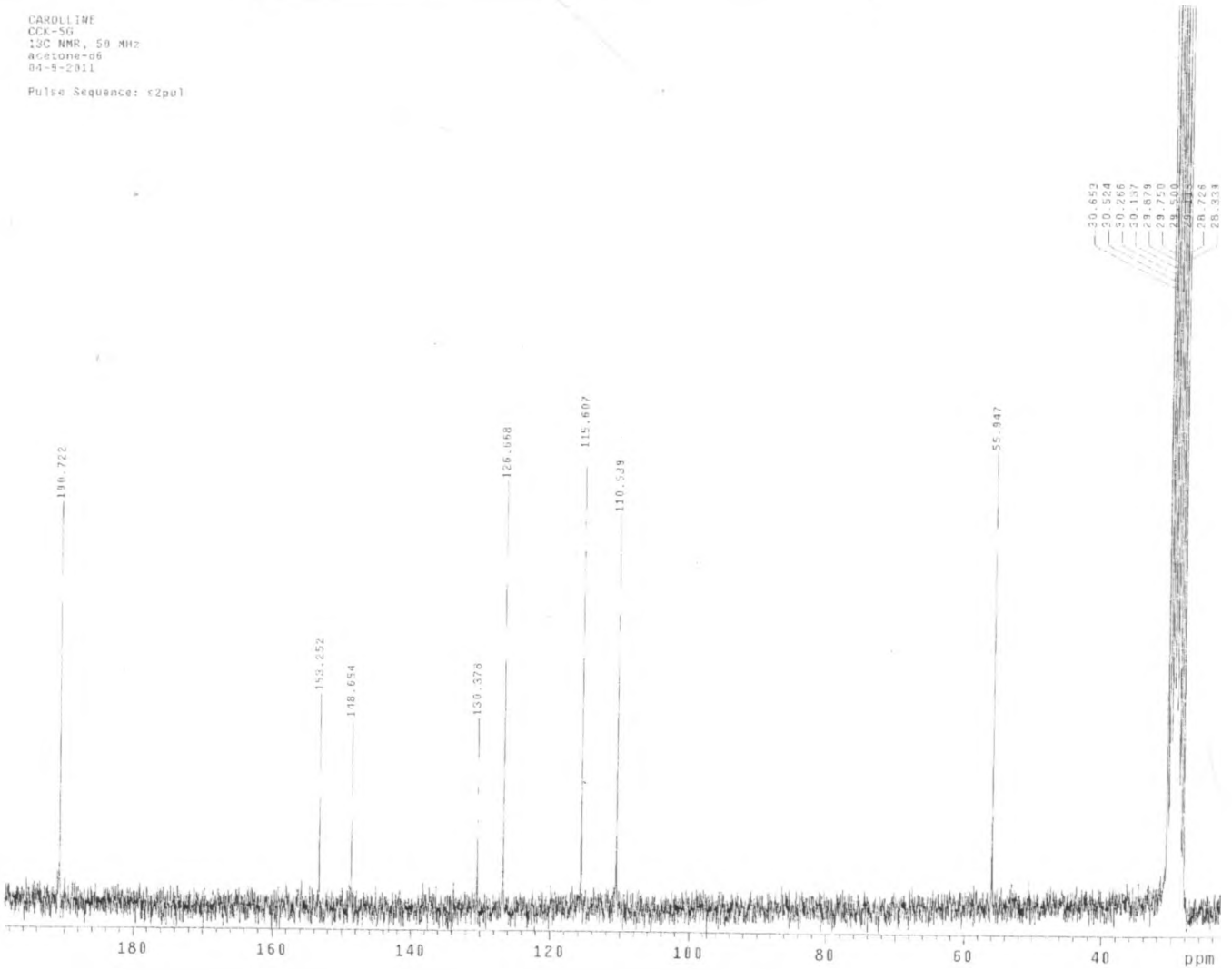

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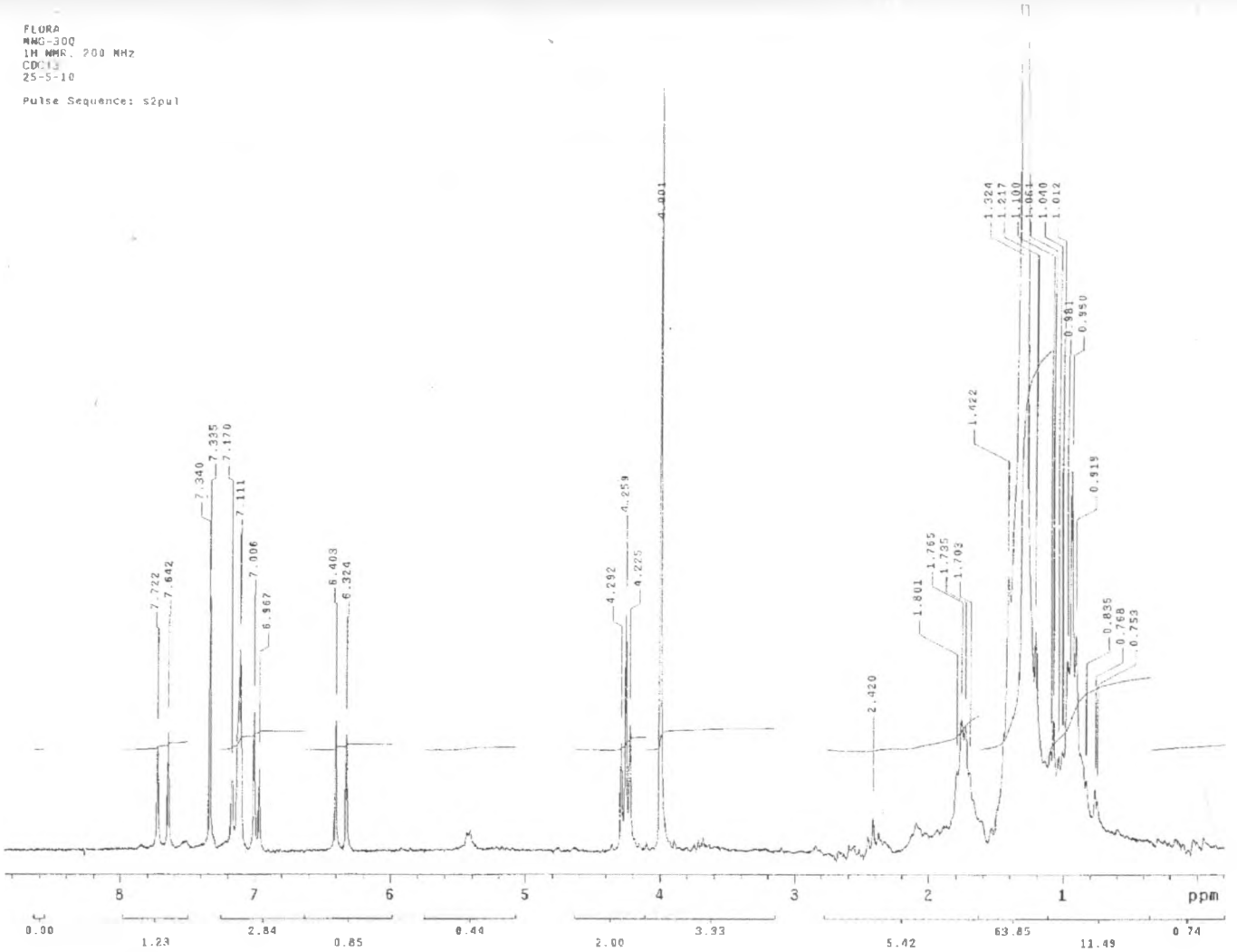

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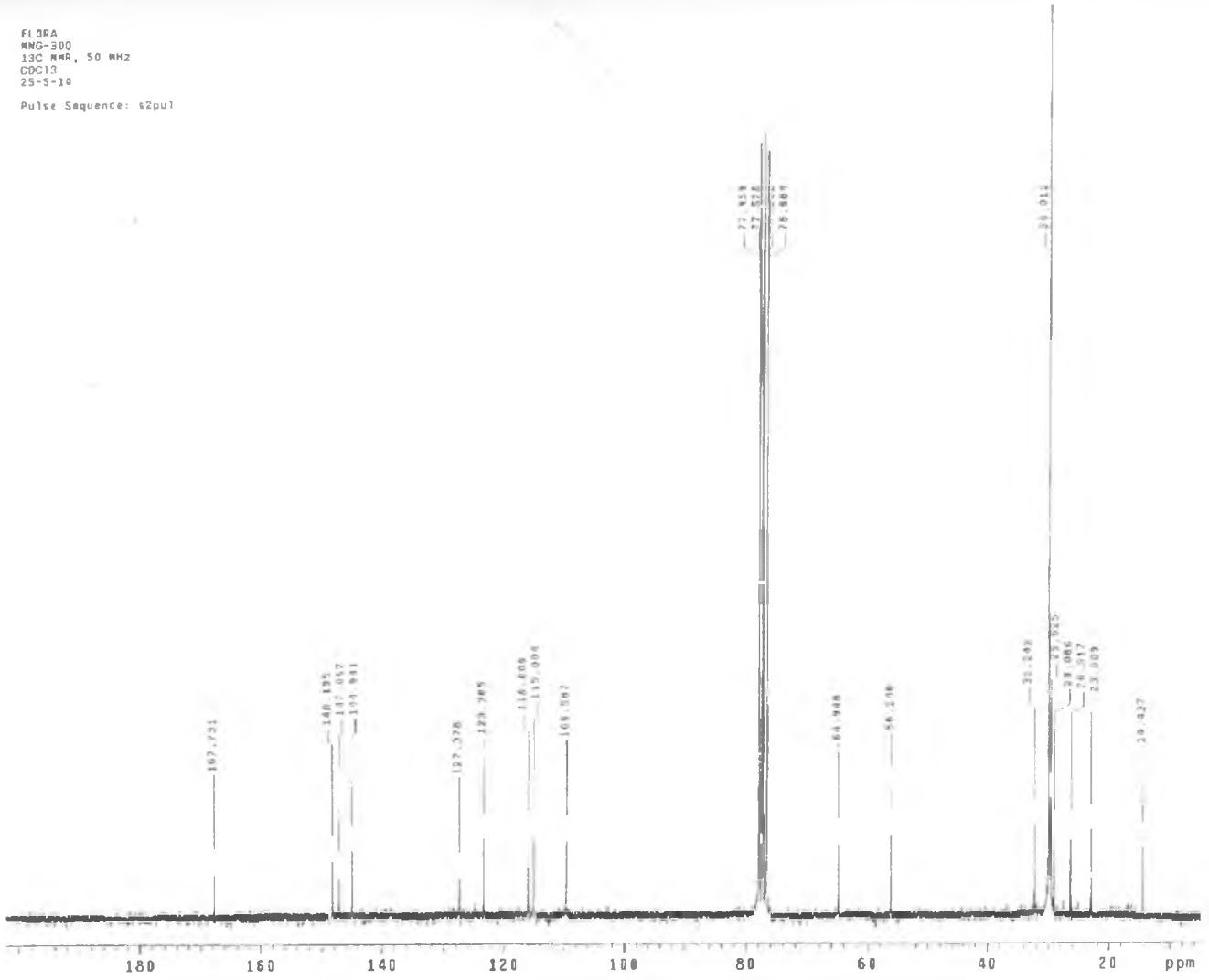

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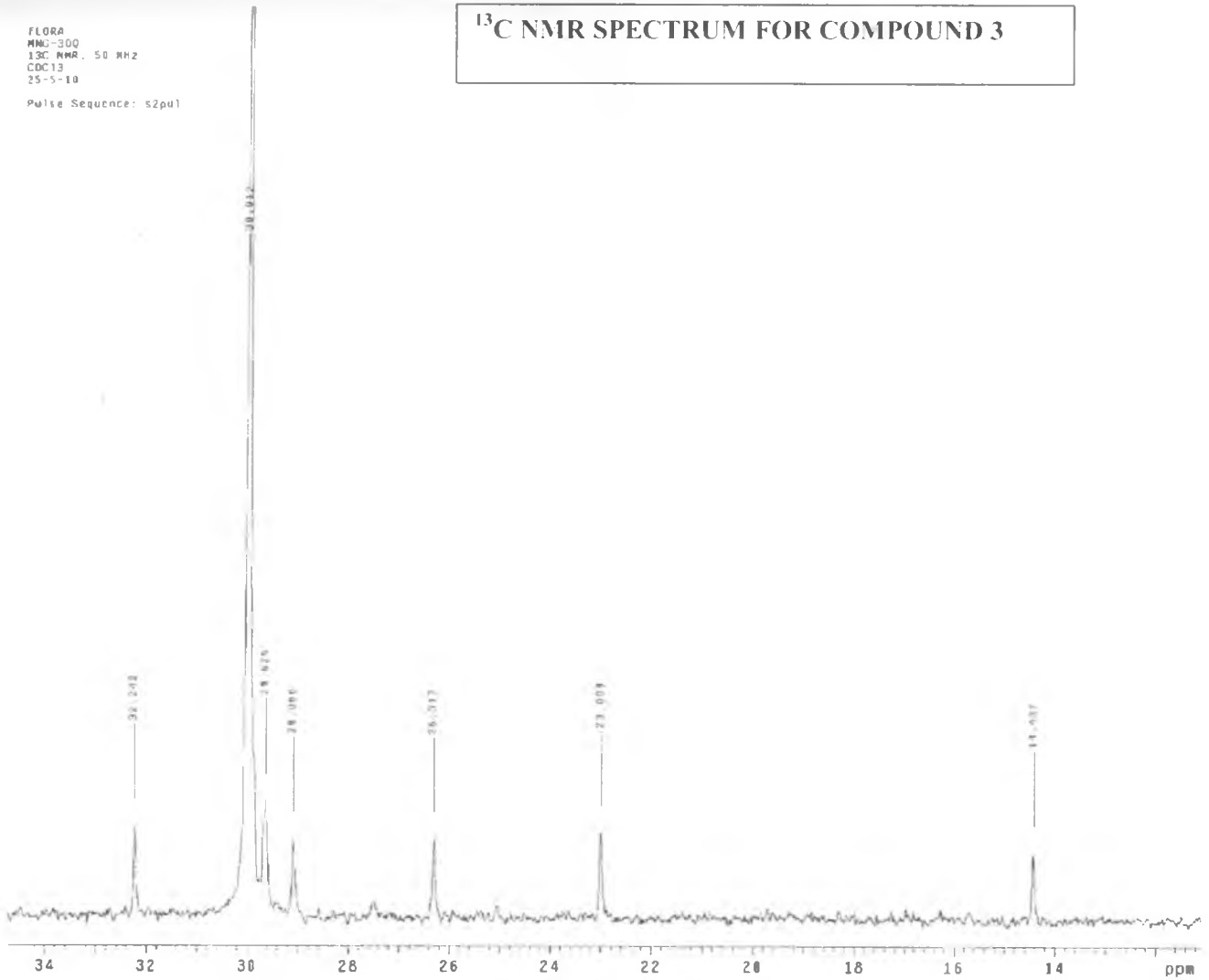
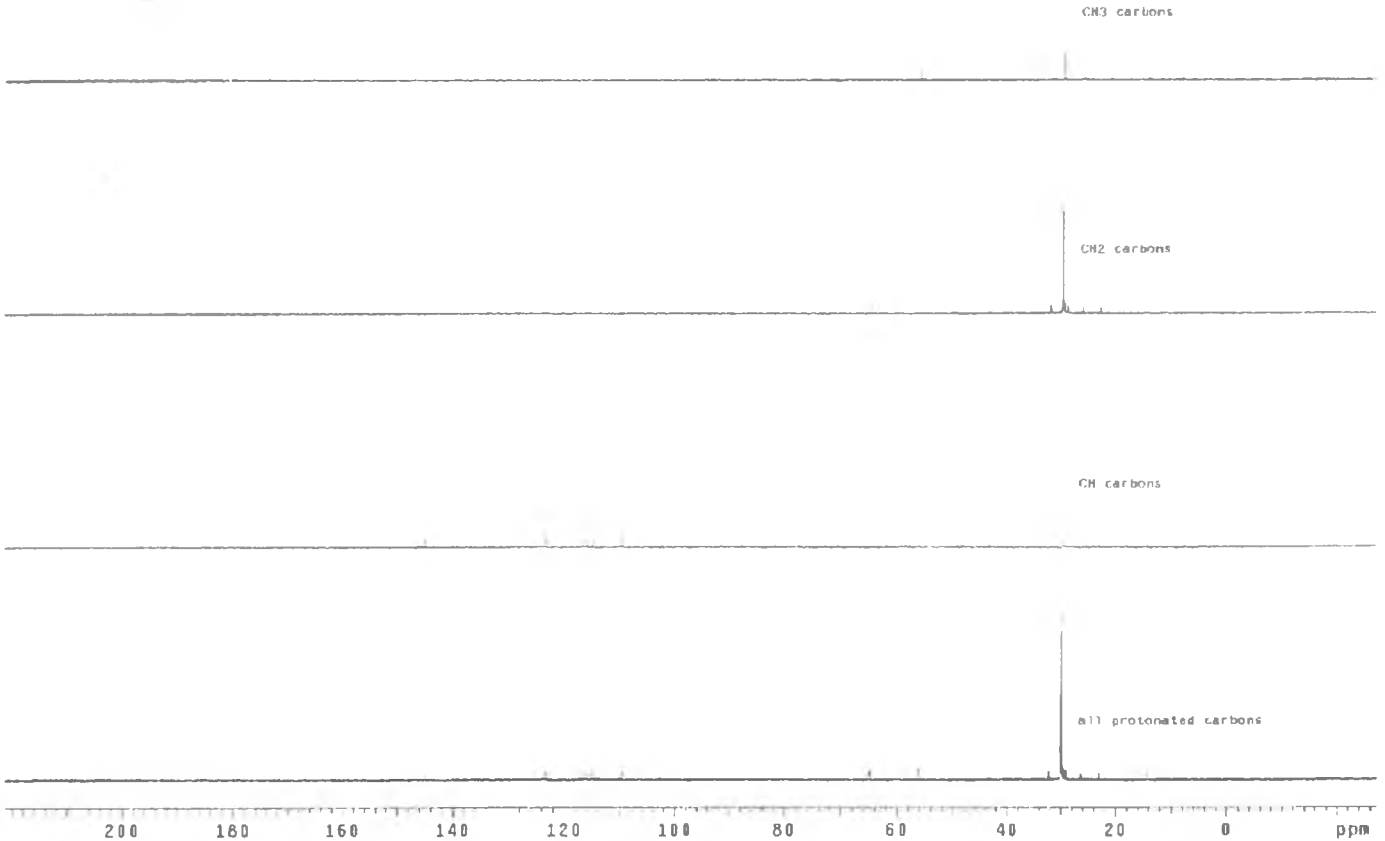


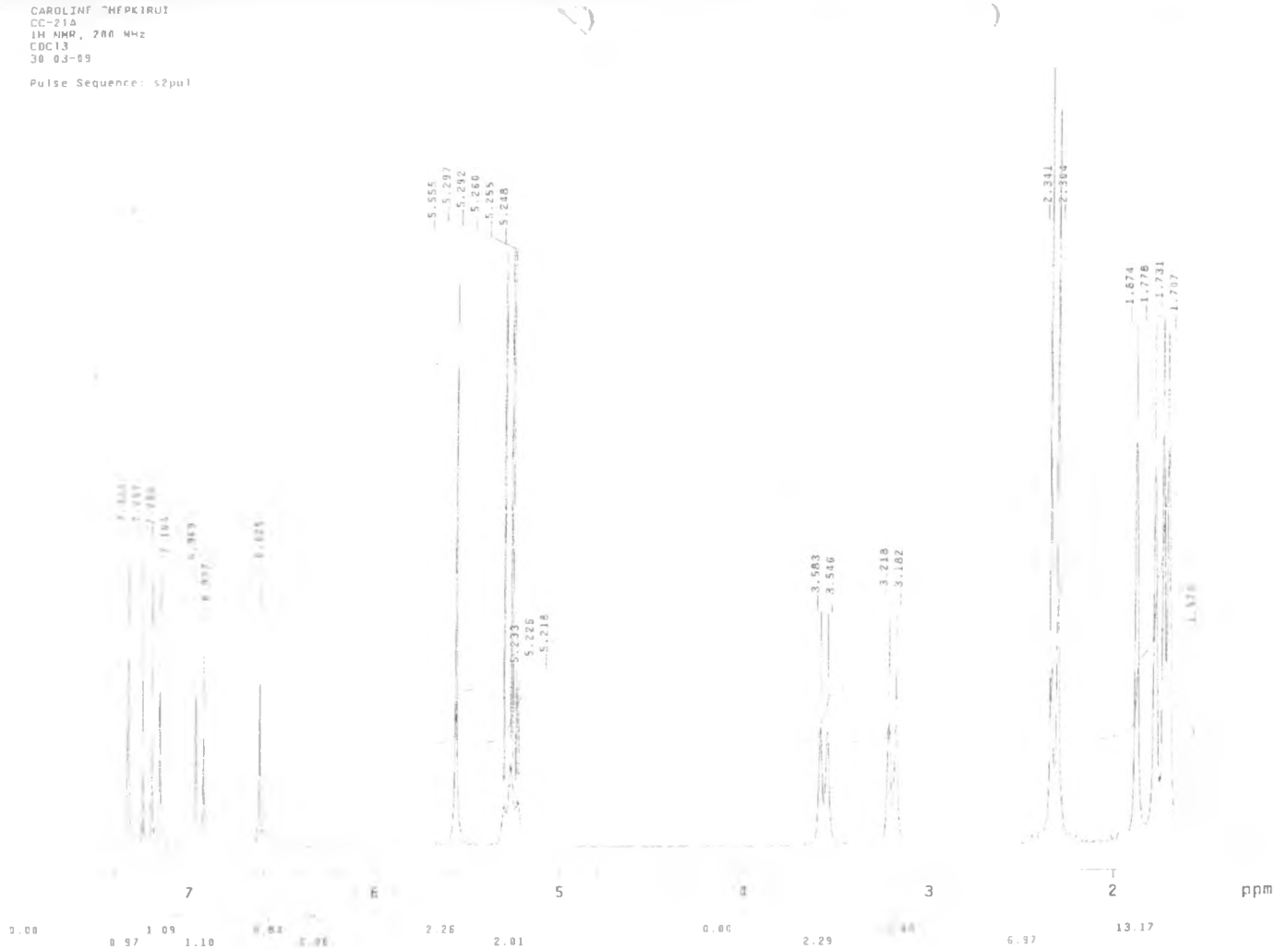
FLORA
MNG-3DQ
DEPT
26/5/10

DEPT SPECTRUM FOR COMPOUND 3



SPECTRA FOR COMPOUND 4

^{1}H NMR SPECTRUM FOR COMPOUND 4



^{1}H NMR SPECTRUM FOR COMPOUND 4

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CDCl3
30-03-09

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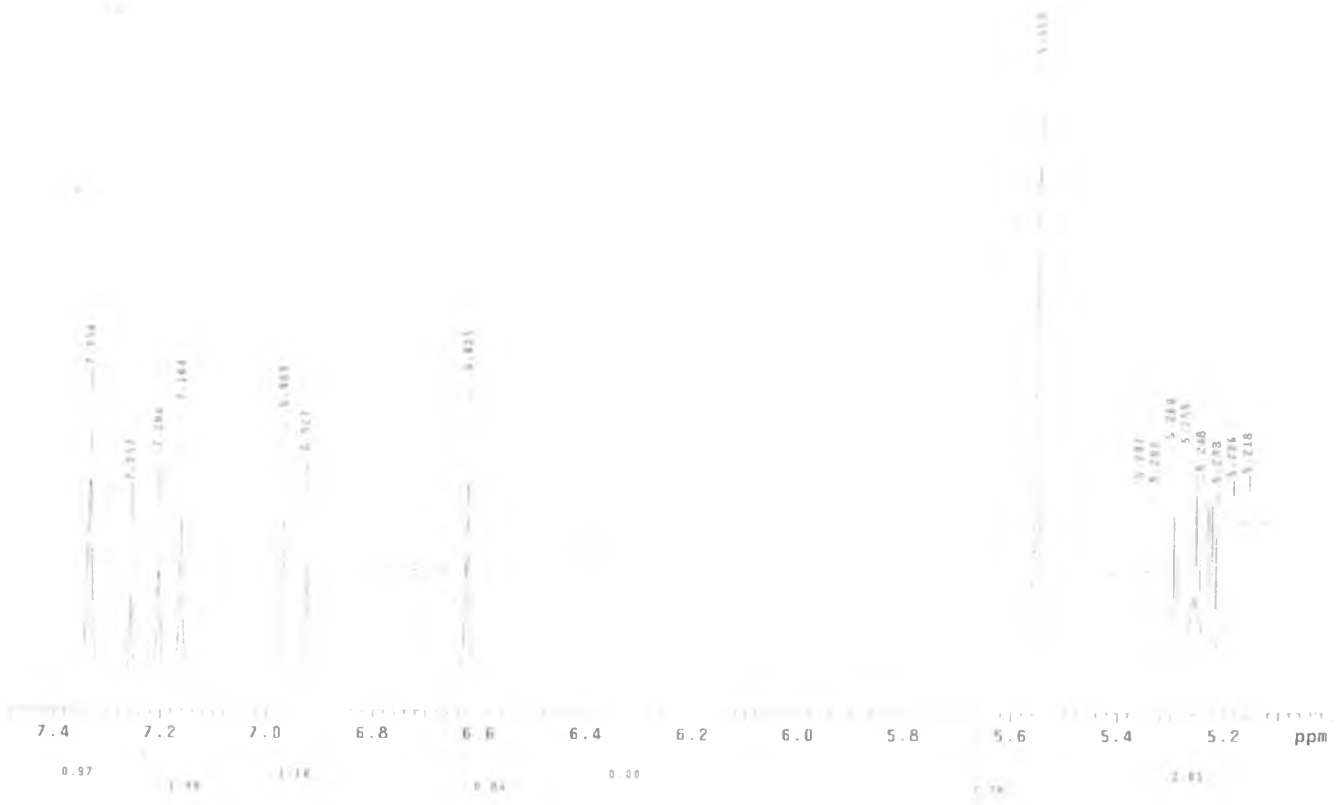

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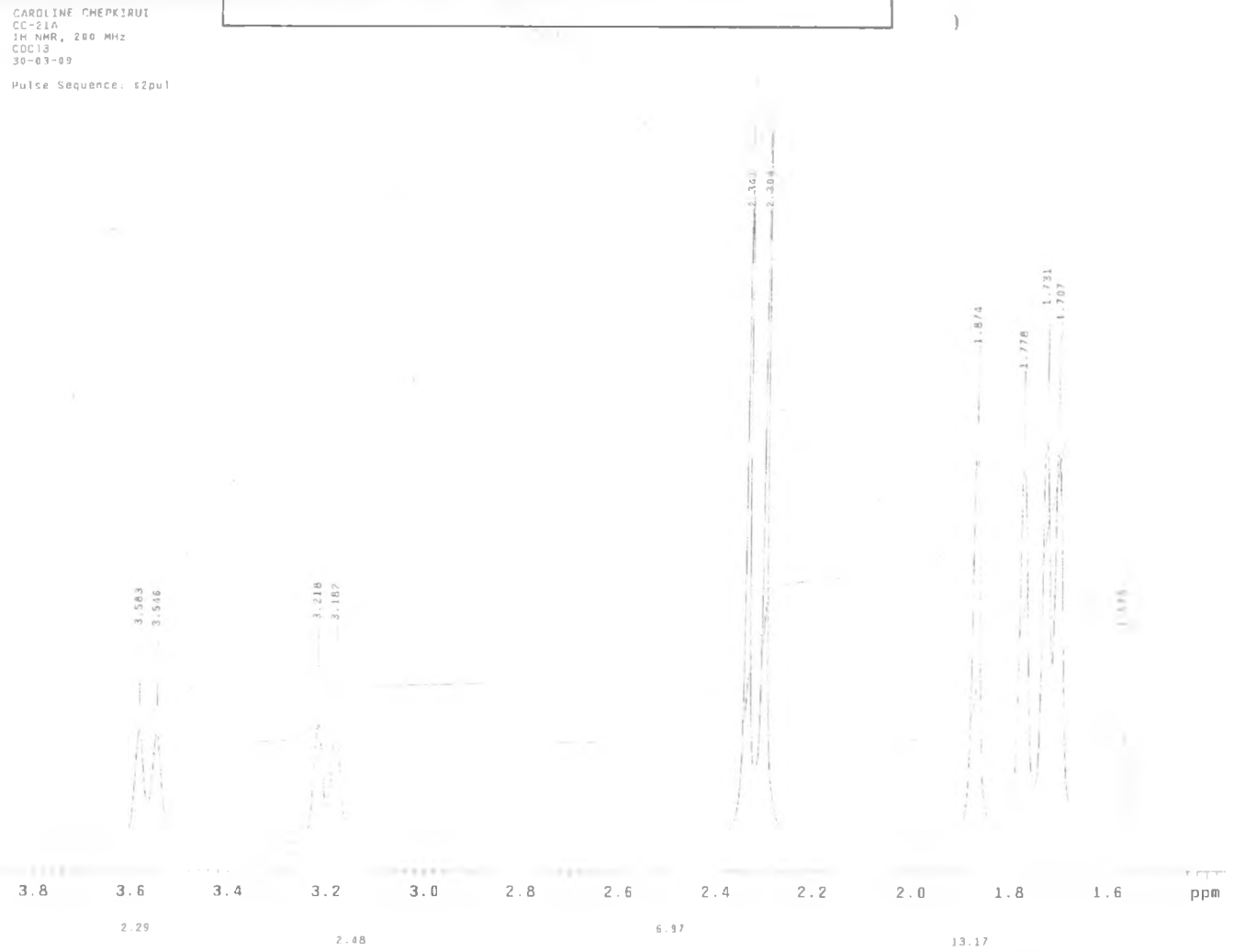

^{13}C NMR SPECTRUM FOR COMPOUND 4

^{13}C NMR SPECTRUM FOR COMPOUND 4

CAROLINE CHEPKIRUI
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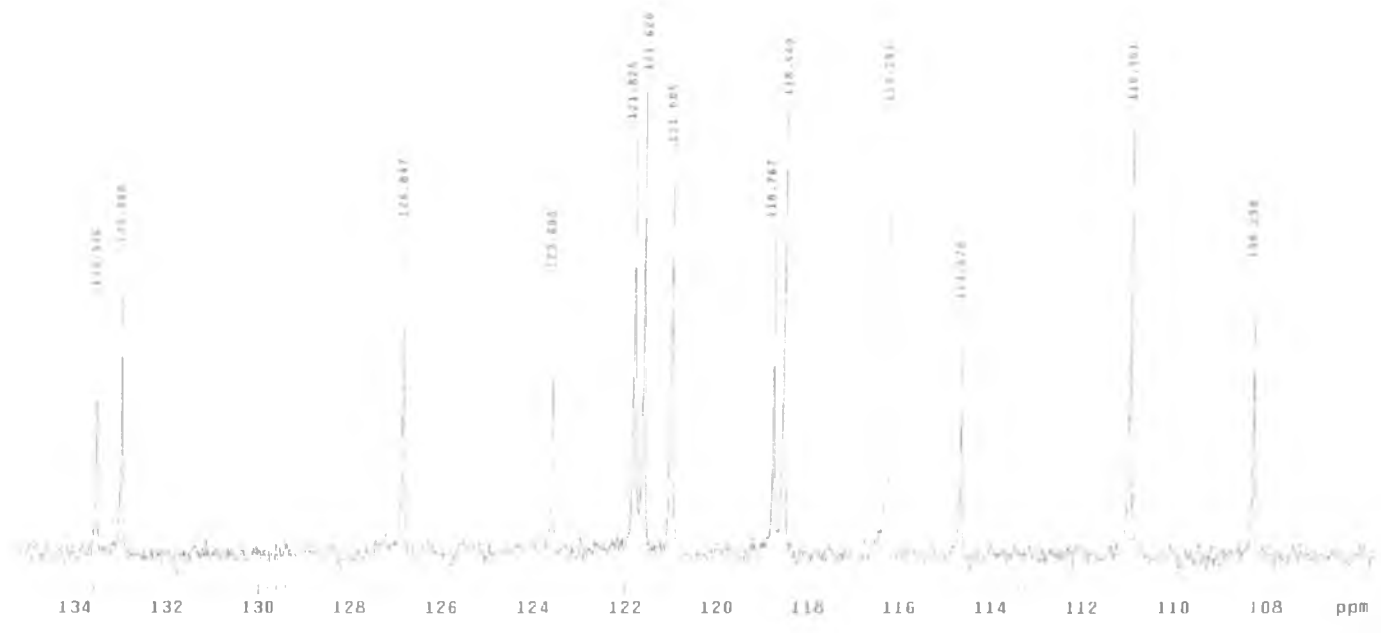
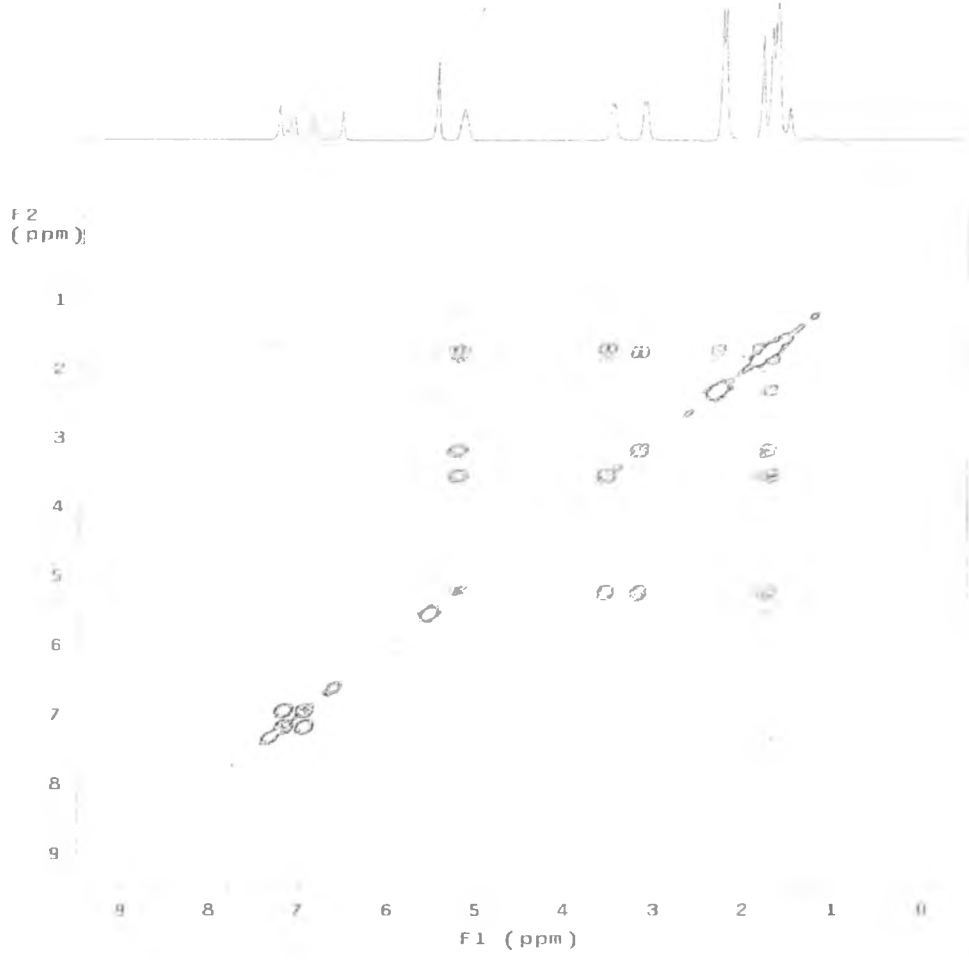
13C NMR, 50 MHz
CDCl3
20-03-09

Pulse Sequence: s2pul



COSY SPECTRUM FOR COMPOUND 4



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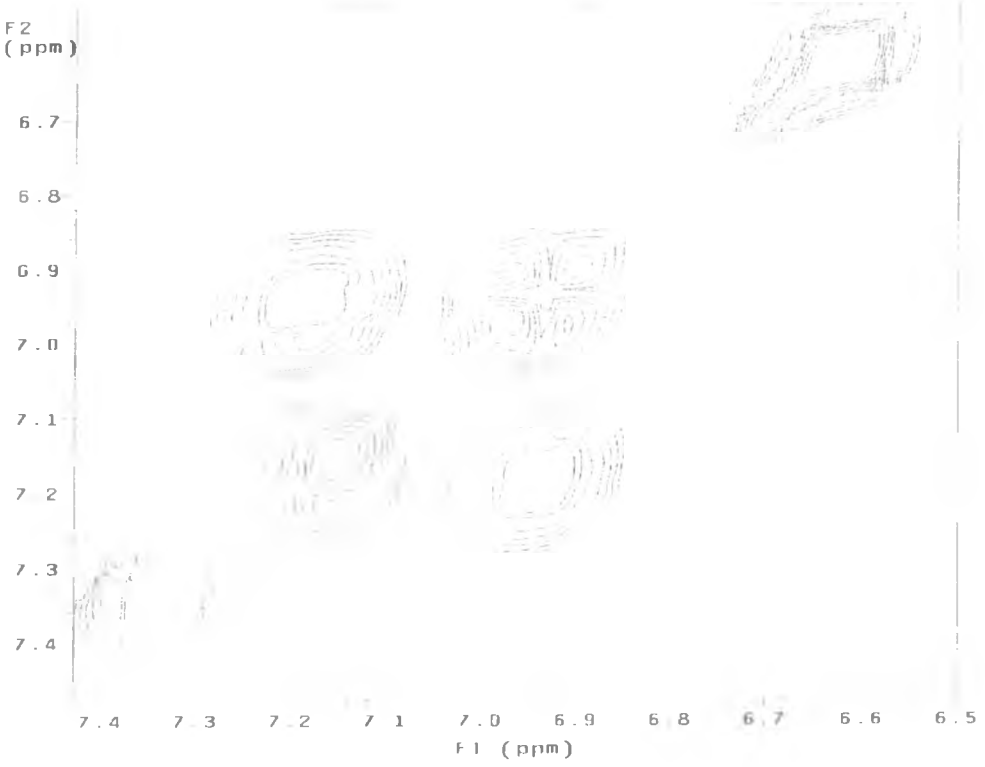

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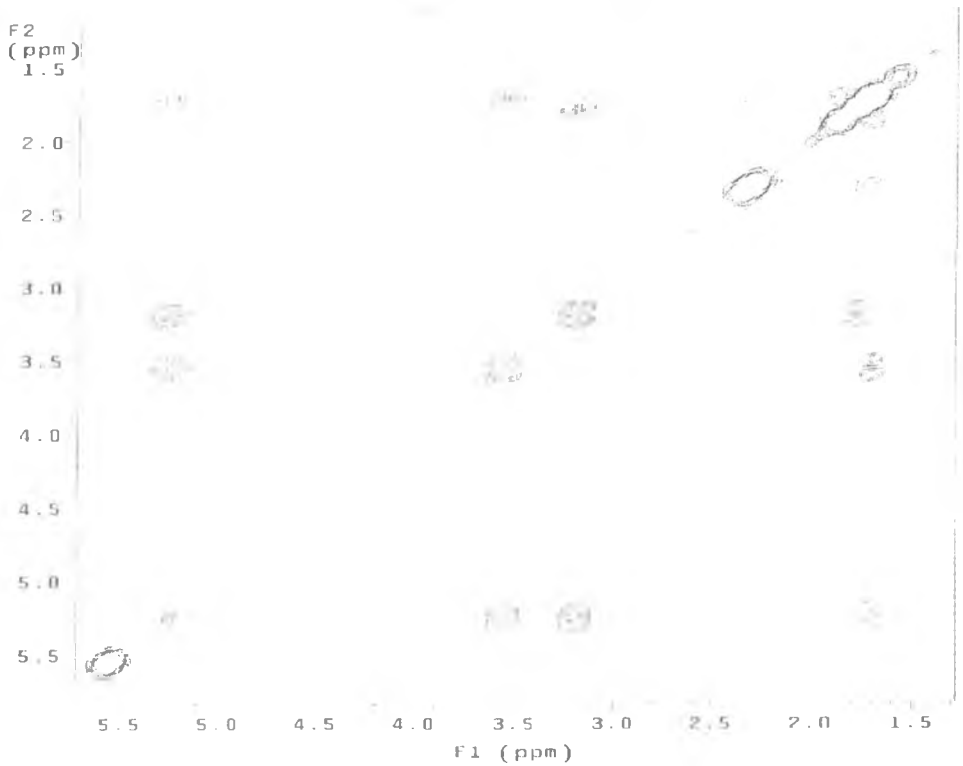
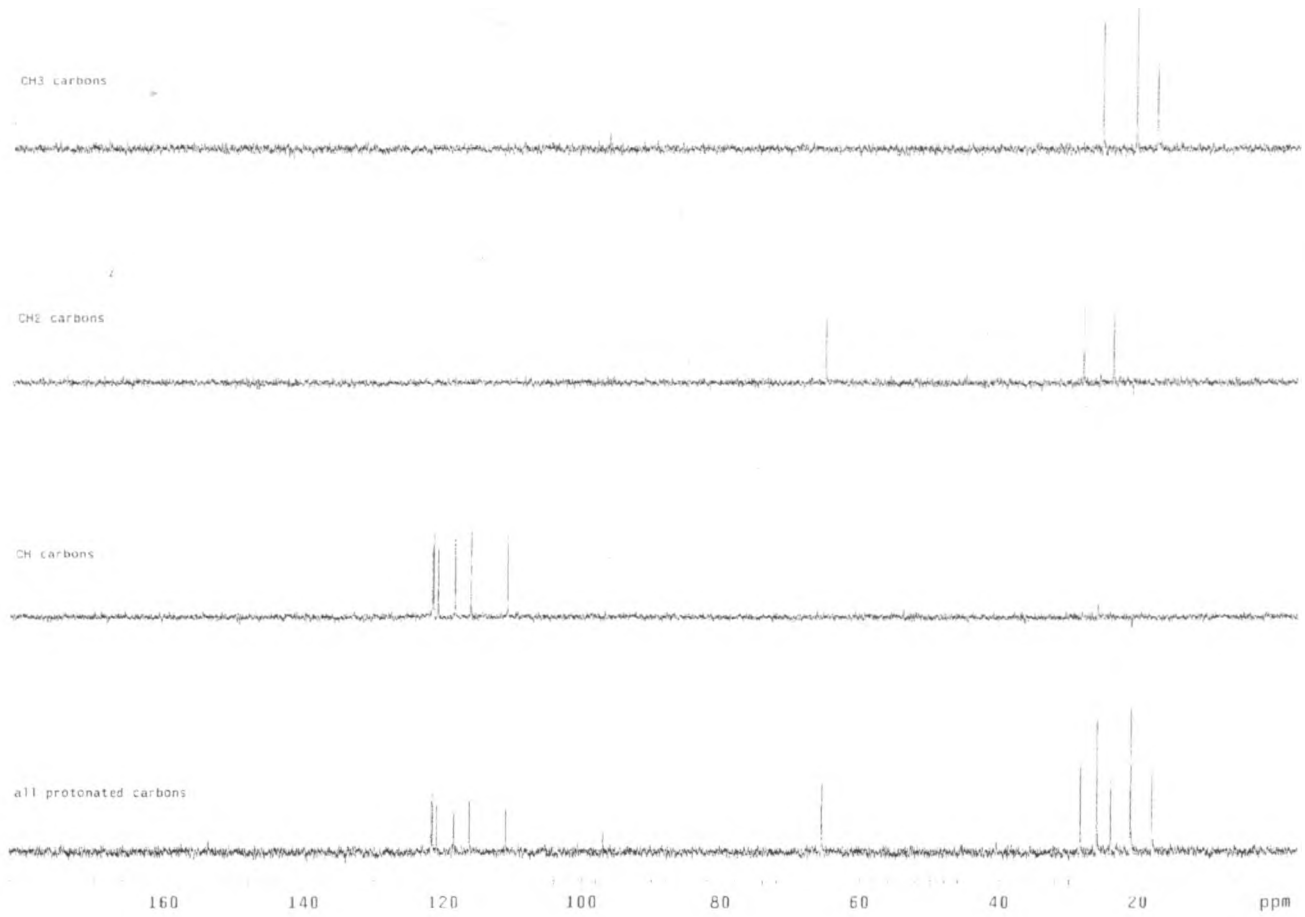


DEPT SPECTREAL FOR COMPOUND 4

CAROLINE CHEPKIECH
CC-214
DEPT
3/10/3109



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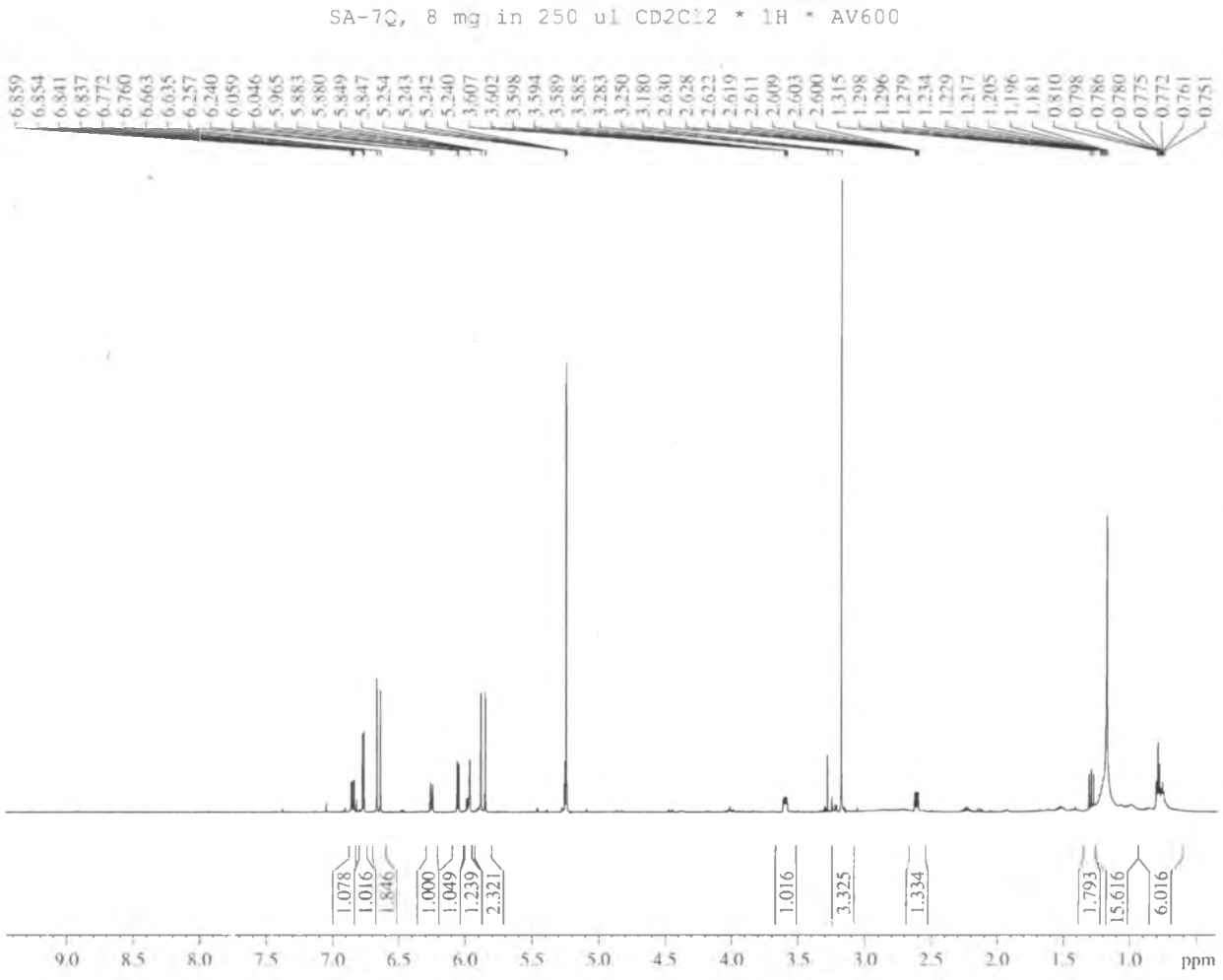

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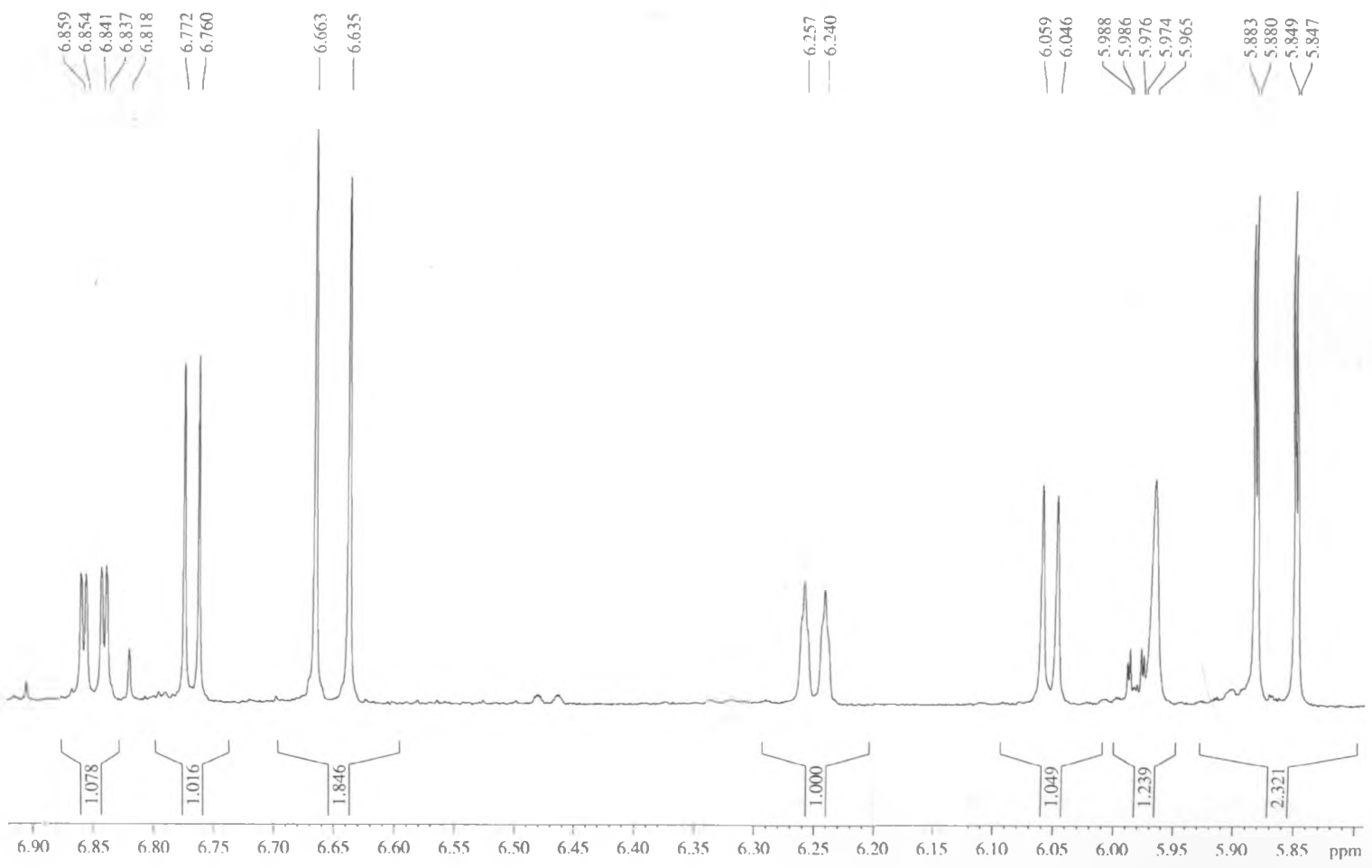
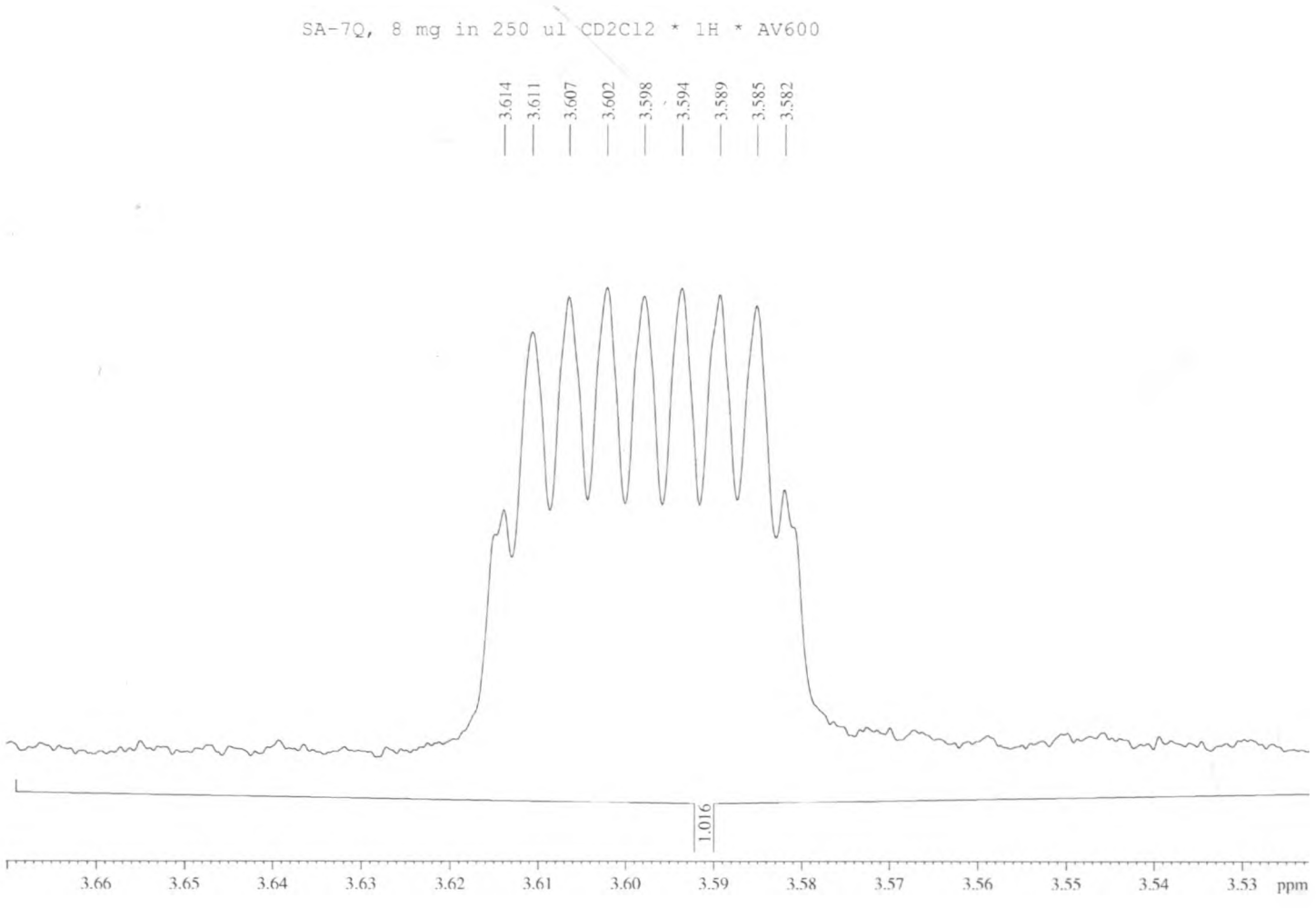


^{1}H NMR SPECTRA FOR COMPOUND 5



^{1}H NMR SPECTRA FOR COMPOUND 5

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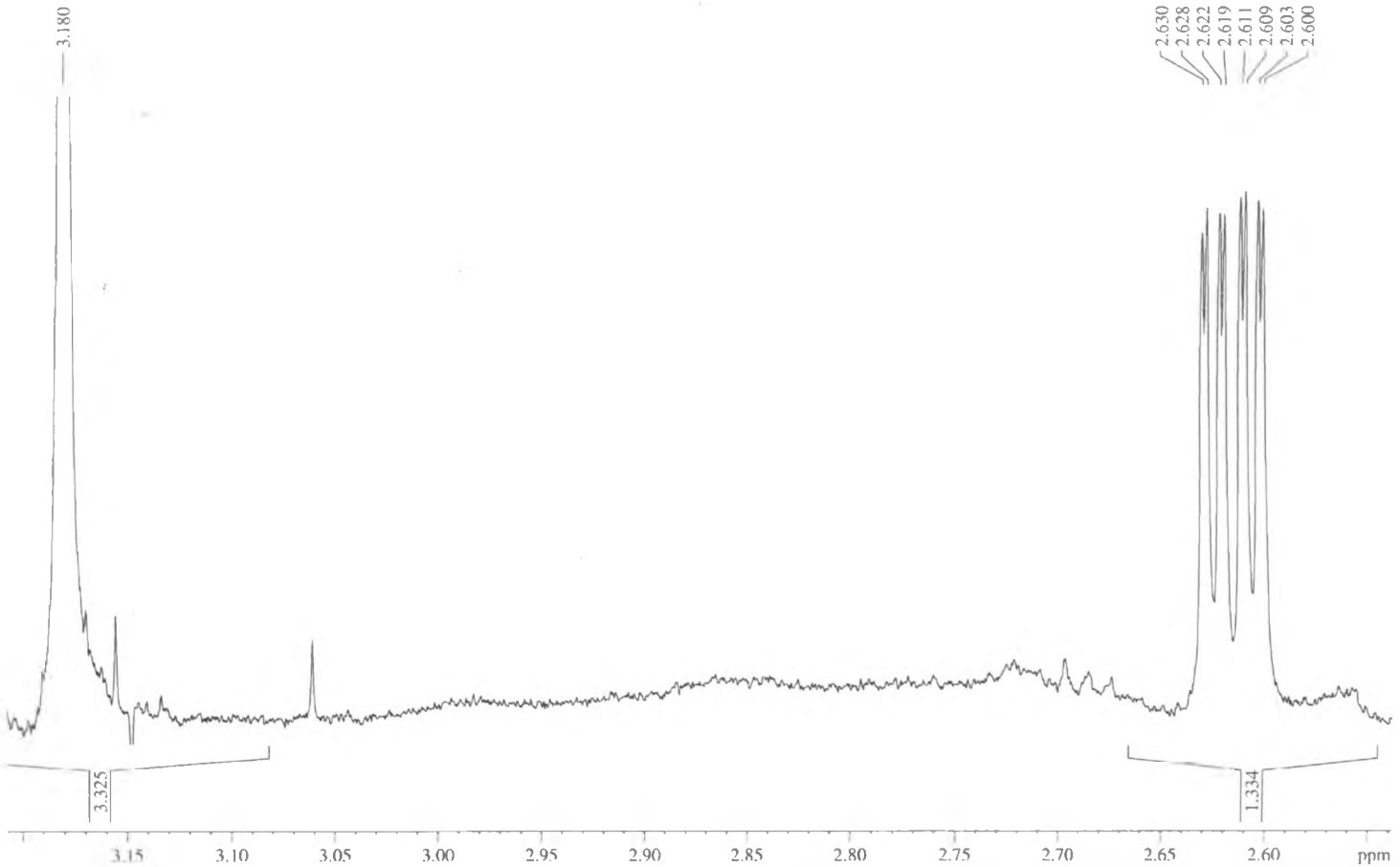
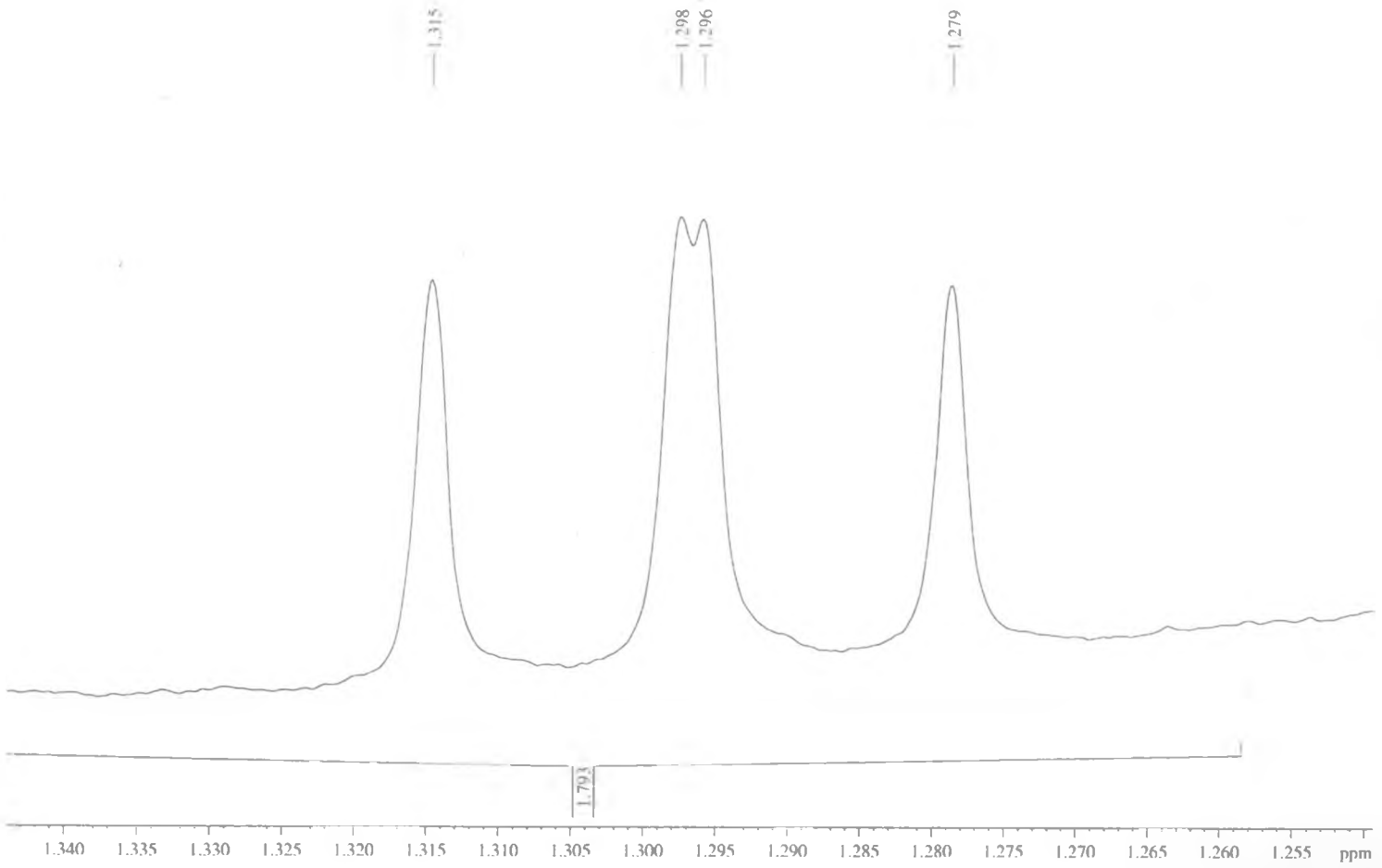
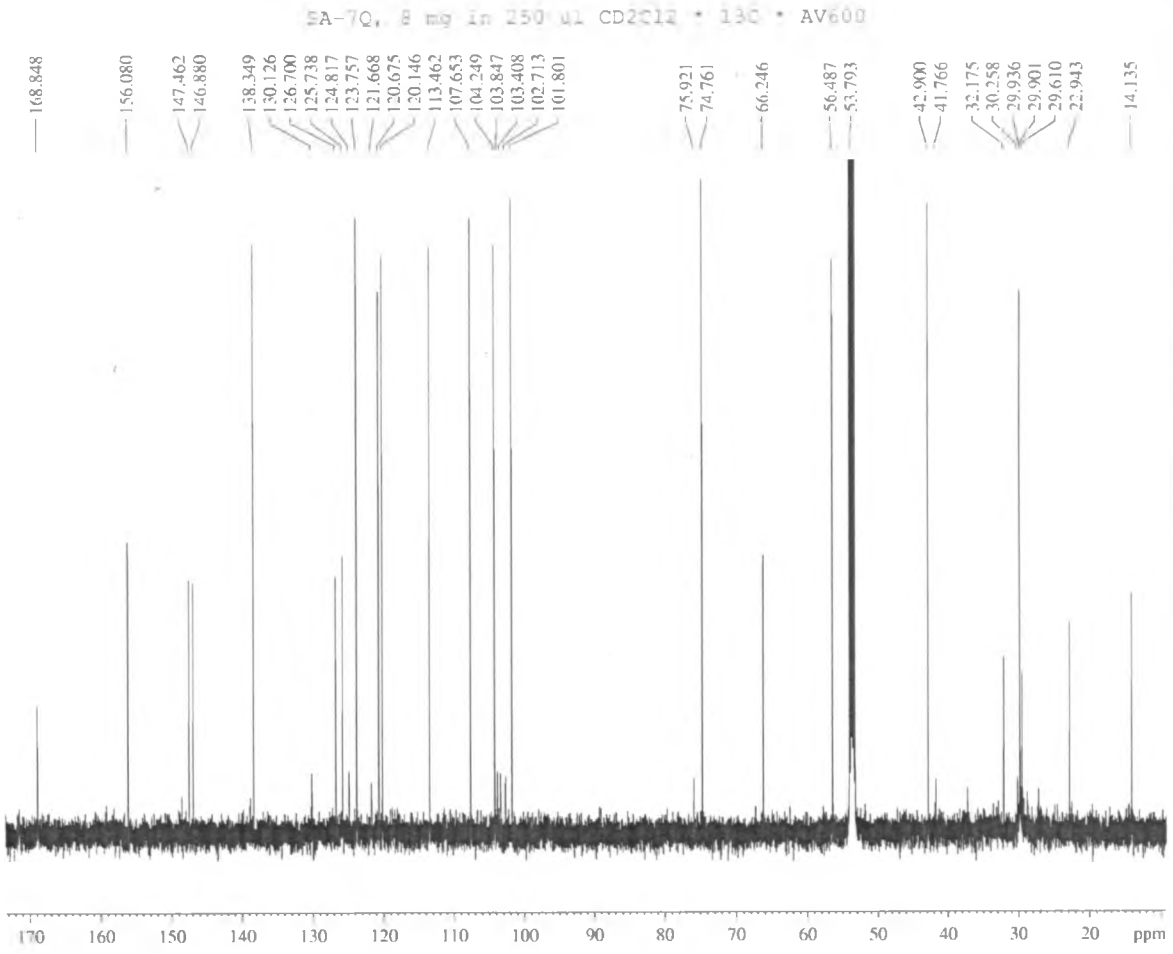


^{1}H NMR SPECTRA FOR COMPOUND 5

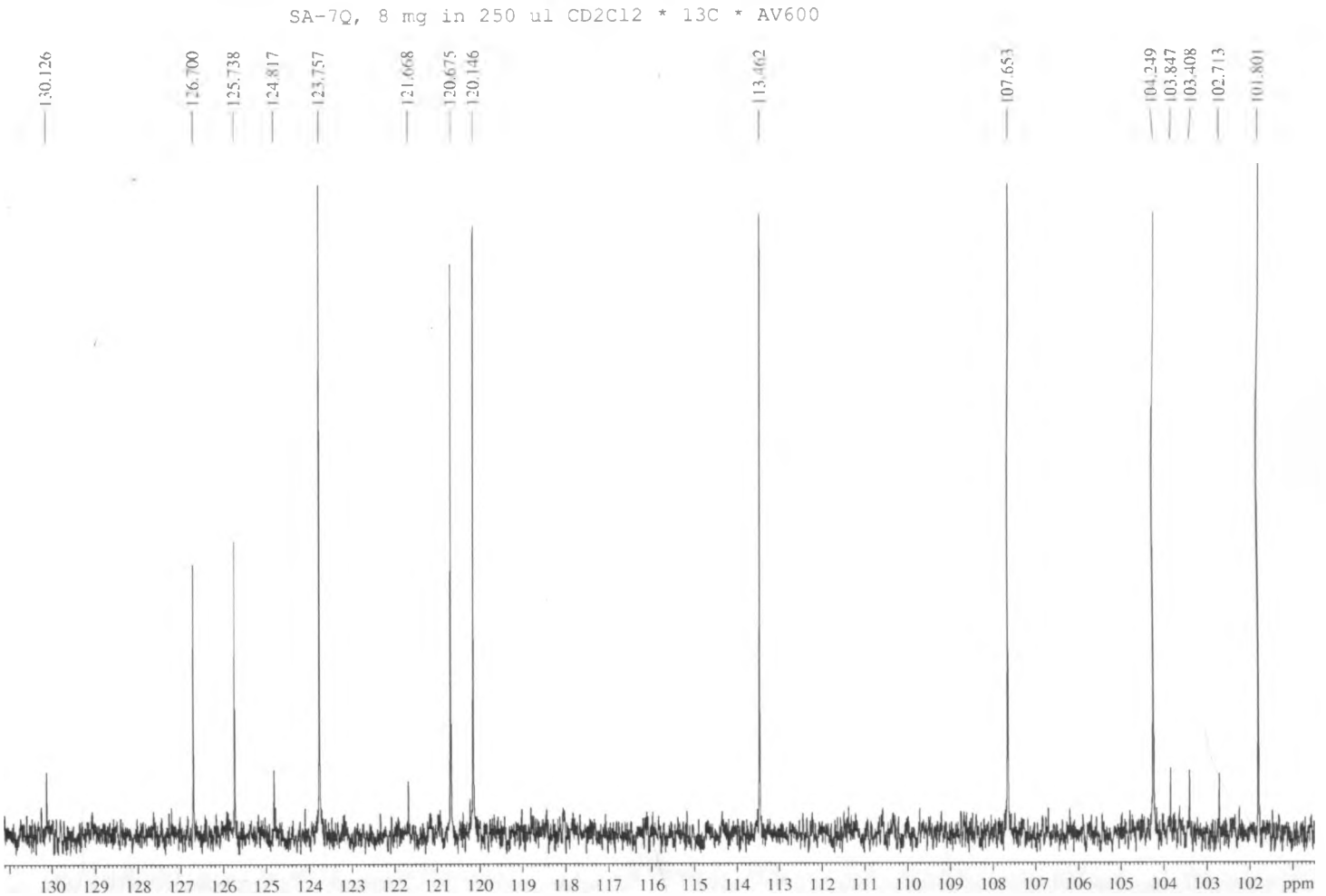
SA-7Q, 8 mg in 250 ul CD2Cl2 * 1H * AV600



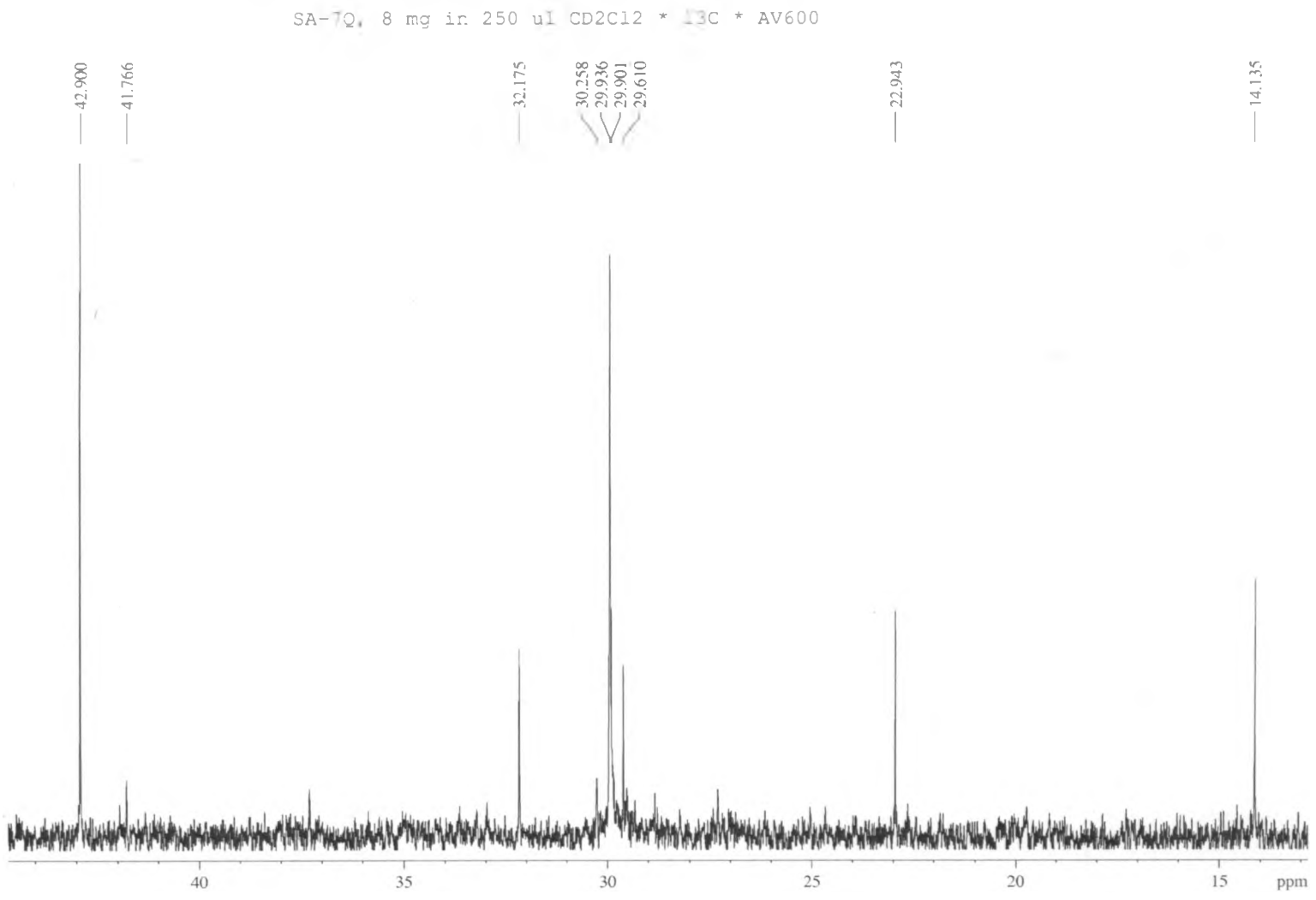
^{13}C NMR SPECTRUM FOR COMPOUND 5



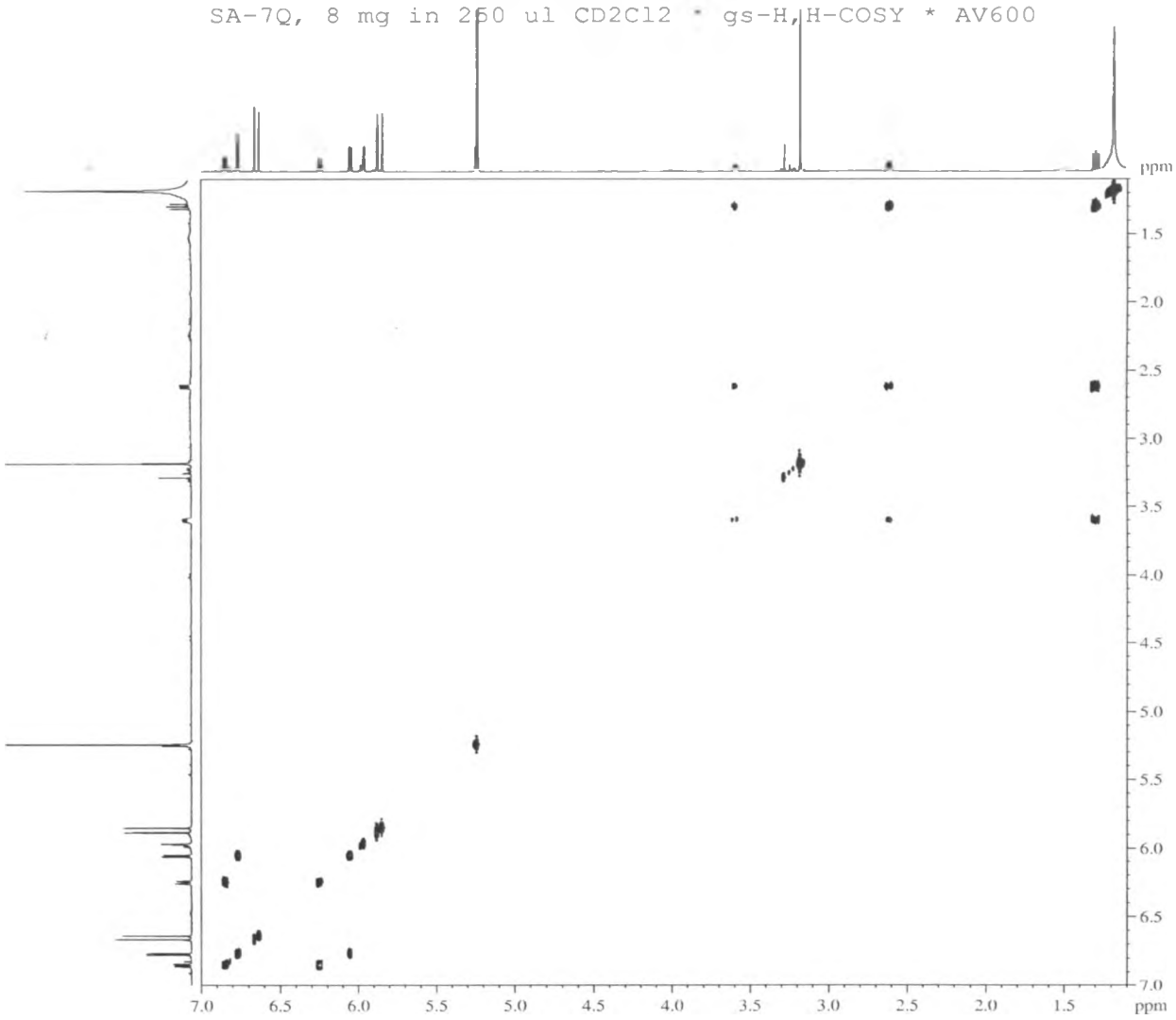
^{13}C NMR SPECTRUM FOR COMPOUND 5



^{13}C NMR SPECTRUM FOR COMPOUND 5

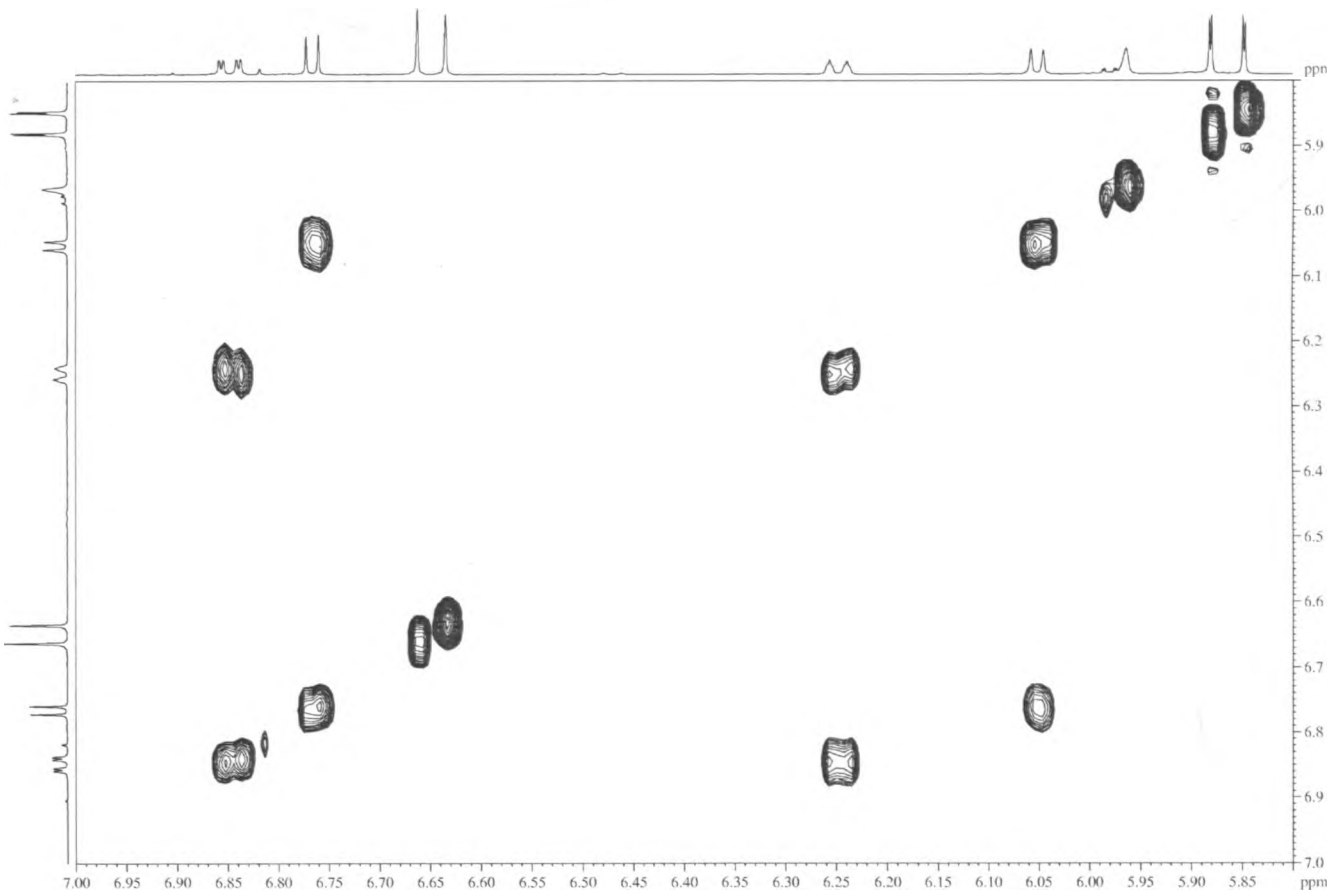


COSY SPECTRUM FOR COMPOUND 5

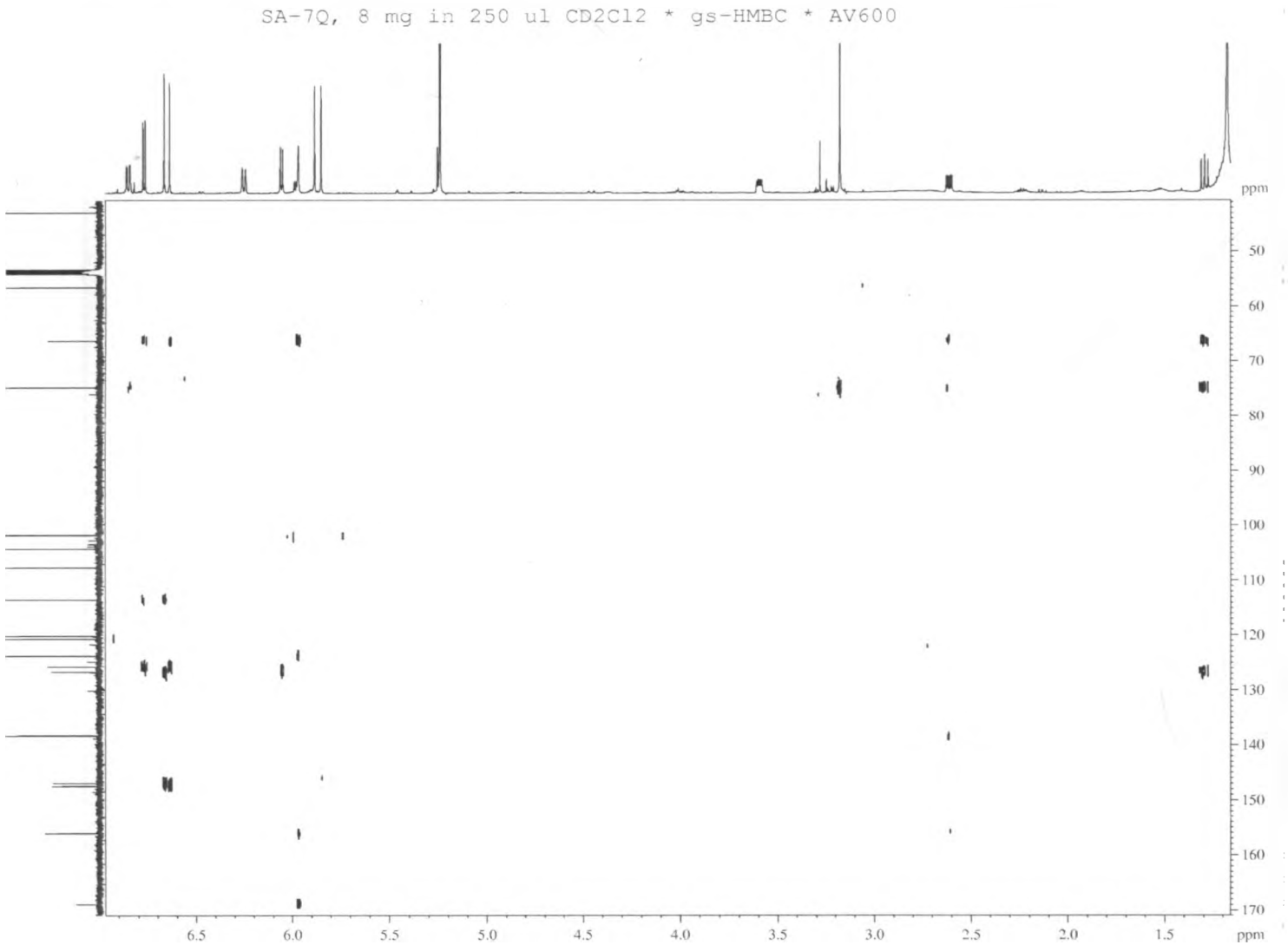


COSY SPECTRUM FOR COMPOUND 5

SA-7Q, 8 mg in 250 ul CD2Cl2 * gs-H,H-COSY * AV600

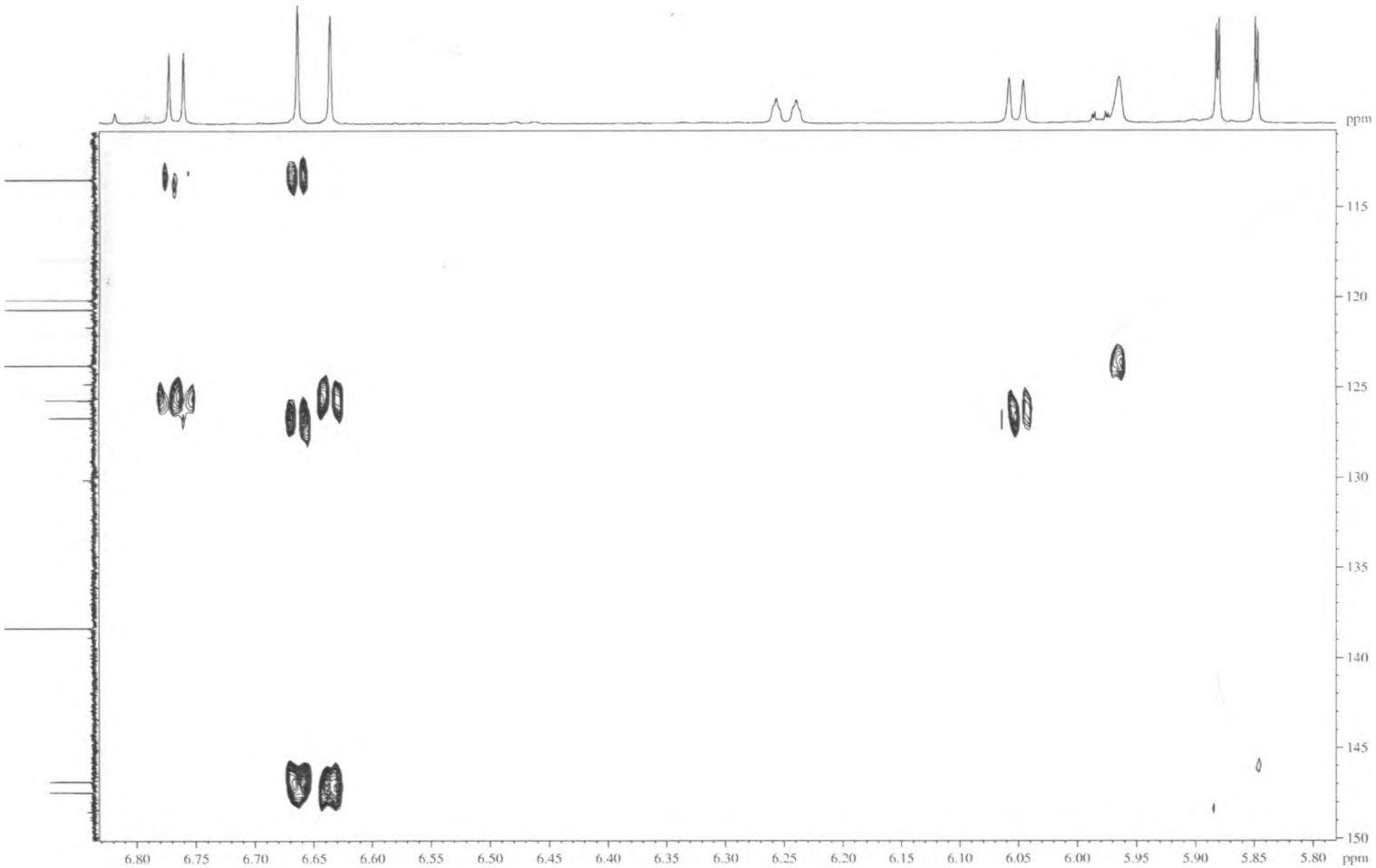


HMBC SPECTRUM FOR COMPOUND 5

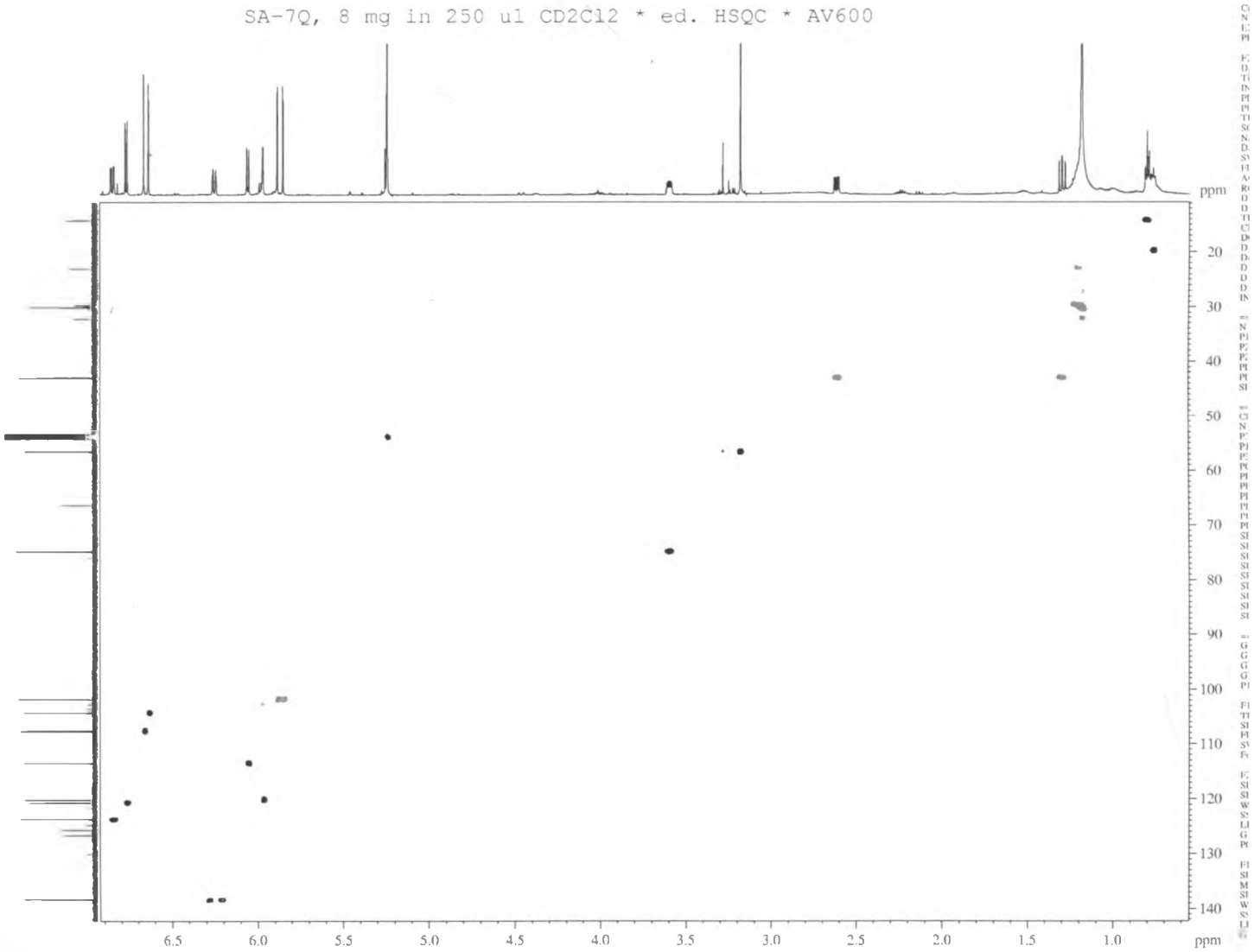


HMBC SPECTRUM FOR COMPOUND 5

SA-7Q, 8 mg in 250 ul CD2Cl2 * gs-HMBC * AV600

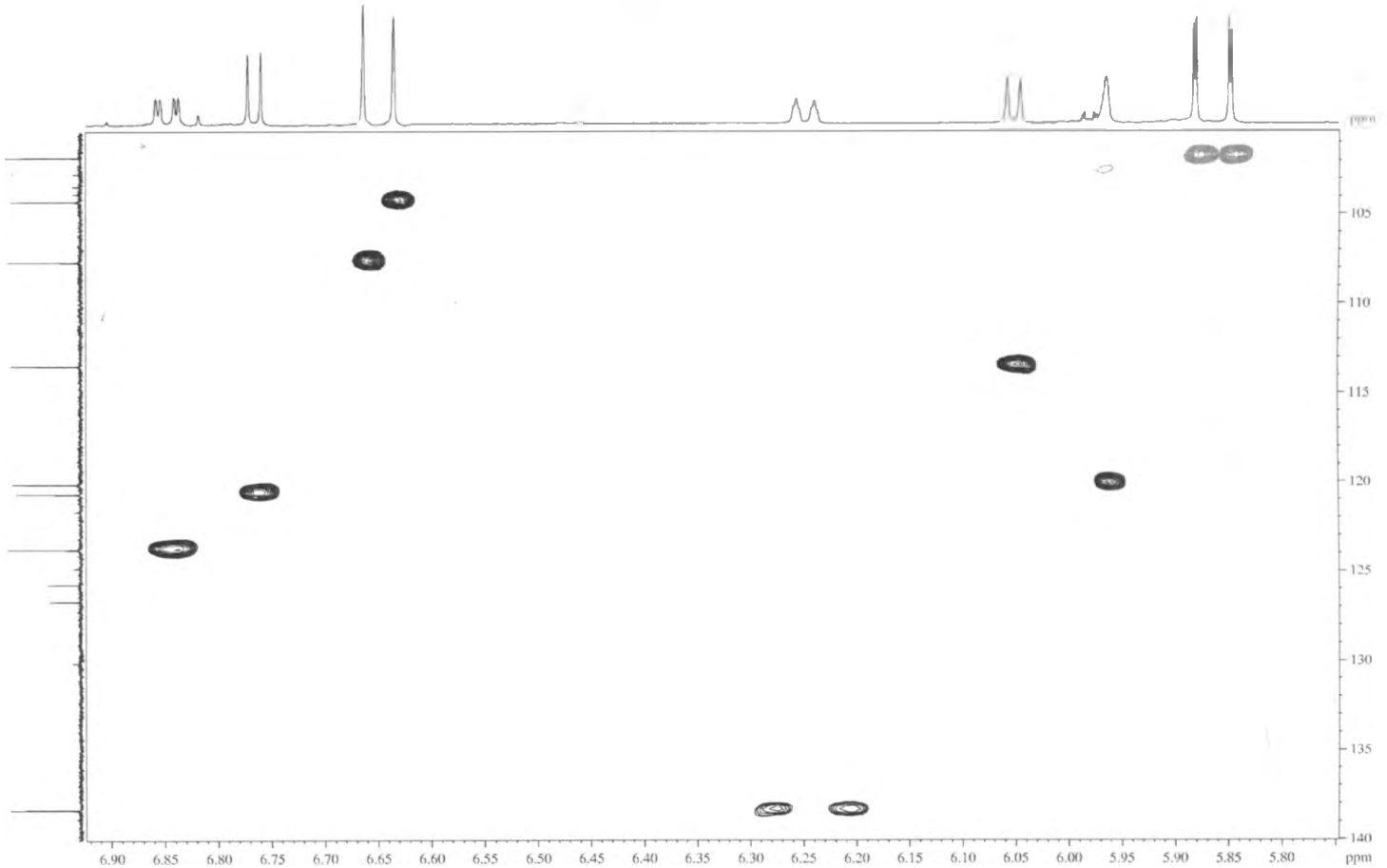


HSQC SPECTRUM FOR COMPOUND 5

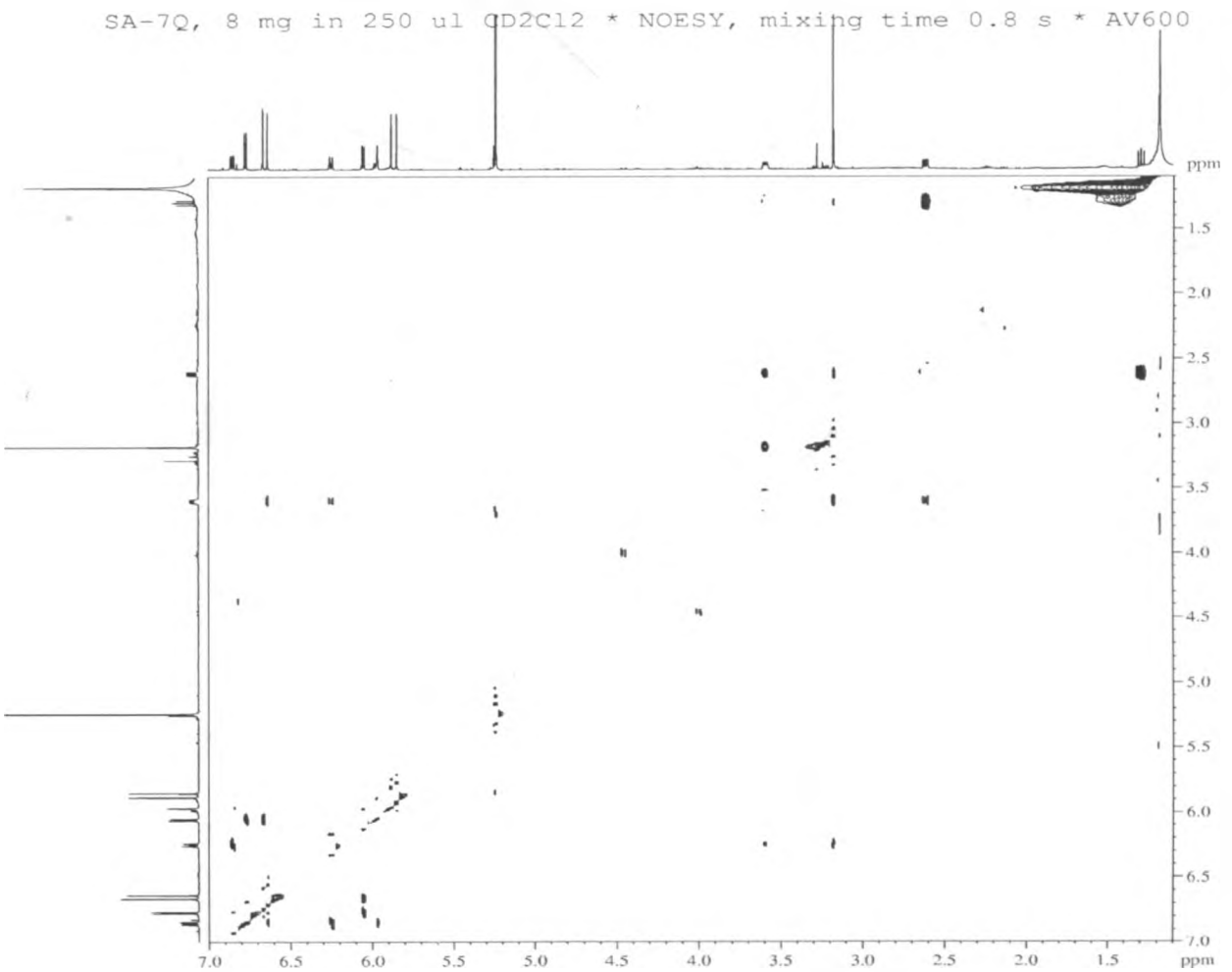


HSQC SPECTRUM FOR COMPOUND 5

SA-7Q, 8 mg in 250 ul CD2Cl2 * ed. HSQC * AV600

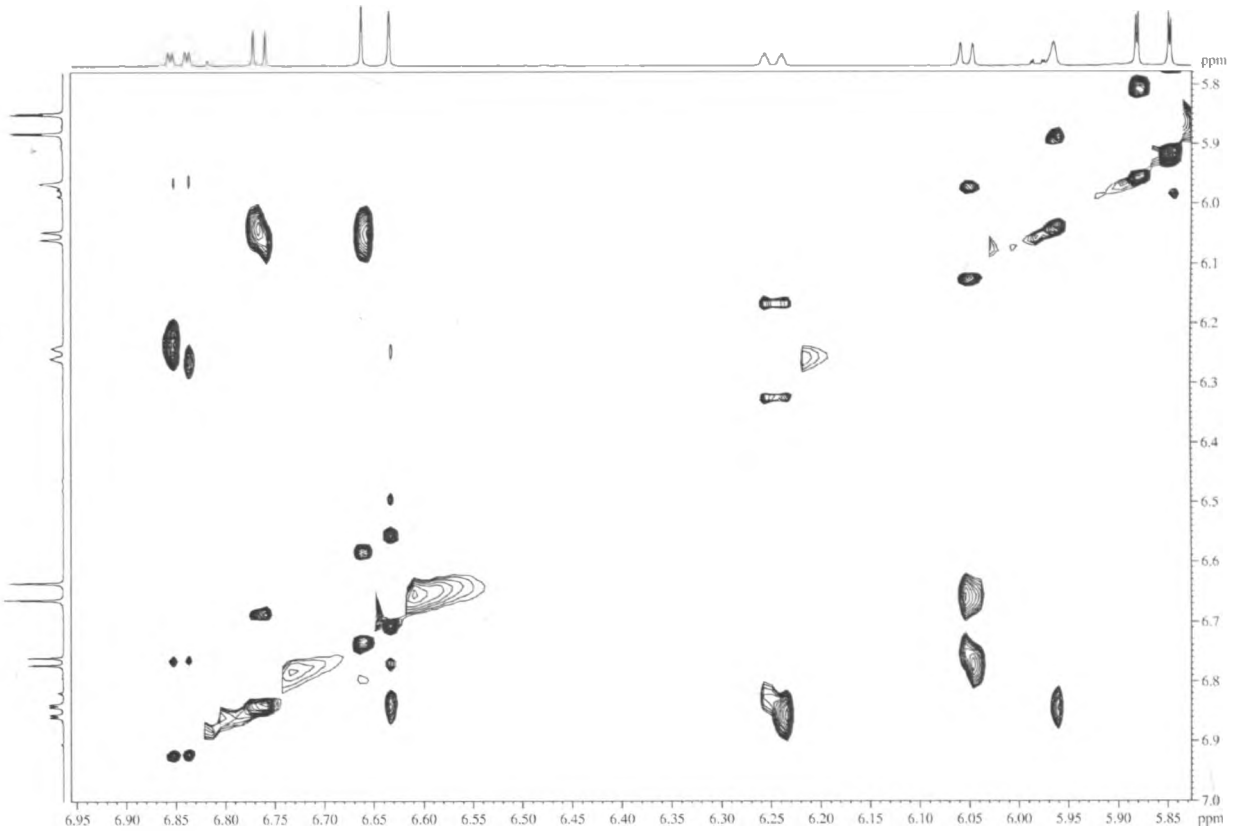


NOESY SPECTRUM FOR COMPOUND 5

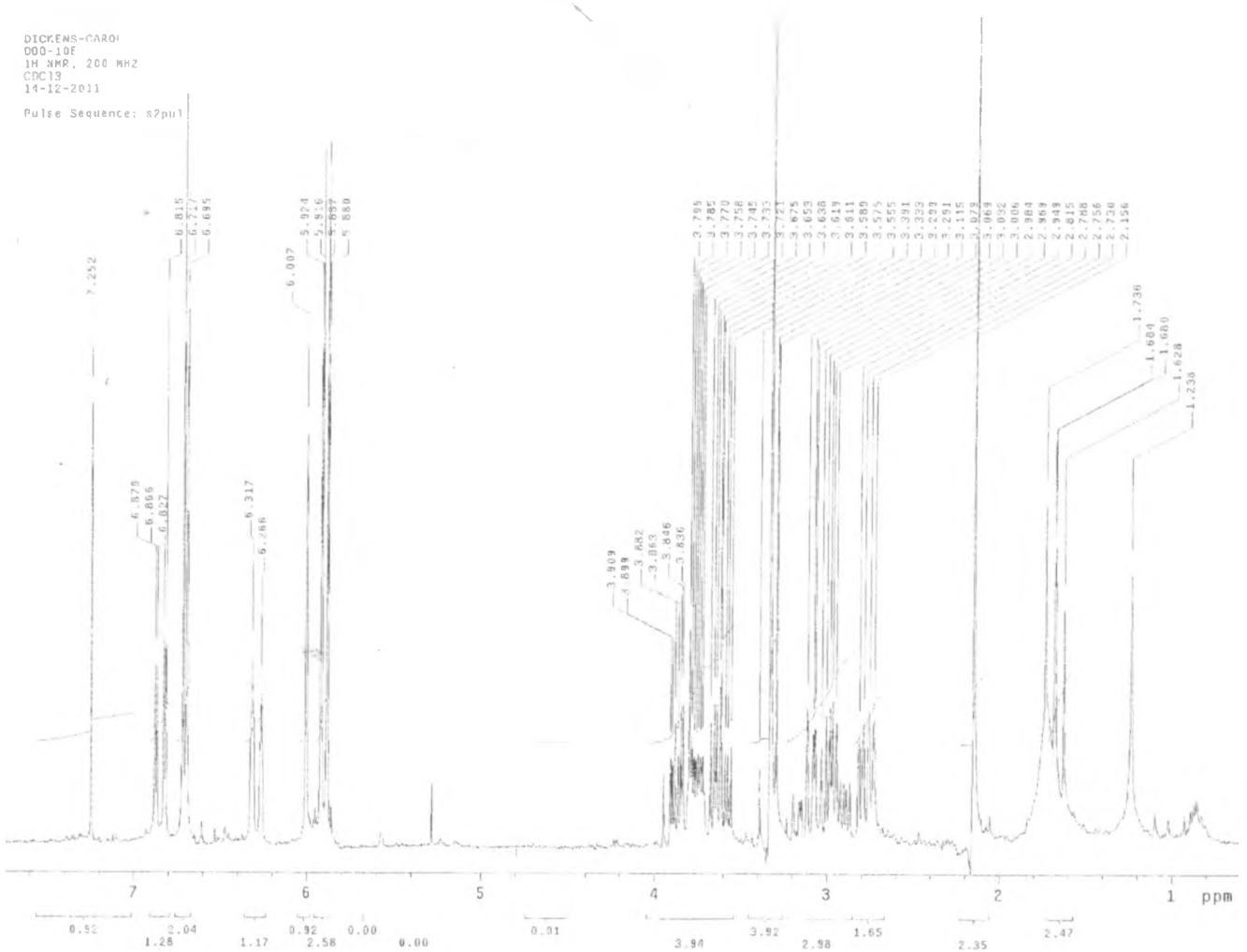


NOESY SPECTRUM FOR COMPOUND 5

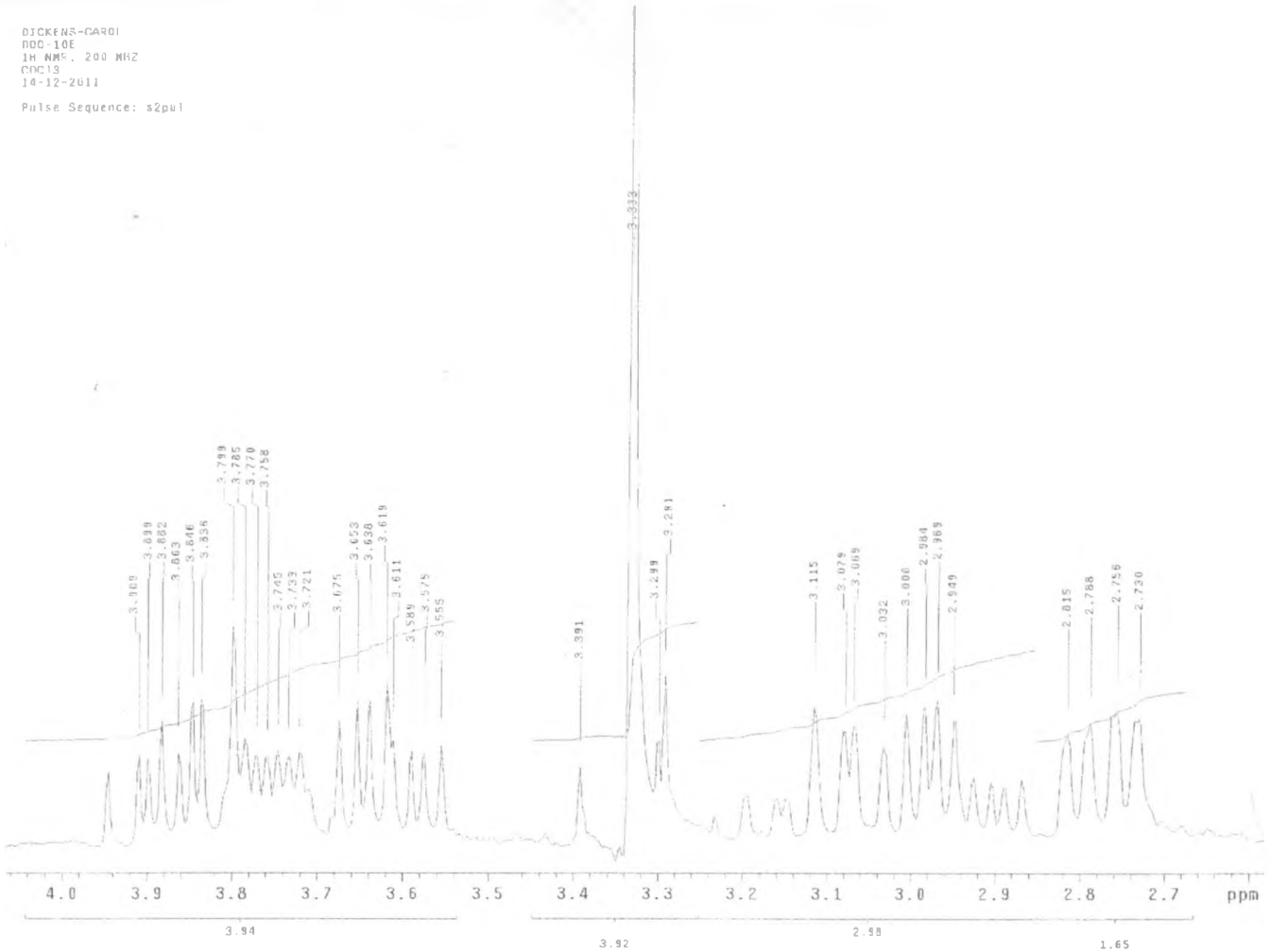
SA-7Q, 8 mg in 250 ul CD2Cl2 * NOESY, mixing time 0.8 s * AV600



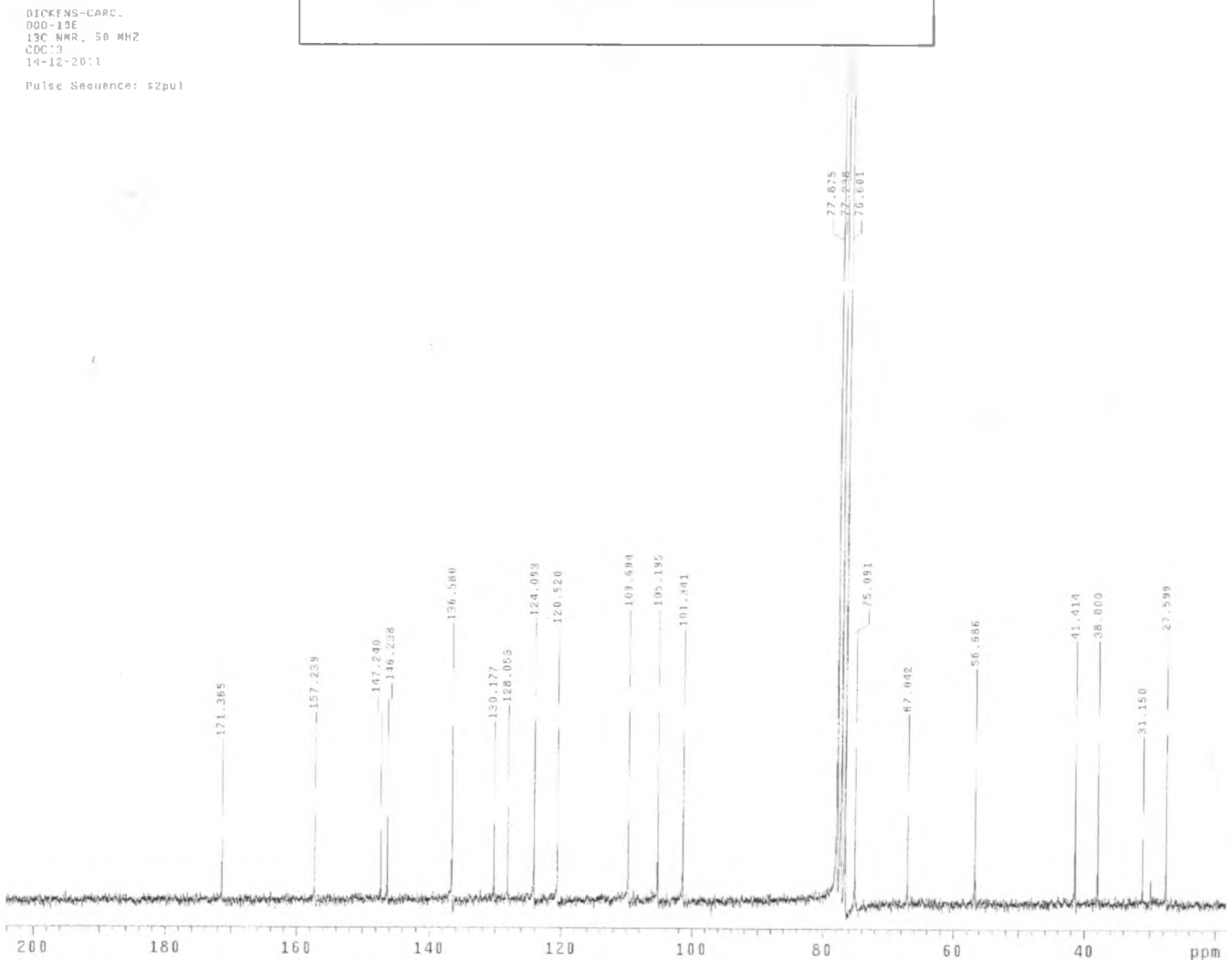
^{1}H NMR SPECTRUM FOR COMPOUND 6



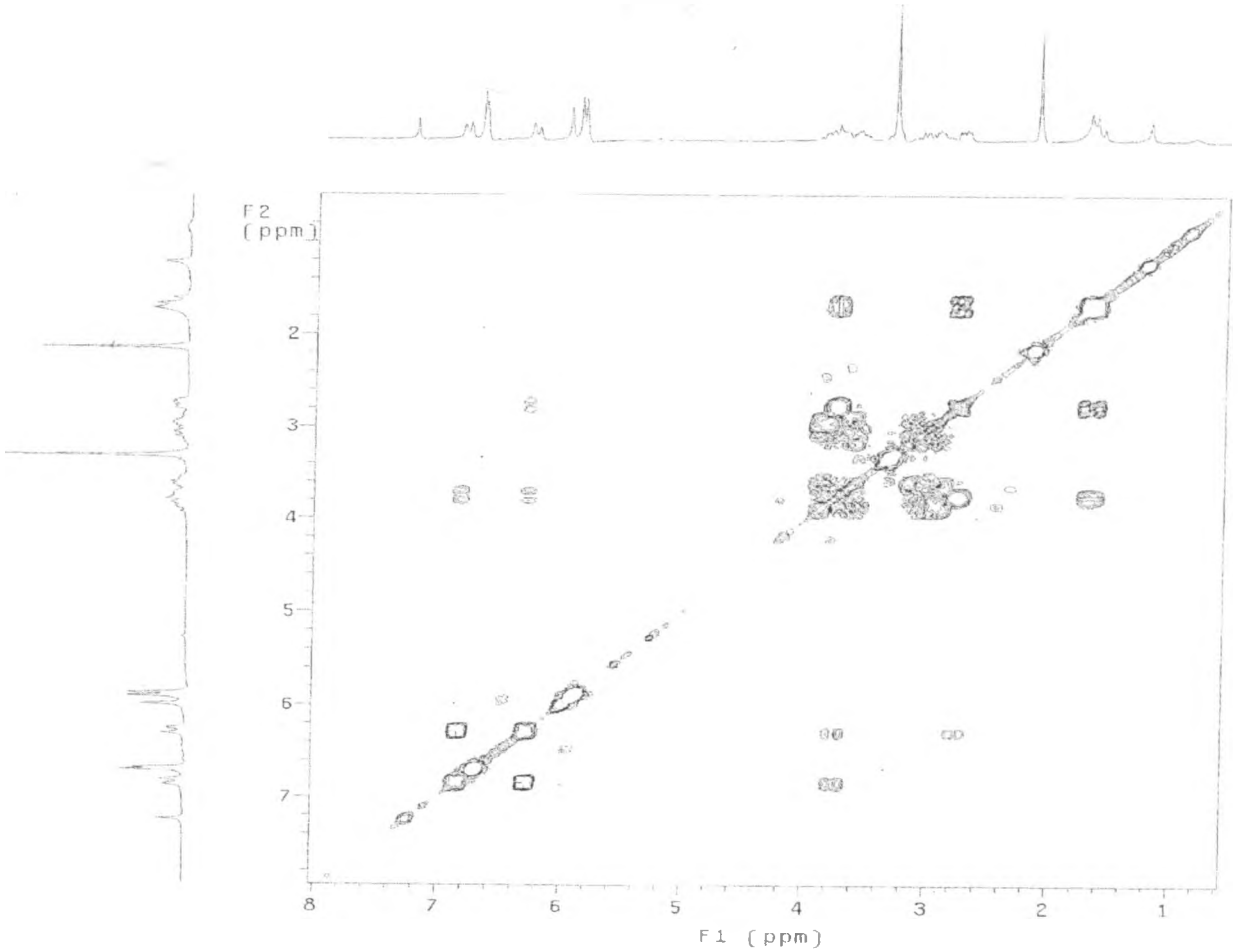
^1H NMR SPECTRUM FOR COMPOUND 6



^{13}C NMR SPECTRUM FOR COMPOUND 6

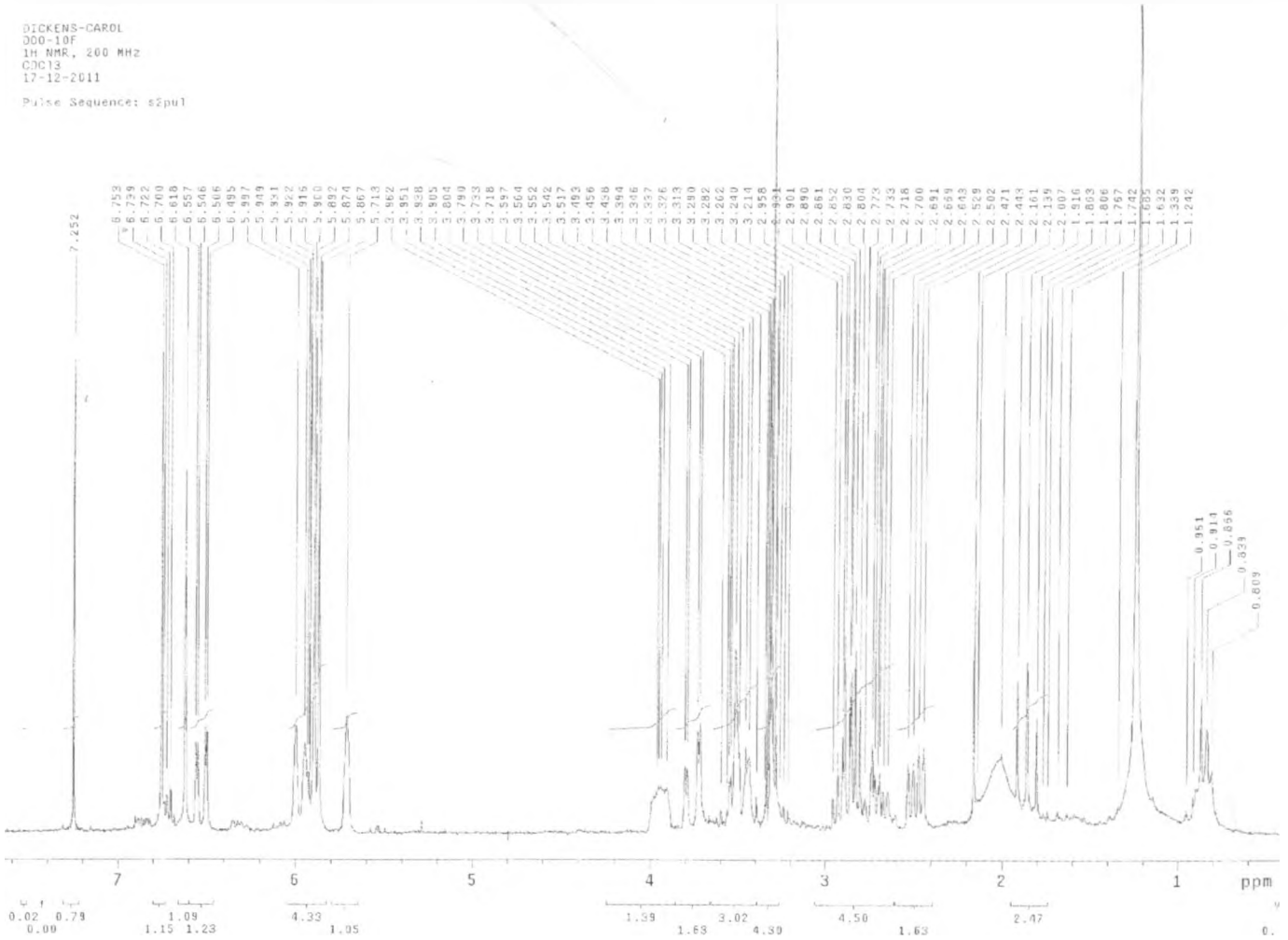


COSY SPECTRUM FOR COMPOUND 6

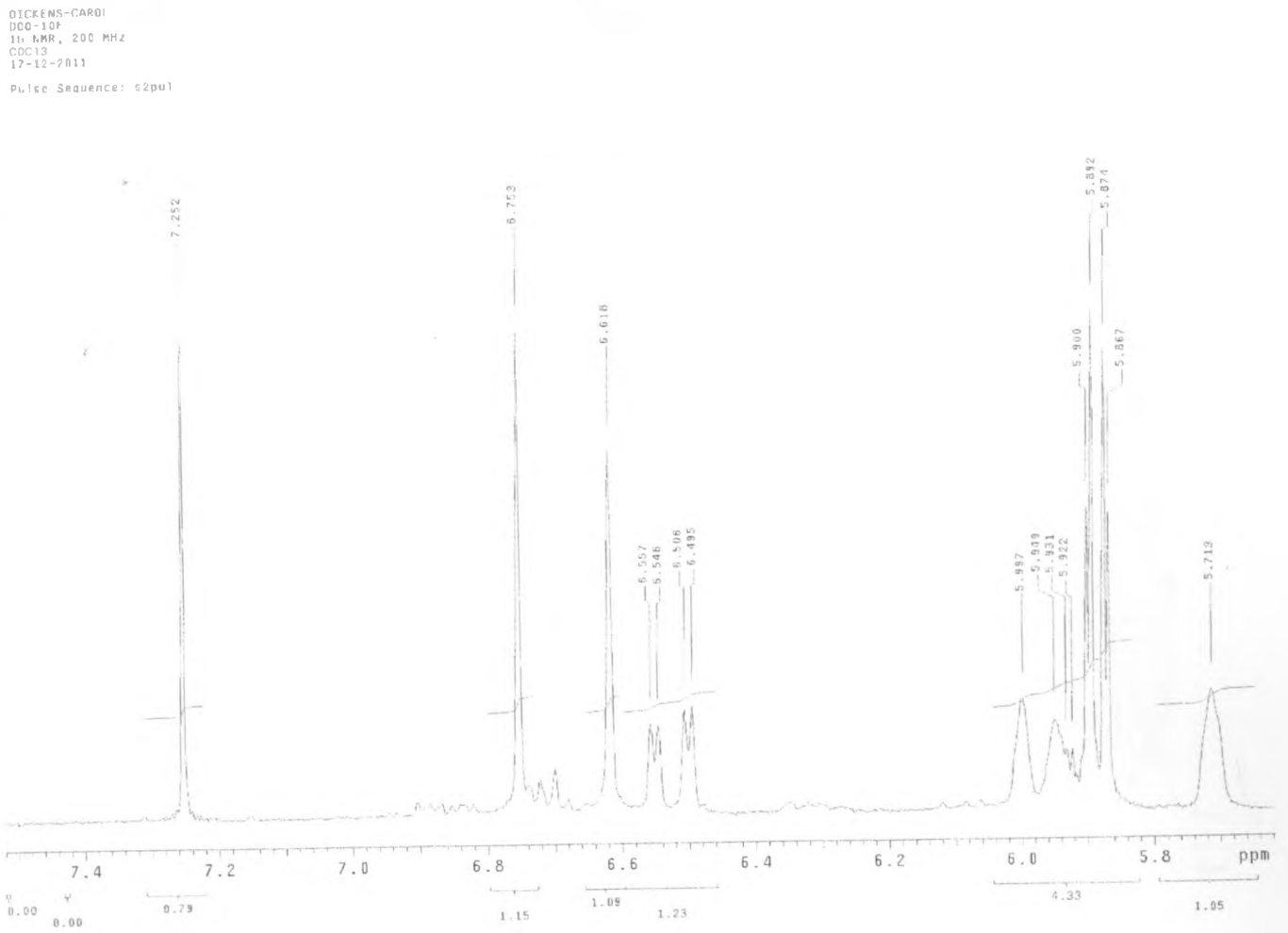


SPECTRA FOR COMPOUND 7

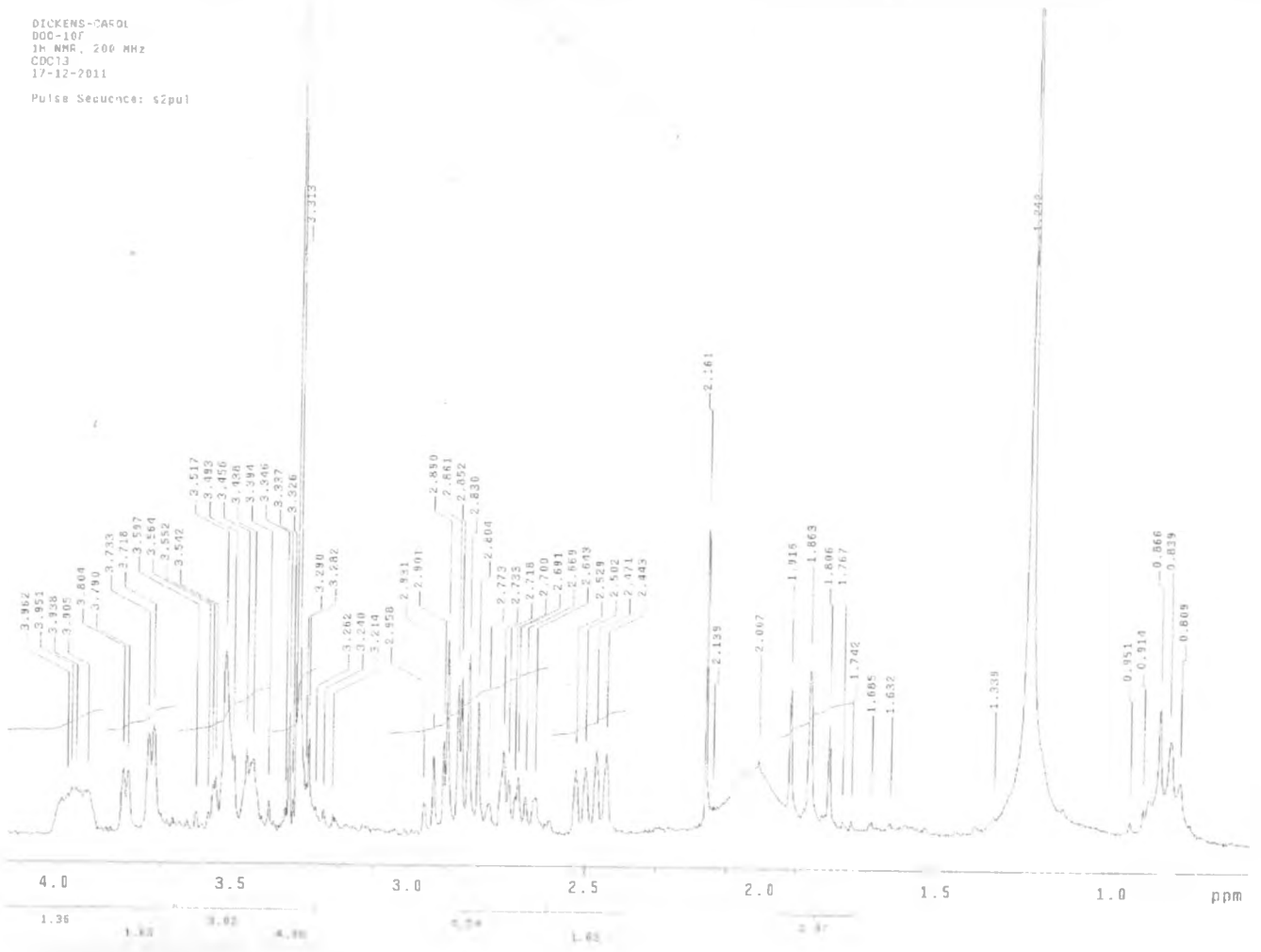
^{1}H NMR SPECTRUM FOR COMPOUND 7



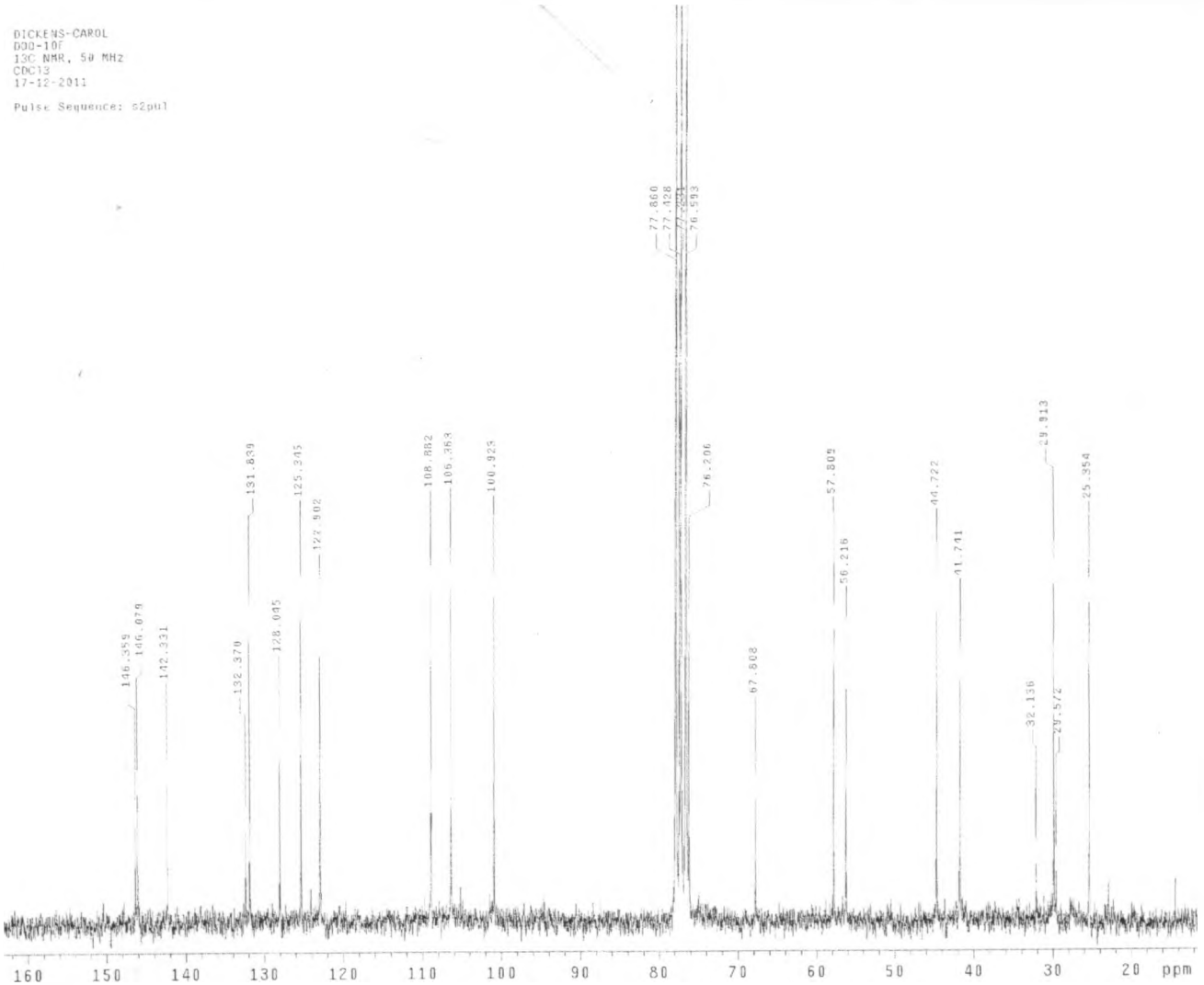
^{1}H NMR SPECTRUM FOR COMPOUND 7



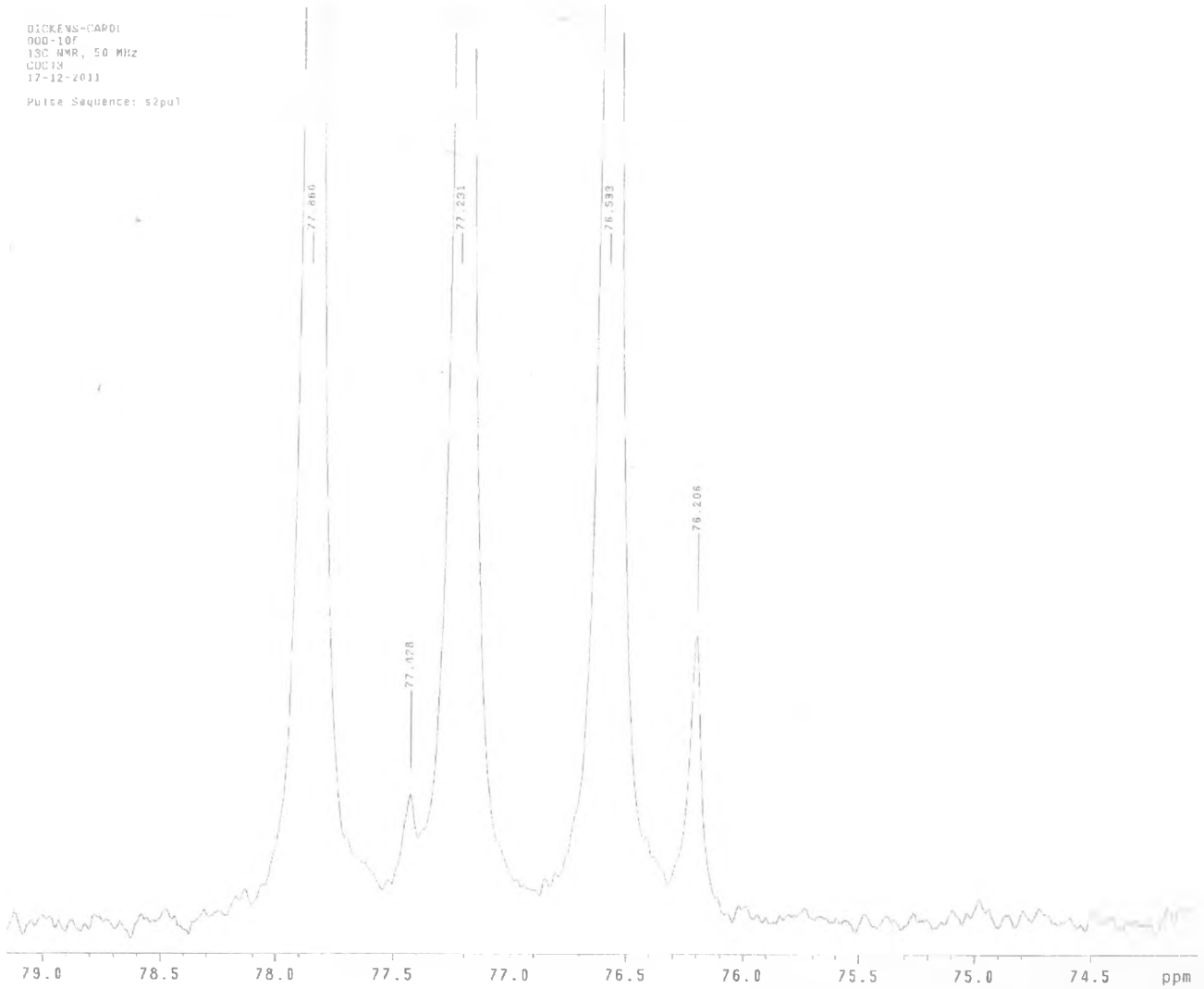
^{1}H NMR SPECTRUM FOR COMPOUND 7



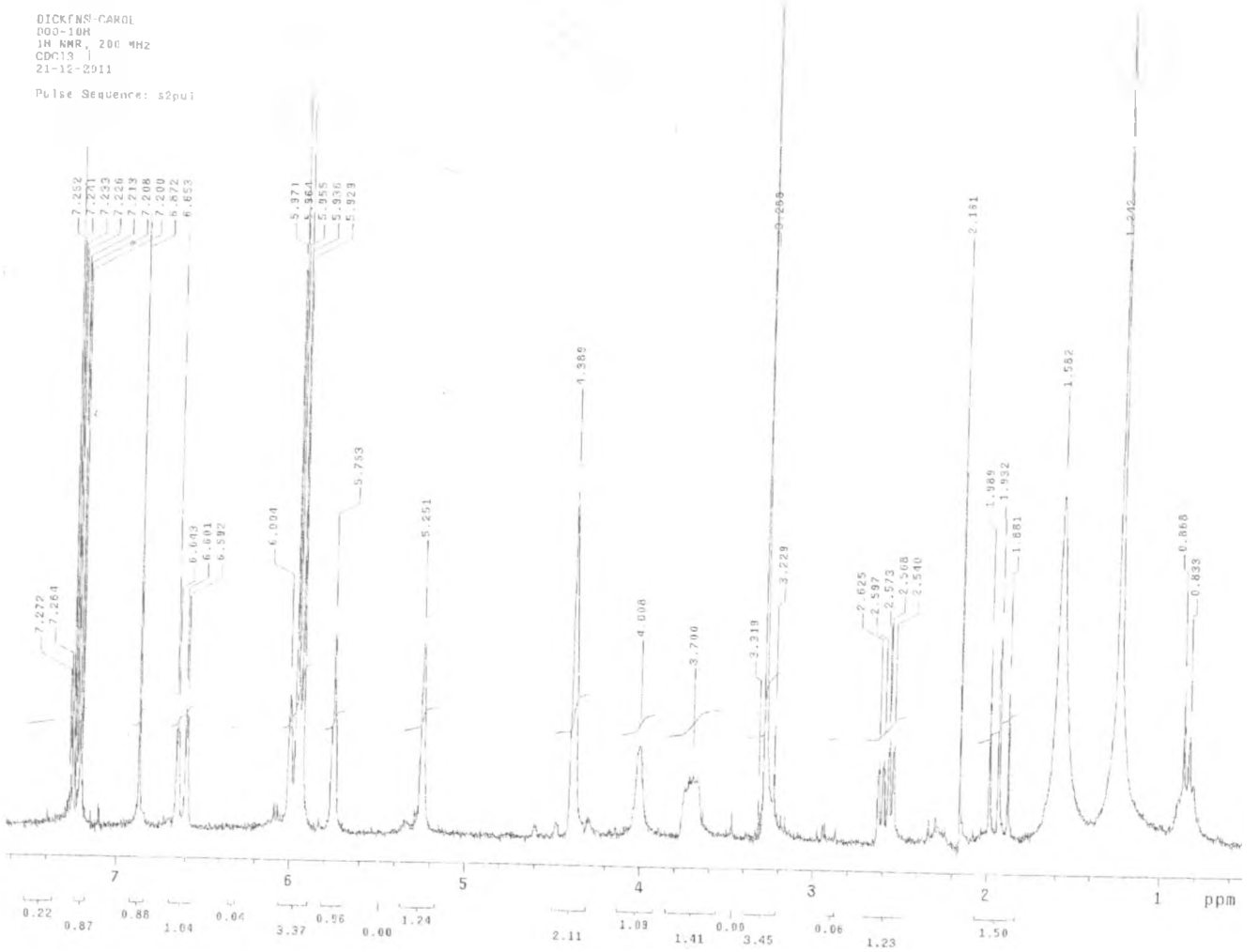
^{13}C NMR SPECTRUM FOR COMPOUND 7



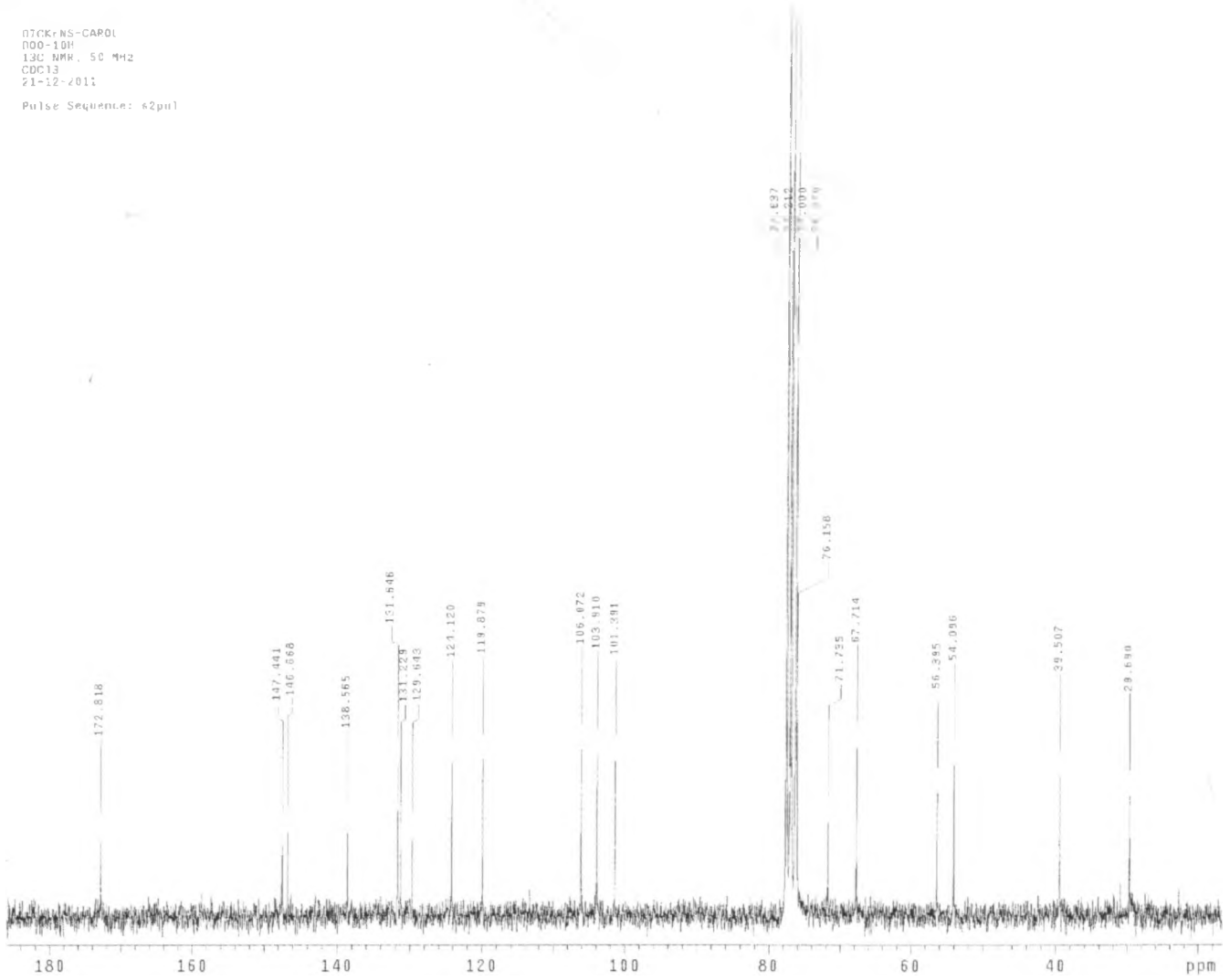
^{13}C NMR SPECTRUM FOR COMPOUND 7



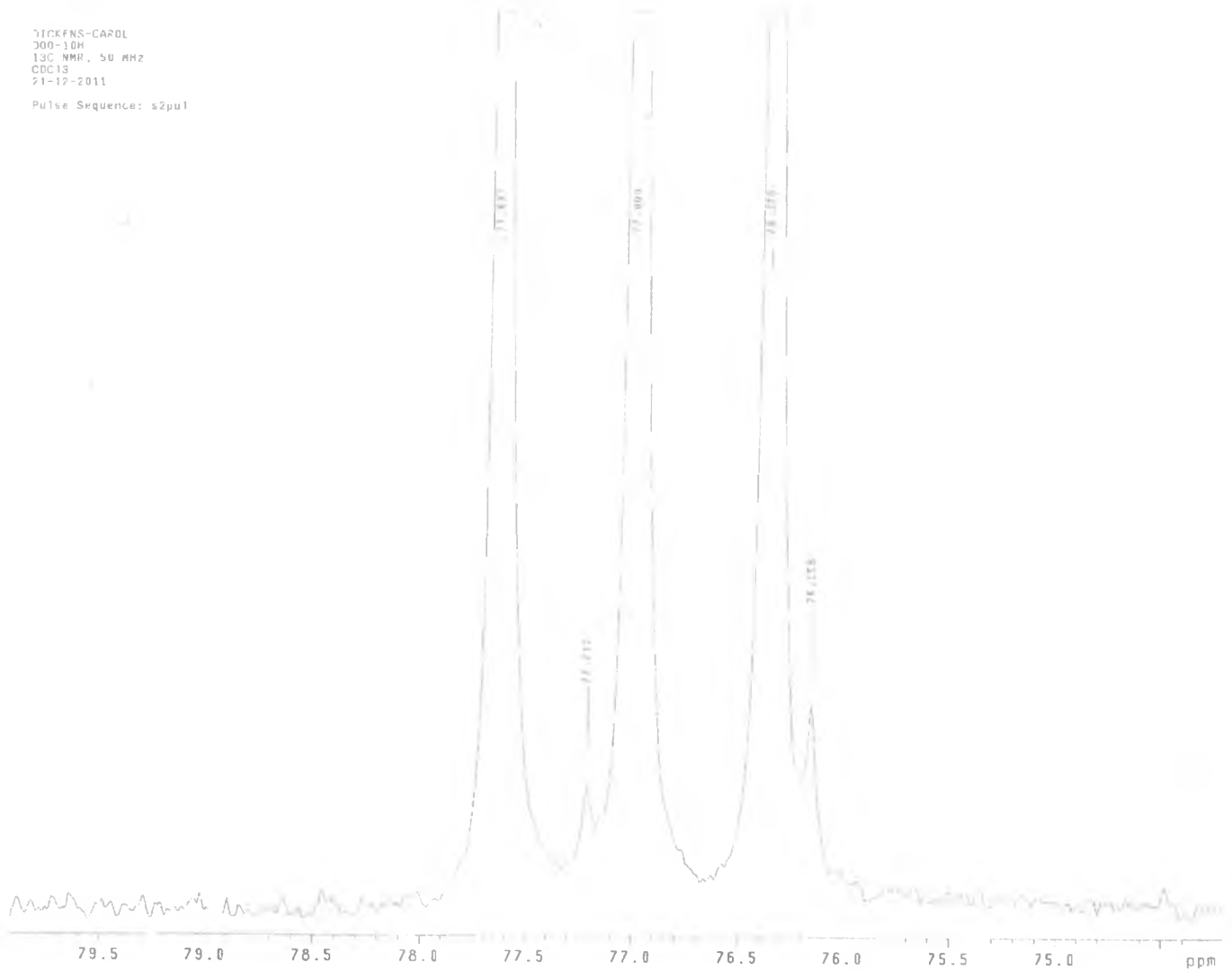
^{1}H NMR SPECTRUM FOR COMPOUND 8



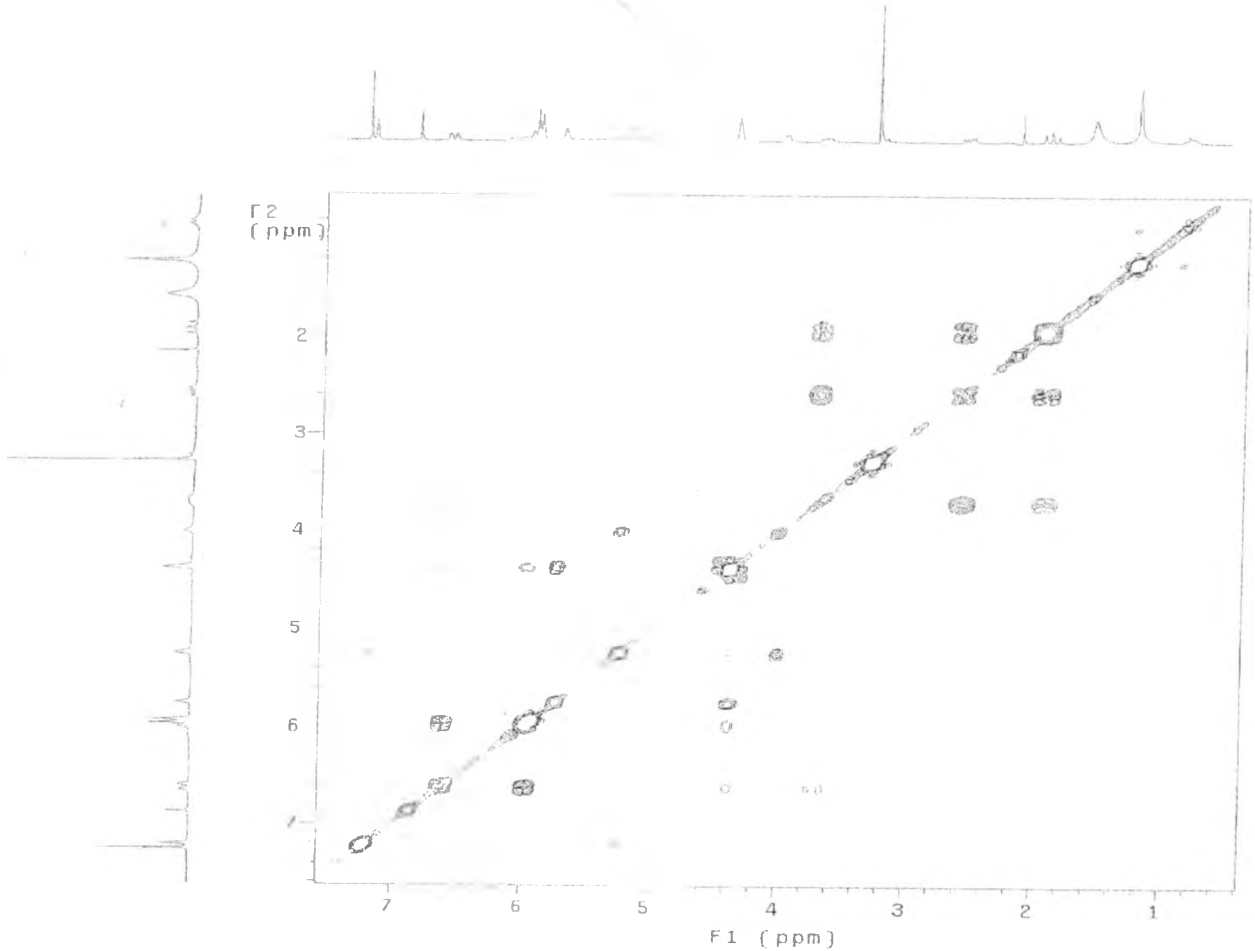
^{13}C NMR SPECTRUM FOR COMPOUND 8



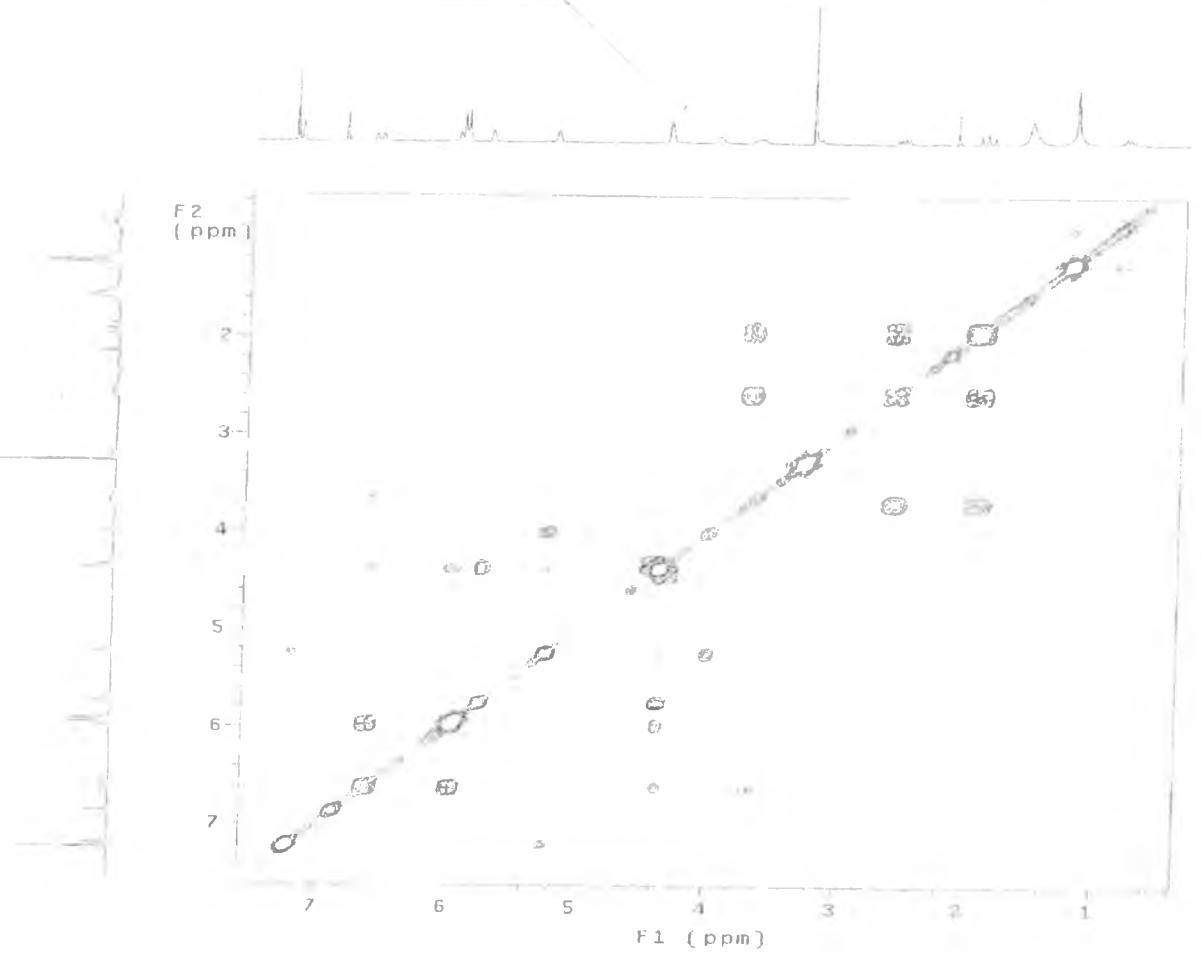
^{13}C NMR SPECTRUM FOR COMPOUND 8



COSY SPECTRUM FOR COMPOUND 8

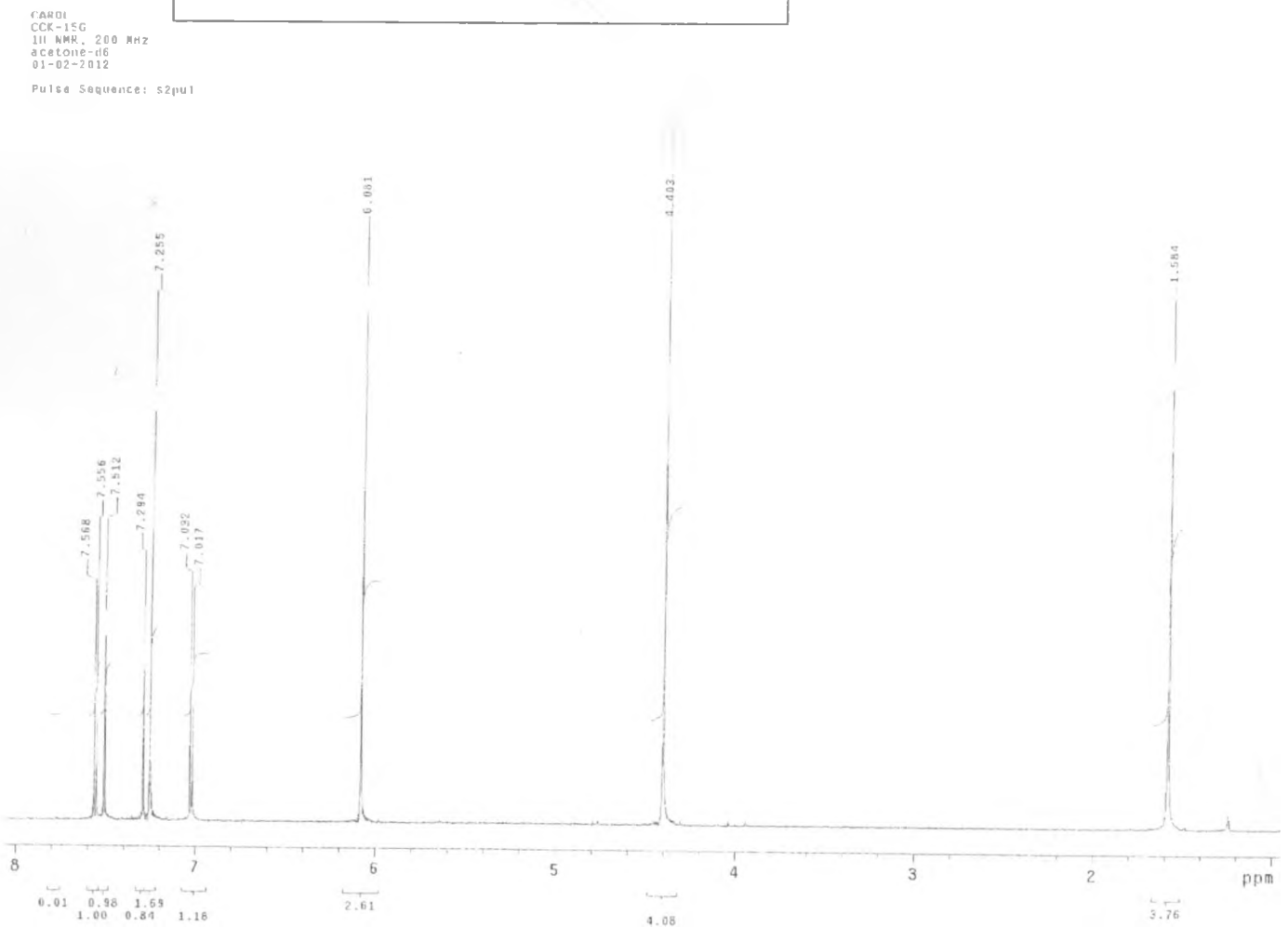


COSY SPECTRUM FOR COMPOUND 8

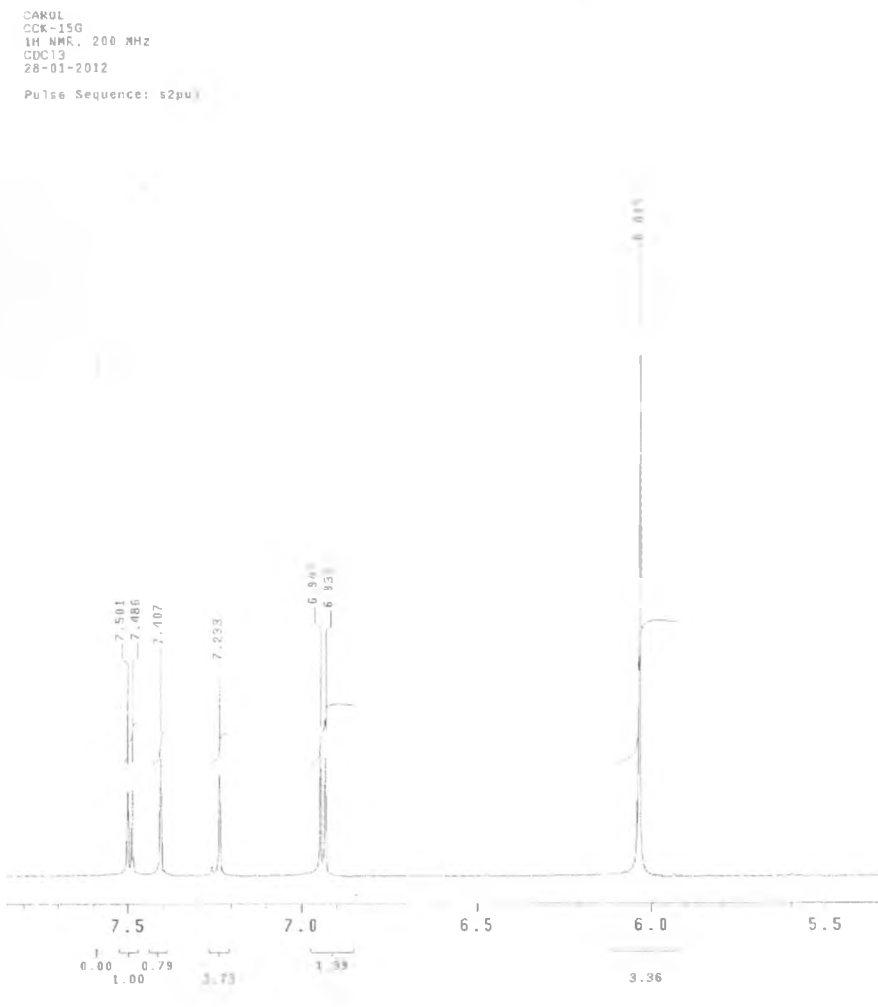


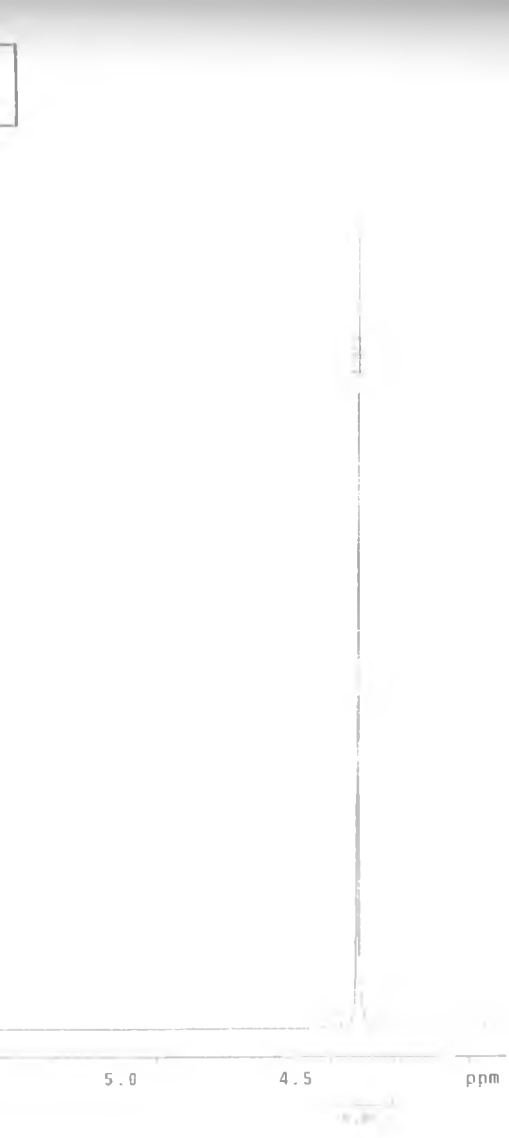
SPECTRA FOR COMPOUND 9

^{1}H NMR SPECTRUM FOR COMPOUND 9

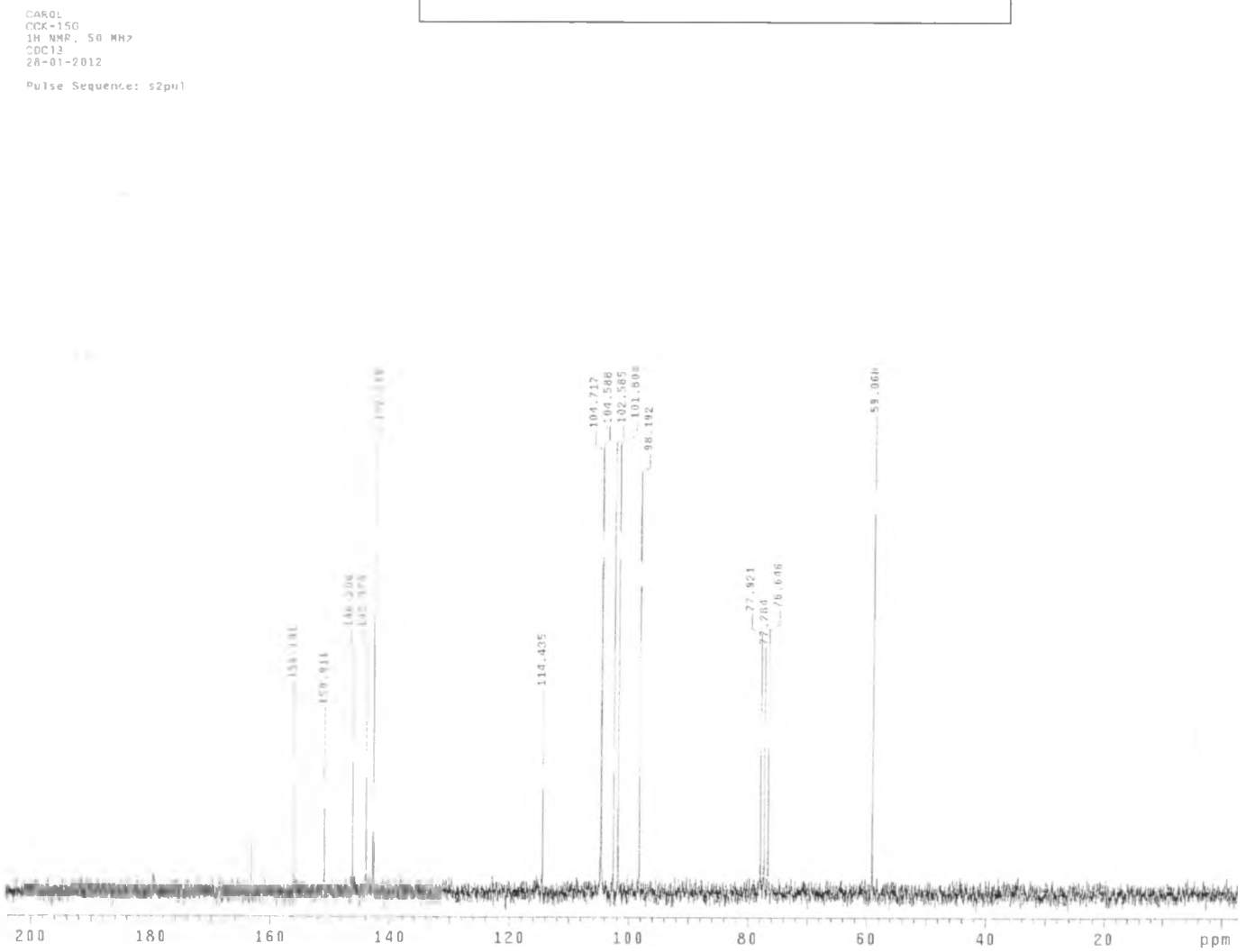


^{1}H NMR SPECTRUM FOR COMPOUND 9



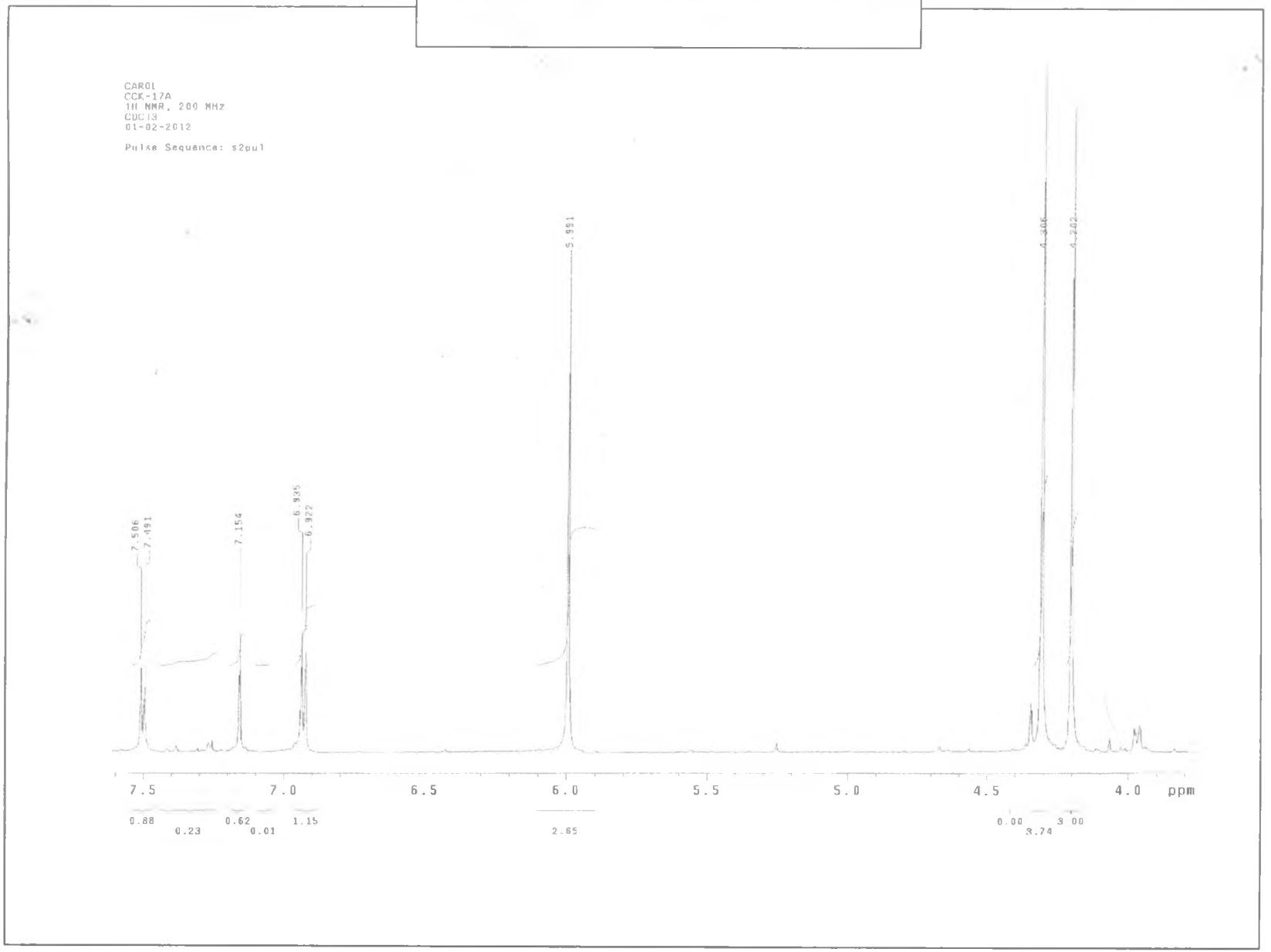

5.0
4.5
ppm

^{13}C NMR SPECTRUM FOR COMPOUND 9



SPECTRA FOR COMPOUND 10

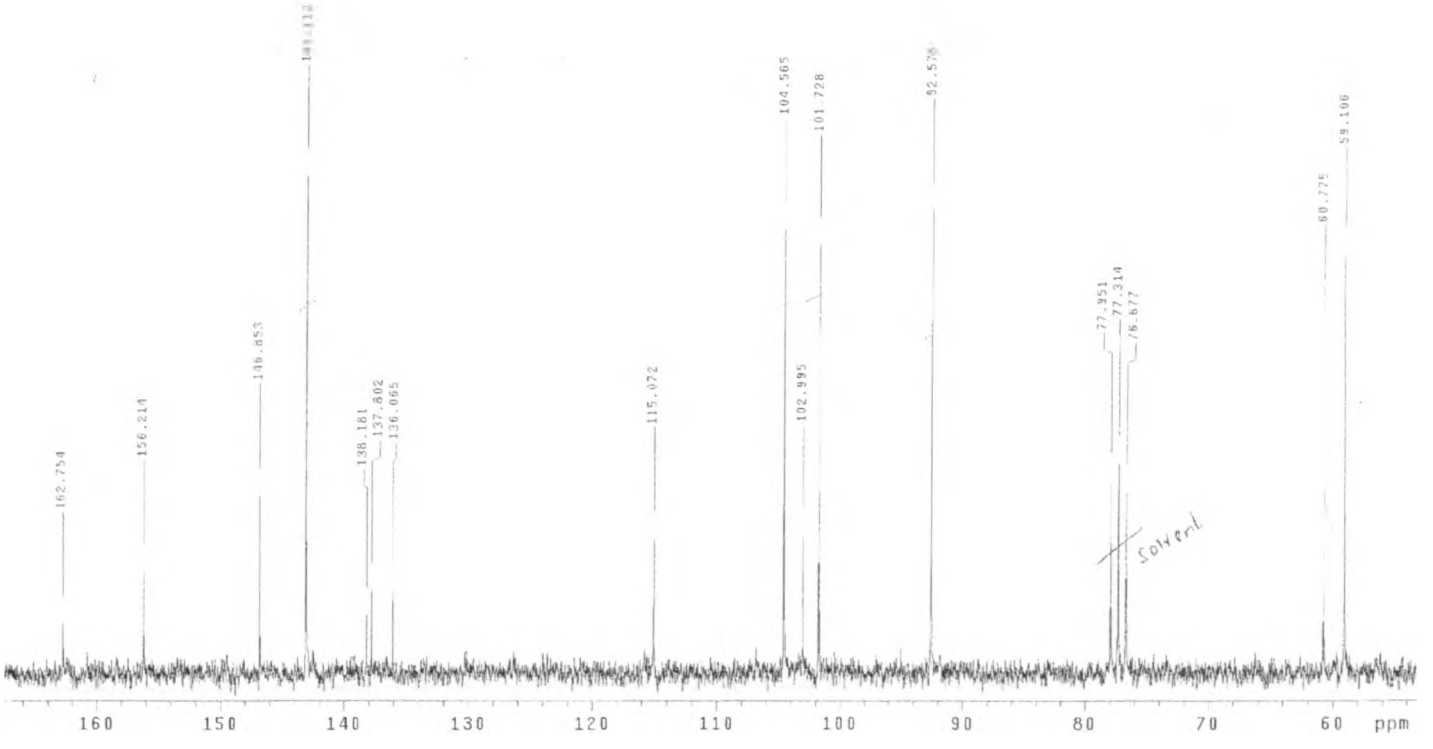
^{1}H NMR SPECTRUM FOR COMPOUND 10



^{13}C NMR SPECTRUM FOR COMPOUND 10

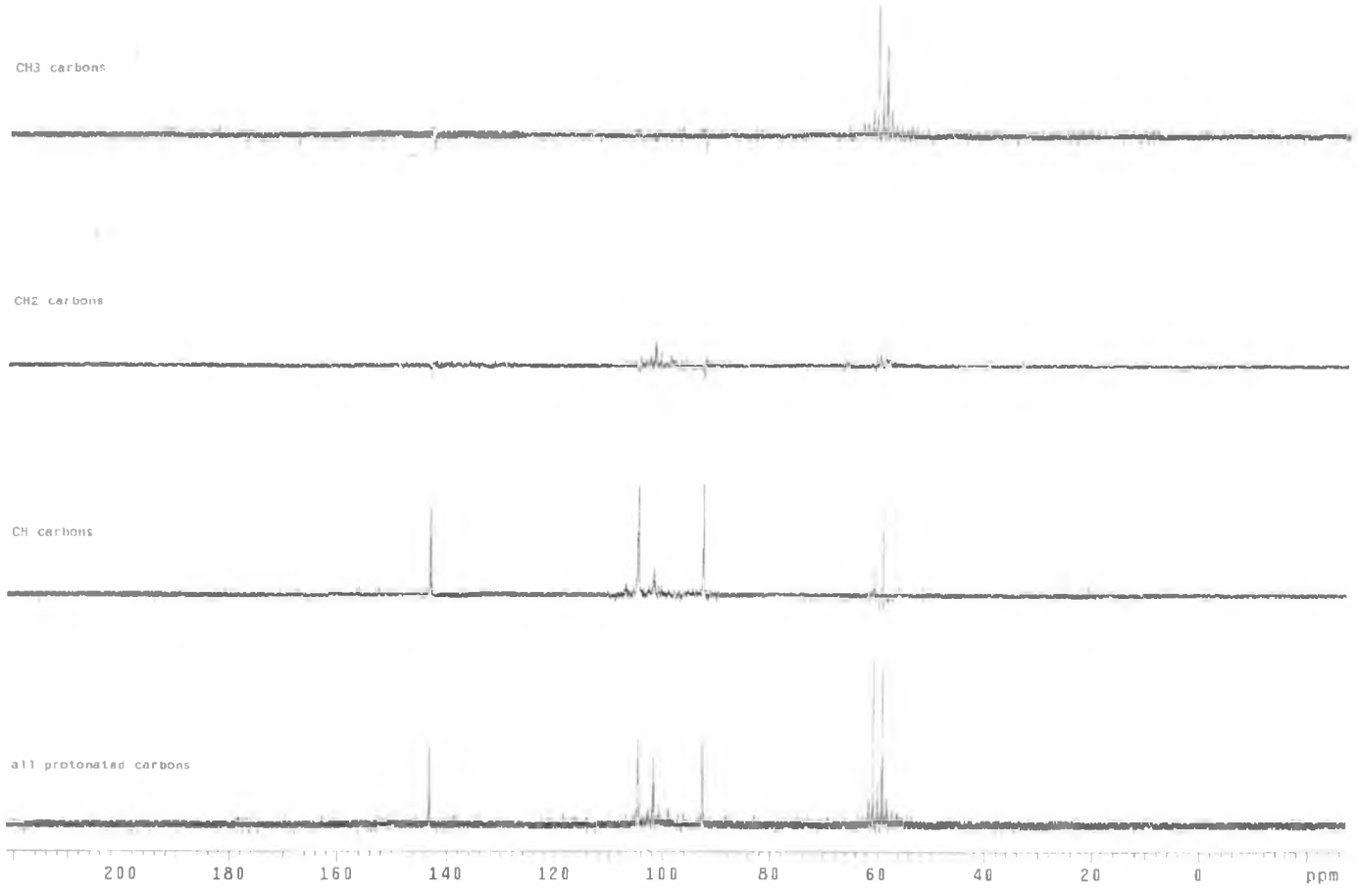
CAROL
CCK-17A
13C NMR, 50 MHz
CDCl3
01-02-2012

Pulse Sequence: s2pul



DEPT SPECTRUM FOR COMPOUND 10

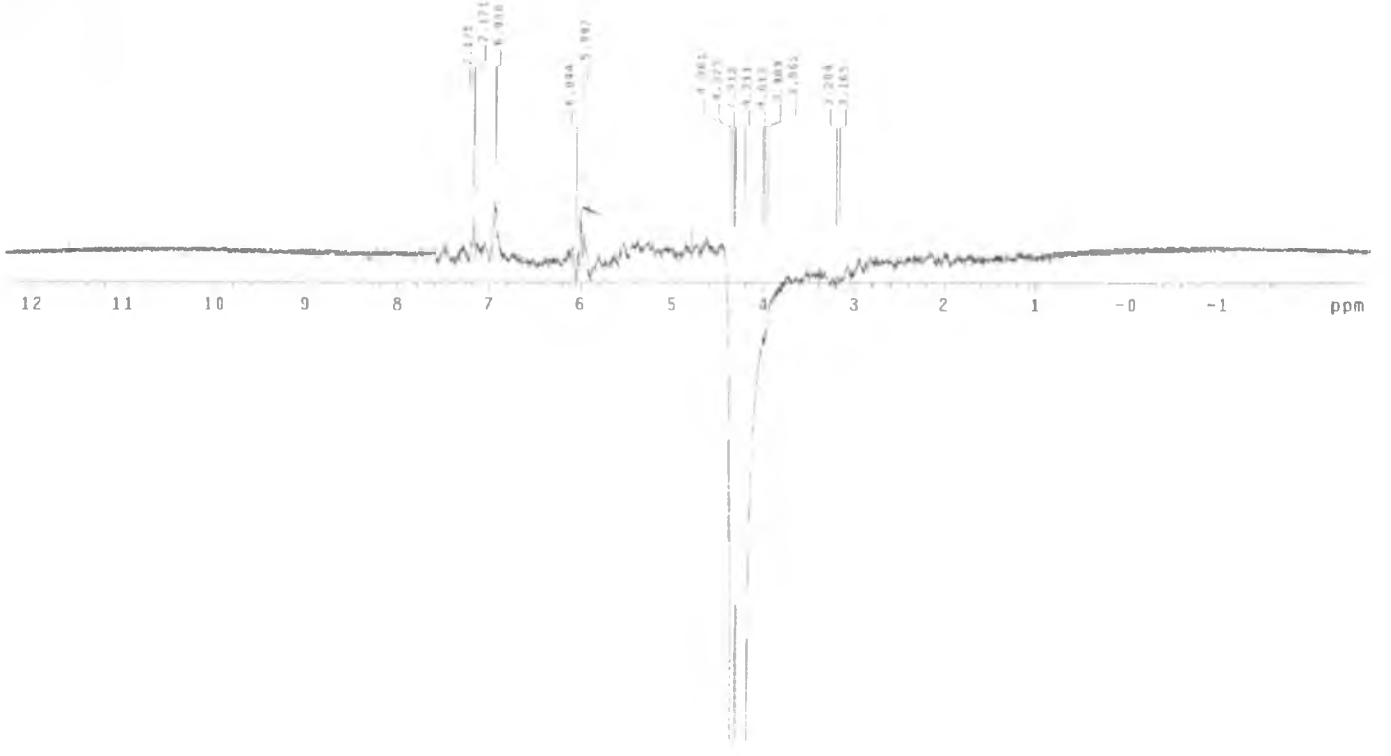
CAROL
CCK-17A
CDCl3
02/02/2012



NOEDIF NMR SPECTRUM FOR COMPOUND 10

CARDI
CCK-17A
NOEDIFF
CDCl3, 200 MHz
02-02-2012

Pulse Sequence: noedif



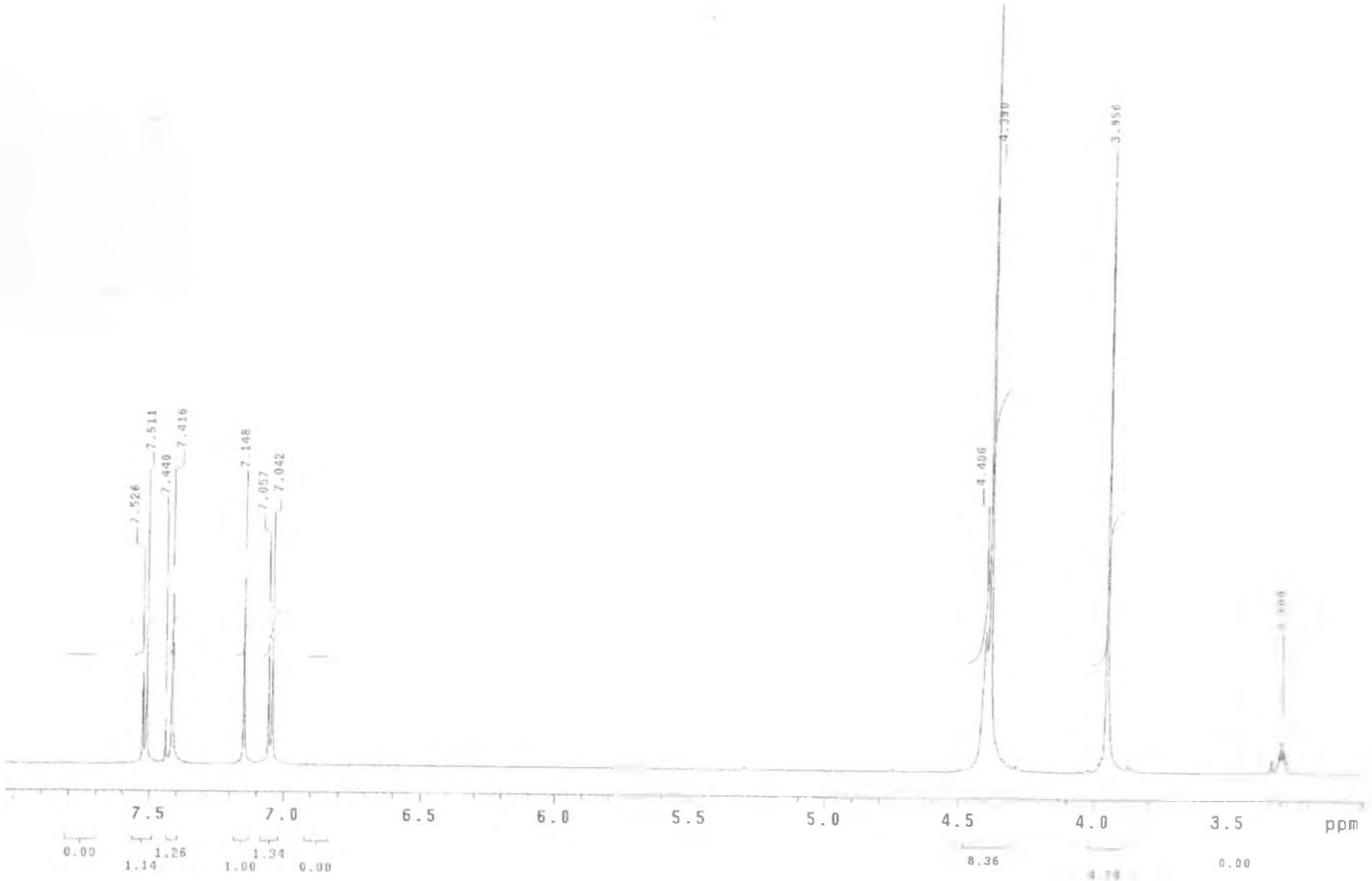
SPECTRA FOR COMPOUND 11

^{1}H NMR SPECTRUM FOR COMPOUND 11

CAROLINE — 218

STANDARD 1H OBSERVE

Pulse Sequence: s2pul

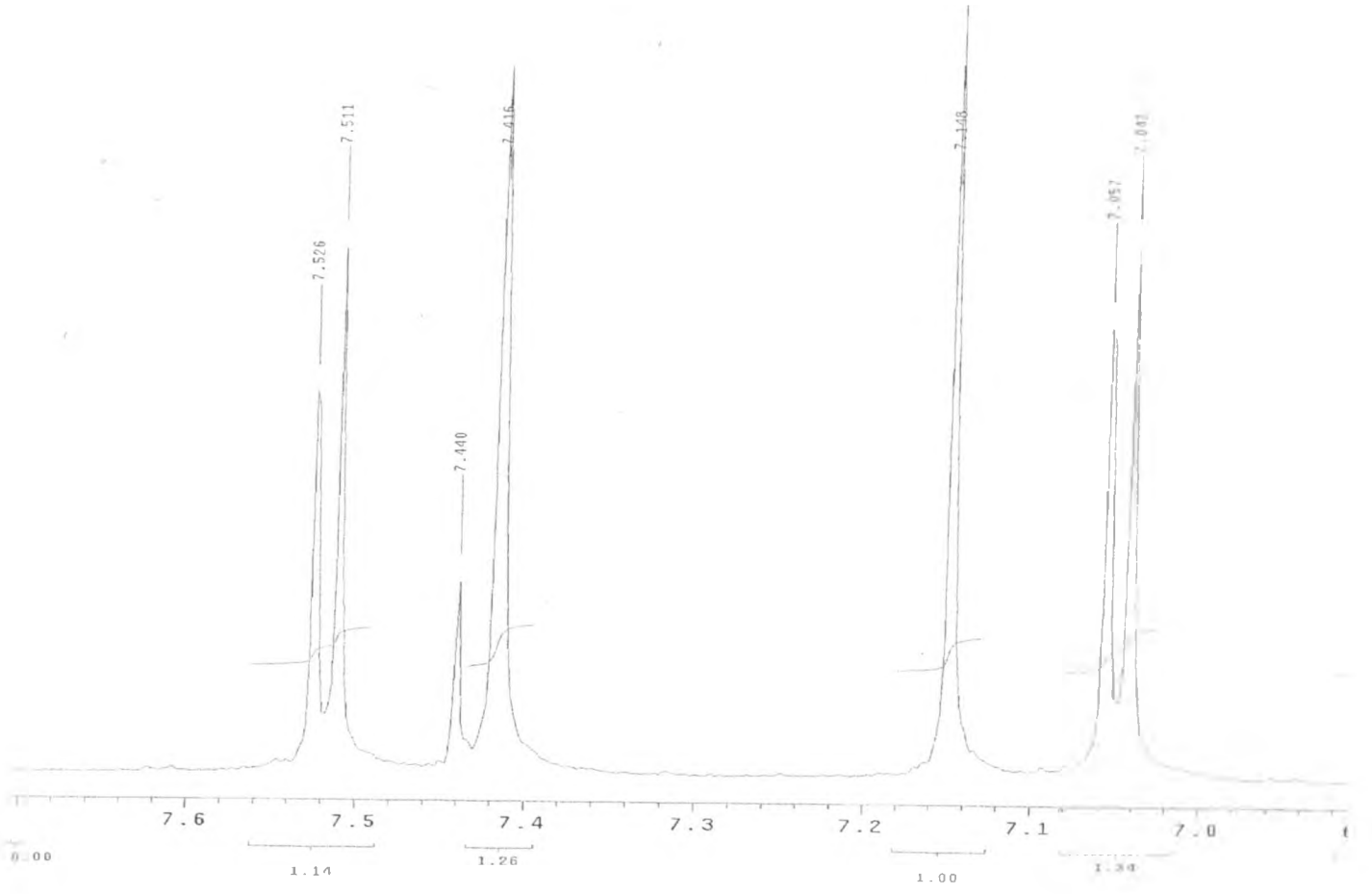


^{1}H NMR SPECTRUM FOR COMPOUND 11

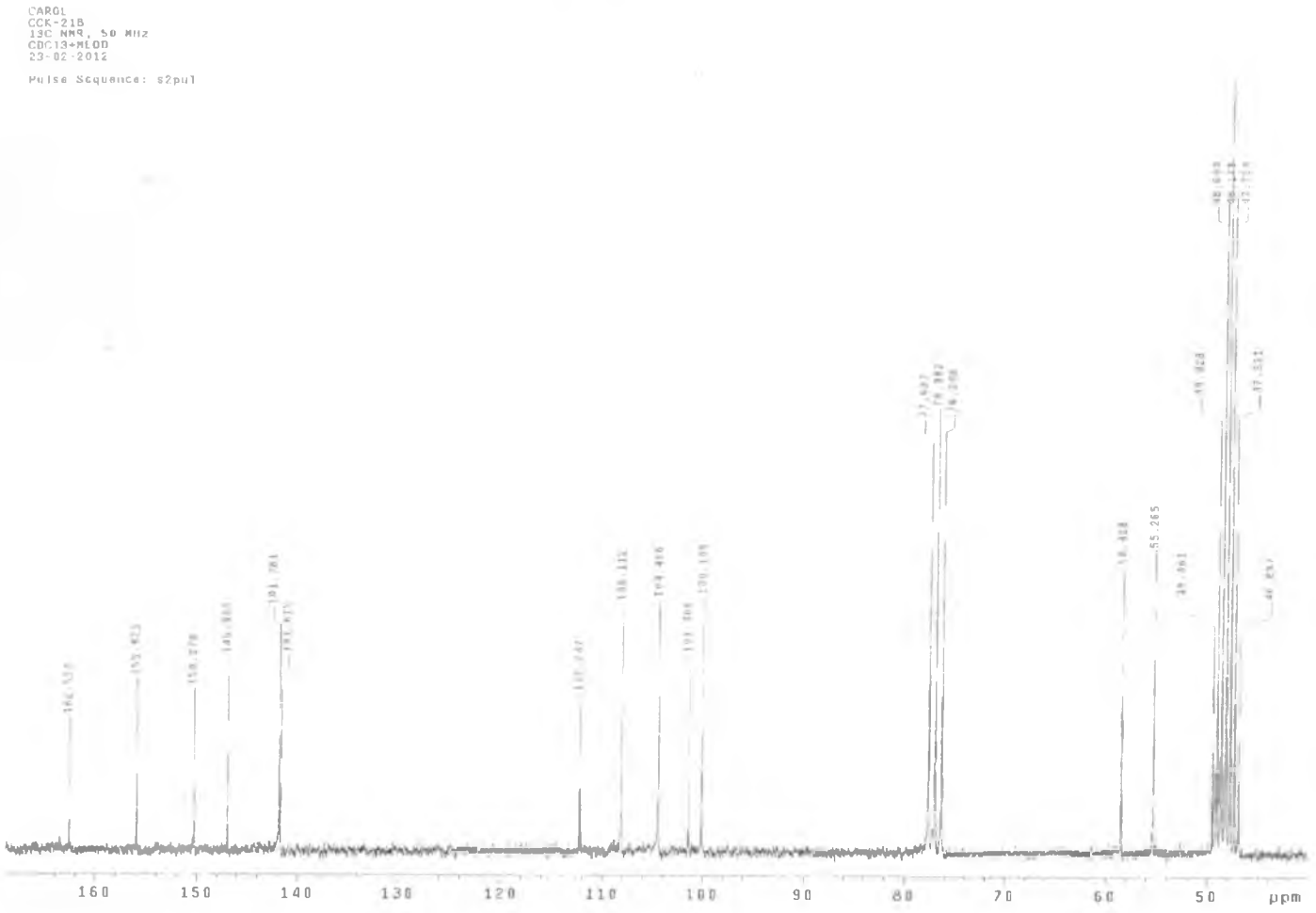
CAROLINE – R1

STANDARD 1H OBSERVE

Pulse Sequence: s2pul

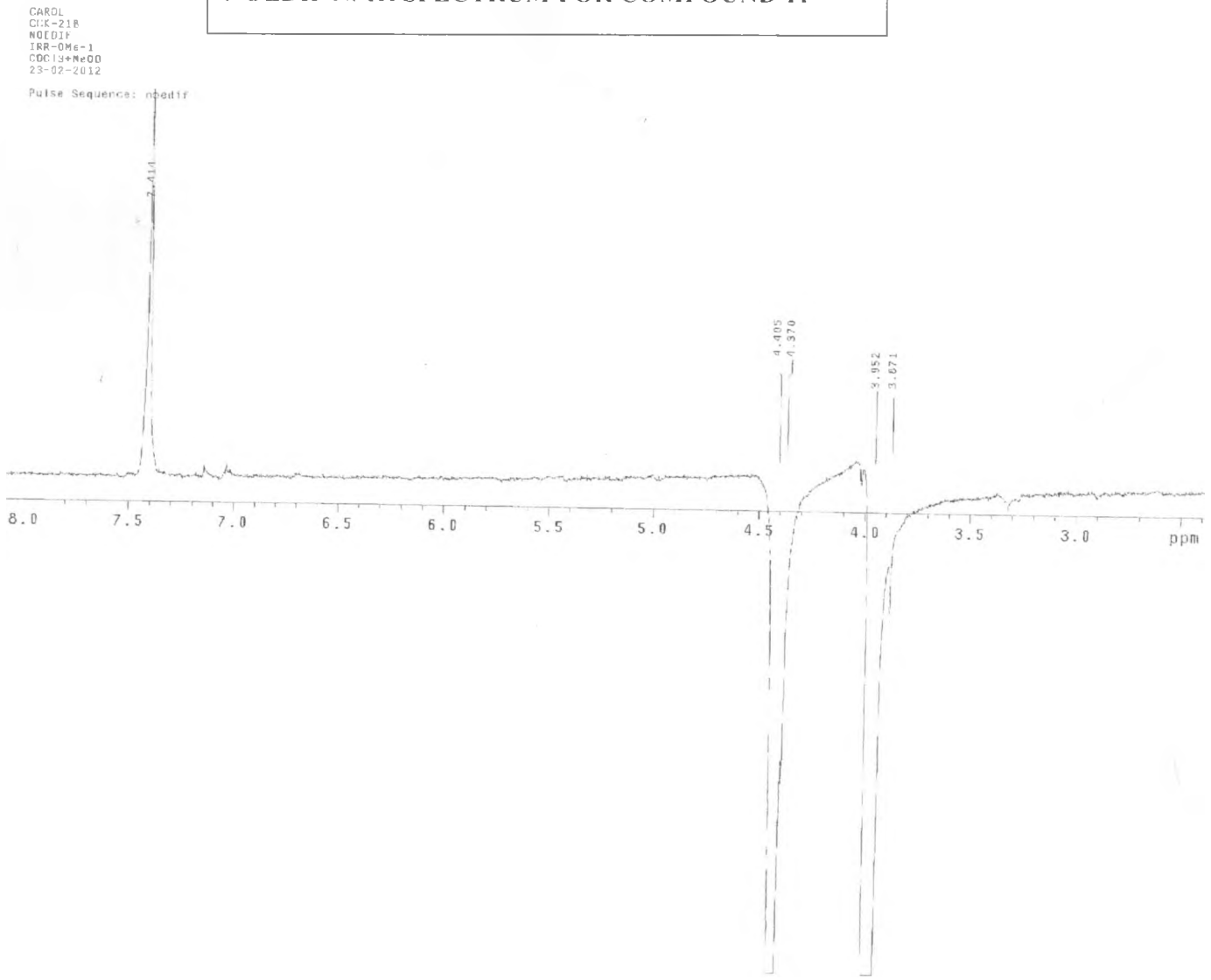


^{13}C NMR SPECTRUM FOR COMPOUND 11



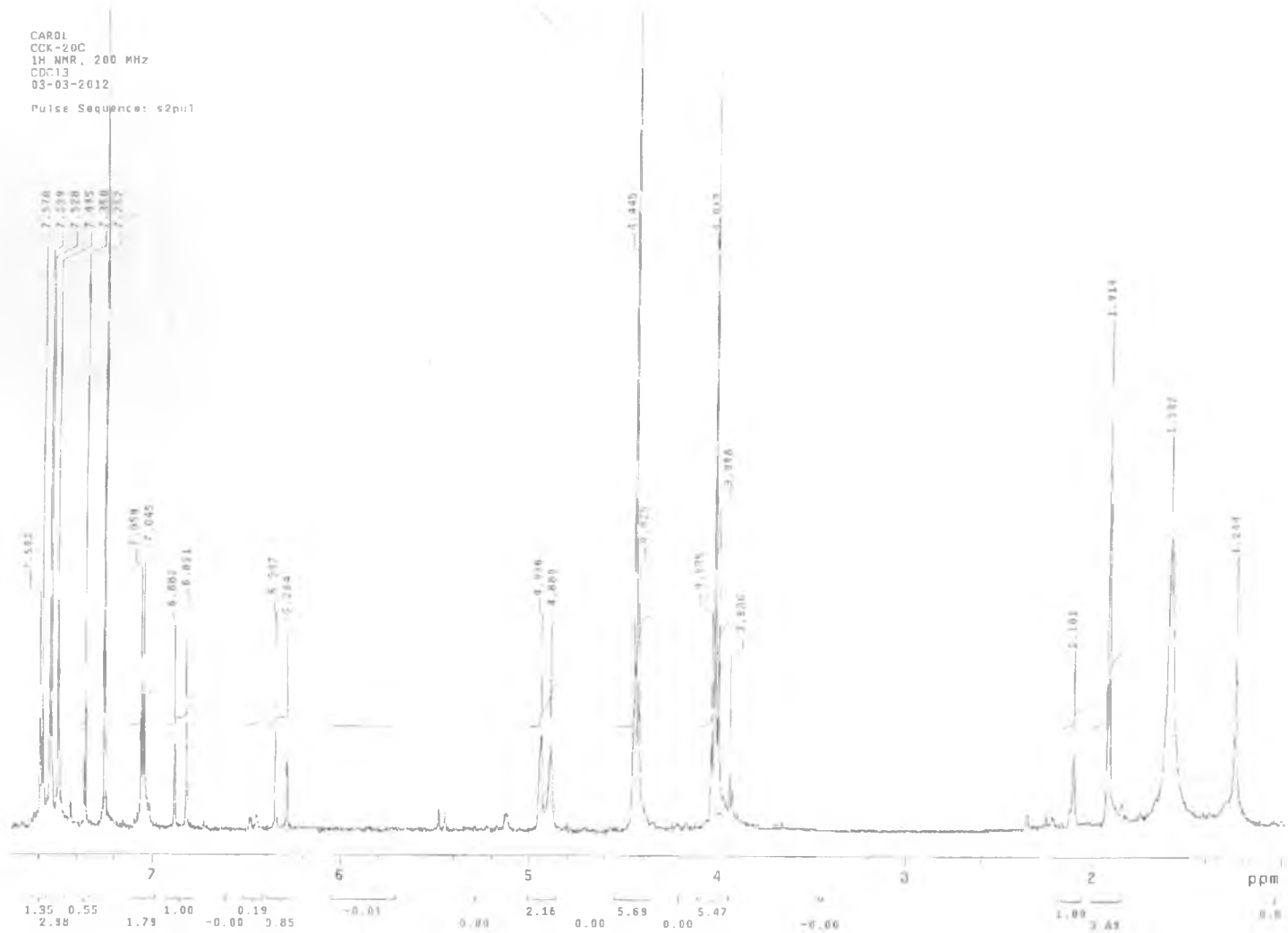
NOEDIF NMR SPECTRUM FOR COMPOUND 11

NOEDIF NMR SPECTRUM FOR COMPOUND 11

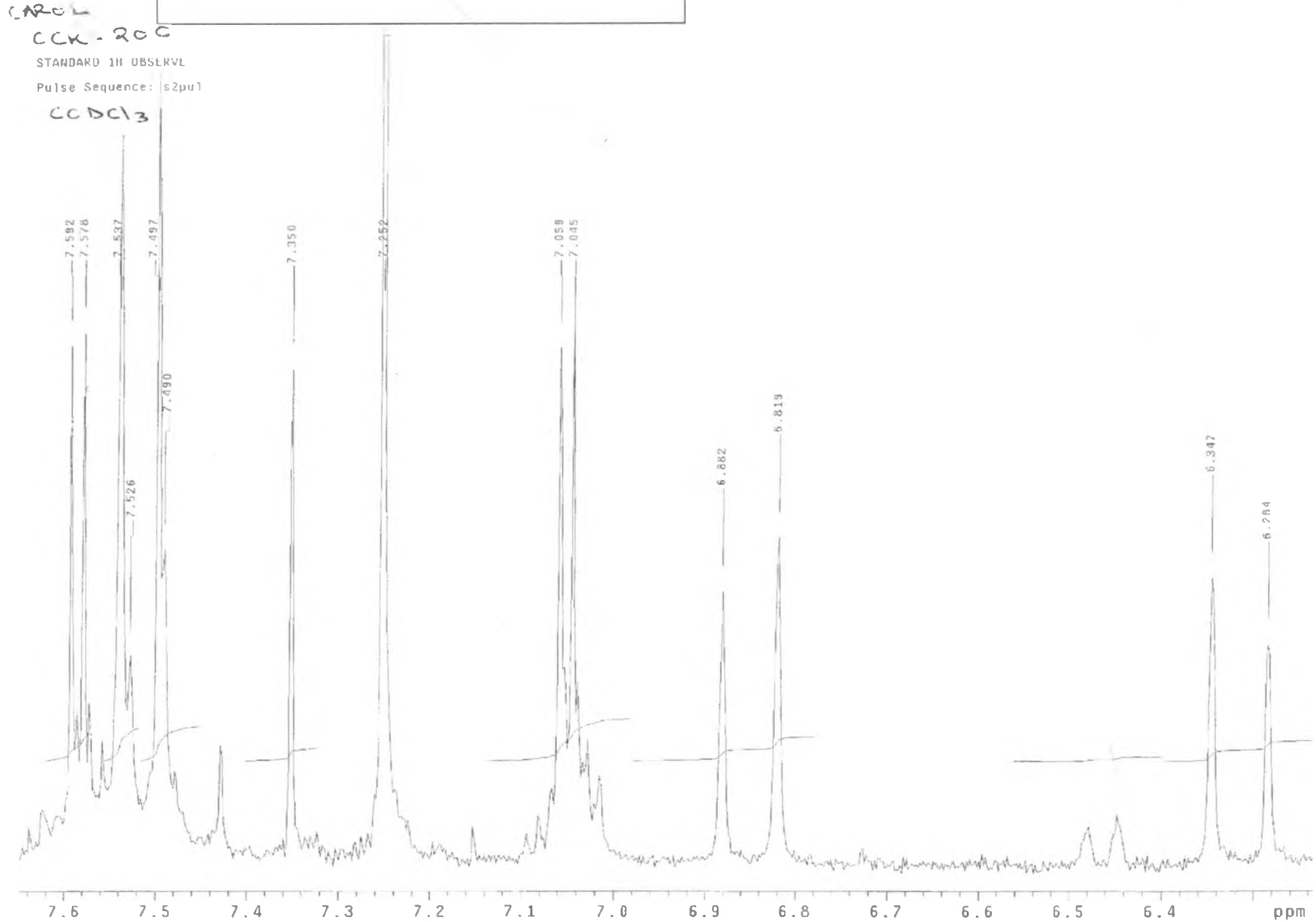


SPECTRA FOR COMPOUND 12

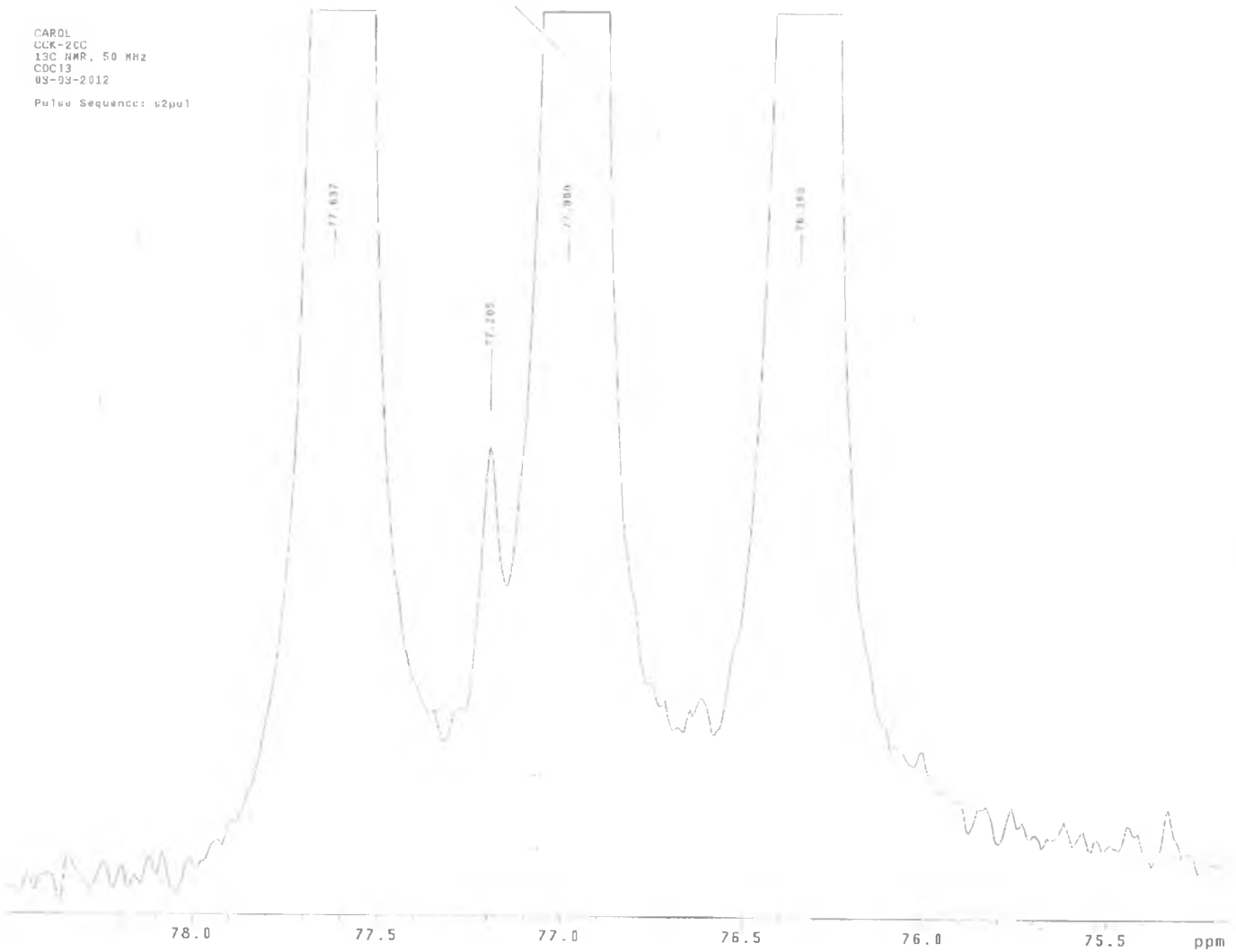
H NMR SPECTRUM FOR COMPOUND 12



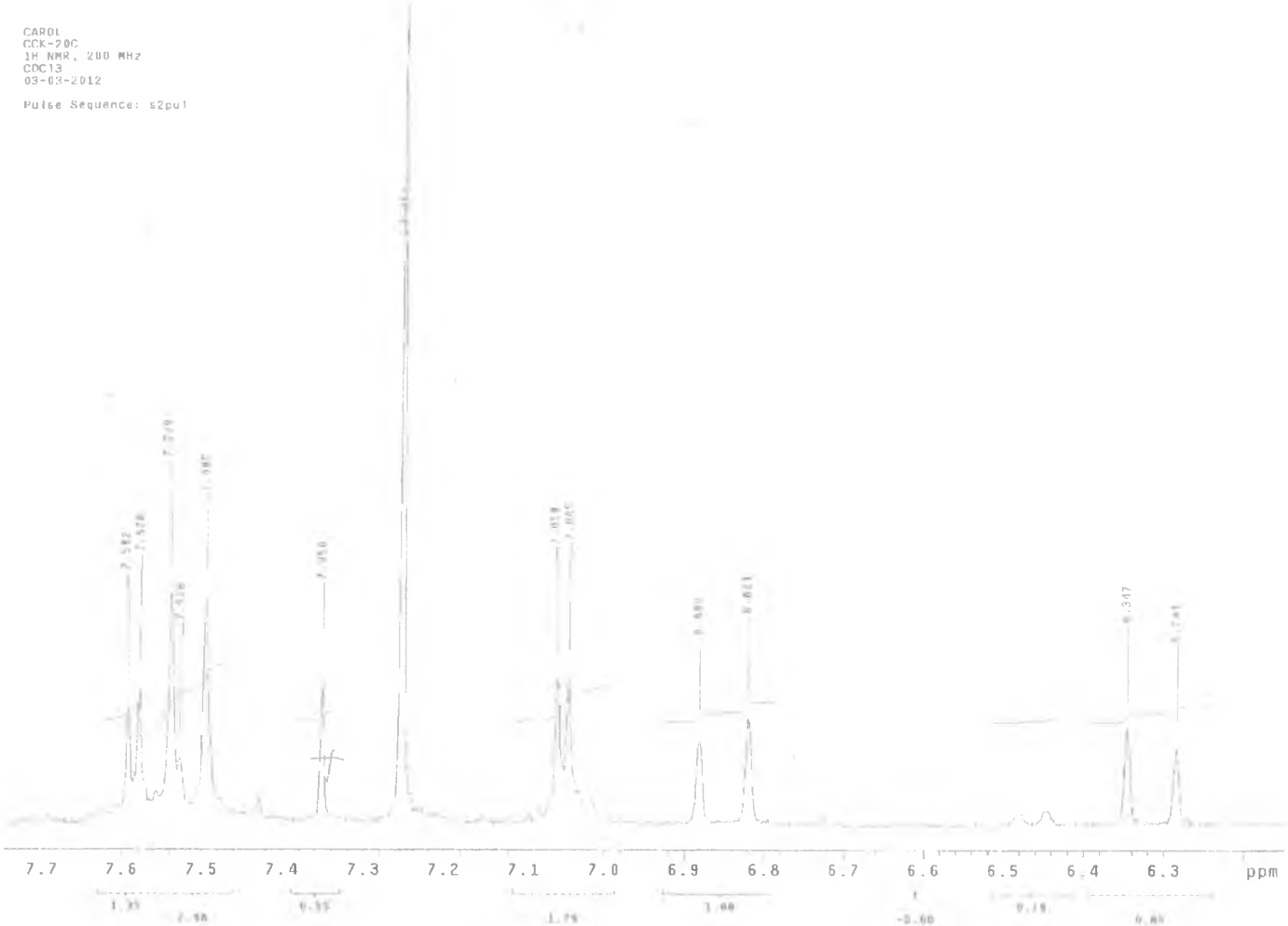
^{1}H NMR SPECTRUM FOR COMPOUND 12



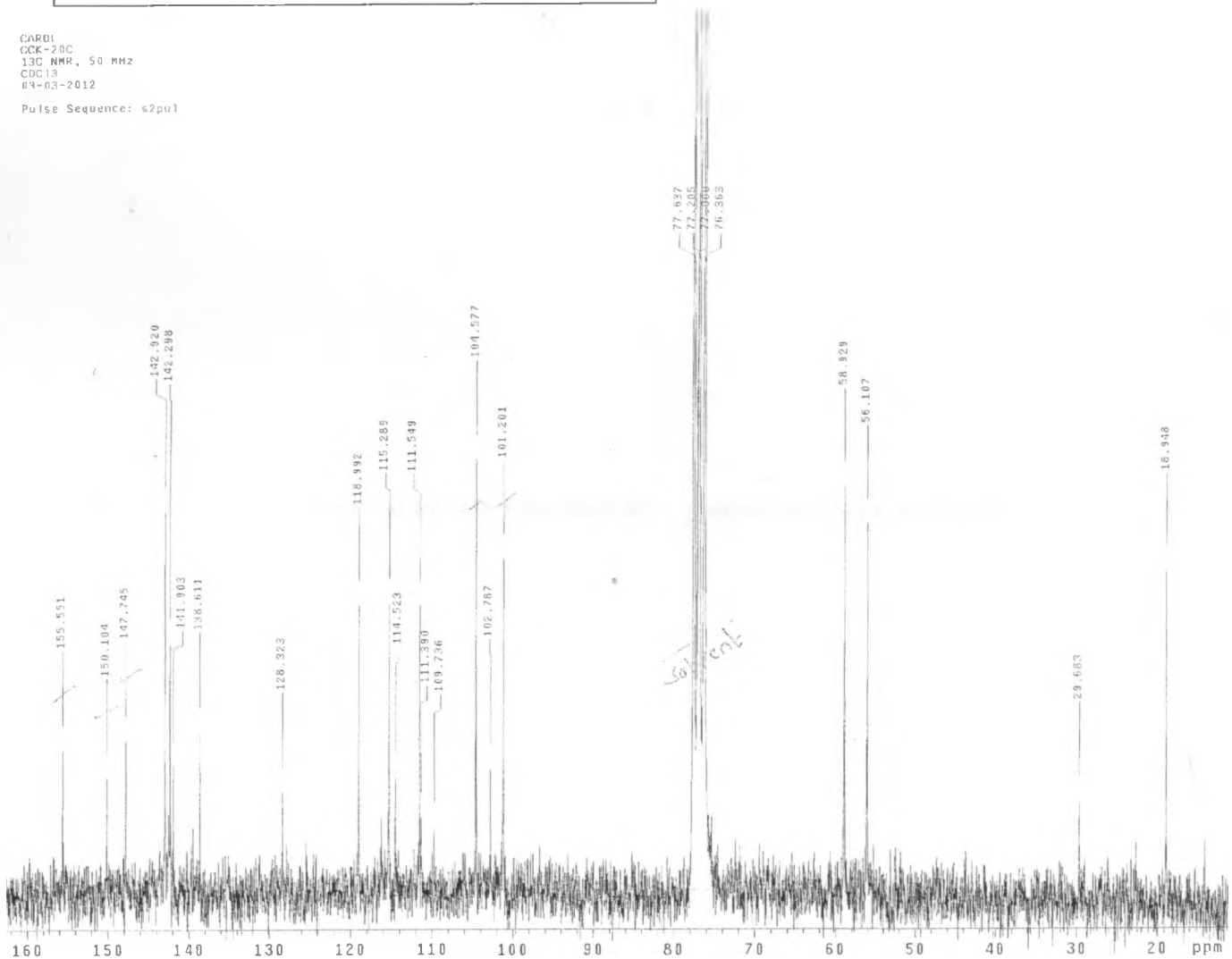
^{1}H NMR SPECTRUM FOR COMPOUND 12



^{1}H NMR SPECTRUM FOR COMPOUND 12



^{13}C NMR SPECTRUM FOR COMPOUND 12



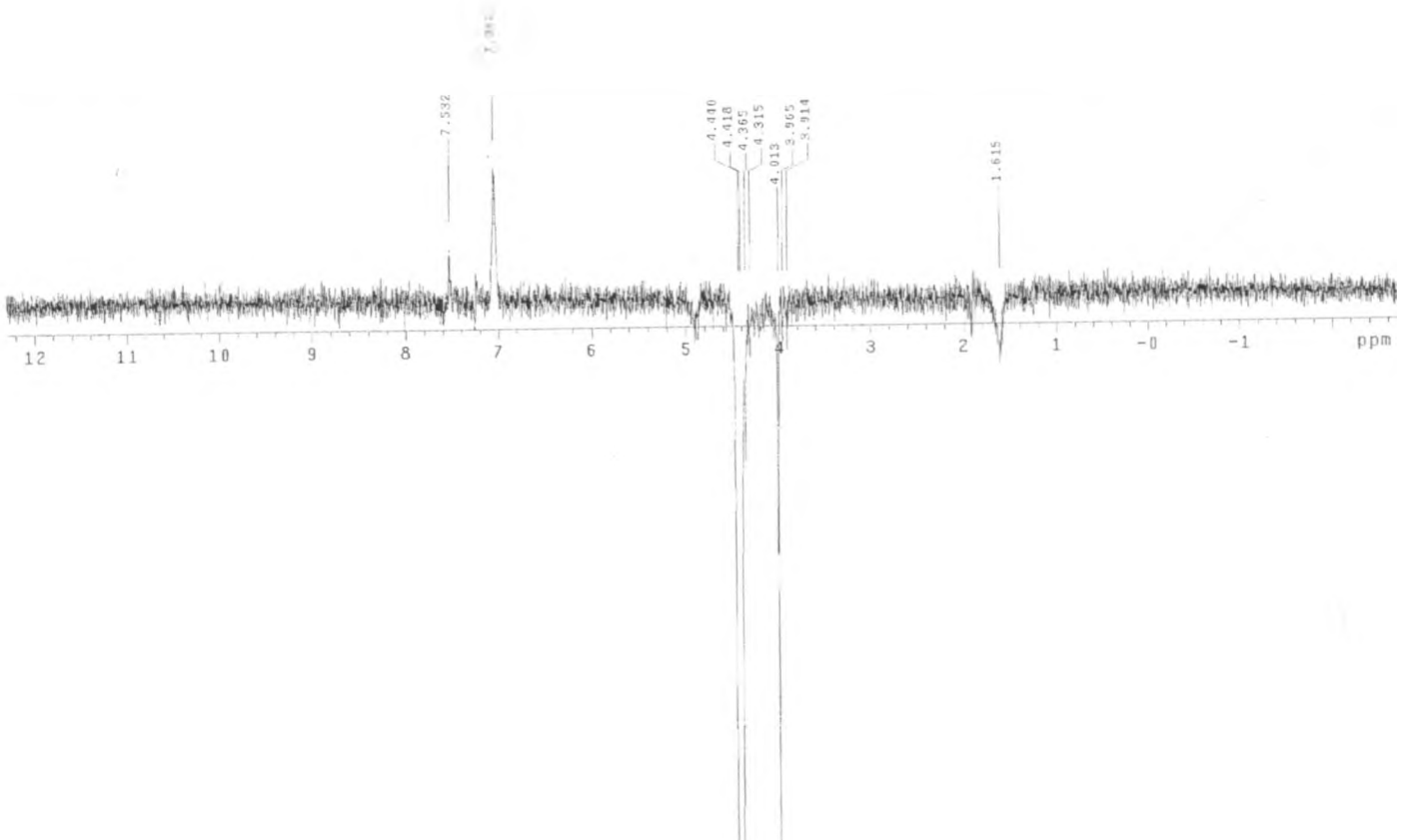
CAROL CCK-20C

STANDARD 1H OBSERVE

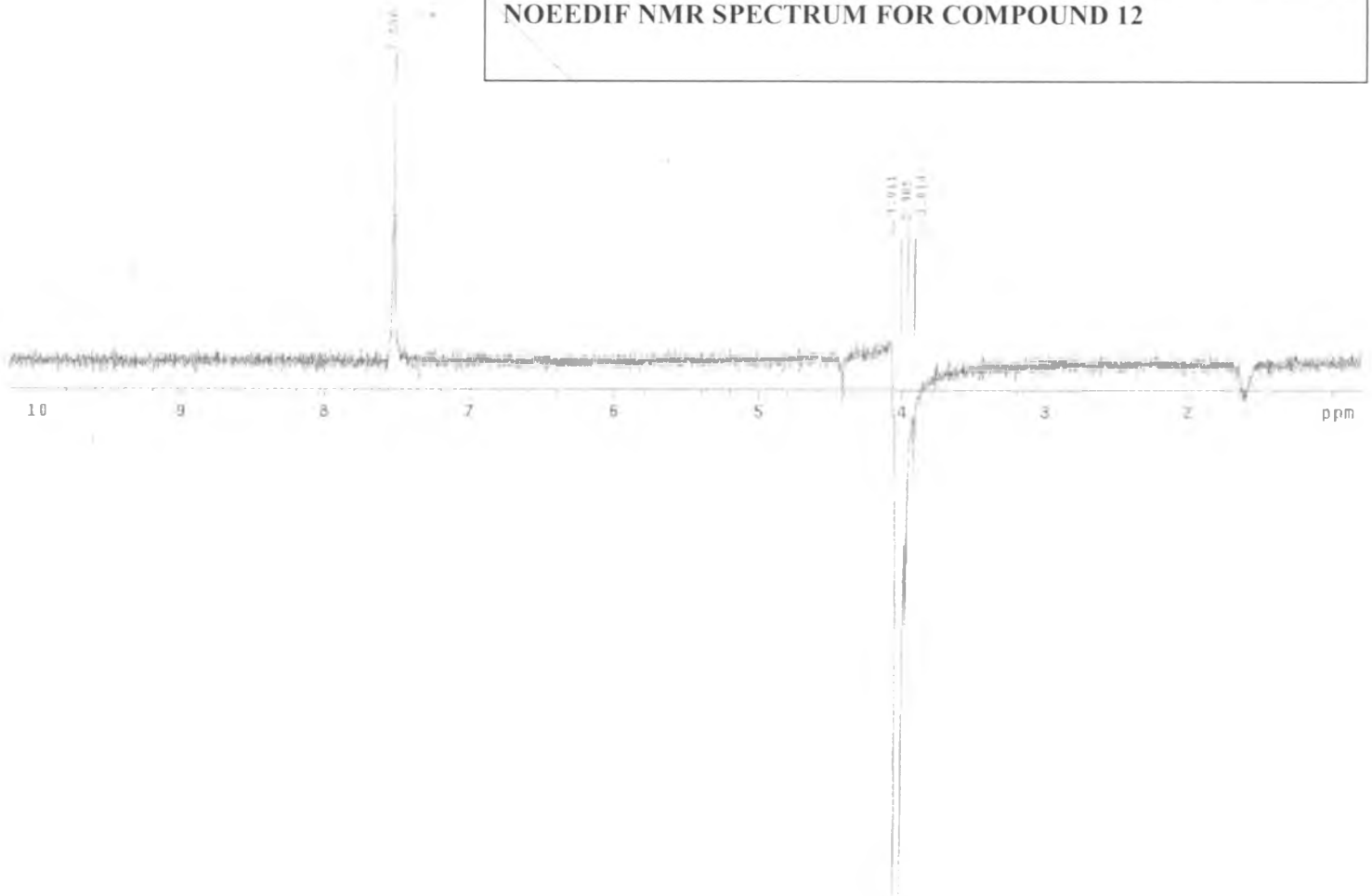
Pulse Sequence: noedif

NOE-DIF

NOEDIF NMR SPECTRUM FOR COMPOUND 12

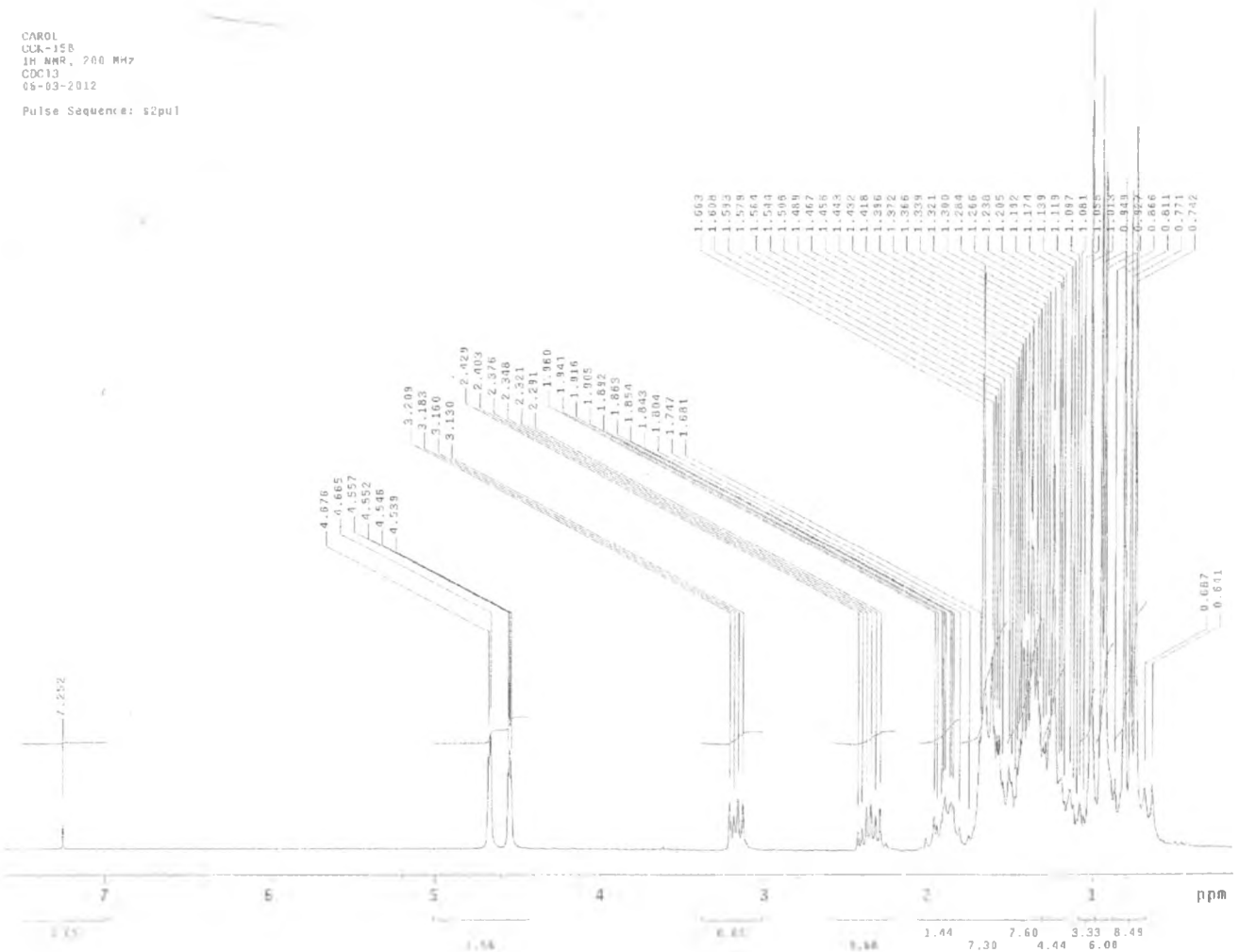


NOEEDIF NMR SPECTRUM FOR COMPOUND 12

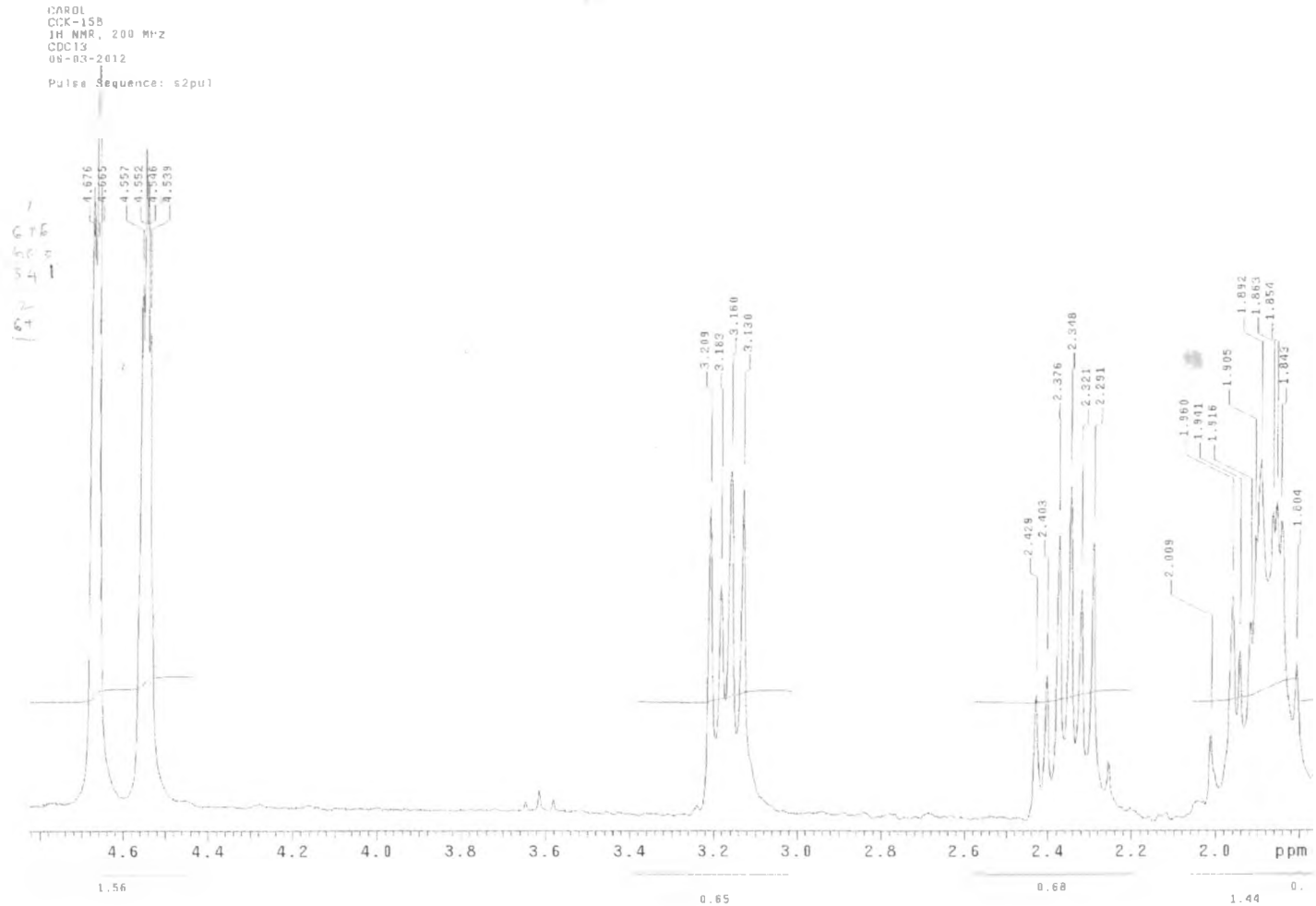


SPECTRA FOR COMPOUND 13

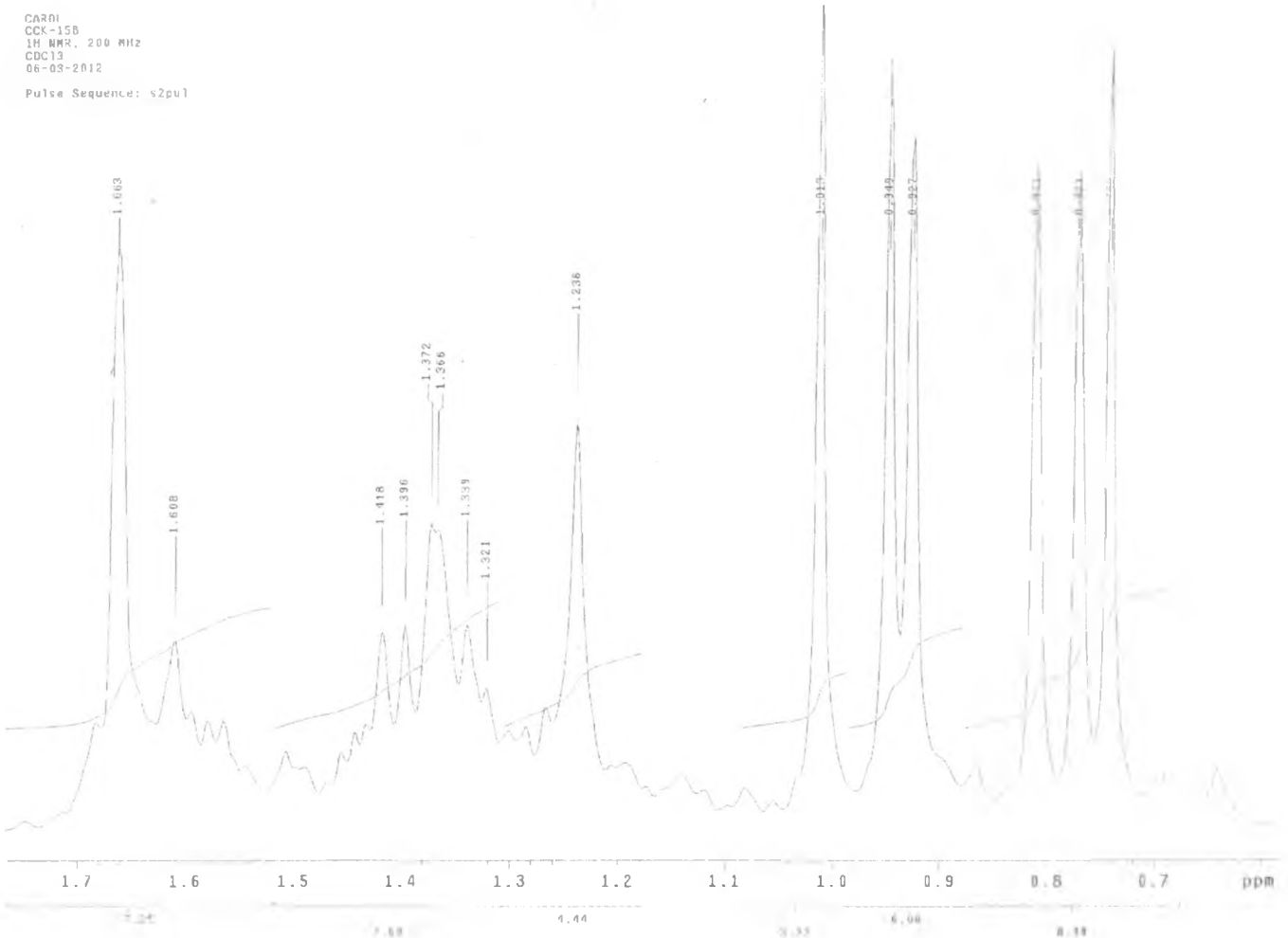
^{1}H NMR SPECTRUM FOR COMPOUND 13



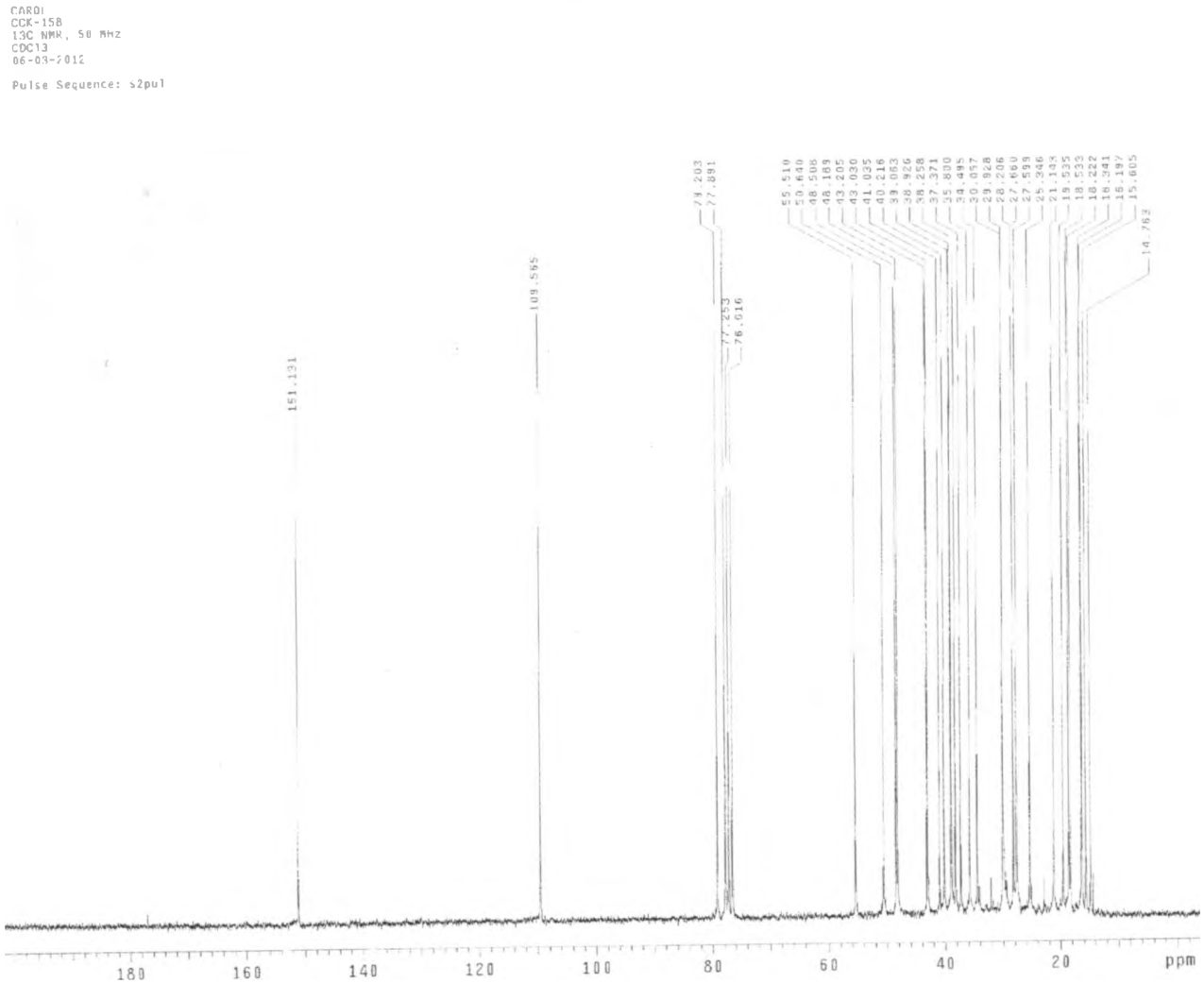
^{1}H NMR SPECTRUM FOR COMPOUND 13



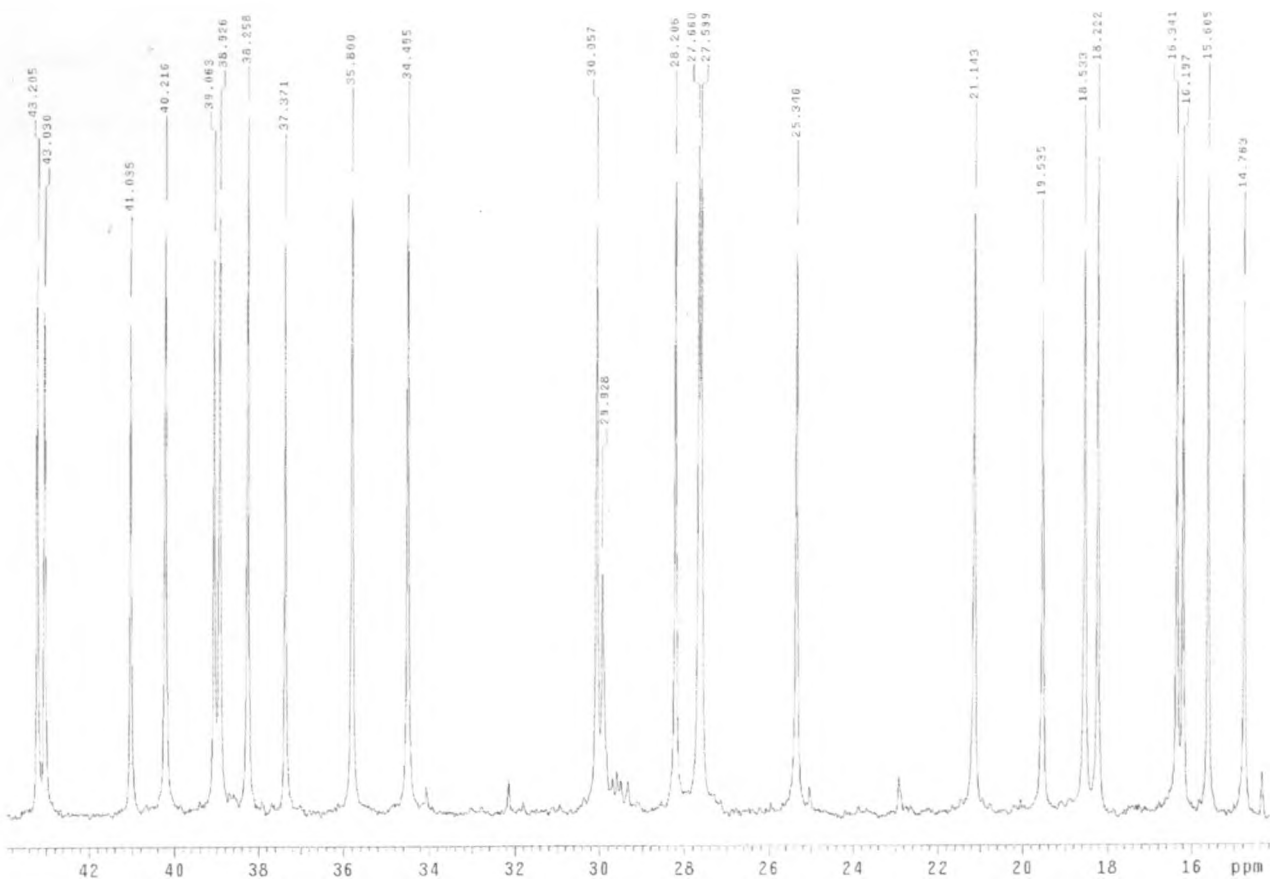
^{1}H NMR SPECTRUM FOR COMPOUND 13



^{13}C NMR SPECTRUM FOR COMPOUND 13

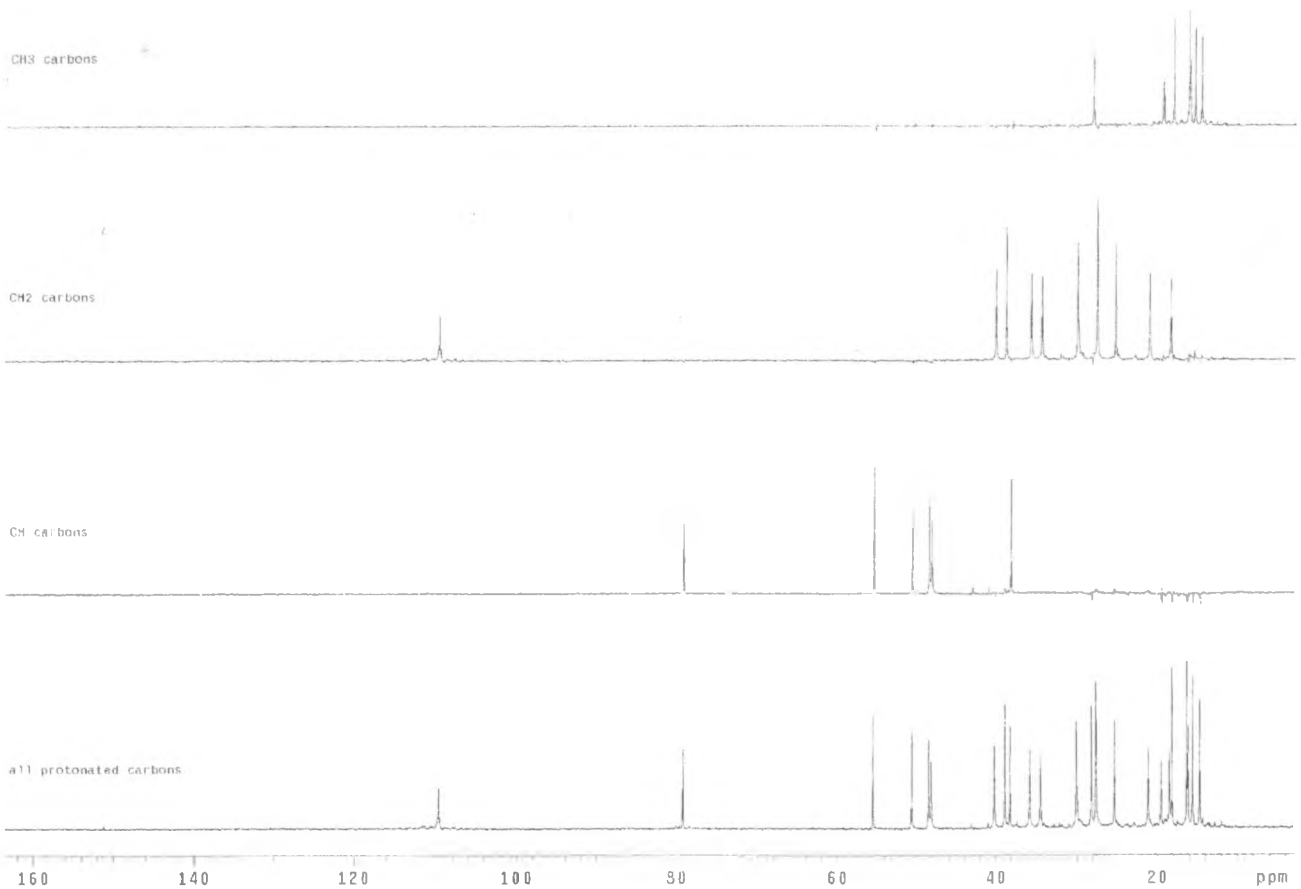


^{13}C NMR SPECTRUM FOR COMPOUND 13



CAROL
CCK-15B
CDCl3
DEPT
07/13/12

DEPT SPECTRUM FOR COMPOUND 13



DEPT SPECTRUM FOR COMPOUND 13

CAROL
CCK-15B
CDCl3
DEPT
07/3/12